



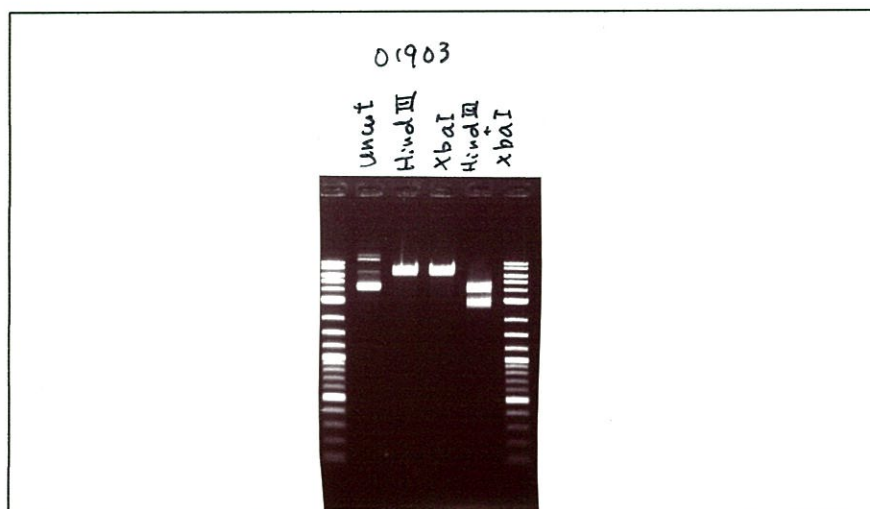
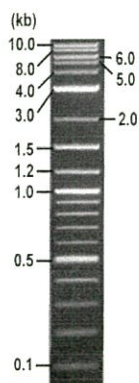
RIKEN DNA BANK

clone name : pCAUT

- Clone ID : RDB _ 01903
- Lot : 13439 _ A5Fi
- 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

2-Log DNA Ladder
(NEB#N3200L),
125 ng/well



Electrophoresis : 41 nanogram DNA per lane ; 1% agarose gel , 1 x TAE Buffer

Restriction enzyme	Expected size of fragment
<u>HindIII</u>	<u>7.1</u> kbp
<u>XbaI</u>	<u>7.1</u> kbp
<u>HindIII + XbaI</u>	<u>4.2, 3.0</u> kbp
	kbp
	kbp

● Confirmation of the insertion sequence

Sequence name	Primer name	Sequence name	Primer name
Sequence - A	pAxCA_F1	Sequence - E	-
Sequence - B	HSV TK pA_R	Sequence - F	-
Sequence - C	Reverse2	Sequence - G	-
Sequence - D	SV40pA3'Element Reverse	Sequence - H	-



APPROVED BY :

S/N G:106 A:85 T:62 C:77
 KB.bpp
 KB 1.4.1.8 Cap:17

01903_13439_A5Fi_paxCA_F1_F05_17
 01903_13439_A5Fi_paxCA_F1
 KB_3500_POP7_BDTV3.mob
 PIs 1204 to 13284 Pk1 Loc:1181
 Version 6.0 HiSQV Bases: 1024

