

# Freezing Human ES Cells:

Thomson Lab, 2004

1. Collagenase cells for approximately 7 minutes at 37°C (until edges of colonies are curling up).
2. With a 5 ml pipet, gently pipet and scrape colonies from plate. Add cell suspension to a 15 ml centrifuge tube and GENTLY break up colonies. It is important to be gentle in this step as “chunkier” colonies will thaw out better than single cells. Ideally, colonies meant for freezing are left slightly larger than they would be for splitting.
3. Spin 5 minutes at 1000 rpm.
4. Resuspend pellet (gently) in 3 ml ES media to wash away collagenase.
5. Spin 5 minutes at 1000 rpm.
6. Resuspend pellet (again, gently!) in 0.25 ml Resuspension Medium per vial (This is one half the final volume required for freezing).
7. Dropwise, add an equivalent volume (0.25 ml per vial) of Cryopreservative Medium and mix. Your DMSO concentration is now 10%.
8. Place 0.5 ml of cells in each freezing vial.
9. Rapidly transfer the cells to a freezing container and place at -70°C overnight (cells don't like to be in DMSO at room temperature for long periods of time).
10. Transfer cells to liquid nitrogen the next day for long-term storage.

## Notes:

- *Pre-label all cryovials appropriately with the following information:*

*Cell line*

*Passage number*

*Number or surface area of cells frozen*

*Date*

*Initials*

- *If 6 well plate is fairly confluent, the usual number of cells to be frozen is 3 vials/well*
- *Remember to fill out a freeze/thaw form*