

**RIKEN Clone ID : IRAK019K19**

Vector : pCMV-SPORT6

Gene	PHB2	
Accession No.	BC014766.1	1342 bp
	<i>CDS</i>	900 bp

● Plasmid DNA purification

Date : 2020.10.13

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

Date : 2020.10.14

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

● Digestion by restriction enzyme/Concentration calibration

Date : 2020.10.14

DNA concentration (O.D.): 322 ng/ul

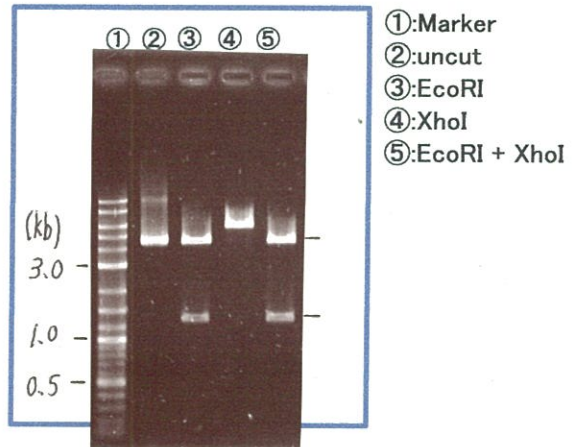
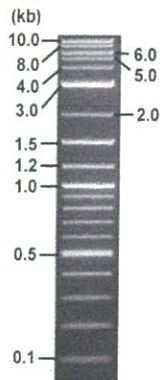
< Size of fragment expected from this clone >

DNA	0.3	ul
Enzyme (EcoRI + XhoI)	0.5 + 0.5	ul
Buffer H	1	ul
dH <sub>2</sub> O	7.7	ul
Total	10	ul

EcoRI	4.4, 1.3 kb
XhoI	5.7 kb
EcoRI + XhoI	4.3kb (Vector)      1.3, 0.1 kb

Electrophoresis : 1% agarose gel, 1x TAE Buffer

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



● Adjust plasmid DNA solution to 25 ng/ul

Date : 2021.06.02

● Confirmation of the insertion sequence

Date : 2020.11.9

DNA ( <u>322</u> ng/ul)	85.0	ul
10x TE	109.4	ul
dH <sub>2</sub> O	900.4	ul
Total	1094.8	ul

Primer A	Reverse2
Primer B	M13
Primer C	poly(A) region primer #0
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:25 A:29 T:20 C:31

