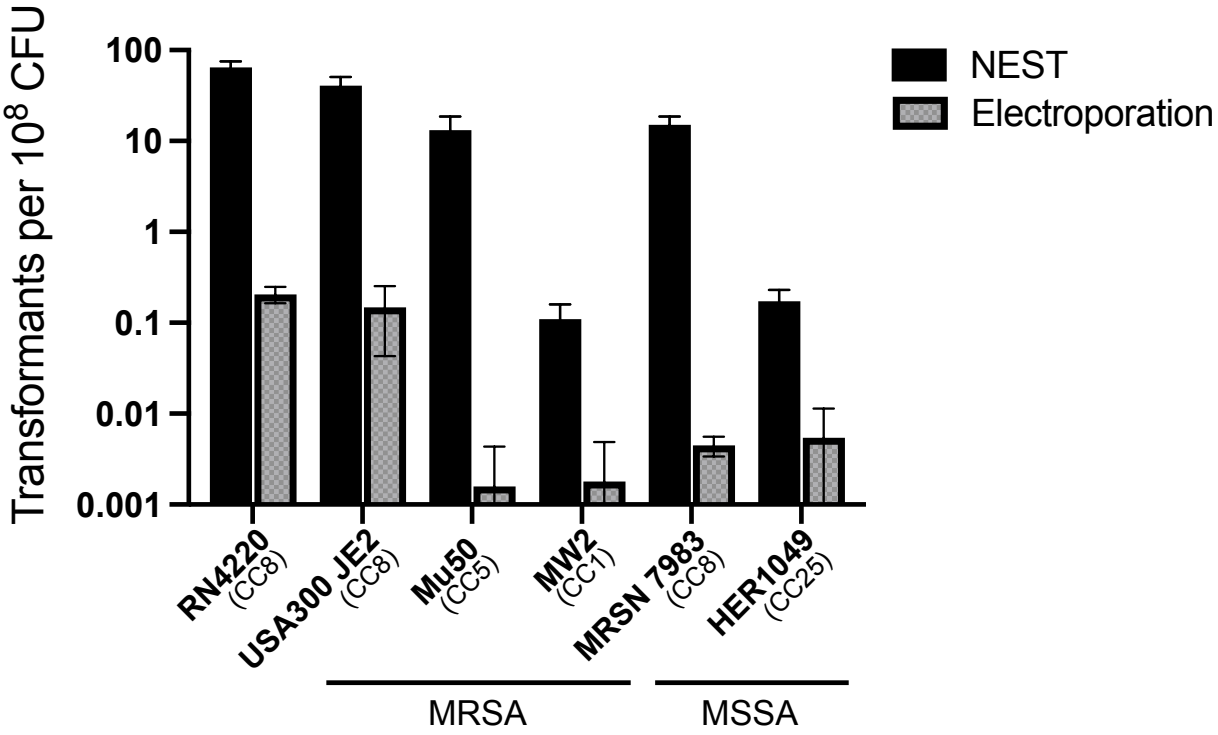


Figure S1. Comparison of NEST vs. Electroporation transformation methods on other *S. aureus* strains. Efficiency of transformation was determined using 300 ng of pCM28 plasmid isolated from *S. aureus* RN4220 transformed into NEST competent (black bars) or electrocompetent (gray bars) cells of clinical and *S. aureus* strains from different clonal complexes: RN4220 (control lab strain, CC8), JE2 (MRSA, CC8), Mu50 (MRSA, CC5), MW2 (MRSA, CC1), MRSN 7983 (MSSA, CC8) and HER1049 (MSSA, CC25). Efficiency of boot-up was determined by calculating the number of transformants per 10^8 CFU. For both methods, ~1×10^{10} CFUs were used in transformation mixes. Each data point represents the mean from three independent experiments, and the error bars indicate standard error.

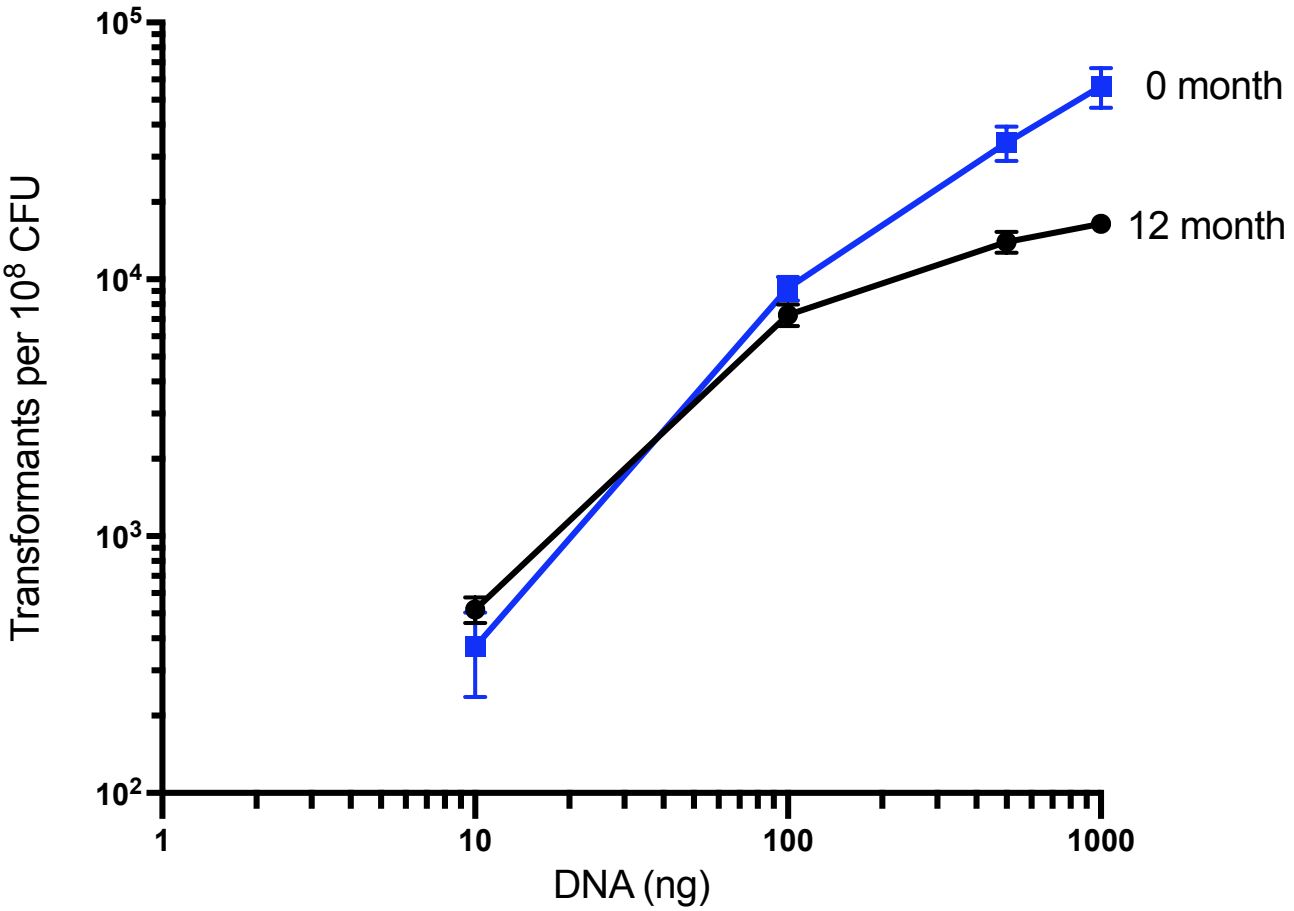


Figure S2. Evaluating the long-term stability of NEST-competent cells. Plasmid pCM28 was transformed into freshly prepared and year old frozen (-80°C) competent cells using the NEST method to evaluate long-term stability. Each data point represents the mean from three independent experiments, and the error bars indicate standard error.

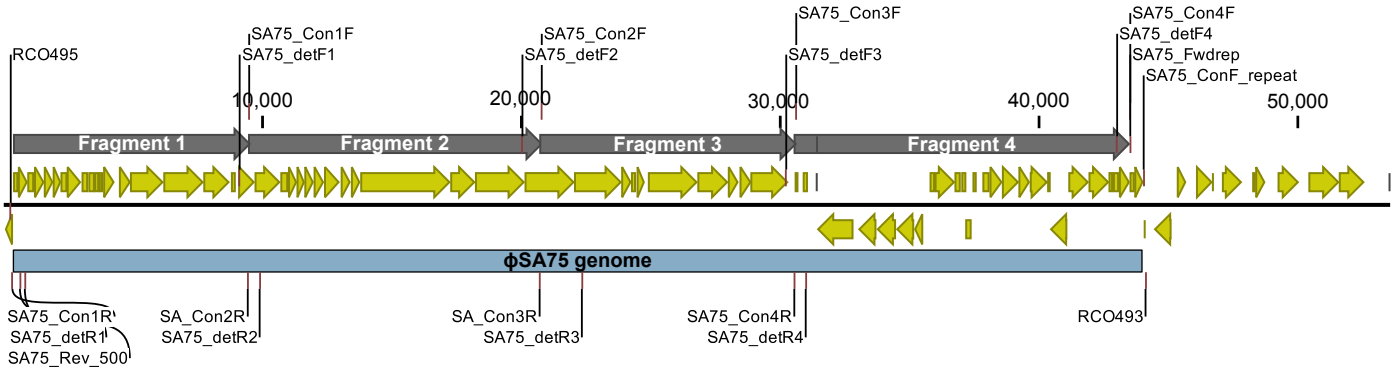
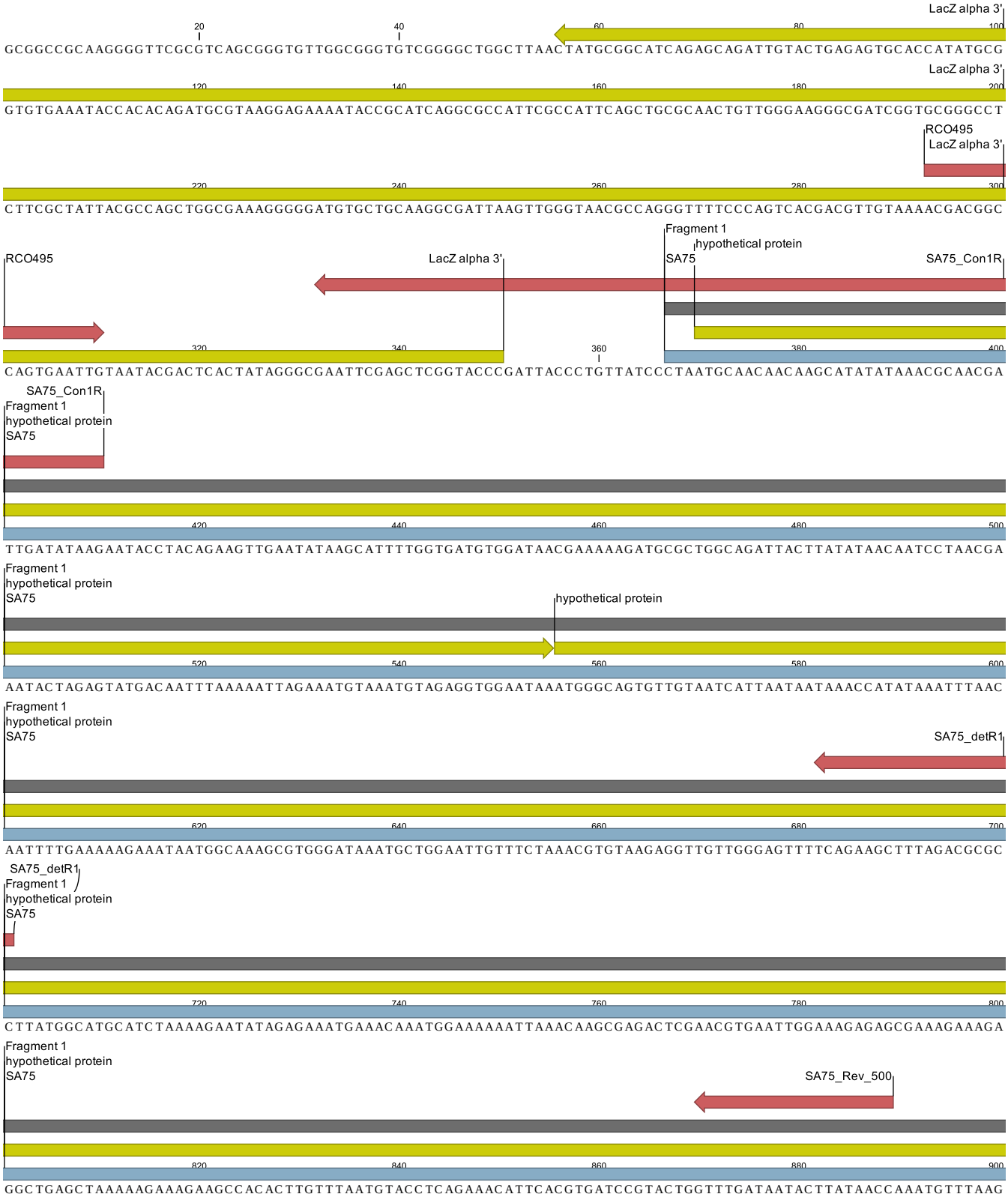


