# CORE FACILTY TRAM

## APPLICATION FOR CONVENTIONAL PRONUCLEAR INJECTION

*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 transgenic mice:
(*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 DNA construct prepared for the injection:

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**4.** Plasmid map of the DNA construct and how to excise the DNA fragment for the injection:
(*Please attach the complete sequence information and picture of the gel with the endonuclease digested DNA construct*)

**5.** Short description of the DNA construct:
(1) Type of gene that needs to be expressed (2) In which tissues and at which developmental stage should the gene be expressed? (3) *Please indicate whether the transgene could affect embryonic lethality*)

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**6.** Genotyping of the founder:
(*On request TRAM will analyze pups born after pronuclear injection. Please provide complete sequence of the plasmid DNA, and indicate 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 the transgenic mice through pronuclear injection.**

TRAM will attempt to generate transgenic mice by introducing a DNA construct into the mouse genome by pronuclear injection. This work will be carried out under the following terms:

1. The customer is obliged to submit a copy of their ethical Committee permission. Without this copy **TRAM will not start the injection!**

2. A DNA construct containing the desired combination of promoter/enhancer and gene is produced by the customer (minimum 300 microgram of plasmid DNA is required). TRAM will perform all necessary purification steps, including CsCl-EtBr gradient centrifugation, isolation of the supercoiled plasmid DNA, excision of the desired DNA fragment and preparing it for the pronuclear injection. The customer is responsible for the construct supplied and TRAM can not be held liable should the DNA construct not perform as expected in the mice. The Customer will provide TRAM with a map of the plasmid where information regarding excision of the DNA construct etc., should be included.

3. The DNA is injected into pronuclei of fertilized FVB/N mouse eggs during one round (150-200 oocytes) of the pronuclear injection. Injected eggs will be transferred to the oviducts of pseudopregnant foster mothers by TRAM.

4. Offspring from the injected eggs are born approximately 20 days later. The offspring will remain in the TRAM animal facility until they are 4 weeks old, i.e. after weaning. Then they will be shipped to the Customer, if nothing else has been agreed upon.

5. If the customer orders mouse genotyping service, the genotyping results will be provided when mice are 4 weeks old. In case no transgenic animals are identified by TRAM, one additional round of pronuclear injection will be performed free of charge.

6. 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.

7. TRAM does not guarantee that transgenic mice will result from the pronuclear injections. Lack of transgenic founder animals could, for example, be due to unexpected lethality during embryogenesis etc. TRAM has, however, succeeded in all previous attempts to generate transgenic mice by pronuclear injection.

8. The Customer is obliged to acknowledge TRAM in the first published paper that describes the resulting genetically engineered mice. **A reprint of such a publication shall also be sent to the facility.**

9. The customer is obliged to inform TRAM about the number of founder animals produced.

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 Signature (Customer)

## TRAM notes (For Internal Use only):

- Number of oocytes injected.....
- Date of injection.....
- Number of embryo transfer made (one or two-cell embryos).....
- Number of pups born.....
- Number of transgenic founders.....