

ES-cell injection into mouse blastocysts

Antonius Plagge

The Babraham Institute, Babraham Hall, Babraham, Cambridge CB2 4AT, UK.

Gavin Kelsey

The Babraham Institute, Babraham Hall, Babraham, Cambridge CB2 4AT, UK.

Nicholas D. Allen

The Babraham Institute, Babraham Hall, Babraham, Cambridge CB2 4AT, UK.

Equipment and reagents

- ◆ Injection apparatus and microinstruments as in [Preparation of microinstruments for injection of ES cells into mouse embryos](#)
- ◆ Blastocyst-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 Place 10–20 recently expanded blastocysts, in a cluster, in the microinjection chamber with the ES cells ([Preparation of ES cells for microinjection into mouse embryos](#)).
- 2 Orient each embryo to be picked up and hold it firmly on the holding pipette at the ICM end.
- 3 Collect 10–20 ES cells into the injection pipette.
- 4 Pierce through the zona pellucida and trophectoderm and deposit 10–20 cells in the blastocoel cavity.
- 5 Withdraw the pipette and release the injected embryo from the holding pipette to a separate part of the slide.
- 6 After injecting all the embryos in the chamber, transfer them into a culture drop of ES-cell culture medium, to allow them to re-expand.
- 7 Transfer re-expanded embryos to the uterine horns of day 3 pseudopregnant foster mothers ([Transfer of morulae and blastocysts to pseudopregnant mothers](#)).