

# **Karolinska Center for Transgene Technologies**

## **The Transgenic Mouse Core Facility at KI**



Information package and ordering forms

13 January 2004



## INTRODUCTION

Welcome to the Karolinska Center for Transgene Technologies!

This information package describes the transgenic mouse core facility (KCTT) at the Karolinska Institute.

In 1991 two independent transgenic core facilities were established at the Karolinska Institute, one at the KI South-Campus at Huddinge University Hospital / Novum, Unit for Embryology and Genetics (formerly MEG) and one at the KI North-Campus at the Department of Cell and Molecular Biology (CMB) named MouseCamp. After an international evaluation of both core facilities, the scientific board of the KI decided to merge the two existing facilities, to increase the capacity and to take better advantage of the existing scientific expertise.

Within the past few years KCTT has developed into one of the major transgenic core facilities within Sweden and Europe.

KCTT is a **non-profit, non-commercial academic core facility** located at Karolinska Campus North and Campus South. KCTT is a state-of-the-art transgenic mice facility that has an outstanding track record in the production of genetically altered mice. The facility has a high production and throughput rate, both for pronuclear and blastocyst injection as well as for ES cell electroporation. The aim of KCTT is to produce transgenic mice by the pronuclear injection and ES cell techniques for research groups at the Karolinska Institute and research groups at other academic institutions. Companies interested in our service should contact Karolinska Research Services AB (KARSAB) for further information about services offered and regulations.

KCTT is one of the largest transgenic core facilities in Scandinavia with a yearly number of 60 - 70 constructs for pronucleus injection, around 30 - 40 electroporations of knockout constructs and 40 - 50 injected ko constructs of targeted ES cells.



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## SERVICES SUPPLIED BY KCTT

1. Transgenic mice generated by the pronuclear injection technique, generated in various genetic backgrounds, depending on the preference of the research group.
2. Transgenic mice generated by the ES cell technique.

This includes:

- a) Electroporation of DNA for homologous recombination in ES cells and the generation of G418-resistant ES cell clones.
  - b) Introduction of successfully targeted ES cells into early mouse embryos and the generation of chimeric (mosaic) mice.
  - c) For groups that already have generated their own ES cells, KCTT offers the injection of these cells to produce chimeric mice.
3. Cleansing of infected mice (KI groups only). Please get in contact with KCTT.
  4. Re-derivation of mice from frozen or unfrozen embryos (KI groups only). Please get in contact with KCTT.

## ACCESS

The Karolinska Center for Transgene Technologies (KCTT) distinguishes between the following categories of customers, as the KCTT receives financial support from the Karolinska Institute (KI).

1. Research groups from KI
2. Academic groups (non-KI)

Companies interested in our service should contact Karolinska Research Services AB (KARSAB) for further information about services offered and regulations.



## ***Waiting List***

### 1. KI customers

The orders from Karolinska Institute, Karolinska Hospital, Huddinge Hospital and other KI-connected research groups will be treated on a strict "first come, first served" basis, i.e. the place in the waiting list will be based on the day KCTT receives the DNA construct. The aim is that the time between receipt of the DNA construct and injection into fertilized mouse eggs should not exceed 1 month (for the pronuclear injection technique). For electroporation the time between receipt of the DNA construct and the electroporation into ES cells should not exceed 2 months. For the injection of ES cells, targeted by the research groups, the waiting time should not be longer than 6 - 8 weeks.

### 2. Non-KI Groups

Like for KI groups, the waiting time for pronucleus injection, electroporation and injection of own targeted ES cells should be in the range of the time given for KI groups. Besides this KCTT can't promise to keep the place in the waiting list in case urgent constructs from KI groups have to be injected. Before changing a date, KCTT will set all efforts to keep the promised schedule.

## **ORDERING SERVICE FROM KCTT**

To place an order for the generation of transgenic mice by the pronuclear injection or ES cell technique, the ordering forms on page 19, 21, 23 and 25 respectively should be used and sent to KCTT. The appendices, belonging to the different forms, should be sent along with the application forms. Incomplete applications cannot be accepted. It is desirable that the research group notifies KCTT as early as possible, to indicate when they would like the project to be carried out. This greatly facilitates the long-term planning for KCTT.



It is normally not possible to pre-book an injection date, only in case of time sensitive experiments have to be done (e.g. taking out embryos at a certain stage) dates can be booked. If a time point for the injection has been set, the DNA construct has to be delivered latest 2 weeks before the date. If the DNA is not delivered in time or the injection is cancelled too late, the costs for the whole injection date will be charged to the group.

KCTT is working on a cost recovery basis and therefore will charge the groups using the service only the arising costs! In addition the Karolinska Institute is forced to take VAT and overhead from non-KI customers. VAT can be reclaimed from the Swedish tax office. The administration will help you in case of questions.

## **ADVISORY BOARD**

An Advisory Board has been appointed by the Board of Research and reviews the activities of KCTT on a yearly basis. For more information please contact KCTT.

