

**RIKEN Clone ID : W01A035E07**

Vector : pENTR-TOPO

Gene	CD40LG	
Accession No.	BC071754.1	1859 bp
	<i>CDS</i>	786 bp
		1..1859
		73..858

● Plasmid DNA purification

Date : 140430

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

Date : 140501

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

● Digestion by restriction enzyme/Concentration calibration

Date : 140501

DNA concentration (O.D.): 114 ng/ul

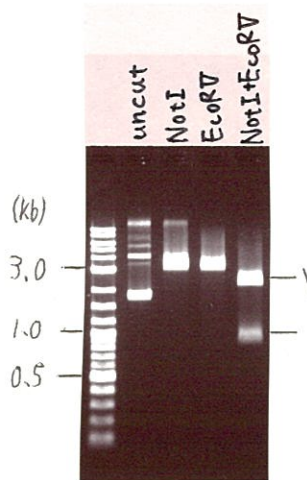
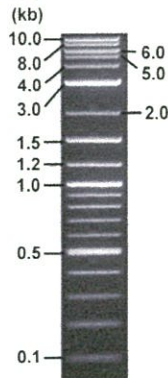
<Size of fragment expected from BC071754.1 >

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	3558 bp
EcoRV	3558 bp
NotI + EcoRV	2553bp (Vector)      1005 bp

Erectrophoresis : 1% agarose gel, 1x TAE Buffer

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



● Adjust plasmid DNA solution to 25 ng/ul

Date : 140507

● Confirmation of the insertion sequence

Date : 140502

DNA ( <u>114</u> ng/ul )	90.0 ul
10x TE	41.0 ul
dH <sub>2</sub> O	279.4 ul
Total	410.4 ul

