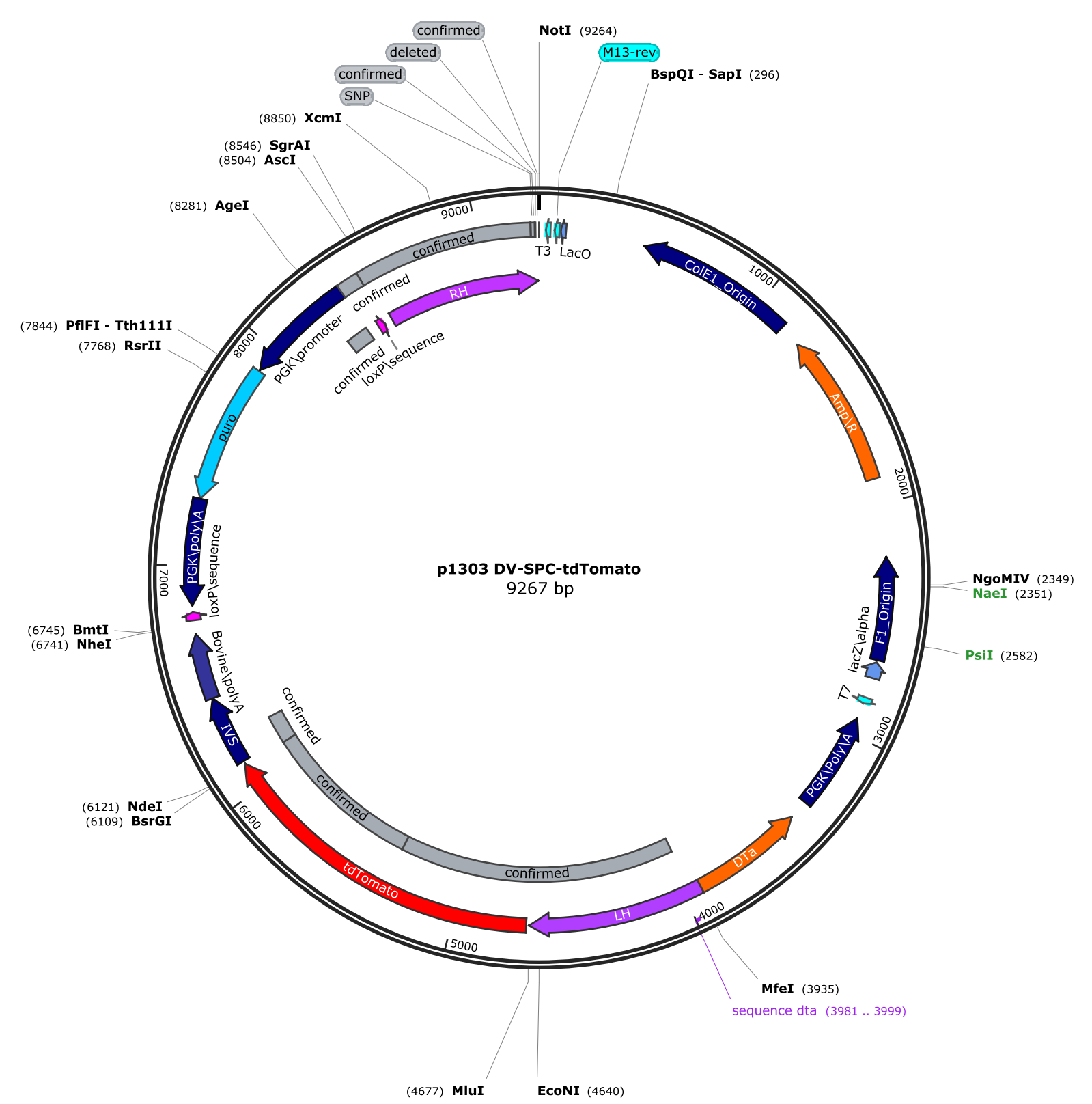
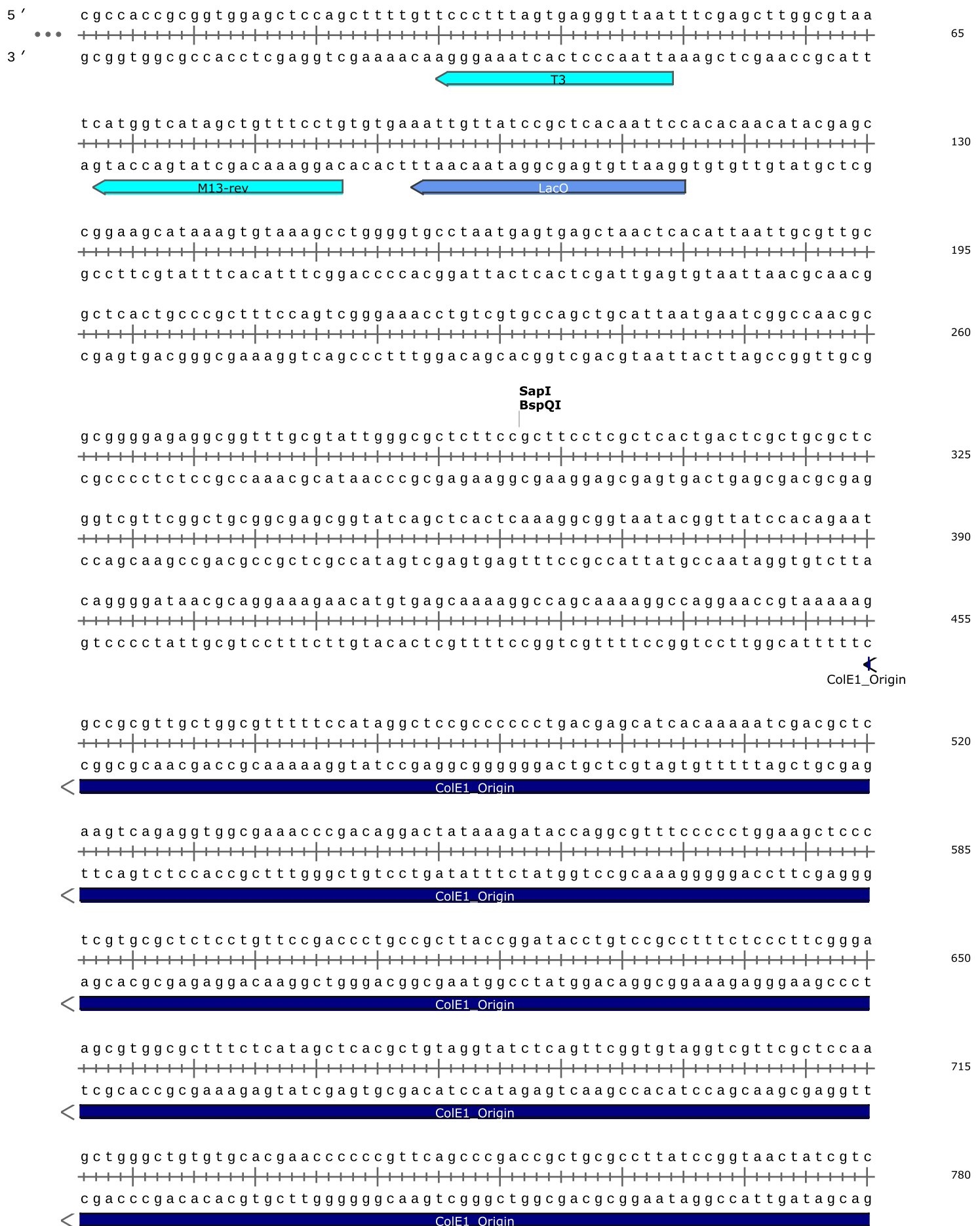
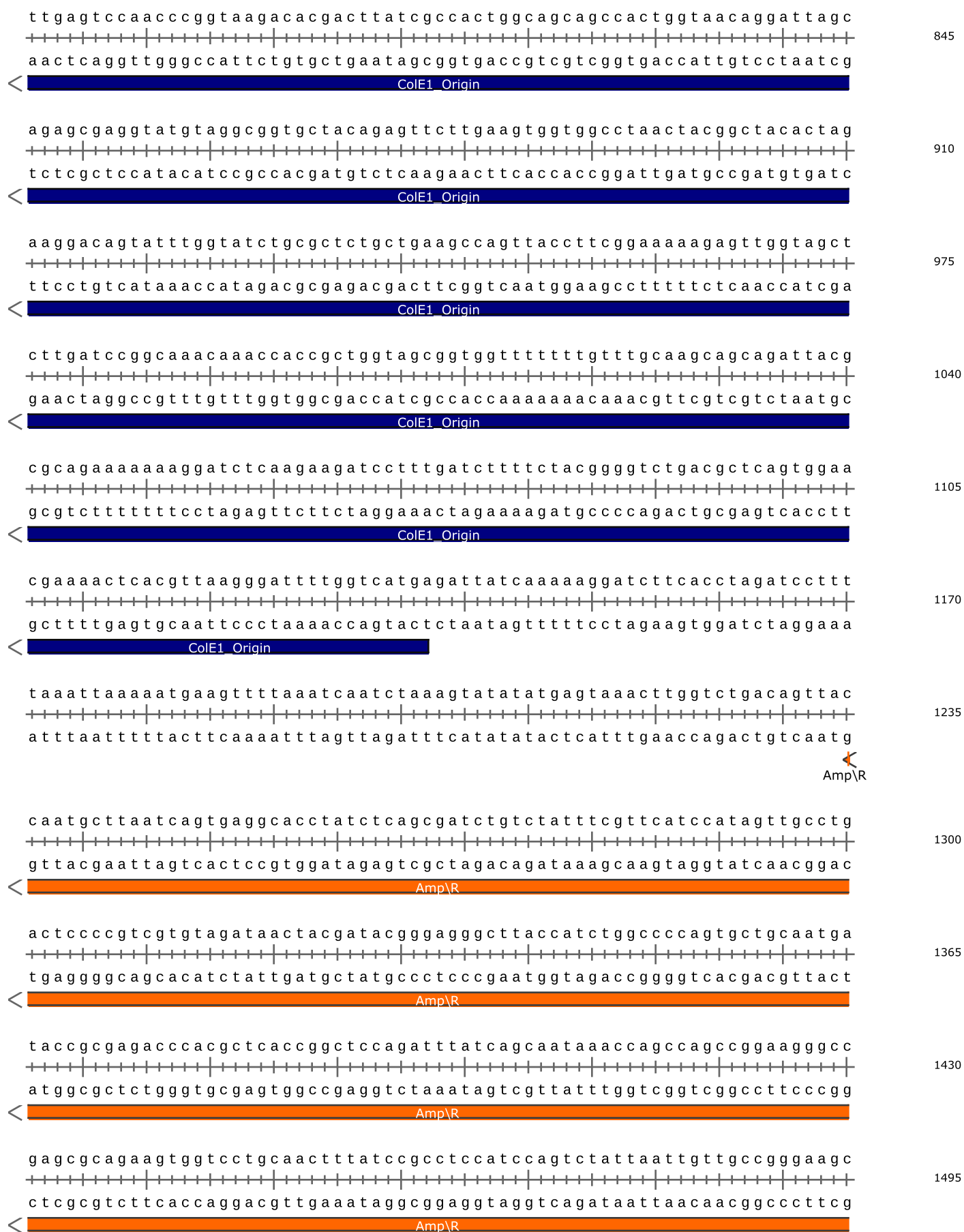
