Figure S1. Nucleotide sequence comparisons of the chromosomal translocation regions in the lager brewing yeast with the corresponding chromosomal regions of *S. cerevisiae* and *S. bayanus*

For sequence alignment analyses, the nucleotide sequences of *S. cerevisiae* and *S. bayanus* were referred to the *Saccharomyces* Genome Database (http://www.yeastgenome.org/). Identical bases are indicated by red letters with gray boxes, and similar bases are indicated by blue or black letters with gray boxes, respectively. Underlining indicates the predicted breakpoints. Details of each alignment are summarized below.

1-1) Scer-chr11: *S. cerevisiae*, chromosome XI, 10149-9160 bp; Sc11-Sc11: lager brewing yeast, ScXI-ScXI type chromosome; Sc15-Sc11: lager brewing yeast, ScXV-ScXI type chromosome; Scer-chr15: *S. cerevisiae*, chromosome XV, 1056621-1057610 bp.

1-2) Sbay-c532: *S. bayanus*, contig 532, 13376-17198 bp; Sb02-Sb04: lager brewing yeast, SbII-SbIV type chromosome; Sb04-Sb02: lager brewing yeast, SbIV-SbII type chromosome; Sbay-c611: *S. bayanus*, contig 611, 48907-52796 bp. In *S. bayanus*, the chromosomal region corresponding to *YDR012W* is recognized as ORF, but not annotated. The translocation breakpoint is not specifically indicated because the

location of the breakpoint could not be determined due to unusual homology between *YBR031W* and *YDR012W*.

1-3) To avoid confusion about the results, these two types of chromosomes, VIII-XV and XV-VIII, are separately aligned. Although chromosomes VIII and XV are thought to be crossed at intergenic regions, *YHR014W*/*YHR015W* and *YOR018W*/*YOR019W*, the location of the breakpoint could not be determined. a) Sbay-c668: *S. bayanus*, contig 668, 27312-32543 bp; Sb08-Sb15: lager brewing yeast, SbVIII-SbXV type chromosome. b) Sbay-c606: *S. bayanus*, contig 606, 22955-32725 bp; Sb15-Sb08: lager brewing yeast, SbXV-nonScVIII type chromosome.

1-4) The chromosomal translocation between ScIII and SbIII was found to occur at the MAT locus. a) Based on the result of blastn analysis, the MAT loci on the ScIII-ScIII type chromosome of lager brewing yeast were predicted to correspond to the Sb and alpha type mating cassette. Scer-chr03: *S. cerevisiae*, chromosome III, 199242-201154 bp; Sc03-Sc03: lager brewing yeast, ScIII-ScIII chromosome; Sbay-chr03: hypothetical nucleotide sequence obtained by connection of *S. bayanus* contig 675, 147973-148289 bp with contig 623, 38628-37011 bp. The former sequence corresponds to *YCR038C*, and the latter, to *YCL067C*, *YCL066W* (the silenced copy of alpha type mating factors), and dubious *YCL065W* (recognized as ORF, but not annotated in *S. bayanus*). b)

Based on the result of blastn analysis, the MAT loci on the SbIII-ScIII type chromosome of lager brewing yeast were predicted to correspond to the Sc and A type mating cassette. Scer-chr03: *S. cerevisiae*, chromosome III, 199222-201147 bp, but the nucleotide sequence corresponding to the alpha type mating cassette, 199542-200965 bp, is replaced with that of the A type mating cassette (accession no. V01313, 878-2196 bp); Sb03-Sc03; lager brewing yeast, SbIII-ScIIII type chromosome; Sbay-chr03: hypothetical nucleotide sequence obtained by connection of *S. bayanus* contig 675, 147953-148174 bp, with contig 666, 22173-23783 bp. Although the genes encoding mating factors are not annotated in *S. bayanus*, blastn and blastx analyses indicate that the former sequence corresponds to *YCR038C* and the latter covers the region from MATa2 to the dubious *YCR041W*.

1-5) Scer-chr07: *S. cerevisiae*, chromosome VII, 178823-179748 bp; Sc07-Sc07: lager brewing yeast, ScVII-ScVII type chromosome; Sc07_Sb07: lager brewing yeast, ScVII_SbVII type chromosome; Sbay_c674: *S. bayanus*, contig 674, 83431-84356 bp.

1-6) Scer-chr10: *S. cerevisiae*, chromosome X, 453366-454521 bp; Sc10-Sc10: lager brewing yeast, ScX-ScX type chromosome; Sc10-Sb10: lager brewing yeast, ScX-SbX type chromosome; Sb10-Sc10: lager brewing yeast, SbX-ScX type chromosome; Sb10-Sb10: lager brewing yeast, SbX-SbX type chromosome; Sbay-c646: *S. bayanus*,

contig 646, 71599-72758 bp.

1-7) Scer-chr11: *S. cerevisiae*, chromosome XI, 353594-354433 bp; Sc11-Sc11: lager brewing yeast, ScXI-ScXI type chromosome; Sc11-Sb11: lager brewing yeast, ScXI-SbXI type chromosome; Sb11-Sb11: lager brewing yeast, SbXI-SbXI type chromosome; Sbay-c652: *S. bayanus*, contig 652, 59787-58948 bp.

1-8) Scer-chr13: *S. cerevisiae*, chromosome XIII, 870225-871229 bp; Sc13-Sc13: lager brewing yeast, ScXIII-ScXIII type chromosome; Sb13-Sc13: lager brewing yeast, SbXIII-ScXIII type chromosome; Sbay-c671: *S. bayanus*, contig 671, 7526-6516 bp.

1-9) Scer-chr16: *S. cerevisiae*, chromosome XVI, 96309-99947 bp; Sc16-Sc16: lager brewing yeast, ScXVI-ScXVI type chromosome; Sc16-Sb16: lager brewing yeast, ScXVI-SbXVI type chromosome; Sbay-c637: *S. bayanus*, contig 637, 7836-4211 bp. Since YPL240C is not annotated in *S. bayanus*, the location of the gene is not demonstrated in the figure.

1-10) Scer-chr16: *S. cerevisiae*, chromosome XVI, 862374-863580 bp; Sc16-Sb16: lager brewing yeast, ScXVI-SbXVI type chromosome; Sb16-Sb16: lager brewing yeast, SbXVI-SbXVI type chromosome; Sbay-c654: *S. bayanus*, contig 654, 16023-17229 bp.

1-11) Scer-chr16: *S. cerevisiae*, chromosome XVI, 917583-922418 bp; Sb16-Sc16: lager brewing yeast, SbXVI-ScXVI type chromosome; Sbay-c654: *S. bayanus*, contig

654, 69146-73739 bp. Since YPR192W is not annotated in *S. bayanus*, the location of the gene is not demonstrated in the figure.

Supplementary Figure S2. Comparison between the 36 predicted chromosomal sequences and the optical mapping

Alignments between the optical maps and DNA sequenced-based maps from the 36 predicted chromosome sequences were created with the MapViewer software (OpGen, Inc., Madison, WI) with default parameters except for ScXII. ScXII was separated into two parts at the rDNA position and aligned to the corresponding opmap using the parameters (OM stderr of 1.0). The names of Sc and Sb show the restriction patterns of 36 provisional chromosome sequences of lager brewing yeast of restriction patterns using the enzyme *Xho*I. The names of Opmap show the restriction patterns using the enzyme *Xho*I obtained from optical mapping. The lines connect the regions of significantly similar restriction patterns.

(A), (B), (C), and (D) are the results of a comparison of chromosomes without translocations, chromosomes with translocations between Sc-type chromosomes, chromosomes with translocations between Sb-type chromosomes, and chromosomes with translocations between Sc- and Sb-type chromosomes, respectively. Green and

white indicate regions displaying similarity and lack of similarity between the provisional chromosome and the optical mapping.

Supplementary Figure S3. Unrooted phylogenetic tree of ITS sequences among *Saccharomyces* species

Sequences of ITS1-5·8S-ITS2 of the Sc- and Sb-types of lager brewing yeast, *S. cerevisiae* S288C, *S. bayanus* CBS 380 (Genbank accession no. Z95945.1), *S. uvarum* CBS 395 (Genbank accession no. Z95946), *S. pastorianus* CBS 1538 (Genbank accession no. Z95949), and *S. carlsbergensis* CBS 1513 (Genbank accession no. Z95950) constructed from Kimura 2 distances and the neighbor-joining algorithm. The scale bar indicates the genetic distance.

Supplementary Figure S4. MtDNA of lager brewing yeast and comparison of *S. cerevisiae* and *S. bayanus*

Detailed gene maps of MtDNA of lager brewing yeast and *S. cerevisiae* are shown. Genes on one strand and the opposing one are indicated by red and blue thick lines, respectively. Introns are indicated by thin black lines. Staggered lines represent genes with overlaps. (C) A gene map of *S. bayanus* determined by the mapping with restriction enzymes[1] is shown.

Supplementary Table S1. Cosmids covering the Sb-type chromosomes and showing chromosomal rearrangements between Sc- and Sb-type sub-genomes

The 11,794 sequences from both ends of the 5,897 cosmids were aligned against the 16 chromosomes of *S. cerevisiae* using NCBI-BLAST BlastN, and the longest alignments were considered to be significant. The Sb-type cosmids in which both end sequences (forward and reverse sequences) have relatively lower similarity (<90) to *S. cerevisiae* were selected. Then, the Sb-type cosmids covering all 16 *S. cerevisiae* chromosomes (from the left to the right telomeric regions) were selected and are shown in (A). Eight cosmids with one end sequence identified as the Sc-type and the other as the Sb-type, which were considered as candidate cosmids for chromosomal rearrangement between Sc- and Sb-type chromosomes, were also selected and are shown in (B).

Chr No; the chromosome number of *S. cerevisiae* match

Start Position and End Position; start and end position in alignment of *S. cerevisiae* chromosome with each cosmid end sequence.

Alignment Length; length in alignment of each cosmid end sequence with the *S. cerevisiae* chromosome (bp).

Identity; the percent nucleotide sequence identity.

Estimated Cosmid Length (kb); cosmid length (kb) estimated by the alignment of the both cosmid end sequences against *S. cerevisiae* chromosome.

Supplementary Table S2.

This file describes the matches of lager brewing yeast predicted ORFs (pORFs) against *S. cerevisiae* S288C and *S. bayanus* CBS7001. In these files, all the matches are reported for every lager brewing yeast ORF.

1. ORF ID; unique identifier of the predicted Open Reading Frame (pORF)
2. chr No; chromosomal number of lager brewing yeast
3. p_len (aa); predicted protein length in amino acids (if single exon protein)
4. category;

   s (single match); pORF in a cluster including only one *S. cerevisiae* ORF was assigned its functional role according to the description of the *S. cerevisiae* ORF

   m (multiple match); pORF in a cluster including more than two types of *S. cerevisiae* ORFs was not a conserved gene synteny.

   p (paralog); a single match pORF in a cluster including one *S. cerevisiae* ORF was not a conserved gene synteny.

   b (match with only *S. bayanus*); pORFs in a cluster including not *S. cerevisiae* ORFs

but *S. bayanus* pORFs.

LS (lager brewing yeast specific pORF)

n (no match with *S. cerevisiae*, *S. bayanus*, or nr)

5. contig ID; supercontig to which the pORF belongs. contigID, supercontig to which the pORF belongs _ ORF _ pORF strand in the contig _ start position in the contig _ pORF end position in the contig _ orf
6. sys.gene; the ORF name of the *S. cerevisiae* match
7. fun.gene; the gene name of the *S. cerevisiae* match
8. Feature Type; feature type field from the SGD
9. AA length (Scer); protein length of *S. cerevisiae* ORF
10. identity (Scer); the percent amino acid identity in the match of lager brewing yeast ORF with *S. cerevisiae* ORF
11. s_sta (Scer); start position in alignment of *S. cerevisiae* ORF with lager brewing yeast pORFs
12. s_end (Scer); end position of alignment of *S. cerevisiae* ORF with lager brewing yeast pORFs
13. q_sta (LBYG); start position in alignment of lager brewing yeast pORF with *S. cerevisiae* ORF

14. q_end (LBYG); end position in alignment of lager brewing yeast pORF with *S. cerevisiae* ORF
15. S.bayORF; *S. bayanus* pORF ID
16. identity (Sbay); the percent amino acid identity in the match of lager brewing yeast ORF with *S. bayanus* pORF
17. s_sta (Sbay); start position in alignment of *S. bayanus* pORF with lager brewing yeast pORFs
18. s_end (Sbay); start position in alignment of *S. bayanus* pORF with lager brewing yeast pORFs
19. q_sta (LBYG); start position in alignment of lager brewing yeast pORFs with *S. bayanus* pORF
20. q_end (LBYG); end position in alignment of lager brewing yeast pORFs with *S. bayanus* pORF
21. orf type; assignment of type depending on whether the pORF was matched higher with either *S. cerevisiae* (Sc) or *S. bayanus* (Sb) pORF

Supplementary Table S3. Other chromosomal features except for large chromosomal translocations (Table 2)

Supplementary Table S4. Nucleotide sequence comparison of genes on lager brewing yeast mtDNA with *S. cerevisiae* and *S. bayanus*

Genes that showed similarities with e-values below e-50 in BLASTN similarity search against mitochondrial genes of S288C and Genbank nonredundant database are listed with the results of analyses. Although some genes had a high level of similarity to more than one sequence, only the subjects that give the longest alignment between queries are shown.

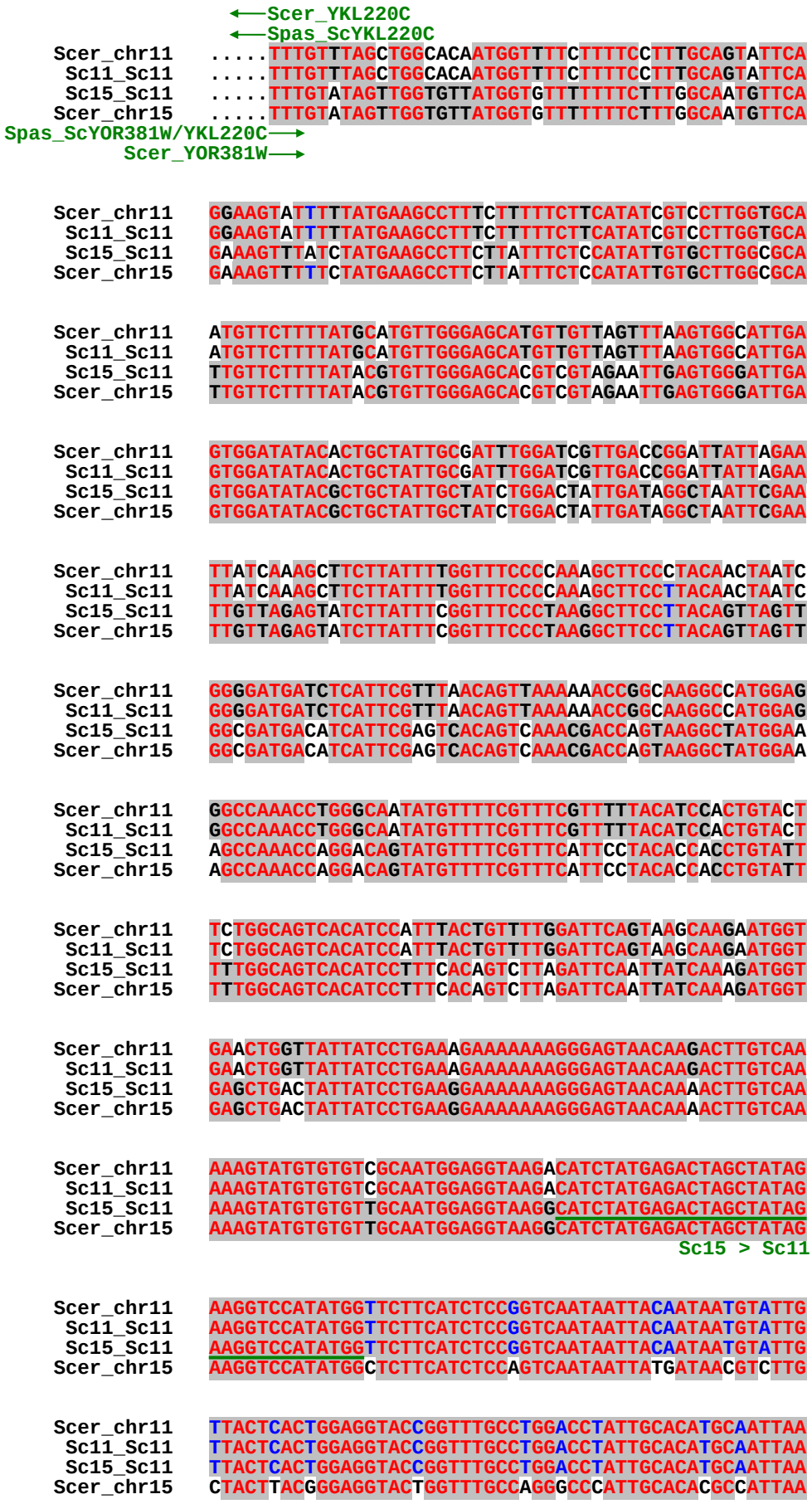
Supplementary Table S5. List of truncated ORFs

ORFs with length less than 75% of that of the corresponding *S. cerevisiae* ORF are shown. 5' ORFs were shown when the genes were split into two ORFs. "Hq" and "confirmed" indicate high-quality ORFs (more than 40 phred sequence values throughout the sequences when each ORF was aligned against the corresponding *S. cerevisiae* ORF) and the ORFs confirmed by PCR amplification and sequencing. GO Annotations (biological process, molecular function, and cellular component) and Systematic deletion fields were retrieved from the SGD. These ORFs were confirmed

to have no sequence polymorphisms by PCR amplification and by sequencing or manual checks because lager brewing yeast is allopolyploid; therefore, two of more alleles are expected to exist. Note that confirmed sequences are listed in SI sequence.

1 Cardazzo, B., Minuzzo, S., Sartori, G., Grapputo, A., and Carignani, G. 1998, Evolution of mitochondrial DNA in yeast: Gene order and structural organization of the mitochondrial genome of *Saccharomyces uvarum*. *Curr Genet.*, **33**, 52-59.

