**Application for CTC cryopreservation of sperm under the BVS contingency agreement**

* Please complete one form for each line to be frozen and submit it to trantec@ed.ac.uk.

What CTC needs:

* 2 males are required per line.
* Males must be **proven** (i.e. have been in a mating which resulted in a pregnancy) in order to be eligible for this contingency sperm cryopreservation programme.
* Sperm quality often declines by 6 months of age, and best results are obtained with sperm from males 10-16 weeks of age (Taft et al., Cold Spring Harb Protoc, 2017). In order to qualify for the free sperm cryo service, the males therefore must be aged between **2-6 months**.
* We would advise that offspring of these males have been genotyped to confirm transmission of the allele (however, this is not a prerequisite to be eligible for the free service).

What CTC offers:

* CTC will perform quality controls (QCs) including an assessment of sperm motility and concentration before and after cryopreservation. If a male scores poorly during the QC process, CTC staff will ask for an additional male to be frozen.
* If a researcher requires quality assurance of specific lines to validate recoverability, an IVF can be requested and paid for by the researcher. This is recommended before a researcher completely closes a colony.
* CTC will store approximately 8 sperm straws per male in long term liquid N2 storage.
* CTC will cover the costs of freezing and the subsequent storage of the sperm samples. The samples will not be distributed without the original owner’s consent.
* If the owner leaves the University, samples can be exported to the new work place or an archiving institution, transferred to another UoE researcher who takes on ownership, or need to be discarded (<https://www.ed.ac.uk/bioresearch-veterinary-services/transgenics/cryopreservation/cryostorage>).
* The sperm frozen under the cryopreservation programme is not intended for global distribution. If a line is expected to be popular with a lot of distribution demand, we recommend that additional sperm is frozen at a repository such as the National Mouse Archive – NMA (<https://www.har.mrc.ac.uk/services/archiving-and-distribution/>) or the European Mouse Mutant Archive – EMMA (<https://www.infrafrontier.eu/emma/>).

**I agree with the conditions stated above:**

**Name of Principal Investigator:** Click or tap here to enter text.

**Electronic signature:**

**Date:** Click or tap here to enter text.

Please supply all requested information in full using a new form for each line.

**Part A: Contact details**

|  |  |  |  |
| --- | --- | --- | --- |
| **Project leader:** |  | **e-mail:** |  |
| **Lab contact:** |  | **e-mail:** |  |
| **TR number (if originally generated or re-derived by CTC):** |  |

**Part B: Details of line to be frozen**

|  |  |
| --- | --- |
| **Location of animals:** | [ ]  ASH[ ]  CRM[ ]  GSQ[ ]  HRB[ ]  IRR[ ]  LFR[ ]  MAR[ ]  ROS[ ]  WGH[ ]  other: |
| **Tick@lab line name:** |  |
| **Tick@lab TEAM name:** |  |
| **Background strain:** | [ ]  C57BL/6J (Charles River)[ ]  C57BL/6JOlaHsd[ ]  C57BL/6NCrl[ ]  B6CBAF1/Crl[ ]  Crl:CD1(ICR)[ ]  Hsd:ICR (CD-1)[ ]  BALB/cAnNCrl[ ]  BALB/cAnNHsd[ ]  BALB/cOlaHsd[ ]  FVB/NCrl[ ]  FVB/NHanHsd[ ]  mixed (please give further details)[ ]  other: |
| **Traits/genetic modification** (short description of changes made to this transgenic/mutant line in plain text): |  |
| **MGI number** https://www.informatics.jax.org/ |  |
| **Technical scientific name** (e.g. B6.129P2-Apoa1tm1Unc/J); please refer to https://www.informatics.jax.org |  |
| **Originating establishment/Source:** |  |
| **Number of backcrosses** (if applicable): |  |
| **Coat colour:** |  |
| **Citation/PubMed ID** (if available): |  |

**Part C: Identification of males to be frozen**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tick@lab ID** | **DOB (age range 2-6 months)** | **Genotype** | **Proven Y/N****MANDATORY** | **Transmits allele****Y/N****ADVISORY** |
|  |  |  |  |  |
|  |  |  |  |  |