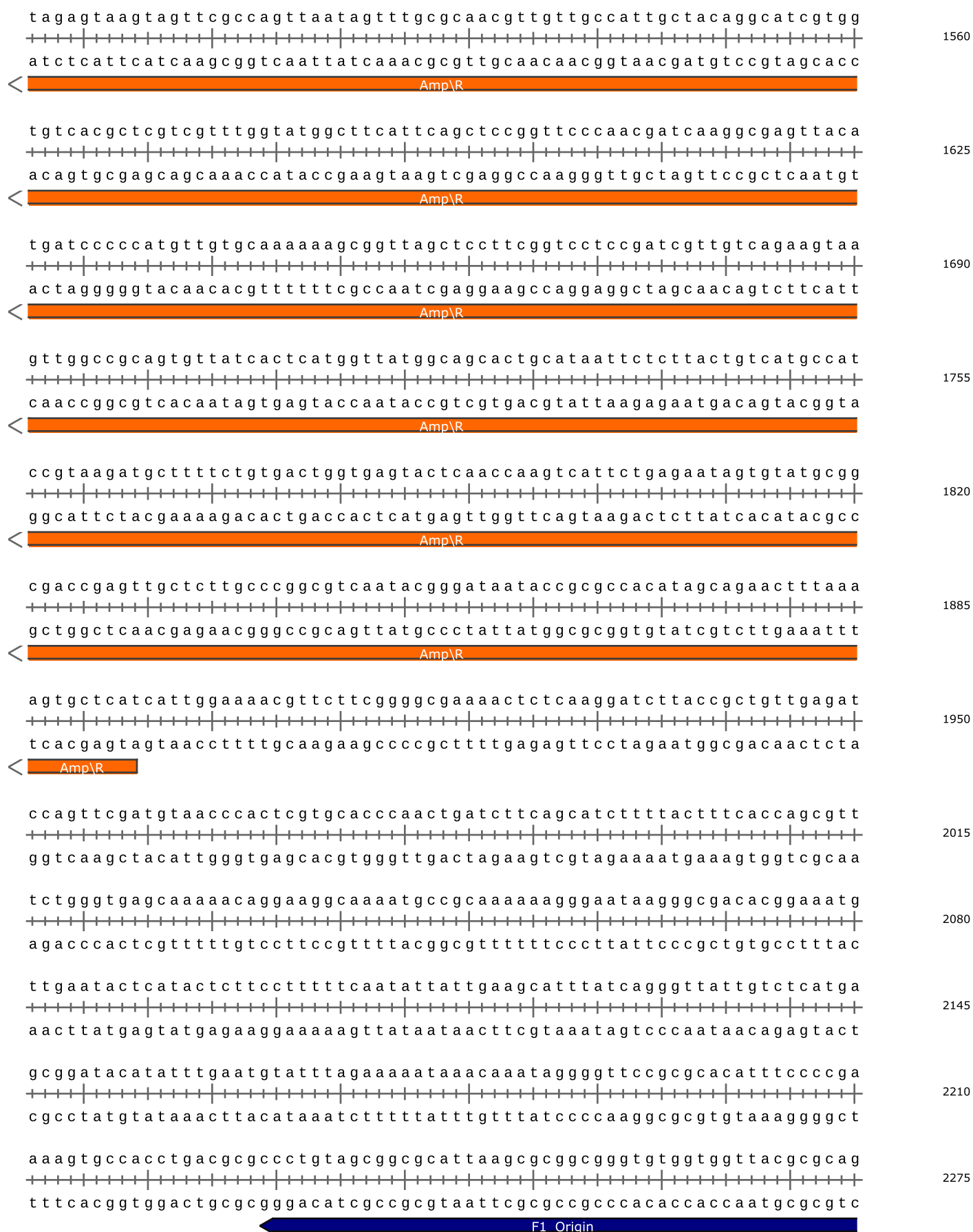
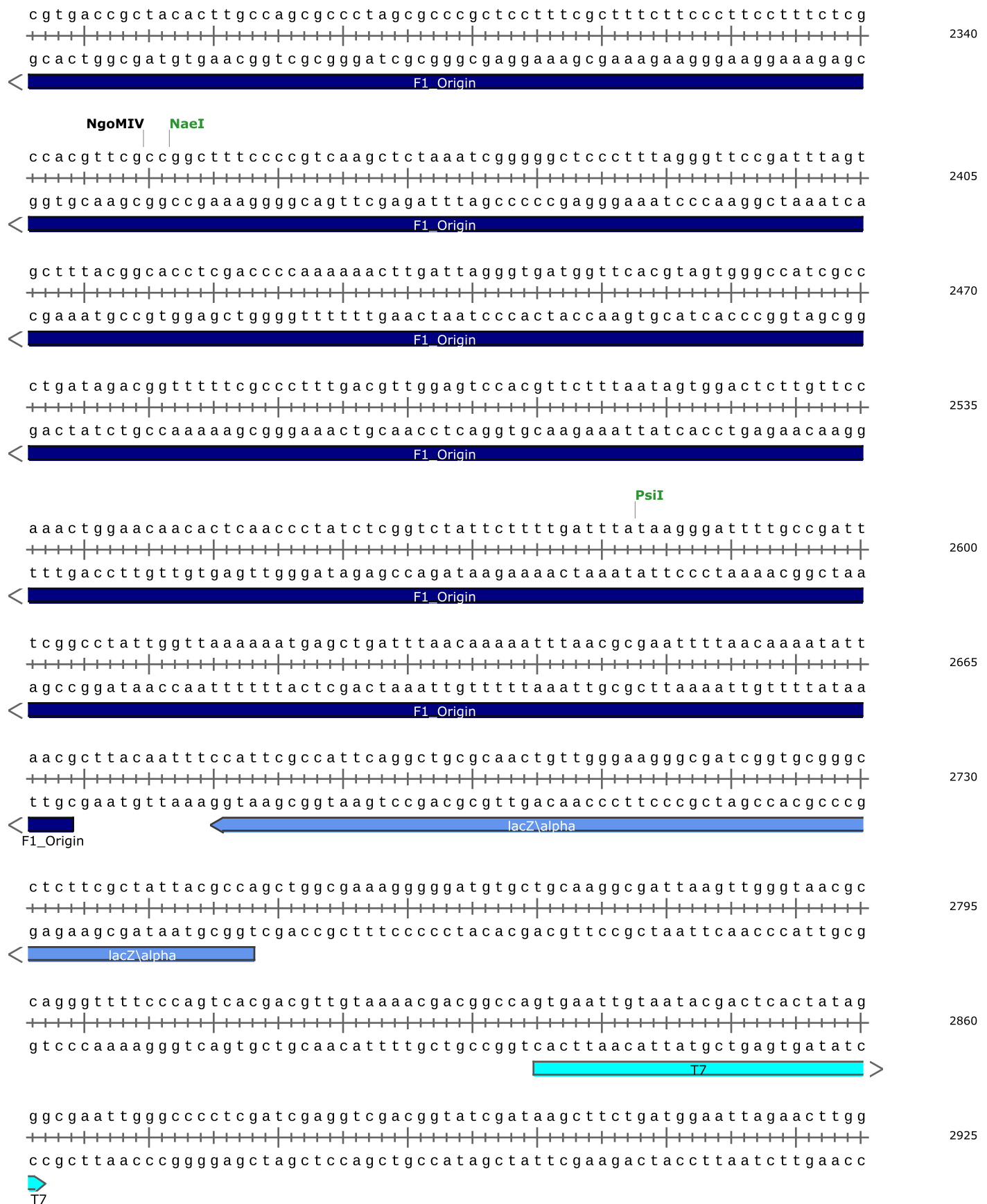


