

RIKEN Clone ID : IRAK174B04

Vector : pCMV-SPORT6

| | | |
|---------------|------------|--------|
| Gene | CTRB2 | |
| Accession No. | BC073145.1 | 960 bp |
| | CDS | 792 bp |

● Plasmid DNA purification

Date : 150629

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

Date : 150630

Purification : QIAGEN Miniprep kit -> dH₂O 100 ul

● Digestion by restriction enzyme/Concentration calibration

Date : 150630

DNA concentration (O.D.): 238 ng/ul

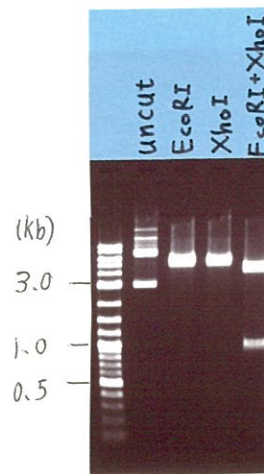
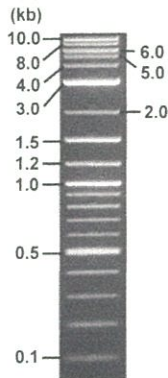
< Size of fragment expected from this clone >

| | | |
|-----------------------|-----------|-------|
| DNA | 0.2 | ul |
| Enzyme (EcoRI + XhoI) | 0.5 + 0.5 | ul |
| Buffer H | | 1 ul |
| dH ₂ O | 7.8 | ul |
| Total | | 10 ul |

| | |
|--------------|----------------------------|
| EcoRI | 5.3 kb |
| XhoI | 5.3 kb |
| EcoRI + XhoI | 4.3kb (Vector) 1.0 kb |

Electrophoresis : 1% agarose gel, 1x TAE Buffer

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



● Adjust plasmid DNA solution to 25 ng/ul

Date : 150701

| | |
|-------------------------|-----------------|
| DNA (<u>238</u> ng/ul) | <u>85.0</u> ul |
| 10x TE | <u>80.9</u> ul |
| dH ₂ O | <u>643.3</u> ul |
| Total | <u>809.2</u> ul |

● Confirmation of the insertion sequence

Date : 150630

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

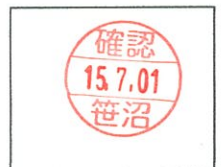
● Shipping

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

Conc. : ng/ul, Volume : ul

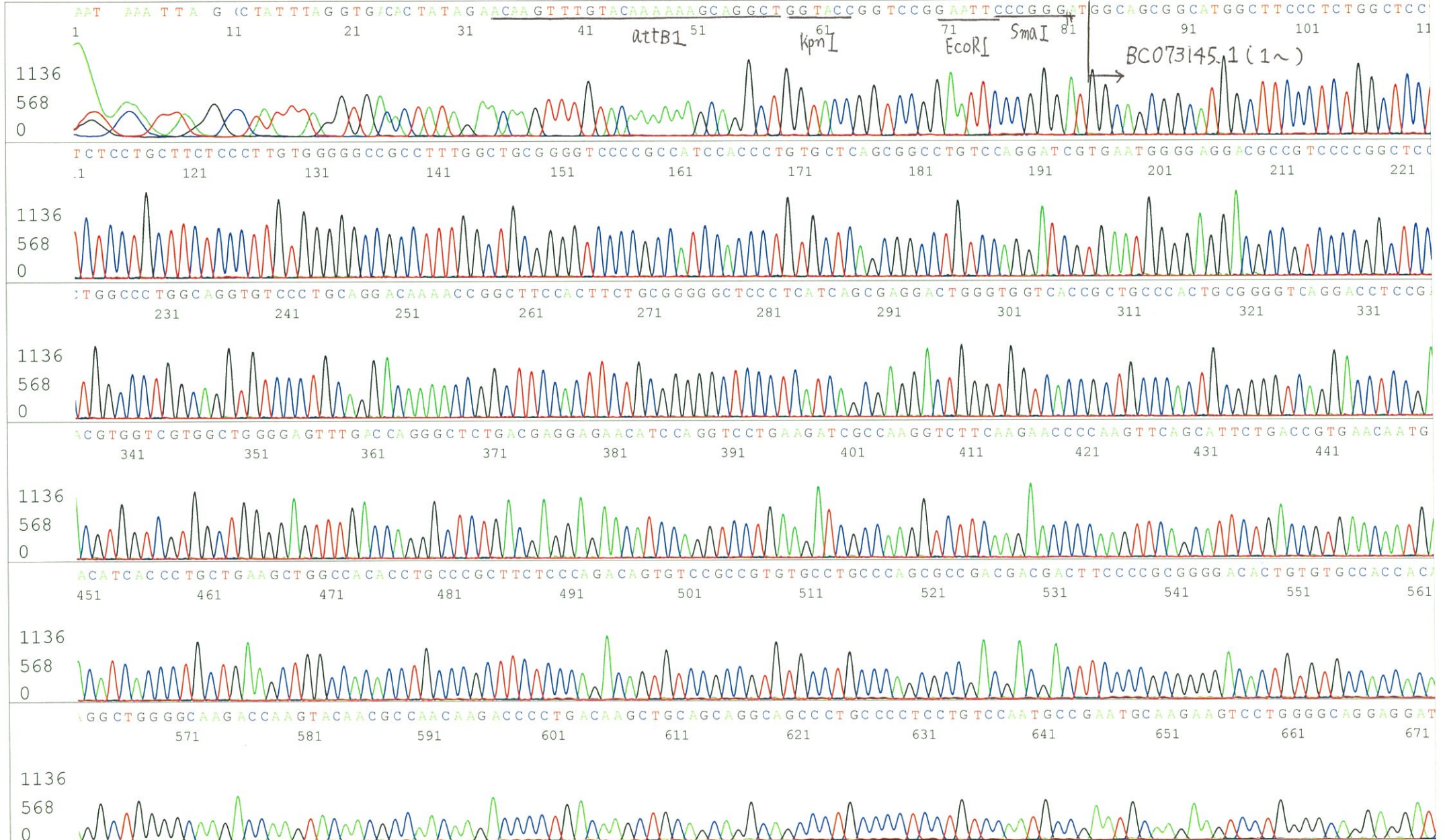
APPROVED BY :

