

## 1\_pCAP2005\_01\_pLGR-dual-guide-puro-mycNLS-eGFP ...

SgrDI

GTCGACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAG  
CAGCTGCCTAGCCCTCTAGAGGGCTAGGGGATACCACGTGAGAGTCATGTTAGACGAGACTACGGCGTATCAATTTCGGTCATAGACGAGGGACGAACACACAACCTC

10

20

30

40

50

60

70

80

90

100

NruI

GTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGA  
CAGCGACTCATCACGCGCTCGTTTTAAATTCGATGTTGTTCCGTTCCGAAGTGGCTGTTAACGTACTTCTTAGACGAATCCCAATCCGCAAAACGCGACGAAGCGCT

110

120

130

140

150

160

170

180

190

200

210

MluI

SpeI

AseI

pU6 5'-CMV

TGTACGGGCCAGATATACGCGTTGACATTGATTATTGACTtagttattaatagtaataacacggggcattagttcatagcccatatatggagttccgcgttacat  
ACATGCCCGTCTATATGCGCAACTGTAATAAAGTatcaataattatcattagttaatgcccagtaatacagtatcgggtatatacctcaaggcgcaatgta

pU6 5'-CMV

CMV enhancer

220

230

240

250

260

270

280

290

300

310

320

pU6 5'-CMV

aacttacggtaaatggccgcctggctgaccgccaacgacccccgccattgacgtcaataatgacgtatgttcccatagtaacccaatagggactttccattga  
ttgaatgccatttacccggcgaccgactggcgggttgctggggcgggtaactgcagttattactgcatacaagggtatcattgcggttatccctgaaaggtaact

pU6 5'-CMV

CMV enhancer

330

340

350

360

370

380

390

400

410

420

NdeI

pU6 5'-CMV

cgtcaatgggtggagtatttacggtaaactgcccacttggcagttacatcaagtgtatcatatgccaagtacccccctattgacgtcaatgacggtaaatggccgc  
gcagttaccacctcataaatgccatttgacgggtgaaccgtcatgttagttcacatagtatacggttcatgcgggggataactgcagttactgccatttacccggcg

pU6 5'-CMV

CMV enhancer

430

440

450

460

470

480

490

500

510

520

530

SnaBI

BsaAI

NcoI

CspCI

pU6 5'-CMV

ctggcattatgccagttacatgaccttatgggactttcctacttggcagttacatctacgtatttagtcacgtattaccatggtgatgcggttttggcagttacatca  
gaccgtaatacgggtcatgtactggaataccctgaaaggatgaaccgtcatgttagatgcataatcagtagcgataatgtaccactacgcaaaaccgtcatgtagt

