

SUPPLEMENTARY MATERIALS

Article

***De novo* assembly of the *Salvia miltiorrhiza* mitochondrial genome using Pacbio long reads provides the first evidence of multi-chromosomal mitochondrial DNA structure of *Salvia* species**

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Table S1 Summary of sequence data generated by PacBio RS and Illumina platform.

Sequencing platform	PacBio RS	Illumina
Total number of nucleotides (raw data, bp)	19,434,039,998	16,380,908,800
Total Number of Reads	1,408,786	163,809,088
N50 Read Length	19,289	NA
Mean Read Length (bp)	13,794	100
Mean Read Quality Score	0.82	NA
Total Number of Mapped Reads	23,959 (MC1), 13,431(MC2)	3,291,507 (MC1), 1,021,009(MC2)
Average Coverage Depth	327.77 (MC1), 396.13 (MC2)	969.556 (MC1), 903.15 (MC2)

Table S2 SSRs in the *S. multiorrhiza* mitogenome. “MC1/2”: mitogenome chromosome 1/2.

[illegible]

Table S3 Summary of SSRs in the mitogenome of Lamiales.

accession number	species	Genome size	mononucleotides	dinucleotides	trinucleotides	tetranucleotides	pentanucleotides	hexanucleotides	Total
NC_023103	<i>Ajuga reptans</i>	352069	7	15	16	36	6	0	80
MW553042	<i>Scutellaria tsinyunensis</i>	354073	15	15	16	41	3	0	90
MN585275.1/6.1	<i>Salvia miltiorrhiza</i>	414114	15	29	20	39	5	4	112
NC_049064	<i>Rothea serrata</i>	482114	14	19	17	34	8	3	95
NC_031806	<i>Castilleja paramensis</i>	495499	21	29	15	46	6	0	117
NC_023209	<i>Salvia miltiorrhiza</i>	499236	17	27	22	43	6	3	118
NC_016741	<i>Dorcocheras hygrometricum</i>	510519	22	34	9	33	5	1	104
NC_018041	<i>Erythranthe guttata</i>	525671	36	17	14	44	8	1	120
NC_031323	<i>Hesperelaea palmeri</i>	658522	50	40	28	64	8	0	190
NC_034982	<i>Utricularia reniformis</i>	857234	36	33	26	63	4	2	164

Table S4 Tandem repeats in the mitogenome of the Lamiales.

accession_number	Indices	Period size(bp)	Copy Number	Consensus Size (bp)	Percent Matches	Percent Indels	Score	Bases number				Entropy (0–2)
								A	G	C	T	
MN585276.1	194570-194609	15	2.5	16	88	8	55	70	17	2	10	1.27
MN585275.1	358379-358424	21	2.1	22	88	11	69	50	0	0	50	1
MN585275.1	402285-402332	16	2.9	16	76	14	53	33	20	12	33	1.9
NC_016741	5295-5330	17	2.1	17	89	0	54	41	11	5	41	1.64
NC_016741	208537-208578	19	2.2	20	87	12	61	52	7	26	14	1.67
NC_016741	237481-237523	21	2	21	95	0	77	27	11	32	27	1.92
NC_016741	283955-283988	16	2	16	88	11	50	58	0	0	41	0.98
NC_016741	364263-364297	18	2	17	88	5	52	5	11	11	71	1.3
NC_018041	9641-9681	20	2	20	100	0	82	24	12	19	43	1.85
NC_018041	31116-31183	18	3.8	18	98	0	127	38	22	16	23	1.93
NC_018041	65710-65739	16	1.9	15	93	6	51	43	13	30	13	1.82
NC_018041	94432-94509	40	1.9	42	81	5	97	17	20	21	39	1.92
NC_018041	104253-104302	24	2.1	24	96	0	91	40	18	20	22	1.92
NC_018041	120892-120935	14	3.1	14	90	6	63	72	6	2	18	1.17
NC_018041	163482-163508	13	2.1	13	100	0	54	14	22	0	62	1.31
NC_018041	180754-180796	21	2	21	100	0	86	32	37	18	11	1.87
NC_018041	222539-222579	20	2	20	100	0	82	43	19	12	24	1.85
NC_018041	234667-234691	12	2.1	12	100	0	50	44	8	32	16	1.76
NC_018041	241974-242009	18	1.9	19	88	5	56	36	33	2	27	1.72
NC_018041	270916-271069	69	2.2	69	84	2	202	25	18	18	38	1.93
NC_018041	270941-271089	69	2.2	69	86	4	203	24	21	15	38	1.92

NC_018041	327462-327529	34	2	34	94	0	118	20	32	17	29	1.96
NC_018041	331088-331149	31	2	31	90	0	97	11	22	16	50	1.76
NC_018041	332369-332410	21	2	21	95	0	75	9	40	19	30	1.83
NC_018041	334170-334225	30	2	28	86	13	80	26	12	44	16	1.83
NC_018041	341742-341805	21	3	21	100	0	128	28	29	9	32	1.88
NC_018041	346877-346912	18	2	18	100	0	72	38	0	44	16	1.48
NC_018041	351394-351431	18	2.1	18	95	0	67	34	18	18	28	1.95
NC_018041	376089-376127	20	2	20	84	0	51	0	28	10	61	1.28
NC_018041	406948-406991	20	2.2	20	100	0	88	31	13	22	31	1.93
NC_018041	472313-472338	13	2	13	100	0	52	38	0	15	46	1.46
NC_018041	479695-479737	21	2	21	90	0	68	39	20	23	16	1.92
NC_018041	481207-481232	12	2.2	12	100	0	52	26	34	15	23	1.94
NC_031323	168064-168145	36	2.3	36	100	0	164	23	21	30	24	1.99
NC_031323	180046-180072	14	1.9	14	100	0	54	66	11	22	0	1.22
NC_031323	182710-182752	21	2	21	86	8	61	34	25	20	18	1.96
NC_031323	298153-298182	11	2.7	11	94	0	51	40	0	0	60	0.97
NC_031323	333953-333989	18	2.1	18	100	0	74	62	0	27	10	1.28
NC_031323	379363-379401	18	2.2	18	95	0	69	30	7	25	35	1.84
NC_031323	405342-405383	2	22.5	2	81	13	54	47	2	0	50	1.14
NC_031323	407129-407168	19	2.1	19	90	0	62	35	7	12	45	1.7
NC_031323	452885-452923	20	2	20	89	0	60	28	15	30	25	1.96
NC_031323	479717-479747	15	2.1	15	93	0	53	45	16	6	32	1.72
NC_031323	491285-491325	21	2	21	95	0	73	34	9	26	29	1.88
NC_031323	541075-541103	14	2.1	14	100	0	58	58	6	6	27	1.5
NC_031323	552386-552442	24	2.4	24	100	0	114	8	29	22	38	1.85
NC_031323	552386-552452	24	2.8	24	93	0	98	8	26	20	43	1.82

NC_031806	110400-110425	13	2	13	100	0	52	46	15	0	38	1.46
NC_031806	237194-237259	24	2.8	24	92	0	114	48	7	18	25	1.74
NC_031806	312354-312413	18	3.3	18	97	0	111	33	26	10	30	1.89
NC_031806	312354-312423	18	4.1	17	92	5	81	35	25	8	30	1.86
NC_031806	330781-330817	18	2.1	18	94	0	65	29	24	18	27	1.98
NC_031806	331981-332011	15	2.1	15	100	0	62	35	25	0	38	1.56
NC_031806	332124-332170	24	2	24	82	0	58	23	14	31	29	1.95
NC_031806	350079-350144	24	2.8	24	95	0	114	22	21	15	40	1.9
NC_031806	350132-350195	18	3.6	18	89	0	92	20	34	17	28	1.95
NC_031806	402577-402606	15	2	15	100	0	60	40	6	13	40	1.71
NC_031806	440298-440340	19	2.6	15	75	25	50	25	6	11	55	1.6
NC_031806	440304-440341	19	2	19	100	0	76	26	5	10	57	1.53
NC_031806	457267-457310	22	2	22	95	0	79	31	29	27	11	1.91
NC_031806	481432-481456	12	2.1	12	100	0	50	8	24	28	40	1.83
NC_034982	263418-263464	24	2	24	86	0	67	29	21	25	23	1.99
NC_034982	264919-264970	28	2	25	85	14	70	17	42	11	28	1.84
NC_034982	337073-337105	16	2.1	16	94	0	57	36	18	27	18	1.94
NC_034982	427450-427497	25	2	23	84	15	62	37	12	33	16	1.86
NC_034982	681553-681610	21	2.8	21	80	10	57	44	15	27	12	1.82
NC_023103	110693-110742	25	2	25	100	0	100	28	28	4	40	1.74
NC_023103	208003-208043	21	2	21	100	0	82	46	4	14	34	1.66
NC_023103	246827-246860	16	2.1	17	88	5	52	47	8	5	38	1.59
NC_023103	246807-246864	19	2.9	21	71	23	52	41	8	8	41	1.66
NC_023103	342398-342426	14	2.1	14	100	0	58	27	6	13	51	1.66
NC_023209	21035-21082	16	2.9	16	76	14	53	33	12	20	33	1.9
NC_023209	147782-147836	24	2.5	22	85	8	67	14	23	12	49	1.78

NC_023209	147804-147871	21	3.2	21	77	8	75	11	22	10	55	1.65
NC_023209	150188-150242	18	3.1	18	91	0	83	29	9	25	36	1.87
NC_023209	172593-172632	15	2.5	17	88	8	57	10	17	2	70	1.27
NC_023209	295019-295071	21	2.5	22	87	9	74	49	0	0	50	1
NC_023209	371146-371177	16	2	16	93	0	55	25	21	15	37	1.93
NC_049064	81772-81806	16	2.3	16	90	5	54	2	37	14	45	1.59
NC_049064	132247-132300	15	3.6	15	94	0	90	40	11	29	18	1.85
NC_049064	160514-160561	6	8	6	100	0	96	33	33	0	33	1.58
NC_049064	185885-185910	13	2	13	100	0	52	46	15	0	38	1.46
NC_049064	269642-269742	24	4.2	24	96	0	175	35	3	35	24	1.74
NC_049064	283791-283836	23	2	23	91	8	76	13	26	13	47	1.78
NC_049064	334317-334395	26	3	26	100	0	158	26	18	15	39	1.91
NC_049064	340274-340318	23	1.9	24	90	4	74	51	15	26	6	1.68
NC_049064	422418-422487	6	11.7	6	100	0	140	17	31	0	51	1.45
NC_049064	470684-470718	18	1.9	18	88	0	52	54	8	8	28	1.6
MW553042	24043-24082	15	2.5	16	88	8	55	70	2	17	10	1.27
MW553042	33394-33438	22	2	22	82	0	54	20	22	15	42	1.89
MW553042	100394-100439	23	2	23	91	8	76	47	13	26	13	1.78
MW553042	162197-162267	37	1.9	37	94	0	124	14	14	50	21	1.77
MW553042	216408-216447	20	2	20	95	0	71	0	32	10	57	1.32
MW553042	320051-320098	24	2	24	91	0	78	33	20	39	6	1.78

Table S5 PCR primers used to validate recombination products of the nine repeats in the *S. miltiorrhiza* mitogenome.

Primer Name	Primer Sequence
sami-r01-F1	CAGTCTATTTGCCCCTGTCC
sami-r01-R1	TAATCGACGGCTGCTAACTCG
sami-r01-F2	AGGTTTACATTCCTCCCTGCA
sami-r01-R2	ACCCTTATTCCGGTGTCCCT
sami-r02-F1	ATGGGGTGGAAGTGGAGATG
sami-r02-R1	TCCCATCTTGATTTGCCCTCT
sami-r02-F2	TTGACCGGAACTGGAGTCTC
sami-r02-R2	CGCTGATCTGATCCTCCCTT
sami-r03-F1	CACCAGTAGCCAAGGAAGGA
sami-r03-R1	GAAAACACGCCCTCTTTGGT
sami-r03-F2	TCCTCAACCTCATCTGCAGC
sami-r03-R2	GGAAGAGCACGAACAAGAGC
sami-r04-F1	CAACGTCGCTTGGATCAGAG
sami-r04-R1	AGCGCCGAGAGATCTGTAA
sami-r04-F2	GCTTTATGGTCCCTGTCCCT
sami-r04-R2	TGGTGGTTGAGTAAGGGCAT
sami-r05-F1	GTCTTCGAGCCAGTACCCTT
sami-r05-R1	TTGGGCATGAATGGAGCTTG
sami-r05-F2	TAACCATCACAGGACCCGAC
sami-r05-R2	CCCCAGAGTGTGCCTTTCTT
sami-r06-F1	TGAACCGAGGACTCTTGCTT
sami-r06-R1	CAAGTGAGTGAGTGGCAGTG
sami-r06-F2	TCTTTCCCCTCACAAGCCAT
sami-r06-R2	CCAGGCCCATCAGACCATAA
sami-r07-F1	CTACGCGCGTATACTGGTTG
sami-r07-R1	CGTAGTTGATGATTCCGCCG
sami-r07-F2	CGCCCGGATACAAAGGAATC
sami-r07-R2	CTCCCCATATTCCCCACTCA
sami-r08-F1	CTGAGGGTAGGGTTAAGGGC
sami-r08-R1	GCCCCAAGAAGCATCAAACA
sami-r08-F2	CATCTCCGCTACTCCACTC
sami-r08-R2	ACCTATCATCGCCGGCATAA
sami-r09-F1	ACACCTCAGGCCCCCTTTATC
sami-r09-R1	CCCGGGTTAACTCTTCCAT
sami-r09-F2	GCTCTGAAAACCACCTCAAATC
sami-r09-R2	AGAAAGTATGAGGCCCCGTCC

Table S6 Chloroplast DNA insertions in the *S. miltiorrhiza* mitogenome. The three longest regions (sami-mtpt-001, sami-mtpt-002, and sami-mtpt-012) similar to the mitogenome sequence are indicated with “*”. “MC1/2”: mitogenome chromosome 1/2, frag: fragment.

MTPT ID	Name of Circular molecule	Identity (%)	Alignment Length (bp)	Number of Mismatches	Number of Gap openings	Positions on the Chloroplast Genome		Positions on the Mitochondrial Genome		Genes Located in the MTPT Fragments	E value	Score
						Start	End	Start	End			
sami-mtpt01	MC1	98.10	1949*	23	6	70841	72786	72166	70229	<i>psbB</i> -frag	0	3382
sami-mtpt-002	MC1	99.71	1737*	2	1	63951	65684	308153	309889	<i>petL,petG,trnW-CCA,trnP-UGG</i> -frag	0	3177
sami-mtpt-003	MC1	100	623	0	0	32783	33405	230470	229848	<i>psbC</i> -frag	0	1151
sami-mtpt-004	MC1	100	135	0	0	32376	32510	264504	264638	<i>psbD</i> -frag	2.06E-65	250
sami-mtpt-005	MC1	75.22	452	102	9	10441	10887	119207	118761	<i>atpA</i> -frag	4.52E-52	206
sami-mtpt-006	MC1	94.03	134	7	1	40981	41113	264689	264822	<i>psaA</i> -frag	5.85E-51	202
sami-mtpt-007	MC1	87.64	178	11	6	45376	45553	298972	299138	<i>rps4</i> -frag	2.72E-49	196
sami-mtpt-008	MC1	94.78	115	6	0	44704	44818	298852	298966	<i>trnS-GGA</i>	2.74E-44	180

sami- mtpt-009	MC1	91.04	134	2	3	132622	132754	164757	164881	<i>trnI</i> -GAU-frag	4.59E-42	172
sami- mtpt-010	MC1	90.07	131	12	1	29796	29925	298382	298252	<i>trnD</i> -GUC	5.93E-41	169
sami- mtpt-011	MC2	99.92	4987*	3	1	51184	56169	78467	73481	<i>trnM</i> -CAU, <i>atpE</i> , <i>atpB</i> , <i>rbcL</i>	0.00E+00	9186
sami- mtpt-012	MC2	99.52	632	3	0	37959	38590	23985	24616		0.00E+00	1151
sami- mtpt-013	MC2	98.79	332	4	0	23320	23651	30454	30785		7.69E-169	592
sami- mtpt-014	MC2	88.73	355	33	2	717	1070	21738	22086		2.30E-119	427
sami- mtpt-015	MC2	100	214	0	0	83019	83232	53309	53522		6.47E-110	396
sami- mtpt-016	MC2	76.37	127	30	0	40230	40356	24032	24158		1.60E-11	69.4

Figure S1. Comparison of the sequences of the plastome assembled in this study and NC_020341.1 of *S. miltiorrhiza*. The X-axis shows the nucleotide sequence of plastome assembled in this study, and the Y-axis displays the nucleotide sequence of NC_020341.1.

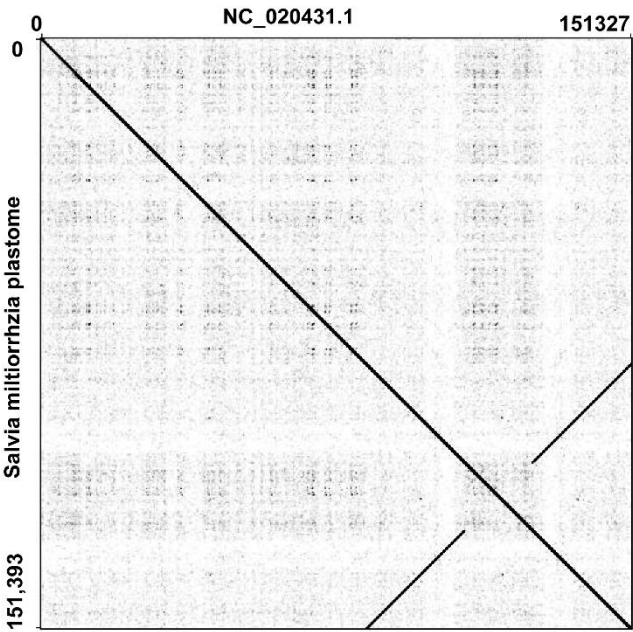
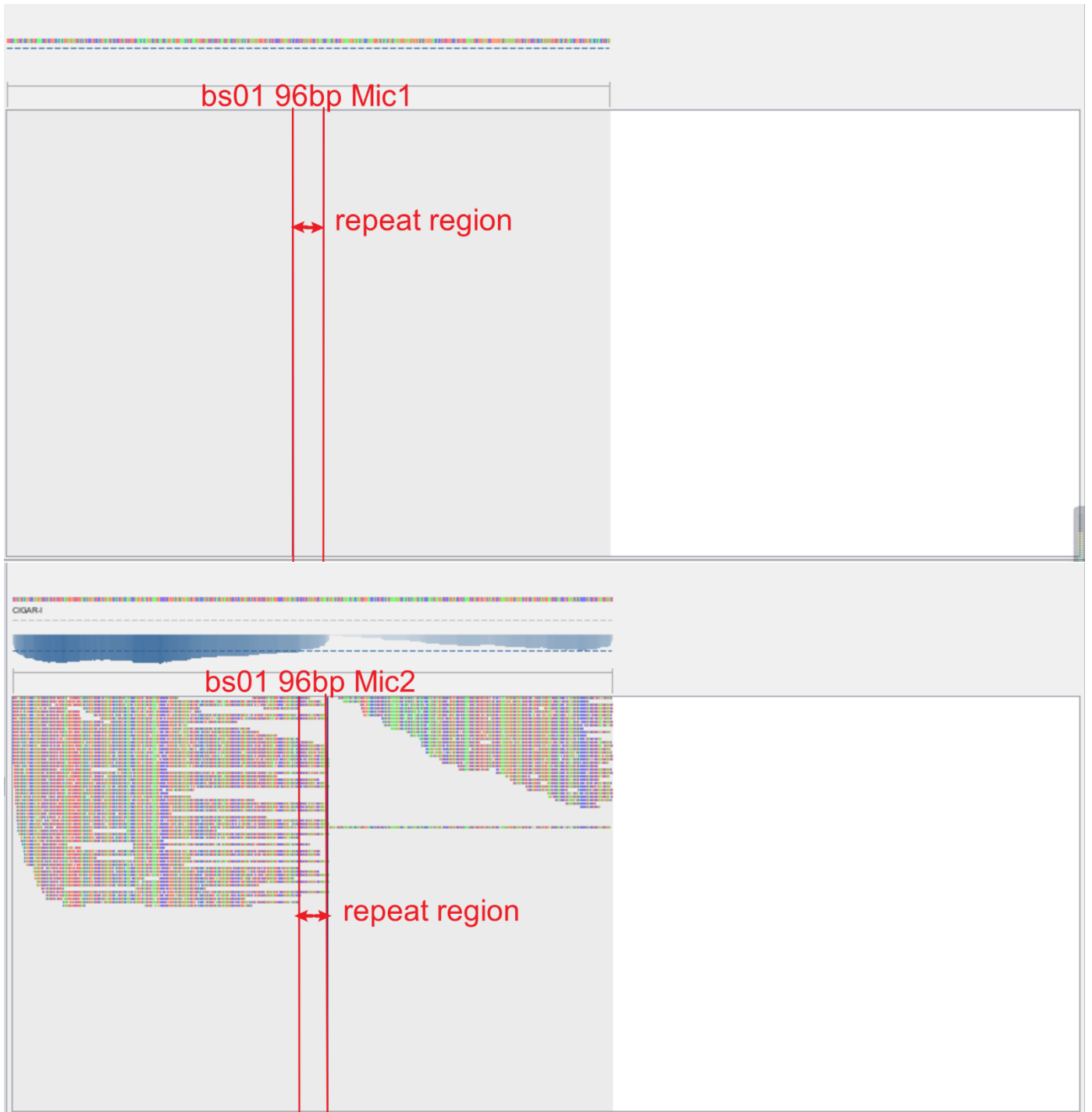


Figure S2. Alignments of long PacBio reads to the four conformations of the seven DBS (bs01–bs07) found in the unitig graph. The unitig graph was generated using Unicycler from Illumina reads, which were filtered with GetOrganelle for mitochondrial reads. Mac corresponds to the connection of shared contigs and their flanking contigs found in MC1 and MC2 (Fig. 1B). Mic corresponds to the alternative connection of the shared contigs and their flanking contigs that are not found in MC1 and MC2. Panels a–g show Mac1 and Mac2 and Mic1 and Mic2 of bs01–bs07, respectively. The figure in each panel can be divided into the top and bottom parts. The top part shows a bird's eye view, whereas the bottom part reveals a base-level view. At the bottom part of the figure, the DBS ID, length of shared contig, and conformation name are shown. The boundaries of shared contig are indicated with red vertical lines. The contigs in the DBS are shown as the red line having arrows at each end. It was labeled as the “repeat region”.

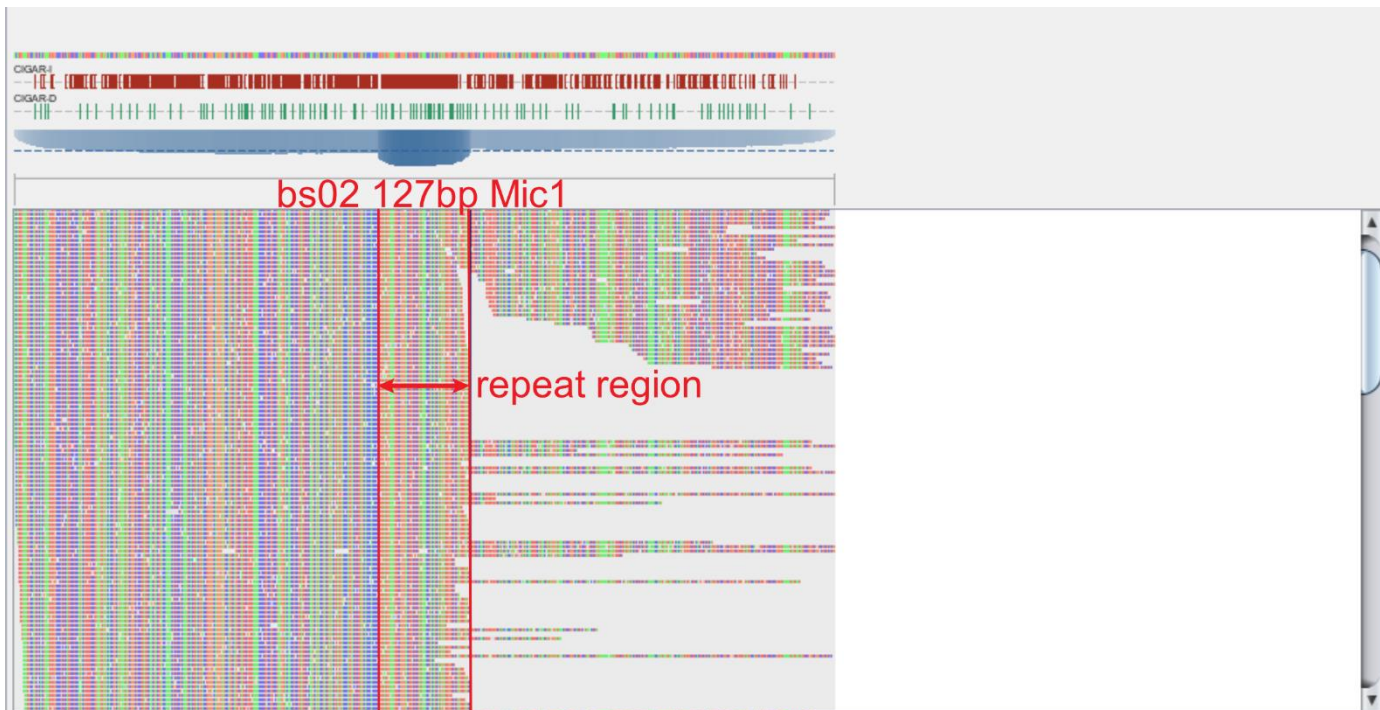
a





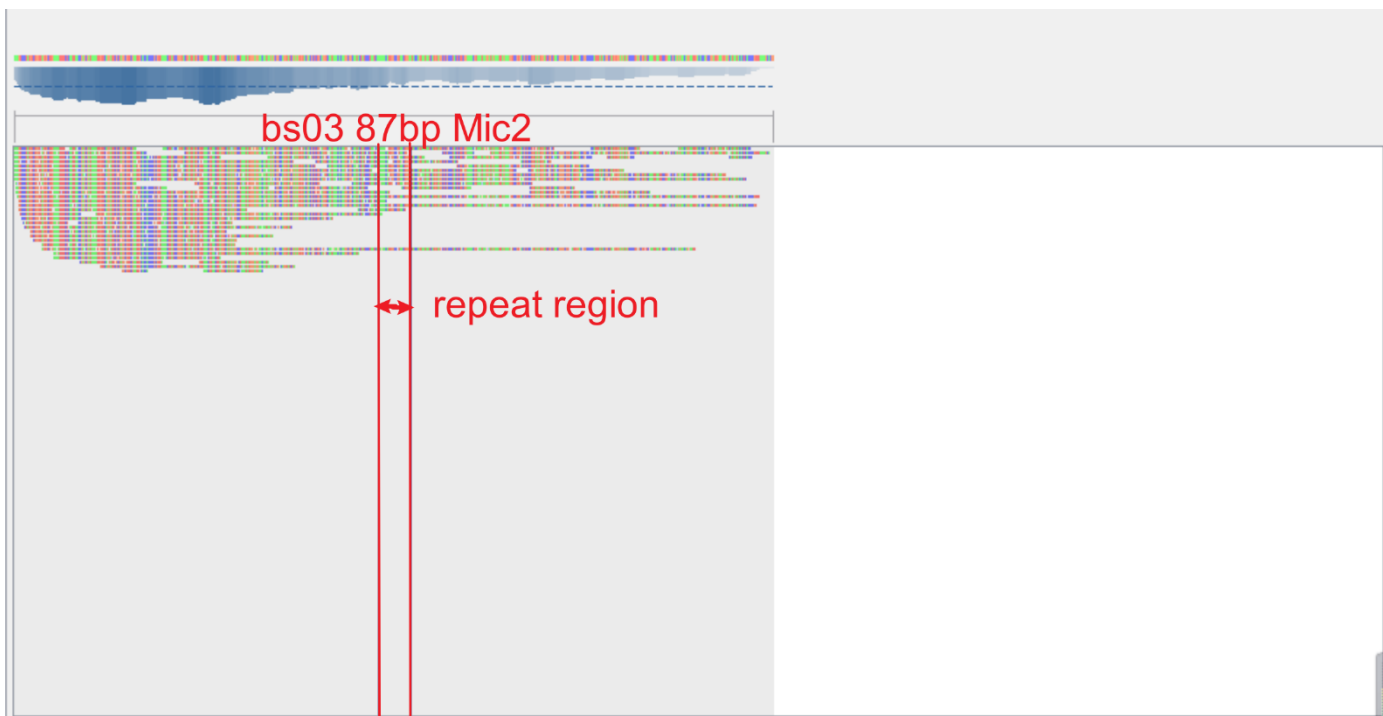
b





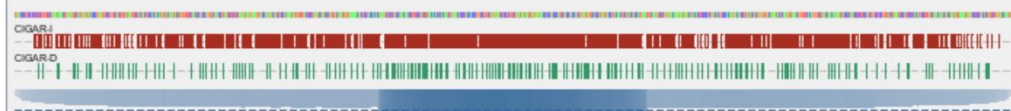
c



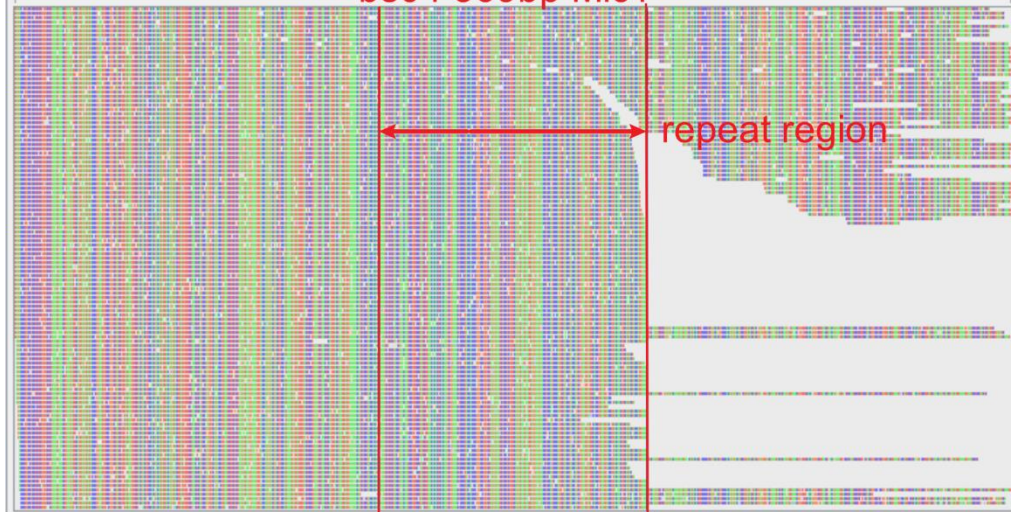


d





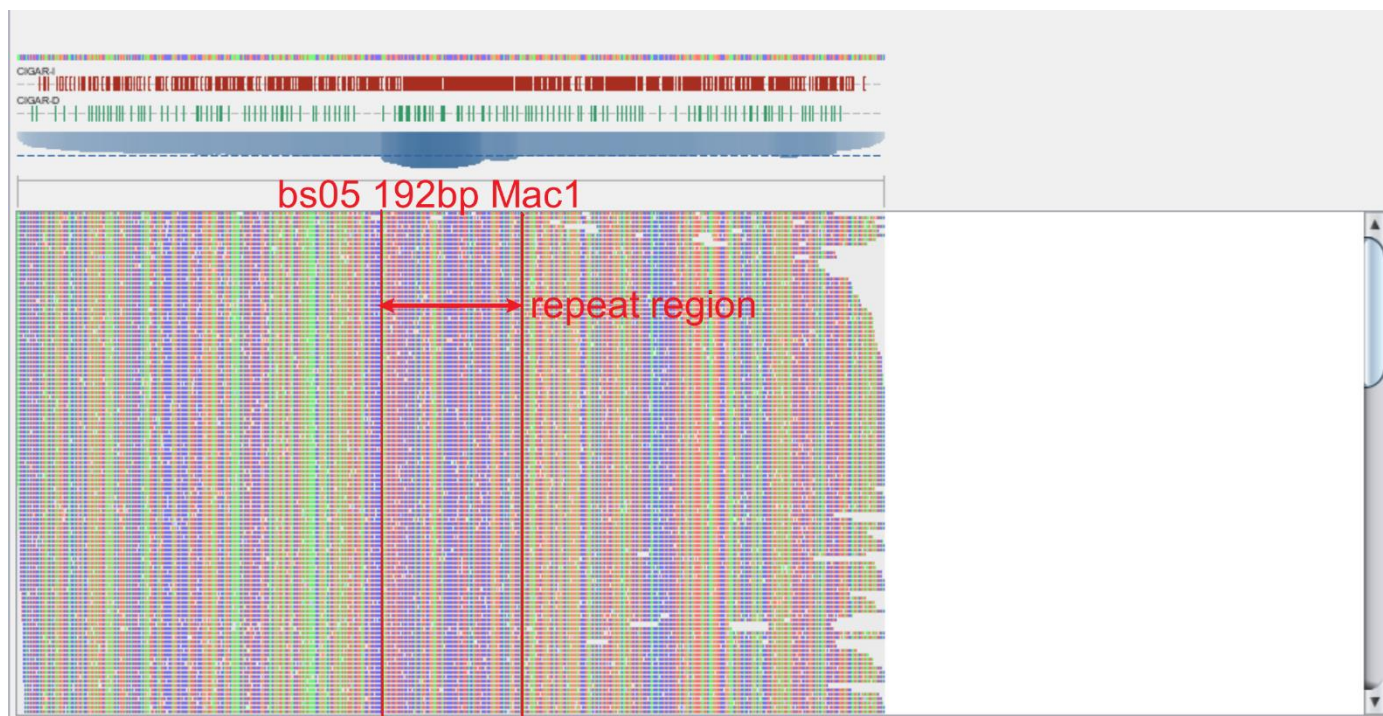
bs04 369bp Mic1

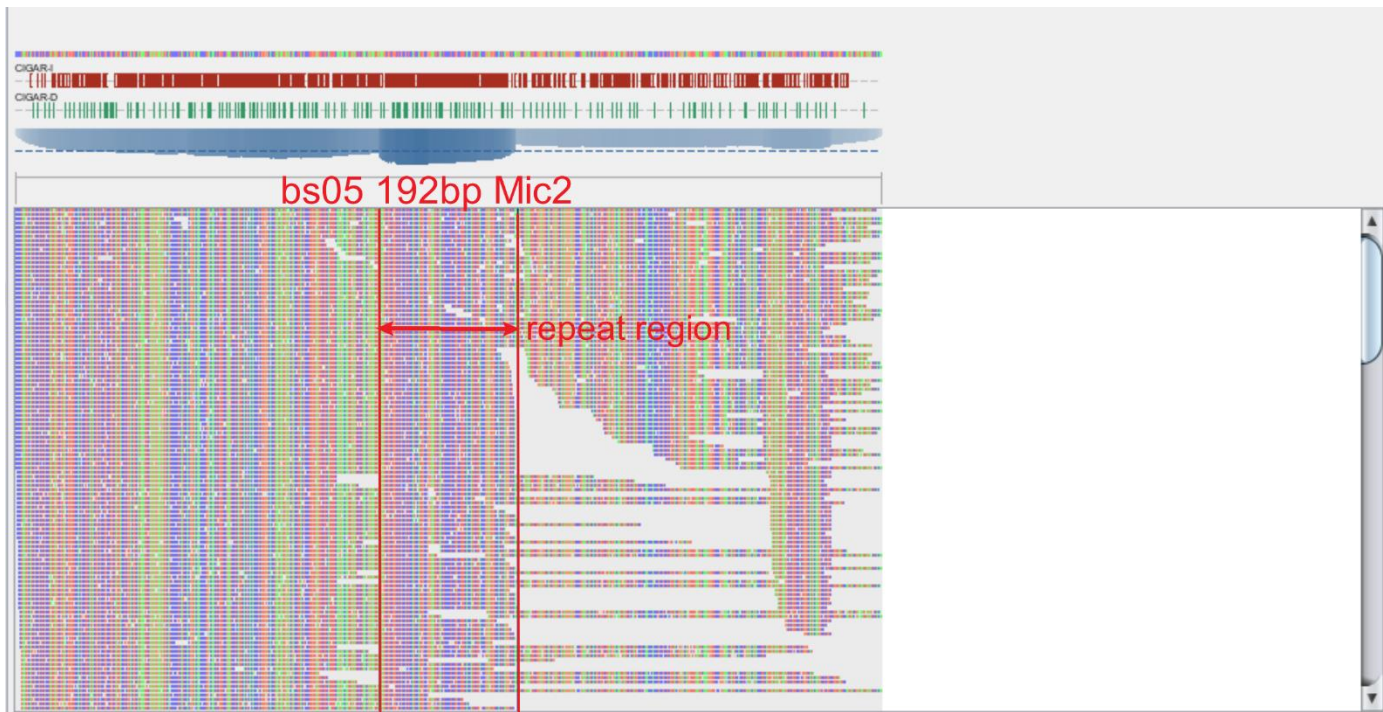


bs04 369bp Mic2



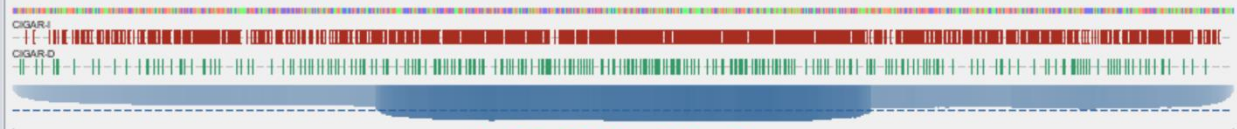
e



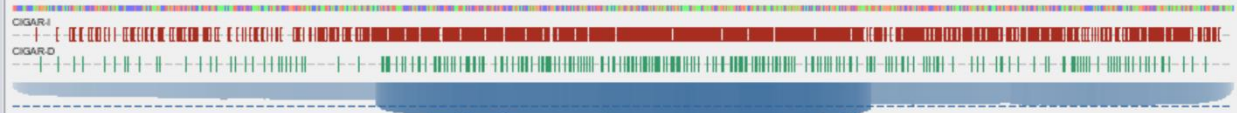
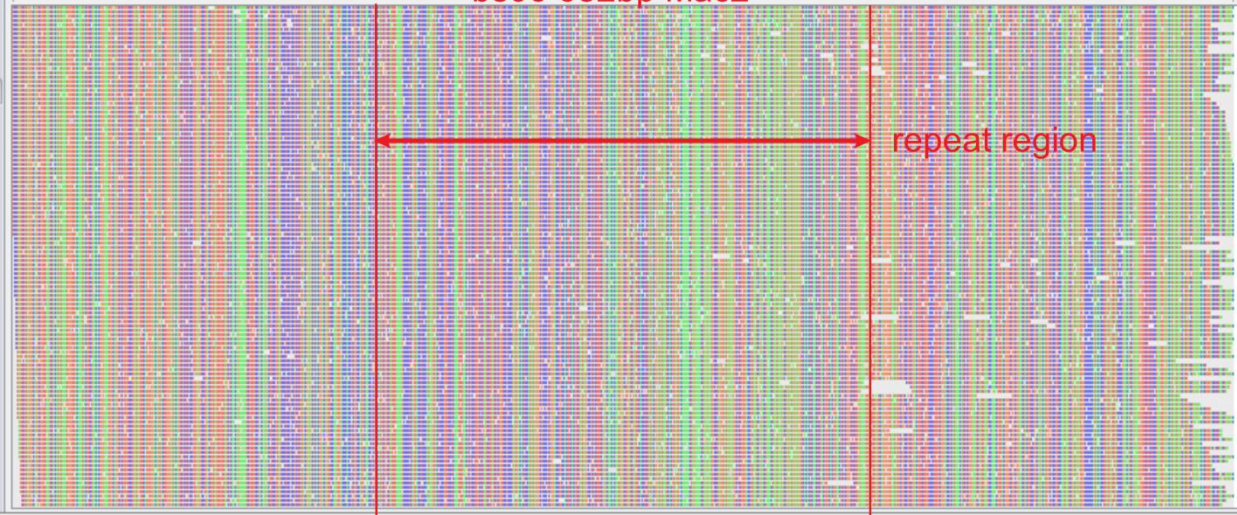


