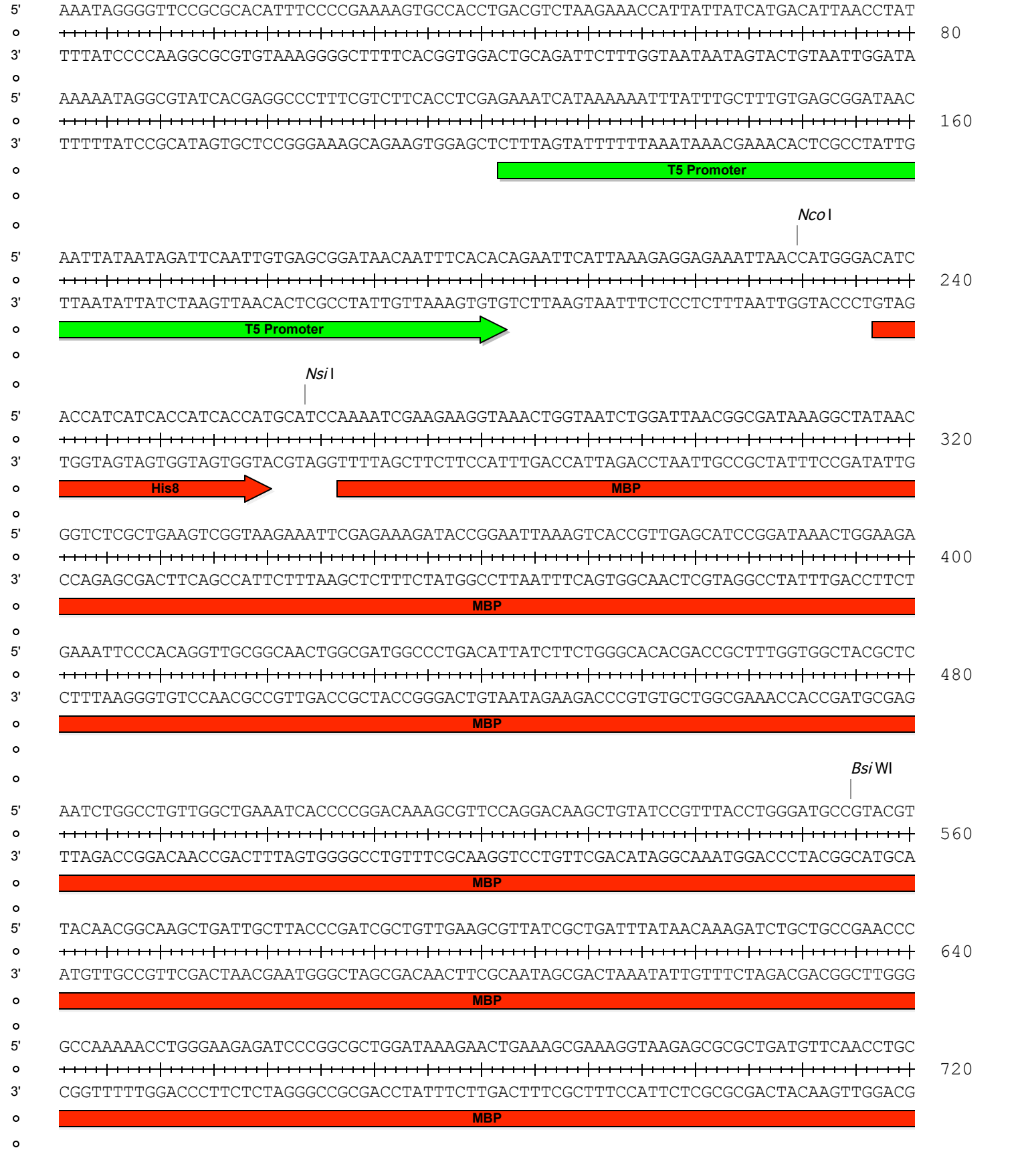
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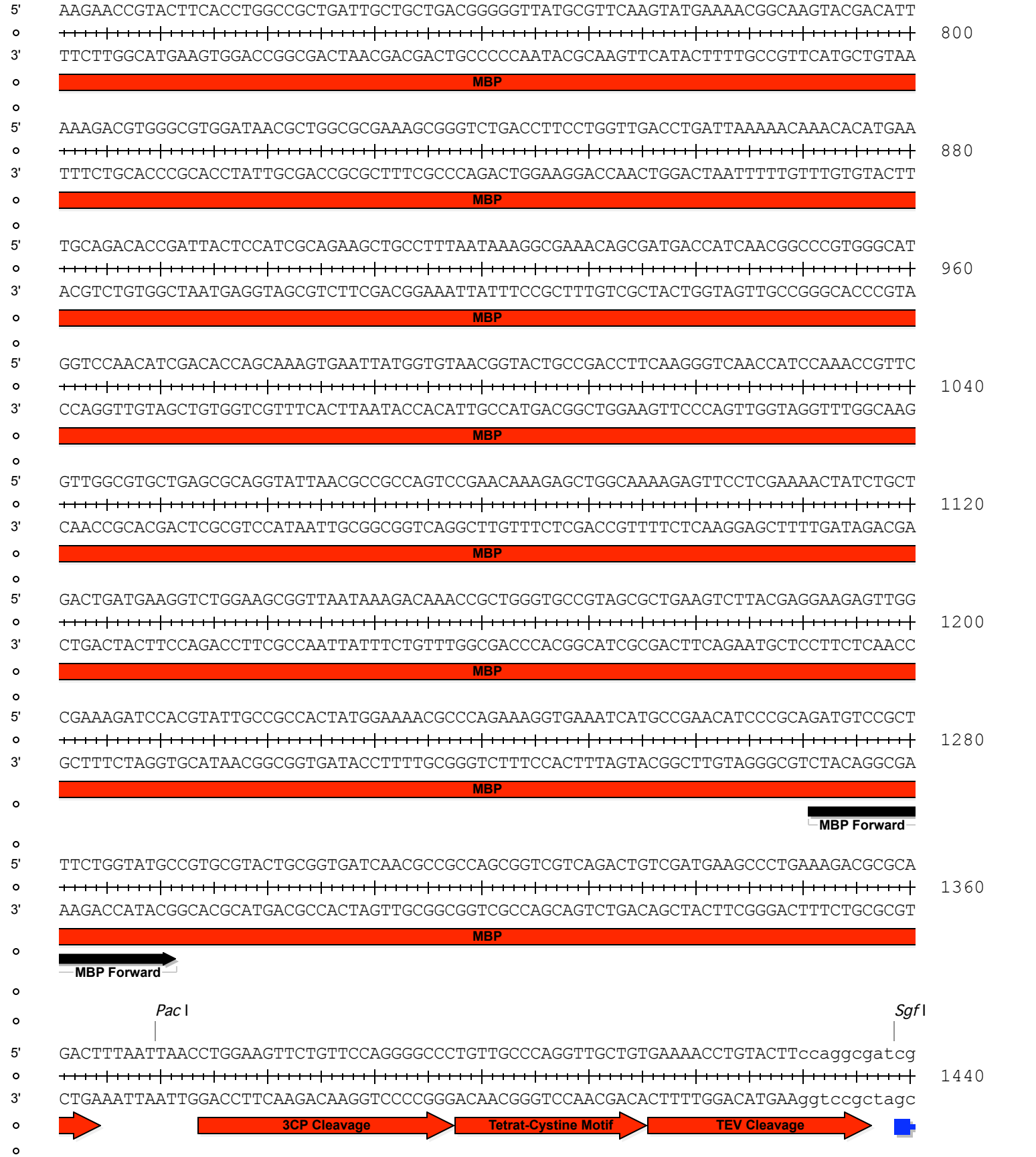
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MBP Forward
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Pac I
Sgf I
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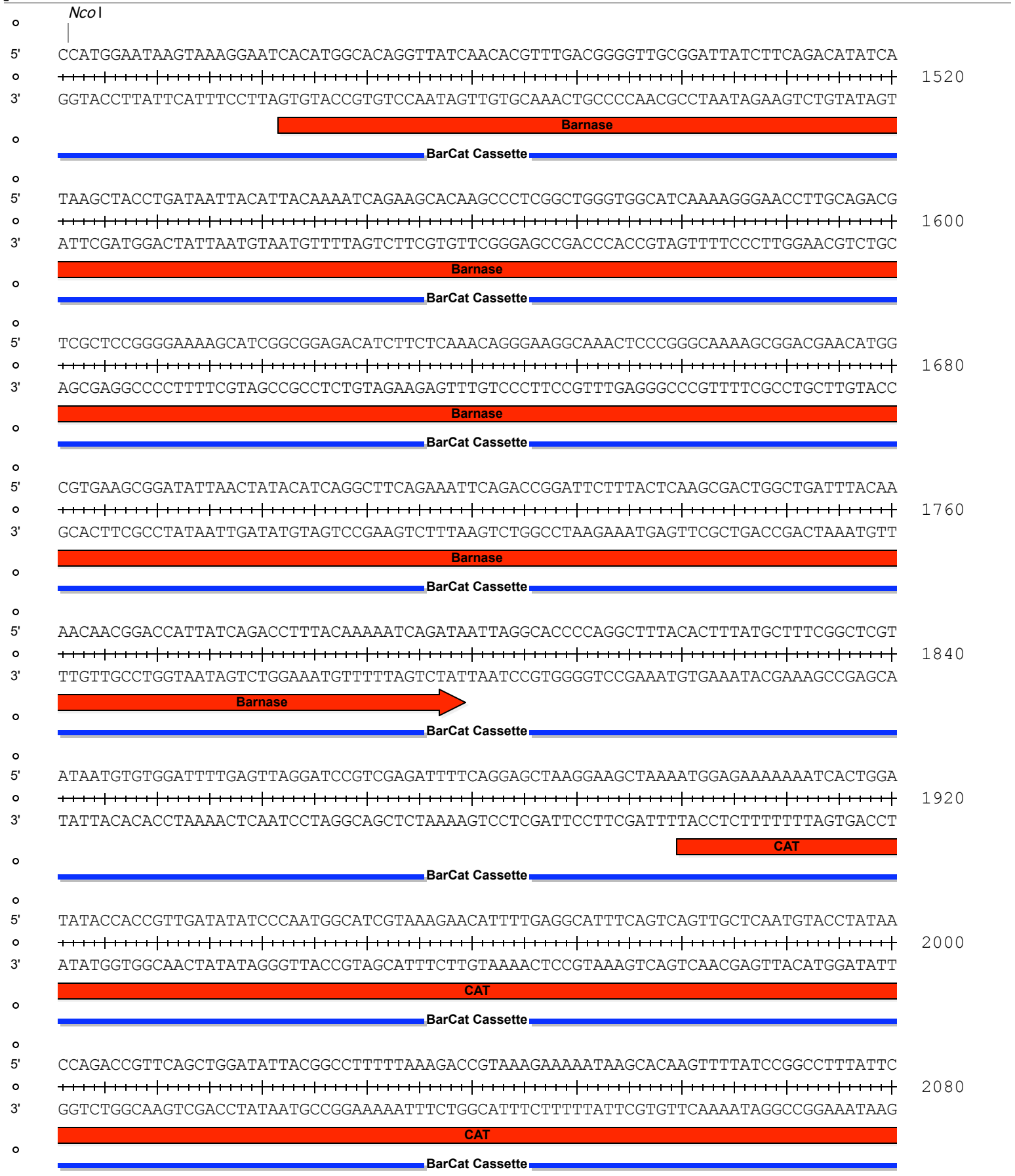