## **STAFF**

The management of KCTT is composed of Johannes Wilbertz (Director), situated at the Campus-North and Stephan Teglund (Vice-Director), situated at Campus-South). Together with the chairman of the scientific board they are heading the activities.

The staff from the KCTT carries out the pronuclear and blastocyst injections as well as all other techniques in the transgenic field. The ES cell culturing is carried out by the technicians of the ES Cell Lab at KCTT. In addition, personnel from the animal facilities provide help with the animal care taking, superovulation, plug checks etc.



## **Animal Health Status (North Campus)**

The latest animal health status from KCTT North can be found on the KCTT homepage (<http://www.mousecamp.ki.se/mousecamp/dok/HealthRep.htm>). KCTT tries to keep the animals free from pathogens. All mice are bought from Charles River Germany and will therefore represent their actual health status.

The animals for transfer and the operated mice are kept in IVC racks (Tecniplast / Italy) to avoid cross-contamination.

The animal health report can be faxed upon request.

## **Animal Health Status (South Campus)**

The latest animal health report can be faxed upon request.

All mice are bought from Scanbur BK Sweden and will therefore represent their actual health status.

## **APPROVAL FROM ETHICAL COMMITTEES**

Each research group is responsible for obtaining an approval from the local ethical committee for the transgenic experiment.

Together with the application form and the respective appendix for pronucleus or blastocyst injection, the research group has to send in a copy of their ethical permission. **Without this copy and the signed forms KCTT can not start the injections.**

## **CONFIDENTIALITY**

Information about projects carried out by KCTT will be treated as strictly confidential and not released to a third party.



## **PATENTS (TRANSGENIC ANIMALS + OTHERS)**

For discussing the possibility of patenting a transgenic mouse strain and other patents affected, please consult with Karolinska Innovations AB.

## **FURTHER INFORMATION**

Please consult Johannes Wilbertz or Stephan Teglund if further information is needed.

<p>Johannes Wilbertz Karolinska Institutet CMB / KCTT von Eulers väg 3 171 77 Stockholm Phone: + 46 8 524 87318 Fax: + 46 8 30 83 74 e-mail: johannes.wilbertz@cmb.ki.se</p>	<p>Stephan Teglund Karolinska Institutet Dept. of Biosciences at Novum, CNT  141 57 Huddinge Phone: + 46 8 608 33 32 Fax: + 46 8 608 15 01 e-mail: stephan.teglund@cnt.ki.se</p>
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## **Contact Information KARSAB**

<p><b>KARSAB</b> Phone: +46 8 5088 45 80 Fax: +46 8 7567277 e-mail: staffan.soderstrom@kab.ki.se</p>
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## **FLOW CHART FOR THE PRONUCLEAR INJECTION TECHNIQUE**

The pronuclear injection technique is suitable for adding genes to the genome in situations where the endogenous genetic makeup is not important, for example when dominant effects or promoter/enhancer activities are studied. For overexpression, a promoter/enhancer that directs expression to a particular organ or timepoint during development is coupled to the gene or cDNA of interest, and for gene regulation studies, the promoter/enhancer of interest is coupled to a reporter gene.

A purified DNA construct is injected into fertilized mouse eggs at the one-cell stage. DNA integration in the genome is random, and the number of integrated copies ranges from one to more than a hundred. In most cases, multiple copies of the introduced DNA are integrated in tandem at a single chromosomal site.

After injection, eggs are transferred into oviducts of pseudopregnant females, where they develop further, and offspring is born approximately 20 days later. Mice that have the transgene integrated in their genome are identified by DNA analysis. For a more detailed account of the pronuclear injection technique, please look at the literature list at the end.



Below follows a schematic representation of a pronuclear injection project carried out by KCTT:

Carried out by the research group (Customer):      Carried out by KCTT:

Production of 60µg of construct DNA

Verification that the DNA cleaves with the appropriate restriction enzymes (photo documentation). Only if cleavage and cleaning is done by KCTT!

Cleavage of DNA construct and final purification of DNA (optional). DNA should be send in 70% EtOH.

Cleavage of DNA construct and final purification of DNA (optional).

Pronuclear injection of DNA into fertilized mouse eggs.

Offspring is born and kept at KCTT until they are 4 weeks old.

The mice are shipped to the Customer for further analysis, including tail biopsy, further breeding etc.



## FLOW CHART FOR THE ES CELL TECHNIQUE

The key features of the ES (embryonic stem) cell technique can briefly be summarized as follows.

Specific mutations, for example inactivation or modification of a gene, can be introduced into the genome by homologous recombination, which leads to the replacement of the endogenous gene fragment by the introduced, engineered fragment.

Because homologous recombination is a rare event, special selection and screening strategies are needed. Usually a specific selectable marker gene is included in the targeting DNA construct.

