The complex intron landscape and massive intron invasion in a picoeukaryote provides insights into intron evolution

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Supplementary Information

Supplementary Methods

Introner Element (Remnant) Prediction

Starting from handpicked example Introner Elements (IEs), we delineated common motifs (pattern blocks) and assembled them into class-specific pattern files (IEA-1 example listed below). We used PatScan (Dsouza, et al. 1997) to scan the *Micromonas* genomes. For each class, multiple pattern files were constructed, ranging from strict to degenerate. When overlapping matches were detected, only the match belonging to the strictest pattern file was kept. EST and protein alignments were generated using GenomeThreader (Gremme, et al. 2005) (v1.4.6; -minalignmentscore 0.95 –mincoverage 0.89) and the splicing information was used in the automated curation of the final set of predicted IEs i.e. adjusting IE start and stop coordinates to match the exon/intron boundaries.

```
p1=GTGCGT
0...15
p2=ACTGGTYCCCRTACGACC[5,0,0]
0...80
p3=STTTCAAT[2,0,0]
0...40
p4=GCCTTTCAACTC[3,0,0]
0...100
p5=AG
```

IE remnants were detected using BLASTN (v2.2.17; -e 1e-05) (Altschul, et al. 1990) using the previously built set of (complete) IEs. For each class, we also built a multiple sequence alignment (MSA), constructed a profile HMM (HMMer v2.3.2), and used it to detect additional instances of degenerated IEs. This HMM approach was also used for members of the IE-B / IE-D class.

Micromonas Re-annotation

IEs were added as an extra evidence track when performing the annotation (EuGene v3.6 (Schiex, et al. 2001)). EST and protein libraries of all Mamiellophyceae were used, as well as extensive sets of manually curated gene models. RNA genes were predicted using tRNAscan-SE (Lowe and Eddy 1997) and Infernal (Griffiths-Jones, et al. 2003). Genes encoding selenoproteins were manually corrected. When compared to the old annotation, the new annotation features fewer but larger gene models. This is mainly due to the IEs that help to span stop codons, allowing adjacent gene models to be merged into one continuous model.

Gene Ontology analysis of IE genes

GO terms for all *Micromonas* proteins were derived using InterPro2GO (Mulder and Apweiler 2007), and GO term over/under-representation of genes carrying IEs, using the GO terms of the entire *Micromonas* proteome as a background, was analysed using the Cytoscape plugin BiNGO (Maere, et al. 2005) (hypergeometric test + FDR correction; significance level 0.05). This GO analysis was only performed on CCMP1545, as the low number of IEs in RCC299 makes the analysis insignificant.

Spliceosomal Components

Spliceosomal components were detected through homology with *A. thaliana* proteins in the Splicing Related Gene Database (http://www.plantgdb.org/SRGD) and through the detection of splicing-related GO labels.

Metagenomic Sequence Analysis

When aligning metagenomic sequences (MSs) to the *Micromonas* genomes, the presence or absence of IEs in both the query (the MS) and the genomic sequence can present too big a gap in the alignment for 'regular' alignment programs to cope with. As such, the environmental sequences were aligned to the genomes using a seed-and-align procedure, initiated by a regular BLASTN. Starting from the best-hit, we expanded the genomic space with neighbouring hits. In the end, we used the outer coordinates to extract the corresponding genomic region, and re-aligned it to the environmental sequence using a SMITH-WATERMAN alignment (EMBOSS (Rice, et al. 2000): water).

To be able to draw accurate conclusions on IE presence/absence polymorphisms (PAPs), we performed a quality filtering step. We only continued with alignments that have more than 100 nucleotides labelled as 'non-IE', an identity percentage of more than 50%, and a coverage of more than 60%. After careful consideration, we also decided to leave aside all metagenomic sequences labelled as 'JCVI', as they were assemblies of smaller metagenomic sequences.

RNA secondary structure analysis

For each IE class, a consensus sequence was constructed (EMBOSS (Rice, et al. 2000): cons). Secondary structures were predicted using RNAfold (Vienna RNA (Hofacker 2003)). Secondary structures were compared between classes, but no general model could be obtained.

Protein evidence for IE-D

The protein sequences of genes containing IE-D sequences and their selected orthologs were aligned using Clustal W (default settings: GONNET weight matrix) (Thompson, et al. 1994).

Visualisation tools

For visualisation purposes, we also employed the following tools: seqlogo (Crooks, et al. 2004) and R (v2.13.0) (R Development Core Team 2008).

Supplementary References

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Mulder N, Apweiler R 2007. InterPro and InterProScan: tools for protein sequence classification and comparison. Methods in molecular biology 396: 59-70.

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Rice P, Longden I, Bleasby A 2000. EMBOSS: the European Molecular Biology Open Software Suite. Trends in genetics : TIG 16: 276-277.

Schiex T, Moisan A, Rouzé P. 2001. EuGène: An Eukaryotic Gene Finder That Combines Several Sources of Evidence. Selected papers from the First International Conference on Computational Biology, Biology, Informatics, and Mathematics: Springer-Verlag.

Thompson JD, Higgins DG, Gibson TJ 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673-4680. doi: 10.1093/nar/22.22.4673

Supplementary Figures

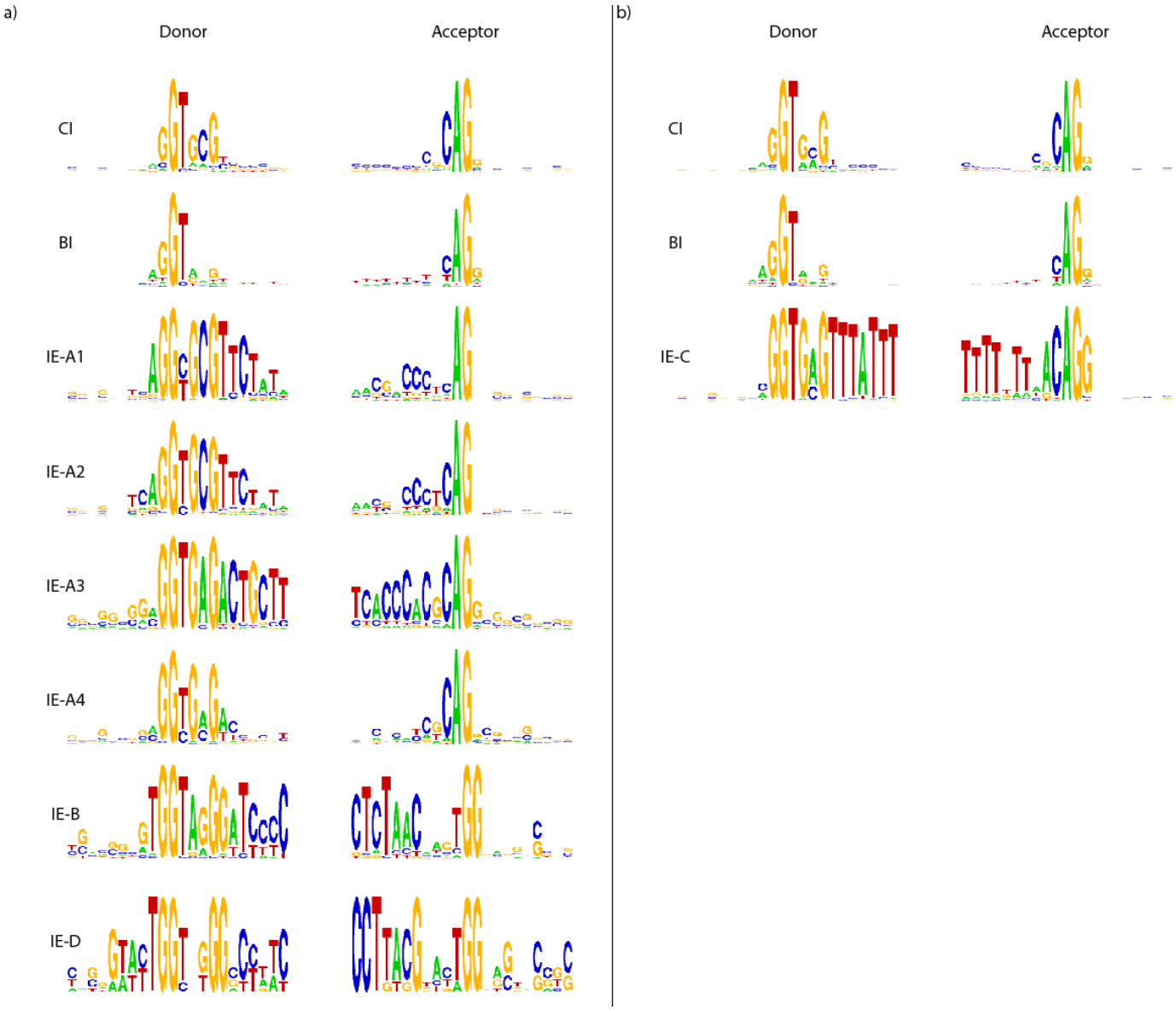

Figure S1. *Micromonas* splice site signals for all intron classes. Shown here are sequence logos for the donor/aceptor site (10 nucleotides upstream and downwstream) for CCMP1545 (**a**) and RCC299 (**b**).

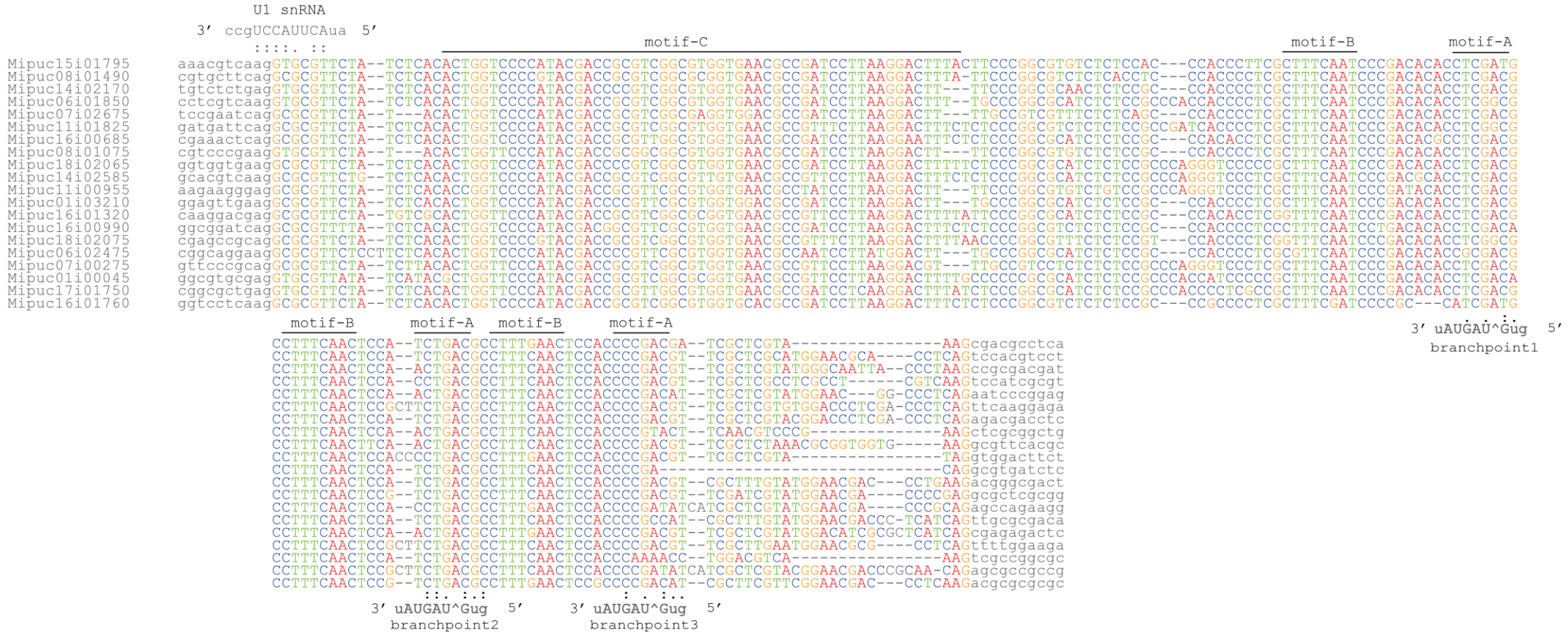

Figure S2. Alignment of 20 random IE-A1 sequences. The motifs (motif-A = branchpoint motif; motif-B: branch-point companion motif; motif-C) are marked, as are the splicing signals (donor site + branch-point) and their base-pairing information from the corresponding spliceosomal RNAs.

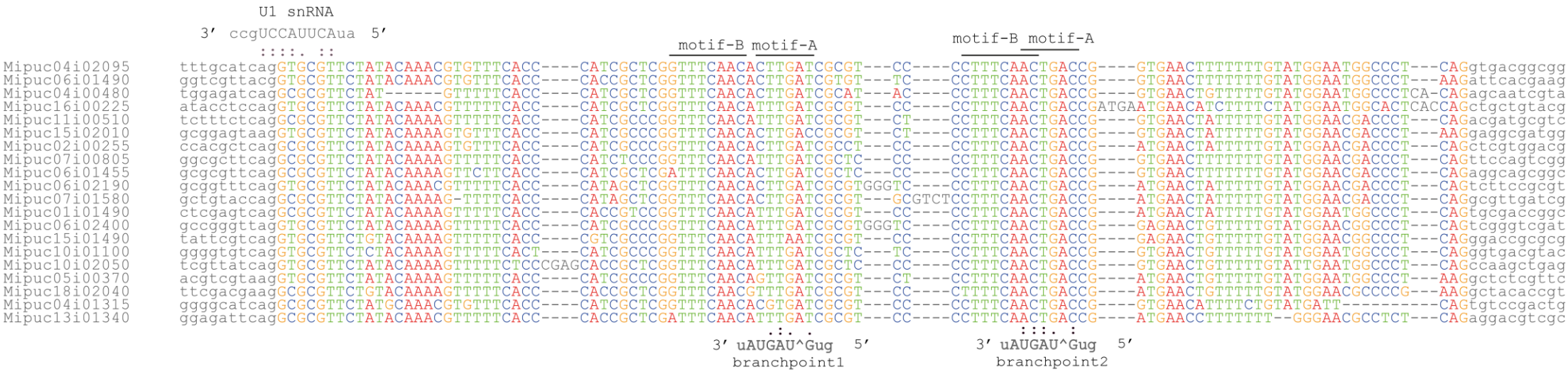

Figure S3. Alignment of 20 random IE-A2 sequences. The motifs (motif-A = branchpoint motif; motif-B: branch-point companion motif; motif-C) are marked, as are the splicing signals (donor site + branch-point) and their base-pairing information from the corresponding spliceosomal RNAs**.**

Figure S4. Alignment of 20 random IE-A3 sequences. The motifs (motif-A = branchpoint motif; motif-B: branch-point companion motif; motif-C) are marked, as are the splicing signals (donor site + branch-point) and their base-pairing information from the corresponding spliceosomal RNAs..