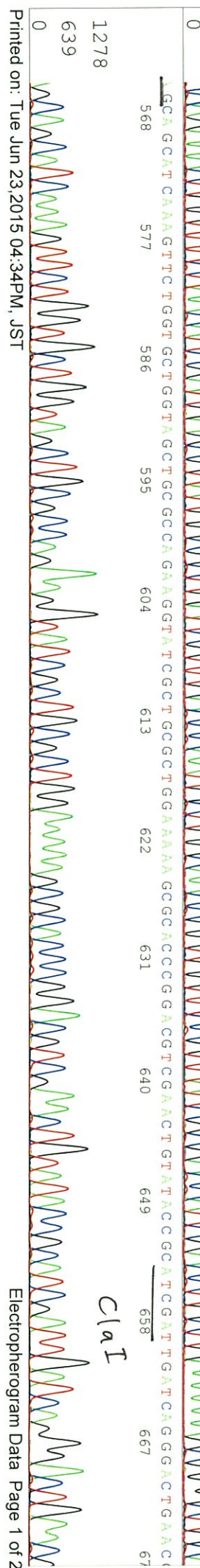
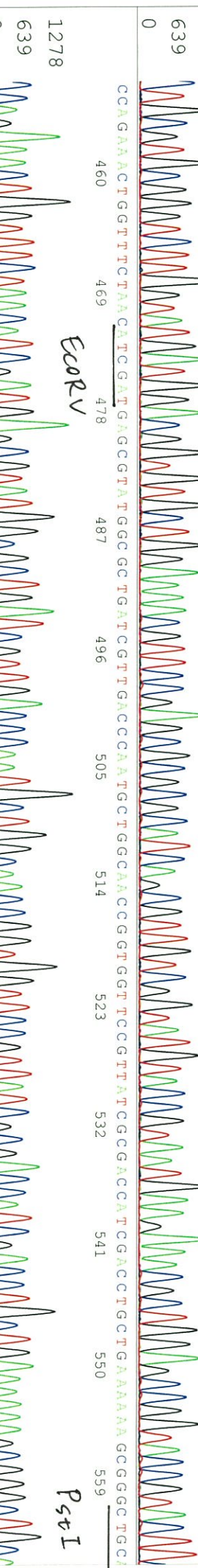
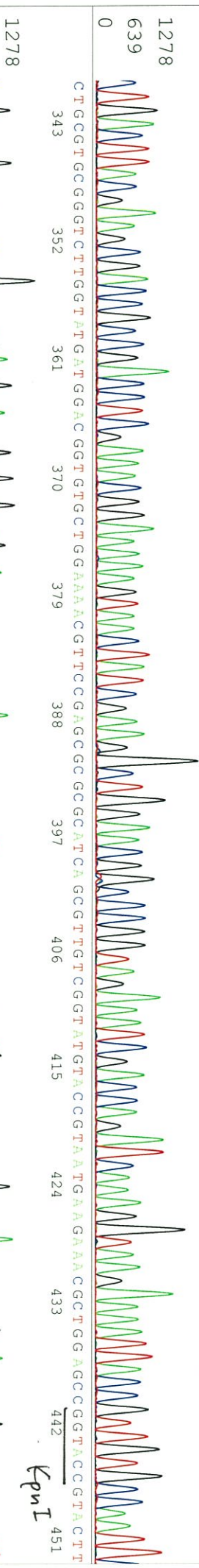
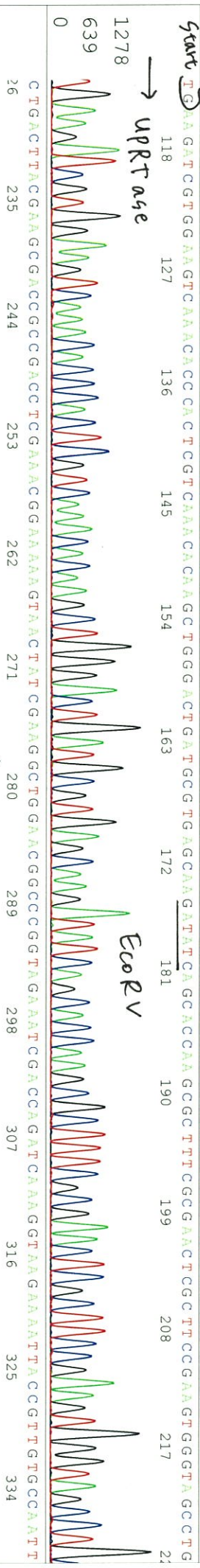
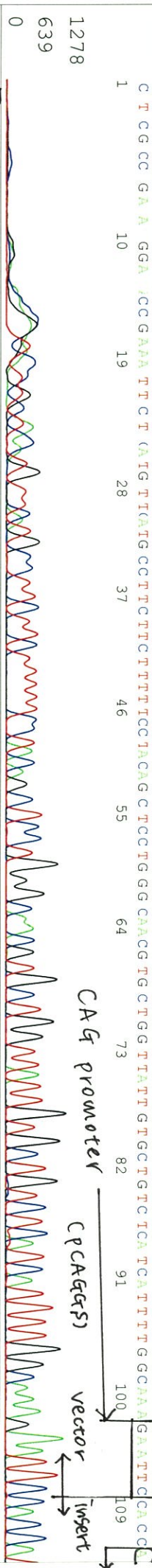
Inst Model/Name 3500/3500 Instrument
 Jun 23, 2015 02:02PM, JST
 Jun 23, 2015 02:36PM, JST
 Spacing:11.78 PIs/Panel1350
 Plate Name: 20150623_bank

primer name A : paxCA_F1
 5-GGCTTCTGGCGGTGACCGGC-3'