Figure S3. Sequence of the synthetic yeast-assembled SA75 phage genome. The complete sequence of SA75YA was determined on an Illumina NovaSeq 6000 Sp, 300 cycles (2×150) Xp workflow (see below). Above is a linear illustration of assembled SA75 genome within the yeast Ycp vector. Predicted protein-coding sequences are denoted as yellow arrows. A light blue arrow marks the location of the phage SA75 genome within the yeast Ycp/BAC vector while the dark gray arrow delineates the position of each of the four cloned SA75 fragments.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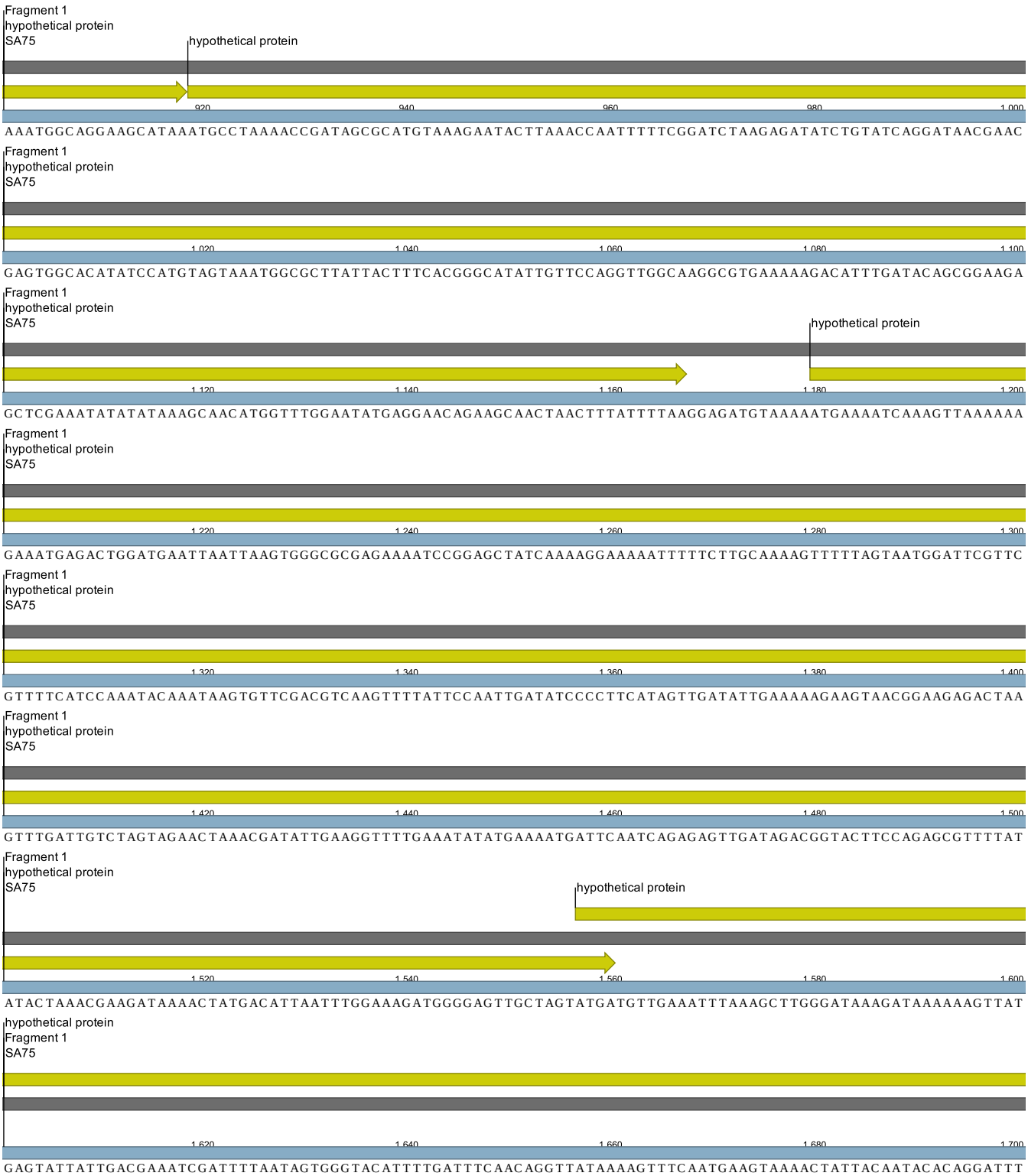
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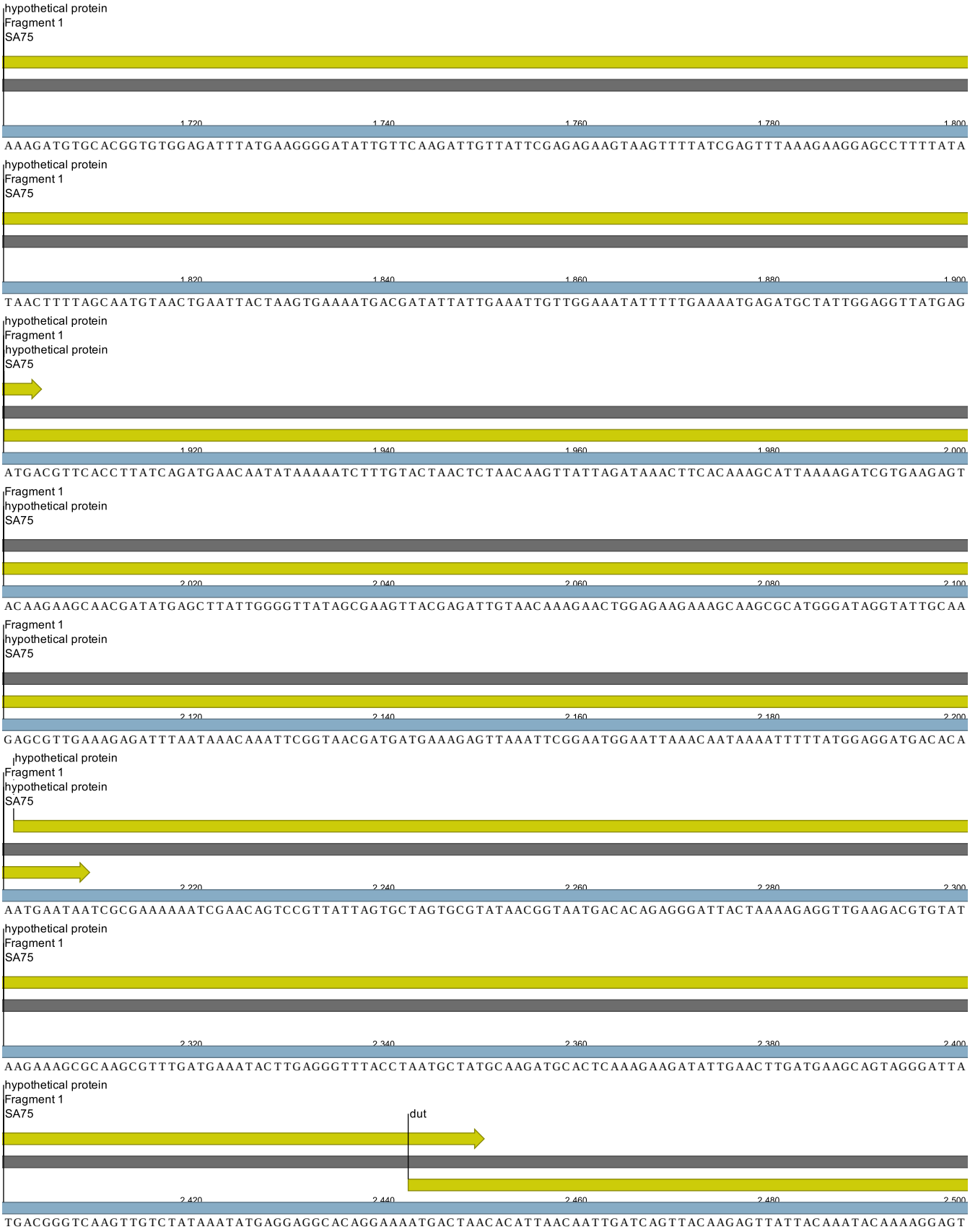
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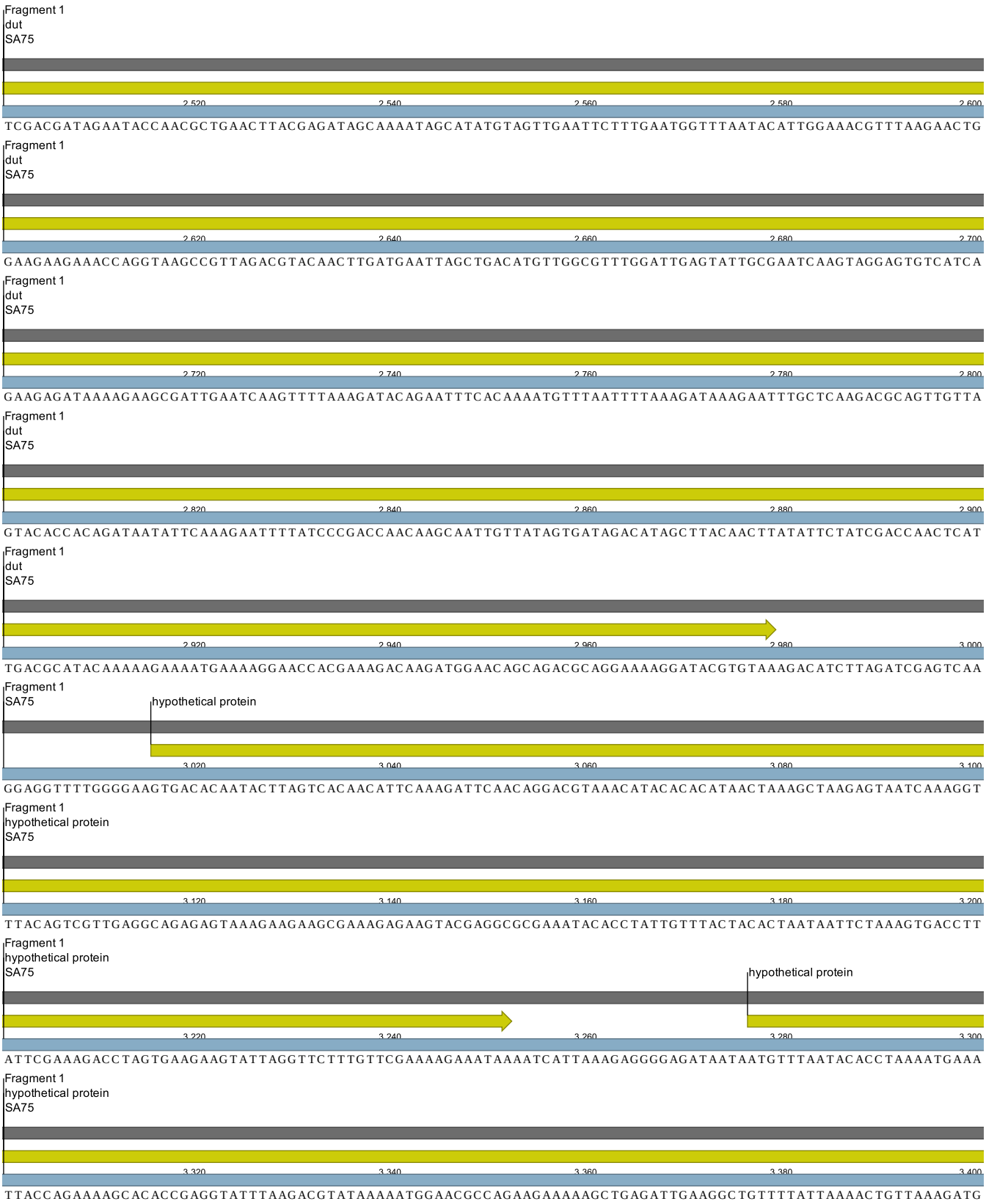
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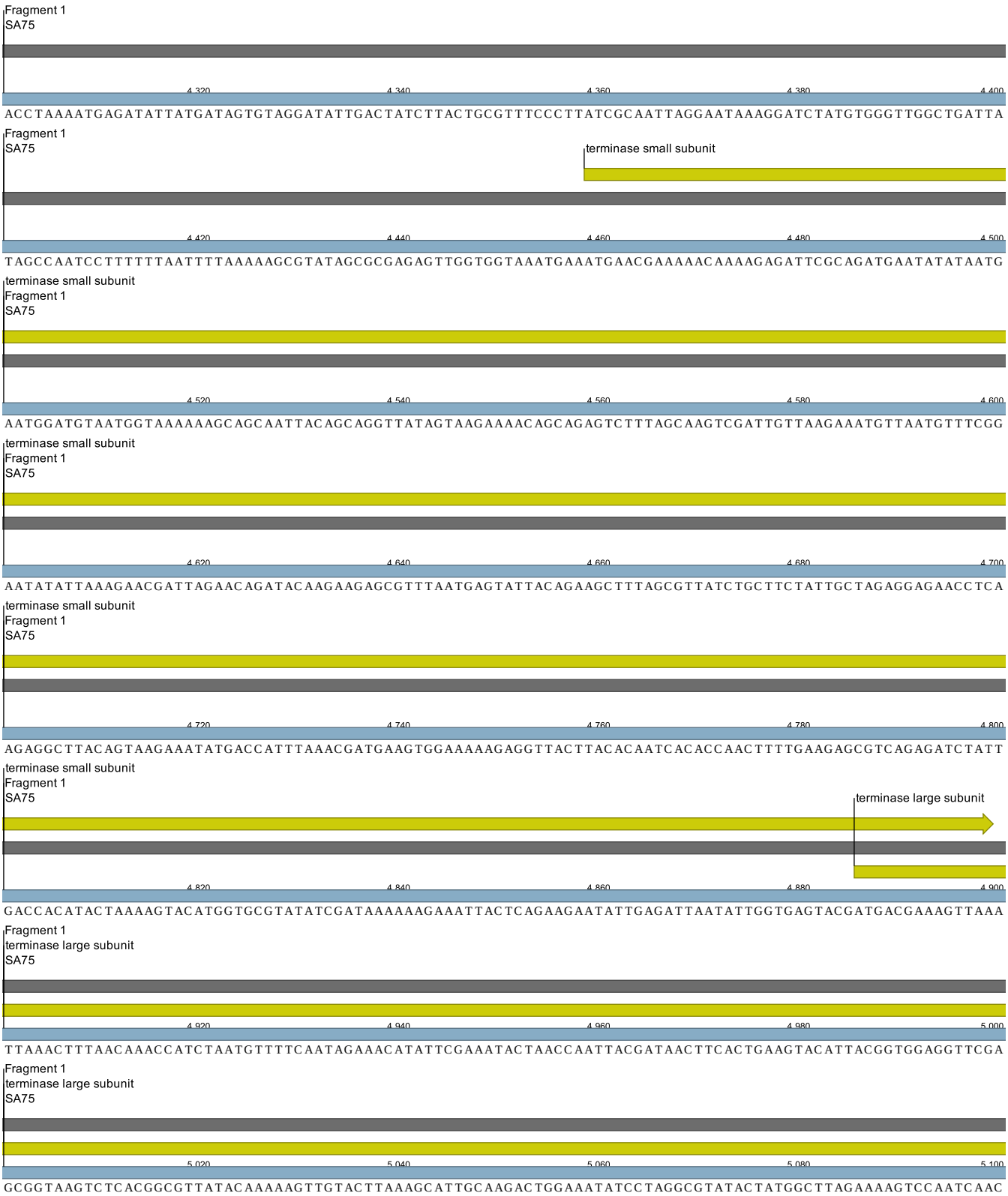
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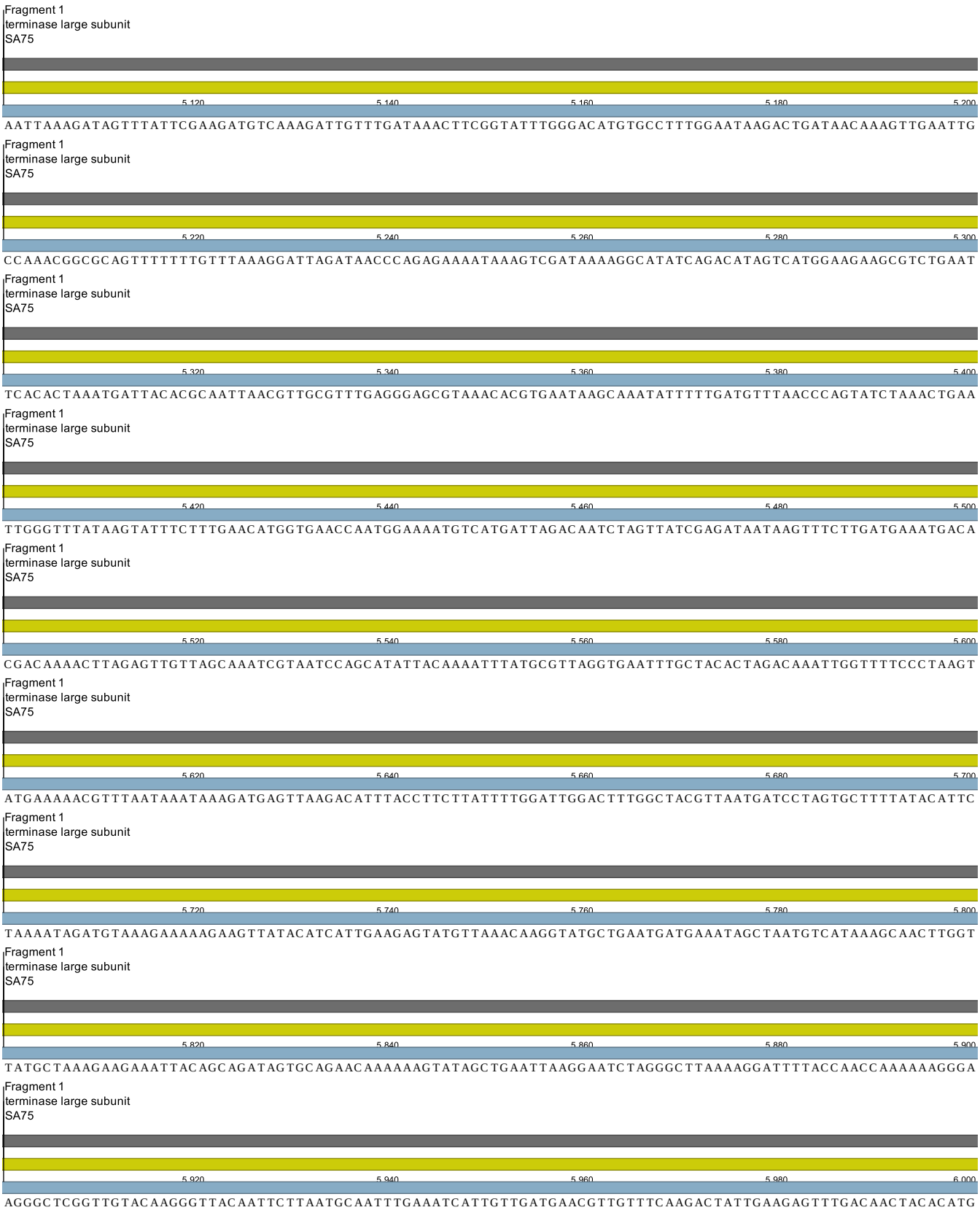
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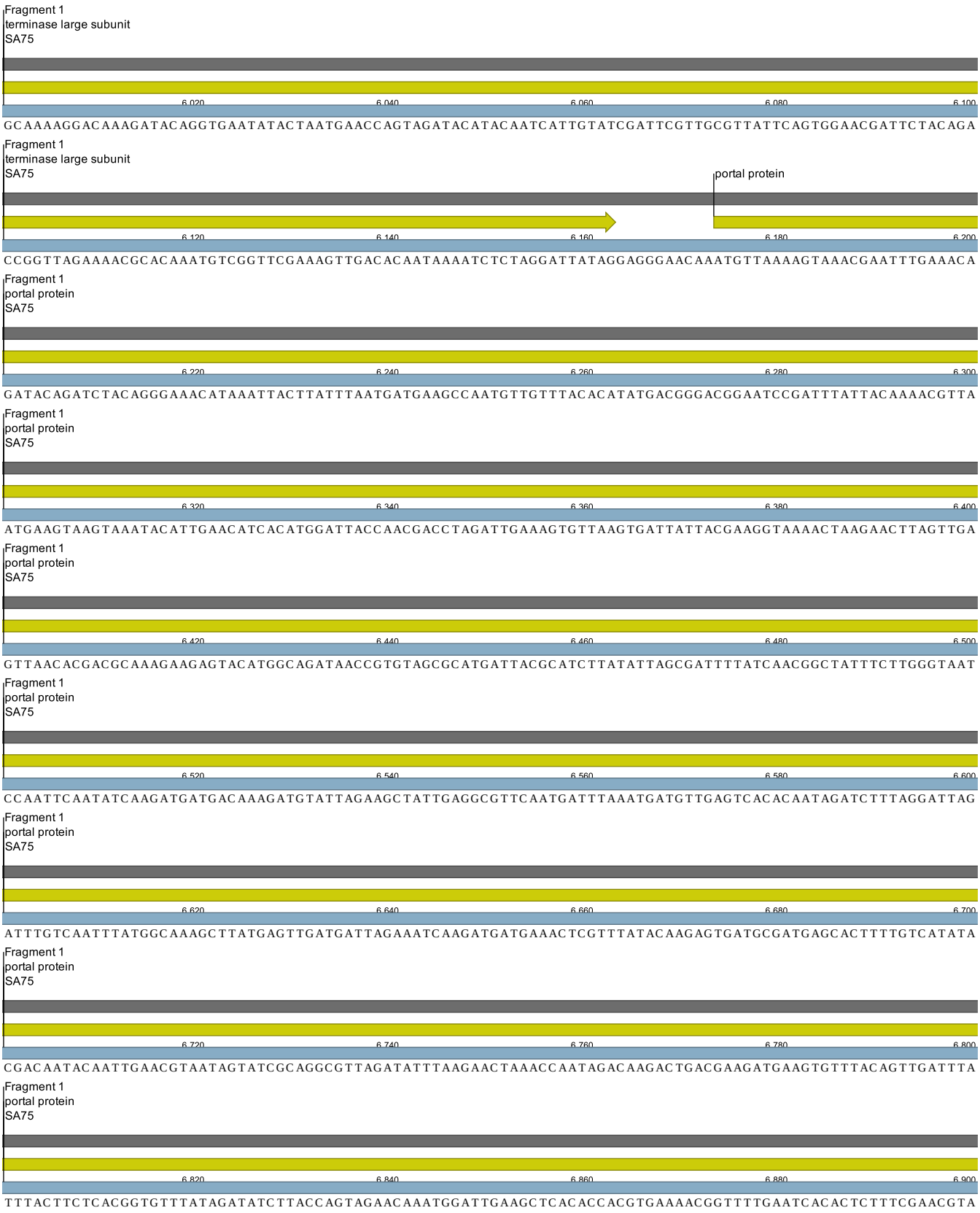
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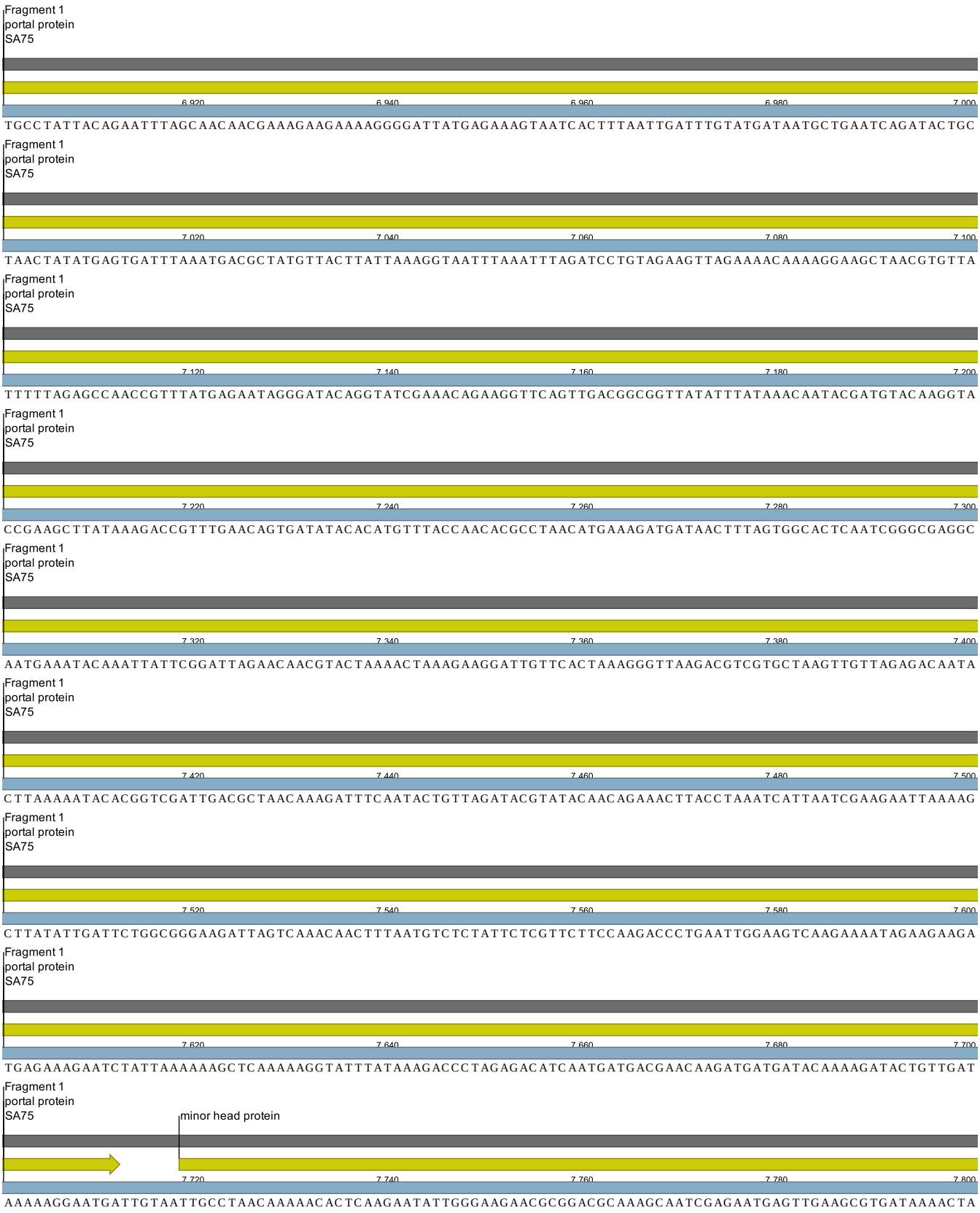
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SA75
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7,440
7,460
7,480
7,500
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Fragment 1
portal protein
SA75
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7,540
7,560
7,580
7,600
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Fragment 1
portal protein
SA75
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7,640
7,660
7,680
7,700
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portal protein
SA75
minor head protein
7,720
7,740
7,760
7,780
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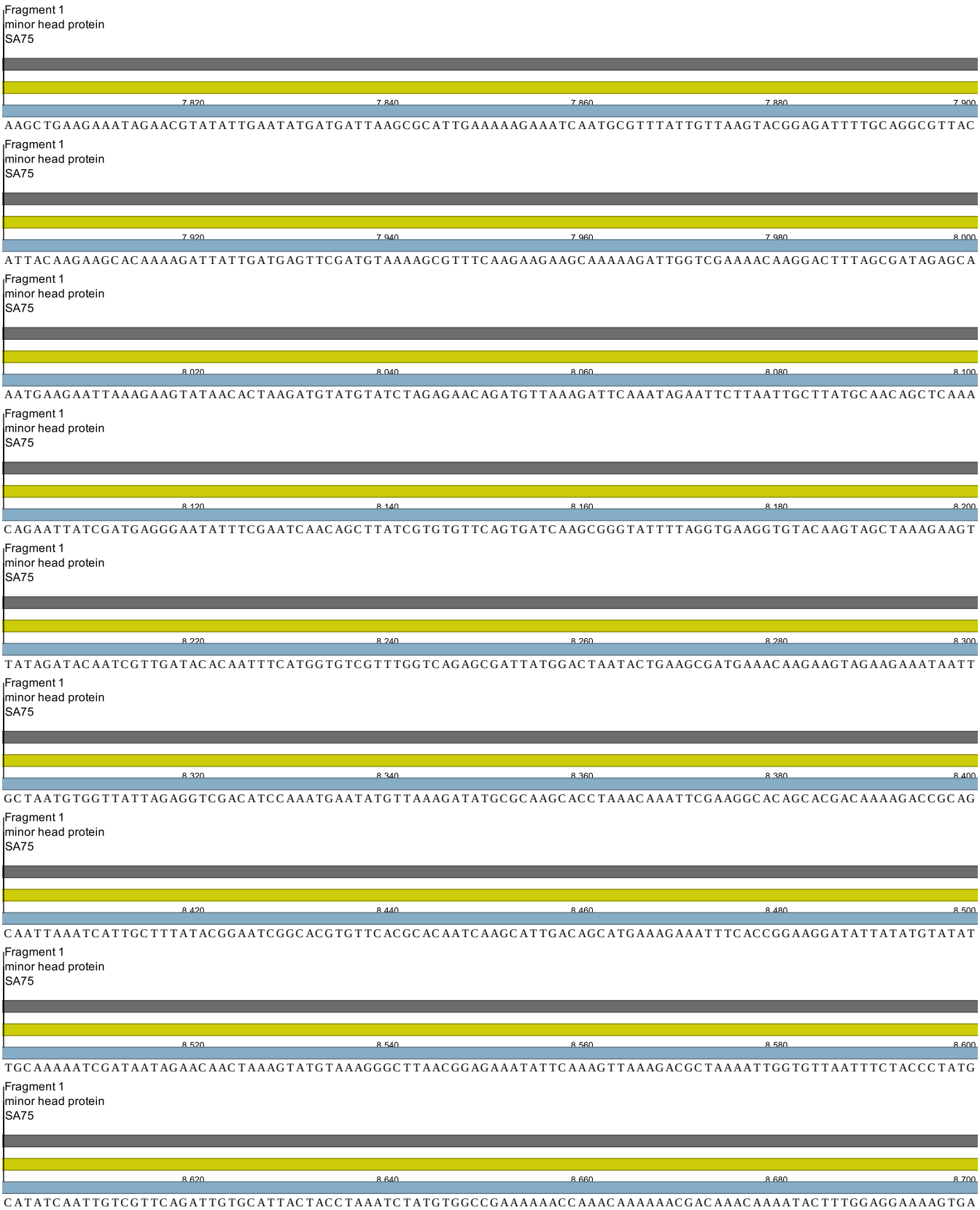
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SA75
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Fragment 1
minor head protein
SA75
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7,940
7,960
7,980
8,000
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minor head protein
SA75
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8,060
8,080
8,100
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minor head protein
SA75
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8,160
8,180
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minor head protein
SA75
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8,240
8,260
8,280
8,300
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Fragment 1
minor head protein
SA75
8,320
8,340
8,360
8,380
8,400
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Fragment 1
minor head protein
SA75
8,420
8,440
8,460
8,480
8,500
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minor head protein
SA75
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8,540
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8,600
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Fragment 1
minor head protein
SA75
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8,640
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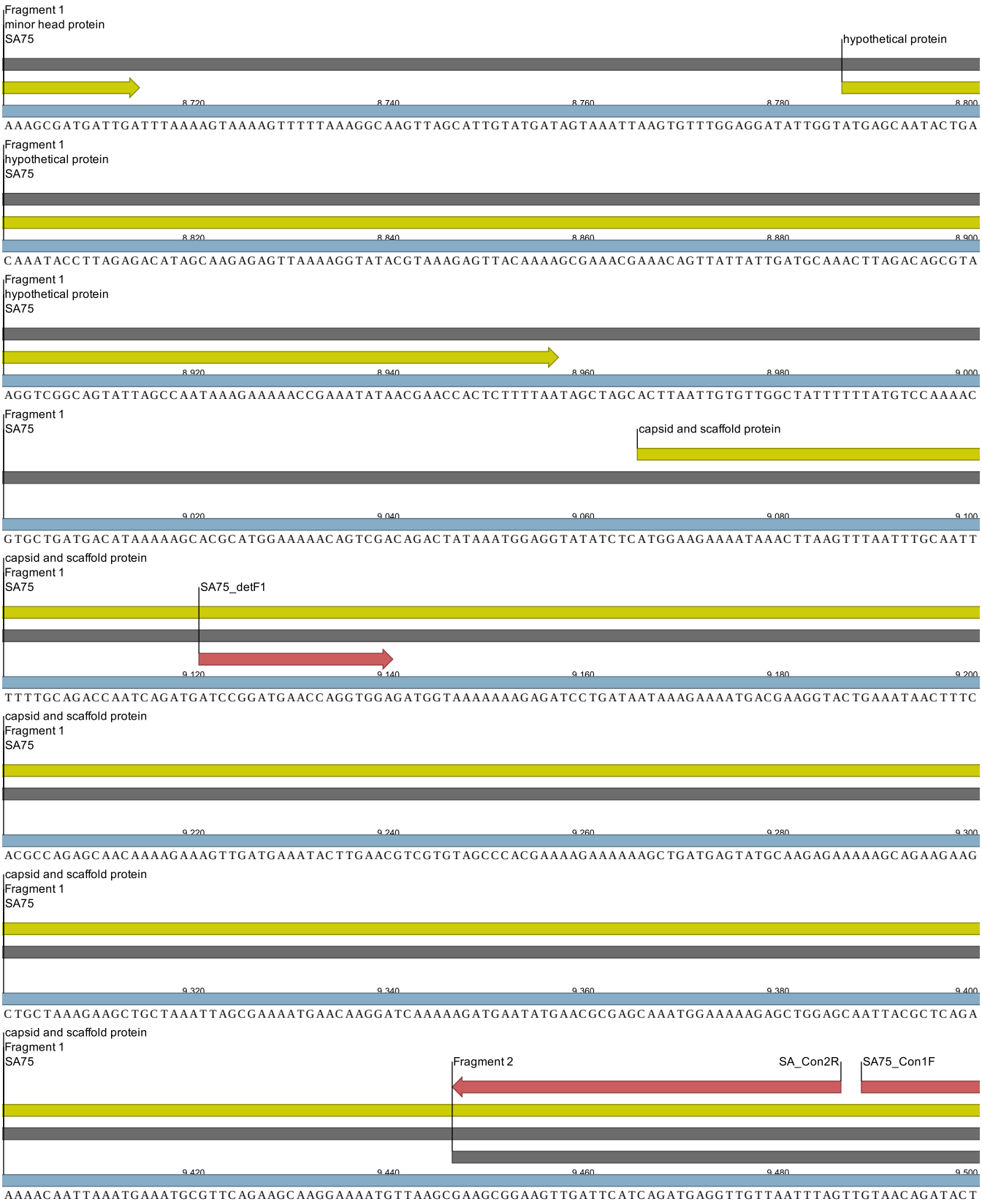
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Fragment 1
hypothetical protein
SA75
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8,860
8,880
8,900
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hypothetical protein
SA75
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capsid and scaffold protein
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capsid and scaffold protein
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SA75
Fragment 2
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SA75_Con1F
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SA75_Con1F
capsid and scaffold protein
Fragment 1
Fragment 2
SA75
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9,560
9,580
9,600
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capsid and scaffold protein
Fragment 2
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major capsid protein
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9,680
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major capsid protein
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9,740
9,760
9,780
9,800
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major capsid protein
Fragment 2
SA75
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9,840
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9,880
9,900
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major capsid protein
Fragment 2
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10,100
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major capsid protein
Fragment 2
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Fragment 2
SA75
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10,300
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major capsid protein
Fragment 2
SA75
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10,340
10,360
10,380
10,400
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major capsid protein
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10,500
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major capsid protein
Fragment 2
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10,600
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major capsid protein
Fragment 2
SA75
hypothetical protein
10,620
10,640
10,660
10,680
10,700
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Fragment 2
SA75
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10,780
10,800
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hypothetical protein
Fragment 2
SA75
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Fragment 2
SA75
hypothetical protein
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Fragment 2
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11,060
11,080
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Fragment 2
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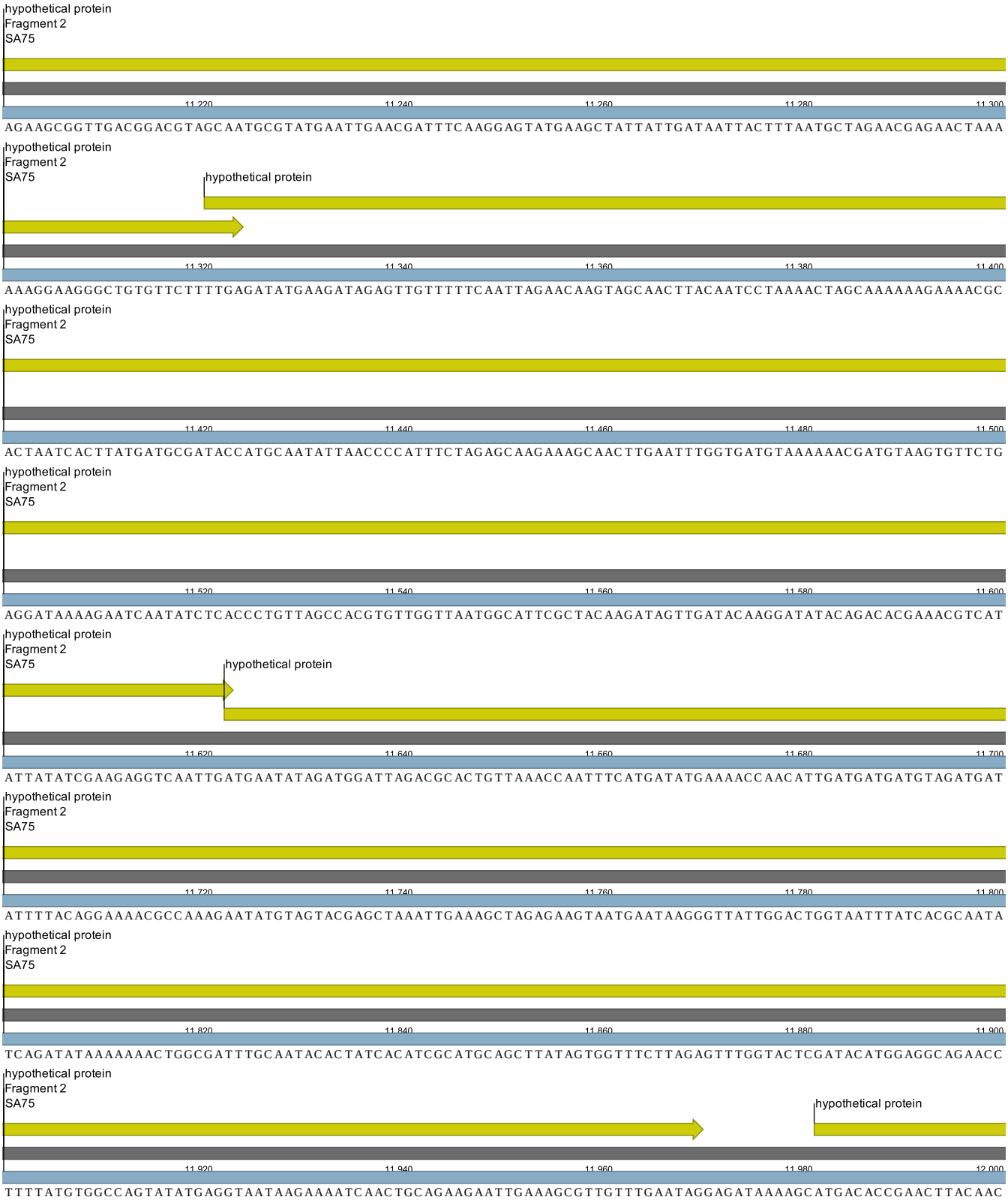
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11,280
11,300
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Fragment 2
SA75
hypothetical protein
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11,340
11,360
11,380
11,400
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Fragment 2
SA75
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11,480
11,500
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11,600
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hypothetical protein
Fragment 2
SA75
hypothetical protein
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11,640
11,660
11,680
11,700
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Fragment 2
SA75
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11,800
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Fragment 2
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Fragment 2
SA75
hypothetical protein
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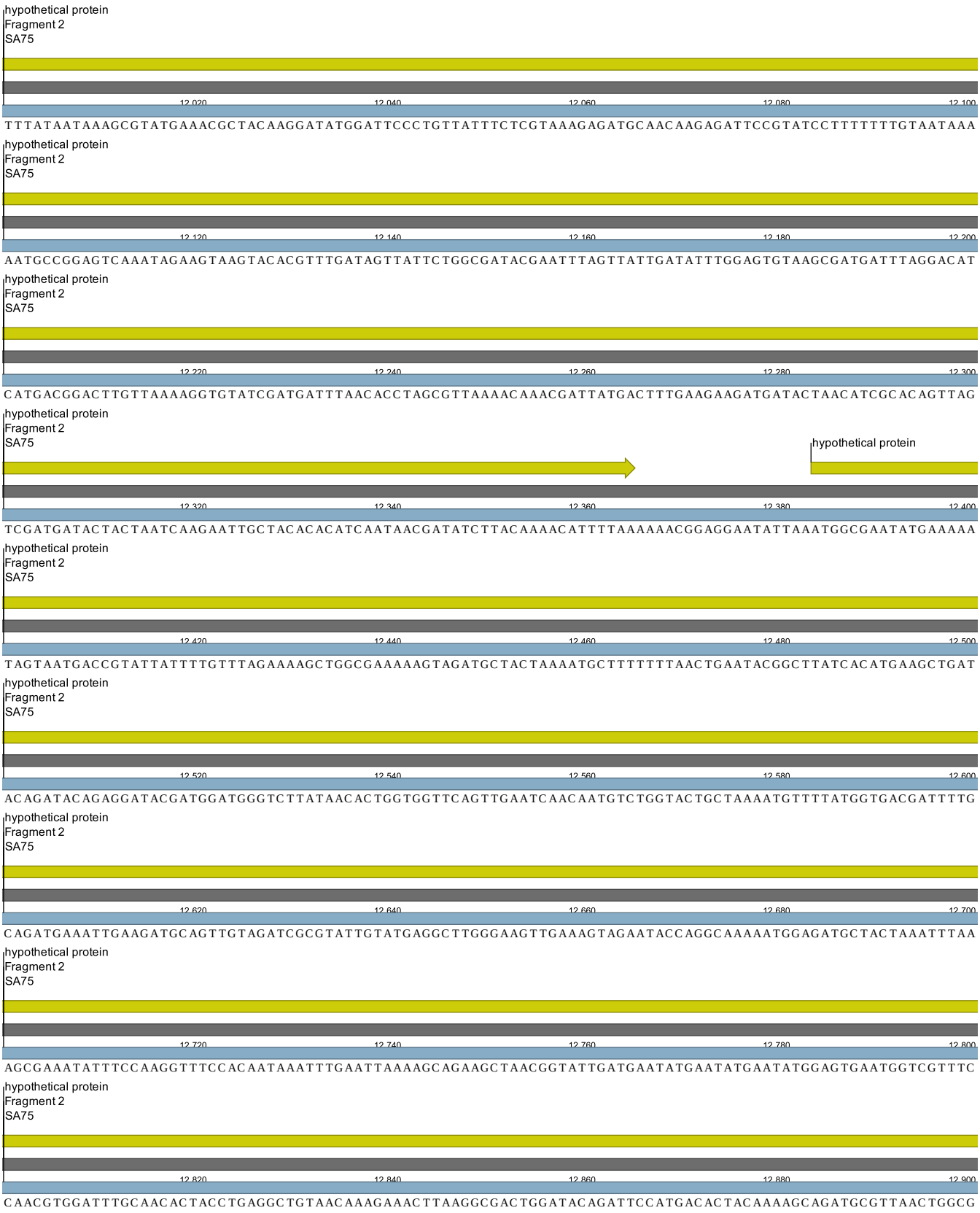
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Fragment 2
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12,280
12,300
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hypothetical protein
Fragment 2
SA75
hypothetical protein
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12,400
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Fragment 2
SA75
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12,460
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12,500
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Fragment 2
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SA75
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12,840
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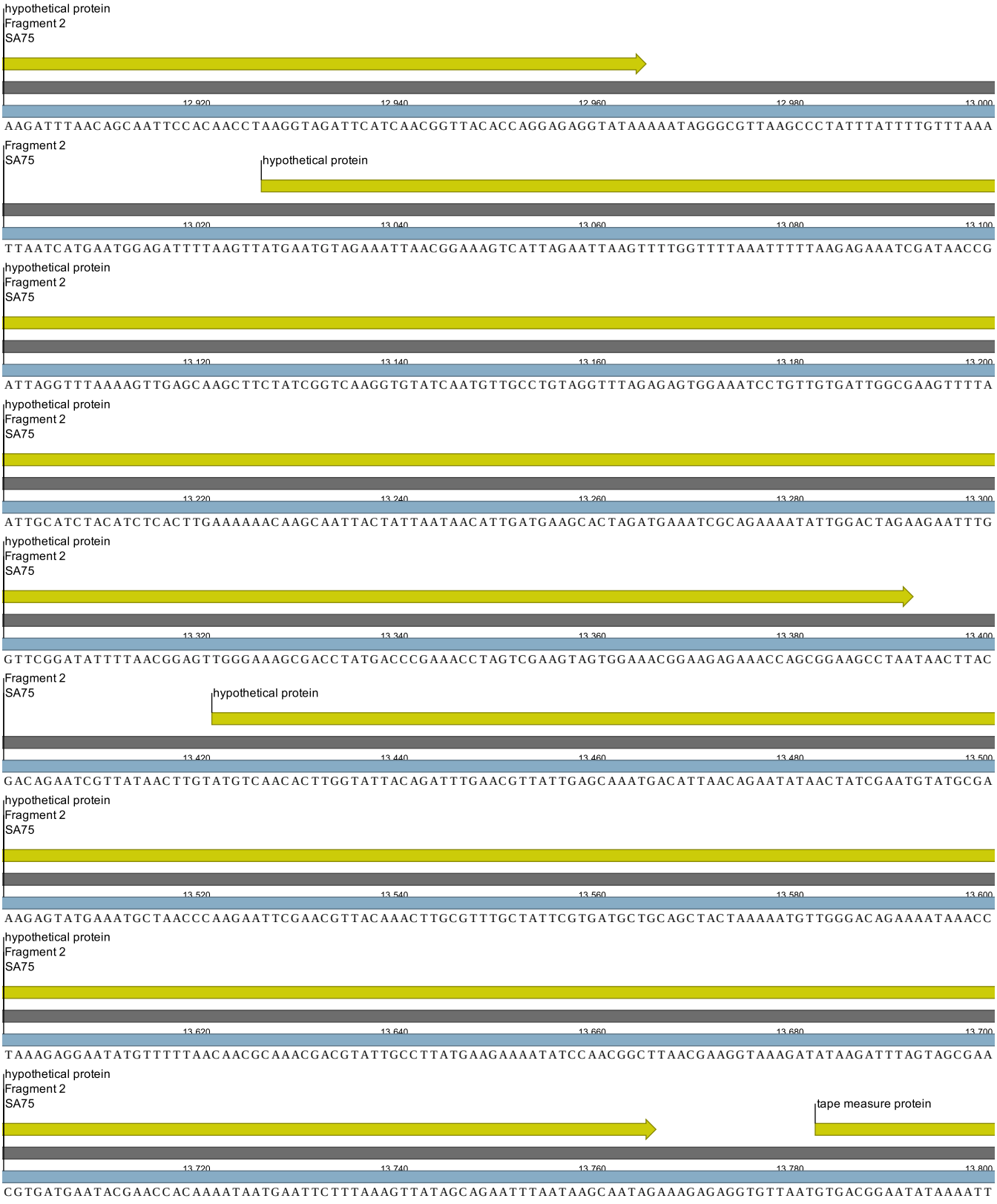
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Fragment 2
SA75
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13,360
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13,400
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Fragment 2
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hypothetical protein
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13,480
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13,600
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Fragment 2
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13,700
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Fragment 2
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tape measure protein
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13,760
13,780
13,800
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tape measure protein
Fragment 2
SA75

