EMMA ID: *04608*
Gene: *Pknox*
Common name: *EUCJ0079_D10*
Allele: *Pknox1* $^{Gt(EUCJ0079d10)Hmgu}$

Genotyping Information

Genotyping by end-point PCR based on gel is composed of a genespecific short range PCR using primers on wild type allele and a mutant allele-specific short range PCR. The combined results show the genotype of the mice. For example: mutant positive, wild type positive = Heterozygous.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wildtype	EUCJ79_D10-F-5'arm	EUCJ79_D10-R-3'arm	427
Mutant	EUCJ79_D10-F-5'arm	Sanger5'	338

Primer sequences

Primer Name	Sequence 5' --> 3'
EUCJ79_D10-F-5'arm	ACATAGTCACTCCTAGACTGC
EUCJ79_D10-R-3'arm	CTGTCTCCAAGAGTACACACC
Sanger5'	GTCCTCCGATTGACTGAGTCGC

PCR setup (Qiagen, Hot Start Plus)

Component	Volume (µl) 1x	Final conc.
DNA (~ 50-100 ng)	2	
Q-Solution (5x)	2,5	0,5
PCR-Buffer (10x)	2,5	1
DNTP mix (10 mM)	0,5	0,2
$MgCl_2$ (25 mM)	1,5	1,5
Primer 1 (10 pmol/µl)	1	0,4
Primer 2 (10 pmol/µl)	1	0,4
Taq Polymerase (5 U/µl)	0,3	0,06
H_2O*	13,7	
Final volume	25	

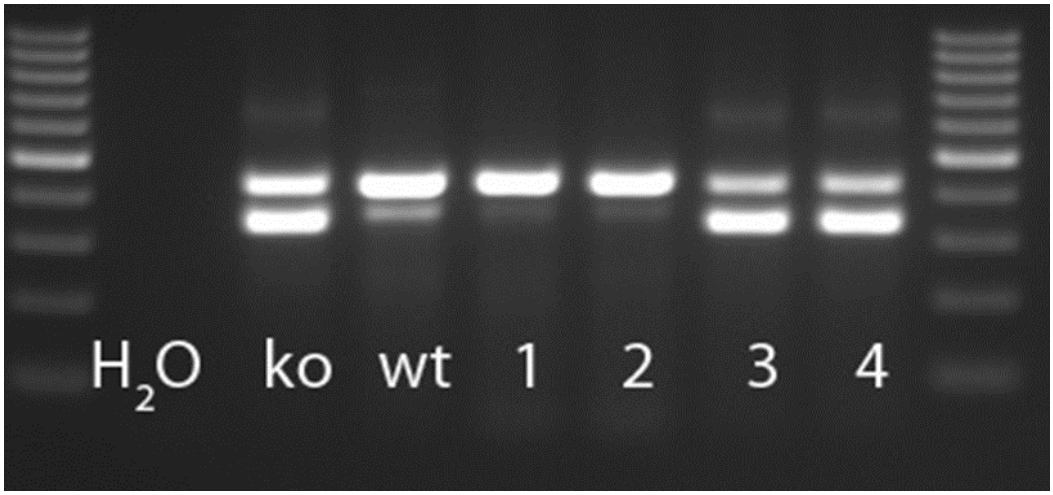
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 55°C 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	12°C	hold	1

These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.

Gel Image



Separated by gel electrophoresis on a 2% agarose gel.

Gene Report

Pknox1 MGI:1201409 ENSMUSG00000006705 OTTMUSG00000040106

Program: KOMP Pipeline Progress: ES Cells - Targeting Confirmed Publicly Reported: Public

Duplicate Project

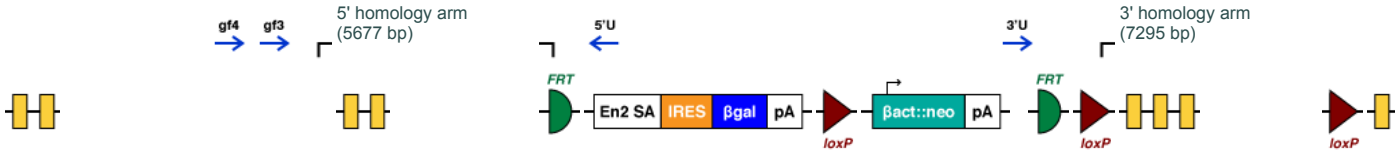
Reset gene to redesign

Pipeline Stage:	Pre-pipeline	Designs	Vectors	ES Cells	Mice
				ES Cells - Targeting Confirmed	