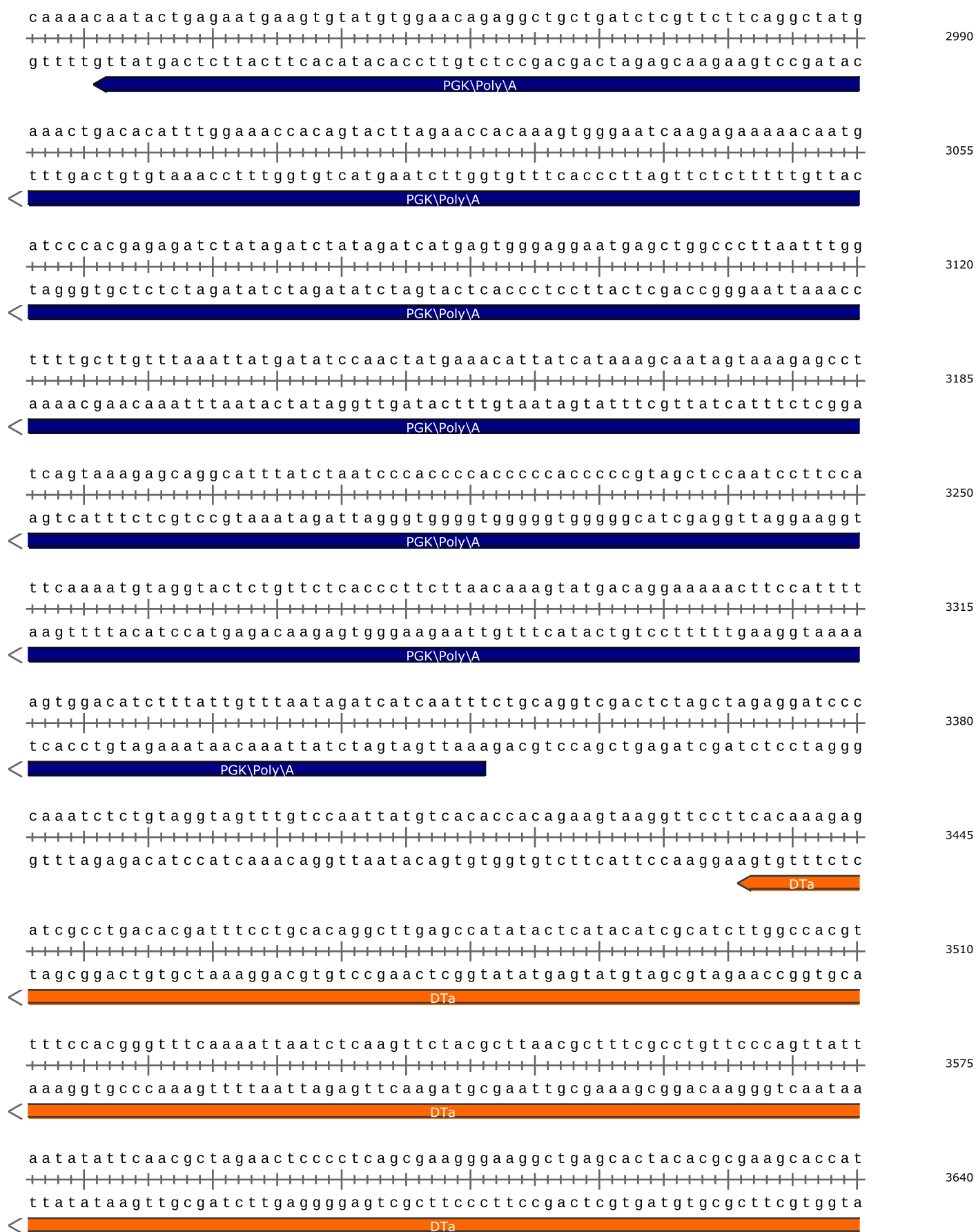


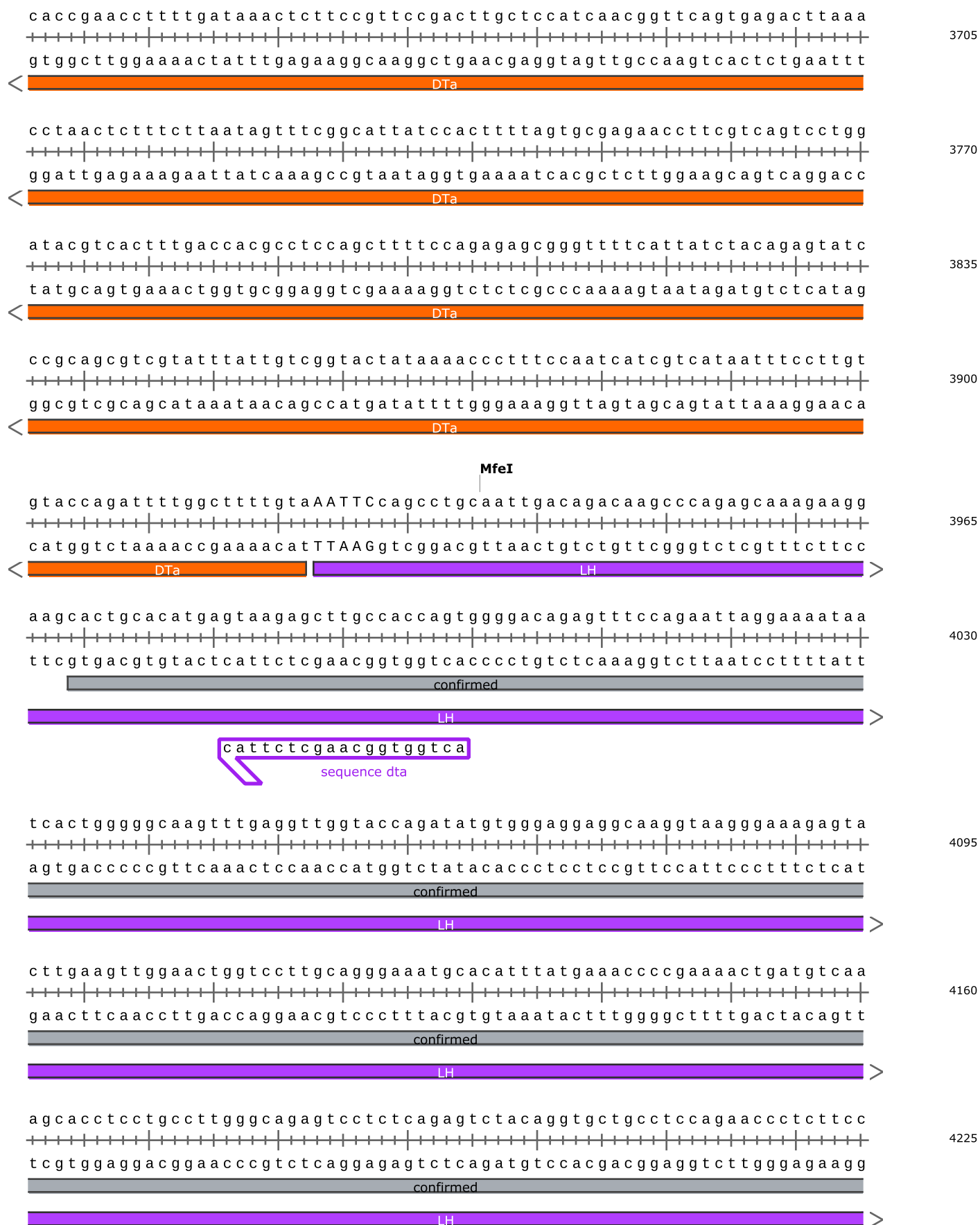












tggagcgcacccctatgtatctagaaattctgctgggaaatatgatggtcagacccttggccacc
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 acctcgcgtagggatacatagatctttaagacgaccctttatactaccagtcctgggaaccggtgg
 confirmed

