Gene Engineering Division
RIKEN BioResource Center

RIKEN Clone ID : RBdS013P01

Vector : pGCAP10

Gene	PDE6A	
RefSeq_mRNA	NM_000440.2	5659 bp	1..5659
	CDS	2583 bp	121..2703
	5'-terminal sequence	5659 bp	1..5659

● Plasmid DNA purification

Date : 130121

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

Date : 130122

Purification : QIAGEN Miniprep kit -> dH_2O 100 ul

● Digestion by restriction enzyme/Concentrarion calibration

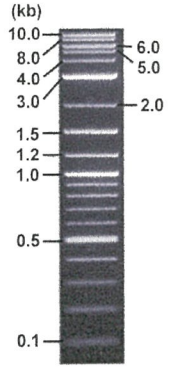
Date : 130122

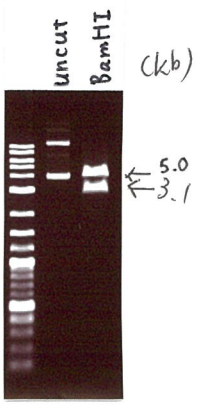
DNA concentration (O.D.): 195 ng/ul

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

Erectrophoresis : 1% agarose gel, 1x TAE Buffer

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





<Expected size of fragment from RBdS013P01 >

4981, 3138, 879 bp (inference)

● Confirmation of the insertion sequence

Date : 130123

Primer A	pGCAP-F
Primer B	pGCAP-R
Primer C	Poly(A) region primer
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/ul

Date : 130129

DNA (195 ng/ul)	86.0 ul
10x TE	67.1 ul
dH_2O	517.7 ul
Total	670.8 ul

確認 13.1.31 笹沼

APPROVED BY :