[ ]  **I do want a quality control IVF to be performed. Please note that this service occurs at a fee**. Contact m.koerner@ed.ac.uk for further information, and indicate the relevant grant holder below:

|  |  |
| --- | --- |
| **Tick@lab project code to be charged for QC-IVF:** |  |

**Replacement male(s)** (in case QC for one of the original males fails): Please only complete this part if a repeat is necessary (upon advice by transgenic technicians):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tick@lab ID** | **DOB (age range 2-6 months)** | **Genotype** | **Proven Y/N****MANDATORY** | **Transmits allele****Y/N****ADVISORY** |
|  |  |  |  |  |
|  |  |  |  |  |

**Part D: GA appendix**

Please copy/paste from your existing GA appendix. Update if necessary. For information about GA appendices, please see: <https://www.ed.ac.uk/bioresearch-veterinary-services/home-office-guidance/ga-appendices>

|  |
| --- |
| **Background: Please give details about the specific genetic alteration/mutation and how the gene of interest fits with the scientific aims:** |
|  |
| **Objectives: Please provide information about how this strain is used to achieve the scientific objectives outlined in the relevant PPL/programme of work and what potential benefits might derive from this work:** |
|  |
| **Phenotypes/adverse effects: List all phenotypes, including adverse effects and observable traits/abnormalities that have potential welfare implications, together with welfare assessment advice (if appropriate) and a time scale of when these effects can be observed. Examples include developmental and behavioural defects; physical abnormalities; homozygous lethality, or incidence of unexpected death and immune status (if immunocompromised, or susceptible to specific parasites, bacteria etc.):** |
|  |
| **Care/Remedial Actions Required: List any additional care/husbandry requirements that are required for this line:** |
|  |
| **Severity limit of breeding protocol:** | [ ]  Mild[ ]  Moderate[ ]  Severe |

**Part E: Genotyping conditions**

|  |  |
| --- | --- |
| **PCR primer sequences:** |  |
| **PCR reaction setup:** |  |
| **Thermocycling conditions:** |  |
| **Fragment size wildtype/mutant allele:** |  |
| **Description of genotyping method if other than PCR:** |  |

**Part F: Genotyping/genetic monitoring (Transnetyx)**

[ ]  **I do want ear biopsies to be taken after Schedule 1 and submitted to Transnetyx for genotyping.** Please share your genotyping assay with trantec@ed.ac.uk . Standard Transnetyx genotyping charges will apply.

[ ]  **I do want genetic monitoring (MiniMUGA) to be carried out by Transnetyx.** If no specific genotyping assay is available, a generic assay will be used for sample QC prior to genetic monitoring. Standard Transnetyx genotyping/genetic monitoring charges will apply.

|  |  |
| --- | --- |
| **Tick@lab project code to be charged for genotyping/genetic monitoring:** |  |

**Genotyping/genetic monitoring results**

|  |  |  |  |
| --- | --- | --- | --- |
| **Tick@lab ID** | **Genotype as expected**  | **Background strain as expected** | **Comments** |
|  |  |  |  |
|  |  |  |  |

**For CTC internal use only**

|  |  |
| --- | --- |
| CTC Cryopreservation Number |  |
| Additional comments: |  |

**Quality control sperm**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  | **Pre-freezing** | **Post-freezing** |
| **Date** | **CTC technician** | **Male ID** | **Signs of clinical disease? (Y/N)[[1]](#footnote-1)** | **Motility** | **Conc.** | **Motility** | **Conc.** |
|  |  |  |  |  |  |  |  |
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|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |

**\* best male to use for IVF**

**Sperm motility scoring criteria**

|  |  |
| --- | --- |
| Excellent (Ex) | Majority of sperm swim forward, fast and in a straight line. |
| Very good (VG) | Majority swim fast/forward but a small fraction is immotile or travels in a slow/crooked motion. Still high fertilisation potential. |
| Good (G) | Some very good motility but ~50% immotile/don’t progress. Reasonable fertilisation potential. |
| Poor (P) | Tails move but some sperm do not progress forward or move very slowly. |
| Very poor (VP) | Sperm fail to move at all or very slow tail pulses without any progression. |

**Sperm concentration scoring criteria**

|  |  |
| --- | --- |
| Excellent (Ex) | Concentration confidently enough for successful fertilisation. |
| Very good (VG) | Concentration expected to give successful fertilisation. |
| Good (G) | Concentration probably enough for successful fertilisation. |
| Poor (P) | Concentration probably not good enough for fertilisation. Ideally: Select a new male or note for use with ICSI. |

**Quality control IVF (only if requested and paid for)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Date** | **Male ID** | **# embryos** | **% eggs to 2-cell stage (fertilisation rate)** |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

1. If Y, please give comments [↑](#footnote-ref-1)