Aggregation of ES cells with mouse morula-stage embryos

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

- 6 cm bacterial culture dish
- M2 medium + 1 mg/ml BSA (Sigma)
- M16 medium + 1 mg/ml BSA (Sigma)
- Blunt-ended darning needle
- Single-cell suspension of ES cells (Preparation of ES cells for microinjection into mouse embryos)
- Sterile mouth pipette (for handling cells and embryos) drawn from Pasteur pipettes or other suitable glass capiliaries (the internal diameter of the pipettes should be ~100µm)
- Eight-cell embryos from superovulated females (Preparation of mouse embryos for microinjection of ES cells)
- Tyrode's solution, acidic (Sigma)
- CO_2 incubator

A. Preparation of aggregation wells

1. Place six drops of M16 culture medium + BSA in a bacteriological-grade culture dish and cover with mineral oil.
2. Make a series of 12–15 small depressions beneath each drop by pressing a round or blunt-ended darning needle, washed with ethanol, onto the plastic surface, through the oil and medium.
3. Place the dish in the incubator to pre-equilibrate the culture medium with CO_2

B. ES-cell preparation

1. Plate the single-cell suspension of ES cells in ES-cell medium in a bacterial culture dish, and incubate for 1–2 h. During this time they will aggregate into small clumps of five to ten cells.

2. Using a hand-pulled, narrow-bore mouth pipette, place one clump (five to ten cells) of ES cells into each aggregation well.

C. Embryo preparation and aggregation

1. Flush eight-cell-stage embryos from the oviducts of day 2.5 superovulated or naturally mated mice in M2 medium (Preparation of mouse embryos for microinjection of ES cells).
2. Wash the embryos by transferring through three drops of M2 medium
3. Remove the zona pellucidae of the embryos by brief exposure to acid Tyrode's solution; using a mouth pipette release 10–20 embryos at the top of a drop of acid Tyrode's, keep the embryos suspended in the solution and observe them until the zona pellucida is seen to dissolve. Take care not to let the embryos fall to the bottom of the drop and touch the plastic, as they will tend to stick irretrievably.
4. Return the embryos to a drop of M2.
5. Wash the embryos through four drops of M16 + BSA medium.
6. Place one embryo in contact with each ES-cell clump in the pre-prepared aggregation wells.
7. Return the dish to the incubator and culture overnight.
8. Transfer compacted or early cavitating morulae to the uterine horns of day 3 pseudopregnant recipients (Transfer of morulae and blastocysts to pseudopregnant mothers).