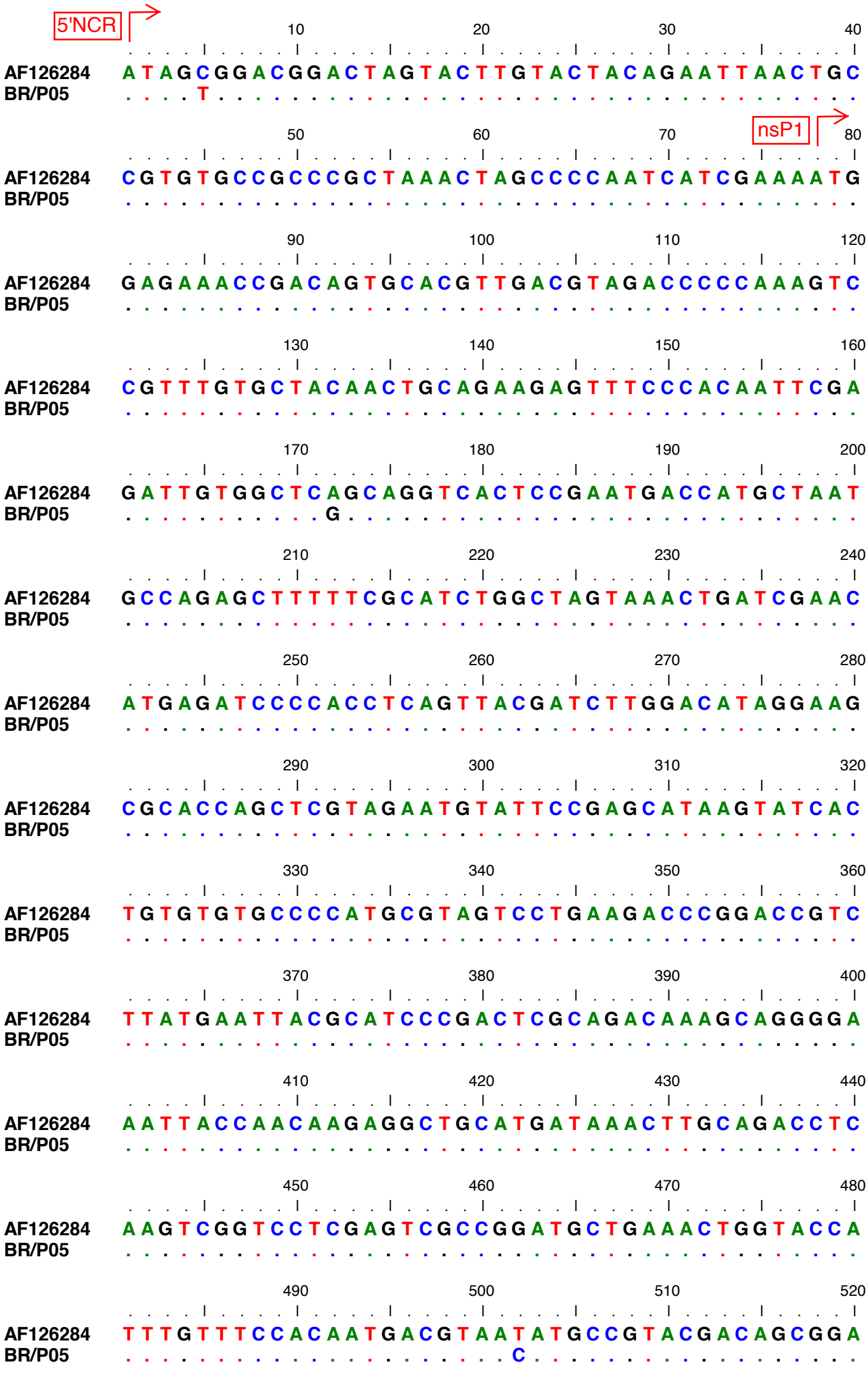
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BR/P05
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.....T.....................................
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GATTGTGGCTCAGCAGGTCACTCCGAATGACCATGCTAAT
.....G...............................
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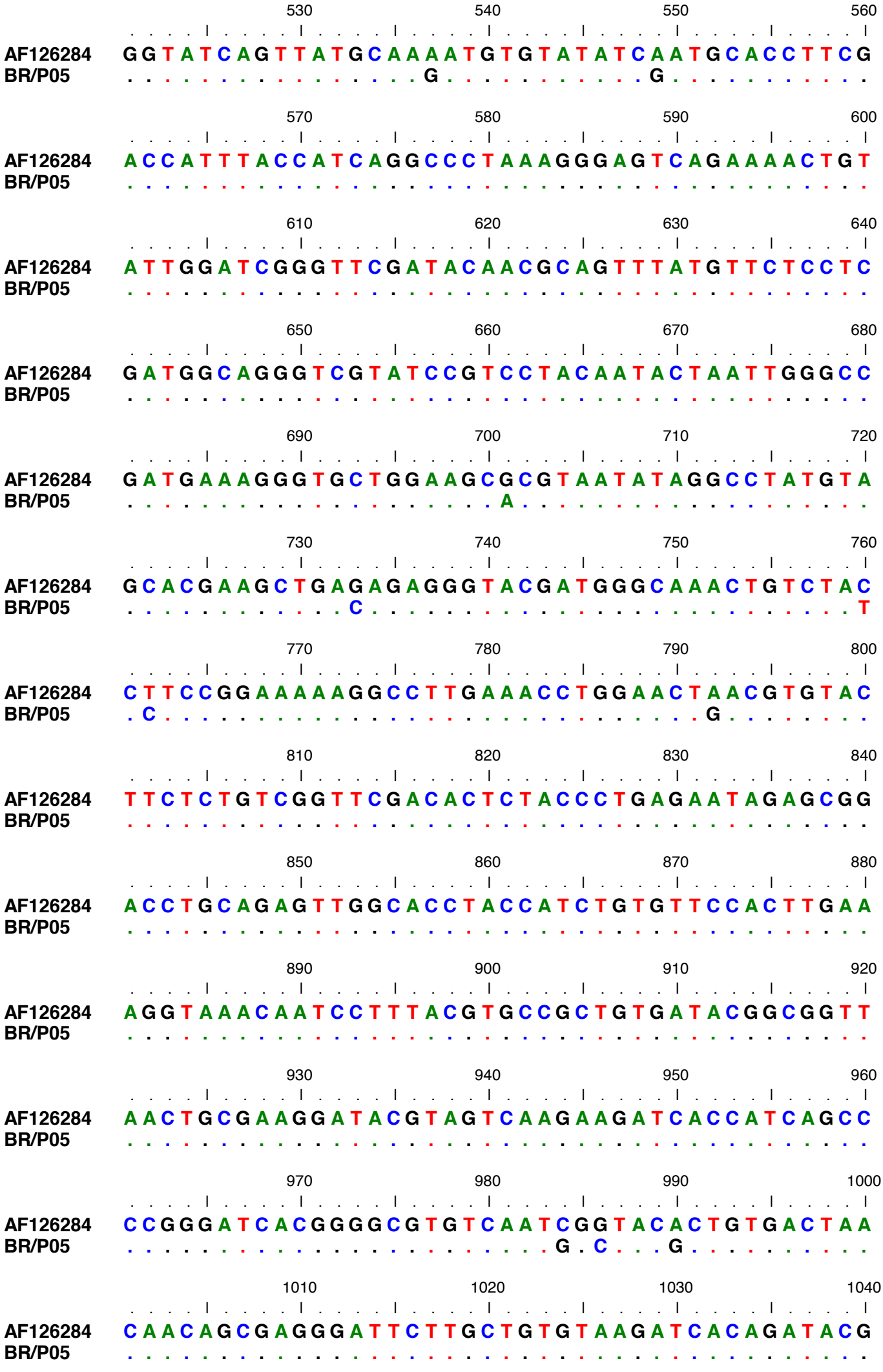
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BR/P05 .....
610 620 630 640
AF126284 ATTGGATCGGGTTCGATACAACGCAGTTTATGTTCTCCTC
BR/P05 .....
650 660 670 680
AF126284 GATGGCAGGGTCGTATCCGTCCTACAATACTAATTGGGCC
BR/P05 .....
690 700 710 720
AF126284 GATGAAAGGGTGCTGGAAGCGCGTAATATAGGCCTATGTA
BR/P05 .....A.....
730 740 750 760
AF126284 GCACGAAGCTGAGAGAGGGTACGATGGGCAAACTGTCTAC
BR/P05 .....C.....T
770 780 790 800
AF126284 CTTCCGGAAAAAAGGCCTTGAAACCTGGAACTAACGTGTAC
BR/P05 .C.....G.....
810 820 830 840
AF126284 TTCTCTGTCGGTTCGACACTCTACCCTGAGAATAGAGCGG
BR/P05 .....
850 860 870 880
AF126284 ACCTGCAGAGTTGGCACCTACCATCTGTGTTCCACTTGAA
BR/P05 .....
890 900 910 920
AF126284 AGGTAAACAATCCTTTACGTGCCGCTGTGATACGGCGGTT
BR/P05 .....
930 940 950 960
AF126284 AACTGCGAAGGATACGTAGTCAAGAAGATCACCATCAGCC
BR/P05 .....
970 980 990 1000
AF126284 CCGGGATCACGGGGCGTGTCAATCGGTACACTGTGACTAA
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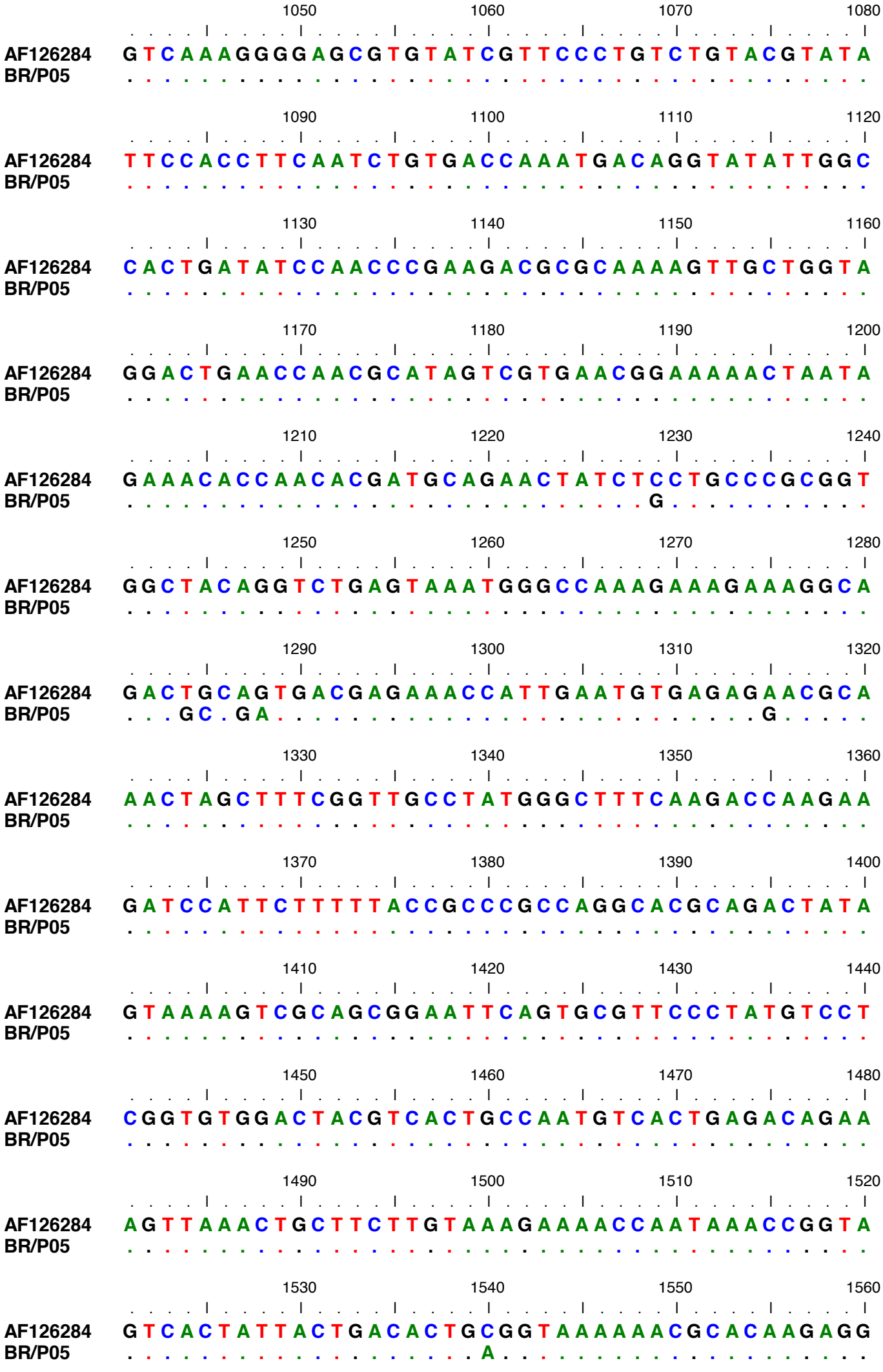
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BR/P05 ....
1130 1140 1150 1160
AF126284 CACTGATATCCAACCCGAAGACGCGCAAAAAGTTGCTGGTA
BR/P05 ....
1170 1180 1190 1200
AF126284 GGACTGAACCAACGCATAGTCGTGAACGGAAAAAACTAATA
BR/P05 ....
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AF126284 GAAACACCAACACGATGCAGAACTATCTCCTGCCCGCGGT
BR/P05 ....G.....................
1250 1260 1270 1280
AF126284 GGCTACAGGTCTGAGTAAATGGGCCAAAGAAAGAAAGGCA
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1290 1300 1310 1320
AF126284 GACTGCAGTGACGAGAAACCATTGAATGTGAGAGAACGCA
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1330 1340 1350 1360
AF126284 AACTAGCTTTCGGTTGCCTATGGGCTTTCAAGACCAAGAA
BR/P05 ....
1370 1380 1390 1400
AF126284 GATCCATTCTTTTTACCGCCCGCCAGGCACGCAGACTATA
BR/P05 ....
1410 1420 1430 1440
AF126284 GTAAAAGTCGCAGCGGAATTCAGTGCGTTCCCTATGTCCT
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1450 1460 1470 1480
AF126284 CGGTGTGGACTACGTCACTGCCAATGTCACTGAGACAGAA
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AF126284 AGTTAAACTGCTTCTTGTAAAGAAAACCAATAAACCGGTA
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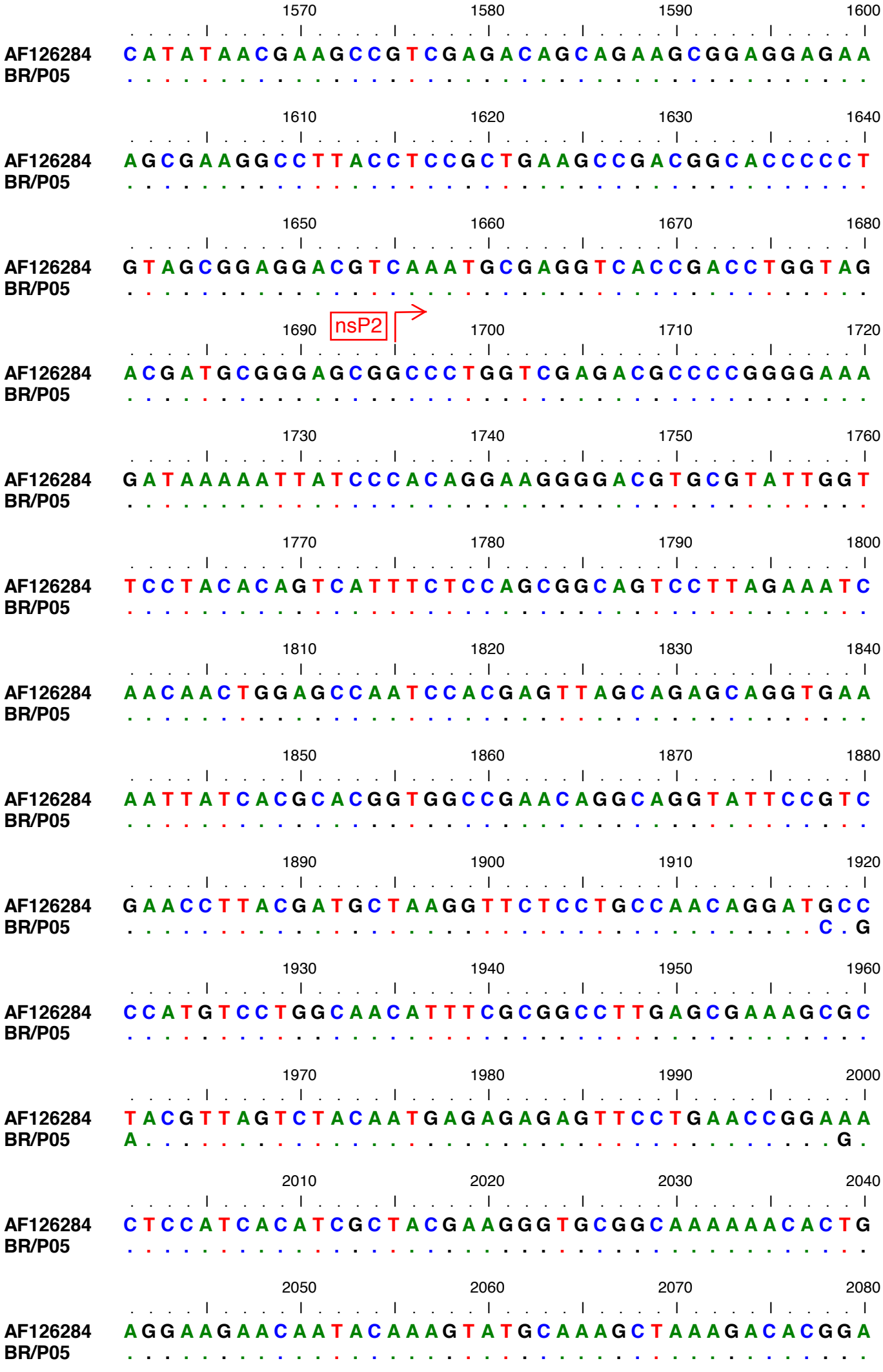
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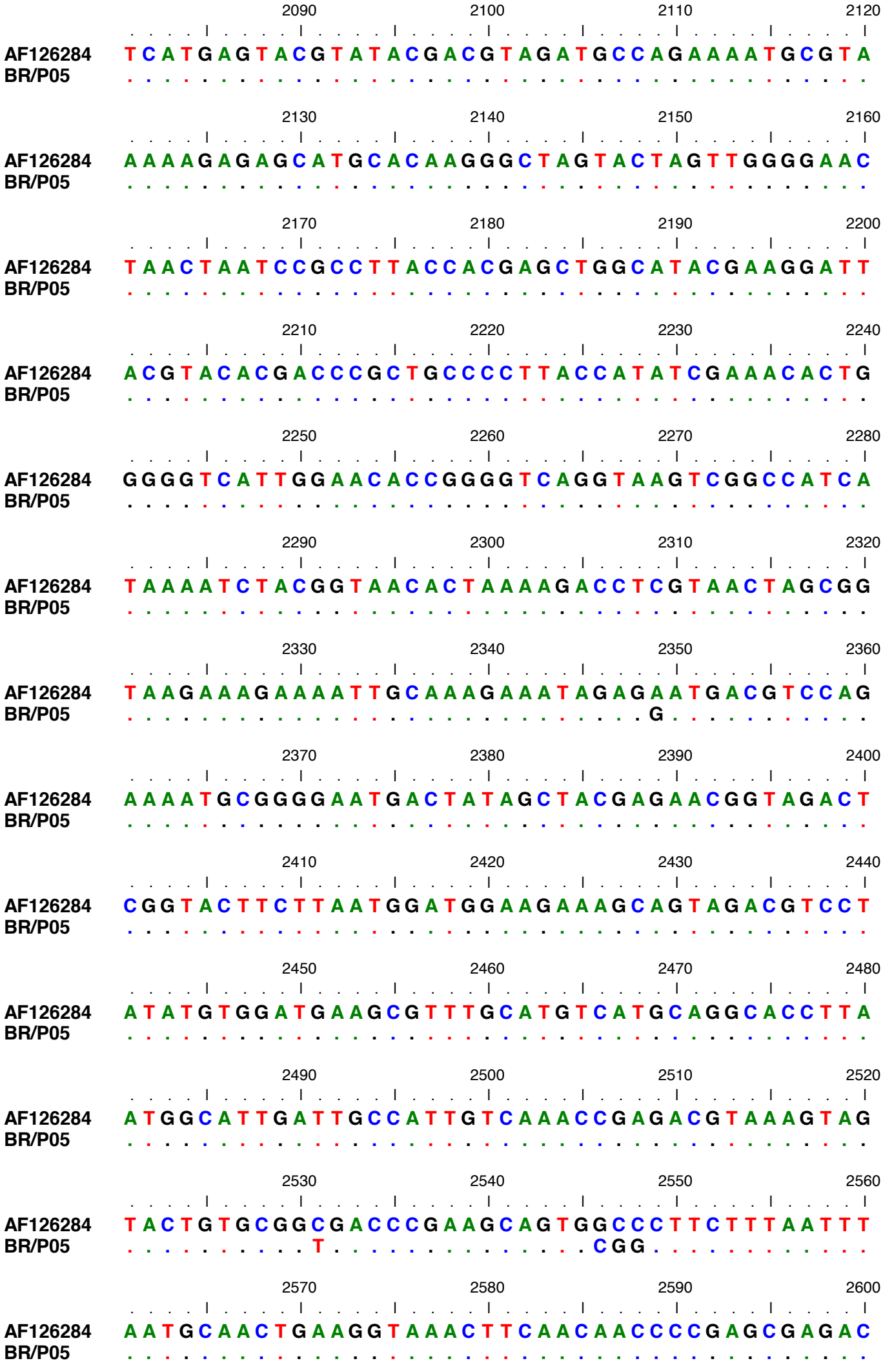
AF126284 AGCGAAGGCCTTACCTCCGCTGAAGCCGACGGCACCCCCT
BR/P05 ....
1650 1660 1670 1680
AF126284 GTAGCGGAGGACGTCAAATGCGAGGTCACCGACCTGGTAG
BR/P05 ....
1690 nsP2 1700 1710 1720
