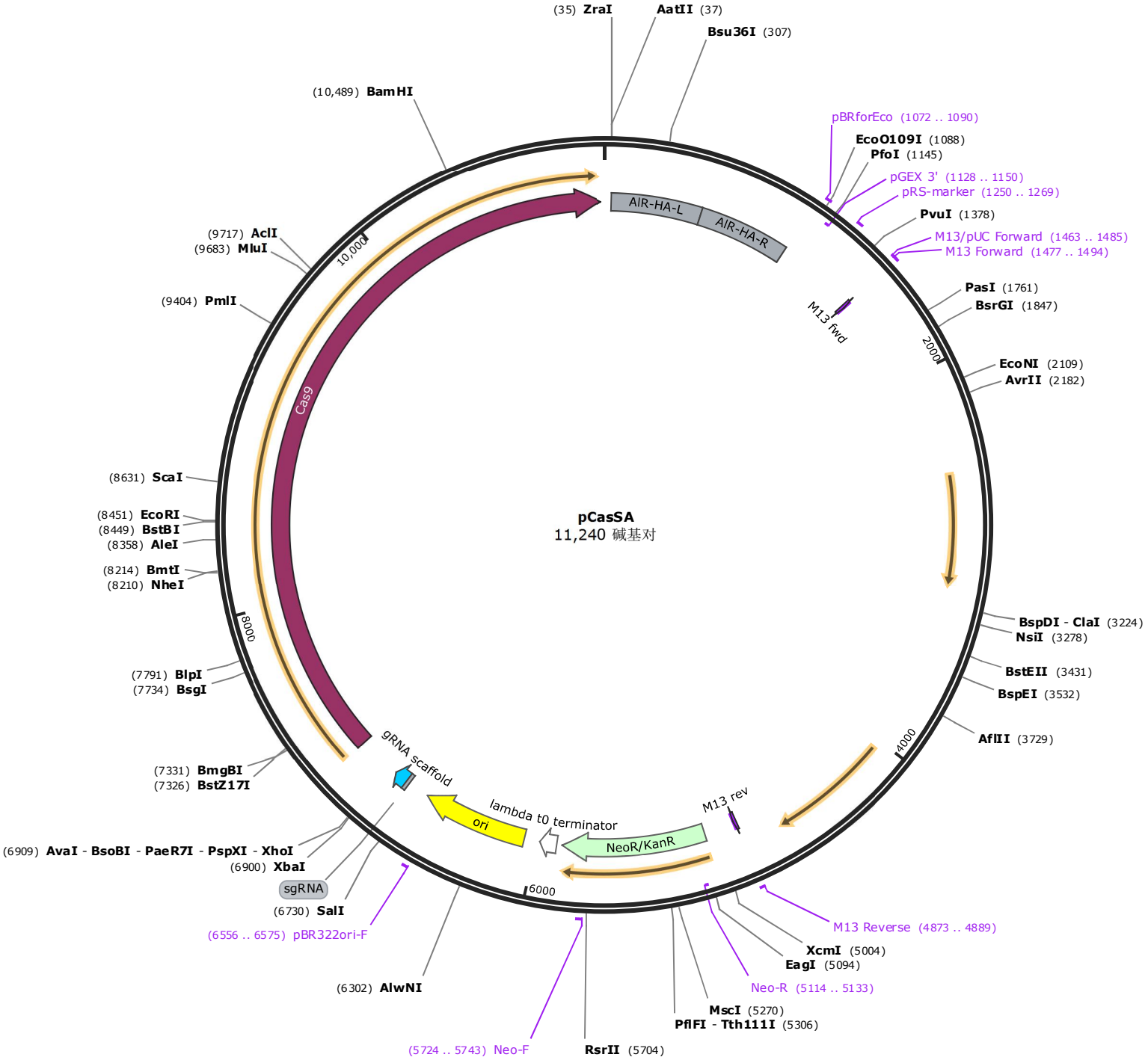
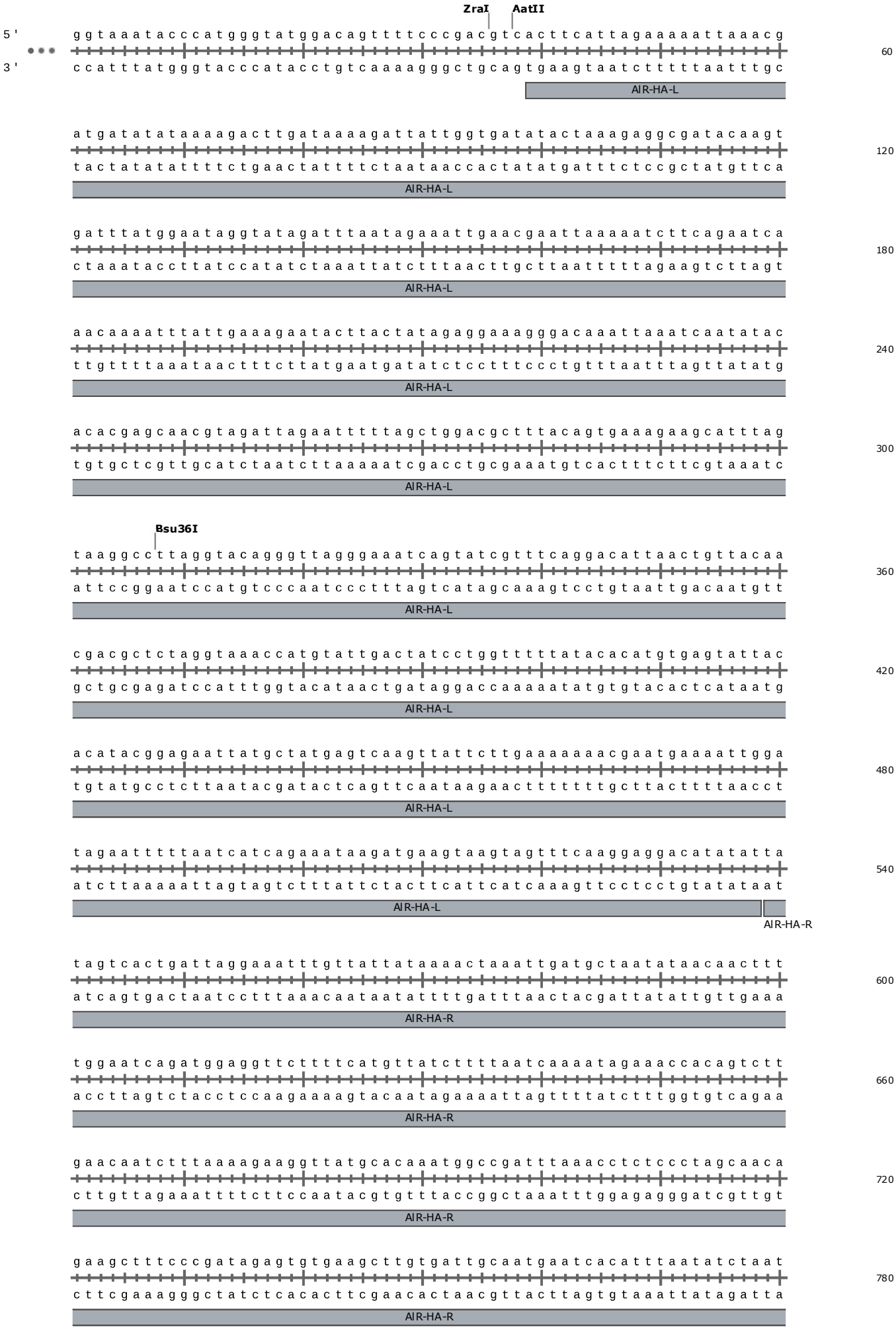
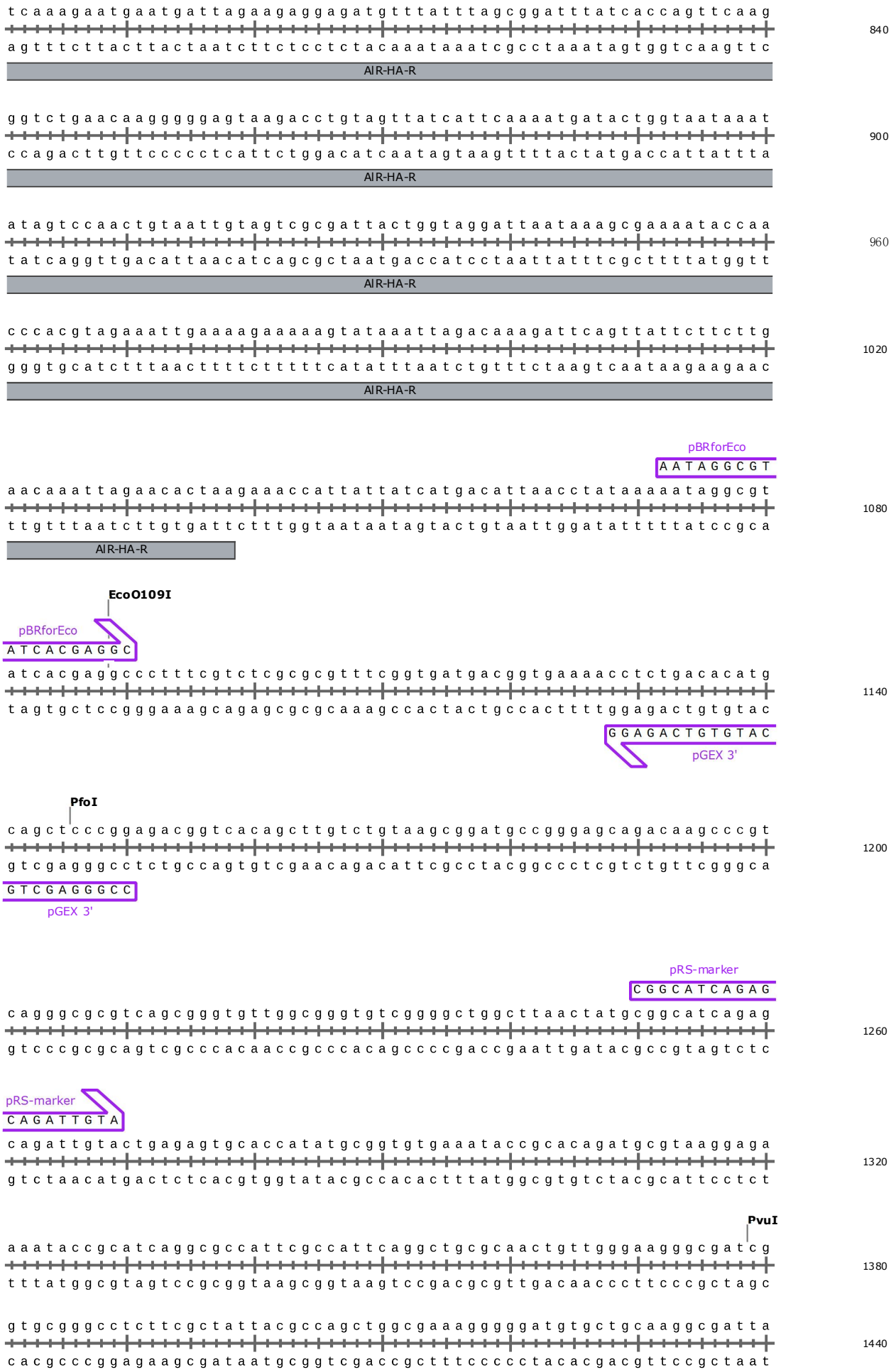
CRISPR-based E. coli/Staphylococcus aureus temperature-sensitive plasmid for genome editing in Staphylococcus aureus using S. pyogenes Cas9.