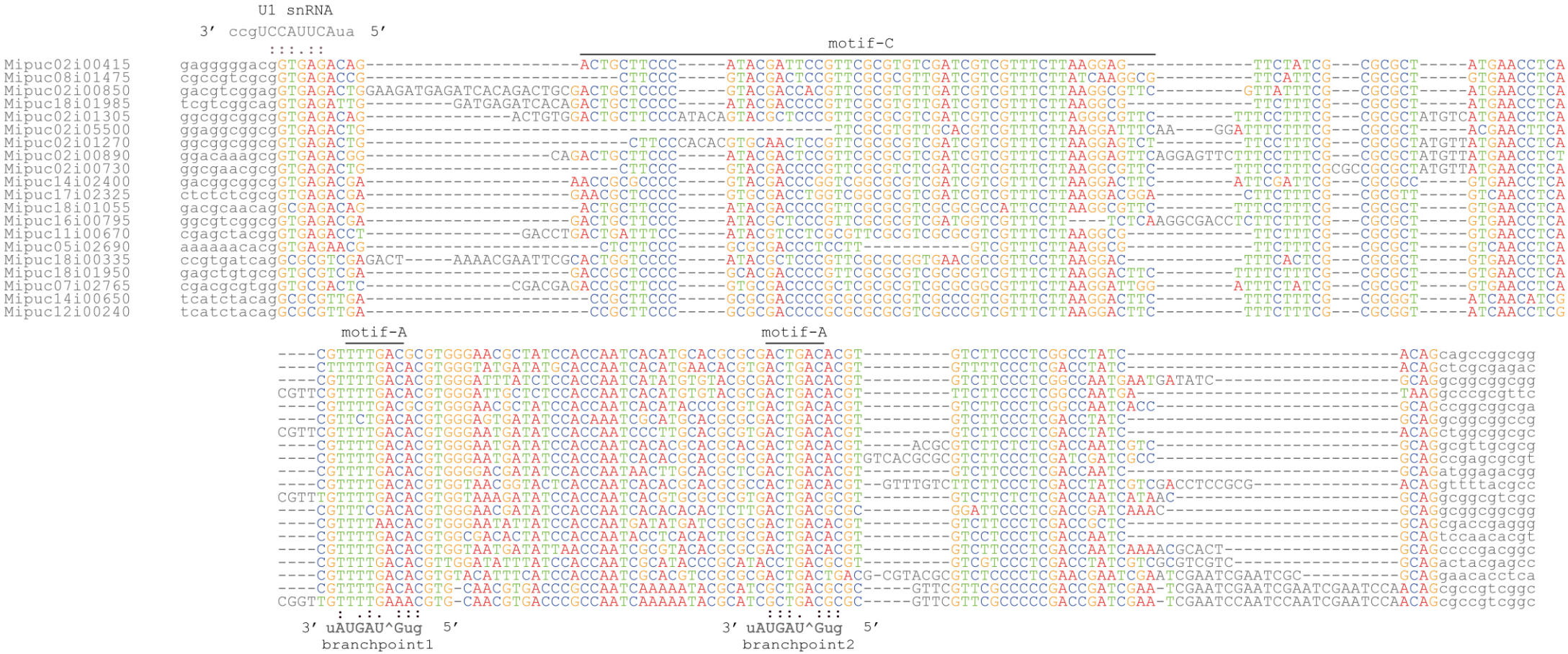

Figure S5. Alignment of 20 random IE-A4 sequences. The motifs (motif-A = branchpoint motif; motif-B: branch-point companion motif; motif-C) are marked, as are the splicing signals (donor site + branch-point) and their base-pairing information from the corresponding spliceosomal RNAs.

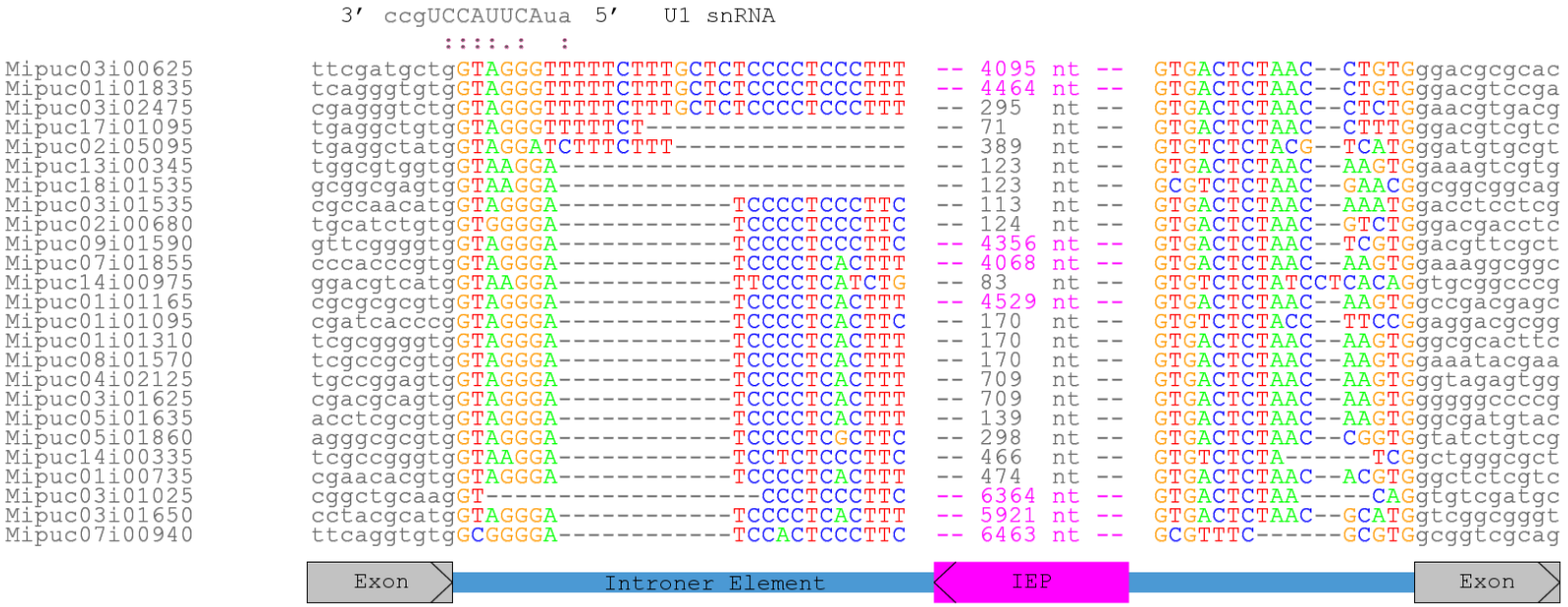

Figure S6. Alignment of all 25 IE-B sequences showing the splice site regions in detail. The structure and orientation of exonic regions (grey) and Intron-Encoded Proteins (purple) is represented schematically beneath the alignment. Base-pairing information regarding the donor site (U1 snRNA) is also provided.

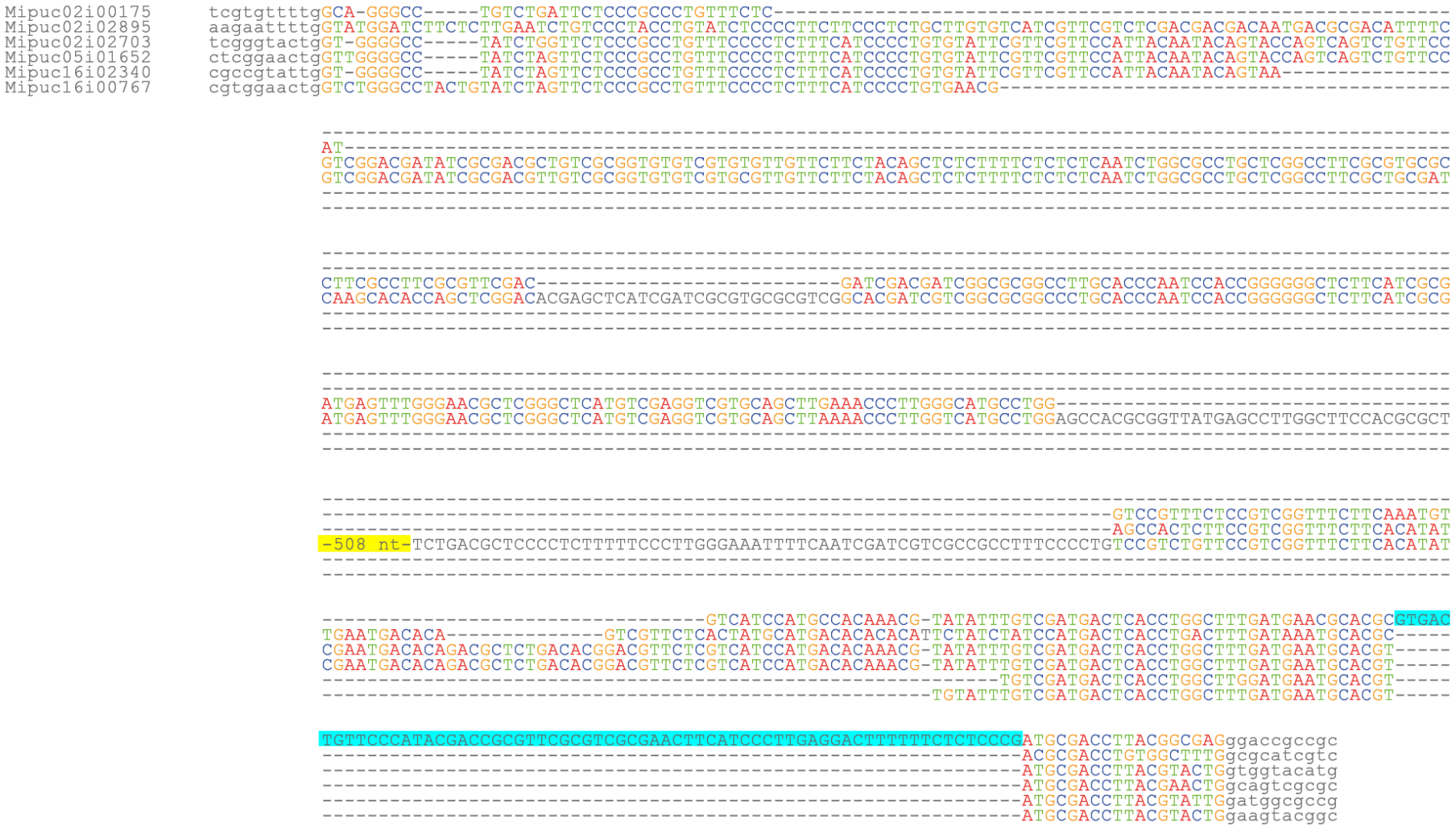

Figure S7. Alignment of all six IE-D sequences showing the splice site regions in detail. The IE-A remnant inside the chimeric Mipuc02i00175 has been highlighted in blue.

Figure S8. Protein evidence supporting IE-D introner elements (in lack of any EST evidence). Canonical introns (light blue), IE-A (green), IE-D (dark blue), and chimeric IEs (red) have been highlighted accordingly. Intron phases are indicated on top of the intron positions. Gene IDs correspond to the following organisms: *Ostreococcus lucimarinus* (Ol*), *Ostreococcus sp.* RCC809 (Od*), *Ostreococcus tauri* (Ot*), *M. pusilla* CCMP1545 (Mipuc*), *M.sp.* RCC299 (Mipur*), *Bathycoccus prasinos* (Bathy*), *Physcomitrella patens* (*PHYPHA), *Arabidopsis thaliana* (*ARATH) and *Dictyostelium discoideum* (*DICDI). a) Alignment of WD89 proteins. b) Alignment of Jumonji-1 proteins (Jmj-N and Jmj-C Jumongi domains are highlighted in grey, while the Zn-finger domain has been highlighted in yellow), c) Alignment of sepA-like Serine/Threonine protein kinase, d) Alignment of U5 snRNP helicase, e) Alignment of UAA1 proteins (UDP-N-acetylglucosamine; UMP antiporters).

