



Leiden University
Medical Center



University of
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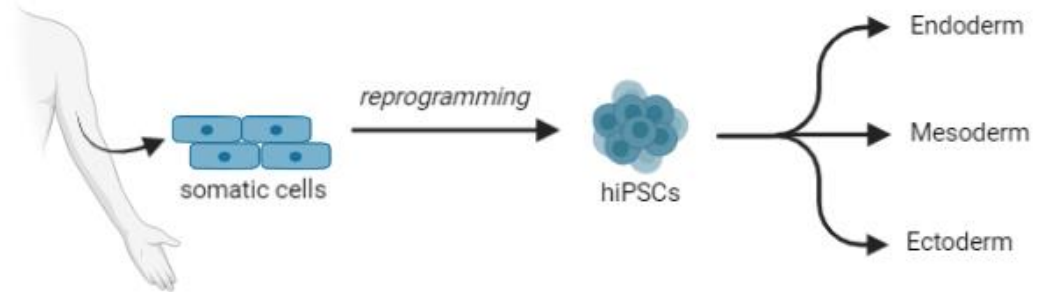
Generating & characterizing pathogenic KCNH2 variants in hiPSCs to study LQT2 syndrome

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Anatomy and Embryology
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hiPSCs and cardiac disease modeling

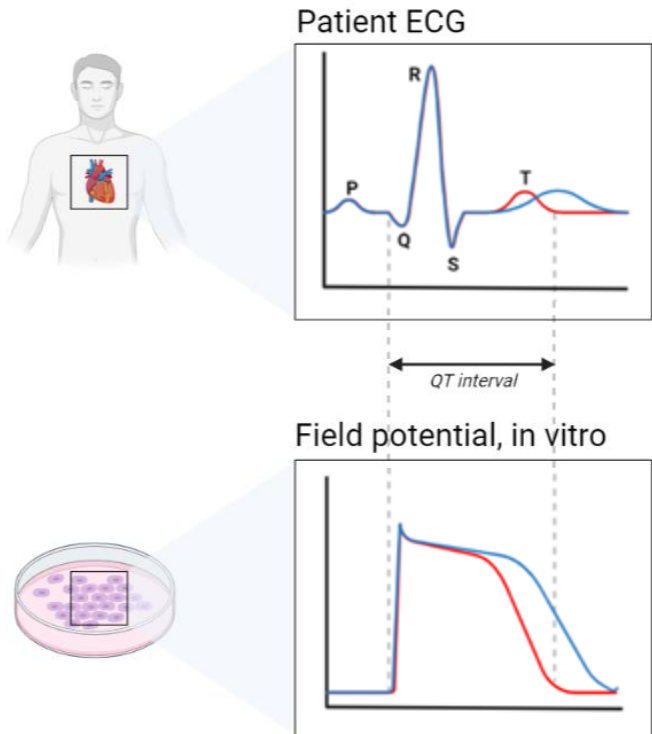
Davis group

- > Cardiac diseases, and the underlying genetics
- > Disease modeling in human induced pluripotent stem cells (hiPSCs)



- > Disease modeling
- > Regenerative medicine
- > Drug studies and personalized treatments
- > Patient specific cell lines
- > Gene editing

