小鼠IVF生物净化申请表

请认真阅读填表说明

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| AP |  | 实验室 |  | 申请人 |  |
| 申请日期 |  | 联系电话 |  |
| 小鼠编号 | 品系全称 | 品系简称 | 基因型 | 遗传背景 | 数量 | 雌/雄 | 出生日期 |
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| 小鼠健康状况 |  □ 好 □ 一般 □ 差  |
| 净化方案选择（详情见填表说明） | □方案一 □方案二 |
| 净化后代是否有数量要求 | □是，具体数量 □否 |
| 是否需要本中心代做基因型鉴定 | □ 是 □ 否 |
| 是否作为脑中心公用品系 | □ 是 □ 否 |
| 备注： |
| 申请人（签字） 日期： |
| 邮件发送并抄送PI 日期： |
| 以下由实验动物中心填写： |
| 收表日期 |  | 技术员姓名 |  |
| 实验动物中心主任（签字）： 日期： |
| 净化后代数量结果（由胚胎操作技术员填写） |  |
| 净化质量结果（由兽医填写） |  |

**请附带基因型鉴定方案，例：**

|  |
| --- |
| **Name:** **B6.Cg-*Gt(ROSA)26Sortm14(CAG-tdTomato)Hze*/J** |
| **Description:** Ai14 mice hemizygous for this Rosa-CAG-LSL-tdTomato-WPRE::deltaNeo conditional allele are viable and fertile. A loxP-flanked STOP cassette prevents transcription of the downstream red fluorescent protein variant (tdTomato). When bred to mice that express Cre recombinase, the resulting offspring will have the STOP cassette deleted in the cre-expressing tissue(s); resulting in expression of tdTomato. Because this CAG promoter driven reporter construct was targeted for insertion into the Gt(ROSA)26Sor locus, tdTomato expression is determined by which tissue(s) express Cre recombinase. Ai14 mice are not expected to express tdTomato prior to introduction of Cre recombinase and tdTomato expression following exposure to cre is expected to be detectable by fluorescence and mRNA (in situ hybridization). These Ai14 mice are useful as a Cre reporter strain; expressing the red fluorescent protein variant, tdTomato, following Cre-mediated recombination.**Generation:**The Rosa-CAG-LSL-tdTomato-WPRE targeting vector was designed with (from 5' to 3') a CMV-IE enhancer/chicken beta-actin/rabbit beta-globin hybrid promoter (CAG), an FRT site, a loxP-flanked STOP cassette (with stop codons in all 3 reading frames and a triple polyA signal), tdTomato sequence (a non-oligomerizing DsRed fluorescent protein variant with a 12 residue linker fusing two copies of the protein (tandem dimer)), a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE; to enhance the mRNA transcript stability), a polyA signal, and an attB/attP-flanked PGK-FRT-Neo-polyA cassette. This entire construct was inserted between exons 1 and 2 of the Gt(ROSA)26Sor locus via electroporation of (129S6/SvEvTac x C57BL/6)F1-derived G4 embryonic stem (ES) cells. Correctly targeted ES cells (clone Ai9) were then transiently transfected with a PhiC31-expressing plasmid. The resulting ES cells (clone Ai14) with the PGK-FRT-Neo-polyA cassette deleted (leaving a single attL site) were injected into recipient blastocysts. High percentage chimeric males were sent to The Jackson Laboratory. Upon arrival, mice were bred to C57BL/6J inbred mice (Stock No. [000664](http://jaxmice.jax.org/strain/000664.html)) to establish the mutant colony (as Stock No. [007908](http://jaxmice.jax.org/strain/007908.html)). Next, Ai14 mice were further backcrossed with C57BL/6J inbred mice using a marker-assisted approach to generate this congenic colony (Stock No. 007914). |
| **Location:** SPF 102# in Peking university |
| **Source:** Jackson Laboratory |
| **Reference:**Madisen L; Zwingman TA; Sunkin SM; Oh SW; Zariwala HA; Gu H; Ng LL; Palmiter RD; Hawrylycz MJ; Jones AR; Lein ES; Zeng H. 2010. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat Neurosci 13(1):133-40. [PubMed: [20023653](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Abstract&list_uids=20023653)]  [MGI Ref ID [J:155793](http://www.informatics.jax.org/searches/reference.cgi?156886)]  |
| **Protocol Primers:****Ai14-1：AAG GGA GCT GCA GTG GAG TA****Ai14-2：CCG AAA ATC TGT GGG AAG TC****Ai14-3：GGC ATT AAA GCA GCG TAT CC****Ai14-4：CTG TTC CTG TAC GGC ATG G** |
| **Reaction：(20ul)****Reaction component Volume (µL)** ddH2O 102xTaq PCR StarMix 7Primer: 2Template DNA 1 |
| **Cycling :**Step      Temp     Time         Note 1           94 °C     3 min 2           94 °C     20 sec 3           61 °C     30 sec      4           72 °C     30 sec      Go to step 2, 35cycles5          72 °C     2 min 6            4 °C     forever |
| Expected Results:WT: 297 bpTM: 196 bpHom: KI/ki Het: KI/wt WT:WT/wtGel Information:Separated by gel electrophoresis on a 3.0%agarose gel. |

