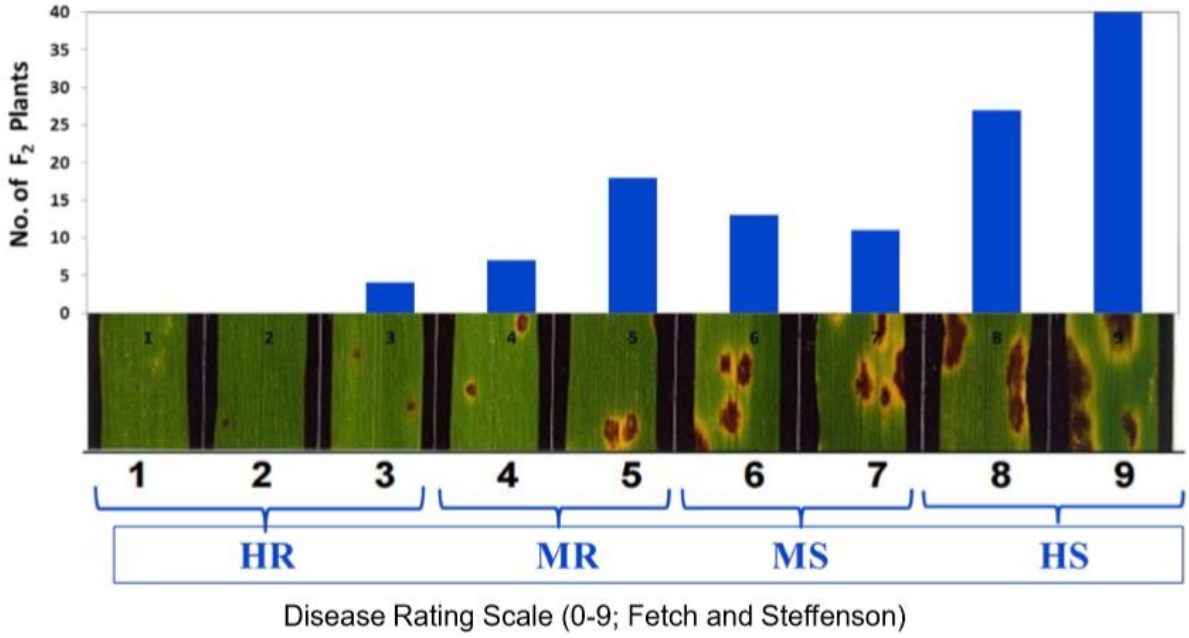
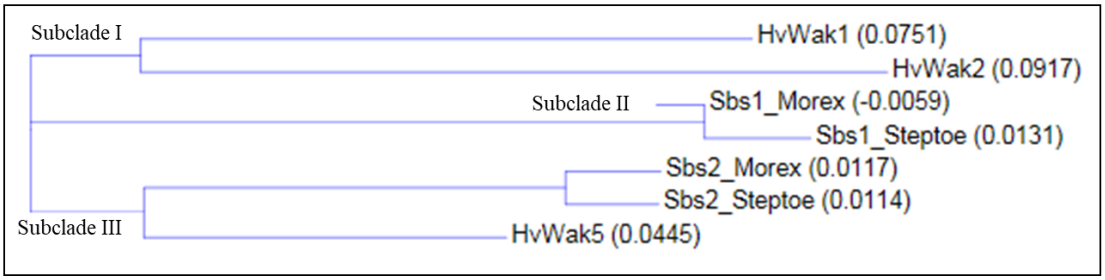
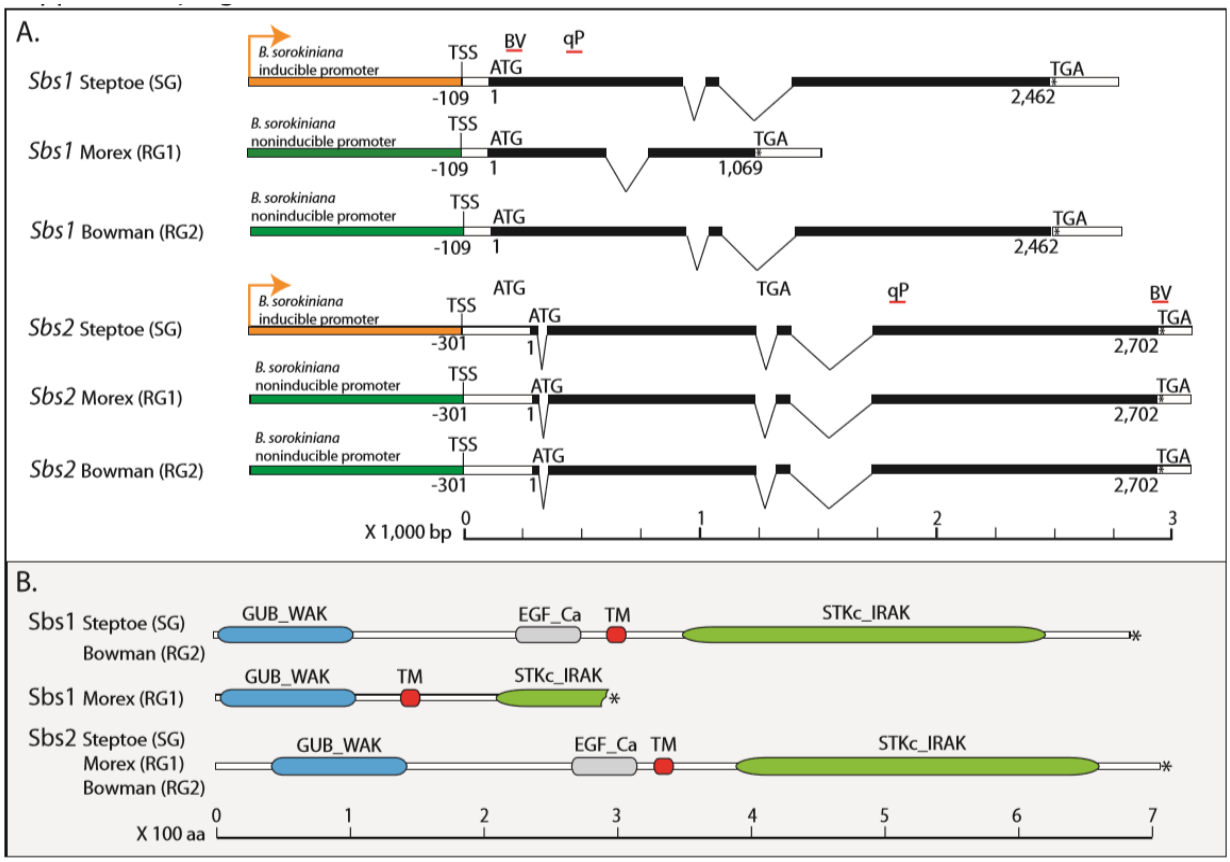


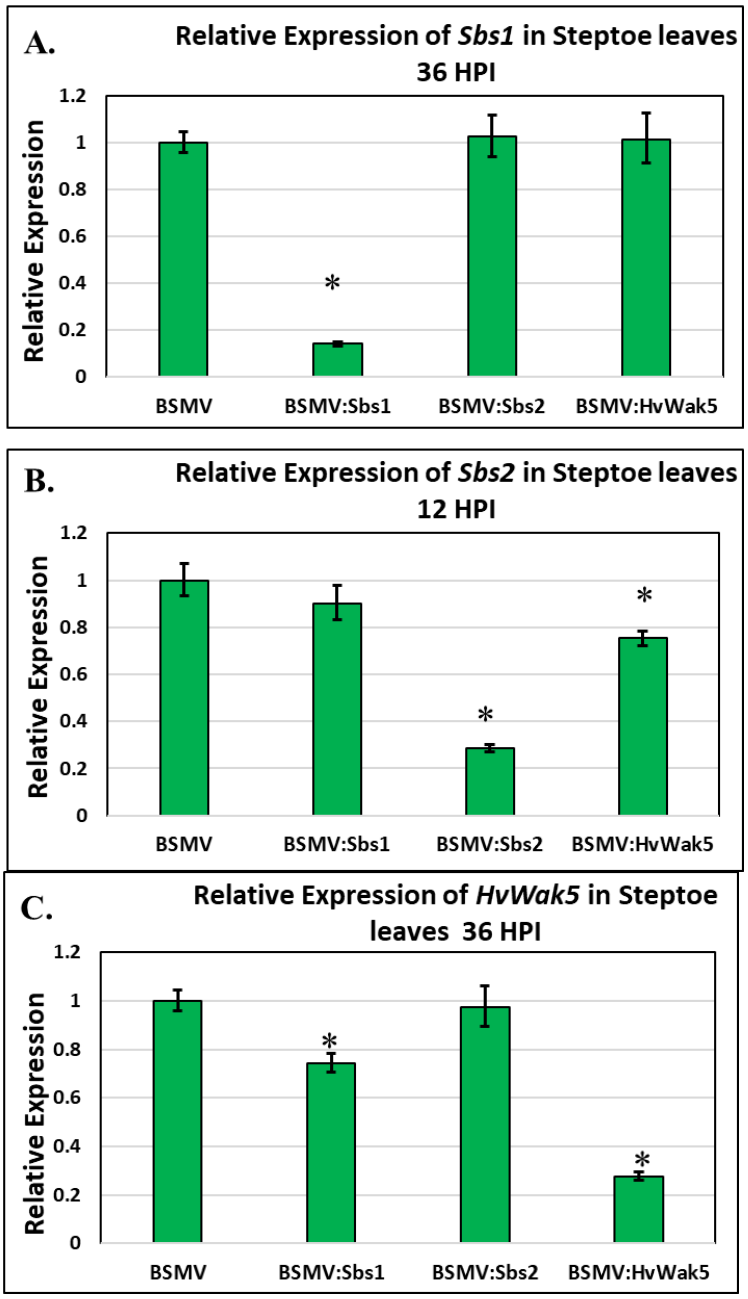
Supplementary Figure 1. Frequency distribution for the mean of the spot blotch disease response of *Bipolaris sorokiniana* isolate ND85F as observed on the 120 F_2 progeny of the Morex /Steptoe population, along with the corresponding rating scale developed by Steffenson and Fetch.



Supplementary Figure 2. Homology of the five spot blotch disease susceptibility candidates Wall-associated Kinases (WAKs) in barley**.**

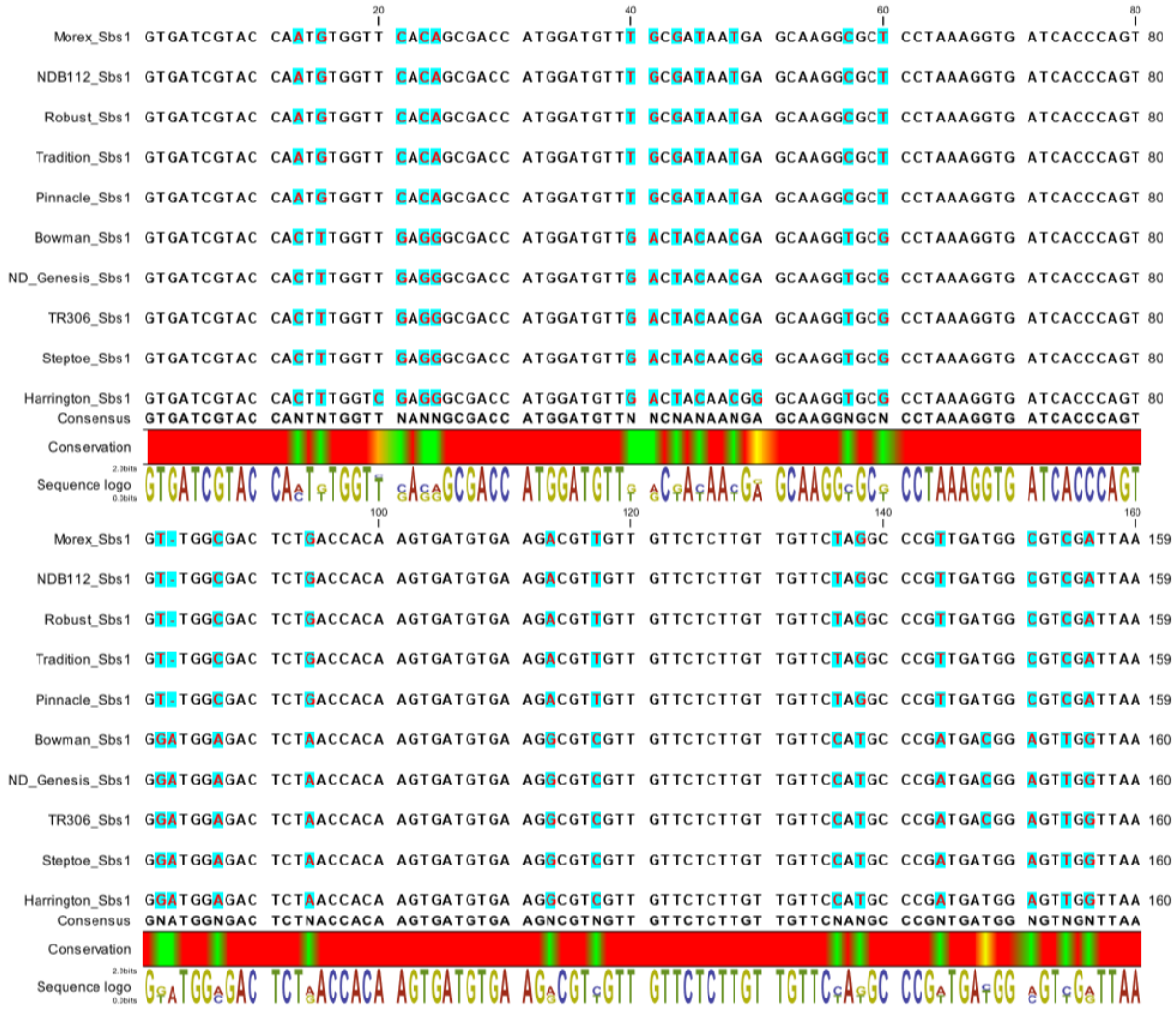


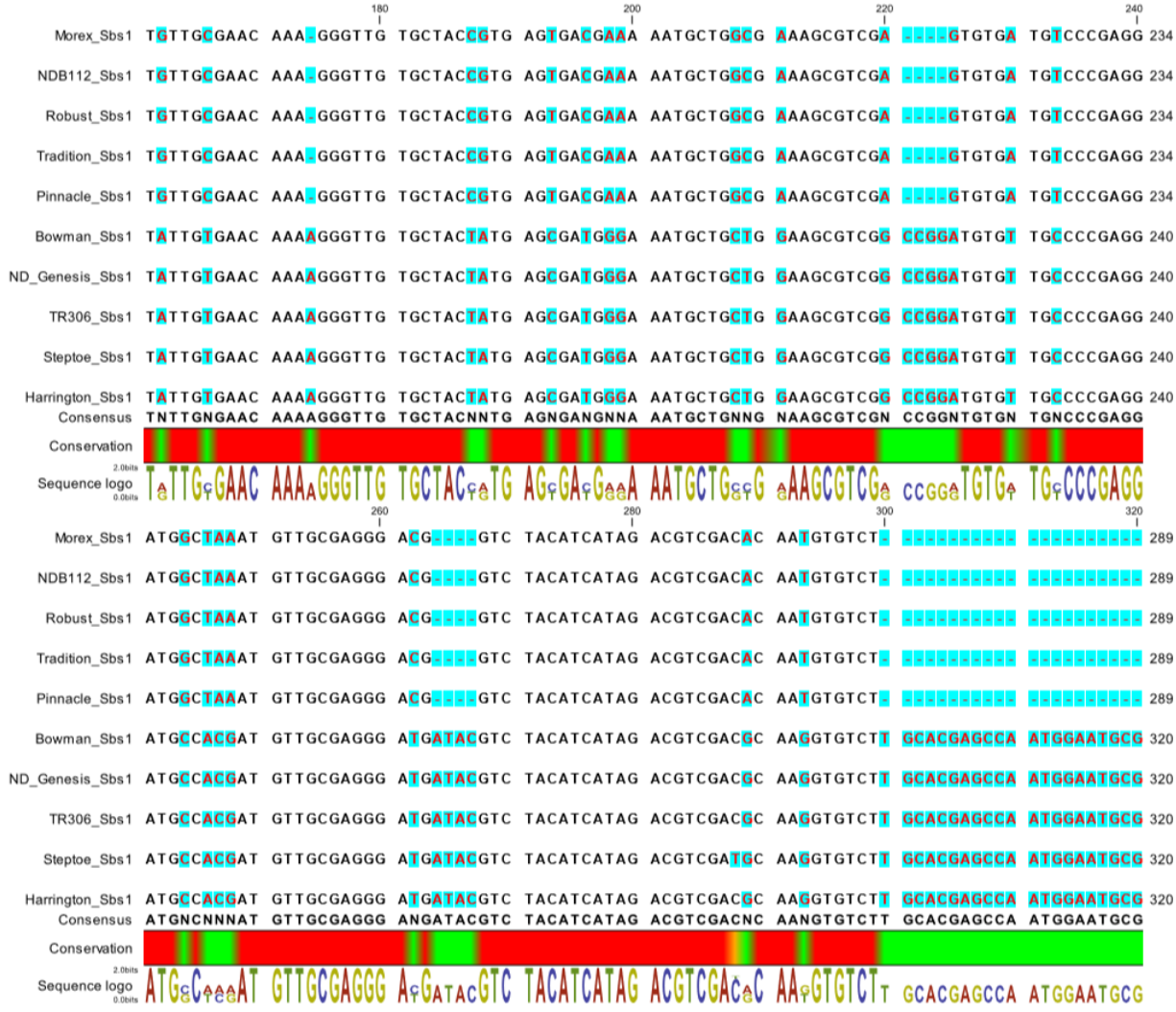
Supplementary Figure 3. The *Sbs1* and *Sbs2* predicted gene and protein structures from spot blotch susceptible and resistant barley cultivars. 3A) The genomic and cDNA gene structures for *Sbs1* and *Sbs2* with cultivar (cv) Steptoe representing the susceptibility group (SG), and resistant cvs Morex and Bowman representing resistant group 1 (RG1) and resistant group 2 (RG2), respectively. The genomic and predicted mRNA structures are represented to scale with exon (black bars), intron (black Vs), UTR (white bars), inducible promoter (yellow bars), noninducible promoter (green bars) transcription start site (TSS), start codon (ATG), and stop codon (TGA). The position of the TSS, start codon and stop codon are provided reletive to the start codon. The positions of the BSMV-VIGS and qPCR amplicons are represented as red bars labeled BV and qP, respectively. 3B) The Sbs1 and Sbs2 protein structures are shown for cvs Steptoe (SG), Morex (RG1)and Bowman (RG2). The white bar represents the full-length predicted proteins with blue, grey, red and green bar represents the GUB (galacturonic acid binding), EGF_Ca (epidermal growth factor –Calcium binding), TM (transmembrane) and STKc_IRAK (Serine/Threonine kinases, Interleukin-1 Receptor Associated Kinases) domains, respectively. The Sbs1 in Steptoe and Bowman encodes a full length protein, however Sbs1 in Morex encodes a protein with missing EGF_Ca domain and a truncated STPKc_IRAK. The Sbs2 in all three cultivars encode a full length wall-associated kinase protein.



Supplementary Figure 4. Transcript analysis of 4A) *Sbs1*, 4B) *Sbs2* and 4C) *HvWak5* was analyzed at 36, 12 and 36 hours post inoculation (hpi), respectively, during the infection process of *Bipolaris sorokiniana* in the BSMV:*Sbs1*, BSMV:*Sbs2* and BSMV:*HvWak5* silenced plants of cultivar Steptoe along with the BSMV-VIGS control (n=3). The reference gene *HvSnoR14* expression in each sample at each time point was used to normalize the transcripts (X-axis). Error bars depict SEM±1(n=3). BSMV-VIGS control was used as control sample for relative expression analysis (Y-axis).

Supplementary Figure 5A.