A targeting construct, which is made from mouse 129 strain DNA, is introduced into totipotent mouse embryonic stem (ES) cells (isolated from 129 mice). Cells that have integrated the introduced DNA in their genome are selected using a drug that kills cells which have not taken up the marker gene. Selected clones are then analyzed by restriction enzyme digestion and Southern blot or by PCR analysis to identify the clones where homologous recombination has occurred. Positive clones are then expanded.

ES cells from correctly targeted clones are introduced into mouse embryos at the blastocyst stage by injection, and injected blastocysts are transferred into the uteri of pseudopregnant females. Chimeric (mosaic) mice consisting of cells originating from the blastocyst and from ES cells are born approximately 17 days after transfer. Chimeric mice are then tested for their ability to transmit the introduced mutation to the next generation (germline transmission).

For a more detailed account of the pronuclear injection technique, please look at the literature list at the end.



Below follows a schematic representation of an ES cell project carried out by KCTT:

Carried out by the research group (Customer):      Carried out by KCTT:

Production of 60µg of construct DNA and delivery of the construct in 70% EtOH to KCTT

Electroporation of DNA into ES cells

Selection of G418 (+ Gancyclovir optional) resistant ES cell clones (250 clones)

ES cells for DNA analysis

Freezing of ES cell clones

DNA analysis of ES cell clones (Southern or PCR). Identification of correct ES cell clones

Thawing of named clones.  
DNA preparation for confirmation

DNA analysis of thawed clone.  
Confirming results.

Injection of correct ES cell clones into blastocysts

Generation of chimeric mice

Crosses of mosaic mice to establish germ line transmission

Further biological analysis of the transgenic mice



## **CHARGES FOR THE INJECTION SERVICE FROM KAROLINSKA CENTER FOR TRANSGENE TECHNOLOGIES**

Because the KCTT is a non-business, non-profit academic research institution, we ask the groups using our service to reimburse our expenses like media, animals used, salary costs, technical costs etc. KCTT is working at a cost recovery basis, but as we get funding from several sources, the costs differ from group to group. The prices given for non-KI groups are **without VAT** but **include overhead!**

**Charges for industry have to be discussed with KARSAB.**

For more information about the cost calculations, please get in contact to KCTT.

The costs are as follows for the different categories:

### ***Pronuclear Injection***

A) One-day pronuclear injection of DNA in hybrid B6CBA mice (DNA cleaned by customer; injection of at least 210 embryos, and a minimum of 8 transfers in pseudopregnant females; animals delivered direct after weaning<sup>#</sup>):

KI = 19 500 Swedish crowns

non-KI (academic) = 26 195 Swedish crowns

!!!For injection of very large DNA fragments (e.g. P1, BAC, PAC) a minimum of 210 eggs will be injected. Because of the higher mortality of the eggs, the 8 transfers can however not be guaranteed!!! For further information please contact KCTT.



B) One-day pronuclear injection of DNA in outbred B6CBA mice (DNA cleaned by customer; injection of at least 210 embryos, respectively 8 transfers in pseudopregnant females; animals analyzed during embryo stages):

KI = 17 000 Swedish crowns

non-KI (academic) = 22 890 Swedish crowns

C) One-day pronuclear injection of DNA in **inbred** C57Bl/6N or FVB/N mice (DNA cleaned by customer; injection of at least 210 embryos; animals delivered direct after weaning<sup>#</sup>). Because of the higher lethality of the oocytes after injection, 8 transfers can't be guaranteed. We strongly recommend at least two injection days for one construct due to the lower number of oocytes and born pups:

KI = 20 150 Swedish crowns

non-KI (academic) = 27 202 Swedish crowns

D) One-day pronuclear injection of DNA in **inbred** C57Bl/6N or FVB/N mice (DNA cleaned by customer; injection of at least 210 embryos; animals analyzed during embryo stages). Because of the higher lethality of the oocytes after injection, 8 transfers can't be guaranteed. We strongly recommend at least two injection days for one construct due to the lower number of oocytes and embryos:

KI = 17 700 Swedish crowns

non-KI (academic) = 23 900 Swedish crowns

!!!For injection into other inbred strains, please contact KCTT for further information and prices!!!



E) Cleavage and cleaning of normal DNA constructs (non P1, BAC, PAC, plasmids) for pronuclear injection:

KI = 5 790 Swedish crowns

non-KI (academic) = 7 820 Swedish crowns

!!!Customers with very large constructs (P1, BAC, PAC, and plasmids) are asked to cleave and clean the constructs themselves prior to injection. In case a very special restriction enzyme has to be used for cleavage, the prices will be higher. Ask KCTT for further information and prices!!!

**The price for two days of pronuclear injection is twice the price for a single day injection.**



## ***Blastocyst Injection***

One day blastocyst injection of one clone in inbred C57BL/6 mice. Produced chimeras will be delivered direct after weaning<sup>#</sup>:

KI = 16 140 Swedish crowns

non-KI (academic) = 21 790 Swedish crowns

!!! KCTT recommends at least two days of blastocyst injection (respectively two clones) for one knockout construct. In case two days of injection will result only in a low number (less than 4) of chimeras or only low chimeras, KCTT will inject a third time without further costs.

