

RIKEN Clone ID : IRAL019K10

Lot#: 7566_B4Ac

Vector : pDNR-LIB

Gene	CART1	
Accession No.	BC010923.1	1326 bp
	<i>CDS</i>	981 bp

● Plasmid DNA purification

Date : 2024/01/15

Culture : LB (25 ug/ml Chloramphenicol) 5 ml → at 37 deg C over night

Date : 2024/01/16

Purification : QIAGEN Miniprep kit → dH₂O 100 ul**● Digestion by restriction enzyme/Concentrarion calibration**

Date : 2024/01/16

DNA concentration (O.D.): 273 ng/ul

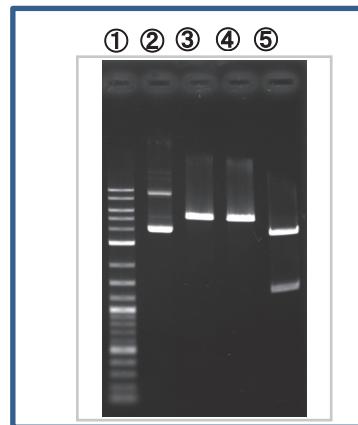
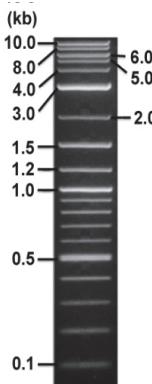
< Size of fragment expected from this clone >

DNA	<u>0.35</u> ul
Enzyme (EcoRI + XhoI)	<u>0.5 + 0.5</u> ul
Buffer H	<u>1</u> ul
dH ₂ O	<u>7.65</u> ul
Total	<u>10</u> ul

EcoRI	5.3 kb
XhoI	5.3 kb
EcoRI + XhoI	3.9kb (Vector) 1.4 kb

Electrophoresis : 1% agarose gel, 1x TAE Buffer

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



- ①:Marker
- ②:uncut
- ③:EcoRI
- ④:XhoI
- ⑤:EcoRI + XhoI

● Adjust plasmid DNA solution to 25 ng/ul

Date : 2024/01/19

● Confirmation of the insertion sequence

Date : 2024/01/18

DNA (<u>273</u> ng/ul)	<u>84</u> ul
10x TE		<u>92</u> ul
dH ₂ O		<u>742</u> ul
Total		<u>917</u> ul

Primer A	T7 long
Primer B	M13 Reverse
Primer C	poly(A) region primer #0
List of Sequencing Primers	http://dna.brc.riken.jp/en/GNPclone3en.html

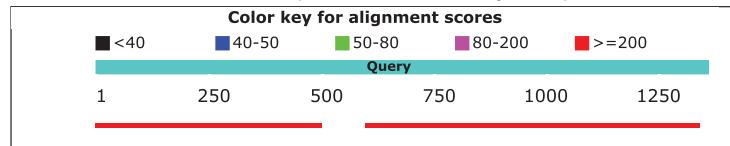
● Shipping

Conc. : 25 ng/ μl, Volume : 40 μl

Conc. : ng/ μl, Volume : μl

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



[Questions/comments](#)**BLAST Results****Job title:** gb|BC010923.1|**RID** UH2MMGYD114 (Expires on 01-19 09:19 am)**Query ID** BC010923.1**Description** Homo sapiens ALX homeobox 1, mRNA
(cDNA clone MGC:13497
IMAGE:4278460), complete cds**Subject ID** 3 subjects[See details](#)**Molecule type** dna**Subject Length** 1384**Program** BLASTN 2.15.0+**Molecule type** nucleic acid**Query Length** 1326**Blast 2 sequences****Job title:** gb|BC010923.1|**RID** UH2MMGYD114 (Expires on 01-19 09:19 am)**Query ID** BC010923.1**Description** Homo sapiens ALX homeobox 1, mRNA
(cDNA clone MGC:13497
IMAGE:4278460), complete cds**Subject ID** 3 subjects[See details](#)**Molecule type** dna**Subject Length** 1384**Program** BLASTN 2.15.0+**Molecule type** nucleic acid**Query Length** 1326**Graphic Summary****Distribution of the top 2 Blast Hits on 2 subject sequences****Descriptions**

