

**RIKEN Clone ID : W01A049O20**

Vector : pENTR-TOPO

Gene	PRRT2	
Accession No.	BC011405.1	2372 bp
	<i>CDS</i>	1023 bp
		1..2372
		193..1215

● Plasmid DNA purification

Date : 131126

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

Date : 131127

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

● Digestion by restriction enzyme/Concentration calibration

Date : 131128

DNA concentration (O.D.): 119 ng/ul

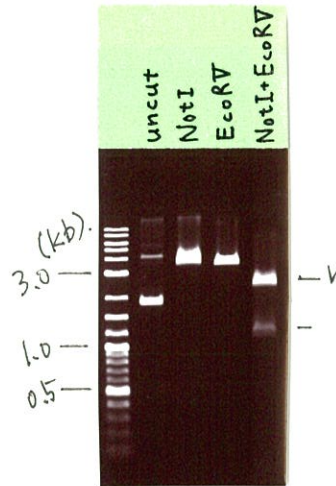
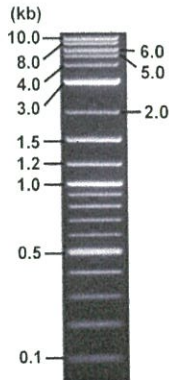
<Size of fragment expected from BC011405.1 >

DNA	0.4 ul
Enzyme (NotI + EcoRV)	0.5 + 0.5 ul
Buffer H	1 ul
dH <sub>2</sub> O	7.6 ul
Total	10 ul

NotI	3795 bp
EcoRV	3795 bp
NotI + EcoRV	2553bp (Vector)      1242 bp

Erectrophoresis : 1% agarose gel, 1x TAE Buffer

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



● Confirmation of the insertion sequence

Date : 131129

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>

● Adjustment of DNA concentration

Shipping amount : 40 ul

Concentration at the time of preparation of plasmid DNA : 25 ng/ul

Date : 131129

DNA ( <u>119</u> ng/ul )	85.0 ul
10x TE	40.5 ul
dH <sub>2</sub> O	279.1 ul
Total	404.6 ul



APPROVED BY :

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

S/N G:18 A:13 T:9 C:11

Primer A : M13

W01A049O20\_A3Kp\_M13(-40)

Nov 29,2013 12:57PM, JST

KB.bcp

5' GTTTTCCCAGTCACGACGTTGTA 3'

