

**RIKEN Clone ID : IRAK050G10**

Lot#: 7893\_A3lr

Vector : pCMV-SPORT6

Gene	RORC	
Accession No.	BC031554.1	2150 bp
		1..2150
	<i>CDS</i>	1557 bp
		27..1583

● Plasmid DNA purification

Date : 130930

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

Date : 131001

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

● Digestion by restriction enzyme/Concentration calibration

Date : 131001

DNA concentration (O.D.): 131 ng/ul

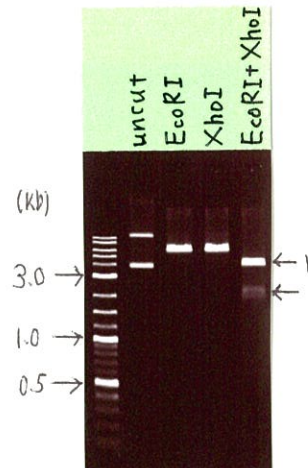
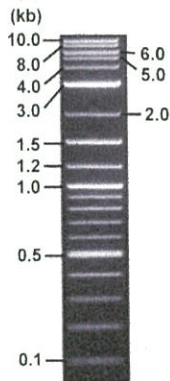
< Size of fragment expected from IRAK050G10 >

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	6532	bp
XhoI	6532	bp
EcoRI + XhoI	4339bp (Vector)	2193 bp

Erectrophoresis : 1% agarose gel, 1x TAE Buffer

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



● Confirmation of the insertion sequence

Date : 131002

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>

● Adjustment of DNA concentration

Shipping amount : 40 ul

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

Date : 131003

DNA ( <u>131</u> ng/ul)	83.0	ul
10x TE	43.5	ul
dH <sub>2</sub> O	308.4	ul
Total	434.9	ul



APPROVED BY :

