

mu6-mClover-gRNA-Sp-GFP-optlig (8655 bp)

gaagatcctttgatcttttctacggggtctgacgctcagtggaacgaaaactcacgttaaggattttggatcatgagattatcaaaaaggatcttcacctagatcct
cttctaggaaactagaaaagatgccccagactgacgagtcaccttgcttttgagtgcaattccctaaaaccagtactctaatagtttttcctagaagtggatctagga

10 20 30 40 50 60 70 80 90 100

tttaattaaaaatgaagttttaaataaatcaatctaaagtatatatgagtaaacttggctgacagttaccaatgcttaatcagtgaggcacctatctcagcgatctgtc
aatttaatttttacttcaaaaattagttagatttcatatatactcatttgaaccagactgtcaatggttacgaattagtcactccgtggatagagtcgtagacag



110 120 130 140 150 160 170 180 190 200 210

tatttcggtcatccatagttgctgactccccgctggtgtagataactacgatacgggagggttaccatctggccccagtgctgcaatgataccgagagaccacgc
ataaagcaagtaggtatcaacggactgaggggcagcacatctattgatgctatgccctcccgaatggtagaccggggtcagacgttactatggcgctctgggtgcg



220 230 240 250 260 270 280 290 300 310 320

tcaccggctccagatttatcagcaataaaccagccagccggaagggccgagcgcagaagtggctcgaactttatccgctccatccagtcctattaattggtgccg
agtggccgaggtctaaatagtcgttatttggctggctcgccctcccggctcgctcttaccaggacgttgaatagcggaggtaggtcagataattaacaacg



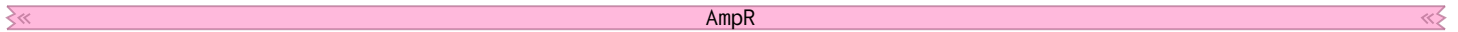
330 340 350 360 370 380 390 400 410 420

ggaagctagagtaagtagttcggcagttaatagtttgcgcaacgttgttgccattgctacaggcatcgtgggtgcacgctcgtctgtttggatggcttcattcagct
ccttcgatctcattcatcaacggctcaattatcaaacggttgaacaacggtaacgatgtccgtagcaccacagtgcgagcagcaaacctaccgaagtaagtcga



430 440 450 460 470 480 490 500 510 520 530

ccggttccaacgatcaaggcagttacatgatccccatgttgtgcaaaaaagcggttagctccttcggctcctccgatcgttgtcagaagtaagttggccgagtg
ggccaaggttgctagttccgctcaatgtactaggggtacaacacgttttttcgccaatcgaggaagccaggaggtagcaacagtcctcattcaaccggcgtcac



540 550 560 570 580 590 600 610 620 630 640

PvuI



ttactcactatggttatggcagcactgcataattcttactgtcatgccatccgtaagatgcttttctgtgactggtagtactcaaccaagtcattctgagaata
aatagtgagtaccaataccgctcgtgacgtattaagagaatgacagtagcggtaggcattctacgaaaagacactgaccactcatgagttggttcagtaagactcttat

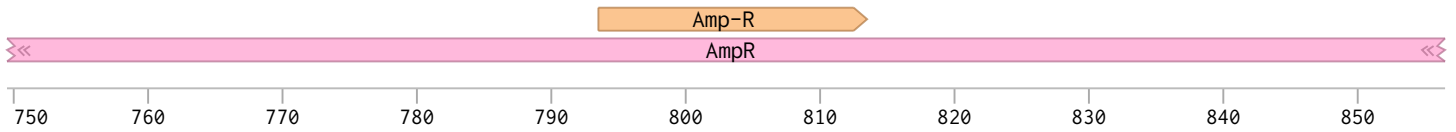


650 660 670 680 690 700 710 720 730 740

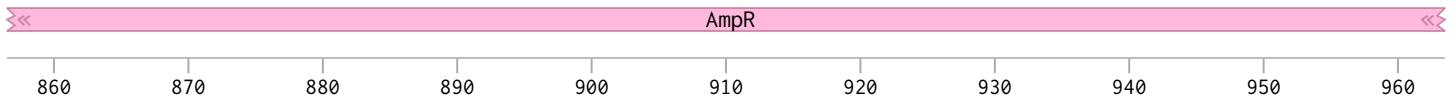
ScaI



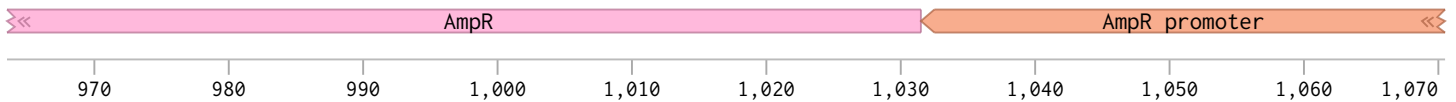
gtgtatgCGGCGaccgagttgctcttGCCGGCGtcaatacGGGataataccgCGccacatagcagaactttaaagtGctcatcattggaaaacgttcttcggggc
 cacatacCGcgtggctcaacgagaacGGGCGcagttatGCCctattatggCGcgggtgatcgtcttgaattttcacgagtagtaacctttgcaagaagccccg



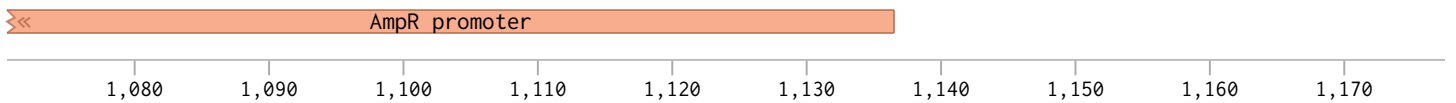
gaaaactctcaaggatcttaccgctgttgagatccagttcgatgtaaccactcgtgcacccaactgatcttcagcatcttttactttcaccagcgttcttggtga
 cttttgagagttcctagaatggcgacaactctaggtcaagctacattgggtgagcacgtgggttgactagaagtcgtagaaaatgaaagtggctgcaagaccact



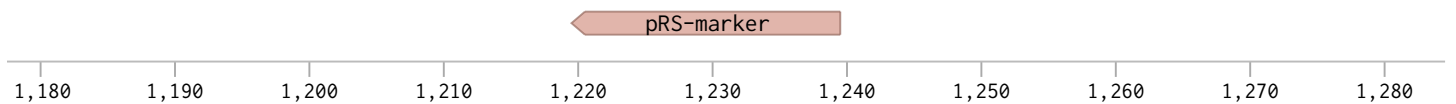
gaaaaacaggaaggcaaaatGCCGaaaaaaggaataagggCGacacggaatgttgaatactcatacttctcttttcaatattatgaagcatttatcaggg
 cgtttttgctctccgttttacggcgtttttcccttatcccgctgtgcctttacaactatgagtagagaaggaaaagtataataacttcgtaaatagtc



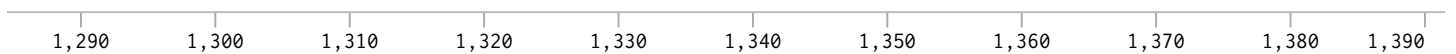
ttattgtctcatgagcggatacatattgaatgtatttagaaaaataaacaataggggttccgCGcacatttccccgaaaagtccacctgacgcttaagcggctg
 aataacagagtactcgcctatgtataaactacataaatctttttattgtttatcccgaaggCGcgtgtaaaggggcttttcacggtggactcggaattcgccagc