Because of the different ES cell handling of customer produced clones, KCTT can not do this for customer derived clones!!!

If ES cells from the customers are used, additional costs for the preparation prior to injection will be charged (see ES cell service).

### **NOTE!**

<sup>#</sup> If mice can not be transferred to the customer KCTT can not guarantee space for continued housing. If space is available in the animal facility, the costs for housing will be charged to the customer. If no space is available, the customer will be asked to take care about the produced mice within the next 4 weeks. After this period the mice will be euthanized. All accrued costs will be charged to the customer.

The full shipping costs for the animals will be charged to the customer.

**All prices without VAT.**



## **CHARGES FOR THE ES CELL SERVICE FROM THE KAROLINSKA CENTER FOR TRANSGENE TECHNOLOGIES**

A fixed price for an entire ES cell project can not be given in advance; it depends on the success in the various steps. We have chosen to specify the costs for each individual step, to give the Customer a better view of how costs are distributed within the project. The costs for the injection of clones are listed above (see: Charges for the injection service from the KCTT).

### A) Electroporation of a DNA construct:

ES-cells, media, pre-tested fetal calf serum, mouse embryo fibroblasts from neo<sup>R</sup> transgenic mice, leukemia inhibitory factor (LIF), tissue culture plastics, pre-testing of reagents, cage costs for neo<sup>R</sup> transgenic mice, maintenance and replacement of equipment, picking of 250 clones (single selection) for screening, preparation of the clones for ES cell injection, technical costs:

KI = 27 255 Swedish crowns

non-KI (academic) = 36 791 Swedish crowns

### B) Preparation of ES cells sent from the customer for blastocyst injection:

Media, pre-tested fetal calf serum, mouse embryo fibroblasts from neo<sup>R</sup> transgenic mice, leukemia inhibitory factor (LIF), tissue culture plastics, pre-testing of reagents, cage costs for neo<sup>R</sup> transgenic mice, maintenance and replacement of equipment, technical costs:

KI = 5 720 Swedish crowns

non-KI (academic) = 7 722 Swedish crowns

### **Note!**

Full shipping costs for the lysed clones will be charged to the customer.

**All prices without VAT.**



## ORDERING FORM FOR TRANSGENIC MICE BY THE PRONUCLEAR INJECTION TECHNIQUE

Date: .....

Research group ordering the mice: .....  
(complete address, fax and e-mail) .....

Responsible person for project: .....  
(name and e-mail) .....

Shipping address for mice: .....  
.....  
.....  
.....

Address for invoice: .....  
.....  
.....  
.....

VAT Number: .....  
(for non-Swedish groups)

Approval from the local Ethical Committee (date, number):

**No injection without approval! Copy has to be added!**

.....



Name of the DNA construct that will be injected:

.....

Short description of the DNA construct:

.....

.....

Cleavage and cleaning of the construct:

KCTT .....  
.....

Customer .....  
.....

Construct delivered in:

Injection buffer .....  
.....

70% EtOH .....  
.....

Genetic background in which the transgenic mice will be made:

F2 (CBA X C57Bl6) .....  
.....

inbred (C57Bl6) .....  
.....

inbred (FVB/N) .....  
.....

How many days of pronuclear injections do you want:

1 day .....  
.....

2 days .....  
.....

The offspring from the pronuclear injections will be analyzed at:

embryo stages .....  
.....

adult .....  
.....

Phenotype expected?:

Yes .....  
.....

No .....  
.....





## ORDERING FORM FOR TRANSGENIC MICE BY THE ES CELL INJECTION (ES CELLS MADE BY KCTT)

Date: .....

Research group ordering the mice: .....

(complete address, fax and e-mail) .....

.....

.....

Responsible person for project: .....

(name and e-mail) .....

Shipping address for mice: .....

.....

.....

.....

Address for invoice: .....

.....

.....

.....

VAT Number: .....

(for non-Swedish groups)

Approval from the local Ethical Committee (date, number):

**No injection without approval! Copy has to be added!**

.....





## ORDERING FORM FOR ELECTROPORATION

Date: .....

Research group ordering the mice: .....  
(complete address, fax and e-mail) .....

.....  
.....

Responsible person for project: .....  
(name and e-mail) .....

Shipping address for DNA: .....

.....  
.....  
.....

Address for invoice: .....

.....  
.....  
.....

VAT Number: .....

(for non-Swedish groups)

Name of the DNA that will be electroporated:

.....





**Please add Appendix 2 and send to:**

Johannes Wilbertz  
Karolinska Institutet  
CMB / KCTT  
von Eulers väg 3  
171 77 Stockholm  
Fax: + 46 8 30 83 74

**or**

Stephan Teglund  
Karolinska Institutet  
Dept. of Biosciences at Novum, CNT  
141 57 Huddinge  
Fax: + 46 8 608 15 01



## ORDERING FORM FOR TRANSGENIC MICE BY THE ES CELL INJECTION (ES CELLS MADE BY THE CUSTOMER)

Date: .....