AF126284 ACGATGCGGGAGCGGCCCTGGTCGAGACGCCCCGGGGAAA
BR/P05 ....
1730 1740 1750 1760
AF126284 GATAAAAATTATCCCACAGGAAGGGGACGTGCGTATTGGT
BR/P05 ....
1770 1780 1790 1800
AF126284 TCCTACACAGTCATTTCTCCAGCGGCAGTCCTTAGAAATC
BR/P05 ....
1810 1820 1830 1840
AF126284 AACAACTGGAGCCAATCCACGAGTTAGCAGAGCAGGTGAA
BR/P05 ....
1850 1860 1870 1880
AF126284 AATTATCACGCACGGGTGGCCGAACAGGCAGGTATTCCGTC
BR/P05 ....
1890 1900 1910 1920
AF126284 GAACCTTACGATGCTAAGGTTCTCCTGCCAACAGGATGCC
BR/P05 ....C.G
1930 1940 1950 1960
AF126284 CCATGTCCTGGCAACATTTCGCGGCCTTGAGCGAAAGCGC
BR/P05 ....
1970 1980 1990 2000
AF126284 TACGTTAGTCTACAATGAGAGAGAGTTCCTGAACCGGAAA
BR/P05 A..............G.
2010 2020 2030 2040
AF126284 CTCCATCACATCGCTACGAAGGGTGCGGCAAAAAACACTG
BR/P05 ....
2050 2060 2070 2080
AF126284 AGGAAGAACAATACAAAGTATGCAAAGCTAAAGACACGGA
BR/P05 ....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AF126284 TCATGAGTACGTATACGACGTAGATGCCAGAAAATGCGTA
BR/P05 .....G.....
2130 2140 2150 2160
AF126284 AAAAGAGAGCATGCACAAGGGCTAGTACTAGTTGGGGAAC
BR/P05 .....
2170 2180 2190 2200
AF126284 TAACTAATCCGCCTTACCACGAGCTGGCATACGAAGGATT
BR/P05 .....
2210 2220 2230 2240
AF126284 ACGTACACGACCCGCTGCCCCTTACCATATCGAAACACTG
BR/P05 .....
2250 2260 2270 2280
AF126284 GGGGTCATTGGAACACCGGGGTCAGGTAAGTCGGCCATCA
BR/P05 .....
2290 2300 2310 2320
AF126284 TAAAATCTACGGTAACACTAAAAGACCTCGTAACTAGCGG
BR/P05 .....
2330 2340 2350 2360
AF126284 TAAGAAAGAAAATTGCAAAGAAATAGAGAATGACGTCCAG
BR/P05 .....G.....
2370 2380 2390 2400
AF126284 AAAATGCGGGGAATGACTATAGCTACGAGAACGGTAGACT
BR/P05 .....
2410 2420 2430 2440
AF126284 CGGTACTTCTTAATGGATGGAAGAAAGCAGTAGACGTCCT
BR/P05 .....
2450 2460 2470 2480
AF126284 ATATGTGGATGAAGCGTTTGCATGTCATGCAGGCACCTTA
BR/P05 .....
2490 2500 2510 2520
AF126284 ATGGCATTGATTGCCATTGTCAAACCGAGACGTAAAGTAG
BR/P05 .....
2530 2540 2550 2560
AF126284 TACTGTGCGGCGACCCGAAGCAGTGGCCCTTCTTTAATTT
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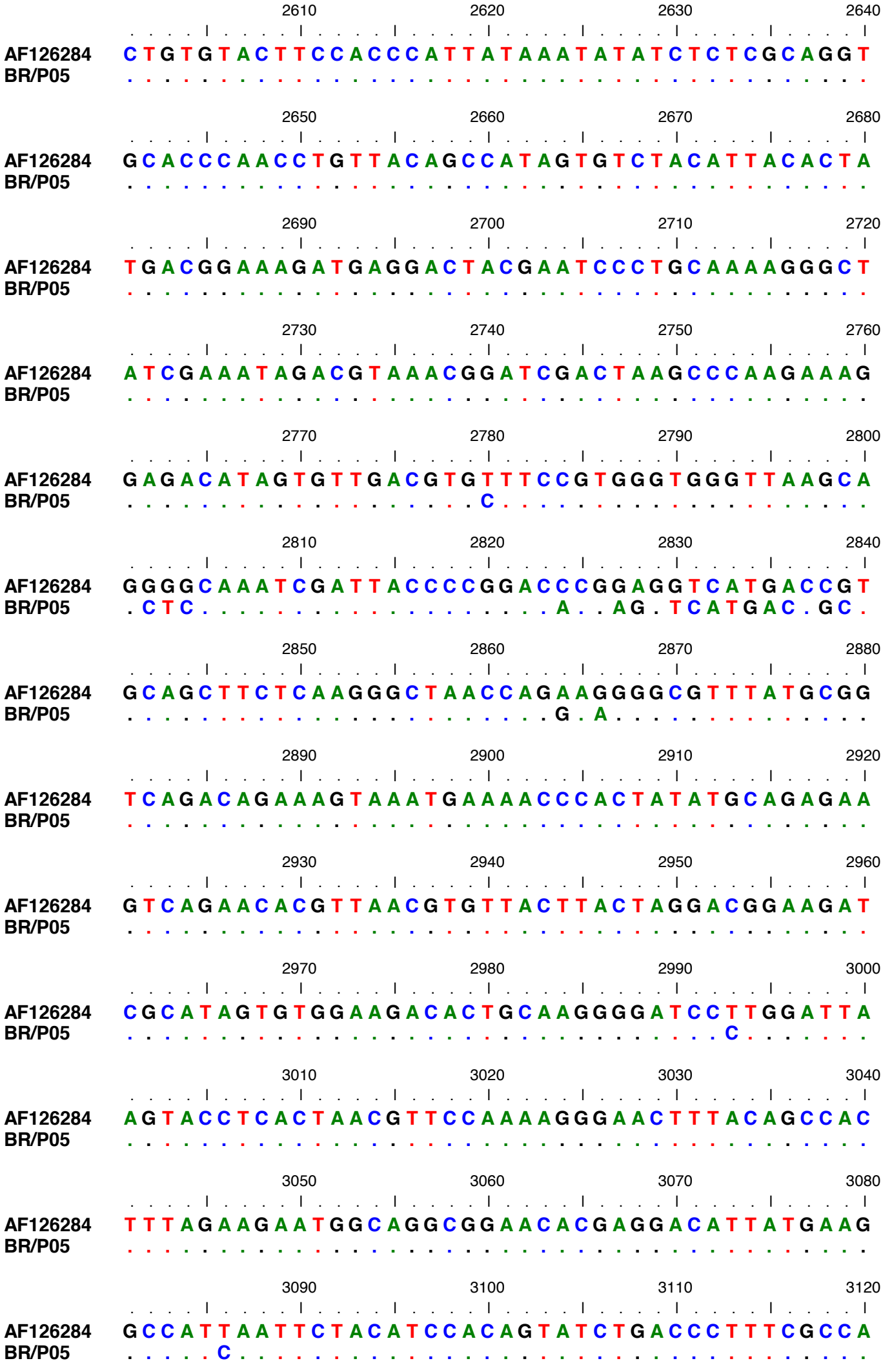
2570 2580 2590 2600
AF126284 AATGCAACTGAAGGTAAACTTCAACAACCCCGAGCGAGAC
BR/P05 .....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AF126284 CTGTGTACTTCCACCCATTATAAATATATCTCTCGCAGGT
BR/P05 .....C.....
2650 2660 2670 2680
AF126284 GCACCCAACCTGTTACAGCCATAGTGTCTACATTACACTA
BR/P05 .....
2690 2700 2710 2720
AF126284 TGACGGAAAGATGAGGACTACGAATCCCTGCAAAAGGGCT
BR/P05 .....
2730 2740 2750 2760
AF126284 ATCGAAATAGACGTAAACGGATCGACTAAGCCCAAGAAAG
BR/P05 .....
2770 2780 2790 2800
AF126284 GAGACATAGTGTTGACGTGTTTCCGTGGGTGGGTTAAGCA
BR/P05 .....C.....
2810 2820 2830 2840
AF126284 GGGGCAAATCGATTACCCCGGACCCGGAGGTCATGACCGT
BR/P05 .CTC.....A..AG.TCATGAC.GC.
2850 2860 2870 2880
AF126284 GCAGCTTCTCAAGGGCTAACCAGAAGGGGCGTTTATGCGG
BR/P05 .....G.A.....
2890 2900 2910 2920
AF126284 TCAGACAGAAAGTAAATGAAAACCCCACTATATGCAGAGAA
BR/P05 .....
2930 2940 2950 2960
AF126284 GTCAGAACACGTTAACGTGTTACTTACTAGGACGGAAGAT
