

RBRC 02661

Strain name A.AKITA-Ins2<Akita>

Primer	primer_name	primer_seq	length	Reference
1	02661_common	GGAGGTGCCAGGGAGCCAGGAG	23	RIKEN BRC
2	02661_Wild	AGCGTGGCATTGTAGATCAGTGCAG	25	RIKEN BRC
3	02661_Mut	AGCGTGGCATTGTAGATCAGTGCAA	25	RIKEN BRC

**Reaction Components**

Enzyme

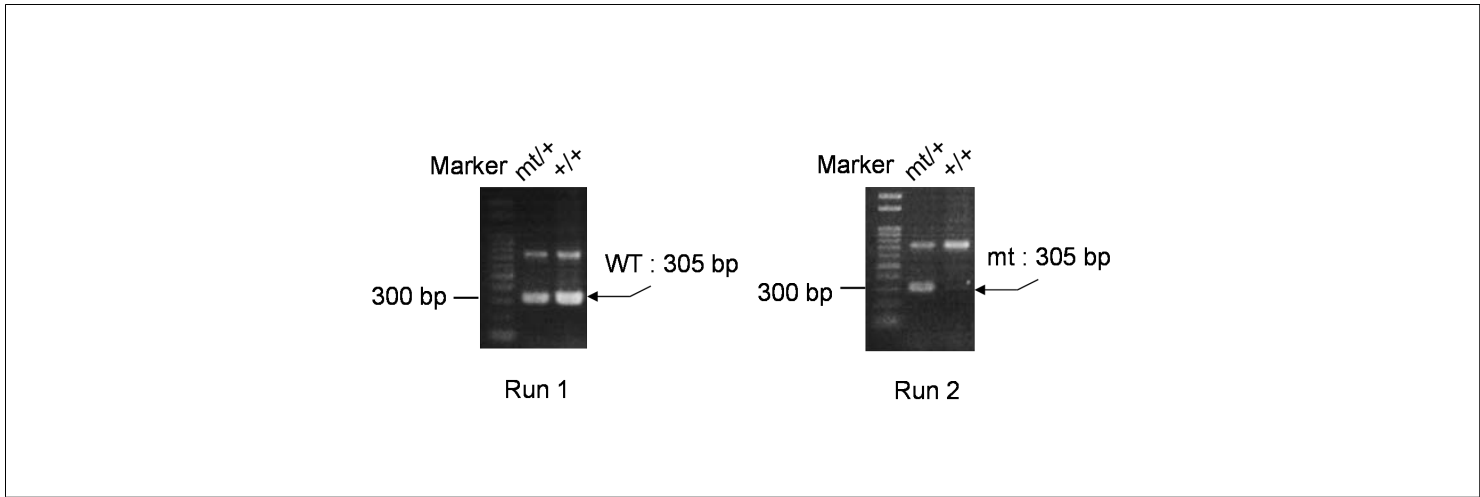
Reagent	Conc	Reaction 1	2	3	4
H2O		2.0	2.0		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Master Mix	2 X	5.0	5.0		
<b>total</b>		10	10		

(μL)

**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	95	900	
2	94	30*	
3	65	90*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

<b>Marker</b>	100 bp ladder (Invitrogen, Cat. 15628-050)
<b>Gel</b>	2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1	primer 2	305 bp WT
primer 1	primer 3	305 bp mutant

Comment Allele specific PCR (ASP)

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**Reaction Components**

Enzyme

**Reaction Conditions**

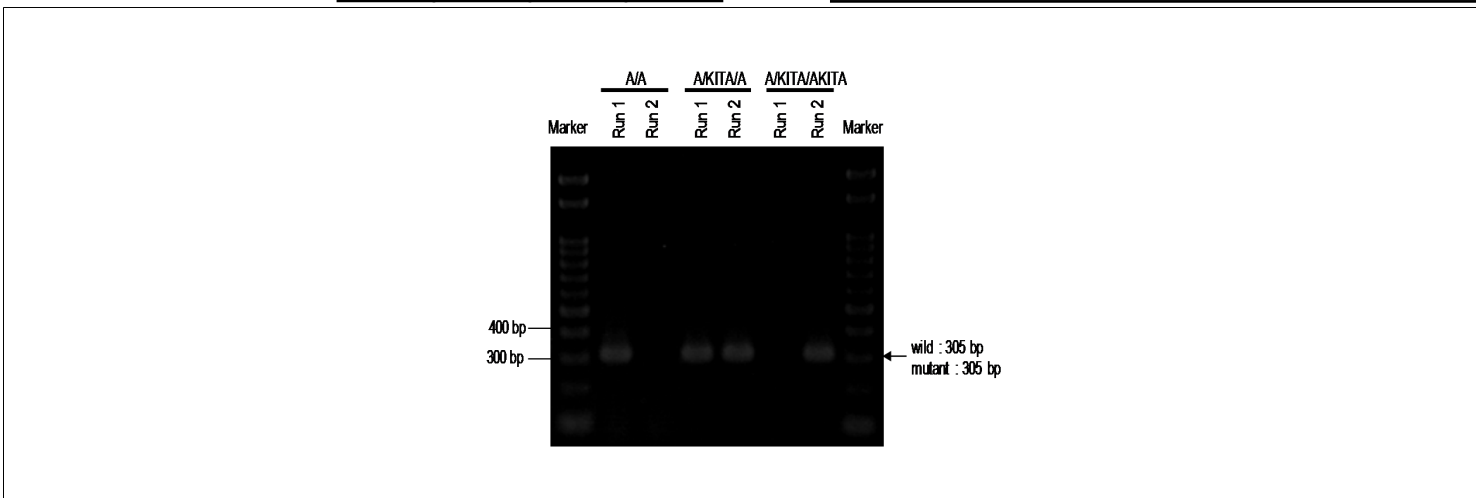
step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	65	30*	
4	72	60*	*Repeat for 35 cycles
5	72	300	

Reagent	Conc	Reaction 1	2	3	4
H2O		17.3	17.3		
PCR buffer	10 X	2.5	2.5		
dNTP	2.5 mM	2.0	2.0		
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		
Taq		0.2	0.2		
<b>total</b>		25	25		

(μL)

**Marker**

**Gel**



primer_set	product_size	product (allele type)	
primer 1	primer 2	305 bp	WT
primer 1	primer 3	305 bp	mutant

**Comment** Allele specific PCR (ASP)