

**RIKEN Clone ID : IRAL004D12**

Lot#: 7566\_A5Kk

Vector : pOTB7

Gene	GCDH	
Accession No.	BC002579.2	1843 bp
	CDS	1317 bp

● Plasmid DNA purification

Date : 15/201

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

Date : 15/202

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

● Digestion by restriction enzyme/Concentration calibration

Date : 15/202

DNA concentration (O.D.): 255 ng/ul

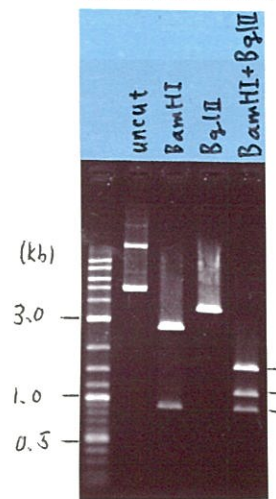
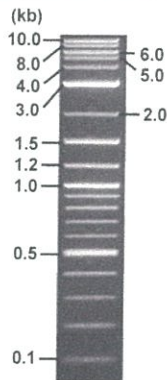
< Size of fragment expected from this clone >

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

BamHI	2.7, 0.90 kb
BglII	3.6 kb
BamHI + BglII	1.6kb (Vector) 1.1, 0.90kb

Electrophoresis : 1% agarose gel, 1x TAE Buffer

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



● Adjust plasmid DNA solution to 25 ng/ul

Date : 15/204

DNA ( 255 ng/ul)	82.0 ul
10x TE	83.6 ul
dH <sub>2</sub> O	670.8 ul
Total	836.4 ul

