Feeding iPSCs
Warm-up media (MTeSR1)
Sterilize hood
Aspirate media from cell culture
Replace with media at 36oC, 2.5 ml/6-well well if colonies sparce, increase volume as needed

Passaging iPSCs
Coat cell culture plates (Dissolve 100 ul aliquot of Geltrex in 10 ml of COLD media (usually Knock-out DMEM), immediately put on plates, incubate for 1 hour at 37oC, then 10 min at room temperature
Warm-up media (MTeSR1)
Warm-up Accutase
Aspirate media from cell culture
Wash cells with PBS, remove PBS
Add accutase (~ 1ml per 6-well plate well) and place in incubator
Wait until cells ball-up, but not until they peel off
Add about 1.5 ml media per well, if cells do not come off, use a scraper
Transfer to 15 ml falcon tube
Spin at 200G (1000 rpm in cell culture centrifuge)
Remove media/accutase solution
Gently resuspend in MTeST with ROCK inhibitor (10mM, use 1 ul per 1 ml of media)
Label plate with cell line name, date and passage number (increase passage number by 1)
Replace with MTeSR media without ROCK inhibitor in 24 hours or less.

Note: Rock inhibitor - Y27632 (dihydrochloride) is a highly potent ATP-competitive inhibitor of Rho– associated coiled-coil forming protein serine/threonine kinase (ROCK). It has been shown to prevent dissociation-induced apoptosis in human embryonic stem cells (hES cells). Y27632 enhances the survival and cloning efficiency of dissociated hES cells without affecting their pluripotency.
Soluble in DMSO. Used as a 10mM stock solution.

Cell culture rules:
Spray hood with 70% ethanol, expose surface for 1 min, wipe
Whatever enters hood needs to be sprayed with 70% ethanol (except cell culture plates)
Never leave items on grid
Always spray anything coming from water bath
Sterilize hood in between different experiments, even if done one right after another.

Change pipettes when:
1. going back into stock solution (never use the same pipette to go back into stock solution, to prevent contamination)

2. between cell lines (a faster growing cell line can overtake slower one in a few passages (also change aspirator tube)
3. always change a pipette or aspirator tube if it touched anything except the inside of a culture dish