Splitting cells for iPS colony derivation
(Samantha Hastie and Yen Bui, Sept. 2012)

Initial Picking:

Use a 48-well plate prepared with matrigel:
1. Make a 1:100 dilution of matrigel (stored in 4°C) into DMEM media, mix well, and aliquot 200 uL/well.
2. Incubate at 37°C for 1 hour prior to use.
3. When ready to add colonies, aspirate out the matrigel/DMEM media and add 200 uL of a 1:1 mixture of mTeSR media with hESC media supplemented with a 1:1000 dilution of Rok inhibitor (keeps single cell iPSC colonies from dying during the picking process) to each well.

You are ready to pick once your initial clones (after reprogramming) have been identified with nice sharp round borders without much differentiation. Use a 200 uL pipette tip with sterile filter to encircle around a clone, scrape into sections, and suck up. Put each clone into an individual well of the prepared matrigel 48-well plate. Pick about 20 clones per cell line.

- You can use 100% mTeSR media (without Rok inhibitor) to change media 24 hours after isolating the first iPS colony.
- Change media every day.
- Look at cells daily to see when they are ready to split. Split at 80% confluency.

Splitting Cells:

To split from a 48-well plate, split 1:2 into a 24-well plate. When ready to split, split 1:2 into a 12-well plate, then when ready split 1:2 into a 6-well plate.

For example, to split from a 48-well plate:
1. Prepare the 24-well plate you are splitting into with matrigel/DMEM as above.
2. Aspirate media from the original plate with iPS colonies.
3. Wash 1x with PBS.
4. Add 200 uL acutase per well (incubate 30 sec for a 48-well plate).
5. Add 0.5 mL mTeSR media per well (with 1:1000 dilution Rok inhibitor) to deactivate acutase.
6. Use a 200 uL sterile pipette tip with filter to scrape around the edges of the well in a circular motion then zigzag to get cells in the center of the well.
7. Aspirate out matrigel/DMEM from 24-well plate.
8. Use a 1 mL pipette to transfer cell suspension to one well of the 24-well plate.
9. Repeat for any other clones you are splitting.

Check cells daily for confluency and split if necessary.