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

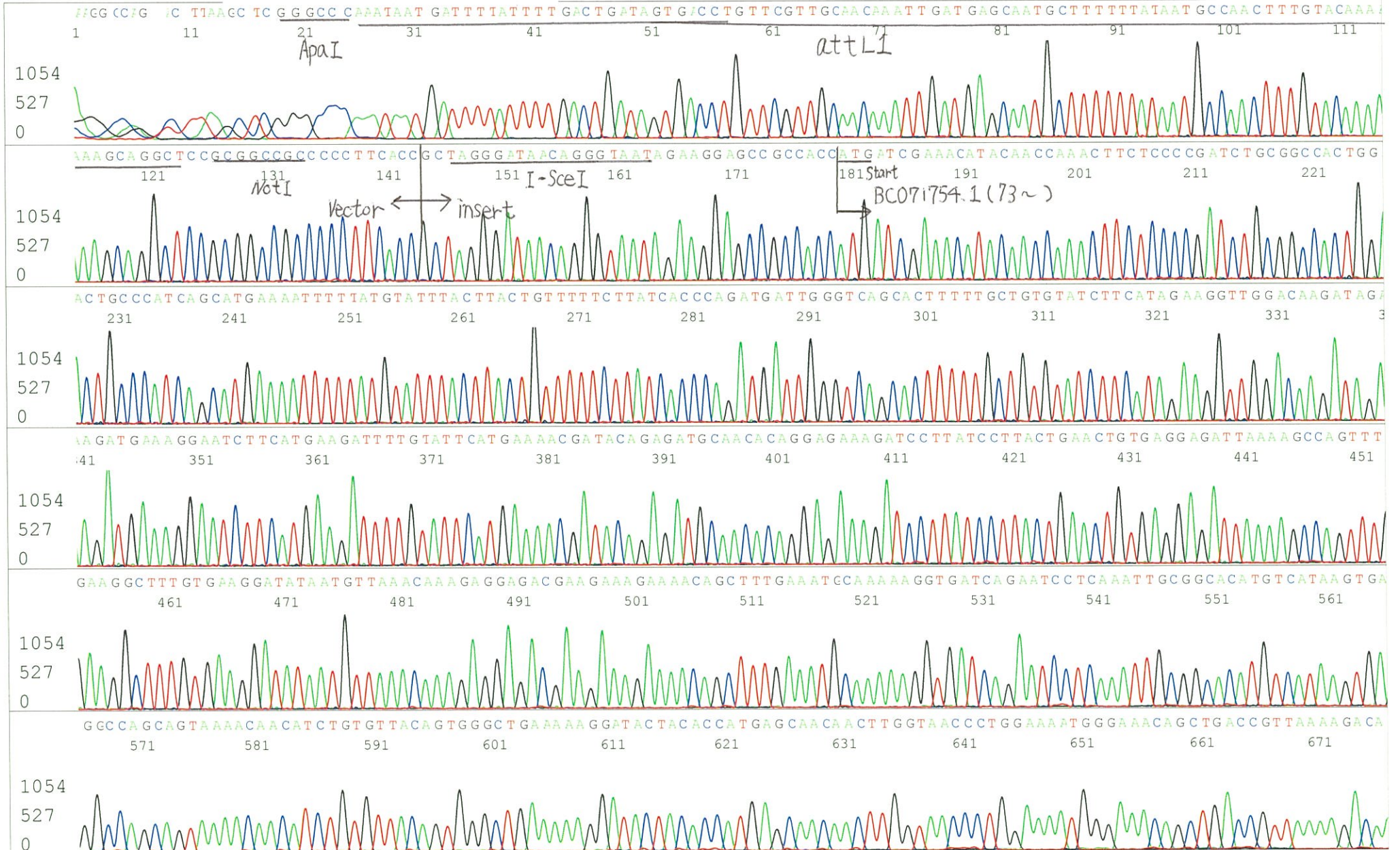
Conc. : 25 ng/ul, Volume : 40 ul

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

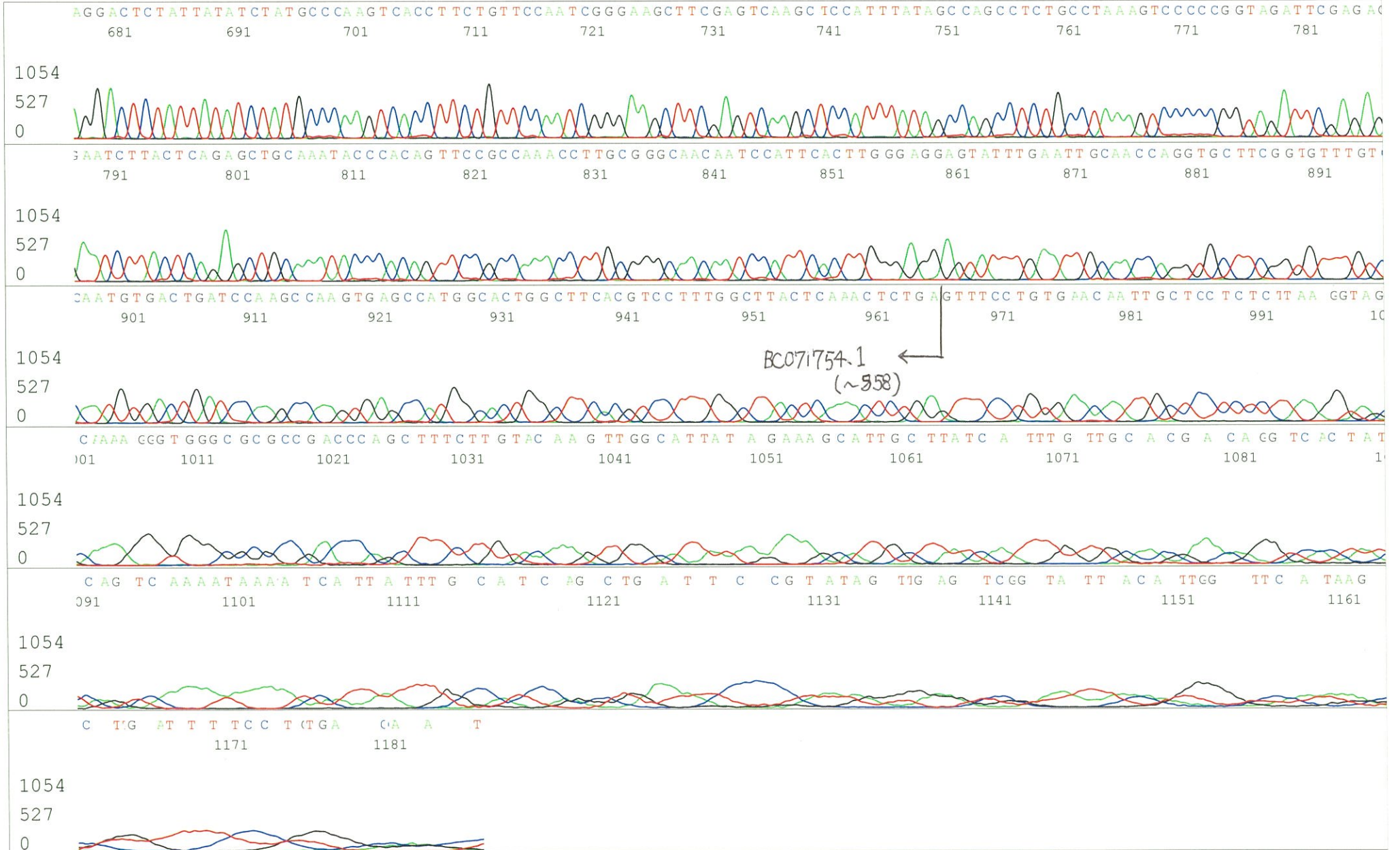


APPROVED BY : <http://dna.brc.riken.jp/index.html>

S/N G:114 A:138 T:103 C:116  
KB.bcp  
KB 1.4.1.8 Cap:15  
Primer A : M13  
5' GTTTTCCCAGTCACGACGTTGTA 3'



