Initiating culture of frozen fibroblasts:

Number of cells in vial either written on label of cryovial, or should be in paperwork if purchasing commercial cells.

1. Using total number of viable cells, determine how much SA can be innoculated to achieve an initial seeding density of ~2500-4000 cells/cm^2 (for fibroblasts).
2. Prepare flasks – add 5mL complete growth media per 25 cm^2 of surface area. Place flasks in 37degC incubator and allow media to pre-equilibrate to temperature and pH for 30 minutes prior to adding cells.
3. While culture flasks equilibrate, remove a vial of cells from storage and thaw by gentile agitation in a 37 degC water bath. **Keep O ring and cap out of water** to avoid contamination. Thawing should be rapid (no more than 2 minutes).
4. Watch carefully until the last sliver of ice melts. Remove as soon as contents are thawed and decontaminate with 70% ethanol.
5. Add appropriate volume of complete media (volume = 1mL x number of flasks to be seeded – 1 mL) into sterile conical tube. Transfer cells from cryovial to conical tube. Gently pipette solution cells to homogenize suspension. Do not centrifuge.
6. Transfer 1mL of cell suspension to each of the pre-equilibrated culture flasks prepared in steps 1-3 of Handling procedure for frozen cells and initiation of culture. Pipette several times then cap and gently rock each flask to evenly distribute cells.
7. Place seeded culture flasks in incubator at 37 degC with 5% CO2 atmostphere. Incubate at least 24h before processing further.