

**RIKEN Clone ID : IRAK004A12**

Vector : pCMV-SPORT6

|               |            |         |
|---------------|------------|---------|
| Gene          | SGK        |         |
| Accession No. | BC001263.1 | 2382 bp |
|               | <i>CDS</i> | 1296 bp |

● Plasmid DNA purification

Date : 190109

Culture : LB (100 ug/ml Ampicillin) 5 ml → at 37 deg C over night

Date : 190110

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

● Digestion by restriction enzyme/Concentration calibration

Date : 190110

DNA concentration (O.D.): 199 ng/ul

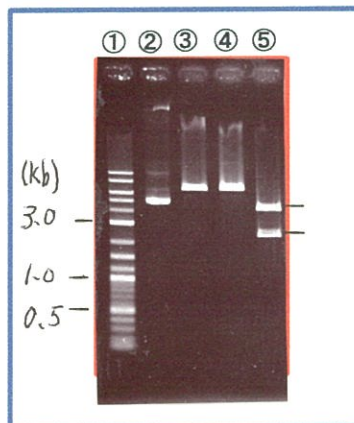
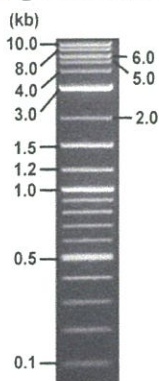
< Size of fragment expected from this clone >

|                       |               |
|-----------------------|---------------|
| DNA                   | <u>0.5</u> ul |
| Enzyme (EcoRI + XhoI) | 0.5 + 0.5 ul  |
| Buffer H              | 1 ul          |
| dH <sub>2</sub> O     | <u>7.5</u> ul |
| Total                 | 10 ul         |

|              |                            |
|--------------|----------------------------|
| EcoRI        | 6.7 kb                     |
| XhoI         | 6.7 kb                     |
| EcoRI + XhoI | 4.3kb (Vector)      2.4 kb |

Erectrophoresis : 1% agarose gel, 1x TAE Buffer

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



- ①:Marker
- ②:uncut
- ③:EcoRI
- ④:XhoI
- ⑤:EcoRI + XhoI

● Adjust plasmid DNA solution to 25 ng/ul

Date : 190121

|                         |                 |
|-------------------------|-----------------|
| DNA ( <u>199</u> ng/ul) | <u>86.0</u> ul  |
| 10x TE                  | <u>68.4</u> ul  |
| dH <sub>2</sub> O       | <u>530.1</u> ul |
| Total                   | <u>684.5</u> ul |

● Confirmation of the insertion sequence

Date : 190115

|                            |   |
|----------------------------|---|
| Primer A                   | Reverse2  |
| Primer B                   | M13   |
| Primer C                   | poly(A) region primer #0  |
| List of Sequencing Primers | <a href="http://dna.brc.riken.jp/en/GNPclone3en.html">http://dna.brc.riken.jp/en/GNPclone3en.html</a> |

