Standard operational procedure

Freeze hiPS cells from E8 culture using TeSR-E8 + 10% DMSO

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LUMC iPSC line:______________________________
Passage number:______________________________
Date of freezing:______________________________

Ampoule sticker:

Materials:

- TeSR-E8 *(iPS_SOP_0034.2)*
  Prepared on: ____________________
- DMSO (Sigma /D2650)
  Lot. __________ exp.: __________
- UltraPure™ 0,5 M EDTA, pH 8,0 (Invitrogen/15575)
  Lot. __________
- DPBS -CaCl2, -MgCl2 (Gibco/14190)
  Lot. __________ exp.: __________
- Cell scraper (Greiner/541-070)
- Mr. Frosty or CoolCell

Method:

- ☐ Remove all differentiated areas from the colonies using a small pipet tip.
- ☐ Dilute EDTA in PBS (1:1000, final concentration 0,5 mM EDTA).
- ☐ Aspirate medium from the wells.
- ☐ Add 1 ml/6-well EDTA and incubate at RT.
  NOTE: *Incubation time will differ per clone, always check in between to see whether the cells have been incubated long enough!*
- ☐ Aspirate EDTA from the wells and immediately add 1ml/well TeSR-E8.
- ☐ Gently detach colonies by scraping with a cell-scraper.
- ☐ Transfer the detached cells aggregates to a 15ml tube using a P1000.

- ◻ If you want you can rinse the well with an additional 2ml TeSR E8 and add this to the 15 ml tube.
- ◻ Spin 2 min. at 600 rpm.
- ◻ Carefully aspirate the supernatant without disturbing the pellet.
- ◻ Be aware to keep the break-up of cell aggregates to a minimal with the next steps!
- ◻ Prepare TeSR-E8 with 20 % DMSO.
- ◻ Very gently resuspend the pellet in TeSR-E8, add an equal volume of TeSR-E8 + 20% DMSO, use 0,5-1ml TeSR-E8 + 10% DMSO for each vial you will make.
- ◻ Transfer cell aggregates in TeSR-E8 + 10% DMSO to a pre-cooled and properly labeled vial.

  LUMC hiPS number; Passage; Freezing medium; size start-up culture dish+Medium/coating; Date; Initials; LUMC iPS core facility
- ◻ Freeze vials using a Mr. Frosty or CoolCell.

Note: ____

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