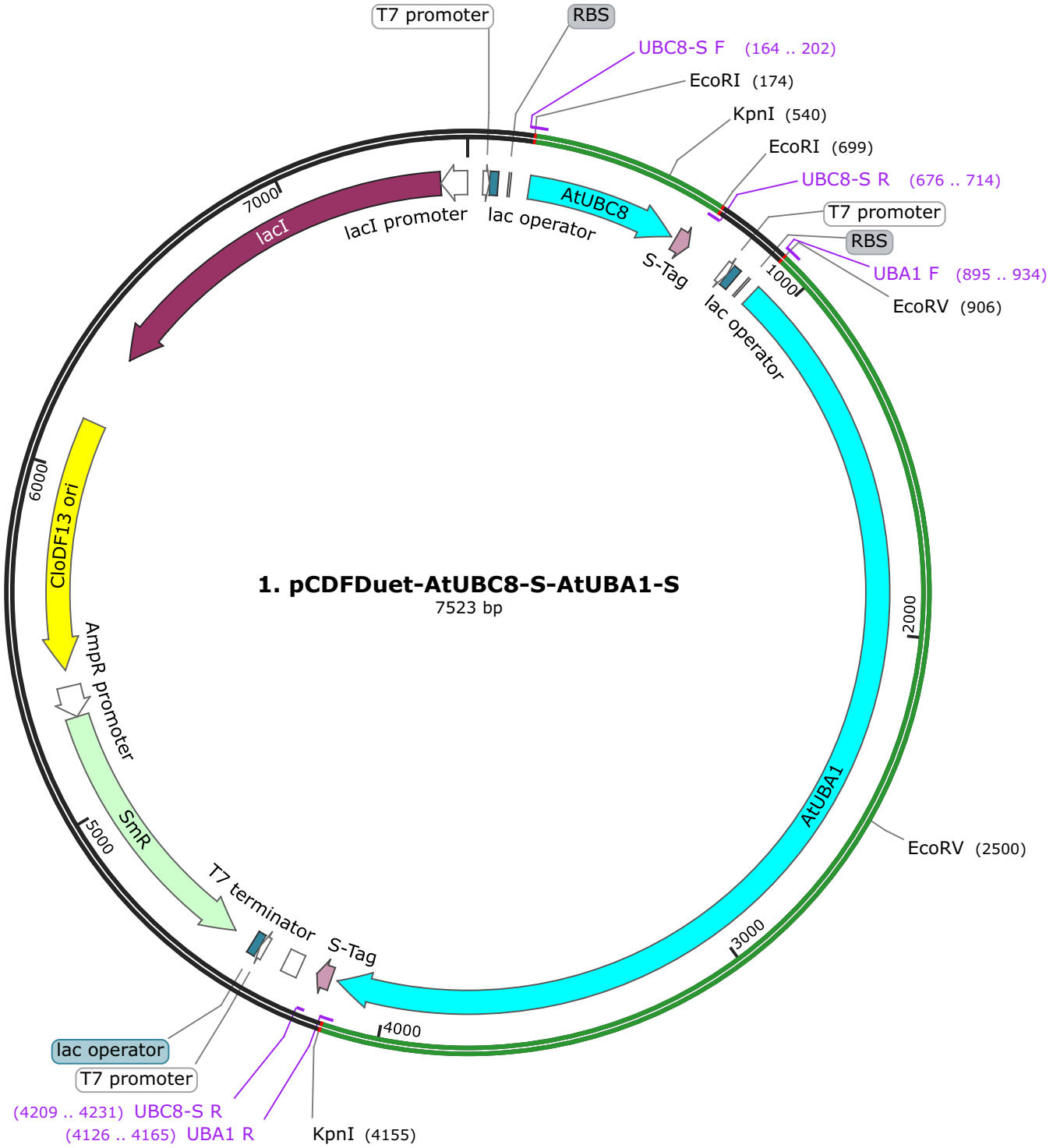
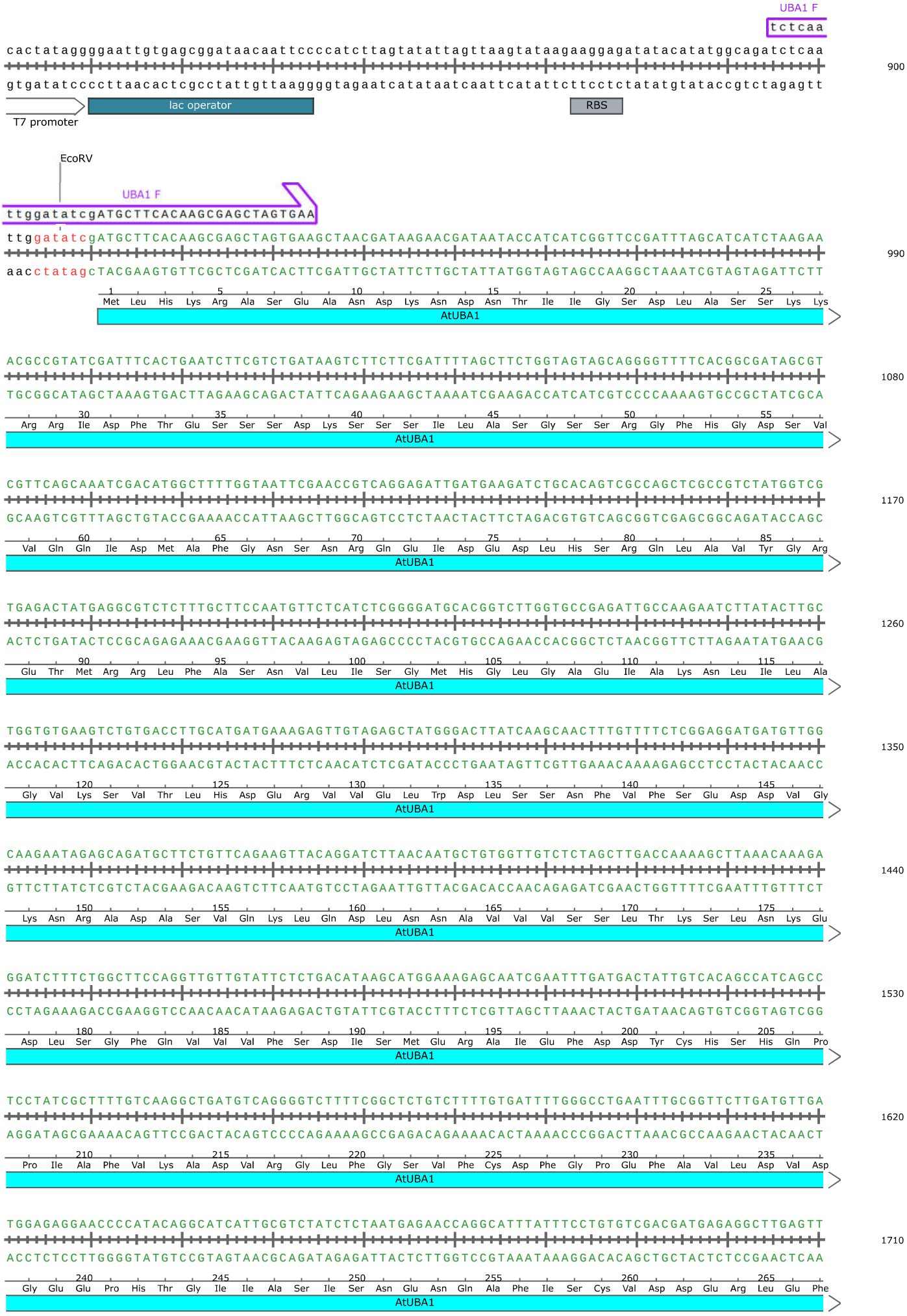
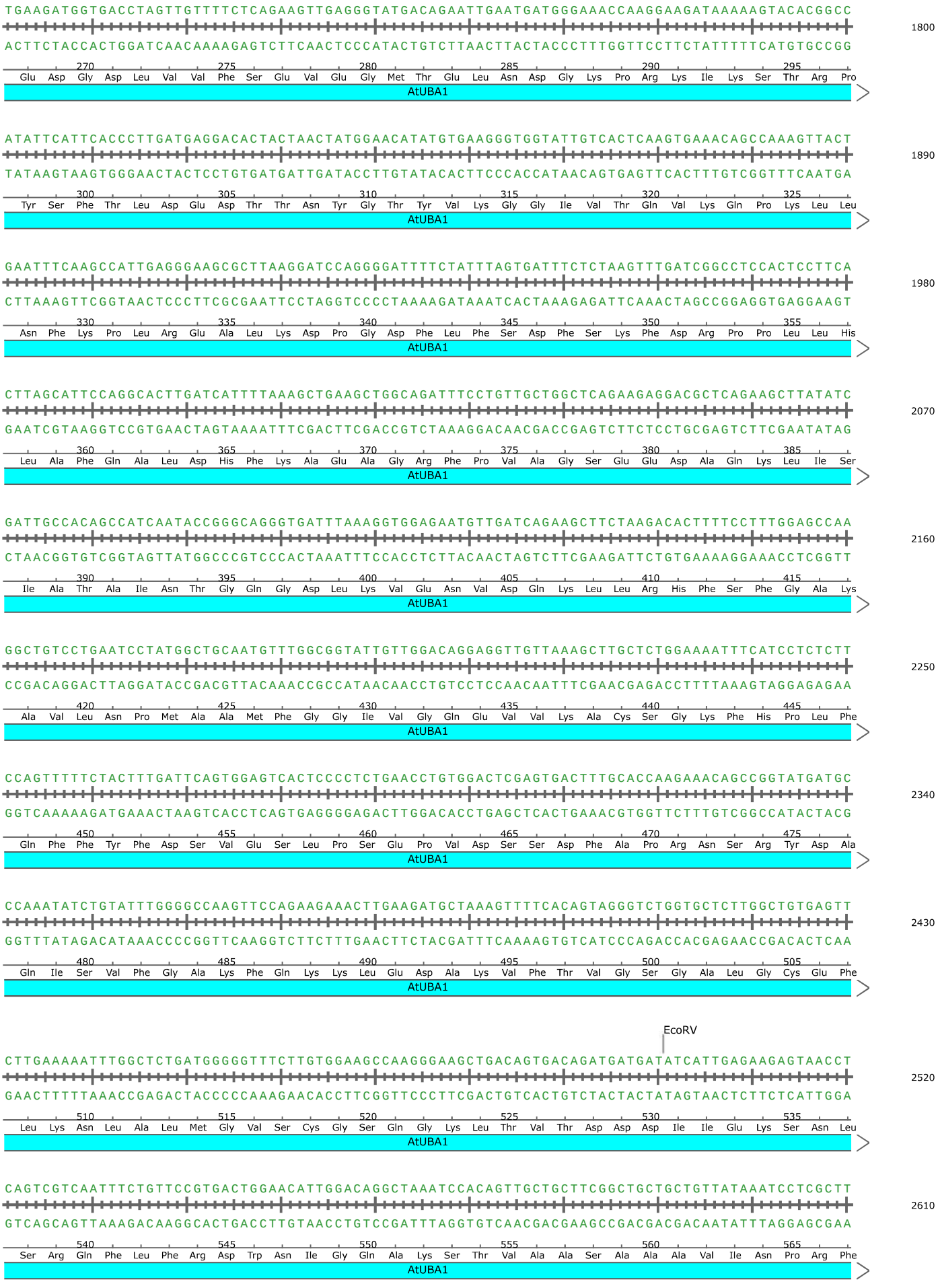
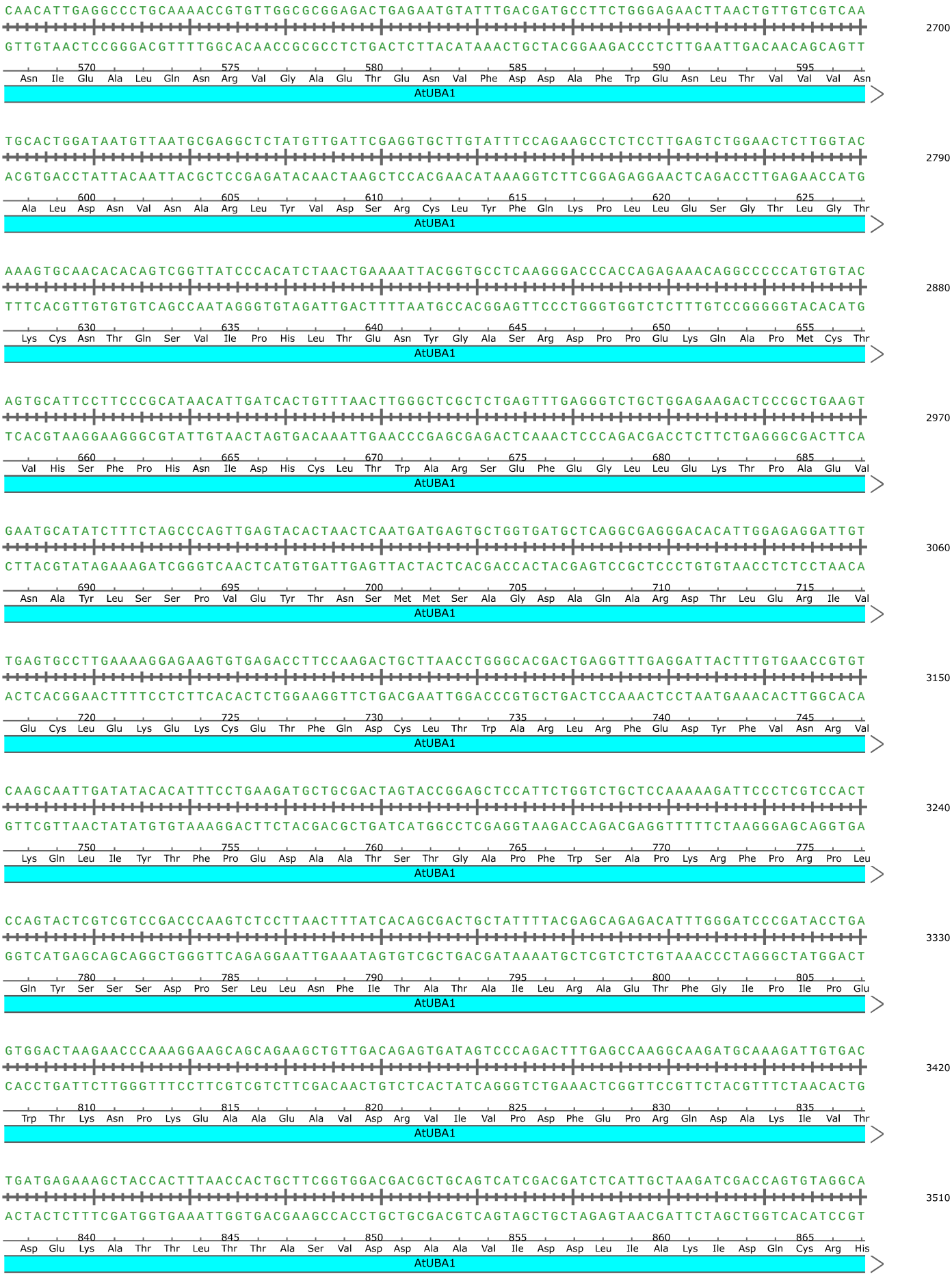
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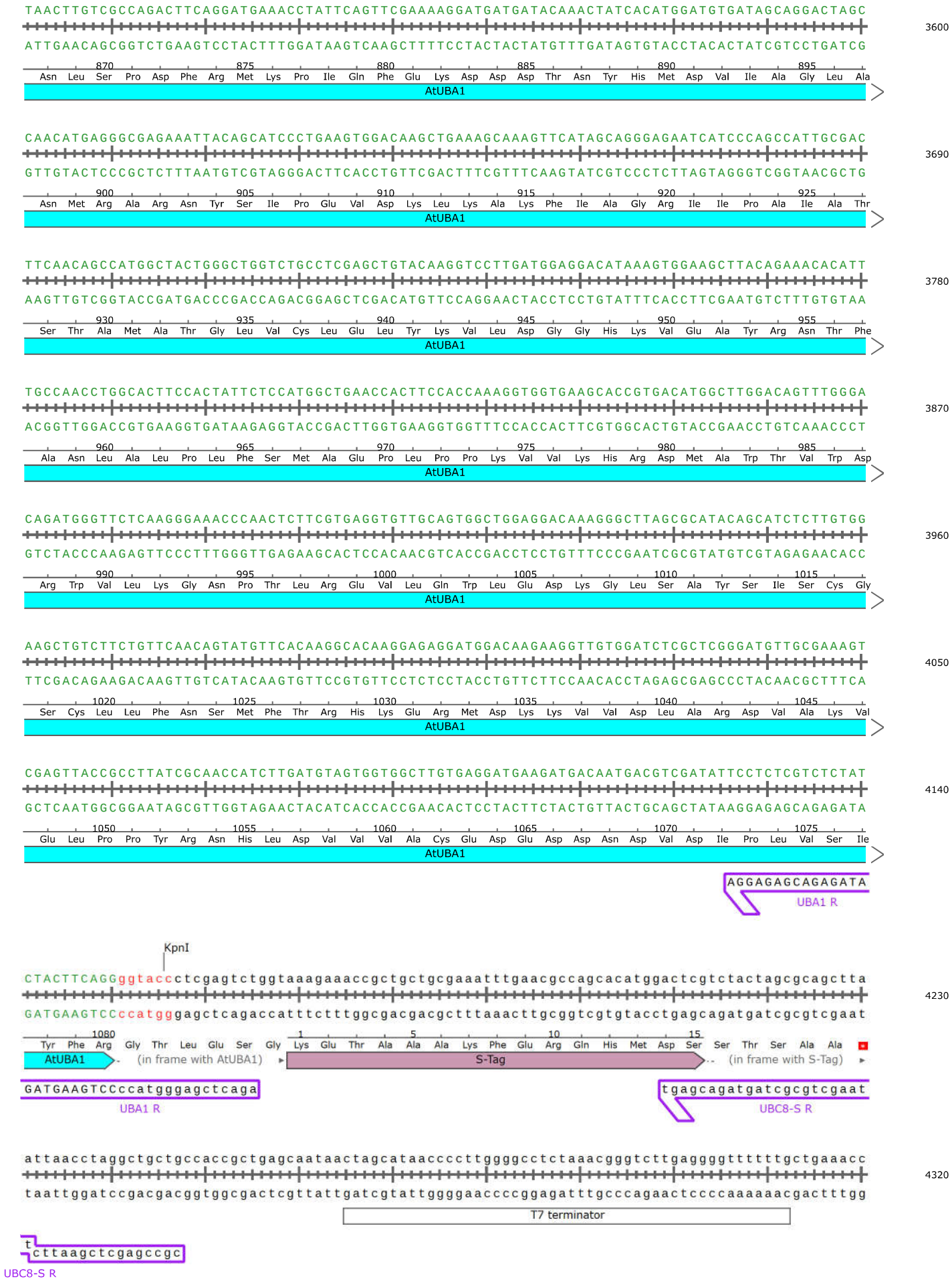


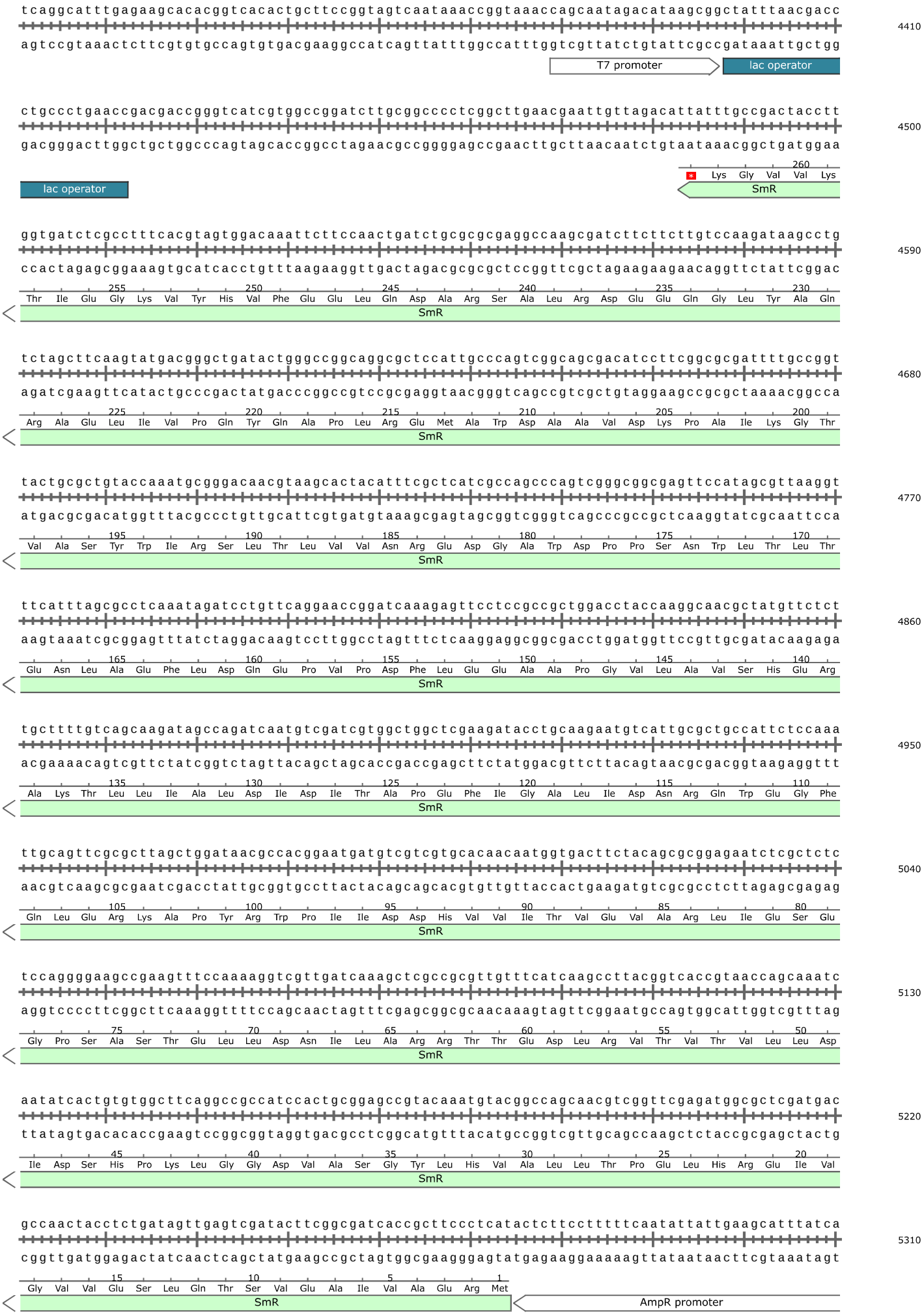

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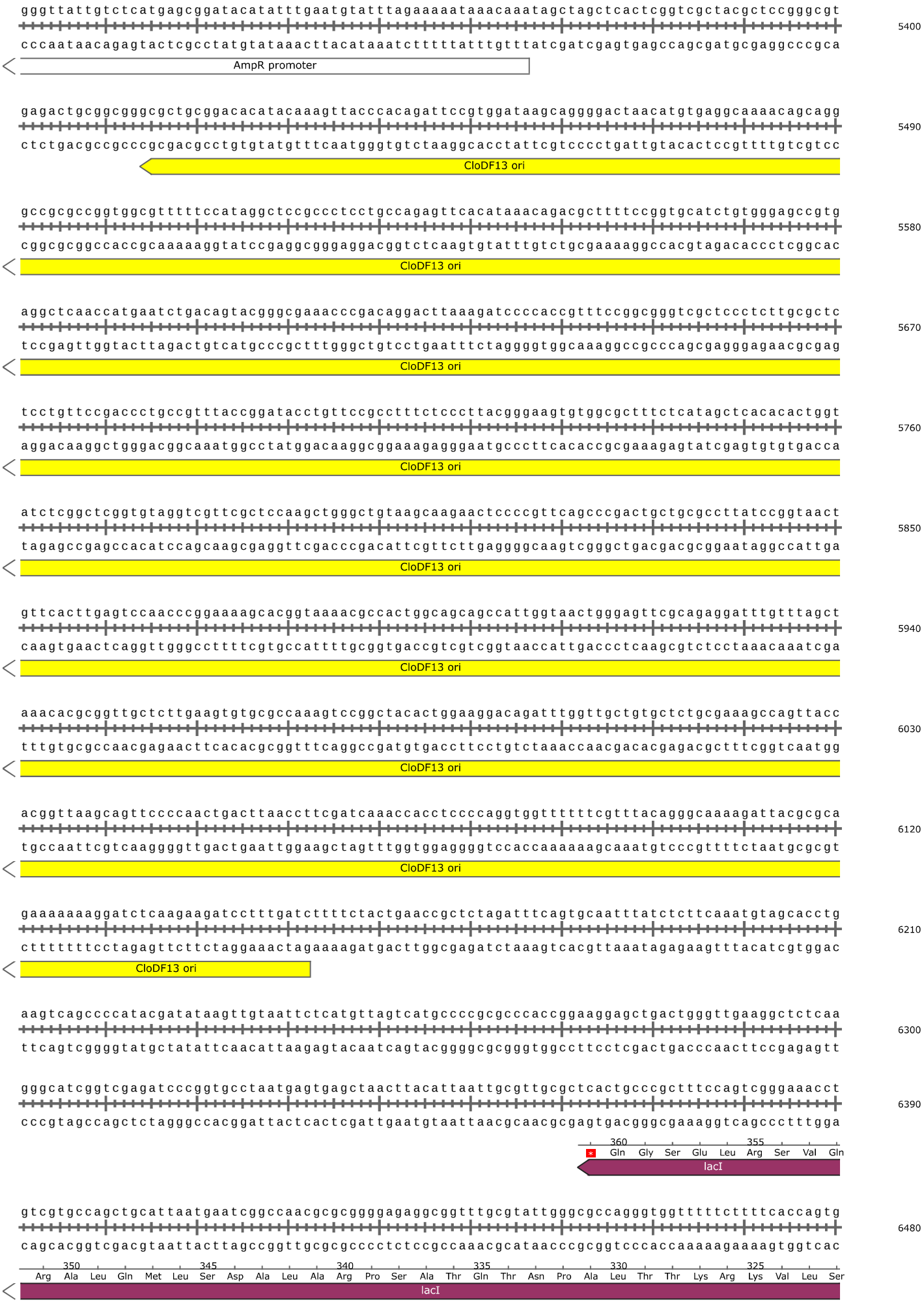

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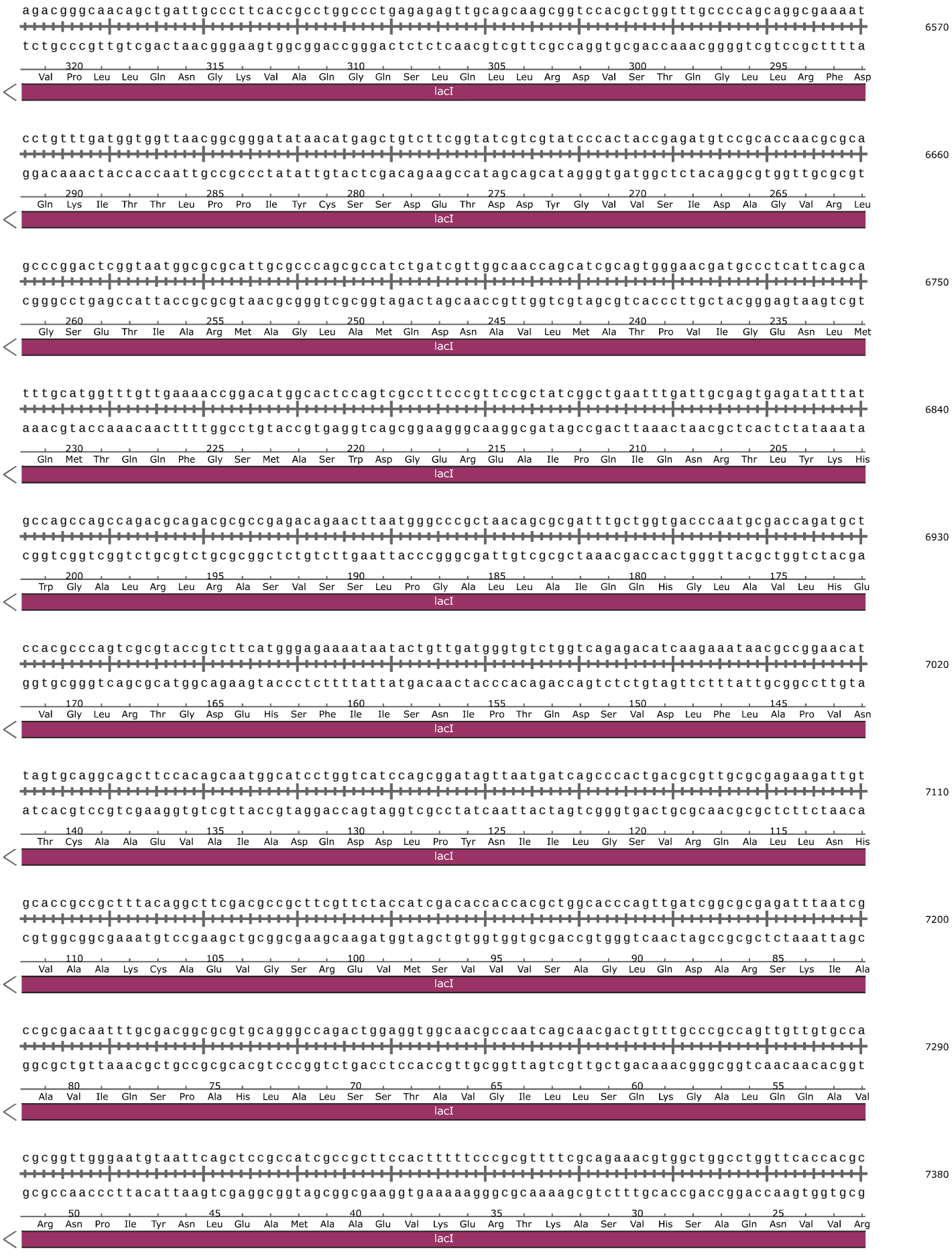

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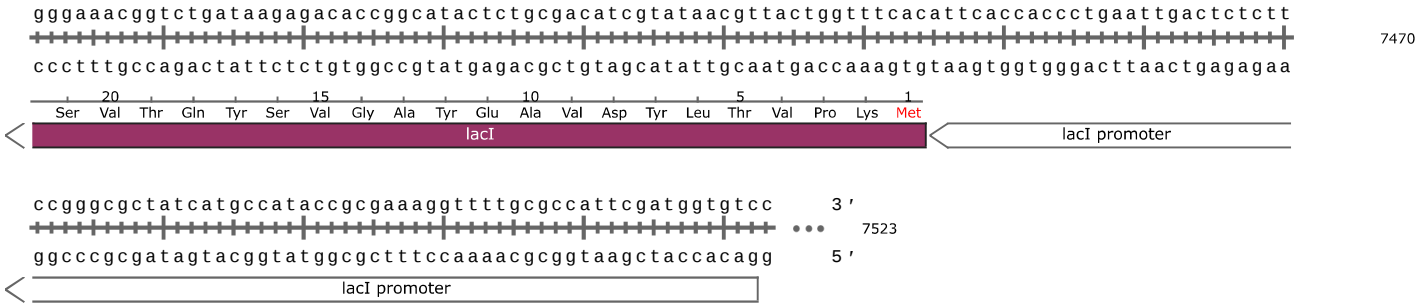

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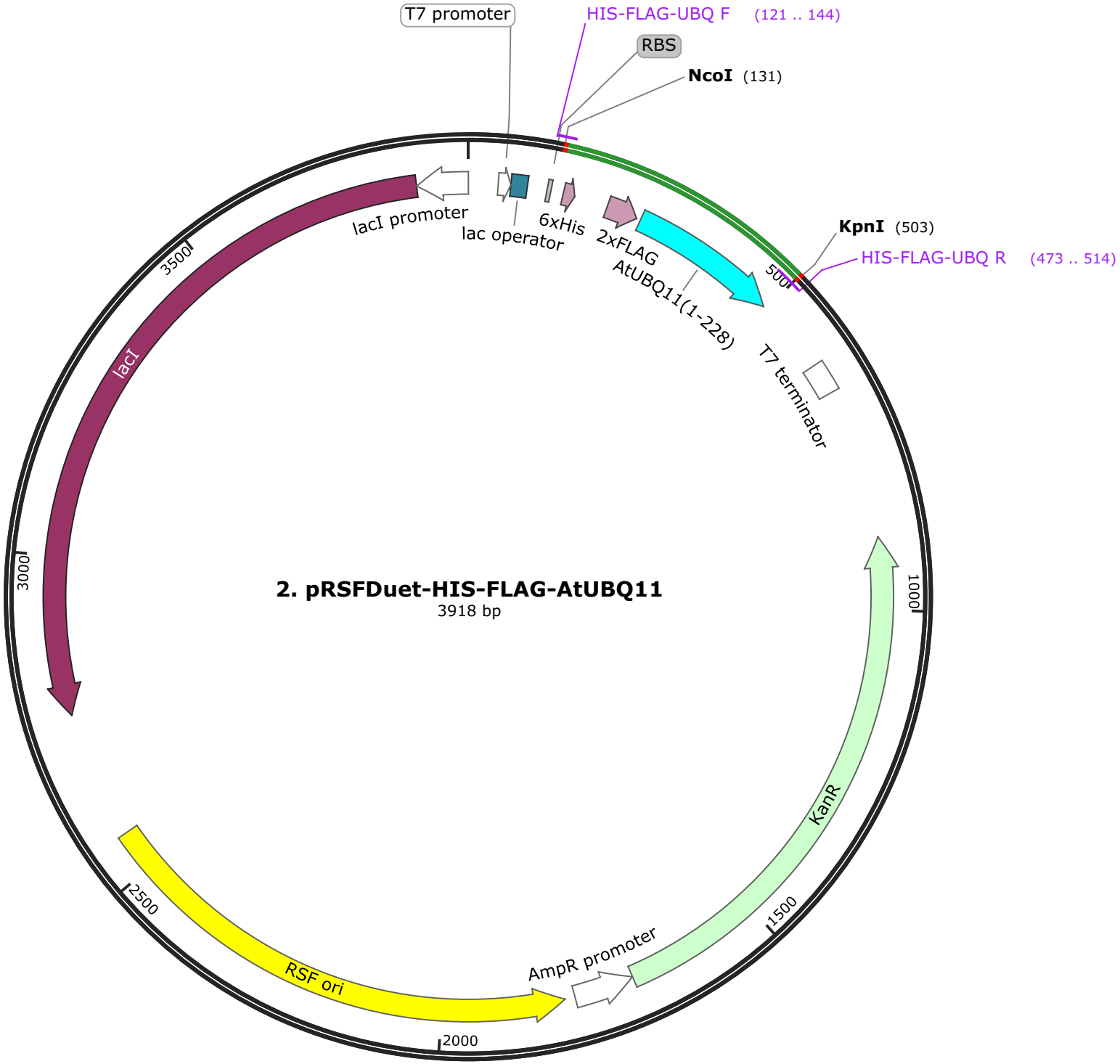

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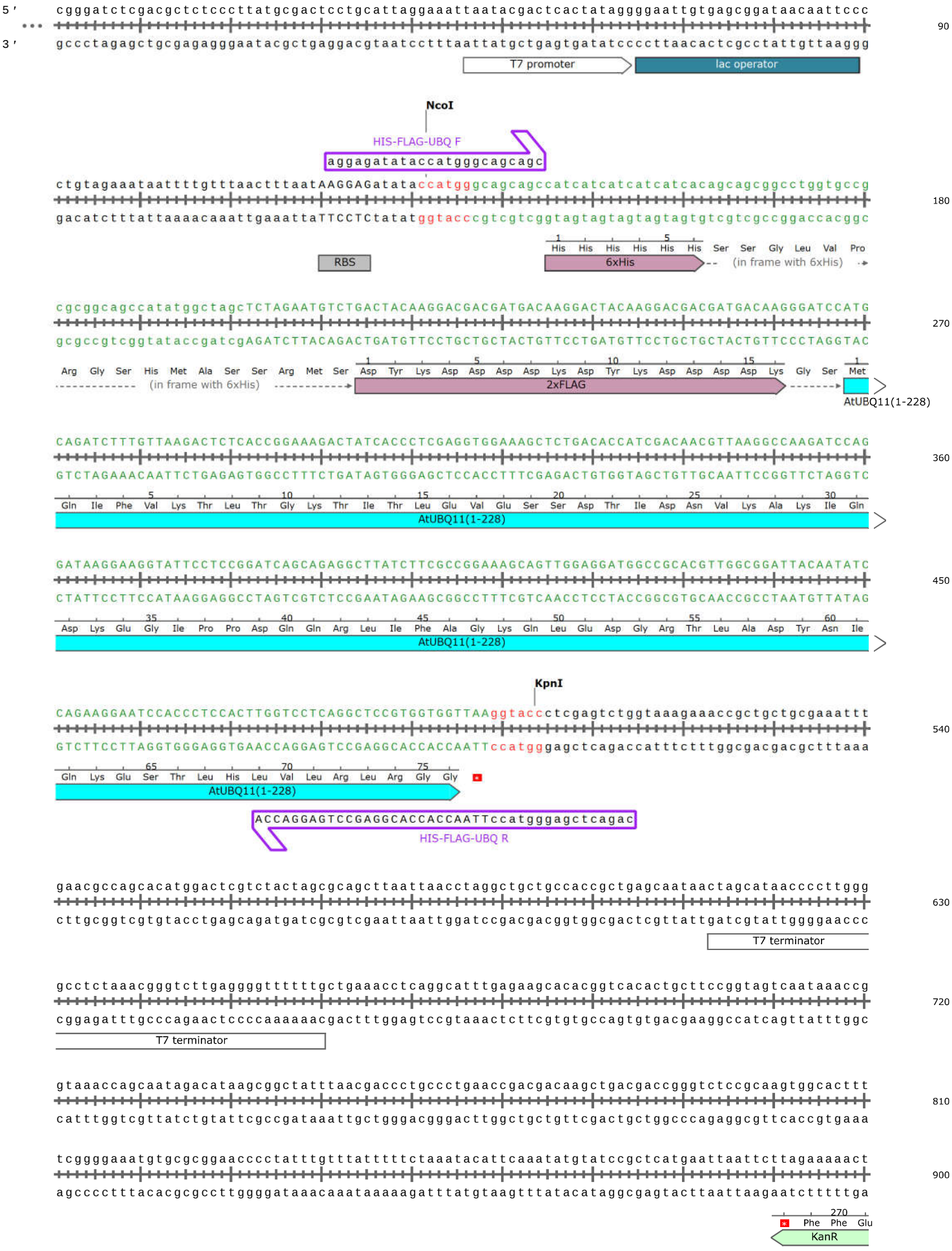