1-1) ScXI/ScXV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Sc15_Sc11 .....TTTGTATAGTTGGTGTTATGGTGTTTTTTTCTTTGGCAATGTTCA
Scer_chr15 .....TTTGTATAGTTGGTGTTATGGTGTTTTTTTCTTTGGCAATGTTCA
Spas_ScYOR381W/YKL220C—→
Scer_YOR381W—→
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 Sc15_Sc11    TTGCAGTTAGAGGATTCGACGTACTCGAGGCTTATAAGCCGGAGTTGATG
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Scer_chr11    TGTTTAGAAAATCTGAATGTACAGCTTCACATCTACAACACAATGGAAGT
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Scer_chr11    TAAGGAACTTCTACATGAAGCGGCTGAATTGAGTGGCTCATTATCTGTGG
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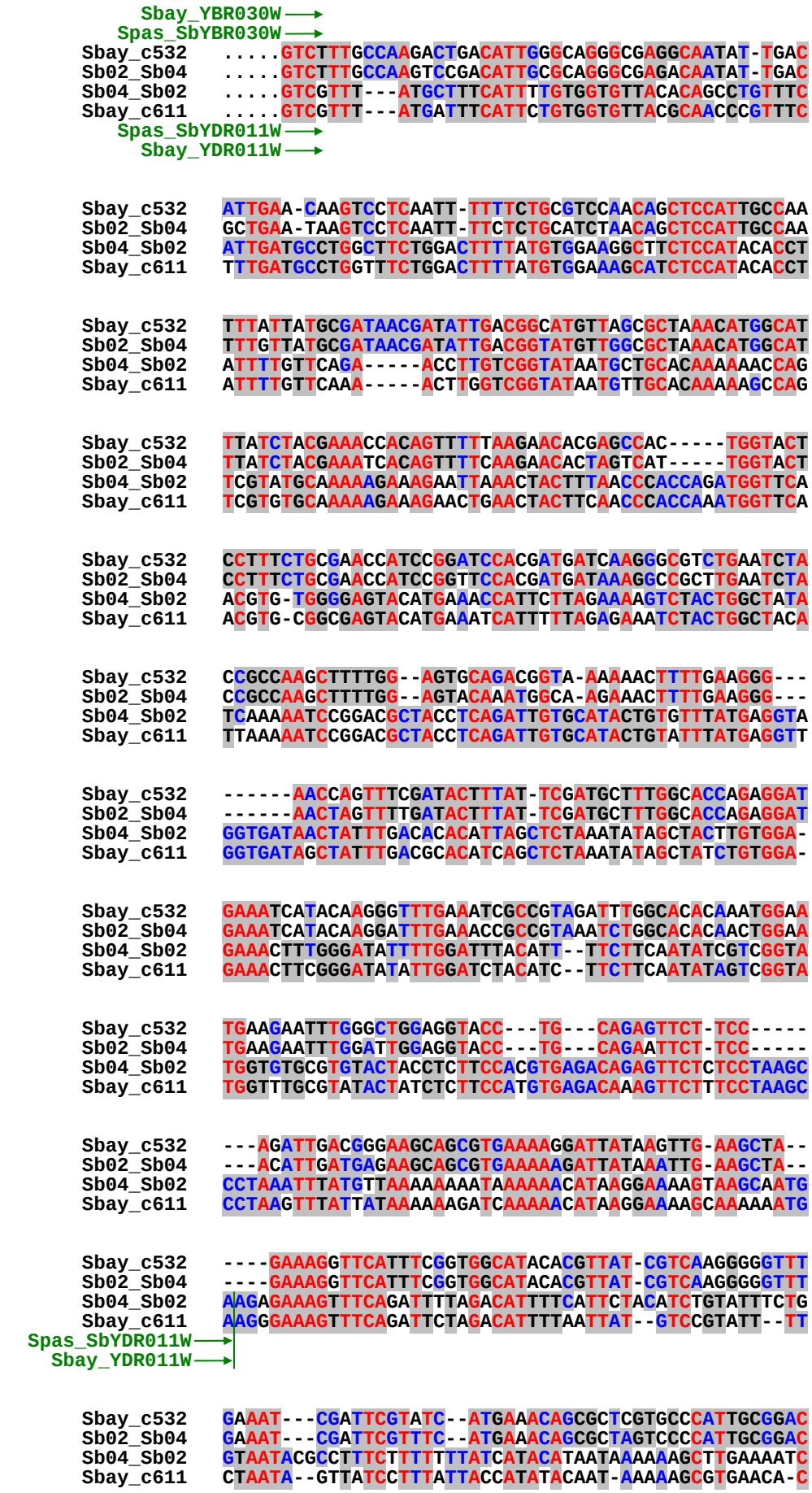
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Sbay_YDR011W
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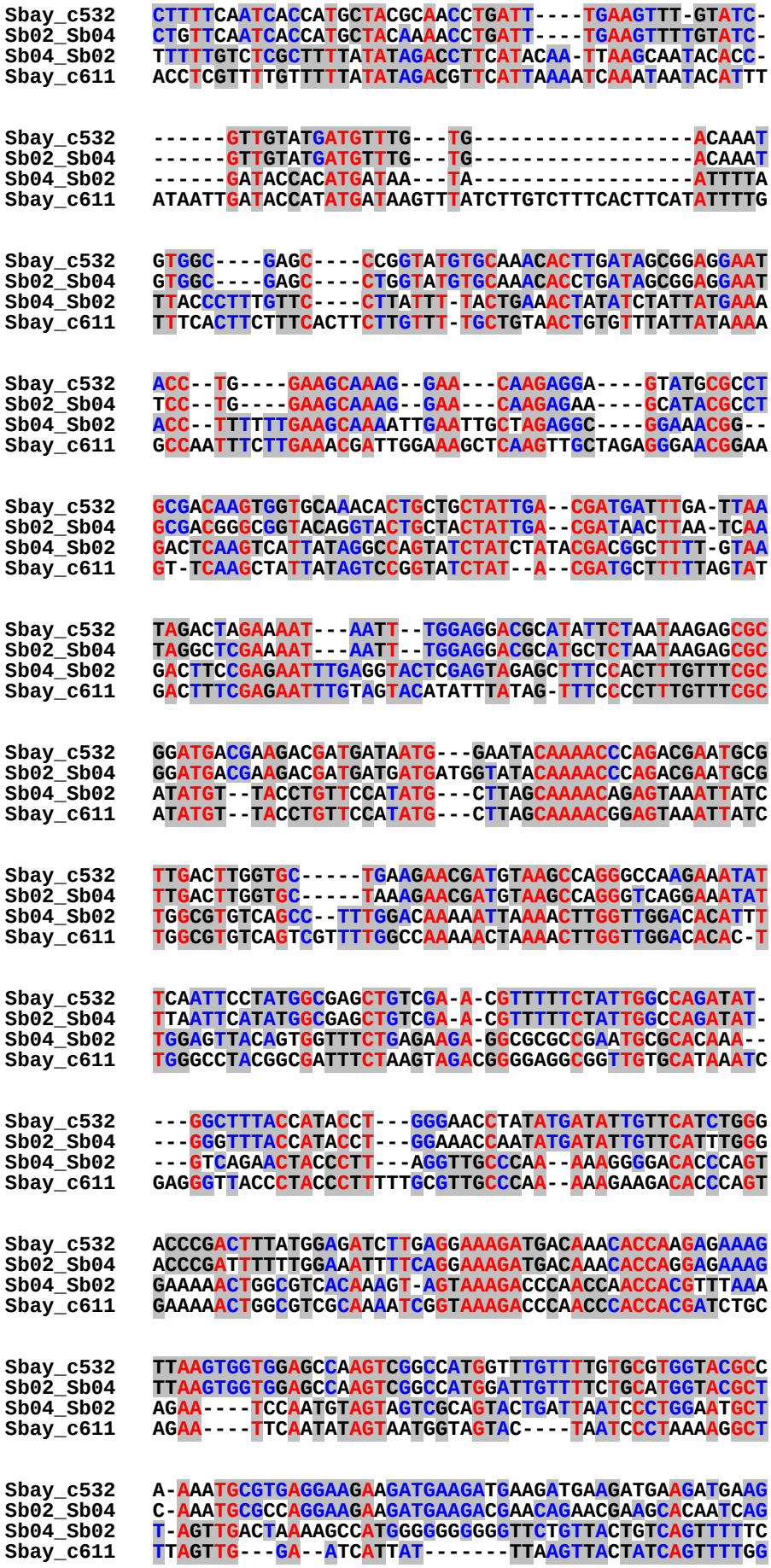
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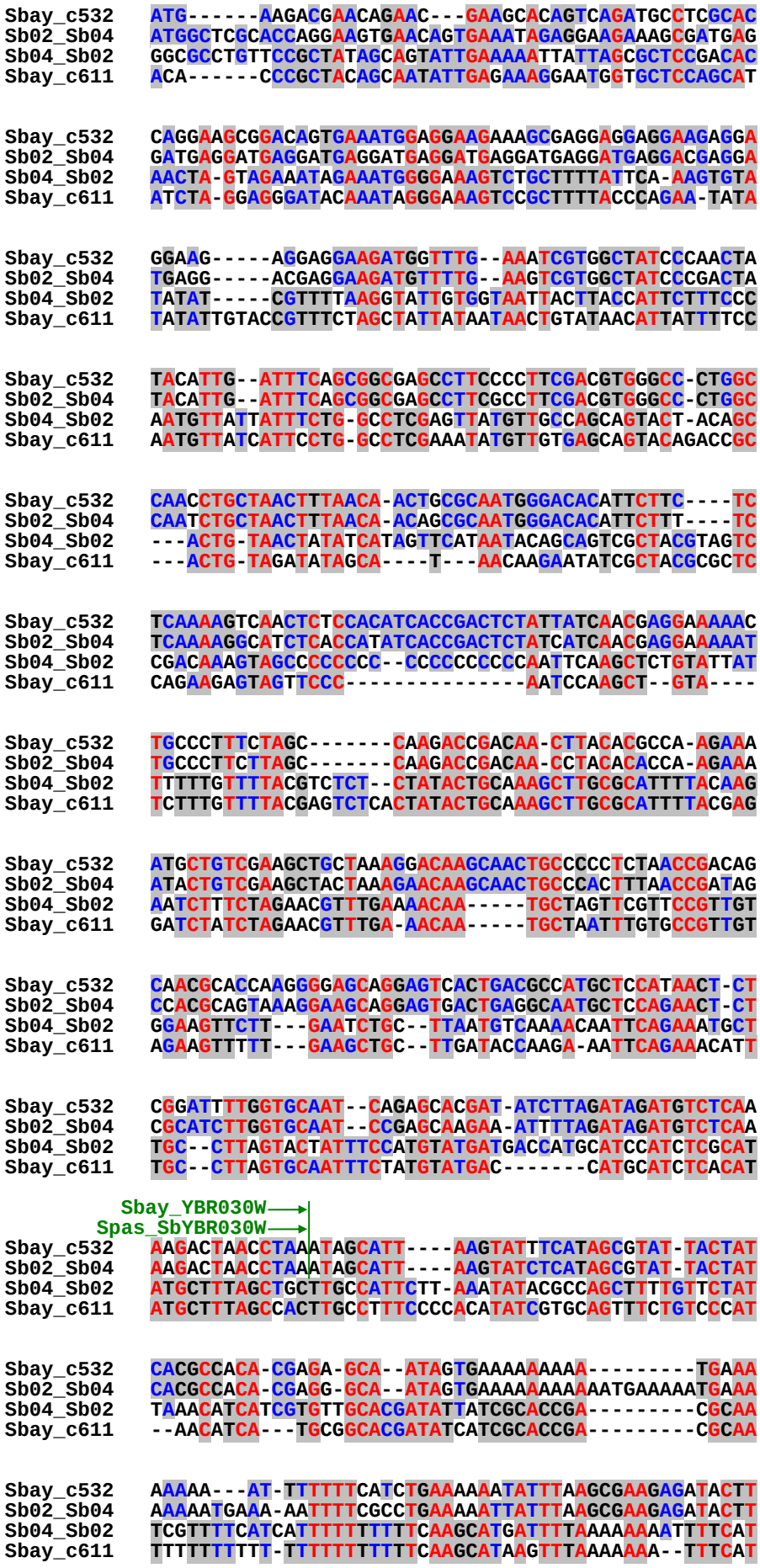
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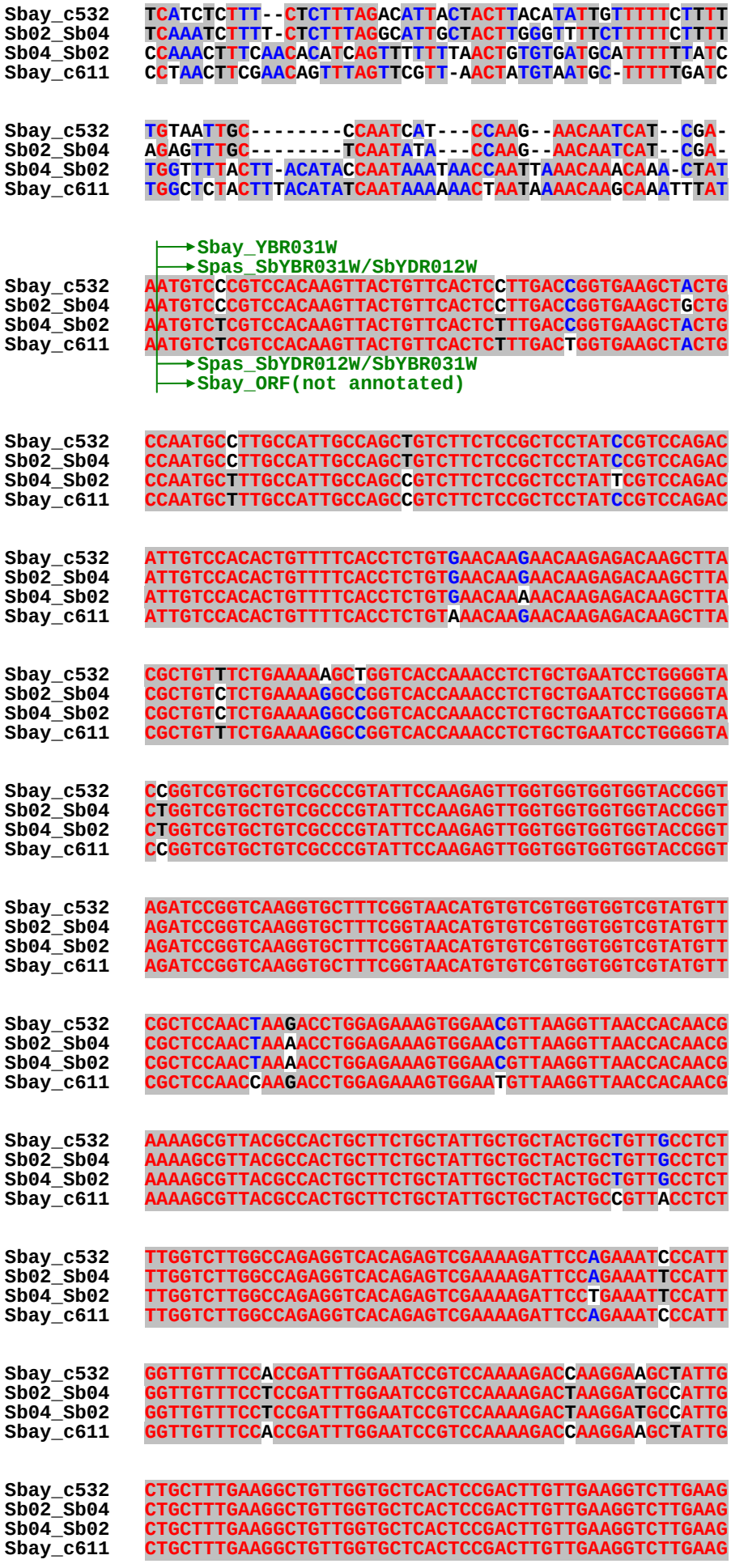
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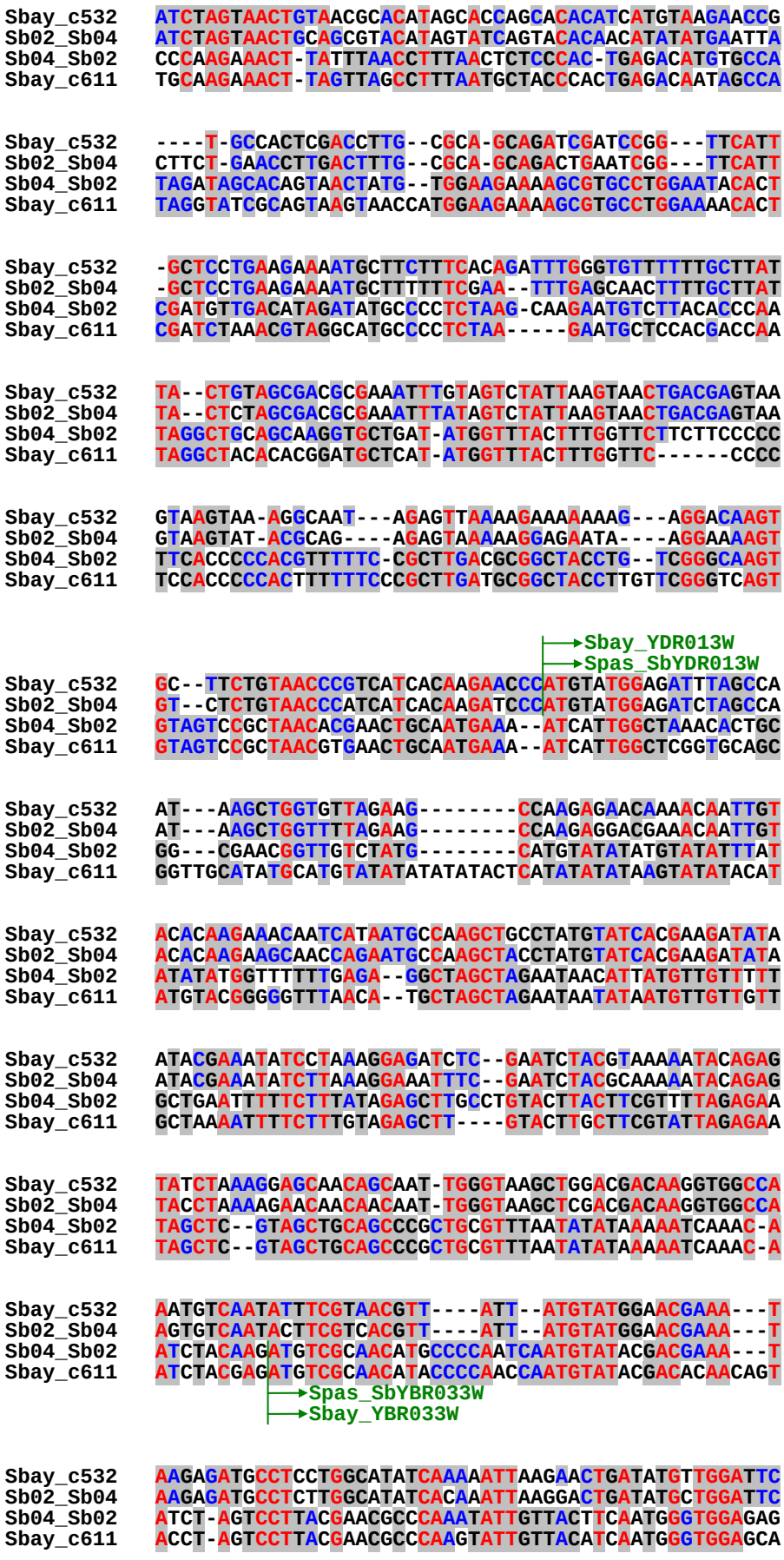
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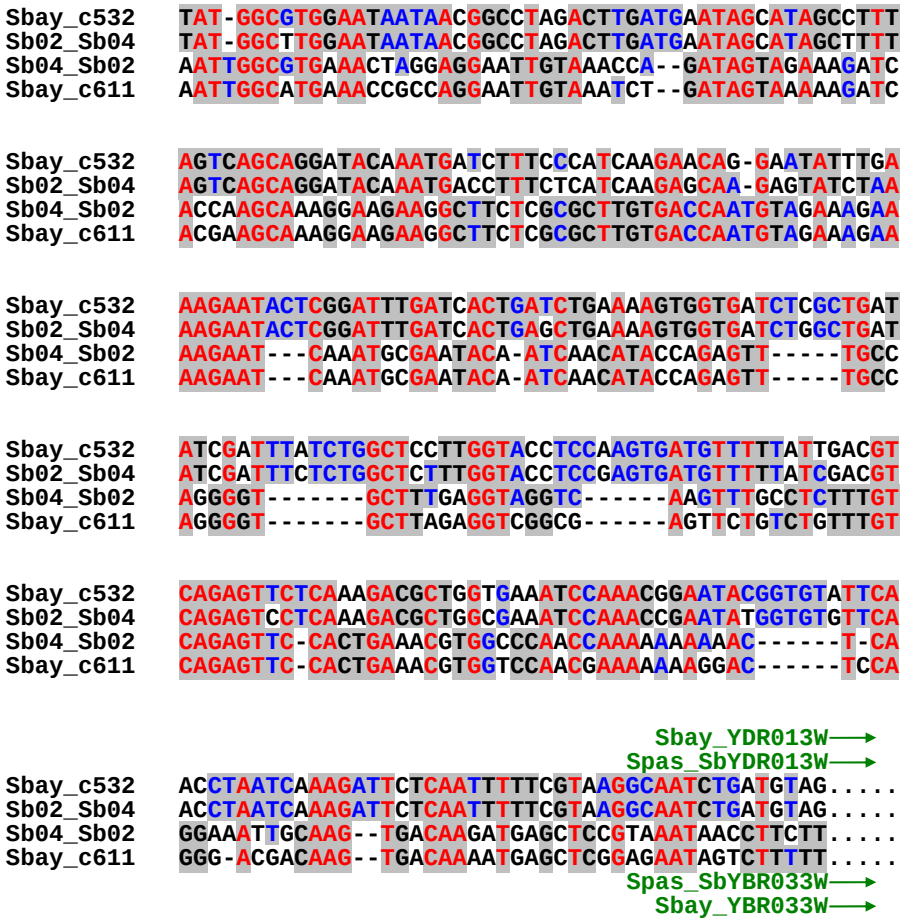
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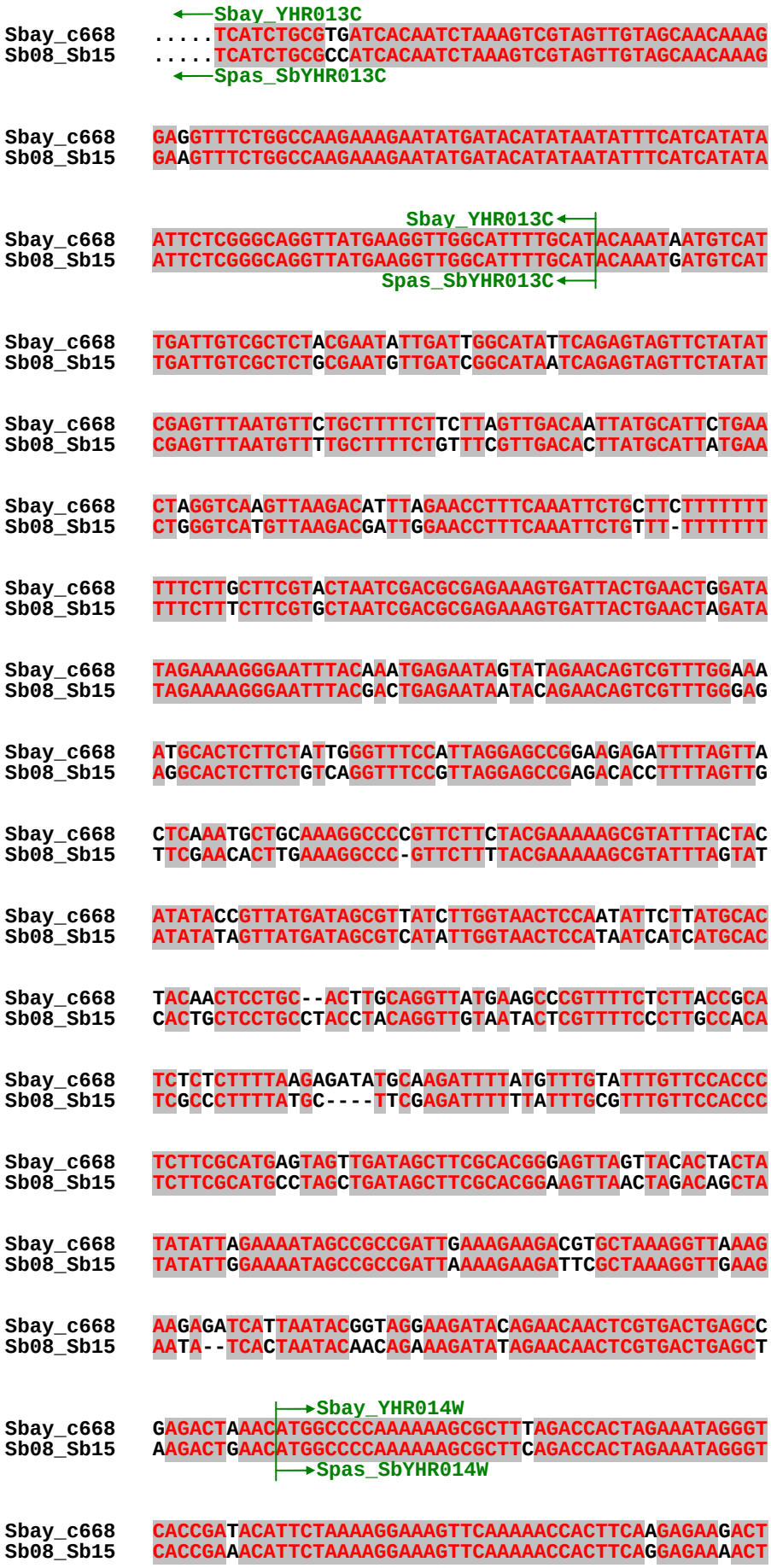

