Mouse House Protocols

C57BL/6 donors for blastocyst injection:

- Ordering of C57BL6 females as donors for blastocyst injection on Tuesdays by phone (Supplier: Charles River, Kundennummer …….., phone number …….). Fill-in the official ordering form, get a PSP-number and a signature from Thomas Magin and send it *via* house mail to Frau Kirchner, Materialwirtschaft, Uniklinikum Bonn.
- Shipment of **15** B6 females, 3-weeks old, on Fridays, used for superovulation on Tuesday.
- Unpacked females are supposed to get a cage card containing the following information: strain name, date of shipment, supplier, date of birth, number of females per cage.
- On Tuesday, PMSG i.p. injection of **15** 3-weeks-old B6 females at 13:00-13:30 (german winter time; light-dark cycle 6am-6pm) or 14:00-14.30 (german summer time; light-dark cycle 7am-7pm), respectively. Indicate date of injection and time of hormone application on the cage card. Fill-in the blastocyst injection documentation sheet.
- On Thursday, hCG injection at 12:00-12:30 (german winter time; light-dark cycle 6am-6pm) or 13:00-13.30 (german summer time; light-dark cycle 7am-7pm), **exactly 47 hours later**. Indicate date of injection and time of hormone application on the cage card. Fill-in the blastocyst injection documentation sheet.
- On the same day (on Thursday) setting-up 1:1 matings with C57BL/6 males between 15:00 and 17:30.
- Plug-check next morning between 8:00 and 10:00 and subsequent separation of the females from the males. Plug-positive females are collected and moved to a labelled cage; date of the plug needs to be added to the cage card information. The day of the plug corresponds to stage E0.5 during embryonic development. Plug-negative females are collected in an extra cage with an appropriate cage card containing all necessary information. Fill-in the blastocyst injection documentation sheet.
- At day E3.5 (usually on Mondays) plug-positive females are sacrificed between 8:00 and 10:00 and blastocysts and morula stage embryos are collected by flushing of the uterus horns. Numbers of obtained embryos, their developmental stage (blastocyts *versus* morulae) and their quality needs to be entered to the blastocyst injection documentation sheet.

C57BL/6 studs:

- **15** C57BL/6 males, used to mate to superovulated females are housed in individual cages. Every three months one third of them are replaced by newly shipped young males in order to maintain a young colony.
- Every individual gets a cage card containing the following information: strain name, date of shipment, supplier, date of birth.
- Ideally, notes should be added to the cage card if the male is not producing a plug; those individuals should be identified and substituted by new reproductive males.

NMRI foster females:

- The NMRI females used to generate fosters for blastocyst injection are housed in groups of four individuals per cage. The pool of females should be about 20-28 individuals. The age should be rather young, i.e. 2-3 months old (max. 4 months). Corresponding cage cards should contain the following information: strain name, date of shipment, supplier, date of birth.
- In order to produce foster females as recipients for injected blastocysts, **20** 2-3 months old NMRI females are mated to **10** vasectomized, sterile NMRI males in a 1:2 ratio.
- Matings are set up at the day of the C57BL/6 donor plugs, i.e. usually on Fridays between 15:00 and 17:30 in order to produce fosters for blastocyst injection on Mondays.
- Plug-check next morning (usually on Saturdays) between 8:00 and 10:00 and subsequent separation of the females from the males. Plug-positive females are collected and moved to a labelled cage; cage card information should include date of birth, date of the plug, indicated date of blastocyst injection and number of individuals. The day of the plug corresponds to stage E0.5 during embryonic development. Plug-negative females are moved back to their original cages; if necessary update cage card information, e.g. number of individuals per cage.
- Fill-in the blastocyst injection documentation sheet.
- Plug-positive NMRI females are used at day 2.5 (usually corresponding to Mondays) as recipients for injected blastocysts.
- Plug-positive NMRI females that have **not** been used as fosters for injected blastocyts can be moved back to the original NMRI female pool after 2 weeks of the produced plug.
- In order to provide fosters on a routine basis, the steady state pool of NMRI females that are ready to be mated to the vasectomized males need to contain about 24-28 (minimum 20) individuals. Therefore, the number of females needs to be monitored routinely and if necessary every second week an order for the shipment of 4-8 young (7-8 weeks old) females has to be placed right in time.

Vasectomized, sterile NMRI males:

- In Nov./Dec. 2006 a total number of **15** young NMRI males have been vasectomized. They are housed in individual cages and are currently used to produce pseudo-pregnant recipients for injected blastocysts.
- Every three months about **5** new, young NMRI males are vasectomized in order to generate substitute for future experiments. They will be used always to replace the oldest studs.
- If necessary, 4-5 weeks old NMRI males have to be ordered right in time for that purpose.
- Between one and two weeks after the surgery, these young vasectomized males are subjected to test matings; i.e. one female is added to each candidate for about 7-10 days. After that, the matings are separated and potential pregnancies and litters are monitored for **additional three weeks**. Separated females need to be tagged in order to identify the corresponding males, if necessary. Sucessfully vasectomized males should **not** produce any offspring.
- Vasectomized males that produce offspring need to be killed immediately.

- Cage card information should include strain name, date of shipment, supplier, date of birth, date of vasectomy.

Housing and monitoring of foster mothers after embryo transfer:

- After embryo transfer, 2-3 foster females can be housed in one cage for the subsequent 2 weeks. Individual housing from the very beginning is only recommended if different ES cell lines have been used for microinjection.
- Cage cards of the foster females should include the following information, date of birth of the foster mother, day of the plug, day of embryo transfer, ES cell line used for blastocyst injection, investigators name (if necessary).
- Pregnancies should be monitored and indicated on the blastocyst injection documentation sheet.
- Minimum 3 days before the calculated day of birth, females should be housed individually in order to give them time to built a nest for their offspring; nest material should be provided. Prevent the usage of too much tissue paper per cage, since the contact of it with the water bottle pipette could result in leakiness of the water bottle.
- At the calculated day of birth carefully check the presence of the litter; **do not disturb the pups!** Indicate date of birth on the cage card and on the blastocyst injection documentation sheet.
- After 10-14 days of birth, the size and the composition of the litter should be monitored, i.e. number of individuals, black *versus* chimeric, males *versus* females and indicated on the blastocyst injection documentation sheet.
- Offspring is supposed to be weaned between 3 and 4 weeks of age. Non-chimeric offspring and foster mothers can be killed. Chimeras can be housed together with their littermates. Cage cards of the chimeric offspring should contain date of birth, injected cell line, number of individuals, investigators name (if necessary).

Maintaining genetically manipulated mouse lines:

- Mouse cages need to be labelled with appropriate cage cards containing the following information: name of the mouse line, number of individuals, ID of the individuals (i.e. ear tag number), gender, date of birth, parents ID (optional), genotype. Cage card information should be up-dated on a routine basis.
- Offspring should be weaned between 3 and 4 weeks after birth.
- Tail tips for genotyping are taken after ear-tagging of individuals.
- A mouse list as a computer file should be maintained, containing all necessary information, including genotyping results, date of killing or loss of individuals.
- It is recommended to backcross the line always to their intended genetic background in order to prevent a genetic drift.