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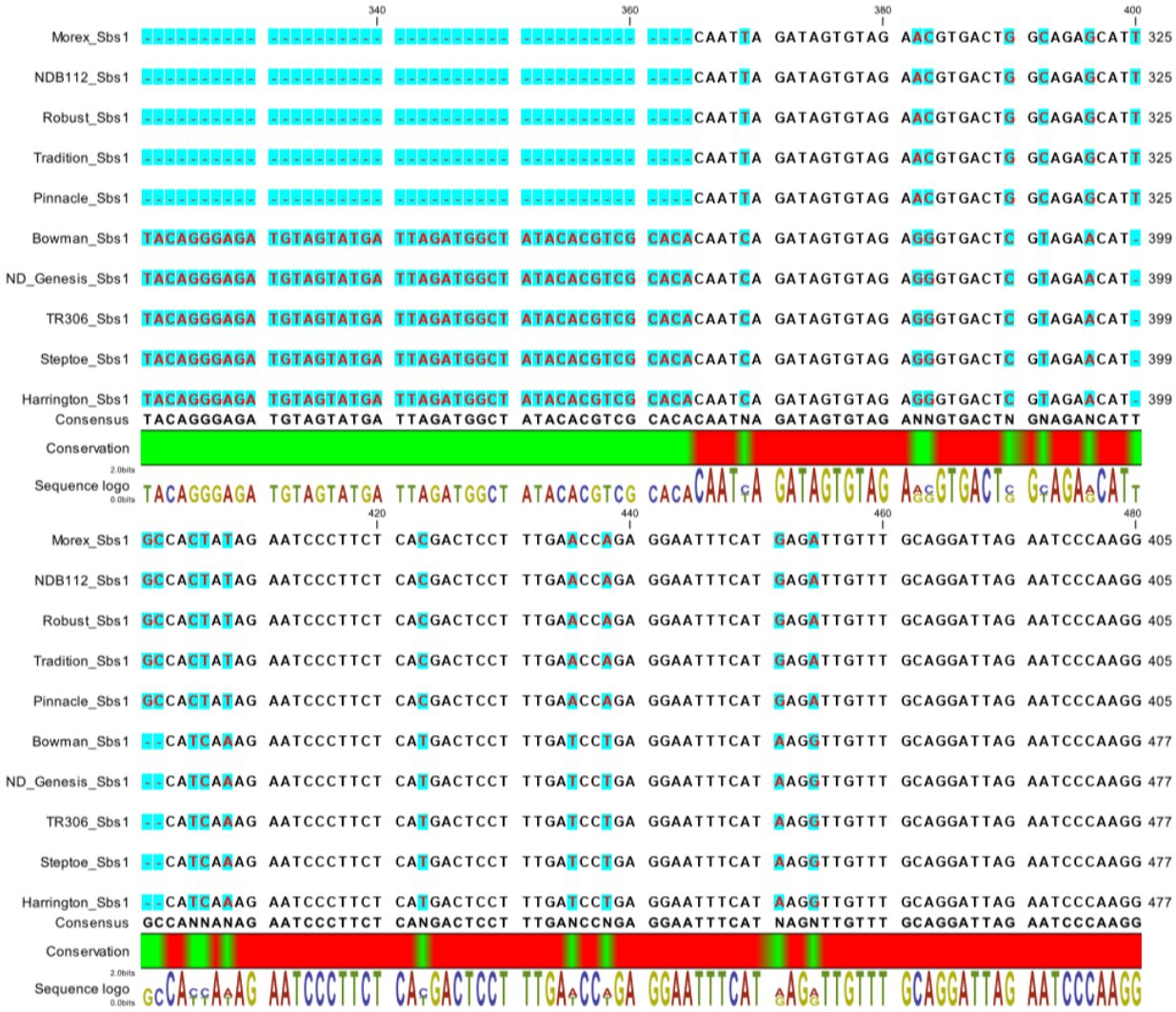
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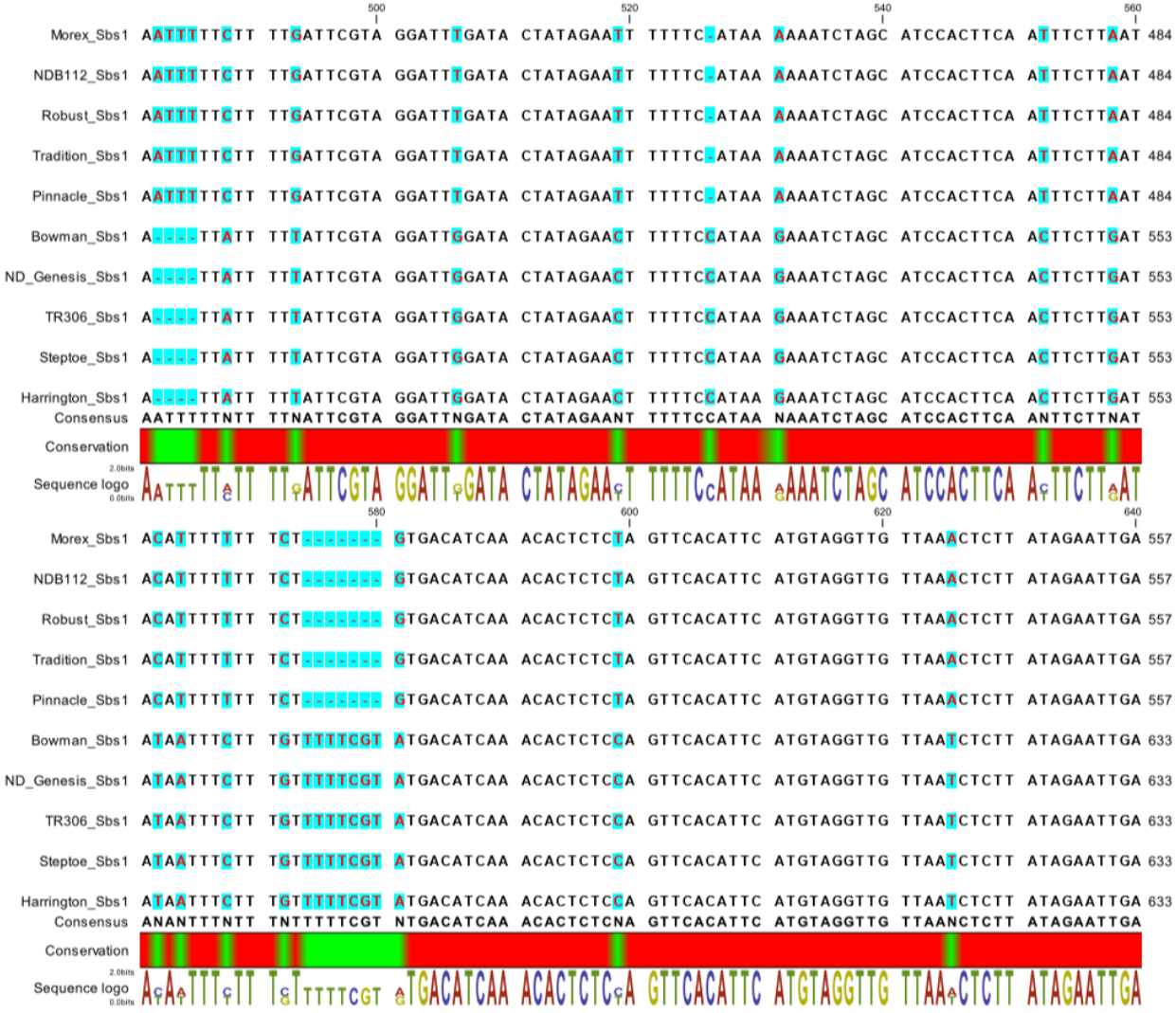
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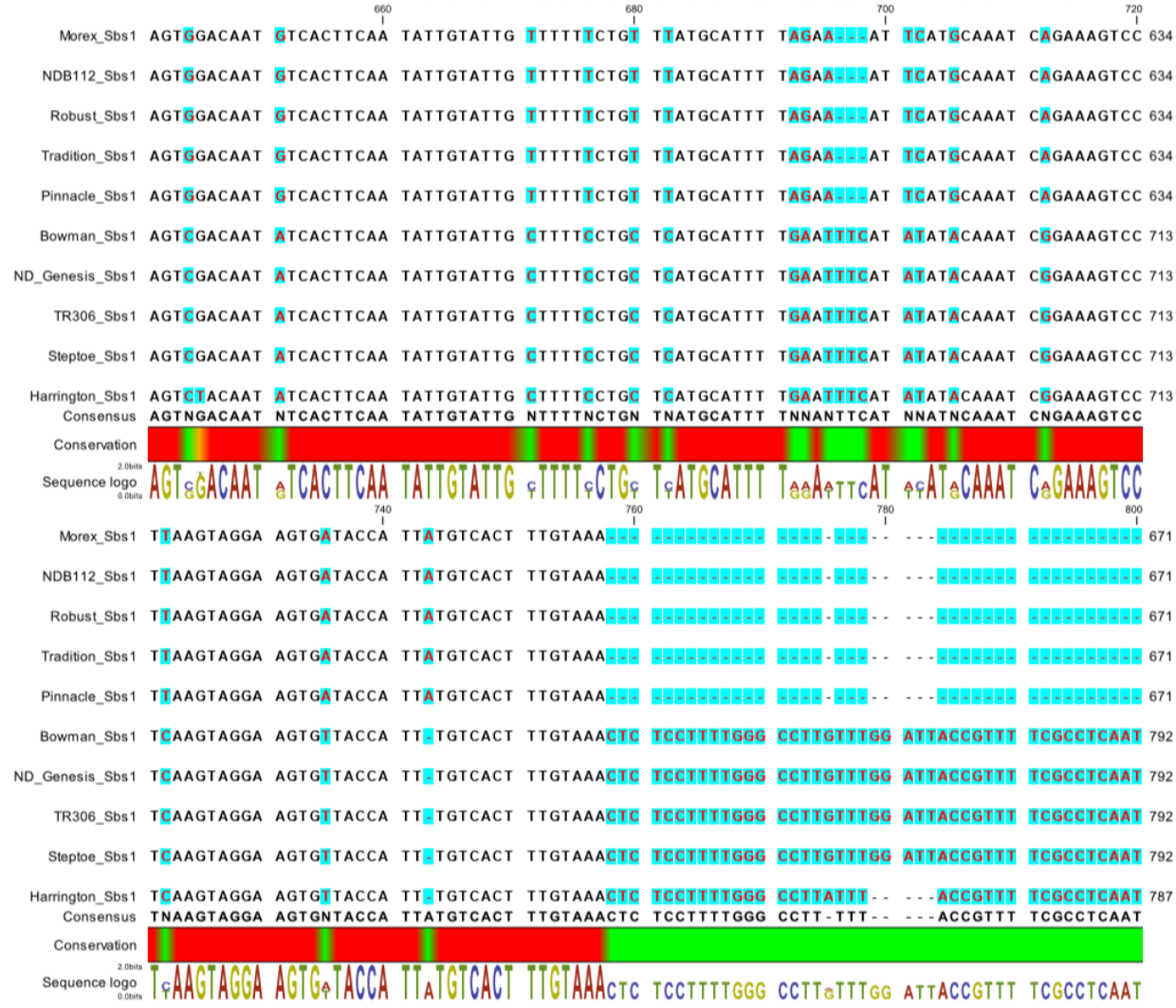
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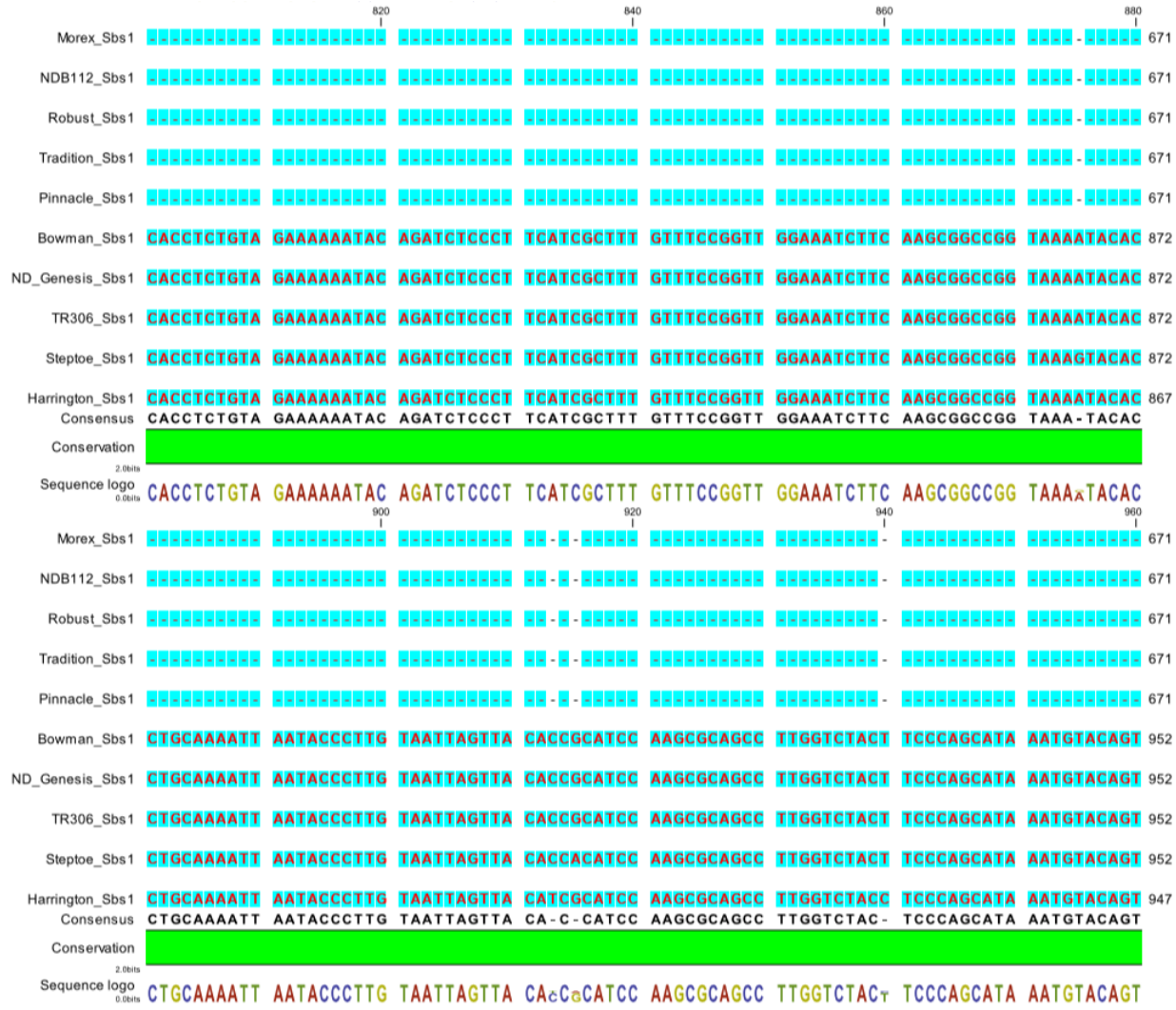
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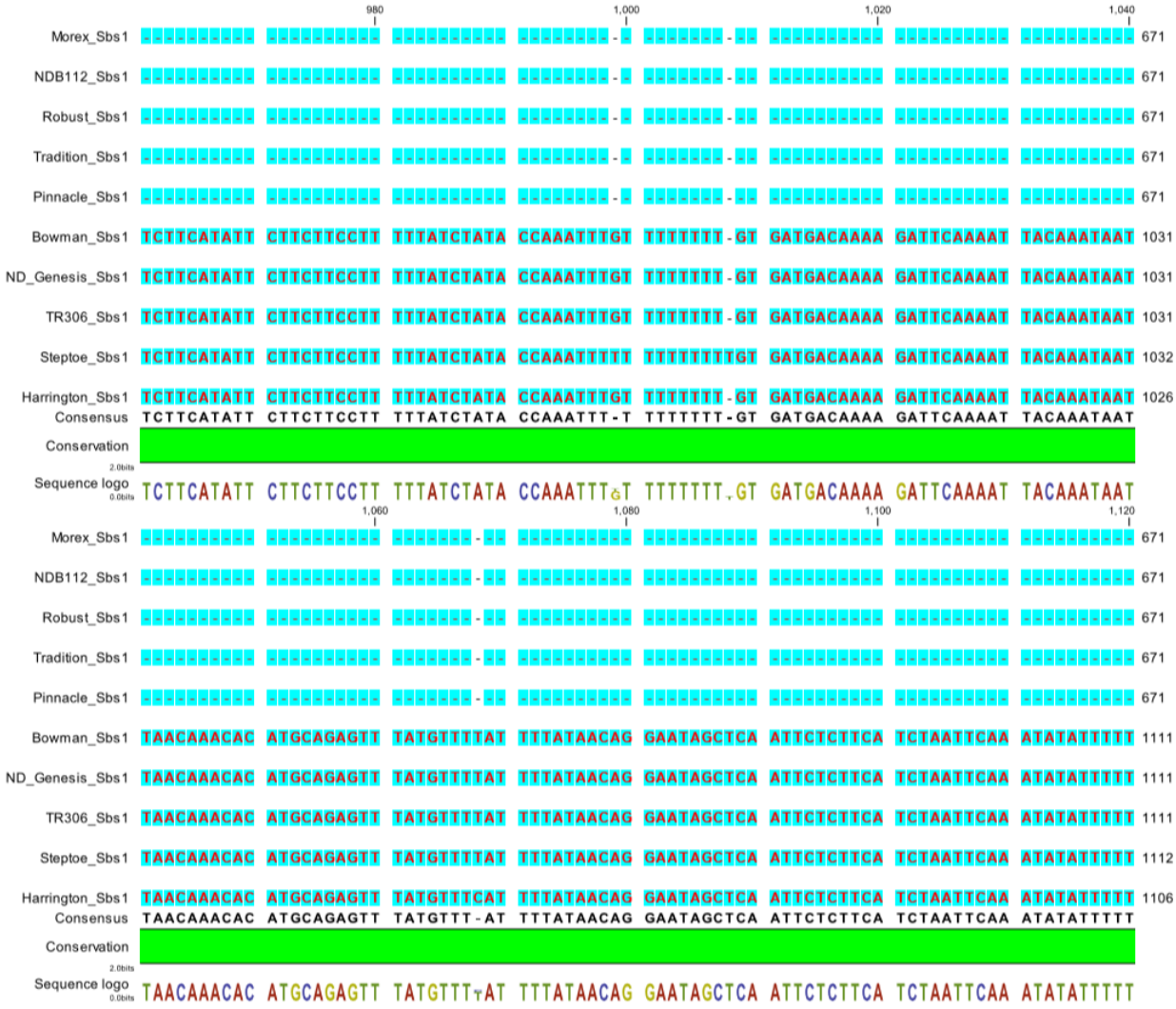
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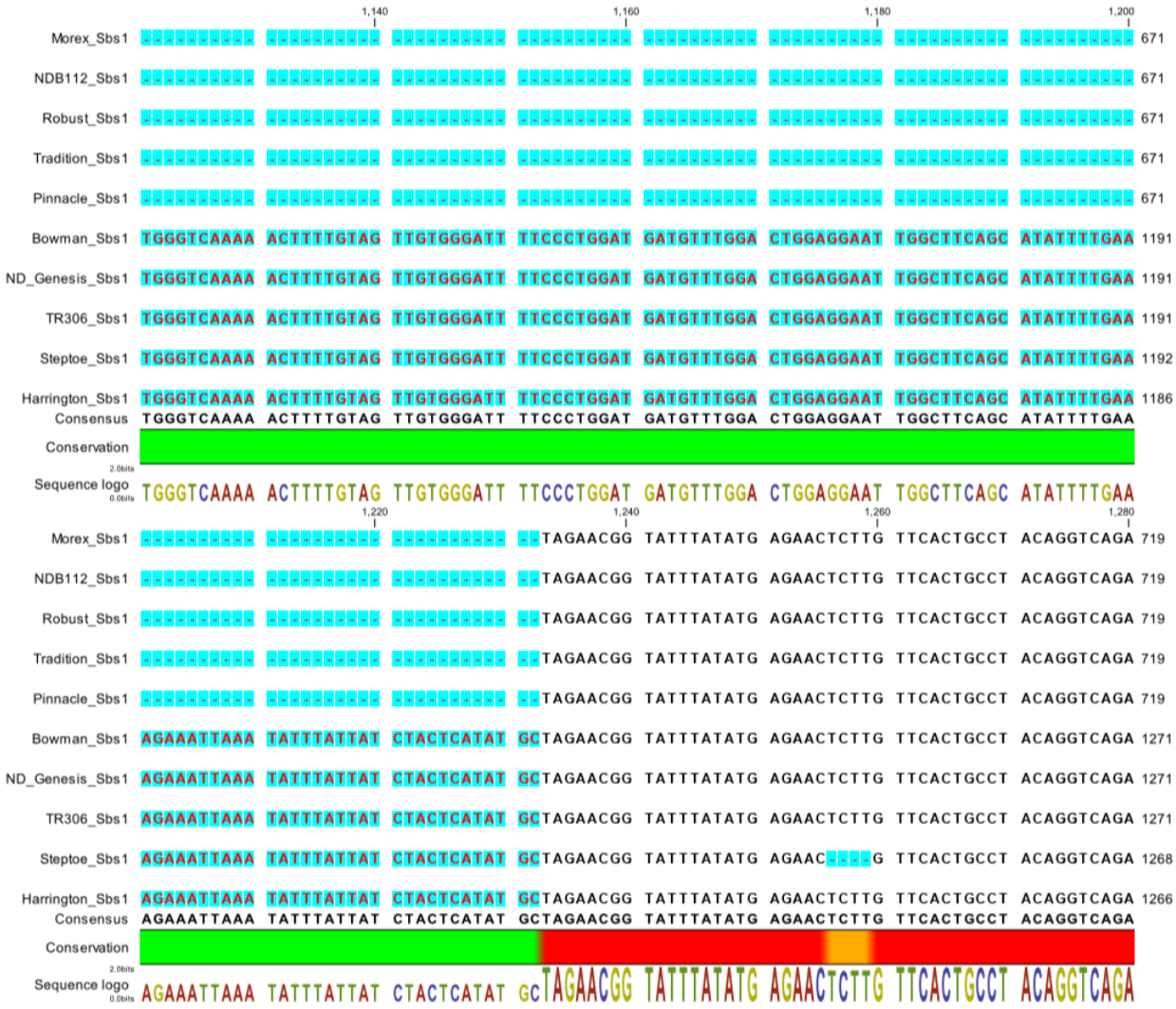
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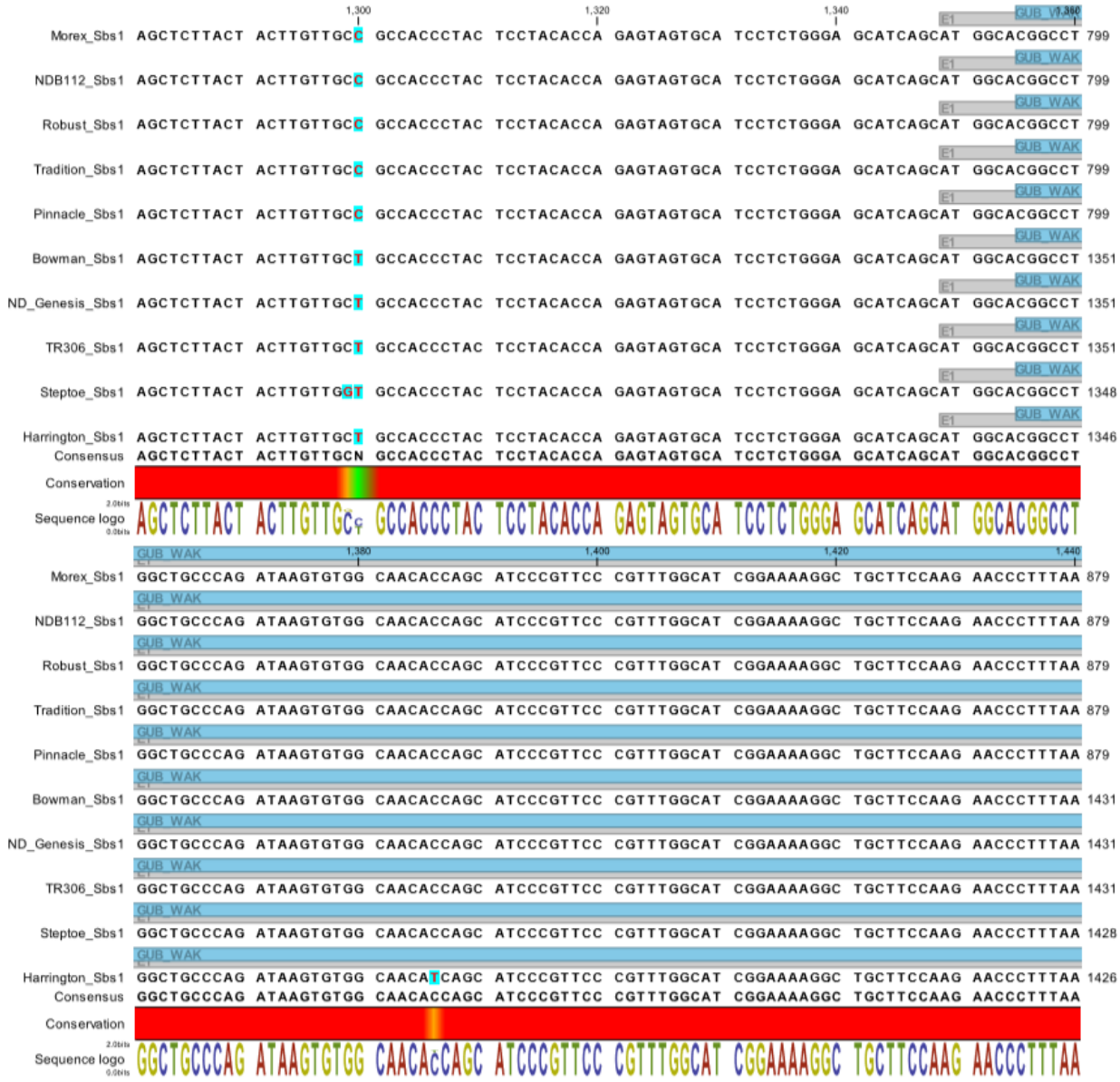
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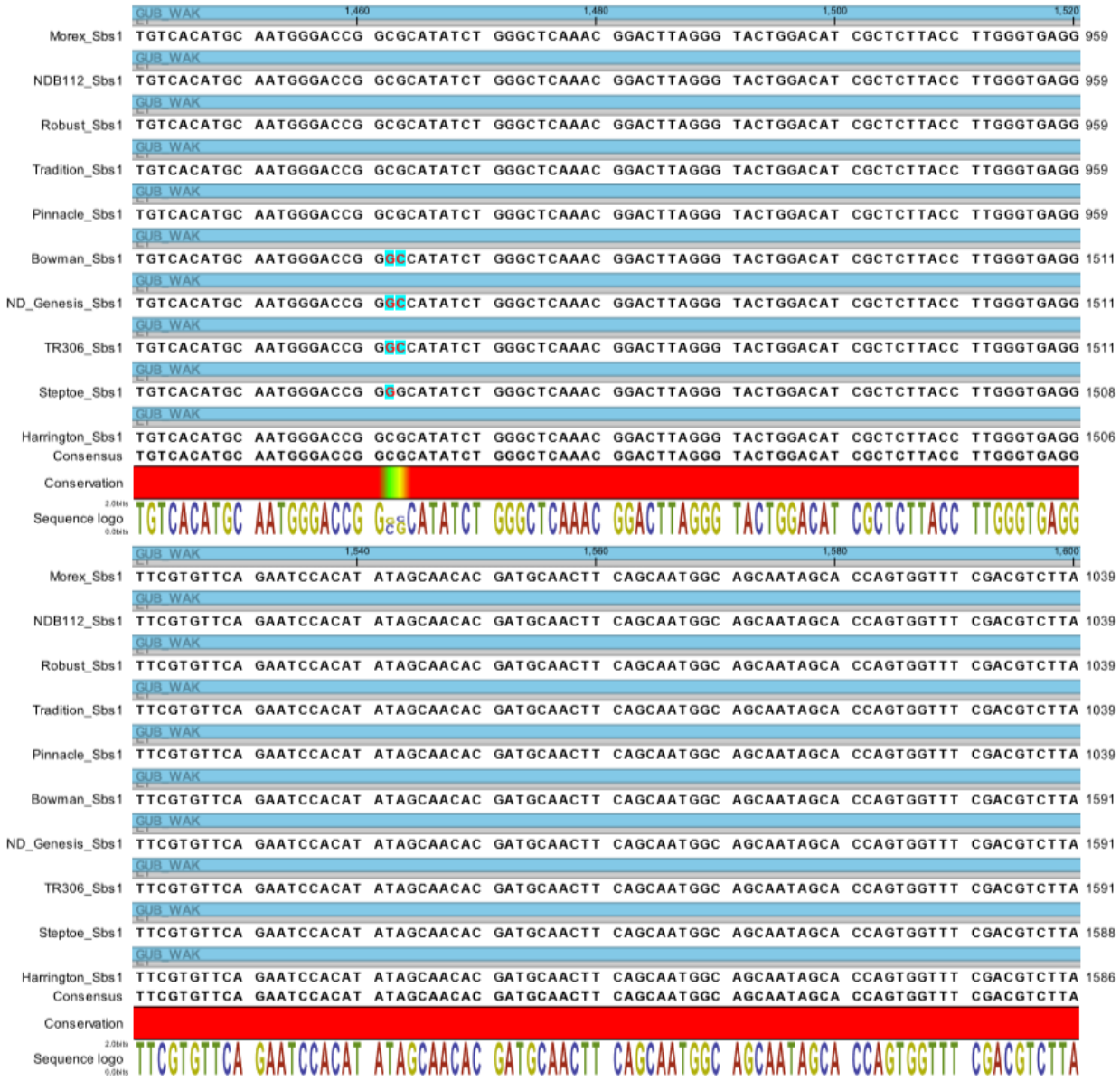
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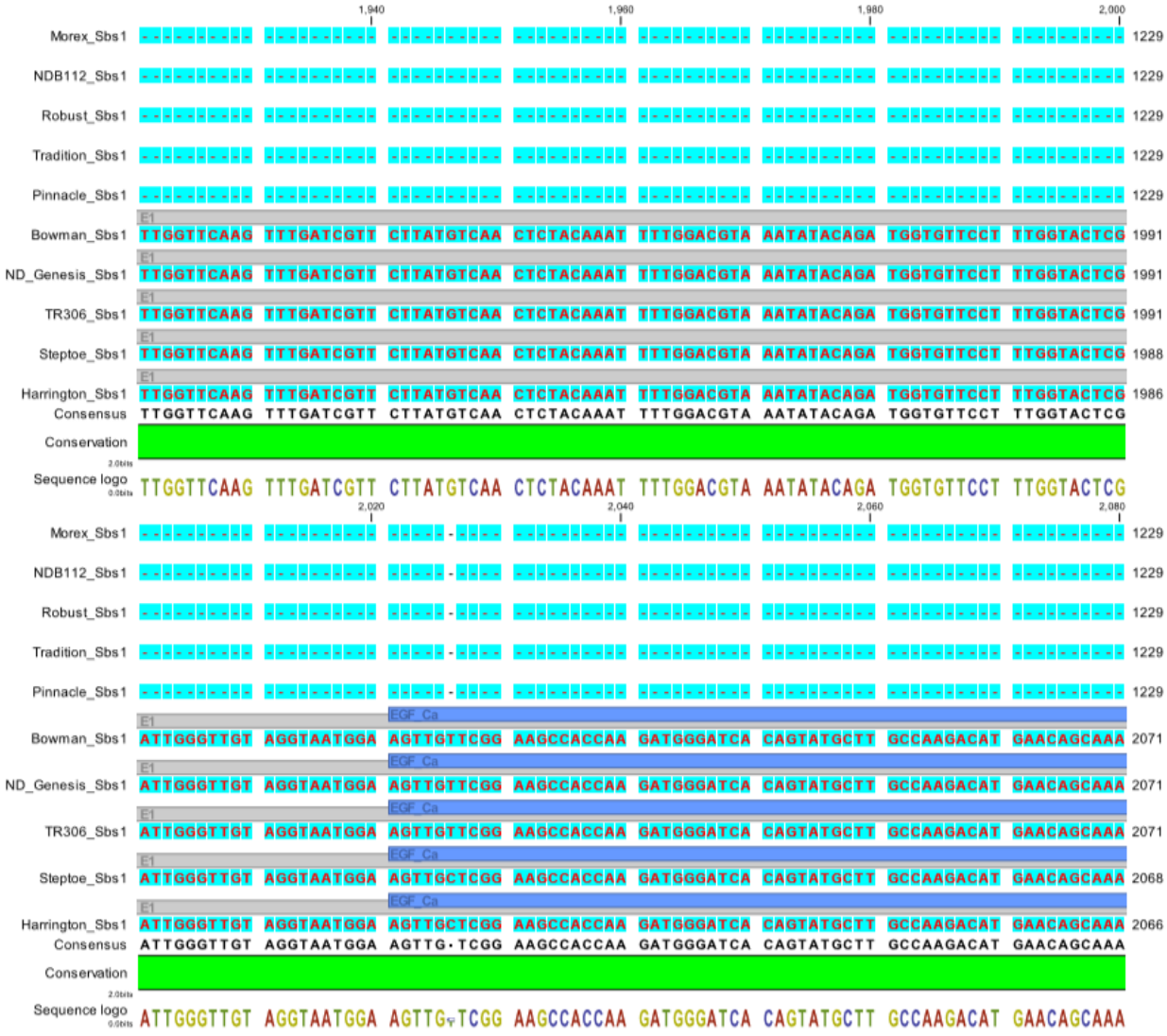
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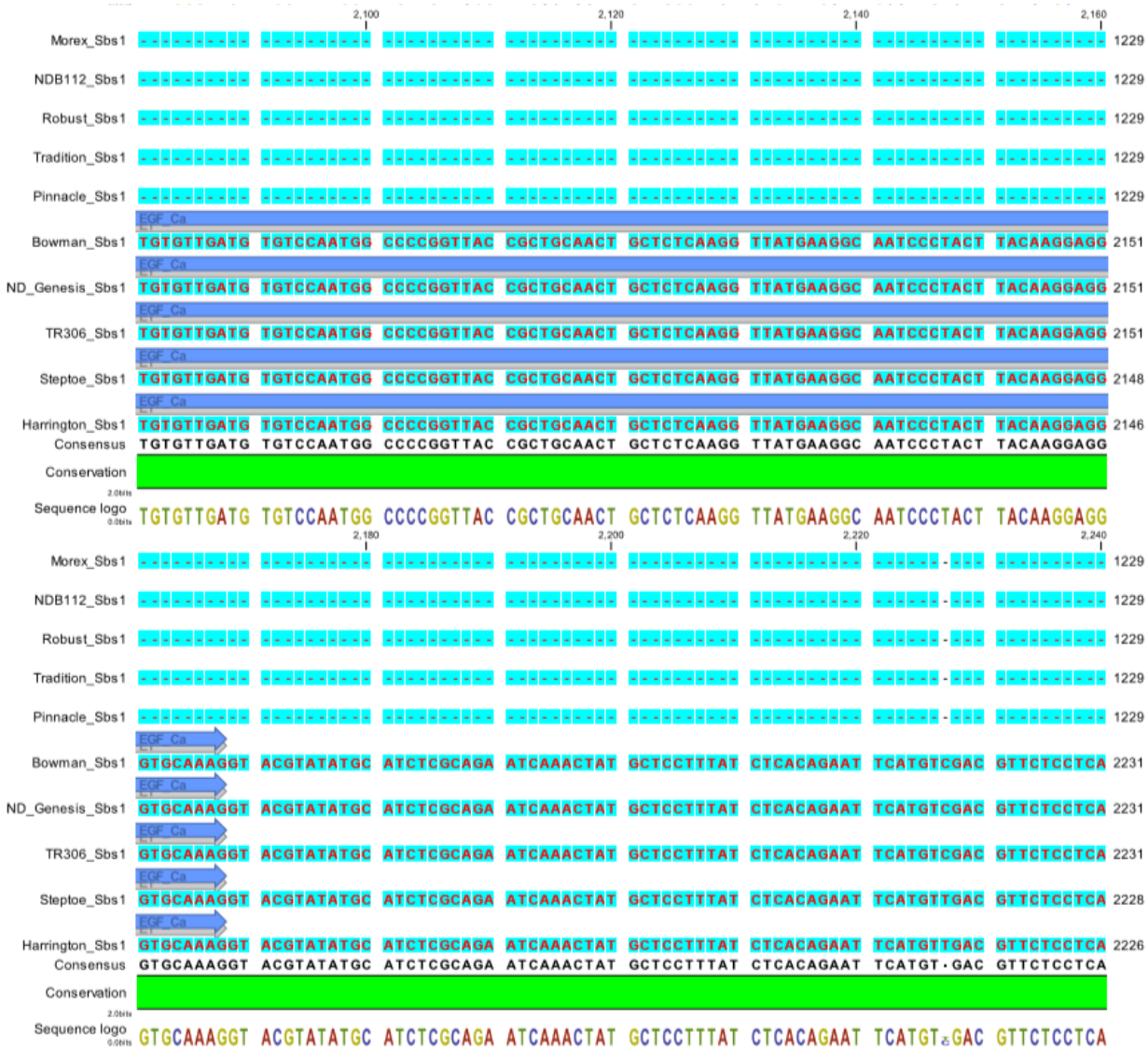
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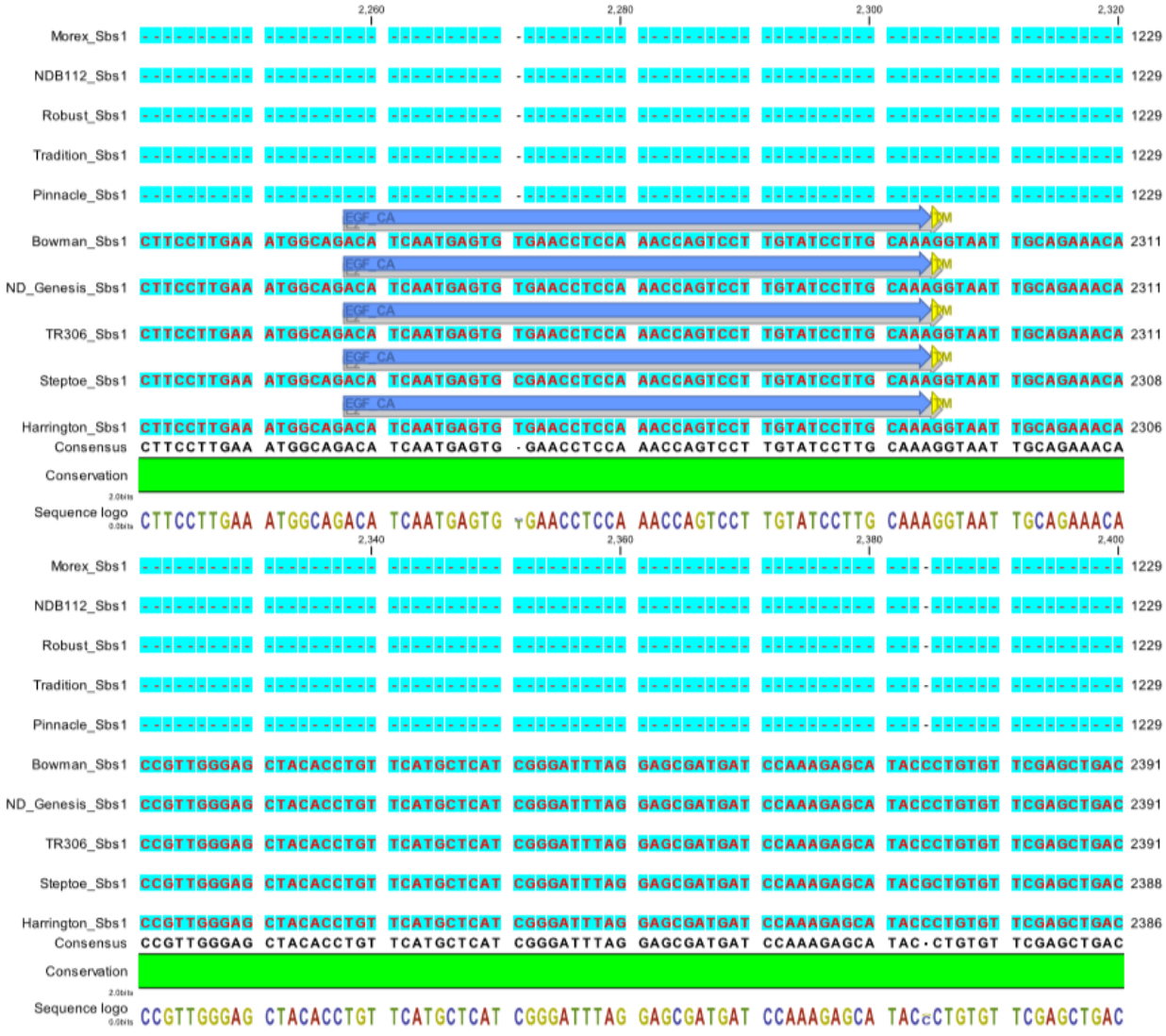
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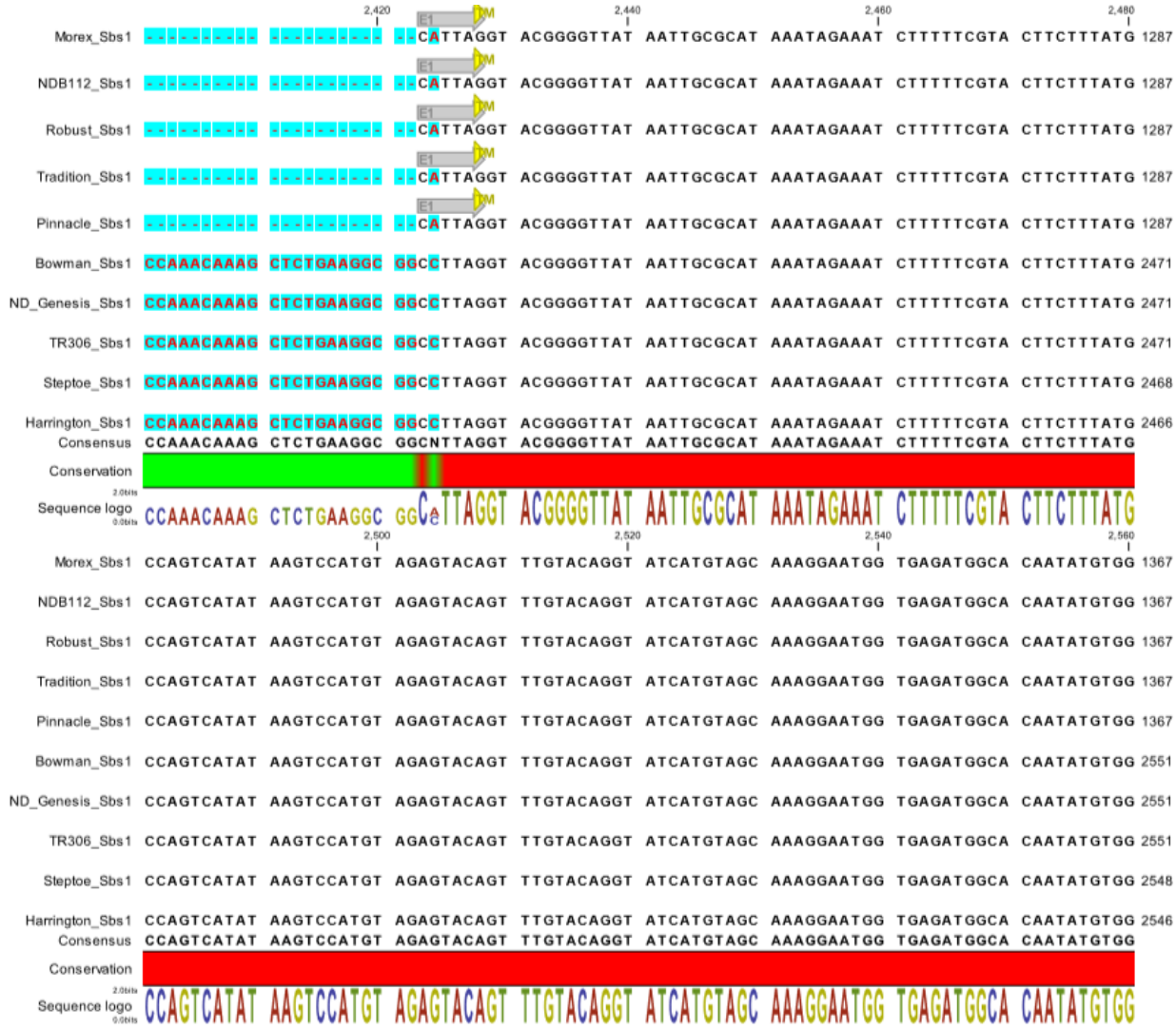
