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RNA-seq for human iPSC-CM following GSK3 inhibition

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We use this protocol and it's working

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Abstract

This protocol describes RNA-seq methods in human iPSC-CM following growth with or without GSK3 inhibition using CHIR99021.

Troubleshooting

Matrigel coating tissue culture plates

1 Prepare Matrigel-coated vessels as needed, following the manufacturer's instructions. Use coated vessels within two weeks.

2 Thaw Matrigel aliquot and dilute 1:30 in cold DMEM/F12.

3 Add Matrigel solution to plates quickly (2 ml for a 6-well, 12 ml for a 10-cm, 32 ml for a 15-cm).

4 Incubate at 37°C for a minimum of 1 hour. Use plates before the medium evaporates or within two weeks.

iPSC to cardiomyocyte (iPSC-CM) differentiation with WTC11 cell line

5 Aspirate media and add dPBS to wash. Then, aspirate dPBS. The differentiation protocol is based on previously published work. (**1**)

6 Add Accutase™ to cells to dissociate them (1 ml for a 6-well, 3 ml for a 10-cm). Incubate at 37°C for 3 to 5 minutes.

7 Add 1:1 warm DMEM/F12 to the Accutase™ and pipette gently 2 to 3 times to promote a single-cell suspension. Transfer cells to a conical tube and centrifuge at 300g for 5 minutes.

8 Aspirate medium and resuspend cells in mTeSR+™ (mTeSR+™ basal medium + supplement) + Rock inhibitor (10uM final concentration).

9 Seed cells in Matrigel coated plates quickly so that in 72hrs cells are 70% to 80% confluent. (2ml for 6-well, 12ml for a 10-cm of mTeSR+™)

10 Change medium to RB- (RPMI 1640 + 50x B-27™ supplement - insulin) + CHIR99021 (10 µM final concentration) (3 ml for a 6-well, 18 ml for a 10-cm). Volumes per tissue culture plate will remain consistent during differentiation.

11 After 48 hours, change medium to RB- + IWP2 (7.5 µM final concentration). Change media as close to 48 hours as possible, within +/- 5 minutes.

12 After another 48 hours, change medium to RB-. Change media as close to 48 hours as possible, within +/- 5 minutes.

13 After another 48 hours, change medium to RB+ (RPMI 1640 + 50x B-27™ supplement with 100 U/ml penicillin and 100 µg/ml streptomycin). Change media as close to 48 hours as possible, within +/- 5 minutes.

14 After another 48 hours, change medium to RB+. Change media as close to 48 hours as possible, within +/- 5 minutes. Check for cell beating.

15 After another 48 hours, check for cell beating. If beating has not occurred, replace with RB+ until beating occurs. If beating has occurred, change medium to NG+ (RPMI 1640 - glucose + 50x B-27™ supplement with 100 U/ml penicillin, 100 µg/ml streptomycin, 500 µg/ml recombinant human albumin, 213 ng/ml ascorbic acid, and 0.748 µl/ml 60% w/w sodium lactate solution), based on CDM3 medium. (**2**)

16 After another 48 hours, change medium to NG+.

17 After 48 hours, replate cells in an appropriate dish and density (3 × 10^6 cells for a 6-well, 1.8 × 10^7 cells for a 10-cm). To do this, aspirate media, add dPBS to wash, and then aspirate dPBS. Differentiation is complete at this point. Cells are now referred to as iPSC-CMs.

18 Add trypsin-EDTA (0.05%) to cells to dissociate them (1 ml for a 6-well, 3 ml for a 10-cm). Incubate at 37°C for 3 minutes.

19 Add a 1:1 mixture of stop media (DMEM + 5% FBS + DNase at 20 µg/ml final concentration) to quench the trypsin-EDTA (0.05%). Pipette gently to dislodge cells from the tissue culture plate and form a single-cell suspension, limiting to 3 to 5 repetitions. Transfer cells to a conical tube and centrifuge at 300g for 5 minutes.

20 Resuspend cells in RB+ + Rock inhibitor (10 µM final concentration). Seed cells in a 6-well plate over six wells (3 × 10^6 cells per well).

21 After 24 hours, change medium to RB+ (3 ml for a 6-well). Repeat every 48 hours for 144 hours.

Induce proliferation and developmental phenotypes in iPSC-CMs

22 Change the medium to RB+ for half the wells. For the remaining wells, change to RB+ + CHIR99021 (4 µM final concentration). Repeat every 48 hours for 168 hours.

RNA-seq

23 iPSC-CMs were cultured for 28 days following the initiation of differentiation.

24 Isolate RNA using the Total RNA Purification Plus Kit (Norgen) and submit to Azenta for standard RNA-seq with ribosomal RNA depletion.

25 Trim reads using Trimmomatic v0.32 to remove adapters, and then align to GRCh38 using the STAR aligner v2.4.1a. (**3,4**)

26 Obtain gene counts with featureCounts from the subread package (version 1.4.6-p4) using the comprehensive gene annotation in Gencode v22. (**5**)

27 Conduct differential expression analysis with DESeq2, where gene counts are fitted to a negative binomial generalized linear model, and a Wald test identifies significantly differentially expressed genes. (**6**)

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