pU6 5'-CMV

CMV enhancer

CMV promoter

540

550

560

570

580

590

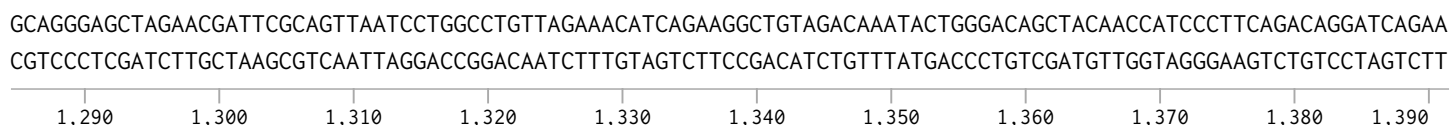
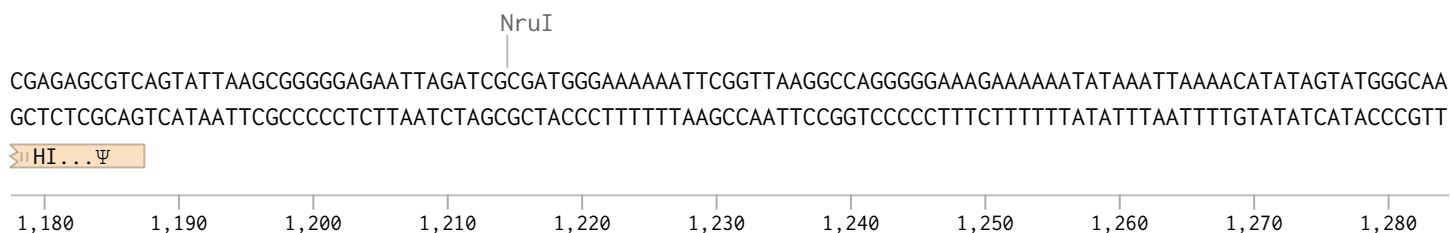
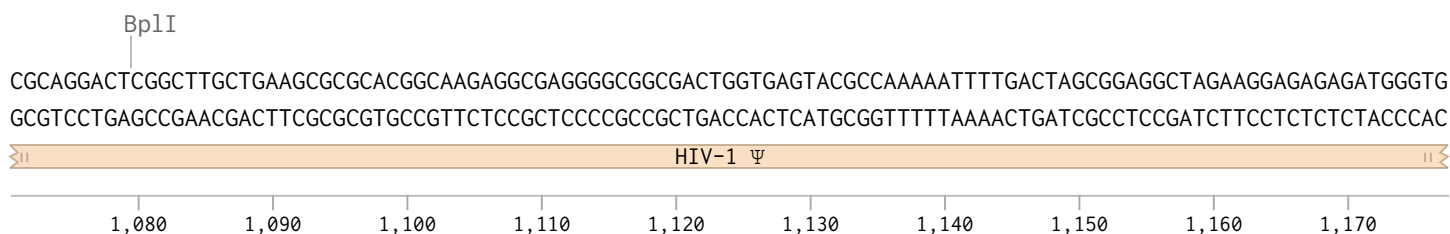
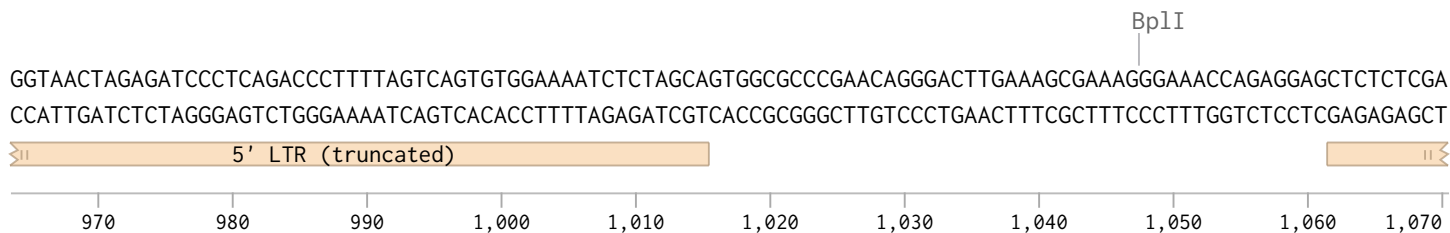
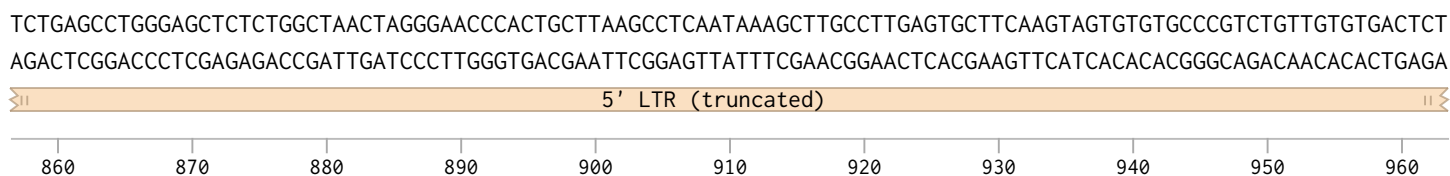
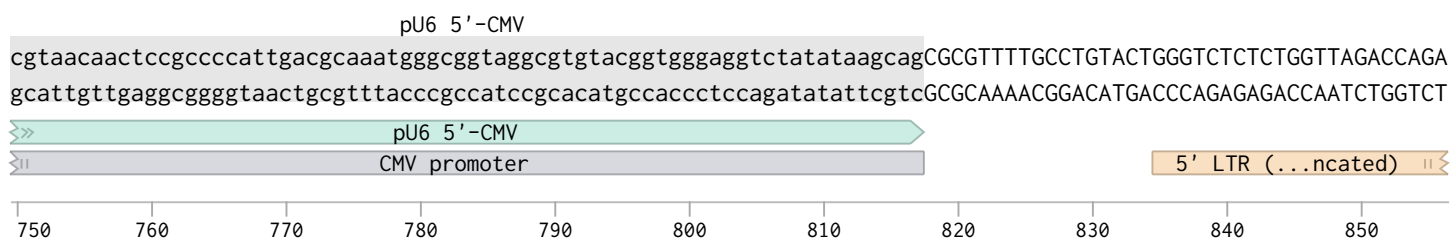
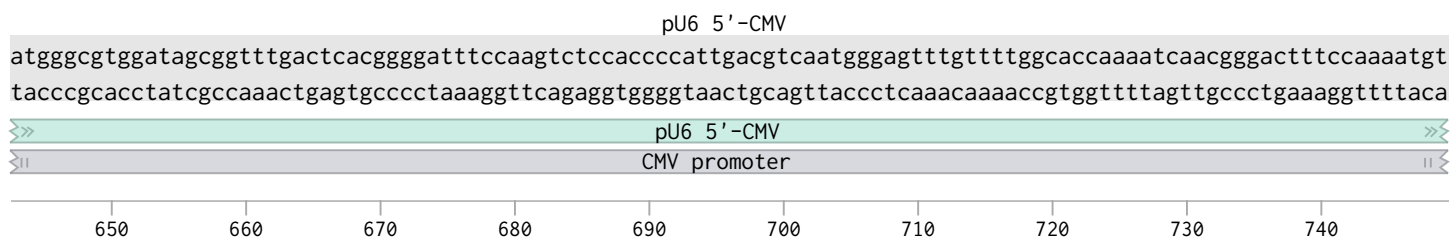
600