ZraI
AatII
5'
3'
60
AIR-HA-L
120
180
240
300
Bsu36I
360
420
480
540
AIR-HA-R
600
660
720
780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AIR-HA-R
900
AIR-HA-R
960
AIR-HA-R
1020
AIR-HA-R
pBRforEco
1080
AIR-HA-R
EcoO109I
pBRforEco
1140
pGEX 3'
PfoI
1200
pGEX 3'
pRS-marker
1260
pRS-marker
1320
PvuI
1380
1440


M13 Forward
TGTAAAACGACGGCCAGT
M13/pUC Forward
CCCAGTCACGACGTTGTAAAACG
agttgggtaacgccagggttttcccagtcacgacgttgtaaaacgacggccagtgccaag
tcaacccattgcggtcccaaaagggtcagtgctgcaacatttttgctgccggtcacggttc
M13 fwd
1500
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gaacgtacggacgtcttgcctaacaactactaatgctttttataattctcgtgtctgataa
1560
acacagaaaatcaagaattaaaaaaaacgtagagagagtttgaaagaagtagtgaatacat
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1620
ggaaagaggggtatcacgaaaaaaagtaaagaggttaataaattaaagcgagagaatgata
cctttctccccatagtgctttttttcatttctccaattatttaatttcgctctcttactat
1680
gtttgaatgagcagttgaatgtatcagagaaatttcaagatagtacagtgactttatatc
caaacttactcgtcaacttacatagtctctttaaagttctatcatgtcactgaaatatag
1740
PasI
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cacgacgctcccgcttaaagggacccaaactctttcccaaattatccgaatttctcttta
1800
BsrGI