填表说明：

1）表格所有填写内容请用签字笔填写或电脑打印(签名处除外)。申请人、PI、及实验动物中心主任签字栏签字。

2）请提前十个工作日提交电子版发送邮箱至Liuxiaojing@cibr.ac.cn并抄送至PI，方便实验动物中心工作人员进行准备工作。

3）生物净化供鼠选择与要求：

方案一：只提供雄鼠：健康雄鼠至少2只，3-6月龄（超龄可以尝试），品系背景为C57BL/6J、C57BL/6N、CBA/Caj，且为单基因修饰动物。

方案二：雄雌均提供：健康雄鼠至少2只，3-6月龄（超龄可以尝试），雌鼠3-4周龄，5只以上。品系背景不常见，且为多基因修饰动物。

4）净化所需时间：因实验动物品系不同，其排卵、受孕等操作存在较大差异，因此，净化所需时间每个品系有所不同。一般为2-3个月后可以拿到4周龄的后代。净化实验中雌鼠都会被处死取胚胎，但并不是每个小鼠都能取到发育正常的胚胎。所以，请留够雌鼠用于繁殖，不要出现断种的情况。

5）需要本中心代做基因型鉴定的请提供相关的引物以及PCR反应体系。

6）**若该品系可作为脑中心公用品系，则本中心不收取该品系净化费用。如需后代，则每只鼠按照LARC野生型小鼠收费。**

7）联系电话：18031067609

 邮箱：Liuxiaojing@cibr.ac.cn

The application for mice pathogen free rederivation by IVF

Please read the instructions carefully

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| AP |  | Dept |  | Applicant |  |
| Date |  | Phone  |  |
| Mouse No. | The full name of the strain | The short name of the strain | Genotype | Genetic Background | Number | F/M | DOB |
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|  |  |  |  |  |  |  |  |
| Health of mice |  □ Best □ Normal □ Bad  |
| The rederivation plan（Details are shown in the instructions） | □ Plan A □ Plan B |
| Whether there is a requirement of the number of offspring | □ Yes，The specific quantity is □ No |
| Whether LARC needs to do genotyping | □ Yes □ No |
| Whether it is a common strain of CIBR | □ Yes □ No |
| Note： |
| The applicant（Signature） Date： |
| Send by E-mail and copy to PI Date： |
| The following is completed by LARC’S staff： |
| Receipt of date |  | Staff’s name |  |
| LARC’s director（Signature）： Date： |
| The number of offspring  |  |
| The quality of offspring |  |

Instructions:

1）Please fill in the form by gel pen or computer（except the signature）.

2）Please submit the electronic application by e-mail to zhangyizhong@cibr.ac.cn (c.c. to PI simultaneously) 10 working days in advance to facilitate the preparation by the staff in the Center.

3）Selection and requirements of the donor mice:

Plan A: Only males are provided: at least 2 healthy males, 3-6 months old (older can be tried), the genetic background of the strains is C57BL/6J, C57BL/6N, CBA/Caj and is a single gene modified animal.

Plan B: Males and females are provided: at least 2 healthy males, 3-6 months old (older can be tried), 3-4 weeks old female, 5 or more. The genetic background of the strain is uncommon or is a multi-gene modified animal.

4) Time required for rederivation: Due to the different strains of experimental animals, there are large differences in the operations such as ovulation and conception. Therefore, the time required for rederivation varies from strain to line. It is generally 2-3 months later to get 4 weeks old offspring. In the experiment, the females are sacrificed to obtain embryos, but not every mouse can obtain normal embryos. Therefore, please leave enough females for breeding and not to break the seeds.

5）If you need LARC to do genotyping, please provide primers and PCR reaction system.

**6）If the strain can be used as the common strain of CIBR, the IVF wil be free.The offspring will be charged as wild-type.**

7）Phone number:18526280938（Yizhong Zhang）

 E-mail:zhangyizhong@cibr.ac.cn