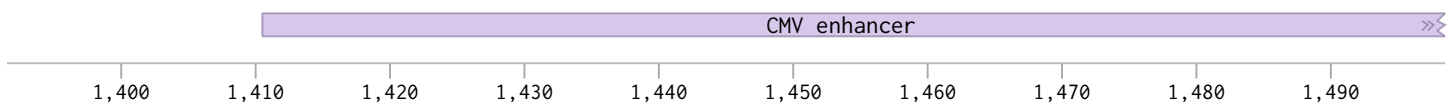
acggatcgggagatctcccgatcccctatggtgcaactctcagtacaatctgctctgatgccgcatagttaagccagtatctgctccctgcttggtgttgagggtcg
 tgccctagccctctagagggttaggggataccacgtgagagtcagtttagacgagactacggcgtatcaattcgggtcatagacgagggacgaacacacaacctccagc



ctgagtagtgcgCGagcaaaatttaagctacaacaaggcaaggcttgaccgacaattgcatgaagaatctgcttagggttaggcgttttgcgctgcttcgcatgta
 gactcatcacgCGctcgttttaaatcagatgttgttccgttccgaactggctgttaactgacttcttagacgaatcccaatccgcaaaacgCGacgaagcgtacat



cggccagatatacCGgttgacattgattattgactagttattaatagtaataattacggggtcattagttcatagccatataatggagtccgCGttacataact
 gccCGtctatatgCGcaactgtaactaataactgatcaataattatcattagttaatgccccagtaatacaagtatcgggtatatactcaaggCGcaatgtatga



tacggtaaatggcccgcctggctgaccgccaacgacccccgccattgacgtcaataatgacgtatgttccatagtaacccaataggactttccattgacgtc
atgccatttacggggcggaccgactggcgggttgctggggcgggtaactgcagttattactgcatacaagggtatcatgcggttatccctgaaaggtaactgcag

» CMV enhancer »

1,500 1,510 1,520 1,530 1,540 1,550 1,560 1,570 1,580 1,590 1,600

aatgggtggagtatttacggtaaacctgccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacggtaaatggcccgcctgg
ttaccacctcataaatgccatttgacgggtgaaccgtcatgtagttcacatagtagcgttcatgcggggataactgcagttactgccatttacggggcggacc

» CMV enhancer »

1,610 1,620 1,630 1,640 1,650 1,660 1,670 1,680 1,690 1,700 1,710

cattatgcccagttacatgacctatgggactttcctacttggcagttacatctacgtatttagtcatcgctattaccatgggtgatgcggttttggcagttacatcaatgg
gtaatacgggtcatgtactggaataacctgaaaggatgaaccgtcatgtagatgcataatcagtagcgataatggtaccactacgcaaaaaccgtcatgtagtacc

» CMV enhancer » CMV promoter »

1,720 1,730 1,740 1,750 1,760 1,770 1,780 1,790 1,800 1,810

gcgtggatagcggttgactcacgggatitccaagtctccacccatgacgtcaatgggagttgttttggcaccaaaatcaacgggactttccaaaatgtcgta
cgcacctatcgcaaaactgagtgccccaaagggtcagagggtgggtaactgcagttaccctcaaaaacacgggtgttttagttgccctgaaaggtttacagcat

» CMV promoter »

1,820 1,830 1,840 1,850 1,860 1,870 1,880 1,890 1,900 1,910 1,920

acaactccgccccattgacgcaaatggcggttagcgtgtacggtgggaggtctatataagcagcgcgttttgcctgtactgggtctctctggttagaccagatctg
tgttagggcgggtaactgcgtttaccgccatccgcacatgccacctccagatatattcgctcgcgcaaaaacggacatgaccagagagaccaatctggtctagac

» CMV promoter » 5' LTR (truncated) »

1,930 1,940 1,950 1,960 1,970 1,980 1,990 2,000 2,010 2,020 2,030

agcctgggagctctctggctaacttaggaaccactgcttaagcctcaataaagcttgcccttgagtgttcaagtagtgtgcccgtctgttgtgtgactctggta
tcggaccctcgagagaccgattgatcccttgggtgacgaattcggagtatttcgaacggaactcagaagtcatcacacacgggcagacaacacactgagaccat

» 5' LTR (truncated) »

2,040 2,050 2,060 2,070 2,080 2,090 2,100 2,110 2,120 2,130 2,140

actagagatccctcagacccttttagtcagttgtgaaaatctctagcagttggcggcggcgaacaggacttgaagcgaagggaaccagagagactctctcgacgca
tgatctctaggagctctggaaaatcagtcacaccttttagagatcgtcaccgcggtgtcctcgaacttcgctttccctttggtctcctcgagagagctgcgt

» 5' LTR (truncated) » HIV...si »

2,150 2,160 2,170 2,180 2,190 2,200 2,210 2,220 2,230 2,240

ggactcggcttgctgaagcgcgcacggcaagaggcggggcgcgactggtagtacgcaaaaatgactagcggaggctagaaggagagatgggtgcgag
cctgagccgaacgacttcgcgctgcccgttctccgctccccgctgaccactcatgcggttttaaaactgatcgctccgatcttctctctaccacgctc

»» HIV-1 Psi »»

2,250 2,260 2,270 2,280 2,290 2,300 2,310 2,320 2,330 2,340 2,350

NruI

agcgtcagtattaagcgggggagaattagatcgcgatgggaaaaaatcggttaaggccaggggaaagaaaaatataaattaaacatatagtatgggcaagcag
tcgagtcataattcggcccctctaatctagcgtaccctttttaagcaattccggtcccccttctttttatatttaattttgtatatcatacccgttcgctc

»»

2,360 2,370 2,380 2,390 2,400 2,410 2,420 2,430 2,440 2,450 2,460

ggagctagaacgattcgcagttaatcctggcctgtagaacaatcagaaggcttagacaatactgggacagctacaaccatcccttcagacaggatcagaagaac
cctcgatcttgtaagcgtcaattaggaccggacaatctttagtagcttccgacatctgtttatgacctgtcgatgttgtagggaagtctgtcctagtcttcttg

2,470 2,480 2,490 2,500 2,510 2,520 2,530 2,540 2,550 2,560

SapI

ttagatcattatataatacagtagcaaccctctattgtgtgcatcaaaggatagagataaaagacaccaaggaagctttagacaagatagaggaagagcaaaacaaa
aatctagtaataatattatgtcatcgttgggagataaacacacgtagtttctatctctattttctgtggttcttcgaaatctgttctatctccttctcgttttgttt

2,570 2,580 2,590 2,600 2,610 2,620 2,630 2,640 2,650 2,660 2,670

EcoNI

MfeI

agtaagaccaccgcacagcaagcggccggcgcgctgatctcagacctggaggaggagatatgagggacaattggagaagtgaattatataaatataaagtagtaa
tcattctggtggcgtgctgcttcgccggccggcgcgactagaagtctggacctcctcctataactccctgttaacctctcacttaataatattatattcatcatt

2,680 2,690 2,700 2,710 2,720 2,730 2,740 2,750 2,760 2,770 2,780

aaatgaaccattaggagtagcaccaccaaggcaagagaagagtgggtgcagagagaaaaagagcagtgggaaataggagcttgttcttgggttcttgggagca
tttaacttggtaatcctcatcgtgggtggttccgtttcttcttaccacgtctcttttttctcgtcaccttatcctcgaacaaggaaccaagaacctcgt

RRE

»»

2,790 2,800 2,810 2,820 2,830 2,840 2,850 2,860 2,870 2,880

gcaggaagcactatggcgagcgtcaatgacgctgacggtacaggccagacaattattgtctggtatagtcgagcagcagaacaatttgctgagggtattgaggc
cgtccttctgatacccgctcgcagttactgagctgcatgtccggtctgtaataacagaccatatcacgctcgtcttgttaaagactcccgataactccg

»» RRE »»

2,890 2,900 2,910 2,920 2,930 2,940 2,950 2,960 2,970 2,980 2,990

BstAPI

gcaacagcatctgttcaactcacagtctggggcatcaagcagctccaggcaagaatcctggctgtggaaagatacctaaggatcaacagctcctggggatttggg
cgttctgtagacaacgttgagtgtagacccccgtagttcgtcagagtcctgcttcttaggaccgacaccttctatggatttctagttgtcgaggaccttaaccc

»» RRE »»