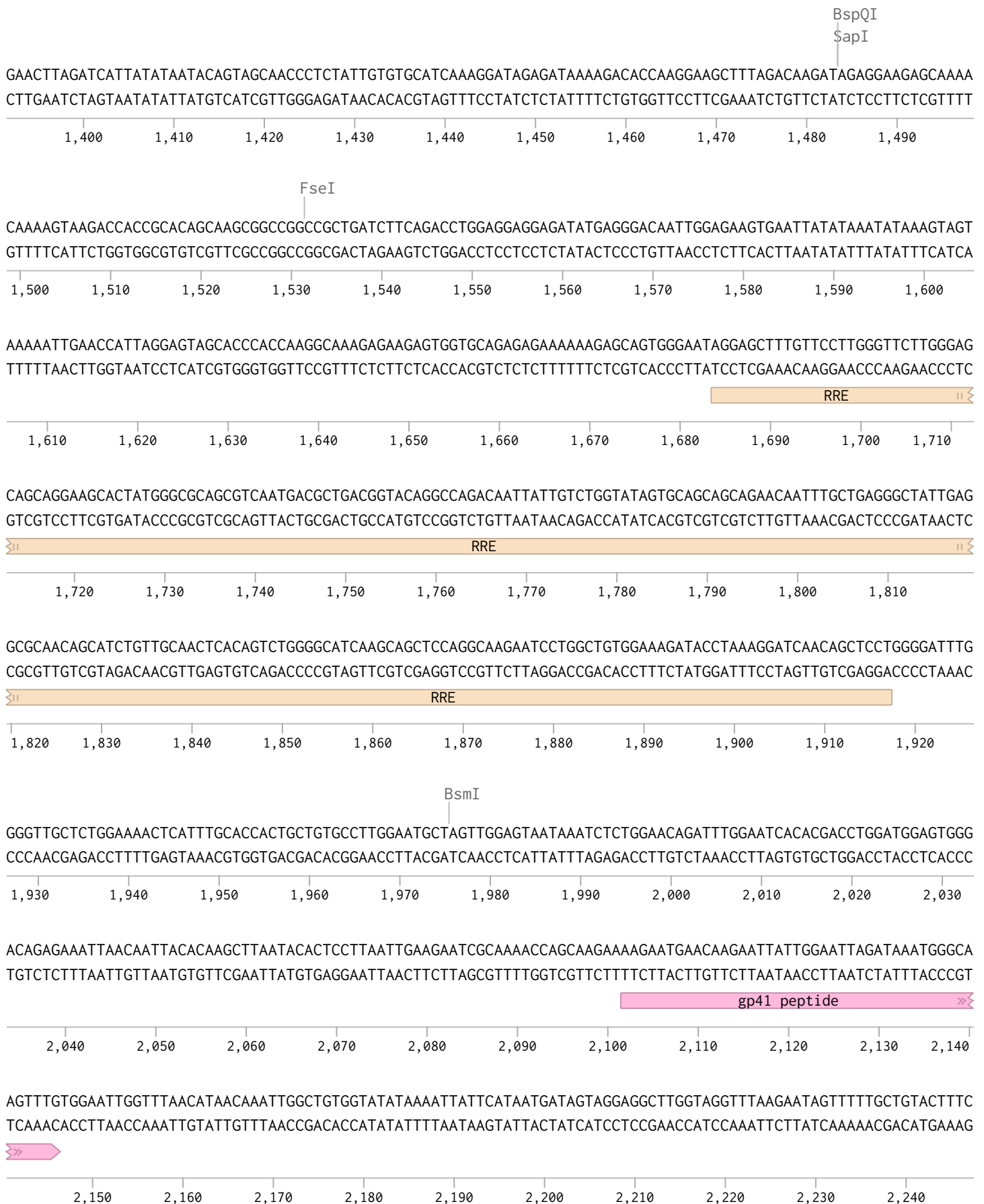
610

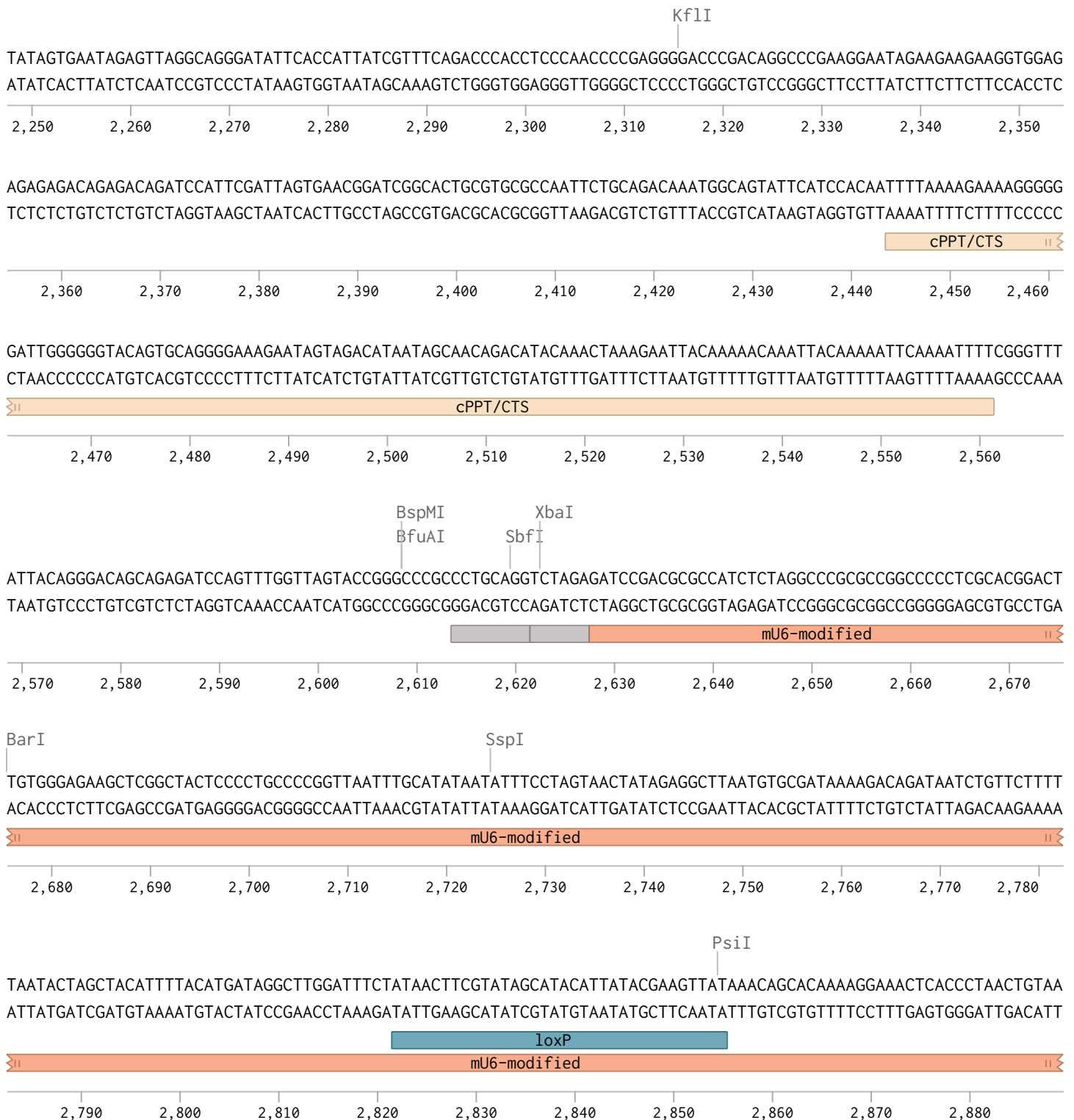
620

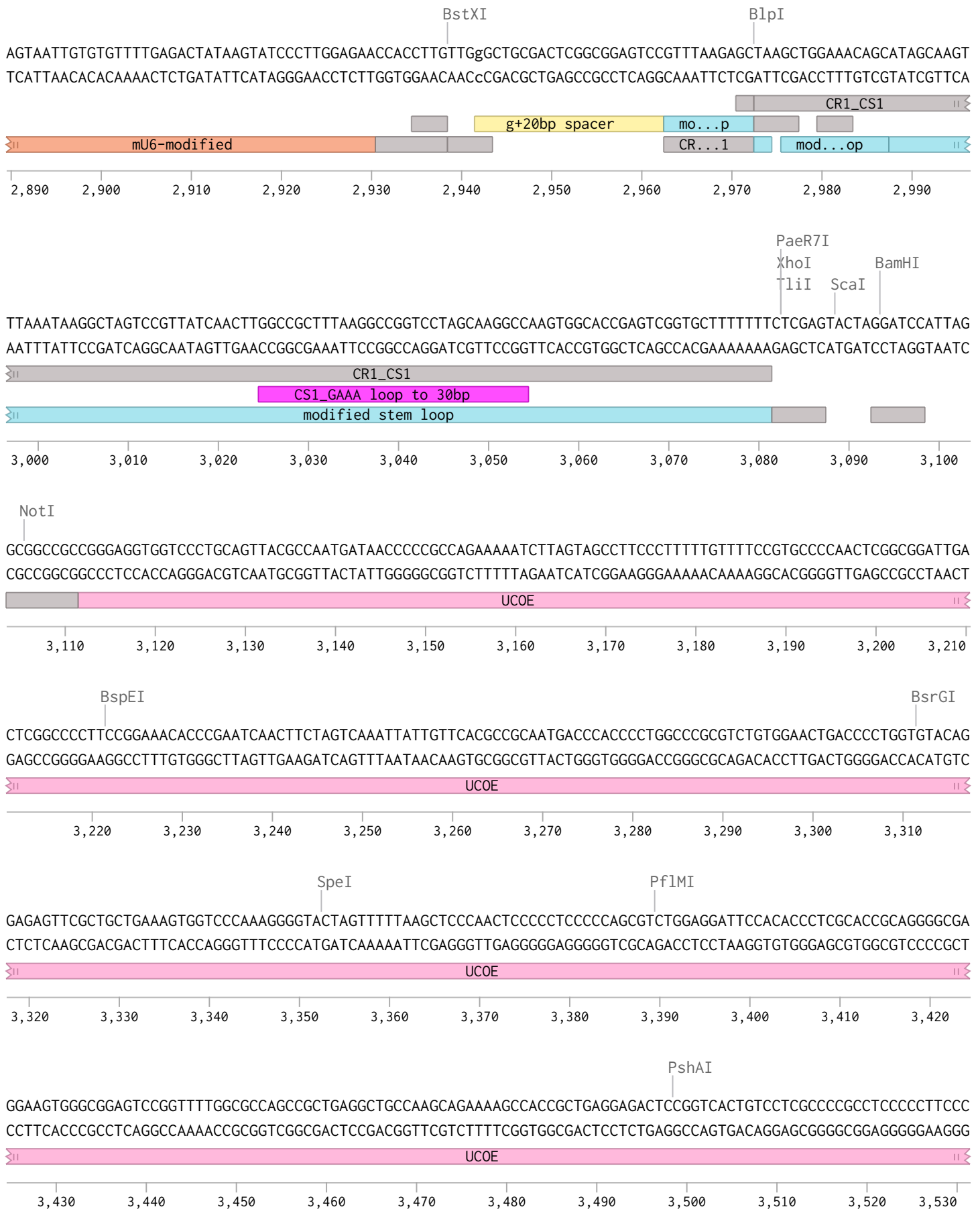
630

640











TTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCGCTGCGAATCTGGTGGCACCTTCGCGCTGTCTCGTGCTTTTCGATAAGTCTCTAGCCA  
AAGCGGAGCACGAACTCAACTCCGGACCGGACCCGCGACCCGGCGCGCACGCTTAGACCACCGTGGAAGCGCGGACAGAGCGACGAAAGCTATTAGAGATCGGT

EF-1 $\alpha$  intron A

4,180 4,190 4,200 4,210 4,220 4,230 4,240 4,250 4,260 4,270 4,280

BspMI  
BfuAI

TTTAAATTTTGGATGACCTGCTGCGACGCTTTTTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCCGCGGGC  
AAATTTTAAAACTACTGGACGACGCTGCGAAAAAAGACCGTTCTATCAGAACATTTACGCCCGGTTCTAGACGTGTGACCATAAAGCCAAAACCCCGCGCGCCG

