PCR protocol

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RBRC 06298 **Strain name** CAnPt.Cg-Aicda<tm2.1Hon>

Primer	primer_name	primer_seq	length		
1	AID17640F	GCGCTGGGGTCCAGATCGGAT	21		Depositor's primer
2	AID17900R	AGATTGATGTATACTTGTAATT	22		Depositor's primer
3	04892Common	CAGAGCTGAGGGATTAAAGGGCTG	24		RIKEN BRC
4	04892WT	AATGTCCGCTGGGCCAAGGGACGG	24		RIKEN BRC
5	04892MT	AATGTCCGCTGGGCCAAGTCTAGA	24		RIKEN BRC

Reaction Components

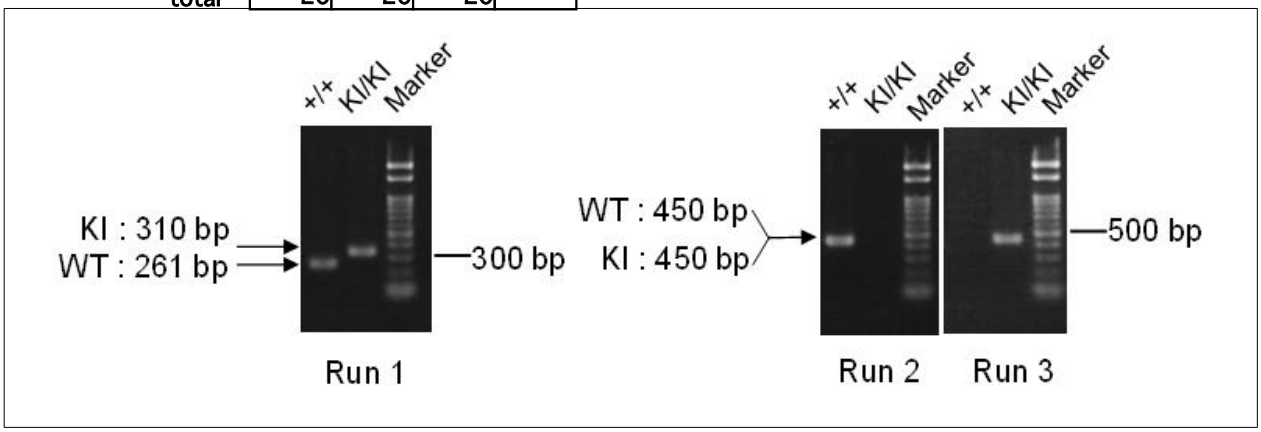
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.8	2.8	2.8	
PCR buffer	2 X	10.0	10.0	10.0	
dNTP	2 mM	4.0	4.0	4.0	
primer 1	10 pmol/μl	1.0	0	0	
primer 2	10 pmol/μl	1.0	0	0	
primer 3	10 pmol/μl	0	1.0	1.0	
primer 4	10 pmol/μl	0	1.0	0	
primer 5	10 pmol/μl	0	0	1.0	
DNA		1.0	1.0	1.0	
Taq	1 U/μl	0.2	0.2	0.2	
	total	20	20	20	

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	55	30	*
4	68	60	* *Repeat for 30 cycles

Allele specific PCR (ASP)



Marker

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

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	261 bp	WT
primer 1	primer 2	310 bp	KI
primer 3	primer 4	450 bp	WT
primer 3	primer 5	450 bp	KI

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