

Genotyping protocol

EMMA ID: EM:09786

Gene symbol: Shb

Strain designation: STOCK Shb^{tm1a(EUCOMM)Hmgu/Oulu}

Primers:

Primer name	Primer sequence (5'- 3')
ShbF2	GGC ACT TCT GGA GAG CAG TAG A
ShbR3	ACA GTG TGA GGC GAT CTC TGG
Construct primer	TCG TGG TAT CGT TAT GCG CC

Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	ShbF2	ShbR3	741
Mutant	ShbF2	Construct primer	436

Reaction mix:

Component	Volume (µl)
Genomic DNA (~50-100 ng)	2.0
Primer 1 (10 µM)	1.0
Primer 2 (10 µM)	1.0
10x Buffer (15 mM MgCl ₂)	3.0
MgCl ₂ (25 mM)	1.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.05
H ₂ O	21.7
Total	30.0

PCR program:

Time	Temperature (+ °C)	Cycles
5 min	95	
1 min	94	30
1 min	60	
1 min	72	
7 min	72	
∞	4	