

Murine Stem Cell Laboratory Requisition
Mouse Biology Program
University of California
Davis, California USA 95618

ES Cell Electroporation Request Form

PI DETAILS:

PI's first name:

PI's last name:

Phone #:

Fax#:

E-mail address:

Address:

Membership: Cancer Center _____ CEHS _____ None _____

CONTACT DETAILS:

Contact's first name:

Contact's last name:

Phone #:

Fax#:

E-mail address:

ACCOUNT DETAILS:

Billing contact:

Phone#:

Email address:

For UCD Campus PI: please provide 4 Digit recharge number:

For other UC campus PI: please provide Intercampus String:

For off Campus PI: please provide Purchase Order (PO) #:

AND fax a copy to MSCL (530-757-3284). If you want to pay by credit card, please contact Brandy Thomas (brthomas@ucdavis.edu, phone: 530-757-3322, Fax 530-757-3277)

ALL FIELDS MUST BE COMPLETED BEFORE WE CAN PROCESS YOUR ORDER

For MSCL USE ONLY:

Billing date:

Order number:

GENERAL INFO:

Construct Name:

Preferred ES cell line: R1 (129/Sv) ___ ; Bruce4 (C57BL/6) _____ ; E14 (129/01a) _____ ; JM8 (C57BL/6) _____.

Genomic Library Origin: 129/SvJ ___ 129/SvEv _____ C57BL/6J _____ Other _____

Genomic Library Format: BAC _____ λ -library _____ Source _____

Transgene Origin (knock-in construct): Mouse ___ Human ___ Chicken ___ Other _____

Nature of Construct: Knock-in _____ Knock-out _____ conditional _____ Other _____

Expected phenotype (heterozygous) : Potential lethal ___ Unknown ___ Other _____

CONSTRUCT INFO:

Gene location: Chromosome _____

Circular plasmid preparation method: CsCl₂ Banding _____ Qiagen column _____ Other _____

Construct size:

Long arm size:

Short arm size:

Enzyme for linearization:

Positive Selection: PGK-neo _____ Mc1-neo _____ Other _____ (include promoter)

Negative Selection : PGK-DT _____ PGK-TK _____ Other _____ (include promoter)

GENOTYPING INFO:

ES cell screening will be done by: ___ PI's lab ___ MGAL

Genotyping method: ___ PCR ___ Southern Blot

For PCR, please provide the following information:

___ estimated positive band size _____ bp

___ estimated endogenous band size _____ bp

___ positive control quantity used in PCR _____ pg

___ negative control (suggested negative control : 0.3 ug WT genomic DNA)

___ confirmation of the DNA sequence from positive PCR product

For Southern Blot;

___ estimate positive band size _____ bp

___ estimate wild type band size _____ bp

PLEASE ATTACH THE FOLLOWING INFORMATION:

1. A brief description of the experiment.

2. Please provide the construct map with the following info:
 - ___ construct size
 - ___ vector location and size
 - ___ long arm location and size
 - ___ short arm location and size
 - ___ positive and negative selection cassette location, including promoter and orientation
 - ___ size and location of the endogenous fragment to be replaced
 - ___ linearization enzyme cut site
 - ___ genotyping primer or probe location

3. Copy of a southern blot or PCR analysis showing detection of the expected bands.

4. Photo of linearized DNA
 - ___ Gel electrophoresis should be clear and all the fragments should be fully separated
 - ___ Gel photo should be large and include MW marker, uncut circular plasmid, linearized fragment (100 to 500 ng).
 - ___ All fragments should be clearly indicated by size and name.

5. If PI's lab is responsible for genotyping, please provide shipping address for cells for genotyping and PI's FedEx account number.

TERMS AND CONDITIONS:

1. The investigator must submit the request form with a Recharge number, UC Intercampus Recharge number, or Purchase Order; to schedule the electroporation date. If you prefer to use credit card, please contact Brandy Thomas (brthomas@ucdavis.edu) (phone: 530-757-3322), please DO NOT send your credit card info with the request.

2. Please send the request form to Carole Kurahara or Sasha Wirth (Fax: 530-757-3284, Email: cgkurahar@ucdavis.edu or sswirth@ucdavis.edu) when your construct is ready, but do not prepare the DNA for electroporation until the electroporation date is confirmed. Please see attachment for DNA purification instructions.