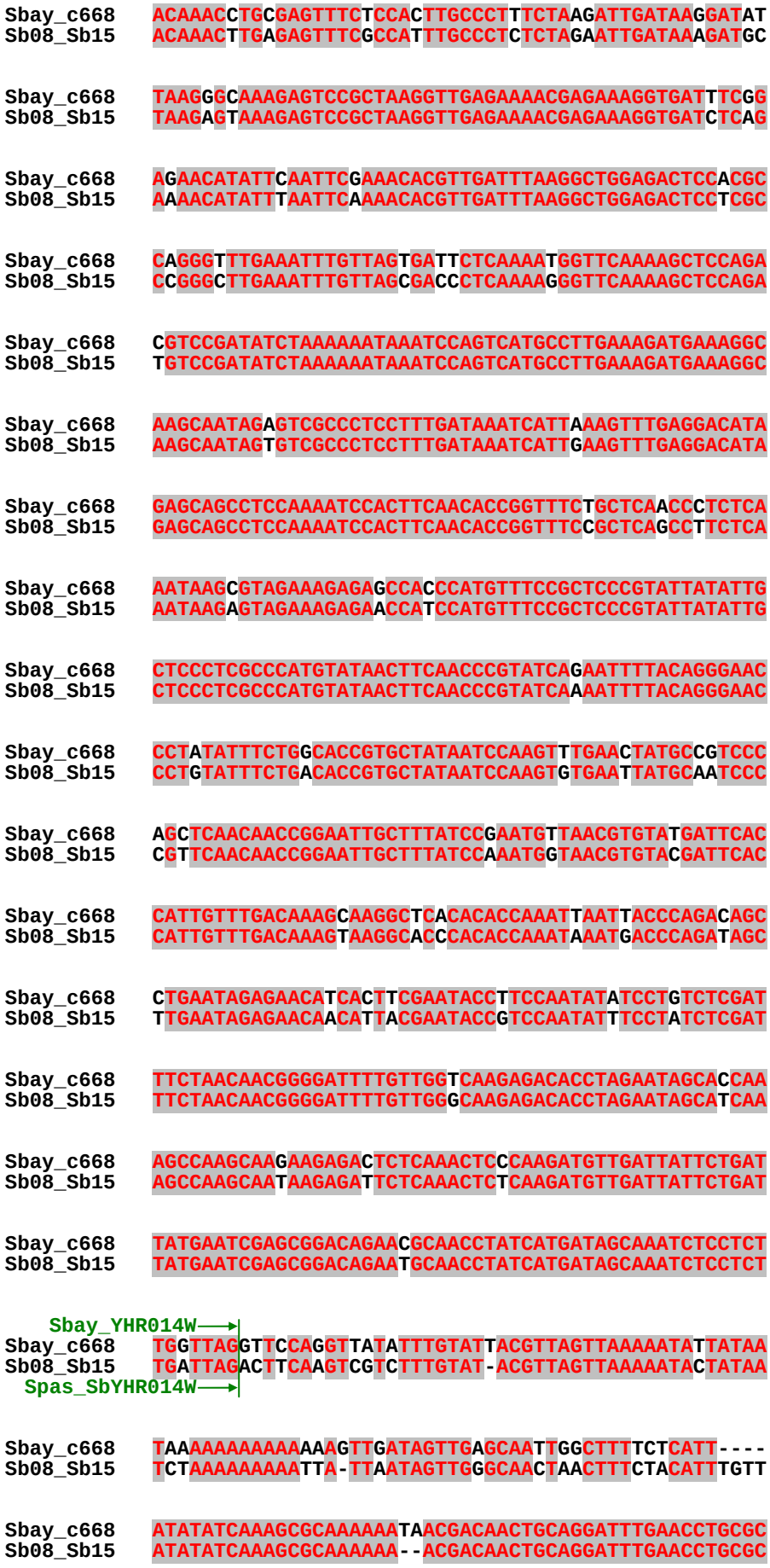
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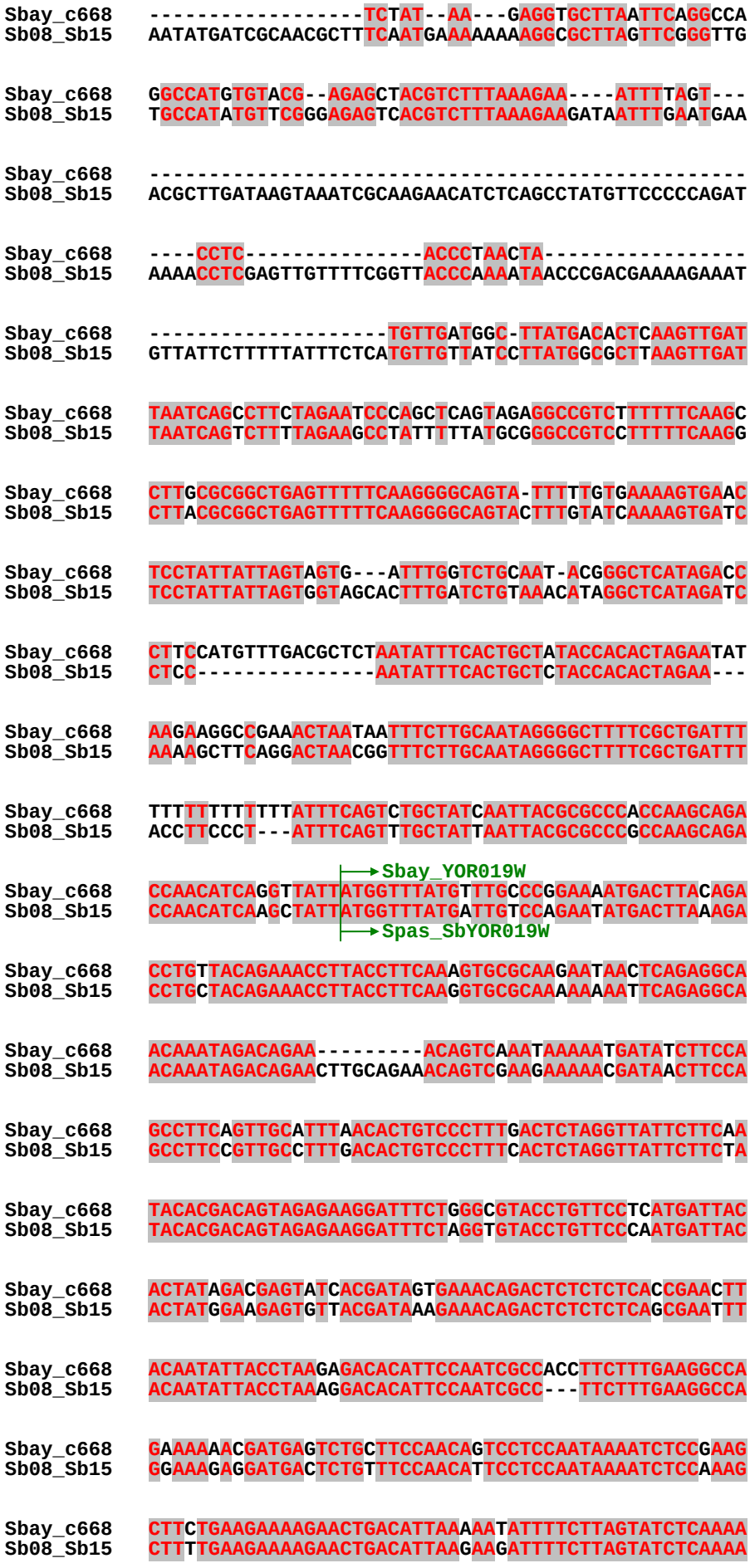
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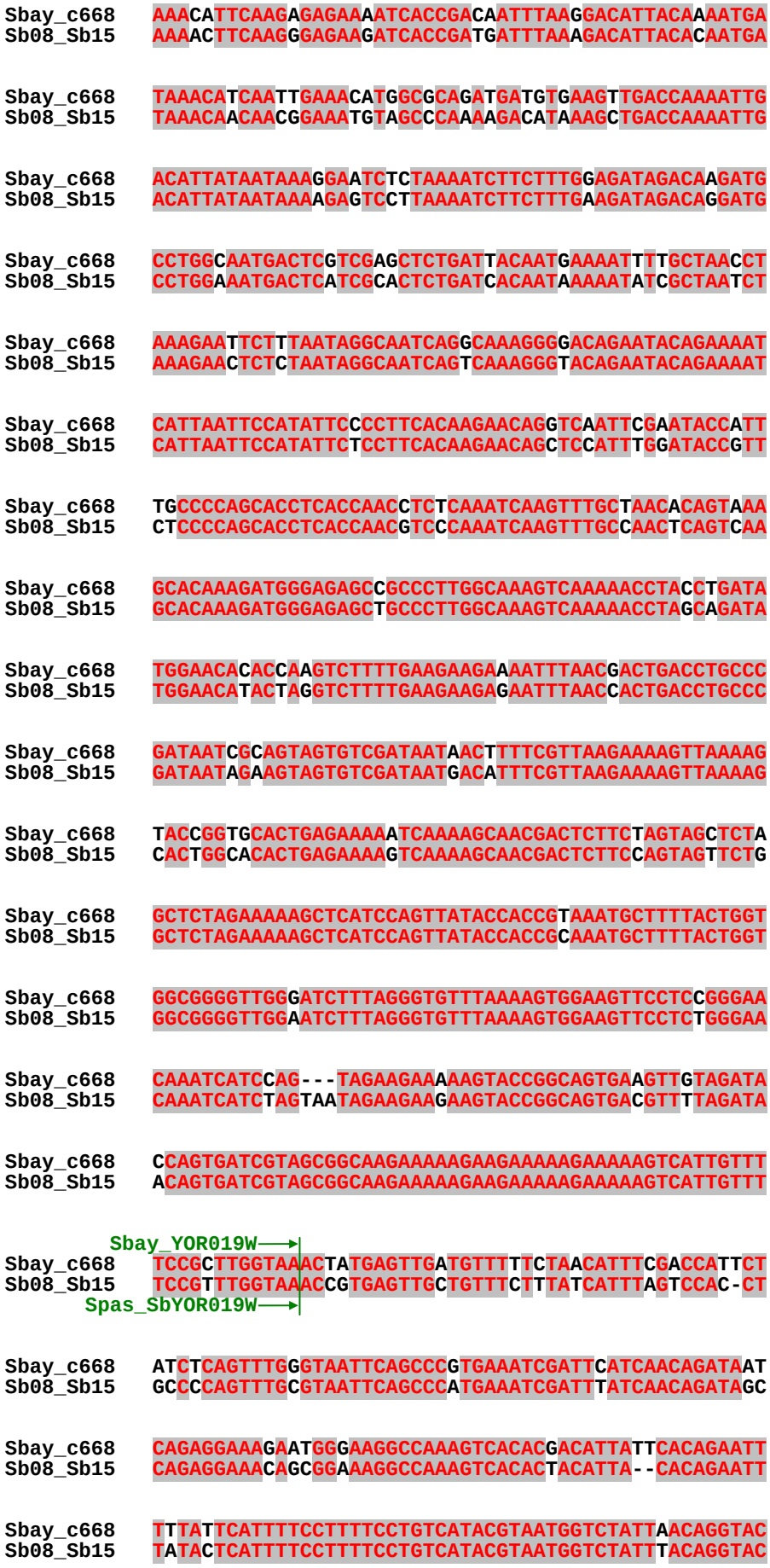
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1-3-b) SbXV > SbVIII

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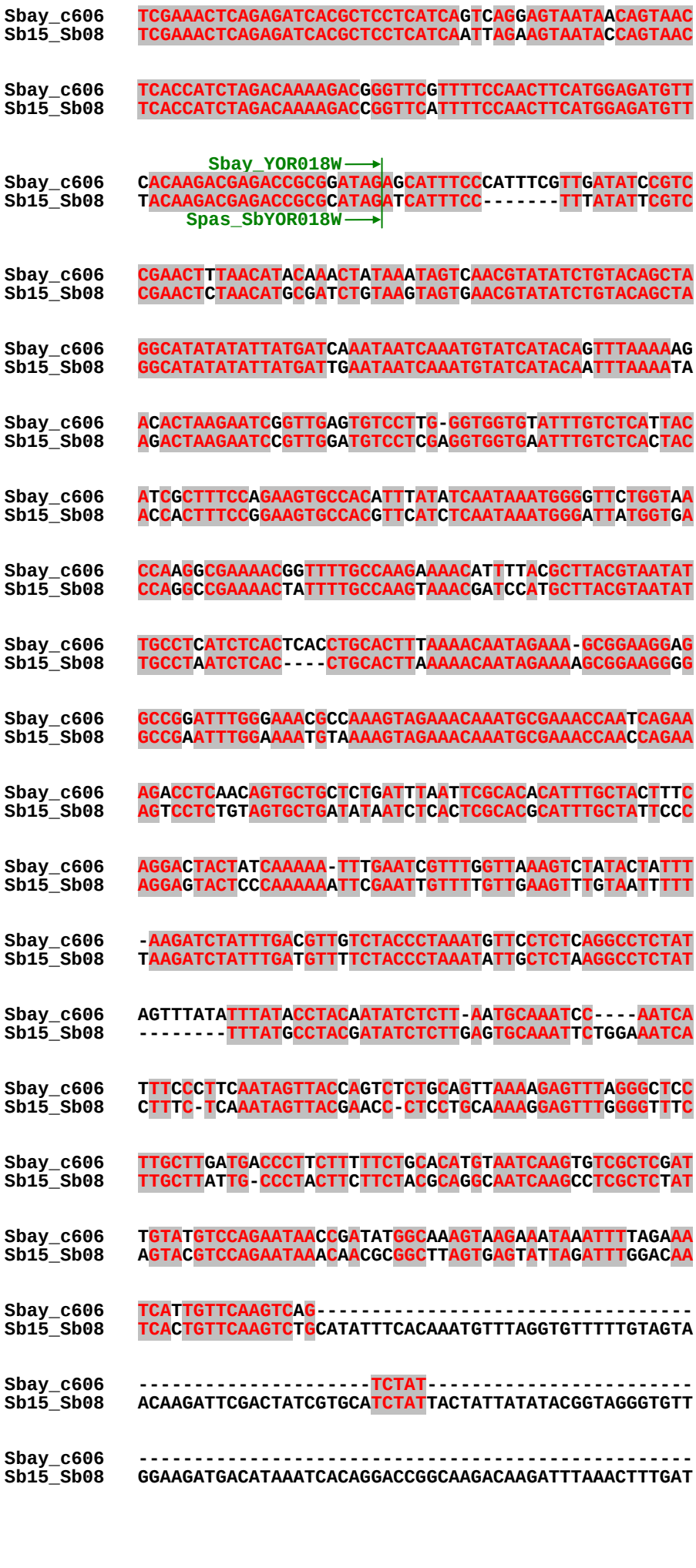
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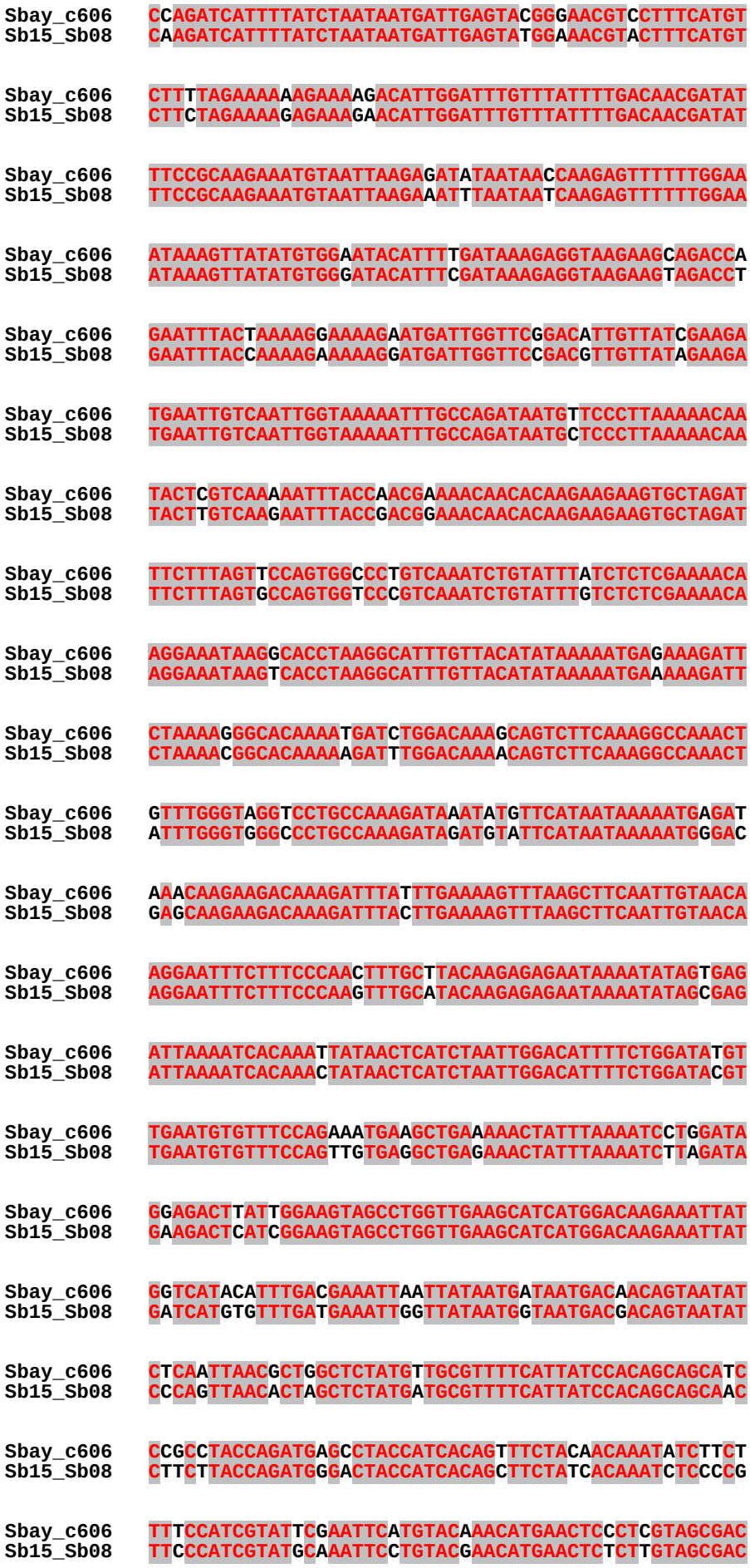
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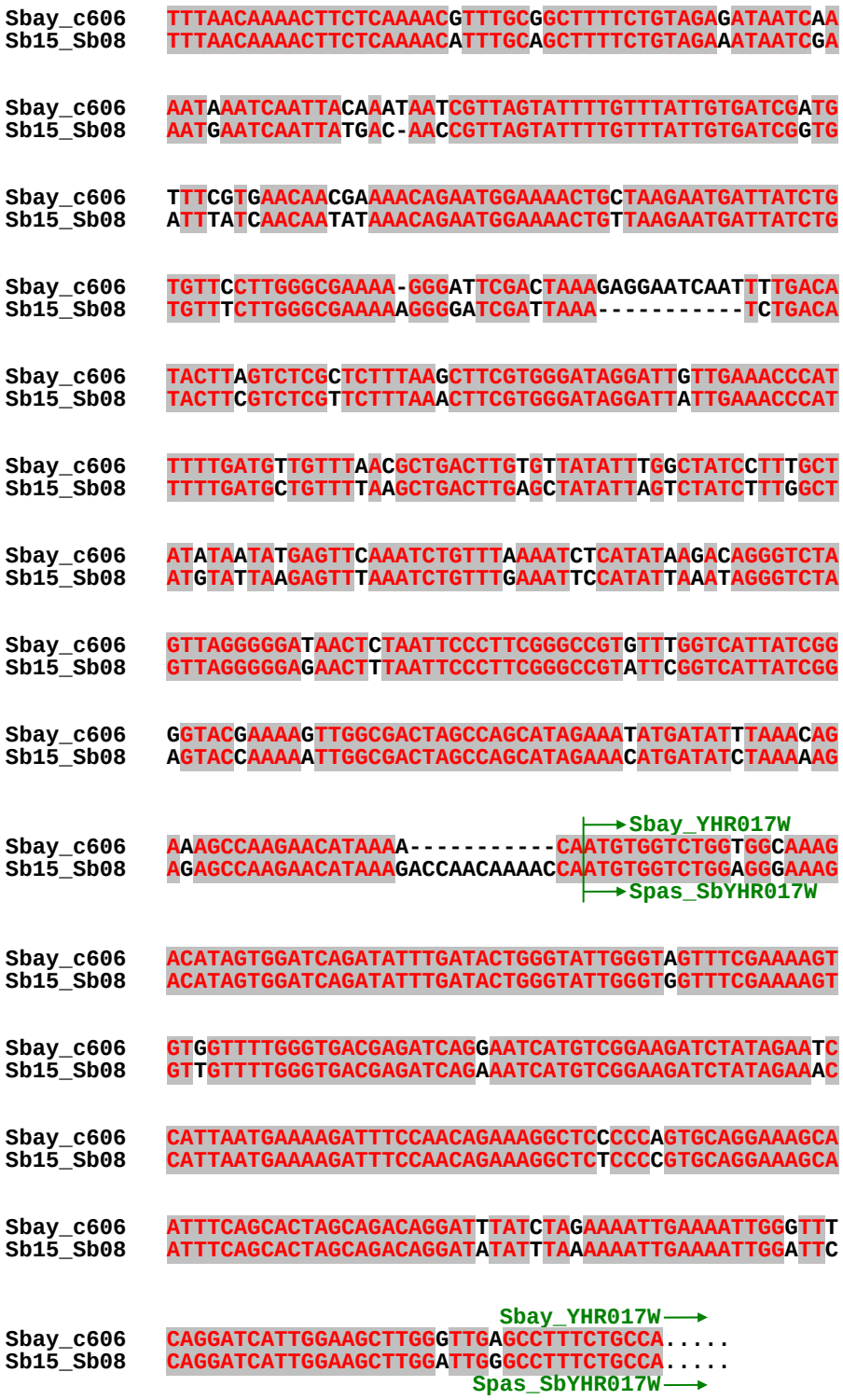
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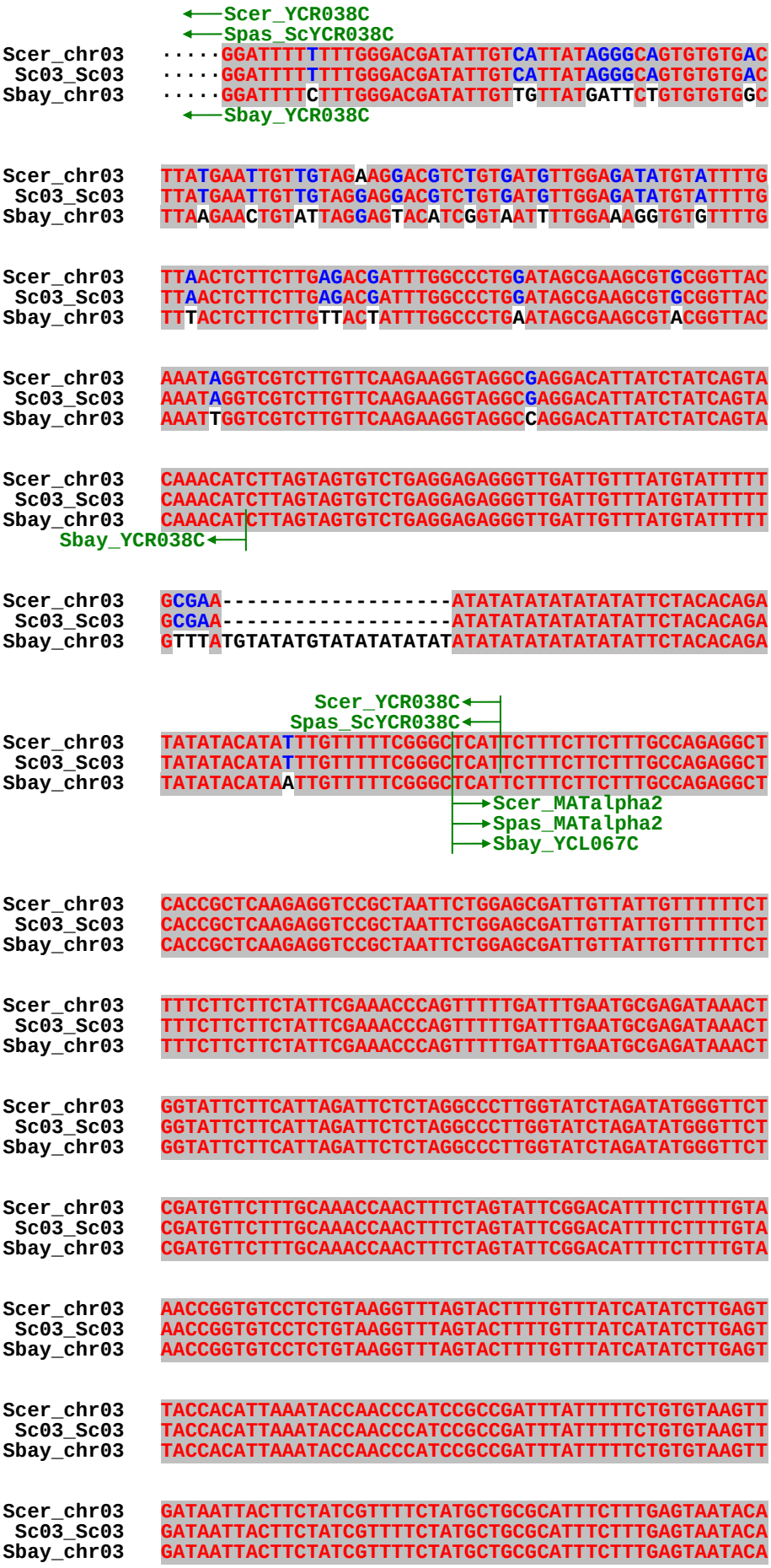

