

**RIKEN Clone ID : IRAL006G22**

Lot#: 7566\_A6Ku

Vector : pOTB7

Gene	HSPA2	
Accession No.	BC001752.1	2496 bp
	<i>CDS</i>	1920 bp

● Plasmid DNA purification

Date : 161205

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

Date : 161206

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

● Digestion by restriction enzyme/Concentration calibration

Date : 161206

DNA concentration (O.D.): 64 ng/ul

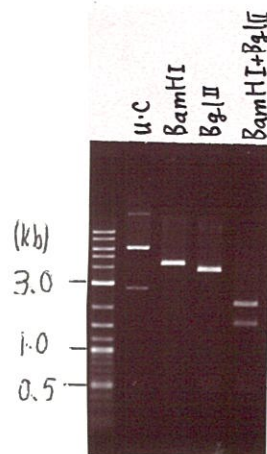
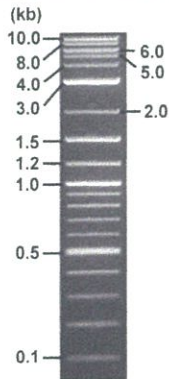
< Size of fragment expected from this clone >

DNA	1.5	ul
Enzyme (BamHI + BglII)	0.5 + 0.5	ul
Buffer H		1 ul
dH <sub>2</sub> O	6.5	ul
Total		10 ul

BamHI	4.3 kb	
BglII	3.8, 0.50 kb	
BamHI + BglII	1.6kb (Vector)	2.2, 0.50 kb

Electrophoresis : 1% agarose gel, 1x TAE Buffer

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



● Adjust plasmid DNA solution to 25 ng/ul

Date : 161209

● Confirmation of the insertion sequence

Date : 161208

DNA ( <u>64</u> ng/ul)	<u>80.0</u>	ul
10x TE	<u>20.5</u>	ul
dH <sub>2</sub> O	<u>104.3</u>	ul
Total	<u>204.8</u>	ul

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 :

