

RBRC 10054

Strain name C57BL/6N-Aqp4^{tm1Tсна}

Primer	primer_name	primer_seq	length	Reference
1	A4e3F	GAAACTGGGCAAACCACTG	19	Depositor's primer
2	AQP4 in 3R	CAGCTGCTGGTGAAGACCTAG	21	Depositor's primer
3	AQP4 in 2F	CAGGGCTGGCTTTAAGCAC	19	Depositor's primer

Reaction Components

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

Reaction Conditions

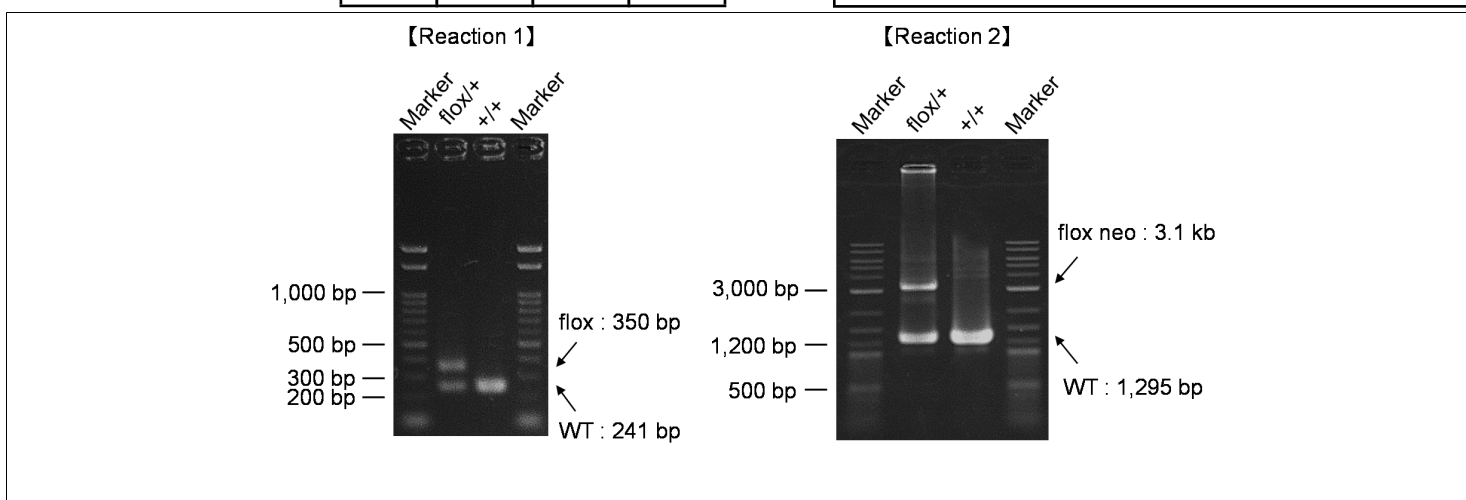
step	temp (°C)	time (sec)	note
1	94	120	[Reaction 1]
2	98	10*	
3	60	30*	
4	68	60*	*Repeat for 30 cycles
1	94	120	[Reaction 2]
2	98	10*	
3	60	30*	
4	68	240*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8	2.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	1.0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
total		20	20		

(μL)

Marker marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker Run2: 2-Log DNA Ladder (NEB, Cat. N3200)

Gel Run1: 2% agarose in 1 X TAE (100 V, 30 min) Run2: 1% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)	
primer 1	primer 2	241 bp	WT
primer 1	primer 2	350 bp	flox
primer 3	primer 2	1,295 bp	WT
primer 3	primer 2	3.1 kb	flox neo
primer 3	primer 2	480 bp	null (After cre mediated excision)

Reaction 1 +/+ : 241 bp
flox/+ : 241 bp, 350 bp

Reaction 2 +/+ : 1,295 bp
flox/+ : 1,295 bp, 3.1 kb

Comment Standard PCR

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