Preparing Matrigel

*BD Matrigel, GFR (Cat# 354230) arrives at a volume of 10mls in varying concentrations from BD Bioscience. Prior to use, Martigel should be aliquoted into 3 different concentrations - 0.5mg, 1mg and 2mg.*

Materials
- Cold 0.5ml eppendorf tubes
- Cold eppendorf tube racks
- Cold 200ul and 1000ul pipette tips
- Thawed bottle of Matrigel**

**Thaw Matrigel by submerging the bottle in ice (using an ice bucket and lid) and storing in the 4° overnight.

Protocol
1. The day before aliquoting, place autoclaved 0.5ml eppendorff tubes, tube racks and unfiltered tips in the -80° and thaw Matrigel.
2. Match the Lot # on the bottle to the Lot # on the spec sheet. Note the concentration.
3. Based on the concentration calculate the volume needed to get 0.5mg, 1mg and 2mg
   a. i.e. if the concentration is 8mg/ml the calculations would look like this:
      i. 0.5mg: (8mg) (Volume) = (0.5mg) (1000ul)
        1. Volume = 62ul of Matrigel to give [0.5mg]
      ii. 1mg: (8mg) (Volume) = (1mg) (1000ul)
          1. Volume = 124ul of Matrigel
      iii. 2mg: (8mg) (Volume) = (2mg) (1000ul)
           1. Volume = 250ul of Matrigel
4. Calculate how many 0.5mg’s, 1mg’s and 2mg’s you can get out of 10mls
5. Using the cold tips, aliquot the appropriate volume of Matrigel into the cold eppendorf tubes.
   a. Make sure to change to a new cold pipette tip after a few tubes to keep it cold.
6. Label the tubes and store in the -20°
Coating tissue culture plates with Matrigel

*Resuspend 0.5mg of Matrigel in 6mls of media. This can coat:

- 1-6 well plate (1ml/well)
- 1-12 well plate (.5ml/well)
- ½-24 well plate (.5ml/well)
- 3-60mm dishes (2ml/dish)
- 1-10cm dish
- 1-T-75 flask

Materials

- Basal media (DMEM, DMEM/F12, or F12)
- Cold 200ul pipette tips
- Cold 15ml/50ml conical tubes
- Cold 5/10ml serological pipettes
- Matrigel at desired concentration

Protocol

1. Grab a cold conical, cold tips and cold pipette from -80° freezer
2. Quickly add an appropriate volume of cold basal media into your cold conical using a regular pipette (not cold)
3. Grab appropriate amount of Matrigel from -20°
4. Using the cold tips, quickly use cold media to thaw frozen Matrigel by gently pipetting up and down.
   a. Take care to keep fingertips above the Matrigel level
   b. Continue changing to cold tips as needed.
5. Transfer Matrigel to conical with cold media. Repeat until all Matrigel is added into cold media.
6. Using the cold pipette, transfer media and Matrigel into your TC dish
   a. 6 well plates = 1ml/well
   b. 12 well plates = 0.5ml/well
   c. 24 well plates = 0.5ml/well
   d. 60mm dishes = 2ml/dish
   e. 10cm dish = 6ml/dish
   f. T-75 flask = 6ml/flask
7. Incubate at room temperature for at least 1 hour.
   a. If you are not using Matrigel coated dishes on the same day as coating, wrap dishes in parafilm, and store in 4° for up to 1 week. Make sure wells do not dry out.
Protocol for passaging hESC/iPSCs on Matrigel

mTeSR Media

- 400ml mTeSR Basal Media + 100ml mTeSR 5x Supplement (StemCell Technologies, Cat # 05850)

Materials

- 6 well Tissue Culture dish
- Matrigel, (0.5mg/plate)
- The StemPro® EZPassage™ Disposable Stem Cell Passaging Tool (Invitrogen, Cat # 23181-010)

