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Towards the cloning of the human heat shock protein twenty-seven multigene family

Ann A Ohiaeri

University of Nevada, Las Vegas

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TOWARDS THE CLONING OF THE HUMAN
HEAT SHOCK PROTEIN TWENTY-SEVEN
MULTIGENE FAMILY

by

Ann A. Ohiaeri

Bachelor of Science
University of Nevada, Las Vegas
1995

A thesis submitted in partial fulfillment
of the requirements for the degree of

Master of Science

in

Chemistry

**Department of Chemistry
University of Nevada, Las Vegas
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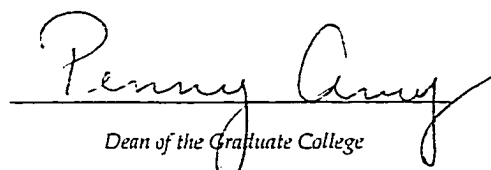
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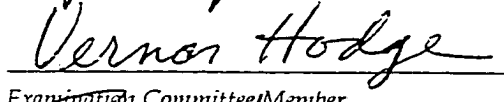
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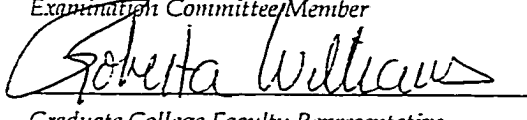
Master Of Science in Chemistry


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ABSTRACT

Towards the Cloning of the Human Heat Shock Protein Twenty-Seven Multigene Family

by

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Dr. Stephen Carper, Examination Committee Chair
Associate Professor of Chemistry
University of Nevada, Las Vegas

We attempted to clone closely-related members of the human heat shock protein 27 (hsp 27) multigene family. Genomic DNA was obtained from human A549 lung cancer cells. Colony hybridization and the Polymerase chain reaction identified the hsp 27 genes. Positive clones were subcloned into pGEM-3Z. The DNA sequence information for three of the clones was determined, and analyzed using DNAsis and Blast Algorithms. DNA Alignments of the inserts contained in three positive clones showed a homology of 29-51% to the human hsp 27 gene. Our data suggests that these inserts are distant members of the human hsp 27 multigene family. Blast searches did not determine the identity of these inserts in GenBank and SwissProt databases (11/1/98). This result indicated that the inserts are novel sequences. Hence, the nucleotide information for the inserts was submitted to GenBank (Accession Numbers: AF120329, AF120330, AF120331, AF120332, and AF120333).

TABLE OF CONTENTS

ABSTRACT	iii
LIST OF FIGURES	ix
LIST OF TABLES	x
ACKNOWLEDGMENTS	xi
CHAPTER 1 INTRODUCTION	1
Cancer	1
Heat Shock Proteins	2
Human hsp 27 - Protein	3
Human hsp 27 - Gene	3
Other Genes Related To Human hsp 27	5
Objective of The Study	6
CHAPTER 2 MATERIALS AND METHODS	7
General Overview	7
Bacterial Strains and Growth Conditions	16
Parental Plasmids	17
Enzymes	18
Genomic Library Construction	19
Colony Hybridization	19
Subcloning	21
PCR Analysis	23
Sizing of Fragments	25
Restriction Mapping	26
DNA Sequencing	27
DNAsis Software Analysis	27
Blast Analysis	30
CHAPTER 3 RESULTS	32
Overview	32
Colony Hybridization	33

Subcloning	35
PCR Analysis	35
Sizing Of Fragments	37
Restriction Mapping	39
DNA Sequence Analysis	40
DNAsis Analysis - DNA Level	43
Blast Analysis - DNA Level	48
DNAsis Analysis - Protein Level	50
Blast Analysis - Protein Level	51
PCR Primer Location Analysis	54
Other Positive Clone Information	56
DNA 1 - Size And Restriction Map	56
DNA 1 - DNA Sequence	56
DNA 1 - DNAsis Analysis (DNA Level)	59
DNA 1 - Blast Analysis (DNA Level)	63
DNA 1 - DNAsis Analysis (Protein Level)	63
DNA 1 - Blast Analysis (Protein Level)	73
DNA 1 - PCR Primer Location Analysis	78
DNA 2 - Size And Restriction Map	78
DNA 2 - DNA Sequence	78
DNA 2 - DNAsis Analysis (DNA Level)	80
DNA 2 - Blast Analysis (DNA Level)	83
DNA 2 - DNAsis Analysis (Protein Level)	83
DNA 2 - Blast Analysis (Protein Level)	90
DNA 2 - PCR Primer Location Analysis	95
Summary	95
DNA 3	95
DNA 5	98
DNA 1 vs. DNA 2	98
DNA 4 vs. DNA 2/1	99
Nucleotide Sequence Accession Numbers	101
CHAPTER 4 DISCUSSION	102
APPENDIX I	108
Plasmid Map For pGEM-3Z	109
APPENDIX II	110
DNA Sequence For pGEM-3Z	111
DNA Sequence For Human hsp 27 cDNA	114
DNA Sequence For The Human hsp 27 Gene	115

DNA Sequence For DNA 4/T7	118
DNA Sequence For DNA 4/SP6	119
Complete DNA Sequence For DNA 4	120
DNA Sequence For DNA 1/T7	122
DNA Sequence For DNA 1/SP6	124
DNA Sequence For DNA 1/HIND3/SP6	125
Partial DNA Sequence For DNA 1/Region 3+2	127
DNA Sequence For DNA 2/T7	128
DNA Sequence For DNA 2/SP6	129
 APPENDIX III	 130
Amino Acid Sequence For Human hsp 27	131
 APPENDIX IV	 132
Alignment Of DNA 4-1 (Normal Strand), And DNA 4-2 (Complementary Strand)	133
Alignment Of Human hsp 27 cDNA, And The Human hsp 27 Gene	135
Alignment Of DNA 4 (Normal Strand), And The Human hsp 27 Gene	139
Alignment Of DNA 4 (Complementary Strand), And The Human hsp 27 Gene	143
Alignment Of DNA 1/Region 1 (Normal Strand), And The Human hsp 27 Gene	147
Alignment Of DNA 1/Region 1 (Complementary Strand), And The Human hsp 27 Gene	151
Alignment Of DNA 1/Region 2 (Normal Strand), And The Human hsp 27 Gene	155
Alignment Of DNA 1/Region 2 (Complementary Strand), And The Human hsp 27 Gene	159
Alignment Of DNA 1/Region 3 (Normal Strand), And The Human hsp 27 Gene	163
Alignment Of DNA 1/Region 3 (Complementary Strand), And The Human hsp 27 Gene	167
Alignment Of DNA 2/Region 1 (Normal Strand), And The Human hsp 27 Gene	171
Alignment Of DNA 2/Region 1 (Complementary Strand), And The Human hsp 27 Gene	175
Alignment Of DNA 2/Region 2 (Normal Strand), And The Human hsp 27 Gene	179
Alignment Of DNA 2/Region 2 (Complementary Strand),	

And The Human hsp 27 Gene	183
APPENDIX V	187
Alignment Of DNA 4 (Normal Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	188
Alignment Of DNA 4 (Complementary Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	190
Alignment Of DNA 1/Region 1 (Normal Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	192
Alignment Of DNA 1/Region 1 (Complementary Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	194
Alignment Of DNA 1/Region 2 (Normal Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	196
Alignment Of DNA 1/Region 2 (Complementary Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	198
Alignment Of DNA 1/Region 3 (Normal Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	200
Alignment Of DNA 1/Region 3 (Complementary Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	202
Alignment Of DNA 2/Region 1 (Normal Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	204
Alignment Of DNA 2/Region 1 (Complementary Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	206
Alignment Of DNA 2/Region 2 (Normal Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	208
Alignment Of DNA 2/Region 2 (Complementary Strand), And The Most Similar-sized, Homologous Area Of Human hsp 27	210
APPENDIX VI	212

Alignment Of DNA 1/Region 1 (Normal Strand), And DNA 2/Region 1 (Normal Strand)	213
Alignment Of DNA 1/Region 2 (Normal Strand), And DNA 2/Region 2 Normal Strand)	215
Alignment Of DNA 4 (Normal Strand), And DNA 2/Region 1 (Normal Strand)	217
Alignment Of DNA 4 (Normal Strand), And DNA 2/Region 2 (Normal Strand)	219
REFERENCES	221
VITA	224

LIST OF FIGURES

Figure 1.1	General Overview Flowchart	8
Figure 1.2	Construction Of A Genomic Library	9
Figure 1.3	Colony Hybridization	11
Figure 1.4	Subcloning	12
Figure 1.5	Polymerase Chain Reaction	14
Figure 1.6	Hybridization Sites For PCR Primers	24
Figure 2.1	Colony Hybridization Results	34
Figure 2.2	Gel Electrophoresis Results For PCR Analysis Of DNA 4	36
Figure 2.3	Gel Electrophoresis Results For Sizing Of DNA 4	38
Figure 2.4	Plasmid Map For pDNA 4	41
Figure 2.5	Sequencing Strategy For DNA 4	42
Figure 2.6	Homologous DNA Areas Between DNA 4-1 (Normal Strand), And DNA 4-2 (Complementary Strand)	45
Figure 2.7	Homologous DNA Areas Between DNA 4 (Normal And Complementary Strands), And The Human hsp 27 Gene	47
Figure 3.1	Plasmid Map For pDNA 1	57
Figure 3.2	Sequencing Strategy For DNA 1	58
Figure 3.3	Homologous DNA Areas Between Regions Of DNA 1 (Normal And Complementary Strands), And The Human hsp 27 Gene	62
Figure 4.1	Plasmid Map For pDNA 2	79
Figure 4.2	Sequencing Strategy For pDNA 2	81
Figure 4.3	Homologous DNA Areas Between Regions Of DNA 2 (Normal And Complementary Strands), And The Human hsp 27 Gene	84
Figure 5.1	Plasmid Map For pDNA 3	97
Figure 6.1	Comparison Between DNA 1 And DNA 2	100

LIST OF TABLES

Table 1.1	Blastn Results For DNA 4	49
Table 1.2	Open Reading Frames For DNA 4	52
Table 1.3	Amino Acid Alignments Between DNA 4 And Human hsp 27	53
Table 1.4	Blastx Results For DNA 4	55
Table 2.1	DNA Alignments Between Areas Of DNA 1 And The Human hsp 27 Gene	61
Table 2.2	Blastn Results For DNA 1/Region 1	64
Table 2.3	Blastn Results For DNA 1/Region 2	65
Table 2.4	Blastn Results For DNA 1/Region 3	66
Table 2.5	Open Reading Frames For DNA 1/Region 1	68
Table 2.6	Amino Acid Alignments Between DNA 1/Region 1 And Human hsp 27	70
Table 2.7	Open Reading Frames For DNA 1/Region 2	71
Table 2.8	Amino Acid Alignments Between DNA 1/Region 2 And Human hsp 27	74
Table 2.9	Open Reading Frames For DNA 1/Region 3	75
Table 2.10	Amino Acid Alignments Between DNA 1/Region 3 And Human hsp 27	77
Table 3.1	DNA Alignments Between Regions Of DNA 2 And The Human hsp 27 Gene	82
Table 3.2	Blastn Results For DNA 2/Region 1	85
Table 3.3	Blastn Results For DNA 2/Region 2	86
Table 3.4	Open Reading Frames For DNA 2/Region 1	88
Table 3.5	Amino Acid Alignments Between DNA 2/Region 1 And Human hsp 27	89
Table 3.6	Open Reading Frames For DNA 2/Region 2	91
Table 3.7	Amino Acid Alignments Between DNA 2/Region 2 And Human hsp 27	93
Table 3.8	Blastx Results For DNA 2/Region 2	94
Table 4.1	Summary Of DNA And Amino Acid Alignments Between Human hsp 27 And DNA 4, DNA1 And DNA 2	96

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CHAPTER 1

INTRODUCTION

Cancer

Cancer is a disease characterized by the uncontrollable proliferation of cells. Cancer is the second leading cause of death in this country, today. According to studies conducted by the American Cancer Society in 1998, over 180,000 women will be diagnosed with breast cancer. Of these women, 44,000 will die from the disease. We desire to increase the number of women surviving breast cancer. However, chemotherapy has been hampered by the expression of certain proteins that enable cancer cells to survive the adjuvant therapies designed to destroy them. In order to develop better treatments for breast cancer, more studies examining the proteins involved in protecting breast cancer cells from death are required. Specifically, more information regarding the genes involved in the expression of these proteins is needed.

Human hsp 27 can be found at high levels in human breast tumor cells (1). Reports indicated that in these human breast tumors that express high levels of hsp 27, tumor cells come back sooner (2). Hence, identifying and studying all of the genes involved in the

expression of hsp 27 should ultimately lead to the development of more effective therapies for the treatment of breast cancer.

Heat Shock Proteins

Heat shock proteins are found in all living cells. Following the exposure of cells to stresses, there is an increase in the synthesis of these proteins. The production of heat shock proteins (hsps) protect cells from a wide variety of stresses such as a sudden increase in temperature, heavy metals, oxygen deprivation, and metabolic poisons (3-4). Other stresses such as the exposure to hormones, growth factors, and viruses also induce the heat shock response.

There are five major classes of heat shock proteins that are expressed in humans. These include hsp 110, hsp 90, hsp 70, hsp 60 and hsp 27. These heat shock proteins are classified according to their apparent molecular weight in kilodaltons. Hsp 110 is involved in the import of proteins into the mitochondrial compartment of a cell (5). Hsp 90 associates with steroid hormone receptors, and maintains them in an inactive state until they bind to their hormone (6). The ability of Hsp 70 to unfold proteins, via an ATP-dependent mechanism, allows for the import of these proteins into sub-cellular locations of the cell (7-8). Hsp 70 also associates with newly-synthesized proteins, assisting in their correct folding at desirable times. Hsp 60 possesses an ATPase activity. Hsp 60 and hsp 10 assist in the folding of proteins into the correct conformation through a barrel-shaped structure (9). Hence, the common feature amongst these classes of heat shock proteins is that they function as molecular chaperones in protein folding.

Human hsp 27 - Protein

Human hsp 27 is approximately 27,000 Da in size. Several functions for human hsp 27 have been reported. These include the development of thermotolerance following a heat shock (10-11), and the ability to function as an actin cap-binding protein (12). Human hsp 27 has also been reported to function as a molecular chaperone (13), and confers drug-resistance to cytotoxic drugs (14).

Human hsp 27 - Gene

Genes are located on chromosomes within the nucleus of a cell. A gene is a piece of double-stranded DNA that contains all the information necessary for the synthesis of a functional protein. A gene is made up of a promoter region (located at the 5'-end, and responsible for gene regulation), exons (that code for the synthesis of a protein), and introns (non-coding intervening regions). For a gene to synthesize a protein, it must first be converted into RNA (transcription). Transcription begins at a site known as the TATA box. RNA processing occurs, which involves the removal of introns, the joining of the exon regions (splicing), the addition of a 7-methylguanosine cap at the 5'-end, and the addition of a poly-A tail at the 3'-end. Following the poly-A tail, there is a 3'-untranslated region. The RNA is then further converted into a protein (translation). Translation usually begins at the first ATG site.

It has been suggested that human hsp 27 is made up of a multigene family. Studies conducted by Hickey et al. (15) indicated the presence of four genes encoding human hsp 27, including two linked genes and one pseudogene. Linked genes are located close to each other on a chromosome. Pseudogenes are genes that have evolved over time and lost their function.

Other findings by McGuire et al. (16) reported the presence of at least three genes encoding for human hsp 27. Our research confirmed the presence of three hsp 27 genes in the human lung cancer cell line A549, and the presence of four hsp 27 genes in the human breast cancer cell line MCF-7 (17). The three human hsp 27 genes are located on chromosomes 3, 9 and X (16).

The genomic DNA and pseudogene that encode human hsp 27 have been sequenced (15). The human hsp 27 gene contains three exons, and two introns. The 5'-promoter region contains two TATA sequences (hence, two transcription start sites), and an SP1 binding site. The SP1 binding site is a sequence that a transcription factor binds to, resulting in an increase in transcriptional efficiency. The promoter region also contains a heat shock element (HSE). The HSE contains a pentameric DNA regulatory motif (GAAnnTTC), that is responsible for the induction of the gene following a heat shock. The pseudogene does not possess promoter elements or intervening sequences. The complementary DNA (cDNA), encoding hsp 27 has also been sequenced (17). Carper et al. (17) reports a mistake in the gene sequence of hsp 27, as reported by Hickey et al. (1986). The corrected amino acid sequence for human hsp 27 is 100% identical to the previous amino acid sequence in the first 193 amino acids.

Additional human hsp 27 promoter region information has been reported by Oesterreich et. al (18). Hickey et al. (1986) previously reported only 200 base pairs of 5'-information in the promoter region of the gene. In the promoter region there are also two CAAT boxes, a G/C region, an additional SP1 site, two AP2 sites, and an estrogen responsive element half-sites (ERE). The AP2 binding site is a sequence that a transcription factor binds to, resulting in an increase in transcriptional efficiency. The ERE is a palindromic, regulatory DNA motif (GGTCA), located near the first TATA box. It has been

proposed that the ERE, and the proximal TATA box are responsible for the regulation of human hsp 27 by the hormone, estrogen.

Other Genes Related To Human Hsp 27

In addition to the genes that make up the human hsp 27 multigene family, there are other genes that are related to human hsp 27. These include the alpha-crystallins, murine hsp 27, and rat hsp 27.

Alpha-crystallins are proteins which polymerize to form large aggregates. In mammals, alpha-crystallins are a major component of the eye lens. The aggregation undergone by alpha-crystallins, plays a crucial role in determining the properties of the eye lens. The alpha-crystallins can act as molecular chaperones (19). The hsp 27 multigene family is related to the alpha-crystallins (20).

The structure and organization of the genes encoding murine hsp 27 has been determined (21-22). Unlike human hsp 27 which has at least three genes encoding for hsp 27, only two genes have been reported to encode hsp 27 in the mouse genome - one gene, and a pseudogene. Murine hsp 27 contains one more HSE than is found in human hsp 27. However, murine hsp 27 is similar to human hsp 27, as it is also made up of three exons, and two introns. The amino acid sequence of murine hsp 27 is 81% homologous to the predicted amino acid sequence for human hsp 27 (17).

The structure and organization of the genes encoding rat hsp 27 has been determined (23). Unlike human hsp 27, only one gene has been reported to encode rat hsp 27. This gene has three exons, and two introns. The promoter region of rat hsp 27 possesses more heat

shock elements, and SP1 sites than in seen in human hsp 27. The amino acid sequence of rat hsp 27 is 81% homologous to the predicted amino acid sequence of human hsp 27 (17).

Objective Of The Study

As discussed previously, identifying and studying all the human hsp 27 genes should lead to better treatments for breast cancer. The first step in this process involves finding all the members of the multigene family. The second step involves studying the regulation of these genes. The final step will examine methods to decrease the level of expression of these genes, in order to lead to better treatments for breast cancer patients. At this point in time, only the human hsp 27 gene and pseudogene members of the family, have been identified and studied. The linked genes have been mentioned, but have not been reported on in any subsequent studies. The present study was aimed at cloning the closely-related, linked gene members of the human hsp 27 multigene family. Our results, however, indicated that we had cloned three distantly-related members of the family. Searches within the GenBank database indicated that these inserts were unique sequences. Hence, the DNA sequences for these three inserts have been deposited with GenBank.

CHAPTER 2

MATERIALS AND METHODS

General Overview

The individual steps in recombinant DNA technology that were used to clone the genes are outlined in the general overview diagram shown in Figure 1.1 (p. 8). The process of cloning the DNA fragments began with the construction of a DNA library. This step was carried out in our lab by Michael Wright. The genomic DNA Library used in this study contained all the DNA from the human lung cancer cell line A549. Figure 1.2 (p. 9) depicts the individual steps involved in the construction of the genomic library. The DNA library was generated by partially digesting the genomic DNA with a specific restriction enzyme, producing restriction fragments of different sizes. The DNA was subjected to partial rather than complete digestion so that the genomic library contained intact representatives of all human genes, including those whose sequences contained restriction sites. The DNA fragments were separated by centrifugation in a gradient. DNA fragments of 15-23 kilobase pairs (kb) were then packaged into a lambda phage vector, which had also been digested with the same restriction enzyme.

Subsequent steps involved in the cloning of the genes were carried out by joint efforts

Genomic Library Construction



Colony Hybridization



Subcloning



PCR Analysis



Sizing of Fragments



Restriction Mapping



DNA Sequencing



DNAsis Software Analysis



Blast Algorithm Analysis



GenBank Submission

Figure 1.1 General Overview Flowchart. The steps involved in the cloning of the human hsp 27 multigene family are shown.

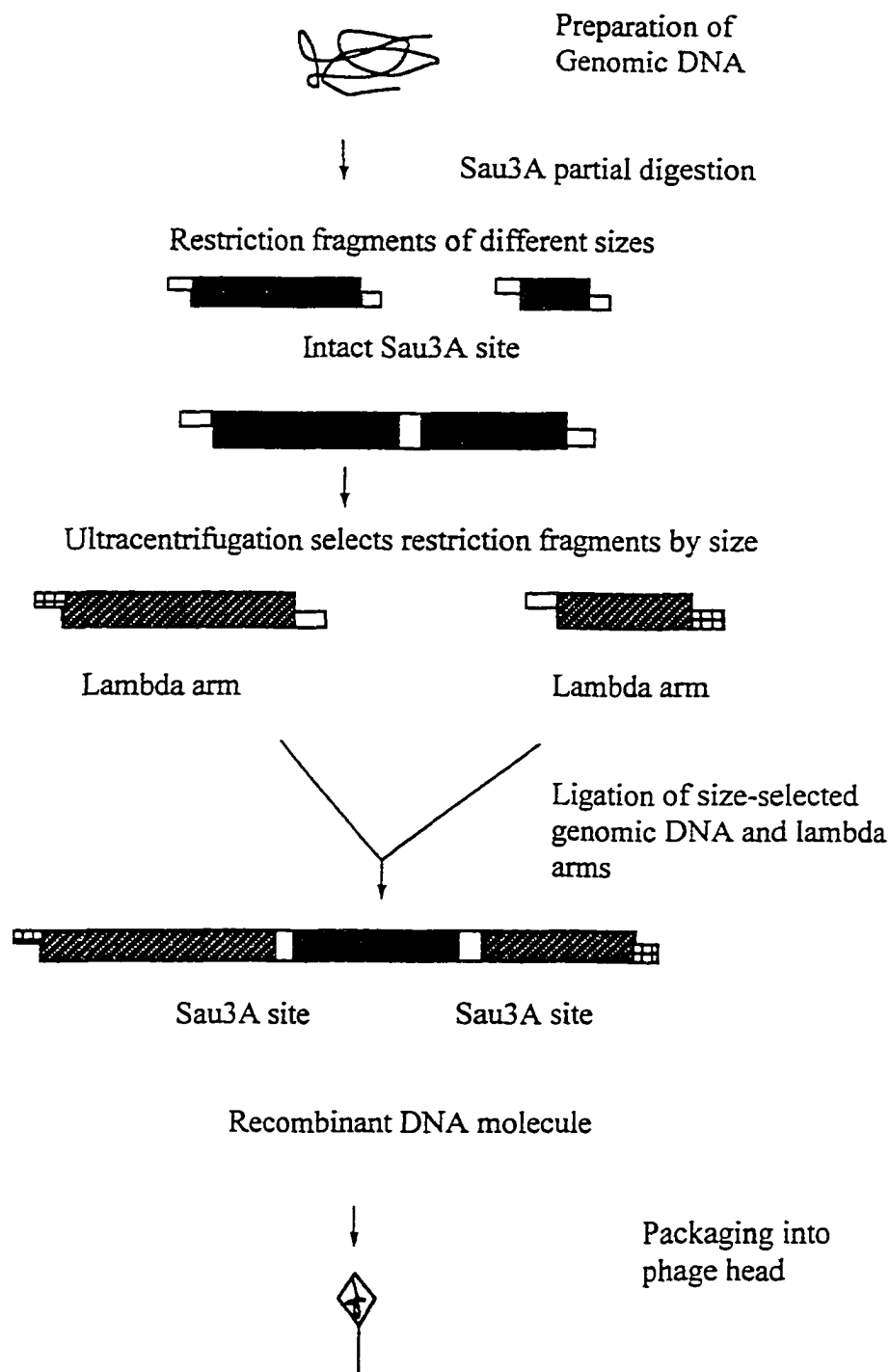


Figure 1.2 Construction Of A Genomic Library. The cloning of foreign DNA in lambda phage.

made by Dr. Santosh Dubey, Daniel Mazur, and myself. The human genomic library constructed in lambda phage was screened for hsp 27 genes. The lambda EMBL3 phage containing the genomic DNA from the A549 cell line was initially used to infect bacterial cells, which were plated on a petri dish. Colony hybridization was then used to identify the phage vectors that contained the desired DNA. Figure 1.3 (p. 11) depicts the individual steps involved in colony hybridization. Plaques (areas of clear lawn, signifying the location of the phages) were transferred to a filter. The filter was then treated to expose the DNA contained in the plaques. A probe, a DNA molecule specifically designed to bind to our desired DNA, was later allowed to anneal to the filter. Blackening on the filter identified plaques that had hybridized to our probe, and hence, contained our desired DNA.

The phage DNA containing the desired pieces of genomic DNA (the positive plaques), were isolated and purified. The phage DNA was removed, and the genomic DNA was enzymatically cut into smaller fragments. These small fragments were ligated into a plasmid vector for further analysis (subcloning). In this way, we narrowed our search for the hsp 27 genes from within larger pieces of DNA of 15-23 kb, to amongst smaller pieces of DNA (of about 5 kb in size). Figure 1.4 (p. 12) shows the individual steps involved in subcloning. The insert DNA fragment was cleaved with a specific restriction enzyme, to yield insert DNA fragments possessing this particular type of ends. The vector (the circular, double-stranded DNA that would hold the insert DNA), was also cleaved with the same restriction enzyme, producing vector DNA with identical ends. The complimentary ends of the insert DNA and the vector DNA, specifically associated under certain conditions, and covalently attached to each other via the action of the enzyme DNA ligase (ligation). This produced a recombinant plasmid.

Colonies containing phages

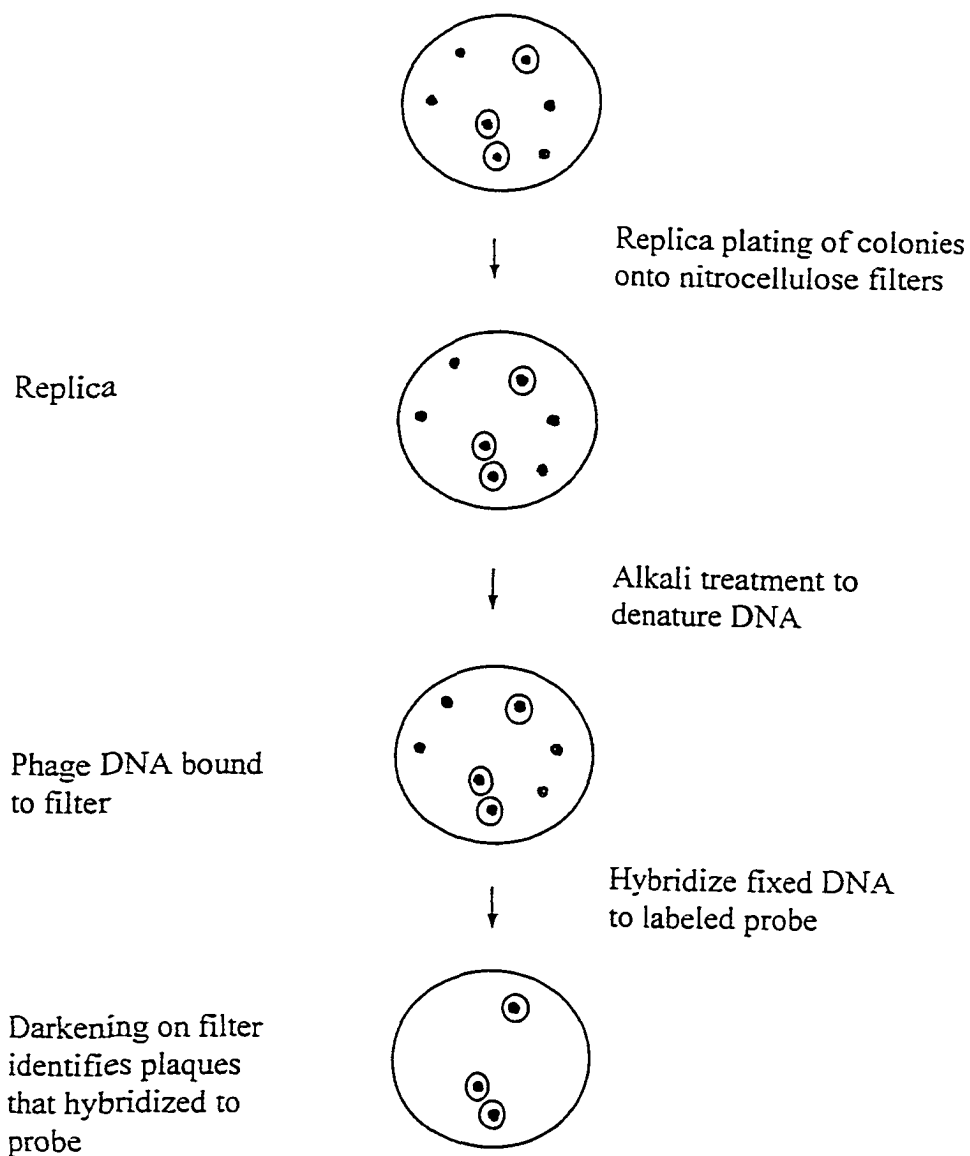


Figure 1.3 Colony Hybridization. This technique identifies the clones containing the desired DNA.

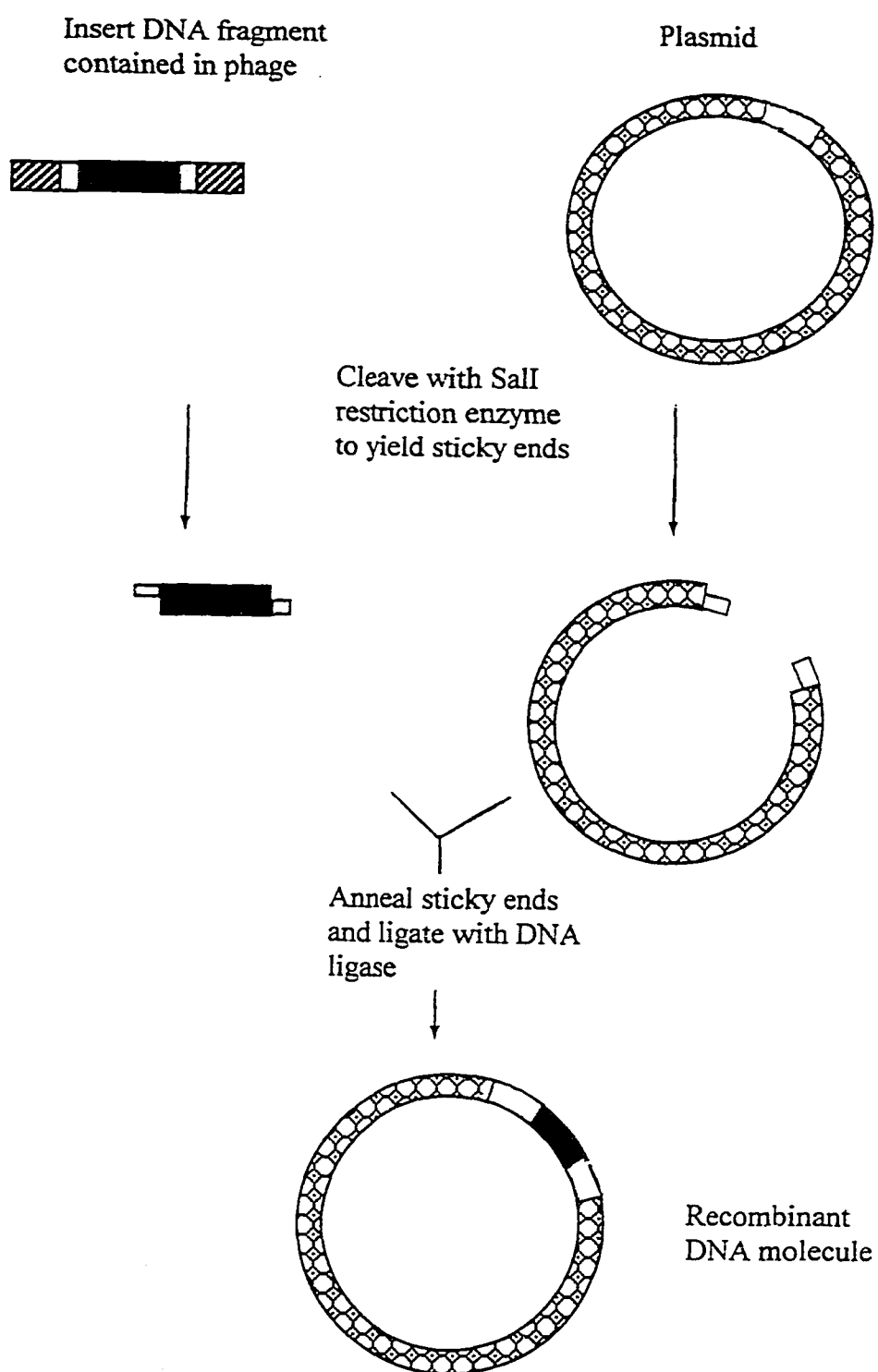


Figure 1.4 Subcloning. The construction of a recombinant DNA molecule through the insertion of an enzyme-digested-insert into a vector's corresponding restriction site.

The polymerase chain reaction (PCR), was used as a second technique to further determine if the DNA inserts in the recombinant plasmids were the desired hsp 27 genes. Figure 1.5 (p. 14) shows the individual steps involved in PCR. Heat was initially used to denature and separate the double-stranded DNA duplex into single strands. Then, a primer was added and allowed to anneal to the single-stranded DNA. The primer was a short DNA molecule specifically made to bind to a region on our desired DNA. When the enzyme DNA polymerase was later added, it catalyzed the extension of the primer, resulting in the synthesis of a new identical DNA molecule. In this way, thousands and thousands of copies of the DNA were made. It is important to note here that each cycle of PCR only amplified our desired hsp 27-related DNA. If the plasmid vector contained hsp 27 DNA, then a single 363 base pair (bp) PCR product was obtained, and the plasmid insert was further characterized.

Restriction endonucleases were used to determine the sizes of the DNA inserts contained within the positive clones. The positive clones were cleaved with restriction enzymes, and the fragments were separated on an agarose gel (gel electrophoresis). In gel electrophoresis, an electric field was used to separate our DNA molecules by allowing the molecules to migrate through a porous agarose gel. Smaller molecules migrated faster relative to the larger ones. A DNA size ladder was used to identify the molecular range of the fragments. Restriction maps showing the sites where particular enzymes cleaved the insert contained in the positive clones, were also generated with the information.

To determine the identity of the DNA sequence information, the positive clones were

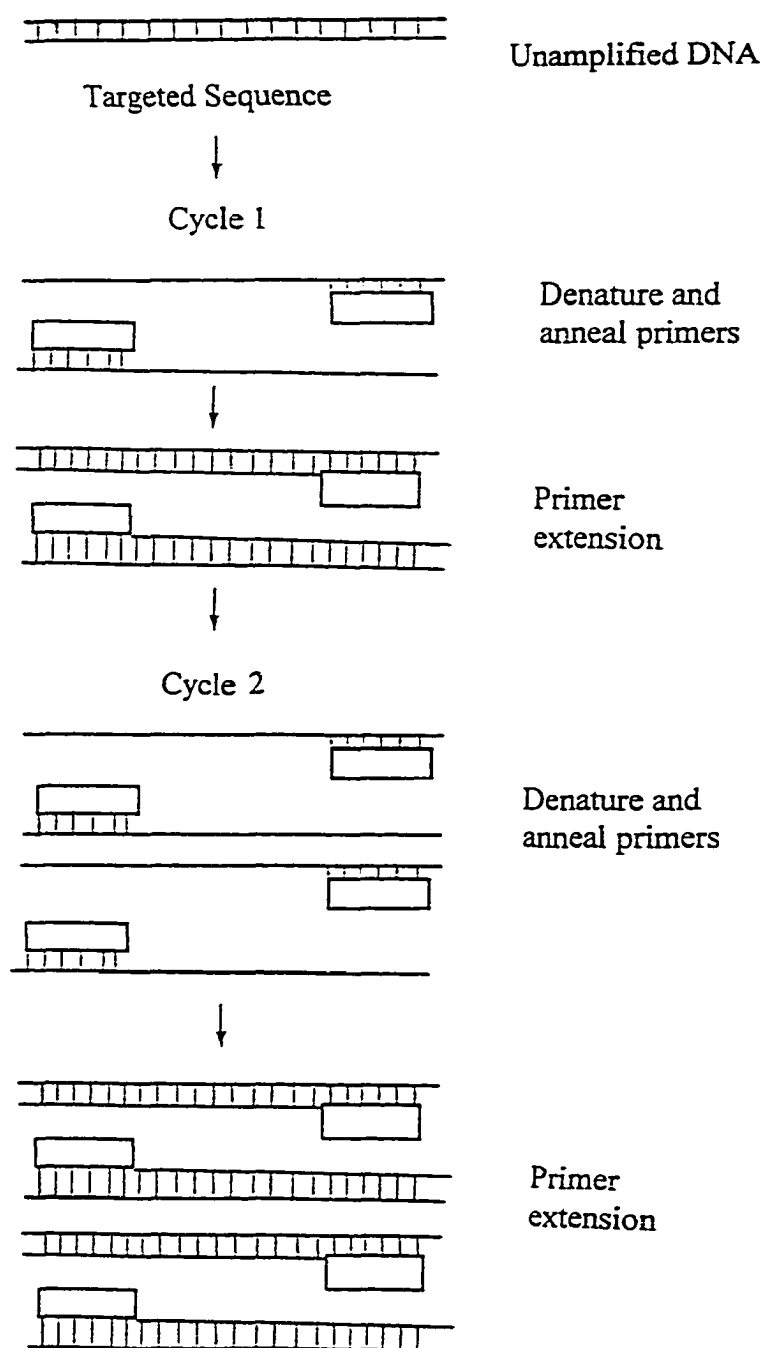


Figure 1.5 Polymerase Chain Reaction. In each cycle of the reaction, the double-stranded DNA is separated by heat denaturation, synthetic DNA primers anneal to a complementary segment on each strand, and the primers are extended by DNA Polymerase. The process is then repeated for numerous cycles.

transformed into bacterial cells, to make thousands of copies of the DNA. The plasmid DNA was extracted from the bacterial cells, purified and subjected to automated DNA sequencing.

The sequence information for the DNA inserts was initially analyzed using the DNAsis computer software (DNAsis Maximum Matching Alignment). This computer algorithm compared the similarity between two DNA sequences. The program was used to determine if the isolated DNA fragments were similar to hsp 27 in their nucleotide sequences.

In addition, we desired to compare the predicted amino acid sequences of the DNA inserts to the amino acid sequence of hsp 27. Therefore, we searched for sites in the DNA sequence that would indicate the coding strand for the amino acid sequence. This was accomplished using a modification of the DNAsis Restriction Enzyme Search Function. The determination of the open reading frame was performed using the DNAsis Open Reading Frame Function. The information was used to identify the corresponding predicted amino acid sequences using the DNAsis DNA-To-Protein Translation Algorithm. The amino acid sequences of the inserts were used for later amino acid comparisons between the inserts and hsp 27. This was done using the DNAsis Maximum Matching Alignment.

In order to further determine if the inserts were closely-related to human hsp 27, we wanted to identify the location where the PCR primers should have bound to the probe. Hence, the corresponding DNA sequence motifs for the forward and reverse PCR primers were searched for, within the DNA sequence of the inserts contained in the positive clones.

The DNA sequence information was also analyzed using the Blast Algorithm (Basic Local Alignment Search Tool). This computer algorithm searched for a query sequence in a database. The program was used to determine the identity of the isolated DNA inserts.

Finally, the nucleotide sequence information for the inserts contained in the positive clones was deposited with the GenBank database.

Bacterial Strains And

Growth Conditions

Escherichia coli (*E. coli*) JM109 competent cells were utilized in the subcloning of the hsp 27 genes (Promega Corporation, Madison, WI). These bacterial cells carry certain genetic markers that were useful for our cloning purposes. These genetic markers included endA1 (an endonuclease mutation that improved the quality of plasmid DNA isolation), recA1 (a mutation in recombination that prevented the recombination of introduced DNA with host DNA, ensuring the stability of inserts), hsdR17 (which allowed cloning without the cleavage of DNA by endogenous restriction endonucleases), and lacZ-D-M15 on an F' (which when used with an appropriate vector, made it possible to distinguish between colonies that had a vector containing an insert from those that had a vector lacking an insert).

Bacterial cultures were grown with shaking in Luria Bertani Medium (LB) containing ampicillin (Amp; 50 mg/ml) at 37°C. LB was made using 5 g of yeast extract, 10 g of tryptone and 10 g of sodium chloride (all from Fisher Biotech., Santa Clara, CA). The final solution was made up to 1000 ml using deionized water (pH 7.0). LB + Amp plates were prepared by the addition of 7.5 g of Bacto agar (Difco, Detroit, Michigan) to 500 ml of LB. To this solution 0.5 ml of ampicillin (50 mg/ml; Sigma Chemical Company, St. Louis, MO), was added to select and maintain JM109 transformants. Cultures (100µl) were placed in 850 µl of 80% Glycerol stock solution. The 4 ml glycerol stock vials (VWR Scientific, San Francisco, CA) were stored at -70°C.

Parental Plasmids

Lambda EMBL3 phage was used as a vector for cloning large pieces of genomic DNA from the A549 lung cancer cell line (2×10^6 pfu/mg; Stratagene, La Jolla, CA). This vector was chosen as it is very useful for cloning Sau3A partial digests, since the BamHI sites in the vectors are flanked by EcoRI and SalI restriction sites. Lambda EMBL3 phage was supplied as a BamHI-digested lambda EMBL3 phage product. The BamHI-digestion removed the 13.7 kb stuffer fragment (which was unnecessary for the propagation of the vector), and allowed for the replacement of the stuffer fragment with an insert. Therefore, it was possible to insert a piece of Sau3A-digested genomic DNA into the phage via ligation to the BamHI-digested phage product. Also, it was possible to ultimately remove the cloned genomic DNA fragments from the recombinant phage when necessary, by digestion with SalI restriction enzyme. Recombinant phages were Spi-negative, and were therefore selected on the P2-lysogenic host, *E. coli* XL-1 blue cells.

pGEM-3Z, a 2,743 bp plasmid was used in subcloning and DNA sequencing experiments (Promega Corporation, Madison, WI). This vector carried the lacZ gene and multiple cloning site arrangement from pUC18. pGEM-3Z contained both the SP6 and T7 RNA polymerase promoters flanking the multiple cloning site. pGEM-3Z is capable of complementing the product of the lacZ-delta-M15 gene to produce functional beta-galactosidase. Hence, in *E. coli* JM109 cells (that contain the lacZ-delta-M15 on an F'), pGEM-3Z allowed for the visual screening of recombinant transformants when plated on ampicillin (50 mg/ml; Sigma Chemical Co., St. Louis, MO), X-Gal (5-bromo-4-chloro-3-indoyl-Beta-D-galactopyranoside in 2% DMF solution); (Promega Corporation, Madison, WI), and IPTG (isopropyl-Beta-D-thiogalactopyranoside; 100 mM; Promega Corporation,

Madison, WI). The parental pGEM-3Z vector contains a lacZ gene with a multiple cloning site for inserting foreign DNA. When the parental pGEM-3Z vector (with the intact lacZ gene) was grown in the presence of X-gal (5-bromo-4-chloro-3-indoyl-b-D-galactopyranoside), the lacZ gene encoded for beta-galactosidase, an enzyme which hydrolyzed the colorless X-gal, producing a blue product (5-bromo-4-chloro-3-hydroxyindole) and beta-D-galactose. IPTG (isopropyl-b-D-thiogalactopyranoside) was used with X-gal as it induced beta-galactosidase activity more than 10-fold above background levels in these *E. coli* cells. Ampicillin was also used as a selection marker in order to exclude bacteria that have not taken up any plasmid, which would otherwise also form white colonies in the presence of X-gal. Cells containing the recombinant vector will have an insert present in the lacZ gene, which interrupts the gene, preventing the production of beta-galactosidase and resulting in white colonies. Bacterial colonies containing the parental pGEM-3Z plasmid have no insert, which leaves the lacZ gene intact and produces beta-galactosidase, resulting in blue colonies. The plasmid map for the parental pGEM-3Z plasmid is shown in Appendix I (p. 109). The nucleotide sequence for the parental pGEM-3Z plasmid is shown in Appendix II (p. 111).

Enzymes

The enzymes SmaI, XbaI, PstI, ScaI, HincII, KpnI, EcoRI, BamHI, SalI and HindIII were obtained from New England Biolabs (Beverly, MA). Sau3A restriction enzyme was ordered from the Promega Corporation (Madison, WI). Vent DNA polymerase I large klenow fragment and calf intestinal alkaline phosphatase (CIP) were also obtained from New England Biolabs (Beverly, MA).

Genomic Library Construction

A genomic DNA library from the human A549 lung cancer cell line was previously constructed in our lab. The genomic DNA (100 µg) was partially digested with Sau3A restriction endonuclease (1 unit) at 37°C for 2 hours (24). The Sau3A-cut genomic DNA (0.127 µg/µl) containing the hsp 27-related sequences was recovered in 500 µl aliquots, by centrifugation over a 1-15% glycerol gradient at 24,300 rpm for 21.3 hours in a Sorvall AH-627 rotor (Dupont Company, Newton, CT). The 15-23 kb genomic DNA fragments (1 µl) were ligated into lambda EMBL3 phage (6.7 µl of 0.127 µg/µl) at 16°C for 6 hours. Specifically, BamHI-cut and dephosphorylated lambda EMBL3 phage was ligated to the Sau3A-cut genomic DNA using T4 DNA Ligase (4 units), 10X Ligase buffer (1.1 µl) and 10 mM ATP (1.1 µl). The lambda EMBL3 phages were amplified in *E. coli* XL-1 blue cells (Stratagene, La Jolla, CA), using a concentration of 1.2×10^{10} pfu/ml titre, and the recombinant phages were later used in colony hybridization.

Colony Hybridization

The human genomic library constructed in lambda EMBL3 phage was screened for hsp 27 genes, using full-length human hsp 27 cDNA obtained from our lab (17). First, the lambda EMBL3 phage containing the genomic library was plated on NZY agar plates, using 150 mm petri dishes (24). NZY agar plates (pH 7.0), were made using NZ amine (10 g), sodium chloride (5 g), yeast extract (5 g), and 950 ml of distilled water. The lambda phage lysate was subjected to a 1×10^{-4} dilution in SM Buffer before plating on the NZY agar plates. SM Buffer was made using 5.8 g of sodium chloride, 2 g of magnesium sulfate (Sigma Chemical Co., St. Louis, MO), 50 mg of 1 M Trizma-base (pH 7.5; Sigma Chemical

Co., St. Louis, MO), and 5 ml of gelatin (2% solution). The final volume was made up to 1,000 ml using deionized water. *E. coli* XL-1 blue cells were grown at 37°C for 3-4 hours until an optical density of 0.5 was obtained (600 nm). The diluted phage (200 µl) was mixed with the *E. coli* cells XL-1 blue cells (600 µl) and incubated at 38°C for 15 minutes. To this mixture, melted top agar was added (7.5 ml). Melted top agar was made using agarose (0.8 g), 1 M magnesium sulfate (1mg), and LB (100 ml). This solution was plated on NZY agar plates, which were later incubated at 37°C for 6-7 hours.

The plaques were then transferred to nitrocellulose filters that were obtained from Stratagene (La Jolla, CA) (24). Prior to colony hybridization, it was necessary to remove the human hsp 27 cDNA from the recombinant plasmid pTRI/62Y.16.7, via an EcoRI digestion, producing a 762 bp EcoRI-cDNA fragment to be used as the probe. The nucleotide sequence for human hsp 27 cDNA is assigned the GenBank accession number X54079 and is shown in Appendix II (p. 114).

A Nonradioactive DIG DNA Labeling and Detection System (Boehringer Mannheim Biochemicals, Indianapolis, IN) was used to determine the sites of hybridization of the probe to the hsp 27-related sequences. The prehybridization step was carried out at 68°C for 1 hour, using 20 ml of hybridization solution (without labeled DNA). Hybridization solution was made using 0.25 g of blocking reagent, 50 mg of N-Lauroyl Sarcosine, 100 mg of SDS, 12.5 ml of 20X SSC, and 37.5 ml of distilled water. The hybridization step was carried out overnight at 68°C, using 26 ng of denatured probe, and 20 ml of hybridization solution. The hybridized nitrocellulose filters were washed twice, for 5 minutes at 65°C using 50 ml of wash buffer 1 (2X SSC and SDS 0.1% w/v). The filters were rewashed twice, for 15 minutes at 65°C, using 50 ml of wash buffer 2 (0.2X SSC and SDS 0.1% w/v).

Positive plaques were evident as dark spots on the nitrocellulose filter. These spots indicated the sites of hybridization of the human hsp 27 genes to the human hsp 27 cDNA probe. As a positive control for colony hybridization, we used *E. coli* cells with the plasmid pTRI/62Y.16.7, because they contained full-length hsp 27 cDNA and would hybridize to the probe.

The number of positive plaques was then determined. A pasteur pipette was then used to pick up the agar plugs containing the positive plaques, and these plaques were eluted in SM buffer (100 μ l) overnight at 4°C. Three different rounds of colony hybridization were performed on three different sets of plates. After each of the screening steps, the positive plaques were eluted in SM Buffer, and the plates were stored at 4°C. These recombinant phages were later digested into smaller fragments (subcloning), and analyzed using PCR.

Subcloning

The positive plaques identified from colony hybridization were isolated following the protocol for the isolation of phage DNA (24). Subcloning was used to narrow the search for the hsp 27 genes from within larger fragments of DNA (15-23 kb), to within smaller fragments (of about 5 kb). In the subcloning step, the positive plaques were digested with SalI (1 unit) at 37°C for one hour to remove the phage arms, and to simultaneously cut the genomic DNA into smaller fragments. The SalI site was used as an insertion site instead of the BamHI or EcoRI sites, as these latter sites were lost once the larger genomic DNA fragments were previously inserted into BamHI-cut lambda EMBL3 phage. The SalI enzyme was then heat-inactivated at 65°C for 20 minutes. The pGEM-3Z vector DNA (Promega

Corporation, Madison, WI) was also digested with the SalI enzyme at 37°C for 2 hours. The SalI enzyme was once again heat-inactivated at 65°C for 20 minutes.

The digested pGEM-3Z vector DNA (16 µl) was dephosphorylated with 10X calf intestinal phosphatase enzyme (2 µl), and 10X alkaline phosphatase buffer (2 µl) at 37°C for 30 minutes (24). This was done to prevent the resealing of the digested parental pGEM-3Z vector ends before the insertion of a DNA fragment. The SalI-cut genomic DNA (10 µl) and the dephosphorylated-SalI-cut pGEM-3Z vector DNA (4 µl) were ligated together at 4°C overnight using T4 DNA ligase (4 unit/µl), 10X ligase buffer (5 µl), riboATP (5 µl) and distilled water (25.5 µl). The resulting recombinant plasmids contained smaller genomic DNA fragments from the lung cancer A549 cell line, than the 15-23 kb genomic fragments that were previously packaged into lambda EMBL3 phage. These smaller fragments of about 5 kb in length, were inserted into the SalI site, in the multiple cloning site of plasmid pGEM-3Z.

These resulting recombinant plasmids were transformed into *E. coli* JM109 cells, and the culture was plated on LB + Amp + X-gal + IPTG plates (24). This method of selecting recombinant plasmids was based on a selectable antibiotic marker, and a color-producing substrate. The white colonies were picked with a sterile toothpick, and grown overnight with shaking at 37°C in 3 ml of LB media containing 3 µl of ampicillin (50 mg/ml). The plasmid DNA of the positive clones were isolated from the *E. coli* JM109 host cells using a Plasmid DNA Extraction Miniprep Kit (Promega Corporation, Madison, WI). The manufacturer's instructions were followed, and the appropriate centrifugation steps were carried out in either a Micro Centrifuge Model 235C (Fisher Scientific, Santa Clara, CA), or in a Sorvall RC2B

Centrifuge (Dupont Company, Newton, CT). The plasmid DNA was resuspended in distilled water (50 μ l), and further analyzed using PCR.

PCR Analysis

PCR was used to further identify positive clones containing the hsp 27 genes. In this reaction, synthetic oligonucleotide primers were used to amplify the first exon of hsp 27-related DNA. We initially deduced that the first exon region on the DNA would be a conserved region present in all members of the hsp 27 gene family. Figure 1.6 (p. 24) shows an illustration of the hybridization sites for the forward and reverse primers in the first exon region of the human hsp 27 gene sequence.

The primers were synthesized by an Applied Biosystems 380A DNA synthesizer (Integrated DNA Technologies Inc., Coralville, IA). The forward primer (19 bp) and the reverse primer (20 bp) contained the following nucleotide sequences:

Forward Primer- 5'-GAGCGCCGCGTCCCCTTCT-3'

Reverse Primer- 5'-ATCTCCACCACGCCATCCTT-3'

PCR was carried out in a Perkin Elmer Thermal Cycler (Perkin-Elmer Express, Foster City, CA). The 100 μ l reaction mixture contained forward and reverse primers (50 pmoles each), dNTP's (2.5 mM each), 5X PCR reaction buffer (pH 10), and a single wax bead coated with magnesium chloride (1.5 mM), all obtained from Invitrogen (San Diego, CA). Also, the reaction mixture contained Vent DNA polymerase (1 unit; New England Biolabs, Beverly, MA). As a positive control for PCR, we used the plasmid pTRI/62Y.16.7 (5 μ l), because it contained the full-length hsp 27 cDNA with the first exon region. As a negative control for

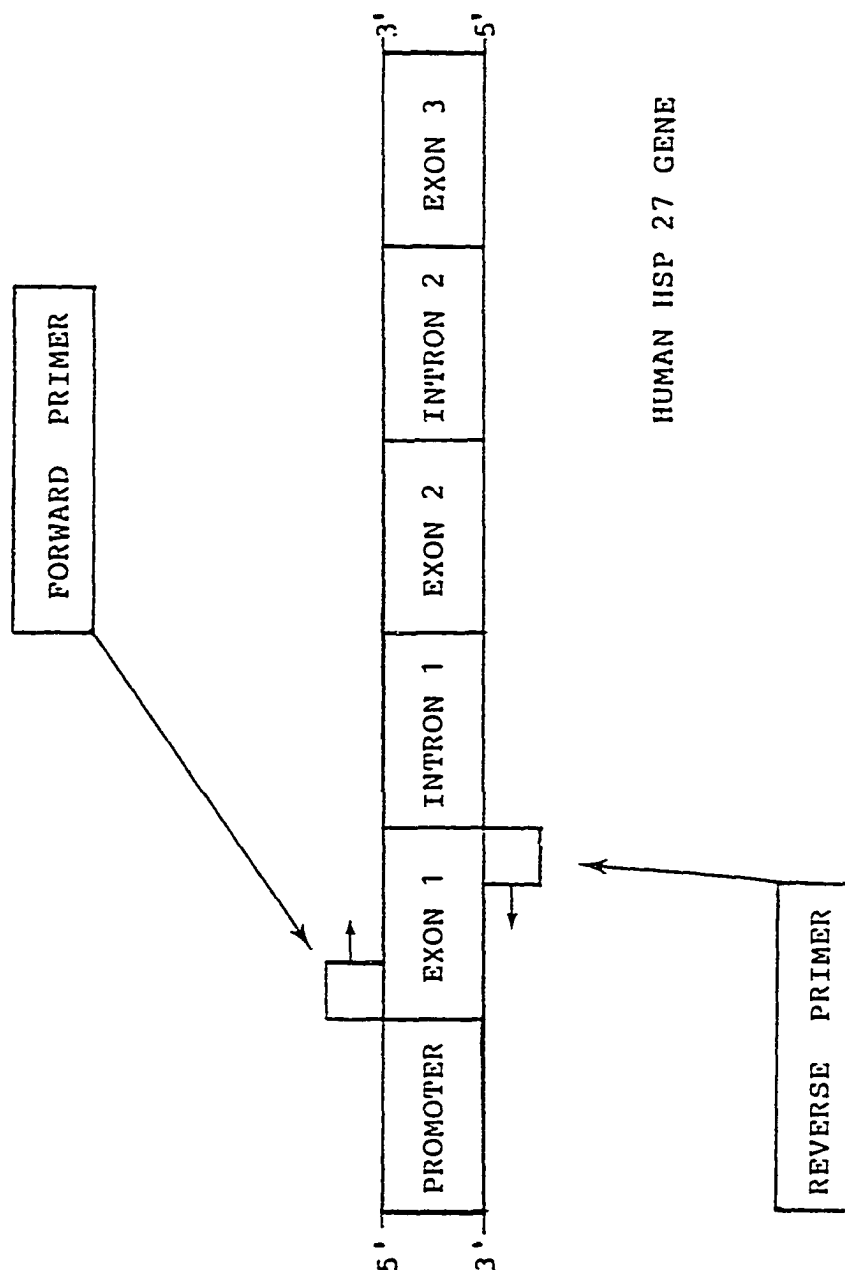


Figure 1.6 Hybridization Sites For PCR Primers. The forward and reverse PCR primers bind to the first exon region of the hsp 27 gene sequence.

PCR, we used wild-type lambda EMBL3 phage (5 μ l), as it lacked the first exon region of hsp 27 cDNA.

The plasmid DNA sample (5 μ l) from each clone that was analyzed underwent 30 cycles of denaturation, annealing and extension. In each cycle of the PCR, the DNA was denatured at 94°C for one minute, to separate the double-stranded DNA into single strands. The primers were then allowed to anneal to each of the single-stranded DNA at 60°C for one minute. Then, new double-stranded DNA was synthesized by DNA polymerase in the extension reaction that occurred at 72°C for one minute. To ensure that all of the DNA was in the double-stranded form, an extra 10 minute extension reaction was performed at 72°C.

The PCR products (5 μ l) were separated by 2% agarose gel electrophoresis (3:1; low-melting agarose: normal agarose) in Tris acetate EDTA buffer (1X). The gel was run at 70 V for an hour, and visualized by ultraviolet transillumination of ethidium bromide-stained DNA (24). A 100 bp DNA size ladder (Gibco-BRL, Grand Island, NY) was used to identify the molecular range of the fragments. The presence of a 363 bp fragment (the size of the first exon of hsp 27 DNA), depicted a clone that was PCR-positive. The DNA was photographed using Polaroid 664K film (Polaroid Corporation, Cambridge, MA). The sizes of the inserts contained in these positive clones was then determined.

Sizing Of Fragments

The inserts contained in the positive clones were sized by cutting the DNA at specific sites using EcoRI, BamHI and HindIII restriction endonucleases. The DNA was digested at 37°C for 1.5 hours using a variety of endonucleases (1 unit each). The DNA

products were separated by 1% agarose gel electrophoresis in Tris acetate EDTA buffer (1X), at 78 V for 2 hours, and visualized by ultraviolet transillumination of ethidium bromide-stained DNA (24). A 1 kb DNA size ladder (Gibco-BRL, Grand Island, NY) was used to identify the molecular range of the fragments. The sizes of the fragments were compared to the 2,743 bp parental pGEM-3Z plasmid (Promega Corporation, Madison, WI). The sizes of the inserts in each positive clone were determined using the digestion patterns, and subtracting the total sizes of the fragments from the 2,743 bp parental pGEM-3Z plasmid. The DNA was photographed using Polaroid 664K film (Polaroid Corporation, Cambridge, MA). The restriction sites available on the inserts of the positive clones was then determined.

Restriction Mapping

The restriction maps (or plasmid maps), of the insert contained in the positive clones were determined by cutting the DNA at specific sites with the restriction enzymes SmaI, XbaI, PstI, ScaI, HincII, KpnI, EcoRI, BamHI, and HindIII. The DNA was digested at 37°C for 1.5 hours using 1 unit of these various endonucleases. The DNA products were separated by 1% agarose gel electrophoresis in Tris acetate EDTA buffer (1X), at 70 V for 2.5 hours, and visualized by ultraviolet transillumination of ethidium bromide-stained DNA (24). A 1 kb DNA size ladder (Gibco-BRL, Grand Island, NY) was used to identify the molecular range of the fragments. The sizes of the fragments were compared to the 2,743 bp parental pGEM-3Z plasmid (Promega Corporation, Madison, WI), and plasmid maps were generated on the basis of the restriction enzyme digestion patterns. The DNA was photographed using Polaroid 664K film (Polaroid Corporation, Cambridge, MA). The nucleotide sequences of the inserts contained in the positive clones were then determined.

