

Primer	primer_name	primer_seq	length		Reference
1	MC4R-U22008	GTGTGGGCGTTATGAATTGAC	21		Depositor's primer
2	MC4R-L22308	CAGCAAGGATAATAGGCAAGC	21		Depositor's primer
3	MC4R-U20424	CTCCCAGCAGTACAGCGAGTC	21		Depositor's primer

Reaction Components

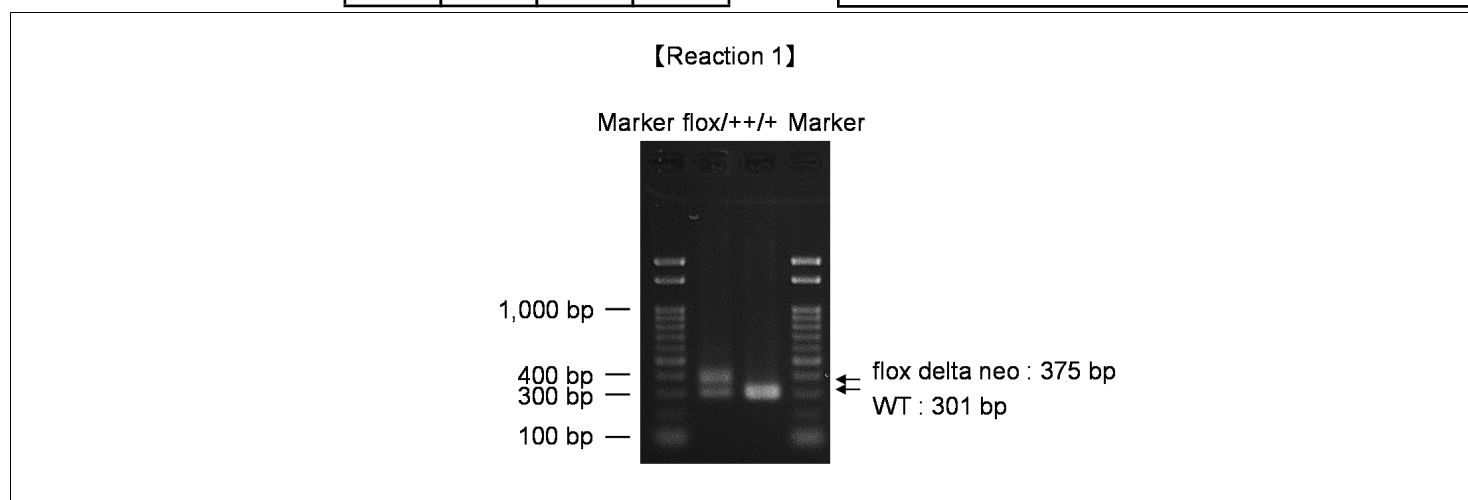
Enzyme

Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8	2.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/µl	1.0	0		
primer 2	10 pmol/µl	1.0	1.0		
primer 3	10 pmol/µl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/µl	0.2	0.2		
total		20	20		

Marker marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker Run2: 2-Log DNA Ladder (NEB, Cat. N3200)
Gel Run1: 2% agarose in 1 X TAE (100 V, 30 min) Run2: 1% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)	
primer 1	primer 2	301 bp	WT
primer 1	primer 2	375 bp	flox delta neo
primer 2	primer 3	1,885 bp	WT
primer 2	primer 3	1,993 bp	flox delta neo
primer 2	primer 3	376 bp	null (After cre mediated excision)
			Reaction 1 +/+ (wild-type): 301 bp flox/+ (heterozygous): 301 bp and 375 bp

Comment Standard PCR

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