

# CORE FACILITY TRAM

## APPLICATION FOR CONVENTIONAL ES CELLS INJECTION INTO THE BLASTOCYST CAVITY

*This application form must be filled out, signed and returned to the Core Unit before work will begin on the project.*

**1. Principal Investigator ordering the ES blastocysts injection:**  
(Complete address, Fax and e-mail of contact person)

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**2. Approval from the local Ethical Committee:**  
(Date, Number, please attach a copy of the approval)

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**3. Name of the ES cells and targeted gene:**

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**4. Map of the genomic DNA modified through the targeted mutagenesis:**  
(Please attach the complete sequence information and results of the Southern blot analysis)

**5. Short description of the gene targeted:**

*(Please indicate whether the planned alteration is likely to be embryonic lethal)*

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**6. Short description of ES cells, their karyotype analysis and health status:**

*(Mycoplasma contamination)*

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**7. Breeding of the chimaeric mice:**

*(If you would like us to breed your chimaeric animals and analyze germ-line transmission of the targeted gene, please provide the complete sequence of the targeting DNA vector, and indicate here possible PCR primer sequence information)*

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# AGREEMENT

**Between the Transgenic Mouse facility (hereafter called TRAM) and \_\_\_\_\_ (hereafter called Customer) for production of chimaeric mice through ES cell blastocyst injection.**

TRAM will attempt to generate chimaeric mice by injection of targeted ES cells into blastocysts of wild-type animals. This work will be carried out under the following terms:

1. The customer is obliged to submit a copy of the Ethical Committee permission. Without this copy **TRAM will not start the injection!**
2. Frozen ES cells, containing the targeted gene of interest have to be produced by the customer. TRAM will perform all necessary steps to expand, characterize and prepare ES cells for their injection into the blastocyst cavity. The customer is responsible for the correctly targeted ES clones. TRAM can not be held liable should the chimaeric mice not transmit the desired gene-targeted mutation to the germ-line. The customer will provide TRAM with a map of the plasmid and targeted genomic locus.
3. The ES cells will be introduced into blastocysts by injection into the blastocyst cavity (blastocyst injection). For each ES clone one round of blastocyst injection (1 clone per day) in B6D2F1 blastocysts will be carried out. Injected embryos will be transferred to the oviducts/uteri of pseudopregnant foster mothers by TRAM.
4. Offspring from the injected embryos are born approximately 18 days later. TRAM will score the offspring for chimaerism by inspection of coat color, and chimaeric mice will be shipped to the Customer when they are four weeks old (i.e. after weaning), if nothing else has been agreed upon.
5. The customer accepts with his/her signature the health status of the TRAM animals as given in the health report. The health reports can be faxed on request.
6. TRAM does not guarantee that chimaeric mice will be produced. Lack of germ-line transmission could, for example, be due to unexpected lethality during embryogenesis, or diminished capabilities of ES cells to colonize the germ-line, etc. TRAM has, however, succeeded in all previous attempts to generate chimaeric mice, and germ line transmission has been accomplished for all targeting constructs.
7. The Customer is obliged to acknowledge TRAM in the first published paper that describes the genetically engineered mice. **A reprint of such a publication shall also be sent to the facility.**

The Customer certifies that all figures above are correct and that the Customer has read and understood the conditions listed:

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Place, Date

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Signature (Customer)

**TRAM notes (For Internal Use only):**

- Number of blastocysts injected.....
- Date of injection.....
- Number of embryo transfers made.....
- Number of pups born.....
- Number and % of chimaeric mice.....