DNA Sequencing

Automated DNA sequencing was chosen as the tool for determining the nucleotide sequences of the inserts contained in the positive clones. Hence, it was necessary to use plasmid DNA that was isolated using a Qiagen Maxiprep (Qiagen Incorporated, Chatsworth, CA), to provide a clean plasmid to be used in an automated DNA sequencer. Prior to using the Qiagen Maxiprep, the glycerol stock solutions containing the positive clones (10 μ l) were grown overnight with shaking at 37°C in 3 ml of LB media containing 3 μ l of ampicillin (50 mg/ml). This culture was used to inoculate a 200 ml LB media containing 200 μ l of ampicillin (50 mg/ml). This 200 ml culture was further grown with shaking at 37°C overnight, and was then used to isolate plasmid DNA via the Qiagen Maxiprep. The plasmid DNA containing the positive clones was resuspended in distilled water (9.5 μ l), prior to automated DNA sequencing.

Fluorescent dye dideoxy chain-terminating DNA sequencing was performed by a DNA Sequencing Laboratory (Biotechnology Division, University of Arizona, AZ), on an Applied Biosystems Automated Sequencer Model 377. The DNA sequence reaction was conducted using suitable synthetic oligonucleotide primers for the T7 and SP6 promoters, and 1 μ g DNA samples for each reaction.

DNAsis Software Analysis

Nucleotide sequence comparisons were performed using the DNAsis computer software's Maximum Matching Alignment (Hitachi Software Engineering Company Limited, Yokohama, Japan). This function analyzed the similarity between two sequences according to the Needleman-Wunsch Algorithm (25). It aligned two sequences side by side, assigning

a score to the most similar alignment. If the score was less than a specific value, the function went back over the aligned sequence and inserted gaps, and rescored the new alignment. Gaps were typically inserted into the shorter of the two sequences, and a penalty was given for gaps. The highest score was determined and reported in relation to a homology percentage. In the illustrations of the alignments, gaps were designated as hyphens. Matching regions were indicated by vertical bars. If one of the sequences had been shifted relative to the other, vacant positions were indicated by dots.

In the initial analysis of the DNA sequence data, the nucleotide sequences of the inserts contained in the positive clones (and the complementary strand DNA sequences) were compared to the human hsp 27 gene using the Maximum Matching Alignment. The nucleotide sequence of the human hsp 27 gene is assigned the GenBank accession number XO3900, and is shown in Appendix II (p. 115).

We desired to compare the predicted amino acid sequences of the inserts to the amino acid sequence of the human hsp 27 gene. Therefore, we searched for sites in the DNA sequence that would indicate the coding strand that would translate the DNA sequence into a predicted amino acid sequence. This was accomplished using a modification of the Restriction Enzyme Search Function. This function was intended to be used to search for restriction enzyme sites within a nucleotide sequence, but was modified for our purposes. The excision sites for an intron 5'-splice site is AA!GUAAGU (26). The excision sites for an intron 3'-splice site is UNCAG!G, with a polypyrimidine string preceding the 3'-splice sites (26). The symbol, '!', denotes the site of actual bond cleavage. The 5'-splice site, and the 3'-splice site were searched for as a motif, within the DNA sequences of the inserts. The

sequence CCCCC, and TTTTT were used here to search for the string of polypyrimidines preceding the 3'-splice site.

If 5' and 3'-splice sites were found on a particular strand of an insert, this strand was used as the coding strand. Hence, the introns (the intervening regions between the 5' and 3'-splice sites), were removed from the DNA sequences of the inserts, and the remaining DNA information (exons) were joined together. The exon information from the coding strand, was translated into predicted amino acid sequences in three reading frames. If no introns were found within an insert, we could not determine the coding strand. Therefore, the insert was analyzed in all six reading frames, by obtaining a variety of open reading frames for that insert, that would result in the synthesis of a variety of predicted amino acid sequences.

The determination of the open reading frame was performed using the Open Reading Frame Function. Each strand of a DNA molecule can be read in one of three reading frames, for the synthesis of one of three possible proteins. Hence, a double-stranded DNA molecule can be translated into six possible proteins. The open reading frame is the one particular reading frame out of the six, that is the most likely reading frame for the synthesis of the appropriate protein.

The Open Reading Frame Function is based on the Fickett Algorithm (27). The Fickett Algorithm scans a query sequence for codons known to be associated with the initiation (ATG) and termination of transcription (TAA, TAG and TGA). In this way, the algorithm distinguishes true protein coding sequences from false open reading frames. This function indicated the predicted open reading frame by displaying the sizes of the proteins produced by each reading frame (in Daltons).

Once the open reading frames were determined, the translation of the nucleotide

sequences into predicted amino acid sequences was performed using the DNA-To-Protein Translation Function. The predicted amino acid sequences of the inserts contained in the positive clones were compared to the amino acid sequence of the human hsp 27 gene, using the Maximum Matching Alignment. The amino acid sequence information of human hsp 27 is assigned the GenBank accession number XO3900, and is shown in Appendix III (p. 131).

In order to further determine if our inserts were the closely-related, linked human hsp 27 genes, we searched for regions in the DNA sequence of the inserts, that would indicate sites to which the primers should have bound during PCR. If the inserts were closely-related to human hsp 27, we predicted that within the nucleotide sequences of the inserts, we would identify the motifs for the corresponding hybridization sites for the forward and reverse primer PCR primers. The motifs of the complementary sequences for the forward and reverse PCR primers (to the first exon of the human hsp 27 gene), were searched for using a modification of the Restriction Enzyme Search Function.

Blast Analysis

The Blast Algorithm was used to determine the identity of the inserts with known databases. Homology searches using the nucleotide sequences of the insert contained in the positive clones were conducted by using the Blast Algorithm at GenomeNet, Japan (Basic local alignment search tool at www.blast.genome.ad.jp). This algorithm is derived from the Lipman-Pearson Algorithm (28), and facilitated the search for similarities between these newly determined sequences, and already available databases. The Blast Algorithm selected DNA sequences in two DNA databases, that were similar to the query sequence (Blastn). It also used the DNA sequence of a query, translated it into six possible amino acid sequences,

and searched for these six sequences in two protein database (Blastx). By translating each strand of double-stranded DNA into three reading frames for the synthesis of six possible proteins, comparisons were made at the protein level that allowed for mutations in DNA. Protein comparisons were used to identify weak similarities encoded in nucleic acids that were not apparent from just looking at similarities between two nucleotide sequences.

The sequences of the insert contained in the positive clones were searched for in the GenBank nucleotide database, GenBank nucleotide updated database, SwissProt protein database and SwissProt protein updated database. The BLOSUM62 scoring matrix was selected for the Blastx searches. No scoring matrix was required for the Blastn searches. The results were displayed as the names of the "sequences producing high scoring segment pairs", and shown as visual homology regions and identity percentages.

CHAPTER 3

RESULTS

Overview

Cloning was used to isolate specific DNA fragments of linked human hsp 27 genes. The process of cloning the DNA fragments began with the construction of a genomic library. The DNA fragments from the human lung cancer cell line A549 genomic library were ligated into lambda phage, a DNA virus that can accommodate large inserts. The human genomic library constructed in lambda phage was screened for hsp 27 genes. Colony hybridization was used to identify the phages that contained the hsp 27-related genes, via the use of a probe. The hsp 27-related genes contained in lambda phage (the positive plaques), were enzymatically cut into smaller fragments. These small fragments were ligated into a plasmid vector for further analysis (subcloning). In this way, we narrowed our search for the hsp 27 genes from within larger pieces of DNA to amongst smaller pieces of DNA.

PCR was used as a second technique, to further determine if the DNA inserts in the recombinant plasmids were positive clones containing the desired Hsp 27 genes. Restriction endonucleases were used to determine the sizes of the DNA fragments (inserts), contained in the positive clones. To determine the DNA sequence information of the inserts, the inserts

were subjected to automated DNA sequencing. To determine if the inserts contained in the positive clones were similar to hsp 27 in their nucleotide sequences and predicted amino acid sequences, the DNAsis software program, and the Blast Algorithm were used. Finally, the nucleotide sequence information for the inserts contained in three of the positive clones were deposited in the GenBank database.

In presenting the results of this study, I will initially show the specific results for a particular positive clone (DNA 4), from the stage of colony hybridization until the results for the Blast algorithm analysis. Then, I will discuss the results for four other positive clones.

Colony Hybridization

The human genomic library constructed in lambda EMBL3 phage was screened for the human hsp 27 genes, as outlined in the Materials and Methods section. A total of three rounds of colony hybridization screenings were performed. It was necessary to perform three screenings in order to isolate unique positive plaques that may contain the hsp 27 genes. Plaques were purified before further analysis (24). We obtained 100,000 plaques from each round of colony hybridization. Of these 300,000 total plaques, 50 were positive plaques.

One of the plates used in colony hybridization (Plate 9), contained 8 positive plaques, and is shown in Figure 2.1 (p. 34). This figure also shows the positive control that was used for colony hybridization. We used *E. coli* cells containing the plasmid pTRI/62Y.16.7 as a positive control, because this plasmid contained full-length hsp 27 cDNA and would hybridize to the probe. The positive plaques were then subjected to subcloning.

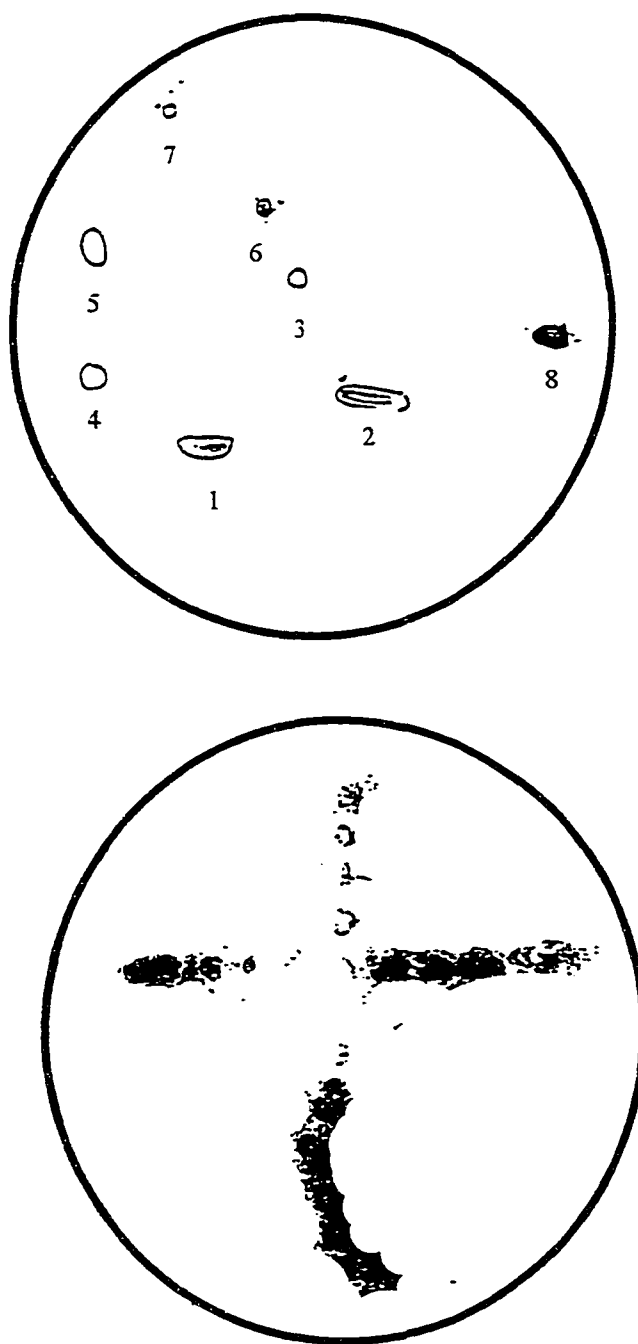


Figure 2.1 Colony Hybridization Results. The upper filter shows positive clones from plate 9. Dark spots on the nitrocellulose paper are the sites of hybridization to the human hsp 27 cDNA probe. Positive clones have been circled for further emphasis. The lower filter shows the positive control (the plasmid pTRJ/62Y.16.7), containing human hsp 27 cDNA that served as the probe.

Subcloning

The positive plaques identified from colony hybridization were purified, and subcloned into pGEM-3Z, as stated in the Materials and Methods section. Using Colony hybridization, a positive plaque was chosen from Plate 9, and called 9.1. Following the purification of plaque 9.1, subcloning into pGEM-3Z, transformation into *E. coli* JM109 cells, and plating on LB + Ampicillin + X-gal + IPTG media (on Plate 9.1), 24 recombinants were selected as white colonies. These white colonies were selected as they indicated plasmids containing DNA inserts.

PCR Analysis

PCR was used as a second technique to further determine if the DNA inserts in the recombinant plasmids contained hsp 27 genes. We amplified the first exon region of human hsp 27 cDNA (363 bp), using forward and reverse primers to the first exon of human hsp 27 cDNA. If human hsp 27 genes were present in a particular recombinant plasmid, after gel electrophoresis analysis, a band indicative of the 363 bp first exon region of the hsp 27 gene would be present.

Of the 24 white recombinant colonies that were screened from plate 9.1 using PCR amplification, two colonies, 9.1/1 and 9.1/2 were found to be PCR-positive. Figure 2.2 (p. 36) shows the gel electrophoresis result for the positive clones obtained from the PCR analysis of plate 9.1. In this picture, a positive clone is identified as a 363 bp amplified band on the gel. Lane 2 is the positive control, which contains a 363 bp band, and is indicated by an arrow. Lane 2 also shows a smear of large DNA fragments that were also amplified. The

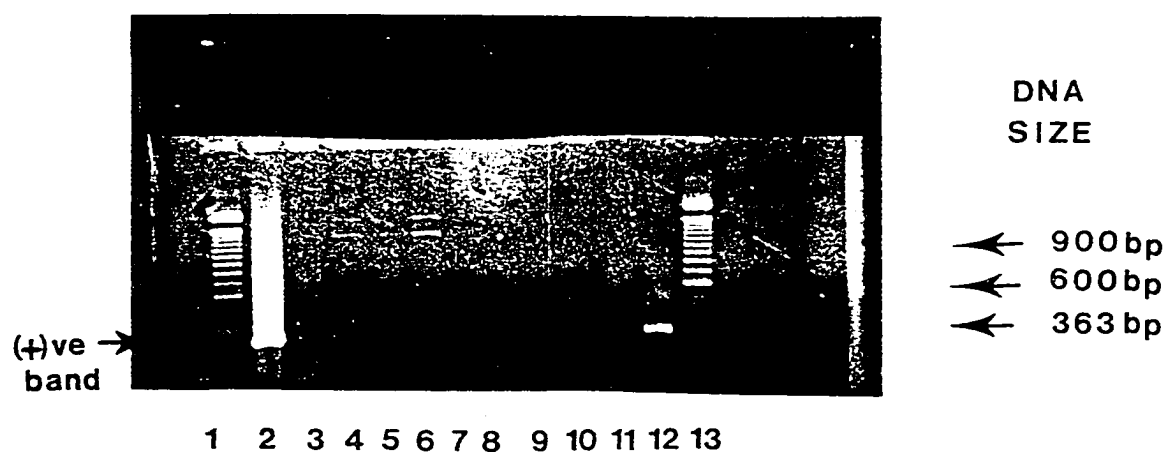


Figure 2.2 Gel Electrophoresis Results For PCR Analysis Of DNA 4. Lane 1 contains the 100 bp DNA mass ladder. Lane 2 is the positive control (the plasmid pTRI/62Y.16.7, containing human hsp 27 cDNA, and hence shows the 363 bp amplified band). Lanes 3-10 show the negative results for other clones. Lane 11 shows the amplified 363bp band from Plate 9.1/1 (a positive clone). Lane 12 shows the amplified 363bp band from Plate 9.1/2 (a positive clone). Lane 13 contains the 100 bp DNA mass ladder.

positive clone shown in Lane 12, 9.1/2, was picked and chosen for further study. This positive clone was named pDNA 4. pDNA 4 contained the insert, DNA 4.

We conducted two more rounds of colony hybridization, which yielded four other positive clones, that were chosen for further analysis. The four additional positive clones were designated as pDNA 1 (containing the insert, DNA 1), pDNA 2 (containing the insert, DNA 2), pDNA 3 (containing the insert, DNA 3) and pDNA 5 (containing the insert, DNA 5). It was then necessary to obtain more information, regarding the inserts contained in the positive clones.

Sizing Of Fragments

The inserts contained in the positive clones were sized by cutting the DNA at specific sites using EcoRI, BamHI and HindIII restriction endonucleases and separating the fragments on an agarose gel. These restriction enzymes were chosen because they possessed cleavage sites within the multiple cloning site of pGEM-3Z. Hence, after cleavage with these enzymes, the total sizes of the fragments obtained could be used to determine the size of the insert. The sizes of the inserts in each positive clone were determined using the digestion patterns, and subtracting the total sizes of the fragments from the 2,743 bp parental pGEM-3Z plasmid. Figure 2.3 (p. 38) shows a representation of an agarose gel showing the digestion patterns for pDNA 4. In Lane 2, we observe pDNA 4 that has been cut with EcoRI. Lane 2 shows the single fragment that results from this digestion - a 3,900 bp fragment. In addition, Lane 3 shows the pDNA 4 digestion fragment from restriction enzyme BamHI. Lane 3 also contains the 3,900 bp fragment. The actual size of DNA 4 was calculated by subtracting the 2,743 bp pGEM-3Z vector DNA from the 3,900 bp size of the band in Lane 2. The restriction

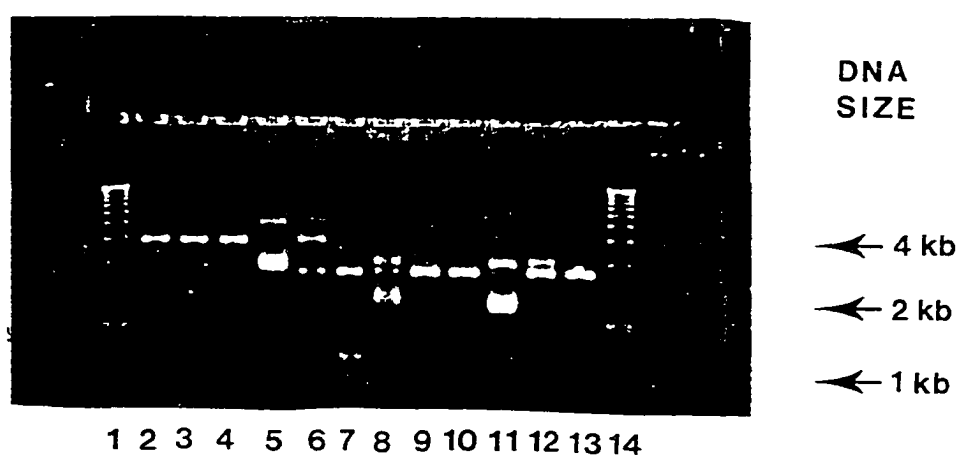


Figure 2.3 Gel Electrophoresis Results For Sizing Of DNA 4. Lane 1 = 1 kb DNA mass ladder. Lane 2 = EcoRI-cut pDNA 4. Lane 3 = BamHI-cut pDNA4. Lane 4 = HindIII-cut pDNA 4. Lane 5 = Undigested pDNA 4. Lane 6 = EcoRI-cut + HindIII-cut pDNA 4. Lane 7 = BamHI-cut + HindIII cut pDNA 4. Lanes 8-13 = pDNA 5 digests. Lane 14 = 1 kb DNA mass ladder.

analysis of pDNA 4 indicated that we had cloned a fragment, (DNA 4) that was approximately 1,200 bp long.

Restriction Mapping

Restriction digestion sites in pDNA 4 were examined by cutting the DNA at specific sites using the restriction enzymes EcoRI, PstI, ScaI, BamHI and HindIII. The restriction enzymes EcoRI and PstI were chosen because they cleaved within the human hsp 27 gene sequence. Since we were looking for hsp 27 genes, it was possible that one of these restriction enzymes would also cleave within the inserts of our positive clones. The restriction enzymes BamHI and HindIII were chosen because they cleaved within the multiple cloning site of pGEM-3Z. The restriction enzyme ScaI was chosen randomly.

If no restriction site was present in DNA 4, but was present in the multiple cloning site, a single fragment would be identified as a single band on the gel. This is because the enzyme would cut the plasmid in the multiple cloning site, thereby linearizing the plasmid, and producing a single fragment. If a single restriction site was present in DNA 4, and also present in the multiple cloning site, two fragments would be identified as two bands on the gel. This is because the enzyme would cut the plasmid in the multiple cloning site, as well as within the insert region. Hence, x bands identified on the gel would be indicative of $(x-1)$ locations at which the particular restriction enzyme cleaved within the insert. This reasoning was true for all single digestions involving enzymes that cleaved within the multiple cloning site of the plasmid.

A plasmid map was generated for pDNA 4, on the basis of the restriction enzyme

digestion patterns. The plasmid map for pDNA 4 is shown in Figure 2.4 (p. 41). The plasmid map indicated that there were no sites in DNA 4 for EcoRI, PstI, ScaI, BamHI or HindIII.

DNA Sequence Analysis

Since the plasmid map for pDNA 4 showed that the size of DNA 4 was approximately 1,200 bp in length, and since automated DNA sequencing can determine the nucleotide sequence of about 1,000 bp in a single run, it was apparent that a large portion of the nucleotide sequence information for DNA 4 could be obtained from just two rounds of automated DNA sequencing (using the T7 and SP6 promoters). The sequencing strategy for regions of DNA 4 is shown in Figure 2.5 (p. 42).

An appropriate primer to the T7 RNA polymerase promoter was used to determine the nucleotide sequence of the top strand of DNA 4 (DNA 4/T7) shown in Appendix II (p. 118). DNA 4/T7 contains 942 bp of sequence information. To obtain more sequence information for DNA 4, another appropriate primer to the SP6 RNA polymerase promoter was used to determine the nucleotide sequence of the complementary strand of DNA 4. The nucleotide sequence encoding DNA 4 using the SP6 promoter (DNA 4/SP6) is shown in Appendix II (p. 119) and contains 944 bp of sequence information.

The DNA sequences for both DNA 4/T7 and DNA 4/SP6 contain a small number of unknown nucleotides designated as "N". Unknown nucleotides are sometimes present in sequence information obtained from automated DNA sequencing. Unknown nucleotides occur when the automated DNA sequencer is unable to distinguish the overlapping signals obtained as it encounters more than one nucleotide, during a single point of analysis.

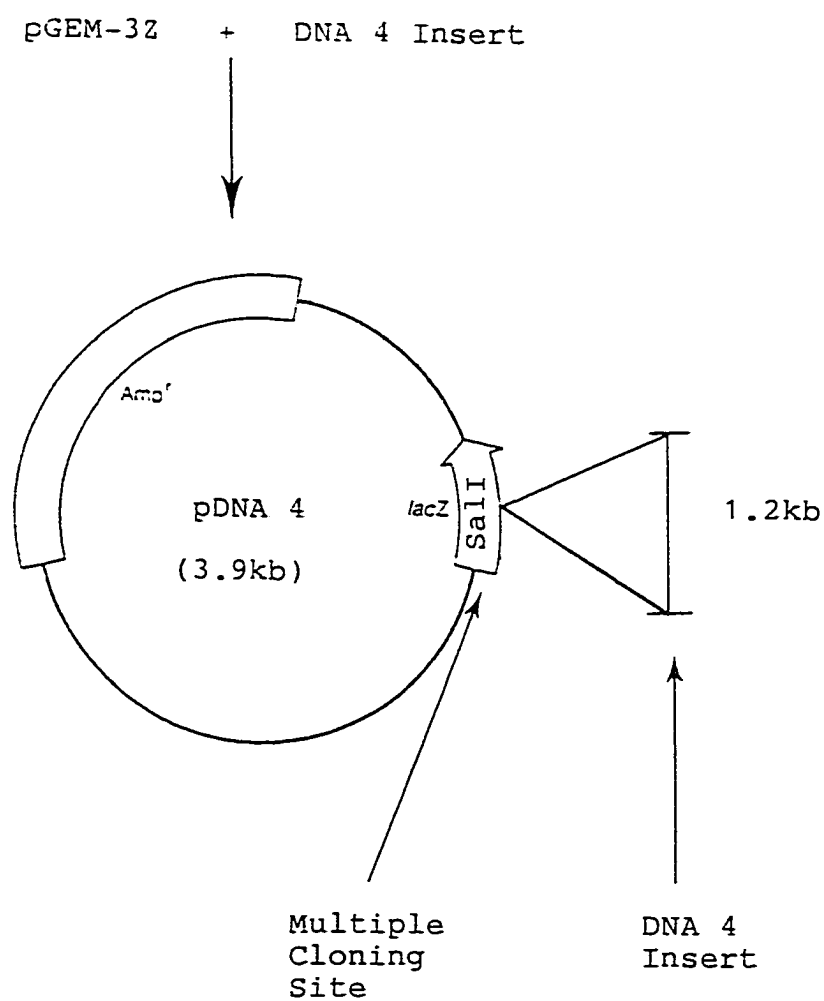


Figure 2.4 Plasmid Map For pDNA 4.

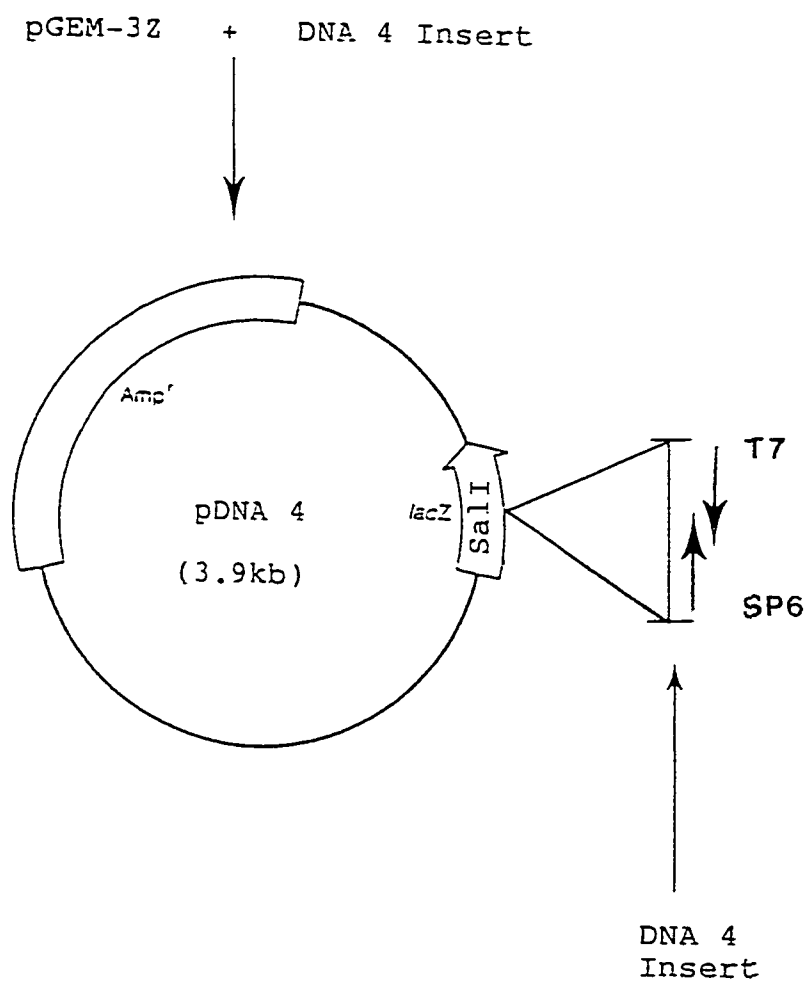


Figure 2.5 Sequencing Strategy For DNA 4.

DNAsis Analysis - DNA Level

Before DNAsis Software analysis was used, it was necessary to eliminate any pGEM-3Z vector nucleotide information that was present in the DNA 4/T7 nucleotide sequence. This was done by visually comparing the initial information of DNA 4/T7 with that of pGEM-3Z, and manually eliminating the short homologous region (1-29 bp). Once this was done, the DNA 4/T7 information that lacked any pGEM-3Z information was redesignated as DNA 4-1. The nucleotide information removed from DNA 4/T7 to obtain DNA 4-1 is underlined in Appendix II (p. 118). The DNA 4-1 nucleotide information was the partial sequence information for the insert contained in pDNA 4 using the T7 promoter. The nucleotide sequence for DNA 4-1 was used in subsequent analysis.

It was also necessary to eliminate any pGEM-3Z vector nucleotide information present in the DNA 4/SP6 nucleotide sequence. This was done by visually comparing the initial information of DNA 4/SP6 with that of pGEM-3Z, and manually eliminating the short homologous region (1-26 bp). Once this was done, the DNA 4/SP6 information that lacked any pGEM-3Z information was redesignated as DNA 4-2. The nucleotide information removed from DNA 4/SP6 to obtain DNA 4-2 is underlined in Appendix II (p. 119). The DNA 4-2 nucleotide information was the partial sequence information for the insert contained in pDNA 4 using the SP6 promoter. The nucleotide sequence for DNA 4-2 was used in subsequent analysis.

In order to obtain the complete sequence information for the approximately 1,200 bp DNA 4 insert, we required the 5'-sequence information for DNA 4-1 (normal strand), the 5'-information for DNA 4-2 (complementary strand), and the overlapping regions between these two sequences. To obtain the overlapping sequence information, the Maximum

Matching Alignment was used to compare the similarity between regions of the DNA 4-1 sequence information (normal strand), and regions of the DNA 4-2 sequence information (complementary strand). The results for this comparison are shown in Appendix IV (p. 133). A graphical illustration of the homologous regions between these two DNA sequences is shown in Figure 2.6 (p. 45).

Since we had more confidence in the 5'-DNA 4-1 information (as it was closer to the T7 sequencing primer), it was used to start the complete sequence of DNA 4. We had more confidence in the 5'-DNA 4-2 information (as it was closer to the SP6 sequencing primer), hence, it was used to finish the DNA 4 complete sequence. When a gap, or unknown nucleotide in either of the sequences was encountered, it was replaced with the appropriate nucleotide from the other strand. The new complete sequence information for DNA 4 was 1,147 bp in length, and is shown in Appendix II (p. 120).

The DNAsis computer software was used to compare the similarity between two DNA sequences. A homology of greater than 50% represented a good homology. In the positive control for the Maximum Matching Alignment, the DNA sequence for the human hsp 27 gene was aligned against itself and produced a 100% homology. Then, the DNA sequence of human hsp 27 cDNA (789 bp) was aligned against the DNA sequence of the entire human hsp 27 gene (2,496 bp). A 48% homology was evident, and is shown in Appendix IV (p. 135). This alignment of the human hsp 27 cDNA with the human hsp 27 gene, should have produced a homology percentage much greater than 48%, as these two sequences are definitely related. Visual analysis of the homology regions indicated that certain segments of the alignments showed better homologies than were indicated by the total homology percentage. Thus, when the cDNA of human hsp 27 was aligned against the most

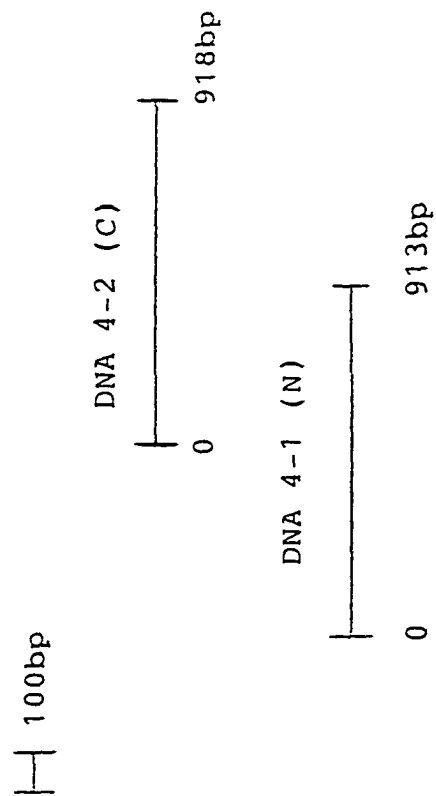


Figure 2.6 Homologous DNA Areas Between DNA 4-1 (Normal Strand), And DNA 4-2 (Complementary Strand).

homologous, similar-sized region of the human hsp 27 gene (198-418 bp region of the first exon), an increased homology of 100% was shown. This indicated that the pattern of alignment was just as important as the homology percentage, in determining relatedness.

The Maximum Matching Alignment was used to determine if the nucleotide sequence of DNA 4 (the normal strand) was similar to the nucleotide sequence of the human hsp 27 gene. In this comparison, a 38% homology was indicated by the results shown in Appendix IV (p. 139).

Alignments were performed using DNA 4 (normal strand), and similar-sized regions of the human hsp 27 gene. The alignment of the normal strand of DNA 4, and a similar-sized region of the human hsp 27 gene (1-1,147 bp), produced a 50% homology. The alignment of the normal strand of DNA 4, and another similar-sized region of the human hsp 27 gene (1,147-2,294 bp), produced a 51% homology. The alignment of the normal strand of DNA 4, and the most homologous, similar-sized region of the human hsp 27 gene (1,349-2,496 bp), is shown in Appendix V (p. 188). A homology percentage of 51% was indicated. A graphical illustration of this comparison is shown in Figure 2.7 (p. 47).

At this point, we were unaware of which strand of the double-stranded DNA 4 (the normal or complementary strand) would lead to the synthesis of the appropriate protein. Hence, the DNAsis Maximum Matching Alignment was used to determine if the complementary strand of DNA 4 was similar to the nucleotide sequence of the human hsp 27 gene. In this comparison, a 38% homology was indicated by the results shown in Appendix IV (p. 143).

Alignments were performed using DNA 4 (complementary strand), and similar-sized regions of the human hsp 27 gene. The alignment of the complementary strand of DNA 4,

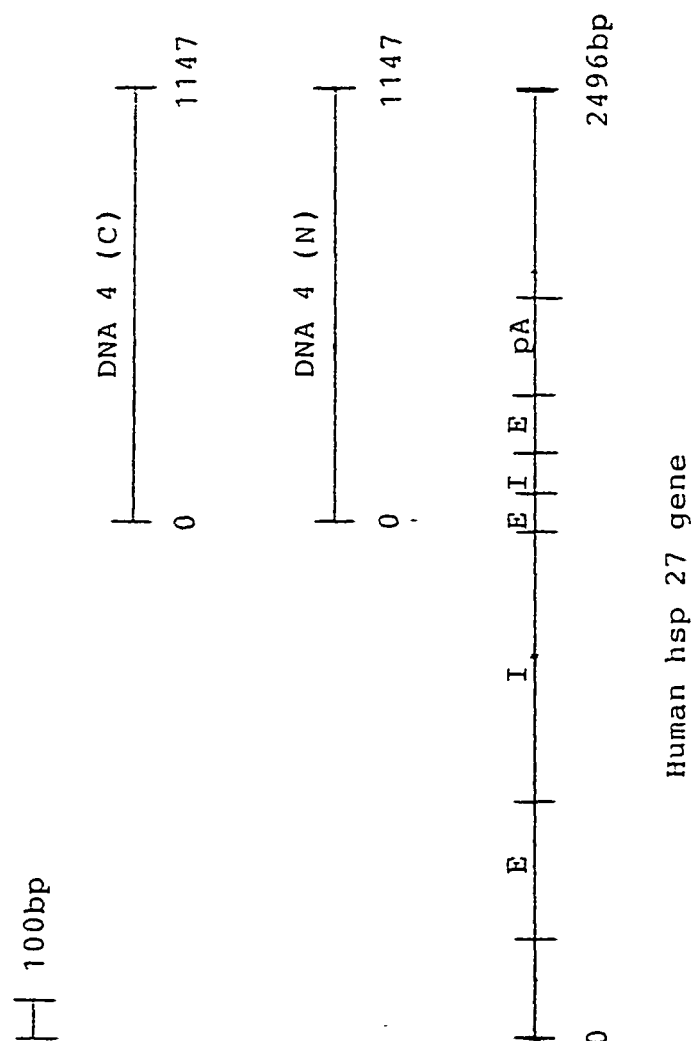


Figure 2.7 Homologous DNA Areas Between DNA 4 (Normal And Complementary Strands), And The Human hsp 27 Gene. The exon (E), intron (I), and polyA (pA) regions of the human hsp 27 gene are shown.

and a similar-sized region of the human hsp 27 gene (1-1,147 bp), produced a 49% homology. The alignment of the complementary strand of DNA 4, and another similar-sized region of the human hsp 27 gene (1,147-2,294 bp), produced a 51% homology. The alignment of the complementary strand of DNA 4, and the most homologous, similar-sized region of the human hsp 27 gene (1,349-2,496 bp), is shown in Appendix V (p. 190). A homology percentage of 51% was indicated. A graphical illustration of this comparison is shown in Figure 2.7 (p. 47).

Blast Analysis - DNA Level

In order to determine if DNA 4 was similar to any known DNA sequence, the DNA sequence information for DNA 4 was analyzed using the Blast Algorithm (Basic Local Alignment Search Tool). This computer algorithm searched for DNA 4 in the GenBank nucleotide database and the GenBank nucleotide updated database (Blastn).

An identity percentage of greater than 50% represented a good homology. In the control for the Blastn Algorithm, the DNA sequence for the human hsp 27 gene was searched for, and found to be 99% homologous to itself in the GenBank nucleotide database. The human hsp 27 gene was also found to be 75-90% homologous to the mouse hsp 27 nucleotide sequence, and 72-88% homologous to the rat hsp 27 nucleotide sequence.

We attempted to search for the nucleotide sequence of DNA 4 (normal strand), using the Blastn Algorithm. The results were displayed as the names of the "sequences producing high scoring segment pairs", and were shown as visual homology regions and identity percentages. The results for the Blastn searches using the nucleotide sequence of DNA 4 (normal strand) are shown in Table 1.1 (p. 49). Only 2 DNA sequences were identified as

Table 1.1 Blastn Results For DNA 4.

<u>Homolog</u>	<u>GenBank Accession Number</u>	<u>Homology</u>	<u>Length</u>
Homo sapiens cDNA clone NhHMPu	AI042521	75%	47 bp
Homo sapiens Chromosome 15q26.1 PAC clone	AC005319	75%	47 bp

as being related to this query sequence. The human hsp 27 gene sequence was not identified as being homologous to the nucleotide sequence for DNA 4 (normal strand).

DNAsis Analysis - Protein Level

Due to the fact there is a wobble in the genetic code, allowing for the translation of more than one codon into the same amino acid, it was possible that two DNA sequences that differed slightly at the nucleotide level, might show a greater homology at the protein level. Therefore, we looked for homologies at the protein level, that may not have been apparent at the DNA level. In order to compare the predicted amino acid sequence of DNA 4 to the amino acid sequence of the human hsp 27 gene, it was necessary to determine the coding strand for DNA 4, that would ultimately translate the DNA sequence into a predicted amino acid sequence. Therefore, we attempted to identify the coding strand by determining which strand of DNA 4 contained exons and introns, by looking at the 5' and 3'-splice sites, as outlined in the Materials and Methods section.

DNA 4 (normal strand) showed the presence of 2 potential 3'-splice sites at positions 84 and 841. The second potential splice site was preceded by a string of polypyrimidines (TTTTT) at position 487. No 5'-splice sites were identified. DNA 4 (complementary strand) showed the presence of one potential 3'-splice site at position 175. The potential splice site was not preceded by a string of polypyrimidines, nor was it preceded by any 5'-splice sites. Since there were 3'-splice sites on both strands of DNA 4, we were unable to determine which strand of DNA 4 was the coding strand. Therefore, we used a more random approach that involved the identification of predicted open reading frames that were present in all six reading frames.

The Open Reading Frame Function was used to translate the nucleotide sequence of DNA 4 into a variety of predicted amino acid sequences. This was done by translating the nucleotide sequence in both orientations, and in all three reading frames. The results for the open reading frames for both the normal and complementary strands of DNA 4 are shown in Table 1.2 (p. 52). In order to determine which DNA regions to translate into predicted amino acid sequences, we considered 20 amino acid residues (aa) to be unique. Since an amino acid is about 110 Daltons in size, a 20 amino acid sequence is 2,200 Da in size. Therefore, we picked at least one sequence from every reading frame (that did not contain an unknown nucleotide), and would result in a predicted amino acid sequence of greater than 2,200 Da in size. Then, the selected Open Reading Frames were translated into proteins using the DNA-To-Protein Translation Function. The Maximum Matching Alignment was used to compare these predicted amino acid sequences to the amino acid sequence for the human hsp 27 gene. Table 1.3 (p. 53) shows the results for these comparisons.

Blast Analysis - Protein Level

In order to determine if DNA 4 was similar to any known amino acid sequence, the amino acid sequence information for DNA 4 was analyzed using the Blast Algorithm (Basic Local Alignment Search Tool). This computer algorithm searched for DNA 4, as a protein in all six reading frames, in the SwissProt protein database and the SwissProt protein updated database (Blastx). The program also determined if the DNA insert in this positive clone would select hsp 27 as a homologous sequence in these databases.

As a control for the Blastx algorithm, the human hsp 27 gene sequence was searched for and found to be 100% homologous to the human hsp 27 amino acid sequence in the

Table 1.2 Open Reading Frames For DNA 4.

NORMAL

A	1 Frame		
	Start	End	MW
	1	46	1704.85
	112	124	563.62
	160	265	3916.38
	394	601	7751.61
B	1054	1144	3333.62
	2 Frame		
	Start	End	MW
	2	8	271.31
	425	506	3094.49
	524	788	8993.43
C	713	788	2493.75
	896	935	1603.83
	1001	1145	5520.39
	3 Frame		
	Start	End	MW
	3	426	15678.73
D	72	426	13438.36
	306	426	4442.85
	402	426	1041.15
	492	501	395.51
	540	558	706.78

COMPLEMENTARY

E	1 Frame		
	Start	End	MW
	1	40	1840.07
	616	646	1175.45
F	841	850	347.42
	2 Frame		
	Start	End	MW
	2	359	13402.20
	623	641	796.93
G	878	911	1280.57
	1025	1145	4658.17
	3 Frame		
	Start	End	MW
	3	150	5319.89
	6	150	5163.71
	18	150	4745.27
	48	150	3700.12
	726	828	4059.54

Table 1.3 Amino Acid Alignments Between DNA 4 And Human hsp 27.

<u>Sequence</u>	<u>Strand</u>	<u>Homology To hsp 27</u>	<u>Length</u>
A	Normal	17%	69 aa
B	Normal	25%	88 aa
C	Normal	30%	141 aa
D	Normal	28%	118 aa
E	Complementary	7%	13 aa
F	Complementary	27%	119 aa
G	Complementary	19%	49 aa

SwissProt protein database. It was also found to be 86% homologous to the mouse hsp 27 amino acid sequence, and 87% homologous to the rat hsp 27 amino acid sequence. The human hsp 27 gene sequence was also found to be 55% homologous to the human alpha-crystallin A-chain amino acid sequence, and 45% homologous to the human alpha-crystallin B-chain amino acid sequence.

The Blastx Algorithm was then used to search for the nucleotide sequence of DNA 4 (normal strand). Only 3 proteins were identified as being homologous to this query sequence. The results for the homologs are shown in Table 1.4 (p. 55). The human hsp 27 protein was not identified as being homologous to the nucleotide sequence of DNA 4 (normal strand).

PCR Primer Location Analysis

In order to further determine if DNA 4 was one of the closely-related hsp 27 genes, we attempted to identify sites where the PCR primers should have bound to the DNA 4 nucleotide sequence (normal and complementary strands). A modification of the DNAsis Restriction Enzyme Search Function was used to search for the motifs of the complementary strands of the forward and reverse PCR primers to the first exon of human hsp 27 gene. The results indicated that the PCR primers did not bind to any region on the DNA 4 nucleotide sequence.

Table 1.4 Blastx Results For DNA 4.

<u>Homolog</u>	<u>GenBank Accession Number</u>	<u>Homology</u>	<u>Length</u>
<i>E.coli</i> Phospho- ribosyl AMP	HIS2ECOLI	31%	7 aa
Rat Cytochrome P450	CPC7RAT	40%	23 aa
SHFL Phospho-	HIS2SHIFL	29%	41aa

Other Positive Clone Information

DNA 1 - Size And Restriction Map

The positive clone, pDNA 1, was sized by cutting the DNA at specific sites, using restriction endonucleases, and separating the fragments on an agarose gel. The restriction analysis of pDNA 1 indicated that we had cloned an insert, (DNA 1) that was approximately 4,000 bp long. Restriction digestion sites in the positive clone, pDNA 1, were examined by cutting the DNA at specific sites using all the restriction enzymes mentioned in the Materials and Methods section. A plasmid map was then generated for pDNA 1, and is shown in Figure 3.1 (p. 57).

DNA 1 - DNA Sequence

Since the plasmid map for the positive clone pDNA 1 showed that the size of DNA 1 was approximately 4,000 bp in length, it was apparent that only about 2,000 bp of the nucleotide sequence information for DNA 1 could be obtained from two rounds of automated DNA sequencing (using the T7 and SP6 promoters). The sequencing strategy for regions of DNA 1 is shown in Figure 3.2 (p. 58).

The nucleotide sequence encoding DNA 1 using the T7 promoter (DNA 1/T7) is shown in Appendix II (p. 122). DNA 1/T7 contains 1,122 bp of sequence information. The nucleotide sequence encoding DNA 1 using the SP6 promoter (DNA 1/SP6) is shown in Appendix II (p. 124). DNA 1/SP6 contains 958 bp of sequence information.

Manipulation of the positive clone pDNA 1 was necessary to obtain more nucleotide information for DNA 1. Since the plasmid map for pDNA 1 indicated the presence of a

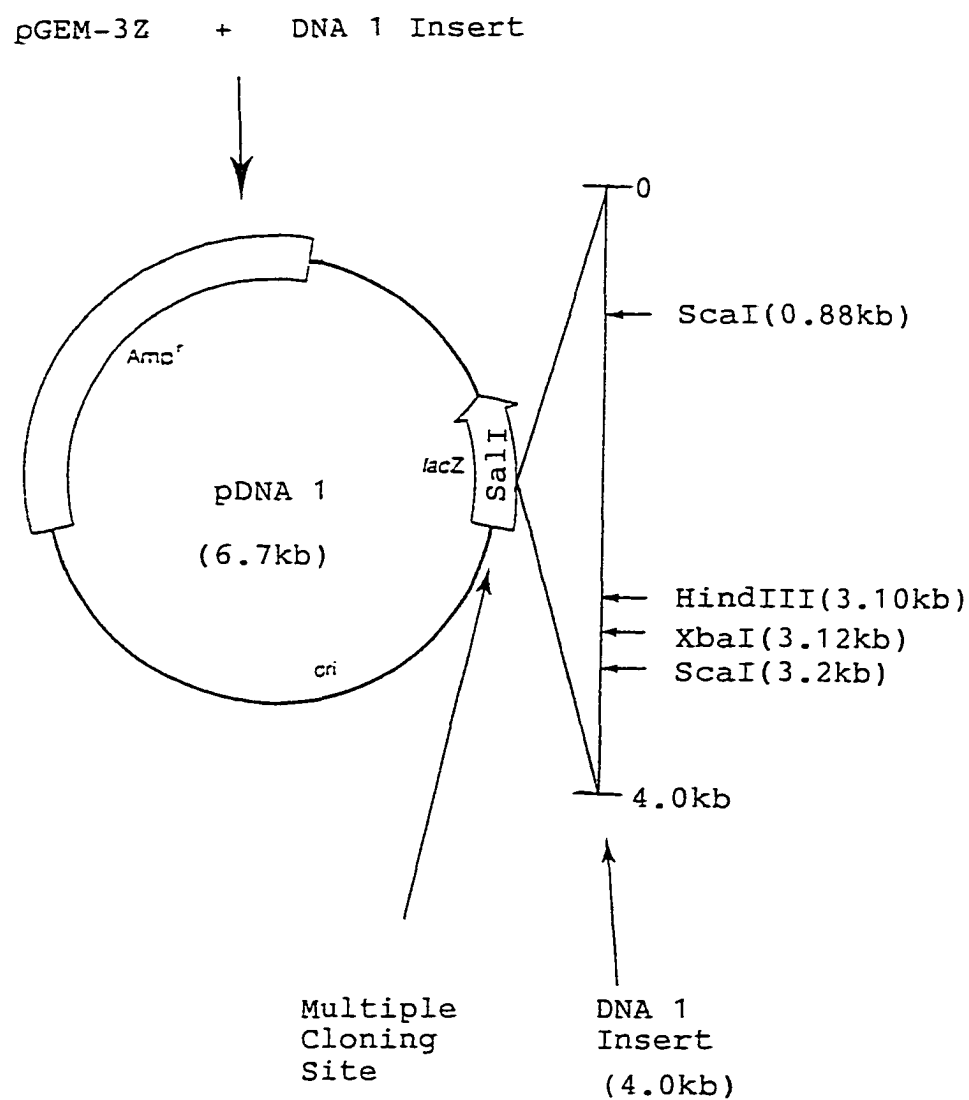


Figure 3.1 Plasmid Map For pDNA 1.

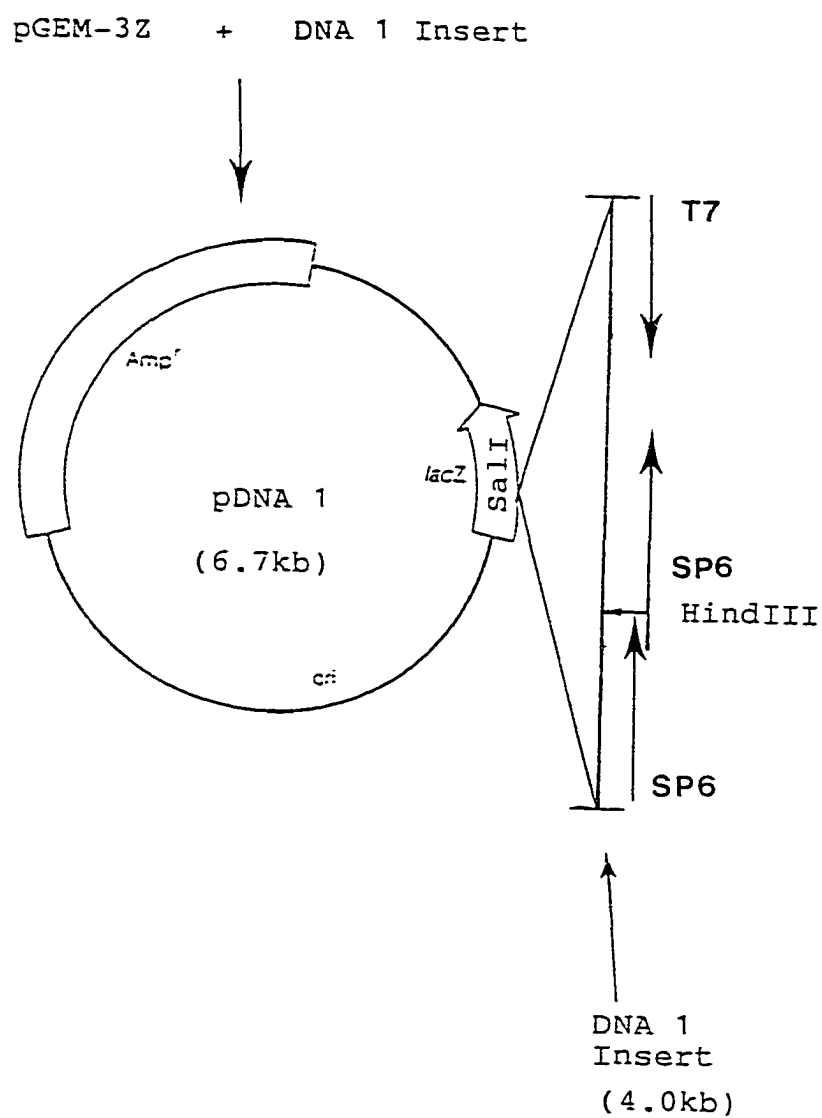


Figure 3.2 Sequencing Strategy For DNA 1.

HindIII site within DNA 1, it was apparent that a HindIII restriction digestion to remove a 1,000 bp fragment from DNA 1, followed by a ligation step to reseal the remaining 3,000 bp of information would enable more information to be determined from DNA sequencing via the SP6 promoter. The sequencing strategy for this region of DNA 1 is also shown in Figure 3.2 (p. 58).

Hence, following the required restriction digestion and ligation steps, an appropriate primer to the SP6 RNA polymerase promoter was used to determine the additional nucleotide information. This new partial DNA sequence for DNA 1 (DNA 1/HIND3/SP6) provided an additional 1,000 bp of information. The nucleotide sequence encoding DNA 1/HIND3/SP6 is shown in Appendix II (p. 125). DNA 1/HIND3/SP6 contains 1,032 bp of additional sequence information.

DNA 1 - DNAsis Analysis

(DNA Level)

Before DNAsis Software analysis was used, it was necessary to eliminate any pGEM-3Z vector nucleotide information that was present in the nucleotide sequence for DNA 1/T7, DNA 1/SP6, and DNA 1/HIND3/SP6. The nucleotide information for DNA 1/T7 that lacked any pGEM-3Z information (with 1-61 bp removed) was designated as DNA 1/Region 1. The nucleotide information removed from DNA 1/T7 to obtain DNA 1/Region 1 is underlined in Appendix II (p. 122).

The nucleotide information for DNA 1/SP6 that lacked any pGEM-3Z information (with 1-27 bp removed) was designated as DNA 1/Region 2. The nucleotide information removed from DNA 1/SP6 to obtain DNA 1/Region 2 is underlined in Appendix II (p. 124).

The nucleotide information for DNA 1/HIND3/SP6 that lacked any pGEM-3Z information (with 1-9 bp removed) was designated as DNA 1/Region 3. The nucleotide information removed from DNA 1/HIND3/SP6 to obtain DNA 1/Region 3 is underlined in Appendix II (p. 125).

The DNAsis Maximum Matching Alignment was used to determine if the nucleotide sequence of DNA 1 /Region 1, DNA 1/Region 2 and DNA 1/Region 3 (normal and complementary strands), was similar to the nucleotide sequence of the human hsp 27 gene. The alignments are shown in Appendix IV (p. 147, 151, 155, 159, 163, and 167). A summary of the alignments is shown in Table 2.1 (p. 61).

The DNAsis Maximum Matching Alignment was used to determine if the nucleotide sequence of DNA 1 /Region 1, DNA 1/Region 2 and DNA 1/Region 3 (normal and complementary strands), were similar to the nucleotide sequence of the most homologous, similar-sized region of the human hsp 27 gene. The alignments are shown in Appendix V (p. 192, 194, 196, 198, 200 and 202). A graphical illustration of these comparisons is shown in Figure 3.3 (p. 62).

In order to obtain partial sequence information for the approximately 4,000 bp DNA 1 insert, we required the 5'-sequence information for DNA 1/Region 3 (complementary strand), the 5'-information for DNA 1/Region 2 (complementary strand), and the overlapping regions between these two sequences. To obtain the overlapping sequence information, the Maximum Matching Alignment was used. The new partial sequence information for DNA 1 (DNA 1/Region 3+2) was 991 bp in length, and is shown in Appendix II (p. 127).

Table 2.1 DNA Alignments Between Areas Of DNA 1 And The Human hsp 27 Gene.

<u>Clone</u>	<u>Strand</u>	<u>Homology</u>
DNA 1/Region 1	Normal	34%
DNA 1/Region 1	Complementary	35%
DNA 1/Region 2	Normal	31%
DNA 1/Region 2	Complementary	32%
DNA 1/Region 3	Normal	32%
DNA 1/Region 3	Complementary	32%

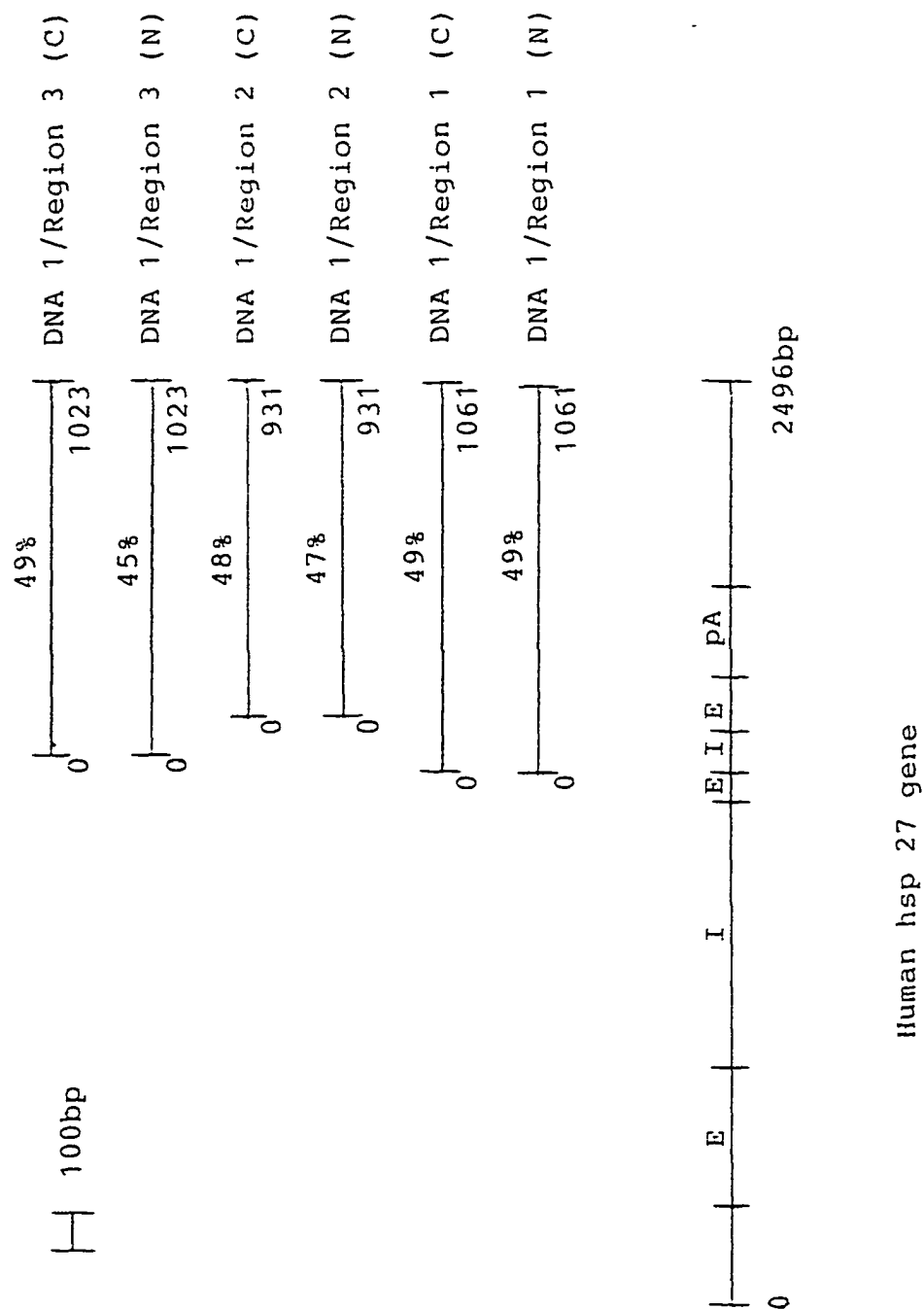


Figure 3.3 Homologous DNA Areas Between Regions Of DNA 1 (Normal And Complementary Strands), And The Human hsp 27 Gene. The exon (E), intron (I), and polyA (pA) regions of the human hsp 27 gene are shown.

DNA 1 - Blast Analysis

(DNA Level)

In order to determine if DNA 1 was similar to any known DNA sequence, the DNA sequence information for regions of DNA 1 was analyzed using the Blastn Algorithm. The results for the Blastn search using the nucleotide sequence of DNA 1/Region 1 (normal strand) are shown in Table 2.2 (p. 64). Some of the human sequences have been listed, out of the 46 total sequences identified for DNA 1/Region 1 (normal strand). The Blastn searches did not identify the human hsp 27 gene as a homologous sequence.

The results for the Blastn search using the nucleotide sequence of DNA 1/Region 2 (normal strand) are shown in Table 2.3 (p. 65). Some of the human sequences have been listed, out of the approximately 200 total sequences identified for DNA 1/Region 2 (normal strand). The Blastn searches did not identify the human hsp 27 gene as a homologous sequence.

The results for the Blastn search using the nucleotide sequence of DNA 1/Region 3 (normal strand) are shown in Table 2.4 (p. 66). Some of the human sequences have been listed, out of the 87 total sequences identified for DNA 1/Region 3 (normal strand). The Blastn searches did not identify the human hsp 27 gene as a homologous sequence.

DNA 1 - DNAsis Analysis

(Protein Level)

In order to compare the predicted amino acid sequences for DNA 1 to the amino acid sequence of the human hsp 27 gene, we attempted to identify the coding strands for regions of DNA 1, by determining which strands contained exons and introns. DNA 1/Region 1

Table 2.2 Blastn Results For DNA 1/Region 1.

