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SJSU Undergraduate Research Grants

Production of Integrase Deficient Lentiviral Vector for Gene Editing

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Abstract

Hematopoietic stem cells are ideal cellular targets for *ex vivo* genome-editing applications because of 1) their ability to self-renew, 2) their tremendous cellular output, and 3) their well established isolation and transplantation procedures. However, achieving adequate targeting rates in HSCs remains challenging[1]. The presence of exogenous DNA required for gene addition can induce a type-I interferon (INF) response, triggering the host immune system and effectively eliminating modified gene corrected HSCs[2,3]. Therefore, we intend to mask our exogenous DNA repair template inside of an integrase deficient lentiviral vector (IDLV) in order to avoid detection via the INF response. IDLV is an attractive option as a DNA delivery vehicle in that the genomic content of the vector will not disrupt the integrity of the host genome[4,5]. Thus, to this aim we have identified four IDLV construction plasmids. HEK-293T fibroblasts were then transfected and used to produce IDLV. We are currently optimizing the transfection protocol to identify lipofectamine or polyethylenimine (PEI) as the optimal transfection reagent for high viral production efficiency.

Research Questions

Aim 1. Evaluate IDLV production efficiency using two different transfection reagents.

Which transfection reagent is the most efficient?

- Polyethylenimine (PEI) or Lipofectamine 2000

Aim 2. Evaluate the production of IDLV using Addgene plasmids vs. a proprietary plasmid mix with Lipofectamine.

Aim 3. Evaluate the production of IDLV using Addgene plasmids vs. a proprietary plasmid mix with PEI.

Identification of IDLV Production Plasmids

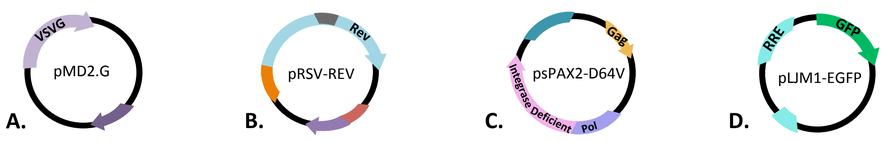


Figure 1. Integrase Deficient Lentiviral Vector Production Plasmids
A) pMD2.G is the envelope plasmid containing the VSVG envelope capsid in order to pseudotype IDLV for ubiquitous mammalian cell transduction. **B)** pRSV-REV is the packaging plasmid encoding REV, a gene that facilitates the production of viral proteins. **C)** psPAX2-D64V is a packaging plasmid which encodes for a core structural protein, GAG, and a viral transcriptase, POL. A single amino acid change was performed in the Pol gene to produce an integrase deficient transcriptase. **D)** pLJM1-EGFP, the transferring plasmid, contains the ψ recognition sequence denoting the genetic content to be incorporated into the viral capsid.

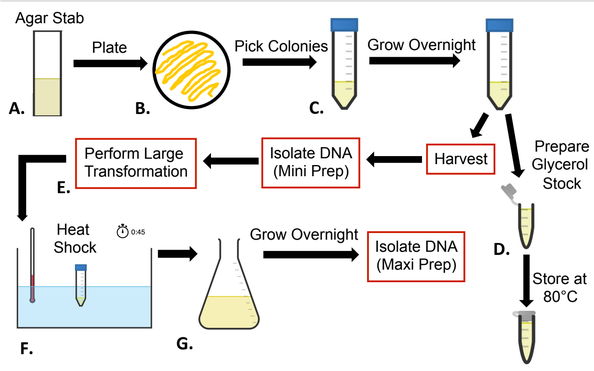
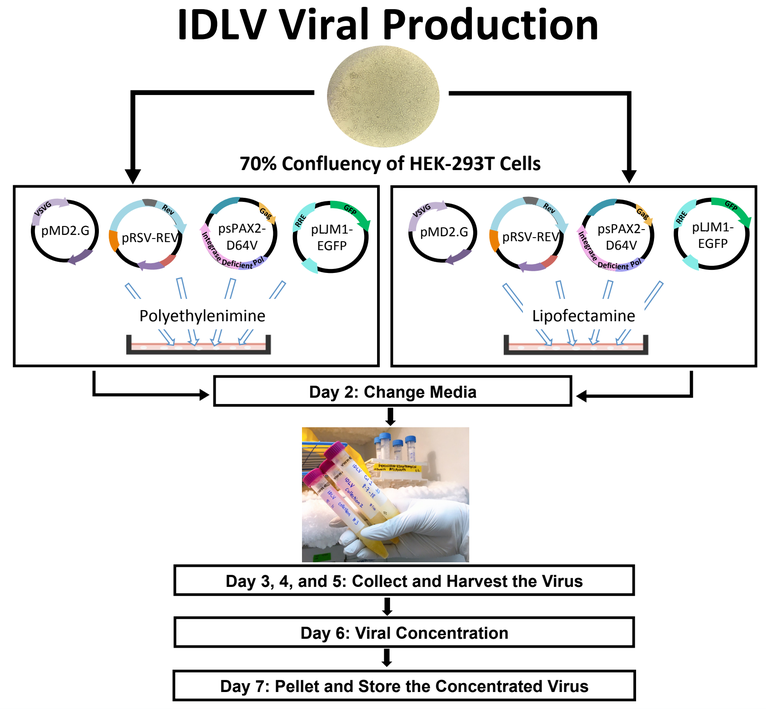