CAG promoter

(CPAGGS)

vector insert



3 A G C A C G G A T A C A T T A T T C C C G G G C C T C G G C G A T G C C G G T G A C A A A A T C T T T G G T A C G A A A G G A G G A T C C C G G C G G G G G C G G T G G A G G A G G G G G T A T G G C T T C G T A C C C C T G
 6 685 694 703 712 721 730 739 748 757 766 775 784



MluI

VPRtase

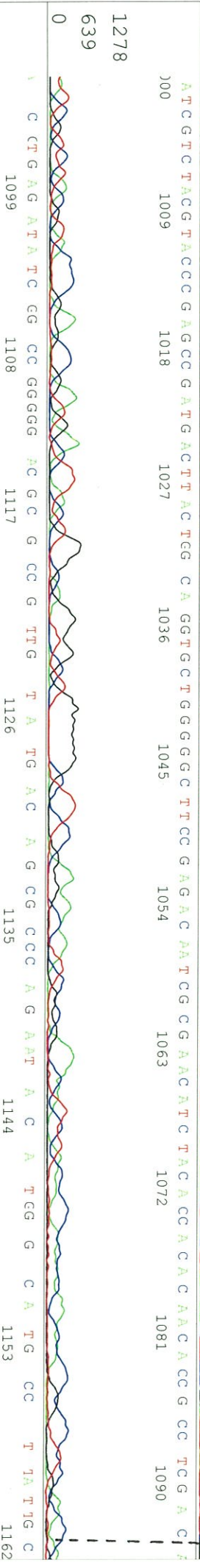
BamHI

HSV TK

C C A T C A A C A C G G T C T G C G T T C G A C C A G G C T G C G C G T T C T C G C G G C C A T A G C A A C C G A A C G T A C G G C G T G C G C C C T C G C C G G C A G C A A G A A G C C A C G G A A G T C C G C T G
 793 802 811 820 829 838 847 856 865 874 883 892

G A G C A G A A A A T G C C C A C C G T A C T G C G G G T T A T A T A G A C G G T C C T C A C G G G A T G G G G A A A A C C A C C A C C A C G C A A C T G C T G G T G G C C C T G G G T T C G C G C G A C G A T
 901 910 919 928 937 946 955 964 973 982 991 1000

A T C G T C T A C G T A C C C G A G C C G A T G A C T T A C T G G C A G G T G C T G G G G C T T C C G A G A C A A T C G C G A A C A T C T A C A C C A C A A C A C C G C C T C G A C A
 1000 1009 1018 1027 1036 1045 1054 1063 1072 1081 1090 1100



C C G T G A G A T A T C G G C C G G G G G A C G C G C C G T T G T A T G A C A G C G C C C A G A A T A C A T G G G C A A T G C C T T G C C T T G C C T T G C C T T G C C
 1099 1108 1117 1126 1135 1144 1153 1162



SN G:582 A:342 T:275 C:512

primer name β : HSV TK pA_R

KB_3500_POP7_BDTv3.mob

Jun 26, 2015 09:58AM, JST

KB.bcp

5'-TTTATTCTGTCTTTATTGC-3'