<u>Homolog</u>	<u>GenBank Accession Number</u>	<u>Homology</u>	<u>Length</u>
Human DNA sequence from clone 757P12 on Chromosome Xp22.11-22	HS757P12	68%	59 bp
Homo sapiens NFKB1 gene	HSNFX1112	77%	43 bp
Homo sapiens Chromosome 17	AC005883	65%	59 bp
Homo sapien Chromosome 5	AC004629	68%	59 bp
Homo sapiens NFKB1 gene	HSNFX1112	77%	43 bp

Table 2.3 Blastn Results For DNA 1/Region 2.

<u>Homolog</u>	<u>GenBank Accession Number</u>	<u>Homology</u>	<u>Length</u>
Homo sapiens DNA sequence on Chromosome Xq25-q27	HS119E23	80%	39 bp
Homo sapiens Chromosome 16	HUAC004525	62%	59 bp
Human Lambda DNA for Immunoglobulin light chain	D87004	64%	59 bp
Homo sapiens LIPA gene	HSLIPA2	72%	64 bp

Table 2.4 Blastn Results For DNA 1/Region 3.

<u>Homolog</u>	<u>GenBank Accession Number</u>	<u>Homology</u>	<u>Length</u>
Homo sapiens Chromosome 17	AC004448	75%	51 bp
Homo sapiens Chromosome 21	AP000035	74%	36 bp
Homo sapiens T-cell Receptor	HUAE000658	60%	61 bp

(normal strand) showed the presence of one potential 5'-splice site at position 781. No 3'-splice sites were identified. DNA 1/Region 1 (complementary strand) showed the presence of 2 potential 3'-splice sites at positions 1154 and 174. The potential splice sites were preceded by a string of polypyrimidines. Hence, since there was a 5'-splice site on one strand, and a 3'-splice site on the other strand, we were unable to determine which strand of DNA 1/Region 1 was the coding strand. Therefore, we used a more random approach that involved the identification of a variety of Open Reading Frames, within all six reading frames.

The results for the open reading frames for both the normal and complementary strands of DNA 1/Region 1 are shown in Table 2.5 (p. 68). The DNAsis Maximum Matching Alignment was used to compare a number of these predicted amino acid sequences to the amino acid sequence for the human hsp 27 gene. Table 2.6 (p. 70) shows the results for these comparisons.

DNA 1/Region 2 (normal strand) showed the presence of 2 potential 3'-splice sites at positions 7 and 91. Neither of the potential splice sites were preceded by a string of polypyrimidines. No 5'-splice sites were identified. DNA 1/Region 1 (complementary strand) showed the presence of 8 potential 3'-splice sites at positions 1, 7, 9, 19, 158, 171, 588 and 598. The last five 3'-splice sites were preceded by a string of polypyrimidines. Hence, since there were 3'-splice sites on both strands, we were unable to determine which strand of DNA 1/Region 2 was the coding strand. This necessitated the search for a variety of open reading frames, within all six reading frames.

The results for the open reading frames for both the normal and complementary strands of DNA 1/Region 2 are shown in Table 2.7 (p. 71). The DNAsis Maximum Matching Alignment was used to compare a number of these predicted amino acid sequences

Table 2.5 Open Reading Frames For DNA 1/Region 1.

NORMAL

A	1 Frame		
	Start	End	MW
	1	37	1450.67
	472	556	3519.78
	481	556	3126.34
	538	556	807.75
	592	631	1468.65
	598	631	1238.33
B	865	910	1792.28
	2 Frame		
	Start	End	MW
	2	32	1276.43
	614	620	335.41
	626	638	491.54
	641	692	1962.66
	851	890	1674.15
C	1046	1058	585.66
	3 Frame		
	Start	End	MW
	3	42	1502.66
	243	267	904.01
	633	666	1371.61
	645	666	907.07

COMPLEMENTARY

D	1 Frame		
	Start	End	MW
	1	16	527.27
	463	526	2670.93
	523	526	149.21
	553	694	5510.89
	589	694	4183.40
	634	694	2416.40
	700	706	263.31
	712	784	2734.96
	829	832	149.21
	916	949	1351.57

E	2 Frame		
	Start	End	MW
	2	119	4522.78
	608	662	2085.12
	683	698	580.59
	749	845	3611.15
F	812	845	1343.52
	3 Frame		
	Start	End	MW
	3	144	5507.02
	108	144	1466.33
	177	369	7548.13
	210	369	6267.36
	549	561	524.66
	879	906	1075.24

Table 2.6 Amino Acid Alignments Between DNA 1/Region 1 And Human hsp 27.

<u>Sequence</u>	<u>Strand</u>	<u>Homology To hsp 27</u>	<u>Length</u>
A	Normal	11%	28 aa
B	Normal	11%	17 aa
C	Normal	7%	13 aa
D	Complementary	16%	47 aa
E	Complementary	17%	39 aa
F	Complementary	18%	53 aa

Table 2.7 Open Reading Frames For DNA 1/Region 2.

NORMAL

A	1 Frame		
	Start	End	MW
	1	112	4372.19
	25	112	3543.26
	70	112	1792.06
	151	178	1138.22
	253	346	3491.90
	301	346	1662.89
	340	346	246.32
	469	487	764.98
	568	580	537.70
	604	610	296.38
	670	685	709.80
B	2 Frame		
	Start	End	MW
	2	26	793.85
	182	398	8251.63
C	3 Frame		
	Start	End	MW
	3	99	3865.16
	102	105	149.21
	822	858	1410.19
	828	858	1160.12

COMPLEMENTARY

D	1 Frame		
	Start	End	MW
	1	58	2186.44
	109	238	5105.26
	331	355	964.97
	373	391	712.82
	421	472	1996.44
	667	679	392.41
	760	772	478.55

E	2 Frame		
	Start	End	MW
	2	32	1219.12
	251	344	3649.19
	410	422	537.70
	428	431	149.21
	515	551	1536.68
	571	761	3480.98
F	3 Frame		
	Start	End	MW
	3	120	4435.66
	447	480	1271.46
	630	663	1353.49
	741	753	565.67
	885	927	1761.13
	906	927	902.14

to the amino acid sequence for the human hsp 27 gene. Table 2.8 (p. 74) shows the results for these comparisons.

DNA 1/Region 3 (normal strand) showed the presence of one potential 3'-splice site at position 992. The potential 3'-splice site was preceded by a string of polypyrimidines. No 5'-splice sites were identified. DNA 1/Region 3 (complementary strand) showed the presence of 4 potential 3'-splice sites at positions 35, 98, 222 and 586. The last three 3'-splice sites were preceded by a string of polypyrimidines. Hence, since there were 3'-splice sites on both strands, we were unable to determine which strand of DNA 1/Region 3 was the coding strand. Therefore, we used a more random approach to determine the coding strand, that involved the identification of an open reading frame.

The results for the open reading frames for both the normal and the complementary strands of DNA 1/Region 3 are shown in Table 2.9 (p. 75). The DNAsis Maximum Matching Alignment was used to compare a number of these predicted amino acid sequences to the amino acid sequence for the human hsp 27 gene. Table 2.10 (p. 77) shows the results for these comparisons.

DNA 1 - Blast Analysis

(Protein Level)

In order to determine if DNA 1 was similar to any known amino acid sequence, the amino acid sequence information for regions of DNA 1 was analyzed using the Blastx Algorithm. i.e. the Blastx Algorithm was used to search for the nucleotide sequences of DNA 1/Region 1 (normal strand), DNA 1/Region 2 (normal strand), and DNA 1/Region 3 (normal

Table 2.8 Amino Acid Alignments Between DNA 1/Region 2 And Human hsp 27.

<u>Sequence</u>	<u>Strand</u>	<u>Homology To hsp 27</u>	<u>Length</u>
A	Normal	12%	37 aa
B	Normal	18%	72 aa
C	Normal	12%	32 aa
D	Complementary	15%	43 aa
E	Complementary	12%	31 aa
F	Complementary	6%	14 aa

Table 2.9 Open Reading Frames For DNA 1/Region 3.

NORMAL

A	1 Frame		
	Start	End	MW
	1	46	1829.16
	40	46	305.39
	214	268	1982.85
B	382	451	2651.23
	622	688	2639.92
	2 Frame		
	Start	End	MW
	2	2	18.02
C	65	71	277.38
	116	167	1970.39
	122	167	1740.07
	236	317	3135.47
	260	317	2205.50
	461	494	1269.32
	482	494	390.48
	554	602	2147.29
	563	602	1715.80
	3 Frame		
	Start	End	MW
	3	24	788.95
	285	288	149.21
	330	342	519.61
	546	564	580.70
	672	678	206.26
	855	1020	6463.49

COMPLEMENTARY

D	1 Frame		
	Start	End	MW
	1	79	2770.05
	460	496	1361.61
	493	496	149.21
	541	547	268.09
	604	616	523.61
	607	616	392.42
	796	829	1370.31
	820	829	425.53

E	2 Frame		
	Start	End	MW
	2	86	3085.56
	575	605	1129.00
	644	647	149.21
	653	716	2495.93
F	857	914	2259.67
	3 Frame		
	Start	End	MW
	3	171	6297.79
	312	339	969.87
	591	642	2065.48
	615	642	1031.30
	738	810	2838.45
	939	960	988.20
	945	960	709.84

Table 2.10 Amino Acid Alignments Between DNA 1/Region 3 And Human hsp 27.

<u>Sequence</u>	<u>Strand</u>	<u>Homology To hsp 27</u>	<u>Length</u>
A	Normal	15%	22 aa
B	Normal	10%	27 aa
C	Normal	9%	7 aa
D	Complementary	7%	12 aa
E	Complementary	18%	28 aa
F	Complementary	15%	24 aa

strand). The results for the Blastx searches using these nucleotide sequences did not identify any protein as being related to this query sequence, including human hsp 27.

DNA 1 - PCR Primer Location Analysis

In order to further determine if DNA 1 was a human hsp 27 gene, we attempted to identify sites where the PCR primers should have bound to the DNA 1/Region 1, DNA 1/Region 2 and DNA 1/Region 3 nucleotide sequences (normal and complementary strands). The results indicated that the PCR primers did not bind to any of these regions on the DNA 1 nucleotide sequence.

DNA 2 - Size And Restriction Map

The positive clone pDNA 2 was sized by cutting the DNA using specific restriction endonucleases and separating the fragments on an agarose gel. The restriction analysis of pDNA 2 indicated that we had cloned a fragment, (DNA 2) that was approximately 4,300 bp long. Restriction digestion sites in pDNA 2 were examined by cutting the DNA 2 using all the restriction enzymes mentioned in the Materials and Methods section. A plasmid map was then generated for pDNA 2, as shown in Figure 4.1 (p. 79).

DNA 2 - DNA Sequence

Since the plasmid map for the positive clone, pDNA 2, showed that the size of DNA 2 was approximately 4,300 bp in length, it was apparent that only about 2,000 bp of the nucleotide sequence information for DNA 2 could be obtained from two rounds of automated

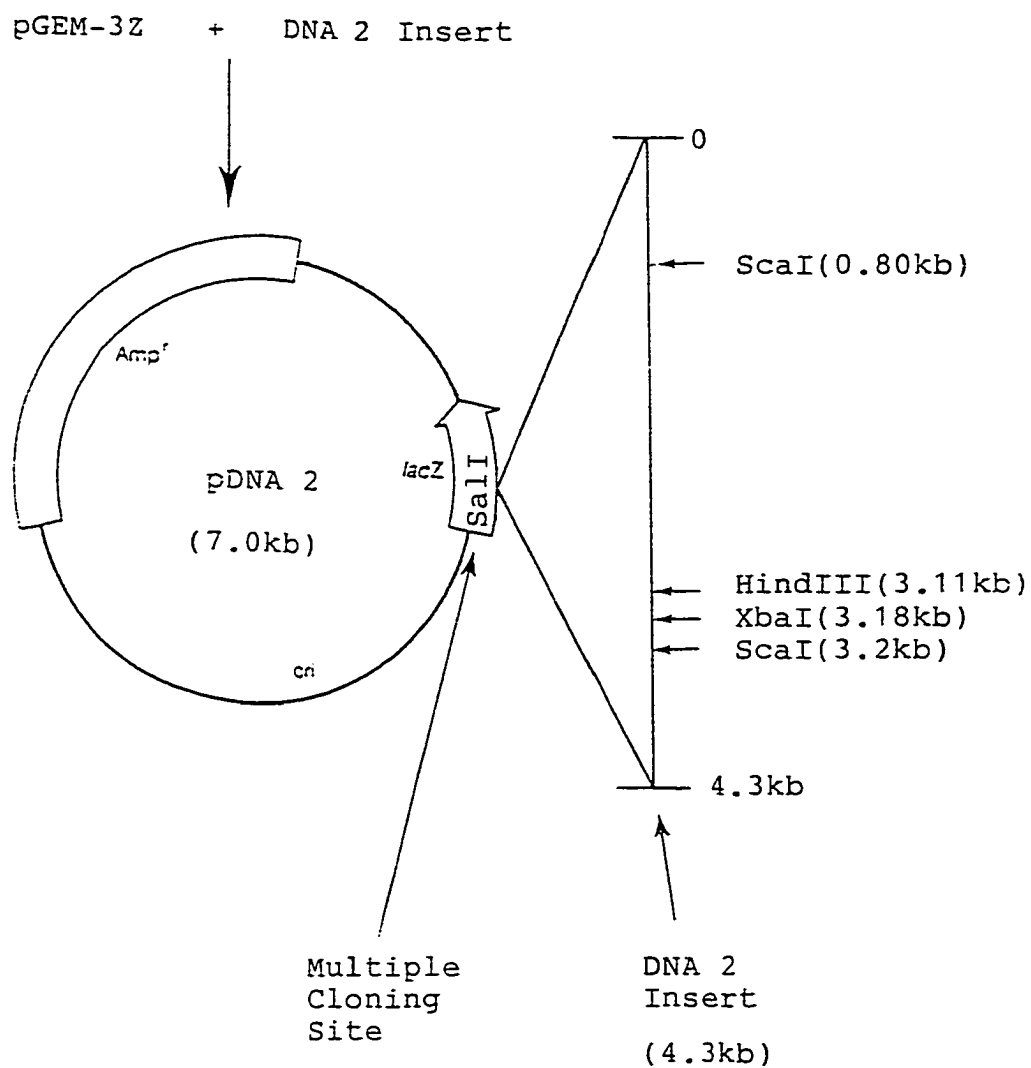


Figure 4.1 Plasmid Map For pDNA 2.

DNA sequencing (using the T7 and SP6 promoters). The sequencing strategy for regions of DNA 2 is shown in Figure 4.2 (p. 81).

The nucleotide sequence encoding DNA 2 (892 bp) using the T7 promoter (DNA 2/T7), is shown in Appendix II (p. 128). The nucleotide sequence encoding DNA 2 (977 bp) using the SP6 promoter (DNA 2/SP6), is shown in Appendix II (p. 129).

DNA 2 - DNAsis Analysis

(DNA Level)

Before DNAsis Software analysis was used, it was necessary to eliminate any pGEM-3Z vector nucleotide sequence that was present in the nucleotide sequence for DNA 2/T7 and DNA 2/SP6. The modified information of DNA 2/T7 that lacked any pGEM-3Z information (with 1-35 bp removed) was redesignated as DNA 2/Region 1. The nucleotide information removed from DNA 2/T7 to obtain DNA 2/Region 1 is underlined in Appendix II (p. 128).

The modified information of DNA 2/SP6 that lacked any pGEM-3Z information (with 1-27 bp removed) was redesignated as DNA 2/Region 2. The nucleotide information removed from DNA 2/SP6 to obtain DNA 2/Region 2 is underlined in Appendix II (p. 129).

The DNAsis Maximum Matching Alignment was used to determine if the nucleotide sequence of DNA 2 /Region 1 and DNA 2/Region 2 (normal and complementary strands), were similar to the nucleotide sequence of the human hsp 27 gene. The alignments are shown in Appendix IV (p. 171, 175, 179, and 183). A summary of the alignments is shown in Table 3.1 (p. 82).

The DNAsis Maximum Matching Alignment was used to determine if the nucleotide

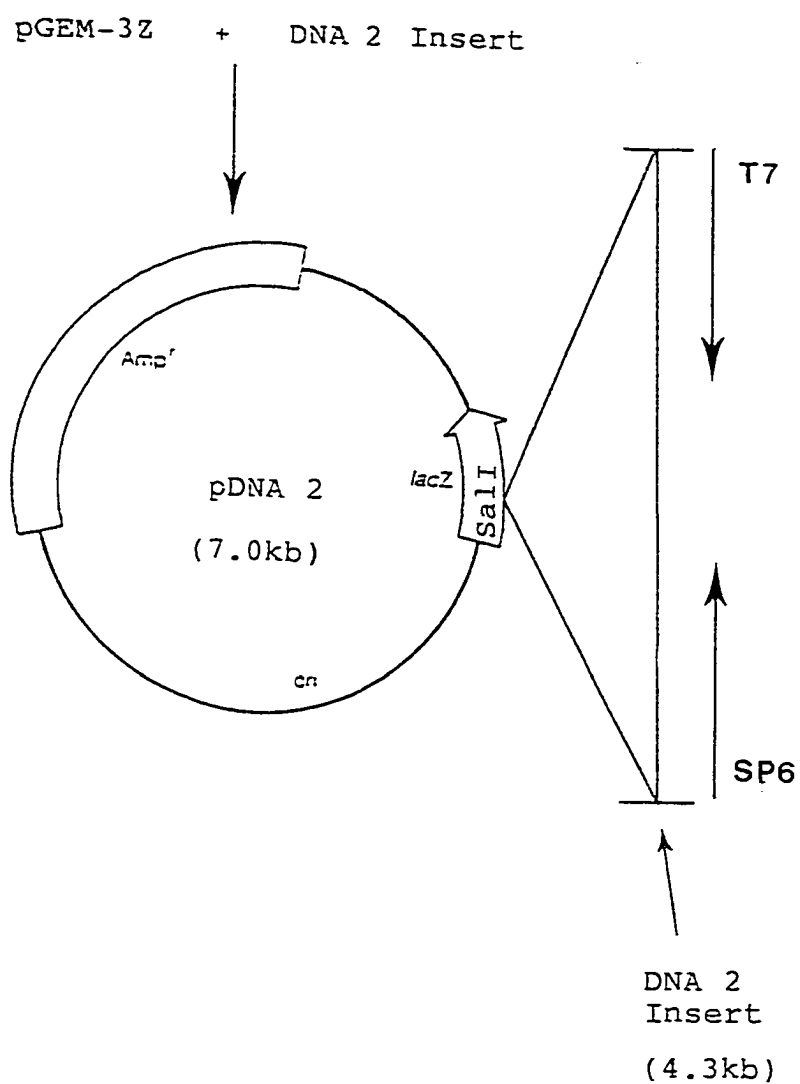


Figure 4.2 Sequencing Strategy For DNA 2.

Table 3.1 DNA Alignments Between Regions Of DNA 2 And The Human hsp 27 Gene.

<u>Clone</u>	<u>Strand</u>	<u>Homology</u>
DNA 2/Region 1	Normal	29%
DNA 2/Region 1	Complementary	29%
DNA 2/Region 2	Normal	31%
DNA 2/Region 2	Complementary	32%

sequence of DNA 2 /Region 1 and DNA 2/Region 2 (normal and complementary strands), were similar to the nucleotide sequence of the most homologous, similar-sized region of the human hsp 27 gene. The alignments are shown in Appendix V (p. 204, 206, 208, and 210). A graphical illustration of these comparisons is shown in Figure 4.3 (p. 84).

DNA 2 - Blast Analysis

(DNA Level)

In order to determine if DNA 2 was similar to any known DNA sequence, the DNA sequence information for regions of DNA 2 was analyzed using the Blastn Algorithm. The results for the Blastn search using the nucleotide sequence of DNA 2/Region 1 (normal strand) are shown in Table 3.2 (p. 85). Some of the human sequences have been listed, out of the 17 total sequences identified for DNA 2/Region 1 (normal strand). The Blastn searches did not identify the human hsp 27 gene as a homologous sequence.

The results for the Blastn search using the nucleotide sequence of DNA 2/Region 2 (normal strand) are shown in Table 3.3 (p. 86). Some of the human sequences have been listed, out of the approximately 125 total sequences identified for DNA 2/Region 2 (normal strand). The Blastn searches did not identify the human hsp 27 gene as a homologous sequence.

DNA 2 - DNAsis Analysis

(Protein Level)

In order to compare the predicted amino acid sequence for DNA 2 to the amino acid sequence of the human hsp 27 gene, it was necessary to determine the coding strands for

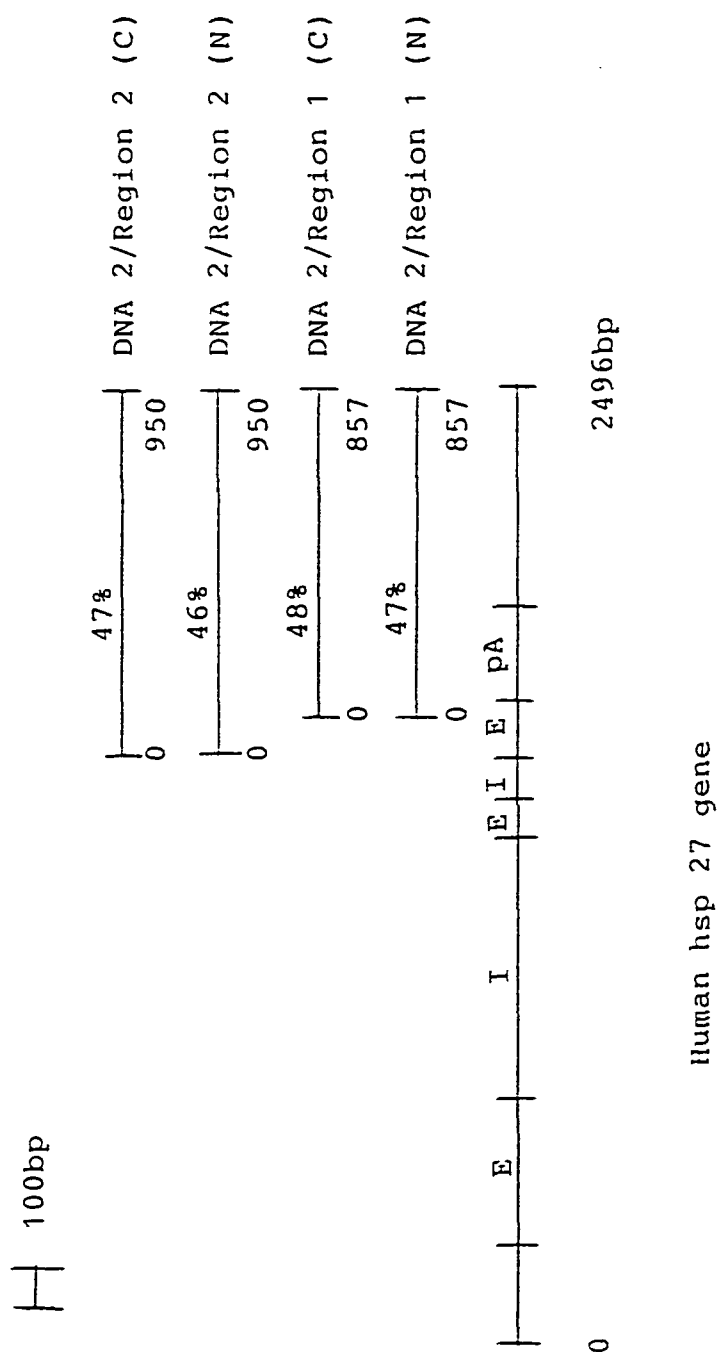


Figure 4.3 Homologous DNA Areas Between Regions Of DNA 2 (Normal And Complementary Strands), And The Human hsp 27 Gene. The exon (E), intron (I), and polyA (pA) regions of the human hsp 27 gene are shown.

Table 3.2 Blastn Results For DNA 2/Region 1.

<u>Homolog</u>	<u>GenBank Accession Number</u>	<u>Homology</u>	<u>Length</u>
Human DNA sequence region on ChromosomeX	HSU65A4	72%	50 bp
Homo sapiens NFKB1 gene	HSNFX1112	77%	43 bp
Human Genomic Sperm Library	AO170454	62%	39 bp
Homo sapiens Chromosome 20	AC004499	73%	52 bp

Table 3.3 Blastn Results For DNA 2/Region 2.

<u>Homolog</u>	<u>GenBank Accession Number</u>	<u>Homology</u>	<u>Length</u>
Homo sapiens Serine Threonine Kinase Receptor	HUMSKR2	82%	38 bp
Homo sapiens Chromosome 21 Down Syndrome	AP000015	76%	45 bp
Homo sapiens Chromosome 4	AC004056	70%	59 bp
Homo sapiens DNA sequence on Chromosome Xq25-q27	HS119E23	80%	39 bp

regions of DNA 2. Therefore, we attempted to identify the coding strands by determining which strands of regions of DNA 2 contained exons and introns.

DNA 2/Region 1 (normal strand) indicated the presence of 2 potential 3'-splice sites at positions 717 and 809. Both of the 3'-splice sites were preceded by a string of polypyrimidines. No 5'-splice sites were identified. Interestingly, DNA 2/Region 1 (complementary strand) showed the presence of one potential 5'-splice site at 16. There were also 4 potential 3'-splice sites at positions 10, 67, 68, and 253. Only the last three potential splice sites were preceded by a string of polypyrimidines. Hence, since there was a 5'-splice site and a 3'-splice site on this strand, we predicted that the normal strand of DNA 2/Region 1 was the coding strand, which would result in the synthesis of the predicted amino acid sequence. Therefore, an intron region of the normal strand of DNA 2/Region 1 (16-68 bp) was excised, and the remaining exons were joined together.

The exon information was used to determine the coding strand for the Open Reading Frame. Table 3.4 (p. 88) shows the open reading frames for the normal strand of DNA 2/Region 1. Then, the DNAsis Maximum Matching Alignment was used to compare three of these predicted amino acid sequences to the amino acid sequence for the human hsp 27 gene. Table 3.5 (p. 89) shows the results for these comparisons.

DNA 2/Region 2 (normal strand) showed the presence of 3 potential 3'- splice sites at positions 7 and 91 and 907. Only one of the potential splice sites was preceded by a string of polypyrimidines. No 5'-splice sites were identified. DNA 2/Region 2 (complementary strand) showed the presence of 2 potential 3'-splice sites at positions 607 and 617. Both of the 3'-splice sites were preceded by a string of polypyrimidines. Since there were 3'-splice sites on both strands, we were unable to determine which strand of DNA 2/Region 2 was the

Table 3.4 Open Reading Frames For DNA 2/Region 1.

NORMAL

A	1 Frame		
	Start	End	MW
	1	43	1814.03
	427	460	1280.26
	493	583	3514.54
B	539	700	4364.23
	2 Frame		
	Start	End	MW
	2	11	418.47
	197	221	904.01
C	437	512	3006.77
	569	587	725.83
	3 Frame		
	Start	End	MW
	3	15	403.46

Table 3.5 Amino Acid Alignments Between DNA 2/Region 1 And Human hsp 27.

<u>Sequence</u>	<u>Strand</u>	<u>Homology To hsp 27</u>	<u>Length</u>
A	Normal	15%	37 aa
B	Normal	11%	25 aa
C	Normal	26%	4 aa

coding strand. Therefore, we used a more random approach to that involved all six reading frames.

The results for the open reading frames for both the normal and complementary strands of DNA 2/Region 2 are shown in Table 3.6 (p. 91). The DNAsis Maximum Matching Alignment was used to compare a number of these predicted amino acid sequences to the amino acid sequence for the human hsp 27 gene. Table 3.7 (p. 93) shows the results for these comparisons.

DNA 2 - Blast Analysis

(Protein Level)

In order to determine if DNA 2 was similar to any known amino acid sequence, the amino acid sequence information for regions of DNA 2 was analyzed using the Blastx Algorithm. Initially, the Blastx Algorithm was used to search for the nucleotide sequences of DNA 2/Region 1 (normal strand). The results for the Blastx search using this nucleotide sequence identified only one homolog as being homologous to this query sequence. The homolog was the 40 aa Pyruvate Flavodoxin Oxidoreductase Protein (NIFJ ENTAG), and was found to be 32% homologous. The Blastx search did not identify the human hsp 27 protein as a homologous sequence. The Blastx Algorithm was used to search for the nucleotide sequences of DNA 2/Region 2 (normal strand). Only four proteins were identified as being homologous to the query sequence. Some of the human sequences have been listed in Table 3.8 (p.94). The Blastx searches did not identify the human hsp 27 protein as a homologous sequence.

Table 3.6 Open Reading Frames For DNA 2/Region 2.

NORMAL

A	1 Frame		
	Start	End	MW
	1	112	4372.19
	25	112	3543.26
	70	112	1792.06
	151	178	1138.22
	253	346	3491.90
	301	346	1662.89
	340	346	246.32
B	469	487	764.98
	2 Frame		
	Start	End	MW
	2	26	793.85
	182	398	8251.63
	227	398	6710.95
	569	572	149.21
C	671	686	649.70
	824	836	496.33
	3 Frame		
	Start	End	MW
	3	99	3865.16
	102	105	149.21
	603	609	296.38
	681	711	1151.06
	819	849	1309.68

COMPLEMENTARY

D	1 Frame		
	Start	End	MW
	1	67	2772.64
	391	436	1813.12
	466	499	1271.46
	649	682	1353.49
	760	772	565.67
	904	946	1761.18
	925	946	902.14

E	2 Frame		
	Start	End	MW
	2	179	6855.27
	131	179	1806.68
	269	281	472.51
	440	491	2016.19
	686	698	392.41
	779	791	478.55
F	3 Frame		
	Start	End	MW
	3	147	5776.97
	63	147	3410.63
	159	363	7808.52
	351	363	537.66
	429	441	537.70
	447	450	149.21
	534	570	1536.68
	690	780	3480.98
	738	780	1631.92

Table 3.7 Amino Acid Alignments Between DNA 2/Region 2 And Human hsp 27.

<u>Sequence</u>	<u>Strand</u>	<u>Homology To hsp 27</u>	<u>Length</u>
A	Normal	12%	37 aa
B	Normal	18%	72 aa
C	Normal	12%	32 aa
D	Complementary	10%	15 aa
E	Complementary	8%	17 aa
F	Complementary	12%	30 aa

Table 3.8 Blastx Results For DNA 2/Region 2.

<u>Homolog</u>	<u>GenBank Accession Number</u>	<u>Homology</u>	<u>Length</u>
Insulin	INS ANSAN	75%	16 aa
Insulin	INS LEPS	60%	17 aa

DNA 2 - PCR Primer Location Analysis

In order to further determine if DNA 2 was a linked human hsp 27 gene, we attempted to identify sites where the PCR primers should have bound to the DNA 2/Region 1 and DNA 2/Region 2 nucleotide sequences (normal and complementary strands). The results indicated that the PCR primers did not bind to any of these regions on the DNA 2 nucleotide sequence.

Summary

Table 4.1 (p. 96) shows a summary of the homologies between DNA 4, and human hsp 27 that were obtained from the DNAsis Maximum Matching Alignment. The same table shows the summary of the homologies between regions of DNA 1 and human hsp 27, as well as the homologies between regions of DNA 2 and human hsp 27.

DNA 3

The positive clone pDNA 3 was sized by cutting the DNA using specific restriction endonucleases and separating the fragments on an agarose gel. The restriction analysis of pDNA 3 indicated that we had cloned a fragment, (DNA 3) that was approximately 1,200 bp long.

Restriction digestion sites in pDNA 3 were examined by cutting the DNA at specific sites using the restriction enzymes EcoRI, PstI, BamHI, HindIII, and ScaI. A plasmid map was then generated for pDNA 3, on the basis of the restriction enzyme digestion patterns. The plasmid map for pDNA 3 is shown in Figure 5.1 (p. 97). There were no restriction sites

Table 4.1 Summary Of DNA And Amino Acid Alignments Between Human hsp 27 And DNA 4, DNA 1 And DNA 2.

<u>DNA Clone</u>	<u>Nucleotide Homology To hsp 27</u>	<u>Amino Acid Homology To hsp 27</u>
DNA 4	38-51%	7-30%
DNA 1/Region 1	35-49%	7-18%
DNA 1/Region 2	31-48%	6-18%
DNA 1/Region 3	32-45%	3-18%
DNA 2/Region 1	29-48%	11-26%
DNA 2/Region 2	31-47%	8-18%

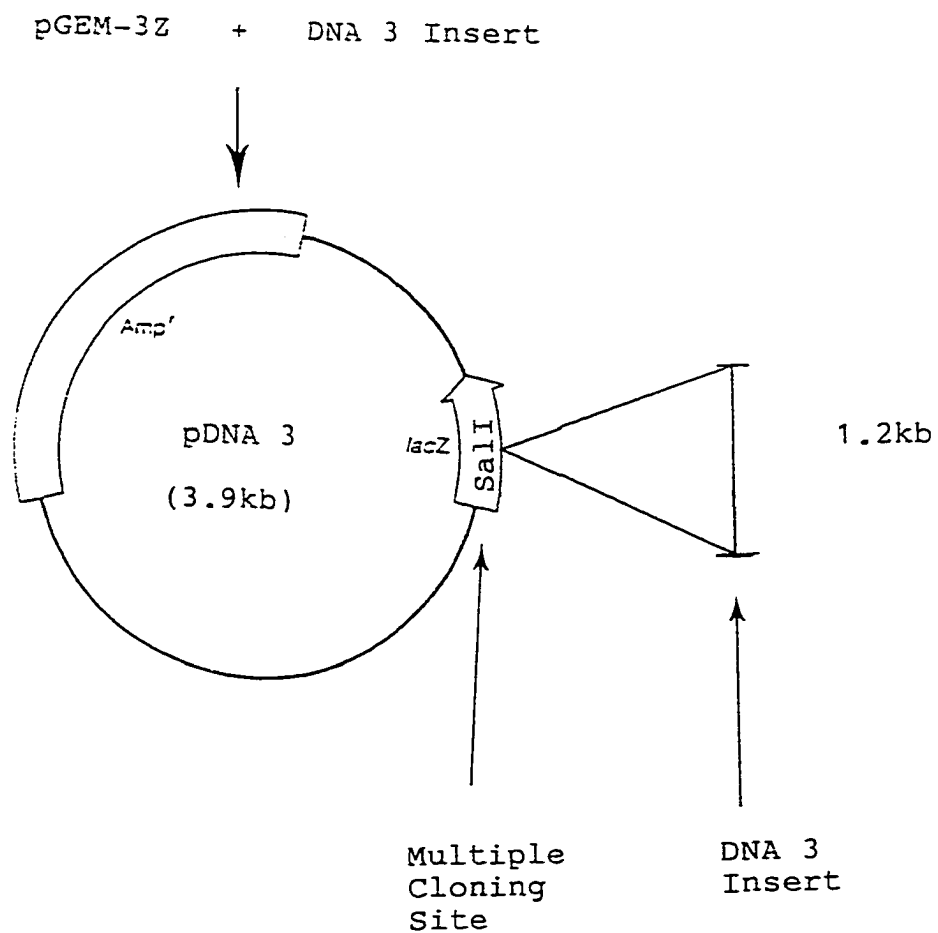


Figure 5.1 Plasmid Map For pDNA 3.

for any of these enzymes within the DNA 3 insert. The plasmid map for pDNA 3 showed that we had cloned a fragment that was approximately 1,200 bp in size.

Since the plasmid maps of pDNA 3 and pDNA 4 showed similarities in the digestion patterns for DNA 3 and DNA 4 (i.e. no restriction sites for five enzymes), and since DNA 3 was identical in size to DNA 4, this raised the possibility that DNA 3 and DNA 4 were identical pieces of DNA. Comparisons between the nucleotide information for DNA 3 and DNA 4 may have confirmed the similarity between these two inserts. However, at this point in time, no further analysis has been conducted using pDNA 3.

DNA 5

The positive clone pDNA 5 was sized by cutting the DNA using specific restriction endonucleases and separating the fragments on an agarose gel. The total size of the digestion product was approximately 2,700 bp. i.e. it was the size of the parental pGEM-3Z plasmid. The restriction analysis of pDNA 5 indicated that this clone contained no DNA insert. Hence, no further analysis was conducted using pDNA 5.

DNA 1 vs. DNA 2

The nucleotide sequence of DNA 2 was submitted to the GenBank database, because it was the largest-sized insert (of approximately 4,300 bp). Before the submission of DNA 1, it was necessary to determine if DNA 1 came from a larger-sized DNA 2, i.e. it was important to determine if DNA 1 and DNA 2 contained identical sequences. This possibility arose since the plasmid map for pDNA 2 seemed to contain identical restriction sites as in pDNA 1, and the inserts that these plasmids contained were both similar in size (DNA 1 -

4,000 bp and DNA 2- 4,300 bp). In order to determine if DNA 1 and DNA 2 contained similar sequences, the nucleotide sequences of regions of DNA 1 were compared to the nucleotide sequences of regions of DNA 2.

The DNAsis Maximum Matching Alignment was used to determine if the nucleotide sequence of DNA 1/Region 1 (normal strand), was similar to the nucleotide sequence of DNA 2/Region 1 (normal strand). In this comparison, a 70% overall identity was indicated (some regions are 100%), by the results shown in Appendix VI (p. 213). The DNAsis Maximum Matching Alignment was used to compare the nucleotide sequence of DNA 1/Region 2 (normal strand), with the nucleotide sequence of DNA 2/Region 2 (normal strand). In this comparison, an 86% identity was indicated by the results shown in Appendix VI (p. 215).

Hence, due to the high identities in the starting and ending regions of DNA 1 and DNA 2, it was possible that DNA 1 is related to DNA 2 (with DNA 2 containing about 300 bp of additional unsequenced information that was not contained in DNA 1). The extra 300 bp of information may lie in the unsequenced region between the SP6 and T7 information. Figure 6.1 (p. 100) shows the comparison between the insert DNA 1, and the insert DNA 2. Hence, it was possible that DNA 1 and DNA 2 were different pieces of DNA. Therefore, the nucleotide sequence of DNA 1 was submitted to the GenBank database.

DNA 4 vs. DNA 2/1

Before submission of DNA 4 to the GenBank database, it was necessary to determine if DNA 4 came from a larger-sized DNA 2 or DNA 1 (DNA 2/1). In order to determine if DNA 4 and DNA 2/1 contained identical nucleotide sequences, the nucleotide sequence of

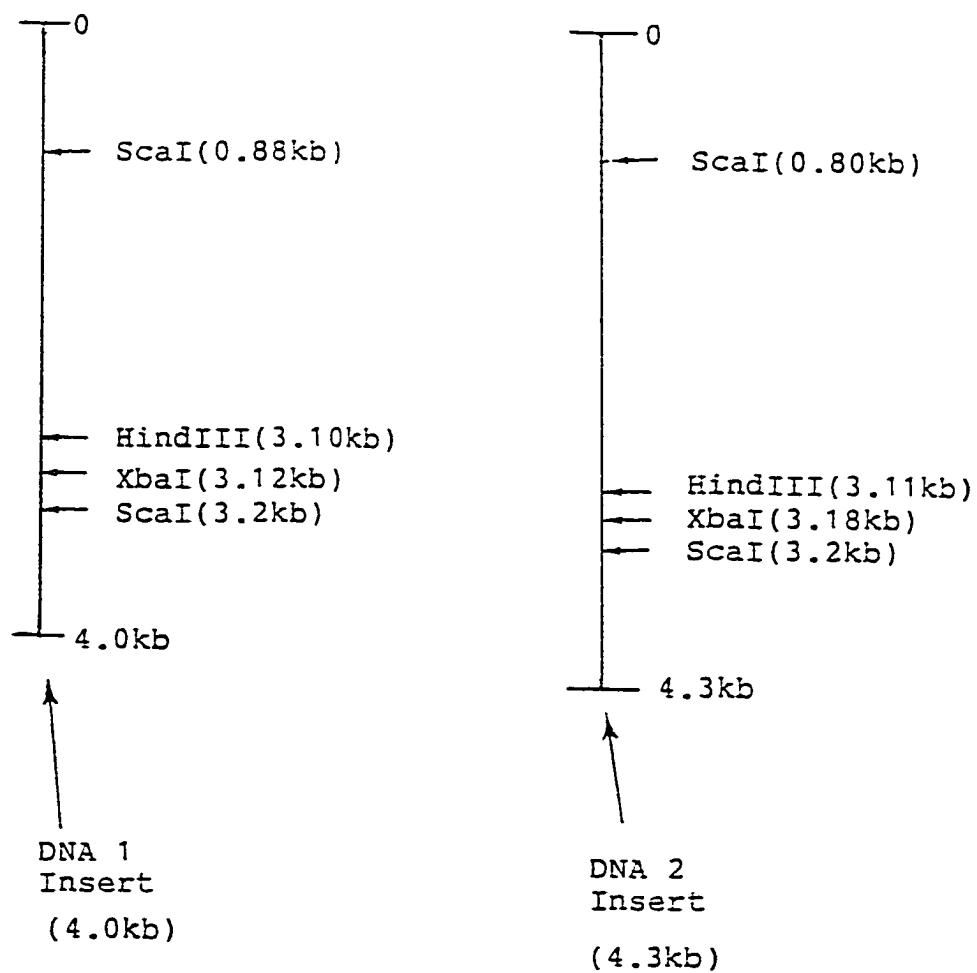


Figure 6.1 Comparison Between DNA 1 And DNA 2.

DNA 4 (normal strand) was compared to the nucleotide sequence for DNA 2/Region 1 (normal strand), using the DNAsis Maximum Matching Alignment. In this comparison, a 48% identity was indicated by the results shown in Appendix VI (p. 217).

The nucleotide sequence of DNA 4 (normal strand) was compared to the nucleotide sequence for DNA 2/Region 2 (normal strand), using the DNAsis Maximum Matching Alignment. In this comparison, a 47% identity was indicated by the results shown in Appendix VI (p. 219).

Due to these low identity results, it was possible that DNA 4 was a unique sequence. Hence, it was possible that DNA 4 and DNA 2/1 were different pieces of DNA. Therefore, the nucleotide sequence of DNA 4 was submitted to the GenBank database.

Nucleotide Sequence

Accession Numbers

The nucleotide sequence for DNA 1, the nucleotide information for DNA 2, and the complete nucleotide information for DNA 4 were deposited with GenBank under the accession numbers: AF120329, AF120330, AF120331, AF120332, and AF120333.

CHAPTER 4

DISCUSSION

In order to find the gene members of the human hsp 27 multigene family, five positive clones were identified following colony hybridization and PCR analysis (pDNA 1, pDNA 2, pDNA 3, pDNA 4, and pDNA 5). Four of these clones contained DNA inserts (DNA 1, DNA 2, DNA 3 and DNA 4). pDNA 5 did not contain an insert, and no further analysis was conducted on pDNA 5. The insert contained in pDNA 3 was also eliminated from further analysis, as discussed in the Materials and Methods section. Hence, the inserts DNA 4, DNA 1, and DNA 2 were subjected to subsequent analysis to determine if they were the linked gene members of the human hsp 27 multigene family.

Since human hsp 27 cDNA is 762 bp in length, and the promoter region of the human hsp 27 gene is minimally 200 bp in length (15), we predicted that the sizes of the DNA inserts contained in the inserts must be greater than 1,000 bp, in order to contain the complete information for the genes. The restriction digestion results indicated that the inserts DNA 1, and DNA 2, were better candidates for full-length members of the human hsp 27 multigene family, as they were both significantly greater than 1,000 bp in length (DNA 1 and DNA 2 were approximately 4,000 bp and 4,300 bp long). The size of the insert, DNA 4 (approximately 1,200 bp), was too small to be a full-length member of the family.

Further considerations determining if the inserts were the closely-related, gene members of the human hsp 27 multigene family, were based on the fact that any closely-related member should be homologous to hsp 27 in its amino acid sequence. Murine hsp 27 is closely-related to human hsp 27 (23). Blastx search results indicated that murine hsp 27 exhibited an 86% homology to human hsp 27 at the protein level. Rat hsp 27 is also closely-related to human hsp 27 (23). Blastx search results indicated that rat hsp 27 exhibited an 87% homology to human hsp 27 at the protein level. The distantly-related alpha-crystallins (21) were found to be 45-55% homologous to human hsp 27 at the amino acid level (Blastx search results). Therefore, we predicted that our inserts would be greater than 50% homologous to human hsp 27, at the protein level, if they were the closely-related, gene members of the human hsp 27 multigene family. Align algorithm results using the amino acid sequences of the inserts indicated that neither DNA 1, DNA 2, nor DNA 4 appeared to be closely-related gene members of the human hsp 27 multigene family. These inserts were less than 50% homologous to human hsp 27 at the amino acid level, from all predicted open reading frames. See Table 4.1 (p. 96).

It is important to note the manner in which we selected our amino acid sequences. The coding strand for the synthesis of an amino acid sequence was determined from the identification of 5' and 3'-splice sites. In the identification of a potential 3'-splice site from a preceding polypyrimidine string, only the strings CCCCC and TTTTT were used. An exhaustive search of potential 3'-splice sites would have included all possible polypyrimidine strings such as CTTTT, CCTTT, TCTCT and CTCTC. Hence, it may appear that the omission of a potential 3'-splice site may have affected our results. However, in our analysis, a coding strand was chosen when both 5' and 3'-splice sites were identified. If a strand

contained only a 5'-splice site, it was not chosen as the coding strand, and both strands of DNA were used to code for predicted amino acid sequences. Therefore, if we did indeed miss a potential 3'-splice site, it should not have affected our results.

PCR primer location analysis also indicated that one of the inserts (DNA 4), was not closely-related to the human hsp 27 multigene family. This was due to the fact that a sequence search for the hybridization sites of the PCR primers, could not identify the corresponding motif in the DNA 4 nucleotide sequence. Therefore, since DNA 4 underwent PCR analysis specifically to amplify this region, this indicated that DNA 4 was a false positive. Hence, DNA 4 could not be one of the members of the gene family that we desired. When the sequenced regions of DNA 1, and DNA 2 were analyzed for the corresponding PCR primer motif, no such motif was found. However, we cannot conclude that DNA 1 and DNA 2 are also false positives, because the motif may be present in the unsequenced regions of these inserts. DNA 5 was also a false positive, as it contained no insert, and should not have been amplified by PCR. It is possible that PCR false positives may have been avoided, if we had used pGEM-3Z as a negative control, rather than the wild-type lambda EMBL3 phage.

We went on to determine if the inserts were distantly-related to the human hsp 27 multigene family at the DNA Level. An alignment of a closely-related member of the human hsp 27 multigene family, should be homologous to hsp 27 in its nucleotide sequence. Murine hsp 27 is closely-related to human hsp 27, and was found to exhibit an 75-90% homology to hsp 27 at the DNA level (Blastn search results, data not shown). Rat hsp 27 is also closely-related to human hsp 27, and was found to exhibit an 72-88% homology to hsp 27 at the DNA level (Blastn search results, data not shown). Hence, we determined that closely-related

members of the human hsp 27 multigene family, would be greater than 50% homologous to human hsp 27, at the DNA level. Therefore, we predicted that distantly-related members of the human hsp 27 multigene family would be less than 50% homologous to the human hsp 27 gene.

Align algorithm results using the nucleotide sequences of the inserts contained in the positive clones indicated that neither DNA 1, DNA 2, nor DNA 4 appeared to be closely-related members of the human hsp 27 multigene family. These inserts were not greater than 50% homologous to human hsp 27 at the DNA level. However, these inserts were very close to 50% homologous to hsp 27 at the DNA level. See Table 4.1 (p. 96). Hence, these results indicated that our inserts may be distantly-related to the human hsp 27 multigene family.

It is interesting to note that the nucleotide sequences of both the normal and complementary strands of an insert, aligned with the same region of the human hsp 27 gene. These similarities should not have occurred, as the normal and complementary strands of a DNA sequence contain different nucleotide information. However, the graphical illustrations shown in the Results section indicated that some of the normal and complementary strand information (for a particular insert), lined up with the 3'-untranslated region of the human hsp 27 gene, as well as other regions near the 3'-end of the gene. Hence, it seems that both the normal and complementary strands contain some similarity in the 3'-regions, which caused the entire sequences of the strands, to align with that region of the human hsp 27 gene (with the greatest homology percentage). The 3'-untranslated region of the human hsp 27 gene contains Alu sequences. Alu sequences are short repetitive sequences (SINES), that range from 100-500 bp in length. They are present in about 9% of the human genome, and have no specific function. The presence of these repetitive sequences can cause an artificial

increase in the homology percentage obtained for alignments (29). Hence, the presence of repetitive Alu sequences in the 3'-end of the human hsp 27 gene may have caused the normal and complementary strands of the inserts to line up with the same regions of the human hsp 27 gene. Therefore, these alignments showed an artificially higher homology percentage than alignments with other regions of the human hsp 27 gene.

We attempted to determine the identity of the proposed distantly-related human hsp 27 genes (i.e. the inserts). Blast Algorithm searches of the GenBank nucleotide database, and the SwissProt protein database indicated that the inserts were novel sequences. This is because the DNA sequences, and the amino acid sequences of the inserts showed homologies to very short regions of other known genes (regions of about 36-64 bp). These short regions of other genes, showed high homologies to our inserts, however, the regions of similarity might not actually be true homologies, but instead sites of short repeating secondary structures (such as alpha-helices and beta-pleated sheets), that most proteins typically possess (29).

In conclusion, in our attempts to isolate the closely-related members of the human hsp 27 multigene family (i.e. the linked-genes)(15), three positive clones containing genomic DNA from the human A549 cell line were identified. Subsequent Align algorithm analysis of the nucleotide sequences and the predicted amino acid sequences of the positive clones suggested that we had in fact, isolated distantly-related members of the human hsp 27 multigene family. A closer examination of the individual steps employed in our research may explain our results.

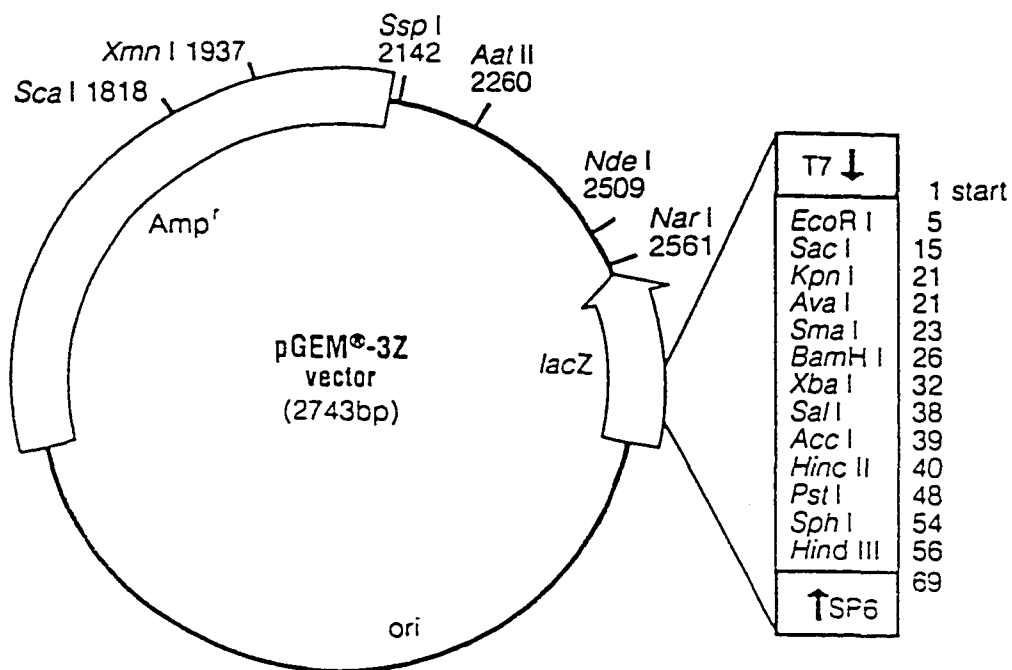
Firstly, it is possible that the distantly-related genes are present in a higher copy number in the genomic DNA (from the human A549 cell line), than the linked human hsp

27 genes. In this case, a greater proportion of the recombinant plaques selected from colony hybridization would contain the distantly-related genes. We obtained 300,000 total plaques from colony hybridization. Since we only analyzed 5 out of 50 positive plaques, it may appear that we may have statistically reduced our chances of isolating the linked genes. Hence, it may seem that further screenings of more plaques, may have enabled us to obtain the linked human hsp 27 genes. However, the human genome contains 6×10^9 bp of information. Since our plaques contained fragments of approximately 20 kb in length, it would require the examination of 300,000 plaques in order to find a single copy gene. Therefore, our examination of 5 of 50 positive plaques, obtained from 300,000 total plaques, should have enabled us to find at least one human hsp 27 gene.

Secondly, it is possible that the conditions chosen for the PCR analysis, may also have reduced our chances of finding the linked genes. The PCR conditions (i.e. the ideal reaction mixtures), were designed to amplify human hsp 27 cDNA. These conditions may have allowed our primers to hybridize to non-specific sites in the distantly-related genes, which had less than 100% homology to human hsp 27. It is possible that if the project is repeated, and the conditions for PCR amplification are designed to work best for the human hsp 27 gene sequence, we may isolate the desired linked genes. However, the focus of our laboratory has shifted from cloning these genes to other areas in apoptosis. Therefore, at this time, we have suspended further attempts to clone, and study the regulation of the linked human hsp 27 genes. Our efforts have concluded with the submission of the novel nucleotide sequences for the distantly-related genes to the GenBank nucleotide database. We will resume our studies involving the regulation of the linked gene members of the human hsp 27 multigene family, after the genes have been cloned by the Human Genome Project.

APPENDIX I

Plasmid Map For pGEM-3Z



APPENDIX II

DNA Sequence For pGEM-3Z

(The T7 and SP6 promoters are underlined).

10	20	30	40	50	60
GGGCGAATTC	GAGCTCGGTA	CCCGGGGATC	CTCTAGAGTC	GACCTGCAGG	CATGCAAGCT
70	80	90	100	110	120
TGAGTATTCT	<u>ATAGTGTAC</u>	<u>CTAAATAGCT</u>	TGGCGTAATC	ATGGTCATAG	CTGTTTCCTG
SP6 PROMOTER					
130	140	150	160	170	180
TGTGAAATTG	TTATCCGCTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	ATAAAGTGTA
190	200	210	220	230	240
AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	TCAGTCCCCG
250	260	270	280	290	300
CTTTCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA
310	320	330	340	350	360
GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG	CTTCCTCGCT	CACTGACTCG	CTGCGCTCGG
370	380	390	400	410	420
TCGTTTCGGCT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG
430	440	450	460	470	480
AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC
490	500	510	520	530	540
GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCG	ATAGGCTCCG	CCCCCCTGAC	GAGCATCACA
550	560	570	580	590	600
AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT
610	620	630	640	650	660
TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC
670	680	690	700	710	720
TGTCCGCTTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTTCTCA	TAGCTCACGC	TGTAGGTATC
730	740	750	760	770	780
TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC
790	800	810	820	830	840
CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGTA	AGACACGACT
850	860	870	880	890	900
TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG
910	920	930	940	950	960
CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTTGGTA

970	980	990	1000	1010	1020
TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA
1030	1040	1050	1060	1070	1080
AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA
1090	1100	1110	1120	1130	1140
AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAAACG
1150	1160	1170	1180	1190	1200
AAAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC
1210	1220	1230	1240	1250	1260
TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG
1270	1280	1290	1300	1310	1320
ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTCAT
1330	1340	1350	1360	1370	1380
CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT	ACGGGAGGGC	TTACCATCTG
1390	1400	1410	1420	1430	1440
GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	TTATCAGCAA
1450	1460	1470	1480	1490	1500
TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	TCCGCCTCCA
1510	1520	1530	1540	1550	1560
TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCGCCAGTT	AATAGTTTGC
1570	1580	1590	1600	1610	1620
GCAACGTTGT	TGGCATTGCT	ACAGGCATCG	TGGTGTACAG	CTCGTCGTTT	GGTATGGCTT
1630	1640	1650	1660	1670	1680
CATTGAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGTGCAAAA
1690	1700	1710	1720	1730	1740
AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAGTTGGCC	GCAGTGTTAT
1750	1760	1770	1780	1790	1800
CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	GTAAGATGCT
1810	1820	1830	1840	1850	1860
TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGCGACCGA
1870	1880	1890	1900	1910	1920
GTTGCTCTTG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAAG
1930	1940	1950	1960	1970	1980
TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GAAACTCTC	AAGGATCTTA	CCGCTGTTGA
1990	2000	2010	2020	2030	2040
GATCCAGTTC	GATGTAACCC	ACTCGTGCAC	CCAACTGATC	TTCAGCATCT	TTTACTTTCA

2050	2060	2070	2080	2090	2100
CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAAG	GGATAAAGCG
2110	2120	2130	2140	2150	2160
CGACACGGAA	ATGTTGAATA	CTCTACTCT	TCCTTTTCA	ATATTATTGA	AGCATTTATC
2170	2180	2190	2200	2210	2220
AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG
2230	2240	2250	2260	2270	2280
GGGTTCCGCG	CACATTTCCC	CGAAAAGTGC	CACCTGACGT	CTAAGAAACC	ATTATTATCA
2290	2300	2310	2320	2330	2340
TGACATTAAC	CTATAAAAT	AGGCGTATCA	CGAGGCCCTT	TCGTCTCGCG	CGTTTCCGGT
2350	2360	2370	2380	2390	2400
ATGACGGTGA	AAACCTCTGA	CACATGCAGC	TCCCGGAGAC	GGTCACAGCT	TGTCTGTAAG
2410	2420	2430	2440	2450	2460
CGGATGCCGG	GAGCAGACAA	GCCCGTCAGG	GCGCGTCAGC	GGGTGTTGGC	GGGTGTCGGG
2470	2480	2490	2500	2510	2520
GCTGGCTTAA	CTATGCGGCA	TCAGAGCAGA	TTGTACTGAG	AGTGCACCAT	ATGCGGTGTG
2530	2540	2550	2560	2570	2580
AAATACCGCA	CAGATGCGTA	AGGAGAAAAT	ACCGCATCAG	GCGCCATTTC	CCATTTCAGG
2590	2600	2610	2620	2630	2640
TGCGCAACTG	TTGGGAAGGG	CGATCGGTGC	GGGCCTCTTC	GCTATTACGC	CAGCTGGCGA
2650	2660	2670	2680	2690	2700
AAGGGGGATG	TGCTGCAAGG	CGATTAGTT	GGGTAACGCC	AGGGTTTTCC	CAGTCACGAC
2710	2720	2730	2740	2750	2760
GTTGTAAAAC	GACGGCCAGT	GAATTGTAAT	AGGACTCACT	ATA.....

T7 PROMOTER

DNA Sequence For Human hsp 27 cDNA

(GenBank Accession Number X54079)

10	20	30	40	50	60
GCCCAGCGCC	CCGCACTTTT	CTGAGCAGAC	GTCCAGAGCA	GAGTCAGCCA	GCATGACCGA
70	80	90	100	110	120
GCGCCGCGTC	CCCTTCTCGC	TCCTGCGGGG	CCCCAGCTGG	GACCCCTTCC	GCGACTGGTA
130	140	150	160	170	180
CCCGCATAGC	CGCCTCTTCG	ACCAGGCCCTT	CGGGCTGCCC	CGGCTGCCGG	AGGAGTGGTC
190	200	210	220	230	240
GCACTGGTTA	GGCGGCAGCA	GCTGGCCAGG	CTACGTGCGC	CCCCTGCCCC	CCGCCGCCAT
250	260	270	280	290	300
CGAGAGCCCC	GCACTGGCCG	CGCCCGCCTA	CAGCCGCGCG	CTCAGCCGGC	AACTCAGCAG
310	320	330	340	350	360
CGGGGTCTCG	GAGATCCGGC	ACACTGCGGA	CCGCTGGCGC	GTGTCCCTGG	ATGTCAACCA
370	380	390	400	410	420
CTTCGCCCCG	GACGAGCTGA	CGGTCAAGAC	CAAGGATGGC	GTGGTGGAGA	TCACCGGCAA
430	440	450	460	470	480
GCACGAGGAG	CGGCAGGACG	AGCATGGGTA	CATCTCCCGG	TGCTTCACGC	GGAAATACAC
490	500	510	520	530	540
GCTGCCCCCC	GGTGTGGACC	CCACCCAAGT	TTCTCTCTCC	CTGTCCCCTG	AGGGCACACT
550	560	570	580	590	600
GACCGTGGAG	GCCCCCATGC	CCAAGCTAGC	CACGCAGTCC	AACGAGATCA	CCATCCCAGT
610	620	630	640	650	660
CACCTTCGAG	TCGCGGGCCC	AGCTTGGGGG	CCCAGAAAGT	GCAAAATCCG	ATGAGACTGC
670	680	690	700	710	720
CGCCAAGTAA	AGCCTTAGCC	CGGATGCCCA	CCCCTGCTGC	CGCCACTGGC	TGTGCCTCCC
730	740	750	760	770	780
CCGCCACCTG	TGTGTTCTTT	TGATACATTT	ATCTTCTGTT	TTTCTCAAAT	AAAGTTCAAA
790	800	810	820	830	840
GCAACCACC.

