

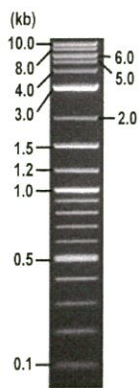


RIKEN DNA BANK

clone name : **HELLSpx330**

- Clone ID : RDB_ 17381
- Lot : 17381_ A9Fh
- 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

1 kb Plus DNA Ladder
(NEB#N3200L),
250 ng/well



Electrophoresis : 100 nanogram DNA per lane ; 1% agarose gel , 1 x TAE Buffer

| Restriction enzyme | Expected size of fragment |
|----------------------|---------------------------|
| EcoRI | 8.5 kbp |
| EcoRV | 8.5 kbp |
| EcoRI + EcoRV | 6.2, 2.3 kbp |
| | kbp |

● **Confirmation of the insertion sequence**

Please be sure to check our sequence analysis results before your request.

| Sequence name | Primer ID | Primer name | Confirmed feature |
|---------------|-----------|-------------------------|--|
| Sequence - A | Pr0108 | SV40pA3'element reverse | U6 pro, gRNA (HELLS) gRNA scaffold, CBh pro 5' |
| Sequence - B | Pr0650 | Cas9-N_R1 | Cas9 5', SV40 NLS, 3xFLAG, CBh pro 3' |
| Sequence - C | Pr0606 | BGH_rev2 | bGH p(A), nucleoplasmin NLS, Cas9 3' |
| Sequence - D | - | - | - |
| Sequence - E | - | - | - |
| Sequence - F | - | - | - |
| Sequence - G | - | - | - |
| Sequence - H | - | - | - |

APPROVED BY :



S/N G:33 A:36 T:33 C: primer name A: SV40pA3'Element Reverse

KB.bcp KB_3500_POP7_BDTV3.mob

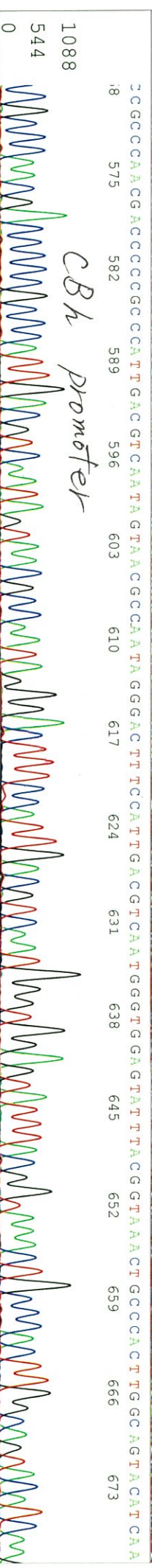
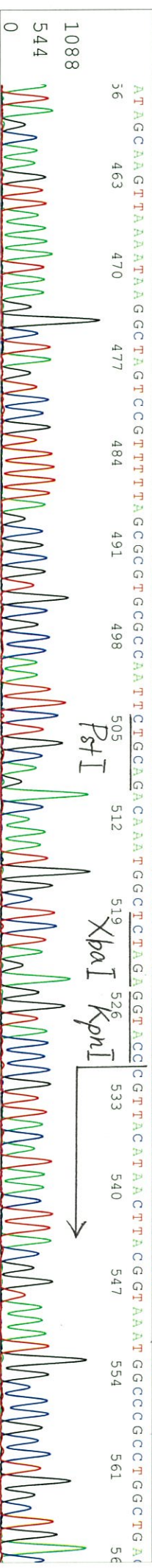
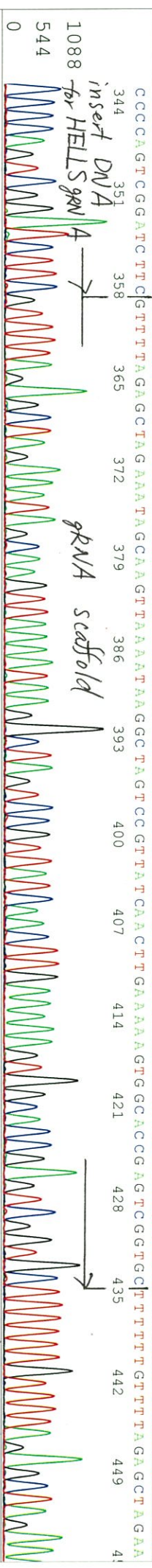
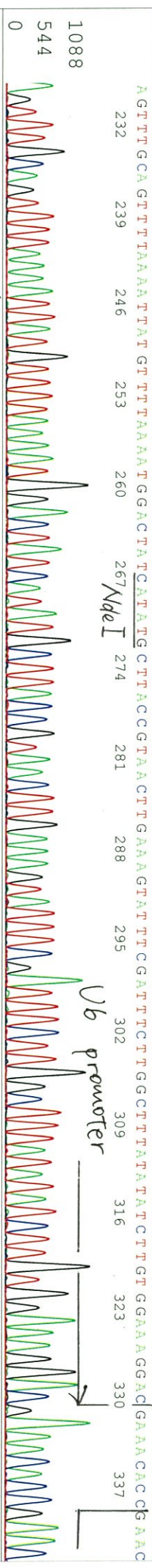
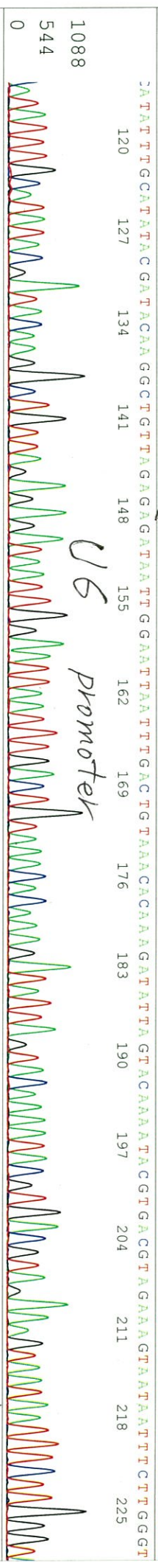
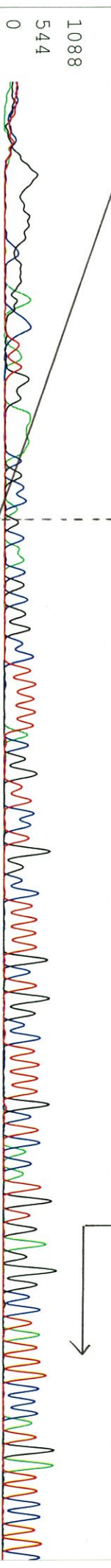
Jul 04, 2019 02:34PM, JST

