

**RIKEN Clone ID : W01A070C13**

Vector : pENTR-TOPO

Gene	GLS2	
Accession ID of Origin	AK000467.1	2403 bp
	<i>CDS</i>	1809 bp

● Plasmid DNA purification

Date : 22.02.08

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

Date : 22.02.09

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

● Digestion by restriction enzyme/Concentration calibration

Date : 22.02.09

DNA concentration (O.D.): 144 ng/ul

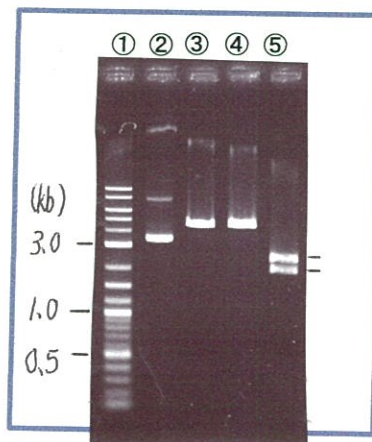
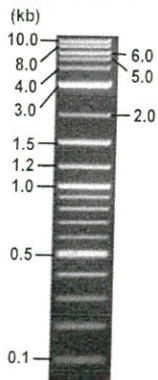
< Size of fragment expected from this clone >

DNA	<u>0.7</u> ul
Enzyme (NotI + EcoRV)	<u>0.5 + 0.5</u> ul
Buffer H	<u>1</u> ul
dH <sub>2</sub> O	<u>7.3</u> ul
Total	<u>10</u> ul

NotI	4.5 kb
EcoRV	4.5 kb
NotI + EcoRV	2.5kb (Vector) 2.0 kb

Electrophoresis : 1% agarose gel, 1x TAE Buffer

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



- ①:Marker
- ②:uncut
- ③:NotI
- ④:EcoRV
- ⑤:NotI+EcoRV

● Adjust plasmid DNA solution to 25 ng/ul

Date : 22, 02, 10

DNA ( <u>144</u> ng/ul)	<u>84.0</u> ul
10x TE	<u>98.3</u> ul
dH <sub>2</sub> O	<u>351.5</u> ul
Total	<u>483.8</u> ul

● Confirmation of the insertion sequence

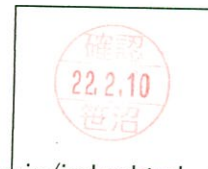
Date : 22, 02, 10

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/μl, Volume : 40 μl
- Conc. :      ng/μl, Volume :      μl

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



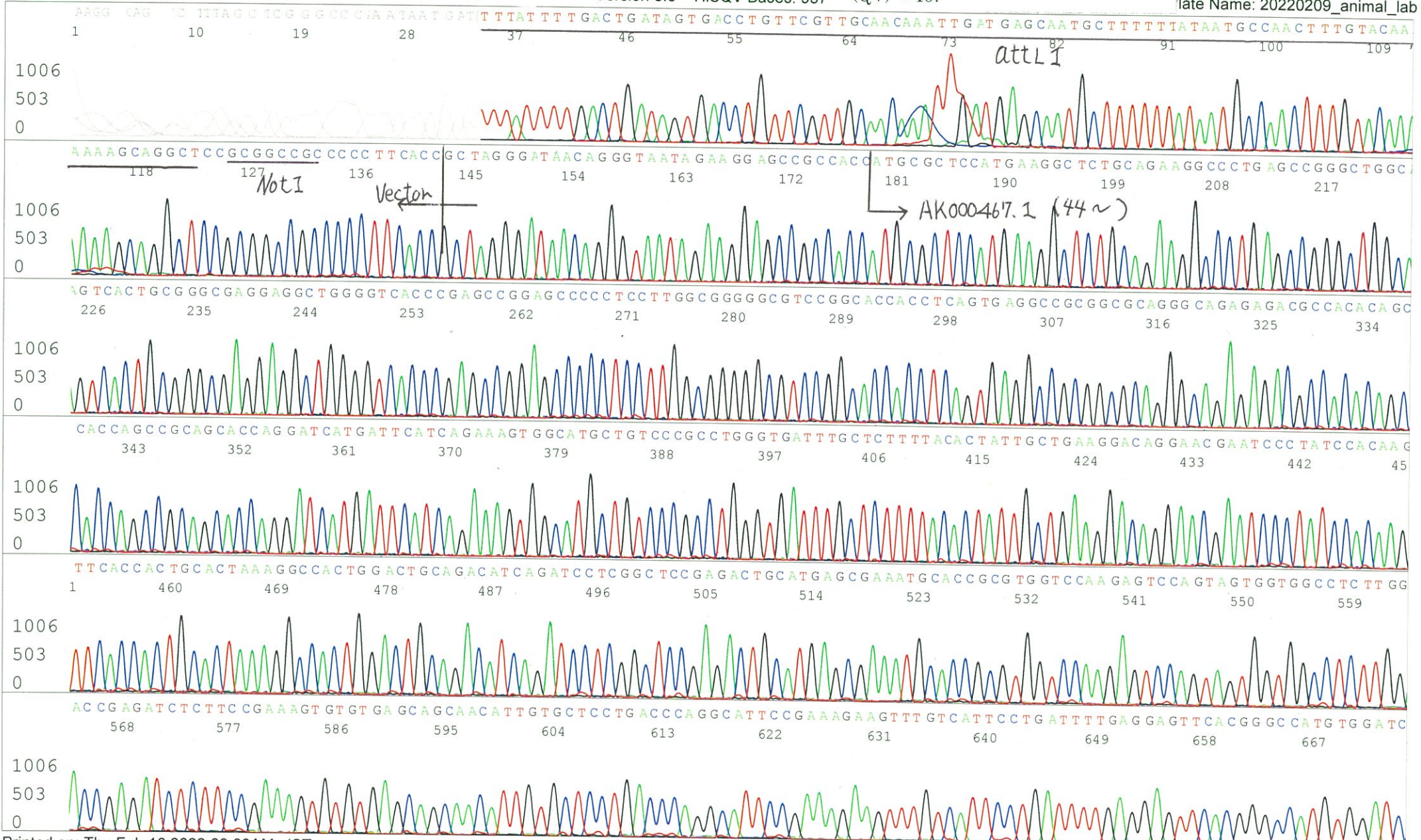


S/N G:161 A:188 T:109 C:18  
KB.bcp  
KB 1.4.1.8 Cap:1

Primer A : M13

5' GTTTTCCCAGTCACGACGTTGTA 3'

Gray line indicates quality value





S/N G:9 A:10 T:8 C:11  
KB.bcp  
KB 1.4.1.8 Cap:4

Primer B: T7 long

5' CGCCAAGCTCTAATACGACTCACTATAGGG 3'

Gray line indicates quality value  
(QV) < 40.

