

Primer	primer_name	primer_seq	length	Reference
1	Obif-51	GATTCTGGGAGCCTGGCCGGATAAC	25	Depositor' s primer
2	OBIF-WT31	GCTTCCCCTTGGCCCTCACTGGTGGCA	27	Depositor' s primer
3	MC-3	CAATATCACGGGTAGCCAACGC	22	Depositor' s primer

Reaction Components

Enzyme

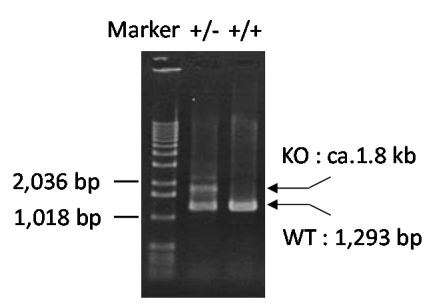
Reagent	Conc	Reaction 1	2	3	4	(μ L)
H2O		1.8				
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/ μ l	1.0				
primer 2	10 pmol/ μ l	0.5				
primer 3	10 pmol/ μ l	1.5				
DNA		1.0				
Taq		0.2				
total		20				

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	180*	*Repeat for 30 cycles

Marker

Gel



primer_set	product_size	product (allele type)
primer 1 primer 2	1,293 bp	WT
primer 1 primer 3	ca 1.8 kb	KO

Comment

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.