EF-1 $\alpha$  intron A

4,290 4,300 4,310 4,320 4,330 4,340 4,350 4,360 4,370 4,380

PciI

FseI

GGCGACGGGGCCCGTGCCTCCAGCGCACATGTTTCGGCGAGGCGGGGCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCT  
CCGCTGCCCGGGCAGCAGGGTCCGCTGTACAAGCCGCTCCGCCCGGACGCTCGCGCCGGTGGCTCTTAGCCTGCCCCATCAGAGTTCGACCGGCCGGACGAGA

EF-1 $\alpha$  intron A

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

PasI

GGTGCCTGGCCTCGCGCCGCGTGTATCGCCCCGCCCTGGGCGGAAGGCTGGCCCGGTGCGCACCAAGTTCGCTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTG  
CCACGGACCGGAGCGCGGCGGCACATAGCGGGCGGGACCCGCCGTTCCGACCGGGCCAGCCGTGGTCAACGCACTCGCCTTTCTACCGCGAAGGGCCGGGACGAC

EF-1 $\alpha$  intron A

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

CAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAAGTACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCC  
GTCCCTCGAGTTTTACCTCCTGCGCCGCGAGCCCTCTCGCCCGCCCACTAGTGGGTGTGTTTCTTTCCCGGAAAGGCAGGAGTCGGCAGCGAAGTAACTGAGG

EF-1 $\alpha$  intron A

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

ACGGAGTACCGGGCGCGTCCAGGCACCTCGATTAGTTCTCGCGCTTTTGAGTACGTGCTTTAGGTTGGGGGGAGGGGTTTATGCGATGGAGTTTCCACAC  
TGCCTCATGGCCCGCGCAGGTCCGTGGAGCTAATCAAGAGCGGAAAACCTCATGCAGCAGAAATCCAACCCCTCCCAAAATACGCTACCTCAAAGGGGTGTG

