Gene Engineering Division
RIKEN BioResource Center

RIKEN_Clone_ID : ARe69D07

Vector : pKA1U5

Gene	NEO1		
RefSeq_mRNA	NM_002499.3	7088 bp	1..7088
	CDS	4386 bp	193..4578
	5'-terminal sequence	7018 bp	71..7088

● Plasmid DNA purification

Date : 130627 Culture : LB (100 ug/ml Ampicillin) 5 ml -> at 37 deg C O/N

Date : 130628 Purification : QIAGEN Miniprep kit -> dH_2O 100 ul

● Digestion by restriction enzyme/Concentrarion calibration

Date : 130628

DNA concentration (O.D.): 250 ng/ul

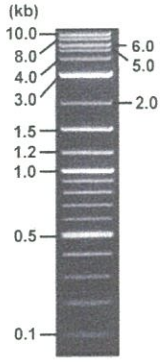
DNA	0.2	ul
Enzyme (BamHI)	1	ul
Buffer H	1	ul
dH_2O	7.8	ul
Total	10	ul

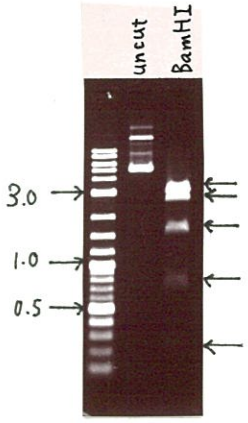