● Shipping

Conc. : 25 ng/μl, Volume : 40 μl

Conc. :      ng/μl, Volume :      μl

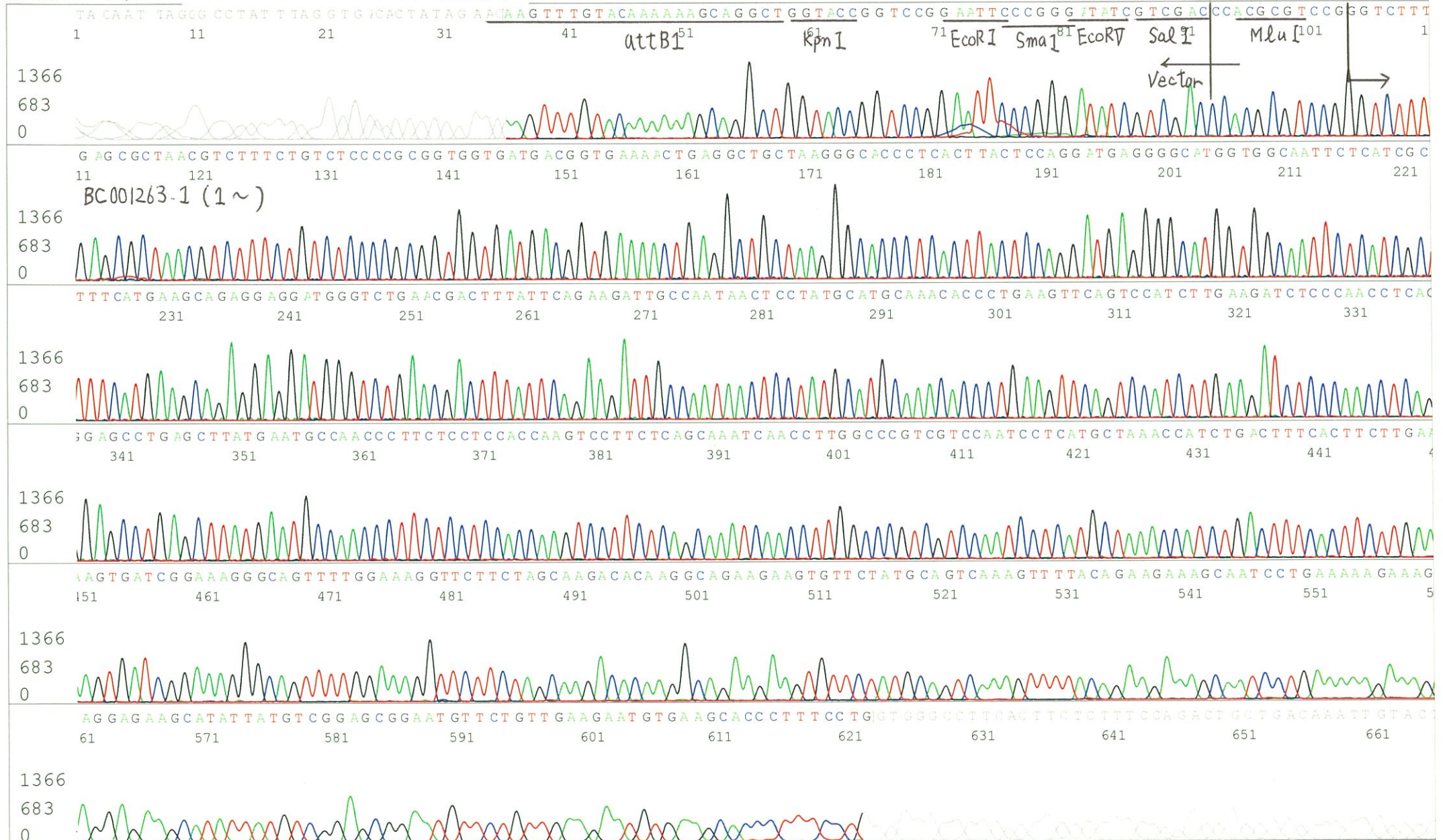


APPROVED BY :

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

S/N G:74 A:88 T:80 C:12 Primer A : Reverse2  
 KB.bcp  
 KB 1.4.1.8 Cap:6 5' GCGGATAACAATTTTCACACAGG 3'