KB 1.4.1.8 Cap:15 5'-CTCTGACCTTGAGCGCTCGATTT-3'

Pis 1353 to 12965 Pk1 Loc:1330

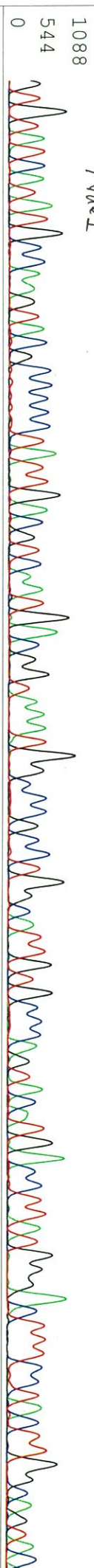
Spacing:11.6 Pts/Panel1350

Version 6.0 HISQV Bases: 911



GTGTAATCATTTGCCAAGTACGCCCCCTATTGTGACGTCAATGACGGTAAATGGCCCGCTGGCATTGTGCCCAGTACATGACCTTATGGGACTTTCCTACTTGCCAGTAC
680 687 694 701 708 715 722 729 736 743 750 757 764 771 778 785

Nde I



Nco I

CBh promoter



ATCTACGTTATTAGTCAATCGCTATTACCATGGTCCGAGGTGAGCCCCACGTTCTGCTTCCACTCTCCCATCTCCCCCTCCCAATTTGTTATTATTA
792 799 806 813 820 827 834 841 848 855 862 869 876 883 890



TTTTTTNATTAATTGTCGCA~~NC~~ATN~~GGGGCGGGGGGGGGG~~GNN
897 904 911 918 925 932 939



S/N G:90 A:77 T:93 C:121

primer name C : BGH_rev2

KB_3500_POP7_BDTV3.mob

Jul 08, 2019 04:13PM, JST

KB 1.4.1.8 Cap:5

5'-TATTAGGAAGGACAGTGGG-3'

Pls 1486 to 13459 PK1 Loc:1463

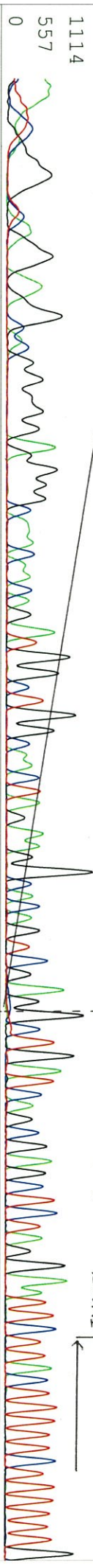
Jul 08, 2019 04:41PM, JST

Version 6.0 HiSQV Bases: 983

Spacing:12.17 Pts/Panel1350

Plate Name: 20190708_mix

1 10 19 28 37 46 55 64 73 82 91 100 109



1114
557
0
CCTGCCCGCCCTTTTCGTGGCCGCCCGCCGCTTTTGTCCGCTCCCAAGCTGAGACAGT CGATCCGTGTCCTCGTACAGGCCGGT GATGCTCTGGATCAGGGTGGCCGCTCCAAG
118 127 136 145 154 163 172 181 190 199 208 217
nucleoplasm NLS ← Cas9

1114
557
0
CACCTCTTGGTGGTGTGTACCTCTCCGGTCGATGGTGTCAAGTACTTGAAGCCAGCCAGGGCTCCAGATTGTCAGGGTAACAGGTGGATGATATCTCCGGCC
226 235 244 253 262 271 280 289 298 307 316 325 334

1114
557
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343 352 361 370 379 388 397 406 415 424 433 442 45

1114
557
0
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1 460 469 478 487 496 505 514 523 532 541 550 559

1114
557
0
3GGCAGGGCCAGTTCGTTCCCTTCTGCAAGTCCGCGCAGAGGCCAGCATTTCTTCCAGCTTCGAACAAGGAGTACTTAGGCACTTGATGATCAGGTC
568 577 586 595 604 613 622 631 640 649 658 667
PstI