Primer A : Reverse2

7893\_IRAK019K19\_B0J8\_1\_Reverse2

Oct 15,2020 09:52AM, JST

KB.bcp

KB\_3500\_POP7\_BDTv3.mob

Gray line indicates quality value

Oct 15,2020 10:07AM, JST

KB 1.4.1.8 Cap:3

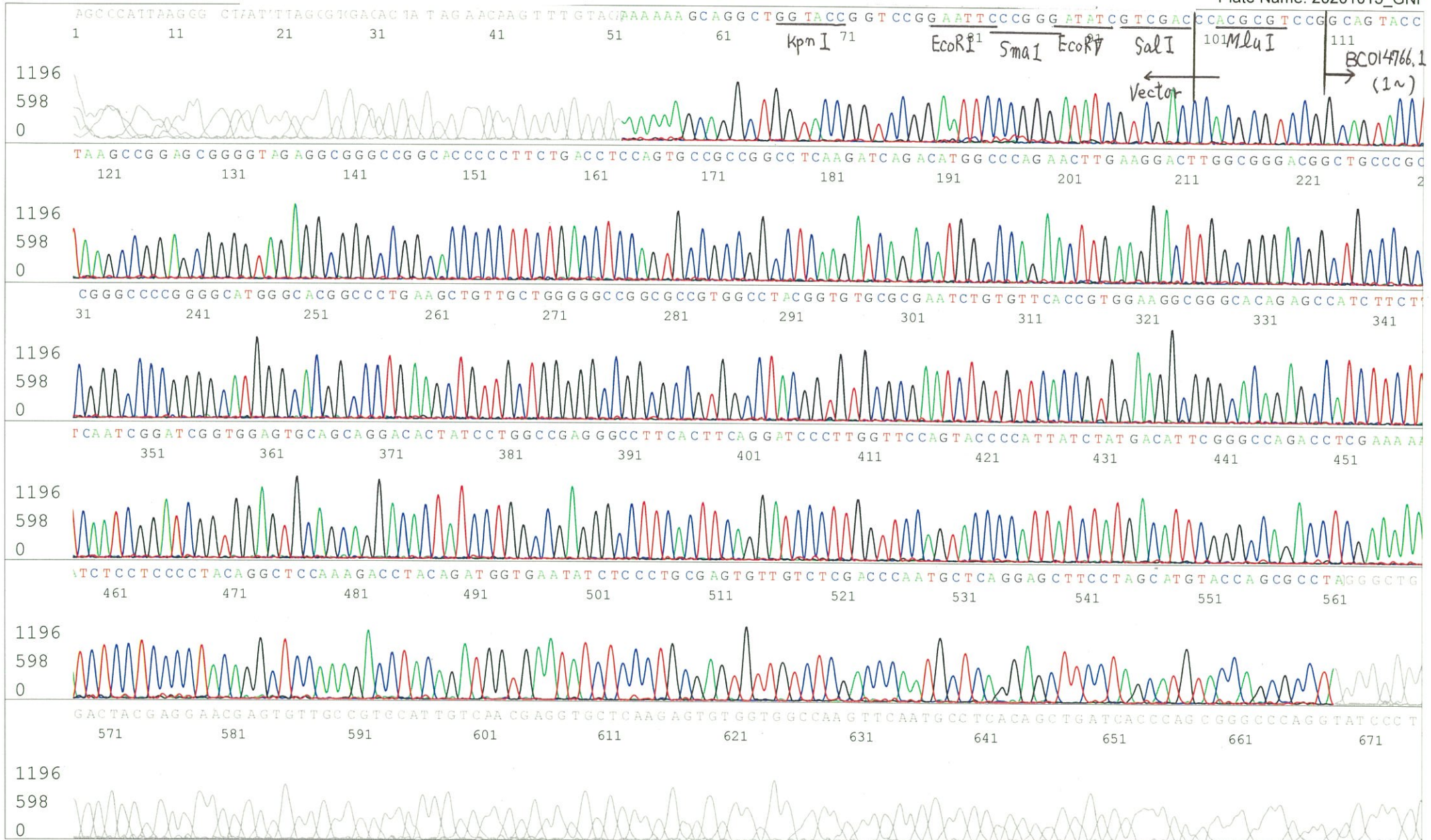
5' GCGGATAACAATTTTCACACAGG 3'

Pts 1803 to 8707 Pk1 Loc:1771

(QV) < 40.

Spacing:10.79 Pts/Panel1350

Plate Name: 20201015\_GNP



S/N G:126 A:149 T:119 C:134 Primer B : M13

7893\_IRAK019K19\_B0J8\_1\_M13(-40)

Oct 16,2020 10:32AM, JST

KB.bcp

KB\_3500\_POP7\_BDTv3.mob

Gray line indicates quality value

Oct 16,2020 10:49AM, JST

KB 1.4.1.8 Cap:3

5' GTTTCCAGTCACGACGTTGTA 3'