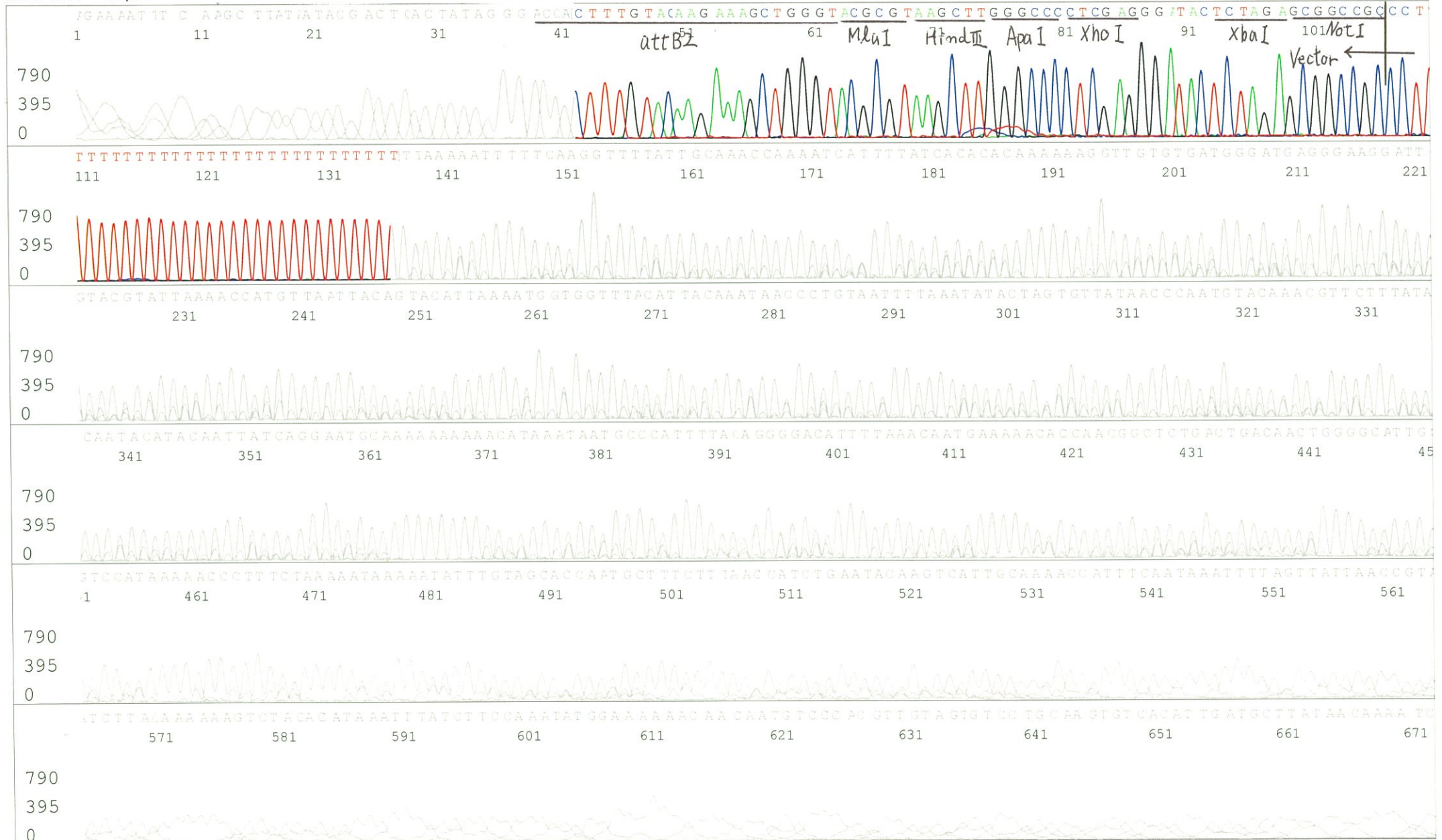
Gray line indicates quality value  
 (QV) < 40.



S/N G:46 A:68 T:71 C:68  
KB.bcp  
KB 1.4.1.8 Cap:7

Primer B : M13  
5' GTTTCCAGTCACGACGTTGTA 3'

Gray line indicates quality value  
(QV) < 40.



S/N G:8 A:9 T:9 C:10

KB.bcp

KB 1.4.1.8 Cap:8

Primer C : Poly(A) region primer #0

5' TTTTTTTTTTTTTTTTTTTTTTNNN 3'

IRAK004A12\_A9A8\_GNP Mixture

KB\_3500\_POP7\_BDTv3.mob

Pts 1920 to 8704 Pk1 Loc:1888

Version 6.0 HiSQV Bases: 544

Gray line indicates quality value

(QV) < 40.

Jan 15,2019 10:00AM, JST

Jan 15,2019 10:31AM, JST

Spacing:11.0 Pts/Panel1350

Plate Name: 20190115\_GNP