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Fragment 2
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tape measure protein
Fragment 2
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tape measure protein
Fragment 2
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tape measure protein
Fragment 2
SA75

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Fragment 2
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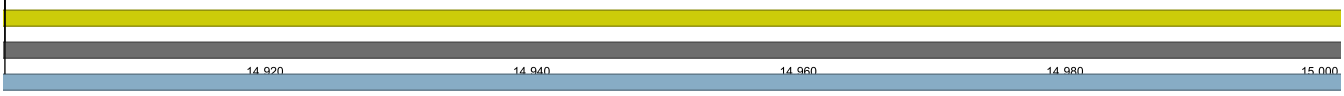

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tape measure protein
Fragment 2
SA75

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Fragment 2
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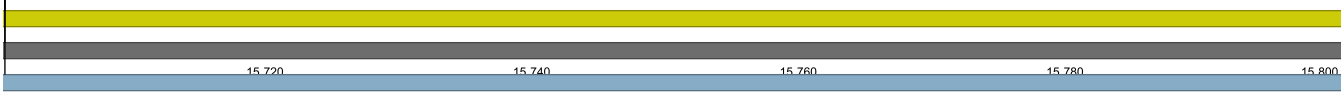

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Fragment 2
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Fragment 2
SA75

TTCACGACGATAATGACAATAGTTATGACGATATGGACAGCTATTTGGACATTCTTAAGTACAATCTGGAACACGATAATTACAATCGCTACTACGATTT

tape measure protein
Fragment 2
SA75

GGAATTTGTTAGTCACTGTAATAACTACAGTGTTTACCACAATTATGACTATCGCAATGACAATTTGGAACGCTATTTGGACGTTCTTACAAACGTTGTG

tape measure protein
Fragment 2
SA75

GAACACTATAGTTACTGTGGCAACTAAGGTTTGGAACGCTATCACTACAGCTATATCTACTGCGTTACAAGCGGCATGGAGTTTTATTTCTAATATATGG

tape measure protein
Fragment 2
SA75
16,520
16,540
16,560
16,580
16,600
AATACGATTTGGAGTTTCTTATCTGGTATATTAACGACAATTTGGAATAAAGTTGTAAGCATATTCACACAAGTTGTTTCAACTATATCAGACAAAATGT
tape measure protein
Fragment 2
SA75
16,620
16,640
16,660
16,680
16,700
CTCAAGCTTGGAACTTCATTGTCACTAAAGGTATGCAATGGGTATCTACTATAACAAGTACGCTAATTAACTTTGTTAATAGAGTTATTCAAGGATTCGT
tape measure protein
Fragment 2
SA75
16,720
16,740
16,760
16,780
16,800
TAATGTTGTAAACAAAGTTAGTCAAGGTATGACAAATGCAGTAAATAAAATAAAAAGCTTTATAGGAGATTTTGTGTCTGCAGGTGCTGATATGATCCGT
tape measure protein
Fragment 2
SA75
16,820
16,840
16,860
16,880
16,900
GGTTTAATTAGAGGTATTGGACAAATGGCTGGTCAATTAGTAGATGCAGCTAAAAATGTTGCTAAGAAAGCTTTAGATGCAGCTAAAAGTGCTTTGGGTA
tape measure protein
Fragment 2
SA75
16,920
16,940
16,960
16,980
17,000
TTCACTCACCTTCACGTGAATTCATGGATGTTGGTATGTATTCAATGCTAGGTTTCGTTAAAGGTATAGATAATCATTCAAGTAAAGTTATCCGTAATGT
tape measure protein
Fragment 2
SA75
17,020
17,040
17,060
17,080
17,100
TTCTAATGTTGCAGATAAAGTAGTTGATGCATTTCAACCTACATTAAACGCACCTGACATTTCTAGTATTACAGGAAACTTAAGTAATTTAGGTGGAAAT
tape measure protein
Fragment 2
SA75
17,120
17,140
17,160
17,180
17,200
ATAAATGCGCAAGTACAACACACACATTCTATTGAAACATCACCGAACATGAAAACTGTTAAAGTTGAATTCGATGTTAATAACGATGCGCTTACTAGTA
tape measure protein
Fragment 2
SA75
putative distal tail protein
17,220
17,240
17,260
17,280
17,300
TTGTTAACGGCAGAAATGCTAAACGCAATTCTGAGTATTACTTATAAAGGAGGTTACAAATGGACATAGAATTAACAAAAAAAGATGGTACTGTAATCAA
putative distal tail protein
Fragment 2
SA75
17,320
17,340
17,360
17,380
17,400
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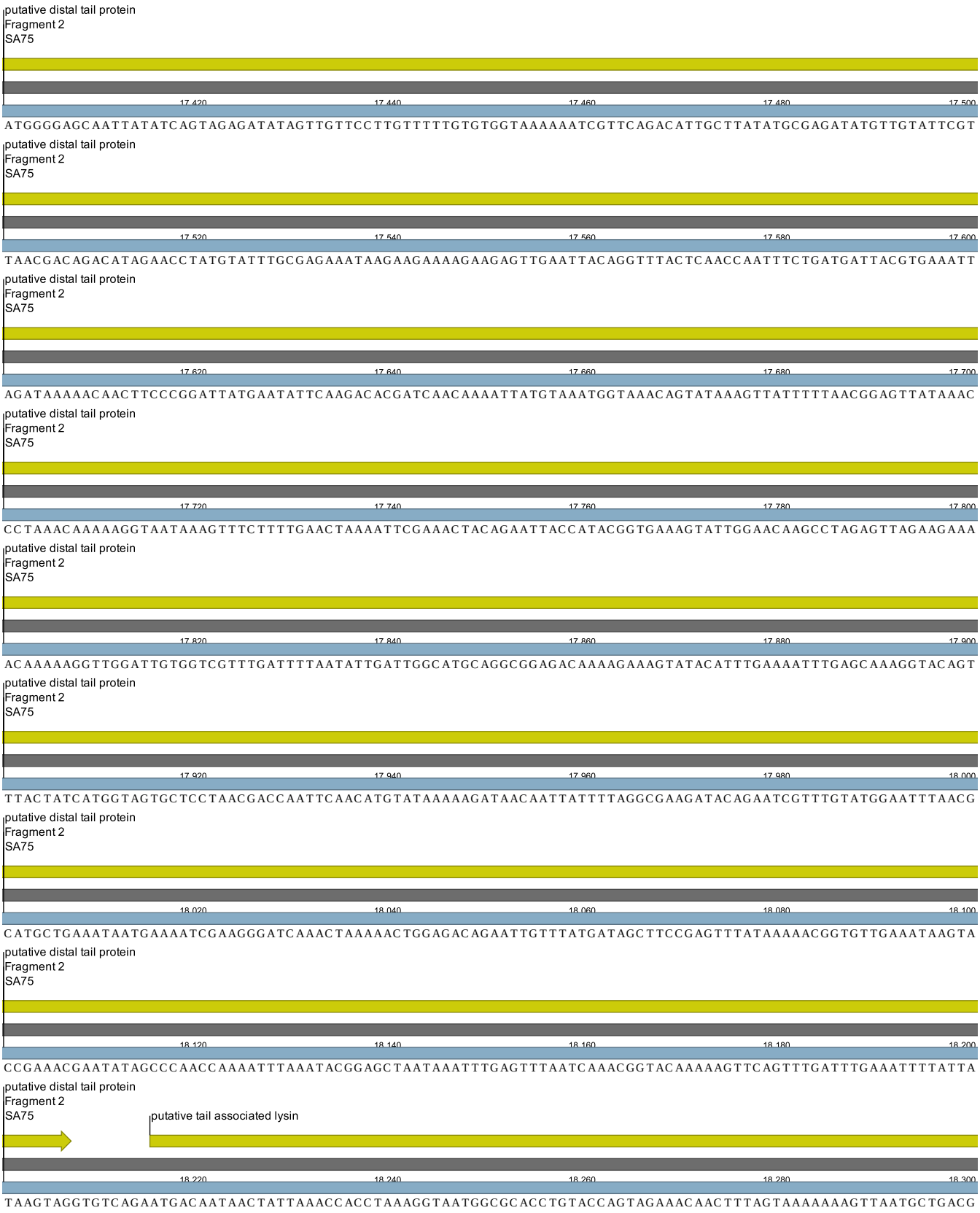
putative distal tail protein
Fragment 2
SA75
17,420
17,440
17,460
17,480
17,500
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putative distal tail protein
Fragment 2
SA75
17,520
17,540
17,560
17,580
17,600
TAACGACAGACATAGAACCTATGTATTTGCGAGAAATAAGAAGAAAAGAAGAGTTGAATTACAGGTTTACTCAACCAATTTCTGATGATTACGTGAAATT
putative distal tail protein
Fragment 2
SA75
17,620
17,640
17,660
17,680
17,700
AGATAAAAACAACTTCCCGGATTATGAATATTCAAGACACGATCAACAAAATTATGTAAATGGTAAACAGTATAAAGTTATTTTTAACGGAGTTATAAAC
putative distal tail protein
Fragment 2
SA75
17,720
17,740
17,760
17,780
17,800
CCTAAACAAAAAGGTAATAAAGTTTCTTTTGAACTAAAATTCGAAACTACAGAATTACCATACGGTGAAAGTATTGGAACAAGCCTAGAGTTAGAAGAAA
putative distal tail protein
Fragment 2
SA75
17,820
17,840
17,860
17,880
17,900
ACAAAAAGGTTGGATTGTGGTCGTTTGATTTTAATATTGATTGGCATGCAGGCGGAGACAAAAGAAAGTATACATTTGAAAATTTGAGCAAAGGTACAGT
putative distal tail protein
Fragment 2
SA75
17,920
17,940
17,960
17,980
18,000
TTACTATCATGGTAGTGCTCCTAACGACCAATTCAACATGTATAAAAAGATAACAATTATTTTAGGCGAAGATACAGAATCGTTTGTATGGAATTTAACG
putative distal tail protein
Fragment 2
SA75
18,020
18,040
18,060
18,080
18,100
CATGCTGAAATAATGAAAATCGAAGGGATCAAACTAAAAACTGGAGACAGAATTGTTTATGATAGCTTCCGAGTTTATAAAAACGGTGTTGAAATAAGTA
putative distal tail protein
Fragment 2
SA75
18,120
18,140
18,160
18,180
18,200
CCGAAACGAATATAGCCCAACCAAAATTTAAATACGGAGCTAATAAATTTGAGTTTAATCAAACGGTACAAAAAGTTCAGTTTGATTTGAAATTTTATTA
putative distal tail protein
Fragment 2
SA75
putative tail associated lysin
18,220
18,240
18,260
18,280
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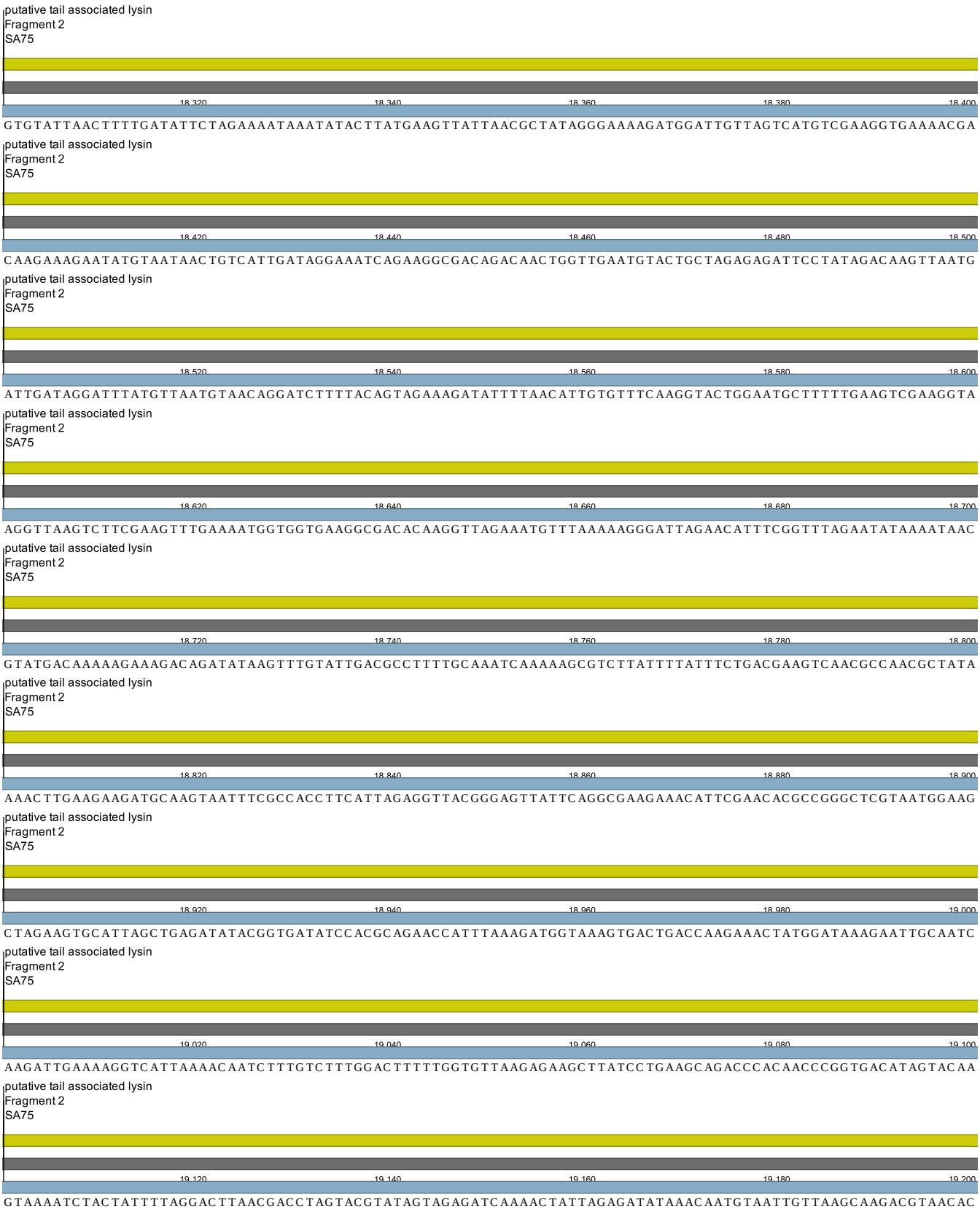
putative tail associated lysin
Fragment 2
SA75
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18,340
18,360
18,380
18,400
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putative tail associated lysin
Fragment 2
SA75
18,420
18,440
18,460
18,480
18,500
CAAGAAAGAATATGTAATAACTGTCATTGATAGGAAATCAGAAGGCGACAGACAACTGGTTGAATGTACTGCTAGAGAGATTCCTATAGACAAGTTAATG
putative tail associated lysin
Fragment 2
SA75
18,520
18,540
18,560
18,580
18,600
ATTGATAGGATTTATGTTAATGTAACAGGATCTTTTACAGTAGAAAGATATTTTAACATTGTGTTTCAAGGTACTGGAATGCTTTTTGAAGTCGAAGGTA
putative tail associated lysin
Fragment 2
SA75
18,620
18,640
18,660
18,680
18,700
AGGTTAAGTCTTCGAAGTTTGAAAATGGTGGTGAAGGCGACACAAGGTTAGAAATGTTTAAAAAGGGATTAGAACATTTCGGTTTAGAATATAAAATAAC
putative tail associated lysin
Fragment 2
SA75
18,720
18,740
18,760
18,780
18,800
GTATGACAAAAAGAAAGACAGATATAAGTTTGTATTGACGCCTTTTGCAAATCAAAAAGCGTCTTATTTTATTTCTGACGAAGTCAACGCCAACGCTATA
putative tail associated lysin
Fragment 2
SA75
18,820
18,840
18,860
18,880
18,900
AAACTTGAAGAAGATGCAAGTAATTTCGCCACCTTCATTAGAGGTTACGGGAGTTATTCAGGCGAAGAAACATTCGAACACGCCGGGCTCGTAATGGAAG
putative tail associated lysin
Fragment 2
SA75
18,920
18,940
18,960
18,980
19,000
CTAGAAGTGCATTAGCTGAGATATACGGTGATATCCACGCAGAACCATTTAAAGATGGTAAAGTGACTGACCAAGAAACTATGGATAAAGAATTGCAATC
putative tail associated lysin
Fragment 2
SA75
19,020
19,040
19,060
19,080
19,100
AAGATTGAAAAGGTCATTAAAACAATCTTTGTCTTTGGACTTTTTGGTGTTAAGAGAAGCTTATCCTGAAGCAGACCCACAACCCGGTGACATAGTACAA
putative tail associated lysin
Fragment 2
SA75
19,120
19,140
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19,180
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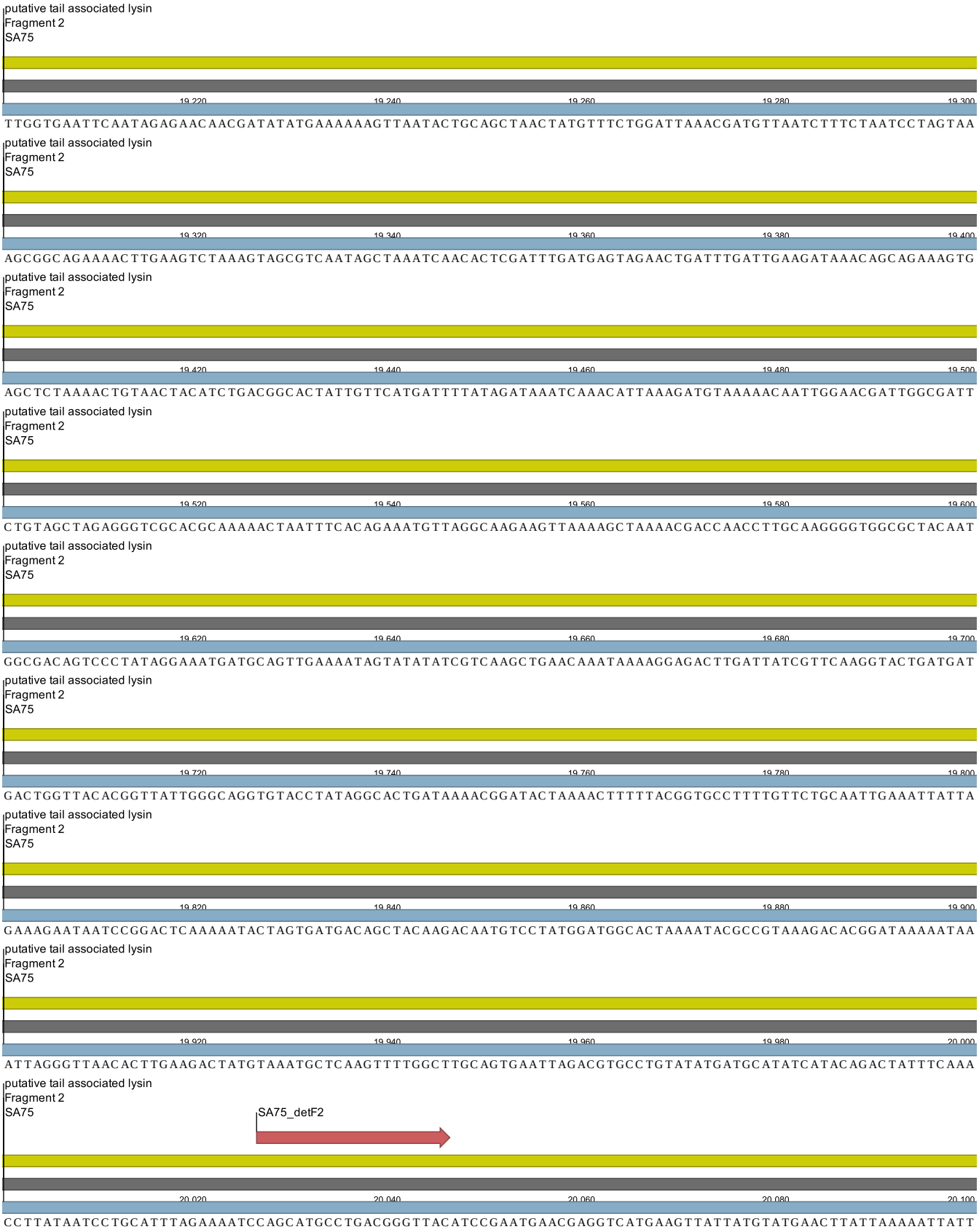
putative tail associated lysin
Fragment 2
SA75
19,220
19,240
19,260
19,280
19,300
TTGGTGAATTCAATAGAGAACAACGATATATGAAAAAAGTTAATACTGCAGCTAACTATGTTTCTGGATTAAACGATGTTAATCTTTCTAATCCTAGTAA
putative tail associated lysin
Fragment 2
SA75
19,320
19,340
19,360
19,380
19,400
AGCGGCAGAAAACTTGAAGTCTAAAGTAGCGTCAATAGCTAAATCAACACTCGATTTGATGAGTAGAACTGATTTGATTGAAGATAAACAGCAGAAAGTG
putative tail associated lysin
Fragment 2
SA75
19,420
19,440
19,460
19,480
19,500
AGCTCTAAAACTGTAACTACATCTGACGGCACTATTGTTCATGATTTTATAGATAAATCAAACATTAAAGATGTAAAAACAATTGGAACGATTGGCGATT
putative tail associated lysin
Fragment 2
SA75
19,520
19,540
19,560
19,580
19,600
CTGTAGCTAGAGGGTCGCACGCAAAAACTAATTTCACAGAAATGTTAGGCAAGAAGTTAAAAGCTAAAACGACCAACCTTGCAAGGGGTGGCGCTACAAT
putative tail associated lysin
Fragment 2
SA75
19,620
19,640
19,660
19,680
19,700
GGCGACAGTCCCTATAGGAAATGATGCAGTTGAAAATAGTATATATCGTCAAGCTGAACAAATAAAAGGAGACTTGATTATCGTTCAAGGTACTGATGAT
putative tail associated lysin
Fragment 2
SA75
19,720
19,740
19,760
19,780
19,800
GACTGGTTACACGGTTATTGGGCAGGTGTACCTATAGGCACTGATAAAACGGATACTAAAACTTTTTACGGTGCCTTTTGTTCTGCAATTGAAATTATTA
putative tail associated lysin
Fragment 2
SA75
19,820
19,840
19,860
19,880
19,900
GAAAGAATAATCCGGACTCAAAAATACTAGTGATGACAGCTACAAGACAATGTCCTATGGATGGCACTAAAATACGCCGTAAAGACACGGATAAAAATAA
putative tail associated lysin
Fragment 2
SA75
19,920
19,940
19,960
19,980
20,000
ATTAGGGTTAACACTTGAAGACTATGTAAATGCTCAAGTTTTGGCTTGCAGTGAATTAGACGTGCCTGTATATGATGCATATCATACAGACTATTTCAAA
putative tail associated lysin
Fragment 2
SA75
SA75_detF2
20,020
20,040
20,060
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20,100
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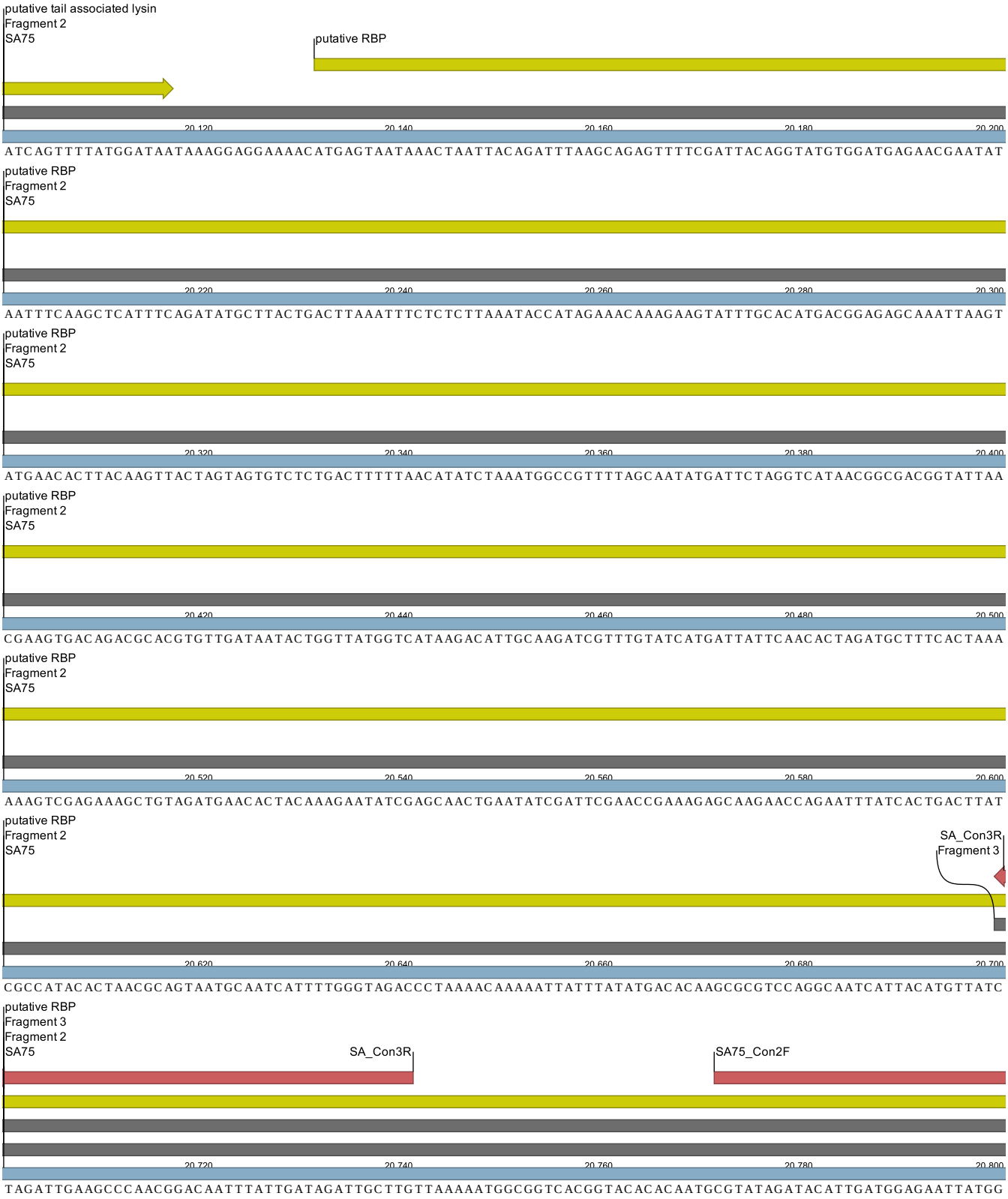
putative tail associated lysin
Fragment 2
SA75
putative RBP
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20,140
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20,180
20,200
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putative RBP
Fragment 2
SA75
20,220
20,240
20,260
20,280
20,300
AATTTCAAGCTCATTTCAGATATGCTTACTGACTTAAATTTCTCTCTTAAATACCATAGAAACAAAGAAGTATTTGCACATGACGGAGAGCAAATTAAGT
putative RBP
Fragment 2
SA75
20,320
20,340
20,360
20,380
20,400
ATGAACACTTACAAGTTACTAGTAGTGTCTCTGACTTTTTAACATATCTAAATGGCCGTTTTAGCAATATGATTCTAGGTCATAACGGCGACGGTATTAA
putative RBP
Fragment 2
SA75
20,420
20,440
20,460
20,480
20,500
CGAAGTGACAGACGCACGTGTTGATAATACTGGTTATGGTCATAAGACATTGCAAGATCGTTTGTATCATGATTATTCAACACTAGATGCTTTCACTAAA
putative RBP
Fragment 2
SA75
20,520
20,540
20,560
20,580
20,600
AAAGTCGAGAAAGCTGTAGATGAACACTACAAAGAATATCGAGCAACTGAATATCGATTCGAACCGAAAGAGCAAGAACCAGAATTTATCACTGACTTAT
putative RBP
Fragment 2
SA75
SA_Con3R
Fragment 3
20,620
20,640
20,660
20,680
20,700
CGCCATACACTAACGCAGTAATGCAATCATTTTGGGTAGACCCTAAAACAAAAATTATTTATATGACACAAGCGCGTCCAGGCAATCATTACATGTTATC
putative RBP
Fragment 3
Fragment 2
SA75
SA_Con3R
SA75_Con2F
20,720
20,740
20,760
20,780
20,800
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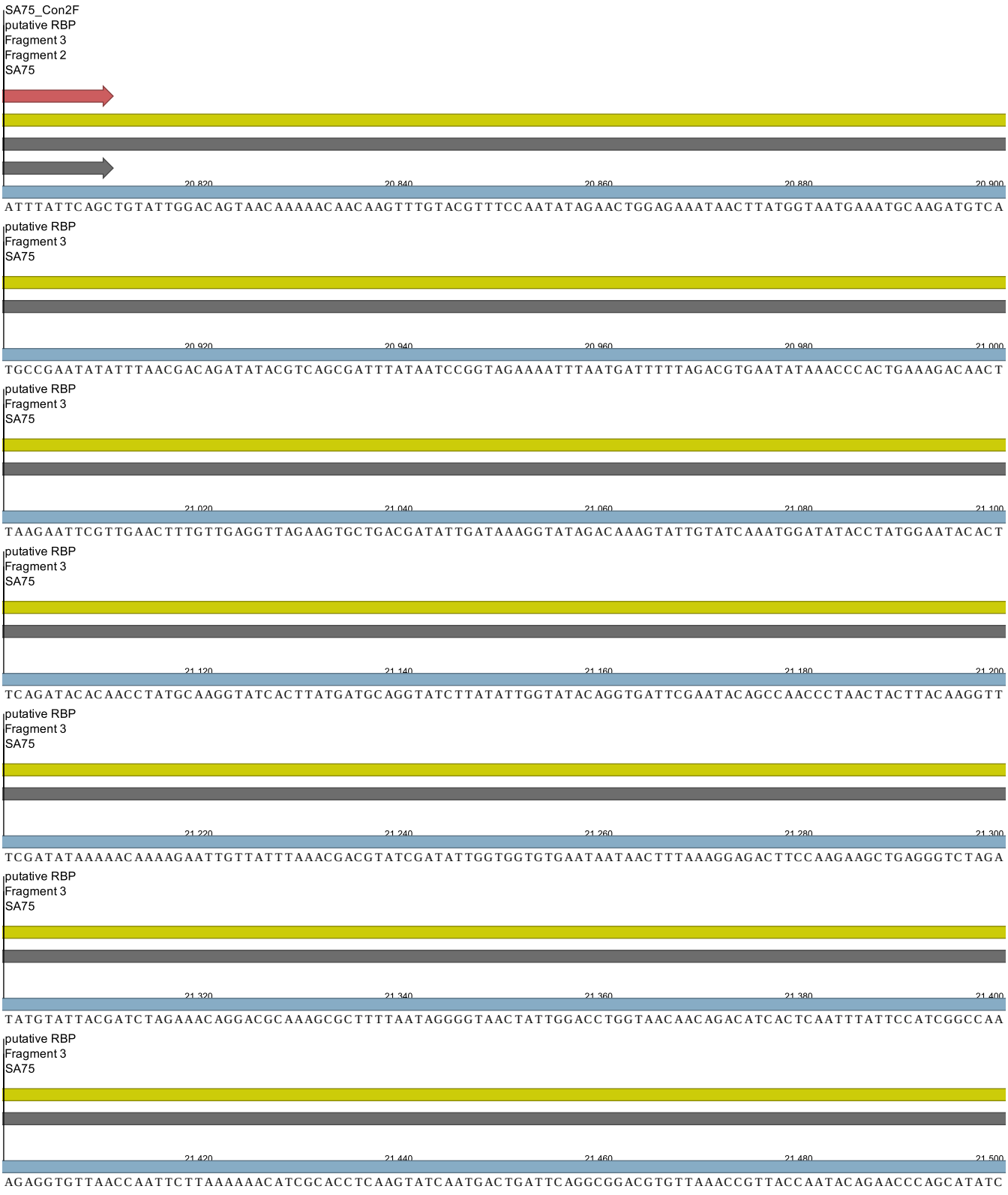
SA75_Con2F
putative RBP
Fragment 3
Fragment 2
SA75
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20,840
20,860
20,880
20,900
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putative RBP
Fragment 3
SA75
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20,960
20,980
21,000
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putative RBP
Fragment 3
SA75
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21,060
21,080
21,100
TAAGAATTCGTTGAACTTTGTTGAGGTTAGAAGTGCTGACGATATTGATAAAGGTATAGACAAAGTATTGTATCAAATGGATATACCTATGGAATACACT
putative RBP
Fragment 3
SA75
21,120
21,140
21,160
21,180
21,200
TCAGATACACAACCTATGCAAGGTATCACTTATGATGCAGGTATCTTATATTGGTATACAGGTGATTCGAATACAGCCAACCCTAACTACTTACAAGGTT
putative RBP
Fragment 3
SA75
21,220
21,240
21,260
21,280
21,300
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putative RBP
Fragment 3
SA75
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21,360
21,380
21,400
TATGTATTACGATCTAGAAACAGGACGCAAAGCGCTTTTAATAGGGGTAACTATTGGACCTGGTAACAACAGACATCACTCAATTTATTCCATCGGCCAA
putative RBP
Fragment 3
SA75
21,420
21,440
21,460
21,480
21,500
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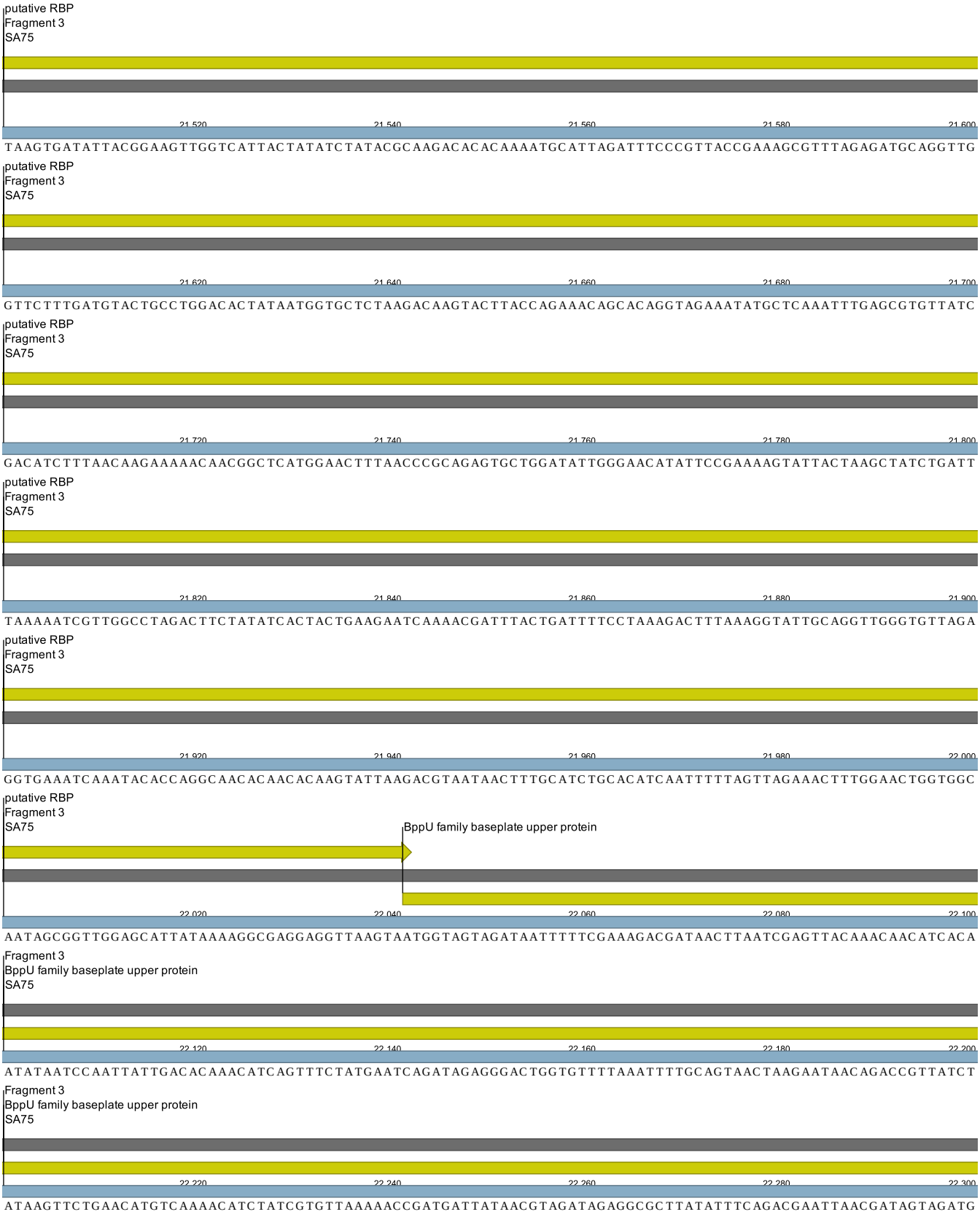
putative RBP
Fragment 3
SA75
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21,600
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Fragment 3
SA75
21,620
21,640
21,660
21,680
21,700
GTTCTTTGATGTACTGCCTGGACACTATAATGGTGCTCTAAGACAAGTACTTACCAGAAACAGCACAGGTAGAAATATGCTCAAATTTGAGCGTGTTATC
putative RBP
Fragment 3
SA75
21,720
21,740
21,760
21,780
21,800
GACATCTTTAACAAGAAAAACAACGGCTCATGGAACTTTAACCCGCAGAGTGCTGGATATTGGGAACATATTCCGAAAAGTATTACTAAGCTATCTGATT
putative RBP
Fragment 3
SA75
21,820
21,840
21,860
21,880
21,900
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putative RBP
Fragment 3
SA75
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21,960
21,980
22,000
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putative RBP
Fragment 3
SA75
BppU family baseplate upper protein
22,020
22,040
22,060
22,080
22,100
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Fragment 3
BppU family baseplate upper protein
SA75
22,120
22,140
22,160
22,180
22,200
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Fragment 3
BppU family baseplate upper protein
SA75
22,220
22,240
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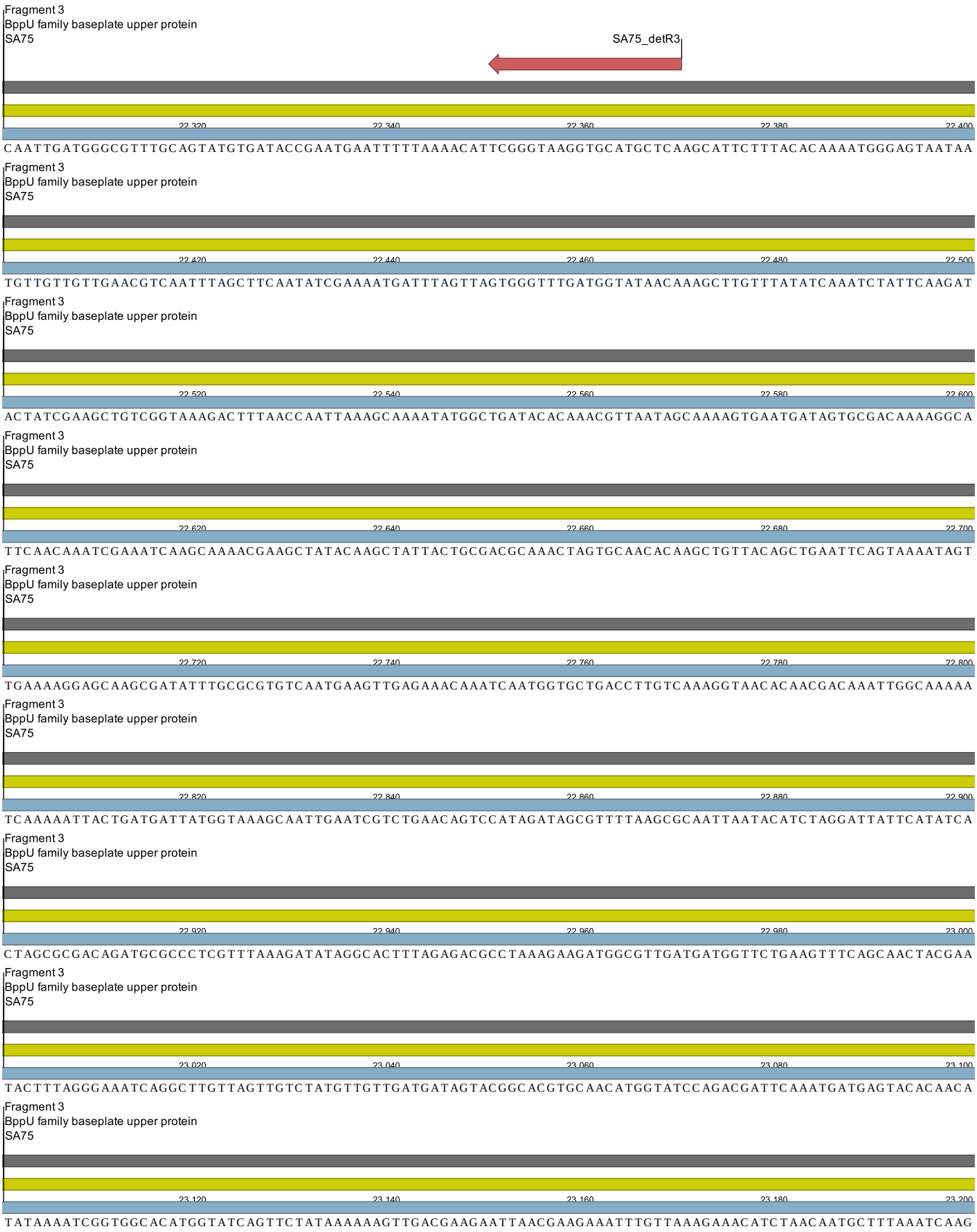
Fragment 3
BppU family baseplate upper protein
SA75
SA75_detR3
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Fragment 3
BppU family baseplate upper protein
SA75
22,420
22,440
22,460
22,480
22,500
TGTTGTTGTTGAACGTCAATTTAGCTTCAATATCGAAAATGATTTAGTTAGTGGGTTTGATGGTATAACAAAGCTTGTTTATATCAAATCTATTCAAGAT
Fragment 3
BppU family baseplate upper protein
SA75
22,520
22,540
22,560
22,580
22,600
ACTATCGAAGCTGTCGGTAAAGACTTTAACCAATTAAAGCAAAATATGGCTGATACACAAACGTTAATAGCAAAAGTGAATGATAGTGCGACAAAAGGCA
Fragment 3
BppU family baseplate upper protein
SA75
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22,640
22,660
22,680
22,700
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Fragment 3
BppU family baseplate upper protein
SA75
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22,740
22,760
22,780
22,800
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Fragment 3
BppU family baseplate upper protein
SA75
22,820
22,840
22,860
22,880
22,900
TCAAAAATTACTGATGATTATGGTAAAGCAATTGAATCGTCTGAACAGTCCATAGATAGCGTTTTAAGCGCAATTAATACATCTAGGATTATTCATATCA
Fragment 3
BppU family baseplate upper protein
SA75
22,920
22,940
22,960
22,980
23,000
CTAGCGCGACAGATGCGCCCTCGTTTAAAGATATAGGCACTTTAGAGACGCCTAAAGAAGATGGCGTTGATGATGGTTCTGAAGTTTCAGCAACTACGAA
Fragment 3
BppU family baseplate upper protein
SA75
23,020
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23,080
23,100
TACTTTAGGGAAATCAGGCTTGTTAGTTGTCTATGTTGTTGATGATAGTACGGCACGTGCAACATGGTATCCAGACGATTCAAATGATGAGTACACAACA
Fragment 3
BppU family baseplate upper protein
SA75
23,120
23,140
23,160
23,180
23,200
TATAAAATCGGTGGCACATGGTATCAGTTCTATAAAAAAGTTGACGAAGAATTAACGAAGAAATTTGTTAAAGAAACATCTAACAATGCTTTAAATCAAG

