

**RIKEN Clone ID : IRAL005F01**

Lot#: 7566\_A9Kp

Vector : pOTB7

|               |            |         |
|---------------|------------|---------|
| Gene          | GNB3       |         |
| Accession No. | BC002454.2 | 1715 bp |
|               | CDS        | 1023 bp |

● Plasmid DNA purification

Date : 191127

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

Date : 191128

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

● Digestion by restriction enzyme/Concentration calibration

Date : 191128

DNA concentration (O.D.): 222 ng/ul

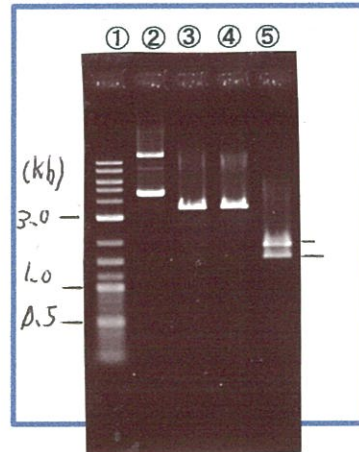
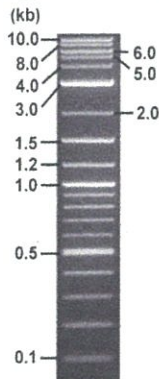
< Size of fragment expected from this clone >

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

|               |                            |
|---------------|----------------------------|
| BamHI         | 3.5 kb                     |
| BglII         | 3.5 kb                     |
| BamHI + BglII | 1.6kb (Vector)      1.9 kb |

Electrophoresis : 1% agarose gel, 1x TAE Buffer

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



- ①:Marker
- ②:uncut
- ③:BamHI
- ④:BglII
- ⑤:BamHI + BglII

● Adjust plasmid DNA solution to 25 ng/ul

Date : 191202

|                         |                 |
|-------------------------|-----------------|
| DNA ( <u>222</u> ng/ul) | <u>72.0</u> ul  |
| 10x TE                  | <u>63.9</u> ul  |
| dH <sub>2</sub> O       | <u>503.4</u> ul |
| Total                   | <u>639.3</u> ul |

● Confirmation of the insertion sequence

Date : 191129

|                            |   |
|----------------------------|---|
| Primer A                   | pOTB7_F   |
| Primer B                   | pOTB7_R   |
| 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/ul, Volume : 40 ul

Conc. : ng/ul, Volume : ul

APPROVED BY :

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



S/N G:242 A:256 T:173 C:33

Primer A: pOTB7-F

7566\_IRAL005F01\_A9Kp\_2\_pOTB7\_F

KB\_3500\_POP7\_BDTv3.mob

Gray line indicates quality value

Nov 29, 2019 01:47PM, JST

KB.bcp

5' AACGCGGCTACAATTAATACATAACC 3'

Pts 1760 to 8704 Pk1 Loc:1728

(QV) < 40.

