

**RIKEN Clone ID : SpYFH38C10**

Vector : pDual-YFH1c

Systematic Name	SPAC17A5.07c
ORF length	2376 bp

● Plasmid DNA purification

Date : 121206

Culture : LB (100 ug/ml Ampicillin) 5 ml -> 37°C O/N

Date : 121207

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

● Digestion by restriction enzyme/Concentration calibration

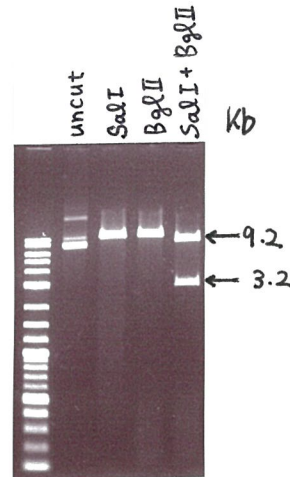
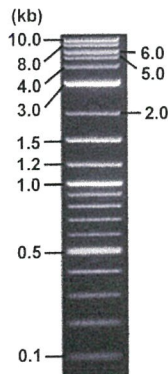
Date : 121207

DNA concentration (O.D.): 305 ng/ul

DNA	0.1 ul
Enzyme (SalI + BglII)	0.5 + 0.5 ul
Buffer H	1 ul
dH <sub>2</sub> O	7.9 ul
<b>Total</b>	<b>10 ul</b>

Electrophoresis : 0.8% agarose gel, 1x TAE Buffer

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



<Expected digestion pattern from SpYFH38C10 >

9117. 3226 bp

● Adjust plasmid DNA solution to 25 ng/ul ~preparation for shipping~

Date : 121212

Shipping amount : 40 ul

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

DNA ( <u>305</u> ng/ul )	85.0	ul
10x TE	103.7	ul
dH <sub>2</sub> O	848.3	ul
<b>Total</b>	<b>1,037.0</b>	<b>ul</b>

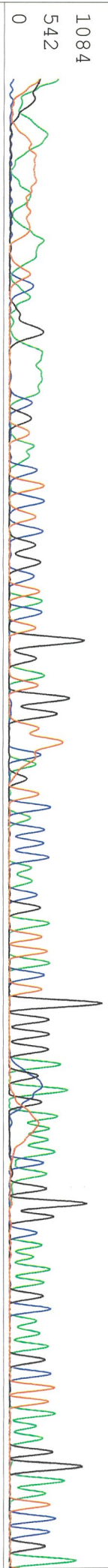
APPROVED BY :



Primer A : nmt1-F

5' GCTGTAAAACACACCGAGAGAC 3'

CTA AATTTTATTTA A TCA G G AATAAC G TAACT C TCG G C TACTG G FTG GTTCA G TCA C CCA ACG A T TACT G G G G A G A G A A A C A G G C C A A A G C A A A G C T T A A A G G A A T C C G A



TT GTCATTC GGCAATGT GCA GCG A A A C T A A A A A C C G G A T A A T G G A C C T T T T A A T C G A A A C A T T G A A G A T A T A A A G G A A G A G A A T C C T G G C A T T C A T T G A A T T A A G



TT GAATTAAT TATTTCAA TCTCA TTTCT CACTTTCT GACT TATAGTCGCT TTTGTTAAAGCTA GCGTCGACA GGCCTGGATCCGG ATATCA CAA GTTTGTACAA A A A A A G C A G G C

nmt promoter ←

Nhe I 291 SaI I

BamH I 301

atfB1 321

TC TCA TAT G CCG T A T G T T A T T C T T T C G T T T C T T T T C T T T A C T A A C T A T T T T T T A G C T G A T G A T A A A T C A G G G T C A T T C A C T A G T C T T C T T C C C C G T T T G G A A A C A A A G

← Vector → insert

3 T T T G A C T T T A C C T C G T C T T T T A T T C A C A T T C C T A T A T T A A T A T T T T T A C C A A T C T A G A G G T A C T A G C C C A A A T G A T G C T A T T C C T A T T A A G A G C C G C T A G C C

A A T A G T G T A A C A T C C C C G G A A A A G C C A A C G G T T C G A A C T G C T A T A C A G A A G G A T T C A C C A A G G C G G A A G C A G A T A G A C G A T G A T C A A A C A C C T C C T A A G C A C T T A A A A C G C T

