

RBRC 06363

Strain name B6.Cg-Tg(KRT5-cre)1Tak Fgf18<tm1Tri>

Primer	primer_name	primer_seq	length	Reference
1	int3_rv	GTTGTCATGACCTGATGGCAACG	23	RIKEN BRC
2	int3f	CTGAGGAGTGTGGACAGGGAGG	22	RIKEN BRC
3	K5 promoter	ATGCCAATGCCCCCTCAGTTCCT	23	CANCER RESEARCH 63, 674 – 681 (2003).
4	1-CN3-2	TGTTTCACTATCCAGGTTACGGA	23	RIKEN BRC

Reaction Components

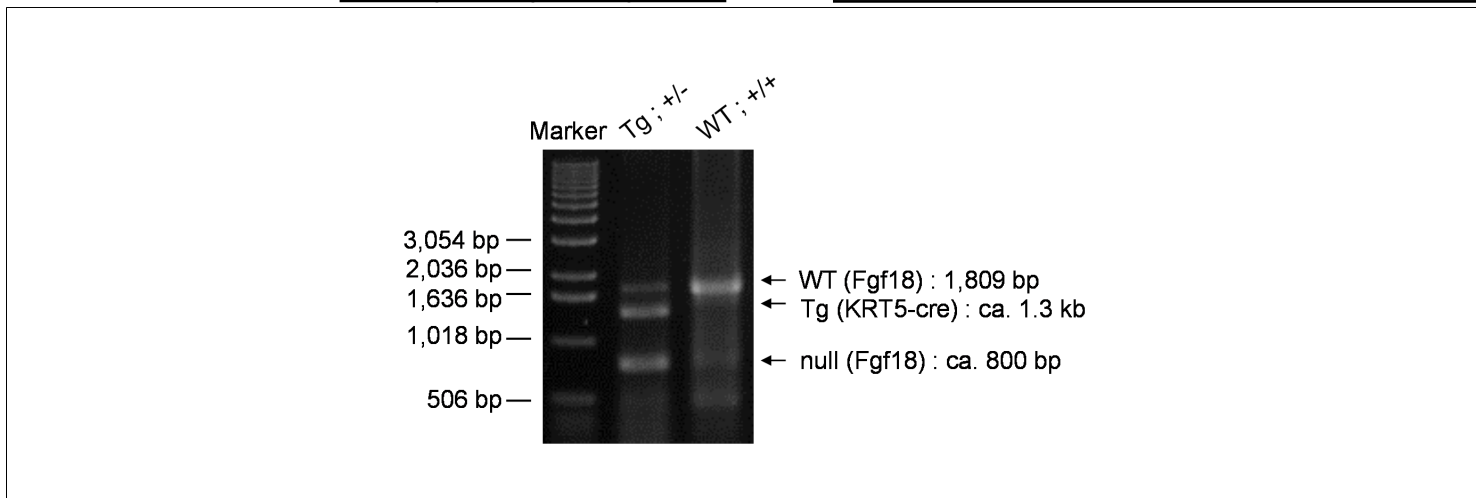
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	180*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4	(μL)
H2O		0.8				
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.5				
primer 2	10 pmol/μl	1.5				
primer 3	10 pmol/μl	0.5				
primer 4	10 pmol/μl	0.5				
DNA		1.0				
Taq	1 U/μl	0.2				
total		20				

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	1,809 bp WT (Fgf18)
primer 1	primer 2	ca. 800 bp null (Fgf18)
primer 3	primer 4	ca. 1.3 kb Tg (KRT5-cre)

Comment

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