# Preparation of ES cells for microinjection into mouse embryos

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## Equipment and reagents

- 6 cm tissue culture dish
- ES-cell culture medium (see Chapter 3 (Matise, Auerbach and Joyner), Gene Targeting 2e)
- ES cells
- Phosphate-buffered saline (PBS; Sigma)
- Injection medium: Hepes-buffered DMEM + 10% fetal calf serum (ICN)
- Trypsin: 0.05% trypsin, 0.02% EDTA in modified Puck's saline A (Gibco/BRL)
- Depression slide (BDH)
- Embryo-tested mineral oil (Sigma)
- 50 ml polypropylene tubes (e.g. Falcon)
- Bench-top centrifuge

## A. Preparation of ES cells for injection

1 Plate $10^4$ cells for microinjection or aggregation 2 days before use on a 6 cm dish and culture in ES-cell culture medium.

2 One hour prior to use, harvest the cells. First, wash the cells with PBS, then add enough trypsin to cover the bottom of the dish (about 0.5 ml for a 6 cm dish). Watch the cells under a tissue-culture microscope until the colonies begin to detach, and then add 5 vols (2.5 ml) of ES-cell culture medium. Disperse the colonies to single cells by repeated pipetting.[a]

3 Harvest the cell suspension, by centrifugation at 1000 r.p.m. for 3 min; resuspend the ES cells in injection medium and place on ice.

## B. Microinjection chamber

**Note:** Microinjection is performed in depression slides.

1. Wash the slides and rinse thoroughly with water followed by ethanol.
2. Place a small drop (50 μl) of injection medium in the depression and cover with mineral oil to prevent evaporation.
3. Transfer 10–20 embryos to this injection chamber and add enough ES cells to form a sparse lawn on the bottom of the chamber. If too many cells are added to the chamber they may clog the microinjection instruments. The ES cells will soon settle to the bottom; because they are small they will be in a slightly different focal plane from the embryos.

## Notes

a If the ES-cell line used is grown on feeder cells, a significant proportion of feeders can be removed from the cell suspension by replating the cells onto a culture dish for up to 1 h. In this time feeder cells will adhere to the culture plate, leaving the majority of ES cells still in suspension.