Fragment 3
BppU family baseplate upper protein
SA75
23,220
23,240
23,260
23,280
23,300
CTAAGCAGTATGTAGATGATAAATTCGGAACAACGAGTTGGCAACAACATAAGATGACAGAGGCGAACGGTCAATCAATACAAGTTAACTTAAACAATGC
Fragment 3
BppU family baseplate upper protein
SA75
23,320
23,340
23,360
23,380
23,400
GCAAGGCGATTTAGGCTATTTAACTGCTGGTAATTACTATGCAACAAGAGTGCCGGATTTACCAGGTAGTGTTGAAAGTTATGAGGGTTATTTATCTGTA
Fragment 3
BppU family baseplate upper protein
SA75
23,420
23,440
23,460
23,480
23,500
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Fragment 3
BppU family baseplate upper protein
SA75
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23,540
23,560
23,580
23,600
GGACTGTACCAAATGAGTATAAAAAAGCGGTTTTATTTGATGGCGCGGCTAACGGAGTTGGTACAACACTTAACTTAACTGAATCATATCAAAACTATTC
Fragment 3
BppU family baseplate upper protein
SA75
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23,660
23,680
23,700
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Fragment 3
BppU family baseplate upper protein
SA75
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23,740
23,760
23,780
23,800
GATAGTGATGGCAACGGTGGTGGCTTATATGAATGTTCTGTTTCTAAAACTAGCAATACTACATTCAGAATCGACGTTGATATCCTATATGACATCGGTA
Fragment 3
BppU family baseplate upper protein
SA75
hypothetical protein
23,820
23,840
23,860
23,880
23,900
AAAGTGCGGGTTCTGGTGCAAATGCAAACAAAATTACTATTAAACGTATTGAGGGGTGGAAGTAATGAAAATCACAGTAAACGATAAAAACGAAGTTATC
hypothetical protein
Fragment 3
SA75
23,920
23,940
23,960
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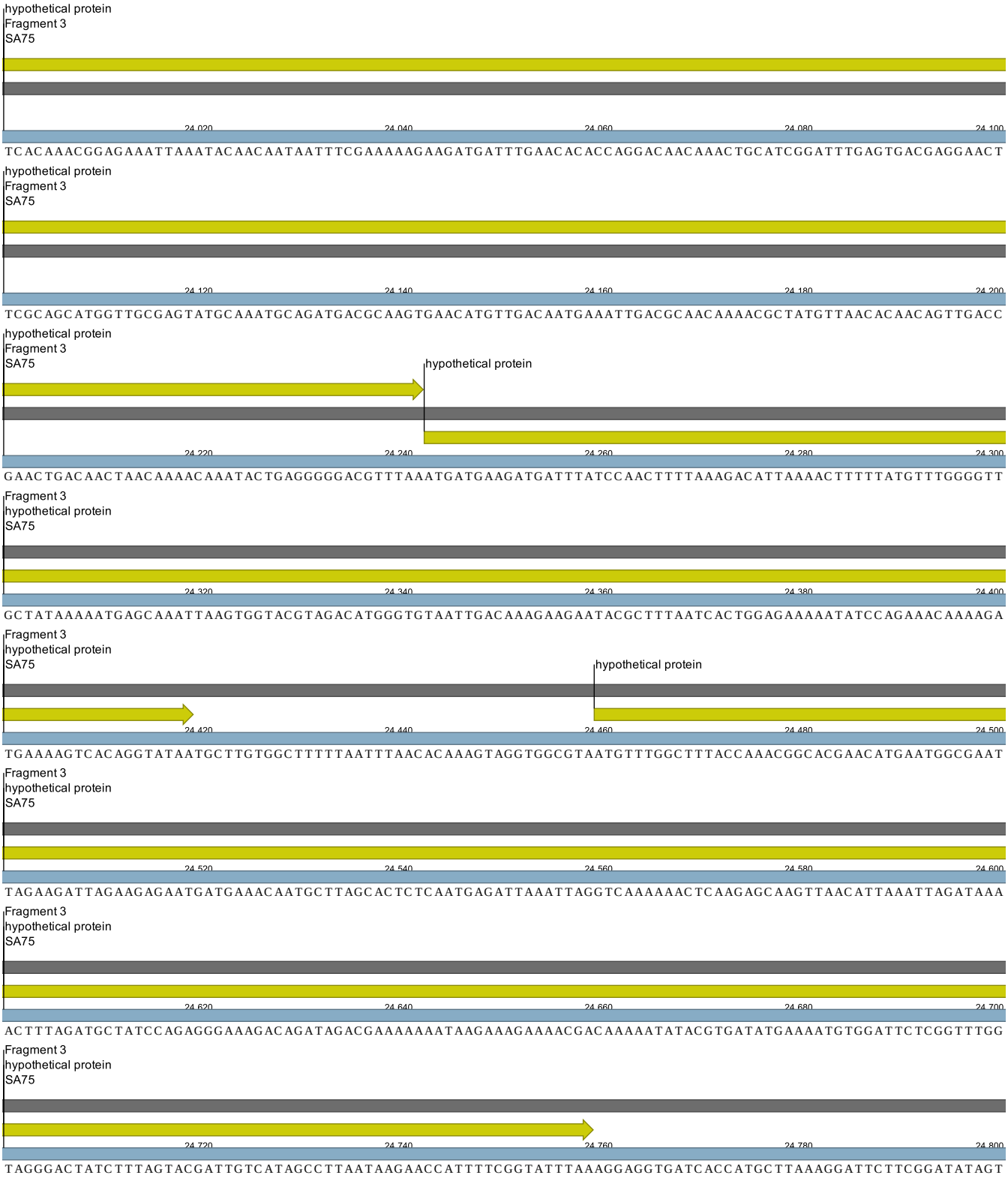
hypothetical protein
Fragment 3
SA75
24,020
24,040
24,060
24,080
24,100
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hypothetical protein
Fragment 3
SA75
24,120
24,140
24,160
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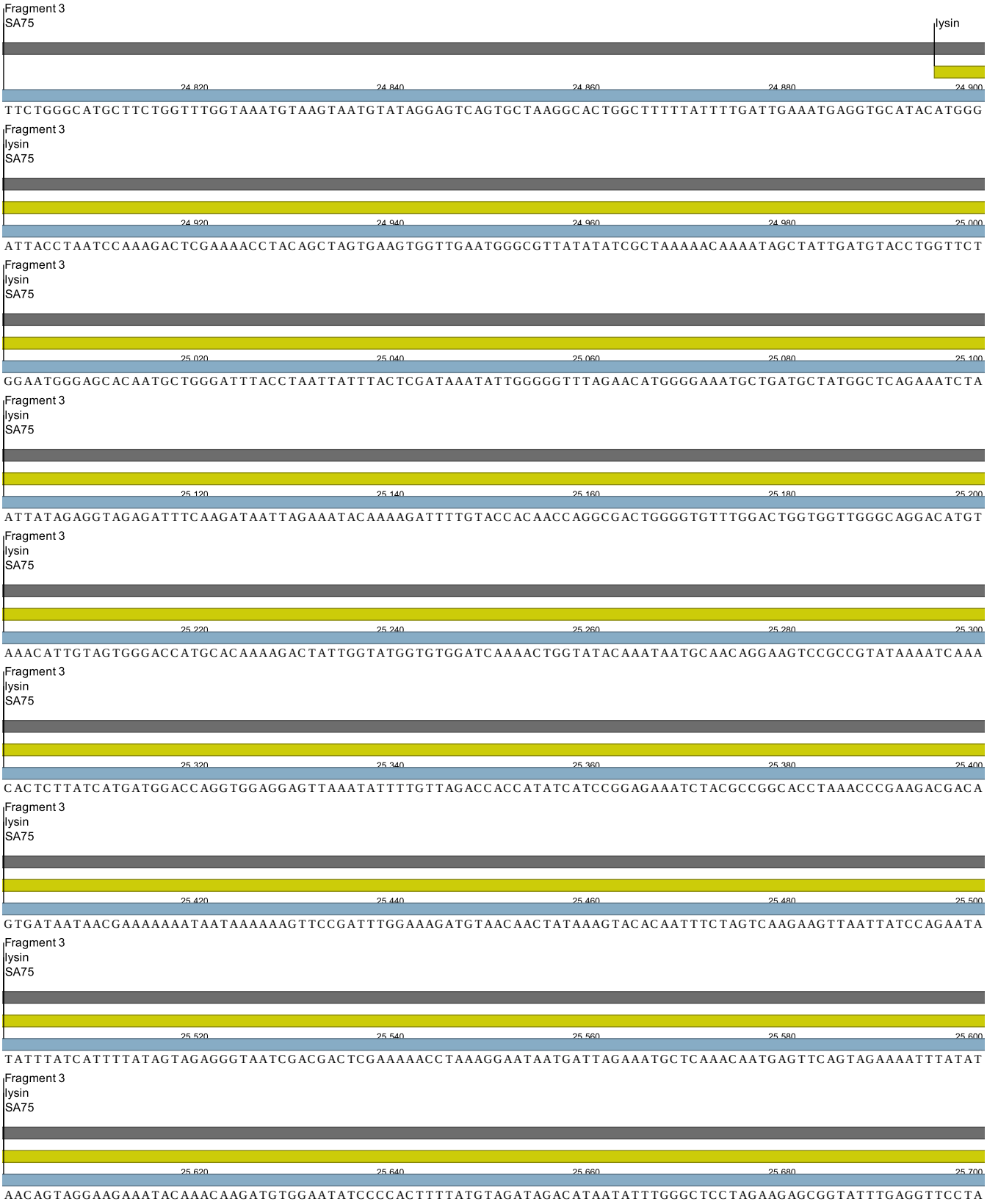
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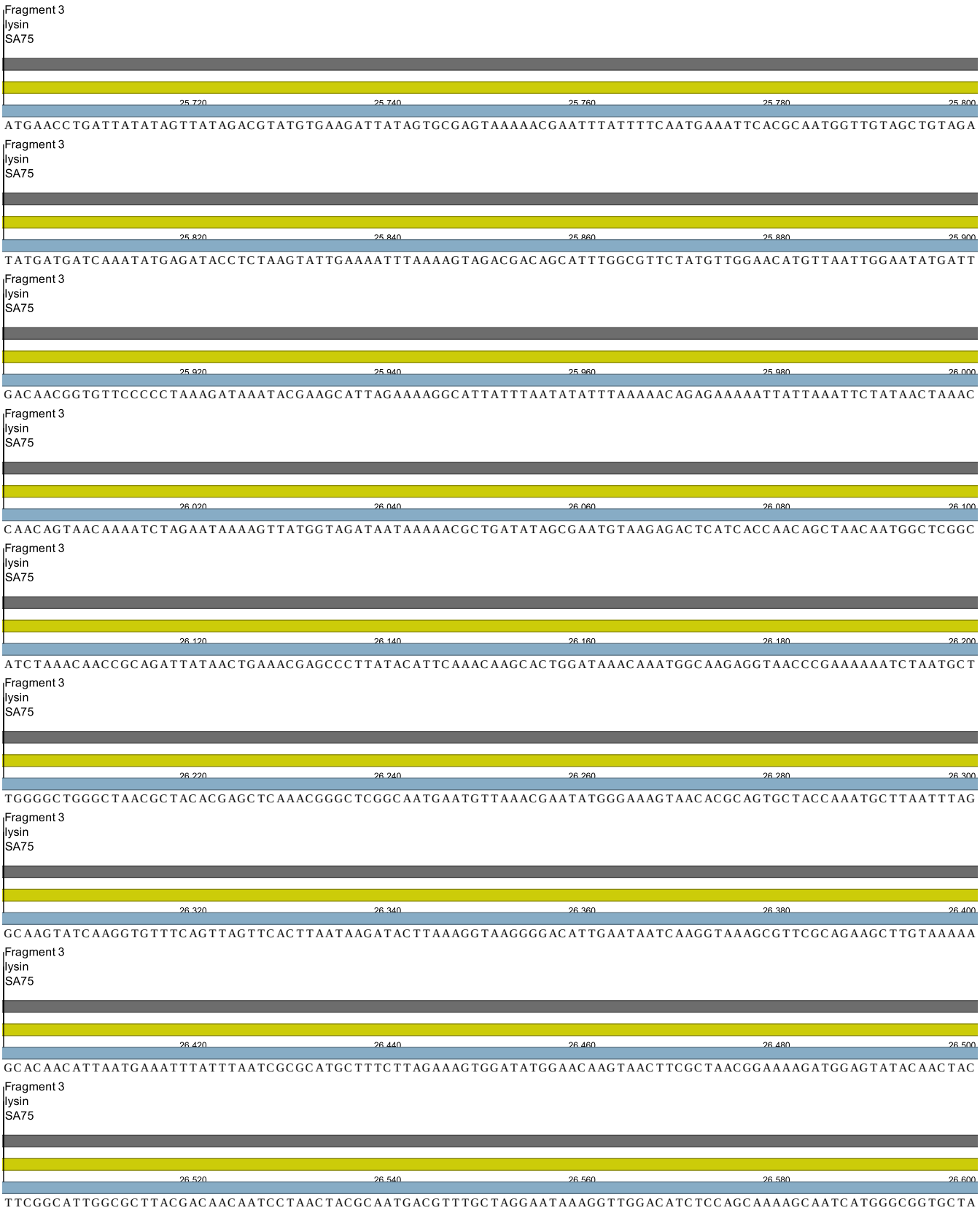
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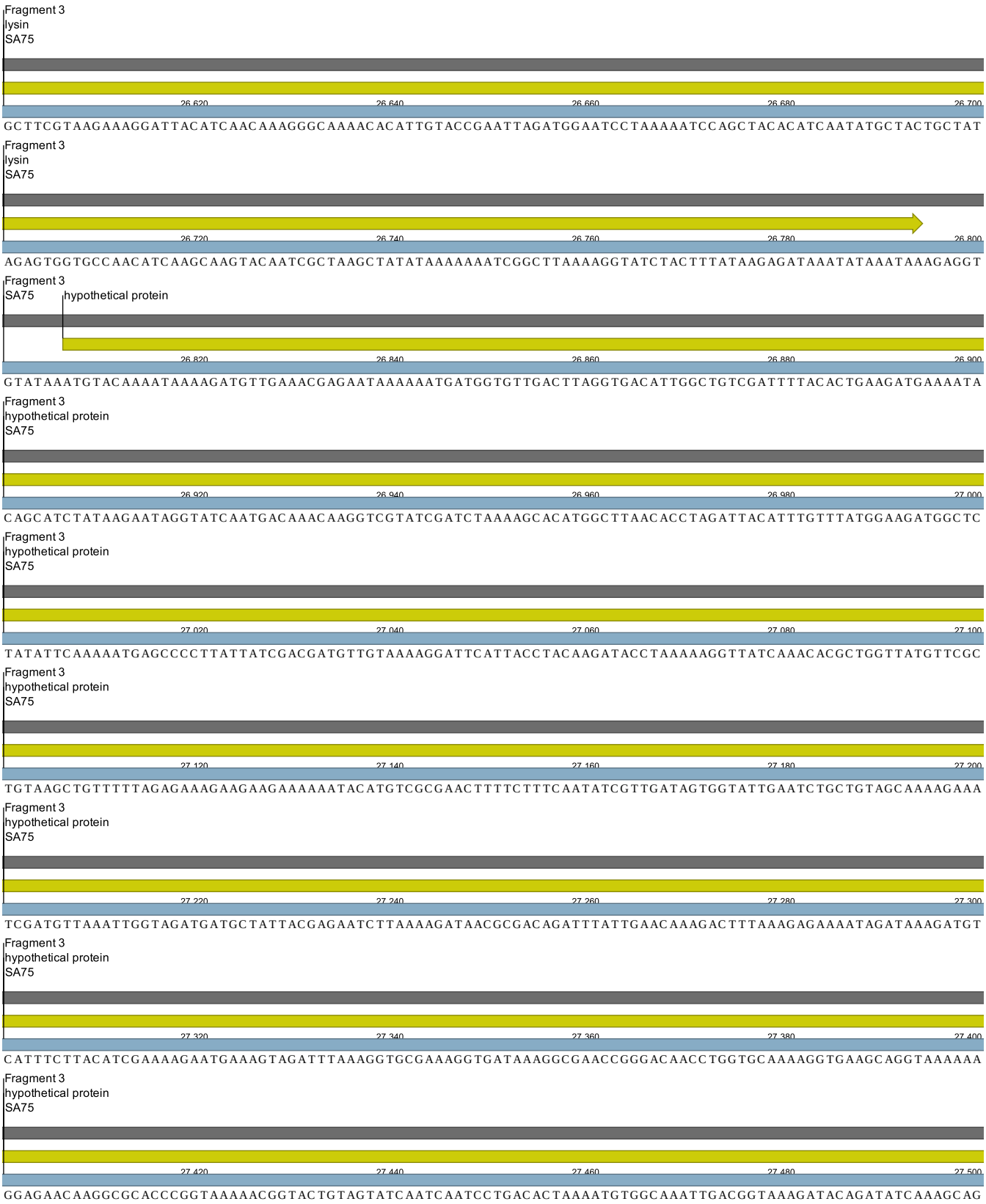
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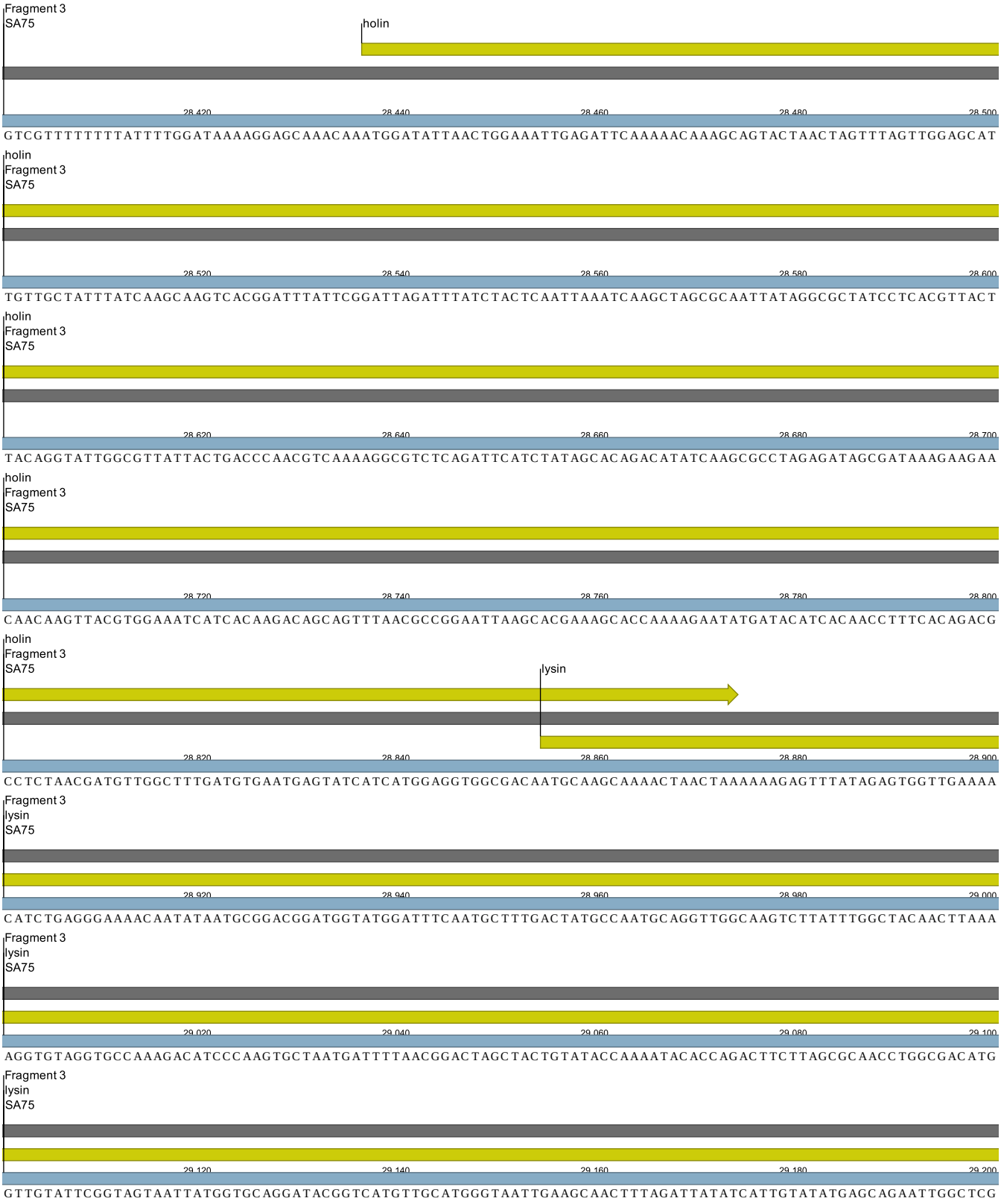
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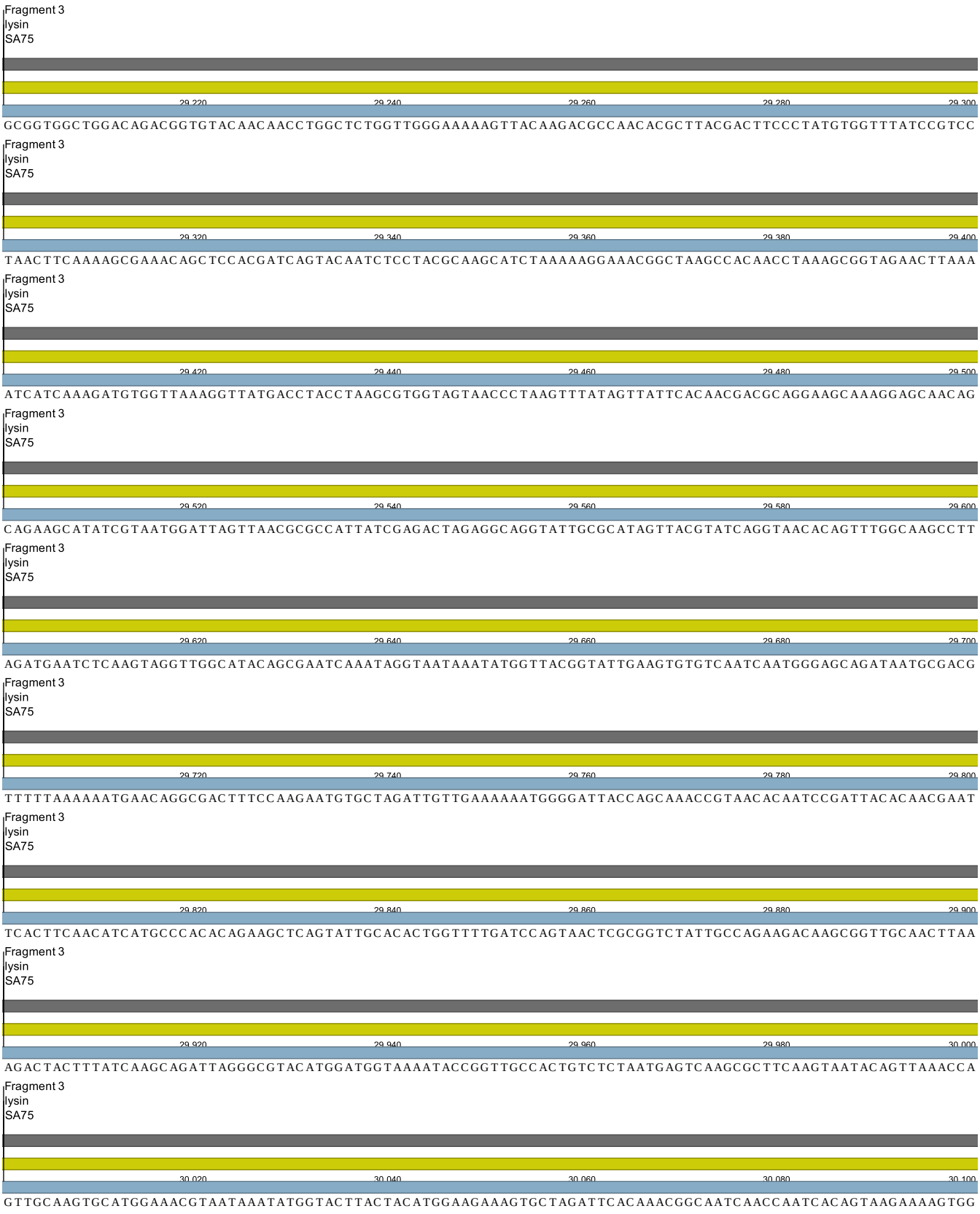
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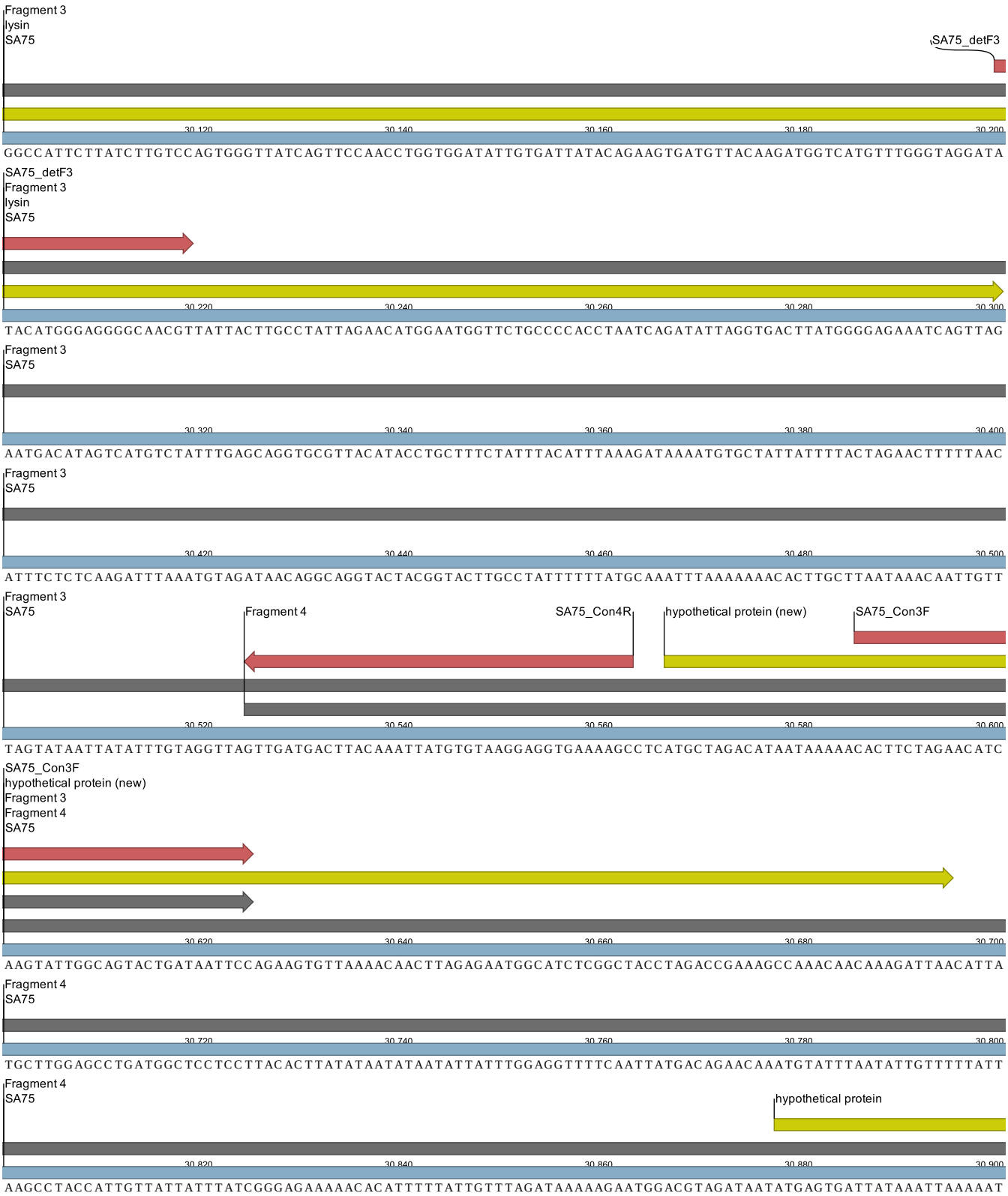
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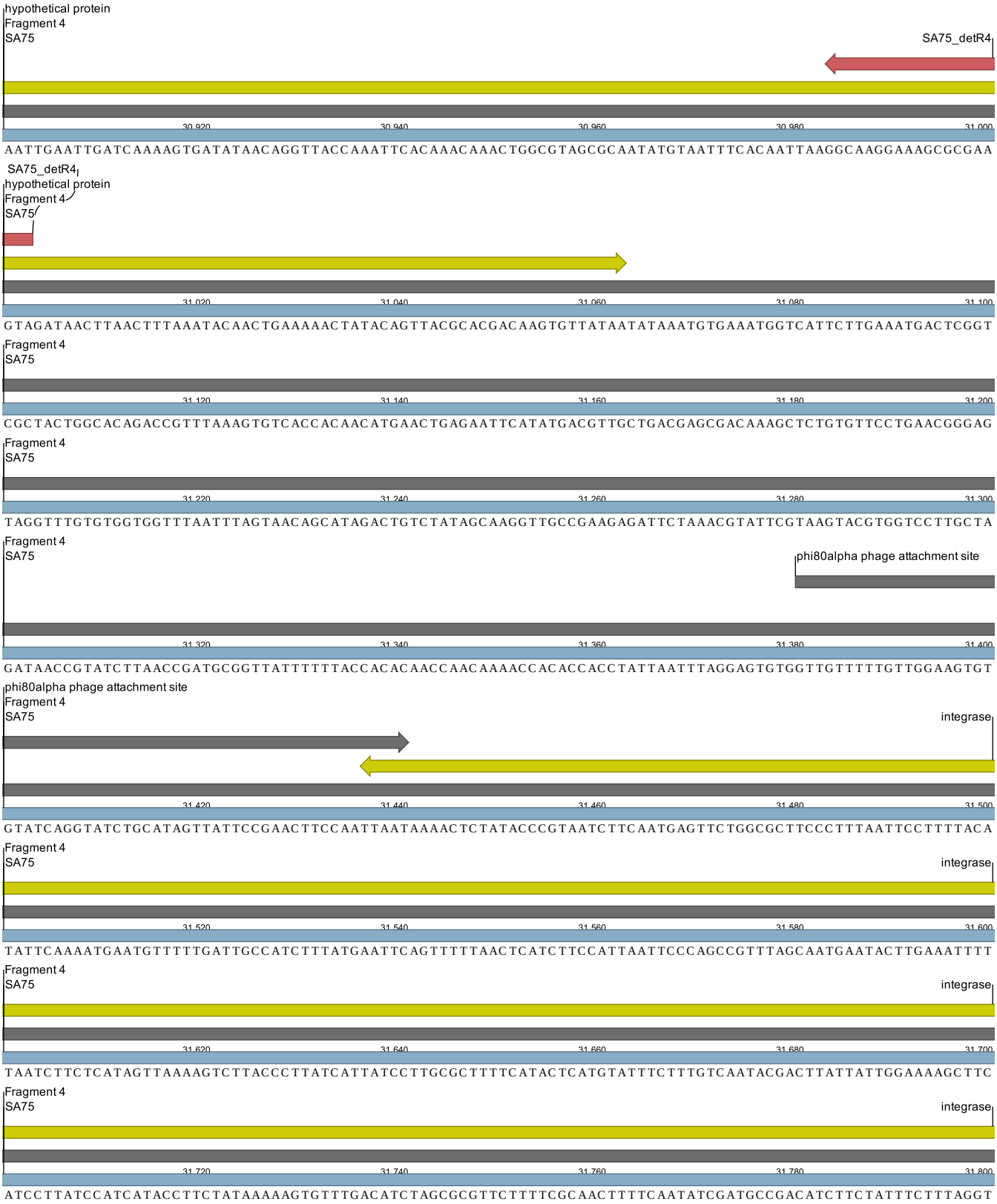
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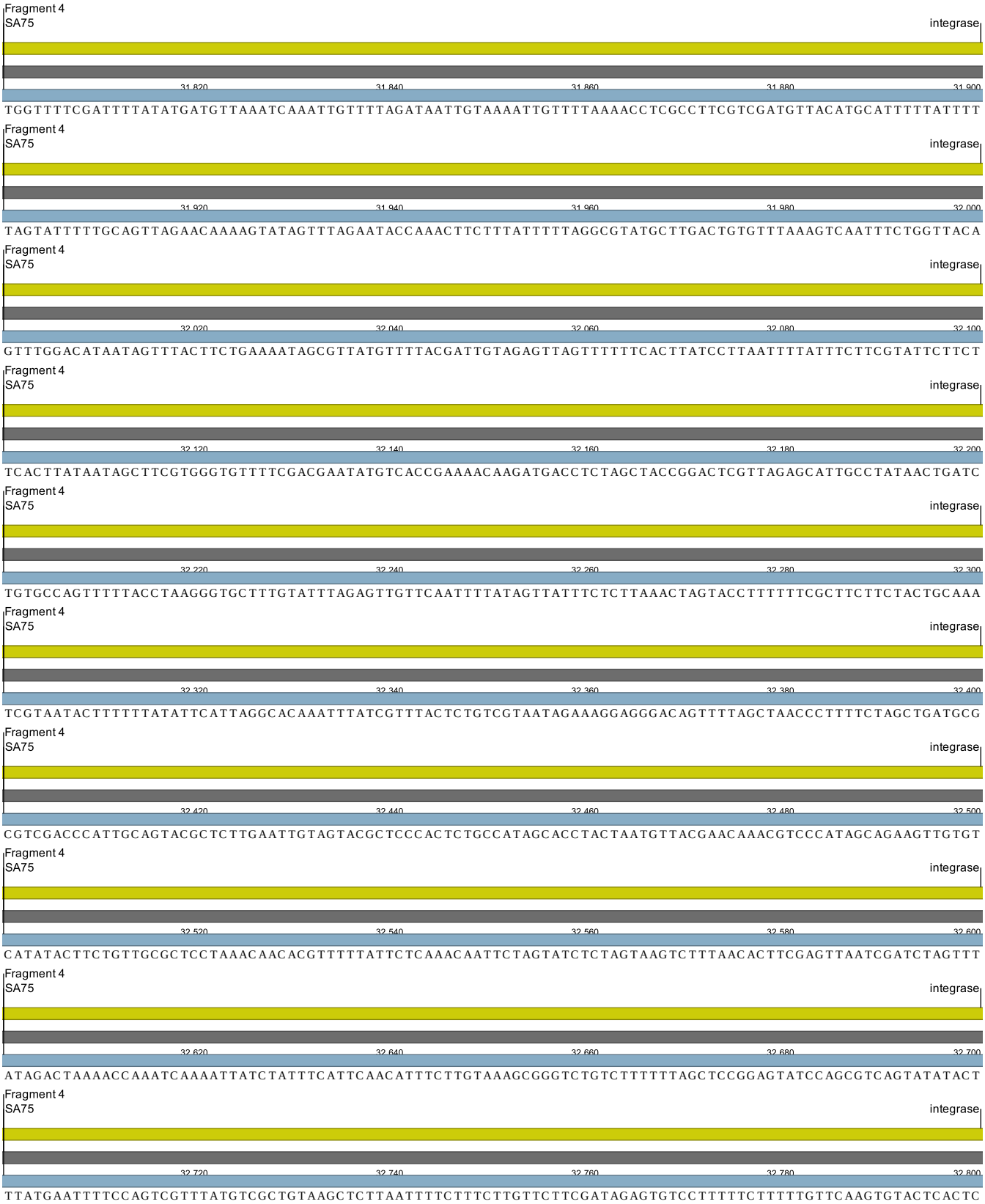
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Fragment 4
SA75
integrase
32,420
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32,500
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Fragment 4
SA75
integrase
32,520
32,540
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32,600
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Fragment 4
SA75
integrase
32,620
32,640
32,660
32,680
32,700
ATAGACTAAAACCAAATCAAAATTATCTATTTCATTCAACATTTCTTGTAAAGCGGGTCTGTCTTTTTTAGCTCCGGAGTATCCAGCGTCAGTATATACT
Fragment 4
SA75
integrase
32,720
32,740
32,760
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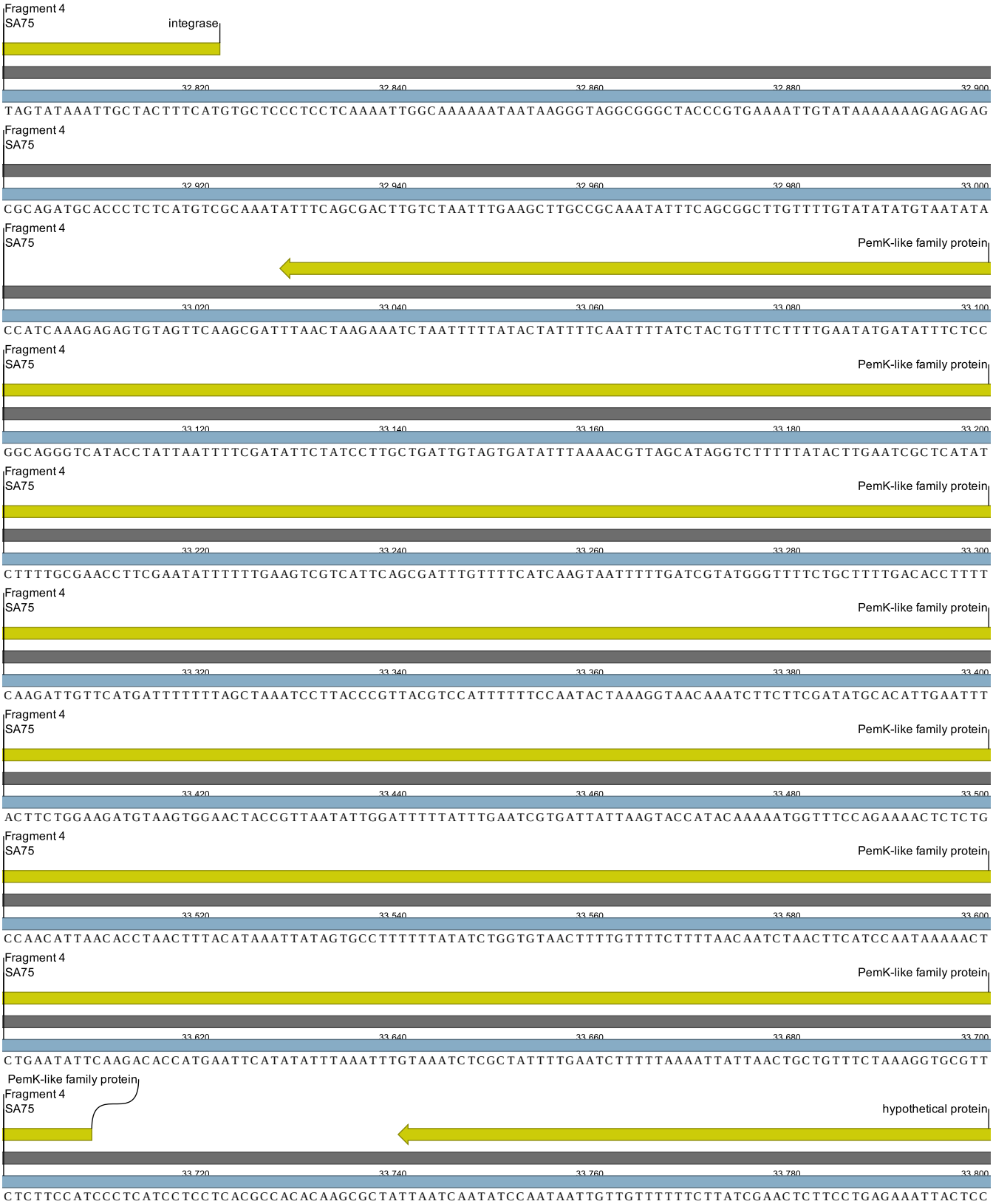
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SA75
PemK-like family protein
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33,060
33,080
33,100
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Fragment 4
SA75
PemK-like family protein
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33,140
33,160
33,180
33,200
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SA75
PemK-like family protein
33,220
33,240
33,260
33,280
33,300
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SA75
PemK-like family protein
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33,340
33,360
33,380
33,400
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Fragment 4
SA75
PemK-like family protein
33,420
33,440
33,460
33,480
33,500
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Fragment 4
SA75
PemK-like family protein
33,520
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33,580
33,600
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Fragment 4
SA75
PemK-like family protein
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33,680
33,700
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PemK-like family protein
Fragment 4
SA75
hypothetical protein
33,720
33,740
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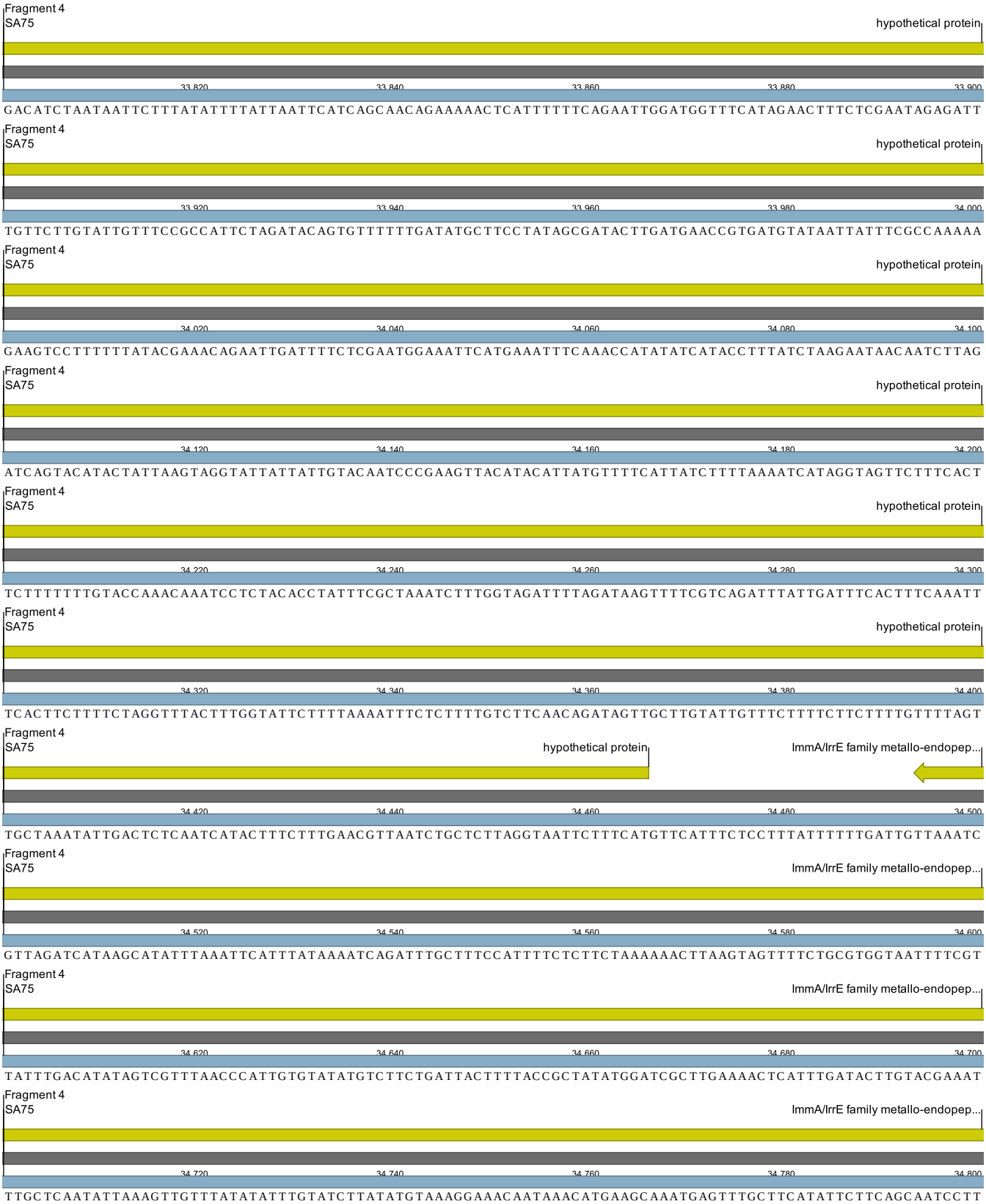
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hypothetical protein
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Fragment 4
SA75
hypothetical protein
