Genotyping protocol

General information:

Strain name	B6N;129-Kap<tm1Msgr>/Cnbc

Primers:

Name	Sequence	Primer type
LOX1	CTA GTG GGC TGG TGA GAT AGC TTA G	
MANO 4	CCT GAA GCA CCT AAG CCC AAT AC	
A1	TCC TGA CCA TTG TGT CTG GGT TAC	
F7	GGA ACT TCG CTA GAC TAG TAC GCG TG	

In case more than two primers are introduced, please indicate how they should be combined:

	Forward primer	Reverse primer
LoxP	LOX1	MANO 4
mutant	A1	F7

Reaction mix:

ddH_2O	18,5	µl
PCR Buffer 15mM $MgCl_2$	2,5	µl
dNTPs 2,5mM	2,5	µl
Primer 100µM	0,125	µl
Primer 100µM	0,125	µl
Taq Polymerase 5U/µl	0,250	µl
DNA	1	µl
Final volume	25	µl

PCR program LoxP:

99	°C	5	min	
95	°C	30	sec	X30
55	°C	60	sec	
72	°C	60	sec	
72	°C	10	min	

PCR program Mutant:

99	°C	10	min	
95	°C	30	sec	X30
55	°C	60	sec	
72	°C	165	sec	
72	°C	10	min	

Expected fragment size:

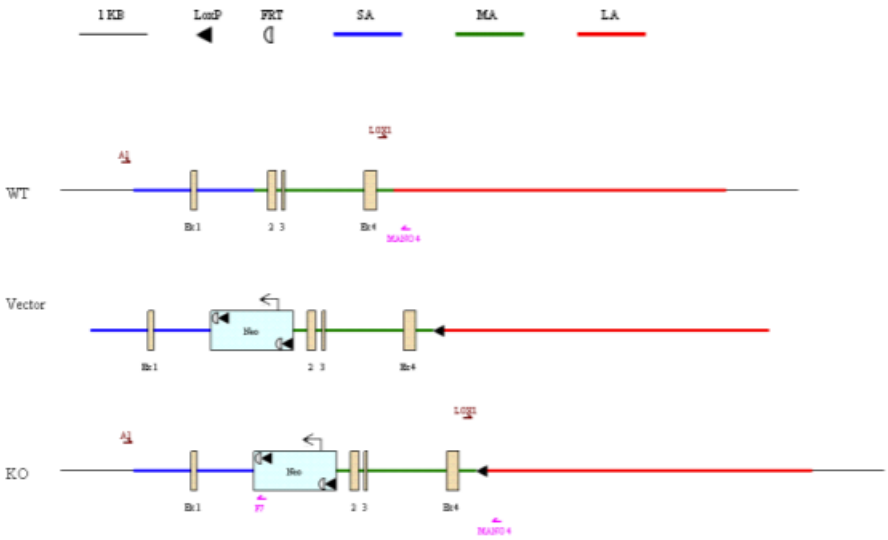
LoxP wt	259	bp
LoxP mut	73	bp
Mutant A1/F7	2.66	Kb

Comments/Additonal information:

inGenious Targeting Laboratory
www.genetargeting.com
laronov@genetargeting.com
phone: (631) 468 – 8530 ext. 103

1. Schematic and Information

Targeted iTL BA1 (C57BL/6N x 129/SvEv) hybrid embryonic stem cells were microinjected into C57BL/6 blastocysts. Resulting chimeras with a high percentage agouti coat color were mated to wild-type C57BL/6N mice to generate F1 heterozygous offspring. Tail DNA was analyzed as described below from pups with agouti or black coat color.



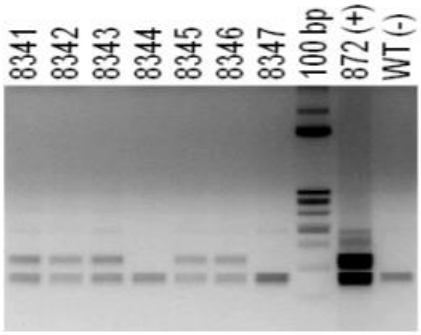
Primers for PCR Screening

```
LOX1:    5′- CTA GTG GGC TGG TGA GAT AGC TTA G -3′
MANO 4:  5′- CCT GAA GCA CCT AAG CCC AAT AC -3′
A1:      5′- TCC TGA CCA TTG TGT CTG GGT TAC -3′
F7:      5′- GGA ACT TCG CTA GAC TAG TAC GCG TG -3′
```

laronov@genetargeting.com
phone: (631) 468 – 8530 ext. 103

2. Screening for Distal LoxP Site

A PCR was performed to detect presence of the distal LoxP site using the LOX1 and MANO 4 primers. This reaction amplifies a wild type product 259 bp in size. The presence of a second PCR product 73 bp greater than the wild type product indicates a positive LoxP PCR.

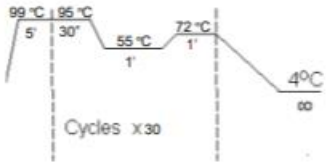


LOX1/MANO 4 (259 bp/332 bp)

PCR Parameters for LOX1/MANO 4:

The Exo- PCR System (Roche catalog# 04738420001)

1.5 µL DNA
200 µM dNTP
20% GC Rich Solution
1 µM Primer
2.5 µL PCR Buffer with 15mM $MgCl_2$
Add ddH_20 to final volume 25µL

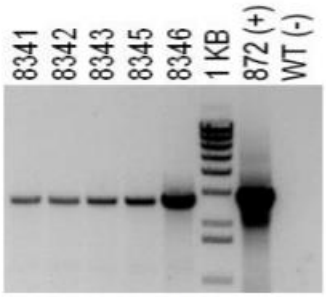


After a 5 minute hot start at 99°, 0.125µL of Taq polymerase was added to each PCR sample followed by a layer of 2 drops mineral oil. The PCR product was run on a 2% gel with a 100 bp ladder as reference. The expanded ES clone, which was used as a positive control, is denoted by a (+) in the gel photograph above.

inGenious Targeting Laboratory
www.genetargeting.com
laronov@genetargeting.com
phone: (631) 468 – 8530 ext. 103

3. Confirmation of Short Homology Arm Integration

Tail DNA samples from mice were amplified with primers A1 and F7. F7 is located inside the Neo cassette and A1 is located upstream of the short homology arm, outside the region used to create the targeting construct. A1/F7 amplifies a fragment of 2.66 kb in length.

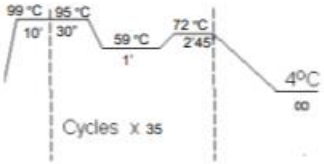


A1/F7 (2.66 kb)

PCR Parameters for A1/F7:

Expand High Fidelity PCR System (Roche catalog # 04 738 276 001)

1.5 μL DNA
200 μM dNTP
4% DMSO
1 μM Primer
2.5 μL PCR Buffer with 15mM $MgCl_2$
Add ddH_20 to final volume 25 μL



After a 10 minute hot start at 99°, 0.125 μL of Taq polymerase was added to each PCR sample followed by a layer of 2 drops mineral oil. The PCR product was run on a 0.8% gel with a 1 KB ladder as reference. The expanded ES clone, which was used as a positive control, is denoted by a (+) in the gel photograph above.