4290

LH >

tgaaagggttcaggggtggtagaagaaaaaggaaagccacagggcagcaggggcaggtgccagcaag
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 actttccaagtcccaccatcttctttttcctttcggtgtcccgctgctcccggtccacgggtcgttc
 confirmed

4355

LH >

gaaggcaggcacgccaggaagacacccatggtgagaagtgcagatggcccgagggcaagtttgct
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 cttccgtccgtgcggtccttctgtgggtaccactcttcacgtctaccgggctcccggttcaaacga
 confirmed

4420

LH >

caactcaccaggtttgctcttgctggggccaagaggactcatgtgccagggccaagggcccttg
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 gttgagtgggtccaaacgagaacgaccccggttctcctgagtacacggtcccggttcccggaac
 confirmed

4485

LH >

ggggctctcacaggggggttatctgggcttcggttctggagggccaggaacaaacaggcttcaa
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 ccccgagagtgtccccgaatagaccggaagccaagacctcccggtccttgtttgtccgaagttt
 confirmed

4550

LH >

gccaaagggttggtggcacacagggggcttggtccttcacctctgtccctctccctacggaca
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 cggttcccgaaaccgaccgtgtgtccccgaaccaggaagtggagacaggggagagggatgcctgt
 confirmed

4615

LH >

catataagaccctggtcacacctgggagaggaggagaggagagcatagcacctgcagcaagAcgc
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 gtatatcttgggaccagtgtggaccctctcctcctcctcctcgtatcgtggacgtcgttcTgcg
 confirmed

4680

LH >

gtgGTACCATGGTGAGCAAGGGCGAGGAGgtcatcaaagagttcatgcgcttcaagggtgcgcatg
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 caccATGGTACCACTCGTTCCCGCTCCTCcagtagtttctcaagtacgcgaagttccacgcgtac

4745

1 Met Val Ser Lys 5 Gly Glu Glu Val Ile 10 Lys Glu Phe Met Arg 15 Phe Lys Val Arg Met
 confirmed

tdTomato >

g a g g g c t c c a t g a a c g g c c a c g a g t t c g a g a t c g a g g g c g a g g g c g a g g g c g c c c t a c g a g g g
c t c c c g a g g t a c t t g c c g g t g c t c a a g c t c t a g c t c c c g c t c c c g c t c c c g g c g g g g a t g c t c c c
20 25 30 35 40
Glu Gly Ser Met Asn Gly His Glu Phe Glu Ile Glu Gly Glu Gly Arg Pro Tyr Glu Gly
tdTomato

confirmed

c a c c c a g a c c g c c a a g c t g a a g g t g a c c a a g g g c g g c c c c t g c c t t c g c c t g g g a c a t c c t g t
 g t g g g t c t g g c g g t t c g a c t t c c a c t g g t t c c c g c c g g g g a c g g g a a g c g g a c c c t g t a g g a c a
 Thr Gln Thr Ala Lys Leu Lys Val Thr Lys Gly Gly Pro Leu Pro Phe Ala Trp Asp Ile Leu
 tdTomato

confirmed

c c c c c a g t t c a t g t a c g g c t c c a a g g c g t a c g t g a a g c a c c c c g c c g a c a t c c c c g a t t a c a a g
+ + + + + | + + + + + | + + + + + | + + + + + | + + + + + | + + + + + | + + + + + | + + + + + | + + + + + | + + + + +
g g g g g g t c a a g t a c a t g c c g a g g t t c c g c a t g c a c t t c g t g g g g c g g c t g t a g g g g c t a a t g t t c

Ser Pro Gln Phe Met Tyr Gly Ser Lys Ala Tyr Val 75 Lys His Pro Ala Asp Ile Pro Asp Tyr Lys

tdTomato

>

confirmed

aagctgtccttcccgagggttcagtgggagcgcgtgatgaacttcgaggacggcgggtctgggt
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
ttcgacaggaaggggtcccgaagttcacccctcgcgcactacttgaaagctcctgccgccagacca
85 90 95 100 105
Lys Leu Ser Phe Pro Glu Gly Phe Lys Trp Glu Arg Val Met Asn Phe Glu Asp Gly Gly Leu Val

tdTomato

confirmed

[illegible]

confirmed

c c a a c t t c c c c c g a c g g c c c c g t a a t g c a g a a g a a g a c c a t g g g c t g g g a g g c c t c c a c c g a g
+
g g t t g a a g g g g g g g c t g c c g g g g c a t t a c g t c t t c t t c t g g t a c c g a c c c t c c g g a g g t g g c t c

130 135 140 145

Thr Asn Phe Pro Pro Asp Gly Pro Val Met Gln Lys Lys Thr Met Gly Trp Glu Ala Ser Thr Glu >

tdTomato

confirmed

c g c t t g t a c c c c g c g a c g g c g t g c t g a a g g g c g a g a t c c a c c a g g c c t g a a g c t g a a g g a c g g
+ + + + +
g c g g a c a t g g g g g c g c t g c c g c a c g a c t t c c c g c t c t a g g t g g t c c g g g a c t t c g a c t t c c t g c c

150 155 160 165 170
Arg Leu Tyr Pro Arg Asp Gly Val Leu Lys Gly Glu Ile His Gln Ala Leu Lys Leu Lys Asp Gly

tdTomato >

5200

confirmed

cggccactacctggtggagttcaagaccatctacatggccaagaagcccggtgcaactgcccggct
5265
gccggtgatggaccacctcaagttctggtagatgtaccggttcttcgggcacgttgacgggccga

175 180 185 190
Gly His Tyr Leu Val Glu Phe Lys Thr Ile Tyr Met Ala Lys Lys Pro Val Gln Leu Pro Gly
tdTomato
confirmed

actactacgtggacaccaagctggacatcacctcccacaacgaggactacaccatcgtggaacag
5330
tgatgatgcacctgtggttcgacctgtagtggagggtgttgctcctgatgtggtagcaccttgtc

195 200 205 210
Tyr Tyr Tyr Val Asp Thr Lys Leu Asp Ile Thr Ser His Asn Glu Asp Tyr Thr Ile Val Glu Gln
tdTomato
confirmed confirmed

tacgagcgctccgagggccgccaccacctgttcctggggcatggcaccggcagcaccggcagcgg
5395
atgctcgcgaggctcccggcggtggtggacaaggaccccggtaccgtggcgcgtcgtggccgctcgcc

215 220 225 230 235
Tyr Glu Arg Ser Glu Gly Arg His His Leu Phe Leu Gly His Gly Thr Gly Ser Thr Gly Ser Gly
tdTomato
confirmed

cagctccggcaccgcctcctccgaggacaacaacatggccggtcatcaaagagttcatgcgcttca
5460
gtcgaggccgtggcggaggaggctcctgttgttgtaaccggcagtagtttctcaagtacgcgaagt

240 245 250 255
Ser Ser Gly Thr Ala Ser Ser Glu Asp Asn Asn Met Ala Val Ile Lys Glu Phe Met Arg Phe
tdTomato
confirmed

agggtgcgatggagggctccatgaacggccacgagttcgagatcgagggcgagggcgagggccgc
5525
tccacgcgtacctcccagggtacttgccggtgctcaagctctagctcccgcgtcccggcg

260 265 270 275
Lys Val Arg Met Glu Gly Ser Met Asn Gly His Glu Phe Glu Ile Glu Gly Glu Gly Arg
tdTomato
confirmed

ccctacgagggcaccagaccgccaagctgaaggtgaccaaggcgcccccctgccttgcctg
5590
gggatgctcccgtgggtctggcgggttcgacttccactggttcccgcgggggacgggaagcggac

280 285 290 295 300
Pro Tyr Glu Gly Thr Gln Thr Ala Lys Leu Lys Val Thr Lys Gly Gly Pro Leu Pro Phe Ala Trp
tdTomato
confirmed

ggacatcctgtccccccagttcatgtacggctccaaggcgtacgtgaagcaccgccgacatcc
5655
cctgtaggacaggggggtcaagtacatgccgaggttccgcatgcacttcgtggggcggcgtgtagg