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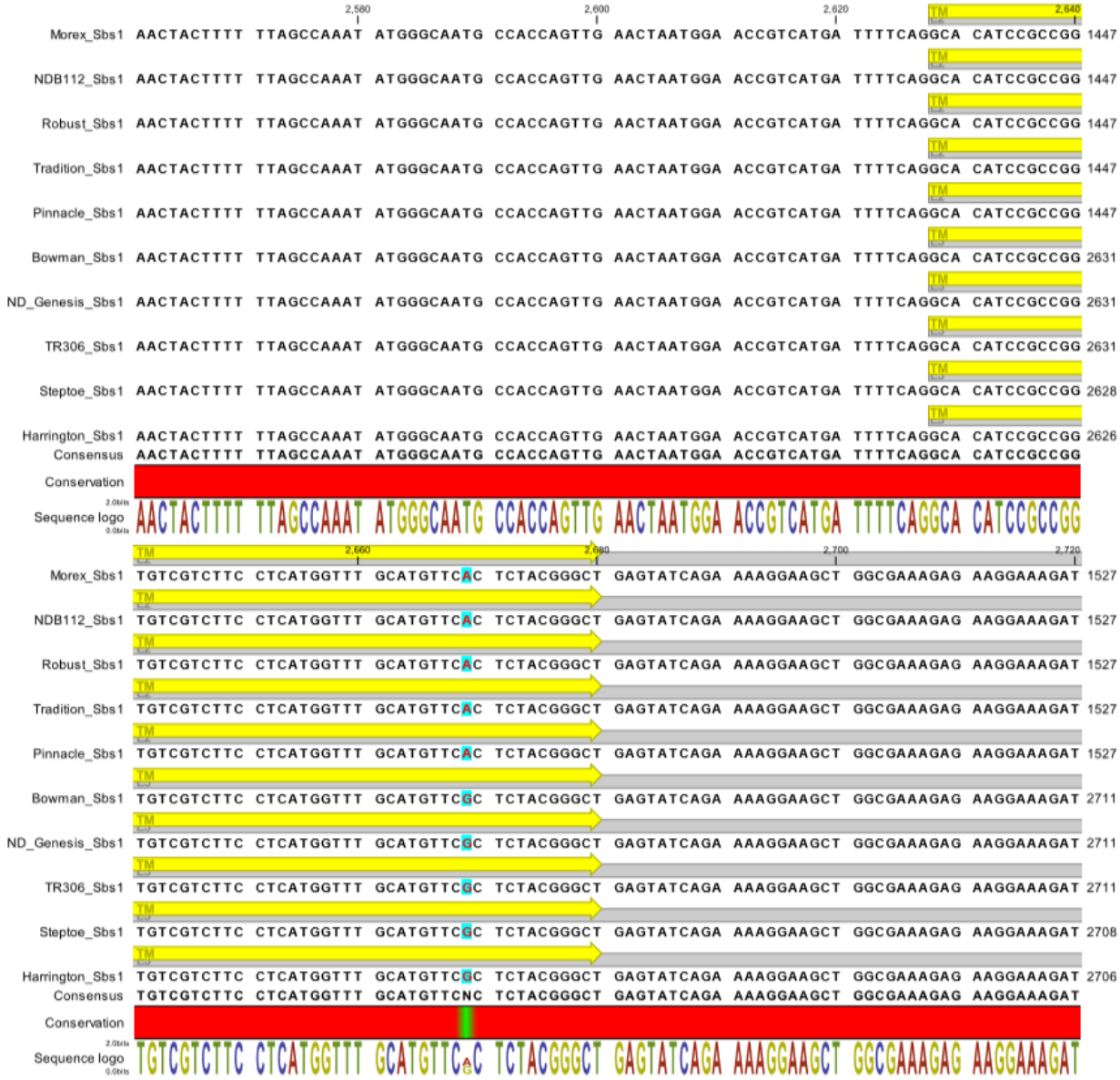
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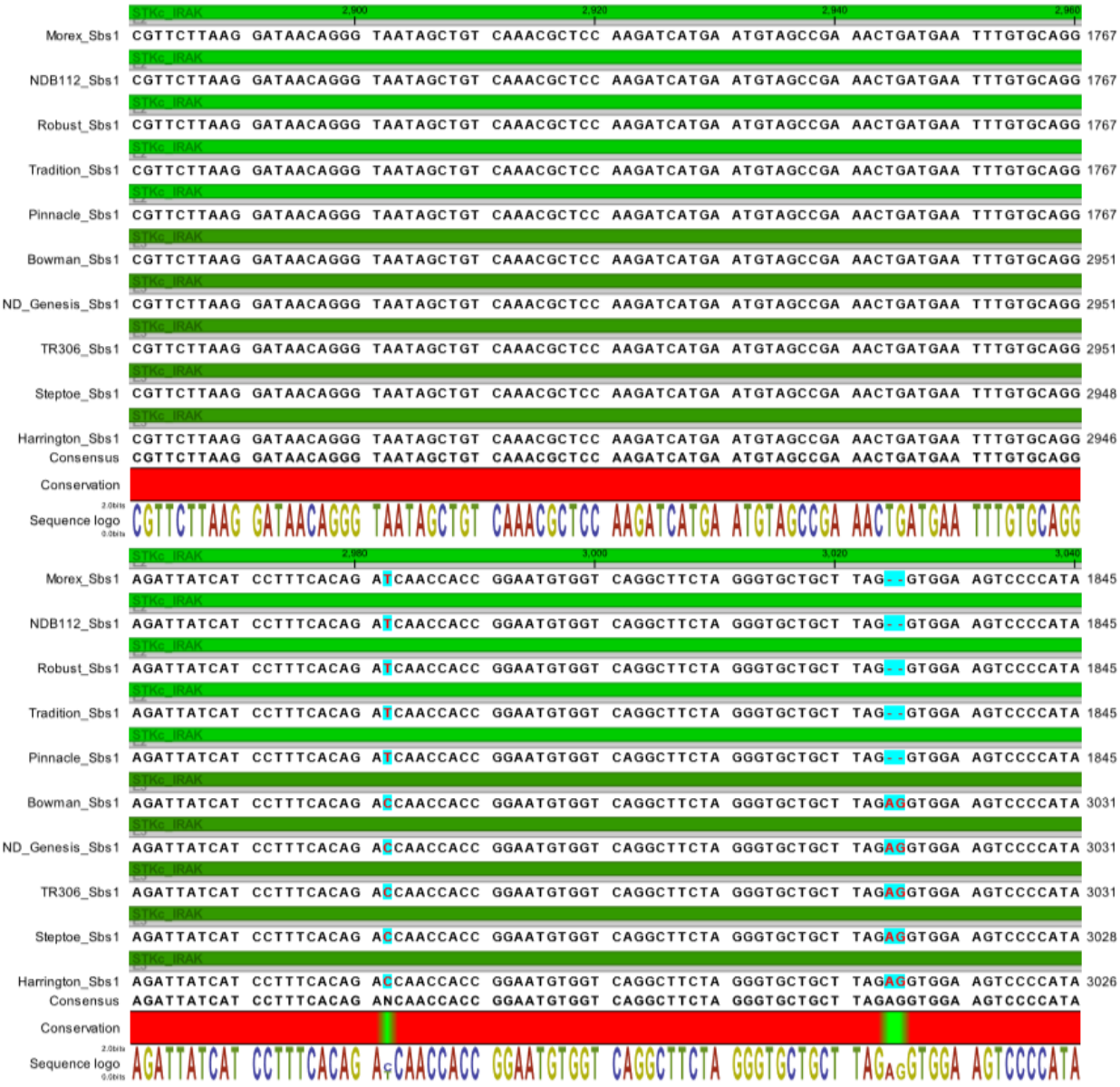
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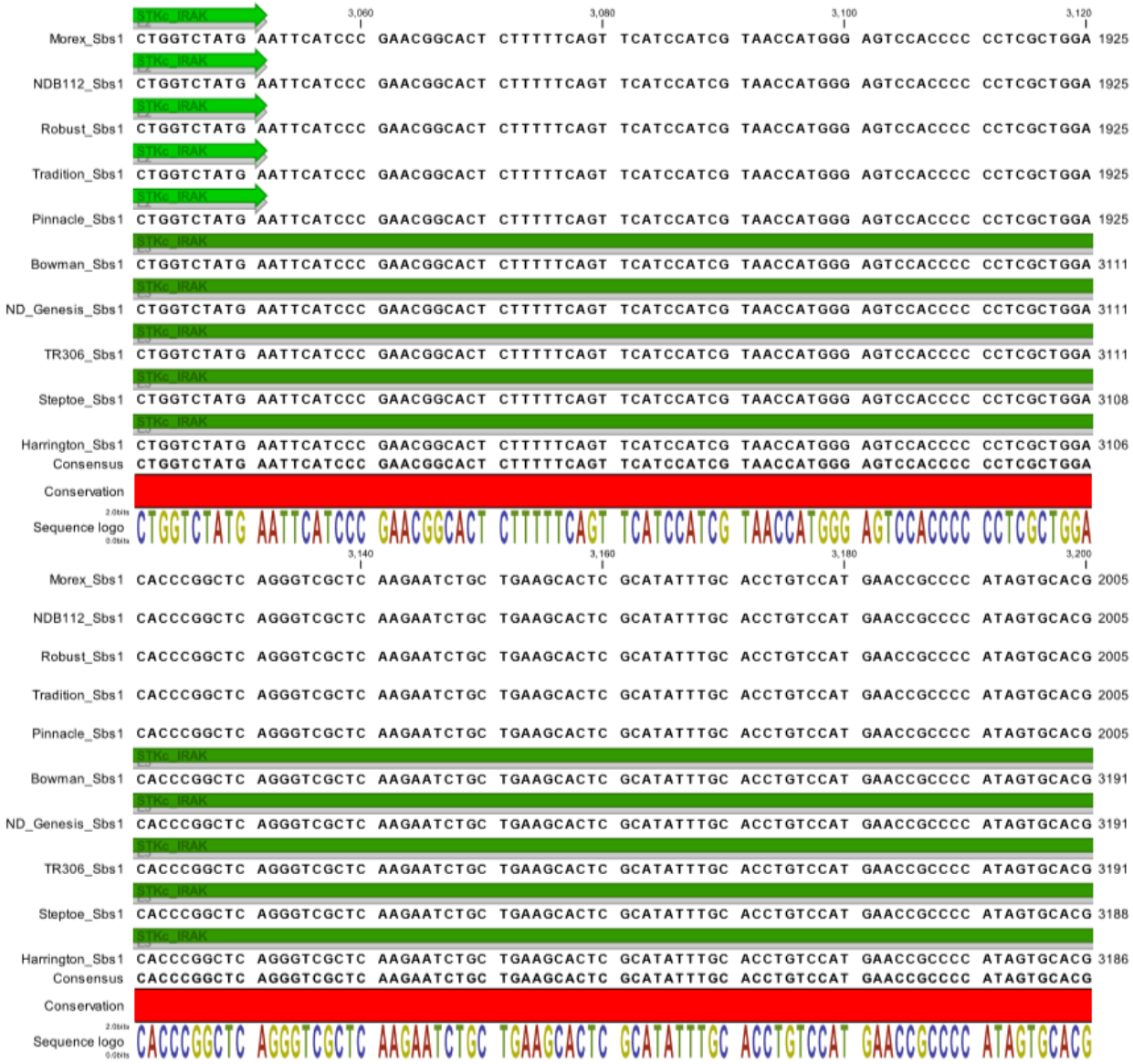
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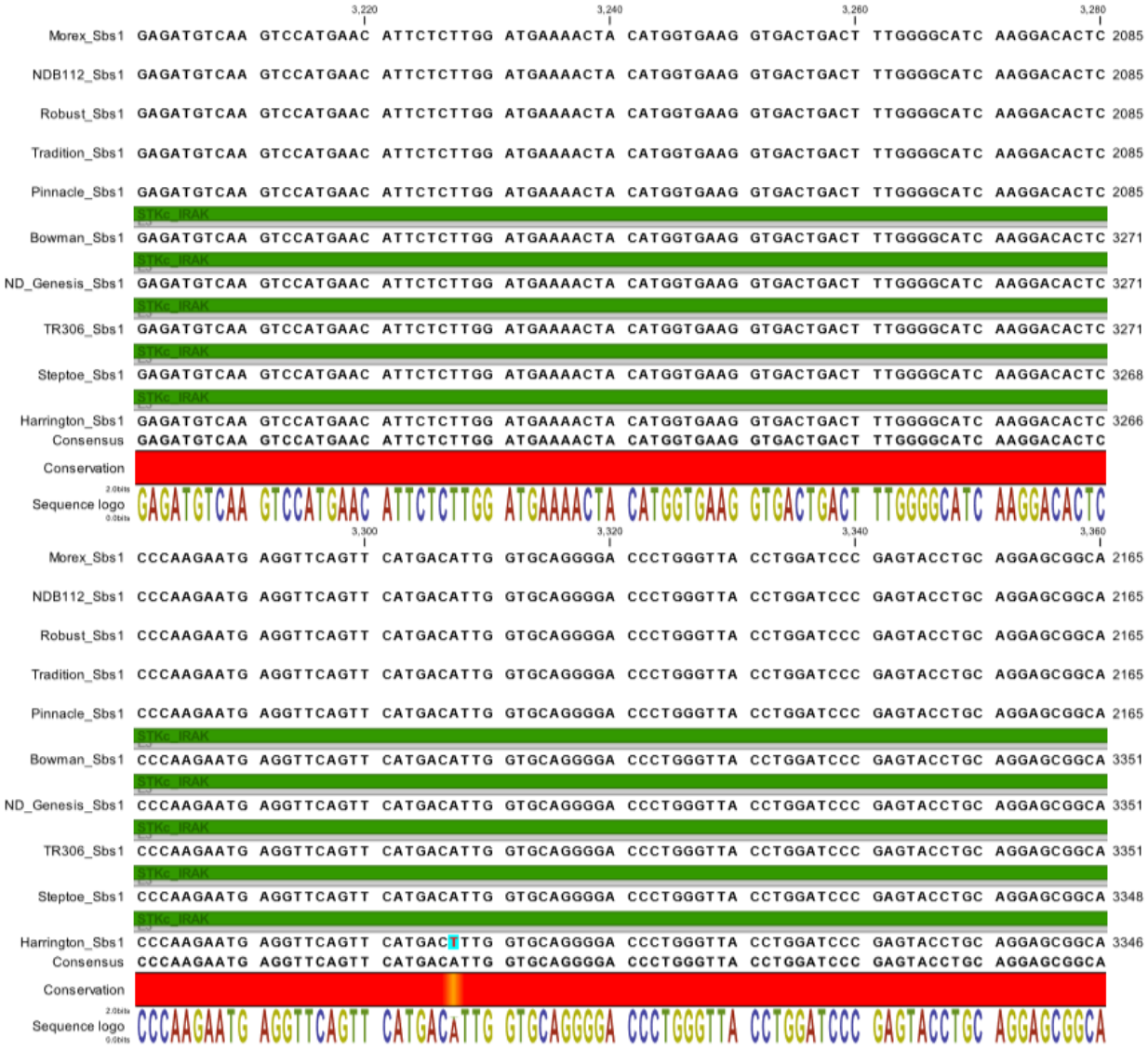
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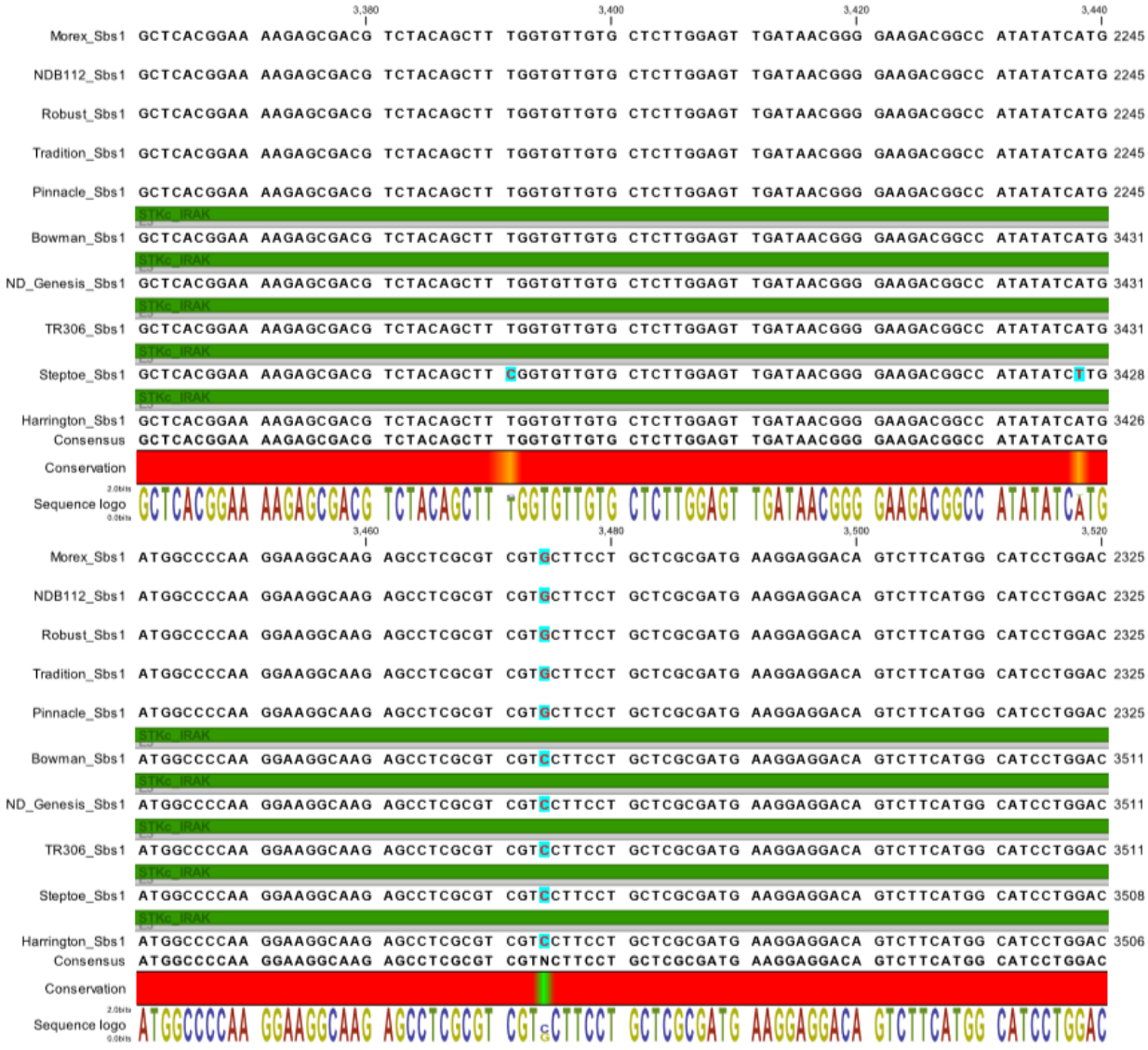
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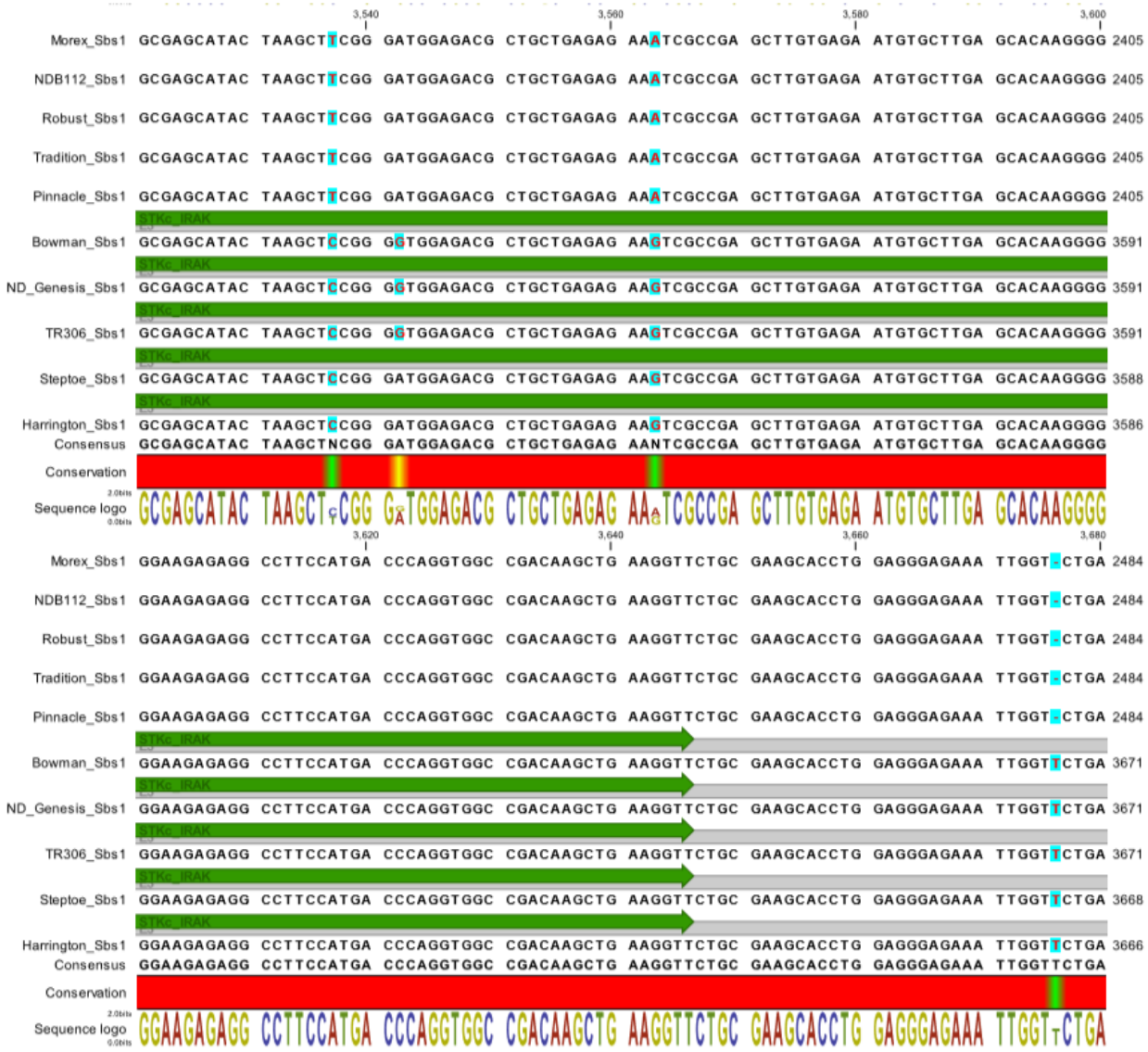
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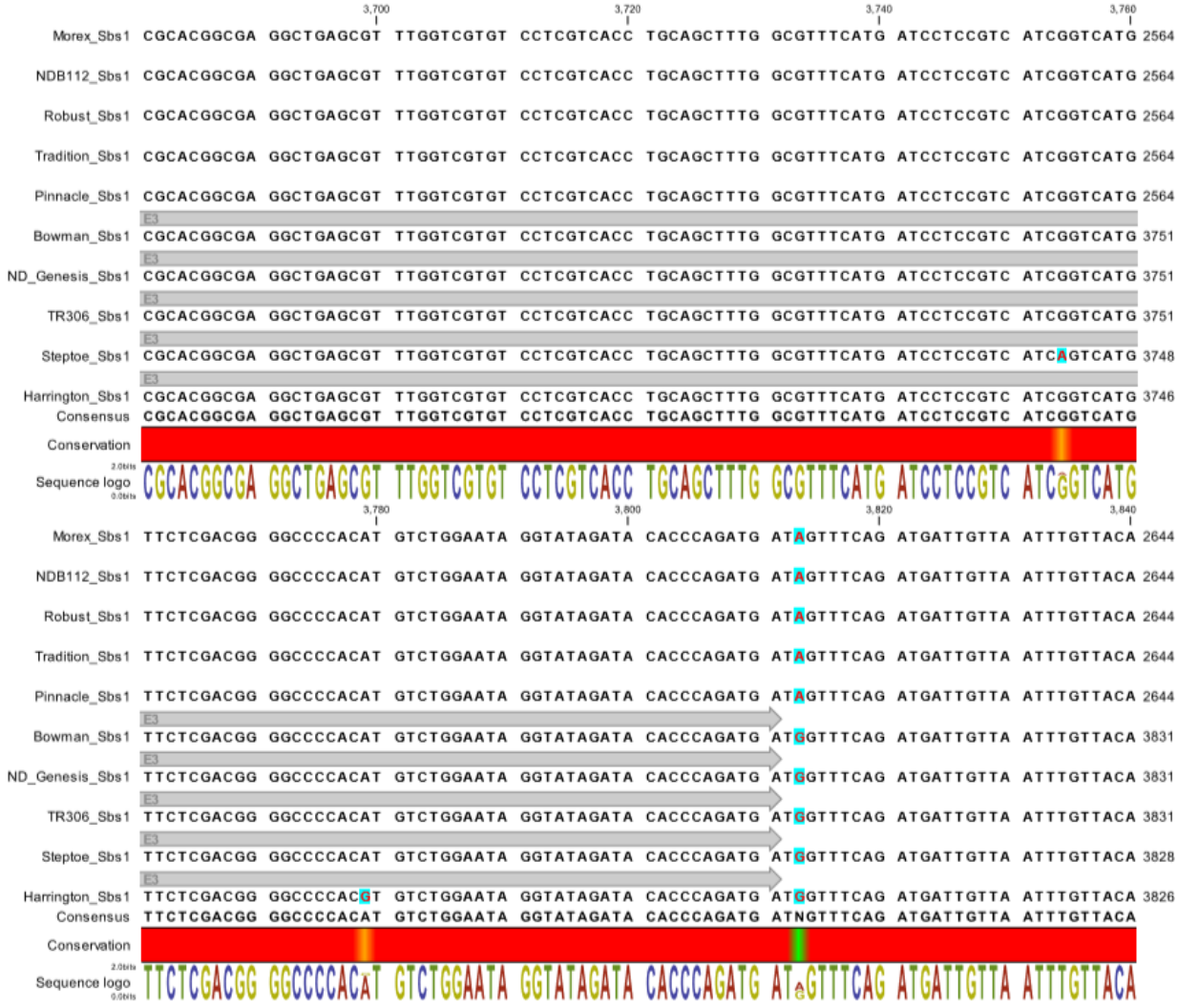
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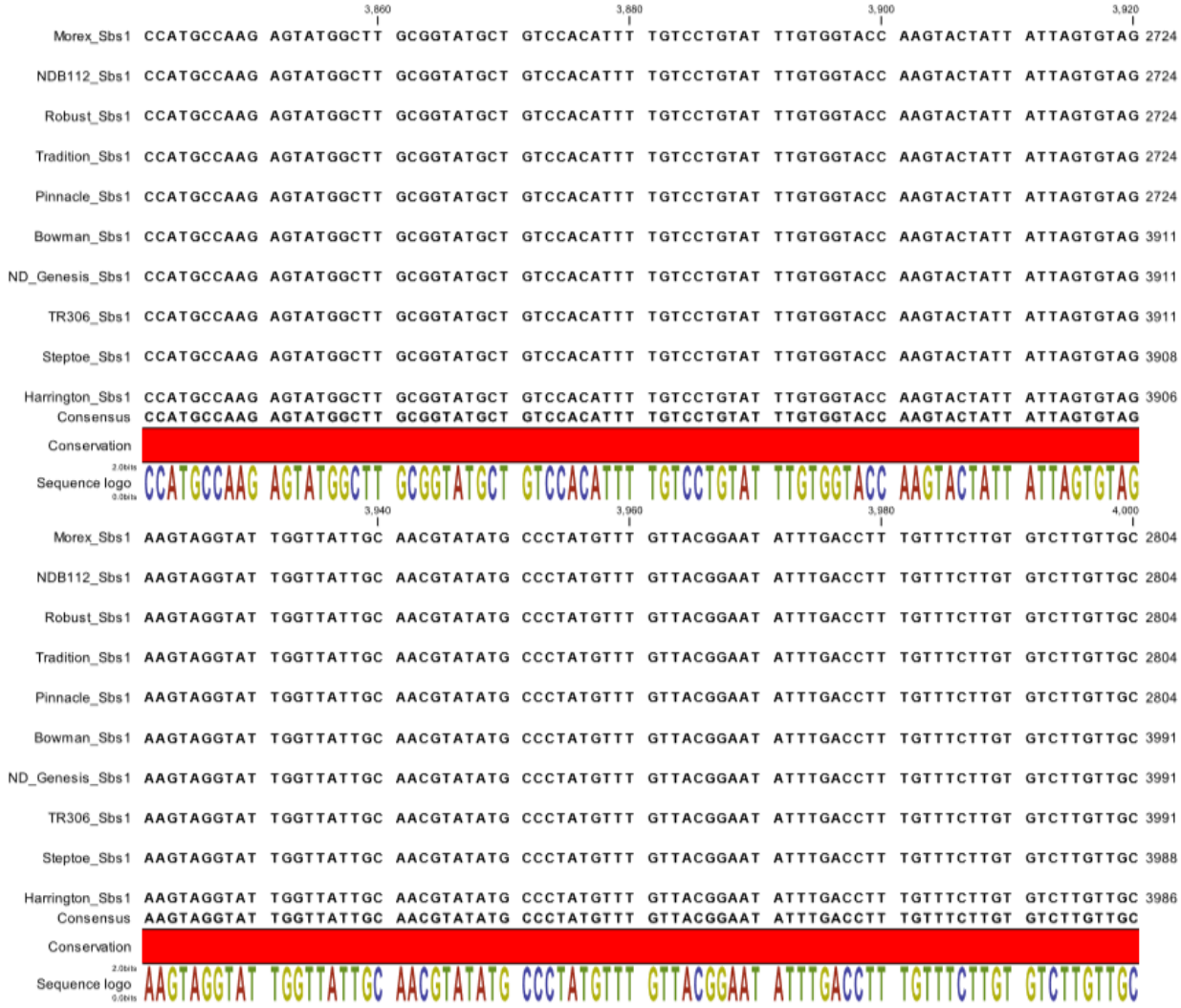
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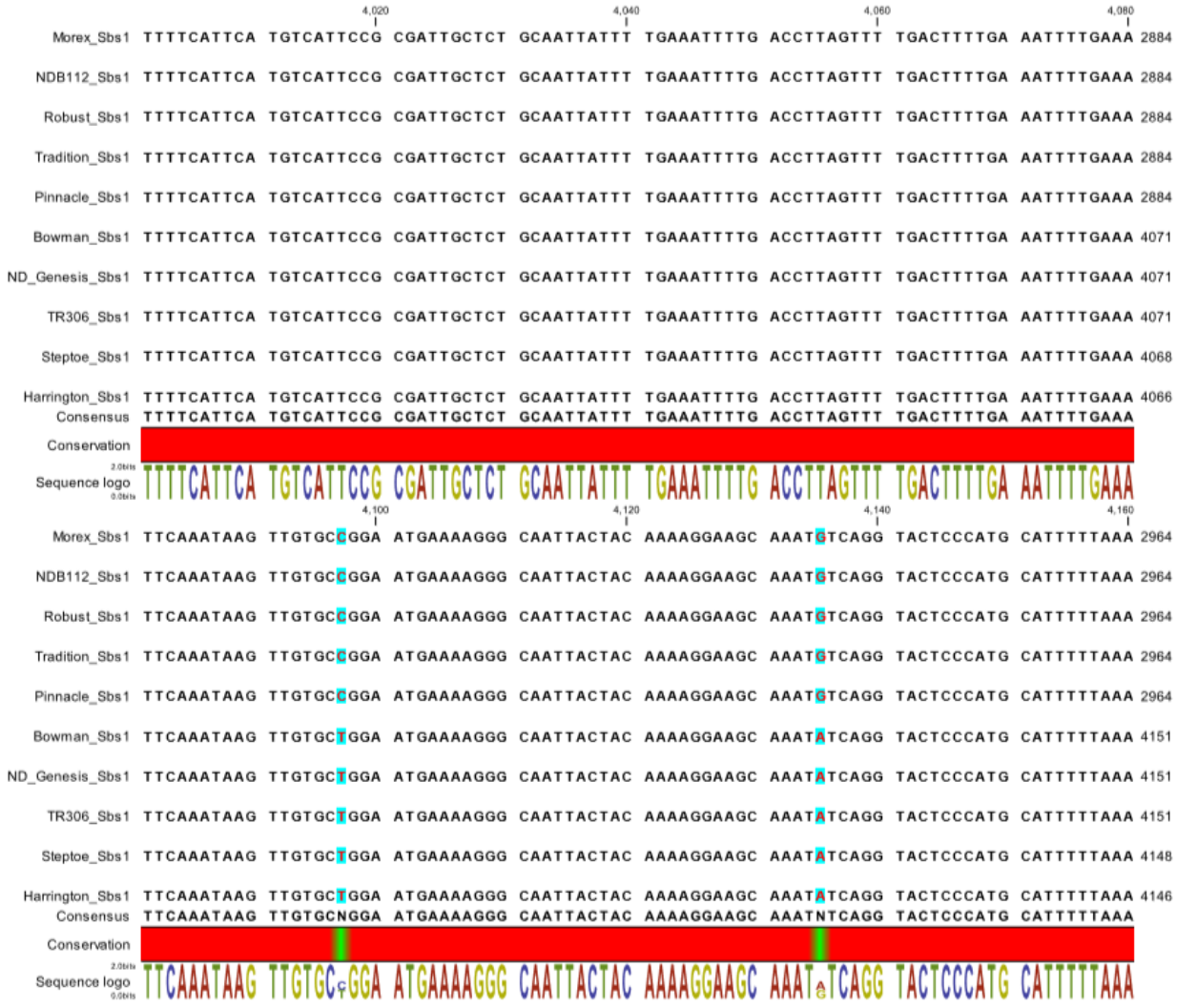
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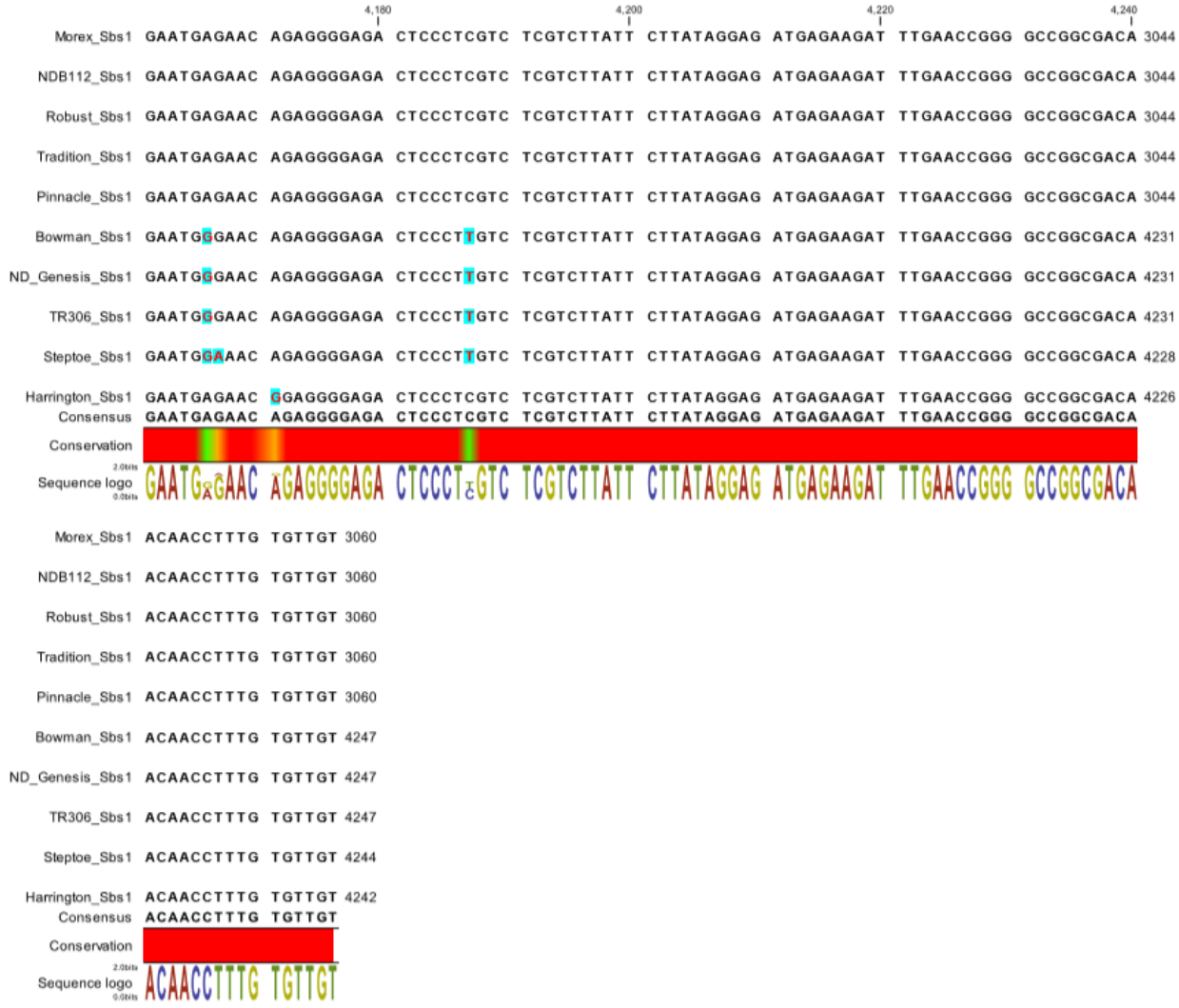
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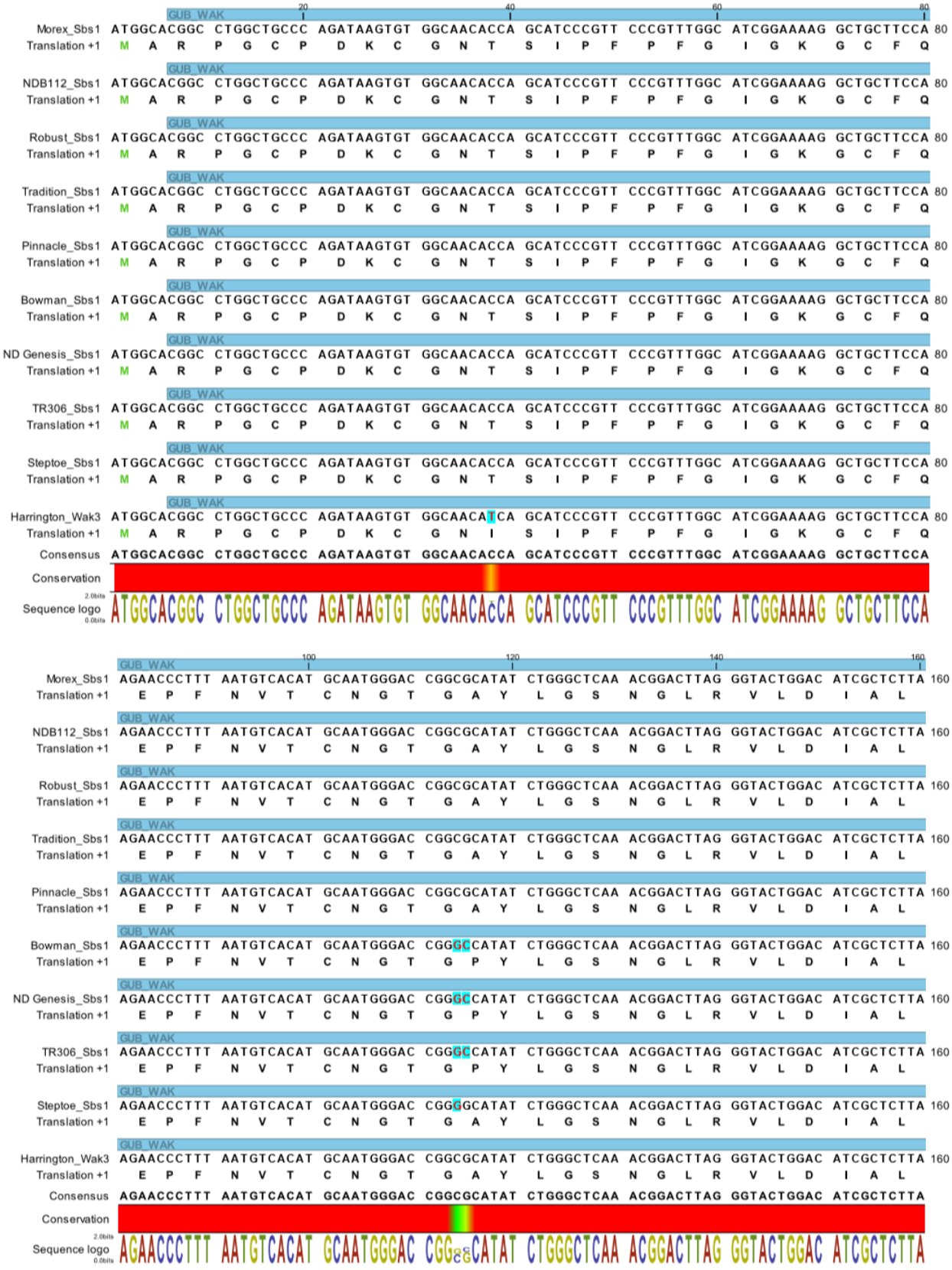

