Automated Generation of 600 Polyclonal Knock-In Lines
Leads to Discovery of Highly Active Promoter-Locus Combinations in *iPSCs*

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Background

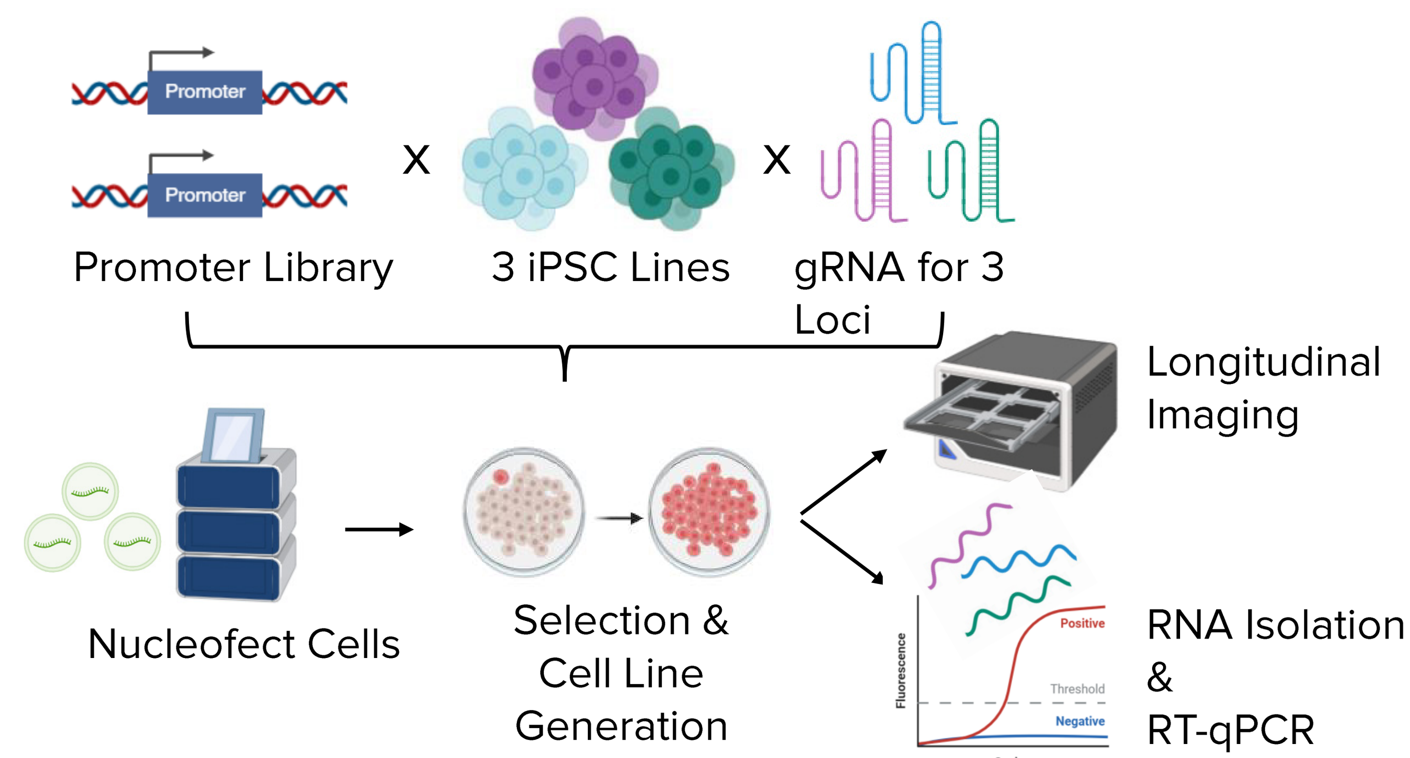
Promoter screening in induced pluripotent stem cells (iPSCs) is essential for deciphering the intricate mechanisms governing gene expression in these versatile cells. iPSCs, reprogrammed from adult cells, harbor the potential to differentiate into various cell types, serving as valuable models for developmental biology, disease modeling, and drug discovery. Understanding the regulatory elements dictating gene expression in iPSCs is crucial for harnessing their full potential.

Diverse promoter sequences drive gene expression with varying efficiencies and specificities, impacting cellular phenotypes and functions. Additionally, the genomic context surrounding these promoters influences their activity, underscoring the need for comprehensive screening approaches. Moreover, cell line variability further complicates gene expression studies, necessitating systematic evaluation across multiple cell lines.

While transient expression methods provide initial insights, stable cell lines offer greater stability and reproducibility, crucial for long-term studies and therapeutic applications. Arrayed screening allows precise interrogation of individual promoters, facilitating in-depth characterization and comparison of their activities.