Sequences producing significant alignments:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
primerB (Reverse)	1358	1358	55%	0.0	100.00%	Query_4997383
primerA (Forward)	917	917	37%	0.0	100.00%	Query_4997382

Alignments

primerB (Reverse)

Sequence ID: Query_4997383 Length: 796 Number of Matches: 1
Range 1: 62 to 796

Score	Expect	Identities	Gaps	Strand	Frame
1358 bits(735)	0.0()	735/735(100%)	0/735(0%)	Plus/Minus	
Features:					
CDS:ALX homeobox 1 [195	592	Q I Q Q A K S H F A A T Y D I S V L P R			651
Query		CCAAATACAACAGCGAAAAGCCATTGGCTGCCACCTATGATATATCAGTTTGCAG			
Sbjct	796	CCAAATACAACAGCGAAAAGCCATTGGCTGCCACCTATGATATATCAGTTTGCAG			737
CDS:ALX homeobox 1 [215	652	T D S Y P Q I Q N N L W A G N A S G G S			711
Query		GACTGACAGCTACCCACAGATTCAAGACAATTGTGGCAGGAAATGCAAGTGGTGT			
Sbjct	736	GACTGACAGCTACCCACAGATTCAAGACAATTGTGGCAGGAAATGCAAGTGGTGT			677
CDS:ALX homeobox 1 [235	712	V V T S C M L P R D T S S C M T P Y S H			771
Query		TGTGGTTACTTCATGCATGTTACCAAGTGACACTCTCTCTGTATGACACCTTATCTCA			
Sbjct	676	TGTGGTTACTTCATGCATGTTACCAAGTGACACTCTCTCTGTATGACACCTTATCTCA			617
CDS:ALX homeobox 1 [255	772	S P R T D S S T G F S N H O N O F S H			831
Query		CTGGCTCGGACAGATTCCAGTTACACGGGTTCTAAACCCACAGAACCTTCAAGCA			
Sbjct	616	CTGGCTCGGACAGATTCCAGTTACACGGGTTCTAAACCCACAGAACCTTCAAGCA			557
CDS:ALX homeobox 1 [275	832	V P L N N F T D S S L T G A T N G H A			891
Query		CGTGGCCCTCAACAAATTCTACTGACTCTCTCTACTGGGCAACCAATGGACATGC			
Sbjct	556	CGTGGCCCTCAACAAATTCTACTGACTCTCTCTACTGGGCAACCAATGGACATGC			497
CDS:ALX homeobox 1 [295	892	F E T K P E F E R R S S S I A V L R M K			951
Query		ATTGAAACAAAGCAGAGTTGAAGAGGAGTCTTCAGATATCGCAGTTCTCGAATGAA			
Sbjct	496	ATTGAAACAAAGCAGAGTTGAAGAGGAGTCTTCAGATATCGCAGTTCTCGAATGAA			437
CDS:ALX homeobox 1 [315	952	A K E H T A N L I S W A M			1011
Query		AGCCAAGGAGCACCGGCCAATTTCATGGCCCATGTAACATACAGTACTCTTATT			
Sbjct	436	AGCCAAGGAGCACCGGCCAATTTCATGGCCCATGTAACATACAGTACTCTTATT			377
Query	1012	TCTTTAAATAGCAAAGTTAACATTCTTATTCATATTAAAGGATAACAAATAAG			1071
Sbjct	376	TCTTTAAATAGCAAAGTTAACATTCTTATTCATATTAAAGGATAACAAATAAG			317
Query	1072	CTGCTGTGTGGAAATTGCTAAAGGTCAAGATATTAGTAGAGACAGCTTAAATGAA			1131
Sbjct	316	CTGCTGTGTGGAAATTGCTAAAGGTCAAGATATTAGTAGAGACAGCTTAAATGAA			257
Query	1132	TTGTATTAAACATTAAATCTAAGATGAACCTCTGAAAGACTAAATAGTTACCAT			1191
Sbjct	256	TTGTATTAAACATTAAATCTAAGATGAACCTCTGAAAGACTAAATAGTTACCAT			197
Query	1192	GTGCCAGTCTCCACAAACCCGTGTTAGTAGTAAAGGTTCTTCTTCTTCTTCTT			1251
Sbjct	196	GTGCCAGTCTCCACAAACCCGTGTTAGTAGTAAAGGTTCTTCTTCTTCTTCTT			137
Query	1252	AATGAAATATGATCAGCAACTTAAAGAATAATGTGTAAAaaaaaaaaaaaaaa			1311
Sbjct	136	AATGAAATATGATCAGCAACTTAAAGAATAATGTGTAAAaaaaaaaaaaaaaa			77
Query	1312	aaaaaaaaaaaaaaaaaaaaaaa 1326			
Sbjct	76	aaaaaaaaaaaaaaaaaaaaaaa 62			

primerA (Forward)