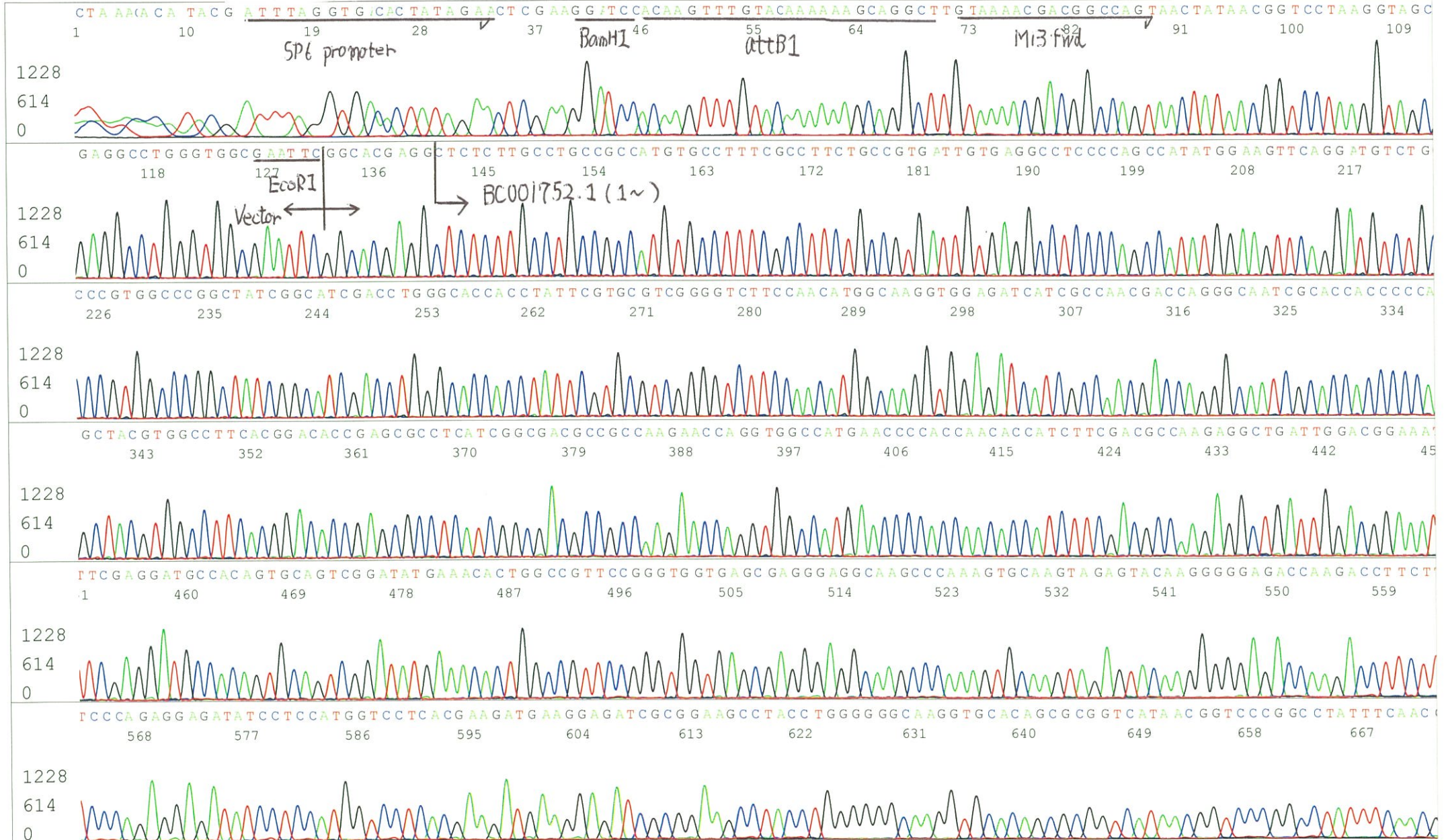
<http://dna.brc.riken.jp/index.html>



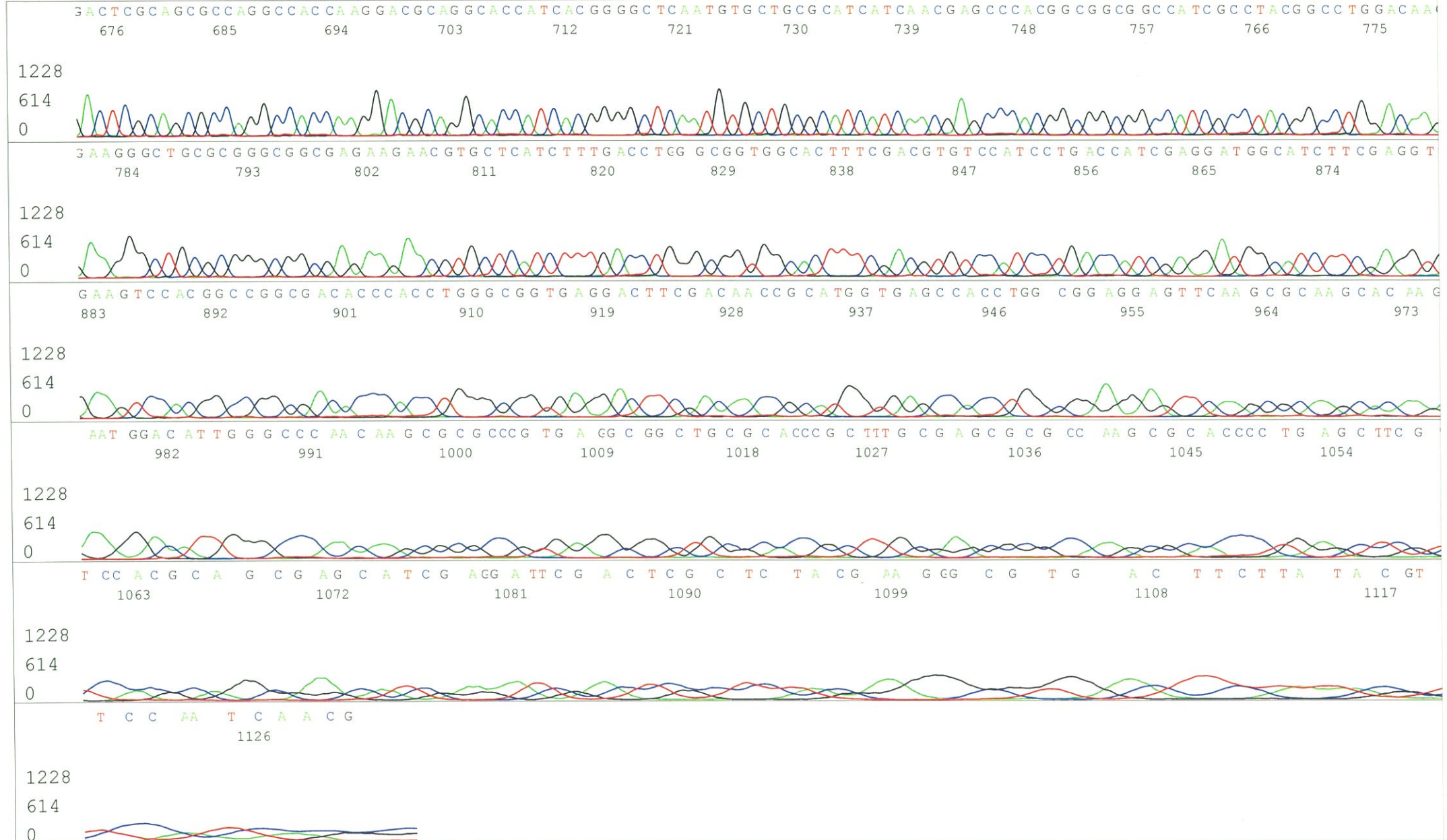
S/N G:409 A:463 T:344 C:640  
KB.bcp  
KB 1.4.1.8 Cap:10

