Vitrification method

Thawing and culturing procedures for human ES/iPS cells cryopreserved by vitrification method

CAUTION !

Some of human ES/iPS cell lines sent from our bank have been cryopreserved by vitrification method.

Its thawing procedure is totally different from by the slow-cooling method, which is most commonly used in cell culture. If you thaw the cells without following the procedure below, all cells will die and we can not guarantee the result.

Reference

Fujioka T et al. A simple and efficient cryopreservation method for primate embryonic stem cells. Int J Dev Biol. 48(10):1149-54 (2004) PMID: 15602701, DOI: 10.1387/ijdb.041852tf

*Prepare all necessary materials and reagents before starting procedure

Required Materials

Human ES/iPS medium
15mL centrifuge tube
0.1% gelatin coated dish with feeder cells
(All plate should be coated with 0.1% gelatin prior to plating feeder cells)
LN_2 reservoir
1000 µl micropipette

1. Prepare 10mL of the media in 15ml centrifuge tube and warm it in a 37°C water bath.

NOTE: Always use liquid nitrogen (NOT dry-ice) to transfer the cryotube. Place LN_2 reservoir close to the safety cabinet for subsequent manipulation.

2. Set up the pre-warmed media prepared at step 1, and you need to start thawing procedure before the media cool down.

NOTE: It is required to complete the following thawing steps as quick as possible.

3. Remove the frozen vial of ES/iPS cells from liquid nitrogen, transfer immediately to the safety cabinet.
4. Using a 1000 µl micropipette, take 1mL of the pre-warmed media from the tube previously prepared at STEP 1, then immediately add to the frozen vial by pippeting up and down repeatedly until the cells have completely thawed.

NOTE: It is important to avoid ice crystal formation in cryopreserved ES/iPS cells during this thawing procedure.

5. Transfer cell suspension into the pre-warmed media in the 15 mL tube.
6. Centrifuge at 1000RPM (200g) for 3 minutes at room temperature.
7. Aspirate supernatant from the tube and gently resuspend pellet with 4 to 5 ml fresh ES/iPS medium.
8. Place suspension into ONE gelatin-coated 60mm culture dish containing feeder cells.
9. Next day, replace medium. (Change medium daily)

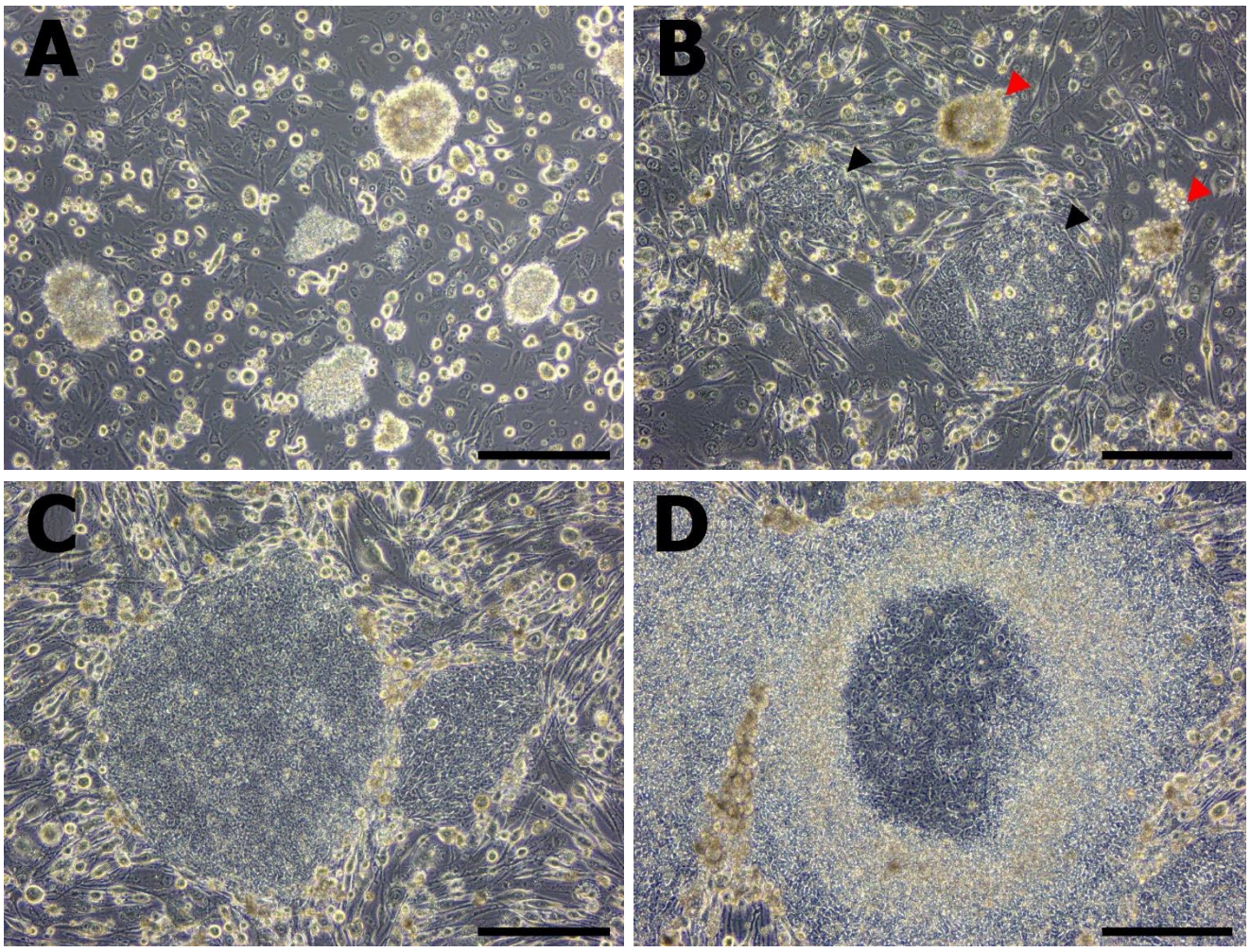

Fig.3 A human iPS cells after thawing（Scale bar 200μm）
B Next day （Viable colonies（Black）and Dead cells（Red））
C human iPS colonies
D Morphology of the differentiated iPS colony 5 days after thawing

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