

**RIKEN Clone ID : IRAK110K09**

Lot#: 7893\_A5C6

Vector : pCMV-SPORT6

|               |            |            |
|---------------|------------|------------|
| Gene          | ARHGEF11   |            |
| Accession No. | BC057394.1 | 6928 bp    |
|               |            | 1..6928    |
|               | CDS        | 4689 bp    |
|               |            | 1065..5753 |

● Plasmid DNA purification

Date : 150309

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

Date : 150310

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

● Digestion by restriction enzyme/Concentration calibration

Date : 150310

DNA concentration (O.D.): 330 ng/ul

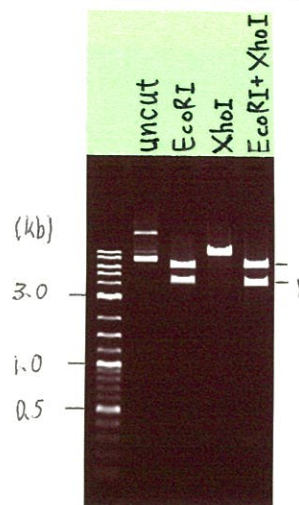
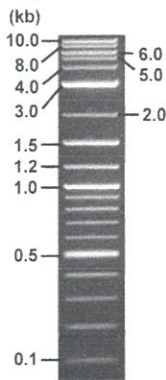
< Size of fragment expected from IRAK110K09 >

|                       |           |       |
|-----------------------|-----------|-------|
| DNA                   | 0.15      | ul    |
| Enzyme (EcoRI + XhoI) | 0.5 + 0.5 | ul    |
| Buffer H              |           | 1 ul  |
| dH <sub>2</sub> O     | 7.75      | ul    |
| Total                 |           | 10 ul |

|              |                              |
|--------------|------------------------------|
| EcoRI        | 6680, 4630 bp                |
| XhoI         | 11310 bp                     |
| EcoRI + XhoI | 4339bp (Vector) 6680, 291 bp |

Electrophoresis : 1% agarose gel, 1x TAE Buffer

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



● Adjust plasmid DNA solution to 25 ng/ul

Date : 150316

|                   |        |    |
|-------------------|--------|----|
| DNA ( 330 ng/ul)  | 86.0   | ul |
| 10x TE            | 113.5  | ul |
| dH <sub>2</sub> O | 935.7  | ul |
| Total             | 1135.2 | ul |

● Confirmation of the insertion sequence

Date : 150313

|                            |   |
|----------------------------|---|
| Primer A                   | Reverse2  |
| Primer B                   | M13   |
| 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> |

● Shipping

Conc. : 25 ng/ul, Volume : 40 ul

Conc. : \_\_\_\_\_ ng/ul, Volume : \_\_\_\_\_ ul

APPROVED BY :