● Confirmation of the insertion sequence

Date : 15/203

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>

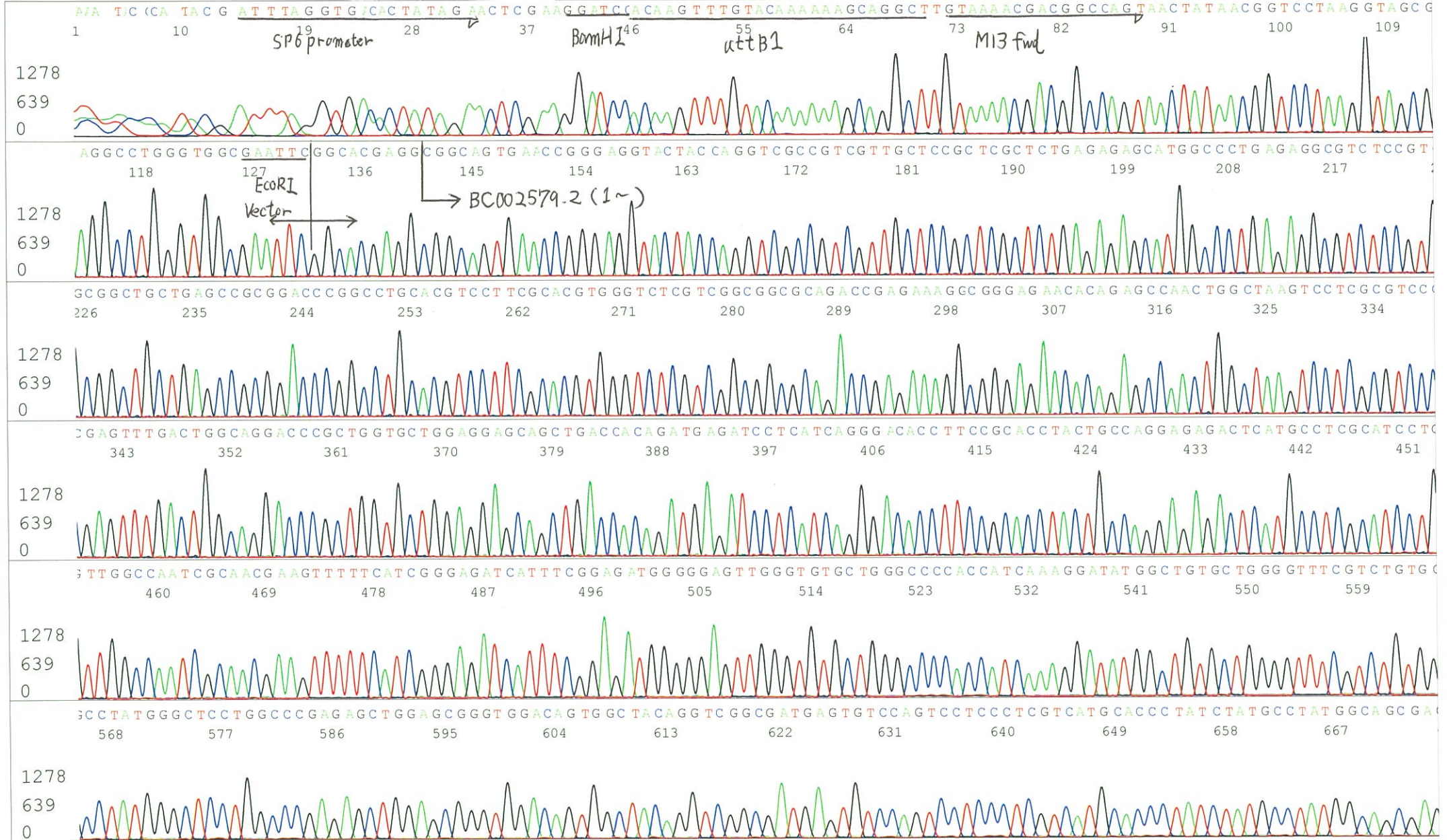


S/N G:380 A:350 T:282 C:318  
KB.bcp  
KB 1.4.1.8 Cap:1

Primer A: pOTB7-F  
5' AACGCGGCTACAATTAATACATAACC 3'

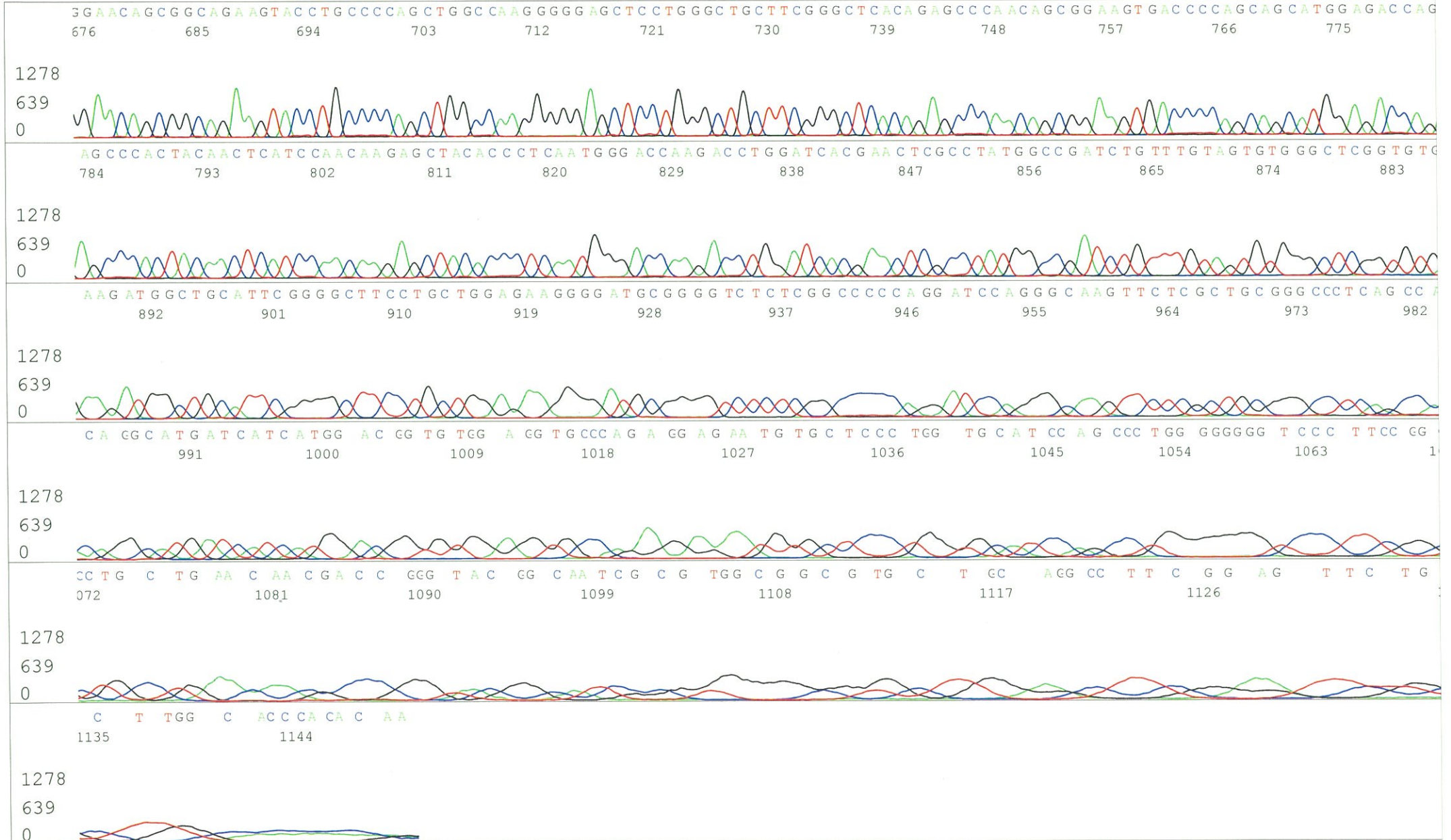
IRAL004D12\_A5Kk\_6\_pOTB7\_F\_A07\_01  
IRAL004D12\_A5Kk\_6\_pOTB7\_F  
KB\_3500\_POP7\_BDTv3.mob  
Pts 1350 to 12836 Pk1 Loc:1327  
Version 6.0 HiSQV Bases: 1004