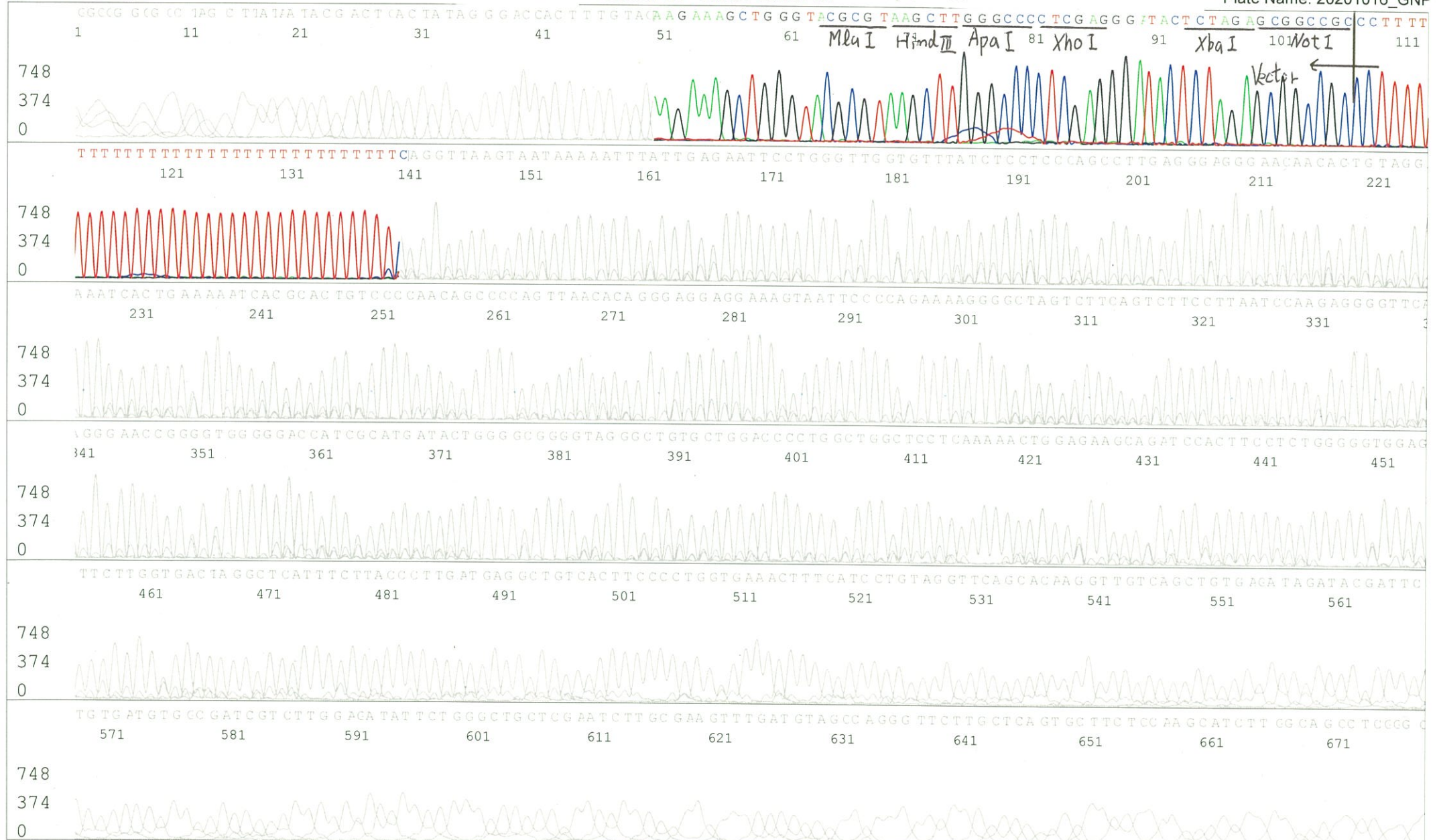
Pts 1767 to 8706 Pk1 Loc:1735

(QV) < 40.

Spacing:10.49 Pts/Panel1350

Version 6.0 HiSQV Bases: 437

Plate Name: 20201016\_GNP



S/N G:40 A:30 T:21 C:25

7893\_IRAK019K19\_BOJ8\_1\_GNPmixture

Oct 16,2020 10:32AM, JST

KB.bcp

Primer C : Poly(A) region primer #0

KB\_3500\_POP7\_BDTv3.mob

Gray line indicates quality value

Oct 16,2020 10:49AM, JST

KB 1.4.1.8 Cap:9

5' TTTTTTTTTTTTTTTTTTTTTNNN 3'

Pts 1743 to 8706 Pk1 Loc:1711

(QV) < 40.

Spacing:10.47 Pts/Panel1350

Plate Name: 20201016\_GNP