305 310 315 320
Asp Ile Leu Ser Pro Gln Phe Met Tyr Gly Ser Lys Ala Tyr Val Lys His Pro Ala Asp Ile
tdTomato
confirmed

ccgattacaagaagctgtccttccccgagggcttcaagtgggagcgcgtgatgaacttcgaggac
 5720
 ggctaattgttcttcgacaggaaggggctcccgaagttcacccctcgcgcactacttgaagctcctg
 325 330 335 340
 Pro Asp Tyr Lys Lys Leu Ser Phe Pro Glu Gly Phe Lys Trp Glu Arg Val Met Asn Phe Glu Asp

tdTomato
confirmed

ggcgggtctgggtgaccgtgaccaggactcctccctgcaggacggcacgctgatctacaaggtgaa
 5785
 ccgccagaccactggcactgggtcctgaggaggacgtcctgcctgctgcgactagatgttccactt
 345 350 355 360 365
 Gly Gly Leu Val Thr Val Thr Gln Asp Ser Ser Leu Gln Asp Gly Thr Leu Ile Tyr Lys Val Lys

tdTomato
confirmed

gatgcgcggcaccaacttccccccgacggcccgtaatgcagaagaagaccatgggctgggagg
 5850
 ctacgcgcctgtggttgaaggggggctgccggggcattacgtcttcttcttggtacccgacctcc
 370 375 380 385
 Met Arg Gly Thr Asn Phe Pro Pro Asp Gly Pro Val Met Gln Lys Lys Thr Met Gly Trp Glu

tdTomato
confirmed

cctccaccgagcgcctgtacccccgcgacggcgtgctgaagggcgagatccaccaggccctgaag
 5915
 ggagggtggctcgcggacatggggcgctgcccgcacgacttcccgctctagggtgggtccgggacttc
 390 395 400 405
 Ala Ser Thr Glu Arg Leu Tyr Pro Arg Asp Gly Val Leu Lys Gly Glu Ile His Gln Ala Leu Lys

tdTomato
confirmed

ctgaaggacggcggccactacctgggtggagttcaagaccatctacatggccaagaagcccgtgca
 5980
 gacttccctgccgcgggtgatggaccacctcaagttcttggtagatgtaccggttcttcgggcacgt
 410 415 420 425 430
 Leu Lys Asp Gly Gly His Tyr Leu Val Glu Phe Lys Thr Ile Tyr Met Ala Lys Lys Pro Val Gln

tdTomato
confirmed

actgcccggctactactacgtggacaccaagctggacatcacctcccacaacgaggactacacca
 6045
 tgacgggccgatgatgatgcacctgtggttcgacctgtagtggagggtgttgctcctgatgtggt
 435 440 445 450
 Leu Pro Gly Tyr Tyr Tyr Val Asp Thr Lys Leu Asp Ile Thr Ser His Asn Glu Asp Tyr Thr

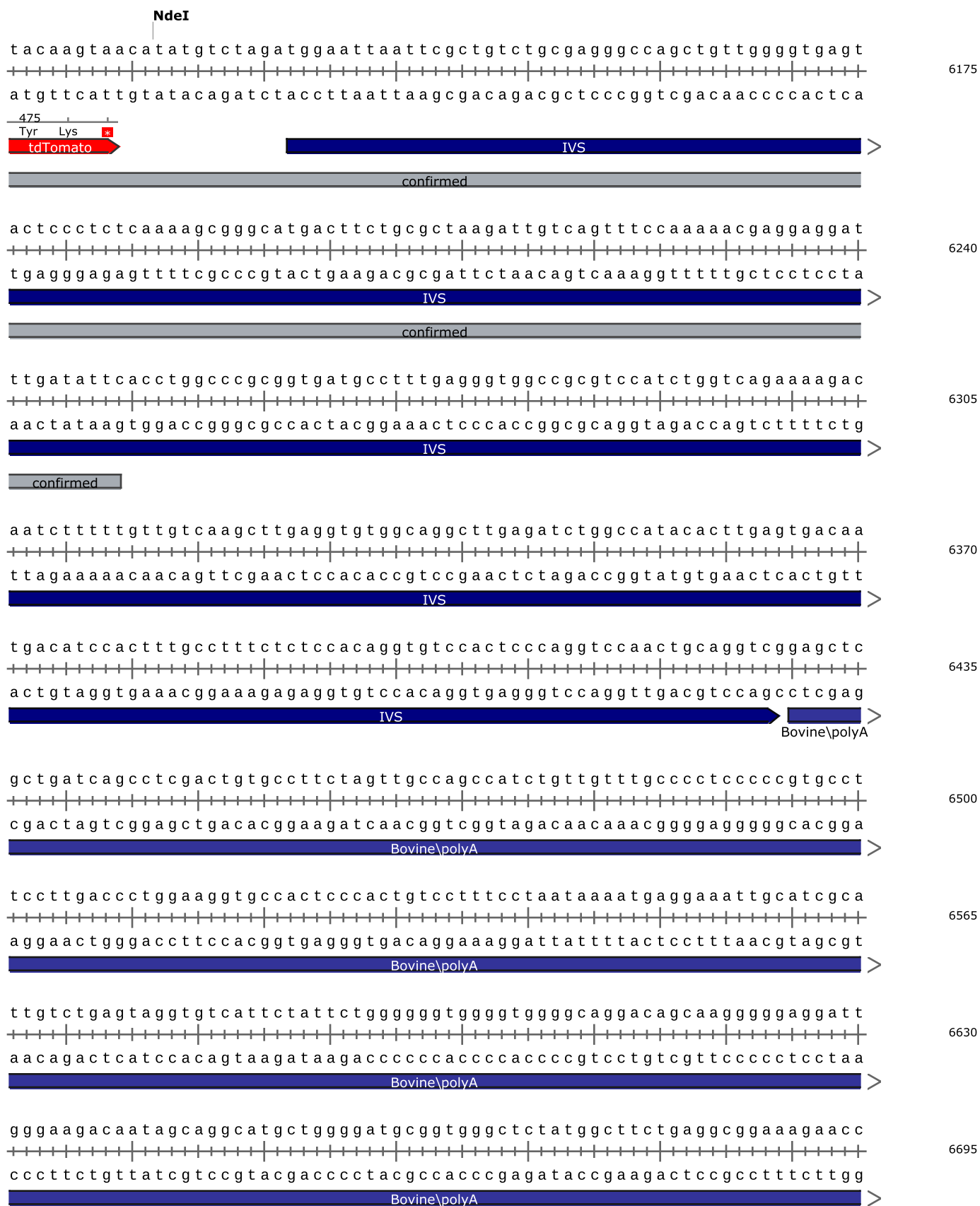
tdTomato
confirmed

tcgtggaacagtacgagcgcctccgagggccgccaccacctgttctctgtacggcatggacgagctg
 6110
 agcaccttgatcatgctcgcgaggctcccggcggtggtggacaaggacatgcccgtacctgctcgac
 455 460 465 470
 Ile Val Glu Gln Tyr Glu Arg Ser Glu Gly Arg His Leu Phe Leu Tyr Gly Met Asp Glu Leu

