PREPARATION OF ES CELLS FOR BLASTOCYST INJECTION

To exclude artefacts, at least two germline chimeras of two independent knock-out clones have to be generated for studying the phenotypic effects of hetero- or homozygous knock-out mice.

The ES clones have been

- subcloned or checked for correct stoichiometry by Southern hybridization
- checked for morphology
- checked by karyotyping
- expanded to 20 cm^2 each
- frozen down in 5 vials of 4 cm^2 each

Of each independent knock-out clone 5 implanted fosters are generated carrying a total number of ±75 injected blastocysts. In 1-2 days of injection this number of fosters can be achieved.
Since on 3 days per week (wednesday to friday) ES cells are injected into blastocysts, the aim is to inject 2 independent ES clones in one week.

Preferentially at least two days before injection one vial of knock-out ES cells has to be brought into culture on 2x a 4-well dish with feederlayer cells :

- well nr 1: 50%=2 cm^2 ES cells
- well nr 2: 1/3 of ES cells of well nr 1
- well nr 3: 1/3 of ES cells of well nr 2
- well nr 4: 1/3 of ES cells of well nr 3

Procedure:

- pipet 0.6 ml of medium into wells 1, 2 and 3, and 0.3 into well 4
- pipet with blue tip 0.3 ml of ES cells (2 cm^2) in well 1
- with same tip: 0.3 ml from 1 into 2
- 0.3 from 2 into 3
- 0.3 from 3 into 4

The ES cells have to be refed the day after thawing and spreading, since DMSO leaks out of the cells that first day of culturing.

The person who delivers the cells for injection into blastocysts on wednesday, aliquots medium for the injection procedure for <u>all</u> 3 days: 3 tubes with 25 ml and 3 tubes with 7.5 ml complete medium <u>without</u> β-mercaptoethanol and <u>without</u> LIF.