NRCD Human Full-Length cDNA Clone

Gene Engineering Division
RIKEN BioResource Research Center

RIKEN Clone ID : RBd23E05

Lot# : 7677_B3Jr

Vector : pGCAP10

Gene	COL18A1	
RefSeq_mRNA	NM_130445.4	5399 bp

● Plasmid DNA purification

Date : 2023/10/26 Culture : LB (100 ug/ml Ampicillin) 5 ml -> at 37 deg C O/N

Date : 2023/10/27 Purification : QIAGEN Miniprep kit -> dH_2O 100 ul

● Digestion by restriction enzyme/Concentrarion calibration

Date : 2023/10/27

DNA concentration (O.D.): 237 ng/ul

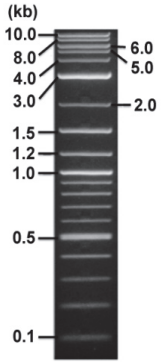
DNA	0.4	ul
Enzyme (BamHI)	1	ul
Buffer H	1	ul
dH_2O	7.6	ul
Total	10	ul

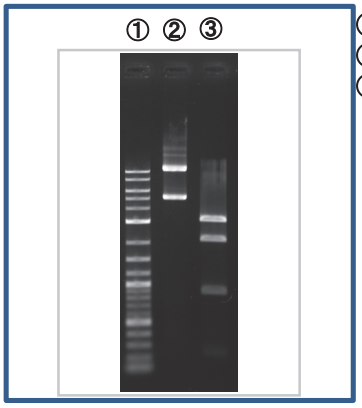
< Size of fragment expected from this clone >

BamHI 3.1kb (vector), 3.2, 0.9, 0.2 kb (unsure)

Erectrophoresis : 1% agarose gel, 1x TAE Buffer

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





①:Marker
②:uncut
③:BamHI

● Confirmation of the insertion sequence

Date : 2023/11/06

Primer A	pGCAP-F
Primer B	pGCAP-R
Primer C	Poly(A) region
List of Sequencing Primers	http://dna.brc.riken.jp/en/NRCDhum2en.html

● Adjust plasmid DNA solution to 25 ng/ul

Date : 2023/12/13

DNA (237 ng/ul)	78 ul
10x TE	74 ul
dH_2O	587 ul
Total	739 ul

● Shipping

☑ Conc. : 25 ng/μl, Volume : 40 μl

☐ Conc. : ___ ng/μl, Volume : ___ μl

APPROVED BY : 検 '23.12.13 笹沼

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

BLAST Results

Questions/comments

Blast 2 sequences

Job title: ref|NM_130445.4|

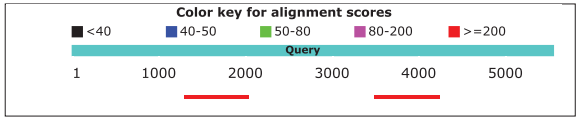
RID M3RDXT62114 (Expires on 11-02 13:10 pm)

Query ID NM_130445.4
Description Homo sapiens collagen type XVIII alpha 1 chain (COL18A1), transcript variant 2, mRNA
Molecule type rna
Query Length 5399

Subject ID 3 subjects
Description See details
Molecule type dna
Subject Length 1525
Program BLASTN 2.14.1+

Graphic Summary

Distribution of the top 2 Blast Hits on 2 subject sequences



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'23.11.06
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Descriptions

Sequences producing significant alignments:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
primerC (Reverse)	1362	1362	13%	0.0	100.00%	Query_125451
primerA (Forward)	1358	1358	13%	0.0	99.86%	Query_125450

Alignments

primerC (Reverse)

Sequence ID: Query_125451 Length: 737 Number of Matches: 1
Range 1: 1 to 737

Score	Expect	Identities	Gaps	Strand	Frame
1362 bits(737)	0.0()	737/737(100%)	0/737(0%)	Plus/Minus	

Features:

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CDS:collagen alpha-1  1127  E P Q P Y P G A P H H S S Y V H L R P A
Query                 3446  agccccagcccctaccccgcagccccGCACCACAGCTCCTACGTGCACCTGCGGCCGGCGC  3505
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                  737  AGCCCCAGCCCTACCCCGGAGCCCCGCACCACAGCTCCTACGTGCACCTGCGGCCGGCGC   678