a) WD89

```
                    10        20        30        40        50        60        70        80        90       100
                     |         |         |         |         |         |         |         |         |         |
Ol03g00040      M--TRTRDDDAHDDDDRRGARALVDDSGAPNVFENDDYVEPTPESDEALTFTVRGG-----------RASTTAMSLRATSMTSTAVDKDAPYNTHIASN
Od03g03130      M----SRDD--------------LVDDSGAPNAFENDGYDEPGPESDEALTHALPAAGT--------TASDVDAVALVARSHASTSVKGDAPYNVHVATN
Ot03g00170      M-RDATVEDD-------------FVDRSGAPSAFENDGYDEPDATNDEALTFQCARG------------GGDGSAALVARATRTTAREGDAPYNTHLSVS
Mipuc02g00100   M------PS-------------------RLAPACTTVAAFG---PSHDPCYVMALAPRSVRARLVRVPPHVPPTLRSARVDRSLAPTRLLPPPPSPLPRRS
Mipur03g06080   MYRPASNPSP--------------CH-HLAPVSTTVAAFG---PQNDPCYVMAIASR------------YV--AR---AHPTPSPAAPLSLPPS------
                          .                  **       :    .  * . .                          .    :.:     * .

                   110       120       130       140       150       160       170       180       190       200
                     |         |         |         |00       |         |         |         |         |         |
Ol03g00040      HDGTSCAVATTSGRVRVYARERATGAFAHG--------------EREIRLNASVNECEFGSAADPFSIVCACGDGTTRVYDARSQ-DDRPTASFRAPFN
Od03g03130      HDGGVVAVVTTSGRAVVYERRGTDGGLETET-------------ARDVEVGCVVNECEFGPREDPYSLVCACGDGTVRVYDARCG-DSRAVASFRAPAG
Ot03g00170      ADGAMCAVTTTSGRVRIIARDGGSGALGDDAR------------EFVPGHGCAVNECEFGSASDPHAIVLACGDGTVRVYDVRTK-DDRATAVFRAPYG
Mipuc02g00100   PDGAHLAASLSTNAVKIYARGDAPSGGDFGGGGGGGGHLGTLRAVTELSAHDGAVTDCAFPIPSEPWTVLTSSADATIRAWDLRQG-GERPCASYVAPFA
Mipur03g06080   SDGKYLATSLSTQAVKVYQR--------TED--G------GIAALVELTGHGAAVTDVTIPLPHEPCTVCSSSLDGTVRLWDCRAAEGQREVMKFVAGFA
                 **   *.  ::  . :  *                          .  *.:  :   :* ::  :. *.* * :* *   ..*    : *
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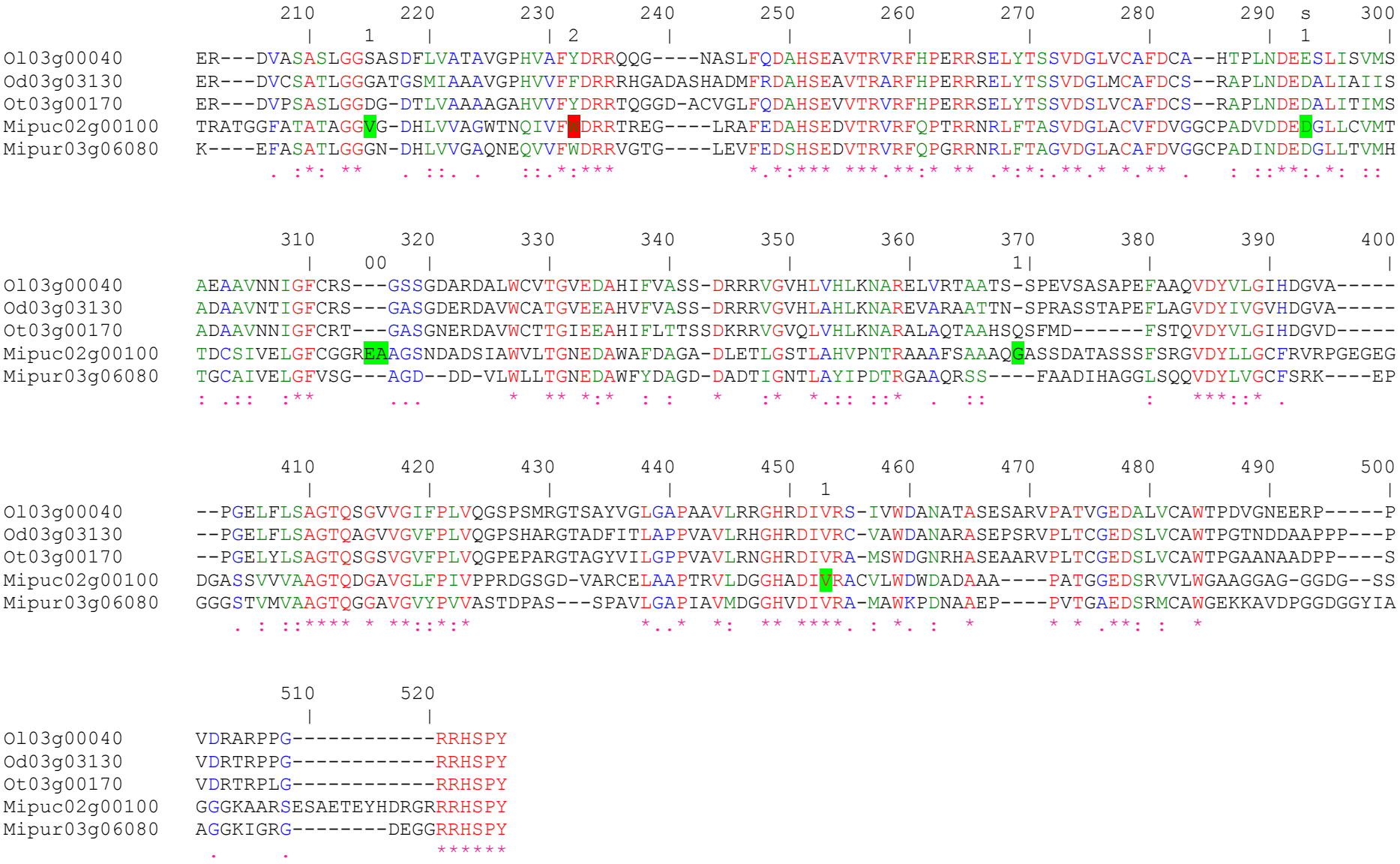
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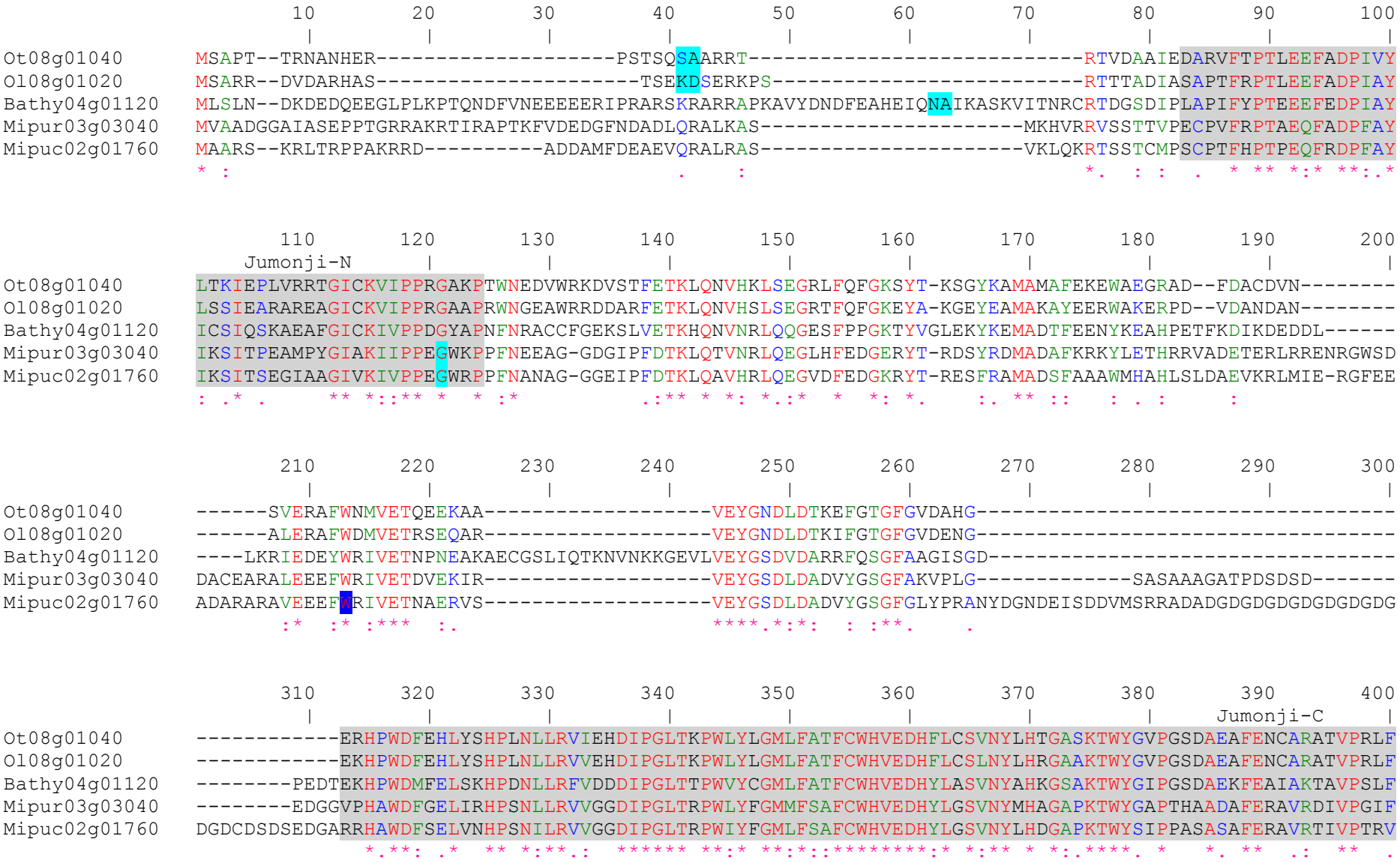
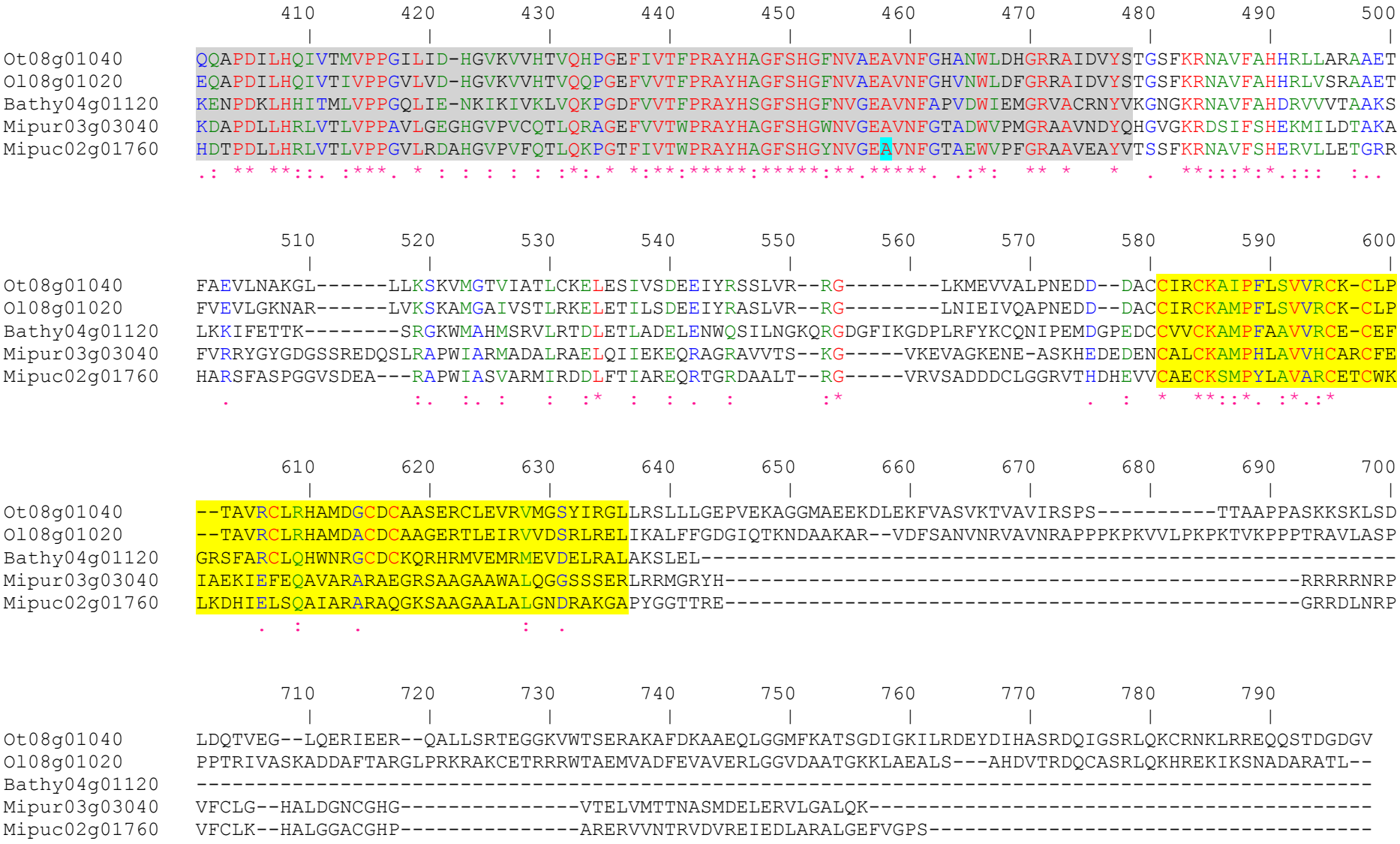

Jumonji-N
Jumonji-C
Ot08g01040
