

RBRC 02338

Strain name B6;129P2-p130Cas<tm2.1Homy>

Primer	primer_name	primer_seq	length	Reference
1	Cas15	AAAGATTGACTGGGGTCAACTCCACTTTGC	30	Hepatology, 52(3), 1089-1099 (2010).
2	Cas20	CTATCAGGCCCTGAGAGAGACTGTCTTGTGA	30	Hepatology, 52(3), 1089-1099 (2010).
3	3'	TGGAGGTCAGGGTCACCCCTTG	21	RIKEN BRC

Reaction Components

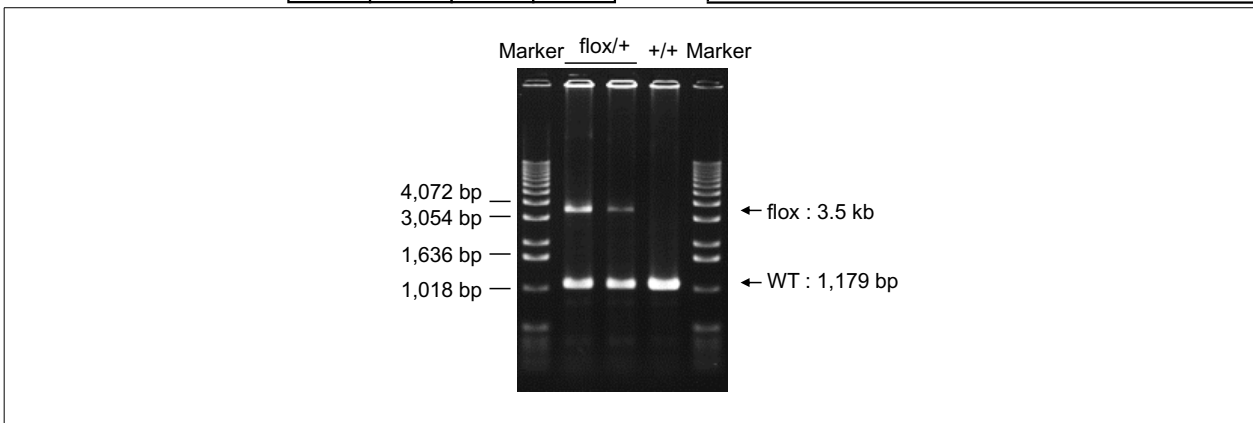
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reagent	Conc	Reaction 1	2	3	4
H ₂ O		0.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/ μ l	2.0			
primer 2	10 pmol/ μ l	0.5			
primer 3	10 pmol/ μ l	1.5			
DNA		1.0			
Taq	1 U/ μ l	0.2			
total		20			

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	300*	*Repeat for 30 cycles

Marker	1 kb DNA ladder (Invitrogen, Cat. 15615-024)
Gel	1% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)	
primer 1	primer 2	1,179 bp	WT
primer 1	primer 3	3.5 kb	flox

+ /+ (wild-type): 1,179 bp
flox /+ (heterozygous): 1,179 bp and 3.5 kb

Comment

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

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Primer	primer_name	primer_seq	length	Reference
1	Cas15	AAAGATTGACTGGGGTCAACTCCACTTTGC	30	Hepatology, 52(3), 1089-1099 (2010).
2	Cas16	CAGAATCTTGAGGCGTTACCAGGCACAAT	30	Am. J. Physiol. Cell Physiol., 304(6):C541-7 (2013).

Reaction Components

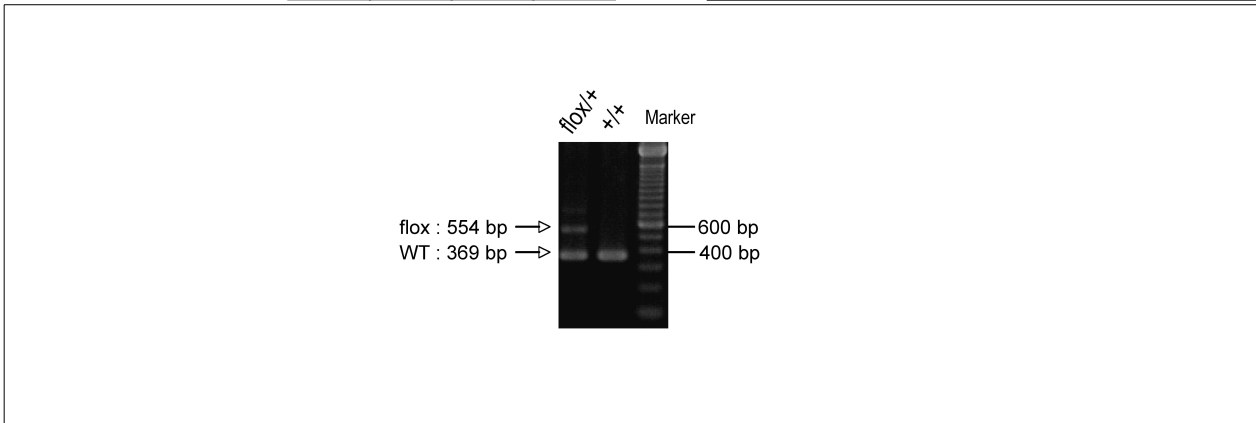
Enzyme Roche Taq (Cat. No. BM1418432)

Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4	(μ L)
H2O		17.3				
PCR buffer	10 X	2.5				
dNTP	2.5 mM	2.0				
primer 1	10 pmol/ μ l	1.0				
primer 2	10 pmol/ μ l	1.0				
DNA		1.0				
Taq		0.2				
total		25				

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



primer_set	product_size	product (allele type)	
primer 1	primer 2	369 bp	WT
primer 1	primer 2	554 bp	flox

Comment [Standard PCR](#)