

RIKEN Clone ID : IRAK014N06

Vector : pCMV-SPORT6

Gene	EZH2		
Accession No.	BC010858.2	2655 bp	1..2655
	<i>CDS</i>	2256 bp	123..2378

● Plasmid DNA purification

Date : 121029

Culture : LB (100 ug/ml Ampicillin) 5 ml -> at 37 deg C over night

Date : 121030

Purification : QIAGEN Miniprep kit -> dH₂O 100 ul

● Digestion by restriction enzyme/Concentration calibration

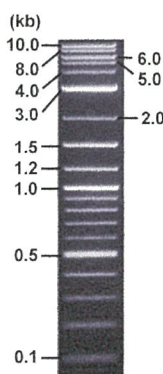
Date : 121030

DNA concentration (O.D.): 147 ng/ul

DNA	1	ul
Enzyme (EcoRI + XhoI)	0.5 + 0.5	ul
Buffer H	1	ul
dH ₂ O	7	ul
Total	10	ul

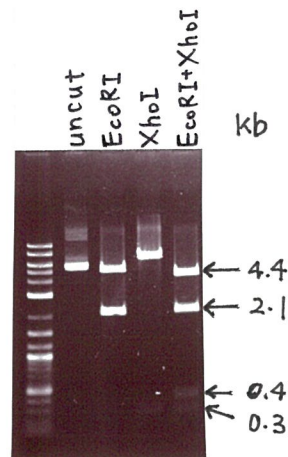
Erectrophoresis : 1% agarose gel, 1x TAE Buffer

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



<Expected size of fragment from BC010858.2 >

4339, 2065, 389, 244 bp



● Confirmation of the insertion sequence

Date : 121101

Primer A	Reverse2
Primer B	M13
Primer C	-
List of Sequencing Primers	http://dna.brc.riken.jp/en/GNPclone3en.html

● Adjustment of DNA concentration

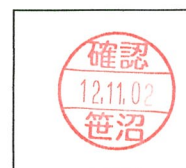
Shipping amount : 40 ul

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

Date : 121102

DNA (<u>147</u> ng/ul)	82.0	ul
10x TE	48.2	ul
dH ₂ O	352.0	ul
Total	482.2	ul

APPROVED BY :



S/N G:108 A:80 T:57 C:66
KB.bcp
KB 1.4.1.8 Cap:19

Primer A : Reverse2

5' GCGGATAACAATTTTCACAGCAGG 3'

1 CTT AACCG TTA CG CC TAT TTA G GT G FICACTA TA G AACAA G TTT T TTA CAAA AAAAGCAGG C T GGT ACC G GT C CCG AATT TCCC G G GATAT C G T C G A C C C A G G C G T C C C G G