Sequence ID: Query_4997382 Length: 584 Number of Matches: 1
Range 1: 89 to 584

Score	Expect	Identities	Gaps	Strand	Frame
917 bits(496)	0.0()	496/496(100%)	0/496(0%)	Plus/Plus	
Features:					
CDS:ALX homeobox 1	1	M E F L S E K F A L K S P P S K N			
Query	1	GGGGAGGATTATGGAGTTCTGAGCGAGAAAGTTGCCCTCAAGAGCCCTCCGAGTAAAAA			60
Sbjct	89	GGGGAGGATTATGGAGTTCTGACGGAGAAAGTTGCCCTCAAGAGCCCTCCGAGTAAAAA			148
CDS:ALX homeobox 1	18	S D F Y M G A G G P L E H V M E T L D N			
Query	61	CAGTGACTTTACATGGGCCAGGAGGTCTCTGGAGCACGTTATGGAGACGCTGGACAA			120
Sbjct	149	CAGTGACTTTACATGGGCCAGGAGGTCTCTGGAGCACGTTATGGAGACGCTGGACAA			208
CDS:ALX homeobox 1	38	E S F Y S K A S A G K C V Q O A F G P L P			
Query	121	TGAGTCCCTTACAGCAAAGCCTCTGCAGGCAAATGGGTGCAGGCCTCGGACCCCTGCC			180
Sbjct	209	TGAGTCCCTTACAGCAAAGCCTCTGCAGGCAAATGGGTGCAGGCCTCGGACCCCTGCC			268
CDS:ALX homeobox 1	58	R A E H H V R L E T S P C Q D S S V N			
Query	181	CCGGCCGAGCATCACGTGGCTTGAGAGGACCTGGCCCTTCAGGACAGCAGCGTGA			240
Sbjct	269	CCGGCCGAGCATCACGTGGCTTGAGAGGACCTGGCCCTTCAGGACAGCAGCGTGA			328
CDS:ALX homeobox 1	78	Y G I T K V E G Q P L H T E L N R A M D			
Query	241	CTATGGATCACTAAAGTAGAAGGACAGCCCTTCACACGGAACTGAATAGAGCTATGGA			300
Sbjct	329	CTATGGATCACTAAAGTAGAAGGACAGCCCTTCACACGGAACTGAATAGAGCTATGGA			388
CDS:ALX homeobox 1	98	N C N S L R M S P V K G M O E K G E L D			
Query	301	CAACTGTAACAGTCTCGGAATGTCTCCGTTGAAAGGGATGCAAGAGAAAGGAGACTGGA			360
Sbjct	389	CAACTGTAACAGTCTCGGAATGTCTCCGTTGAAAGGGATGCAAGAGAAAGGAGACTGGA			448
CDS:ALX homeobox 1	118	E L G D K C D S N V S S S K K R R H R T			
Query	361	TGAACTGGGATAATGTGATAGCAATGTATCCACCACTAAAGAACGGAGCCACCGAAC			420
Sbjct	449	TGAACTGGGATAATGTGATAGCAATGTATCCACCACTAAAGAACGGAGCCACCGAAC			508
CDS:ALX homeobox 1	138	T F T S L Q L E E L E K V F Q K T H Y P			
Query	421	CACCTCACCACTTGAGCTAGAGGAAGCTGGAGAAAAGTCCTCAGAAAACCTTACCC			480
Sbjct	509	CACCTCACCACTTGAGCTAGAGGAAGCTGGAGAAAAGTCCTCAGAAAACCTTACCC			568
CDS:ALX homeobox 1	158	D V Y V R			
Query	481	GGATGTGTATGTCAGA	496		
Sbjct	569	GGATGTGTATGTCAGA	584		

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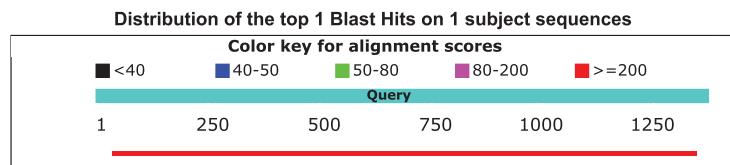
[Questions/comments](#)**BLAST Results****Blast 2 sequences**

Job title: NM_006982:Homo sapiens ALX homeobox 1 (ALX1),...

