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Genomic engineering of iPSCs

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Ellen Hertz[1], Yu Chen[1], Joel Blanchard[2], Ellen Sidransky[1]

[1]National Institutes of Health; [2]Icahn School of Medicine at Mount Sinai

Ellen Hertz
National Institutes of Health

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We use this protocol and it's working

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Abstract

Genomic engineering of iPSCs as in Hertz et al 2025

Prepare cells for transfection

1 Grow iPSCs in Essential 8 Medium (Cat.# A1517001 Thermo Fisher) until ca 70% confluency

2 Wash cells with PBS

3 Dissociate iPSC into single cells with 1 ml StemPro-Accutase (Cat.# A1110501; Gibco)

4 Incubate at 37 C for 5 min

5 Resuspend cells in DMEM and spin down at 300 x g for 3 min

6 Resuspend cells in Essential 8 Medium with Y-27632 and seed at a density of 200,000 cells/cm^2

7 Let cells settle for 2 hours at 37 degrees

Transfection

8 Mix Lipofectamine Stem Transfection Reagent (Cat.# STEM00001; Invitrogen) and plasmids in OPTI-MEM (Cat # 31985062, Thermo) according to instuctions

9 Incubate for 5 min at RT

10 Add drop wise to the cells

11 Incubate over night at 37 C and 5% CO

Selection of transfected cells

12 Reseed cells (as described above) and resuspend cells in StemFlex (Cat.# A3349401; Thermo
Scientific), supplemented with Y-27632

13 Incubate 24 h

14 Start selection with 0.25 mg/ml puromycin (Cat.# A11138-03, Thermo Scientific)

15 Change media (including puromycin) every 2-3 days until the selected clones are almost confluent (days-weeks)

CRE-mediated excision of the selection cassette

16 Transfection of plasmid encoding GFP-tagged CRE-recombinase to
remove the selection cassette as above. Incubate 24 h in cell incubator

17 Coat 96 well plate with laminin-521 overnight in incubator

18 FACS sort individual clones into laminin coated plate with Stemflex supplemented with 10 µM Y-27632, 5 µM Emricasan (Cat.# 7310, R&D), 1x penicillin-streptomycin, 0.7 µM ISRIB (Cat.# 5284, R&D) and 1x polyamines (Cat.# P8483, Sigma)

19 Incubate cells 3 days

20 Change 1/2 media with Stemflex supplemented with penicillin-streptomycin, ISRIB and polyamines

21 Incubate 3 days

22 Change 1/2 media with Stemflex every 3 days until clones reach ca 50% confluency.

23 Expand clones