Chimera production

- A. Prerequisite: Before placing an order for chimera production service, the following conditions should be fulfilled.
 - 1. ES genotyping completed upon applying for TCF services.
 - 2. Method and proof to identify targeted genes.
 - 3. Space to maintain mice once being generated.
 - 4. Understanding that not every chimera will give germ line transmission.
 - 5. It is the user's obligation to notify TCF of germ-line transmission of all given gene targeting mouse lines.
- B. TCF performs chimera production via blastocyst microinjection. For chimera production using TCF-generated ES clones please refer to <u>Blastocyst</u> <u>Microinjection Application Form</u>. For chimera production using non-TCF ES cells, please refer to <u>Blastocyst Microinjection Application Form(non-TCF cells)</u>.
- C. TCF uses C57BL/6 and C57BL/6-C2J strain as host embryo donor for blastocyst injection of 129 and C57BL/6 ES cells, respectively..
- D. The quality of the individual ES clones varies in their toltipotency, which correlates well with the degree of ES contribution to embryonic development and subsequent chance of germ line transmission.
- E. For each request, TCF will expand multiple clones and select better ones for injection. Should the quality of selected ES clone permits, TCF will provide at least four male chimeras (with more than 70% chimerism for 129 ES cells or 50% chimerism for C57BL/6 ES cells estimated by coat color) for each targeted clone. However, when the quality of injected ES clone falls under preferred condition, TCF will promptly inform user after primary injection and prepare other clone(s) for injection.
- F. After exhausting all candidate clones, if less than two positive clones to yield guaranteed quality and quantity of chimera(s), additional gene targeting experiment will be arranged to obtain more positive clones.
- G. H. TCF will not be responsible for long-term maintenance of chimeric mice.

All chimeric mice will be released after weaning. Upon receiving chimera, an <u>animal release form</u> will be asked by TCF staff to sign.

- H. All targeted clones will be released when the case is closed. To avoid unexpected cell quality changes after releasing, TCF will not process injection for any released clones.
- I. Please be advised that normally more than one independent mouse lines is needed for a given gene targeting experiment for its legitimacy.
- J. Specifications/protocols available upon request:
 - 1. Tissue DNA extraction for Southern blot or dot blot.
 - 2. Tissue DNA extraction for PCR.
- K. Materials available upon request: Genomic DNA from the R1(129X1/SvJ x 129S1/Sv) and C57BL/6 (Open Biosystem v26.2) ES cells used by TCF.
- L. Charges.
 - 1. NT\$ 30,000 for blastocyst microinjection one ES clones.
 - 2. Charge will be bill to the order of requester at the beginning of service.
 - All service charges for orders from within IMB or other Institutes will be the same. All service charges cover some material cost and animal fee only. However, the IMB transgenic committee will subject price and service type to change according to the decision.