f

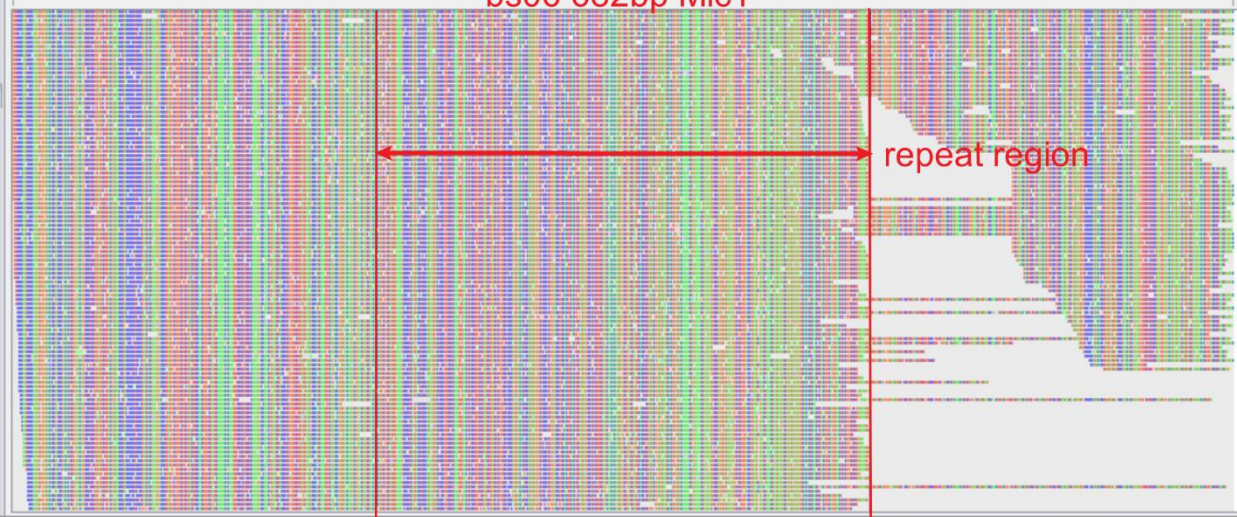


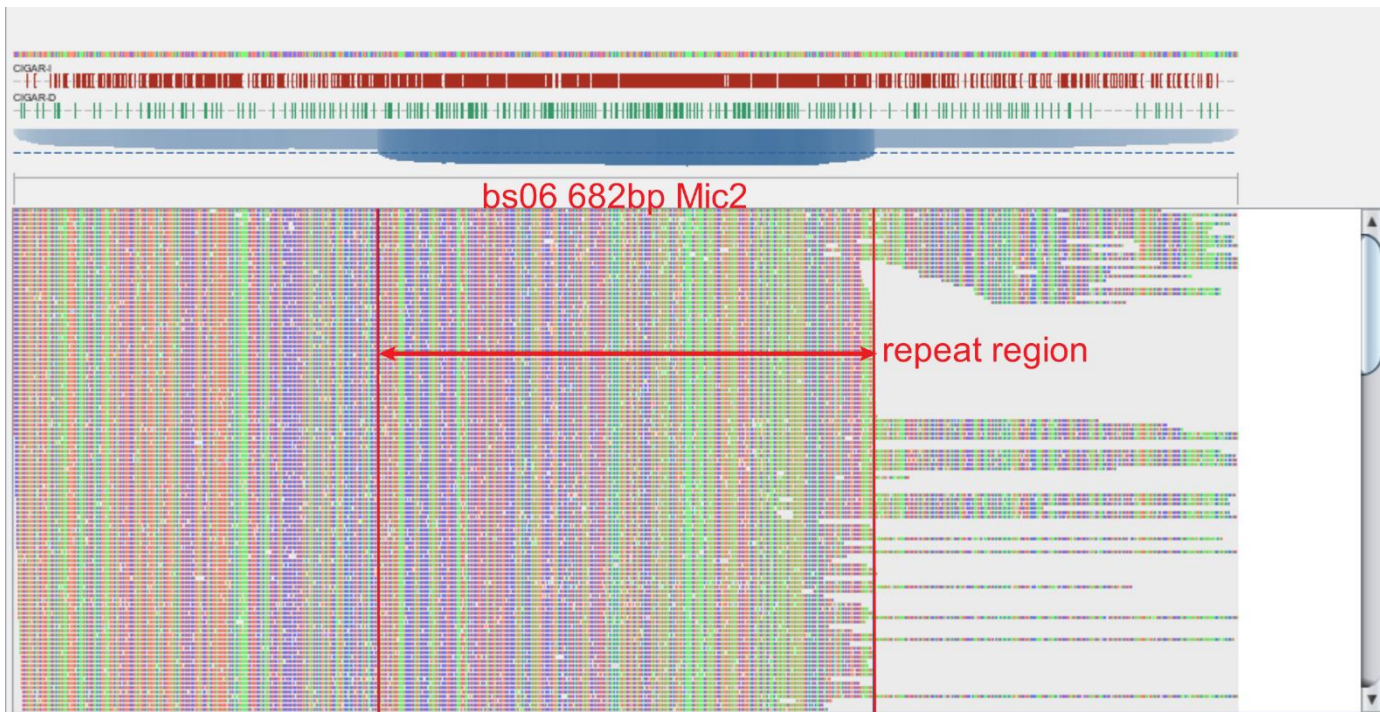


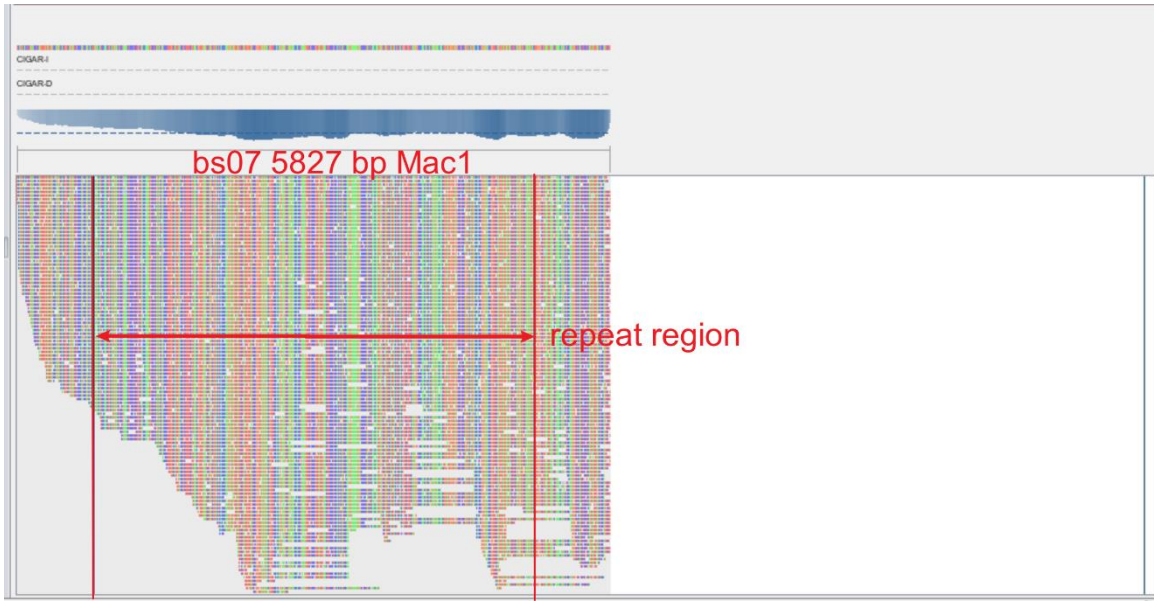
bs06 682bp Mac2



bs06 682bp Mic1









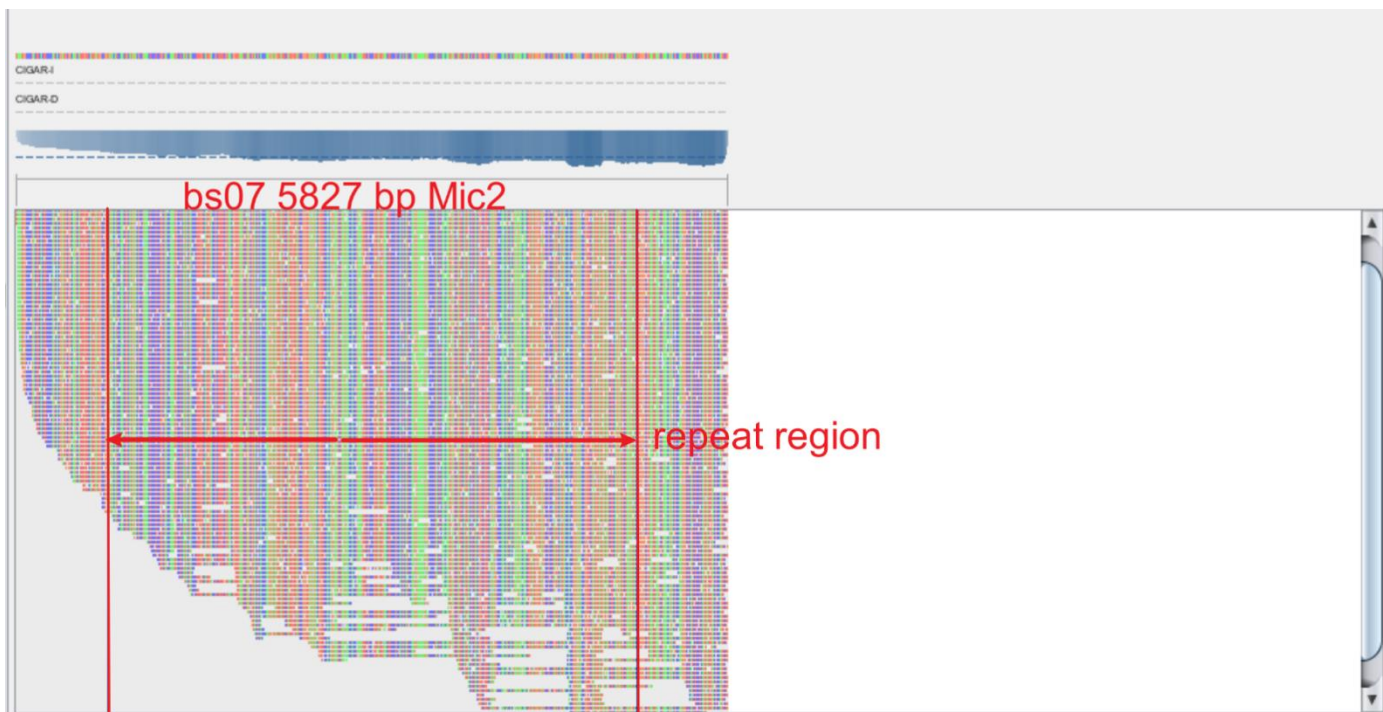


Figure S3. Mapping results of PacBio and Illumina reads to the MC1 and MC2 of *S. miltiorrhiza*. Panels a and b show the mapping results of PacBio reads to MC1 and MC2, respectively. Panels c and d show the mapping results of all Illumina reads to MC1 and MC2, respectively. The X- and Y-axis show the nucleotide position and corresponding coverage depth, respectively.

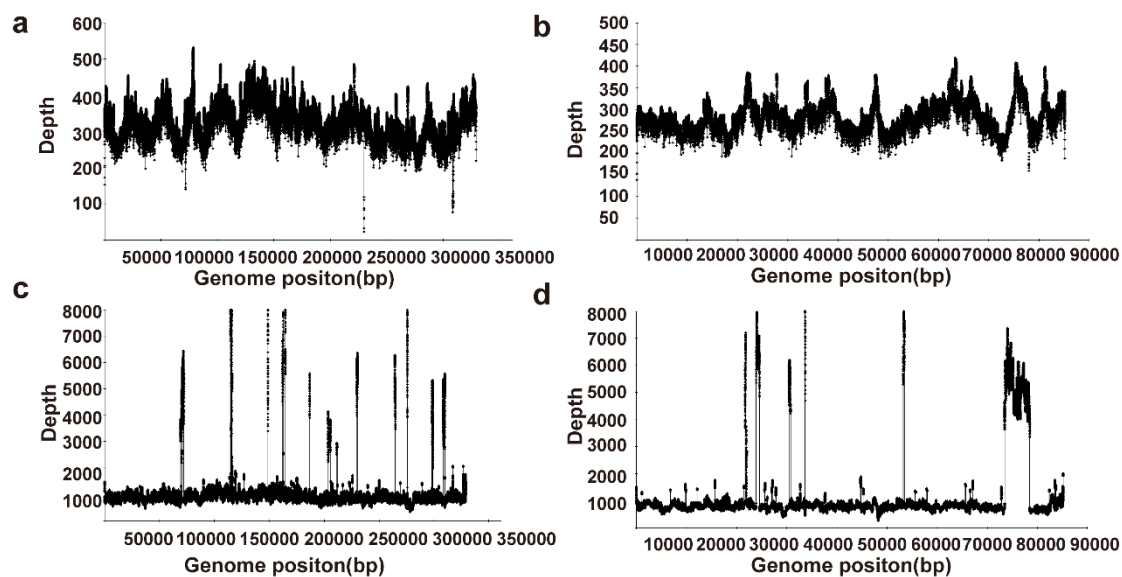


Figure S4. Comparison of the two assemblies of the mitogenome of *S. miltiorrhiza*. Comparison of the sequences of MC1 (A), MC2 (B), and NC_023209.1. The X-axis shows the nucleotide sequence of MC1, and the Y-axis presents the nucleotide sequence of NC_023209.1. The largest collinear block between them was 66,778 bp in length and is highlighted with a red circle.

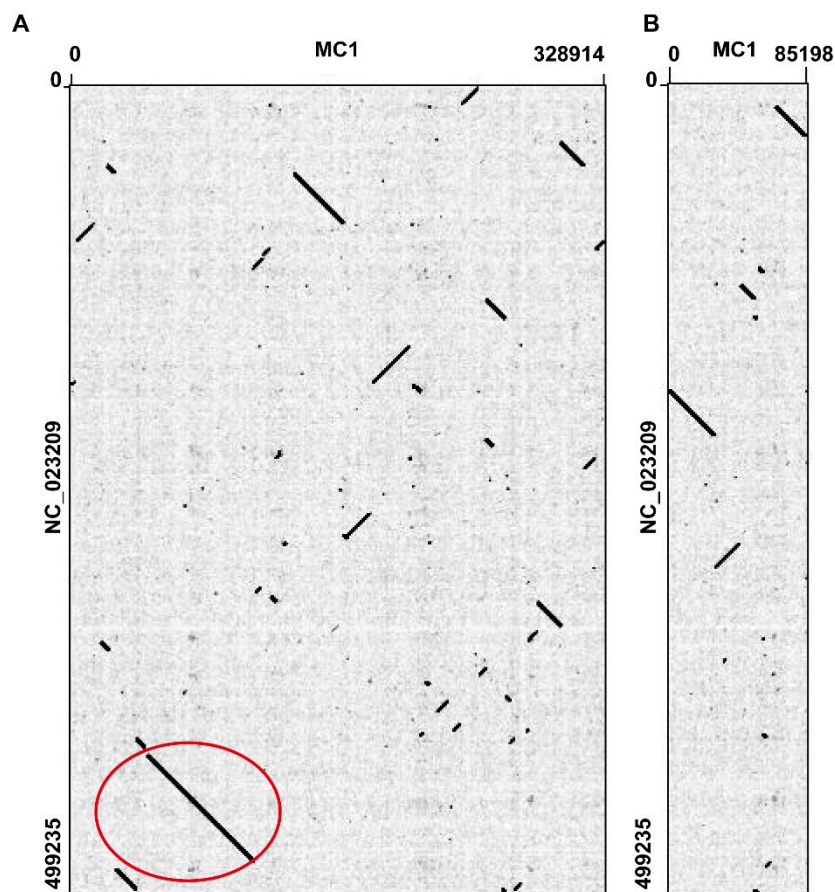


Figure S5. Mapping results of PacBio long reads to the mitogenome of *S. miltiorrhiza* in GenBank (NC_023209.1). The X-axis shows the nucleotide position, and the Y-axis reveals the corresponding coverage depth. Three large regions not supported by the long reads in this study are highlighted with red squares.

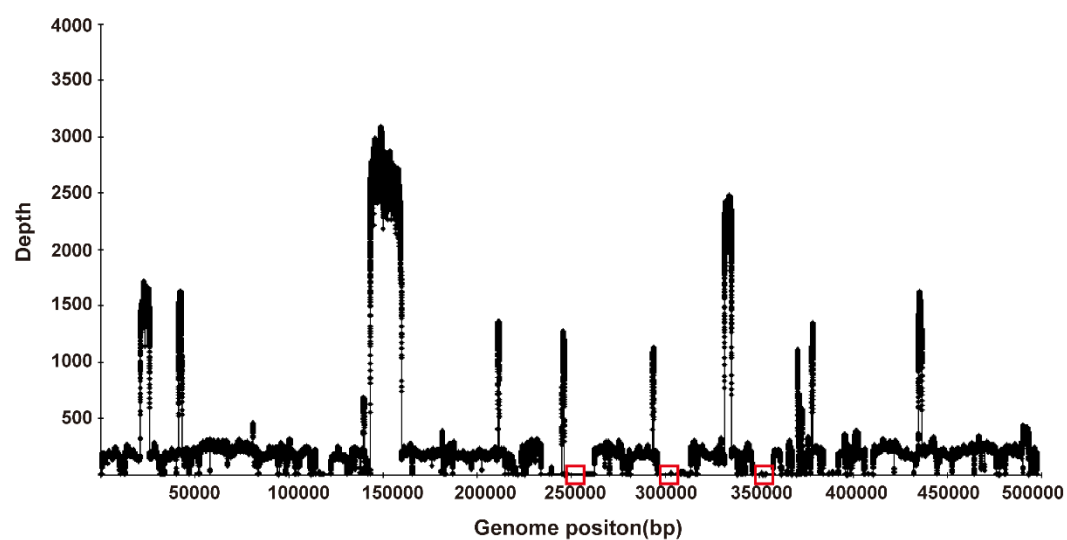
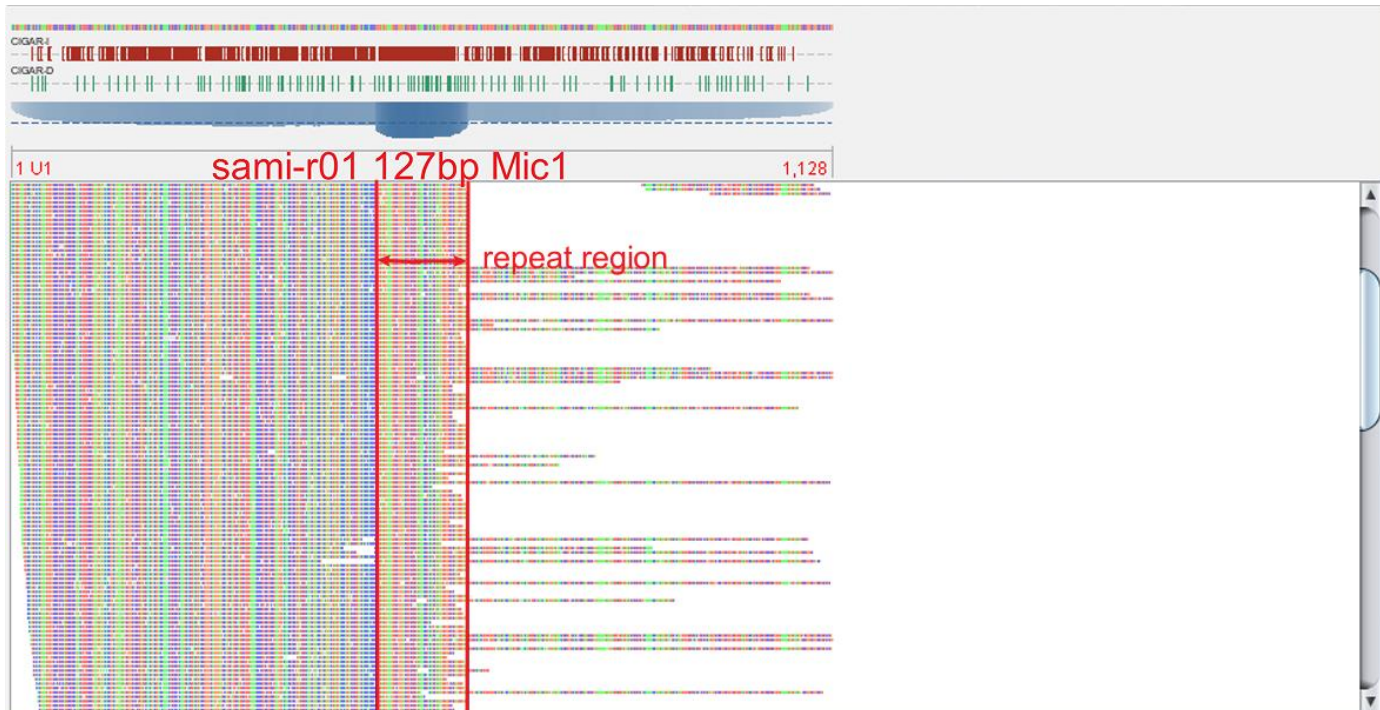


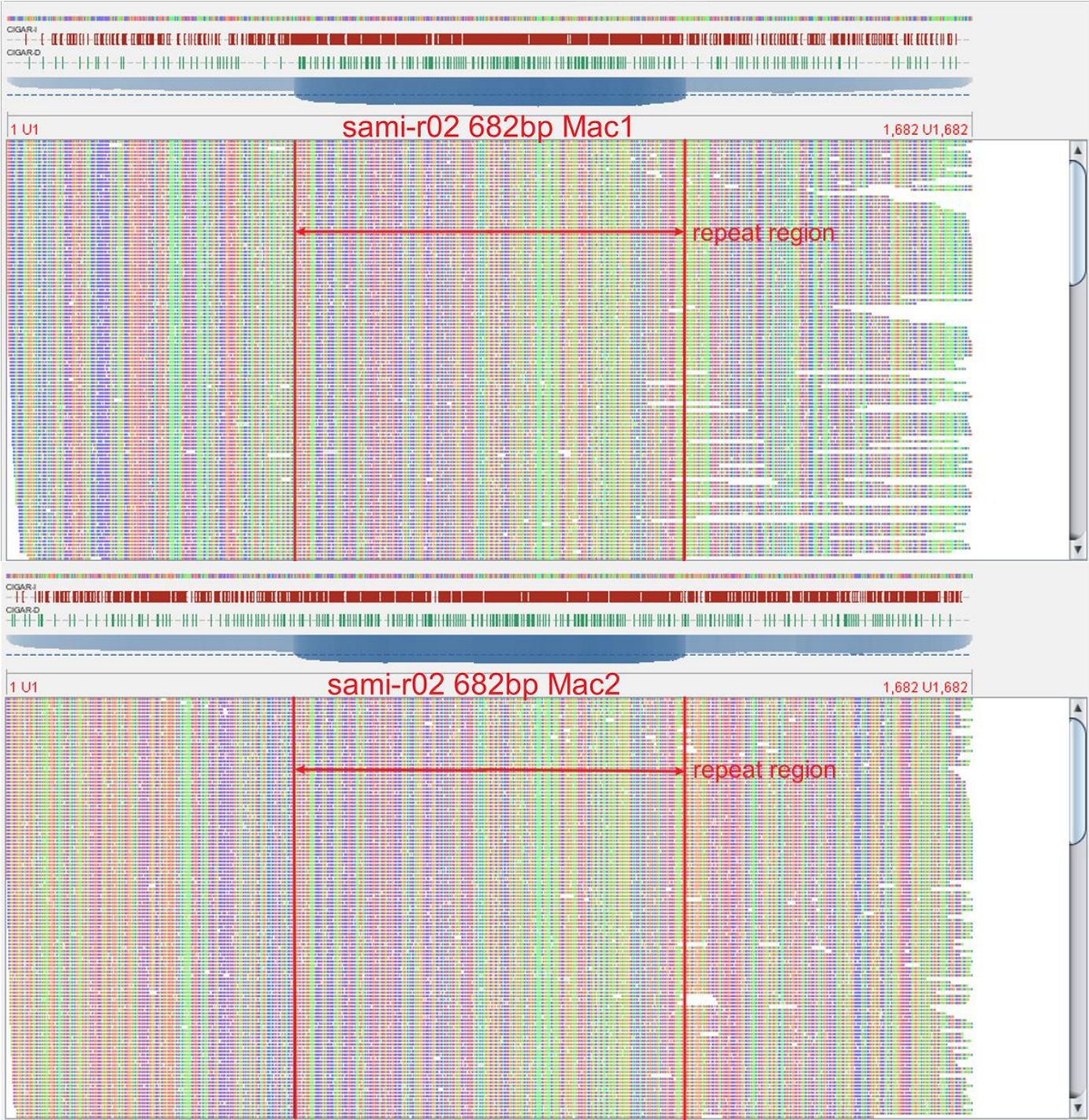
Figure S6. Alignments of long PacBio reads to the Mac and Mic resulted from nine pairs of HSPs (sami-r01 to sami-r09). The nine HSPs were identified using BLASTn with the e-value = 1e-6. The alignment of two HSPs generated a DBS, similar to the ones observed in the unitig graph (Fig. 1). In the presence of homologous recombination, the DBSs formed by the alignment of HSPs and their flanking sequences will also have four conformations, similar to those shown in Fig. 1B. We constructed the sequences corresponding to the four conformations of each DBS for each HSP. We then mapped the long PacBio reads to these sequences. The results are shown in panels a - i for sami-r01 to sami-r09, respectively. We named the conformations having the most supporting reads as Mac1 and Mac2. The reference sequences of Mac1 and Mac2 are reverse-complementary to each other. The alternative conformations were named as Mic1 and Mic2. The figure in each panel can be divided into the top and bottom parts. The top part shows a bird's eye view, whereas the bottom part reveals a base-level view. At the bottom part of the figure, the HSP ID, length of HSP, and conformation name are shown. The boundaries of HSPs are indicated with red vertical lines. The HSPs are shown as the red line having arrows at each end. They were labeled as “repeat region”.

a





b



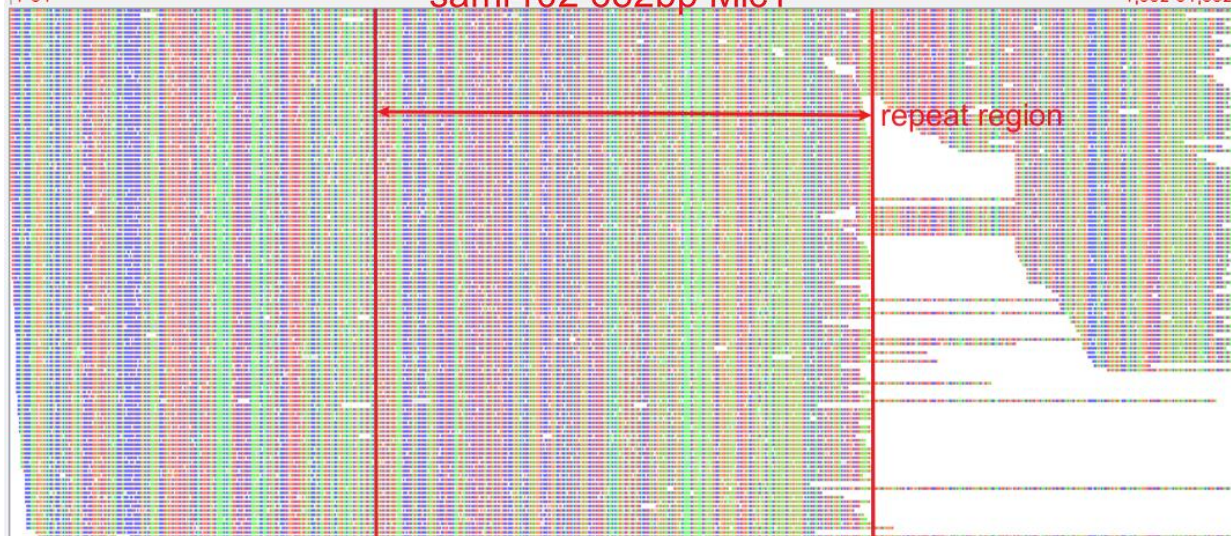
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CIGAR:D

1 U1

sami-r02 682bp Mic1

1,682 U1,682



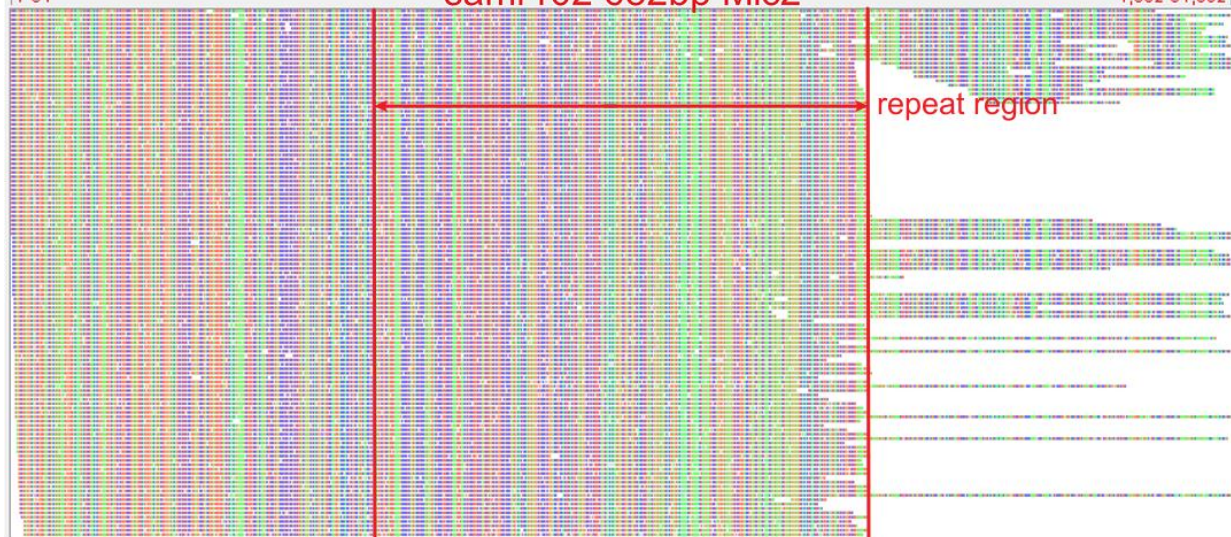
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CIGAR:D

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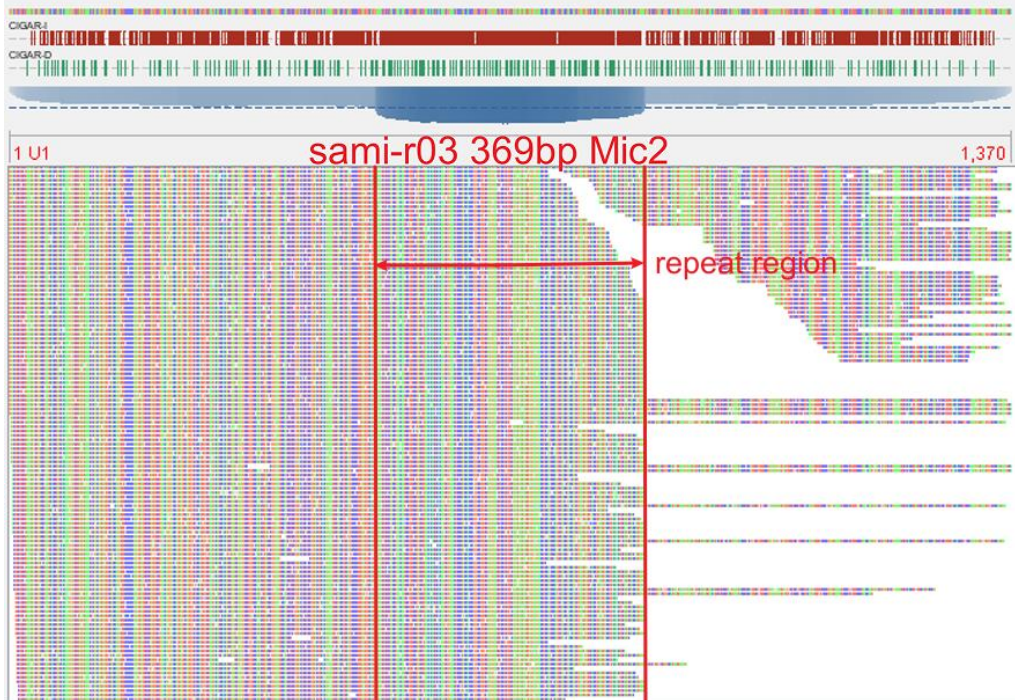
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1,682 U1,682



c



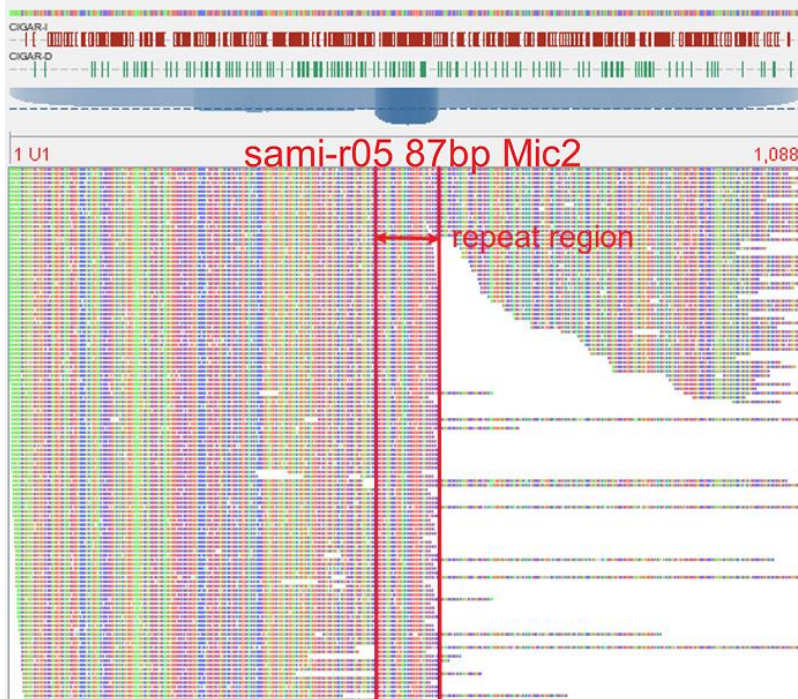
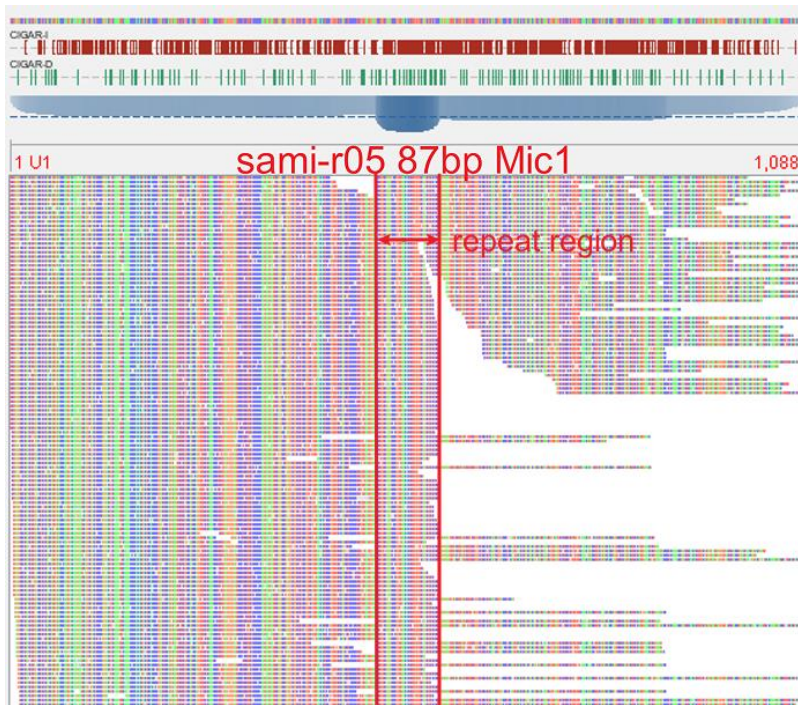


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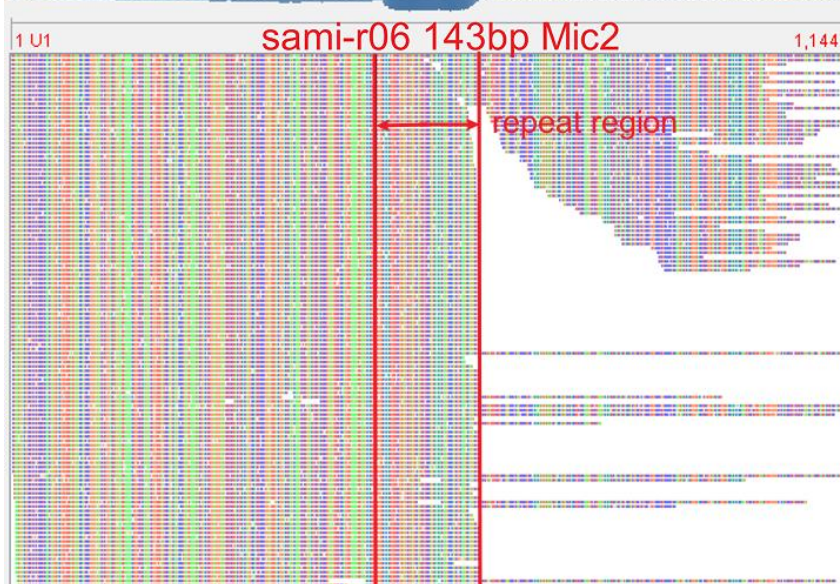
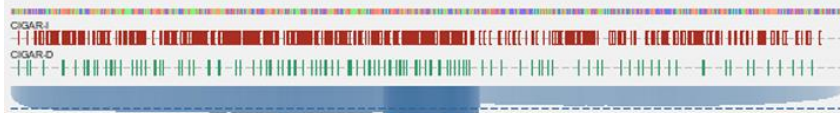
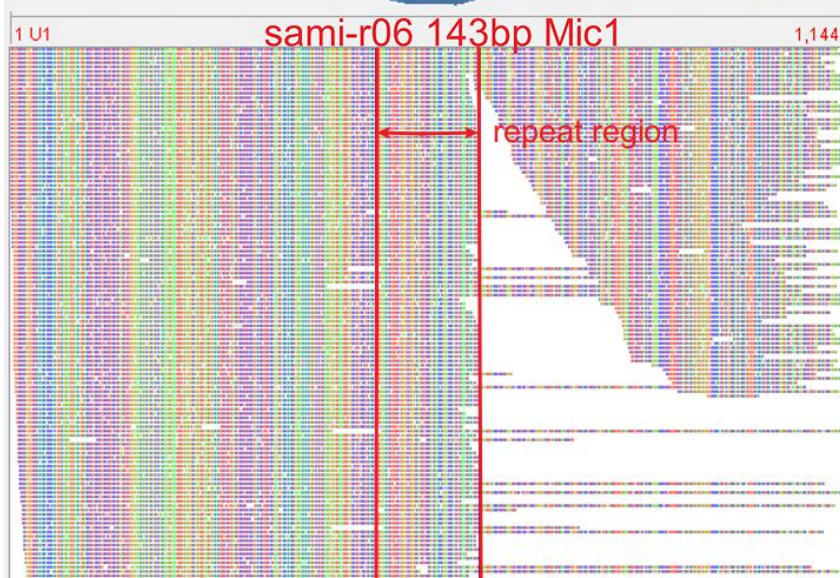
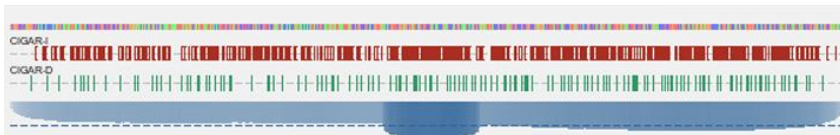
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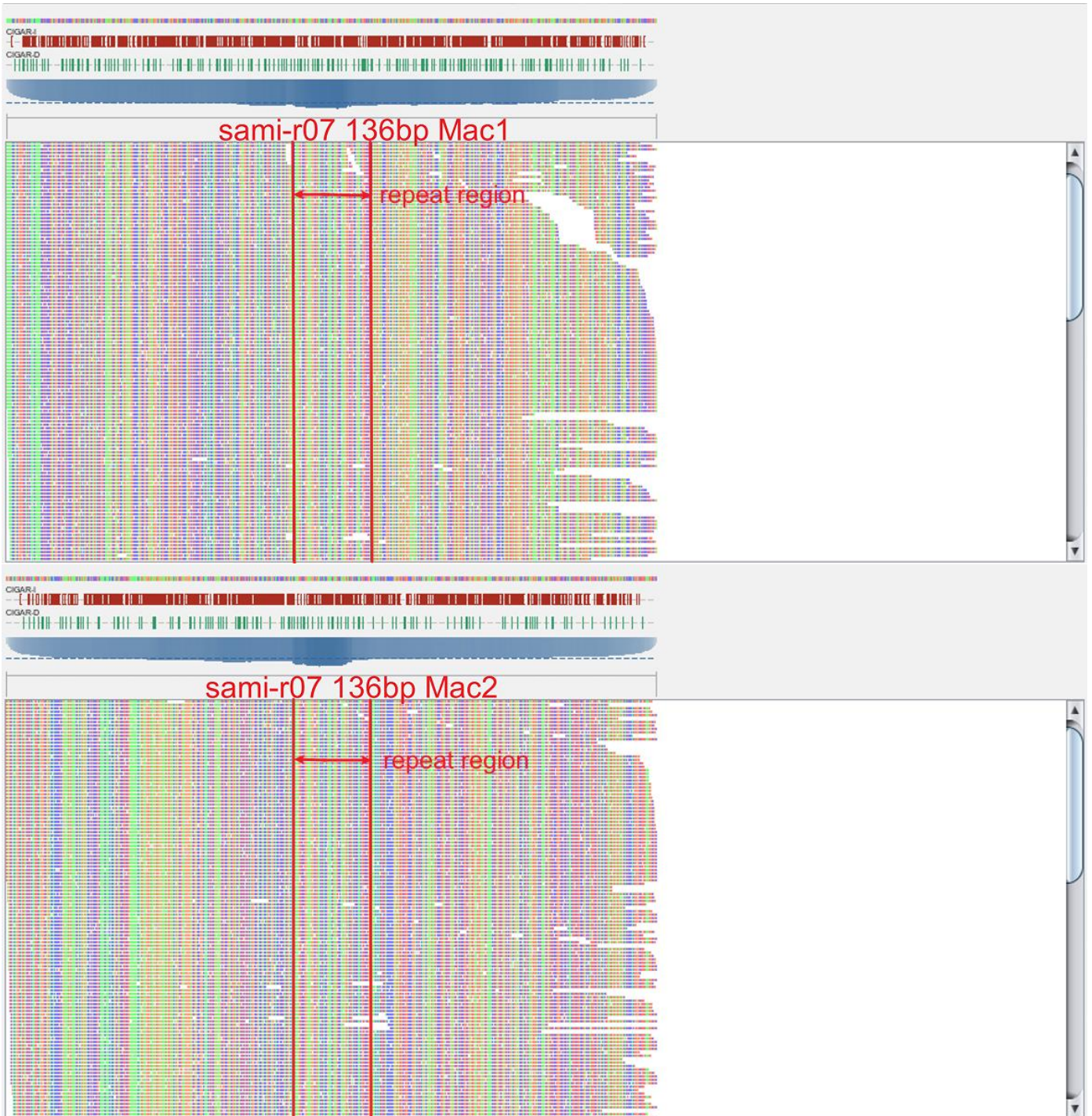


