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# pDEST FG12-CMV DsRed Vector

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Melanoma is the most rapidly increasing malignancy among young people in the United States. If detected early, the disease is easily treated; however, once the disease has metastasized it is largely refractory to conventional therapies and is associated with a high mortality rate. The development of human cancer from a pre-malignant primary tumor to a metastatic lesion that develops at secondary sites is thought to be a multi-step process, requiring many genetic and epigenetic events that provide a growth advantage to cells. It is still unclear which of the many genetic changes in human cancers are required for metastasis. Therefore, it is critical to evaluate each step in the metastatic process. To this end, we will generate novel lentiviral vectors containing fluorescent reporter genes to better understand the metastatic potential of melanoma cells. Vectors containing green fluorescent protein (GFP) have already been generated while vectors containing red fluorescent protein (RFP) and yellow fluorescent protein (YFP) will be cloned. Viruses will be generated and used to infect syngeneic explanted tumor cells. Since each vector will be marked with a reporter gene of a different color, we will be able to track the movement of these cells *in vivo* and determine the source of each metastatic tumor. Whole body fluorescence will be detected using the FluorVivo Imaging System (INDEC BioSystems, Santa Clara, CA). The experiments proposed will contribute to an increased understanding of the biology of melanoma, which has the potential to identify specific molecular targets and promote the development of more effective therapies for advanced stages of this disease.

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## Abstract

Melanoma is the most rapidly increasing malignancy among young people in the United States. If detected early, the disease is easily treated; however, once the disease has metastasized it is largely refractory to conventional therapies and is associated with a high mortality rate. The development of human cancer from a pre-malignant primary tumor to a metastatic lesion that develops at secondary sites is thought to be a multi-step process, requiring many genetic and epigenetic events that provide a growth advantage to cells. It is still unclear which of the many genetic changes in human cancers are required for metastasis. Therefore, it is critical to evaluate each step in the metastatic process. To this end, we will generate novel lentiviral vectors containing fluorescent reporter genes to better understand the metastatic potential of melanoma cells. Vectors containing green fluorescent protein (GFP) have already been generated while vectors containing red fluorescent protein (RFP) and yellow fluorescent protein (YFP) will be cloned. Viruses will be generated and used to infect syngeneic explanted tumor cells. Since each vector will be marked with a reporter gene of a different color, we will be able to track the movement of these cells *in vivo* and determine the source of each metastatic tumor. Whole body fluorescence will be detected using the FluorVivo Imaging System (INDEC BioSystems, Santa Clara, CA). The experiments proposed will contribute to an increased understanding of the biology of melanoma, which has the potential to identify specific molecular targets and promote the development of more effective therapies for advanced stages of this disease.

## Background

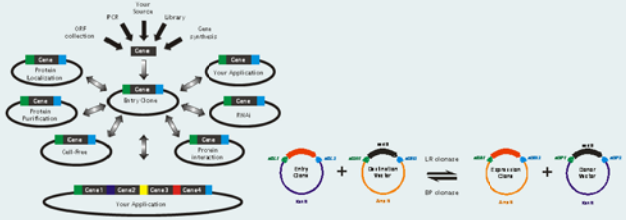
• Lentiviruses: are retroviruses that can affect both diving cells and non dividing cells. These viruses are effective because of their durability, which can penetrate the intact membrane of the nucleus of whatever their target cells are. They are highly effective to the point that they can manipulate the gene of the host cell that they are affecting up to six months.

• Retroviruses: are RNA virus strands that replicated inside a host cell using an enzyme reverse transcriptase to produce new DNA strands using the RNA virus strand as its new template. Retroviruses on the other hand can only replicate when the cell that they are infecting is dividing.

• David Baltimore and Howard Temin were awarded the Nobel Prize in 1975 for their discovery of reverse transcriptase. Reverse transcriptase transcribes a single strand of RNA into a single strand of DNA, which is why it is given the name "reverse."

• The FG12 CMV DsRed Vector is a lentiviral vector that can be used in just about any kind of cancer cell. We are constructing it to express the DsRed gene to allow it to fluoresce a red color while inside a tumor cell. Once the Lentiviral vector infects the cancer cell it will replicate with the tumor cells allowing us to see the cancer in action as it moves to different parts of the body.

• Gateway Cloning: is a major component of our project. This type of cloning is by far one of the least complex ways to insert our target gene into our entry clone. Some advantages that the gateway cloning system has includes: directional cloning, no need for restriction enzymes, no ligation, no resequencing, and reverse reactions. Some of the major benefits include easy shuttling the inserted DNA from on expression plasmid to another.



