

**RIKEN Clone ID : W01A091D06**

Vector : pENTR-TOPO

Gene	RHOA	
Accession ID of Origin	BC005976.1	1439 bp
	<i>CDS</i>	582 bp

● Plasmid DNA purification

Date : 160623

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

Date : 160624

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

● Digestion by restriction enzyme/Concentration calibration

Date : 160624

DNA concentration (O.D.): 99 ng/ul

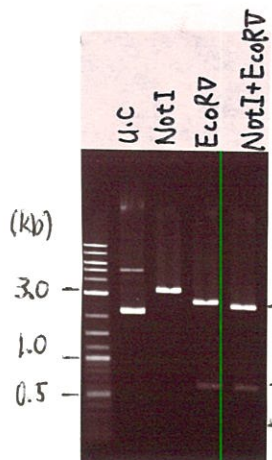
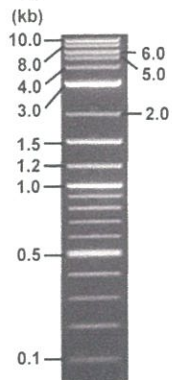
< Size of fragment expected from this clone >

DNA	1	ul
Enzyme (NotI + EcoRV)	0.5 + 0.5	ul
Buffer H	1	ul
dH <sub>2</sub> O	7	ul
Total	10	ul

NotI	3.3	kb
EcoRV	2.7, 0.61	kb
NotI + EcoRV	2.5kb (Vector)	0.61, 0.19 kb

Erectrophoresis : 1% agarose gel, 1x TAE Buffer

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



● Adjust plasmid DNA solution to 25 ng/ul

Date : 16.7.4

DNA ( <u>99</u> ng/ul)	<u>77.0</u>	ul
10x TE	<u>30.5</u>	ul
dH <sub>2</sub> O	<u>197.4</u>	ul
Total	<u>304.9</u>	ul