3,000 3,010 3,020 3,030 3,040 3,050 3,060 3,070 3,080 3,090 3,100

gttgccttggaactcatttgaccactgctgtgccttggaatgctagtgtgagtaataaatctctggaacagatttgaatcacacgacctggatggagtgggac
caacgagaccttttgagtaaacgtggtgacgacacggaaccttacgatcaacctcattattagagaccttgctaaaccttagtgtgctggacctacctcacctg

3,110 3,120 3,130 3,140 3,150 3,160 3,170 3,180 3,190 3,200 3,210

agagaaattaacaattacacaagcttaatacactccttaattgaagaatcgcaaaaccagcaagaaagaatgaacaagaattatggaatagataaatgggcaag
tctcttaattgttaattgtgtcgaattatgtgaggaattaacttcttagcgttttggctgtcttttcttactgttcttaataaccttaattctattaccgctc

3,220 3,230 3,240 3,250 3,260 3,270 3,280 3,290 3,300 3,310

tttgtggaattggtttaaatacaaaattggctgtggtatataaaaattattcataatgatagtaggaggcttggtaggttaagaatagttttgctgtactttcta
aaacaccttaacaaattgtattgtttaaccgacaccatataatttaataagtattactatcatctccgaacctccaattcttatcaaaaacgacatgaaagat

3,320 3,330 3,340 3,350 3,360 3,370 3,380 3,390 3,400 3,410 3,420

BsaXI

tagtgaatagagttaggcaggatattcaccattatcgtttcagaccacctccaacccgaggggacccgacaggccgaaggaatagaagaagaaggtggagag
atcattatctcaatccgtccctataagtggtaatagcaaagctctgggtggagggttggggctcccctgggctgtccgggcttcttattcttcttccacctctc

3,430 3,440 3,450 3,460 3,470 3,480 3,490 3,500 3,510 3,520 3,530

PpuMI

agagacagagacagatccattcgattagtgaaacggatcggcactgctgctgccaattctgcagacaaatggcagttatccacaattttaaagaaaagggggga
tctctgtctctgtctagtaagctaatacttgcctagccgtgacgcacgcggttaagacgtctgtttaccgtcataagtaggtgttaaaatttctttcccccct

cPPT/CTS >>

3,540 3,550 3,560 3,570 3,580 3,590 3,600 3,610 3,620 3,630

ttgggggtacagtgacggggaagaatagtagacataatgaacagacatacaaaactaagaattacaaaacaaattacaaaattcaaaatttctgggttat
aaccctcatgtcacgtccccttcttattcatctgtattatcgttgctgtatgtttgatcttctaatgttttgtttaatgttttaagttttaaagcccaata

>> cPPT/CTS >>

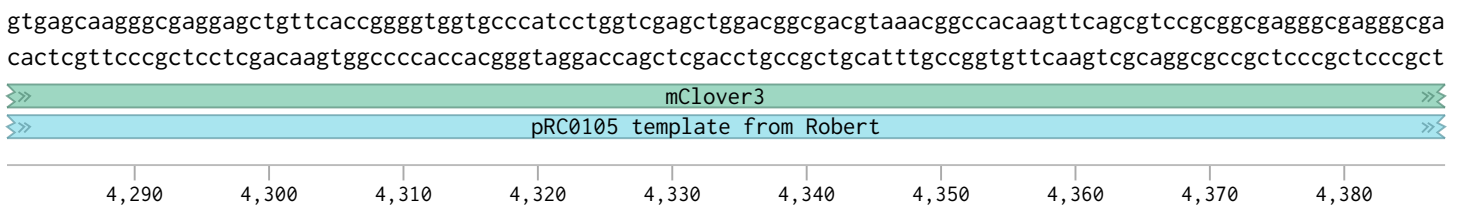
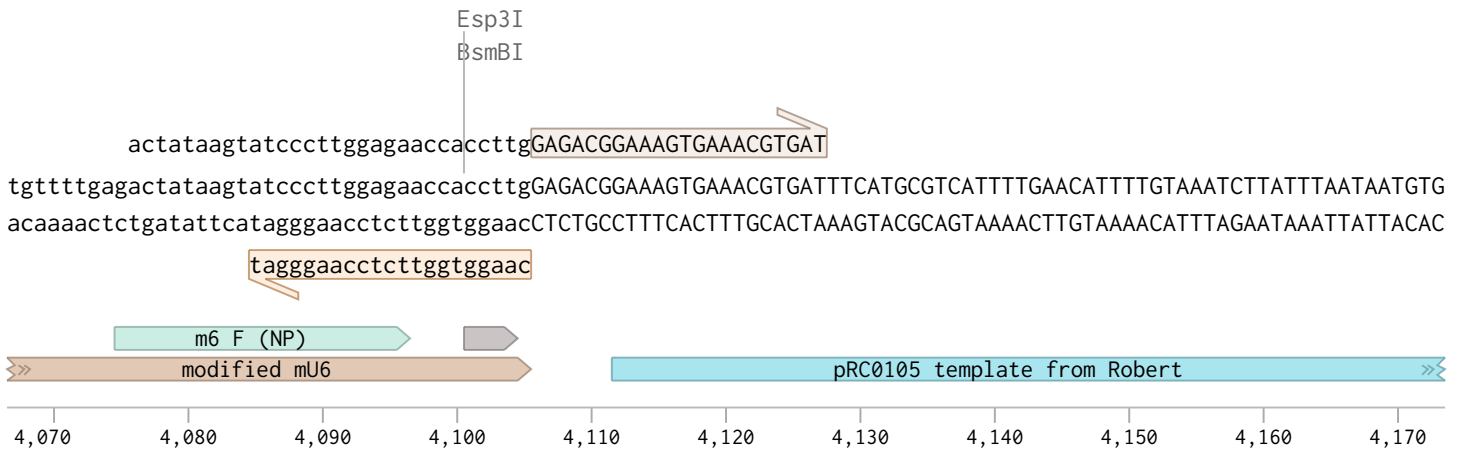
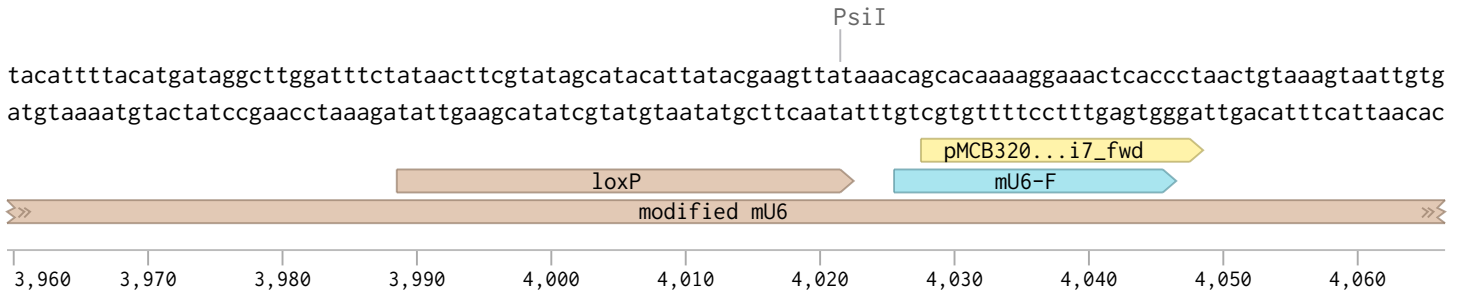
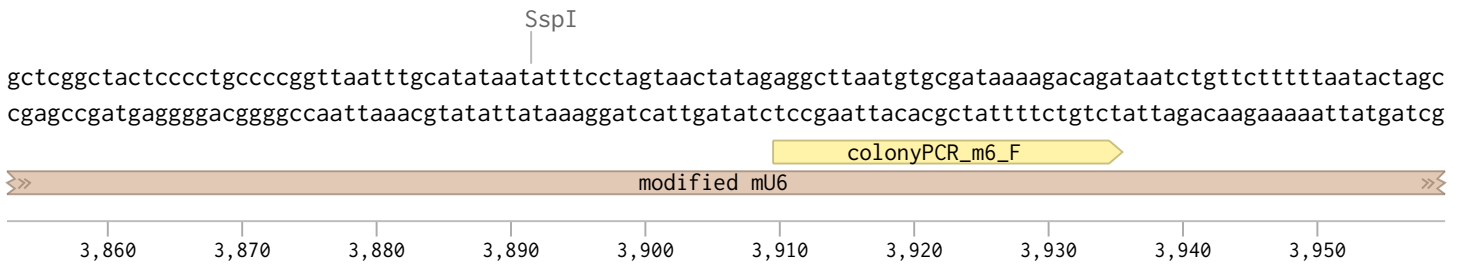
3,640 3,650 3,660 3,670 3,680 3,690 3,700 3,710 3,720 3,730 3,740

tacaggacagcagagatccagtttggttagtaccgggcccgtctagagatccgacgcgccatctctaggcccgcgcccctcgcacggacttgtgggagaa
atgtccctgtcgtctctaggtcaaaccaatcatgcccggcgagatctctaggtgctgctgtagagatccggcgcgccgggggagcgtgcctgaacacctctt

