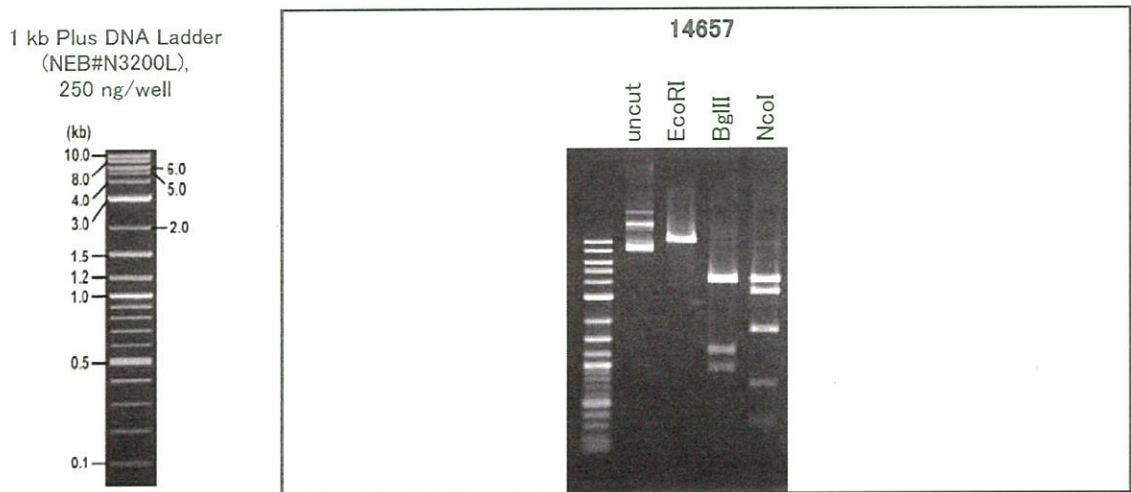




**RIKEN DNA BANK**

clone name : T7-NLS-hCAS9-NLS-P2A-EGFP-pA

- Clone ID : RDB \_ 14657
- Lot : 14657 \_ A7Cs
- DNA Concentration : 25 nanogram/microliter
- Volume : 40 microliter
- Form : DNA solution in TE buffer
- Host : DH5 alpha
- Culture : LB medium
- Antibiotics : 100 microgram/ml Ampicillin
- Purification : QIAGEN QIAprep Spin Miniprep kit
- Digestion by restriction enzyme



Electrophoresis : 200 nanogram DNA per lane ; 1% agarose gel , 1 x TAE Buffer

Restriction enzyme	Expected size of fragment	
<b>EcoRI</b>	<b>10.4</b>	kbp
<b>BglII</b>	<b>4.2, 4.1, 1.2, 0.9</b>	kbp
<b>NcoI</b>	<b>4.2, 3.3, 1.8, 0.7, 0.3</b>	kbp
		kbp

● Confirmation of the insertion sequence

Sequence name	Primer name	Sequence name	Primer name
Sequence - A	CMV-Forward	Sequence - E	HSV_TK_pA_R
Sequence - B	EGFP-N	Sequence - F	-
Sequence - C	EGFP-C2	Sequence - G	-
Sequence - D	SV40pro_F	Sequence - H	-