Channelopathies and LQT syndrome



Disfunctions in ion channels: Channelopathies

hiPSC models > rodent models

Different expression of ion channels

Ion channels: regulation contraction

Long QT syndromes: prolonged QT interval

-> Prolonged duration of action potential

-> Delayed repolarization

Stress/exercise -> arrhythmias/cardiac arrest

Classification and stratification KCNH2 variants

Long QT 2 syndrome

Loss-of-function mutations in *KCNH2*
hERG channel

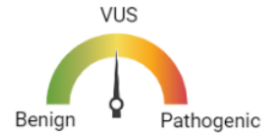
> 500 mutations

Type and location of mutation

≠ disease phenotype

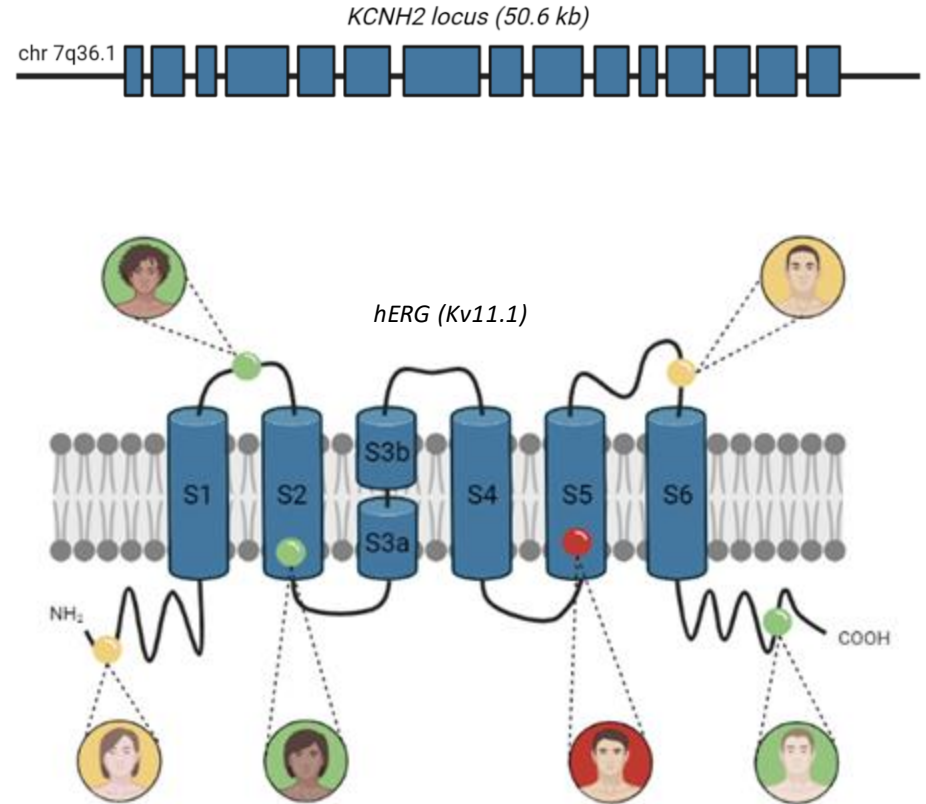
≠ disease severity

≠ drug response



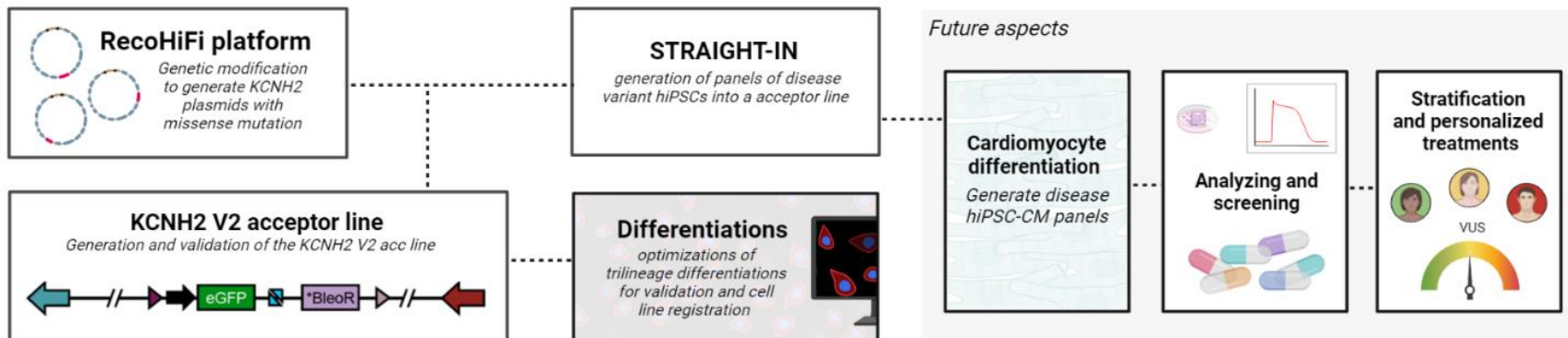
Stratification of mutations

Determine pathogenicity

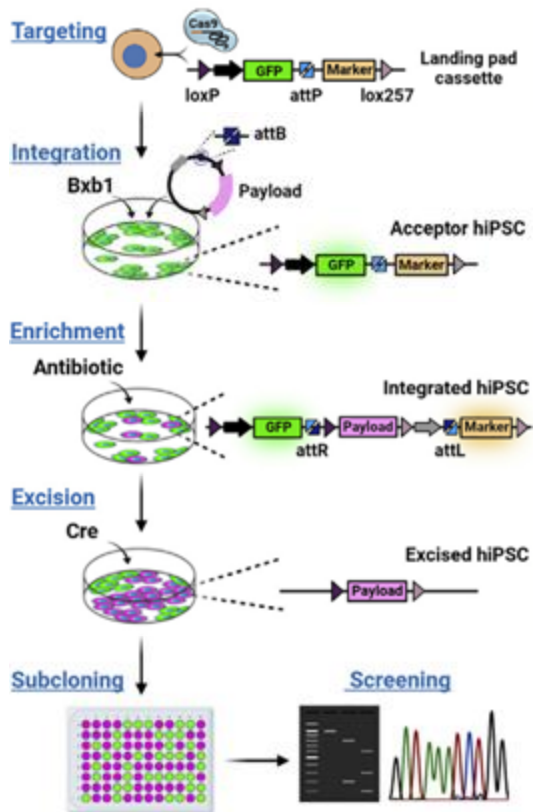


Generate a library of disease lines harboring mutations in the KCNH2 gene that are associated with LQT2

