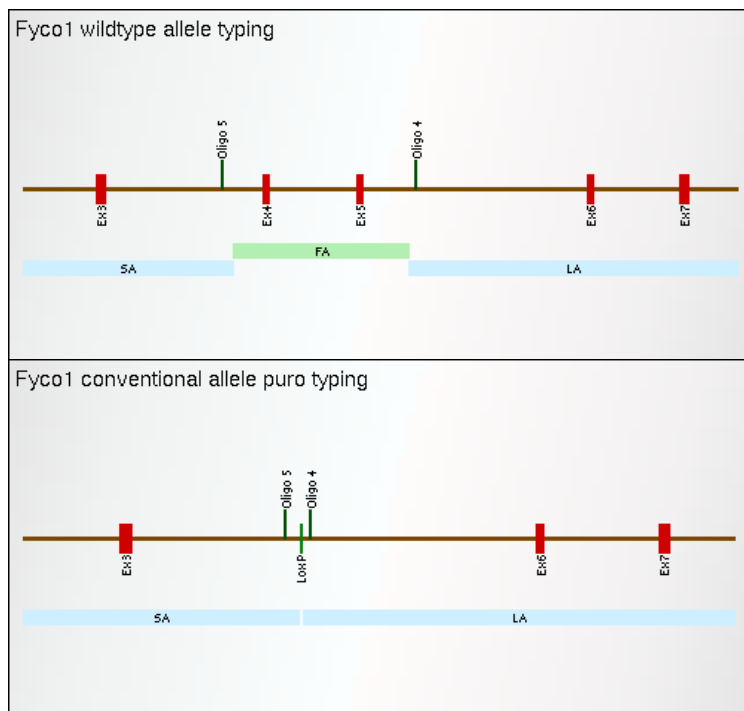


EMMA ID: 05509

Gene: *Fyco1*

Common name: *Csemp1 KO conv*

Allele Information



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)
control band	1260_1	1260_2	585
Mutant	1646_31	1645_28new	370

Primer sequences

Primer Name	Sequence 5' --> 3'
1646_31	GATTCCTAGATTCCATGTTGC
1645_28new	GGAAGGGAGTTATTGGTGATGG
1260_1	GAGACTCTGGCTACTCATCC
1260_2	CCTTCAGCAAGAGCTGGGGAC

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	

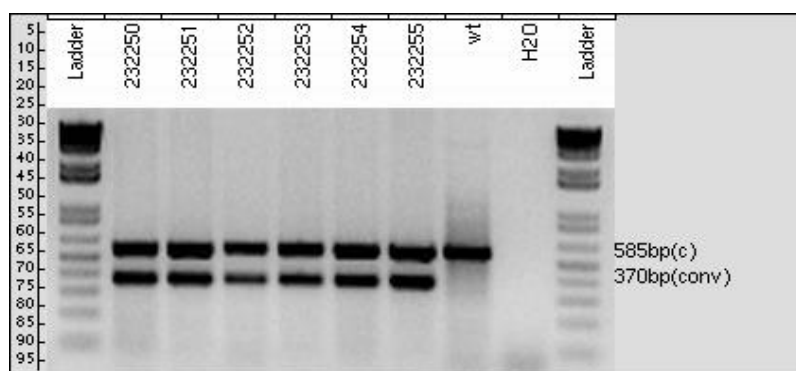
* The amount of H₂O is adjusted with the number of primer.

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cycles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 60°C 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

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

Gel Image



The fragment amplified with oligos 4
 (1645_28new: GGAAGGGAGTTATTGGTGATGG)
 + 5 (1646_31: GATTCCTAGATTCCATGTTGC)
 detects heterozygous/homozygous
 conventional alleles. The wildtype fragment will
 not be detected applying the conditions
 described above.

Separated by gel electrophoresis on a 2% agarose gel.