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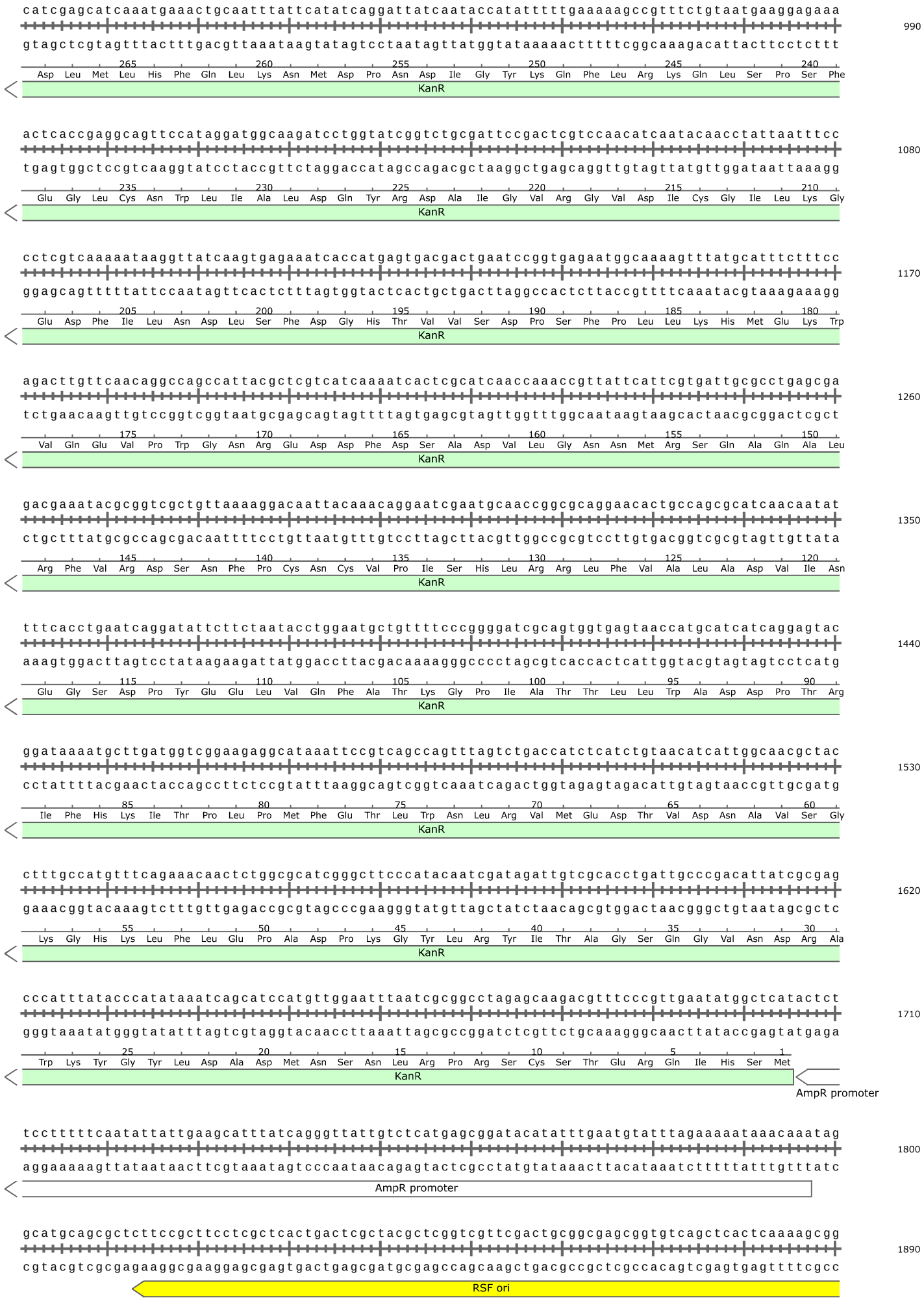

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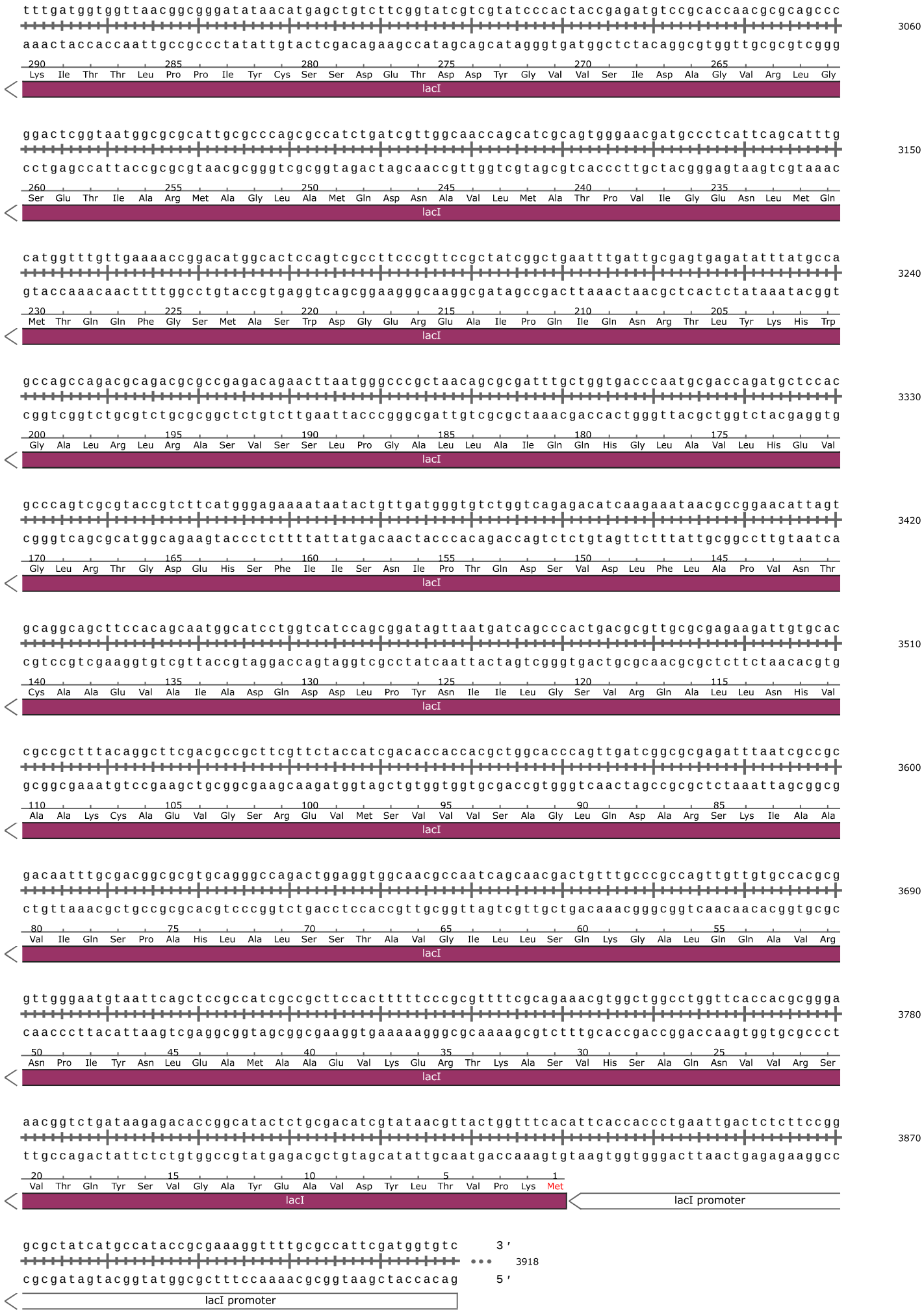
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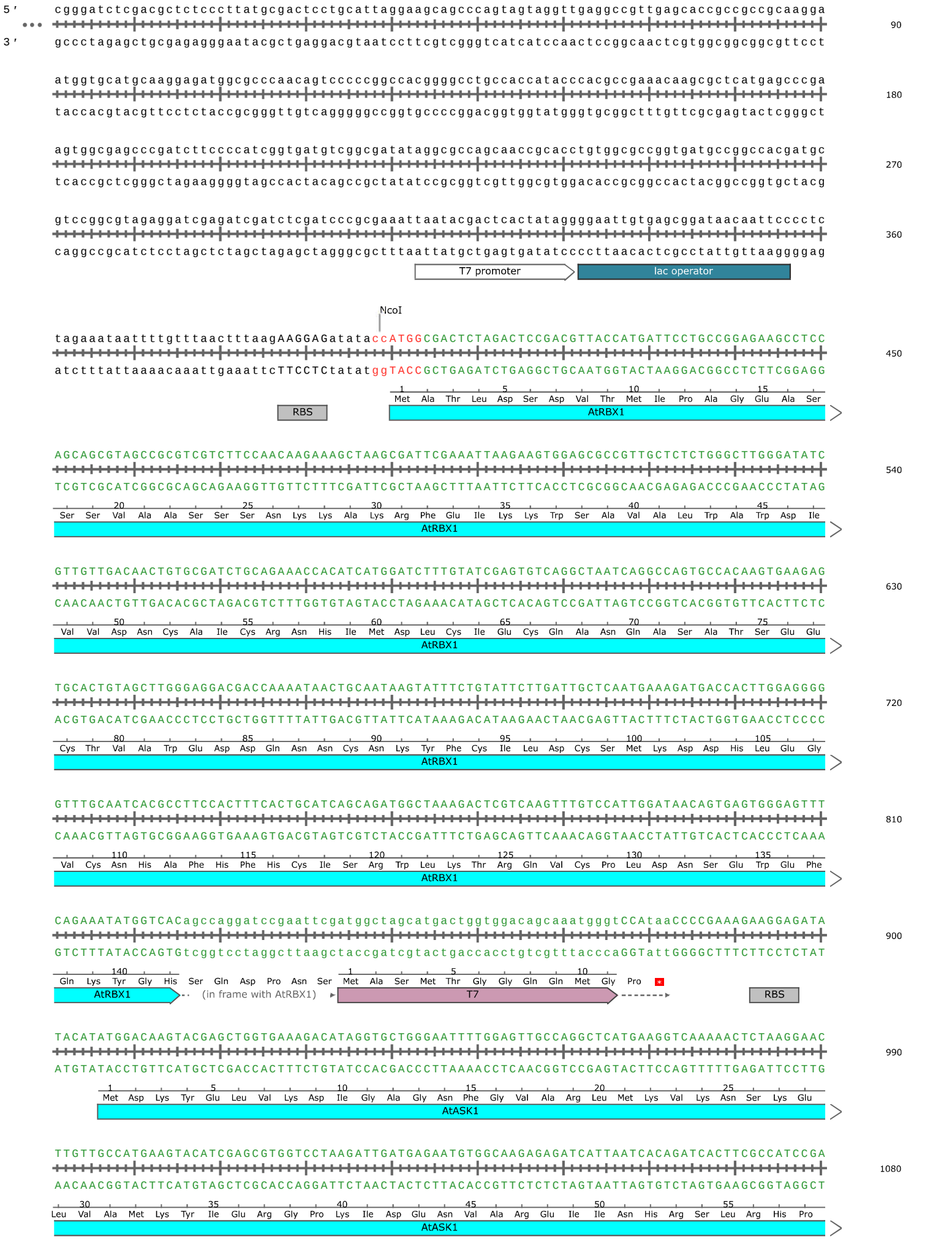

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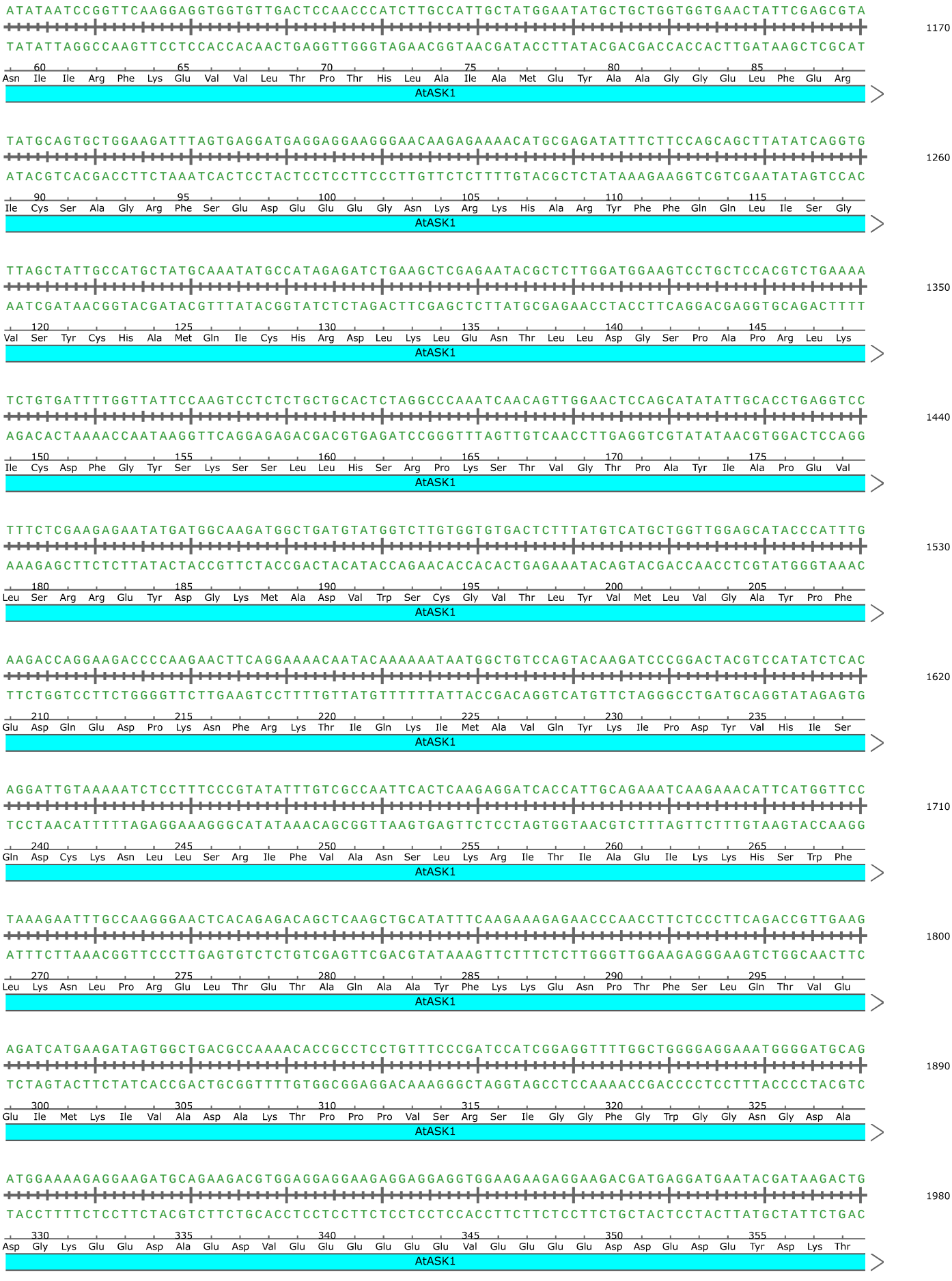

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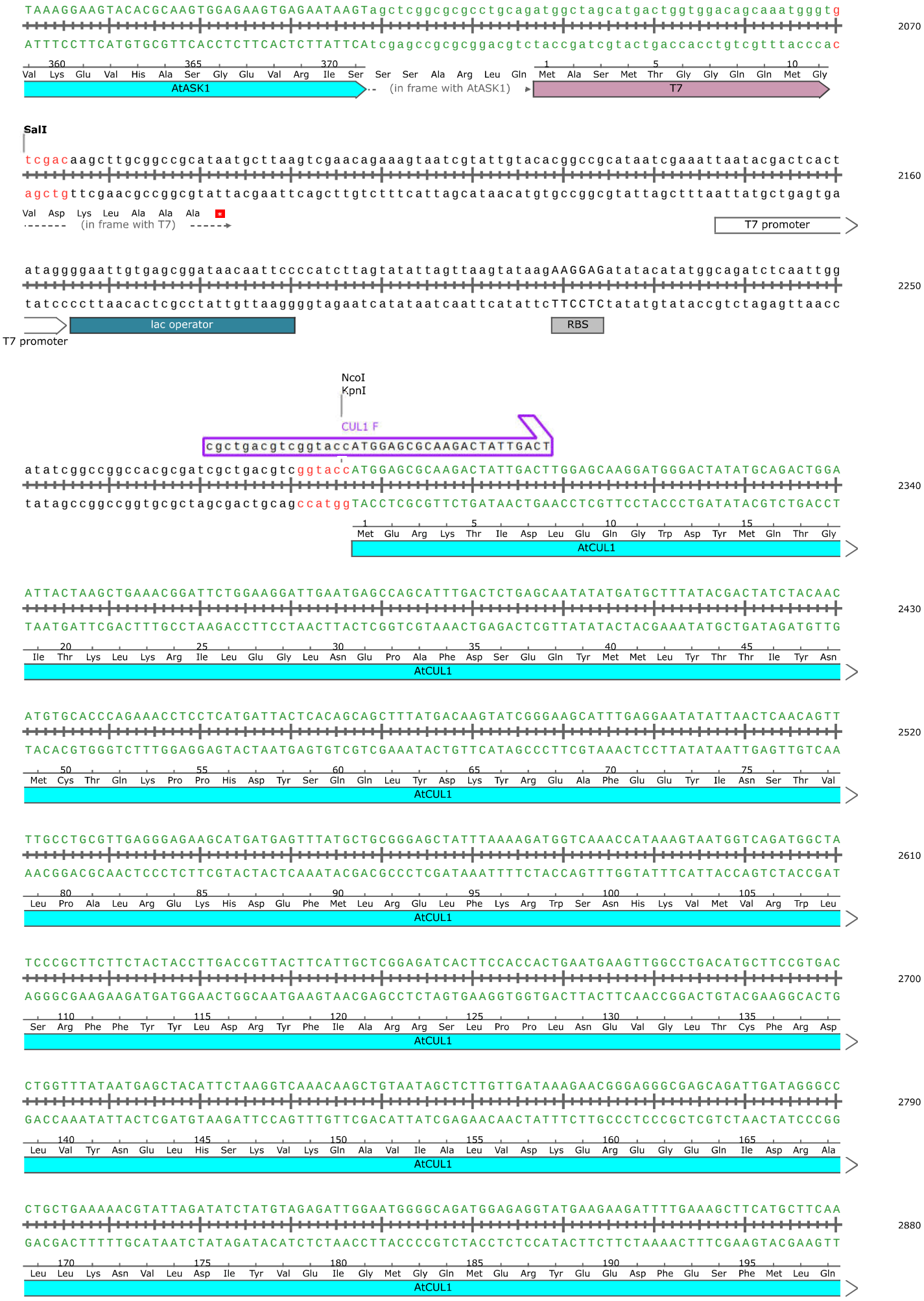

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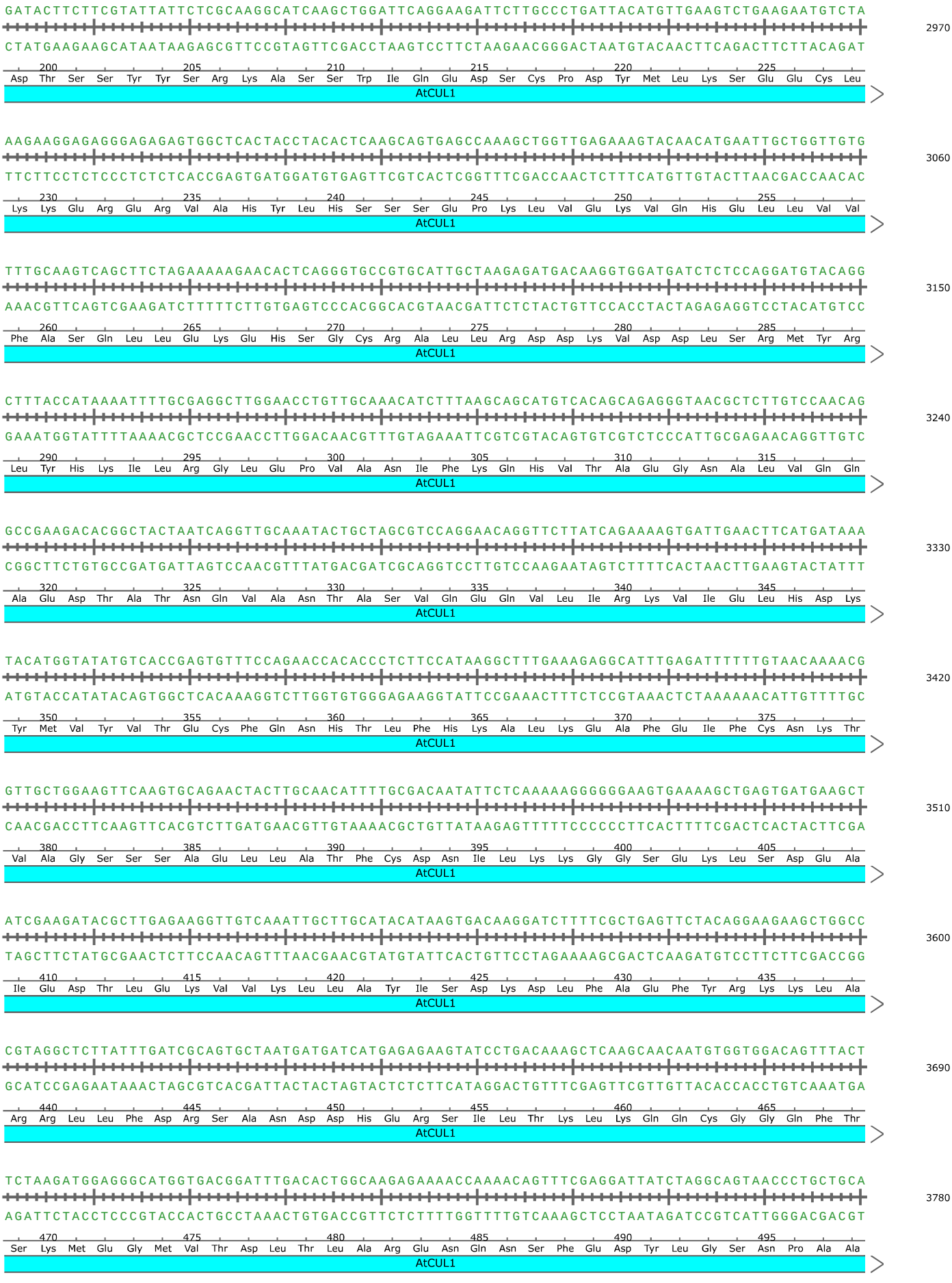
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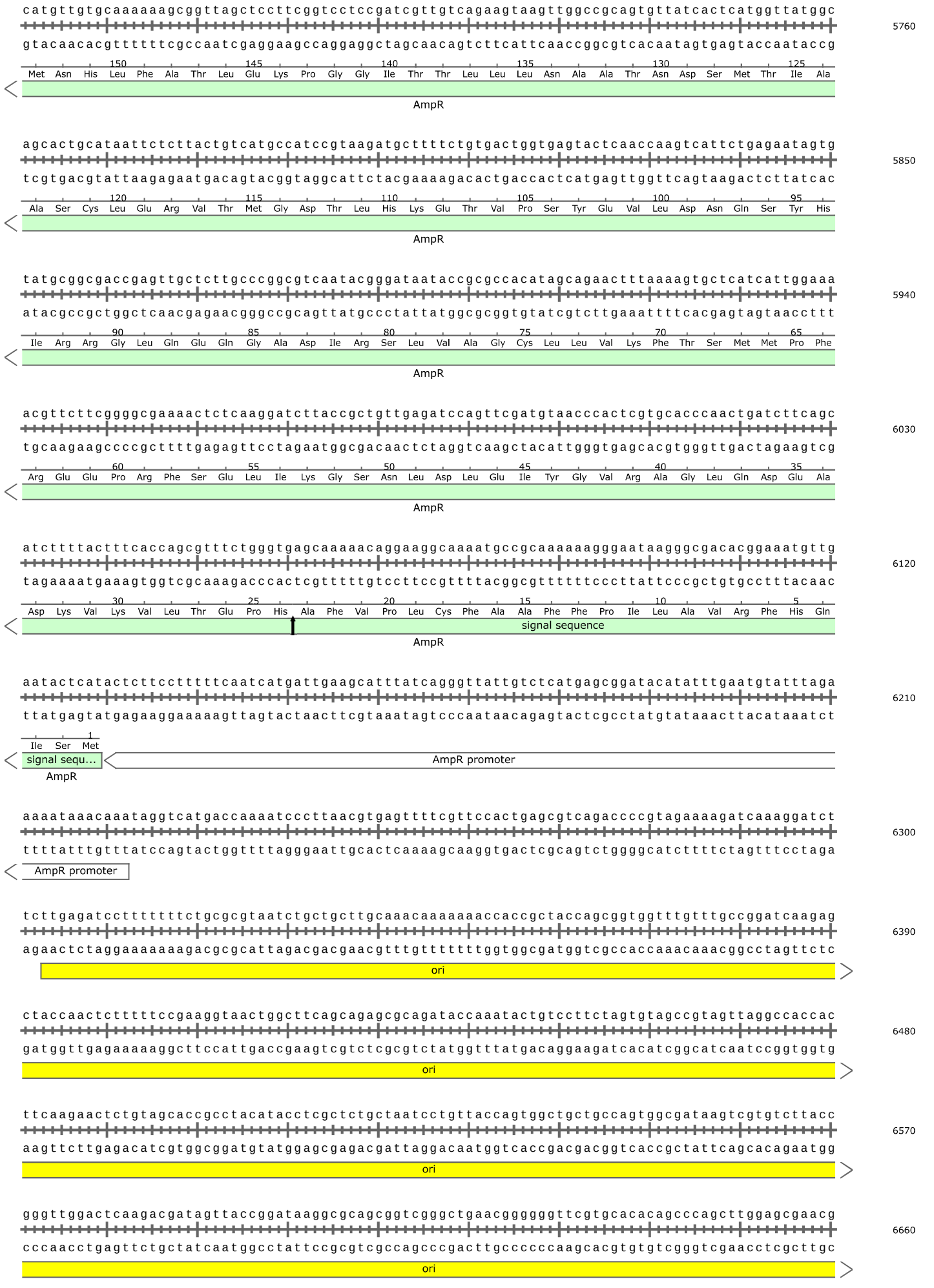

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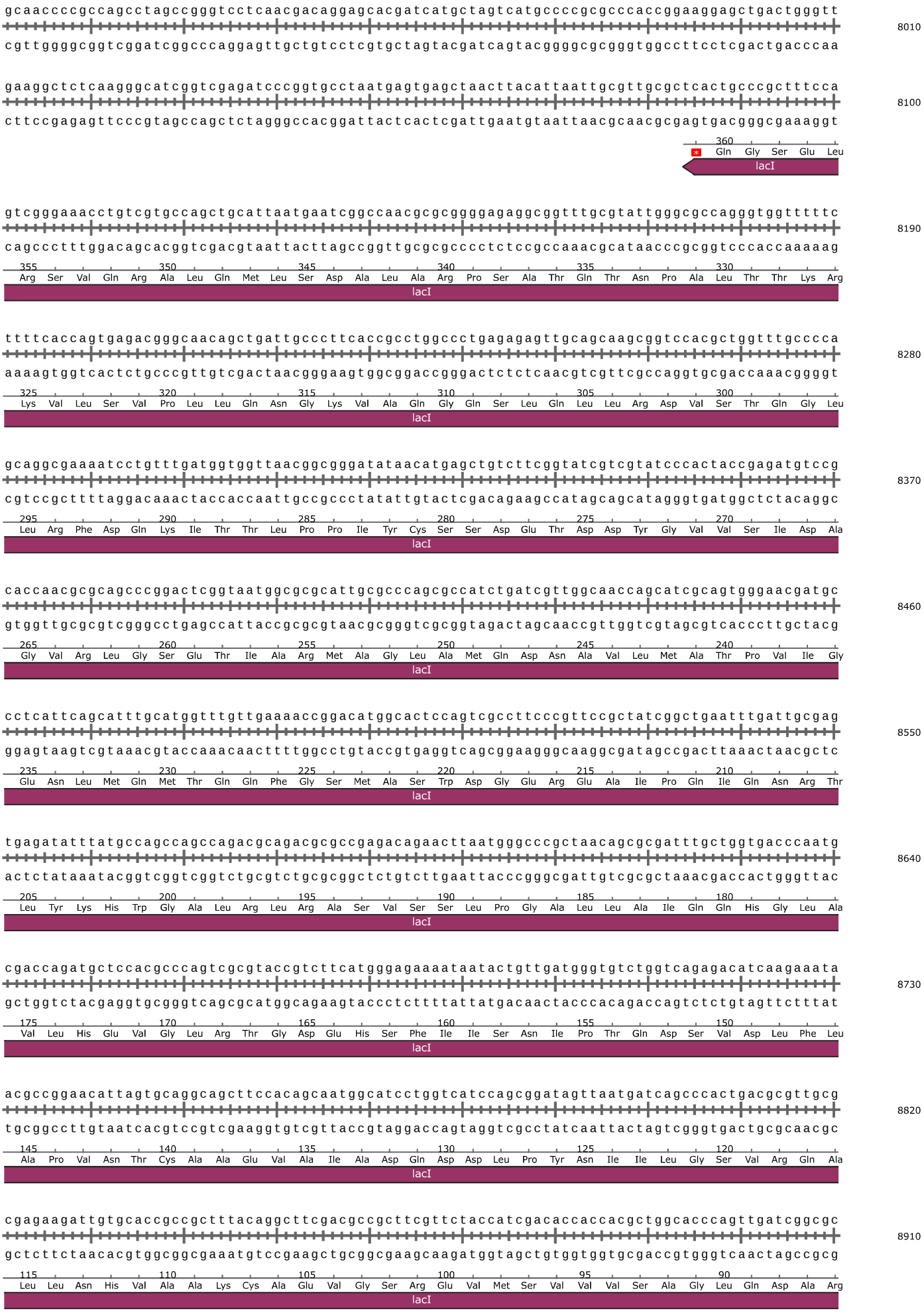

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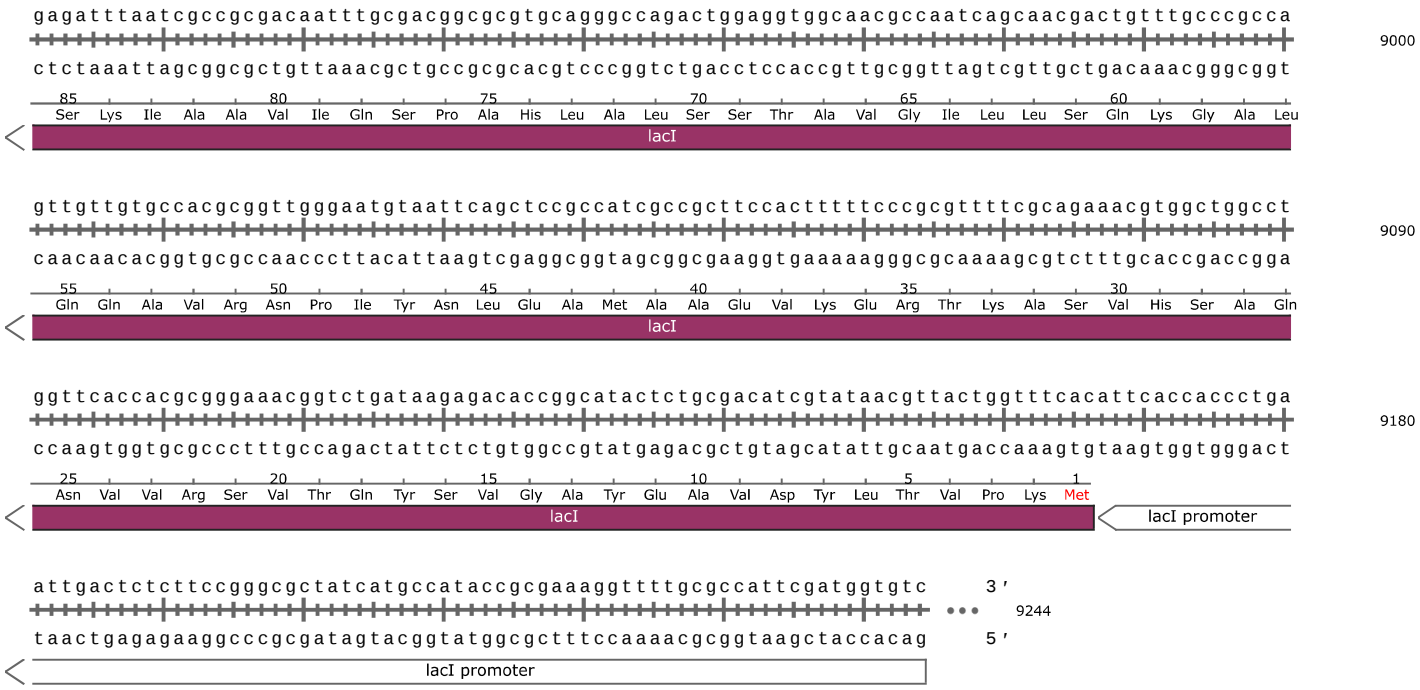

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(in frame with S)
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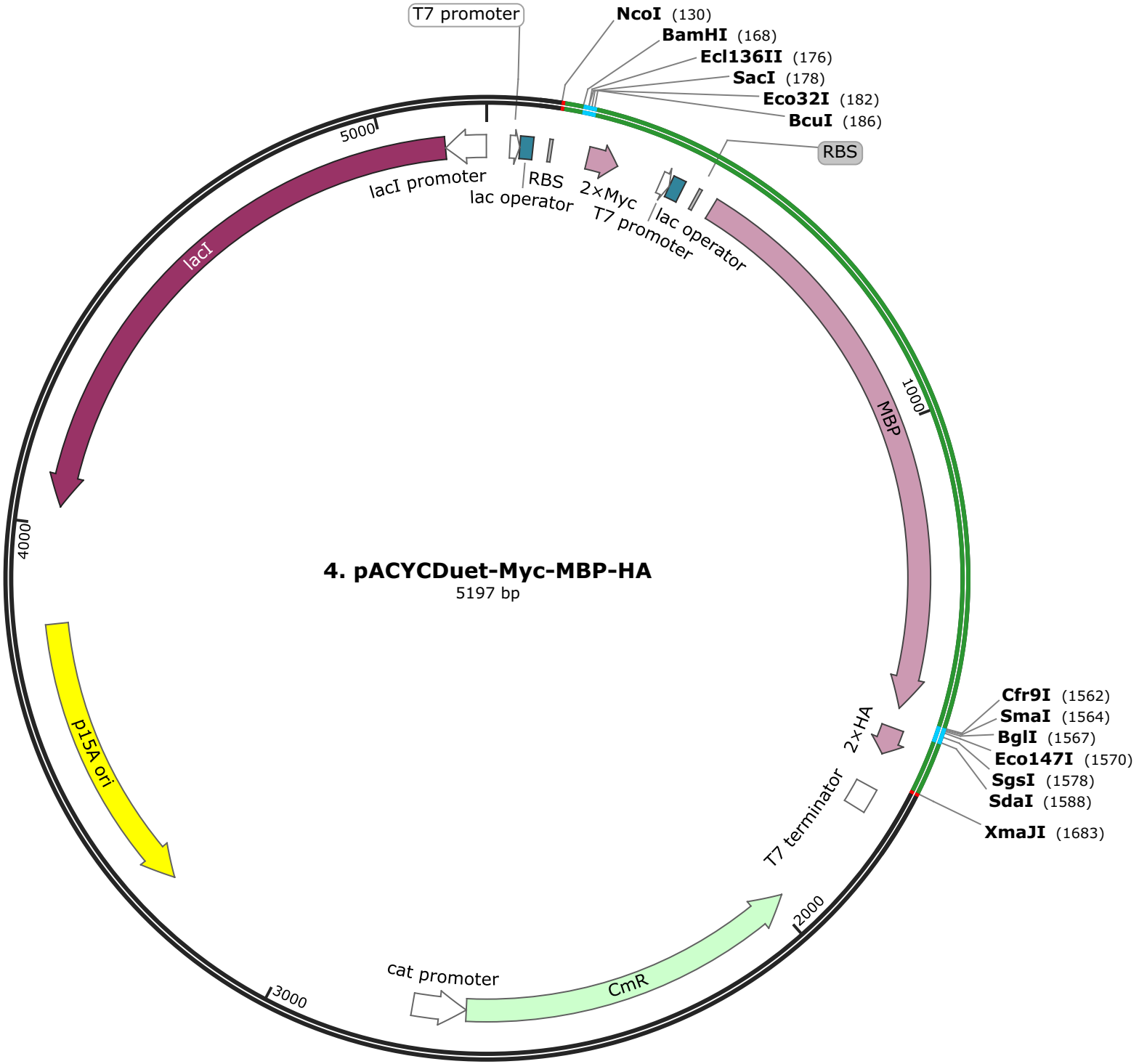