Primer A: pOTB7-F

5' AACGCGGCTACAATTAATACATAACC 3'



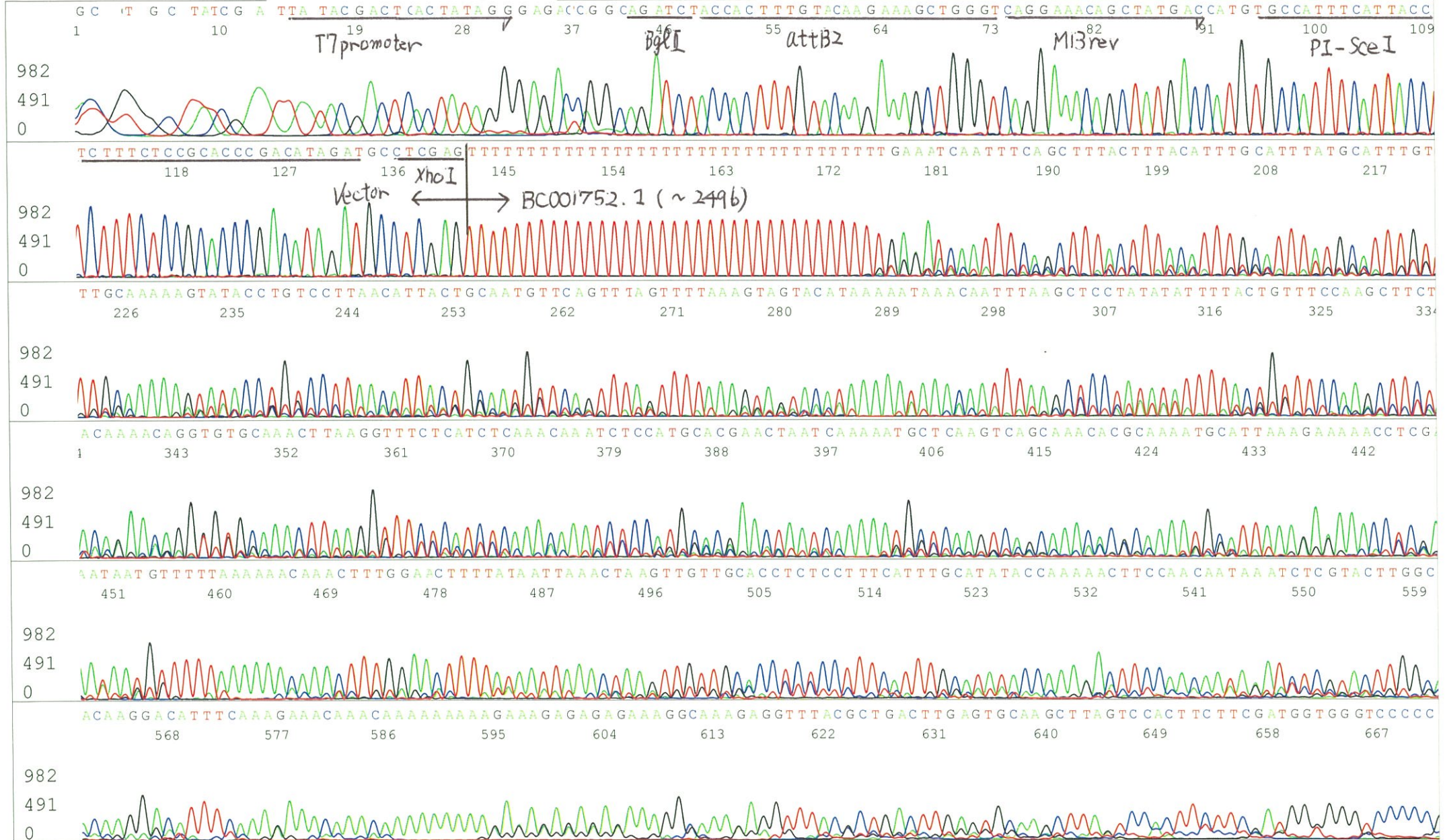