Pts 1336 to 13396 Pk1 Loc:1313

Jun 26, 2015 10:25AM, JST

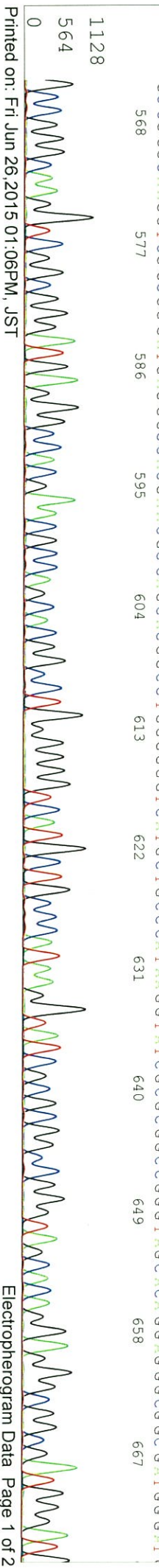
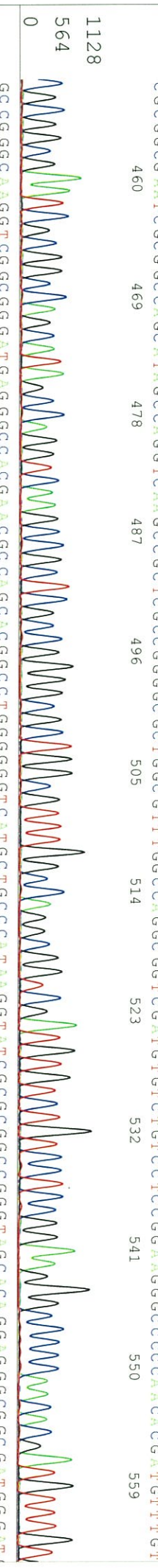
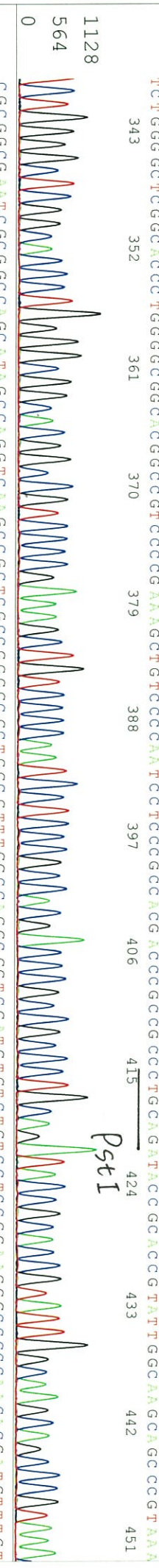
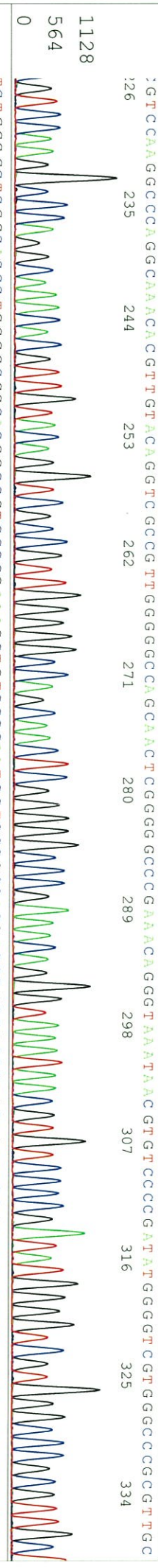
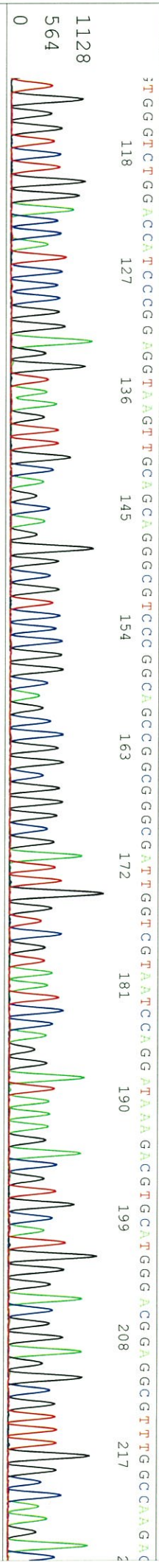
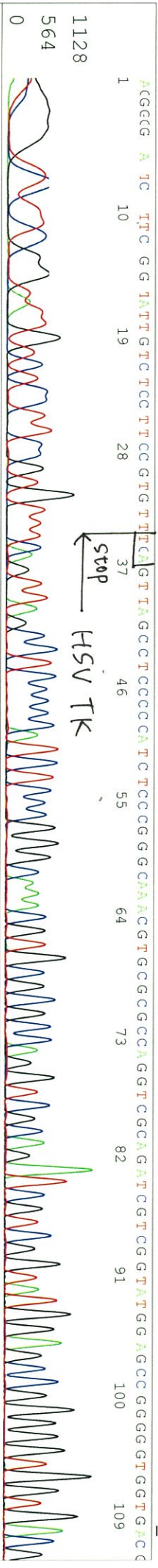
KB 1.4.1.8 Cap:24