CDS:collagen alpha-1  1147  R P T S P P A H S H R D F Q P V L H L V
Query                 3506  GACCCACAAGCCCACCCGCCCACAGCCACCCGCGACTTCCAGCCGGTGCTCCACCTGGTTG  3565
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                  677  GACCCACAAGCCCACCCGCCCACAGCCACCCGCGACTTCCAGCCGGTGCTCCACCTGGTTG   618

CDS:collagen alpha-1  1167  A L N S P L S G G M R G I R G A D F Q C
Query                 3566  CGCTCAACAGCCCCCTGTCAGGCGGCATGCGGGGCATCCGCGGGGGCCGACTTCCAGTGCT  3625
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                  617  CGCTCAACAGCCCCCTGTCAGGCGGCATGCGGGGCATCCGCGGGGGCCGACTTCCAGTGCT   558

CDS:collagen alpha-1  1187  F Q Q A R A V G L A G T F R A F L S S R
Query                 3626  TCCAGCAGGCGCGGGGCCGTGGGGCTGGCGGGCACCTTCCGCGCCTTCCTGTCCTCGCGCC  3685
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                  557  TCCAGCAGGCGCGGGGCCGTGGGGCTGGCGGGCACCTTCCGCGCCTTCCTGTCCTCGCGCC   498

CDS:collagen alpha-1  1207  L Q D L Y S I V R R A D R A A V P I V N
Query                 3686  TGCAGGACCTGTACAGCATCGTGCGCCGTGCCGACCGCGCAGCCGTGCCCATCGTCAACC  3745
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                  497  TGCAGGACCTGTACAGCATCGTGCGCCGTGCCGACCGCGCAGCCGTGCCCATCGTCAACC   438

CDS:collagen alpha-1  1227  L K D E L L F P S W E A L F S G S E G P
Query                 3746  TCAAGGACGAGCTGCTGTTTCCCAGCTGGGAGGCTCTGTTCTCAGGCTCTGAGGGTCCGC  3805
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                  437  TCAAGGACGAGCTGCTGTTTCCCAGCTGGGAGGCTCTGTTCTCAGGCTCTGAGGGTCCGC   378

CDS:collagen alpha-1  1247  L K P G A R I F S F D G K D V L R H P T
Query                 3806  TGAAGCCCGGGGCACGCATCTTCTCCTTTGACGGCAAGGACGTCCTGAGGCACCCCACCT  3865
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Sbjct                  377  TGAAGCCCGGGGCACGCATCTTCTCCTTTGACGGCAAGGACGTCCTGAGGCACCCCACCT   318

CDS:collagen alpha-1  1267  W P Q K S V W H G S D P N G R R L T E S
Query                 3866  GGCCCCAGAAGAGCGTGTGGCATGGCTCGGACCCCAACGGGCGCAGGCTGACCGAGAGCT  3925
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Sbjct                  317  GGCCCCAGAAGAGCGTGTGGCATGGCTCGGACCCCAACGGGCGCAGGCTGACCGAGAGCT   258

CDS:collagen alpha-1  1287  Y C E T W R T E A P S A T G Q A S S L L
Query                 3926  ACTGTGAGACGTGGCGGACGGAGGCTCCCTCGGCCACGGGCCAGGCCTCCTCGCTGCTGG  3985
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                  257  ACTGTGAGACGTGGCGGACGGAGGCTCCCTCGGCCACGGGCCAGGCCTCCTCGCTGCTGG   198

CDS:collagen alpha-1  1307  G G R L L G Q S A A S C H H A Y I V L C
Query                 3986  GGGGCAGGCTCCTGGGGCAGAGTGCCGCGAGCTGCCATCACGCCTACATCGTGCTCTGCA  4045
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                  197  GGGGCAGGCTCCTGGGGCAGAGTGCCGCGAGCTGCCATCACGCCTACATCGTGCTCTGCA   138