Ol08g01020
Bathy04g01120
Mipur03g03040
Mipuc02g01760


410 420 430 440 450 460 470 480 490 500
Ot08g01040 QQAPDILHQIVTMVPPGILID-HGVKVVHTVQHPGEFIVTFPRAYHAGFSHGFNVAEAVNFGHANWLDHGRRAIDVYSTGSFKRNAVFAHHRLLARAAET
Ol08g01020 EQAPDILHQIVTIVPPGVLVD-HGVKVVHTVQQPGEFVVTFPRAYHAGFSHGFNVAEAVNFGHVNWLDFGRRAIDVYSTGSFKRNAVFAHHRLVSRAAET
Bathy04g01120 KENPDKLHHITMLVPPGQLIE-NKIKIVKLVQKPGDFVVTFPRAYHSGFSHGFNVGEAVNFAPVDWIEMGRVACRNYVKGNGKRNAVFAHDRVVVTAAKS
Mipur03g03040 KDAPDLLHRLVTLVPPAVLGEGHGVPVCQTLQRAGEFVVTWPRAYHAGFSHGWNVGEAVNFGTADWVPMGRAAVNDYQHGVGKRDSIFSHEKMILDTAKA
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510 520 530 540 550 560 570 580 590 600
Ot08g01040 FAEVLNAKGL------LLKSKVMGTVIATLCKELESIVSDEEIYRSSLVR--RG--------LKMEVVALPNEDD--DACCIRCKAIPFLSVVRCK-CLP
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610 620 630 640 650 660 670 680 690 700
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Ol08g01020 --TAVRCLRHAMDACDCAAGERTLEIRVVDSRLRELIKALFFGDGIQTKNDAAKAR--VDFSANVNRVAVNRAPPPKPKVVLPKPKTVKPPPTRAVLASP
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710 720 730 740 750 760 770 780 790
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Bathy04g01120 ----------
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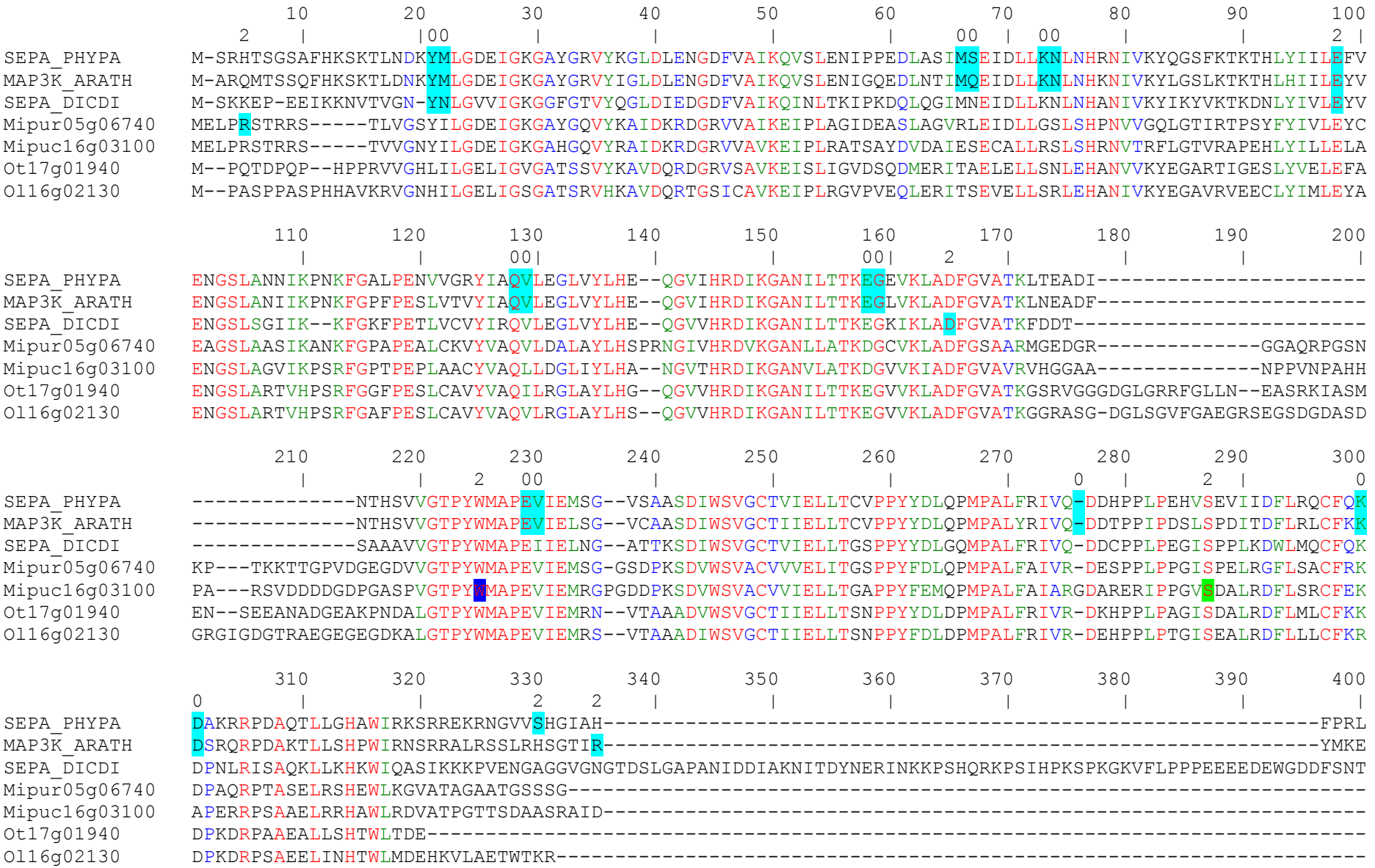
c) SepA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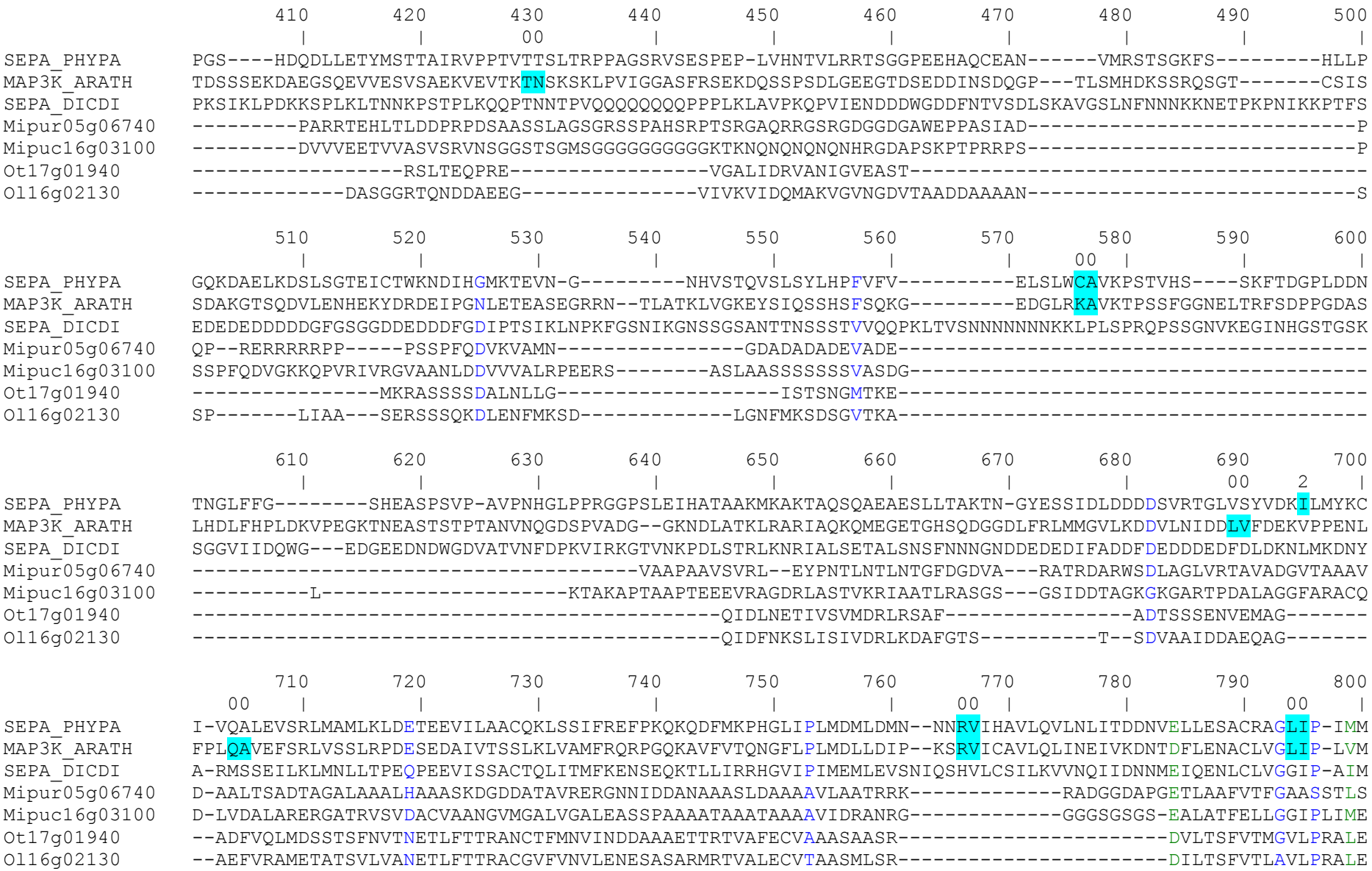
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MAP3K_ARATH TDSSSEKDAEGSQEVVESVSAEKVEVTKTNSKSKLPVIGGASFRSEKDQSSPSDLGEEGTDSEDDINSDQGP---TLSMHDKSSRQSGT-------CSIS
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Ol16g02130 ----------DASGGRTQNDDAEEG----------VIVKVIDQMAKVGVNGDVTAADDAAAAN----------S
510 520 530 540 550 560 570 580 590 600
SEPA_PHYPA GQKDAELKDSLSGTEICTWKNDIHGMKTEVN-G---------NHVSTQVSLSYLHPFVFV----------ELSLWCAVKPSTVHS----SKFTDGPLDDN
MAP3K_ARATH SDAKGTSQDVLENHEKYDRDEIPGNLETEASEGRRN--TLATKLVGKEYSIQSSHSFSQKG---------EDGLRKAVKTPSSFGGNELTRFSDPPGDAS
SEPA_DICDI EDEDEDDDDDGFGSGGDDEDDDFGDIPTSIKLNPKFGSNIKGNSSGSANTTNSSSTVVQQPKLTVSNNNNNNNKKLPLSPRQPSSGNVKEGINHGSTGSK
Mipur05g06740 QP--RERRRRRPP-----PSSPFQDVKVAMN----------GDADADADEVADE----------