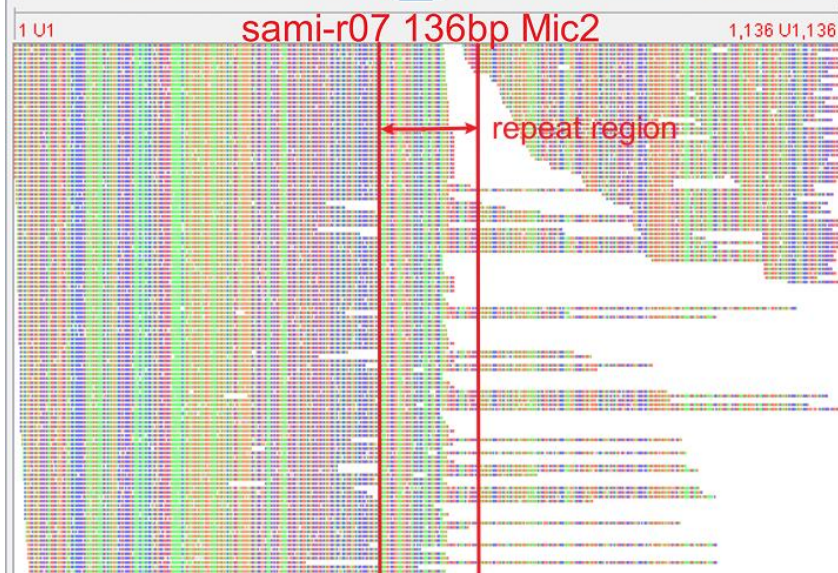
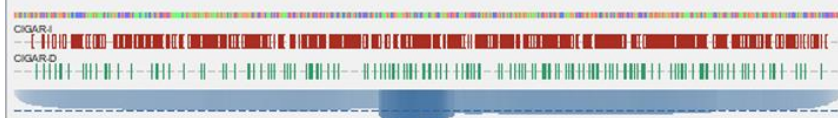
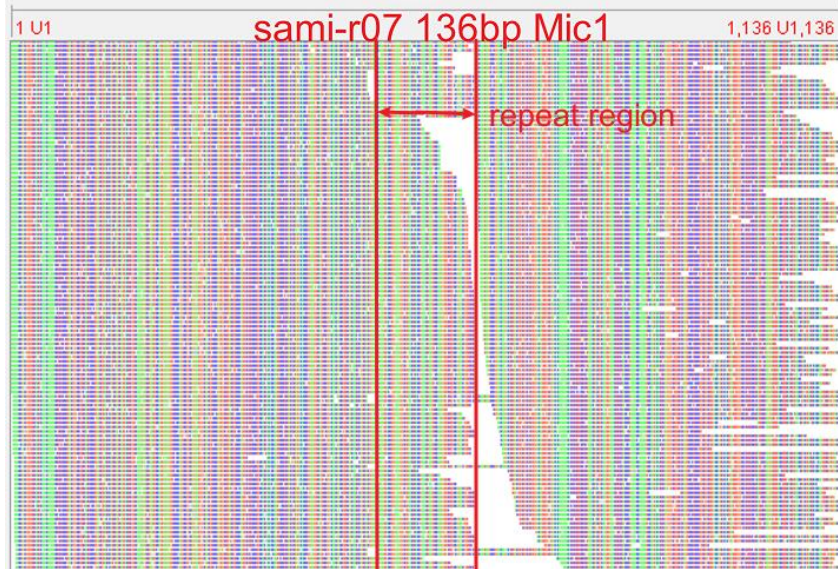
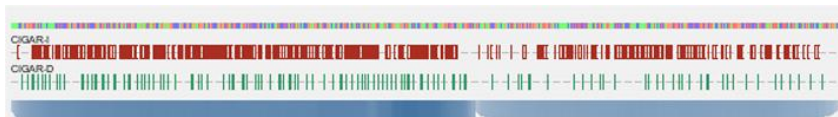


f

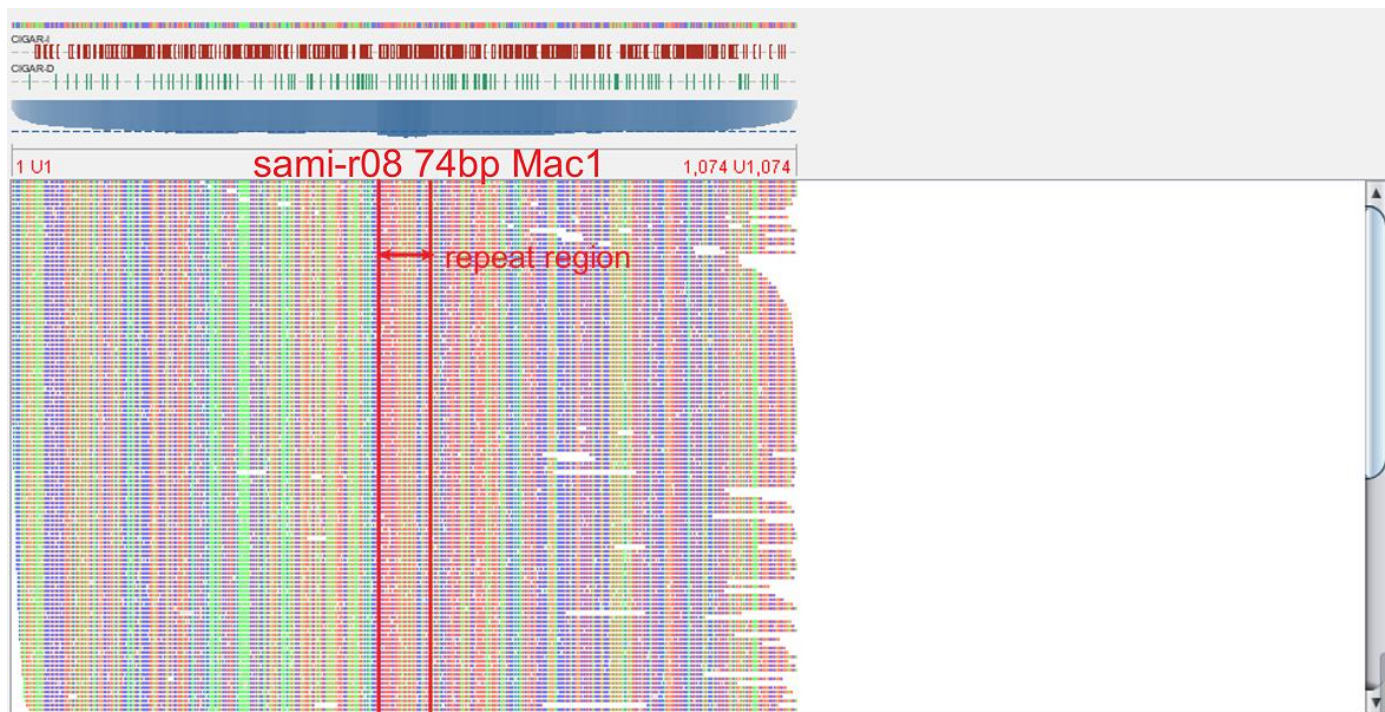


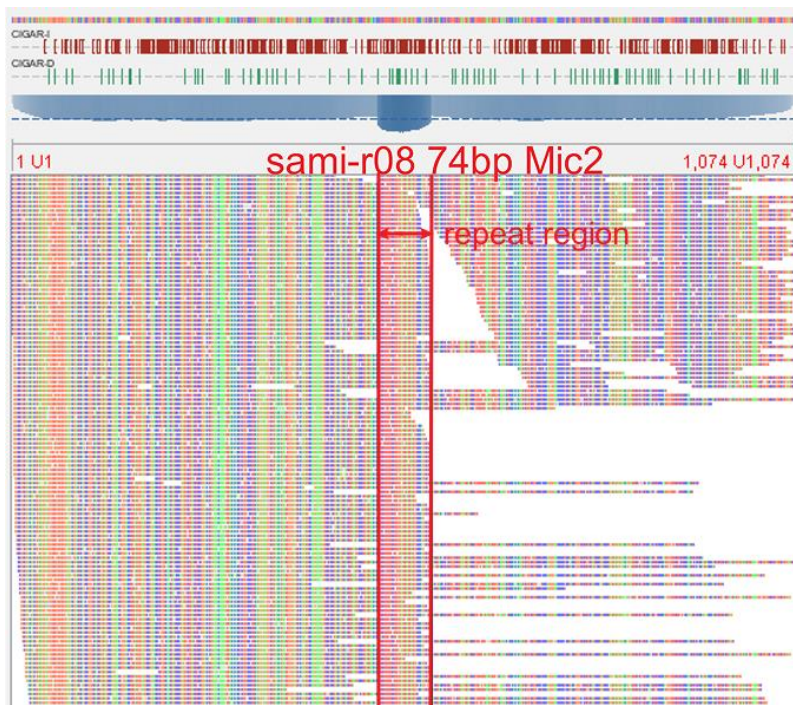


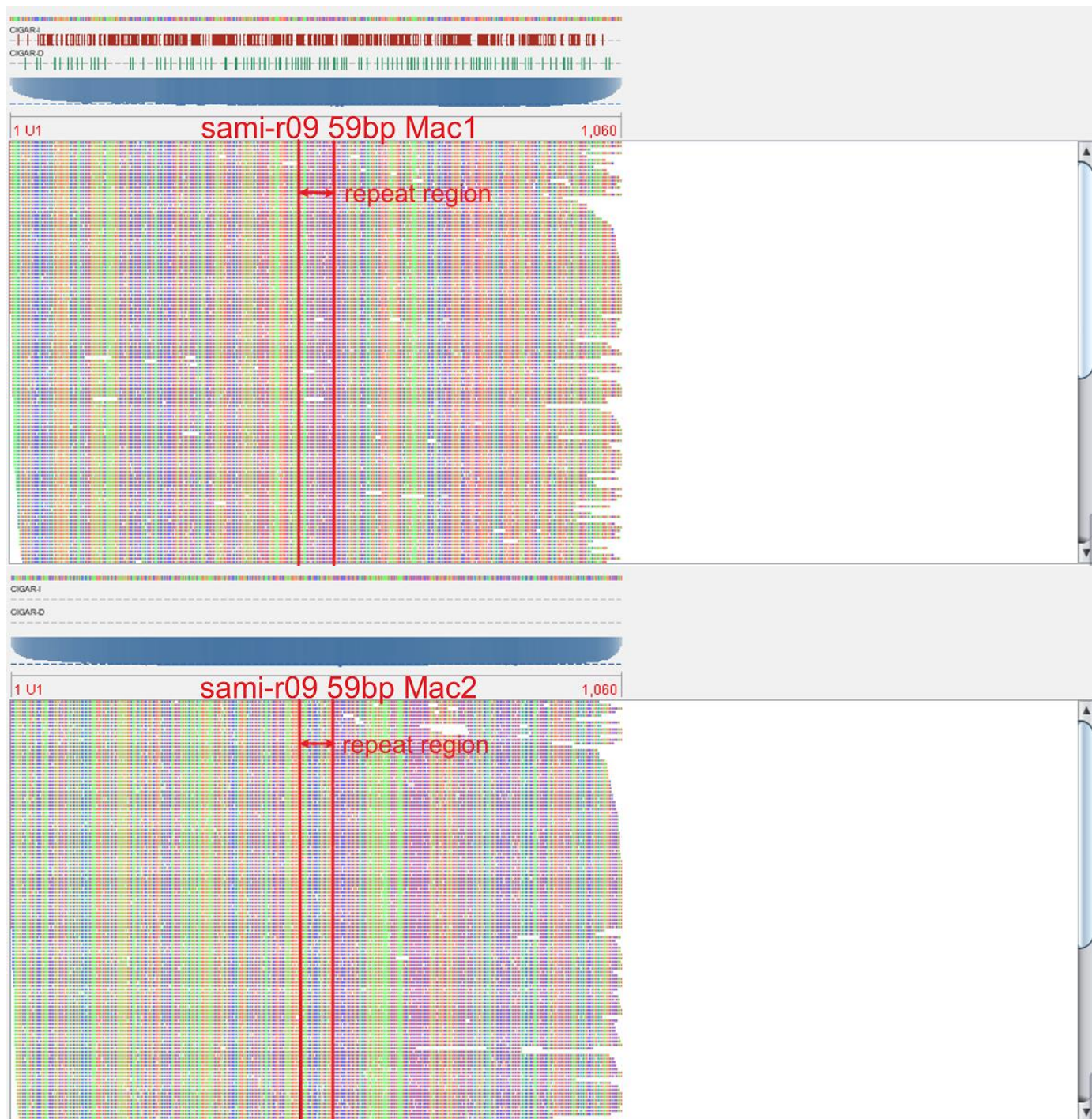




h







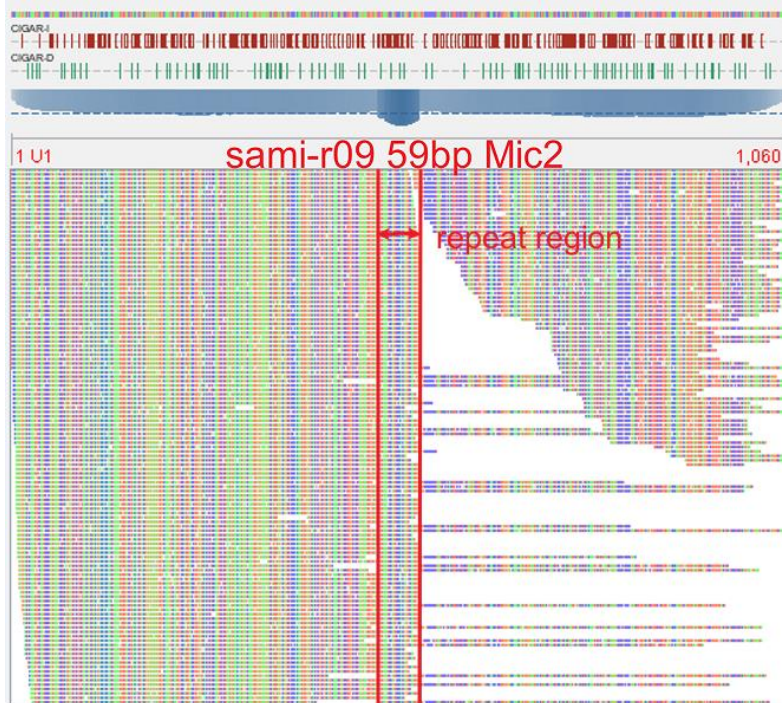
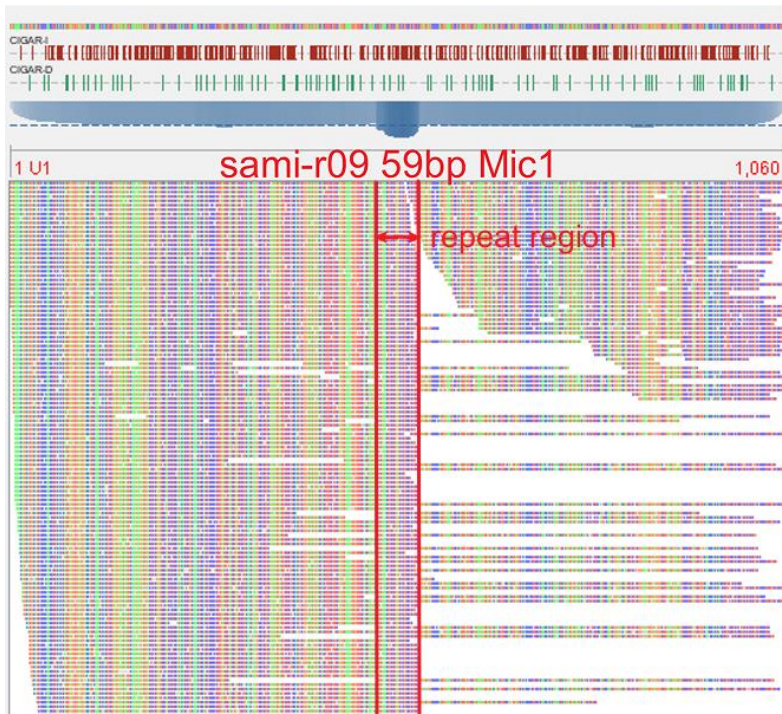
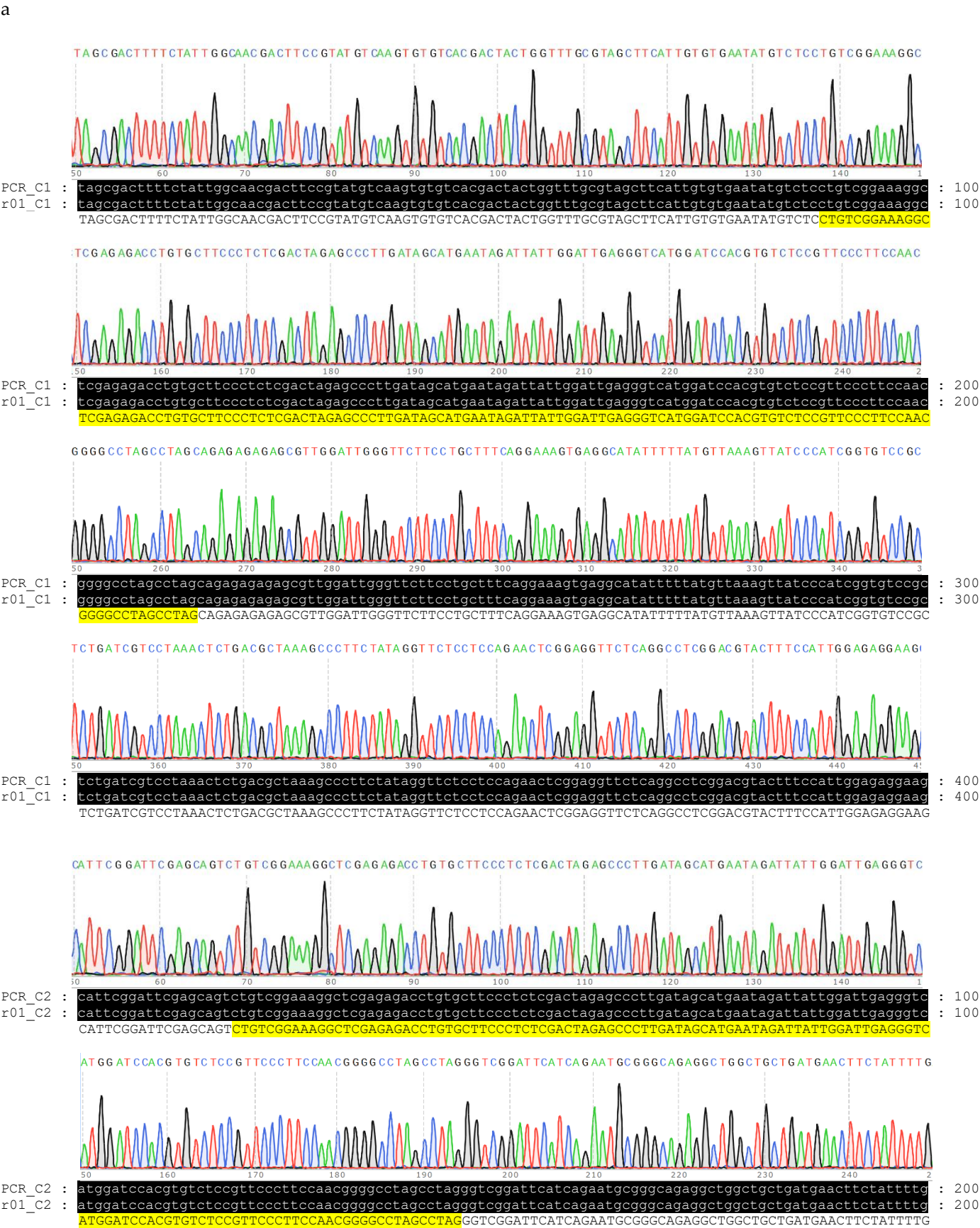
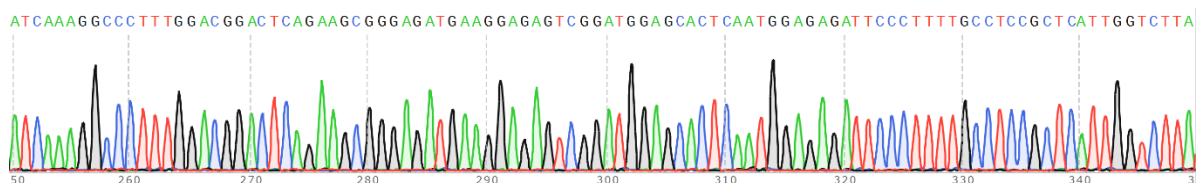
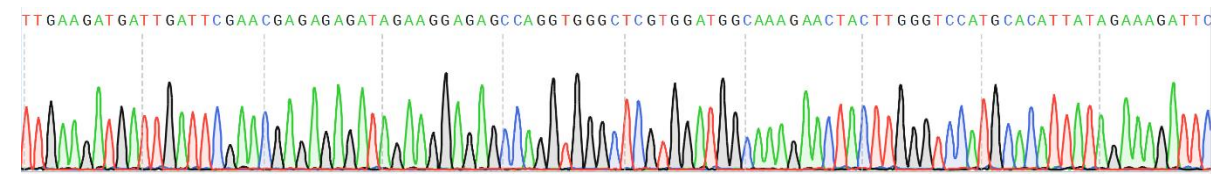


Figure S7. Validation of four conformations corresponding to the recombination products mediated by the nine repeats. PCR primers were designed based on the four conformations. The genomic DNAs were then amplified by PCR, and PCR products were subjected to Sanger sequencing. The sequencing chromatogram (top), Sanger sequencing results (labeled with “PCR” and conformation number), expected sequences (labeled with repeat ID and conformation number), and consensus sequences are shown from top to bottom. Panels a–i correspond to repeats of the r01 to r09 results, respectively. The sequences of r01–r09 are highlighted in yellow.

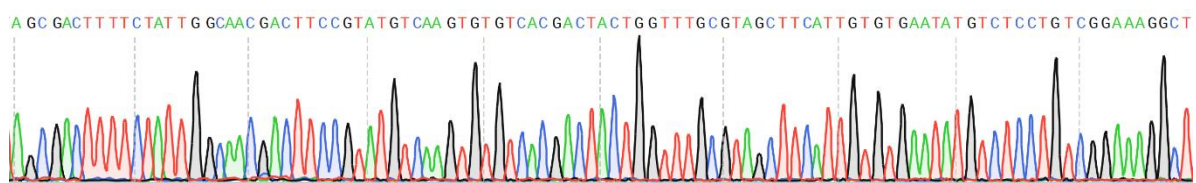




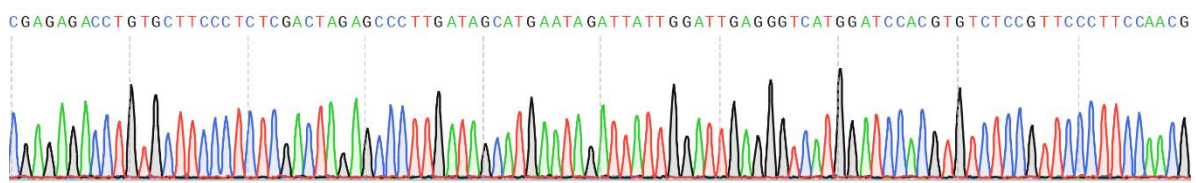
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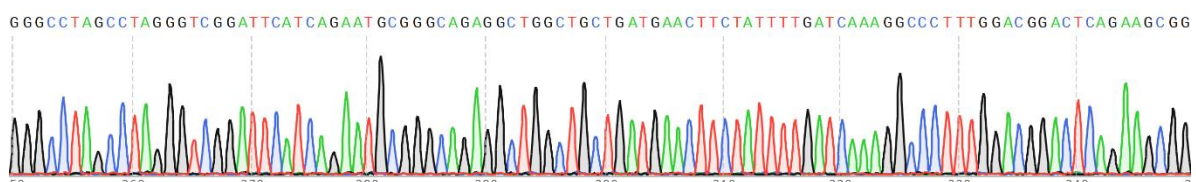
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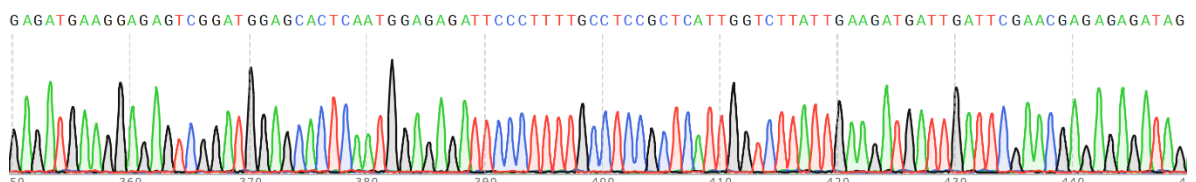
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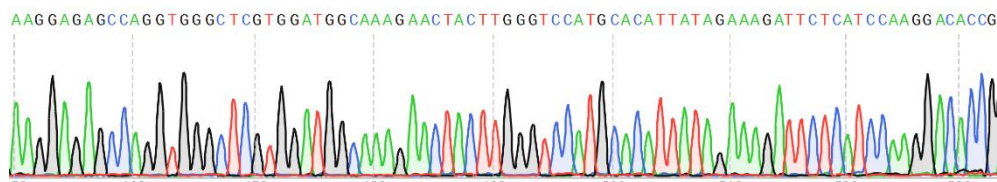
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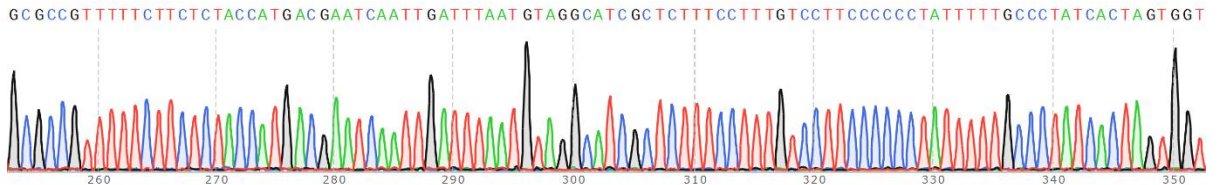
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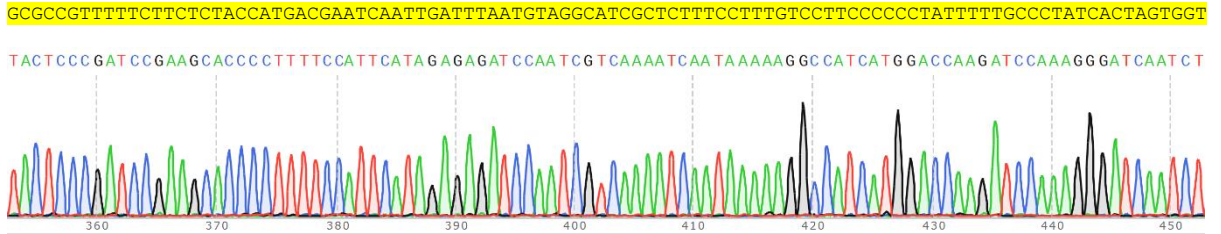
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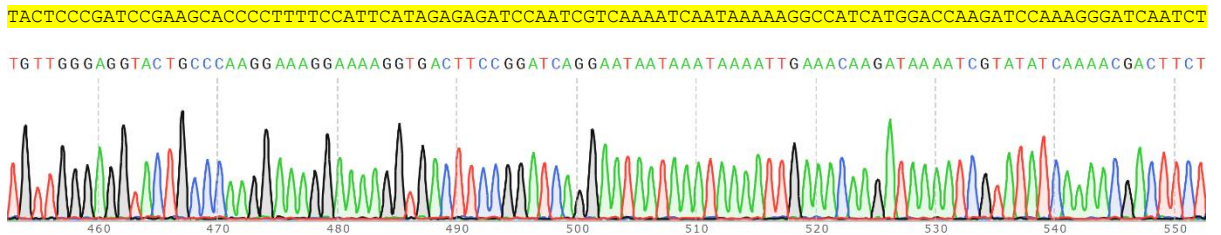
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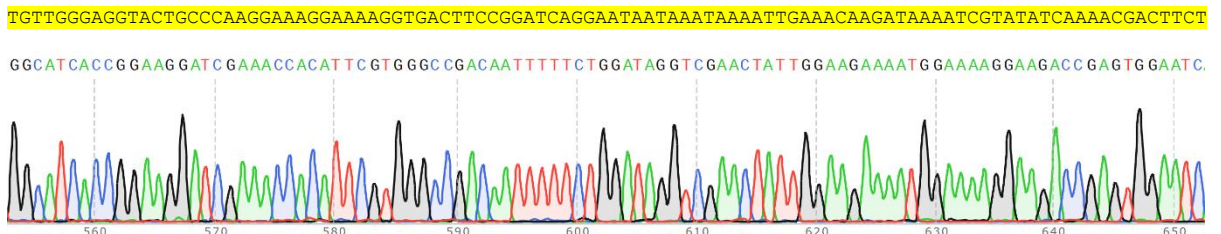
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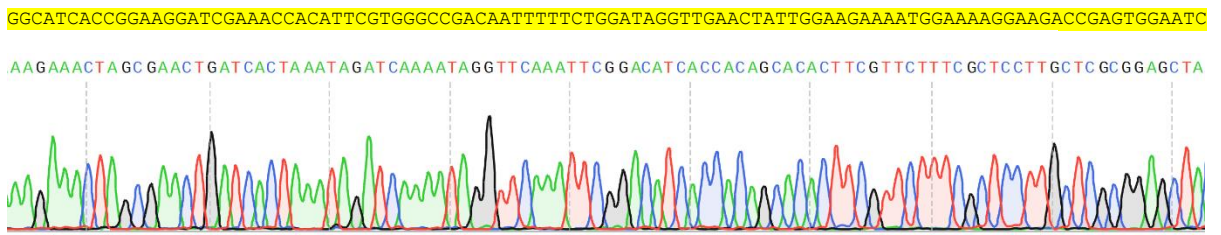
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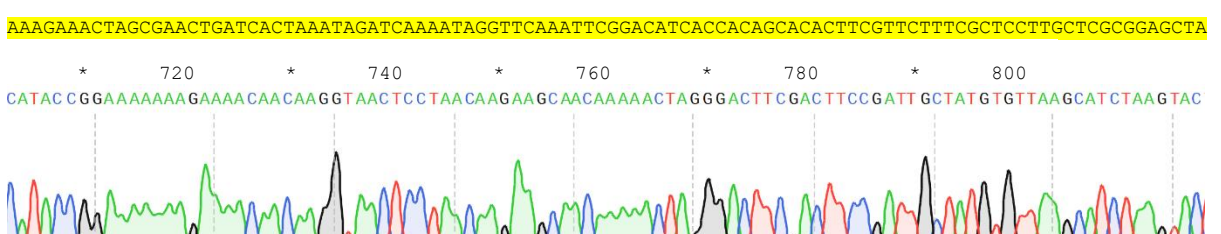
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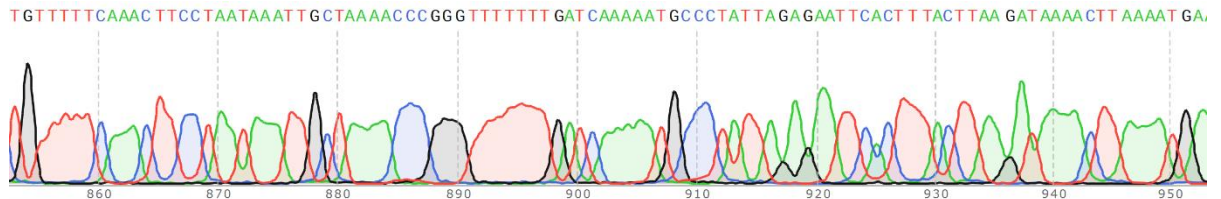


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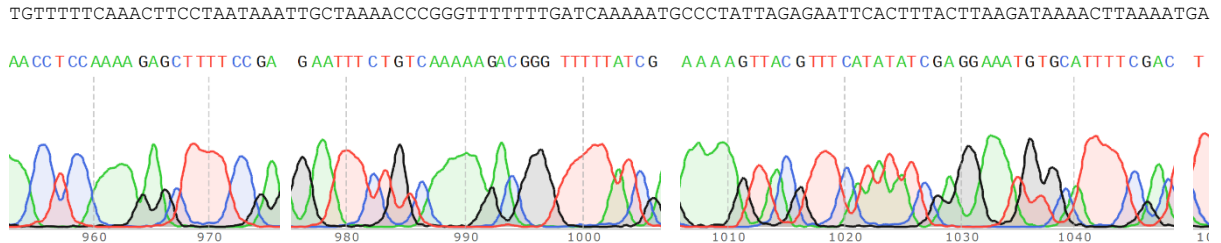


