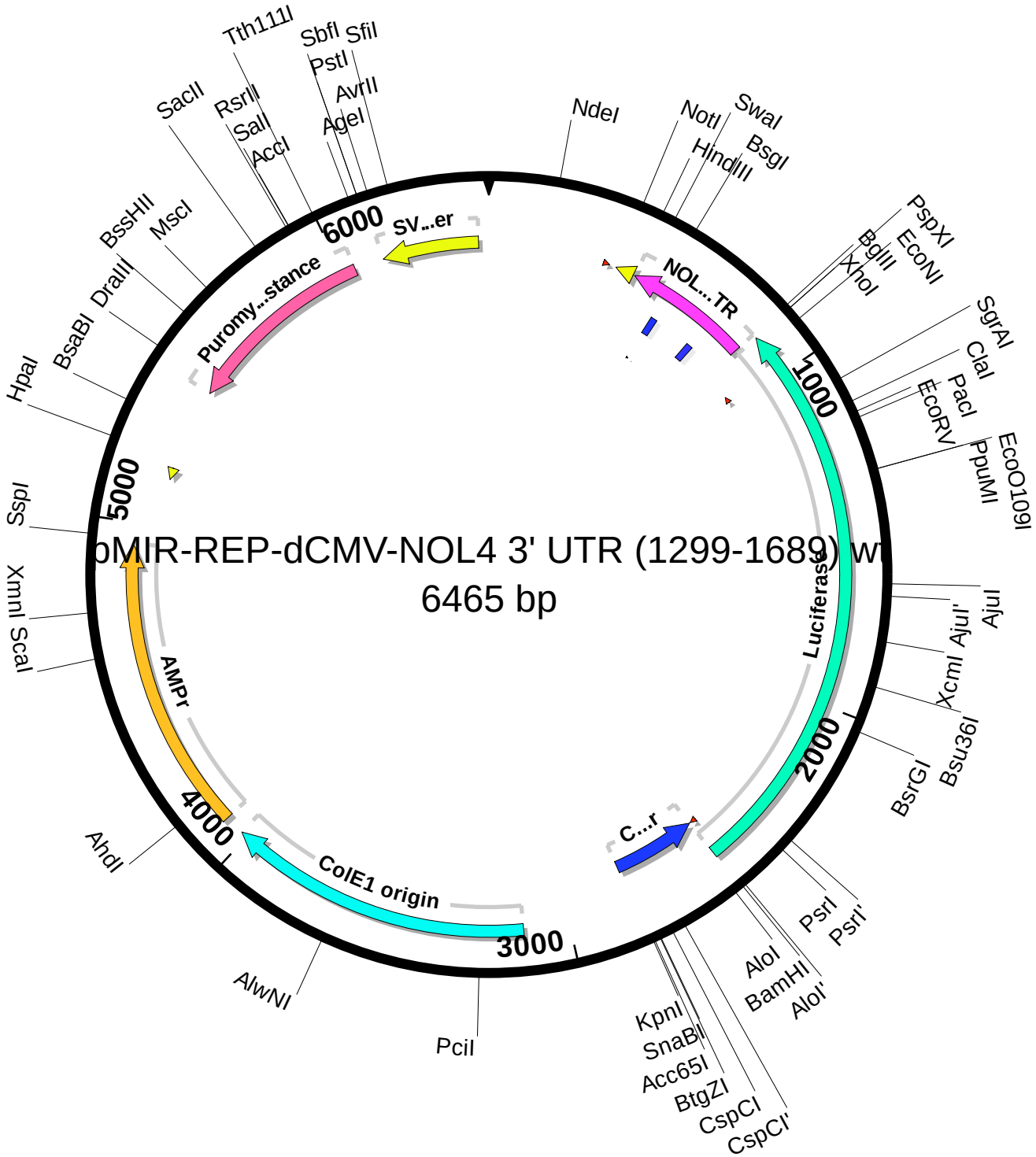
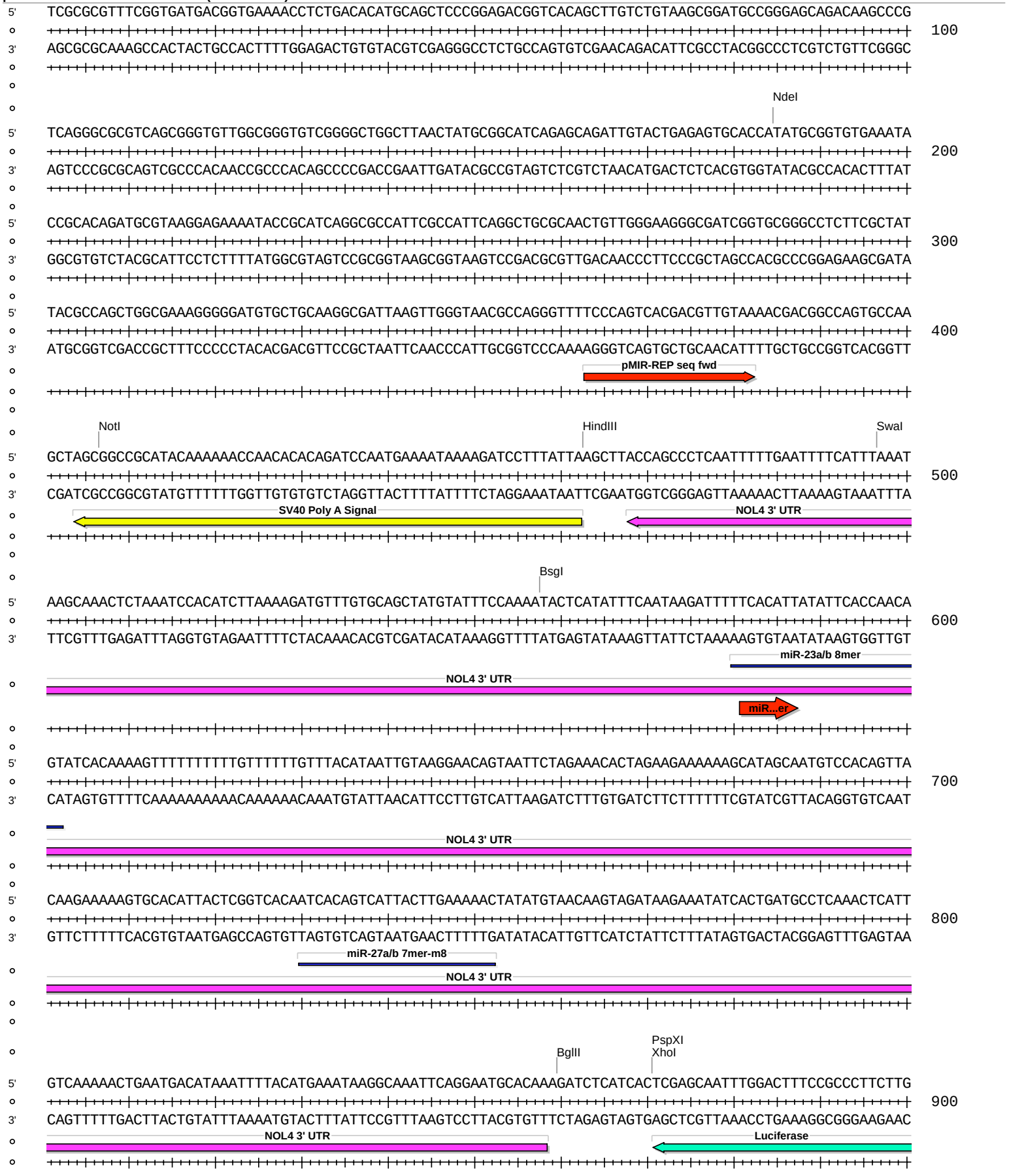
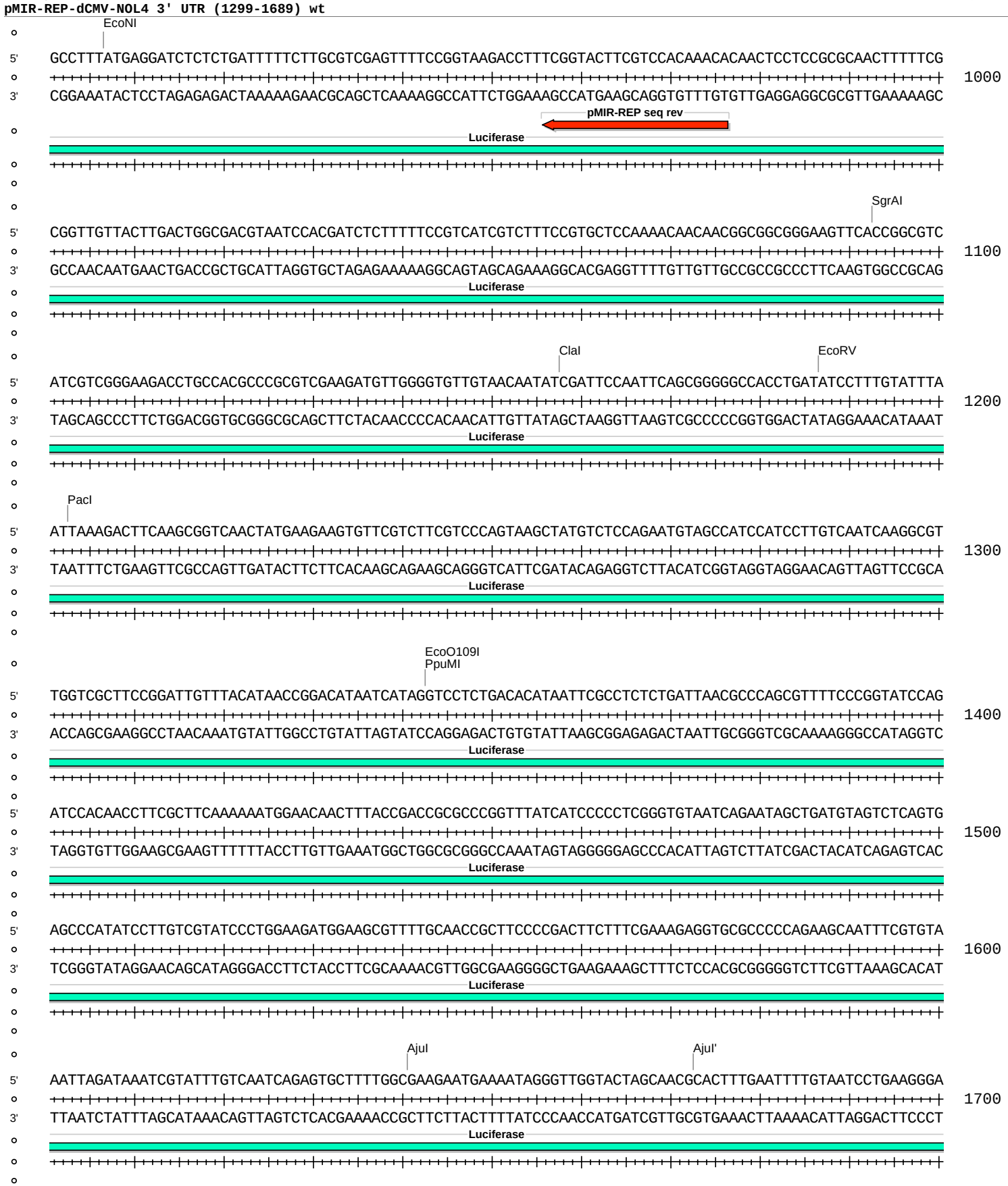

pMIR-REP-dCMV-NOL4 3' UTR (1299-1689) wt
6465 bp
1000
2000
3000
4000
5000
6000
Luciferase
AMPr
ColE1 origin
Puromy...stance
SV...er
NOL...TR
C...r
Tth111I
SbfI
SfiI
PstI
AvrII
AgeI
SacII
RsrII
SalI
AccI
NdeI
NotI
SwaI
HindIII
BsgI
PspXI
EcoNI
BglII
XhoI
SgrAI
ClaI
PacI
EcoRV
PpuMI
EcoO109I
AjuI
AjuI'
XcmI
Bsu36I
BsrGI
PsrI
PsrI'
AloI
BamHI
AloI'
KpnI
SnaBI
Acc65I
BtgZI
CspCI
CspCI'
PciI
AlwNI
AhdI
ScaI
XmnI
SspI
HpaI
BsaBI
DraIII
BssHII
MscI

Absent Sites	0	AarI,AbsI,AfeI,AflII,AleI,ApaI,AscI,AsiSI,BaeI,BaeI',BarI,BarI',BbvCI,BclI,BlpI,BmgBI,Bpu10I,BstXI,BstZ17I, EcoICRI,FalI,FalI',FseI,FspAI,MauBI,MluI,MreI,NaeI,NgoMIV,NruI,PasI,PflMI,PmeI,PmlI,PshAI,PspOMI,SacI, SanDI,SgrDI,SpeI,SrfI
Acc65I	1	2795 (6465)
AccI	1	5930 (6465)
AgeI	1	6098 (6465)
AhdI	1	4152 (6465)
AjuI	1	1641 (6465)
AjuI'	1	1673 (6465)
AloI	1	2554 (6465)
AloI'	1	2522 (6465)
AlwNI	1	3675 (6465)
AvrII	1	6149 (6465)
BamHI	1	2530 (6465)
BglII	1	860 (6465)
BsaBI	1	5314 (6465)
BsgI	1	558 (6465)
BsrGI	1	2031 (6465)
BssHII	1	5582 (6465)
Bsu36I	1	1909 (6465)
BtgZI	1	2780 (6465)
ClaI	1	1158 (6465)
CspCI	1	2744 (6465)
CspCI'	1	2709 (6465)
DraIII	1	5473 (6465)
EcoNI	1	907 (6465)
EcoO109I	1	1343 (6465)
EcoRV	1	1187 (6465)
HindIII	1	463 (6465)