<Expected size of fragment from ARe69D07 >

BamHI	3183	3445, 2693, 838, 256bp (inference)

Erectrophoresis : 1% agarose gel, 1x TAE Buffer

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





● Confirmation of the insertion sequence

Date : 130703

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

● Adjustment of DNA concentration

Shipping amount : 40 ul

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

Date : 130703

DNA (250 ng/ul)	85.0	ul
10x TE	85.0	ul
dH_2O	680.0	ul
Total	850.0	ul

APPROVED BY : 確認 13.7.03 笹沼

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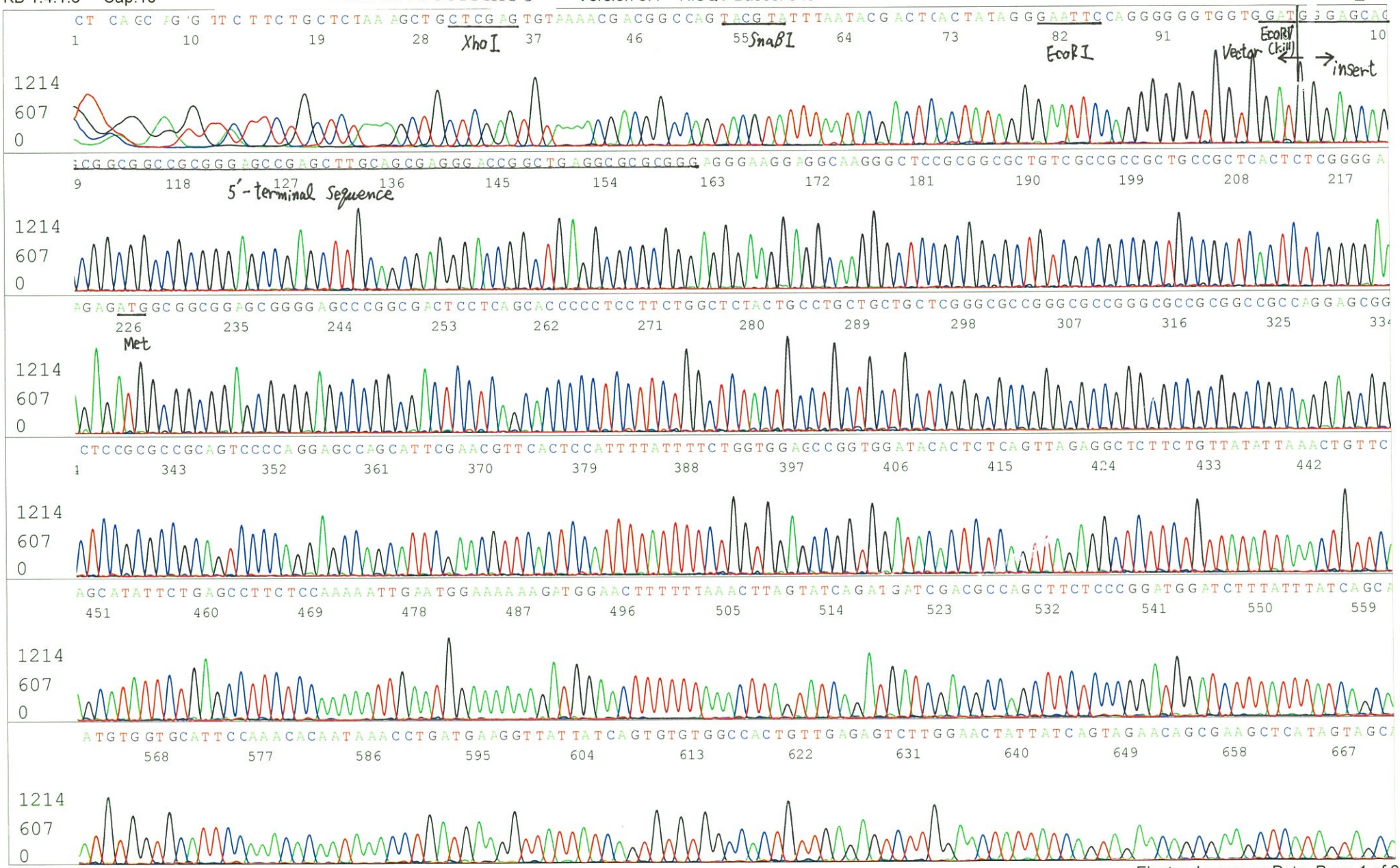
S/N G:291 A:241 T:171 C:21(
KB.bcp
KB 1.4.1.8 Cap:10