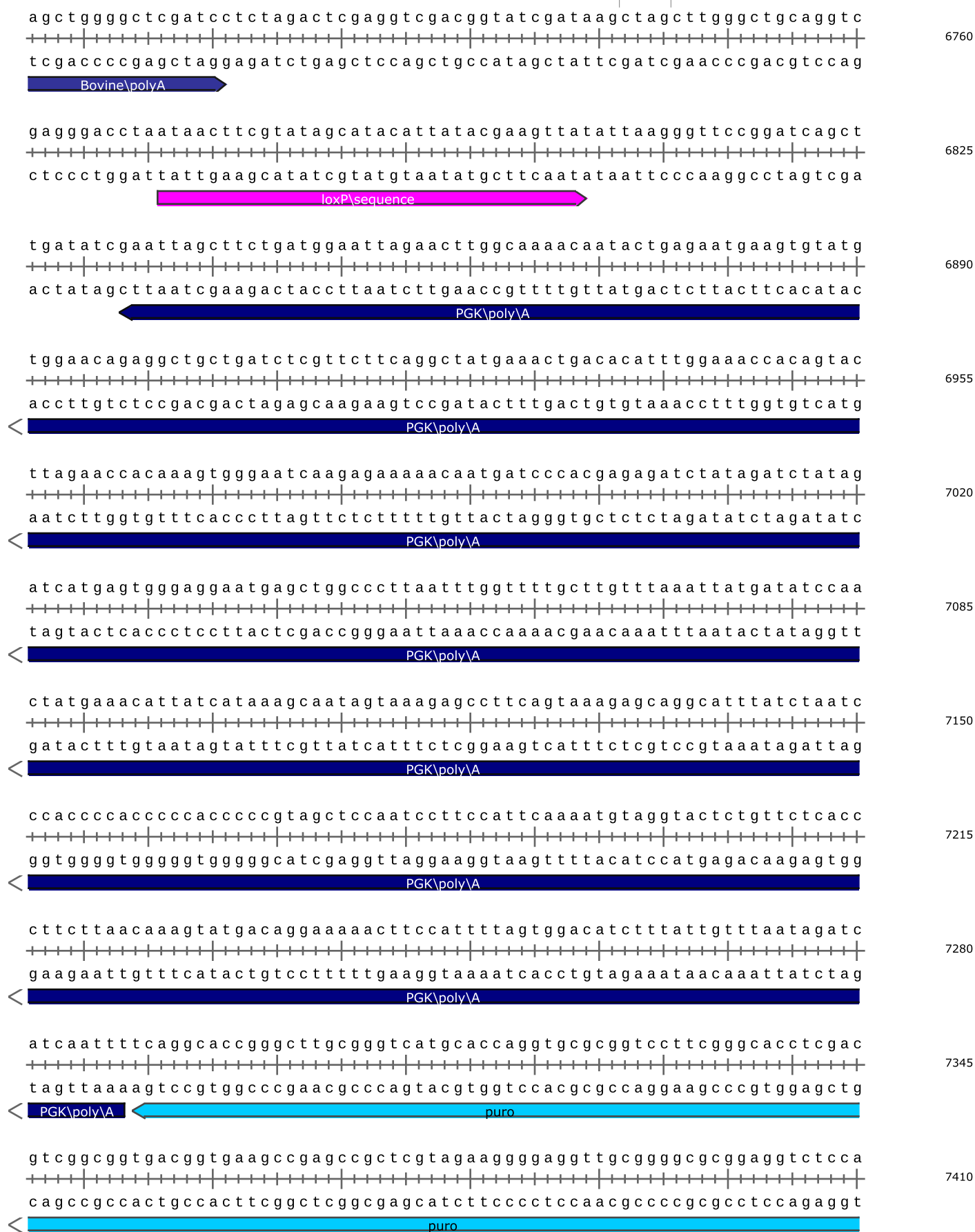
tdTomato
confirmed

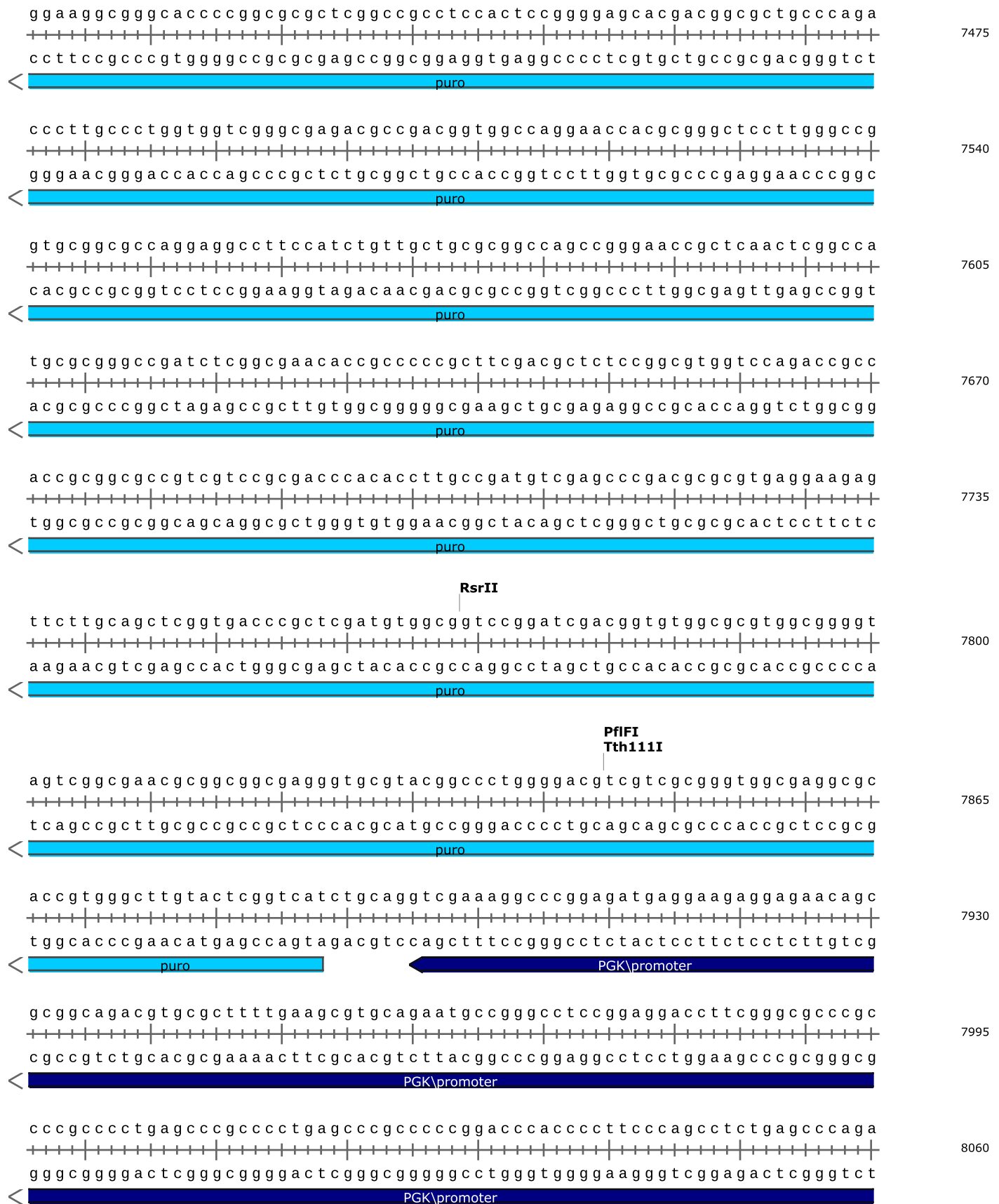
BsrGI

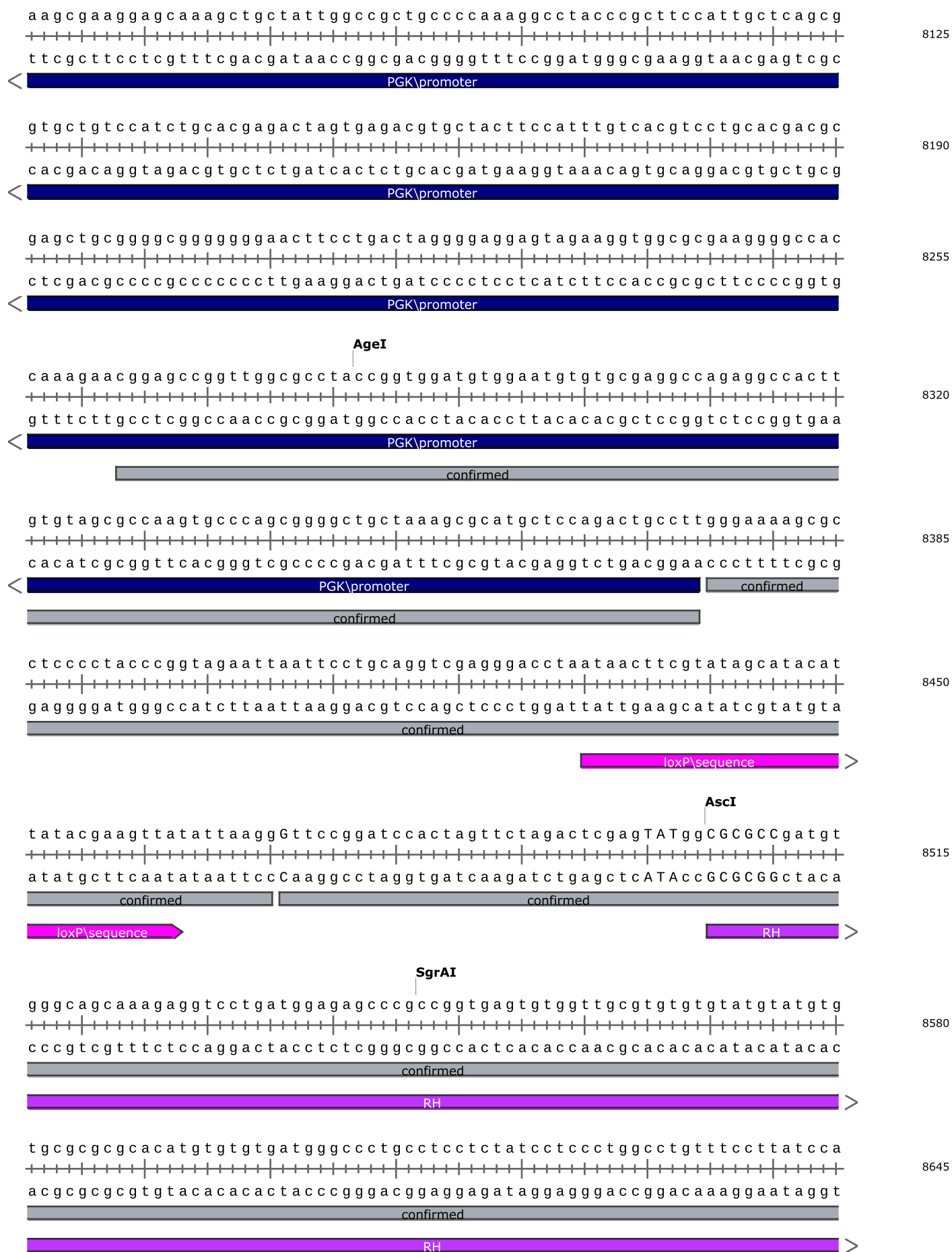
confirmed

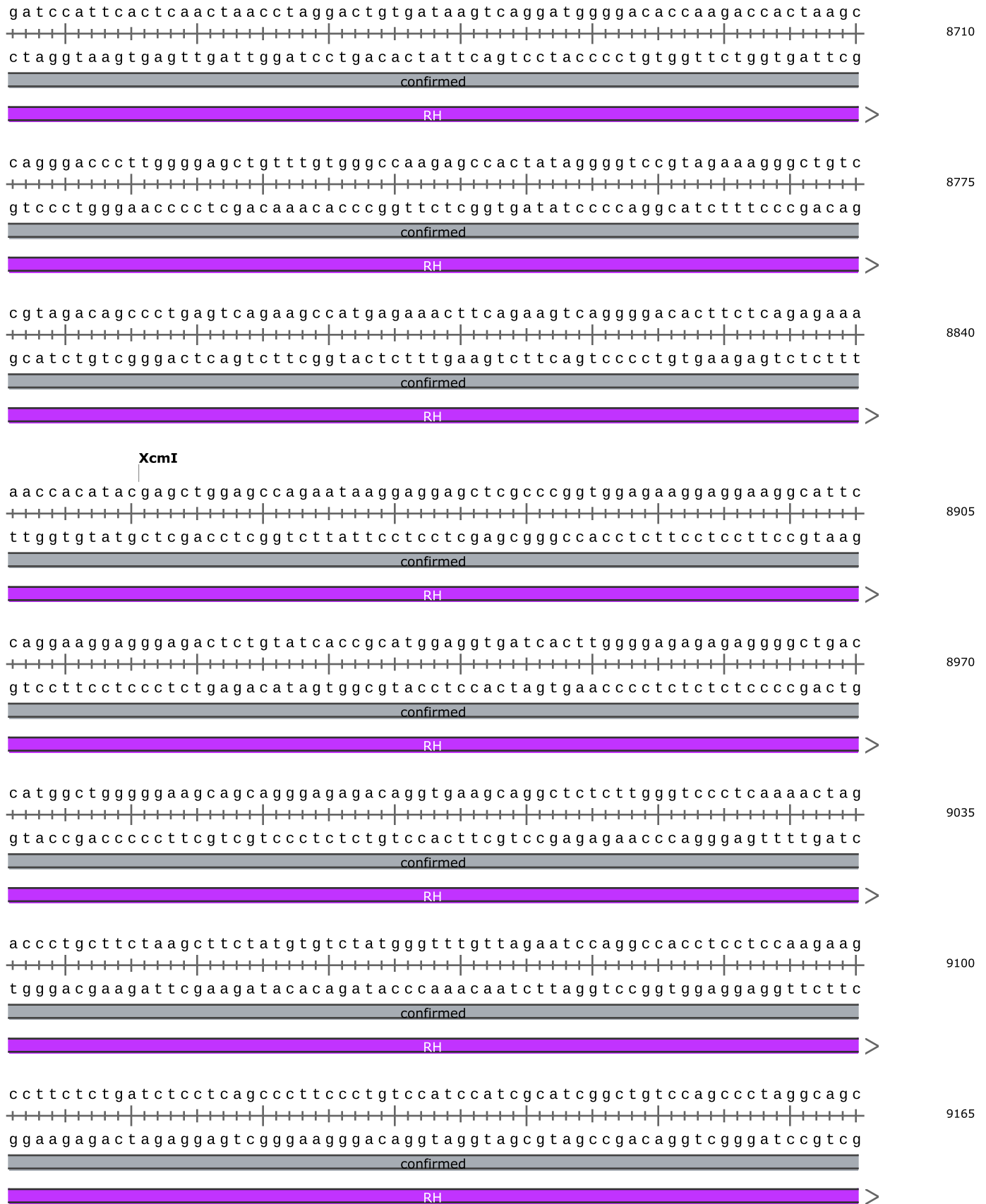


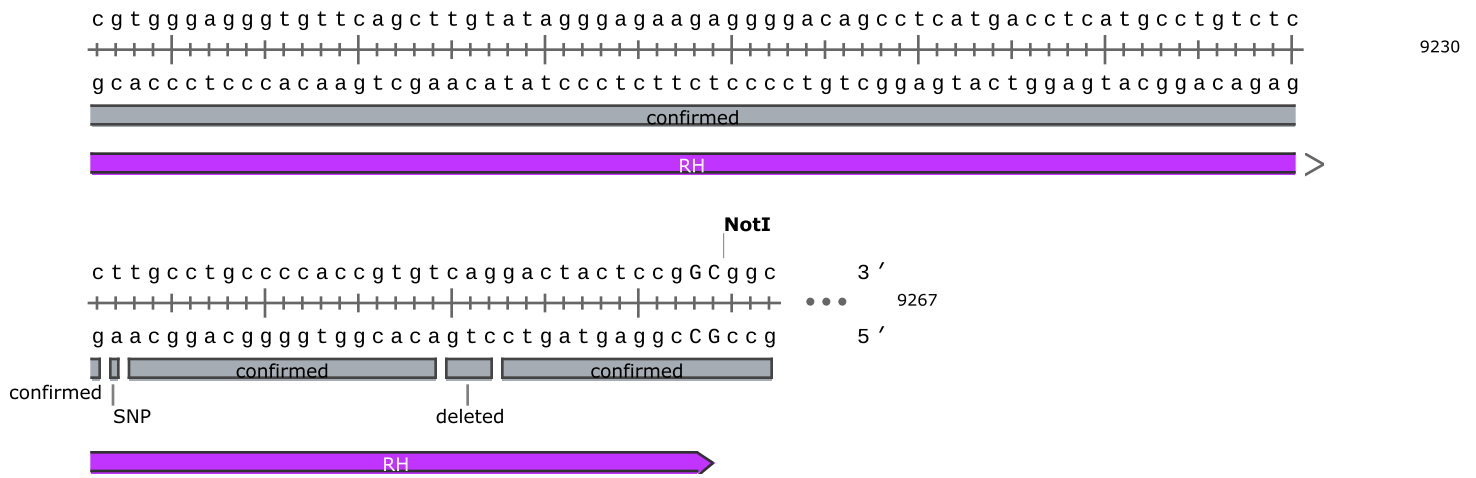
NheI BmtI











| Enzymes | Sites | |
|----------------|-------|------|
| AgeI | 1 | 8281 |
| AscI | 1 | 8504 |
| BmtI | 1 | 6745 |
| BspQI | 1 | 296 |
| BsrGI | 1 | 6109 |
| EcoNI | 1 | 4640 |
| MfeI | 1 | 3935 |
| MluI | 1 | 4677 |
| NaeI | 1 | 2351 |
| NdeI | 1 | 6121 |
| NgoMIV | 1 | 2349 |
| NheI | 1 | 6741 |
| NotI | 1 | 9264 |
| PfiFI | 1 | 7844 |
| PsiI | 1 | 2582 |
| RsrII | 1 | 7768 |
| SapI | 1 | 296 |
| SgrAI | 1 | 8546 |
| Tth111I | 1 | 7844 |
| XcmI | 1 | 8850 |

