

## Preparation of ESCs for a Blastocyst Injection

We typically grow our Embryonic Stem Cells (ESCs) in 6 well plates. If our stock is frozen, we prefer thawing, growing in vitro for 3-4 days and passing once before the injection. We usually thaw our cells about 1 week (depending on how many were frozen) before the injection day, grow them for 3-4 days and then dissociating them using 0.25% Trypsin in Hepes-EDTA.

One well of a 6-well plate usually provides a sufficient number of cells for an injection. It is important that the cells are dissociated to a single-cell state, to facilitate a smooth injection.

### On the day of an ESC injection, we prepare ECS as following:

1. Aspirate out the medium from the well that you will be dissociating.
2. Wash the well with PBS or HEPES and aspirate out the medium.
3. Add 0.5 mL of trypsin to the well and put the plate in your incubator. We use our common stock trypsin, which is: 0.25% in HEPES+EDTA (EDTA conc. = 0.1 mM).
4. Periodically check in on the dissociation process by taking the plate out of the incubator, tapping the sides and gently tilting & tapping the plate to assist dissociation. Observe under a microscope to assess the process. If you take the plate out be sure to return it to the incubator. No, or few, clumps that don't disperse with tapping (observe under a microscope) should be present before stopping the process (the process shouldn't exceed 20-30 min depending on the cell background).
5. After the colonies break up enough so that a majority are single cells, add 2-3 mL of mouse embryonic stem cell medium (mESC medium) to deactivate the trypsin. Collect this volume and put it in a 15 mL falcon tube.
6. Centrifuge the falcon tube for 4 minutes at 1,000 rpm. Then, aspirate out the supernatant, leaving only the cell pellet on the bottom.
7. Draw up approximately 1-2 mL of mESC medium using a plugged Pasteur pipette and re-suspend the cell pellet. The suspension should be dense.
8. Label the tube with the name of your cell line and clone.
9. Put the falcon tube on ice, the cells are ready to inject.