Version 6.0 HSQV Bases: 1039

Plate Name: 20150626_mix

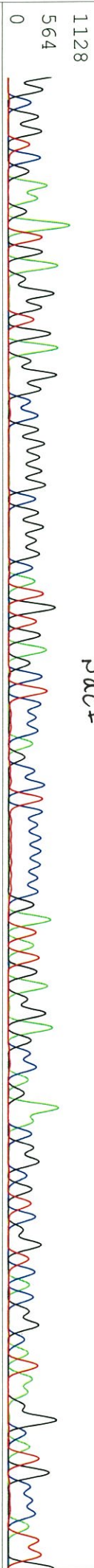
Spacing: 11.92 Pts/Panel1350

Stop HSV TK



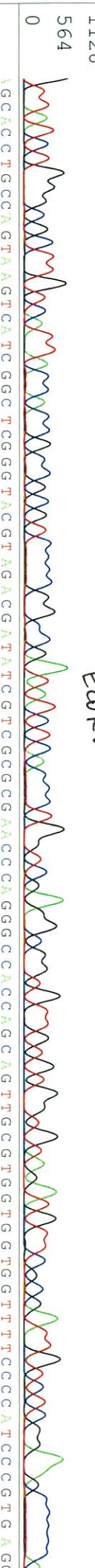
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SacI



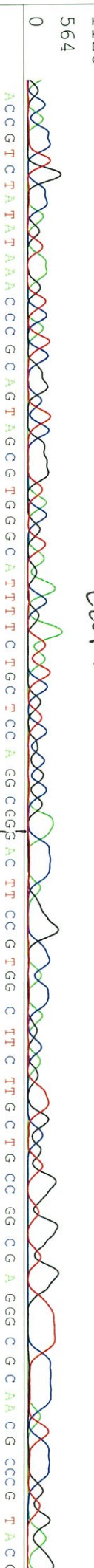
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EcoRV



AGCACCTGGCCAATAAGTCAATCGGCTCGGGTACGTAGACGATATCTGTCGGCCGAAACCCAGGGCCCAAGCAGTTGCTGGTGGTGGTGTTCCTCCCAATCCCGTGA

EcoRV



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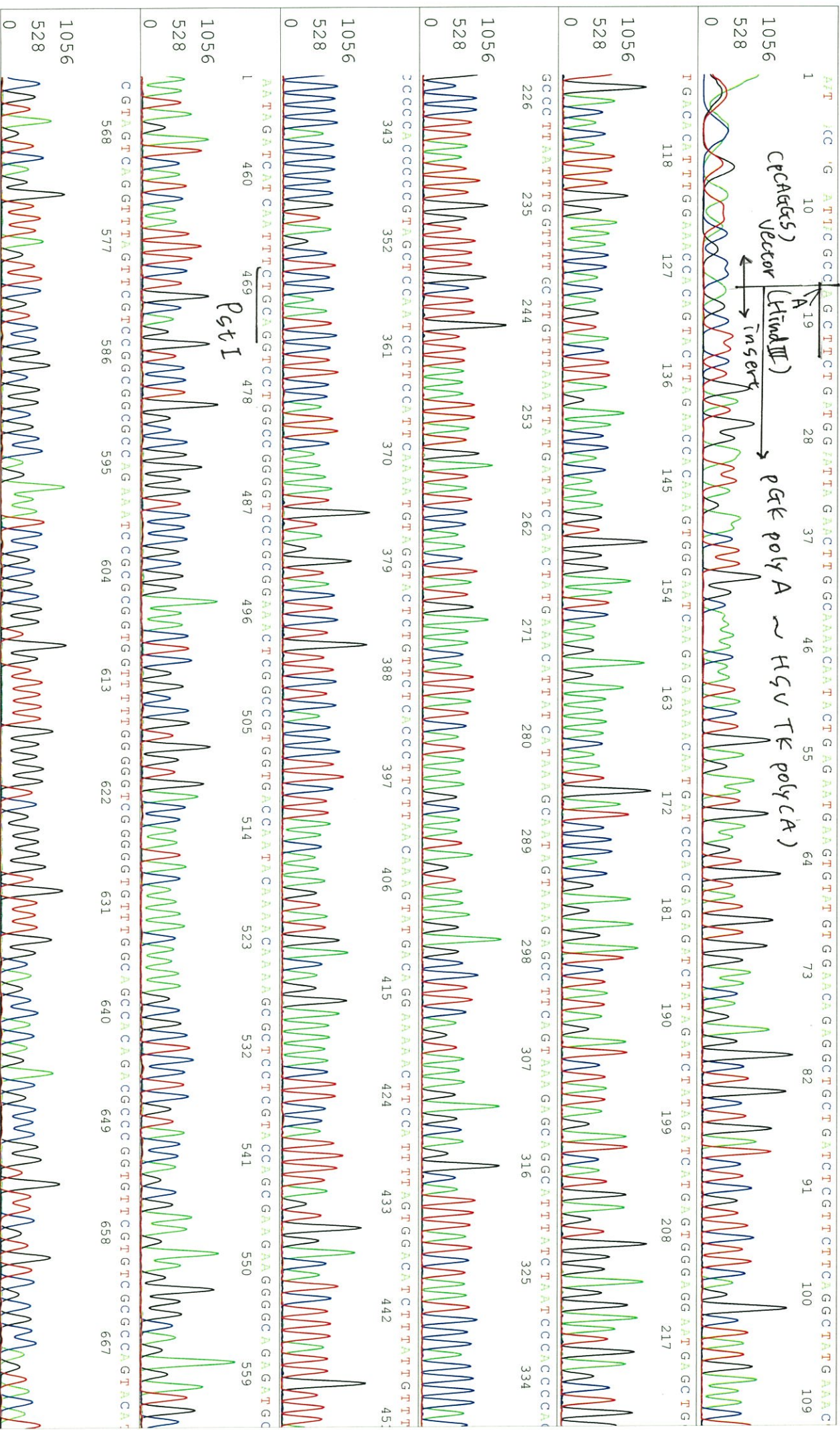