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

S/N G:257 A:210 T:167 C:1

KB.bcp

KB 1.4.1.8 Cap:3

Primer A : Reverse2

IRAK050G10\_A3lr\_Reverse2

KB\_3500\_POP7\_BDTv3.mob

Pts 1348 to 13397 Pk1 Loc:1325

Version 5.4 HiSQV Bases: 1027

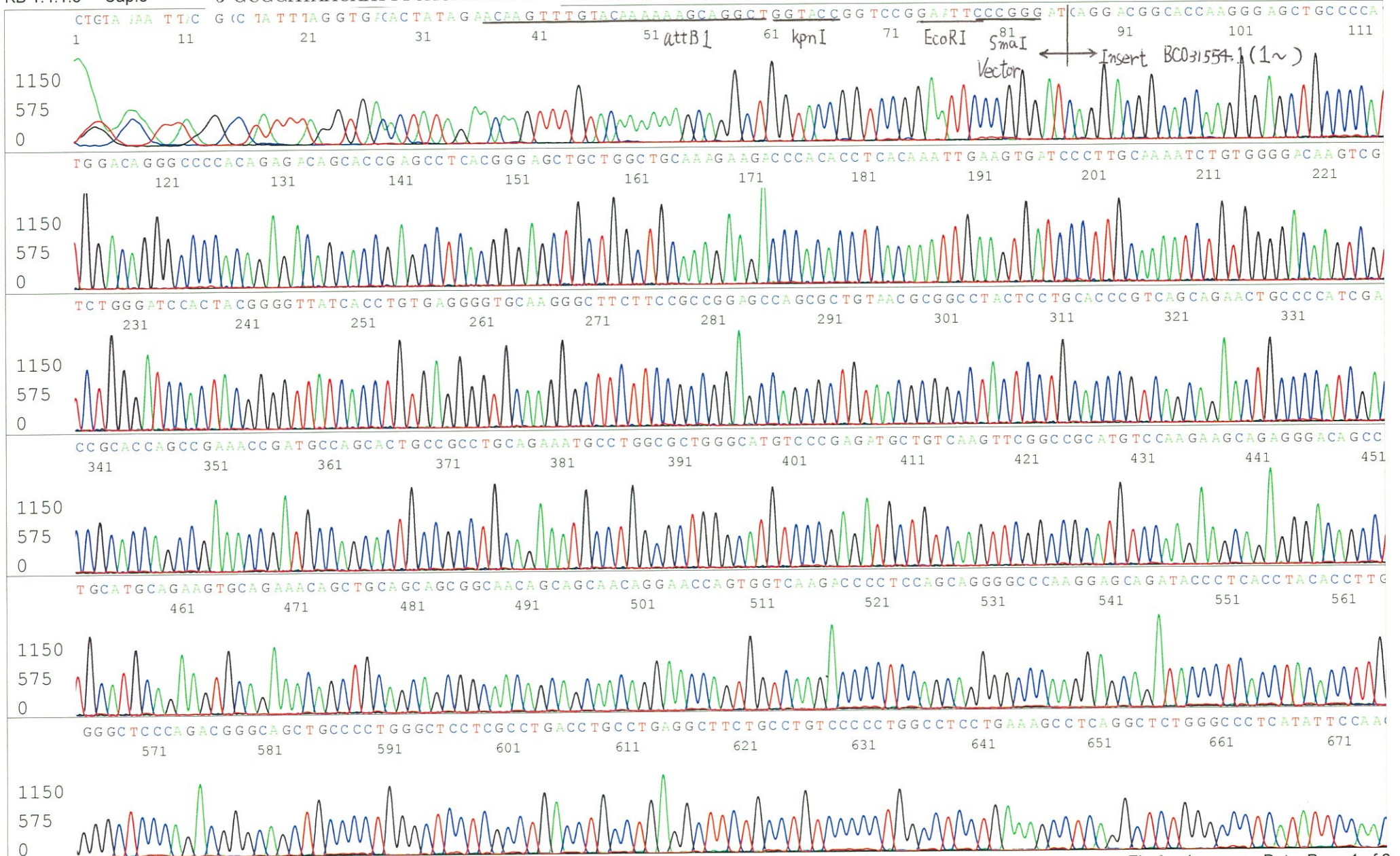
Oct 02,2013 10:37AM, JST

Oct 02,2013 11:03AM, JST