RID [U9EH2BMR114](#) (Expires on 01-16 11:52 am)

Query ID [NM_006982.3](#)
Description Homo sapiens ALX homeobox 1 (ALX1), mRNA
Molecule type nucleic acid
Query Length 1332

Subject ID [BC010923.1](#)
Description Homo sapiens ALX homeobox 1, mRNA (cDNA clone MGC:13497 IMAGE:4278460), complete cds
[See details](#)
Molecule type nucleic acid
Subject Length 1326
Program BLASTN 2.15.0+

Graphic Summary**Dot Matrix View****Descriptions**

Sequences producing significant alignments:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Homo sapiens ALX homeobox 1, mRNA (cDNA clone MGC:13497 IMAGE:4278460), complete cds	2394	2394	97%	0.0	100.00%	BC010923.1

Alignments

Homo sapiens ALX homeobox 1, mRNA (cDNA clone MGC:13497 IMAGE:4278460), complete cds
Sequence ID: **BC010923.1** Length: 1326 Number of Matches: 1

[See 1 more title\(s\)](#)

Range 1: 5 to 1300

Score	Expect	Identities	Gaps	Strand	Frame
2394 bits(1296)	0.0()	1296/1296(100%)	0/1296(0%)	Plus/Plus	
Features:					
CDS:ALX homeobox pro	1	M E F L S E K F A L K K S P P S K N S			
Query	37	AGGATTATGGAGTTCTGAGGGAGAAAGTTGCCCTCAAGAGCCCTCCGAGTAAAAACAGT			96
Sbjct	5	AGGATTATGGAGTTCTGAGGGAGAAAGTTGCCCTCAAGAGCCCTCCGAGTAAAAACAGT			64
CDS:ALX homeobox 1	1	M E F L S E K F A L K K S P P S K N S			
CDS:ALX homeobox pro	19	D F Y M G A G G P L E H V M E T L D N E			
Query	97	GACTTTTACATGGGGCAGAGGTCTCTCTGGAGCACGTTATGGAGACCCCTGGACAAATGAG			156
Sbjct	65	GACTTTTACATGGGGCAGAGGTCTCTCTGGAGCACGTTATGGAGACCCCTGGACAAATGAG			124
CDS:ALX homeobox 1	19	D F Y M G A G G P L E H V M E T L D N E			
CDS:ALX homeobox pro	39	S F Y S K A S A G G K C V Q O A F G P L P R			
Query	157	TCCCTTTACAGCAAAGCGTCAGGAGCAAATGGCTCAGGCGCTTCGGACCCCTGGCCCGC			216
Sbjct	125	TCCCTTTACAGCAAAGCGTCAGGAGCAAATGGCTCAGGCGCTTCGGACCCCTGGCCCGC			184
CDS:ALX homeobox 1	39	S F Y S K A S A G G K C V Q O A F G P L P R			
CDS:ALX homeobox pro	59	A E H H V R L E R T S P C Q D S S V N Y			