TCGCTTGCTATGGCCGCGAGAAACGCGCAGCTTAATTCGGACGGCAAGACGGCCGTGTGTGAATGGCCAGGGGATAACTG



AGCCATAATACCCCTCAATCGT







S/N G:451 A:491 T:336 C:417 primer name Φ : SV40pA3:Element Reverse

01903_13439_A5F1_SV40pA3:ElementReverse

Jun 24, 2015 01:58PM, JST

KB.bcp 5'-CTCTGACTTGAGCGTGCATT-3'

KB_3500_POP7_BDTV3.mob

Jun 24, 2015 02:32PM, JST

KB 1.4.1.8 Cap:18

5'-CTCTGACTTGAGCGTGCATT-3'

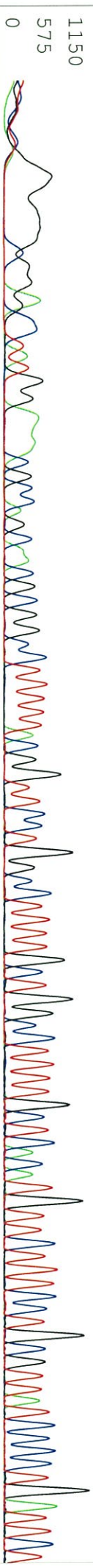
Pis 1321 to 13432 Pk1 Loc:1298

Spacing:11.94 Pts/Panel1350

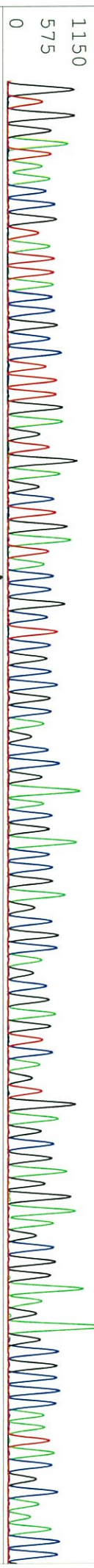
Plate Name: 20150624_Mix

Version 6.0 HISQV Bases: 1023

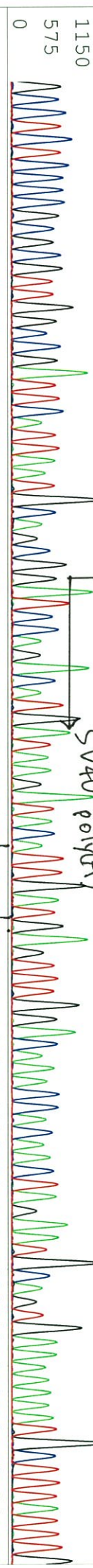
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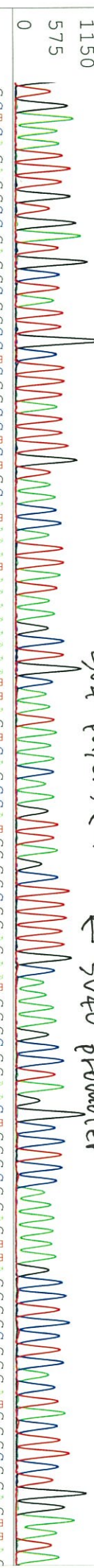
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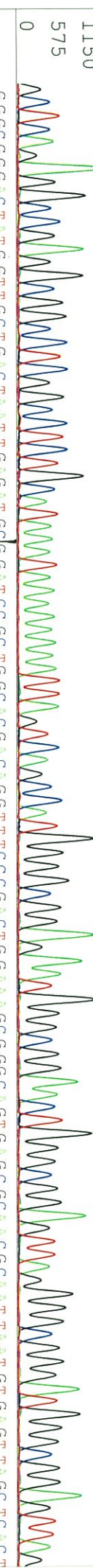
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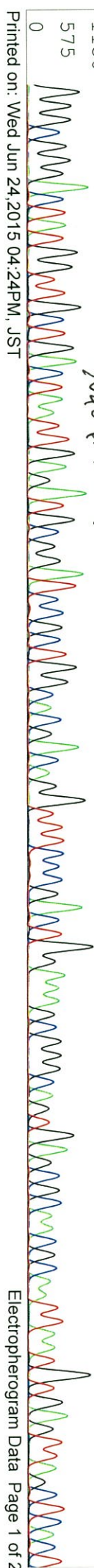
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G C T C A G A G G C C G A G G C G G C C T C G C C T C T G C A T A A A T A A A A A A A A T T A G T C A G C C A T G G G C C G A A A T G G C C G A A C T G G G C G G A G T T A G G G C C G G A T G G G C G A G T T A G G G C C G G A T G G C G A G T T A C