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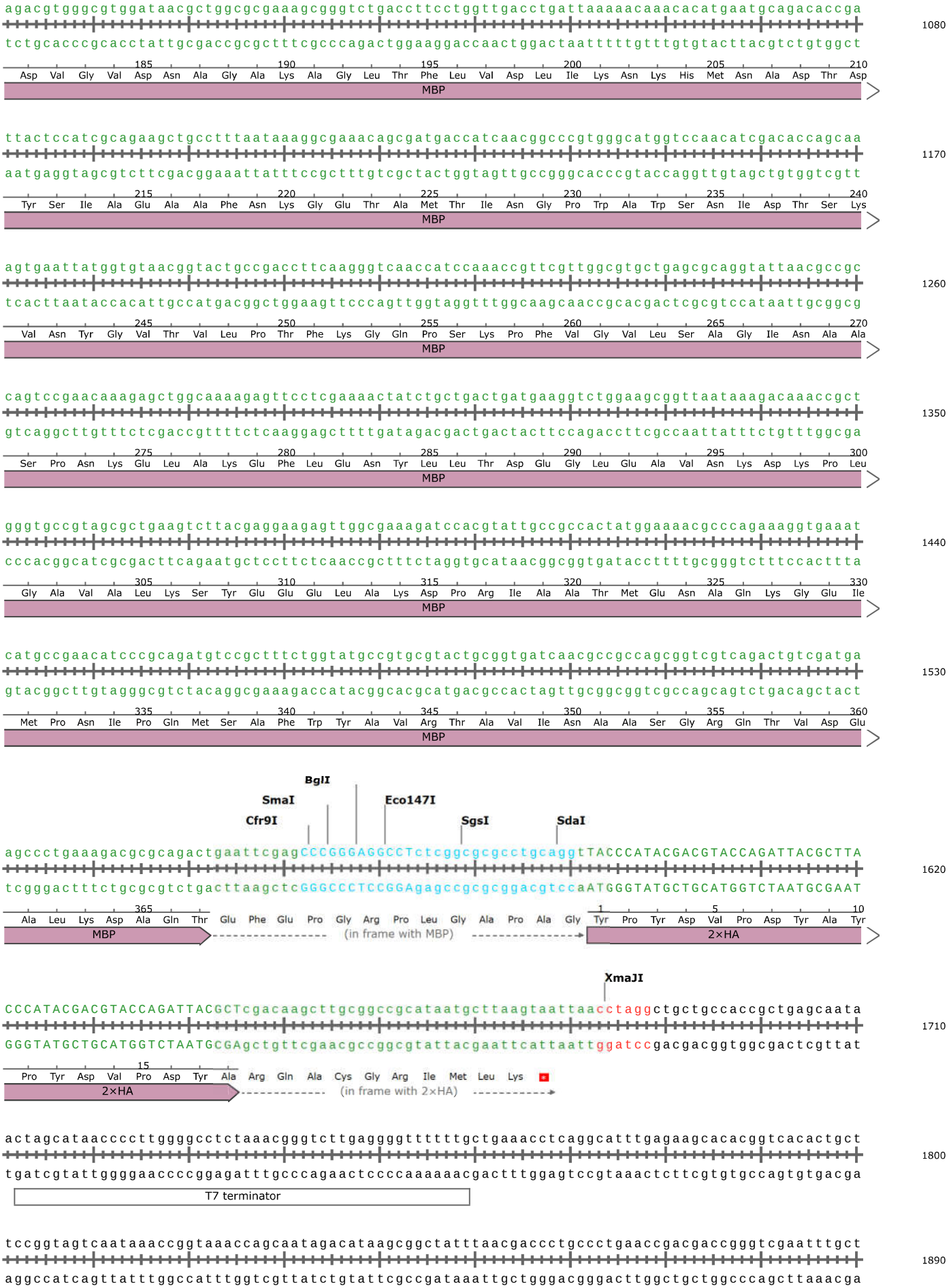

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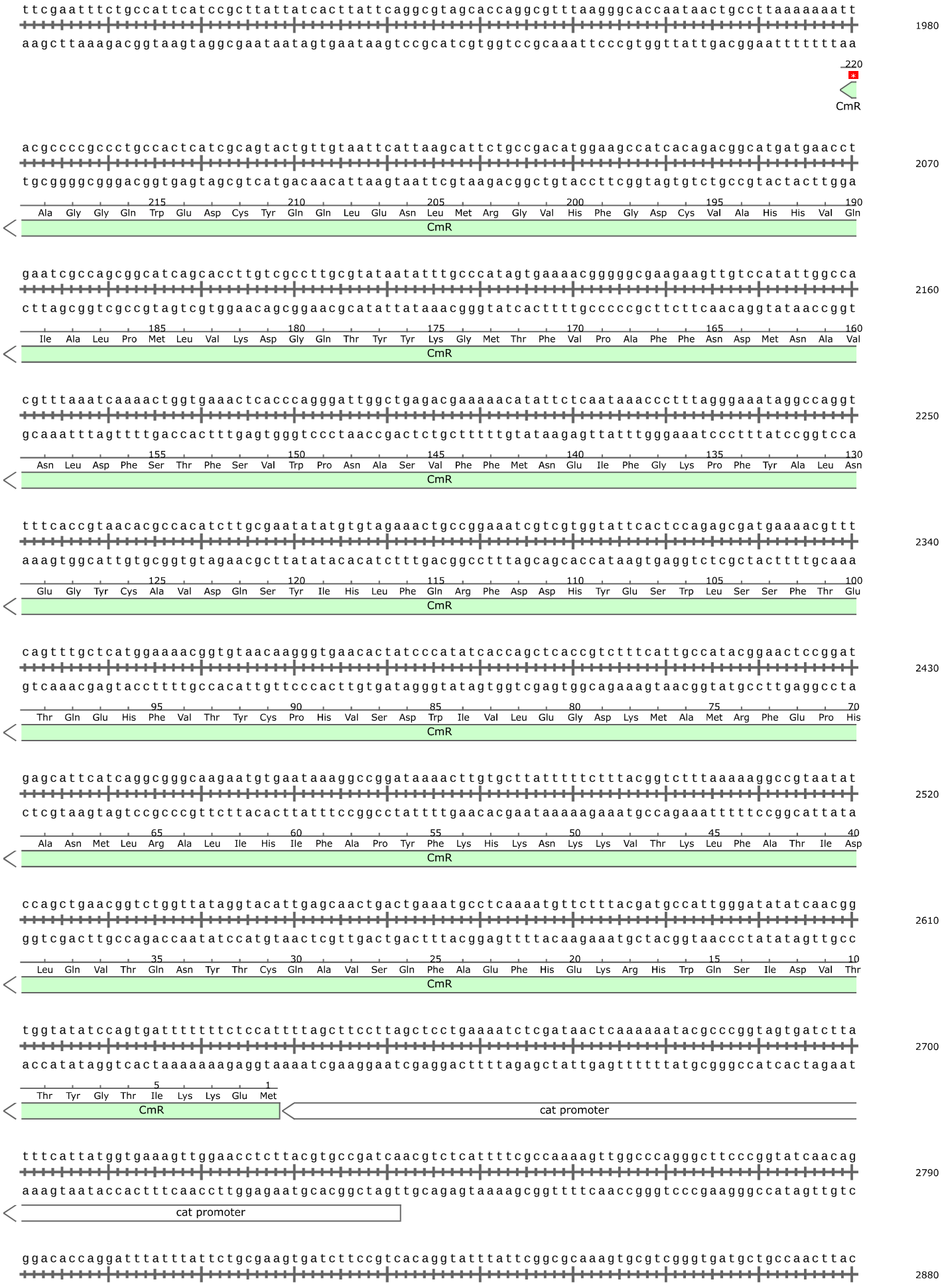

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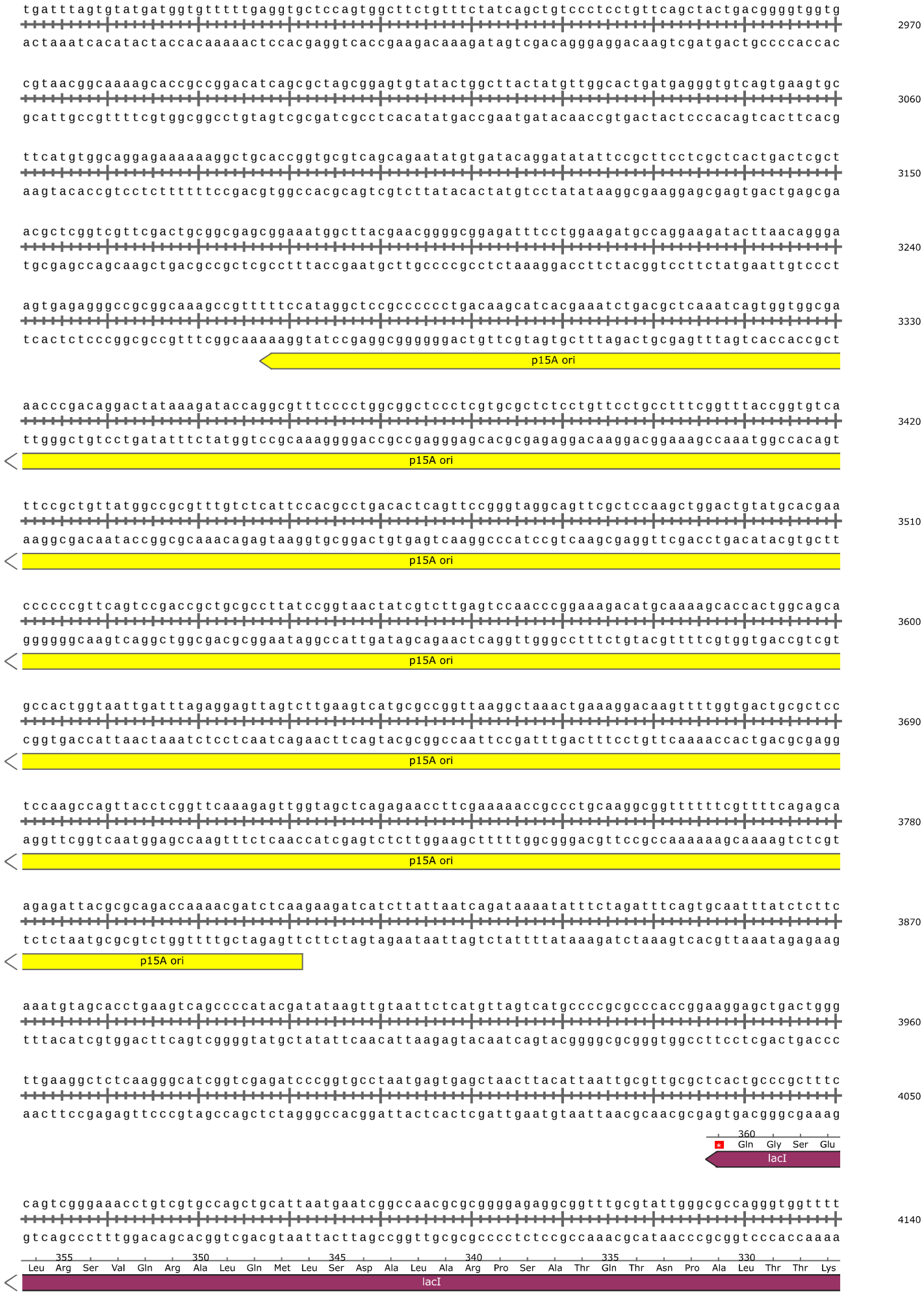
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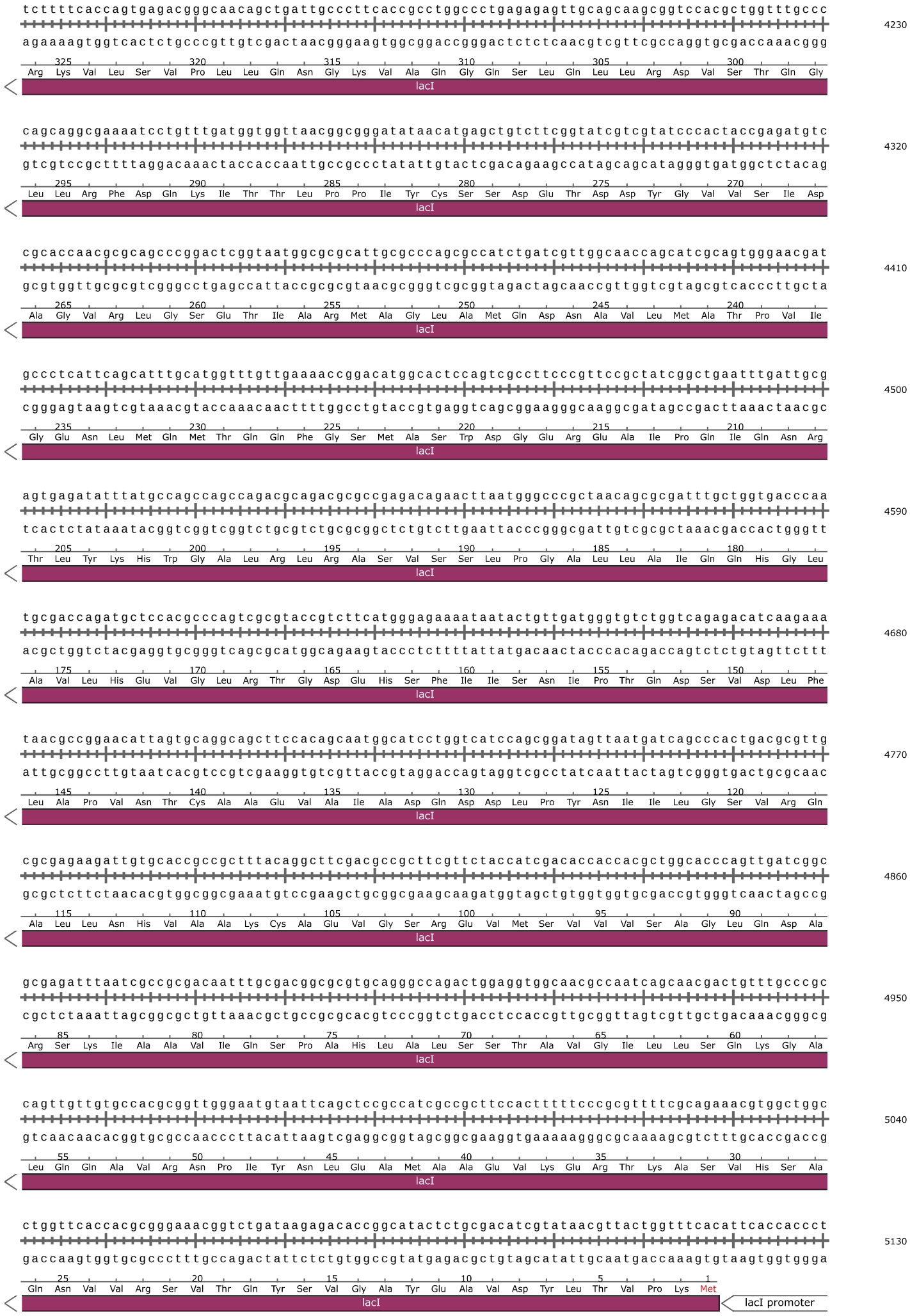


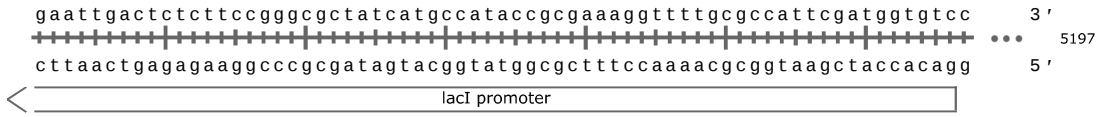

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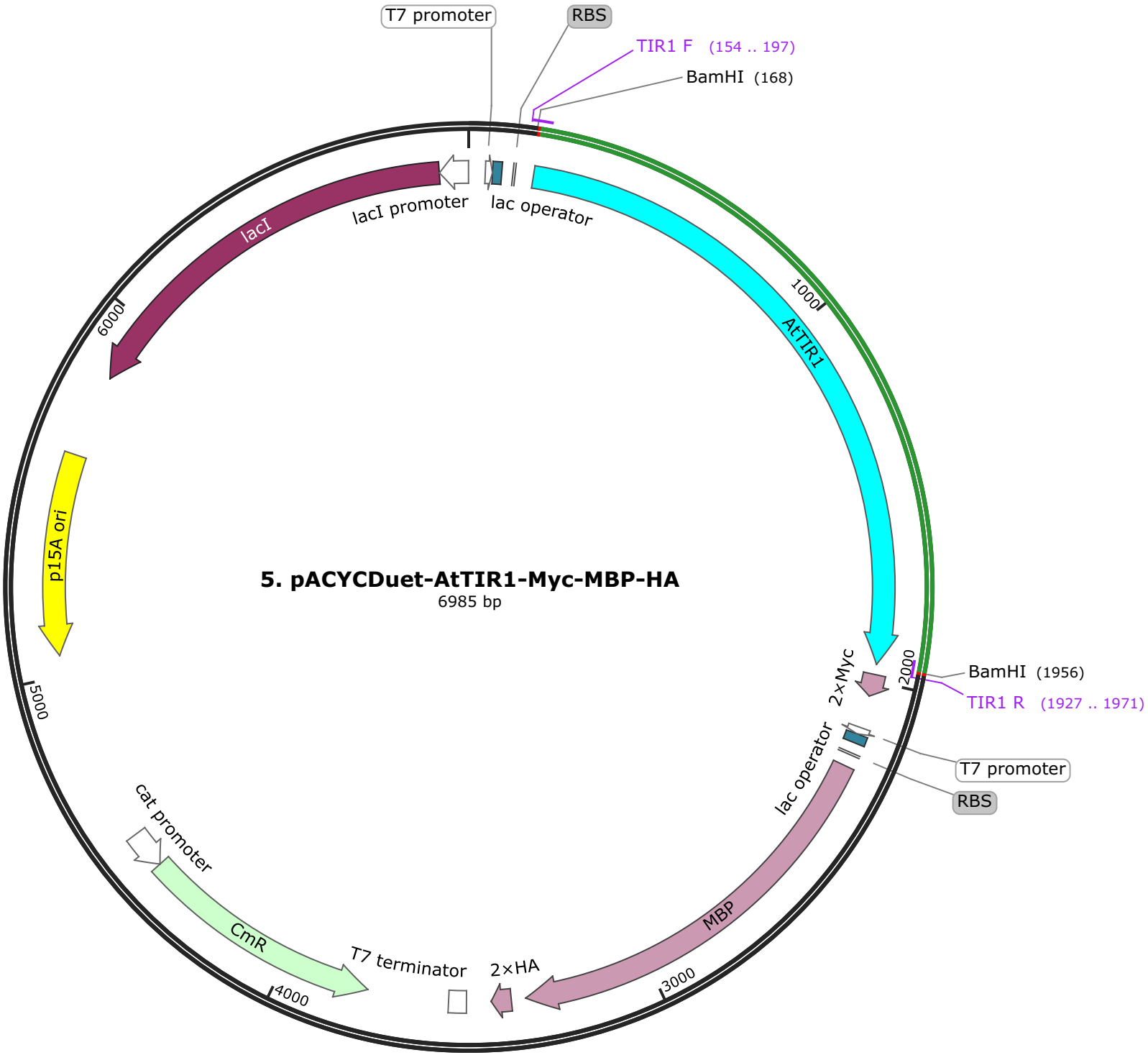

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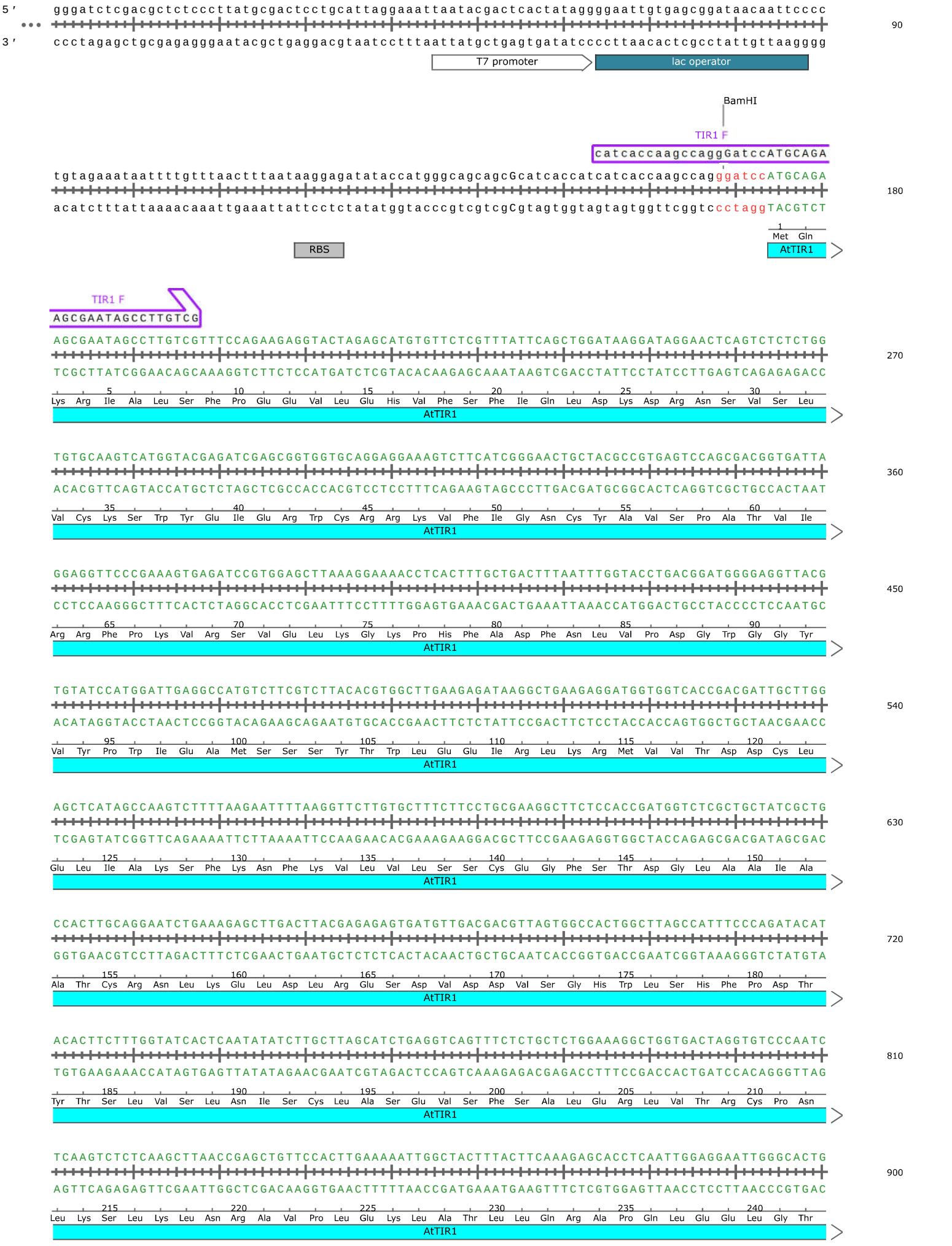

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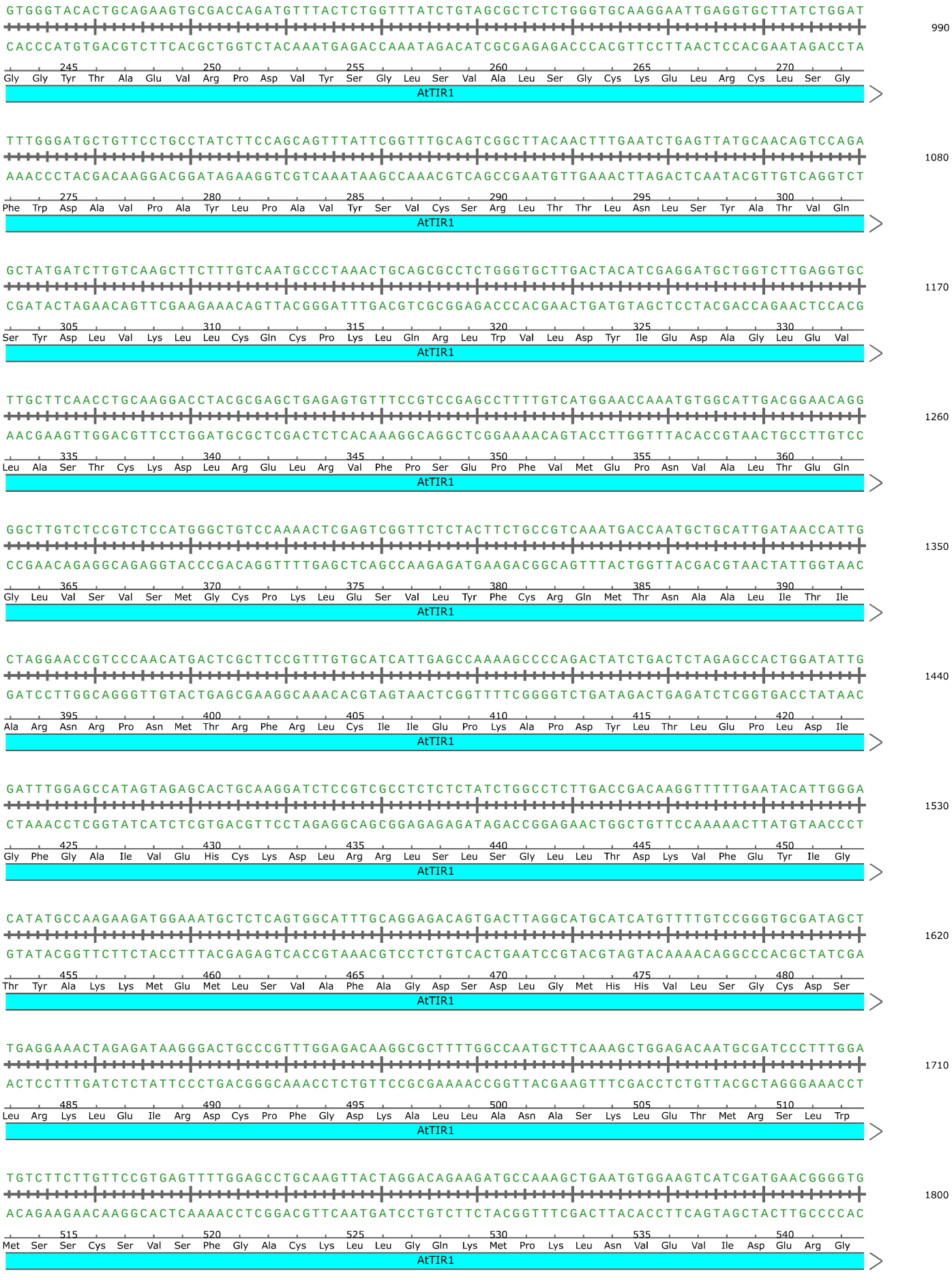

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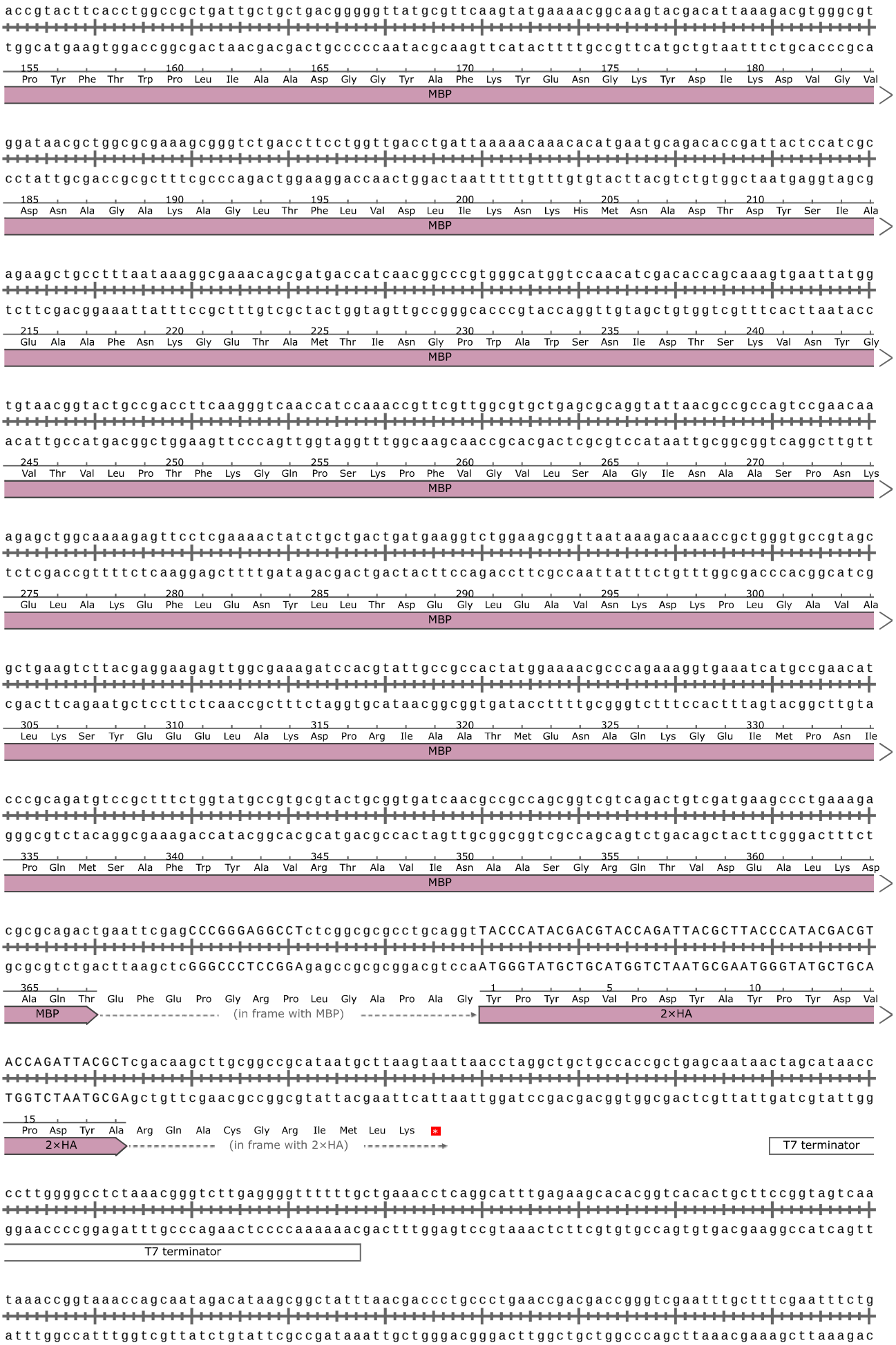
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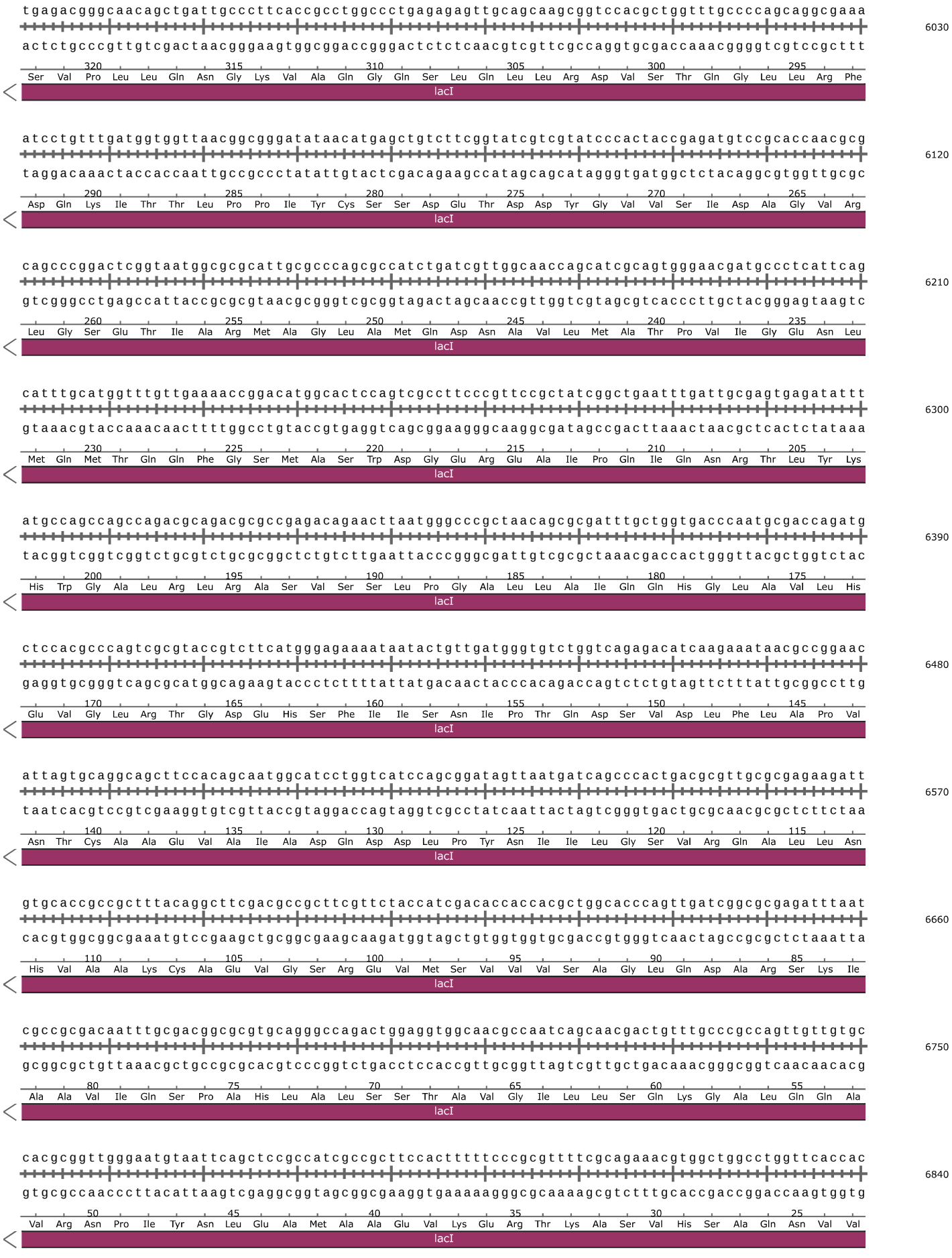

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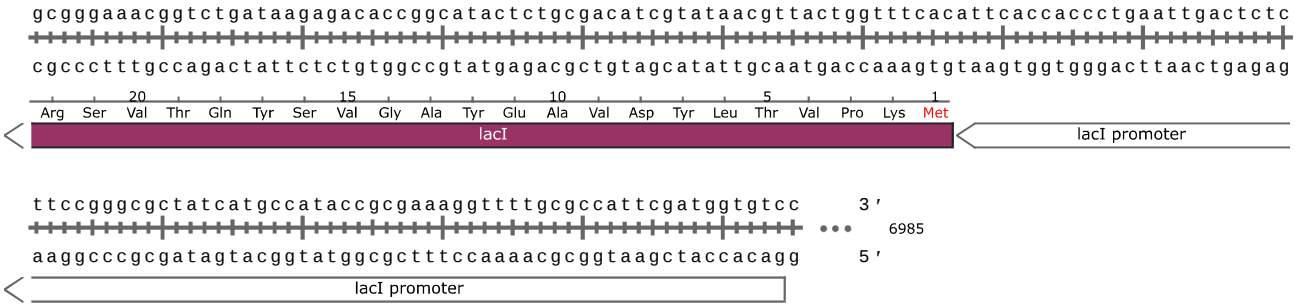

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TIR1 R
2×Myc
(in frame with 2×Myc)
T7 promoter
lac operator
RBS
MBP


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3690
MBP
(in frame with MBP)
2×HA
(in frame with 2×HA)
T7 terminator


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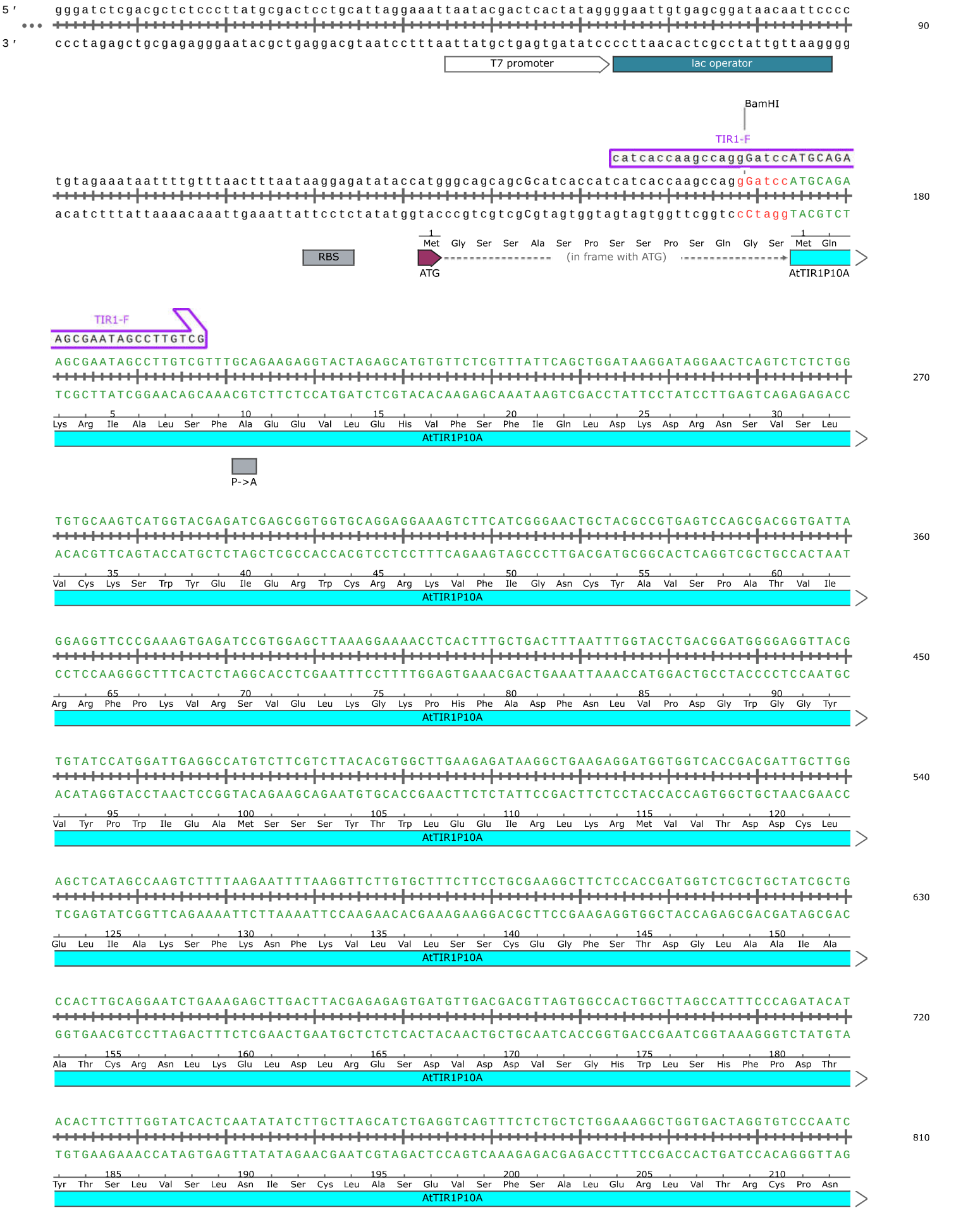

