

**RIKEN Clone ID : IRAL025B06**

Vector : pOTB7

Gene	ANKH	
Accession No.	BC009835.2	1892 bp
	<i>CDS</i>	1479 bp
		257..1735

● Plasmid DNA purification

Date : 140310

Culture : LB (25 ug/ml Chloramphenicol) 5 ml -> at 37 deg C over night

Date : 140312

Purification : QIAGEN Miniprep kit -> dH<sub>2</sub>O 100 ul

● Digestion by restriction enzyme/Concentration calibration

Date : 140312

DNA concentration (O.D.): 50 ng/ul

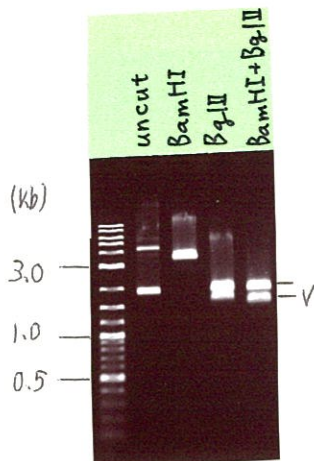
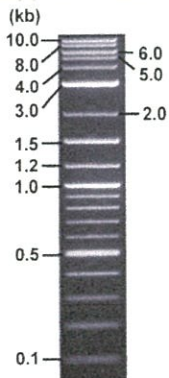
< Size of fragment expected from IRAL025B06 >

DNA	1	ul
Enzyme (BamHI + BglII)	0.5 + 0.5	ul
Buffer H		1 ul
dH <sub>2</sub> O	7	ul
Total		10 ul

BamHI	3537, 156 bp
BglII	1725, 1968 bp
BamHI + BglII	1615bp (Vector) 1922, 110, 46 bp

Electrophoresis : 1% agarose gel, 1x TAE Buffer

Marker : 2-Log DNA Ladder (NEB#N3200L)



● Confirmation of the insertion sequence

Date : 140314

Primer A	pOTB7_F
Primer B	pOTB7_R
Primer C	-
List of Sequencing Primers	<a href="http://dna.brc.riken.jp/en/GNPclone3en.html">http://dna.brc.riken.jp/en/GNPclone3en.html</a>

● Adjustment of DNA concentration

Shipping amount : 40 ul

Concentration at the time of preparation of plasmid DNA : 25 ng/ul

Date : 140317

DNA ( <u>50</u> ng/ul)	<u>76.0</u>	ul
10x TE	<u>15.2</u>	ul
dH <sub>2</sub> O	<u>60.8</u>	ul
Total	<u>152.0</u>	ul

APPROVED BY :