Figure 2. Mass Production of IDLV Plasmids
A) The plasmid was obtained from Addgene in the form of a bacterial stab. This is created by piercing a transformed bacterial colony on LB agar with a pipet tip. **B)** The bacteria was streaked onto a plate and colonies were picked following overnight incubation. **C)** Bacterial clones were grown in LB media overnight. **D)** Glycerol stocks were prepared for long term storage of the plasmids for future applications. **E)** A large bacterial transformation was performed to produce more of our known IDLV production plasmids after isolating and screening the DNA. A bacterial transformation allows for modification of a bacterial cell to uptake foreign plasmid DNA into a chemically competent bacterial cell. **F)** A crucial step of a bacterial transformation is the heat shock step as it is performed to cause a formation of pores in the bacterial membrane, through which plasmid DNA molecules can pass. **G)** Bacteria is then grown overnight followed by isolation of large amounts of plasmid DNA via a MaxiPrep.

IDLV Viral Production



Results

Optimal Polyethylenimine Concentration

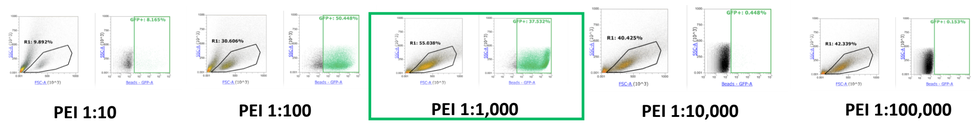


Figure 7. Optimal Polyethylenimine Concentration
To determine the optimal PEI concentration for transfection of HEK-293T cells, serial dilutions of PEI were used producing a dose response. The transfection protocol was followed to include pMAX, a GFP expression plasmid, into HEK-293T cells. The 1:1,000 dilution of PEI was most tolerated by the HEK-293T cells with a viability of 55.038% and 37.5% GFP positive cells.

GFP+ Expression with Lipofectamine

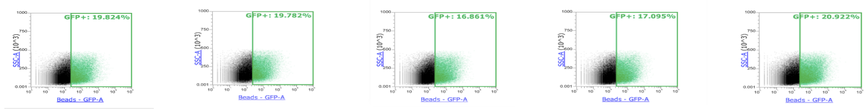


Figure 8. GFP Positive Expression Readings Using Flow Cytometry
Upon transfection, the collected virus was utilized to infect the HEK293T cells to achieve successful GFP expression. GFP expression was determined via flow cytometry. The percent of GFP positive cells indicate the amount of GFP expression achieved in viable cells.

PLASMID	YIELD
pLJM1-EGFP	458.0 ng/uL
psPAX2-D64V	132.5 ng/uL
pMD2.G	927.5 ng/uL
pRSV-Rev	488.5 ng/uL

Figure 3. Maxi Prep Yields of IDLV Production Plasmids
The maxi prep allowed for the large amounts of plasmid DNA to be extracted from bacteria. Yields were obtained via spectrophotometery. Yields were taken into consideration when performing calculations for transfection (mass of plasmid divided by concentration of plasmid).

References

1. Yu K.R., Natanson, H., & Dunbar C. E. (2016) Gene Editing of Human Hematopoietic Stem and Progenitor Cells: Promise and Potential Hurdles. *Human Gene Therapy*. 729-740.
2. Sun, L., Wu, J., Du, F., Chen, X., & Chen, Z. J. (2012) Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science*. 339(6121) 786-91.
3. Hoffman B.E., Ertl H.C., Terhorst C. High K.A., & Herzog R.W. (2012) Gene therapy research at the frontiers of viral immunology. *Frontiers in microbiology*. 3:182.
4. Dull T., Zufferey R., Kelly M., Mandel R.J., Nguyen M., Trono D., & Naldini, L. (1998) A third generation lentivirus vector with a conditional packaging system. *Journal of virology*. 72(11):8463-8471.
5. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P. (2003) LMO2- associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science*. 302:415-9.
6. Raj D., Davidoff A.M., & Nathwani A.C. (2011) Self-complementary adeno-associated viral vectors for gene therapy of hemophilia B: progress and challenges. *Expert review of hematology*. 4(5), 539-49.
7. Rogers G.L., & Herzog R.W. (2015) Gene therapy for hemophilia. *Frontiers in bioscience*. 20:556-603.
8. Lombardo A., Genovese P., Beausejour C.M., Colleoni S., Lee Y.L., Kim K.A., & Holmes, M. C. (2007) Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. *Nature biotechnology*. 25(11):1298.