

# PCR protocol

RBRC 09907

Strain name 129.Cg-Braf<tm1Tumg>

Primer	primer_name	primer_seq	length	
1	mBr1-Fw	GTGTTGTTCTGCCCATACTTACTGC	25	Hum. Mol. Genet., 23, 6553-6566 (2014).
2	mBr1-Rv	GTGACTTAATGTACAGCATGGATCA	25	Hum. Mol. Genet., 23, 6553-6566 (2014).

**Reaction Components**

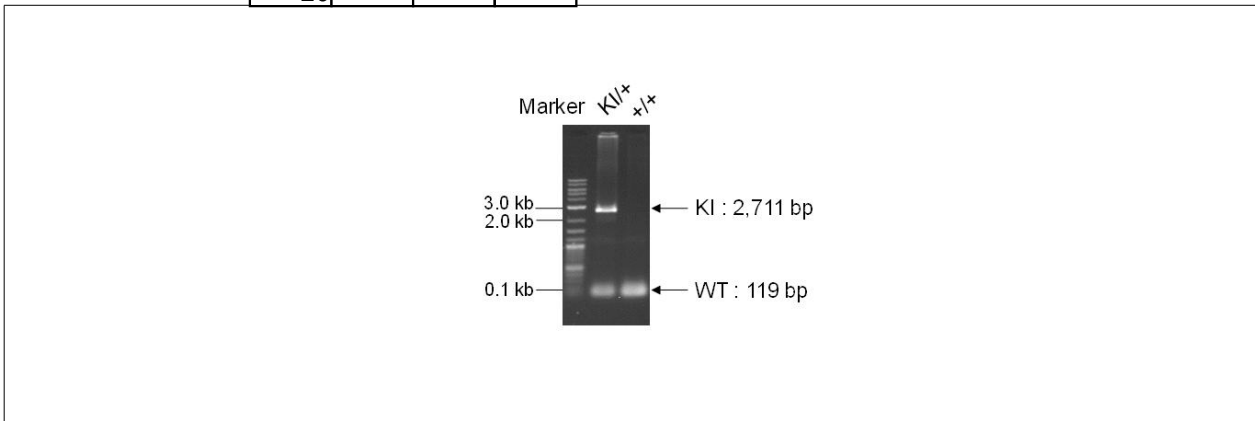
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.8			
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			
DNA		1.0			
Taq	1 U/ $\mu$ l	0.2			
<b>total</b>		<b>20</b>			

**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	240*	*Repeat for 30 cycles

Standard PCR



**Marker**  
2-Log DNA Ladder (NEB, Cat. N3200)

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

PCR products	primer_set	product_size	product
primer 1	primer 2	119 bp	WT
primer 1	primer 2	2,711 bp	KI

**Comment**

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