Query	217	GCCGAGCATCACGTGGCTGGAGAGGACCTGGCCCTGCAAGCACGGAGGTGAACAT			276
Sbjct	185	GCCGAGCATCACGTGGCTGGAGAGGACCTGGCCCTGCAAGCACGGAGGTGAACAT			244
CDS:ALX homeobox 1	59	A E H H V R L E R T S P C Q D S S V N Y			
CDS:ALX homeobox pro	79	G I T K V E G Q P L H T E L N R A M D N			
Query	277	GGGATCACTAAAGTAGAAGGACAGCCCCCTCACACGGAACCTGAATAGAGCTTGGAACAC			336
Sbjct	245	GGGATCACTAAAGTAGAAGGACAGCCCCCTCACACGGAACCTGAATAGAGCTTGGAACAC			304
CDS:ALX homeobox 1	79	G I T K V E G Q P L H T E L N R A M D N			
CDS:ALX homeobox pro	99	C N S L R M S P V K G M Q E K G E L D E			
Query	337	TGTAAAGCTTCGGAAATGTCCTCCGTAAAGGGATGCAAGAGAAAGGGAGAGCTGGATGAA			396
Sbjct	305	TGTAAAGCTTCGGAAATGTCCTCCGTAAAGGGATGCAAGAGAAAGGGAGAGCTGGATGAA			364
CDS:ALX homeobox 1	99	C N S L R M S P V K G M Q E K G E L D E			
CDS:ALX homeobox pro	119	L G D K C D S N V P S S S K K R R H R T T			
Query	397	CTTGGGGATAAAATGTAGCAATGTATCCAGCAGTAAGAAACGGAGCCACCAACACC			456
Sbjct	365	CTTGGGGATAAAATGTAGCAATGTATCCAGCAGTAAGAAACGGAGCCACCAACACC			424
CDS:ALX homeobox 1	119	L G D K C D S N V P S S S K K R R H R T T			
CDS:ALX homeobox pro	139	F T S L Q L E E L E K V F Q K T H Y P D			
Query	457	TTCACCAAGTTGGAGCTAGAGGAGCTGGAGAAAGCTTCAAGAAAACCTTACCCGGAT			516
Sbjct	425	TTCACCAAGTTGGAGCTAGAGGAGCTGGAGAAAGCTTCAAGAAAACCTTACCCGGAT			484
CDS:ALX homeobox 1	139	F T S L Q L E E L E K V F Q K T H Y P D			
CDS:ALX homeobox pro	159	V Y V R E Q L A L R T E A R V Q V			
Query	517	GTGTATGTCAAGAGAACAGCTGGCTCTGGAGCACAGCTACTGGGGCAGGGTCCAGGGT			576
Sbjct	485	GTGTATGTCAAGAGAACAGCTGGCTCTGGAGCACAGCTACTGGGGCAGGGTCCAGGGT			544
CDS:ALX homeobox 1	159	V Y V R E Q L A L R T E A R V Q V			
CDS:ALX homeobox pro	179	W F Q N R R A K W R K R E R Y G O I O O			
Query	577	TGGTTCAAAATCGAAGGGCAAATGGAGAAAAGGGAAACGTTATGGCAAATACAACAA			636
Sbjct	545	TGGTTCAAAATCGAAGGGCAAATGGAGAAAAGGGAAACGTTATGGCAAATACAACAA			604
CDS:ALX homeobox 1	179	W F Q N R R A K W R K R E R Y G Q I Q Q			