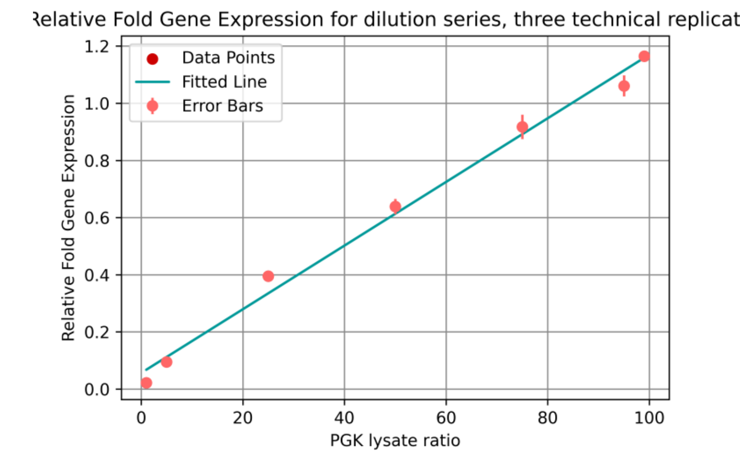
In this study we generated and characterized stably integrated iPSC lines with 12 different promoter sequences at 3 therapeutically relevant loci of AAVS1, TRAC and B2M in three independent lines.

Workflow



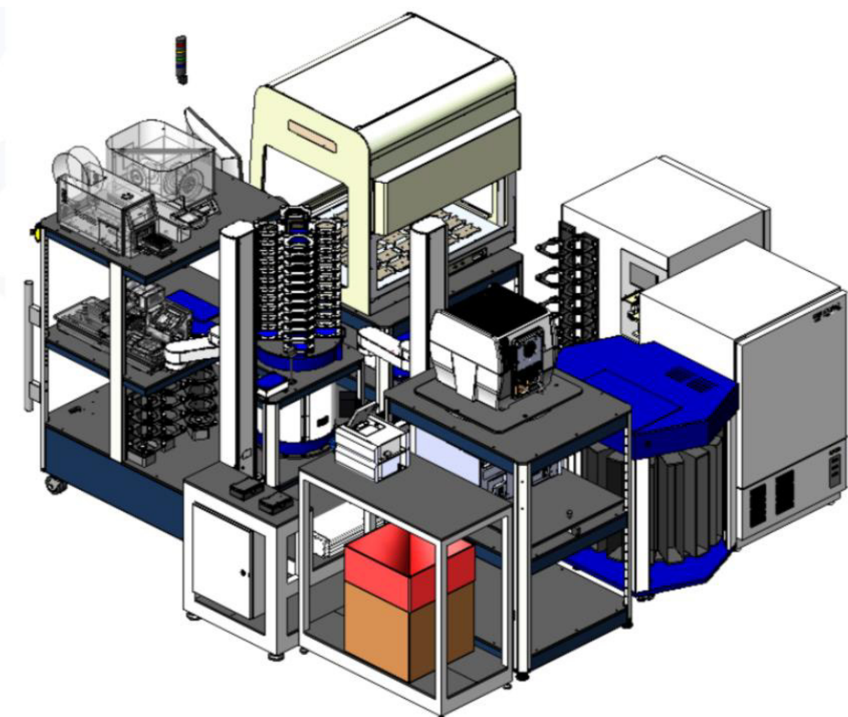
Assay Development

RT-qPCR assay development involved serial dilution of lysate from a PGK-GFP control cell-line. The resulting linear correlation had high quality scores: R-squared = 0.9919, MSE = 0.0014, MAE = 0.03323. These metrics validate assay precision and sensitivity in quantifying gene expression levels, ensuring reliability for further analyses.



