PCR design for RIKEN ENU-based gene-driven mutagenesis Ver. 9 April 5, 2010

About the target gene:
We will make the “gene name”, “gene location” and "progress" open to public in our web site, http://www.brc.riken.go.jp/lab/mutants/genedriven_name.htm, when we update the site. The identified base substitutions will be open to public in an appropriate timing as described in the Application Form. The RIKEN ENU-based gene-driven mutagenesis system in the mouse is still under development and does not guarantee to provide mutants of the target gene. I appreciate your opinions and suggestions.

Please make sure your target gene(s) is **not on X chromosome**. Our archive is constructed from G1 male sperms so that they do not carry any G0 X chromosome which carries ENU-induced mutations.

PCR condition
Template DNA: genomic DNA from C57BL/6J, equal to or less than **10 ng** in a 10 μl reaction.
Polymerase: we use TaKaRa EX Taq Hot Start Version.
Thermal cycler: We conduct PCR in 96-well plates by using Biometra T1 thermocycler or Biometra T-Gradient.

The size of your PCR amplicons: The appropriate size of the PCR product is about 300 bp. The genetic background of G1 samples is DBA/2JXC57BL/6JF1 or C3H/HeJ/C57BL/6JF1. ENU was administered to C57BL/6J paternal G0 males so that the ENU induced mutations located on C57BL/6J chromosomes. Design the PCR primers of the target sequence(s) and confirm the specific PCR amplification by using C57BL/6J genomic DNA.

Standard PCR reaction
One reaction in 10ul per well of 96-well plate:

	Final conc.	stock solution	Amount
Buffer with 20mM MgCls	1X	10XBuffer	1.0 μl
$MgCl_2$ (optional)	---	---	-- μl
dNTP	200 μM	2.5 mM dNTP	0.8 μl
primer F	0.2 μM	5 μM PrimerF	0.4 μl
primer R	0.2 μM	5 μM PrimerR	0.4 μl
Taq(TakaraEx Hot Start Version)	0.25U	5U/μl	0.05 μl
Template DNA	0.01 μg	0.01 μg/μl	1.0 μl
distilled water	to 10 μl		6.35 μl

Example of PCR cycles
step1, 94°C, 4 min.
step2, 94 °C, 30 sec.
step3, XX °C, 1 min. (annealing temp)
step4, 72°C, 1 min.
step5, Go to step 2, 29times (30 cycles)
step6, 72°C, 4 min.
step7, End
Then, electrophoresis 2μl to confirm a single clear band with no background noise.

Please send us your primers (more than 200 μl of 100 μM) and the photocopy of your PCR

amplicon(s) in an electrophoresis gel with the exact condition for the PCR. Please fulfill the "PCRSheet.xls" for the necessary information about your gene(s) and PCR condition(s) and send it to us as an electric file so that we can copy and paste the information to our database. We will check the PCR by ourselves and will compare the data with yours to see the reproducibility of the PCR.

Please feel free to ask any questions. Suggestions are also welcome and appreciated.

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