



## Preparation of ES cells for blastocyst or morula microinjection

It is important to maintain optimal ES cell culture conditions all the time but particularly for ES cell clones for generation of chimeras. ES cells are grown in recommended ES cell medium at sub-confluent state, i.e. passage is done at densities that are neither high nor low, most of the time splitting (1:3 to 1:6) every other day and used when growing exponentially.

Thaw a vial of ES cells on MEF two to three days prior to microinjection. If necessary, thaw the cells earlier to ensure their timely recovery and the quantity required for experiment but use cells at as low passage as possible. Change the medium next day after thawing. Aim to have sub-confluent plates on the day of injections. One sub-confluent 60 mm plate per clone is more than sufficient but at least two plates at different confluency may be prepared to have the choice for the optimum cell density. Change the medium in the morning or at least one hour before the injections (optional but highly recommended step).

### On a day of microinjections:

- About 45 minutes before the cells are required for injections, remove the medium and wash the cells with PBS. Add trypsin and incubate for 5 minutes at 37 C. Add ES cell medium and resuspend the cells by gentle pipetting. It is very important to generate single cell suspension. Pipette well but avoid foaming.
- Pre-plating (if cells are grown on MEF): Leave the cell suspension in the original plate and incubate for 15 minutes at 37 C to allow MEF to reattach. Carefully remove the plate from the incubator, tilt it and collect the medium with ES cell suspension. DO NOT swirl or wash the plate, most feeder cells should remain attached. If the last passage was done on the gelatin this pre-plating step is not necessary.
- Spin down the cell suspension (200 g, 5 minutes), remove the supernatant and resuspend the pellet in cold 1 ml of 20 mM HEPES buffered DMEM + 15% FBS (if DMEM+HEPES is not available, regular DMEM can be used). Leave the tube for half an hour on ice.
- Discard the floating dead cells by carefully removing the top 1/4 of the medium (~250  $\mu$ l) and leaving settled cells at the bottom of the tube. Transfer the middle 0.5 ml of single cell suspension to the sterile eppendorf tube and place it on ice - cells are now ready for microinjections. For transfer between labs, place the eppendorf tube inside 15 or 50 ml tube, mark clearly with the clone ID and transport on ice.

## References

1. Gene Targeting: A Practical Approach, 2nd Edition. Joyner AL, ed. 2000. IRL Press at Oxford University Press. New York.
2. Methods in Enzymology. Guide to Techniques in Mouse Development. 1993. Edited by Paul M. Wassarman and Melvin L. DePamphilis. Vol. 225.
3. Manipulating the Mouse Embryo: A Laboratory Manual. 3<sup>rd</sup> Ed. (2003) by Nagy, A, Gertsenstein, M, Vintersten, K, Behringer, R. Cold Spring Harbor Laboratory Press. New York.

