BLAKE WALL

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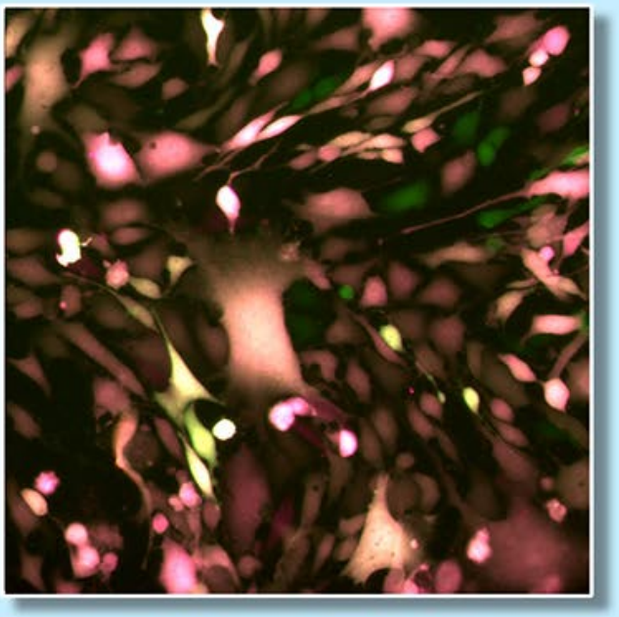

Mouse Embryonic Fibroblasts Expressing Three Fluorescent Proteins from a Polycistronic Vector

Gene Position Affects Protein Concentration in a 2A Polycistronic Vector System

Cellular reprogramming of fibroblasts to cardiomyocytes requires a stoichiometric balance of transcriptions factors within the nucleus of the cell. In order for a cell to express these novel proteins, the genes must be introduced. Traditional methods required multiple plasmids to be transfected into a cell for reprogramming. A problem is that cells vary in their ability to take in multiple target genes, which decreases reprogramming efficiency. Polycistronic vectors are attractive due to the ability to introduce one plasmid with desired genes for expression into a cell, as opposed to multiple plasmids. The challenge of polycistronic vectors is that eukaryotic cells do not have the cellular machinery to process polycistronic proteins during translation. However, viral oligopeptides (called 2A peptides) have been identified that enable ribosomes to cleave and skip at a certain peptide bond, allowing for cleavage of polycistronic proteins. Despite this advantage, widespread adoption of 2A polycistronic vectors has been limited due to the lack of systematic comparison of the different 2A sequences in a polycistronic system. This project sought to characterize the effect of gene position on protein expression in tricistronic 2A constructs containing fluorescent protein genes in varying positions. To investigate a 2A polycistronic system, mouse embryonic fibroblasts (MEF-T) were infected with retroviruses carrying the polycistronic vector. These transduced cells were then analyzed via fluorescent microscopy and flow cytometry in order to quantify the expression of each fluorescent protein. It was determined that the gene in the first position achieved the highest level of expression, followed by the third position, with the second position yielding the lowest protein expression. Such a finding is important because it not only shows that gene order matters, but will hopefully help further the utilization of 2A polycistronic systems for cellular reprogramming.