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

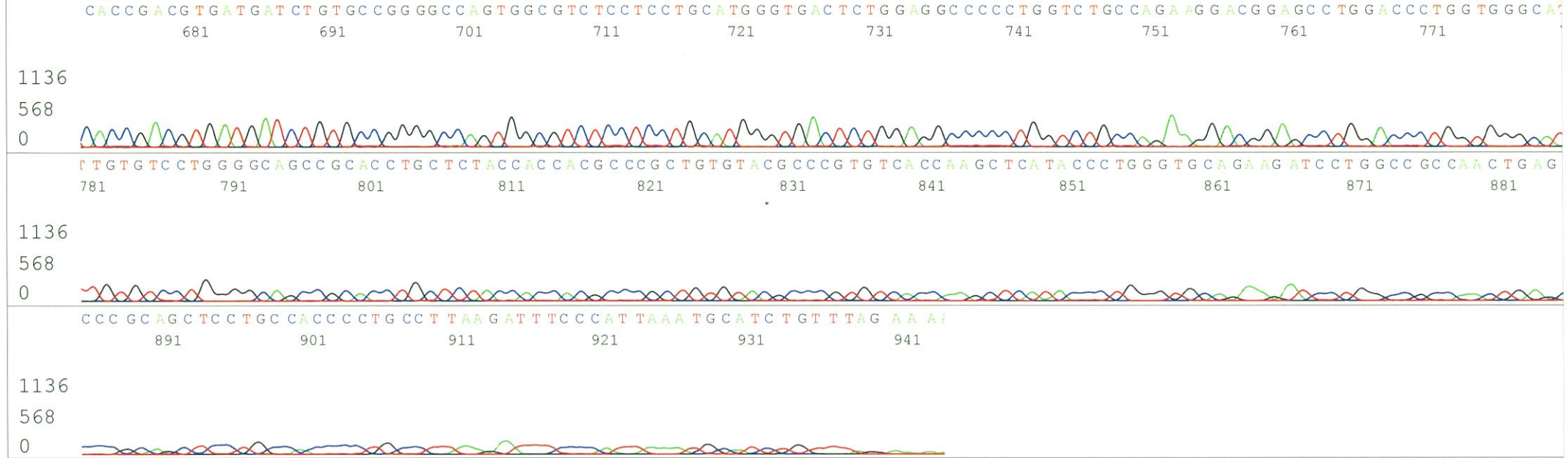


S/N G:366 A:183 T:152 C:240
KB.bcp
KB 1.4.1.8 Cap:7

Primer A : Reverse2
5' GCGGATAACAATTTTCACACAGG 3'