Primer A : pGCAP-F

5' CTCAGTGGATGTTGCCTTTAC 3'

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7672_ARe69D07_A3Fr_pGCAP_F
KB_3500_POP7_BDTv3.mob
Pts 1503 to 13066 Pk1 Loc:1480
Version 5.4 HiSQV Bases: 949

Inst Model/Name 3500/3500 Instrument
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Jul 03,2013 11:23AM, JST
Spacing:11.56 Pts/Panel1350
Plate Name: 20130703_mix

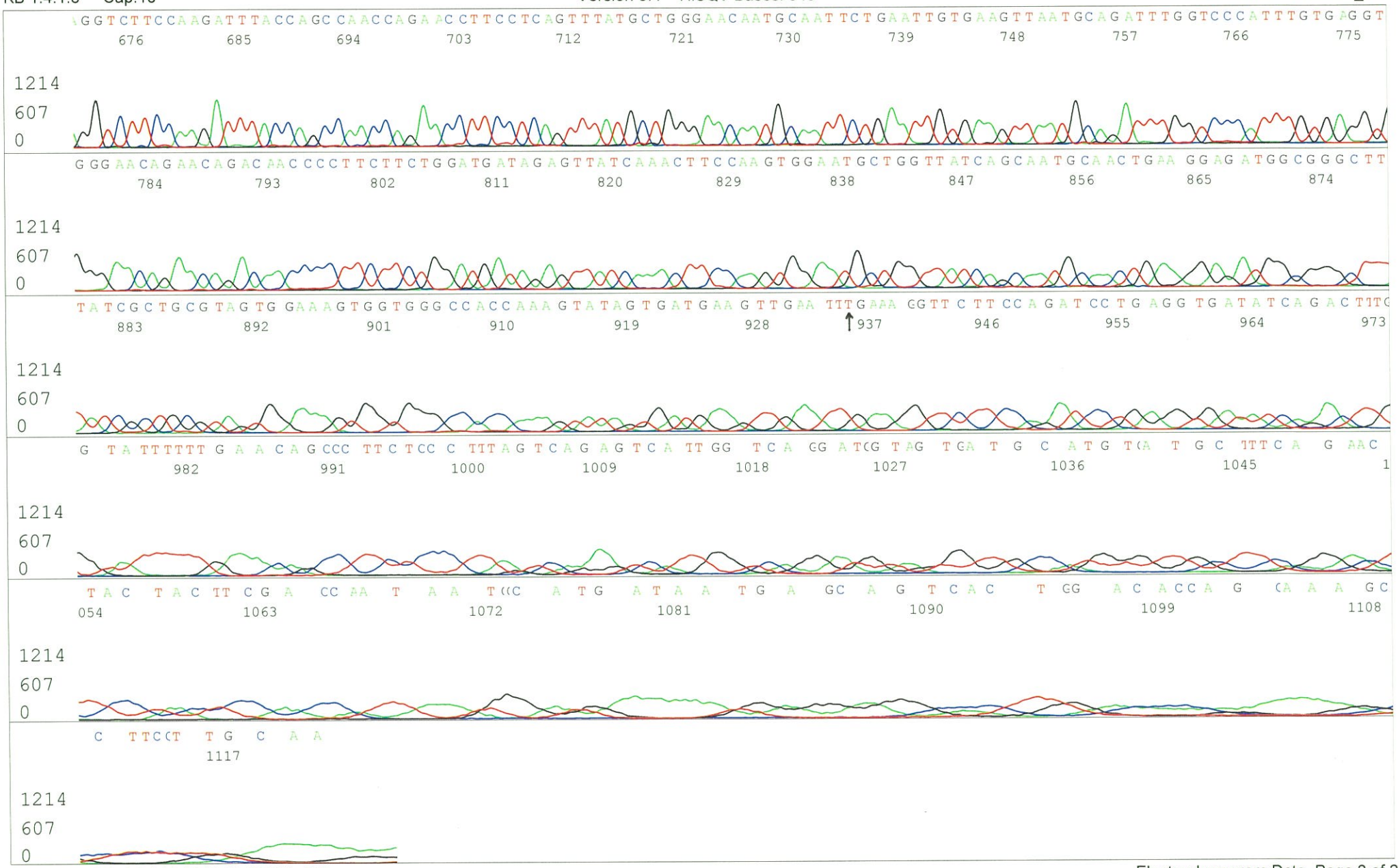


Applied Biosystems

S/N G:291 A:241 T:171 C:210
KB.bcp
KB 1.4.1.8 Cap:10

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KB_3500_POP7_BDTv3.mob
Pts 1503 to 13066 Pk1 Loc:1480
Version 5.4 HiSQV Bases: 949

Inst Model/Name 3500/3500 Instrument
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Applied Biosystems