3. The electroporation will NOT be scheduled if the info required on the request form is not provided.

4. The investigator is responsible for the construct preparation and purification if the construct is made in the PI's lab. A minimum of 150µ l of 200ng/µ l linearized construct is required for each project. The construct has to be in sterile PBS, and prepared no longer than 14 days before the electroporation date. When you submit the DNA construct, you need to provide gel photo. MMCL has the right to reject the construct if the construct is not clean or the concentration is not correct.

5. The PI must demonstrate proof of a working genotyping protocol before commencement of project. If not in place, we must insist that a Long-Short-Arm electroporation is performed before electroporation of the complete construct. When PCR screening strategy is optimize with LSA clones, we will schedule electroporation of the complete construct. Murine Genetic Analysis Laboratory (MGAL) provides consultation and PCR/Southern Blot development services, please contact Brandon Willis (email: bjwillis@ucdavis.edu, phone: 530-757-3353) for assistance.

6. MSCL will pick the specified number of clones (~300 or as requested by the investigator). The service does NOT guarantee any homologous recombinant clones by PCR or Southern blot. If the PI wants to repeat electroporate the construct due to lack of homologous clones, the PI has to pay for another set of electroporations.

7. MSCL maintains a separate laboratory space fully equipped and staffed to perform the planned procedures. The cost for each set of electroporation will be quoted in an estimate sent to investigator. This covers all costs associated with ES cell electroporation, drug selection and picking ~300 clones, and providing one set of 96-well plates for genotyping. Unless otherwise advised; quotes do not include variables such as expansion of positive clones, Southern screening and Karyotyping. If the PI requests another set of 96-well plates for genotyping, MSCL will charge *as per quoted*.
8. MSCL only guarantees successful expansion of positive clones within 3 months after the master plates were frozen down. The lab that does genotyping should be ready for genotyping as soon as the electroporation is done and request expanding positive clones as soon as the positive clones are identified. Delays in genotyping can cause the failure of expanding the potential positive clones.
9. **For non-UC investigators, a 44.4% NUD will be added too all prices quoted.**

The investigator requesting Electroporation understands and agrees to the terms and conditions outlined above.

Signature:

Date:

(The request from will not be accepted without the investigators signature.

DNA Purification for Electroporation Instructions: (CeCl₂ banding is also acceptable.)

1. Purify the DNA targeting vector using [Qiagen EndoFree Plasmid Maxi](#) kit or a CsCl gradient.
2. Linearize the purified DNA construct using a unique restriction site present only in the vector or in the plasmid polylinker. Do not digest the DNA such that the targeting vector itself is disrupted. It is not necessary to remove vector sequences for ES cell targeting.
3. Run a small sample of the digest on a gel to verify that digestion is complete. Incomplete digestion will result in reduced electroporation efficiency (Please submit the minigel picture with your construct).

DNA preparation for Electroporation

1. EtOH precipitate the linearized construct overnight
2. Pelletize DNA in centrifuge – high speed, 5 minutes
3. Wash DNA with 70% EtOH
4. Pelletize DNA in centrifuge – high speed, 5 minutes
5. Wash DNA with 100% EtOH
6. Pelletize DNA in centrifuge – high speed, 5 minutes
7. Air-dry pellet
8. Resuspend pellet with 150ul Sterile PBS
9. Please compare linearized DNA to plasmid by running an aliquot of purified linearized DNA with a ladder as well as original circularized vector for control. Please send gel picture along with aliquot of linearized DNA with concentration listed.

Optimal DNA Concentration (200-250ng/ul)

4. If the construct is made by off campus PI, please send the construct on ice by FedEx overnight service to the following address and send the tracking number to Carole by email (cgkurahara@ucdavis.edu).

Carole Kurahara
ES Cell Laboratory (MSCL)
Mouse Biology Program
University of California
2795 2nd St, Suite 400
Davis, California 95618, USA

Please send your request form to Sasha Wirth or Carole Kurahara (Fax:530- 757-3284, Email: sswirth@ucdavis.edu or cgkurahara@ucdavis.edu).

Thank you