Spacing:11.87 Pts/Panel1350

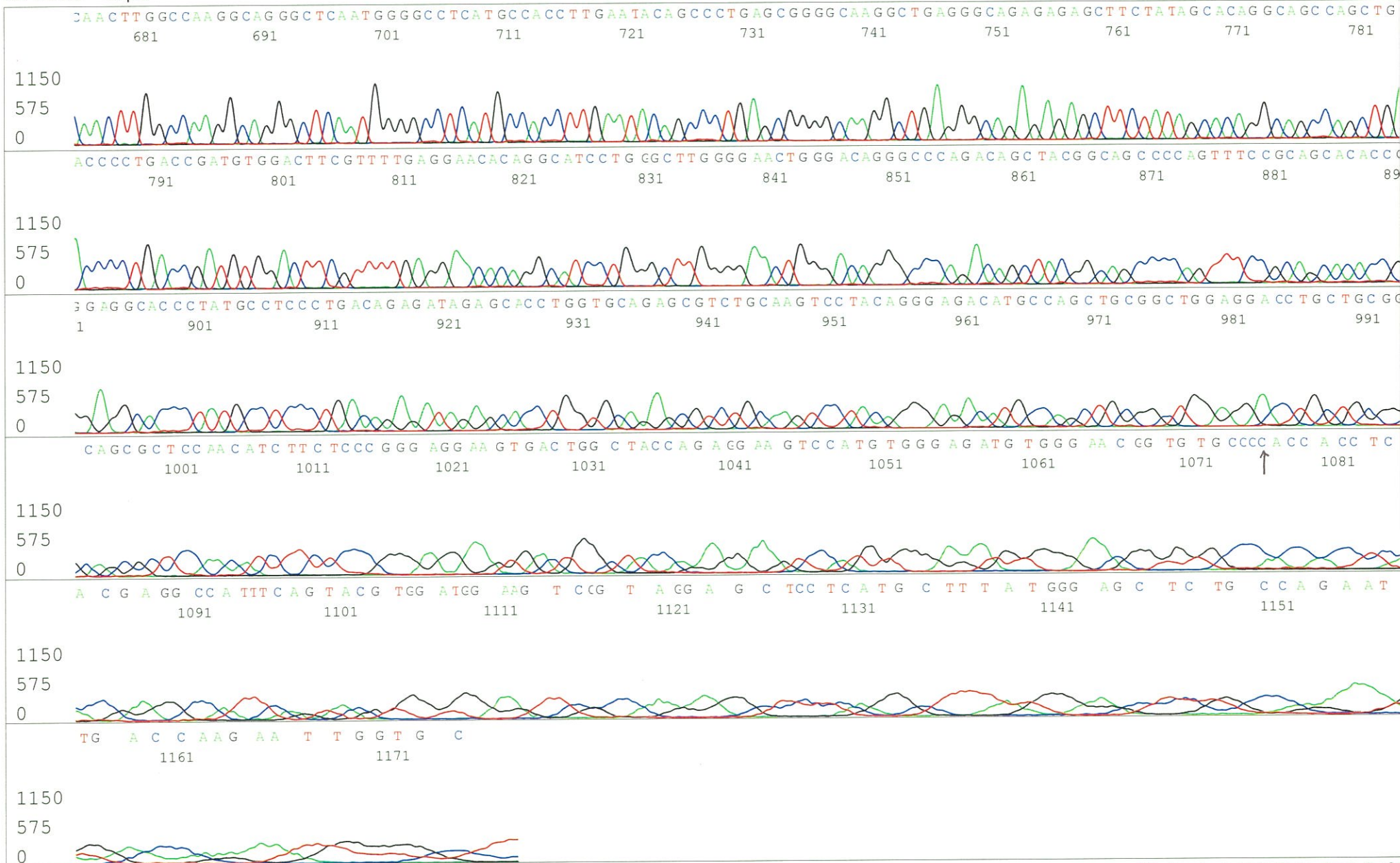
Plate Name: 20131002\_Mix

5' GCGGATAACAATTTTCACACAGG 3'





S/N G:257 A:210 T:167 C:160  
KB.bcp  
KB 1.4.1.8 Cap:3



S/N G:96 A:72 T:121 C:56 Primer B : M13

KB.bcp

KB 1.4.1.8 Cap:6

5' GTTTTCCAGTCACGACGTTGTA 3'

IRAK050G10\_A3lr\_M13(-40)