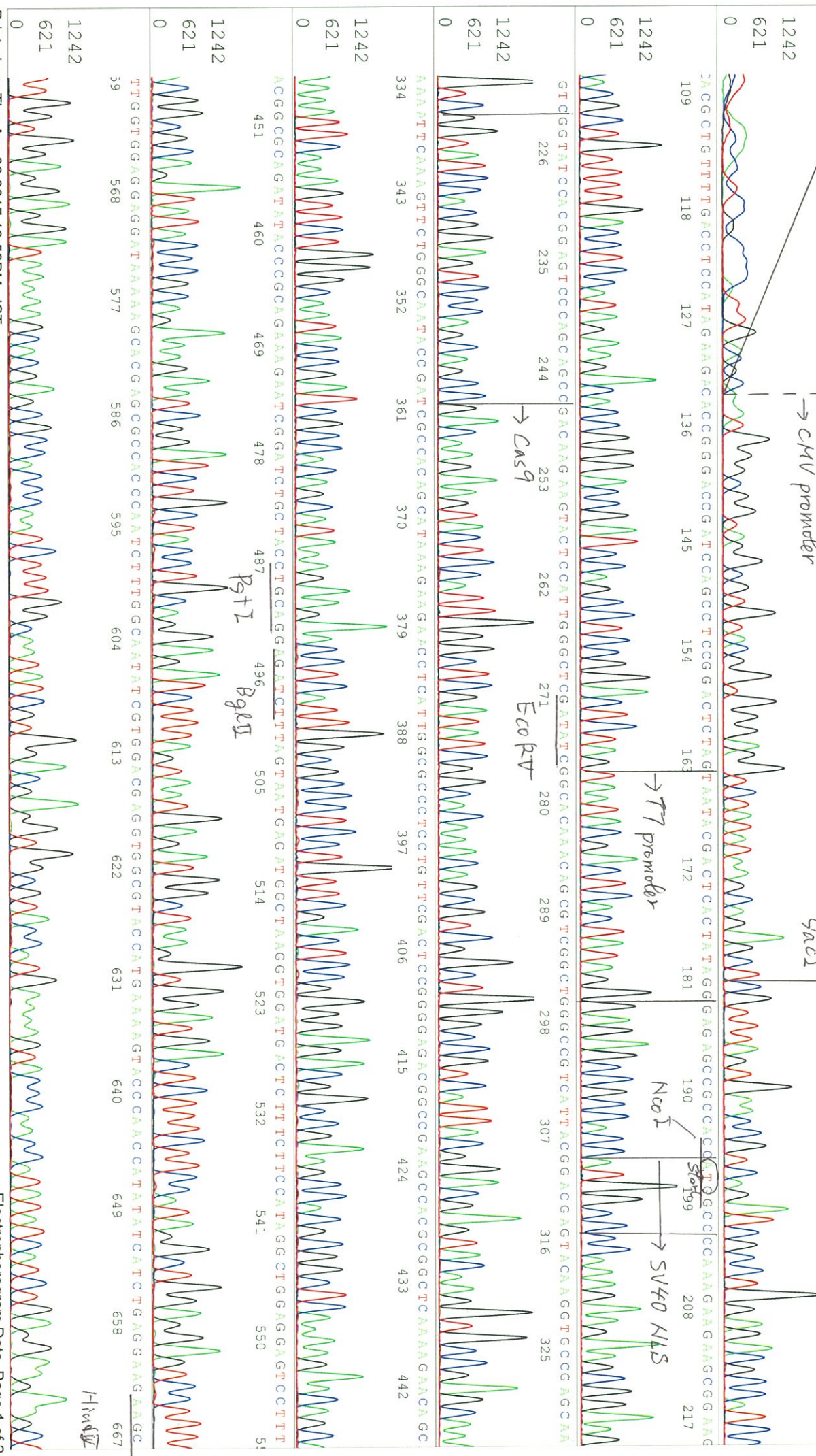
APPROVED BY :



S/N G:140 A:172 T:111 C:165 primer name A : CMV Forward

KB:bcp KB\_3500\_POP7\_BDTV3.mob Version 6.0 HISQV Bases: 943

KB 1.4.1.8 Cap:13 5-GCACCAAAATCAACGGGACTT-3'