TEV Cleavage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BarCat Cassette
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1600
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Barnase
BarCat Cassette
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1680
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Barnase
BarCat Cassette
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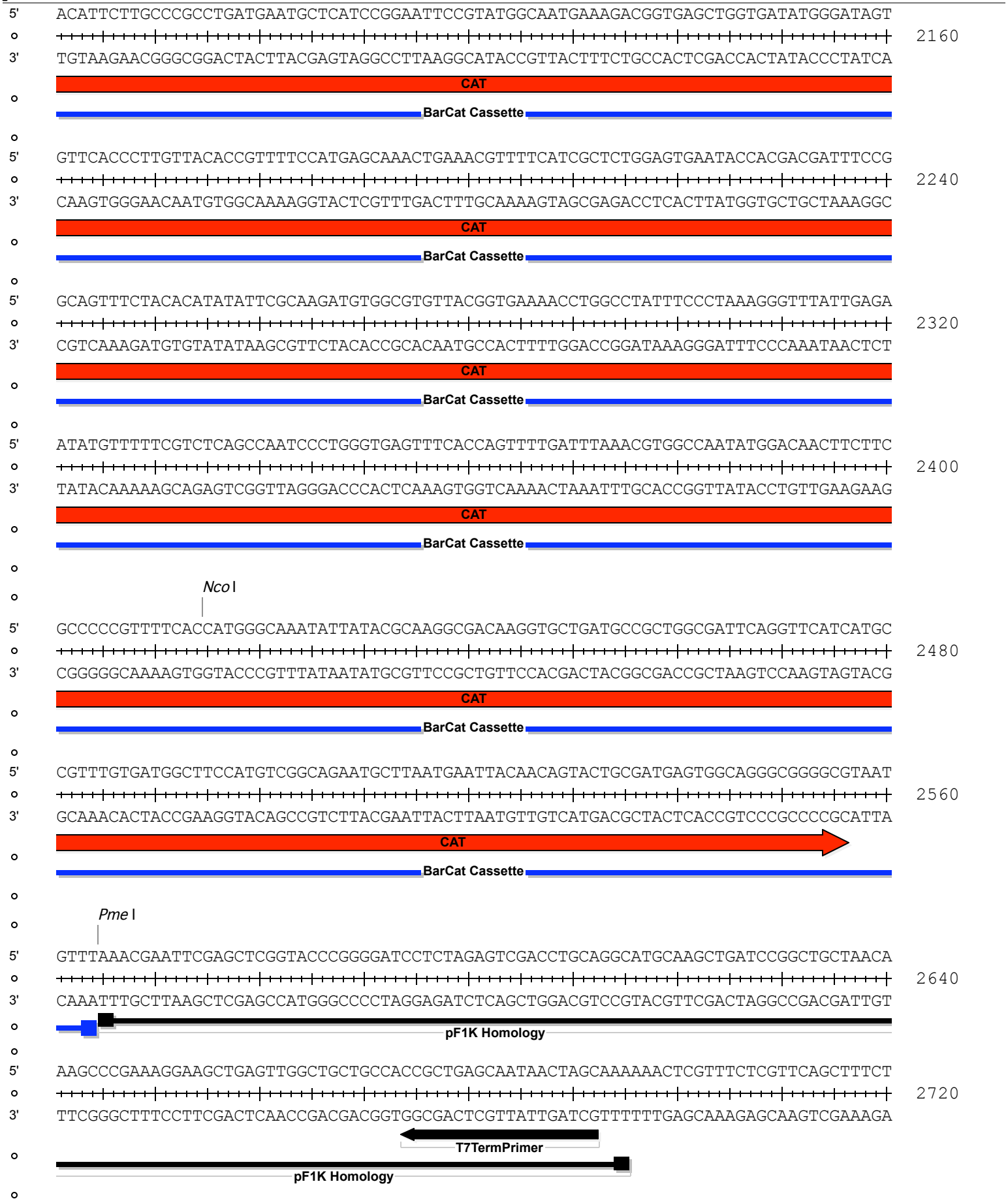
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2320
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BarCat Cassette
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2400
CAT
BarCat Cassette
NcoI
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BarCat Cassette
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3' TTCGGGCTTTCCTTCGACTCAACCGACGACGGTGGCGACTCGTTATTGATCGTTTTTTGAGCAAAGAGCAAGTCGAAAGA
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T7TermPrimer
pF1K Homology