KB\_3500\_POP7\_BDTv3.mob

Pts 1344 to 13560 Pk1 Loc:1321

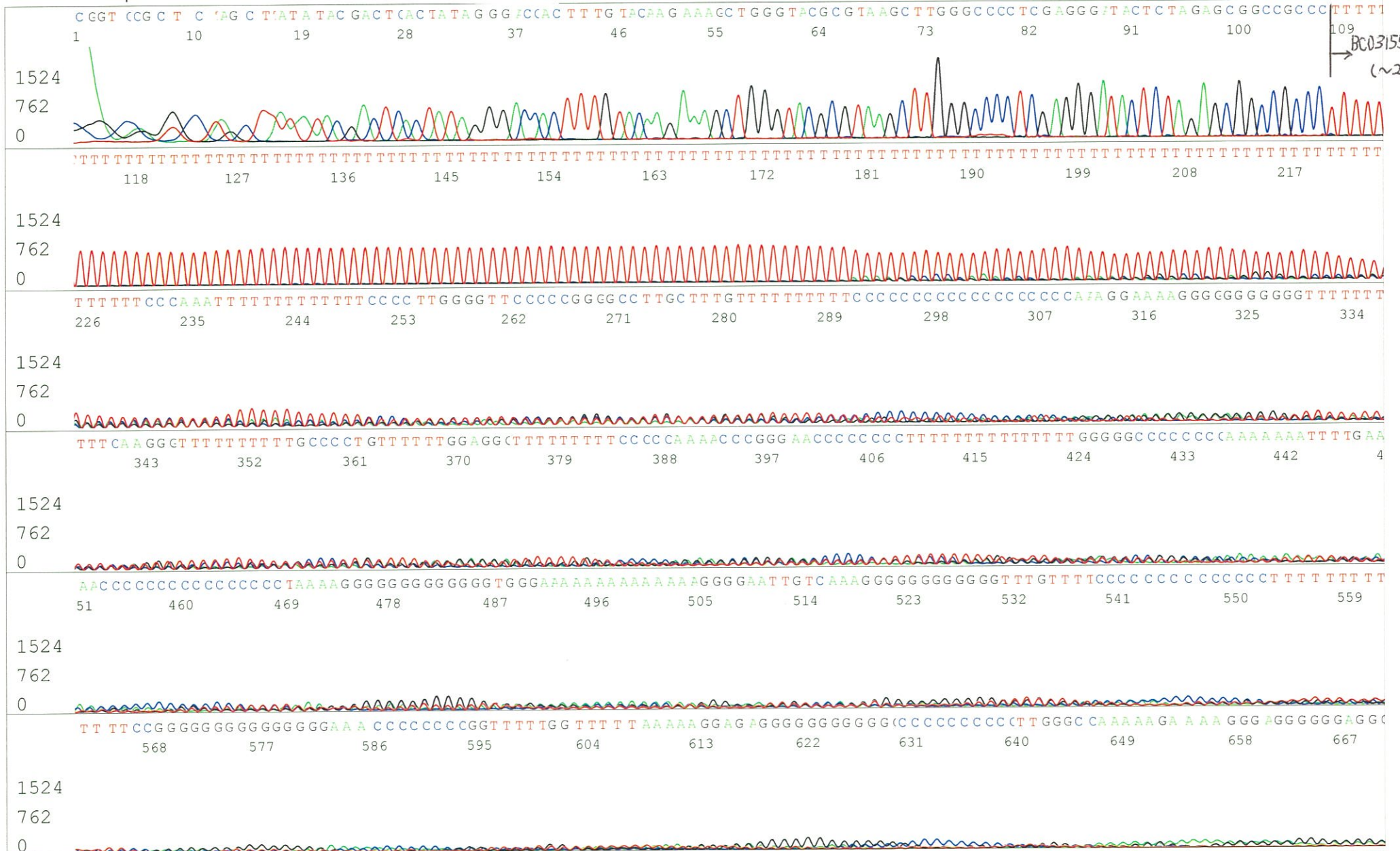
Version 5.4 HiSQV Bases: 242

Oct 02,2013 10:37AM, JST

Oct 02,2013 11:03AM, JST

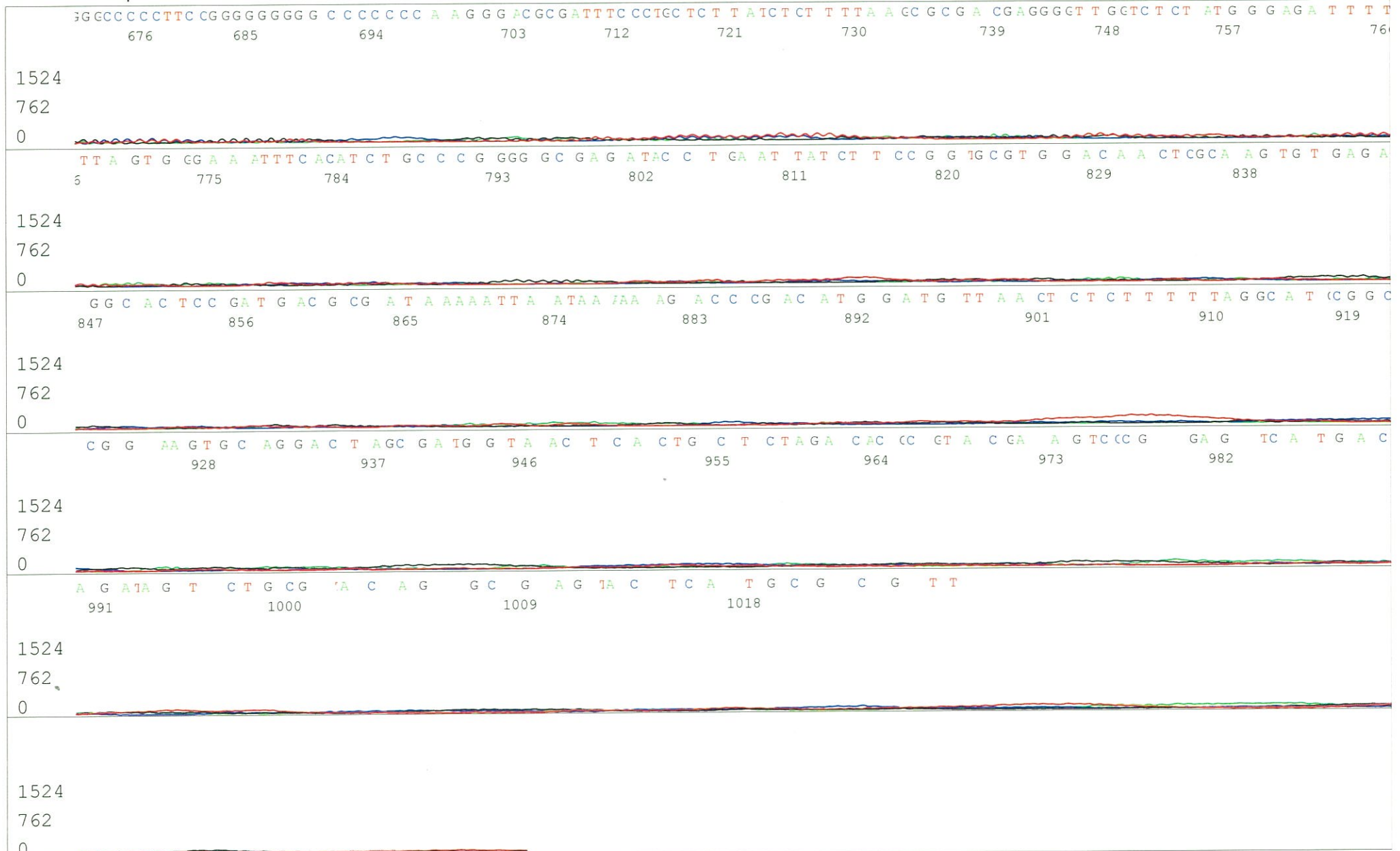
Spacing:12.17 Pts/Panel1350

Plate Name: 20131002\_Mix





S/N G:96 A:72 T:121 C:56  
KB.bcp  
KB 1.4.1.8 Cap:6



S/N G:38 A:24 T:21 C:18

Primer C : Poly(A) region primer #0

KB.bcp

KB 1.4.1.8 Cap:9

5' TTTTTTTTTTTTTTTTTTTTTTNNN 3'