DNA Sequence For The

Human hsp 27 Gene

(GenBank Accession Number X03900)

10	20	30	40	50	60
GAATTCATTT	GCTTTTCCTT	AACGAGAGAA	GGTTCCAGAT	GAGGGCTGAA	CCCTCTTCGC
70	80	90	100	110	120
CCCCCCCACG	CCCCCTGAAC	GCTGGGGGAG	GAGTGCATGG	GGAGGGGCGG	CCCTCAAACG
130	140	150	160	170	180
GGTCATTGCC	ATTAATAGAG	ACCTCAAACA	CCGCCTGCTA	AAAATACCCG	ACTGGAGGAG
190	200	210	220	230	240
CATAAAAGCG	CAGCCGAGCC	CAGCGCCCCG	CACTTTTCTG	AGCAGACGTC	CAGAGCAGAG
250	260	270	280	290	300
TCAGCCAGCA	TGACCGAGCG	CCGCGTCCCC	TTCTCGCTCC	TGCGGGGCCC	CAGCTGGGAC
<u>EXON 1</u>					
310	320	330	340	350	360
CCCTTCCGCG	ACTGGTACCC	GCATAGCCGC	CTCTTCGACC	AGGCCTTCGG	GCTGCCCCGG
370	380	390	400	410	420
CTGCCGAGG	AGTGGTCGCA	GTGGTTAGGC	GGCAGCAGCT	GGCCAGGCTA	CGTGCGCCCC
430	440	450	460	470	480
CTGCCCCCGG	CCGCCATCGA	GAGCCCCGCA	GTGGCCGCGC	CCGCCTACAG	CCGCGCGCTC
490	500	510	520	530	540
AGCCGGCAAC	TCAGCAGCGG	GGTCTCGGAG	ATCCGGCACA	CTGCGGACCG	CTGGCGCGTG
550	560	570	580	590	600
TCCCTGGATG	TCAACCACTT	CGCCCCGGAC	GAGCTGACGG	TCAAGACCAA	GGATGGCGTG
610	620	630	640	650	660
GTGGAGATCA	CCGGTGAGCC	CCCCTGCTCC	TGCAGGGGAG	AGGAGGAGGC	TAGCAGGGCG
670	680	690	700	710	720
GGCAGGGCCG	GGGGCGTGCG	GTTGAAACGG	GGGTCCCGGG	GGCCTGGGGA	GTTAAACGTT
730	740	750	760	770	780
GGCCCAGCAC	CGGGAAAAAC	AGGACTCCTG	ATTCCCTTGC	TCAGGAATTG	GGAGTGCGGG
790	800	810	820	830	840
TCGCTTCTAA	GGGCGCTTTC	TGCTCTGTAA	TCCCAGCGCT	TTGGGAGGCC	GAGACGGGAG
850	860	870	880	890	900
GATCGCTTGA	GGCCAGGAGT	TCAAGACTAG	CCTGGGCAAC	ATAGCGAGAC	GCGCCCCCCC
910	920	930	940	950	960
GCCCCGACCC	CGCGCCATTA	CAAAAAAATA	GCAAACAAAA	ATTTTTTTTAA	AGATCATCGA

970	980	990	1000	1010	1020
TGAAGAGAGA	AAATGCGCTT	TTCTACAGAG	TCCCCTTCCC	ACCCACAGCC	CCATCCCCAG
1030	1040	1050	1060	1070	1080
ATAAGCGGGG	AGTTCCCTGG	CGCGGTGCCA	GTTTCTAGCC	GCTGAGTGGG	CGTGTGCGCG
1090	1100	1110	1120	1130	1140
GCTCCAAGTG	CGCCTGCGTA	CTGCTCACTC	CCCAGCTCCG	CGCCCTGCTC	CGTTCCTCCC
1150	1160	1170	1180	1190	1200
AAAACCTCTGA	ATCGAAGAAC	TTTCCGGAAG	TTTCTGAGAG	CCCAGACCGG	CGGGCACGCC
1210	1220	1230	1240	1250	1260
CCCATCCCCA	ACCCCCTCTG	TTAATCCCTA	CCAGCCTGCA	GTCCTGGCTG	CTTCCAAGCA
1270	1280	1290	1300	1310	1320
GGAGGTGGGG	CCTCTGGCTA	GCGGGGCCGA	AAAAGTCCCC	TCCCCCGCAT	GTCTGATTTT
1330	1340	1350	1360	1370	1380
CCTCTTCCCC	CCAAAGGCAA	GCACGAGGAG	CGGCAGGACG	AGCATGGCTA	CATCTCCCCG
EXON 2					
1390	1400	1410	1420	1430	1440
TGCTTCACGC	GGAAATACAC	GTGAGTCCTG	GCGCCAGGTC	GGGGTGGGTG	GGTGGCGTGG
1450	1460	1470	1480	1490	1500
GGGTGGGGTC	AGGGAAGAGG	GCACAGGGAC	CCACCCGGTG	TGTAATGTAA	CGCTTGCCTT
1510	1520	1530	1540	1550	1560
TCCTCTCTGC	ACGTCCAGGC	TGCCCCCCCG	TGTGGACCCC	ACCCAAGTTT	CCTCCTCCCT
EXON 3					
1570	1580	1590	1600	1610	1620
GTCCCCTGAG	GGCACACTGA	CCGTGGAGGC	CCCCATGCCC	AAGCTAGCCA	CGCAGTCCAA
1630	1640	1650	1660	1670	1680
CGAGATCACC	ATCCCAGTCA	CCTTCGAGTC	GCGGGCCCAG	CTTGGGGGCA	GAAGCTGCAA
1690	1700	1710	1720	1730	1740
AATCCGATGA	GACTGCCGCC	AAGTAAAGCC	TTAGCCCGGA	TGCCCACCCC	TGCTGCCGCC
1750	1760	1770	1780	1790	1800
ACTGGCTGTG	CCTCCCCCGC	CACCTGTGTG	TTCTTTTGAT	ACATTTATCT	TCTGTTTTTC
1810	1820	1830	1840	1850	1860
TCAAATAAAG	TTCAAAGCAA	CCACCTGTCA	CTGGCCCAGG	CCCTGGTGTT	TGTGGAAGGA
1870	1880	1890	1900	1910	1920
AGCCTCAGGC	ACCTGCCATT	TGCTGGCTTT	CAGGAGTCAT	CTTTGCTCAG	GCCCCGTGCTG
1930	1940	1950	1960	1970	1980
GGCCATGTGG	GTACACTGGT	GTAGGTTGCT	GGACACAGGC	TGACTCACAT	CCATAAAGAC
1990	2000	2010	2020	2030	2040
AGAGGTCPTA	GGGCCGGGCG	CAGTGGCTCA	TACCTACAAT	CCCAGCACTT	TGGGGGGTTG

2050	2060	2070	2080	2090	2100
AAGCAGGAGG	AGTGCTTGAA	GCCAAGAGTT	CTAGACCAGC	CTGGACAACA	TAGTAAGACT
2110	2120	2130	2140	2150	2160
GTCTCTAAAA	AATAAAAATT	AGGCAGGGTG	GTACTGCACG	CCTGTAGTCC	CAGCTACTCA
2170	2180	2190	2200	2210	2220
GGAGGCTGAG	GCAGGAGGAT	CGCTTGAGCC	CAGAGTTGTG	AAGGTACAGT	GAGCTAACAT
2230	2240	2250	2260	2270	2280
CGTGCCATTG	CACTCCAGCC	TGGGCAACAG	AACAAGATCC	TGTCTCAAAA	CAACCAAAAG
2290	2300	2310	2320	2330	2340
CCCAGAGAGA	AAGAGTGAGA	CCCCATCTTT	AAAAGAAAAA	AAAAAAAGGT	CATGATTGCA
2350	2360	2370	2380	2390	2400
AGGTCACGAT	TGCAATTAAA	ACTGTAAGGT	GGGGAAGGAG	GAGGAAATAA	GAGAAGCACC
2410	2420	2430	2440	2450	2460
TGAGGCTTGA	GTTCTCAGGA	GCACCTAGGT	TGGGTCCCAG	GTGAAGGGCC	ACAGAGGTAA
2470	2480	2490	2500	2510	2520
TTGCACCTCA	GAGCTGATGG	GAGGATTACT	ATGTCA....

DNA Sequence For DNA 4/T7

(The pGEM-3Z information that was removed is underlined).

10	20	30	40	50	60
CATTCGAGTC	GGTACCGGGG	ACCTCTAGAT	CGACCTTGAA	TACGGAGCTT	CAAGCCAGGC
70	80	90	100	110	120
TCCAGCTGGC	GTTTTAGGTG	TGGTGCAAGC	AANGCTGGGT	ANGTATTTCC	AGGAAGCAGT
130	140	150	160	170	180
AGANGAGCTC	GCCGTGCAAT	ATGAATTATC	ATTAGCAGCA	AGGAAATTCCG	TCAACGATTT
190	200	210	220	230	240
GTTGGTAAAT	GAATTTCTCTG	TCAGATATCC	AGTTGCTTGG	GAGAACGCTG	CCTTTGCGCC
250	260	270	280	290	300
TCCTGCGGAC	GGCTCAATCT	GGCTTAAGTA	CGACTACACA	GAAGTTGACA	CAGTAACATA
310	320	330	340	350	360
CGGGTTAAGC	AGGAAGTGCA	AATACTACGT	TGGCATGGTG	CAGATTTTCAG	TGTTCTTCAG
370	380	390	400	410	420
TCCGGGGACT	GGGATTGATA	AGCCGAGACA	AATAGCTAAT	CAATTGGCAG	AATCTATCGT
430	440	450	460	470	480
TGATGGTACA	ATGCTTGACA	GCGGGACCAT	TTATGANTCT	GGAGTTGTTA	ACCCGGTTAT
490	500	510	520	530	540
CAAATCCAAG	TCTGGGTGGT	TTATCCCGGT	TCGTTTTTAT	GTTCGTCTAN	ACTAACAAAA
550	560	570	580	590	600
GGAAAAATAC	ATGGCCCATC	TCAGCAATGG	CACGCAGGTC	TTCGTNNAAA	GCTCTCGCGG
610	620	630	640	650	660
AACTGCAATC	GACNTAACTG	CAATTTCTAA	CGCAGTTACC	CCTGTTTTTAA	CTGTATCTGA
670	680	690	700	710	720
CCNCTGGTT	TGGTCNTTGG	TGAATACCTG	CTTTTCANCT	CTTCTGCTTC	AACGCTTCTA
730	740	750	760	770	780
ACTGANAANC	AANTTCNAAT	GACTGCAATC	CCTGGTACNT	CAGTAACTGT	TGAAAGGTTT
790	800	810	820	830	840
GATACCTCCC	ACCACCNCAA	AATTCCCNNG	TNGGCTTAAC	TGGGTGAAAT	TTTCAAAATC
850	860	870	880	890	900
ACCCCGGGT	TCCAAATTTT	CNTGCGNTTC	AGGAANTTTC	NACNGAAAGG	GGGGGAAANA
910	920	930	940	950	960
ANCANTTCCC	TTAACTTCCC	ATTGCTTGTT	CNAANNAAAC	GG.....

DNA Sequence For DNA 4/SP6

(The pGEM-3Z information that was removed is underlined).

10	20	30	40	50	60
CAAGCTTGCA	TGCCTGCAGG	GTCGACCACG	AATGGATACC	GCGATGGATA	CCGTTTCGAT
70	80	90	100	110	120
TTCGTTAACA	CCAATGGTAG	GGGTGTCGTT	GAAAGCGATA	GTACCGGACT	GCAAGCGCAT
130	140	150	160	170	180
TTCGCTAGCT	CGAGGTACGA	ACAGACGAAT	CGCAACAACC	TGGCCAGACT	CATCGTAGTT
190	200	210	220	230	240
ACGCAGAAC	GGATATACAG	GGTTGGTGTA	CTCGTGAGCG	AAGGTGAAGG	TGTTAGTTAC
250	260	270	280	290	300
CGCAGATTTA	TAGGTCGGAA	TCTGCTGTTC	ACGGTCATCG	GACAAGCACT	GGAAGTTAAC
310	320	330	340	350	360
GAATTGCTGT	TCGCCACCGT	CAGTAGATAC	GTCCTGAACG	CACGGAACTT	CGAACCAGGA
370	380	390	400	410	420
GGTGATCTTG	ACAACCTCAC	CAGTTAAGCC	AGCCGGGAAC	TTTGTGGTGC	TGGAGGTATC
430	440	450	460	470	480
AATACCTTCA	ACAGTTACTG	ACGTACCAGT	GATTGCAGTC	ACTCNAACTT	GCTTGTCAGC
490	500	510	520	530	540
TAGAANCGTT	GAAGCAGAA	AAGTGAAAAG	CAGGTAATCA	CCAACGACCA	AACCAGACGC
550	560	570	580	590	600
GTCAGATACA	GTAAAACAG	GGGTAAGTGC	GTTAGAAATT	GCAGTTACGT	CNATTGCAGC
610	620	630	640	650	660
TCCNCTNNAN	CCTTCTACNA	AAAAGTGCCT	GCCATTGCTG	AAATGGGCCA	GTTTTTTTCC
670	680	690	700	710	720
CTTTGTTAAT	CTAGACAACA	TAAAAACGAA	CCNGGAATAA	ACCCCCNAAC	TGGATTGTGAN
730	740	750	760	770	780
AACCGGGTTA	CAACNCCAAA	CNCATAAATG	GTCCCCTGTT	CAACATGTTT	CCNCACNAAA
790	800	810	820	830	840
AATCCGCCCT	TGAATAACTA	TTTTTTTCGNN	TACAANCCAT	CCCCGGACTG	AAAAACTGAA
850	860	870	880	890	900
ATCGCCCCNG	CCACTTTTTT	TTCCCTCCCC	CTNACCCTTT	TTCCGNGTCA	CTCCGTTTTN
910	920	930	940	950	960
CCTACTNAAC	CNAATAACCC	CCNGGAAGCC	AANGGGNTTC	NCCT.....

Complete DNA Sequence For DNA 4

10	20	30	40	50	60
TCGACCTTGA	ATACGGAGCT	TCAAGCCAGG	CTCCAGCTGG	CGTTTTAGGT	GTGGTGCAAG
70	80	90	100	110	120
CAAGGCTGGG	TATGTATTTC	CAGGAAGCAG	TAGANGAGCT	CGCCGTGCAA	TATGAATTAT
130	140	150	160	170	180
CATTAGCAGC	AAGGAAATTC	GTCAACGATT	TGTTGGTAAA	TGAATTCCT	GTCAGATATC
190	200	210	220	230	240
CAGTTGCTTG	GGAGAACGCT	GCCTTTGCGC	CTCCTGCGGA	CGGCTCAATC	TGGCTTAAGT
250	260	270	280	290	300
ACGACTACAC	AGAAGTTGAC	ACAGTAACAT	ACGGGTTAAG	CAGGAAGTGC	AAATACTACG
310	320	330	340	350	360
TTGGCATGGT	GCAGATTTC	GTGTTCTTCA	GTCCGGGGAC	TGGGATTGAT	AAGCCGAGAC
370	380	390	400	410	420
AAATAGCTAA	TCAATTGGCA	GAATCTATCG	TTGATGGTAC	AATGCTTGAA	CAGCGGGACC
430	440	450	460	470	480
ATTTATGAGT	CTGGAGTTGT	TAACCCGGTT	ATCAAATCCA	AGTCTGGGTG	GTTTATTCCC
490	500	510	520	530	540
GGTTCGTTTT	TATGTTTCGTC	TAGACTAACA	AAAGGAAAAA	TACATGGCCC	ATCTCAGCAA
550	560	570	580	590	600
TGGCACGCAG	GTCTTCGTAG	AAAGCTCTCG	CGGAACTGCA	ATCGACGTAA	CTGCAATTTTC
610	620	630	640	650	660
TAACGCAGTT	ACCCCTGTTT	TAAGTGTATC	TGACGCGTCT	GGTTTGGTCG	TTGGTGAATA
670	680	690	700	710	720
CCTGCTTTTC	ACCTCTTCTG	CTTCAACGCT	TCTAACTGAC	AANCAAGTTC	GAATGACTGC
730	740	750	760	770	780
AATCCCTGGT	ACGTCAGTAA	CTGTTGAAGG	TTTGATACCT	CCAGCACCAC	AAAGTTCCCCG
790	800	810	820	830	840
GCTGGCTTAA	CTGGTGAAGT	TGTCAAGATC	ACCTCCTGGT	TCGAAGTTCC	GTGCGTTCAG
850	860	870	880	890	900
GACGTATCTA	CTGACGGTGG	CGAACAGCAA	TTCGTTAAGT	TCCAGTGCTT	GTCCGATGAC
910	920	930	940	950	960
CGTGAACAGC	AGATTCCGAC	CTATAAATCT	GCGGTAAGTA	ACACCTTCAC	CTTCGCTCAC

970	980	990	1000	1010	1020
GAGTACACCA	ACCCTGTATA	TCCGGTTCTG	CGTAACTACG	ATGAGTCTGG	CCAGGTTGTT
1030	1040	1050	1060	1070	1080
GCGATTGTC	TGTTTCGTACC	TCGAGCTAGC	GAAATGCGCT	TGCAGTCCGG	TACTATCGCT
1090	1100	1110	1120	1130	1140
TTCAACGACA	CCCCTACCAT	TGGTGTTAAC	GAAATCGAAA	CGGTATCCAT	CGCGGTATCC
1150	1160	1170	1180	1190	1200
ATTTCGTG...

DNA Sequence For DNA 1/T7

(The pGEM-3Z information that was removed is underlined).

10	20	30	40	50	60
GNNNNNGNNN	NNNNNNNNNN	NNNCATTNGA	GCTCGGGTAC	CGGGGGACCT	CTAGAGTCGA
70	80	90	100	110	120
CTATCTGGTA	CTCTATACCT	CTACACACCT	TTTAAAGTGA	ATTGACCTAT	TTGCTTTTTC
130	140	150	160	170	180
TAGAATTTTT	AAAATTTTTG	TTCCACCGTT	AAACCCCAGT	TGATTCTCGA	CTCAACTGAA
190	200	210	220	230	240
AACTATCTT	CTTTCGAAA	CCTTCATTGA	CTATTCAAAG	TTCTTCTTTA	TTCTTTAGTG
250	260	270	280	290	300
CCATCCTCTG	TATATTACCC	TTATTACCCT	TCAAACCTACC	TATTGTAAC	ACATATTGTT
310	320	330	340	350	360
TTTATGTCCA	TCTCCCCTAA	TAAACAGTGA	GCTCCTTCAA	GAAAGAGTAT	CCTTCCACCT
370	380	390	400	410	420
CCCTTTTTC	GCATCACTAG	AACAGGGTAG	GTAGGCACTG	ATTAAGTACA	TTATTCAGTT
430	440	450	460	470	480
CATCACTCAG	TATTGGACAT	ATATTTCTTG	AGAATTTACT	AAAGGCCTTG	CTTGCGTGGT
490	500	510	520	530	540
TCTANGCATC	GTGAATTGAG	TGGTGAACAA	AACATTTAAA	CCCCTTGTC	TCATGGACTT
550	560	570	580	590	600
TATGTTCCCTG	GGGACAGAAT	TAGATAACAT	ACATATAAAA	ACACAGTGTT	ATTTTACAT
610	620	630	640	650	660
GGTCNTAAGA	AGAAAATAAA	GATAGATAAG	GAAGTGAAG	TATTTAGGTG	GAATGGTCAT
670	680	690	700	710	720
GGAAGGTCTC	TTTAATGTGG	TGACAAATGA	TTAATGACTG	AATGTATGTT	AGTGAGAAAT
730	740	750	760	770	780
CAATTTTGAN	AACACCTGTT	GATAGANTGT	CCTAATTTGA	AGAAACAGCA	AGCAGAAGGN
790	800	810	820	830	840
AGAAAAAGCA	GTGGGGTTTG	AAGTTATTTT	GAAAANGGTC	ACTGTATCTG	GGANACANAA
850	860	870	880	890	900
AGTNANTTAA	AGGTCGAANA	AAAAAACAGA	AGGTCAGATT	TCCNNACGGT	ACAAGGGAAT

910	920	930	940	950	960
TTGAAATCCA	CATGTTCCCG	AATAAATGGA	GAAANGAATT	NANCATCCCC	TGAATTTGTT
970	980	990	1000	1010	1020
GAATAATANC	TGAAAGTTAC	CCCCTTTCAA	CTGGTTCCNA	ATTAAAAATT	CTCATTAACC
1030	1040	1050	1060	1070	1080
TTGAACCCCT	CAANCNTTTT	TTTGAAAAGT	GGATAAAATT	CAAGAACTTC	NCCCNGGCCA
1090	1100	1110	1120	1130	1140
AAAATTTGGG	AAACCCCCCT	ATNTTTATGC	CCCAAAATCC	NC.....

DNA Sequence For DNA 1/SP6

(The pGEM-3Z information that was removed is underlined).

(The HindIII site is double-underlined).

10	20	30	40	50	60
<u>CTCNAGCTTG</u>	CATGCCTGCA	GGTCGACCTG	CAGGTCAACG	GGATCTTTGG	CATGATATTT
70	80	90	100	110	120
ATACACTGTC	TCATTTTCTT	CGTTTGTATA	TTGGGGATGT	TTATAATTAT	TTTGCAGGAT
130	140	150	160	170	180
TATTTTGAAT	GTGAGAAATA	ATATCTTTAA	TTTGATTTTC	TTTTGTTAGC	TAATTAAATG
190	200	210	220	230	240
GTAACAACT	TTTATTCATA	TAATTAGTAT	GGTCTCATCA	CACAAGGCCC	TTTGCAACAT
250	260	270	280	290	300
TGCTCCTACT	TCTATGTCTA	AGTTCTGCTT	CACTTTCCTA	TGCTCCATCC	ATTTCAATCA
310	320	330	340	350	360
CCCCAGACGT	CTCTGTATAA	TTTGGCCATG	TCCTATCACA	ACTCTAATCT	TGTTTGTATT
370	380	390	400	410	420
CCTGGAATGC	CCTGAAAAAC	TCCTACTCAG	ACTTAAAAAG	CCAGCTCAAA	TATTGTGTTC
430	440	450	460	470	480
TTCGTGATTC	TTTTCCTTCT	TCATTGANGT	AGANTCTAAC	ACTTCTTCAC	ACACCACTAA
490	500	510	520	530	540
CAAACAGGT	TTGAAATGTT	ACTTTTGTAC	ATTTAAGAAC	CTTTTTTACA	TTTTTCATTT
550	560	570	580	590	600
TAAAAACATT	TTAATAGCAA	AAATATTATA	ACAGAGAAGA	ATACATACTA	ACGAATGTTA
610	620	630	640	650	660
TTTAAATAAG	ANTTATTGGT	ATAAACATAG	ATGTTTAAAT	AACTGGTAAT	CTGGAAAAAG
670	680	690	700	710	720
AAATAGAACA	CAGCCTGGTA	TCACTGAAAC	TCCTCTATGT	TCTTCCATGA	ATAACCACCT
730	740	750	760	770	780
ATCCCCGTGC	CTCCTTGGAA	TTAATAACNA	ATTTTGAATT	TTGGTGAANA	TTTTTTTAA
790	800	810	820	830	840
AAANTTGCCT	NAATTTTAAT	CCNGAAACCT	TTGGAAAAAA	TACGTTTTTTT	CTATTTTAA
850	860	870	880	890	900
ACNGGCNCAT	GNAAATGGGA	AGGTNTGCTG	GAAAAAAATT	TTTTTGANAT	AATTCCTTT
910	920	930	940	950	960
AACTGGGTCT	<u>NGNAAAAAAT</u>	NTTTTAAANN	AAANNTTNCC	CTGAACACCC	CNNTNTNN..

DNA Sequence For DNA 1/HIND3/SP6

(The pGEM-3Z information that was removed is underlined).

10	20	30	40	50	60
<u>CNCAAGCTTA</u>	TAAAAGCAAA	GTTTCTATTA	AGTGACCAGT	GTATTTATAT	GCGATAAGAA
70	80	90	100	110	120
GATACTACTT	TTAATGAAAT	AAATACATAA	ACATTTTGAA	CTGTCTACAG	AAAAACTCAG
130	140	150	160	170	180
AGAGATGGTA	ATGAAAACAC	TCTGTTTATA	TAAAAGAACT	TCTTCACTAG	TCCCATAGAA
190	200	210	220	230	240
TAGAGTGAAT	AGAGTCTTGT	CTTAATACAG	CATTGATTTA	TTATGTNATA	TATCCATATT
250	260	270	280	290	300
CCCGATGATC	AAGATCTTGC	TGGTGTNTAT	GGAGGCTGAG	GGCCGCTTGG	CTCATGTGAA
310	320	330	340	350	360
GAAAGATAAA	TTTTTTATTG	TGTCTTGAGT	GTTTTAATAT	GAAACAAAAC	TAAAAAAGAA
370	380	390	400	410	420
AAAGCCTCTG	TCTTAAGCAT	CACTTACATT	ATGTNGAAAG	AAGCAATTTT	AAAATCATTC
430	440	450	460	470	480
GTTTCATCATA	TAAGAGTATC	ATCAACNCNG	AATCCCATNT	AGAGTGATAA	TGAAGATCTG
490	500	510	520	530	540
TTTTCACNCC	ATGGGAGCTA	TTTGAGTATT	CTTTCCTTAA	CTGCTTCTGG	CAGTTCACAT
550	560	570	580	590	600
TGTCAGCGAA	GTTAATGCTG	GGATGTGGAA	CATGATTCCT	CTCTTTTTTT	TCCNCCAATT
610	620	630	640	650	660
TATTTTTTTTT	TAGATCCACA	GATAAAATTT	ATGTTTTTCT	CNNGTTTGAC	TGAAGGTTTG
670	680	690	700	710	720
GAAATNTINTT	ACNTTGTTGA	ATGGGTAA	GTTAGCTAAT	TNACTTTGTT	TTACCTGACA
730	740	750	760	770	780
TNNTTATCCT	TTTTGAAGGT	NGGGACACTT	TNAATTGAAT	TNGGTTNNAA	NAANNTTGAC
790	800	810	820	830	840
CTTNAANAAA	ANANTNNTAN	GGGCCTTTGC	CCNNAATNGG	GAAANTTNAC	TGCCCCGGGG
850	860	870	880	890	900
AAAAAANANT	NCAATTACCT	TAAATGCCCC	CNTTNTTCCN	CCCNAANTTN	CCCTTGAATN

910	920	930	940	950	960
TTTNAAGNTT	TTTNAAAAAT	NNTTNCNCN	TTNCCCNAN	NANTTTAANT	CCTAAAANCC
970	980	990	1000	1010	1020
CCCGNCCCN	NTTGGCGGGG	NGAATTTCCC	NNTTTTTNMN	NTANGGGTNA	TTCCGGGGGN
1030	1040	1050	1060	1070	1080
TNACCCGGGN	TN.....

Partial DNA Sequence For

DNA 1/Region 3+2

10	20	30	40	50	60
CTGCAGGTCA	ACGGGATCTT	TGGCATGATA	TTTATACACT	GTCTCATTTT	CTTCGTTTGT
70	80	90	100	110	120
ATATTGGGGA	TGTTTATAAT	TATTTTGCAG	GATTATTTTG	AATGTGAGAA	ATAATATCTT
130	140	150	160	170	180
TAATTTGATT	TTCTTTTGTT	AGCTAATTAA	ATGGTAACTA	ACTTTTATTC	ATATAATTAG
190	200	210	220	230	240
TATGGTCTCA	TCACACAAGG	CCCTTTGCAA	CATTGCTCCT	ACTTCTATGT	CTAAGTTCTG
250	260	270	280	290	300
CTTCACTTTC	CTATGCTCCA	TCCATTTCAA	TCACCCCAGA	CGTCTCTGTA	TAATTTGGCC
310	320	330	340	350	360
ATGTCCTATC	ACAACTCTAA	TCTTGTTTGT	ATTCCTGGAA	TGCCCTGAAA	AACTCCTACT
370	380	390	400	410	420
CAGACTTAAA	AAGCCAGCTC	AAATATTGTG	TTCTTCGTGA	TTCTTTTCCT	TCTTCATTGA
430	440	450	460	470	480
GGTAGANTCT	AACACTTCTT	CACACACCAC	TAACAAACTA	GGTTTGAAAT	GTTACTTTTG
490	500	510	520	530	540
TACATTTAAG	AACCTTTTTT	ACATTTTCA	TTTTAAAAAC	ATTTTAATAG	CAAAAATATT
550	560	570	580	590	600
ATAACAGAGA	AGAATACATA	CTAACGAATG	TTATTTAAAT	AAGAATTATT	GGTATAAACA
610	620	630	640	650	660
TAGATGTTTT	AATAACTGGT	AATCTGGAAA	AAGAAATAGA	ACACAGCCTG	GTATCACTGA
670	680	690	700	710	720
AACTCCTCTA	TGTTCTTCCA	TGAATAACCA	CCTATCCCCG	TGCCTCCTTG	GAATTAATAA
730	740	750	760	770	780
CAAATTTTGA	ATTTTCGGTG	AATATTTTTA	TATTACATAA	AAATCAATGC	CTGAATTTTA
790	800	810	820	830	840
ATCCAAGACT	CTATTCACTC	TATTCTATGG	AACTAGTGAA	GATACGTTTT	TTCTATTTTT
850	860	870	880	890	900
AAACAGAGCG	TTTTTCATGTA	ACATGGCTCT	GAAGGTTTGC	TGGAGACAGT	TCAAAATTTT
910	920	930	940	950	960
TTTGATATATA	ATTTCTTTTA	ACTGGGTCTA	GTAAAAAACT	TCTTATCGCA	TATAAATACA
970	980	990	1000	1010	1020
TTGCTCACTT	AATAGAACAC	CCCCTTTTA	T.....

DNA Sequence For DNA 2/T7

(The pGEM-3Z information that was removed is underlined).

10	20	30	40	50	60
CGATTCGAGC	TCGGTACCGG	GGATCCTCTA	<u>GAGTCGACTA</u>	TCTGGTAAGT	ACTCTATAACC
70	80	90	100	110	120
TCTACACACC	TTTTAAAGTG	AATTGACCTA	TTTGCTTTTT	CTAGAATTTT	TAAAATTTTT
130	140	150	160	170	180
GTTCCACCGT	TAAACCCAG	TTGATTCTCG	ACTCAACTGA	AACTATTCT	TCTTTCGGAA
190	200	210	220	230	240
ACCTTCATTG	ACTATTCAA	GTTCTTCTTT	ATTCTTTAGT	GCCATCCTCT	GTATATTACC
250	260	270	280	290	300
CTTATTACCC	TTCAAACACTAC	CTATTGTAAC	TACATATTGT	TTTTATGTCC	ATCTCCCCTA
310	320	330	340	350	360
ATAAACAGTG	AGCTCCTTCA	AGAAAGANTA	TCCTTCCACC	TCCCTTTTTTC	CGCATCACTA
370	380	390	400	410	420
AAACAGGGTA	GGTAGGCACT	GATTAAGTAC	ATTATTCAGT	TCATCACTCA	GTATTGGACA
430	440	450	460	470	480
TATATTTCTT	GAGAATTTAC	TAAAGGCCTT	GCTTGCGTGG	TTCTAGGCAT	CGTGAAATTG
490	500	510	520	530	540
AGTGGTGAAC	AAAACATTTA	AACCCCTTGT	CCTCATGGGA	CTTTATGTTT	CTGGGGANAG
550	560	570	580	590	600
AATTAGATAA	CATACATATA	AAAACACAGT	GTATTTTAC	ATGGTCCTMN	GAAGAAAATT
610	620	630	640	650	660
AAGATANATA	AGGAAGTGGA	AGTATTTAGG	TGGAANTGTC	CTGGAAGGTC	NCTTNAATGT
670	680	690	700	710	720
NGGNGACCAN	TGANTAATGA	ACTTGAANTG	TTTTGTNTTG	AAGAAACNAC	TTTTTGAAAA
730	740	750	760	770	780
CCCCGGTGGA	ANAAAATTTT	CNCNTTTGNA	GGAAACCNCC	CCCCAAAGGN	NAAAAACCC
790	800	810	820	830	840
NTGGGTTTGA	AANTTCTTTT	NAAAAANGNC	CNCGTNNCTG	GGGANNNNNA	ANCTCATTTT
850	860	870	880	890	900
NAGGTCNAAN	AAAAAANANA	ANNCTNATNT	CNCNNACNNT	GCCNNGGAAN	CN.....

DNA Sequence For DNA 2/SP6

(The pGEM-3Z information that was removed is underlined).

10	20	30	40	50	60
NCCAAGCTTG	CATGCCTGCA	GGTCGACCTG	CAGGTCAACG	GGATCTTTGG	CATGATATTT
70	80	90	100	110	120
ATACACTGTC	TCATTTTCTT	CGTTTGTATA	TTGGGGATGT	TTATAATTAT	TTTGCAGGAT
130	140	150	160	170	180
TATTTTGAAT	GTGAGAAATA	ATATCTTTAA	TTTGATTTTC	TTTTGTTAGC	TAATTAAATG
190	200	210	220	230	240
GTAACATACT	TTTATTCATA	TAATTAGTAT	GGTCTCATCA	CACAAGGCCC	TTTGCAACAT
250	260	270	280	290	300
TGCTCCTACT	TCTATGTCTA	AGTTCTGCTT	CACCTTCCTA	TGCTCCATCC	ATTTCAATCA
310	320	330	340	350	360
CCCCAGACGT	CTCTGTATAA	TTTGGCCATG	TCCTATCACA	ACTCTAATCT	TGTTTGTATT
370	380	390	400	410	420
CCTGGAATGC	CCTGAAAAAC	TCCTACTCAG	ACTTAAAAAG	CCAGCTCAAA	TATTGTGTTC
430	440	450	460	470	480
TTCGTGATTG	TTTTCCTTCT	TCATTGAGGT	AGAGTCTAAC	ACTTCTTCAC	ACACCACTAA
490	500	510	520	530	540
CAAACTAGGT	TTGAAATGTT	ACTTTTGTAC	ATTTAAGAAN	CTTTTTTACA	TTTTTCATTT
550	560	570	580	590	600
TAAAAACATT	TTANTAGCNA	AAATATTNTA	ACCGAANAGG	AGTACATNCT	AACGAATGTA
610	620	630	640	650	660
ATTTAAATAA	GATTATTGTA	TAAACATAGA	TGTTTTTAATA	ACTGGTAATC	TGAAAAAAG
670	680	690	700	710	720
AAATANAACA	CAGCCTGGTA	TCACTGAAGC	NCCTCTAATG	TCCTTCCATG	AATAACCACC
730	740	750	760	770	780
TAATCCCGNT	GCCTCCTTGA	ANTTAANAAA	TAATTTTGAN	TTTGGTGAAN	ANTTTTTTTTT
790	800	810	820	830	840
ANAAATTTCG	CNANNTTTAA	TCCGAATCTT	TGGAAACATA	CGTTTTTTCT	ATTTTNAAAC
850	860	870	880	890	900
TGCCCATGNA	ATGGAGGTTN	GCTGAANNAA	ATTTTGTAAA	ANTTCTTTTN	ACTGGTCTGN
910	920	930	940	950	960
AAAAANTTTT	AACATTNTTC	NCCGACCNNC	CANNTNCTCC	TNAGCCAAAA	AAAAANGNAA
970	980	990	1000	1010	1020
ATNNTTTTNT	NNCNNTT...

APPENDIX III

Amino Acid Sequence For The
Human hsp 27 Gene
 (GenBank Accession Number X03900)

10	20	30	40	50	60
MTERRVPFSL	LRGPSWDFFR	DWYPHSRLFD	QAFGLPRLPE	EWSQWLGGSS	WPGYVRPLPP
70	80	90	100	110	120
AAIESPAVAA	PAYSRALSRQ	LSSGVSEIRH	TADRWVRVSLD	VNHFAPDELT	VKTKDGVVEI
130	140	150	160	170	180
TGKHEERQDE	HGYISRCFTR	KYTLPPGVDP	TQVSSSLSPE	GTLTVEAPMP	KLATQSNEIT
190	200	210	220	230	240
IFVTFESRAQ	LGGRSCKIR.

APPENDIX IV

Alignment Of DNA 4-1 (Normal Strand),

And DNA 4-2 (Complementary Strand)

(The top row shows the DNA 4-1 nucleotide sequence).

(The bottom row shows the DNA 4-2 nucleotide sequence).

```

1 TCGACCTTGAATACGGAGCTTCAAGCCAGGCTCCAGCTGGCGTTTATAGGT 50
      | | | | | | | | | |
-11 .....A-GGNG---AANCCCN--T-----TGGC-TT----- 38

51 GTGGTGCAGCAANGCTGGGTANGTATTTCCAGGAAGCAGTAGANGAGCT 100
      | | | | | | | | | |
39 -----C---CN-GG---GGGT-T---ATTN---GG-----T---TNAG-T 88

101 CGCCGTGCAATATGAATTATCATTAGCAGCAAGGAAATTCGTCAACGATT 150
      | | | | | | | | | |
89 AG--GN--AA-A--A-----C---G--G-A-G---T--GACN-CG--- 138

151 TGTGGTAAATGAATTTCTGTGATATCCAGTTGCTTGGGAGAACGCT 200
      | | | | | | | | | |
139 -G-----AAA-AA-----G--G-----GTN-----AG--G-- 188

201 GCCTTTGCGCCTCTCGCGACGGCTCAATCTGGCTTAAGTACGACTACAC 250
      | | | | | | | | | |
189 G-----G-G-----A-GG-----G---AA--A--A--A--A-- 238

251 AGAAGTTGACACAGTAACATACGGGTTAAGCAGGAAGTGCAAATACTACG 300
      | | | | | | | | | |
239 A-A-GT-G-----G---CN---GGG---GC-G-A--T-----T--T-C- 288

301 TTGGCATGGTGAGATTTTCTGTTCTTCTAGTCCGGGGAAGTGGGATTGAT 350
      | | | | | | | | | |
289 ----A-G-T-----TTT---T---C---AGTCCGGGGA-TGGN-TTG-T 338

351 AAGCCGAGACAAATAGCTAATCAATTGGCAGAATCTATCGTTGATGGTAC 400
      | | | | | | | | | |
339 ANNC-GAAA-AAATAGTTATTCAAG-GCGGATTTT-TNGT-GNGGGAAC 388

401 AATGCTTGA-CAGCGGACCATTTATGANTCTGGAGTTGTTAACCCGGTT 450
      | | | | | | | | | |
389 A-TG-TTGAACAG-GGGACCATTTATGNGTTTGGNGTTGT-AACCCGGTT 438

451 ATCAAATCCAAGTCTGGGTGGTTTAT-CCCGGTTCTGTTTATGTTTCGTC 500
      | | | | | | | | | |
439 NTCAAATCCA-GTTNGGG-GGTTTATTCNGGTTCTGTTTATGTT-GTC 488

501 TANACTAACAAAAGGAAAAATACATGGCCCATCTCAGCAATGGCAGCAG 550
      | | | | | | | | | |
489 TAGATTAAACAAAGGAAAAAAC-TGGCCCATTTTCAAGCAATGGCAGCAG 538

551 GTCTTCGTNNAAAGCTCTCGCGGAAGTCAATCGACNTAACTGCAATTTTC 600
      | | | | | | | | | |
539 TTTTNGTAGAAGGNTNTCGNGGAGCTGCAATNGACGTAAGTCAATTTTC 588

601 TAACGCAGTTACCCCTGTTTAACTGTATCTGAC-CNTCTGGTTTGGTCN 650
      | | | | | | | | | |
589 TAACGCAGTTACCCCTGTTTAACTGTATCTGACCGTCTGGTTTGGTCG 638

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651	TTGGTGAATACCTGCTTTTCANCTCTTCTGCTTCAACGCTTCTAACTGAN	700
639	TTGGTGATTACCTGCTTTTCACTTNTTCTGCTTCAACGNTTCTAGCTGAC	688
701	AANCAANTTCNAATGACTGCAATCCCTGGTACNTCAGTAACTGTTGAAAG	750
689	AAGCAAGTTNGAGTGACTGCAATCACTGGTACGTCAAGTAACTGTTGAA-G	738
751	GT-TTGATACCTCC--CACCAC-----CNC-----AA-----AA	800
739	GTATTGATACCTCCAGCACCACAAAGTTCCCGCTGGCTTAACTGGTGAA	788
801	-TT--C-----C-CN----GGTN-G--G--C--T--T--A--AC-T---	850
789	GTGTGCAAGATCACCTCCTGGTTGGAAGTTCCGTGCGTTACAGGACGTATC	838
851	----G--GGTG---AA-A----TT--TTCAAATC-A---C---CC---	900
839	TACTGACGGTGGCGAACAGCAATTCGTT-AACTTCCAGTGCTTGTCCGAT	888
901	--CCG-G-----G----TTCC-A---A-A--T-T---T--C---CN--TG	950
889	GACCGTGAACAGCAGATTCCGACCTATAAATCTGCGGTAACACACCTT	938
951	CGN-TTC-----A-G-G-A-AN-----T-T-T--CN-----A-CN	1000
939	CACCTTCGCTCACGAGTACACCAACCCTGTATATCCGGTTCTGCGTAACT	988
1001	--GA--A-----AGG--G--G-G---G---G-----A-----A---	1050
989	ACGATGAGTCTGGCCAGGTTGTTGCGATTCTGTTCTGTACCTCGAGCT	1038
1051	AN--AAN--CANTT-C---CCT-TA--A-C--TT-C--C--CAT---TGC	1100
1039	AGCGAAATGCGCTTGCAGTCCGGTACTATCGCTTTCAACGACACCCCTAC	1088
1101	--TTG-T-T---CNAANN--AAACGG.....	1150
1089	CATTGGTGTAAACGAAATCGAAACGGTATCCATCGCGGTATCCATTCTGTG	1138

Alignment Of Human hsp 27 cDNA,

And The Human hsp 27 Gene

(The top row shows the human hsp 27 cDNA nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

-196	-147
1 GAATTCATTTGCTTTTCCTTAACGAGAGAAGGTTCCAGATGAGGGCTGAA	50
-146	-97
51 CCCTCTTCGCCCCCGCCACGGCCCTGAACGCTGGGGGAGGAGTGCATGG	100
-96	-47
101 GGAGGGGCGGCCCTCAAACGGGTCATTGCCATTAATAGAGACCTCAAACA	150
-46	3
151 CCGCCTGCTAAAAATACCCGACTGGAGGAGCATAAAAGCGCAGCCGAGCC	200
4 CAGCGCCCCGCACTTTTCTGAGCAGACGTCCAGAGCAGAGTCAGCCAGCA	53
201 CAGCGCCCCGCACTTTTCTGAGCAGACGTCCAGAGCAGAGTCAGCCAGCA	250
54 TGACCGAGCGCGCGCTCCCTTCTCGCTCCTGCGGGGCCCCAGCTGGGAC	103
251 TGACCGAGCGCGCGCTCCCTTCTCGCTCCTGCGGGGCCCCAGCTGGGAC	300
104 CCCTTCCGCGACTGGTACCCGCATAGCCGCCTCTTCGACCAGGCCTTCGG	153
301 CCCTTCCGCGACTGGTACCCGCATAGCCGCCTCTTCGACCAGGCCTTCGG	350
154 GCTGCCCCGGCTGCCGAGGAGTGGTCGCAGTGGTTAGGCGGCAGCAGCT	203
351 GCTGCCCCGGCTGCCGAGGAGTGGTCGCAGTGGTTAGGCGGCAGCAGCT	400
204 GGCCAGGCTACGTGCGCCCCCTGCCCCCGCCGCGCATCGAGAGCCCCGCA	253
401 GGCCAGGCTACGTGCGCCCCCTGCCCCCGCCGCGCATCGAGAGCCCCGCA	450
254 GTGGCCGCGCCCGCTACAGCCGCGCGCTCAGCCGGCAACTCAGCAGCGG	303
451 GTGGCCGCGCCCGCTACAGCCGCGCGCTCAGCCGGCAACTCAGCAGCGG	500
304 GGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGCGTGTCCCTGGATG	353
501 GGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGCGTGTCCCTGGATG	550

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354 TCAACCACTTCGCCCCGGACGAGCTGACGGTCAAGACCAAGGATGGCGTG 403
|||||
551 TCAACCACTTCGCCCCGGACGAGCTGACGGTCAAGACCAAGGATGGCGTG 600

404 GTGGAGATCACC GG-----C-----A-----AG-----C 453
|||||
601 GTGGAGATCACC GG TGAGCCCCCTGCTCCTGCAGGGGAGAGGAGGAGGC 650

454 -A-C-G-----AGG-----A-----G-----C--GG- 503
| | |
651 TAGCAGGGCGGGCAGGGCCGGGGCGTGC GGTTGAAACGGGGGTCCCGGG 700
504 --C-----AG-----G-----A-C--G-A-----G--C----- 553
| |
701 GGCCTGGGGAGTTAAACGTTGGCCCAGCACC GGGAACAGGACTCCTG 750

554 AT-----G---G-----C---T-----A---C----- 603
||
751 ATTCCCTTGCTCAGGAATTGGGAGTGCGGGTTCGCTTCTAAGGGCGCTTTC 800

604 -----A-TC-----T-----CC---CGG-----T-GCTT-- 653
| |
801 TGCTCTGTAATCCAGCGCTTTGGGAGGCCGAGACGGGAGGATCGCTTGA 350

654 --C-A-----C-G-C--G---G---AA-ATA-C-A--CGC----- 703
| |
851 GGCCAGGAGTTCAAGACTAGCCTGGGCAACATAGCGAGACGCGCCCCCCC 900

704 -----T-----GC---C----- 753
|
901 GCCCCGACCCCGCGCCATTACAAAAAAGCAAAACAAAATTTTAA 950

754 ---C--C-----C-C-----G-GT----- 803
| |
951 AGATCATCGATGAAGAGAGAAAATGCGCTTTTCTACAGAGTCCCCTTCCC 1000

804 -----G-----T-----G-----A---CCC---C-----A 853
|
1001 ACCCACAGCCCCATCCCCAGATAAGCGGGGAGTTCCCTGGCGCGGTGCCA 1050

854 ---C---CC---A-----A-GT-----T---T- 903
| |
1051 GTTTCCTAGCCGCTGAGTGGGCGTGTGCGCGGCTCCAAGTGCGCCTGCGTA 1100

904 C--CTC-CTCCC---T--G-----T-C-CC---C-T-----GA 953
| |
1101 CTGCTCACTCCCCAGCTCCGCGCCCTGCTCCGTTCTCCCAAACTCTGA 1150

954 ---G--G-----G-----C-A---C-A-C----- 1003
| |
1151 ATCGAAGAACTTTCGGAAGTTCTGAGAGCCCAGACCGGGCGGCACGCC 1200

1004 ---T-----G--A-CC-----G--TG--G----- 1053
|
1201 CCCATCCCCAACCCCTCTGTTAATCCCTACCAGCCTGCAGTCTCTGGCTG 1250

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1054 ----A-G-G-----CC-C--C--C-----A---T----- 1103
      | | |         | | | | | | | | | | |
1251 CTTCCAAGCAGGAGGTGGGGCCTCTGGCTAGCGGGGCCGAAAAAGTCCCC 1300

1104 -----GC---C-----C-----AA-G-C----- 1153
      || | | | | | | | | | | | | | | |
1301 TCCCCCGCATGTCTGATTTCCTCTTCCCCCAAGGCAAGCACGAGGAG 1350

1154 -----T---A-----G---C--CACGC--A----- 1203
      | | | | | | | | | | | | | | | |
1351 CGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCACGCGGAAATACAC 1400

1204 GT---CC-----A----- 1253
      || || | | | | | | | | | | |
1401 GTGAGTCCTGGGCCAGGTGCGGGTGGGTGGGTGGCGTGGGGGTGGGGTC 1450

1254 A-----C--G--A-----G-----A-T---C----- 1303
      | | | | | | | | | | | | | | |
1451 AGGGAAGAGGGCACAGGGACCCACCCGGTGTGTAATGTAACGCTTGCCTT 1500

1304 -----AC--C-A---T-CCC-----A-----GT-- 1353
      || | | | | | | | | | | | | | |
1501 TCCTCTCTGCACGTCCAGGCTGCCCCCGGTGTGGACCCACCCAAGTTT 1550

1354 C-----A---C-C--T-----T-----C-----G--- 1403
      | | | | | | | | | | | | | | | |
1551 CCTCCTCCCTGTCCCCTGAGGGCACACTGACCGTGGAGGCCCCCATGCC 1600

1404 A-G-T--C---GC-G-----G-G--C-CCA-----G-C---TT-G-G-- 1453
      | | | | | | | | | | | | | | | |
1501 AAGCTAGCCACGCAGTCCAACGAGATCACCATCCCACTCACCTTCGAGTC 1650

1454 G-GG-CCCAG-----A-A-GCTGCAAAATCCGATGAGACTGCCGCC 1503
      | | | | | | | | | | | | | | | |
1651 GCGGGCCCAGCTTGGGGGCAGAAGCTGCAAAATCCGATGAGACTGCCGCC 1700

1504 AAGTAAAGCCTTAGCCCGGATGCCACCCCTGCTGCCGCCACTGGCTGTG 1553
      | | | | | | | | | | | | | | | |
1701 AAGTAAAGCCTTAGCCCGGATGCCACCCCTGCTGCCGCCACTGGCTGTG 1750

1554 CCTCCCCCGCCACCTGTGTGTTCTTTTGATACATTTATCTTCTGTMTTTC 1603
      | | | | | | | | | | | | | | | |
1751 CCTCCCCCGCCACCTGTGTGTTCTTTTGATACATTTATCTTCTGTMTTTC 1800

1604 TCAAATAAAGTTCAAAGCAACCACC..... 1653
      | | | | | | | | | | | | | | |
1801 TCAAATAAAGTTCAAAGCAACCACCTGTCACTGGCCCAGGCCCTGGTGTT 1850

1654 ..... 1703

1851 TGTGGAAGGAAGCCTCAGGCACCTGCCATTTGCTGGCTTTCAGGAGTCAT 1900

```

1704	1753
1901	CTTTGCTCAGGCCCGTGCTGGGCCATGTGGGTACACTGGTGTAGGTTGCT	1950
1754	1803
1951	GGACACAGGCTGACTCACATCCATAAAGACAGAGGTCTTAGGGCCGGGCG	2000
1804	1853
2001	CAGTGGCTCATACCTACAATCCCAGCACTTTGGGGGGTTGAAGCAGGAGG	2050
1854	1903
2051	AGTGCTTGAAGCCAAGAGTTCTAGACCAGCCTGGACAACATAGTAAGACT	2100
1904	1953
2101	GTCTCTAAAAAATAAAAATTAGGCAGGGTGGTACTGCACGCCTGTAGTCC	2150
1954	2003
2151	CAGCTACTCAGGAGGCTGAGGCAGGAGGATCGCTTGAGCCCAGAGTTGTG	2200
2004	2053
2201	AAGGTACAGTGAGCTAACATCGTGCCATTGCACTCCAGCCTGGGCAACAG	2250
2054	2103
2251	AACAAGATCCTGTCTCAAAACAACCAAAAGCCCAGAGAGAAAGAGTGAGA	2300
2104	2153
2301	CCCCATCTTTAAAGAAAAAAGGTCATGATTGCAAGGTCACGAT	2350
2154	2203
2351	TGCAATTAAACTGTAAGGTGGGGAAGGAGGAGGAAATAAGAGAAGCACC	2400
2204	2253
2401	TGAGGCTTGAGTTCTCAGGAGCACCTAGGTTGGGTCCCAGGTGAAGGGG	2450
2254	2303
2451	ACAGAGGTAATTGCACCTCAGAGCTGATGGGAGGATTACTATGTCA....	2500

Alignment Of DNA 4 (Normal Strand),

And The Human hsp 27 Gene

(The top row shows the DNA 4 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

-3	...TC---GA---CCTT---GA-ATA---C--G--GAG--CT---	46
1	GAATTCATTTGCTTTTCCTTAACGAGAGAAGGTTCCAGATGAGGGCTGAA	50
47	---TCAA-GCC-----A-GGCTCC--A--GCTGGCGTTTATAG--TG--	96
51	CCCTCTTCGCCCCCGCCACGGCCCTGAACGCTGGGGG---AGGAGTGCA	100
97	TGGTGCAAG--C-----AA--GG--C-TGGG--T-A-T-G-----T-A	146
101	TGGGG-AGGGGCGGCCCTCAAACGGGTCATTGCCATTAAATAGAGACCTCA	150
147	-----T--T-----T-CC--A--GGA--AGCAGTA---G---AN--	196
151	AACACCGCCTGCTAAAAATACCCGACTGGAGGAGCA-TAAAAGCGCAGCC	200
197	GAGCTC-GC-CGT-GCAATAT---GA--ATTA--TC-ATTAGCAG---CA	246
201	GAGCCCAGCGCCCCGCACTTTTCTGAGCAG-ACGTCCAG-AGCAGAGTCA	250
247	---AGGA--A--A-----T-----T-C--G-TCAA-CG-----A-	296
251	GCCAGCATGACCGAGCGCCGCGTCCCTTCTCGCTCCTGCGGGGCCCGAG	300
297	-T-----TT--GT--TGGTA-----A-A-----T---GA--AT-	346
301	CTGGGACCCCTTCCGCGACTGGTACCCGCATAGCCGCCTCTTCGACCAGG	350
347	--TTC---CTG-----T-C---AG-A-TA-TC-CAGTTGCTT-GG-GA	396
351	CCTTCGGGCTGCCCCGCGTGCCTGAGGAGTGGTCGAGTGG-TTAGGCG-	400
397	GAA-C-GCTG-CCT---T---TGCGCTCTCTGC-----G--G--A-CG-G	446
401	GCAGCAGCTGGCCAGGCTACGTGCGCCCCCTGCCCCCGCCGCCATCGAG	450
447	--CTCA--A-T--CTG-GCT---TA-AG-----T-A-C-G--A-CT	496
451	AGCCCCGAGTGGCCGCGCCCGCTACAGCCGCGCGCTCAGCCGGCAACT	500
497	-A-CA-C-----AGA-----A---G-----T-----TGA	546
501	CAGCAGCGGGGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGCGTGT	550
547	CAC---A-GT-AAC-A-TACG---GG-----T-----T-AAG-C-A-G	596
551	CCCTGGATGTCAACCACTTCGCCCCGACGAGCTGACGGTCAAGACCAAG	600
597	GAAG---TGCA--A-AT-AC---T-A-C-----G-T--TG--GC--AT-	646
601	GATGGCGTGGTGGAGATCACCGGTGAGCCCCCTGCTCCTGCAGGGGAGA	650

647 GGTG----C-AG-ATTTC----AGTGT-----T-C--TTCA--G-- 696
 || | | | | | | | | | | | | | | | |
 651 CGAGGAGGCTAGCAGGGCGGGCAGGGCCGGGGCGTGGCGTTGAAACGGG 700
 697 --TCC-GGGGAC-TGGG-A-TTGATAA-G---CCGAG-AC---AAATA 746
 ||| |||| | | | | | | | | | | | | | |
 701 GGTCCCGGGGGCCTGGGGAGTT-A-AACGTTGGCCACGACCGGGAAAAA 750
 747 --G--CTA---AT-C-----A--A-TTGGCAG-----A 795
 | | | | | | | | | | | | | | | |
 751 CAGGACTCCTGATTCCCTTGCTCAGGAATTGGGAGTGCGGGTCGCTTCTA 800
 797 A-----T--CT-----A-TC---G---TT-G--ATG--G----- 846
 | | | | | | | | | | | | | | | |
 801 AGGGCGCTTTCTGCTCTGTAAATCCAGCGCTTTGGGAGGCCGAGACGGGA 850
 847 ---T-----A--C-A--A-T---G-CT---TG---AACA--GCG-G- 896
 | | | | | | | | | | | | | | | |
 851 GGATCGCTTGAGGCCAGGAGTTCAAGACTAGCCTGGGCAACATAGCGAGA 900
 897 -G-----ACC-----ATTT-AT-----G-A----- 946
 | | | | | | | | | | | | | | | |
 901 CGCGCCCCCGCCCGACCCCGCCGCAATTACAAAAAAGCAACAAA 950
 947 -----G-TC-T-G--GA-GT-----TG---TT---A-A-- 996
 | | | | | | | | | | | | | | | |
 951 AATTTTAAAGATCATCGATGAAGAGAGAAATGCGCTTTTCTACAGA 1000
 997 --CCCGGTT--ATC-A-A-----ATCC--A-A---G-----T-C--T 1046
 ||| || | | | | | | | | | | | | | |
 1001 GTCCCC-TTCCACCCACAGCCCATCCCCAGATAAGCGGGGAGTTCCCT 1050
 1047 GG-G---TG---GTTT--A-----T---T---C-----C-CGG-T----- 1096
 || | | | | | | | | | | | | | | | |
 1051 GGCGCGGTGCCAGTTTCTAGCCGCTGAGTGGCGGTGTGCGCGGCTCCAAG 1100
 1097 T-CG--T---T--T--T---T---A--T--G-----T--TC-GT-C-T- 1146
 | | | | | | | | | | | | | | | |
 1101 TGCGCCTGCGTACTGCTCACTCCCCAGCTCCGCGCCCTGCTCCGTTCTC 1150
 1147 --AGA-CT---AA-C-AA-AA-----GGAA-----A-A---ATACA 1196
 | | | | | | | | | | | | | | | |
 1151 CCAAACTCTGAATCGAAGAACTTTCCGGAAGTTTCTGAGAGCCAGACC 1200
 1197 TG-G--C-C-C---ATCTC-AGC-----AATGGC-AC--GCA-G 1246
 | | | | | | | | | | | | | | | |
 1201 GGCGGGCACGCCCCCATCCCCAACCCCTCTGTTAATCCCTACCAGCCTG 1250
 1247 --GTCTT--C-G-T---A-GAA--AG-----C-TCT--C--GCGG---- 1296
 ||| | | | | | | | | | | | | | |
 1251 CAGTCCTGGCTGCTTCCAAGCAGGAGGTGGGGCCTCTGGCTAGCGGGGCC 1300
 1297 -AA-----C---T-----GCAA-TC-GA---CG-T-----AACTGC 1346
 || | | | | | | | | | | | | | | | |
 1301 GAAAAAGTCCCTCCCCCGCATGTCTGATTTCCTCTTCCCCCAAAGGC 1350
 1347 AATTTCTA--A-CG-CAG-----T---TAC--C-CCTG-T--TT--- 1396
 || | | | | | | | | | | | | | | | |
 1351 AAGCACGAGGAGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCAC 1400
 1397 -----TA-ACTGT-A-TC-TGACGC--G-TCTGGTT---TGG-T--CG 1446
 || | | | | | | | | | | | | | | | |
 1401 GCGGAAATACAC-GTGAATCCTGGCGCCAGGTCGGGGTGGGTGGGTGGCG 1450