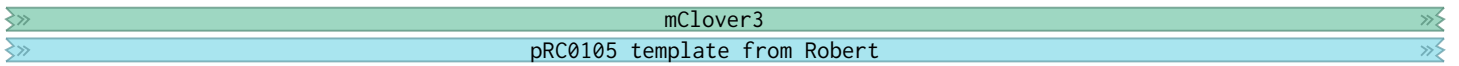
modified mU6 >>

3,750 3,760 3,770 3,780 3,790 3,800 3,810 3,820 3,830 3,840 3,850

XbaI
ApaI

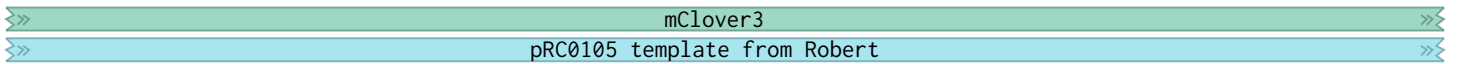


tgccaccatcggcaagctgacctgaagttcatctgcaccaccggcaagctgccctggcccaccctcgtgaccaccttcggctacggcgtggcctgcttca
acggtgtagccgttcgactgggacttcaagtagacgtggtggccgttcgacgggcacgggaccgggtgggagcactggtggaaccgatgccgcaccggacgaagt



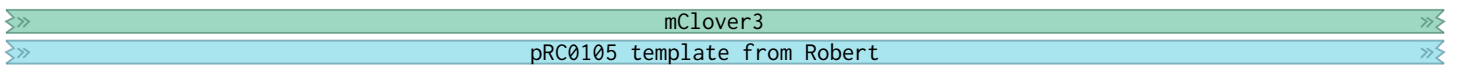
4,390 4,400 4,410 4,420 4,430 4,440 4,450 4,460 4,470 4,480 4,490

gccgtacccgaccacatgaagcagcagcacttcttcaagtcgccatgcccgaaggctacgtccaggagcgcaccatctacttcaaggacgacggtacctacaag
cggcgatggggctggtgtacttctgtctgctgaagaagttcaggcgttacggccttccgatgcaggtcctcgcgtggtagatgaagttcctgctgccatggatgttc



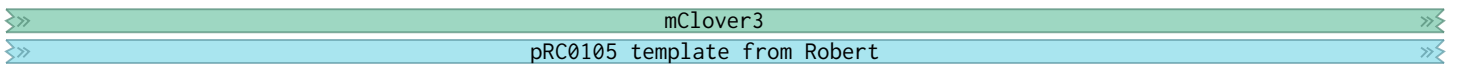
4,500 4,510 4,520 4,530 4,540 4,550 4,560 4,570 4,580 4,590 4,600

accgcgccgaggtgaagttcagggcgacaccctggtgaaccgcacgagctgaaggcgcacttcaaggaggacggcaacatcctggggcacaagctggagta
tggcgcgggctccacttcaagctcccgtgtgggaccacttggcgtagctcacttcccgtagctgaagttcctcctgccgtttagtagcccggtgttcgacctat



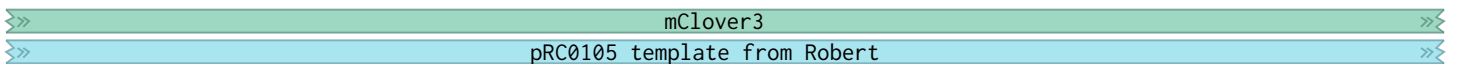
4,610 4,620 4,630 4,640 4,650 4,660 4,670 4,680 4,690 4,700

caacttcaacagccactacgtctatatcacggccgacaagcagaacaacagcatcaaggctaacttaccatccgccacaacggttaggacggcagcgtgcagctcg
gttgaagttgtcggtagtcagatatagtgccggctgttcgtcttgttgcgtagttccgattgaagtggttaggcggttgaactcctgccgtgcacgtcgagc



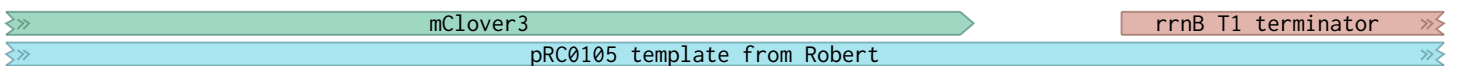
4,710 4,720 4,730 4,740 4,750 4,760 4,770 4,780 4,790 4,800 4,810

ccgaccactaccagcagaacacccccatcggcgacggccccgtgctgctgcccacaaccactacctgagccatcagtcgccctgagcaagacccaacgagaag
ggctggtgatggtcgtcttgtggggtagccgctgccggggcacgacgacggcgtgttggtagtgactcggtagtcaggcgggactcgttctgggggtgctcttc

