

RBRC 11138

Strain name B6.Cg-Blnk<tm1Dkit> Ptprc<a>

Primer	primer_name	primer_seq	length	Reference
1	BLNK endo	GTGTTCTCGAAGGGACTATGCAT	24	Depositor's primer
2	BLNK common	ATGCTTGACAGTGTGGGCTTCTGT	24	Proc. Natl. Acad. Sci. USA, 97(6):2755-60 (2020).
3	olMR0214	CCGGAGAACCTGCGTGCAATCC	22	JAX Genotyping Protocol

### Reaction Components

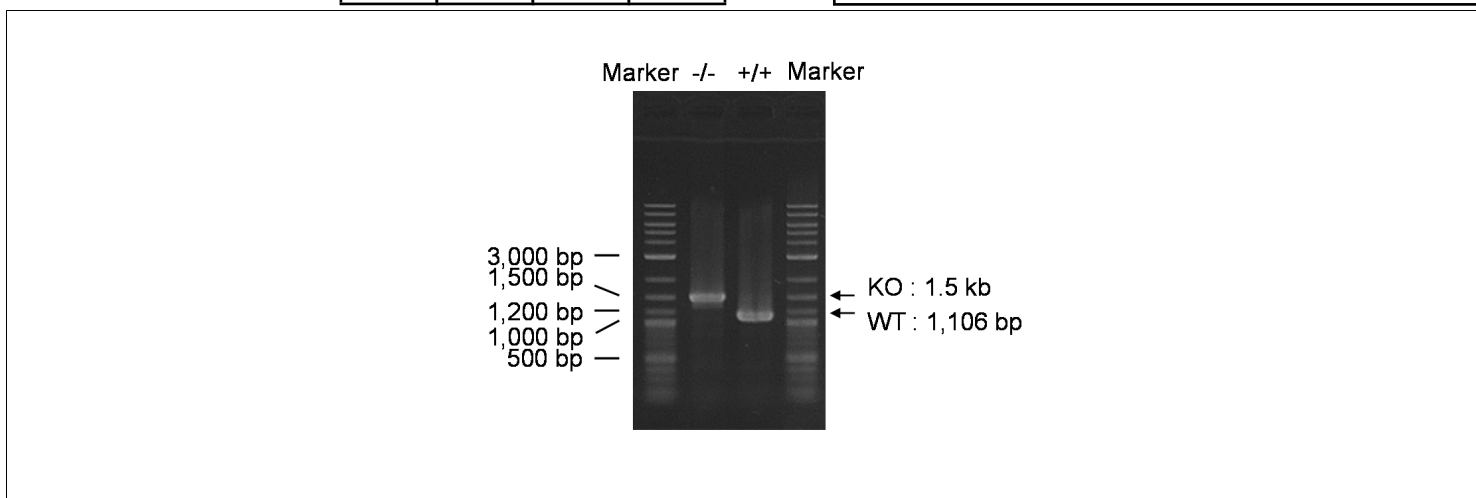
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reagent	Conc	Reaction 1	2	3	4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
<b>total</b>		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 2-Log DNA Ladder (NEB, Cat. N3200)  
Gel 1% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1	primer 2	1,106 bp WT
primer 2	primer 3	1.5 kb KO

Comment Standard PCR

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

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Strain name B6.Cg-Blnk&lt;tm1Dkit&gt; Ptpcr&lt;a&gt;

Primer	primer_name	primer_seq	length	Reference
1	common	CAGGTCCACTGAAACAGAGGATG	23	RIKEN BRC
2	Ptpcr_a	CTGAGCCTGCATCTAAACCTGATC	24	RIKEN BRC
3	Ptpcr_b	CTGAGCCTGTATCTAAACCTGAGT	24	RIKEN BRC

**Reaction Components**

Enzyme

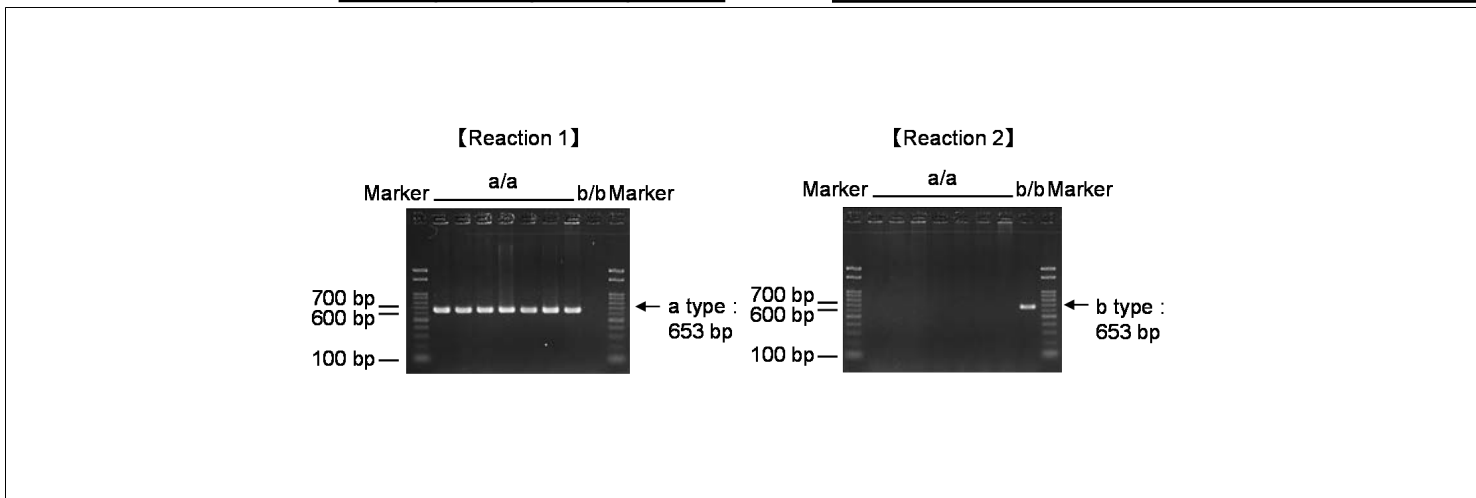
**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	95	900	
2	94	30*	
3	60	90*	
4	72	60*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4
H2O		2.0	2.0		
Master Mix	2 X	5.0	5.0		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
<b>total</b>		10	10		

Marker

Gel



primer_set	product_size	product (allele type)
primer 1 / primer 2	653 bp	a type
primer 1 / primer 3	653 bp	b type

Comment [Allele specific PCR \(ASP\)](#)