Developing a CRISPR-based Prime Editing Workflow for Human Induced Pluripotent Stem Cells to Model Cardiac Arrhythmogenic *TNNT2* Variants

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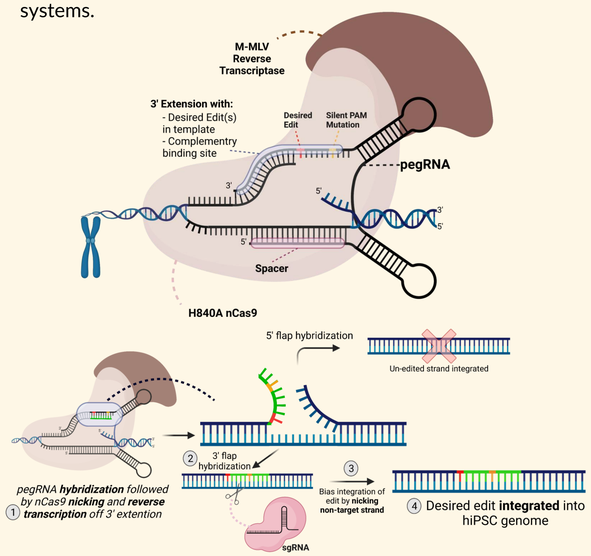
Background

Hypertrophic cardiomyopathy (HCM) is the most prevalent inherited cardiovascular disease.

- Affect 1:500 individuals and the primary cause of sudden cardiac arrest (SCA) in youth and young adults, including elite athletes.
- Interestingly, some patient specific ***TNNT2* variants** related HCM develop varying degrees of HCM yet demonstrate a disproportionally high incidence of SCA.

CRISPR-based prime editing builds on established genome editing protocols with the benefits of using a **nicking Cas9 endonuclease** circumventing the array of issues arising from the double-strand breaks (DSBs) of traditional CRISPR/Cas9 systems.



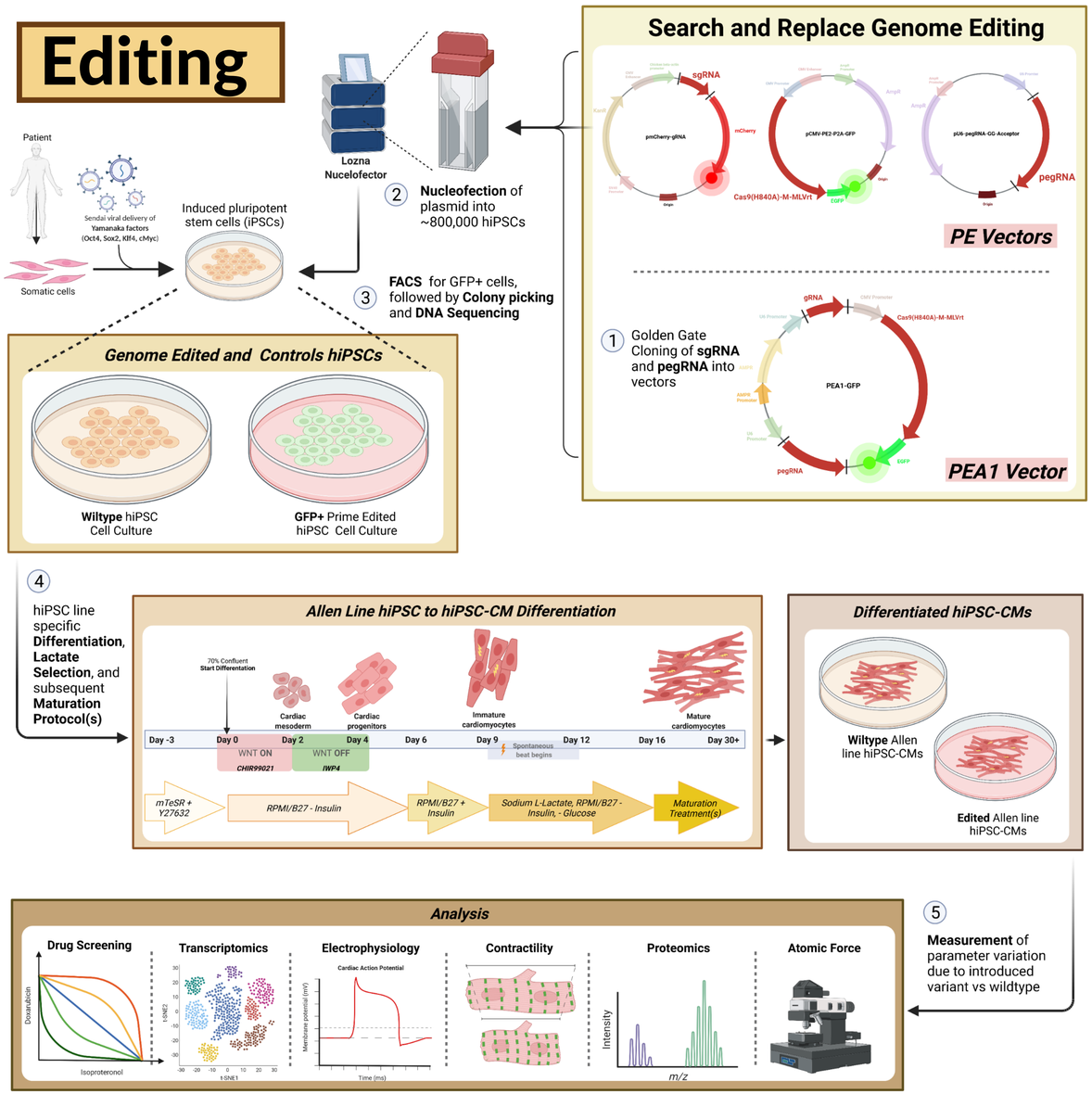
3 components of PE3b system:

