**Title –** Exchanging of selection markers in pLKO.1 DNA plasmids through cloning technique

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**Background –** The technique of cloning plasmids provides scientists the opportunity to study the effects of proteins and pathways in various organisms by easily modifying or changing the expression of a target. Cloning a plasmid, a circular piece of DNA that is separate from the chromosomal DNA and can replicate independent from the rest of the cell, is used for the purpose of transferring genetic information into another organism. The organism will then use its own mechanisms to express the inserted plasmid. After the plasmid is expressed, this expression can be further studied to understand gene function, investigate promoters, small RNAs, and other genetic elements.

**Goals –** Our goal was to successfully change the antibiotic selection marker from puromycin to blasticidin in pLKO.1 DNA plasmids.

**Materials and Methods –** In order to extract the parental vector from our plasmid we conducted a restriction enzyme digestion using specific restriction enzymes to target the appropriate sites on the plasmid. We used restriction enzymes, KpnI and BamHI, to remove the puromycin segment. We then performed gel electrophoresis on our digest reactions using a 1% agarose gel in order to separate the products. We cut out the top band from the gel, and we gel purified the sample in order to extract the parental vector without puromycin. These same steps were repeated in order to obtain the blasticidin segment from a different parental vector. We then proceeded to join the insert (blasticidin) and the parental vector via a ligation reaction. We also performed a vector-only ligation reaction as a negative control. The ligation reactions were then transformed into *E. coli* competent cells and plated on LB and ampicillin agar plates to be incubated overnight at 37**°**C. Colonies were selected and grown overnight in LB + ampicillin broth. We then extracted the DNA from the colonies by conducting a quick and dirty miniprep. To confirm that our ligation joined the correct two DNA segments, we digested the DNA extracted from individual colonies with restriction enzymes and performed a gel electrophoresis on the digestion products.

**Results –** We have successfully cloned multiple plasmids to now contain a selectable marker of blasticidin instead of puromycin. Upon diagnostic analysis, the correct plasmids will have a band around 6500 base pairs and another band around 400 base pairs in size. Diagnostic analysis of colonies that produces a smear on the gel or does not have a band around 400 base pairs is incorrect.

**Conclusion –** This work is still in progress as certain plasmids are more difficult to clone than others. These newly constructed plasmids will be used to produce lentivirus and infect cells in order to manipulate a particular gene expression in human cell lines.