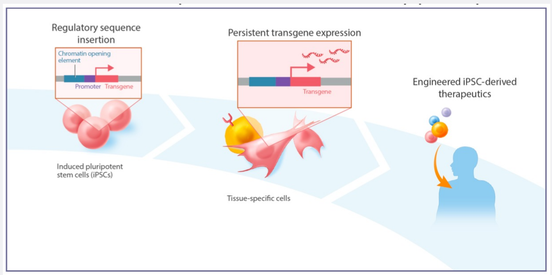
Sustained Transgene Expression in Engineered iPSC-Derived Tissue-Specific Cells

Taeyun Kim[1], Raven D. Hinkel[1], Ismet C. Tanrikulu[1], Christopher B. Rohde[1], Matthew Angel[1]

1Factor Bioscience Inc., Cambridge MA

Summary

Induced pluripotent stem cells (iPSCs) provide an ideal starting point for the generation of gene-edited tissue-specific cells for cell therapy. In particular, engineered iPSC-derived mesenchymal stem cells (iMSCs) combine the advantages of tissue-derived MSCs with gene editing, presenting a new pathway for the development of cell therapies. However, transgene expression can be unstable during the differentiation of iPSCs to EiMSCs. To enable sustained transgene expression upon differentiation, we explored the use of universal chromatin-opening elements (UCOEs) in transgenic cassettes that express green fluorescent protein (GFP) under the EF1α promoter. Cassettes with and without a UCOE were inserted into the adeno-associated virus integration site 1 (AAVS1) safe-harbor locus in iPSCs through the use of mRNA encoding UltraSlice™ gene-editing endonucleases and single-stranded DNA (ssDNA) repair templates. Clonal iPSC lines were isolated by the single-cell deposition of GFP-sorted cells and GFP expression was monitored during differentiation toward EiMSCs. While only ~36% of EiMSCs engineered without a UCOE were GFP-positive, use of a UCOE yielded in uniform GFP expression (> 99%). We applied our established workflow to knock-in expression cassettes encoding IL7-IL15 fusion proteins into iPSCs and differentiated them to clonal EiMSC lines that stably express IL7-IL15 fusion protein without using reporter component.



Conclusions

We demonstrated that high knock-in efficiency obtained from the use of UltraSlice™ and ssDNA repair template enable establishment of transgene expressing iPS cell line with or without reporter component. Furthermore, we have shown that incorporating UCOE with EF1α promoter not only increases the transgene expression, but also maintains stable transgene expression throughout iPSC to iMSC differentiation. We are currently investigating in vivo use of IL7-IL15 expressing iMSCs as well as establishing other EiMSC lines that express immunomodulatory proteins such as IL4-IL10 fusion proteins.

1. Transgene insertion into AAVS1 locus in iPSCs

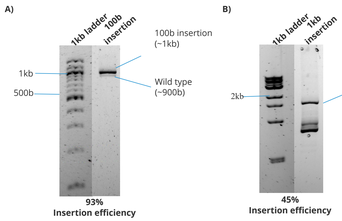


Figure 1. UltraSlice™ gene editing mRNA enables high eifficiency insertion of ssDNA repair templates into AAVS1 locus in iPSCs. AAVS1 PCR amplicon shows that co-transfection of UltraSlice™ mRNA and ssDNA repair templates including noncoding 100 base sequence resulted in 93% insertion efficiency **A)** and 45% for 1kb sequence insertion **B)**.

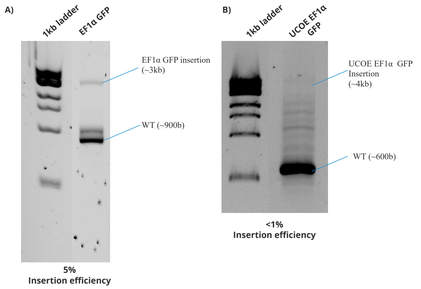


Figure 2. EF1α GFP and UCOE EF1α GFP ssDNA repair template insertion into AAVS1 locus in iPSCs. AAVS1 PCR amplicon shows successful insertion of EF1α GFP ssDNA repair template **(A)** and UCOE EF1α GFP ssDNA repair template **(B)** in iPSCs. Different primers set was used between **(A)** and **(B)**, resulting different WT band size.

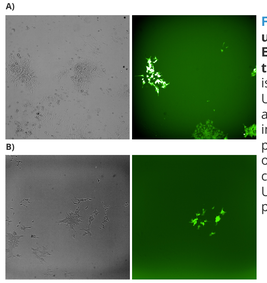


Figure 3. GFP expression upon direct insertion of EF1α and UCOE EF1α GFP transgene into iPSCs. GFP is expressed under non-UCOE EF1α promoter **(A)** as well as UCOE incorporated EF1α promoter **(B).** The intensity of GFP expression was comparable between UCOE and non-UCOE EF1α promoter.

2. Cell Line Development

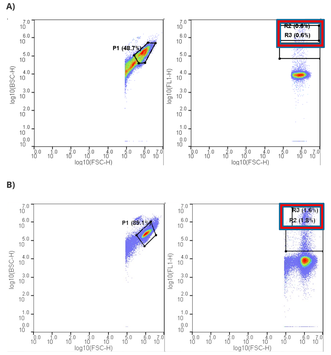


Figure 4. Isolation of iPSCs by GFP expression. GFP expressing iPSCs under EF1α promoter **(A)** and UCOE associated EF1α promoter **(B)** were sorted by single cells and was deposited into 96 well plate. Cells that express strong enough GFP signal were selected for sorting as indicated in the red box.

