

**EMMA ID:** 04054

**Gene:** *Golm1*

**Common name:** *EUC0047A08*

**Allele:** *Golm1*<sup>Gt(*EUC0047a08*)Hmgu</sup>

## 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	EUC47A08-F	EUC47A08-R	680
Mutant	EUC47A08-F	Sanger5'seq	440

### Primer sequences

Primer Name	Sequence 5' --> 3'
EUC47A08-F	GCTCTGTTCTGGGAGCAGTCGCTG
EUC47A08-R	CCTTAAGAGACAGAACTGGTGCCT
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 <sub>2</sub> (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 <sub>2</sub> O*	13,7	
Final volume	25	

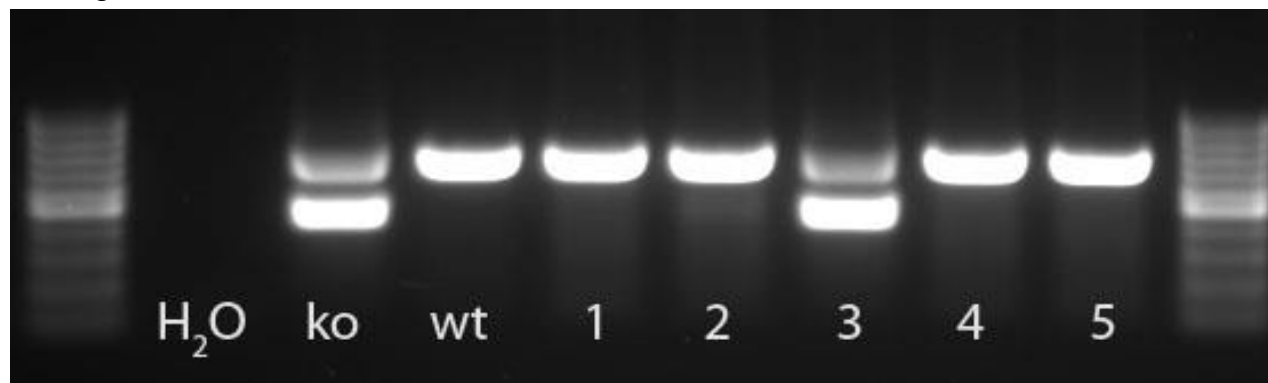
\* The amount of H<sub>2</sub>O is adjusted with the number of primer.

### 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.

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

[Home](#) > [Report](#) > Gene Report

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## Gene Report

### Go1m1 MGI:1917329 ENSMUSG0000021556

E	K	M	N	ET	R	ETS	ETCs	S	TPP	MB	Gene	Chromosome	Status	Design	Cassette	Backbone
	K										Go1m1		ES Cells - Targeting Confirmed	182775 (199_E08)	L1L2_Bact_P (promoter)	R3R4_pBR_DTA+
							ETCs				Go1m1		ES Cells - No QC Positives	182775 (199_E08)	pL1L2_GT1_LF2A_nEGFP0_T2A_CreERT_puro (promoterless)	L3L4_pD223_DT/
	K										Go1m1		Vector Complete	182775 (199_E08)	pL1L2_GT1_LF2A_nEGFP0_T2A_CreERT_puro (promoterless)	L3L4_pD223_DT/
	K										Go1m1		Vector - Initial Attempt Unsuccessful	182775 (134_C04)	L1L2_Bact_P (promoter)	L3L4_pZero_DTA
	K										Go1m1		Vector Construction in Progress	182775 (199_E08)	pL1L2GT1_LF2A_nEGFPOT2A_CreERT2_pAroxbActNeopAro (promoterless)	L3L4_pD223_DT/
	E										Go1m1		ES cells - Conditional Gene Trap	Not Applicable	FlpROSBetaGeo	

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

[Duplicate Project](#)

Pipeline Stage: Pre-pipeline Designs Vectors ES Cells Mice

[Reset gene to redesign](#)