CDS:collagen alpha-1  1327  I E N S F M T A S K
Query                 4046  TTGAGAACAGCTTCATGACTGCCTCCAAGTAGCCACCGCCTGGATGCGGATGGCCGGAGA  4105
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Sbjct                   77  GGACCGGCGGCTCGGAGGAAGCCCCCACCGTGGGCAGGGAGCGGCCGGCCAGCCCCTGGC   18

Query                 4166  CCCAGGACCTGGCTGCC  4182
                            |||||||||||||||||
Sbjct                   17  CCCAGGACCTGGCTGCC  1
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primerA (Forward)

Sequence ID: Query_125450 Length: 784 Number of Matches: 1
Range 1: 47 to 784

Score	Expect	Identities	Gaps	Strand	Frame
1358 bits(735)	0.0()	737/738(99%)	0/738(0%)	Plus/Plus	

Features:

```
CDS:collagen alpha-1  408   G D P G E D G K P G D T G P Q G F P G T
Query                 1287  GGGCGACCCCGGTGAAGACGGAAAGCCGGGCGACACCGGGCCACAAGGCTTCCCCGGGAC  1346
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                   47  GGGCGACCCCGGTGAAGACGGAAAGCCGGGCGACACCGGGCCACAAGGCTTCCCCGGGAC   106

CDS:collagen alpha-1  428   P G D V G P K G D K G D P G V G E R G P
Query                 1347  TCCAGGGGACGTAGGTCCCAAGGGCGACAAGGGAGACCCTGGGGTTGGAGAGAGAGGGCC  1406
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                  107  TCCAGGGGACGTAGGTCCCAAGGGCGACAAGGGAGACCCTGGGGTTGGAGAGAGAGGGCC   166

CDS:collagen alpha-1  448   P G P Q G P P G P P G P S F R H D K L T
Query                 1407  CCCAGGACCCCAAGGGCCTCCAGGGCCCCCAGGACCCTCCTTCAGACACGACAAGCTGAC  1466
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                  167  CCCAGGACCCCAAGGGCCTCCAGGGCCCCCAGGACCCTCCTTCAGACACGACAAGCTGAC   226

CDS:collagen alpha-1  468   F I D M E G S G F G G D L E A L R G P R