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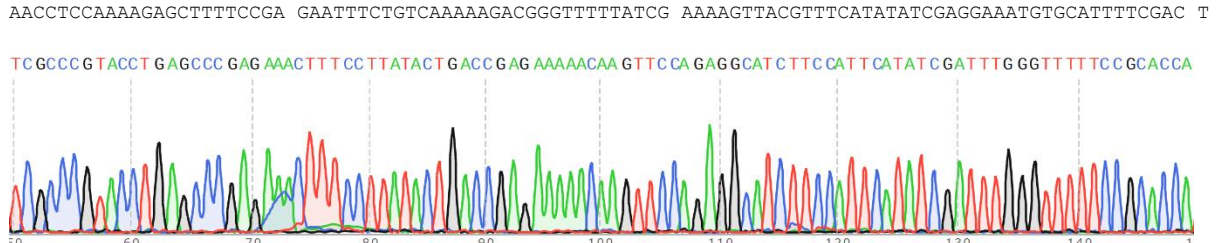
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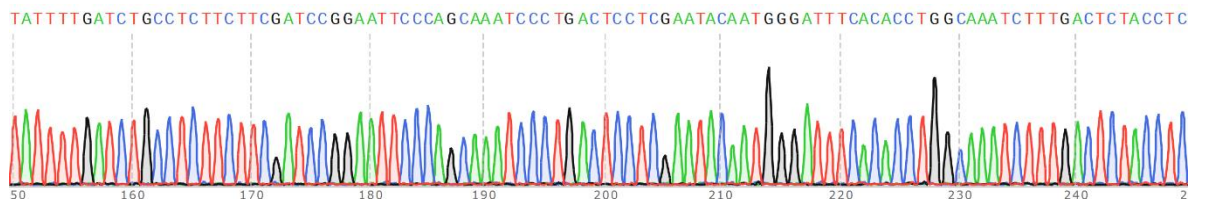
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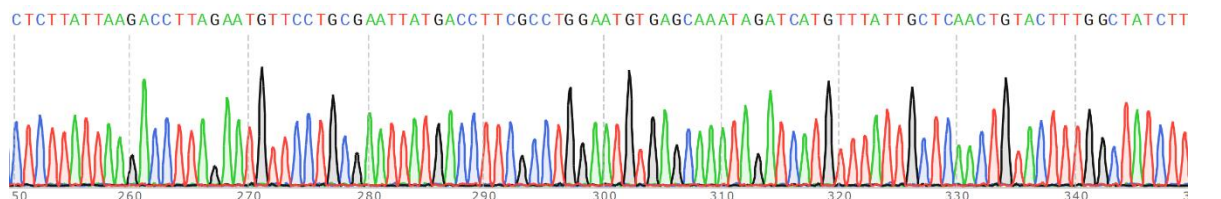
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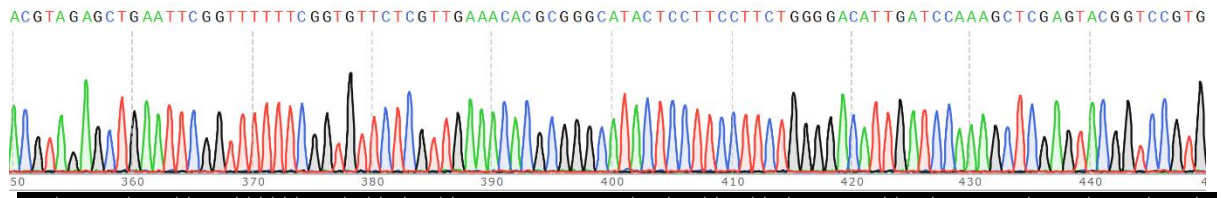
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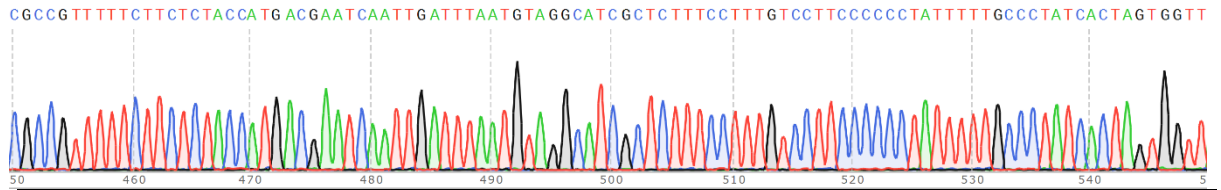
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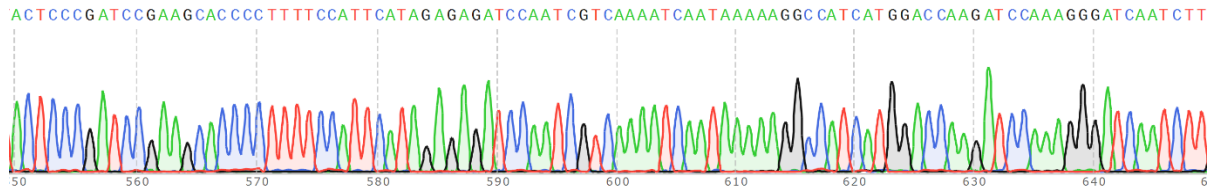
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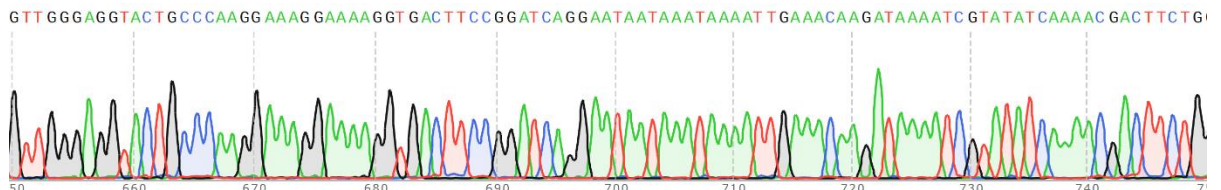
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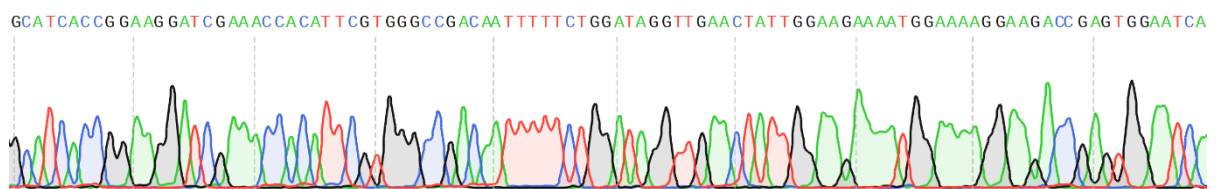
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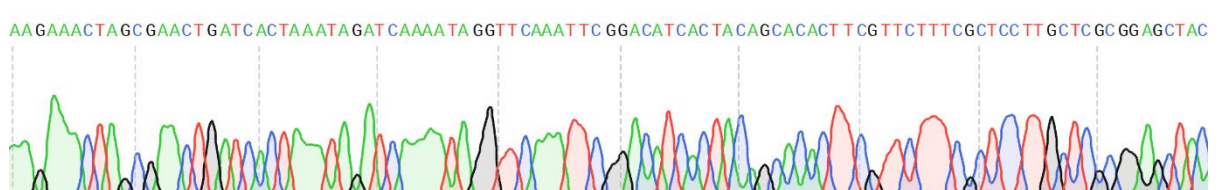
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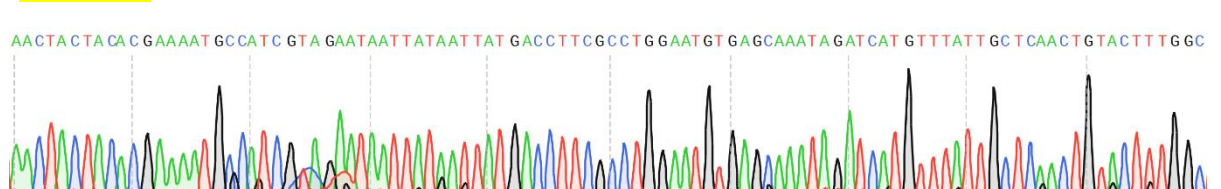
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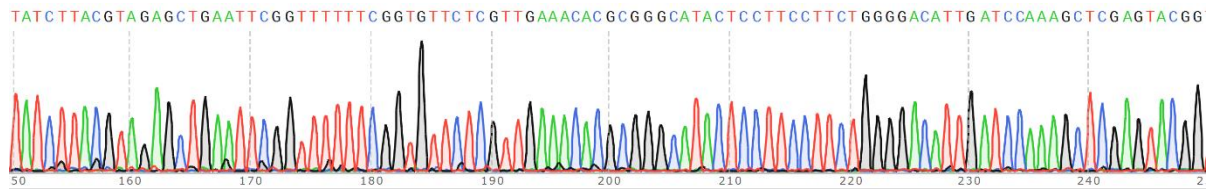
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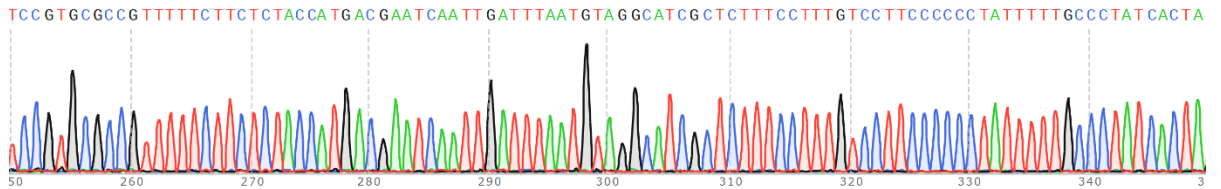
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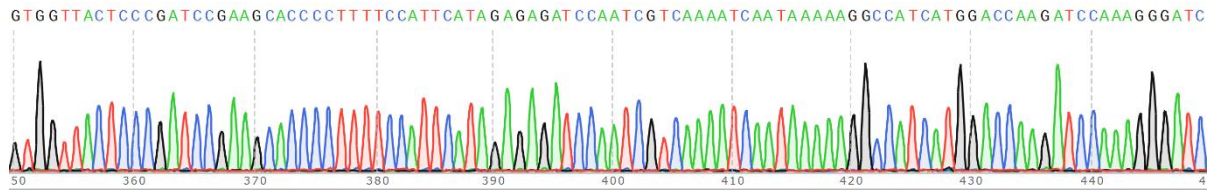
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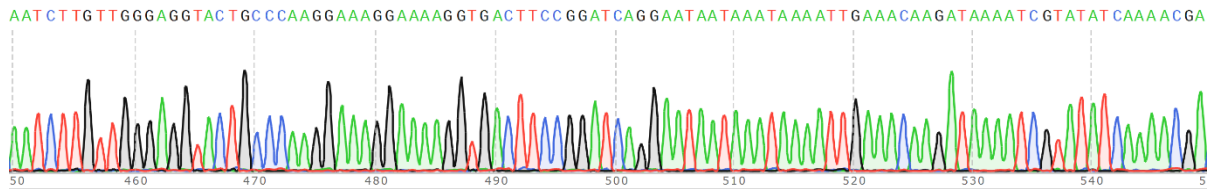
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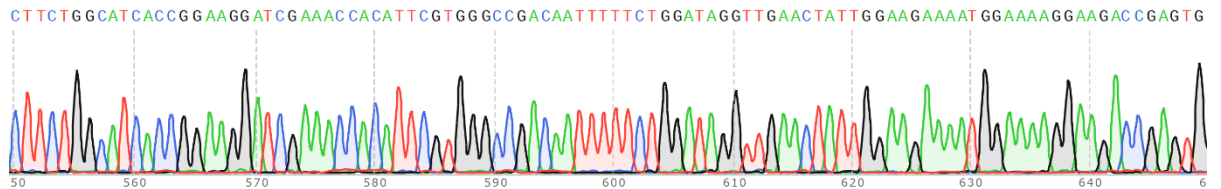
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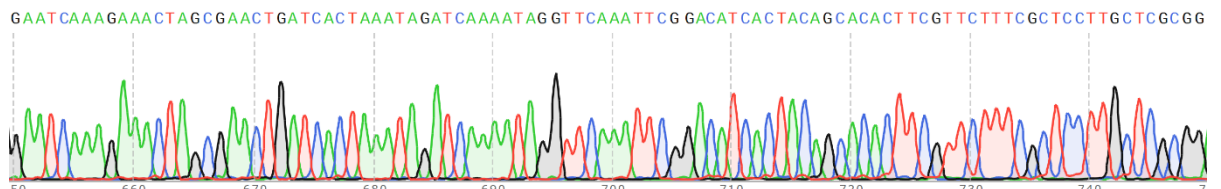
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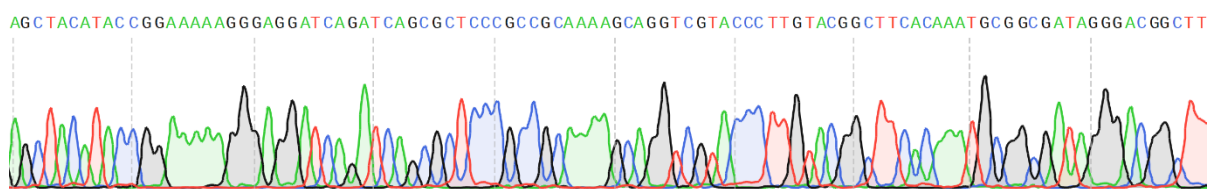
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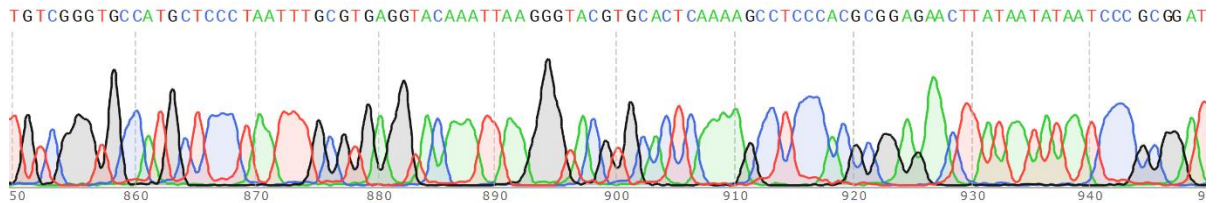
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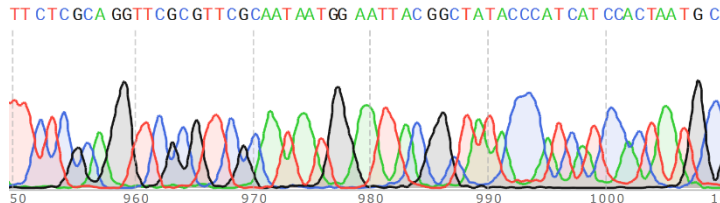
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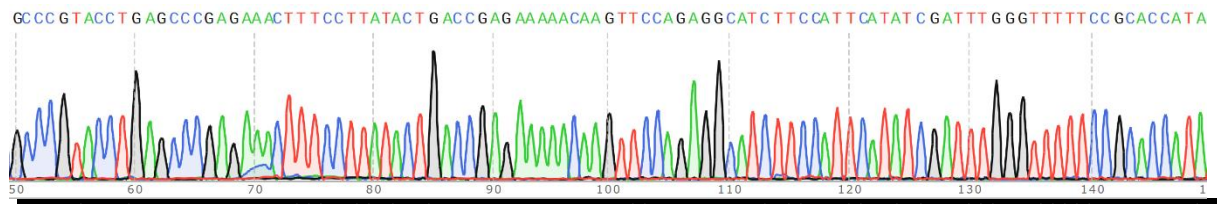
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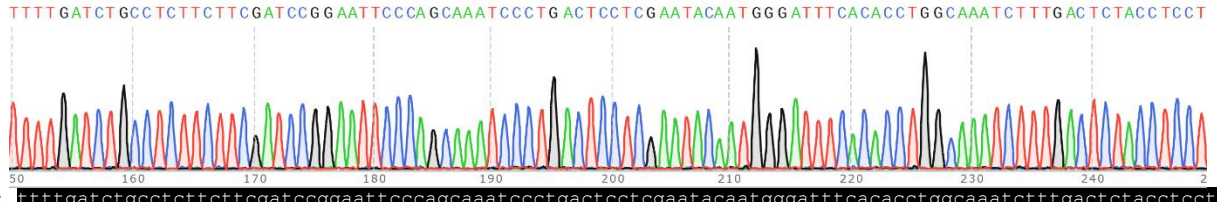


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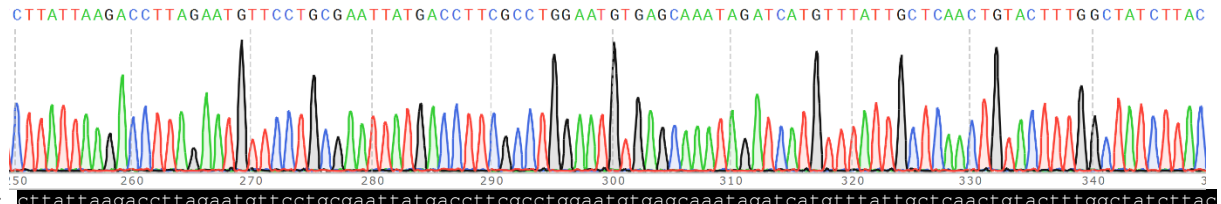
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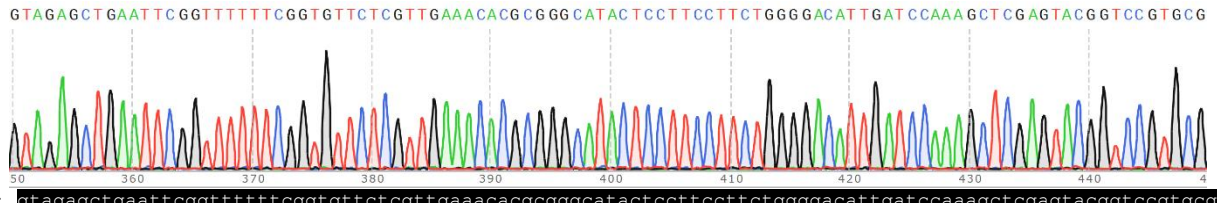
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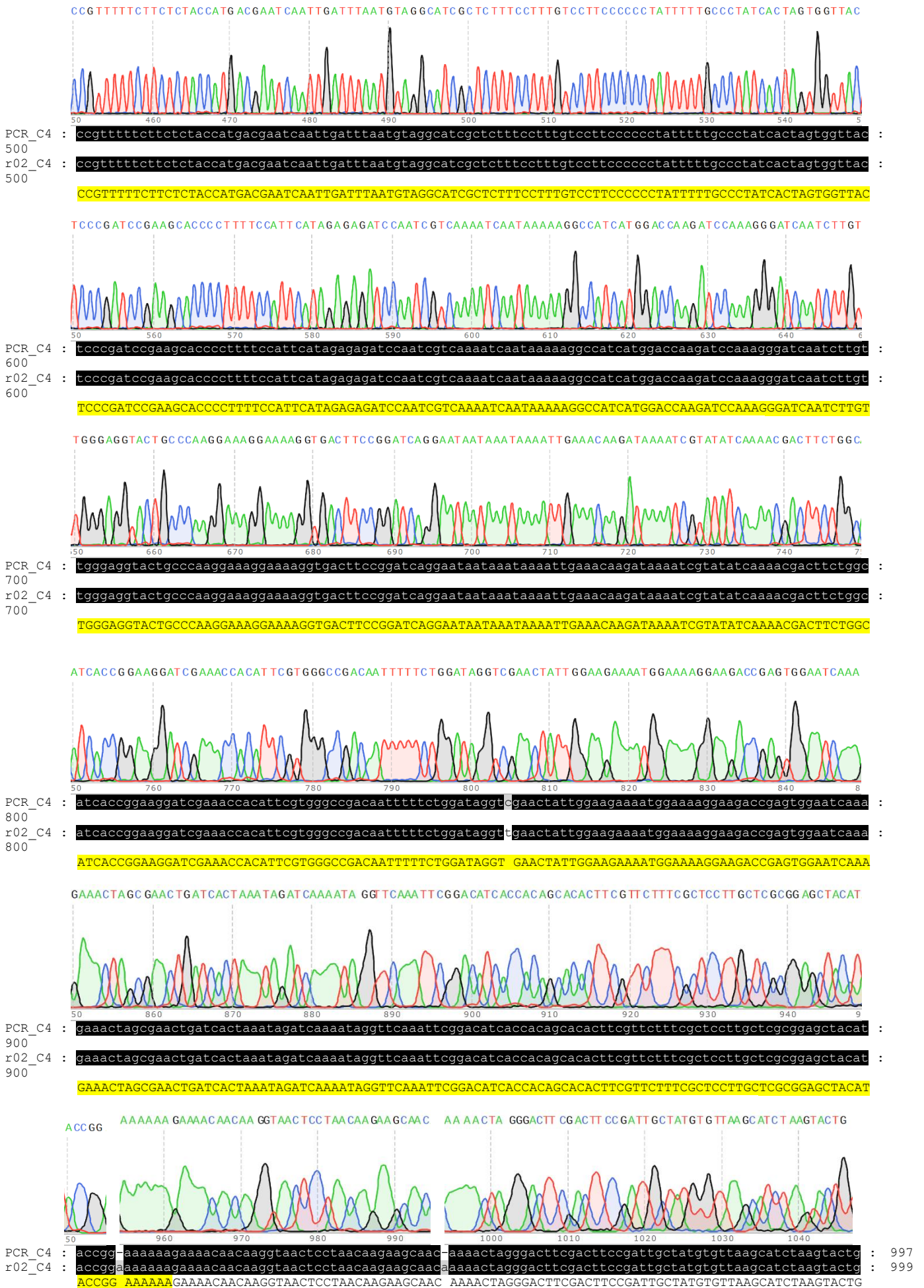
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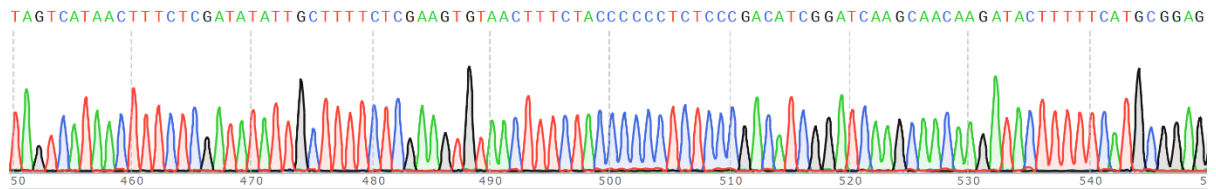
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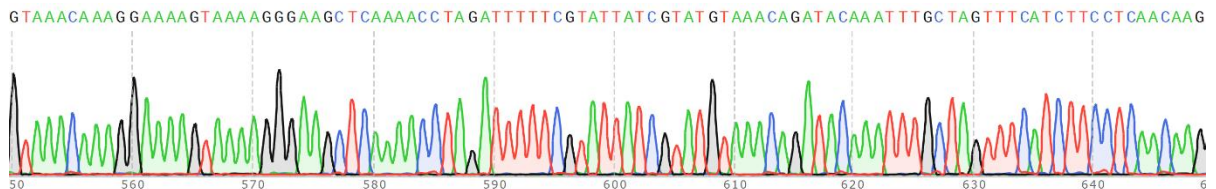
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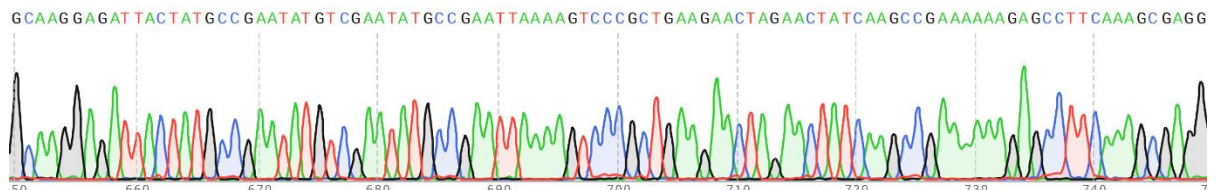




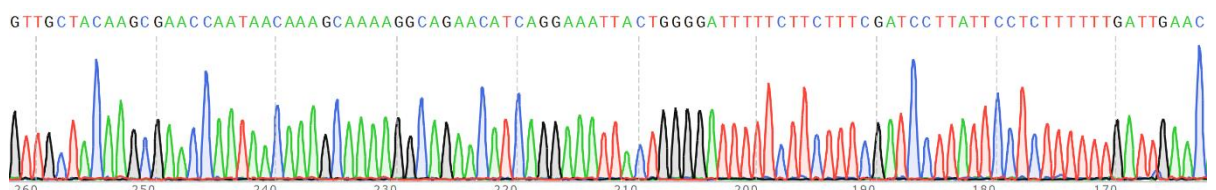
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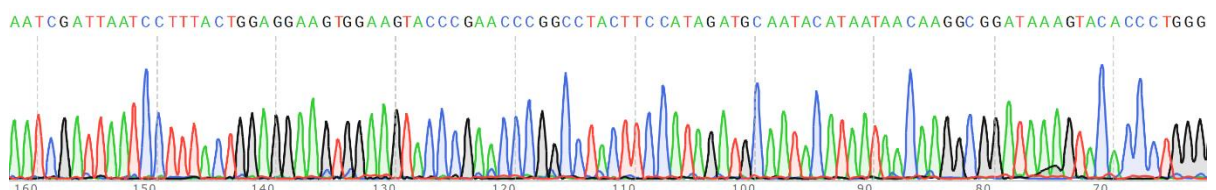
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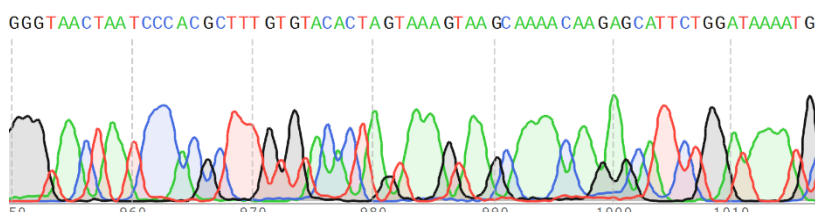
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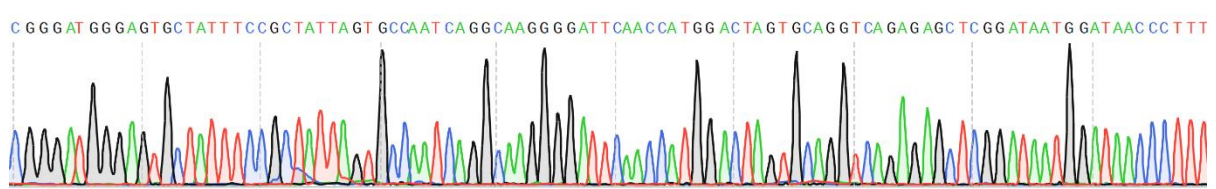
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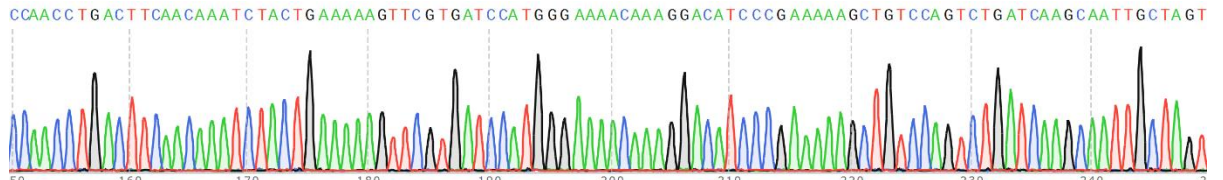
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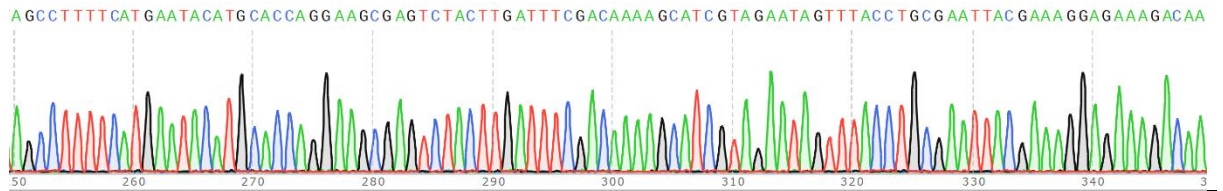
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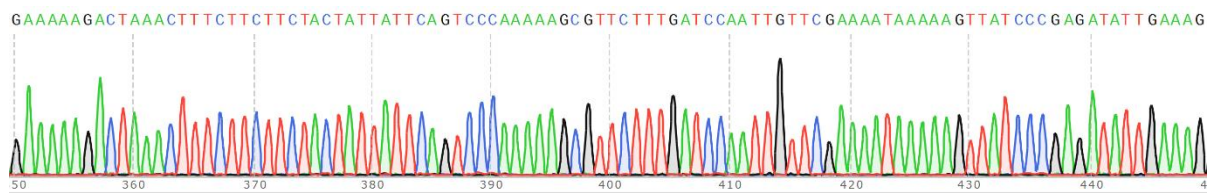
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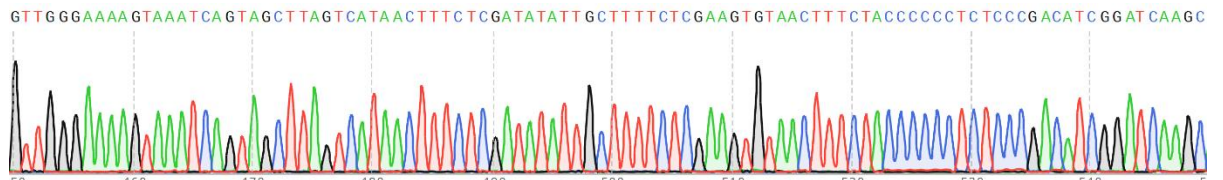
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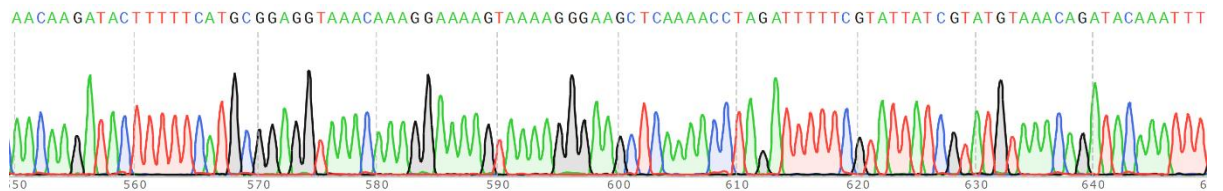
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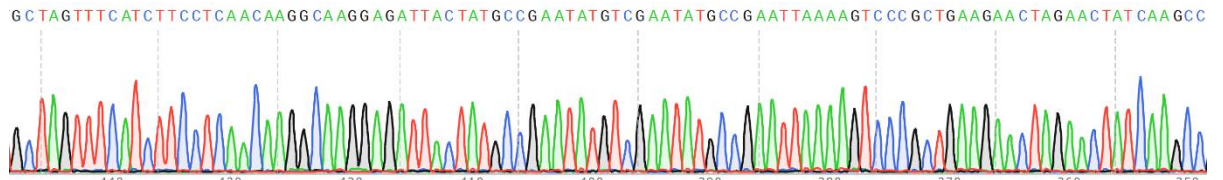
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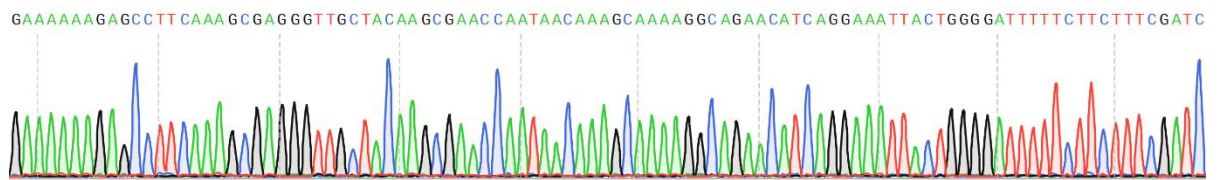
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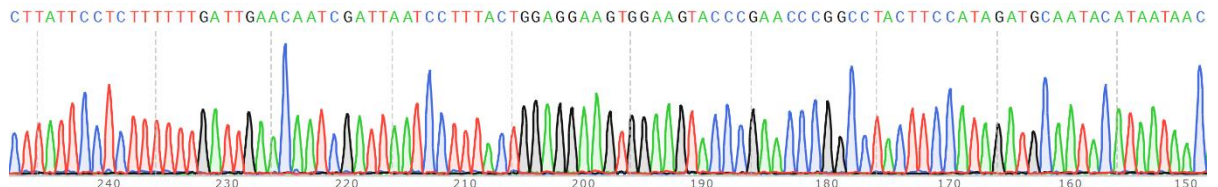
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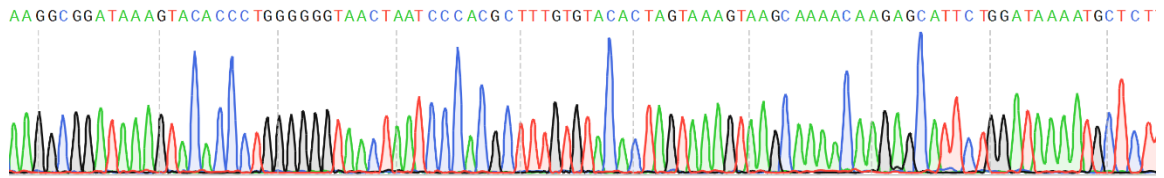
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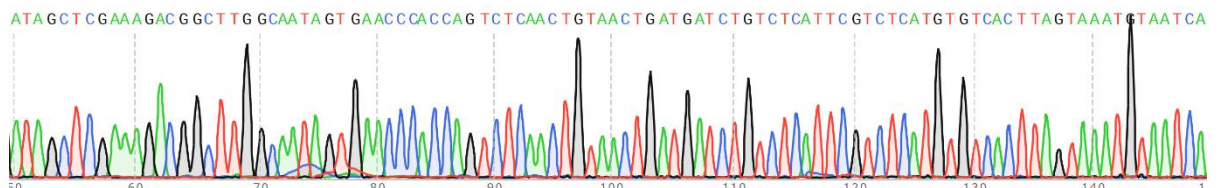
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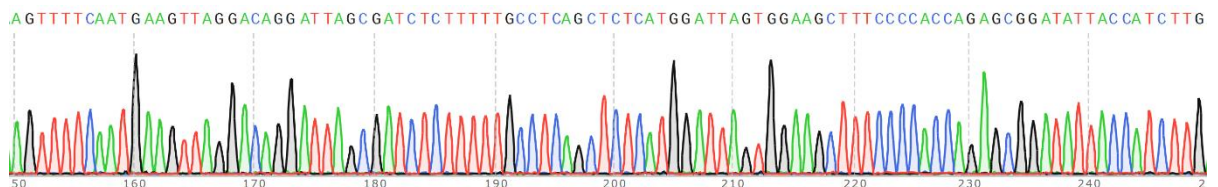
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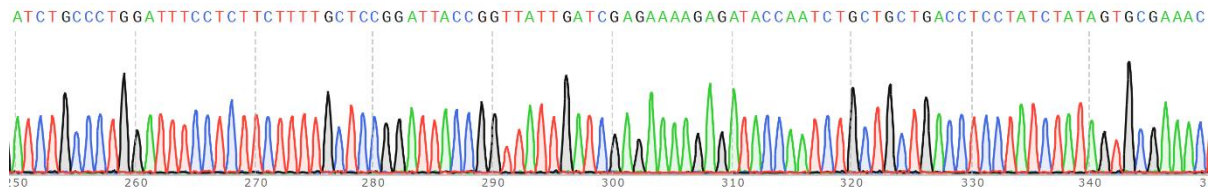
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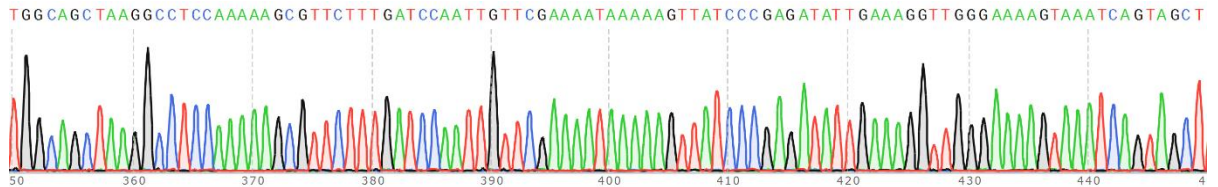
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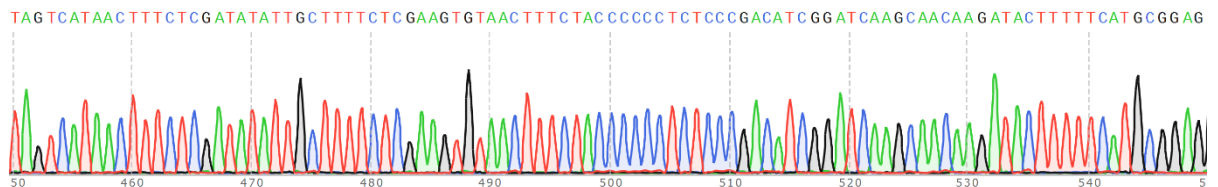
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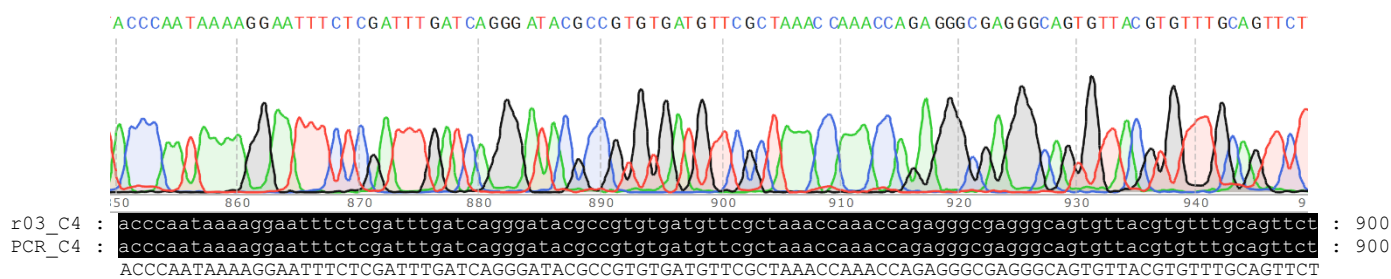
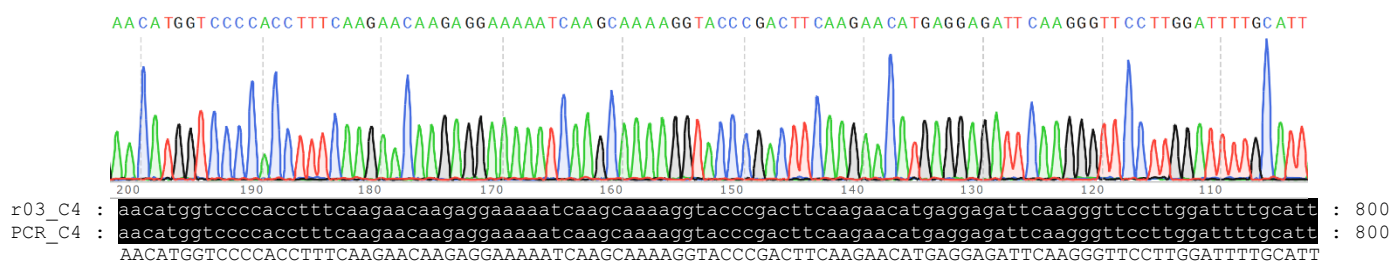
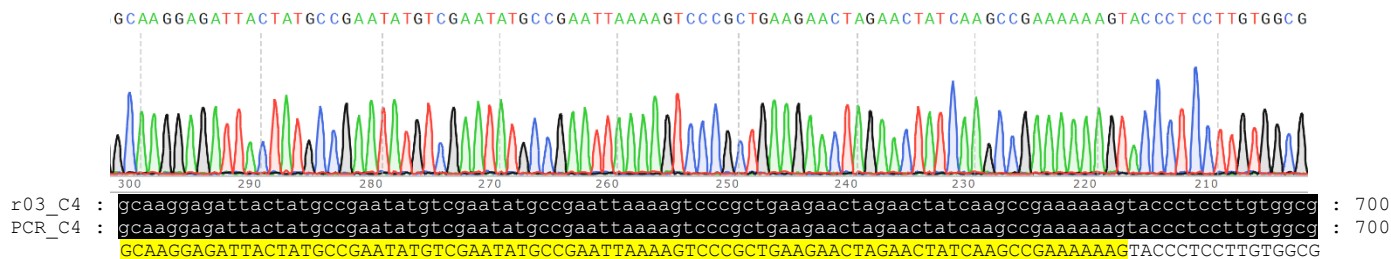
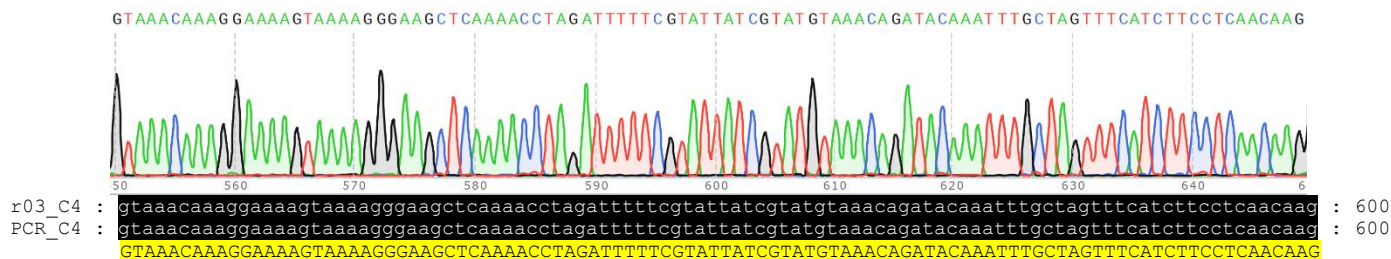
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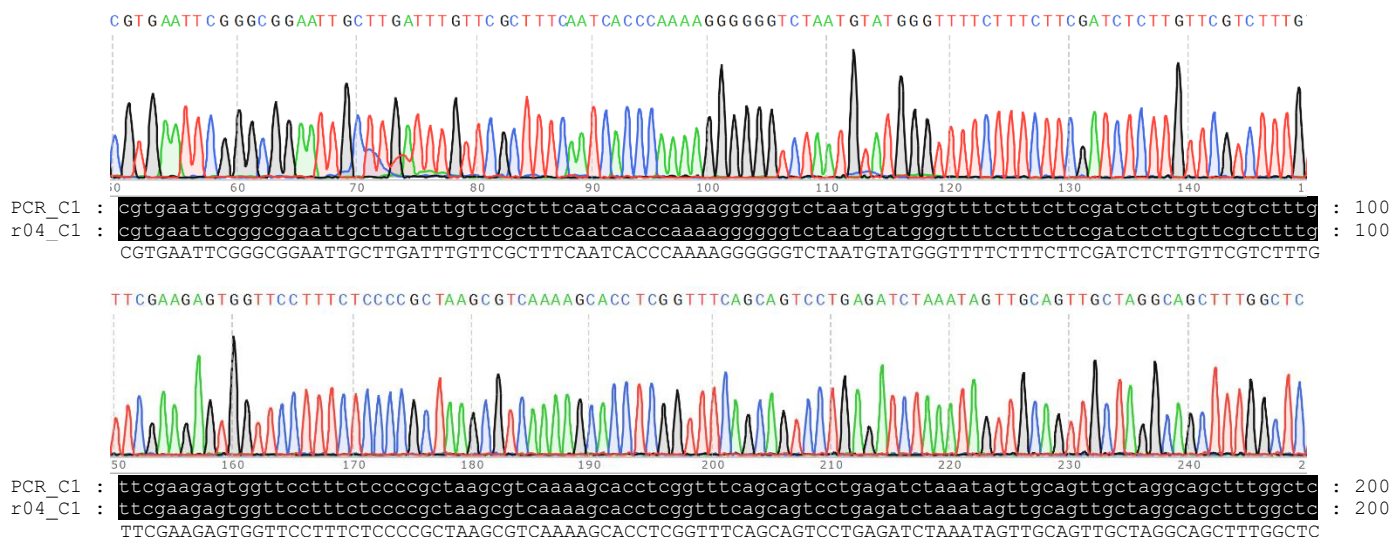
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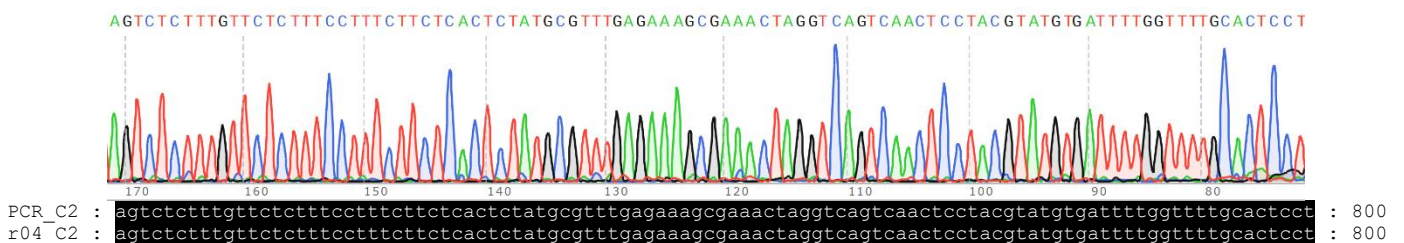
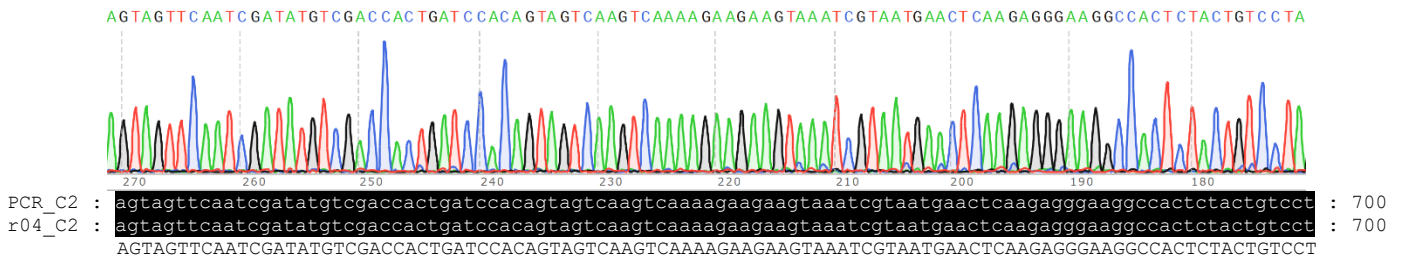
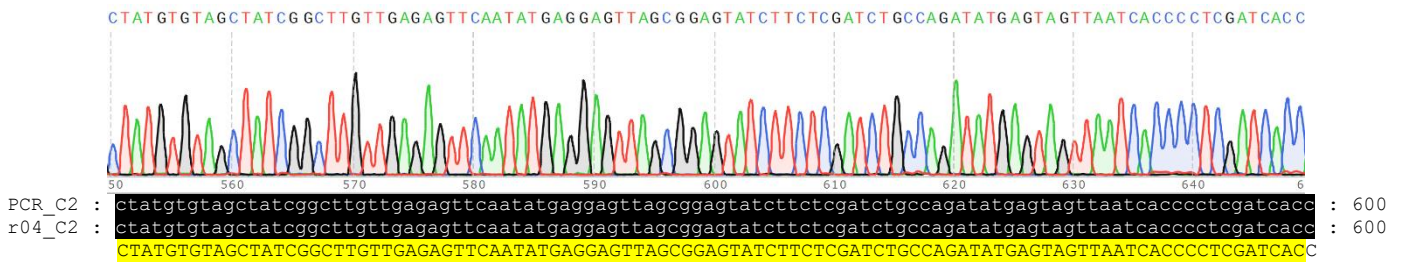
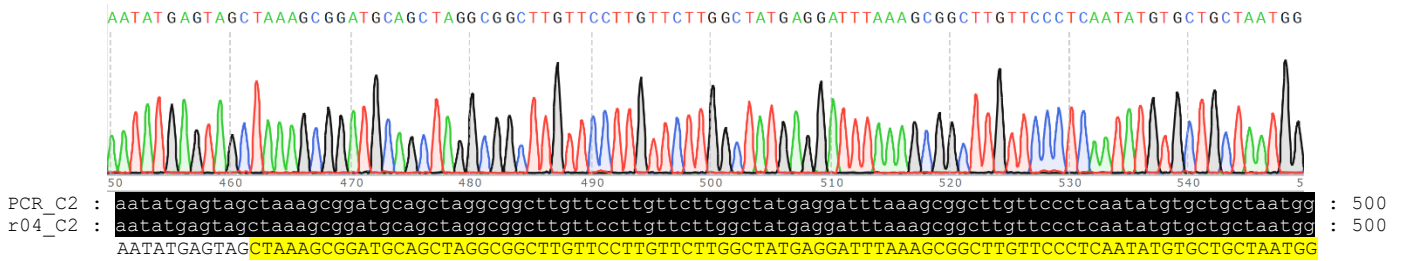
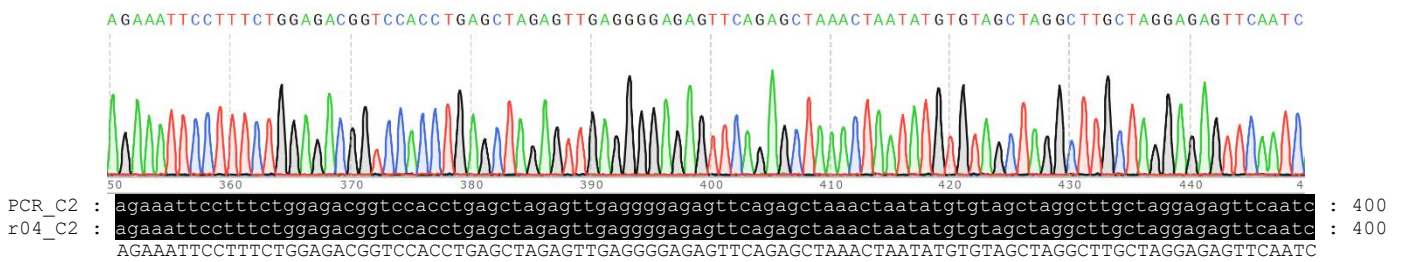
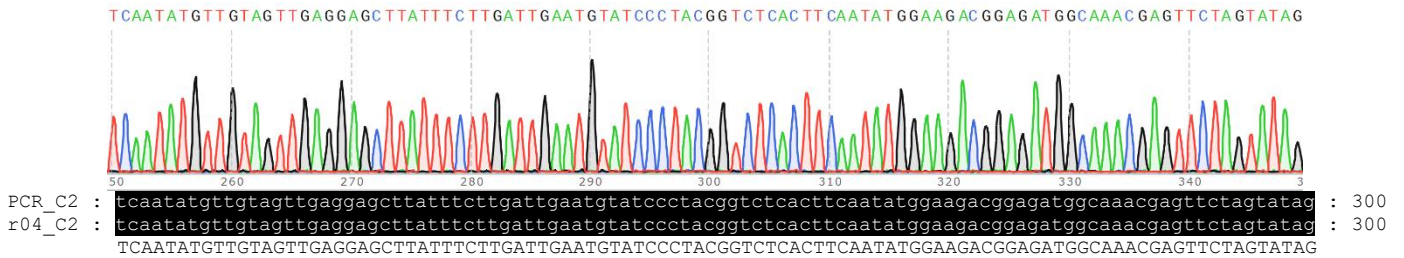
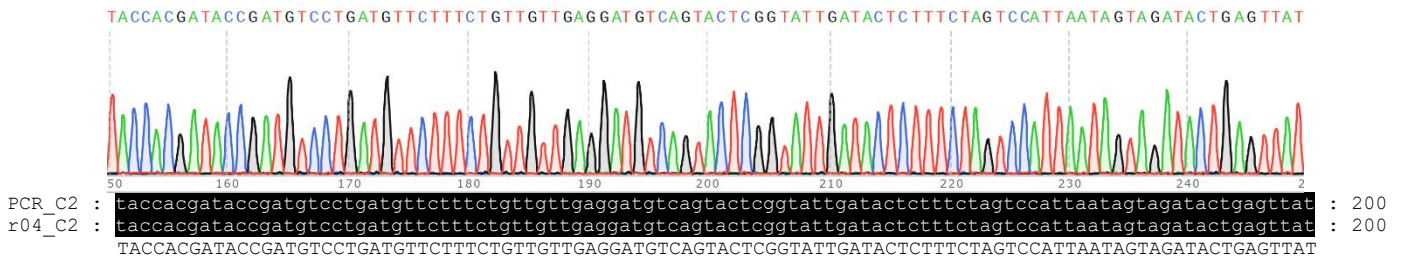


