THE UNIVERSITY OF IOWA

Gene Targeting Request Form

Principal Investigator: _____ Department: _____

Address: _____ E-mail _____

Telephone: _____ FAX: _____

MFK Number _____

Contact Person: _____ E-mail: _____

Telephone: _____ FAX _____

DNA Construct Name: _____ DNA Concentration (μg/μl): _____

Construct Size: _____ Total Amount (μl) _____

Length of 5' Arm: _____ Length of 3' Arm: _____

Positive Selectable Maker: neo ☐ puro ☐ hygro ☐ HPRT ☐ Other _____

Negative Selectable Maker: tk ☐ dta ☐ None ☐ Other _____

Map of gene, vector and targeted gene provided: Yes ☐ No ☐

Plasmid linearized: Yes ☐ No ☐

Southern blot or other screening method provided: Yes ☐ No ☐

Map must be provided before the work can start.
DNA must be supplied in linear form.
Autoradiogram or PCR must be provided for inspection.

Other special considerations: _____

_____

_____

Recombinant DNA registration: Yes ☐ Approval Date _____ Number _____

No ☐ Pending ☐

ACURF Approval: Yes ☐ Approval Date _____ Number _____

No ☐ Pending ☐

GTCF Staff Only	Received by: _____	Date: _____	Project code: _____

DNA: Yes ☐ No ☐ Map: Yes ☐ No ☐ Screening Strategy Yes ☐ No ☐

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Things we need before we can get the project started:

- An account number.
- A map of the targeting strategy including the endogenous gene locus, the targeting vector showing the selectable makers, arm lengths and locations, and the deletion region, and the targeted locus.
- A screening strategy, including the diagnostic enzymes and probes for Southern blot analyses, PCR primer sequences and locations, and the size of the amplified region for PCR screening (Southern blot is still needed for final confirmation).
- We would like to inspect an autoradiograph or a PCR gel.
- Linearized targeting vector, about 100 μg in no more than 200 μl. DNA does not need to be further purified after digestion with restriction enzyme(s).
- A recombination DNA registration form is needed for our file (contact Carol Showalter at HPO, 5-9553, for information). It is a good idea to start ACURF (contact Tina Neill-Hudson, 5-7985, for information), although it is not needed at this stage. We have protocol to cover the making of KO mice.

What will happen after we receive your DNA?

- We will assign a project code for you. Each code is targeting construct specific, and repeated targeting of the same gene with different construct will result in different code. All ES clones and all mice generated will bear this code name. All communication needs to refer this code.
- We will electroporate R1 cells, or other cells you prefer, with your targeting construct, usually within two weeks, and you will be notified if longer waiting is foreseen. We will select drug-resistant cells.
- Drug resistant colonies will be picked. We will pick 500 colonies (5 X 96) if clones can be screen in 96-well format, and 200 colonies (2 X 96) will be picked if large quantity of DNA (half of a 6-well) is desired.
- One portion of the colonies will be frozen at -80°C, and the other portion will be used for DNA preparation (by investigator).

What will happen after positive clones have been found?

- Putative positive clones will be expanded from -80°C freezer to 100-mm plate. One sixth of the cells will be sent back to investigator for confirmation, two thirds of the cells will be frozen in four aliquots in liquid nitrogen, and the remaining one sixth will be used for karyotyping. Not all positive clones will be karyotyped.
- Upon the confirmation of the positive clones, one vial will be expanded from liquid nitrogen and will be ready for microinjection (40 embryos each ES clone) and uterine transfer to generate chimeras.
- Chimeras will be bred for germline transmission.

How long does it take to get a line of targeted mice?

- It can take up to 18 mo even if everything goes as planed, not including making the construct, although there were instances that we got it done within 12 mo.
- Keep in mind that the gestation period for mouse is three wks (21 days) and male mouse will need 8 to 10 wks (56 to 70 days) before sexually mature and is ready for breeding.

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