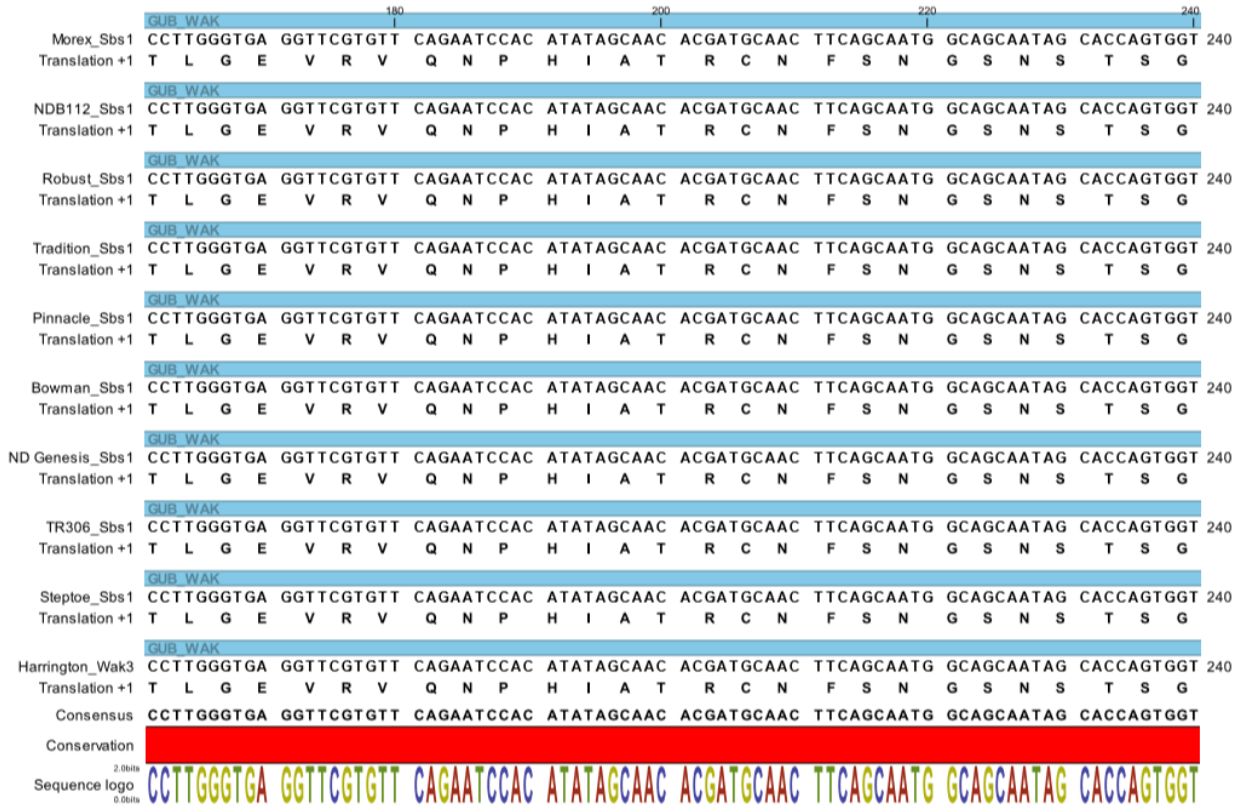
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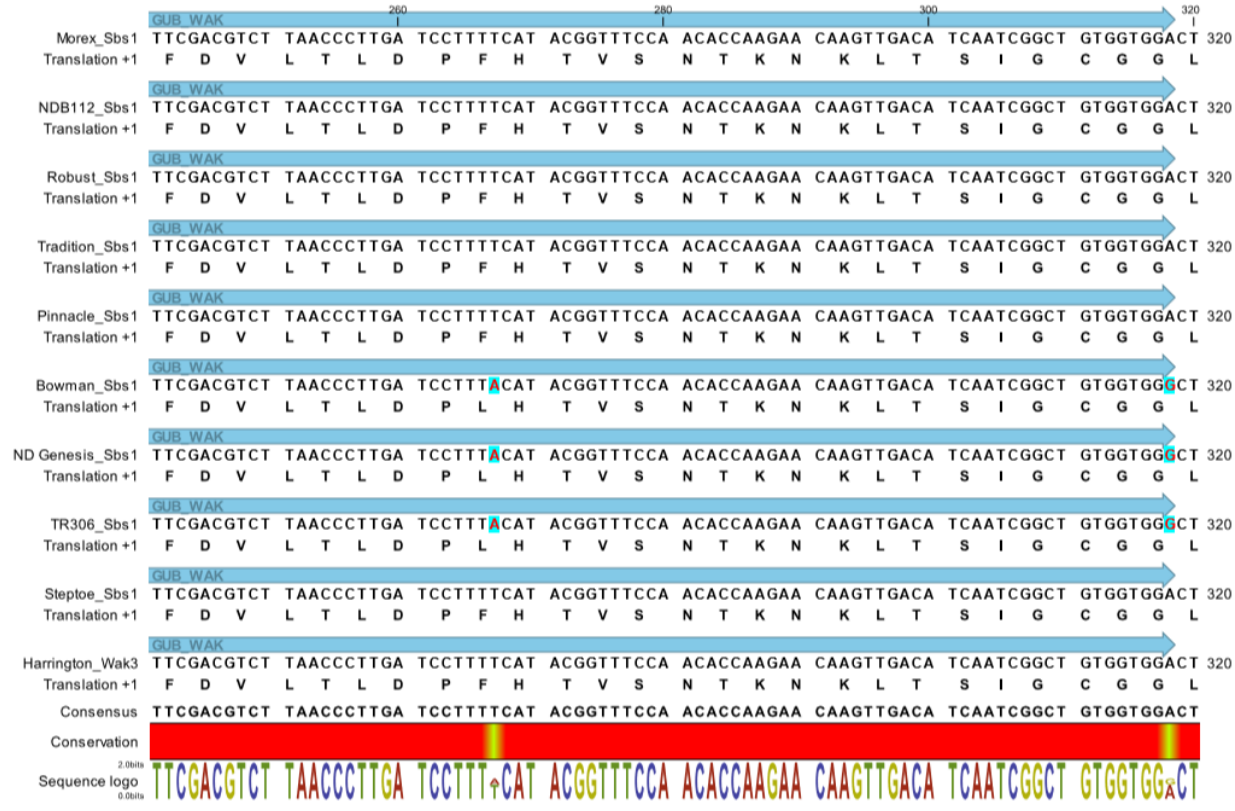
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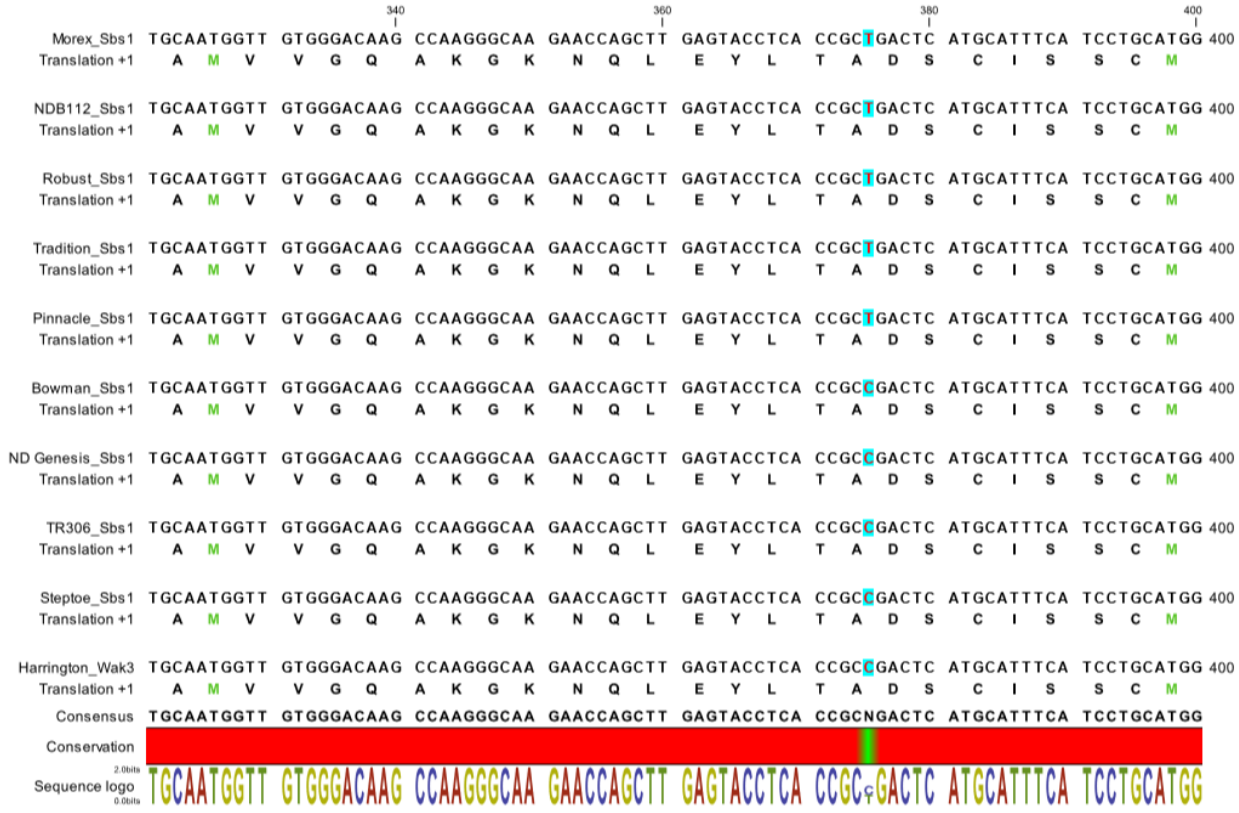
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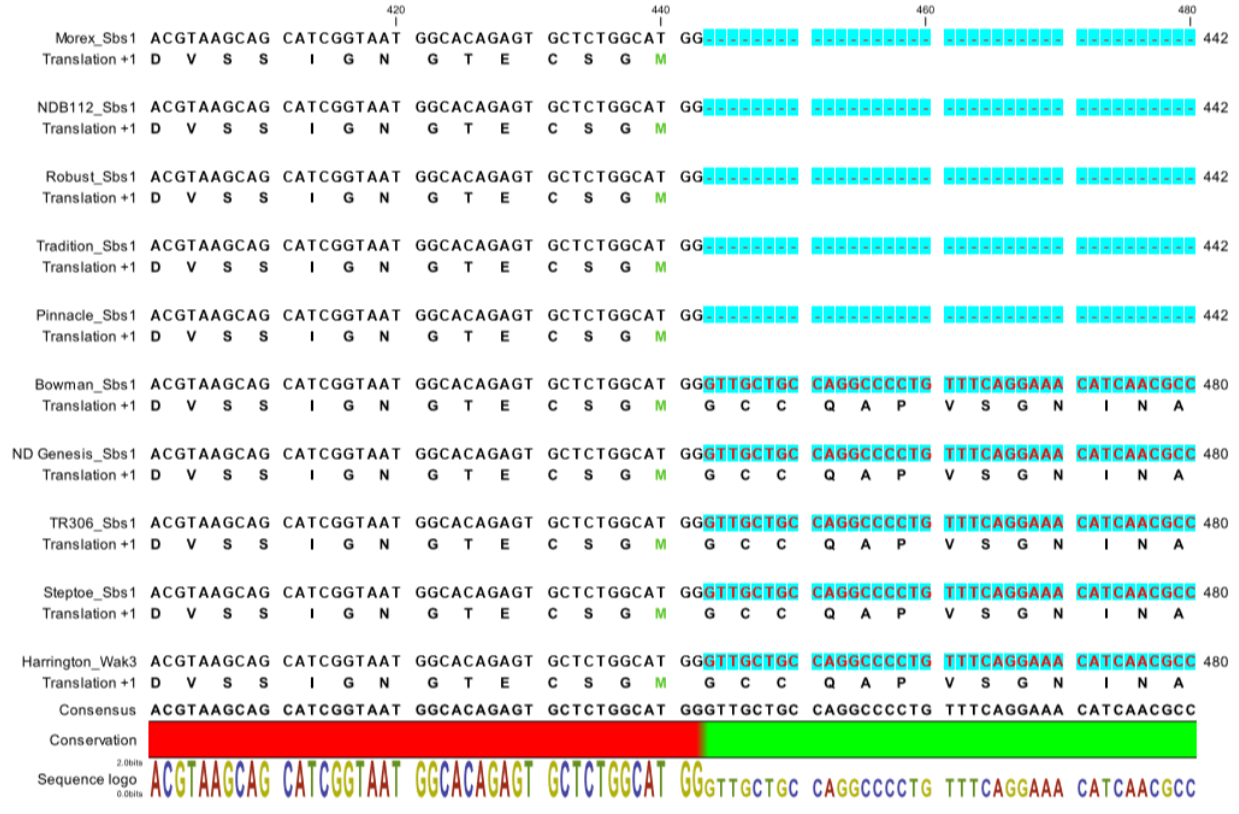
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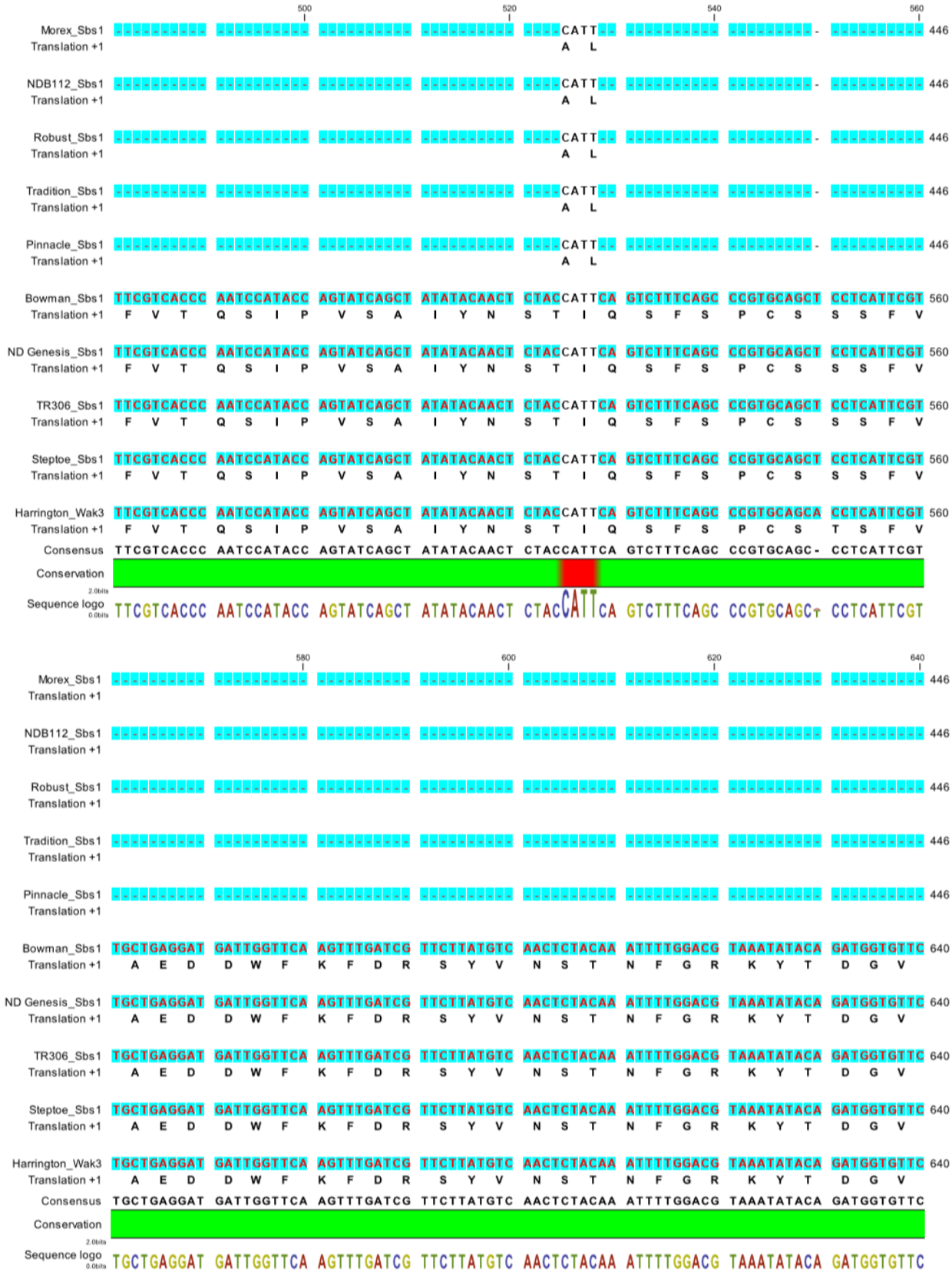
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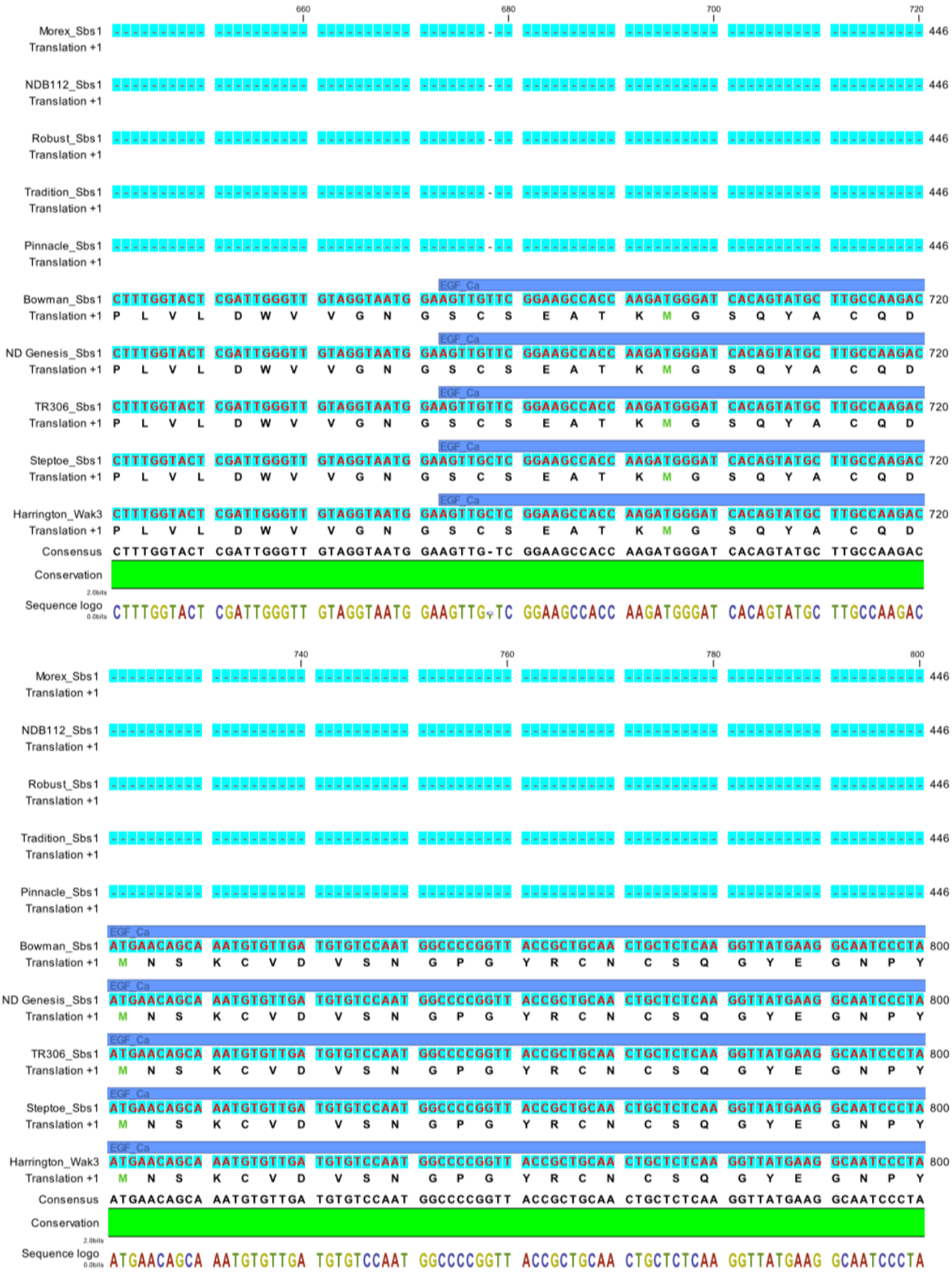
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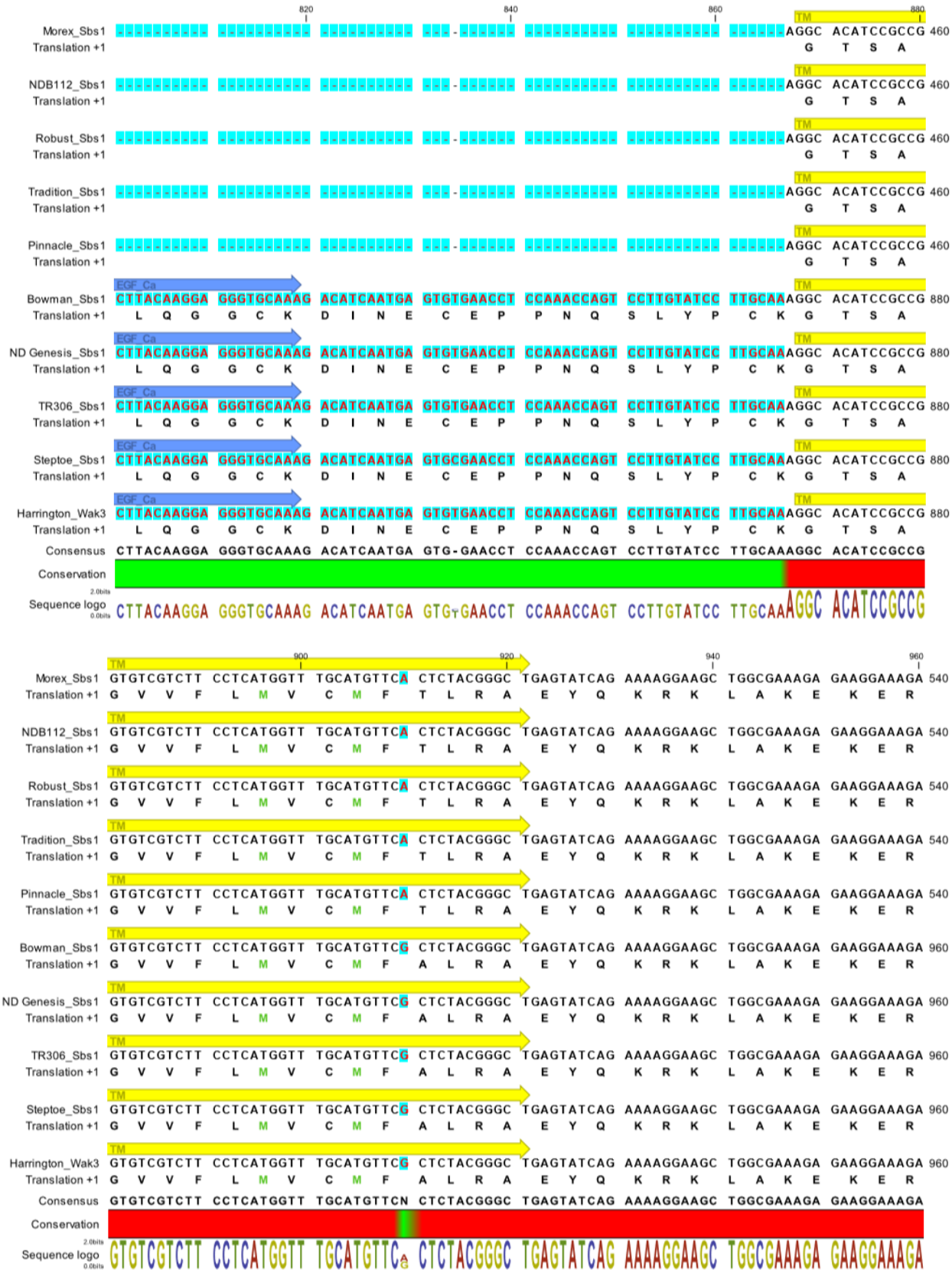
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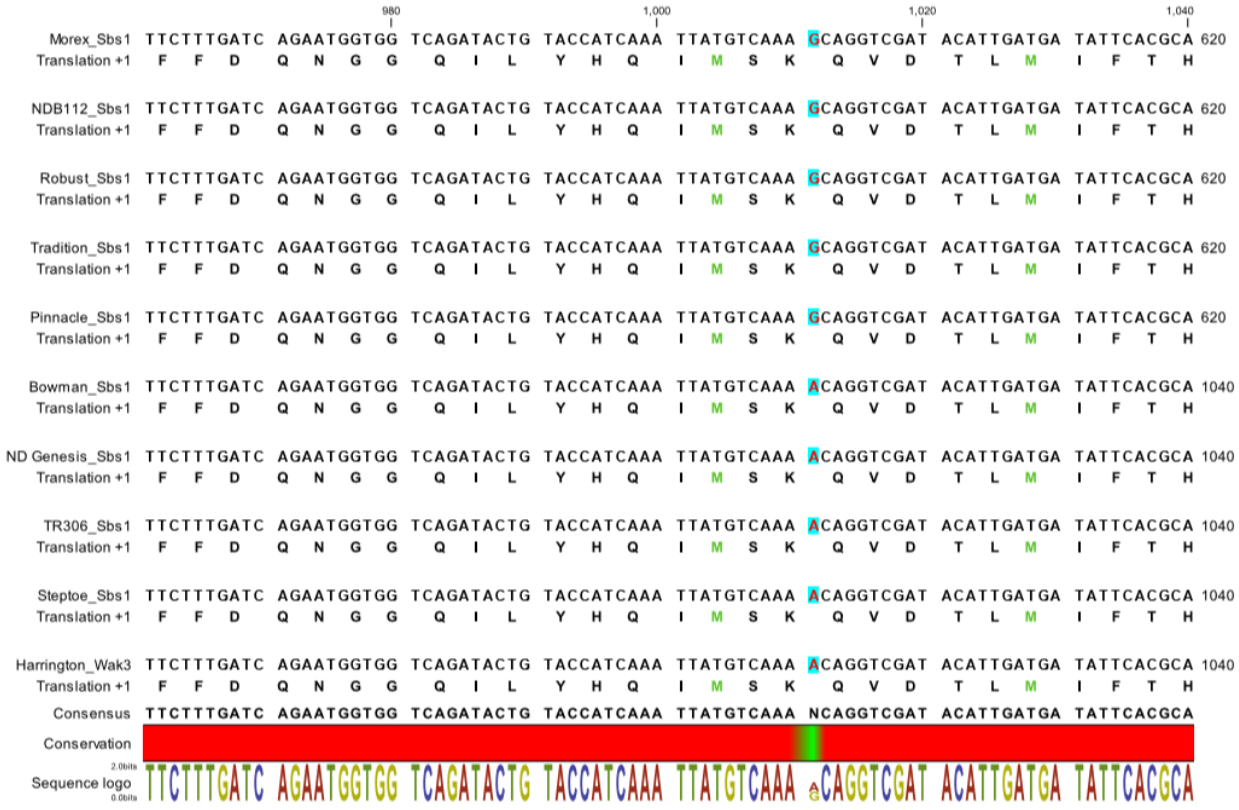
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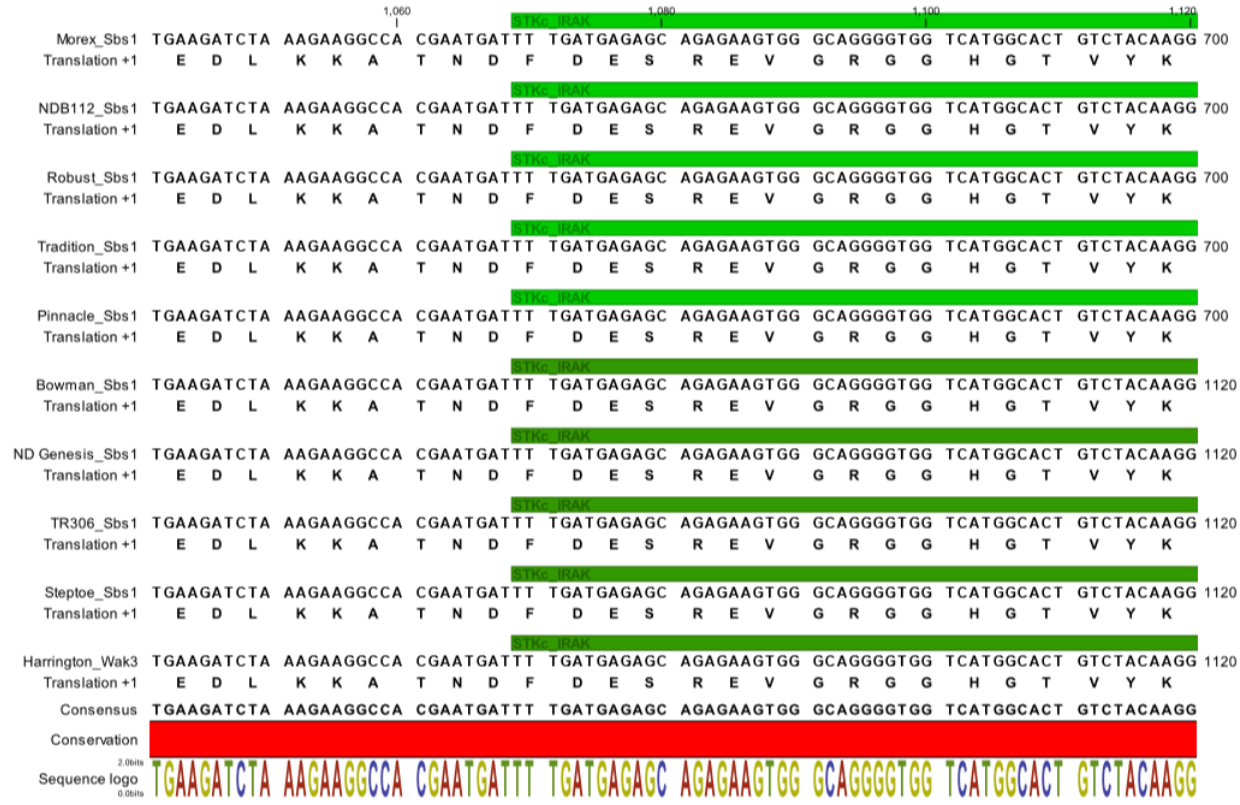
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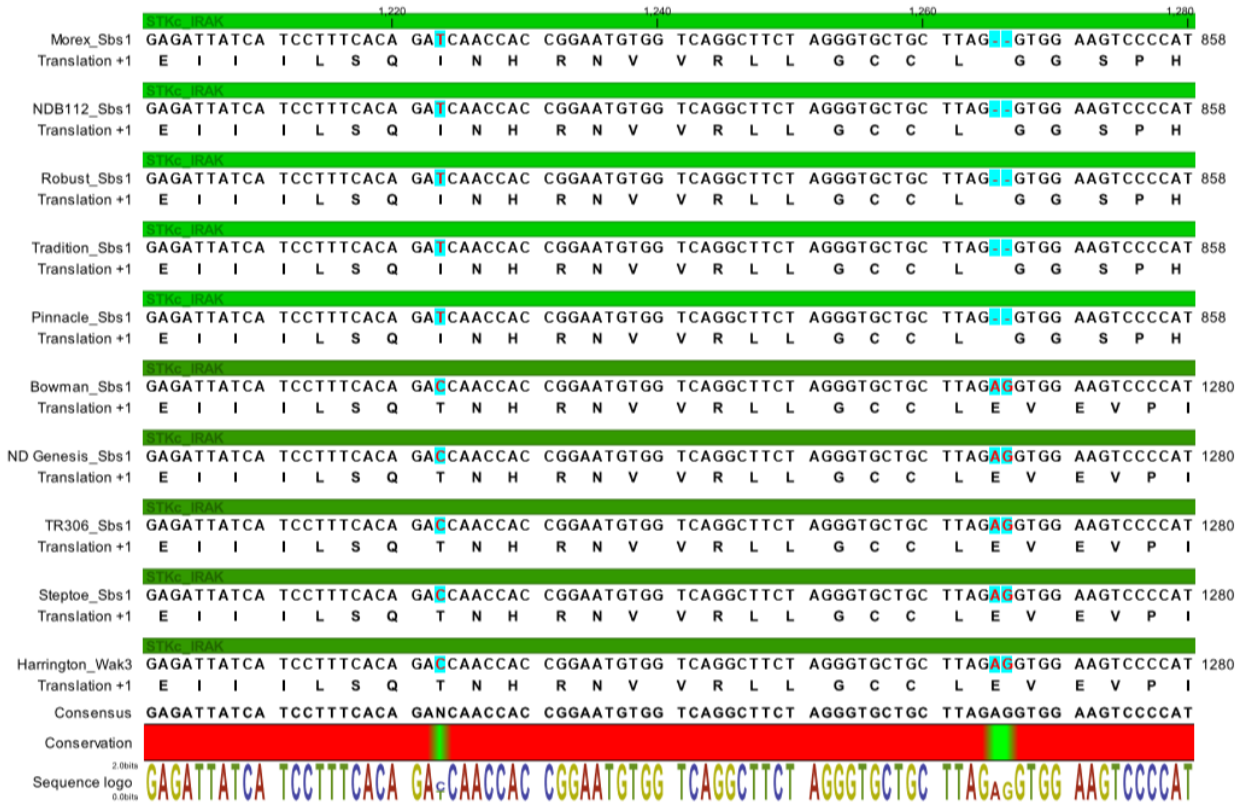
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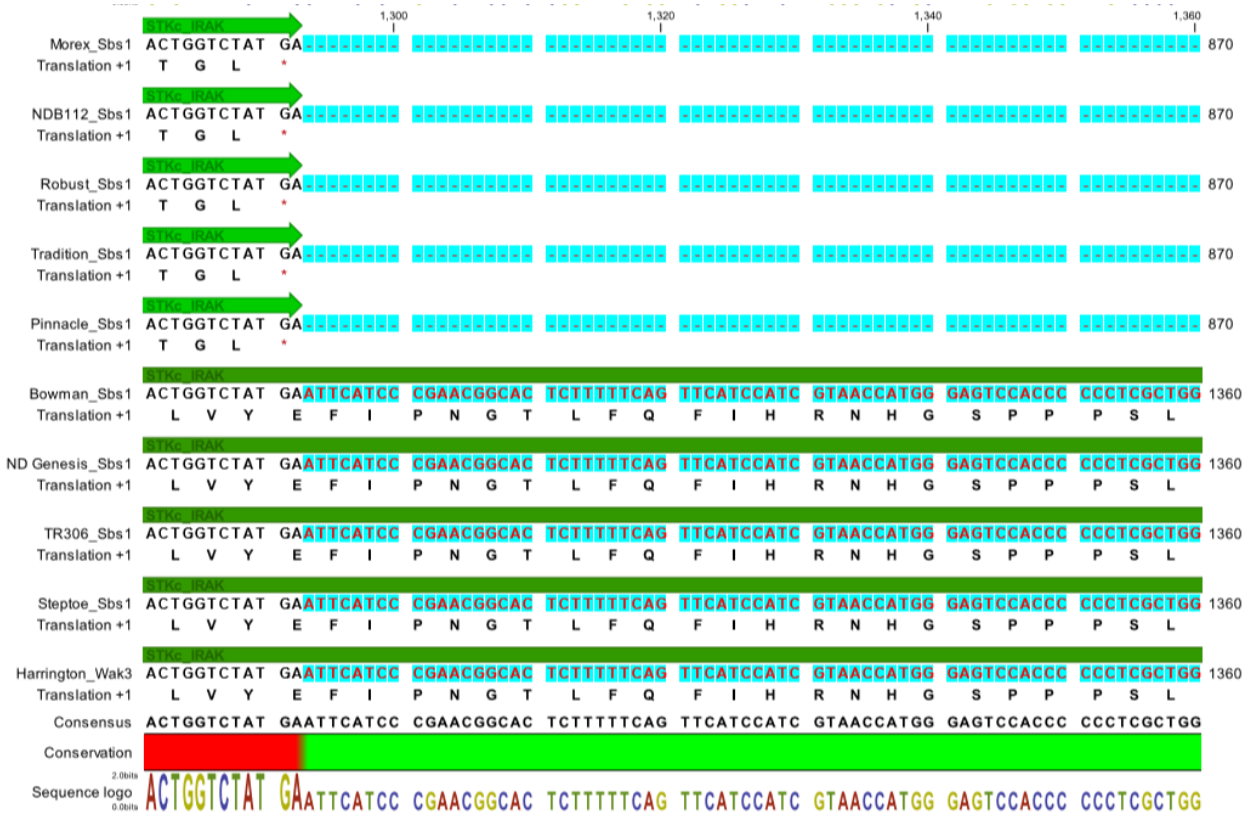
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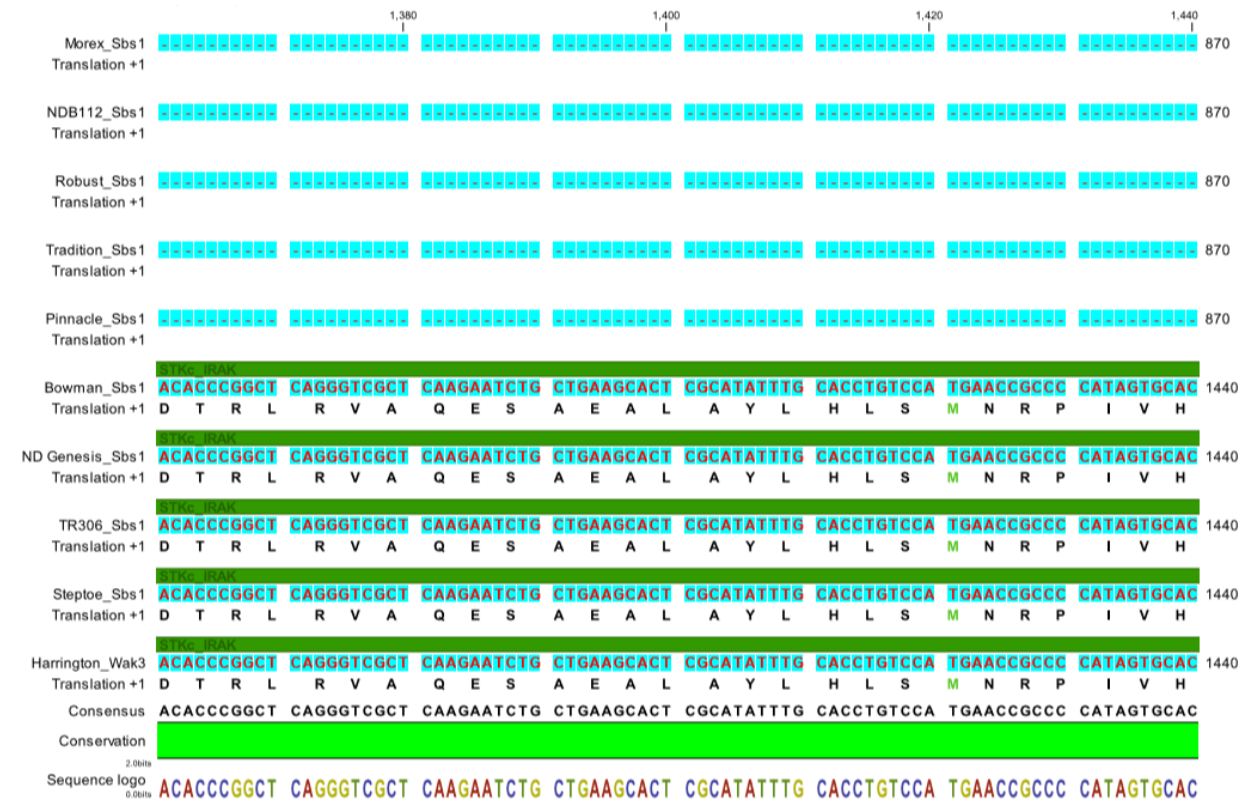
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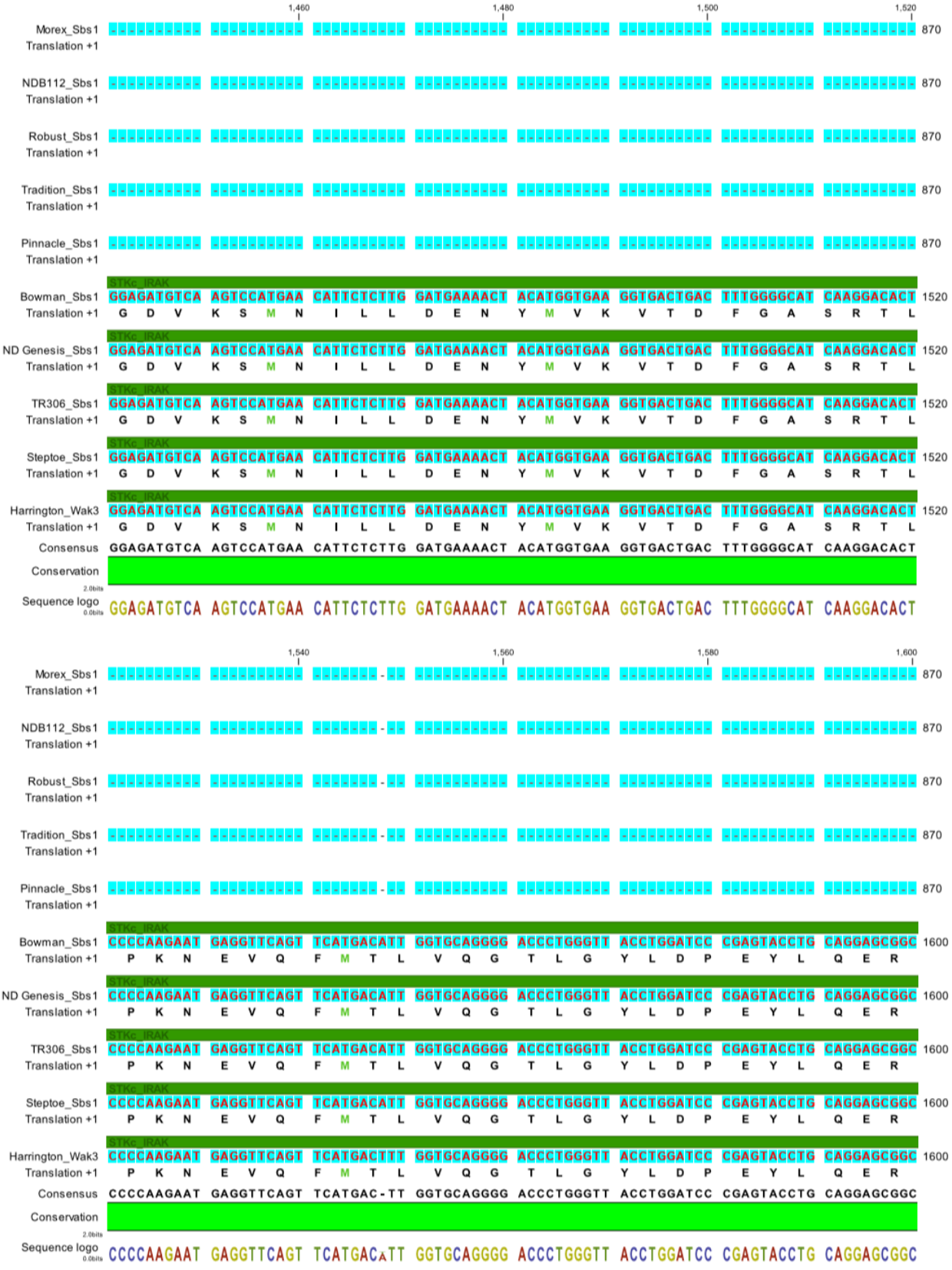
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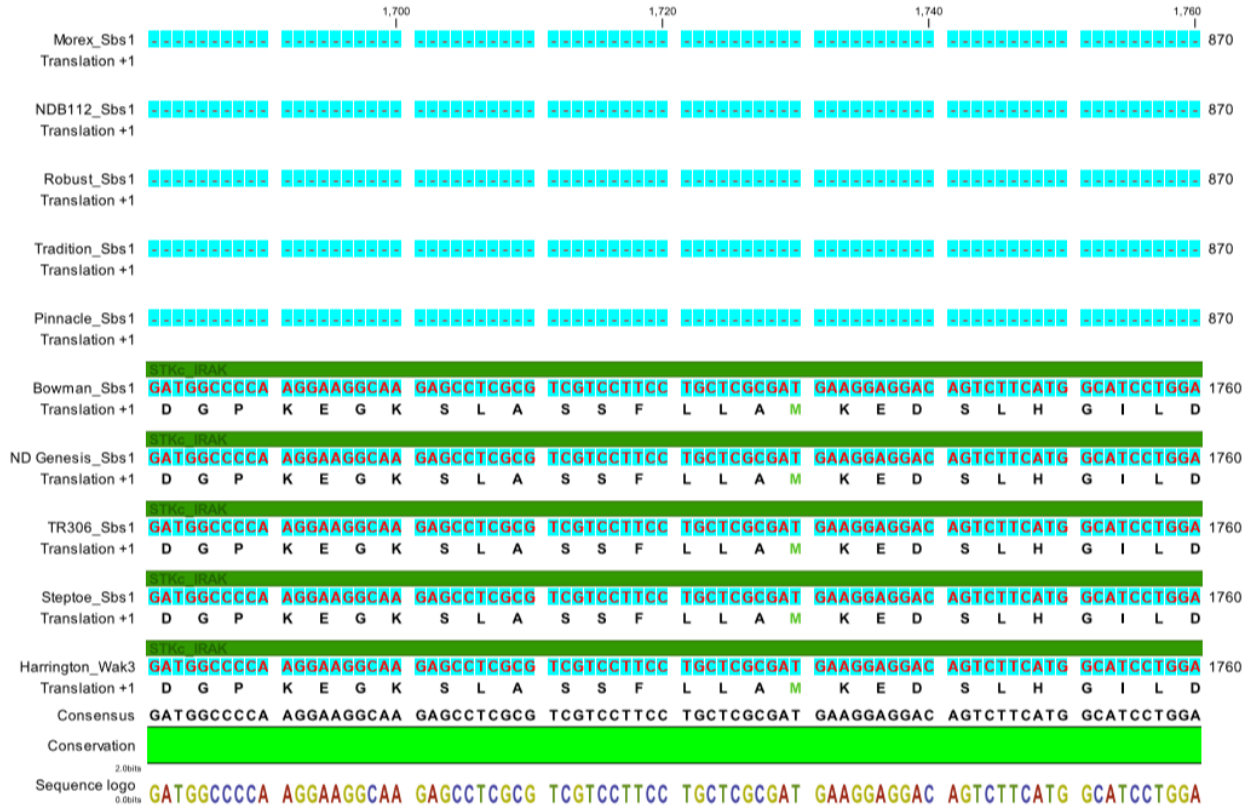
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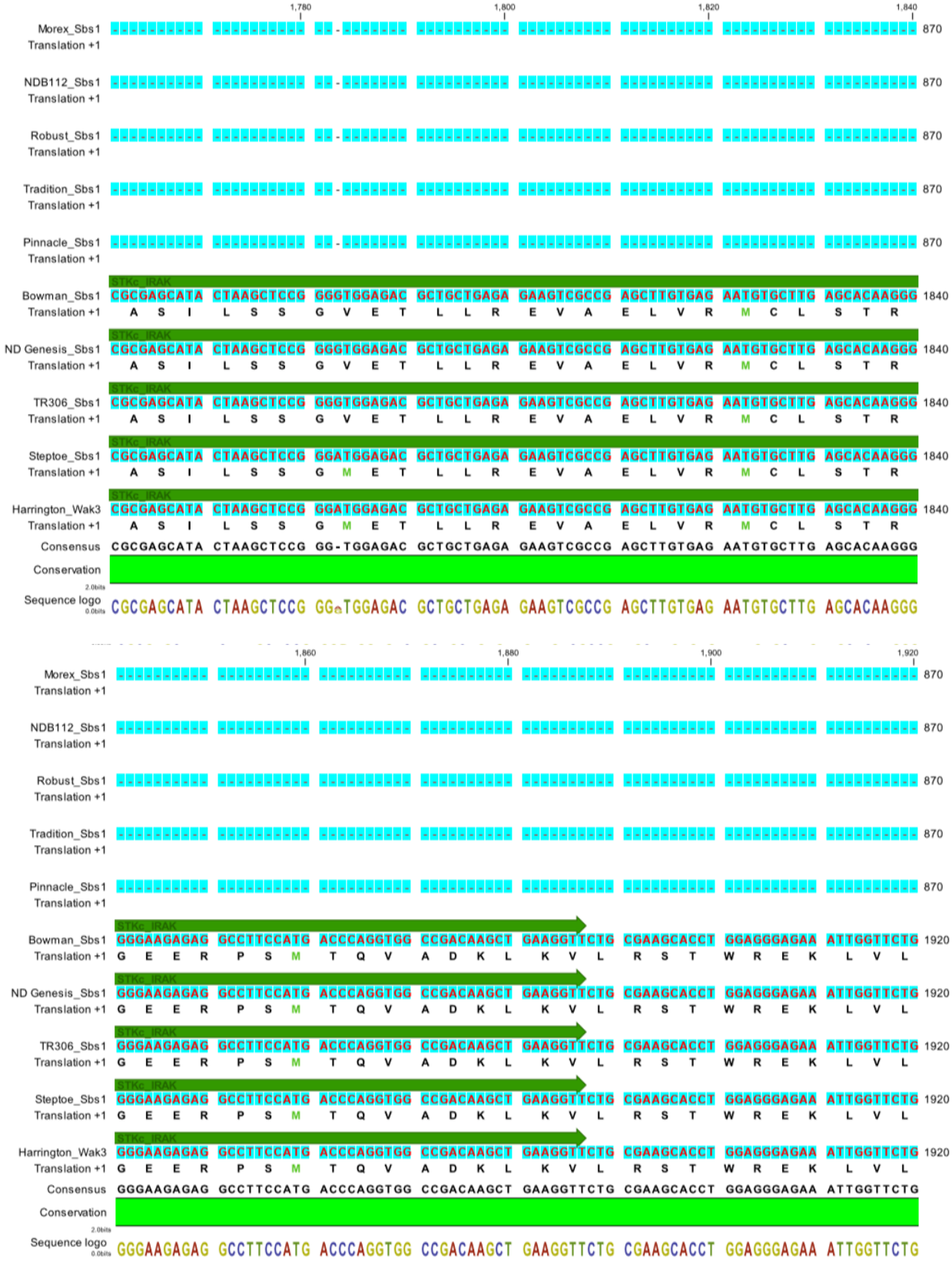
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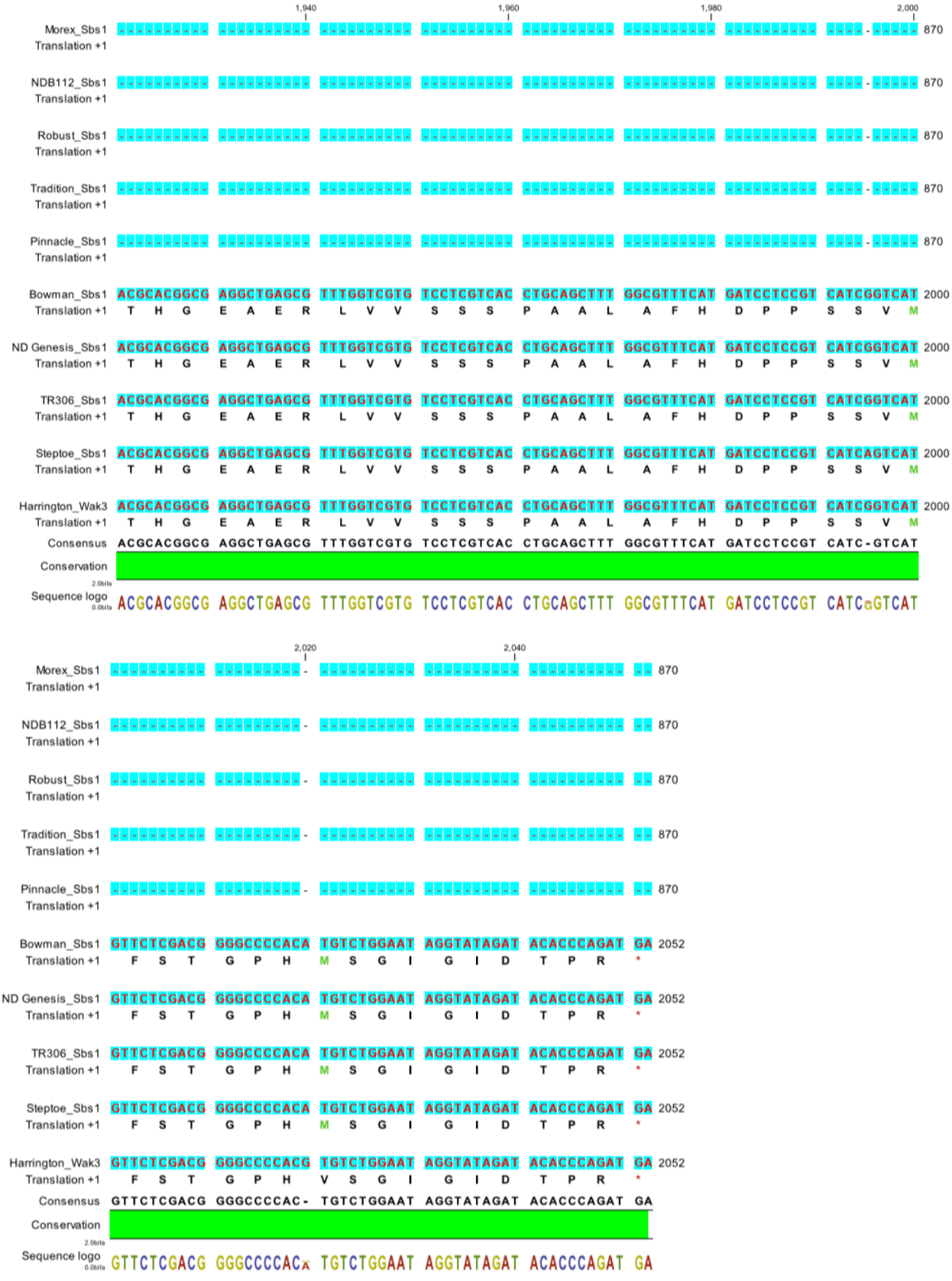
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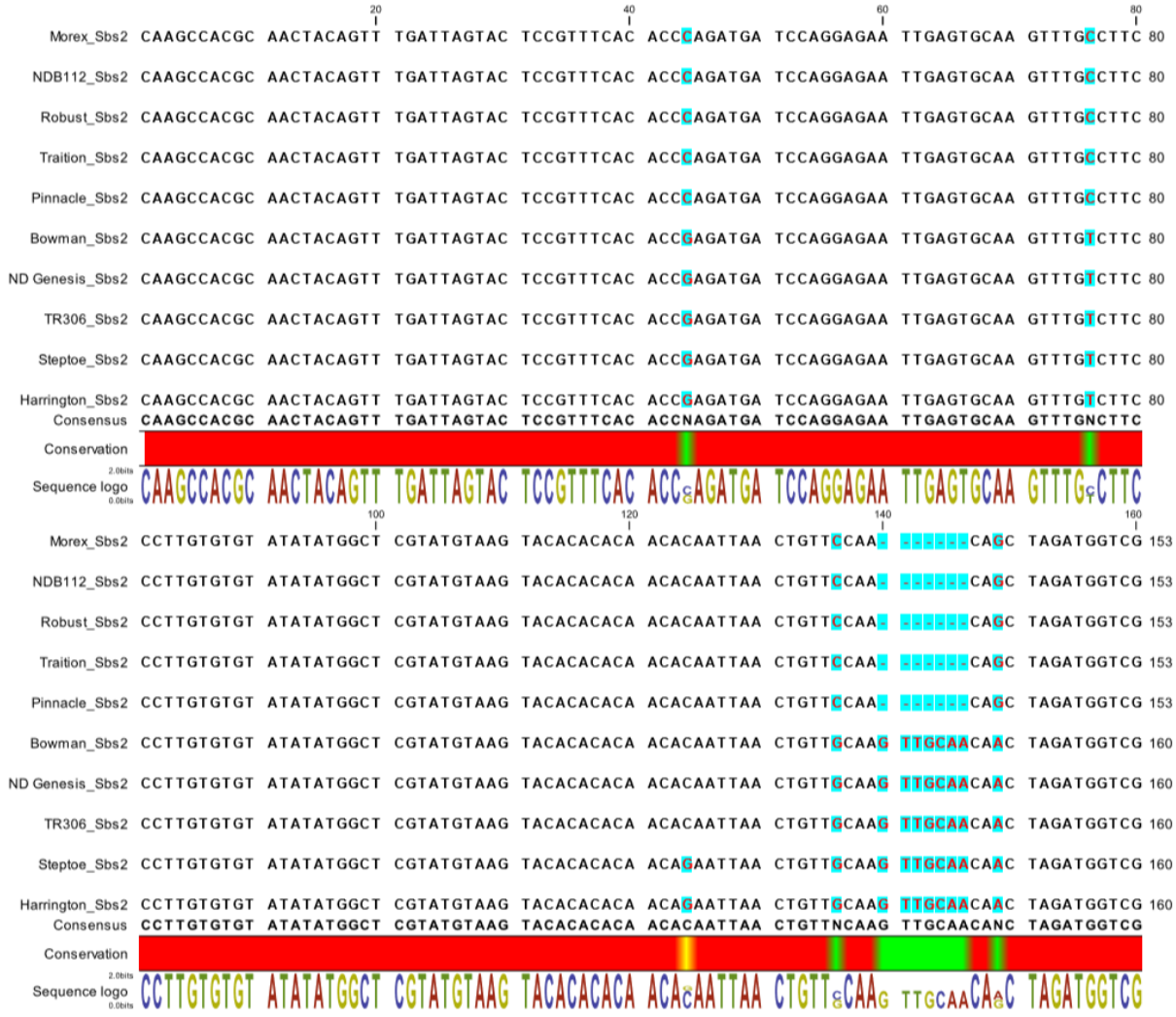
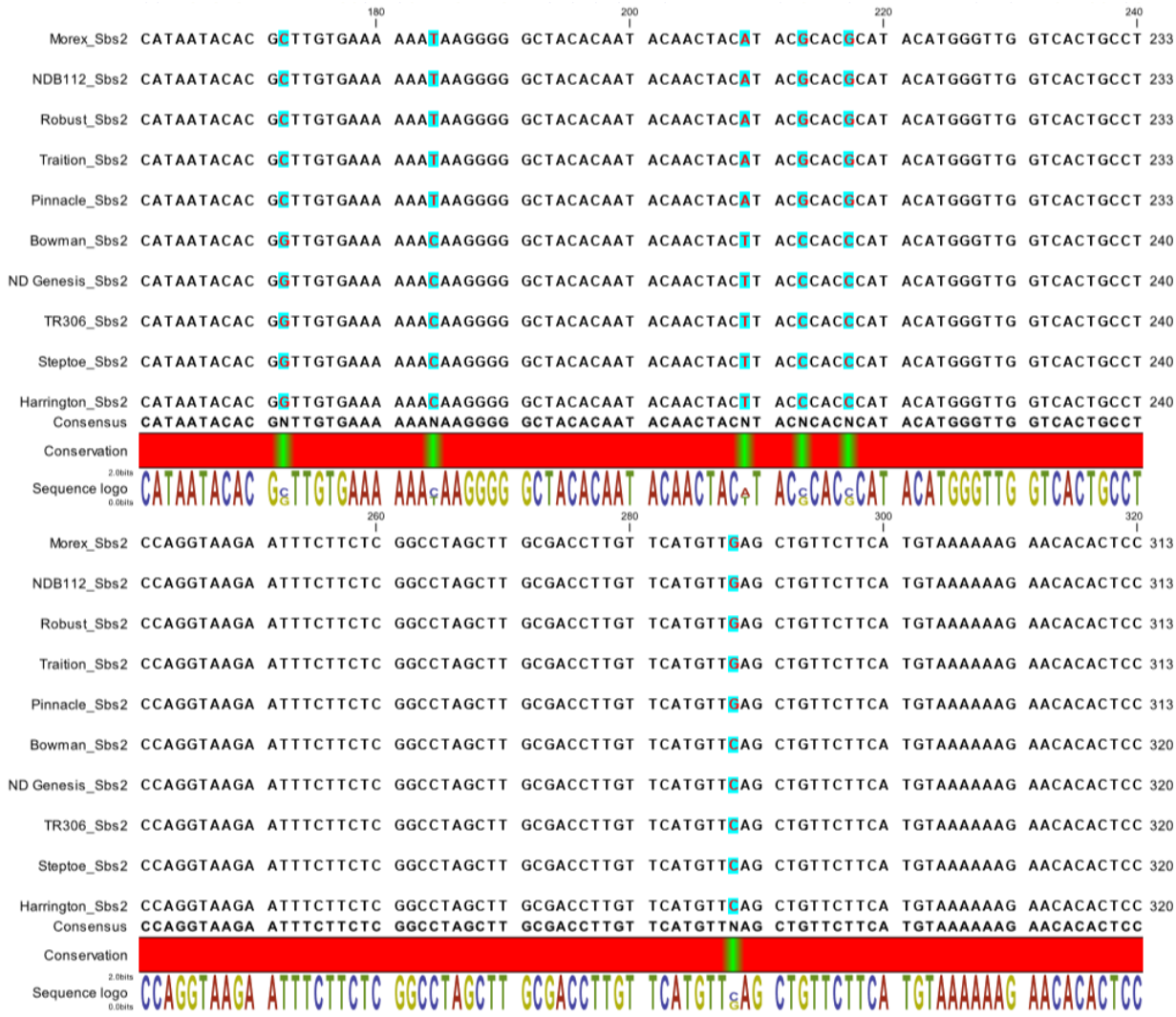
Supplementary Figure 6A.