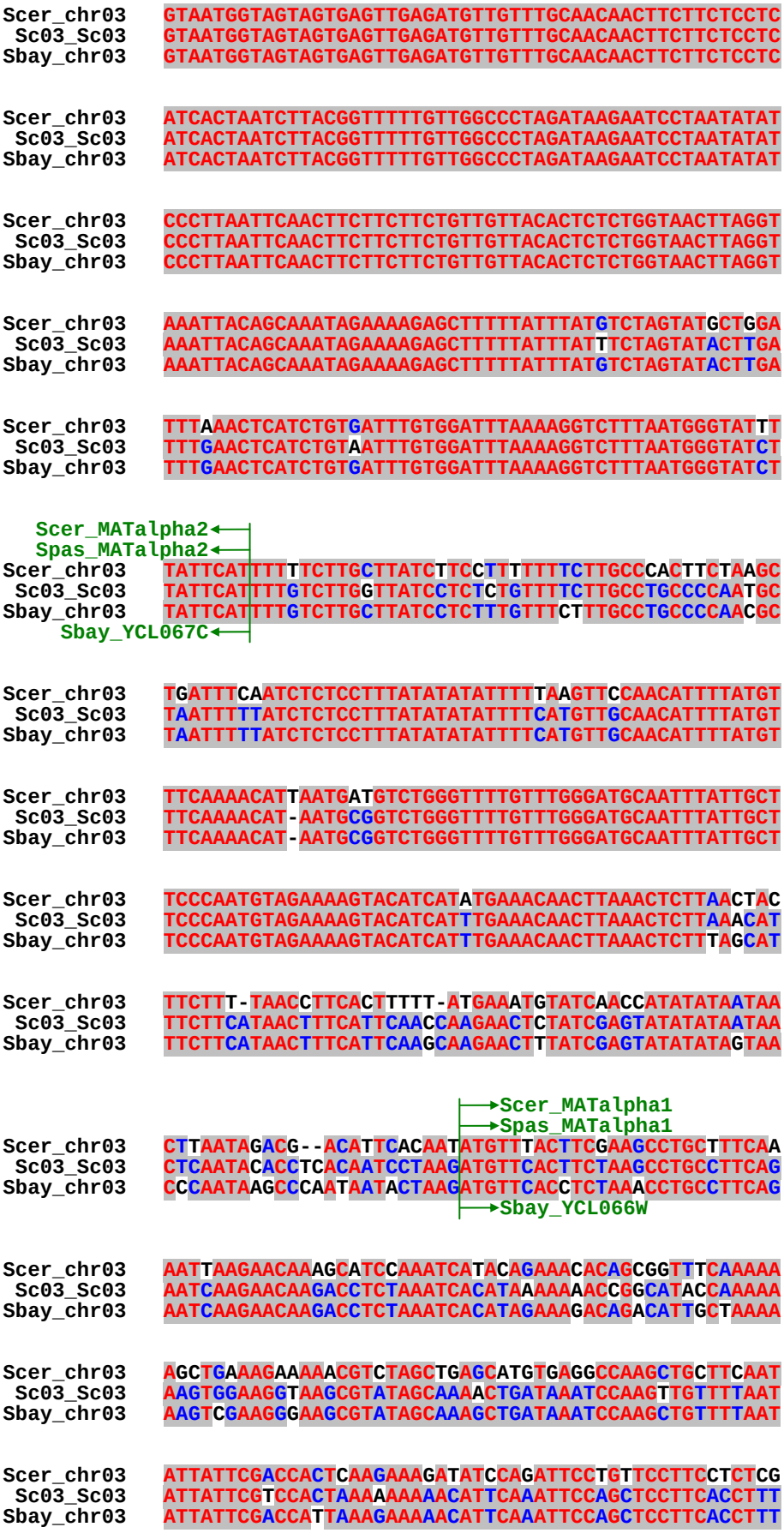
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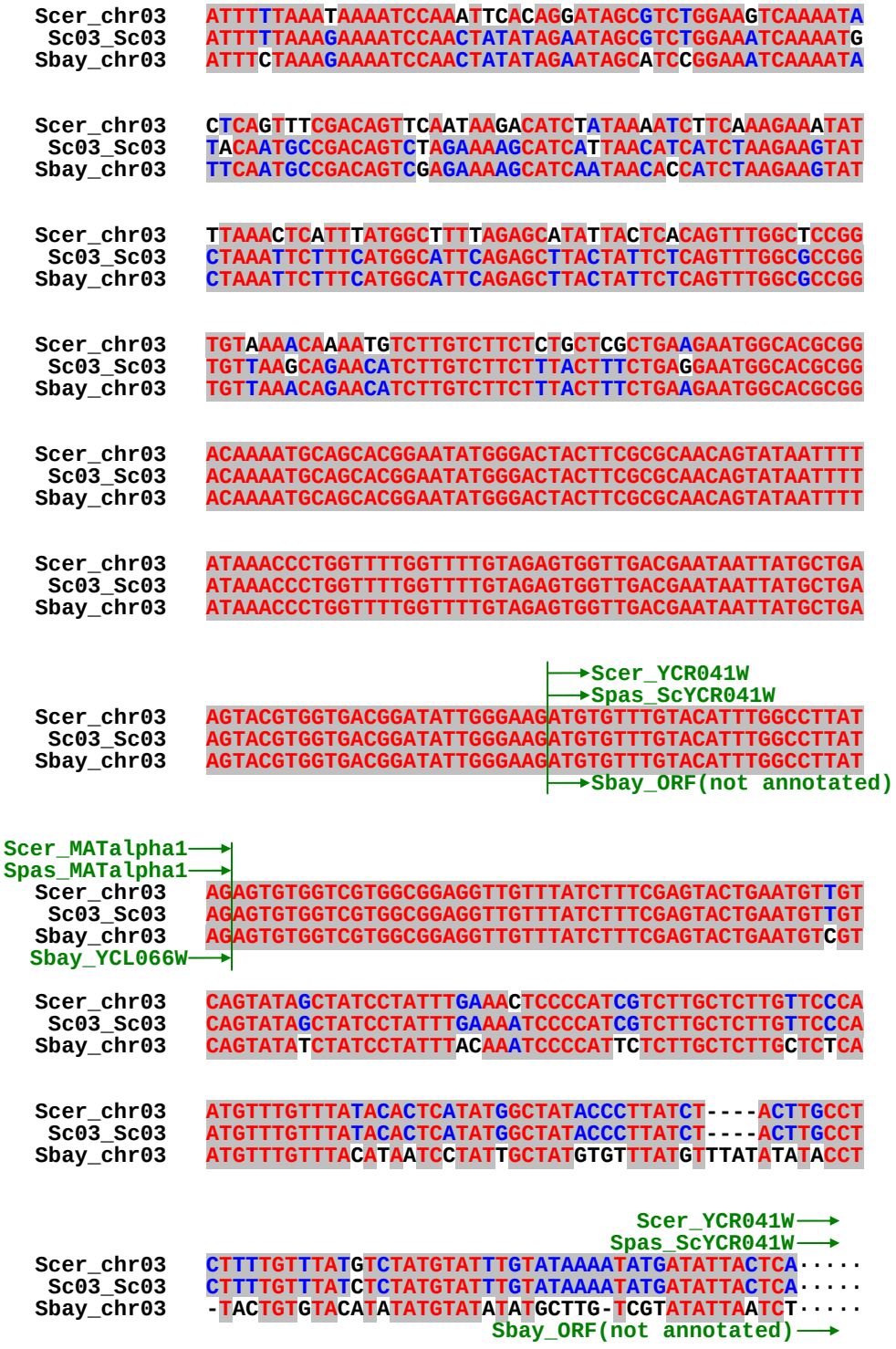

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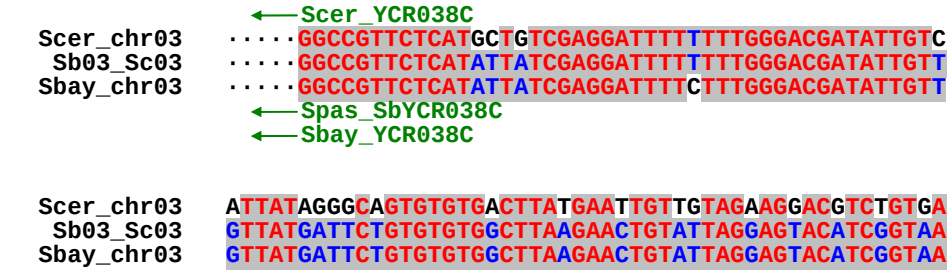

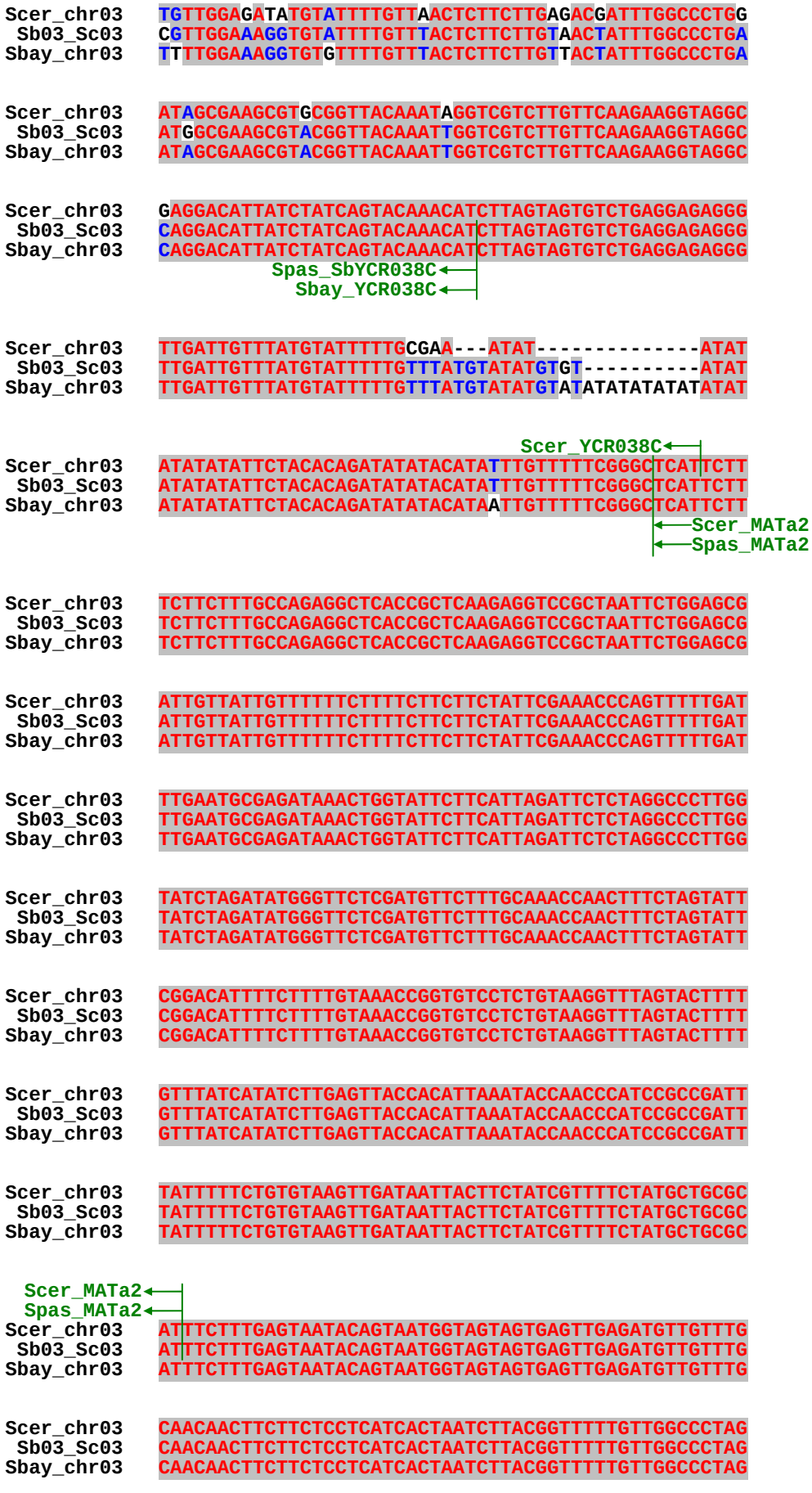
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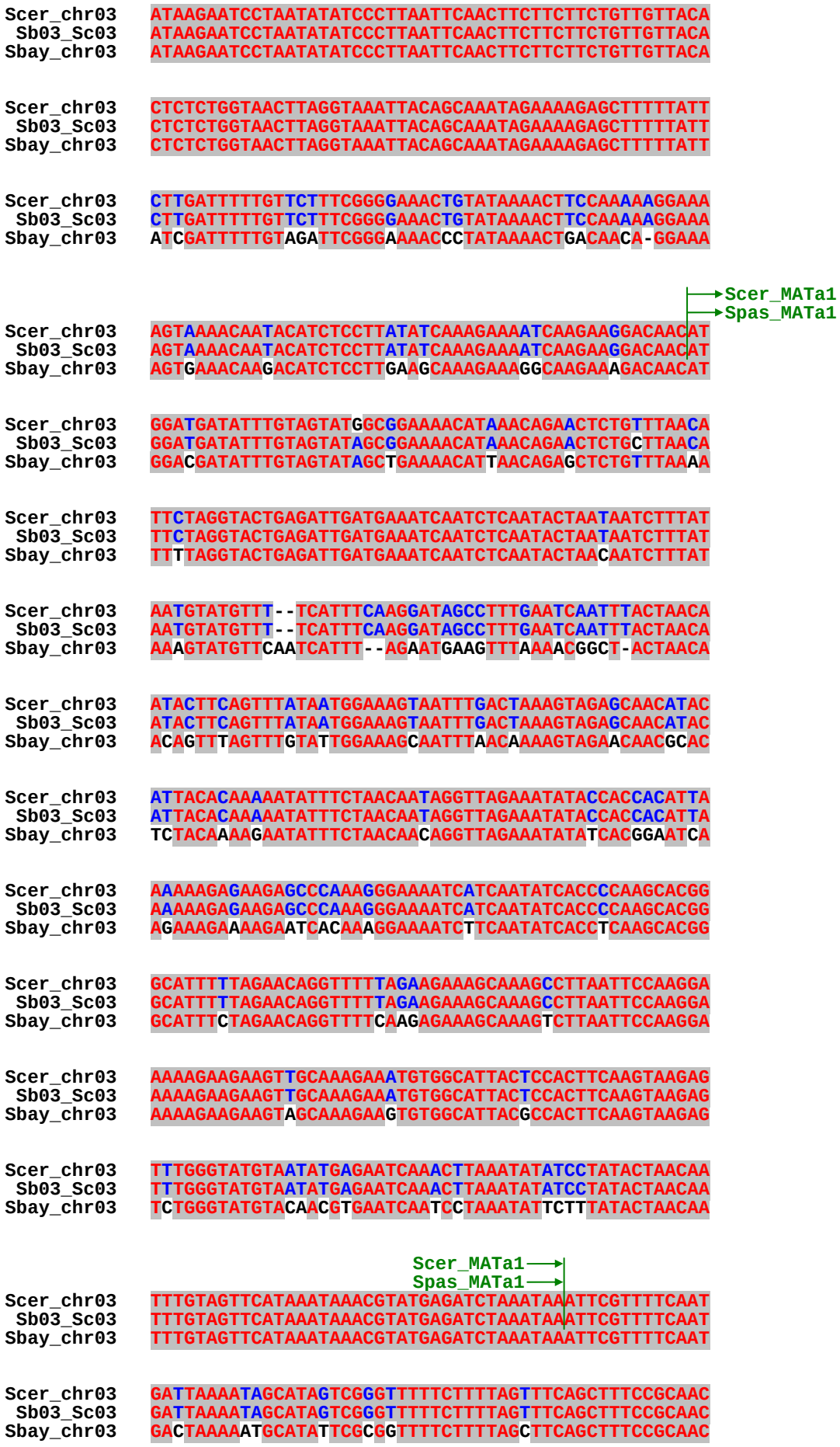
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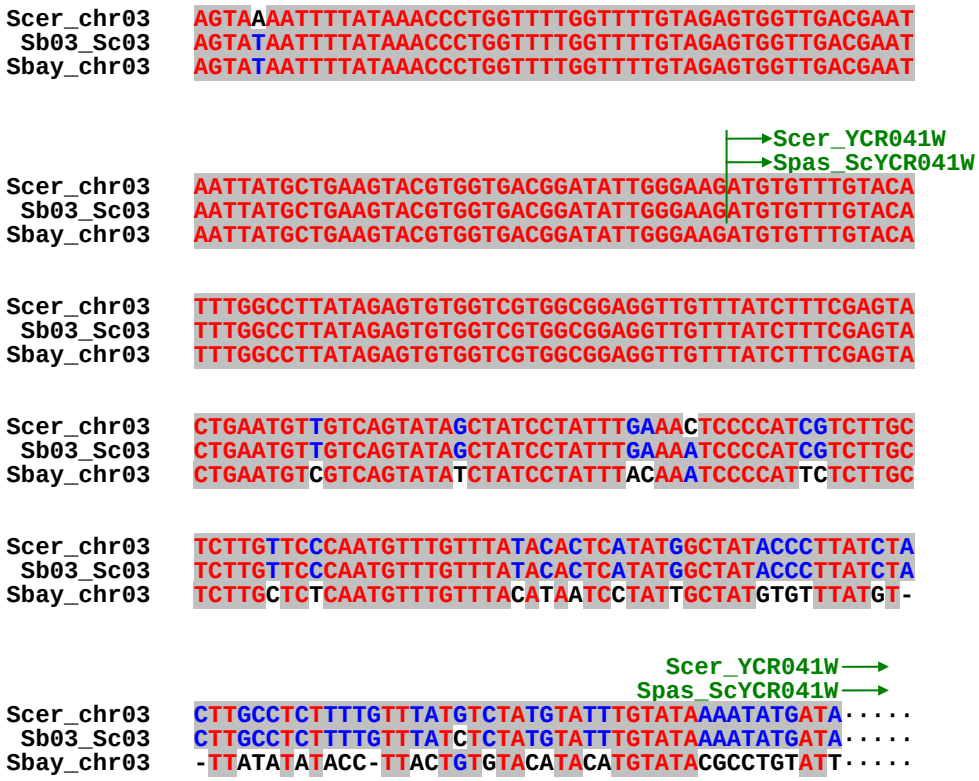
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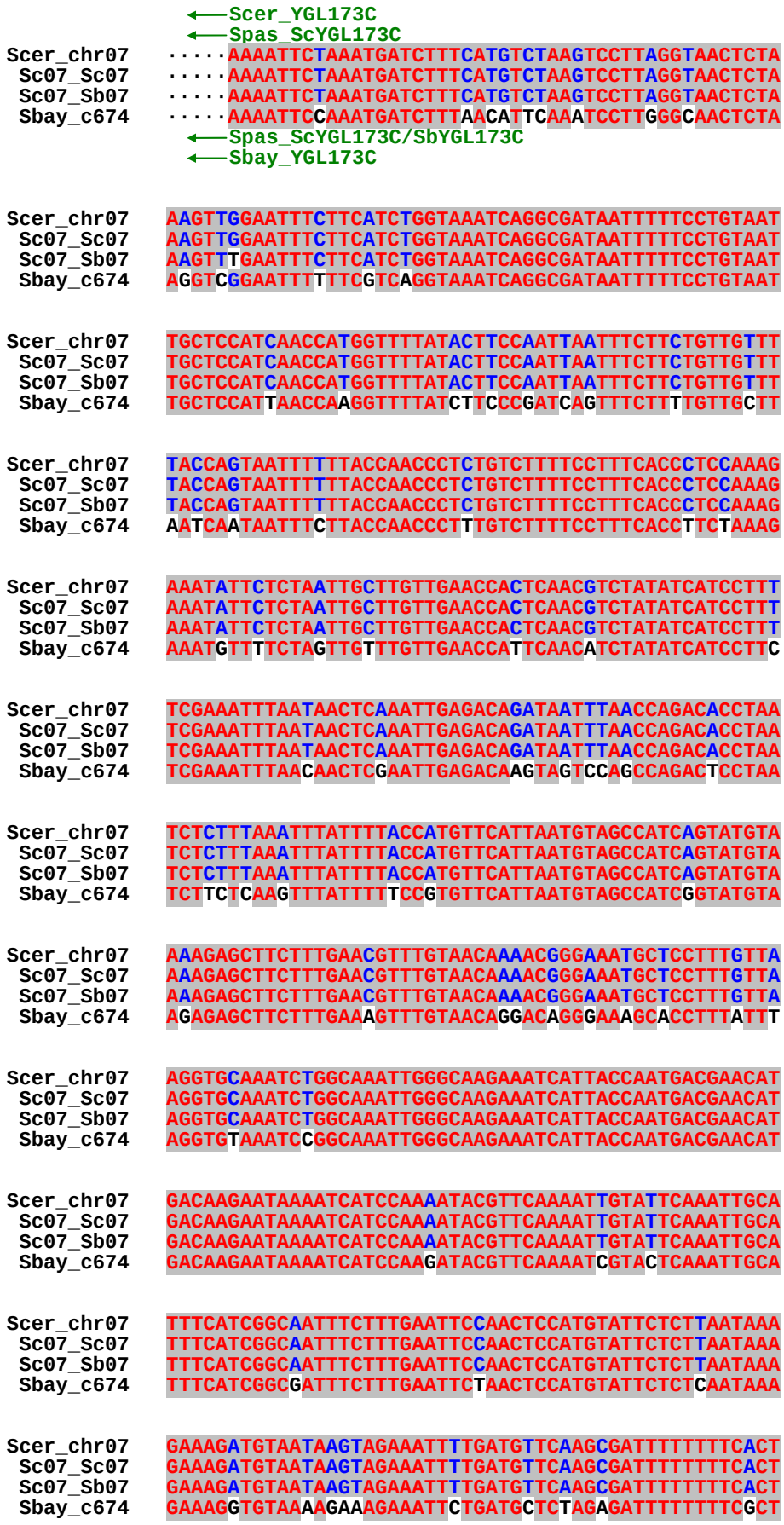

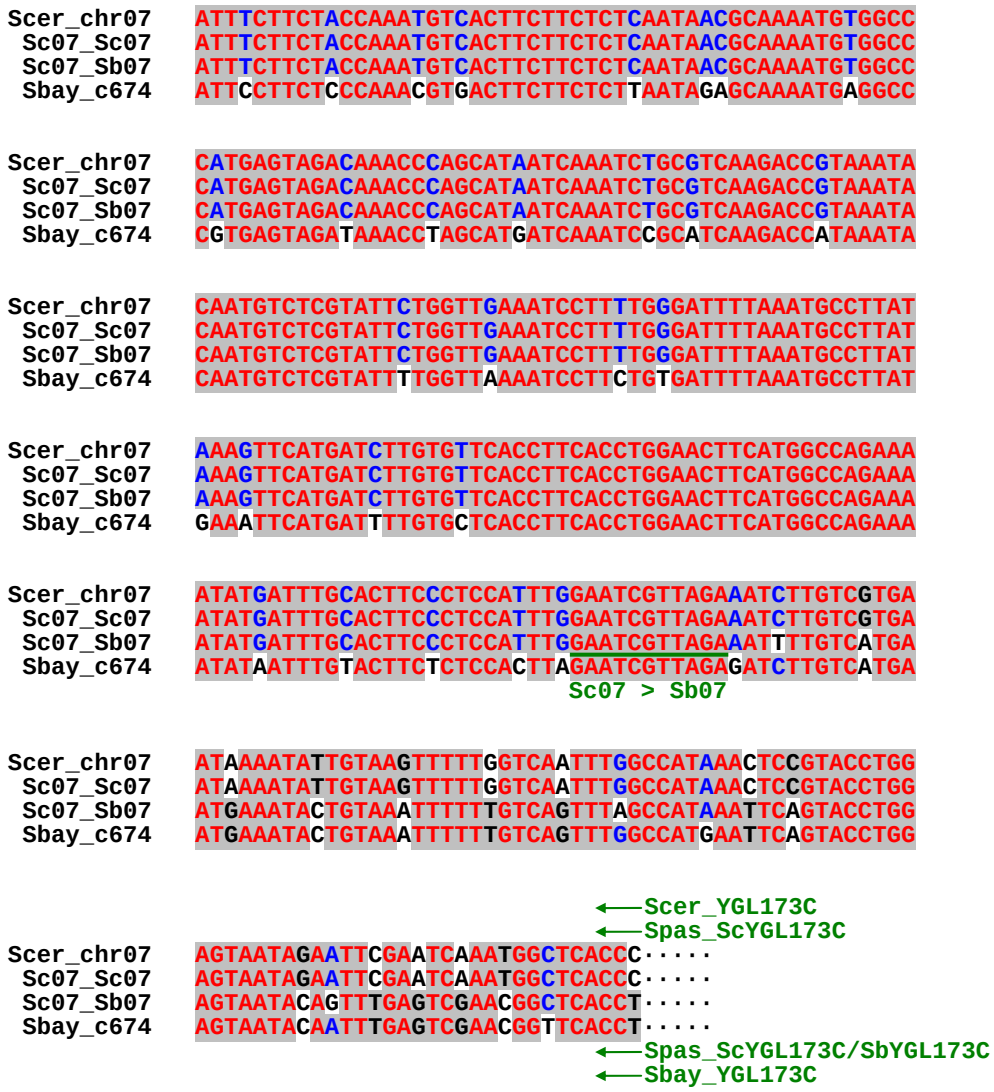
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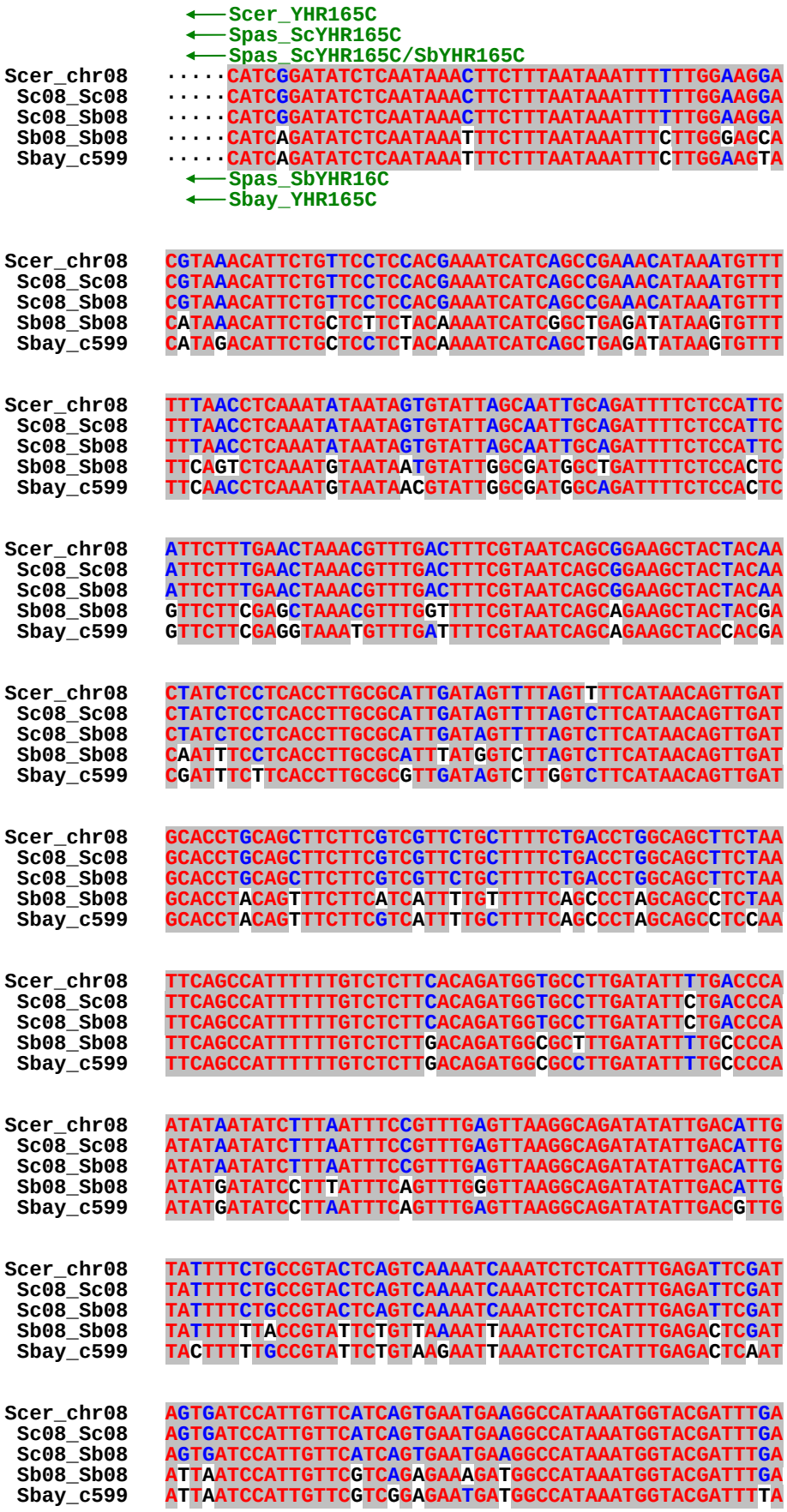