HpaI	1	5213 (6465)
KpnI	1	2799 (6465)
MscI	1	5666 (6465)
NdeI	1	185 (6465)
NotI	1	407 (6465)
PacI	1	1203 (6465)
PciI	1	3259 (6465)
PpuMI	1	1343 (6465)
PspXI	1	871 (6465)
PsrI	1	2392 (6465)
PsrI'	1	2360 (6465)
PstI	1	6121 (6465)
RsrII	1	5922 (6465)
SacII	1	5829 (6465)
SalI	1	5929 (6465)
SbfI	1	6121 (6465)
ScaI	1	4632 (6465)
SfiI	1	6203 (6465)
SgrAI	1	1093 (6465)
SnaBI	1	2778 (6465)
SspI	1	4956 (6465)
SwaI	1	497 (6465)
Tth111I	1	5998 (6465)
XcmI	1	1791 (6465)
XhoI	1	871 (6465)
XmnI	1	4751 (6465)

pMIR-REP-dCMV-NOL4 3' UTR (1299-1689) wt

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1000
3' CGGAAATACTCCTAGAGAGACTAAAAAGAACGCAGCTCAAAAGGCCATTCTGGAAAGCCATGAAGCAGGTGTTTGTGTTGAGGAGGCGCGTTGAAAAAGC
pMIR-REP seq rev
Luciferase
SgrAI
5' CGGTTGTTACTTGACTGGCGACGTAATCCACGATCTCTTTTTCCGTCATCGTCTTTCCGTGCTCCAAAACAACAACGGCGGCGGGAAGTTCACCGGCGTC
1100
3' GCCAACAATGAACTGACCGCTGCATTAGGTGCTAGAGAAAAAGGCAGTAGCAGAAAGGCACGAGGTTTTGTTGTTGCCGCCGCCCTTCAAGTGGCCGCAG
Luciferase
ClaI
EcoRV
5' ATCGTCGGGAAGACCTGCCACGCCCGCGTCGAAGATGTTGGGGTGTTGTAACAATATCGATTCCAATTCAGCGGGGGCCACCTGATATCCTTTGTATTTA
1200
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Luciferase
PacI
5' ATTAAAGACTTCAAGCGGTCAACTATGAAGAAGTGTTCGTCTTCGTCCCAGTAAGCTATGTCTCCAGAATGTAGCCATCCATCCTTGTCAATCAAGGCGT
1300
3' TAATTTCTGAAGTTCGCCAGTTGATACTTCTTCACAAGCAGAAGCAGGGTCATTCGATACAGAGGTCTTACATCGGTAGGTAGGAACAGTTAGTTCCGCA