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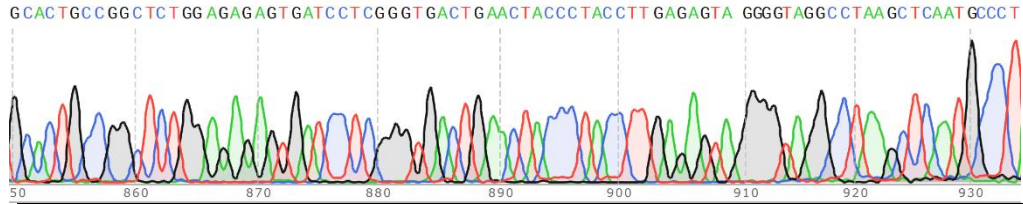


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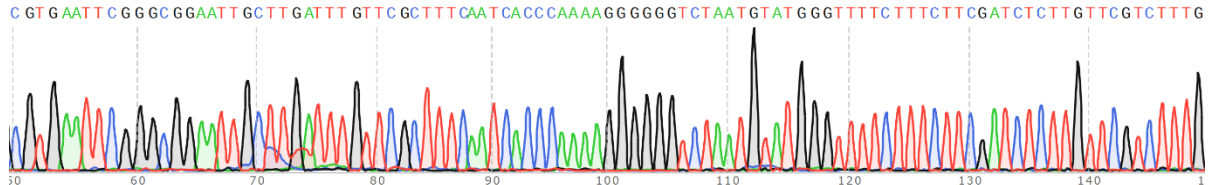




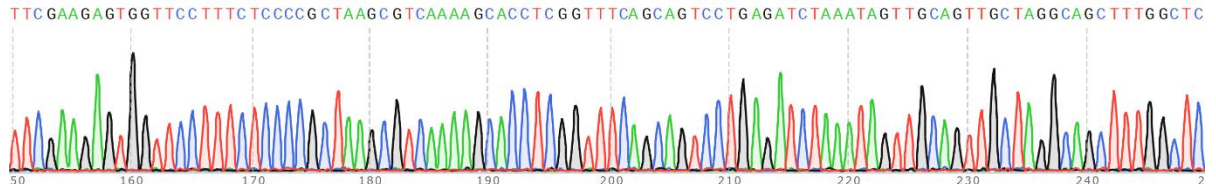
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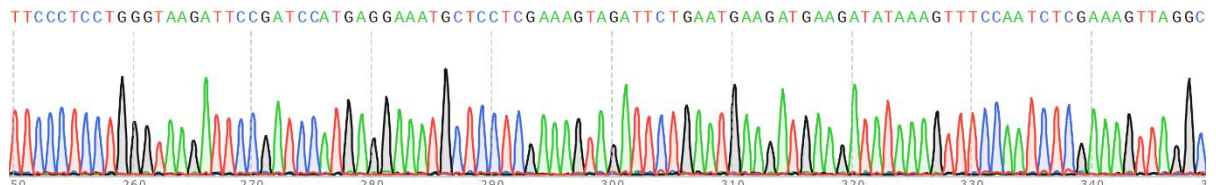
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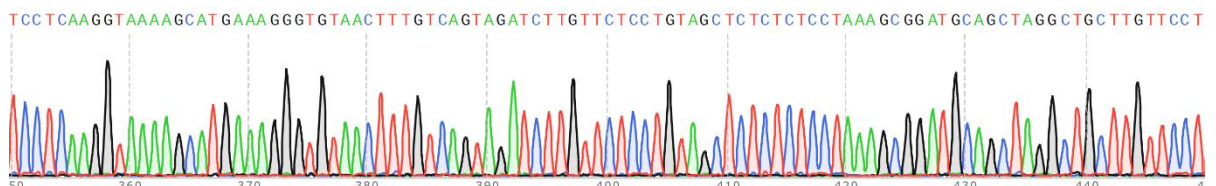
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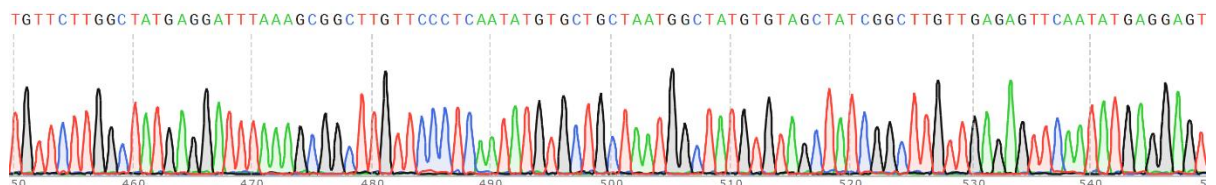
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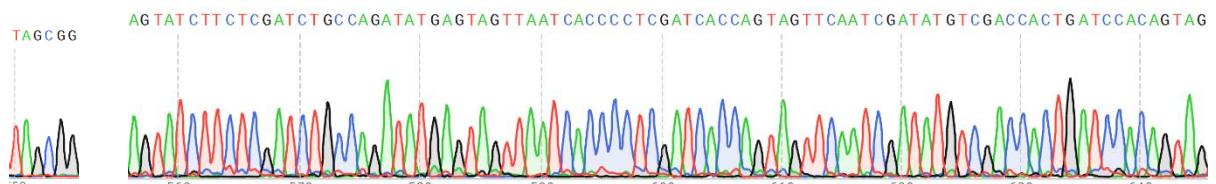
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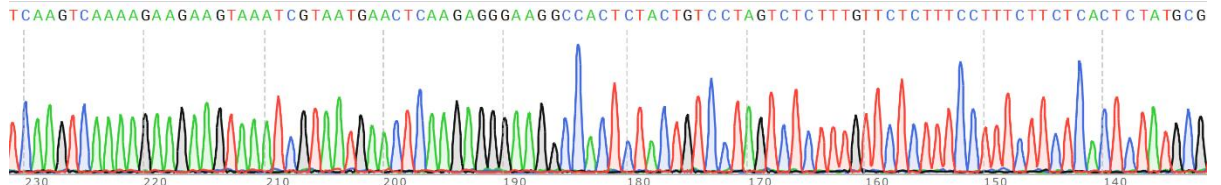


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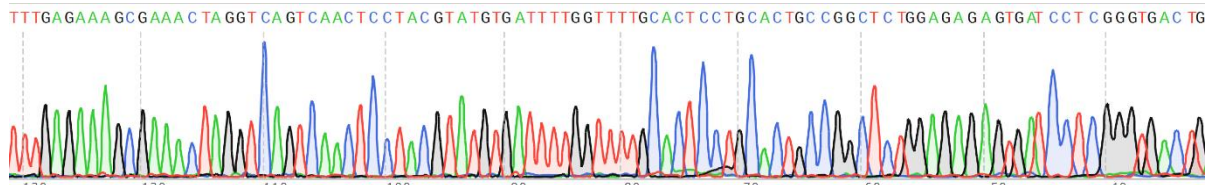


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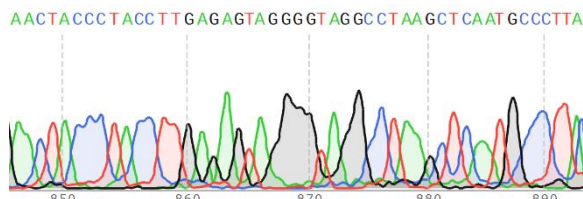
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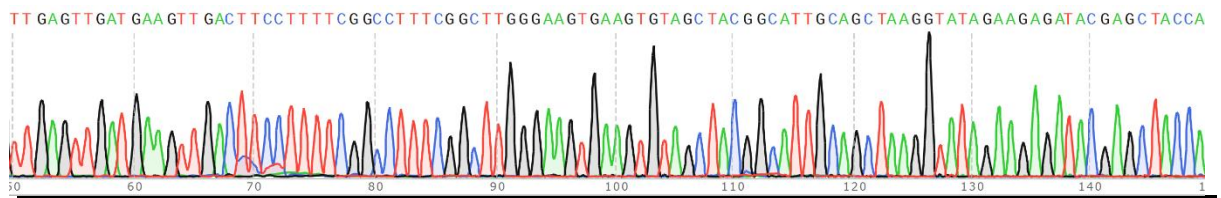
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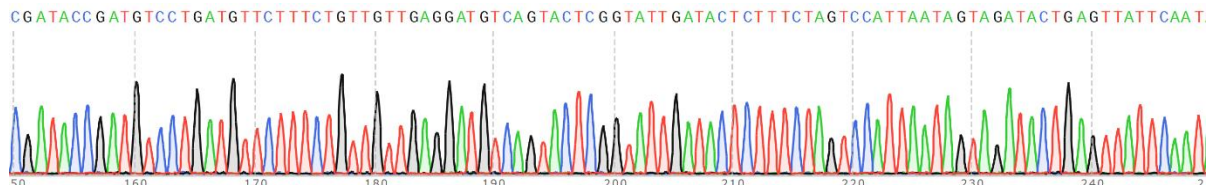
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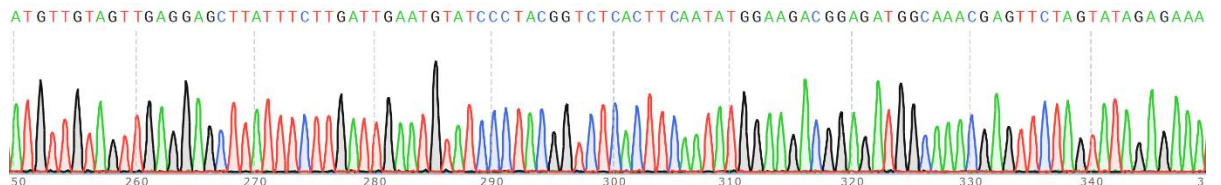
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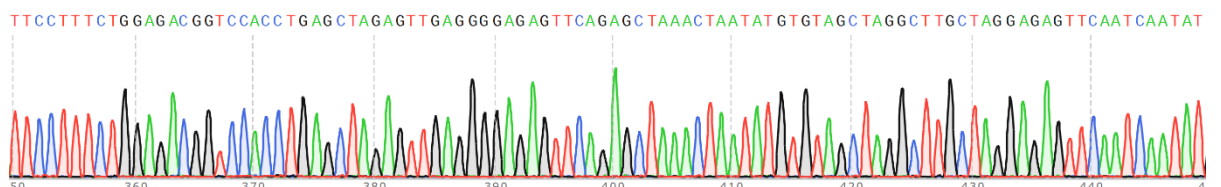
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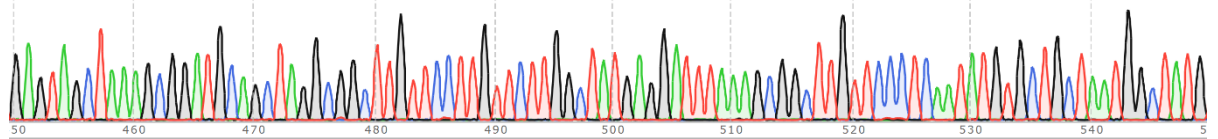
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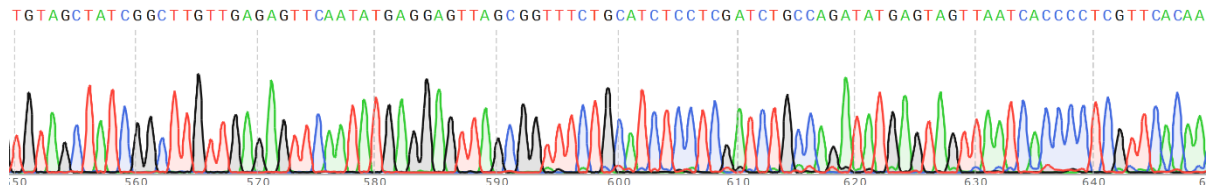
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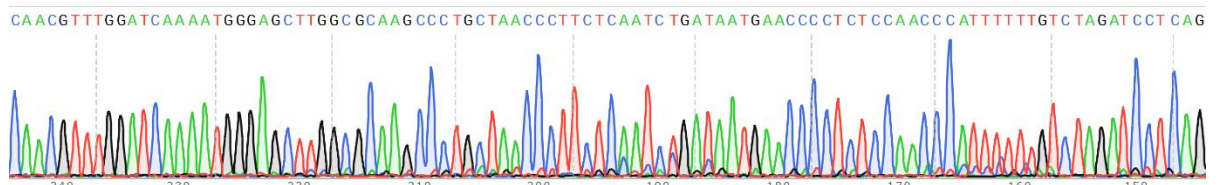
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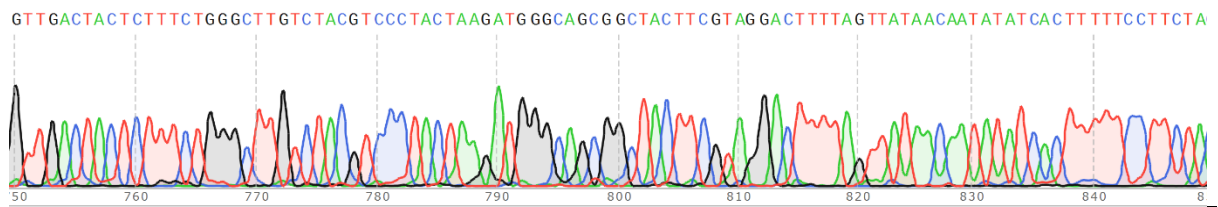
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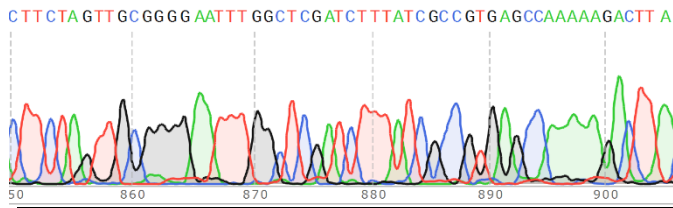
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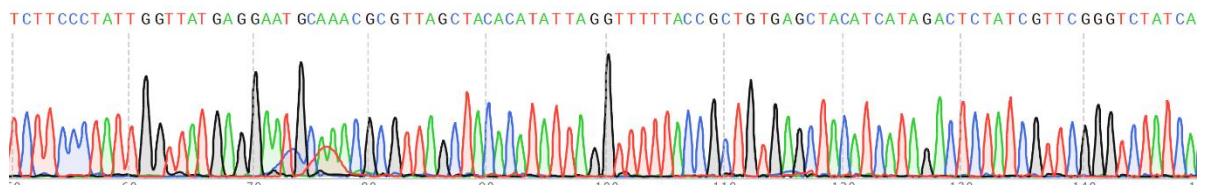


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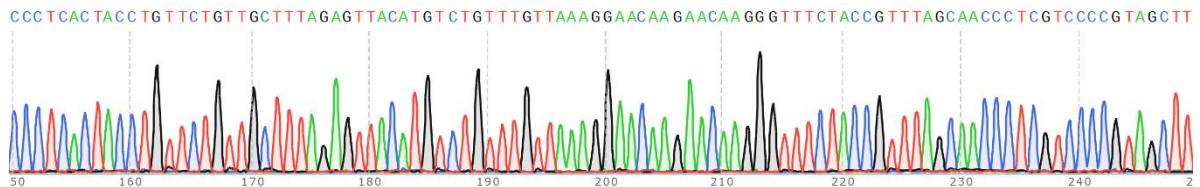


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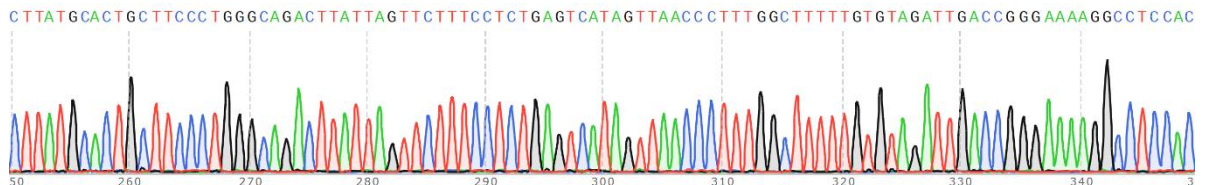
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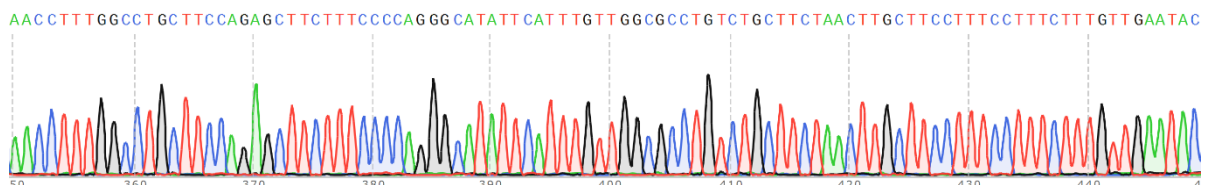
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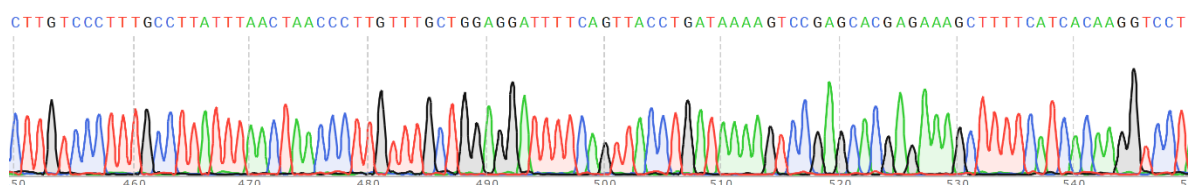
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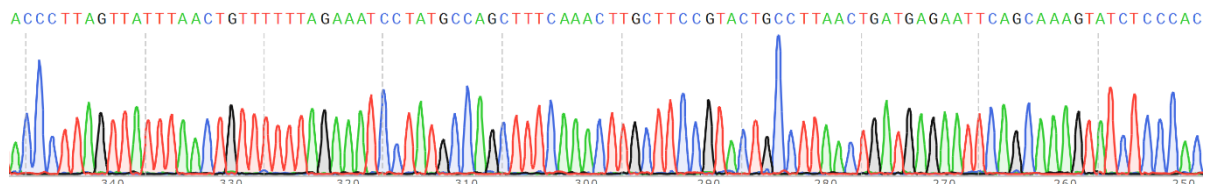
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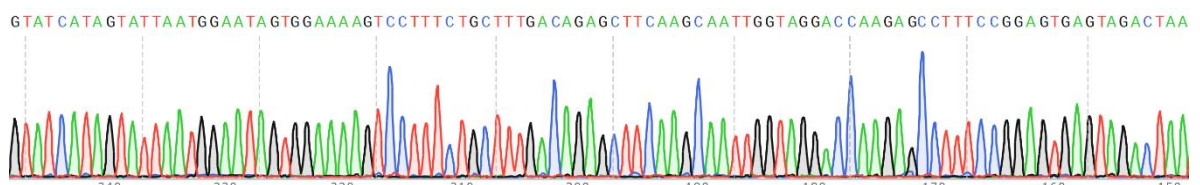
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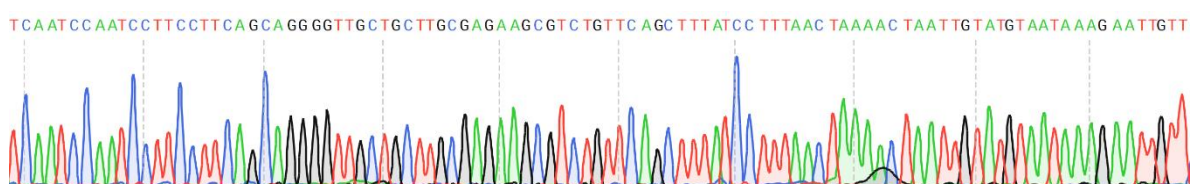
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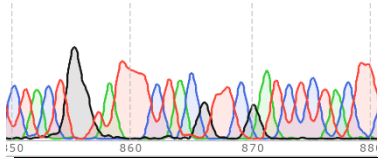


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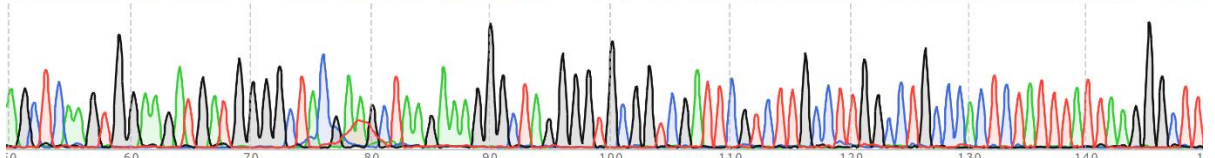
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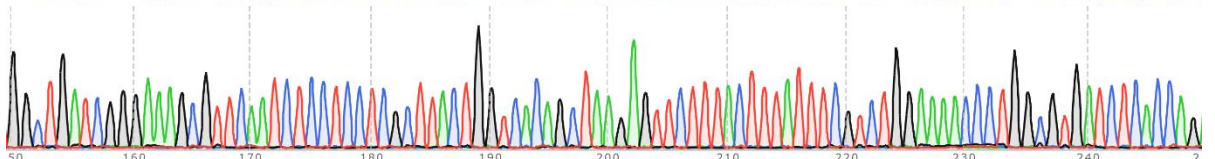
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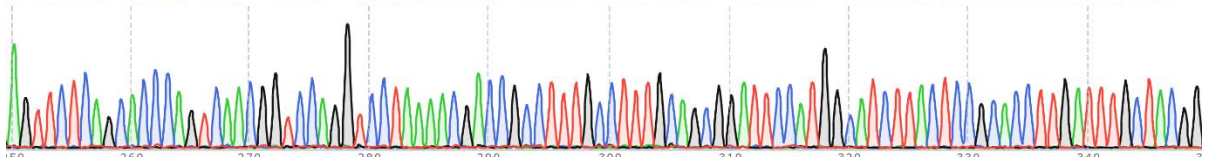
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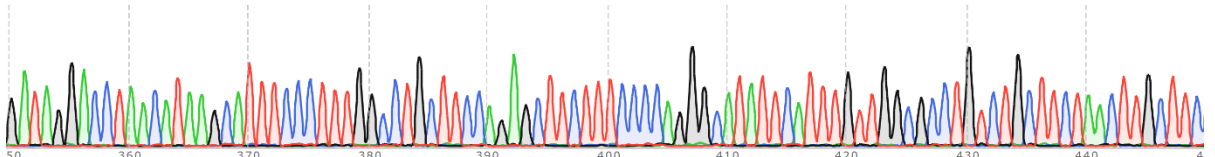
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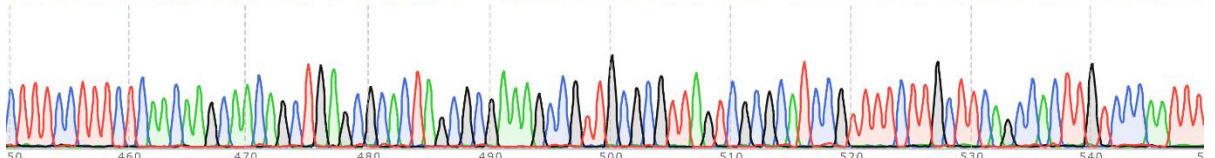
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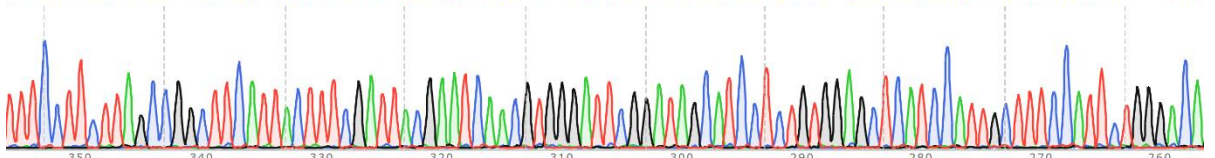
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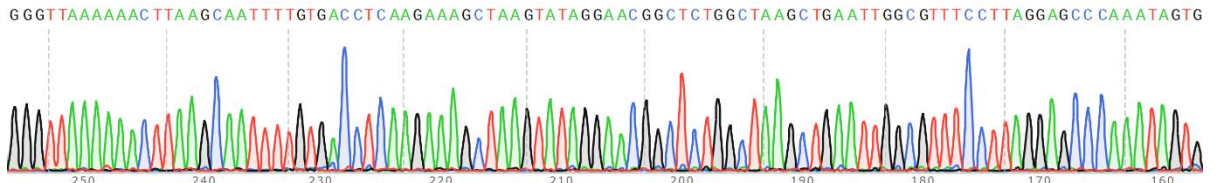


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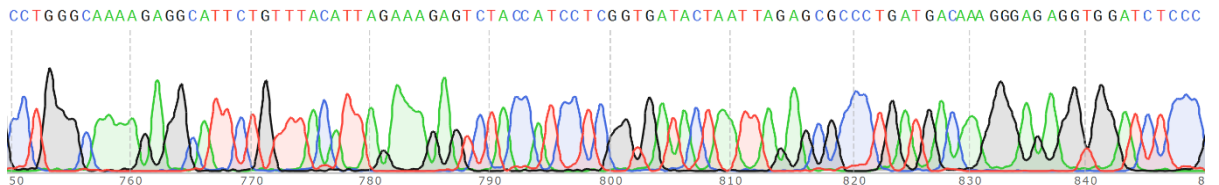
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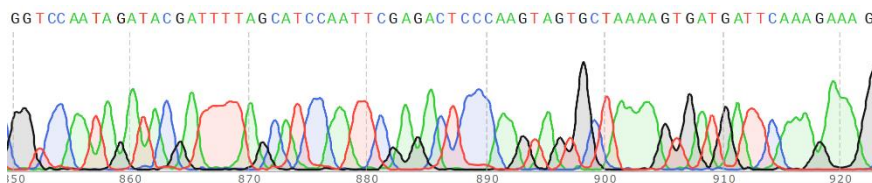
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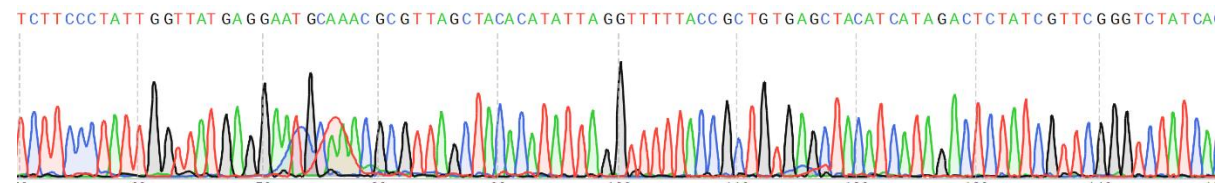
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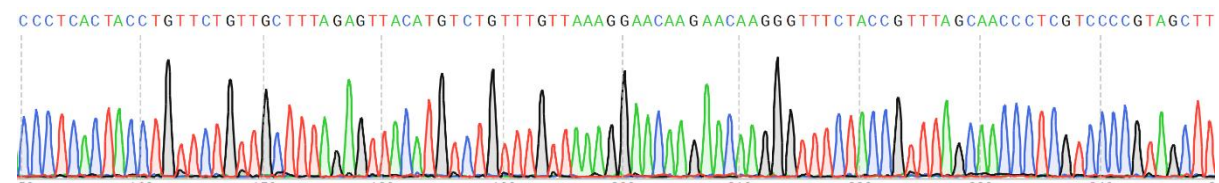
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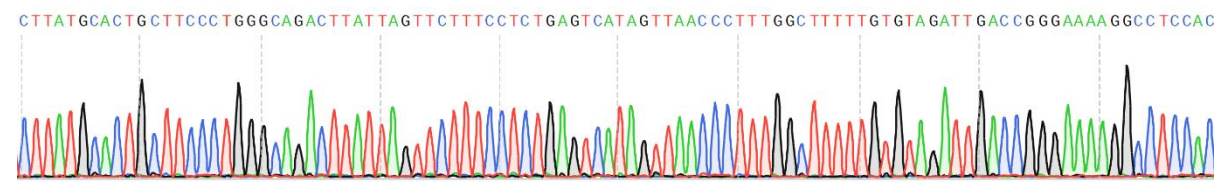
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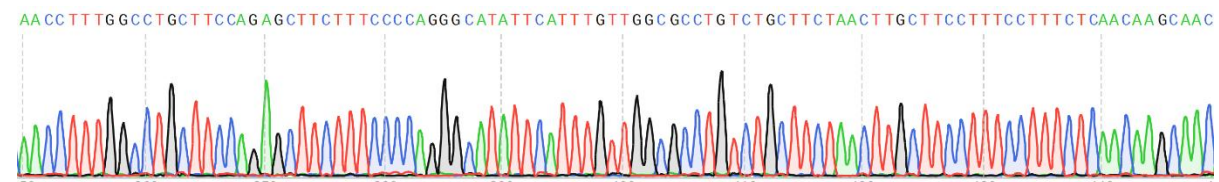
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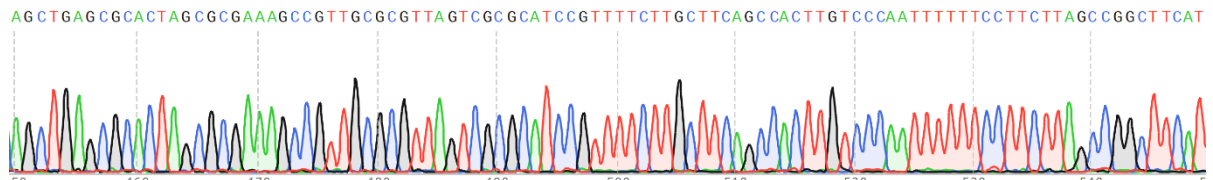
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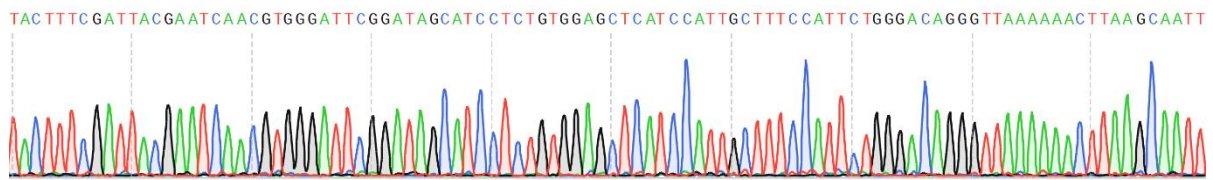
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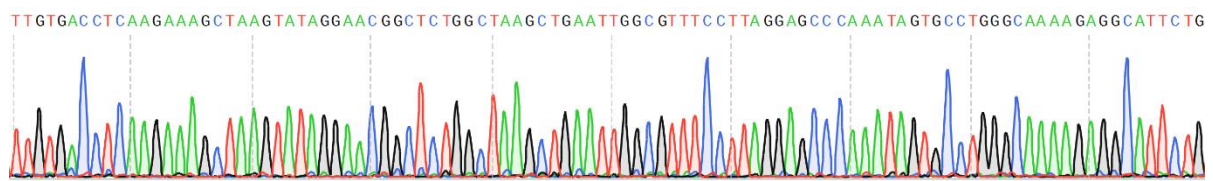
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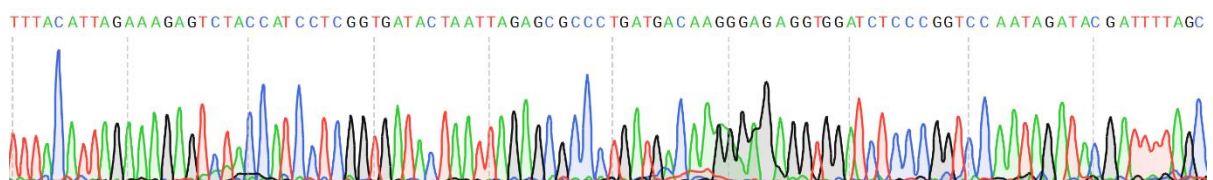
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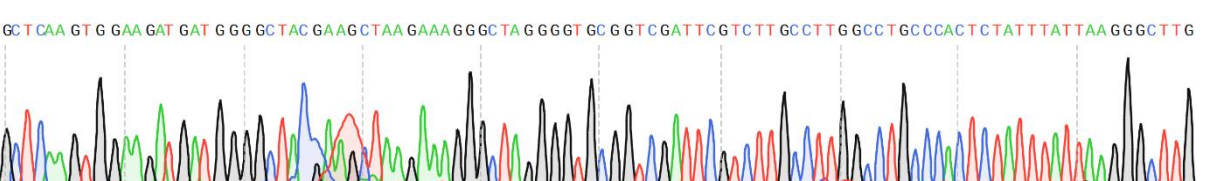
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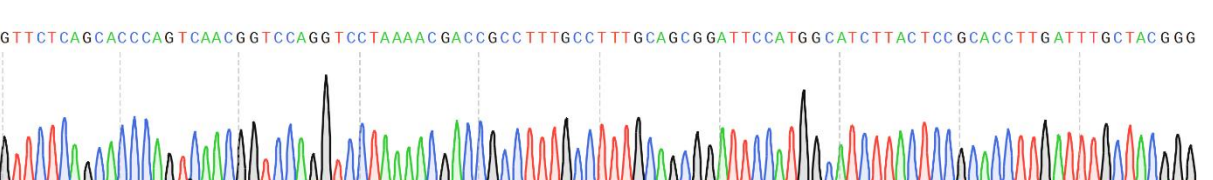
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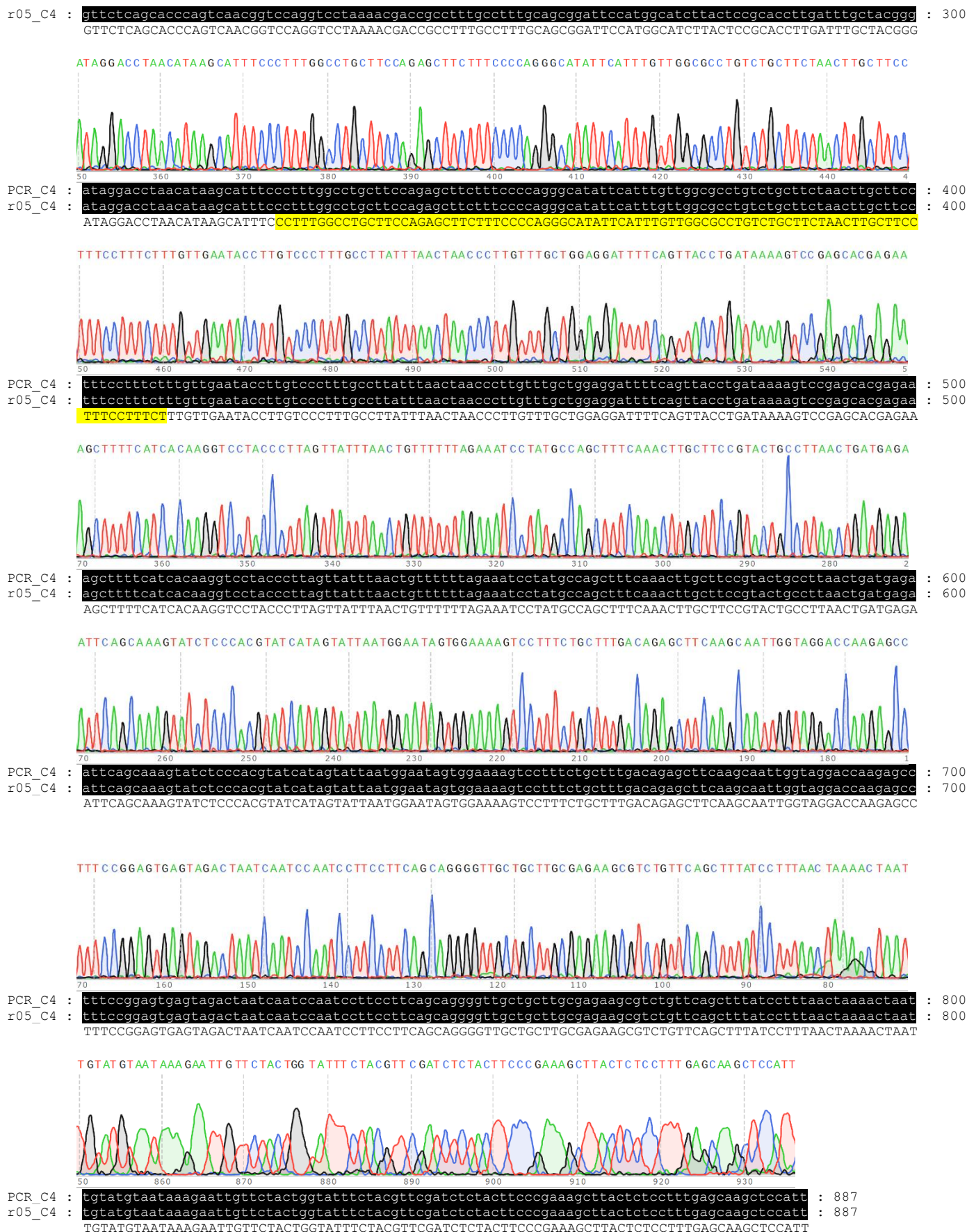
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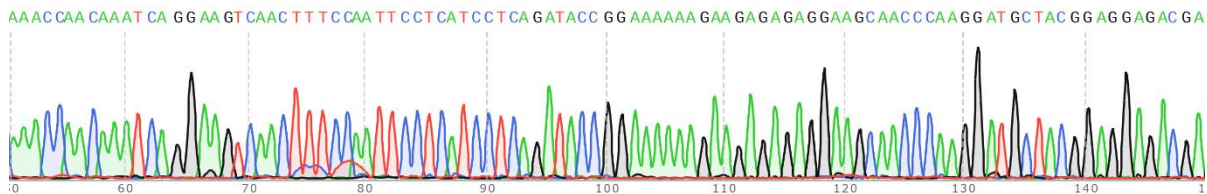