Hin dIII

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o  +---+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+  3120
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o  +---+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+  3280
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o
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5'  TATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCTTCG
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Nco I

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5'  GTTTGTGATGGCTTCCATGTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAATT
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5'  CAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCCTCTAG
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o
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o
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o
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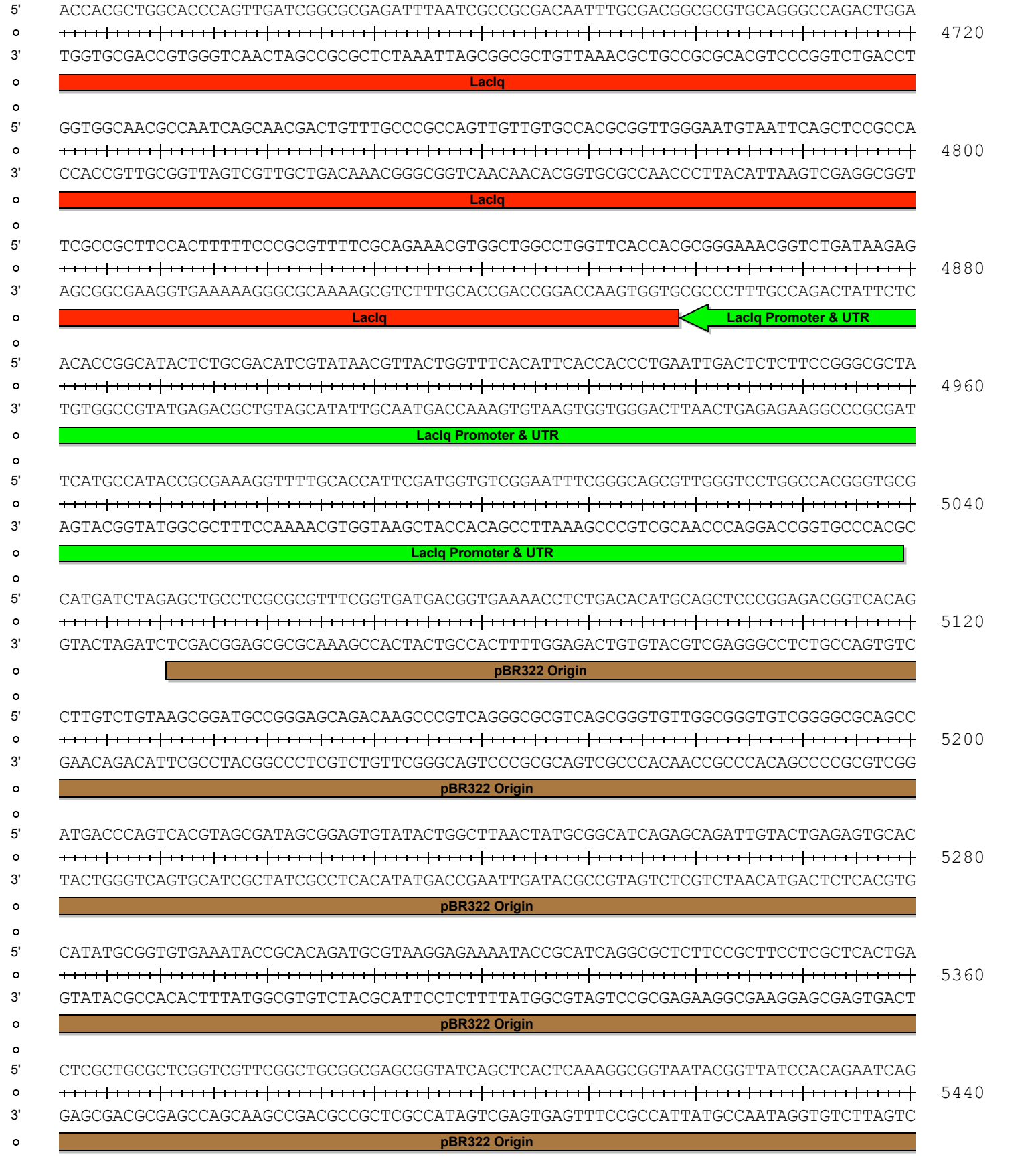
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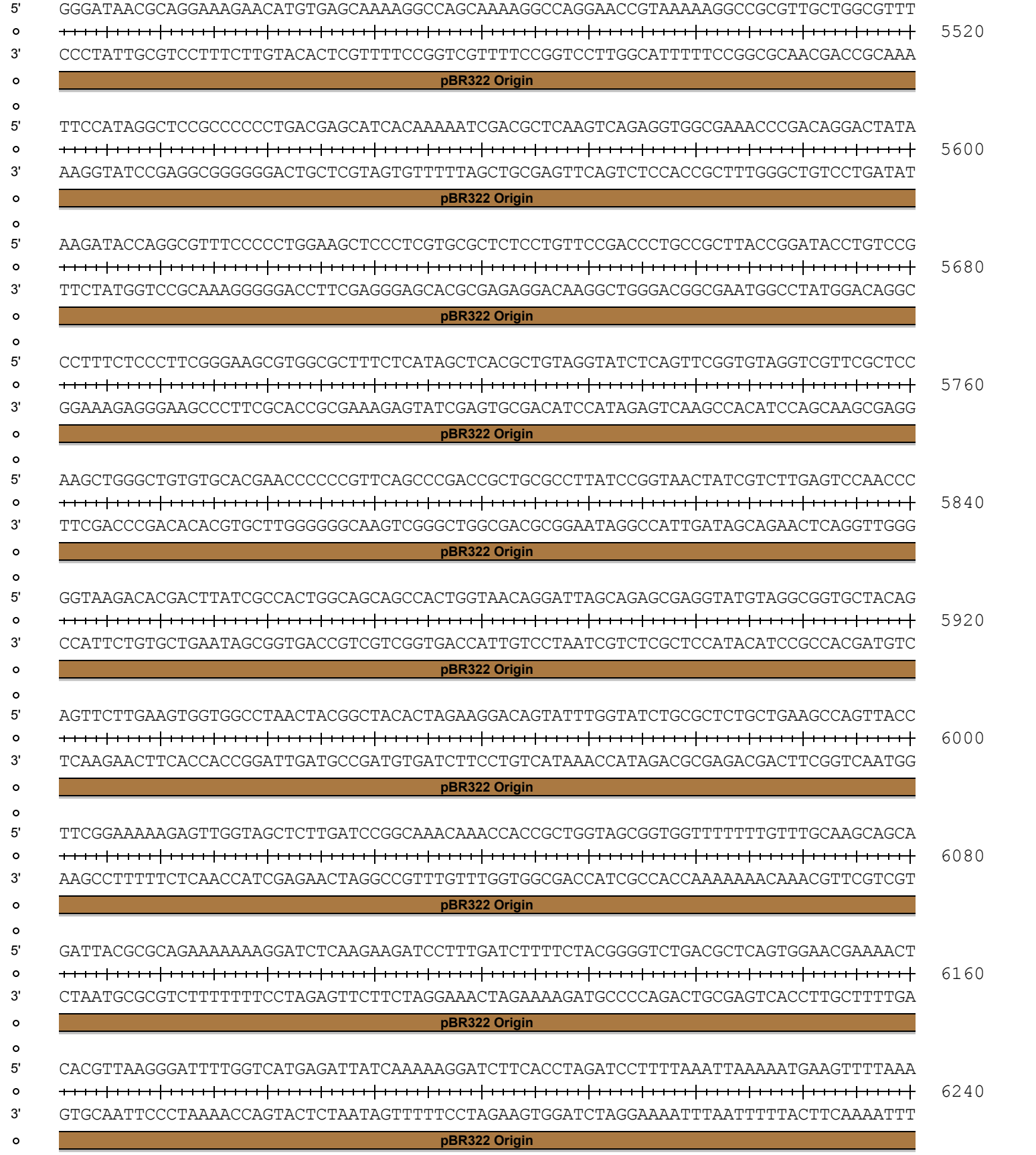
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pBR322 Origin
5520
5' TTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATA
3' AAGGTATCCGAGGCGGGGGGACTGCTCGTAGTGTTTTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCCTGATAT
pBR322 Origin
5600
5' AAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCG
3' TTCTATGGTCCGCAAAGGGGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACGGCGAATGGCCTATGGACAGGC
pBR322 Origin
5680
5' CCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCC
3' GGAAAGAGGGAAGCCCTTCGCACCGCGAAAGAGTATCGAGTGCGACATCCATAGAGTCAAGCCACATCCAGCAAGCGAGG
pBR322 Origin
5760
5' AAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCC
3' TTCGACCCGACACACGTGCTTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAACTCAGGTTGGG
pBR322 Origin
5840
5' GGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAG
3' CCATTCTGTGCTGAATAGCGGTGACCGTCGTCGGTGACCATTGTCCTAATCGTCTCGCTCCATACATCCGCCACGATGTC
pBR322 Origin
5920
5' AGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACC
3' TCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTTCCTGTCATAAACCATAGACGCGAGACGACTTCGGTCAATGG
pBR322 Origin
6000
5' TTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCA
3' AAGCCTTTTTCTCAACCATCGAGAACTAGGCCGTTTGTTTGGTGGCGACCATCGCCACCAAAAAAACAAACGTTCGTCGT
pBR322 Origin
6080
5' GATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACT
3' CTAATGCGCGTCTTTTTTTCCTAGAGTTCTTCTAGGAAACTAGAAAAGATGCCCCAGACTGCGAGTCACCTTGCTTTTGA
pBR322 Origin
6160
5' CACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAA
3' GTGCAATTCCCTAAAACCAGTACTCTAATAGTTTTTCCTAGAAGTGGATCTAGGAAAATTTAATTTTTACTTCAAAATTT
pBR322 Origin
6240

