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iPSC-derived Hepatocyte TF Perturbseq

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes methods for performing TF Perturbseq in iPSC-derived hepatocytes.

Materials

accutase (Sigma-Aldrich, SCR005)

mTeSR Plus (Stemcell Technologies, 100-0276)

Rock inhibitor (Stemcell Technologies, 72304)

RPMI-1640 (Gibco, 11875093)

B-27 supplement minus insulin (Gibco, A1895601)

1X Penicillin-Streptomycin (Gibco, 15140122)

CHIR99021 (Stemcell Technologies, 72054; Cayman Chemical, 13122)

Recombinant Human/Murine/Rat Activin A (Peprotech, 120-14P)

versene (Gibco, 15040066)

B-27 supplement (Gibco, 17504044)

DMSO (Sigma-Aldrich, D2650)

BMP4 (Peprotech, 120-05-50UG)

bFGF (Peprotech, 100-18B)

HGF (Peprotech, 100-39H)

HCM Hepatocyte Culture Medium BulletKit plus media (Lonza, CC-3198)

recombinant human oncostatin M (Peprotech, 300-10)

100 nM Dexamethasone (Sigma-Aldrich, D4902)

Troubleshooting

iPSC-derived hepatocytes

- 1 Day 0: Using accutase (Sigma-Aldrich, SCR005), WTC11 iPSCs were passaged and lentivirally transduced at a high MOI (~3) onto matrigel-coated plates at a density of 10,000 cells/cm² in mTeSR Plus (Stemcell Technologies, 100-0276) supplemented with 10 uM Rock inhibitor (Stemcell Technologies, 72304)
- 2 Day 1: Media was changed with mTeSR Plus (Stemcell Technologies, 100-0276)
- 3 Days 2-3: Media was changed with mTeSR Plus (Stemcell Technologies, 100-0276) supplemented with 1 ug/mL puromycin
- 4 Day 4: Using accutase (Sigma-Aldrich, SCR005), hPSCs were passaged onto matrigel-coated plates at a density of 20,000 cells/cm² in mTeSR Plus (Stemcell Technologies, 100-0276) supplemented with 10 uM Rock inhibitor (Stemcell Technologies, 72304)
- 5 Days 5-7: Within the first 16 hours, media was replaced and maintained for 3 days with definitive endoderm media consisting of RPMI-1640 (Gibco, 11875093) supplemented with 2% B-27 supplement minus insulin (Gibco, A1895601), 1X Penicillin-Streptomycin (Gibco, 15140122), 3 uM CHIR99021 (Stemcell Technologies, 72054; Cayman Chemical, 13122), and 100 ng/mL Recombinant Human/Murine/Rat Activin A (Peprotech, 120-14P)
- 6 Day 8: Wells were briefly (~30 seconds) rinsed with versene (Gibco, 15040066) before adding hepatic specified media consisting of RPMI-1640 (Gibco, 11875093) supplemented with 2% B-27 supplement (Gibco, 17504044), 1X Penicillin-Streptomycin (Gibco, 15140122), 0.5% DMSO (Sigma-Aldrich, D2650), 20 ng/ml BMP4 (Peprotech, 120-05-50UG), and 10 ng/ml bFGF (Peprotech, 100-18B)
- 7 Days 9-12: Media was changed with hepatic specified media
- 8 Days 13-17: Cells were then maintained for 5 days with daily media changes in hepatoblast media made of RPMI-1640 (Gibco, 11875093) supplemented with 2% B-27 supplement (Gibco, 17504044), 1X Penicillin-Streptomycin (Gibco, 15140122), 0.5% DMSO (Sigma-Aldrich, D2650), and 20 ng/ml HGF (Peprotech, 100-39H)
- 9 Days 18-22: Cells were maintained for 5 days with daily media changes in mature hepatocyte media consisting of HCM Hepatocyte Culture Medium BulletKit plus media (Lonza, CC-3198) omitting EGF supplemented with 0.5% DMSO (Sigma-Aldrich, D2650), 20 ng/mL recombinant human HGF (Peprotech, 100-39H), 20 ng/mL recombinant human oncostatin M (Peprotech, 300-10), and 100 nM Dexamethasone (Sigma-Aldrich, D4902)



Prepare cells for scRNAseq

- 10 Day 23:
- o Washed 48 wells 1X with PBS
 - o Added 1 mL of Tryple-express to each well
 - o Let sit 25 minutes at 37C
 - o Resuspended with gentle pipetting into 50 mL tube filled with mature hepatocyte media
 - o Filtered through 70 um filter
 - o Spun at 0.3 RCF for 5 minutes
 - o Resuspended in 3 mL 0.04% BSA (made from 10% stock and PBS) and counted
 - o Spun again at 0.3 RCF for 5 minutes
 - o Resuspended with 0.04% BSA and filtered through 30 uM filter