

RBRC 05156

Strain name B6;129S6-Gt(ROSA)26Sor<tm1(CAG-ChR2/mCherry)Imayo>

Primer	primer_name	primer_seq	length	
1	R26F2	AAAGTCGCTCTGAGTTGTTAT	21	Depositor's primer
2	oIMR0316	GGAGCGGGAGAAATGGATATG	21	JAX Genotyping Protocol
3	oIMR0315	GCGAAGAGTTTGTCTCAACC	21	JAX Genotyping Protocol
4	LFcheck-common-fw	AATTCCTCGACGGGGAATTCGGG	23	Depositor's primer
5	LFcheck-ChR2-rv	CATCCGGCACAGTAACATTGATCCTCAG	28	Depositor's primer
6	mCherry-300bp-fw	GTTTCATGTACGGCTCCAAGGCCTACG	26	Depositor's primer
7	mCherry-300bp-rv	TTCAGCCTCTGCTTGATCTCGCCCTTC	27	Depositor's primer

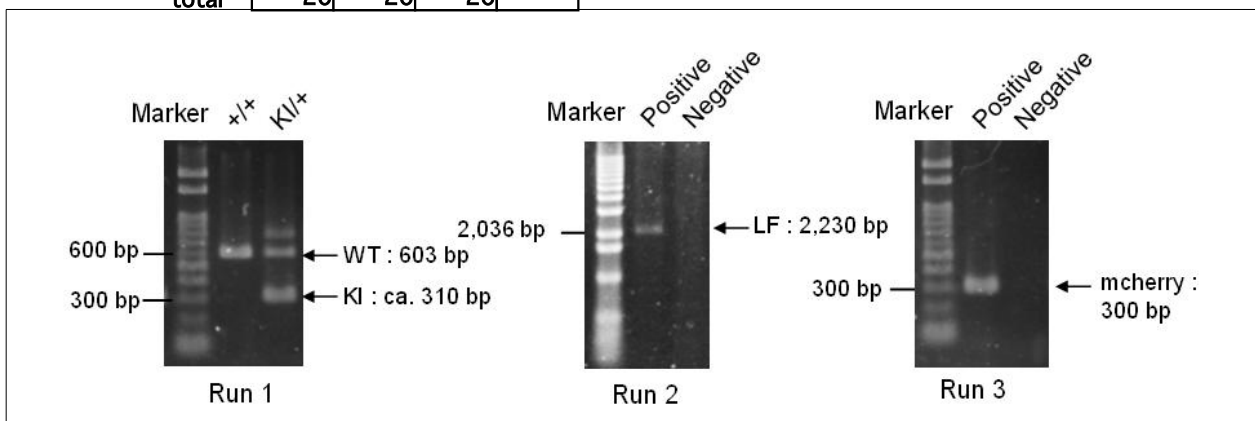
Reaction Components

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

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	63	30*	
4	68	120*	*Repeat for 30 cycles

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8	2.8	2.8	
PCR buffer	2 X	10.0	10.0	10.0	
dNTP	2.0 mM	4.0	4.0	4.0	
primer 1	10 pmol/μl	1.0	0	0	
primer 2	10 pmol/μl	1.0	0	0	
primer 3	10 pmol/μl	1.0	0	0	
primer 4	10 pmol/μl	0	1.0	0	
primer 5	10 pmol/μl	0	1.0	0	
primer 6	10 pmol/μl	0	0	1.0	
primer 7	10 pmol/μl	0	0	1.0	
DNA		1.0	1.0	1.0	
Taq	1 U/μl	0.2	0.2	0.2	
total		20	20	20	



Marker

marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker

Gel

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

PCR products

primer_set	product_size	product
primer 1	603 bp	WT
primer 1	ca. 310 bp	KI
primer 4	2,230 bp	LF
primer 6	300 bp	mCherry

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