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agaaattactaaggtttaagctcgcacaccctgtcaaatacctacaacatgtcctattac
1860
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1920
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tgaaaatacggctctttttgaaaaacgcacactgtcaggaatttttatatgaatctcgcatt
1980
gcgaaagtagtagcgacagctattaactttcggttgcaaagctctaggatttttaatgga
cgctttcatcatcgctgtcgataattgaaagccaacgtttcgagatcctaaaaattacct
2040
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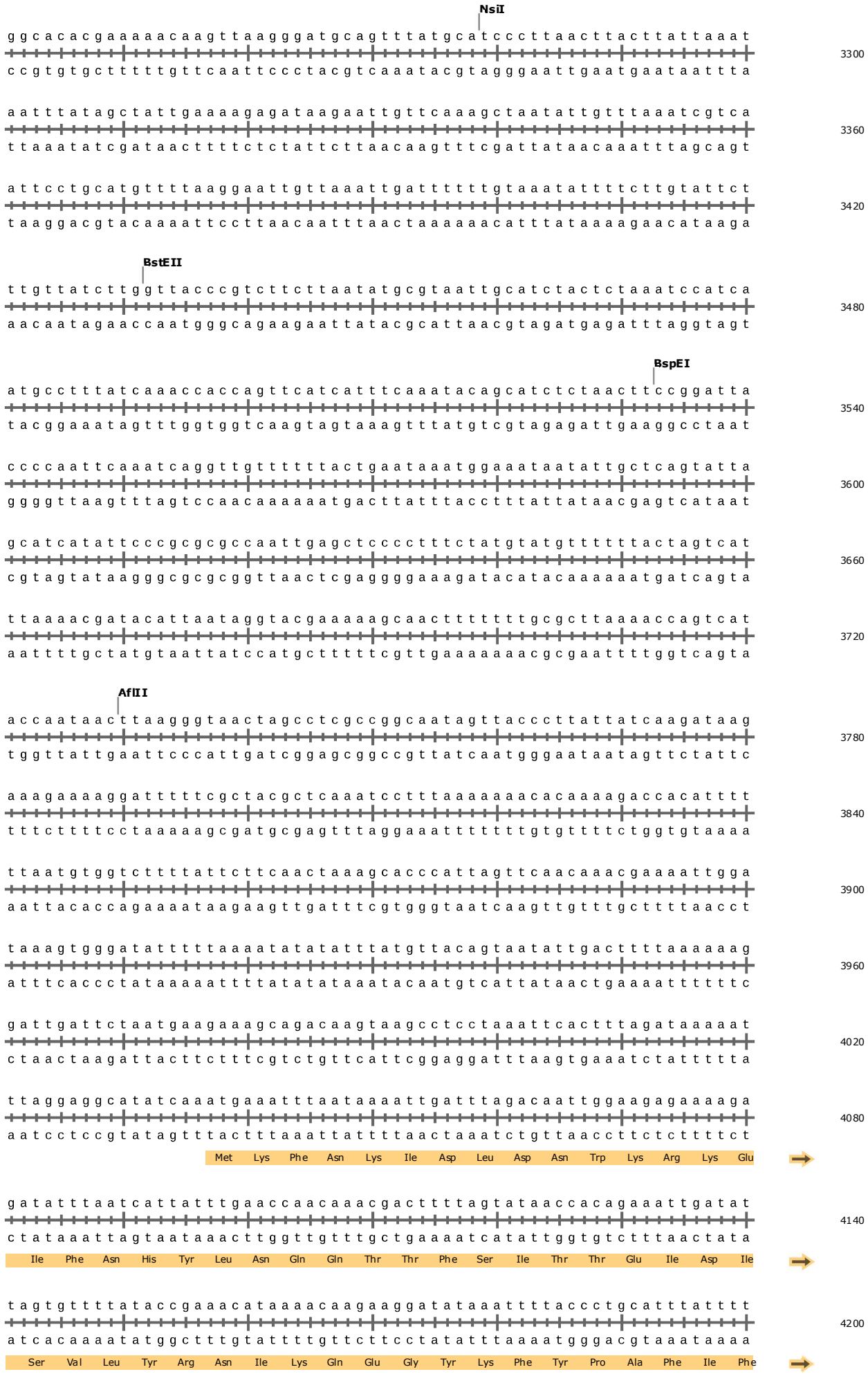
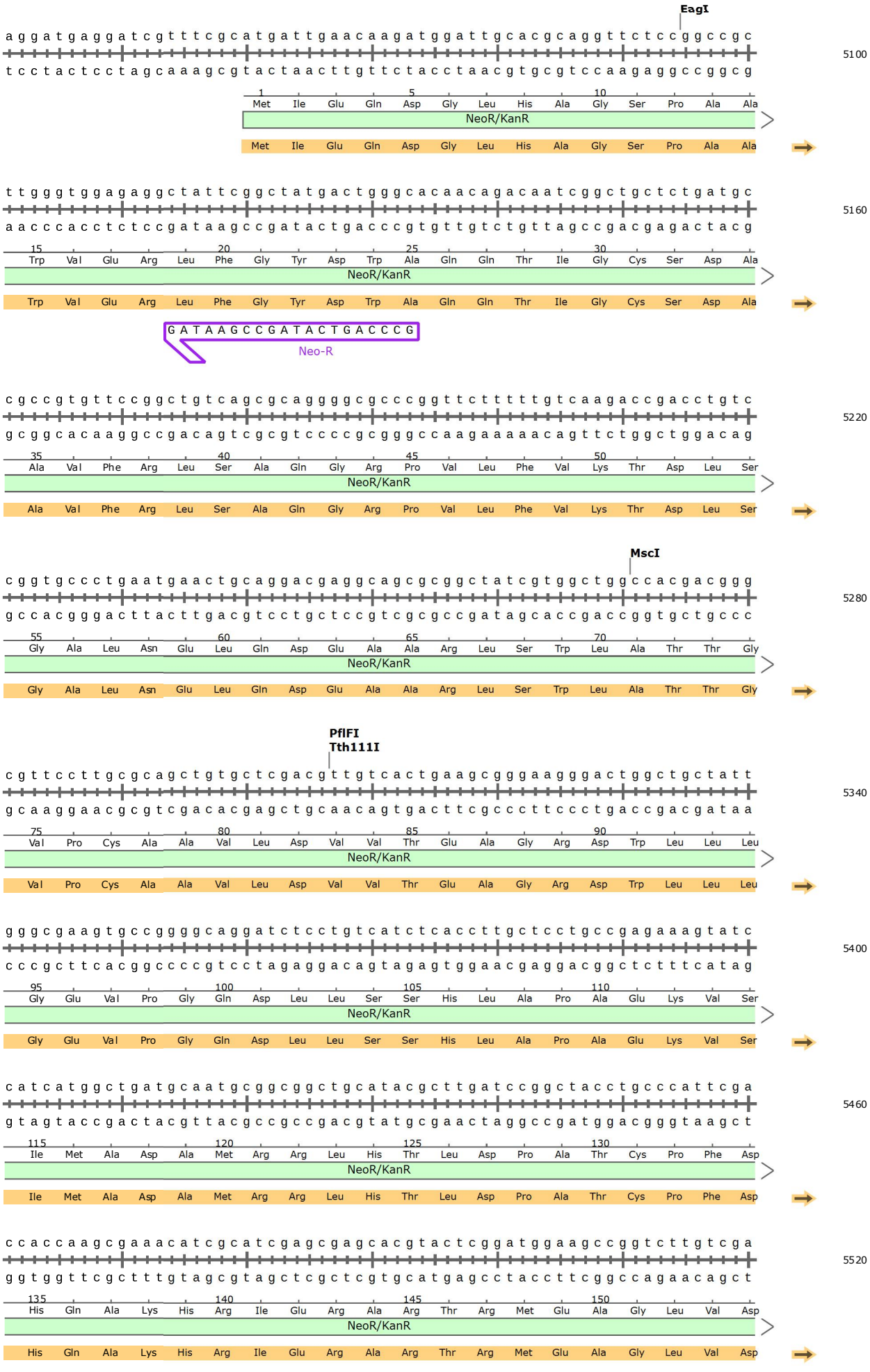
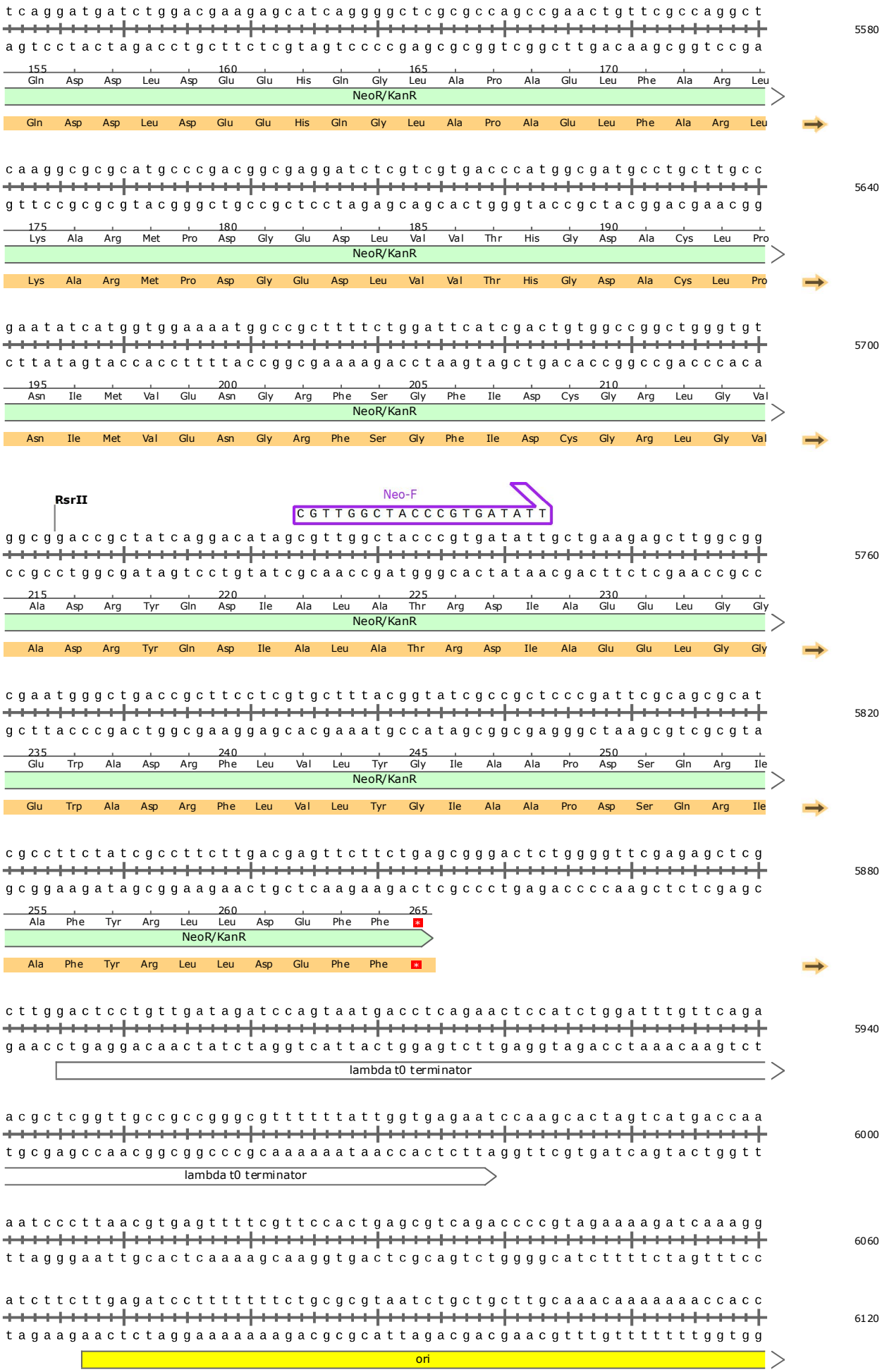
2100
EcoNI
aagacctttttgaggtctttttttcttagatttttggggttatttaggggagaaaacata
ttctggaaaaactccagaaaaaaaagaatctaaaaaccccaataaatccccctcttttgtat
2160
AvrII
ggggggtactacgacctcccccctaggtgtccattgtccattgtccaaacaaataaataa
ccccccatgatgctggaggggggatccacaggtaacaggtaacaggtttgtttatttatt
2220
atattgggttttaatgttaaaaggttgttttttatgttaaagtgaaaaaaacagatgtt
tataacccaaaaattacaatttttccaacaaaaaatacaatttcacttttttttgtctacaa
2280
gggaggtacagtgatggttgtagatagaaaagaagagaaaaaagttgctgttactttaag
ccctccatgtcactaccaacatctatctttttcttctctttttttcaacgacaatgaaattc
2340
acttacaacagaagaaaatgagatatttaaatagaatcaaagaaaaatataatattagcaa
tgaatgttgtcttctttttactctataatttatcttagtttcttttttatattataatcgtt
2400


