**Sperm Cryopreservation**

The cryopreservation of murine sperm and subsequent *In vitro* fertilisation methodology has progressed significantly over the past decade. The technique now offers a quick, efficient and robust method to archive mutant mouse models on a range of genetic backgrounds, using a minimal number of mice.

After harvesting sperm from two males we expect to freeze 20 straws, each containing one aliquot of sperm which will be housed in the BSU storage tanks (unless otherwise requested. Freezing 20 straws will also allow for distribution to collaborators if required. The price for a sperm harvest and cryopreservation attempt is £466.00.

It is recommended to perform a quality control (QC) check on the cryopreserved sperm to check that the line is recoverable and the correct mutation has been cryopreserved. The level of QC should be tailored by the requester. Guidance on the QC options is given in Appendix 1. Please note that the “Basic” level of QC is included in the standard sperm cryopreservation price.

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.

**Note:** Sperm freezing may not be appropriate if a particular line is from a mixed or unknown background that needs to be maintained, also if there are homozygous or multiple mutations which need to be maintained. Normally when a line is recovered a wildtype background strain will be used to provide donor oocytes, this has the same effect as a single generation of backcrossing.

If you would like to request the cryopreservation of sperm from any of your lines please provide the information requested below and return to tolga.oralman@kcl.ac.uk. Do get in touch if you need any further information about sperm cryopreservation or if you would like to discuss the application of any cryopreservation or assisted reproductive techniques (ART).

**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)Or, opt for a Full QC for a Rederivation  |  |
| Colony Comments |  |
| Colony Location | BSU:  | Room: |

**Sperm Harvest Male Check List**

|  |  |  |  |
| --- | --- | --- | --- |
| Comments | Requirement | ID #1 | ID #2 |
| Freezing sperm from two males significantly improves the likelihood of recovering the line, increasing the density of sperm whilst avoiding any unexpected issues associated with one particular male. | 2 males |  |  |
| Preferably both males designated for sperm harvest will have proven reproductive capability *in vivo*. Avoiding any issues associated with infertility. | Proven males? Y/N |  |  |
| Males should be separated from any mating’s for at least 5 days prior to sperm being harvested. | Separated (date) |  |  |
| Males should be between 8 and 16 weeks of age for optimum harvest. | Age |  |  |
| Confirming the genotype of the males allocated for sperm freezing will prevent the recovery of unexpected genotypes during the QC process. | Genotype confirmed? |  |  |
| It is important to know the genetic background of the sperm harvest males to ensure the correct integrity is maintained upon recovery. (e.g., C57BL6/**J**, C57BL6/**N**, C57BL6/**Ola-Hsd** etc.) | Genetic background |  |  |

**For BSU/GEEC Staff use**

|  |  |
| --- | --- |
| Date of Harvest  |   |
| CPA Batch # |   |
| Pre-Freeze Motility Assessment  |   |
| Storage Location/s |   |
| Completed By |   |
| General Comments  |   |
| LN2 Depth |  |

**Appendix 1**

**Sperm Cryopreservation Quality Control (QC)**

It is important to carry out an appropriate quality control process before considering any cryopreservation attempt successful, and before removing a particular line as a live resource.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Level of QC** | **Description** | **Time from freezing** | **Price** | **Number of mice required** | **Comments** |
| Basic: | Post freeze motility check only. | < 1 week | Included | 1-2 | Sperm will be graded post freeze/thaw to assess progressive motility/morphology and density (key factors in whether the sperm will generate viable embryos in an IVF). Less than 3% of lines with motile sperm upon thawing fail to produce viable embryos in an IVF. |
| Part: | IVF attempt using appropriate female donors to generate embryos and achieve a predetermined level of fertilisation. Resulting embryos are cultured to check normal pre-implantation development to expanded blastocyst. | 2 weeks | £582 | 2-5 | The generation of embryos that successfully culture through to expanded blastocyst stage confirms the viability of the frozen sperm (>80 must develop to blastocyst stage), and rules out parthenogenetic activation.Please supply the PPL and Protocol number for “Superovulation”. |
| IVF attempt using appropriate female donors to generate embryos and achieve a predetermined level of fertilisation. 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. Any additional embryos not required for culture will be cryopreserved at the 2 cell stage. | Various 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 “Superovulation”.If you wish to recover live born pups for genotyping, you will also need to supply the appropriate Protocol number for “Embryo Transfer”. |
| Full: |  |  | 6-7 weeks |  £980 | 12-15 |
| IVF attempt using appropriate female donors to generate embryos and achieve a predetermined level of fertilisation. Resulting embryos are surgically transferred into ~2 Pseudopregnant recipients. Live born pups will be used to confirm recovery and provide tissue for genotype confirmation. Any additional embryos not required for embryo transfer will be cryopreserved at the 2 cell stage. |