AflB1 50

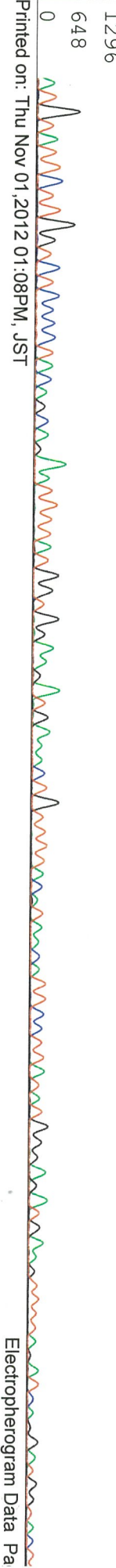
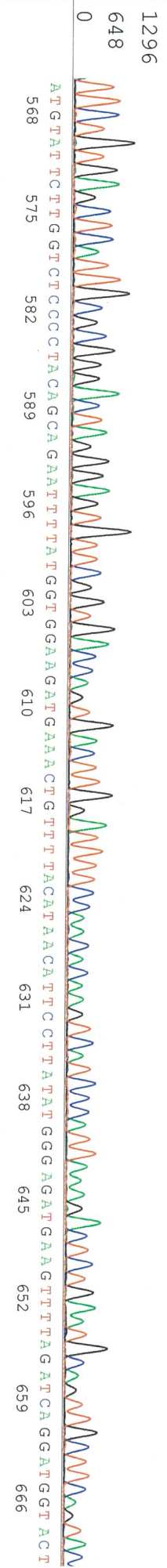
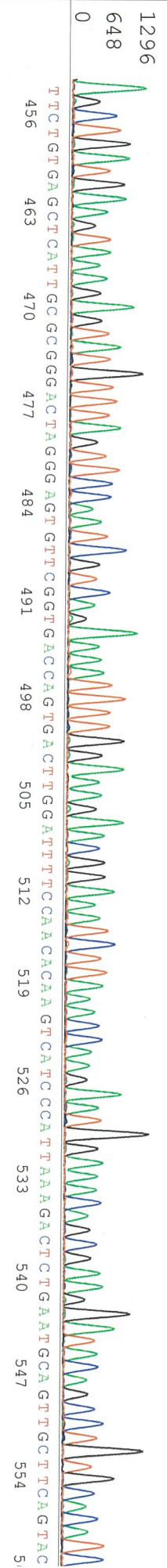
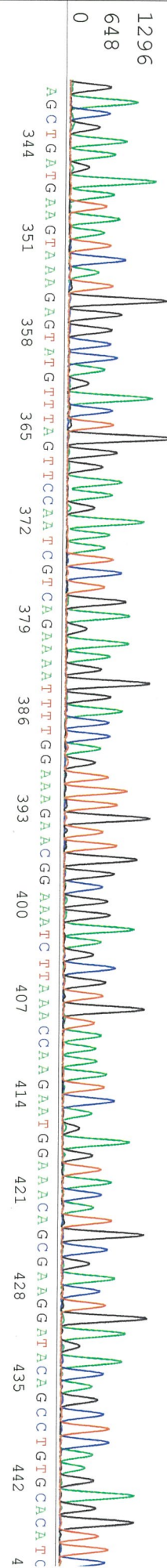
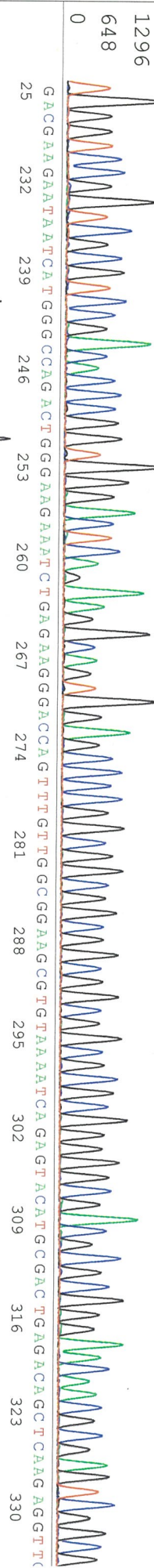
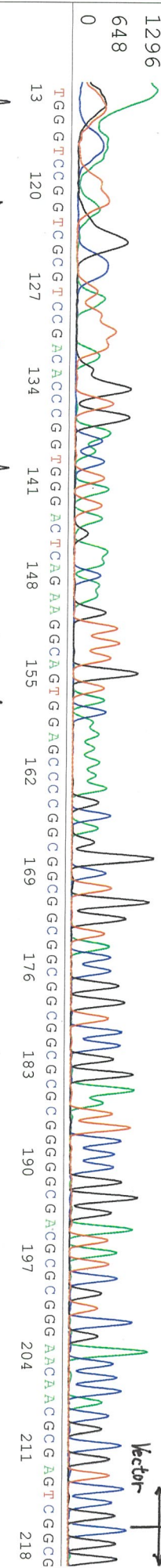
KpnI 64