● Confirmation of the insertion sequence

Date : 16.7.1

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>

● Shipping

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

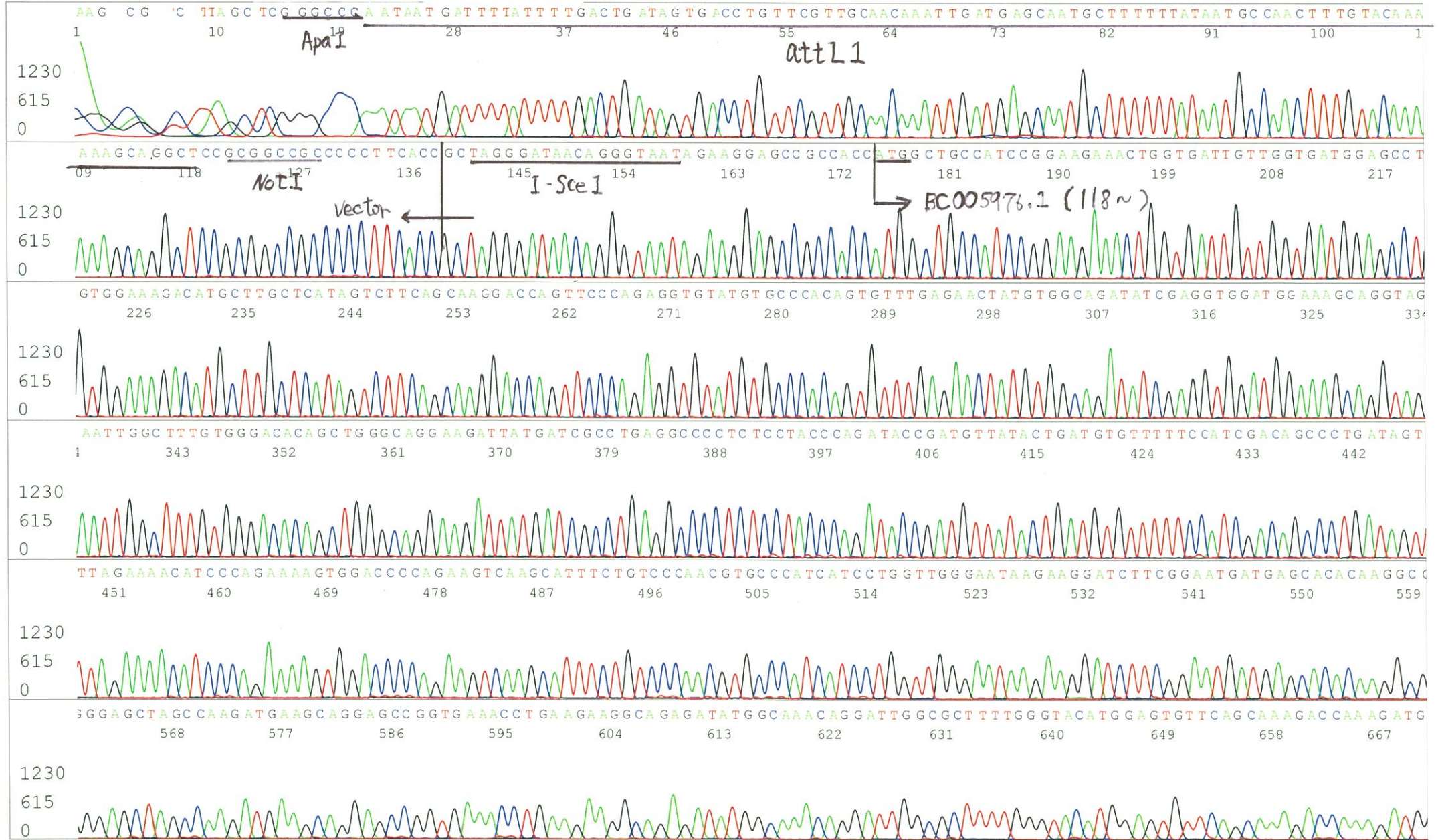


APPROVED BY : <http://dna.brc.riken.jp/index.html>

S/N G:708 A:560 T:354 C:372  
KB.bcp  
KB 1.4.1.8 Cap:4

Primer A : M13

5' GTTTTCCCAGTCACGACGTTGTA 3'

