Growing Melanoma cells from frozen stock

- Pre-Warm DMEM medium in water bath at 37°C
- In the hood, place 10ml of media into a T75
- Place this flask with medium into the incubator for at least 15 minutes so that the medium can reach its correct/normal pH
- Thaw frozen cells by gently pipetting up and down with warm media until the frozen pellet has completely thawed
- Place media and cells in the flask, gently rock to mix with media and distribute evenly across flask.
- Incubate at 37°C, 5%CO₂. Leave the cells overnight then change the medium.

Changing media

- Pre-Warm DMEM media and sterile 1X PBS in water bath at 37°C
- Aspirate existing media off
- Add 3-5 ml PBS to rinse, rock gently then aspirate off
- Add appropriate volume of media to the flask.

Splitting cells (when cells reach 75-80% confluence)

- Pre-Warm DMEM media, sterile 1X PBS, and trypsin in water bath at 37°C
- In the hood, place 10ml of media into a T75 flask, or 20ml in a T150
- Place this flask with medium into the incubator for at least 15 minutes so that the medium can reach its correct/normal pH
- Aspirate existing media off of cells
- Add 3-5 ml PBS to rinse, rock gently then aspirate off
- Add 1ml trypsin to T75 or 2 ml to T150 and incubate at 37°C for 3-5 minutes
- Check with microscope that cells are no longer adhered
- Add ~5ml DMEM media, then pipette into a conical tube and centrifuge. Aspirate off media and trypsin
- Re--suspend cells with 2ml media and transfer appropriate volume into the new T75 or T150 flask
- Swirl and incubate at 37°C, 5%CO₂

Freezing cells

- Detach cells according to the protocol listed above
- Add DMEM medium to deactivate trypsin
- Pellet cells by centrifugation
- Resuspend in cold PBS to wash
- Count cells
- Pellet cells again
- Resuspend in freezing media (Usually 1E6-5E6 cells/ml freezing media)