G G G G G G A C T A T G G T T G C C T G A C T A A T T G A G A T G C G G A T C C G C C T G G C A C G A C A G G T T T C C C G A C T G G A A A G C C G G C A G T G A G C C G C A A C C A A T T A A T G T G A G T T A G C T C A C T



SV40 promoter ← Bam HI

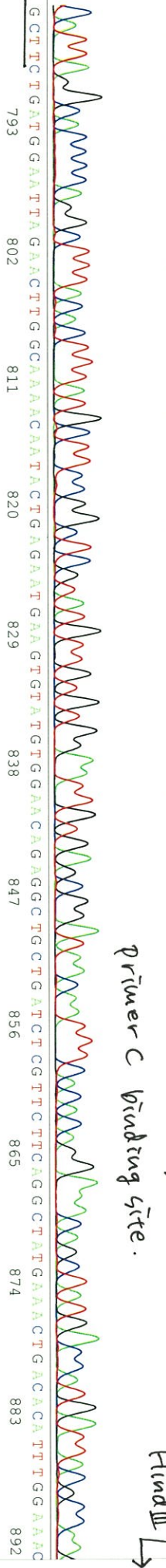
Bam HI SV40 poly(A) SV40 poly(A) SV40 promoter

Nco I

Primer C binding site.

HindIII

CATTAAGGACCCCGAGGCTTACACCTTATGCTTCCGGCTCCGATATGTTGTGTGAATTTGACCGCGATAACAAATTCACCAAGAAACAGCTATGACCATGATTACGCCA



PKKp(A) ~ HSV TKp(A)

GCTTCGTGATGAAATTA GAACCTTG GCAAAACAACTACTGAGAAT GAA GTGTA TGTG GAA CAA GAGGC TGTG ATCT CGTTC TTCAGGCTATGAAA CTGACACA TTGGAAAC

ScaI

BglII BglII

CACAGTACTTAGAACCACCAAAAGTGGAAATCAA GAGAAAACAATGATCCCACGA GAGATCTATAGATCTATA GATCATGAGTGGGA GGAATGAGACTGG CCCTTA

ATTTGCTTTTGC TTGTT AAAATTATGATATCC A C TATG AAA CATTATCAT A A GC A TAG TAA A GAG C TTCAG TAA GAGCA GCA TTTATCT A

TTCC ACCC A CCCC A CCCC G TAG C T C AATCC TTCCAA TTC A A TG TAA GG TAC TC GG TTC TCA C C TTCCT

AA CAAAG TAT TGA AAC AAGGG A
1162 1171 1181