Research group ordering the mice: .....  
(complete address, fax and e-mail) .....

.....  
.....

Responsible person for the project: .....  
(name and e-mail) .....

Shipping address for mice: .....

.....  
.....  
.....

Address for invoice: .....

.....  
.....  
.....

VAT Number: .....

(for non-Swedish groups)

Approval from the local Ethical Committee (date, number):

**No injection without approval! Copy has to be added!**

.....





## APPENDIX 1a:

### AGREEMENT FOR INTRODUCTION OF DNA CONSTRUCTS BY PRONUCLEAR INJECTION INTO FERTILIZED MOUSE EGGS

\_\_\_\_\_  
Project name

#### Agreement

between the Karolinska Center for Transgene Technologies (hereafter called KCTT, and the following research group (hereafter called Customer):

\_\_\_\_\_  
Name

\_\_\_\_\_  
Affiliation

\_\_\_\_\_  
Department

\_\_\_\_\_  
Address

\_\_\_\_\_  
Phone# and Fax#

\_\_\_\_\_  
e-mail

\_\_\_\_\_  
DNA construct

\_\_\_\_\_  
Date

KCTT 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 send in a copy of their ethical permission. **Without this copy KCTT will not start the injections.**

2. A DNA construct containing the desired combination of promoter/enhancer and gene is produced by the Customer. If possible, KCTT always recommend that the construct is tested for expression in a cell transfection assay before mice are produced. The Customer is responsible for the construct supplied and KCTT can not be held liable should the DNA construct not perform as expected in the mice. The Customer will provide KCTT with a map of the plasmid where information regarding excision of the DNA construct etc., should be included. The plasmid DNA must be purified by a commercial DNA purification kit, e.g. Qiagen or equivalent, or by at least one round of CsCl-EtBr gradient purification.
  
3. Purifying the DNA construct will then be performed by the Customer or the Customer request the service from KCTT (see ordering form). If it is done by the Customer, the vector backbone is removed and the linear DNA construct is purified. Approved protocols for DNA purification can be obtained from KCTT. The Customer will provide KCTT with at least 5 µg of purified DNA construct dissolved in 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA at a concentration of approximately 50-250 ng/µl or as pellet in 70% EtOH. Also, a photo of a diagnostic agarose gel showing the purified fragment alongside DNA size markers and an indication how much is loaded must be provided to KCTT. KCTT will make the final dilution of the construct with pre-tested microinjection buffer to around 2 ng/µl. If the purification service is requested from KCTT, the Customer should provide information what restriction enzyme(s) to use for removal of the construct from the plasmid. A photo of a test agarose gel showing the restriction digest of the plasmid must be provided to KCTT. At least 60 µg of plasmid DNA should be provided. If unusual and expensive restriction enzymes is needed, extra charges may apply for ordering these enzyme(s). Consult with KCTT in advance.
  
4. The DNA is injected into pronuclei of fertilized mouse eggs. Depending on the agreement with the customer, one, two or more days of pronuclear injection will be carried out by KCTT. The genotype of the fertilized mouse eggs can be F2 between two inbred strains or inbred, depending on the preference of the Customer. Injected eggs will be transferred to the oviducts of pseudopregnant foster mothers by KCTT.



5. Offspring from the injected eggs is born approximately 20 days later. The offspring will remain in the KCTT 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.
6. The customer accepts with his signature the health status of the KCTT animals as given in the health report. The health reports can be faxed on request or can be checked in the KCTT homepage (KCTT North Campus only).
7. The Customer is responsible for further analysis of the offspring, i.e. analysis of transgenicity from DNA from tail biopsies, and all biological analyses related to the project.
8. KCTT makes **NO** promises 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. KCTT has, however, succeeded in all previous attempts to generate transgenic mice by pronuclear injection.
9. The Customer is obliged to acknowledge KCTT in the first published paper that describes the resulting transgenic mice. **A reprint of such a publication shall also be sent to the facility.**
10. The customer is obliged to inform KCTT about the number of founder animals produced. The customer is asked to send back the filled in Appendix 1b (information about produced founder animals) to KCTT after screening.
11. Title and rights to all constructs and produced mice ("Material") under this Agreement remain vested in Customer. Title to any subject matter of any patent or patent application generated or developed through the use of this Material shall be governed by the rules of inventorship, provided, that the parties agree any invention relating to a transgenic animal model incorporating the Material shall be deemed a sole invention of Customer. Each party shall cooperate with each other in order to perfect each party's interest as set forth herein, including execution of



any assignment documentation to document a party's ownership rights. Customer shall have the exclusive right to negotiate an exclusive license to any invention conceived in part or whole by KCTT.

