SMYD3-KO – Genotyping protocol

Primers

GEOF3731	5' – GTG CCA ATG AAT CGT CTG ACC – 3'
GEOR3887	5' – ATA CAG CGC GTC GTG ATT AGC – 3'

PCR Reaction Mix

200ng	Genomic mouse tail DNA
5µl	10x Taq Buffer
4µl	$MgCl_2$ (25mM)
1µl	dNTPs (10mM)
1µl	Taq Pol
10µl	Betaine 5M
2µl	Forw Primer (10mM stock) GeoF3731
2µl	Rev Primer (10mM stock) GeoR3887
	H_2O
50µl	Total Volume

PCR conditions

1.	94.0	5 min
2.	94.0	30 sec
3.	58.0	30 sec
4.	72.0	30 sec, back to step 2 for 35 cycles
5.	72.0	5 min (extension)
		END

PCR respective product band sizes are:

~190bp SMYD3-KO

SMYD3-KO – QPCR Genotyping protocol

Primers

GEOF3731	5’ – GTG CCA ATG AAT CGT CTG ACC – 3’
GEOR3887	5’ – ATA CAG CGC GTC GTG ATT AGC – 3’
GAPDH-F	5' – CCA ATG TGT CCG TCG TGG ATC T – 3'
GAPDH-R	5' – TTG AAG TCG CAG GAG ACA ACC – 3'

Q-PCR Reaction Mix

4,5 µl	Genomic mouse tail DNA (5 ng/µl)
2 µl	10 x Buffer
2,8 µl	MgCl2 25 mM
0,5 µl	dNTPs 10 mM
0,4 µl	Taq Pol
0,5 µl	Syber green 1/8 (when stock diluted in 1.5mM Tris gives 0,4+/-0,01 at 495nm)
4 µl	Betaine 5M
0,75 µl	Forw Primer (6 µM)
0,75 µl	Rev Primer (6 µM)
3,8 µl	H_2O
20 µl	Total Volume

PCR conditions

95.0	5 min	
95.0	15 sec	
58.0	10 sec	
72.0	15 sec	40 cycles
Melting curve		

Always Run samples together: homozygous + heterozygous (+ wt optional)

Homozygous PCR products are present in twice the amount of heterozygous products in quantitative QPCR
FOR NORMALISATION, GAPDH MUST ALWAYS BE RUN IN THE SAME PLATE (GAPdH product 200bp)