2460
2520
2580
Met Ser Glu Asn Val Ile Lys Glu Thr Glu Asn Lys Lys
2640
Asn Ser Arg Gly Arg Asn Trp Thr Phe Val Leu Tyr Pro Glu Ser Ala Lys Ala Glu Trp
2700
Leu Glu Tyr Leu Lys Glu Leu His Ile Gln Phe Val Val Ser Pro Leu His Asp Arg Asp
2760
Thr Asp Thr Glu Asp Arg Met Lys Lys Glu His Tyr His Ile Leu Val Met Tyr Glu Gly
2820
Asn Lys Ser Tyr Glu Gln Ile Lys Ile Ile Thr Glu Glu Leu Asn Ala Thr Ile Pro Gln
2880
Ile Ala Gly Ser Val Lys Gly Leu Val Arg Tyr Met Leu His Met Asp Asp Pro Asn Lys
2940
Phe Lys Tyr Gln Lys Glu Asp Met Ile Val Tyr Gly Gly Val Asp Val Asp Glu Leu Leu
3000
Lys Lys Thr Thr Thr Asp Arg Tyr Lys Leu Ile Lys Glu Met Ile Glu Phe Ile Asp Glu
3060
Gln Gly Ile Val Glu Phe Lys Ser Leu Met Asp Tyr Ala Met Lys Phe Lys Phe Asp Asp
3120
Trp Phe Pro Leu Leu Cys Asp Asn Ser Ala Tyr Val Ile Gln Glu Tyr Ile Lys Ser Asn
3180
Arg Tyr Lys Ser Asp Arg *
ClaI
BspDI
3240

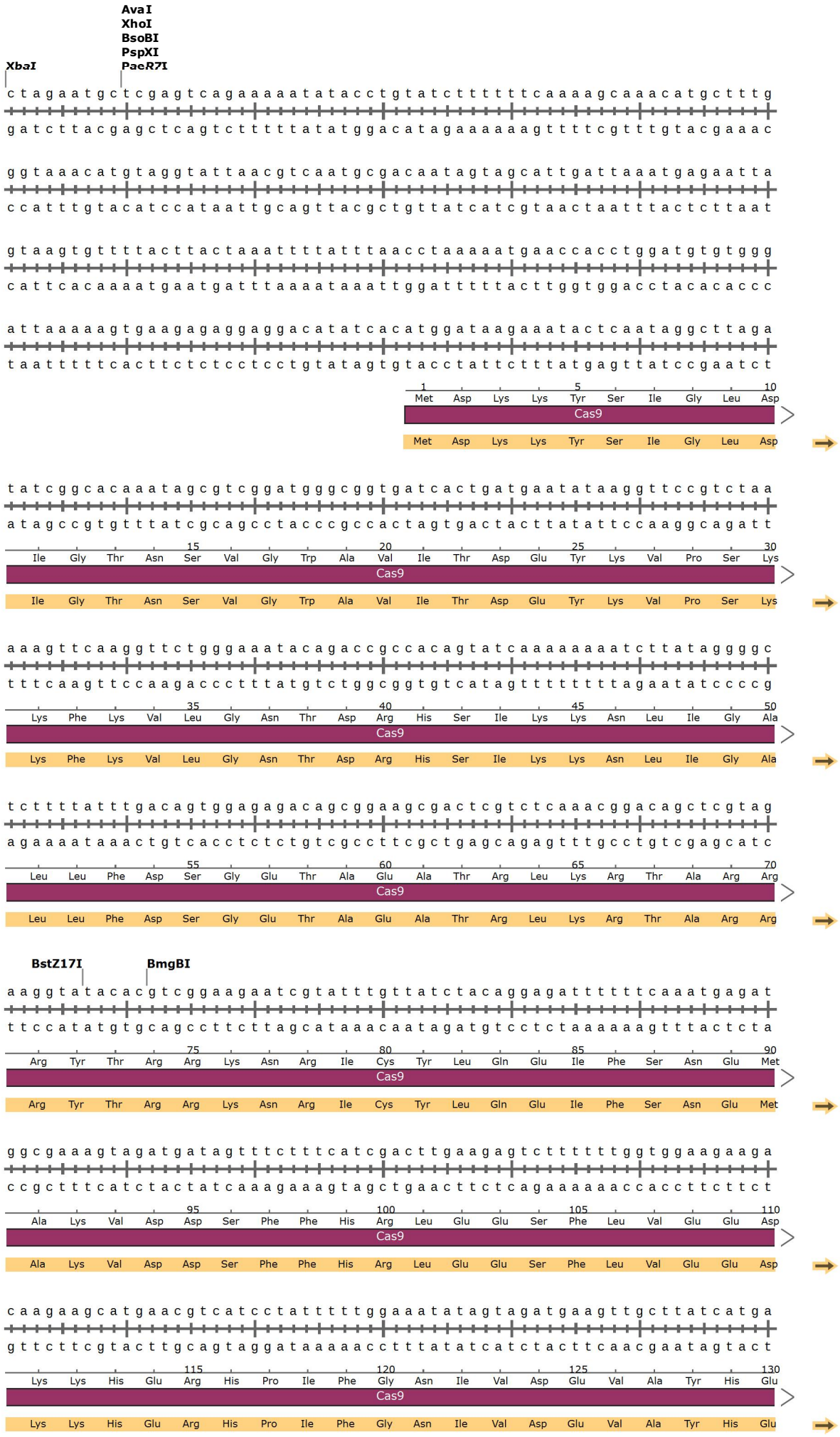

NsiI
BstEII
BspEI
AflII
3300
3360
3420
3480
3540
3600
3660
3720
3780
3840
3900
3960
4020
4080
4140
4200
Met Lys Phe Asn Lys Ile Asp Leu Asp Asn Trp Lys Arg Lys Glu
Ile Phe Asn His Tyr Leu Asn Gln Gln Thr Thr Phe Ser Ile Thr Thr Glu Ile Asp Ile
Ser Val Leu Tyr Arg Asn Ile Lys Gln Glu Gly Tyr Lys Phe Tyr Pro Ala Phe Ile Phe