Luciferase
EcoO109I
PpuMI
5' TGGTCGCTTCCGGATTGTTTACATAACCGGACATAATCATAGGTCCTCTGACACATAATTCGCCTCTCTGATTAACGCCCAGCGTTTTCCCGGTATCCAG
1400
3' ACCAGCGAAGGCCTAACAAATGTATTGGCCTGTATTAGTATCCAGGAGACTGTGTATTAAGCGGAGAGACTAATTGCGGGTCGCAAAAGGGCCATAGGTC
Luciferase
5' ATCCACAACCTTCGCTTCAAAAAATGGAACAACTTTACCGACCGCGCCCGGTTTATCATCCCCCTCGGGTGTAATCAGAATAGCTGATGTAGTCTCAGTG
1500
3' TAGGTGTTGGAAGCGAAGTTTTTTACCTTGTTGAAATGGCTGGCGCGGGCCAAATAGTAGGGGGAGCCCACATTAGTCTTATCGACTACATCAGAGTCAC
Luciferase
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1600
3' TCGGGTATAGGAACAGCATAGGGACCTTCTACCTTCGCAAAACGTTGGCGAAGGGGCTGAAGAAAGCTTTCTCCACGCGGGGGTCTTCGTTAAAGCACAT
Luciferase
AjuI
AjuI'
5' AATTAGATAAATCGTATTTGTCAATCAGAGTGCTTTTGGCGAAGAATGAAAATAGGGTTGGTACTAGCAACGCACTTTGAATTTTGTAATCCTGAAGGGA
1700
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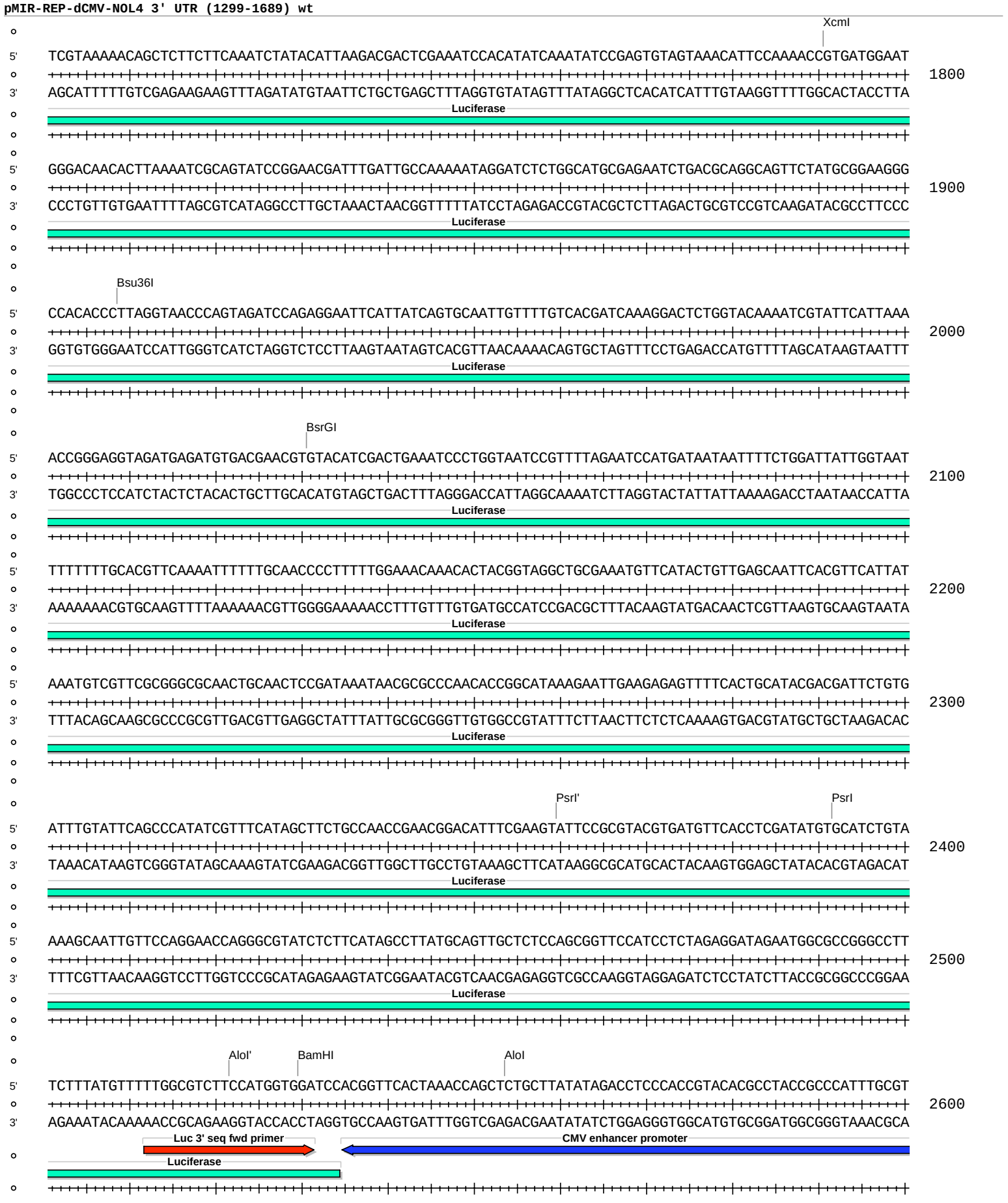
Luciferase


XcmI
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1800
3' AGCATTTTTGTCGAGAAGAAGTTTAGATATGTAATTCTGCTGAGCTTTAGGTGTATAGTTTATAGGCTCACATCATTTGTAAGGTTTTGGCACTACCTTA
Luciferase
5' GGGACAACACTTAAAATCGCAGTATCCGGAACGATTTGATTGCCAAAAATAGGATCTCTGGCATGCGAGAATCTGACGCAGGCAGTTCTATGCGGAAGGG
1900
3' CCCTGTTGTGAATTTTAGCGTCATAGGCCTTGCTAAACTAACGGTTTTTATCCTAGAGACCGTACGCTCTTAGACTGCGTCCGTCAAGATACGCCTTCCC
Luciferase
Bsu36I
