Lentivirus Transduction on MiaPaCa-2 Cells

Taylor Gogolen; Jerome Irianto Ph.D.
Florida State University, College of Medicine

Introduction

-In our lab, we are currently working on a research project to manipulate the upregulation and down-regulation of 5 integrins (ITGA6, ITGA10, ITGB4, ITGB6, and ITGB8) in MiaPaCa-2 cells.

-The MIA PaCa-2 cell line is a human pancreatic cancer cell line used in research to come up with new drug therapies and treatment methods.

-CRISPR is a gene editing system that includes the Cas9 protein and a guide RNA that is used to insert or delete a sequence of DNA.

-CRISPRi is another form of CRISPR but with an inhibitor in the promoter region of the DNA which inhibits gene expression. This inhibitor works alongside the dead CAS9 protein to repress the expression of DNA. (“CRISPRa and CRISPRi” n.d.)

-CRISPRa is a form of CRISPR in which the system is used to modify gene expression. CRISPRa has an activator in the promoter region of the DNA which helps increase gene expression. (“CRISPRa and CRISPRi” n.d.)

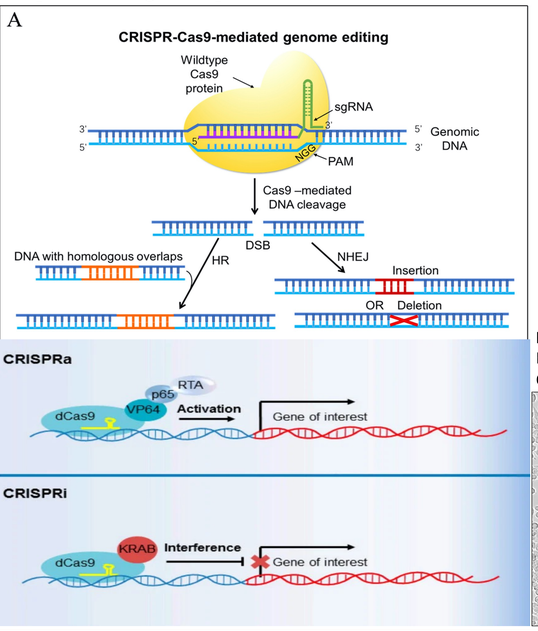
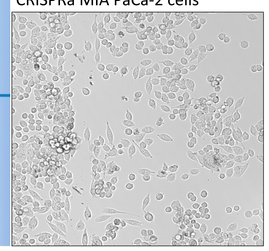
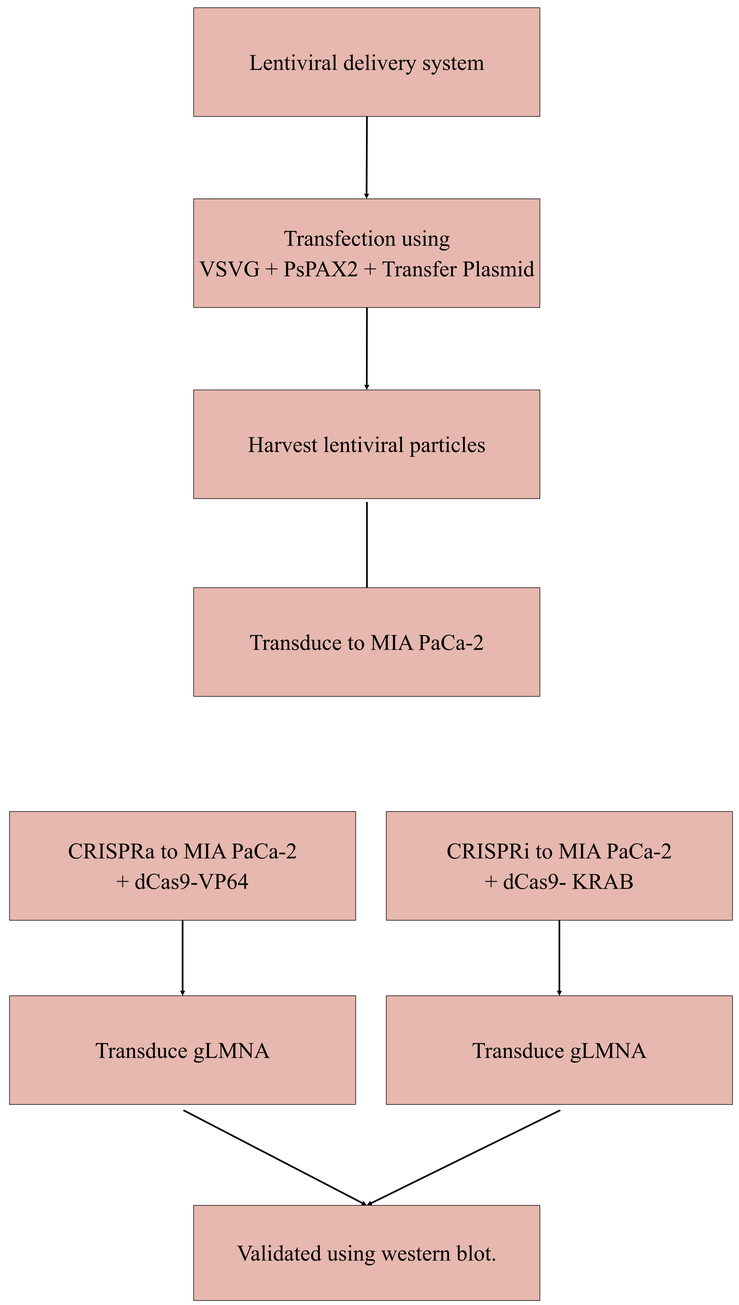


