

EMMA ID: *EM:14919*

Gene: *Nbea*

Common name: *Nbea_K1*

Allele: *Nbea^{em1Kili}*

Allele Information

This knock-in mouse line carries a single amino acid replacement in exon 22 (amino acid 1085) of its endogenous neurobeachin gene (*Nbea*). The L1085A (TTG to GCG) mutation was introduced into exon 22 by homology-directed repair. An additional silent mutation (GCC to GCG) was introduced nearby to prevent the binding and re-cutting of the sequence by gRNA after homology-directed repair.

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. In addition to the expected product, the mutant assay may also amplify the endogenous wild type sequence, which will appear as a larger band on an agarose gel. The presence of this extra band will depend on the size of the original deletion.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wild type	Ex22_NBEA_L1085A	NBEA_L1085A_R	305
Mutant	Ex22_NBEA_L1085A	Ex22_NBEA_L1085A	301

Primer sequences

Primer Name	Sequence 5' --> 3'
Ex22_NBEA_L1085A_WT_F	CTGACCCTTGTTGGAAGTGGAGTCCTT
Ex22_NBEA_L1085A_KI_F	CGCTTGTGGAAGTGGAGTCCGC
NBEA_L1085A_R	GCAAGCAACCCAAGATCATCACT

PCR setup (AllTaq)

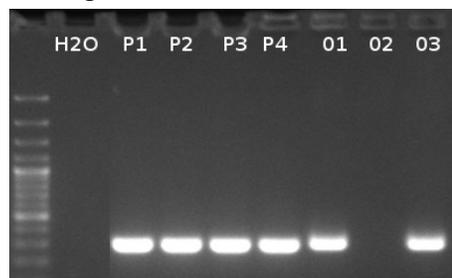
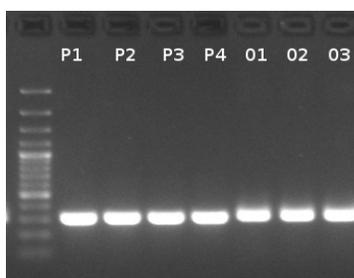
Component	Volume (µl) 1x	Final conc.	
DNA (~ 50-100 ng)		2	
Q-Solution (5x)		2,5	0,5
PCR-Buffer (5x)		5	1
DNTP mix (10 mM)		0,5	0,2
MgCl ₂ (25 mM)		1,5	1,5
Primer 1 (10 pmol/µl)		1	0,4
Primer 2 (10 pmol/µl)		1	0,4
Primer 3 (10 pmol/µl)		1	0,4
Taq Polymerase (5 U/µl)		0,5	2,5 U/rxn
H ₂ O*		10	
Final volume		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
	62°C	45 sec	
	72°C	45 sec	
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

Touch-Down cycling protocol: first 10 cycles anneal at 65°C, decreasing 1°C per cycle, next 30 cycles anneal at 55°C. These PCR conditions have been optimized for our methods and preparation

Gel Image

mutant PCR

wt PCR

P1, P2, P3, P4	het
1, 3	het
2	wt

Separated by gel electrophoresis on a 2% agarose gel.