BR/P05 .....
2970 2980 2990 3000
AF126284 CGCATAGTGTGGAAGACACTGCAAGGGGATCCTTGGATTA
BR/P05 .....C.....
3010 3020 3030 3040
AF126284 AGTACCTCACTAACGTTCCAAAAGGGAACTTTACAGCCAC
BR/P05 .....
3050 3060 3070 3080
AF126284 TTTAGAAGAATGGCAGGCGGAACACGAGGACATTATGAAG
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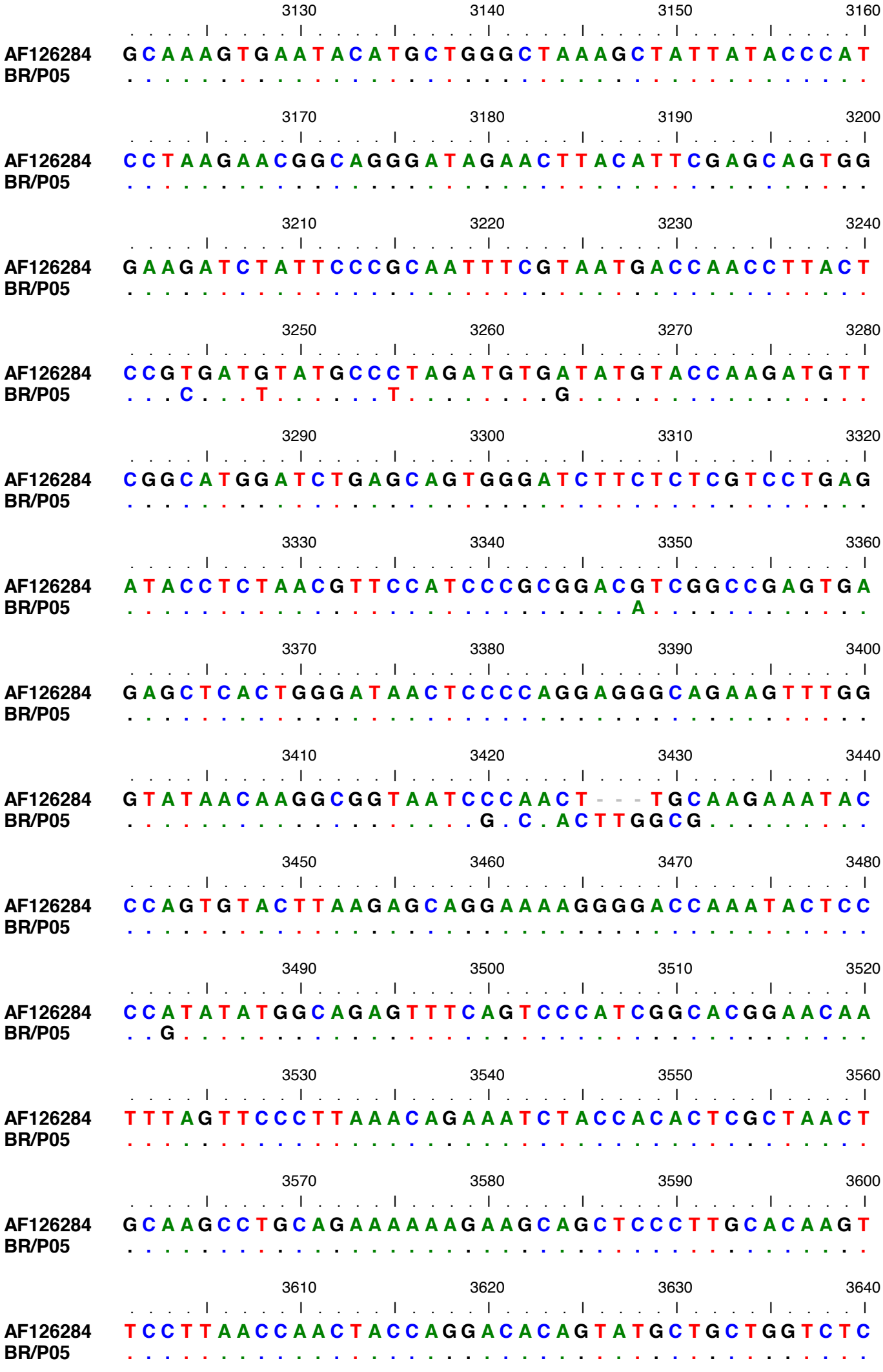
BR/P05 ....C.....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AF126284 GCAAAGTGAATACATGCTGGGCTAAAGCTATTATACCCAT
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3170 3180 3190 3200
AF126284 CCTAAGAACGGCAGGGATAGAACTTACATTCGAGCAGTGG
BR/P05 ....
3210 3220 3230 3240
AF126284 GAAGATCTATTCCCGCAATTTCGTAATGACCAACCTTACT
BR/P05 ....
3250 3260 3270 3280
AF126284 CCGTGATGTATGCCCTAGATGTGATATGTACCAAGATGTT
BR/P05 ...C...T.....T.......G.....................
3290 3300 3310 3320
AF126284 CGGCATGGATCTGAGCAGTGGGATCTTCTCTCGTCCTGAG
BR/P05 ....
3330 3340 3350 3360
AF126284 ATACCTCTAACGTTCCATCCCGCGGACGTCGGCCGAGTGA
BR/P05 ....A..........................
3370 3380 3390 3400
AF126284 GAGCTCACTGGGATAACTCCCCAGGAGGGCAGAAGTTTGG
BR/P05 ....
3410 3420 3430 3440
AF126284 GTATAACAAGGCGGTAATCCCAACT---TGCAAGAAATAC
BR/P05 ..........G.C.ACTTGGCG..........
3450 3460 3470 3480
AF126284 CCAGTGTACTTAAGAGCAGGAAAAGGGGACCAAATACTCC
BR/P05 ....
3490 3500 3510 3520
AF126284 CCATATATGGCAGAGTTTCAGTCCCATCGGCACGGAACAA
BR/P05 ..G..
3530 3540 3550 3560
AF126284 TTTAGTTCCCTTAAACAGAAATCTACCACACTCGCTAACT
BR/P05 ....
3570 3580 3590 3600
AF126284 GCAAGCCTGCAGAAAAAAGAAGCAGCTCCCTTGCACAAGT
BR/P05 ....
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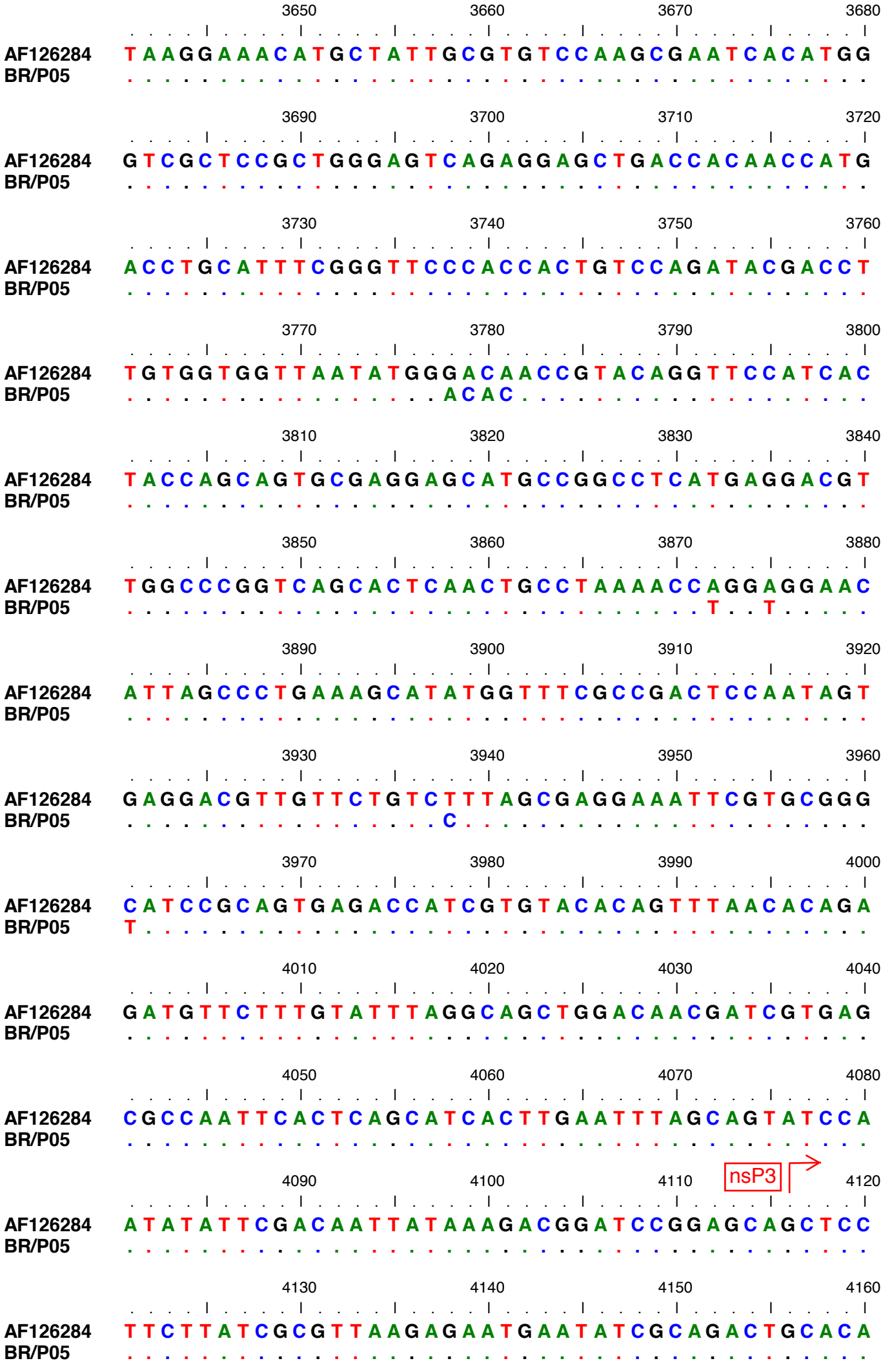
AF126284 TCCTTAACCAACTACCAGGACACAGTATGCTGCTGGTCTC
BR/P05 ....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3680
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3720
AF126284 GTCGCTCCGCTGGGAGTCAGAGGAGCTGACCACAACCATG
BR/P05 ....
3730
3740
3750
3760
AF126284 ACCTGCATTTCGGGTTCCCACCACTGTCCAGATACGACCT
BR/P05 ....
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3780
3790
3800
AF126284 TGTGGTGGTTAATATGGGACAACCGTACAGGTTCCATCAC
BR/P05 ....ACAC................
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3820
3830
3840
AF126284 TACCAGCAGTGCGAGGAGCATGCCGGCCTCATGAGGACGT
BR/P05 ....
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3870
3880
AF126284 TGGCCCGGTCAGCACTCAACTGCCTAAAACCAGGAGGAAC
BR/P05 ..................T..T..............
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3900
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3920
AF126284 ATTAGCCCTGAAAGCATATGGTTTCGCCGACTCCAATAGT
BR/P05 ....
3930
3940
3950
3960
AF126284 GAGGACGTTGTTCTGTCTTTAGCGAGGAAATTCGTGCGGG
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3980
3990
4000
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BR/P05 T...................................
4010
4020