<http://dna.brc.riken.jp/index.html>



S/N G:58 A:63 T:55 C:72

Primer A: pOTB7-F

IRAL025B06\_A4Cb\_pOTB7\_F

Mar 14, 2014 10:36AM, JST

KB.bcp

KB\_3500\_POP7\_BDTv3.mob

Mar 14, 2014 11:05AM, JST

KB 1.4.1.8 Cap:14

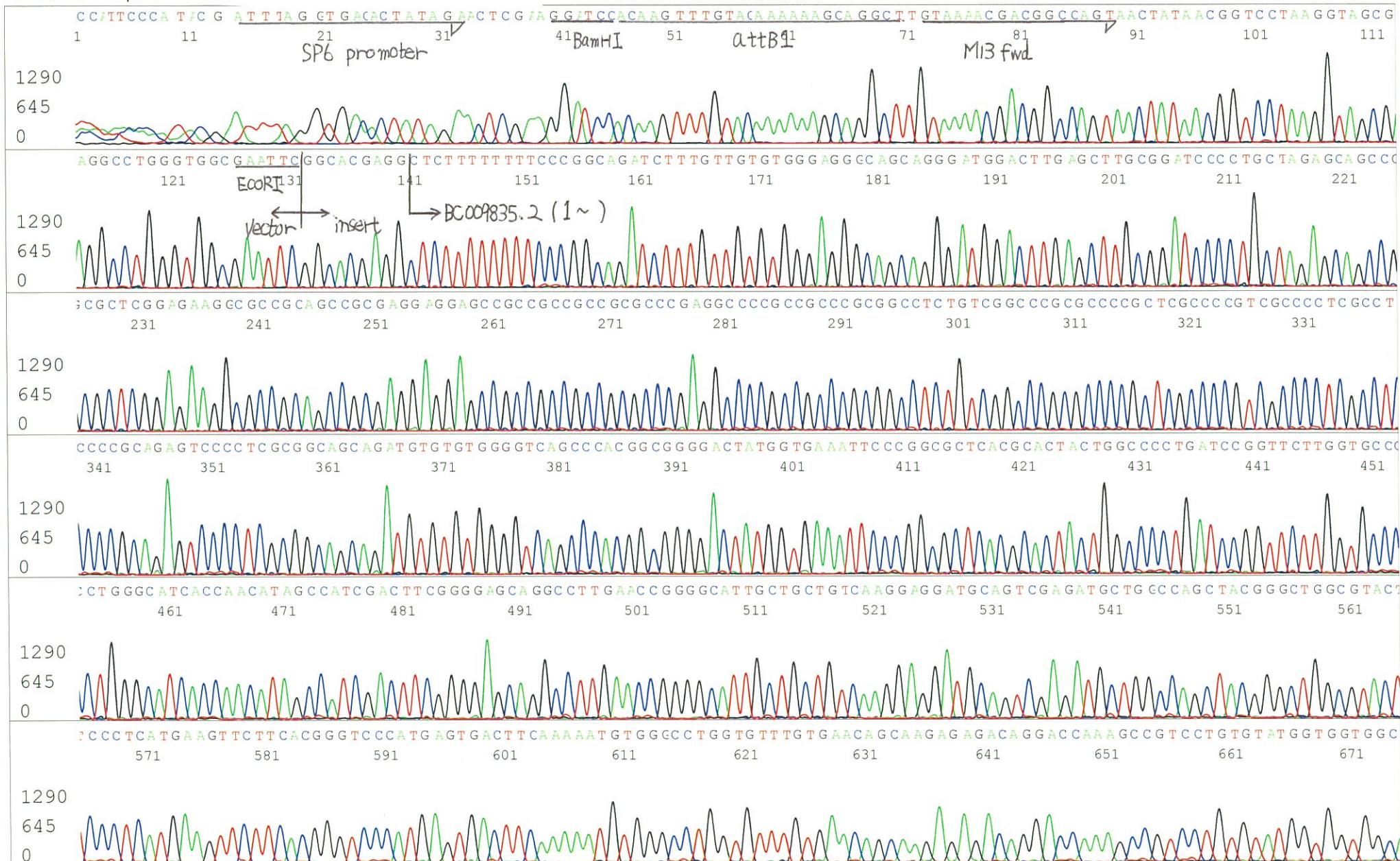
5' AACGCGGCTACAATTAATACATAACC 3'