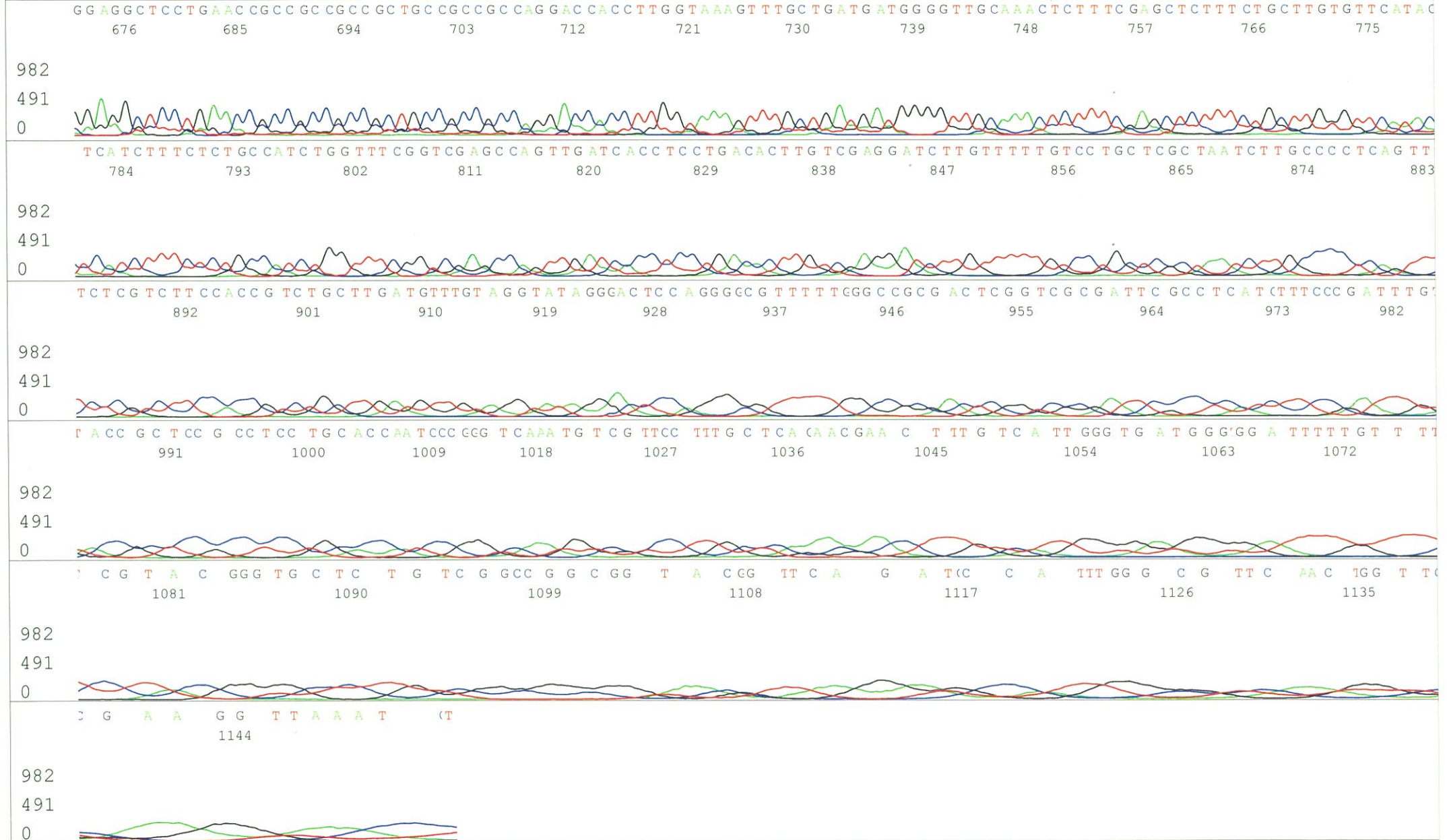
S/N G:240 A:332 T:415 C:388  
KB.bcp  
KB 1.4.1.8 Cap:13