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33,960
33,980
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Fragment 4
SA75
hypothetical protein
34,020
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34,080
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Fragment 4
SA75
hypothetical protein
34,120
34,140
34,160
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Fragment 4
SA75
hypothetical protein
34,220
34,240
34,260
34,280
34,300
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Fragment 4
SA75
hypothetical protein
34,320
34,340
34,360
34,380
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Fragment 4
SA75
hypothetical protein
ImmA/IrrE family metallo-endopep...
34,420
34,440
34,460
34,480
34,500
TGCTAAATATTGACTCTCAATCATACTTTCTTTGAACGTTAATCTGCTCTTAGGTAATTCTTTCATGTTCATTTCTCCTTTATTTTTTGATTGTTAAATC
Fragment 4
SA75
ImmA/IrrE family metallo-endopep...
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34,560
34,580
34,600
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Fragment 4
SA75
ImmA/IrrE family metallo-endopep...
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34,640
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34,680
34,700
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Fragment 4
SA75
ImmA/IrrE family metallo-endopep...
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34,740
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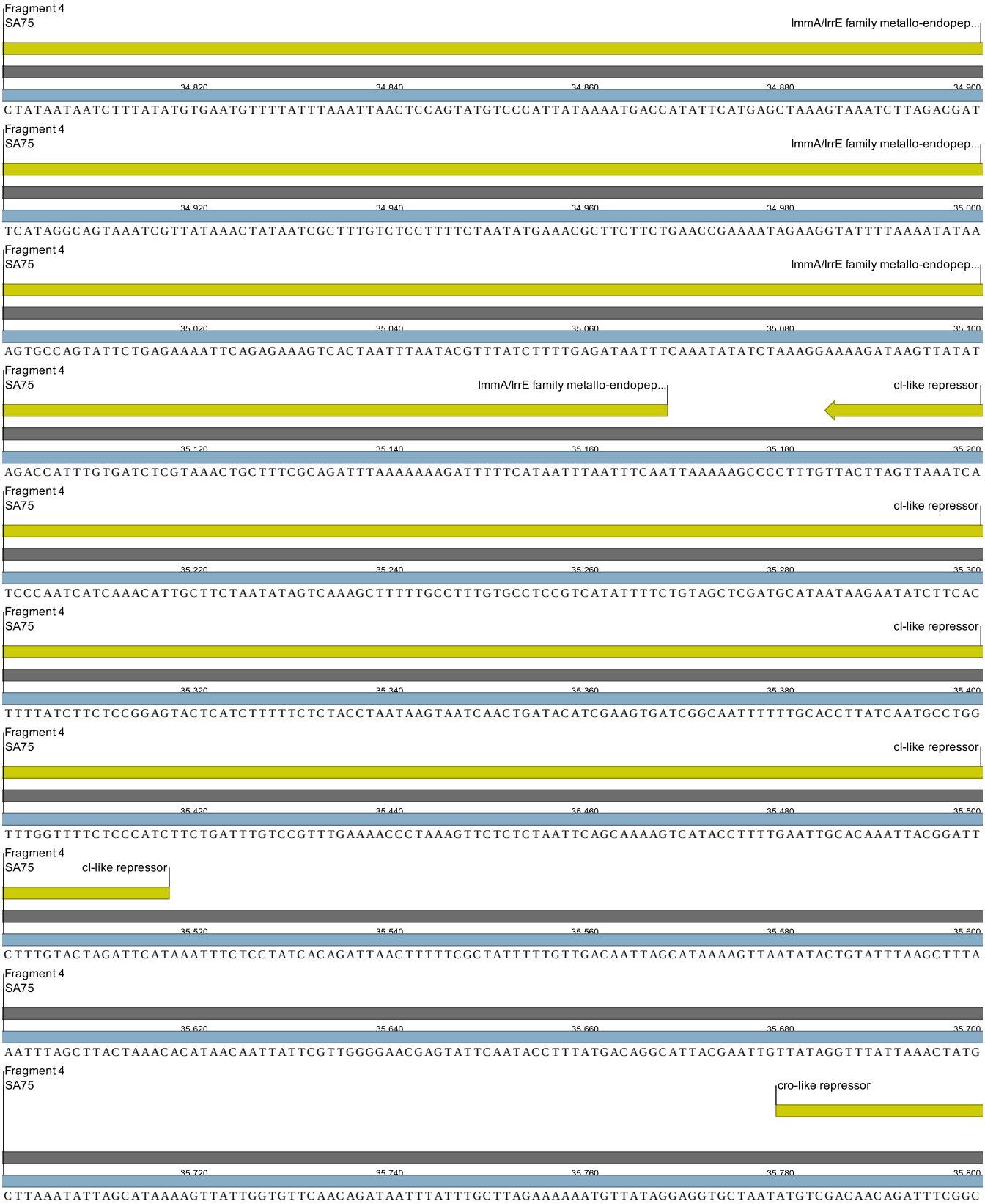
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Fragment 4
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34,980
35,000
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Fragment 4
SA75
ImmA/IrrE family metallo-endopep...
35,020
35,040
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35,080
35,100
AGTGCCAGTATTCTGAGAAAATTCAGAGAAAGTCACTAATTTAATACGTTTATCTTTTGAGATAATTTCAAATATATCTAAAGGAAAAGATAAGTTATAT
Fragment 4
SA75
ImmA/IrrE family metallo-endopep...
cI-like repressor
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35,140
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35,180
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Fragment 4
SA75
cI-like repressor
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35,300
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Fragment 4
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Fragment 4
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cI-like repressor
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35,700
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35,740
35,760
35,780
35,800
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cro-like repressor
Fragment 4
SA75
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35,860
35,880
35,900
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cro-like repressor
Fragment 4
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antirepressor protein
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35,960
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antirepressor protein
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SA75
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Fragment 4
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SA75
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36,400
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36,600
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antirepressor protein
Fragment 4
SA75
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36,700
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antirepressor protein
Fragment 4
SA75
hypothetical protein
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36,800
TGACTGAATTCGGTTATGAAGCTAATTTAGGGGGAGCGTAAATGACACCAGAACAAAAAGAAAAGCTAAACAATATAGTATTAACACTTTATGCAGTTAA
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Fragment 4
SA75
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36,900
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Fragment 4
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Fragment 4
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Fragment 4
SA75
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Fragment 4
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Fragment 4
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hypothetical protein
Fragment 4
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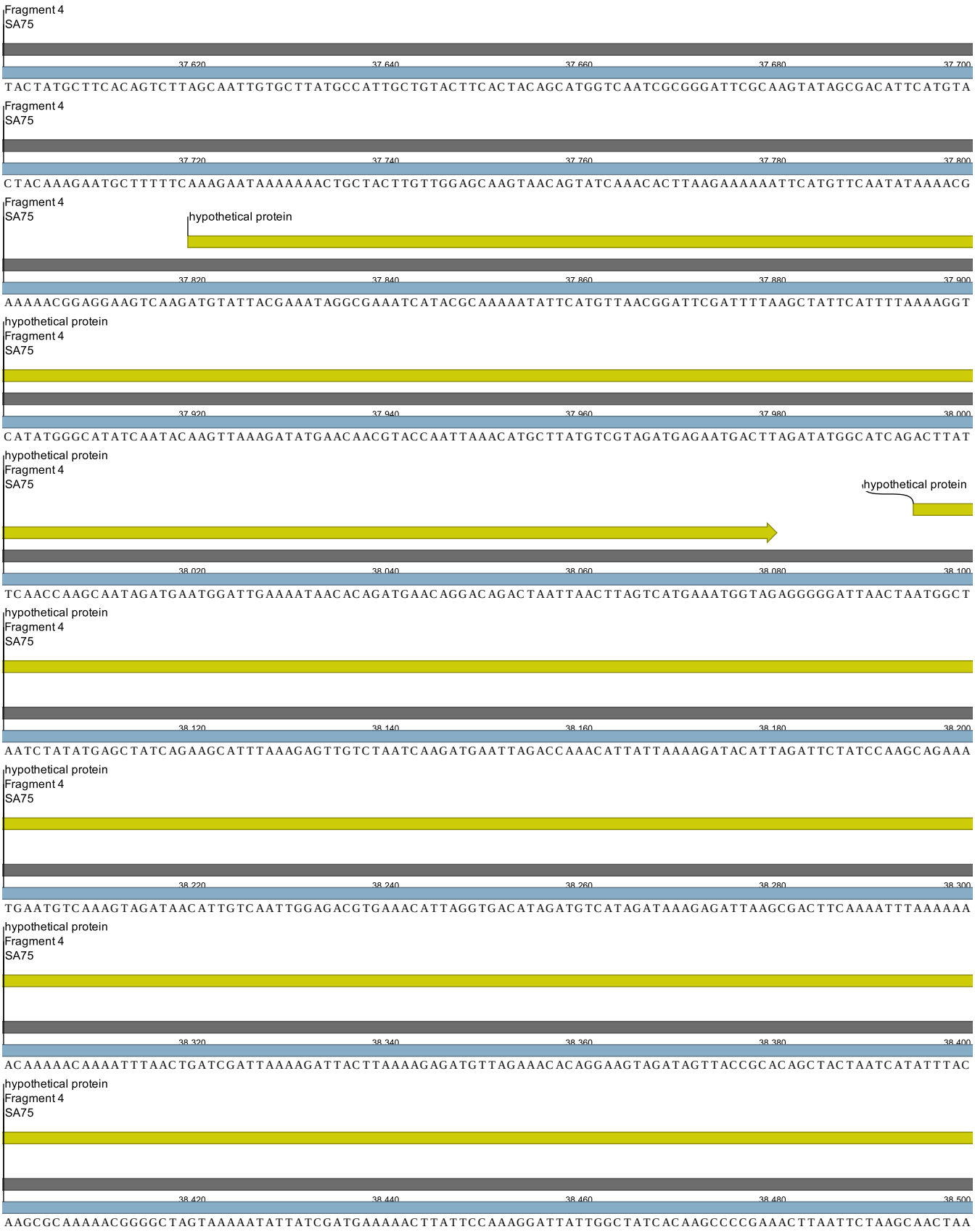
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Fragment 4
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37,780
37,800
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Fragment 4