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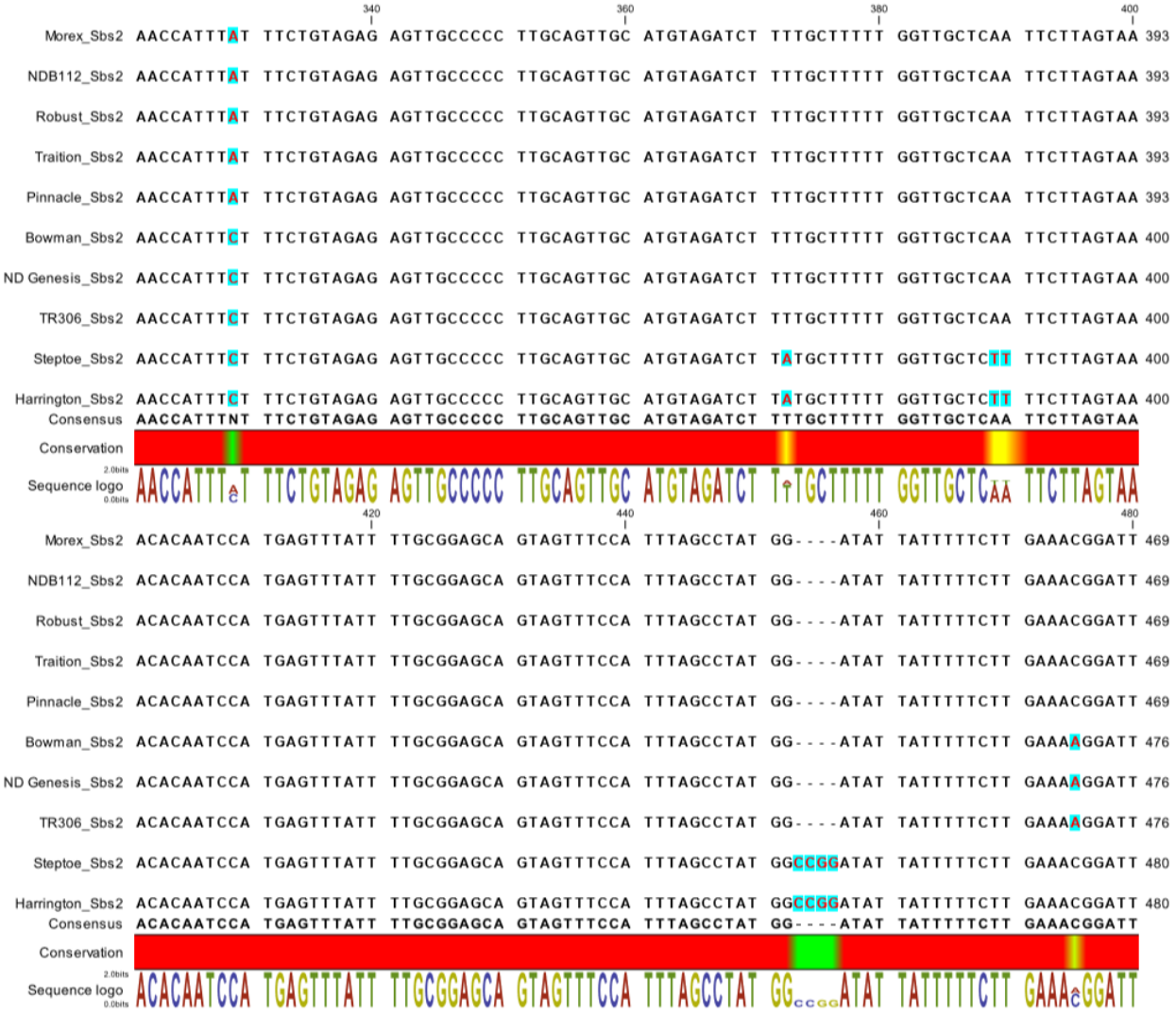
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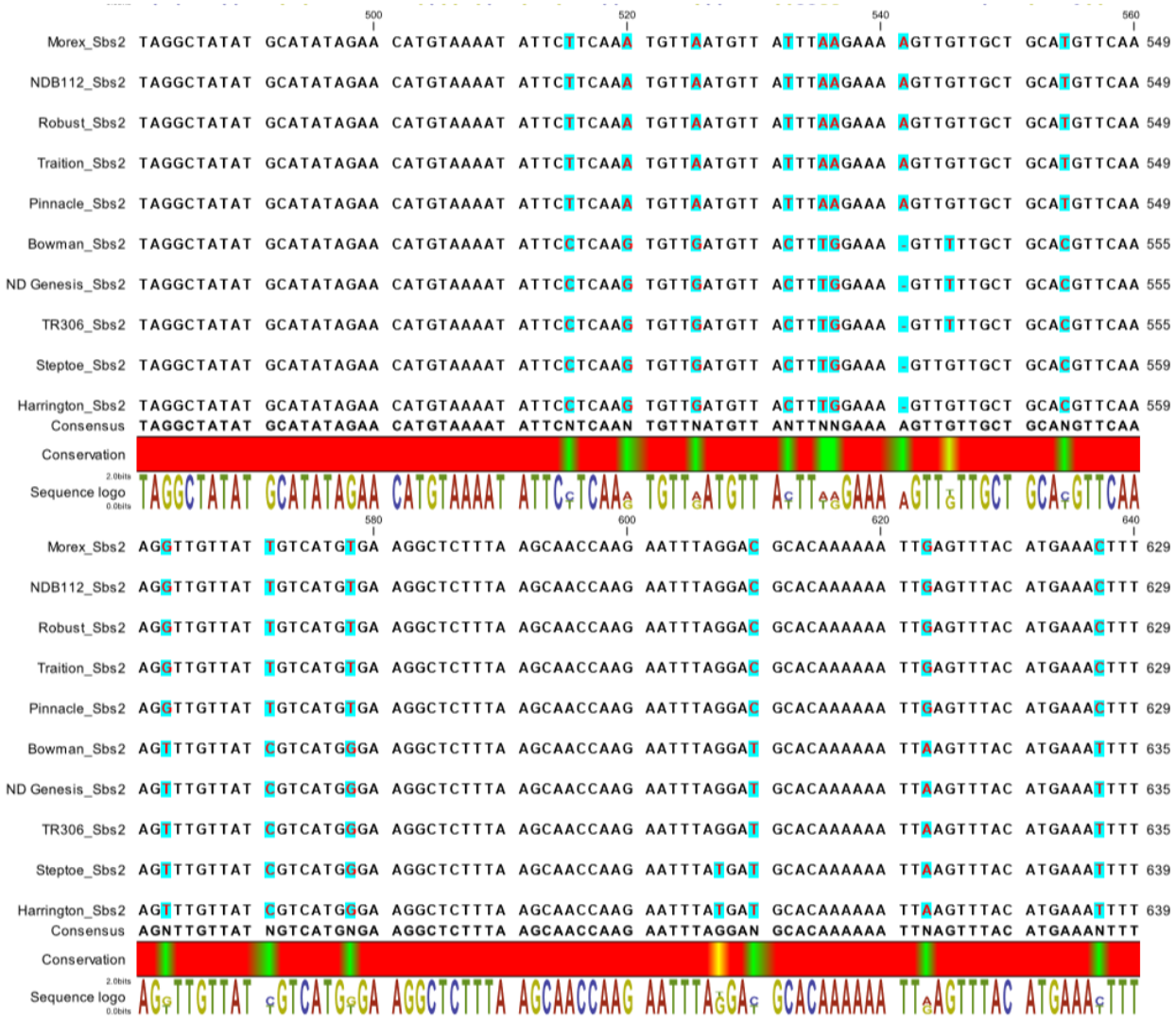
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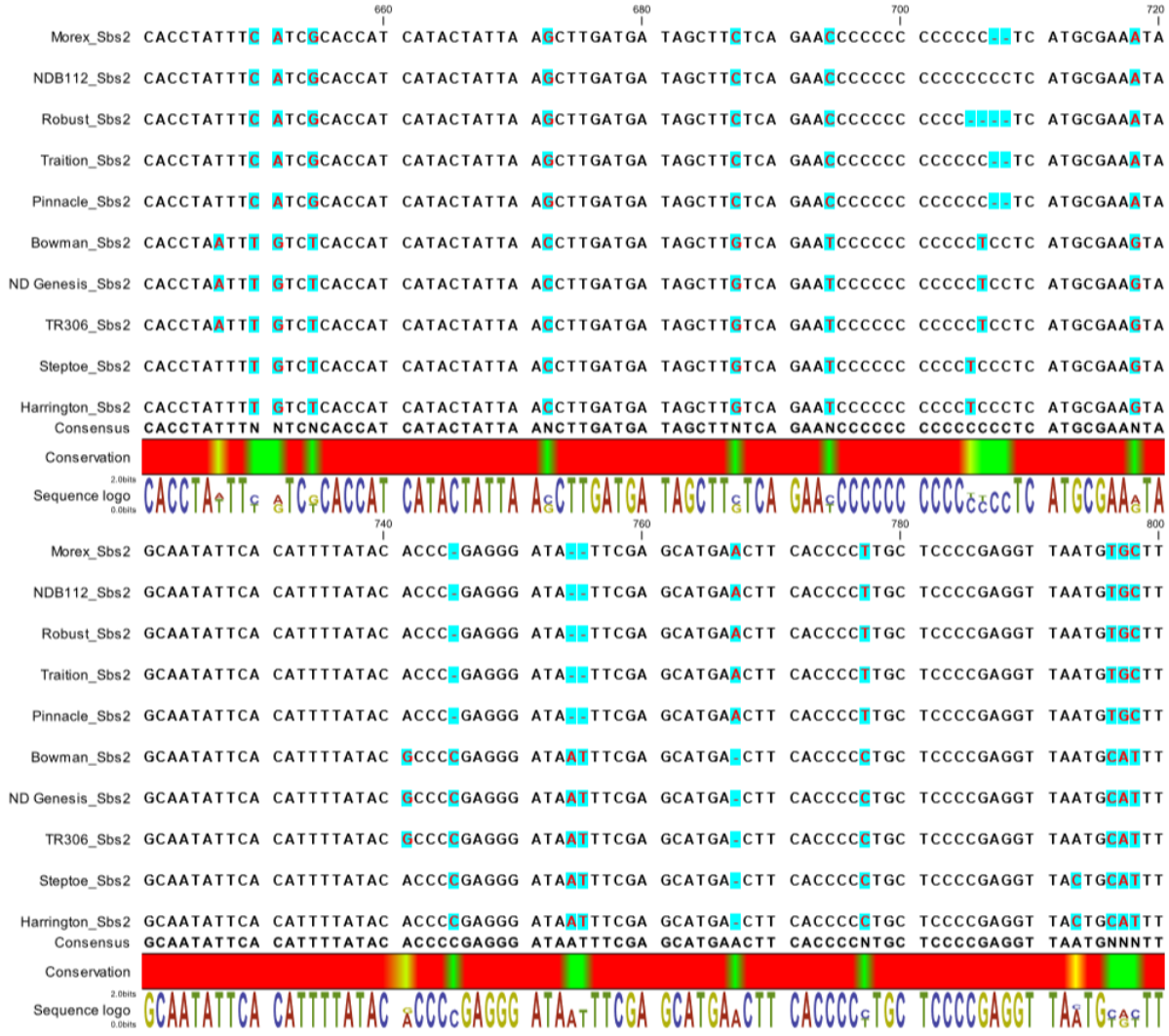
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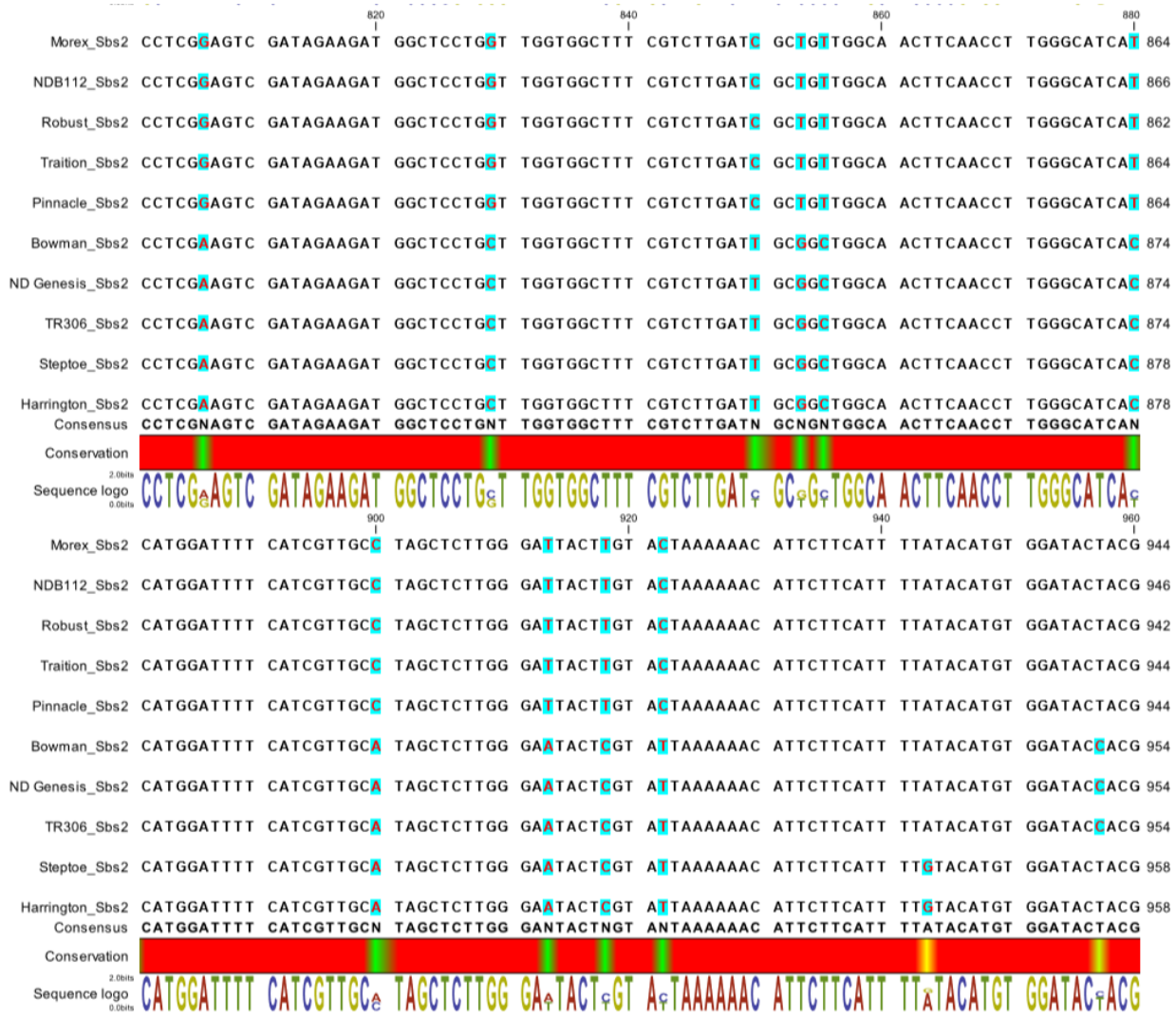
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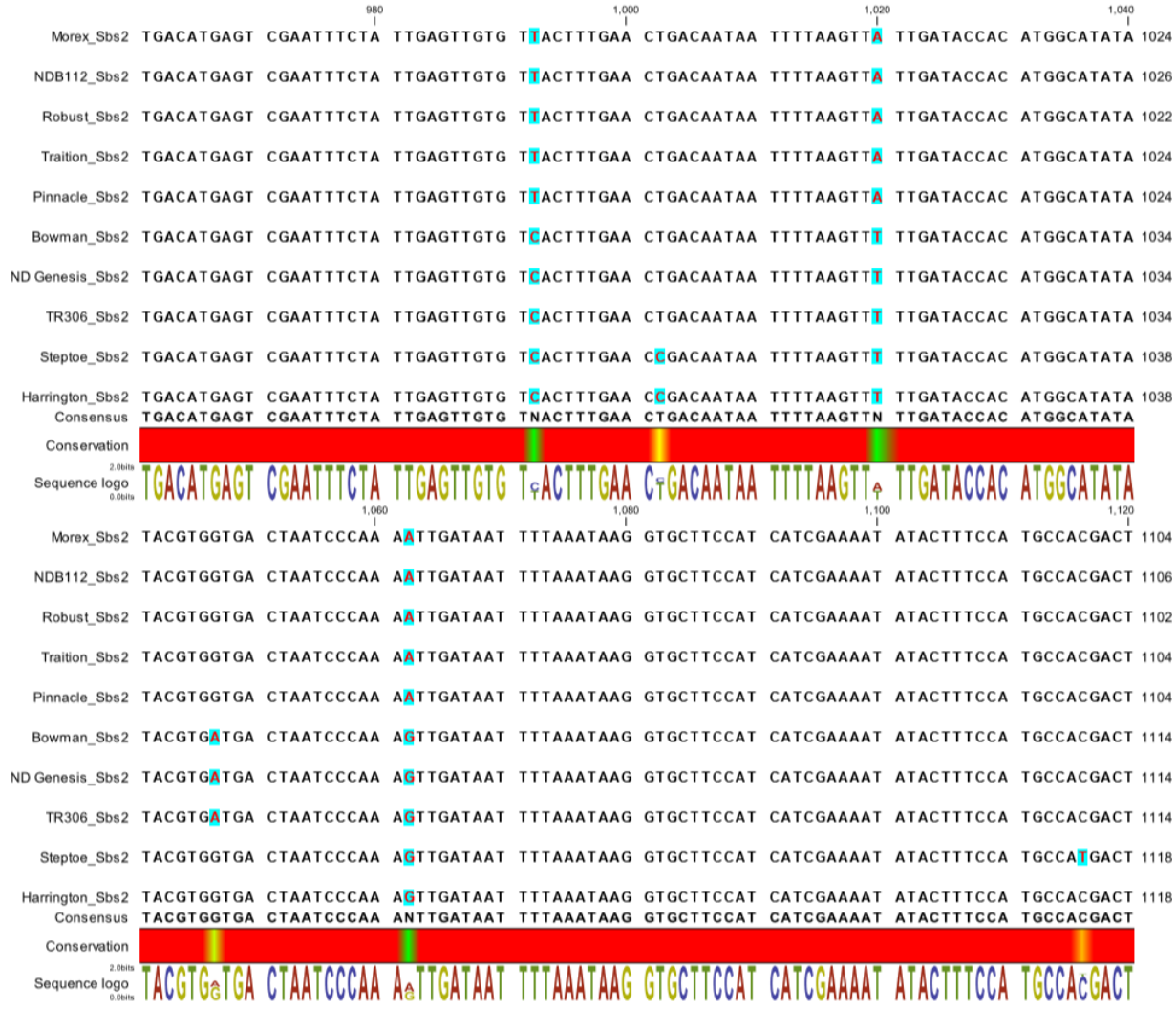
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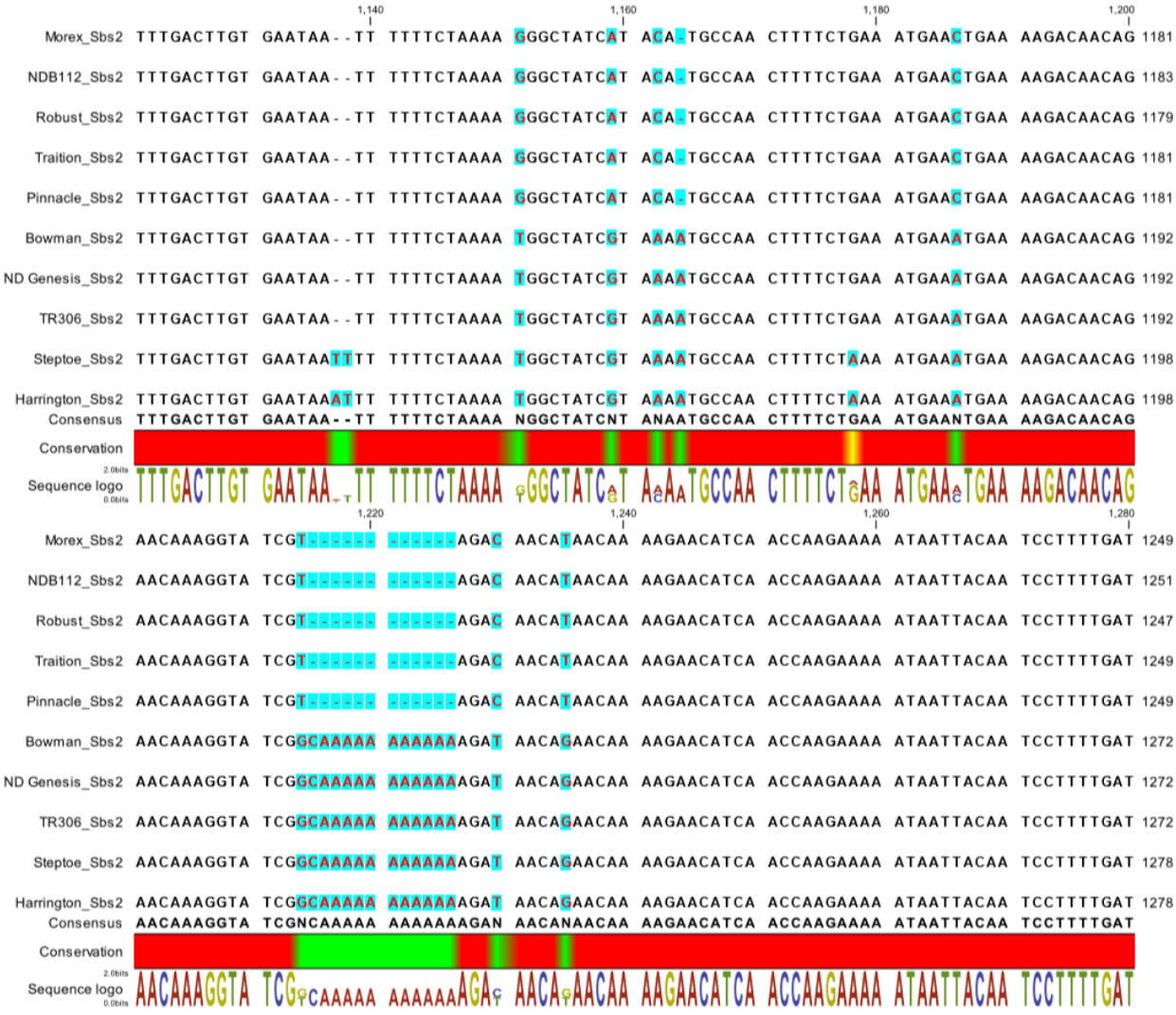
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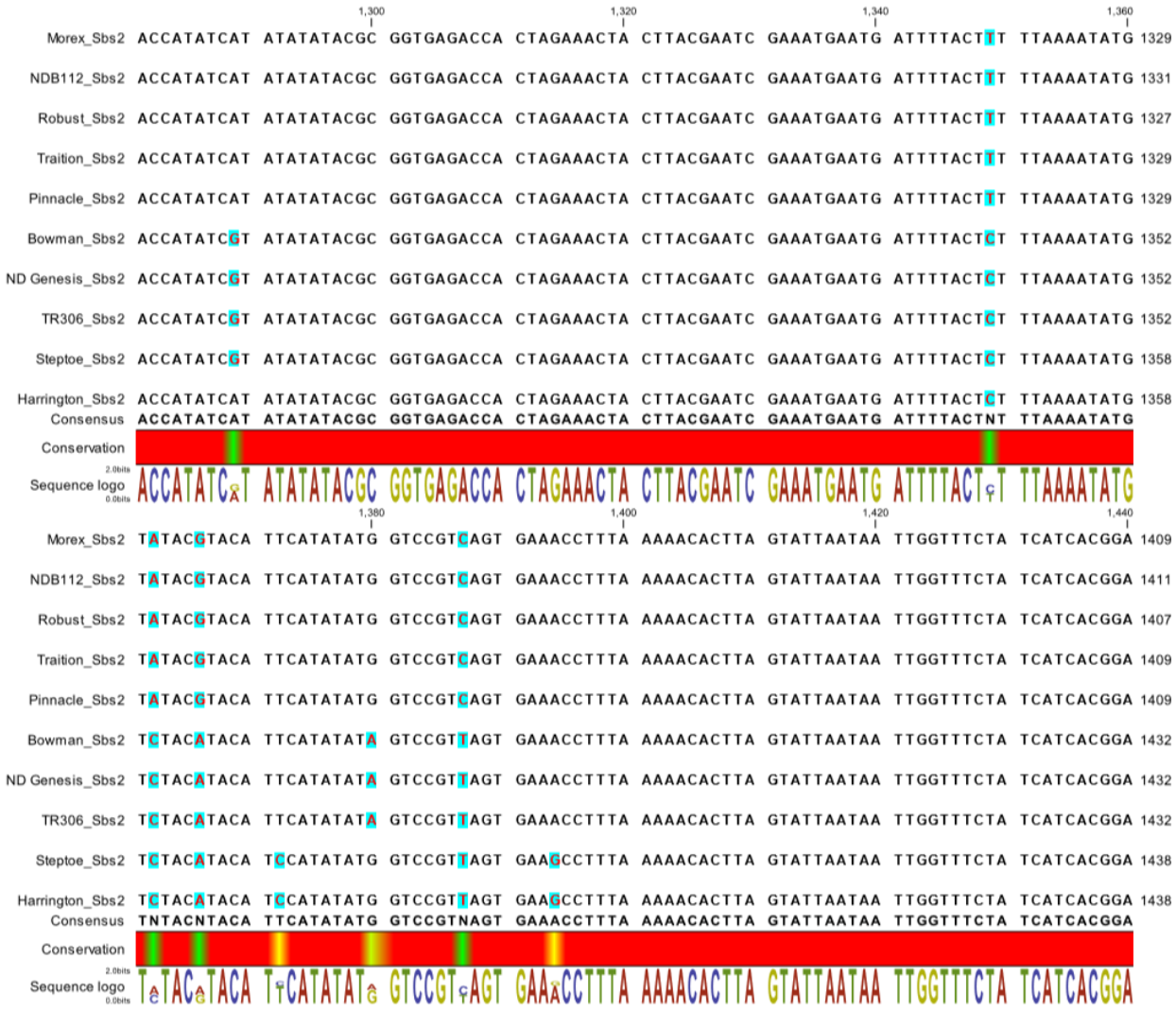
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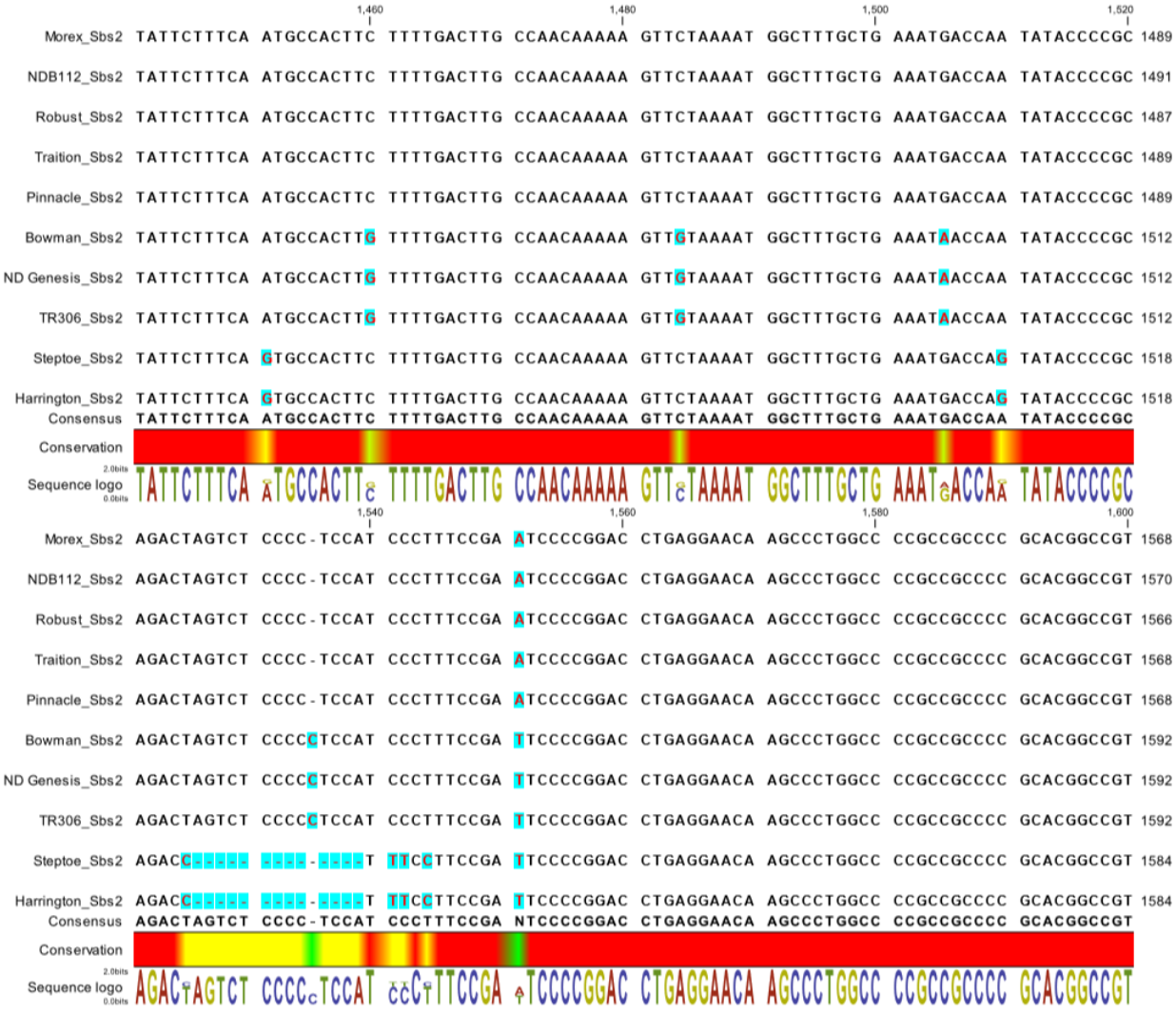
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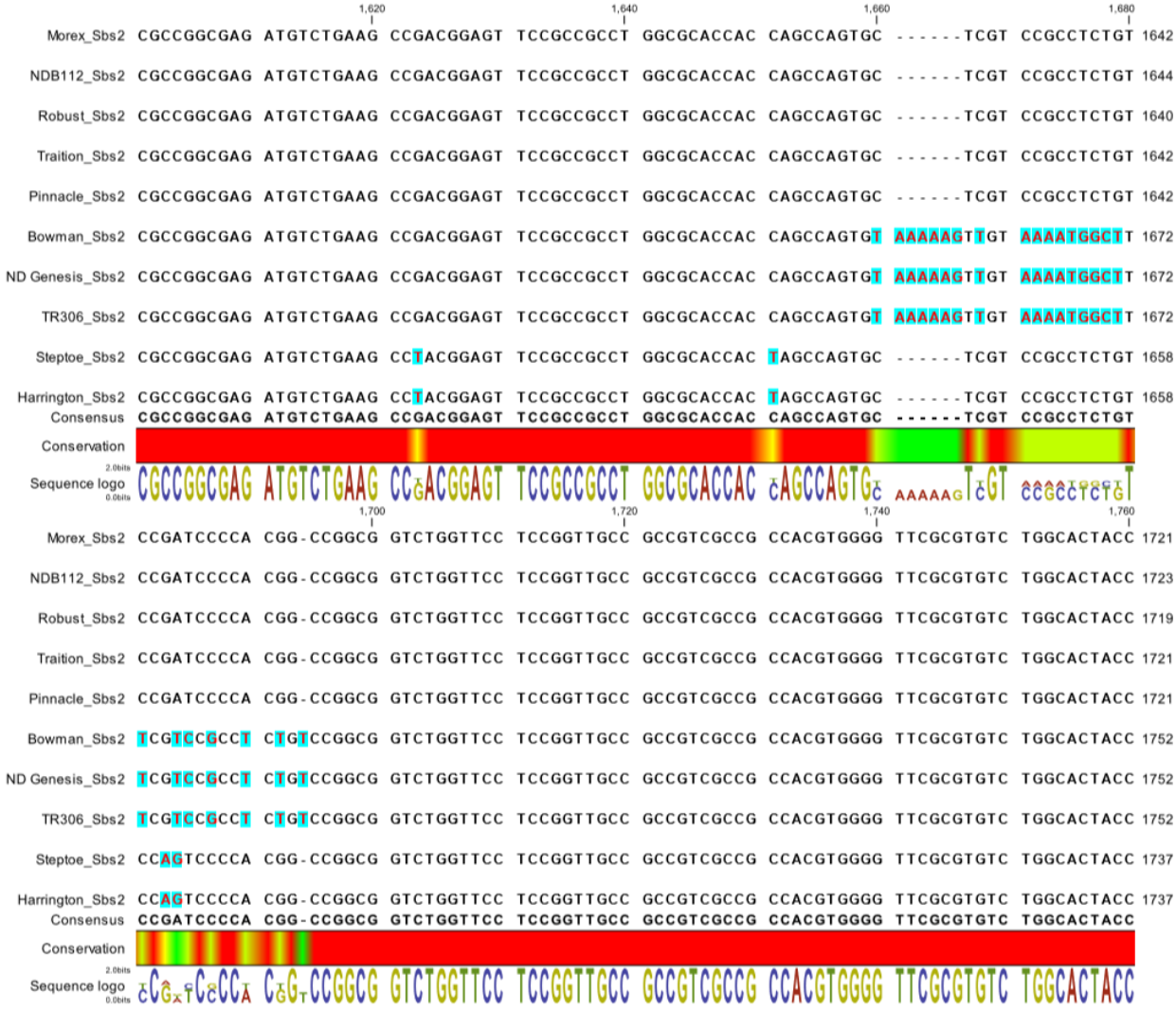
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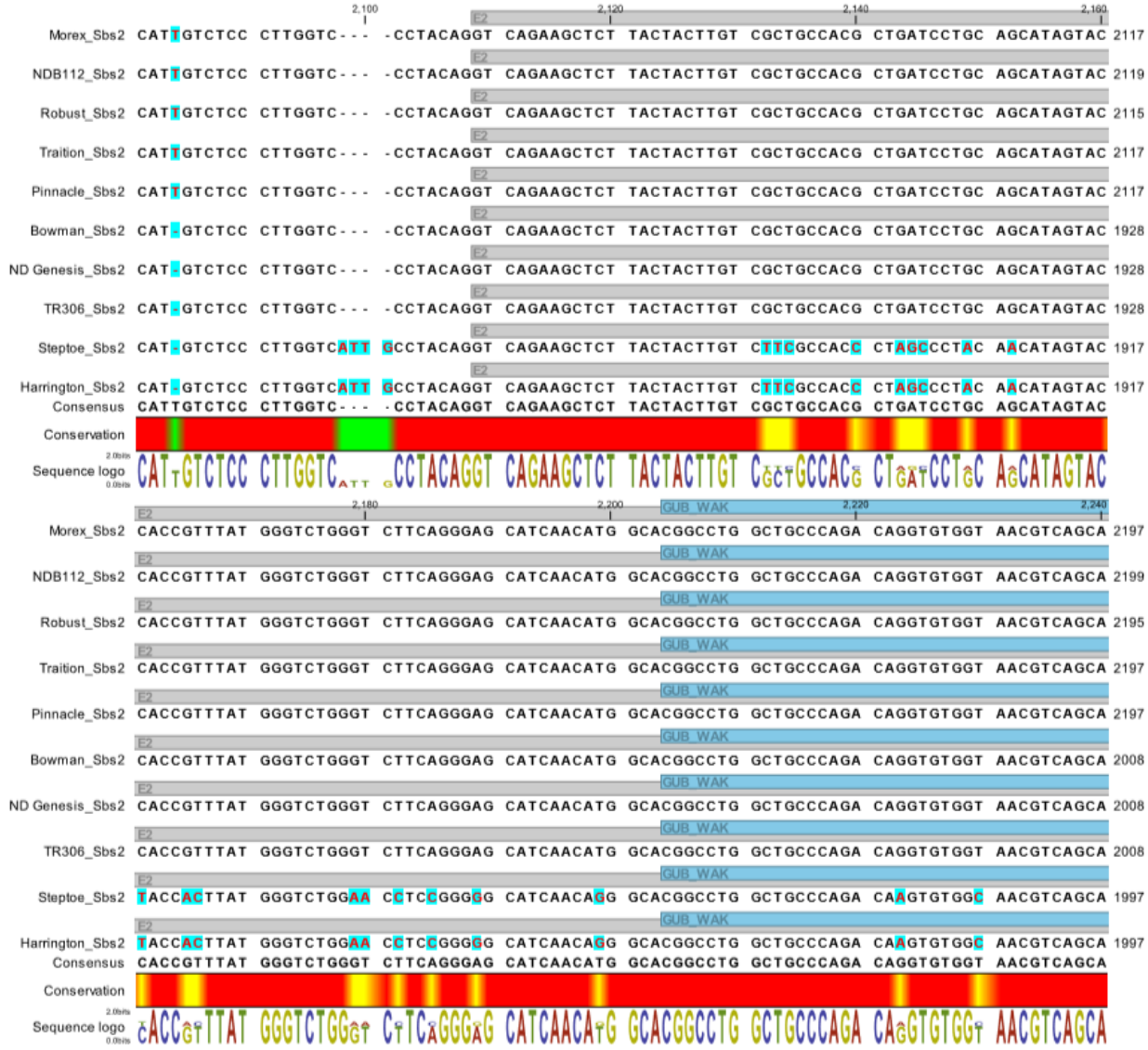
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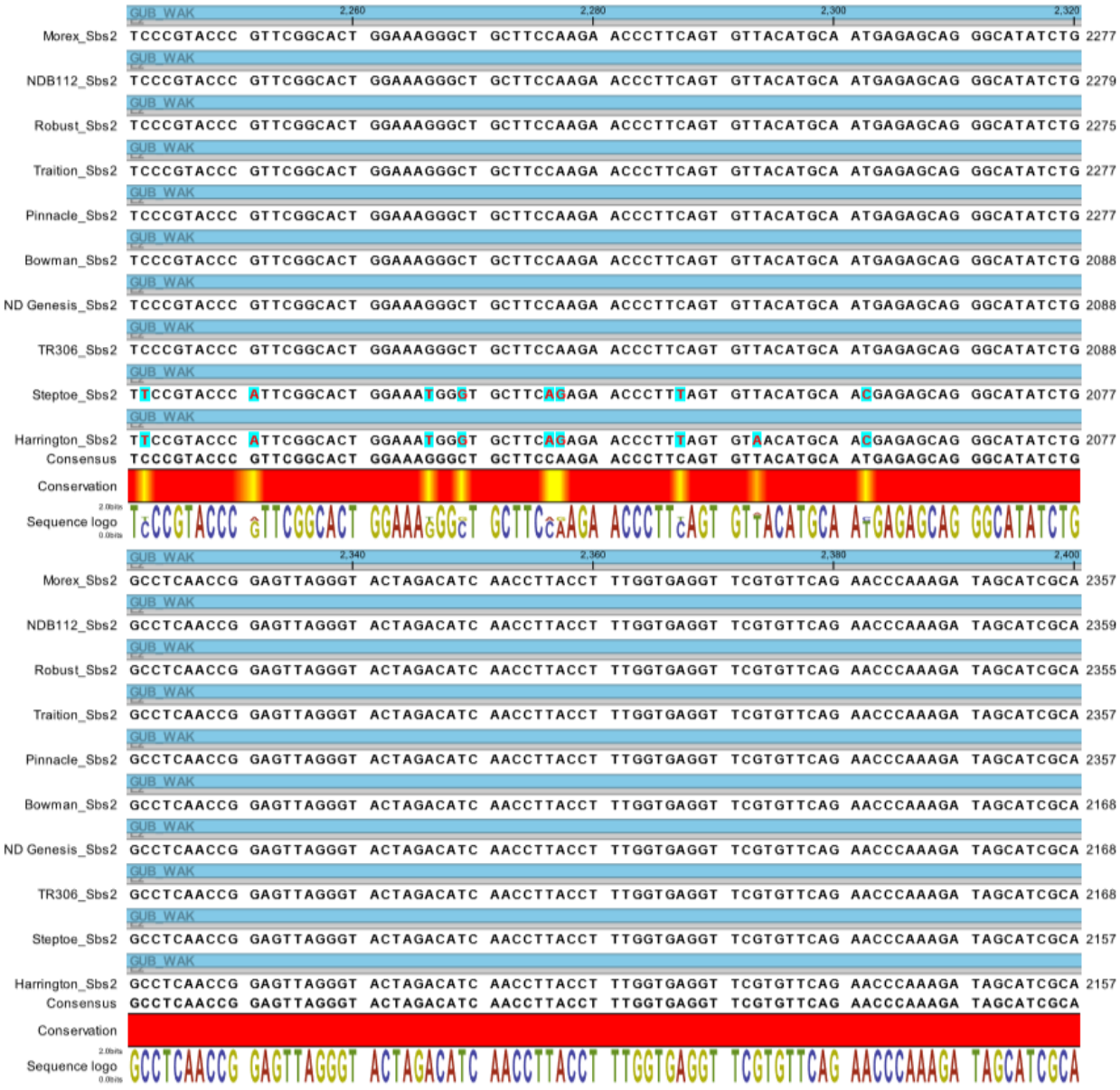
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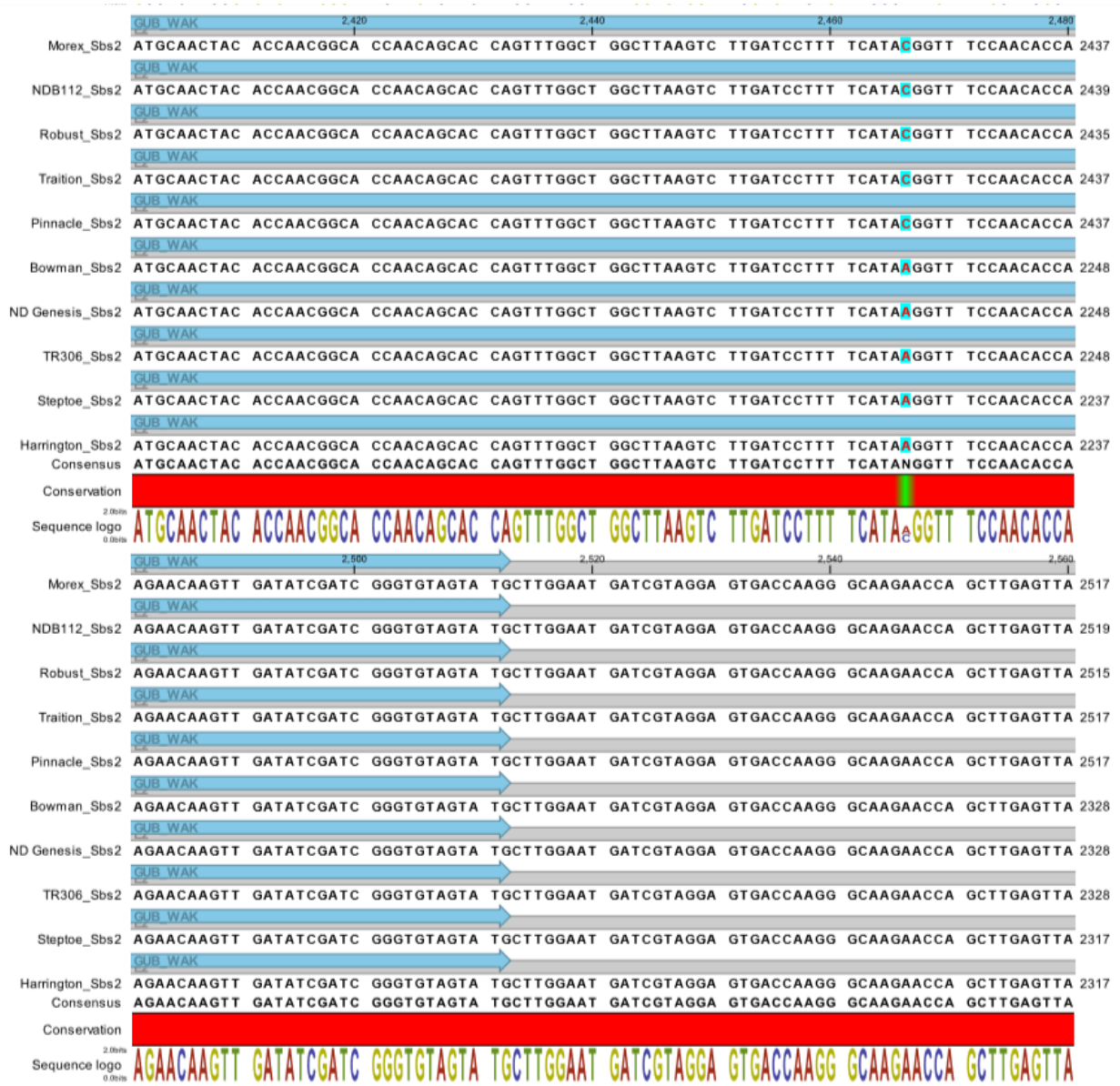
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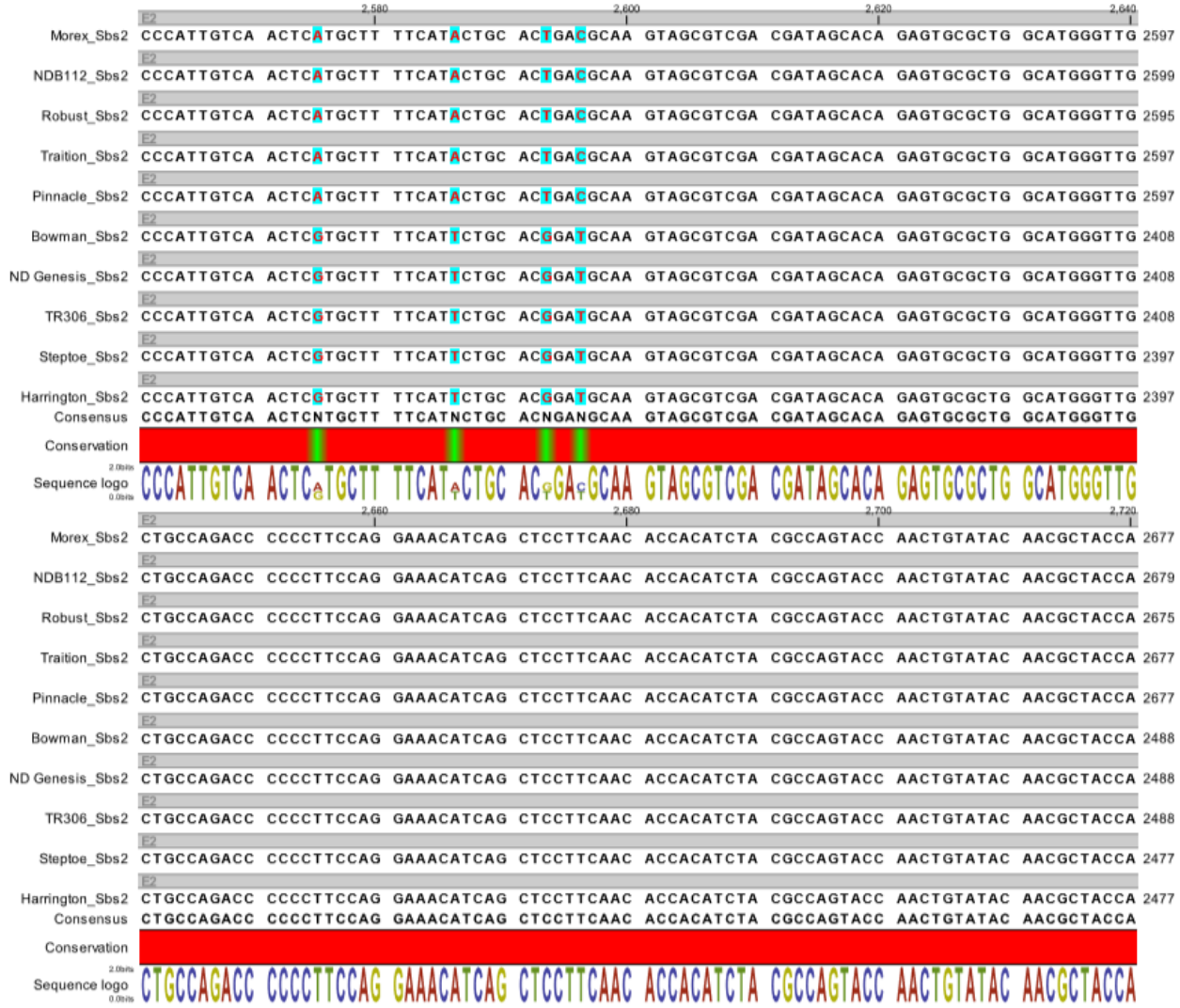
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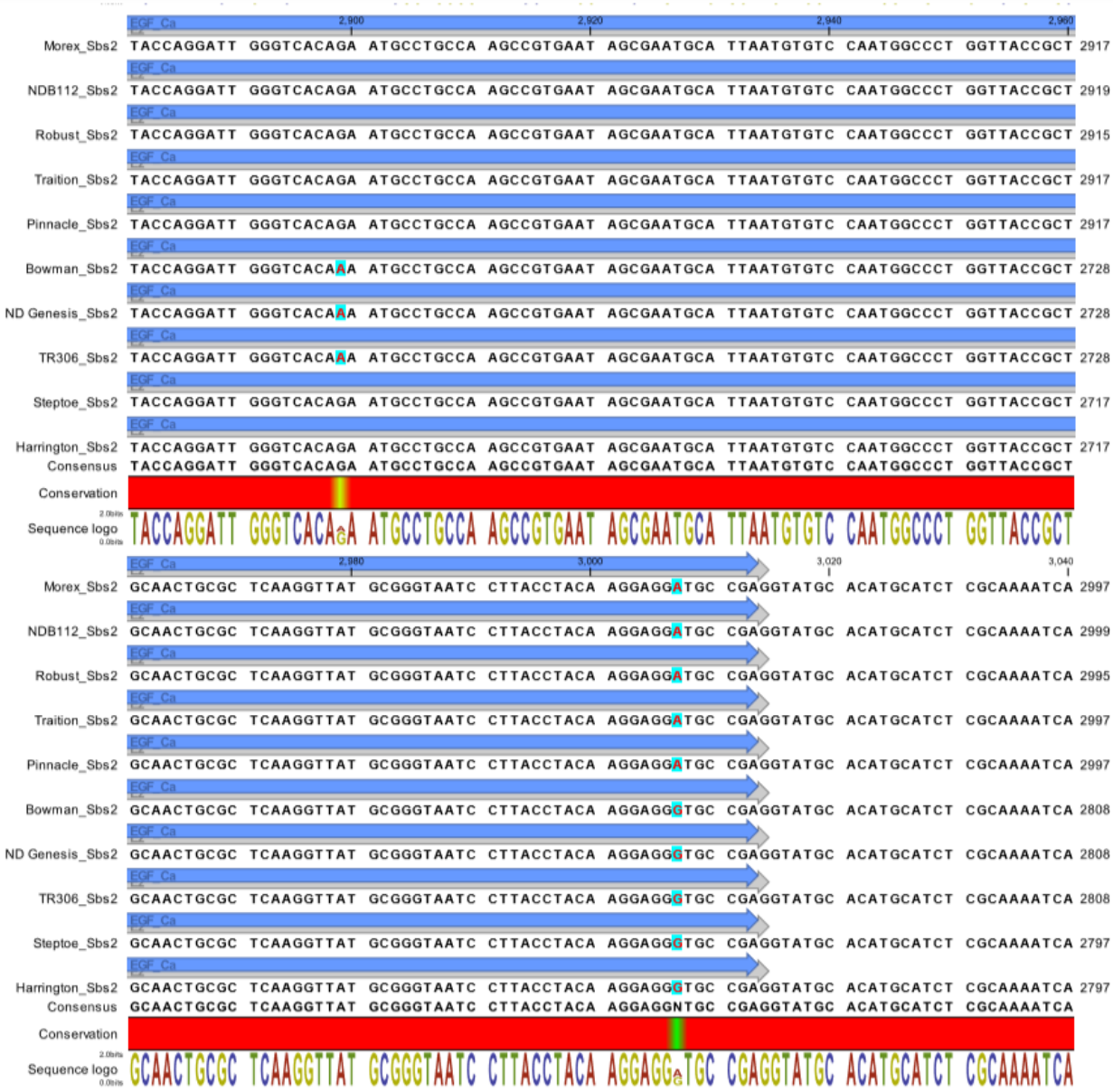
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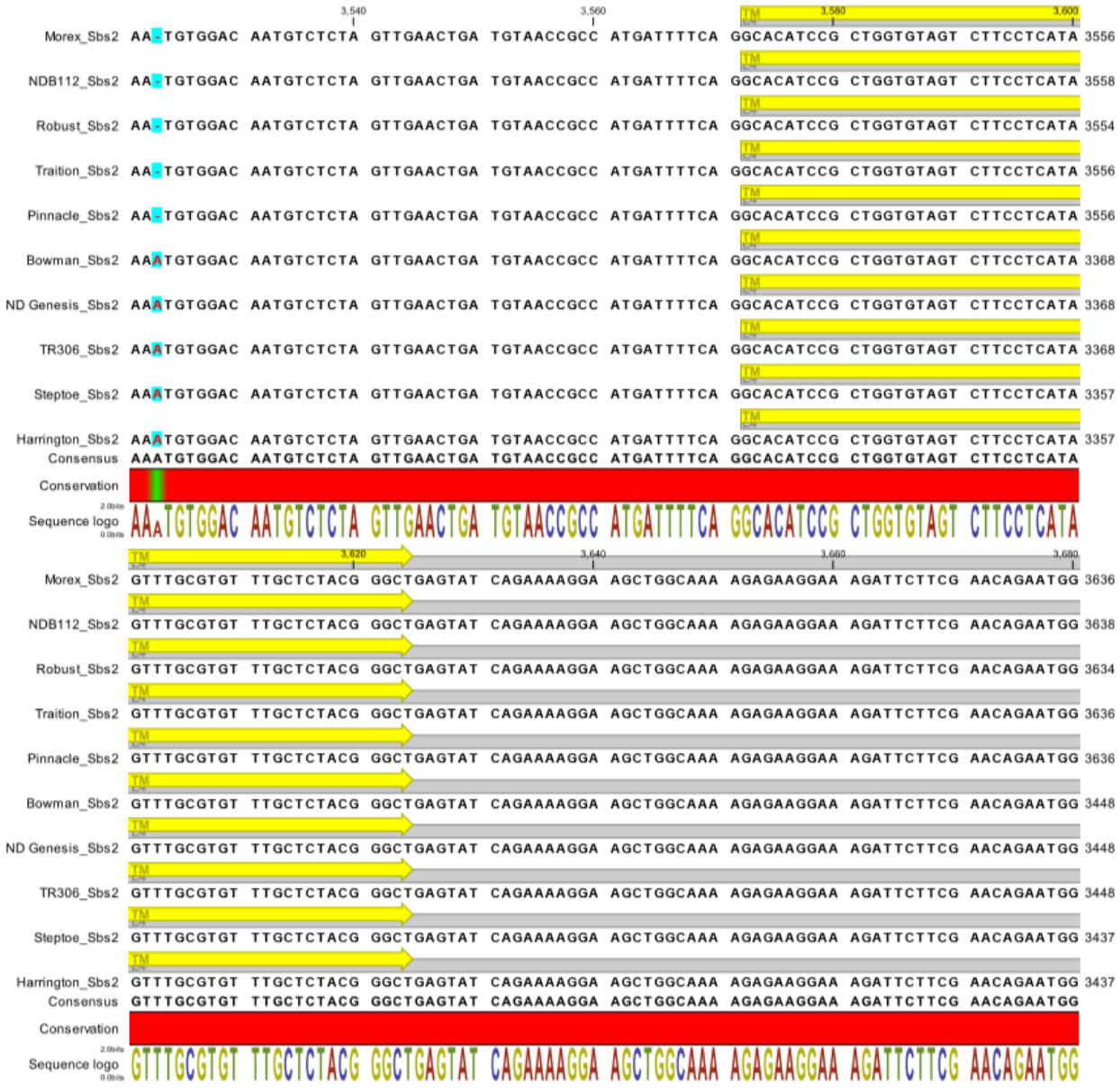
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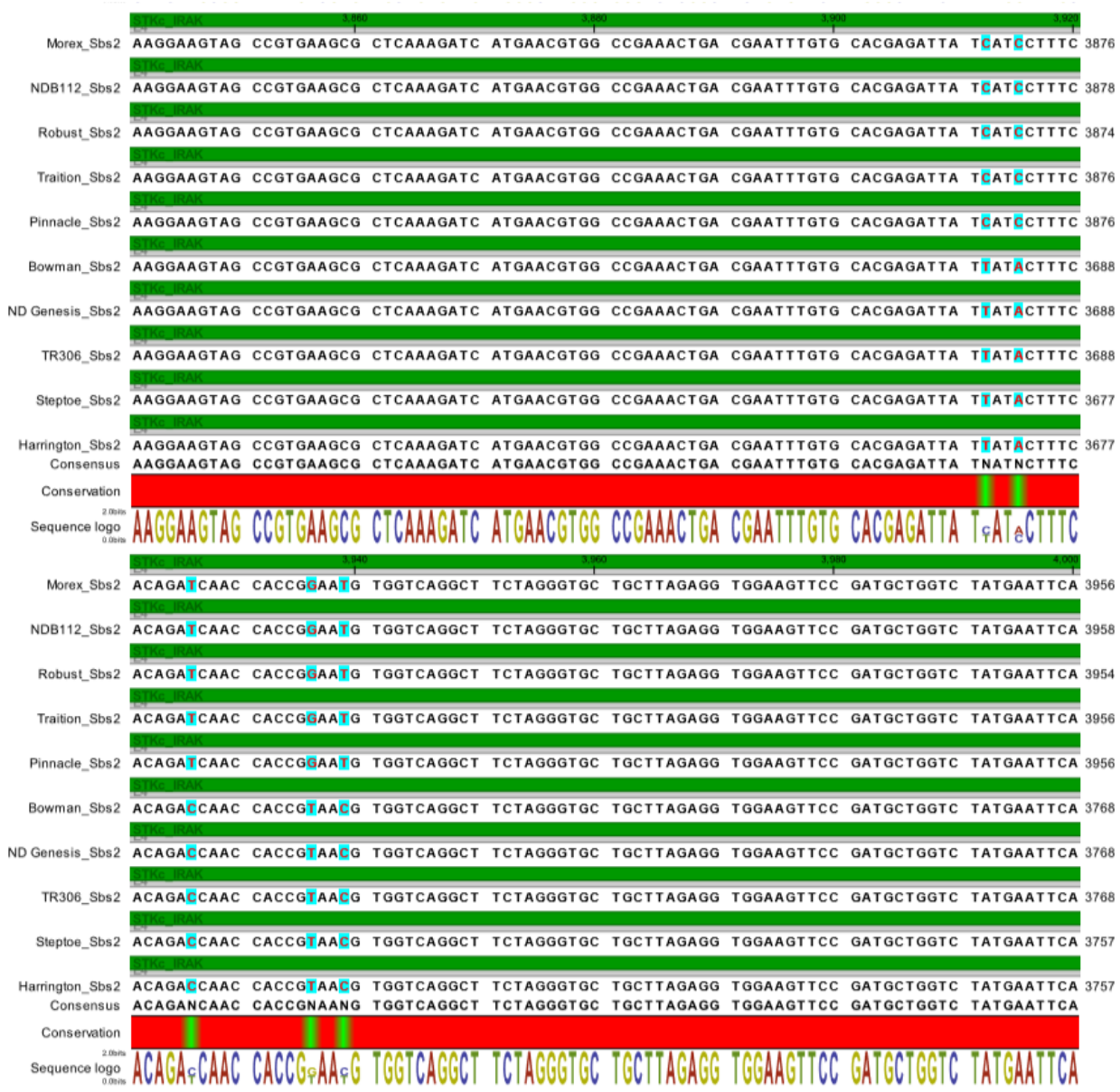
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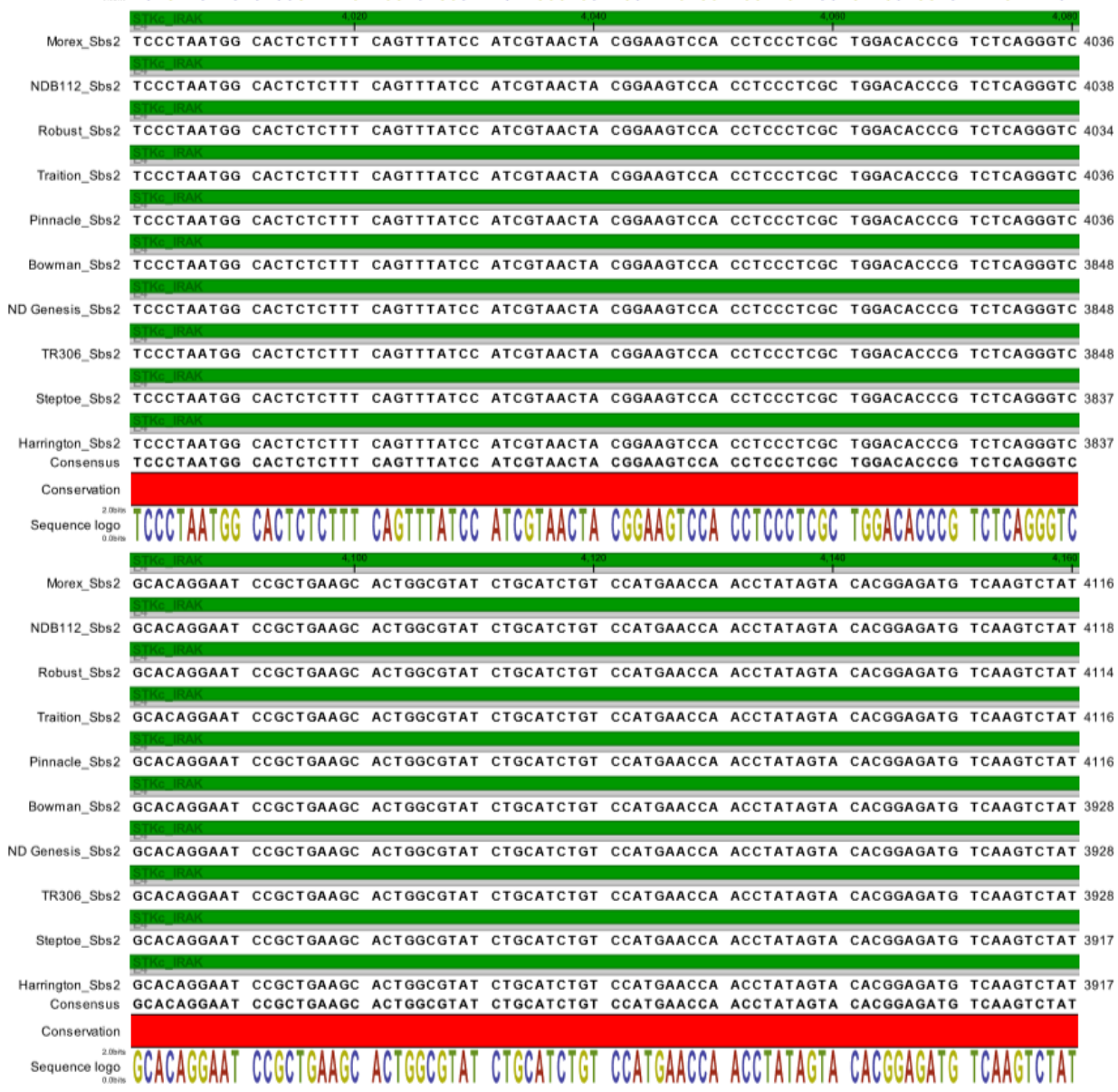
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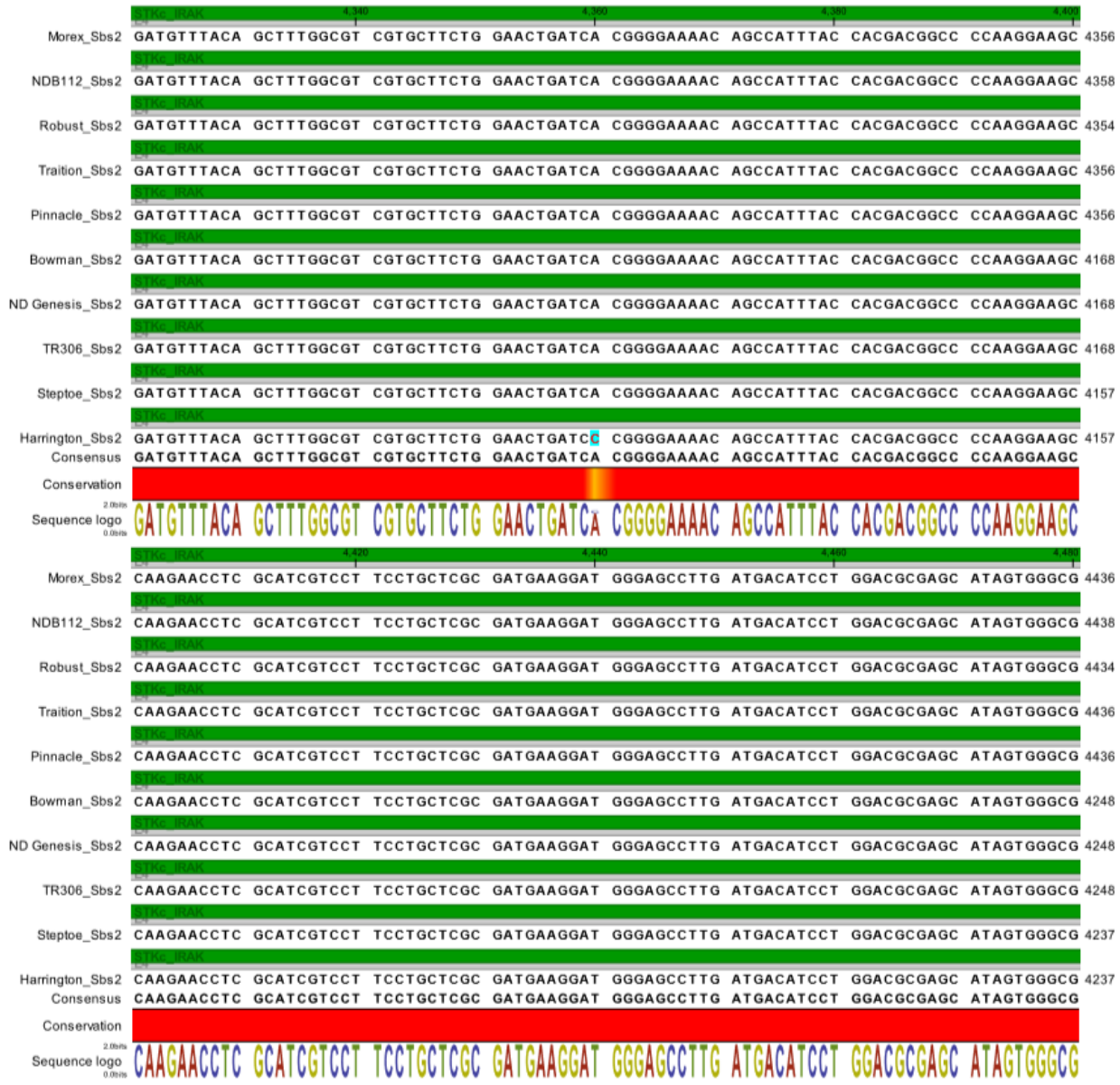
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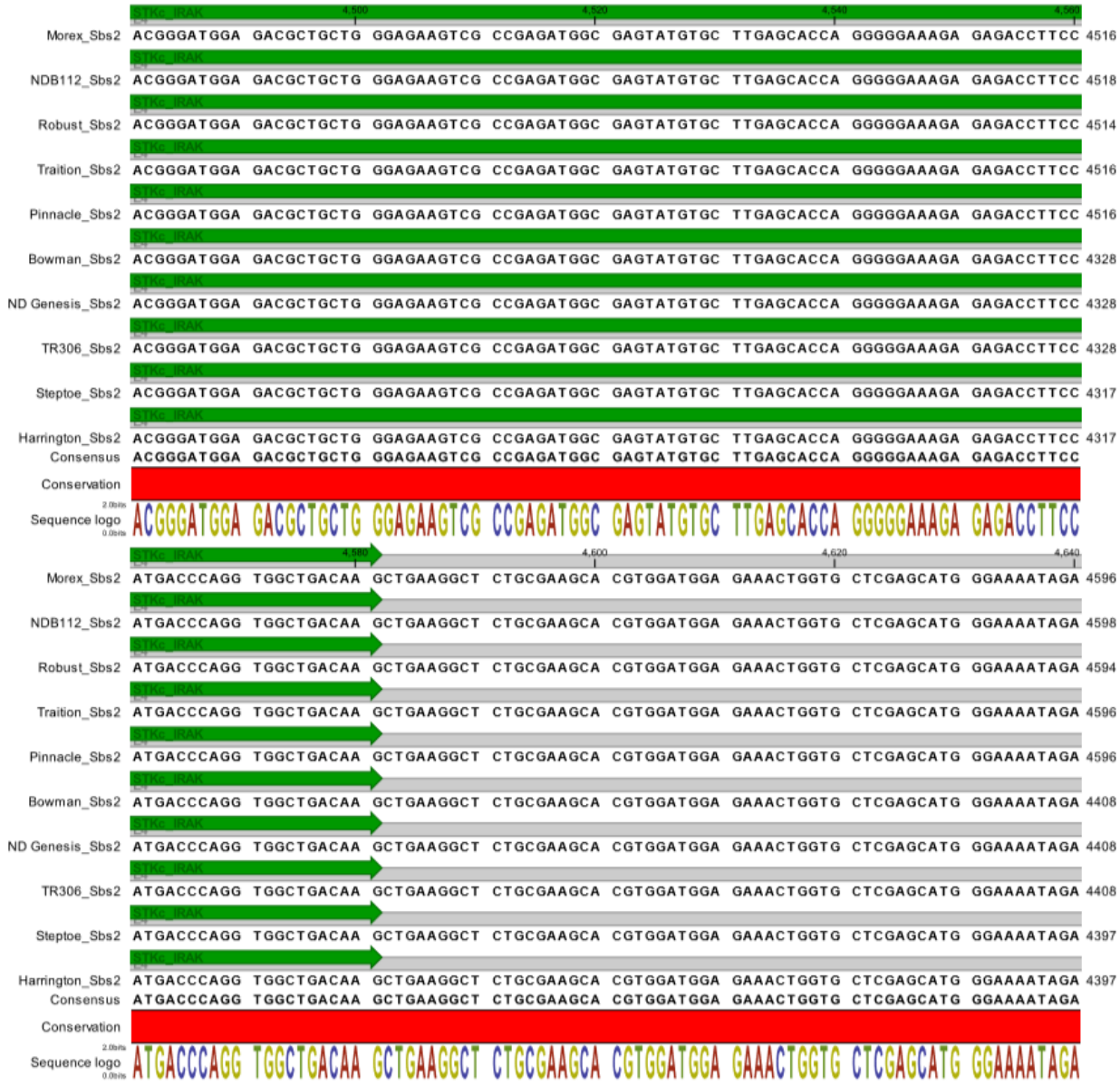
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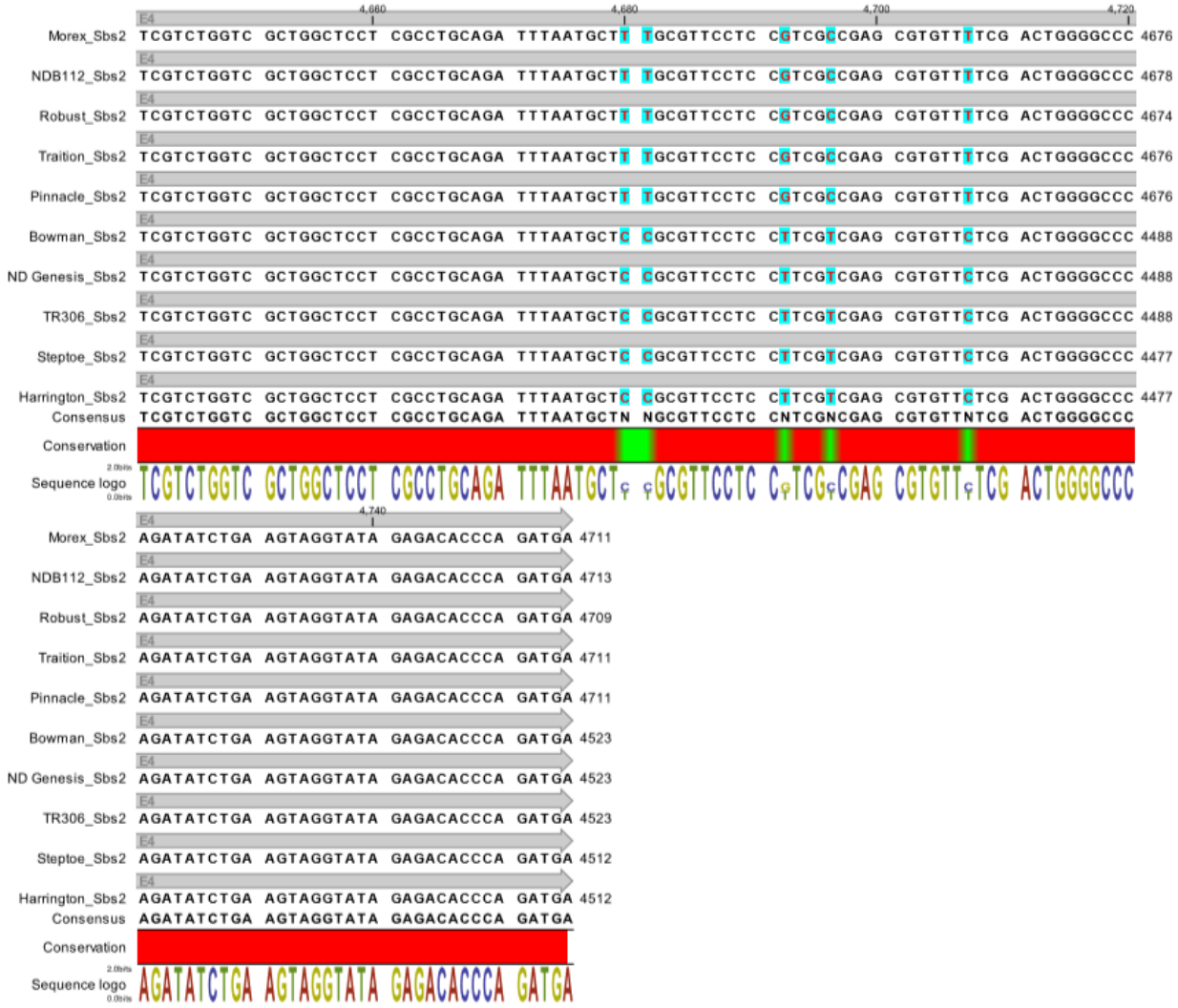
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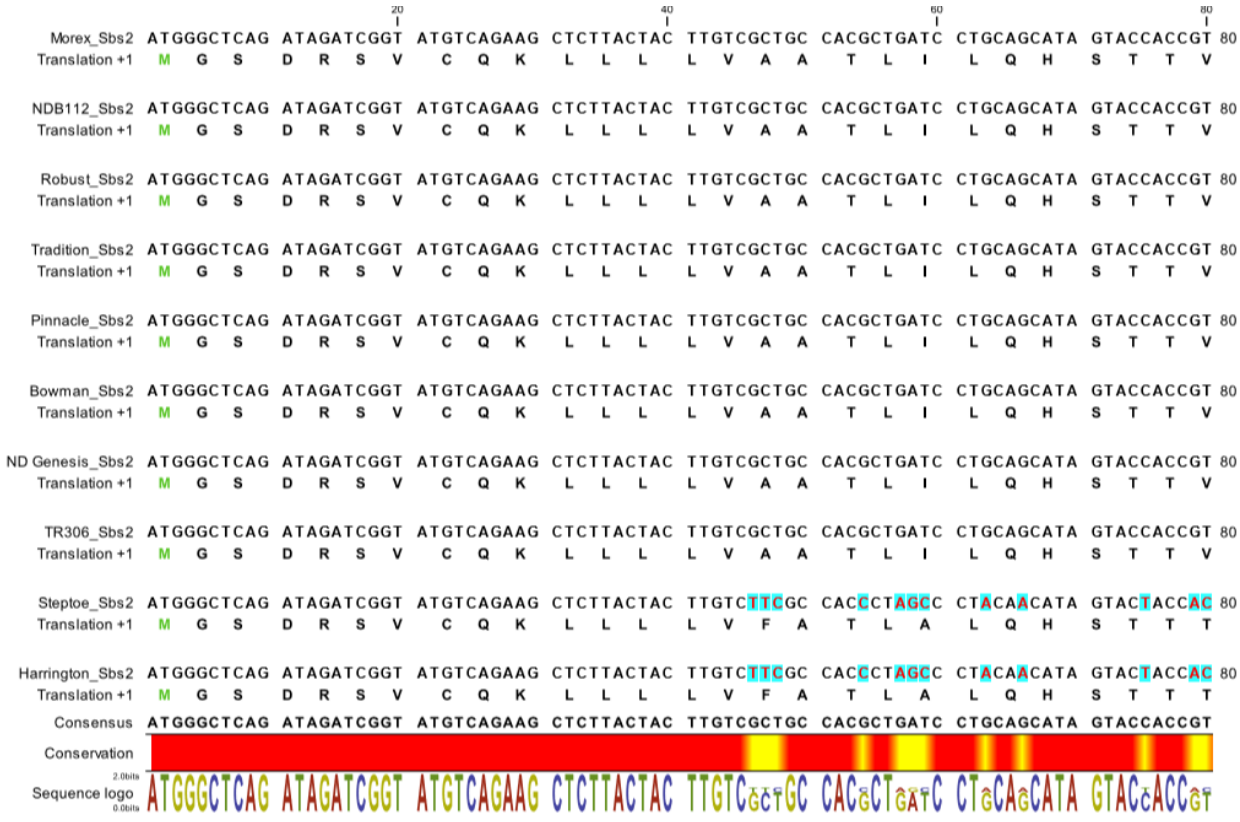
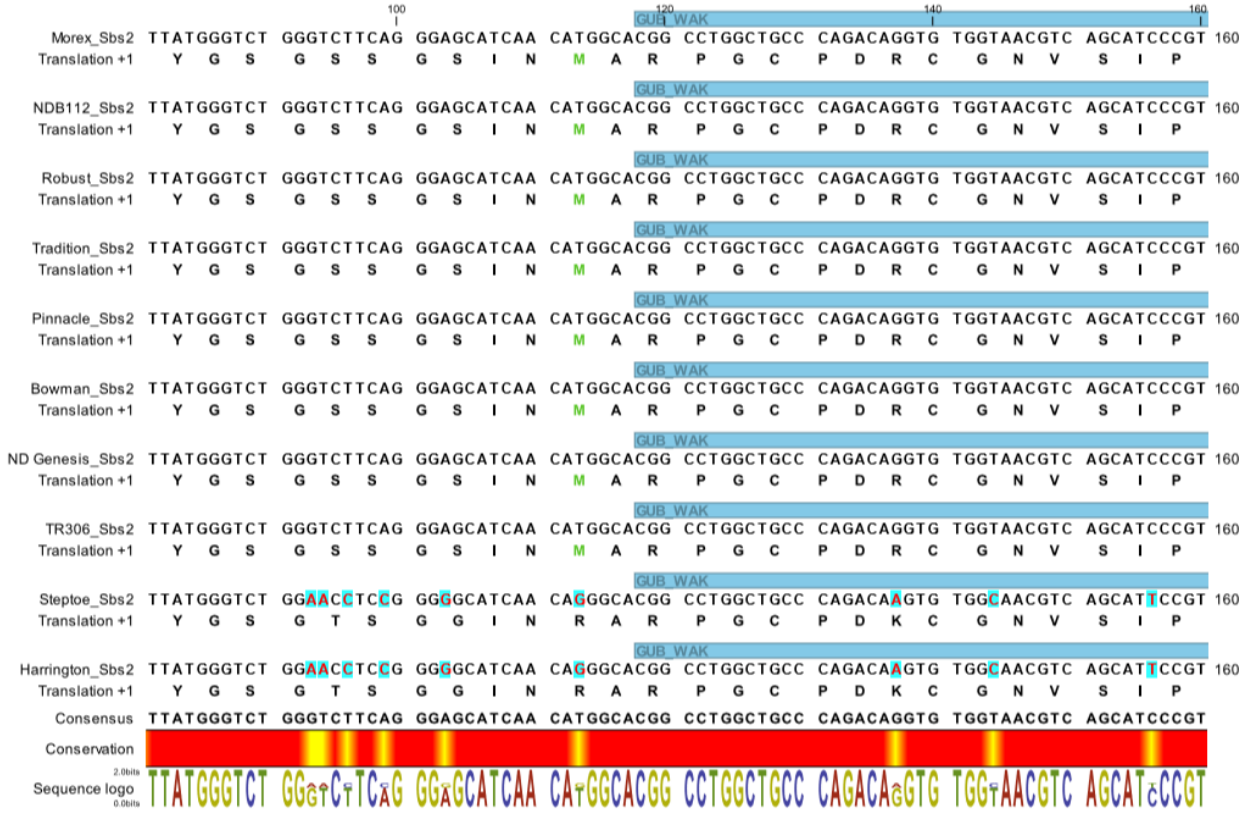
Supplementary Figure 6B.