12. Upon completion of the project, and in the absence of further agreement of the parties, KCTT shall cease all use and make no further use of the Material, and the Material and any material related thereto shall be returned to Customer or destroyed upon written request.

13. KCTT will not utilize the Material for commercial purposes, nor will the data or results of research performed with the Material be disseminated or distributed to any third party without the express written consent of Customer.

For KCTT:

For the Customer:

\_\_\_\_\_  
Name

\_\_\_\_\_  
Name

\_\_\_\_\_  
Affiliation

\_\_\_\_\_  
Affiliation

\_\_\_\_\_  
Address

\_\_\_\_\_  
Address

\_\_\_\_\_  
Phone# and Fax#

\_\_\_\_\_  
Phone# and Fax#

\_\_\_\_\_  
e-mail

\_\_\_\_\_  
e-mail

\_\_\_\_\_  
Date

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Signature



## APPENDIX 1b:

### INFORMATION ABOUT PRODUCED FOUNDER ANIMALS

As core facility KCTT is very much dependent on the feed back of the groups using our service. It is not only important for us to improve our techniques but also to report the number of produced founder to the Board of Science of the KI and to the local authorities controlling us.

We would be very grateful if you could fill in the form after screening and fax it back to the following address:

**To:**

Dr. Johannes Wilbertz  
Karolinska Institutet / KCTT

Fax: +49 8 308374

**From:**

Research group: .....

(complete address, fax and e-mail) .....

.....

.....

Name of DNA injected: .....

Date of birth of the mice: .....

Number of mice received: .....

Total number of pups screened: .....

Total number of positive mice: .....

Signature (Customer): .....



## APPENDIX 2:

### **AGREEMENT FOR HOMOLOGOUS RECOMBINATION IN ES CELLS**

A prerequisite for achieving homologous recombination in embryonic stem (ES) cells is a careful design of targeting vectors, good quality of DNA used in electroporation, and good routines in the tissue culture facility. For retaining the totipotency of ES cells, special care has to be taken in culturing them. This means daily feeding and use of pre-tested media and reagents, which is very tedious and labor intensive, even during optimal conditions. To increase the chances of the project to succeed and to ensure a maximal throughput in our service for gene targeting projects, we kindly ask our Customers to agree upon the points detailed below.

#### **Agreement**

KCTT will attempt to achieve homologous recombination in ES cells of the E14 or GSI-1 line with a targeting vector supplied by the Customer under the following terms:

_____	_____
Name	Affiliation
_____	_____
Department	Address
_____	_____
Phone# and Fax#	e-mail
_____	_____
DNA construct	Date

KCTT will attempt to achieve homologous recombination in ES cells of the E14 line with a targeting vector supplied by the Customer under the following terms:



1. Previously characterized and well functioning cassettes conferring resistance to neomycin will be used for construction of the targeting vector.
2. The design of the targeting vector is to be presented to and approved by KCTT in advance.
3. The targeting construct will be made with genomic DNA from the mouse 129 line. Libraries of mouse 129 DNA in phage lambda, P1 or BAC vectors are commercially available. To achieve the highest target frequency, the DNA library should be of the same 129 substrain as the target ES cell line.
4. The Customer presents, in advance, experimental data that validate the correctness of the targeting construct to be used. This includes, if considered necessary by KCTT, southern blot data with genomic mouse 129 DNA and restriction maps and photographs of restriction enzyme cleaved targeting DNA.
5. Evidence for a pre-tested strategy for identification of correctly targeted ES clones that have undergone homologous recombination shall be presented by the Customer to KCTT prior to the beginning of all ES cell work.
6. The Customer will provide KCTT with 60 $\mu$ g of plasmid encoding the targeting vector. This DNA must be purified by at least one round of CsCl-EtBr gradient purification or appropriate other methods. The DNA must be cleaved to completion with the appropriate restriction enzyme prior to delivery. The volume may not exceed 100 $\mu$ l. If the customer negotiated a date for the electroporation, the DNA to be electroporated has to be shipped to KCTT at least 8 days prior to the electroporation date.



7. KCTT will make at most two attempts to isolate and freeze down 250 neomycin-resistant, individual ES cell clones with matching preparations of cell lysates for preparation of DNA. Cell lysates will be delivered to the Customer about 5 weeks after initiation of the ES cell work. The Customer is responsible for identifying any ES cell clones that have undergone homologous recombination.
8. The frozen ES cell clones will be stored by KCTT for a maximum of 2 months after delivery of the cell lysates.
9. KCTT will thaw and expand maximally 10 ES clones that have been identified as correctly targeted by the Customer, and freeze duplicates of these clones for later use. KCTT will also provide new cell lysates from these clones for confirmatory DNA analysis.
10. Upon unambiguous identification, KCTT will thaw and expand maximally 10 ES clones for subsequent injection into blastocysts.
11. KCTT makes **NO** promises that homologous recombination will take place, or that targeted ES clones will participate in germline transmission. KCTT will, however, use batches of ES cells that previously have consistently given germline transmission.
12. KCTT will only initiate ES cell work after receipt of the targeting vector.
13. The customer can cancel the agreement within the first 5 days of delivery of the DNA to KCTT. Cancellations at later stages in the process will be charged to the Customer, and the amount is based on in which step of the process the cancellation occurred.
14. The Customer is obliged to acknowledge KCTT in the first published paper that describes the ES cells and mice generated thereof. **A reprint of such a publication shall also be sent to the facility.**