1. RecoHiFi: Generate KCNH2 donor vectors with mutations and Tags
2. Validating the LU99 KCNH2 v2 acc line
3. Testing and optimizing trilineage differentiations protocols
4. STRAIGHT-IN to generate disease KCNH2 hiPSC panels



STRAIGHT-IN platform



STRAIGHT-IN enables high-throughput targeting of large DNA payloads in human pluripotent stem cells

Blanch-Asensio A et al. STRAIGHT-IN enables high-throughput targeting of large DNA payloads in human pluripotent stem cells. *Cell Rep Methods*. 2022 Sep 22;2(10):100300. doi: 10.1016/j.crmeth.2022.100300. PMID: 36313798; PMCID: PMC9606106

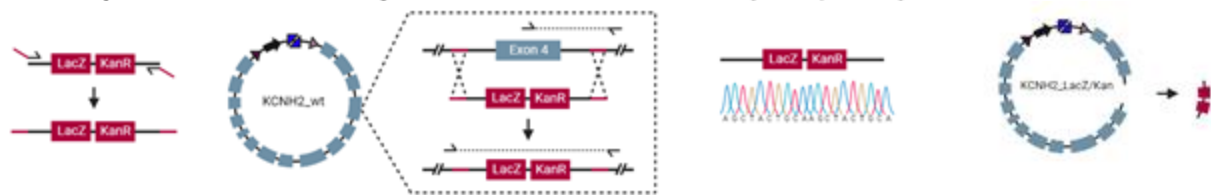
- > Integration of large genomic fragments into hiPSCs
- > Simultaneous generation of panels of disease variants

1. **Targeting** -> CRISPR cas9, homologous recombination, LP cassette
2. **Integrating** -> Serine recombinases (Bxb1) -> attP and attB site
3. **Enrichment** -> EF1 α promoter -> Zeo selection
4. **Excising** -> Tyrosine recombinases (Cre) -> Lox sites

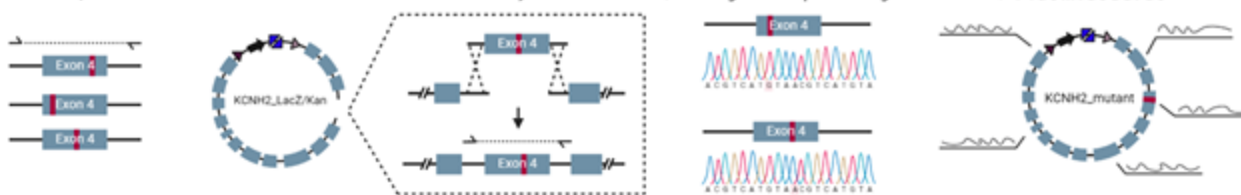
Generation of a plasmid library

RecoHiFi platform: Simultaneously generate a library of plasmids

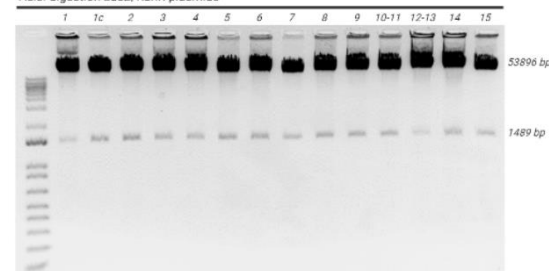
Cassette amplification → Homologous recombination → Sanger sequencing → Restriction digest



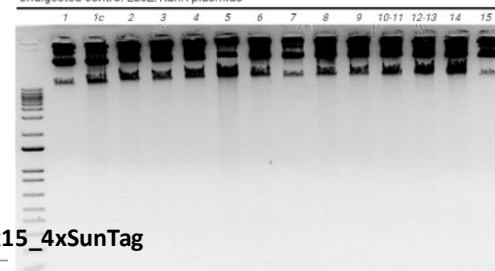
eBlock amplification → HiFi DNA assembly → Sanger Sequencing → Plasmidsaurus