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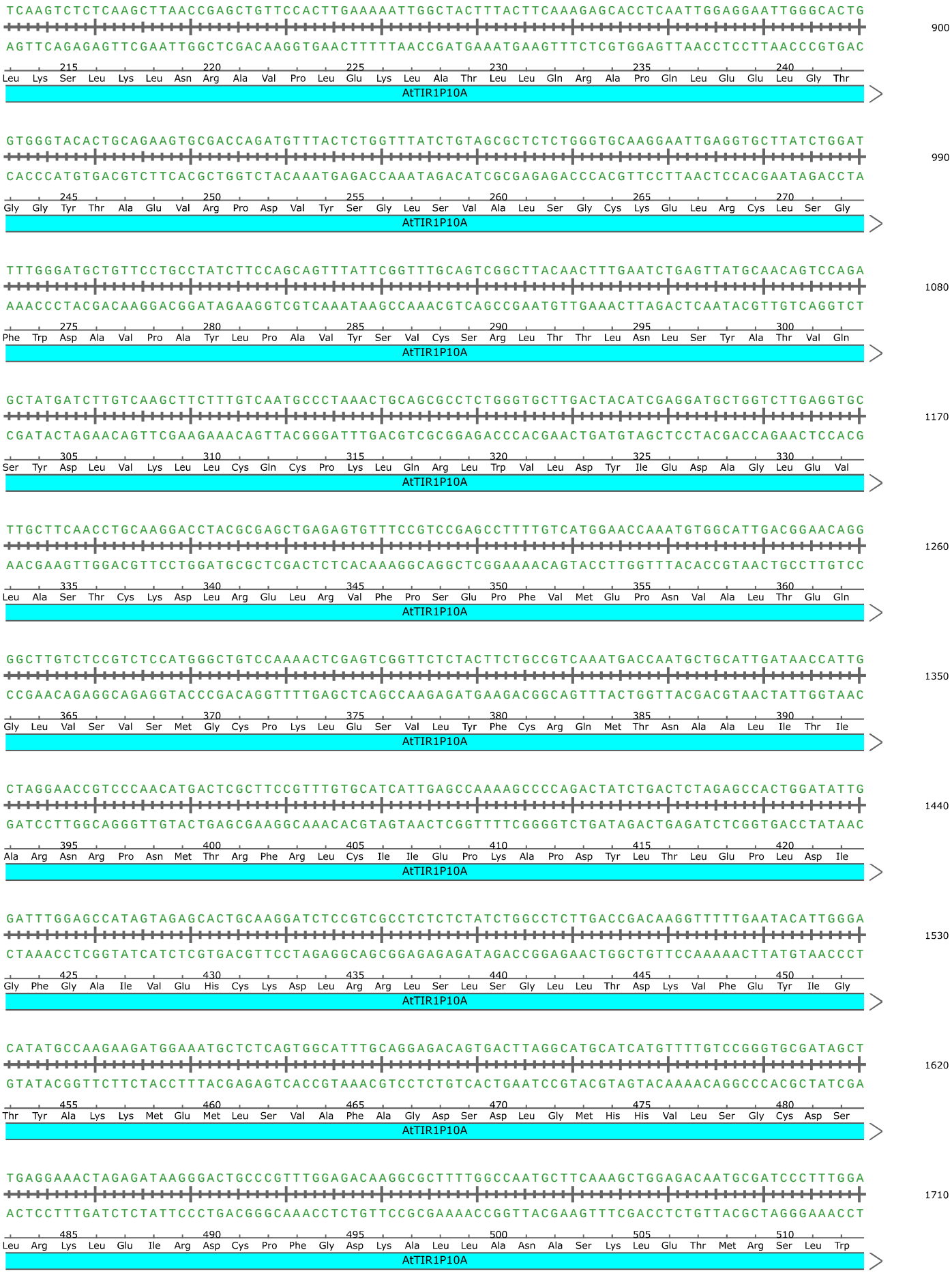

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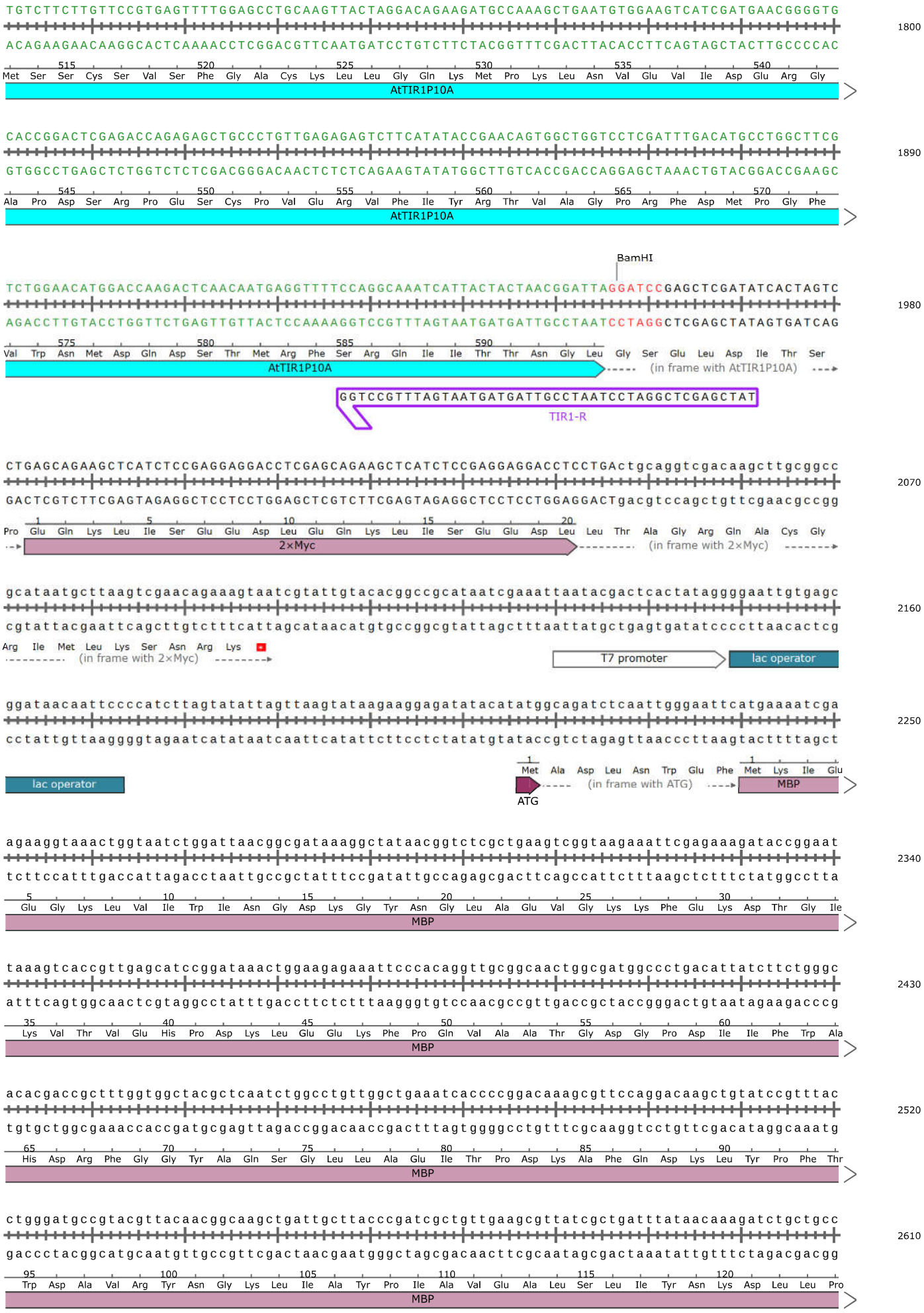
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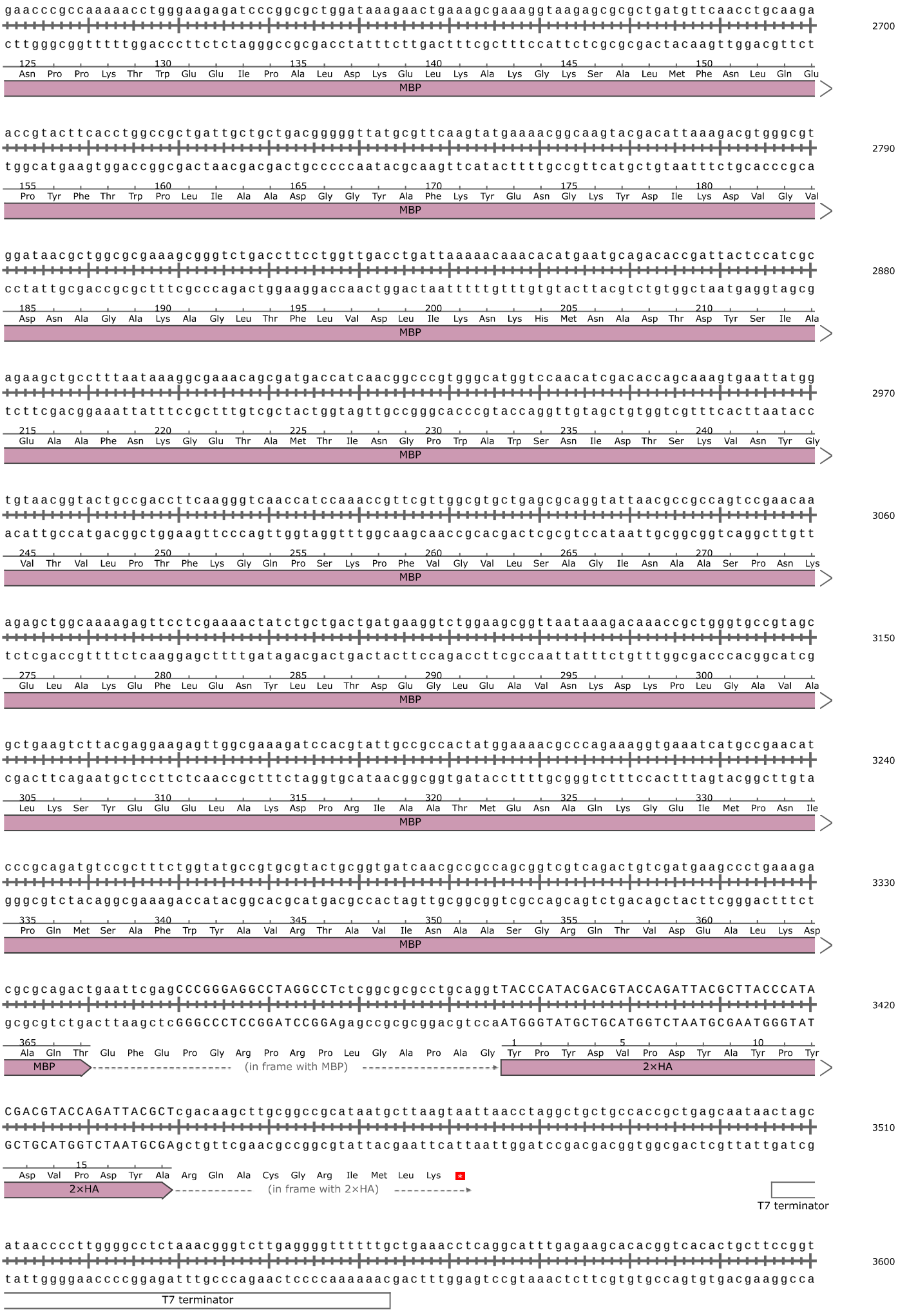
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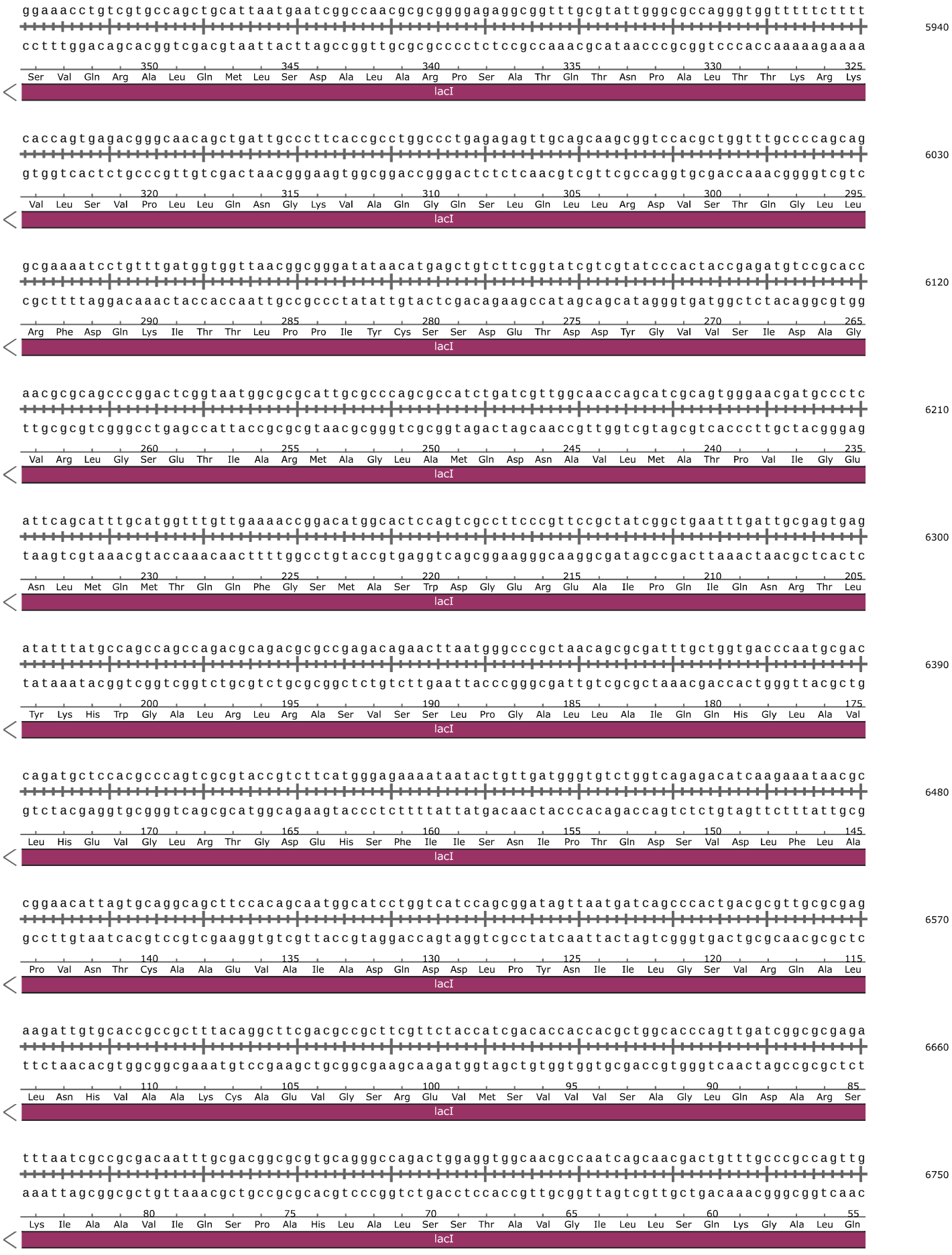

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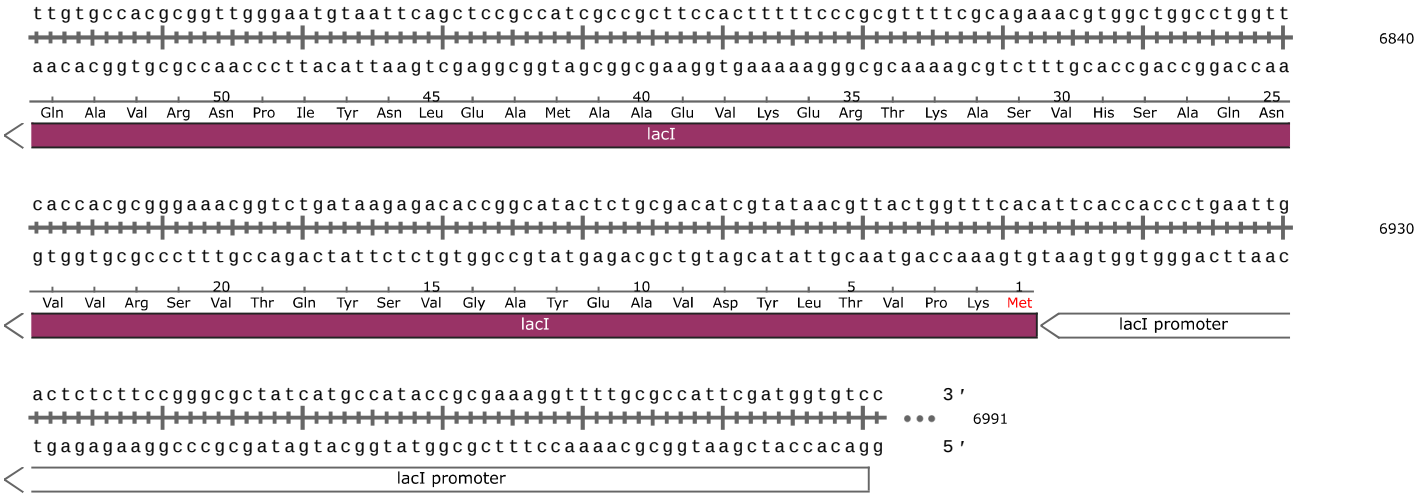

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(in frame with 2×Myc)
T7 promoter
lac operator
(in frame with ATG)
MBP
ATG


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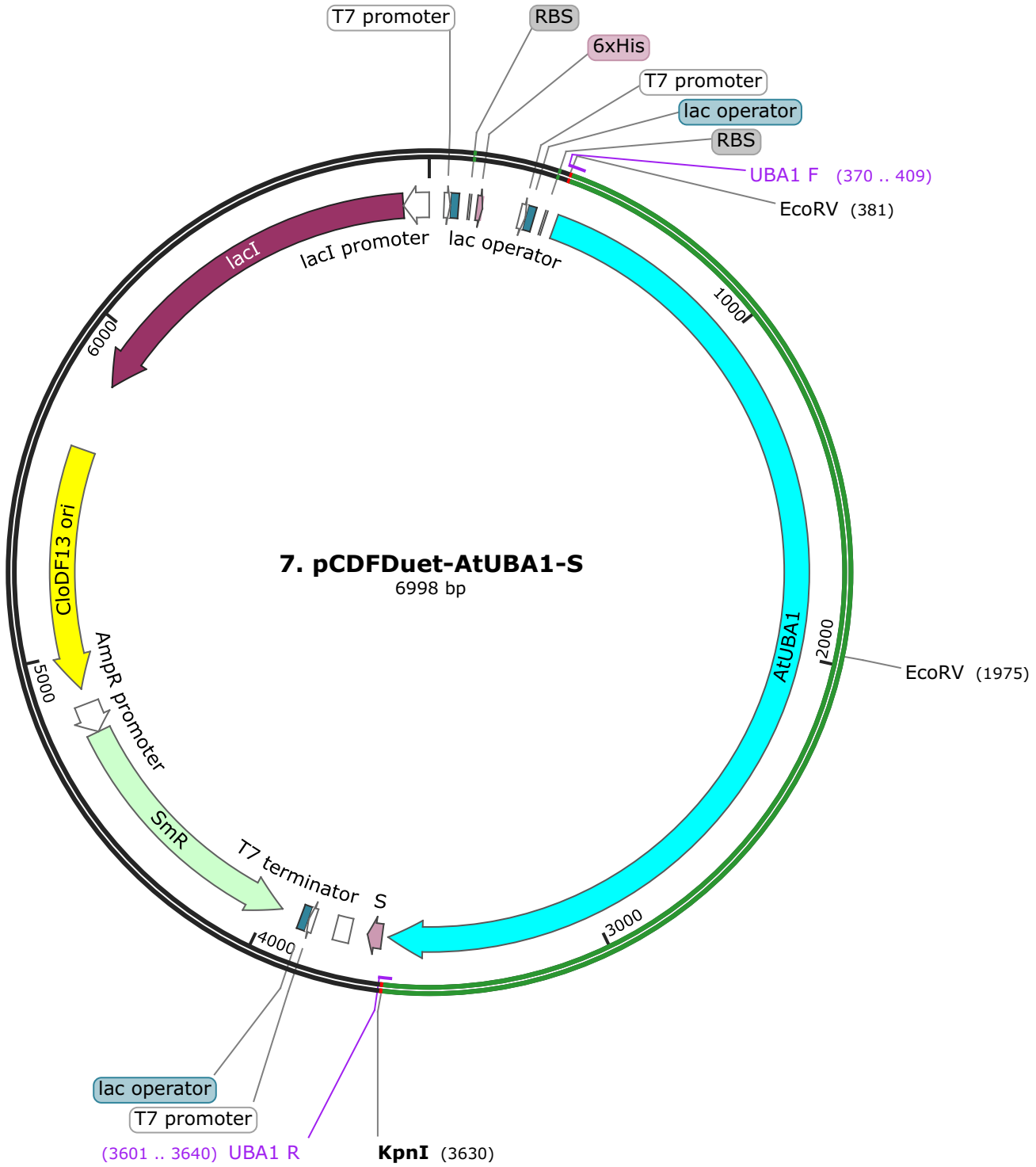

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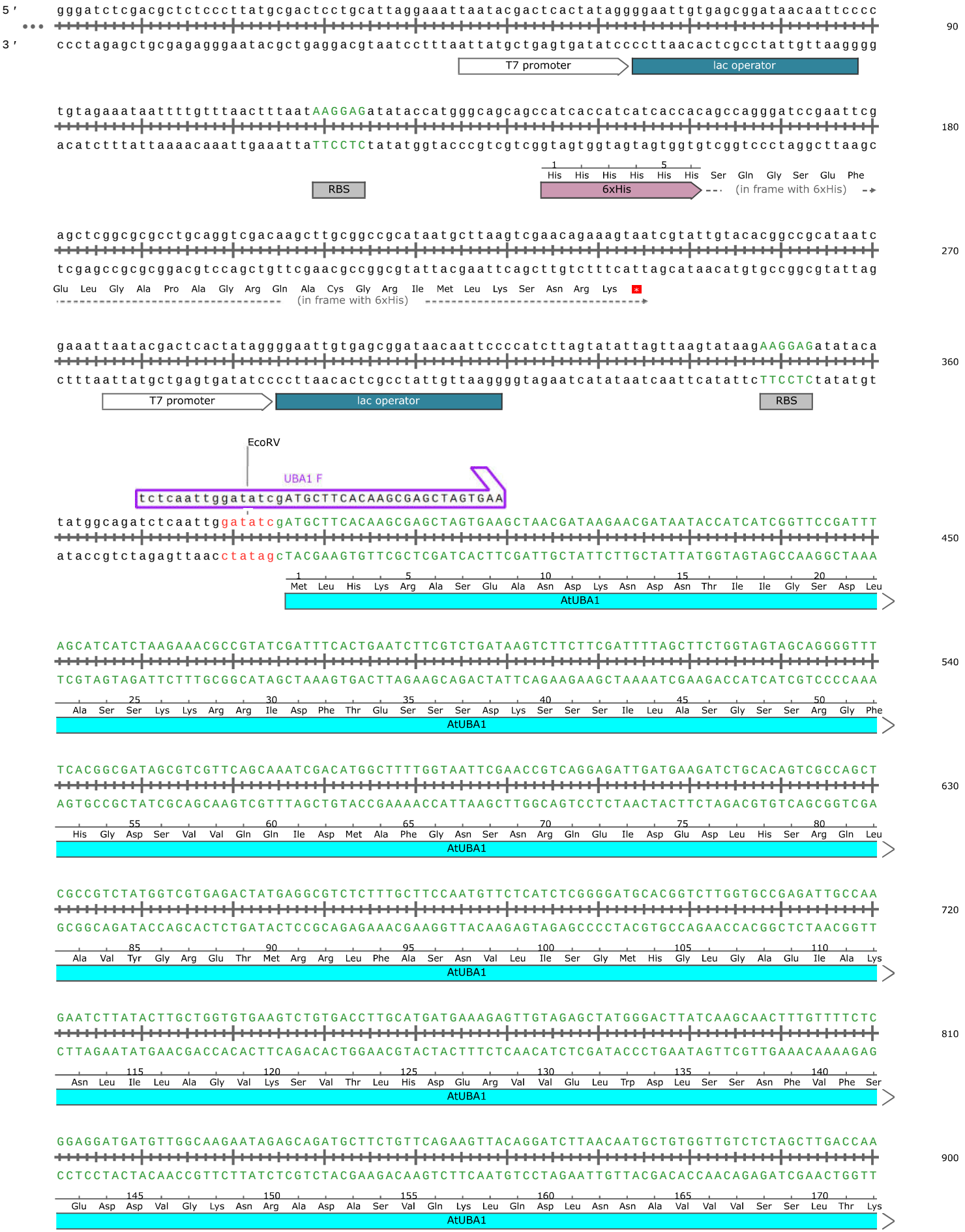

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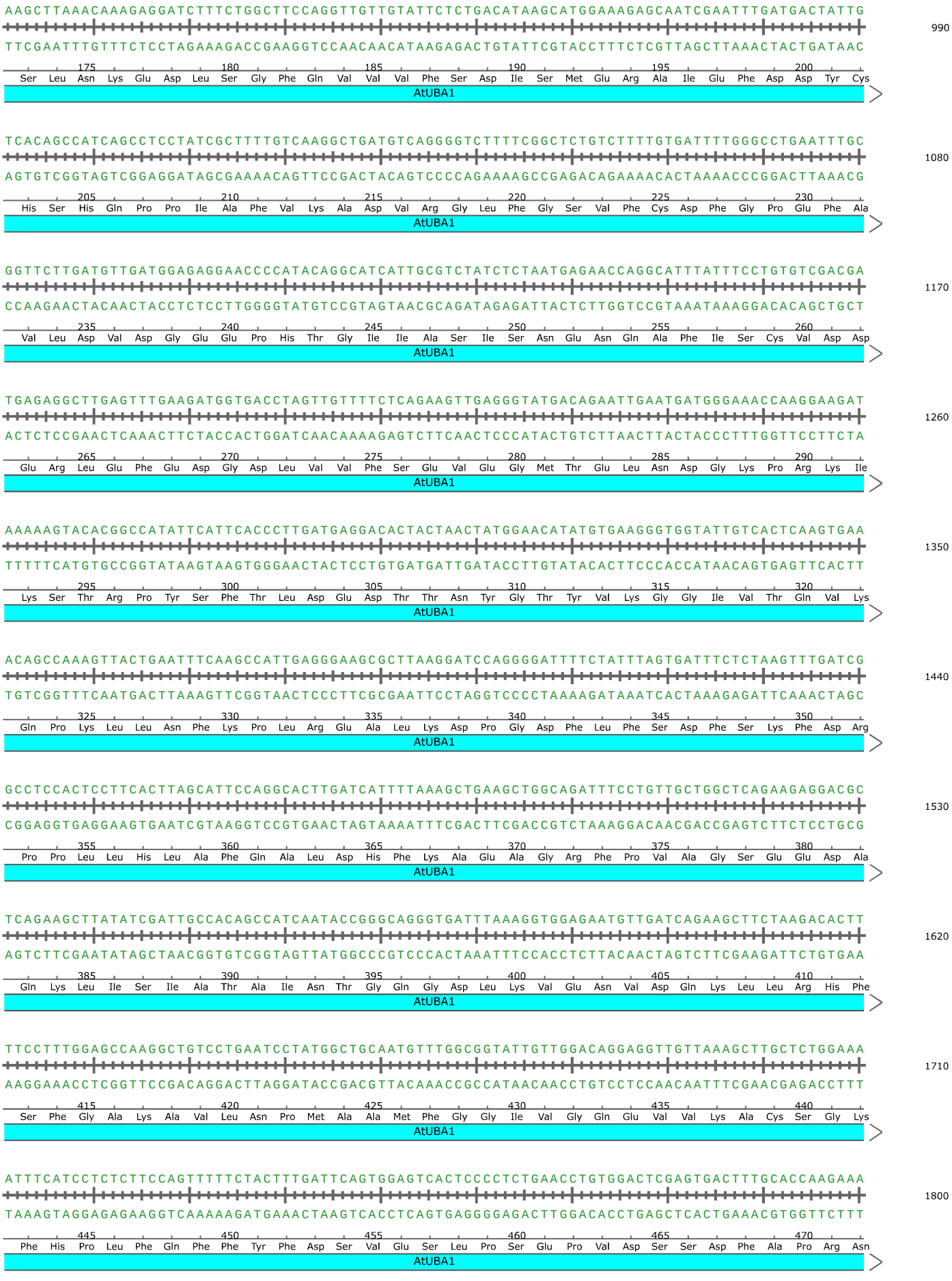

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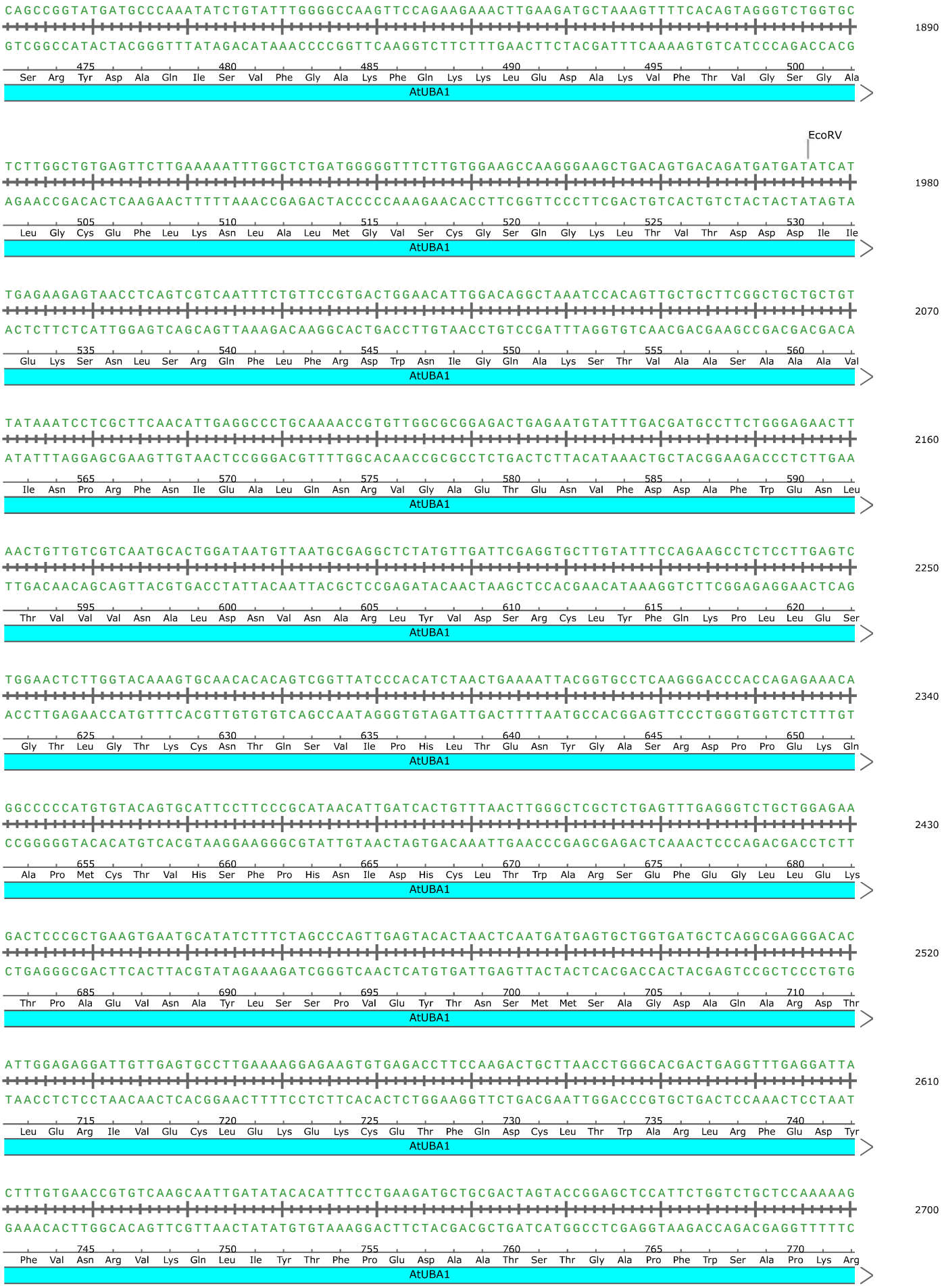

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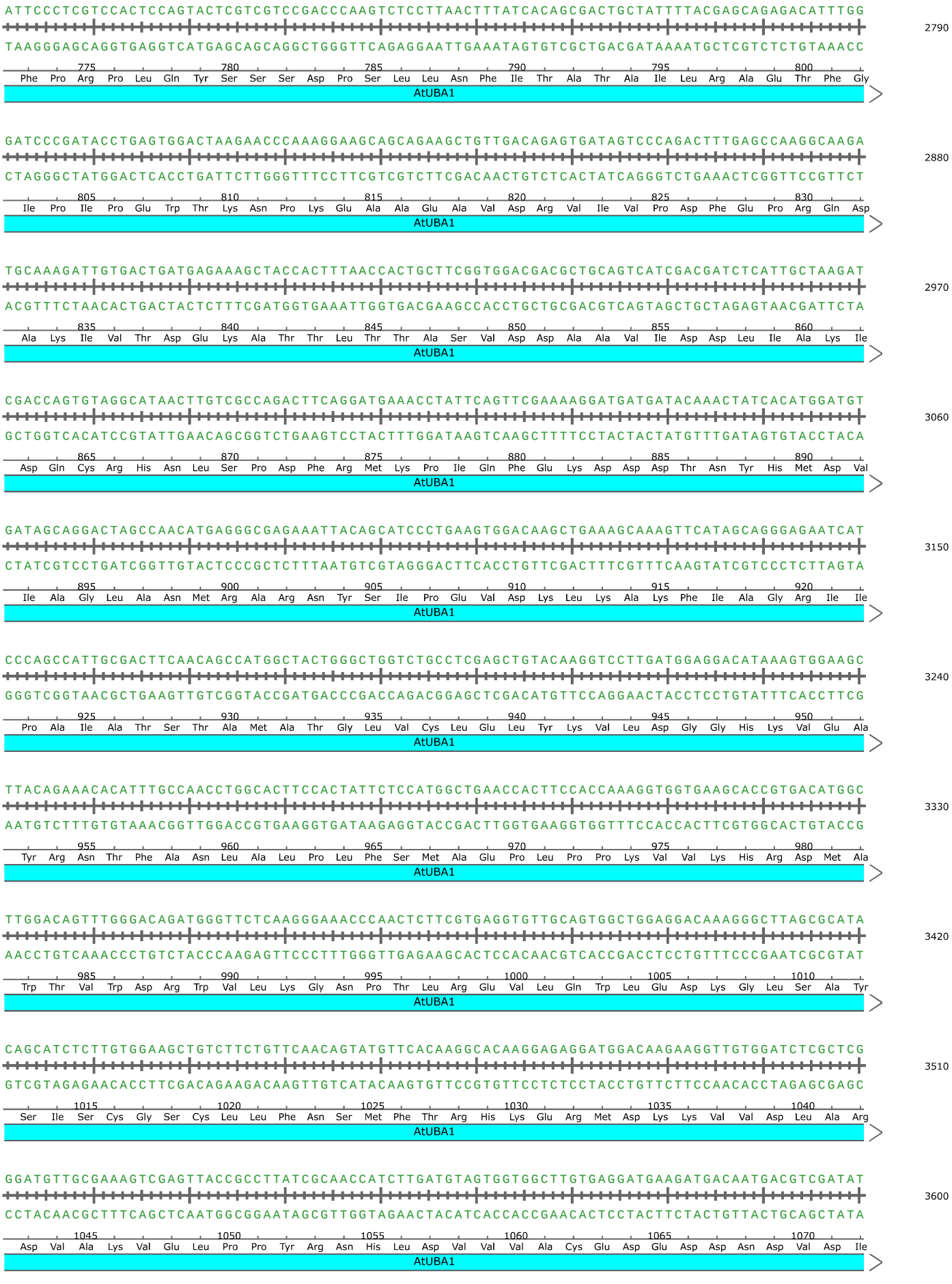
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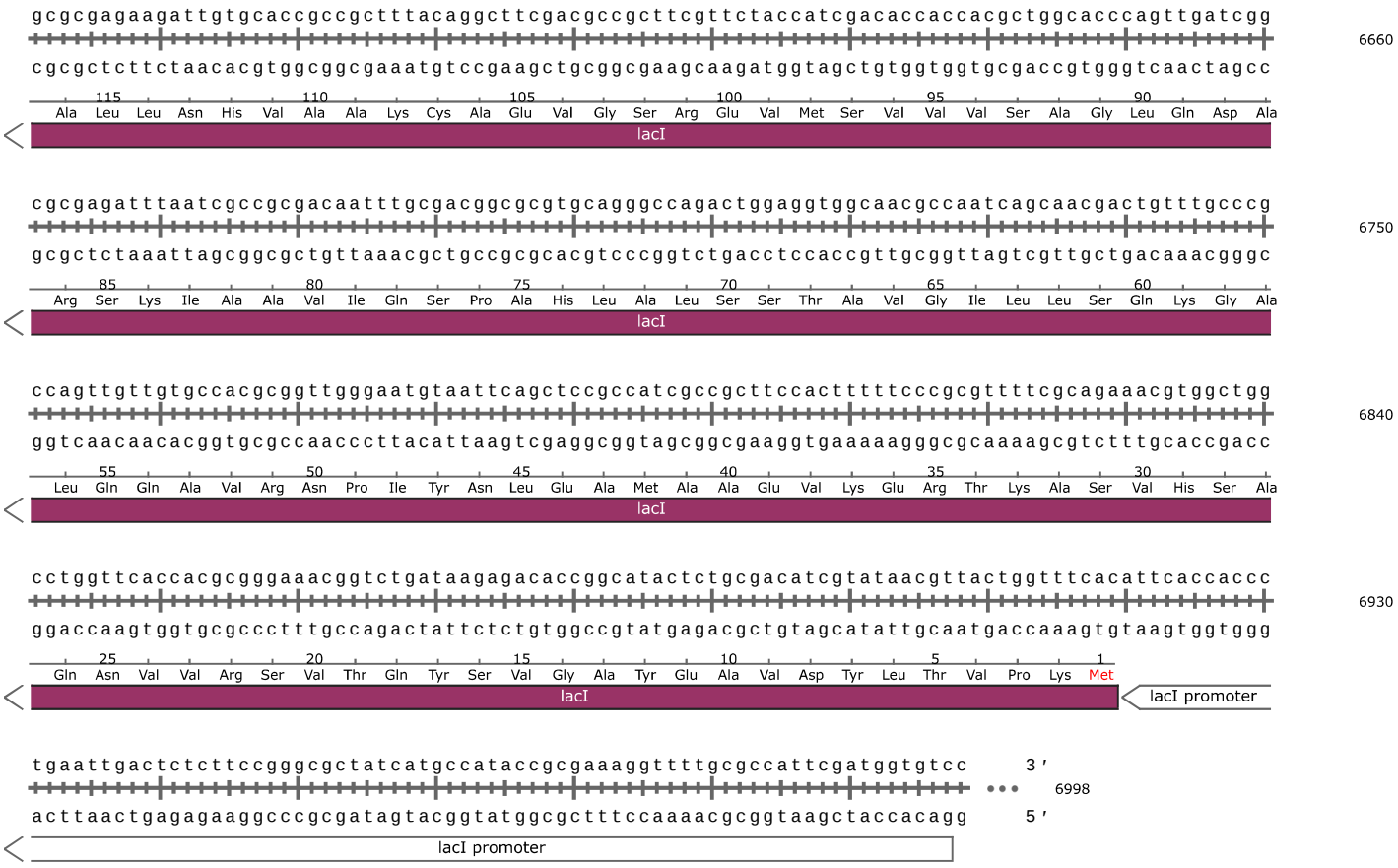