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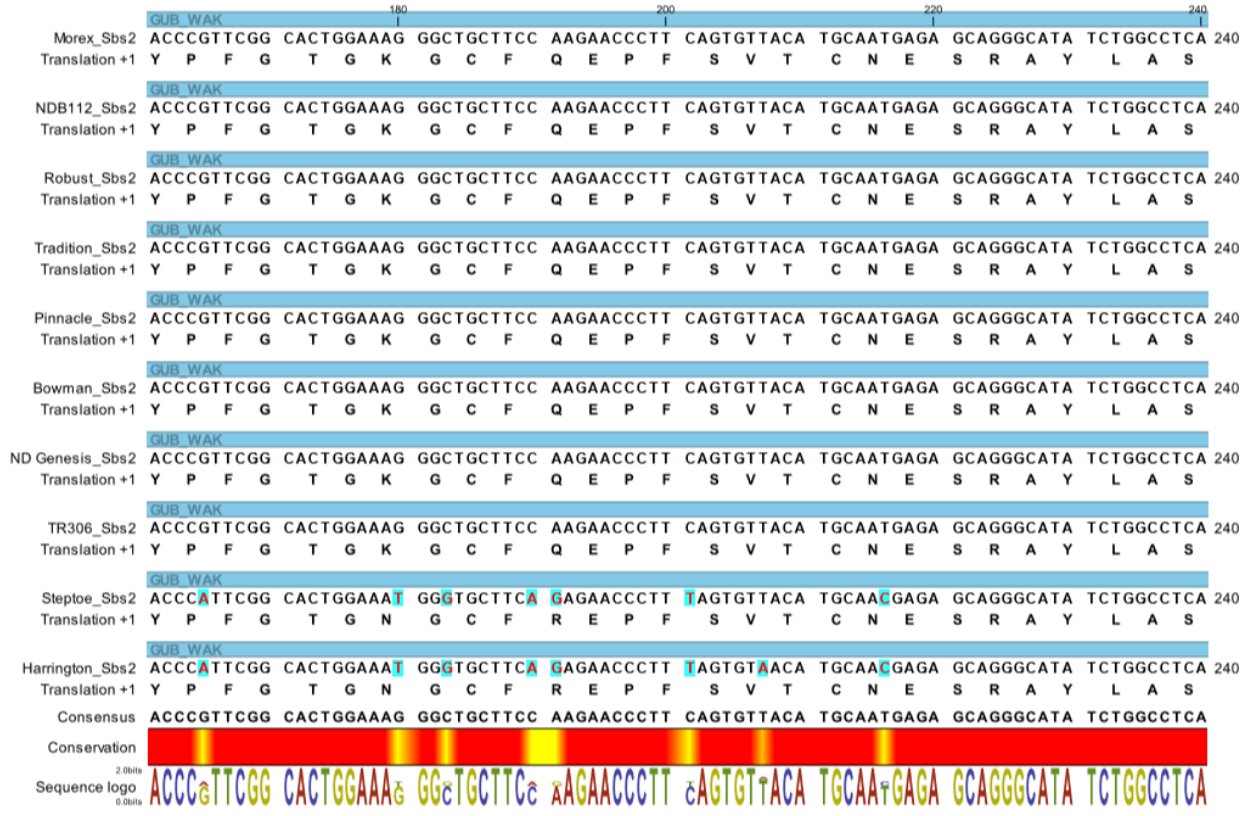
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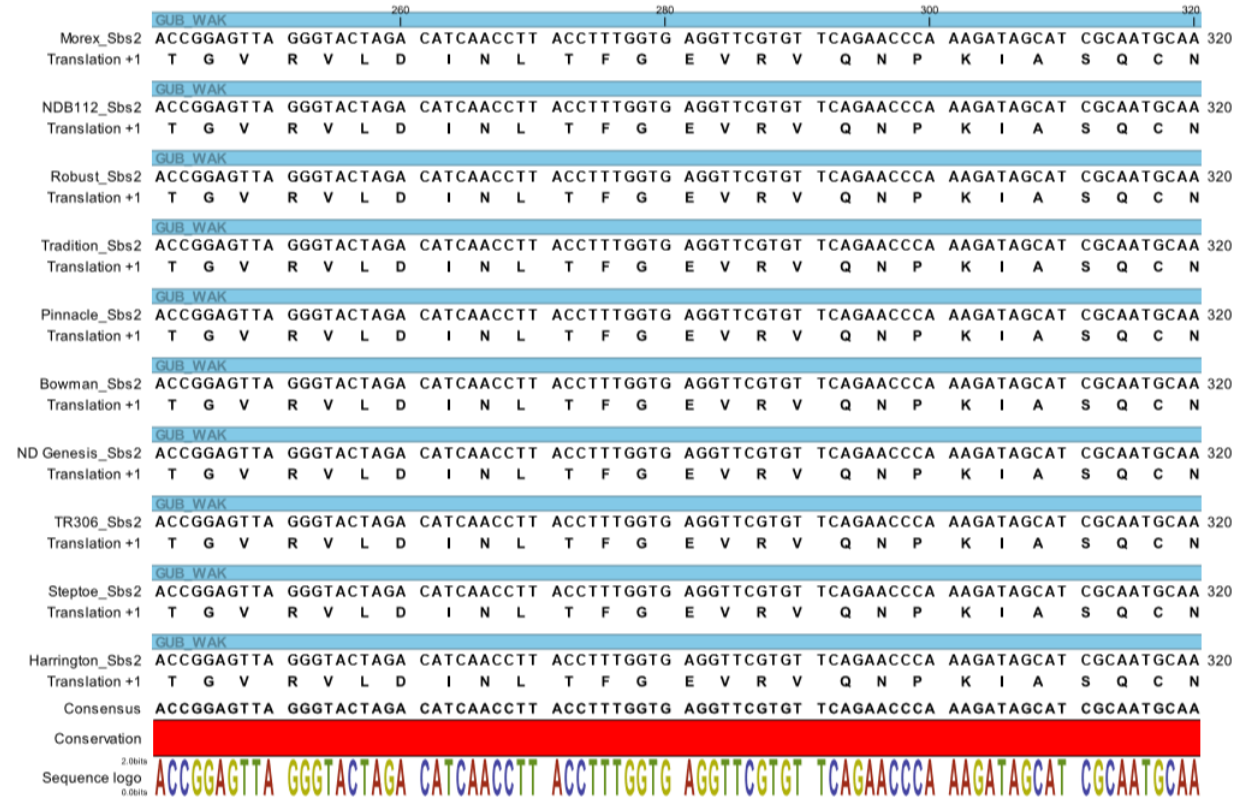
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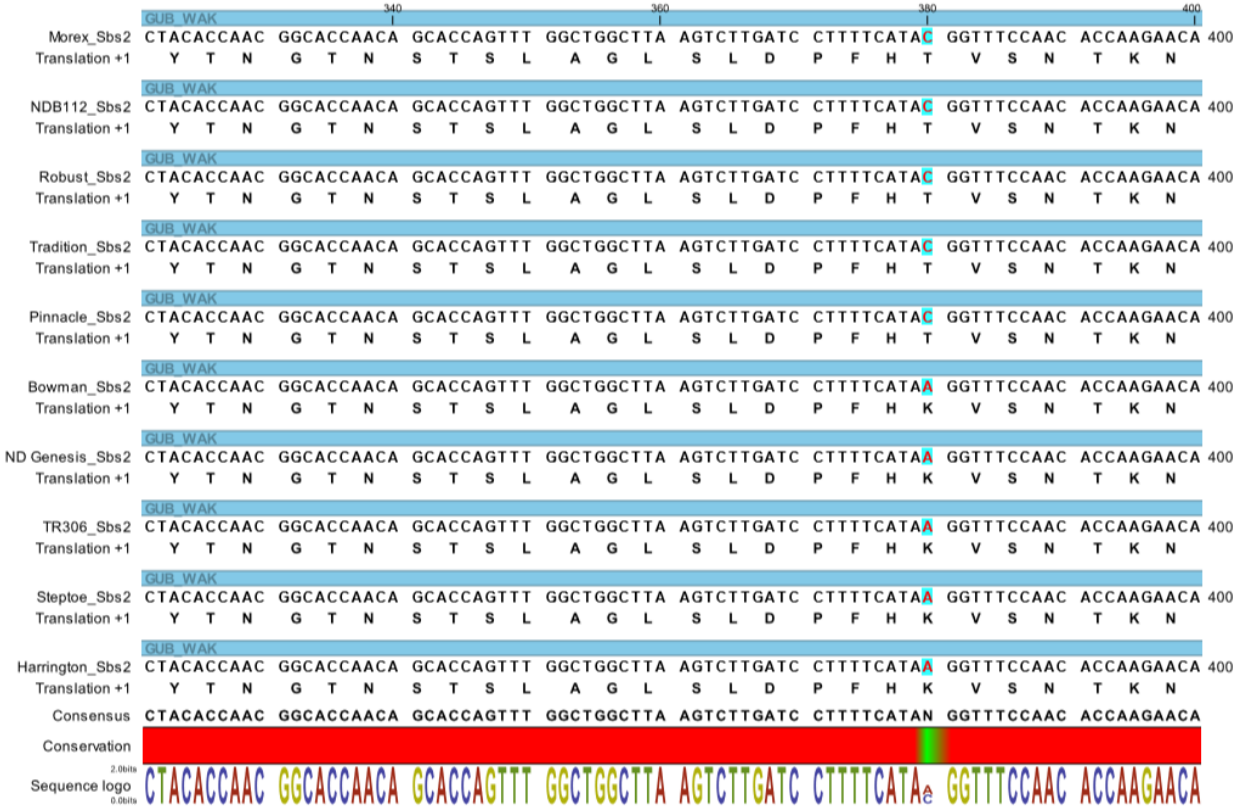
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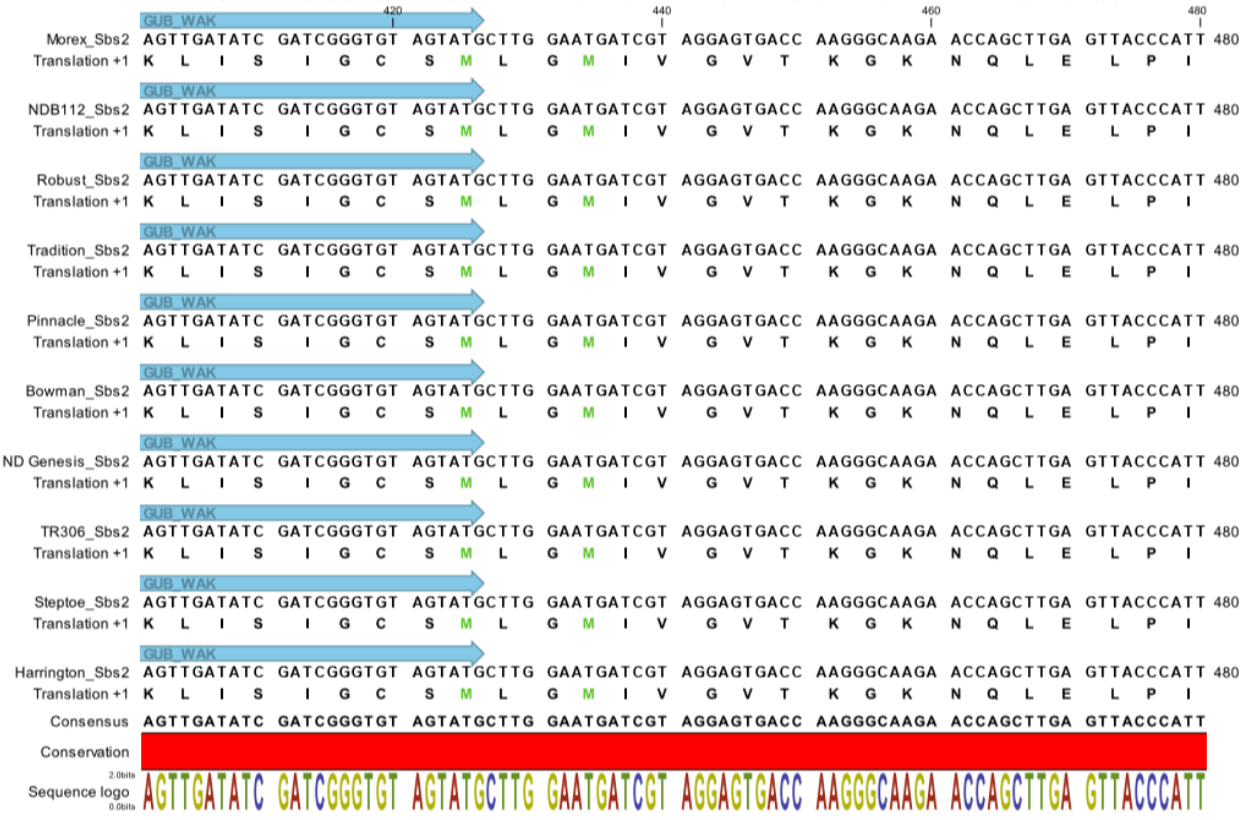
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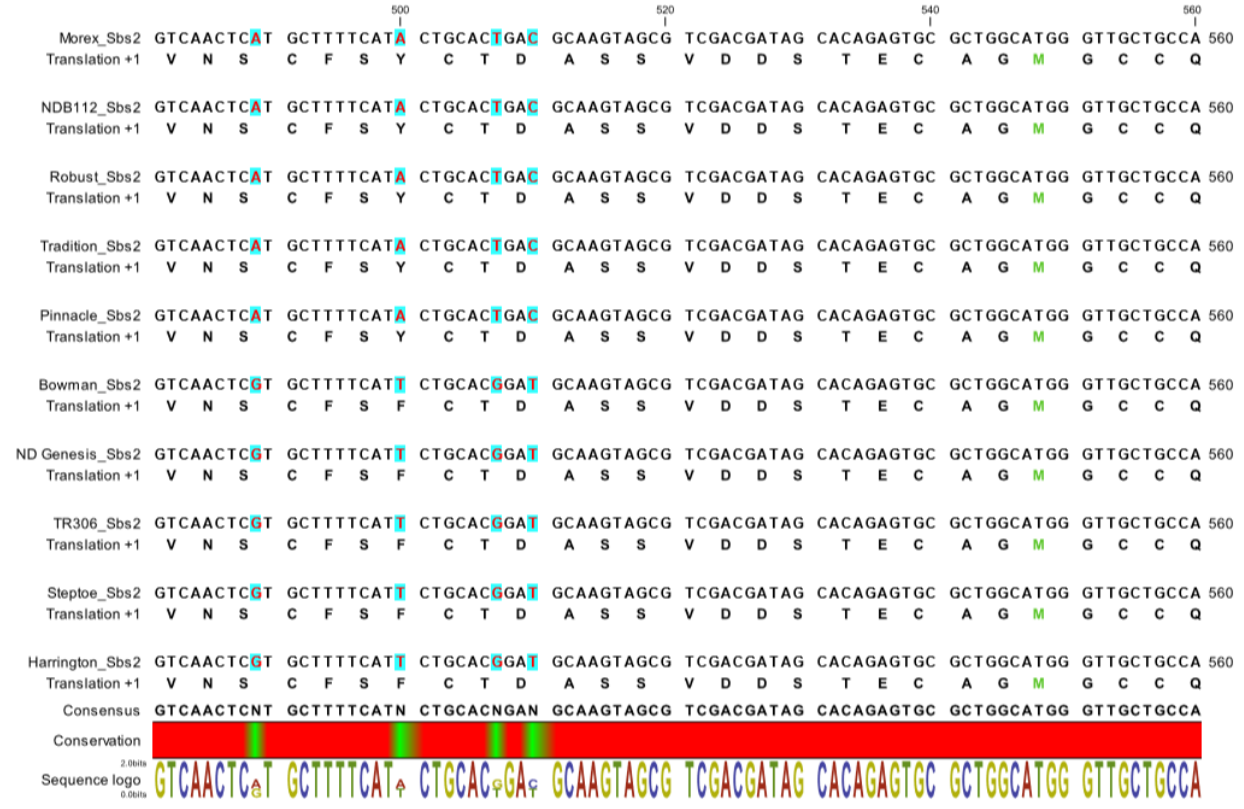
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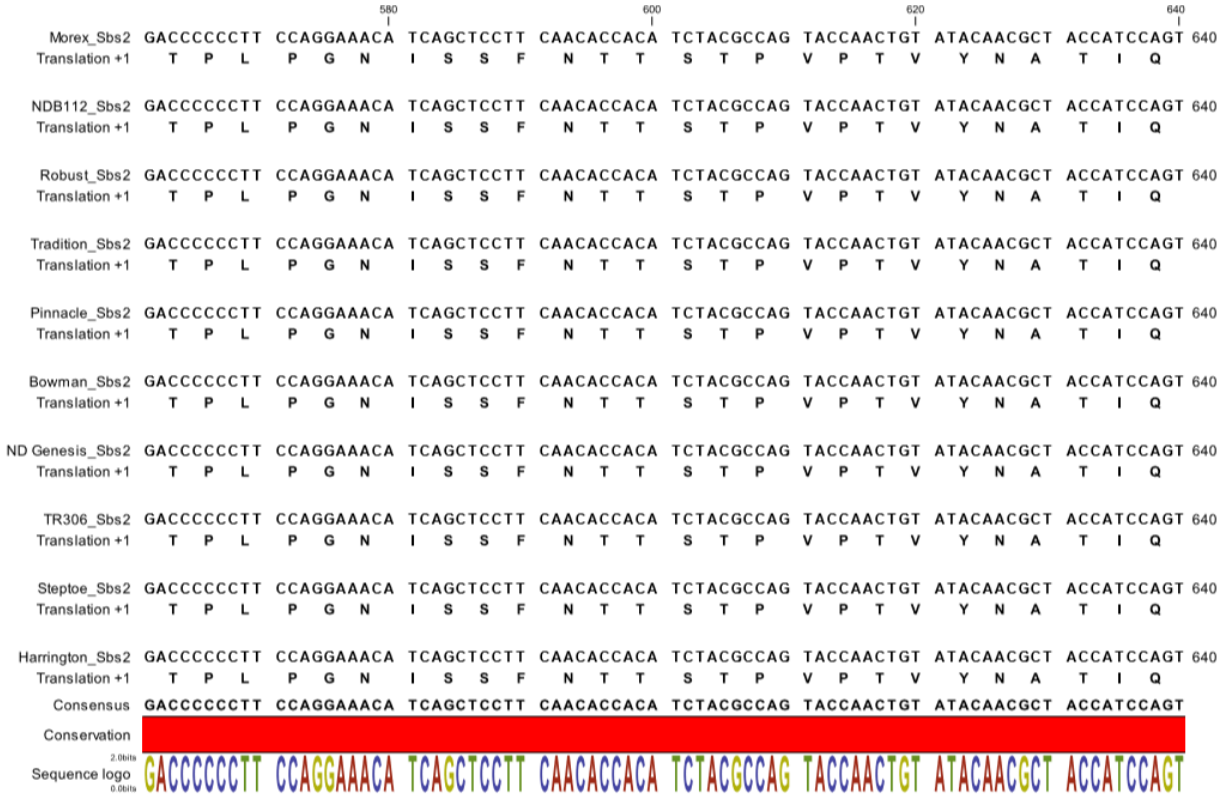
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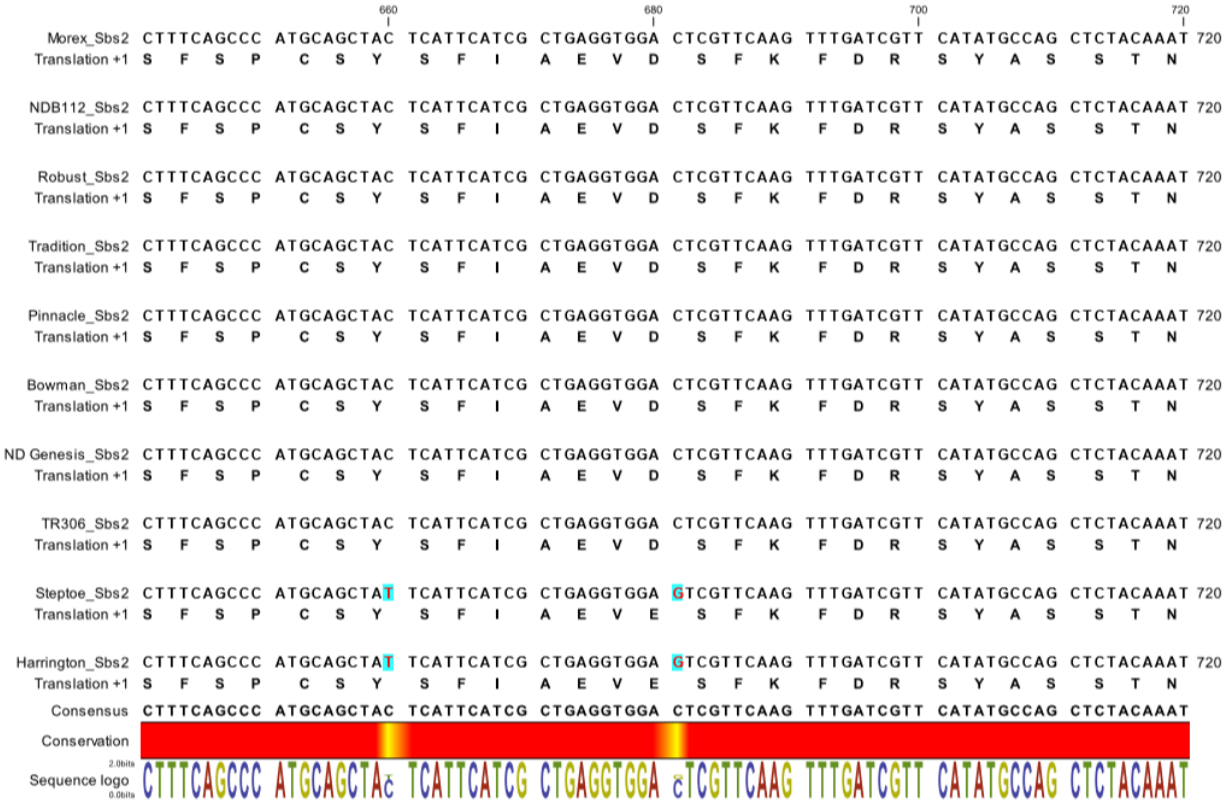
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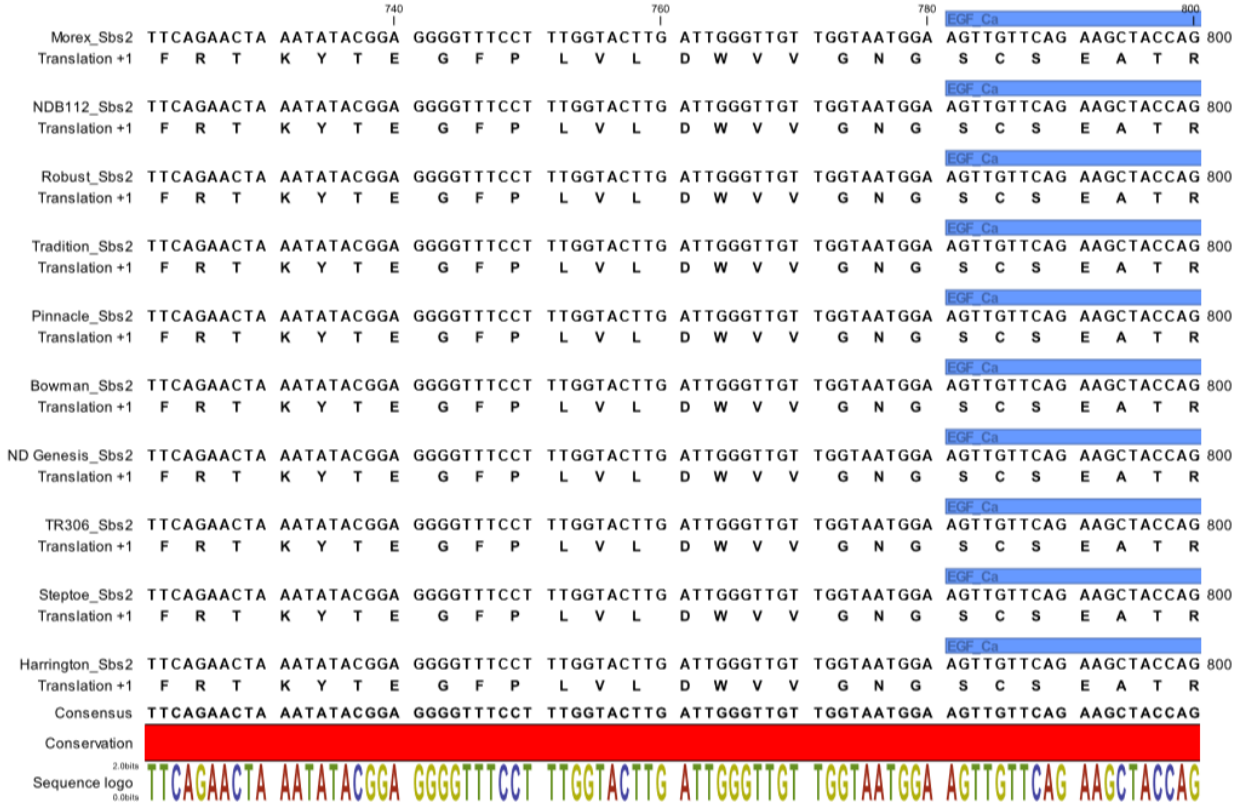
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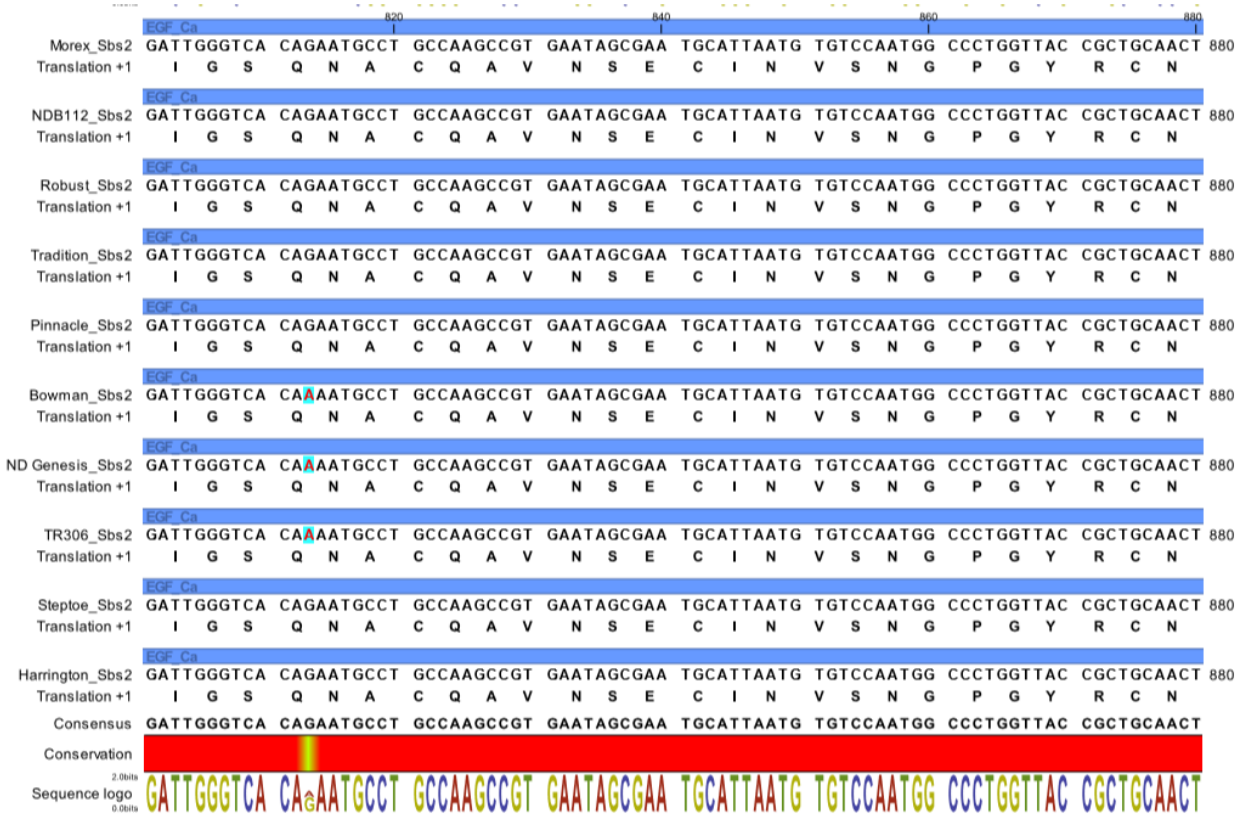
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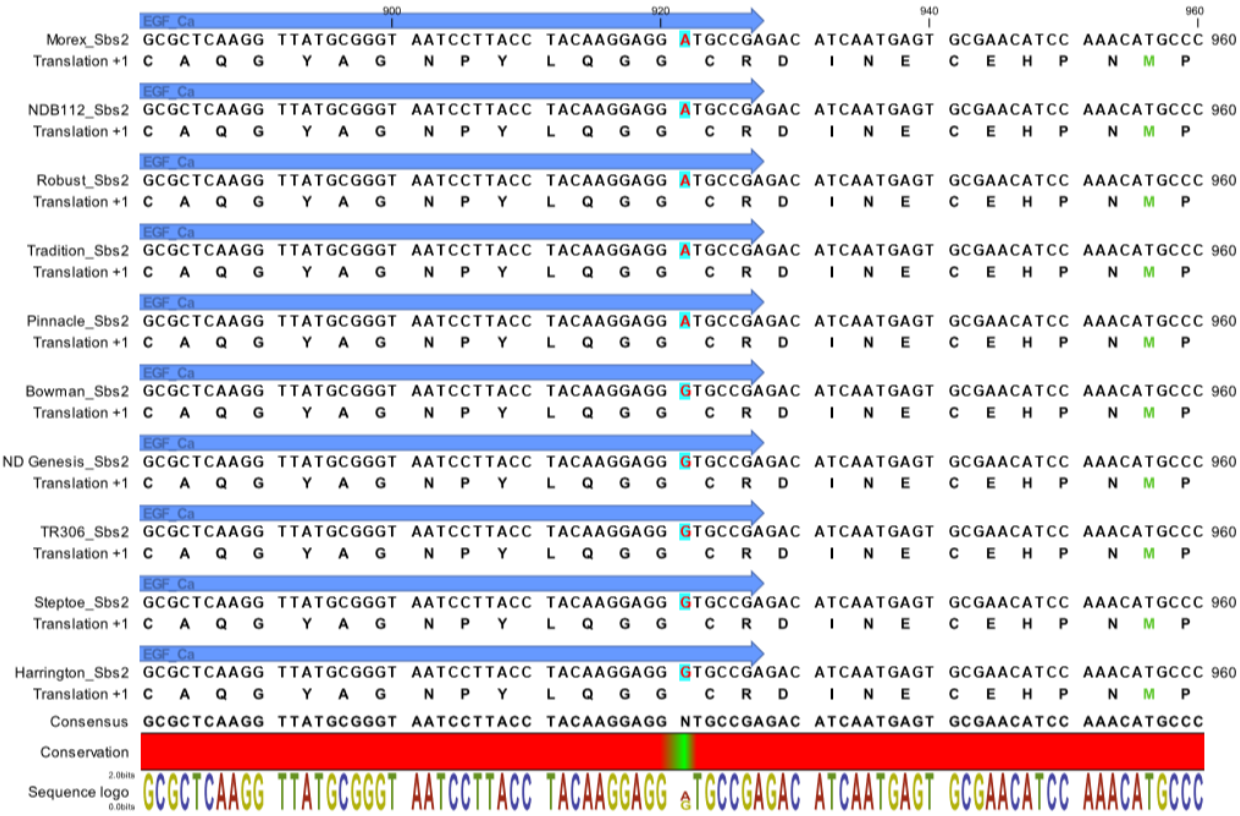
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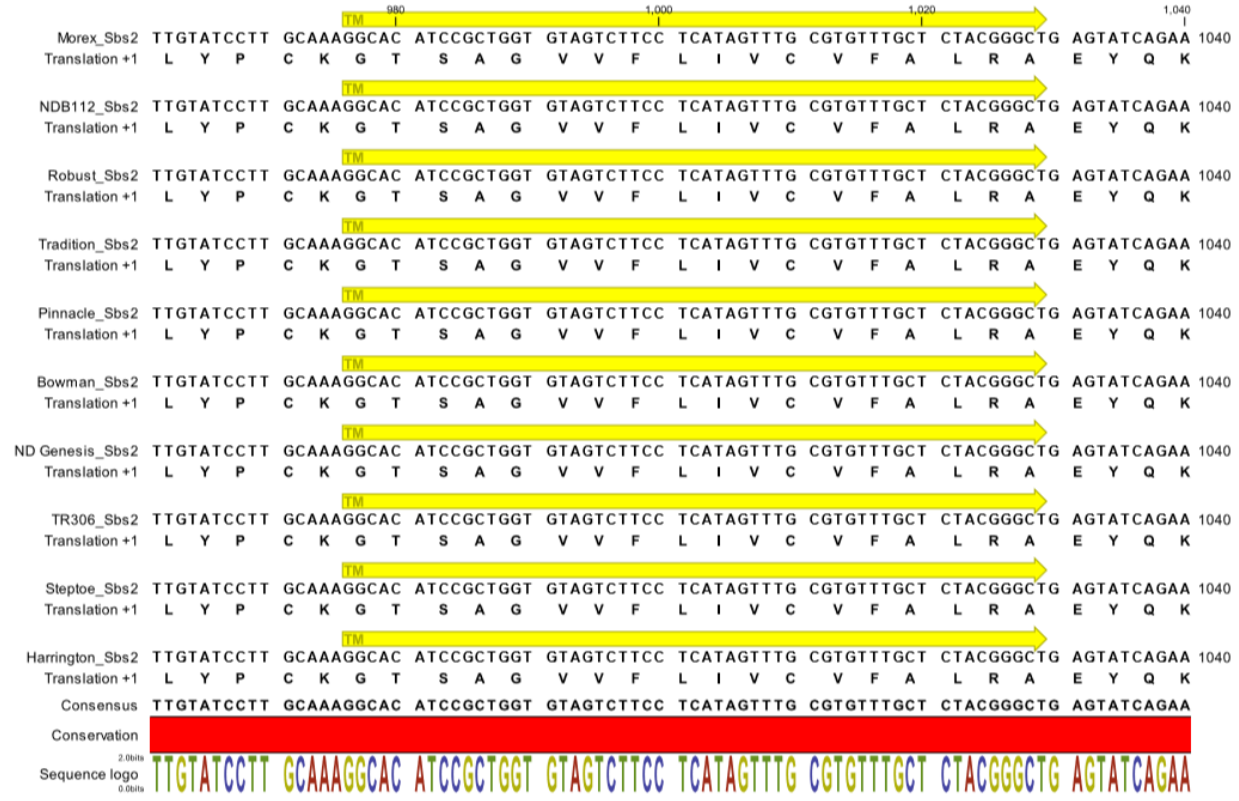
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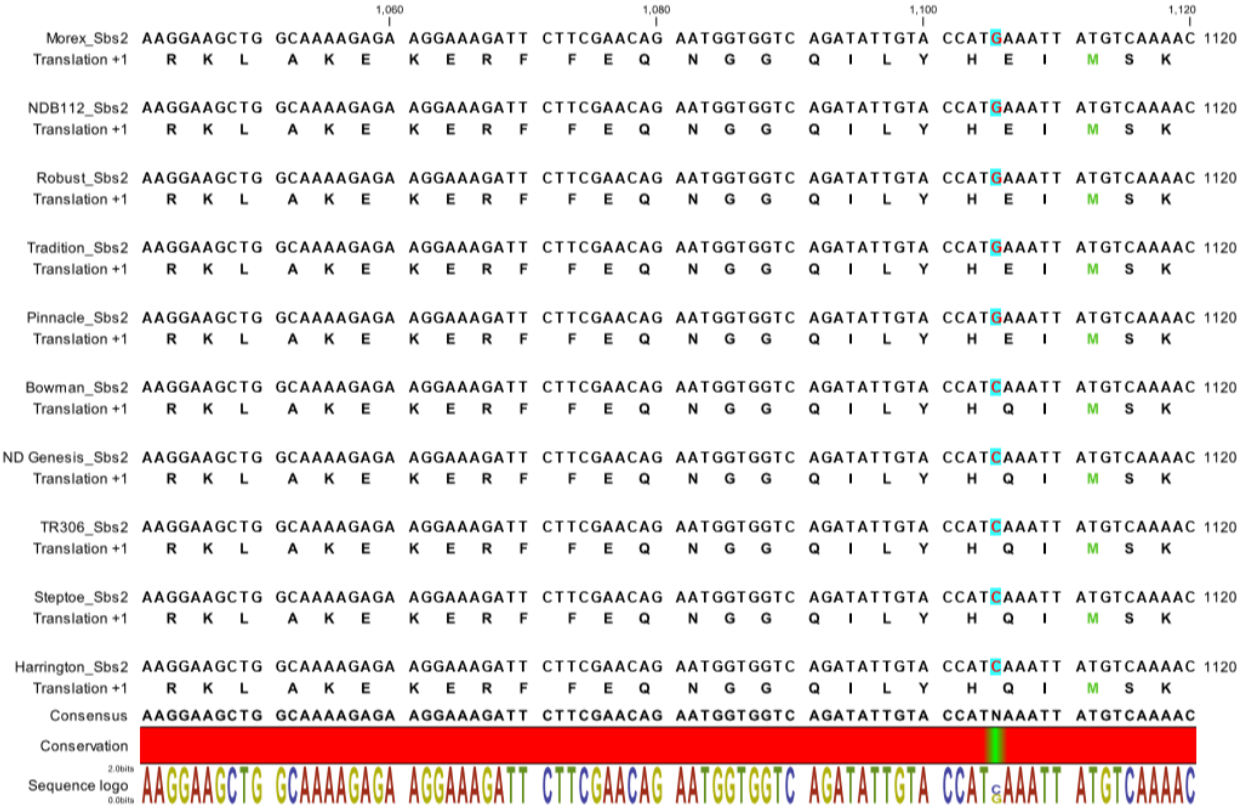
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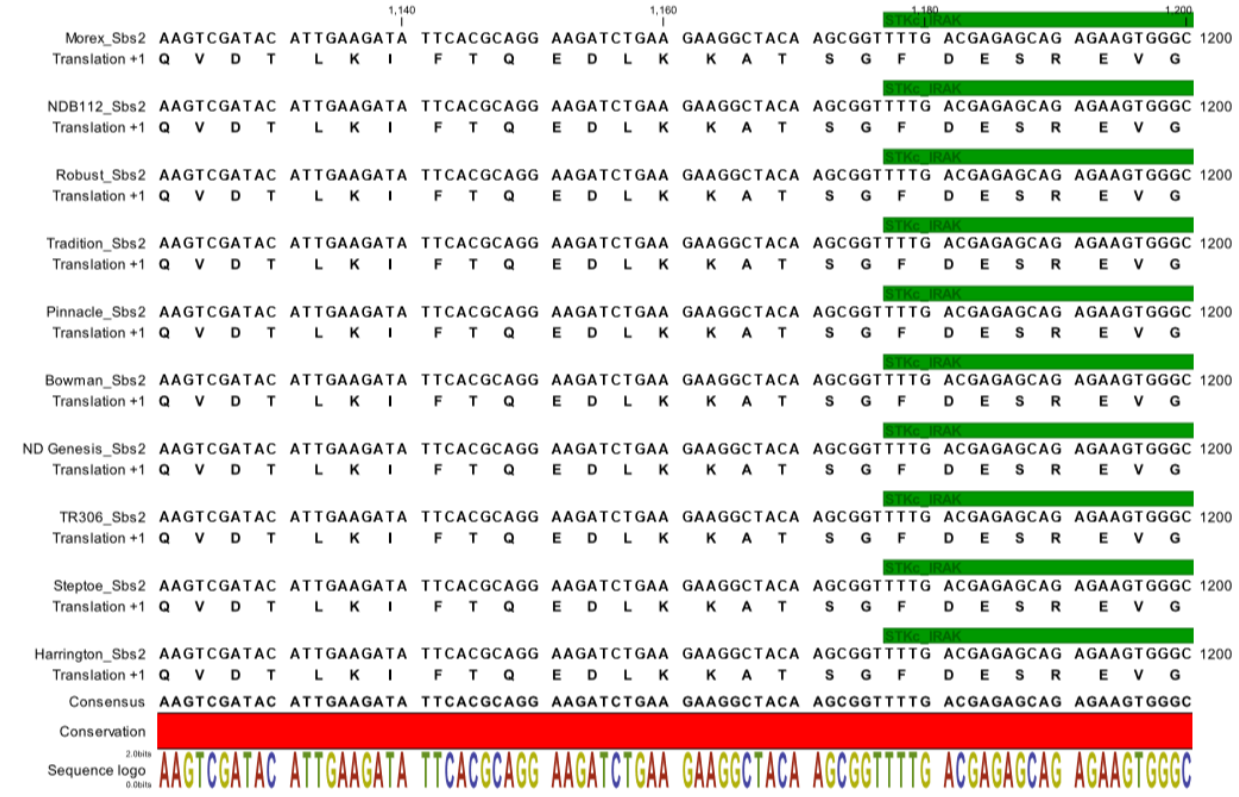