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hypothetical protein
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37,840
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37,880
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Fragment 4
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hypothetical protein
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hypothetical protein
Fragment 4
SA75
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38,240
38,260
38,280
38,300
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hypothetical protein
Fragment 4
SA75
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38,340
38,360
38,380
38,400
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hypothetical protein
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SA75
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38,440
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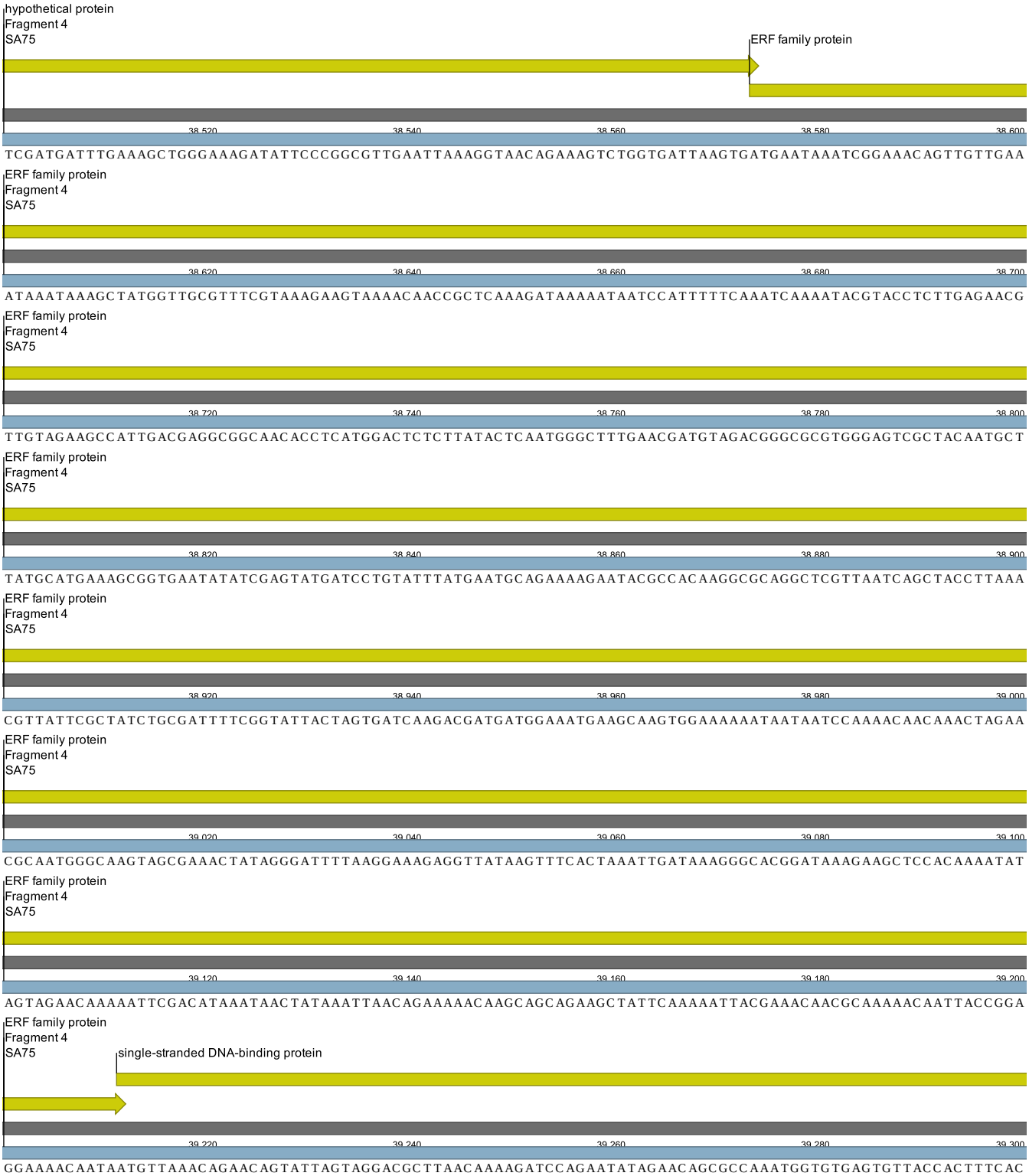
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ERF family protein
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38,700
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ERF family protein
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SA75
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38,800
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ERF family protein
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38,900
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ERF family protein
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SA75
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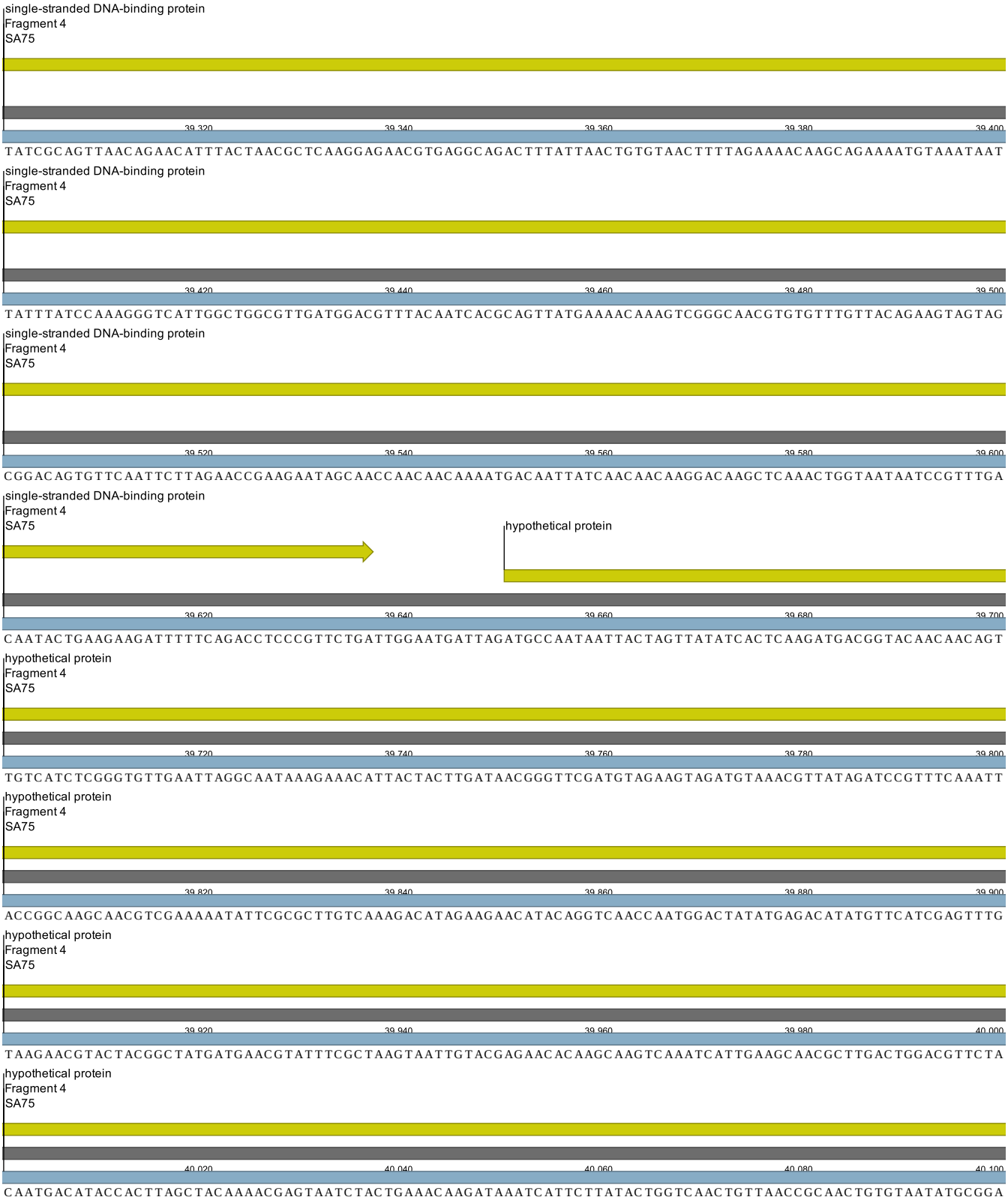
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Fragment 4
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39,500
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Fragment 4
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39,580
39,600
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single-stranded DNA-binding protein
Fragment 4
SA75
hypothetical protein
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hypothetical protein
Fragment 4
SA75
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39,760
39,780
39,800
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hypothetical protein
Fragment 4
SA75
39,820
39,840
39,860
39,880
39,900
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hypothetical protein
Fragment 4
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hypothetical protein
Fragment 4
SA75
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40,080
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hypothetical protein
Fragment 4
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hypothetical protein
Fragment 4
SA75
hypothetical protein
40,320
40,340
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40,400
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hypothetical protein
Fragment 4
SA75
hypothetical protein
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Fragment 4
SA75
hypothetical protein
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Fragment 4
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hypothetical protein
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40,900
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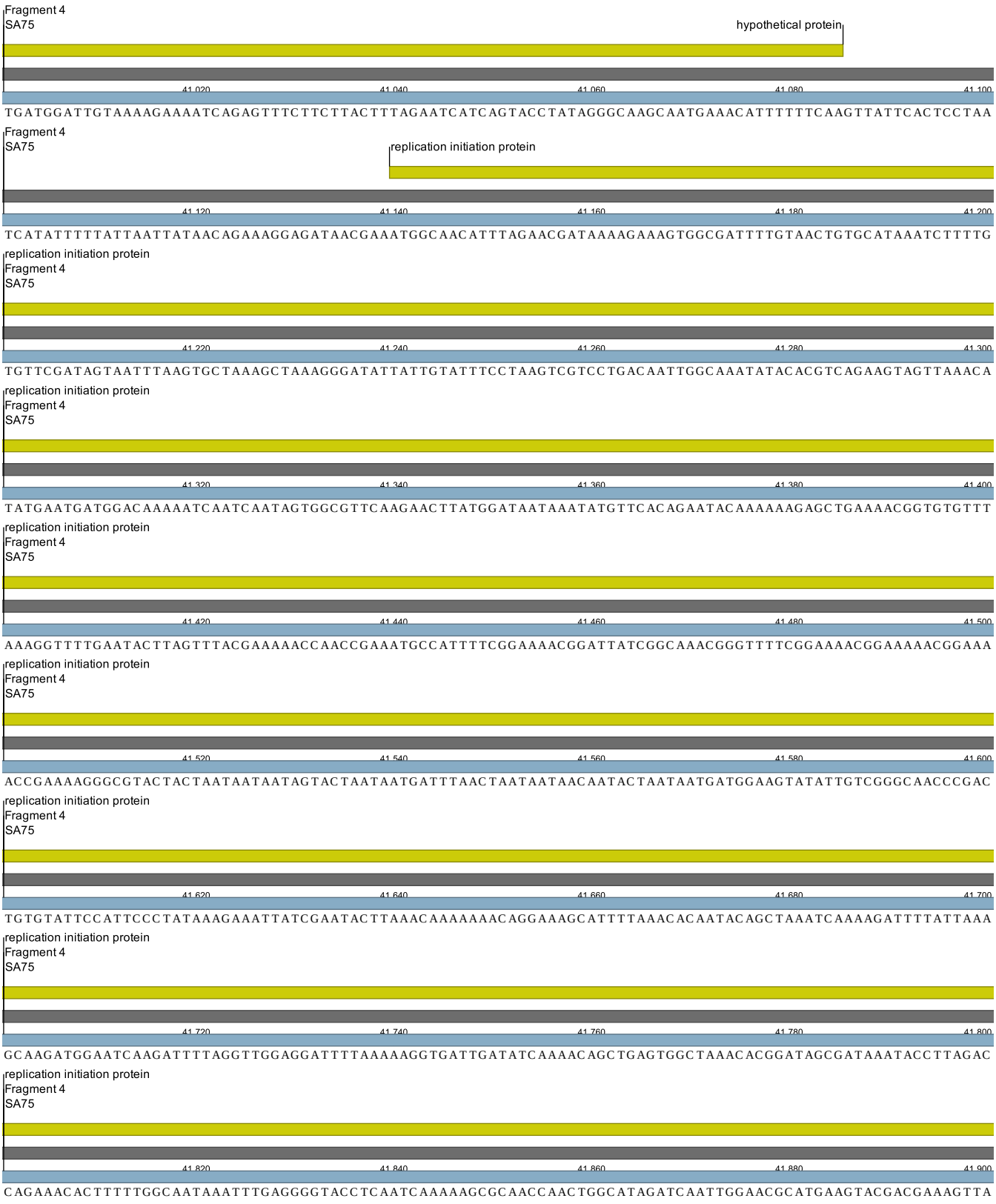
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SA75
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Fragment 4
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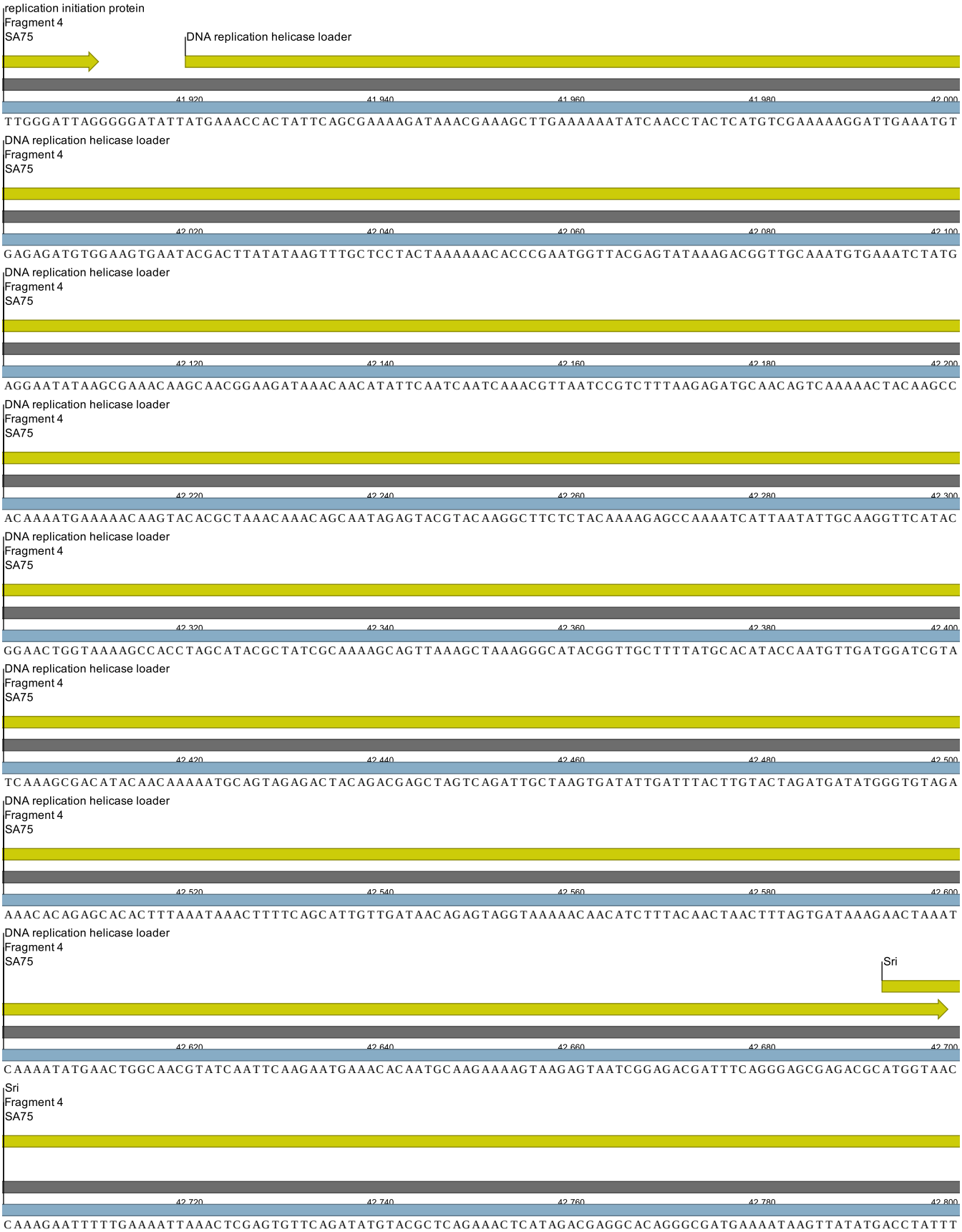
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DNA replication helicase loader
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DNA replication helicase loader
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Fragment 4
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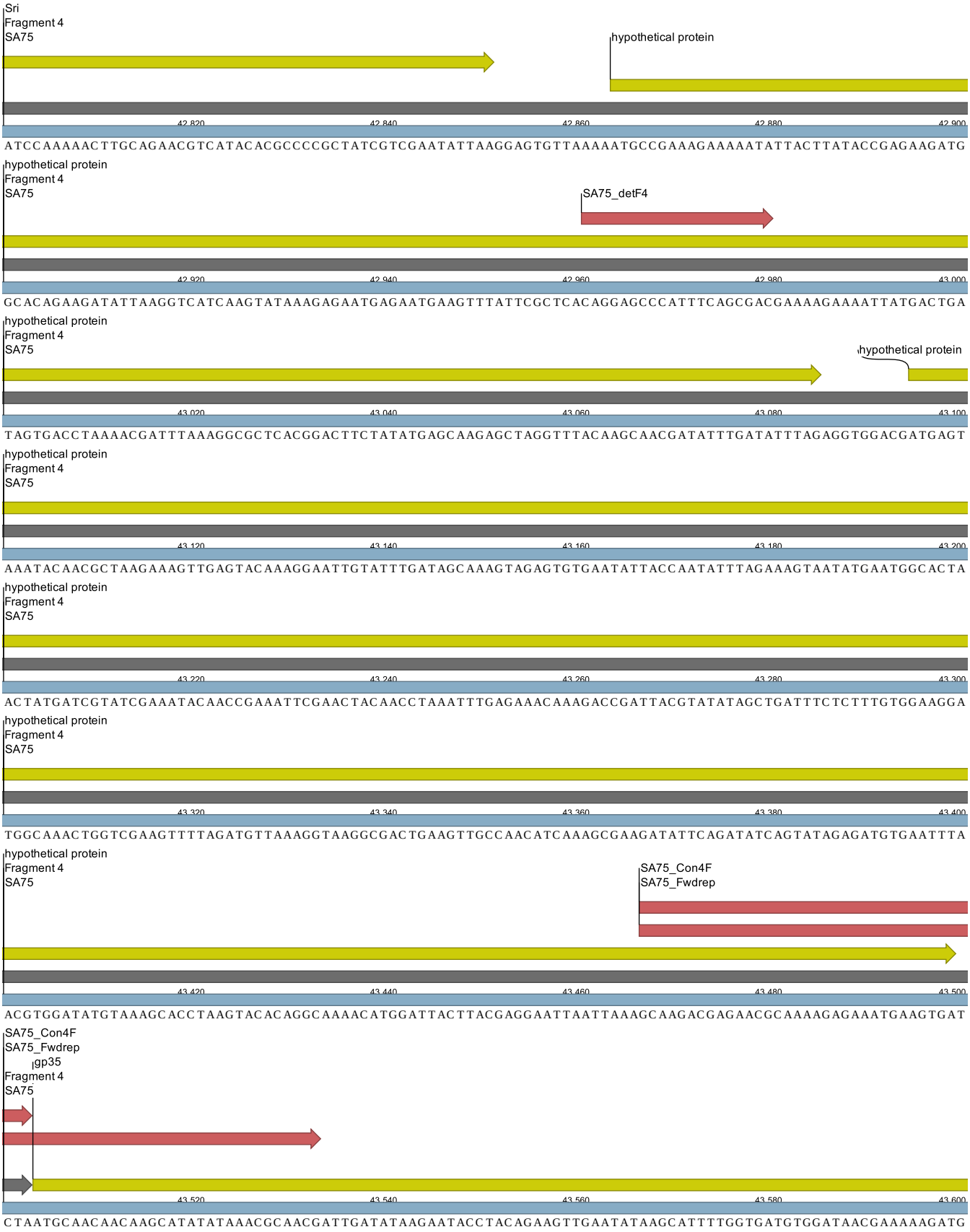
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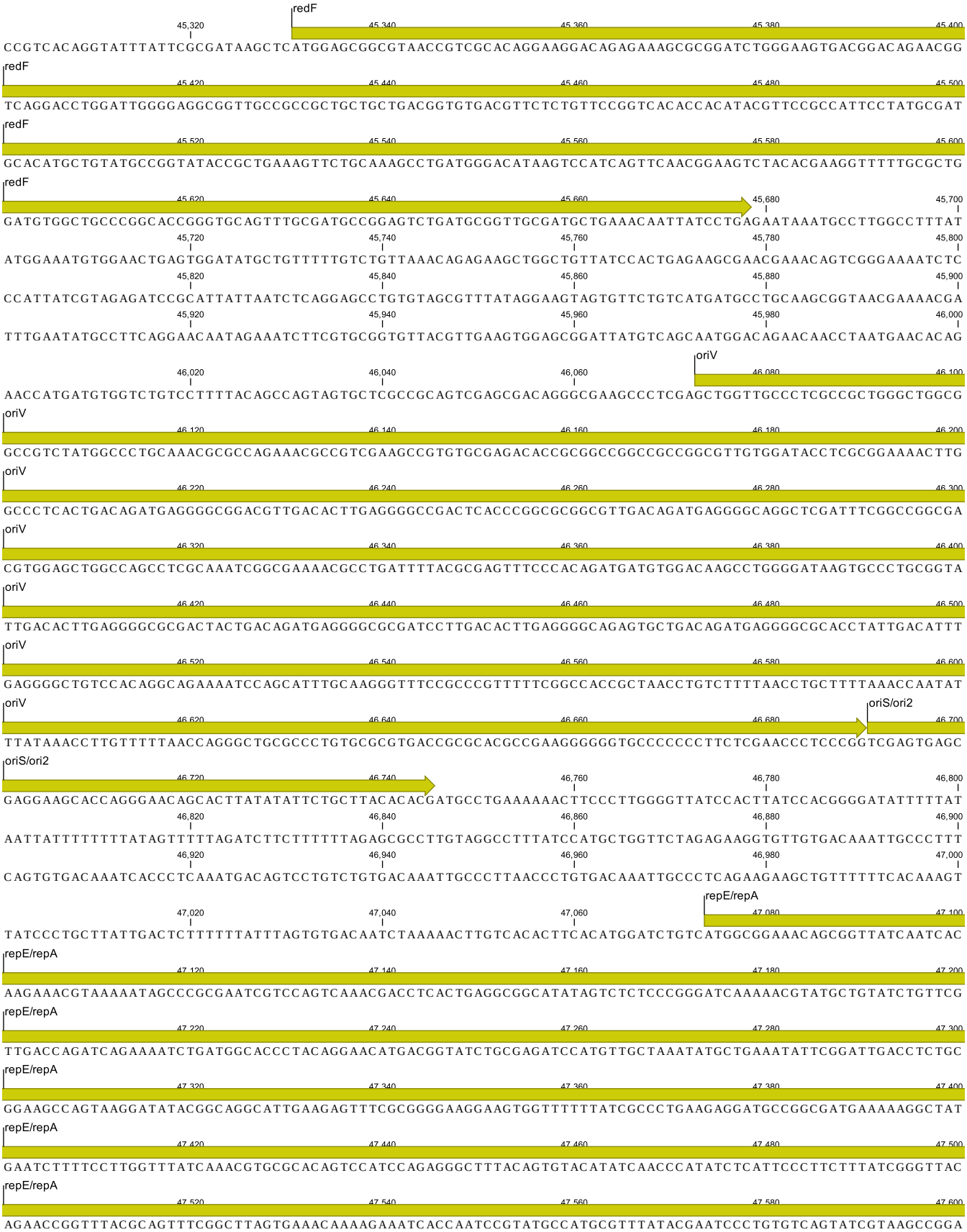
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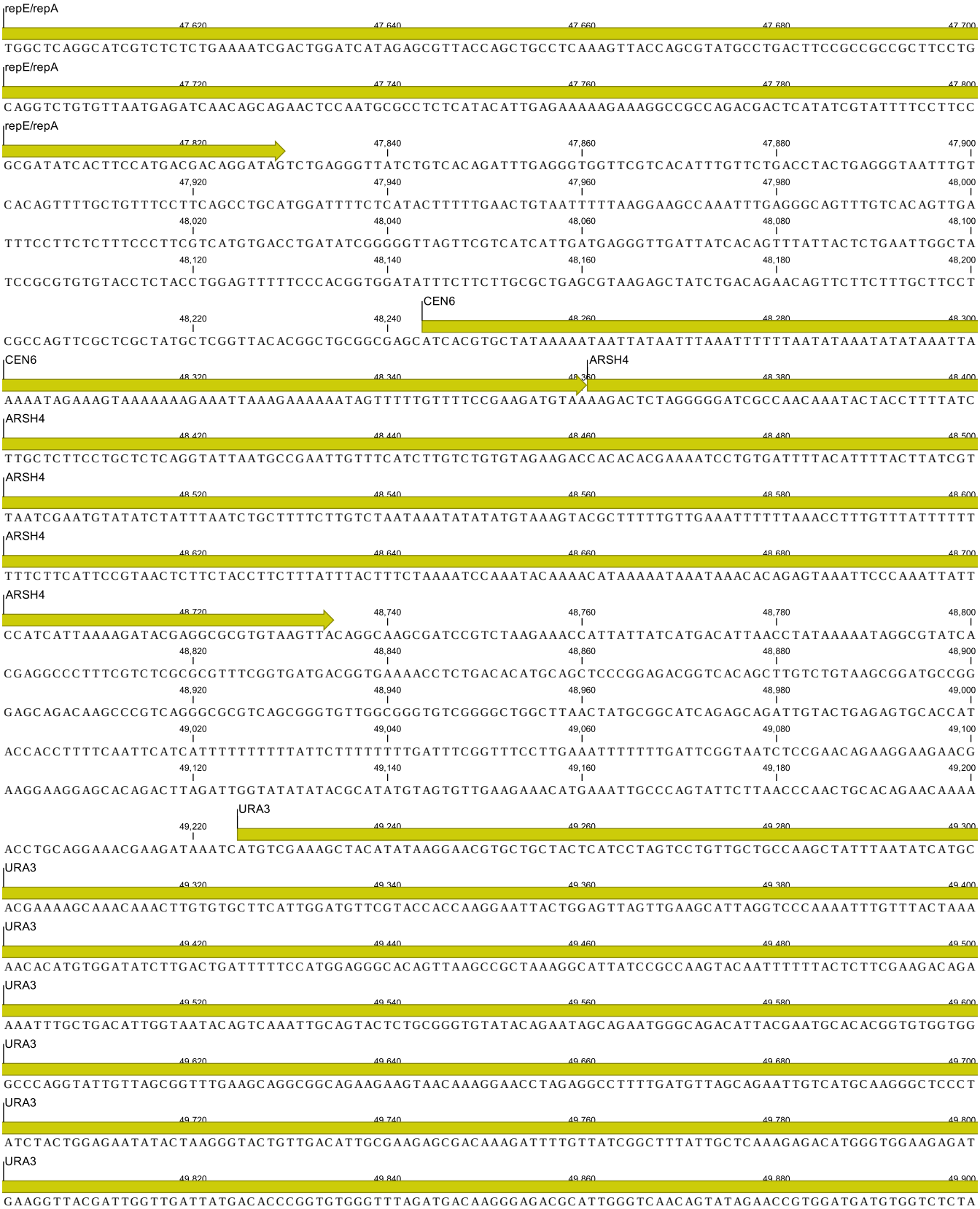
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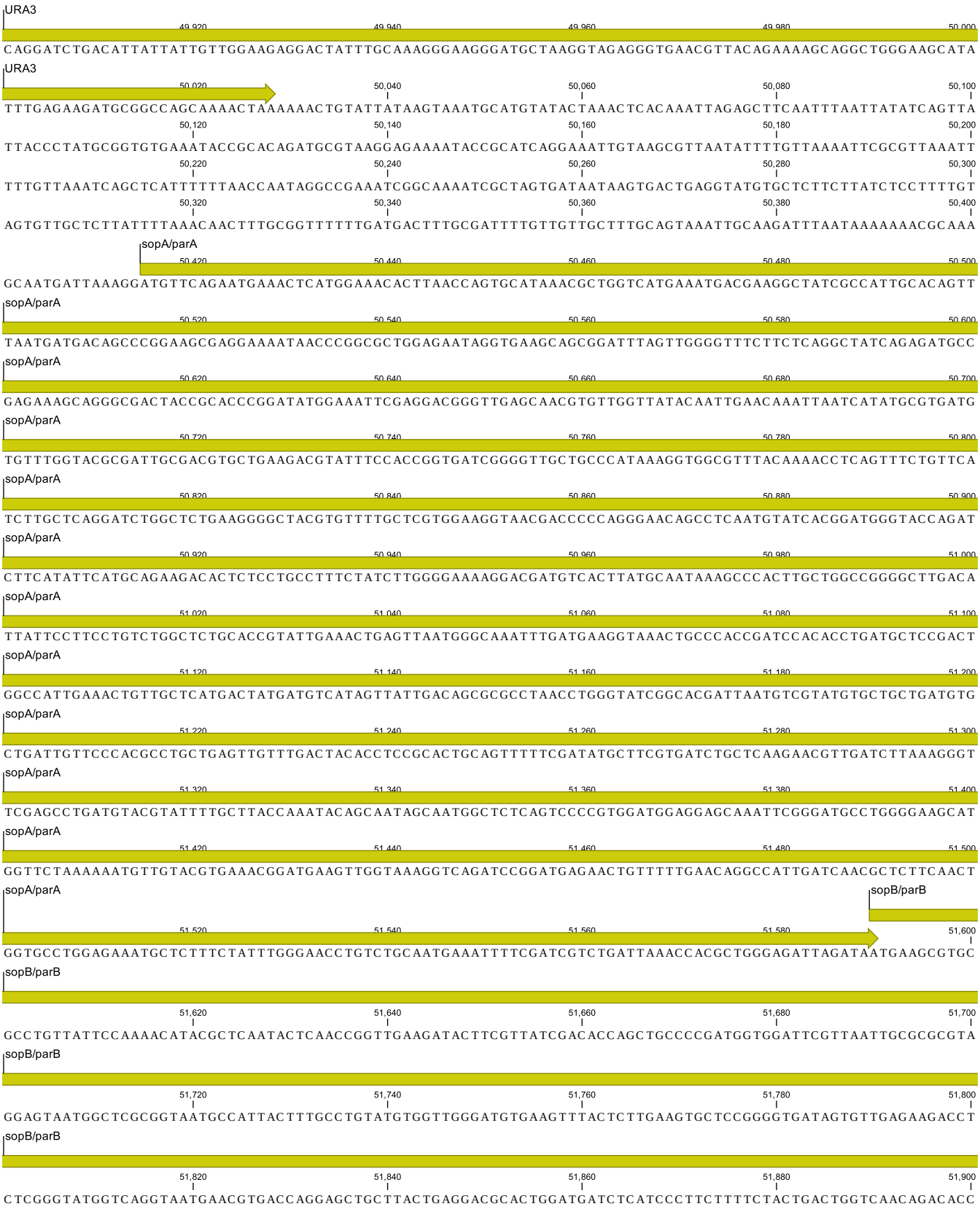
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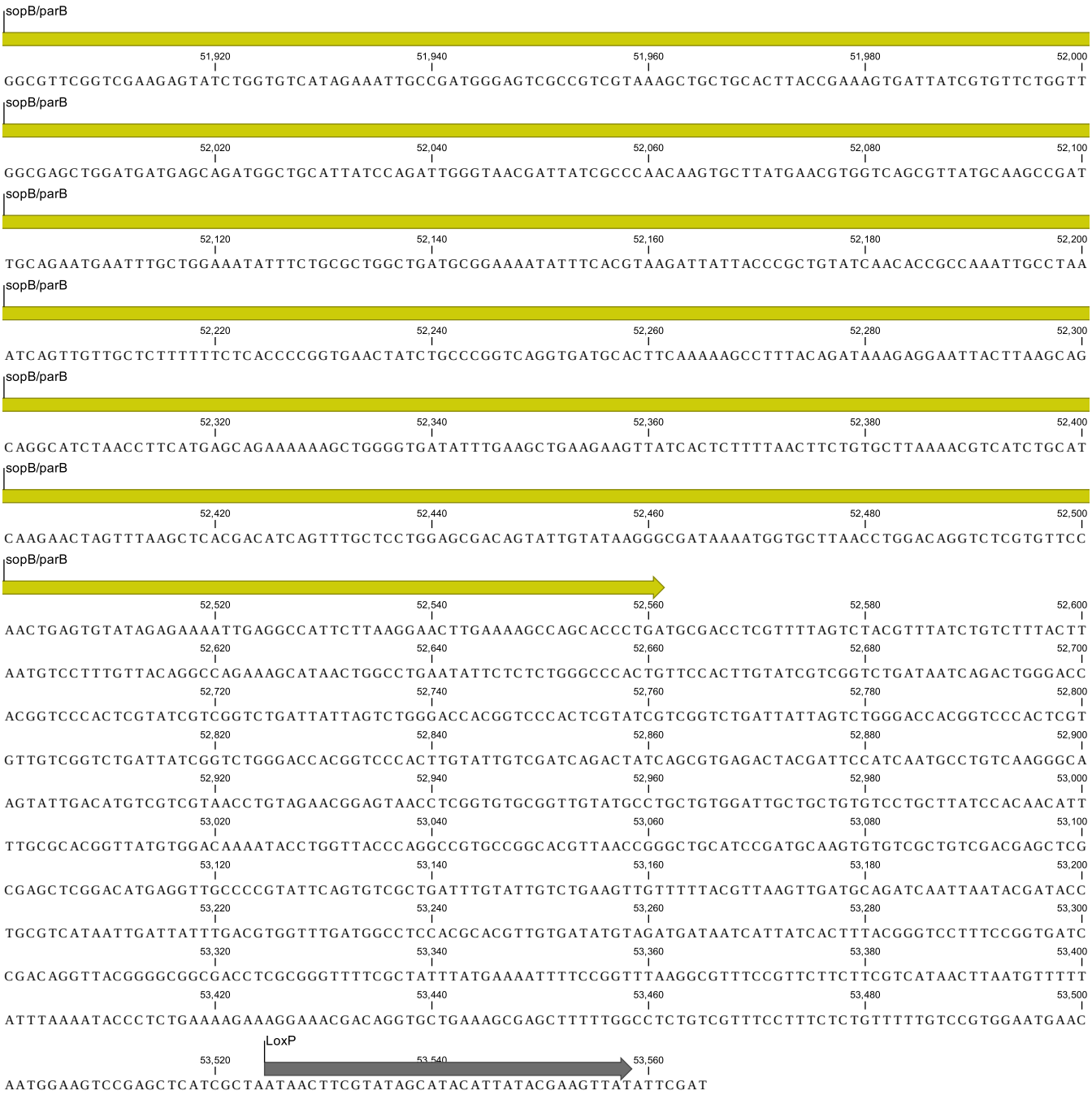
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Table S1. Primers used in this study. This includes oligonucleotide primers used for constructing each fragment and final assembly of the SA75 genome via TAR-cloning as well as primers used for detection of each fragment junction and primers used to generate the 500 bp direct repeat at the 3' end of the cloned phage genome.