1447 T-----TGG--T--G--AATA---C-CTG---C-----T-T-T--TC 1496
 | | | | | | | | | | | | | | | | | | | | | |
 1451 TGGGGGTGGGGTCAGGGAAGAGGGCACAGGGACCCACCCGGTGTGTAATG 1500
 1497 -A-C-CT--C-TT-C-TGCT-T-CAA--C--G-CTTCTAAC---TG---A 1546
 | | | | | | | | | | | | | | | | | | | | | |
 1501 TAACGCTTGCCTTTCTCT-CTCTGCACGTCCAGGCTGCCCCCGGTGTGGA 1550
 1547 CA--ANC--AAGTT-C-----G-----A---A---TGAC--TGC 1596
 | | | | | | | | | | | | | | | | | | | | | |
 1551 CCCCACCAAGTTTCTCTCCTCCCTGTCCCTGAGGGGACACTGACCGTGG 1600
 1597 AATCCC---TG-----G-TA-C---GTCAGT--AACT-GTTGA--AG--- 1646
 | | | | | | | | | | | | | | | | | | | | | |
 1601 AGGCCCCCATGCCCAAGCTAGCCACG-CAGTCCAACGAGATCACCATCCC 1650
 1647 -GT-----TT-GA-TAC-CT--CC-AGC-----A---C---CACAAAG 1696
 | | | | | | | | | | | | | | | | | | | | | |
 1651 AGTCACCTTCCAGT-CGCGGGCCAGCTTGGGGGCAGAAGCTGCA-AAA- 1700
 1697 TTCCC--G-G-CTG--GCT---TAA--C-T--G---G--TGA--AGT--T 1746
 | | | | | | | | | | | | | | | | | | | | | |
 1701 T-CCGATGAGACTGCCGCCAAGTAAAGCCTTAGCCCCGATGCCACCCCT 1750
 1747 G-T-CAAG--A-T--CA---CCTCC-----TG-GT-T-C---GA- 1796
 | | | | | | | | | | | | | | | | | | | | | |
 1751 GCTGCC--GCCACTGGCTGTGCCTCCCCGCCACCTGTGTGTTCTTTGAT 1800
 1797 AG-TT---C--C-GTG---C-----GTTTCA-G-A-C-----GT-A 1846
 | | | | | | | | | | | | | | | | | | | | | |
 1801 ACATTTATCTTCTGTTTCTCAAATAAAGTTCAAAGCAACCACCTGTCA 1850
 1847 -T--CT-A--C--TGACG---GTGGC-G-AA-C---AG-CA-----ATT 1896
 | | | | | | | | | | | | | | | | | | | | | |
 1851 CTGGCCAGGCCCTGGTGTGTTGTGGAAGGAAGCCTCAGGCACCTGCCATT 1900
 1897 --C-G--TT--A--A--C-T-T---C-CAG-----TGCT-----TGT-- 1946
 | | | | | | | | | | | | | | | | | | | | | |
 1901 TGCTGGCTTTCAGGAGTCATCTTTGCTCAGGCCCCGTGCTGGGCCATGTGG 1950
 1947 ---C-C-G---A--T-G---AC-C-G--TGA---ACA-----G-C 1996
 | | | | | | | | | | | | | | | | | | | | | |
 1951 GTACACTGGTGTAGGTTGCTGGACACAGGCTGACTCACATCCATAAGAC 2000
 1997 AGA--T-T-----CCG-----A---C-C-TA--TA-AATCT--GC--- 2046
 | | | | | | | | | | | | | | | | | | | | | |
 2001 AGAGGTCTTAGGGCCGGGCGCAGTGGCTCATACCTACAATCCAGCACTT 2050
 2047 -GG-----T--AA-CTA--AC-AC--CTTCA--CC-----TTC--G-CTCA 2096
 | | | | | | | | | | | | | | | | | | | | | |
 2051 TGGGGGGTTGAAGC-AGGAGGAGTGCTTGAAGCCAAGAGTTCTAGAC-CA 2100
 2097 -C--GAGTACACCA-ACC---CTGT---A-----TA-----TC---C-G 2146
 | | | | | | | | | | | | | | | | | | | | | |
 2101 GCCTG-G-ACAACATAGTAAGACTGTCTCTAAAAAATAAAAAATTAGGCAG 2150
 2147 G-T--T-CTGC--G--T--A-----A-CTAC---G-A---TGAG----- 2196
 | | | | | | | | | | | | | | | | | | | | | |
 2151 GGTGGTACTGCAGCCTGTAGTCCCAGCTACTCAGGAGGCTGAGGCAGGA 2200
 2197 ---TC--T-G-GCC-AG-GTTGTT--G---C-G--AT-T--CGTC-TG-- 2246
 | | | | | | | | | | | | | | | | | | | | | |
 2201 GGATCGCTTGAGCCCAGAGTTGTGAAGGTACAGTGAGCTAACATCGTGCC 2250

2247	-TT-CG-T--A-CCT---C---GA---G--C-T-----A-----	2296
2251	ATTGCACTCCAGCCTGGGCAACAGACAAGATCCTGTCTCAAAACAACCA	2300
2297	---GC---GA-A-A-----TCCG-C-----T-T-----GCA-----	2346
2301	AAAGCCCAGAGAGAAAGAGTGAGACCCCATCTTTAAAAGAAAAAAAAA	2350
2347	-G-TC-----C--GGT-AC--T---A-T-----C-GCT---T-----	2396
2351	AGGTCATGATTGCAAGGTCACGATTGCAATTAAACTG-TAAGGTGGGGA	2400
2397	-----TCAACGACAC-C-CCT-AC-CATTG-GTGT-TAACGA-	2446
2401	AGGAGGAGGAAAT-AA-GAGAAGCACCTGAGGC-TTGAGT-TCTCAGGAG	2450
2447	-A---A--T-----C---G---AA-----AC-G-G-TA-TC-CATCGC-G	2496
2451	CACCTAGGTTGGGTCCCAGGTGAAGGGGCACAGAGGTAATTGCACCTCAG	2500
2497	-G-T-ATCC-A---TT-CG-TG.....	2546
2501	AGCTGATGGGAGGATTACTATGTCA.....	2550

Alignment Of DNA 4 (Complementary Strand), And The Human hsp 27 Gene

(The top row shows the DNA 4 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

-16C--A--CGA--AT--GGATACC--GC--GATGGAT--A	33
1	GAATTCATTTGCTTTTCCTTAACGAGAGAAGGTT--CCAGATGACGGCTGA	50
34	--CCGT--TTCG-----ATTTCGT----T--AACACC-----A--A--TGG	83
51	ACCCTCTTCGCCCCGCCA---CGGCCCTGAACGCTGGGGGAGGAGTGC	100
84	-T----AGGGGTG----TC----G--T--T-G--A--AA--GCEA--T--A	133
101	ATGGGGAGGGGCGGCCCTCAAACGGGTCATTGCCATTAATAGAGACCTCA	150
134	-----G--T--A-----CCGGA CTG-----CA--A---GCGCATTTTC	193
151	AACACCGCCTGCTAAAAATACCCGACTGGAGGAGCATAAAAGCGCAGC--C	200
184	G--CT--AGC-----T---C--GAG--GTACGAACAGA--C--GAATC--G	233
201	GAGCCCAGCGCCCCGCACTTTTCTGAGCAG--ACGTCCAGAGCAGAGTCAG	250
234	CAA--CA--ACCT--G--GCCA--GAC---T--CATCG--T-----AG	283
251	CCAGCATGACCGAGCGCCGCGTCCCCCTTC--TCGCTCCTGCGGGCCCCAG	300
284	TT---AC-----GC--A--GA--ACCGG--ATA-----T-----AC--AGG	333
301	CTGGGACCCCTTCCGCGACTGGTACCCGCATAGCCGCTCTTCGACCAGG	350
334	G--TT--GG--TG-----TAC-----T---CG---TG---AG--CGA--	383
351	CCTTCGGGCTGCCCCGGCTGCCGGAGGAGTGGTCGCAGTGGTTAGGCGGC	400
384	AG--G--TGA--AGG--T--GTTAG-----T-----T--A---	433
401	AGCAGCTGGCCAGGCTACGTGCGCCCCCTGCCCCCGCCGCGCATCGAGAG	450
434	CC--GCAGATT-----TATAG--G-----TC--G--G--AA--TC	483
451	CCCCGCAG--TGGCCGCGCCCGCTACAGCCGCGGCTCAGCCGGCAACTC	500
484	TGCTG-----T--TC--A---C--GG-----T--C--A---T--CG--G----	533
501	AGCAGCGGGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGCGTGTC	550
534	-----A---CAAGCACT--G-----G--A--AG--T-----T--AA--CGAAT--	583
551	CCTGGATGTCAACCACTTCGCCCCGACGAGCTGACGGTCAAGACCAAGG	600
584	-TG--C--TG--T-----TCGCC-----A--CC-----G--TC-----AGT--AGA--	633
601	ATGGCGTGGTGGAGATCACCAGGTGAGCCCCCTGCTCCTGCAGGGGAGAG	650

634 -----TA-C-GT-C---C-----TG-----AA-CG--- 683
 || | | | | || || ||
 651 GAGGAGGCTAGCAGGGCGGCAGGGCCGGGGCGTGCAGTTGAAACGGGG 700
 684 --CACGG-----A---A-C-TTCG---A--ACCAGGA-----G 733
 | || | | | | | | | | || | || |
 701 GTCCCGGGGGCCTGGGGAGTTAAACGTTGGCCAGCACCGGGAACACAG 750
 734 G--T---GAT-C---TTGA-CA--A-----C---T---T-C-A--- 783
 | | || | | || | | | | | | | | |
 751 GACTCCTGATTCCCTTGCTCAGGAATTGGGAGTGCGGGTCGCTTCTAAGG 800
 784 -C-CA----G-T-T--AAGCC-AGC-C---GGGA-----A--CTTTGTGG 833
 | | | | | | | | | | | | | | | | | | | |
 801 GCGCTTTCTGCTCTGTAATCCACGCTTTGGGAGGCCGAGACGG-GAGG 850
 834 -T-GCT-G-G---AGGTA-T-CAA-AC---C-T-----T--C-A-AC 883
 | | | | | | | | | | | | | | | | | | | |
 851 ATCGCTTGAGGCCAGG-AGTTCAAGACTAGCCTGGGCAACATAGCGAGAC 900
 884 -----A---G---TTACT-----G-A--CG--- 933
 | | || | | | | |
 901 GCGCCCCCCCCCGCCCGACCCCGGCCATTACAAAAAAGCAAAACAAA 950
 934 -T-----A-----C-C-A-G--G-GAT---TGC-----A--G-- 983
 | | | | | | | | | | | | | | | |
 951 ATTTTAAAGATCATCGATGAAGAGAGAAAATGCGCTTTTCTACAGAG 1000
 984 TCA--TTCG-A--AC-----T-----T--GN-----TT---G- 1033
 || || | | || | | | | | | | | | | | |
 1001 TCCCTTCCCAACCCACAGCCCATCCCAAGCGGGAGTTCCCTGG 1050
 1034 -----T-C-AGTT---AGA-----AG-----CGT-TGAAGCAG---AAGA 1083
 | | | | | | | | | | | | | | | | | | | |
 1051 CGCGGTGCCAGTTTCTAGCCGCTGAGTGGGCGTGTGC-GCGGCTCCAAGT 1100
 1084 G-G--TGA--A-----A-----AGCA--G-G---TA-T---T-C--- 1133
 | | || | | | || | | | | | | | | |
 1101 GCGCCTGCGTACTGCTCACTCCCAGCTCCGCGCCCTGCTCCGTTCTCC 1150
 1134 -A---C-C--AA-CGA---C---C--AA-----A--CC-AGAC-G 1183
 | | | | | | | | | | | | | | | | | | |
 1151 CAAAACCTCTGAATCGAAGAACTTTCCGGAAGTTTCTGAGAGCCAGACCG 1200
 1184 -CGT-CA-G-----ATAC--A-----GTAA-----A--A-C--- 1233
 || || | | || | | | | | | | | | | | |
 1201 GCGGGCACGCCCCCATCCCAACCCCTCTGTTAATCCCTACCAGCCTGC 1250
 1234 AG---GG--G-T---AA-C-----TGCG---T-T---AG----- 1283
 || || | | | | | | | | | | | | | | |
 1251 AGTCCTGGCTGCTTCCAAGCAGGAGGTGGGCGCTCTGGCTAGCGGGCCG 1300
 1284 AAA---T---T-----GCA-GT-T-A---CG-TC-----G--A 1333
 || | | | | | | | | | | | | | | | | |
 1301 AAAAAGTCCCTCCCCCGCATGTCTGATTTCCTCTTCCCCCAAGGCA 1350
 1334 TTGCA-GTT---C--C-G--CGAG-A-G-CT---T-TC---T-----AC 1383
 || | | | | | | | | | | | | | | | | |
 1351 A-GCAGGAGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCAC 1400
 1384 G---AAG-AC-C-TGCGT---G-C-C-A--T-----TG-----C-T 1433
 | | | | | | | | | | | | | | | | | |
 1401 GCGGAAATACACGTGAGTCCTGGCGCCAGGTCGGGGTGGGTGGGTGGCGT 1450

1451 GGGGGTGGGGTCAGGGAAGAGGGCACAGGGACCCACCCGGTGTGTAATGT 1500
 1484 -----TT-CCTTT--TGT-T--A-GTCTAGAC-GA-----AC- 1533
 || ||||| | | | | ||| || | | ||
 1501 AACGCTTGCCTTTCTCTCTGCACGTCCAGGCTGCCCCCGGTGTGGACC 1550
 1534 --AT--AA-----A---A-AC-GA-----A- 1583
 | || | | || | |
 1551 CCACCCAAGTTTCCTCCTCCCTGTCCCTGAGGGCACACTGACCGTGGAG 1600
 1584 -CC-----G-----G---G-A--A-T--AA--A--C-C-A-CCCAG- 1633
 || | | | | | | | | |
 1601 GCCCCCATGCCCAAGCTAGCCACGCAGTCCAACGAGATCACCATCCAGT 1650
 1634 -AC-TTGA-T-----TTG-----A-----T--AA--CCG-- 1683
 || || || | ||| | | |||
 1651 CACCTTCGAGTCGCGGGCCAGCTTGGGGGCAGAGCTGCAAAATCCGAT 1700
 1684 G-GT-T-----AAC-AA--C-T---CC---A-G---AC---T-C----- 1733
 | | | || || | | || | | || | |
 1701 GAGACTGCCCAAGTAAAGCCTTAGCCCGGATGCCACCCCTGCTGCCG 1750
 1734 --A-TAAATG-G--TCCC--GC---TGT-T---C-----A-AGCATTGT 1783
 | | || | || | || | | | ||| |
 1751 CCACTGGCTGTGCCTCCCCCGCCACCTGTGTGTCTTTTGATA-CATT-T 1800
 1784 A-C--CA-T---C--AACGA--T--A--G-A-----T-TC--TG-CC 1833
 | | | | | | | | | | | | | | | | | | | |
 1801 ATCTTCTGTTTCTCAAATAAAGTTCAAAGCAACCACCTGTCACTGGCC 1850
 1834 AA-----T--TGATT---A-G---C-T-A-----T----TTGT-CTC 1883
 | | || || | | | | | | | || ||
 1851 CAGGCCCTGGTGTGTTGTGGAAGGAAGCCTCAGGCACCTGCCATT-TGCT- 1900
 1884 GGCTTATCA--A-TC--C---C--AGTCCC---C-GGAC--TGAAGA-A 1933
 ||||| || | | | | | | ||| | || | | |
 1901 GGCTT-TCAGGAGTCATCTTTGCTCAGGCCCGTGTGGGCCATGTGGGTA 1950
 1934 CACTGAA--A--T--CTGCAC-CA---TG-C-CA-A-CG-TA--GT-A-- 1983
 ||||| | | ||| || | || | | | | | | | | |
 1951 CACTGGTGTAGGTTGCTGGACACAGGCTGACTCACATCCATAAAGACAGA 2000
 1984 --T-TT-G--CACTT-C-C--TG-CT--TA---AC---CC--GTA-T--G 2033
 | || | | | | | | | | | | | | | | | | | | |
 2001 GGTCTTAGGGC-CGGGCGCAGTGGCTCATACCTACAATCCCAGCACTTTG 2050
 2034 -----TT-A--CTGT-G---T-C---AA-C-----TTCT-G-----T 2083
 || | | | | | | | | | | | | | | | |
 2051 GGGGGTTGAAGCAGGAGGAGTGCTTGAAGCCAAGAGTTCTAGACCAGCCT 2100
 2084 G-----TAGT-----C-GTA-CT-----TAA-----GCCAGA-T--T 2133
 | |||| | | | | || | | || | | |
 2101 GGACAACATAGTAAGACTGTCTCTAAAAAATAAAAAATTAGGCAGGGTGGT 2150
 2134 ---G-A-GCC-GT---CC--GC-A---GGAGGC-G---CAA-AGG--C- 2183
 | | ||| || | | | | ||||| | || ||| |
 2151 ACTGCACGCCTGTAGTCCCAGCTACTCAGGAGGCTGAGGCAGGAGGATCG 2200
 2184 ----AGC---G--TTCTCC-----CA---AGC-AAC-T-G-G--AT---A 2233
 ||| | || | || ||| ||| | | | |
 2201 CTTGAGCCCAGAGTTGTGAAGGTACAGTGAGCTAACATCGTGCCATTGCA 2250

2234	-TC-----TG---A-CAGGA-AAT-TCAT-T-T-AC--CAAC-AAAT-C-	2283
2251	CTCCAGCCTGGGCAACAGACAAGATCCTGTCTCAAAACAACCAAAAGCC	2300
2284	--GTTGACGAATT--T----CCT--TGCTGCTAAT-GATAAT-----	2333
2301	CAGA-GA-GAAAGAGTGAGACCCCAT-CTT-TAAAAAGAAAAAAAAAAG	2350
2334	-TCAT-ATTGCACGG-CGA-GCT--CN-TCTA---CTGCTTCC-TGG--A	2383
2351	GTCATGATTGCAAGGTC-ACGATTGCAAT-TAAAACTG-TAAGGTGGGGA	2400
2384	A--AT-AC-A--TA-----C-CC--AGCCTTGC-TTG-CACCA-CACC	2433
2401	AGGAGGAGGAATAAGAGAAGCACCTGAGGCTTGAGTTCTCAGGAGCACC	2450
2434	TA-----A-----AACG--C-CAGCTGG-A---GC-C-T--G-GC	2483
2451	TAGGTGGGTCCCAGGTGAAGGGGCACAGA-GGTAATTGCACCTCAGAGC	2500
2484	T--TGA-AGCTCCGTATT-CAAGGTCGA.....	2533
2501	TGATGGGAG----G-ATTACTATGTC-A.....	2550

Alignment Of DNA 1/Region 1 (Normal Strand), And The Human hsp 27 Gene

(The top row shows the DNA 1/Region 1 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

-3	...T-ATCTGGT-----A-C-----T-C---T-A---T-A-	46
1	GAATTCATTGCTTTTCCTTAACGAGAGAAGGTTCCAGATGAGGGCTGAA	50
47	CC-TCTACAC-----AC--CTTTTAAA-G-TG---A--A-T---TG-	96
51	CCCTCTTCGCCCCGCCCCACGGCCCCCTGAACGCTGGGGGAGGAGTGCATGG	100
97	--A---C--C--T-A-----T--TTGCTTTTCTAGA-ATTTTAAA-	146
101	GGAGGGGCGGCCCTCAAACGGGTCATTGCCATTATAGAGACCTC-AAAC	150
147	ATTTT-TG-T-----T-CC--ACCGT-----TAAA--C-C--CC-AGT	196
151	ACCGCCTGCTAAAAATACCCGACTGGAGGAGCATAAAAGCGCAGCCGAGC	200
197	T--G-----A-TT--CT---C-GAC-TC-A-A-CTGAA--A--A-C	246
201	CCAGCGCCCCGCACTTTTCTGAGCAGACGTCAGAGCAGAGTCAGCCAGC	250
247	-T-AT-----TC--TTCT---T--T-CGGA-----A-----A	296
251	ATGACCGAGCGCCGCTCCCTTCTCGCTCCTGCGGGGCCCGAGCTGGGA	300
297	CC--TTC---ATTG--AC-----TA-----T-T-CAA--AGTTCTTCT	346
301	CCCCTTCGCGACTGGTACCCGCATAGCCGCTCTTCGACCAGGCCTTCG	350
347	---T-----T-----A---T--TC---T--TLAGT-G-C--CA--	396
351	GGCTGCCCCGCTGCCGAGGAGTGGTCGCAGTGGTTAGCGGCAGCAGC	400
397	T--CC---T-C-TG-----T-----AT--ATTA-CCCTT	446
401	TGGCCAGGCTACGTGCGCCCCCTGCCCCCGCCGCGCATCGAG-AGCCCCG	450
447	-A-T-----TAC--CCT-----TCA-----AACT-A-C--C	496
451	CAGTGGCCGCGCCCGCTACAGCCGCGGCTCAGCCGGCAACTCAGCAGC	500
497	TA--T-T-GTA-A-CT---ACA-T---ATTGTT-----T-T---T--A	546
501	GGGGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGCGTGTCCCTGGA	550
547	TGTC--C-A-T-CTCCCCT-A--A--T-A-----AA--C-A-G--TG---	596
551	TGTCAACCACTTCGCCCCGGACGAGCTGACGGTCAAGACCAAGGATGGCG	600
597	-----AG--C-----T--CC---T--TCA---AG---A-A--AG-AG	646
601	TGGTGGAGATCACCGGTGAGCCCCCTGCTCCTGCAGGGGAGAGGAGGAG	650

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647 --TATCCTT-C---CA---CC-----T-C-----C-----C--- 696
    || | | | | | | | | | | | | | | | | | | | |
651 GCTAGCAGGGCGGGCAGGGCCGGGGCGTGCCTTGAAACGGGGGTCCCG 700
697 -----T-----TT-----TT--CC--CCA-----TC- 746
    | | | | | | | | | | | | | | | | | | | | |
701 GGGGCCCTGGGGAGTTAAACGTTGGCCCAGCACCGGGAAAAACAGGACTCC 750
747 --A--C--T-----AG-AACAGGG--TA-GG-T-----A-GG-CACT- 796
    | | | | | | | | | | | | | | | | | | | | |
751 TGATTCCCTTGCTCAGGAATTGGGAGTGCGGGTTCGCTTCTAAGGGCGCTT 800
797 ---GAT-T--AA-----G---T-----A-C-A-----T---T- 846
    | | | | | | | | | | | | | | | | | | | | |
801 TCTGCTCTGTAATCCCAGCGCTTTGGGAGGCCGAGACGGGAGGATCGCTT 850
847 -A-----TTCA-G--T---T---CA---T-C-A--CTC----- 896
    | | | | | | | | | | | | | | | | | | | | |
851 GAGGCCAGGAGTTCAAGACTAGCCTGGGCAACATAGCGAGACCGGCCCCC 900
897 -----A---GT---ATTG-----G-A--CATATATTT--- 946
    | | | | | | | | | | | | | | | | | | | | |
901 CCGCCCCGACCCCGCGCCATTACAAAAAAAAGCAAAACAAAAATTTT 950
947 -----C-T---TGA-GA-A-----T---TTA-CTA-A-AG----- 996
    | | | | | | | | | | | | | | | | | | | | |
951 AAAGATCATCGATGAAGAGAGAAATGCCCTTTTCTACAGAGTCCCCTTC 1000
997 -----GCC---T-----T--GC-----TT---G-CG---TG- 1046
    ||| | | | | | | | | | | | | | | | | | |
1001 CCACCCACAGCCCCATCCCCAGATAAGCGGGGAGTCCCTGGCGCGGTGC 1050
1047 --GTT-CTAN--GC--A-T---CGTGAATT-G-----A-GTG-G--TG 1096
    ||| ||| | | | | | | | | | | | | | | | |
1051 CAGTTTCTAGCCGCTGAGTGGGCGTG--TGCGCGGCTCCAAGTCGCCTG 1100
1097 AACAAAACATT-TAA-----A-C-CC-C---T--T--GT-CCTC--A- 1146
    | | | | | | | | | | | | | | | | | | | | |
1101 --CGTA-C-TGCTCACTCCCAGCTCCGCGCCCTGCTCCGTTCTCCCAA 1150
1147 ---T--G---GA---CTTT---ATGTTCTTG-G-G---GAC----- 1196
    | | | | | | | | | | | | | | | | | | | | |
1151 AACTCTGAATCGAAGAACTTTCCGGAAGTTTCTGAGAGCCCAGACCGGCG 1200
1197 ---A-G-----A-----A-----T-TAGATAA-CA-TAC-A---T--A- 1246
    | | | | | | | | | | | | | | | | | | | | |
1201 GGCACGCCCCCATCCCCAACCCCTCT-GTTAATCCCTACCAGCCTGCAG 1250
1247 TA-----AA--A--A-----C-----A-C-----A- 1296
    | | | | | | | | | | | | | | | | | | | | |
1251 TCCTGGCTGCTTCCAAGCAGGAGGTGGGCGCTCTGGCTAGCGGGCCGAA 1300
1297 ---GT-----G--T-T---ATTT---T-TAC-----AT-GGTCNT 1346
    || | | | | | | | | | | | | | | | | | | |
1301 AAAGTCCCTCCCGCATGTCTGATTTCCTCTTCCCCCAAAGG-CA- 1350
1347 A--A-GA--AG---A--A--A-ATA---A-A-----GAT-----A-G 1396
    | | | | | | | | | | | | | | | | | | | | |
1351 AGCACGAGGAGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCACG 1400
1397 ---A--TA-A-G-GA-----AG-T-GGAA-G--TA--T---T- 1446
    | | | | | | | | | | | | | | | | | | | | |
1401 CGGAAATACACGTGAGTCCTGGCGCCAGGTCGGGGTGGGTGGGCGTG 1450

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2247 CC--CCTTT-CAACTG-----GTTCCNAAT-T-AAAA-ATTCTCATTAA- 2296
    || ||| |||| | | ||| | | |||| | | || ||
2251 CCAGCCTGGGCAACAGAACAGATCCTG-TCTCAAAACAA-C-CAA-AAG 2300

2297 CCT-----TGA-ACCCC-TC---AANCN-----T 2346
    || ||| |||| | | || | |
2301 CCCAGAGAGAAAGAGTGAGACCCCATCTTTAAAGAAAAAAAAAAAAAGGT 2350

2347 --T--TT-----T-----TTG-AA--AA-----GT--GG-----AT--A- 2396
    | || | | ||| || || || || | |
2351 CATGATTGCAAGGTCACGATTGCAATTAAACTGTAAGGTGGGGAAGGAG 2400

2397 -A--AATTCA-AGAACTTCNCC-----CN-G-G--C-CAAAA--A--T-- 2446
    | || | | |||| | || | | | | | | | |
2401 GAGGAAATAAGAGAAG--CACCTGAGGCTTGAGTTCTCAGGAGCACCTAG 2450

2447 -TTGGG----A-----AACCCCC-CTATNTTTA-T-GC-CC-CA-A----A 2496
    |||| | | | | | | | | | | | | | |
2451 GTTGGGTCCCAGGTGAAGGGGCAC-AGAGGTAATTGCACCTCAGAGCTGA 2500

2497 ----A---TC-CN----C..... 2546
    | | | |
2501 TGGGAGGATTACTATGTCA..... 2550

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Alignment Of DNA 1/Region 1

(Complementary Strand), And

The Human hsp 27 Gene

(The top row shows the DNA 1/Region 1 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

51	-----T-TTC-CC-----A-----AATT-T-----T----	100
51	AACCCCTCTTCGCCCCCGCCACGGCCCTGAACGCTGGGGGAGGAGTGCAT	100
101	GGCCNCGGN-G-----AA--GT-TC-TTGA-ATTT-TA-----TC---	150
101	GGGGAGGGCGGCCCTCAACGGGTCATTGCCATTAATAGAGACCTCAAA	150
151	CACTTT-T-CAAAAA-AN--GNTTG-AGGGGT-TCAA-G-GTTAAT-GA	200
151	CACCGCTGCTAAAAATACCCGACTGGAGGAGCATAAAGCGC-AGCCGA	200
201	G--A-----A-TTTT-T-A--AT---TN--G-G-A-A--C--C-A	250
201	GCCCAGCGCCCGCACTTTTCTGAGCAGACGTCCAGAGCAGATCAGCCA	250
251	GT-TGAAAG-G-G--G-GTAAC-TT-TC-----AGNT--	300
251	GCATGACCGAGCGCGCGTCCCTTCTCGCTCCTGCGGGCCCCAGCTGG	300
301	-A-----TT-----A-T--T-CAA-CAAA-----T-T-C-A--GG----	350
301	GACCCCTTCGCGACTGGTACCCGCATAGCCGCTCTTCGACCAGGCCTT	350
351	-GGA-TGN-----TN-----A--A-T--TCN---T--TT---CT-C--CA	400
351	CGGGCTGCCCCGGCTGCCGGAGGAGTGGTCGAGTGGTTAGCGGCAGCA	400
401	T-T-----TA--T-----T-C-----G--G-----GA-A-C---	450
401	GCTGGCCAGGCTACGTGCGCCCCCTGCCCCCGCCGCATCGAGAGCCCC	450
451	--A-TGT--G-GAT---T-----TCA-----AATTC--C--	500
451	GCAGTGGCCGCGCCCGCTACAGCCGCGCTCAGCCGGCAACTCAGCAG	500
501	CTTG-TAC-CG-----TNNGGAA-A-T-CTGACCT-T--CT-GTTTT--T-	550
501	CGGGGT-CTCGGAGATCCGGCAGCTGCGGACCGCTGGCGCGTGTCCCTG	550
551	--T-TN-----TTCG-----AC---CTT---T-AANTN-A-----	600
551	GATGTCAACCACTTCGCCCCGACGAGCTGACGGTCAAGACCAAGGATGG	600
601	C-T--T-----TN-----TG-----TN-TCC--CAG--A-----	650
601	CGTGGTGGAGATCACCGGTGAGCCCCCTGCTCCTGCAGGGGAGAGGAGG	650

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651 ----TA-CAGTG-----A---CCNT-----T----TTCAAA----- 700
      || ||| | | | | | | | | | | | | | | | | | | | | |
651 AGGCTAGCAGGGCGGGCAGGGCCGGGGCGTGCCTTGAACCGGGGTCC 700

701 -----A-T-AA-C-TT--CAAACC-CC---A---CTG--CT 750
      | | | | | | | | | | | | | | | | | | | | | | | |
701 CGGGGGCCTGGGGAGTTAAACGTTGGCCCAGCACCGGGAACAGGACT 750

751 T-T--TTC--TN-C-C-----TT-----C---T-GCTTGCT--G---- 800
      | ||| | | | | | | | | | | | | | | | | | | | |
751 CCTGATTCCCTTGCTCAGGAATTGGGAGTGCGGGTCGCTT-CTAAGGGCG 800

801 -TTTCT--TCA--AATT--AG-GACANTCT---AT-C--A-AC---AGG- 850
      |||| | | | | | | | | | | | | | | | | | | | |
801 CTTTCTGCTCTGTAATCCAGCG-C-TT-TGGGAGGCCGAGACGGGAGGA 850

851 T-G-TTNT--CAAA-A-TT---GATTTCTCA-C-T---AACATA-C-AT 900
      | | | | | | | | | | | | | | | | | | | | | | | |
851 TCGCTTGAGGCCAGGAGTTCAAGA---CT-AGCCTGGGCAACATAGCGAG 900

901 TC-----A---GT-C-ATTA-AT-----CA----- 950
      | | | | | | | | | | | | | | | | | | | | | |
901 ACGCGCCCCCGCCCCGACCCCGCGCCATTACAAAAAAGCAACAA 950

951 ---TTTGTGTC-ACC-A-CAT---T-AA-AGAGA-----C-CTT--C--CAT 1000
      ||| | | | | | | | | | | | | | | | | | | | | |
951 AAATTTTTTTAAAGATCATCGATGAAGAGAGAAAATGCGCTTTTCTACA- 1000

1001 GA--CCA-TTCC-ACCTA-A-----AT---A-----C-----TTCCA 1050
      || || ||| ||| | | | | | | | | | | | | | | | |
1001 GAGTCCCCTTCCCACCCACAGCCCCATCCCCAGATAAGCGGGGAGTTCC- 1050

1051 CT-----T-CC--TTA-T-C---T-A-T---C-T-T-----T--A 1100
      || | | | | | | | | | | | | | | | | | | | | | |
1051 CTGGCGCGGTGCCAGTTTCTAGCCGCTGAGTGCGCGTGTGCGCGGTCCA 1100

1101 TTT-----T-C-T-T-T-CT---T-----AN----GA-CCATG-TA----- 1150
      | | | | | | | | | | | | | | | | | | | | | | | |
1101 AGTGGCGCTGCGTACTGCTCACTCCCCAGCTCCGCGCCCTGCTCCGTTCC 1150

1151 ----AAAA-T---AA-C-A---CTGT--GT---TTT-T-ATAT---G- 1200
      |||| | | | | | | | | | | | | | | | | | | | |
1151 TCCCAAACTCTGAATCGAAGAACTTCCGGAAGTTTCTGAGAGCCGAGA 1200

1201 -----T---A-----T--GTTA-TC--TA--A--- 1250
      | | | | | | | | | | | | | | | | | | | | | |
1201 CCGGGGGGCACGCCCCCATCCCCAACCCCTCTGTTAATCCCTACCAGCC 1250

1251 T----TC-TG--T-C--CC---CAGGA-----A-C----- 1300
      | | | | | | | | | | | | | | | | | | | | | |
1251 TGCACTCCTGGCTGCTTCCAAGCAGGAGGTGGGGCCTCTGGCTAGCGGG 1300

1301 ---ATAAAGTCC-----ATG---A-----G 1350
      | ||||| | | | | | | | | | | | | | | | | | |
1301 CCGAAAAAGTCCCCTCCCCCGCATGTCTGATTTCCTCTCCCCCAAG 1350

1351 G-A--CA--AGG-G-GTTT--A--A--ATG--T---T-T-----TG-TTC 1400
      | | || ||| | | | | | | | | | | | | | | | | |
1351 GCAAGCACGAGGAGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTC 1400

1401 AC-C--AC-T-CA----A-T--T--C---A---CGA--TG-----C 1450
      || | | | | | | | | | | | | | | | | | | | | |
1401 ACGCGAAATACACGTGAGTCCTGGCGCCAGGTGCGGGTGGGTGGGTGGC 1450

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1451 NT-----AG--AAC---CAC-G--C--A-----A--      1500
      |           ||  ||           |||  |  |  |
1451 GTGGGGGTGGGGTCAGGGAAGAGGGCACAGGGACCCACCCGGTGTGTAAT      1500

1501 GCAA-G---GCCTTT-----A-GT--A-----A--      1550
      |  ||  |  |||||           |  ||  |
1501 GTAACGCTTGCCCTTCCTCTCTGCACGTCCAGGCTGCCCCCGGTGTGGA      1550

1551 ----A-----TT-C-TC-----A---A---GA-----      1600
      |           ||  ||  ||           |  |  ||
1551 CCCCACCAAGTTTCCTCCTCCCTGTCCCCTGAGGGCACACTGACCGTGG      1600

1601 A-----AT---A--TA-----T---G---TC-C-A---A--      1650
      |           ||  |  ||           |  |  |||  ||
1601 AGCCCCCATGCCAAGCTAGCCACGCAGTCCAACGAGATCACCATCCCA      1650

1651 -T-AC-T--GAGT-G-----A--T-G-----A-A--CTG-AA--T---      1700
      |  ||  |  ||||  |  |  |  |  |  |  |||  ||
1651 GTCACCTTCGAGTCGCGGGGCCAGCTTGGGGGCAGAAGCTGCAAAATCCG      1700

1701 A--A---TG-----TA---C-TTA-----AT-C--A-----G-TGC      1750
      |  |  ||           ||  |||  |  ||  |  |  |  |||
1701 ATGAGACTGCCGCCAAGTAAAGCCTTAGCCCCGATGCCCCACCCCTGCTGC      1750

1751 CT--AC---CTA--CC-C-----TGT-T---CTAGTGATGC-----      1800
      |  ||  ||  ||  |  |  |  |  |  |  |||  |||  |
1751 CGCCACTGGCTGTGCCTCCCCCGCCACCTGTGTGTTCTTTTGATACATTT      1800

1801 -----G-----G---AAA-AA-----G---GG--      1850
      |           |  |||  ||           |  ||
1801 ATCTTCTGTTTTTCTCAAATAAAGTTCAAGCAACCACCTGTCACTGGCC      1850

1851 -AGG---TGG-----AAGGA---T-A--C---T-C--TTT-CTTG      1900
      |||  |||  ||||  |  |  |  |  |  |||  ||
1851 CAGGCCCTGGTGTGTGTGGAAGGAAGCCTCAGGCACCTGCCATTGTGCTGG      1900

1901 A---AGGAG-C-TC-----A--C---TG-T-----T-T---A---      1950
      |||||  |  ||           |  |  ||  |  |  |
1901 CTTTCAGGAGTCATCTTTGCTCAGGCCGCTGCTGGGCCATGTGGGTACAC      1950

1951 T--T--AGG--G--G-AGAT-GG---AC--A--T--A-AAA-ACA-A--T      2000
      |  ||  |||  |  ||  ||  ||  ||  ||  |||  |||  |
1951 TGGTGTAGGTTGCTGGACACAGGCTGACTCACATCCATAAAGACAGAGGT      2000

2001 ---A-----TG--T-AGT---TACAAT---AGG--TA-GT--      2050
      |           ||  ||  ||  |||||  ||  |  |
2001 CTTAGGGCCGGGCGCAGTGGCTCA-TACCTACAATCCCAGCACTTTGGGG      2050

2051 --TTGAAG--GGTA--A-T-----AAG---G-GT---A-AT-A---T--      2100
      |||||  ||  |  |  |  |||  |  ||  |  |  |
2051 GGTGGAAGCAGG-AGGAGTGCTTGAAGCCAAGAGTTCTAGACCAGCCTGG      2100

2101 ACAG-A--G---GA-TGGCACTAAAGAATAAAGA--AGA-AC--T--T--      2150
      |||  |  |  ||  ||  ||||  |||||  ||  |  |  |
2101 ACAACATAGTAAGACTGTCTCTAAAAAATAAAATTAGGCAGGGTGGTAC      2150

2151 TG-A-----A-T---AG-T-C--A---TGA---AGG---T---T      2200
      ||  |  |  |  |  |  |  |  |||  |||  |  |
2151 TGCACGCCTGTAGTCCCAGCTACTCAGGAGGCTGAGGCAGGAGGATCGCT      2200

2201 T---CC--GA-----AAG--A-AGA-A--TA-----GT---TT---T      2250
      |  ||  ||  ||  ||  ||  ||  |  ||  ||  ||  |
2201 TGAGCCCAGAGTTGTGAAGGTACAGTGAGCTAACATCGTGCCATTGCACT      2250

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2251 C-AGT-TG---A---GT-C--GA----G----AAT-CAACT---G---- 2300
      | | | | | | | | | | | | | | | | | | | | | |
2251 CCAGCCTGGGCAACAGAACAGATCCTGTCTCAAAACAACCAAAAGCCCA 2300
      | | | | | | | | | | | | | | | | | | | | | |
2301 G-G-GTTTAACG-GTG-GA----A-C---AAAA-A-----T- 2350
      | | | | | | | | | | | | | | | | | | | | | |
2301 GAGAGA--AA-GAGTGAGACCCCATCTTTAAAGAAAAAAAAAAGGTC 2350
      | | | | | | | | | | | | | | | | | | | | | |
2351 -T--TTA-AA----A--ATT-C--T-A-----G-AA-----AA--AGC 2400
      | | | | | | | | | | | | | | | | | | | | | |
2351 ATGATTGCAAGGTCACGATTGCAATTAAACTGTAAGGTGGGGAAGGAGG 2400
      | | | | | | | | | | | | | | | | | | | | | |
2401 A--AA-TA-G-GT--CA----A---TTCACCTT-T-A--A--A---AGGT- 2450
      | | | | | | | | | | | | | | | | | | | | | |
2401 AGGAAATAAGAGAAGCACCTGAGGCTTGAGTTCTCAGGAGCACCTAGGTT 2450
      | | | | | | | | | | | | | | | | | | | | | |
2451 G--T---G-T-A-G---A--G-G-TA-T---A-----GAG-T-ACC-- 2500
      | | | | | | | | | | | | | | | | | | | | | |
2451 GGGTCCCAGGTGAAGGGGCACAGAGGTAATTGCACCTCAGAGCTGATGGG 2500
      | | | | | | | | | | | | | | | | | | | | | |
2501 AG-AT-A..... 2550
      | | | |
2501 AGGATTACTATGTCA..... 2550

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Alignment Of DNA 1/Region 2 (Normal Strand), And The Human hsp 27 Gene

(The top row shows the DNA 1/Region 2 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

-4C-T--GC-----A--G-G-----T-C-A-ACG-GGA-T---	45
1	GAATTCATTTGCTTTTCCTTAACGAGAGAAGGTTCCAGATGAGGGCTGAA	50
46	C--T-TT-G---GC--A-----TGA---T-----ATT--T--AT--	95
51	CCCTCTTCGCCCCGCCCCACGGCCCCCTGAACGCTGGGGGAGGAGTGCATGG	100
96	--A---CA-C--T-----G--TC-T--C-ATTT-T-----CTTC-----	145
101	GGAGGGGCGGCCCTCAACGGGTCATTGCCATTAATAGAGACCTCAAACA	150
146	--GTTTG-TATA--T-----TGG-GGA---T---GTTTAT--A---	195
151	CCGCCTGCTAAAAATACCCGACTGGAGGAGCATAAAAGCGCAGCCGAGCC	200
196	-ATT-----A-TTTT--G--CAG--G--A-----T-----	245
201	CAGCGCCCCGCACTTTCTGAGCAGACGTCAGAGCAGAGTCAGCCAGCA	250
246	T-A-----T---TT-T-GA-----A--TG----	295
251	TGACCGAGCGCCGCTCCCTTCTCGCTCCTGCGGGCCCCAGCTGGGAC	300
296	---T---G--A--G--A-----A-A-----T-----A--A-----TATCT	345
301	CCCTTCCGCGACTGGTACCCGCATAGCCGCCTCTTCGACCAGGCCT-TCG	350
346	---T-----T-----A--ATT--T-G-A-T--TT-----	395
351	GGCTGCCCCGGCTGCGGAGGAGTGGTCGCAGTGGTTAGGCGGCAGCAGC	400
396	T-C-----T--T-----T-----T-G-----	445
401	TGGCCAGGCTACGTGCGCCCCCTGCCCCCGCGCCATCGAGAGCCCCGC	450
446	--T-----TA--GC-----T-A-----AT-TAA--A---	495
451	AGTGGCCGCGCCCGCTACAGCCGCGGCTCAGCCGGCAACTCAGCAGCG	500
496	---T---GG--T-----A-ACTA--AC--T-----T-T---T--AT	545
501	GGGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGCGTGTCCCTGGAT	550
546	-TCA-----T-----A-----T-A-----A-----T---T	595
551	GTCAACCACTTCGCCCCGACGAGCTGACGGTCAAGACCAAGGATGGCGT	600
596	-----AG-T-A-----TG-G-----T-CTCAT-CA-----	645
601	GGTGGAGATCACCGGTGAGCCCCCTGCTCCTGCAGGGGAGAGGAGGAGG	650
646	C-A-CA-----AGG-CC-----C-T---TTGCAAC-----	695
651	CTAGCAGGGCGGGCAGGGCCGGGGCGTGGGTTGAAACGGGGGTCCCCG	700

696	-----A-TT---G---C-----TCCT	745
701	GGGCCTGGGGAGTTAAACGTTGGCCCGAGCACCAGGAAAAACAGGACTCCT	750
746	-A--C--TT-CT-A---T-G---T-C---T-----AAG---TT-	795
751	GATTCCTTGCTCAGGAATTGGGAGTGCGGTCGCTTCTAAGGGCGCTTT	800
796	CTGCT-T-----C--A-C--TTT-----CCTA-----T-GCT--	845
801	CTGCTCTGTAATCCCAGCGCTTTGGGAGGCCGAGACGGGAGGATCGCTTG	850
846	---CCA---TCCAT---T---T---CAA--T--C-A--C-C-CCA---	895
851	AGGCCAGGAGTTCAAGACTAGCCTGGGCAACATAGCGAGACGCCCCCCC	900
896	-G-----AC---GT-C--T--CT-----GTATA-A---TTTG----	945
901	CGCCCCGACCCCGGCCATTACAAAAAAAAGCAAACAAAAATTTTTTTA	950
946	--G--C--C-ATG-----T-C-CT-----A-----TC-----	995
951	AAGATCATCGATGAAGAGAGAAAATGCGCTTTTCTACAGAGTCCCTTCC	1000
996	-AC--A-A-C---TC-----TAA-----T-C--T-----TG--	1045
1001	CACCCACAGCCCCATCCCCAGATAAGCGGGAGTTCCCTGGCGCGGTGCC	1050
1046	---TTT---G---T-A-T-----T---C-CTGG---AA-TGC-CCTGAA	1095
1051	AGTTTCTAGCCGCTGAGTGGGCGTGTGGC--GGCTCCAAGTGGCGCTGCG	1100
1096	-A-----A-----A-CTCC-----TACTCAG-----A---CT-T	1145
1101	TACTGCTCACTCCCCAGCTCCGCGCCCTGCTCCGTTCTCCCAAACTCT	1150
1146	AAA---AAG--C---C---A-G---CT-----C--A-A-----A--	1195
1151	GAATCGAAGAACTTTCGGAAGTTTCTGAGAGCCCAGACCGGCGGCACG	1200
1196	-----T---A-----T-TGIGT--TCT-T-C--G--TG-ATTC-T--	1245
1201	CCCCCATCCCCAACCCCTCTGT-TAATCCCTACCAGCCTGCAGTCCTGG	1250
1246	-T--TTCC-----T-----TCT---T--C-----A-----T-	1295
1251	CTGCTTCCAAGCAGGAGGTGGGCTCTGGCTAGCGGGGCCGAAAAGTC	1300
1296	---T-----G-ANGTA-GANTCTAACACTTCTTCACAC-A---C---CA	1345
1301	CCCTCCCCCGCATGTCTGATT-TC-C-CT-CTTCCCCCAAAGGCAAGCA	1350
1346	CTA--A-C---A--A--A-C-T---A-----GGT--TT---G---A	1395
1351	CGAGGAGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCACGCGGA	1400
1396	AAT---GTTA--C-T-----T-----T---TG--TA-CAT-----	1445
1401	AATACACGTGAGTCCTGGCGCCAGGTCGGGTGGGTGGGTGGCGTGGGGG	1450

1446 T----T-A---A-GA----AC-----CT-----T-T-T--T-TA-CA- 1495
 | | | | | | | | | | | | | | | | | | | | | |
 1451 TGGGGTCAGGGAAGAGGGCACAGGGACCCACCCGGTGTGTAAATGTAACGC 1500
 1496 TT---TTTCAT-T-T-----T-A-----A---A-- 1545
 | | | | | | | | | | | | | | | | | | | | | |
 1501 TTGCCTTTCTCTCTGCACGTCCAGGCTGCCCCCGGTGTGGACCCACCC 1550
 1546 -AA---CAT--T---T-TAA--T-AG--CA-A---A-----A----- 1595
 | | | | | | | | | | | | | | | | | | | | | |
 1551 CAAGTTTCTCTCTCTGCTGCTGAGGGCAGACTGACCGTGGAGGCCCC 1600
 1596 -AT---ATTA--TAAC-A-G-AG---AA-GA-AT-AC-ATACTA---AC 1645
 | | | | | | | | | | | | | | | | | | | | | |
 1601 CATGCCCA--AGCTAGCCACGAGTCCAACGAGATCACCATCCAGTCAC 1650
 1646 ----GAAT-GTT-----AT-TT-----A-AA--T--AAGANTT--AT-- 1695
 | | | | | | | | | | | | | | | | | | | | | |
 1651 CTTGAGTTCGCGGGCCAGCTTGGGGGCGAAGCTGCAA-AATCCGATGA 1700
 1696 ---TG--GT-A--TAAA-CAT-AG-----ATG-----T--T----- 1745
 | | | | | | | | | | | | | | | | | | | | | |
 1701 GACTGCCGCCAAGTAAAGCCTTAGCCCGGATGCCCAACCCCTGCTGCCGCC 1750
 1746 --T---TAA---TA-----AC-TG-GTAATCTG--GAAA-A---A--- 1795
 | | | | | | | | | | | | | | | | | | | | | |
 1751 ACTGGCTGTGCCTCCCCCGCCACCTGTGTGTTCTPTTGATACATTTATCT 1800
 1796 ---G-----AAATA--G---AA--CA-C-AGCCTGGT-A-T--C--A 1845
 | | | | | | | | | | | | | | | | | | | | | |
 1801 TCTGTTTTTCTCAAATAAAGTTCAAAGCAACCA-CCTG-TCAGTGGCCCA 1895
 1846 --C--TG-----AA--A--C-TC---CTC-T---ATGT--T--CT 1900
 | | | | | | | | | | | | | | | | | | | | | |
 1851 GGCCCTGGTGTGTTGTGGAAGGAGCCTCAGGCACCTGCCATTTGCTGGCT 1945
 1896 TCCATGAATAACCACCTAT-C-C---CC-GTGCCT---CC-T-TGG--A- 1950
 | | | | | | | | | | | | | | | | | | | | | |
 1901 TTCAGGAGT--C-ATCTTTGCTCAGGCCCCGTGC-TGGGCCATGTGGGTAC 1995
 1946 A-T--TA-A--TAACN--A-A-----T---T-----T---T---GA-AT-- 2000
 | | | | | | | | | | | | | | | | | | | | | |
 1951 ACTGGTGTAGGTTGCTGGACACAGGCTGACTCACATCCATAAAGACAGAG 2045
 1996 -T-TT-GGT--GA-----AN-----AT---T-----T-----TTT--- 2050
 | | | | | | | | | | | | | | | | | | | | | |
 2001 GTCTTAGGGCCGGGCGCAGTGGCTCATACCTACAATCCCAGCACTTTGGG 2095
 2046 ---TT-AA--A--A--ANT--T-G---CCTNA-ATTT-TA-ATC--CN-G 2100
 | | | | | | | | | | | | | | | | | | | | | |
 2051 GGGTTGAAGCAGGAGGAGTGTGTAAGCC-AAGAGTTCTAGACCAGCCTG 2145
 2096 -A-AACCTT-T--G---G-----AAAAATA-----C-G--T--T- 2150
 | | | | | | | | | | | | | | | | | | | | | |
 2101 GACAACATAGTAAGACTGTCTCTAAAAATAAAATTAGGCAGGGTGGTA 2195
 2146 -T-----T-T--TC-----TA-T-----T-----T----- 2200
 | | | | | | | | | | | | | | | | | | | | | |
 2151 CTCACGCCTGTAGTCCCAGCTACTCAGGAGGCTGAGGCAGGAGGATCGC 2245
 2196 TT-A---A-A-----CNG-G--CN--CAT-GN---A---A- 2250
 | | | | | | | | | | | | | | | | | | | | | |
 2201 TTGAGCCCAGAGTTGTGAAGGTACAGTGAGCTAACATCGTGCCATTGCAC

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2246 ---A---TGGG-AA--G-----G-TN-TG-CTG-----G--- 2295
      |   | | | | | | | | | | | | | | | | | | |
2251 TCCAGCCTGGGCAACAGAACAAGATCCTGTCTCAAAACAACCAAAAGCCC 2300
2296 A-A-A-AAA-ATTT-----T-TTT-----GANA-----T-A- 2345
      | | | | | | | | | | | | | | | | | | | |
2301 AGAGAGAAAGAGTGAGACCCCATCTTTAAAAAGAAAAAAAAAAGGTCAT 2350
2346 -ATT-----TC-C--TT-----T-AA--CTG---GGTCTNGNA--A--A- 2395
      | | | | | | | | | | | | | | | | | | | |
2351 GATTGCAAGGTCACGATTGCAATTAAACTGTAAGGTGGGGAAGGAGGAG 2400
2396 -AAATN-----T-----TT---TTA--ANNA--A---ANNTTN- 2445
      | | | | | | | | | | | | | | | | | | | |
2401 GAAATAAGAGAAGCACCTGAGGCTTGAGTTCTCAGGAGCACCTAGGTTGG 2450
2446 --CCC---TGAA-----CAC-----C-CCNN-----TN-TNN... 2495
      | | | | | | | | | | | | | | | | | | | |
2451 GTCCCAGGTGAAGGGGCACAGAGGTAATTGCACCTCAGAGCTGATGGGAG 2500
2496 ..... 2545
2501 GATTACTATGTCA..... 2550

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Alignment Of DNA 1/Region 2

(Complementary Strand), And

The Human hsp 27 Gene

(The top row shows the DNA 1/Region 2 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

-3	...NNAN-----ANNG-G-G--GTGTTC-AG--G-GN----	46
1	GAATTCATTGCTTTTCCTTAACGAGAGAAG-GTTCAGATGAGGGCTGA	50
47	ANN-T-TTNN-----T-----T-----A--A--A--	96
51	ACCTCTCTCGCCCCCGCCACGGCCCTGAACGCTGGGGGAGGAGTGCATG	100
97	---A-----ANA---T--TT---TT---TNCNAGACC-CAGT	146
101	GGGAGGGGCGCCCTCAAACGGGTCAATGCCATTAATA-GAGACCTCA--	150
147	TAA-A--G---G---AAAT-TATNT--C---A--A--A-AAAATTTTPTTC	196
151	-AACACCGCCTGCTAAAAATACCGACTGGAGGAGCATAAAAG-----C	200
197	-CAGC--AN---A-C-CTTCC-CA-TTNCATGNGCCN--GTTTAAA--A	246
201	GCAGCCGAGCCAGCGCC-CCGCACTTTTC-TGAGCAGACGTCCAGAGCA	250
247	-A-T-AGAAA--A--A--A-CGTA---T---TT-T---T-T-C----	296
251	GAGTCAGCCAGCATGACCAGCGCGCGCTCCCTTCTCGCTCCTGCGGGG	300
297	CAA-AGGT-----TTCNG-GA-T--TAA-----A-A-----T-TN--	346
301	CCCAGCTGGGACCCCTTCCGCGACTGGTACCCGCATAGCCGCCTCTTCG	350
347	A---GGC-----A--ANT--T-----T--TTA	396
351	ACCAGGCCTTCGGGCTGCCCGGGCTGCCGGAGAGTGGTCGCAGTGGTTA	400
397	-----A--A-----A--A-----A--	446
401	GGCGGCAGCAGCTGCCAGGCTACGTGCGCCCCCTGCCCCCGCCGCCAT	450
447	--A-A-----TN-----T-----TCA-CC---	496
451	CGAGAGCCCCGAGTGGCCGCGCCCGCTACAGCCGCGGCTCAGCCGGC	500
497	AA---A--A-----T-TC--A-A-----A-A-T-----TNG---	546
501	AACTCAGCAGCGGGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGC	550
547	-T-T-----AT-T-AA-----TTC-C-----A--AG--GA-GG-CA---C	596
551	GTGTCCCTGGATGTCAACCACTTCGCCCCGACGAGCTGACGGTCAAGAC	600
597	---GG--GG-----A--T-A--GGTG-GT-----TA-T--T-CATGG	646
601	CAAGGATGGCGTGGTGGAGATCACCGGTGAGCCCCCTGCTCCTGCAGGG	650

647 -A-AG-A--A--CATAG-AGG-----AG-----T---TTCA- 696
 | | | | | | | | | | | | | | | | | | | | | |
 651 GAGAGGAGGAGGC-TAGCAGGGCGGGCAGGGCCGGGGCGTGGCGTTGAA 700
 697 --G---T---G-----A-T-A--C---C--AG---G--- 746
 | | | | | | | | | | | | | | | | | | | | | |
 701 ACGGGGGTCCCGGGGGCCTGGGGAGTTAAACGTTGGCCAGCACCGGGAA 750
 747 ---C---T---G-T-----G-T-----T-----C---T--- 796
 | | | | | | | | | | | | | | | | | | | | | |
 751 AAACAGGACTCCTGATTCCCTTGCTCAGGAATTGGGAGTGGCGGTGCGCTT 800
 797 --A-----TTTCT--T-T-T--TCC-AGA--TT---A--CC-AG--- 846
 | | | | | | | | | | | | | | | | | | | | | |
 801 CTAAGGGCGCTTCTGCTCTGTAATCCCAGCGCTTTGGGAGGCCGAGACG 850
 847 -----T---T--A-----TTAAA-AC-ATC-T---A---T-GT- 896
 | | | | | | | | | | | | | | | | | | | | | |
 851 GGAGGATCGCTTGAGGCCAGGAGTTCAAGACTAGCCTGGGCAACATAGCG 900
 897 -----TTATA-----C---C 946
 | | | | | | | | | | | | | | | | | | | | | |
 901 AGACGCGCCCCCGCCCCGACCCCGCGCCATTACAAAAAAAAGCAAAC 950
 947 AATAANTCTTATTTAAATA--A-C-AT-----T-CG-TT---- 996
 | | | | | | | | | | | | | | | | | | | | | |
 951 AAAAAAT-TT-TTAAAGATCATCGATGAAGAGAGAAAATGCGCTTTTCT 1000
 997 A--G--TA---T-----GT---AT-----T---C-----TT 1046
 | | | | | | | | | | | | | | | | | | | | | |
 1001 ACAGAGTCCCCTTCCCACCCACAGCCCATCCCAGATAAGCGGGGAGTT 1050
 1047 C--T--C---TG---TTA-TAA---T-A-T---T-T-----T- 1096
 | | | | | | | | | | | | | | | | | | | | | |
 1051 CCCTGGCGCGGTGCCAGTTTCTAGCCGCTGAGTGGCGTGTGCCCGGCTC 1100
 1097 ---TGC---T---A-T--TAAA-----A--T--G---T--T---TT 1146
 | | | | | | | | | | | | | | | | | | | | | |
 1101 CAAGTGCCTGCGTACTGCTCACTCCCCAGCTCCGCGCCCTGCTCCGTT 1150
 1147 --T---AAAA-T--GAAAAAT-GTAA-AA-----AAGGTTCTTAAAT 1196
 | | | | | | | | | | | | | | | | | | | | | |
 1151 CCTCCCAAACTCTGAA---TCG-AAGAACTTTCCGGAAGTTTCTGAGA- 1200
 1197 GTACAAA-----A-G-----T-----AACA---T-T-TCAA----- 1246
 | | | | | | | | | | | | | | | | | | | | | |
 1201 GCCCAGACCGGGCGGGCACGCCCCCATCCCAACCCCTCTGTTAATCCCT 1250
 1247 ACC-----T--AGT--T---TG-TT--A-GT-GG---TG-----T--G--T 1296
 | | | | | | | | | | | | | | | | | | | | | |
 1251 ACCAGCCTGCAGTCTTGGCTGCTTCCAAGCAGGAGGTGGGGCTCTGGCT 1300
 1297 -G-----AAGAAGT-----G--T-TA-GANT-C--TACNTC- 1346
 | | | | | | | | | | | | | | | | | | | | | |
 1301 AGCGGGGCGGAAAAAGTCCCCTCCCCCGCATGTCTGATTTCCTT-CTTCC 1350
 1347 ----AATG--AAG-A--AGGA-----A--A--AG-A---A--TC---- 1396
 | | | | | | | | | | | | | | | | | | | | | |
 1351 CCCCAAAGGCAAGCACGAGGAGCGGCAGGACGAGCATGGCTACATCTCCC 1400
 1397 -----ACG---AAGA-ACACA--A-T-----A--T-----T-- 1446
 | | | | | | | | | | | | | | | | | | | | | |
 1401 GGTGCTTCACGCGGAA-ATACACGTGAGTCTGGCGCCAGGTCGGGGTGG 1450

1447 -TGAG---C-TGG-----C----- 1496
 || | | || |
 1451 GTGGGTGGCGTGGGGTGGGGTCAGGGAAGAGGGCACAGGGACCCACCCG 1500
 1497 -T-T-T--T-TAA-G-T--C-T-----G-A-GT--AGG----- 1546
 | | | | || | | | | | | | | |
 1501 GTGTGTAATGTAACGCTTGCCCTTCCTCTCTGCACGTCCAGGCTGCCCCC 1550
 1547 -----A-----GTTT--T--TC-----AGGGCA--- 1596
 | | | | | | | | | |
 1551 CGGTGTGGACCCACCCAAGTTTCCTCCTCCCTGTCCCTGAGGGCACAC 1600
 1597 T-----T-----CC---A-G-----G-A--A-----T--A-C-A-A-- 1646
 | | | | | | | | | | | | | | | |
 1601 TGACCGTGGAGGCCCCCATGCCAAGCTAGCCACGCAGTCCAACGAGATC 1650
 1647 AC-A----AG--A--TT--AG-----AG-TTG-----TG 1696
 || | || | | | | | | | | | | |
 1651 ACCATCCCAGTCACCTTCGAGTCGCGGGCCAGCTTGGGGGCAGAAGCTG 1700
 1697 -A---T---A-G-GACATG--GCCAAATTA----T-A-CA-G-A-G---A 1746
 | | | | | | | | | | | | | | | |
 1701 CAAATCCGATGAGAC-TGCCGCAAGTAAAGCCTTAGCCCGGATGCCCA 1750
 1747 CG--T-CTG--G-----GG-TGAT--T-----GAAA--TG-GATG---- 1796
 | | | | | | | | | | | | | | | |
 1751 CCCCTGCTGCCGCCACTGGCTG-TGCCTCCCCGCCACCTGTG-TGTTCT 1800
 1797 ---GAG-CAT--AG-----G-----AAAGTGAAG--CA--G-AAC-- 1846
 || || | | | | | | | | | | | |
 1801 TTTGATACATTTATCTTCTGTTTCTCTCAA-TAAAGTTCAAAGCAACCA 1850
 1847 --T-T-A--GAC--A-----T-----A-G-AAG--T-AGG-A-- 1896
 | | | | | | | | | | | | | | | |
 1851 CCTGTCACTGGCCAGGCCCTGGTGTGTGTGGAAGGAAGCCTCAGGCACC 1900
 1897 -GCAATGT--TG-C---A--A--A-----G---GGCC--T--TG--- 1946
 || || | | | | | | | | | | | |
 1901 TGCCATTTGCTGGCTTTTCAAGAGTCATCTTTGCTCAGGCCCGTGTGGGC 1950
 1947 --TGTG---A---TG---AG-----AC-CA---T-ACT-A-AT--- 1996
 || | | | | | | | | | | | | | |
 1951 CATGTGGGTACACTGGTGTAGGTGCTGGACACAGGCTGACTCACATCCA 2000
 1997 TA-----T---A-----TG---A-A--TA-AA--- 2046
 || | | | | | | | | | | | | | |
 2001 TAAAGACAGAGGTCTTAGGGCCGGGCGAGTGGCTCATACCTACAATCCC 2050
 2047 AG---TTAG-----TT-AC-CA-----T--TT-AA-----TT--A 2096
 || || | | | | | | | | | | | |
 2051 AGCACTTTGGGGGTGAAGCAGGAGGTGCTTGAAGCCAAGAGTTCTA 2100
 2097 G-C-----T--A-A-CA-A--AAGA-----AAA---TCAAA-TTAA- 2146
 | | | | | | | | | | | | | | | |
 2101 GACCAGCCTGGACAACATAGTAACACTGTCTCTAAAAAATAAAATTAGG 2150
 2147 -AG-----A-T--A---T-TA-T-----T--TC-----T---CA 2196
 || | | | | | | | | | | | | | |
 2151 CAGGGTGGTACTGCACGCCTGTAGTCCCAGCTACTCAGGAGGCTGAGGCA 2200
 2197 -----C-----A-----TT-----CA---A--AA--T--- 2246
 | | | | | | | | | | | | | | | |
 2201 GGAGGATCGCTTGAGCCCAGAGTTGTGAAGGTACAGTGAGCTAACATCGT 2250