Inst Model/Name 3500/3500 Instrument  
Dec 03,2015 02:03PM, JST  
Dec 03,2015 02:29PM, JST  
Spacing:11.44 Pts/Panel1350  
Plate Name: 20151203\_mix





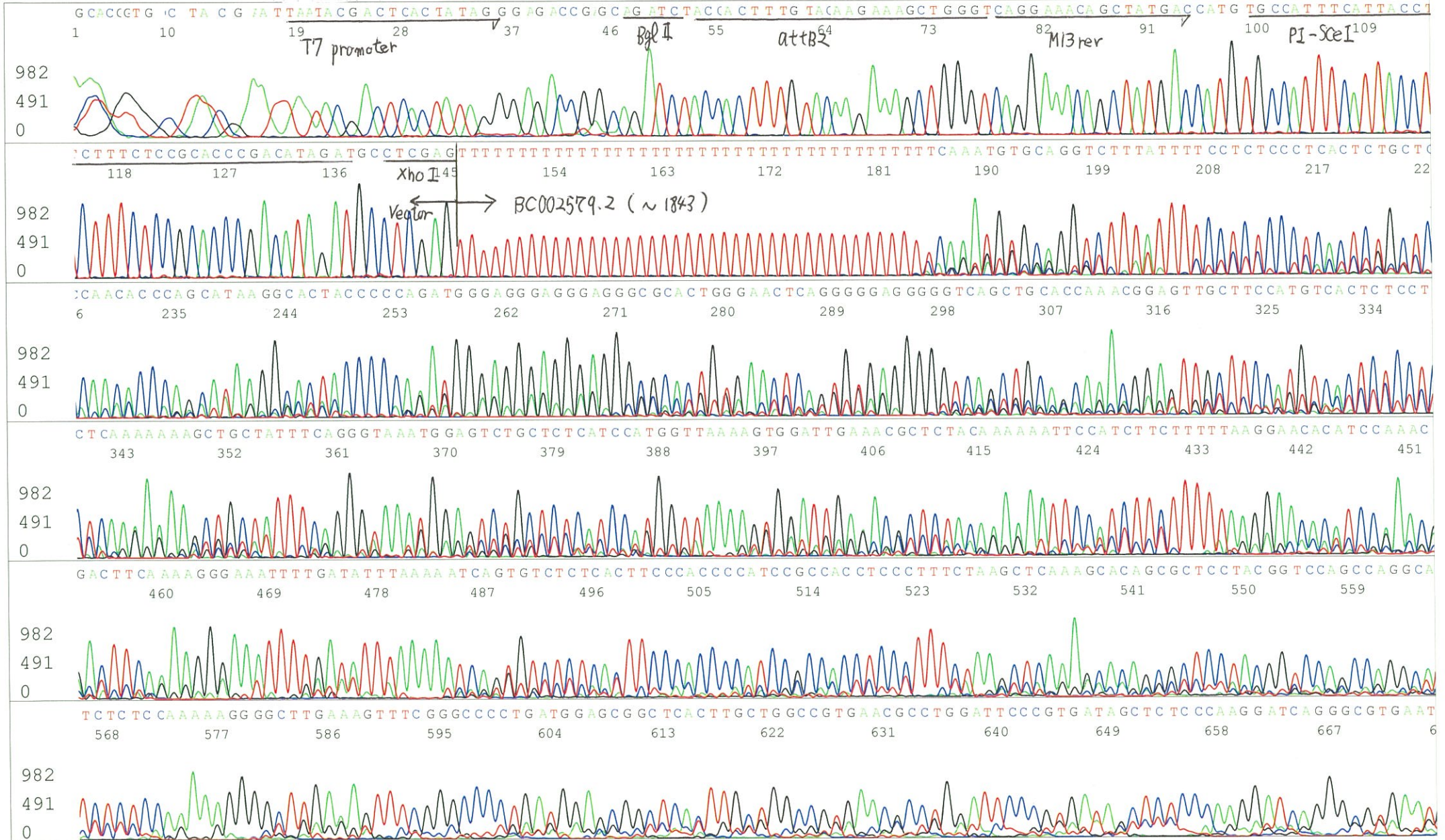
S/N G:380 A:350 T:282 C:318  
KB.bcp  
KB 1.4.1.8 Cap:1