Figure 1
Effectively transduced CRISPRa MIA PaCa-2 cells



Methods



Results

Figure 2

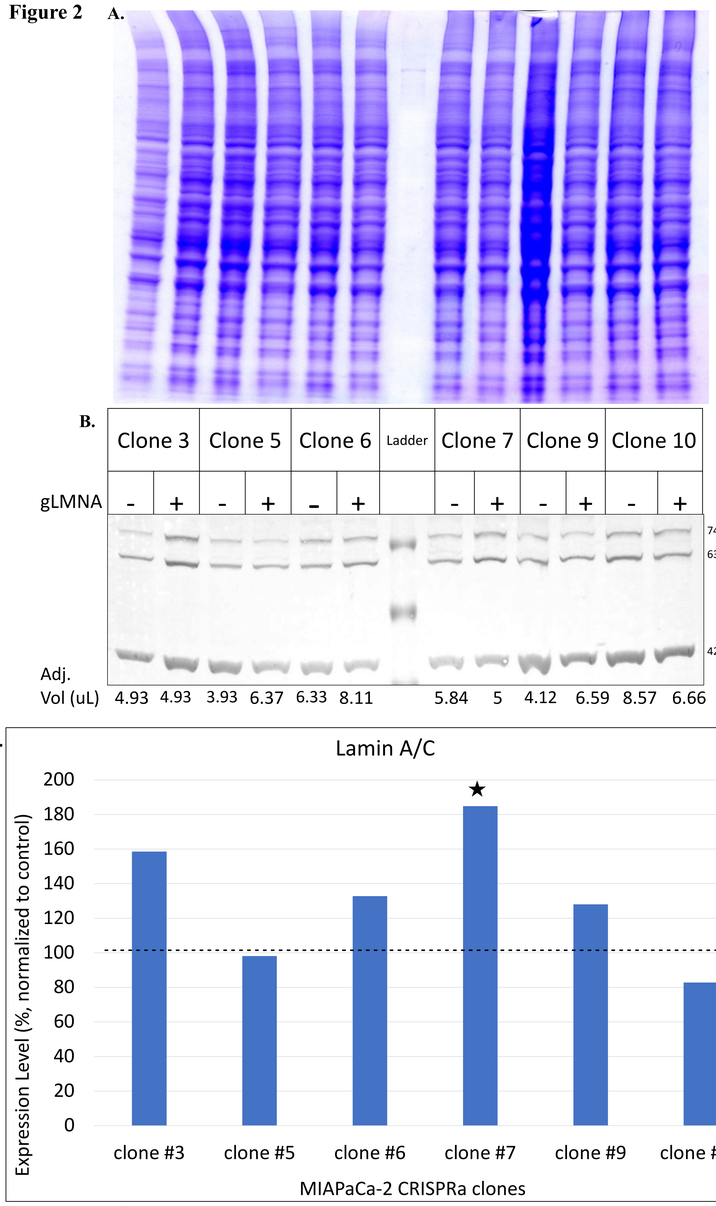


Figure 2 A. allows us to compare the width of the bands which reflect the amount of protein expression each of our clones had. Figure 2 B. is the western blot results which showed us that our transduced MiaPaCa-2 cells had more protein expression. Figure 2 C. is the quantification of our results as it showed us that clone #7 LMNA is the best working clone as it has the most protein expression. This validates the lentivirus production and transduction done as we would expect an increase in protein expression as the cells are transduced with the CRISPRa system which would increase the amount of expression.

References

- *CRISPRa and CRISPRi*. Synthego. (n.d.). Retrieved March 3, 2023, from https://www.synthego.com/guide/crispr-methods/crispri-crispra
- Maestre, L. (n.d.). Transient transfection protocol for HEK293T cells. Retrieved March 3, 2023, from https://www.euromabnet.com/protocols/transient-transfection-HEK293T-cells.php
- *Puromycin faqs*. AG Scientific. (n.d.). Retrieved March 3, 2023, from https://agscientific.com/blog/puromycin-faqs.html#:~:text=Puromycin%20is%20an%20antibiotic%20researchers,suitable%20for%20research%20purposes%20only.
- *Validation of gene transfection/transduction*. Validation of Gene Transfection/Transduction | Thermo Fisher Scientific - US. (n.d.). Retrieved March 3, 2023, from https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-applications/real-time-pcr-protein-analysis/protein-expression-taqman-assays/validation-transfection-transduction.html#:~:text=Transfection%20is%20the%20process%20of,foreign%20gene%20into%20host%20cells.
- 7, J. (2022, June 7). *Lentivirus production for gene delivery*. Lentivirus Production for Gene Delivery | Azenta Life Sciences. Retrieved March 3, 2023, from https://www.azenta.com/blog/lentivirus-production-gene-delivery

Acknowledgements

I would like to thank Jerome Irianto for all of his help and guidance while working on this project. I would also like to thank Hyeje Sumajit, Gabriela De Jesus, and Dhenu Patel for their assistance and advice throughout this process.

Future work

My project is a small portion of a larger project that is looking to manipulate the upregulation and down-regulation of the integrins ITGA6, ITGA10, ITGB4, ITGB6, and ITGB8 in different extracellular matrices.