S/N G:366 A:183 T:152 C:240
KB.bcp
KB 1.4.1.8 Cap:7



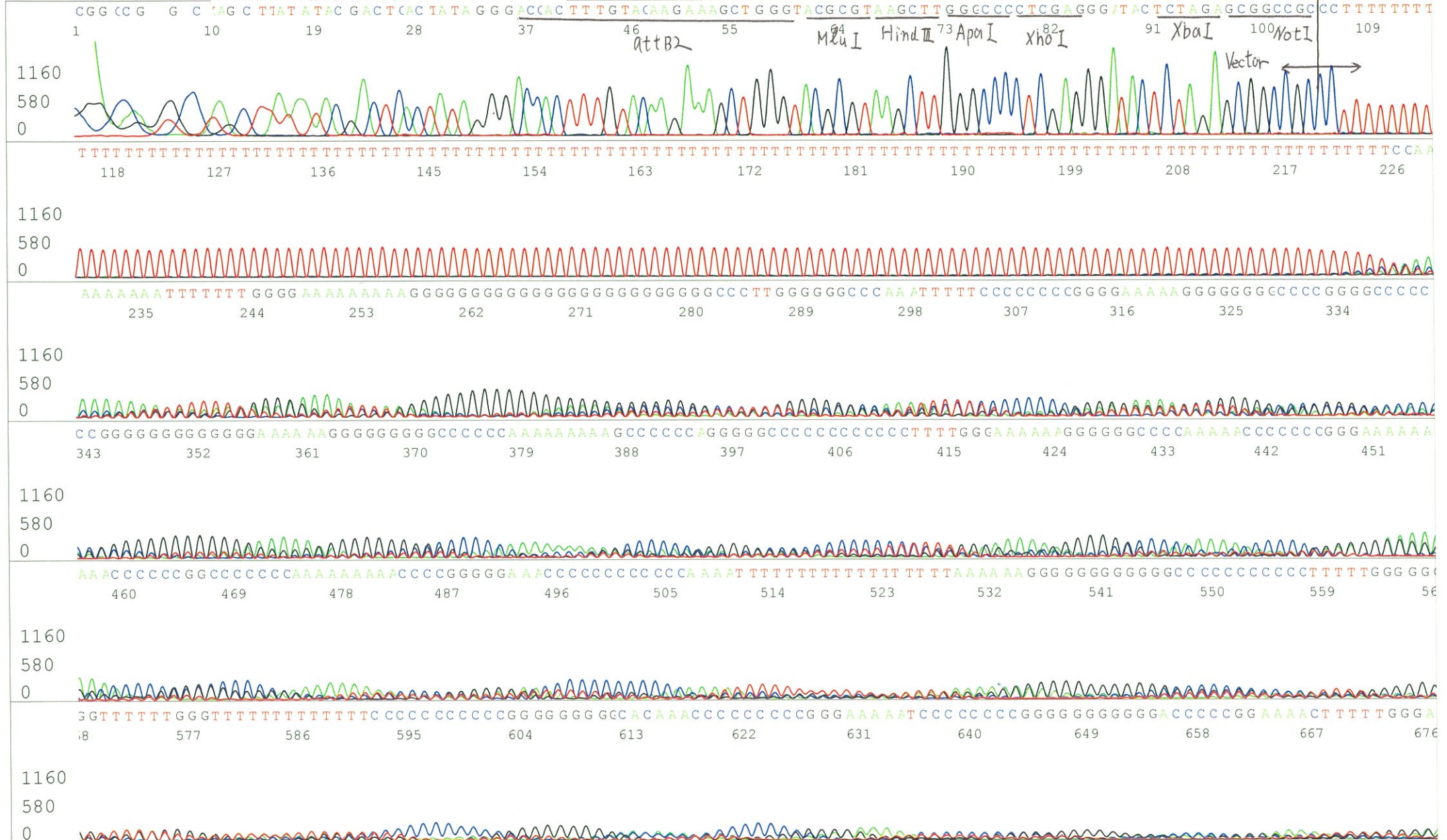
S/N G:64 A:31 T:39 C:29
KB.bcp
KB 1.4.1.8 Cap:10

Primer B : M13

5' GTTTTCCCAGTCACGACGTTGTA 3'

IRAK174B04_A5Ft_M13(-40)_D04_10
IRAK174B04_A5Ft_M13(-40)
KB_3500_POP7_BDTv3.mob
Pts 1355 to 13000 Pk1 Loc:1332
Version 6.0 HiSQV Bases: 309