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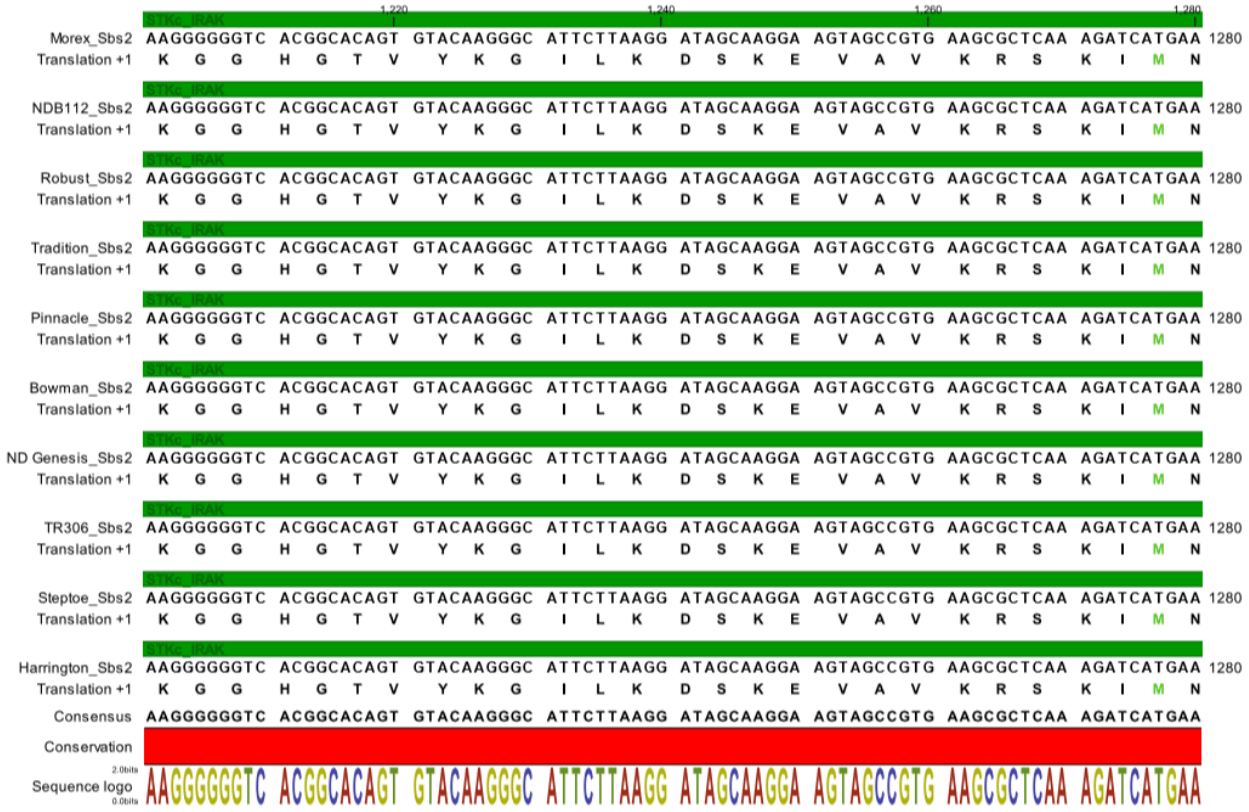
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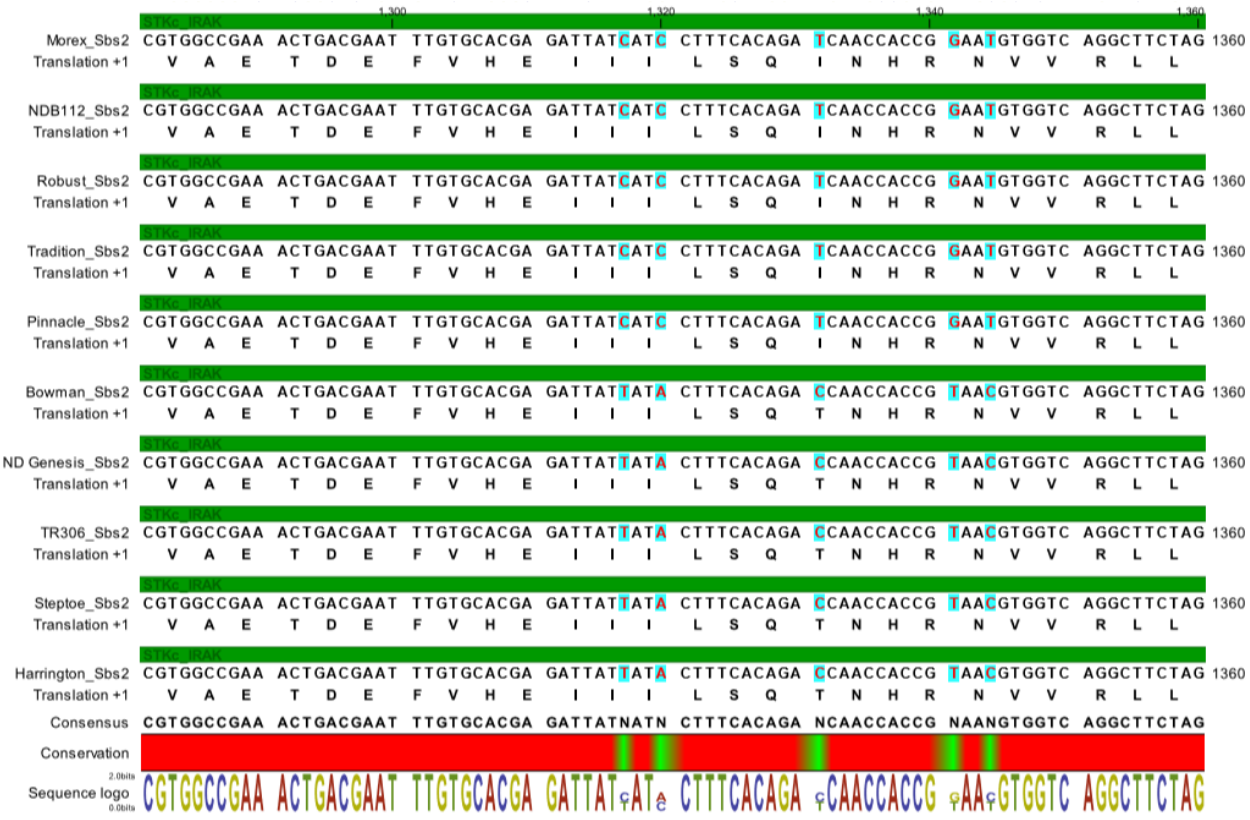
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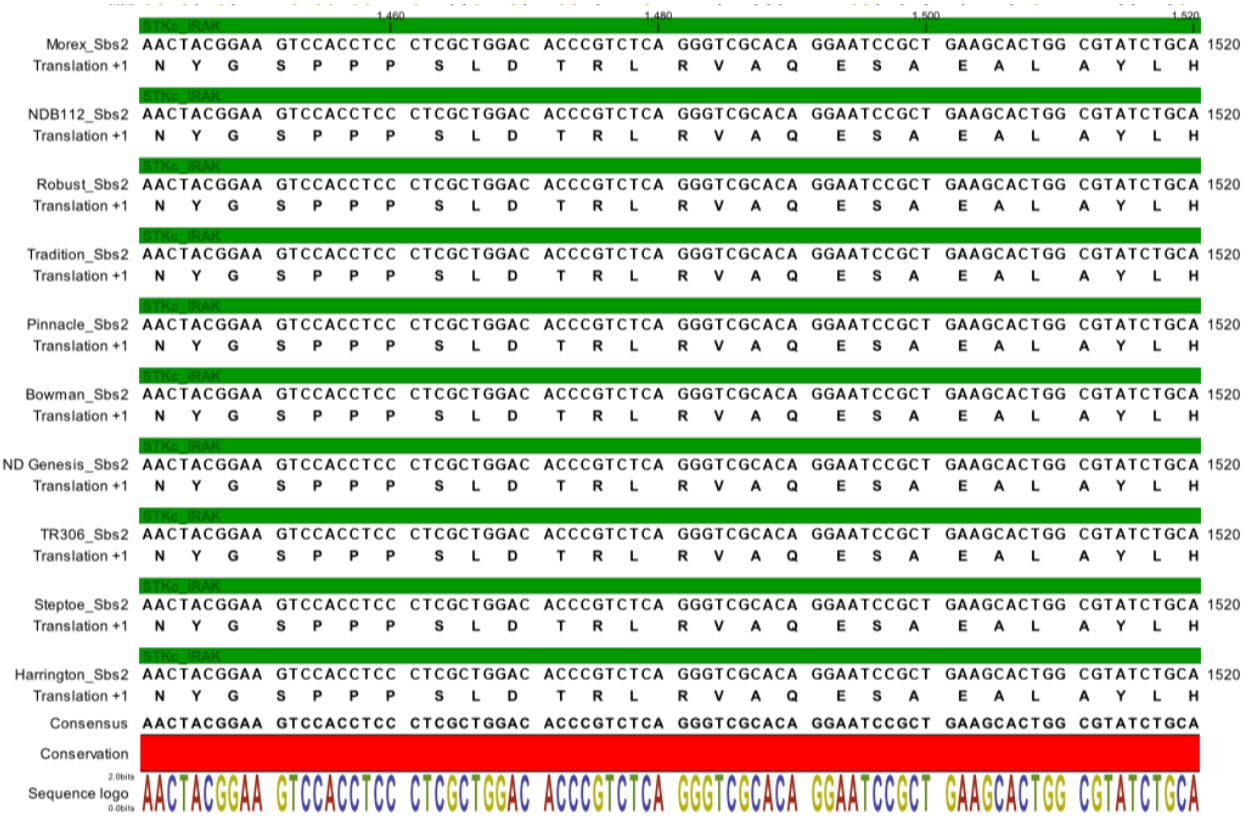
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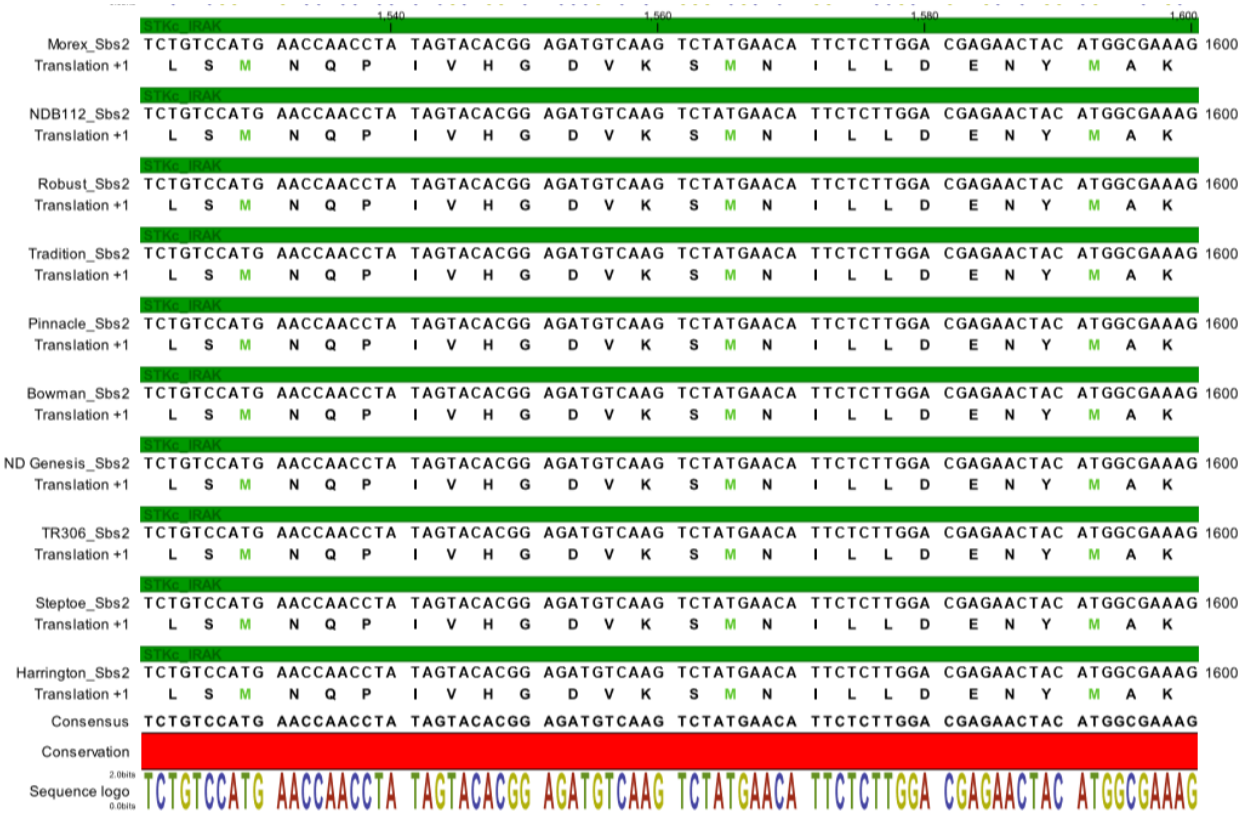
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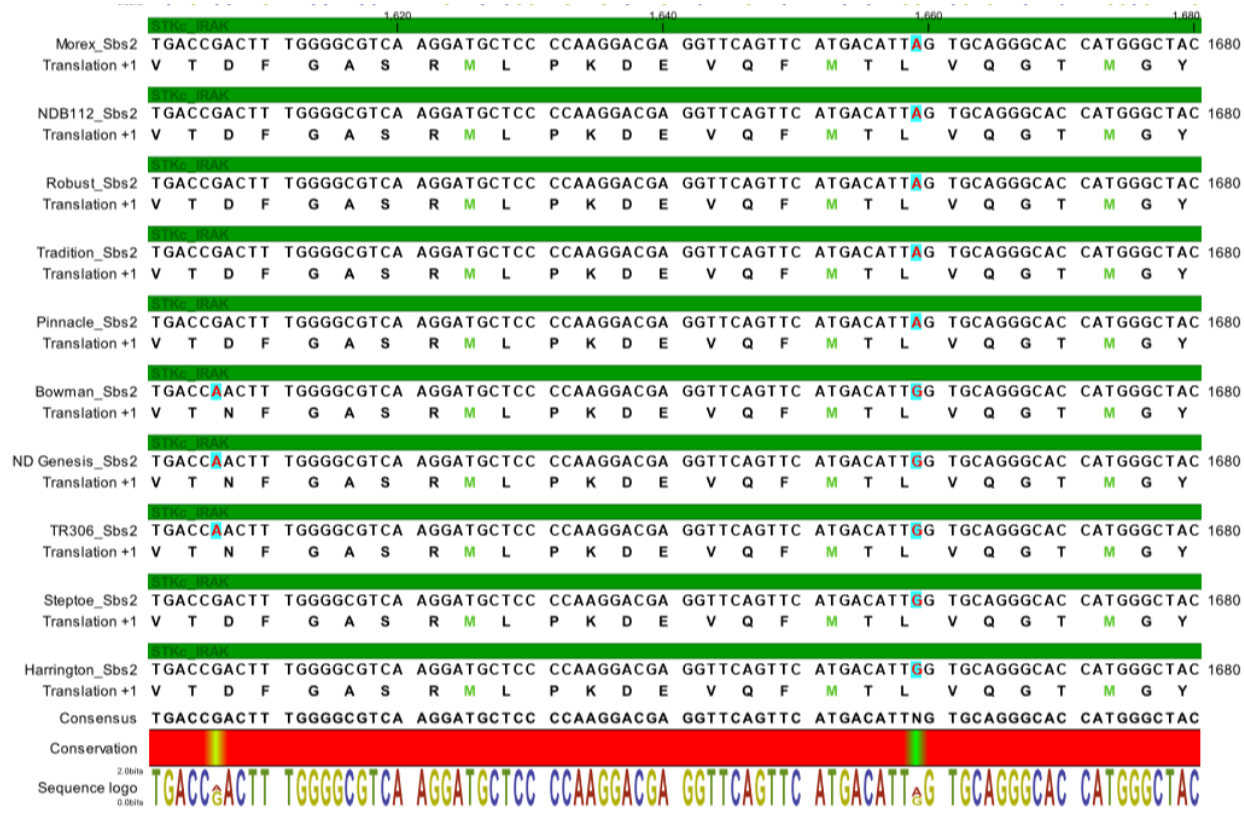
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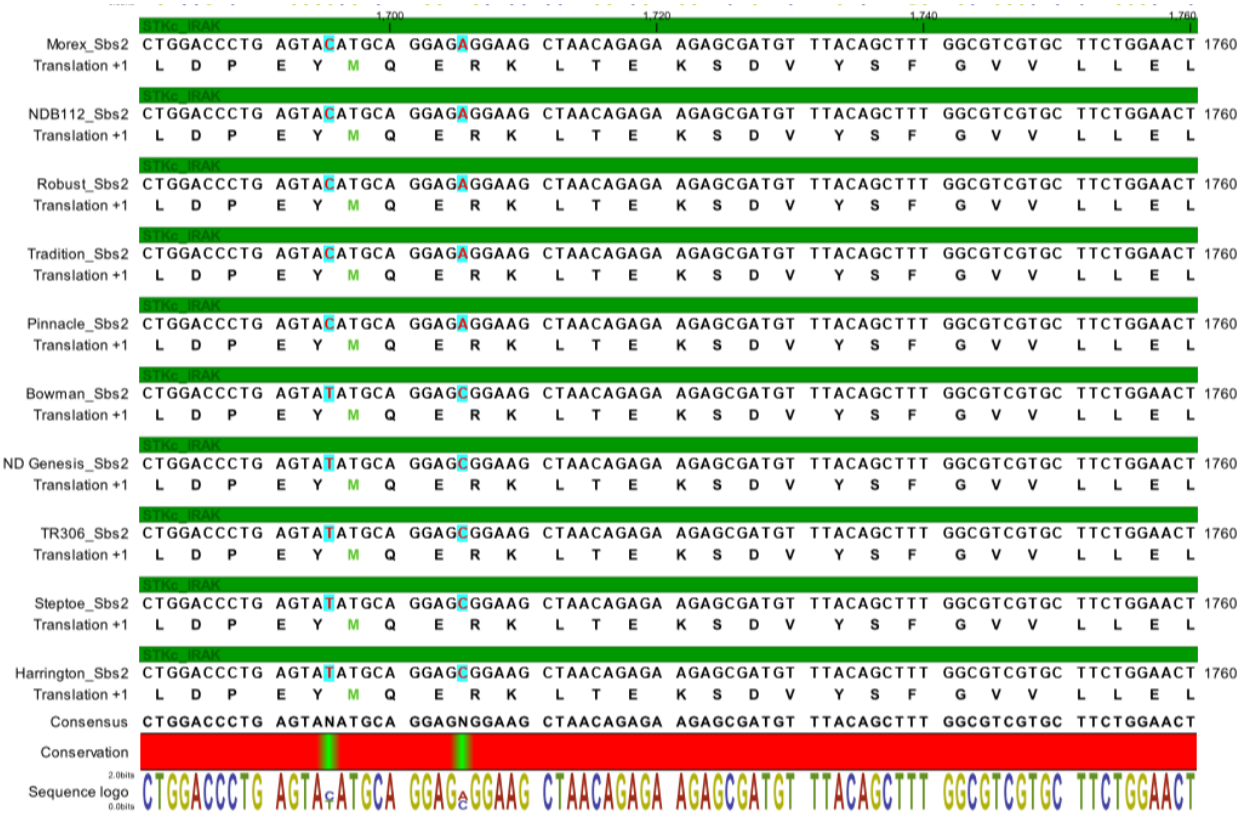
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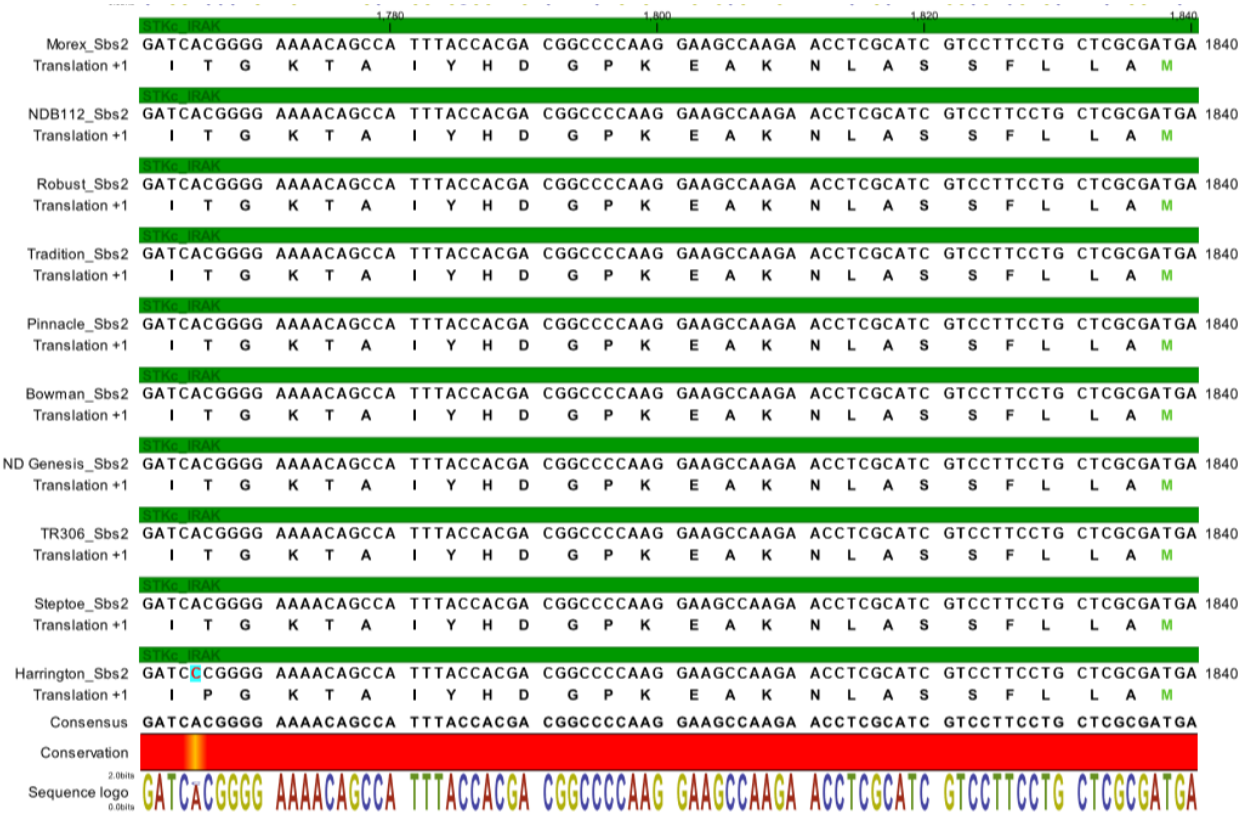
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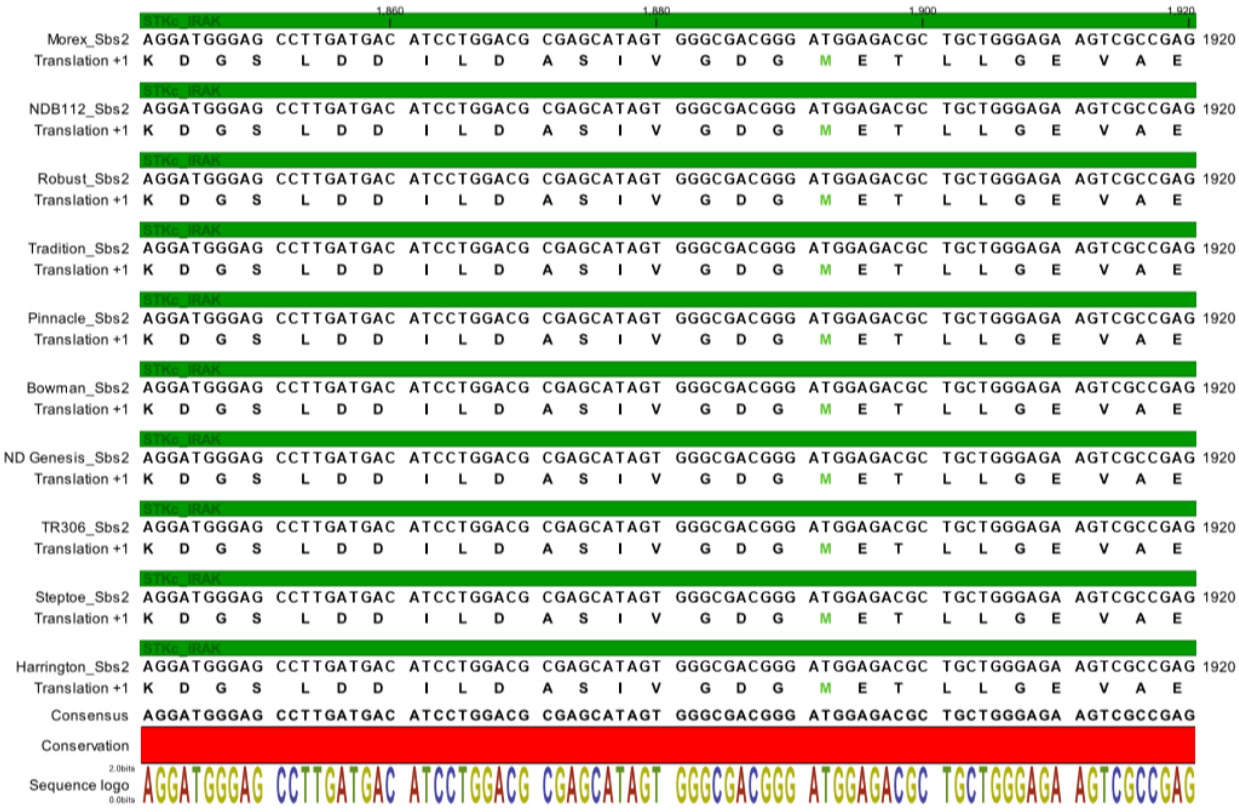
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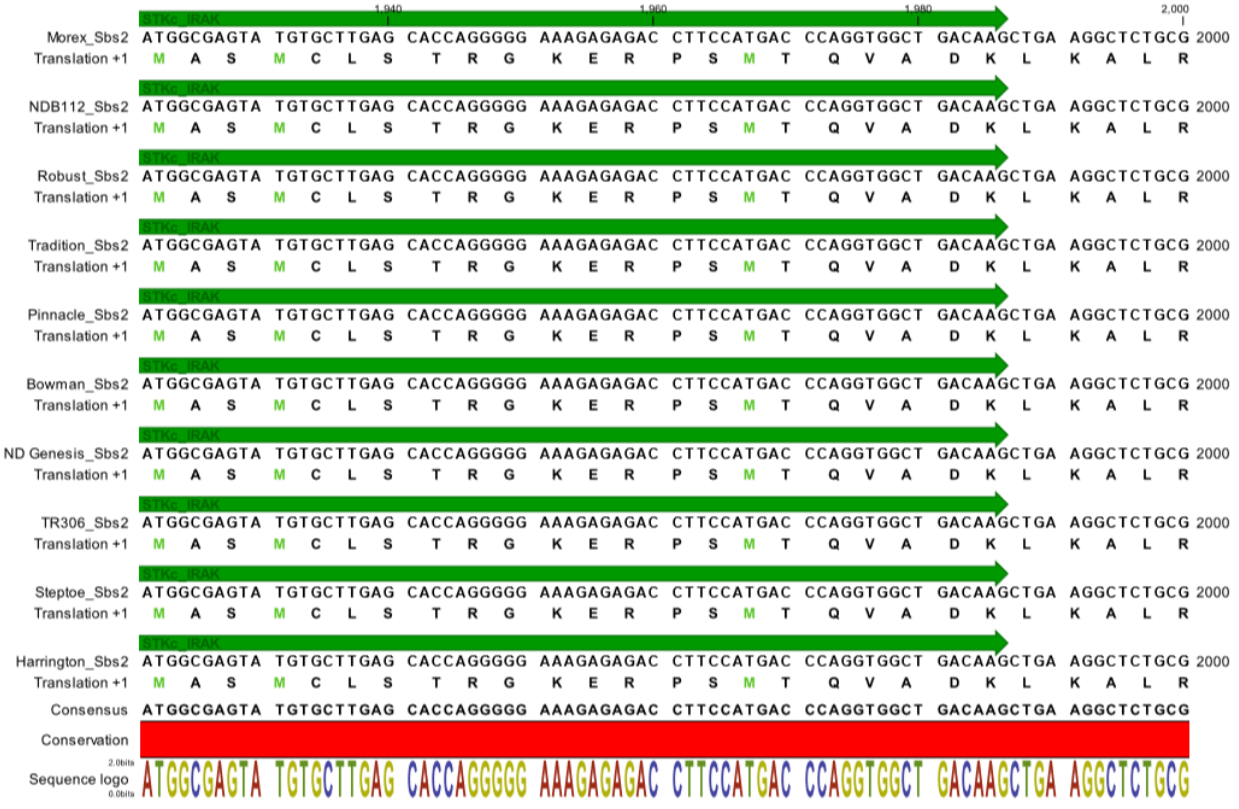
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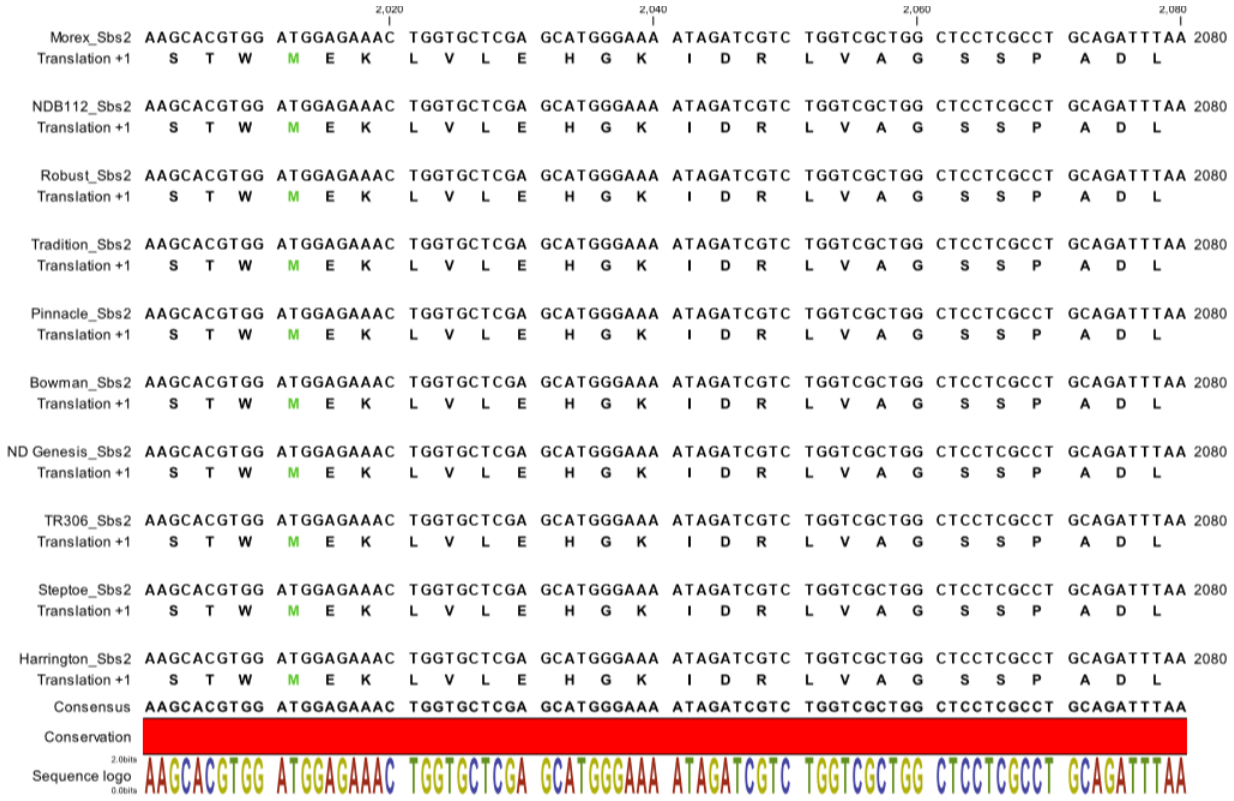
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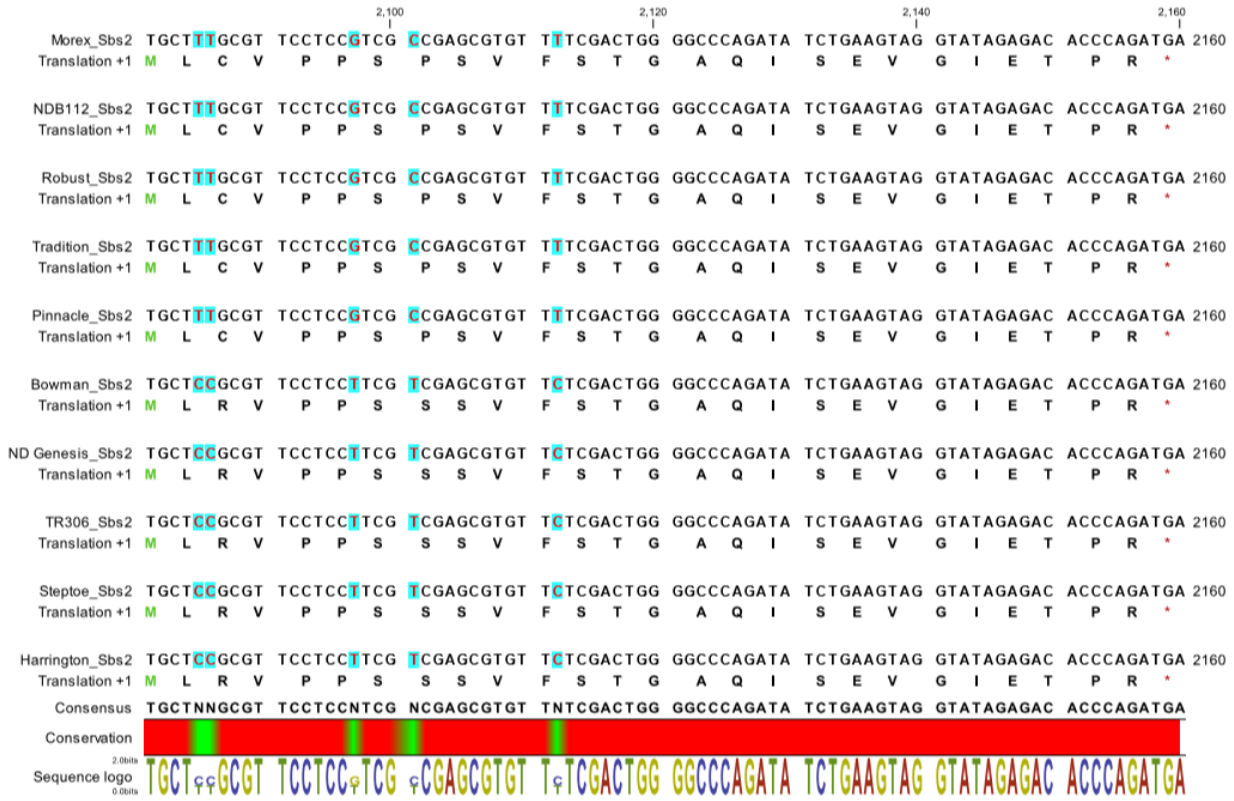
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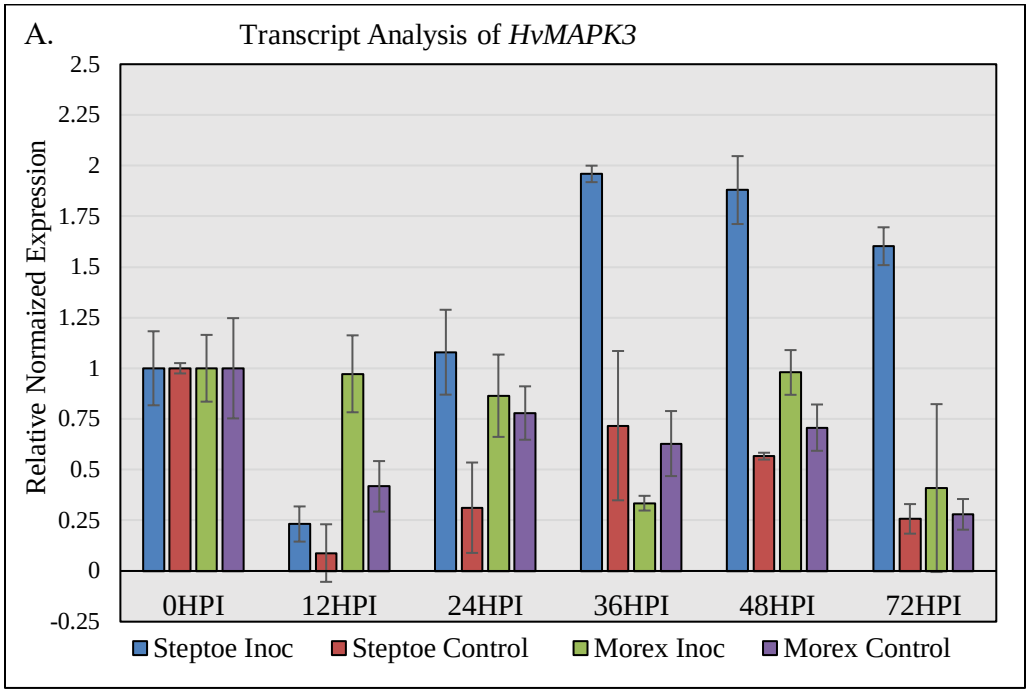
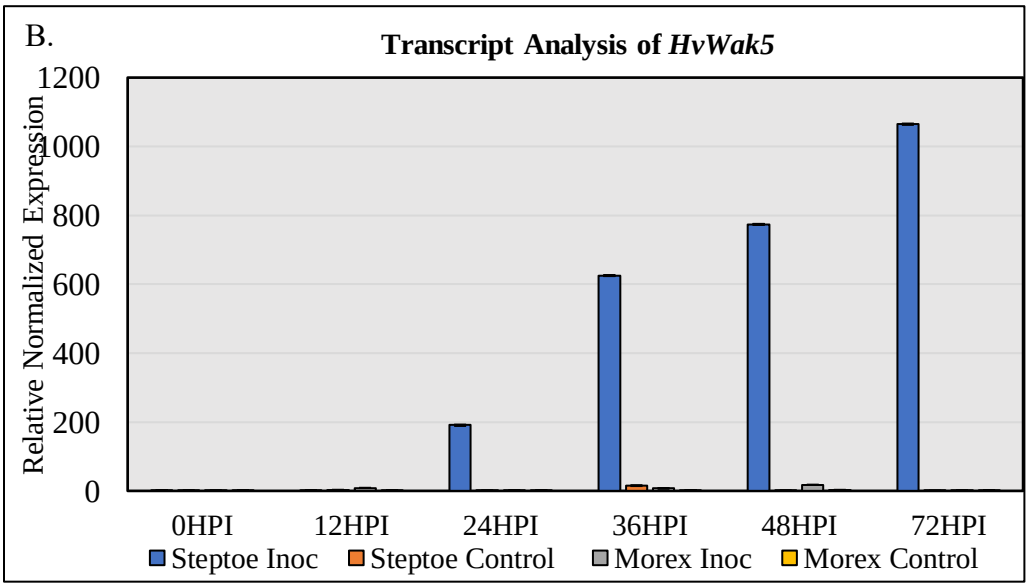
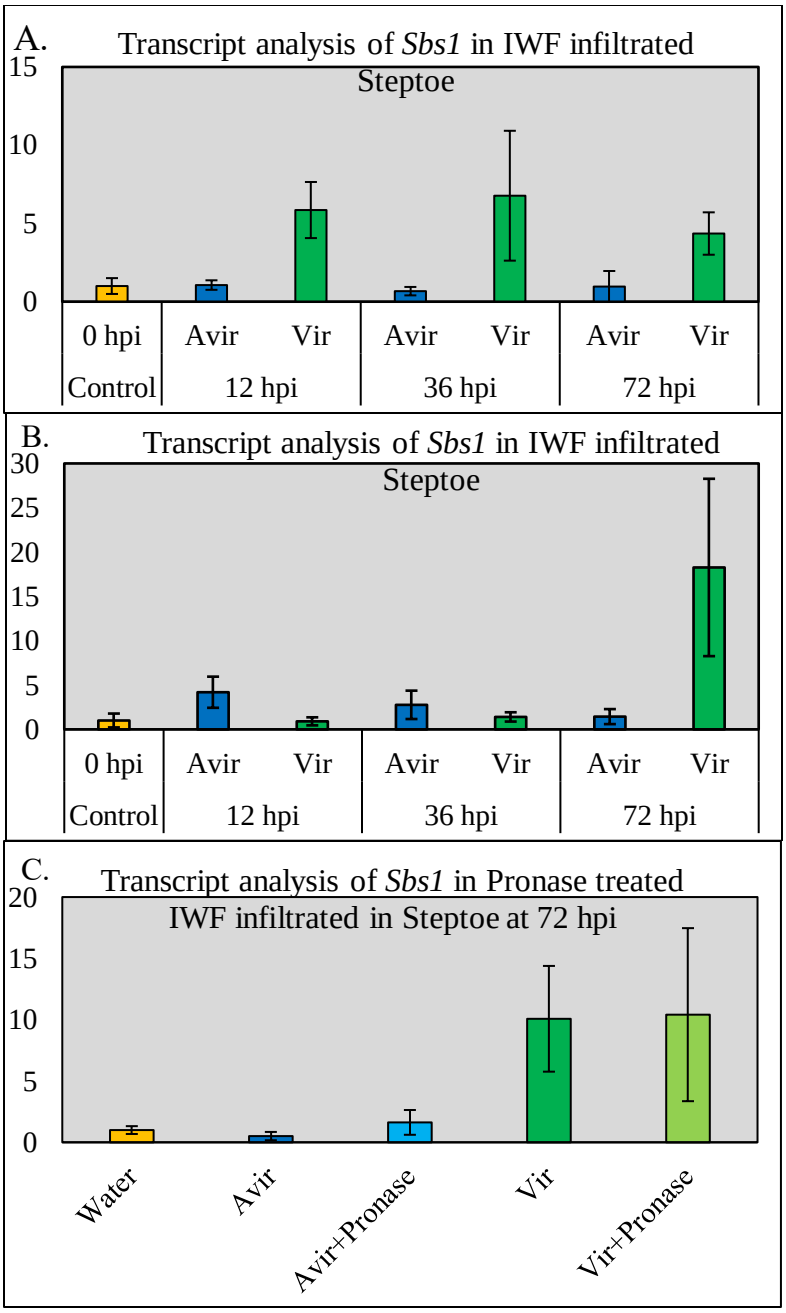
Supplementary Figure 7.

