



**THE NORTHWESTERN UNIVERSITY  
TRANSGENIC AND TARGETED MUTAGENESIS LABORATORY**

**Preparing ES Cells For Blastocyst Injection**

NOTE: If the cells are on MEFs, start procedure about 45 minutes before you want to bring them upstairs for injection. If they are on gelatin, 30 minutes is sufficient.

1. Feed at least 2hr before you begin to prep the cells. They should be 60-80% confluent and dividing exponentially.
2. After two hours, wash plate 2X with warm PBS.
3. Add 0.05% Trypsin (+ 1% heat-inactivated chicken serum, optional) and incubate for 3 minutes. Tilt (do not swirl) the plate to check to see if cells lift off the dish. If they do not, incubate for 2 additional minutes and check again. Cells should be up by this point, but if they are not, continue incubation in 2-minute increments.
4. When cells are sufficiently trypsinized, stop the trypsin with media. Gently distribute with a pipet and then with a P1000 to break up clumps and obtain a good single cell suspension.
5. If cells are on MEFs, place the cells that will be injected onto an uncoated dish and incubate for at least 15 minutes (skip this step if cells are not on MEFs).
  - a. Spin and freeze the remaining cells, if desired.
6. When the MEFs have attached after 15 minutes, carefully pipet the cells off the plate using a P1000 and spin in a total of 10mls of media.
7. Re-suspend the cells in 10mls PBS and spin again. Repeat.
8. Suction off PBS and, gently re-suspend the cells in 300ul cold injection buffer (see below) using a P1000. Place on ice. The cells are now ready for injection.

Injection Buffer = 5mls ES cell growth media without G418, supplemented with 100 $\mu$ l of 1M HEPES (Gibco/Invitrogen, cat #15630-106)