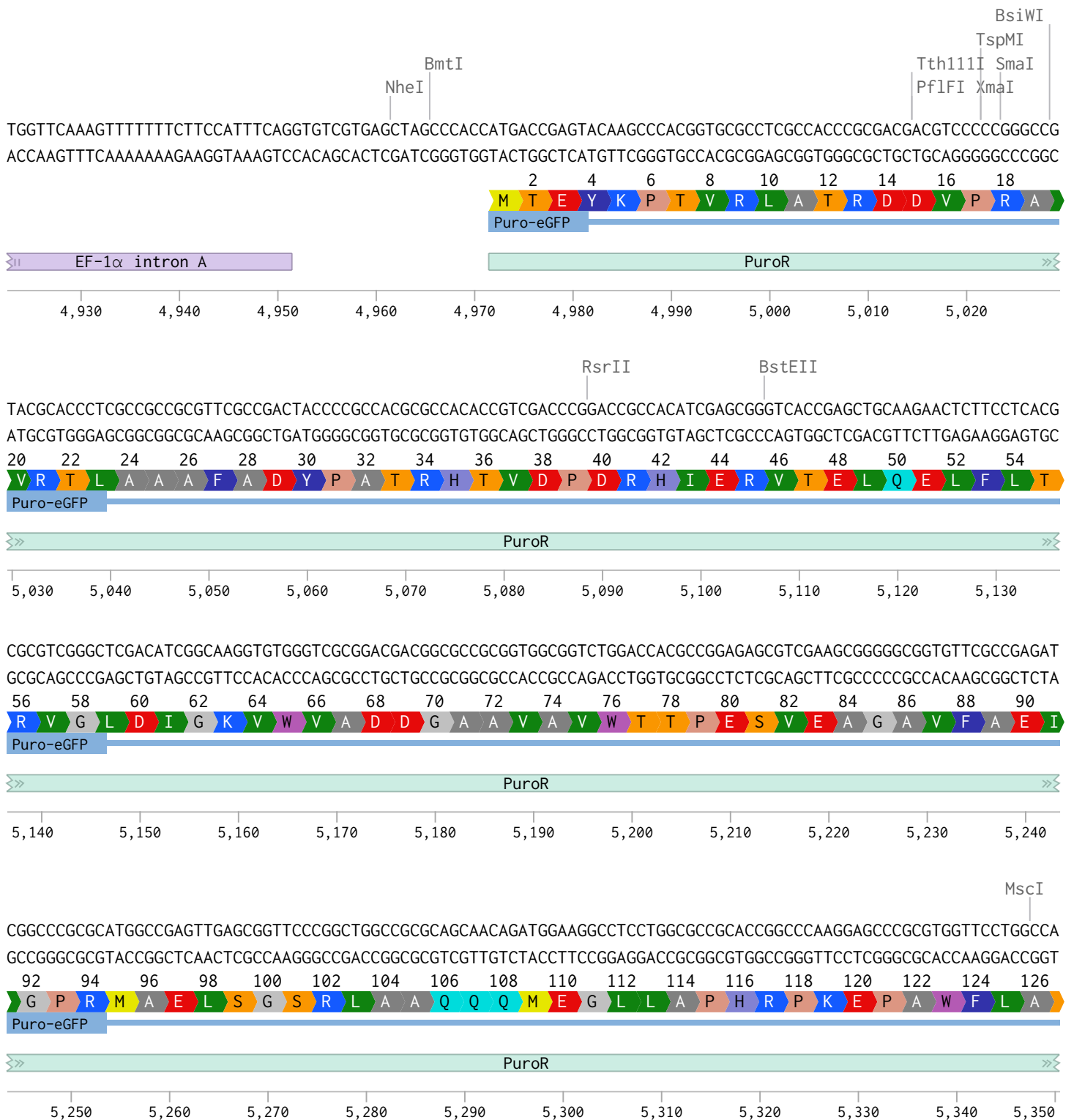
EF-1 $\alpha$  intron A

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

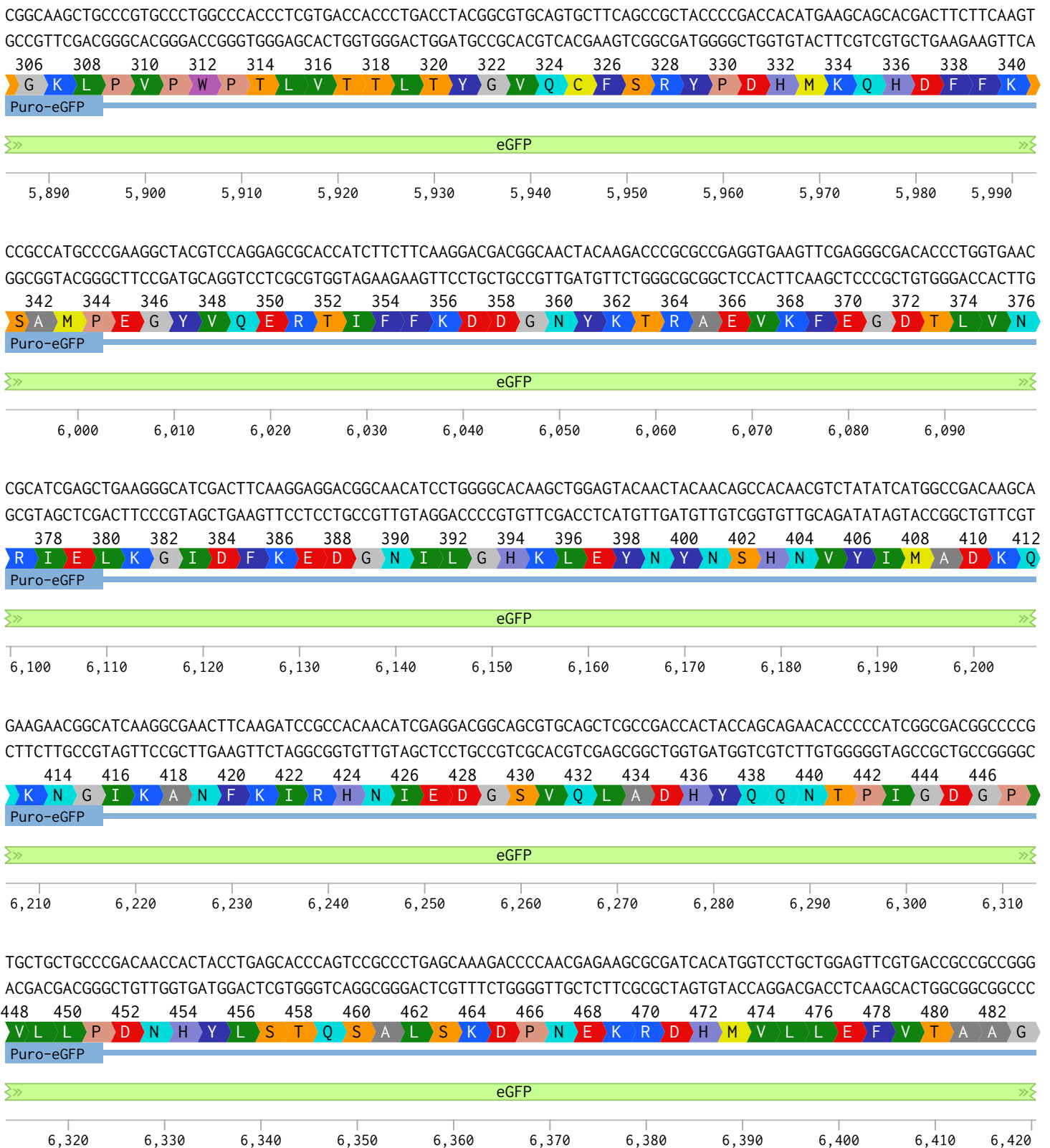
TGAGTGGGTGGAGACTGAAGTTAGCCAGCTTGGCACTTGATGTAATTCTCCTTGAATTTGCCCTTTTGGAGTTGGATCTTGGTTTCTCAAGCCTCAGACAG  
ACTCACCCACCTCTGACTTCAATCCGGTGAACCGTGAACCTAATTAAGAGGAACCTTAAACGGGAAAACTCAAACCTAGAACCAAGTAAGAGTTTCGGAGTCTGTC

