PCR protocol

Sheet ID PS_07210
Release date : 4/20/2017
Print date : 4/20/2017

RBRC 00522 Strain name C57BL/6-Rag2^{tm1Fwa}-IL2rb^{tm1Mak}

Primer	primer_name	primer_seq	length		
1	Neo10	ATCAGAGCAGCCGATTGTCTGTTG	24		Depositor's primer
2	RG2-1 for RAG	TTAATTCAACCAGGCTTCTCACTT	24		Depositor's primer
3		GCCTGCTTATTGTCTCCTGGTATG	24		Depositor's primer
4	IL-2RβF1	TGGCCTTGTCCGAAAGGTCA	20		Depositor's primer
5	PrF4	TGCCTGCTTGCCGAATATCATGG	23		Depositor's primer
6	IL-2RβHomo	CCAGGTCTCTCACTACATTG	20		Depositor's primer

Reaction Components

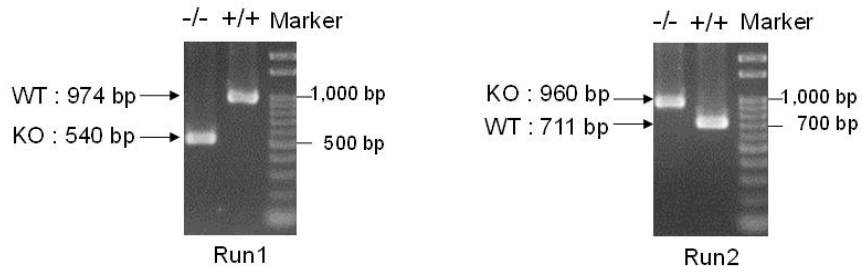
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8	1.8		
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	0		
primer 3	10 pmol/μl	1.0	0		
primer 4	10 pmol/μl	0	1.0		
primer 5	10 pmol/μl	0	1.0		
primer 6	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	90	* *Repeat for 30 cycles

Separated PCR



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 3	540 bp	KO (Rag2)
primer 2	primer 3	974 bp	WT (Rag2)
primer 4	primer 5	960 bp	KO (IL2rb)
primer 4	primer 6	711 bp	WT (IL2rb)

Comment

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

PCR protocol

Sheet ID PS_01923
Release date : 1/30/2017
Print date : 4/20/2017

RBRC 00522 Strain name C57BL/6-Rag2^{tm1Fwa}-IL2rb^{tm1Mak}

Primer	primer_name	primer_seq	length		
1	Neo10	ATCAGAGCAGCCGATTGTCTGTTG	24		Depositor's primer
2	RG2-1 for RAG	TTAATTCAACCAGGCTTCTCACTT	24		Depositor's primer
3	RG2-3 for RAG	GCCTGCTTATTGTCTCCTCCTGGTATG	27		Depositor's primer
4	IL-2RβF1	TGGCCTTGTCCGAAAGGTCA	20		Depositor's primer
5	PrF4	TGCCTGCTTGCCGAATATCATGG	23		Depositor's primer
6	IL-2RβHomo	CCAGGTCTCTCACTACATTG	20		Depositor's primer

Reaction Components

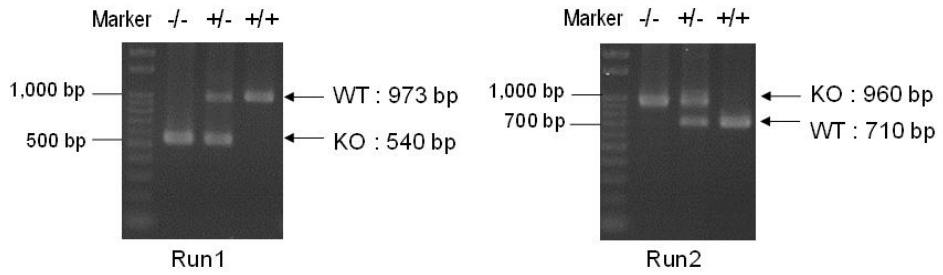
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8	1.8		
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	0		
primer 3	10 pmol/μl	1.0	0		
primer 4	10 pmol/μl	0	1.0		
primer 5	10 pmol/μl	0	1.0		
primer 6	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	90	* *Repeat for 30 cycles

Separated PCR



Marker

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

PCR products	primer_set	product_size	product
primer 1	primer 3	540 bp	KO (Rag2)
primer 2	primer 3	973 bp	WT (Rag2)
primer 4	primer 5	960 bp	KO (IL2rb)
primer 4	primer 6	710 bp	WT (IL2rb)

Comment

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