15. Title and rights to all Material under this Agreement remain vested in Customer. Title to any subject matter of any patent or patent application generated or developed through the use of this Material shall be governed by the rules of inventorship, provided, that the parties agree any invention relating to a knockout animal model incorporating the Material shall be deemed a sole invention of Customer. Each party shall cooperate with each other in order to perfect each party's interest as set forth herein, including execution of any assignment documentation to document a party's ownership rights. Customer shall have the exclusive right to negotiate an exclusive license to any invention conceived in part or whole by KCTT.
  
16. Upon completion of the project, and in the absence of further agreement of the parties, KCTT shall cease all use and make no further use of the Material, and the Material and any materials related thereto shall be returned to Customer or destroyed upon written request.
  
17. KCTT will not utilize the Material for commercial purposes, nor will the data or results of research performed with the Material be disseminated or distributed to any third party without the express written consent of Customer.



For KCTT:

\_\_\_\_\_  
Name

\_\_\_\_\_  
Affiliation

\_\_\_\_\_  
Address

\_\_\_\_\_  
Phone# and Fax#

\_\_\_\_\_  
e-mail

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

For the Customer:

\_\_\_\_\_  
Name

\_\_\_\_\_  
Affiliation

\_\_\_\_\_  
Address

\_\_\_\_\_  
Phone# and Fax#

\_\_\_\_\_  
e-mail

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature



## APPENDIX 3:

### AGREEMENT FOR INTRODUCTION OF ES CELLS PRODUCED BY KCTT INTO MICE BY BLASTOCYST INJECTION

#### Agreement

between the Karolinska Center for Transgene Technologies (hereafter called KCTT), and the following research group (hereafter called Customer):

_____	_____
Name	Affiliation
_____	_____
Department	Address
_____	_____
Phone# and Fax#	e-mail
_____	_____
DNA construct	Date

KCTT will attempt to introduce ES cells into early mouse embryos with the aim of generating chimeric (mosaic) mice that can be used for germ line transmission of specific gene alterations. This work will be carried out under the following terms:

1. The customer is obliged to send in a copy of their ethical permission. **Without this copy KCTT will not start the injections.**
2. Frozen ES cells, which have been identified by the Customer to carry the correct genetic alteration, will be thawed and cultured under conditions, which keep them undifferentiated.



3. The ES cells will be introduced into blastocysts by injection into the blastocyst cavity (blastocyst injection). For each targeting construct two days of blastocyst injection (1 clone per day) in C57Bl6 blastocysts will be carried out.
4. Injected blastocysts will be transplanted to the uterus of pseudopregnant fostermothers.
5. KCTT will score the offspring for chimerism by inspection of coat color, and chimeric mice will be shipped to the Customer when they are four weeks old (i.e. after weaning).
6. The customer accepts with his signature the health status of the KCTT animals as given in the health report. The health reports can be faxed on request or can be checked in the KCTT homepage (KCTT North Campus only).
7. Subsequent crosses to achieve germ line transmission will be carried out by the Customer.
8. KCTT makes **NO** promises that chimeric mice will be produced. KCTT has, however, succeeded in nearly all previous attempts to generate chimeric mice, and germ line transmission has been accomplished for all targeting constructs.
9. The Customer is obliged to acknowledge KCTT in the first published paper that describes the resulting transgenic mice. **A reprint of such a publication shall also be sent to the facility.**
10. Title and rights to all ES-cells and chimeras ("Material") under this Agreement remain vested in Customer. Title to any subject matter of any patent or patent application generated or developed through the use of this Material shall be governed by the rules of inventorship, provided, that the parties agree any invention relating to a knockout animal model incorporating the Material shall be deemed a sole invention of Customer. Each party shall cooperate with each other



in order to perfect each party's interest as set forth herein, including execution of any assignment documentation to document a party's ownership rights. Customer shall have the exclusive right to negotiate an exclusive license to any invention conceived in part or whole by KCTT.

11. Upon completion of the project, and in the absence of further agreement of the parties, KCTT shall cease all use and make no further use of the Material, and the Material and any materials related thereto shall be returned to Customer or destroyed upon written request.

12. KCTT will not utilize the Material for commercial purposes, nor will the data or results of research performed with the Material be disseminated or distributed to any third party without the express written consent of Customer.

