Injection of ES cells into morulae

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Equipment and reagents

- Injection apparatus and microinstruments as in Preparation of microinstruments for injection of ES cells into mouse embryos
- Morula-stage embryos (Preparation of mouse embryos for microinjection of ES cells)
- ES cells (Preparation of ES cells for microinjection into mouse embryos)
- M16 medium + 1 mg/ml BSA (Sigma)

Method

1. Mount the holding and injection pipettes onto the micromanipulators.
2. Place 10–20 embryos into the depression chamber and cluster them into a single group (Preparation of ES cells for microinjection into mouse embryos).
3. Pipette ES cells into the depression chamber to form a sparse lawn surrounding the embryos.
4. Suck one embryo from the group onto the holding pipette and hold it firmly under gentle pressure.
5. Adjust the focal plane and suck a number of ES cells into the injection pipette. Take care to select the best, small, round ES cells, excluding larger differentiated cells or feeder cells.
6. Raise the injection pipette until the tip of the pipette and the zona pellucida of the embryo are in the same focal plane.
7. Gently pierce through the zona with the tip of the injection pipette and expel three to five cells under the zona pellucida.
8. Gently remove the injection pipette.
9. Release the injected embryo and move to a distinct part of the injection chamber to form a group of injected embryos, taking care not to mix injected and uninjected morulae as they are not easy to distinguish.

10 Repeat the injection procedure for all remaining embryos.

11 Collect the injected embryos from the injection chamber with a mouth pipette, wash through four to six drops of M16 medium + 1 mg/ml BSA and return them to the incubator until ready for transfer.

12 Manipulated morulae are best transferred directly to the oviducts of day 1 pseudopregnant recipient foster mothers (up to eight embryos per oviduct) (Transfer of injected oocytes into the oviduct of pseudopregnant mice to produce transgenic animals). Alternatively the morulae may be cultured overnight in M16 medium + 1 mg/ml BSA and then transferred to the uterine horns of day 3 pseudopregnant recipient foster mothers (Transfer of morulae and blastocysts to pseudopregnant mothers).