CDS:ALX homeobox pro	199	A K S H F A A T Y D I S V L P R T D S Y	696
Query	637	GCGAAAAGCCATTGCTGCCACCTATGATAATACAGTTGCCAAGGACTGACAGCTAC	
Sbjct	605	GCGAAAAGCCATTGCTGCCACCTATGATAATACAGTTGCCAAGGACTGACAGCTAC	664
CDS:ALX homeobox 1 [199	A K S H F A A T Y D I S V L P R T D S Y	
CDS:ALX homeobox pro	219	P Q I Q N N L W A G N A S G G G S V V T S	756
Query	697	CCACAGATTCAAGAACAAATTGTGGCAGGAAATGCAAGTGGTGGTTCTGTGGTTACTTCA	
Sbjct	665	CCACAGATTCAAGAACAAATTGTGGCAGGAAATGCAAGTGGTGGTTCTGTGGTTACTTCA	724
CDS:ALX homeobox 1 [219	P Q I Q N N L W A G N A S G G G S V V T S	
CDS:ALX homeobox pro	239	C M L P R D T S S C M T P Y S H S P R T	816
Query	757	TGCATGTTACCACTGACACTTCTCTCTGATGACACCTTATTCTCACTCCCTCGGACA	
Sbjct	725	TGCATGTTACCACTGACACTTCTCTCTGATGACACCTTATTCTCACTCCCTCGGACA	784
CDS:ALX homeobox 1 [239	C M L P R D T S S C M T P Y S H S P R T	
CDS:ALX homeobox pro	259	D S S Y T G F S N H O N O F S H V P L N	876
Query	817	GATTCAGTTACAGGGGTCTCAACCACCCAGAACCCAGTTCAGCCAGTGCCCTAAC	
Sbjct	785	GATTCAGTTACAGGGGTCTCAACCACCCAGAACCCAGTTCAGCCAGTGCCCTAAC	844
CDS:ALX homeobox 1 [259	D S S Y T G F S N H Q N Q F S H V P L N	
CDS:ALX homeobox pro	279	N F F T D S L L T G A T N G H A F E T K	936
Query	877	AATTTTCACTGACTCTCTTACTGGCCAACCAATGGACATGCAATTGAACAAAG	
Sbjct	845	AATTTTCACTGACTCTCTTACTGGCCAACCAATGGACATGCAATTGAACAAAG	904
CDS:ALX homeobox 1 [279	N F F T D S L L T G A T N G H A F E T K	
CDS:ALX homeobox pro	299	P E F E R R S S S I A V L R M K A K E H	996
Query	937	CCAGAGTTGAAGGAGGTCTCCAGATCCAGTCTCCGAATGAAGCCAGGAC	
Sbjct	905	CCAGAGTTGAAGGAGGTCTCCAGATCCAGTCTCCGAATGAAGCCAGGAC	964
CDS:ALX homeobox 1 [299	P E F E R R S S S I A V L R M K A K E H	
CDS:ALX homeobox pro	319	T A N I S W A M	1056
Query	997	ACCGCCAATATTGATGGCCATGTAACATACAGTACTCTTATTTCTTTAATAGC	
Sbjct	965	ACCGCCAATATTGATGGCCATGTAACATACAGTACTCTTATTTCTTTAATAGC	1024
CDS:ALX homeobox 1 [319	T A N I S W A M	
Query	1057	AAAGTTAACATTCTTATTCTCATATTAAAGGATACCCAAATAAGCTGGTGTGTGG	1116
Sbjct	1025	AAAGTTAACATTCTTATTCTCATATTAAAGGATACCCAAATAAGCTGGTGTGTGG	1084
Query	1117	AATTGCTAAAGGTCAAGATATTCACTGAGACCCAGCTAAATGAATAGTTGTTAAC	1176
Sbjct	1085	AATTGCTAAAGGTCAAGATATTCACTGAGACCCAGCTAAATGAATAGTTGTTAAC	1144
Query	1177	TTAAAAATCTAAGAATGAACCTCTGAAAAAGACTAAATAGGTTACCATGTGCCAGTC	1236
Sbjct	1145	TTAAAAATCTAAGAATGAACCTCTGAAAAAGACTAAATAGGTTACCATGTGCCAGTC	1204
Query	1237	CAAACCCGTTTAGTAGTAAGGTTCTTCTTCTATTGTACAAGTCATGAATATGAT	1296
Sbjct	1205	CAAACCCGTTTAGTAGTAAGGTTCTTCTATTGTACAAGTCATGAATATGAT	1264
Query	1297	CACGCCAACTTAAAGAATAATGTGTTAACAAA	1332
Sbjct	1265	CACGCCAACTTAAAGAATAATGTGTTAACAAA	1300

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S/N G:303 A:555 T:305 C:490 Primer A : T7 long