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

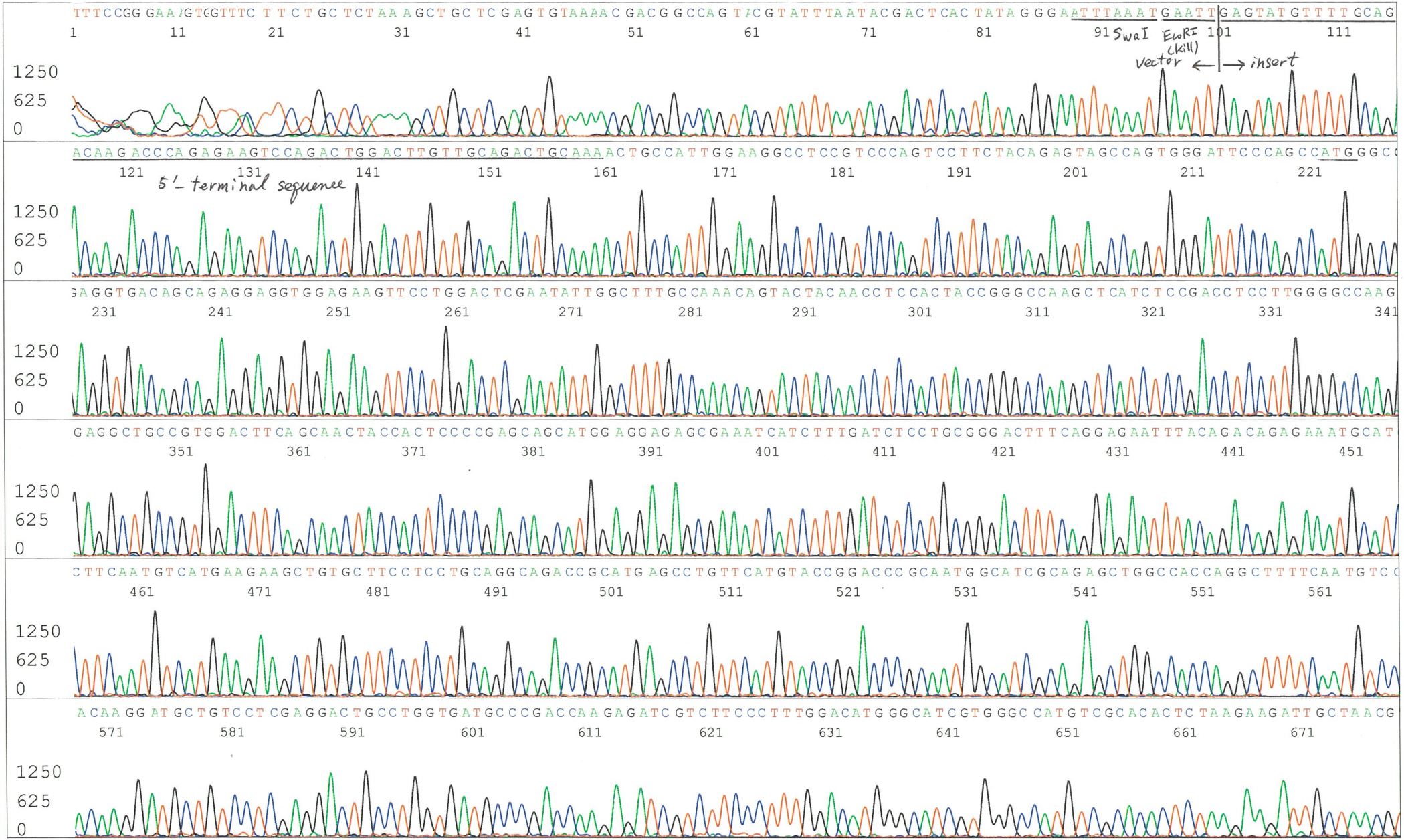
Applied Biosystems

S/N G:26 A:16 T:18 C:17
KB.bcp
KB 1.4.1.8 Cap:15

Primer A : pGCAP-F
5' CTCAGTGGATGTTGCCTTTAC 3'

6608_RBdS013P01_pGCAP_F_E12_15
6608_RBdS013P01_pGCAP_F
KB_3500_POP7_BDTv3.mob
Pts 1406 to 14323 Pk1 Loc:1383
Version 5.4 HiSQV Bases: 1026

Inst Model/Name 3500/3500 Instrument
Jan 23,2013 11:03AM, JST
Jan 23,2013 11:45AM, JST
Spacing:12.64 Pts/Panel1350
Plate Name: 20130123_MIX