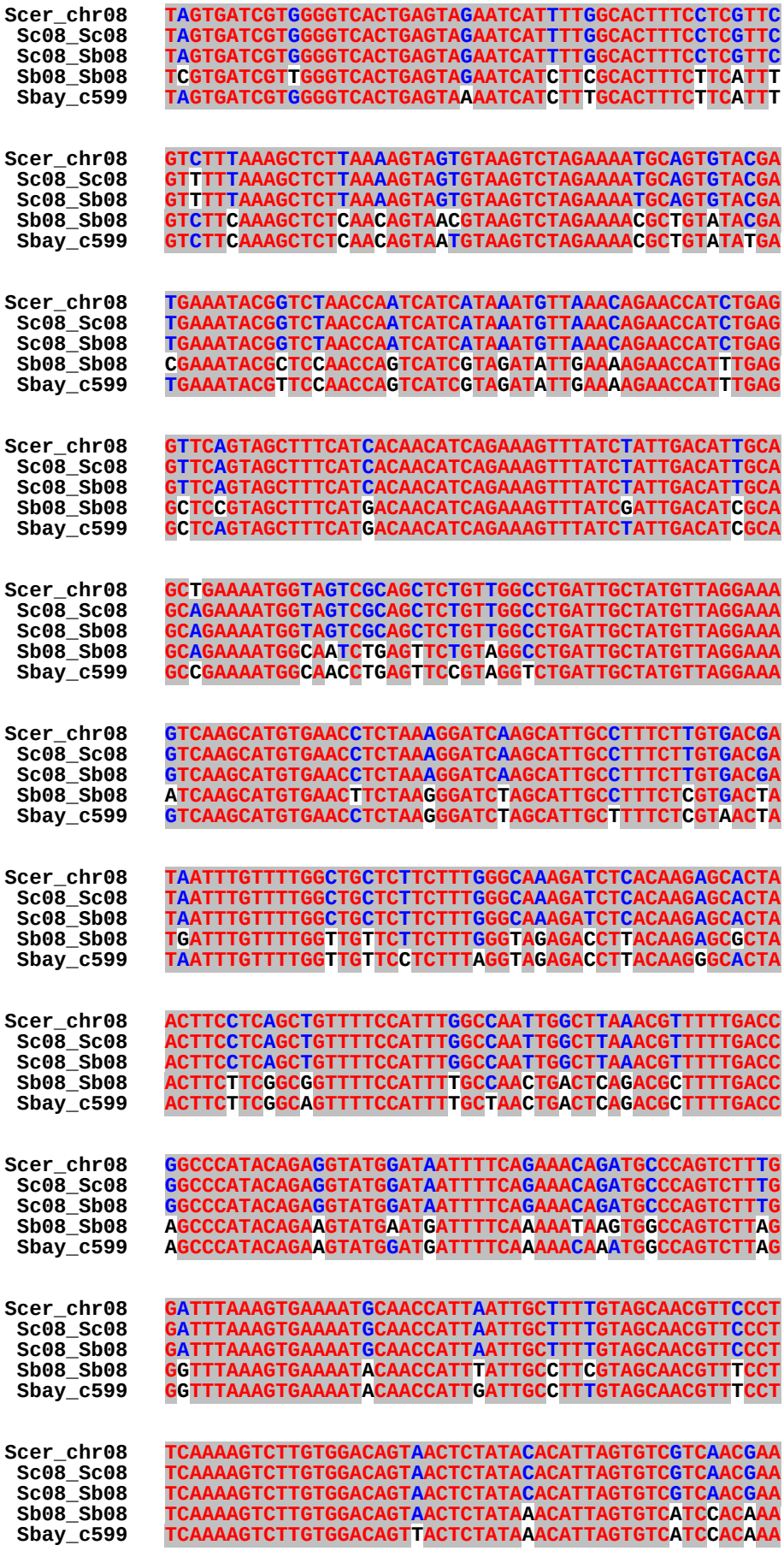
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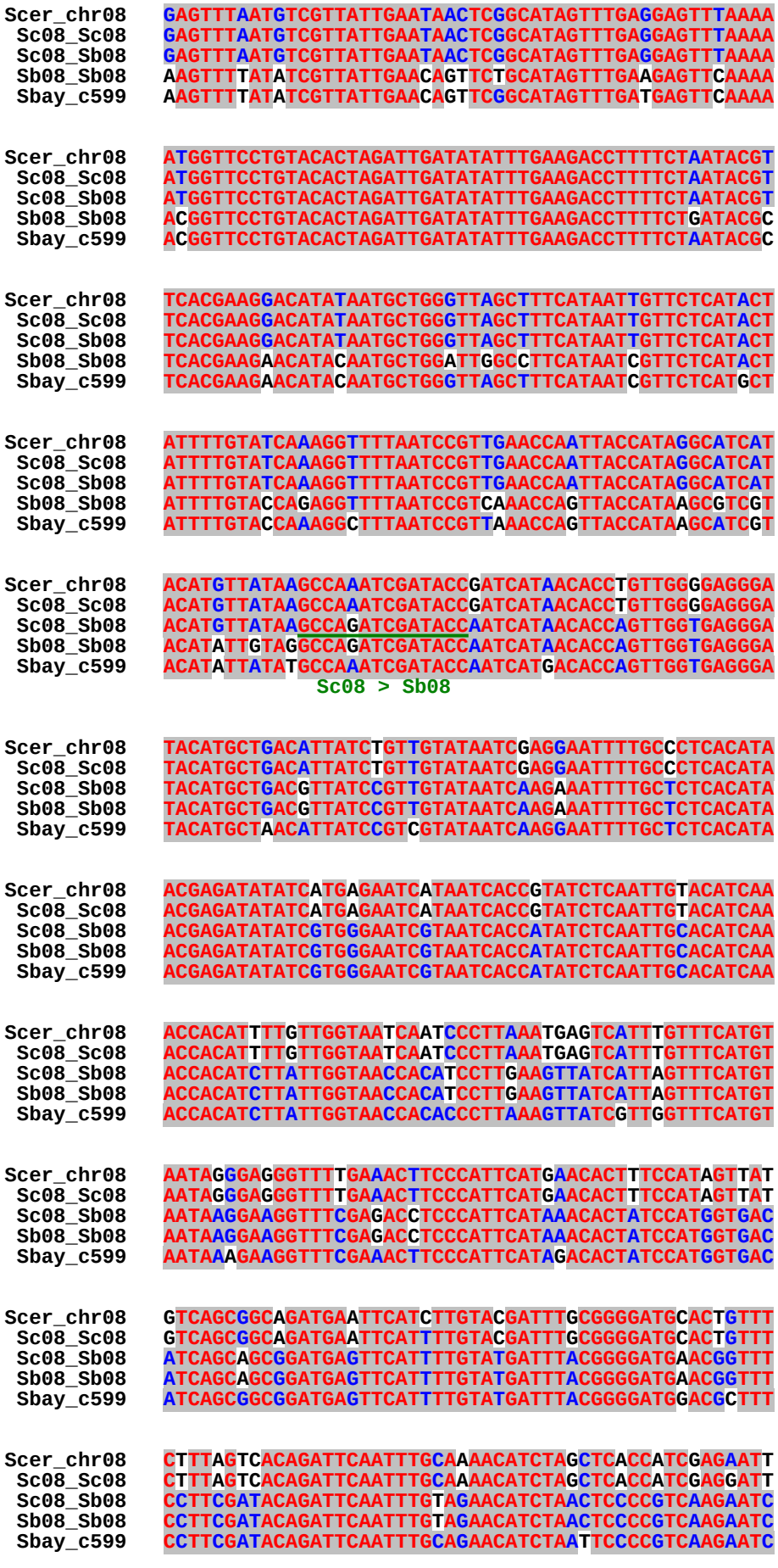
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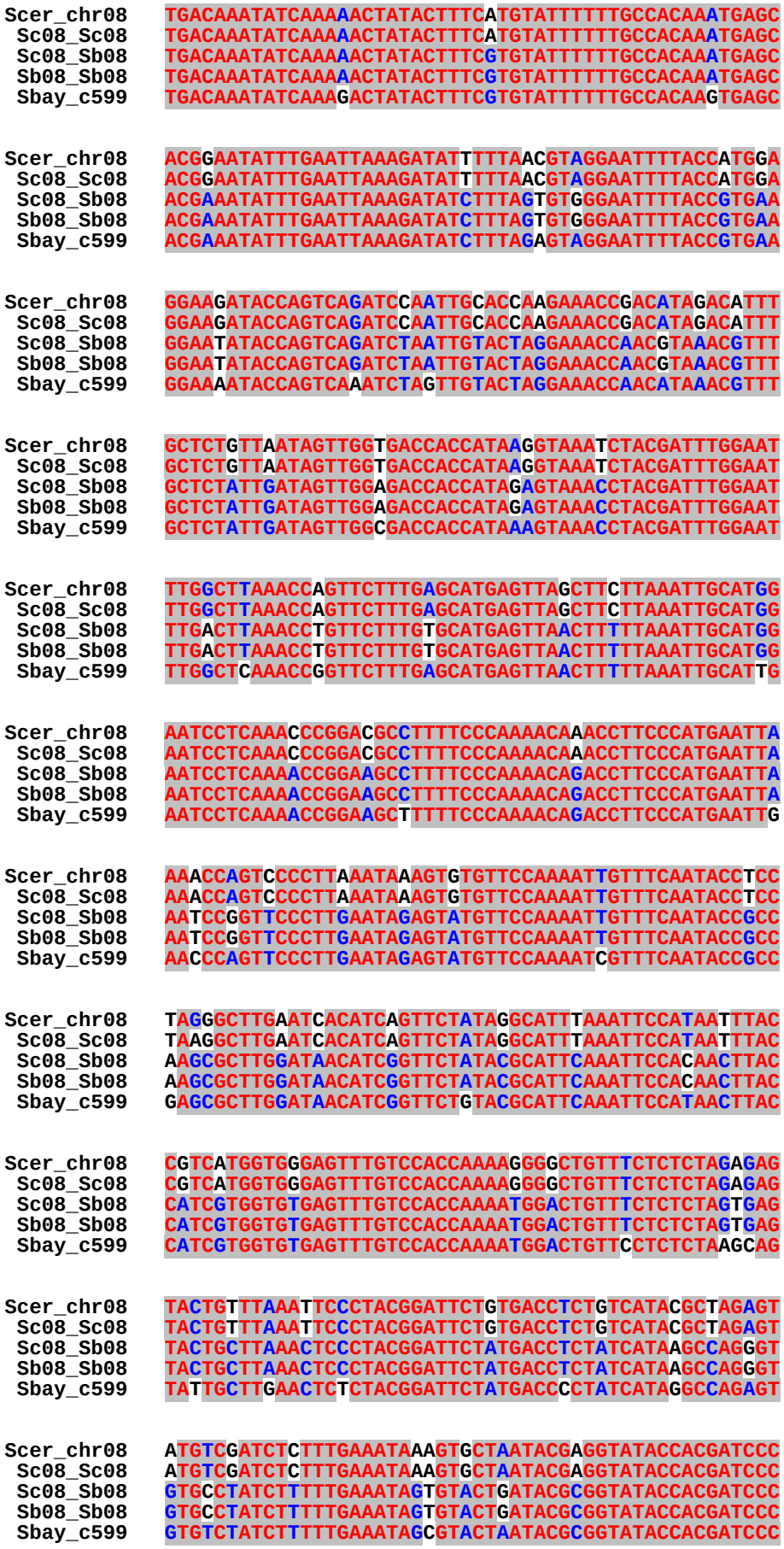
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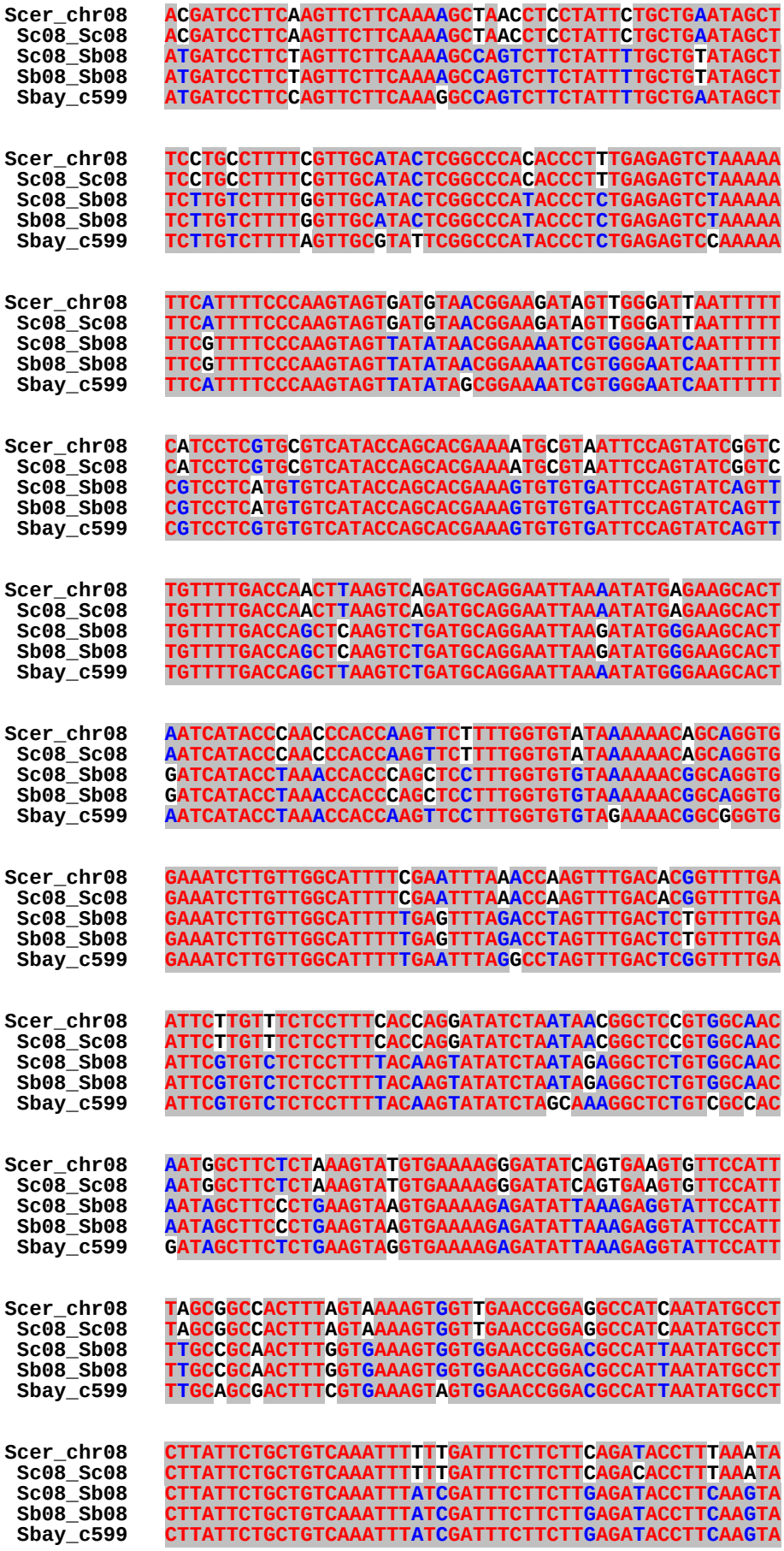
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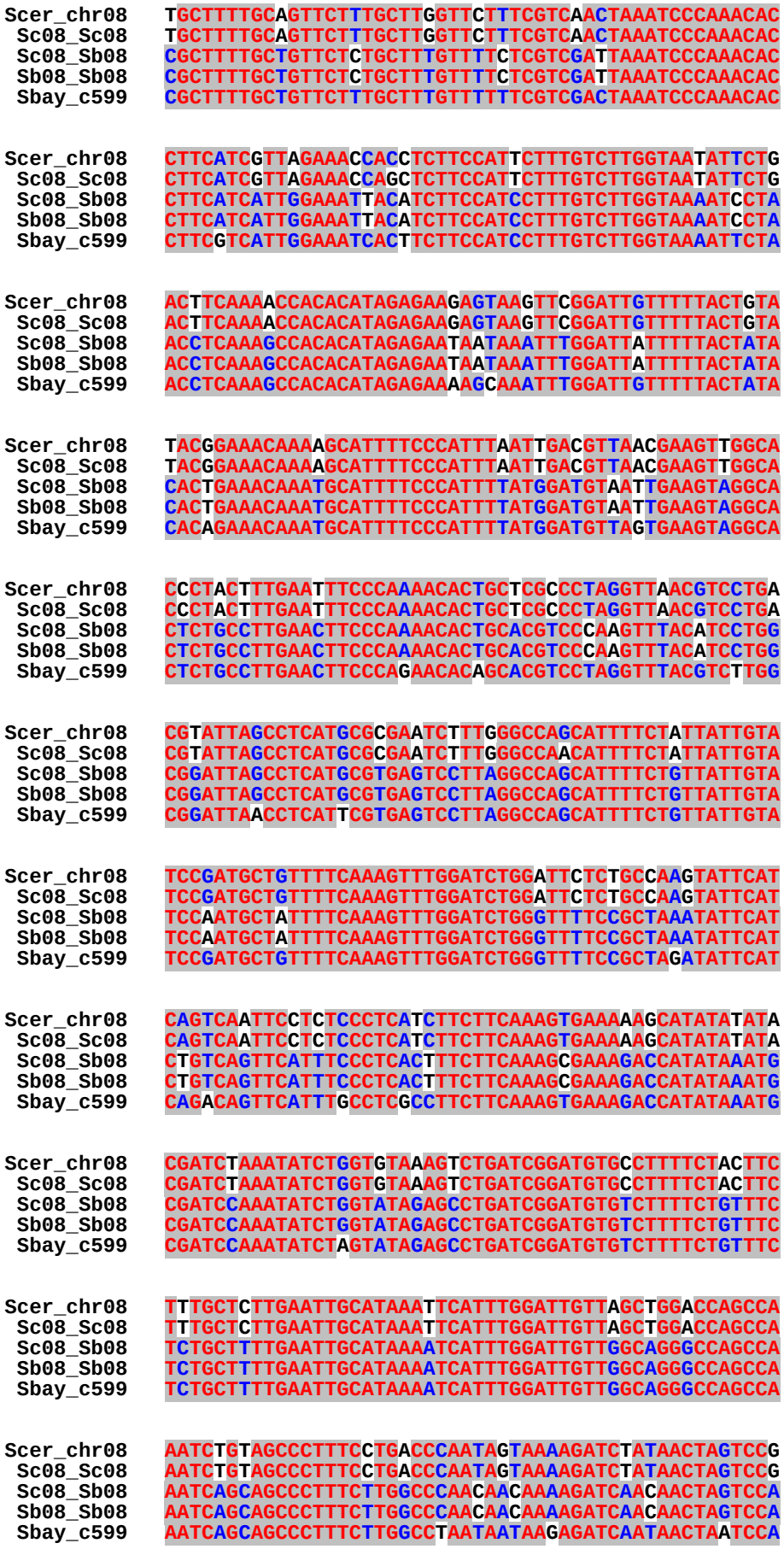
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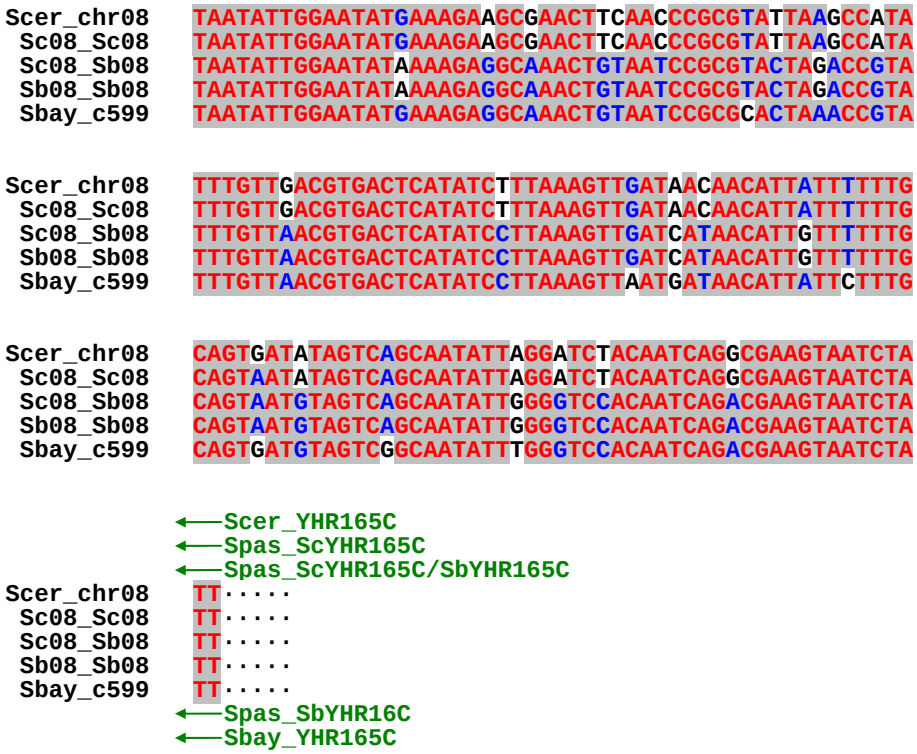
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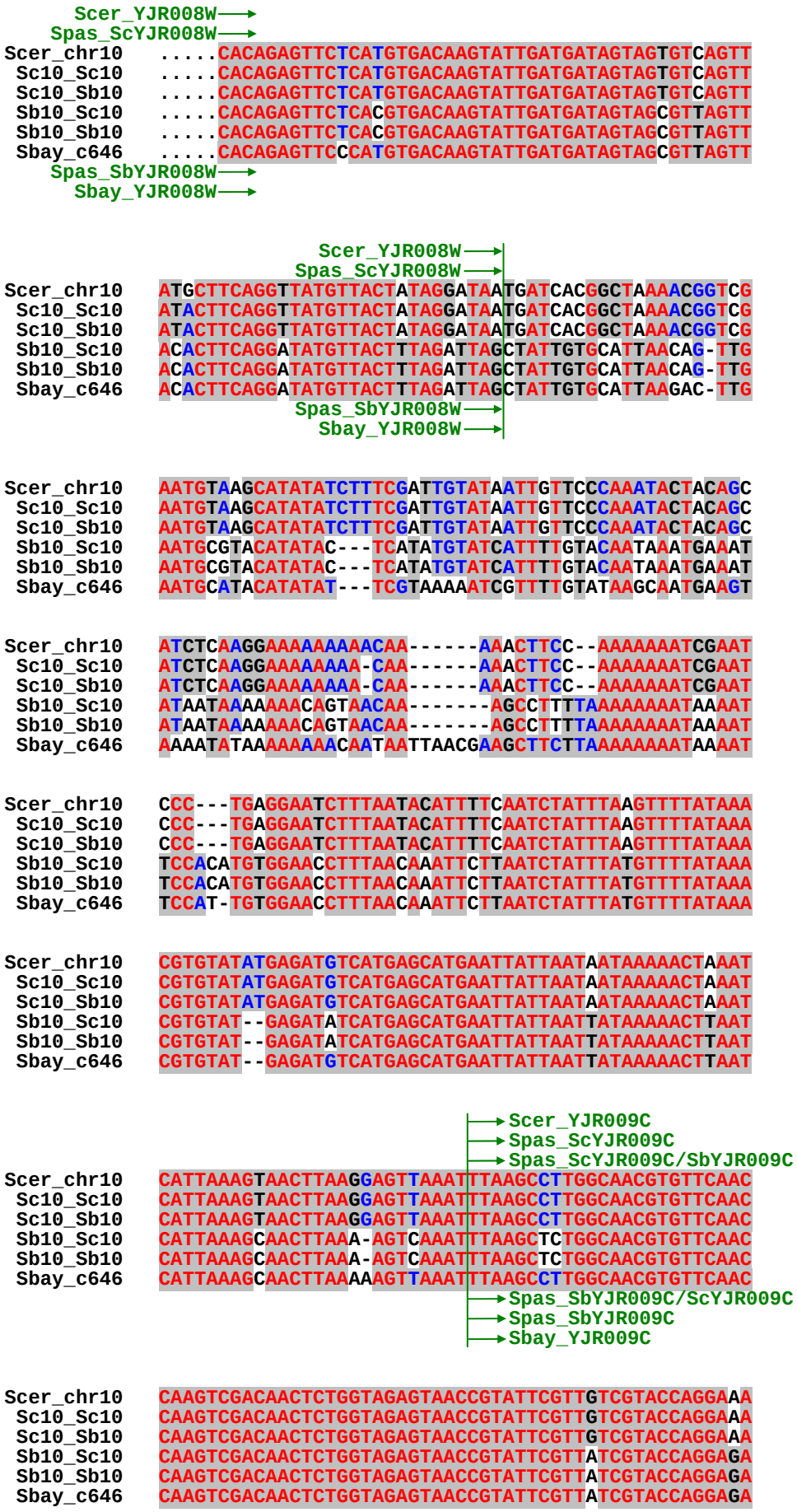
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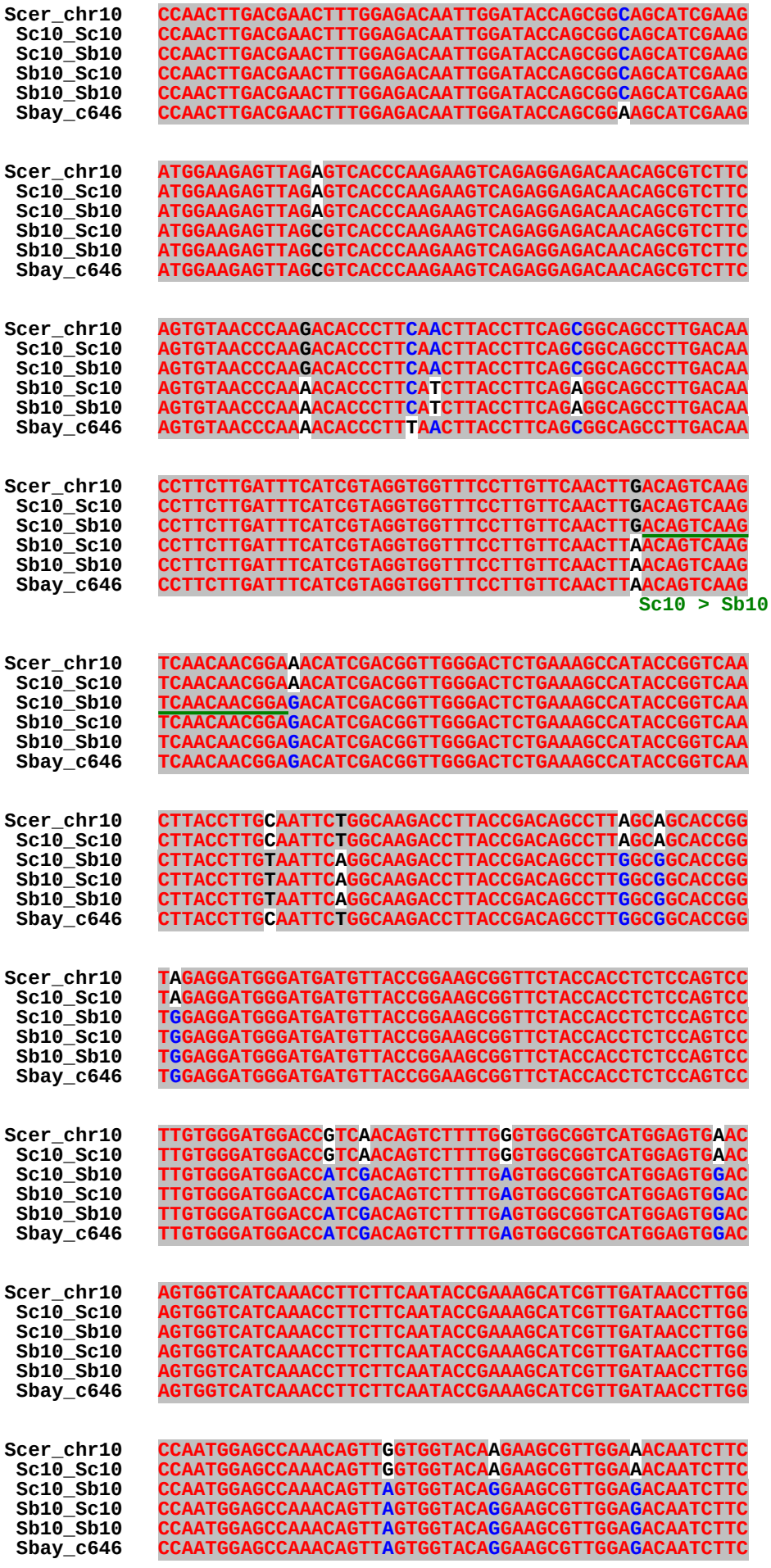
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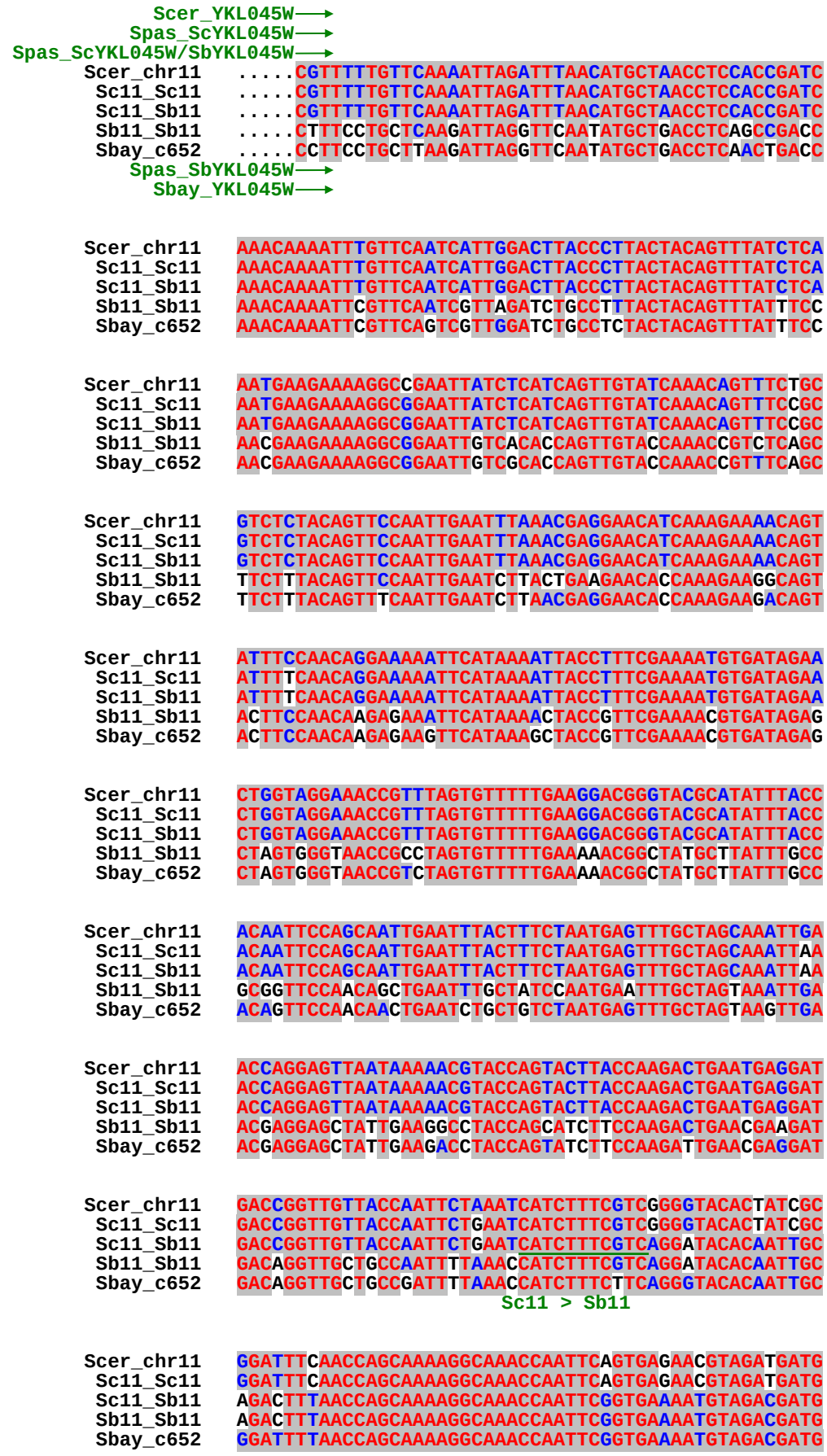
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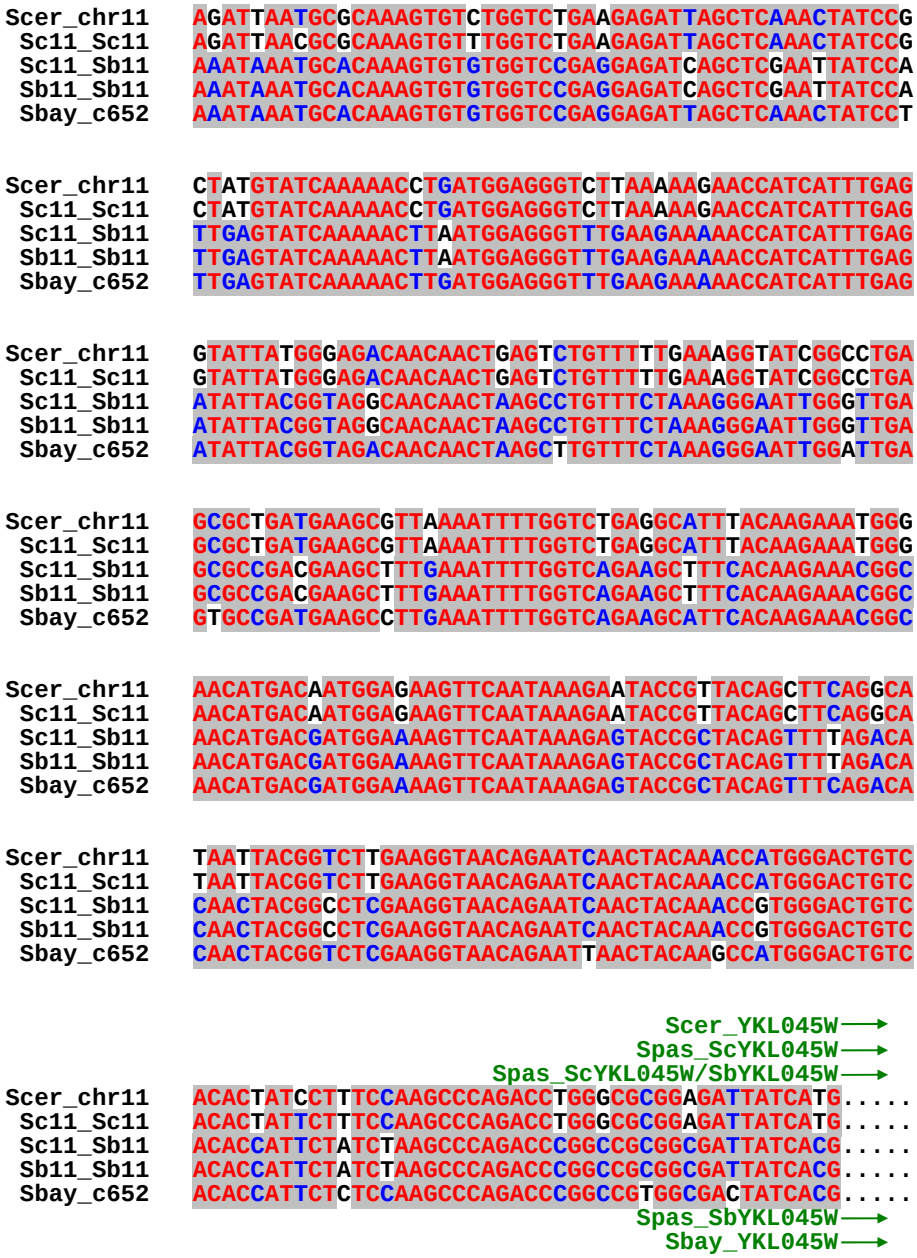
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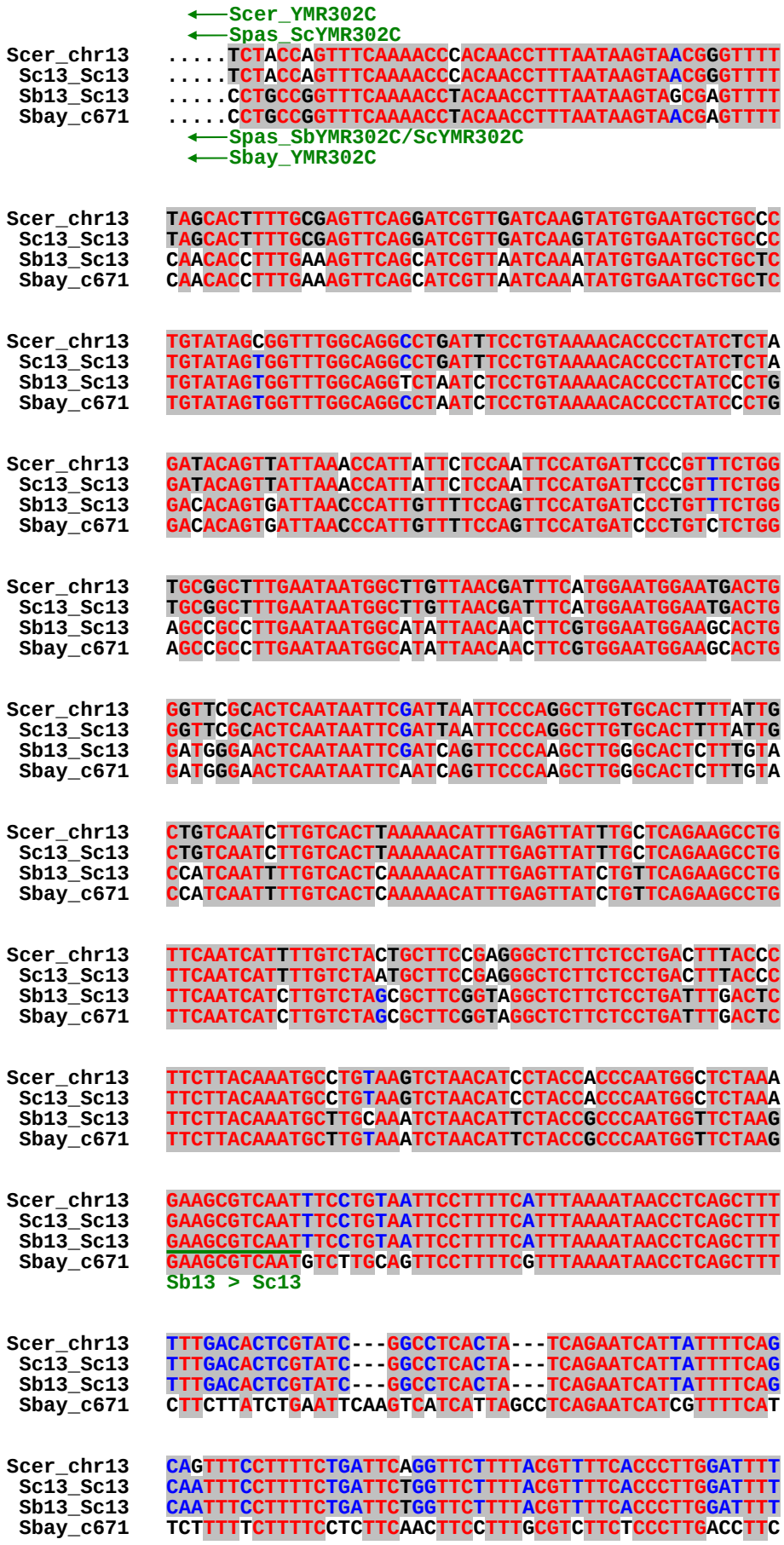