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4040
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4060
4070
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AF126284 CGCCAATTCACTCAGCATCACTTGAATTTAGCAGTATCCA
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AF126284 ATATATTCGACAATTATAAAGACGGATCCGGAGCAGCTCC
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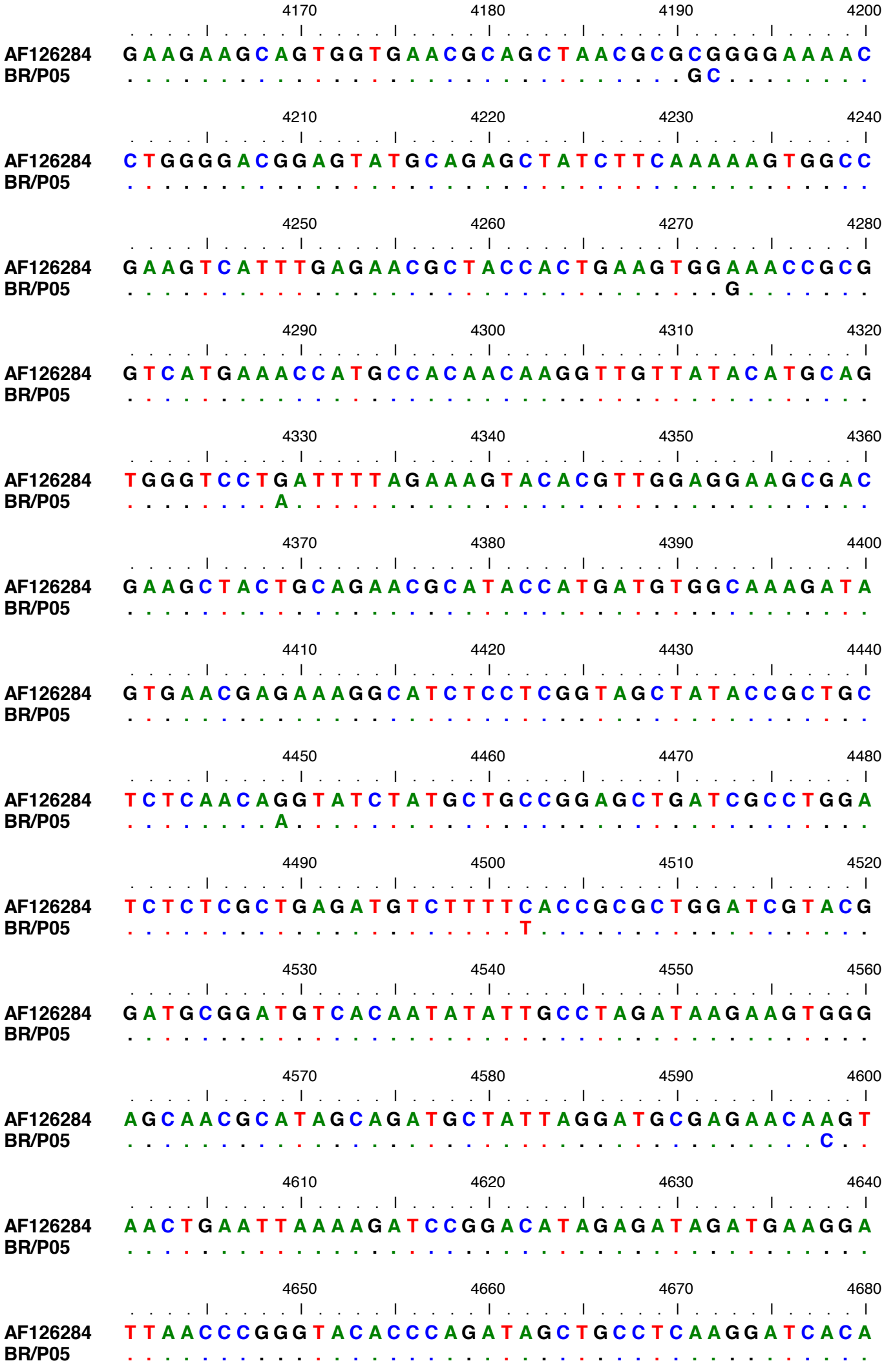
BR/P05 ....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AF126284 CTGGGGACGGAGTATGCAGAGCTATCTTCAAAAAGTGGCC
BR/P05 .....
4250 4260 4270 4280
AF126284 GAAGTCATTTGAGAACGCTACCACTGAAGTGGAAACCGCG
BR/P05 .....G.....
4290 4300 4310 4320
AF126284 GTCATGAAACCATGCCACAACAAGGTTGTTATACATGCAG
BR/P05 .....
4330 4340 4350 4360
AF126284 TGGGTCCTGATTTTAGAAAGTACACGTTGGAGGAAGCGAC
BR/P05 .....A.....
4370 4380 4390 4400
AF126284 GAAGCTACTGCAGAACGCATACCATGATGTGGCAAAGATA
BR/P05 .....
4410 4420 4430 4440
AF126284 GTGAACGAGAAAGGCATCTCCTCGGTAGCTATACCGCTGC
BR/P05 .....
4450 4460 4470 4480
AF126284 TCTCAACAGGTATCTATGCTGCCGGAGCTGATCGCCTGGA
BR/P05 .....A.....
4490 4500 4510 4520
AF126284 TCTCTCGCTGAGATGTCTTTTCACCGCGCTGGATCGTACG
BR/P05 .....T.....
4530 4540 4550 4560
AF126284 GATGCGGATGTCACAATATATTGCCTAGATAAGAAGTGGG
BR/P05 .....
4570 4580 4590 4600
AF126284 AGCAACGCATAGCAGATGCTATTAGGATGCGAGAACAAGT
BR/P05 .....C..
4610 4620 4630 4640
AF126284 AACTGAATTAAAAGATCCGGACATAGAGATAGATGAAGGA
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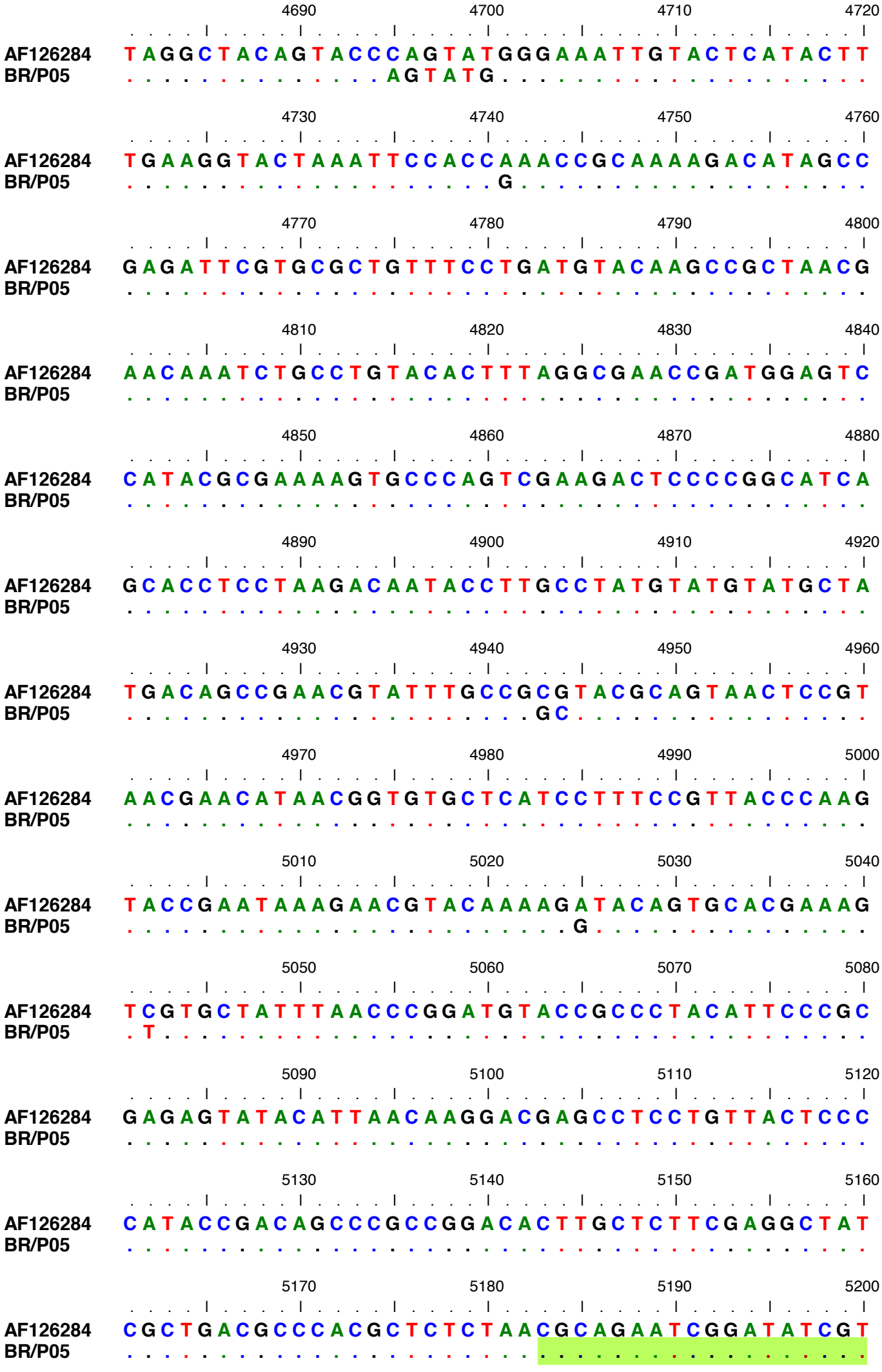
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AF126284 TGAAGGTACTAAATTCCACCAAACCGCAAAAGACATAGCC
BR/P05 ....G..........
4770 4780 4790 4800
AF126284 GAGATTCGTGCGCTGTTTCCTGATGTACAAGCCGCTAACG
BR/P05 ....
4810 4820 4830 4840
AF126284 AACAAATCTGCCTGTACACTTTAGGCGAACCGATGGAGTC
BR/P05 ....
4850 4860 4870 4880
AF126284 CATACGCGAAAAGTGCCCAGTCGAAGACTCCCCGGCATCA
BR/P05 ....
4890 4900 4910 4920
AF126284 GCACCTCCTAAGACAATACCTTGCCTATGTATGTATGCTA
BR/P05 ....
4930 4940 4950 4960
AF126284 TGACAGCCGAACGTATTTGCCGCGTACGCAGTAACTCCGT
BR/P05 ....GC..........
4970 4980 4990 5000
AF126284 AACGAACATAACGGTGTGCTCATCCTTTCCGTTACCCCAAG
BR/P05 ....
5010 5020 5030 5040
AF126284 TACCGAATAAAGAACGTACAAAAGATACAGTGCACGAAAG
BR/P05 ....G..........
5050 5060 5070 5080
AF126284 TCGTGCTATTTAACCCGGATGTACCGCCCTACATTCCCGC
BR/P05 .T..........
5090 5100 5110 5120
AF126284 GAGAGTATACATTAACAAGGACGAGCCTCCTGTTACTCCC
BR/P05 ....
5130 5140 5150 5160
AF126284 CATACCGACAGCCCGCCGGACACTTGCTCTTCGAGGCTAT
BR/P05 ....
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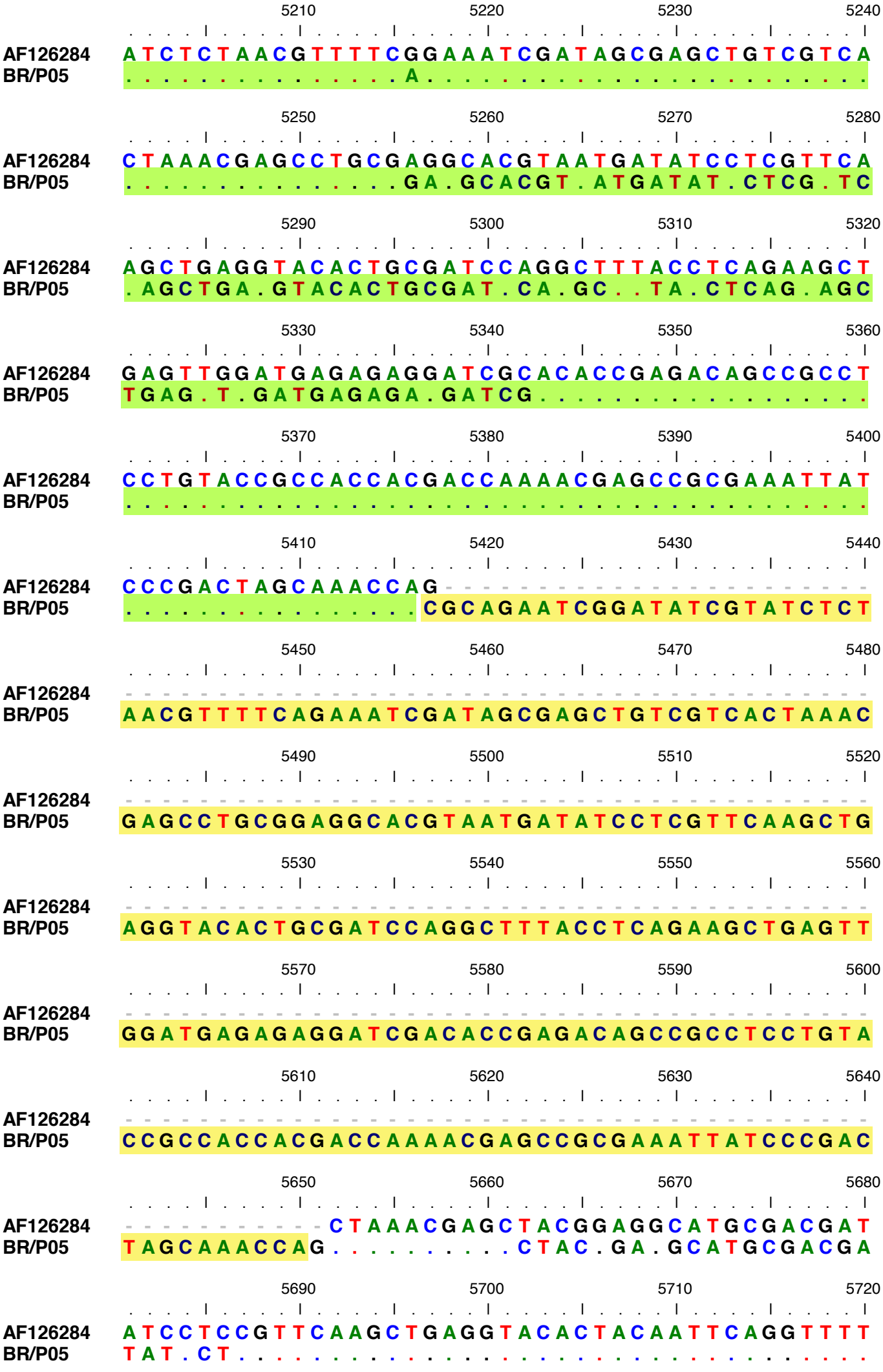
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5250 5260 5270 5280
AF126284 CTAAACGAGCCTGCGAGGCACGTAATGATATCCTCGTTCA
BR/P05 .....GA.GCACGT.ATGATAT.CTCG.TC
5290 5300 5310 5320
AF126284 AGCTGAGGTACACTGCGATCCAGGCTTTACCTCAGAAGCT
BR/P05 .AGCTGA.GTACACTGCGAT.CA.GC..TA.CTCAG.AGC