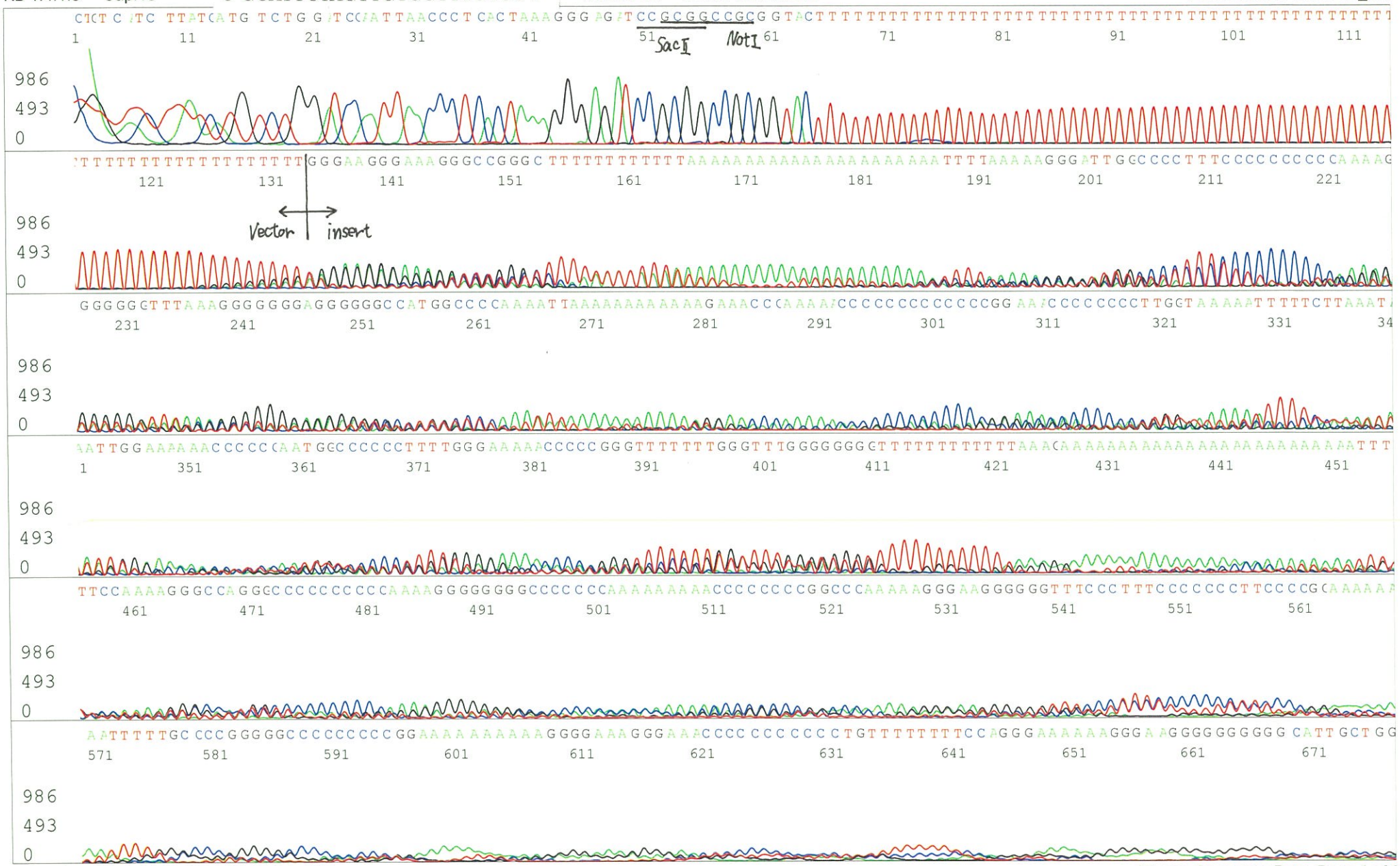
S/N G:92 A:107 T:96 C:86
KB.bcp
KB 1.4.1.8 Cap:13

Primer B : pGCAP-R

5' GCATTCTAGTTGTGGTTTGTCC 3'

7672_ARe69D07_A3Fr_pGCAP_R_E04_13
7672_ARe69D07_A3Fr_pGCAP_R
KB_3500_POP7_BDTv3.mob
Pts 1532 to 13291 Pk1 Loc:1509
Version 5.4 HiSQV Bases: 215

Inst Model/Name 3500/3500 Instrument
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Jul 03,2013 11:23AM, JST
Spacing:11.82 Pts/Panel1350
Plate Name: 20130703_mix