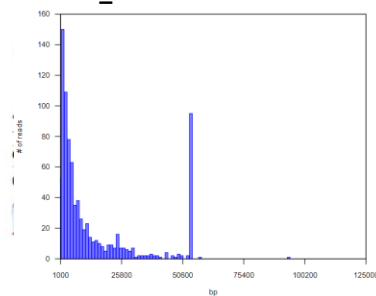
Asist digestion LacZ/KanR plasmids



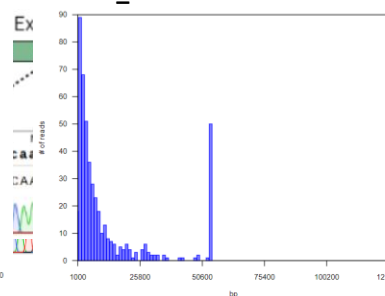
Undigested control LacZ/KanR plasmids



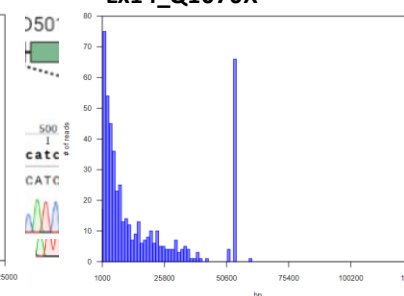
Ex3_P114S



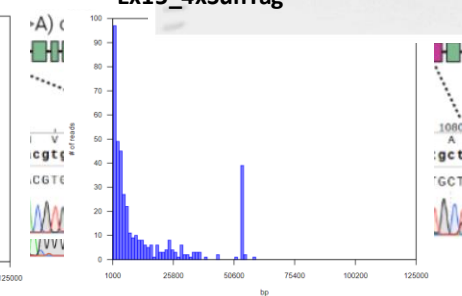
Ex7_T634S



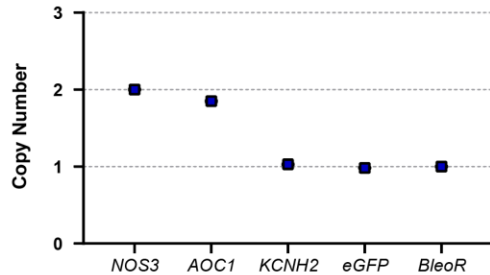
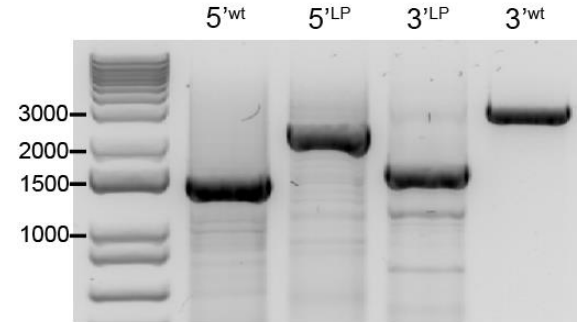
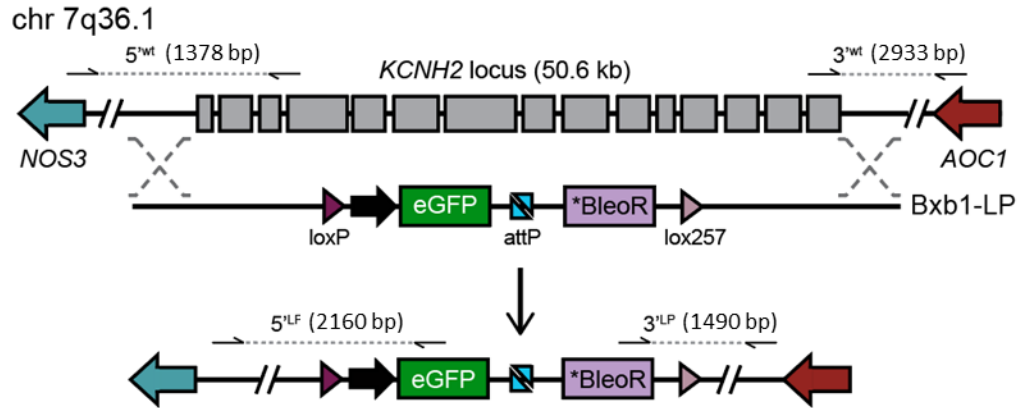
Ex14_Q1070X