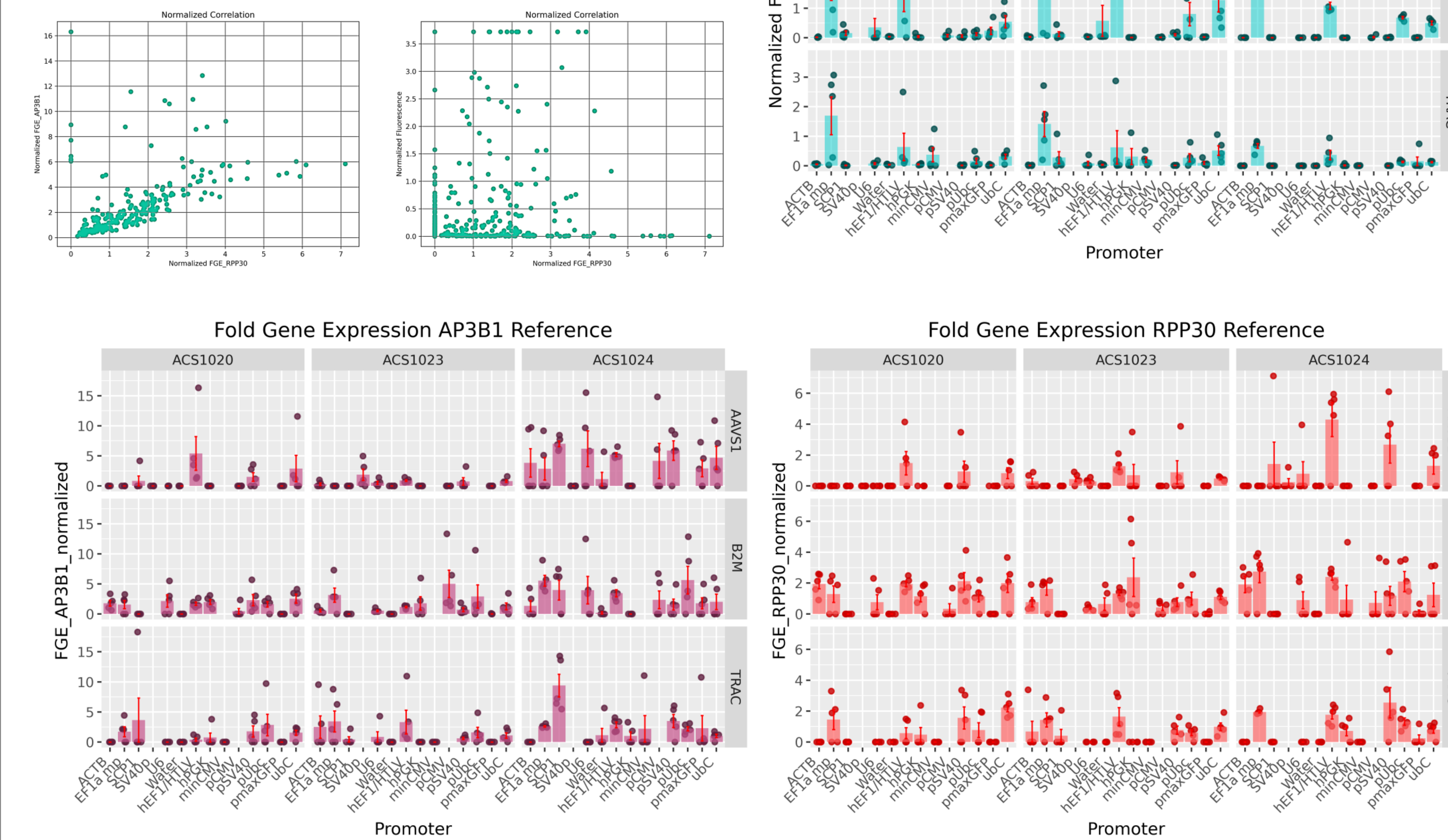
Automated Cell Line Generation and Screening

In this study, we employed rigorous characterization, including RT-qPCR and live-cell longitudinal imaging, to quantify gene expression. By automating cell culture, selection, and expression analysis, we ensured high-throughput and reproducible screening of promoter activities, enabling the characterization of 600 iPSC lines across passages. This systematic approach provides a robust foundation for understanding gene regulation in iPSCs, with implications for advancing regenerative medicine and disease modeling.