1. **Cas9 endonuclease (H840A Cas9n)** fused to a **Moloney murine leukemia modified reverse transcriptase (M-MLV-RT)**
2. **Prime editing guide RNA (pegRNA)**
3. **Nicking guide RNA (ngRNA)**

Research Objective

Develop a robust prime editing protocol in hiPSCs to study pro-arrythmogenic gene variants in differentiated hiPSC-CMs

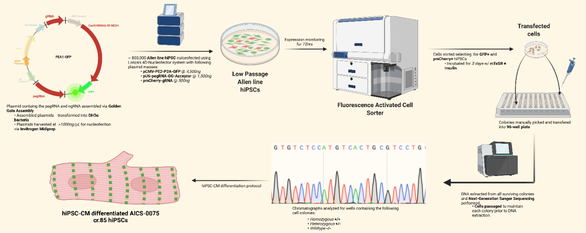
A **high-fidelity** and **efficient** work-flow for developing variant hiPSC-CMs using **PE3b-based Prime Editing**



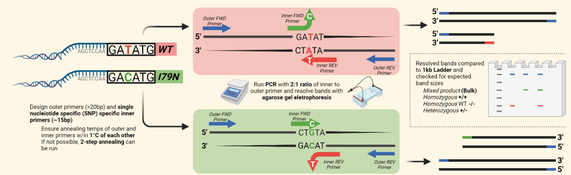
Diagrams made using BIORENDER

Methods

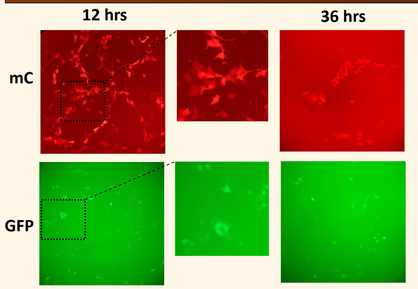
hiPSC Prime Editing workflow. AICS-0075 cl.85 hiPSCs from the Allen Institute for Cell Sciences nucleofected with PE vectors/PEA1 vector. Cells sorted for GFP+ & pmCherry/GFP+ using FACS. Individual colonies manually picked to genotype variant lines.



Amplification-Refractory Mutation System (ARMS-PCR). Variant and wildtype specific primers designed for I79N$^{+/-}$, and R92Q$^{+/-}$, detection. Primer optimization for WT detection.



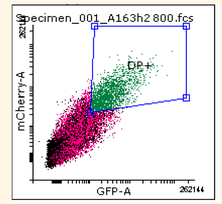
Results

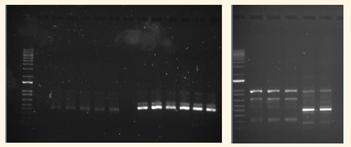


Cell Imaging: GFP+/pmCherry+ (mC) hiPSCs imaged using Leica SP8 Confocal microscope

- **Fluorescence diminished** and **cell death increased** across 36hr monitoring period

Fluorescence Activated Cell Sorting (FACS): Successfully nucleofected pmCherry+ and GFP+ (DP+) PE vector in AICS-0075 cr.85 hiPSCs *(right plot)*. PEA1 vector failed to express adequately following nucleofection for viable FACS





ARMS-PCR: Primer annealing optimized for R92 and I79 WT gene locus. 2-step annealing required for R92 designed primers

Summary

- Preliminary work established effective PE vectors & PEA1 nucleofection-based transfection and FACS based cell sorting for identifying and individualizing potentially edited hiPSCs
  - Waning expression and lack of effective PE expression due to rapid promoter silencing remain obstacles
- ARMS-PCR optimized for WT hiPSCs at I79 and R92 *TNNT2* gene locus
  - High degree of variant specific primer optimization trial questions usability in research-based applications

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References

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