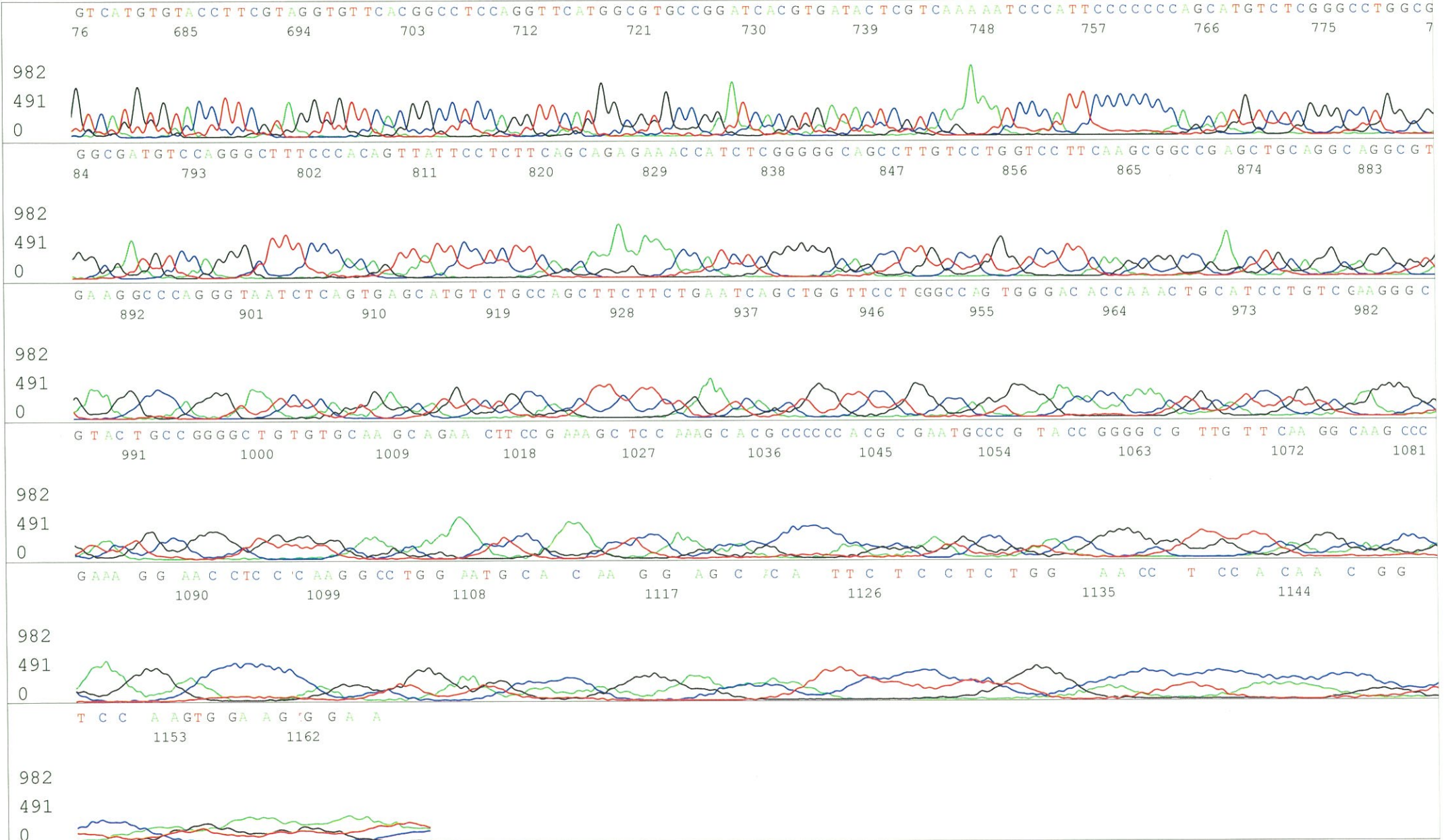
S/N G:88 A:91 T:94 C:89  
KB.bcp  
KB 1.4.1.8 Cap:4

