hiPS Cell Culture protocols.

Thawing:

- Transfer the cryovial from liquid nitrogen or -80°C storage to a container with dry ice or ice.
- Thaw the cryovial in a 37°C water bath until a small piece of ice remains.
- Carefully transfer the contents into a 15mL tube.
- Slowly add 2mL of cold Essential 8(E8) media drop by drop using a pipette.
- Centrifuge at 200g for 3-4 minutes.
- Aspirate the supernatant and resuspend the pellet in media, then immediately seed into a Vitronectin-coated dish.
- OPTIONAL: use 10-20 µM ROCK inhibitor to improve cell recovery

Passage:

- Prepare a new Vitronectin-coated dish with fresh E8 media.
- Wash the cells with PBS.
- Add 0.5mM EDTA in PBS.
- Incubate for 2-3 minutes at 37°C.
- Aspirate the EDTA in PBS.
- Add E8 media and gently remove colonies by pipetting.
- Seed the cells into a new dish at a ratio based on plating efficiency (1:5 to 1:20).

Freezing:

- Passage the cells enzymatically or manually and centrifuge them at 200g for 4 minutes.
- Prepare and label cryovials with the name, clone, passage number, cultivation conditions, and date.
- Aspirate the supernatant and add 500µL of Synth-a-Freeze per vial, then gently resuspend the cells.
- Quickly place the cryovials into Cryo-boxes and transfer them to -80°C.
- Keep the Cryo-boxes at -80°C for 24-48 hours, then transfer the vials to liquid nitrogen for long-term storage.