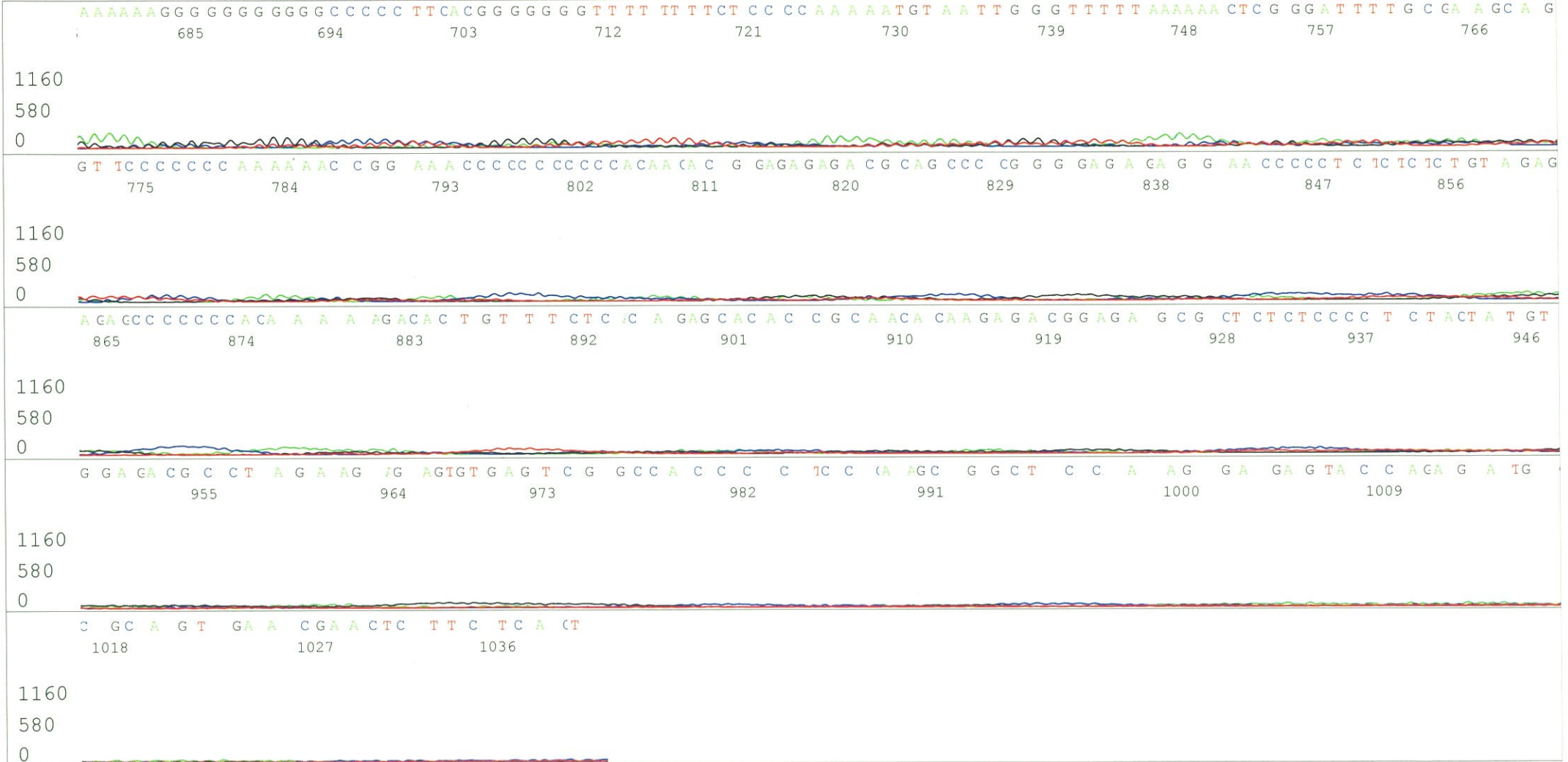
Inst Model/Name 3500/3500 Instrument
Jun 30,2015 01:55PM, JST
Jun 30,2015 02:22PM, JST
Spacing:13.25 Pts/Panel1350
Plate Name: 20150630_mix