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2247 ---A---A-TCC---TG---CAA-A-AT-AA--T--TAT---AAA-CA- 2296
      |   |   |   |   |   |   |   |   |   |   |   |
2251 GCCATTGCACTCCAGCCTGGGCAACAGAACAAGATCCTGTCTCAAAACAA 2300

2297 -----T---CCCCA---A----- 2346
      |   |   |   |   |   |
2301 CCAAAGCCCAAGAGAGAAAGAGTGAGACCCCATCTTTAAAGAAAAAAA 2350

2347 -----T-AT-A---CAA---ACGA---A-----G-AA----- 2396
      |   |   |   |   |   |   |   |   |   |   |
2351 AAAAGGTCATGATTGCAAGGTCACGATTGCAATTAAACTGTAAAGGTGGG 2400

2397 -AA-----T--GAGA--CAG-TG---T--A-T---A--A--A 2446
      ||   |   |   |   |   |   |   |   |   |   |
2401 GAAGGAGGAGGAAATAAGAGAAGCACCTGAGGCTTGAGTTCTCAGGAGCA 2450

2447 --TA--T-----C--A--TGC-----CA-A-AG--A-T--C-CC---G-- 2496
      ||   |   |   |   |   |   |   |   |   |   |
2451 CCTAGGTTGGGTCCCAGGTGAAGGGGCACAGAGGTAATTGCACCTCAGAG 2500

2497 -T--TG--AC-----CT--G-CAG..... 2546
      |   |   |   |   |   |
2501 CTGATGGGAGGATTACTATGTCA..... 2550

```

Alignment Of DNA 1/Region 3 (Normal Strand), And The Human hsp 27 Gene

(The top row shows the DNA 1/Region 3 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

-1	..AT--A-----AA--AGCA-AAG-TT-----T-----CT-A	48
1	GAATTCATTGCTTTTCCTTAACGAG-AGAAGGTTCCAGATGAGGGCTGA	50
49	---T-TA-----A-G-----TGA-C-C-----AGT-GT--ATT	98
51	ACCCTCTTCGCCCCGCCACGGCCCTGAACGCTGGGGGAGGAGTGCATG	100
99	-----T-A-----T-AT-GCGAT-AAGA-AGA--T-A--C	148
101	GGGAGGGGCGGCCCTCAAACGGGTCAATGCCATTAATAGAGACCTCAAAC	150
149	TACT---T--T-----TA---A-TG-A--A--ATAAATA-CATA----A	198
151	-ACCGCCTGCTAAAAATACCCGACTGGAGGAGCATAAA-AGCGCAGCCGA	200
199	---A-C-----A-TTIT--GA--A--CTGTCTACAGAA-A-A---A-	248
201	GCCCAGCGCCCGCACTTTTCTGAGCAGAC-GTC--CAGAGCAGAGTCAG	250
249	CTCAG-A-GA--GA-----T-----G-----G-----	298
251	C-CAGCATGACCGAGCGCCGCTCCCTTCTCGCTCCTGCGGGGCCCCAG	300
299	-T--A-----A-TG--A-----A-A-----AC-A--	348
301	CTGGGACCCCTTCCGCGACTGGTACCCGCATAGCCGCCTCTTCGACCAGG	350
349	C-T-C-----TG-----T-----T--T--A-TA-TAAAA-G--	398
351	CCTTCGGGCTGCCCCGCTGCCGGAGGAGTGGTCGCAGTGGTTAGGCGGC	400
399	A--A-CTT-CTTCA--CTA-GT-C-CC-----AT--AG	448
401	AGCAGCTGGC--CAGGCTACGTGCCGCCCTGCCCGCCGCCATCGAG	450
449	A-----A-TAG-----A--G-----T--G-----AA-T	498
451	AGCCCCGCAGTGGCCGCCCGCCTACAGCCGCGGCTCAGCCGGCAACT	500
499	-AG-AGTCTTG-TCTT--A-ATACAGCAT--TG---AT---T-----TA	548
501	CAGCAG-CGGGGTCTCGGAGATCCGGCAGCTGCGGACCGCTGGCGCGTG	550
549	T---T--ATGTNATATATCCATATTC-CC--G-A-----TGA---TCAAG	598
551	TCCCTGGATGTCA-A---CCAC-TTCGCCCCGACGAGCTGACGGTCAAG	600
599	A-----T--C-T--TG-----C-----TG-G-----TG-TN-T--AT	648
601	ACCAAGGATGGCGTGGTGGAGATCACCGGTGAGCCCCCTGCTCCTGCAG	650

649 GG-AG-G-----CT-G-AGGGC---C-G--CT-----TG-G----- 698
 || || | || | |||| | | | || |
 651 GGGAGAGGAGGAGGCTAGCAGGGCGGGCAGGGCCGGGGCGTGCGGTGA 700
 699 --C-----TC-----A-T-----GT-G---A--A--G--A 748
 | || || | | | | | | | | | |
 701 AACGGGGGTCCCGGGGGCCTGGGGAGTTAAACGTTGGCCAGCACCAGGA 750
 749 AA---G-A-T---A-----A--A-TT---T---T---T 798
 || | | | | | | | | | | | |
 751 AAAACAGGACTCCTGATTCCCTTGCTCAGGAATTGGGAGTGCGGGTCGCT 900
 799 T--A-----TT---G-T--GT---C-----TT-G--AG----- 848
 | | | | | | | | | | | |
 801 TCTAAGGGCGCTTTCTGCTCTGTAATCCAGCGCTTTGGGAGGCCGAGAC 850
 849 -----T-G-TT-----TT-AA---TA---TGA--AACA-A-- 898
 | | | | | | | | | | | |
 851 GGGAGGATCGCTTGAGGCCAGGAGTTCAAGACTAGCCTGGGCAACATAGC 900
 899 -A-ACT-----A-----A--A-AAAGAAAAGC--- 948
 | || | | | | | | | | | | |
 901 GAGACGCGCCCCCGCGCCCGACCCCGCGCCATTACAAAAAAAAGCAAA 950
 949 C-----TCT-----G-TC-T---T-AAGC-A-----T-CACTT---A 998
 | | | | | | | | | | | |
 951 CAAAAATTTTTTAAAGATCATCGATGAAGAGAGAAAATGCGCTTTTCTA 1000
 999 CA---T---T---AT-----G-----TN---GA-AAG-----A--- 1048
 || | | | | | | | | | | | |
 1001 CAGAGTCCCCCTCCACCCACAGCCCCATCCCCAGATAAGCGGGGAGTTC 1050
 1049 -----AG---C-A-----A-T-----T-T-----T-- 1098
 || | | | | | | | | | | | |
 1051 CCTGGCGCGGTGCCAGTTTCTAGCCGCTGAGTGGGCGTGTGCGCGGCTCC 1100
 1099 AA-----A-----A-TC---A--T-----T-C---GTTC 1148
 || | | | | | | | | | | | |
 1101 AAGTGCCTGCGTACTGCTCACTCCCCAGCTCCGCGCCCTGCTCCGTTC 1150
 1149 ATC--ATA--T---AA--GA-GTA-T--C---A--T--C--A-A-CNCNG 1198
 || | | | | | | | | | | | |
 1151 CTCCCAAACTCTGAATCGAAGAACTTTCCGGAAGTTTCTGAGAGCCCAG 1200
 1199 A-----AT-CCC--ATNT--AGAG---T--GATAATG---A--AG 1248
 | || | | | | | | | | | | |
 1201 ACCGGCGGGCAGCCCCCATCCCCA-ACCCCTCTGTTAATCCCTACCAG 1250
 1249 -----A-TC-TGT-T--TTC-ACNCC--A--TGGGAGC-TATT--T-G-- 1298
 | || | | | | | | | | | | |
 1251 CCTGCAGTCTTGCTGCTTCCAAGCAGGAGGTGGG-GCCTCTGGCTAGCG 1300
 1299 -----A---GT-----AT-TCT--TT-CC-T-T-----A 1348
 | || | | | | | | | | | | |
 1301 GGGCCGAAAAGTCCCCTCCCCCGCATGTCTGATTTCCCTCTTCCCCCA 1350
 1349 A---CT-GCTT-----CTG--G--C-AGT-T--C-ACAT-T---G-T-C 1398
 | | | | | | | | | | | |
 1351 AAGGCAAGCAGGAGGCGGCGAGGACGAGCATGGCTACATCTCCCGGTGC 1400
 1399 ---A-GCG-AAGT-----T-A-----A--T-G----- 1448
 | || | | | | | | | | | | |
 1401 TTCACGCGGAAATACACGTGAGTCTGGCGCCAGGTCGGGGTGGGTGGGT 1450

1449 --C-TGGGA-TG---T--GG-AA-----CA-----TG--- 1498
 | | | | | | | | | | | | | | | | | | | | | |
 1451 GGCCTGGGGGTGGGGTCAGGGAAGAGGGCACAGGGACCCACCCGGTGTGT 1500
 1499 A-T-T--C-CT--C-TCT--T-T-T-----T-----T-----T-T 1548
 | | | | | | | | | | | | | | | | | | | | | |
 1501 AATGTAACGCTTGCCCTTCTCTCTGCACGTCCAGGCTGCCCCCGGTGT 1550
 1549 ---CCNC-C--AA-TTTATTT-T---T-T---T-----T----- 1598
 | | | | | | | | | | | | | | | | | | | | | |
 1551 GGACCCCAACCAAGTTCTCTCTCCCTGTCCCTGAGGGCACACTGACCG 1600
 1599 T--AG-----AT-CC-A--C-AG--A-----T--AA--A-ATTT--AT- 1648
 | | | | | | | | | | | | | | | | | | | | | |
 1601 TGGAGGCCCCCATGCCCAAGCTAGCCACGCACTCCAACGAGATCACCATC 1650
 1649 ---GTTT--TTC---TCNNGT-----TTGA---CTGAAGGT-----T 1698
 | | | | | | | | | | | | | | | | | | | | | |
 1651 CCAGTCACCTTCGAGTCGGGGGCCAGCTTGGGGGCAGAAGCTGCAAAAT 1700
 1699 ----TG-GA-----AA-TNTN---TTA-CNT---TG-----T-- 1748
 | | | | | | | | | | | | | | | | | | | | | |
 1701 CCGATGAGACTGCCGCCAAGTAAAGCCTTAGCCCGGATGCCCAACCCCTGC 1750
 1749 TGA----A-TGG--GT---TAA-----A---GT-TAG--CT----A-A- 1798
 | | | | | | | | | | | | | | | | | | | | | |
 1751 TGCCGCCACTGGCTGTGCTCCCCGCCACCTGTGT-GTCTTTTGATAC 1800
 1799 -TTNA-CTT-TGTTTAC-C---T---G---A---CATNN---T-T-A-T 1848
 | | | | | | | | | | | | | | | | | | | | | |
 1801 ATTTATCTTCTGTTTCTCAAATAAAGTTCAAAGCAACCACCTGTCAC 1850
 1849 --CC-----T--T-TTG---AAGGTNG-----GG-AC-----A--- 1898
 | | | | | | | | | | | | | | | | | | | | | |
 1851 GGCCCAAGGCCCTGGTGTTTGTGGAAGGAAGCCTCAGGCACCTGCCATTTG 1900
 1899 CT---TTN-A--A-T-T---GA--A-----T--TNGG---T-TNNA- 1948
 | | | | | | | | | | | | | | | | | | | | | |
 1901 CTGGCTTTCAGGAGTCATCTTGTCTCAGGCCCGTGTCTGGGCCATGTGGGT 1950
 1949 ANA----ANNTTG----AC-C---T---TNA-AN--A-AAANAN-- 1998
 | | | | | | | | | | | | | | | | | | | | | |
 1951 ACACTGGTGTAGGTTGCTGGACACAGGCTGACTCAGATCCATAAAGACAG 2000
 1999 ---TNNTANGG--G--C-C--T---T--TGCC--CMN---A--A-TN-G 2048
 | | | | | | | | | | | | | | | | | | | | | |
 2001 AGGTCTTAGGGCCGGCGCAGTGGCTCATACCTACAATCCCAGCACTTTG 2050
 2049 GG-----AA--AN-----T--TN-A--C-----T---G-CC--CCG 2098
 | | | | | | | | | | | | | | | | | | | | | |
 2051 GGGGGTTGAAGCAGGAGGAGTGCTTGAAGCCAAGAGTTCTAGACCAGCCT 2100
 2099 GGGAAAAANANTNCA-A-T-TAC-CT-----TAAA--T--G-C----- 2148
 | | | | | | | | | | | | | | | | | | | | | |
 2101 GG-ACAACATAGTA-AGACTGT-CTCTAAAAAATAAAAAATTAGGCAGGGT 2150
 2149 ----C--C-C-CNT-TNTCCNC-C--CN-A--ANT-TN---C----- 2198
 | | | | | | | | | | | | | | | | | | | | | |
 2151 GGTACTGCACGCCTGTAGTCCAGCTACTCAGGAGGCTGAGGCAGGAGGA 2200
 2199 -C-CTTGA----ATN-TT-TNAAGNTTTT-TNAA--AA-ATNNTNCCN- 2248
 | | | | | | | | | | | | | | | | | | | | | |
 2201 TCGCTTGAGCCCAGAGTTGTGAAGGTACAGTGAGCTAACATCGT-GCCAT 2250

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2249 --CNTNC--CCNN---ANNANTTTAAN-TCCT-----AAAAN--CC--- 2298
      | | | | | | | | | | | | | | | | | | | |
2251 TGCACTCCAGCCTGGGCAACAGAACAGATCCTGTCTCAAAACAACCAAA 2300

2299 --CCC-GN-----CCCMNN-TT-----G-----G 2348
      | | | | | | | | | | | | | | | | | | | |
2301 AGCCCAGAGAGAAAGAGTGAGACCCCATCTTTAAAAGAAAAAAAAAAG 2350

2349 --C--G---G---GN---GA---A-TT-----T----- 2398
      | | | | | | | | | | | | | | | | | | | |
2351 GTCATGATTGCAAGGTCACGATTGCAATTAAACTGTAAGGTGGGGAAGG 2400

2399 -----C-CCNN-----TT---TT-TNNNN-----TAN 2448
      | | | | | | | | | | | | | | | | | | | |
2401 AGGAGGAAATAAGACAAGCACCTGAGGCTTGAGTTCTCAGGAGCACCTAG 2450

2449 G--GG-TN--AT-TCC-GGGG---GN--TNA---C-CC---G-G--GNT 2498
      | | | | | | | | | | | | | | | | | | | |
2451 GTTGGGTCCCAGGTGAAGGGGCACAGAGGTAATTGCACCTCAGAGCTGAT 2500

2499 N..... 2548

2501 GGGAGGATTACTATGTCA..... 2550

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Alignment Of DNA 1/Region 3

(Complementary Strand), And

The Human hsp 27 Gene

(The top row shows the DNA 1/Region 3 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

-18NANC-----CC-G--G-G---TNAN	31
1	GAATTCATTGCTTTTCTTAACGAGAGAAGGTTCCAGATGAGGGCTGAA	50
32	CCC-C--CG---GA--ATNACCCNT-ANNNN-----A--A---A---	81
51	CCCTCTTCGCCCCCGCCACGGCCCTGAACGCTGGGGGAGGAGTGCAATGG	100
82	--A-----ANN-GGG--A---A--A-T-----TCN--C-	131
101	GGAGGGGCGCCCTCAAACGGGTCATTGCCATTAAATAGAGACCTCAAACA	150
132	CC-C--GC-----C---A--..JNNG-----G-GN--C-G-G--	181
151	CCGCCTGCTAAAAATACCCGACTGGAGGAGCATAAAGCGCAGCCGAGCC	200
182	--G-G---GN--TTTT--AG--GANTT--A-A--ANTNNTNNGG--GN	231
201	CAGCGCCCCGCACTTTTCTGAGCAGACGTCCAGAGCAG-AGTCAGCCAGC	250
232	A--AN-GNG-GNAANNA---TT-T---T--TN-----A-----A	281
251	ATGACCGAGCGCCGCTCCCTTCTCGCTCCTGCGGGGCCCCAGCTGGGA	300
282	-----A-----A--AN--C---T-TN--A--A-----	331
301	CCCCCTCCGCGACTGGTACCCGCATAGCCGCTCTTCGACCAGGCCTTCG	350
332	-----AN-A-T--TC--A-----AGG-GN-A--AN-	381
351	GGCTGCCCCGGCTGCCGGAGGAGTGGTTCGAGTGGTTAGGCGGCAGCAGC	400
382	T-----TN-G-G-GN-----G-----G-----A--ANA-----	431
401	TGGCCAGGCTACGTGCGCCCCCTGCCCCCGCCGCCATCGAGAGCCCCGC	450
432	AN-GG--G-G--GCAT-----T-----T-A--AG-G	481
451	AGTGGCCGCGCCCGCTACAGCCGCGCGCTCAGCCGGCAACTCAGCAGCG	500
482	---T-----A-AT-----TGN--AN--TN-----T-T---T---T	531
501	GGGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGCGTGTCCCTGGAT	550
532	-T-----T-C-CCC-GG--G-GC--A-G-TNAAN-----T---T	581
551	GTCAACCACTTCGCCCCGACGAGCTGACGGTCAAGACCAAGGATGGCGT	600
582	--T-----C-CCN---A-----T--TNN-G--GGC-A-A--AGG---	631
601	GGTGGAGATCACCAGTGAGCCCCCTGCTCCTGCAGGGGAGAGGAGGAGG	650

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632 C---C---CNT---ANNANTNTTT---TN---TTNAA---GG---TCAANN      681
    |   |   |   |   |   |   |   |   |   |   |   |   |   |
651 CTAGCAGGGCGGGCAGGGCCGGGGCGGTGCGGTGAAACGGGGGTCCCGG      700

682 ----T-----TN-----TTNN---A---ACCN--AATT-CA--A-T--T      731
    |   |   |   |   |   |   |   |   |   |   |   |   |
701 GGGCCTGGGGAGTTAAACGTTGGCCCAGCACCGGAAAAACAGGACTCCT      750

732 NA-----A--A--G---TG---TC-C--CNA----C-CTT-      781
    |   |   |   |   |   |   |   |   |   |   |   |   |
751 GATTCCCTTGCTCAGGAATTGGGAGTGCGGGTCGCTTCTAAGGGCGCTTT      800

782 CA-----AA---A-----AGG---ATA---ANNAT-G-T--      831
    |   |   |   |   |   |   |   |   |   |   |   |   |
801 CTGCTCTGTAAATCCAGCGCTTTGGGAGGCCGAGACGGGAGGATCGCTTG      850

832 ---C-AGG--TA-AA-ACAAAG--TN---AAT-TAGCTA-ACTTTA-----      881
    |   |   |   |   |   |   |   |   |   |   |   |   |
851 AGGCCAGGAGTTCAAGACTA-GCCTGGGCAACATAGCGAGACGGCCCCC      900

882 -----ACCC-----ATT-CAACAAN---GTAAN-ANA---TTT-----      931
    |   |   |   |   |   |   |   |   |   |   |   |   |
901 CCGCCCCGACCCCGCGCCATTACAAAAAAAAGCAAAACAAAAATTTTTTT      950

932 -----C-C-A-AA-----C-CTT-C-A--G--TCAA-----      981
    |   |   |   |   |   |   |   |   |   |   |   |   |
951 AAAGATCATCGATGAAGAGAGAAAATGCGCTTTTCTACAGAGTCCCCTTC      1000

982 --ACNNG-AG---A-----A-A-AA-C---A-TAA-----      1031
    |   |   |   |   |   |   |   |   |   |   |   |   |
1001 CCACCCACAGCCCCATCCCCAGATAAGCGGGGAGTTCCTGGCGCGGTGC      1050

1032 -A-TTT-TA-----T-----C-TGTG-G-----A-T-C---T---      1081
    |   |   |   |   |   |   |   |   |   |   |   |   |
1051 CAGTTTCTAGCCGCTGAGTGGGCGTGTGCGCGGCTCCAAGTGCCTGCG      1100

1082 -A-----A-----A-----A-----A-----A-----      1131
    |   |   |   |   |   |   |   |   |   |   |   |   |
1101 TACTGCTCACTCCCCAGCTCCGCGCCCTGCTCCGTTCTCCCAAACTCT      1150

1132 -AAT--AA-A--TTGGNGGAAAAA---A-AG---AGA--GG-----A--      1181
    |   |   |   |   |   |   |   |   |   |   |   |   |
1151 GAATCGAAGAACTTTCCGGAAGTTTCTGAGAGCCCAGACCGGCGGCACG      1200

1182 -----ATC---A-----T-GTT---CC--AC-ATCC--CAG-CAT---      1231
    |   |   |   |   |   |   |   |   |   |   |   |   |
1201 CCCCCATCCCAACCCCTCTGTTAATCCCTACCAGCCTGCAGTCCTGGC      1250

1232 T-----AA-C-----T-----TC-G-CT-GA---C--AAT--GT--      1281
    |   |   |   |   |   |   |   |   |   |   |   |   |
1251 TGCTTCCAAGCAGGAGGTGGGGCTCTGGCTAGCGGGGCCGAAAAAGTCC      1300

1282 -----GAA---CTG---CC-----A--G--AAGCA-GTT-      1331
    |   |   |   |   |   |   |   |   |   |   |   |   |
1301 CCTCCCCCGCATGTCTGATTTCCTCTTCCCCCAAGGCAAGCAGGAGG      1350

1332 A-----AGGA--A--A-G---A-ATA-C---T-C---A-----AA-TA-      1381
    |   |   |   |   |   |   |   |   |   |   |   |   |
1351 AGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCACGCGGAAATAC      1400

1382 --G-----C-T--C-CCA--T-GGNGTG-----      1431
    |   |   |   |   |   |   |   |   |   |   |   |   |
1401 ACGTGAGTCCTGGCGCCAGGTCGGGGTGGGTGGGTGGCGTGGGGGTGGGG      1450

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1432 --A---AA-A---CA--G--ATC-----T-TC-A-T-TA----T--CA      1481
      |  | | | | | | | | | | | | | | | | | | | | | | | |
1451 TCAGGGAAGAGGGCACAGGGACCCACCCGGTGTGTAATGTAACGCTTGCC      1500
      |  | | | | | | | | | | | | | | | | | | | | | | |
1482 ---C-TCT----AN----A---TG-----GG-----A-----T      1531
      |  | | | | | | | | | | | | | | | | | | | | | | |
1501 TTTCCTCTCTGCACGTCCAGGCTGCCCGGGTGTGGACCCACCCAAGT      1550
      |  | | | | | | | | | | | | | | | | | | | | | | |
1532 T-CNGNGT---TGA----TGAT---ACTCTTA---T--ATG-----ATG-      1581
      |  | | | | | | | | | | | | | | | | | | | | | | |
1551 TTCCTCCTCCCTGTCCCTGAGGGCACACTGACCGTGGAGGCCCCCATGC      1600
      |  | | | | | | | | | | | | | | | | | | | | | | |
1582 --AA-CGA---ATG-ATTTTAA--A-AT-----T---G-C---TTC---      1631
      |  | | | | | | | | | | | | | | | | | | | | | | |
1601 CCAAGCTAGCCACGCAGTCCAACGAGATCACCATCCAGTCACCTTCGAG      1650
      |  | | | | | | | | | | | | | | | | | | | | | | |
1632 T-----TT-----CN-A--C---ATAAT--G-TAAG--TG---      1681
      |  | | | | | | | | | | | | | | | | | | | | | | |
1651 TCGCGGGCCAGCTTGGGGGCAGAACTGCAAAATCCGATGAGACTGCCG      1700
      |  | | | | | | | | | | | | | | | | | | | | | | |
1682 --A--T---GC-TTAA---G-A--C--A-----G-----A--GGCT-      1731
      |  | | | | | | | | | | | | | | | | | | | | | | |
1701 CCAAGTAAAGCCTTAGCCCGGATGCCACCCCTGCTGCCGCCACTGGCTG      1750
      |  | | | | | | | | | | | | | | | | | | | | | | |
1732 T---T-----T-T-----CTTTT--T---T--AGT-TT-TGTTT      1781
      |  | | | | | | | | | | | | | | | | | | | | | | |
1751 TGCCTCCCCGCCACCTGTGTGTTCTTTTGATACATTTA-TCTTCTGTTT      1800
      |  | | | | | | | | | | | | | | | | | | | | | | |
1782 --C--A--TA---TT-AAA--A-C-AC-T--CAA-GACACAA---T---      1831
      |  | | | | | | | | | | | | | | | | | | | | | | |
1801 TTCTCAAATAAAGTTCAAAGCAACCACCTGTCACTGGCCAGGCCCTGGT      1850
      |  | | | | | | | | | | | | | | | | | | | | | | |
1832 -----AA-AA-----A---A--T-----TTA--T--CTTTCT----T      1881
      |  | | | | | | | | | | | | | | | | | | | | | | |
1851 GTTTGTGGAAGGAAGCCTCAGGCACCTGCCATTGCTGGCTTTCAGGAGT      1900
      |  | | | | | | | | | | | | | | | | | | | | | | |
1882 CA-CAT-G---AG-CCAA-GC-GGCCC-T-----CA--GC-----      1931
      |  | | | | | | | | | | | | | | | | | | | | | | |
1901 CATCTTTGCTCAGGCCCGTGCTGGGCCATGTGGGTACACTGGTGTAGGTT      1950
      |  | | | | | | | | | | | | | | | | | | | | | | |
1932 -CT---C-CA---T-AN--ACA-CCA----G-CA-AGATCTT-GATCATC      1981
      |  | | | | | | | | | | | | | | | | | | | | | | |
1951 GCTGGACACAGGCTGACTCACATCCATAAAGACAGAGGTCTTAGGGC--C      2000
      |  | | | | | | | | | | | | | | | | | | | | | | |
1982 GGAATA-TGG---ATA--TA---TN--A-CA-T-----AAT-A      2031
      |  | | | | | | | | | | | | | | | | | | | | | | |
2001 GGGCGCAGTGGCTCATACCTACAATCCAGCACTTTGGGGGGTTGAAGCA      2050
      |  | | | | | | | | | | | | | | | | | | | | | | |
2032 --A--A-T-C---AATGCT--GTA-TT--A-A---G-----ACAA---G      2081
      |  | | | | | | | | | | | | | | | | | | | | | | |
2051 GGAGGAGTGCTTGAA-GCCAAG-AGTTCTAGACCAGCCTGGACAACATAG      2100
      |  | | | | | | | | | | | | | | | | | | | | | | |
2082 -A---CT--CTAT-----TCAC--TCTA-----T--T-CT--A---      2131
      |  | | | | | | | | | | | | | | | | | | | | | | |
2101 TAAGACTGTCTCTAAAAAATAAAAT-TAGGCAGGGTGGTACTGCACGCC      2150
      |  | | | | | | | | | | | | | | | | | | | | | | |
2132 TG--G-----G--ACT-AG-----TGA---AG-A--A--G-TT---CT--      2181
      |  | | | | | | | | | | | | | | | | | | | | | | |
2151 TGTAGTCCAGCTACTCAGGAGGCTGAGGCAGGAGGATCGCTTGAGCCCA      2200
      |  | | | | | | | | | | | | | | | | | | | | | | |
2182 ---TT-T-A---TA---T-A---AACAGAGTGTT-TT-CATTACCATC-T      2231
      |  | | | | | | | | | | | | | | | | | | | | | | |
2201 GAGTTGTGAAGGTACAGTGACCTAACATCGTGCCATTGCACT-CCAGCCT      2250

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2232 ---CT-CTGA---GTTT-T-TCTGTAGACA-----GTTCA-A-A-A- 2281
      | | || | | | || | || | | | |
2251 GGGCAACAGAACAAAGATCCTGTCTCAAAACAACCAAAAGCCCAGAGAGAA 2300
2282 ----TG-----T-TT-AT--G-----T-AT--TT--A- 2331
      || | | | | | | | | | | | |
2301 AGAGTGAGACCCCATCTTTAAAAGAAAAAAAAAAGGTCATGATTGCAA 2350
2332 --T-----TT-CA-TTAAAA--GTA-G-T---A-----T--- 2381
      | | | | | | | | | | | | | |
2351 GGTCAAGATTGCAATTAAACTGTAAGGTGGGGAAGGAGGAAATAAG 2400
2382 -----CT--T-----CTT-A-T-C---G---CA--TA--TAA-----A-- 2431
      | | | | | | | | | | | | | |
2401 AGAAGCACCTGAGGCTTGAGTTCTCAGGAGCACCTAGGTTGGGTCCCAGG 2450
2432 T-A-----CACTG-G-T-----CACTT-A-A--T-A-GA-A--ACTTTGC 2481
      | | | | | | | | | | | | | | |
2451 TGAAGGGGCACAGAGGTAATTGCACCTCAGAGCTGATGGGAGGA-TTA-C 2500
2482 TTT-T-AT..... 2531
      | | | |
2501 TATGTCA..... 2550

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**Alignment Of DNA 2/Region 1 (Normal
Strand), And The Human hsp 27 Gene**

(The top row shows the DNA 2/Region 1 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

1	GACT--AT---CT-----G-G-----T---A-A-G---T-A-	50
1	GAATTCATTGCTTTTCCTTAACGAGAGAAGGTTCCAGATGAGGGCTGAA	50
51	C--TCT-----A-----T-A-C-CT-----C-T--	100
51	CCCTCTTCGCCCCCGCCACGGCCCCCTGAACGCTGGGGGAGGAGTGCATGG	100
101	--A---C-----A---C---A---CC-TT--T-----T-AAA--	150
101	GGAGGGGCGGCCCTCAAACGGGTCATTGCCATTAAATAGAGACCTCAAACA	150
151	--G--TG--AA---T-----TG-A---C-----C-----	200
151	CCGCCTGCTAAAAATACCGACTGGAGGAGCATAAAAGCGCAGCCGAGCC	200
201	-----T-----A-----T-----T-----	250
201	CAGCGCCCCGCACTTTTCTGAGCAGACGTCCAGAGCAGAGTCAGCCAGCA	250
251	TG-C-----T---TT-T---TC-TA-GA-----A--T-----	300
251	TGACCGAGCGCCGCTCCCTTCTCGCTCCTGCGGGCCCCAGCTGGGAC	300
301	---TT-----T---TAA---A-A-----T-TT-----TT-G-	350
301	CCCTTCGCGACTGGTACCGCATAGCCGCTCTTCGACCAGGCCTTCGG	350
351	--T-----T-CC--A-----C-C-GT--T-A-----A--A-C-	400
351	GCTGCCCCGGCTGCCGAGGAGTGGTCGCACTGGTTAGGCGGCAGCAGCT	400
401	--CC---C-A-GT-----TG-----AT-----	450
401	GGCCAGGCTACGTGCGCCCCCTGCCCCCGCCGCCATCGAGAGCCCCGCA	450
451	-T--C-----T-C-G-----A-C-----TCA--A-C--	500
451	GTGGCCGCGCCCGCCTACAGCCGCGGCTCAGCCGCAACTCAGCAGCGG	500
501	--T---G-A-A-----A-ACT---A---T-----T--C--T--T-	550
501	GGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGCGTGTCCCTGGATG	550
551	-C-----TT-----T---CGG--AA-ACCT---T--CAT-	600
551	TCAACCACTTCGCCCCGACGAGCTGACGGTCAAGACCAAGGATGGCGTG	600
601	-TG-A---C-----T-A-----T--TC---A---A-AGTT-----C	650
601	GTGGAGATCACCGGTGAGCCCCCTGCTCCTGCAGGGAGAGGAGGAGGC	650

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651 TT-C-----T-----TT-AT-----TC----- 700
    | |               | | | |
651 TAGCAGGGCGGGCAGGGCCGGGGCGTGCGGTTGAAACGGGGTCCCGGG 700

701 ----T-----TTA---GT-G-CC-A-----TCCT- 750
    |       |||  || | || |
701 GGCCTGGGGAGTTAAACGTTGGCCAGCACCGGGAAAAACAGGACTCCTG 750

751 ---C--T-G-T-AT-A-TT---A---C---C-CTT--A-----TT-- 800
    | | | | | | | | | | | | | | | |
751 ATTCCCTTGCTCAGGAATTGGGAGTGGGGTCGCTTCTAAGGGCGCTTTC 800

801 -----A---CCC-----TT-----C--A-A---A---C--T--A 850
    | |||  || | | | | | | | | |
801 TGCTCTGTAATCCCAGCGCTTTGGGAGGCCGAGACGGGAGGATCGCTTGA 850

851 --CCT--A-TT---G---TAAC-T---A-CATA----- 900
    || | | | | | | | | |
851 GGCCAGGAGTTCAAGACTAGCCTGGGCAACATAGCGAGACGGCCCCCCCC 900

901 -----TT-----G-----TTTTTAT-- 950
    || | | | |
901 GCCCCGACCCCGCGCCATTACAAAAAAAAGCAAAACAAAATTTTTTTAA 950

951 -G-TC--C-AT-----C--T--C--C-----CC--T--- 1000
    | | | | | | | | | | | |
951 AGATCATCGATGAAGAGAGAAAATGCGCTTTTCTACAGAGTCCCTTCCC 1000

1001 A---A-----T---A-A-A--C---AGT-----GA-GC--T-CC- 1050
    | | | | | | | | | | | |
1001 ACCCACAGCCCATCCCCAGATAAGCGGGGAGTTCCTTGGCGCGGTGCCA 1050

1051 -TT-CAAGAAA--GANT-----A--T-C-C-T---T- 1100
    || | | | | | | | | | |
1051 GTTCTAGCCGCTGAGTGGGCGTGTGCGCGGCTCCAAGTGCCTGCGTA 1100

1101 C--C--AC-C-----TCC-C---T--T---TT--TCCGCA---TC-- 1150
    | | | | | | | | | | | |
1101 CTGCTCACTCCCCAGCTCCGCGCCCTGCTCCGTTCTCC--CAAACTCTG 1150

1151 ACT--AA-AACA---GG--GT-----AG-GT--AG---G-C-----ACTG 1200
    | | | | | | | | | | | |
1151 AATCGAAGAACTTTCCGGAAGTTTCTGAGAGCCAGACCGGCGGGCAC-G 1200

1201 -----ATT---AAG---TACA-TTA-T---T-C-AGT-T-CA-TCA--- 1250
    || | | | | | | | | | |
1201 CCCCCATCCCCAACCCCT-CTGTTAATCCCTACCAGCCTGCAGTCCTGG 1250

1251 CT-C---A-GTA-----T-----T--GG--A-C-----A-----T- 1300
    || | | | | | | | | | |
1251 CTGCTTCCAAGCAGGAGGTGGGGCCTCTGGCTAGCGGGGCCGAAAAAGTC 1300

1301 -----AT-----ATTC--T-T-----G--A-G-A--AT 1350
    || | | | | | | | | | |
1301 CCCTCCCCCGCATGTCTGATTTCCTCTTCCCCCAAGGCAAGCACGAG 1350

1351 TTA-CT--A--A--AG---G-C--C-T-T---G---CTT---GCG---T 1400
    | | | | | | | | | | | |
1351 G-AGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCACGCGGAAAT 1400

1401 G---GT---TC-TAG-GC-A--TCG---TGAAA---T---TGAG--TGG 1450
    || | | | | | | | | | |
1401 ACACGTGAGTCCTGGGCCAGGTGCGGGTGGGTGGGTGGCGTGGGGTGG 1450

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1451 --T--G--AACA---A-A---AC--A-----T-T-TAA---A-C-C--- 1500
      | | ||| | | | | | | | | | | | | | | |
1451 GGTCAAGGAAGAGGGACAGGGACCCACCCGGTGTGTAAATGTAACGCTTG 1500

1501 CCTTG--TC-CT-CA--T--GG--G-----AC----- 1550
      ||| | | | | | | | | | | | | | |
1501 CCTTTCCTCTCTGCACGTCCAGGCTGCCCGCCGGTGTGGACCCACCCAA 1550

1551 -TTTA-TG-T---T--CC--TG-GGG-ANA--GA-----A-----T 1600
      ||| | | | | | | | | | | | | | |
1551 GTTTCCTCCTCCCTGTCCCCTGAGGGCACACTGACCGTGGAGGCCCCCAT 1600

1601 -----TAG--A-----T--AAC-A--T-AC-AT---A-T-A----- 1650
      ||| | | | | | | | | | | | | | |
1601 GCCCAAGCTAGCCACGCAGTCCAACGAGATCACCATCCCAGTCACCTTCG 1650

1651 A-----A-----A-A--C---A---C--A-G---TG- 1700
      | | | | | | | | | | | | | | | |
1651 AGTCGCGGGCCAGCTTGGGGGCAGAAGCTGCAAATCCGATGAGACTGC 1700

1701 -----TA-----TT-----T-----T--TAC---A-TGG- 1750
      || | | | | | | | | | | | | | |
1701 CGCCAAGTAAAGCCTTAGCCCGGATGCCACCCCTGCTGCCGCCACTGGC 1750

1751 T---CCTNN---GA-A---G-----A-A-A---AT-T----- 1800
      | ||| | | | | | | | | | | | |
1751 TGTGCCTCCCCGCCACCTGTGTGTCTTTTGATACATTTATCTTCGTGT 1800

1801 -----AAGATANA-T--AAGG-AA-----GT---GGA--AG---TA 1850
      || ||| | | | | | | | | | | |
1801 TTTCTCAA-ATAAAGTCAAAGCAACCACCTGTCACTGGCCCAGGCCCTG 1850

1851 -T-TT-----A-GG-----T--GGAAN-TG---T---C---CT---GGA 1900
      | || | | | | | | | | | | | | |
1851 GTGTTTGTGGAAGGAAGCCTCAGGCACCTGCCATTTGCTGGCTTTCAGGA 1900

1901 ---A-----G---G-----T-CN--C--T-TN---A-A-TG-TN--GG 1950
      | | | | | | | | | | | | | | | |
1901 GTCATCTTTGCTCAGGCCCGTGCTGGGCCATGTGGGTACACTGGTGTAGG 1950

1951 N-G---AC-CAN--TGANT-A-AT-----GA-A---CTT-GA--- 2000
      | | | | | | | | | | | | | | | |
1951 TTGCTGGACACAGGCTGACTCACATCCATAAAGACAGAGGTCTTAGGGCC 2000

2001 -----ANTG--T--T---T---T---G---TNT-----T-GAAGAA 2050
      | || | | | | | | | | | | | | |
2001 GGGCCAGTGGCTCATACCTACAATCCCAGCACTTTGGGGGGTTGAAGCA 2050

2051 --ACNACTTTTGA---AAC---C---CCGG--TGGA-AN-A-A--A 2100
      | | | | | | | | | | | | | | | |
2051 GGAGGAGTGCTTGAAGCCAAGAGTTCTAGACCAGCCTGGACAACATAGTA 2100

2101 AT--T-TC-CNCN----T-----TT-CN-AGGAA---AC--CNC-CC--- 2150
      | | | | | | | | | | | | | | | |
2101 AGACTGTCTCTAAAAATAAAAATTAGGCAGGGTGGTACTGCACGCCTGT 2150

2151 ---CCCA---A---AGGNNNA---A---A--A-----A-CCCN--- 2200
      |||| | | | | | | | | | | | |
2151 AGTCCCAGCTACTCAGGAGGCTGAGGCAGGAGGATCGCTTGAGCCCAGAG 2200

2201 T-G-G--GTT---TGA---AAN-T--T-C--TT---T-----TN--- 2250
      | | | | | | | | | | | | | | | |
2201 TTGTGAAGGTACAGTGAGCTAACATCGTGCCATTGCACTCCAGCCTGGGC 2250

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```

2251 AA-A-AAN--GN-CCNCGTNNCT-----G---G-G-GANNN 2300
      || || || | || || | | | | | | | |
2251 AACAGAACAGATCCT-GTCTCAAAACAACCAAAAGCCCAGAGAGAAAGA 2300

2301 N--A-ANC---TC---A-----T--T--T--CNAGGT 2350
      | | | | | | | | | | | | | |
2301 GTGAGACCCCATCTTTAAAGAAAAAAGGTCATGATTGCAAGGT 2350

2351 CN--A-----AN--AAAA-----AAN-----AN--A--ANN----- 2400
      | | | | | | | | | | | | | |
2351 CACGATTGCAATTAAACTGTAAGGTGGGAAGGAGGAGGAATAAGAGA 2400

2401 --C---TNA---TN---T-CNNN-A-CNN-T-G-----CCNNGG--A 2450
      | | | | | | | | | | | | | |
2401 AGCACCTGAGGCTTGAGTTCTCAGGAGCACCTAGGTTGGGTCCCAGGTGA 2450

2451 AN---CN..... 2500
      | |
2451 AGGGGCACAGAGGTAATTGCACCTCAGAGCTGATGGGAGGATTACTATGT 2500

2501 ..... 2550

2501 CA..... 2550

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Alignment Of DNA 2/Region 1

(Complementary Strand), And

The Human hsp 27 Gene

(The top row shows the DNA 2/Region 1 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

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-8 .....NGNTT--CCNN---G-GC-ANNGTNN--GN-GAN-----A-      41
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
  1 GAATTCATTGCTTTTCCTTAACGAGAGAAGGTTCCAGATGAGGGCTGAA      50

42 ---TN-----A-GNT--TN-----TN-----T--T--      91
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
51 CCCTCTTCGCCCCGCCCACGGCCCTGAACGCTGGGGGAGGAGTGCATGG     100

92 -----T-----T--TTN---TTN---GA--CCTNGAAAT      141
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
101 GGAGGGGCGGCCCTCAAACGGGTCATTGCCATTAATAGAGACCTC-AAAC     150

142 GA--GN-T--TNNNNNT-CCC--C---AGNN--A-----CGN-G--GN-     191
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
151 -ACCGCTGCTAAAAATACCGACTGGAGGAGCATAAAGCGCAGCCGAG      200

192 CN-----TTTT-TNA--A-A-----AGA--ANTTTCAA--A-      241
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
201 CCCAGCGCCCCGCACTTTTCTGAGCAGACGTCCAGAGCAGAGTCAGCCAG     250

242 C---CC-AN-G--G-GT---TT-T--T--TNN---CC-----T---      291
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
251 CATGACCGAGCGCCGCTCCCTTCTCGCTCCTGCGGGGCCCCAGCTGGG     300

292 -----TTG-G-G---GG-----GN---G--G--T-TTC--C-----TNC     341
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
301 ACCCTTCCGCGACTGGTACCGCATAGCCGCTCTTCGACCAGGCCTTC      350

342 AAAN-GN---GGA-----A--ATT--T-----TN-TT---C--CA-C---     391
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
351 GGGCTGCCCGGGCTGCCGGAGGAGTGGTCGAGTGGTTAGGCGGCAGCAG     400

392 C-GG---GGTT---T-----T-CA-----A---A-A-----      441
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
401 CTGGCCAGGCTACGTGCGCCCCCTGCCCGCCGCGCCATCGAGAGCCCCG     450

442 -AGTNG-----T-----T-----TC-----      491
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
451 CAGTGGCCGCGCCCGCTACAGCCGCGGCTCAGCCGGCAACTCAGCAGC     500

492 ---T-TC--A-AN-----ACA-----A-----A-----      541
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
501 GGGGTCTCGGAGATCCGGCAGACTGCGGACCGCTGGCGCGTGTCCCTGGA     550

542 ---A-C-ANTTC-----A--AG-T---TCA-----T---      591
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
551 TGTCAACCACTTCGCCCCGACGAGCTGACGGTCAAGACCAAGGATGGCG     600

592 T-----AN-TCAN---TG-G-----T-CNCCN--A-----      641
   | | | | | | | | | | | | | | | | | | | | | | | | | | |
601 TGGTGGAGATCACCGGTGAGCCCCCTGCTCCTGCAGGGGAGAGGAGGAG     650

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642 -C-A-----T-----TN-AA--GNGA--CC-- 691
 | | | | | | | | | | | | | | | |
 651 GCTAGCAGGGCGGGCAGGGCCGGGGCGTGCGGTTGAAACGGGGGTCCCG 700
 692 -----T-----T-----C-----C--AG---G--A---CANT--TCC 741
 | | | | | | | | | | | | | | | |
 701 GGGGCCTGGGGAGTTAAACGTTGGCCCGCAGCACCGGAAAAACAGGACTCC 750
 742 --A--CC-T-----A--AAT---ACT-----TC-C---A---C--TT 791
 | | | | | | | | | | | | | | | |
 751 TGATTCCCTTGCTCAGGAATGGGAGTGCGGGTCGCTTCTAAGGGCGCTT 800
 792 -C--CT-T--A-TN-----T-----A-----TC--TT 841
 | | | | | | | | | | | | | | | |
 801 TCTGCTCTGTAATCCAGCGCTTTGGGAGGCCGAGACGGGAGGATCGCTT 850
 842 -A---AT---TT-----T--C-T-----T--CNN-A-G-G----- 891
 | | | | | | | | | | | | | | | |
 851 GAGGCCAGGAGTTCAGACTAGCCTGGGCAACATAGCGAGACGCGCCCCC 900
 892 -----ACC-----ATGTA-AAAATA---CA--C-----TGTGTT 941
 | | | | | | | | | | | | | | | |
 901 CCGCCCCGACCCCGCGCCAT-TACAAAAAAAGCAAAACAAAAT-T-TT 950
 942 TTTA-----T-AT-G-T-A-----TG---TTATCTA-AT--TCTN- 991
 | | | | | | | | | | | | | | | |
 951 TTTAAAGATCATCGATGAAGAGAGAAAATGCGCTTTTCTACAGAGTCCCC 1000
 992 T-CCC-C--AG-GA---A-C---A--TAA-----AGT-CCCATGA-G-G 1041
 | | | | | | | | | | | | | | | |
 1001 TTCCACCCACAGCCCCATCCCCAGATAAGCGGGGAGTTCCTCC-TGGCGCG 1050
 1042 -----A---C-A-----AG-GGG--T-T-----T--AA----- 1091
 | | | | | | | | | | | | | | | |
 1051 GTGCCAGTTTCTAGCCGCTGAGTGGGCGTGTCGCGGCTCCAAGTGGCGC 1100
 1092 -----A-TG-T--T-----T-----TG-T---T-C-----A--- 1141
 | | | | | | | | | | | | | | | |
 1101 TGGCTACTGCTCACTCCCGAGCTCCGCGCCCTGCTCCGTTCCCTCCCAAAA 1150
 1142 C-C--A--C-----T--C---AA-TTTC--A---C---GAT-G-C--- 1191
 | | | | | | | | | | | | | | | |
 1151 CTCTGAATCGAAGAACTTTCGGGAAGTTTCTGAGAGCCAGACCGGCGGG 1200
 1192 C-----T---A-----G--AA-CC--AC--GC-----A--- 1241
 | | | | | | | | | | | | | | | |
 1201 CACGCCCCCATCCCCAACCCCTCTGTTAATCCCTACCAGCCTGCAGTCC 1250
 1242 -----A-GCA--AGG---CCT-T---TAGT-----AAA-- 1291
 | | | | | | | | | | | | | | | |
 1251 TGGCTGCTTCCAAGCAGGAGGTGGGCGCTCTGGCTAGCGGGGCCGAAAAA 1300
 1292 -T---TC-----T--C--A-----A--G--AA--AT 1341
 | | | | | | | | | | | | | | | |
 1301 GTCCCTCCCGCATGTCTGATTTCCCTCTTCCCCCAAAGGCAAGCAC 1350
 1342 -A-----T---A--T-----G-T-C--CA-----A- 1391
 | | | | | | | | | | | | | | | |
 1351 GAGGAGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCACGCGGAA 1400
 1392 -TAC---TGAGT---G-----A--T-G----- 1441
 | | | | | | | | | | | | | | | |
 1401 ATACACGTGAGTCCTGGCGCCAGGTCGGGGTGGGTGGGTGGCGTGGGGGT 1450

1442 -----A---A-----CT--G--A---A-----T-----AATGTA-C--T 1491
 | | | | | | | | | |
 1451 GGGGTCAGGGAAGAGGGGCACAGGGACCCACCCGGTGTGTAATGTAACGCT 1500
 1492 TA-----A--TC-AG--TGCC-----T-----ACCT-ACCC 1541
 | | | | | | | | |
 1501 TGCCTTTCCTCTCTGCACGTCCAGGCTGCCCCCGGTGTGGACCCACCC 1550
 1542 T-GTTT--TA-----GT-----GA-----TG-C-G-GAA----- 1591
 | | | | | | | | |
 1551 AAGTTTCCTCCTCCCTGTCCCTGAGGGCACACTGACCGTGGAGGCCCCC 1600
 1592 A-----AAGG--G--A-G--GTGGAA-G-GAT--AN--TC---T---TT 1641
 | | | | | | | | | |
 1601 ATGCCCAAGCTAGCCACGCAGTCCAACGAGATCACCATCCCAGTCACCTT 1650
 1642 C---T-----T-G-----A-A-G--G-A-----G-----CT 1691
 | | | | | | | | |
 1651 CGAGTCGCGGGCCAGCTTGGGGGCAGAGCTGCAAAATCCGATGAGACT 1700
 1692 -CA-C---T---G--TT-----T---A---T--T-----A--G 1741
 | | | | | | | | | |
 1701 GCCGCCAAGTAAAGCCTTAGCCCGGATGCCACCCCTGCTGCCGCCACTG 1750
 1742 G--G-GA-----G--A--TG-G-----A--CAT--A----- 1791
 | | | | | | | | | |
 1751 GCTGTGCCTCCCCGCCACCTGTGTGTCTTTTGATACATTATCTTCTG 1800
 1792 -----AAA-A-----CAAT--A-----TGT-A--G-----T 1841
 | | | | | | | | | |
 1801 TTTTCTCAAATAAAGTTCAAAGCAACCACCTGTCACTGGCCCAGGCCCT 1850
 1842 --T-----A-----C---A--A--T---A---G--G--T---AG- 1891
 | | | | | | | | | |
 1851 GGTGTTTGTGGAAGGAAGCCTCAGGCACCTGCCATTTGCTGGCTTTTCAAG 1900
 1892 --T--T-T--G---A-----A-G-GG-TA-A-T-----A- 1941
 | | | | | | | | | |
 1901 AGTCATCTTTGCTCAGGCCCGTGTGGCCATGTGGGTACACTGGTGTAG 1950
 1942 -----A---GG--G--T-A-AT--ATA---CAGAGG---A--- 1991
 | | | | | | | | | |
 1951 GTTGCTGGACACAGGCTGACTCACATCCATAAAGACAGAGGTCTTAGGGC 2000
 1992 -----TGGC--A--C-TA-AAG---A--A-T-----AA-- 2041
 | | | | | | | | | |
 2001 CGGGCGCAGTGGCTCATACCTACAATCCAGCACTTTGGGGGGTTGAAGC 2050
 2042 AG-A--AG-----AA-C-----TT-T-GAATAG--T---CAA--T-G- 2091
 | | | | | | | | | |
 2051 AGGAGGAGTGCTTGAAGCCAAGAGTTCTAGACCAGCCTGGACAACATAGT 2100
 2092 AAGGTT-TC-CGAAAGAGAGAA---T-AGT-----T--T--T-CA-G--T- 2141
 | | | | | | | | | |
 2101 AAGACTGTCTCTAAAAAATAAAAAATTAGGCAGGGTGGTACTGCACGCCTG 2150
 2142 T-G---AG-T-C---G-AG---A---A-----TCA---A-C----- 2191
 | | | | | | | | | |
 2151 TAGTCCCAGCTACTCAGGAGGCTGAGGCAGGAGGATCGCTTGAGCCCAGA 2200
 2192 -T-G-G--GGT---T---TAAC---G-G---T-G-----G----- 2241
 | | | | | | | | | |
 2201 GTTGTGAAGGTACAGTGAGCTAACATCGTGCCATGCACTCCAGCCTGGG 2250

2242	-AACA-AA-AA--T--T-T-T-AAAA-A-----	2291
2251	CAACAGAACAAAGATCCTGTCTCAAAACCAAAAGCCCAGAGAGAAAGA	2300
2292	-T-----TCT--A---GAAAAA-----G--CAA-AT---A-GGT	2341
2301	GTGAGACCCCATCTTTAAAAAGAAAAAAAAAAGGTCATGATTGCAAGGT	2350
2342	CA--ATT-CA-----CT-T---T---AA--A--AGG---T--G---	2391
2351	CACGATTGCAATTAAACTGTAAGGTGGGAAGGAGGAGGAAATAAGAGA	2400
2392	-----TG---T--AG-----AGGT--A--TAG-----AG-T-A	2441
2401	AGCACCTGAGGCTTGAGTTCTCAGGAGCACCTAGGTTGGGTCCCAGGTGA	2450
2442	-----C-----T--T---ACC--AGA--T-A-G-----T--C.....	2491
2451	AGGGGCACAGAGGTAATTGCACCTCAGAGCTGATGGGAGGATTACTATGT	2500
2492	2541
2501	CA.....	2550

**Alignment Of DNA 2/Region 2 (Normal
Strand), And The Human hsp 27 Gene**

(The top row shows the DNA 2/Region 2 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

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-4 .....C-T--GC-----A--G-G-----T-C-A-ACG-GGA-T---      45
   | | | | |
1 GAATTTCATTGCTTTTCCTTAACGAGAGAAGGTTCCAGATGAGGGCTGAA      50

46 C--T-TT-G---GC--A-----TGA---T-----ATT--T--AT--      95
   | | | | |
51 CCCTCTTCGCCCCGCCACGGCCCCCTGAACGCTGGGGGAGGAGTGCAATGG      100

96 --A---CA-C--T-----G--TC-T--C-ATTT-T-----CTTC-----      145
   | | | | |
101 GGAGGGGCGGCCCTCAAACGGGTCAATGCCATTAATAGAGACCTCAAACA      150

146 --GTTTG-TATA--T-----TGG-GGA---T-----GTTTAT---A---      195
   | | | | |
151 CCGCCTGCTAAAAATACCCGACTGGAGGAGCATAAAAGCGCAGCCGAGCC      200

196 -ATT-----A-TTTT--G--CAG--G---A-----T-----      245
   | | | | |
201 CAGCGCCCCGCACTTTTCTGAGCAGACGTCCAGAGCAGAGTCAGCCAGCA      250

246 T-A-----T-----TT-T-GA-----A--TG-----      295
   | | | | |
251 TGACCGAGCGCCGCGTCCCTTCTCGCTCCTGCGGGGCCCCAGCTGGGAC      300

296 ---T---G--A--G--A-----A-A-----T-----A--A---TATCT      345
   | | | | |
301 CCCTTCCGCGACTGGTACCCGCATAGCCGCTCTTCGACCAGGCCT-TCG      350

346 ---T-----T-----A--ATT--T-G-A-T--TT-----      395
   | | | | |
351 GGCCTGCCCGGCTGCGGAGGAGTGGTCGCAGTGGTTAGGCGGCAGCAGC      400

396 T--C-----T---T-----T-----T-G-----      445
   | | | | |
401 TGGCCAGGCTACGTGCGCCCCCTGCCCCCGCCGCAATCGAGAGCCCCGC      450

446 --T-----TA--GC-----T-A-----AT-TAA--A---      495
   | | | | |
451 AGTGGCCGCGCCCGCCTACAGCCGCGCGCTCAGCCGGCAACTCAGCAGCG      500

496 ---T---GG---T-----A-ACTA---AC---T-----T-T---T--AT      545
   | | | | |
501 GGGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGCGTGTCCCTGGAT      550

546 -TCA-----T-----A-----T-A-----A-----T---T      595
   | | | | |
551 GTCAACCACTTCGCCCCGACGAGCTGACGGTCAAGACCAAGGATGGCGT      600

596 ----AG-T-A---TG-G-----T-CTCAT-CA-----      645
   | | | | |
601 GGTGGAGATCACCGGTGAGCCCCCTGCTCCTGCAGGGGAGAGGAGGAGG      650

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646 C-A-CA-----AGG-CC-----C-T---TTGCAAC----- 695
    | | | | | | | | | | | | | | | | | | | | | |
651 CTAGCAGGGCGGGCAGGGCGGGGGCGTGC GGTTGAAACGGGGTCCCGG 700

696 -----A-TT---G---C-----TCCT 745
    | | | | | | | | | | | | | | | | | | | | | |
701 GGGCCTGGGGAGTTAAACGTTGGCCAGCACC GGAAAAACAGGACTCCT 750

746 -A--C--TT-CT-A---T-G---T-C---T-----AAG---TT- 795
    | | | | | | | | | | | | | | | | | | | | | |
751 GATTCCCTTGCTCAGGAATTGGGAGTGC GGTCGCTTCTAAGGGCGCTTT 800

796 CTGCT-T-----C--A-C---TTT-----CCTA-----T-GCT-- 845
    | | | | | | | | | | | | | | | | | | | | | |
801 CTGCTCTGTAATCCCAGCGCTTTGGGAGGCCGAGACGGGAGGATCGCTTG 850

846 ---CCA---TCCAT---T---T---CAA---T--C-A--C-C-CCA--- 895
    | | | | | | | | | | | | | | | | | | | | | |
851 AGGCCAGGAGTTCAGACTAGCCTGGGCAACATAGCGAGACGCGCCCCC 900

896 -G-----AC---GT-C--T--CT-----GTATA-A---TTTG--- 945
    | | | | | | | | | | | | | | | | | | | | | |
901 CGCCCCGACCCCGCGCCATTACAAAAAAGCAAACAAAATTTTTTTA 950

946 --G--C--C-ATG-----T-C-CT---A-----TC----- 995
    | | | | | | | | | | | | | | | | | | | | | |
951 AAGATCATCGATGAAGAGAGAAATGCGCTTTTCTACAGAGTCCCTTCC 1000

996 -AC--A-A-C---TC-----TAA-----T-C--T-----TG-- 1045
    | | | | | | | | | | | | | | | | | | | | | |
1001 CACCCACAGCCCCATCCCCAGATAAGCGGGGAGTTCCTTGGCGCGGTGCC 1050

1046 --TTT---G---T-A-T-----T---C-CTGG---AA-TGC-CCTGAA 1095
    | | | | | | | | | | | | | | | | | | | | | |
1051 AGTTTCTAGCCGCTGAGTGGGCGTGTGCGC-GCCTCCAAGTGCGCCTGCG 1100

1096 -A-----A-----A-CTCC-----TACTCAG-----A---CT-T 1145
    | | | | | | | | | | | | | | | | | | | | | |
1101 TACTGCTCACTCCCCAGCTCCGCGCCCTGCTCCGTTCCTCCCAAACTCT 1150

1146 AAA---AAG--C---C---A-G---CT-----C--A-A-----A-- 1195
    | | | | | | | | | | | | | | | | | | | | | |
1151 GAATCGAAGAACTTTCCGGAAGTTTCTGAGAGCCCAGACCGGCGGCACG 1200

1196 -----T---A-----T-TGTGT--TCT-T-C--G--TG-ATTC-T-- 1245
    | | | | | | | | | | | | | | | | | | | | | |
1201 CCCCCATCCCCAACCCTCTGT-TAATCCCTACCAGCCTGCAGTCTCTGG 1250

1246 -T--TTCCTT-CT-----T---CAT-TG---AG-GTAG---A---GTC 1295
    | | | | | | | | | | | | | | | | | | | | | |
1251 CTGCTTCCAAGCAGGAGGTGGGGCTCTGGCTAGCGGGGCCGAAAAAGTC 1300

1296 ---TAAC---A---CT--T--C--T-T-CACAC-A---C---CACTA- 1345
    | | | | | | | | | | | | | | | | | | | | | |
1301 CCCTCCCCCGCATGTCTGATTTCCTCTTCCCCCAAGGCAAGCAGAG 1350

1346 -A-C---A--A--A-C-T---A-----GGT--TT---G---AAAT- 1395
    | | | | | | | | | | | | | | | | | | | | | |
1351 GAGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCACGCGGAAATA 1400

1396 ---GTTA--C-T-----T-----T---TG--TA-CAT-----T--- 1445
    | | | | | | | | | | | | | | | | | | | | | |
1401 CACGTGAGTCCTGGCGCCAGGTCCGGGTGGGTGGGTGGCGTGGGGTGGG 1450

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1446 -T-A---A-GA----AN-----CT-----T-T-T--T-TA-CA-TT-- 1495
 | | | | | | | | | | | | | |
 1451 GTCAGGGAAGAGGGCACAGGGACCCACCCGGTGTGTAATGTAACGCTTGC 1500
 1496 -TTTCAT-T-T-----T--A-----A---A---AA- 1545
 | | | | | | | | | | | |
 1501 CTTTCCTCTCTGCACGTCCAGGCTGCCCCCGGTGTGGACCCACCCAAG 1550
 1546 ---CAT--T---T-TAN--T-AG--CN-A---A-----A-----AT- 1595
 | | | | | | | | | | | | | | | | | |
 1551 TTTCCTCTCTCCCTGTCCCTGAGGGCACACTGACCGTGGAGGCCCCCAAG 1600
 1596 ---ATTNTA---AC-C-G---AAN-AGG--A-----GT-AC-----A 1645
 | | | | | | | | | | | | | | | | |
 1601 CCCAAGCTAGCCACGCAGTCCAACGAGATCACCATCCAGTCACCTTCGA 1650
 1646 -TN-C-----T-----A-A--C-G-AA--T--G-T-A-ATT-- 1695
 | | | | | | | | | | | | | | |
 1651 GTCGCGGGCCAGCTTGGGGGCAGAAGCTGCAAAATCCGATGAGACTGCC 1700
 1696 -----TAAA---T-A-----A-G---A---T--T-----A-T---T 1745
 | | | | | | | | | | | | | | | | | |
 1701 GCCAAGTAAAGCCTTAGCCCGGATGCCACCCCTGCTGCCGCCACTGGCT 1750
 1746 GTA--TAAAC----A--TAGA-TGTT-TTA--ATA-A-----CTG--GTA 1795
 | | | | | | | | | | | | | | | | | |
 1751 GTGCCTCCCCGCCACCT-GTGTGTCTTTTGATACATTTATCTTCTGTT 1900
 1796 AT-CTGAAA-AAAG---AAATANA--AC-----AC-----AG-CC-TG 1845
 | | | | | | | | | | | | | | | | | |
 1801 TTTCTC-AAATAAAGTTCAAAGCAACCACCTGTCACTGGCCCAGGCCCTG 1850
 1846 GT-----A-----TCA--C---TGA-A---GCNC-CTCT-A--A 1895
 | | | | | | | | | | | | | | | | | |
 1851 GTGTTTGTGGAAGGAAGCCTCAGGCACCTGCCATTTGCTGGCTTTTCAAG 1900
 1896 -T-----G-TC---C---T--T---CCATG-----A-A-T-----A-- 1945
 | | | | | | | | | | | | | | | | |
 1901 GTCATCTTTGCTCAGGCCGTGCTGGGCCATGTGGGTACACTGGTGTAGG 1950
 1946 -----AC-CA--C---CT-A-ATCC-----C-GNTGCCT-----CC 1995
 | | | | | | | | | | | | | | | | | |
 1951 TTGCTGGACACAGGCTGACTCACATCCATAAAGACAGAGTCTTAGGGCC 2000
 1996 TTGA--ANT---T-A-AN--A-AAT---A--A-TTT-----T-GAN--- 2045
 | | | | | | | | | | | | | | | | | |
 2001 GGGCCGAGTGGCTCATACCTACAATCCCAGCACTTTGGGGGGTTGAAGCA 2050
 2046 -----T--TTG--GT---GA-----ANAN-----T-----T--T- 2095
 | | | | | | | | | | | | | | | | | |
 2051 GGAGGAGTGCTTGAAGCCAAGAGTTCTAGACCAGCCTGGACAACATAGTA 2100
 2096 ----T-T-T-T-----TANAAATTCG-C-----CN--ANN--T-T 2145
 | | | | | | | | | | | | | | | | | |
 2101 AGACTGTCTCTAAAAAATAAAAAATTAGGCAGGTGGTACTGCACGCCTGT 2150
 2146 --T---A---A-TC-----C-GA---A-----TC--TT----- 2195
 | | | | | | | | | | | | | | | | | |
 2151 AGTCCCAGCTACTCAGGAGGCTGAGGCAGGAGGATCGCTTGAGCCAGAG 2200
 2196 T-G-GAA---ACA-T-ACG-T---T--T---TT---TC-----T--- 2245
 | | | | | | | | | | | | | | | | | |
 2201 TTGTGAAGGTACAGTGA-GCTAACATCGTGCCATTGCACTCCAGCCTGGG 2250

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2246 -A-----T--T-T-TNAAA-CTGCCCAT-GN--A-ATG-GA--G      2295
      |           | | | | | | | | | | | | | | | |
2251 CAACAGAACAAGATCCTGTCTCAAAACAACCAAAAGCCCAGA-GAGAAAG      2300
      |           | | | | | | | | | | | | | | | |
2296 -GTTNG-C-----T-----GAANNAAAT-----T--T--TTGAAAAAN      2345
      || | | | | | | | | | | | | | | | |
2301 AGTGAGACCCCATCTTTAAAAGAAAAAAAAAAAAAGGTCATGATTGCAAGG      2350
      |           | | | | | | | | | | | | | | | |
2346 T-----TT-CT-TTNA--CTG---G-TCTGNAA--A--A--ANTTTT-A-      2395
      |           | | | | | | | | | | | | | | | |
2351 TCACGATTGCAATTAAACTGTAAAGGTGGGGAAGGAGGAGGAATAAGAG      2400
      |           | | | | | | | | | | | | | | | |
2396 A--CA--T-----TN---TTCNCCG-ACCNCCANNTNC--TCC----TN      2445
      | | | | | | | | | | | | | | | |
2401 AAGCACCTGAGGCTTGAGTTCTCAGGAGCACCTAGGTTGGGTCCCAGGTG      2450
      |           | | | | | | | | | | | | | | | |
2446 A-GC--CAAAAAA-AAN-GNA----A-A--TNNTTTTN---TNNCNT-      2495
      | | | | | | | | | | | | | | | |
2451 AAGGGGCACAGAGGTAATTGCACCTCAGAGCTGATGGGAGGATTACTATG      2500
      |           | | | | | | | | | | | | | | | |
2496 T.....      2545
      |
2501 TCA.....      2550

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Alignment Of DNA 2/Region 2

(Complementary Strand), And

The Human hsp 27 Gene

(The top row shows the DNA 2/Region 2 nucleotide sequence).

-1	..A---ANN-GNNAN-----AA--A-ANNAT-TTNCNTTTTT-----T---	48
1	GAATTCATTTCGCTTTTCCTTAACGAGAGAAGGTTCCAGATGAGGGCTGAA	50
49	---T-TT-G---GCTNA-GGA-----GNANN-TGGNNG-G---T-C--GG	98
51	CCCTCTTCGCCCCCGCCACGGCCCTGAACGCTGGGGGAGGAGTGCATGG	100
99	NGA-----ANNA-----AT-G---TTAA-A-ANT--T-----	148
101	GGAGGGGGCGCCCTCAAACGGGTCATTGCCATTAATAGAGACCTCAAACA	150
149	-----T--T-----TNCA-GACC--AG-----TNAAAG--A----A---	198
151	CCGCCTGCTAAAAATACCCGACTGGAGGAGCATAAAGCGCAGCCGAGCC	200
199	-AN-----TTTTC--A--A-A-----A-ATTTNNTTCAGCNA--A	248
201	CAGCGCCCCGCACTTTTCTGAGCAGACGTCAGAGCAGAGTCAGCCAGCA	250
249	---CC-----TCCA-TTN-CA-T---G-GG--C---AG-T-----	298
251	TGACCGAGCGCCGCTCCCTTCTCGCTCCTGCGGGGCCCCAGCTGGGAC	300
299	---TTNAAA-A-TAGAA-----A-A-----A--A--C---G-	348
301	CCCTTCGCGACTGGTACCCGCATAGCCGCTCTTCGACCAGGCCTTCGG	350
349	--T-----A---TG-T-----T-T---C--CA--A---	398
351	GCTGCCCCGGCTGCCGAGGAGTGGTCGCAGTGGTTAGGCGGCAGCAGCT	400
399	---AG---A--T-----T-C-----G--G--ATTAA-ANN-----	448
401	GGCCAGGCTACGTGCGCCCCCTGCCCGCCGCGCCATCGAGAGCCCCGCA	450
449	-TNG--GCG-----A-A-----T-----T-----	498
451	GTGGCCGCGCCCGCCTACAGCCGCGGCTCAGCCGGCAACTCAGCAGCGG	500
499	--TNT---A-A-----A-A-----A-----A--	548
501	GGTCTCGGAGATCCGGCACACTGCGGACCGCTGGCGCGTGTCCCTGGATG	550
549	--AANTN-TTCACC-----A--A---AN--TCAA-A--ATT-ATT---TN	598
551	TCAACCACTTCGCCCCGACGAGCTGACGGTCAAGACCAAGGATGGCGTG	600
599	-T-----T-A-----AN-----T--TC---A---AG-G-AGG---C	648
601	GTGGAGATCACCGGTGAGCCCCCTGCTCCTGCAGGGGAGAGGAGGAGGC	650

649 -ANC-GGG-----A-----T---T--A---GGTGGT----- 698
 | | | | | | | | | | | | | | | | | | | | | |
 651 TAGCAGGGCGGGCAGGGCCGGGGCGTGGCGTTGAAACGGGGGTCCCGGG 700
 699 ---T---A-TTCA---T-GG---A--A--GG-ACATTAGAGGN----- 743
 | | | | | | | | | | | | | | | | | | | | | |
 701 GGCCTGGGGAGTTAAACGTTGGCCCAGCACCGGGAAAA-ACAGGACTCCT 750
 749 G---C--TT-C--AGTGA-TACC-AG-GCTG-T-G-TTNTA-----TT 793
 | | | | | | | | | | | | | | | | | | | | | |
 751 GATTCCCTTGCTCAG-GAATGGGAGTGGGGTTCGCTTCTAAGGGCGCTT 800
 799 TCT--T-T-T--T-----T-----CC-AGA-----T---T- 843
 | | | | | | | | | | | | | | | | | | | | | |
 801 TCTGCTCTGTAATCCCAGCGCTTTGGGAGGCCGAGACGGGAGGATCGCTT 850
 849 -A--CCAG---TT-A---T---T---AA-A-A-C-AT-CT----- 898
 | | | | | | | | | | | | | | | | | | | | | |
 851 GAGGCCAGGAGTTCAAGACTAGCCTGGGCAACATAGCGAGACGCGCCCC 900
 899 -----A-----T-----G-----TTTAT-- 943
 | | | | | | | | | | | | | | | | | | | | | |
 901 CCGCCCCGACCCCGCGCCATTACAAAAAAAAGCAAACAAAAATTTT 950
 949 A-----CA---AT-AA-----T-C--TT---A---T---TT- 998
 | | | | | | | | | | | | | | | | | | | | | |
 951 AAAGATCATCGATGAAGAGAGAAATCGCGCTTTTCTACAGAGTCCCCTTC 1000
 999 --A---A-A-----T-----TA--C---A-TTC--G-----T-- 1048
 | | | | | | | | | | | | | | | | | | | | | |
 1001 CCACCCACAGCCCCATCCCCAGATAAAGCGGGGAGTTCCTGGCGCGGTGC 1050
 1049 ---T---AGN-----A-TG---T-----A---C---T-C- 1098
 | | | | | | | | | | | | | | | | | | | | | |
 1051 CAGTTTCTAGCCGCTGAGTGGGCGTGTGGCGGGCTCCAAGTCCGCTGCG 1100
 1099 --CTN-T---TC---G---G---T---T-----ANAA-T-- 1143
 | | | | | | | | | | | | | | | | | | | | | |
 1101 TACTGCTCACTCCCAGCTCCGCGCCTGCTCCGTCCTCCCAAACTCT 1150
 1149 -A-T-----TTTN-G-----CT-ANTA---A-A-----AT 1198
 | | | | | | | | | | | | | | | | | | | | | |
 1151 GAATCGAAGAACTTCCGGAAGTTTCTGAG-AGCCAGACCGCGGGGCAC 1200
 1199 G-----T-----T-T-TTAA-----A--A---TG-A----- 1248
 | | | | | | | | | | | | | | | | | | | | | |
 1201 GCCCCCATCCCCAACCCTCTGTTAATCCCTACCAGCCTGCAGTCCTGG 1250
 1249 -----AA--A--A--TG-----T-----A-----AAAAAGN- 1296
 | | | | | | | | | | | | | | | | | | | | | |
 1251 CTGCTTCCAAGCAGGAGGTGGGGCCTCTGGCTAGCGGGGCCGAAAAAGTC 1300
 1299 ---T-----T--CT--T-----AAATGTA--CA--A- 1343
 | | | | | | | | | | | | | | | | | | | | | |
 1301 CCCTCCCCCGCATGTCTGATTCCTCTTCCCCCAAAGGCAAGCACGAG 1350
 1349 -A-----AGTA--A-CAT---T---TC-----A-----AA--- 1398
 | | | | | | | | | | | | | | | | | | | | | |
 1351 GAGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCACGCGGAAATA 1400
 1399 C-C-T-AGT--T-----T-G---T---TAG-TGG--TG---TG-- 1443
 | | | | | | | | | | | | | | | | | | | | | |
 1401 CACGTGAGTCCTGGCGCCAGGTGCGGGTGGGTGGGTGGCGTGGGGTGGG 1450


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1449 -T--G--AAGA---A--G-----TGT-TA--G-A-C--T--C      1498
      | | | | | | | | | | | | | | | | | | | | | |
1451 GTCAGGGAAGAGGGCACAGGGACCCACCCGGTGTGTAATGTAACGCTTGC      1500

1499 -TA-CCTC-----A-----A--TG-----A-----AG--AAG      1548
      | | | | | | | | | | | | | | | | | | | | | |
1501 CTTTCCTCTCTGCACGTCCAGGCTGCCCCCGGTGTGGACCCACCCCAAG      1550

1549 -----G-----A--A--A--A--G--A-----AT--      1598
      | | | | | | | | | | | | | | | | | | | | | |
1551 TTTCCTCCTCCCTGTCCCTGAGGGCACACTGACCGTGGAGGCCCCCATG      1600

1599 C--A--C--G--A---AG---AAC-A---CA--AT---A-T---TT-GA      1648
      | | | | | | | | | | | | | | | | | | | | | |
1601 CCAAGCTAGCCACGAGTCCAACGAGATCACCATCCAGTCACCTTCGA      1650

1649 G-CT-GG-CTTT--TT-----A-A-G-T-C---T--GA-GT-A--G--      1698
      | | | | | | | | | | | | | | | | | | | | | |
1651 GTCGCGGGCCAGCTTGGGGGCAGAGCTGCAAAATCCGATGAGACTGCC      1700

1699 G--A-GTT-----TT-----T-C--A-----G--G--GC-A-T---T      1748
      | | | | | | | | | | | | | | | | | | | | | |
1701 GCCAAGTAAAGCCTTAGCCCGATGCCACCCCTGCTGCCGCCACTGGCT      1750

1749 ---CC-----A--G-G-----A-A--T--A-C-----      1798
      | | | | | | | | | | | | | | | | | | | | | |
1751 GTGCCTCCCCCGCCACCTGTGTGTTCTTTGATACATTTATCTTCTGTTT      1800

1799 ----AAA-----CAA-G-A-----T-T-A--G---AG---T--T      1848
      | | | | | | | | | | | | | | | | | | | | | |
1801 TTCTCAAAATAAAGTTCAAAGCAACCACCTGTCACTGGCCCAGGCCCTGGT      1850

1849 GT--GAT--A-GGA--CAT--GGC-CAA---ATTA--TA-C---AG-AG      1898
      | | | | | | | | | | | | | | | | | | | | | |
1851 GTTTG-TGGAAGGAAGCCTCAGGCACCTGCCATTGCTGGCTTTCAGGAG      1900

1899 --A-CGT--CT--GG--G-G-TG---AT-TGA--A-A-TGG--A--T      1948
      | | | | | | | | | | | | | | | | | | | | | |
1901 TCATCTTTGCTCAGGCCCGTGTGGGCCATGTGGGTACACTGGTGTAGGT      1950

1949 -G--GAG-CATAGG---A---A-AG---TGAAG-CAGAA--CTTAGA-C-      1998
      | | | | | | | | | | | | | | | | | | | | | |
1951 TGCTG-GACACAGGCTGACTCACATCCATAAAGACAGAGGTCTTAGGGCC      2000

1999 -----A-TAG---A-AG-TAGGAGC--A--A-T--G-----TTGCA--A      2048
      | | | | | | | | | | | | | | | | | | | | | |
2001 GGGCGCAGTGGCTCATACCTACAATCCCAGCACTTTGGGGGGTTGAAGCA      2050

2049 --AGG-GC-CTTGT-GT---GA-TG--AGACCA---T--ACTA-AT--TA      2098
      | | | | | | | | | | | | | | | | | | | | | |
2051 GGAGGAGTGCTTGAAGCCAAGAGTTCTAGACCAGCCTGGACAACATAGTA      2100

2099 T-A-TG-----AA---TAAAGTTAG-----T--TAC--CAT---T-T      2148
      | | | | | | | | | | | | | | | | | | | | | |
2101 AGACTGTCTCTAAAAAATAAAATTAGGCAGGGTGGTACTGCACGCCTGT      2150

2149 AATT--AGCTAA-CAAGAG---A---A--A--ATC-----A---A-A-      2198
      | | | | | | | | | | | | | | | | | | | | | |
2151 AGTCCCAGCTACTCAGGAGGCTGAGGCAGGAGGATCGCTTGAGCCCAAGAG      2200

2199 TT---AA---A-G--A--TA---T--T---ATT---TC-----T---C      2248
      | | | | | | | | | | | | | | | | | | | | | |
2201 TTGTGAAGGTACAGTGAGCTAACATCGTGCCATTGCACTCCAGCCTGGGC      2250

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2249 A-CA-----T--T--C--AAAATAATCCT---GC--A-A-A-A----- 2293
      | | |           | | | | | | | | | | | | | | | |
2251 AACAGAACCAAGATCCTGTCTCAAAACAA-CCAAAAGCCCAGAGAGAAAGA 2300
      | | |           | | | | | | | | | | | | | | | |
2299 -T-A-A-----T-TATAAA-----CAT-----C----- 2343
      | | |           | | | | | | | | | | | | | | | |
2301 GTGAGACCCCATCTTTAAAAGAAAAAAAAAAGGTCATGATTGCAAGGT 2350
      | | |           | | | | | | | | | | | | | | | |
2349 C-C-----CAATATACAAAC-G-AAG-----AA--A--A-----T--GA 2393
      | |           | | | | | | | | | | | | | | | |
2351 CACGATTGCAAT-TA-AAACTGTAAGGTGGGGAAGGAGGAGGAAATAAGA 2400
      | | |           | | | | | | | | | | | | | | | |
2399 GA--CAG-TG----T--A-T----A--A--A--TA--T-----C--A--T 2443
      | | | | | | | | | | | | | | | | | | | | | |
2401 GAAGCACCTGAGGCTTGAGTTCTCAGGAGCACCTAGGTTGGGTCCCAGGT 2450
      | | | | | | | | | | | | | | | | | | | | | |
2443 GC-----CA-A-AG--A-T--C-CC---G---T--TG--AC-----CT-- 2493
      | | | | | | | | | | | | | | | | | | | | | |
2451 GAAGGGGCACAGAGGTAATTGCACCTCAGAGCTGATGGGAGGATTACTAT 2500
      | | | | | | | | | | | | | | | | | | | | | |
2499 G-CAG..... 2543
      | | |
2501 GTCA..... 2550

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APPENDIX V

Alignment Of DNA 4 (Normal Strand),

And The Most Similar-sized,

Homologous Area Of

Human hsp 27

(The top row shows the DNA 4 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

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-3   ....TC-G-AC---CTTG---A-ATA-C--GGAGCTTCAAGCCAGGC--T      46
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1349 AGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCACGC--GGAAAT      1398

      47   -C-C---AG-C-TGGCGTTTtaggt-G---TGG-TGCAAGCAAGGC-TGG      96
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1399 ACACGTGAGTCCTGGCGCC--AGGTCGGGGTGGGTGG--GT--GGCGTGG      1448

      97   GTATGTATTTCCAGG-AAGCAGT--AGANG-AGCTCGCC-G-TGCAATA-      146
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1449 GGGTGGGGT-C-AGGGAAG-AGGGCACAGGGACC-CACCCGGTGTG-TAA      1498

      147  TG-AA---TTA--T--CAT-TA-GCA-G-CAAGGAAATTCGTCAACGATT      196
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1499 TGTAACGCTTGCCTTTCTCTCTGCACGTCCAGGC--TGCCCCC-CGGT-      1548

      197  TGTGTTGTA---ATG-AA-TTTCCTGTGAGATATCCAGTTGCTTG-GGAG      246
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1549 -GT-GG-ACCCACCCCAAGTTTCTCTC-C---TC-CCTGTCCCCTGAGG-G      1598

      247  AACGCTG-CCTTTGCG-CCTCCTGCGGACGGCTCAATCTGGCTTAAGT-A      296
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1599 CACACTGACCGTGGAGGCC-CC--C--ATGCC-CAA---G-CT-A-GCCA      1648

      297  CG-ACTACACA-GAAGTTGAC-A-C--AGTAACATACGGGTTAAGCAGGA      346
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1649 CGCAGTCCA-ACGA-GATCACCATCCCAGTCACCTTCGAGTC--GC-GG-      1698

      347  AGTGCAAATACTACG-TTGGCATGGTGCAGATTTAG-TGTTCTTCAGTC      396
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1699 -GC-C-----C-A-GCTTGG---GG-GCAGA---AGCTG--CAAAA-TC      1748

      397  CGGGGACTGGGATTGA-TAAGCCGAGACAAATAGCTAATCAATTGGCAGA      446
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1749 CG---A-TG--A--GACT--GCCGC--CAAGTA---AAGCC-TTAGC---      1798

      447  ATCTATCGTTGATGGT-ACAA-TGCTTGAACAGCGGGACCATTTATGAGT      496
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1799 --C---CG--GATGCCACCCCTGCT-G--C--CG---CCACT---G-G-      1848

      497  CTGGAGTTGT-TAACCCGGTTATCAAATCCAAGTCTGGGTGGT-TTATTC      546
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1849 CTG---T-GCCTCCCCCGC----CA---CCT-GTGTG--T--TCCTT-TT-      1898

      547  CCGGTTCTGTTTTTATGTTT-GTCTAGACT-AACA-AAAGG--AAA--AAT      596
      | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1899 --GATACATTT--ATCTTCTGTTTTT-CTCAA-ATAAAGTTCAAAGCAAC      1948

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597	-ACATGGCCCATCTCAGCA-ATGGCACGCAGGTCTTCGTAGAA--A-GCT	646
1949	CACCTGTC--A-CTG-GCCCA-GGC-C-CTGGTGTITGTGGAAGGAAGC-	1998
647	CTCGCGGAAC-TGCAAT---C-GACGT--A--ACTGCAAT-TTCTAACGC	696
1999	CTCA-GGCACCTGCCATTGCTGGCTTTCAGGAGT-CA-TCTT-TG-CTC	2048
697	AGTTACCCCTGTTTAACTGT---ATCTGACGCGT-C--TGTTTGGTCG	746
2049	AGG--CCCGTG-----CTGGGCCATGTG--G-GTACACTGCTGTAG--G	2098
747	TGGTGAATAC---CTG-CTTTTCACCTCTTC-T---GCTTCA-ACGCTT	796
2099	TGCTGGACACAGGCTGACT---CACATC--CATAAAGA--CAGAGG-T-	2148
797	CTAA---CTGA-CAANCAAGT---TCGAATGAC-TGCAATCCCTGGTACG	846
2149	CTTAGGGCCGGGC--GCA-GTGGCTC-A-T-ACCTACAATCCCAGC-AC-	2198
847	TCAGTAACTG-----TTGAAG--GTTTGA-TACCTCCAGCACCACAAAGT	896
2199	T---T---TGGGGGGTTGAAGCAGGAGGAGTGCTTGAAGC-C-A-AGAGT	2248
897	TC----CCGGC-TGG-CTTAAC-TGGTGAAGT-TGTC---AAGA--TCAC	946
2249	TCTAGACCAGCCTGGAC--AACATAGT-AAGACTGTCTCTAAAAAATAAA	2298
947	C-TCCT-GGTTGGAAGTCCGTCGT--T-CAGGAC-GTA-TCT-A-CTG	996
2299	AAT--TAGG--C-AGG----GTG-GTACTGCACGCCTGTAGTCCCAGCT-	2348
997	AC---GGTGGC-GAA-CAGCA--ATTCG-TTAA-CTTCCAGTGCTTGTC	1046
2349	ACTCAGGAGGCTGAGGCAGGAGGAT-CGCTTGAGC--CCAGAG-TTGT--	2398
1047	GATG--ACCGTGA---A-CA--G--C-A--G-ATTCCGA-CCT----AT-	1096
2399	GAAGGTACAGTGAGCTAACATCGTGCCATTGCACTCC-AGCCTGGGCAAC	2448
1097	A-AA-----TC-TG-CGGT-AACTAACA-CCTTCA-CCTTC-GCTCACGA	1146
2449	AGAACAAGATCCTGTC--TCAA--AACCAACAAAAGCC--CAG---A-GA	2498
1147	GTACACCA-ACCCTGTATATCCGGTTCTGCGTAACTACGATGA-----	1196
2499	G-A-A--AGAG--TG-AGACCCCAT-CTT--TAAA-A-GAAAAAAAAAAAA	2548
1197	-G-TC-TG---GCCAGGTT--G-TTGGGATTCGT-CTGTTCTG-T----AC	1246
2549	AGGTCATGATTGCAAGGTCACGATTGCAATTAAACTGTAAGGTGGGGAA	2598
1247	CTCGAGCTAGCGAAAT--GCCCTTGCACTCCGGTA--CTATCGC-TT-TC	1296
2599	G--GAGG-AG-GAAATAAGAGAA-GCA--CCTG-AGGCT-T-GAGTTCTC	2648
1297	AACGA-CACCCCTACCATTGGTGTAA-ACGA-AATCG--A-A-ACGGTA-	1346
2649	AG-GAGCACC--TAGG-TTGG-GTCCCAGGTGAAGGGGCACAGA-GGTAA	2698
1347	TC-CATCGC-G-G-T-ATCC-A---TT-CG-TG.....	1396
2699	TGACACCTCAGAGCTGATGGGAGGATTACTATGTCA.....	2748

Alignment Of DNA 4 (Complementary

Strand), And The Most Similar-sized,

Homologous Area Of Human hsp 27

(The top row shows the DNA 4 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