KB\_3500\_POP7\_BDTv3.mob

Nov 29,2013 01:24PM, JST

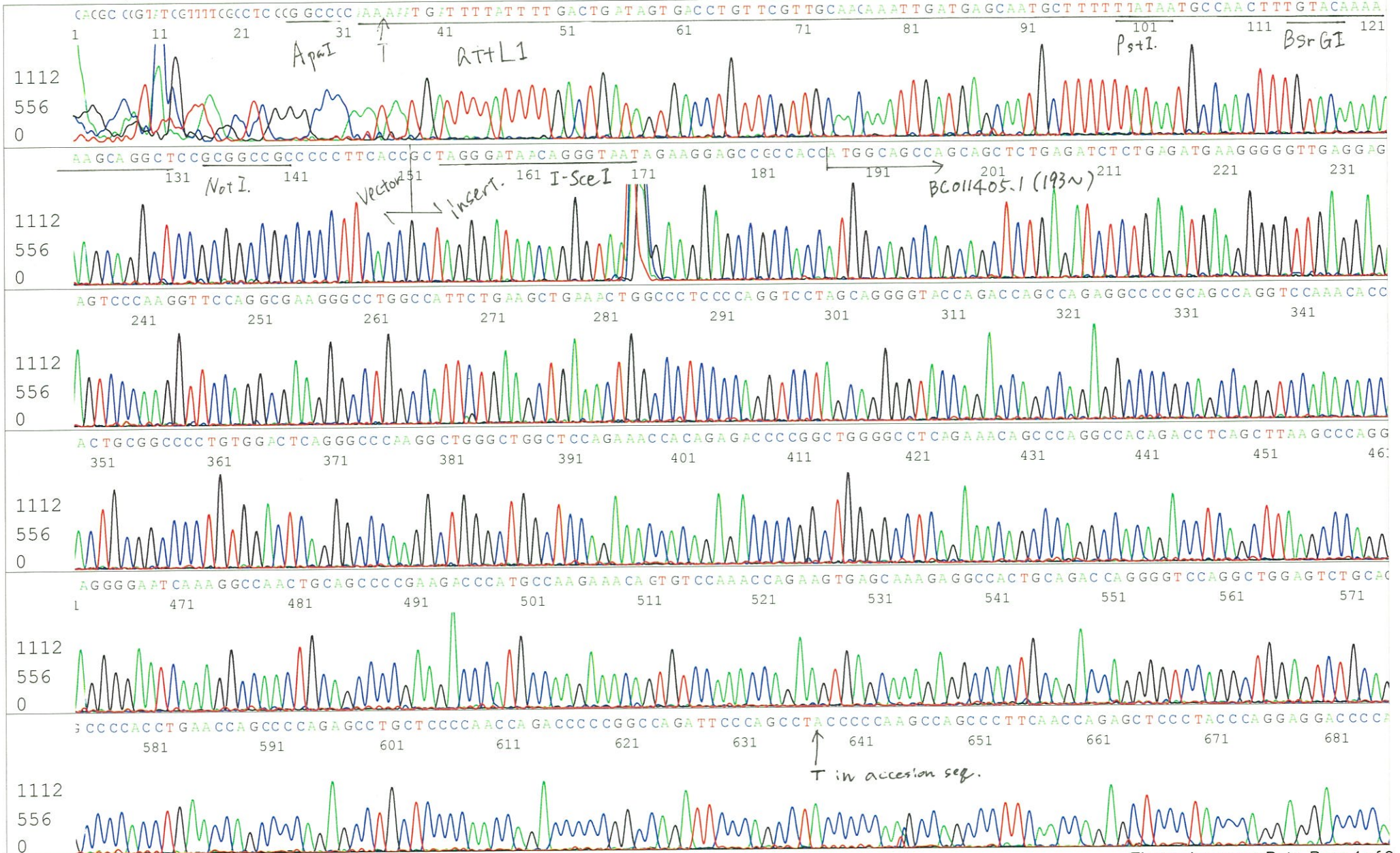
KB 1.4.1.8 Cap:13

Pts 1224 to 12980 Pk1 Loc:1201

Spacing:11.57 Pts/Panel1350

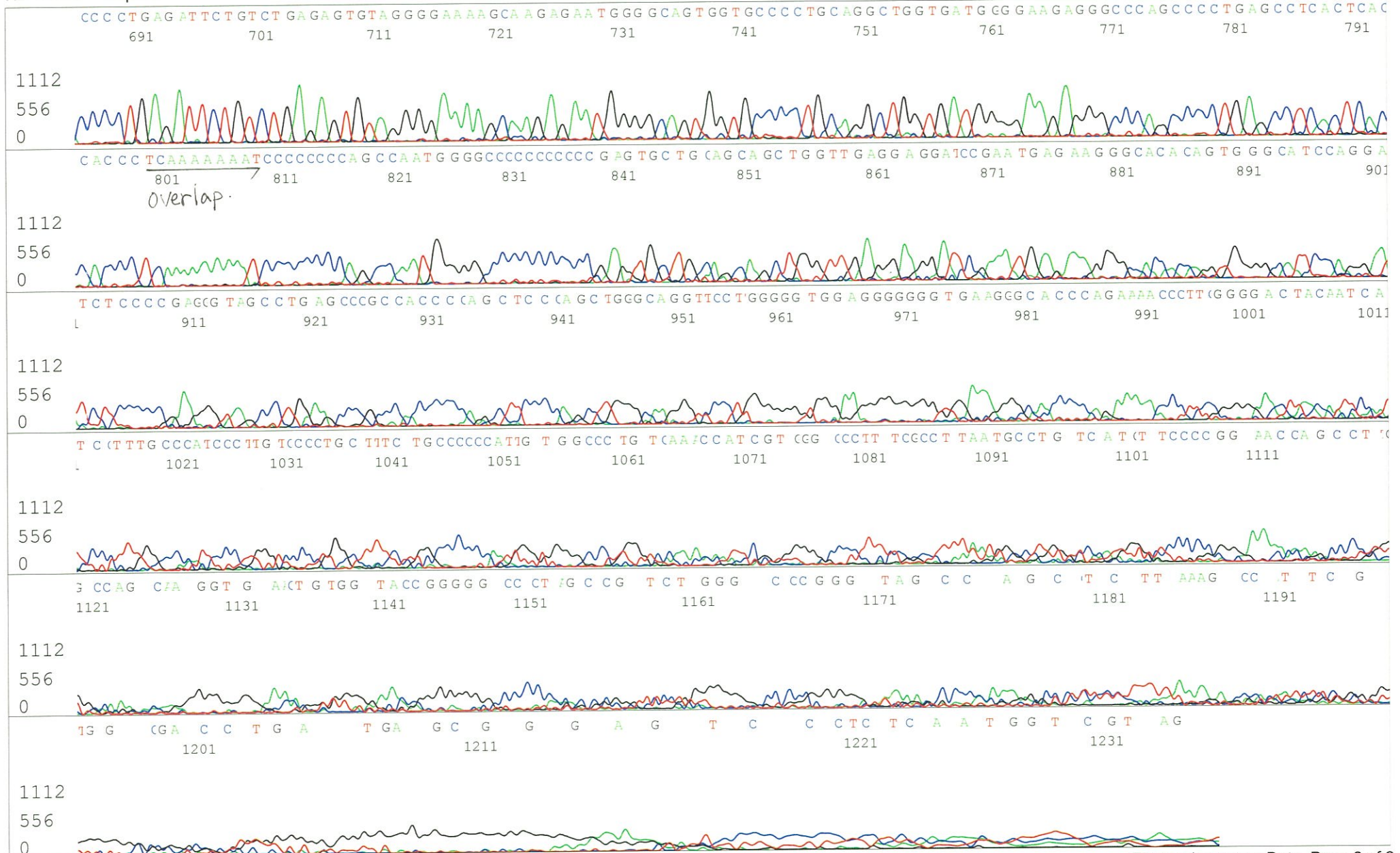
Version 5.4 HiSQV Bases: 1007

Plate Name: 20131129\_mix