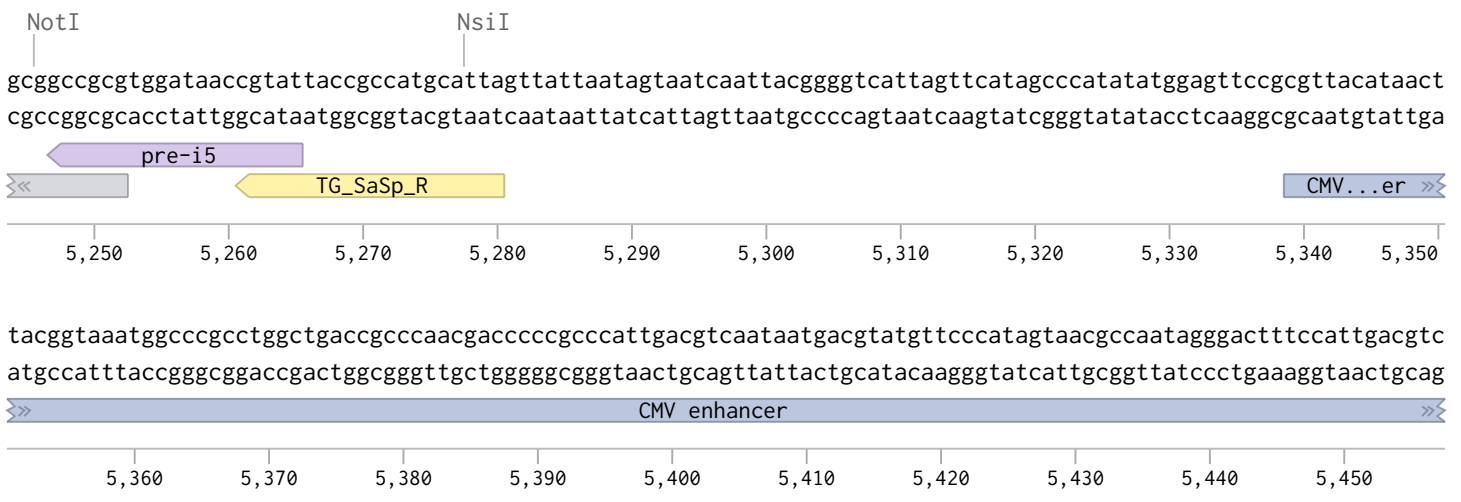
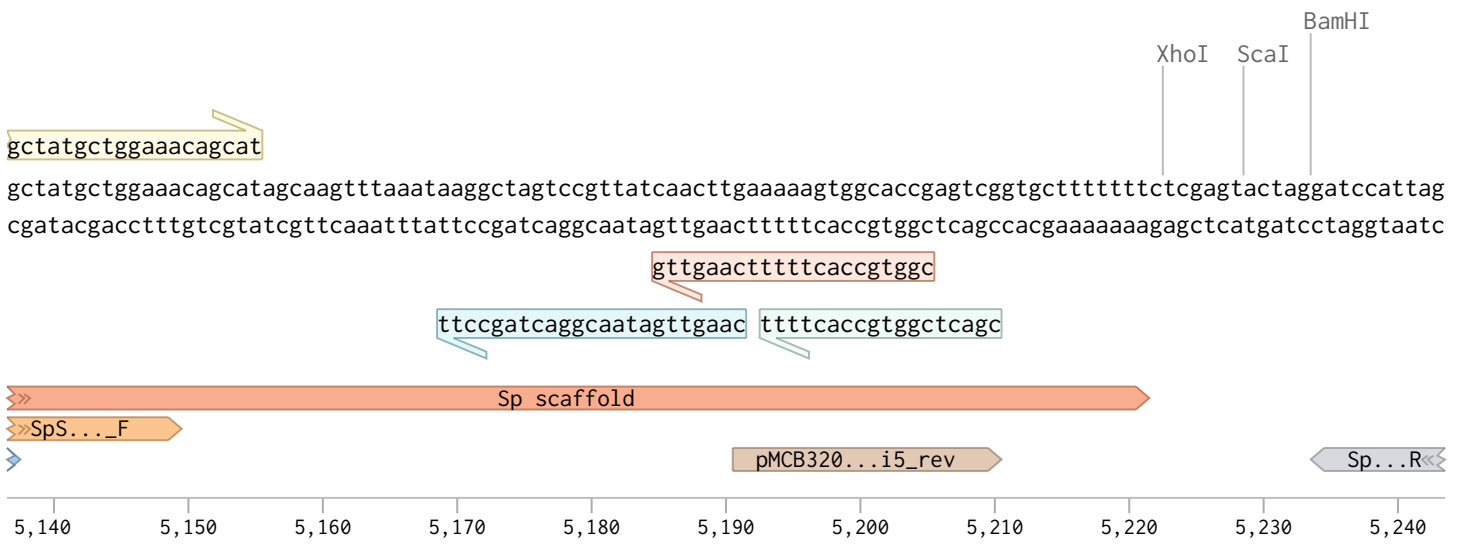
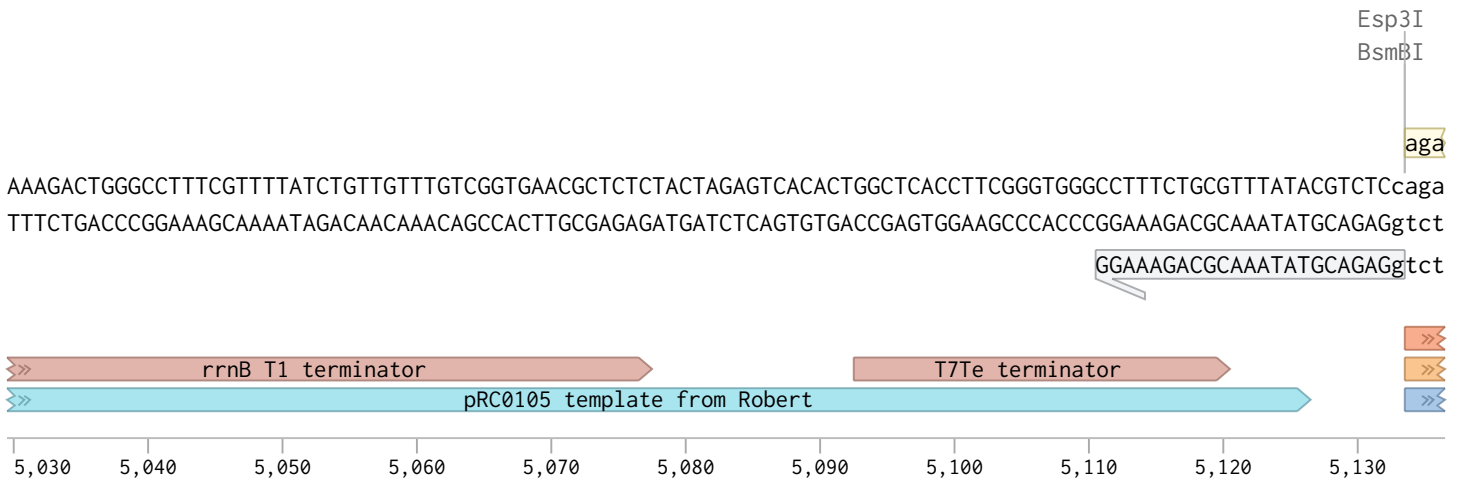