Protocol

1. Coat the tissue culture dish with 0.5mg of Matrigel per plate for 1 hour at temperature.
2. Check colonies in microscope and using a pulled glass pipette or colony marker, remove any areas of differentiation from the culture.
3. Aspirate spent media and add desired amount of fresh mTeSR + Supplement media to each well (we usually add 3mls for 1:6 split).
4. Before cutting cells, aspirate Matrigel from the tissue culture plate and add 2mls mTeSR media to each well (for a total of 2.5mls per well after cells have been added).
5. Using the EZPassage™ tool cut colonies into small squares
   a. Hold the culture vessel in one hand and pull (roll) the StemPro® EZPassage™ Disposable Stem Cell Passaging Tool across the entire plate in one direction (Left to right). Apply enough pressure so the entire roller blade touches the plate and maintain uniform pressure during the rolling action.
   b. Rotate the plate 90° and repeat step a.
6. Use the tip of a 5ml serological pipette to gently dislodge the colonies from the plate. **Be sure to always have media coming out of the pipette.** Continue to pipette up and down gently completely dislodge the cut colonies. Avoid causing bubbles.
7. Add 0.5mls of cells to each well.
8. Shake plate back and forth then side to side to spread out colonies on the plate.
9. Place plate in 37°C incubator with 5% CO₂.
10. Leave cells alone for 24hrs.
Protocol for passaging hESC/iPSCs on Matrigel with Versene

mTeSR Media
- 400ml mTeSR Basal Media + 100ml mTeSR 5x Supplement (StemCell Technologies, Cat # 05850)

Materials
- Tissue Culture dish
- Matrigel, (0.5mg/plate)
- Versene (Gibco)

Protocol
1. Coat the tissue culture dish with 0.5mg of Matrigel per plate for one hour at room temperature.
2. Check colonies in microscope and using a pulled glass pipette or colony marker, remove any areas of differentiation from the culture.
3. Aspirate used media.
4. Rinse cells with a single volume of Versene and aspirate
   a. 1.5 ml for 60mm dish
   b. 1ml for 1well of 6 well plate
5. Add a single volume of Versene to each well
   a. 1.5 ml for 60mm dish
   b. 1ml for 1well of 6 well plate
6. Incubate at 37° for 4-5 minutes
   a. Check cells under microscope after 4-5 minutes to check for breaking up of colonies
7. Gently rinse cells with a single volume of mTeSR
   a. If too many cells have lifted off the plate, add more media collect the cells and spin down for 1min at 1,000rpm. Then move to step 8.
8. Add a volume of mTeSR and collect the iPSCs (3mls for 1:6 split)
9. Pass cells at desired density into new TC dish
10. Remaining cells can either be frozen down or made into a pellet for RNA/protein extraction.
Thawing Induced Pluripotent Stem Cells

*Prior to thawing iPSCs received from the Cedars Sinai iPSC Core Facility, please ensure that you have Matrigel-coated dishes ready beforehand.*

1. Thaw cells quickly in a 37°C water bath until you see a pea sized ball of ice.
2. Using a 2ml pipette, move frozen cells into a 15ml conical.
3. Slowly add cell culture medium to conical drop by drop to dilute CryoStor CS10 (1:10 ratio is recommended).
4. Spin cells down for 1 minute at 1000rpm.
5. While cells are spinning, aspirate Matrigel from dish and add cell culture medium.
6. Aspirate the medium from cells and re-suspend cells to desired volume.
7. Plate cells.

*Note: 1 vial will usually thaw into 1 well of a 6 well plate.*

Freezing iPSCs using CryoStor CS10

1. Using a pulled glass pipette or colony marker, remove any differentiating cells from the culture.
2. Aspirate media.
3. Add 1ml fresh mTeSR to each well.
4. Using a cell scraper, gently lift the colonies from the plate.
5. Collect cells into a 15ml conical.
6. Spin cells down for 1 minute at 1000rpm.
   a. If time allows for it, you may also let the cells settle by gravity.
7. Aspirate media from cells and re-suspend in desired volume of CryoStor CS10 (1ml/cryovial).
8. Add cells to cryovial and freeze using an isopropanol freezing vessel at -80°C overnight then move vials to a liquid nitrogen tank.

*Note: 1-70% confluent well of a 6 well plate can freeze into 2 cryovials.*