Query                 1467  CTTCATTGACATGGAGGGATCTGGCTTCGGGGGCGATCTGGAGGCCCTGCGGGGTCCTCG  1526
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Sbjct                 227  CTTCATTGACATGGAGGGATCTGGCTTCGGGGGCGATCTGGAGGCCCTGCGGGGTCCTCG  286

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Sbjct                 287  AGGCTTCCCTGGACCTCCCGGACCCCCCGGTGTCCCAGGCCTGCCCGGCGAGCCAGGCCG  346

CDS:collagen alpha-1  508    F  G  V  N  S  S  D  V  P  G  P  A  G  L  P  G  V  P  G  R
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CDS:collagen alpha-1  528    E  G  P  P  G  F  P  G  L  P  G  P  P  G  P  P  G  R  E  G
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CDS:collagen alpha-1  548    P  P  G  R  T  G  Q  K  G  S  L  G  E  A  G  A  P  G  H  K
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CDS:collagen alpha-1  568    G  S  K  G  A  P  G  P  A  G  A  R  G  E  S  G  L  A  G  A
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CDS:collagen alpha-1  588    P  G  P  A  G  P  P  G  P  P  G  P  P  G  P  P  G  P  G  L
Query                1827  CCCCGGACCTGCTGGACCACCAGGccccccctgggccccctgggccccccAGGACCAGGACT  1886
                           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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CDS:collagen alpha-1  608    P  A  G  F  D  D  M  E  G  S  G  G  P  F  W  S  T  A  R  S
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CDS:collagen alpha-1  628    A  D  G  P  Q  G  P  P  G  L  P  G  L  K  G  D  P  G  V  P
Query                1947  CGCTGATGGGCCACAGGGACCTCCCGGCCTGCCGGGACTTAAGGGGGATCCTGGCGTGCC  2006
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Query                2007  TGGGCTGCCGGGGGCGAA  2024
