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Protocol: Generation and expansion of myogenic progenitors from iPSCs

Medium and supplements

Myogenic differentiation medium

DMEM/F12

+ 1% P/S

+ 1% L-glutamine

+ 1% Insulin-Transferrin-Selenium-Ethanolamine (ITS-X)

Myogenic progenitor proliferation medium (expansion)

DMEM high glucose

+ 10% fetal bovine serum (FBS)

+ 1% P/S

+ 1% L-glutamine

+ 100 ng/ml FGF2

Maturation medium (differentiation into skeletal muscle cells):

DMEM high glucose

+ 10% fetal bovine serum (FBS)

+ 1% P/S

+ 1% L-glutamine

+ 1xITS-X

+ 1% knockout serum replacement (Gibco)

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Supplements and small molecules:

- CHIR99021 (Axon Medchem)
- FGF2 (Prepotech)
- Revitacell supplement (Gibco)
- ECM (Sigma-Aldrich, E6909)
- 1xITS-X
- 1% knockout serum replacement (Gibco)
- TrypLe reagent (Gibco)

FACS sorting:

- anti-HNK-1-FITC (1:100, Aviva Systems Biology)
- anti-C-MET-APC (1:50, R&D Systems)
- Hoechst (33258, Life Technologies)

Generation and expansion procedure:

Initiate myogenic differentiation after iPSC expansion by changing to myogenic differentiation medium supplemented with 3.5uM CHIR99021 for 5 days (CHIR treatment is toxic test different concentration and duration of treatment between 3-5 days); and then change to myogenic differentiation medium supplemented with 20 ng/ml FGF2 for 14 days. For the last 16 days, culture cells in myogenic differentiation medium only. Refresh medium daily.

CHIR99021 is a critical step, as too low concentration fail to yield myogenic cells, while to high concentration can be toxic. To determine the optimal concentration with CHIR, vary concentration and duration of treatment and score for confluency and PAX7 expression. Perform it for two independent lines.

Feeder-free: 4 days 3 µM CHIR99021 treatment on 0,6 mm sized iPSCs and stain for PAX7 (ab: DSHB, 1:100) after 35-day differentiation.

Purify progenitors of myogenic progenitors using FACS

Wash cell once with PBS, incubate 5 min with TrypLe at 37C and gently detach with pipetboy. Filter the cell suspension through a 40 µM FACS strainer (Falcon) to remove cell aggregates. Centrifuge cells for 4 min at 1, 000 rpm and incubate with anti-HNK-1-FITC (1:100, Aviva Systems Biology) and anti-C-MET-APC (1:50, R&D Systems) antibodies for 30 min on ice in myogenic differentiation medium. Wash cells three times with ice-cold 1% BSA in PBS before FACS sorting. Add Hoechst (33258, Life Technologies) as a viability marker. Sort the c-MET^{+}/Hoechst^{+}/Hnk-1^{-} fraction with a 100 µm nozzle and collect in ice-cold iPSC-myogenic progenitor

proliferation medium. To reduce cell death, supplement medium with 1x Revitacell during collection and the first 24 hours of cell culture. Sort time should not take longer than 20 minutes per well. Coat Plates/well for 30 min at RT with ECM (E6909-5 mL, 1:200 in iPSC-MPC-pro medium, Sigma Aldrich). Depending on the amount of cells, sorted cells are plated either at 40,000 cells in one well of a 48-well plate or at 80,000 cells in one well of a 24-well plate.

Expansion of myogenic progenitors

At day 1 after plating FACS sorted myogenic progenitors, refresh the medium with myogenic proliferation medium on ECM-coated dishes (1:200 diluted). When cells reach 90% confluency, for splitting, detach myogenic progenitors with TrypLe reagent diluted 2x with PBS and plate on ECM-coated plastic.

For cryopreservation, use myogenic proliferation medium supplemented with 10% DMSO.

Maturation of myogenic progenitors into skeletal muscle cells

For differentiation to multinucleated myotubes, when the myogenic progenitors reach 90% confluency, switch to maturation myogenic medium and medium does not need to be refreshed. Fix cells at 6 days, 8 days and 12 days.

Antibodies for myogenic culture characterization

Name	Dilution	Company
Mouse-anti-MF20	1:50	DSHB
Rabbit-anti-Myogenin	1:100	Santa-Cruz (sc-576)
Mouse-anti-PAX7	1:100	DSHB
Mouse-anti-a-Actinin	1:100	Sigma-Aldrich (A7811)
Mouse-anti-Myosin (fast)	1:100	Sigma-Aldrich (M4276)
Mouse-anti-Titin	1:50	DSHB
Mouse-anti-Desmin	1:100	Abcam