[Protocol 1.01.2] Thawing of iPSCs frozen in Bambanker freezing medium

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Protocol 1.01.2 thawing of iPSCs using Bambanker freezing medium
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Media and Reagents:
Appropriate medium with ROCK inhibitorY26732 (final concentration 10 µM)
Geltrex coated cell culture vessel (see *[Protocol 1.05] Geltrex coating of culture vessels*)

Materials and Equipment:
Aspiration Pump
Water bath

1. Introduction and Purpose

This protocol describes the thawing process of induced pluripotent stem cells (iPSC) frozen with Bambanker TM freezing medium.

2. Procedure
1. In case a cryovial contains 1 M cells and is planned to be plated into 2x6W coated with GT (1/3 & 2/3), place 14 ml (9 ml to dilute + 4 ml to feed + 1 ml of buffer) of the appropriate medium (supplemented with ROCK inhibitor) in a 15 ml tube and warm to room temperature.
2. Remove vial from the liquid N2 and perform a quick thaw in the 37°C water bath. Carefully swirl vial in the water, avoid immersing the vial above the level of the cap. Leave a little ice. This should not take longer than 1.5 - 2 min.
3. Sterilize tube by spraying and wiping 70% Ethanol (S1)/Mikrozip (S2) using a tissue.
4. Using a 5 ml serological pipette/1 ml pipette tip remove cells from cryovial and place in a 15 ml conical tube. Be very gentle when using 1 ml pipette tip!!! it's more precise, but the pipetting pressure is higher than serological pipette.
5. Slowly add about 9 ml of medium (supplemented with ROCK inhibitor, RT) to the tube drop-wise, while gently swirling the tube.
6. Centrifuge at 300 x g for 5 minutes and aspirate the supernatant, leaving the cell pellet in the tube.
7. Using a 5 ml pipette gently add 3 ml of medium (supplemented with ROCK inhibitor) to the pellet. Resuspend the cell pellet gently by pipetting up and down. Do not aspirate more than 2 times to avoid breaking the cell clumps into single cells.
8. Add the freshly thawed cells to two wells of a Geltrex coated 6-well plate (1/3 - 1 ml and 2/3 - 2 ml). Add 1 ml of medium supplemented with ROCK inhibitor to the well where 1 ml of cell suspension is plated.
9. Rock the plate side to side, back and forth to spread the cells across the well.
10. Incubate cells at 37°C, 5 % CO2.
11. For further culture refer to *[Protocol 1.01.0] Maintenance of iPSC*

Relevant applicable documents:
Protocol 1.01.0 Maintenance of iPSCs
Protocol 1.03.1 Reconstitution, aliquoting and use of Y26732 ROCK inhibitor
Protocol 1.05 Geltrex coating of culture vessels