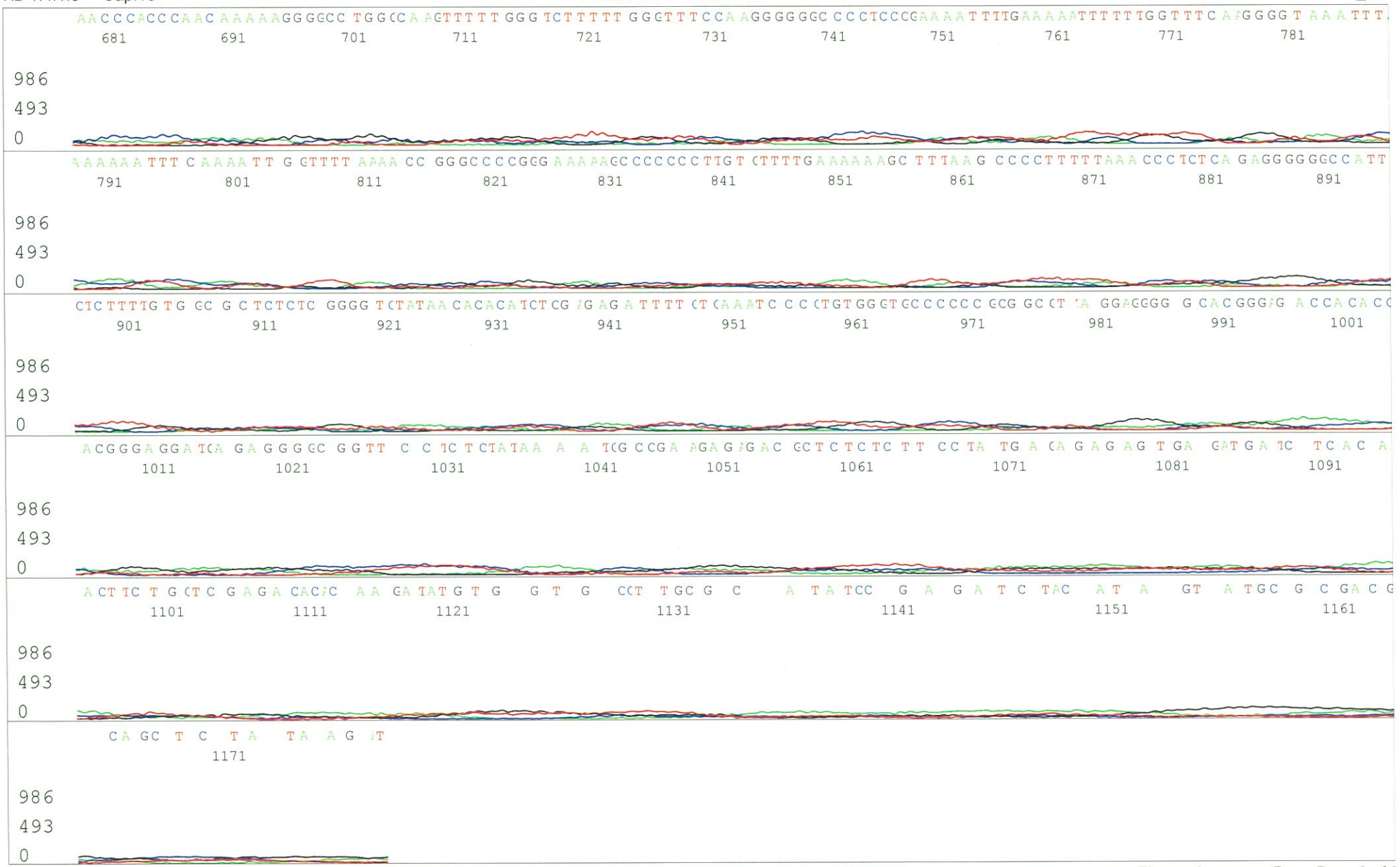


Applied Biosystems

S/N G:92 A:107 T:96 C:86
KB.bcp
KB 1.4.1.8 Cap:13

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7672_ARe69D07_A3Fr_pGCAP_R
KB_3500_POP7_BDTv3.mob
Pts 1532 to 13291 Pk1 Loc:1509
Version 5.4 HiSQV Bases: 215

Inst Model/Name 3500/3500 Instrument
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Jul 03,2013 11:23AM, JST
Spacing:11.82 Pts/Panel1350
Plate Name: 20130703_mix