S/N G:18 A:13 T:9 C:11  
KB.bcp  
KB 1.4.1.8 Cap:13

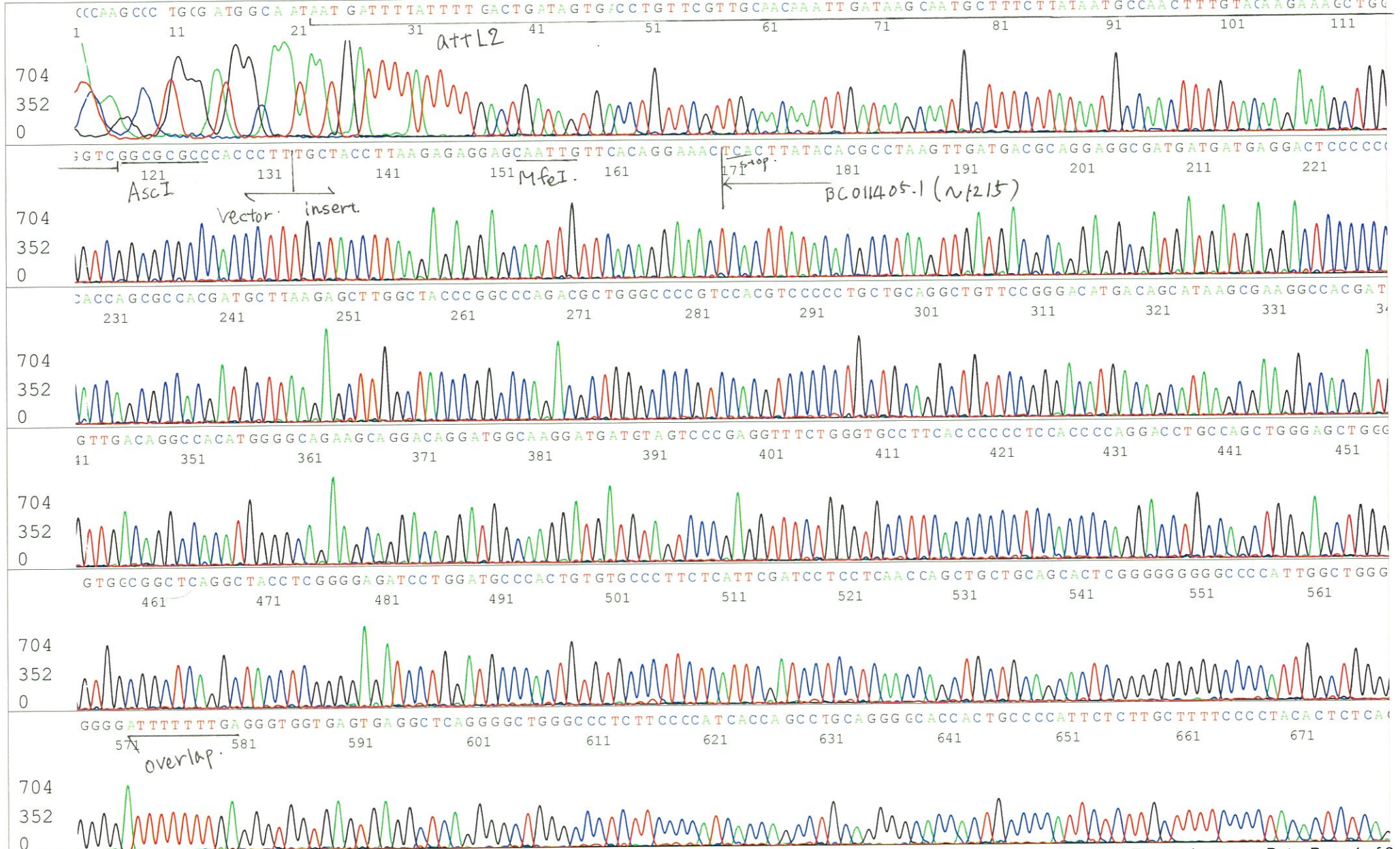




S/N G:40 A:20 T:22 C:17  
KB.bcp  
KB 1.4.1.8 Cap:16

Primer B : T7 long

5' CGCCAAGCTCTAATACGACTCACTATAGGG 3'





S/N G:40 A:20 T:22 C:17  
KB.bcp  
KB 1.4.1.8 Cap:16