S/N G:140 A:172 T:111 C:165

D04203A6\_ATCs\_1\_CMV-Forward

Apr 06,2017 10:37AM, JST

KB.bcp

KB\_3500\_POP7\_BDTV3.mob

Apr 06,2017 11:19AM, JST

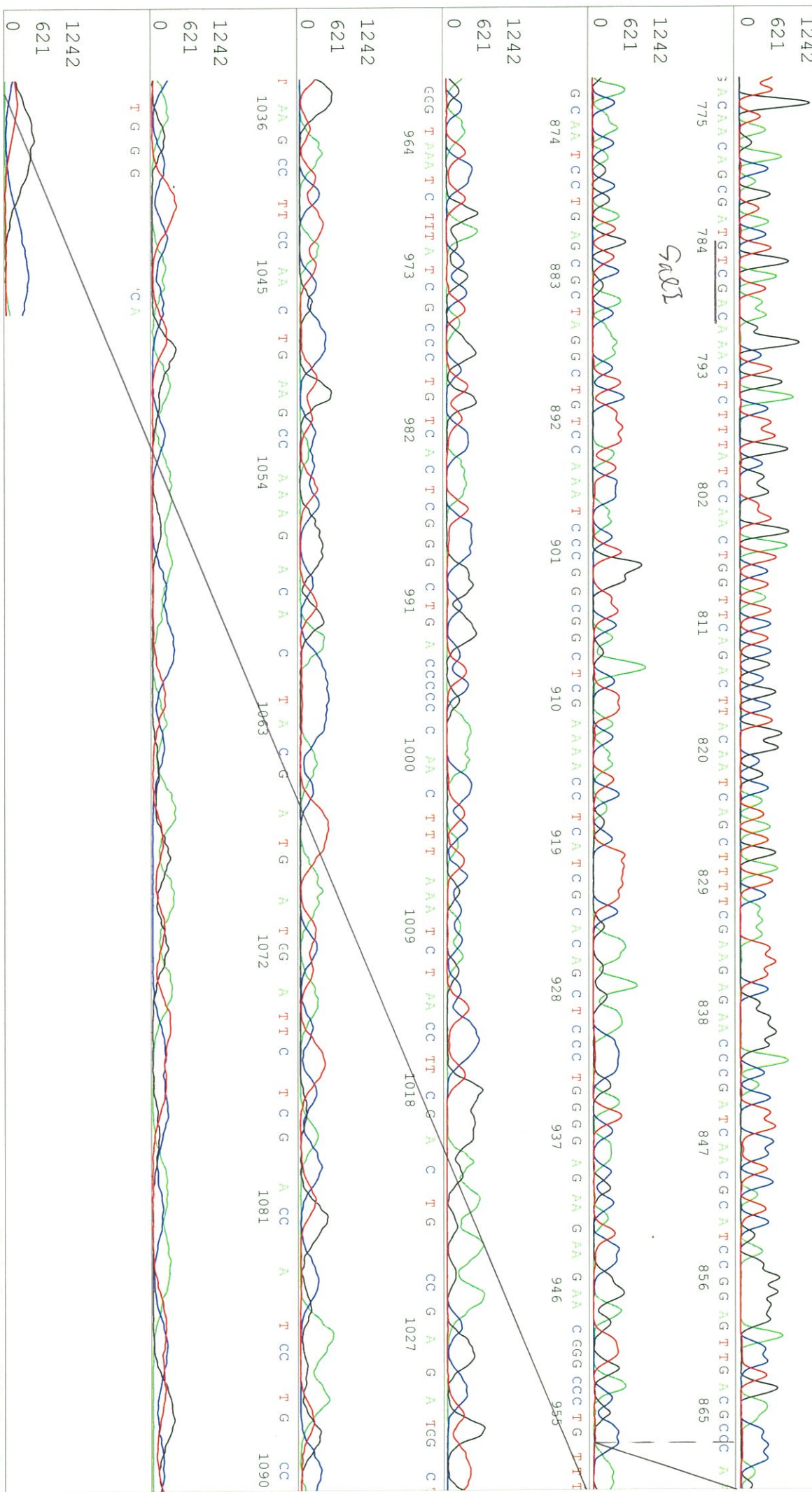
KB 1.4.1.8 Cap:13

Pts 1632 to 13205 Pk1 Loc:1609

Spacing:11.9 Pts/Panel1350

Version 6.0 HiSeqV Bases: 943

Nde I



S/N G:48 A:37 T:42 C:42

primer name B : EGFP-N

KB.bcp

5'-CGTCGCCCGTCCAGCTCGACCAG-3'