5' CCACACCCTTAGGTAACCCAGTAGATCCAGAGGAATTCATTATCAGTGCAATTGTTTTGTCACGATCAAAGGACTCTGGTACAAAATCGTATTCATTAAA
2000
3' GGTGTGGGAATCCATTGGGTCATCTAGGTCTCCTTAAGTAATAGTCACGTTAACAAAACAGTGCTAGTTTCCTGAGACCATGTTTTAGCATAAGTAATTT
Luciferase
BsrGI
5' ACCGGGAGGTAGATGAGATGTGACGAACGTGTACATCGACTGAAATCCCTGGTAATCCGTTTTAGAATCCATGATAATAATTTTCTGGATTATTGGTAAT
2100
3' TGGCCCTCCATCTACTCTACACTGCTTGCACATGTAGCTGACTTTAGGGACCATTAGGCAAAATCTTAGGTACTATTATTAAAAGACCTAATAACCATTA
Luciferase
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2200
3' AAAAAAACGTGCAAGTTTTAAAAAACGTTGGGGAAAAACCTTTGTTTGTGATGCCATCCGACGCTTTACAAGTATGACAACTCGTTAAGTGCAAGTAATA
Luciferase
5' AAATGTCGTTCGCGGGCGCAACTGCAACTCCGATAAATAACGCGCCCAACACCGGCATAAAGAATTGAAGAGAGTTTTCACTGCATACGACGATTCTGTG
2300
3' TTTACAGCAAGCGCCCGCGTTGACGTTGAGGCTATTTATTGCGCGGGTTGTGGCCGTATTTCTTAACTTCTCTCAAAAGTGACGTATGCTGCTAAGACAC
Luciferase
PsrI'
PsrI
5' ATTTGTATTCAGCCCATATCGTTTCATAGCTTCTGCCAACCGAACGGACATTTCGAAGTATTCCGCGTACGTGATGTTCACCTCGATATGTGCATCTGTA
2400
3' TAAACATAAGTCGGGTATAGCAAAGTATCGAAGACGGTTGGCTTGCCTGTAAAGCTTCATAAGGCGCATGCACTACAAGTGGAGCTATACACGTAGACAT
Luciferase
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2500
3' TTTCGTTAACAAGGTCCTTGGTCCCGCATAGAGAAGTATCGGAATACGTCAACGAGAGGTCGCCAAGGTAGGAGATCTCCTATCTTACCGCGGCCCGGAA
Luciferase
AlwI'
BamHI
AlwI
5' TCTTTATGTTTTTGGCGTCTTCCATGGTGGATCCACGGTTCACTAAACCAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGT
