1. Materials

1.1 Reagent: Matrigengel(Cat.082777); ROCK inhibitor (Y-27632); DMEM F12; mTeSR1/E8 medium; Accutase Dissociating solution, DPBS

1.2 Consumables: sterile tips, 6-well plate（or other well number of plates，This protocol will use a 6-well plate as an example）, sterile EP tube.

2. Preparation

2.1. Material pre-cooling

2.1.1 Put the Matrigengel in the ice box and put it in the refrigerator at 4℃ so that the Matrigengel can slowly melt overnight; (Do not allow this product to warm up above 4° C during manipulation. Keep the product on ice and dilute using ice-cold solutions or cell suspensions.)；

2.2.2 Supplies or reagents that come into contact with Matrigengel, such as sterile centrifuge tube, sterile tips and DMEM F12, were be pre-cooled at 4℃ in advance.

2.2. Dilute the Matrigengel(Art no.0827775) at（1:80 - 1:100）(Tip：1:80 - 1:100 are all appropriate,equivalent to Matrigengel concentration of 0.1mg/mL.For iPSC culture, Mogengel recommends coating at a concentration of approximately 0.013 mg/cm², for example, after diluting 12.5 mg/mL of Matrigengel 1:100, the coating volume of each 6-well plate is 1 mL)

2.2.1. Transfer appropriate 25mL of DMEM/F12 at 4℃ into the cooled EP tube；

2.2.2. Use a pipette with the pre-cooled tips to transfer the 1mL DMEM F12 is transferred from the EP tube to the aliquot of Matrigengel, mixed well and then transferred to the other EP tube(kept on ice). Repeat this step several times until the matrix gel is completely pipetted into the EP tube；

2.2.3. The mixture is as a reserve for subsequent coating.

2.3.Plate coating procedure

2.3.1 Add the 1mL/well Matrigengel mixture into the pre-cooled 6-well plate , and gently shake the plate to ensure that the mixture is evenly spread on the plate;

2.3.2 Transfer the 6-well plates to a 37℃ incubator for overnight incubation (the plates can be used after incubating for 1-2 hour, but the coating for overnight incubation is better for cell culture.Coated plates with coating solution can be stored at 4°C and should be used within one week of coating. Coating solution should be aspirated just before using the plates);

2.3.3 Absorb the liquid above the coating before use.

2.4. Configure ROCK inhibitor (Y-27632) working solution: use sterile PBS to dissolve Y-27632 and configure it into 10mM solution (1000X) with working concentration of 10uM;

2.5. Preparation of medium containing ROCK inhibitor: ROCK inhibitor (Y-27632) with 10mM solution was added to mTeSR1/E8 medium until the final concentration was 10uM.

Note :The Matrigengel at 4℃ will gradually polymerization into glue.Please strictly control the operating temperature and the operating time.

3. Cell culture

3.1. iPSC thawing

3.1.1 Remove the ipsc from the liquid nitrogen or dry ice and thaw it in water at 37℃. The thawing should be completed quickly;

3.1.2 Disinfect the frozen tube with 75% alcohol and transfer it to bechtop;

3.1.3 Transfer the cell solution to a new 15ml EP tube and flush the primary tube twice with DMEM F12/DMEM;

3.1.4 Centrifuge the 15ml EP tube at room temperature 300g for 5min(ipsc has good tolerance to 200-300g speed, and 300g is recommended to maximize cell capture, and 200g is recommended in the standard procedure)

3.1.5 Discard the supernatant, gently resuspend the iPSC with 2mL of medium containing ROCK inhibitor, and transfer it to the coated 6-well plate. Shake the plate evenly to distribute the cells (Cell density was adjusted to 1×10^6 cells per well)

3.1.6 Put the 6-well plate back into the 37℃ incubator (please do this immediately after the cells are transferred to avoid increased cell center density).

3.1.7 The ROCK inhibitor was removed the next day, and the cells were cultured with the non-inhibitor medium.

Note: The use of antibiotics in cell culture is not recommended as they can interfere with cells and their differentiation potential. The culture environment should be isolated from other cells, and the mycoplasma should be detected after two passages; If the cryopreservation solution contains DMSO，It's toxic to cells at room temperature and the cell thawing procedure should be completed quickly.

3.2. iPSC passaging

3.2.1 Discard the culture supernatant, rinse with 1mL PBS, and add 1mL Acuutase;

3.2.2 Transfer the plate to a 37℃ incubator for 3 minutes, or observe under a microscope until most cells fall off (if cells are still attached, place the culture plate in your hand and gently place the other hand on the flap which causes the plate to vibrate slightly to make the cells fall off);

3.2.3 Prepare the coated 6-well plate before passage;

3.2.4 Tilt the culture plate and transfer the Accutase solution twice across the surface of the culture layer to separate the clumps and transfer them to the centrifuge tube;

3.2.5 Rinse the surface of the culture layer with DMEM F12 and merge with the cell solution in the tube (wash with DMEM/F12 or PBS, at least 5% is recommended Medium for subsequent granulation and attachment);

3.2.6 Centrifuge the tube 300g at room temperature for 5 min;

3.2.7 Discard the supernatant and resuspend the cells with a medium containing ROCK inhibitor;

3.2.8 Transfer it to the coated 6-well plate. Shake the plate evenly to distribute the cells;

3.2.9 Put the 6-well plate back into the 37℃ incubator for incubation;

Note: iPSCs will rapidly differentiate and die when they grow to a single layer. In order to maintain growth and pluripotency, they should be passed before full growth.

3.3. iPSC cryopreservation

3.3.1 Prepare the cell dissociation solution.

3.3.2 Cell isolation method is similar to the passage, cell counter can be used to cooperate with cell cryopreservation;

3.3.3 Each tube of cells was frozen at the density of $1X10^6$, followed by centrifugation, extraction of the medium, and then resuspended in an appropriate volume of frozen solution;

3.3.4 Add 1ml of the resuspended cell cryoprecipitate to the 1.5ml freezing tube, undergo programmed cooling, and store in the liquid nitrogen for a long time.