Cap:16

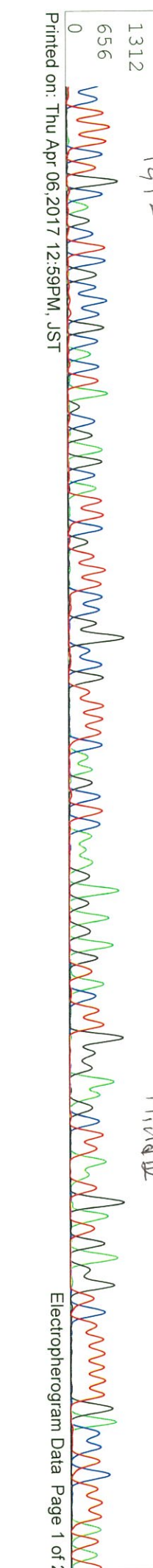
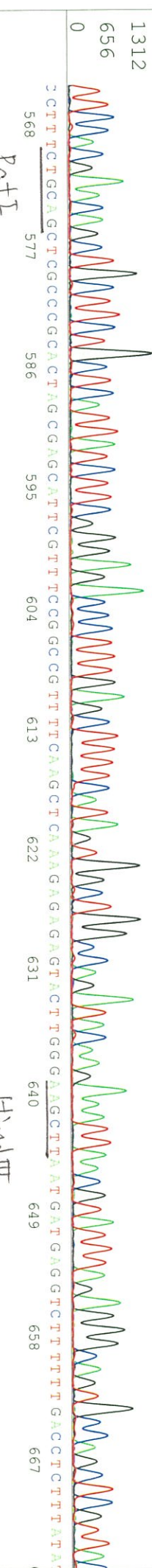
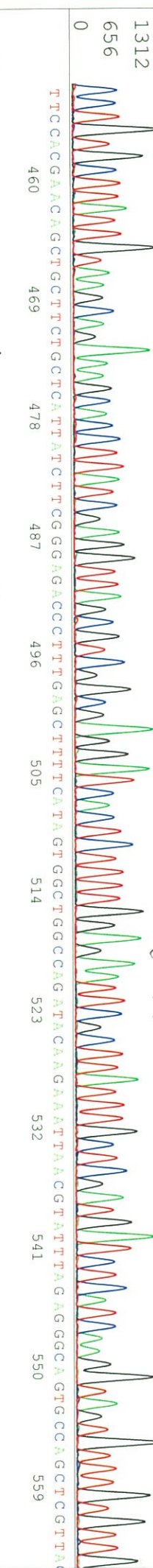
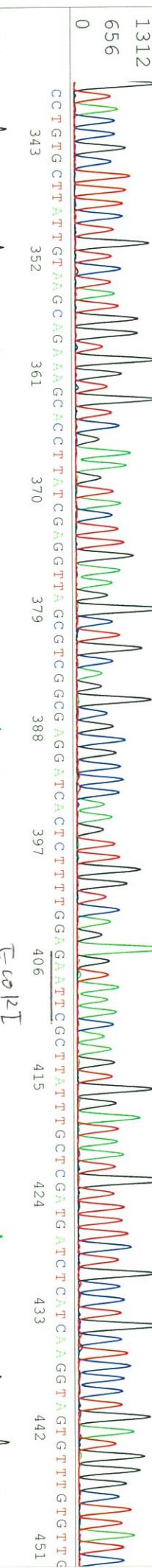
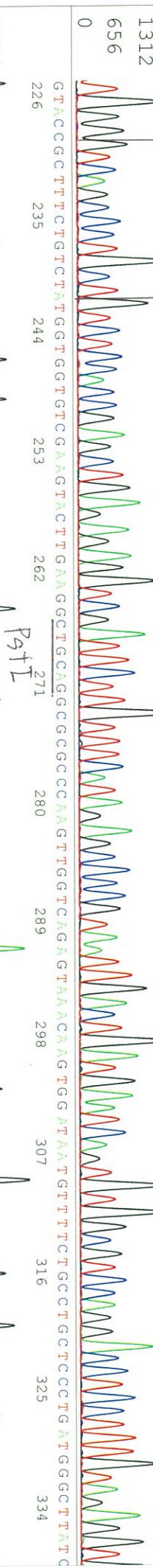
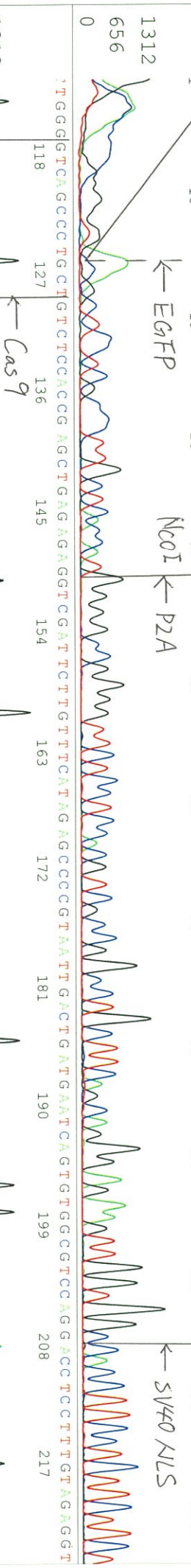
KB 1.4.1.8

