

**RIKEN Clone ID : IRAL003M21**

Vector : pOTB7

Gene	CDC2		
Accession No.	BC014563.1	1119 bp	1..1119
	<i>CDS</i>	894 bp	14..907

● Plasmid DNA purification

Date : 121106

Culture : LB (25 ug/ml Chloramphenicol) 10 ml → at 37 deg C over night

Date : 121107

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

● Digestion by restriction enzyme/Concentration calibration

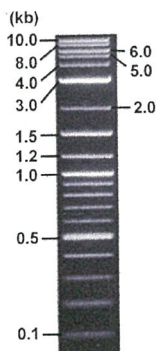
Date : 121107

DNA concentration (O.D.): 99 ng/ul

DNA	0.5	ul
Enzyme (BamHI + BglII)	0.5 + 0.5	ul
Buffer H	1	ul
dH <sub>2</sub> O	7.5	ul
<b>Total</b>	<b>10</b>	<b>ul</b>

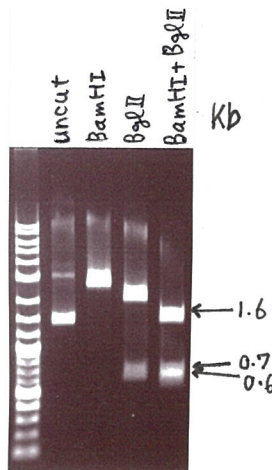
Electrophoresis : 1% agarose gel, 1x TAE Buffer

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



<Expected size of fragment from BC014563.1 >

.....1615, 692, 613 bp.....



● Confirmation of the insertion sequence

Date : 121108

Primer A	pOTB7_F
Primer B	pOTB7_R
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/ul

Date : 121109

DNA ( <u>99</u> ng/ul)	80.0	ul
10x TE	31.7	ul
dH <sub>2</sub> O	205.1	ul
<b>Total</b>	<b>316.8</b>	<b>ul</b>



APPROVED BY :

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

S/N G:127 A:94 T:87 C:69

KB.bcp

KB 1.4.1.8 Cap:1

Primer A: pOTB7-F

5' AACGGCGCTACGATTAAATACATAACC 3'

Version 5.4 HISQV Bases: 1033

IRAL003M21\_pOTB7\_F\_3\_A10\_01

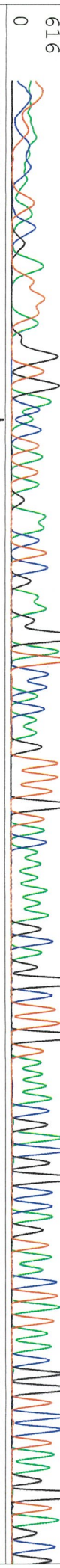
IRAL003M21\_pOTB7\_F\_3

KB\_3500\_POP7\_BDTV3.mob

Pis 1164 to 12323 PK1 Loc:1141

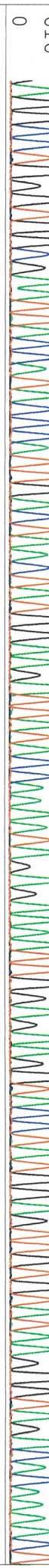
1 ACAATACCA TAC G AT TTA GGTGACACTATA GAACCTCGAAG GATCCACAA GTTTGTACAAA AAAAAACA GGGCTTGTAA AACGACCGCCAG TAACTATAACGGGTCCTAAGGTTAGC

12332 0 616 12332



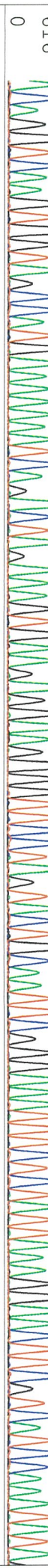
3 AGGCTGGGTGGCGAATTACCA TACCATTTGACTA ACTATG GAAGATTATACC AAAAAATA GAGAAAAATT GGAGAAGGTACC TATGGAGTTGT GTATAAGGGTAGACACAAA ACT

12332 0 616 12332



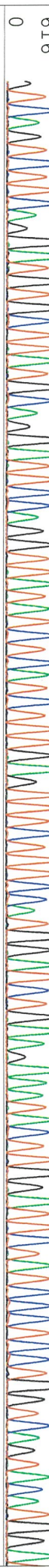
A CAGGTCAA GTGTAGCCATG AAAAAAATCA GACTAGAAA GTGAA GAGGAGGGTT CCTAGTACTGCAATTC GGGAAATTTCTCTATTAAA GGAACCTCGTCA TCCAAA TATTA

12332 0 616 12332



G TCA GTCTT CAGGATGTGCTTATG CAGGATTTCCA GGTTATATCTCA TCTTTGAGTTTCTTCCA TGGATCTG AAGAATACTGGATTCTATCCCTCGTCA GTA CA TGG A

12332 0 616 12332



TTCTTCACCTTGTTAAGAGTTTATTTATACC AAAAACTCTACAGGGGATTGTGTTTGTCACTCTAGAAA GTTCTTCCACAGAGACTTAAAA CCTCAAAA TCTCTTGGATTGATGAC

12332 0 616 12332



AAAGGAACAATTAAACTGGCTGATTTTGGCCCTTGCCA GAGC TTTTGGAAATACCTATCAGAGTATATACACACTGAGGTA GTAACA CACTCTGTACAGATCTCCAGAGTATTG

12332 0 616 12332