(Courtesy of the Invitrogen Corporation)

## Objectives

• Generate an FG12 Vector that expressed the DsRed gene.

• Make the FG12 CMV DsRed Vector Gateway compatible.

• Grow up bacterial colonies containing the expressed FG12-CMV-DsRed Vector.

## Methods

• Transformation: this process allows the DNA to enter the competent cells and replicate by heat shock (icing and heating). The icing allows the DNA to mix with the competent cells until heating. While heating for 30 seconds, the competent cells allow the new DNA products to enter the cell. Icing is again repeated in order for the cell to close. Medium is added to the cells to allow them to grow and replicate. Then after incubation the cells are plated on an antibiotic resistant plate to allow only the resistant cells to grow.

• Ligation: is a process that brings your different enzymes, vectors, and DNA into the circle sequence.

• Gel Extraction: a process that allows us to extract a certain positive band or negative band from a gel and further experiment with it.

• Running Gels: By setting up a gel we can use mixtures of DNA and enzymes to cut the DNA so the gel can separate it by size. Using a positive and negative control we can see if our sample is right or if something we have done to it may not have worked the way it was suppose to.

• Growing Culture: We grow cells in culture and infect them with certain viruses, like the FG12, in hopes that we can better understand what types of capabilities these vectors have in the cell.

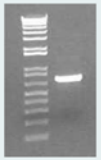
• PCR Machine: this process allows the hydrogen bonds to be broken off the double helix and separated into two strands; which after multiple temperature changes, replicates the sample between the primer sequences. This process continues over twenty five cycles or more depending on your sample.

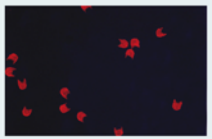
• Nano Drop: used to measure the amount of DNA you have in a sample.

• Maxi Prep: Looking to extract DNA from 500 ml bacterial culture.

• Midi Prep: Looking to extract DNA from 100 ml bacterial culture.

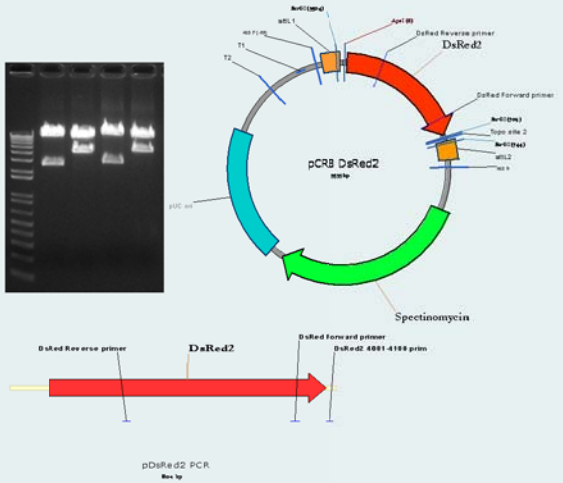
• Mini Prep: Looking to extract DNA from 2 ml of bacterial culture.





*This poster was made possible by NIH Grant Number P20 RR-016464 from the INBRE Program of the National Center for Research Resources.*

Fg12 CMV DsRed Vector Construction







pDsRed2 PCR

## Conclusion

•Through out the whole month of June we transformed, PCR Purified, Ran Gels, and we continued to find that our results kept turning up negative. It wasn't until we did a test digest with the original DNA that we found it to be either too dirty or wrong all together. So we decided to start back at square one and see if we could work with the FG12 DNA and find a way that it could be compatible. A few weeks later during a test digest of a mini prep cutting with Pst I and Not I we found the correct band size that yielded a positive set of results for the FG 12 CMV Vector. (Shown in picture above) The Vector was then grown for Maxi Preps and will be put into the gateway cloning stage.

## Acknowledgements

I would like to thank Dr. Sheri Holmen for allowing me this opportunity to study and learn from her researchers at NVCI in hopes that I will broaden my knowledge in the field of Cancer and maybe one day consider this as a field of study. I would like to thank Kristy Lastwika/ Matt VanBrocklin for working with me personally through out the project and answering any particular questions that came up. I would like to thank the Nevada Cancer Institute for allowing me to be apart of their wonderful work staff and to be apart of all that NVCI has to offer. Lastly I would like to thank the INBRE organization most of all for giving me this wonderful opportunity to learn from these individuals and expand my knowledge in the field of science.

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## Future Note

The FG 12 vector can be cloned using different vectors that will express, and fluoresce different colors in cells. In doing so maybe it will be easier to find certain solutions as to why these cancer cells spread and act the way they do.