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710 720 730 740 750 760 770 780 790 800
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SEPA_DICDI A-RMSSEILKLMNLLTPEQPEEVISSACTQLITMFKENSEQKTLLIRRHGVIPIMEMLEVSNIQSHVLCSILKVVNQIIDNNMEIQENLCLVGGIP-AIM
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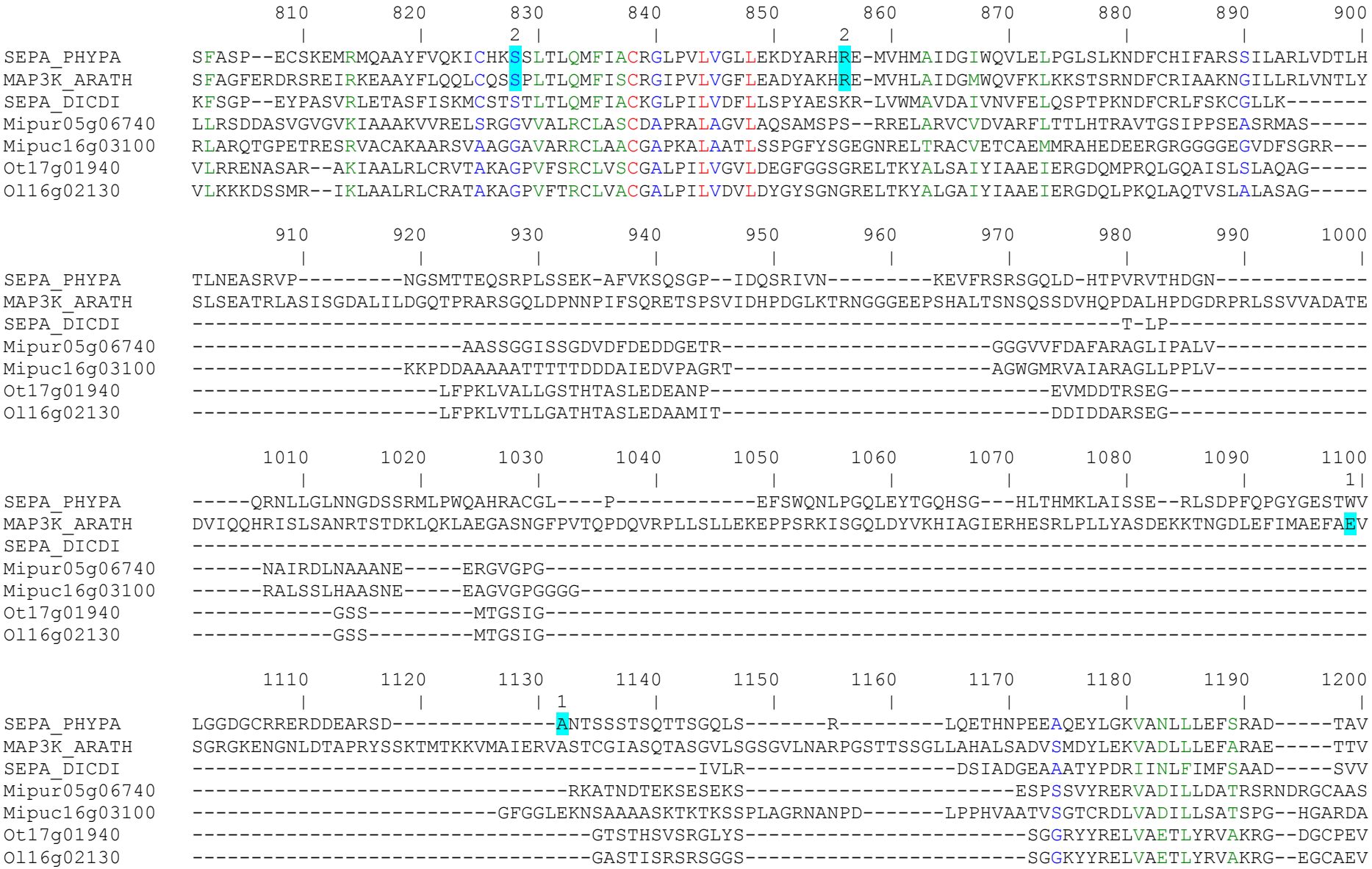
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MAP3K_ARATH SFAGFERDRSREIRKEAAYFLQQLCQSSPLTLQMFISCRGIPVLVGFLEADYAKHRE-MVHLAIDGMWQVFKLKKSTSRNDFCRIAAKNGILLRLVNTLY
SEPA_DICDI KFSGP--EYPASVRLETASFISKMCSTSTLTLQMFIACKGLPILVDFLLSPYAESKR-LVWMAVDAIVNVFELQSPTPKNDFCRLFSKCGLLK-------
Mipur05g06740 LLRSDDASVGVGVKIAAAKVVRELSRGGVVALRCLASCDAPRALAGVLAQSAMSPS--RRELARVCVDVARFLTTLHTRAVTGSIPPSEASRMAS-----
Mipuc16g03100 RLARQTGPETRESRVACAKAARSVAAGGAVARRCLAACGAPKALAATLSSPGFYSGEGNRELTRACVETCAEMMRAHEDEERGRGGGGEGVDFSGRR---
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SEPA_DICDI ----------T-LP----------------
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Ol16g02130 ----------GSS---------MTGSIG----------
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MAP3K_ARATH SGRGKENGNLDTAPRYSSKTMTKKVMAIERVASTCGIASQTASGVLSGSGVLNARPGSTTSSGLLAHALSADVSMDYLEKVADLLLEFARAE-----TTV
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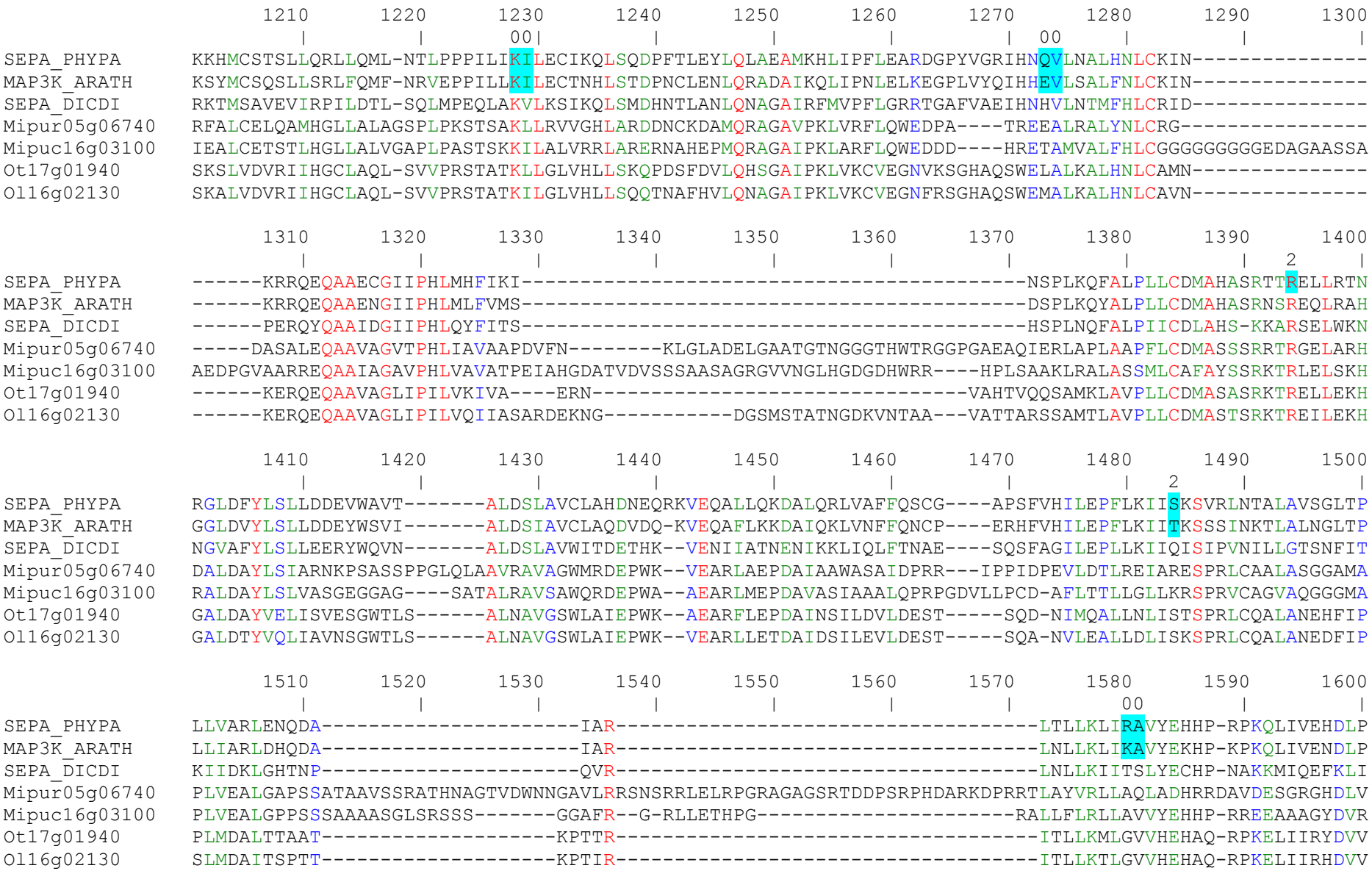
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MAP3K_ARATH        QKLQNLIEERRDGQRSGGQVLVKQMATSLLKALHINTIL
SEPA_DICDI         PIIQKIADT--DKS-----VLVQKMASKLLEAFNANTVI
Mipur05g06740      GRLRGVDDESADSERAAEDVR--TEAERLLAELRR----
Mipuc16g03100      ARLRGVLERGGGGGREAAAAV--DVAEELLRKMR-----
Ot17g01940         ARLKRLIEGVTDER-SSGAVA--QLVDKILRSMRLTRVA
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d) U5 Helicase

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A9RTW1_PHYPA       M------AHLGGGAEAH-----ARFKQYEYRANSS------------LVLTTDTRPRDTHEPTGEPESLYGRIDPRS----FGDRVYHGRA------NDL

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Q9SYP1_ARATH       EDKLKKSKKK---------ERDVVDDMVNIRQSKR-----RRLREESVLTDTDDAVYQPKTKETRAAYEAMLGLIQKQLGGQPP----SIVSGAAD-----
