

RBRC 10268

Strain name B6;129-Mlc1 <tm1.1kftnk>

Primer	primer_name	primer_seq	length	Reference
1	Mlc-80U	AGGGAATGGTGGTCTGAGTCTGTT	24	Glia, 65(1), 150-168 (2016).
2	Mlc159L	GAGAACACCCATGTCTTGTAGCTG	24	Glia, 65(1), 150-168 (2016).
3	TRE_R	CTCGACCCGGGTACCGAGCTC	21	RIKEN BRC

### Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

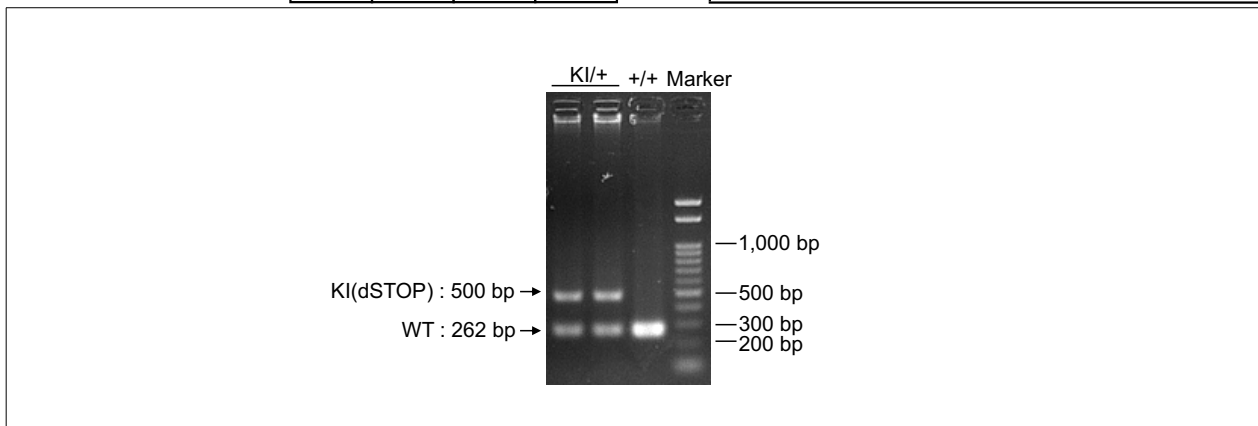
Reagent	Conc	Reaction 1	2	3	4	( $\mu$ L)
H2O		1.8				
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/ $\mu$ l	1.0				
primer 2	10 pmol/ $\mu$ l	1.0				
primer 3	10 pmol/ $\mu$ l	1.0				
DNA		1.0				
Taq	1 U/ $\mu$ l	0.2				
<b>total</b>		20				

### Reaction Conditions

step	temp ( $^{\circ}$ C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	60*	*Repeat for 30 cycles

Marker Gene Ladder 100 (Wako, Cat. 316-06951)

Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)	
primer 1 primer 2	262 bp	WT	KI/+ : 262 bp, 500 bp +/- : 262 bp
primer 1 primer 3	500 bp	KI(dSTOP)	

Comment [Standard PCR](#)

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.  
The extended time was corrected from 300 to 60 on September 03, 2019.