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ATAC-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 Omni ATAC-seq methods in human iPSC-CM following growth with or without GSK3 inhibition using CHIR99021.

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 AccutaseTM 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 AccutaseTM 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+TM (mTeSR+TM 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+TM)
- 10 Change medium to RB- (RPMI 1640 + 50x B-27TM 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-27TM 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-27TM 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.

ATAC-seq

- 23 iPSC-CMs were cultured for 28 days following the initiation of differentiation.
- 24 Add trypsin-EDTA (0.05%) to cells to dissociate them (1 ml for a 6-well plate, 3 ml for a 10-cm plate). Incubate at 37°C for 3 minutes.
- 25 Add an equal volume of stop media (DMEM + 5% FBS + DNase at 20 µg/ml final concentration) to quench the trypsin-EDTA (0.05%). Gently pipette cells to promote dissociation from the tissue culture plate and formation of a single-cell suspension, limiting to 3–5 repetitions. Transfer cells to a conical tube and centrifuge at 300g for 5 minutes.
- 26 Proceed with the Omni-ATAC-seq protocol to minimize mitochondrial reads from the preparations. **(3)**
- 27 Following the final PCR, perform a 0.5x/1.8x double-sided SPRI bead cleanup on the libraries. Assess library quality by determining the cycle count needed to reach 25% of the peak threshold in the diagnostic PCR. Additionally, run the amplified libraries on a High Sensitivity D1000 Tapestation (Agilent) to confirm the expected size, and perform Qubit dsDNA HS assays to determine the final concentration.
- 28 Dilute individual libraries to 10 µM and submit to Azenta for NGS sequencing.
- 29 Adapter sequences were trimmed, and reads were quality-filtered using Trimmomatic (v0.32). **(4)**
- 30 Trimmed reads were aligned to the Hg38 reference genome using Bowtie² (v1.0.0) with parameters allowing up to 2,000 bp fragments (-X 2000), discarding multimapping reads (-m 1), and reporting the best alignments (--best --strata). **(5)**
- 31 ENCODE blacklisted regions and duplicated reads were removed using Picard MarkDuplicates and bedtools (v2.19.1). **(6,7)**
- 32 ATAC peaks were called using the MACS2 (v2.1.1) callpeak function. (8) For visualization, deepTools bamCoverage was used to generate bigwig files with counts per million (CPMs) from deduplicated BAM files. **(9)**
- 33 A union peak set was generated from MACS2 narrowPeak files across all samples. Count files for each sample were produced using featureCounts.

- 34 Differential accessibility within the union ATAC peak set was determined using DESeq2 (Padj < 0.05). (10)

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