

## Genotyping protocol

EMMA ID: EM:08222

Gene symbol: Plod3

Strain designation: B6Ola;129-Plod3<sup>tm2Rmyl</sup>/Oulu

### Primers:

Primer name	Primer sequence (5'- 3')
PLOD3-173	AGG TCC TGA CAA TCC CAG CTT
PLOD3-87	GGA GAT ACC TGA GTG GGA GTT
Neo3	CAT ACA CTT CAT TCT CAG TAT TG

### Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	PLOD3-173	PLOD3-87	365
Mutant	PLOD3-173	Neo3	250

### Reaction mix:

Component	Volume (µl)
Genomic DNA (~50-100 ng)	1.0
Primer 1 (10 µM)	1.5
Primer 2 (10 µM)	1.5
10x Buffer (15 mM MgCl <sub>2</sub> )	3.0
MgCl <sub>2</sub> (25 mM)	1.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.1
H <sub>2</sub> O	21.65
<b>Total</b>	<b>30.0</b>

### PCR program:

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