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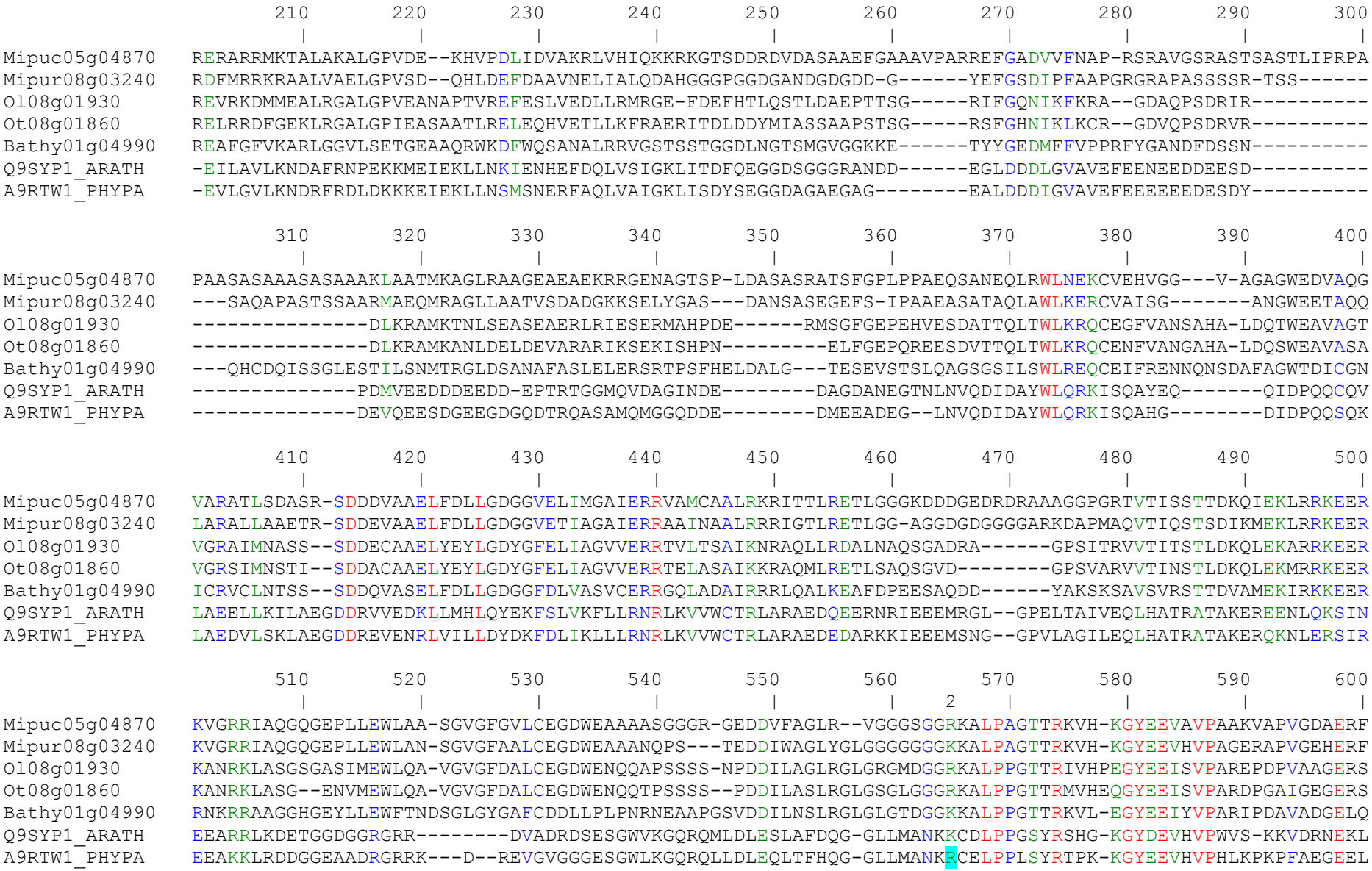
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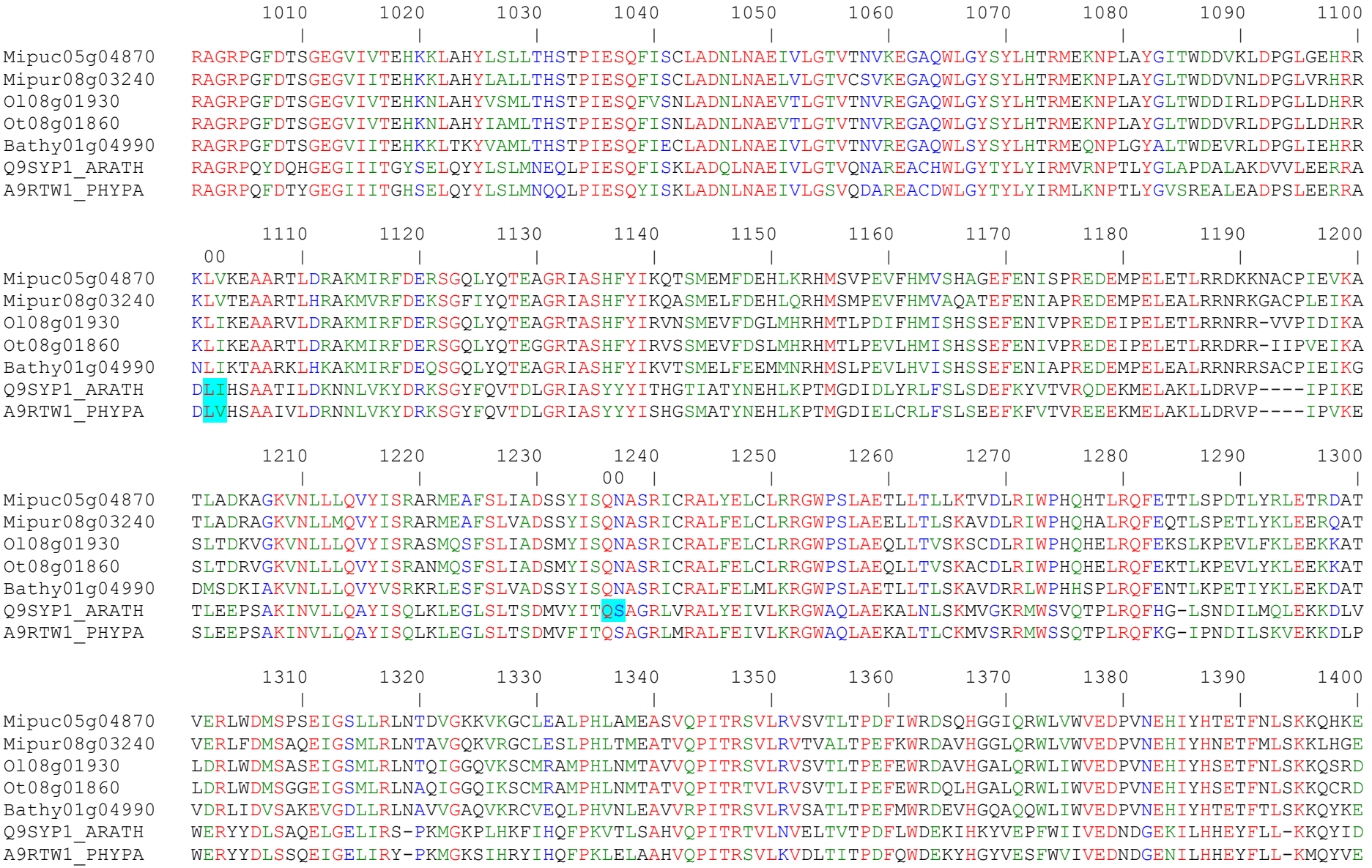
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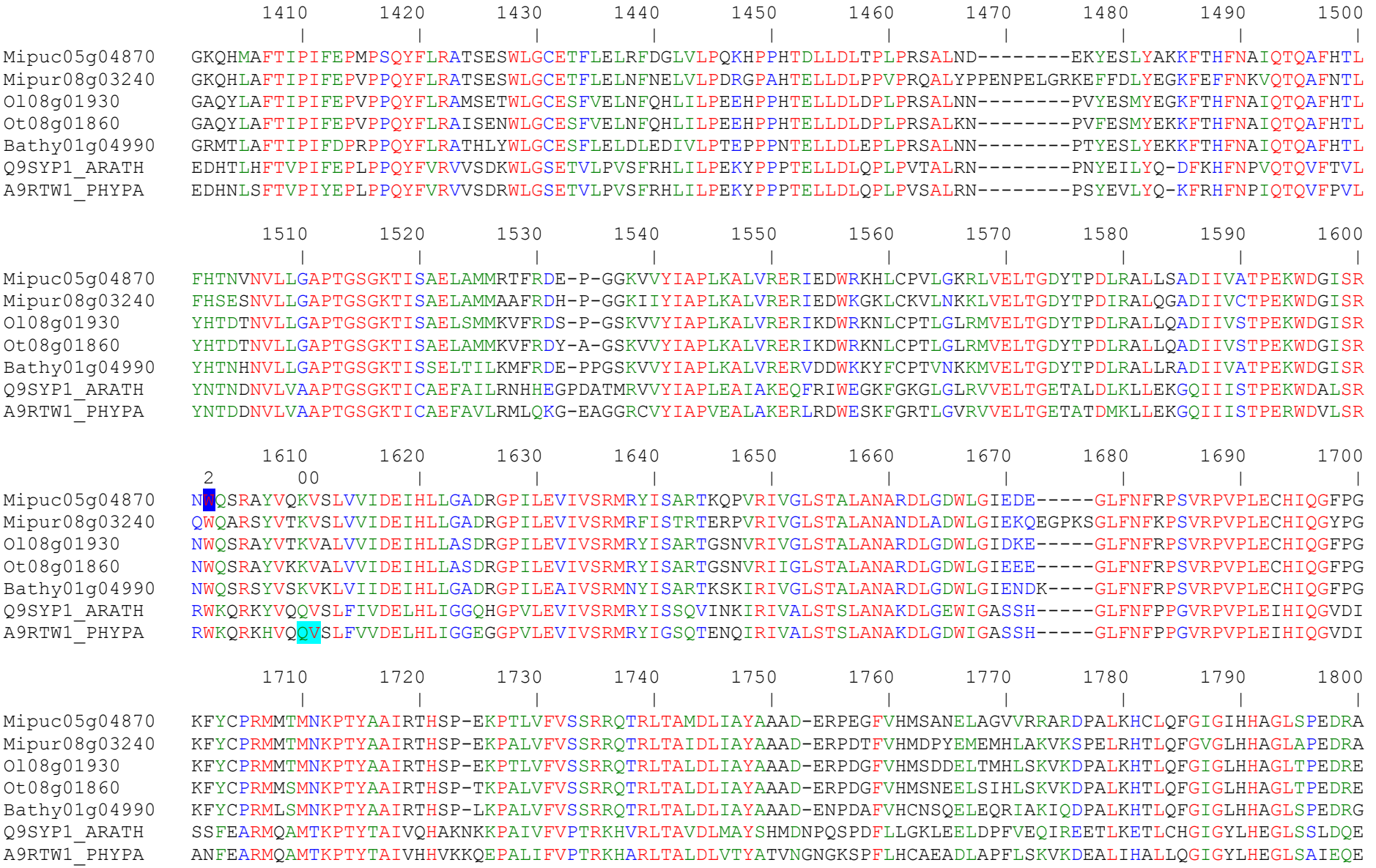
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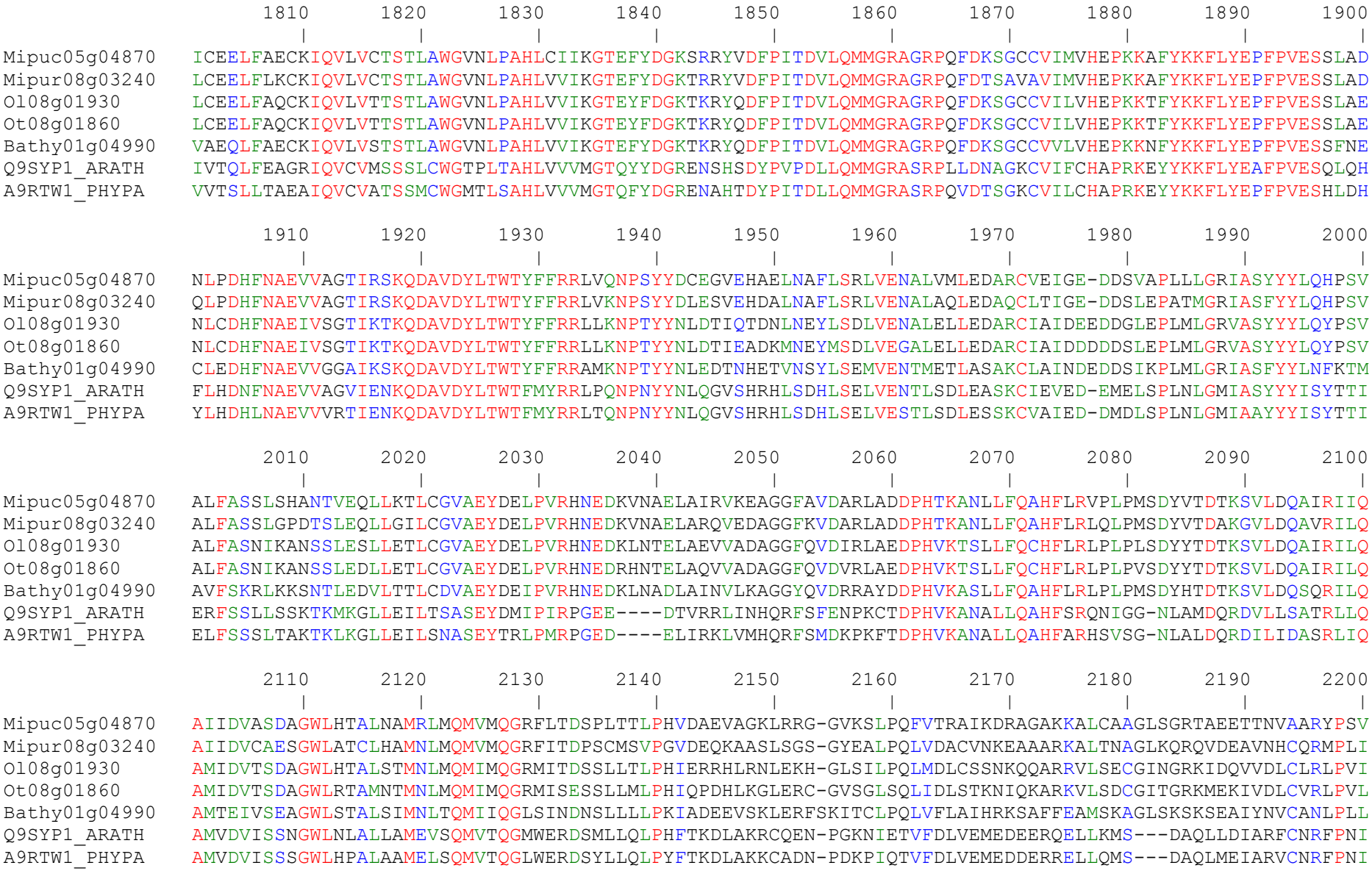
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e) UAA1

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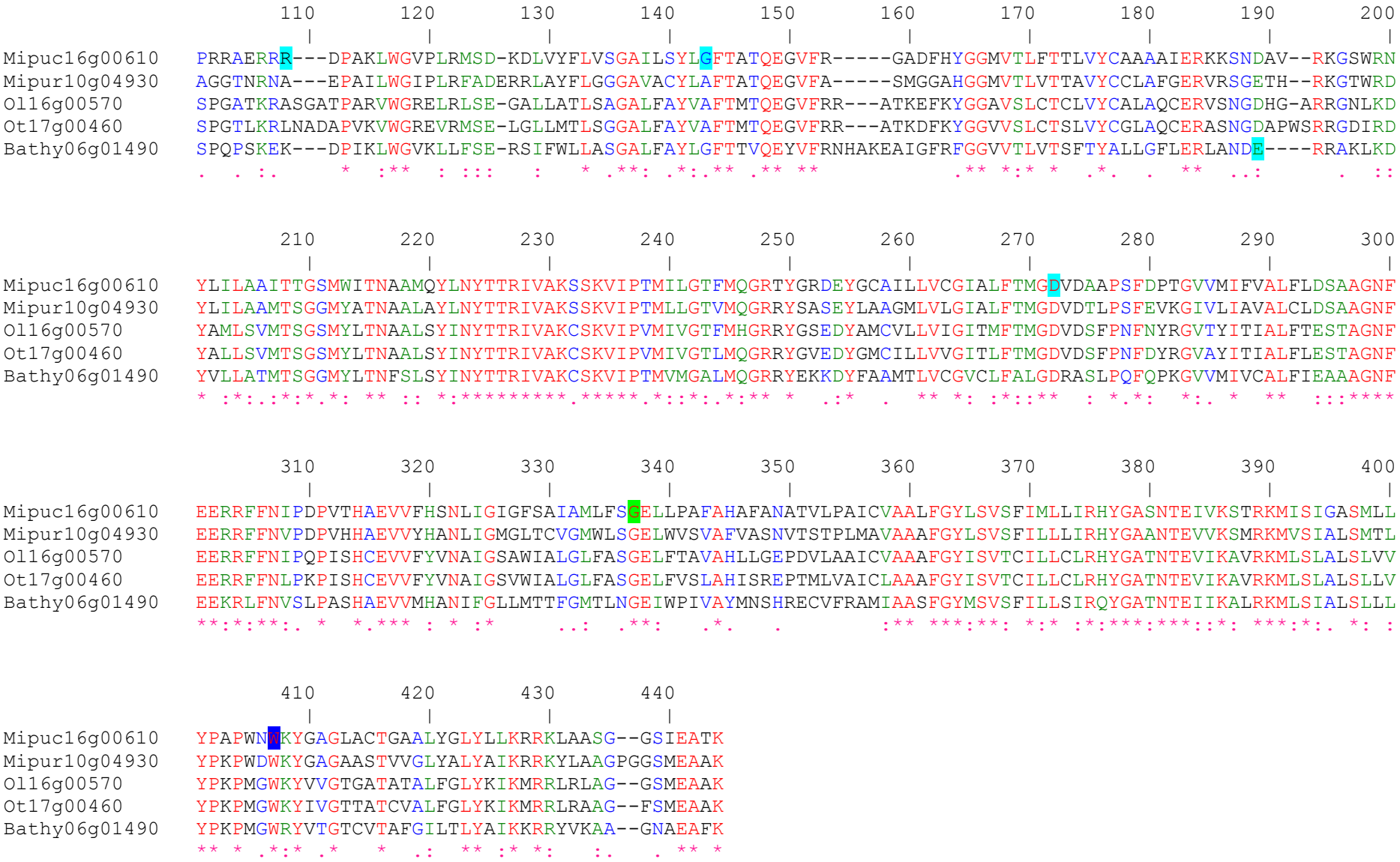
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410 420 430 440
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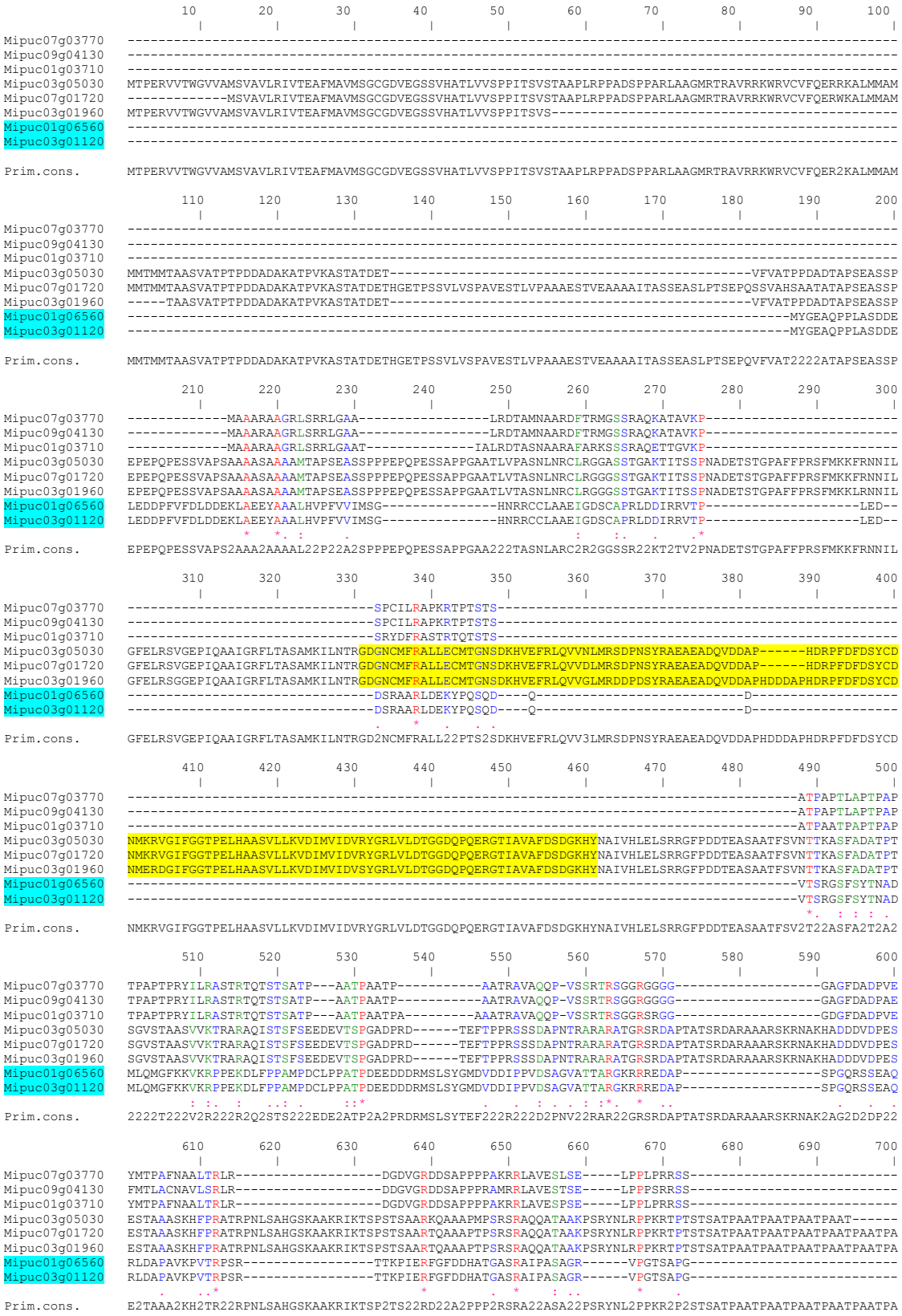
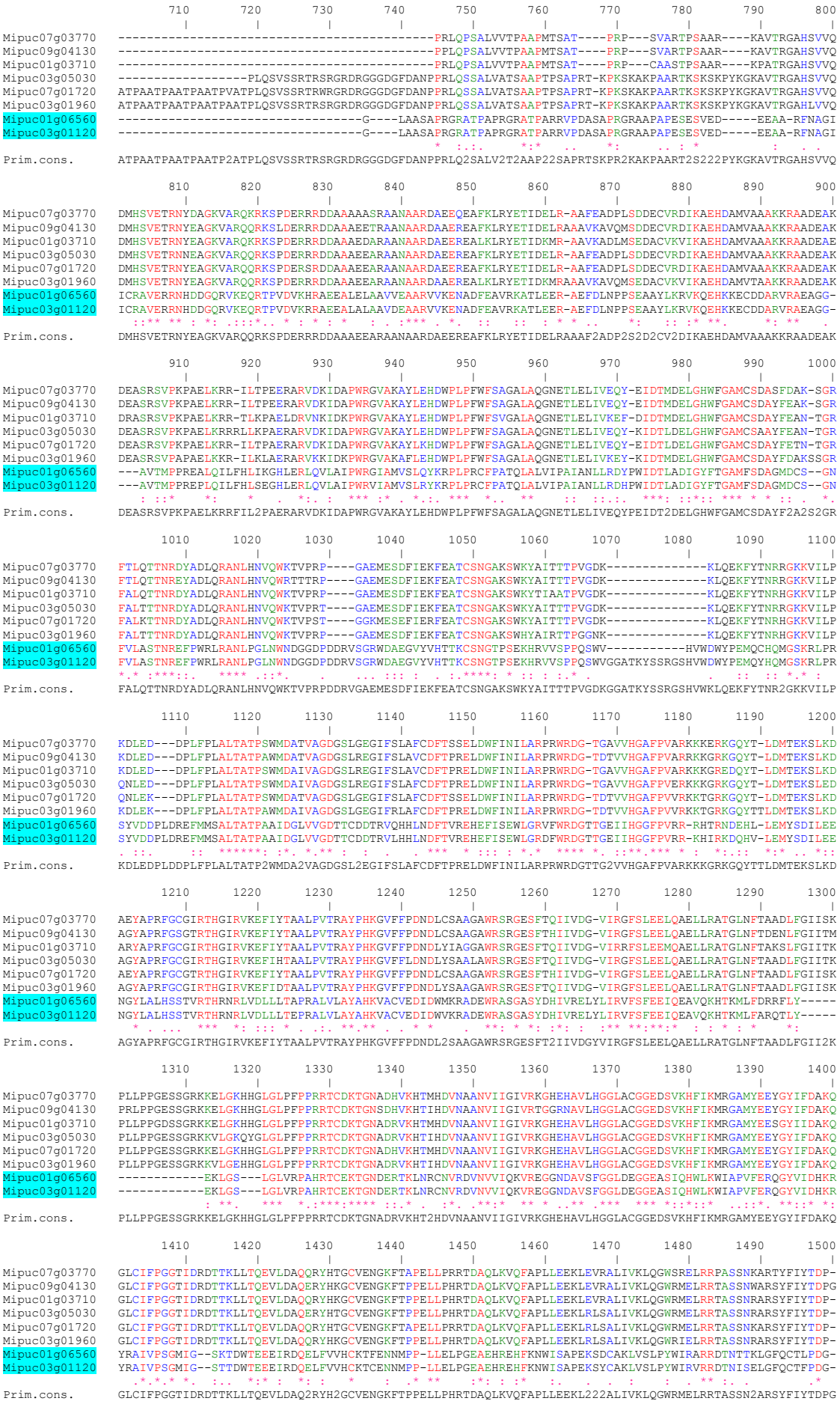
Mipur10g04930 YPKPWDWKYGAGAASTVVGLYALYAIKRRKYLAAGPGGSMEAAK
Ol16g00570 YPKPMGWKYVVGTGATATALFGLYKIKMRRLRLAG--GSMEAAK
Ot17g00460 YPKPMGWKYIVGTTATCVALFGLYKIKMRRLRAAG--FSMEAAK
Bathy06g01490 YPKPMGWRYVTGTCVTAFGILTLYAIKKRRYVKAA--GNAEAFK

Figure S9. Alignment of all intron encoded proteins (IEP) within IE-B sequences. The divergent group of IEPs (blue) and the 'OTU-like cysteine protease domain' (yellow) have been highlighted.





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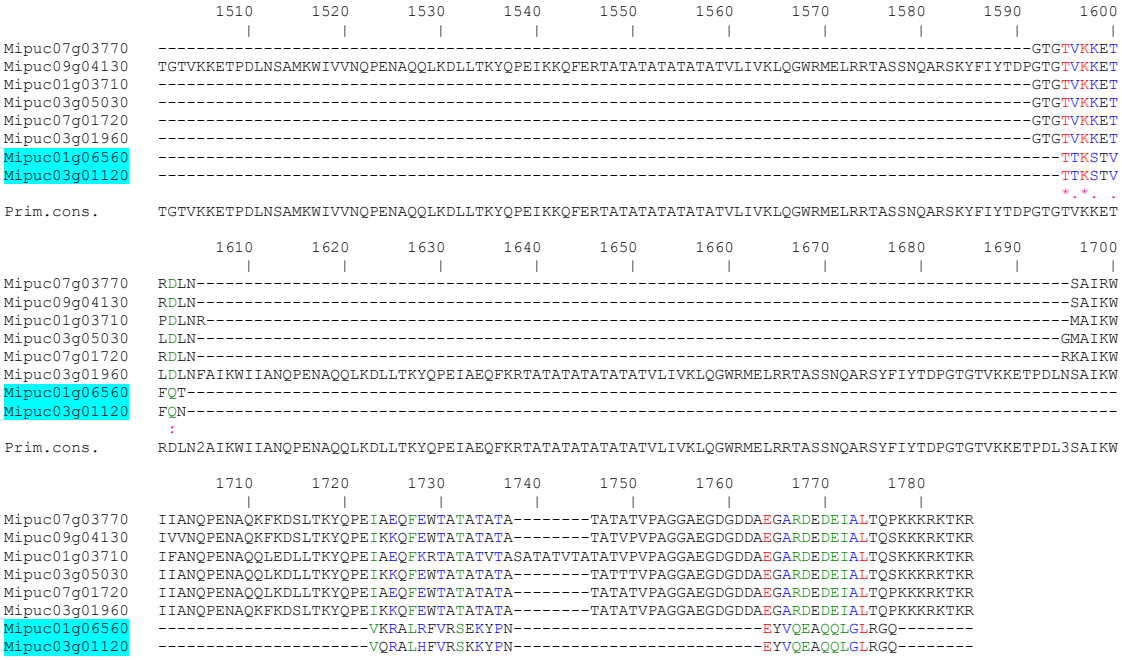
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Mipuc03g05030 ----------GTGTVKKET
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Prim.cons. TGTVKKETPDLNSAMKWIVVNQPENAQQLKDLLTKYQPEIKKQFERTATATATATATATVLIVKLQGWRMELRRTASSNQARSKYFIYTDPGTGTVKKET
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Mipuc03g05030 LDLN----------GMAIKW
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Mipuc01g06560 FQT----------
Mipuc03g01120 FQN----------
Prim.cons. RDLN2AIKWIIANQPENAQQLKDLLTKYQPEIAEQFKRTATATATATATATVLIVKLQGWRMELRRTASSNQARSYFIYTDPGTGTVKKETPDL3SAIKW
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Mipuc01g03710 IFANQPENAQQLEDLLTKYQPEIAEQFKRTATATVTASATATVTATATVPVPAGGAEGDGDDAEGARDEDEIALTQSKKKRKTKR
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Mipuc03g01960 IIANQPENAQKFKDSLTKYQPEIKKQFEWTATATATA--------TATATVPAGGAEGDGDDAEGARDEDEIALTQPKKKRKTKR
