



RIKEN DNA BANK

clone name : BW25113-based RFzero-iy

- **Clone ID** : RDB_ 14427
- **Lot** : 14427_ A9H6
- **Form** : Recombinant in Stab agar
- **Culture** : 37C, LB medium, add 0.1mg/mL 3-Iodo-L-tyrosine
- **Antibiotics** : 17mg/L Chloramphenicol, 25mg/L Zeocin and 7mg/L Gentamicin

● Primers	Primer A	prfAnoATG	5' - AAGCCTTCTATCGTTGCCAAAC - 3'
	Primer B	prfATAARev	5' - TTATTCCTGCTCGGACAACG - 3'
	Primer C	K12-L	5' - TTCCACGGACATGAAGACTACA - 3'
	Primer D	K12-R	5' - ATCCTGCGCACCAATCAACAA - 3'

● **PCR Amplification**

< Condition >

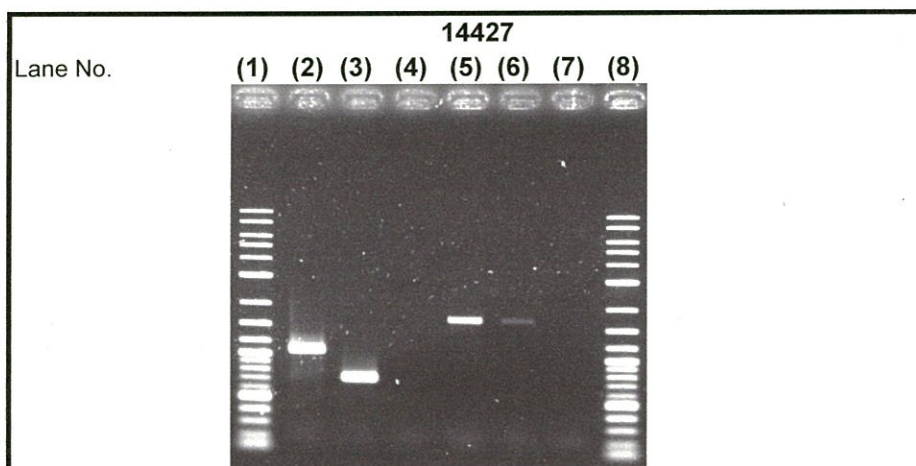
Templete : chelated genomic DNA, 0.5uL
 Enzyme : AmpliTaq Gold® 360 DNA Polymerase (ThermoFisher)
 Volume : 5.0uL

< Reaction program >

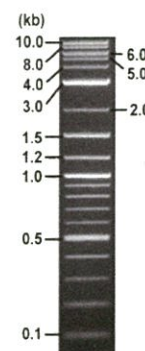
96 degC , 2 min
 [96 degC , 15 sec
 55 degC , 15 sec] × 30 cycle
 72 degC , 1min
 72 degC , 5min
 4 degC , ∞

< Electrophoresis >

2 uL DNA solution per lane ; 1% agarose gel, 1 × TAE buffer



2-Log DNA Ladder
(NEB#N3200L),
250 ng/well



< Lane number and size of fragment >

Primer		delta prfA confirmation	BW25113-based confirmation
		Primer A and B	Primer C and D
host	BW25113*	(1), 1.1 kb	(4), 1.7kb
	RDB14428	(2), 0.73 kb	(5), 1.7kb
No templete		(3), No band	(6), No band

* used as a control.

APPROVED BY :