```

1 CA-CGA-ATGGATACCGCG-ATGGATACCGTTTCGATTTCGTTAACACCA      50
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1348 .AGCGGCA-GGA--C-GAGCATGGCTAC-----AT--C-T---C-CC-      1397

51 ATGGTAGGGGTGTC--GTTGAAAG-CGA--T-AGTACC-GGACTGCAAG-      100
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1398 --GGT-GC--T-TCACGCGGAAATAC-ACGTGAGT-CCTGG-C-GCCAGG      1447

101 -CGCA-T---T---TCGC-TAGC--TCGAGGT-ACGAACAGACGAATCGC      150
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1448 TCGGGGTGGGTGGGTGGCGTGGGGGTGG-GGTCAGGGA-AGAGG----GC      1497

151 AACAACTGGCCAGACTCATC-G-TAGT-TACGCA-G-AACCGGATATAC      200
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1498 A-CA----GG---GACCCACCCGGT-GTGTA---ATGTAAC-GC-T-TGC      1547

201 AGGGTGGTG-TACTCGTG-A-G-CGAAGG-TGAA----GGTGT-AGTT      250
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1548 C---TT--TCCT-CTC-TGCACGTCCA-GGCTGCCCCCGGTGTGGACCC      1597

251 -ACCGCAGATTTATAGGTGCGAATCTGCTGTTACGGTCATCGG-ACAA-      300
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1598 CACC-CA-AGTT-TCC-TCC---TCC-CTGTCCCCTG--AG-GGCACACT      1647

301 GCAC--TGGAAGTTAACGAATTGCT---GTTCCGCCAC-C-GTC-A--GTA      350
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1648 G-ACCGTGGAGGCCCCC-A-T-GCCCAAGCTAGCCACGCAGTCCAACG-A      1697

351 GAT-AC-----GTC-C-T--GAA-CGCACGGA--A-CTTCGAACCAGG      400
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1698 GATCACCATCCAGTCACCTTCGAGTCGCG-GGCCAGCTTGGGGGCAGA      1747

401 AGGTG-A---TCT--TGACAACTTCA-CCA-GTTAAGCC--AGCCGGGA-      450
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1748 AGCTGCAAAATCCGATGAGA-CTGCCGCCAAGTAAAGCCTTAGCCCGGAT      1797

451 ----ACTT-TG-TGGTGC---TGGAGGTA--TCAAACCTTCAACAGTTAC      500
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1798 GCCCACCCTGCTGCCGCCACTGGCTGTGCCTC---CC--C--C-G---C      1847

501 TGACGTACCAG-G-GATTGCAGTCATTCTGA-AC-TTGNT-TGTCAGTTAG      550
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1848 ---C--ACCTGTGTG-TT-CT-T--TT-GATACATTTATCT-TCTGTMTT      1897

551 AAGCGTTGAAGCAGAAGAGGTGAAAAGCAGGTATTCACCA---AC-GACC      600
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1898 T--C-TC-AA---ATAA-AGTTCAAA-GCA---AC-CACCTGTCACTGGCC      1947

601 AAACCAGACGC-G-TCAGATACAGTTAAACAGGGGTAAGTCCGTTAGAA      650
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1948 ---C-AGGCCCTGGT--GTTT--GTGGAA---GG---AA--GCCTCAGGC      1997

```

551 AT-TGC-AGTTACGTCGATTGCGAGTTCCGCGAG--AGCTTT-CTACGAAG 700
 | | | | | | | | | | | | | | | | | | | | | |
 1998 ACCTGCCATTTC-T-G---GCT-TTCAG-GAGTCATCTTTGCT-C-AGG 2047
 701 ACCTGCGTGCCATTGCTGAG--ATG-GGCCATGTATTTTTC-CTTTTGTT 750
 | | | | | | | | | | | | | | | | | | | | | |
 2048 -CC--CGTGC--T-G--G-GCCATGTGG----GTA-----CACTGGTGT- 2097
 751 AG-T--CTAGACGAACA---TAAAA-ACGAACCGGGAATAAACCACCCAG 800
 | | | | | | | | | | | | | | | | | | | | | |
 2098 AGGTTGCTGGAC-A-CAGGCTGACTCAC-ATCC---A-TAAAG-AC--AG 2147
 801 ACTTGGAT-TTGATAACCGGGTTAACAA---CTCCAGACTCATA-AATGG 850
 | | | | | | | | | | | | | | | | | | | | | |
 2148 A---GG-TCTT-AGGGCCGGGCG--CAGTGGCTC-ATAC-C-TACAAT-- 2197
 851 TCCC-GC--TGTTCAAGCATTGTACCATCA--ACGA-TAGATTCT-GCCA 900
 | | | | | | | | | | | | | | | | | | | | | |
 2198 -CCCAGCACT-TTGGGGGGTTG-A--AGCAGGAGGAGT-GCTTGAAGCCA 2247
 901 ATTGA-TTAGCTATTGTCTCGGCTT--ATCAATCCCAGTCCCCGGACTG 950
 | | | | | | | | | | | | | | | | | | | | | |
 2248 A--GAGTT--CTA---GAC-CAGCCTGGA-CAA-CATAGTAA--G-ACTG 2297
 951 -----AAGAACACTGAAA-TCTGCACCATGCCAACGTAGTATTGCACTT 1000
 | | | | | | | | | | | | | | | | | | | | | |
 2298 TCTCTAAAAA-A-TAAAAAT-T--AG---GC-AGGGTGGTACT-GCAGC- 2347
 1001 CCTGCTTAA-CCCGTATGTACT--GTGTCAA-CTT---CTGTGTAG--T 1050
 | | | | | | | | | | | | | | | | | | | | | |
 2348 CCTG-T-AGTCCC--A-GCTACTCAG-G--AGGCTGAGGCAG-G-AGGAT 2397
 1051 CGTACTTAAGCC-AGA-TTG--A-GCCGTCCGCAG-GAGGCGCAA-A--G 1100
 | | | | | | | | | | | | | | | | | | | | | |
 2398 CG--CTTGAGCCCAGAGTTGTGAAG--GTAC--AGTGAG-CT-AACATCG 2447
 1101 -GCAGCGTT-CTC-CCAAGCAACTGG--ATATCTGA-CAGGAAATTCAT- 1150
 | | | | | | | | | | | | | | | | | | | | | |
 2448 TGC--CATTGCACTCCA-GC--CTGGGCA-A-CAGAACAAGA--TCC-TG 2497
 1151 T-T-AC---CAAC-AAAT-C---GTTGACGAATT--T-----CCT--TGCTG 1200
 | | | | | | | | | | | | | | | | | | | | | |
 2498 TCTCAAAACAACCAAAAGCCAGA-GA-GAAAGAGTGAGACCCCAT-CTT 2547
 1201 CTAAT-GATAAT-----TCAT-ATTGCACGG-CGA-GCT--CN-TC 1250
 | | | | | | | | | | | | | | | | | | | | | |
 2548 -TAAAGAAAAAAAAAAAAAGGTCATGATTGCAAGGTC-ACGATTGCAAT- 2597
 1251 TA---CTGCTTCC-TGG--AA--AT-AC-A--TA-----C-CC--AGC 1300
 | | | | | | | | | | | | | | | | | | | | | |
 2598 TAAAACTG-TAAGGTGGGGAAGGAGGAGGAAATAAGAGAAGCACCTGAGG 2647
 1301 CTTGC-TTG-CACCA-CACCTA-----A----AACG--C-CAGC 1350
 | | | | | | | | | | | | | | | | | | | | | |
 2648 CTTGAGTTCTCAGGAGCACCTAGGTTGGGTCCCAGGTGAAGGGGCACAGA 2697
 1351 TGG-A---GC-C-T--G-GCT--TGA-AGCTCCGTATT-CAAGGTCGA.. 1400
 | | | | | | | | | | | | | | | | | | | | | |
 2698 -GGTAATTGCACCTCAGAGCTGATGGGAG----G-ATTACTATGTC-A.. 2747

**Alignment Of DNA 1/Region 1 (Normal
Strand), And The Most Similar-sized,
Homologous Area Of Human hsp 27**

(The top row shows the DNA 1/Region 1 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

```

-8 .....TA---TCTGGTA-----CTCTAT--ACCTCTACACACCTT      41
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1435 GCGTGGGGGTGGGGTCAGGGAAGAGGGGCAC-AGGGACC-C-AC-C-CGGT      1484

      42 -T-TAAAGTGAA---TTGACCTATTTGCTTTTCTAGAAATTTTAAAAATT      91
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1485 GTGTAATGT-AACGCTTG-CCT-TTC-CTCT--CT-GCACGTCCAGGCT-      1534

      92 TTTGTTCCACCG-T-TAAACCCCA-----GTTGAT--TC-TCGACT--CA      141
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1535 ---GCCCC-CCGGTGTGGACCCCAACCAAGTT--TCCTCCTCC-CTGTCC      1584

      142 ACTGAA---A-ACT-ATTCTTCTTTTCGGAAACCTTCATTG---A--CTAT      191
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1585 CCTGAGGGCACACTGA--C--CGT--GGAGGCCCCCAT-GCCCAAGCTAG      1634

      192 TCAA--AGTTCTTCTTTATTCTTTAG-TG-CCATCCTCTGTATATTACCC      241
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1635 CCACGCAGT-C--CA--A--CG--AGATCACCATCC-CAGTC-A--CC-      1684

      242 TT--ATTA-C---CCTTCAACTAC-----CTATTGTAA-CTACATATT--      291
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1685 TTCGAGTCGCGGGCC--CAG-CTTGGGGGC-A--G-AAGCTGCAAAATCC      1734

      292 GTTTTTATGTCCATCT-CC-CCTAA-TAAACAGTGAGCTCCTTCAAGAAA      341
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1735 GATG--A-G---A-CTGCCGCC-AAGTAAA--G---C-C-TT-A-GCCC      1784

      342 GAGTATCCTTCCACCTCCCTTTTCCGCATCACTAGAACAGGGTAGG-TA      391
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1785 G-G-ATGC--CCACC-CC-TGCTGCCGC--CACTGG--CTG--T-GCCTC      1834

      392 G---GC-AC-TGAT-TAAGTACATTATTCAGTTCATC-ACTCAGTAT-TG      441
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1835 CCCC GCCACCTG-TGT--GTC-TT-TTGA-TACATTTA-TC--T-TCTG      1884

      442 GACATATATTTCTTGAGAAT----TTACTAAAGG--CCTTGCTTG-CG-T      491
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1885 ----T-T-TTCTC-A-AATAAAGTT-C-AAAGCAACCAC-CT-GTCACT      1934

      492 GGTTC TANGCA-TCGTGAATTGAGTGGTGAACAAAACATTTAA--ACC--      541
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1935 GGCCC-AGGCCCTGGTGT-TTG--TGG--AAGGAAGCCTC-AGGCACCTG      1984

      542 CC-TT-G-TC-CT--CATG-G--A-CTTAA-T--G-----T--T---CC-      591
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1985 CCATTTGCTGGCTTTCAGGAGTCATCTTTGCTCAGGCCCGTGCTGGGCCA      2034

      592 TG-GGG-ACA--GA---A--TTA--G-ATA-A--C--A-T-ACAT--ATA      641
      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
2035 TGTGGGTACACTGGTGTAGGTTGCTGGACACAGGCTGACTCACATCCATA      2084

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642 AAAACACAGTGTATTATTTTA---CATGGTCNTAAGAAGAAAATA---A-A 691
 || ||| || || || | | | | | | | | | |
 2085 AAGACAGAG-GTC-TT---AGGGCCGGG-CGCA-GTGGCTCATACTACA 2134
 692 GAT---AG-A-TAAGGAAG-TGGAAGTATTTAGGTGGAATGGTCATG--G 741
 || || | | | | | | | | | | | | | | | |
 2135 -ATCCCAGCACTTTGGGGGGTTGAAGCA---GGAGGAGTGCT--TGAAG 2184
 742 --AAG-GTCTCTTTA--ATG--TGGTGACAA-ATGATTAATGACTGAATG 791
 ||| || ||| | | | ||| | | | | | | | | |
 2185 CCAAGAGT-TCTAGACCA-GCCTGG--ACAACAT-AGTAA-GACTGTCTC 2234
 792 TATGTTAGTGAGAAATCAATTTTGANAACACCTGTTGATA--G-ANTGTC 841
 || | | | | | | | | | | | | | | | | | |
 2235 TA----A---A-AAATAAAATT-AGG-CAGG-GTGG-TACTGCAC-G-C 2284
 842 CTAATTTGAAGAAACAGCAAG-CAGAAGGNAGAAAAGCAGTGGGG-T-- 891
 || | | | | | | | | | | | | | | | | | |
 2285 CTG-T---A-GTCCCAGCTACTCAGGAGGCTGAG---GCAG-GAGGATCG 2334
 892 -TTGA-----AGTTATTT-TGAA---A-AN-G-G-T--CA-C-TG---T-- 941
 |||| | | | | | | | | | | | | | | | |
 2335 CTTGAGCCCGAG--AGTTGTGAAGGTACAGTGAGCTAACATCGTGCCATTG 2384
 942 -A-TC-----TGGGANACANAA-A-G-TNANTTAAAGGTGCAANAAAAA 991
 | | | | | | | | | | | | | | | | | | | |
 2385 CACTCCAGCCTGGGCAACAGAAACAAGATC-CT----G-TCT--CAAAACA 2434
 992 AC-AGAAGGTCAGATTTCCNNACG-GTACAAGGGAATTTGAAATCCACAT 1041
 || ||| | | | | | | | | | | | | | | | |
 2435 ACCAAAAG--C-----CCAGA-GAG-A-AAGAG--T--GAGACCC-CAT 2484
 1042 GTTCCC--GAATAAATGGAGAAANGAAT-TNANCATCCCCTG-AATTT-- 1091
 || ||| ||| | | | | | | | | | | | | | |
 2485 CTTTAAAAGAA-AAA---A-AAAA-AAGGTCATGAT----TGCAAGGTCA 2534
 1092 -G-TTG-AAT-AATANCTGAAAGTT----ACCCCTTTTCA--ACTGGTTC 1141
 | ||| ||| || | ||| ||| | | | | | | | |
 2535 CGATTGCAATTAA-AACTGTAAGGTGGGGAAGG-----AGGA--GG--- 2584
 1142 CNAATTAA-A-AATTCTCATTAACCTTGAACCCCTCA--ANCNTTTTT-T 1191
 ||| || | || | | | | | | | | | | | | | |
 2585 -AAAT-AAGAGAAG-CACCTGAGGCTTGAGTTC-TCAGGAGCACCTAGGT 2634
 1192 TGAAAAGT----GGATAA-----ATTCA-AG--AACTT-CNCC-CNG-G 1241
 || || || | | | | | | | | | | | | | | | |
 2635 TGG---GTCCCAGG-TGAAGGGGCA--CAGAGGTAA-TTGCACCTCAGAG 2684
 1242 CCAAAAATTTGGGAA-ACCCCTATNTTTATGCCCCAAAATCCNC.... 1291
 | | | | | | | | | | | | | | | | | | | |
 2685 CTGA---T-GGGAGGAT-----TA-CT--ATG-----TC-A..... 2734

Alignment Of DNA 1/Region 1

(Complementary Strand), And

The Most Similar-sized,

Homologous Area Of

Human hsp 27

(The top row shows the DNA 1/Region 1 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

```

-1 ..GNGGATTTTGGGG-CATA-AANATAGGG----GGGTTTCCCAAATTTT      48
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1435 GCGTGGGGGT-GGGGT-CAGGGAAGA--GGGCACAGGGA--CCCA-----      1484

    49 TGGCCNCG-GNG-AA-GTT-C--TTGAATTT--TATC--CACTTTTCAAA      98
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1485 ---CCCGGTGTGTAATGTAACGCTTGCCTTTCTCTCTGCACGTC-CA--      1534

    99 AAAANGNTTGAGG---GGT-TCAAGGTTAATG-AG--AA-TTT--T--TA      148
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1535 ----GGCT-GCCCCCGGTGT---GG--ACCCACCCCAAGTTTCTCTCTC      1584

    149 A-T-TNG---GAA--C-CAGTTGAAAG-GG-GG-----T-----AA-CTT      198
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1585 CCTGTCCCCTGAGGGCACACT-GACCGTGGAGGCCCCCATGCCCAAGCT-      1634

    199 TCAGNTATT-ATTCAACAAATTCAGGGGATGNTAATTCNTTTCTCCATT      248
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1635 --AGCCACGCAGTC--CAA---C-G---A-GATCAC--CAT--C-CCAGT      1684

    249 TA--TTCG-G--GAACATGTGGATTTCAAATTC--CTTGAC-C-GTNN      298
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1685 CACCTTCGAGTCG--C--G-GG---C-----CCAGCTTGGGGGCAG-AA      1734

    299 G--G-AAA-TCTGACCTT---CTGTTTTTTTNTCGACCTTTAANTNA--      348
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1735 GCTGCAAAATCCGA--TGAGACTG-----C--CG-CC---AAGTAAAG      1784

    349 CTTTNTGTTNTCCCAGATACAGTGACGNTTTTCAAAATAACTTCAAACCCC      398
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1785 CCTTA-G-C-CCG-GATGCCC--ACCC-----C----TG-CTGC---CGCC      1834

    399 ACTG-CTTTTTCTNCCTTCTGCT---TGCTGT-TTCTTCAAATTAGG-AC      448
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1835 ACTGGCTGTGCTTCCCC-C-GCCACCTG-TGTGTTCTT----TT-GATAC      1884

    449 ANTCTATCAACAGGTGTTNTCAAAATTGATTCTCACTAA-CATACATTC      498
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1885 ATT-TATCTTCTGTTTTTCTCAAA-TAAAGTTC--A--AAGCA-AC---C      1934

    499 AGTCATTAATCATTTGTC-A--CCACA--T-TA-----AAGAGA--CCTT      548
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1935 AC-C-TG--TCACTGGCCCAGGCC-CTGGTGTGTTGTGGAAG-GAAGCCT-      1984

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549 CCATG-ACCATTCCACCTAAATACTTCCACTTCCTTATCT--A-TCTTTA 598
 | | | | | | | | | | | | | | | | | | | | | |
 1985 C-AGGCACC-TGCCA--T---T--TGC---TGGCTT-TCAGGAGTC---A 2034
 599 T-TTT-CTT---C---T--TANGACCATGTAAAAATAACACTGTGTTTTT 648
 | | | | | | | | | | | | | | | | | | | | | |
 2035 TCTTTGCTCAGGCCCGTGTGGG-CCATGTGGG--TA-CACTG-GTGT-- 2084
 649 ATATGTATGTT--ATCTA-ATTCTG--TC-C--CCAGGAACATA-A-AG- 698
 | | | | | | | | | | | | | | | | | | | | | |
 2085 AG--GT-TGCTGGA-C-ACAGGCTGACTCACATCCAT-AA-AGACAGAGG 2134
 699 TCCATGAGGACAAGGG-GTTTAAATGTTTTGTTCCACCAC-T-CAATTCAC 748
 | | | | | | | | | | | | | | | | | | | | | |
 2135 TCT-T-AGGGCC-GGGCGC--AG-TG----GCTCAT-ACCTACAATCC-C 2184
 749 -G-A-T--GCN---TAGAACCACGCAAGCAAG-GCCTTTA-GT-AA-A-T 798
 | | | | | | | | | | | | | | | | | | | | | |
 2185 AGCACTTTGGGGGGTTGAAGCAGG-AGG-A-GTGC-TTGAAGCCAAGAGT 2234
 799 TCTC-A--AG-----A-AATATATGTCCAATACTGAGTGATGAAGTGAAT 848
 | | | | | | | | | | | | | | | | | | | | | |
 2235 TCTAGACCAGCCTGGACAACATA-GT--AAGACTGTCTC-TAAAA--AAT 2284
 849 AATGTACTTAATCAG--TGCCTACCT--AC-CCTGT--TCT-AG-TGA-T 898
 | | | | | | | | | | | | | | | | | | | | | |
 2285 AAAA-A-TTAGGCAGGGTGG-TAC-TGCACGCCTGTAGTCCCAGCT-ACT 2334
 899 GC-GGAAAAAGG--GAGGT-GGAAGGATACTCTTT--C-----TTG--A 948
 | | | | | | | | | | | | | | | | | | | | | |
 2335 -CAGGA---GGCTGAGGCAGGA-GGAT-CGCTTGAGCCCAGAGTTGTGA 2384
 949 AGG-A--G----CTCAC-T-GTTT-ATT--AGGGGAGA-TGGACATAAA- 998
 | | | | | | | | | | | | | | | | | | | | | |
 2385 AGGTACAGTGAGCTAACATCGTGCCATTGCACTCCAGCCTGGGCA-ACAG 2434
 999 AACCAATATG-TAGT-TACAATAGGTAGTTTGAAGGGTAATAAGG---GTA 1048
 | | | | | | | | | | | | | | | | | | | | | |
 2435 AACCAAGATCCT-GTCT-CAA-A---AC-----AACC--AA-AAGCCCAG-A 2484
 1049 -ATATACAGAG-GATGGC---A-CT--AAA-GAATAAAGAAGAACTTTGA 1098
 | | | | | | | | | | | | | | | | | | | | | |
 2485 GAGA-A-AGAGTGA-GACCCCATCTTTAAAAGAA-AAA-AA-AA-----A 2534
 1099 ATAG-TCA--AT-G-AAGGTTTC-CGA-----AAG-AAGAA-TAGTTTTC 1148
 | | | | | | | | | | | | | | | | | | | | | |
 2535 A-AAGTCATGATTGCAAGGT--CACGATTGCAATTAA-AACT-GTA---A 2584
 1149 GTTGAGTCGAGAACTCACTGG-GGTT-TAACGGTGGAA-CAAAA-A---T 1198
 | | | | | | | | | | | | | | | | | | | | | |
 2585 GGTG-G--G-GAAGGA---GGAGGAATTA-GA-G-AAGCACCTGAGGCT 2634
 1199 T---TTA--A--A--A---A--TTC--T---AGAAAAAG---CAATAGG 1248
 | | | | | | | | | | | | | | | | | | | | | |
 2635 TGAGTTCTCAGGAGCACCTAGGTTGGGTCCCAGGTGAAGGGGCACAGAGG 2684
 1249 TCAATT-CACTTTAAAAGGTG-TGTAGAGGTATAGAGTACCA-GAT-A.. 1298
 | | | | | | | | | | | | | | | | | | | | | |
 2685 T-AATTGCACCTCAGA-GCTGATGG-GAGG-AT-----TACTATG-TCA.. 2734


```

650 -T-TAATAGCA-AAAAATTATAACAGAGAAGAATACATACTAACGAATG      699
    | | | | | | | | | | | | | | | | | | | | | | | | | | |
2215 CTCTAAAAA-ATAAAA-ATTAGG-CAGGGTGG--TAC-TGC-A-CGCCTG      2264

700 T--T---ATTTA---A--A---T-A---AG-ANTTATTGGTATAAACATA      749
    | | | | | | | | | | | | | | | | | | | | | | | | | | |
2265 TAGTCCCAGCTACTCAGGAGGCTGAGGCAGGAGG-ATCGCT-TGAGCCCA      2314

750 GATGTTTT-AA--TA-ACTG-G-TAATC-TGGAAAAAGAAATAGAACAC-      799
    | | | | | | | | | | | | | | | | | | | | | | | | | | |
2315 GA-GTTGTGAAGGTACAGTGAGCTAA-CATCGT----GCCATTGCACTCC      2364

800 AGCCTGGT-ATCACTGAA-AC--TCCTCTATGTTCTTCCATGAATAACCA      849
    | | | | | | | | | | | | | | | | | | | | | | | | | | |
2365 AGCCTGGGCAACA--GAACAAGATCCT----GT-CT-CAA--AAACAACCA      2414

850 CCTATCCCGTGCCTCCTTGGGAATTA-A-T-A-ACNA-AT-TTGAAATTT      899
    | | | | | | | | | | | | | | | | | | | | | | | | | | |
2415 AA-AGCCAGAGA-----G-AA--AGAGTGAGACCCCATCTTT-AA---      2464

900 TGGTGAANATTTTTTTTAAAAANTTGCCTNAATTTTAA--TCCNGAAACC      949
    | | | | | | | | | | | | | | | | | | | | | | | | | | |
2465 -----AAGA-----AAAAAA-----AAA-----AAGGTCATGA-----      2514

950 TTTGGAaaaaat-ACGTTTTTCTATTTTTTAAACNGGCNCATGNAATGG      999
    | | | | | | | | | | | | | | | | | | | | | | | | | | |
2515 TT-GCAAGG--TCACGATTG--CAATTA--AACTGT-A-A-GG--TGG      2564

1000 G-AAGGTINTGCTGGAAA-AA-A-AT-----T-----TT---TT-TGAN-A      1049
    | | | | | | | | | | | | | | | | | | | | | | | | | | |
2565 GGAAGGAG-GA-GGAAATAAGAGAAGCACCTGAGGCTTGAGTTCTCAGGA      2614

1050 TAATTTCCTTTAACT-GGGTCTN-GNA-AA-----A-A-ATNTTTTAA-A      1099
    | | | | | | | | | | | | | | | | | | | | | | | | | | |
2615 GCA---CCT--AGGTTGGGTCCCAGGTGAAGGGGCACAGAGGTAATTGCA      2664

1100 NNA-A-ANNTTNCCCTG--A--AC-ACCCCNNTNTNN.....      1149
    | | | | | | | | | | | | | | | | | | | | | | | | | | |
2665 CCTCAGAGCTGA---TGGGAGGATTAC---TATGTCA.....      2714

```

Alignment Of DNA 1/Region 2

(Complementary Strand), And

The Most Similar-sized,

Homologous Area Of

Human hsp 27

(The top row shows the DNA 1/Region 2 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

```

1  NNANANNGGGG----TGTTTCAGGGNAANNNTTNTTAAAAANATTTTNTN      50
    |||      || | | | |      ||
1565 CC-T-GAGGGCACA CTGACC-GTGGAGGCC--CC-----CATGC---C      1614
    51  CNAG--A-CC-C--AGTTAAAGGAAATTATNTCAAAAAAATTTTTCCTCA      100
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1615 CAAGCTAGCCACGCAGTCCAACGAGATCA-C-CA-----TC-----CCA      1664
    101 GCANACCTTCCCATTTNCATGNGCCN-GTTTAAAAATAGAAAAAACGTAT      150
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1665 GT-CACCTTCG-AGTCGC--GGGCCAGCTTGGGGGCAGAAG---C-TG-      1714
    151 TTTTTCCAAAGGTTTCNGGATTAAATTNAGGCAANTTTTAAAAAAAAT      200
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1715 -----CAAAA--T--CCG-ATGAGACTGCCGCCAAGT----AAAGC-----      1764
    201 NTTCA-CCAAAATTCAAAATNGTTATTA--ATTCCAAGGAG--GC-ACG      250
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1765 CTT-AGCC-----C-----GG--ATGCCACCCCT-GCTGCCGCCACT      1814
    251 GGGATAGGTGGTTATTCATGGAAGAACATAGAGGAGTTTCAGT-GATACC      300
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1815 GGC-T-G-TGCCCTCCCC-GCCAC--C-T-GTGT-GTT-CTTTGATAC-      1864
    301 AGGCTGTGT-TCTATTTCTTTTTC-CAGATTACCAGTT-ATTAA--AAC-      350
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1865 A---T-T-TATCT-TCTGTTTCTCTCAATAA--AGTTCA--AAGCAACC      1914
    351 ATCTATGTTA-TA-CCAATAANTCT--TATTTA---AAT-AA-CATTTCG      400
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1915 ACCT--GTC-ACTGGCCCAGGCC-CTGGTGTGTTGTGAAGGAAGCCT-C-      1964
    401 TTAGT-A--TGT-ATTCTTCT--CTGTT-ATAA-T-ATTTTGTCT-A---      450
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1965 --AGGCACCTGCCATT-TGCTGGCT-TTCAGGAGTCATCTTTGCTCAGGC      2014
    451 ---T--TAAA--ATGTTTTTAAAAATGAAAAATGTAAAAAAGGTT-CTT-A      500
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
2015 CCGTGCTGGGCCATGTGGGTACACTGG----TGTA-----GGTGTCTGGA      2064
    501 -A-ATG-T-ACAAA-AGTAACATTTCAA-ACCTAGTTTGTGTAGTGGT-GT      550
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
2065 CACAGGCTGACTCACA-TC-CATA--AAGACAGAGGTC-TTAG-GGCCG-      2114

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551 GTGAAGAAGTGT-T-AGANTCTACN-TC--A--A-T--GAAGAAG--GAA 600
 | | | | | | | | | | | | | | | | | | | | | |
 2115 G-GC-GCAGTGGCTCATAC-CTACAATCCCAGCACTTTGGGGG-GTTGAA 2164
 601 A-AG-A--A-T-CACGAAGAACAATA-TT-T-GA---GC-TGG-CTTT 650
 | | | | | | | | | | | | | | | | | | | | | |
 2165 GCAGGAGGAGTGTCTGAAG--C-CAAGAGTTCTAGACCAGCCTGGAC--- 2214
 651 TTAAGTC-TGAGTAGGAGTTTTTCAGGGCAT-TC-C-AGGAATACA-AAC 700
 | | | | | | | | | | | | | | | | | | | | | |
 2215 --AA--CAT-AGTA--AG-----A---C-TGTCTCTAA-AA-A-ATAA-- 2264
 701 AAGATTAG--AGTTGTGATAGG-ACATG---GCCAAAT-TA-TAC-AGAG 750
 | | | | | | | | | | | | | | | | | | | | | |
 2265 AA-ATTAGGCAG--G-G-T-GGTAC-TGCACGCC---TGTAGTCCCAGCT 2314
 751 ACGTCTGG-GG-TGATTGAA--ATGGAT-G---GAGC--ATAG--GA-AA 800
 | | | | | | | | | | | | | | | | | | | | | |
 2315 AC-TCAGGAGGCTGAG-GCAGGA-GGATCGCTTGAGCCCAGAGTTGTGAA 2364
 801 GTG-A-AGC-AG--AAC-T--TAGACATAG-A-----AG--TAGGAGCAAT 850
 | | | | | | | | | | | | | | | | | | | | | |
 2365 G-GTACAGTGAGCTAACATCGT-GCCATTGCACTCCAGCCT-GG-GCAAC 2414
 851 -GTTGCAAAGGGCCTTGTGTGATGAGACCATACTAATTAT--ATGA-AT 900
 | | | | | | | | | | | | | | | | | | | | | |
 2415 AGAA-CAA-GATCCT-GTCTCAA-A-AC-A-ACCAA-AGCCCA-GAGAG 2464
 901 AAA-AGTTAGTTACC--ATTTAATTAGCTAACAAAAGAAAATCAAATTAA 950
 | | | | | | | | | | | | | | | | | | | | | |
 2465 AAAGAGTGAG--ACCCCATCT--TTA---AA-AGAA-AAAA--AAA--AA 2514
 951 AGAT-ATTATTTT---TCAC-ATT-CAA--AATAATCCTGCAAAATAAT 1000
 | | | | | | | | | | | | | | | | | | | | | |
 2515 AGGTCATGATTGCAAGGTCACGATTGCAATTAA-AA-C-TG-----TAAG 2564
 1001 -TAT--AA--AC-ATCCCCAATATACAAACGAAGAAAATGAGAC---AGT 1050
 | | | | | | | | | | | | | | | | | | | | | |
 2565 GTGGGAAGGAGGAGG---AA-ATA-AGA-GAAGCACCTGAGGCTTGAGT 2614
 1051 GTAT-A--A--A--TA--T-----C--A--TGC-----CA-A-AG--A-T 1100
 | | | | | | | | | | | | | | | | | | | | | |
 2615 -TCTCAGGAGCACCTAGGTTGGTCCCAGGTGAAGGGGCACAGAGGTAAT 2664
 1101 --C-CC--G---T--TG--AC-----CT--G-CAG..... 1150
 | | | | | | | | | | | | | | | | | | | | | |
 2665 TGCACCTCAGAGCTGATGGGAGGATTACTATGTCA..... 2714

