

**RIKEN Clone ID : W01A097D18**

Vector : pENTR/D-TOPO

Gene	ESX1	
Accession No.	BC042633.1	1527 bp
	<i>CDS</i>	1221 bp
		31..1251

● Plasmid DNA purification

Date : 121219

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

Date : 121220

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

● Digestion by restriction enzyme/Concentration calibration

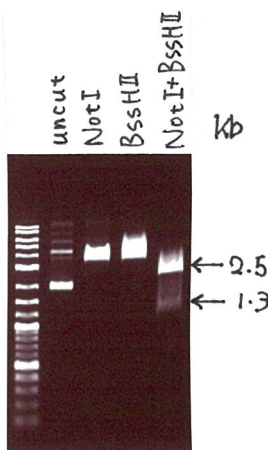
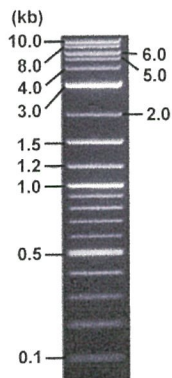
Date : 121220

DNA concentration (O.D.): 79 ng/ul

DNA	0.6 ul
Enzyme (NotI + BssHII)	0.5 + 0.5 ul
Buffer H	1 ul
dH <sub>2</sub> O	7.4 ul
Total	10 ul

Erectrophoresis : 1% agarose gel, 1x TAE Buffer

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



<Expected size of fragment from BC042633.1 CDS>

2553, 1321 bp

● Confirmation of the insertion sequence

Date : 121225

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>

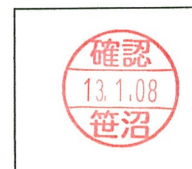
● Adjustment of DNA concentration

Shipping amount : 40 ul

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

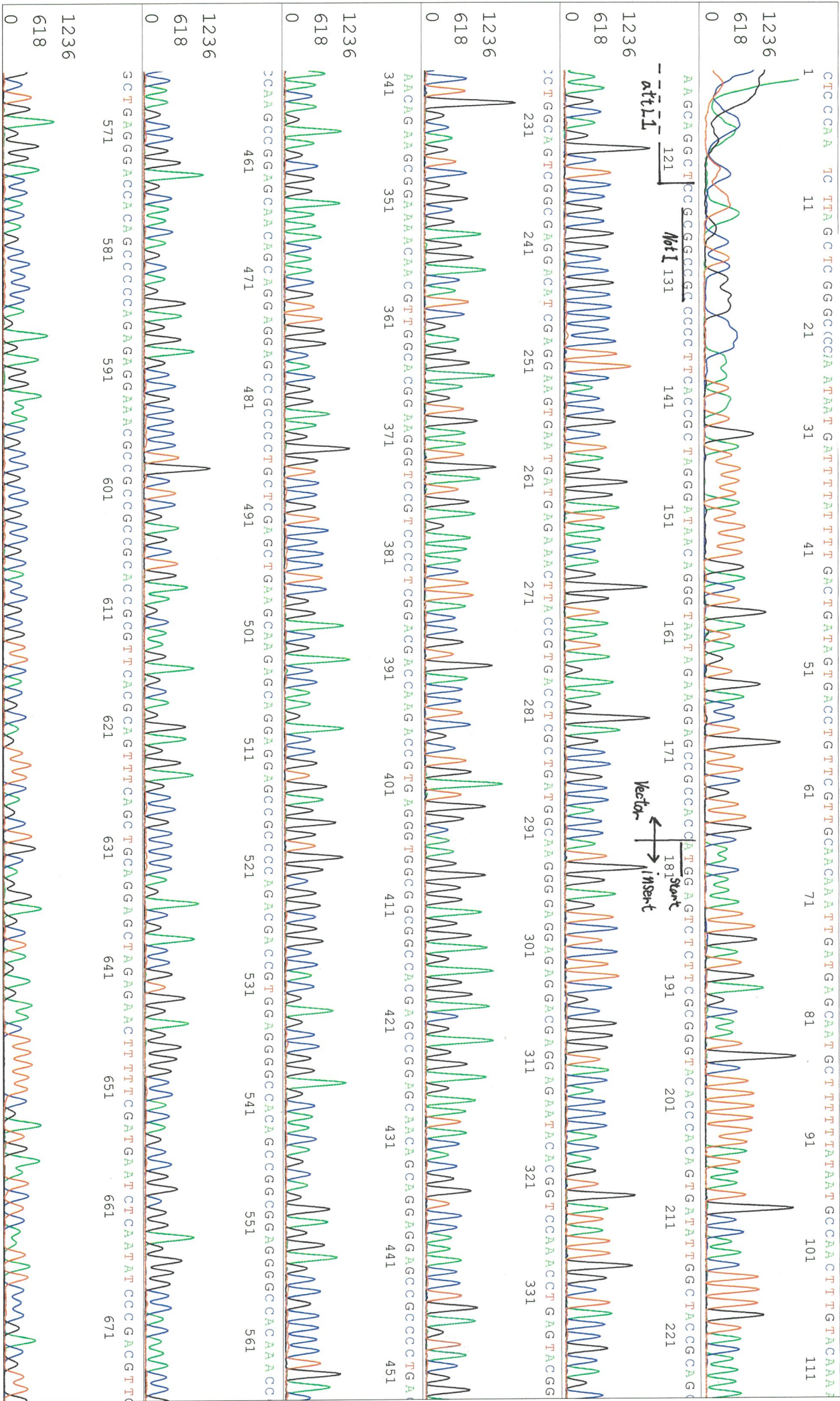
Date : 130108

DNA ( <u>79</u> ng/ul )	85.0	ul
10x TE	26.9	ul
dH <sub>2</sub> O	156.7	ul
Total	268.6	ul



APPROVED BY :

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





SN G:34 A:12 T:16 C:17

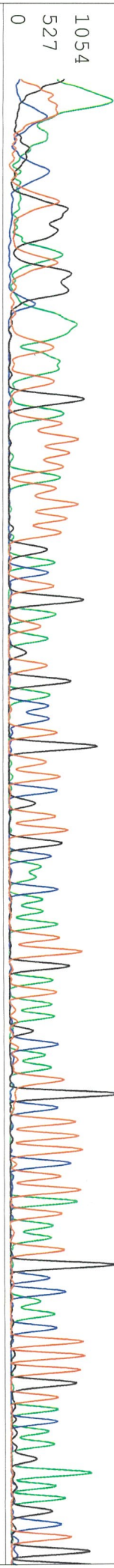
Primer B : T7 long

KB bcp

KB 1.4.1.8 Cap:10

5' CGCCAAAGCTCTAATACGACTCACTATAGGG 3'

CGCCCTGGCA TGGG FTG GCFAT FAT GAT TTTATTT T GACTGATA GT GACCTGT TCGTTGCCAACAAATGATAA GCAATGCTTTCTAT AATGCCAACTTTGTACAA GAAAGCTG



GGTGG GCGGGCCCA C C C T T T G C T A C C C T T A A G A G A G A G C A A T G T T C A C A G G A A A C T C A G A A A A A G G G A C A T G C A T A A C T G T G A T G A C A G G G C C C A T G T G T A C F  
121 BstHI 131 141 151 161 171 Stop Vector insert 181 191 201 211 221 23