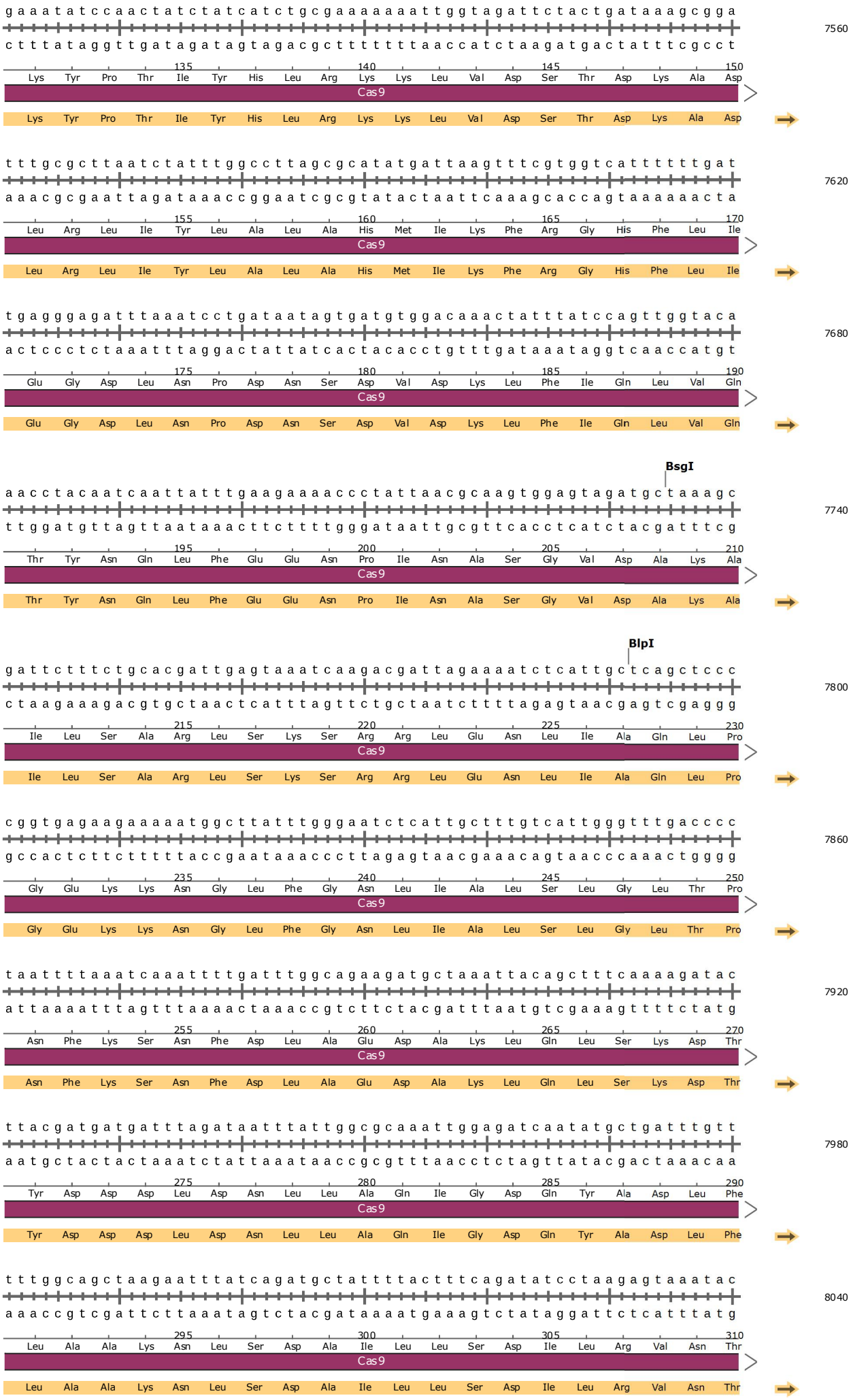

4260
Leu Val Thr Arg Val Ile Asn Ser Asn Thr Ala Phe Arg Thr Gly Tyr Asn Ser Asp Gly
4320
Glu Leu Gly Tyr Trp Asp Lys Leu Glu Pro Leu Tyr Thr Ile Phe Asp Gly Val Ser Lys
4380
Thr Phe Ser Gly Ile Trp Thr Pro Val Lys Asn Asp Phe Lys Glu Phe Tyr Asp Leu Tyr
4440
Leu Ser Asp Val Glu Lys Tyr Asn Gly Ser Gly Lys Leu Phe Pro Lys Thr Pro Ile Pro
4500
Glu Asn Ala Phe Ser Leu Ser Ile Ile Pro Trp Thr Ser Phe Thr Gly Phe Asn Leu Asn
4560
Ile Asn Asn Asn Ser Asn Tyr Leu Leu Pro Ile Ile Thr Ala Gly Lys Phe Ile Asn Lys
4620
Gly Asn Ser Ile Tyr Leu Pro Leu Ser Leu Gln Val His His Ser Val Cys Asp Gly Tyr
4680
His Ala Gly Leu Phe Met Asn Ser Ile Gln Glu Leu Ser Asp Arg Pro Asn Asp Trp Leu
4740
Leu *
4800
4860
M13 Reverse
CAGGAAACAGCTATGAC
4920
M13 rev
4980
XcmI
5040

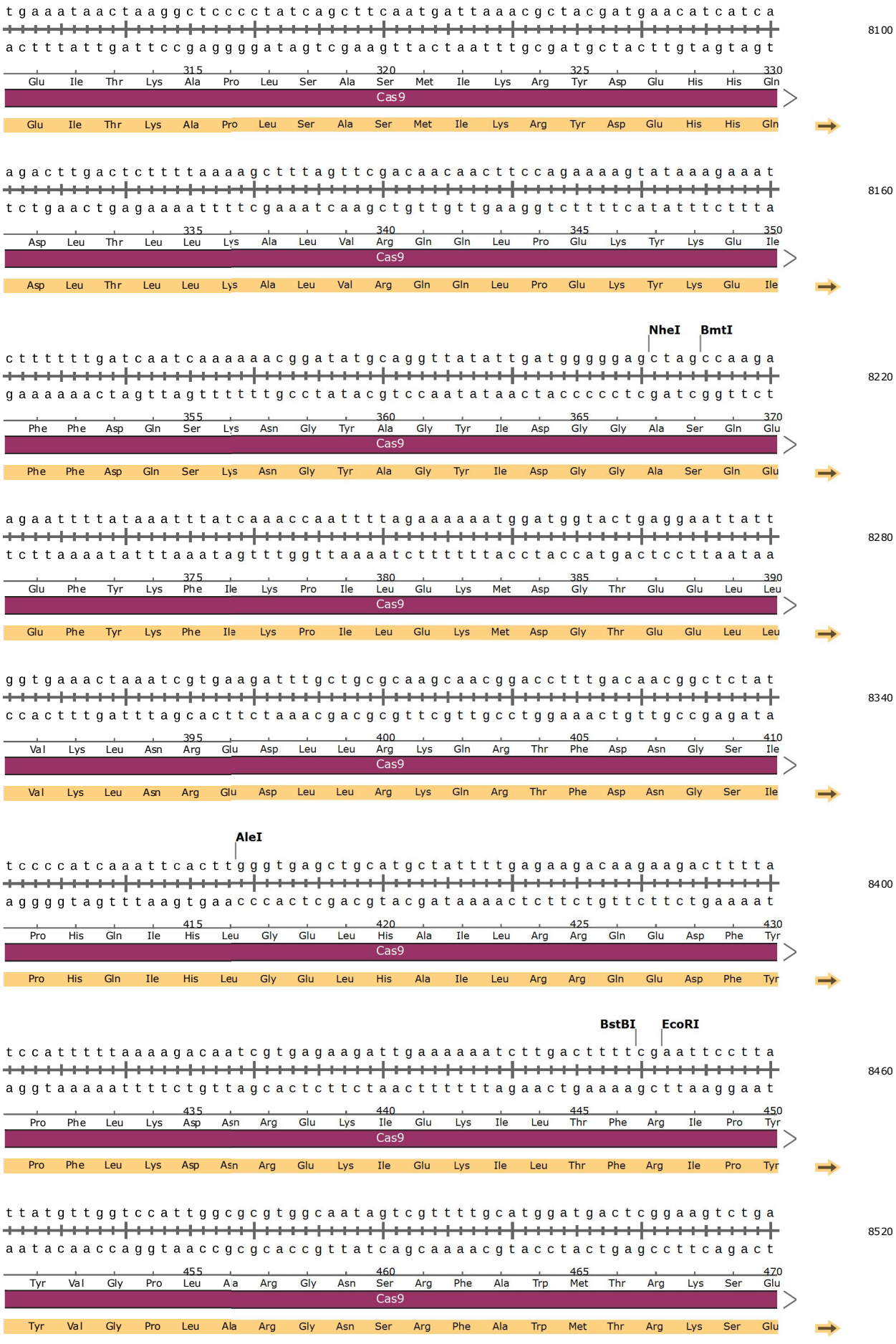

EagI
5100
NeoR/KanR
5160
NeoR/KanR
G A T A A G C C G A T A C T G A C C C G
Neo-R
5220
NeoR/KanR
MscI
5280
NeoR/KanR
PflFI
Tth111I
5340
NeoR/KanR
5400
NeoR/KanR
5460
NeoR/KanR
5520
NeoR/KanR