2600
3' AGAAATACAAAAACCGCAGAAGGTACCACCTAGGTGCCAAGTGATTTGGTCGAGACGAATATATCTGGAGGGTGGCATGTGCGGATGGCGGGTAAACGCA
Luc 3' seq fwd primer
CMV enhancer promoter
Luciferase


5' CAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTTGGTGCCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCC
2700
3' GTTACCCCGCCTCAACAATGCTGTAAAACCTTTCAGGGCAACTAAAACCACGGTTTTGTTTGAGGGTAACTGCAGTTACCCCACCTCTGAACCTTTAGGG
CMV enhancer promoter
CspCI'
CspCI
SnaBI
BtgZI
Acc65I
KpnI
5' CGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCACCATGGTAATAGCGATGACTAATACgtaggttcactGATCTGGTACCT
2800
3' GCACTCAGTTTGGCGATAGGTGCGGGTAACTACATGACGGTTTTGGCGTAGTGGTACCATTATCGCTACTGATTATGcatccaagtgaCTAGACCATGGA
CMV enhancer promoter
5' TGAATTCATGCTTCTCCTCCCTTTAGTGAGGGTAATTCTCTCTCTCTCCCTATAGTGAGTCGTATTAATTCCTTCTCTTCTATAGTGTCACCTAAATCGT
2900
3' ACTTAAGTACGAAGAGGAGGGAAATCACTCCCATTAAGAGAGAGAGAGGGATATCACTCAGCATAATTAAGGAAGAGAAGATATCACAGTGGATTTAGCA
CMV ...oter
5' TGCAATTCGTAATCATGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTG
3000
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5' GGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGC
3100
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5' CAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAG
3200
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ColE1 origin