5330 5340 5350 5360
AF126284 GAGTTGGATGAGAGAGGATCGCACACCGAGACAGCCGCCT
BR/P05 TGAG.T.GATGAGAGA.GATCG.....
5370 5380 5390 5400
AF126284 CCTGTACCGCCACCACGACCAAAACGAGCCGCGAAATTAT
BR/P05 .....
5410 5420 5430 5440
AF126284 CCCGACTAGCAAACCAG
BR/P05 .....CGCAGAATCGGATATCGTATCTCT
5450 5460 5470 5480
AF126284
BR/P05 AACGTTTTCAGAAATCGATAGCGAGCTGTCGTCACTAAAC
5490 5500 5510 5520
AF126284
BR/P05 GAGCCTGCGGAGGCACGTAATGATATCCTCGTTCAAGCTG
5530 5540 5550 5560
AF126284
BR/P05 AGGTACACTGCGATCCAGGCTTTACCTCAGAAGCTGAGTT
5570 5580 5590 5600
AF126284
BR/P05 GGATGAGAGAGGATCGACACCGAGACAGCCGCCTCCTGTA
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AF126284
BR/P05 CCGCCACCACGACCAAAACGAGCCGCGAAATTATCCCGAC
5650 5660 5670 5680
AF126284 .....CTAAACGAGCTACGGAGGCATGCGACGAT
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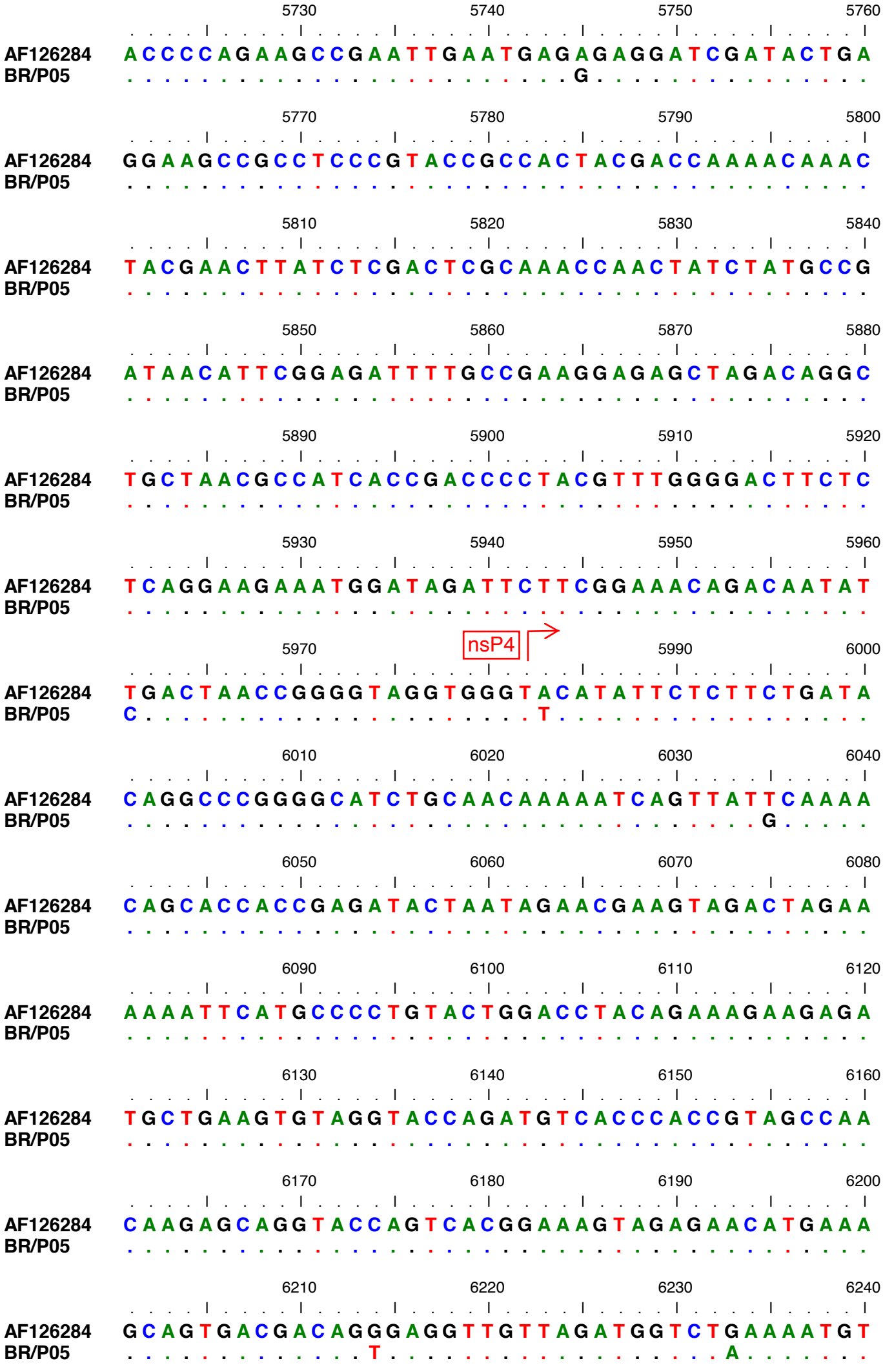
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5750
5760
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5770
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5790
5800
AF126284 GGAAGCCGCCTCCCGTACCGCCACTACGACCAAAACAAAC
BR/P05 .....
5810
5820
5830
5840
AF126284 TACGAACTTATCTCGACTCGCAAACCAACTATCTATGCCG
BR/P05 .....
5850
5860
5870
5880
AF126284 ATAACATTCGGAGATTTTGCCGAAGGAGAGCTAGACAGGC
BR/P05 .....
5890
5900
5910
5920
AF126284 TGCTAACGCCATCACCGACCCCTACGTTTGGGGACTTCTC
BR/P05 .....
5930
5940
5950
5960
AF126284 TCAGGAAGAAATGGATAGATTCTTCGGAAACAGACAATAT
BR/P05 .....
nsP4
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5990
6000
AF126284 TGACTAACCGGGGTAGGTGGGTACATATTCTCTTCTGATA
BR/P05 C.....T.....
6010
6020
6030
6040
AF126284 CAGGCCCGGGGCATCTGCAACAAAAATCAGTTATTCAAAA
BR/P05 .....G.....
6050
6060
6070
6080
AF126284 CAGCACCACCGAGATACTAATAGAACGAAGTAGACTAGAA
BR/P05 .....
6090
6100
6110
6120
AF126284 AAAATTCATGCCCCTGTACTGGACCTACAGAAAGAAGAGA
BR/P05 .....
6130
6140
6150
6160
AF126284 TGCTGAAGTGTAGGTACCAGATGTCACCCACCGTAGCCAA
BR/P05 .....
6170
6180
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6200
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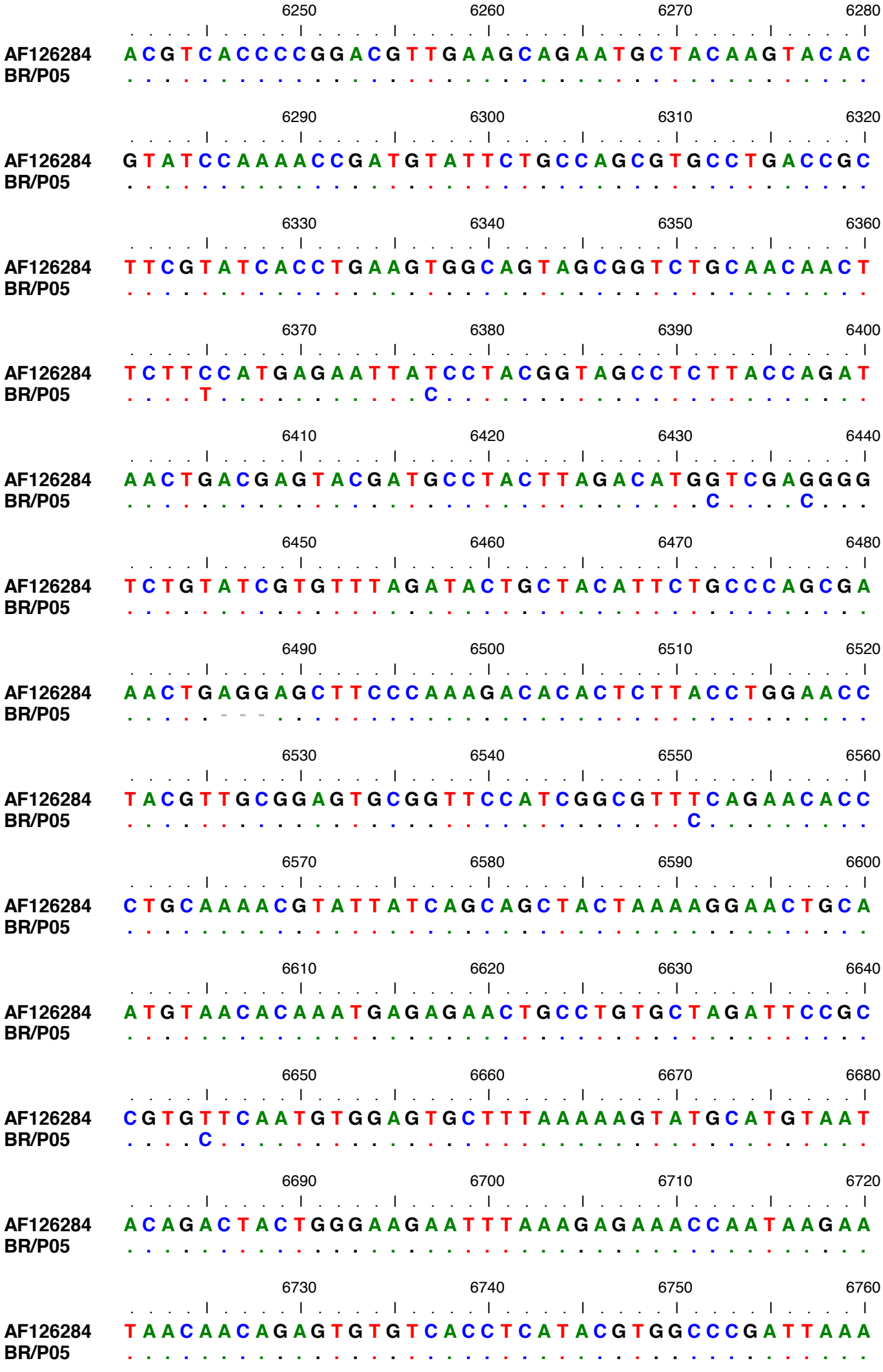
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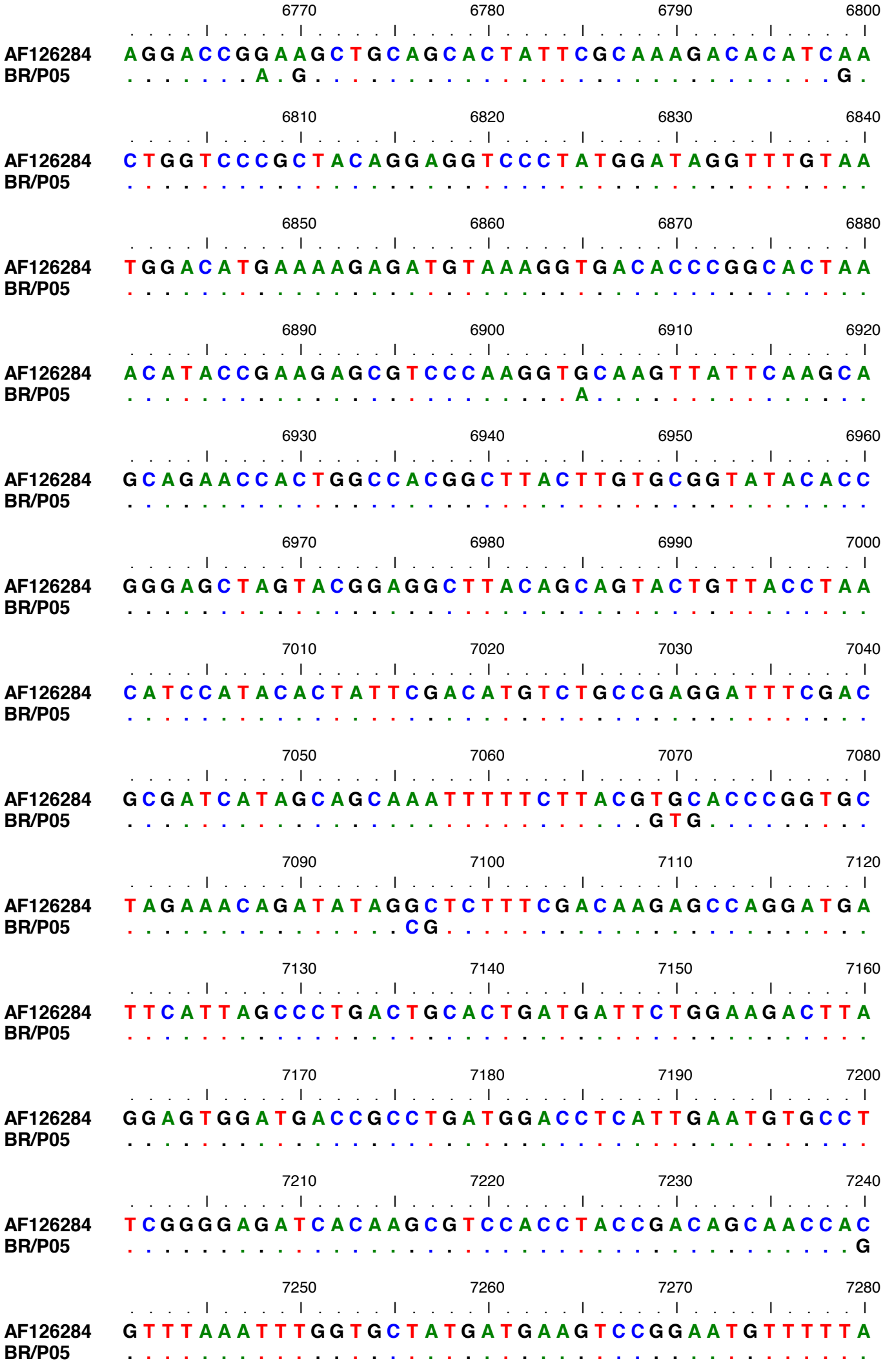
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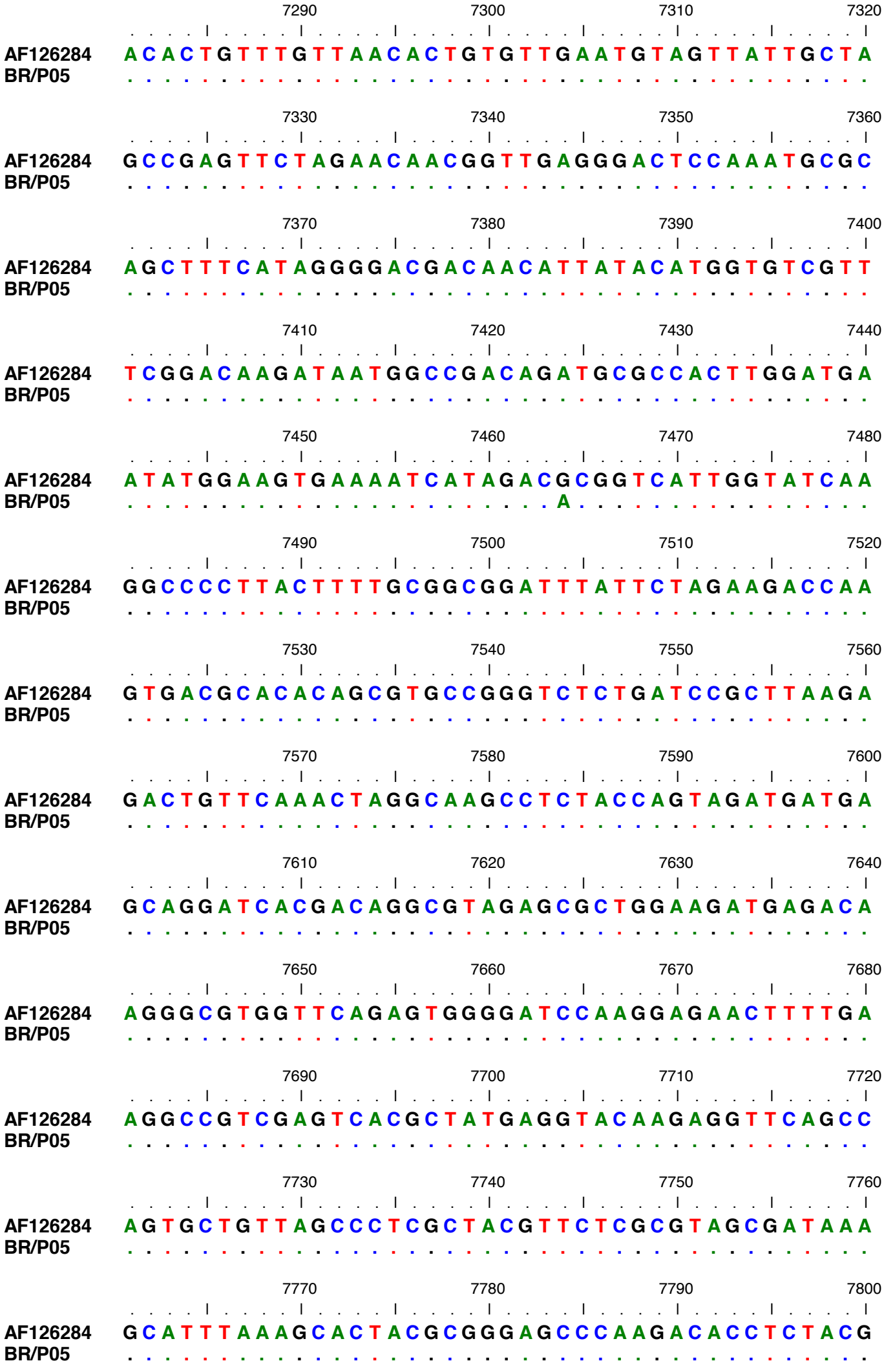
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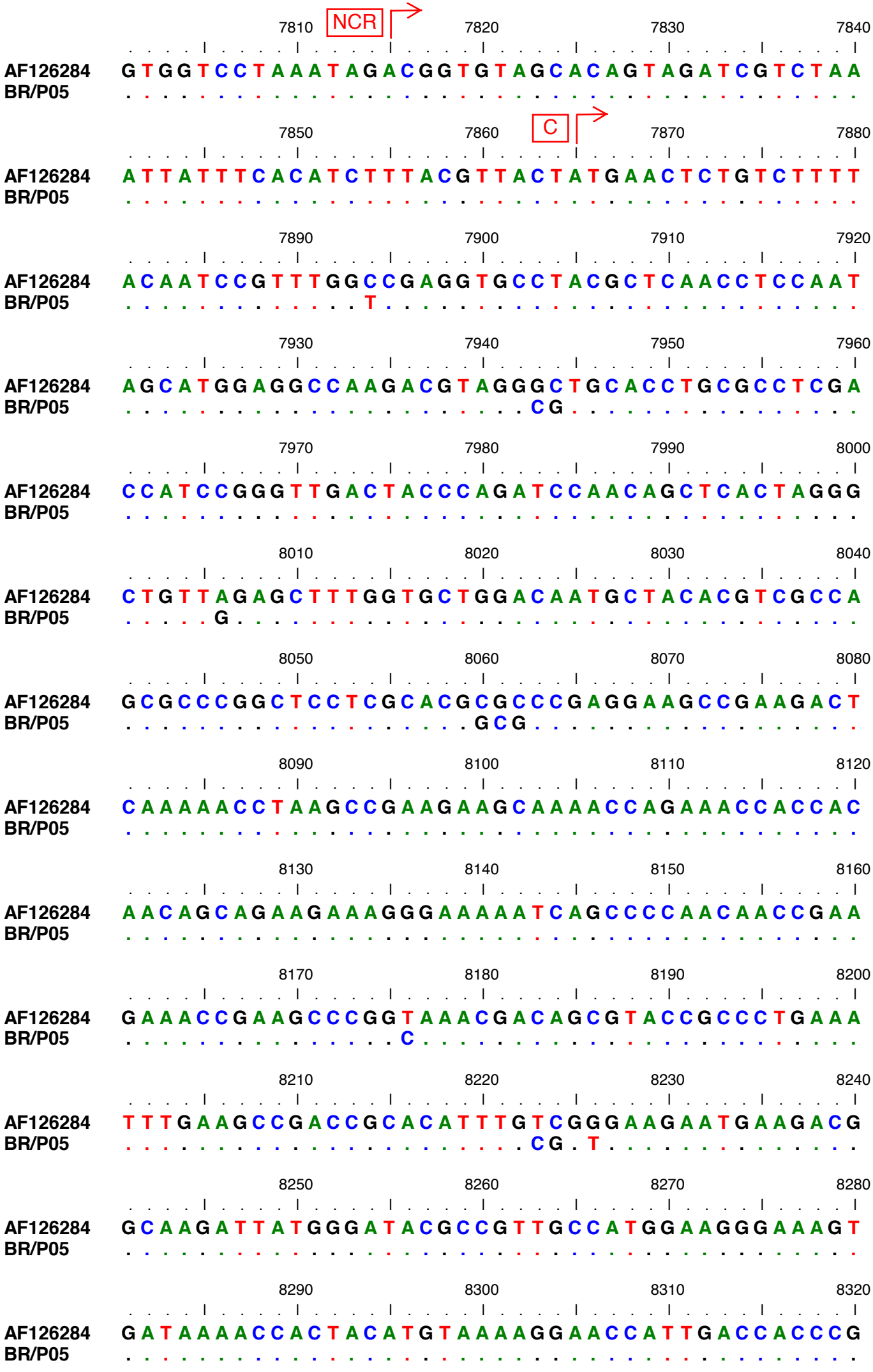
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BR/P05 ....
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BR/P05 ....T.....................
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BR/P05 ....CG..........
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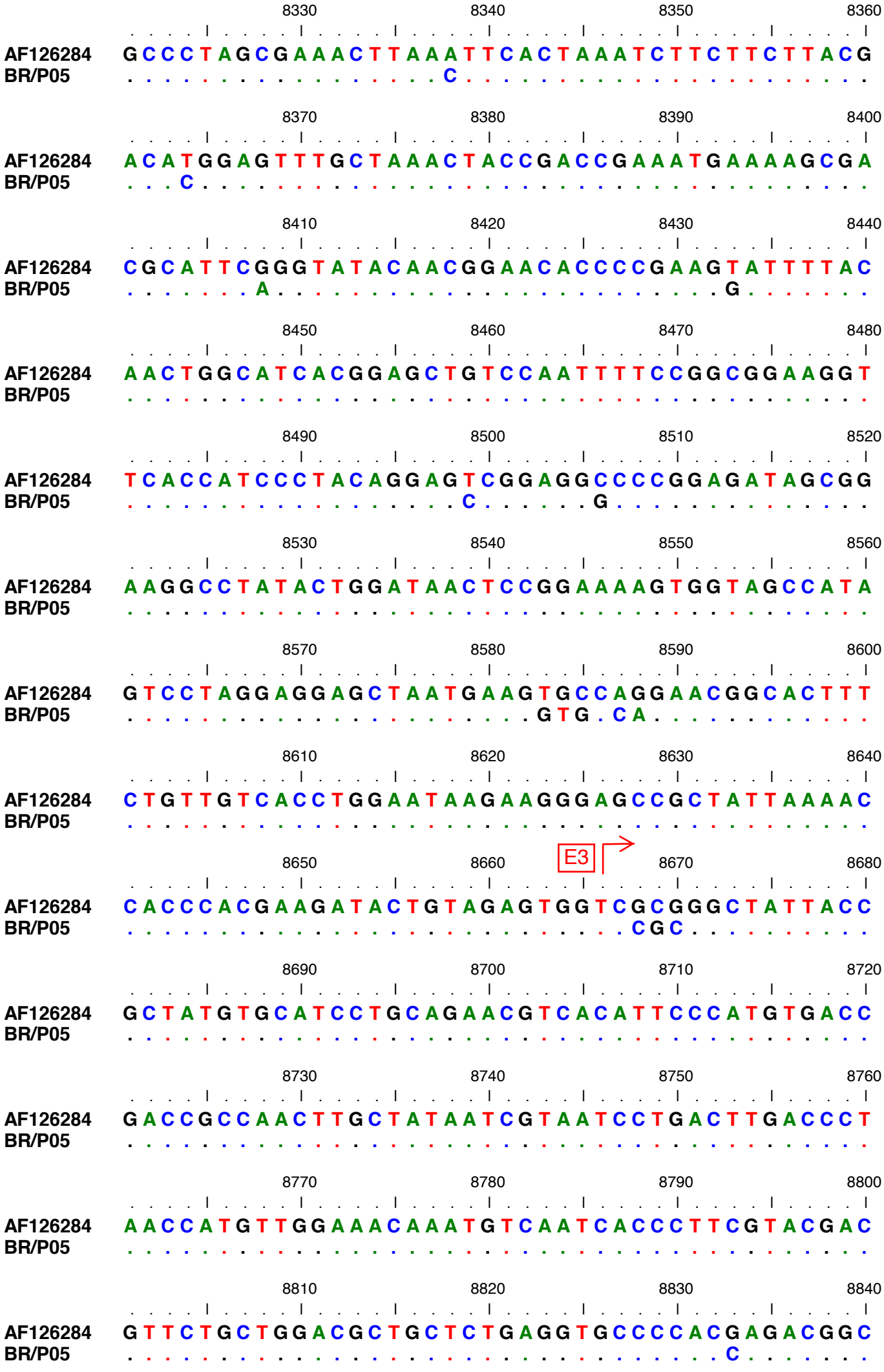
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BR/P05 ...C.....
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BR/P05 ......A.....G.....