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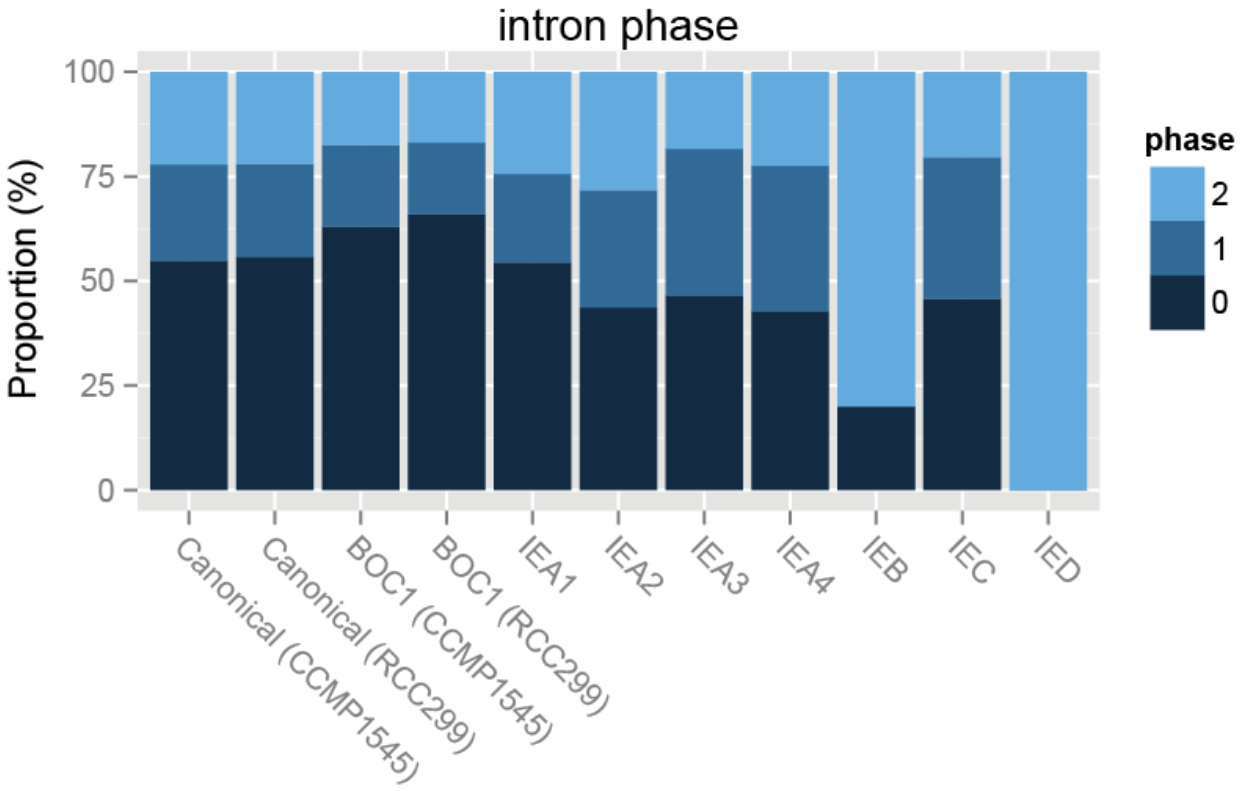


Figure S10. Phase distribution of Introner Elements, BOC1 and canonical introns.

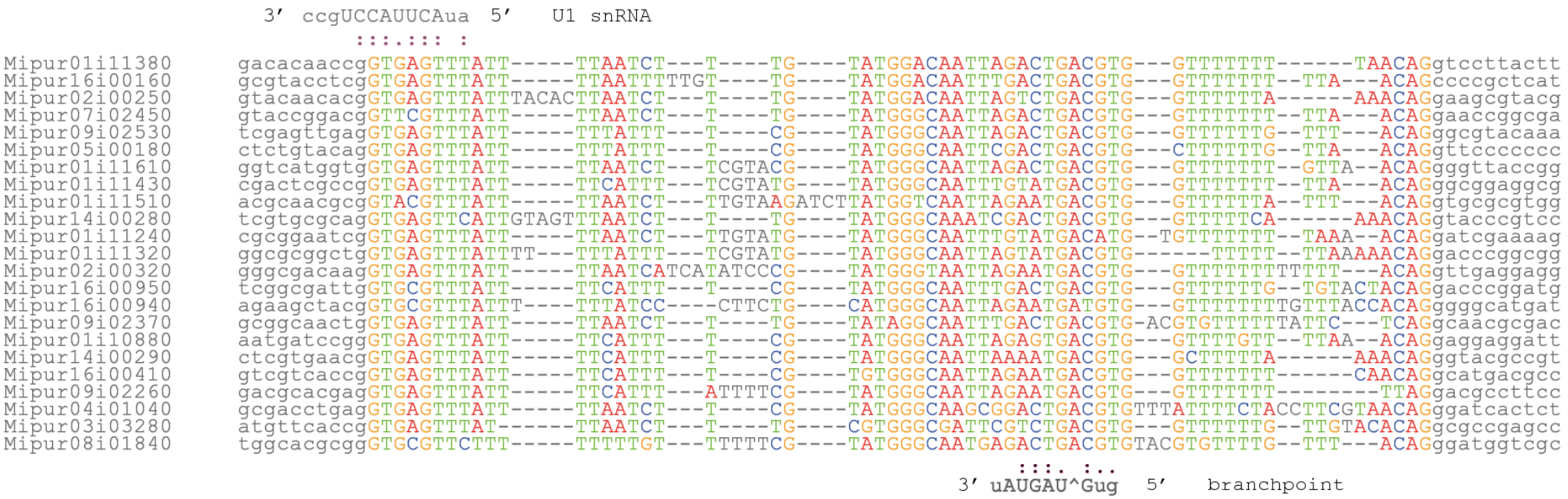


Figure S11. Alignment of typical IE-C sequences, flanked by their exonic regions (grey). Base-pairing information regarding the donor site (U1 snRNA) and the branch-point (U2 snRNA) is also provided.

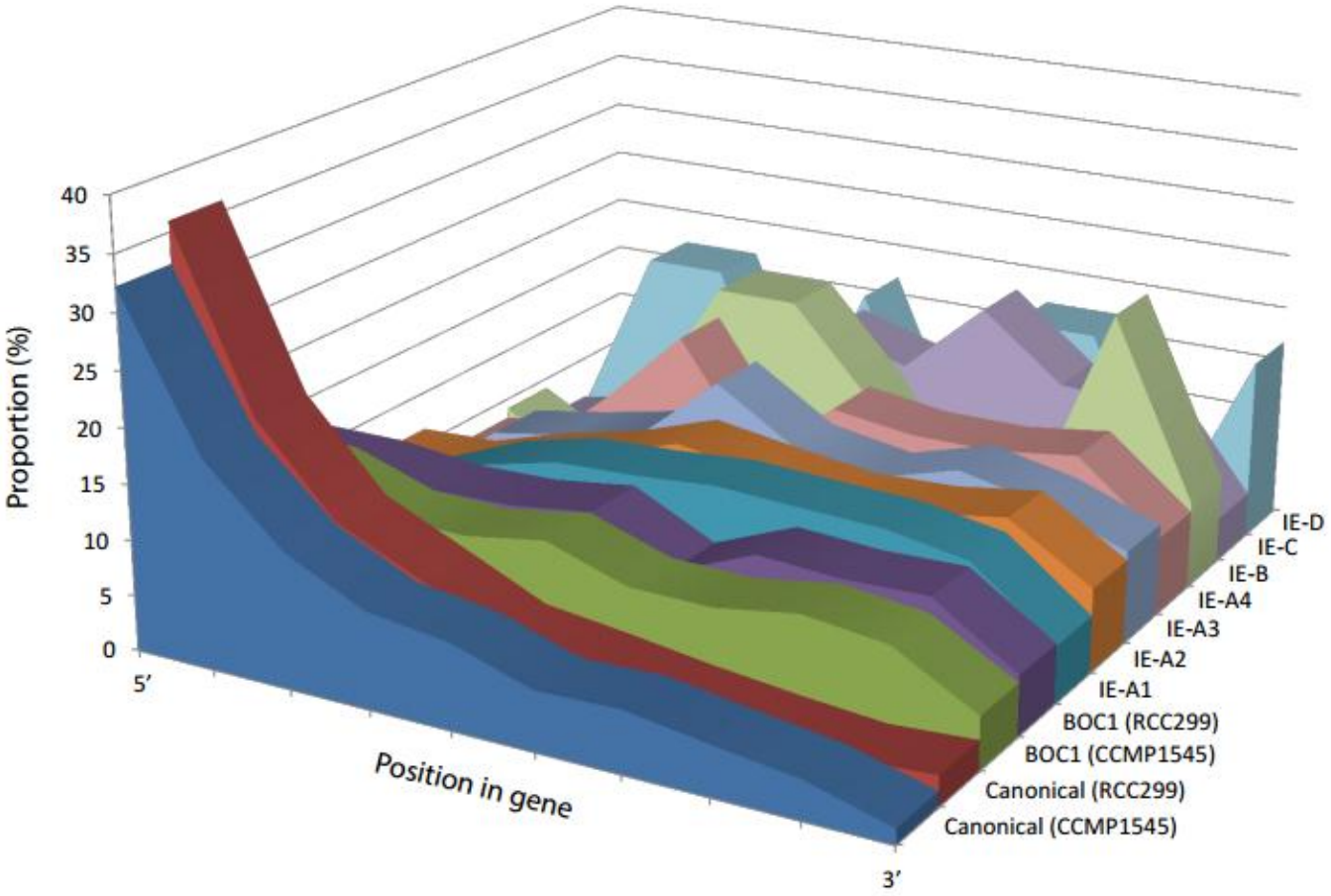
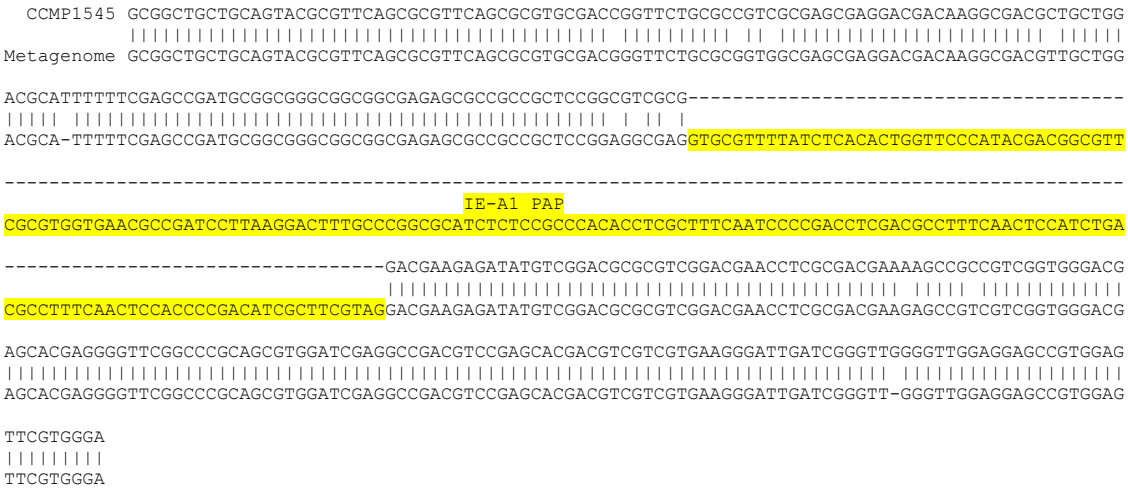


Figure S12. Positioning of Introner Elements, BOC1 and canonical introns inside genes.

Figure S13. Presence/absence polymorphisms in *Micromonas*: IE-A1 PAP (a), IE-A2 PAP (b), IE-A3 PAP (c), and two IE-C PAPs (d).

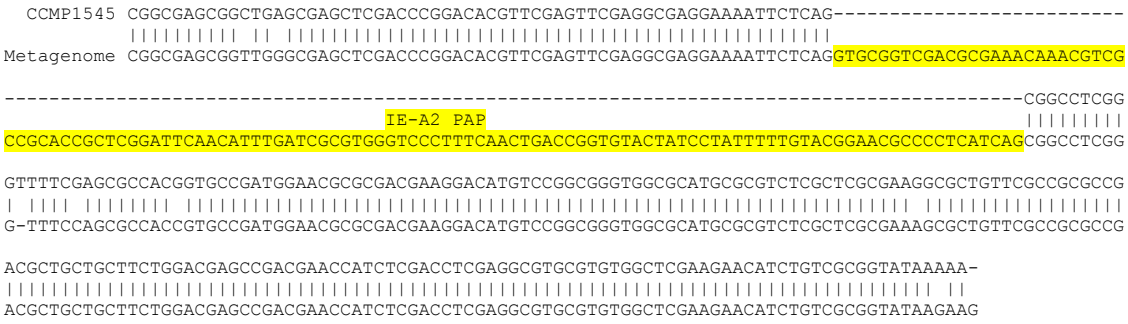
a)

CCMP1545: scaffold_14 (384641..384976)
Metagenomic read: CAM_READ_0212050959 (CAMERA accession)



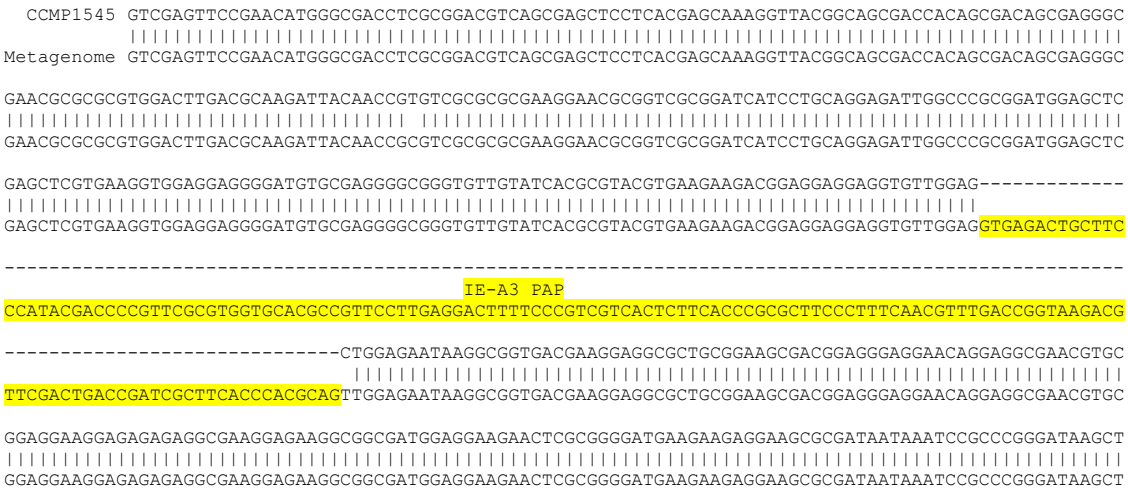
b)

CCMP1545: scaffold_01 (251138..251395)
Metagenomic read: CAM_READ_0212289655 (CAMERA accession)



c)

CCMP1545: scaffold_14 (643394..644184)
Metagenomic read: AACY020815663 (GenBank Accession)



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AGTCAGTACAAGGACAGGGAAG------------------GCGGCGGCGGCGGCGGAAGCGGCGGCGGCGGGAAGAAATCGCCGGCGGGGACGAAGGGCA
|||||||||||||||||||||||                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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|||||||||||||||||||||||||||||||||||||||||||||||||||
GTCTTCAAAAGTTGTCGGGATTGTACATCCGCATCGCCCCCGTCCGGAGCAA
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d)

RCC299: chrom_06 (160647..160930)
Metagenomic read: CCMP1764_READ_00758094 (CAMERA accession)

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Metagenome CTCATCGACGTTCGGTGGGATCGGTGGCGAACCGACGCCGCGGGACGGTACCTGGTCA-CCCACGCGTCGTGTCGCACCTCGCCAACTG

CCACCCGCGGGCTGAGTTCGGGCAGGTGATTCTTTGCATTTTAATCTTTGCATCATGGGCAATCAGATTGACGTGGTTTTTTATTCGCAGGCTGTGCGGC
 ||||||||||||||   |||||                                IE-C PAP                                 |||||||||
TCACCCGCGGGCTGAGCGTCGGCAA-------------------------------------------------------------------ACTGTGCGGC