                           ||||||||||||||||||
Sbjct                 767  TGGGCTGCCGGGGGCGAA  784
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HHS Vulnerability Disclosure

Applied Biosystems

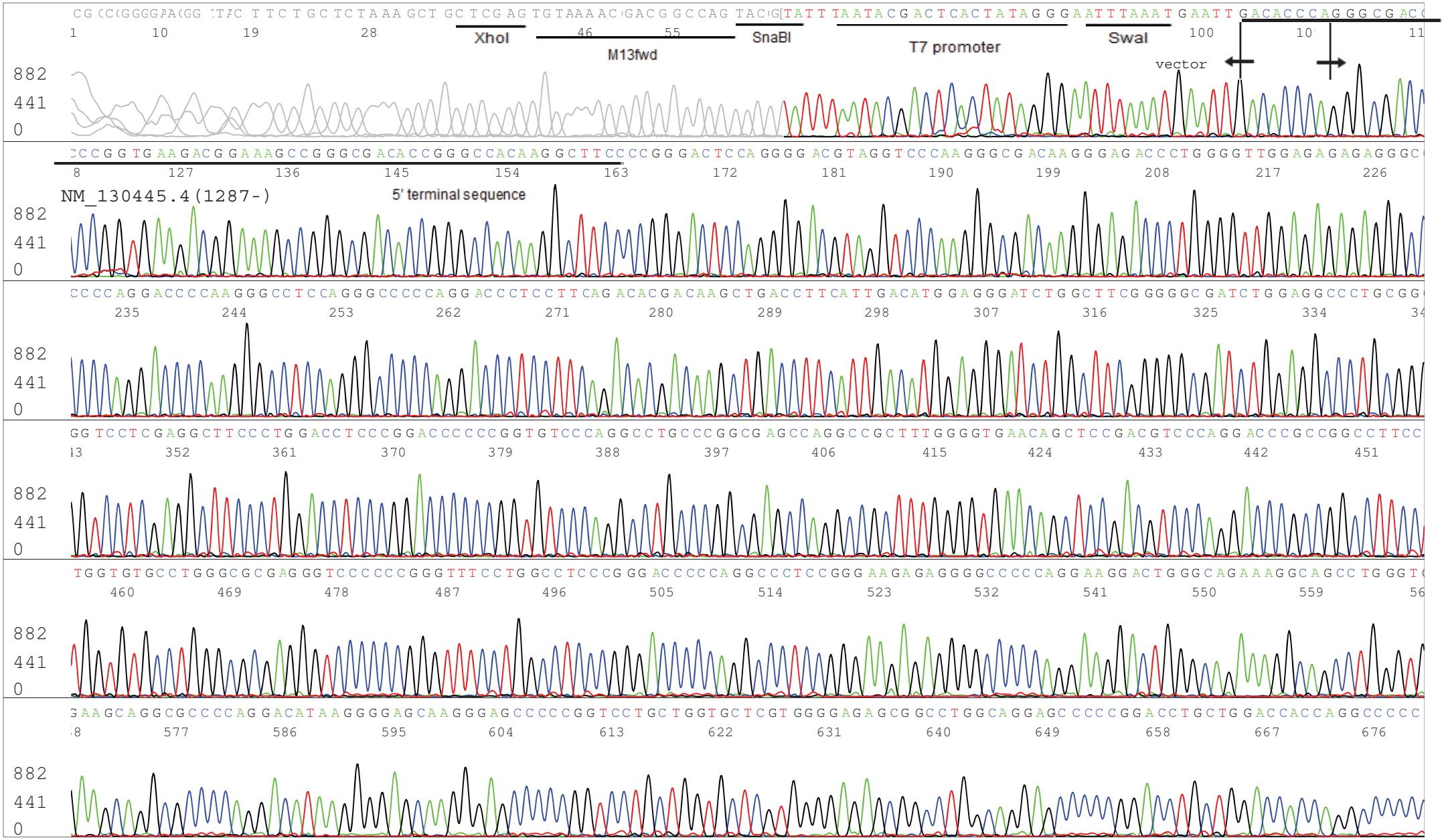
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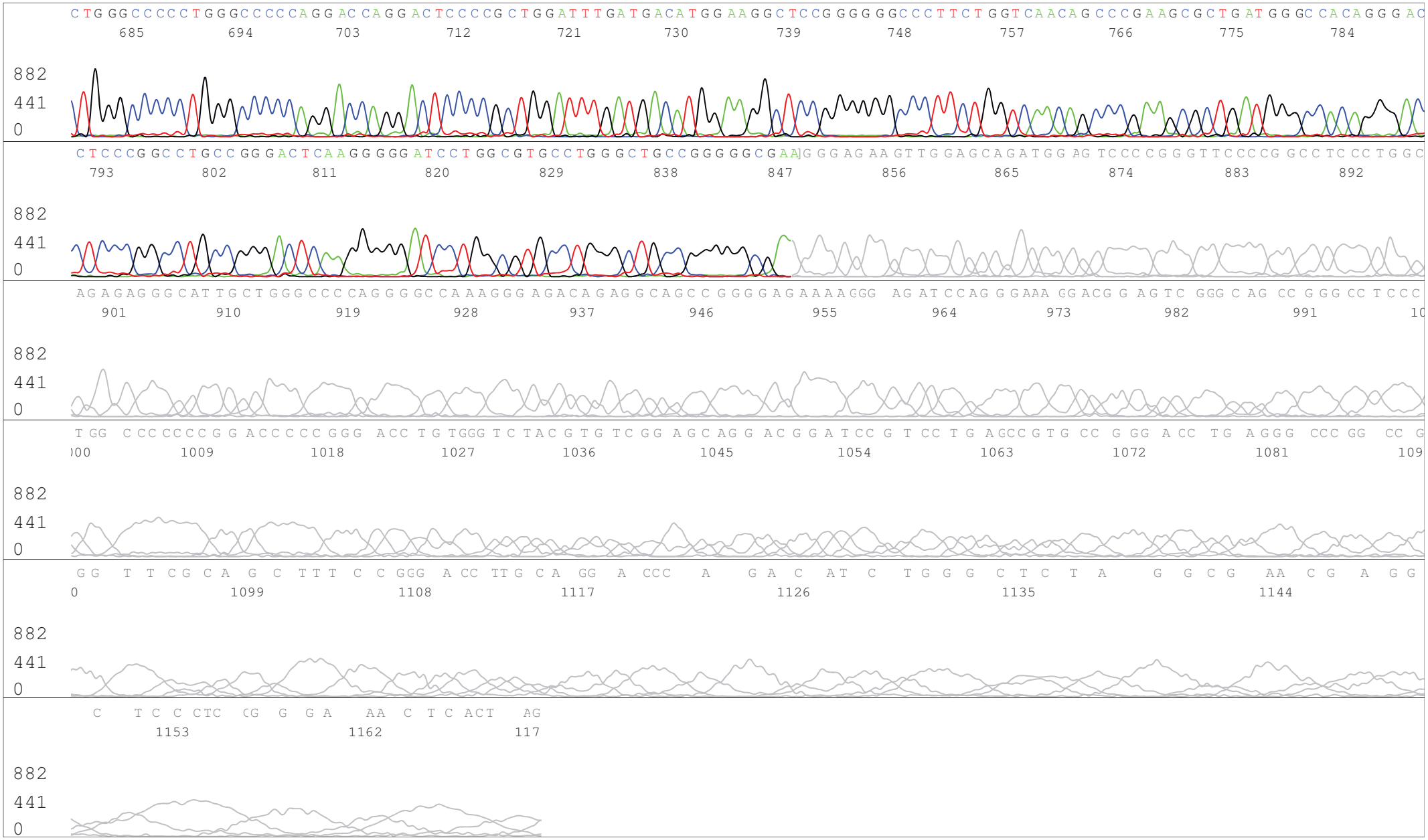
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5' CTCAGTGGATGTTGCCTTTAC 3'

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Pts 1418 to 13631 Pk1 Loc:1395
Version 6.0 HiSQV Bases: 944

Gray line indicates quality value (QV) < 40.

Inst Model/Name 3500/3500 Instrument
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Oct 31,2023 10:41AM, JST
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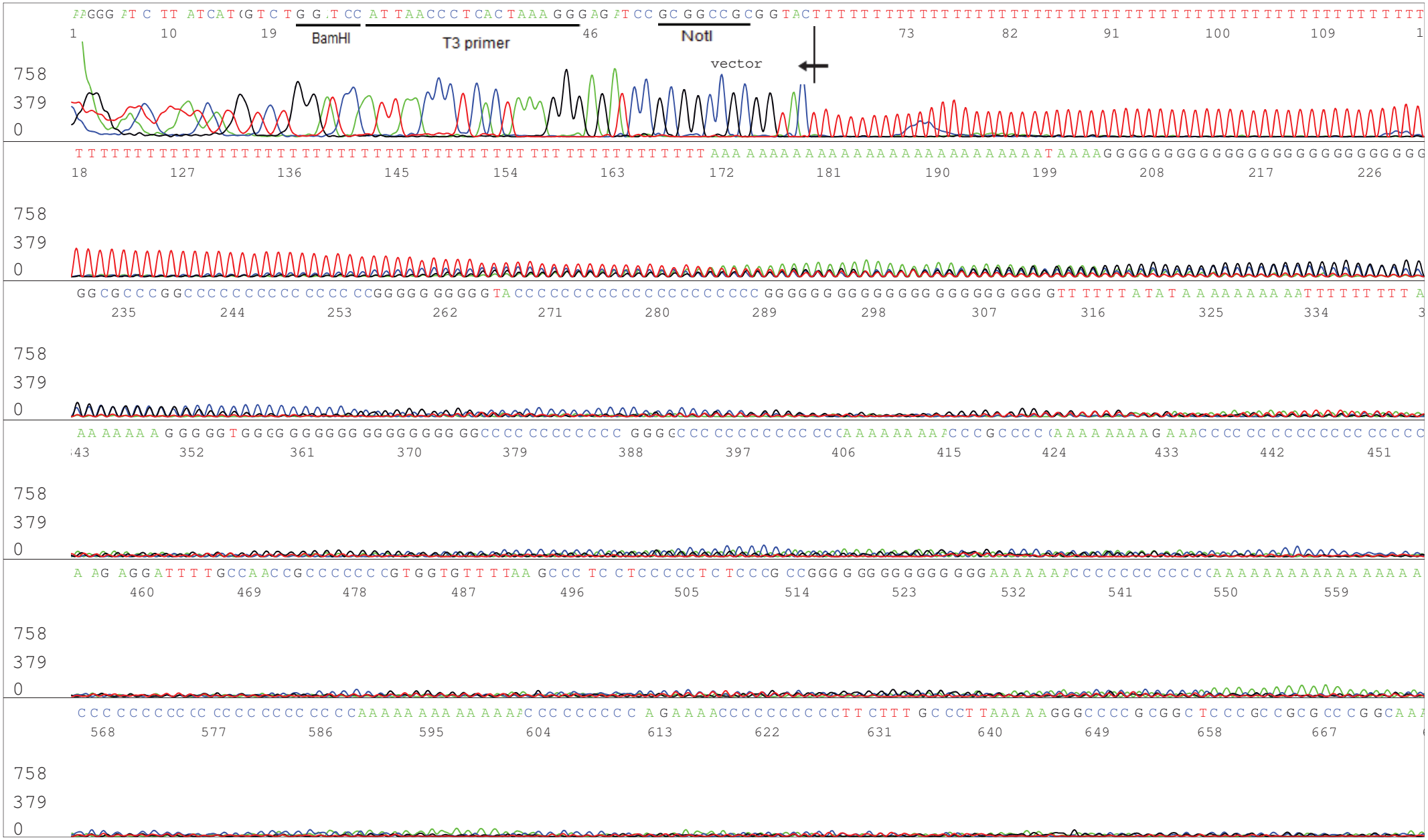
Applied Biosystems

S/N G:13 A:17 T:19 C:19
KB.bcp
KB 1.4.1.8 Cap:2