PciI
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3300
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ColE1 origin
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3400
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ColE1 origin
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3500
3' TCTATGGTCCGCAAAGGGGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACGGCGAATGGCCTATGGACAGGCGGAAAGAGGGAAGCCCTTCGC
ColE1 origin
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3600
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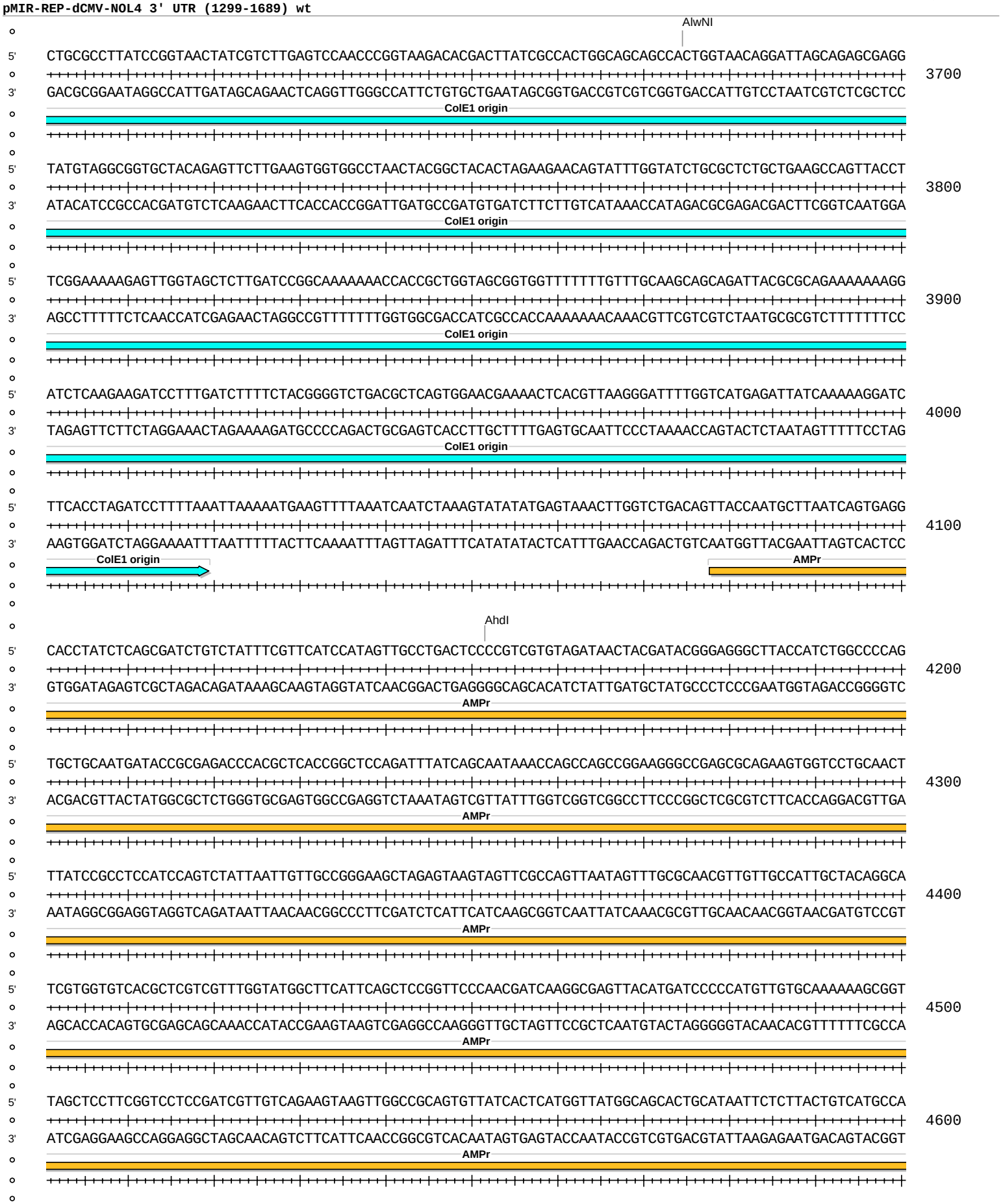
ColE1 origin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ColE1 origin
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3800
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ColE1 origin
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3900
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ColE1 origin
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4000
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ColE1 origin
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4100
3' AAGTGGATCTAGGAAAATTTAATTTTTACTTCAAAATTTAGTTAGATTTCATATATACTCATTTGAACCAGACTGTCAATGGTTACGAATTAGTCACTCC
ColE1 origin
AMPr
AhdI
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4200