For KCTT:

For the Customer:

\_\_\_\_\_  
Name

\_\_\_\_\_  
Name

\_\_\_\_\_  
Affiliation

\_\_\_\_\_  
Affiliation

\_\_\_\_\_  
Address

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Address

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Phone# and Fax#

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Phone# and Fax#

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e-mail

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e-mail

\_\_\_\_\_  
Date

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Signature



## APPENDIX 4:

### AGREEMENT FOR INTRODUCTION OF ES CELLS MADE BY THE CUSTOMER INTO MICE BY BLASTOCYST INJECTION

#### Agreement

between the Karolinska Center for Transgene Technologies (hereafter called KCTT, and the following research group (hereafter called Customer):

_____	_____
Name	Affiliation
_____	_____
Department	Address
_____	_____
Phone# and Fax#	e-mail
_____	_____
DNA construct	Date

KCTT will attempt to introduce ES cells into early mouse embryos with the aim of generating chimeric (mosaic) mice that can be used for germ line transmission of specific gene alterations. This work will be carried out under the following terms:

1. The customer is obliged to send in a copy of their ethical permission. **Without this copy KCTT will not start the injections.**
2. Frozen ES cells, which have been identified by the Customer to carry the correct genetic alteration, will be thawed and cultured under conditions which keep them undifferentiated.



3. The Customer presents, in advance, experimental data that validate that the ES cells shipped were tested for the production of germline chimeric mice. In case no proof can be given KCTT injects the ES cells at the customers own risk.
4. The ES cells will be introduced into blastocysts by injection into the blastocyst cavity (blastocyst injection). For each targeting construct two days of blastocyst injection (1 clone per day) in C57Bl6 blastocysts will be carried out.
5. Injected blastocysts will be transplanted to the uterus of pseudopregnant fostermothers.
6. KCTT will score the offspring for chimerism by inspection of coat color, and chimeric mice will be shipped to the Customer when they are four weeks old (i.e. after weaning).
7. The customer accepts with his signature the health status of the KCTT animals as given in the health report. The health reports can be faxed on request or can be checked in the KCTT homepage (KCTT North Campus only).
8. Subsequent crosses to achieve germ line transmission will be carried out by the Customer.
9. KCTT makes **no** promises that chimeric mice will be produced. KCTT has, however, succeeded in all previous attempts to generate chimeric mice.
10. The Customer is obliged to acknowledge The KCTT in the first published paper that describes the resulting transgenic mice. **A reprint of such a publication shall also be sent to the facility.**
11. Title and rights to all ES-cells and chimeras ("Material") under this Agreement remain vested in Customer. Title to any subject matter of any patent or patent application generated or developed through the use of this Material shall be



governed by the rules of inventorship, provided, that the parties agree any invention relating to a knockout animal model incorporating the Material shall be deemed a sole invention of Customer. Each party shall cooperate with each other in order to perfect each party's interest as set forth herein, including execution of any assignment documentation to document a party's ownership rights. Customer shall have the exclusive right to negotiate an exclusive license to any invention conceived in part or whole by KCTT.

12. Upon completion of the project, and in the absence of further agreement of the parties, KCTT shall cease all use and make no further use of the Material, and the Material and any material related thereto shall be returned to Customer or destroyed upon written request.

13. KCTT will not utilize the Material for commercial purposes, nor will the data or results of research performed with the Material be disseminated or distributed to any third party without the express written consent of Customer.

For KCTT:

For the Customer:

\_\_\_\_\_  
Name

\_\_\_\_\_  
Name

\_\_\_\_\_  
Affiliation

\_\_\_\_\_  
Affiliation

\_\_\_\_\_  
Address

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## Literature

Cid-Arregui, A., García-Carrancá, A. (eds.) 1998. Microinjection and transgenesis: strategies and protocols. Springer-Verlag, Berlin Heidelberg.

Handbook on genetically standardized JAX Mice 1997. The Jackson Laboratory, Bar Harbor.

Nagy, A. et al. 2003. Manipulating the mouse embryo: a laboratory manual. 3<sup>rd</sup> ed. Cold Spring Harbor Press, New York.

Jacksson, I.J. and Abbott, C.M. (eds.) 2000. Mouse genetics and Transgenesis. Oxford University Press, New York.

Joyner, A. (ed.) 2000. Gene targeting - a practical approach. Oxford University Press, New York.

Lyon, M.F., Rastan, S., Brown, S.D.M. 1995. Genetic variants and strains of the laboratory mouse Vol. I+II. Oxford University Press, Oxford.

Müller, Ulrike. 1999. Ten years of gene targeting: targeted mouse mutants, from vector design to phenotype analysis. *Mechanisms of Development* 82, 3 - 21. Elsevier Science Ireland Ltd.

Silver, L.M. 1995. Mouse Genetics. Oxford University Press, New York.

Torres, R.M, Kühn, R. 1997. Laboratory protocols for conditional gene targeting. Oxford University Press, New York.

Wassaman and DePamphilis (eds.) 1993. Guide to techniques in mouse development. *Methods in Enzymology* Vol. 225. Academic Press, San Diego.