| Feature | Location | Size (bp) |  |  | Type |
|---------------------------|---|-----------|---|---|--------------|
| ✓ T3 | 30 .. 49 | 20 |  |  | primer_bind |
| /note | = /ApEinfo_label=T3 - /ApEinfo_fwdcolor=cyan - /ApEinfo_revcolor=green - /vntifkey="28" - /label=T3 - complement(4528..4547) | | | | |
| M13-rev | 67 .. 87 | 21 |  |  | misc_feature |
| /note | = /vntifkey="21" - /label=M13-rev - complement(4565..4585) | | | | |
| ✓ M13-rev | 67 .. 87 | 21 |  |  | primer_bind |
| /note | = /ApEinfo_label=M13-rev - /ApEinfo_fwdcolor=cyan - /ApEinfo_revcolor=green - /vntifkey="28" - /label=M13-rev - complement(4565..4585) | | | | |
| ✓ LacO | 93 .. 115 | 23 |  |  | misc_binding |
| /note | = /ApEinfo_label=LacO - /ApEinfo_fwdcolor=cornflower blue - /ApEinfo_revcolor=cornflower blue - /vntifkey="20" - /label=LacO - complement(4591..4613) | | | | |
| ✓ ColE1_Origin | 455 .. 1137 | 683 |  |  | rep_origin |
| /direction | = LEFT | | | | |
| /note | = /ApEinfo_label=ColE1 origin - /ApEinfo_fwdcolor=gray50 - /ApEinfo_revcolor=gray50 - /vntifkey="33" - /label=ColE1_Origin - complement(4953..5635) | | | | |
| ✓ Amp^R | 1235 .. 1894 | 660 |  |  | CDS |
| /note | = /ApEinfo_label=AmpR - /ApEinfo_fwdcolor=yellow - /ApEinfo_revcolor=yellow - /vntifkey="4" - /label=Amp ^R - complement(5733..6413) | | | | |
| ✓ F1_Origin | 2229 .. 2669 | 441 |  |  | rep_origin |
| /direction | = LEFT | | | | |
| /note | = /ApEinfo_label=F1 ori - /ApEinfo_fwdcolor=gray50 - /ApEinfo_revcolor=gray50 - /vntifkey="33" - /label=F1_Origin - complement(6727..7167) | | | | |
| ✓ lacZ^α | 2680 .. 2748 | 69 |  |  | CDS |
| /note | = /ApEinfo_label=LacZ alpha - /ApEinfo_fwdcolor=cornflower blue - /ApEinfo_revcolor=cornflower blue - /vntifkey="4" - /label=lacZ ^α - complement(7178..7246) | | | | |
| M13-fwd | 2819 .. 2836 | 18 |  |  | misc_feature |
| /note | = /vntifkey="21" - /label=M13-fwd - 7317..7334 | | | | |
| M13-fwd | 2819 .. 2836 | 18 |  |  | primer_bind |
| /note | = /ApEinfo_label=M13-fwd - /ApEinfo_fwdcolor=cyan - /ApEinfo_revcolor=green - /vntifkey="28" - /label=M13-fwd - 7317..7334 | | | | |
| ✓ T7 | 2835 .. 2862 | 28 |  |  | primer_bind |
| /note | = /ApEinfo_label=T7 - /ApEinfo_fwdcolor=cyan - /ApEinfo_revcolor=green - /vntifkey="28" - /label=T7 - 7333..7360 | | | | |
| ✓ PGK\PolyA | 2931 .. 3351 | 421 |  |  | polyA_signal |
| /note | = /ApEinfo_label=PGK pA - /ApEinfo_fwdcolor=#12f0ff - /ApEinfo_revcolor=#026f7e - /vntifkey="25" - /label=PGK\PolyA - complement(7429..7849) | | | | |

| Feature | Location | Size (bp) |  |  | Type |
|---------------------------------------|---|-----------|---|---|--------------|
| ✓ DTa | 3436 .. 3922 | 487 |  |  | CDS |
| /note | = /vntifkey="4" - /label=DTa - /note="Diphtheria Toxin Gene" - complement(7934..8526) | | | | |
| gene | 3436 .. 3922 | 487 |  |  | gene |
| /note | = /ApEinfo_label=DTa - /ApEinfo_fwdcolor=#dcd1dd - /ApEinfo_revcolor=#817e7f - /vntifkey="60" - complement(7934..8526) | | | | |
| ✓ LH | 3923 .. 4677 | 755 |  |  | misc_feature |
| ✓ confirmed | 3969 .. 5322 | 1354 |  |  | misc_feature |
| ✓ tdTomato | 4689 .. 6119 | 1431 |  |  | CDS |
| /product | = tandem dimeric (pseudo-monomeric) derivative of DsRed (Shaner et al., 2004) | | | | |
| /note | = mammalian codon-optimized | | | | |
| /translation | = MVSKEEVIKEFMRFKVRMEGSMNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKKLSFPEGFKWERVMI
LVTVTQDSSLQDGTLYKVKMRGTNFPDPGPMQKKTMGWEASTERLYPRDGLVKGEIHQALKLKDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDIT
TIVEQYERSEGRHHLFLGHGTGSTGSGSSGTASSEDNNMAVIKEFMRFKVRMEGSMNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQ
AYVKHPADIPDYKKLSFPEGFKWERVMNFEDGGLVTVTQDSSLQDGTLYKVKMRGTNFPDPGPMQKKTMGWEASTERLYPRDGLVKGEIHQALKLK
VEFKTIYMAKKPVQLPGYYYVDTKLDITSHNEDYTIVEQYERSEGRHHLFLYGMDELYK* | | | | |
| | 476 amino acids = 54.2 kDa | | | | |
| ✓ confirmed | 5323 .. 6108 | 786 |  |  | misc_feature |
| ✓ confirmed | 6109 .. 6249 | 141 |  |  | misc_feature |
| ✓ IVS | 6132 .. 6429 | 298 |  |  | misc_feature |
| /note | = /ApEinfo_label=IVS - /ApEinfo_fwdcolor=#ff2ddc - /ApEinfo_revcolor=#b3387e - /vntifkey="21" - /label=IVS - 2024..2321 | | | | |
| ✓ Bovine\polyA | 6430 .. 6711 | 282 |  |  | polyA_site |
| /note | = /ApEinfo_label=Bovine pA - /ApEinfo_fwdcolor=#3ff9ff - /ApEinfo_revcolor=#348180 - /vntifkey="26" - /label=Bovine\polyA - 2321..2540 | | | | |
| BpA\min | 6442 .. 6648 | 207 |  |  | polyA_signal |
| /note | = /ApEinfo_label=BpA min - /ApEinfo_fwdcolor=cyan - /ApEinfo_revcolor=#0cb0ab - /vntifkey="25" - /label=BpA\min - 2334..2540 | | | | |
| ✓ loxP\sequence | 6771 .. 6804 | 34 |  |  | misc_recomb |
| /note | = /ApEinfo_label=LoxP - /ApEinfo_fwdcolor=#f94fcb - /ApEinfo_revcolor=#c700bd - /vntifkey="86" - /label=loxP\sequence - 2663..2725 | | | | |
| ✓ PGK\polyA | 6833 .. 7288 | 456 |  |  | polyA_signal |
| /note | = /ApEinfo_label=PGK pA - /ApEinfo_fwdcolor=#12f0ff - /ApEinfo_revcolor=#026f7e - /vntifkey="25" - /label=PGK\polyA - complement(2725..3180) | | | | |
| Puromycin resistance gene_Puro | 7289 .. 7888 | 600 |  |  | CDS |
| /note | = /vntifkey="4" - /label=Puro - /note="Puromycin resistance gene" - complement(3181..3780) | | | | |
| ✓ puro | 7289 .. 7888 | 600 |  |  | gene |
| /note | = /ApEinfo_label=Puro - /ApEinfo_fwdcolor=#b8b5b3 - /ApEinfo_revcolor=#686363 - /vntifkey="60" - complement(3181..3780) | | | | |

| Feature | Location | Size (bp) |  |  | Type |
|---|--------------|-----------|---|---|--------------|
| ✓ PGK\promoter | 7895 .. 8374 | 480 |  |  | promoter |
| /note = /ApEinfo_label=PGK Prom - /ApEinfo_fwdcolor=#ffce10 - /ApEinfo_revcolor=#b79000 - /vntifkey="29" - /label=PGK\promoter - complement(3787..4293) | | | | | |
| ✓ confirmed | 8263 .. 8374 | 112 |  |  | misc_feature |
| ✓ confirmed | 8375 .. 8470 | 96 |  |  | misc_feature |
| ✓ loxP\sequence | 8430 .. 8463 | 34 |  |  | misc_recomb |
| /note = /ApEinfo_label=LoxP - /ApEinfo_fwdcolor=#f94fcb - /ApEinfo_revcolor=#c700bd - /vntifkey="86" - /label=loxP\sequence - 4386..4 | | | | | |
| ✓ confirmed | 8471 .. 9231 | 761 |  |  | misc_feature |
| ✓ RH | 8505 .. 9264 | 760 |  |  | misc_feature |
| ✓ SNP | 9232 .. 9232 | 1 |  |  | misc_feature |
| ✓ confirmed | 9233 .. 9249 | 17 |  |  | misc_feature |
| ✓ deleted | 9250 .. 9252 | 3 |  |  | misc_feature |
| ✓ confirmed | 9253 .. 9267 | 15 |  |  | misc_feature |

Description: We generated this donor vector (p1303 DV-SFTPC-tdTomato) containing the tdTomato coding sequence and a floxed PGK promoter-driven puromycin resistance cassette, flanked by the left and right arms of homology to the human endogenous SFTPC locus, as follows: we first modified the CReM's targeting vector, TVGIP-eGFP-puro (generous gift of Gustavo Mostoslavsky and Cesar Sommer, CReM of Boston University and Boston Medical Center; <http://www.bumc.bu.edu/stemcells>). The GIP-eGFP sequence was replaced with the tdTomato coding sequence. 5' and 3' arms of homology to the SFTPC locus were generated by PCR cloning using gDNA extracts of human ES cells (RUES2) as templates. The 5' arm of homology extends 750 base pairs upstream of the SFTPC ATG start site, and the 3' arm of homology extends 750 base pairs down stream of the ATG start site.

Created: Sep 30, 2014

Last Modified: Sep 19, 2017

Accession Number:

Code Number:

Sequence Author: Trial User

DNA Type: Synthetic DNA

Laboratory Host Organism: Homo sapiens

Bacterial Transformation Strain: DH5α™
Dam⁺ Dcm⁺ EcoKI⁺

Comments:

References: