

EMMA ID: 04086

Gene: *Pml*

Common name: *EUCJ0019c12*

Allele: *Pml*^{Gt(*EUCJ0019c12*)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	E19C12-F2	E19C12-R	600
Mutant	E19C12-F2	Sanger5'seq	520

Primer sequences

Primer Name	Sequence 5' --> 3'
E19C12-F2	GTAGTTCCTTCCAGGGTGAGTATG
E19C12-R	CTCCCTGAAGCCTTGCCTGAAGCG
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 ₂ O*	13,7	
Final volume	25	

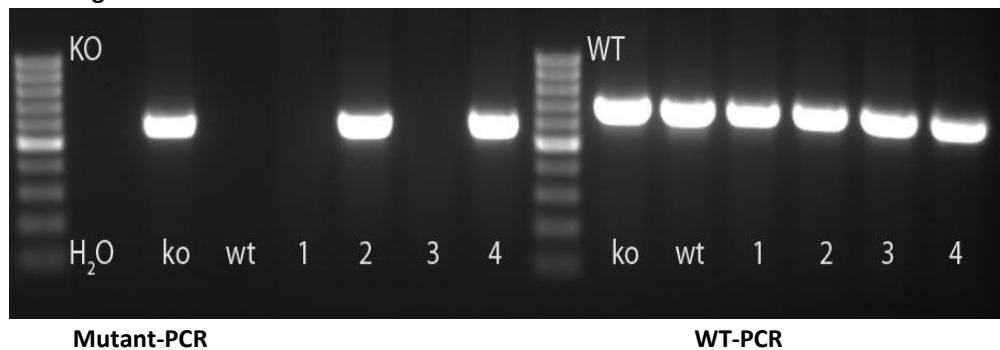
* The amount of H₂O is adjusted with the number of primer.

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cycles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C	30 sec	39
	65°C	45 sec	
	72°C	45 sec	
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.
 ko/wt separate

ConstructQC vector_qc1 esmp_ha
 HTGTDB eucomm_vector esmp_ha

\$Id: header.tt 6045 2011-09-23 14:43:26Z rm7 \$

dp10 htgt1.sanger.ac.uk
 Authenticated username dp10@sanger.ac.uk
 Roles read, edit, eucomm, eucomm_edit

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Gene Report

Pml [MGI:104662](#) [ENSMUSG00000036986](#) [OTTMUSG00000033332](#)

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

[Duplicate Project](#)

Pipeline Pre-pipeline Designs Vectors ES Cells Mice
 Stage:

[Reset gene to redesign](#)

[ES Cells - Targeting Confirmed](#)

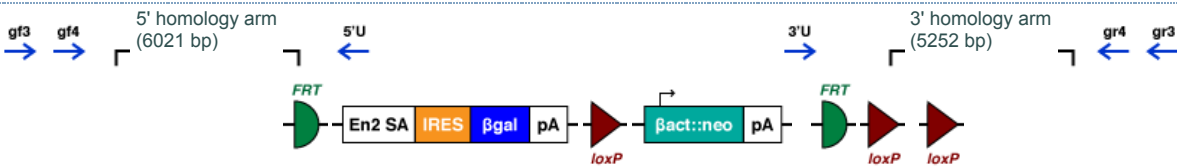
Recovery

Design Tools

ES Cell Clones

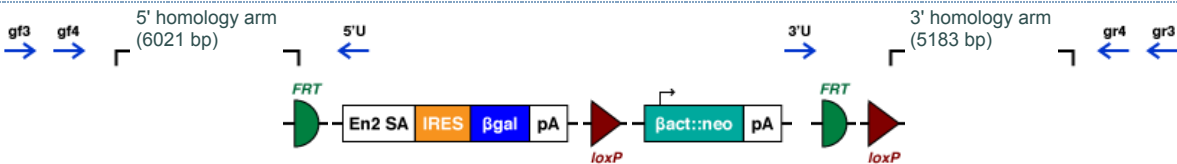
Order	Gene	Targeting Vector	# Colonies Screened	# Knockout First Clones	# Targeted Non-conditional Clones	Ship Date
Pml	PG00193_Z_6_C01	33	15	GenBank File	10	GenBank File
						28-JUN-10 (hzm) 28-JUN-10 (csd)

ES Cell Clones With Conditional Potential



[Promotor-Driven Cassette \(L1L2_Bact_P\)](#)

ES Cell Clones Without Conditional Potential



[Promotor-Driven Cassette \(L1L2_Bact_P\)](#)

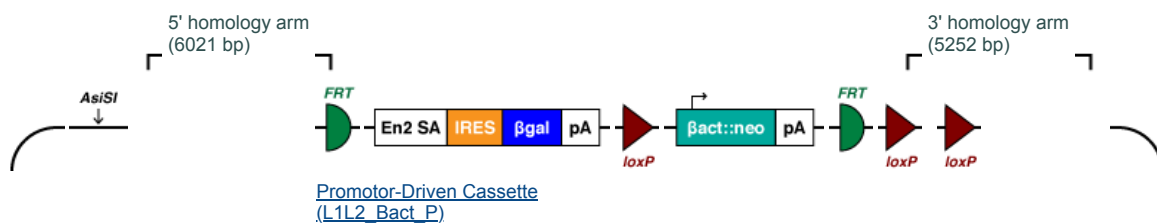
Note: Mutations of type "Targeted Non-conditional" are correctly targeted clones that have lost the 3' loxP site. These mutations cannot be converted into conditional alleles.

LR PCR Genotyping Primers

- 5' Gene Specific (GF3)** CACTCATCAACACCTTCGTTGTGCCTC
- 5' Gene Specific (GF4)** CACTGCTGGTGTTAACACATTCTACTGCG
- 5' Universal (LAR3)** CACAACGGGTTCTTCTGTTAGTCC
- 3' Universal (RAF5)** CACACCTCCCCCTGAACCTGAAAC
- 3' Universal (PNF)** ATCCGGGGGTACCGCGTCGAG
- 3' Universal (R2R)** TCTATAGTCGCAGTAGGCGG
- 3' Gene Specific (GR3)** GCAGGATCCTTCGGCTAGCTGCAG
- 3' Gene Specific (GR4)** CTGCTGAGCAGCTAAGTCCAATACCGCTGC

Targeting Vector

Order	Gene	Design ID	Vector Type	After Flp/Cre	Targeting Vector	Vector Strain	Floxed Exon	Cassette	Backbone	Genbank File
Pml	245128	(193_F07)	Knockout	Frameshift	PG00193_Z_6_C01	C57Bl/6J	ENSMUSE00000254921	L1L2_Bact_P	L3L4_pD223_DTA_T_spec	view



[Promotor-Driven Cassette \(L1L2_Bact_P\)](#)

[DTA Containing Plasmid Backbone](#)

[\(L3L4_pD223_DTA_T_spec\)](#)

Intermediate Vector

Order	Gene	Design ID	Vector Type	Intermediate Vector	Vector Strain	Floxed Exon	Genbank File
	Pml	245128 (193_F07)	Knockout First	PCS00193_A_F07	C57Bl/6J	ENSMUSE00000254921	view

Toolbox

[Short Range Primer Tool](#)

Useful Information

- **Links:**
- - [EUCOMM](#)
 - [EUCOMM repository](#)
 - [WTSI Mouse Resources Portal](#)

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