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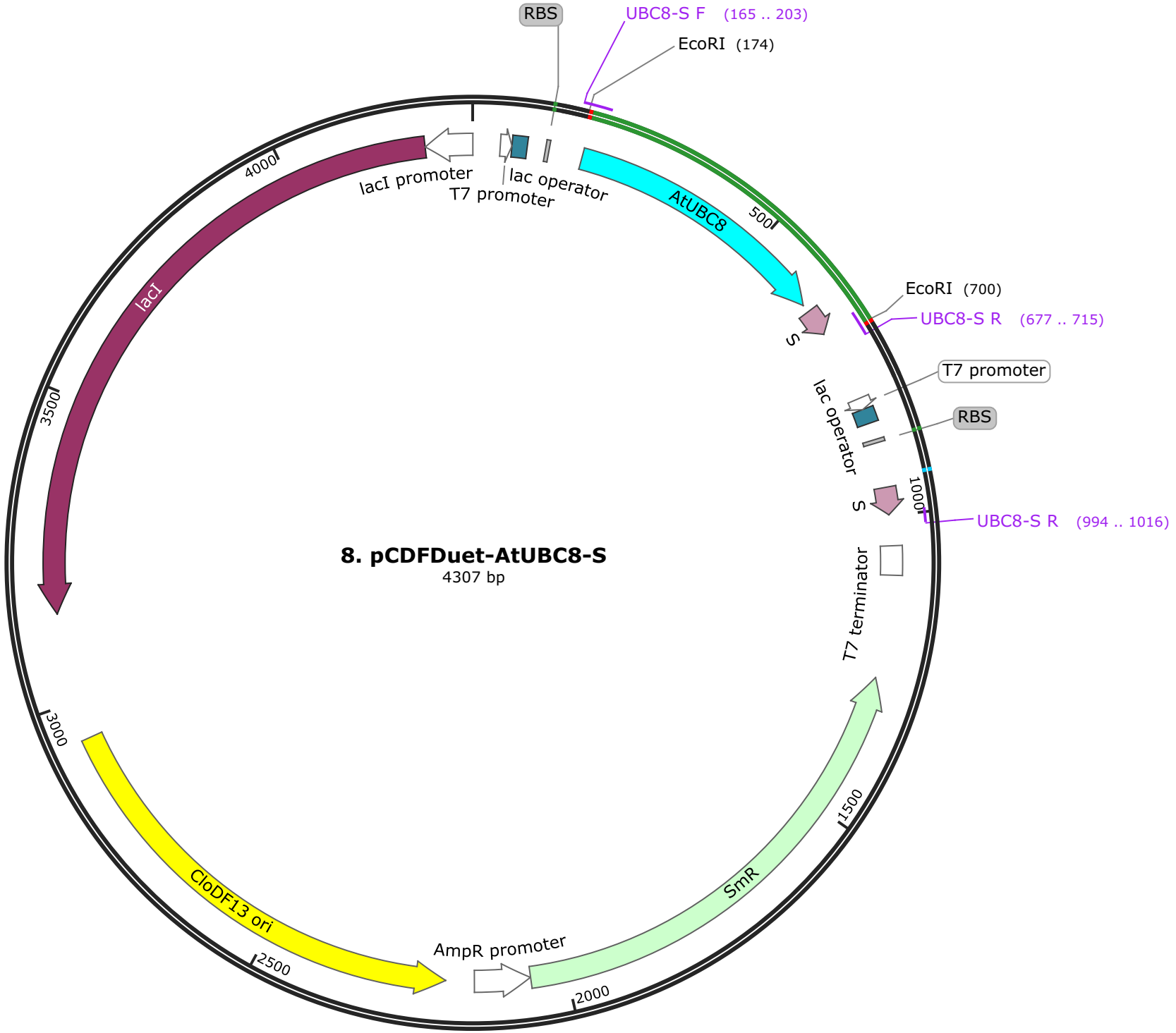

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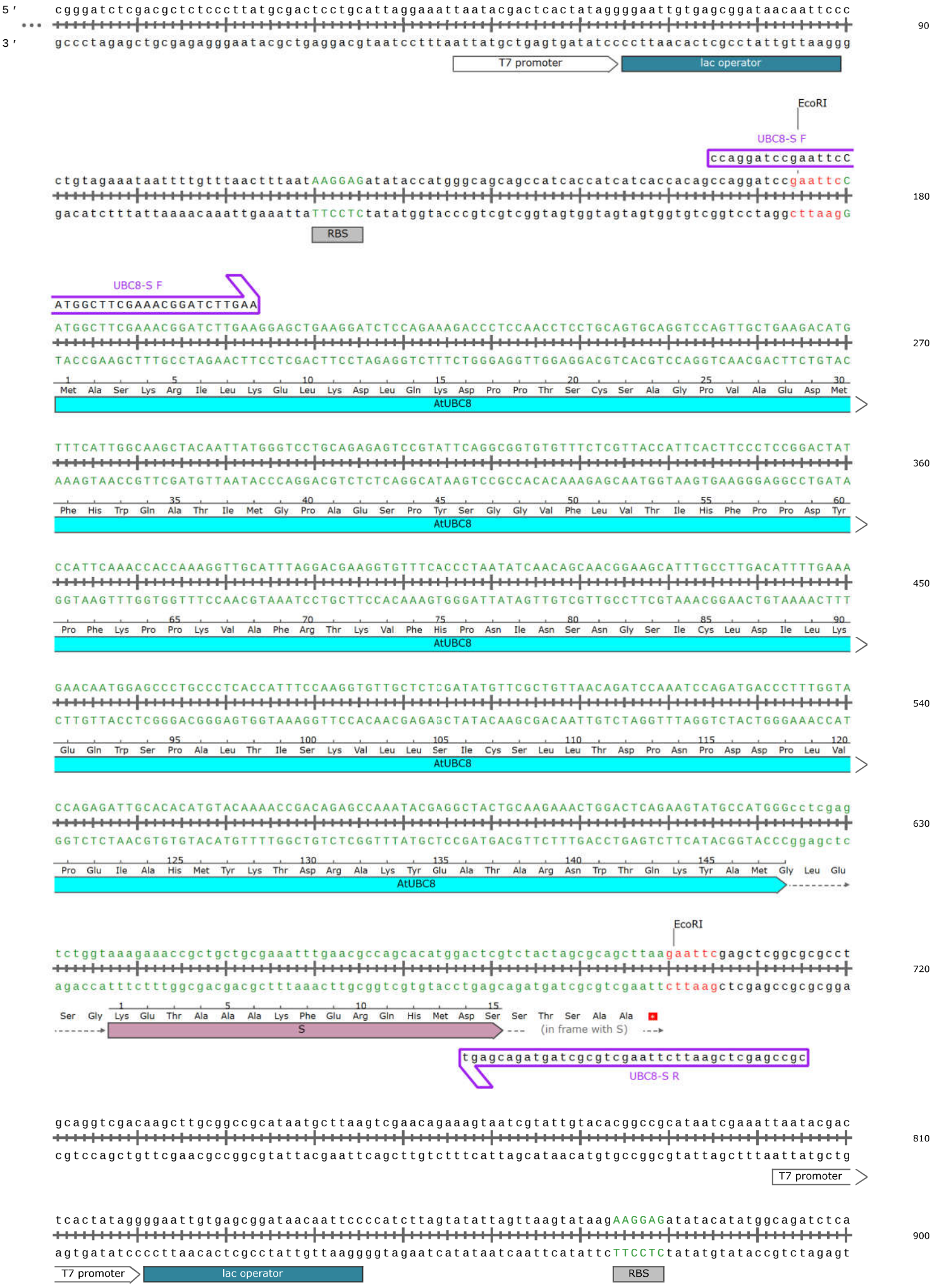

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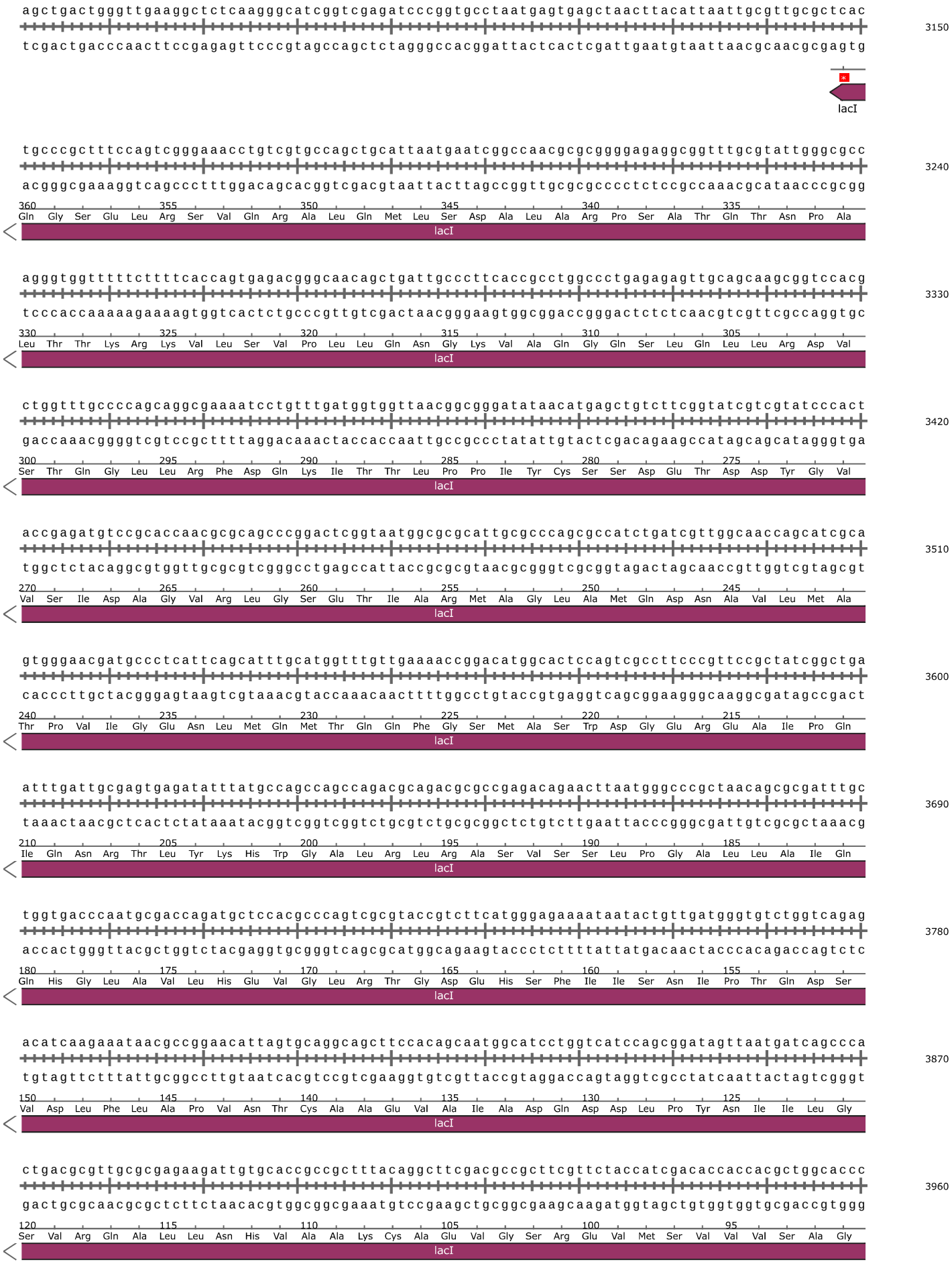
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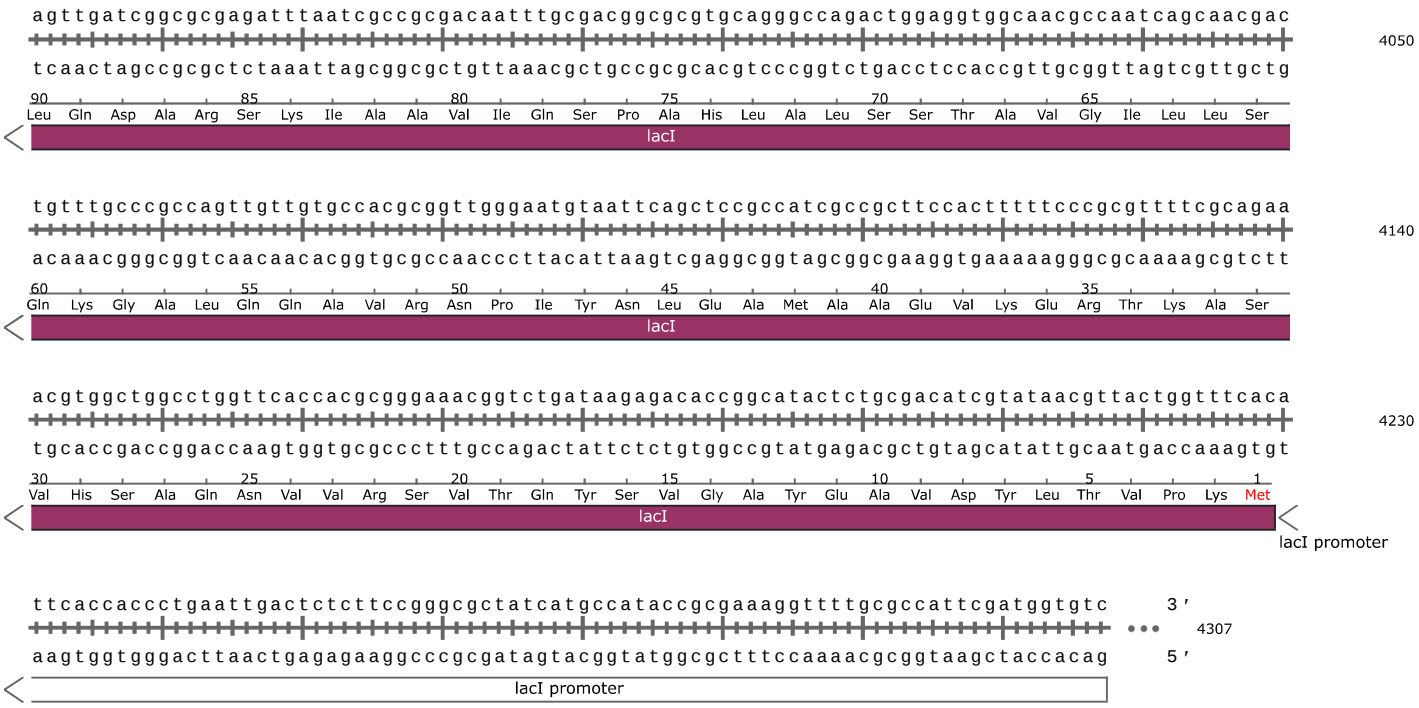



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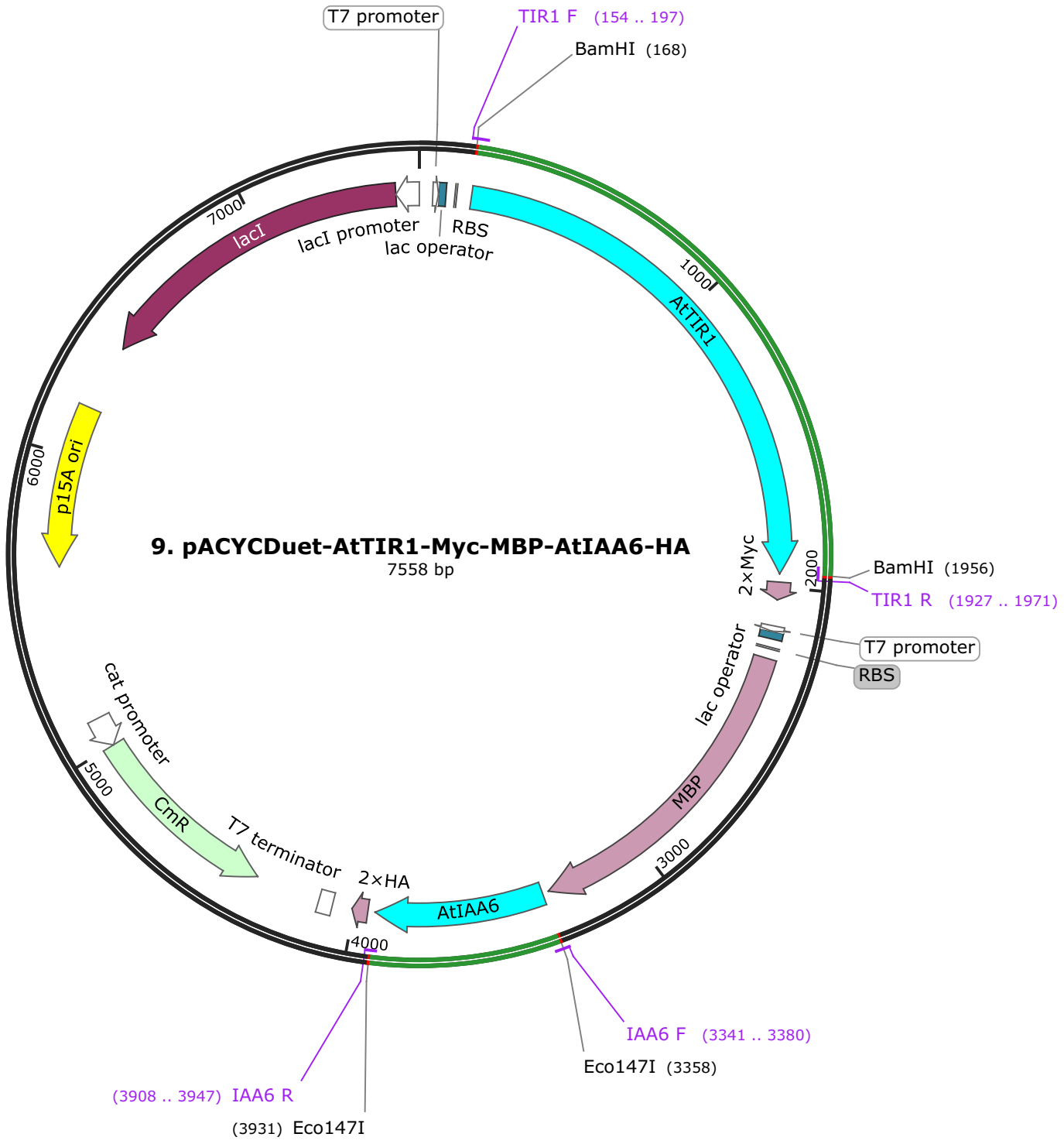

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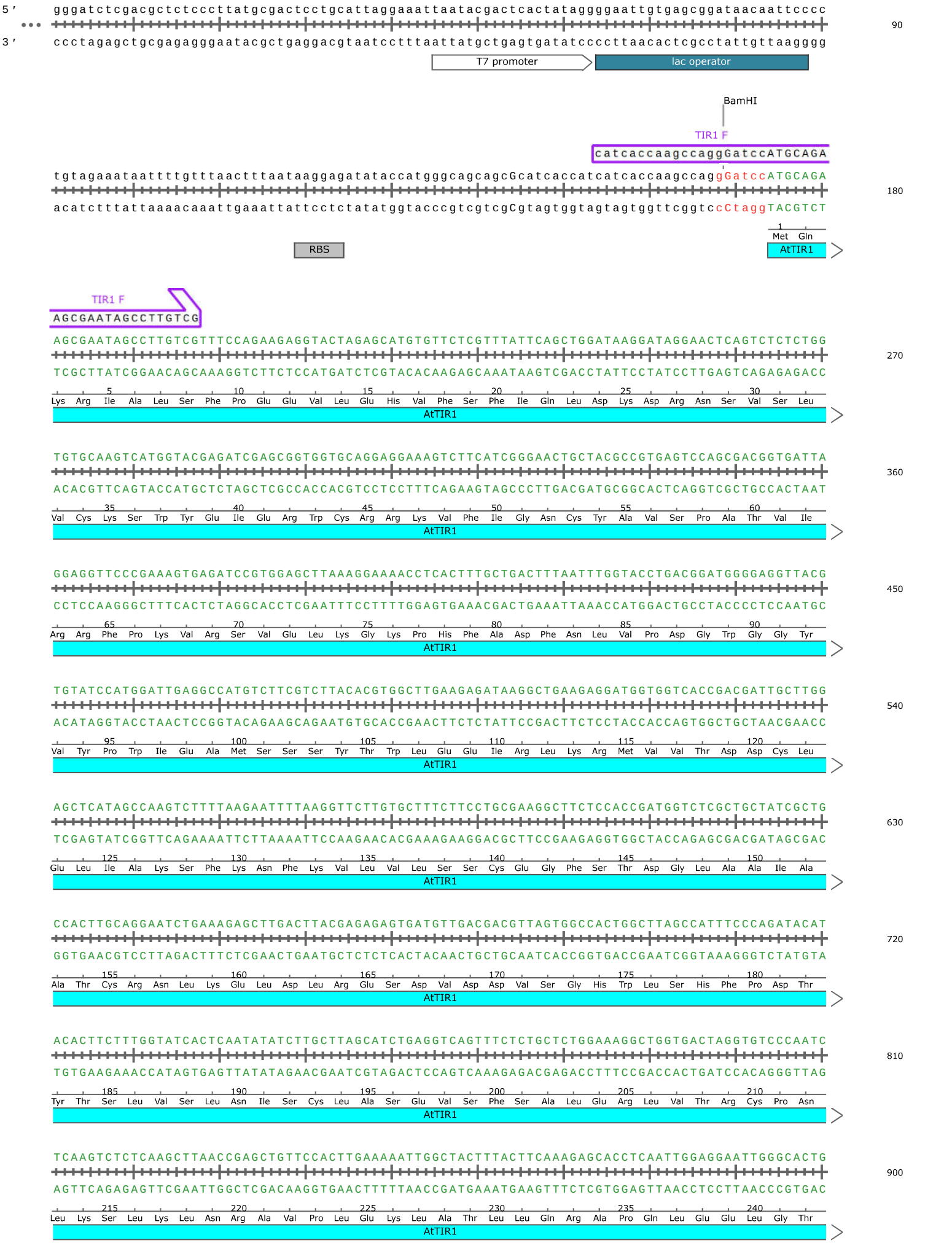

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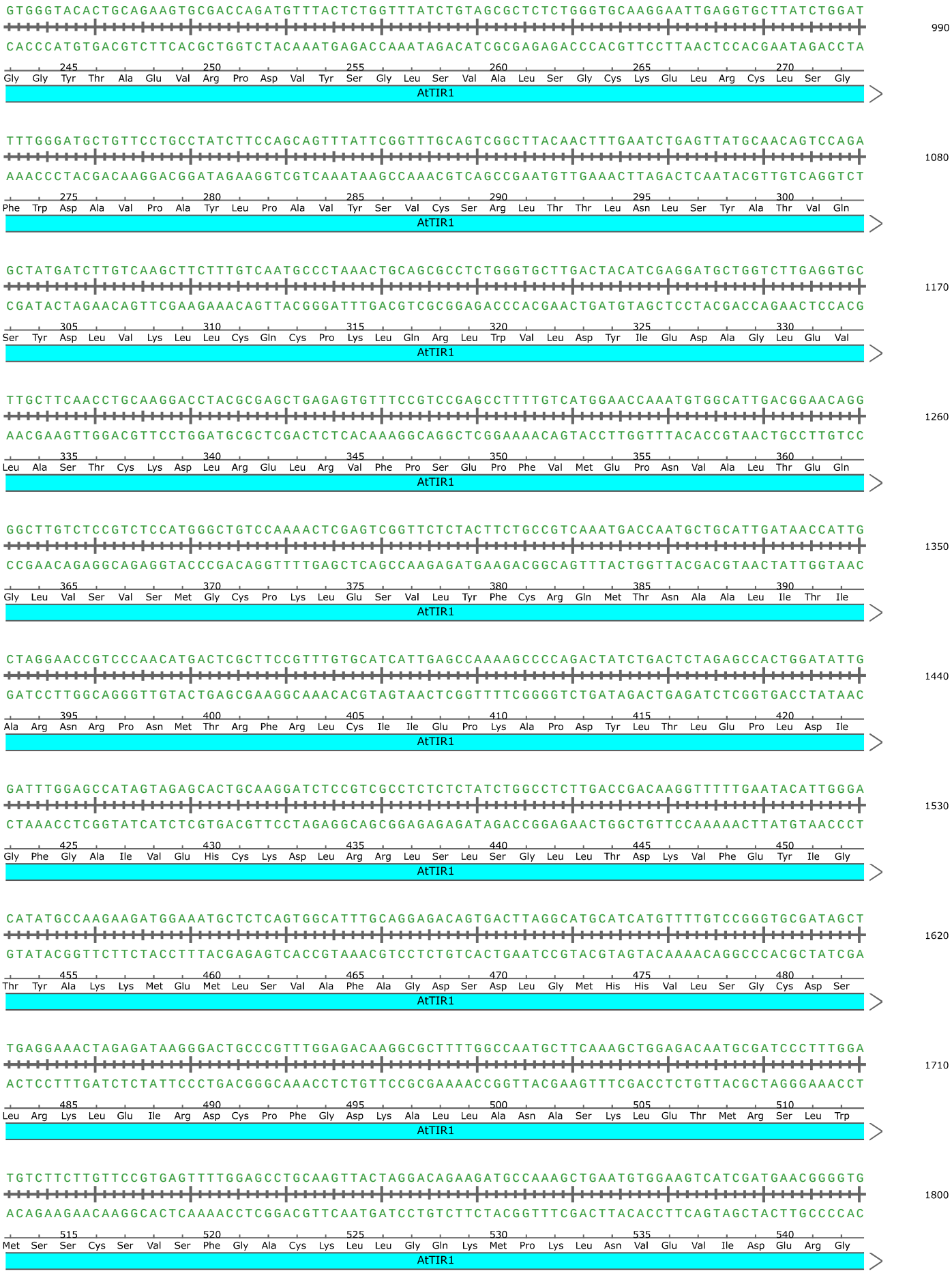

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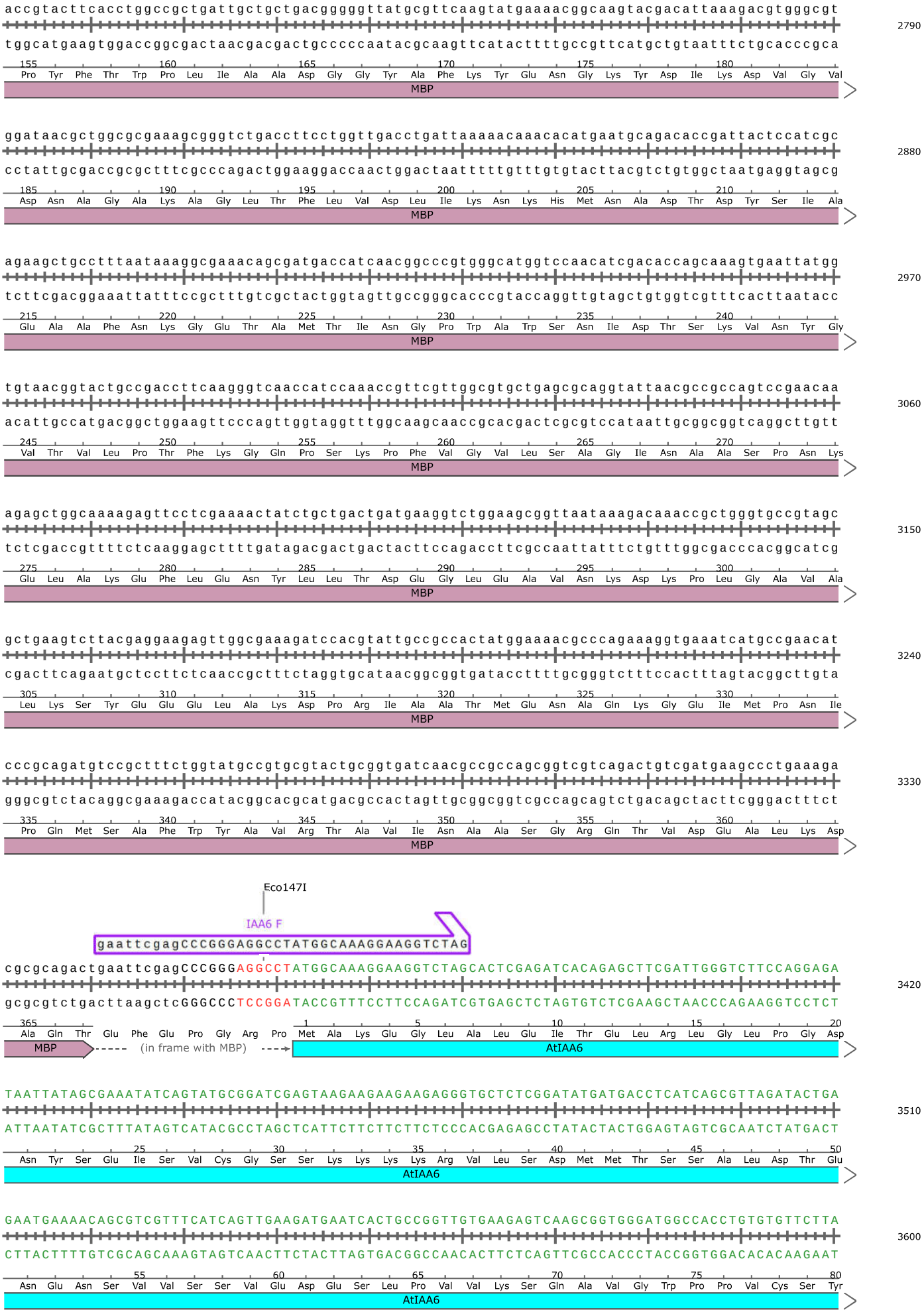
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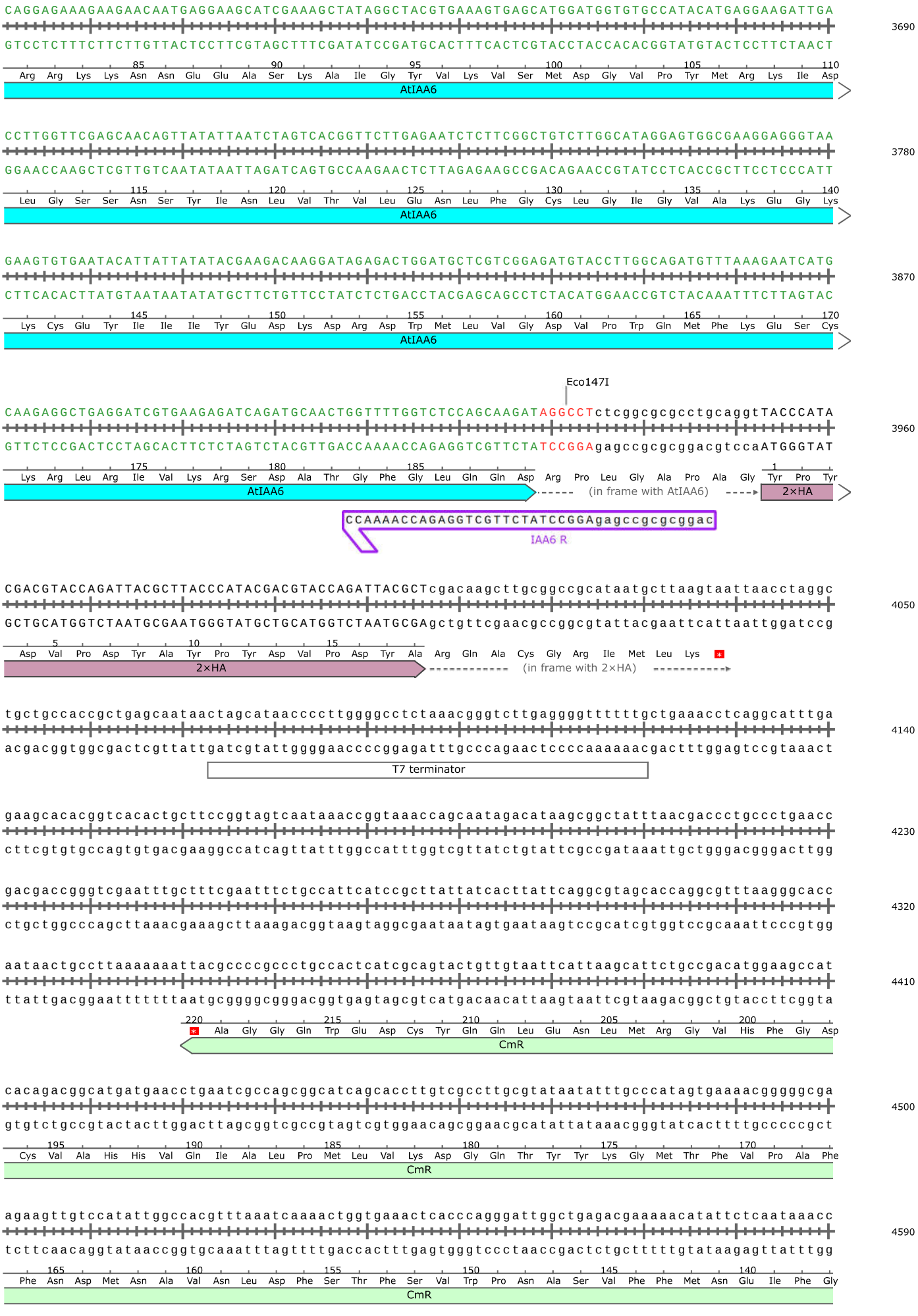