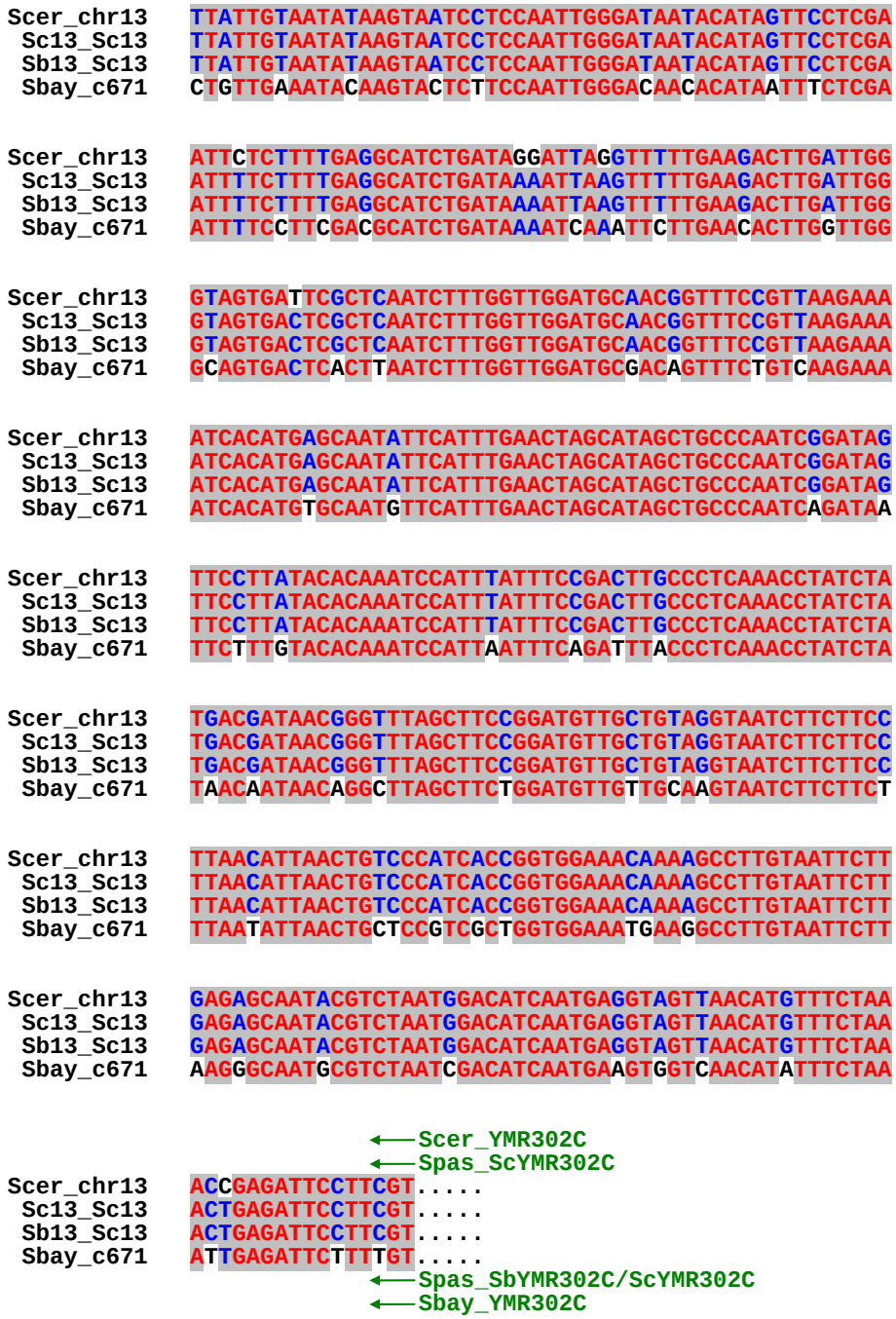
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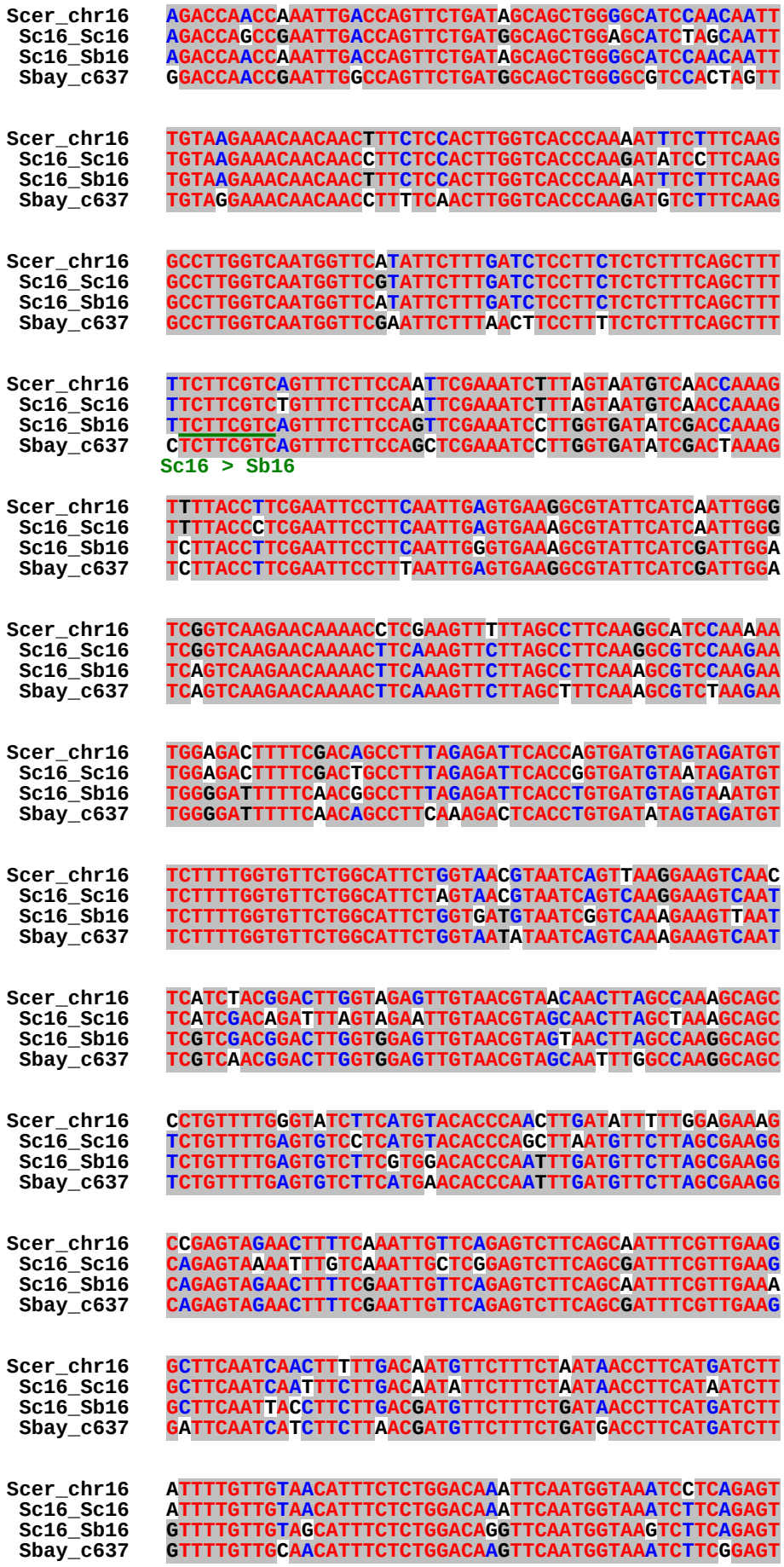
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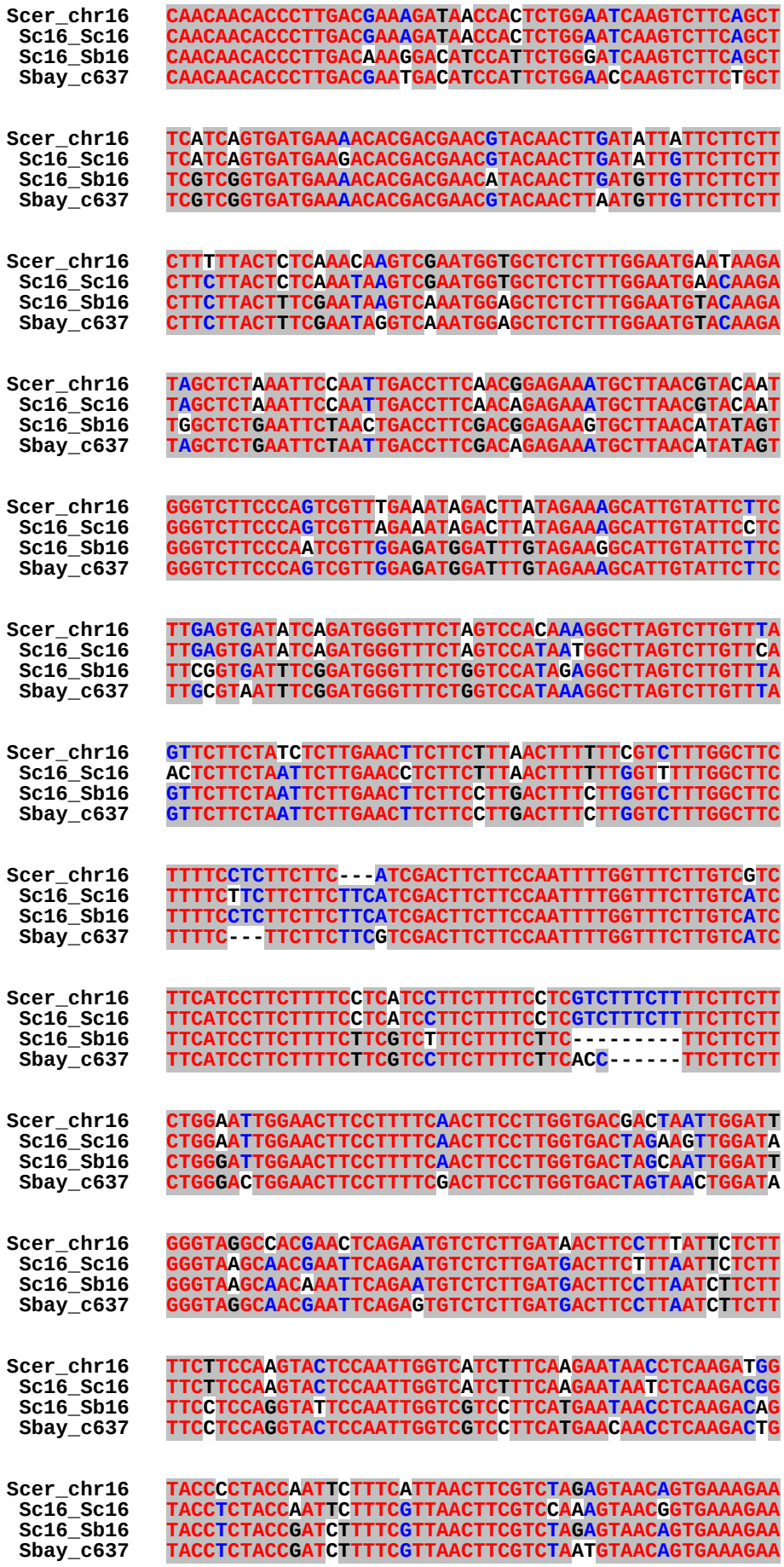
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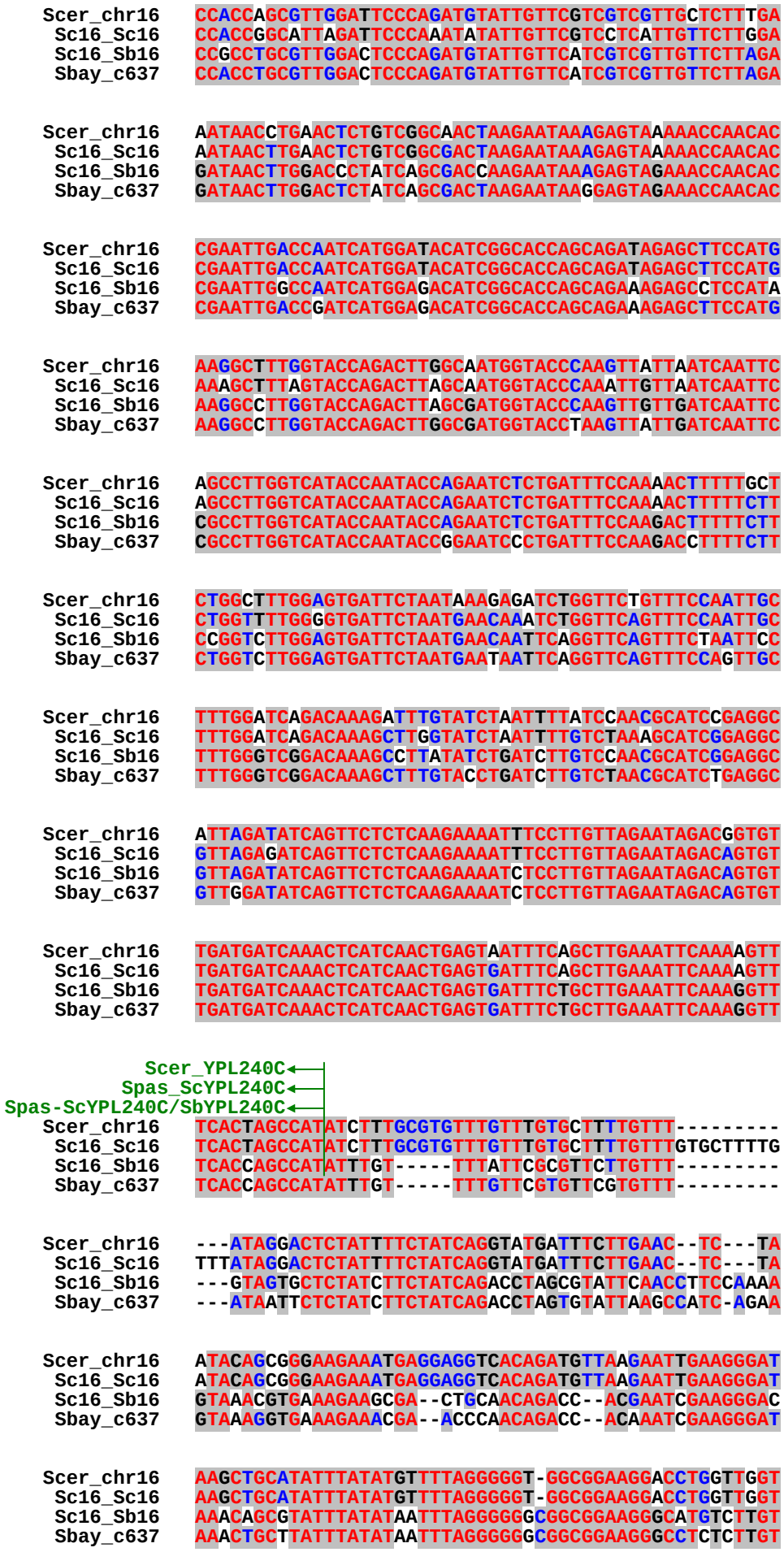
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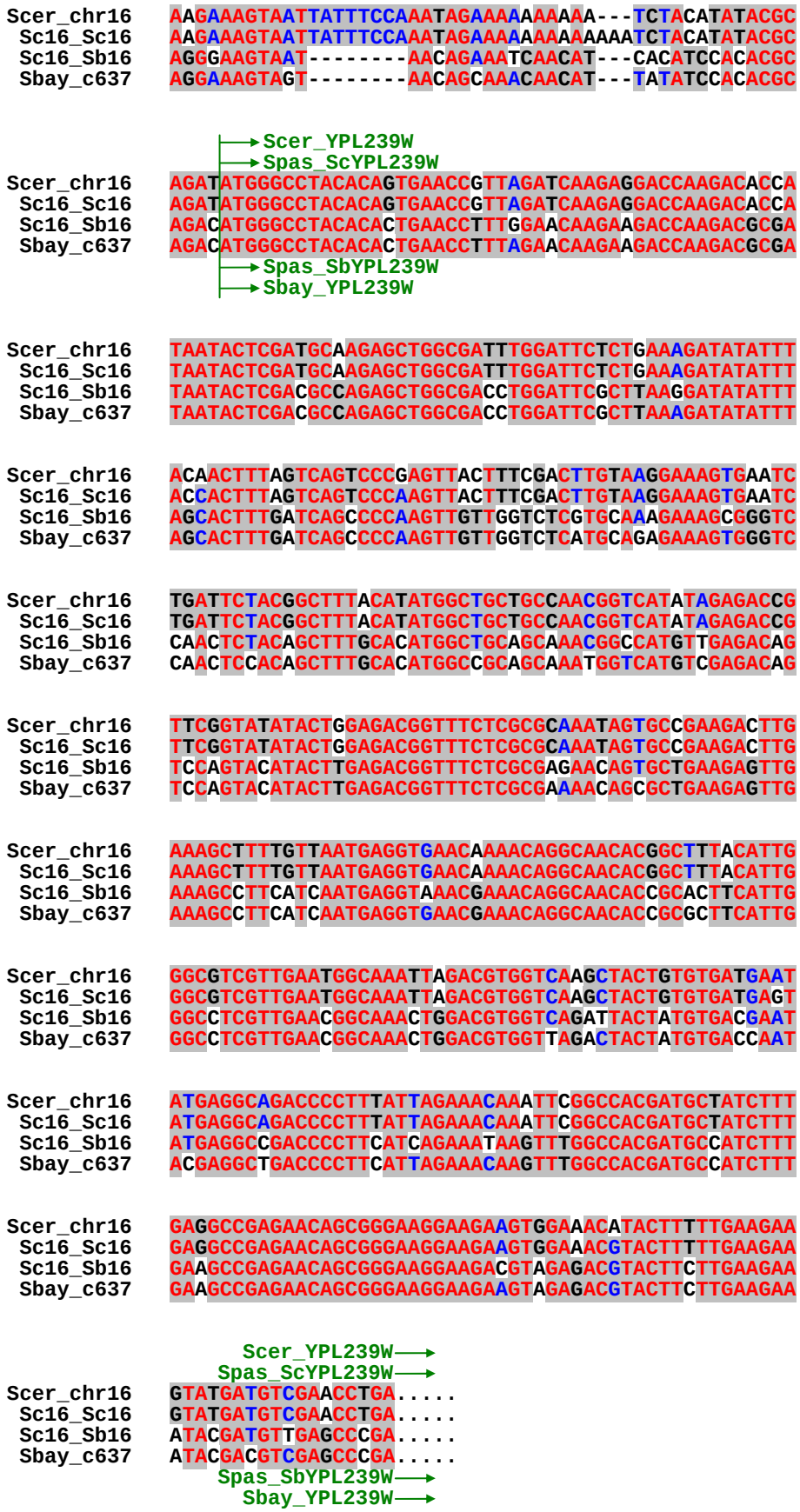
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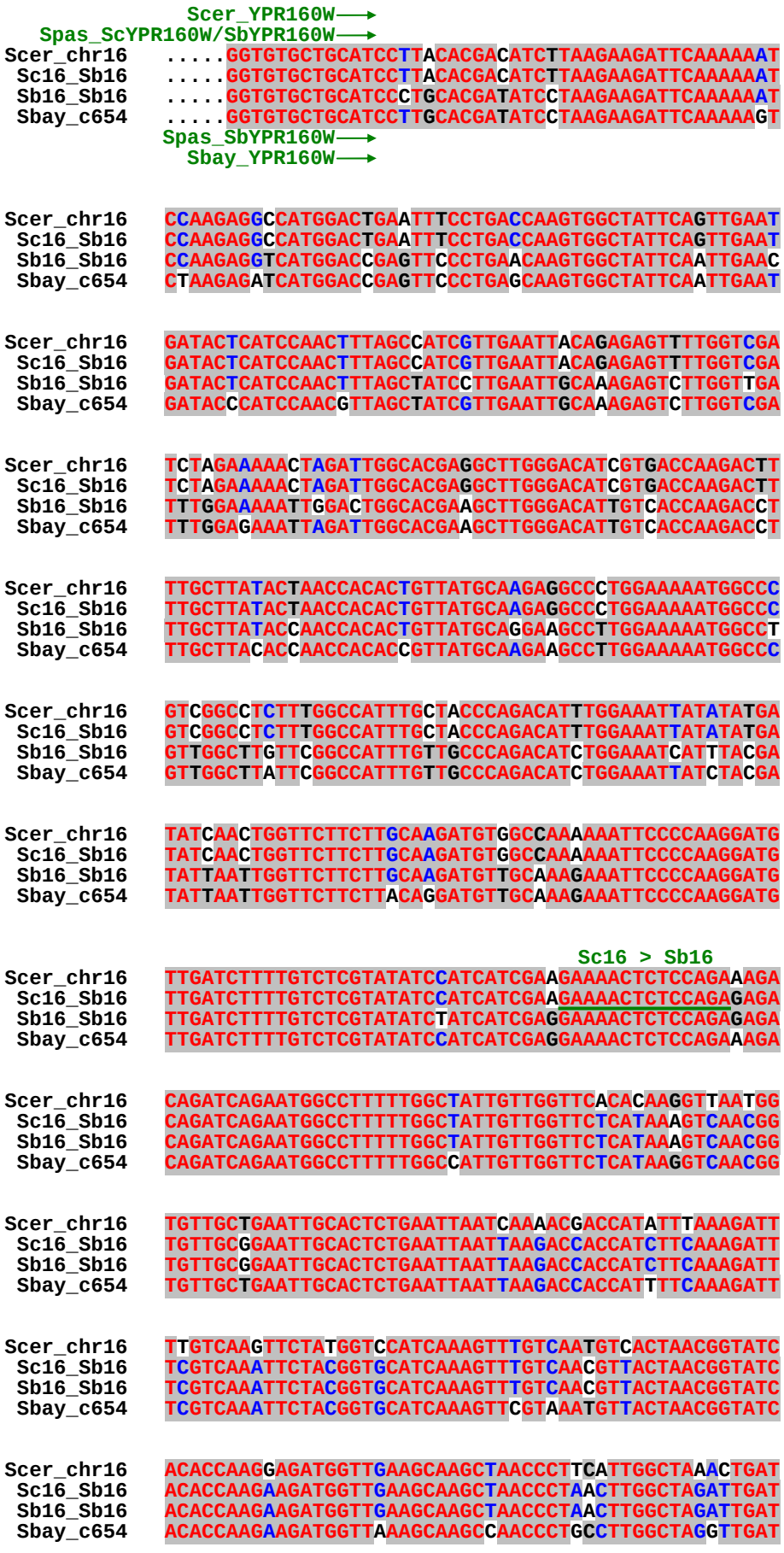