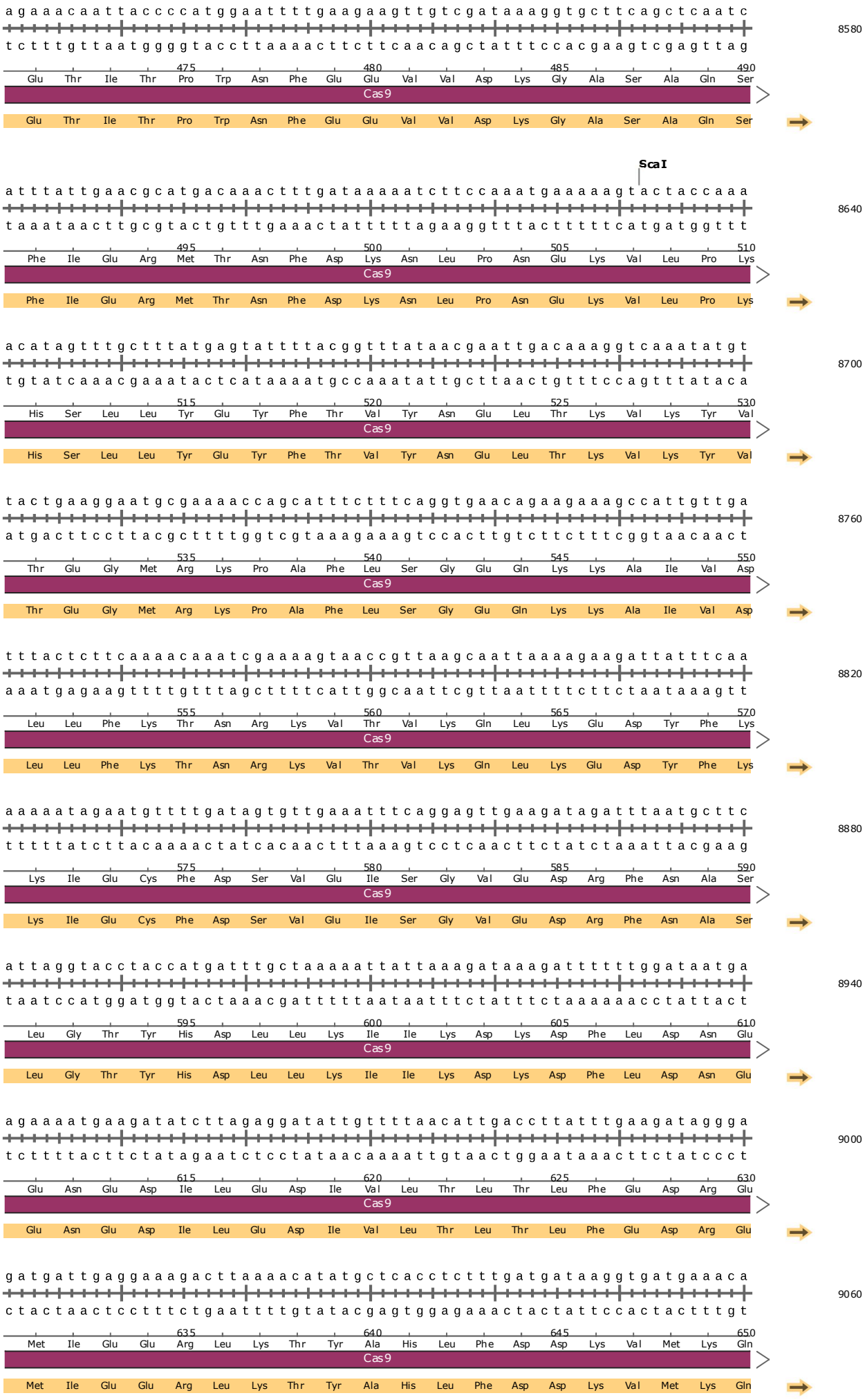

5580
Gln Asp Asp Leu Asp Glu Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu
NeoR/KanR
5640
Lys Ala Arg Met Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala Cys Leu Pro
NeoR/KanR
5700
Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp Cys Gly Arg Leu Gly Val
NeoR/KanR
RsrII
Neo-F
C G T T G G C T A C C C G T G A T A T T
5760
Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala Thr Arg Asp Ile Ala Glu Glu Leu Gly Gly
NeoR/KanR
5820
Glu Trp Ala Asp Arg Phe Leu Val Leu Tyr Gly Ile Ala Ala Pro Asp Ser Gln Arg Ile
NeoR/KanR
5880
Ala Phe Tyr Arg Leu Leu Asp Glu Phe Phe *
NeoR/KanR
5940
lambda t0 terminator
6000
lambda t0 terminator
6060
6120
ori

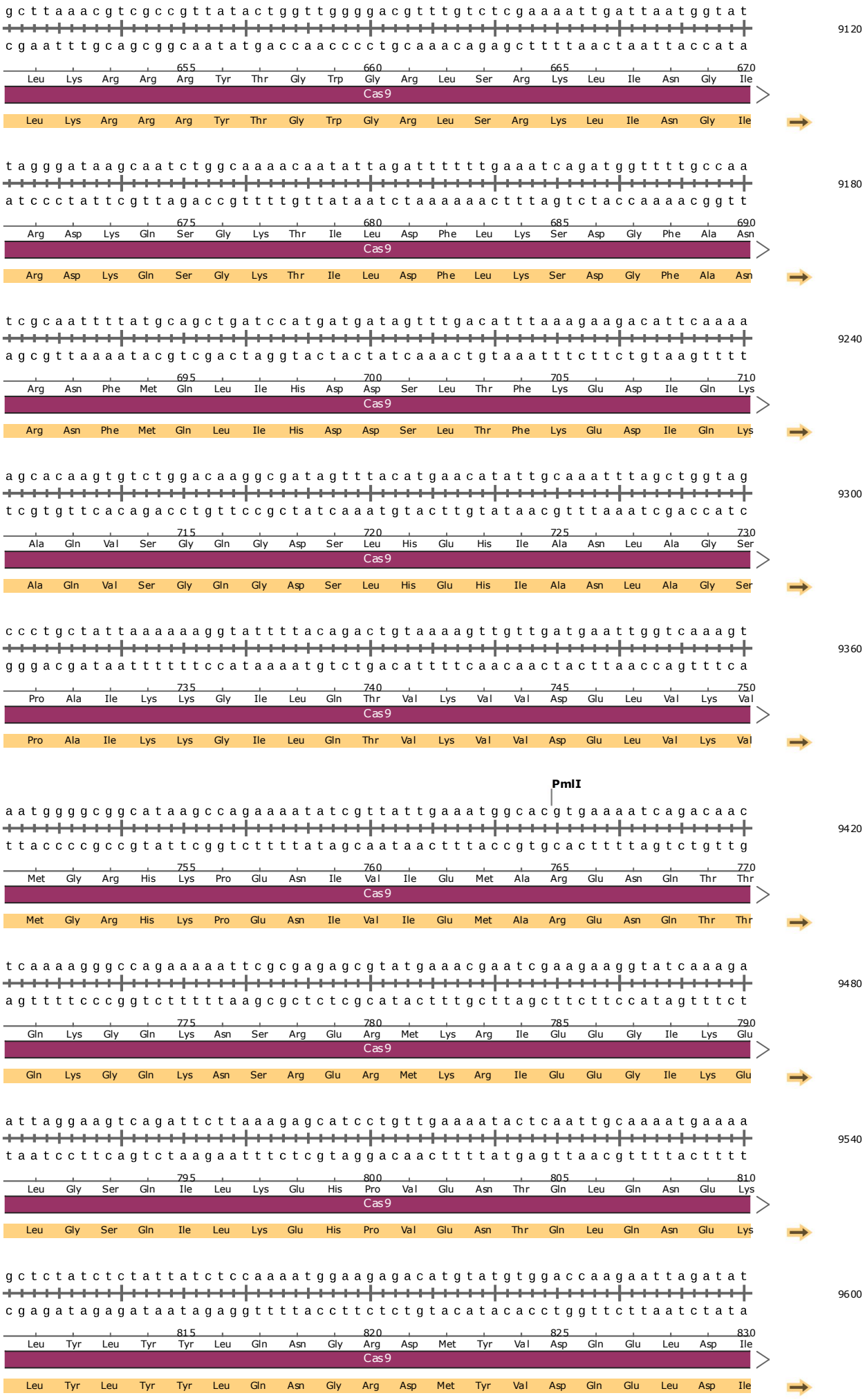

6180
ori
6240
ori
6300
ori
AlwNI
6360
ori
6420
ori
6480
ori
6540
ori
pBR322ori-F
GGGAAACGCCTGGTATCTTT
6600
ori
6660
ori
6720
SalI
6780
sgRNA
6840
sgRNA
gRNA scaffold
6900
gRNA scaffold

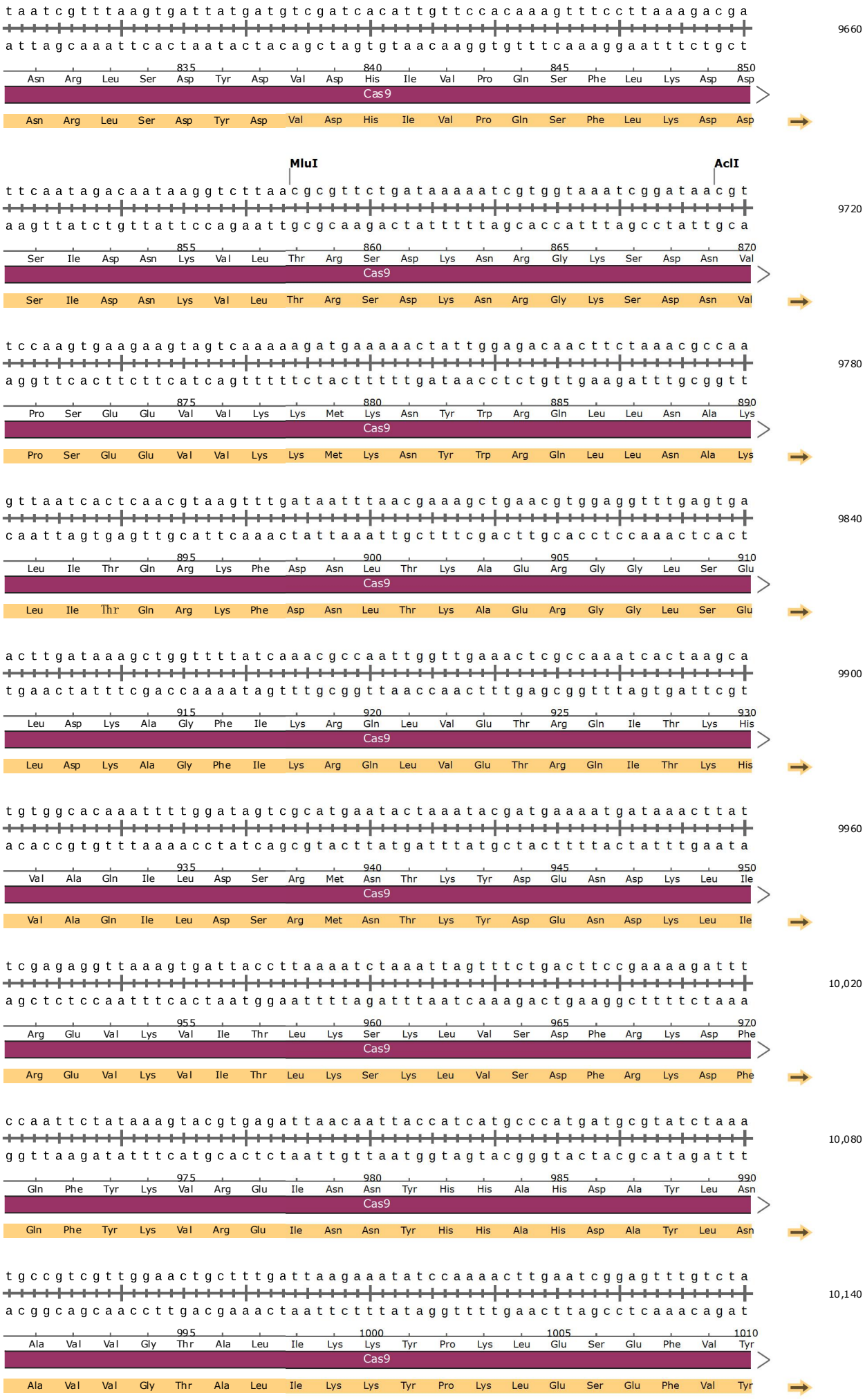

AvaI
XhoI
BsoBI
PspXI
PaeR7I
XbaI
6960
7020
7080
7140
7200
7260
7320
BstZ17I
BmgBI
7380
7440
7500
Cas9