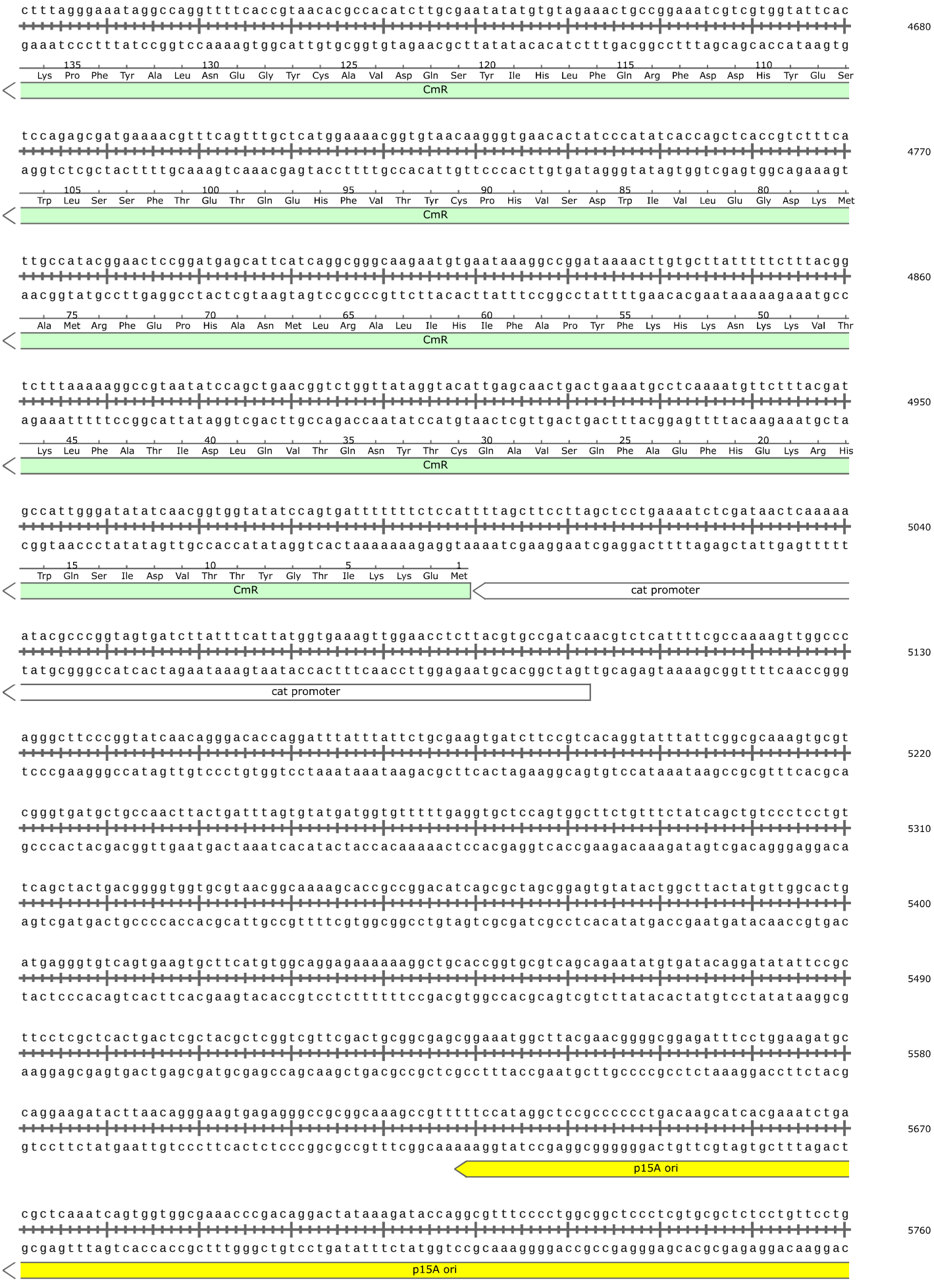

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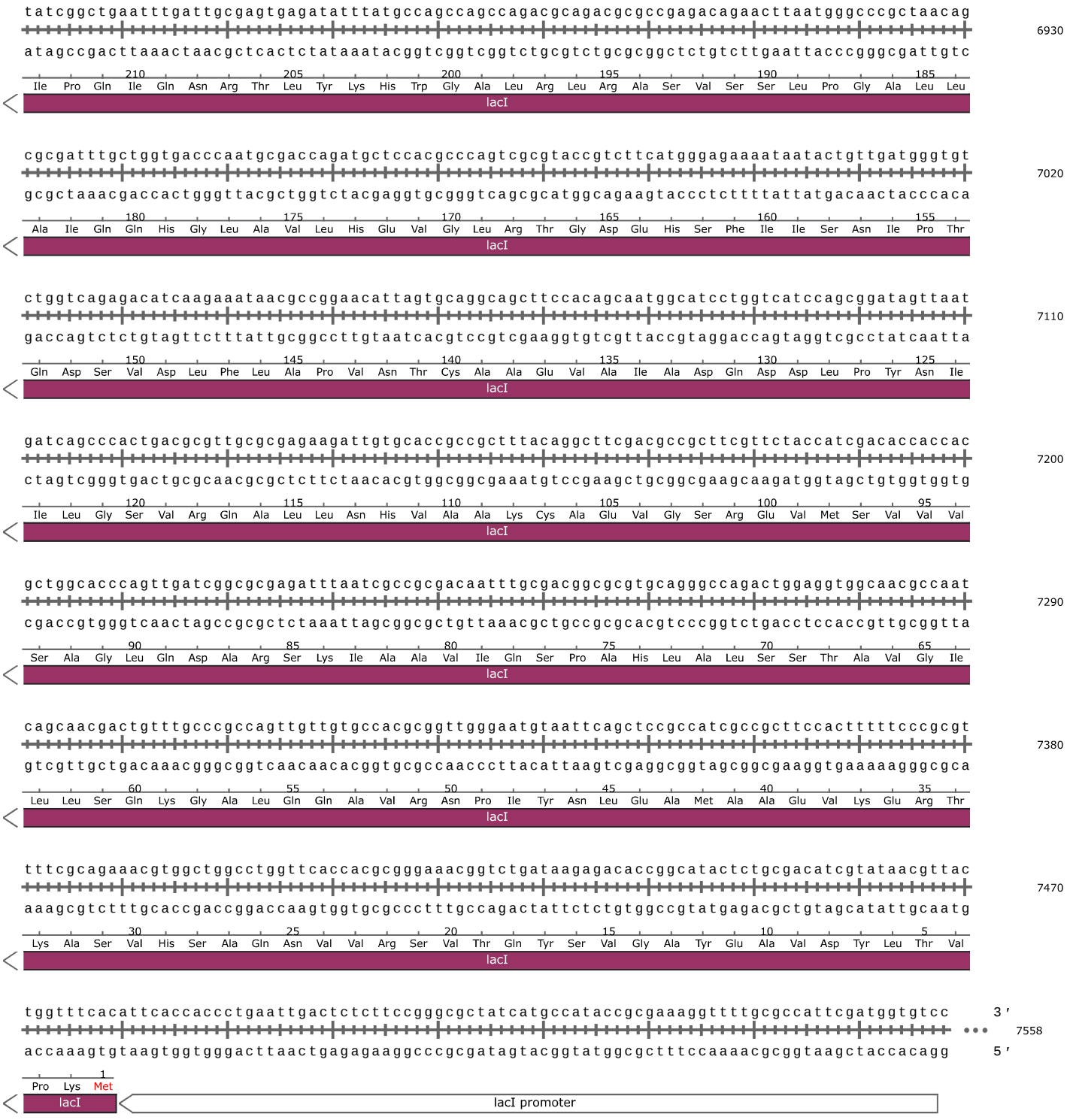

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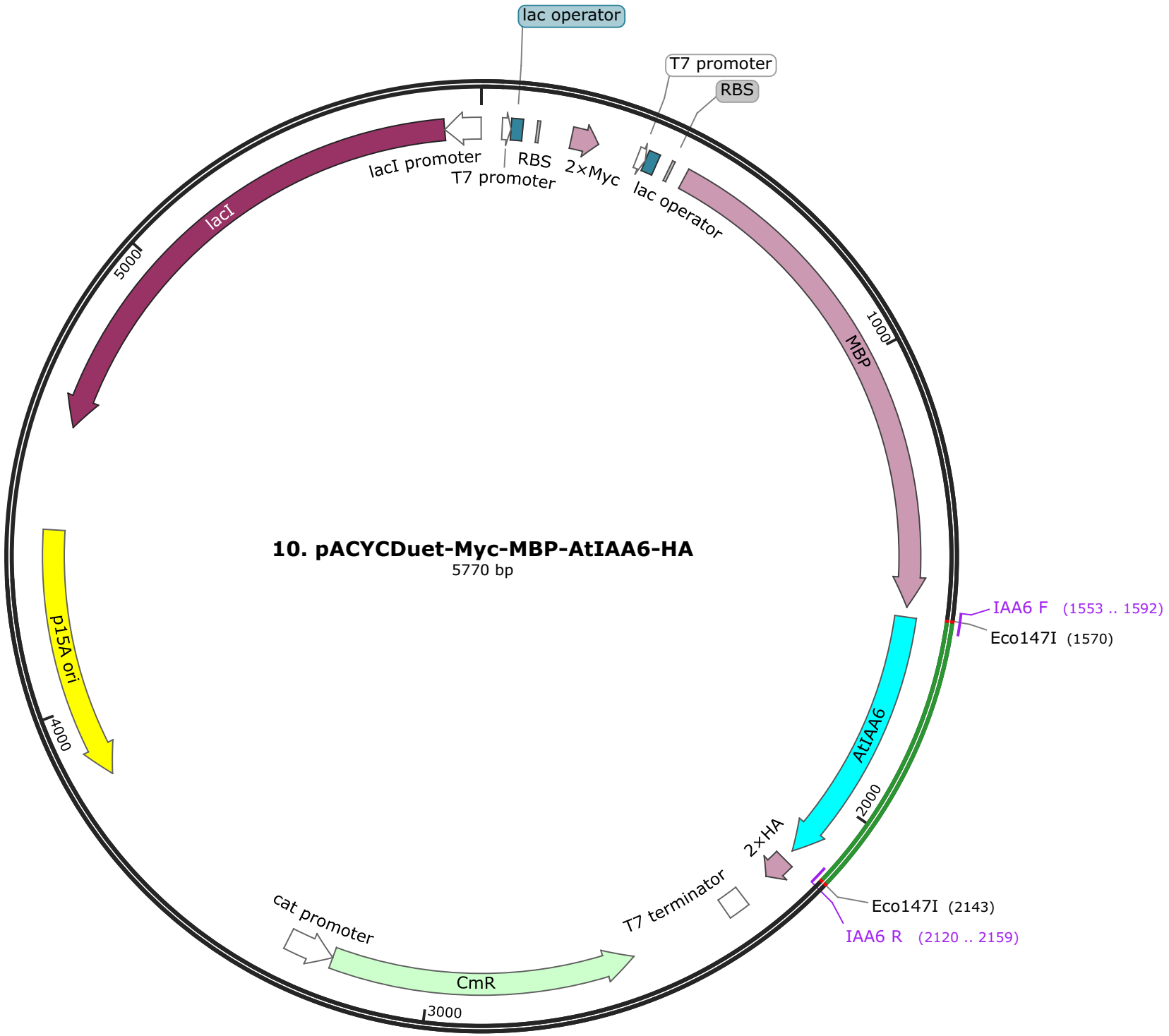

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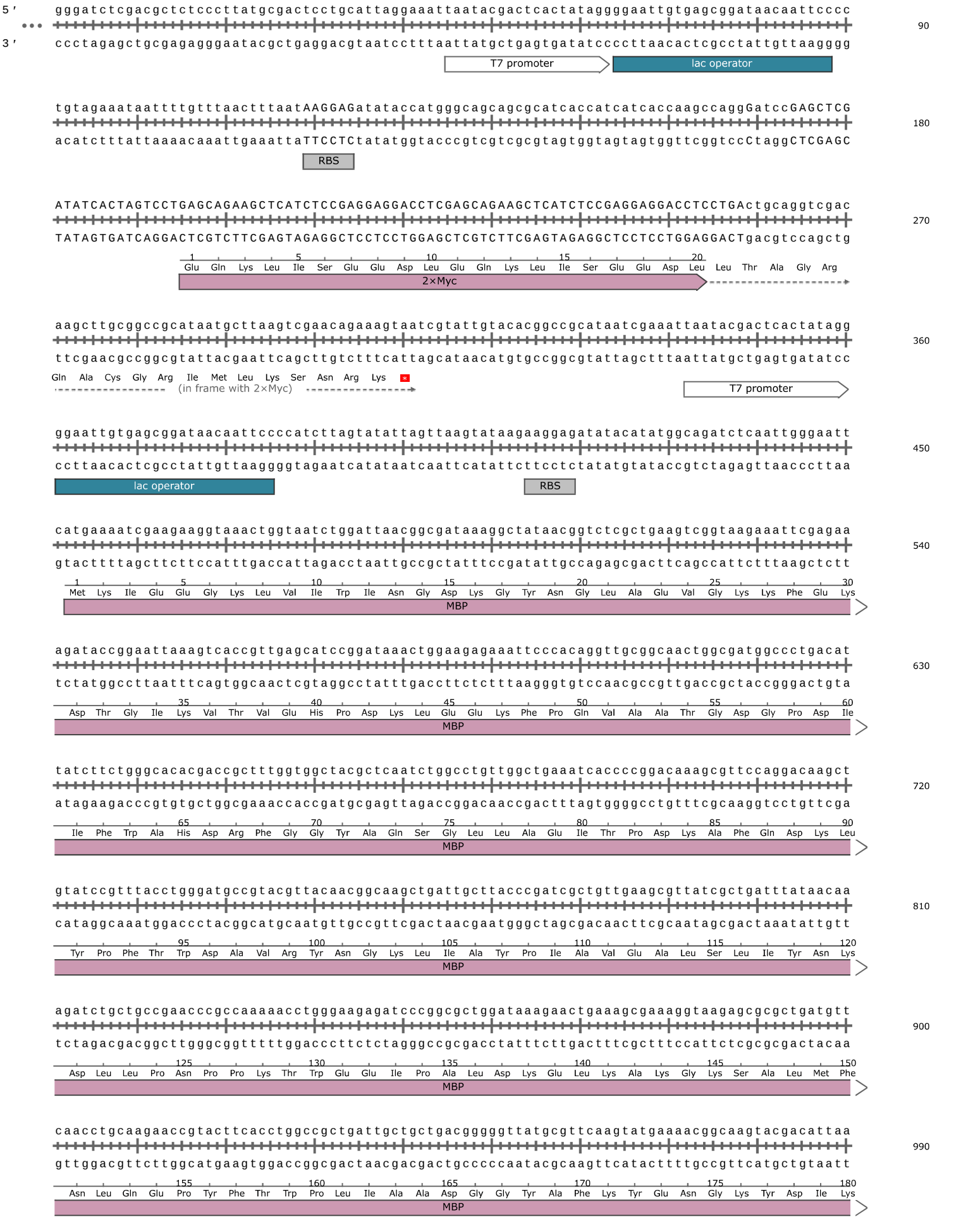

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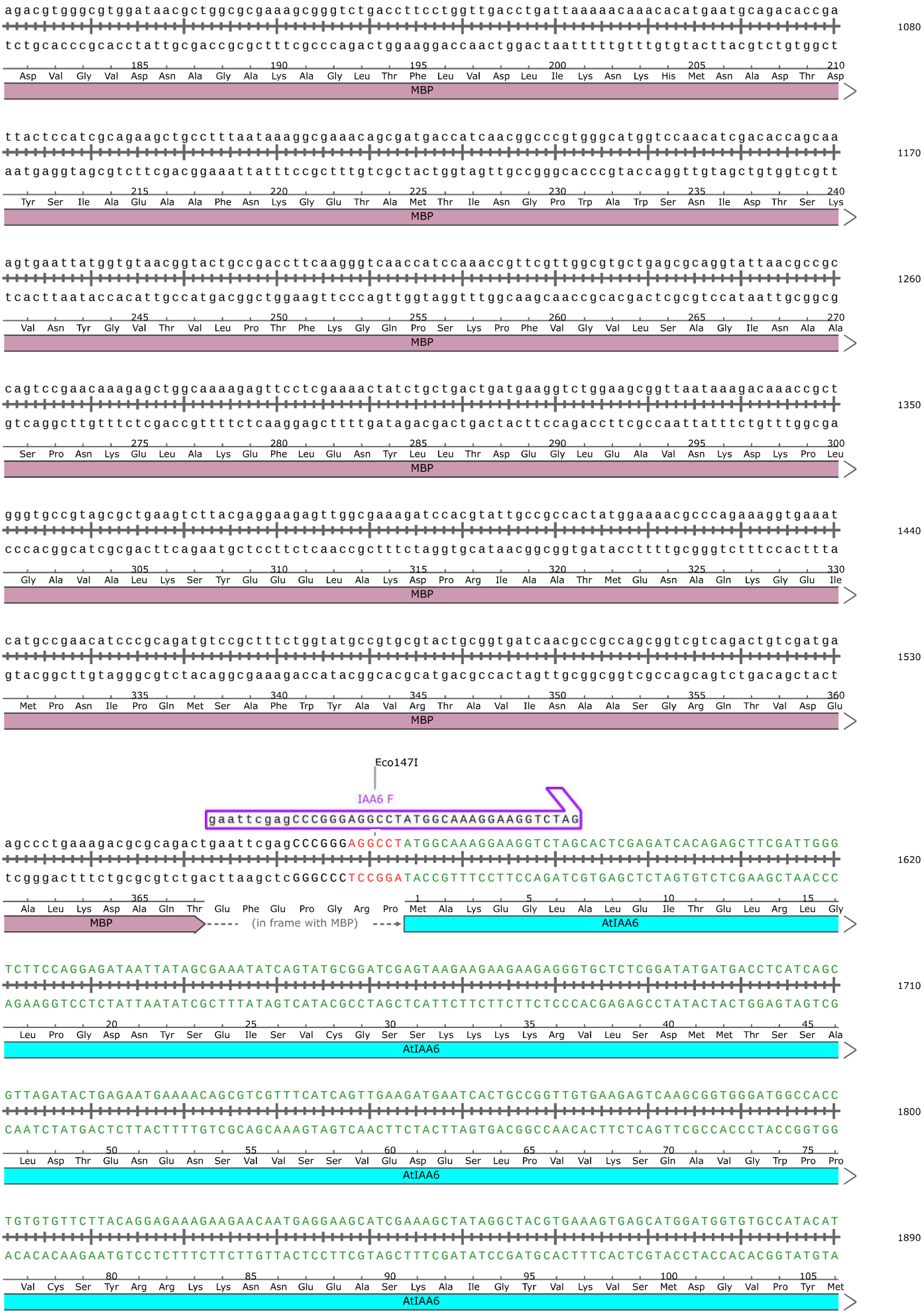

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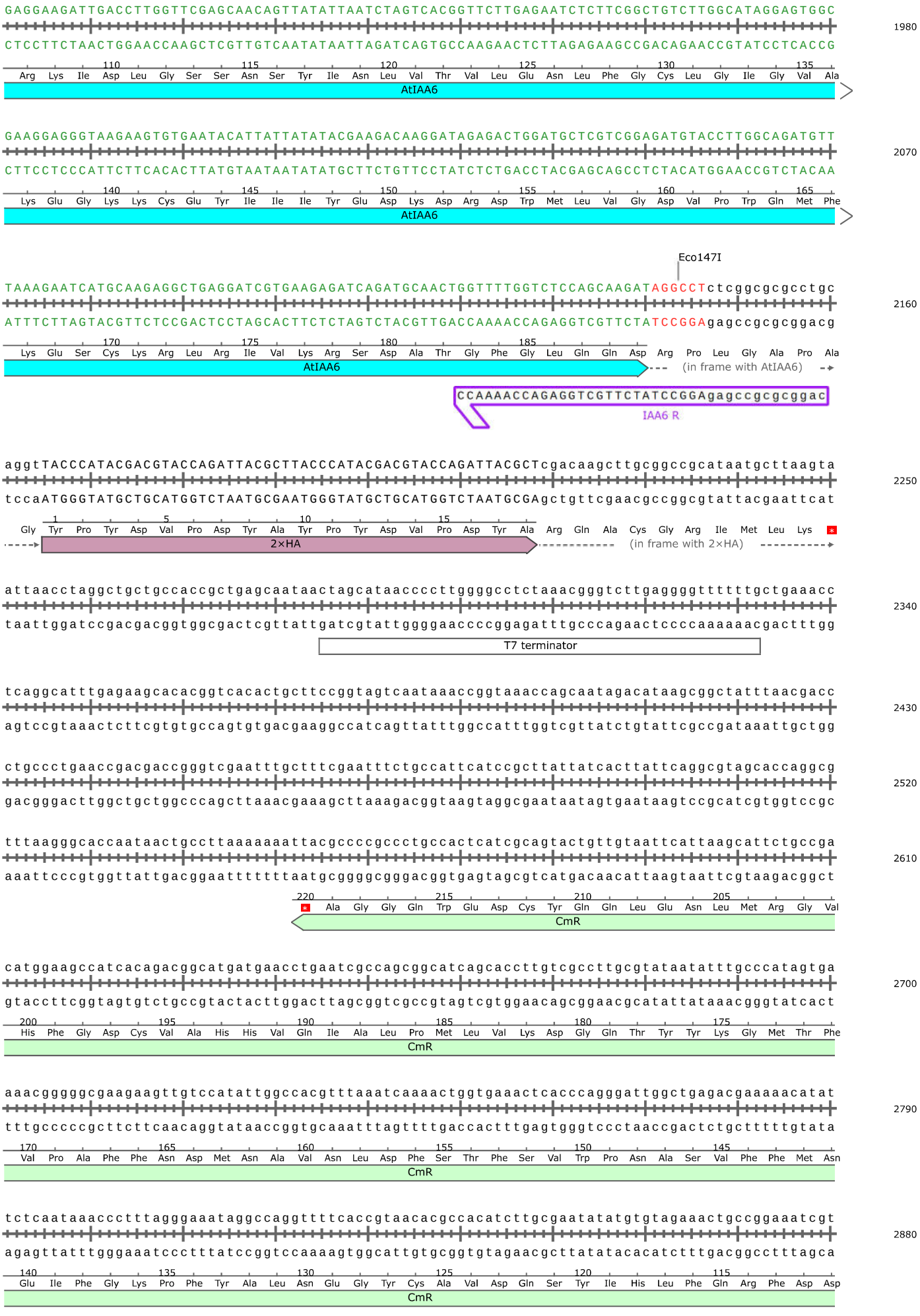

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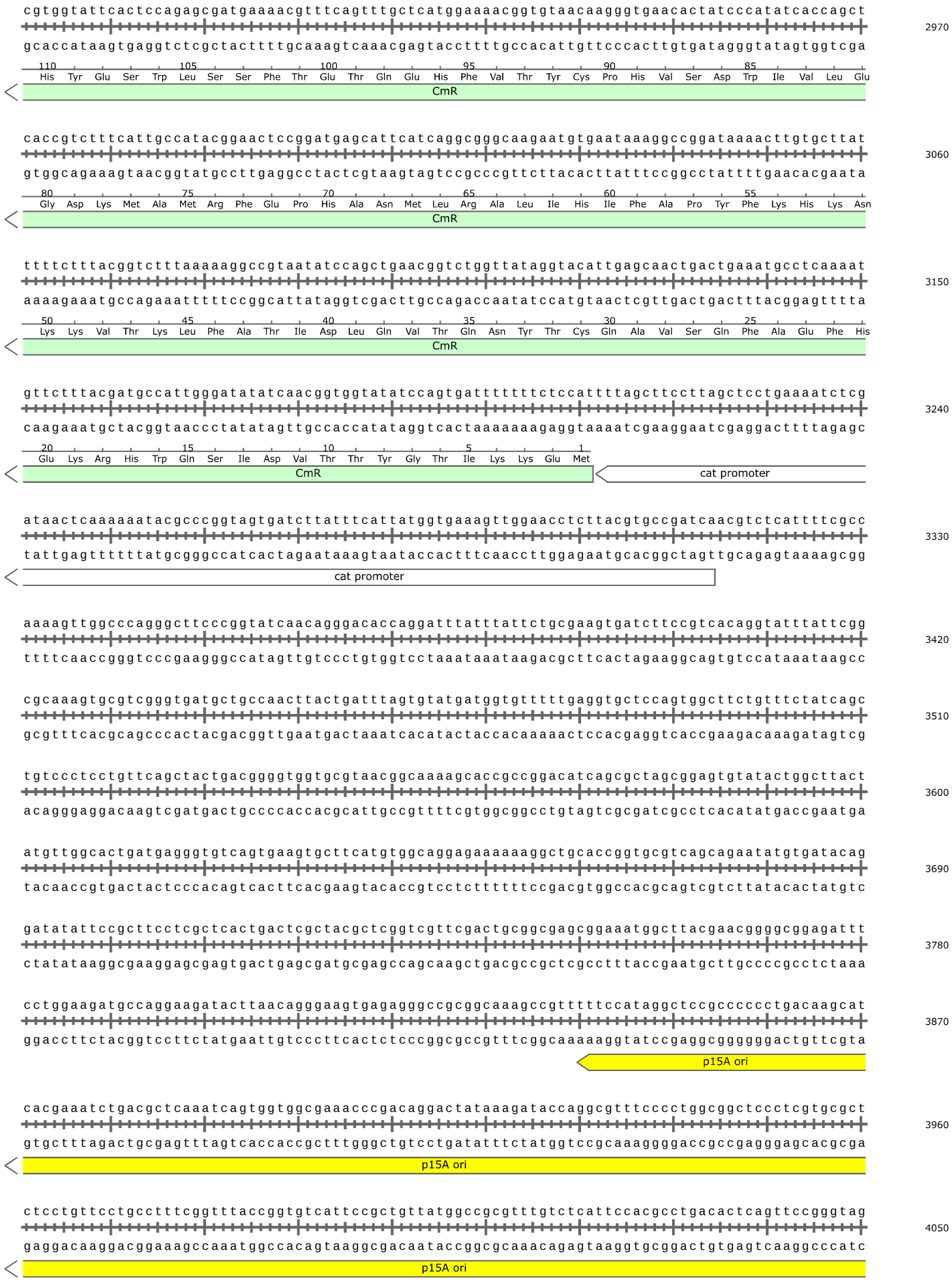
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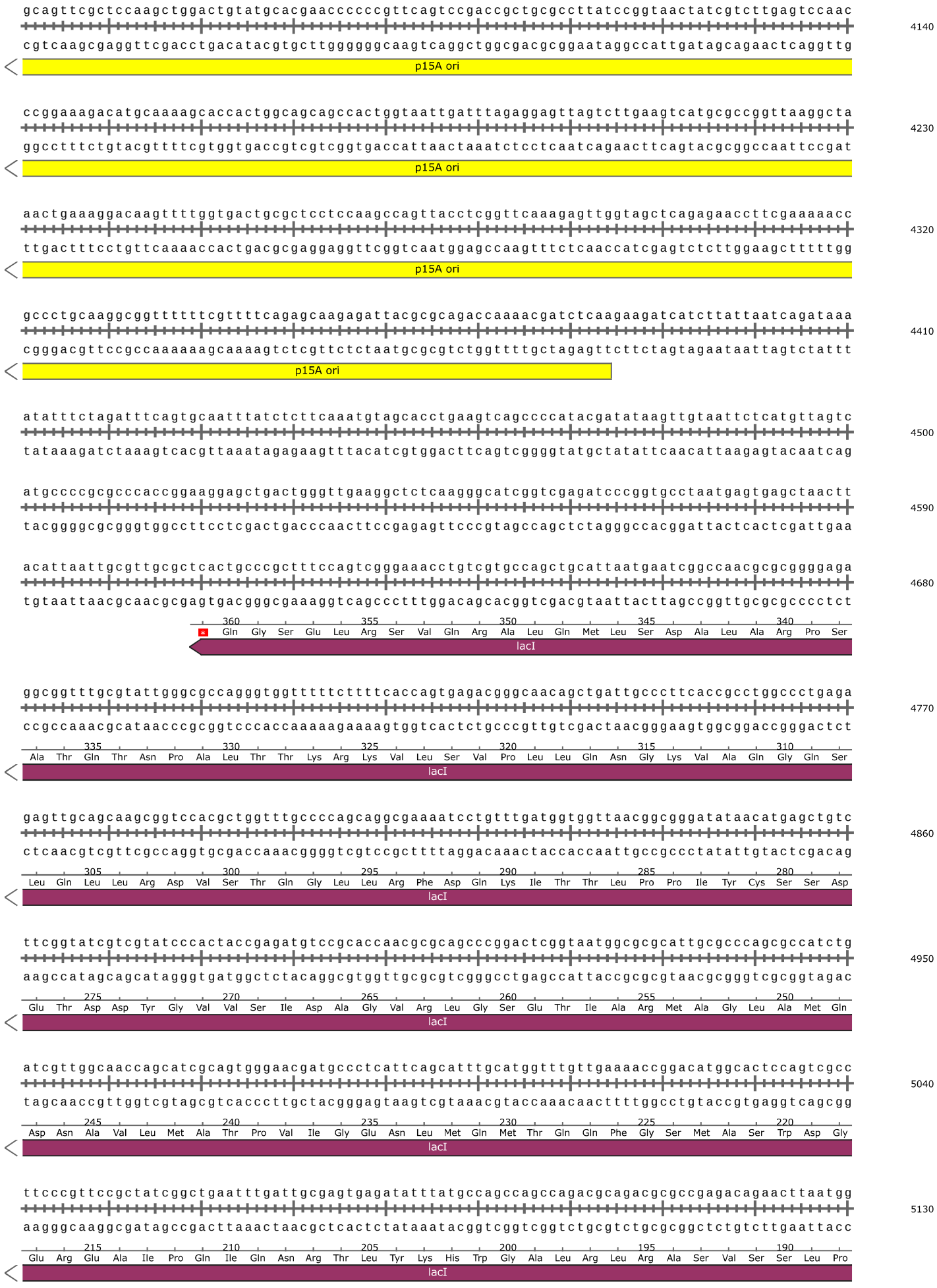


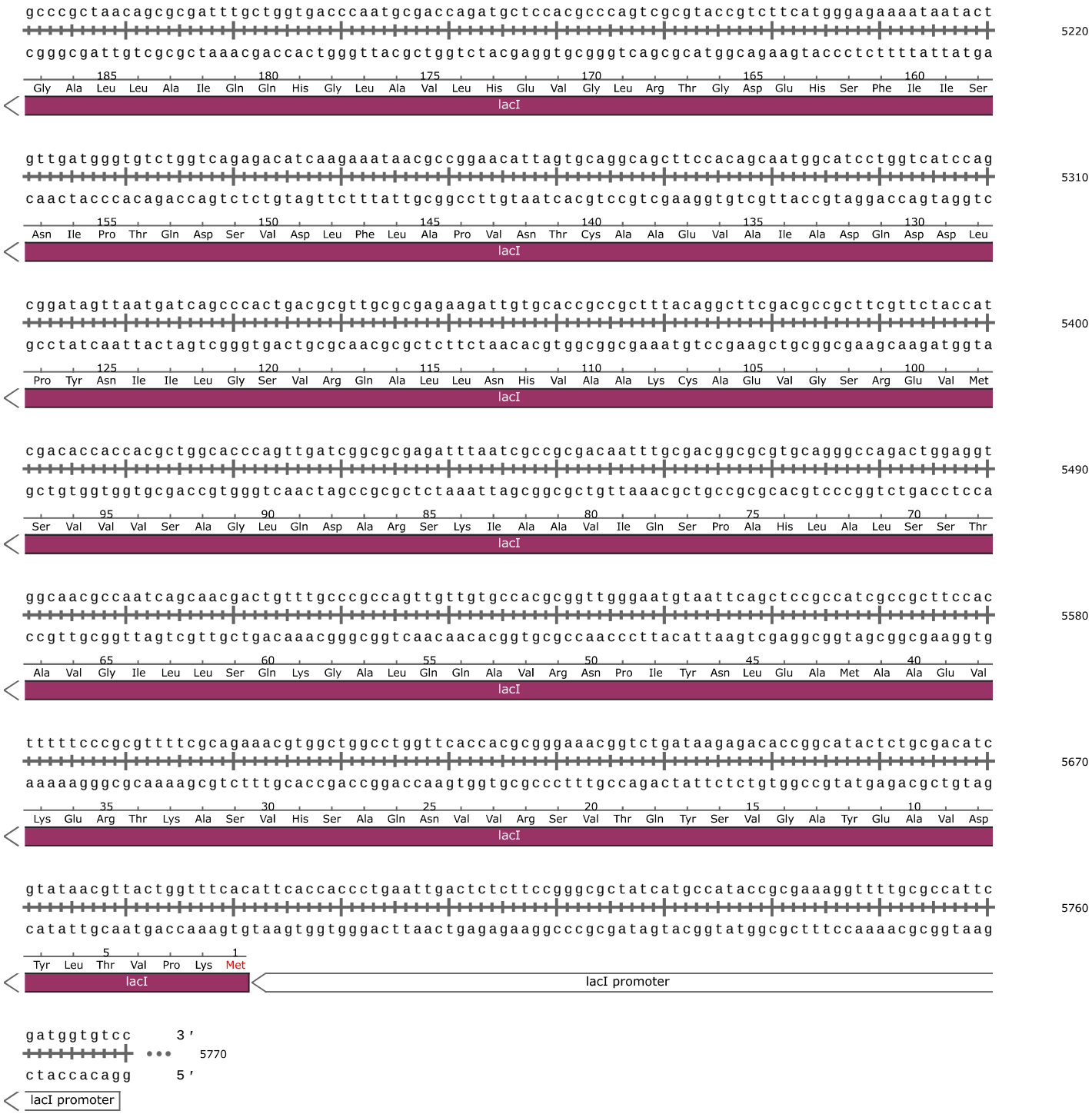

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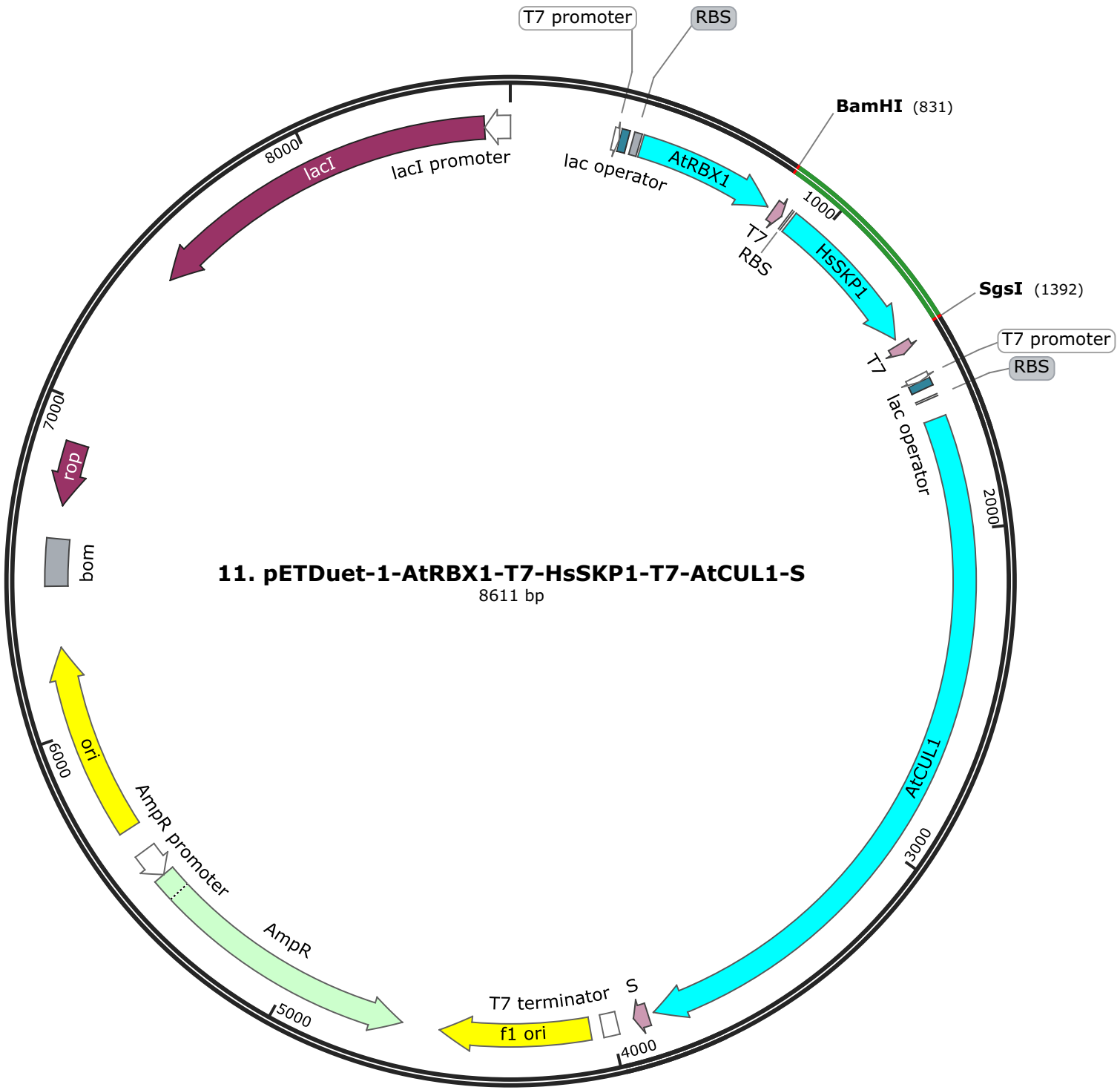

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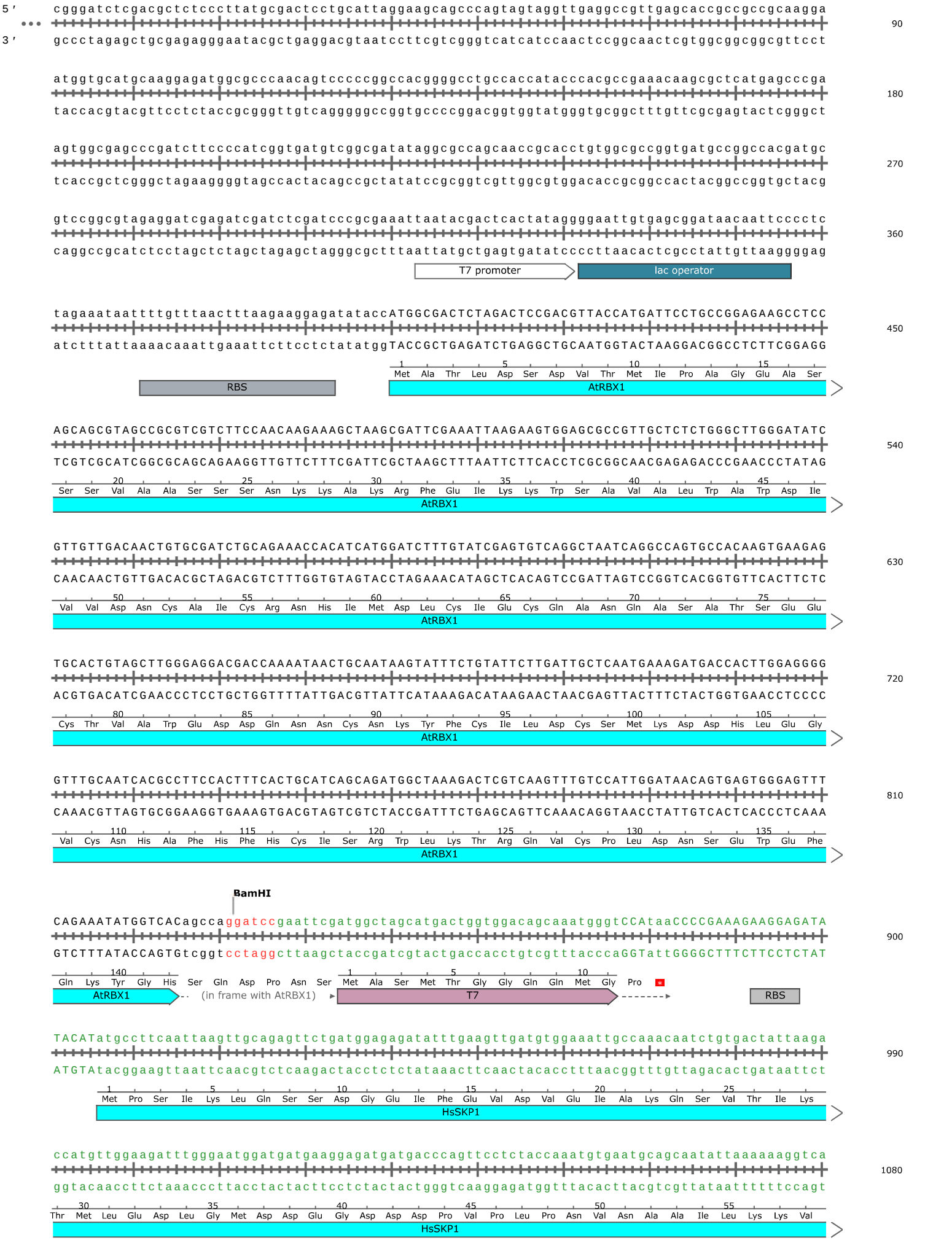