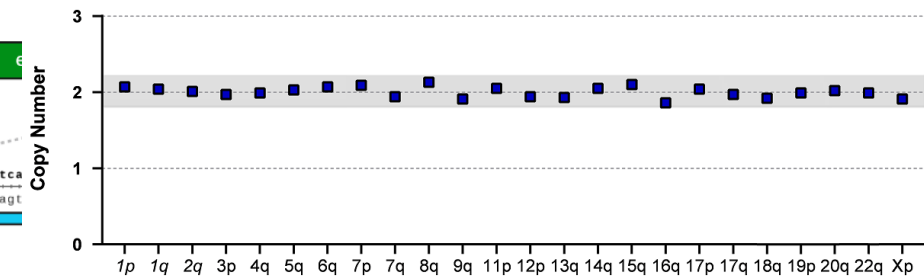
Ex15_4xSunTag



Validation LU99 KCNH2 v2 acc line



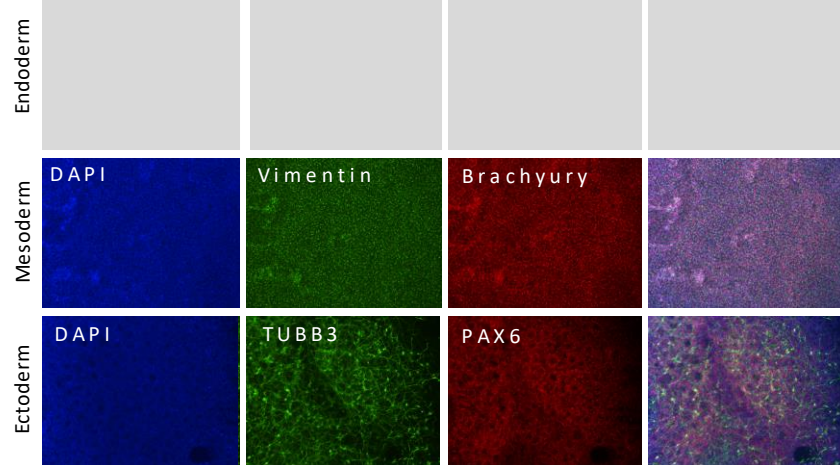
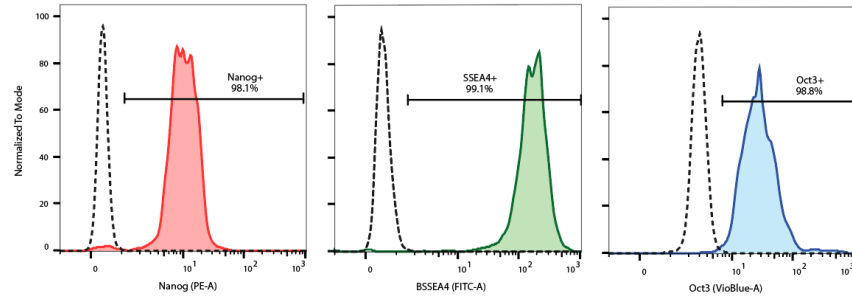
■ *KCNH2*-bxb-v2



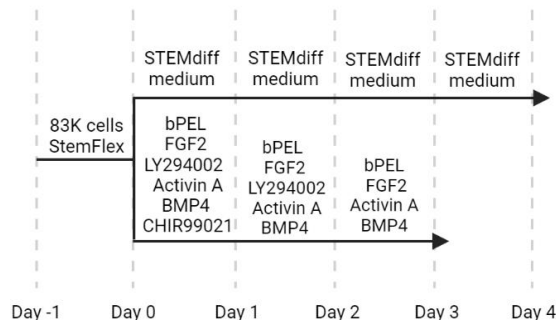
Generation and validation KCNH2 acc line

Pluripotency and differentiation potential

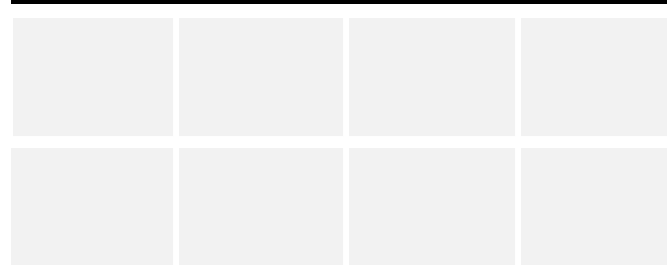
- > Identify abnormalities
- > Reproducible and comparable