7560
7620
7680
BsgI
7740
BlpI
7800
7860
7920
7980
8040
Cas9


8100
8160
NheI
BmtI
8220
8280
8340
AleI
8400
BstBI
EcoRI
8460
8520
Cas9


8580
Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu Val Val Asp Lys Gly Ala Ser Ala Gln Ser
475 480 485 490
Cas9
8640
ScaI
Phe Ile Glu Arg Met Thr Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys
495 500 505 510
Cas9
8700
His Ser Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys Tyr Val
515 520 525 530
Cas9
8760
Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln Lys Lys Ala Ile Val Asp
535 540 545 550
Cas9
8820
Leu Leu Phe Lys Thr Asn Arg Lys Val Thr Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys
555 560 565 570
Cas9
8880
Lys Ile Glu Cys Phe Asp Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser
575 580 585 590
Cas9
8940
Leu Gly Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp Asn Glu
595 600 605 610
Cas9
9000
Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr Leu Phe Glu Asp Arg Glu
615 620 625 630
Cas9
9060
Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala His Leu Phe Asp Asp Lys Val Met Lys Gln
635 640 645 650
Cas9


9120
9180
9240
9300
9360
PmlI
9420
9480
9540
9600
Cas9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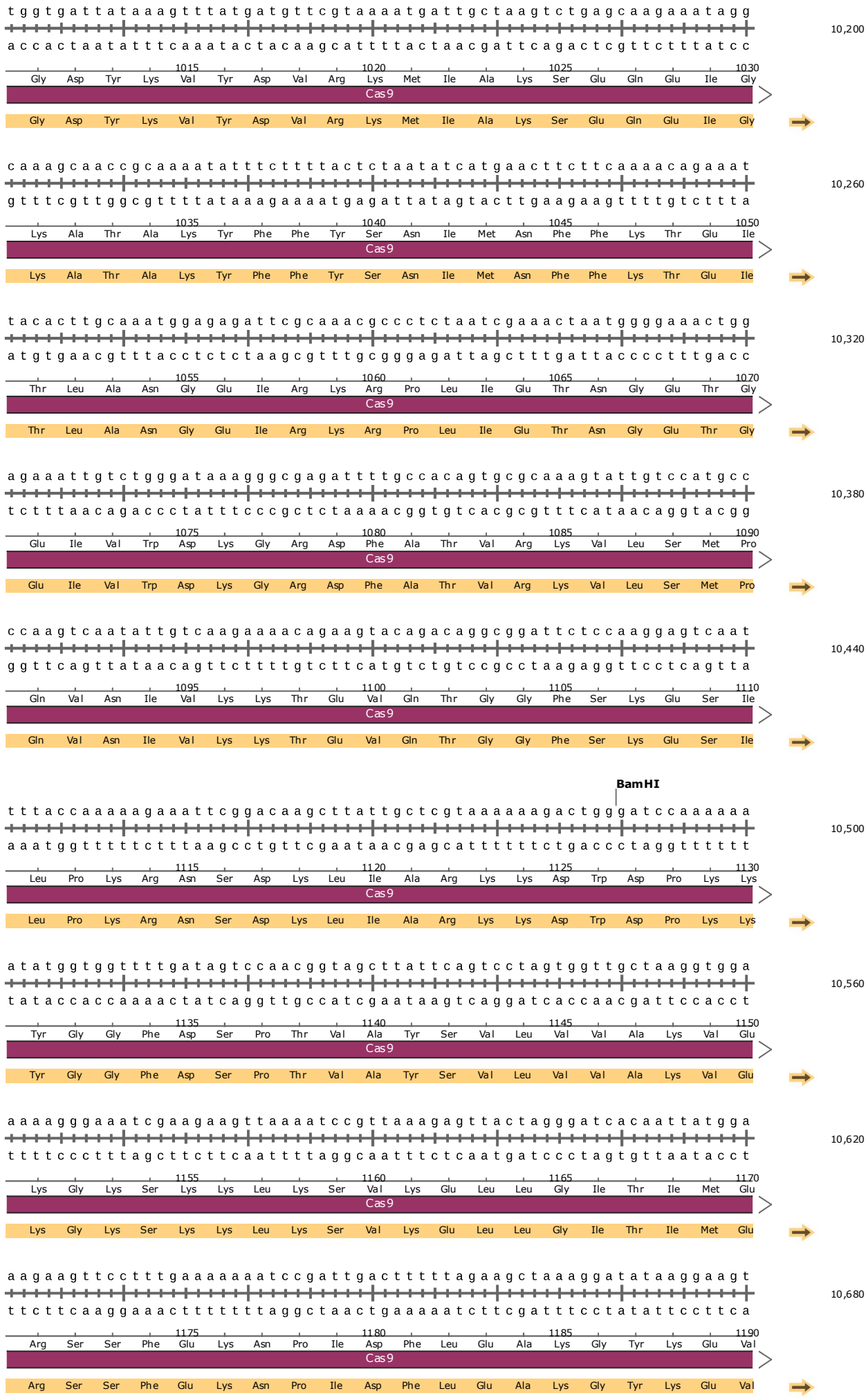
9720
MluI
AclI
9780
9840
9900
9960
10,020
10,080
10,140
Cas9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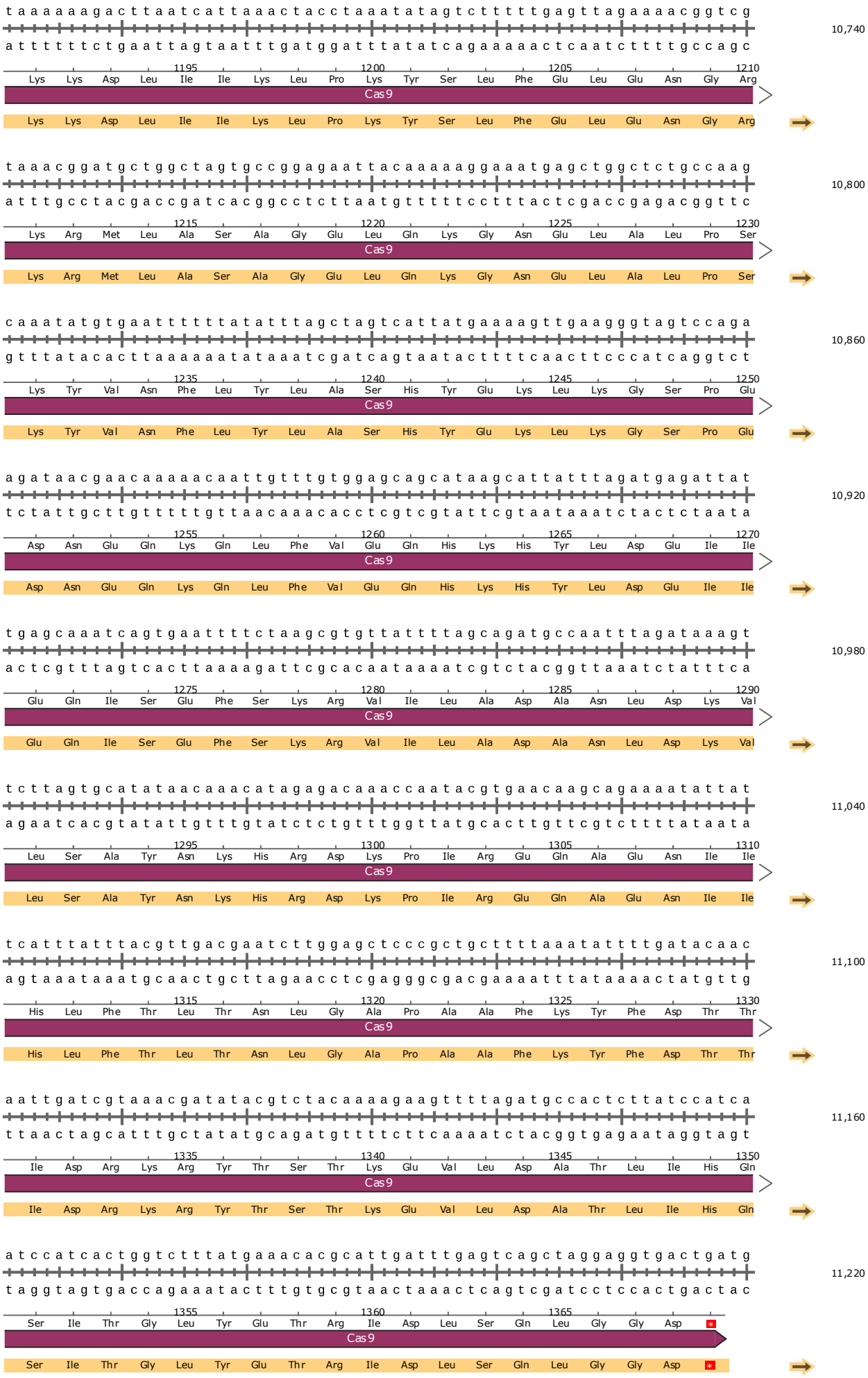
10,440
BamHI
10,500
10,560
10,620
10,680
Cas9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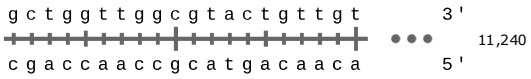
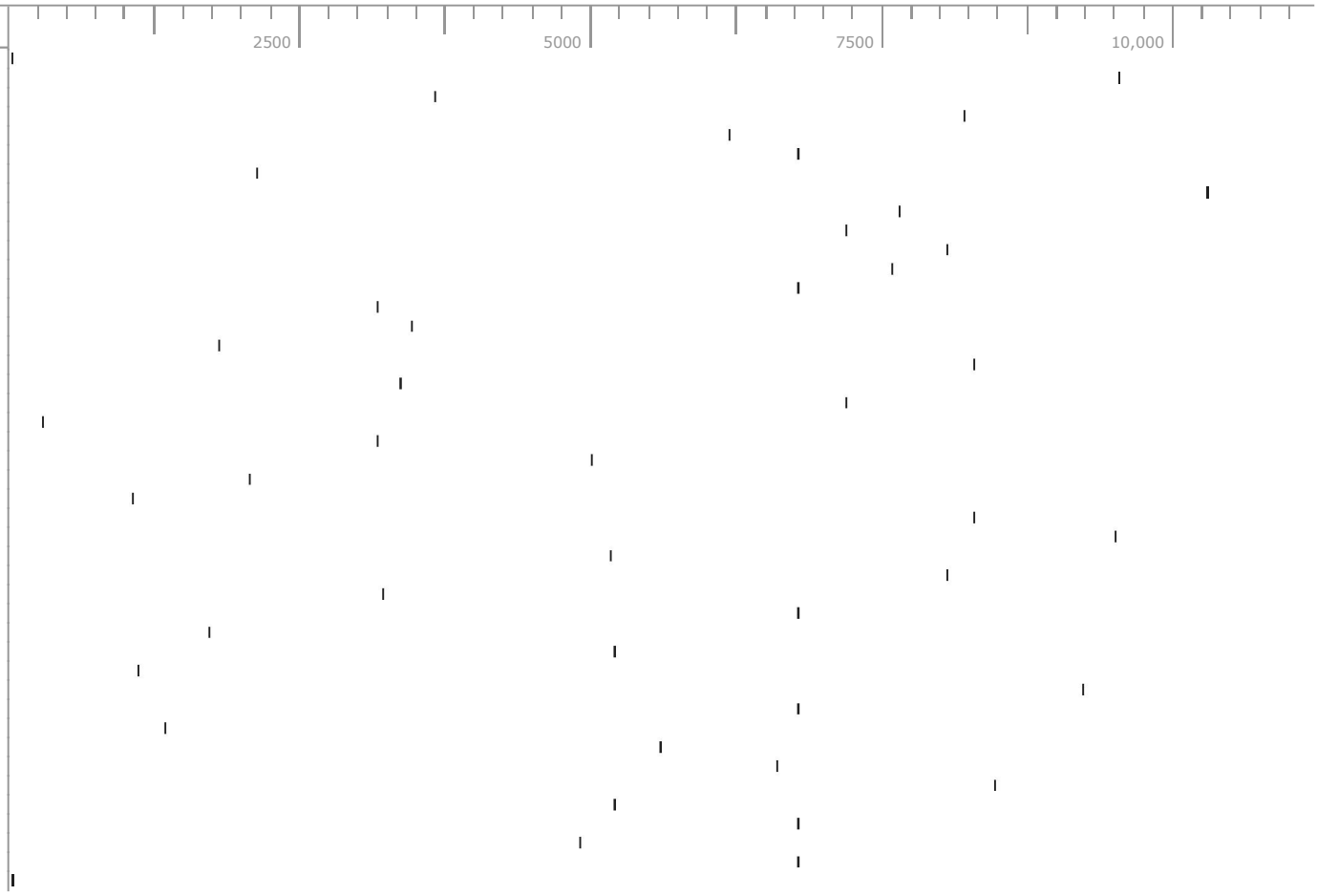
10,800
10,860
10,920
10,980
11,040
11,100
11,160
11,220
Cas9