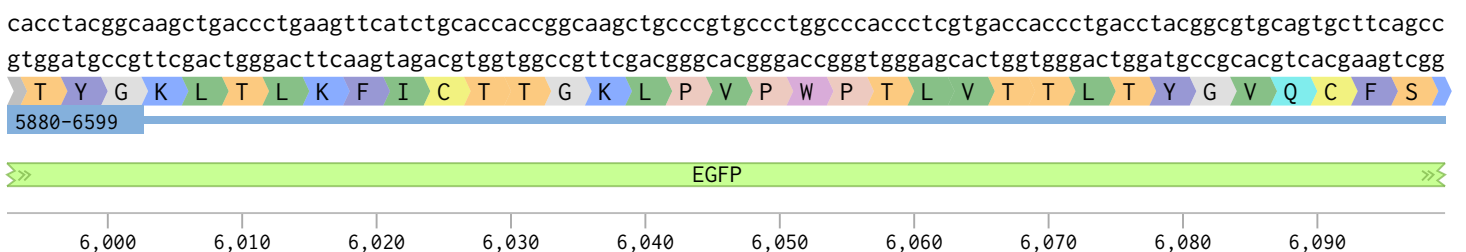
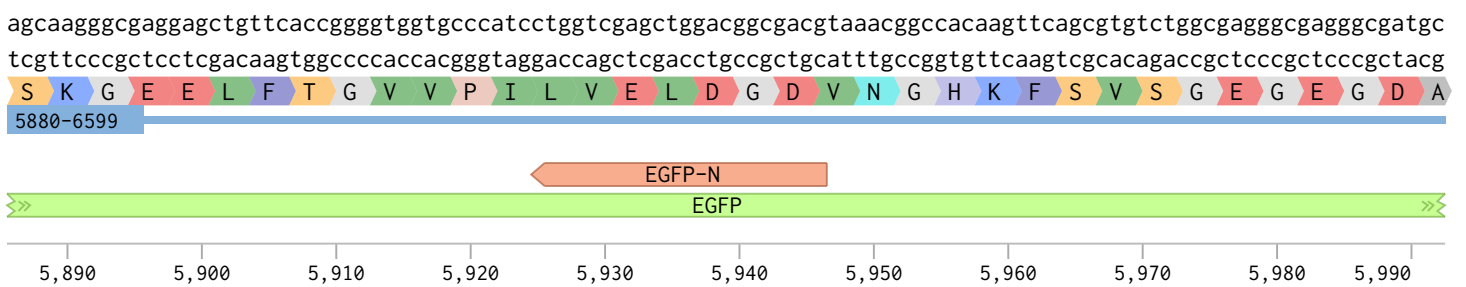
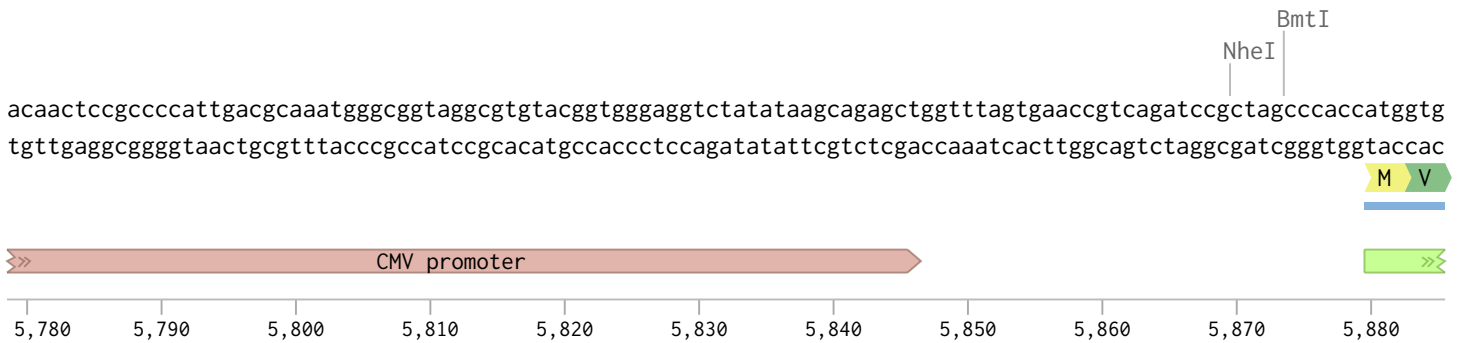
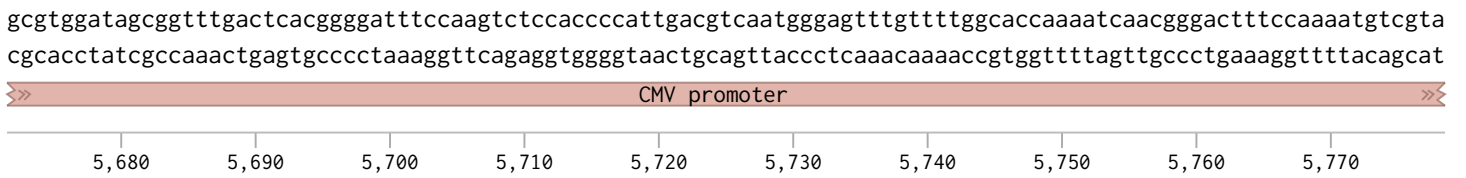
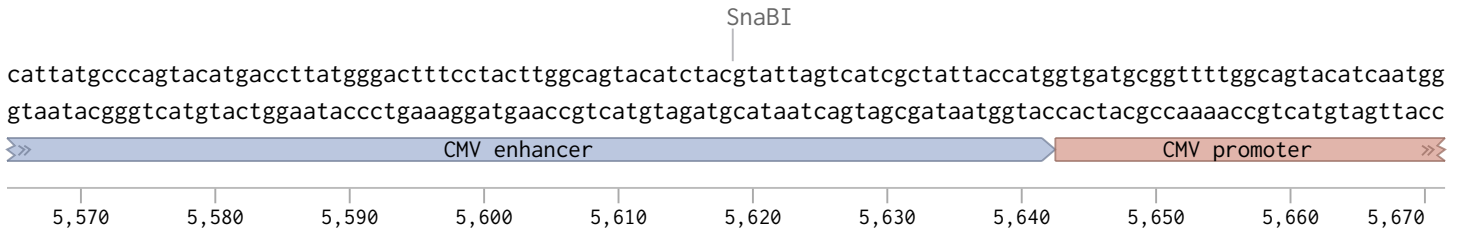
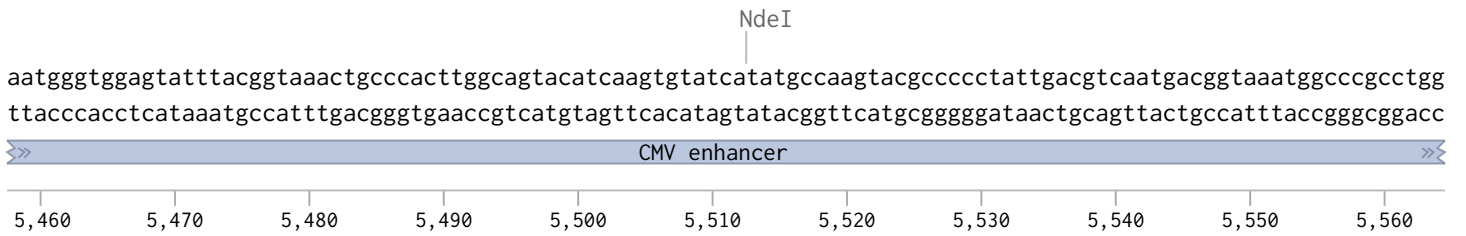
4,820 4,830 4,840 4,850 4,860 4,870 4,880 4,890 4,900 4,910 4,920

cgcgatcacatggtcctgctggagttcgtgaccgccgggattacacatggcatggacgagctgtacaagtaaCCAGGCATCAATAAAAACGAAAGGCTCAGTCG
gcgctagtgtaccaggacgacctcaagcactggcggcgccctaatgtgtaccgtacctgctcgacatgttattGGTCCGTAGTTTATTTGCTTCCGAGTCAGC

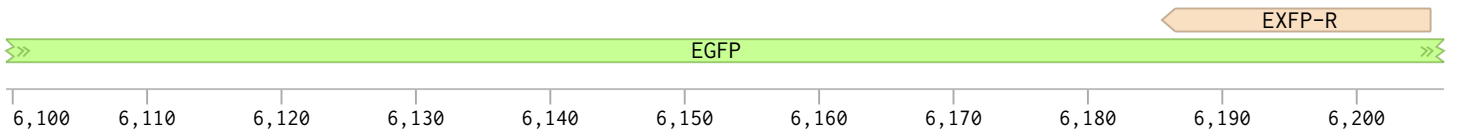


4,930 4,940 4,950 4,960 4,970 4,980 4,990 5,000 5,010 5,020

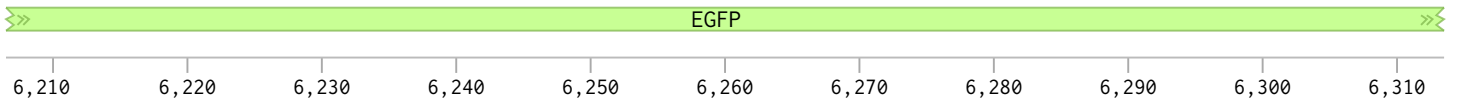




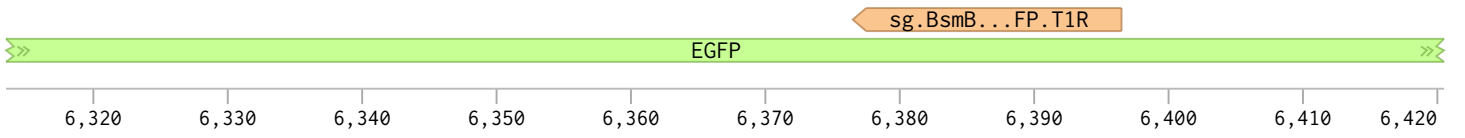
gtagcccgaccacatgaagcagcagcacttcttcaagtcgccatgccgaaggctacgtccaggagcgcaccatcttcttcaaggacgacggcaactacaagacc
 cgatggggctgggtacttctgctgctgaagaagttcaggcggtagcggcttccgatgcaggctctcgctggtagaagaagttcctgctgccgttgatgtctgg
 R Y P D H M K Q H D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T
 5880-6599