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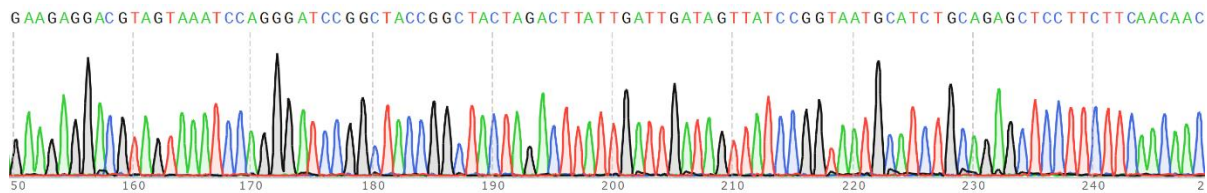


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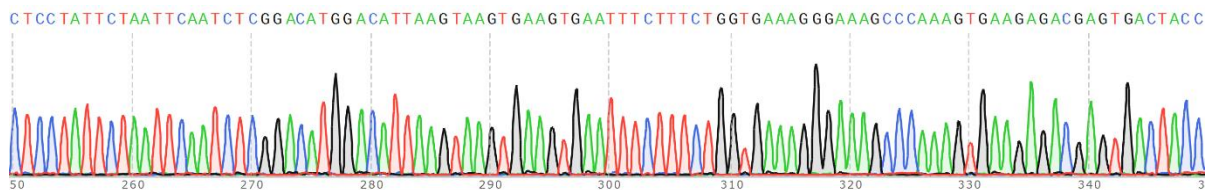




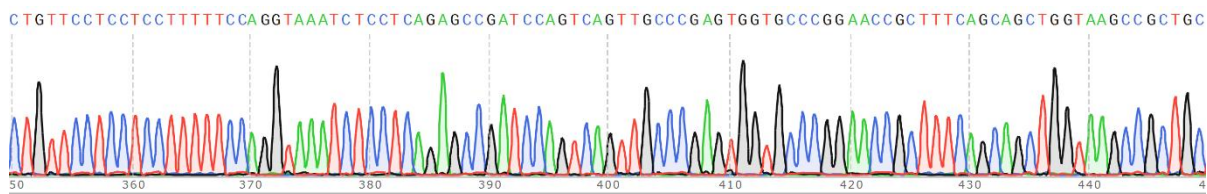
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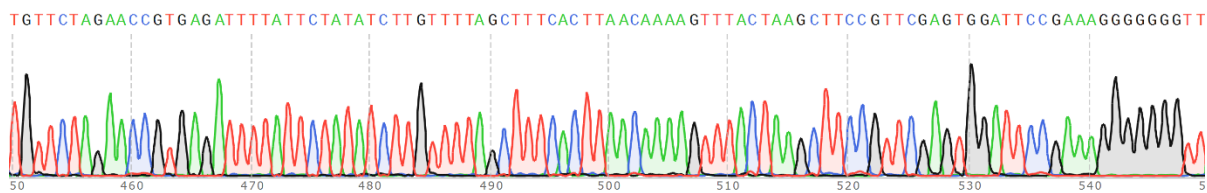
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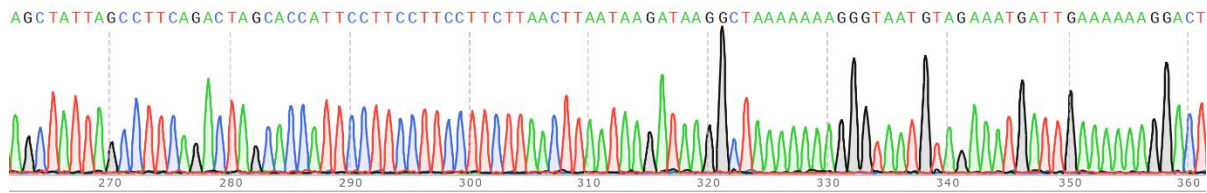
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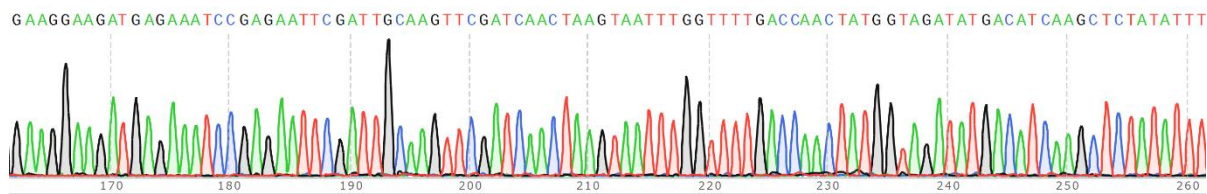
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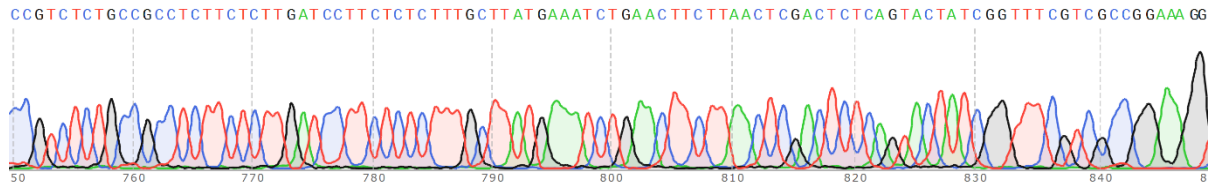
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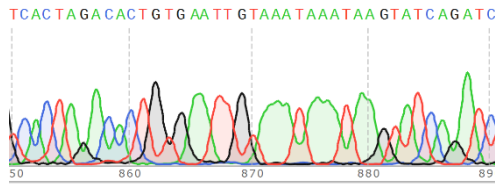
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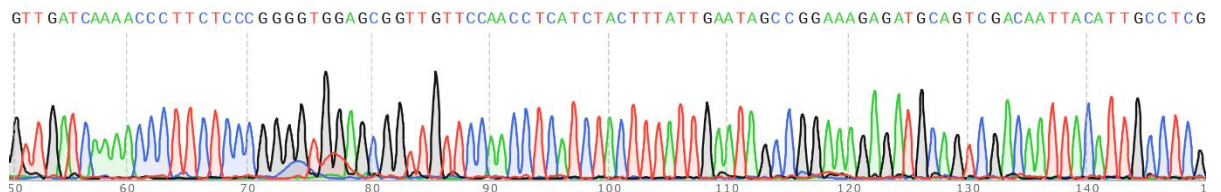
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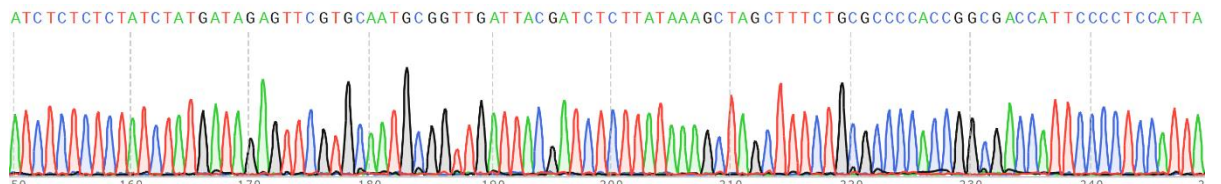
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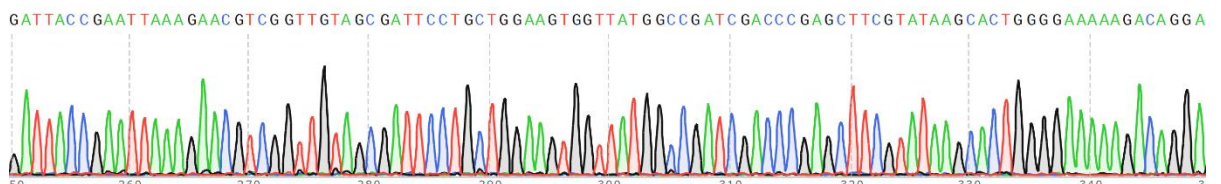
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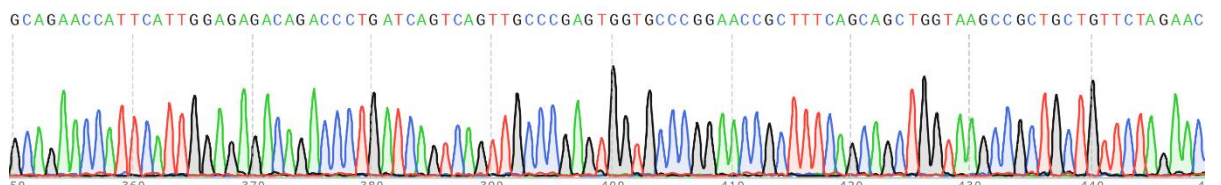
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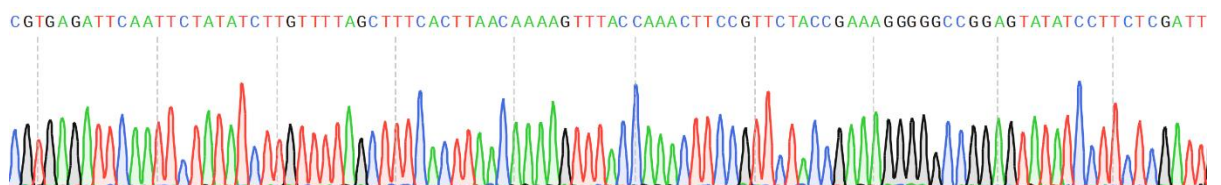
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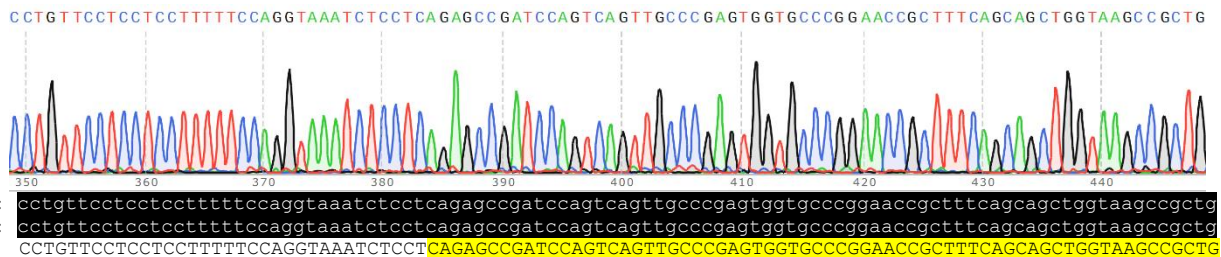
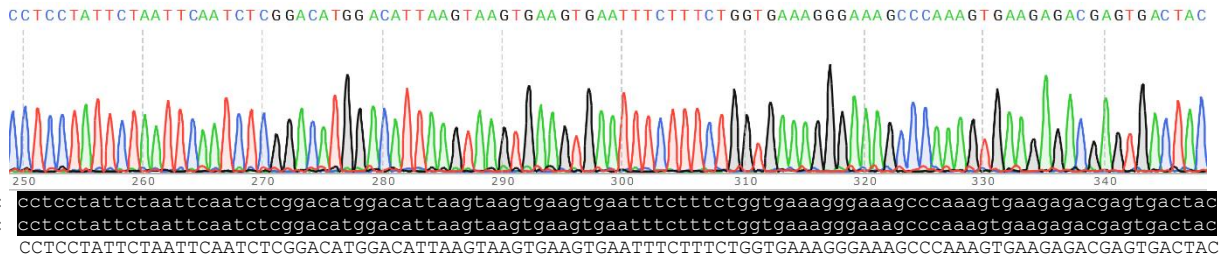
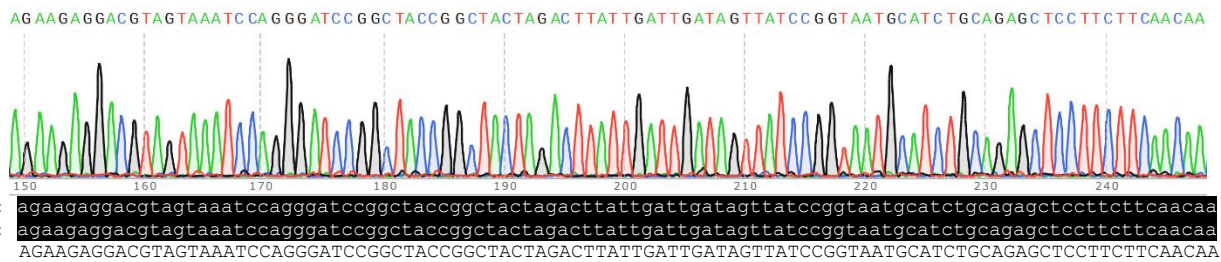
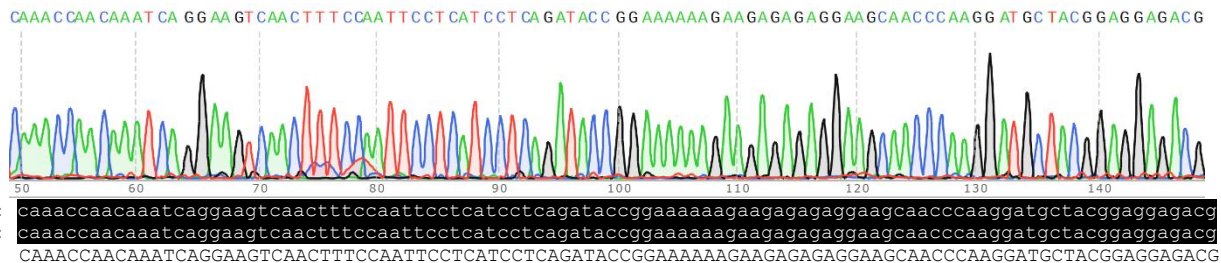
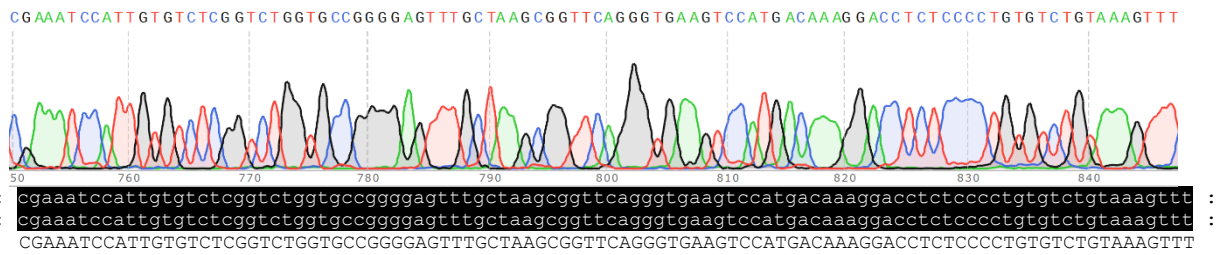
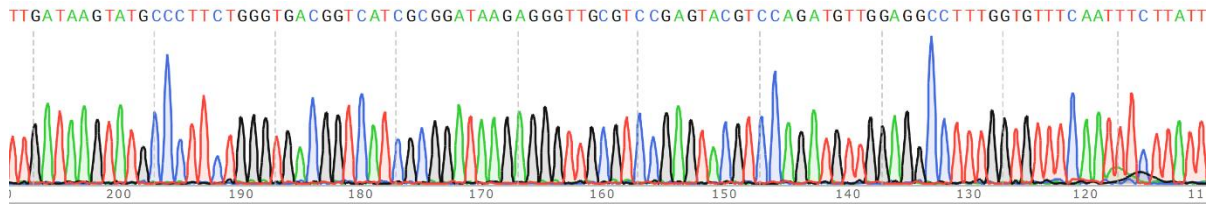
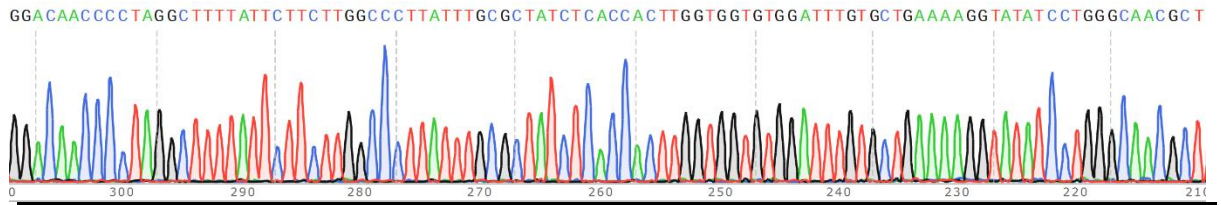
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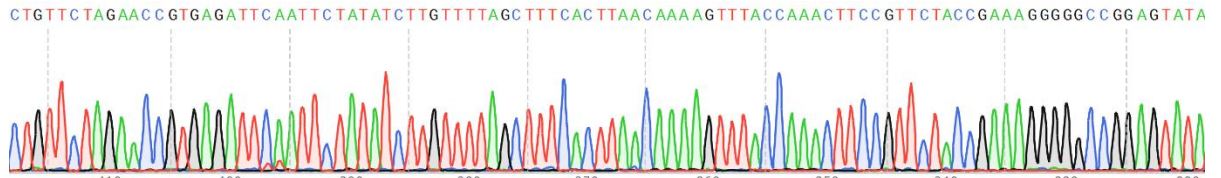


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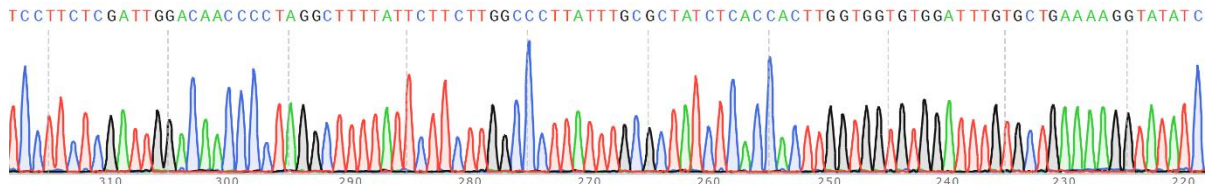


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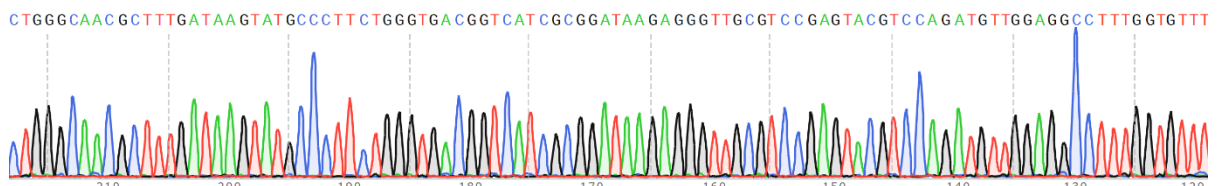




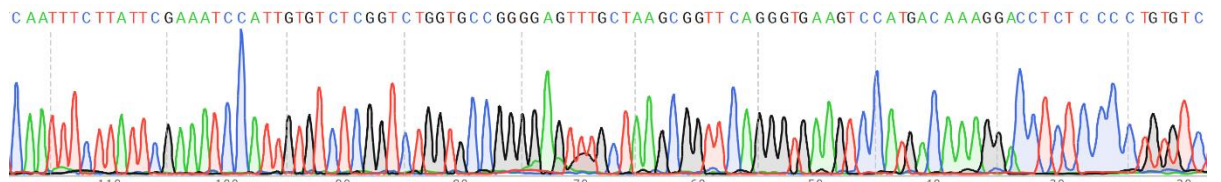
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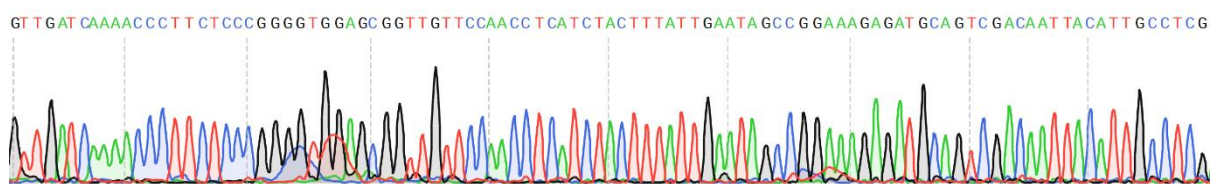
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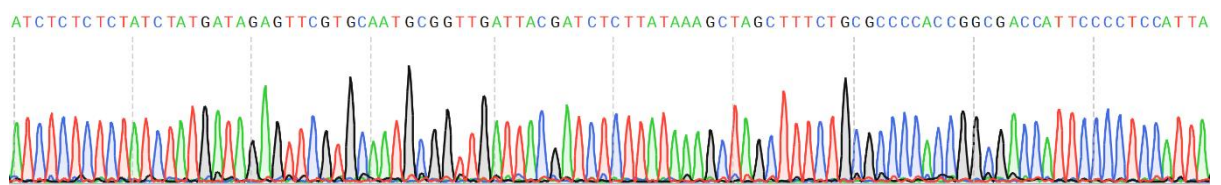
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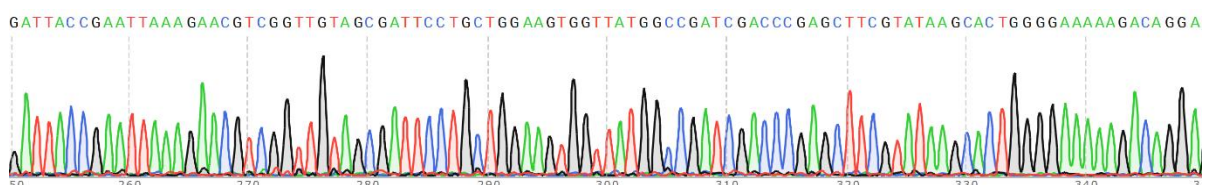
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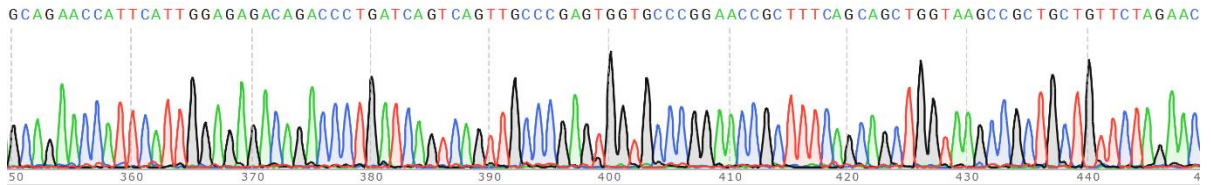


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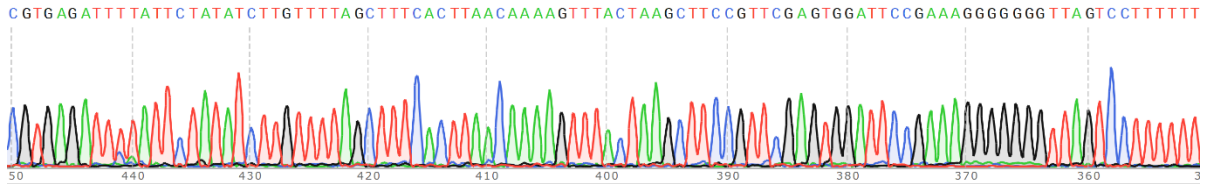


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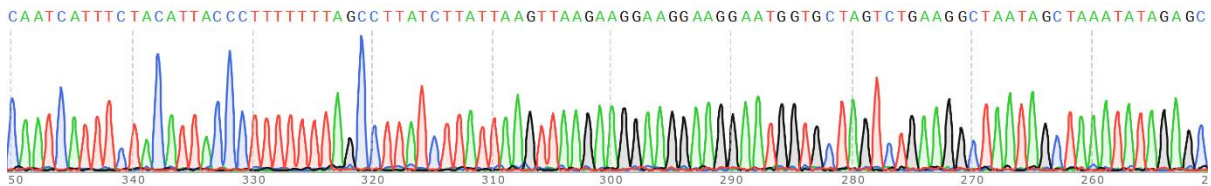
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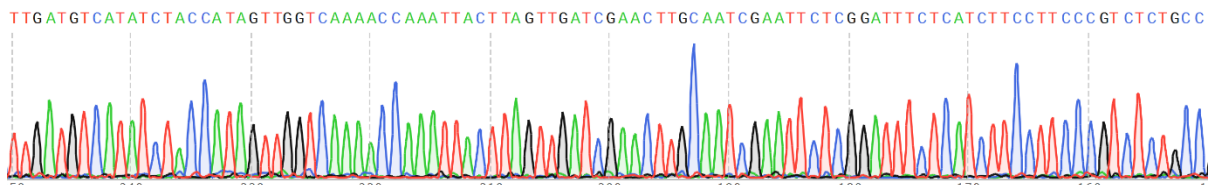
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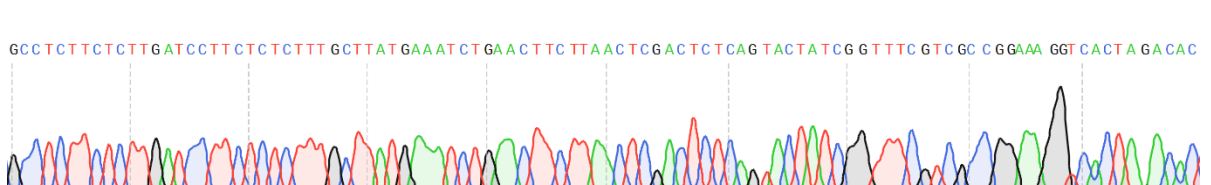
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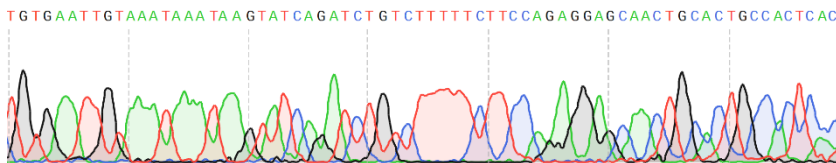
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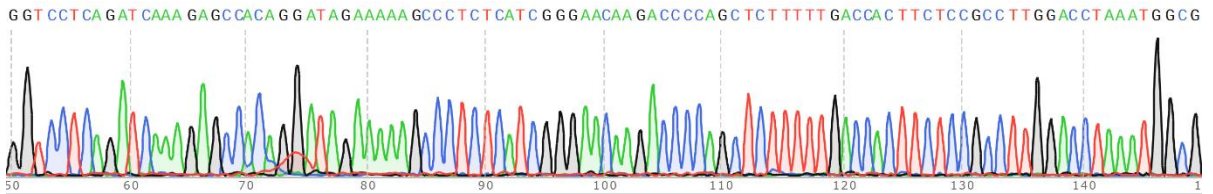
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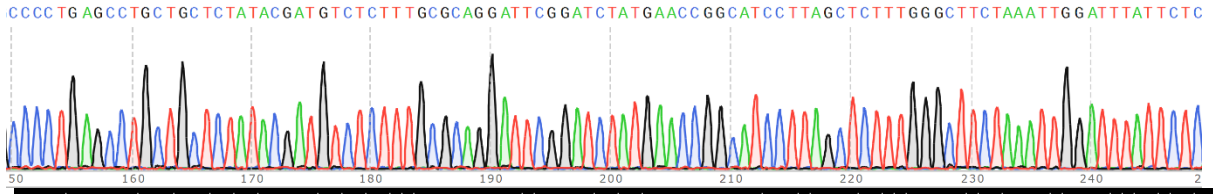
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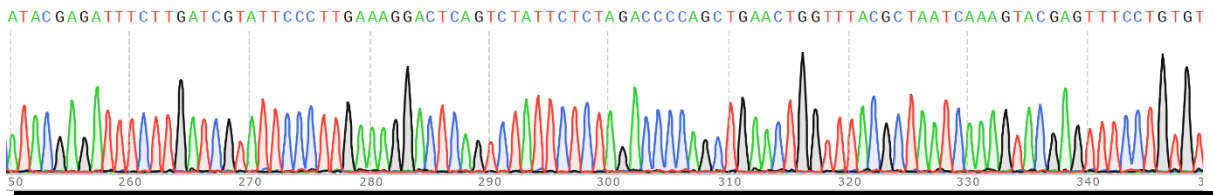
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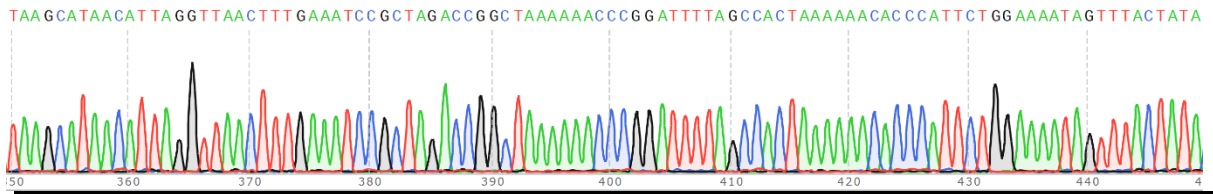
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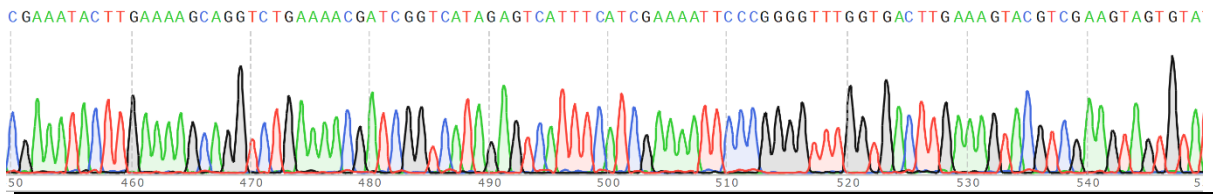
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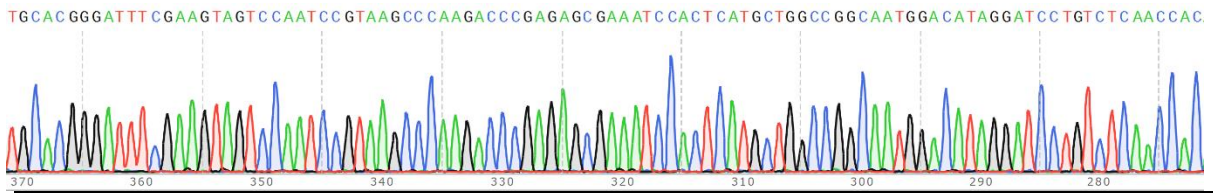
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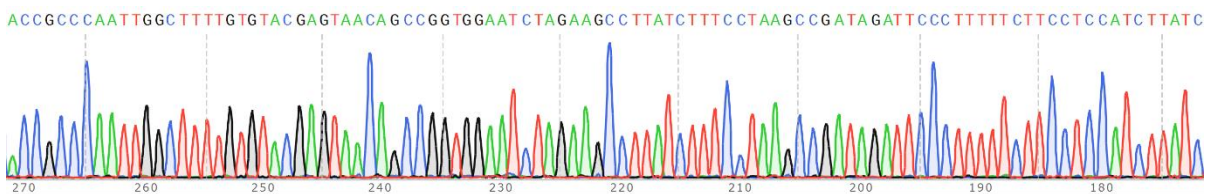
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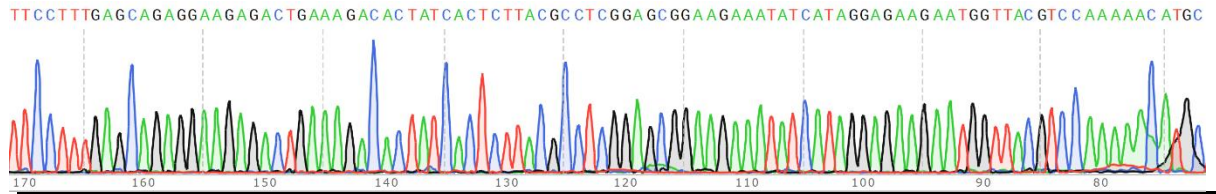
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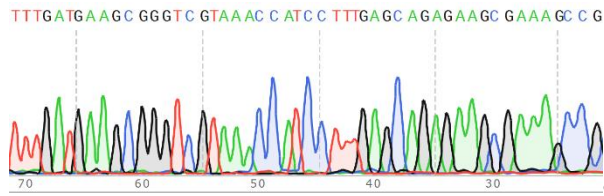
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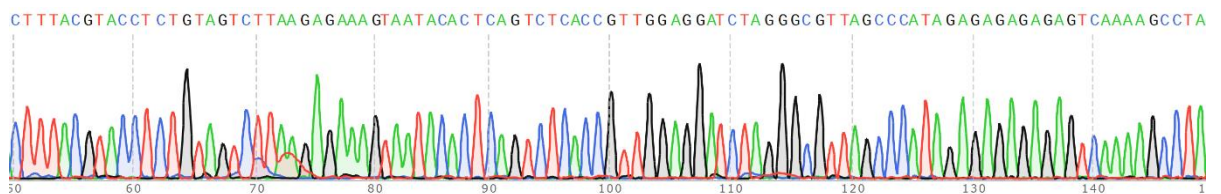
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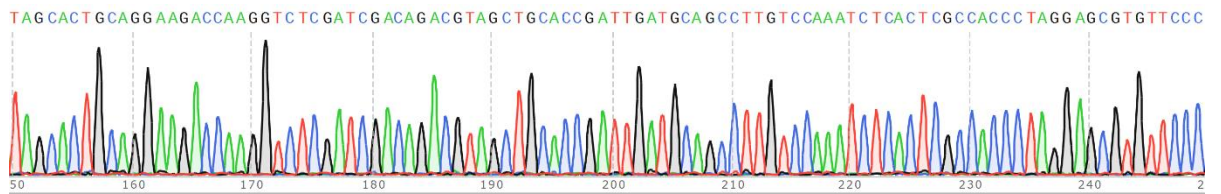
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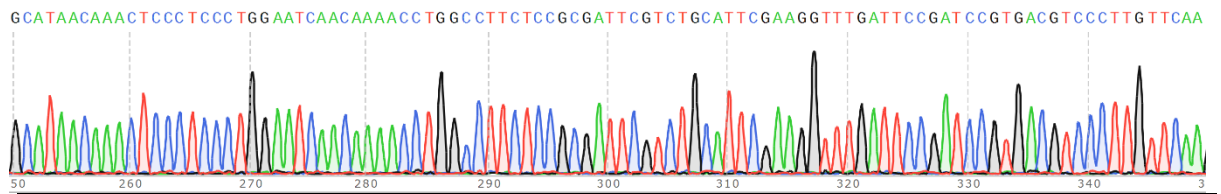
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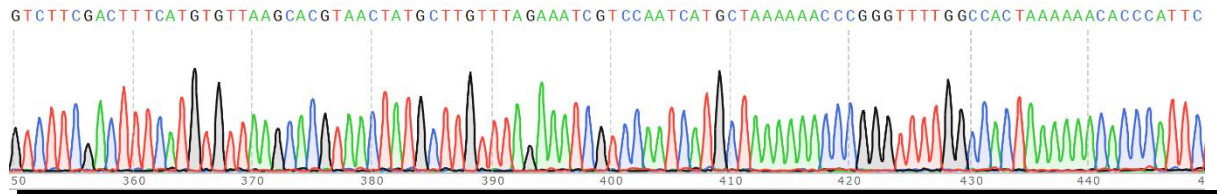
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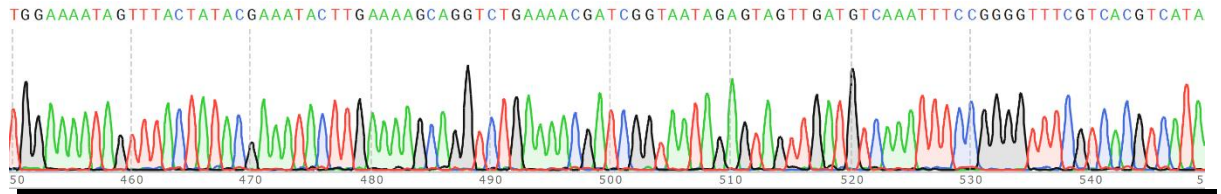
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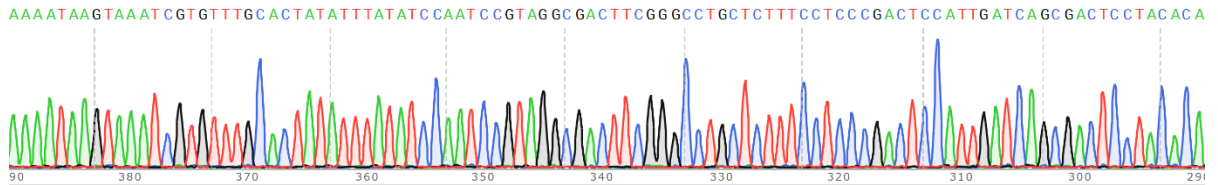
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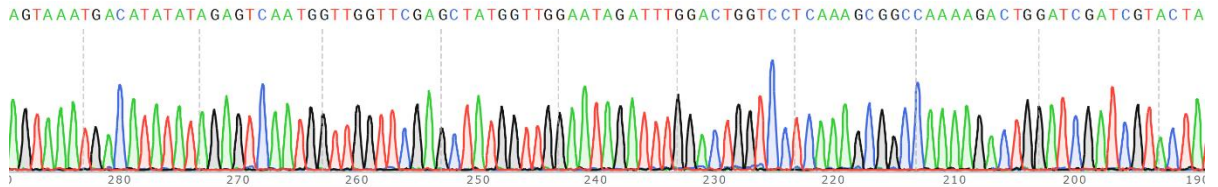
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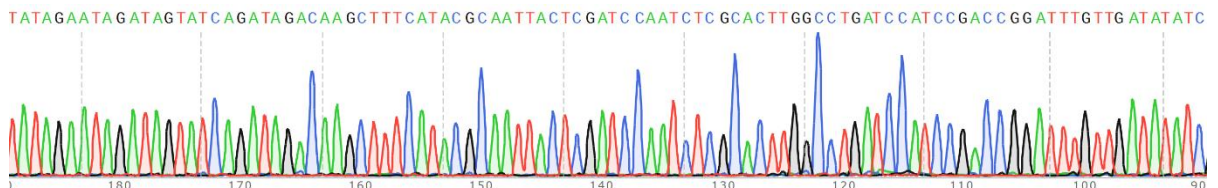
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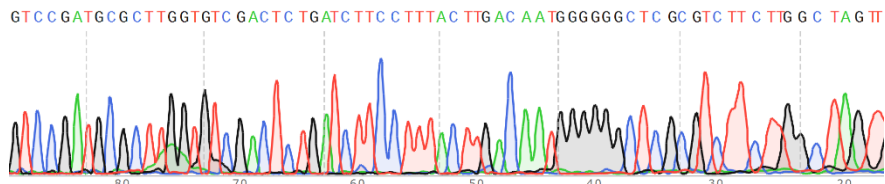
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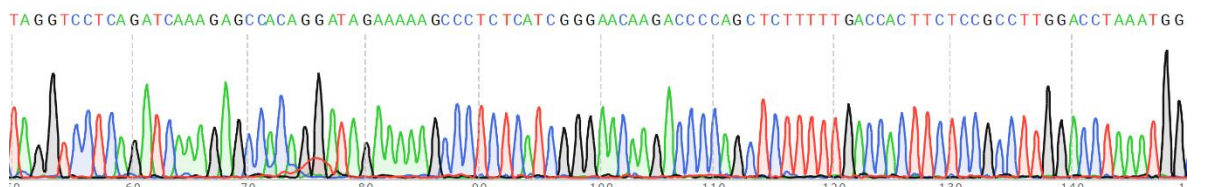
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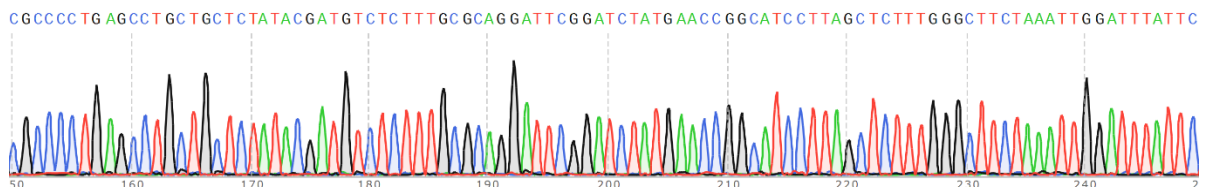
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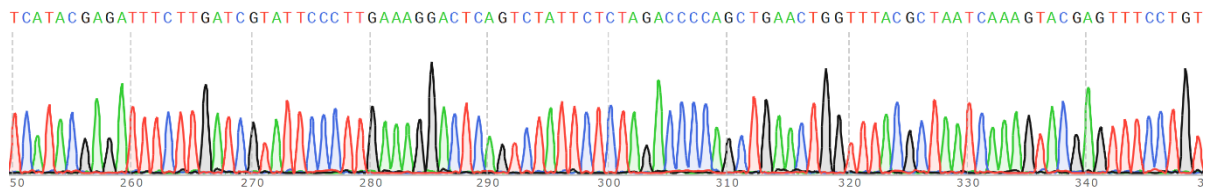
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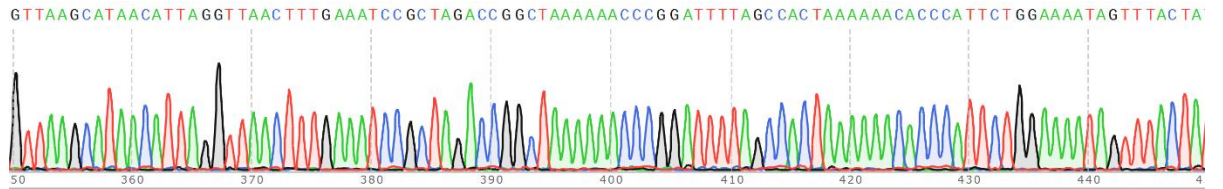