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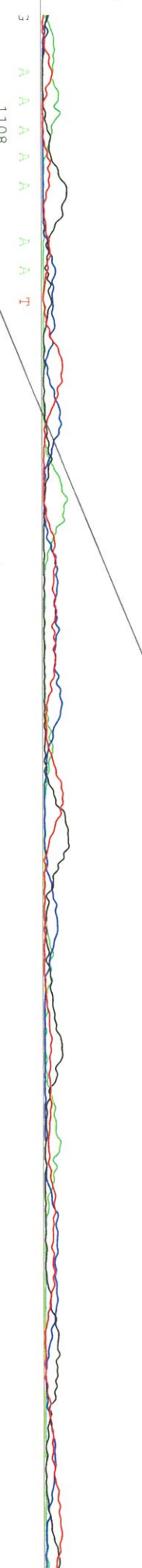
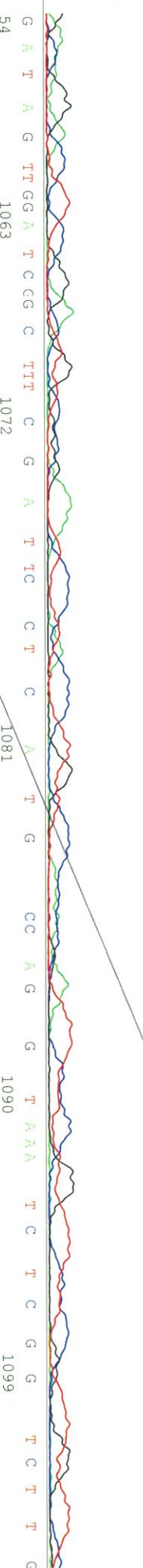
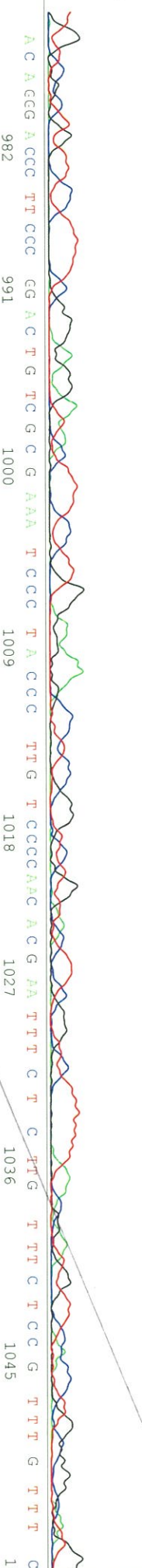
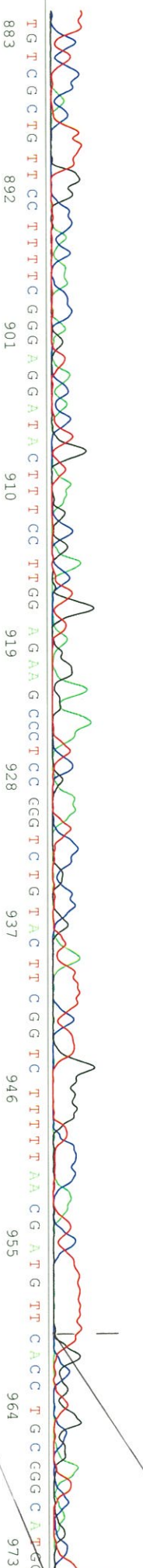
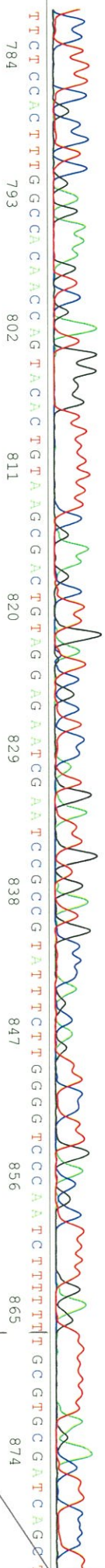
← EGFP