Name	Sequence	Description
SA75_Con1F	GTAACAGATACTGCTGAACAAACTAAATTGAATGTTGAAGCtagggataacagggtaatgatcctctagagtcgacctg	Forward primer to amplify TAR vector 01 from pCC1BAC/YCp (blue) and add I-SceI recognition site (red) as well as SA75 homology (capitals)
SA75_Con1R	CTTATATCAATCGTTGCGTTTATATATGCTTGTTGTTGCATtagggataacagggtaatcgggtaccgagctcgaattc	Reverse primter to amplify TAR vector 01 from pCC1BAC/YCp (blue) and add I-SceI recognition site (red) as well as SA75 homology (capitals)
SA75_Con2F	CGTATAGATACATTGATGGAGAATTATGGATTTATTCAGCtagggataacagggtaatgatcctctagagtcgacctg	Forward primer to amplify TAR vector 02 from pCC1BAC/YCp (blue) and add I-SceI recognition site (red) as well as SA75 homology (capitals)
SA75_Con2R	CTAAATTAACAACCTCATCTGATGAATCAACTTCCGCTTCtagggataacagggtaatcgggtaccgagctcgaattc	Reverse primer to amplify TAR vector 02 from pCC1BAC/YCp (blue) and add I-SceI recognition site (red) as well as SA75 homology (capitals)
SA75_Con3F	CACTTCTAGAACATCAAGTATTGGCAGTACTGATAATTCCtagggataacagggtaatgatcctctagagtcgacctg	Forward primer to amplify TAR vector 03 from pCC1BAC/YCp (blue) and add I-SceI recognition site (red) as well as SA75 homology (capitals)
SA75_Con3R	CAAGCAATCTATCAATAAATTGTCCGTTGGGCTTCAATCTAGtagggataacagggtaatcgggtaccgagctcgaattc	Reverse primer to amplify TAR vector 03 from pCC1BAC/YCp (blue) and add I-SceI recognition site (red) as well as SA75 homology (capitals)
SA75_Con4F	GCAAGACGAGAACGCAAAAGAGAAATGAAGTGATCTAtagggataacagggtaatgatcctctagagtcgacctg	Forward primer to amplify TAR vector 04 from pCC1BAC/YCp (blue) and add I-SceI recognition site (red) as well as SA75 homology (capitals)
SA75_Con4R	GCTTTTCACCTCCTTACACATAATTTGTAAGTCATCAACtagggataacagggtaatcgggtaccgagctcgaattc	Reverse primer to amplify TAR vector 04 from pCC1BAC/YCp (blue) and add I-SceI recognition site (red) as well as SA75 homology (capitals)
SA97_Fwdrep	GCAAGACGAGAACGCAAAAGAGAAATGAAGTGATCTAATGCAACAACAAGCATATATAAACGCAACG	Forward primer to amplify the beginning 500 bp to add a direct repeat to the end of cloned SA75
SA75_Rev_500	tactcaagcttgcatgcctgcaggtcgactctagaggatctagggataacagggtaatGGTTATAAGTATTATCAAAC	Reverse primer to amplify the beginning 500 bp to add a direct repeat to the end of cloned SA75. pCC1BAC/YCp (blue), I-SceI recognition site (red), and SA75 homology (capitals)