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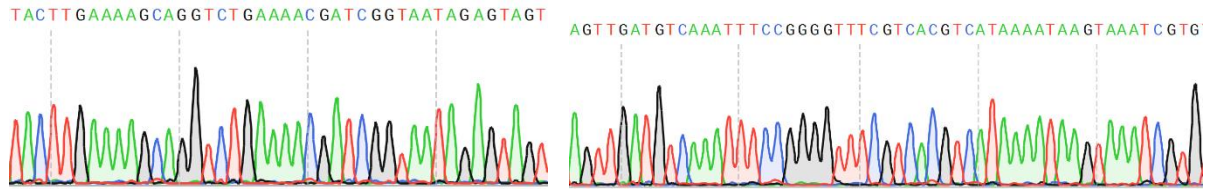


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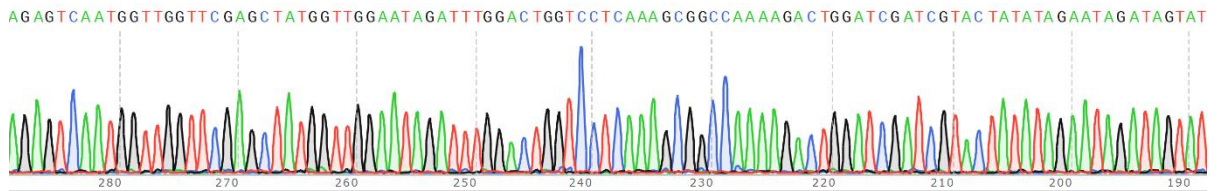
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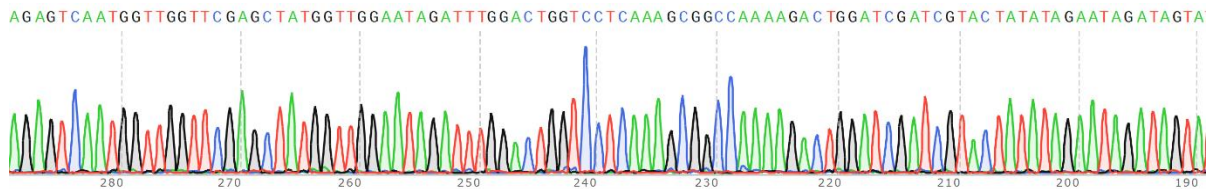
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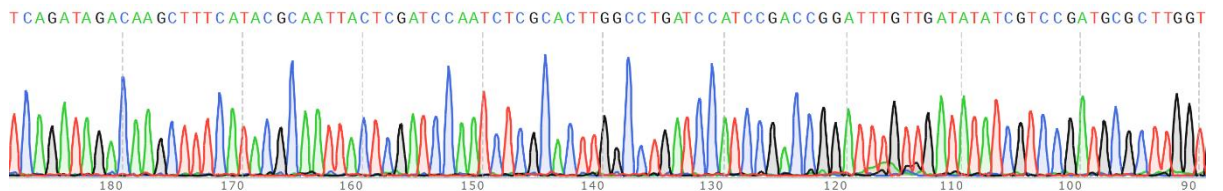
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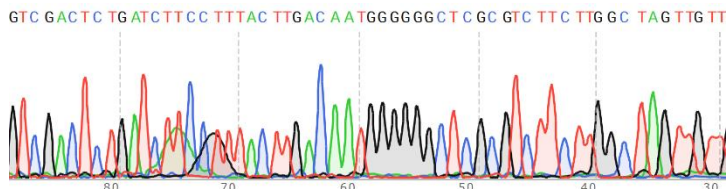
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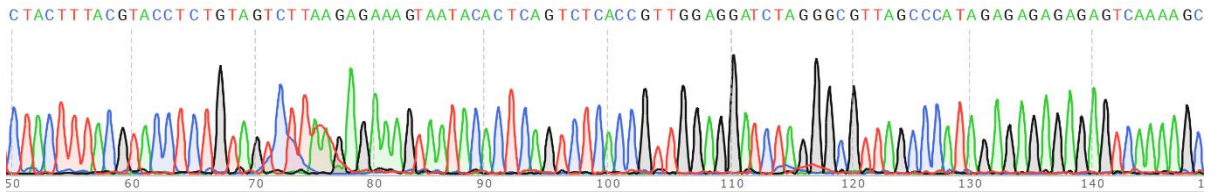
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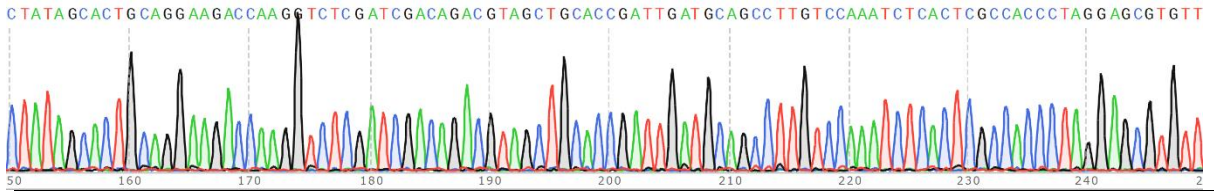
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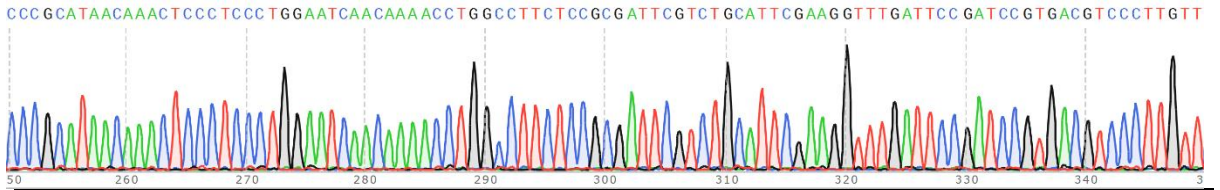
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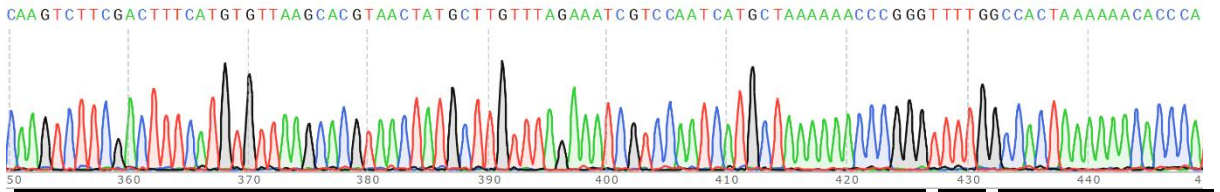
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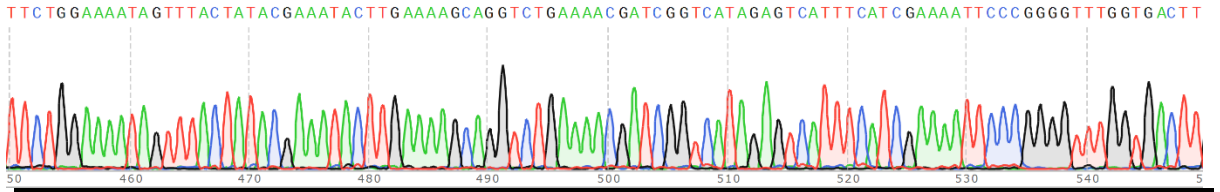
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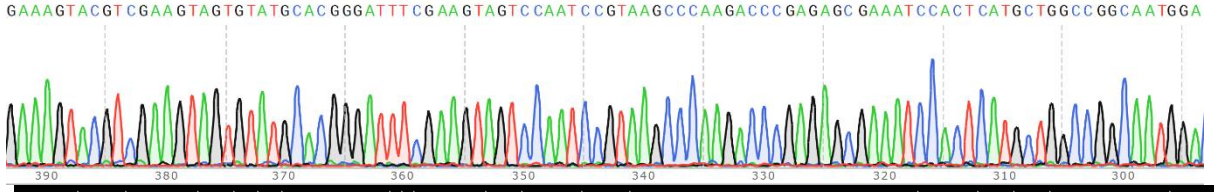
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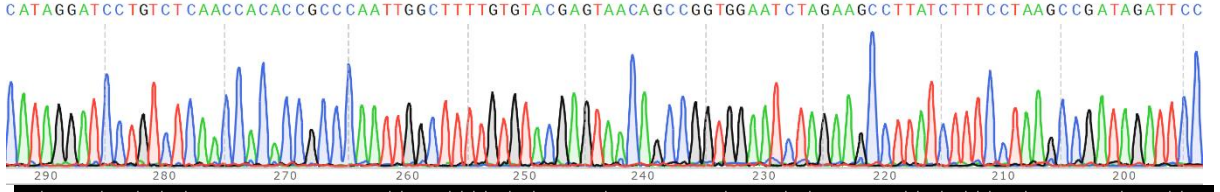
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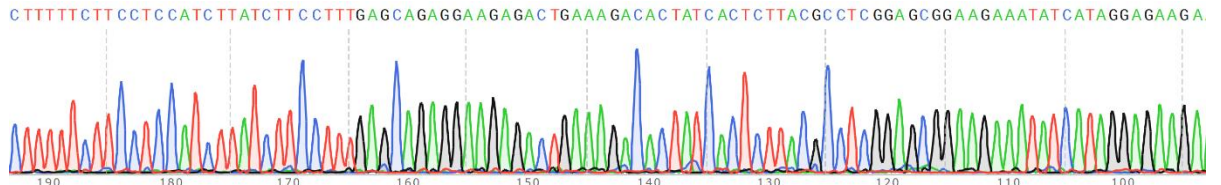
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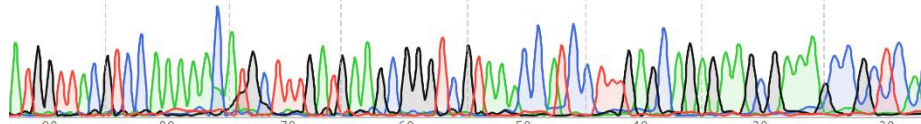


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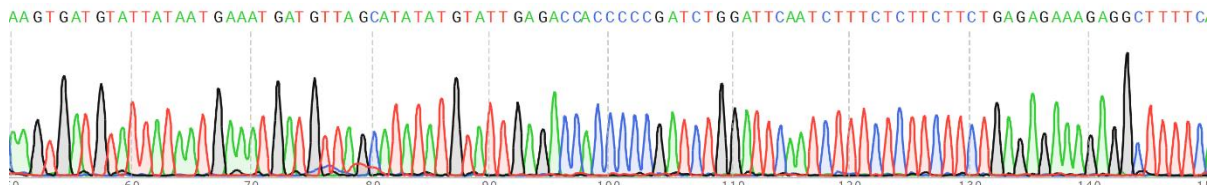
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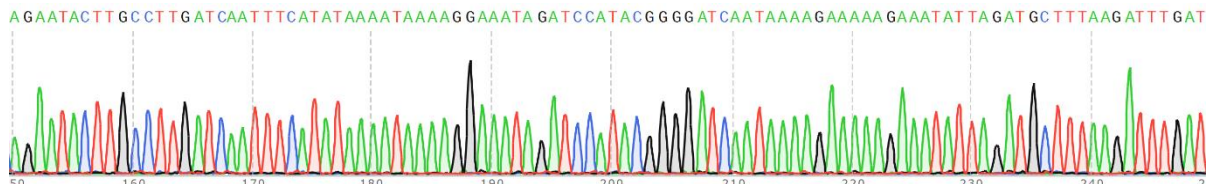


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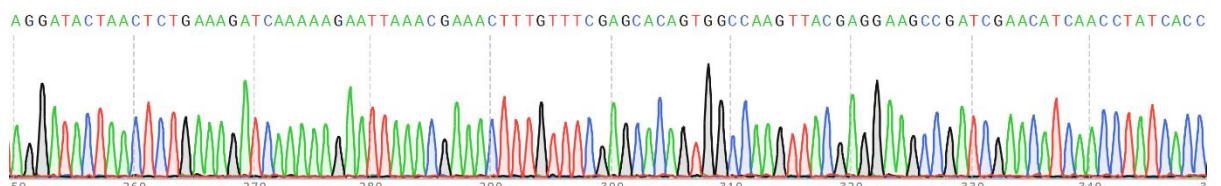
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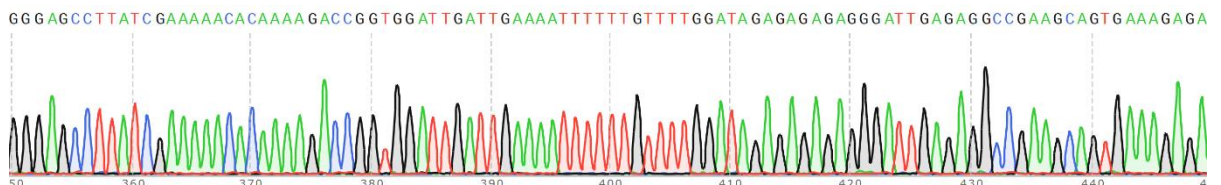
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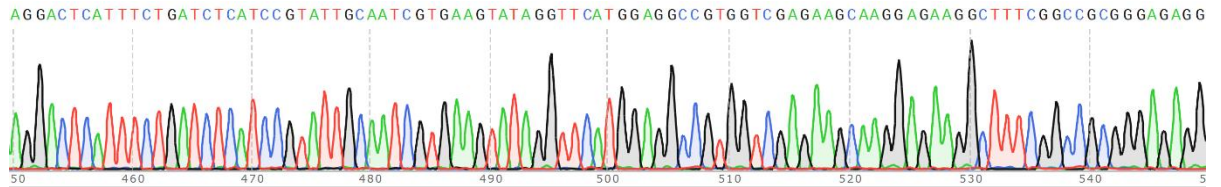
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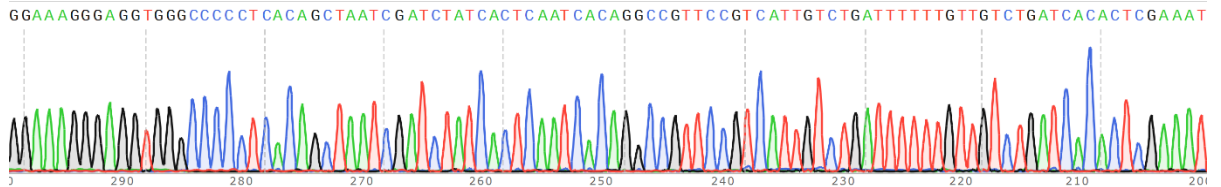
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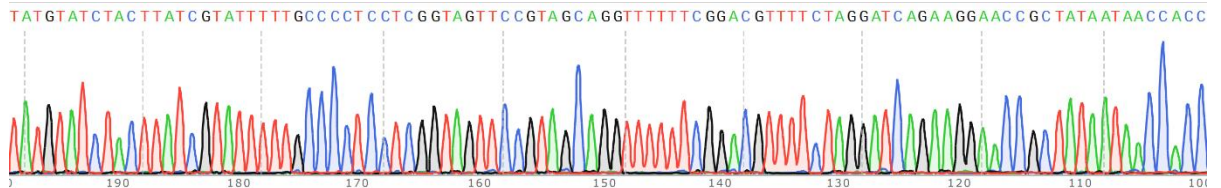
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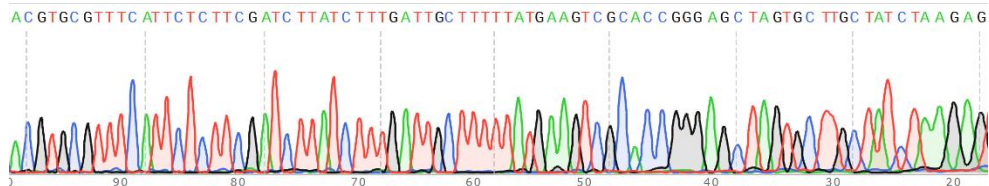
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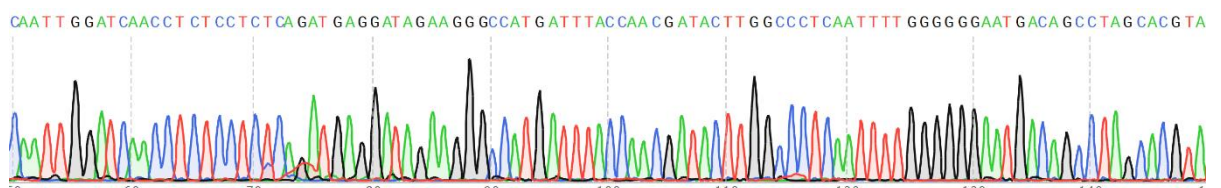
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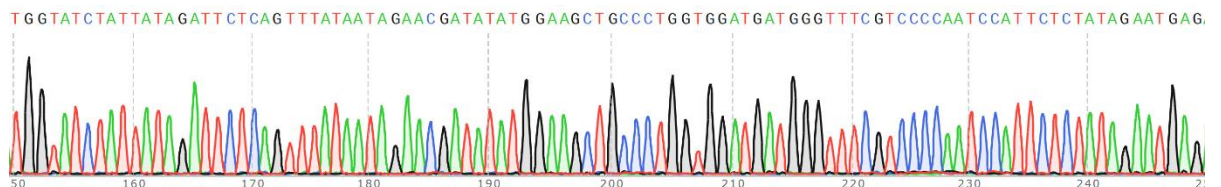
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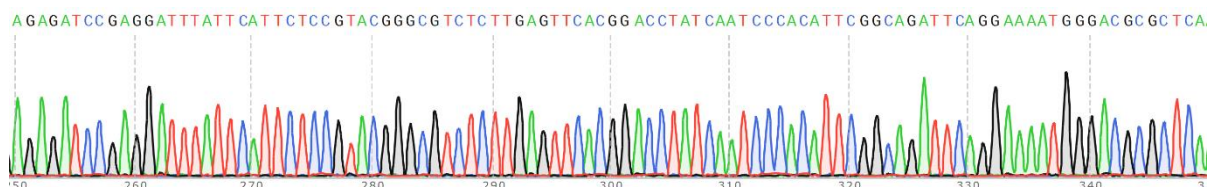
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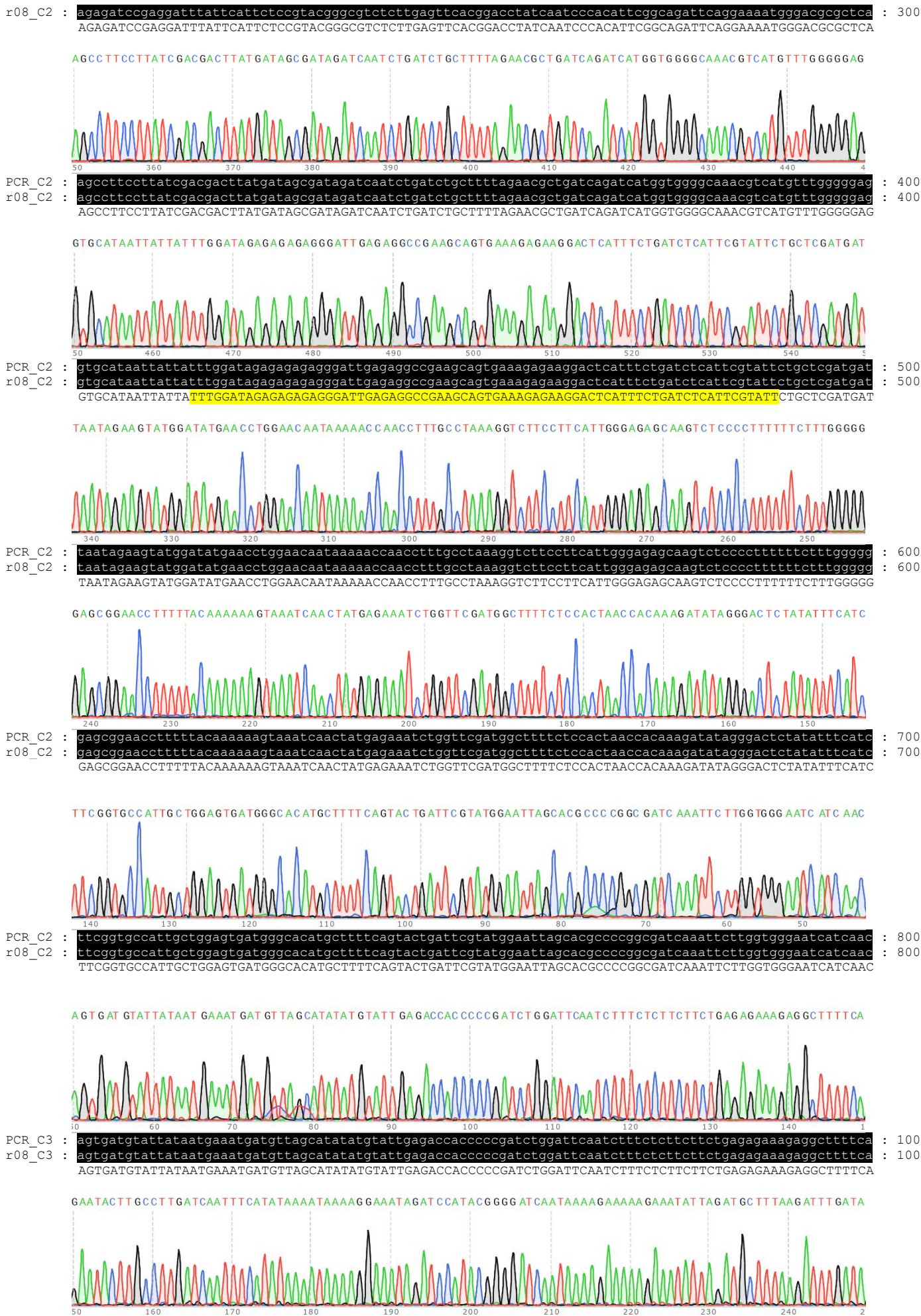
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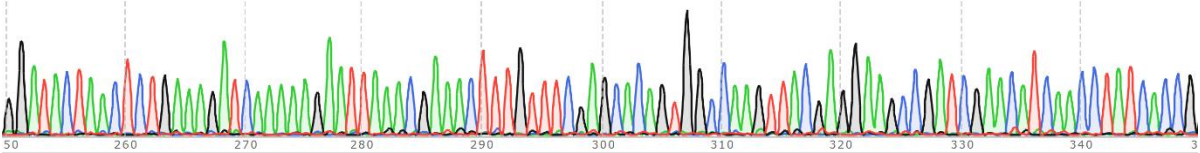


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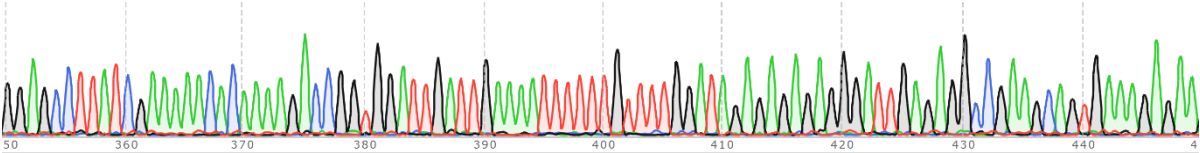
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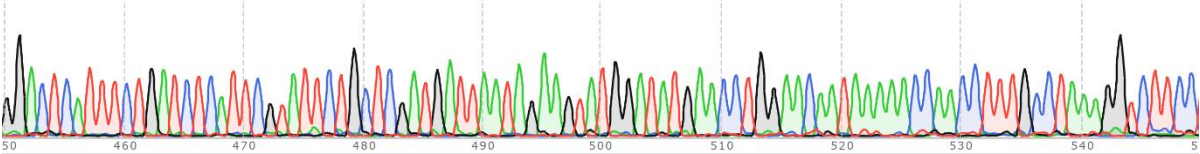
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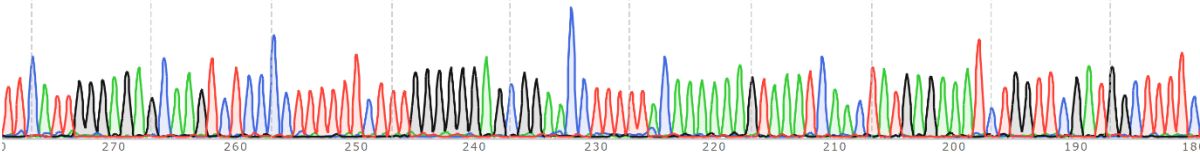
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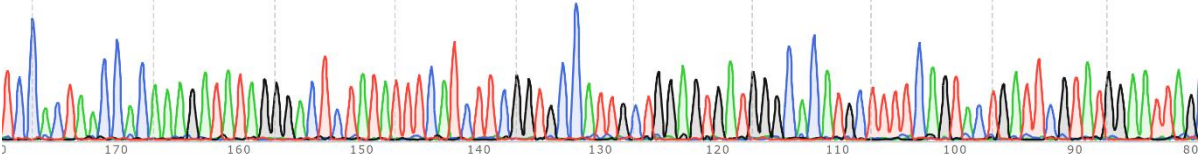
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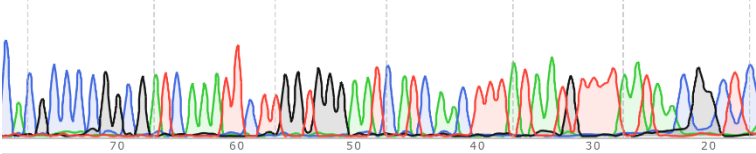
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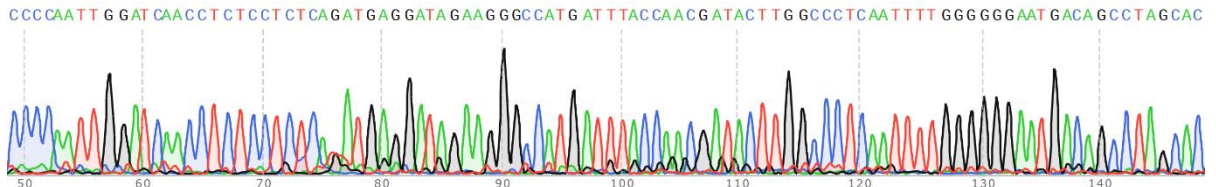


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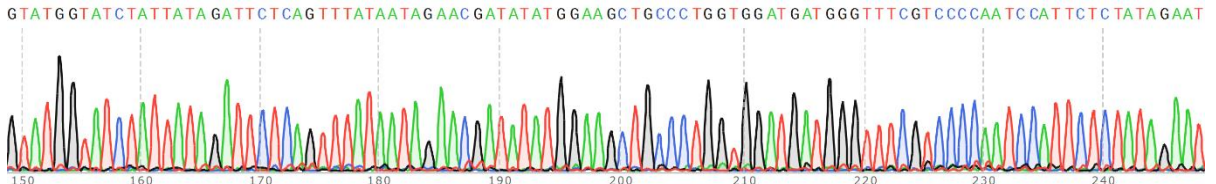
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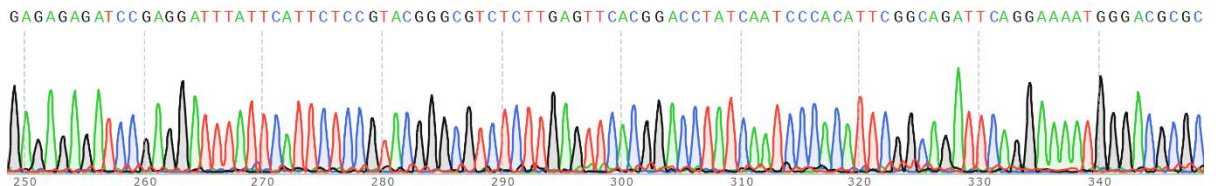
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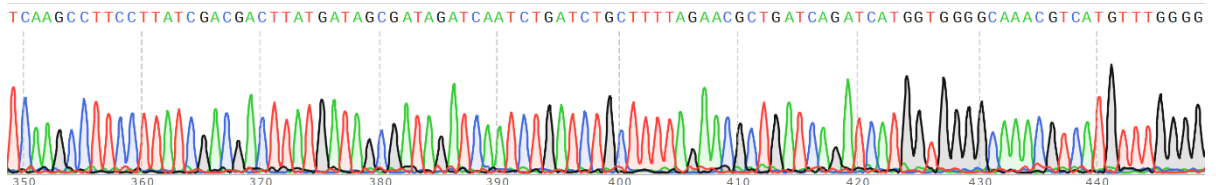
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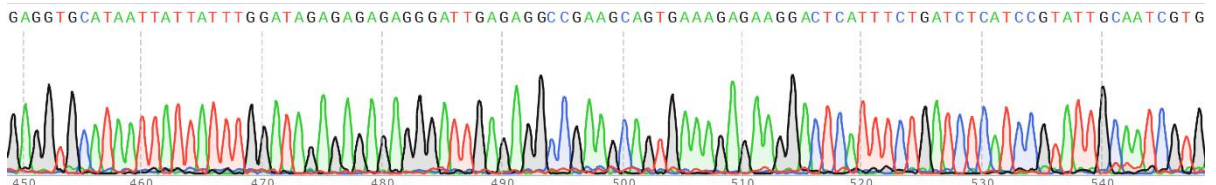
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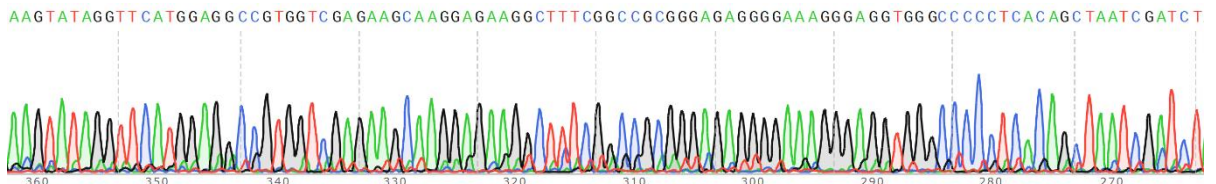
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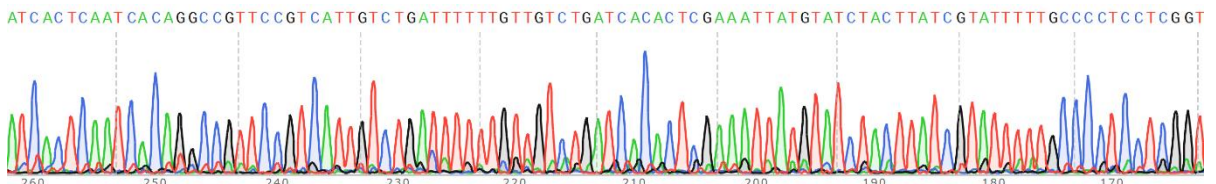
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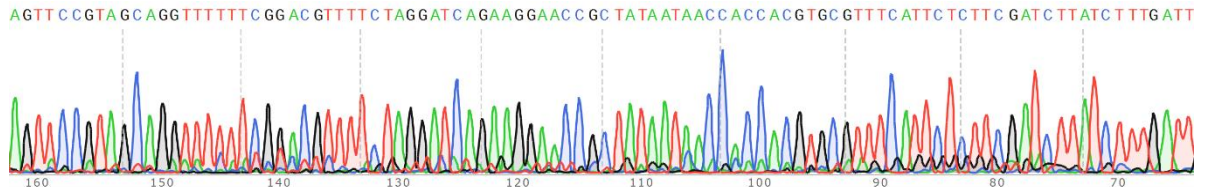
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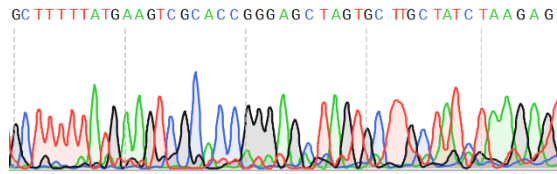
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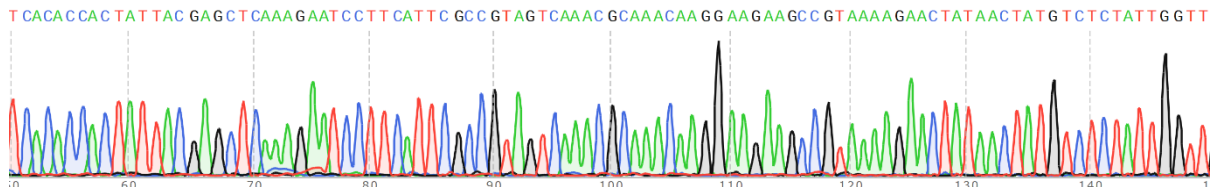


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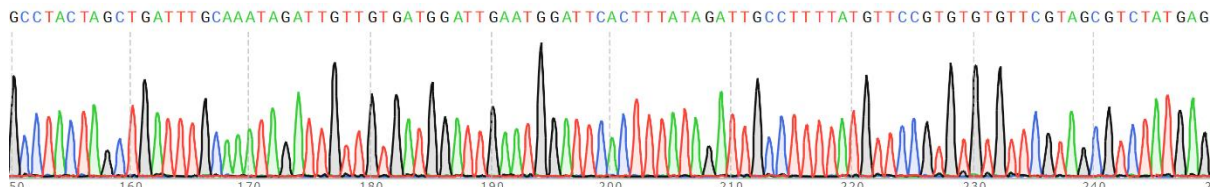


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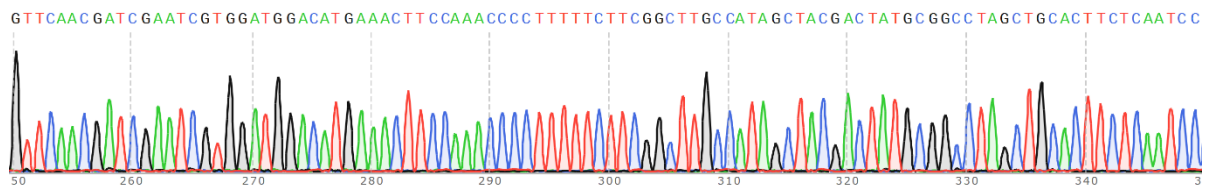
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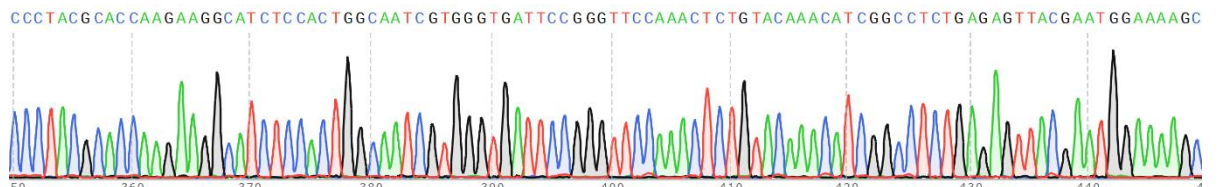
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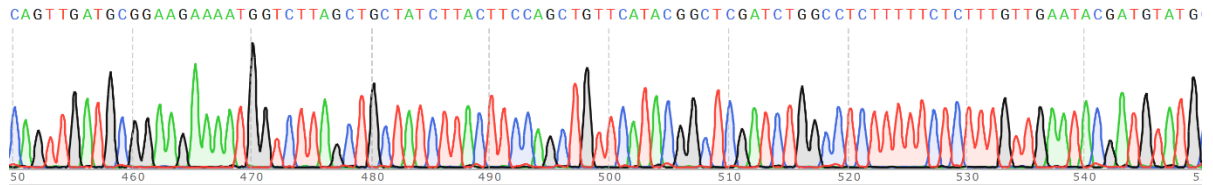
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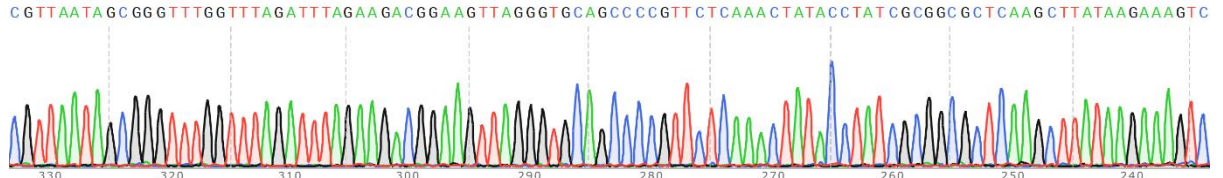
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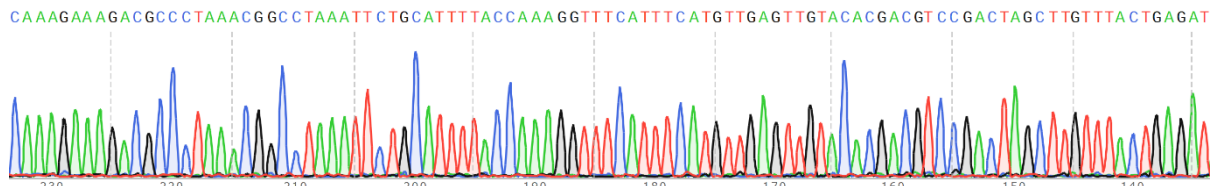
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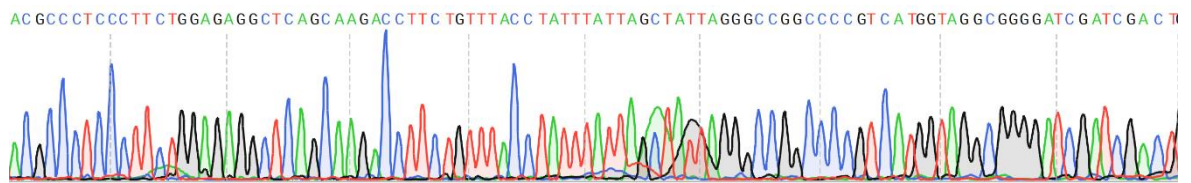
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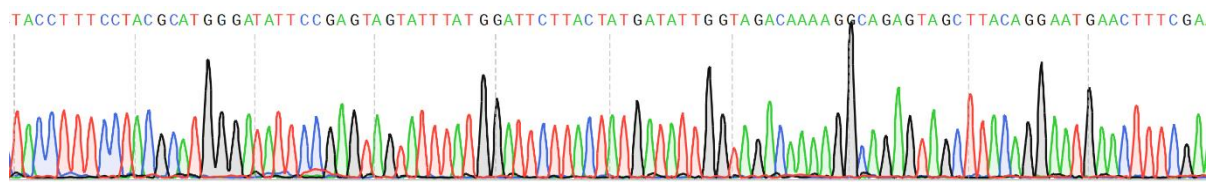
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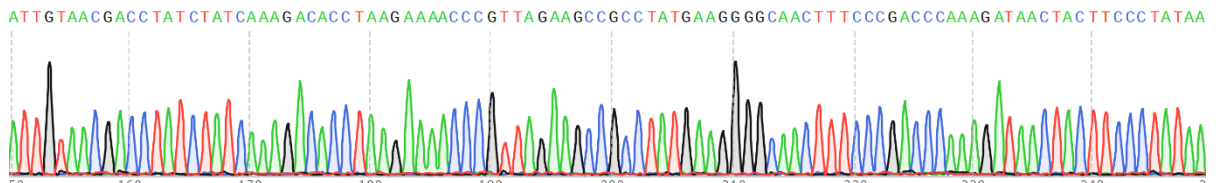
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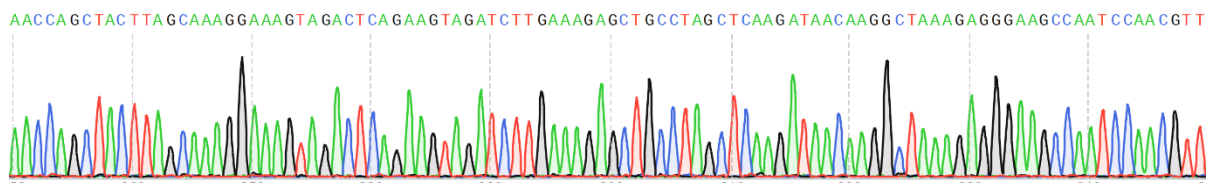
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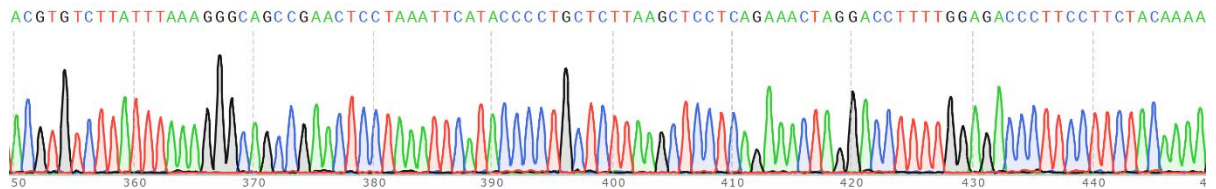
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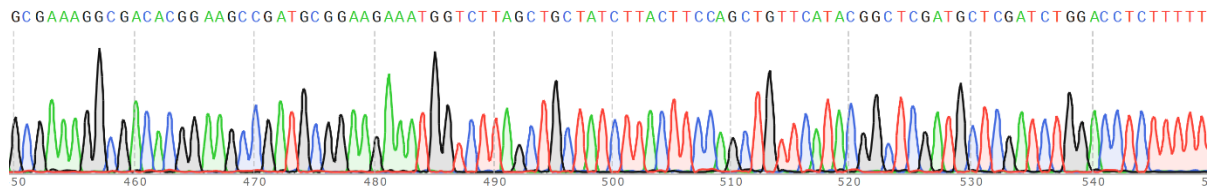
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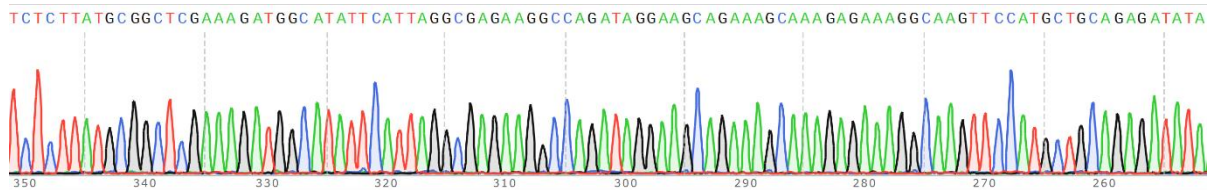
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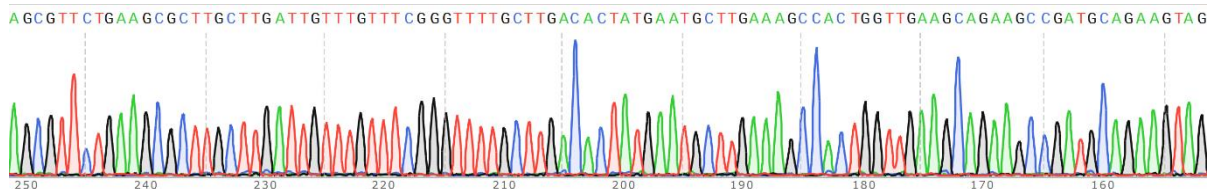
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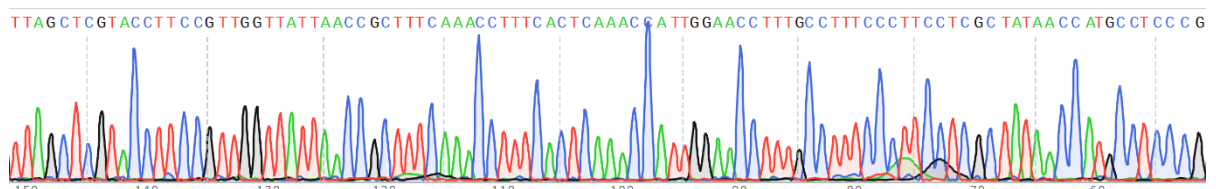
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 r09_C2 : gcgaaaggcgacacggaagccgatgcggaagaaatggtcttagctgctatcttacttcacagctgttcatacggctcgatgctcgatctggacctcttttt : 500
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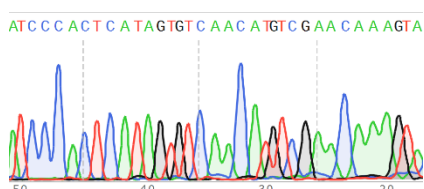
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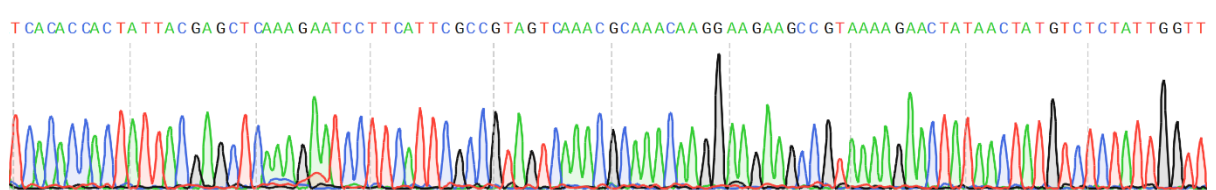
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PCR_C2 : ttagctcgtagcttccggttggttattaaaccgctttcaaacctttcactcaaacattggaacctttgcctttcccttctcgtatataaacatgcctcccg : 800
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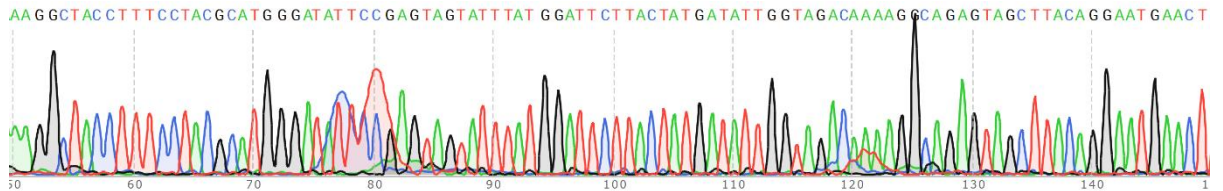