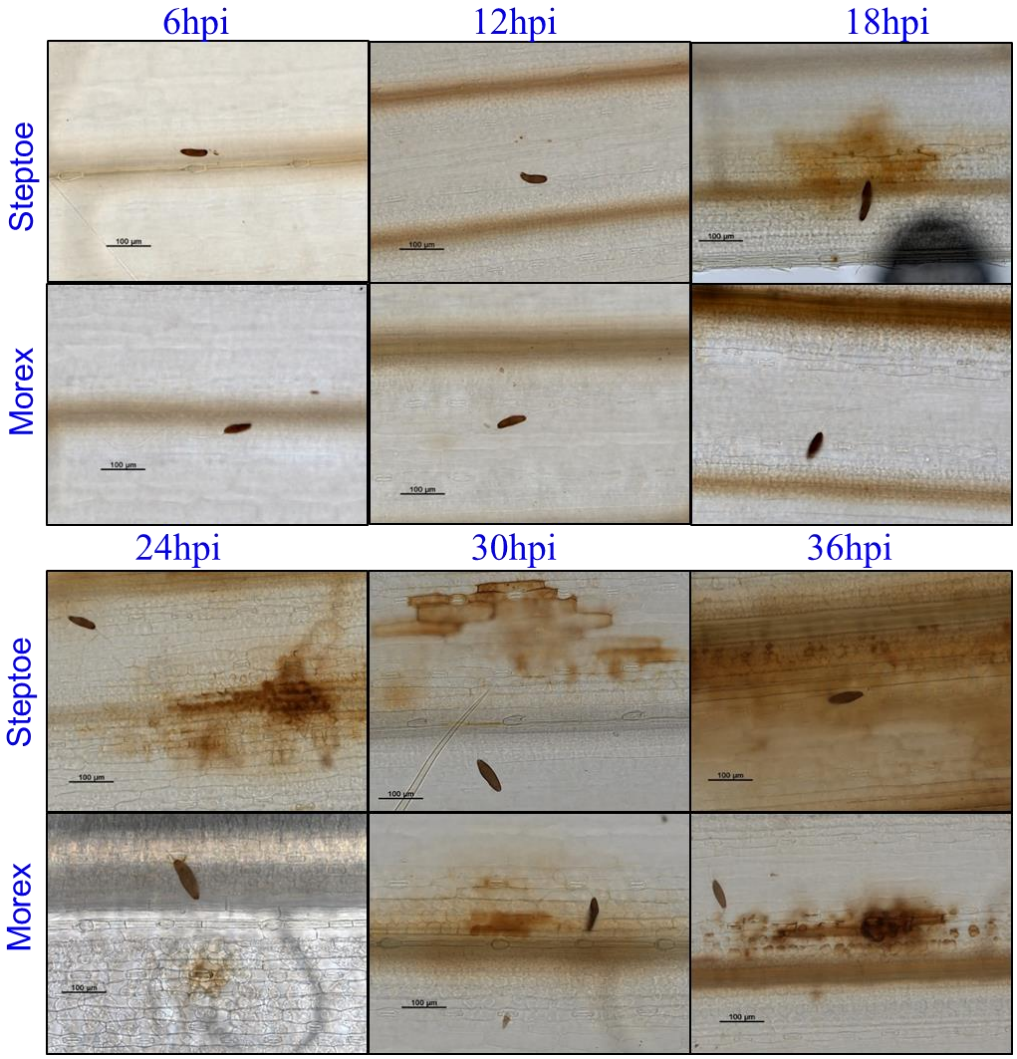




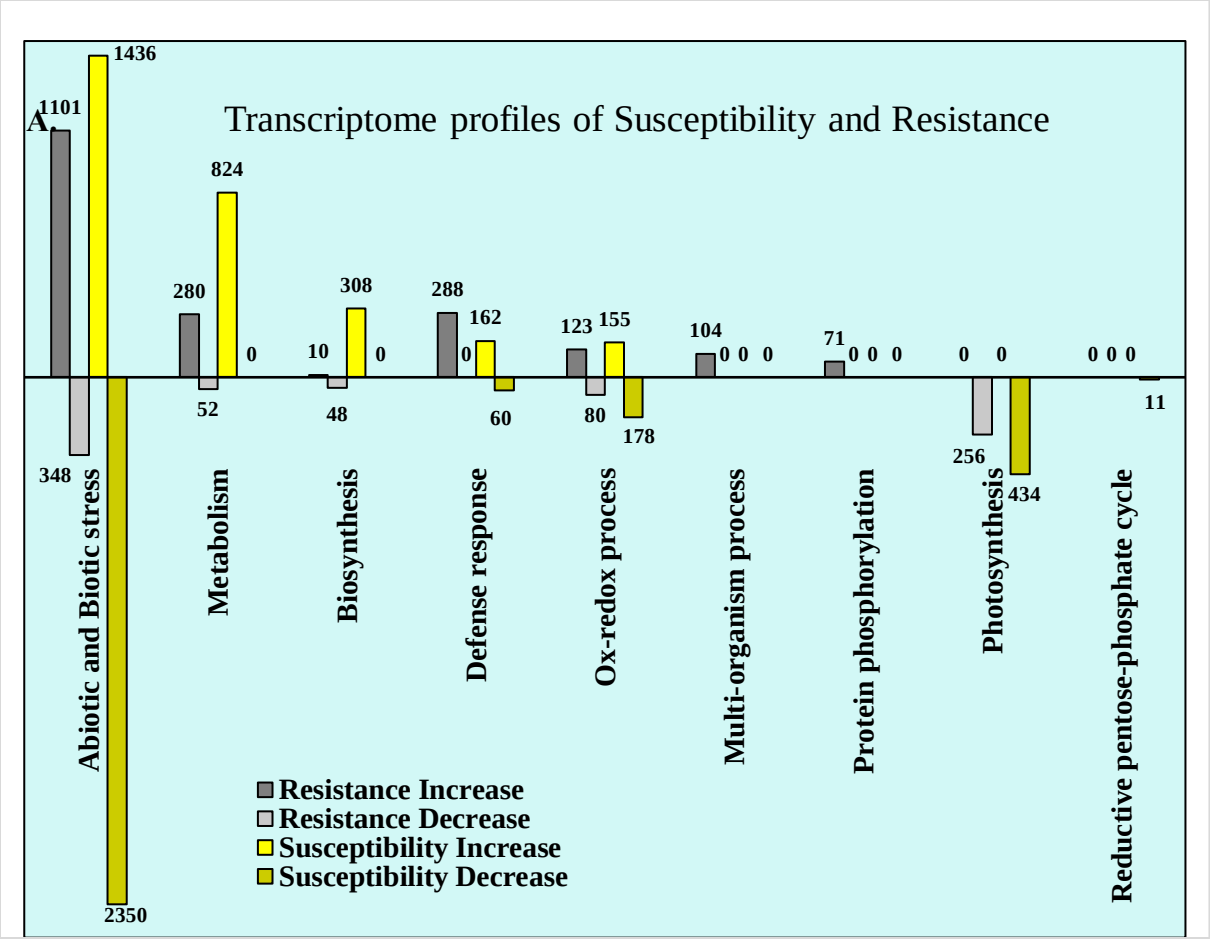
Supplementary Figure 7. Time course qRT-PCR transcript analysis of the spot blotch susceptible cultivar Steptoe and resistant cultivar Morex inoculated with the ND85F isolate of *Bipolaris sorokiniana* and their tween control were analyzed for the expression analysis. 7A) *HvWak5* transcript were analyzed during the spot blotch susceptibility in the barley cultivar Steptoe and Morex. 7B) *HvMAPK3* was required during the infection process of *Bipolaris sorokiniana* in barley. The reference gene *HvSnoR14* expression at each time point was used to normalize the transcripts (X-axis). Error bars depict SEM±1(n=3). Time point 0 HPI was used as control sample for relative expression analysis (Y-axis).



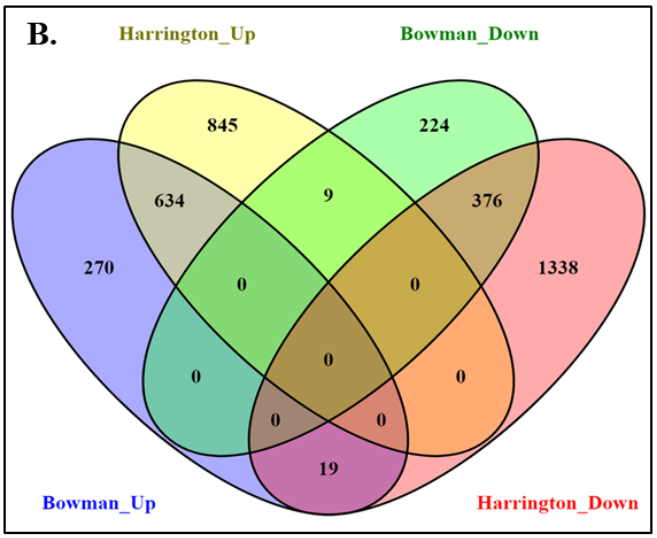
Supplementary Figure 8. Two run of the time course qRT-PCR transcript analysis of the *Sbs1* post infiltration of the extracted Intercellular Fluid Wash (IWF) from avirulent isolate BS035 and virulent isolate ND85F of *Bipolaris sorokiniana* inoculated on cultivar Steptoe (8A and 8B). The reference gene *HvSnoR14* expression at each time point was used to normalize the transcripts (X-axis). Error bars depict SEM±1(n=3). Time point 0 HPI was used as control sample for relative expression analysis (Y-axis). 8C) The qRT-PCR transcript analysis of the *Sbs1* post infiltration of the Pronase treated and non-treated extracted Intercellular Fluid Wash (IWF) from avirulent isolate BS035 and virulent isolate ND85F of *Bipolaris sorokiniana* inoculated on cultivar Steptoe. The reference gene *HvSnoR14* expression at each time point was used to normalize the transcripts (X-axis). Error bars depict SEM±1(n=3). Water infiltrated Steptoe was used as control sample for relative expression analysis (Y-axis).



Supplementary Figure 9. DAB staining to characterize the *Bipolaris sorokiniana* infection timeline, and ROS production in the barley cultivar Steptoe and Morex. The panels show *B. sorokiniana* isolate ND85F infection on Steptoe and Morex from 6 to 36 hours post inoculation (hpi).



Supplementary Figure 10. Differentially expressed genes (DEGs) during the infection process of *Bipolaris sorokiniana* in resistant cultivar Bowman and susceptible cultivar Harrington. 10A) A graphical representation of the DEGs based on their biological function category in the resistant and susceptibility of spot blotch disease. 10B) Venn- diagram showing the common and/or unique DEGs in the yellow and red ellipse representing the susceptibility upregulated (Harrington_up) and downregulated (Harrington_Down); and blue and green ellipse representing resistant upregulated (Bowman_up) and downregulated (Bowman_Down) at 72 hpi with *B. sorokiniana* normalized with their non-inoculated controls DEGs (n=3). 10C) Total reads obtained and mapped to the reference genome of barley in each sample of the DEGs analysis. 10D) Heat Map represents the Log transformed fold changes of the DEGs in the susceptibility and resistance to spot blotch disease.



C.

Genotype	Treatment	Replicate	Total Reads	Reads mapped to barley genome Reads	Rate
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Harrington	Non-Inoc	3	41307906	35272821	85.39
Harrington	72 hpI	1	52518290	41883336	79.75
Harrington	72 hpI	2	54534806	42640765	78.19
Harrington	72 hpI	3	63496548	49501909	77.96
Bowman	Non-Inoc	1	56508226	46726652	82.69
Bowman	Non-Inoc	2	52549749	44241634	84.19
Bowman	Non-Inoc	3	61817167	50881710	82.31
Bowman	72 hpI	1	48707010	42034150	86.3
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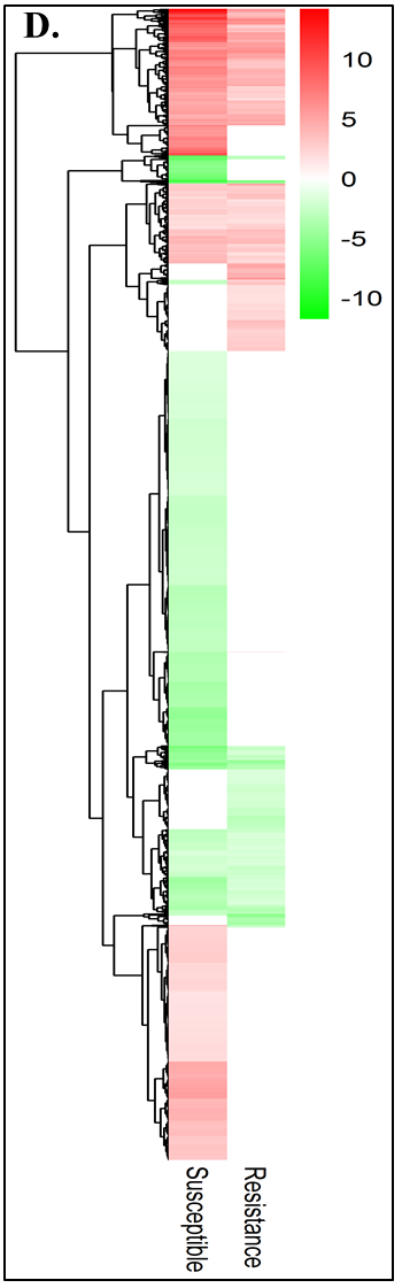
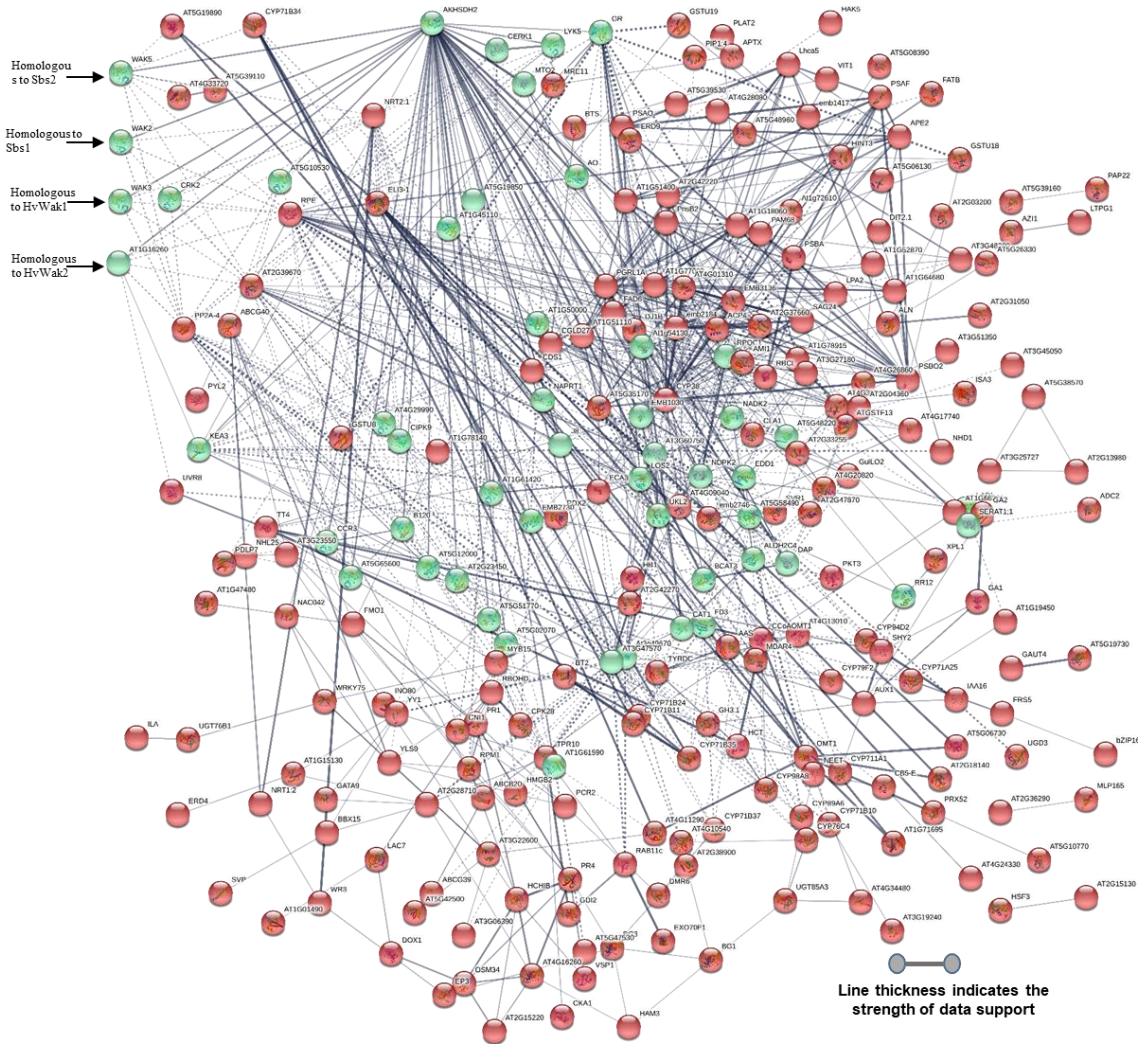
Bowman	72 hpI	3	60830236	51255557	84.26



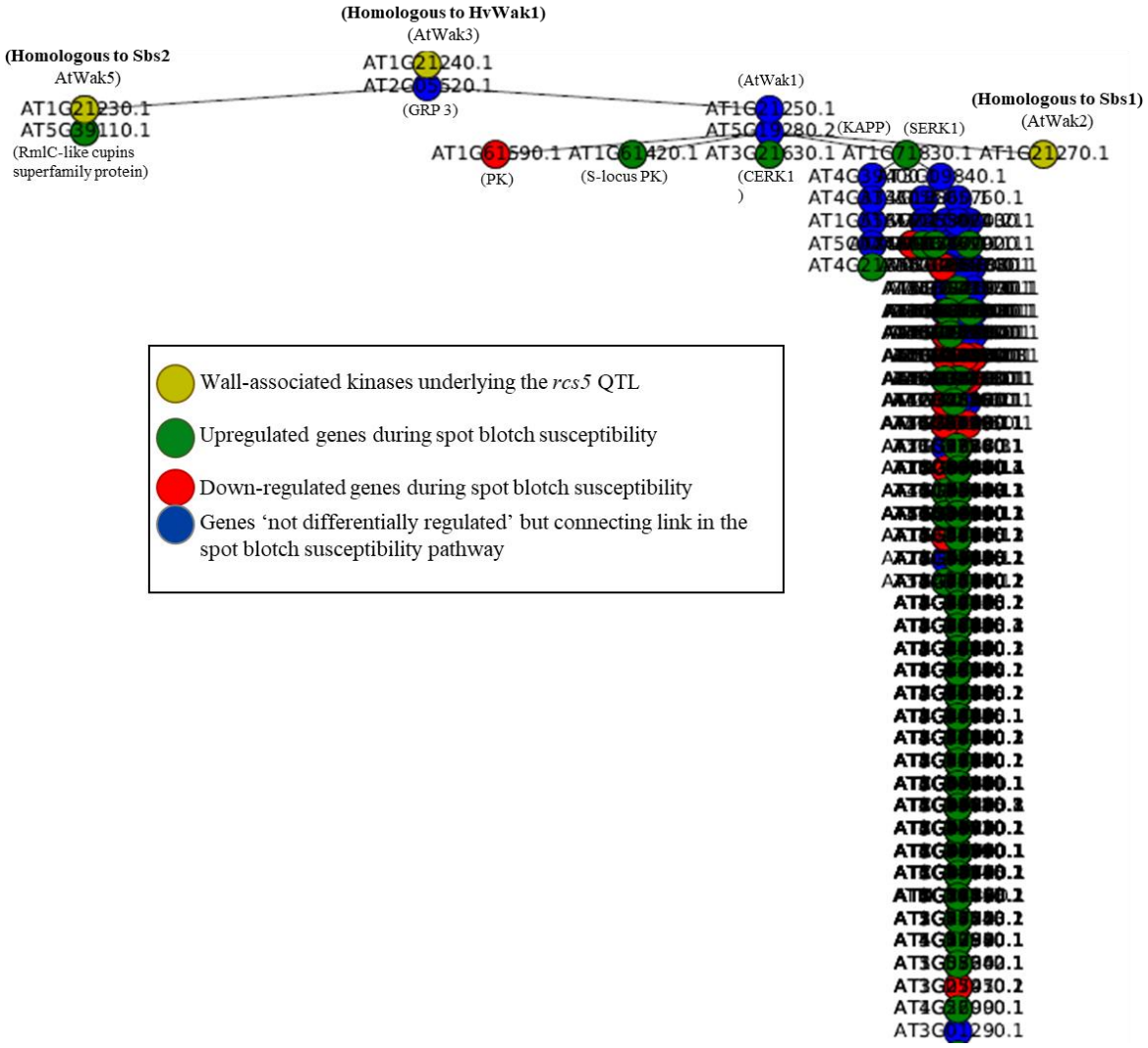
Supplementary Figure 10. Differentially expressed genes (DEGs) during the infection process of *Bipolaris sorokiniana* in resistant cultivar Bowman and susceptible cultivar Harrington (Continued). 10A) A graphical representation of the DEGs based on their biological function category in the resistant and susceptibility of spot blotch disease. 10B) Venn- diagram showing the common and/or unique DEGs in the yellow and red ellipse representing the susceptibility upregulated (Harrington_up) and downregulated (Harrington_Down); and blue and green ellipse representing resistant upregulated (Bowman_up) and downregulated (Bowman_Down) at 72 hpi with *B. sorokiniana* normalized with their non-inoculated controls DEGs (n=3). 10C) Total reads obtained and mapped to the reference genome of barley in each sample of the DEGs analysis. 10D) Heat Map represents the Log transformed fold changes of the DEGs in the susceptibility and resistance to spot blotch disease.

A



Supplementary Figure 11. Protein-protein interaction re-analysis of Arabidopsis homologs of 500 differentially regulated (250 upregulated and 250 downregulated transcripts in Harrington at 72hpi with *Bipolaris sorokiniana*) genes with STRING database (11A). The green color represents upregulated and red color represents downregulated genes in the susceptible cultivar Harrington during spot blotch susceptibility. Line thickness of network edges represent strength of data support and kmeans network clustering method with three cluster group was specified for grouping. The candidate WAKs homologs are represented in the far left in the network. Arabidopsis gene identifiers on each bubble represents the interacting genes in the network. 11B) Prediction of protein-protein interaction of 361 Arabidopsis orthologs for 500 differentially regulated (250 upregulated and 250 downregulated genes in susceptible cultivar Harrington at 72 hpi with *Bipolaris sorokiniana*) along with the Arabidopsis WAK homologs of candidate WAKs in the *rcs5* locus. Yellow color represents the Arabidopsis WAK homologs of *Rcs5* region barley WAK genes added to the list for WAK specific interaction network, green color represents up-regulated, red represents down-regulated, and blue represents not differentially regulated genes introduced in the network for making necessary connections by PPIN analysis from Arabidopsis protein database. Arabidopsis gene identifiers on each bubble represents the interacting genes in the network.

B



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