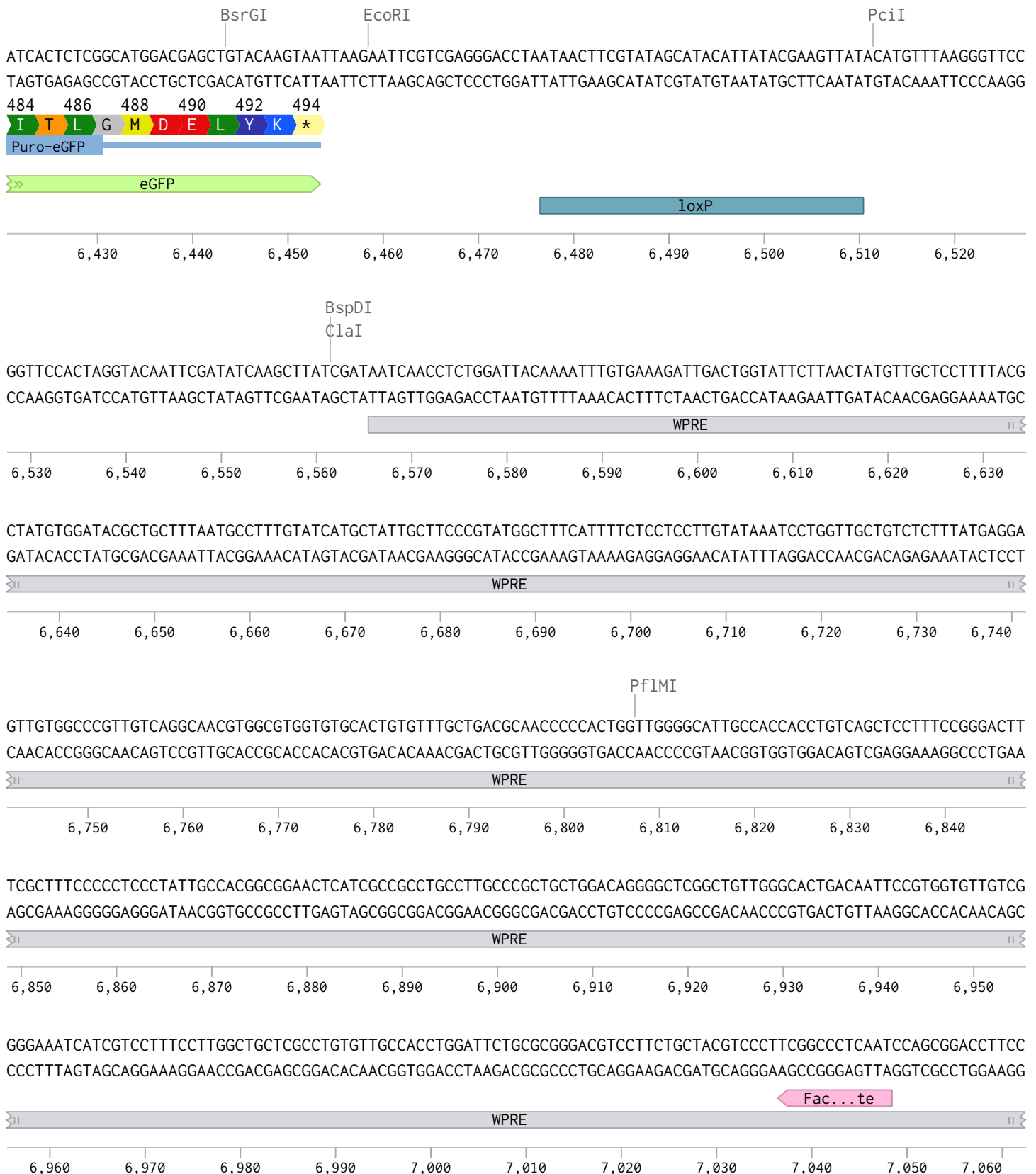
EF-1 $\alpha$  intron A

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











GGTAACTAGAGATCCCTCAGACCCCTTTTAGTCAGTGTGGAATCTCTAGCAGCATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCT  
CCATTGATCTCTAGGGAGTCTGGGAAATCAGTCACACCTTTAGAGATCGTCGTACACTCGTTTTCCGGTCGTTTTCCGGTCCTTGGCATTTTTCCGGCGCAACGA

5' LTR (truncated)

7,820 7,830 7,840 7,850 7,860 7,870 7,880 7,890 7,900 7,910

DrdI

GGCGTTTTTCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTCCCC  
CCGCAAAAGGTATCCGAGGCGGGGGGACTGCTCGTAGTGTTTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCTGATTTCTATGGTCCGCAAGGGG

ori

7,920 7,930 7,940 7,950 7,960 7,970 7,980 7,990 8,000 8,010 8,020

CTGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGT  
GACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACGGCGAATGGCCTATGGACAGGCGGAAAGAGGGAAGCCCTTCGCACCGCGAAAGAGTATCGAGTGCAGACA

ori

8,030 8,040 8,050 8,060 8,070 8,080 8,090 8,100 8,110 8,120 8,130

AGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTC  
TCCATAGAGTCAAGCCACATCCAGCAAGCGAGGTTGACCCGACACACGTGCTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAACTCAG

ori

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

CAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTA  
GTTGGGCCATTCTGTGCTGAATAGCGGTGACCGTCGTGCGTGACCATTTGCTCTAATCGTCTCGCTCCATACATCCGCCACGATGTCTCAAGAACTTCAACACCGGAT

ori

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

ACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAACAAACACCGCT  
TGATGCCGATGTGATCTTCTTGTCATAAACCATAGACGCGAGACGACTTCGGTCAATGGAAGCCTTTTCTCAACCATCGAGAACTAGGCCGTTTGTGTTGGTGGCA

ori

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

GGTAGCGGTGGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAACGA  
CCATCGCCACCAAAAAAACAACGTTCTGCTGCTAATGCGCGTCTTTTTTCTAGAGTTCTTCTAGGAACTAGAAAAAGTGGCCAGACTGCGAGTCACCTTGCT

ori

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

BspHI

AAACTCACGTTAAGGGATTTTGGTCATGAGATTACAAAAAGGATCTTCACCTAGATCCTTTAAATTAATAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGT  
TTTGAGTGCAATTCCTAAACCACTACTCTAATAGTTTTTCTAGAAGTGGATCTAGGAAAATTAATTTTACTTCAAAATTTAGTTAGATTTTATATATACTCA

8,570 8,580 8,590 8,600 8,610 8,620 8,630 8,640 8,650 8,660