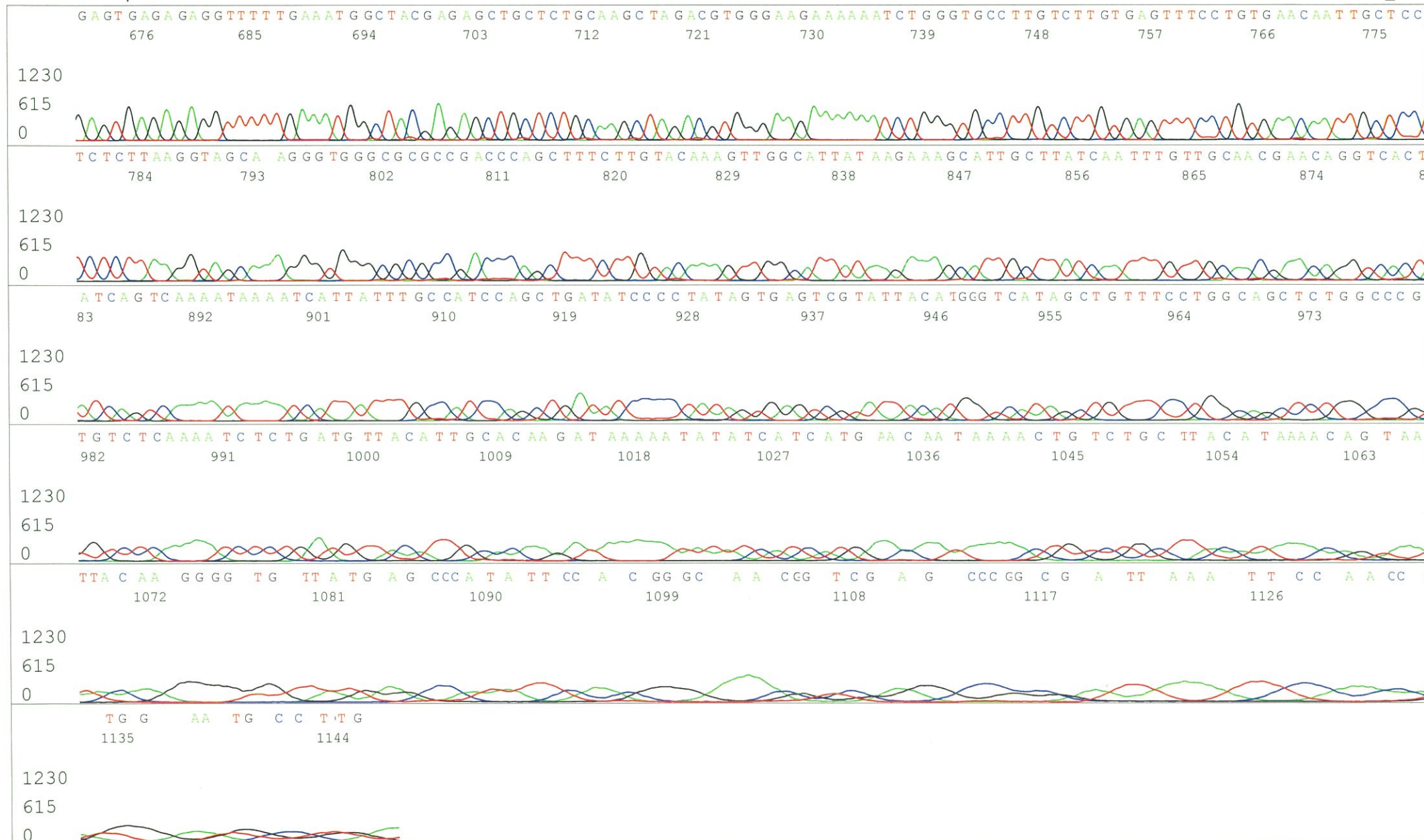




S/N G:708 A:560 T:354 C:372

KB.bcp

KB 1.4.1.8 Cap:4





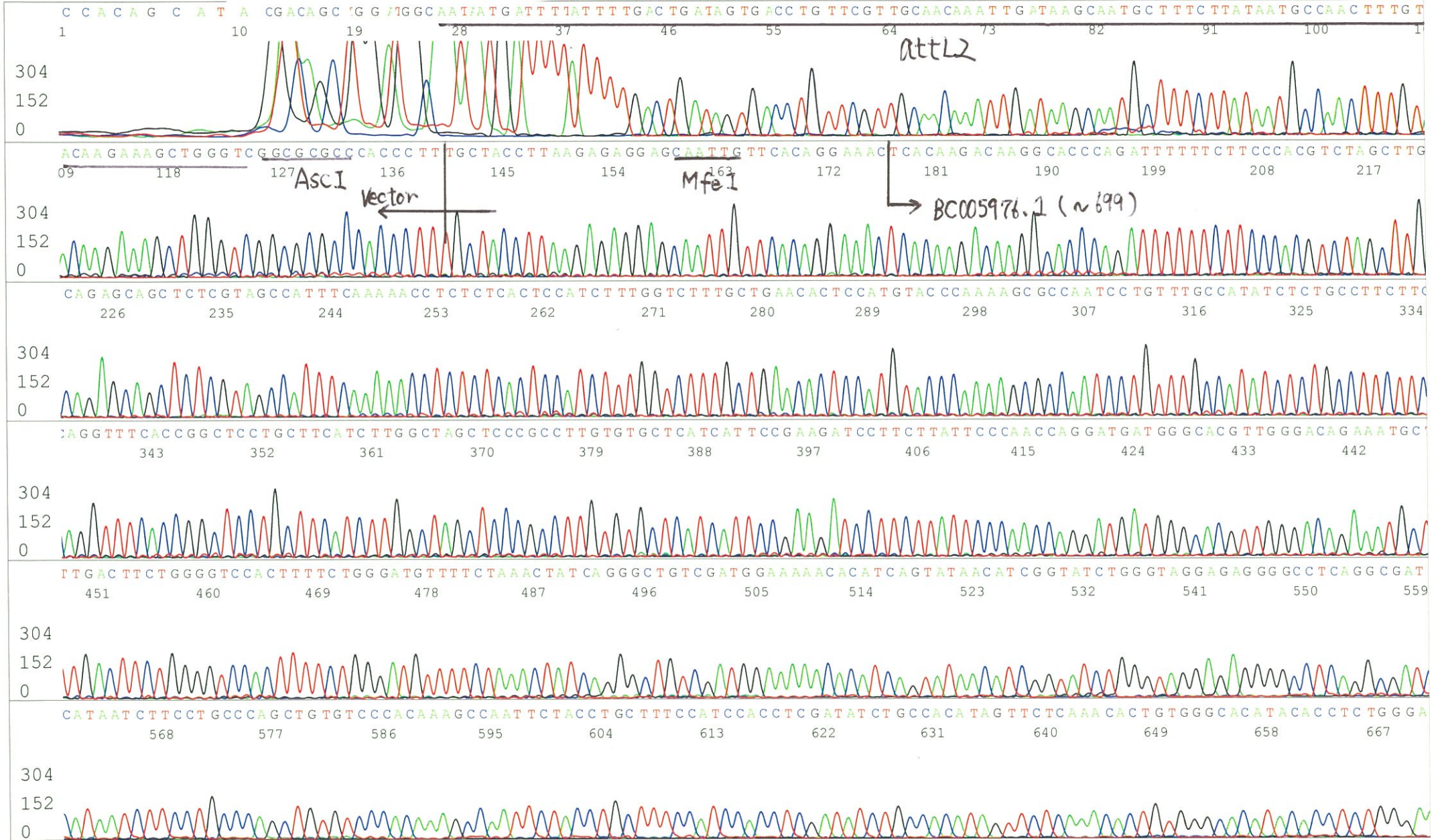
S/N G:850 A:630 T:535 C:524  
KB.bcp  
KB 1.4.1.8 Cap:7

Primer B : T7 long

5' CGCCAAGCTCTAATACGACTCACTATAGGG 3'

W01A091D06\_A6Fm\_T7long\_C01\_07  
W01A091D06\_A6Fm\_T7long  
KB\_3500\_POP7\_BDTv3.mob  
Pts 1260 to 12649 Pk1 Loc:1237  
Version 6.0 HiSQV Bases: 1008

Inst Model/Name 3500/3500 Instrument  
Jul 01,2016 11:46AM, JST  
Jul 01,2016 12:12PM, JST  
Spacing:11.3 Pts/Panel1350  
Plate Name: 20160701\_GNP





S/N G:850 A:630 T:535 C:524  
KB.bcp  
KB 1.4.1.8 Cap:7

W01A091D06\_A6Fm\_T7long\_C01\_07  
W01A091D06\_A6Fm\_T7long  
KB\_3500\_POP7\_BDTv3.mob  
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