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Mouse Tip-like Cell Directed Differentiation Protocol – Kotton Laboratory

INTRO

This protocol describes the methods to differentiate D14 lung progenitors from mouse iPSCs/ESCs into tip-like cells using Lung Progenitor Medium (LPM) from Nichane et al., 2017. This protocol describes how to passage and differentiate these cells. This protocol was published in Cell Stem Cell as part of Herriges et al., 2023 (https://www.cell.com/cell-stem-cell/fulltext/S1934-5909(23)00283-7). See also (https://protocolexchange.researchsquare.com/article/nprot-6247/v1) for methods paper tied to Nichane et al., 2017.

REAGENTS

LPM (Lung Progenitor Medium) (store at 4°C, use before 10days)

- Advanced DMEM/F12 (Thermofisher, 12634010)
- Fgf10 50ng/mL (R&D, 6224-FG-025)
- Fgf9 50ng/mL (R&D, 7399-F9-025)
- Egf 50ng/mL (R&D, 2028-EG-200)
- CHIR99021 3μM (Tocris, 4423)
- BIRB796 1μM (Tocris, 5989)
- Y27632 10μM (Tocris, 1254)
- A8301 1μM (Tocris, 2939)
- Heparin 5μg/mL (Sigma, H3149)
- Insulin 10μg/mL (Roche, 11376497001)
- Transferrin 15μg/mL (Roche, 10652202001)
- PenStrep 1x (Thermofisher, 15140122) or Primocin 1x
- Glutamine 1x (Thermofisher, 25030081) or Glutamax 1x

Reagent	Working Concentration	Dilution Stock:Working	Stock Concentration	Stock Diluent
Insulin	10ug/mL	1:100	1mg/mL	Sterile Water
Glutamax	1x	1:100	100x	N/A
Heparin	5ug/mL	1:200	1mg/mL	PBS
Primocin	1x	1:500	500x	N/A
rmFgf9	50ng/mL	1:1,000	50ug/mL	Sterile Water
rmFgf10	50ng/mL	1:1,000	50ug/mL	0.1% BSA in PBS
CHIR99021	3uM	1:1,000	3mM	DMSO
RI (Y27632)	10uM	1:1,000	10mM	Sterile Water
Transferrin	15ug/mL	1:2,000	30mg/mL	N/A
rmEGF	50ng/mL	1:4,000	200ug/mL	PBS
A 83-01	1uM	1:10,000	10mM (10mg/1.9mL)	DMSO
BIRB796	1uM	1:10,000	10mM (10mg/2.37mL)	DMSO

Dissociation Buffer for Passaging

- Dispase (Thermofisher, 17105041) 0.5mg/mL
- Collagenase IV (Thermofisher, 17104019) 0.5mg/mL
- Papain (Worthington Biochemical Corporation, LS003119) 0.5mg/mL
- Advanced DMEM/F12 (Thermofisher, 12634010)

Matrigel (Corning 356231)
Cryovials (Corning 38053)
ESC FBS (Gibco 16141061)
DMSO (Sigma, C6295)
TrypLE Express Enzyme (Gibco 12604021)
Antibodies
- BioLegend anti-mouse CD326 G8.8 Clone

PROTOCOL

1) D14 Plating
   A. Start with sort purified Nkx2.1-mCherry+, EpCAM+ live cells as described in Mouse Lung Diff Protocol.
   B. Spin down these cells at 300g x 5 min and resuspend pellet in small volume of LPM (100ul-500ul depending on cell number) and place cells on ice.
   C. Count cells on Hemocytometer. Aliquot and spin down the desired number of cells at 300g x 5 min.
   D. Thaw Matrigel on ice.
   E. Aspirate LPM off of spun-down cells and resuspend them in Matrigel such that there are 200 cells/ul of Matrigel.
   F. Slowly pipette the Matrigel + cells into a 6-well plate (~25µl/drops, no more than 200µl per 6-well).
   G. Place the 6-well plate in the incubator for 30 minutes.
   H. Once Matrigel has solidified add 2 ml of LPM to each well. Cells should be fed every other day or daily if the media turns yellow after one day.
2) Splitting (D21-D26)*
   A. Aspirate the media and add 0.9 mL of dissociation buffer to the well. Pipette up and down a few times with a 1 mL pipet to start breaking up the Matrigel.
   B. Incubate plate at 37°C for 30 min, pipette up and down a few times, and return to 37°C for 30 min.
   C. Pipette cells up and down until the majority of cells are single cell. Transfer cell suspension and an additional 9 ml Advanced DMEM/F12 to a 15ml conical tube and spin down at 300g x 5 min.
   D. Aspirate liquid* and flick tube to break up pellet. Add 1 ml of LPM and filter to remove any undigested organoids.
   E. For replating, go to step 1B of this protocol and proceed as instructed. If attempting to differentiate with a media change, still resuspend cells in Matrigel, but after 30 minute settling add 2 ml of new media. If you are transitioning cells to a different media, cells should be changed into media at this point or 2 days after passaging, depending on the media.
3) Freeze/Thawing cells
   A. Perform steps 2A-D from above.
   B. Count cells and determine the desired cell count per frozen vial (recommended to start with ~5e5 cells per cryovial).
   C. Spin down cells at 300g x 5 min and resuspend in ESC FBS with 10% DMSO. Add enough volume to create the desired number of 1 mL aliquots.
   D. Quickly pipet 1 mL of resuspended cells into the desired number of labeled cryovials and transfer to Styrofoam container or other cell freezing container (https://www.premiumvials.com/maccx-1-33-8-cell-freezing-container-for-2ml-cryogenic-vials-12-places-cross-linked-polyethylene-foam-with-a-solid-state-core-cfc012-001/). Immediately move container to -80°C to freeze overnight.
   E. The next day transfer cells to -150°C for long term storage.
   F. When you are ready to thaw cells, take out a tube and immediately thaw using a 37°C water bath or other heat sources. Heat cells until only small amount of ice is left in the tube.
   G. Dilute the thawed cells with 9mL of Advanced DMEM/F12 in a 15mL conical tube.
   H. Continue with steps 1B-H to plate the thawed cells.

COMMENTS

*: Alternatively use 0.9mL of TrypLE in place of the dissociation buffer and incubate cells for 10-15 minutes at 37°C until cells are largely a single cell suspension. Dilute and spin down cells as described for standard dissociation protocol. This method is a little more consistent at producing a single cell suspension for passaging, but does result in significantly more dead cells.

**: Take care when aspirating at this step as the Matrigel may not be fully digested, leading to transparent strings of Matrigel connected to the cell pellet. Do not let the aspirator get within half an inch of the pellet, or you may risk sucking up the pellet.

VERSION HISTORY

2018-09-13	Drafted by Michael Herriges	MJH
2021-09-06	Updates	MJH
2023-09-07	TrypLE update	MJH