NcoI ← P2A

← SV40 NLS



TCCCTTTCGCCCTCGAAGAAAATCGATGGGGTTTTTTTCCGAAAGCTTGATCGCTCCCA TGATTTGATGCCCCAGCAAGTTCCCTTGACCGCTTTTGA GTTTTAA GACTTCCCT  
 576 685 694 703 712 721 730 739 748 757 766 775  
 XhoI HindIII



S/N G:301 A:276 T:203 C:255

primer name C : EGFP-C2

KB\_3500\_POP7\_BDTV3.mob

Apr 06,2017 10:37AM, JST

KB.bcp

5-ACTACTGAGCACCAGTCC-3'

Pls 2255 to 15000 Pk1 Loc:2232

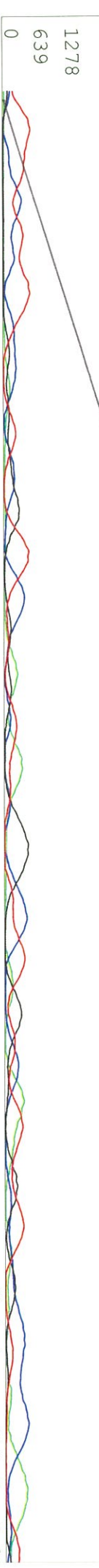
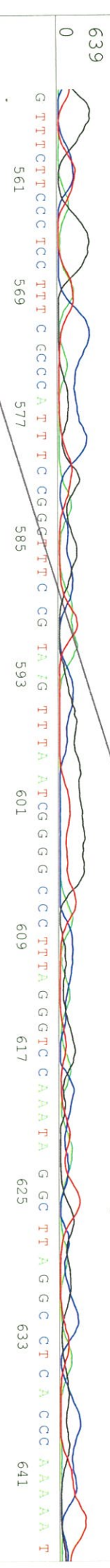
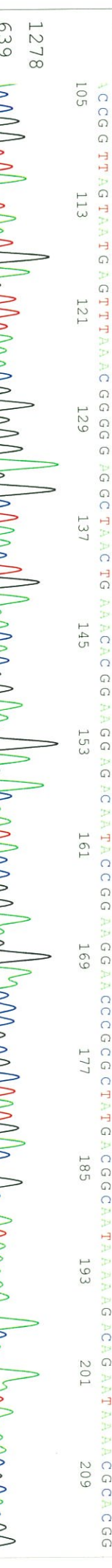
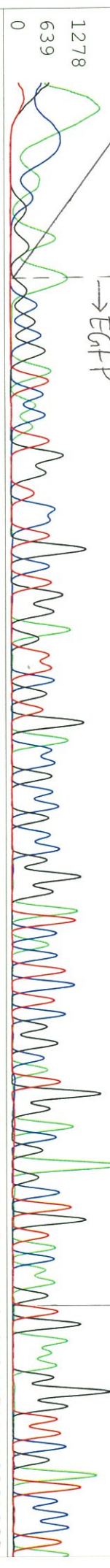
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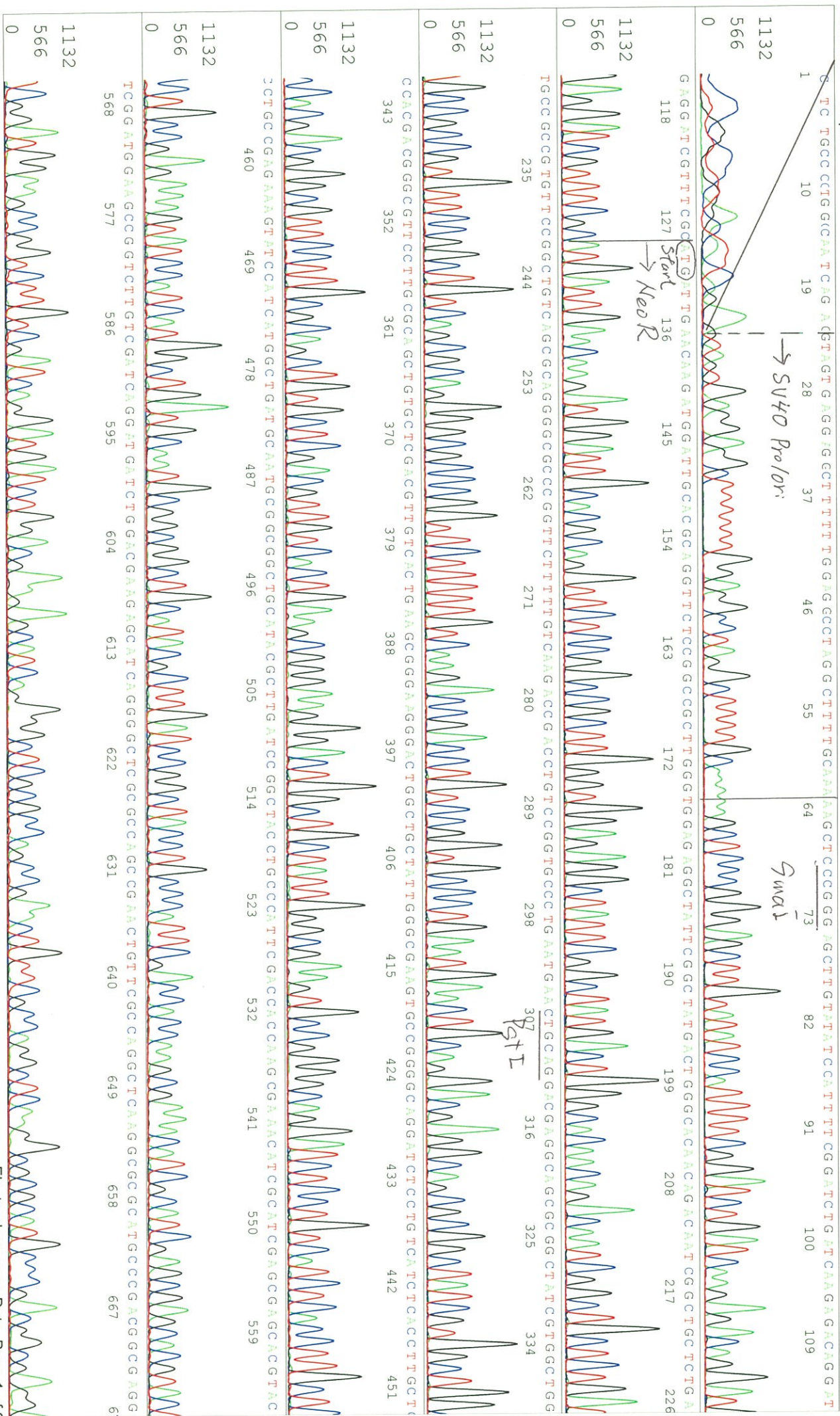
KB 1.4.1.8 Cap.19