KB.bcp

KB 1.4.1.8 Cap:20

7566_IRAL019K10_B4Ac_T7long_G02_20_ABI24

7566_IRAL019K10_B4Ac_T7long

KB_3500_POP7_BDTv3.mob

Pts 1733 to 12383 Pk1 Loc:1710

Version 6.0 HisQV Bases: 757

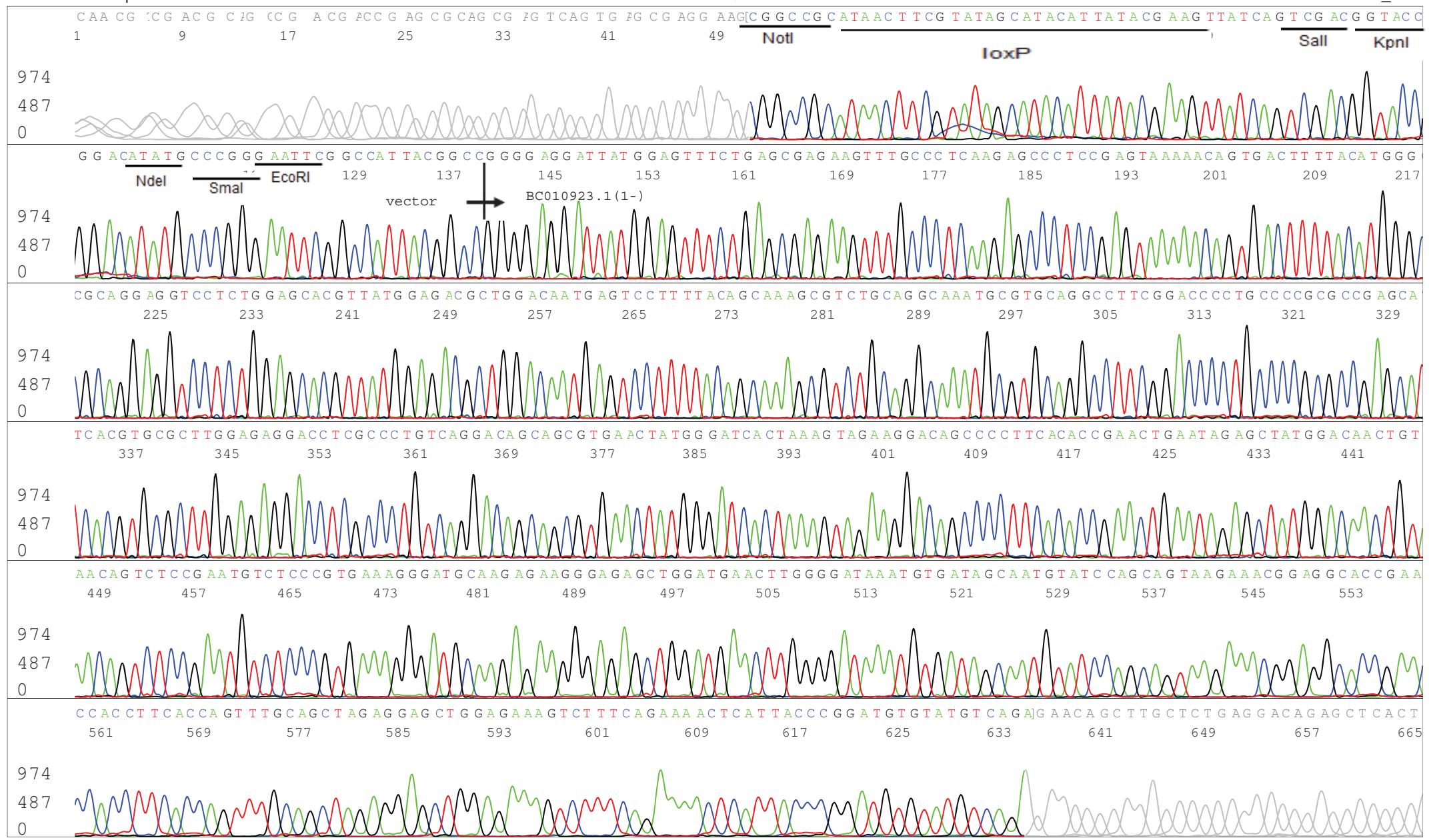
Inst Model/Name 3500/3500 Instrument

Jan 17,2024 09:58AM, JST

Jan 17,2024 10:24AM, JST

Spacing:10.89 Pts/Panel1350

Plate Name: 20240117_GNP



S/N G:164 A:352 T:318 C:290

KB.bcp

KB 1.4.1.8 Cap:23

Primer B : M13 Revers

5' AACAGCTATGACCATGTTCA 3'

7566_IRAL019K10_B4Ac_M13reverse_H02_23_ABI24

7566_IRAL019K10_B4Ac_M13reverse

KB_3500_POP7_BDTv3.mob

Pts 1748 to 12478 Pk1 Loc:1725

Version 6.0 HiSQV Bases: 632

Inst Model/Name 3500/3500 Instrument

Jan 17,2024 09:58AM, JST

Jan 17,2024 10:24AM, JST

Spacing:10.9 Pts/Panel1350

Plate Name: 20240117_GNP