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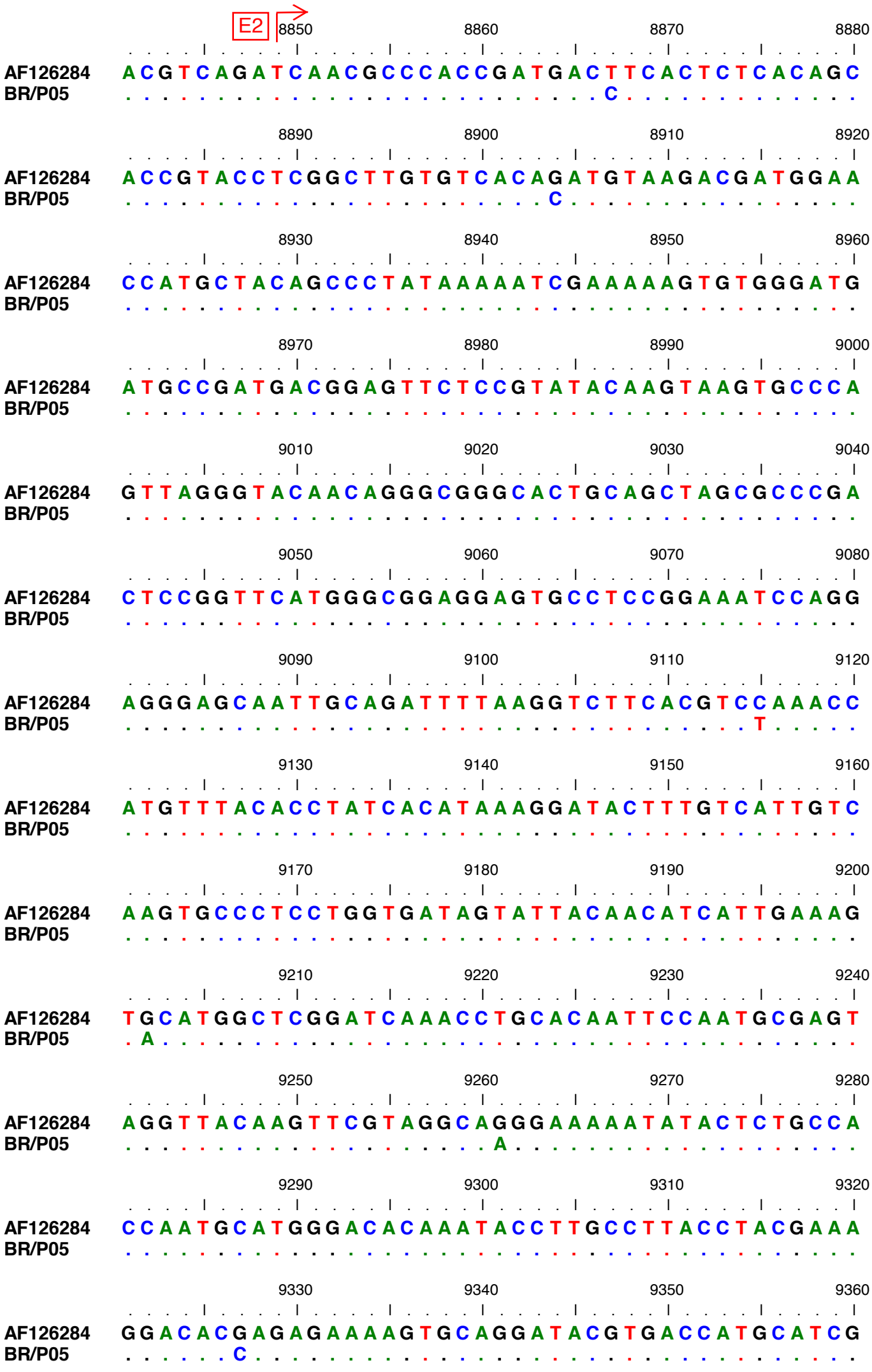
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BR/P05 . . . . . C . . . . . .
8890
8900
8910
8920
AF126284 A C C G T A C C T C G G C T T G T G T C A C A G A T G T A A G A C G A T G G A A
BR/P05 . . . . . C . . . . . .
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8940
8950
8960
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BR/P05 . . . . . .
8970
8980
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9000
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BR/P05 . . . . . .
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BR/P05 . . . . . .
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BR/P05 . A . . . . . .
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9260
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9300
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BR/P05 . . . . . .
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9340
9350
9360
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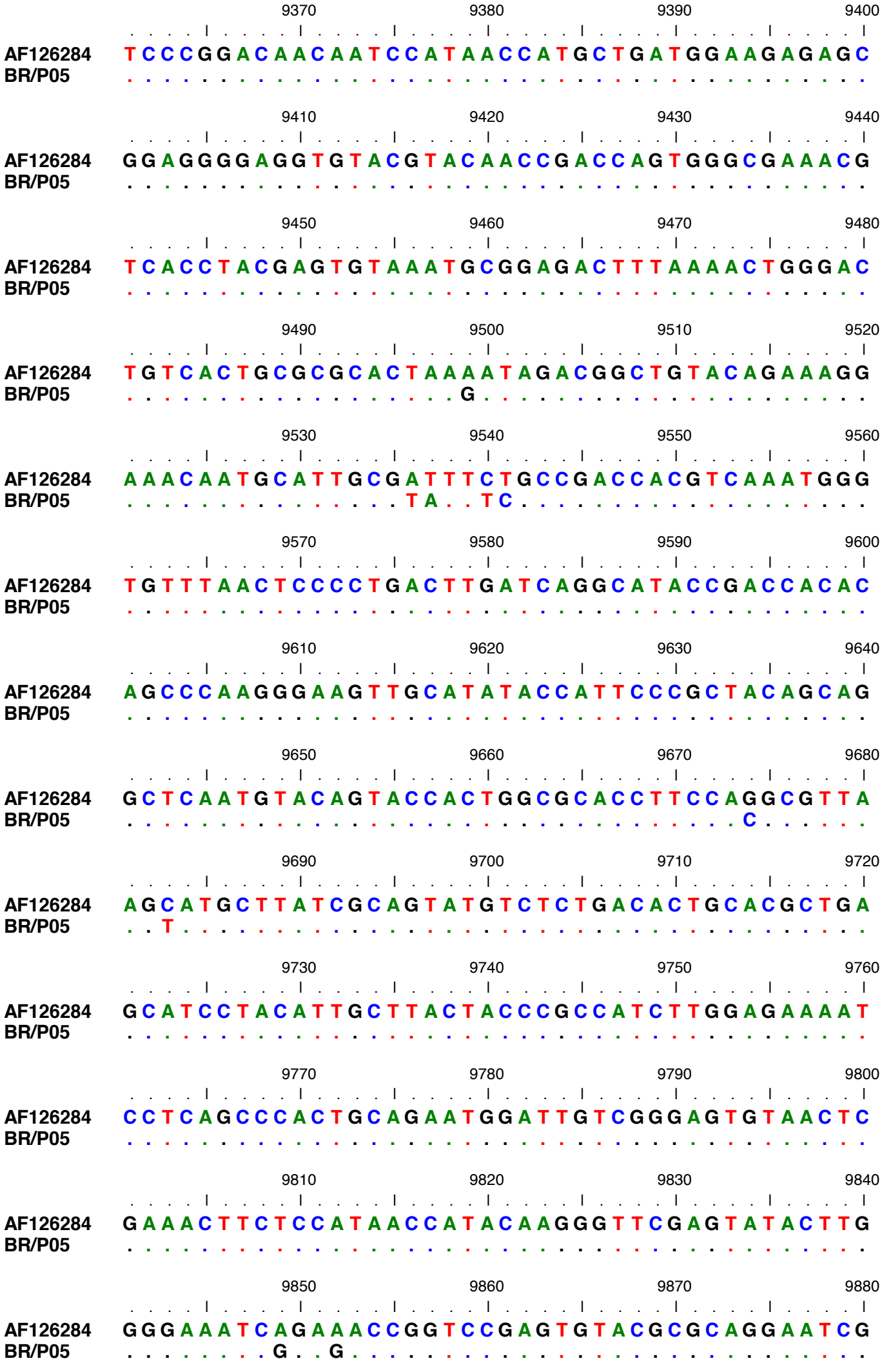
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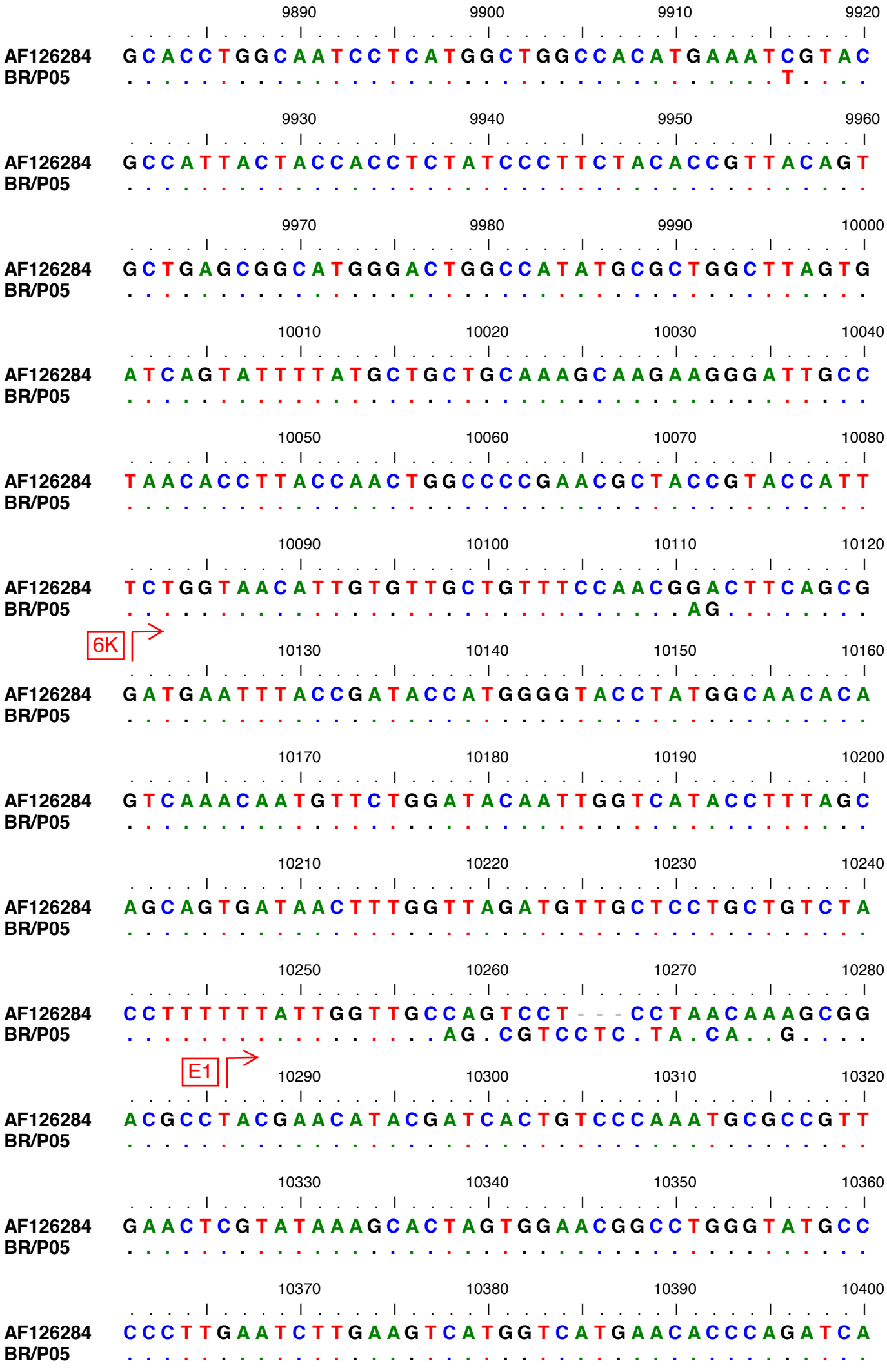
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E1
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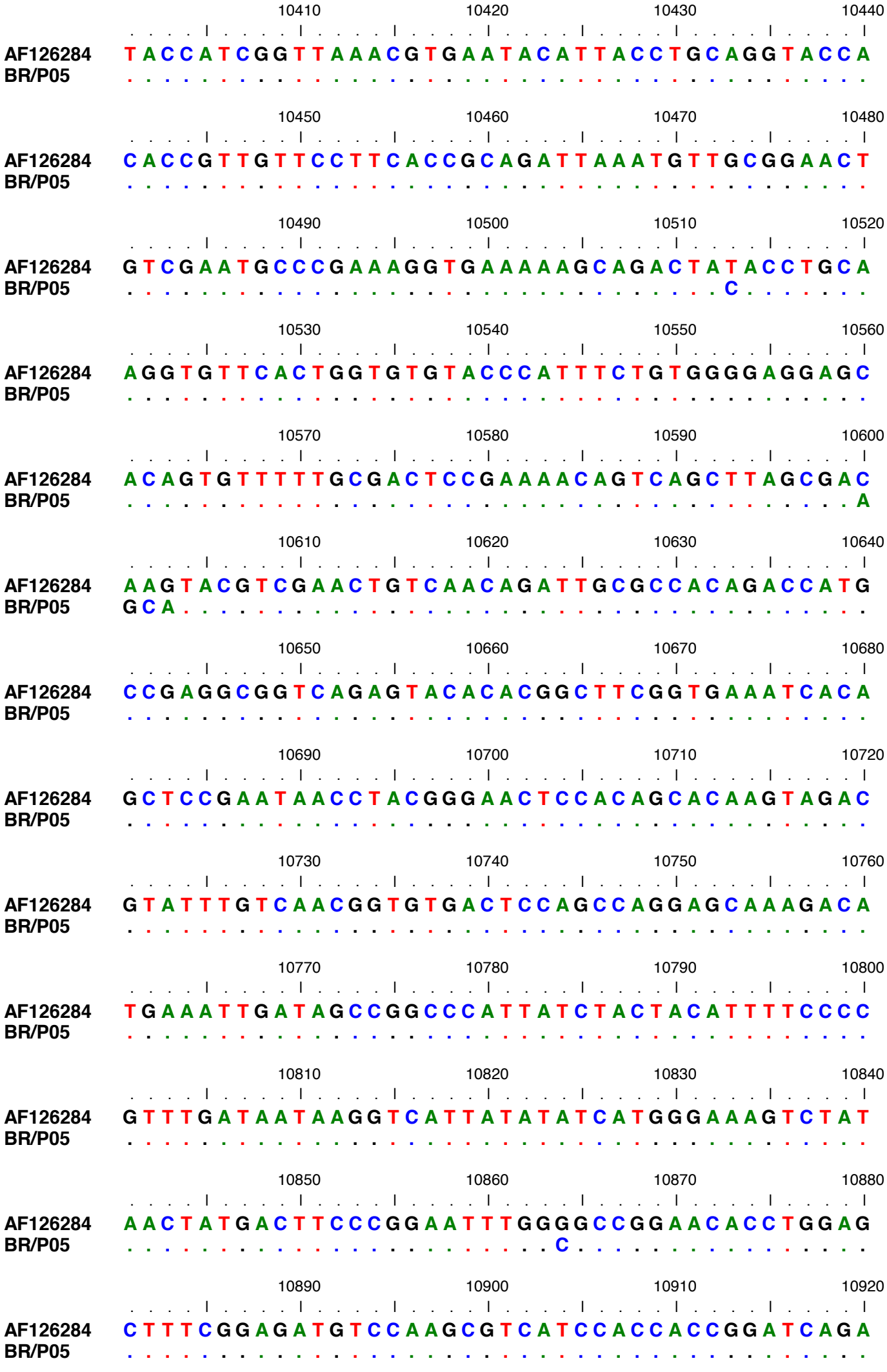
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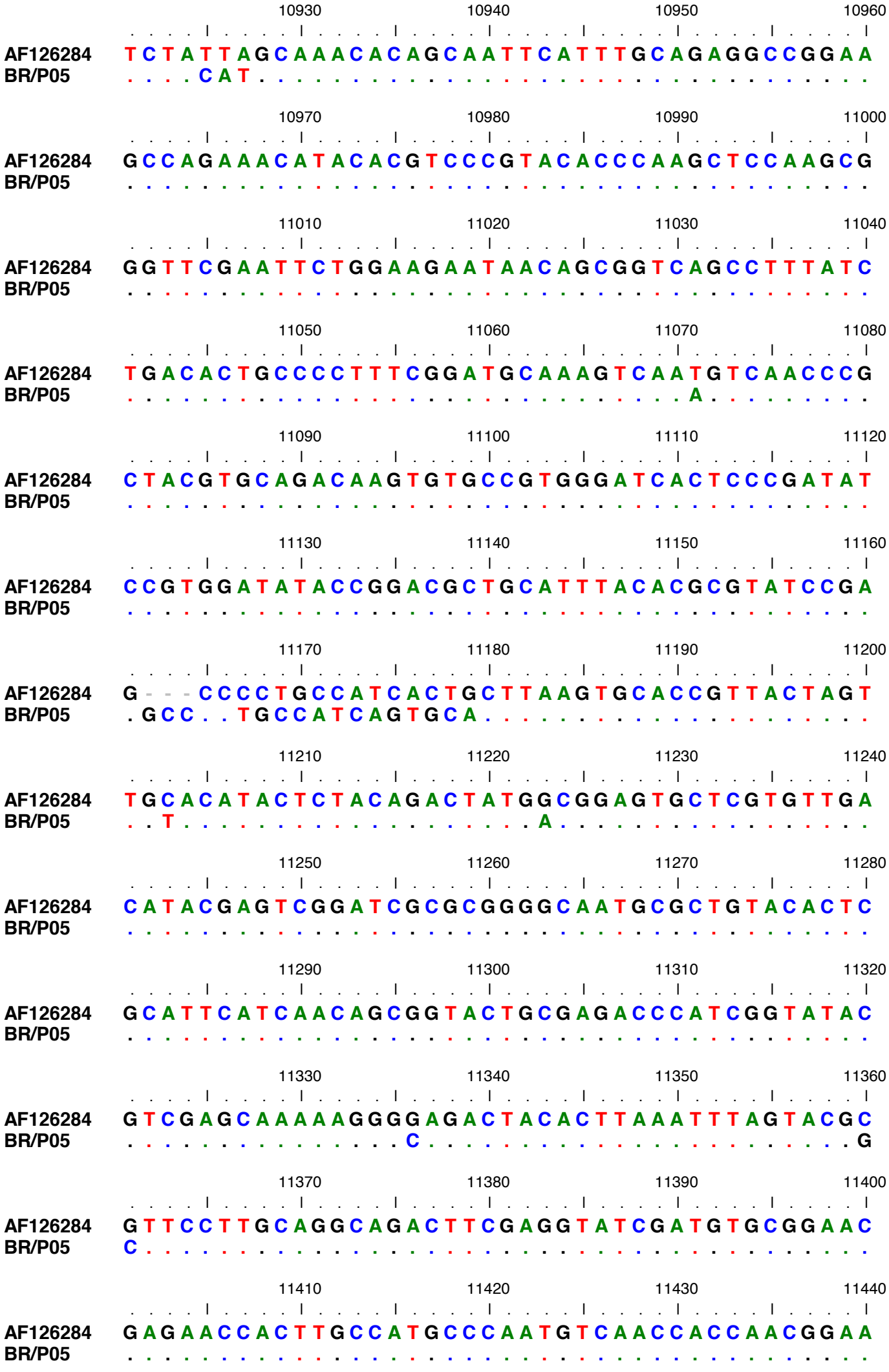
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AF126284 GTCGAGCAAAAAAGGGGAGACTACACTTAAATTTAGTACGC
BR/P05 ....C...........................G
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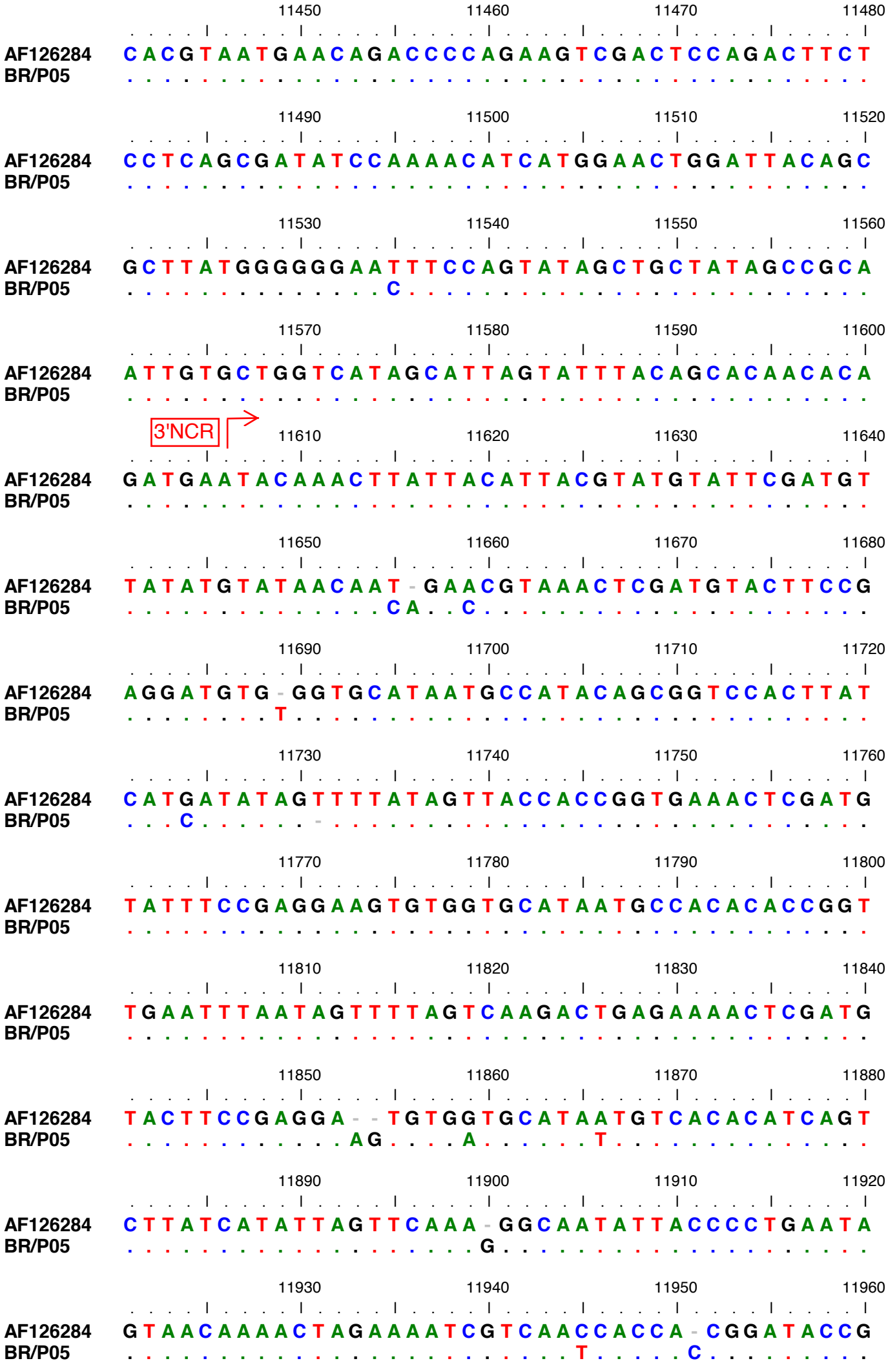
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AF126284 GCTTATGGGGGGAATTTCCAGTATAGCTGCTATAGCCGCA
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3'NCR
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AF126284 TACTTCCGAGGA--TGTGGTGCATAATGTCACACATCAGT
BR/P05 ..........AG....A.....T.....
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AF126284 CTTATCATATTAGTTCAAA-GGCAATATTACCCCTGAATA
BR/P05 ....G.....
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11960
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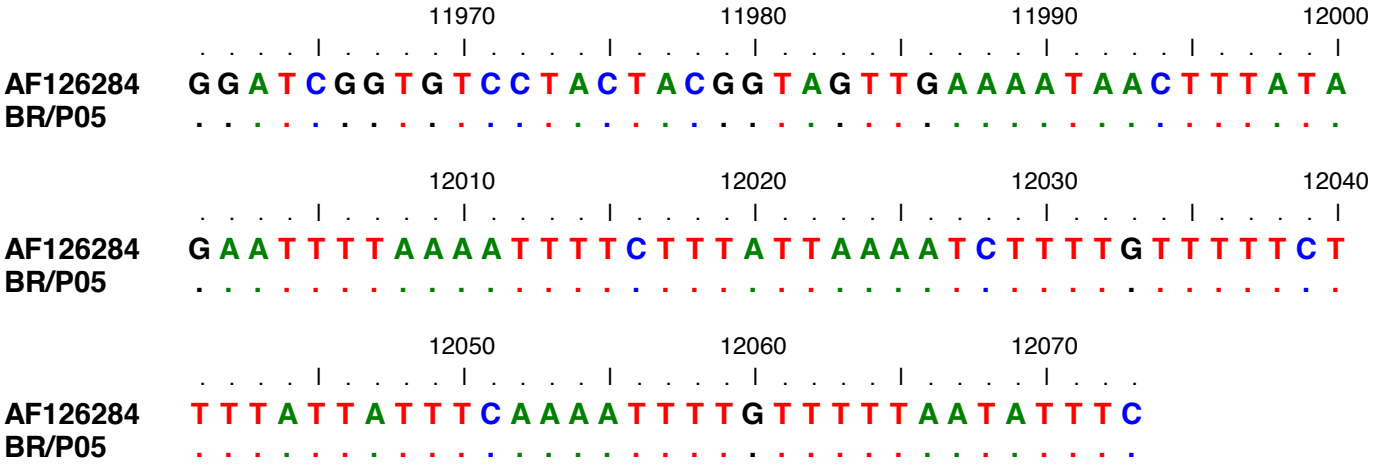
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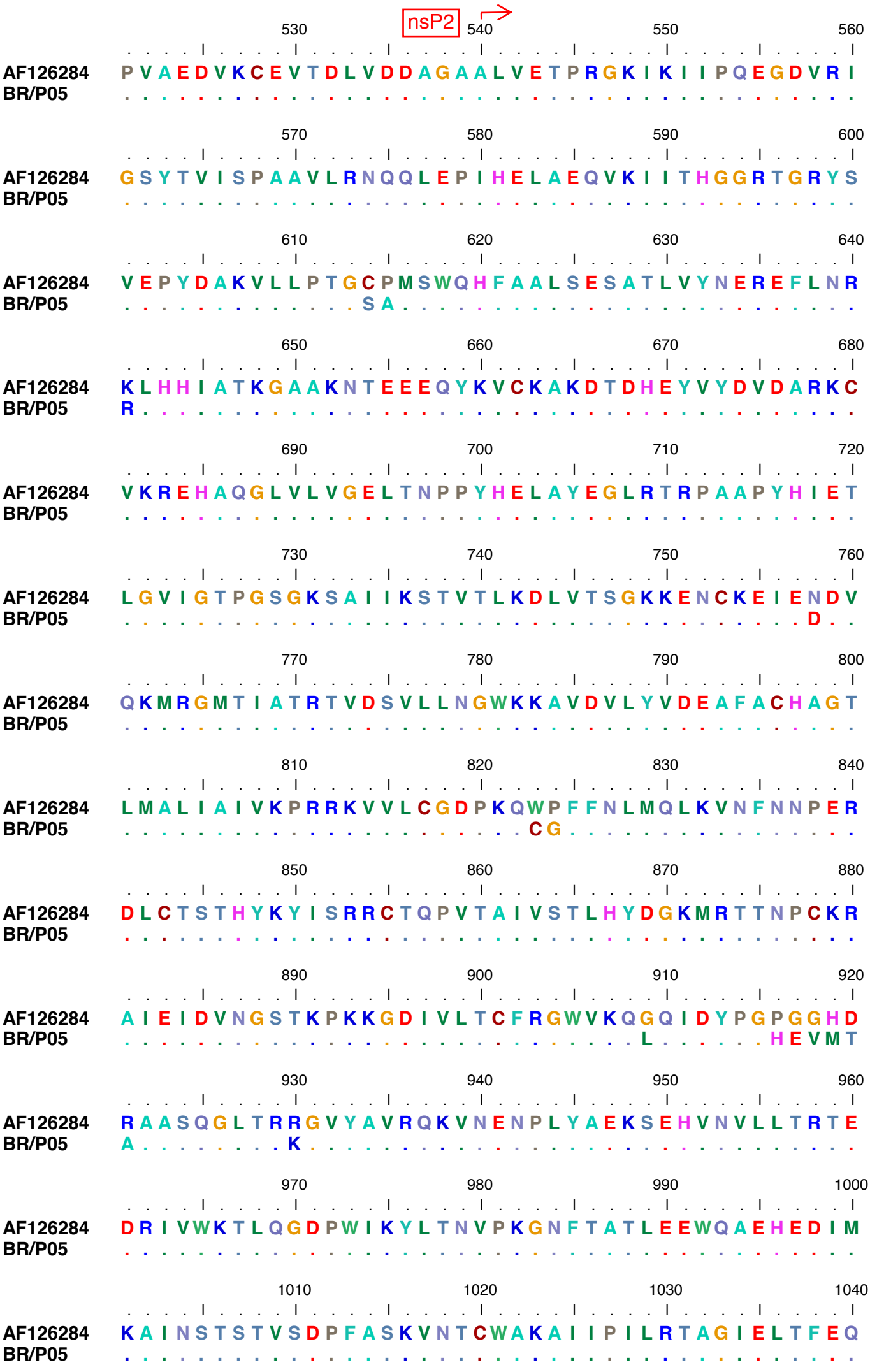
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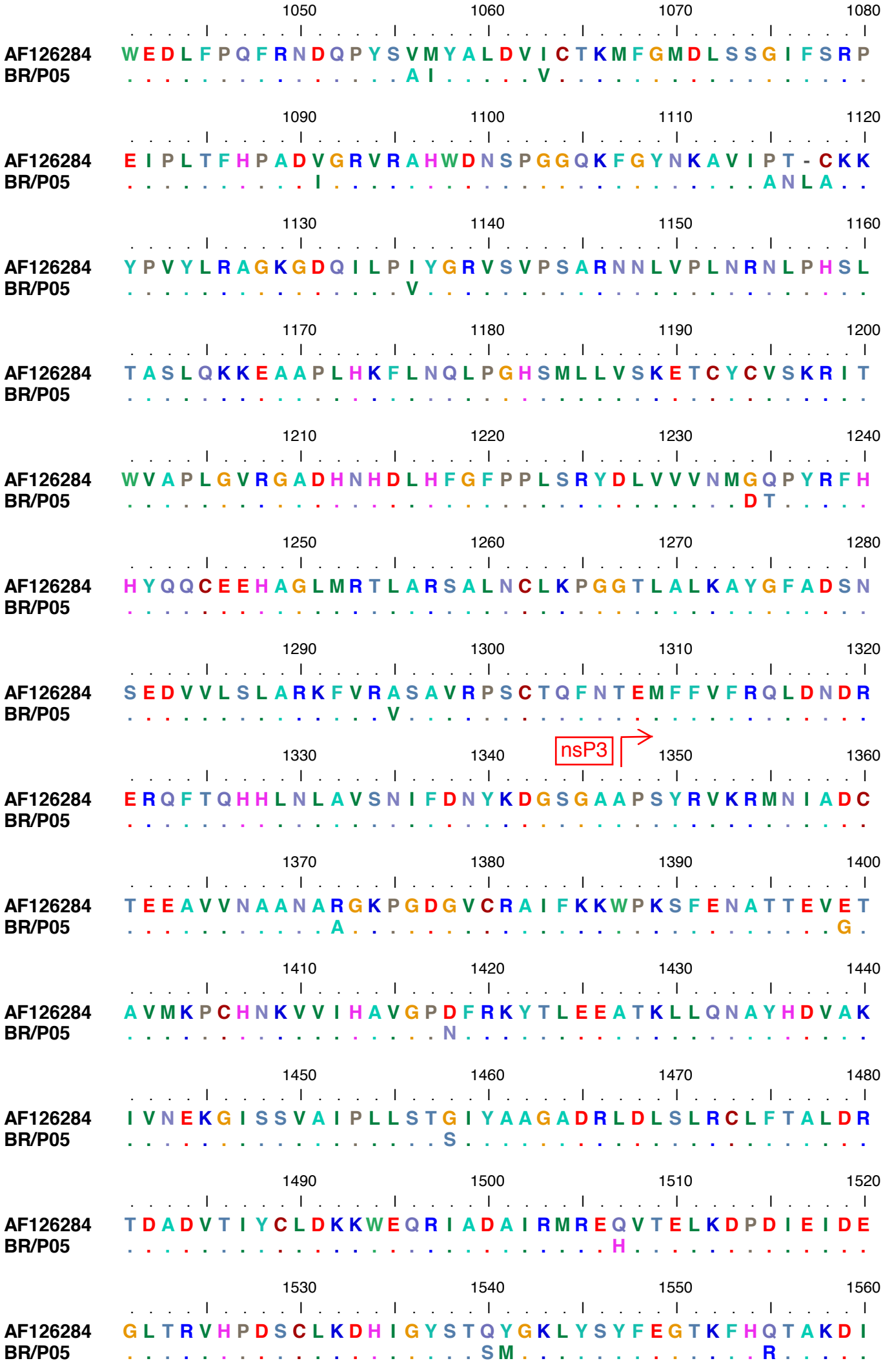
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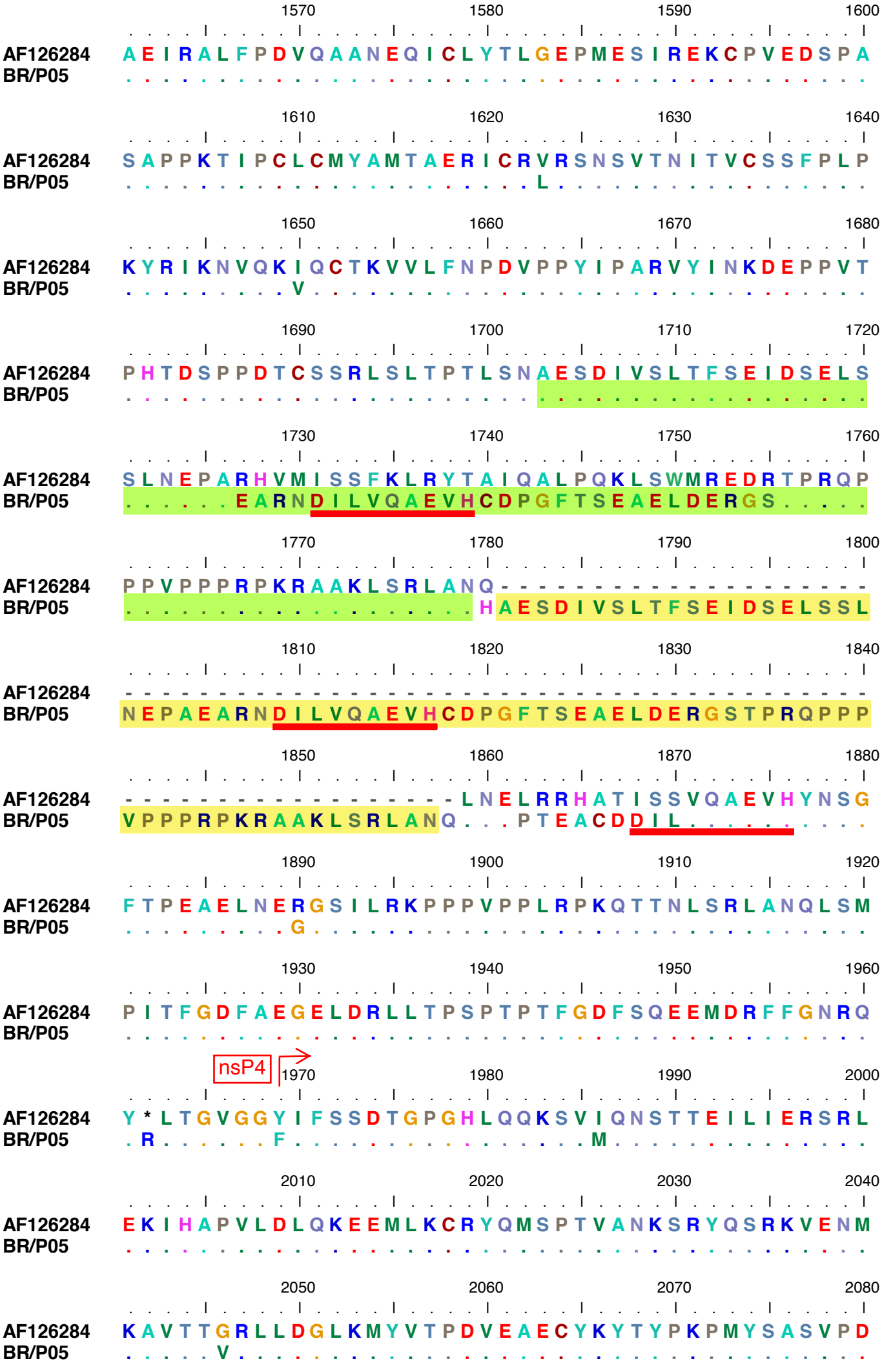
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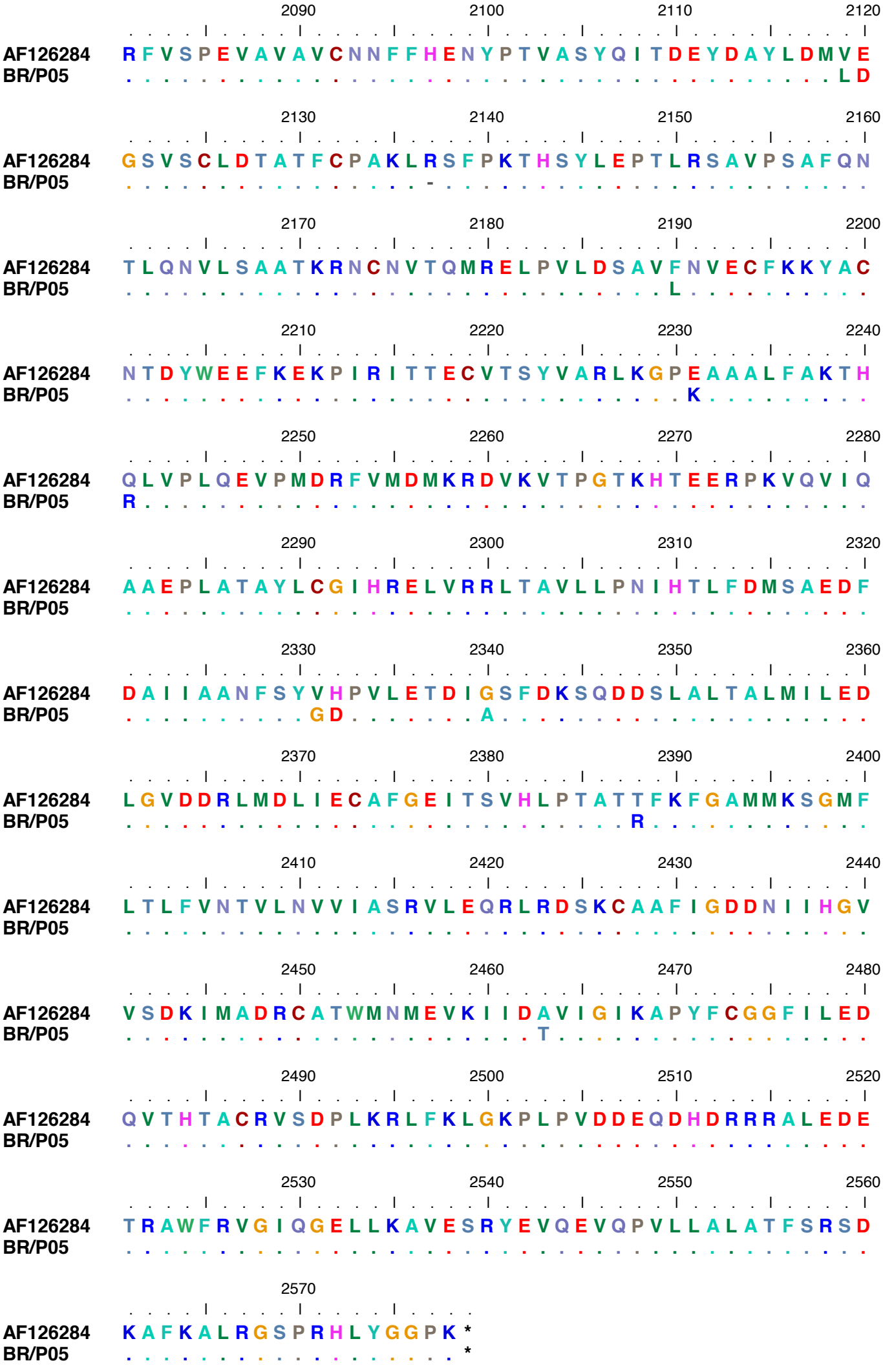
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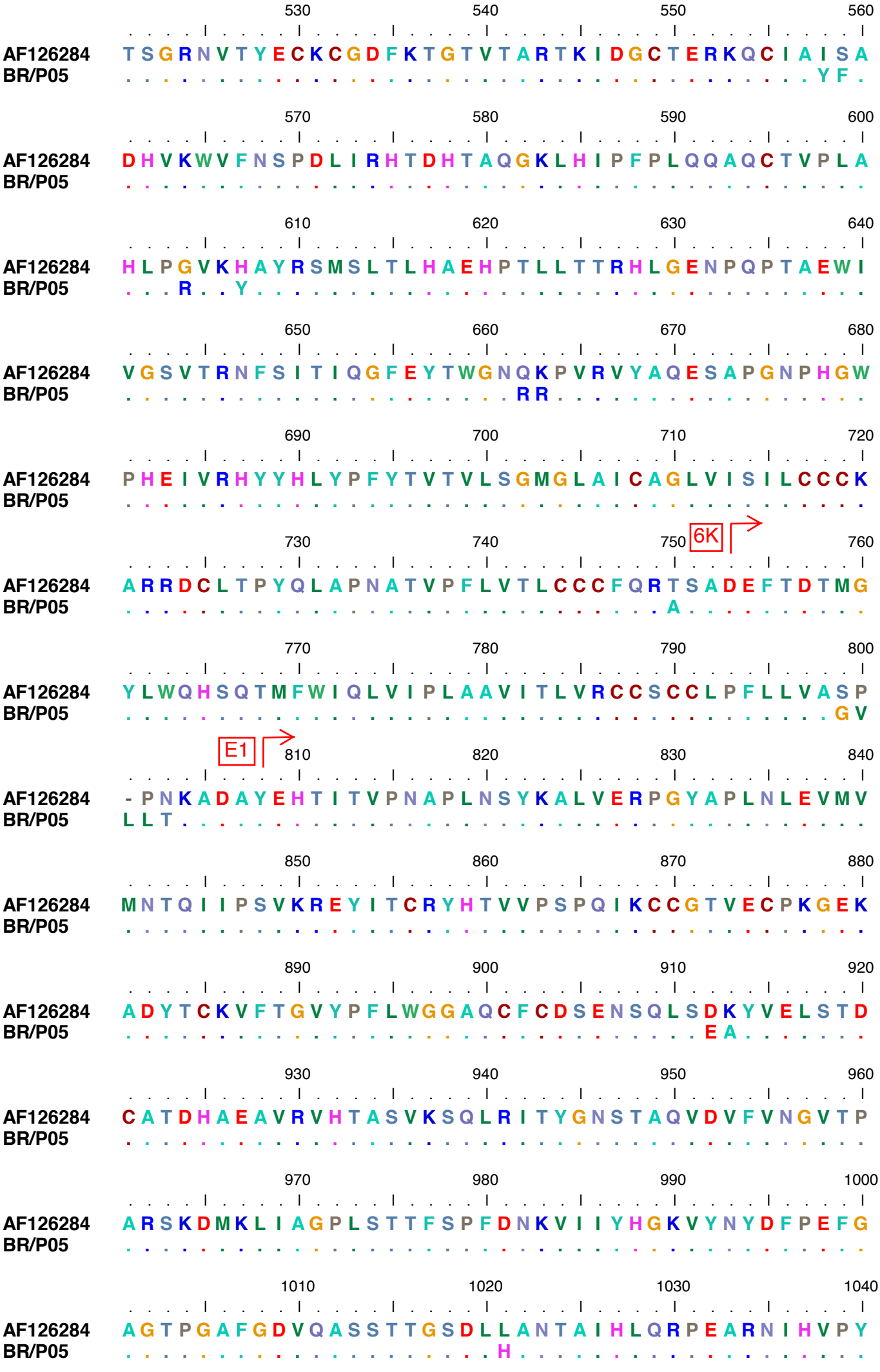
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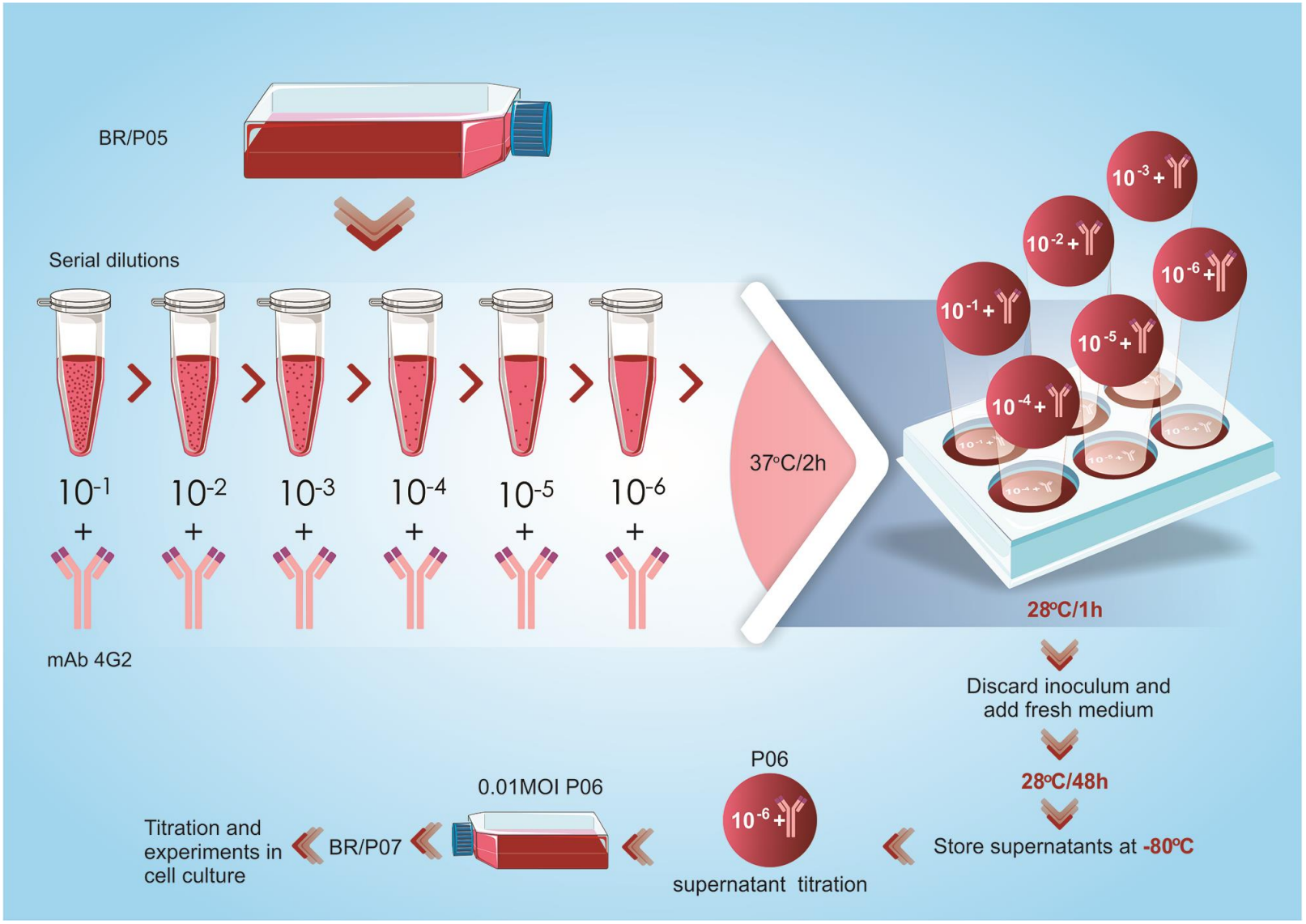