Trilineage diff: Endoderm formation

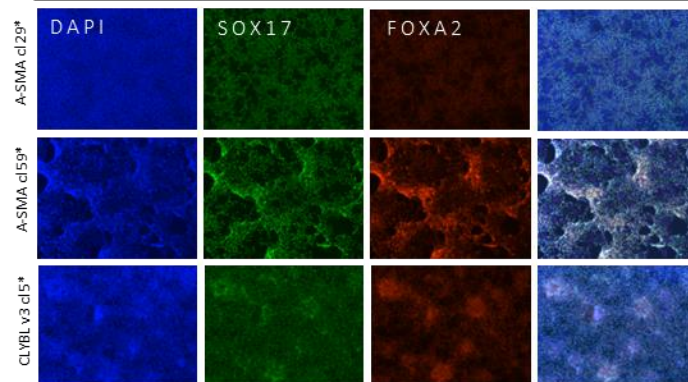


Endoderm: LU99 KCNH2 v2 acc cl17

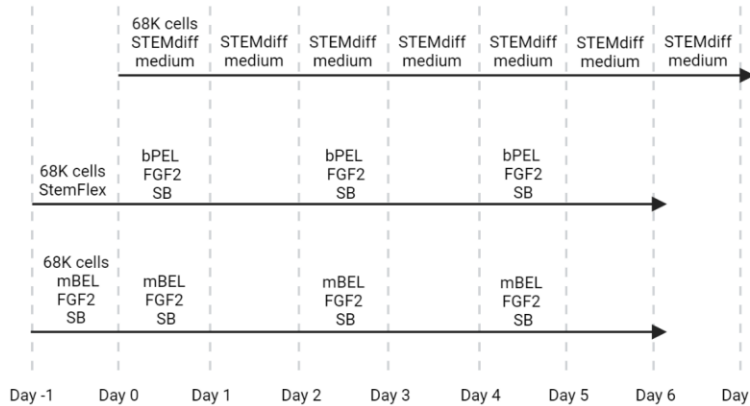


- > Problem with protocol, not with the cell line
- > Problem with the endoderm detaching and clustering
- > Issue with cell viability
- > Optimizing culture conditions and modifying medium

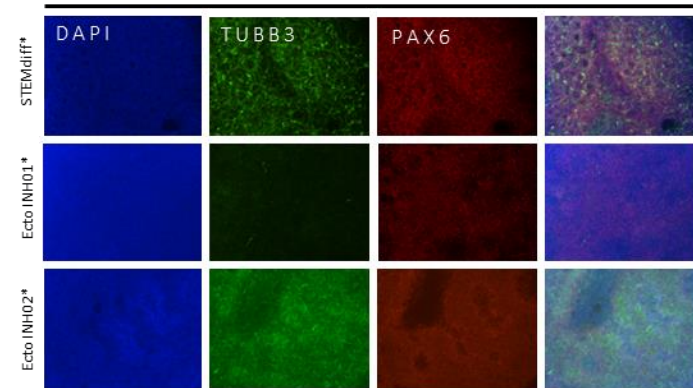
Endo INH3 (3d BPEL/FGF2/LY294002/Activin A/BMP4/CHIR99021)



Trilineage diff: Ectoderm formation

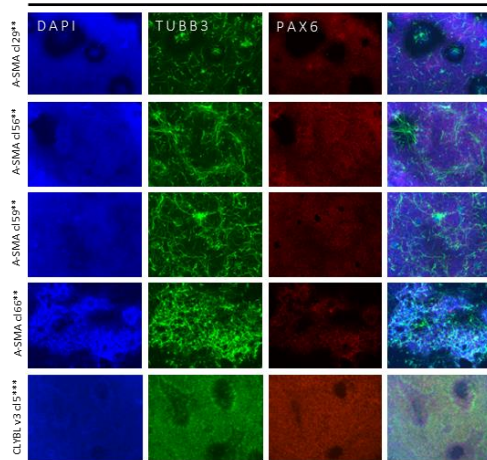


Ectoderm: KCNH2 v2 acc cl17

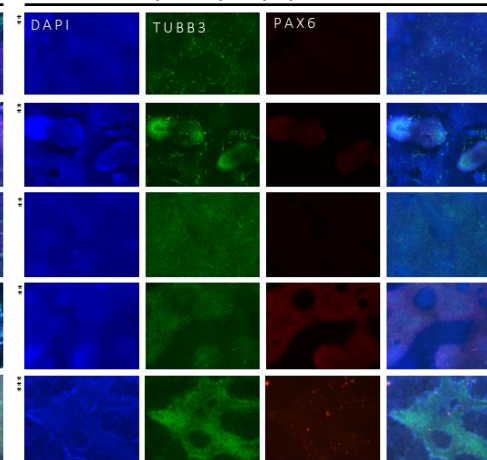


- > STEMdiff: neural rosette formation
- > Variation in mutaration and differentiation stages
- > Overall, robust and reproducible

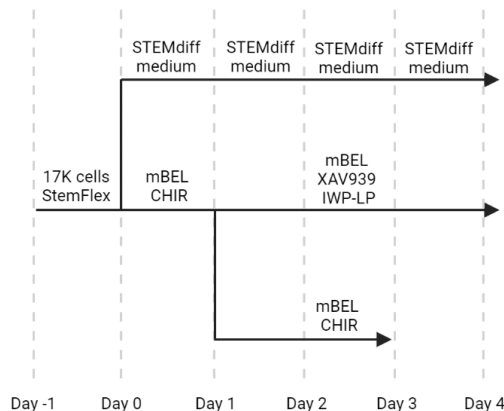
Ectoderm: STEMdiff



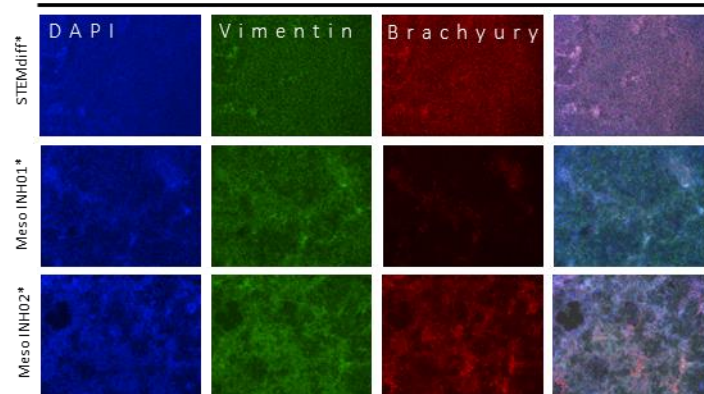
Ectoderm: INH02 (6d mBEL/FGF2/SB)