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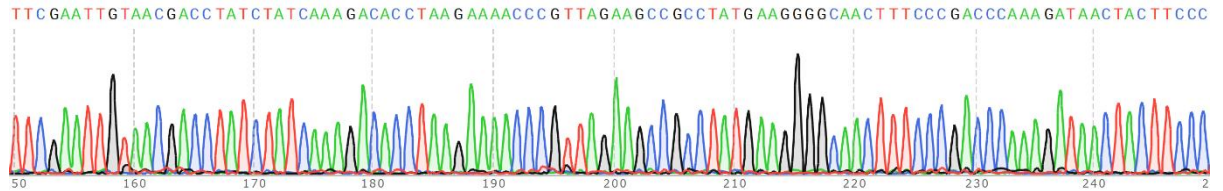


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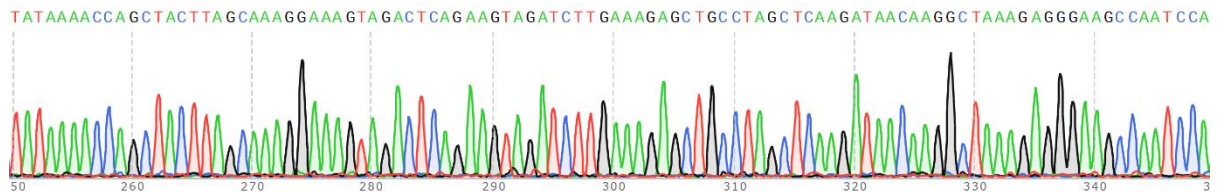
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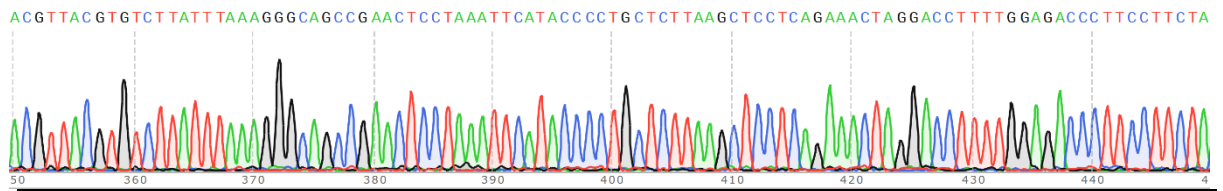
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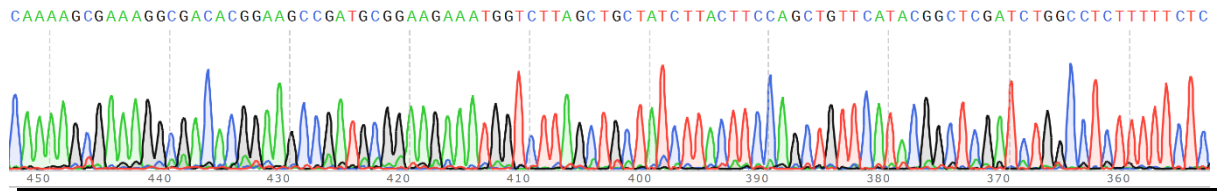
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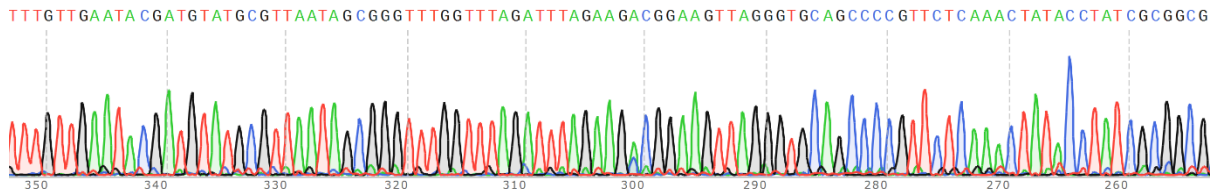
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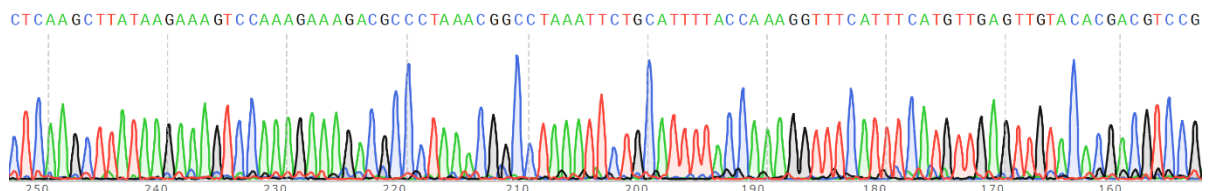
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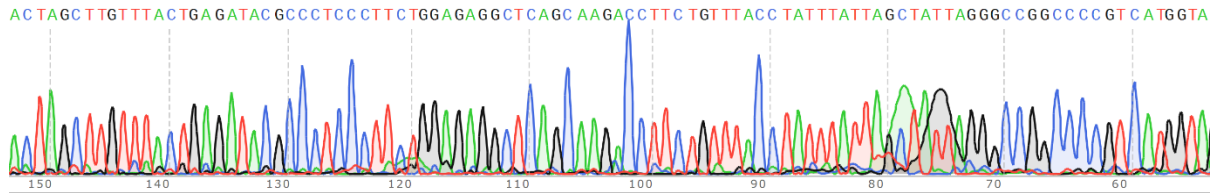
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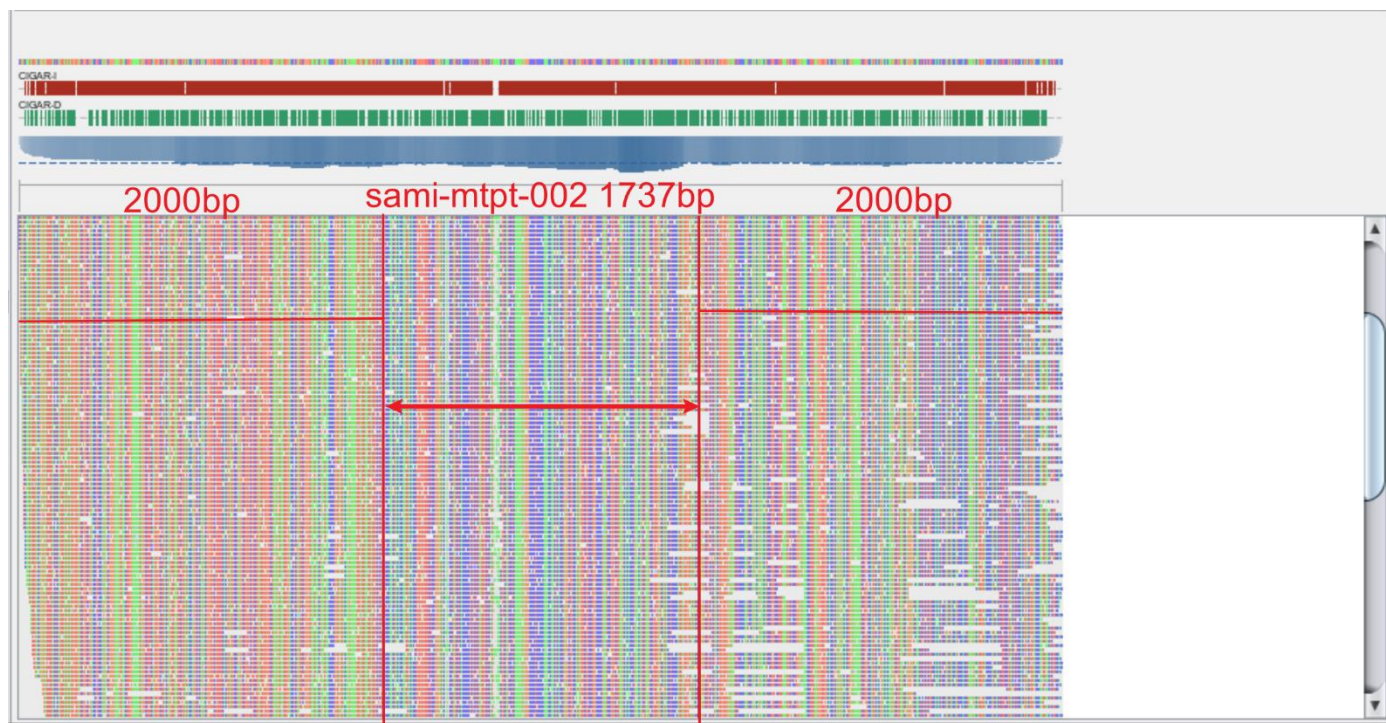
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Figure S8. Alignments of the long PacBio reads to the MTPT fragments in the mitogenome of *S. miltiorrhiza*. Panels a–p show the alignments of long PacBio reads to the ten MTPT fragments in MC1 (sami-mtpt-001 to sami-mtpt-010) and six MTPT fragments in MC2 (sami-mtpt-011 to sami-mtpt-016). The MTPT regions are indicated with the red lines having arrow heads at each end. The 2000 bp-long flanking sequences are indicated with a red line without arrow heads.

a



b



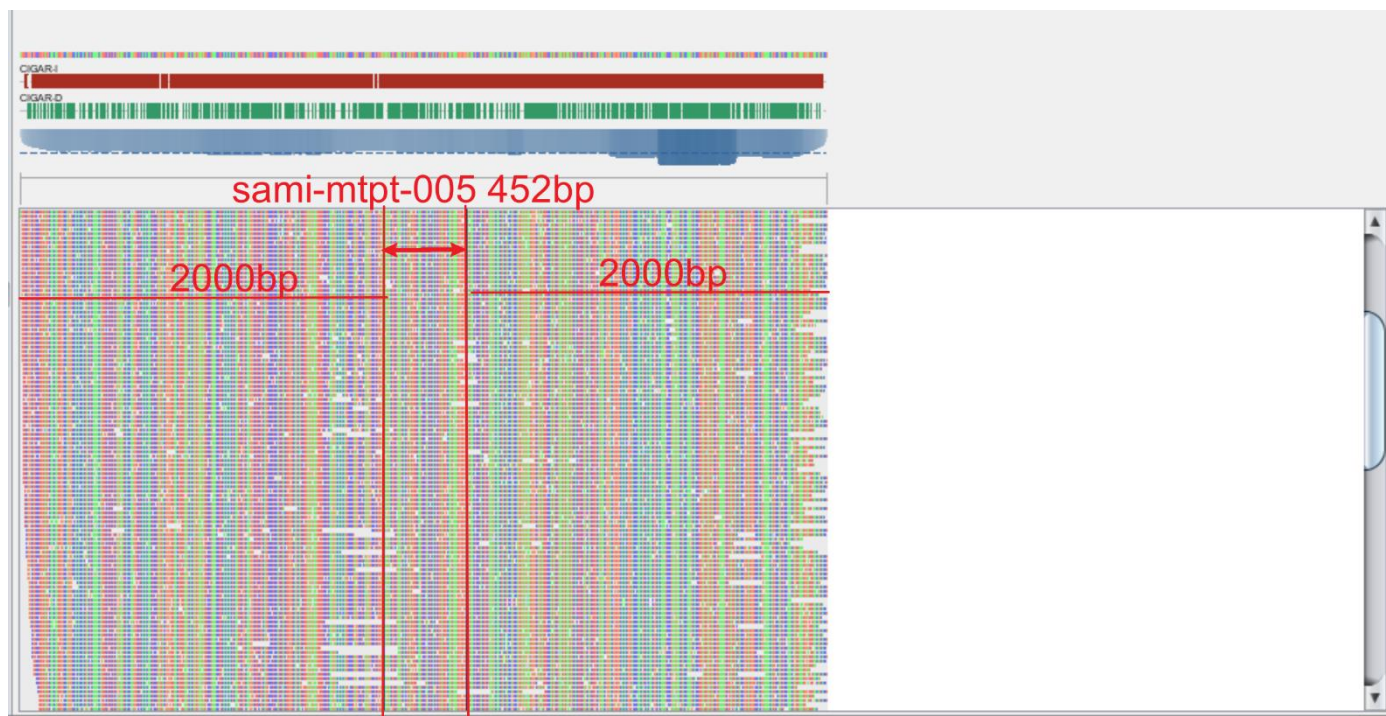
c



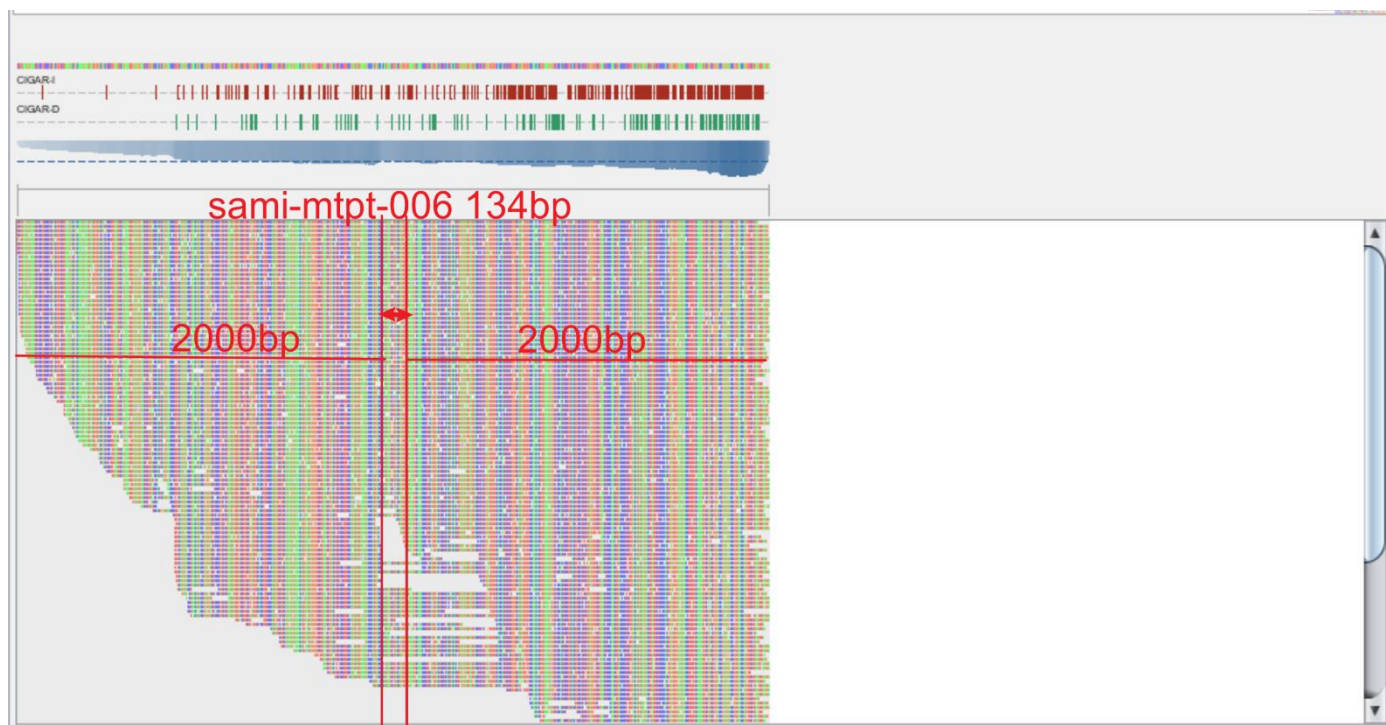
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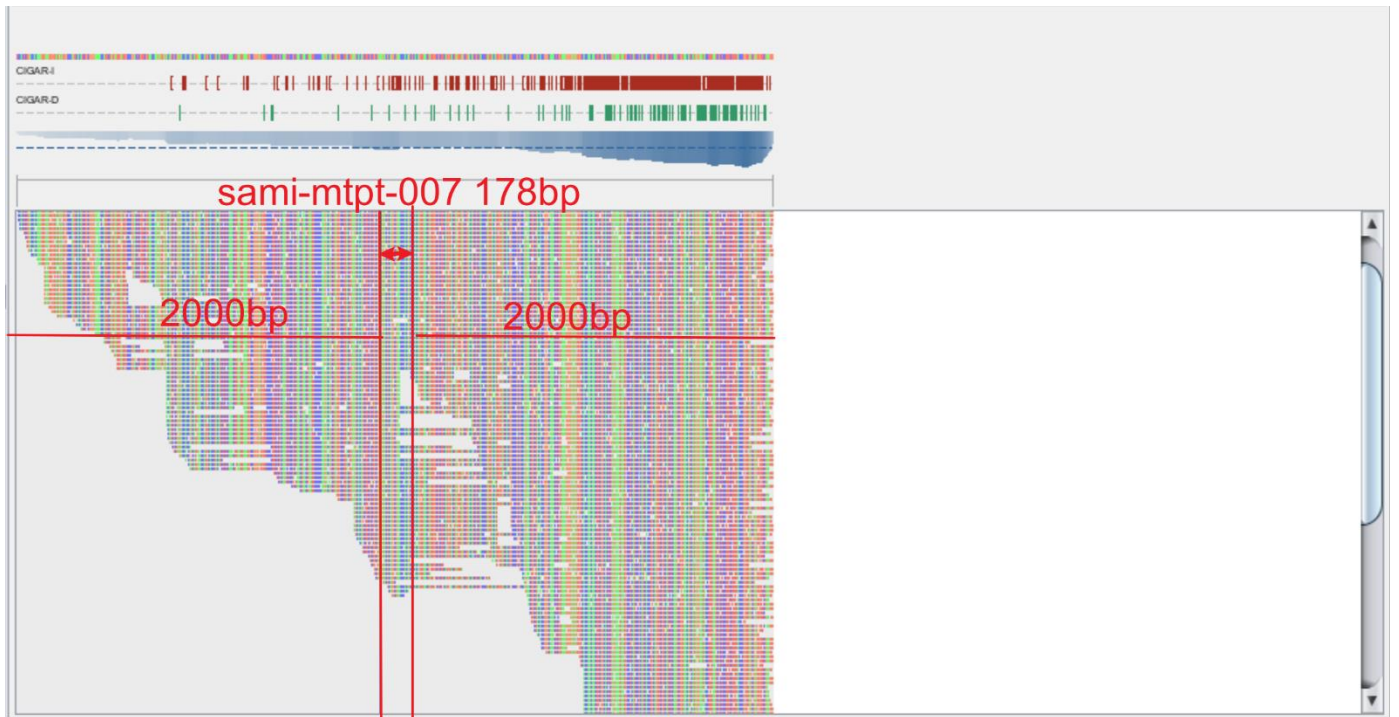


e

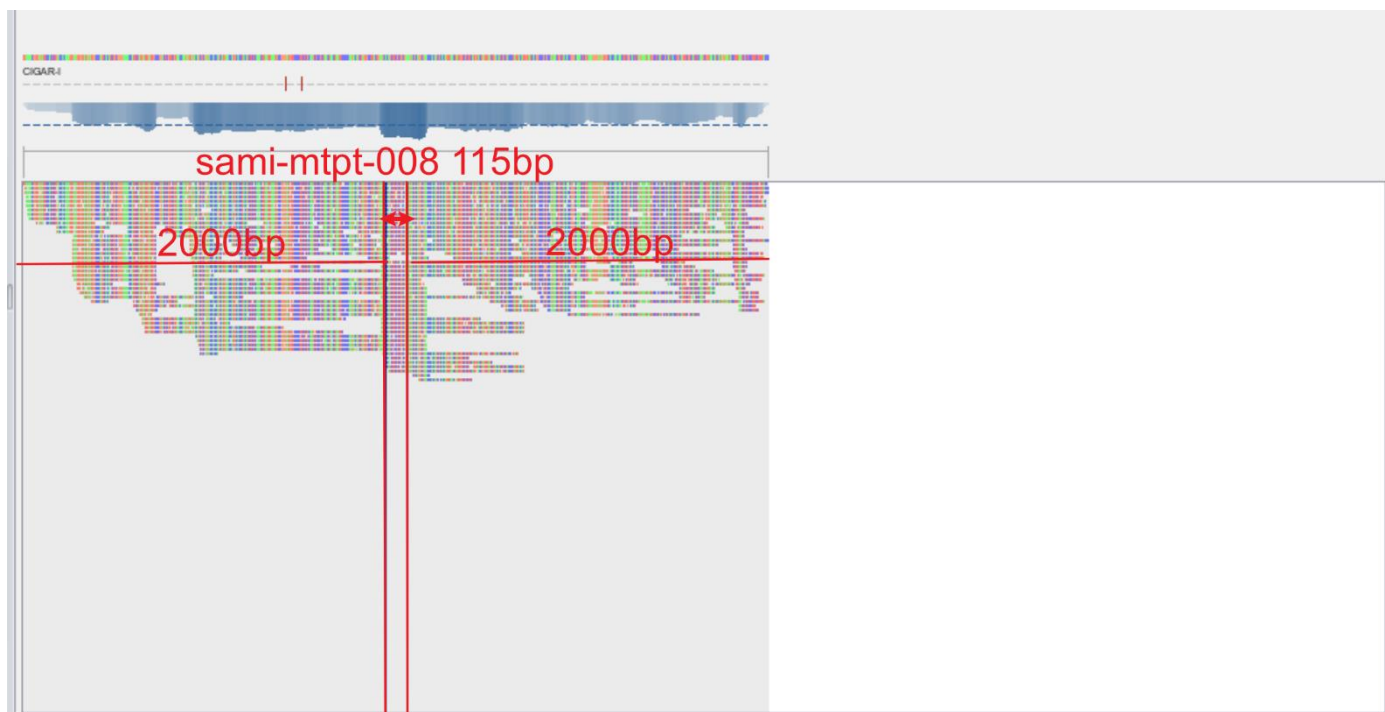


f





h

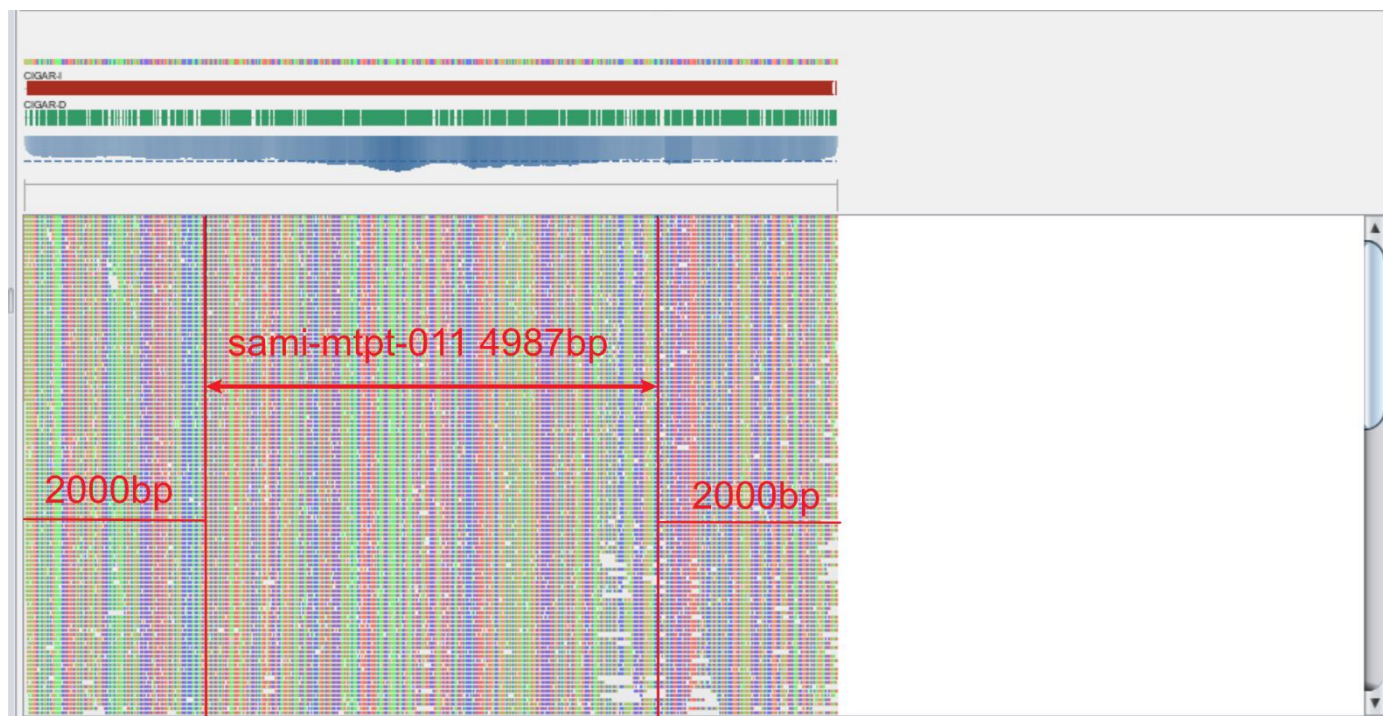




j

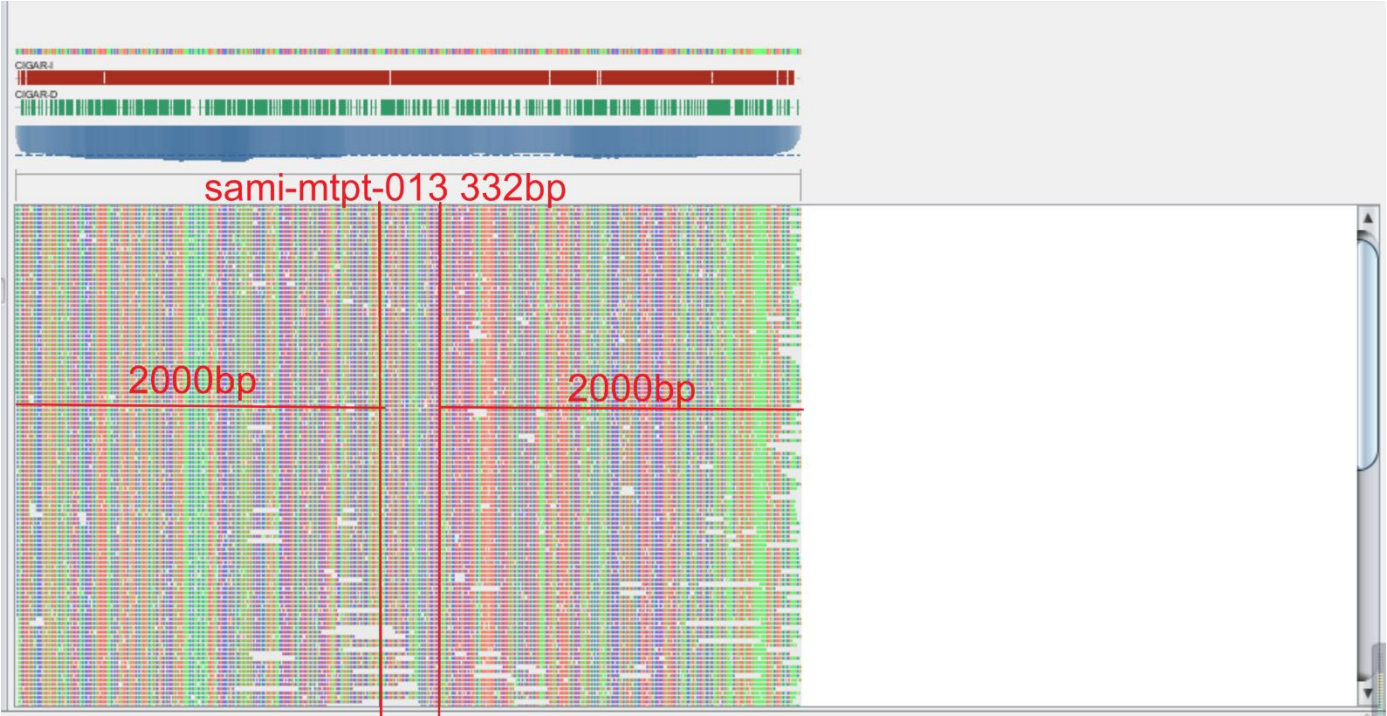


k





m



n



O



p

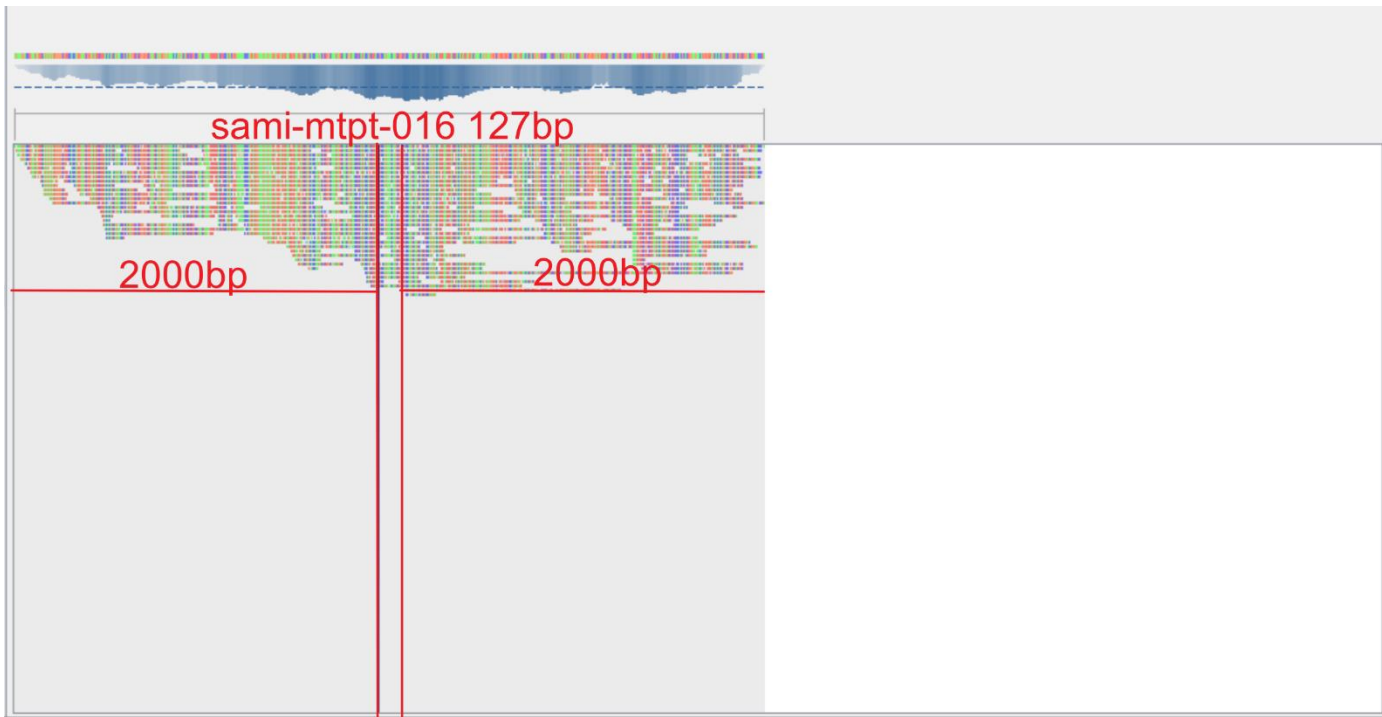


Figure S9. Boxplots of pairwise dN values for mitochondrial genes among the ten Lamiales plants.

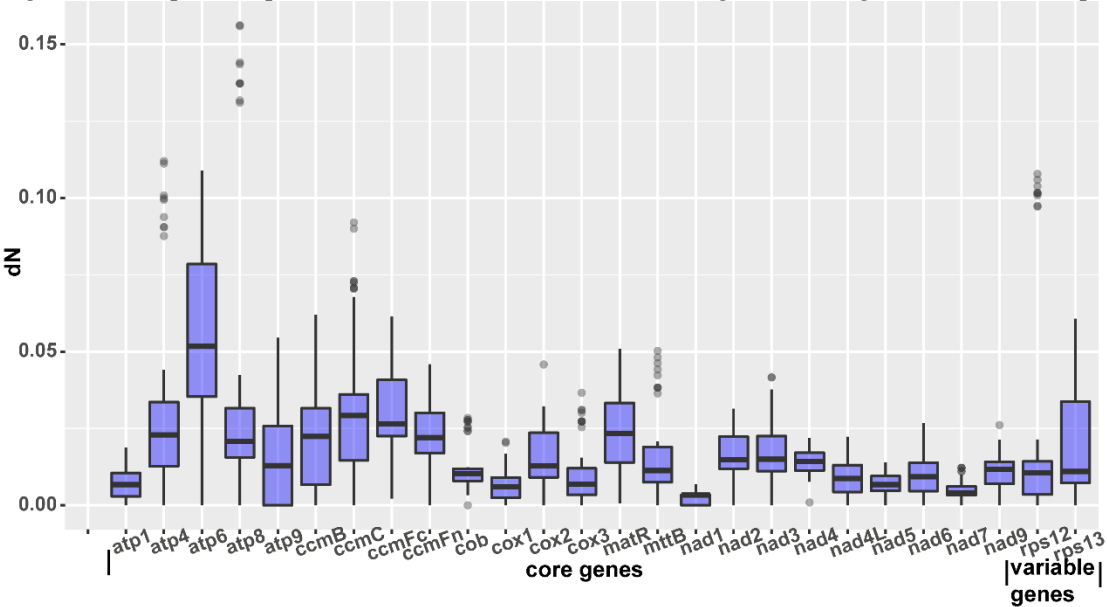
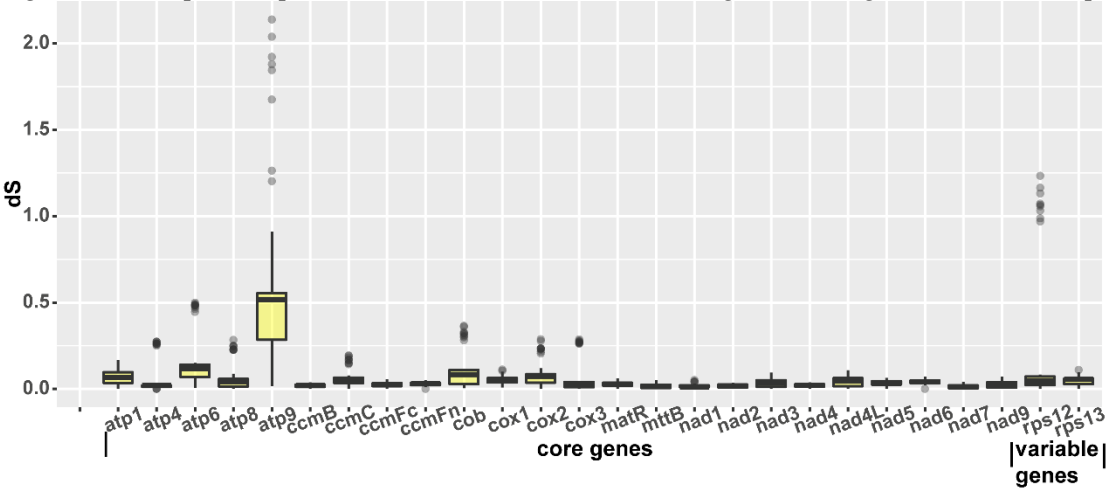


Figure S10. Boxplots of pairwise dS values for mitochondrial genes among the ten Lamiales plants.



Supplementary File S1. DNA sequence of the plastome of *S. miltiorrhiza* assembled using the GetOrganelle toolkit.

Supplementary File S2. DNA sequences of the four possible conformations associated with the eleven HSPs (r01-r11) and their corresponding seven DBSs (bs01-bs07) in the mitogenome of *S. miltiorrhiza*.

Supplementary File S3. The alignment of the sequences of the 26 protein-coding genes of the ten Lamiales mitogenomes and the results of the pairwise dN, dS, and dN/dS values of each two species.