**Embryo Generation for Cryopreservation**

The cryopreservation of preimplantation embryos is a suitable method of archiving GA mouse lines if it’s important to preserve either the genetic background or homozygosity in one or more mutations.

The intended use and zygosity of the cryopreserved material will dictate how many embryos need to be cryopreserved. Freezing 150 embryos will be enough to allow multiple recovery attempts. A bespoke freeze target can be established depending on the individual requirements per colony.

The most efficient way for us to generate embryos is by IVF. We require 1-2 males over the age of 10 weeks. Donor females will need to be provided between 2-12 weeks on the appropriate genetic background and genotype. On average we would hope to generate ~20 viable embryos per donor female and would therefore require between 7-10 donor females to reach the standard cryopreservation target. Generally, it is better to superovulate donor females at 4 weeks of age, however it is possible to use females up to ~12 weeks using a slightly amended superovulation regime.

We are now offering the option to check the genetic background of your line at the point of cryopreservation using Transnetyx's miniMUGA snp array based genetic monitoring service. One mouse from the cryopreservation process will be sent for testing unless users request additional mice. The introductory price for this service is £40/animal tested. Please contact geec@kcl.ac.uk for more information.

If you wish to cryopreserve embryos for a particular line, please contact Tolga Oralman tolga.oralman@kcl.ac.uk with the following information.

The price for generation of embryos via IVF for cryopreservation is £970.00 per session, each session can take up to 10 donor females.

**General Information**

|  |  |
| --- | --- |
| Requester’s Name |  |
| Requester’s Contact Details | Email: |  |
| Phone: |  |
| Department  |   |
| Budget Holder |   |
| Budget Code |   |

**Colony Information**

|  |  |
| --- | --- |
| Colony Name |  |
| Animal Prefix |  |
| PPL |  |
| What level of QC do you require? (See Appendix 1) |  |
| Colony Comments |  |
| Current Colony Location | BSU |  |
| Room |  |

**Stud Male Details/Sperm Import Details**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  Stud # | ID | DOB | Proven (Yes/No)  | Date separated from previous mating | Comments |
| 1 |  |   |  |  |  |
| 2 |  |  |  |   |  |

**Suitable Female Donor Details**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Female # | Colony Prefix | Animal ID | Genotype/s | Comments |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |
| 5 |  |  |  |  |
| 6 |  |  |  |  |
| 7 |  |  |  |  |
| 8 |  |  |  |  |
| 9 |  |  |  |  |
| 10 |  |  |  |  |

**Appendix 1**

**Embryo Cryopreservation Quality Control (QC)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Level of QC** | **Description** | **Time from freezing** | **Price** | **Number of mice required** | **Comments** |
| Basic: | Thaw one straw containing ≥15 embryos and check at least 85% recovery and survival rate. Viable embryos are cultured to check normal pre-implantation development to expanded blastocyst. | 2 weeks | £181  | 0 |  |
| Full: | Thaw one straw containing ≥15 embryos and check at least 85% recovery and survival rate. Resulting embryos are cultured to check normal pre-implantation development to expanded blastocyst, which are then arrayed onto a 96 well plate for subsequent genotyping confirmation by the user.  | 2 weeks | £181 | 0 | Various cryopreserved strains assessed from across the Infrafrontier/European mutant mouse archive (EMMA) over a 4 year period have never failed to pass QC due to non-pregnancy. Therefore an embryo transfer would only ever be necessary if genotype confirmation was essential and blastocyst genotyping failed to work for technical reasons. Please see: http://link.springer.com/article/10.1007/s11248-015-9897-1 "Blastocyst genotyping for quality control of mouse mutant archives: an ethical and economical approach". Please supply the PPL and Protocol number for “Embryo Transfer”. |
|  | Or |
| Thaw one straw containing ~25 embryos and check at least 85% recovery and survival rate. Resulting embryos are surgically transferred into ~2 Pseudopregnant recipients. Live born pups will be used to confirm recovery and provide tissue for genotype confirmation.  | 6-7 weeks | £582 | ~10 |