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AMPr
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4300
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AMPr
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4400
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AMPr
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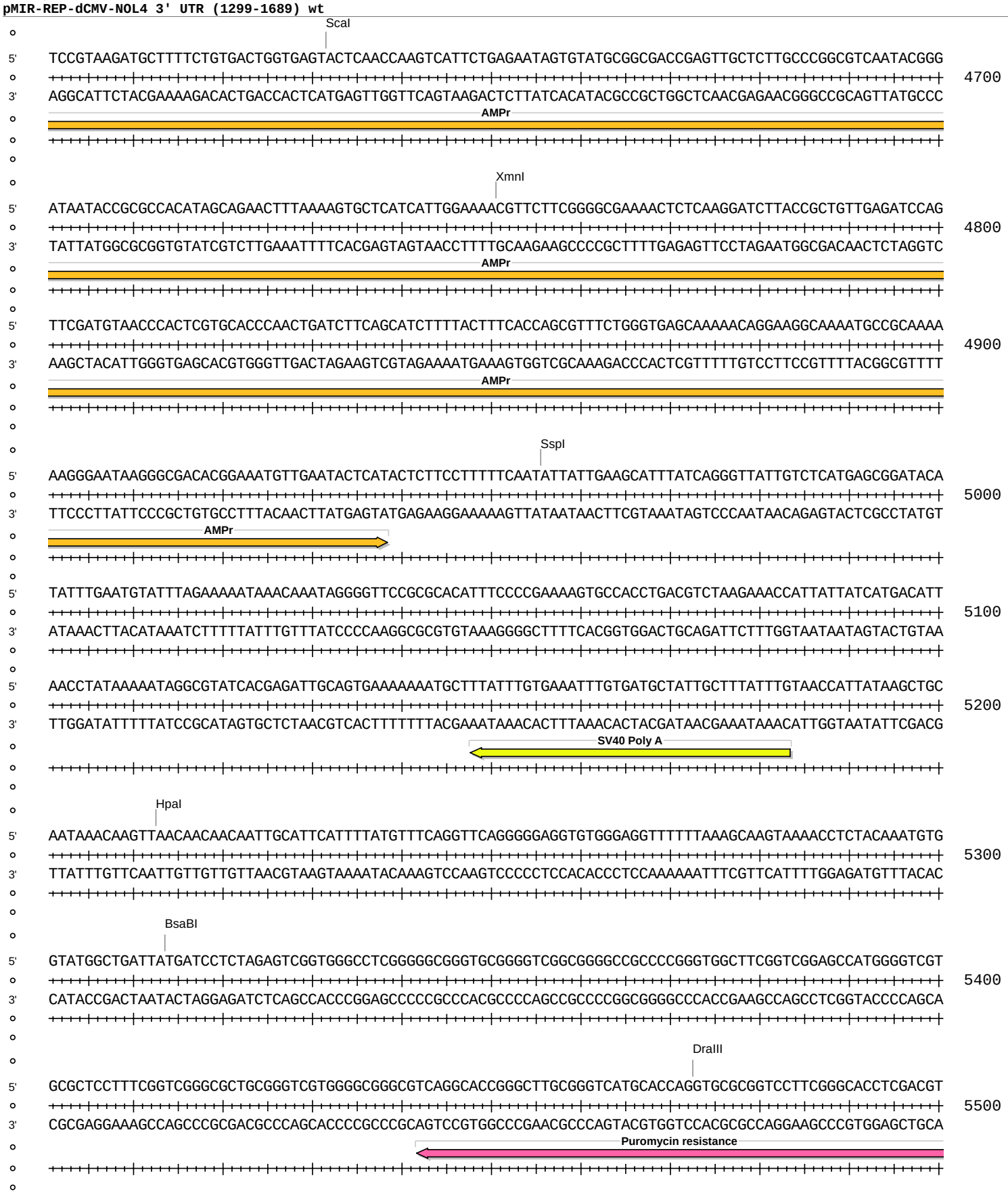
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AMPr
XmnI
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4800
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AMPr
SspI
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AMPr
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5100
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5200
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SV40 Poly A
HpaI
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DraIII
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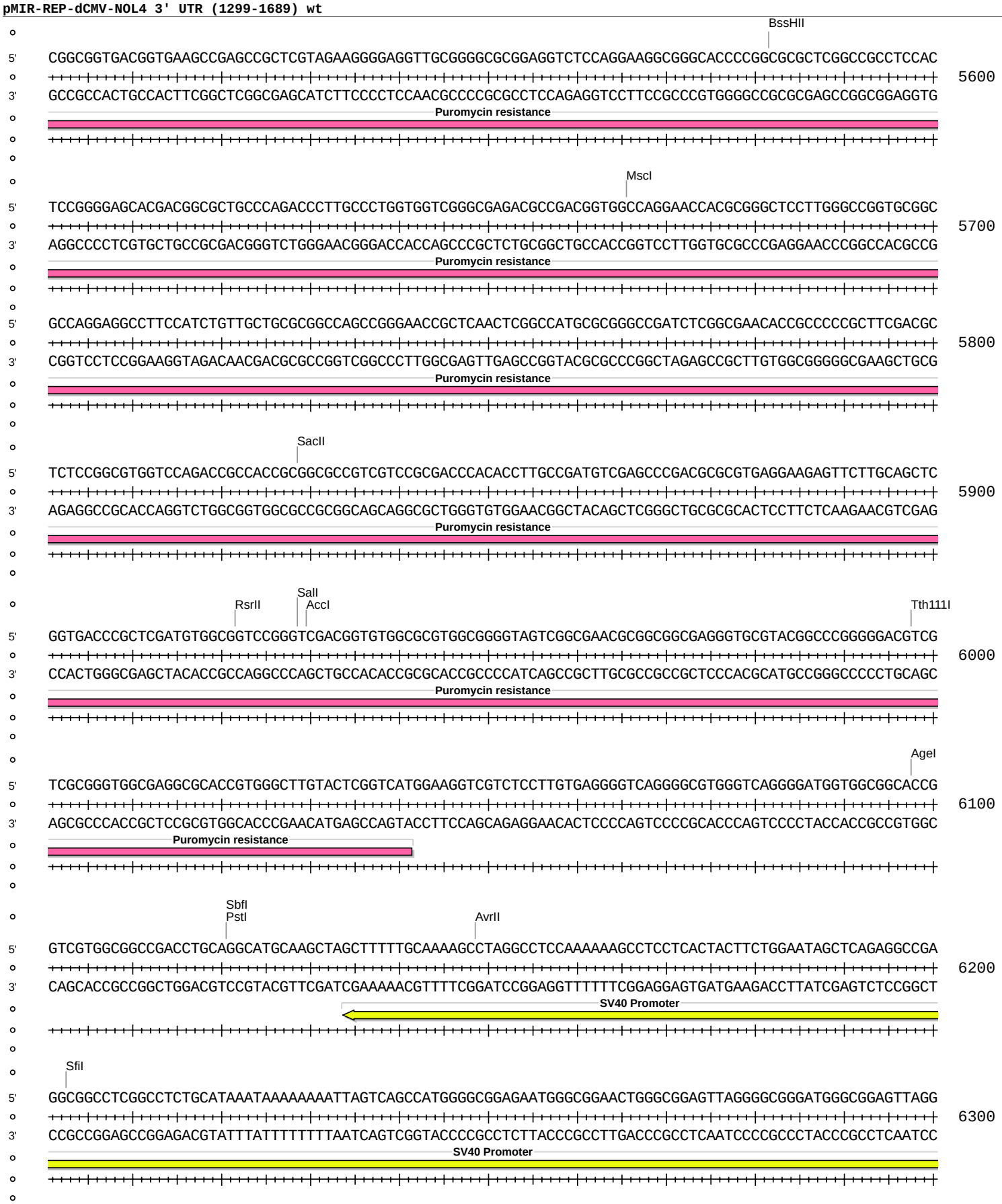
Puromycin resistance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5600
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Puromycin resistance
MscI
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Puromycin resistance
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Puromycin resistance
SacII
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5900