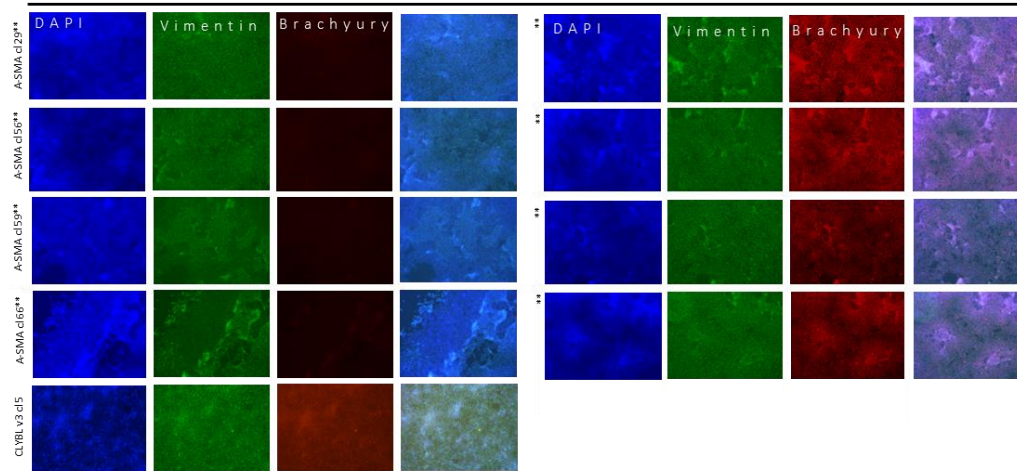
Trilineage diff: Mesoderm formation



Mesoderm: LU99 KCNH2 v2 acc cl17

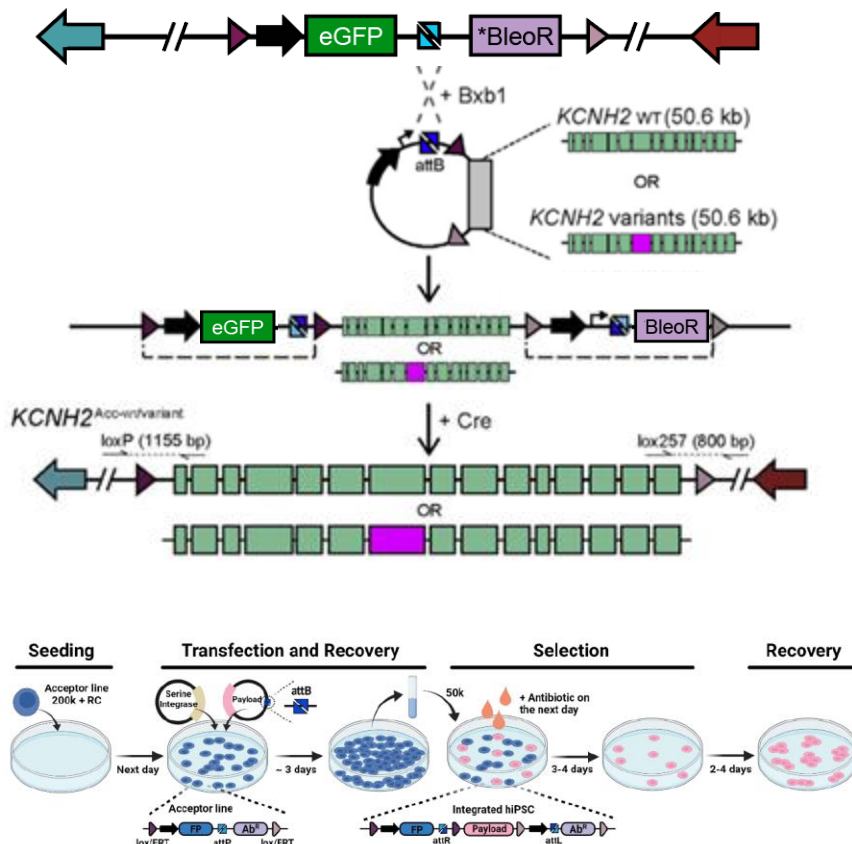


Mesoderm: STEMdiff



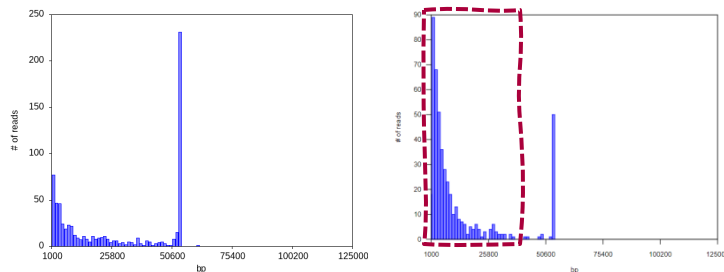
- > Downregulation of Brachyury
- > Irregularities between triplicates a-SMA

STRAIGHT-IN, donor vector integration



Integration (attP x attB -> attL + attR)

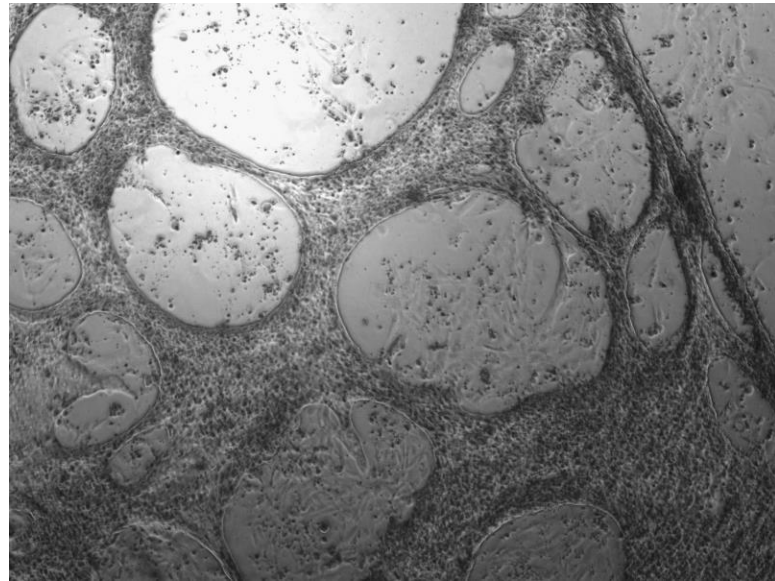
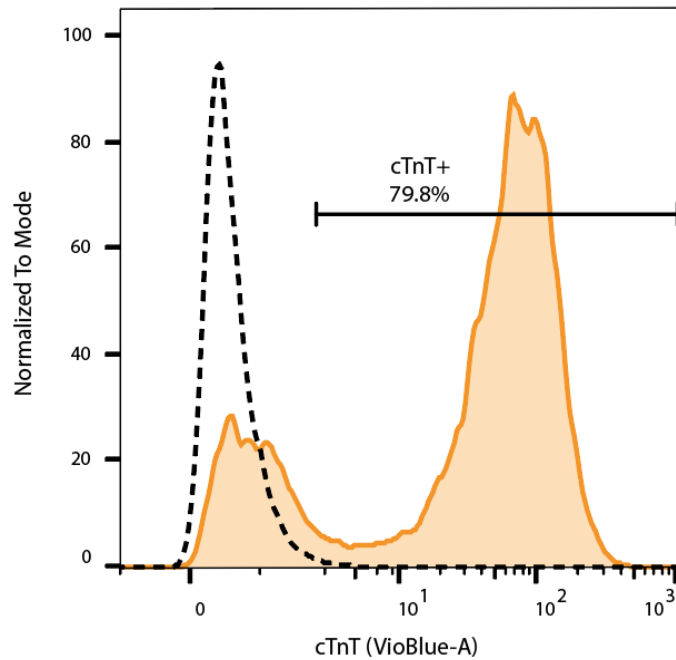
- > Empty vector, CG5 (5kb) -> a lot of colony's (N=3 0.20% efficiency)
- > 12 ex7 KCNH2 variants -> 4 colony's
- > 4 ex7 KCNH2 variants -> 2 colony's
- > Newly made variants -> No colony's



IVT mRNA: Bxb1

- > Reduced plasmid toxicity

Generation and validation KCN_{H2} acc line



Summary

RecoHiFi works well for the KCNH2

Also, for others, the LMNA for example

KCNH2 acc V2

-> CG5 (empty vector) -> 0.20% efficiency, after zeo 100%

With the BsdR V1 this was only 30%

So lower efficiency, but after enrichment 100%

Trilineage differentiations

Commercial diffs work the best overall (meso and ecto)

Endoderm differentiation needs to be optimized

Inhouse protocols give more view and room for optimization for cell line specificity

Acknowledgements

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