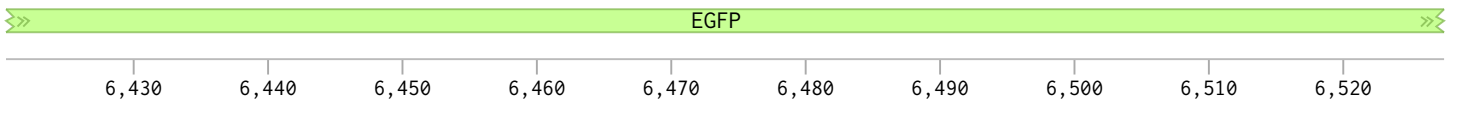
cgcccgagggtgaagttcgagggcgacaccctgggaaccgcatcgagctgaaggcatcgacttcaaggaggacggcaacatcctggggcacaagctggagtacaa
 gcgcggtccacttcaagctcccgctgtgggaccacttggcgtagctcgacttccgtagctgaagttcctcctgcccgtttaggaccccggttcgacctatggt
 R A E V K F E G D T L V N R I E L K G I D F K E D G N I L G H K L E Y N
 5880-6599



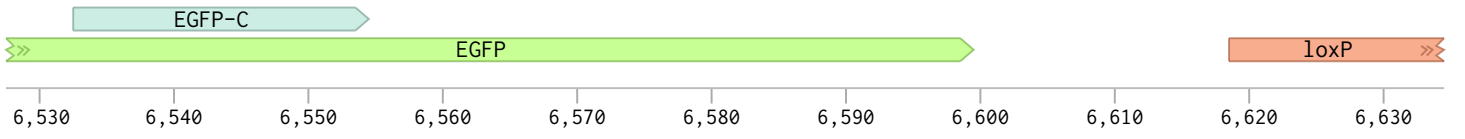
ctacaacagccacaactctatatcatggccgacaagcagaagaacggcatcaaggcgaacttcaagatccgccacaacatcgaggacggcagcgtgcagctcggc
 gatgtgtcgggtgttcagatatagtagccgctgtctgcttcttgcgtagttccgcttgaagttctaggcgggtgttagctcctgccctcgcacgctcgagcggc
 Y N S H N V Y I M A D K Q K N G I K A N F K I R H N I E D G S V Q L A
 5880-6599

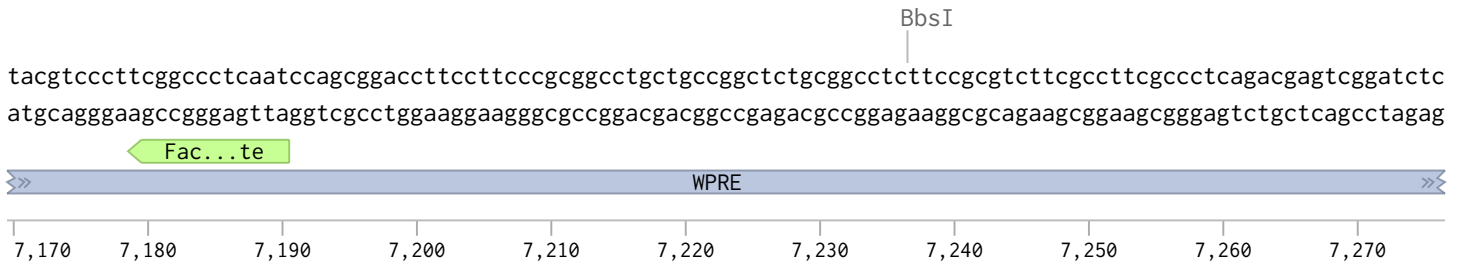
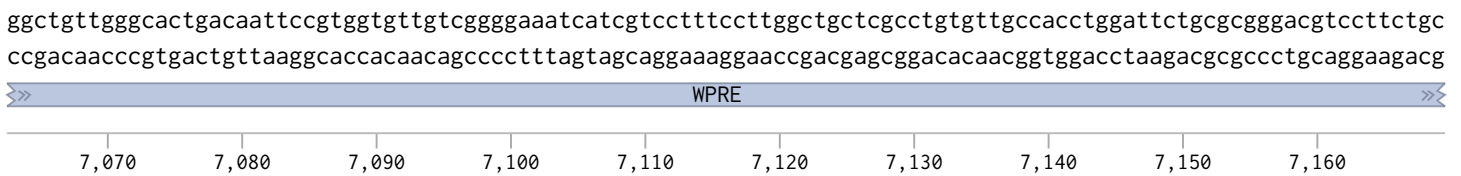
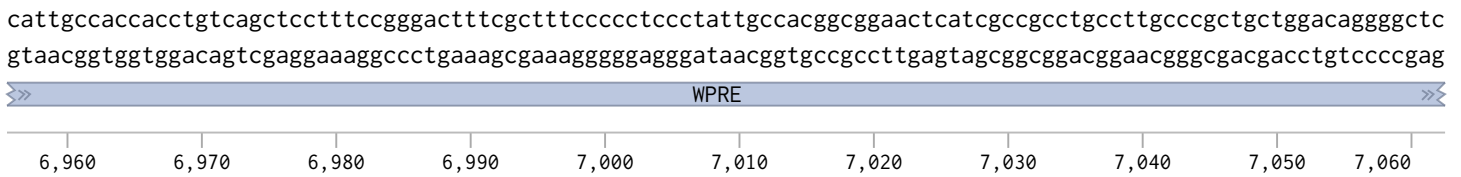
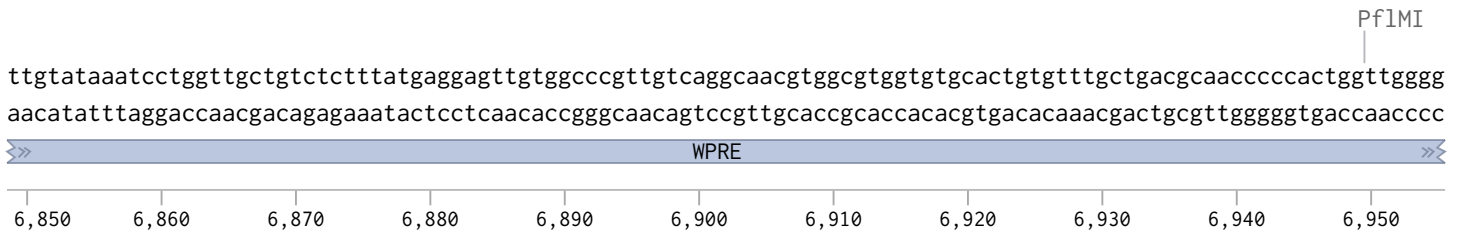
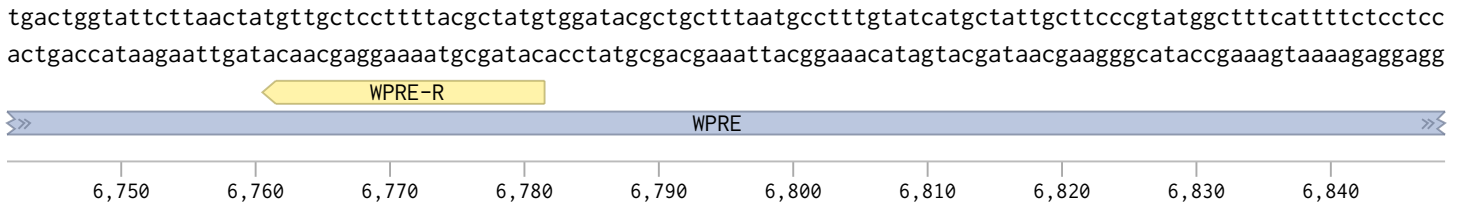
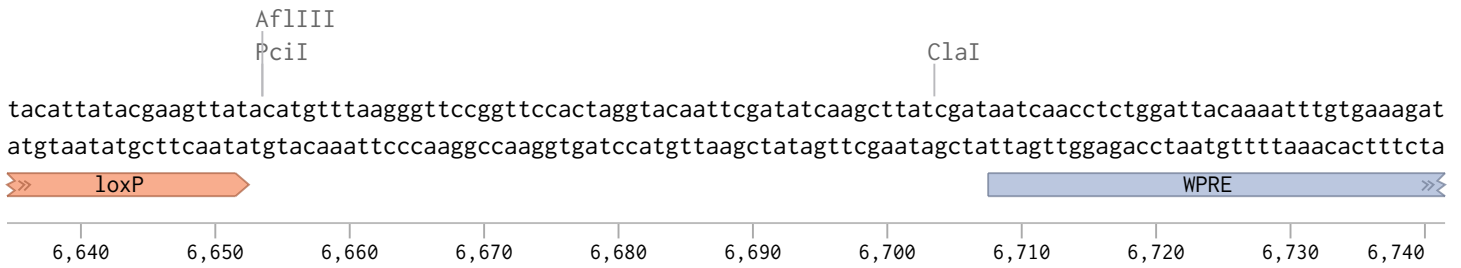