Figure S1. Workflow depicting the protocol used for DENV neutralization and production of the AURAV viral stock (BR/P07) from BR/P05. Different dilutions (10^{-1}-10^{-6}) of the supernatant of BR/P05 were incubated at 37 °C with an anti-flavivirus monoclonal antibody (4G2) for 2 h. This mixture was incubated with C6/36 cells (3.5×10^5 cells/well, seeded the day before in a 6-well plate) for 1 h at 28 °C. The inoculum was discarded, and the cell monolayer washed once with sterile PBS, after which 3 ml/well of medium added. The supernatants were collected at two days post-infection, aliquoted and stored at -80 °C. The supernatant of the infection (P06) that was carried out with the least amount of virus (10^{-6}) was titrated and used to infect C6/36 cells at a multiplicity of infection (MOI) of 0.01 to produce the viral stock (BR/P07) for biological characterization experiments. Figure by Wagner Nagib de Souza Birbeire.

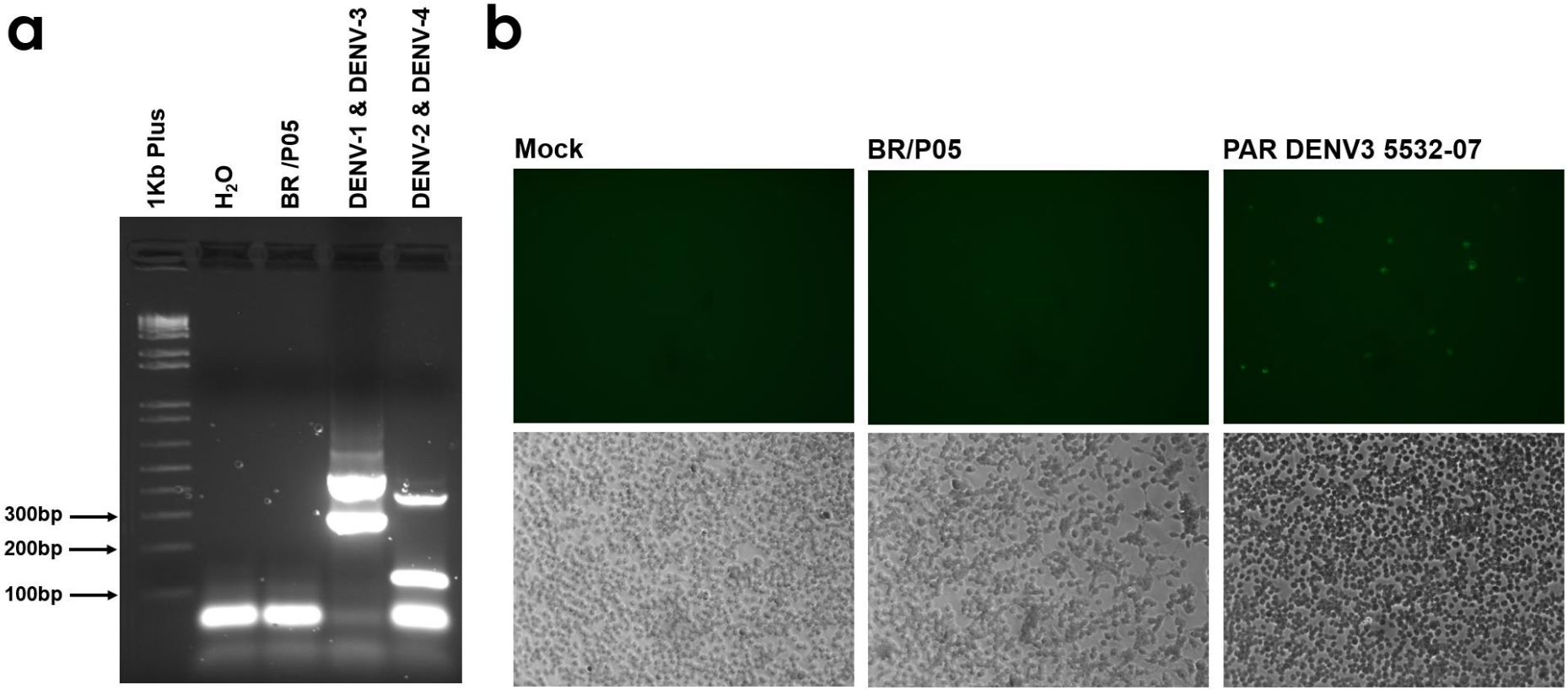


Figure S2. DENV detection in BR/P05. **a** One-step RT-PCR for DENV serotyping. RNA extracted from the supernatant of BR/P05 was used as the template. Water was used as negative control, and RNAs extracted from DENV-1, -2, -3 and 4 were used as positive controls. **b** IFA of C6/36 cells infected with the supernatant of BR/P05 at 2 days post-infection. Cells were fixed with 3% paraformaldehyde, permeabilized with 0.5% triton and incubated with an anti-flavivirus monoclonal antibody (4G2) followed by a goat anti-mouse IgG (H+L), Alexa Fluor 488 conjugate. Images were obtained at 200x magnification using a Nikon Eclipse TE300 inverted microscope attached to a CoolSNAP-ProCf camera and visualized and processed using Image-Pro Plus version 4.5.1.22 software.

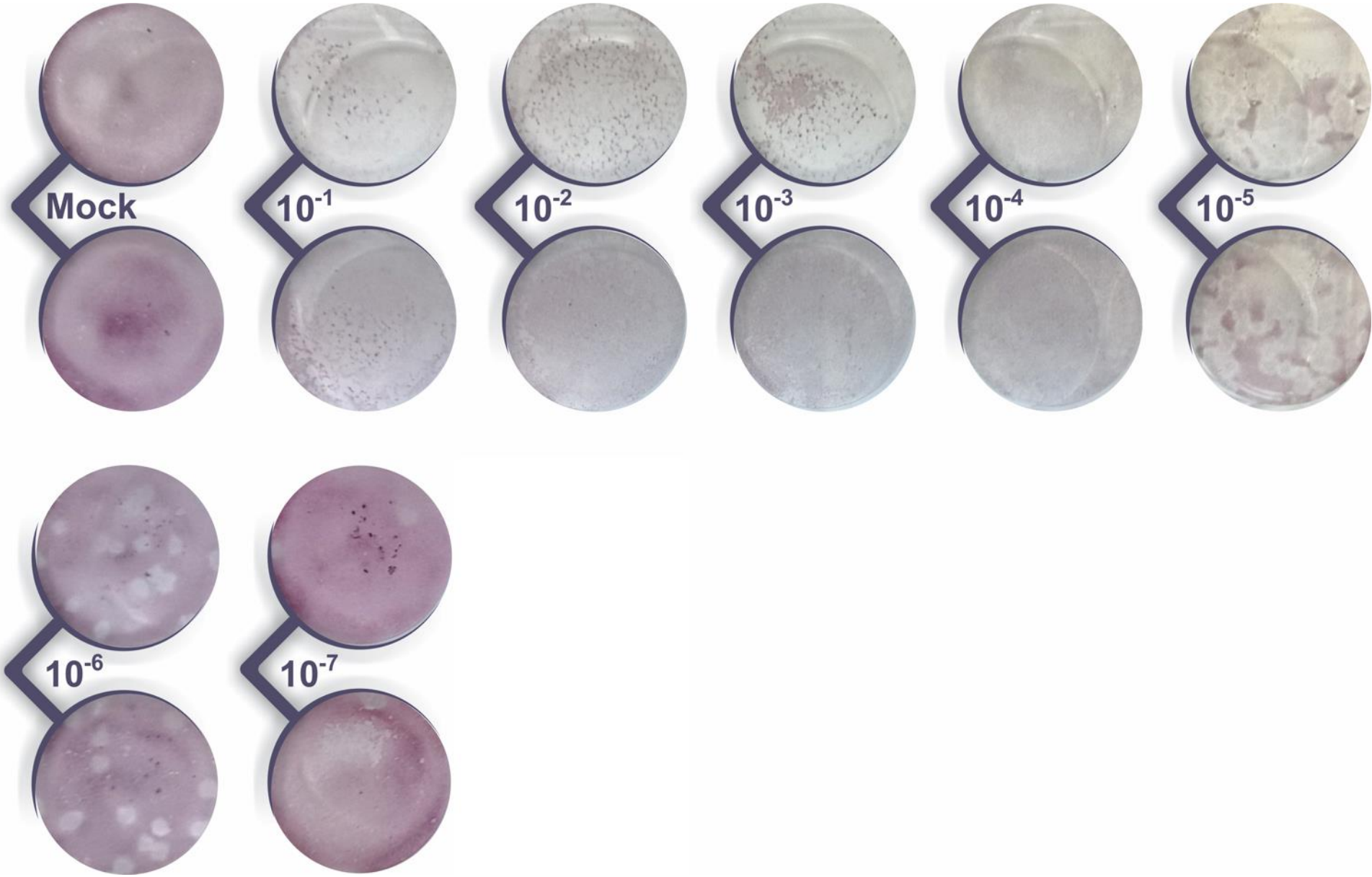


Figure S3. Result of BR/P07 titration by plaque assay in C6/36 cells.

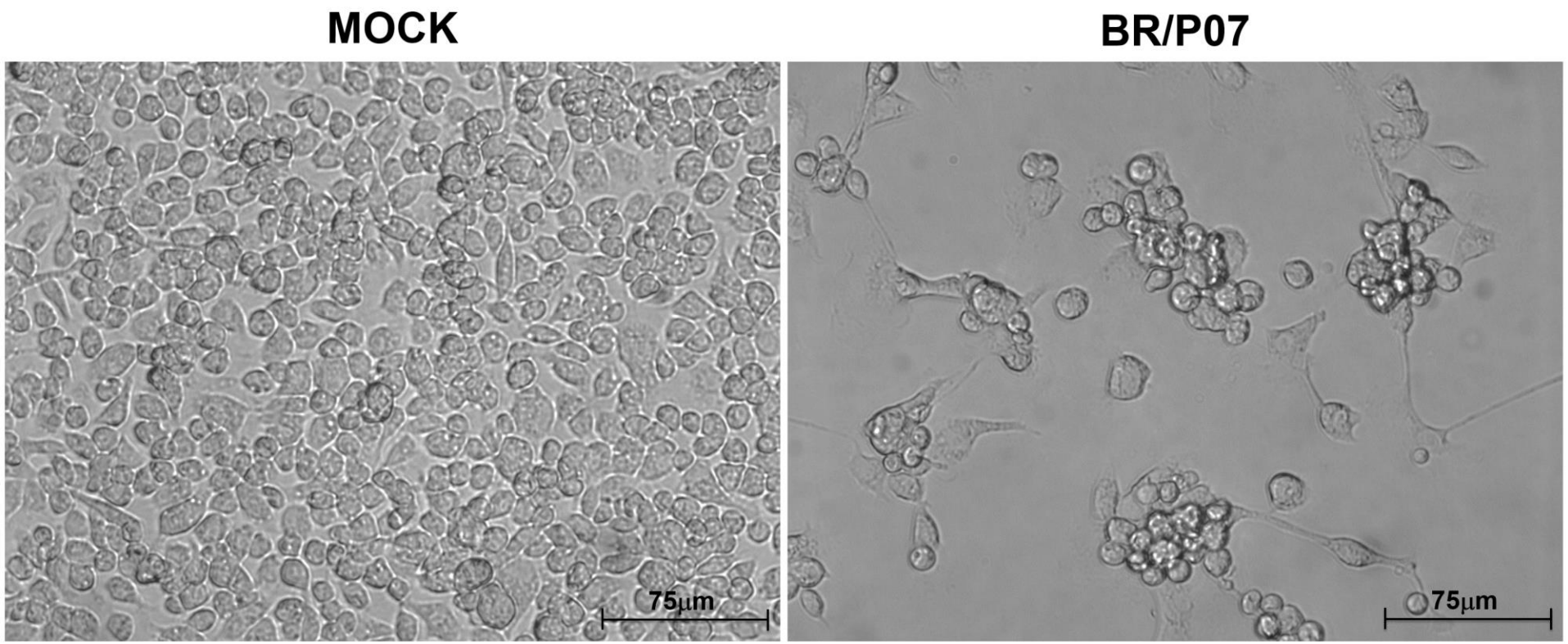


Figure S4. Brightfield images of C6/36 cells infected or not (mock) with 0.1 MOI of BR/P07 at 3 days post-infection. Images were obtained at 400x magnification using a Nikon Eclipse TE300 inverted microscope attached to a CoolSNAP-ProCf camera and visualized and processed using Image-Pro Plus version 4.5.1.22 software.

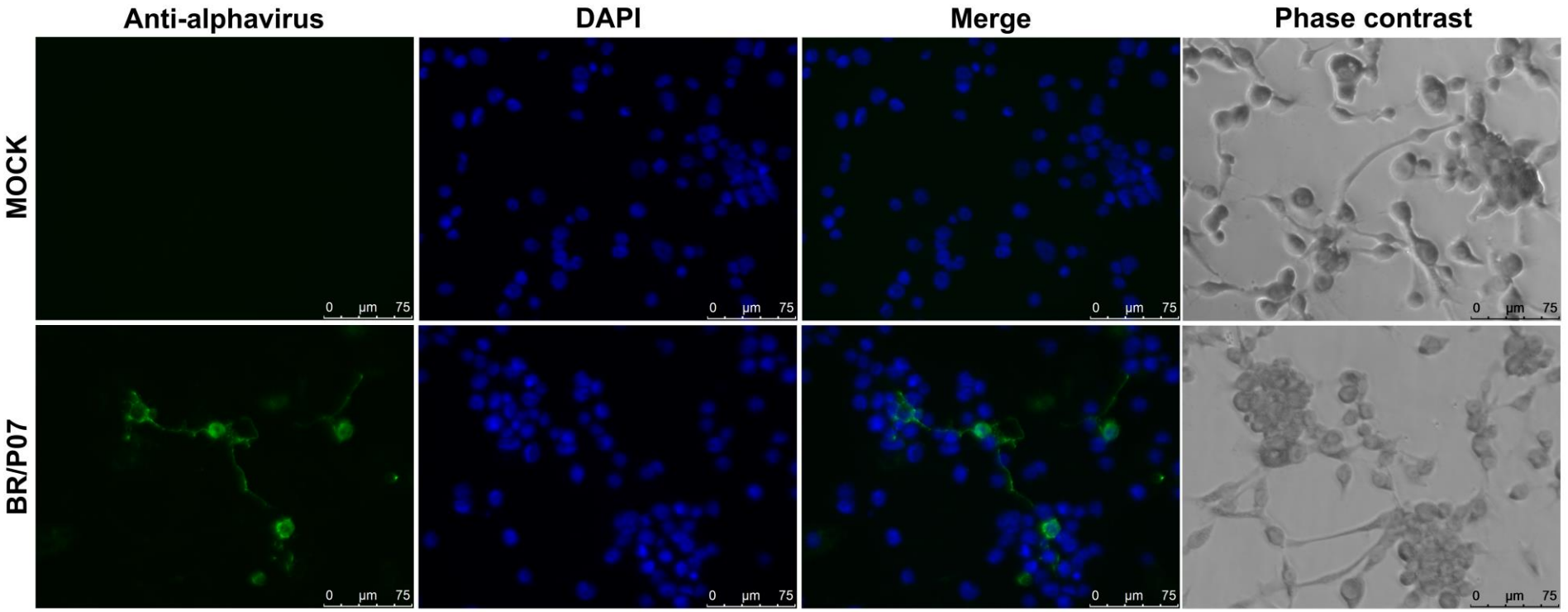


Figure S5. IFA of AP-61 cells infected or not (mock) with 1 MOI of BR/P07. At 3 days post-infection, the cell monolayer was fixed and permeabilized with methanol:acetone (1:1) at -20 ºC and then incubated with an anti-alphavirus monoclonal antibody (mAb) clone 1A4B-6 followed by a goat anti-mouse IgG (H+L), Alexa Fluor 488 conjugate. Images were obtained using a Leica DMI 6000B inverted microscope attached to a Leica DFC365 FX camera and visualized and processed using Leica Application Suite Advanced Fluorescence 3.1.0 software.

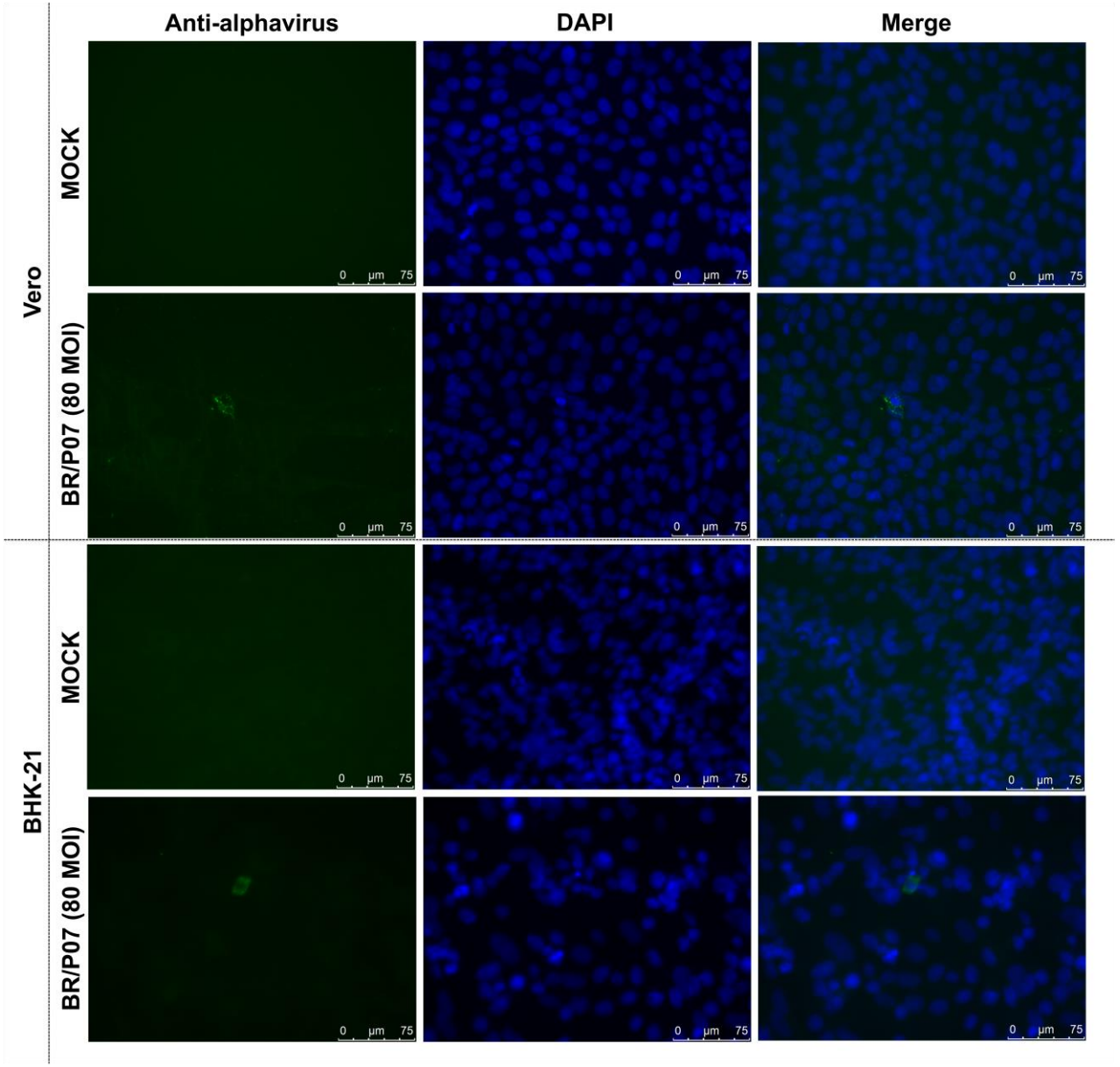


Figure S6. IFA of Vero and BHK-21 cells infected or not (mock) with 80 MOI of BR/P07. At 3 days post-infection, cell monolayers were fixed and permeabilized with methanol:acetone (1:1) at -20 ºC and then incubated with an anti-alphavirus monoclonal antibody (mAb) clone 1A4B-6 followed by a goat anti-mouse IgG (H+L), Alexa Fluor 488 conjugate. Images were obtained using a Leica DMI 6000B inverted microscope attached to a Leica DFC365 FX camera and visualized and processed using Leica Application Suite Advanced Fluorescence 3.1.0 software.

