

UCL Transgenic Service Cryo Request Form

User details

Date Submitted

Owner: _____
Responsible: _____
Department: _____
Email: _____
Telephone: _____

Strain Information

Strain or gene name: _____
Jackson MGI no.(if available) _____
Genetic Background of strain: _____
Genotype (homo, hetero): _____
Backcross generation (if known): _____ Coat colour: _____
Known fertility problems: _____
Adverse Phenotype: _____
Severity limit: _____
Details of mutation: _____

Justification for use:

Genotype/strain of female mice to be used (WT, hetero, hom) _____
No. of males available: _____ DOB of males: _____ Proven fertility? _____
Project licence covering the strain: _____

BSU Contact Information

BSU staff responsible for the line: _____
Phone Number: _____
Contact email: _____

Administrative and financial information, please fill in:

Pyrat project code: _____
PyRat licence code: _____
Animal code: _____
Health report attached? Yes No

The above customer code will be charged for the rederivation on the 01 suffix unless otherwise specified.

CRYOPRESERVATION OF MOUSE STRAINS

Generating Frozen Embryo Stocks

Introduction

Cryopreservation involves freezing and storing pre-implantation stage embryos. This process protects valuable mouse strains against fire, disease, genetic contamination, breeding failure and, importantly, reduces the number of mice on the shelf, thereby minimising costs.

The stocks remain viable indefinitely, enabling strains to be kept in storage for as long as they are not needed for experiments, while always being available should their usage become required again. Frozen embryo stocks also provide an ideal means of exchanging mouse strains with other labs, nationally and internationally.

Home Office licensing

Cryopreservation work at UCL is currently carried out under project licence (PPL: P3A10AFEC) which allows importation and cryopreservation of genetically modified strains, without needing new Home Office approval. Please ensure that (if necessary) your project licence is amended in good time to cover the new strain and/or frozen stock. This must be completed within 4-6 months of starting the importation and/or cryopreservation. Strains will not be kept indefinitely on the importation licence after health screen.

Important – to ensure your strain is included on the project licence, please modify the *Request form* with all details of your strain and email it to tgservice@ucl.ac.uk. This must be done before embryo freezing work can begin.

Animals required for cryopreservation

The aim is to produce 300-400 embryos (8-cell stage) as the cryopreserved stock for each strain. These embryos will be distributed among 15-20 straws in the liquid nitrogen storage (approx. 20-25 embryos per straw). This provides several groups of embryos which could be used, for example, to provide the strain to overseas collaborators while keeping a reserve stock to recover the strain in your lab. Wherever possible, each strain will be stored in two independent liquid nitrogen stores, in order to increase security of storage.

To produce this number of frozen embryos, we need 6-8 single-housed young adult stud males, ideally 2-4 months of age. If fewer males are available, it will require more attempts to accumulate the desired number of frozen embryos. The stud males will be homozygous (if viable) or heterozygous for the genetic modification you wish to preserve.

Ideally these males will be mated to a standard wild type strain (e.g. C57, CD1) which will be supplied by us. However, if you want to preserve the line as homozygote, we will need you to provide around 20-25 females, at 3-4 weeks old. The number of females will depend on how well they respond to superovulation.

Procedure for producing embryos for cryopreservation

Females will be super-ovulated in batches and mated with the stud males, once a week, for about three weeks. Since superovulation is strain-dependent, the yield of 8-cell embryos for freezing is not always predictable. The number of superovulation procedures will be determined individually for each strain. If it proves impossible to get a good yield of embryos by superovulation, valuable strains can be cryopreserved using natural mating, although many more mated females will be needed.

Quality control procedures

Each batch of frozen embryos will be tested to confirm viability by an *in vitro* method. TG Service will defrost embryos from one straw and culture them overnight. If healthy blastocysts are produced, this is a reliable indication that the frozen embryos are of good quality. This *in vitro* quality control procedure is provided free of charge.

If specially requested by the user, TG Service can carry out an *in vivo* quality control assessment. For this, one straw will be thawed and the embryos implanted into pseudo-pregnant recipients to determine whether healthy, live born offspring are produced. Because of the extra work and animal usage involved in this procedure, a charge will be made (see below).

Fees payable by Users

Cryopreservation charge = £2000 per strain, including purchase and maintenance of mice used to generate the embryos (e.g. WT females). The fee will cover all procedures carried out by TG Service to establish the cryopreserved stock (to the embryo numbers indicated above). Cost of subsequent liquid nitrogen storage is also included.

The cost do not cover maintenance of the stud males which will remain under your customer code until the desired number of embryos has been frozen. Additional charges will be applied if a strain which responds poorly to superovulation is used (e.g. 129Sv, Balb/C), the user will be notified after three unsuccessful attempts.

The cost will be charged to the above Customer code with associated grant code unless an alternative is indicated. External customers will need to supply an invoice address and order number.

We cannot initiate a project of rederivation if you do not supply your Customer Code, if unsure, please ask the NACWO of your unit to provide you with the right code.

Recovery of a cryopreserved strain to generate live offspring = £2000 this is the same charge as currently applies to the re-derivation of a strain by pre-implantation embryo transfer, a service also offered by TG Service. This charge will apply whether the procedure is intended to generate live born mice for experiments (i.e. to recover the strain) or whether it is an “*in vivo* quality control” procedure (see above).

Note: Non-UCL based institution and/or scientist will have to add 20% and VAT to the price of the service.