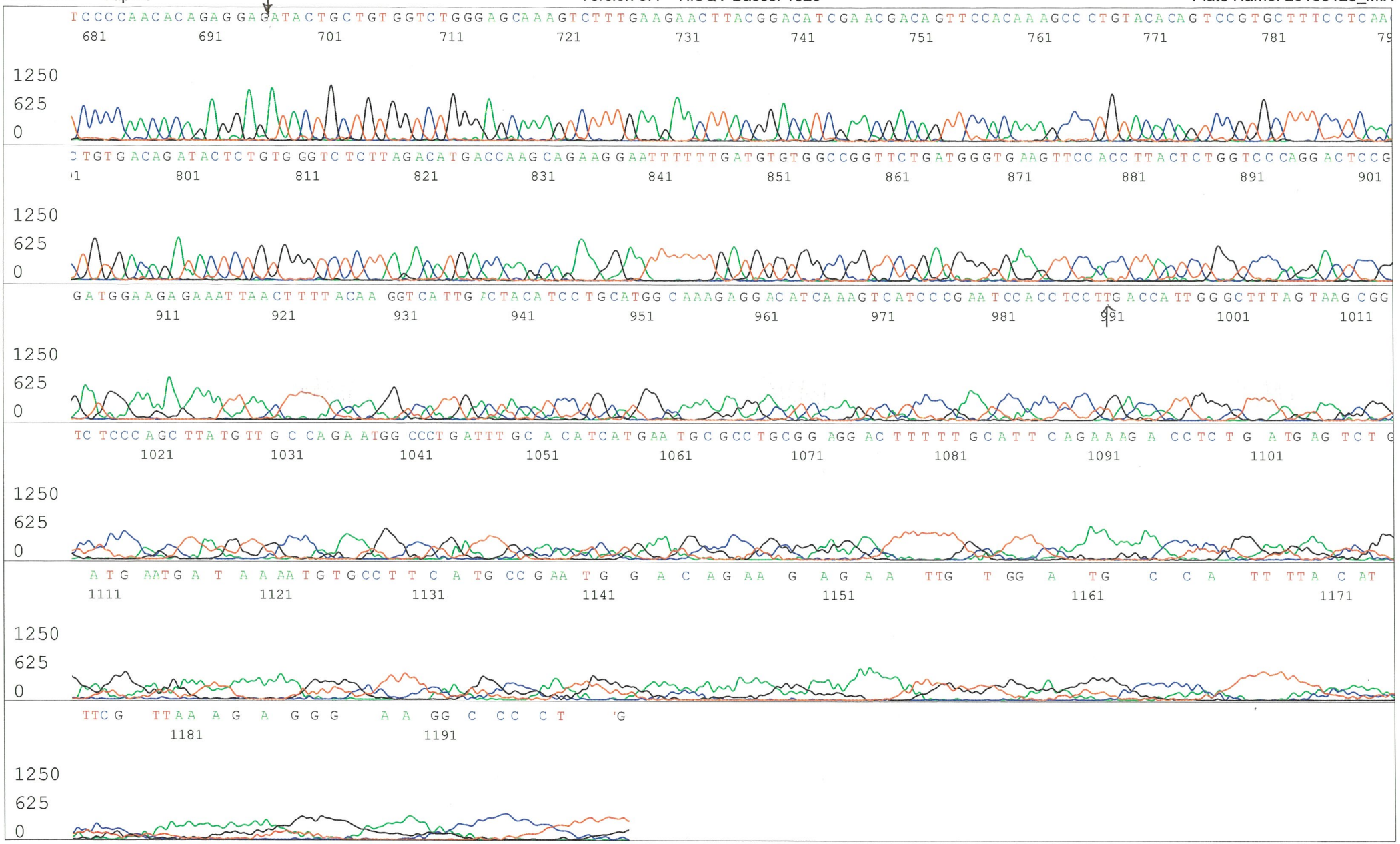


Applied Biosystems

S/N G:26 A:16 T:18 C:17
KB.bcp
KB 1.4.1.8 Cap:15

6608_RBdS013P01_pGCAP_F_E12_15
6608_RBdS013P01_pGCAP_F
KB_3500_POP7_BDTv3.mob
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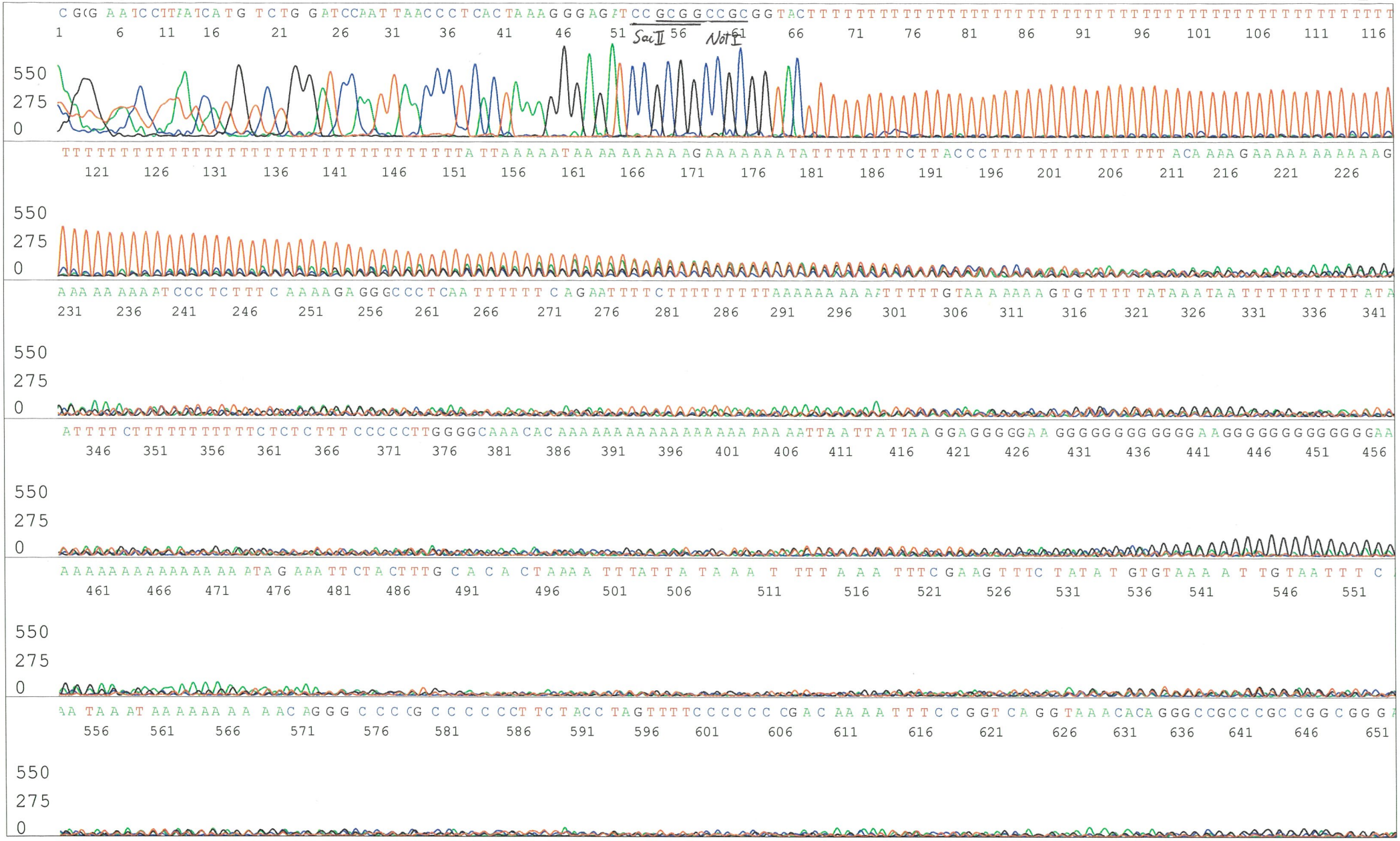
Applied Biosystems

S/N G:12 A:8 T:16 C:7
KB.bcp
KB 1.4.1.8 Cap:18

Primer B : pGCAP-R
5' GCATTCTAGTTGTGGTTTGTCC 3'

6608_RBdS013P01_pGCAP_R_F12_18
6608_RBdS013P01_pGCAP_R
KB_3500_POP7_BDTv3.mob
Pts 1402 to 14632 Pk1 Loc:1379
Version 5.4 HiSQV Bases: 155

Inst Model/Name 3500/3500 Instrument
Jan 23,2013 11:03AM, JST
Jan 23,2013 11:45AM, JST
Spacing:13.01 Pts/Panel1350
Plate Name: 20130123_MIX



Applied Biosystems

S/N G:14 A:10 T:12 C:7
KB.bcp
KB 1.4.1.8 Cap:21

Primer C : Poly(A) region primer #0
5' TTTTTTTTTTTTTTTTTTTTTNNN 3'

6608_RBdS013P01_GNPmixture_G12_21
6608_RBdS013P01_GNPmixture
KB_3500_POP7_BDTv3.mob
Pts 1396 to 14362 Pk1 Loc:1373
Version 5.4 HiSQV Bases: 485

Inst Model/Name 3500/3500 Instrument
Jan 23,2013 11:03AM, JST
Jan 23,2013 11:45AM, JST
Spacing:12.73 Pts/Panel1350
Plate Name: 20130123_MIX