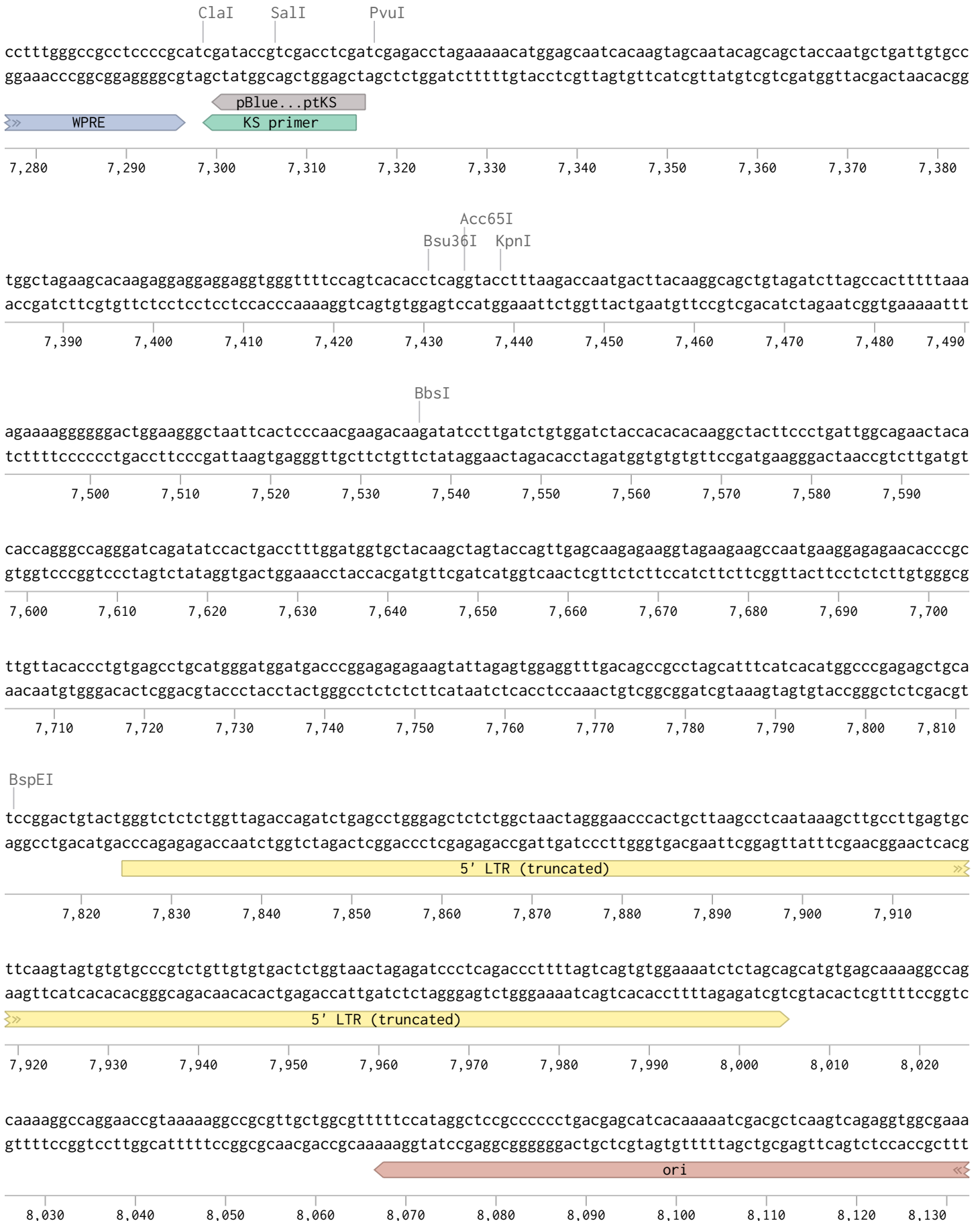
accactaccagcagaacacccccatcggcgacggccccgtgctgctgcccgacaaccactacctgagcaccagtcggccctgagcaaagaccccaacgagaagcgc
 tggatgatgctgcttgtggggtagccgctgccggggcacgacgacgggctgttggatggactcgtgggtcaggcgggactcgtttctggggtgctcttcgcg
 D H Y Q Q N T P I G D G P V L L P D N H Y L S T Q S A L S K D P N E K R
 5880-6599



gatcacatggtcctgctggagtctgtgaccgcccgggatcactctcggcatggacgagctgtacaagtaagaattcgtcagggacctaataactcgtatagca
 ctagtgtaccaggacgacctcaagcactggcggcgccctagtgagagccgtacctgctcgacatgttattcttaagcagctccctggattattgaagcatatcgt
 D H M V L L E F V T A A G I T L G M D E L Y K *
 5880-6599







ccgacaggactataaagataaccaggcgtttcccctggaagctccctcgtgcgctctcctggtccgacctgccgcttaccggatacctgtccgcctttctccctt
 gggctgtcctgatatttctatggctccgcaaagggggaccttcgaggagcacgcgagaggacaaggctgggacggcgaatggcctatggacaggcggaaagaggaa

pBR322ori-F

ori

8,140 8,150 8,160 8,170 8,180 8,190 8,200 8,210 8,220 8,230

cggaagcgtggcgctttctcatagctcacgctgtaggatctcagttcgggtgtaggtcgttcgctccaagctgggctgtgtgcacgaacccccgttcagcccgac
 gcccttcgaccgcgaagagtatcgagtgcgacatccatagagtcaagccacatccagcaagcgaggttcgacccgacacacgtgcttggggggcaagtcgggctg

ori

8,240 8,250 8,260 8,270 8,280 8,290 8,300 8,310 8,320 8,330 8,340

cgctgcgcttatccgtaactatcgtcttgagtccaaccggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcgaggtatgt
 gcgacgcggaataggccattgatagcagaactcaggttgggccattctgtgctgaatagcggtgaccgtcgtcggtgaccattgtcctaactcgtctcgtccataca

ori

8,350 8,360 8,370 8,380 8,390 8,400 8,410 8,420 8,430 8,440 8,450

aggcggctgctacagattcttgaagtggccttaactacggctacactagaagaacagtatttggtatctgcgctctgctgaagccagttaccttcggaaaaagag
 tccgccacgatgtctcaagaacttcaccaccggatgatgccgatgtgatcttctgtcataaacatagacgcgagacgacttcggtcaatggaagcctttttctc

ori

8,460 8,470 8,480 8,490 8,500 8,510 8,520 8,530 8,540 8,550 8,560

ttggtagctcttgatccggcaaaaccaccgctggtagcgggtgttttttgttgaagcagcagattacgcgcagaaaaaaggatctcaa
 aaccatcgagaactaggccgtttgtttggcggcaccatcgccacaaaaaaacaaacgttcgtcgtcctaatacgcgctcttttttcttagagtt

ori

8,570 8,580 8,590 8,600 8,610 8,620 8,630 8,640 8,650