**Alignment Of DNA 1/Region 3 (Normal
Strand), And The Most Similar-sized,
Homologous Area Of Human hsp 27**

(The top row shows the DNA 1/Region 3 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

```

1  ATAAAAGCAAAGTTTCTA-T-TAA-G-T-GACCAGTGTATT-TATATGCG      50
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1468  ....ACCCG-GTGTGTAATGTAACGCTTG-CC--T-T-TCCTCTCTGC-      1517

   51  A--TA-AGAAGATACTACTTT-TAATGAAAT--A---AA-TA-CATAAAC      100
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1518  ACGTCCAG--GCTGCCCCCGGTG-TGGACCCACCCCAAGTTTCCTC--C      1567

   101  AT---T-T----TGAAGTGTCTACA--GAAAACTCAGAGAGATGGTA--      150
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1568  -TCCCTGTCCCTGAGG-G-C-ACACTGA----C-C-GTG-GA-GGCCCC      1517

   151  -ATGA--AA---A-C-ACTCTGTTTATATAAAAGA--ACTTCTTCACTAG      200
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1618  CATGCCCAAGCTAGCCACGCAGTCCA-ACGA--GATCAC--CATCCC-AG      1667

   201  TC-CCATAGAATAGAGTGAATAG---AGTCTTGT---CTTA-ATA-CAGC      250
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1668  TCACCTTCGAGTCGCG-G----GCCCAG-CTTGGGGGC--AGA-AGCTGC      1717

   251  AT--T--GATTTATTA-TGTN---A--TATATCCATATCCCG-ATGATC      300
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1718  AAAATCCGATG-AG-ACTGCCGCCAAGTAAAGCCTTAG-CCCGGATGCC      1767

   301  AAGATCTTGCTGGTGTNTA-TGGAGGCTGAGGG-CCGCTTGGCTCA--TG      350
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1768  ACCC-CT-GCTGCCGCC-ACTGG---CTGTGCCTCCCCC-G-C-CACCTG      1817

   351  TGAAGAAAGATAAAT-TTTT--TA--TTGTGTCTTGAGTGTTTTAATATG      400
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1818  TGT-G-----T---TCTTTGATACATT-TATCTTC--TGTTTT--TCT-      1867

   401  AAACAAAATAAA-----AAAGAAA-AGCCT--C--TGTCTTAAGCATCA      450
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1868  ---CAAA--TAAAGTTCAAAGCAACCA-CCTGTCACTGGCCCAGGC--C-      1917

   451  CT--TACATTATGTNGAAAGAAGCAATTTTAAA-A--T-C-ATTCGTT--      500
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1918  CTGGTG--TT-TGTGGAAGGAAGCC-TC--AGGCACCTGCCATTGCTGG      1967

   501  CATCATATAAG-AGT-ATCAT--CA-A--CNCN-GAATC--CCATNTAGA      550
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1968  C-T--T-TCAGGAGTCATCTTTGCTCAGGCCCGTGC-TGGGCCATGT-G-      2017

   551  GTGATA-A-TGAAGATCT-GTTTTC---ACNCCATGG--GAGCT-AT-T-      600
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
2018  G-G-TACACTG--G-TGTAGGTTGCTGGACAC-A-GGCTGA-CTCACATC      2067

   601  --T---GA--GTAT-TCTTT---CCTTAA-C--TG-CT--T-C-TGGCAG      650
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
2068  CATAAAGACAG-AGGTCTTAGGGCCGGCGCAGTGGCTCATACCTA-CAA      2117

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651 TTC-A-CA-TTGTGAGCGAAGTT-AATGCTGG-G-A-TG-TGGAA-C-AT 700
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2118 TCCCAGCACTT-TGGGGG--GTTGAA-GCAGGAGGAGTGCTTGAAGCCAA 2167
 701 GA-TTCCT---CTCTTTTTTTTCCNC--CAATTTATTTTT--T-T-T-TA 750
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2168 GAGTTC-TAGAC-CAG-----CCTGGACAACATAGTAAGACTGTCTCTA 2217
 751 GATCCACAGATAAAATTTATGTTT--T--T-CT-CNNG--T-T--T---- 800
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2218 AA---A-A-ATAAAATTAGGCAGGGTGGTACTGCACGCCTGTAGTCCCA 2267
 801 G--ACTGA--AGGTTT-GG-A--A--ATNTNTT-A-CN-----TTGTTGA 850
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2268 GCTACTCAGGAGGCTGAGGCAGGAGGATCCCTTGAGCCCAGAGTTGT-GA 2317
 851 ATGGGTTAAAGTTAGCTAATTAATTTGTTTACC-T-GACA-TNNTTAT 900
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2318 A-GG-T-ACAGTGAGCTAA--CA-TC-GTG---CCATTG-CACTCC--AG 2367
 901 CCTTTTTGAAGGTNGGGACACTTTTNAATGAATTNGGT-TNNAAN-AAAN 950
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2368 CCT---G--GGCA---ACAGAA-CAA--GA-TCCTGTCTCAAAACAACC 2417
 951 TTGA-CCTTNA-ANA-AAANANTNNTANGGGCC-T-TTGCCCNRAATNGG 1000
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2418 AAAAGCC--CAGAGAGAAAGAGTGAGACC--CCATCTT----TAAA-AG- 2467
 1001 GAAANTTNACTGCCCCGGGGAAAAAANANTNCAATTACCTTA-AATGCCC 1050
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2468 -AAAA--AA-----AAAAAG-GTC-A-TGA--TTGCAAGGTCA 2517
 1051 CCNTTNTTCCNCCNANTTNCCCTTG-AATNTTIN-AAGNTTTTTNAAA 1100
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2518 CGATTG--CAAT--TAAA--AC--T-GTAAGGTGGGAAGGAGGAGGAAA 2567
 1101 -AATNTTNCNCNNTTNC-CCNANNNANTTTAANTCCTAAA-ANCCCCC- 1150
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2568 TAA-GA--G--A-A--GCACCTGAGG-CTTGAGTTC-TCAGGAGCACCTA 2617
 1151 GN-----CCNNTTGT--GCGGG----GNG--AATTTTC-CCNNTTTTTN 1200
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2618 GGTGCGGTCCCAGGT-GAAG-GGGCACAGAGGTAATTGCACCTC-----A 2667
 1201 NNNTANGGGTNATTCGGGG-GGNTNACCCGGGNTN..... 1250
 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
 2668 GAG--C---TGAT---GGGAGGATTACTATGTC-A..... 2717

Alignment Of DNA 1/Region 3

(Complementary Strand), And

The Most Similar-sized,

Homologous Area Of

Human hsp 27

(The top row shows the DNA 1/Region 3 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

```

1  NANCCCG-G-GTNANC---C-C---CCGGAATNACC-CN-T--ANNNNAA      50
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1472 .ACCCGGTGTGTAATGTAACGCTTGCCT---TT-CCTCTCTGCACGTC--      1521

51  AAANNGGGAAATTCCNCCCGCCAAANNNG-GGNCGGGG-----GNTT--T-      100
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1522 ---CAGGC---T--GCCCC-CC--GGTGTGGACCCACCCAAGTTTCCTC      1571

101 -TAGGANT-TAAANTTNNTNNGGNA-AN-GN--G-GNAANN-----ATTTT      150
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1572 CTCC--CTGTC--C-CCTGAGGGCACACTGACCGTGGAGGCCCCCATGCC      1621

151 TNA--A---AN-CT-TN-AA--ANATTCA--AGGGNA---AN-TTNG-      200
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1622 -CAAGCTAGCCACGAGTCCAACGAGAT-CACCATCCAGTCACCTTCGA      1671

201 G--GNNGAANAAN---GGGGGCATTTAAGGT--AA--T----TCN-ANTN      250
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1672 GTCGCGGGCCAGCTTGGGGGCAG--AAGCTGCAAAATCCGATGAGACTG      1721

251 TTTTTCCTCCGGGGCA-GTNAANT-TT--CCCN-ATTNN-----G--G      300
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1722 -----CCGC-----CAAGTAAAGCCTTAGCCCGGATGCCACCCCTGCTG      1771

301 --GCAA-GGCC---CNTANN-----AN-TNT-T-TTNTTNAAGGT-CA-      350
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1772 CCGCCACTGGCTGTGCTCCCCCGCCACCTGTGTGTCTTTT-GATACAT      1821

351 --ANNTTNT-TNNAAC-CNAATTCAA-TTNAAGGTGCC-CNACCT-TCA      400
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1822 TTATCTTCTGTTTTTCTCAAATA-AAGTTCAAAG---CAACCACCTGTCA      1871

401 AAA----AGGA--TAANNAT-GTCAGGTAA--AA-CAA-AGTNAATTAGC      450
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1872 CTGGCCAGGCCCTGGTGTGTTGT--GG-AAGGAAGCCTCAGGCACCT-GC      1921

451 TAACTTTAACC--CATTCAACAANGTAANANATTT-C-CAAACCTTCAGT      500
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1922 CA--TTTG-CTGGCTTTCAGGA-G-TCAT-C-TTGCTCAGGCC--C-GT      1971

501 CAAACNAGAGAAAAACATA-----A-ATT--T-TA--TCTG-TGGATCTA      550
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1972 G---CTGG-GC---CATGTGGGTACACTGGTGTAGGT-TGCTGGA-C-A      2021

```

551 -A-----A---A-A---A-AAATA-A-A--T-T--CGN--GGAAAAAAA 600
 | | | | | | | | | | | | | | | | | |
 2022 CAGGCTGACTCACATCCATAAAGACAGAGGTCTTAGGGCCGGGC----- 2071

 601 G-AGAGGAATCATGTTCC-ACA-TCCCAGCATTAACTTCGCTGACAATGT 650
 | | | | | | | | | | | | | | | | | |
 2072 GCAGTGGC-TCATA--CCTACAATCCCAGCACT---TTGGGGGG---T-T 2121

 651 GAACTGCCAGAAGCAGT--T--AAGGAAAGAATACTCAAATAG-CTC--C 700
 | | | | | | | | | | | | | | | | | |
 2122 GAA--GC-AGGAGGAGTGCTTGAAGCCAAGAGT--TC---TAGAC-CAGC 2171

 701 CATGGNGTGAA-A-AC-A-GATCT-TCAT-TATCAC-TCTANATG-GGAT 750
 | | | | | | | | | | | | | | | | | |
 2172 C-TGGAC--AACATAGTAAGA-CTGTC-TCTAAAAAATAAAAAATTAGG-- 2221

 751 TCNGNGTTG-A-TGATACTC-T-TA-T---ATGATGAACGAATGATTTT- 800
 | | | | | | | | | | | | | | | | | |
 2222 -CAGGGTGGTACTGC-ACGCCTGTAGTCCCA-GCT-A-CTCAGGAGGCTG 2271

 801 A---A---A---ATGCTT---CTTTCNACA-T----AATGTA-AGTGATGC 850
 | | | | | | | | | | | | | | | | | |
 2272 AGGCAGGAGGATCGCTTGAGC---CCAGAGTTGTGAAGGTACAGTGA-GC 2321

 851 TTAAGACAGAGGCTTTTCTTTTGTAGTTTGTTCATATTAAACACTC 900
 | | | | | | | | | | | | | | | | | |
 2322 T-AA--CA-----T---C-----GT---GC--CAT-TG----CACTC 2371

 901 AAGACA---CAATAAAA-AATTTATCTT-TCTTCA---CATGAGCCAAGC 950
 | | | | | | | | | | | | | | | | | |
 2372 CAGCCTGGGCAACAGAACAG--ATCCTGTCT-CAAAACA--A-CCAAAA 2421

 951 GGCCCTCAGCCTCCATANACACCAGCA---AGA-----TCTTGATCATCG 1000
 | | | | | | | | | | | | | | | | | |
 2422 G-CCC--AG-----AGAGA-A--AG-AGTGAGACCCCATCTT--T-A--- 2471

 1001 GGAATATGGATATATNACATAATAAA--TCAATGCTGTATTAAAGACAAGA 1050
 | | | | | | | | | | | | | | | | | |
 2472 --AA-A-GAA-A-A-AA-A-AA-AAAGGTCA-TG----ATT--G-CAAGG 2521

 1051 -CTCTATT-CACTCTATT-CT--A---TGGG-ACTAGTGAAGAAGTTCTT 1100
 | | | | | | | | | | | | | | | | | |
 2522 TCACGATTGCAAT-TAAACTGTAAAGGTGGGGA--AG-GAGGAGGA---- 2571

 1101 TTATATAAACAGAGTGTTTTCATTACCATCTCTCTGAGTTTCTGTAGA 1150
 | | | | | | | | | | | | | | | | | |
 2572 --A-ATAA---GAG-----A--AGCA-C-CT--GAGG----CT-T-GA 2621

 1151 CAGTTCAAAATGTTTATGTATTTA--TT---TC--AT-T-AA-----A-A 1200
 | | | | | | | | | | | | | | | | | |
 2622 --GTTCTCAG-G---A-GCACCTAGGTTGGGTCCCAGGTGAAGGGGCACA 2671

 1201 GTAG-TATCTT-CTTATCGCATATAAATACACTG-GTCACTTAATAGAA- 1250
 | | | | | | | | | | | | | | | | | |
 2672 G-AGGTAA-TTGC--A-C-C-T-----CA--GAG-C--TGA-TGGGAG 2721

 1251 -ACTTTGCTTT-T-AT..... 1300
 | | | | | | | | | | | | | | | | | |
 2722 GA-TTA-CTATGTCA..... 2771

**Alignment Of DNA 2/Region 1 (Normal
Strand), And The Most Similar-sized,
Homologous Area Of Human hsp 27**

(The top row shows the DNA 2/Region 1 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

```

1 GAC-TATCTGGTAAGTACTCTATACCTCTACACACCTTTT----A-AAG-      50
  || || || || || || || || || || || || || || || || || || ||
1639 CACCT-TC-G--A-GT-CGCGGG-CC-C-AG-C---TTGGGGGCAGAAGC      1688

51 TG-AATTGA-CCTATTTG-CTTTTCTAG--AATTTTAAAAATTTTGTGTT      100
  || || || || || || || || || || || || || || || || || || ||
1689 TGCAAA--ATCCGATGAGACTGC--C--GCCAAGT---AAAGCCTTAG--      1738

101 CCACCGTTAA--ACCCCAAGTTGATTCTCGACTCAACTGAAAACCTATTCTT      150
  || || || || || || || || || || || || || || || || || || ||
1739 CC-CGGATGCCACCCCTGCTG--C-CG-C-CA-CTGG---CTGTGCCT      1788

151 CTTTCGGAAACCTTCATTG--AC-TAT-TCAAAGTTCTTCTTTATTC-TT      200
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1789 C---C-----CC--C---GCCACCTGTGT----GTTCTT-TTGATACATT      1838

201 TAGTGCCATCCTCTGTATATTACCCTTATTACCCTTCAAA-CTACCTAT-      250
  || || || || || || || || || || || || || || || || || || ||
1839 TA-T-C--T--TCTGTTT-TT-CTCAAATAAAG-TTCAAAGCAACC-ACC      1888

251 TGTAAC TA-CATAT----TG-T-TTT-T--ATGTCCATC-TC---C-CCT      300
  || || || || || || || || || || || || || || || || || || ||
1889 TGTCAC TGGCCAGGCCCTGGTGTGTTGTTGGAAGGA-AGCCTCAGGCACCT      1938

301 A--ATAAACAGTGAGCTCCTTCAAGAAAGANT-ATCCTT-C-CA--CC--      350
  || || || || || || || || || || || || || || || || || || ||
1939 GCCATTTGC--TG-GCT--TTCA-G---GAGTCATCTTTGCTCAGGCCCG      1988

351 T-C----CCTTTTCCGCATCACTAAAACAGG-GTAGGTAGGCACTGATT      400
  || || || || || || || || || || || || || || || || || || ||
1989 TGCTGGGCCATGTGG-GTA-CACT-----GGTGTAGGTTG-C--TG---      2038

401 AAGTACATTATTTCAGTTCATCACTCAGTATTGGACATATATTCTTGAGA      450
  || || || || || || || || || || || || || || || || || || ||
2039 --G-ACA-----CAGG-C-TGACTCAC-ATC---CATAAAGA-CA-GAGG      2088

451 ATTTACTAAAGGCCTTGCTTGC-GTGG-T--T-C-TAGGCA-TCGT-GAA      500
  || || || || || || || || || || || || || || || || || || ||
2089 -TCT--TAG-GGCCGGGC--GCAGTGGCTCATACCTA--CAATCCCAGCA      2138

501 ATT-GAGTGGT-GAA-CAAAAC-A-T--TTAAACCCCTT-GTCCTCATGG      550
  || || || || || || || || || || || || || || || || || || ||
2139 CTTTGGGGGTTGAAGCAGGAGGAGTGCTTGAAGCCAAGAGTTCT-A-G-      2188

551 GACTTTATGTTCTTGGGGANAGA-AT--TA-GA-T-----AACATACAT      600
  || || || || || || || || || || || || || || || || || || ||
2189 -ACC--A-G--CCTGG--ACA-ACATAGTAAGACTGTCTCTAA-A-A-A-      2238

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601	ATAAAAAC-A--CAG--TG-TATTTTACATGGTCCTTNGAAGAAAATTA	650
2239	ATAAAAATTAGGCAGGGTGGTACTGC-AC--G--CCTGT-A-G----TCC	2288
651	-ACATANATAAGGAAG-TG-G--A--AGTAT---TT-AGGTG-GAANTGT	700
2289	CAGCTAC-TCAGGAGGCTGAGGCAGGAGGATCGCTTGAGCCCAGAGTTGT	2338
701	CCTGGAAGGT-CNCT----TNA-ATGTNGGNGACCANTG-ANTA-A---T	750
2339	---G-AAGGTACAGTGAGCTAACAT--CG-TG-CCATTGCACTCCAGCCT	2388
751	G---AACTTGAANT-GTTT-TGTNTTGAAGAAACNACTTTTGAACCC	800
2389	GGGCAACA-GAACAAGATCCTGTCTC-AA-AA-CAACC-----AAAAGCC	2438
801	CGGTGGAANAAA-ATTT---CCNCT-TTGNAGGAAACNCCCCCAAAG	850
2439	CAGAG-A-GAAAGAGTGAGACCCATCTT-TA--AAAG-A-----AAA-	2488
851	GNNAAAAACC-CNTG---G---G-T-----TTGAAANTT---CTTT---	900
2489	-AAAAAAGGTGATGATTGCAAGGTCACGATTGCAATTAAACTGTAAG	2538
901	-TN---AA--A--A--AN---GNCCNCGTNNCTG-GG---GANNNN-A	950
2539	GTGGGGAAGGAGGAGGAATAAGAGAA-GCACCTGAGGCTTGAGTTCTCA	2588
951	--ANCTC--A--TT---TCN-AGGTGNAANAAAAANANAANNCTATNT	1000
2589	GGAGCACTAGGTTGGGTCCCAGGT-GAAGGGGCA-CAGA-GG-TAATTG	2638
1001	CNCNN-A---CNN-TGCCNNGGA--AN-----CN.....	1050
2639	CACCTCAGAGCTGATGG-GAGGATTACTATGTCA.....	2688

Alignment Of DNA 2/Region 1

(Complementary Strand), And

The Most Similar-sized,

Homologous Area Of

Human hsp 27

(The top row shows the DNA 2/Region 1 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

```

0 .NGNTTC-----CNNGG-CANNG-TNNGNG--ANA---TN-AGNNTTNTNT      49
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1639 CACCTTCGAGTCGCGGGGCCAGCTTGGGGGCAGAAGCTGCAAAATCCGAT      1588
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
50 TTTTNTTNGACCTN--GA-AA-TGA-GN-TTNNNNN---TCCCCAGNNA      99
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1689 -----G--AGAC-TGCCGCCAAGTAAAGCCTTAGCCCGGATGCCACCCC-      1738
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
100 C-GN-G--GNCNTTTTNNAAAAGAANT-T---TCAAACCCANGGGTTTTT      149
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1739 CTGCTGCCGCCACT---G---G--CTGTGCCTC---CCC-CGC-----      1788
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
150 TNNCCTTTGGGGGGGGTTTCCTNCAAANGNGGA-A-ATTT-TNNTCCAC      199
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1789 -CACCTGTG-----TG-TT-C-TT----T-TG-ATACATTTATCTTCT--      1938
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
200 CGGGGTTTT-TCAAA-AAGTNGTTTCTTCAANACAAAACAN-T-TCAA-GT      249
   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1839 -GTT-TTCTCAAATAA--AGTT-CA--AAG-CAAC-CACCTGTCACTGG      1888
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
250 TCATTANTCANTGGTCNCCACAT-TN-AAGNGA-CCTTCCAGGACANTT      299
   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1889 CCC--AGGCCCTGGTGT--T---TGTGGAAGGAAGCCT-C-AGG-CACCT      1938
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
300 -CCACCTAAATACTTCCACTTCCTTATNTA-TCTTAAT-TTT-CT-----      349
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1939 GCCAT-T---TGCTGGCT-TTC---AGG-AGTC--A-TCTTTGCTCAGGC      1988
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
350 ---T-CNNAGGACCATGTAAAAATACACTGTGTTTTATATGTATGTT--      399
   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
1989 CCGTGCTG-GG-CCATGTGGG--TACACTG-GTGT--AG--GT-TGCTGG      2038
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
400 ATCTA-ATTCTN--TC-C--CCAGGAACATA-A-AG-TCCCATGAGGACA      449
   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
2039 A-C-ACAGGCTGACTCACATCCAT-AA-AGACAGAGGTCTTA-G-GGCC-      2088
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
450 AGGG-GTTTAAATGTTTGTTCACCAC-T-CAATTTACAG-A-T--GCC-      499
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
2089 -GGGCGC--AG-TG----GCTCAT-ACCTACAATCCCA-GCACTTTGGGG      2138
   |||  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
500 --TAGAACCACGCAAGCAAG-GCCTTTA-GT-AA-A-TTCTC-A--AG--      549
   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
2139 GGTGAAGCAGG-AGG-A-GTGC-TTGAAGCCAAGAGTTCTAGACCAGCC      2188

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550 ---A-AATATATGTCCAATACTGAGTGATGAACTGAATAATGTACTTAAT 599
    | | | | | | | | | | | | | | | | | | | | | |
2189 TGGACAACATA-GT--AAGACTGTCTC-TAAAA--AATAAAA-A-TTAGG 2238

600 CAG--TGCCTACCT--AC-CCTGTTTT--AG-TGA-TGC-GGAAAAAGG 649
    | | | | | | | | | | | | | | | | | | | | | |
2239 CAGGGTGG-TAC-TGCACGCCTGTAGTCCAGCT-ACT-CAGGA----GG 2288

650 --GAGGT-GGAAGGATANTCTTT--C-----TTG--AAGG-A--G---- 699
    | | | | | | | | | | | | | | | | | | | | | |
2289 CTGAGGCAGGA-GGATCG-CTTGAGCCCAGAGTGTGAAGGTACAGTGAG 2338

700 CTCAC-T-GTTT-ATT--AGGGGAGA-TGGACATAAA-AACAATATG-TA 749
    | | | | | | | | | | | | | | | | | | | | | |
2339 CTAACATCGTGCCATTGCACTCCAGCCTGGGCA-ACAGAACAAGATCCT- 2388

750 GT-TACAATAGGTAGTTTGAAGGGTAATAAGG---GTA-ATATACAGAG- 799
    | | | | | | | | | | | | | | | | | | | | | |
2389 GTCT-CAA-A---AC----AACC--AA-AAGCCCAG-AGAGA-A-AGACT 2438

800 GATGGC---A-CT--AAA-GAATAAGAAGAACTTTGAATAG-TCAATGA 849
    | | | | | | | | | | | | | | | | | | | | | |
2439 GA-GACCCCATCTTTAAAAGAA-AAA-AA-AA-----AA-AGGTCA-TGA 2488

850 AGGTTTCCGAAAGAAGAAT-A-GTTTTCAAGTTGAGTCGAGAA-TC-AAC- 899
    | | | | | | | | | | | | | | | | | | | | | |
2489 ---TTGC--AA-G--G--TCACGATTGCAATT-A----A-AACTGTAAGG 2538

900 TGGGGTTTAAACGGTGA--ACAA-AA-ATTTTAA--A-----A-----A- 949
    | | | | | | | | | | | | | | | | | | | | | |
2539 TGGGG---AA-GGAGGAGGA-AATAAGAG---AAGCACCTGAGGCTTGAG 2588

950 TTCT-AGAAAAAGCAAATAGGTCAAT---TCACTT-T-AA-----A-AG- 999
    | | | | | | | | | | | | | | | | | | | | | |
2589 TTCTCAGGA---GCACCTAGGT---TGGGTCCAGGTGAAGGGGCACAGA 2638

1000 G-TG-T-G-----T-AGAGGT-ATAG-AGTACTTACCAGATAGTC..... 1049
    | | | | | | | | | | | | | | | | | | | | | |
2639 GGTAAATTGCACCTCAGAGCTGATGGGAGGA-TTACTA--T-GTCA..... 2688

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**Alignment Of DNA 2/Region 2 (Normal
Strand), And The Most Similar-sized,
Homologous Area Of Human hsp 27**

(The top row shows the DNA 2/Region 2 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

```

1 CTGCAGGT-CAACGGGATCTT---TGGCA--TGATATTTATACACTGTCT      50
   || || || || || || || || || || || || || || || || || || ||
1542 ....AGTTTC--C----TCCTCCCTGTCCCCTGAGGGC-A--CACTG---      1591

51 CATTTTCTTCGTTTGTATATTGGGG---ATGTTTATAATTATTTTGC-AG      100
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1592 -A----C--CGTG-G-A----GGCCCCCATGCCCA-AGCTA----GCCAC      1641

101 GATTATTTTGAATGTGAGAAAT-A--AT---A-TCTTTAATTT-GATTTT      150
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1642 GC--AGTCC-AACG--AGA--TCACCATCCCAGTC---ACCTTCGAGTCG      1691

151 CTMTTGTT-AGCTAATTAAATGGTAACTAA-CTTTTATTCATATAATTAG      200
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1692 CGG--GCCCAGCT--TGGG--GGCAG--AAGCTG-----CA-A-AATCCG      1741

201 TATG-GTCT-CATCACACAAG----GCCCTTTGCAAC--ATTGCTCCTAC      250
   || | || | | | | | | | | | | | | | | | | | | | | | | | |
1742 -ATGAGACTGC--CGC-CAAGTAAAGCC-TTAGCC-CGGAT-GC-CC-AC      1791

251 TTCTATGTCTAAGTTCTGCTTCACCTTCCTATGCTCCATCCATTTCAATC      300
   || | || | | | | | | | | | | | | | | | | | | | | | | | |
1792 CCCT--G-CT--GC-C-GC--CACTGGC-TGTGC-C--TCC----C----C      1841

301 ACCCCAGACGTCTCTGTATAAATTGGCCATGTCCTATCACAACCTCTAATC      350
   | || | | | | | | | | | | | | | | | | | | | | | | | | |
1842 -CGCCAC-C-TGTGTGT-TC-TTT-----TG-----AT-ACAT-T-TA-TC      1891

351 TTGT-TTGTATTCCTGGAAT---GCCCTGAAA--AACT-CCT---ACT--      400
   || | || | | | | | | | | | | | | | | | | | | | | | | | |
1892 TTCTGTT-T-TTC-TCAAATAAAGTTC--AAAGCAACCACCTGTCACTGG      1941

401 C--AGAC--T--TA-----AA--AAGCC--AG-C---T-CAAATATTG      450
   | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1942 CCCAGGCCCTGGTGTGTGTGGAAGGAAGCCTCAGGCACCTGCCA-T-TTG      1991

451 -TGTTCTT-C-GTGA-T--TCTTT--TC---CT--T-CTT--C-AT-TGA      500
   || | || | | | | | | | | | | | | | | | | | | | | | | | |
1992 CTGG-CTTTCAG-GAGTCATCTTGTCTCAGGCCCGTGTGGGCCATGTG-      2041

501 GGTA----G----AG-T--CT--A-ACA--CTT-CTTCACACACCACTAA      550
   || | | | | | | | | | | | | | | | | | | | | | | | | | |
2042 GGTACACTGGTGTAGTTGCTGGACACAGGCTGACT-CACAT-CCA-TAA      2091

551 CAAACT-AGGT-TT-GAA-----A-TG--T--TACTTTTGTACATTT      600
   | || | || | | | | | | | | | | | | | | | | | | | | | | |
2092 -AGACAGAGGTCTTAGGGCCGGGCGCAGTGGCTCATACCT----ACAATC      2141

601 A-AGAANCTTTT-----TT-A--CATTTTTCATT--TAAA---AACATT      650
   || | || | | | | | | | | | | | | | | | | | | | | | | | |
2142 CCAGCAC-TTTGGGGGGTTGAAGCAGGAGG-AGTGCTTGAAGCCAAGAGT      2191

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651	T-TANT--AGCN---A-AA-ATATTNTAACC GAANAGGAGTACATNCTAA	700
2192	TCTAGACCAGCCTGGACAACATA--GTAA--GACT-G---T-C-TC-TAA	2241
701	CGAATGTAATTTAAATAAG--A---T--TA-T-----TGTA-TAA-A-	750
2242	AAAAAT--AA---AAATTAGGCAGGGTGGTACTGCACGCCCTGTAGTCCCAG	2291
751	C-A-T-AG-ATGTTTAA---AT-A--A-C--T-G-GT--A-A-TCTG-GA	800
2292	CTACTCAGGAGGCTG-ACGCAGGAGGATCGCTTGAGCCCAGAGT-TGTGA	2341
801	AAAAAGAA-A-T-AN--AACA-CA-GCC-TGGTA-TC-A-C-TGAAGCNC	850
2342	AG---GTACAGTGAGCTAATCATCGTGCCATTCGACTCCAGCCTGG-GCAA	2391
851	CT---CTAATG-TCCT-TCCATGAATAACCACCTAATCCCGNTGCCCTCT	900
2392	CAGAAC-AA-GATCCTGTC--TCAA-AACAACCAAA-----A-GCC-CA-	2441
901	TGAANTTAANAAATAATTTTGANTTTGGTGAANANTTTTPTTTANAAATT	950
2442	-GA-G--A-GAAA-----GAGT--GA-GACCCCATCTTT--AAAA---	2491
951	CGCCNANNTTTAATCCGAA--TC-T--TTGGAAACAT-ACGTTTTTTCTA	1000
2492	-GAAAAAAA-AA---AAGGTCATGATTGCAAGG-TCACGATTG--CAA	2541
1001	TTTTNAAACTGCCCA--TGN--AATGGAGGTNGCTGAANMAA-ATTTTT	1050
2542	TT--AAAAGTGA-AGGTGGGGAA-GGAGG--AG--GAAATAAGA-----	2591
1051	GAAA-ANTTTCTTTNACTGGTCTGNAAAAANTTTT-A--A-CAT-TN--T	1100
2592	GAAGCAC---CT--GA--GG-CTTGA----GTTCTCAGGAGCACCTAGGT	2641
1101	TCNCCGACCNNCCANNTNCTCCTNAGC--CAAAAAA-AAAN-GNA-----A	1150
2642	TGG--G---TCCCAGGTG-----AAGGGGCACAGAGGTAATTGCACCTCA	2691
1151	-A--TNNTTTTN---TNNCNT-T.....	1200
2692	GAGCTGATGGGAGGATTACTATGTCA.....	2741

Alignment Of DNA 2/Region 2

(Complementary Strand), And

The Most Similar-sized,

Homologous Area Of

Human hsp 27

(The top row shows the DNA 2/Region 2 nucleotide sequence).

(The bottom row shows the human hsp 27 gene sequence).

```

1 AANNANANAAAAANNATTTCNTTTTTTTTTGGCTNAGGAGNANN-TGGN 50
  | | | | | | | | | | | | | | | | | | | | | |
1545 .AGTTTCCT-----CC-T--CCCTGTCCC-----CTGAGG-GCACACTGAC 1594
      51 NGGTCGGNGAANA--ATGTTAAANNTTTTNC-A-GACCAGTNAAA-GAAA 100
      | | | | | | | | | | | | | | | | | | | | | |
1595 CG-T-GGAGGCCCCCATGCCCAAGCTAG--CCACG-C-AGTCCAACGAGA 1644
      101 NTTTTCAAAAAATTNTTCAGCNAACCTCC-ATTN-CATGGGC--AGTTT 150
      | | | | | | | | | | | | | | | | | | | | | |
1645 T---CACCA-T--CC--CAGTCA-CCTTCGAGTCGC--GGGCCCAGCTT 1694
      151 NAAAAATAGAAAAAACGTATGTTTCCA-AAGATTGGGATTAAANNTNGCG 200
      | | | | | | | | | | | | | | | | | | | | | |
1695 GG-----G-----G---G---C-AGAAGCTGC--A--AAATC-CG--- 1744
      201 AATTNTAAAAAANNTTTCACCAAANTCAAAATTATTTNTTAANTTCA 250
      | | | | | | | | | | | | | | | | | | | | | |
1745 A-T--G-AGA-----CTGC-CGCCAA-GT-AAAGC-----CTTAGCC-C- 1794
      251 AGGAGGC--ANC---G--G--ATTAGG-TGGTTATTCATGGAAGG-A 300
      | | | | | | | | | | | | | | | | | | | | | |
1795 -GGATGCCCACCCCTGCTGCCGCCACT-GGCTG-TGCCTCCCCC--GCCA 1844
      301 CATTAGAGGNGCTTCAGT-GATACCAGGCTGTGTTNTATTCT-TTTTTC 350
      | | | | | | | | | | | | | | | | | | | | | |
1845 CCT--GTG-TG-TTCTTTTGATAC-A---T-T-T-ATCTT-CTGTTTTTC 1894
      351 -CAGATTACCACTT-ATTAA--AAC-ATCTATGTTTA-TA-CA-ATAATC 400
      | | | | | | | | | | | | | | | | | | | | | |
1895 TCAAATAA--AGTTCA--AAGCAACCACCT--GTC-ACTGGCCCAGGCCC 1944
      401 T--TATTT-----AA--ATTA-CATTCGTTAGNATGTAC-T-CCTNTT-C- 450
      | | | | | | | | | | | | | | | | | | | | | |
1945 TGGTGTTTGTGGAAGGA--AGCCT-C---AGGC---ACCTGCCATTGCT 1994
      451 GG-TTANA--A-T-ATTTTNGCT-ANTAAATGTT-----T-T---TAA 500
      | | | | | | | | | | | | | | | | | | | | | |
1995 GGCTTTCAGGAGTCATCTTTGCTCAGGCCCGTGTGGCCATGTGGGTAC 2044
      501 AATGAAAAATGTAAAAAAGTTT-CCTT-A-A-ATG-T-ACAAA-AGTAACA 550
      | | | | | | | | | | | | | | | | | | | | | |
2045 ACTGG-----TGTA-----GGTTGCTGGACACAGGCTGACTCACA-TC-CA 2094

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551 TTTCAA-ACCTAGTTTGTAGTGGT-GTGTGAAGAAGTGTAGACTC-TA 600
 | | | | | | | | | | | | | | | | | | | |
 2095 TA--AAGACAGAGGTC-TTAG-GGCCG-G-GC-GCAGTG---G-CTCATA 2144

 601 CCT-CAATGA-AGAA----GGA-----AA--AG-A--A-T-CACGAAGA 650
 | | | | | | | | | | | | | | | | | | | |
 2145 CCTACAATCCCAGCACTTTGGGGGTTGAAGCAGGAGGAGTGCTTGAAG- 2194

 651 ACACAATA-TT-T-GA---GC-TGG-CTTTTAAAGTC-TGAGTAGGAGTT 700
 | | | | | | | | | | | | | | | | | | | |
 2195 -C-CAAGAGTTCTAGACCAGCCTGGAC-----AA--CAT-AGTA--AG-- 2244

 701 TTTCAGGGCAT-TC-C-AGGAATACA-AACAAGATTAG--AGTTGTGATA 750
 | | | | | | | | | | | | | | | | | | | |
 2245 ----A---C-TGTCTCTAA-AA-A-ATAA-AA-ATTAGGCAG--G-G-T- 2294

 751 GG-ACATG---GCCAAT-TA-TAC-AGAGACGTCTGG-GG-TGATTGAA 800
 | | | | | | | | | | | | | | | | | | | |
 2295 GGTAC-TGCACGCC---TGTAAGTCCCAGCTAC-TCAGGAGGCTGAG-GCA 2344

 801 --ATGGAT-G---GAGC--ATAG--GA-AAGTG-A-AGC-AG--AAC-T- 850
 | | | | | | | | | | | | | | | | | | | |
 2345 GGA-GGATCGCTTGAGCCCAGAGTTGTGAAG-GTACAGTGAGCTAACATC 2394

 851 -TAGACATAG-A-----AG--TAGGAGCAAT-GTTGCAAAGGGCCTTGTGT 900
 | | | | | | | | | | | | | | | | | | | |
 2395 GT-GCCATTGCACTCCAGCCT-GG-GCAACAGAA-CAA-GATCCT-GTCT 2444

 901 GATGAGACCATACTAATTAT---ATGA-ATAAA-AGTTAGTTACC--ATT 950
 | | | | | | | | | | | | | | | | | | | |
 2445 CAA-A-AC-A-ACCAAA-AGCCCA-GAGAGAAAGAGTGAG--ACCCCATC 2494

 951 TAATTAGCTAACAAAAGAAAATCAAATTAAAGAT-ATTATTTTC----TCA 1000
 | | | | | | | | | | | | | | | | | | | |
 2495 T--TTA---AA-AGAA-AAAA--AAA--AAAGTTCATGATTGCAAGGTCA 2544

 1001 C-ATT-CAA--AATTAATCCTGCAAAATAAT-TAT--AA--AC-ATCCCCA 1050
 | | | | | | | | | | | | | | | | | | | |
 2545 CGATTGCAATTAA-AA-C-TG-----TAAGGTGGGGAAGGAGGAGG---A 2594

 1051 ATATACAAACGAAGAAAATGAGAC---AGTGTAT-A--A--A--TA--T- 1100
 | | | | | | | | | | | | | | | | | | | |
 2595 A-ATA-AGA-GAAGCACCTGAGGCTTGAGT-TCTCAGGAGCACCTAGGTT 2644

 1101 ----C--A--TGC-----CA-A-AG--A-T--C-CC---G---T--TG-- 1150
 | | | | | | | | | | | | | | | | | | | |
 2645 GGGTCCCAGGTGAAGGGGCACAGAGGTAATTGCACCTCAGAGCTGATGGG 2694

 1151 AC-----CT--G-CAG..... 1200
 | | | | |
 2695 AGGATTACTATGTCA..... 2744

APPENDIX VI

**Alignment Of DNA 1/Region 1 (Normal
Strand), And DNA 2/Region 1
(Normal Strand)**

(The top row shows the DNA 1/Region 1 nucleotide sequence).

(The bottom row shows the DNA 2/Region 1 nucleotide sequence).

-2	...TATCTGGTA----CTCTATACCTCTACACACCTTTTAAAGTGAATTG	47
1	GACTATCTGGTAAGTACTCTATACCTCTACACACCTTTTAAAGTGAATTG	50
48	ACCTATTTGCTTTTCTAGAATTTTAAATTTTGTTCACCGTTAAAC	97
51	ACCTATTTGCTTTTCTAGAATTTTAAATTTTGTTCACCGTTAAAC	100
98	CCCAGTTGATTCTCGACTCAACTGAAACTATTCTTCTTCGGAAACCTT	147
101	CCCAGTTGATTCTCGACTCAACTGAAACTATTCTTCTTCGGAAACCTT	150
148	CATTGACTATTCAAAGTTCTTCTTATCTTTAGTGCCATCCTCTGTATA	197
151	CATTGACTATTCAAAGTTCTTCTTATCTTTAGTGCCATCCTCTGTATA	200
198	TTACCCCTTATTACCCCTTCAAACCTACCTATTGTAACCTACATATTGTTTTA	247
201	TTACCCCTTATTACCCCTTCAAACCTACCTATTGTAACCTACATATTGTTTTA	250
248	TGTCCATCTCCCCTAATAAACAGTGAGCTCCTTCAAGAAAGAGTATCCTT	297
251	TGTCCATCTCCCCTAATAAACAGTGAGCTCCTTCAAGAAAGANTATCCTT	300
298	CCACCTCCCCTTTTCCGCATCACTAGAACAGGGTAGGTAGGCACTGATTA	347
301	CCACCTCCCCTTTTCCGCATCACTAAACAGGGTAGGTAGGCACTGATTA	350
348	AGTACATTATTCAAGTTCACTCACTCAGTATTGGACATATATTCTTGAGAA	397
351	AGTACATTATTCAAGTTCACTCACTCAGTATTGGACATATATTCTTGAGAA	400
398	TTTACTAAAGGCCTTGCTTGCGTGGTTCTANGCATCGTGAA-TTGAGTGG	447
401	TTTACTAAAGGCCTTGCTTGCGTGGTTCTAGGCATCGTGAAATTGAGTGG	450
448	TGAACAAACATTTAAACCCCTTGTCCTCATGG-ACTTTATGTTCTGCGG	497
451	TGAACAAACATTTAAACCCCTTGTCCTCATGGACTTTATGTTCTGCGG	500
498	GACAGAATTAGATAACATACATATAAAACACAGTGTTATTTTACATGG	547
501	GANAGAATTAGATAACATACATATAAAACACAGTGT-ATTTTACATGG	550
548	TCNTAAGAAGAAATAAGATAGATAAGGAAGTGGAAGTATTTAGGTGGA	597
551	TCCTNNGAAGAAATAAGATANATAAGGAAGTGGAAGTATTTAGGTGGA	600
598	ATGGTCATGGAAGGTCTCTTAAATGTGG-TGACAAATGATTAATGA-CT-	647
601	ANTGTCCTGGAAGGTCNCTTNAATGTNGNGACCANTGANTAAATGAACCTT	650

**Alignment Of DNA 1/Region 2 (Normal
Strand), And DNA 2/Region 2
(Normal Strand)**

(The top row shows the DNA 1/Region 2 nucleotide sequence).

(The bottom row shows the DNA 2/Region 2 nucleotide sequence).

1	CTGCAGGTCAACGGGATCTTTGGCATGATATTTATACTGTCTCATTTT	50
1	CTGCAGGTCAACGGGATCTTTGGCATGATATTTATACTGTCTCATTTT	50
51	CTTCGTTTGTATATTGGGGATGTTTATAATTATTTTGCAGGATTATTTG	100
51	CTTCGTTTGTATATTGGGGATGTTTATAATTATTTTGCAGGATTATTTG	100
101	AATGTGAGAAATAATATCTTTAATTGATTTTCTTTTGTAGCTAATTAA	150
101	AATGTGAGAAATAATATCTTTAATTGATTTTCTTTTGTAGCTAATTAA	150
151	ATGGTAACTAACTTTTATTCATATAATTAGTATGGTCTCATCACACAAGG	200
151	ATGGTAACTAACTTTTATTCATATAATTAGTATGGTCTCATCACACAAGG	200
201	CCCTTTGCAACATTGCTCCTACTTCTATGTCTAAGTTCTGCTTCACTTTC	250
201	CCCTTTGCAACATTGCTCCTACTTCTATGTCTAAGTTCTGCTTCACTTTC	250
251	CTATGCTCCATCCATTTCAATCACCCAGACGTCTCTGTATAATTTGGCC	300
251	CTATGCTCCATCCATTTCAATCACCCAGACGTCTCTGTATAATTTGGCC	300
301	ATGTCCTATCACAACCTCTAATCTTGTGTTGTATTCCTGGAATGCCCTGAAA	350
301	ATGTCCTATCACAACCTCTAATCTTGTGTTGTATTCCTGGAATGCCCTGAAA	350
351	AACTCCTACTCAGACTTAAAAAGCCAGCTCAAAATATTGTGTTCTTCGTGA	400
351	AACTCCTACTCAGACTTAAAAAGCCAGCTCAAAATATTGTGTTCTTCGTGA	400
401	TTCTTTTCCTTCTTCATGAGGTAGAGTCTAACACTTCTTCACACACCAC	450
401	TTCTTTTCCTTCTTCATGAGGTAGAGTCTAACACTTCTTCACACACCAC	450
451	TAACAACTAGGTTTGAAATGTTACTTTTGTACATTTAAGAACCCTTTT	500
451	TAACAACTAGGTTTGAAATGTTACTTTTGTACATTTAAGAANCCTTTT	500
501	ACATTTTTCATTTTAAAAACATTTTAAATAGCAAAAATATTATAACAGAGA	550
501	ACATTTTTCATTTTAAAAACATTTTANTAGCNAAAATATTNTAACCGAAN	550
551	AGAA-TACATACTAACGAATGTTATTTAAATAAGANTTATTGGTATAAAC	600
551	AGGAGTACATNCTAACGAATGTAATTTAAATTAAGATT-ATTG-TATAAAC	600
601	ATAGATGTTTAAATAACTGGTAATCTGGAAAAA-GAAATAGAACACAGCC	650
601	ATAGATGTTTAAATAACTGGTAATCTGGAAAAAAGAAATANAACACAGCC	650

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651 TGGTATCACTGAAACTCCTCTA-TGTTCTTCCATGAATAACCACTA-TC 700
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
651 TGGTATCACTGAAGCNCCTCTAATGTCCTTCCATGAATAACCACTAATC 700

701 CCCGTGCCTCCTTGGAATTAATAACNAATTTGAATTTTGGTGAANATTT 750
    || ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
701 CCGNTGCCTCCTTGAAANTTAANAATAATTTGANTTTT-GGTGAANANTT 750

751 TTTTAAAAAANTT-GCCTNAATTTTAATCCNGAAACCTTTGGAAAAAATA 800
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
751 TTTTANAAAATTCGCCNANNTTT-AATCCG-AATC-TTTGGAAACA-TA 800

801 CGTTTTTCTATTTTTAAACNGGCNCATGNAAATGGGAAGGTNTGCTGGA 850
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
801 CGTTTTTCTATTTTNAACTG-CCCATGNAA-TGG-A-GGTNTGCTG-A 850

851 AAAAAATTTTTTGANATAATTTCTTTAACTGGGTCTNGNAAAAAATNT 900
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
851 ANNAATTTTTT--GAAA-ANTTTC-TTTNACTGG-TCTGNAAAAANTTTT 900

901 ----TTTTA-ANN-A--AANN-TNCC-C-TGAAC-A-----C-C-- 950
    ||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
901 AACATTNTTCNCCGACCNCCANNNTCTCCTNAGCCAAAAAAAANGNAA 950

951 --CCNNTNTNN..... 1000
    | |
951 ATNNTTTTNTNNCNNTT..... 1000

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**Alignment Of DNA 4 (Normal Strand),
And DNA 2/Region 1 (Normal Strand)**

(The top row shows the DNA 4 nucleotide sequence).

(The bottom row shows the DNA 2/Region 1 nucleotide sequence).

1	TCGACCTTGAATAC-GG-A-G--CTTC-A-AGCCAGGCT-C-CAGCTGGC	50
-1	..GAC-T--A-T-CTGGTAAGTACT-CTATA-CCT--CTACACA-C--C	48
51	GTTTTAG-GTG--TGGTGCAAGCAAGGCTGGGTATG-TATTTCCAGGAAG	100
49	-TTTAAAGTGAAT--TG-AC-CTA---T---T-TGCTTTTCTAG-AAT	98
101	CAGTAGACGA-----GCTCG-CCGTGCAAT---A-T-GAATTATC-ATTA	150
99	TTTTAAA--ATTTTGTTCACCGTTAAACCCCACTGA-TTCTCGACT-	148
151	GCAGCAAGGAAATTCGTCAA-CGATT-TGTT-GGTAAATGAATTCCTGT	200
149	-CAACT-GAAAA--C-T-ATTG--TTCT-TTCGG-AAA-----CCT-T	198
201	CA--GA-TATCCA--GTTGCTTGGGAGAACGCTGCCTTT-GCGCC-TCCT	250
199	CATTGACTATTCAAAGTT-CTTCTTT-AT---T-C-TTTAGTGCCATCCT	248
251	GCGG-ACGGCTCAATCTGGC-TTAAGTACGACTACACAGAAGTTGACACA	300
249	-CTGTA-----T-A-T-TACCCTTAT-TACC-CTTCA-A-AC-T--AC-C-	298
301	GTAACATACGGGTTAAGCAGGAAGTGCAAATACTACGTTGGCATGGTGCA	350
299	-TAT--T--G--T-AA-CT--A-----CA--TA-T---T-G---T--T---	348
351	GATTTTCAGTGTCTTTCAGTCCGGGACTGGGATTGATAAGCCGAGACAAA	400
349	--TTT-A-TGTCCATC--TCCC-----CTA--AT--A-AA-C--AG-----	398
401	T-AGCTAATCAATTGGCA-GAATCTATCGTTGATGGTACAATGCTTGAAC	450
399	TGAGCTC--C--TT--CAAGAA--A--G---AN--TA---TCCTT---C	448
451	AGCGGGACCATTATGAGTCTGGAGTTGTTAACCCGTTATCA--AATCC	500
449	--C---ACC-TCC-----CT-----TT-TT--CC-GC--ATCACTAA---	498
501	AAGTCTGGGT-GGTTTATTCCTGG-TTC-GT---TT-TT-ATGTTCTGCT	550
499	AA--CAGGGTAGGT--AGGCACTGATTAAGTACATTATTCA-GTTCATC-	548
551	AGACTAACAAAA--GGAAAAATA---CATG-GCCCATCTCAGC-AATGGC	600
549	A--CT--CAGTATTGGACATATATTCTTGAGA--ATTT-A-CTAAAGGC	598
601	AC--GCA-G-GTC-TTCGTAGAAAGCTCTCGCGGAACTGCAATCGACGT-	650
599	-CTTGCTGCGTGGTTC-TAG---GCA-TCGTGAAATTG-AGT-G--GTG	648

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651 AACTGCAATTTCTAACGCAGTTA--CCCCTGTTTTAACTGTATCTGACGC      700
    |||  ||      |||  |||  ||||  ||  ||  ||  ||  ||
649 AAC---AA-----AAC--ATTTAAACCCCT-TGTC--CTC-AT--G--G-      698

701 GTCTGGTT-TGGTCGTTGGTGAATACCTGCTTTTCACCTCTTCTGCTTCA      750
    ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
699 GACT--TTATGTTCTCTGGG-GAN-A---GAATT--A-----GAT--A      748

751 ACGCTTCTAACTGACAATCAAGTTCGAATGACTGCAATCCCTGGTACGTC      800
    ||      ||  |||  ||  ||  ||  ||  ||  ||  ||  ||  ||
749 AC-----A--T-ACA-T-A--T---AA--A---AA-CACAG-T--GT-      798

801 AGTAACTGTTGA-AGGTTTGATACCTCCAGCACCACAAAGTTCCCGGCTG      850
    ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
799 ATT---T-TT-ACATG---G-T-CCTNN-GAAG-A-AAA-TTAA-GA-TA      848

851 GCTTAACTGGTGAAGTTGTCAAGATCACCTCCT-GGTTGGAAGT-TCCGT      900
    ||  ||  ||  |||  ||  ||  ||  ||  ||  ||  ||  ||  ||
849 NAT-AA--GG--AAGTGG--AAG-T-AT-T--TAGGTG-GAANTGTCC-T      898

901 GCGTTCAGGACG-TATCTACTGACGGTGGCGAAC-AGCAATTCGTTAACT      950
    ||  |||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
899 G-GA--AGGTCNCT-TNAA-TGTNGNGACCANTGANTAAT--G--AACT      948

951 TCCAGTGCTTGTCCGATGACCGTGAACAGCAGATTCCGACCTAT--AAAT      1000
    ||  |||  ||  ||  ||  |||  ||  ||  ||  ||  ||  ||
949 TGAANTGTTT-T--G-TNT---TGAAGA--A-A--CN-ACTTTTTGAAAA      998

1001 CTGCGGT--AACTAACACCTTC-ACCTTCGCTCACGAGTACACCAACCCT      1050
    ||  |||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||
999 CCCCAGGTGGAANAAA-AT-TCCNCNNTTGN--AGGA--A-ACCNCCCCC      1048

1051 GTATATCCGGTTCTGCGTAACTACGATGAGTCTGGCCA-GGTTGTTGCGA      1100
    ||  ||  ||      ||  ||  ||  ||  ||  ||  ||  ||  ||
1049 CAA-A---GNNA-----AA--A--A--A--C---CNTGGGT-TTGAAA      1098

1101 TTCGTCTGTTTCG----T-ACCTCG-AGCTAGCGAAATGC--GCTTGCAGT      1150
    ||  |||  ||      ||  ||  ||  ||  ||  ||  ||  ||
1099 NT--TCTTTTNAAAAANGNCCNCGTNNCTGGGGANNNNAANCT--CATT      1148

1151 -CC-GGTACTATCGCTTTCAACGACACCCCTACCATTTGGTGTTAACGAAA      1200
    ||  |||  ||  ||  ||  ||  ||  ||  ||  ||  ||
1149 TCNAGGT-CNAANAAAA--AAN-ANAANNCTN--ATN--T-----CN---      1198

1201 TCGAAACGGTATCCATCGCGGTATCCATTCGTG.....      1250
    ||  ||  ||  ||  ||  ||  ||  ||
1199 -CNNA-CNNTG-CCNN-G-G--A--AN-CN.....      1248

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**Alignment Of DNA 4 (Normal Strand),
And DNA 2/Region 2 (Normal Strand)**

(The top row shows the DNA 4 nucleotide sequence).

(The bottom row shows the DNA 2/Region 2 nucleotide sequence).

1	TCGACCTTGAATACGGAGCTTCAAGCCAGGCTCCAGCTGGCGTTTATAGGT	50
-4CT-GCA---GG---T-CAA-CG-GGATCTT--TGGCATGATA--T	45
51	GTGGTGCA-AGCAAGGCTGGGT-ATGTAT-TTCCAG---G-A-A--GCAG	100
46	-T--T--ATA-CA---CTGTCTCATTT-TCTTC--GTTTGTATATTG--G	95
101	TAGACGAGCTCGCCGTGCAATATGAATTATCATTAGCAGCAAGGAA-ATT	150
96	--G--GA--T-GT--T----TAT-AATTATT-TT-GCAG---G-ATTATT	145
151	CGTCAACGATTGTGTG-GTAAATGAATTTCTCTGCAGATATCCAGTTGCT	200
146	T-TGAA---T--GT-GAG-AAAT-AATATCTT-T-A-ATTTG-A-TT--T	195
201	TGGGAGAACGCTGCCTTTGCGCCTCCTGCGGACGGCTCAATC---TGGCT	250
196	T-----C--T--TTTG---T--T----A-G-CT-AATTAAATGG-T	245
251	TAAGTACGACT---A--CACAGAA---GT-TGA-CACAGT-A-ACATACG	300
246	-AACTA--ACTTTTATTCATATAATTAGTATGGTCTCA-TCACACA-A-G	295
301	GG--TTAAGCAGGA-AGTGCAAATACTACGTTGGC-ATGGTGC-AGATTT	350
296	GCCCTTT-GCA--ACATTGC---TCCTAC-TT--CTATG-T-CTA-AGTT	345
351	CAGTGTTCCTCAGTC-CGGGGAAGTGGGATTGATAAGCCGAGACAAATAGC	400
346	C--TG--CTTCACTTTC-----CT---AT-GCT---CC-ATCCA--T---	395
401	TAATCAATTGGCAGAATCTATCGTTGATGGTACAATGCTTGAACAGCGGG	450
396	T--TCAAT---CACCC-C-A--G---ACG-T-C--T-CT-GTATA-----	445
451	ACCATTTATGAGTCTGGAGTTGTTAACCCGGTTATCA-AA-TCCAAGTCT	500
446	A---TTT--G-GCC---A-T-GT---CC---T-ATCACAACCTCTAA-TCT	495
501	GGGTGGTT-TATTCCCGGTTTCGTTTATGTTTCGTCTAGACTAACAA---	550
496	TG-T--TTGTATTCCTGGA-----ATGC-C--CT-GA--AA-AACTC	545
551	--A---AGGAA---AAATACATGGCCCATCTCAGCAATGGCAC-GCAGGTC	600
546	CTACTCAG-ACTTAAA-A-A-G-CC-AGCTCA--AAT---ATTGT-GTTC	595
601	TCGTAGAAAGCTCT-CGCGGAAGTCAATCGACGTA-A--CTG-CAATT	650
596	TCGT-GATT-CTTTTC-CTT--CTTCATT-GAGGTAGAGTCTAACACTT	645

651	TCT--A-ACGC-AGTTACCC-CT-GTTTT-AACTGTATCTGACGCGTCTG	700
646	-CTTCACACACCCTAACAACCTAGGTTTGAAATGT-T---AC---T-T-	695
701	GTTTGGT-CGTTGGTGAATA-CCTGCTTTTCACCTCTT-C-TGCTTCAAC	750
696	-TT-G-TACATT--T-AAGAANCCT-TTTT-ACATTTTTCATT-TTAAAA	745
751	GC-TTCTAACT-GACAATCA-AGTT---C-GAAT-G-ACTGCAATCCCTG	800
746	ACATTTTAN-TAGCNAAC-ATATTNTAACCGAANAGGAGTACA-TNC-TA	795
801	GTACGTCA-GTAACTGTGAAG--G-TT-T-GATACCTCCAGCACCACA-	850
796	--ACGA-ATGTAA-T-TTAAATAAGATTATTG-TA--T--A--A--ACAT	845
851	A-A-GTTCCCGGCTGGCT--TAACTGGTGAAGT-TGTCAA---GA--T-C	900
846	AGATGTT-----T---TAATAACTGGT-AA-TCTGGAAAAAAGAAATAN	895
901	A-C-CT-CCTGGT-TC---GAAGTTCCG-T---G-CGTTT-AGGACGTAT	950
896	AACACAGCCTGGTATCACTGAAGCNCCTCTAATGTCCTTCCATGAA-TAA	945
951	CTAC-TGA-C--GGTGGC-----GAAC--AGCAATTCGTAACTTCCAGT	1000
946	CCACCTAATCCCGNTGCCTCCTTGAANTTAANAAATAATT---TTG-ANT	995
1001	GCTTG-TCCGATGACCGTGA---ACAGCAGATTTC-C-GACCTATAAATCT	1050
996	--TTGGT--GAANANTTTTTTTTANA--A-ATTCGCCNANNTTTAA-TC-	1045
1051	GCGGTAA-CT-----AACACCTTCACCTTCGCTC-ACGAGTACACCAAC--	1100
1046	-CG--AATCTTTGGAA-ACAT--ACGTTTTTTCTATTT-TN-A--AACTG	1095
1101	CC--TGTATATCC-GGTTCTGCGT-AACTACGATGAGTCTGGCCAGGTTG	1150
1096	CCCATGNA-ATGGAGGTTN-GC-TGAANNA--A--A-T-T-----TT-	1145
1151	TTGCGA-TTCGTCTGTTTCGTACCTCGAG-CTAGCGAAATGCGCTTGCACT	1200
1146	T-GAAAANTT-TCT-TTN--AC-T-G-GTCT-GNAAAAAN---TT----T	1195
1201	CCGGTA-CTATCGCTTTCAACGACACCCCTACCATTTGGTG-T--TAA-CG	1250
1196	----TAAC-ATTN-TT-CNCCGAC-CNNC--C-ANN--TNCTCCTNAGCC	1245
1251	AAATCGAAACGGTA-TCCATCGCGGTATCCATTGCTG.....	1300
1246	AAAAAAAANGNAAATNNTTTTN--TNCCNNTT.....	1295

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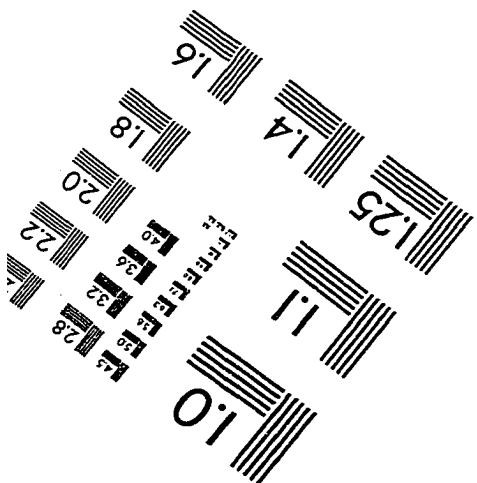
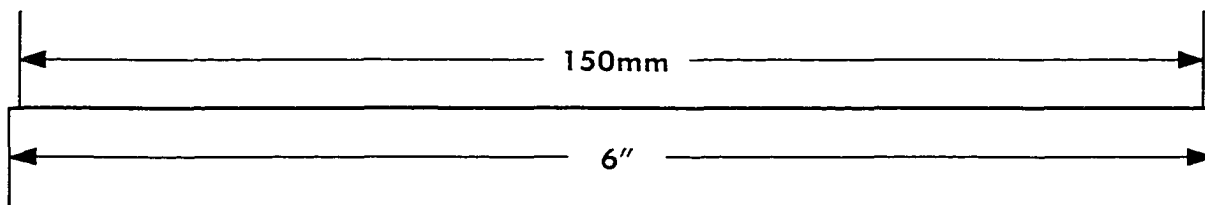
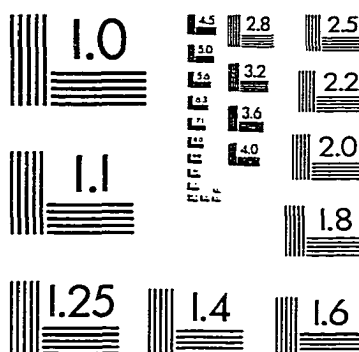
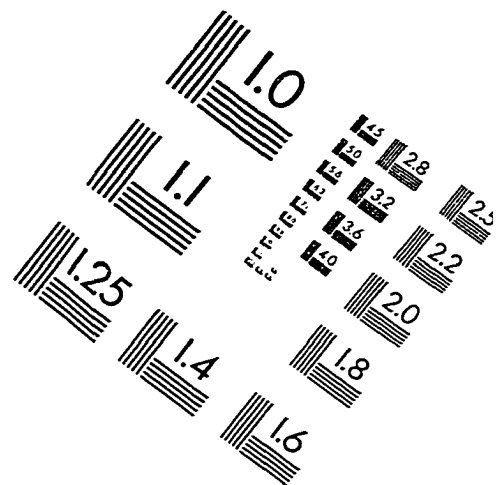
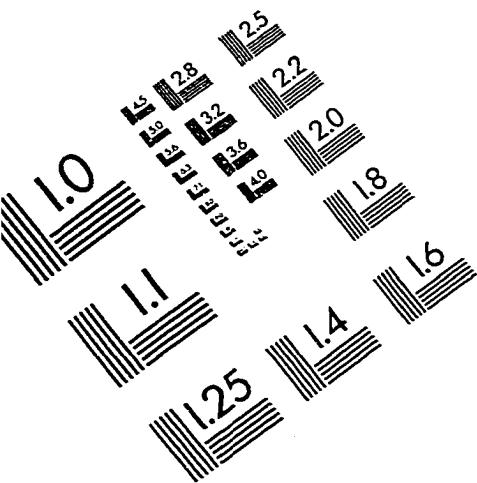
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IMAGE EVALUATION TEST TARGET (QA-3)



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