**Application for CTC cryopreservation of mouse embryos**

* Please complete one form for each line to be frozen and submit it to m.koerner@ed.ac.uk.
* The number of eggs recommended will depend on the genotype of your line (i.e. homozygotes would require less than heterozygotes). For further information, please have a look at the embryo cryopreservation section on our homepage.
* Embryo yield varies, and more than one session may be required to freeze a sufficient amount.
* The researcher is responsible for providing appropriately aged transgenic males and females, wildtype males/females can be organised by CTC.
* For most strains, female mice should be aged between 3-4 weeks at the time of the procedure, in order to obtain optimal embryo yields. However, older female donors can be used as well.
* Males need to be proven (i.e. have produced a pregnancy), and should not be older than 6 months. The number of male mice depends on the method chosen (see below).

There are 2 options for embryo cryopreservation

1. A cohort of up to 20 females is superovulated and eggs collected. 1-2 males are sacrificed and sperm is used for IVF. Fertilised embryos are frozen. This is the preferred method (as long as IVF works well for this background strain), as less animals need to be used, and more embryos are produced.
2. A cohort of 20 (with a minimum of 7) superovulated females are mated with a cohort of singly housed stud males, and the resulting embryos are collected and frozen. This approach usually requires more sessions than the approach in a) and is therefore only used if IVF doesn’t work for this particular line.
* If the owner of the embryos 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>).

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

**Part A: Contact details**

|  |  |  |  |
| --- | --- | --- | --- |
| **Project leader:** |  | **e-mail:** |  |
| **Lab contact:** |  | **e-mail:** |  |
| **Project leader department/UoE location:** |  |
| **Tick@lab project code:** |  |
| **Finance administrator:** |  |

**Part B: Type of service required**

[ ]  IVF

[ ]  Natural matings with collection of eggs

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

|  |
| --- |
| **MALES** |
| [ ]  **transgenics provided by researcher** | **Tick@lab line name:** |  |
| **Tick@lab TEAM name:** |  |
| **Location of animals:** | [ ]  LFR (Chancellor’s Building)[ ]  SCRM[ ]  ASH3 L5[ ]  Western BRF[ ]  Hugh Robson Building[ ]  1 George Square[ ]  Roslin BRF[ ]  other: |
| **Background strain:** | [ ]  C57BL/6J (Charles River)[ ]  C57BL/6JOla[ ]  C57BL/6NCrl[ ]  B6CBAF1[ ]  CD1(ICR)[ ]  BALB/c[ ]  FVB/N[ ]  other: |
| **Coat color:** |  |
| **Tick@lab ID:** |  |
| [ ]  **wt provided by CTC** | **Wildtype strain:** | [ ]  C57BL/6J (Charles River)[ ]  C57BL/6JOlaHsd[ ]  C57BL/6NCrl[ ]  B6CBAF1/Crl[ ]  CD1(ICR)Crl[ ]  CD1(ICR)Hsd[ ]  BALB/cAnNCrl[ ]  BALB/cOlaHsd[ ]  FVB/NCrl[ ]  FVB/NHanHsd[ ]  other: |

|  |
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| **FEMALES** |
| [ ]  **transgenics provided by researcher** | **Tick@lab line name:** |  |
| **Tick@lab TEAM name:** |  |
| **Location of animals:** | [ ]  LFR (Chancellor’s Building)[ ]  SCRM[ ]  ASH3 L5[ ]  Western BRF[ ]  Hugh Robson Building[ ]  1 George Square[ ]  Roslin BRF[ ]  other: |
| **Background strain:** | [ ]  C57BL/6J (Charles River)[ ]  C57BL/6JOla[ ]  C57BL/6NCrl[ ]  B6CBAF1[ ]  CD1(ICR)[ ]  BALB/c[ ]  FVB/N[ ]  other: |
| **Coat color:** |  |
| **Tick@lab ID:** |  |
| [ ]  **wt provided by CTC** | **Wildtype strain:** | [ ]  C57BL/6J (Charles River)[ ]  C57BL/6JOlaHsd[ ]  C57BL/6NCrl (bred inhouse)[ ]  B6CBAF1/Crl[ ]  CD1(ICR)Crl[ ]  CD1(ICR)Hsd[ ]  BALB/cAnNCrl[ ]  BALB/cOlaHsd[ ]  FVB/NCrl[ ]  FVB/NHanHsd[ ]  other: |

**Part D: GA appendix**

Please copy/paste from your existing GA appendix. Update if necessary.

|  |
| --- |
| **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**

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

**For CTC internal use only**

|  |  |
| --- | --- |
| CTC Cryopreservation Number |  |
| CTC Technician freezing/QC: |  |
| Additional comments: |  |

**Quality control media thaw/culture**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Date** | **Media batch** | **QC Strain** | **Morula recovered** | **Viable (%)** | **Cultured to Blasts** | **% to Blasts** | **Pass****Y/N** |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |

**Quality control genetic strain**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Date** | **Media batch** | **QC Strain** | **Morula recovered** | **Viable (%)** | **Cultured to Blasts** | **% to Blasts** | **Pass****Y/N** |
|  |  |  |  |  |  |  |  |
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