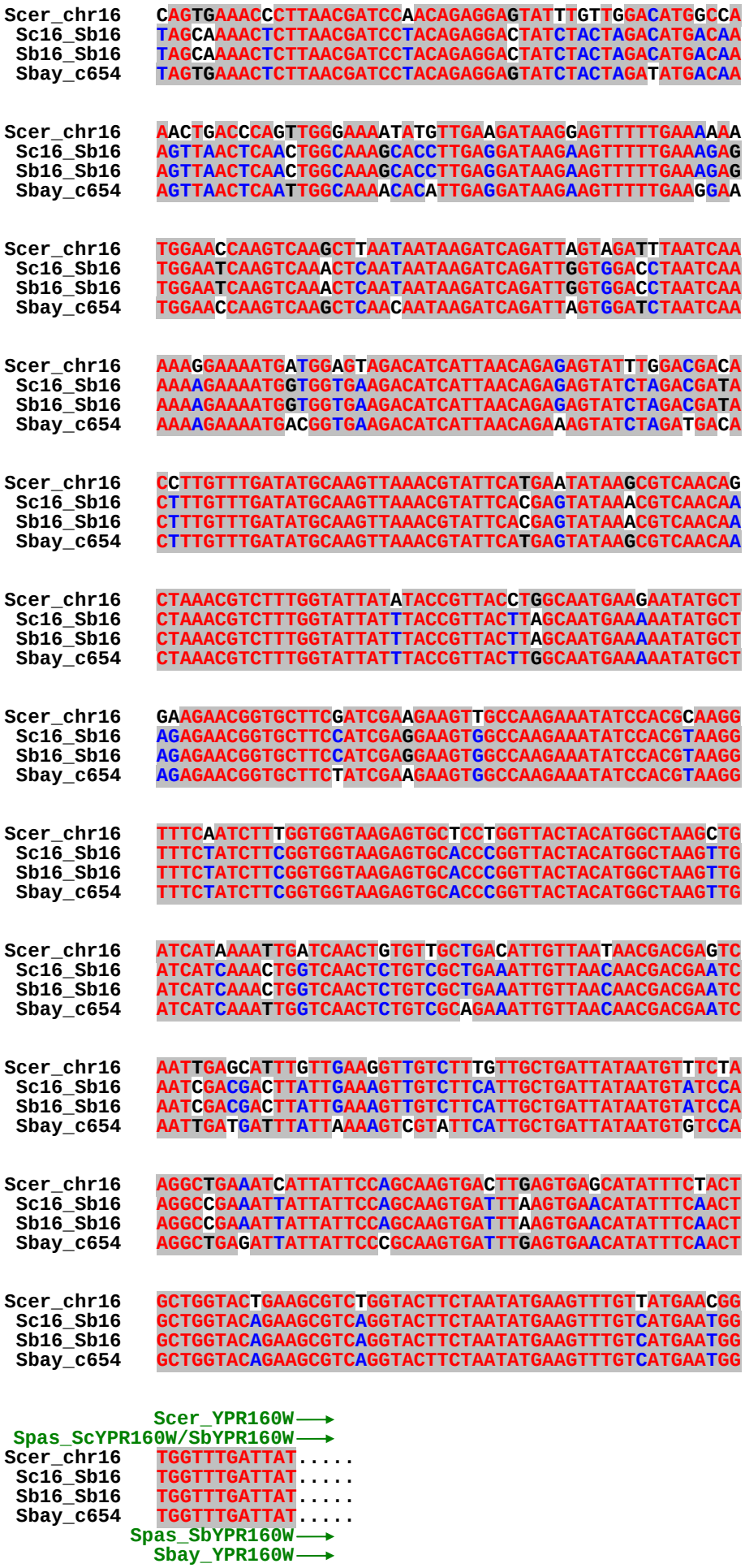
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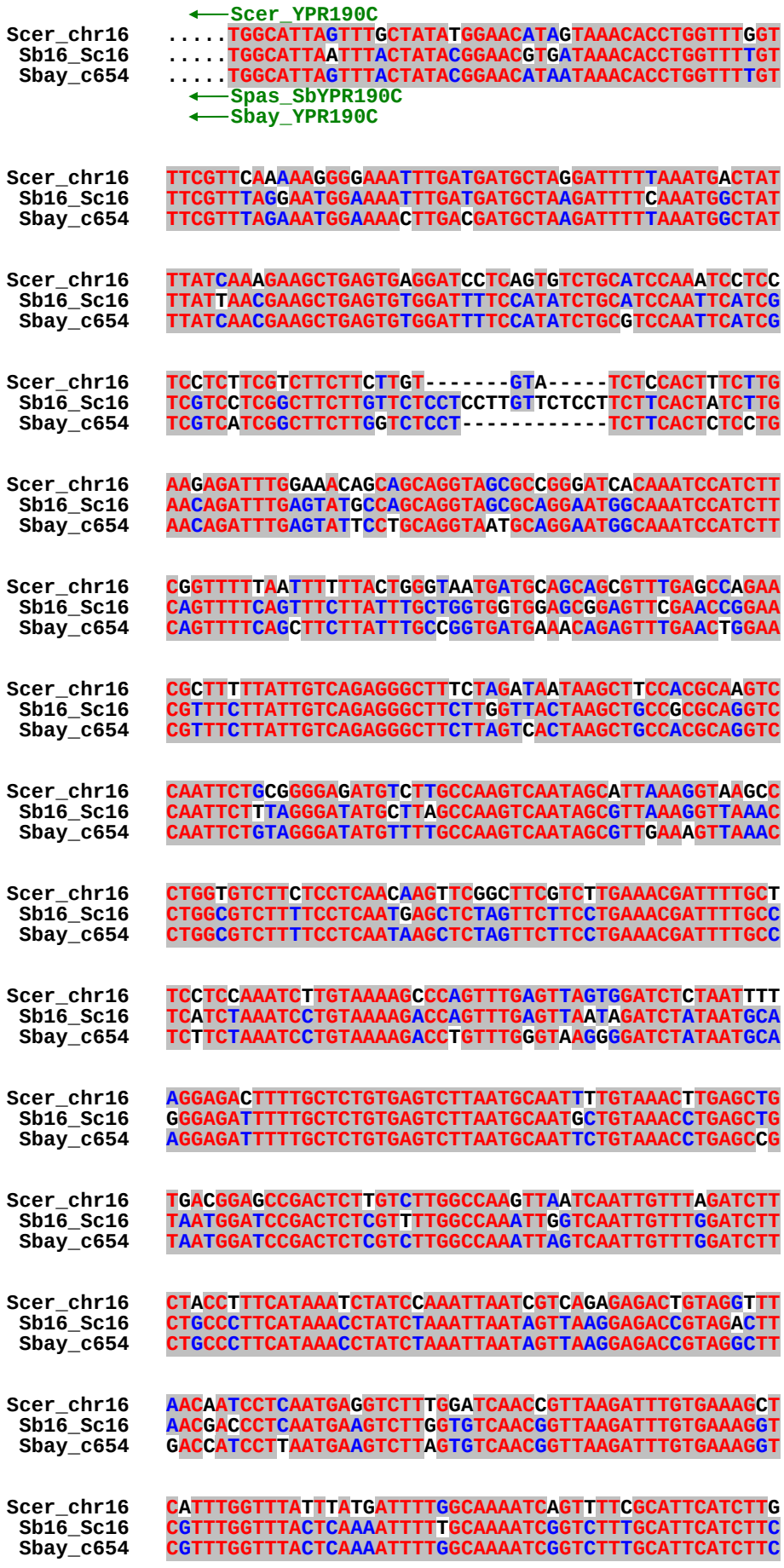