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

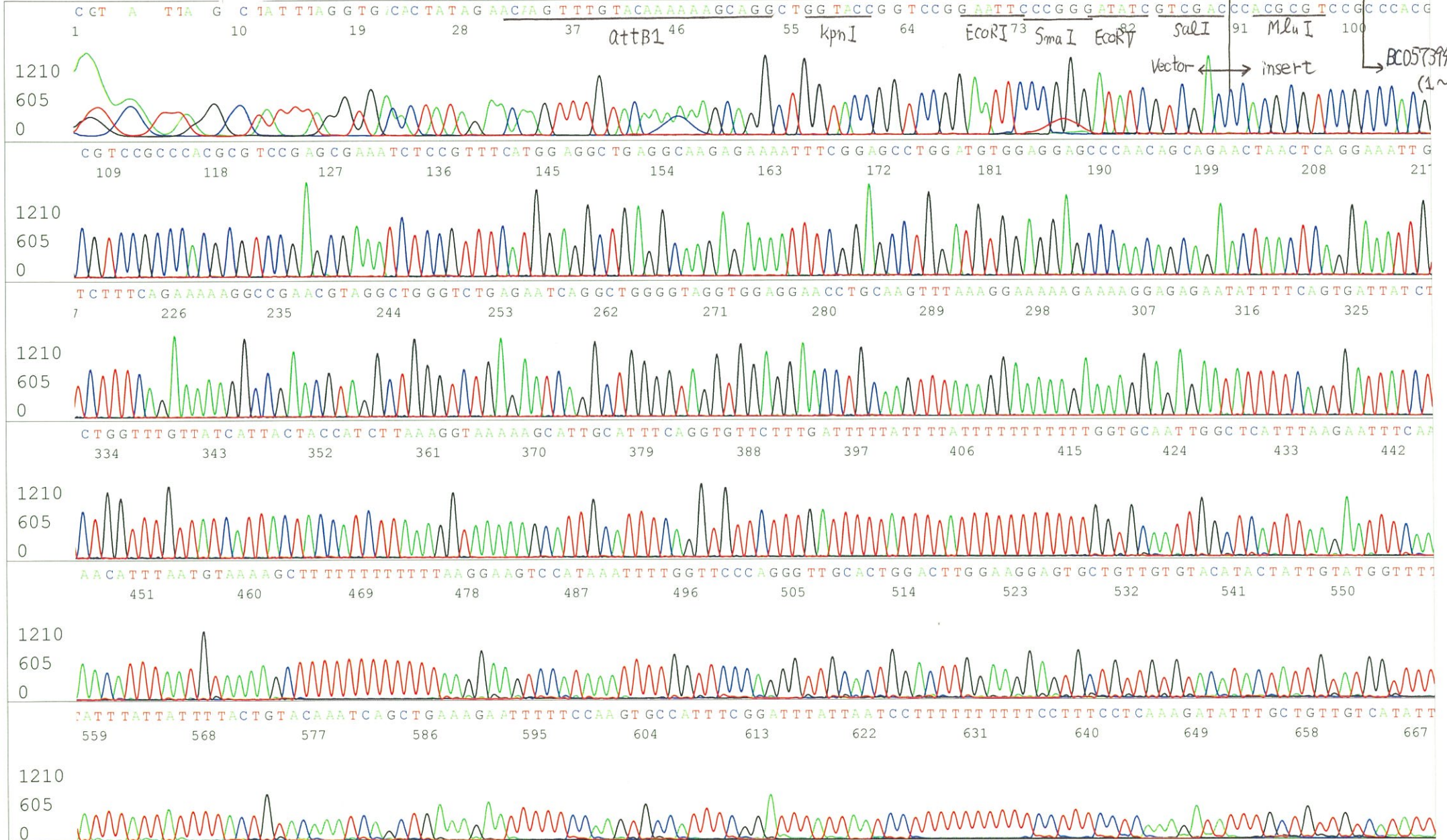


S/N G:997 A:867 T:672 C:564  
KB.bcp  
KB 1.4.1.8 Cap:1

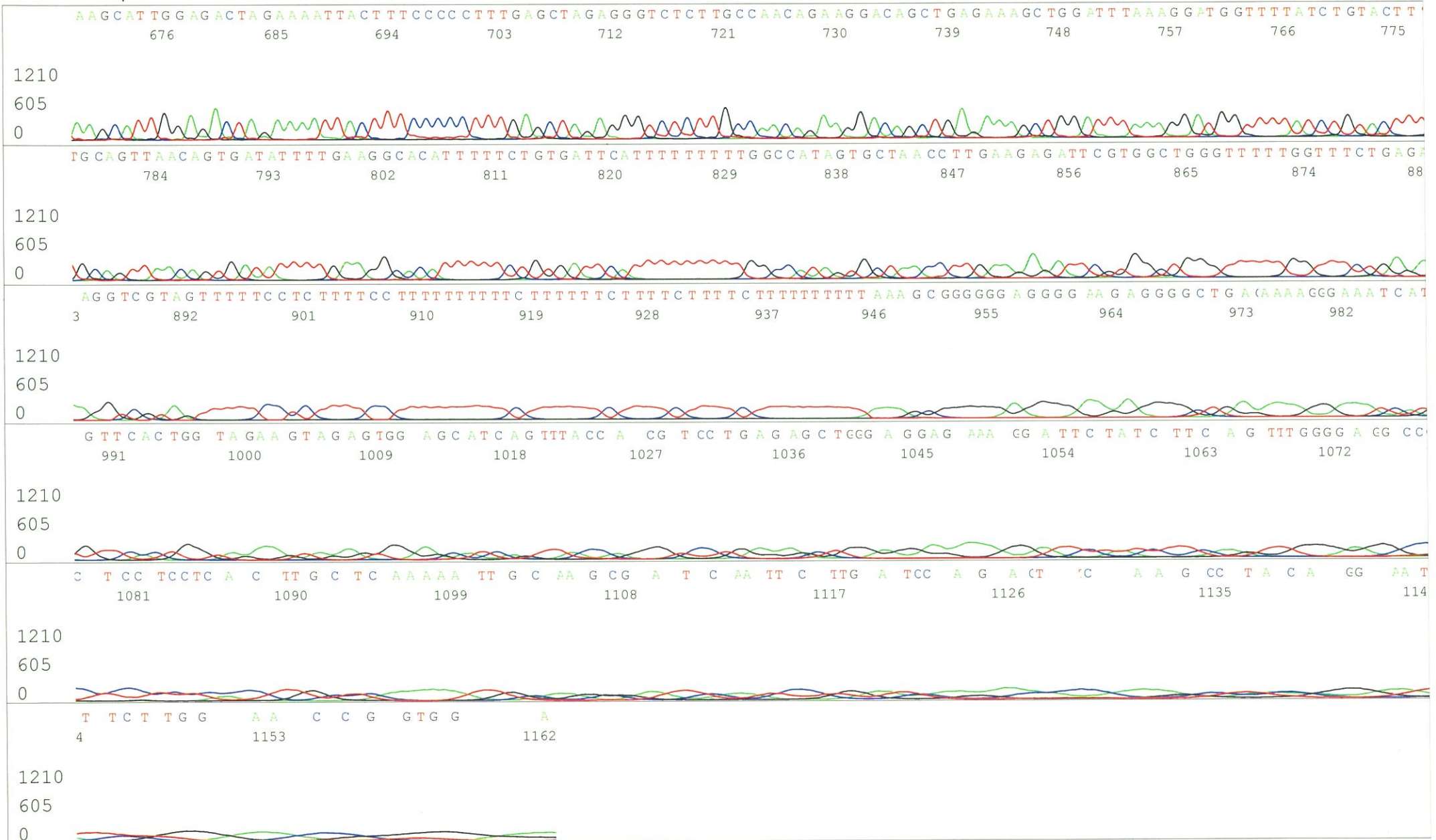
Primer A : Reverse2  
5' GCGGATAACAATTTTCACACAGG 3'

IRAK110K09\_A5C6\_Reverse2\_A10\_01  
IRAK110K09\_A5C6\_Reverse2  
KB\_3500\_POP7\_BDTv3.mob  
Pts 1388 to 13212 Pk1 Loc:1365  
Version 6.0 HiSQV Bases: 1001

Inst Model/Name 3500/3500 Instrument  
Mar 13,2015 10:08AM, JST  
Mar 13,2015 10:34AM, JST  
Spacing:11.34 Pts/Panel1350  
Plate Name: 20150313\_mix