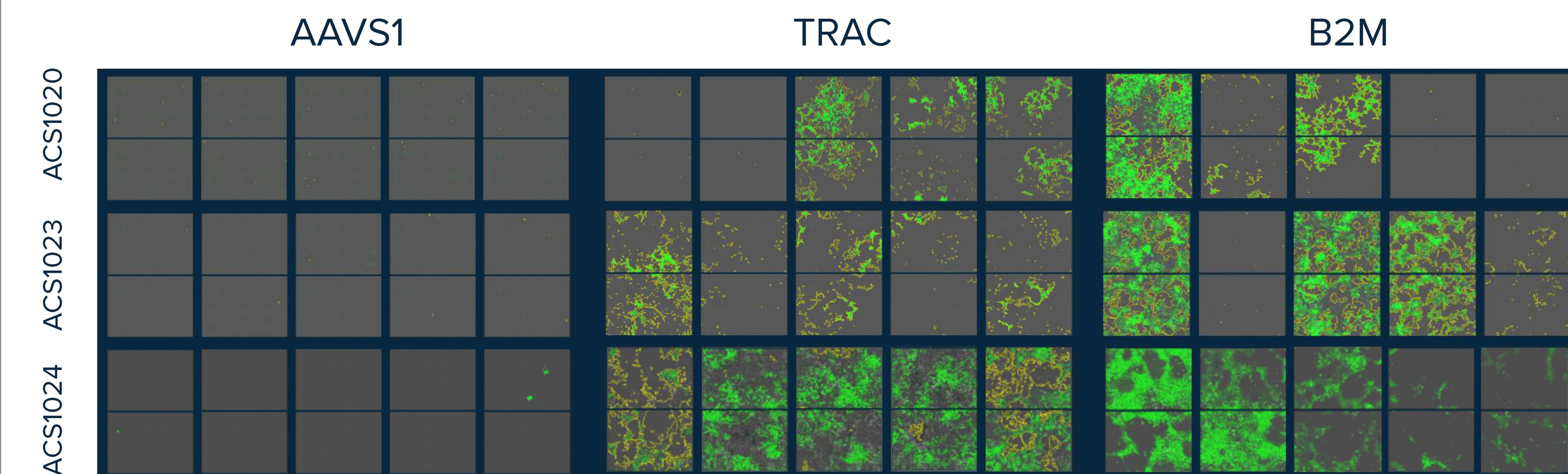


Arrayed generation of 600 polyclonal cell lines enables characterization of promoter strength and effect of genomic loci

Multiplex TaqMan assay includes internal control probes for two ubiquitously expressed genes, RPP30 and AP3B1. Assessing correlation between results normalized to two reference genes and between qPCR and fluorescence data validates assay accuracy across normalization methods.



EF1α is silenced after KI at AAVS1 while it maintains activity at TRAC and B2M

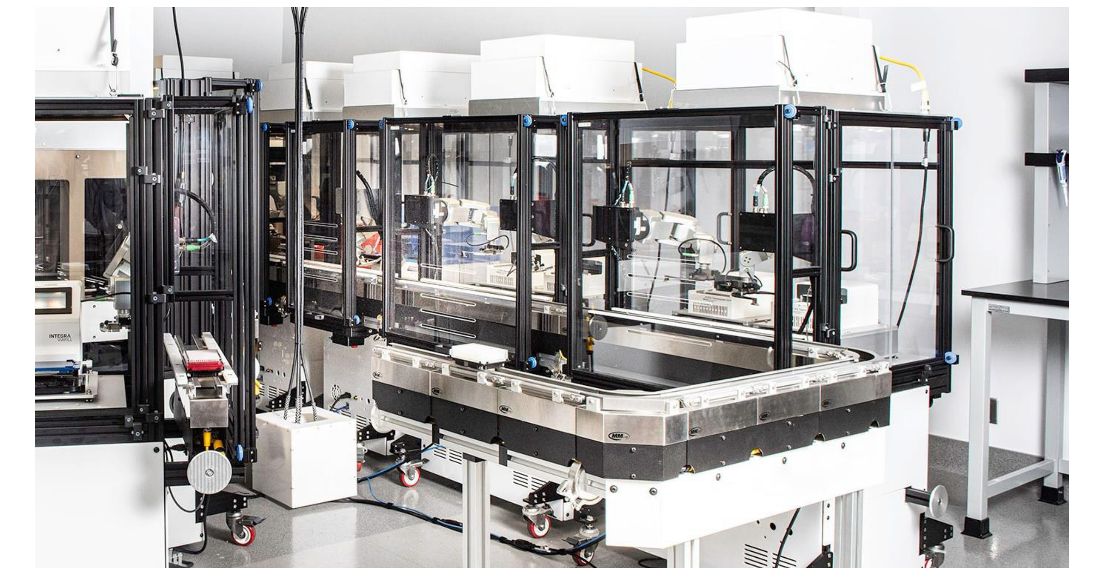


Conclusion

In conclusion, our study elucidates insights into promoter-driven gene expression variability in induced pluripotent stem cells (iPSCs), underlining the importance of comprehensive screening approaches. Notably, we identified promoter-locus combinations associated with exceptionally high expression levels, enhancing our understanding of gene regulation dynamics in iPSCs. Of particular significance was the discovery of divergent expression outcomes linked to the commonly used EF1α promoter at different genomic loci. Our data revealed that while EF1α at AAVS1 consistently resulted in poor expression and loss of cell lines during selection, the same EF1a promoter sequence at TRAC and B2M exhibited robust expression. This finding underscores the critical influence of genomic context on promoter activity, emphasizing the necessity of tailored selection strategies for optimal gene expression outcomes

Overall, our findings contribute valuable insights into the complex regulatory mechanisms governing gene expression in iPSCs, with implications for improving cellular reprogramming strategies and advancing therapeutic applications.

About Ginkgo Bioworks



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