Thawing Human Pluripotent Stem Cells in Feeder-Free conditions

Purpose: This protocol describes steps to thaw human pluripotent stem cells from cryogenic storage conditions. Human pluripotent stems cells commonly suffer low viability when reanimated from cryopreservation. Maintaining intact colonies through minimal pipetting and gentle pellet resuspension and the use of a small-molecule inhibitor of the ROCK signaling pathway can help alleviate viability issue.

Materials

- Matrigel coated 6 well plate
- Dry ice
- TeSR
- ROCK inhibitor – Y27632 (10mM = 1000x stock)
- 15mL conical tube
- Frozen vial of cells
- 37°C water bath
- Table top centrifuge

Procedure

1. Prepare appropriate volume of 1x ROCK inhibitor media (add 1uL 1000x ROCK inhibitor to 1mL TeSR). Approximately 8mL of media is sufficient for thawing one vial.
2. Collect vials to be thawed from cryostorage into dry ice.
3. Place frozen vial into 37°C water bath. It is best to use a floating rack for this step. Avoid shaking/agitating the vial while in the water bath. Remove vial from the water bath when small rice grain-sized piece of ice remains. Do not let the vial thaw completely in the water bath.
4. Transfer thawed cells to 15mL conical tube.
5. Slowly pipette 3-5mL of ROCK inhibitor media into the 15mL conical. Dropwise pipetting while gently tapping the tube is recommended.
6. Wash the vial with an additional 1mL of ROCK inhibitor media and gently add to the 15mL conical.
7. Pellet cells by centrifugation at 1000rpm for 5min.
8. Gently aspirate supernatant leaving 50-100uL of media in the tube bottom. Cap tube and gently tap with finger to resuspend the cell pellet.
9. Aspirate Matrigel solution from 6 well plate.
10. Gently add 2mL of ROCK inhibitor media to resuspended cells and transfer the entire volume to 1 well of 6 well plate. Avoid aggressive pipetting.
11. Place plate in 37°C, 5%CO2 incubator. Allow cells to adhere overnight. After 24hs, replace media with 2mL of fresh TeSR (without ROCK inhibitor).
12. Colonies should appear within one week, allow for two weeks prior to troubleshooting.

Troubleshooting: If experiencing low viability thawing, cells to a 12 well plate is recommended. Plating thawed cells to mitotically inactivated MEFs may also help. Maintaining culture conditions (Matrigel/TeSR, MEFs/KOSR media, etc.…) used prior to thawing is also recommended, as the added stress of adapting cells to new growth conditions during reanimation may contribute to poor viability

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and reduced growth rates. All hESC and hiPSC stocks generated at the Salk Stem Cell facility were cultures in Matrigel/TeSR prior to banking.

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