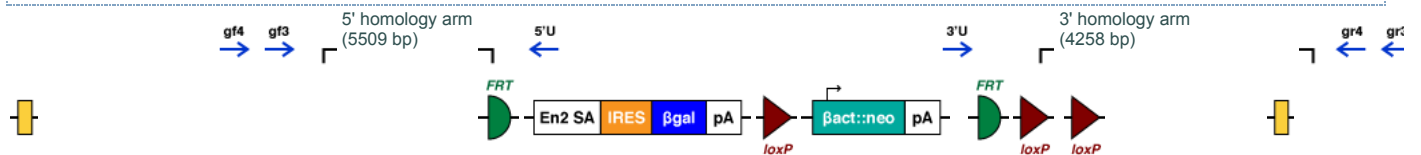
[ES Cells - Targeting Confirmed](#)

### Recovery Design Tools ES Cell Clones

Order Gene Targeting Vector # Colonies Screened # Knockout First Clones

[Go1m1 PG00199\\_Z\\_4\\_E08](#) 33 13 [GenBank File](#)

#### ES Cell Clones With Conditional Potential



[ENSMUSE00000909061](#)

[Promotor-Driven Cassette \(L1L2\\_Bact\\_P\)](#)

[ENSMUSE00000570759](#)

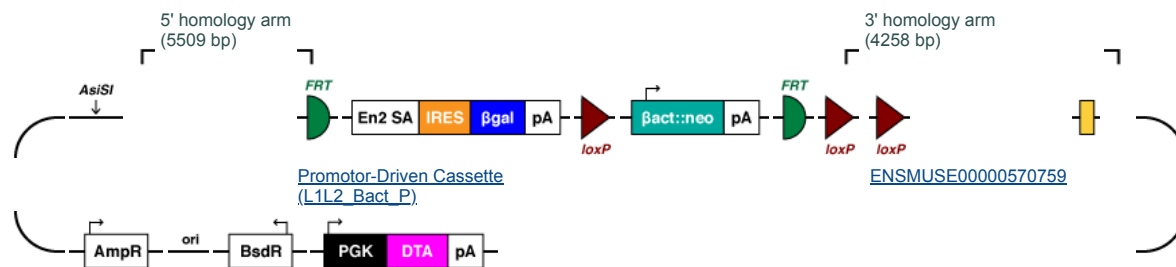
[EN:](#)  
[EN:](#)

### LR PCR Genotyping Primers

- 5' Gene Specific (GF3) CAGCTCAGGAGGCAGGCACTGAATGCACG
- 5' Gene Specific (GF4) CTTGGCTGCTCACAGGCAGCTCACGAGGC
- 5' Universal (LAR3) CACAACGGGTTCTTCTGTTAGTCC
- 3' Universal (RAF5) CACACCTCCCCCTGAACCTGAAAC
- 3' Universal (PNF) ATCCGGGGGTACCGCGTCGAG
- 3' Universal (R2R) TCTATAGTCGAGTAGGCGG
- 3' Gene Specific (GR3) CGAATGGAAGGCTGTGTGGTGGATG
- 3' Gene Specific (GR4) CATGTAGTGATGTTGATAAGATGGCTC


### Targeting Vector

Order	Gene	Design ID	Vector Type	After Flp/Cre	Targeting Vector	Vector Strain	Floxed Exon	Cassette	Backbone	Genbank File
	Go1m1	182775 (199_E08)	Knockout First	Frameshift	PG00199_Z_4_E08	C57Bl/6J	ENSMUSE00000118948	L1L2_Bact_P	R3R4_pBR_DTA+ Bsd_amp	<a href="#">view</a>



[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
	<a href="#">Golm1</a>	<a href="#">182775</a>	(199_E08)	Knockout First	<a href="#">PCTS00199_A_E08</a>	C57Bl/6J	<a href="#">ENSMUSE00000118948</a> <a href="#">view</a>

## Toolbox

[Short Range Primer Tool](#)

## Useful Information

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