Applied Biosystems

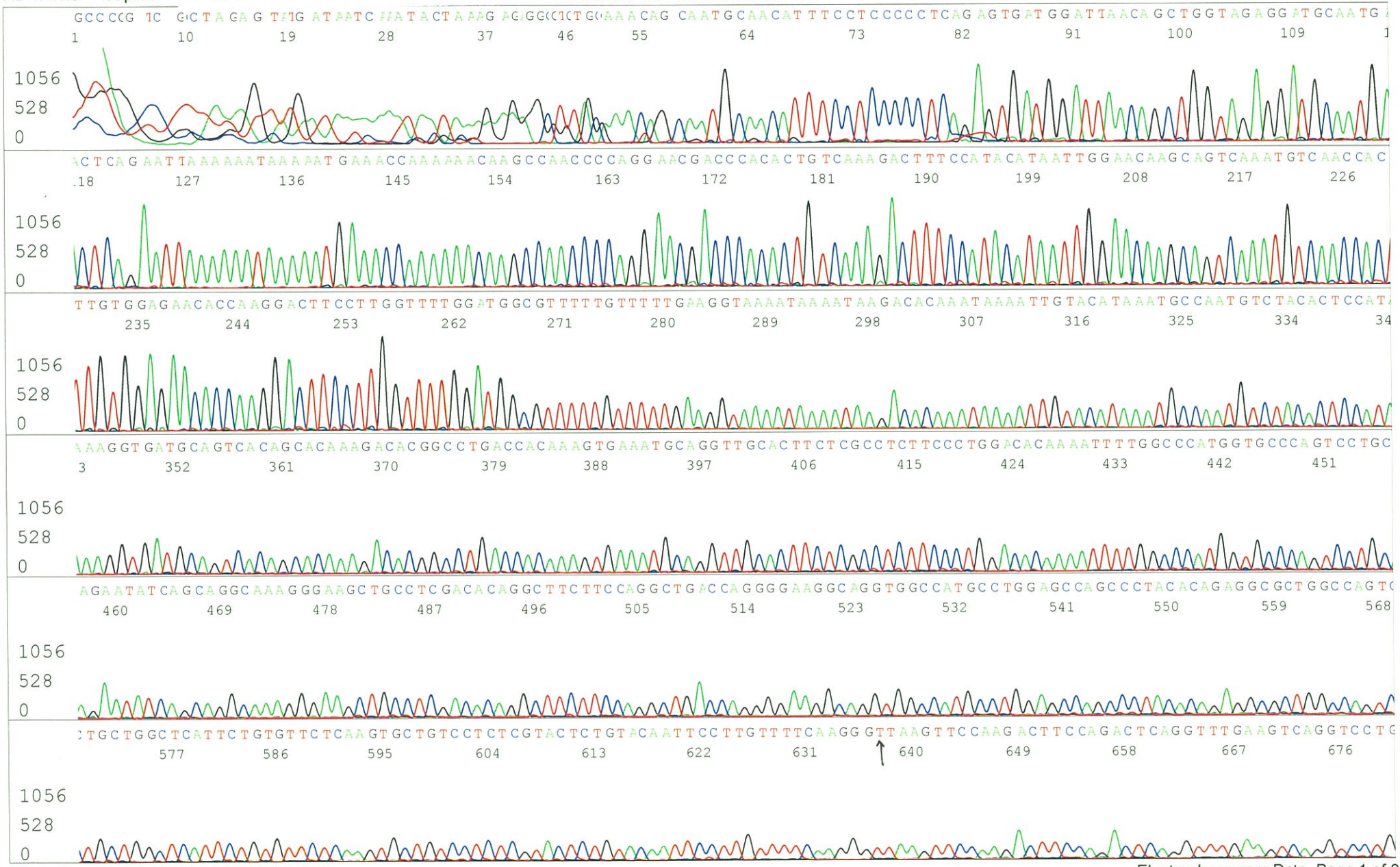
S/N G:129 A:101 T:75 C
KB.bcp
KB 1.4.1.8 Cap:16

Primer C : Poly(A) region primer #0

5' TTTTTTTTTTTTTTTTTTTTTNNN 3'

7672_ARe69D07_A3Fr_GNPmixture_F04_16
7672_ARe69D07_A3Fr_GNPmixture
KB_3500_POP7_BDTv3.mob
Pts 1502 to 13060 Pk1 Loc:1479
Version 5.4 HiSQV Bases: 932

Inst Model/Name 3500/3500 Instrument
Jul 03,2013 10:48AM, JST
Jul 03,2013 11:23AM, JST
Spacing:11.5 Pts/Panel1350
Plate Name: 20130703_mix



Applied Biosystems

S/N G:129 A:101 T:75 C:82
KB.bcp
KB 1.4.1.8 Cap:16

7672_ARe69D07_A3Fr_GNPmixture_F04_16
7672_ARe69D07_A3Fr_GNPmixture
KB_3500_POP7_BDTv3.mob
Pts 1502 to 13060 Pk1 Loc:1479
Version 5.4 HiSQV Bases: 932

Inst Model/Name 3500/3500 Instrument
Jul 03,2013 10:48AM, JST
Jul 03,2013 11:23AM, JST
Spacing:11.5 Pts/Panel1350
Plate Name: 20130703_mix