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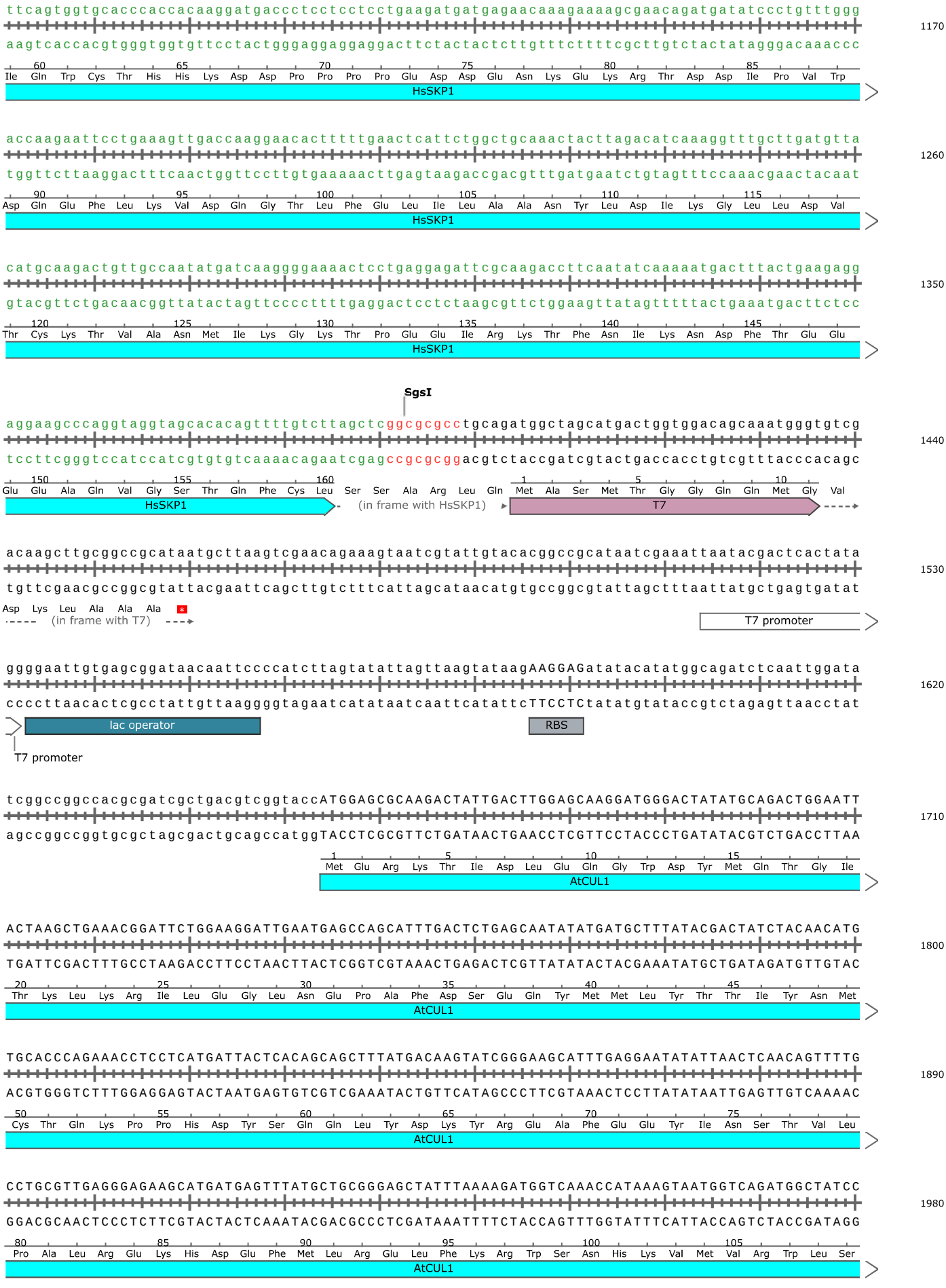

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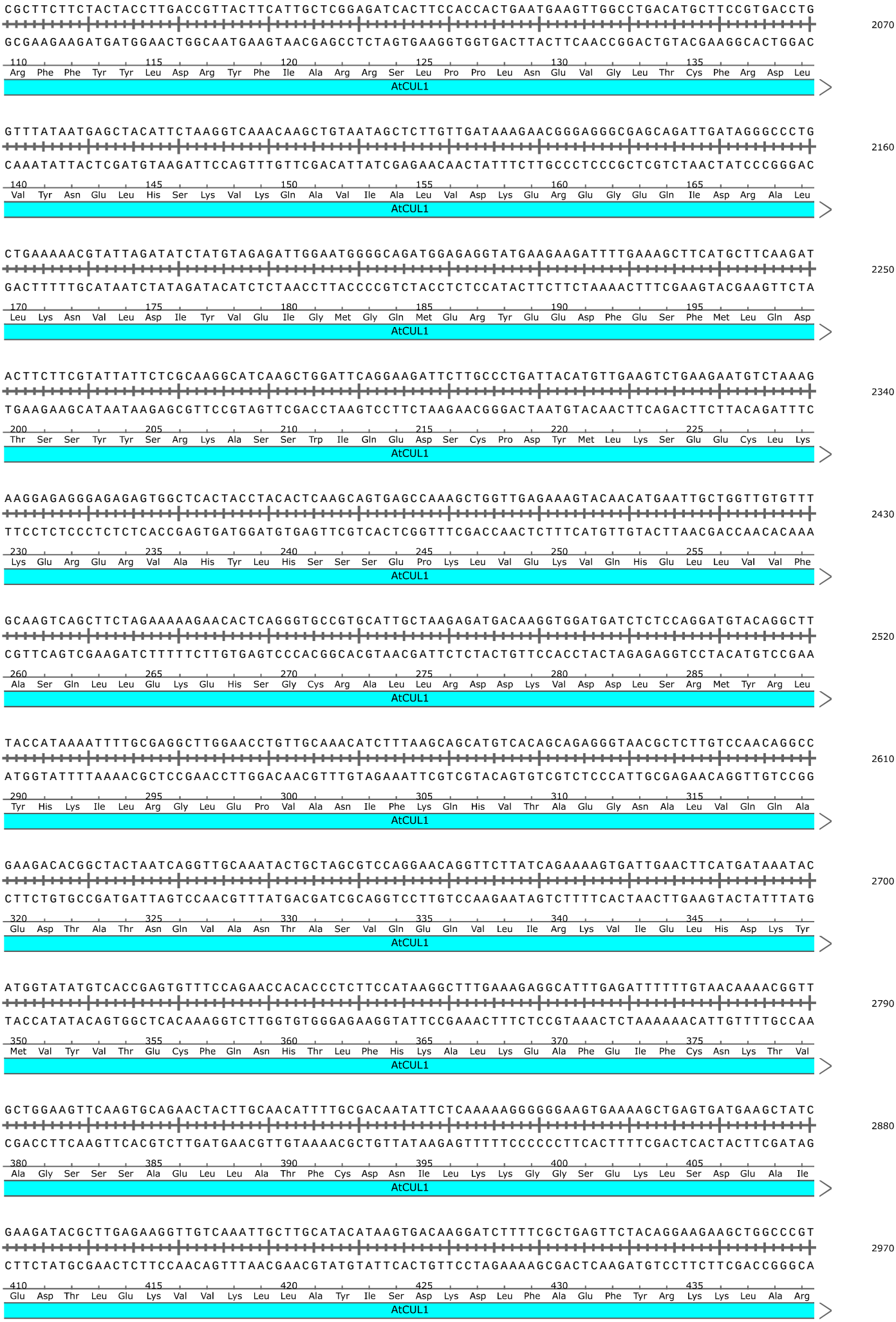

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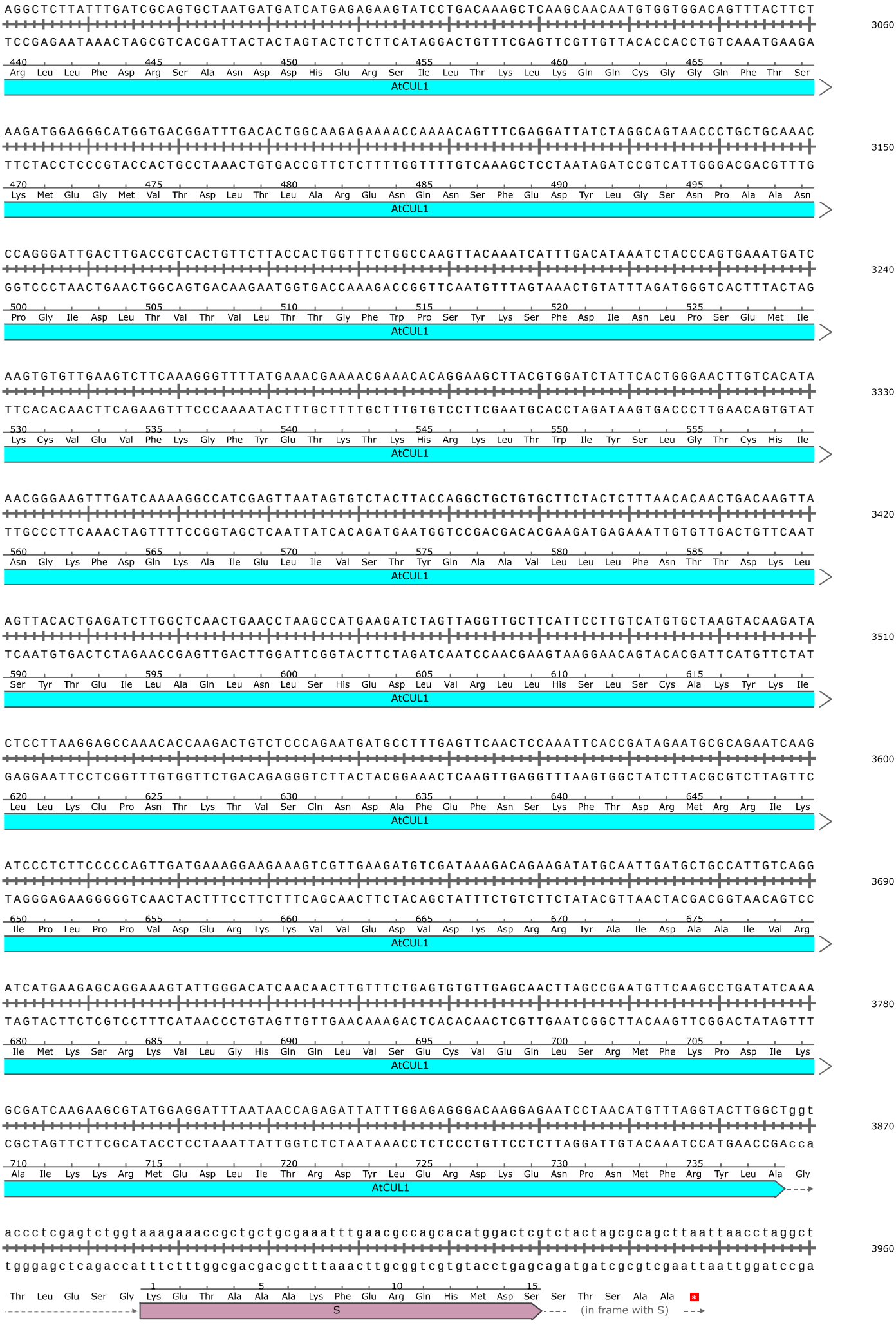
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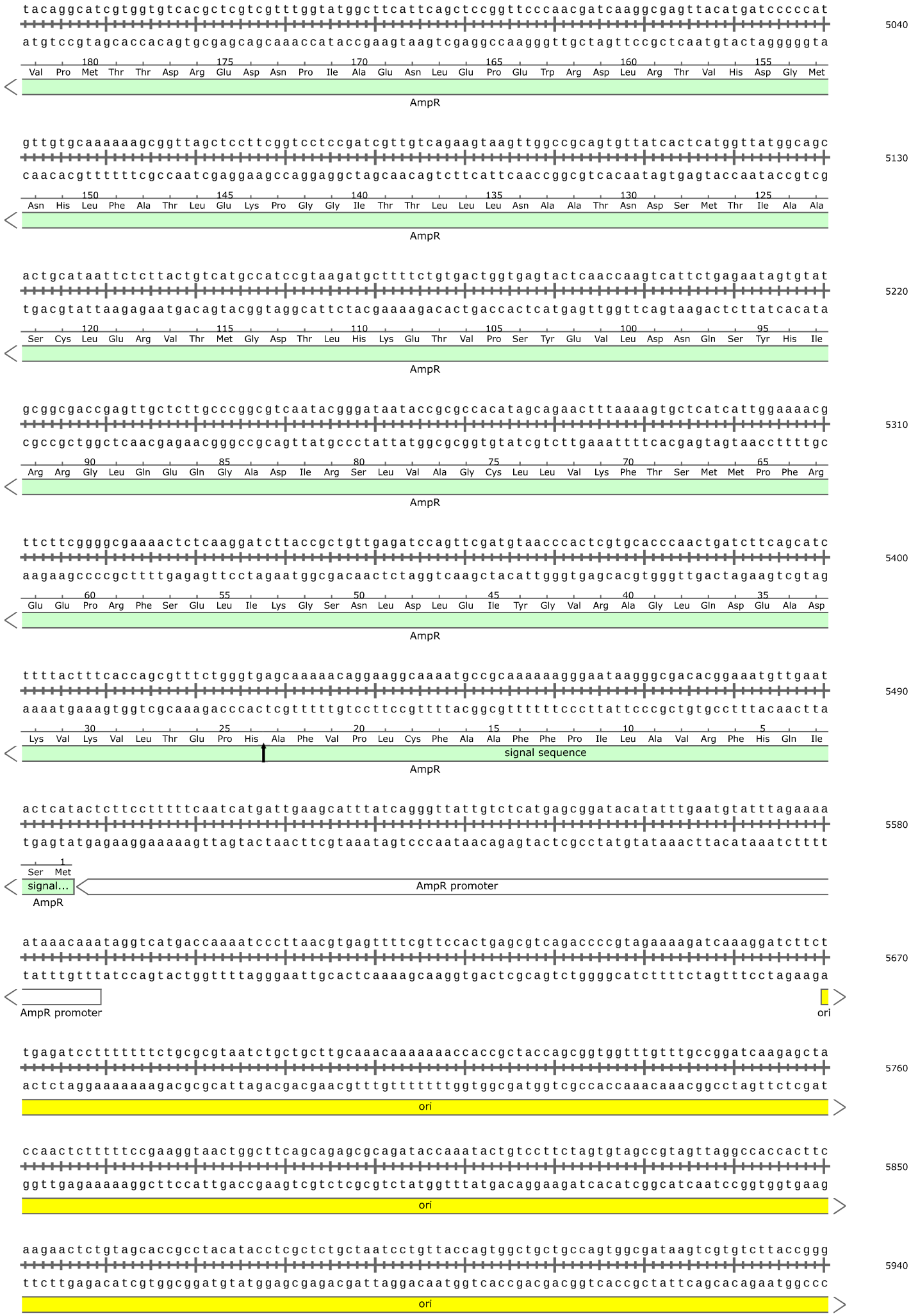

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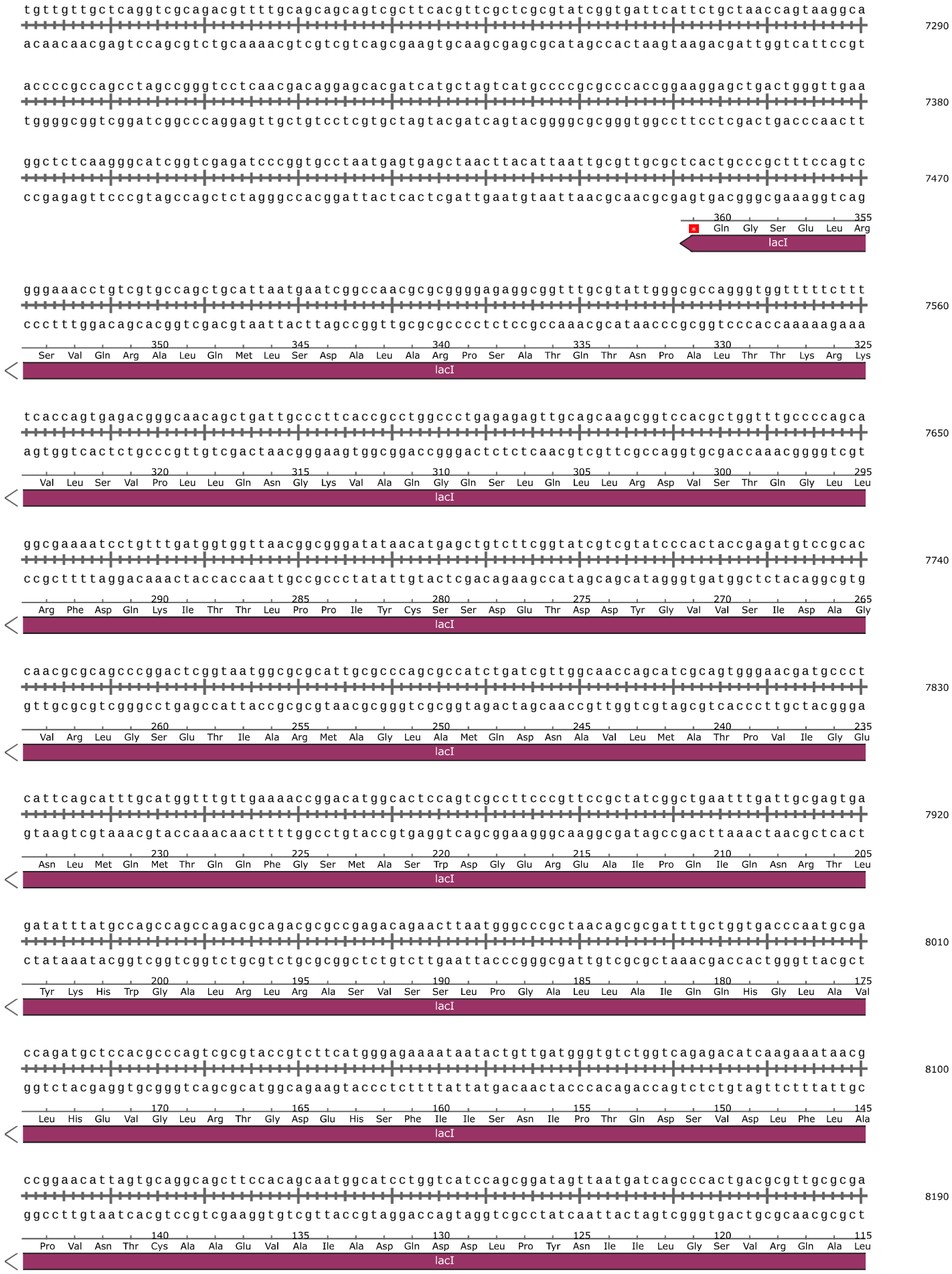

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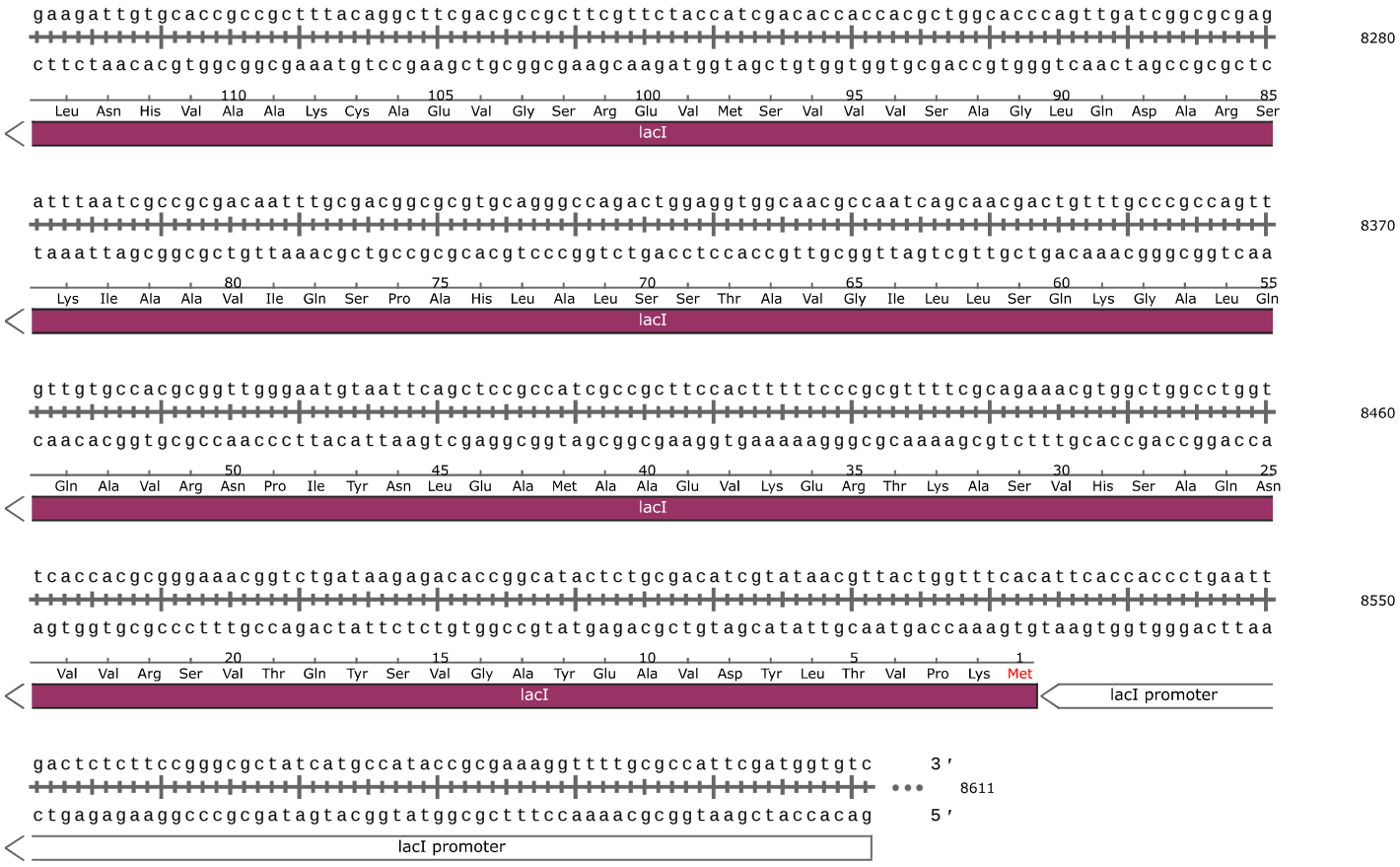

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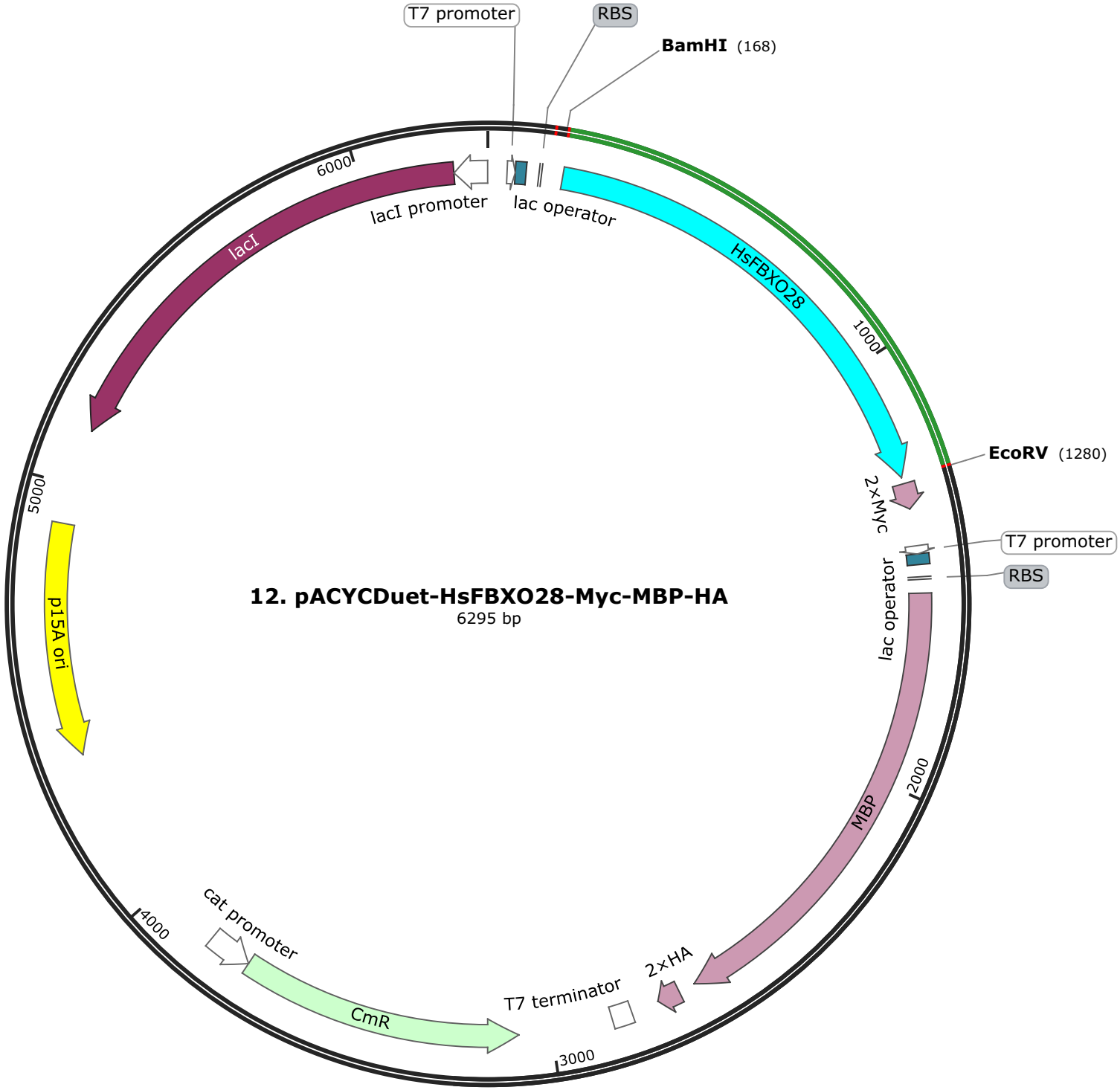

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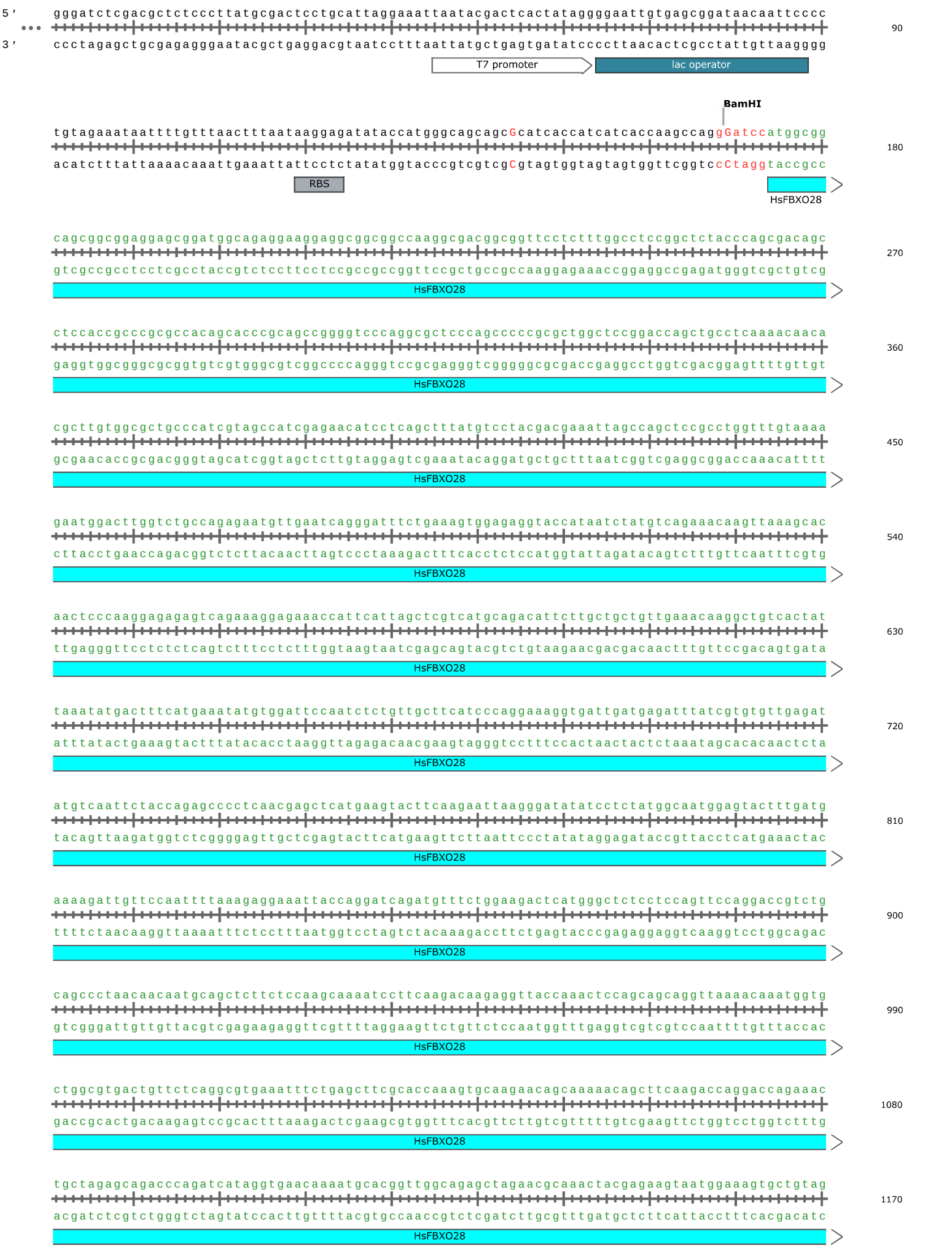

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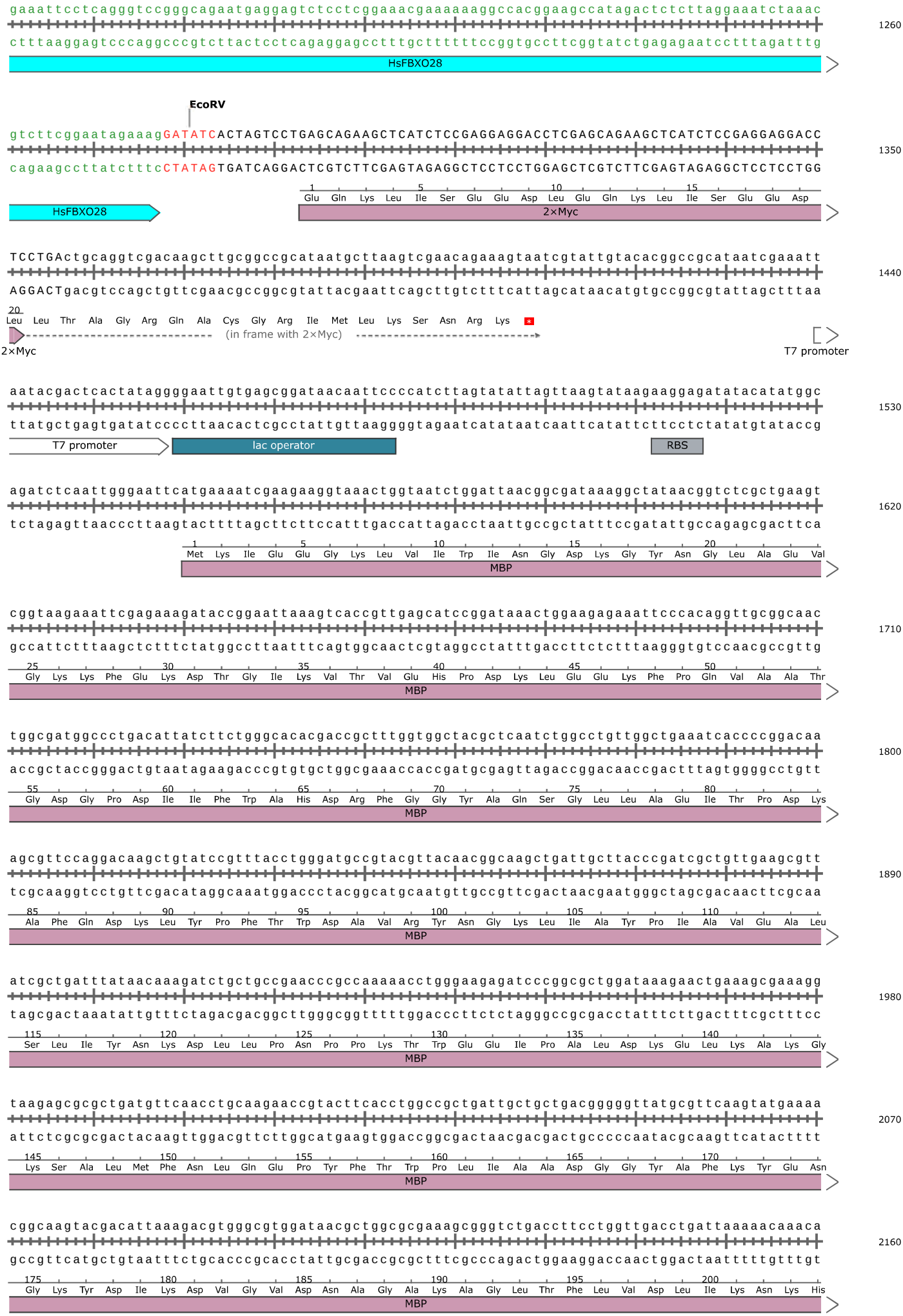