g c t g g t t g g c g t a c t g t t g t
3'
11,240
c g a c c a a c c g c a t g a c a a c a
5'

酶	位点	位置
AatII	1	37
AclI	1	9717
AflII	1	3729
AleI	1	8358
AlwNI	1	6302
AvaI	1	6909
AvrII	1	2182
BamHI	1	10,489
BlpI	1	7791
BmgBI	1	7331
BmtI	1	8214
BsgI	1	7734
BsoBI	1	6909
BspDI	1	3224
BspEI	1	3532
BsrGI	1	1847
BstBI	1	8449
BstEII	1	3431
BstZ17I	1	7326
Bsu36I	1	307
ClaI	1	3224
EagI	1	5094
EcoNI	1	2109
EcoO109I	1	1088
EcoRI	1	8451
MluI	1	9683
MscI	1	5270
NheI	1	8210
NsiI	1	3278
PaeR7I	1	6909
PasI	1	1761
PflFI	1	5306
PfoI	1	1145
PmlI	1	9404
PspXI	1	6909
PvuI	1	1378
RsrII	1	5704
SalI	1	6730
ScaI	1	8631
Tth111I	1	5306
XbaI	1	6900
XcmI	1	5004
XhoI	1	6909
ZraI	1	35

酶	位点
AatII	1
AclI	1
AflII	1
AleI	1
AlwNI	1
AvaI	1
AvrII	1
BamHI	1
BlpI	1
BmgBI	1
BmtI	1
BsgI	1
BsoBI	1
BspDI	1
BspEI	1
BsrGI	1
BstBI	1
BstEII	1
BstZ17I	1
Bsu36I	1
ClaI	1
EagI	1
EcoNI	1
EcoO109I	1
EcoRI	1
MluI	1
MscI	1
NheI	1
NsiI	1
PaeR7I	1
PasI	1
PflFI	1
PfoI	1
PmlI	1
PspXI	1
PvuI	1
RsrII	1
SalI	1
ScaI	1
Tth111I	1
XbaI	1
XcmI	1
XhoI	1
ZraI	1



特征	位置			大小	颜色	方向	类型
AIR-HA-L	39	..	538	500 碱基对		⟼	misc_feature
AIR-HA-R	539	..	1038	500 碱基对		⟼	misc_feature
› **Cas9**	7112	..	11,218	4107 碱基对		→	CDS
› **gRNA scaffold**	6796	..	6871	76 碱基对		→	misc_RNA
› **lambda t0 terminator**	5885	..	5979	95 碱基对		→	terminator
› **M13 fwd**	1478	..	1494	17 碱基对		→	primer_bind
› **M13 rev**	4873	..	4889	17 碱基对		→	primer_bind
› **NeoR/KanR**	5060	..	5854	795 碱基对		→	CDS
› **ori**	6067	..	6655	589 碱基对		→	rep_origin
sgRNA	6776	..	6795	20 碱基对		⟼	misc_feature

引物	长度	颜色	结合位点	方向	温度
M13 Forward	18-mer		1477 .. 1494	→	57°C
M13 Reverse	17-mer		4873 .. 4889	→	49°C
M13/pUC Forward	23-mer		1463 .. 1485	→	61°C
Neo-F	20-mer		5724 .. 5743	→	57°C
Neo-R	20-mer		5114 .. 5133	←	58°C
pBR322ori-F	20-mer		6556 .. 6575	→	56°C
pBRforEco	19-mer		1072 .. 1090	→	57°C
pGEX 3'	23-mer		1128 .. 1150	←	66°C
pRS-marker	20-mer		1250 .. 1269	→	54°C