*Avr*II

```
5'  TCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGcctaggTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGAT
                                                                                6320
3'  AGTTAGATTTCATATATACTCATTTGAACCAGACTGTCggatccAGTCTTCTTGAGCAGTTCTTCCGCTATCTTCCGCTA
```

pBR322 Origin

Kan Resistance

```
5'  GCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATAT
                                                                                6400
3'  CGCGACGCTTAGCCCTCGCCGCTATGGCATTTCGTGCTCCTTCGCCAGTCGGGTAAGCGGCGGTTCGAGAAGTCGTTATA
```

Kan Resistance

```
5'  CACGGGTAGCCAACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCA
                                                                                6480
3'  GTGCCCATCGGTTGCGATACAGGACTATCGCCAGGCGGTGTGGGTCGGCCGGTGTCAGCTACTTAGGTCTTTTCGCCGGT
```

Kan Resistance

```
5'  TTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGAGTCACGACGAGATCCTCACCATCCGGCATACGCGCCTTGAG
                                                                                6560
3'  AAAAGGTGGTACTATAAGCCGTTCGTCCGTAGCGGTACTCAGTGCTGCTCTAGGAGTGGTAGGCCGTATGCGCGGAACTC
```

Kan Resistance

```
5'  CCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCC
                                                                                6640
3'  GGACCGCTTGTCAAGCCGACCGCGCTCGGGGACTACGAGAAGCAGGTCTAGTAGGACTAGCTGTTCTGGCCGAAGGTAGG
```

Kan Resistance

```
5'  GAGTGCGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGC
                                                                                6720
3'  CTCACGCACGAGCGAGCTACGCTACAAAGCGAACCACCAGCTTACCCGTCCATCGGCCTAGTTCGCATACGTCGGCGGCG
```

Kan Resistance

```
5'  ATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAA
                                                                                6800
3'  TAACGTAGTCGGTACTACCTATGAAAGAGCCGTCCTCGTTCCACTCTACTGTCCTCTAGGACGGGGCCGTGAAGCGGGTT
```

Kan Resistance

```
5'  TAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATA
                                                                                6880
3'  ATCGTCGGTCAGGGAAGGGCGAAGTCACTGTTGCAGCTCGTGTCGACGCGTTCCTTGCGGGCAGCACCGGTCGGTGCTAT
```

Kan Resistance

```
5'  GCCGCGCTGCCTCGTCCTGCAGTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCT
                                                                                6960
3'  CGGCGCGACGGAGCAGGACGTCAAGTAAGTCCCGTGGCCTGTCCAGCCAGAACTGTTTTTCTTGGCCCGCGGGGACGCGA
```

Kan Resistance

```
5'  GACAGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAAGC
                                                                                7040
3'  CTGTCGGCCTTGTGCCGCCGTAGTCTCGTCGGCTAACAGACAACACGGGTCAGTATCGGCTTATCGGAGAGGTGGGTTCG
```

Kan Resistance

```
5'   GGCCGGAGAACCTGCGTGCAATCCATCTTGTTCAAGCATGCGAAACGACCGTCATCCTGTCTCTTGATCAGATCTTGATC
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++  7120
3'   CCGGCCTCTTGGACGCACGTTAGGTAGAACAAGTTCGTACGCTTTGCTGGCAGTAGGACAGAGAACTAGTCTAGAACTAG
o    [Kan Resistance]
o
5'   CCCTGCGCCATCAGATCCTTGGCGGCAAGAAAGCCATCCAGTTTACTTTGCAGGGCTTCCCAACCTTACCAGAGGGCGCC
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++  7200
3'   GGGACGCGGTAGTCTAGGAACCGCCGTTCTTTCGGTAGGTCAAATGAAACGTCCCGAAGGGTTGGAATGGTCTCCCGCGG
o
o                                                          BsiWI
                                                             |
5'   CCAGCTGGCAATTCTTTTGAAGCTCACGCTGCCGCAAGCACTCAGGGcgtacg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|+++                          7253
3'   GGTCGACCGTTAAGAAAACTTCGAGTGCGACGGCGTTCGTGAGTCCCgcatgc
o
```