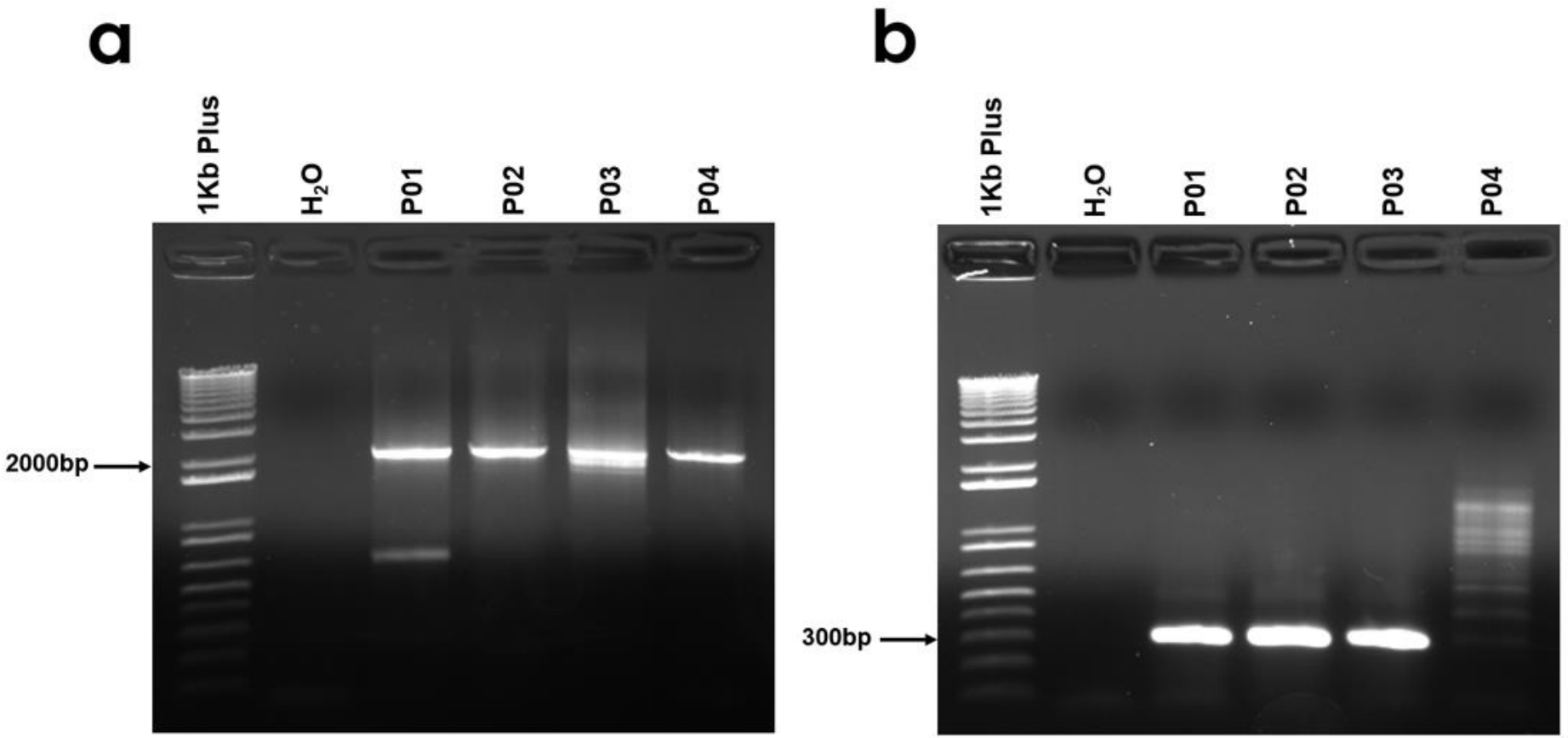
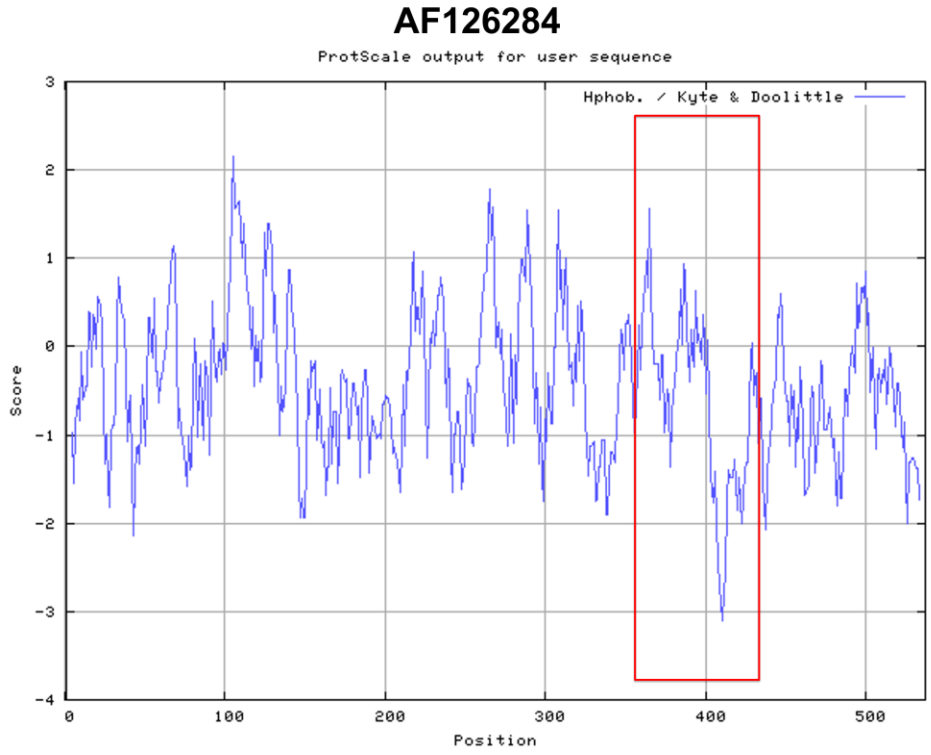


Figure S7. RT-PCR of P01-P04 for **a** AURAV and **b** DENV. RT-PCR was carried out following standard protocols. The primers used for AURAV testing were NsP3F and NsP3R (see Supplementary Table 2 for primer sequence). The primers used for DENV testing were D1 (5'-TCAATATGCTGAAACGCGCGAGAAACCG-3') and TS3 (5'-TAACATCATCATGAGACAGAGC-3'). The expected amplicon size for AURAV and DENV is 1995 bp and 290 bp, respectively.



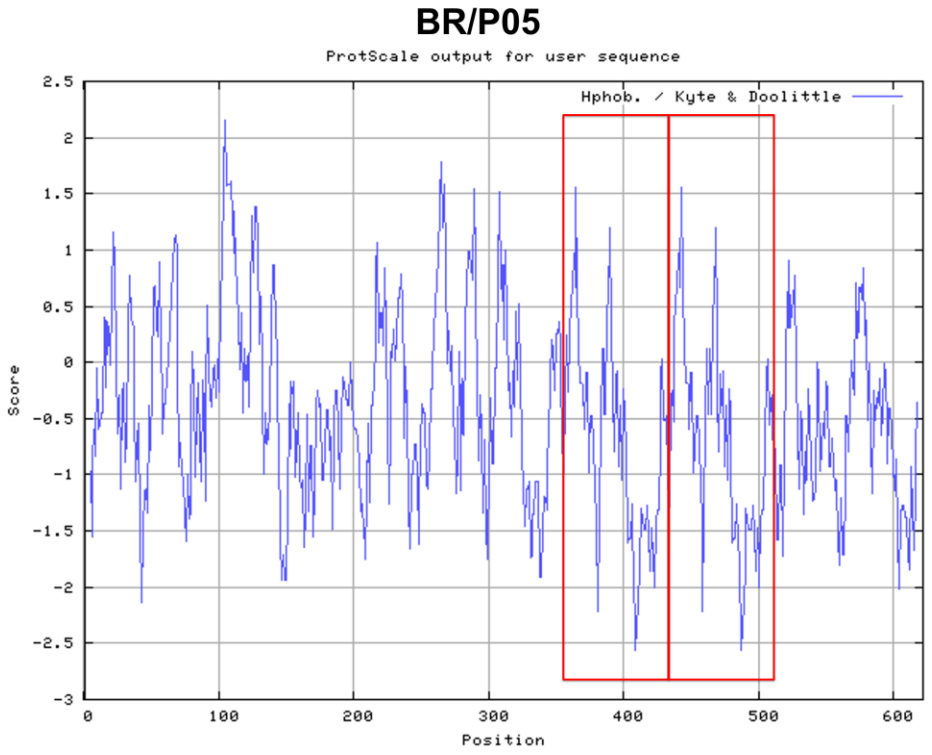


Figure S8. nsP3 hydrophobicity plot of AF126284 and BR/P05. The region corresponding to the duplication is highlighted by the red box. The Kyte & Doolittle hydrophobicity plot was drawn using the default settings, as available at the ProtScale website (http://web.expasy.org/protscale/).

a

Score	Expect	Identities	Gaps	Strand
176 bits(194)	1e-45	149/181(82%)	2/181(1%)	Plus/Plus

```
AF126284  5415  CTAAACGAGCTACGGAGGCATGCGACGATATCCTCCGTTCAAGCTGAGGTACACTACAAT  5474
                |||||||||||     ||||||  |   | |||||||||| ||||||||||||||||||  | ||
AF126284  5238  CTAAACGAGCCTGCGAGGCACGTAATGATATCCTC-GTTCAAGCTGAGGTACACTGCGAT  5296

AF126284  5475  TCAGGTTTTACCCCAGAAGCCGAATTGAATGAGAGAGGATCG-ATACTGAGGAAGCCGCC  5533
                 ||||  ||||||  |||||||  ||  |||  ||||||||||||||  |  ||  |||   |||||||
AF126284  5297  CCAGGCTTTACCTCAGAAGCTGAGTTGGATGAGAGAGGATCGCACACCGAGACAGCCGCC  5356

AF126284  5534  TCCCGTACCGCCACTACGACCAAAACAAACTACGAACTTATCTCGACTCGCAAACCAACT  5593
                |||  ||||||||||  ||||||||||  |  |   ||||  |||||  |||||  ||||||||  ||
AF126284  5357  TCCTGTACCGCCACCACGACCAAAACGAGCCGCGAAATTATCCCGACTAGCAAACCAGCT  5416

AF126284  5594  A  5594
                |
AF126284  5417  A  5417
```

b

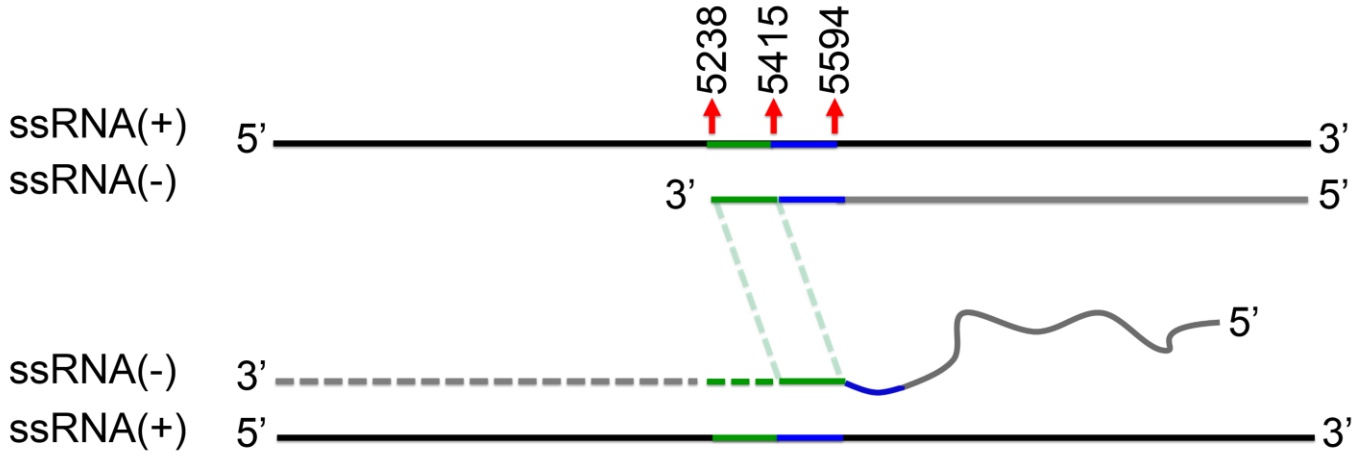


Figure S9. Mechanism of duplication hypothesis. **a** Partial results of BLAST 2 sequences search of AF126284 against itself. **b** Possible mechanism involved in the duplication. During the synthesis of the negative strand, one of these segments in the negative strand (represented in green) might hybridize with the neighboring segment in the positive strand (represented in blue), resulting in duplication of the segment represented in green (green dashed line).

Table S1
BLAST analyses of DOP-PCR sequencing results

Clone	Region of homology with AF126284	Identity	E-value
MP1	6413-6772	99%	8.10^{-176}
MP3	6413-6796 7826-7884	99% 100%	0.0 2.10^{-19}
MP5	8715-9366	99%	0.0
MP7	7463-7884	98%	0.0
MP9	7398-7885	98%	0.0
MP11[1]	-	-	-
MP13	7463-7884	98%	0.0
MP15	6413-6599 10326-10394	98% 94%	9.10^{-85} 1.10^{-19}
MP17	6413-7884	99%	0.0
MP19	7463-7884	98%	0.0
MP21	8715-9366	99%	0.0
MP23	7463-7884	98%	0.0
MP25	8715-9363	99%	0.0
MP27	7463-7885	97%	0.0
MP31	7463-7884	98%	0.0
MP33	4385-5118	98%	0.0
MP35	7409-7886	99%	0.0
MP37	7463-7884	98%	0.0

[1]Blast hits presented low coverage (<17%) and high E-values (>0.11)

Table S2
Primers used in RT-PCR and sequencing

Primer	Sequence (5' → 3')	Position (AF126284)
AURAV1F	ATAGCGGACGGACTAGTACTTGTACTAC	1-28
AURAV2R	TAGGGCCTGATGGTAAATGGTC	581-560
AURAV3F	GGTCAAAGGGGAGCGTGTATCGTTC	1040-1064
AURAV4R	ATAGTTCTGCATCGTGTTGGTGTTTCT	1226-1200
AURAV5F	TAGATGCCAGAAAATGCGTAAAAAGAG	2101-2127
AURAV6R	ATAGTCATTCCCCGCATTTTCTGG	2380-2357
AURAV7F	AATGACCAACCTTACTCCGTGATGT	3225-3249
AURAV8R	GTATTTGGTCCCCTTTTCCTGCTCTTA	3474-3448
AURAV9F	GTTGGAGGAAGCGACGAAGCTA	4343-4364
AURAV10R	ATCTGCTATGCGTTGCTCCCACTT	4574-4551
AURAV11F	AACGAGCTACGGAGGCATGCGACGATA	5418-5444
AURAV12R	CCAAACGTAGGGGTCGGTGATG	5674-5653
AURAV13F	GCAGCACTATTCGCAAAGACACATCGA	6537-6563
AURAV14R	GGTTCTGCTGCTTGAATAACTTGT	6691-6668
AURAV15F	CACTACGCGGGAGCCCAAGACACCT	7534-7558
AURAV16R	TAGTGAGCTGTTGGATCTGGGTAG	7760-7737
AURAV17F	GTTCTGCTGGACGCTGCTCTGA	8564-8585
AURAV18R	CGTCATCGGCATCATCCCACAC	8735-8714
AURAV19F	CACCTCTATCCCTTCTACACCGTTACA	9695-9721
AURAV20R	CATTGTTTGACTGTGTTGCCATAGGTA	9934-9908
AURAV21F	CCGGATCAGATCTATTAGCAAACACAGC	10671-10698
AURAV22R	GGCTCGGATACGCGTGTAAATGCAG	10923-10899
AURAV23F	GCTGCTATAGCCGCAATTGTGCTGG	11303-11327
AURAV24R	GAAATATTAAAAACAAAATTTTGAAATAAT	11824-11795
Nsp3F	GATCGTGAGCGCCAATTCAC	4029-4048
Nsp3R	TTTTTGTTGCAGATGCCCCG	5789-5770
DOP	CCGACTCGAGINNNNNNTGTGG	not applicable

Table S3
Dataset used in the phylogenetic analysis

GenBank Accession number	Abbreviation	Name
AJ316244	SPDV	Salmon pancreas disease virus complete genome, isolate F93125, genomic RNA
AJ316246/NC003433	SDV	Sleeping disease virus complete genome
HM147990	SESV	Southern elephant seal virus, complete genome
U73745	BFV	Barmah Forest virus strain BH2193, complete genome
EF536323	MIDV	Middelburg virus strain MIDV857 non-structural polyprotein and structural polyprotein genes, complete cds
HM147989	NDUV	Ndumu virus, complete genome
AB032553	SAGV	Sagiyama virus genomic RNA, complete genome
AY702913	GETV	Getah virus from South Korea, complete genome
GQ433354	RRV	Ross River virus strain QML 1, complete genome
HM147985	BEBV	Bebaru virus, complete genome
NC_003417	MAYV	Mayaro virus, complete genome
HM147992	UNAV	Una virus non structural polyprotein gene, partial cds; and structural polyprotein gene, complete cds
HM045788	CHIKV	Chikungunya virus strain PO731460, complete genome
AF079456	ONNV	O'nyong-nyong virus strain SG650, complete genome
L01442	VEEV IAB	Venezuelan equine encephalitis virus, complete genome
AF375051	VEEV IC	Venezuelan equine encephalitis virus nonstructural polyprotein and structural polyprotein genes, complete cds
L00930	VEEV ID	Venezuelan equine encephalitis virus strain 3880, complete genome
U34999	VEEV IE	Venezuelan equine encephalitis virus nonstructural and structural polyprotein genes, complete cds
AF075257	VEEV IF	Venezuelan equine encephalitis virus strain 78V-3531, complete genome
AF075251	EVEV	Venezuelan equine encephalitis virus strain Everglades Fe3-7c, complete genome
AF075254	TONV	Venezuelan equine encephalitis virus strain Tonate CaAn 410d, complete genome
AF075253	MUCV	Venezuelan equine encephalitis virus strain Mucambo BeAn 8, complete genome
AF075255	VEE 71D1252	Venezuelan equine encephalitis virus strain 71D-1252, complete genome
AF075256	PIXV	Venezuelan equine encephalitis virus strain Pixuna BeAr 35645, complete genome
AF075259	CABV	Venezuelan equine encephalitis virus strain Cabassou CaAr 508, complete genome
AF075258	RNV	Venezuelan equine encephalitis virus strain AG80-663, complete genome
EF151502	EEEV LinI	Eastern equine encephalitis virus strain FL93-939, complete genome
DQ241303	EEEV LinII	Eastern equine encephalitis virus strain PE-3.0815, complete genome
DQ241304	EEEV_LinIII	Eastern equine encephalitis virus strain PE-0.0155, complete genome
EF151503	EEEV_LinIV	Eastern equine encephalitis virus strain BeAr436087, complete genome

HM147991	TROV	Trocara virus non structural polyprotein gene, partial cds; and structural polyprotein gene, complete CDs
NC_018615	EILV	Eilat virus isolate EO329, complete genome
AF126284	AURAV	Aura virus polyprotein 1 and polyprotein 2 genes, complete CDs
NC001547	SINV	Sindbis virus, complete genome
M69205	SINV Ockelbo	Ockelbo virus strain Edsbyn, complete genome
HM147984	SINV Babanki	Babanki virus, complete genome
AF103728	SINV XJ160	Sindbis virus strain XJ-160, complete genome
AF429428	SINV SW6562	Sindbis virus isolate SW6562, complete genome
HM147993	WHATV	Whataroa virus, complete genome

Supplementary sequence data

In red: pGEM-T-easy sequence

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