SA75_Con4F_repeat	CATTCACGTGATCCGTACTGGTTTGATAA TACTTATAACCtagggataacagggtaat gatcctctagagtcgacctg	Forward to amplify TAR final vector from pCC1BAC/YCp (blue) and add I-SceI recognition site (red) as well as SA75 500 bp repeat homology (capitals)
RCO495	ACGACGGCCAGTGAATTG	Forward primer to detect YCp/SA75 Fragment 1 junction
SA75_detR1	GGCGCGTCTAAAGCTTCTGA	Reverse primer to detect YCp/SA75 Fragment 1 junction
SA75_detF1	ATCCGGATGAACCAGGTGGA	Forward primer to detect SA75 Fragment 1/2 junction
SA75_detR2	GCACCTGGTTTATCAGCCCA	Reverse primer to detect SA75 Fragment 1/2 junction
SA75_detF2	CAGCATGCCTGACGGGTTAC	Forward primer to detect SA75 Fragment 2/3 junction
SA75_detR3	TGAGCATGCACCTTACCCGA	Reverse primer to detect SA75 Fragment 2/3 junction
SA75_detF3	ATACATGGGAGGGGCAACGT	Forward primer to detect SA75 Fragment 3/4 junction
SA75_detR4	TACTTCGCGCTTTCCTTGCC	Reverse primer to detect SA75 Fragment 3/4 junction
SA75_detF4	CAGGAGCCCATTTCAGCGAC	Forward primer to detect SA75 Fragment 4/YCp junction
RCO493	CCAAGCTATTTAGGTGAGAC	Reverse primer to detect SA75 Fragment 4/YCp junction