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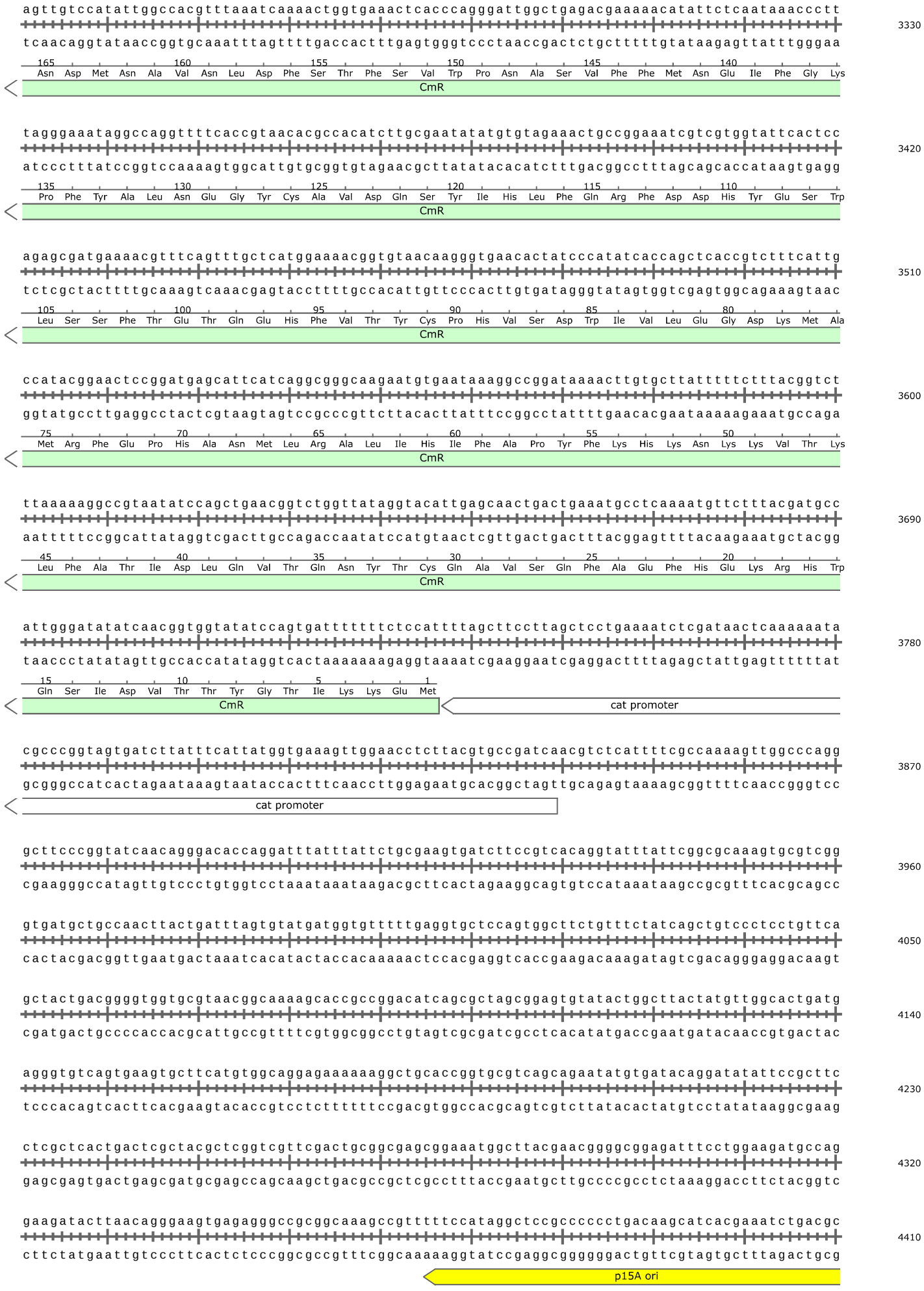
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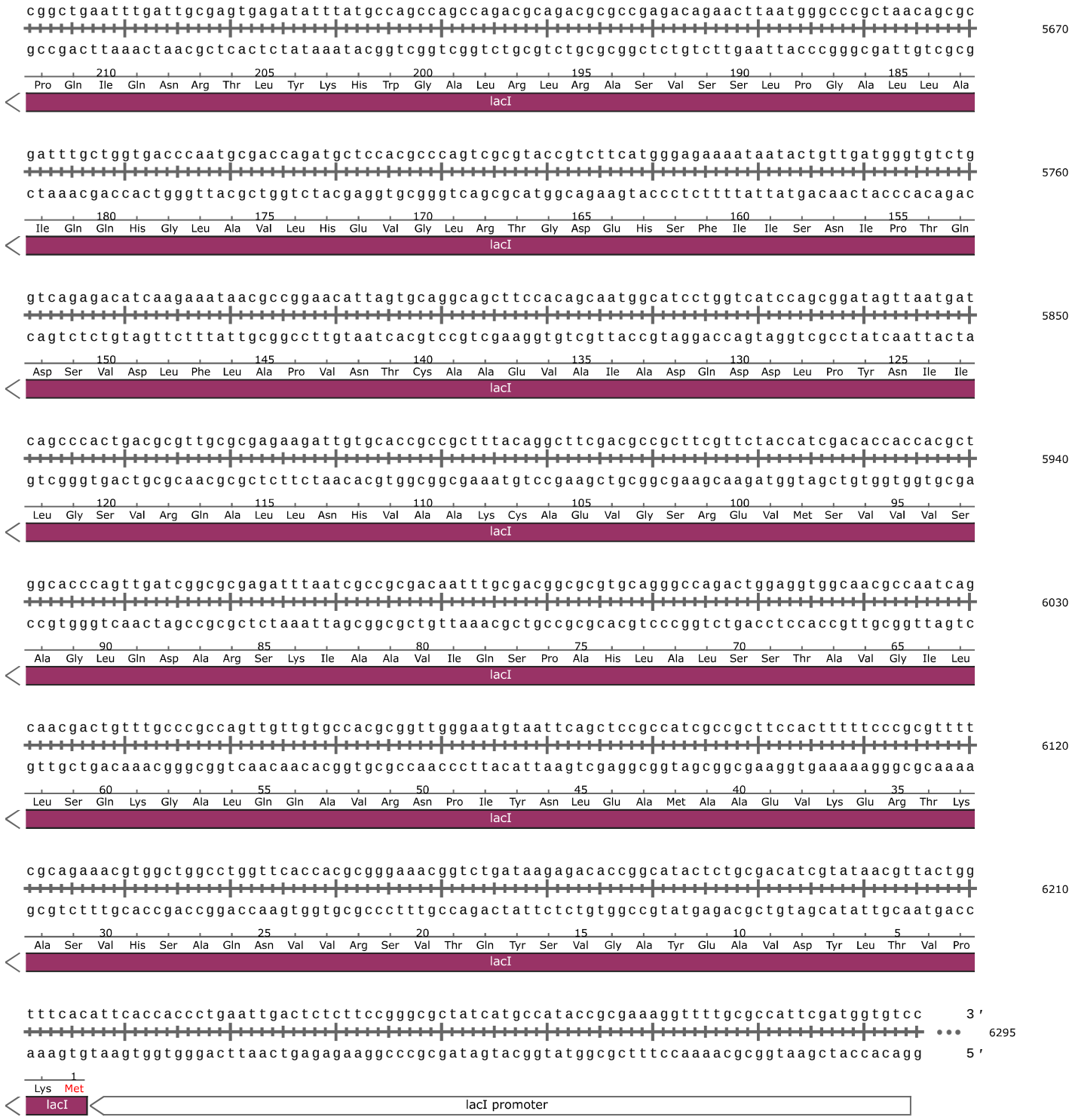

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(in frame with 2×Myc)
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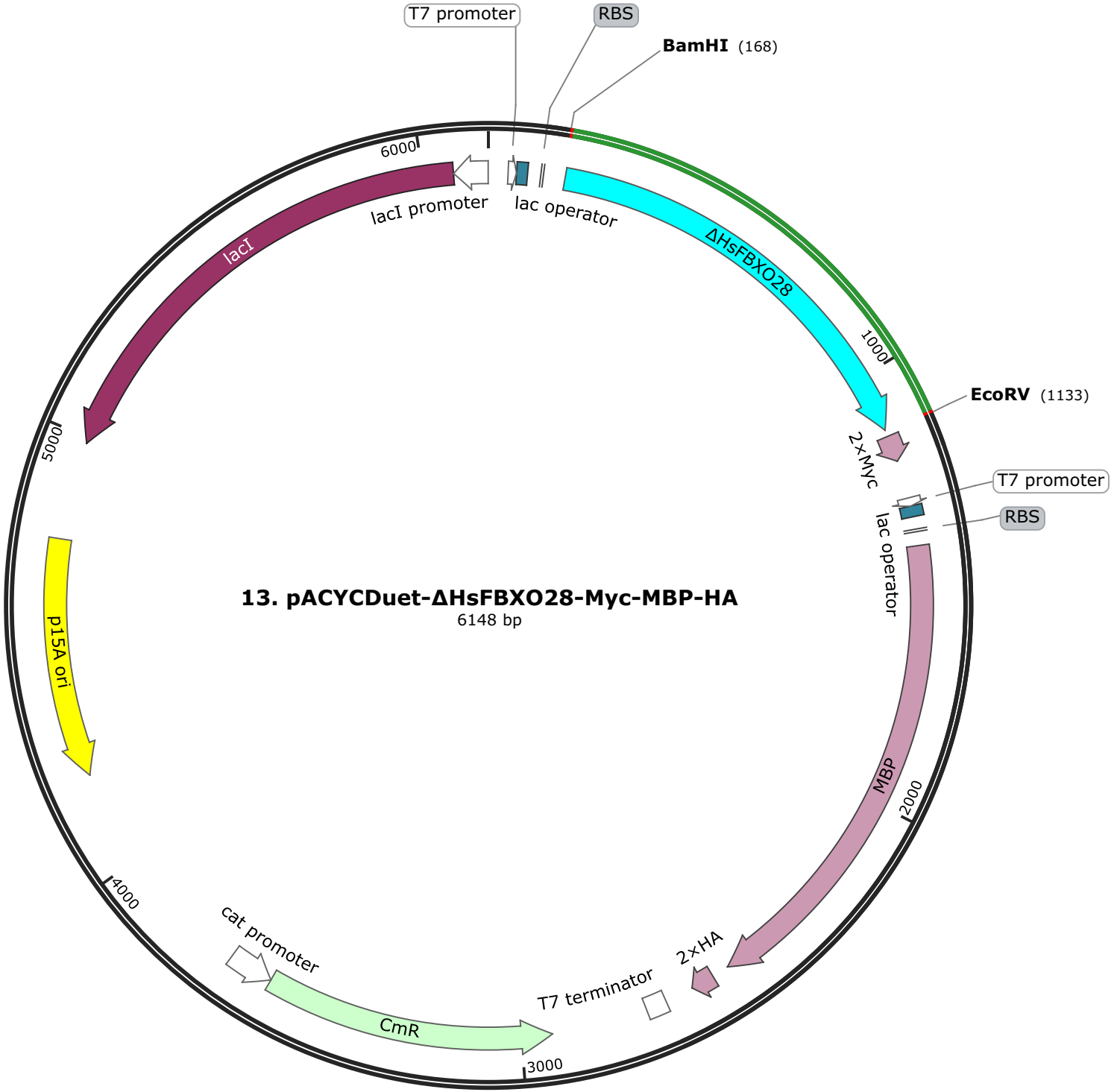

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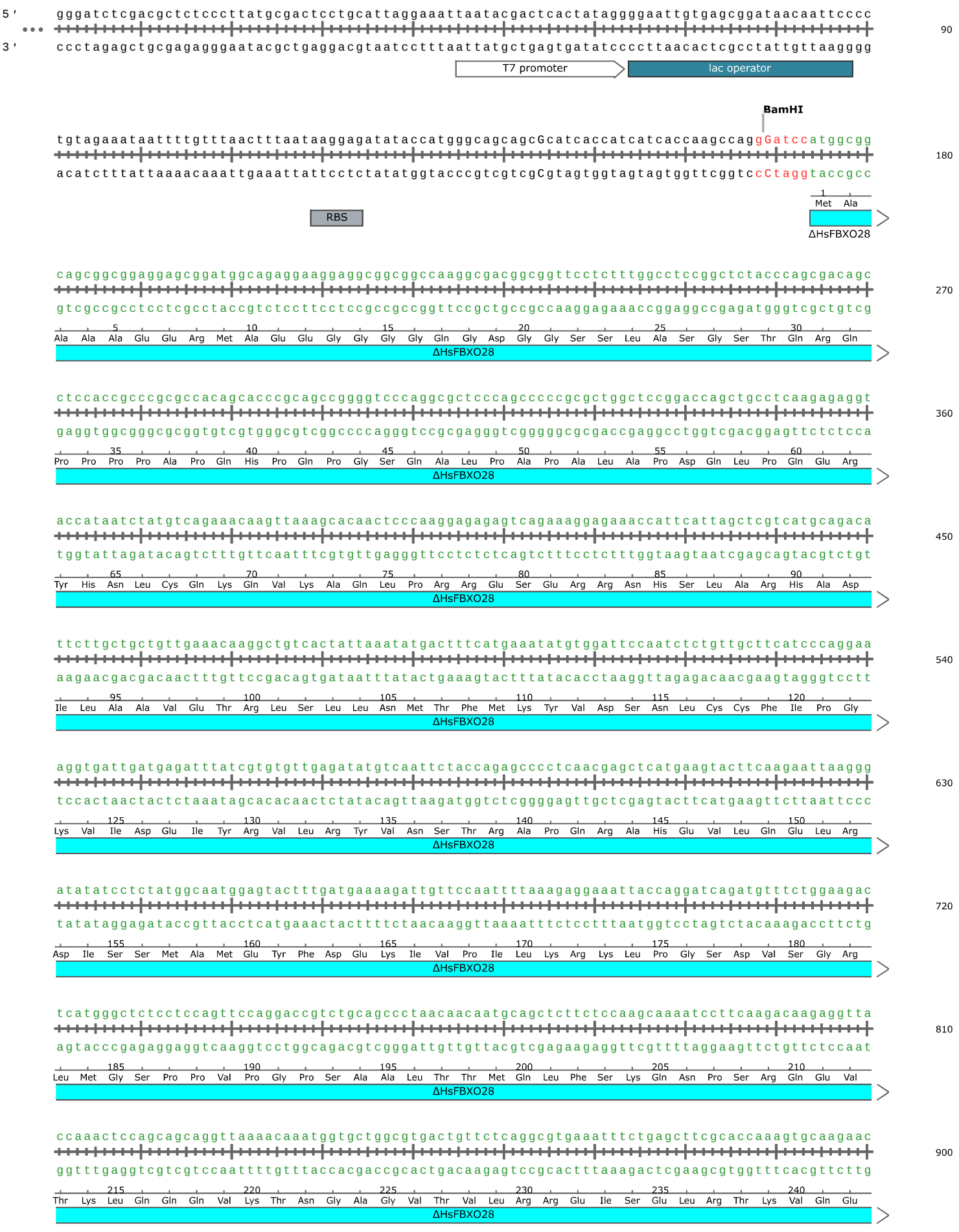

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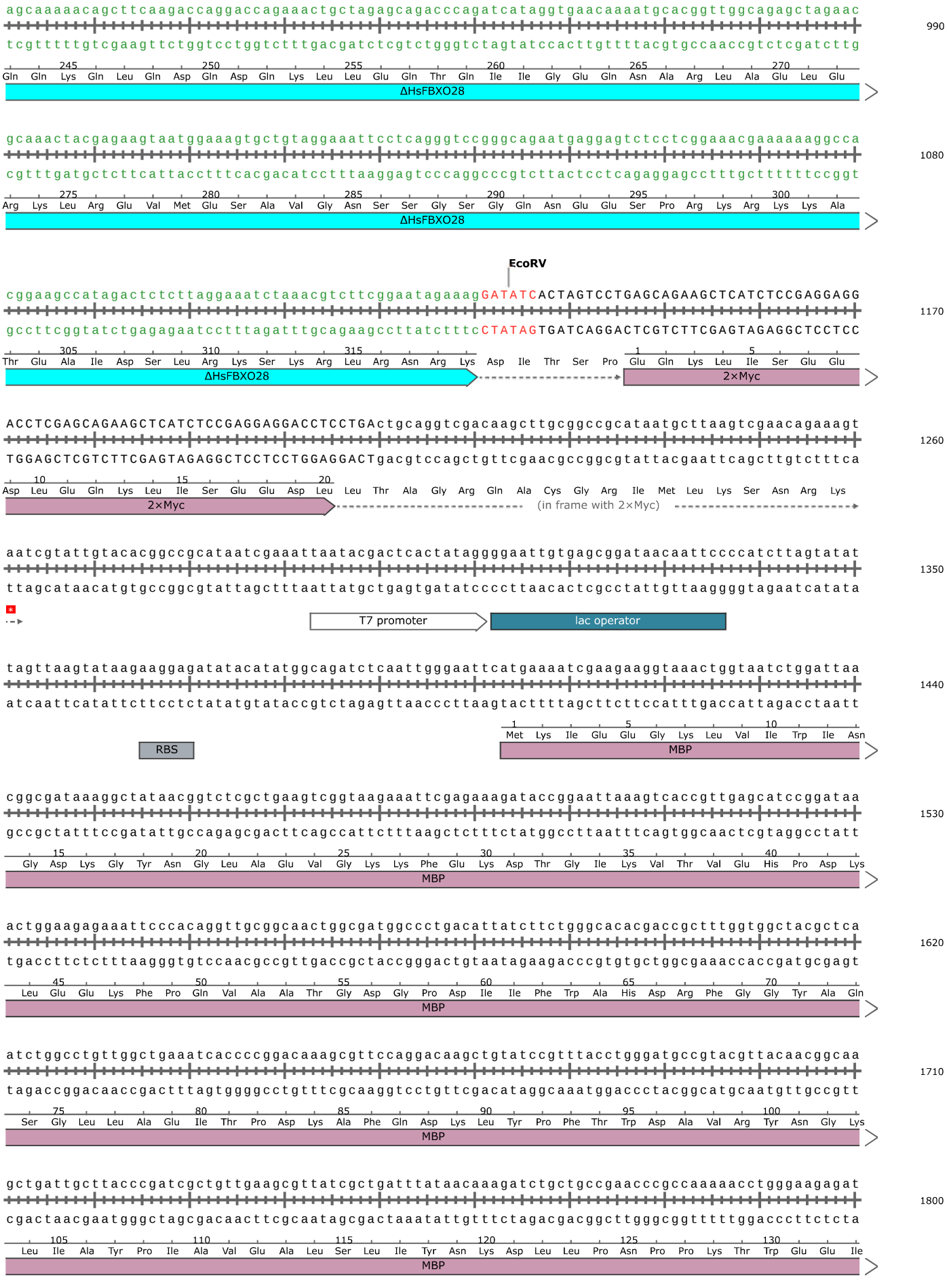

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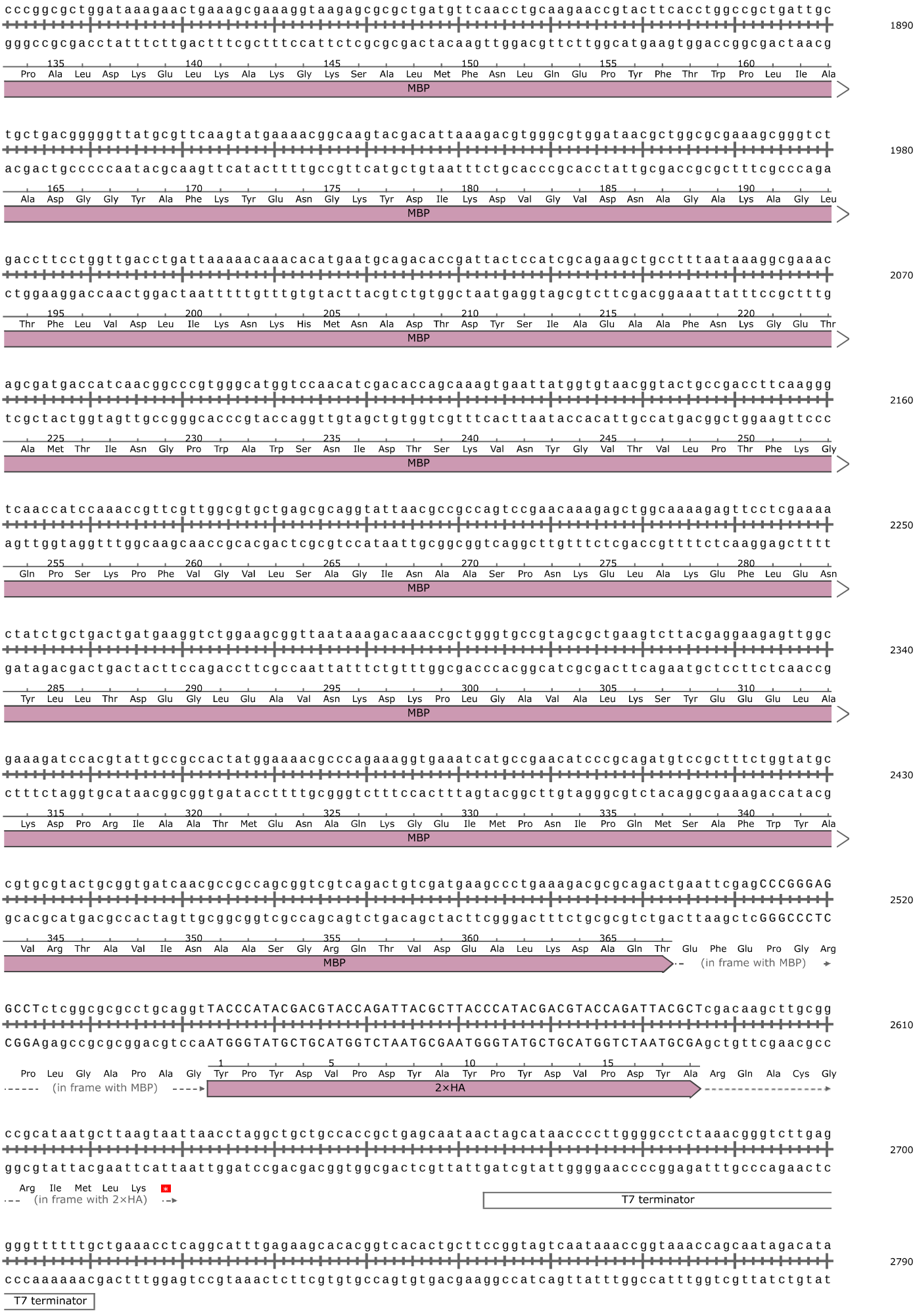

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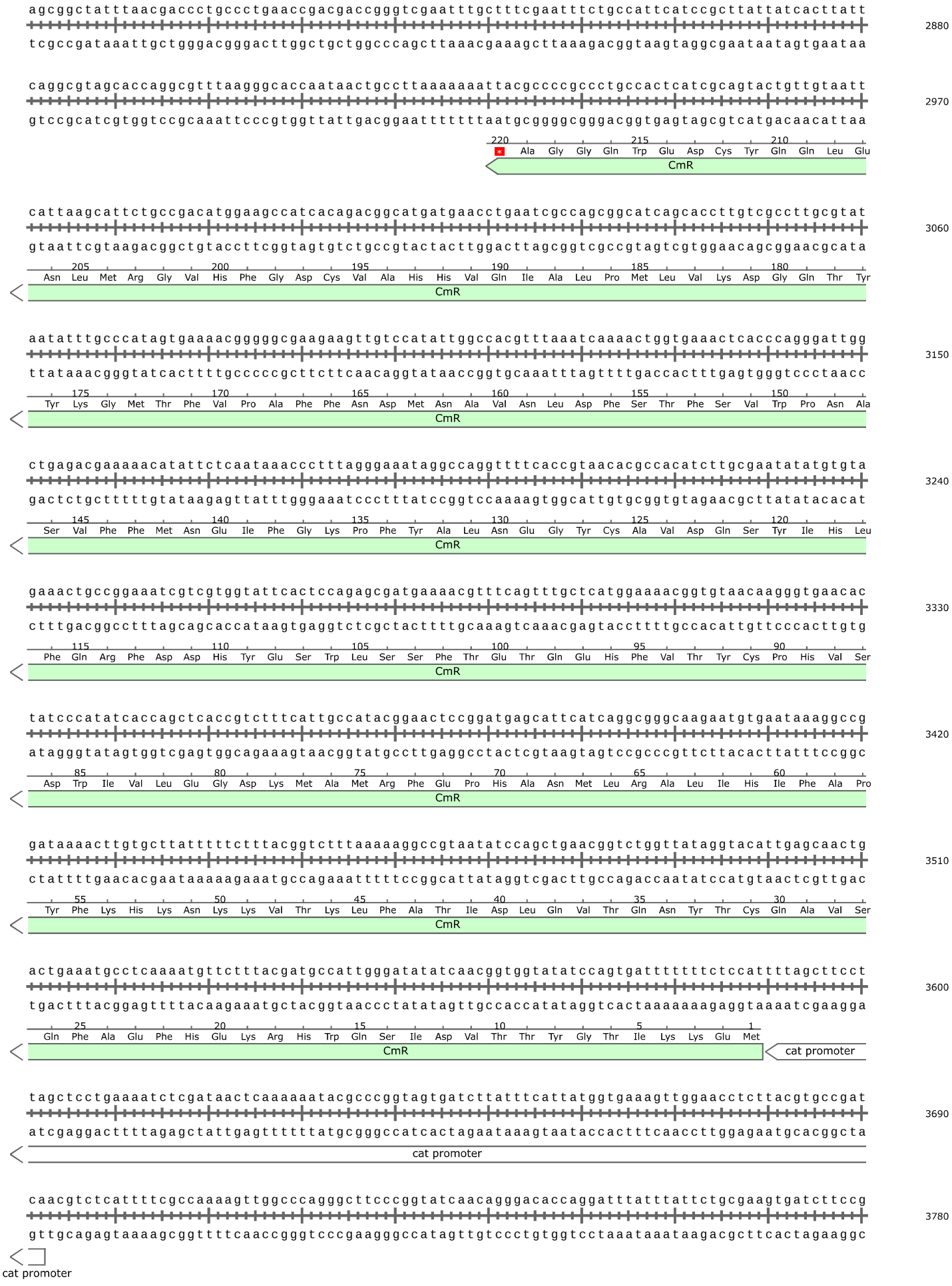
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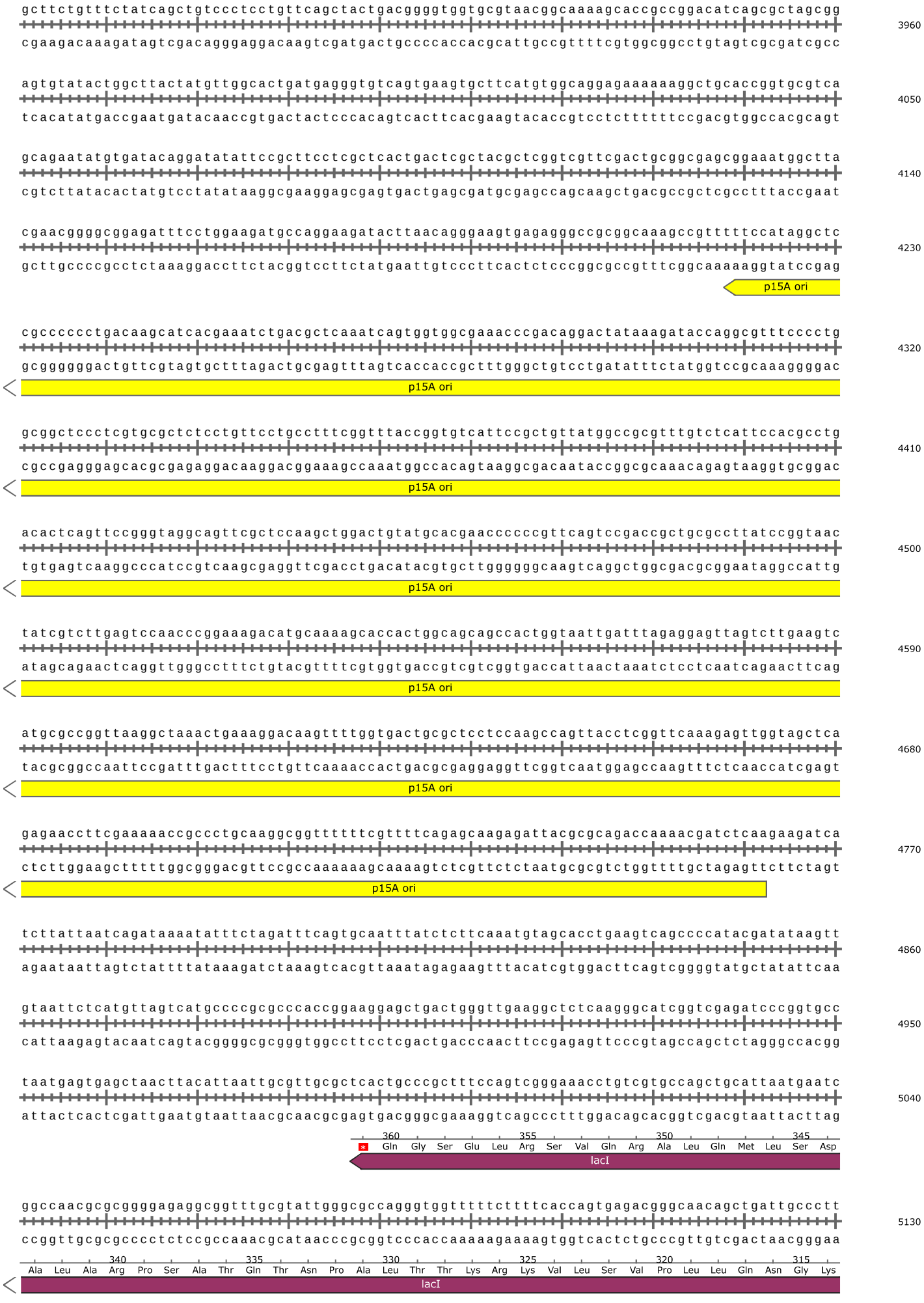


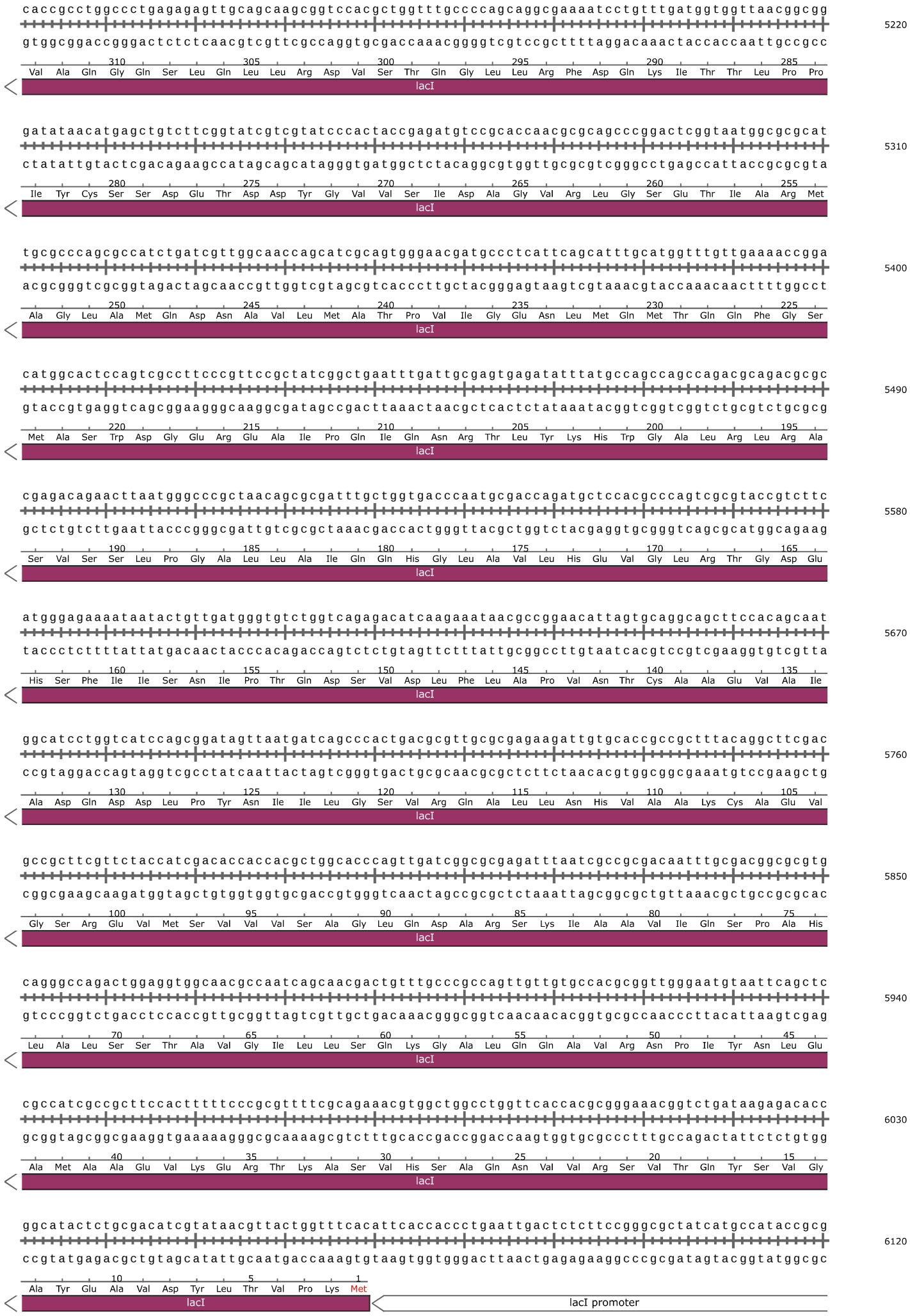

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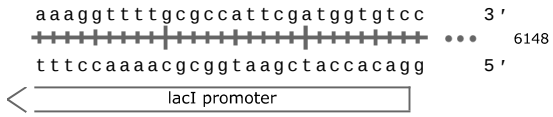

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165 170 175 180 185 190
Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly Leu
MBP
gaccttcctggttgacctgattaaaaacaaacacatgaatgcagacaccgattactccatcgcagaagctgccttaataaaggcgaaac
2070
ctggaaggaccaactggactaatttttgtttgtgtacttacgtctgtggctaatgaggtagcgtcttcgacggaaattatttccgctttg
195 200 205 210 215 220
Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr
MBP
agcgatgaccatcaacggcccgtgggcatggtccaacatcgacaccagcaaagtgaattatggtgtaacggtactgccgaccttcaaggg
2160
tcgctactggtagttgccgggcacccgtaccaggttgtagctgtggtcgtttcacttaataccacattgccatgacggctggaagttccc
225 230 235 240 245 250
Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly
MBP
tcaaccatccaaaccgttcgttggcgtgctgagcgcaggtattaacgccgccagtccgaacaaagagctggcaaaagagttcctcgaaaa
2250
agttggtaggtttggcaagcaaccgcacgactcgcgtccataattgcggcggtcaggcttgtttctcgaccgttttctcaaggagctttt
255 260 265 270 275 280
Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn
MBP
ctatctgctgactgatgaaggtctggaagcggttaataaagacaaaccgctgggtgccgtagcgctgaagtcttacgaggaagagttggc
2340
gatagacgactgactacttccagaccttcgccaattatttctgtttggcgacccacggcatcgcgacttcagaatgctccttctcaaccg
285 290 295 300 305 310
Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Ala
MBP
gaaagatccacgtattgccgccactatggaaaacgcccagaaaggtgaaatcatgccgaacatcccgcagatgtccgctttctggtatgc
2430
ctttctaggtgcataacggcggtgataccttttgcgggtctttccacttttagtacggcttgtagggcgtctacaggcgaaagaccatacg
315 320 325 330 335 340
Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala
MBP
cgtgcgtactgcggtgatcaacgccgccagcggtcgtcagactgtcgatgaagccctgaaagacgcgcagactgaattcgagCCCGGGAG
2520
gcacgcatgacgccactagttgcggcggtcgccagcagtctgacagctacttcgggactttctgcgcgtctgacttaagctcGGGCCCTC
345 350 355 360 365
Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Glu Phe Glu Pro Gly Arg
MBP
(in frame with MBP)
GCCTctcggcgcgcctgcaggtTACCCATACGACGTACCAGATTACGCTTACCCATACGACGTACCAGATTACGCTcgacaagcttgcgg
2610
CGGAgagccgcgcggacgtccaATGGGTATGCTGCATGGTCTAATGCGAATGGGTATGCTGCATGGTCTAATGCGAgctgttcgaacgcc
1 5 10 15
Pro Leu Gly Ala Pro Ala Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Arg Gln Ala Cys Gly
(in frame with MBP)
2×HA
ccgcataatgcttaagtaattaacctaggctgctgccaccgctgagcaataactagcataaccccttggggcctctaaacgggtcttgag
2700
ggcgtattacgaattcattaattggatccgacgacggtggcgactcgttattgatcgtattggggaaccccggagatttgcccagaactc
Arg Ile Met Leu Lys *
(in frame with 2×HA)
T7 terminator
gggtttttgctgaaacctcaggcatttgagaagcacacggtcacactgcttccggtagtcaataaaccggtaaaccagcaatagacata
2790
cccaaaaaacgactttggagtccgtaaactcttcgtgtgccagtgtgacgaaggccatcagttatttggccatttggtcgttatctgtat
T7 terminator


2880
2970
3060
3150
3240
3330
3420
3510
3600
3690
3780
3870
CmR
cat promoter


3960
4050
4140
4230
p15A ori
4320
p15A ori
4410
p15A ori
4500
p15A ori
4590
p15A ori
4680
p15A ori
4770
p15A ori
4860
4950
5040
360 355 350 345
Gln Gly Ser Glu Leu Arg Ser Val Gln Arg Ala Leu Gln Met Leu Ser Asp
lacI
5130
340 335 330 325 320 315
Ala Leu Ala Arg Pro Ser Ala Thr Gln Thr Asn Pro Ala Leu Thr Thr Lys Arg Lys Val Leu Ser Val Pro Leu Leu Gln Asn Gly Lys
lacI


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5220
310 305 300 295 290 285
Val Ala Gln Gly Gln Ser Leu Gln Leu Leu Arg Asp Val Ser Thr Gln Gly Leu Leu Arg Phe Asp Gln Lys Ile Thr Thr Leu Pro Pro
lacI
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5310
280 275 270 265 260 255
Ile Tyr Cys Ser Ser Asp Glu Thr Asp Asp Tyr Gly Val Val Ser Ile Asp Ala Gly Val Arg Leu Gly Ser Glu Thr Ile Ala Arg Met
lacI
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5400
250 245 240 235 230 225
Ala Gly Leu Ala Met Gln Asp Asn Ala Val Leu Met Ala Thr Pro Val Ile Gly Glu Asn Leu Met Gln Met Thr Gln Gln Phe Gly Ser
lacI
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gtaccgtgaggtcagcggaagggcaaggcgatagccgacttaaactaacgctcactctataaatacggtcggtcggtctgcgtctgcgcg
5490
220 215 210 205 200 195
Met Ala Ser Trp Asp Gly Glu Arg Glu Ala Ile Pro Gln Ile Gln Asn Arg Thr Leu Tyr Lys His Trp Gly Ala Leu Arg Leu Arg Ala
lacI
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gctctgtcttgaattacccgggcgattgtcgcgctaaacgaccactgggttacgctggtctacgaggtgcgggtcagcgcatggcagaag
5580
190 185 180 175 170 165
Ser Val Ser Ser Leu Pro Gly Ala Leu Leu Ala Ile Gln Gln His Gly Leu Ala Val Leu His Glu Val Gly Leu Arg Thr Gly Asp Glu
lacI
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taccctctttttattatgacaactacccacagaccagtctctgtagttctttattgcggccttgtaatcacgtccgtcgaaggtgtcgtta
5670
160 155 150 145 140 135
His Ser Phe Ile Ile Ser Asn Ile Pro Thr Gln Asp Ser Val Asp Leu Phe Leu Ala Pro Val Asn Thr Cys Ala Ala Glu Val Ala Ile
lacI
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5760
130 125 120 115 110 105
Ala Asp Gln Asp Asp Leu Pro Tyr Asn Ile Ile Leu Gly Ser Val Arg Gln Ala Leu Leu Asn His Val Ala Ala Lys Cys Ala Glu Val
lacI
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cggcgaagcaagatggtagctgtggtggtgcgaccgtgggtcaactagccgcgctctaaattagcggcgctgttaaacgctgccgcgcac
5850
100 95 90 85 80 75
Gly Ser Arg Glu Val Met Ser Val Val Val Ser Ala Gly Leu Gln Asp Ala Arg Ser Lys Ile Ala Ala Val Ile Gln Ser Pro Ala His
lacI
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5940
70 65 60 55 50 45
Leu Ala Leu Ser Ser Thr Ala Val Gly Ile Leu Leu Ser Gln Lys Gly Ala Leu Gln Gln Ala Val Arg Asn Pro Ile Tyr Asn Leu Glu
lacI
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6030
40 35 30 25 20 15
Ala Met Ala Ala Glu Val Lys Glu Arg Thr Lys Ala Ser Val His Ser Ala Gln Asn Val Val Arg Ser Val Thr Gln Tyr Ser Val Gly
lacI
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6120
10 5 1
Ala Tyr Glu Ala Val Asp Tyr Leu Thr Val Pro Lys Met
lacI
lacI promoter


aaaggttttgcgccattcgatggtgtcc
3′
6148
tttccaaaacgcggtaagctaccacagg
5′
lacI promoter