PCR protocol

Sheet ID PS_06360
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RBRC 02660 **Strain name** A.AKITA-(D6Mit286-D6Mit148)

Primer	primer_name	primer_seq	length		Reference
1	D6Mit286-1	GCCTCCACAAGCACCACTAT	20		Depositor's primer
2	D6Mit286-2	TGCTATTACAGTGTCTTTAAAAAAAAA	27		Depositor's primer
3	D6Mit148-1	TTTCTGCAAGTTTATTTTATTTTACCT	27		Depositor's primer
4	D6Mit148-2	TTCCAGAAGATCAGATGGATCA	22		Depositor's primer

Reaction Components

Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

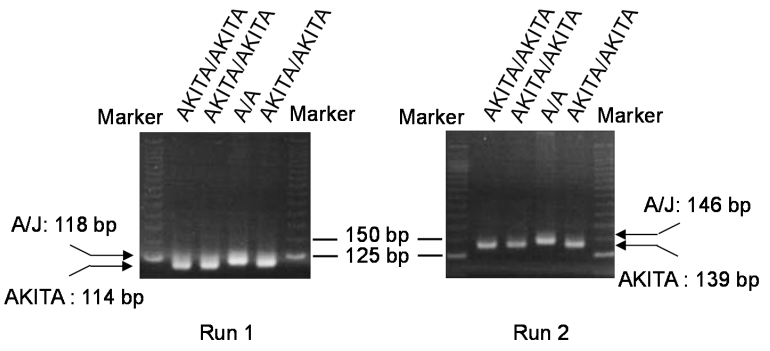
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.0	2.0			(μL)
Master Mix	2 X	5.0	5.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
	total	10	10			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	60	90	*
4	72	60	* *Repeat for 30 cycles
5	60	1800	

Marker 25bp DNA Step Ladder (Wako, Cat. 292-69001)

Gel 4% Nusieve in 0.5 X TBE (100 V, 60 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	114 bp	AKITA
primer 1	primer 2	118 bp	A/J
primer 3	primer 4	139 bp	AKITA
primer 3	primer 4	146 bp	A/J

Comment Separated PCR