Primer B : pGCAP-R
5' GCATTCTAGTTGTGGTTTGTCC 3'

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Pts 1423 to 13000 Pk1 Loc:1400
Version 6.0 HiSQV Bases: 128

Inst Model/Name 3500/3500 Instrument
Oct 31,2023 10:09AM, JST
Oct 31,2023 10:41AM, JST
Spacing:13.25 Pts/Panel1350
Plate Name: 20231031_GNP



Applied Biosystems

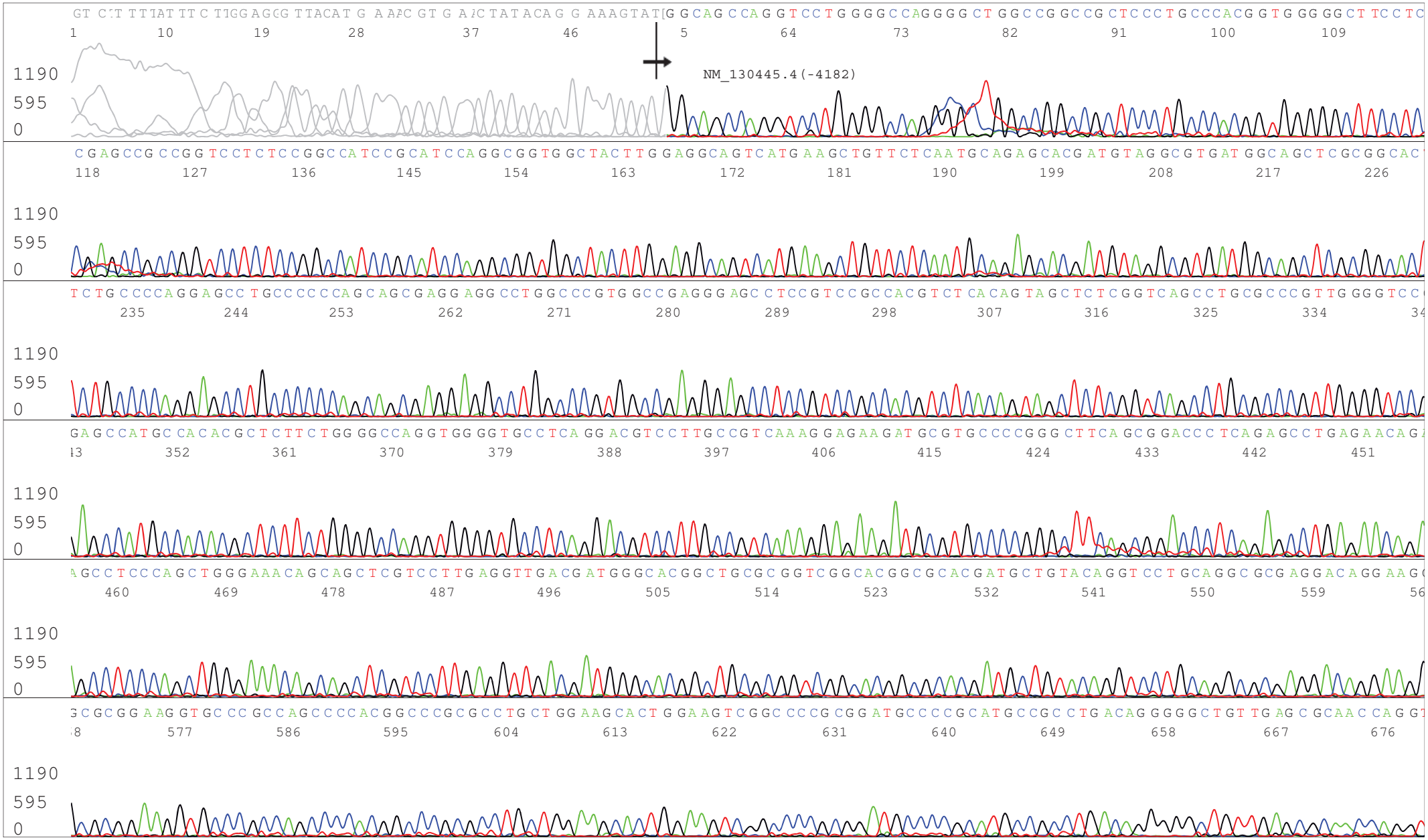
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KB 1.4.1.8 Cap:3

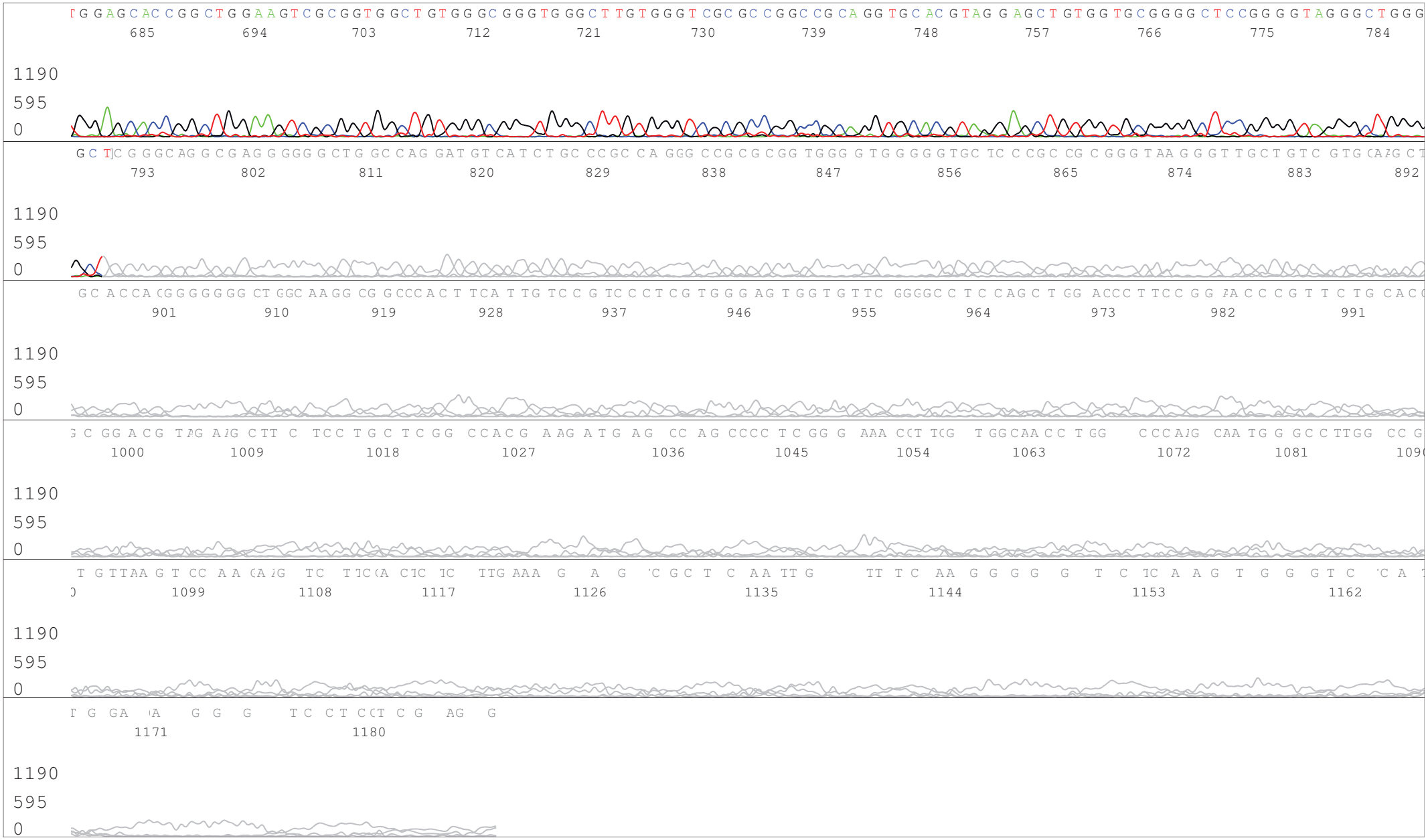
Primer C : Poly(A) region primer #0
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Version 6.0 HiSQV Bases: 843

Gray line indicates quality value (QV) < 40.

Inst Model/Name 3500/3500 Instrument
Oct 31,2023 10:09AM, JST
Oct 31,2023 10:41AM, JST
Spacing:12.1 Pts/Panel1350
Plate Name: 20231031_GNP




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