

PCR Data Sheet: Gm3s <tm1Rlp> WT Line 3259

Oligonucleotide PCR Primers

Primer Name	Primer Sequence (5' → 3')
Gm3sW1	AGC TCA GAG CTA TGC TCA GGA
Gm3sW2	TAC CAC ATC GAA CTG GTT GAG

Optimal PCR Conditions per reaction for the Gm3s<tm1Rlp> WT Genotyping Assay

Reagent	Initial Concentration	Volume per reaction (μL)	Final Concentration
Distilled H ₂ O, DNase, RNase free (Gibco #10977-015)	N/A	Q.S. to 25	N/A
PCR Buffer w/ MgCl ₂ (Qiagen #203205)	10X, 15mM	2.5	10X, 1.5mM
MgCl ₂ (Qiagen #203205)	25mM	1.0	2.5mM (final)
Q-solution (Qiagen #203205)	N/A	N/A	N/A
Deoxynucleotide Mix (dNTPs) (Sigma #D-7295)	10mM	0.5	0.2mM
Gm3sW1 primer (MWG Biotech)	25μM	0.5	0.5μM
Gm3sW2 primer (MWG Biotech)	25μM		0.5μM
HotStarTaq™ DNA Polymerase (Qiagen #203205)	5U/μL	0.25	0.05U/μL
Genomic DNA template	10ng/μL	5.0	2ng/μL
Total reaction volume	N/A	25	N/A

Optimal Thermalcycling Template: Taconic Standard Template 3

Step	Temperature (°C)	Time (min)	Number of Cycles
Hot Start	95	15:00	1
Denature	94	0:45	35
Anneal	60	1:00	
Extension	72	1:00	
Final Extension	72	5:00	1

Reporting nomenclature

Assay	M	T	W
Gm3s<tm1Rlp> KO	~250 bp	~250 bp	No Product
Gm3s<tm1Rlp> WT	No Product	~350 bp	~350 bp

Electrophoresis Conditions:

Run on a 2% TAE/agarose gel at 160V until the loading dye of the DNA sizing ladder migrates a distance of 2.5cm. Load 5μl of PCR product on gel. Score with A.511.