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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 an all-or-none 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 proteins (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 inject syngeneic expanded 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 Fluorimaging System (NIDEC Biodynamics, 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.

Objectives
- Generate an FG12 Vector that expresses 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 homologous recombination. The plasmid allows the DNA to recombine with the competent cells until ligation. By using a different and negative method we can see if our sample is right or if something we have done is not working. We did this by doing a cell to cell culture. Then after inoculation the cells are plated on an antibiotic resistant media to allow 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.
- Finishing Gel: By setting up a gel we can mix tides of DNA and enzymes to cut the DNA so the gel can separate it by size. Using a positive and negative method we can see if our sample is right or if something we have done is not working. We did this by doing a cell to cell culture. Then after inoculation the cells are plated on an antibiotic resistant media to allow the resistant cells to grow.

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References

Future Note
The FG 12 vector can be cloned using different vectors that will express, and this is 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.