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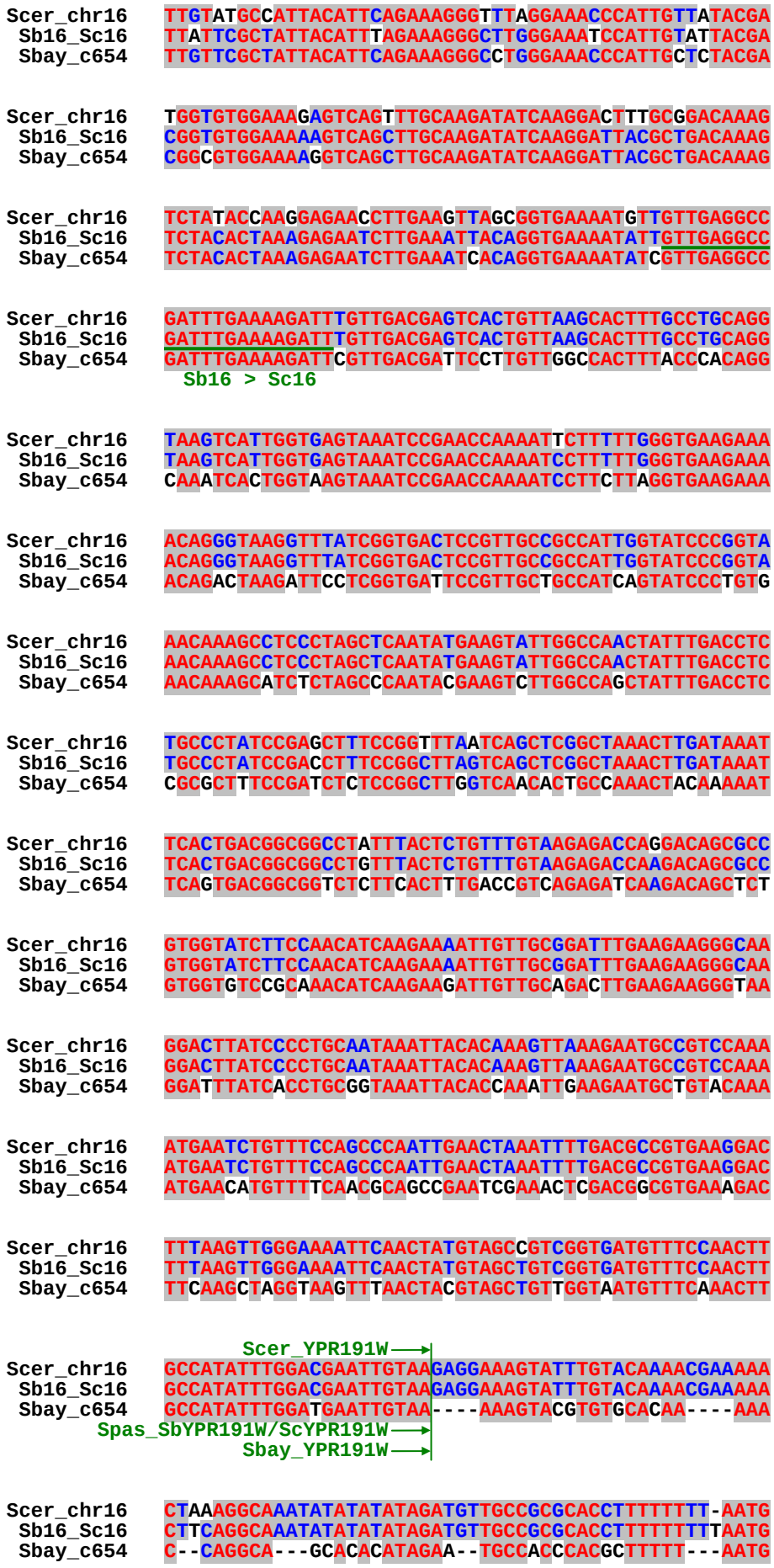
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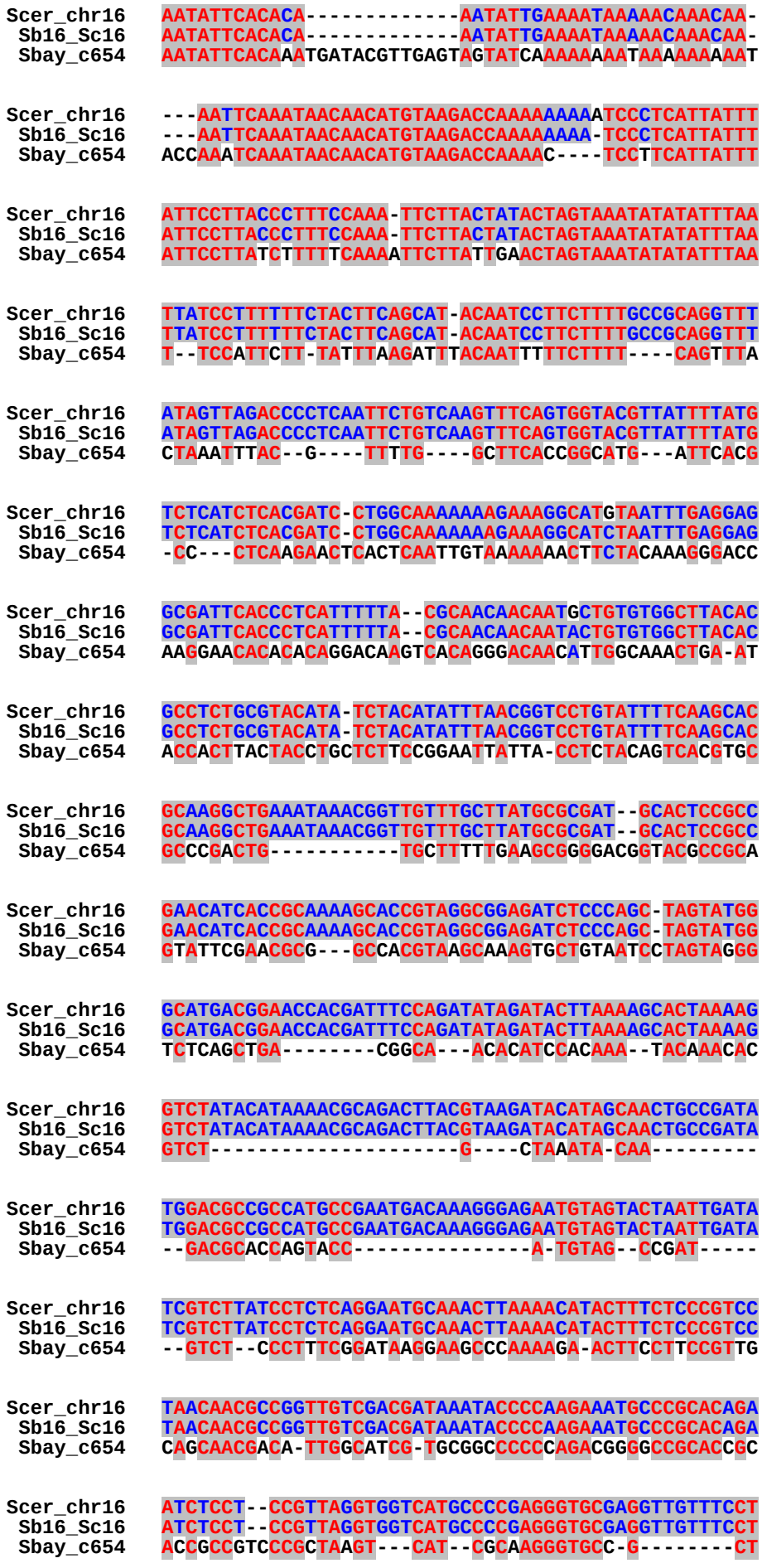
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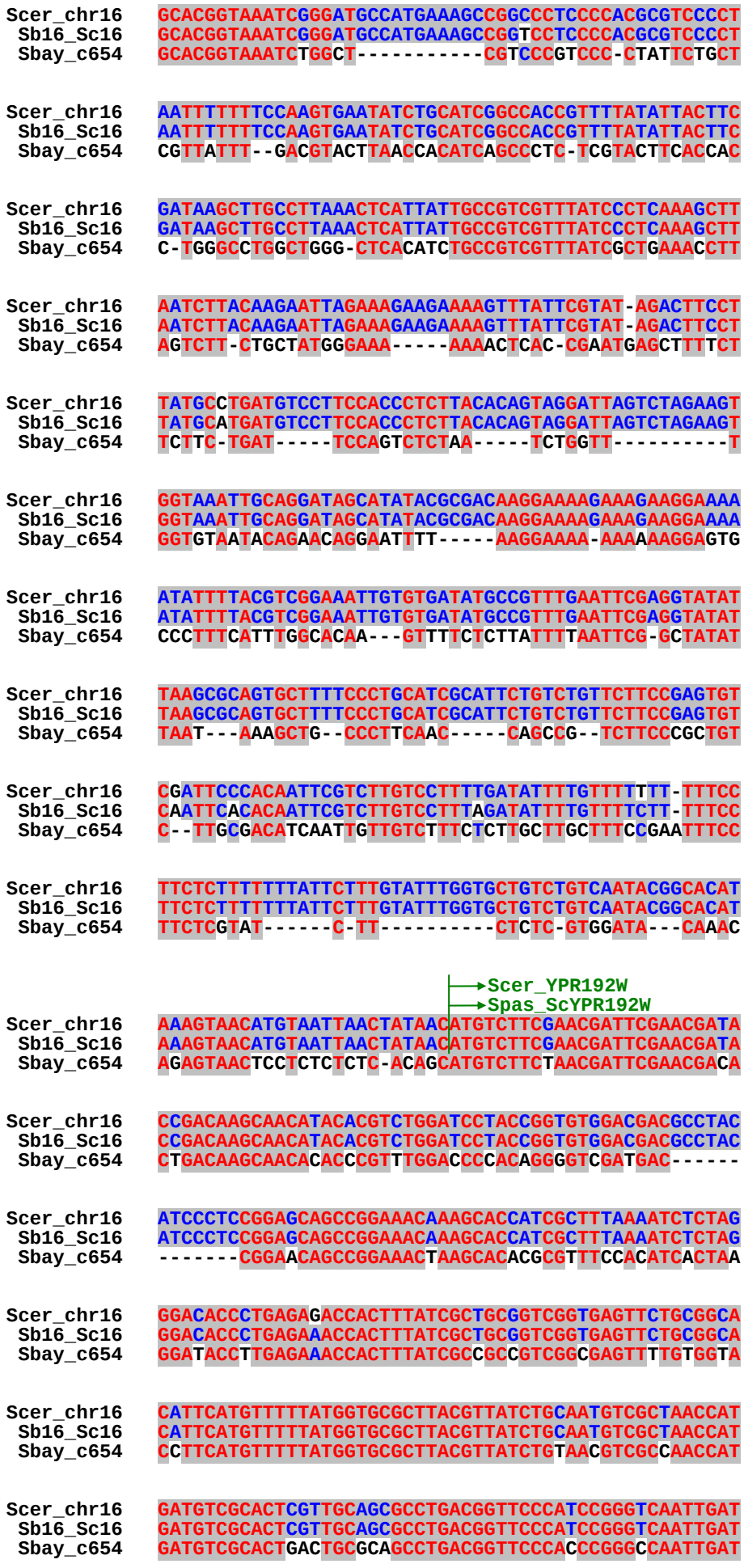
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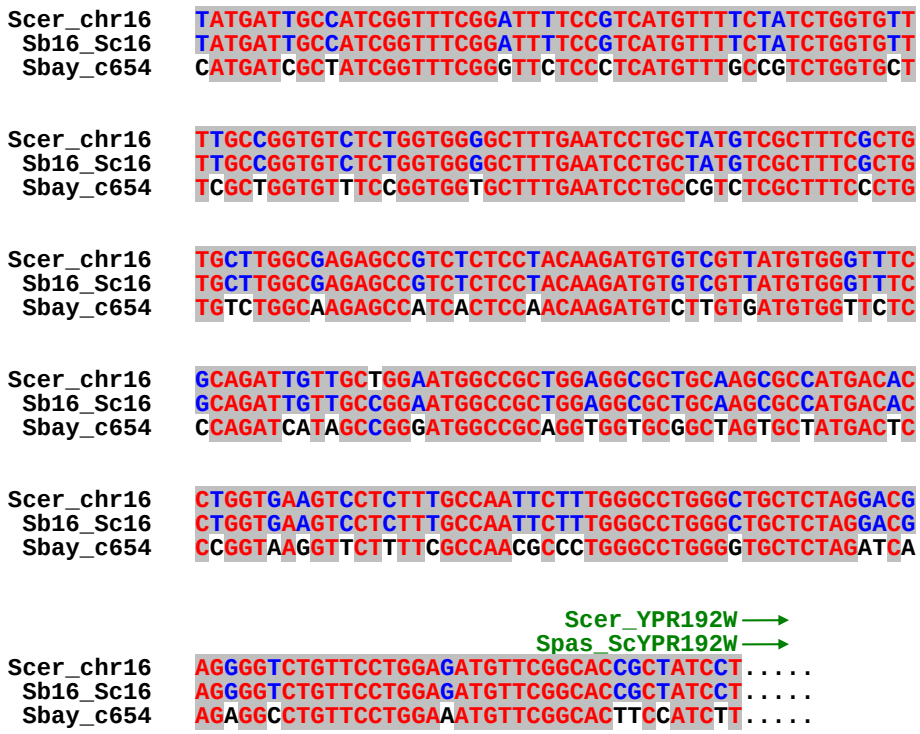

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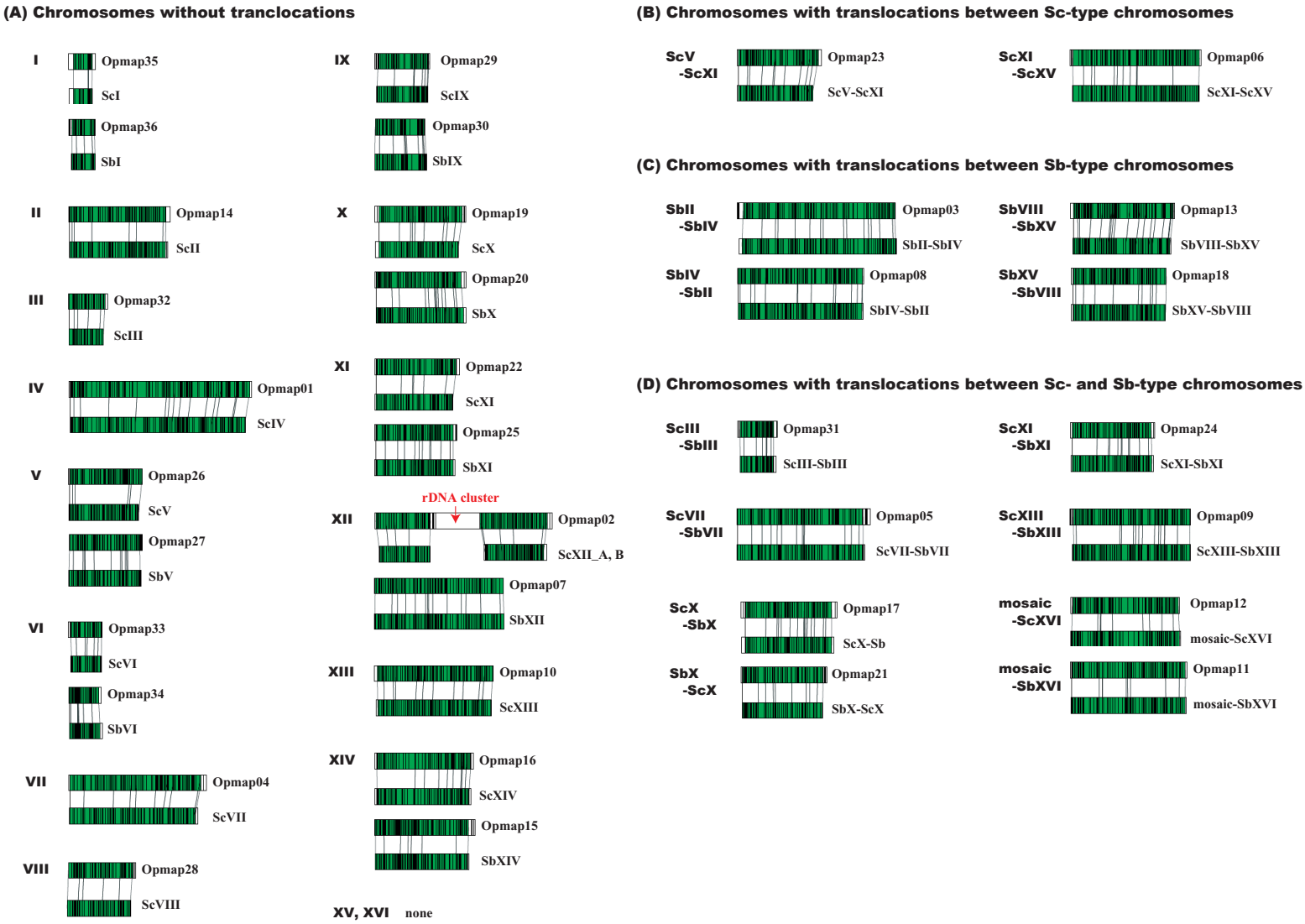

Figure S2, Nakao *et al.*

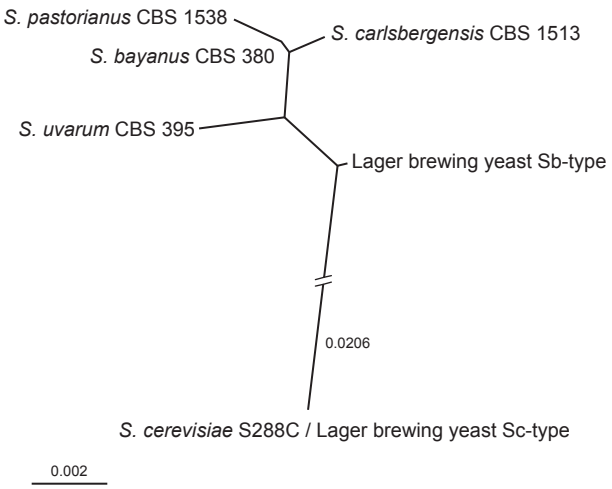

Figure S3, Nakao *et al.*

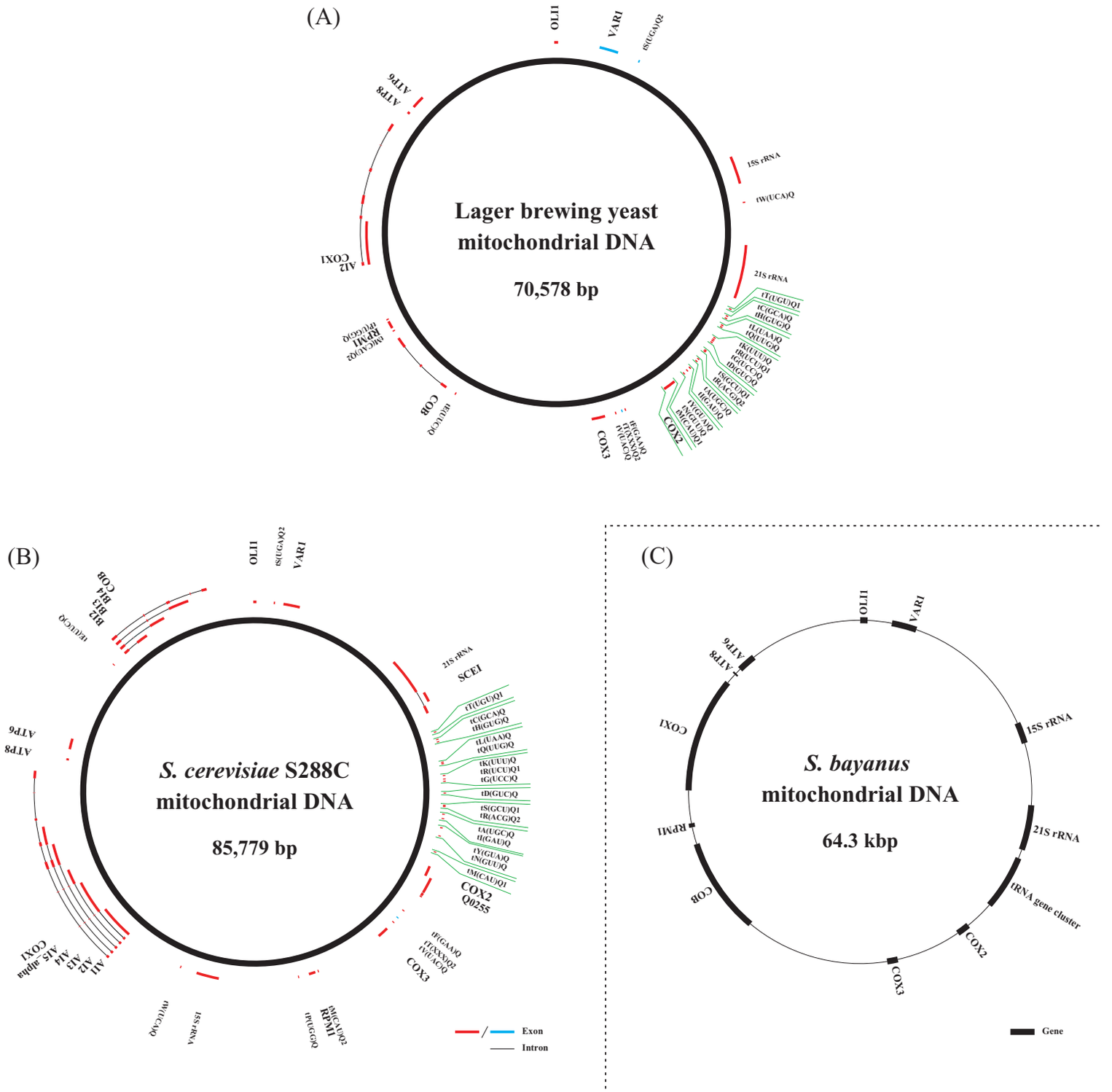

Figure S4, Nakao *et al.*