EMMA ID: *04087*
Gene: *Nfya*
Common name: *EUCJ0004_F10*
Allele: *Nfya* $^{Gt(EUCJ0004f10)Hmgu}$

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	E4F10-F	E4F10-R	520
Mutant	E4F10-F	Sanger5'seq	450

Primer sequences

Primer Name	Sequence 5' --> 3'
E4F10-F	GAGCATTTCTGGGCATTTCAGCTT
E4F10-R	CTGTATTTTACCGCTTCCACTAGC
Sanger5'seq	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	

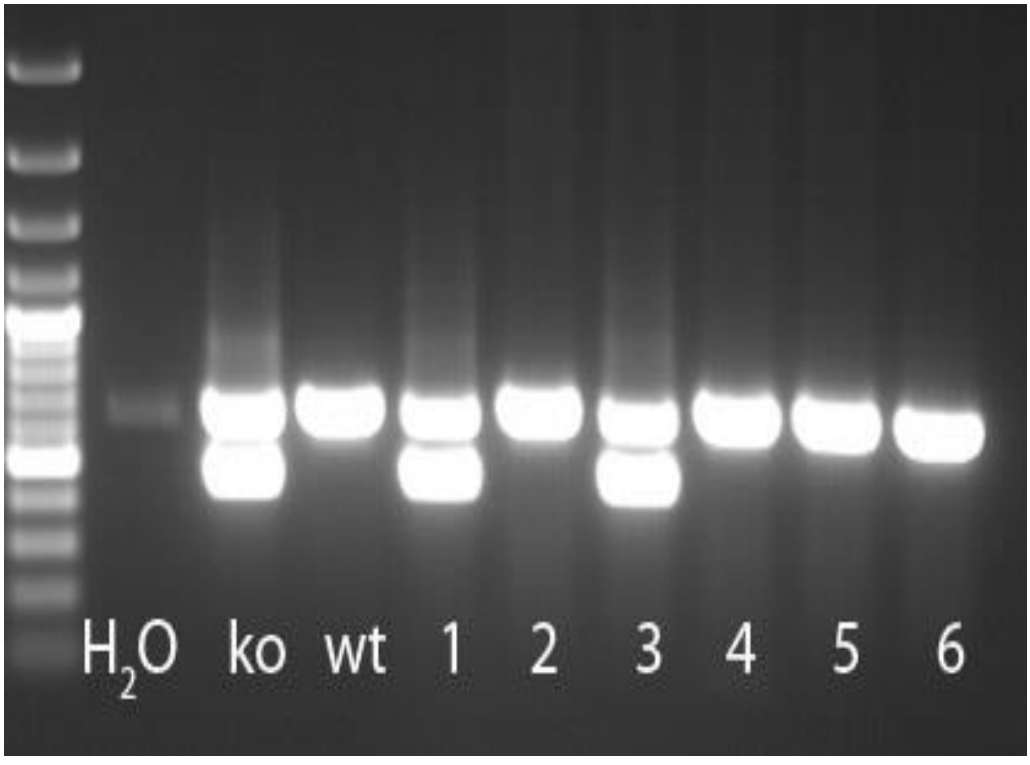
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 65°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