GCCCGAGCCGTCGCCGTCGTCGACGAGACG-------------------------------------------------------------GCGTACGTCGTG
||  |||||||||||||||||||||||||||                       IE-C PAP                           ||||||||||||
GCTCGAGCCGTCGCCGTCGTCGACGAGACGGTGAGTTTATTTGTTATTTCGTATGGGCAATTAGTTTGACATCGTGTTTTGTTCACAGGCGTACGTCGTG

TCGGAGATGTCCAACTCCCTCTCCGTCGTCGTCCTCCCGGCG
||  ||||||||  |||||||||||||||||||||| |||| |||
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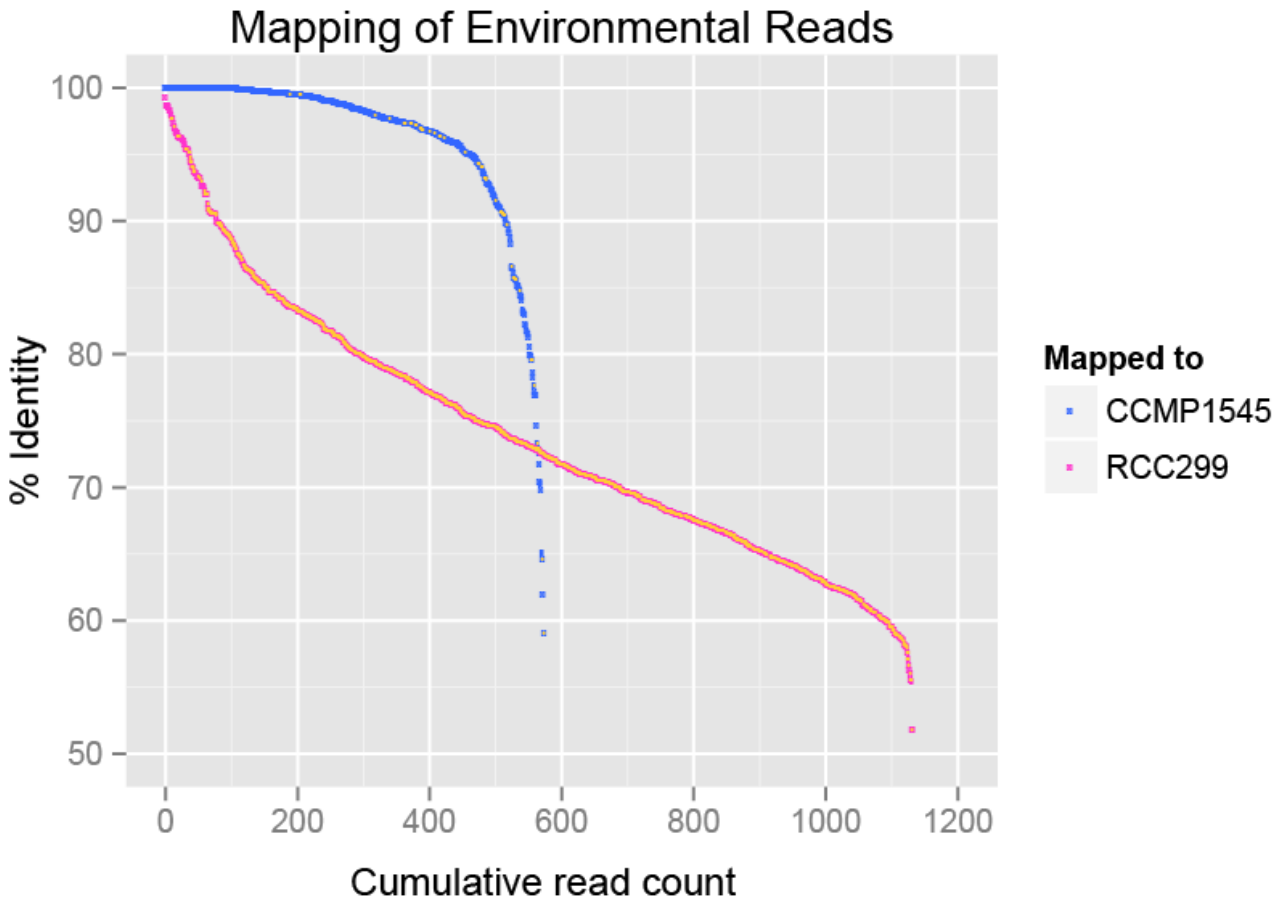

Figure S14. Mapping identities for environmental sequences. Yellow dots indicate alignments showing PAPs.

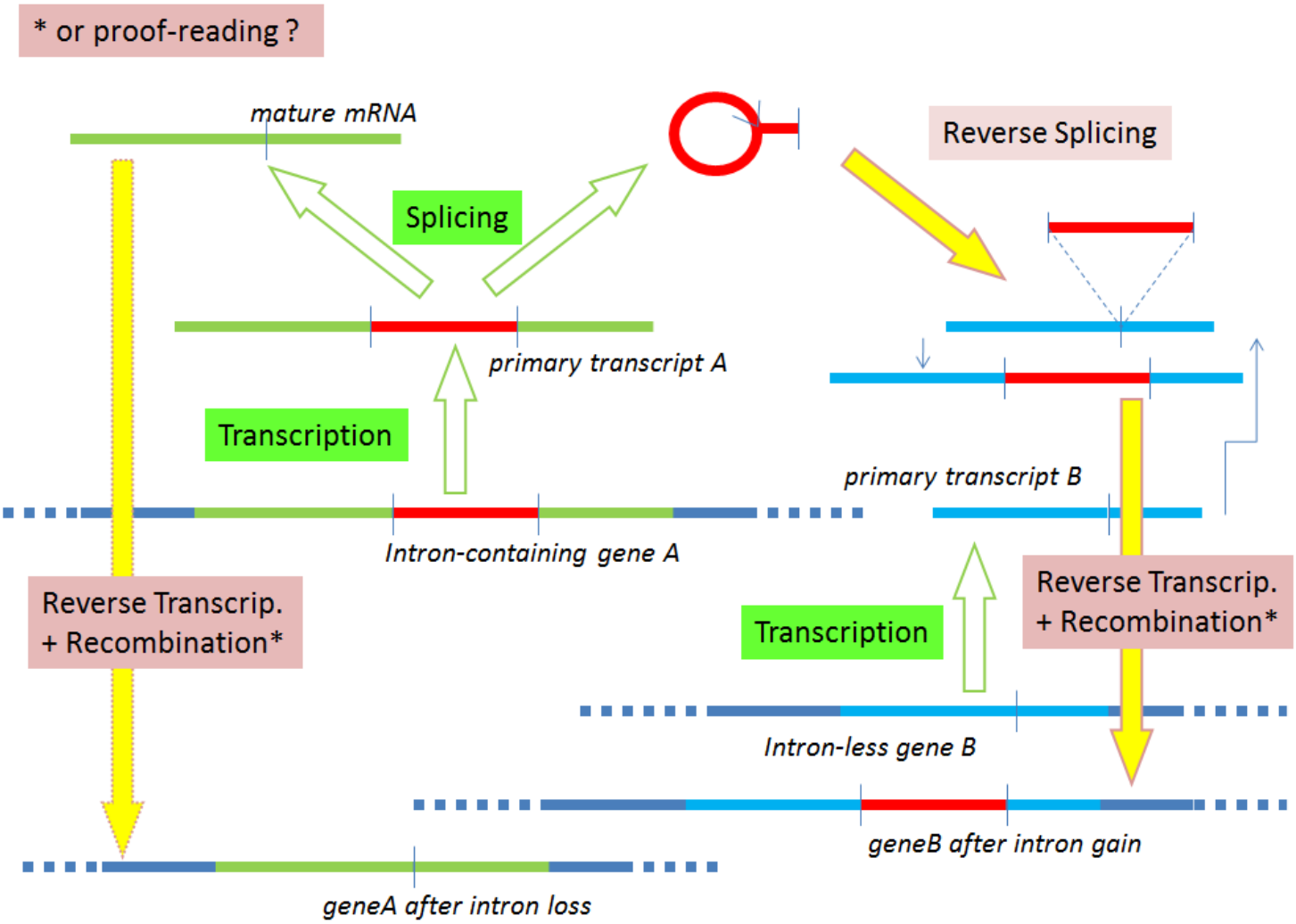

Figure S15. Introner Element replication mechanism.

Mipuc05i01435: IE-A1 + IE-A1

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CGGCGTGCGTTCGTCTCTCTCCGCCCAGGGTCCTTCGGTTCCAATCCCGACGCGCCTCGACGCCTTT
CAACTCCAACAACTGACGCCTTTCAACTTCACCCCGACGCCTTTCAACTTCACCCCCGTCAG

Mipuc02i02490: IE-A1 + IE-A2

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CCGCGTTCGCGTCGTGAACTTCATTCCTCAAGGACTTTCGGCCGATCCTCGCGGACGGACTTCGCCC
GCCCGGCGCGTCTCTCCGCGCACCCCTCGCCGCTTTCGATCCCCCCGACGCGCCTACGCGACGCCTT
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Mipuc16i00710: IE-A1 + IE-A3

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ATGCCTTTCAACTTCAACCGACGCCTTTCAACTCCACCCCGACGTTCATAACTACCCTAAACCCCGT
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ACGTTTGACCGGTAAGACGTTCGACTGACCGATCGCTTCACCCACGCAGGCTCGCGTCGCTCTCCGC
GACGTCGAAG

Mipuc12i00190: IE-A? + IE-A1

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TAATCCCGACGCACCGCGACGCCTCGCGACTTCATCTGACACCCCCTGAACGCCACCCACCCCGACG
TCACCTCGTATGGAAAACGACCCTCAG

Figure S16. Examples of merged Introner Elements. Due to loss of internal splice structures when merging, it is hard to exactly delineate borders.

CCMP1545: scaffold_14; 341989..343470
Metagenomic read: AACY02323272 (GenBank Accession)

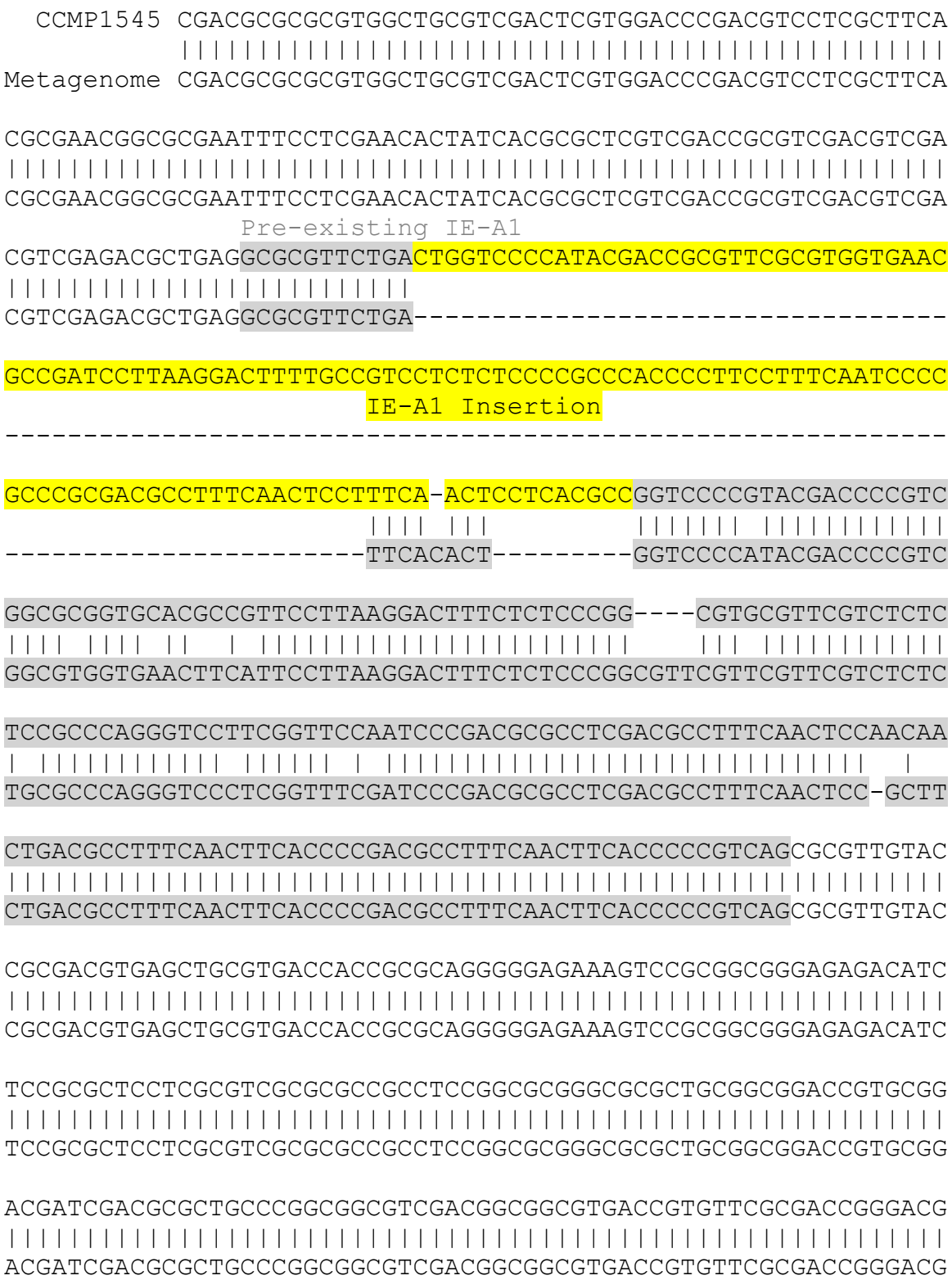

Figure S17. Examples of merged Introner Elements. Due to loss of internal splice structures when merging, it is hard to exactly delineate borders.