

**RIKEN Clone ID : IRAL014M08**

Vector : pOTB7

Gene	CTCF		
Accession No.	BC014267.2	3460 bp	1..3460
	<i>CDS</i>	2184 bp	279..2462

● Plasmid DNA purification

Date : 130110

Culture : LB (25 ug/ml Chloramphenicol) 10 ml -> at 37 deg C over night

Date : 130111

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

● Digestion by restriction enzyme/Concentration calibration

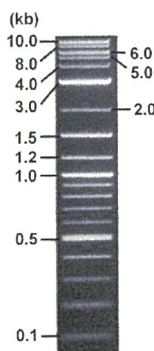
Date : 130111

DNA concentration (O.D.): 182 ng/ul

DNA	0.2 ul
Enzyme (BamHI + BglII)	0.5 + 0.5 ul
Buffer H	1 ul
dH <sub>2</sub> O	7.8 ul
<b>Total</b>	<b>10 ul</b>

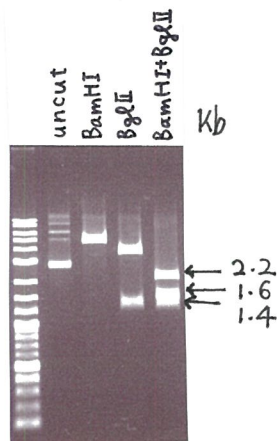
Electrophoresis : 1% agarose gel, 1x TAE Buffer

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



<Expected size of fragment from BC014267.2 >

.....2221, 1615, 1425 bp.....



● Confirmation of the insertion sequence

Date : 130115

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 : 130115

DNA ( <u>182</u> ng/ul)	83.0 ul
10x TE	60.4 ul
dH <sub>2</sub> O	460.8 ul
<b>Total</b>	<b>604.2 ul</b>



APPROVED BY :

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

S/N G:16 A:11 T:10 C:10

Primer A: pOPTB7-F

IRAL014M08\_POTB7\_F\_2  
KB\_3500\_POP7\_BDTV3.mob  
Pts 1489 to 14642 Pk1 Loc:1466

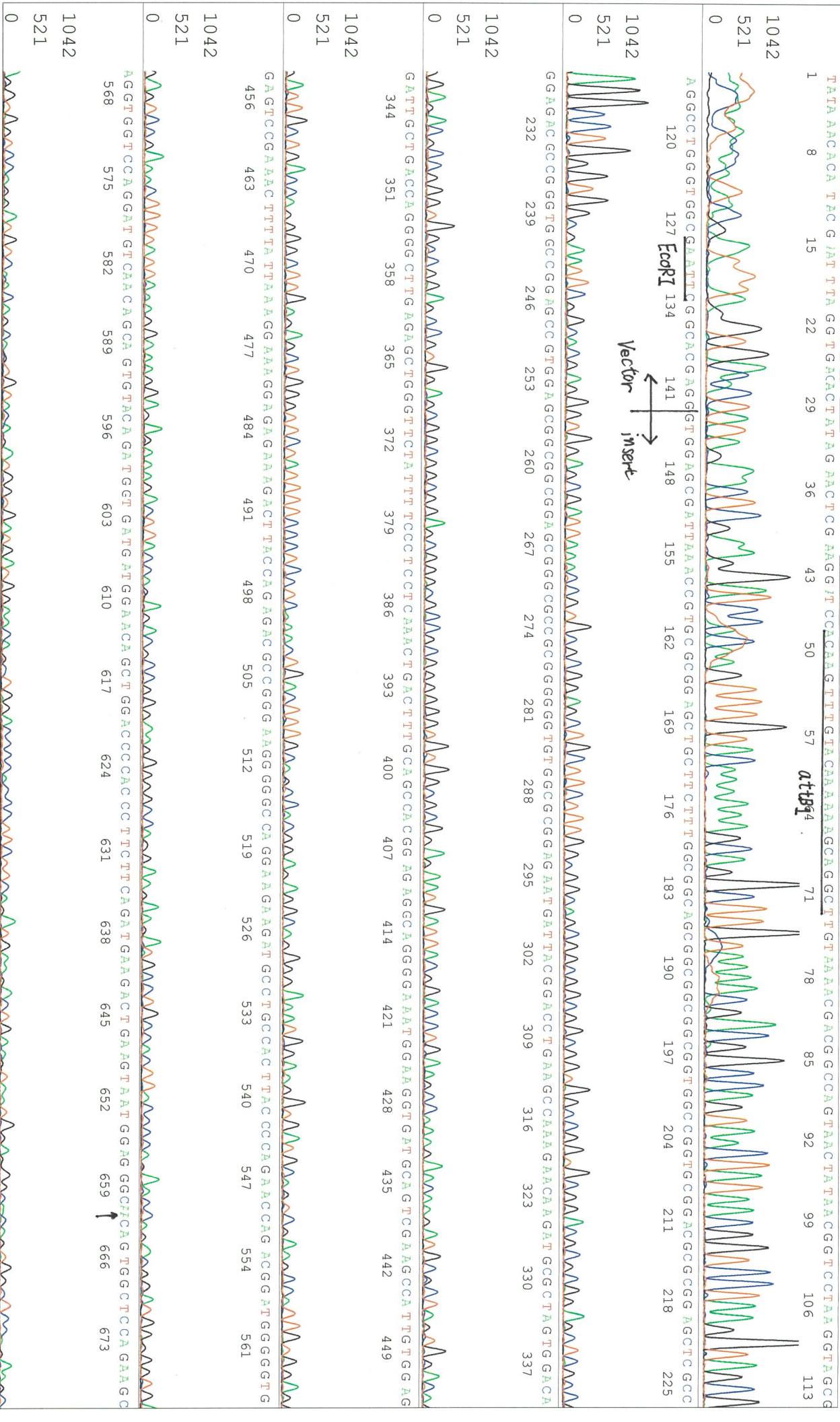
Jan 15,2013 01:40PM, JST  
Jan 15,2013 02:19PM, JST  
Spacing:12.83 Pts/Panel1350

KB.bcp

KB 1.4.1.8 Cap:3 5'AACGGCGCTACAAATTAATACATTAACC 3'

Version 5.4 HiSQV Bases: 747

Plate Name: 20130115\_GNP



S/N:G:214 A:135 T:172 C:113 Primer B: potB7-R  
KB.bcp  
KB 1.4.1.8 Cap:6  
5' GTACTGCAGCCCGATTTCATTAATGC 3'

G A C A T C I C T A T C G A T T A T A C G A C T A C T A T A T G G G A T C G G C A G A T C T A C C A C T T T G T A C A A G A A A G C T G G G T C A G G A A C C A G C T A T G A C C A T G T G C C A T T T C A T T A C C