Nfya MGI:97316 ENSMUSG00000023994 OTTMUSG00000035112

Duplicate Project

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

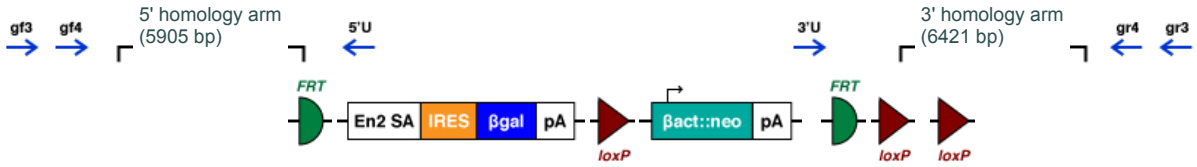
Reset gene to redesign

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

Recovery
Design Tools
ES Cell Clones

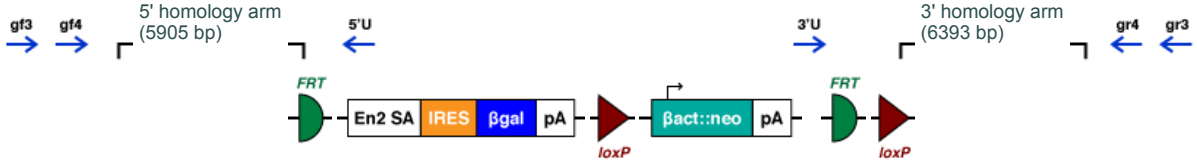
Order	Gene	Targeting Vector	# Colonies Screened	# Knockout First Clones	# Targeted Non-conditional Clones	Ship Date
	Nfya	PRPGS00153_B_C04	33	6 GenBank File	13 GenBank File	29-JUN-09 (hzm) 29-JUN-09 (csd)

ES Cell Clones With Conditional Potential



Promotor-Driven Cassette (L1L2_Bact_P)

ES Cell Clones Without Conditional Potential



Promotor-Driven Cassette (L1L2_Bact_P)

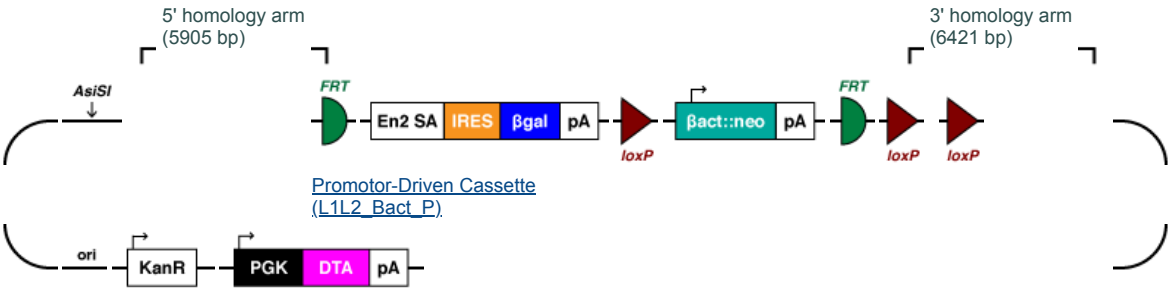
Note: Mutations of type "Targeted Non-conditional" are correctly targeted clones that have lost the 3' loxP site. These mutations cannot be converted into conditional alleles.

LR PCR Genotyping Primers

5' Gene Specific (GF3)	GTGTGTGTATGTATAGTAGGTATCTTAAG
5' Gene Specific (GF4)	CTGTGGAAGGACTTAGACCTAGTCAGAC
5' Universal (LAR3)	CACAACGGGTTCTTCTGTTAGTCC
3' Universal (RAF5)	CACACCTCCCCCTGAACCTGAAAC
3' Universal (PNF)	ATCCGGGGGTACCGCGTCGAG
3' Universal (R2R)	TCTATAGTCGCAGTAGGCGG
3' Gene Specific (GR3)	GCTGAAGCGCTACTGTTAAGTCGGAG
3' Gene Specific (GR4)	GCTGGCTGAAGCGCTACTGTTAAGTCGGAG

Targeting Vector

Order	Gene	Design ID	Vector Type	After Flp/Cre	Targeting Vector	Vector Strain	Floxed Exon	Cassette	Backbone	Genbank File
	Nfya	169999 (153_C04)	Knockout First	Domain Disruption	PRPGS00153_B_C04	C57Bl/6J	ENSMUSE00000137024-ENSMUSE00000137021	L1L2_Bact_P	L3L4_pZero_DTA_kan	view



Promotor-Driven Cassette (L1L2_Bact_P)

DTA Containing Plasmid Backbone (L3L4_pZero_DTA_kan)

Intermediate Vector

Order	Gene	Design ID	Vector Type	Intermediate Vector	Vector Strain	Floxed Exon	Genbank File
	Nfya	169999 (153_C04)	Knockout First	PCS00153_A_C04	C57Bl/6J	ENSMUSE00000137024-ENSMUSE00000137021	view

Toolbox

Short Range Primer Tool

Useful Information

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