Version 6.0 HISQV Bases: 297

Spacing:13.65 Pts/Panel1350

Plate Name: 20170406\_mix





S/N G:67 A:47 T:51 C:63

D04203A6\_ATCs\_1\_SV40pro\_F  
KB\_3500\_POP7\_BDTV3.mob

Apr 11, 2017 09:49AM, JST

KB bcp

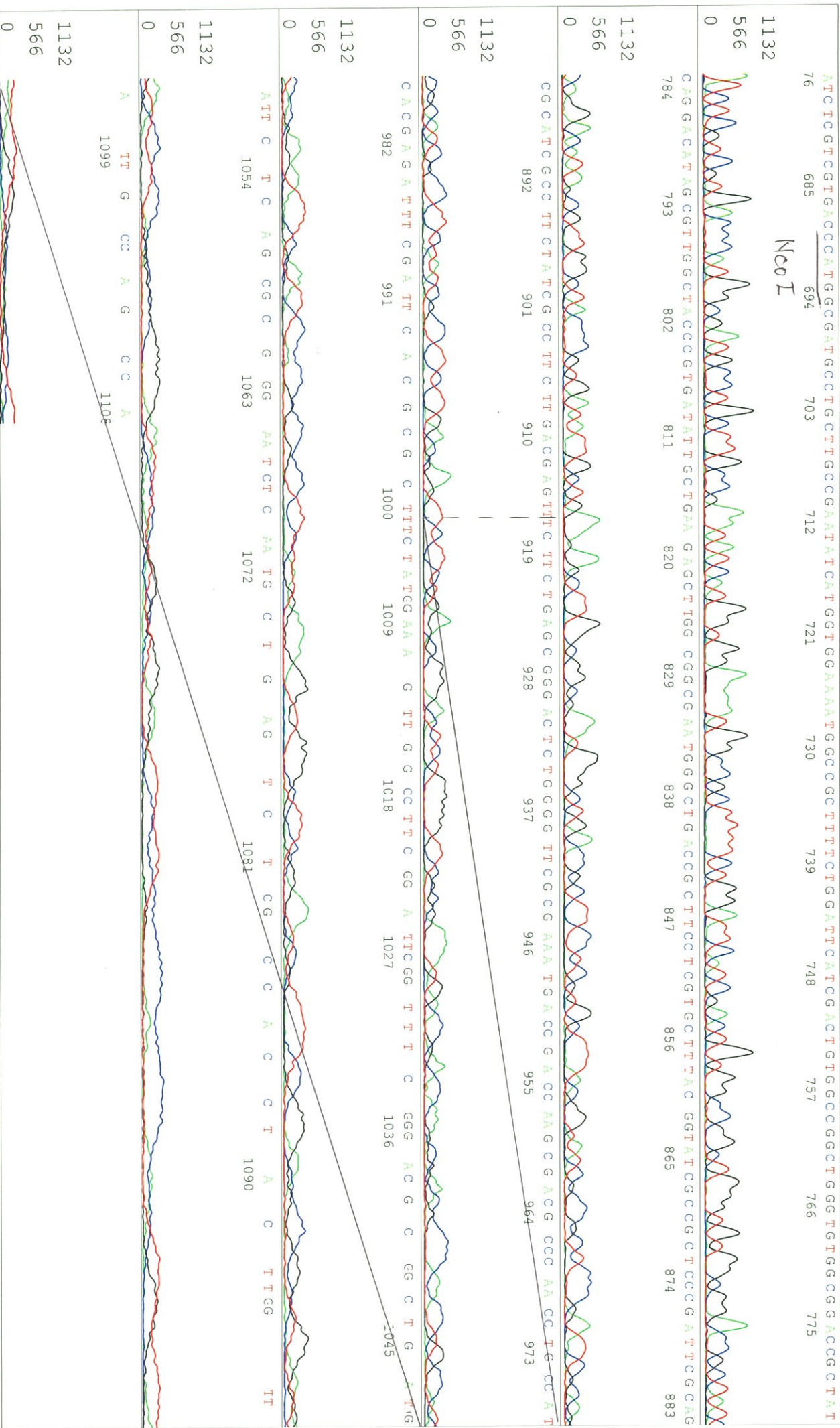
Pls 1552 to 13749 PK1 Loc:1529

Apr 11, 2017 10:15AM, JST

KB 1.4.1.8 Cap:8

Version 6.0 HISQV Bases: 943

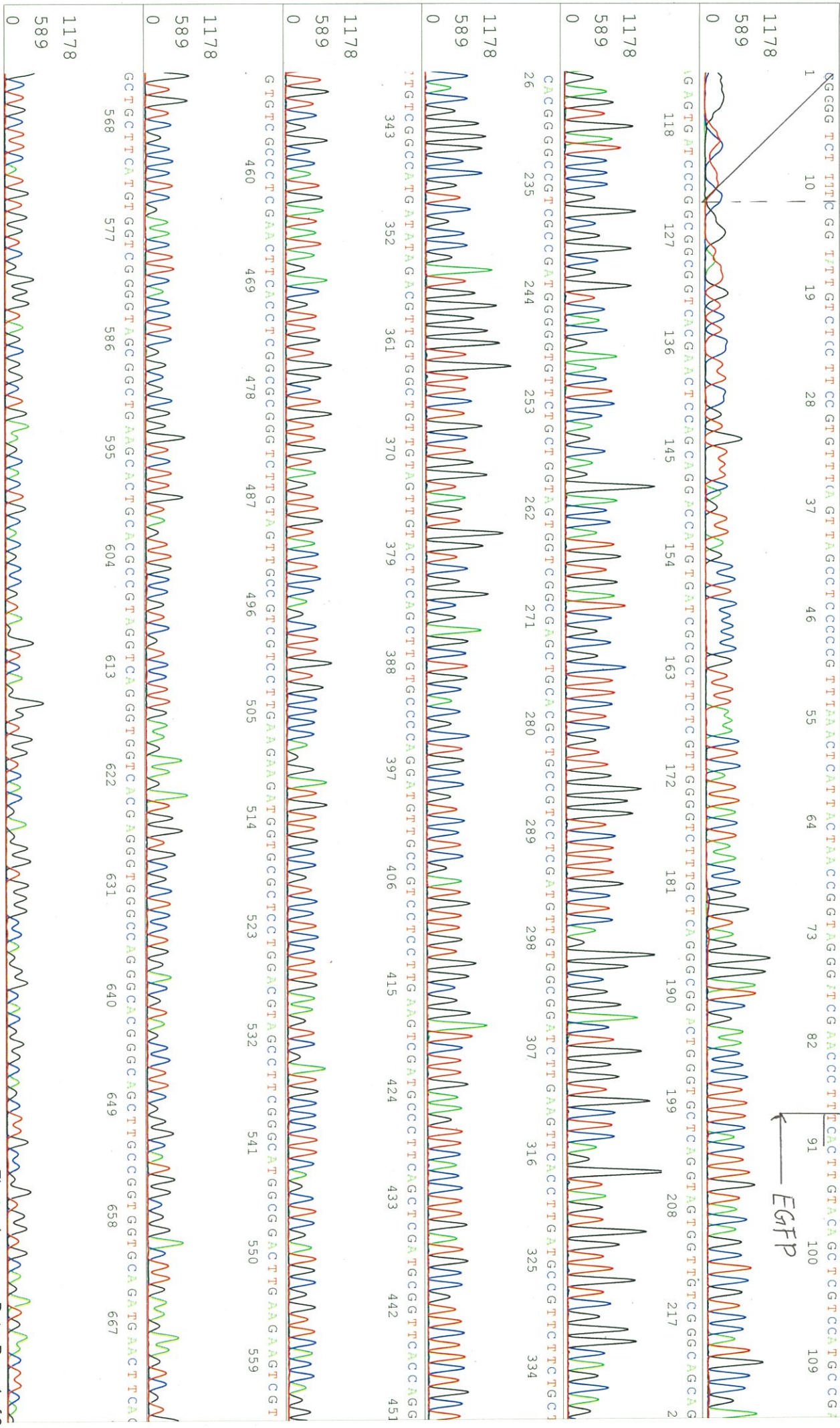
Spacing:12.55 Pts/Panel1350  
Plate Name: 20170411 Kitaku





SIN G:68 A:46 T:61 C:71 primer name **E** : HSV TK pA\_R  
 KB.bcp 5-TTATTCTGCTTTTATTG-C-3'  
 KB 1.4.1.8 Cap.5

14657\_14657\_A9G1F\_1\_HSV\_TK\_pA\_R\_E07\_05\_ABI08  
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 KB\_3500\_POP7\_BDIV3.mob  
 PIs 1526 to 13788 Pk1 Loc:1503  
 Version 6.0 HISQV Bases: 996  