Primer B: pOTB7-R  
5' GTACTGCAGCCGATTCATTAATGC 3'





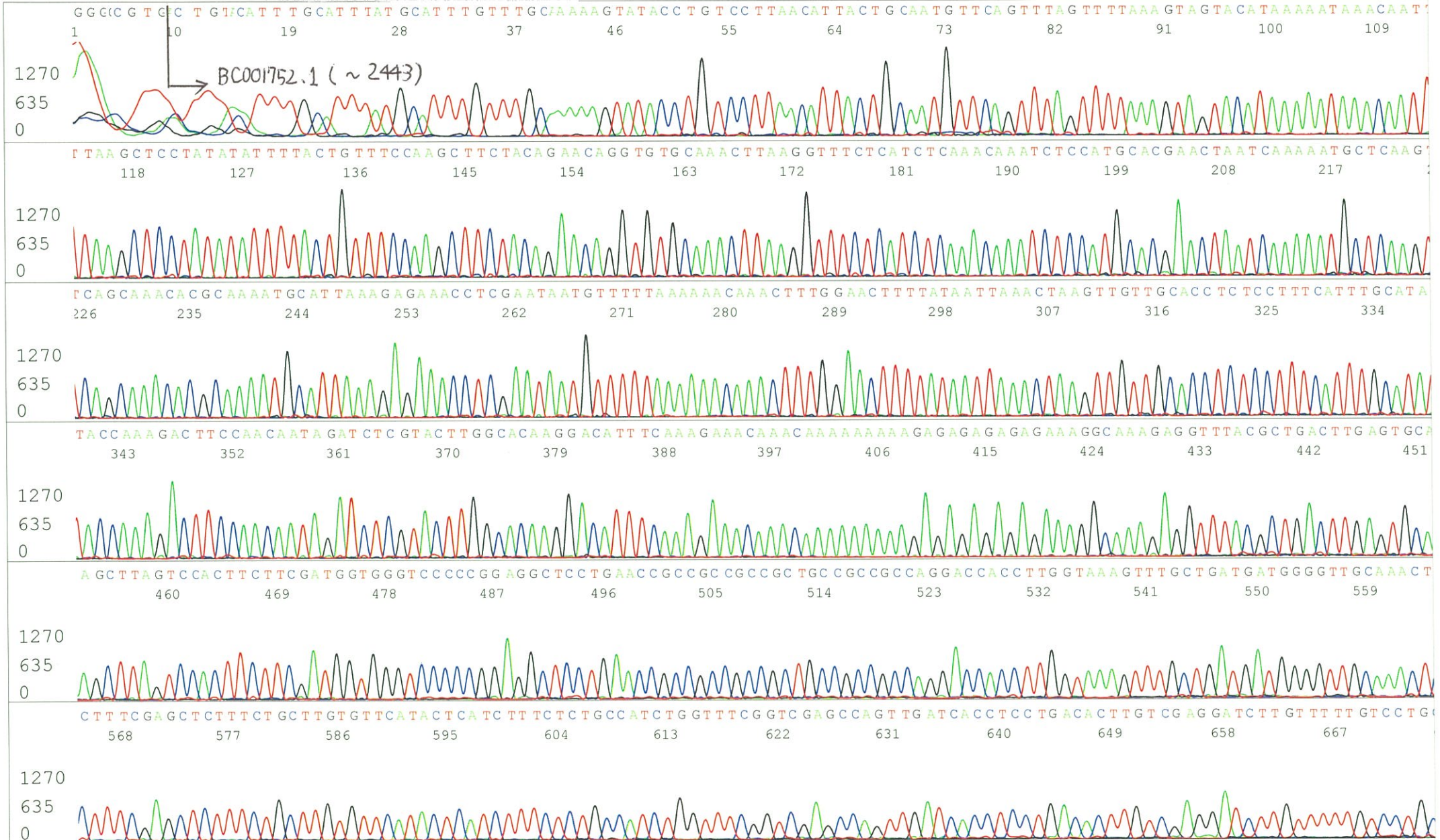
S/N G:240 A:332 T:415 C:388  
KB.bcp  
KB 1.4.1.8 Cap:13





S/N G:92 A:110 T:121 C:130  
KB.bcp  
KB 1.4.1.8 Cap:16

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





S/N G:92 A:110 T:121 C:130  
KB.bcp  
KB 1.4.1.8 Cap:16

