

RBRC 11717

Strain name D2.B6-Cpox<Rgsc0129>/Ohnot

Primer	primer_name	primer_seq	length	Reference
1	Wild	CCATGCTTTTTCTCTGCTGCCCGTTCCA	28	RIKEN BRC
2	Cpox_Ex5R	GGGTGTAGGAGTCATCGCAGTG	22	Exp. Anim., doi: 10.1538/expanim.22-0032.(2022).
3	Cpox_Int4F2	AGAAAACAAAGATACTGCTGGGGTG	25	Exp. Anim., doi: 10.1538/expanim.22-0032.(2022).
4	Mut	AACTATAAAGAAGTAGTCGTACACAC	27	RIKEN BRC

**Reaction Components**

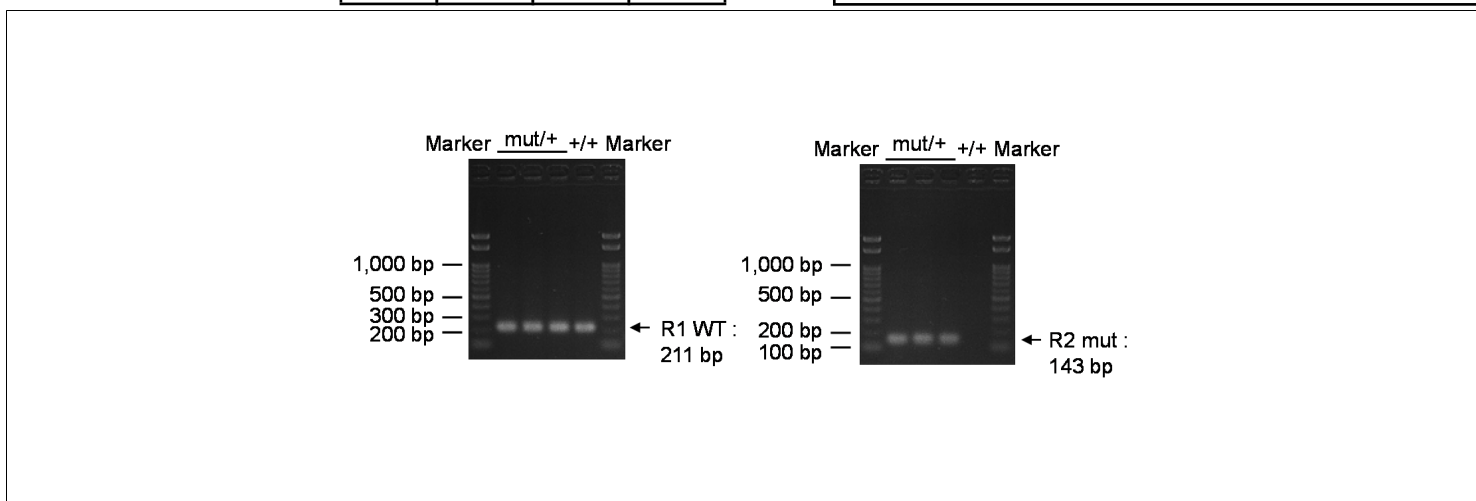
Enzyme: QIAGEN Multiplex PCR Kit (Cat. No. 206143)

**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	(μL)
H2O		2.0	2.0			
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			
Master Mix	2 X	5.0	5.0			
DNA		1.0	1.0			
<b>total</b>		10	10			

Marker: Gene Ladder 100 (Wako, Cat. 316-06951)  
Gel: 2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)	
primer 1	primer 2	211 bp	WT
primer 3	primer 4	143 bp	mutant
Reaction 1			
mut/+ (heterozygous): 211 bp			
+/+ (wild-type): 211 bp			
Reaction 2			
mut/+ (heterozygous): 143 bp			
+/+ (wild-type): none			

Comment: Allele specific PCR (ASP)