Recovery
Design Tools
ES Cell Clones

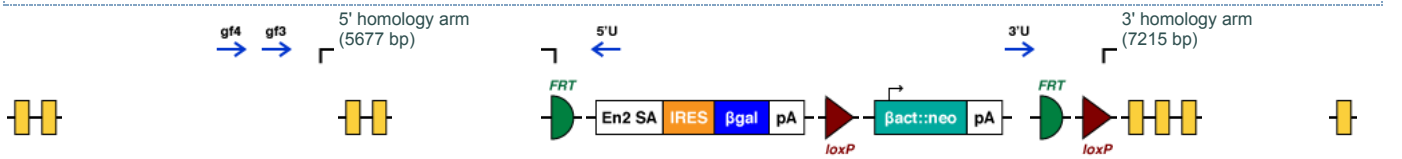
Order Gene	Targeting Vector	# Colonies Screened	# Knockout First Clones	# Targeted Non-conditional Clones
Pknox1	PRPGS00139_C_B12	33	13 GenBank File	10 GenBank File

ES Cell Clones With Conditional Potential



ENSMUSE00000315965 ENSMUSE00000315946 | ENSMUSE00000315919 ENSMUSE00000315882 | Promotor-Driven Cassette (L1L2_Bact_P) | ENSMUSE00000315854 ENSMUSE00000315822 ENSMUSE00000315806 | ENS

ES Cell Clones Without Conditional Potential



ENSMUSE00000315965 ENSMUSE00000315946 | ENSMUSE00000315919 ENSMUSE00000315882 | Promotor-Driven Cassette (L1L2_Bact_P) | ENSMUSE00000315854 ENSMUSE00000315822 ENSMUSE00000315806 | ENSMUS

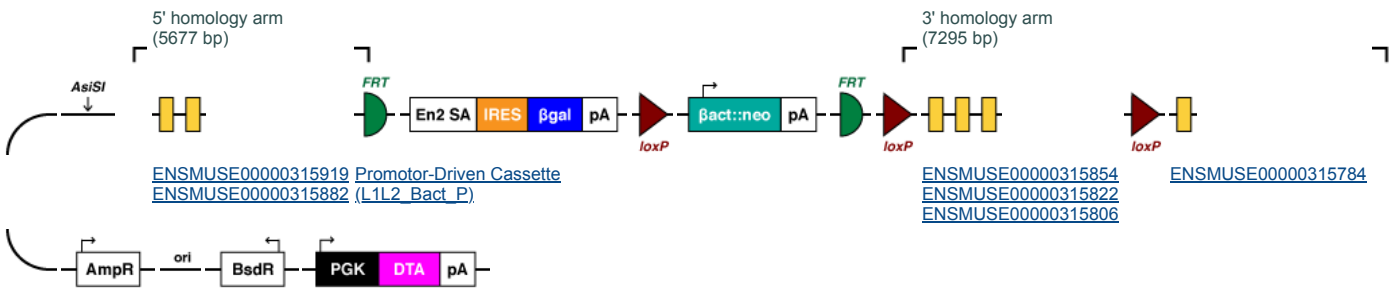
Note: Mutations of type "Targeted Non-conditional" are correctly targeted clones that have lost the 3' loxP site. These mutations cannot be converted into conditional alleles.

LR PCR Genotyping Primers

5' Gene Specific (GF3)	GCACGACGTCTGCCAGCTTCGATGTGGAC
5' Gene Specific (GF4)	GCACTGTGACACTGTGAGCACAGGTATG
5' Universal (LAR3)	CACAACGGGTTCTTCTGTTAGTCC
3' Universal (RAF5)	CACACCTCCCCCTGAACCTGAAAC
3' Universal (PNF)	ATCCGGGGGTACCGCGTCGAG
3' Universal (R2R)	TCTATAGTCGCAGTAGGCGG
3' Gene Specific (GR3)	GTGTGGTGATGGTCACAACGGCACCTGCAG
3' Gene Specific (GR4)	GTGATGGTCACAACGGCACCTGCAGCAGAG

Targeting Vector

Order Gene	Design ID	Vector Type	After Flp/Cre	Targeting Vector	Vector Strain	Floxed Exon	Cassette	Backbone	Genbank File
Pknox1	210458 (139_B12)	Knockout First	Frameshift	PRPGS00139_C_B12	C57Bl/6J	ENSMUSE00000137435-ENSMUSE00000137433	L1L2_Bact_P	R3R4_pBR_DTA+_Bsd_amp	view



ENSMUSE00000315919 ENSMUSE00000315882 | Promotor-Driven Cassette (L1L2_Bact_P) | ENSMUSE00000315854 ENSMUSE00000315822 ENSMUSE00000315806 | ENSMUSE00000315784

DTA Containing Plasmid Backbone (R3R4_pBR_DTA+_Bsd_amp)

Intermediate Vector

Order	Gene	Design ID	Vector Type	Intermediate Vector	Vector Strain	Floxed Exon	Genbank File
	Pknox1	210458 (139_B12)	Knockout First	PCS00139_B_B12	C57Bl/6J	ENSMUSE00000137435-ENSMUSE00000137433	view

Toolbox

Short Range Primer Tool

Useful Information

- **Links:**
  - KOMP project
  - KOMP repository
  - WTSI Mouse Resources Portal