S/N G:64 A:31 T:39 C:29
KB.bcp
KB 1.4.1.8 Cap:10

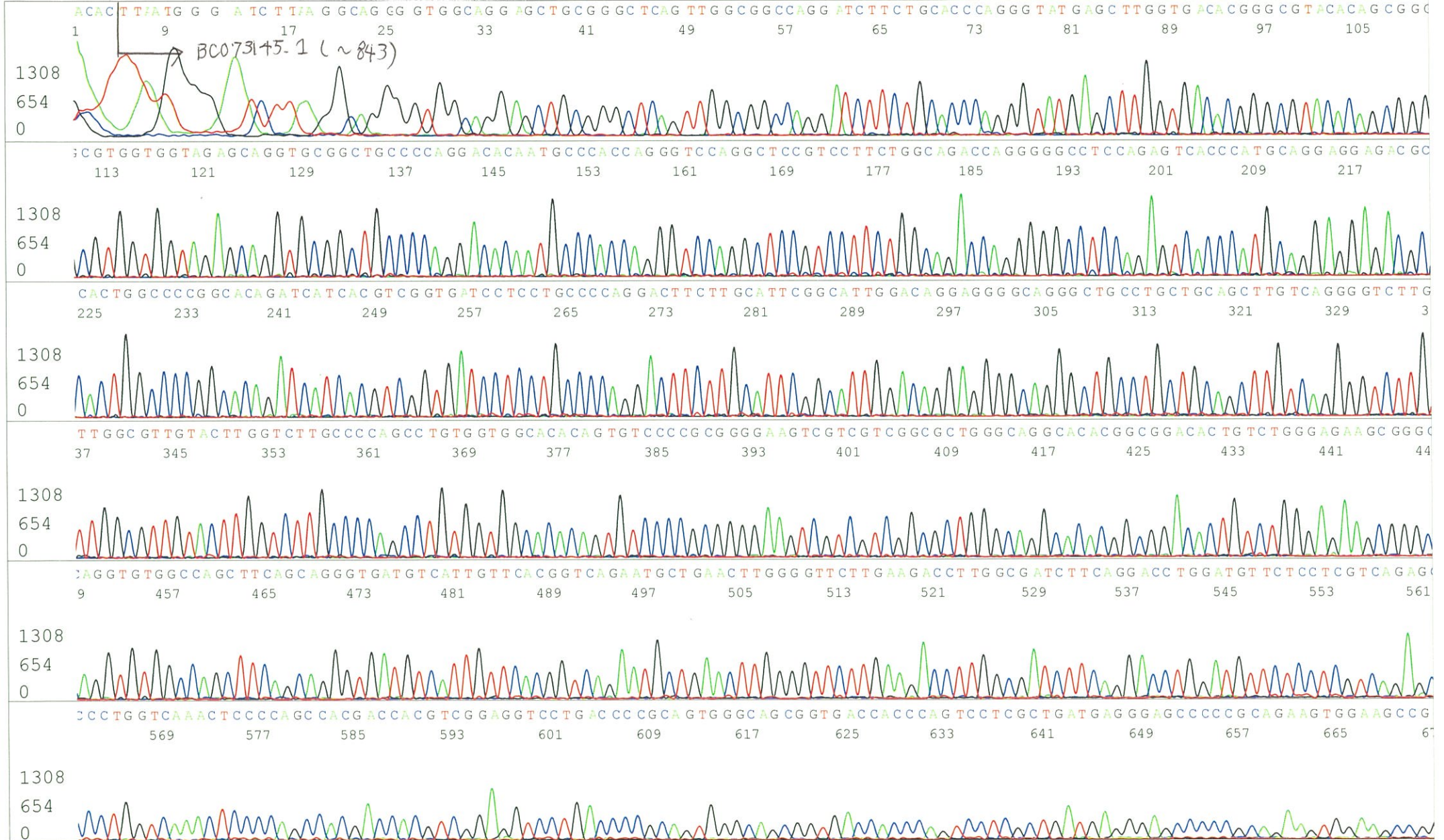
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IRAK174B04_A5Ft_M13(-40)
KB_3500_POP7_BDTv3.mob
Pts 1355 to 13000 Pk1 Loc:1332
Version 6.0 HiSQV Bases: 309

Inst Model/Name 3500/3500 Instrument
Jun 30,2015 01:55PM, JST
Jun 30,2015 02:22PM, JST
Spacing:13.25 Pts/Panel1350
Plate Name: 20150630_mix



S/N G:104 A:35 T:25 C:36
KB.bcp
KB 1.4.1.8 Cap:13

Primer C : Poly(A) region primer #0
5' TTTTTTTTTTTTTTTTTTTTTTNNN 3'



S/N G:104 A:35 T:25 C:36
KB.bcp
KB 1.4.1.8 Cap:13

