Passaging human Embryonic Stem Cells (hESCs)

- HESC colonies should be tight refractile, large and well separated.
- Aspirate media from each well.
- Add 1ml of Trypsin-EDTA for 1 minute.
- Remove trypsin (look at cells under microscope and make sure cells are still stuck to plate). Add 1ml Stop media (IMDM + 50% FCS). If cells are not stuck to plate, do not remove trypsin but instead add 1ml of stop media (*note: cells respond better if you remove trypsin*).
- Scrape the cells with a cell scraper and transfer to a 15 ml tube containing 9mls of WASH MEDIA or IMDM or DMEM with P/S using a 10ml pipette.
- Spin at a 1400 rpm. Resuspend with hESC media using a 1ml pipette.
- Top media up to 600uL or 1200uL or 2400uL depending on whether we choose to do a 1/6, 1/12 or 1/24th split (depending on cell concentration and cell-line). Add 100uL per well containing 2ml of hESC media.
- While putting cells into the incubator make sure to shake it to ensure even distribution of cells.
- Feed every day after the first day with 2 ml of hESC media. If there are a lot of cells, then add 3ml/well. *If splitting 1/12 and do not need more than 6 wells, resuspend pellet in 1ml of hESC media and throw away 0.5ml. With remaining 0.5ml, bring volume up to 12 ml and plate 2ml/well.
- Incubate at 37°C in 5%-6% CO₂ incubator.