 Inst Model/Name 3500/3500 Instrument  
 Jul 01 2019 03:13PM, JST  
 Jul 01 2019 03:55PM, JST  
 Spacing: 12.43 PIs/Panel1350  
 Plate Name: 20190701\_dnabank



3GGTCAAGCTTGGCCGTAGGTGGCATCGCCCTCGCCCTCGCCCGACACCGCTGAAACTGTGGCCGTTTACCGTCGCCGTCCAAGTCGACCAAGATGGGCACCAACCCCGGTGAAC

AGCTCCTCGCCCCCTTGCCTCACCCATGGGGCCGGGGTTCCTCCACCGTCCGCGCTTCAAGCAGGGAGAAATTGGTGGCCACCTTCCCTCTTCTTGCGTCA

4 793 802 811 820 829 838 847 856 865 874 883  
N101 P2A SV40NLS

GCCC TGGT GTCTCCACCGAGCTGAGAGA GGTCCGATTCCTGTTTCAATAGAGCCCGGTTAATTGACTGATGAAATCAGTGTGGCGTCCAGGACCTCC

3TAA GAA GGTGTACCCGCTTCTCTGTTCTATGGGTGGGTGTCGAA GTAC TTGAAA GGC TGC AA GC CGC CCAAG TTGG TCAAG GAG TAA C AA

G TG ATAA TTG TTTCC TGC TTG C TCC TGAA TTGG CC TATCC TGTG CC TA A TGGT TAA CGC CAG

3 AAAAGG GGA ACCCT TA 1144. 1153

0 1178 589

0 1178 589