

**RIKEN Clone ID : W01A004I08**

Vector : pENTR/D-TOPO

Gene	SIRT3		
Accession No.	BC001042.2	1798 bp	1..1798
	CDS	1200 bp	18..1217

## ● Plasmid DNA purification

Date : 130305

Culture : LB (25 ug/ml Kanamycin) 5 ml → at 37 deg C over night

Date : 130306

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

## ● Digestion by restriction enzyme/Concentration calibration

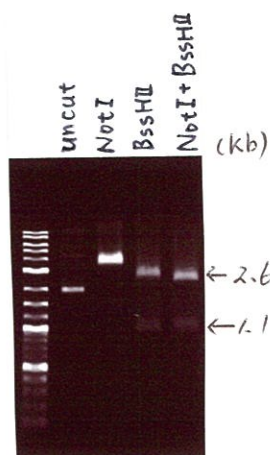
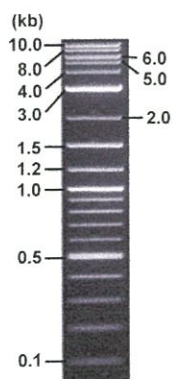
Date : 130307

DNA concentration (O.D.): 142 ng/ul

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

Electrophoresis : 1% agarose gel, 1x TAE Buffer

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



&lt;Expected size of fragment from BC001042.2 CDS&gt;

2553, 1056, 172, 72 bp

## ● Confirmation of the insertion sequence

Date : 130311

Primer A	M13
Primer B	T7 long
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

Date : 130312

DNA ( 142 ng/ul )	92	ul
10x TE	52.3	ul
dH <sub>2</sub> O	378.3	ul
Total	522.6	ul



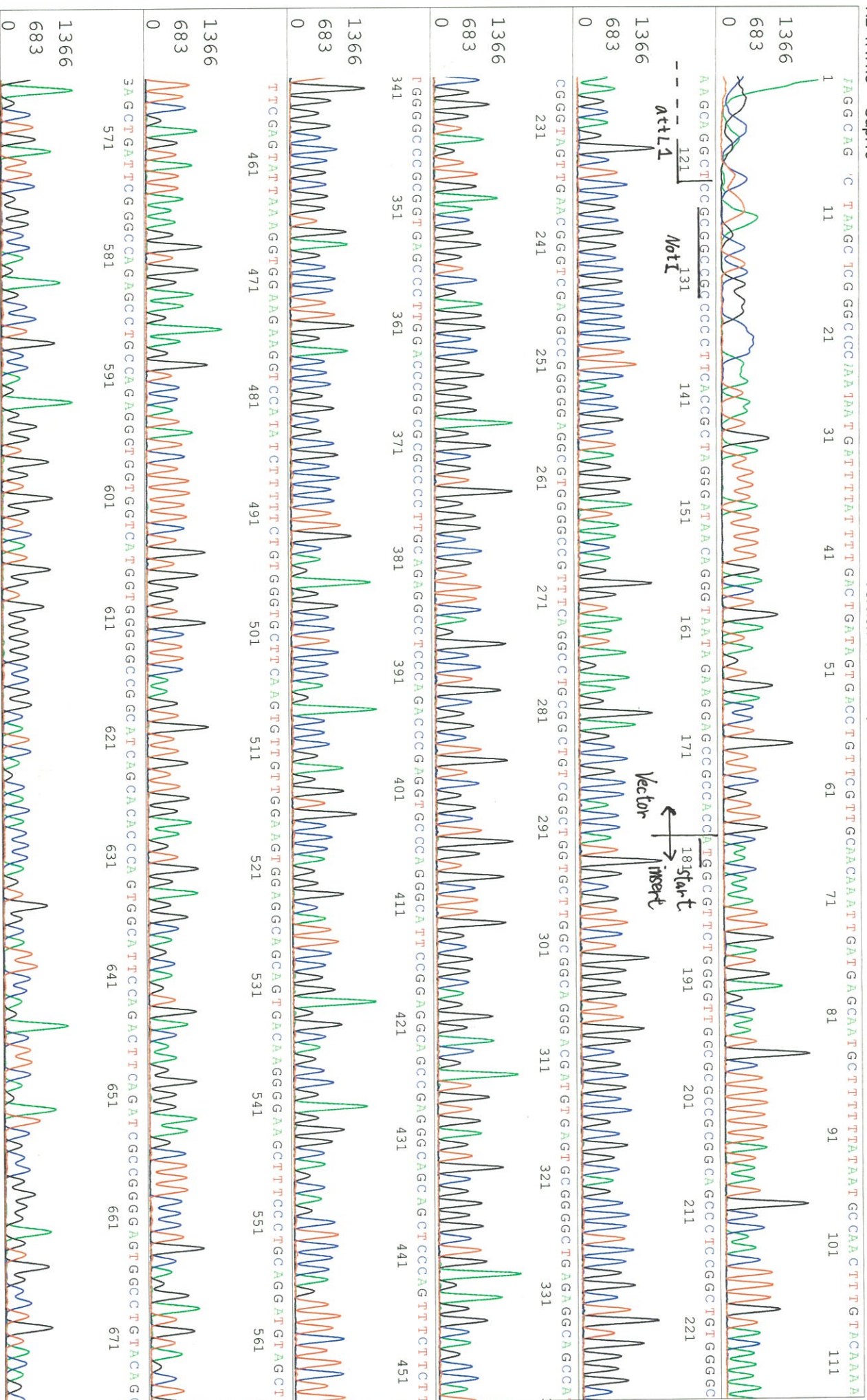
APPROVED BY :

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

Mar 07, 2013 07:40PM, JST

Spacing: 11.76 Pts/Panel 1350

Plate Name: 20130307\_GNP





Mar 07, 2013 08:07PM, JST

Spacing: 11.71 Pts/Panel1350

Plate Name: 20130307\_GNP