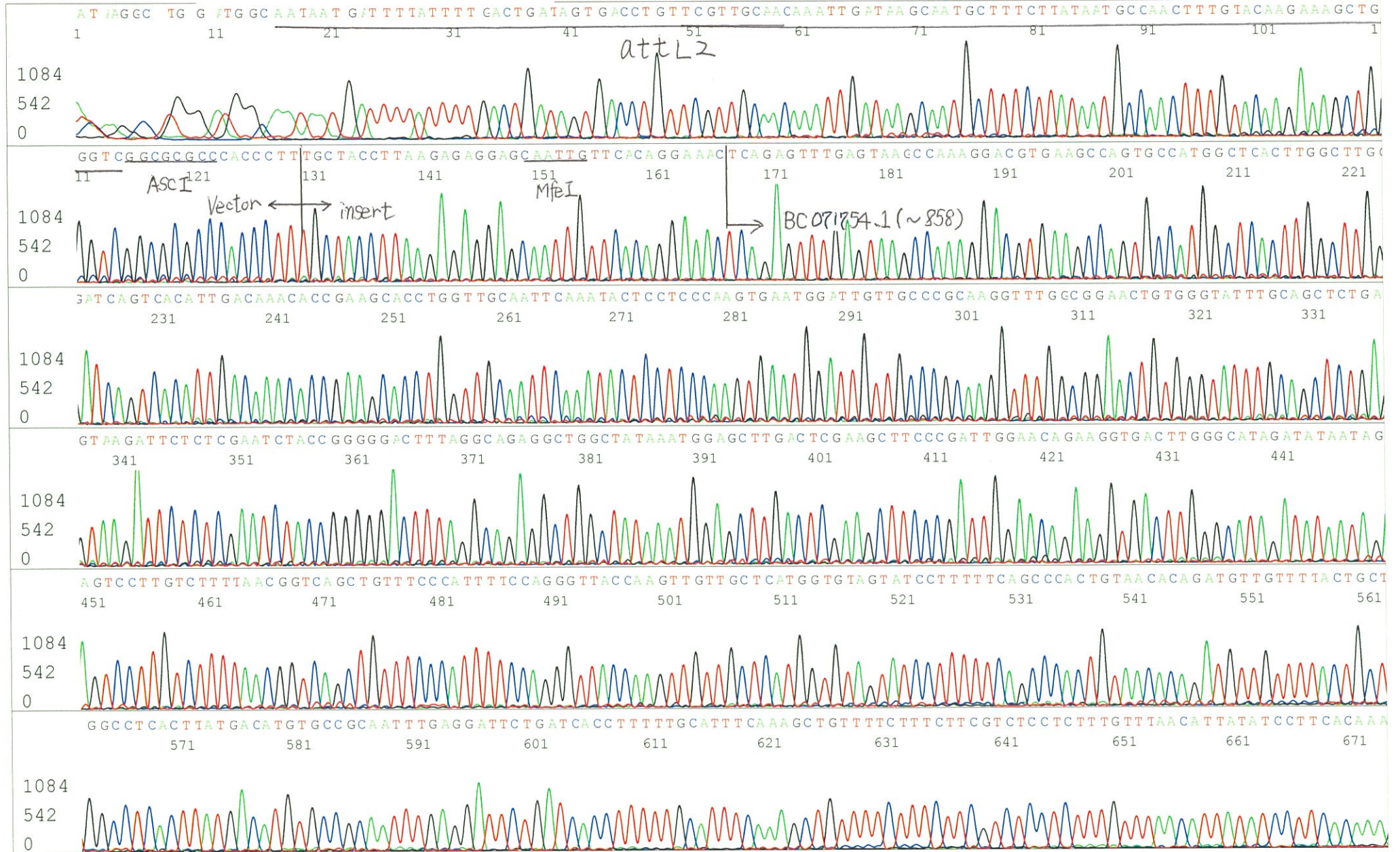
S/N G:114 A:138 T:103 C:116  
KB.bcp  
KB 1.4.1.8 Cap:15



S/N G:98 A:93 T:103 C:99  
KB.bcp  
KB 1.4.1.8 Cap:18

Primer B : T7 long

5' CGCCAAGCTCTAATACGACTCACTATAGGG 3'



S/N G:98 A:93 T:103 C:99  
KB.bcp  
KB 1.4.1.8 Cap:18