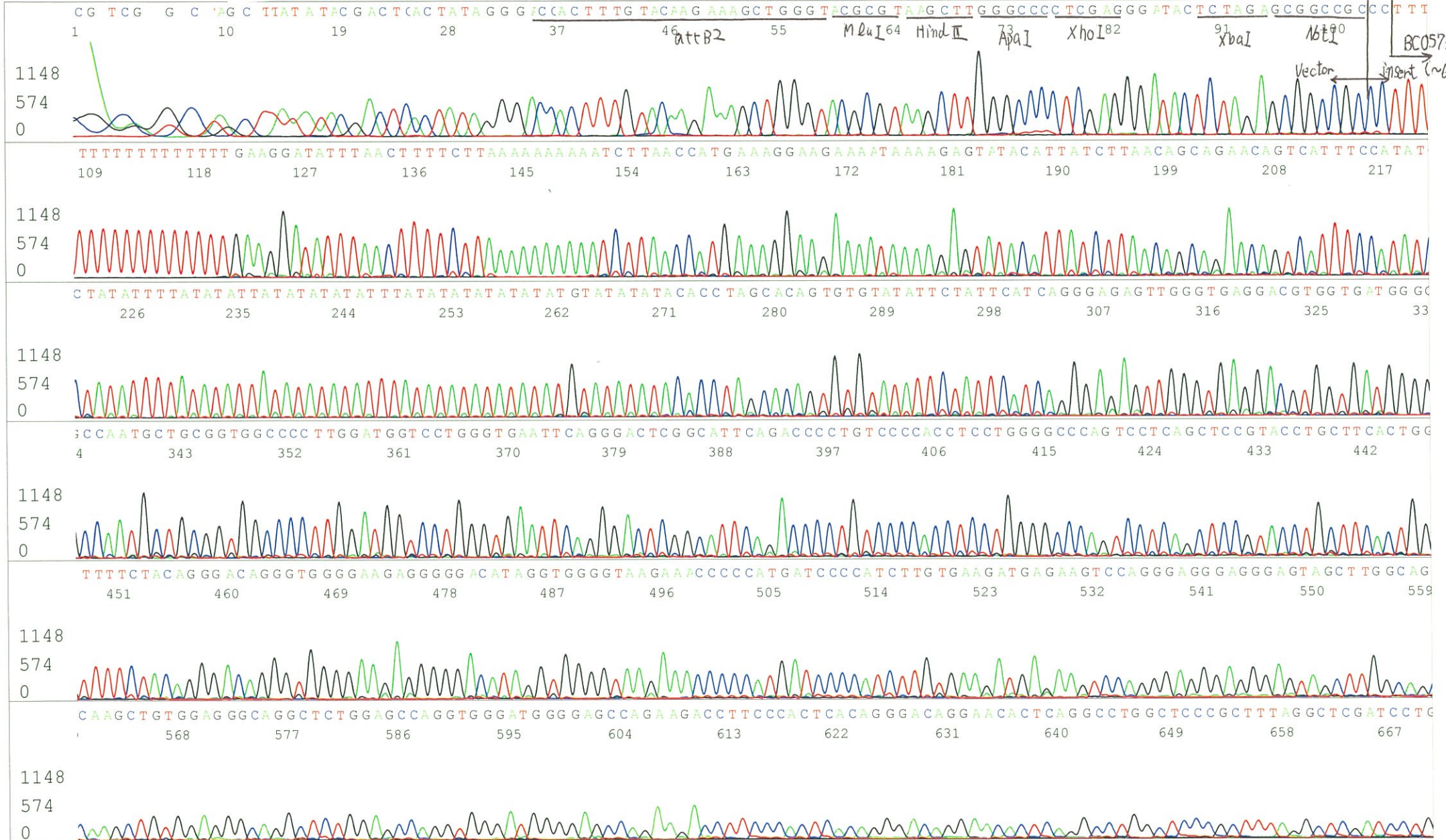


S/N G:997 A:867 T:672 C:564  
KB.bcp  
KB 1.4.1.8 Cap:1



S/N G:560 A:561 T:437 C:404 Primer B : M13  
KB.bcp  
KB.1.4.1.8 Cap:4

5' GTTTTCCCAGTCACGACGTTGTA 3'



S/N G:560 A:561 T:437 C:404  
KB.bcp  
KB 1.4.1.8 Cap:4

IRAK110K09\_A5C6\_M13(-40)\_B10\_04  
IRAK110K09\_A5C6\_M13(-40)  
KB\_3500\_POP7\_BDTv3.mob  
Pts 1388 to 13126 Pk1 Loc:1365  
Version 6.0 HiSQV Bases: 988

Inst Model/Name 3500/3500 Instrument  
Mar 13,2015 10:08AM, JST  
Mar 13,2015 10:34AM, JST  
Spacing:11.6 Pts/Panel1350  
Plate Name: 20150313\_mix