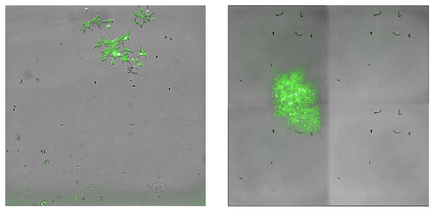


Figure 5. GFP expression post single cell sorting. iPSC colonies that were grown from the single cell were monitored for GFP expression. GFP expression was confirmed for both non-UCOE EF1α promoter **(A)** and UCOE associated EF1α promoter **(B)**, showing that all cells are expressing GFP.

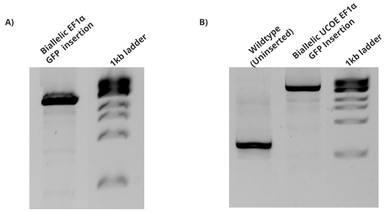


Figure 6. Biallelic transgene insertion into AAVS1 locus. AAVS1 amplicon shows Biallelic insertion of EF1α GFP **(A)** and UCOE EF1α GFP **(B)** into AAVS1 locus. Biallelic insertion AAVS1 amplicon does not show any wildtype band.

3. Monitor GFP expression during iPSC to iMSC differentiation

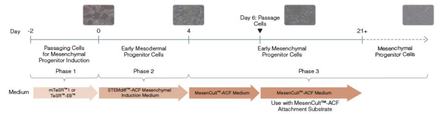


Figure 7. Flowchart of STEMdiff™ Mesenchymal Progenitor kit protocol
(STEMDIFF™ MESENCHYMAL PROGENITAL KIT Catalog #05240 Version 02, Stemcell™ Technologies, 2020, https://www.stemcell.com/stemdiff-mesenchymal-progenitor-kit.html#section-protocols-and-documentation)

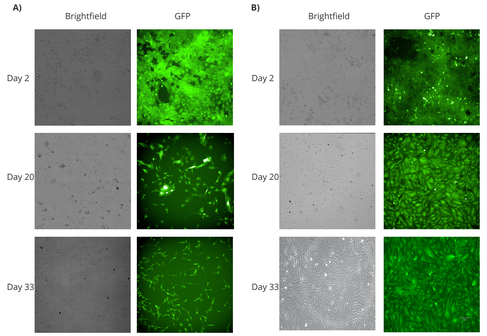


Figure 8. Tracking GFP expression under EF1α promoter and UCOE EF1α promoter during iPSC to iMSC differentiation. Brightfield and GFP images were taken from Day 2 to Day 33 at which point the differentiation was completed. **A)** GFP expression under EF1α promoter was robust until Day 20, but we started to observe loss of GFP expressing cells from Day 20. By Day 33, only ~36% of differentiated iMSCs expressed GFP. **B)** GFP expression under UOCE incorporated EF1α promoter has not shown any silencing and maintained ~99% of cells expressing GFP throughout the entire differentiation process.

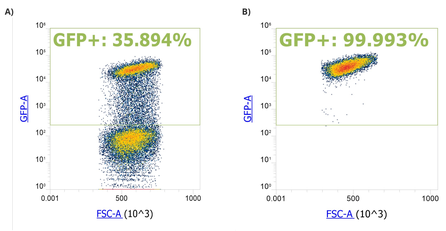


Figure 9. Monitoring GFP expression in iMSCS. Flow cytometry analysis shows that only 36% of non-UCOE Ef1a iMSCs are expressing GFP **(A)** while >99% of iMSCs are expressing GFP under UCOE incorporated EF1α promoter **(B)**.

4. Establish IL7-IL15 fusion protein expressing iMSCs

	Number of colonies sampled	Biallelic colonies confirmed	% Biallelic colonies from sample
UCOE EF1α IL7-IL15	8	1	12,5%
Non-UCOE EF1α IL7-IL15	4	2	50%

Figure 10. Number of Biaellelic colonies obtained from Reporter free single cell deposition of transfected iPSCs. 1 out of 8 colonies harvested for Amplicon verification showed biallelic insertion into AAVS1 locus for UCOE EF1α IL7-IL15 iPS cell line while 2 out 4 colonies showed biallelic insertion, establishing IL7-IL15 fusion protein iPS cell line.

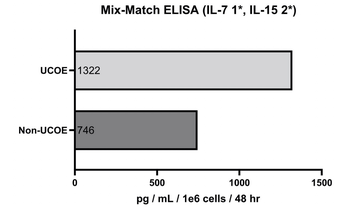


Figure 11. Mix match ELISA shows IL7-IL15 fusion protein expression in iPSCs. After iPS cell lines that express IL7-IL15 fusion protein under EF1α and UCOE incorporated EF1α promoter, mix match ELISA was performed with media supernatant collected 48 hours post passaging of cells. IL7-IL15 fusion was expressed considerably more under UCOE associated EF1α promoter than non-UCOE EF1α promoter in iPSCs.

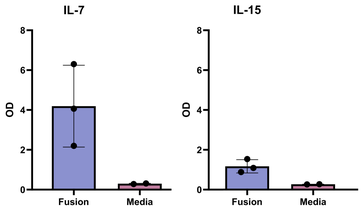


Figure 12. HEK-Blue™ IL7 and IL15 reporter cell assay shows robust IL7-IL15 fusion protein expression. HEK-Blue™ IL7 and IL15 reporter cells were used to quantify IL7-IL15 fusion protein expression in engineered iMSCs. Measured OD value shows solid IL7-IL15 fusion protein expression in differentiated iMSCs under UCOE EF1α promoter.

American Society of Gene + Cell Therapy