Primer B: pOTB7-R  
5' GTACTGCAGCCGATTCATTAATGC 3'





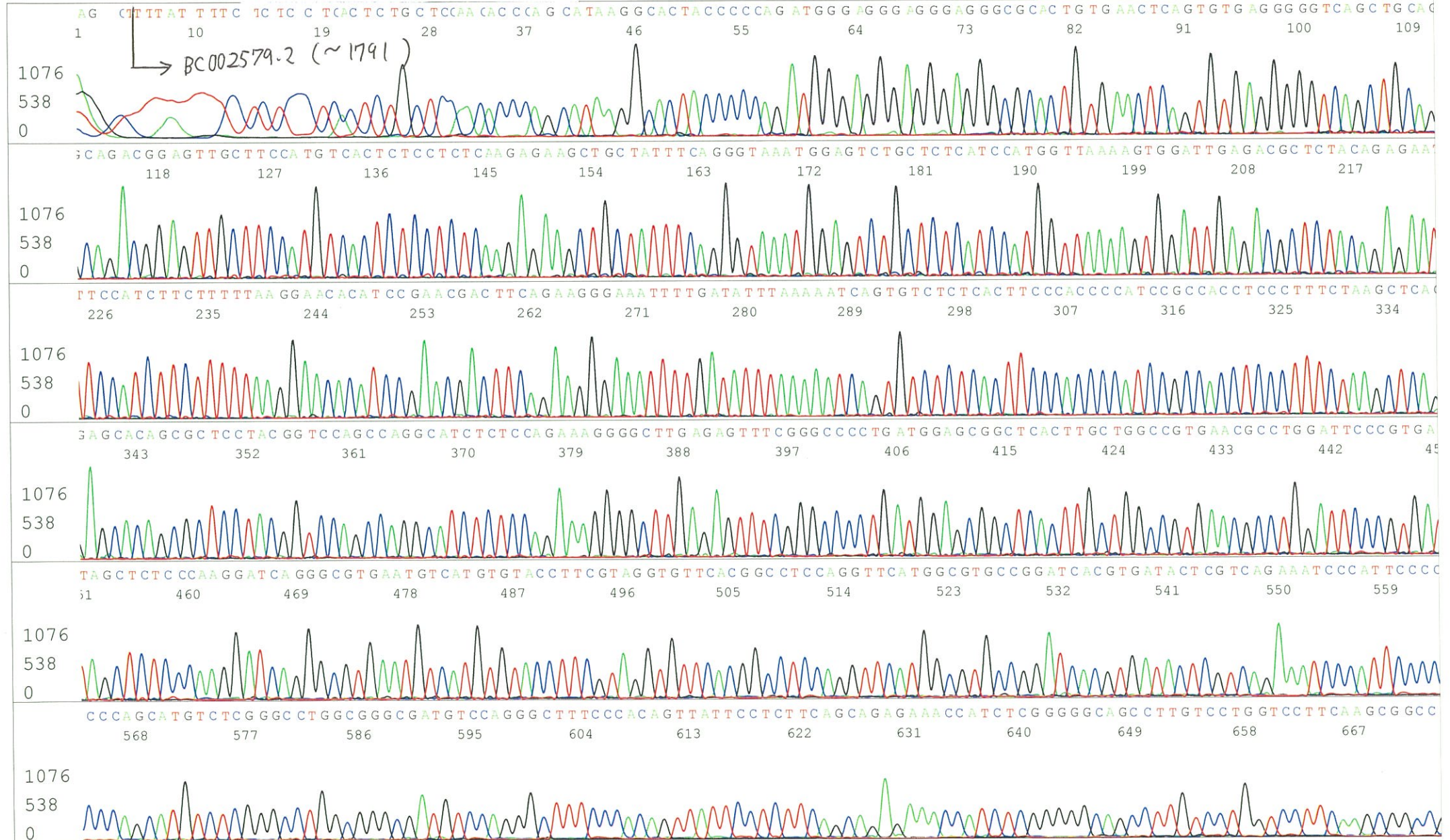
S/N G:88 A:91 T:94 C:89  
KB.bcp  
KB 1.4.1.8 Cap:4





S/N G:610 A:522 T:508 C:561  
KB.bcp  
KB 1.4.1.8 Cap:7

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





S/N G:610 A:522 T:508 C:561  
KB.bcp  
KB 1.4.1.8 Cap:7

