

RBRC 02771

Strain name C.B-17/lcr Prkdc<scid>-Tg(INS-TRECK/DTR)1 Rin

Primer	primer_name	primer_seq	length	
1	scid_Wild	GGCCCCTGCTAACTTTCTCTTAGTA	25	RIKEN BRC
2	scid_Mut	GGCCCCTGCTAACTTTCTCTTAGTT	25	RIKEN BRC
3	common	GATATGTGCCATGTCAGTGTCTGAC	25	RIKEN BRC
4	TRECK-F5	CAACTACATCCTGGTCATCATC	22	Depositor's primer
5	TRECK-R2	CAGACAGATGACAGCACCACAG	22	Depositor's primer
6	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
7	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

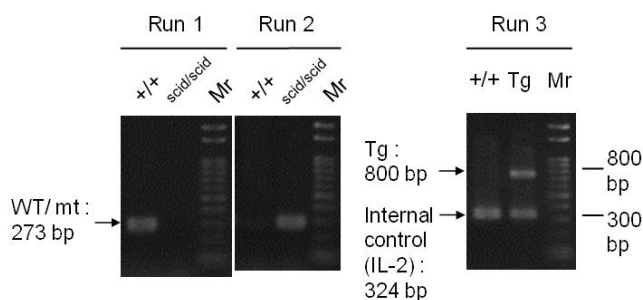
Reaction Components

Enzyme Run1,2:QIAGEN Multiplex PCR Kit (Cat. No. 206143)
Run3:TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	95	900	< Run 1, 2 >
2	94	30*	
3	60	90*	
4	72	60*	*Repeat for 30 cycles
5	72	600	
1	94	120	< Run 3 >
2	98	10*	
3	60	30*	
4	68	60*	*Repeat for 30 cycles

Conc	Run 1	Run 2	Run 3	Run 4
H2O	2.0	2.0	0.8	
primer 1	10 pmol/μl	1.0	0	0
primer 2	10 pmol/μl	0	1.0	0
primer 3	10 pmol/μl	1.0	1.0	0
DNA		1.0	1.0	1.0
Master Mix	2 X	5.0	5.0	0
PCR buffer	2 X	0	0	10.0
dNTP	2.0 mM	0	0	4.0
primer 4	10 pmol/μl	0	0	1.0
primer 5	10 pmol/μl	0	0	1.0
primer 6	10 pmol/μl	0	0	1.0
primer 7	10 pmol/μl	0	0	1.0
Taq	1 U/μl	0	0	0.2
total	10	10	20	



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	273 bp
primer 2	primer 3	273 bp
primer 4	primer 5	800 bp
primer 6	primer 7	324 bp
		WT
		mutant
		Tg
		Internal control (IL-2)

Comment