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Puromycin resistance
RsrII
SalI
AccI
Tth111I
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6000
3' CCACTGGGCGAGCTACACCGCCAGGCCCAGCTGCCACACCGCGCACCGCCCCATCAGCCGCTTGCGCCGCCGCTCCCACGCATGCCGGGCCCCCTGCAGC
Puromycin resistance
AgeI
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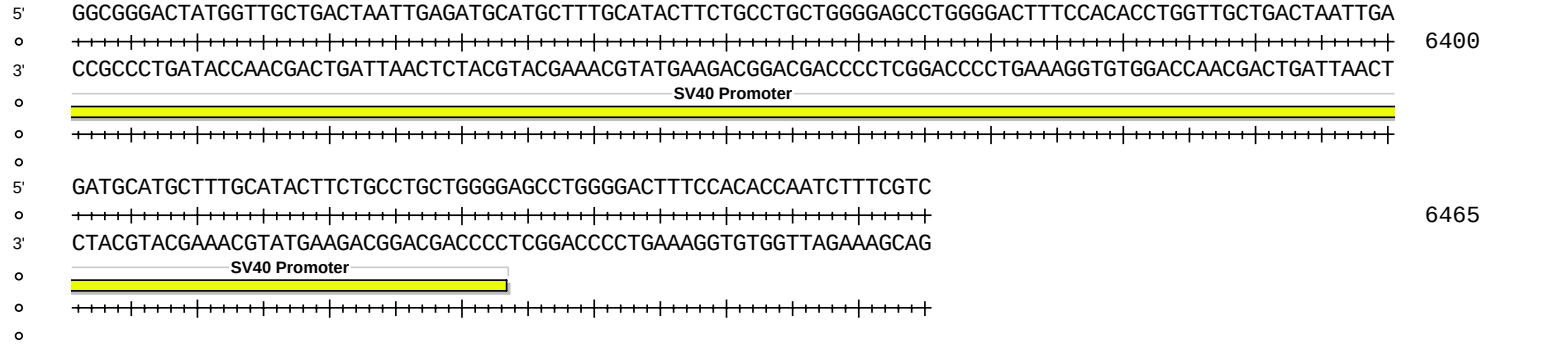
6100
3' AGCGCCCACCGCTCCGCGTGGCACCCGAACATGAGCCAGTACCTTCCAGCAGAGGAACACTCCCCAGTCCCCGCACCCAGTCCCCTACCACCGCCGTGGC
Puromycin resistance
SbfI
PstI
AvrII
5' GTCGTGGCGGCCGACCTGCAGGCATGCAAGCTAGCTTTTTGCAAAAGCCTAGGCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGA
6200
3' CAGCACCGCCGGCTGGACGTCCGTACGTTCGATCGAAAAACGTTTTCGGATCCGGAGGTTTTTTCGGAGGAGTGATGAAGACCTTATCGAGTCTCCGGCT
SV40 Promoter
SfiI
5' GGCGGCCTCGGCCTCTGCATAAATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGATGGGCGGAGTTAGG
6300
3' CCGCCGGAGCCGGAGACGTATTTATTTTTTTTAATCAGTCGGTACCCCGCCTCTTACCCGCCTTGACCCGCCTCAATCCCCGCCCTACCCGCCTCAATCC
SV40 Promoter


5'
GGCGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTTGCTGACTAATTGA
6400
3'
CCGCCCTGATACCAACGACTGATTAACTCTACGTACGAAACGTATGAAGACGGACGACCCCTCGGACCCCTGAAAGGTGTGGACCAACGACTGATTAACT
SV40 Promoter
5'
GATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCAATCTTTCGTC
6465
3'
CTACGTACGAAACGTATGAAGACGGACGACCCCTCGGACCCCTGAAAGGTGTGGTTAGAAAGCAG
SV40 Promoter