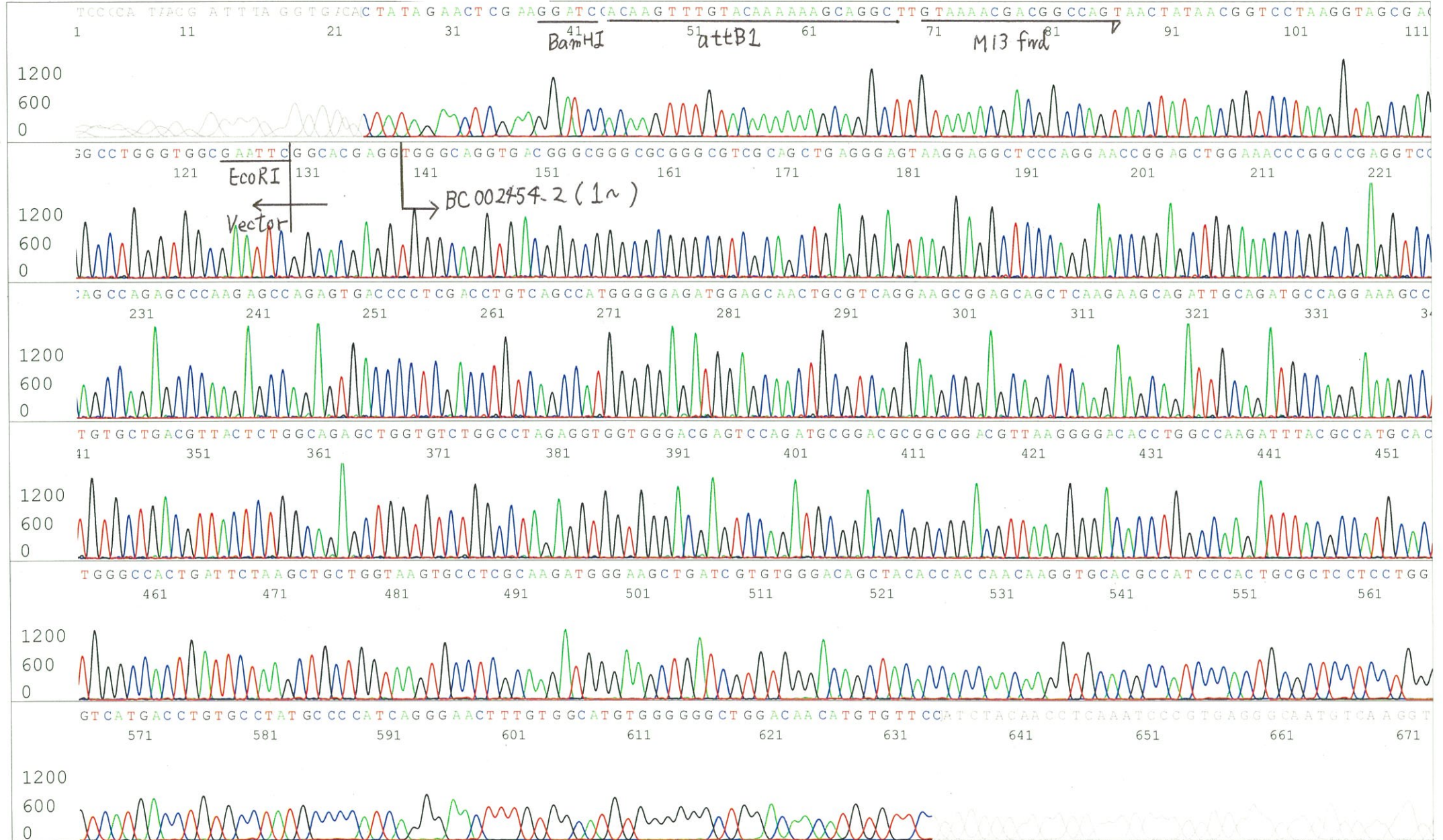
Nov 29, 2019 02:02PM, JST

KB 1.4.1.8 Cap:3

Version 6.0 HiSQV Bases: 688

Spacing:10.55 Pts/Panel1350

Plate Name: 20191129\_Mix



S/N G:60 A:61 T:91 C:79

Primer B: pOTB7-R

7566\_IRAL005F01\_A9Kp\_2\_pOTB7\_R

Nov 29, 2019 01:47PM, JST

KB.bcp

KB\_3500\_POP7\_BDTv3.mob

Gray line indicates quality value

Nov 29, 2019 02:02PM, JST

KB 1.4.1.8 Cap:4

5' GTACTGCAGCCGATTCATTAATGC 3'

Pts 1731 to 8704 Pk1 Loc:1699

Version 6.0 HiSQV Bases: 187 (QV) < 40.

Spacing:10.81 Pts/Panel1350

Plate Name: 20191129\_Mix