IRAK050G10\_A3lr\_GNPmixture

KB\_3500\_POP7\_BDTv3.mob

Pts 1357 to 13418 Pk1 Loc:1334

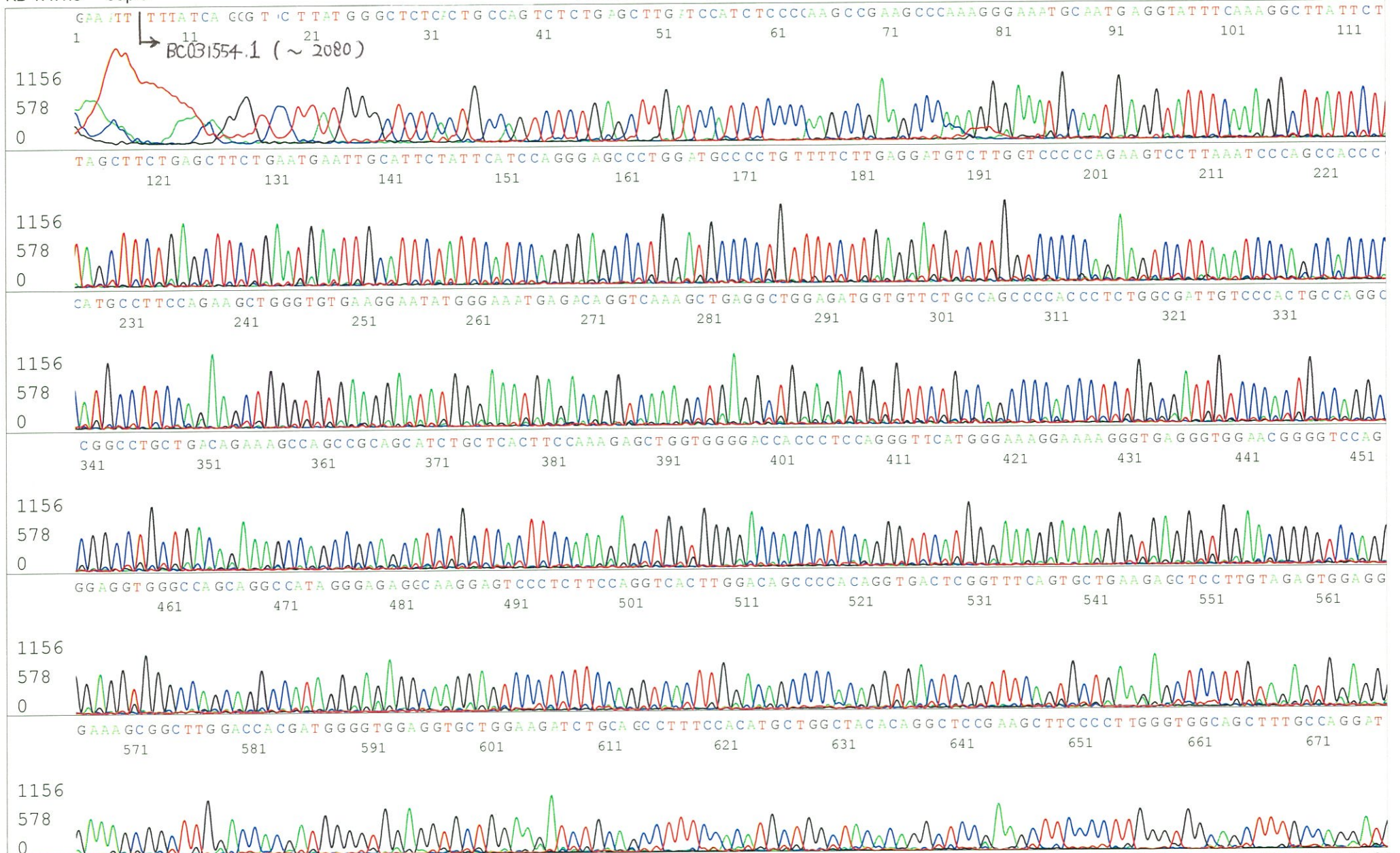
Version 5.4 HiSQV Bases: 1014

Oct 02,2013 10:37AM, JST

Oct 02,2013 11:03AM, JST

Spacing:11.84 Pts/Panel1350

Plate Name: 20131002\_Mix





S/N G:38 A:24 T:21 C:18

KB.bcp

KB 1.4.1.8 Cap:9

IRAK050G10\_A3lr\_GNPmixture

KB\_3500\_POP7\_BDTv3.mob

Pts 1357 to 13418 Pk1 Loc:1334

Version 5.4 HiSQV Bases: 1014

Oct 02,2013 10:37AM, JST

Oct 02,2013 11:03AM, JST

Spacing:11.84 Pts/Panel1350

Plate Name: 20131002\_Mix