Pts 1483 to 13199 Pk1 Loc:1460

Spacing:11.75 Pts/Panel1350

Version 5.4 HiSQV Bases: 989

Plate Name: 20140314\_GNP









S/N G:54 A:77 T:66 C:72

Primer B: pOTB7-R

IRAL025B06\_A4Cb\_pOTB7\_R

Mar 14, 2014 10:36AM, JST

KB.bcp

KB\_3500\_POP7\_BDTv3.mob

Mar 14, 2014 11:05AM, JST

KB 1.4.1.8 Cap:17

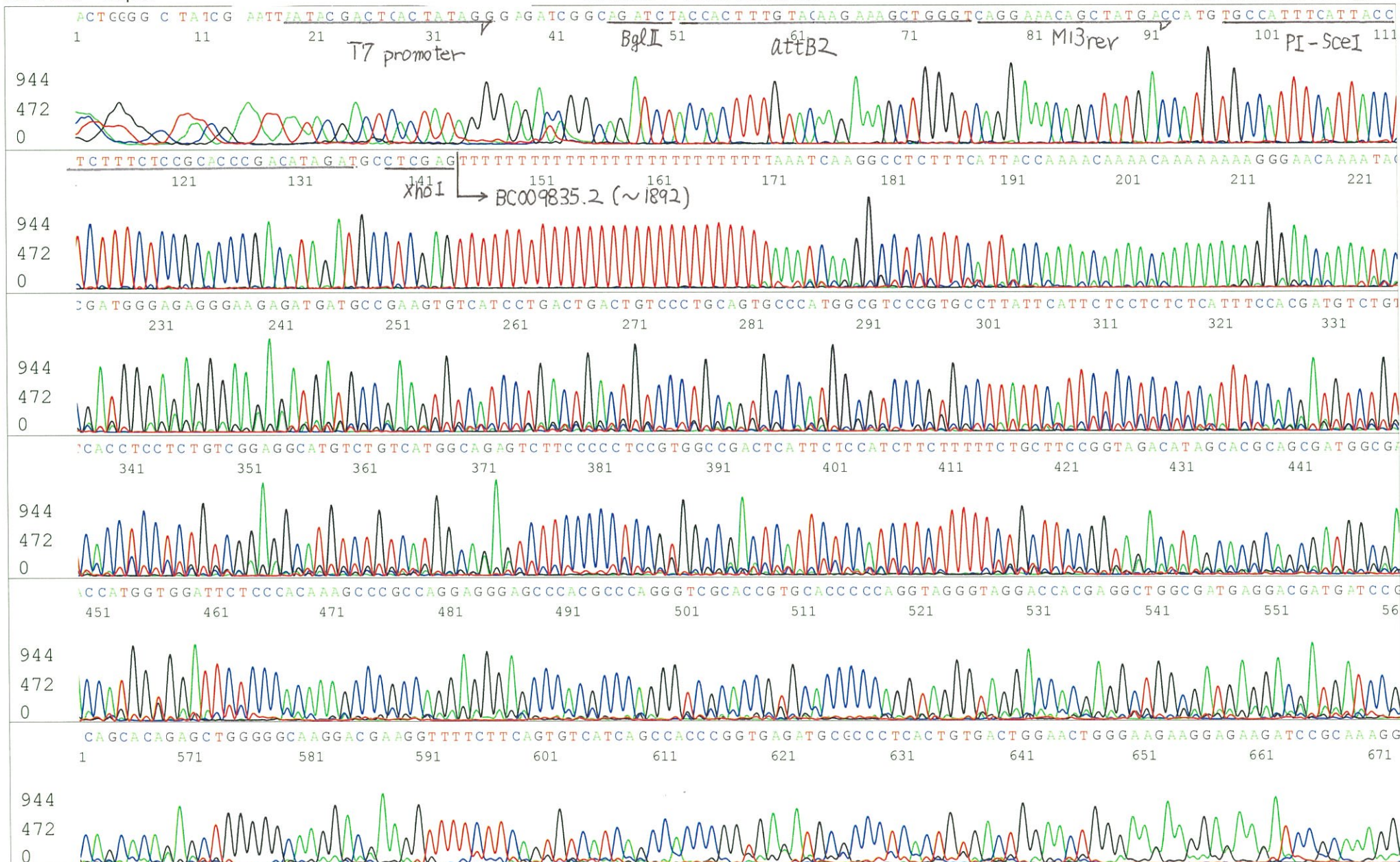
5' GTACTGCAGCCGATTCATTAATGC 3'

Pts 1470 to 13327 Pk1 Loc:1447

Spacing:11.9 Pts/Panel1350

Version 5.4 HiSQV Bases: 945

Plate Name: 20140314\_GNP





S/N G:54 A:77 T:66 C:72

KB.bcp

KB 1.4.1.8 Cap:17