EcoRI 71

SmaI 78

EcoRV 85

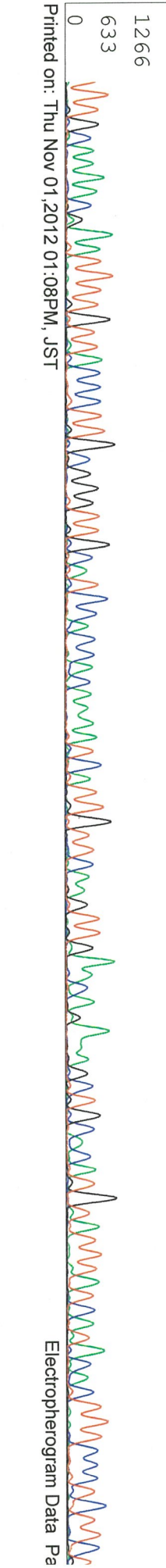
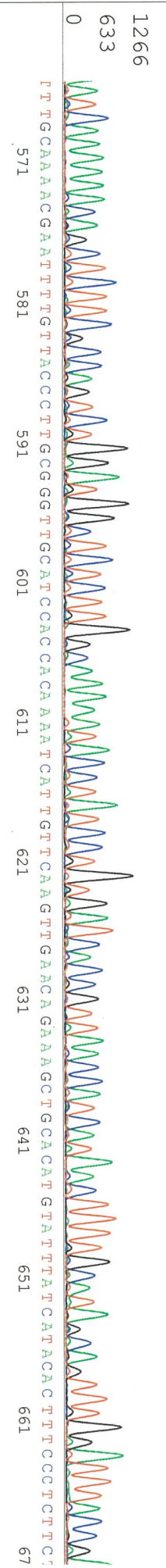
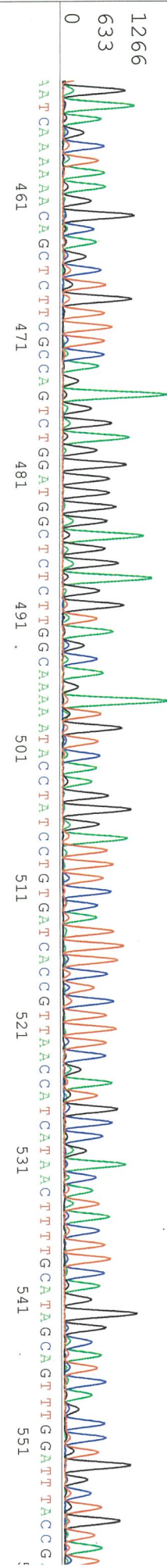
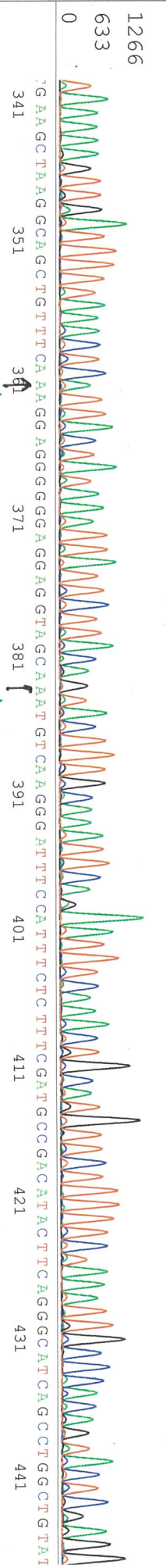
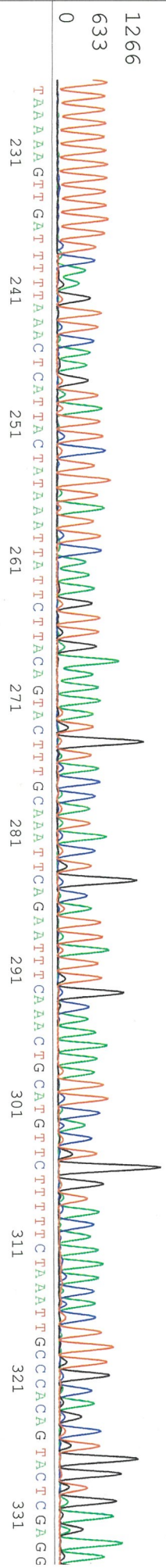
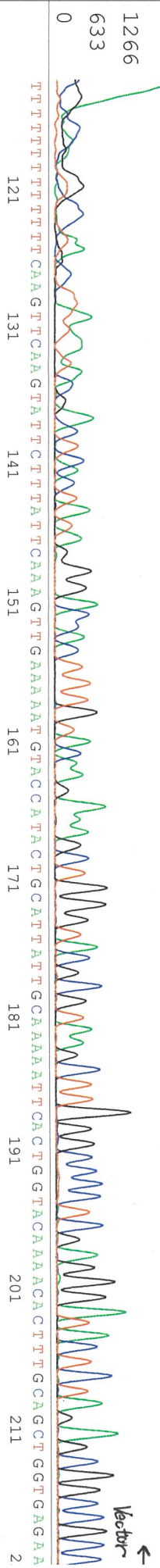
SaI 92



Primer B : M13

5' GTTTTCCAGTCAAGACGGTTGTA 3'

CGCCAAAGGG(C)C(F)AGC(T)TAT(A)TAC(G)ACT(C)ACT(A)TAG(G)ACCA(C)T(T)GTACAA(G)AAAGC(T)GGG(T)ACGC(G)TAAAGC(T)GGGCC(C)TCGAG(G)GATTAC(T)CTAGAG(C)GGCC(G)C
 1 11 21 31 41 51 **atf2** 61 **MluI** 71 **ApaI** 81 **XhoI** 91 **XbaI** 101 **NotI** ←



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Electropherogram Data Pa