SUPPLEMENTARY INFORMATION

Community science designed ribosomes with beneficial phenotypes

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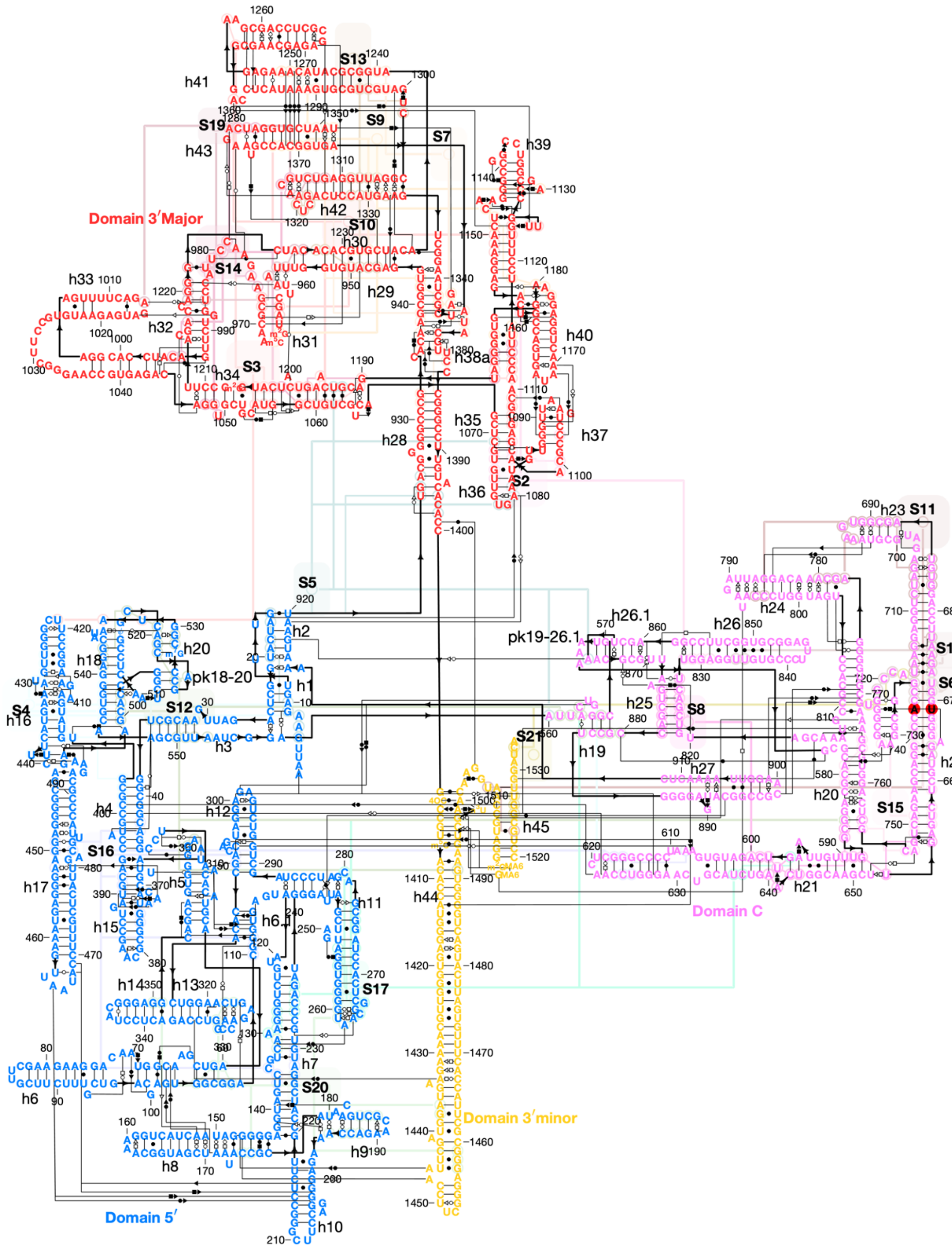

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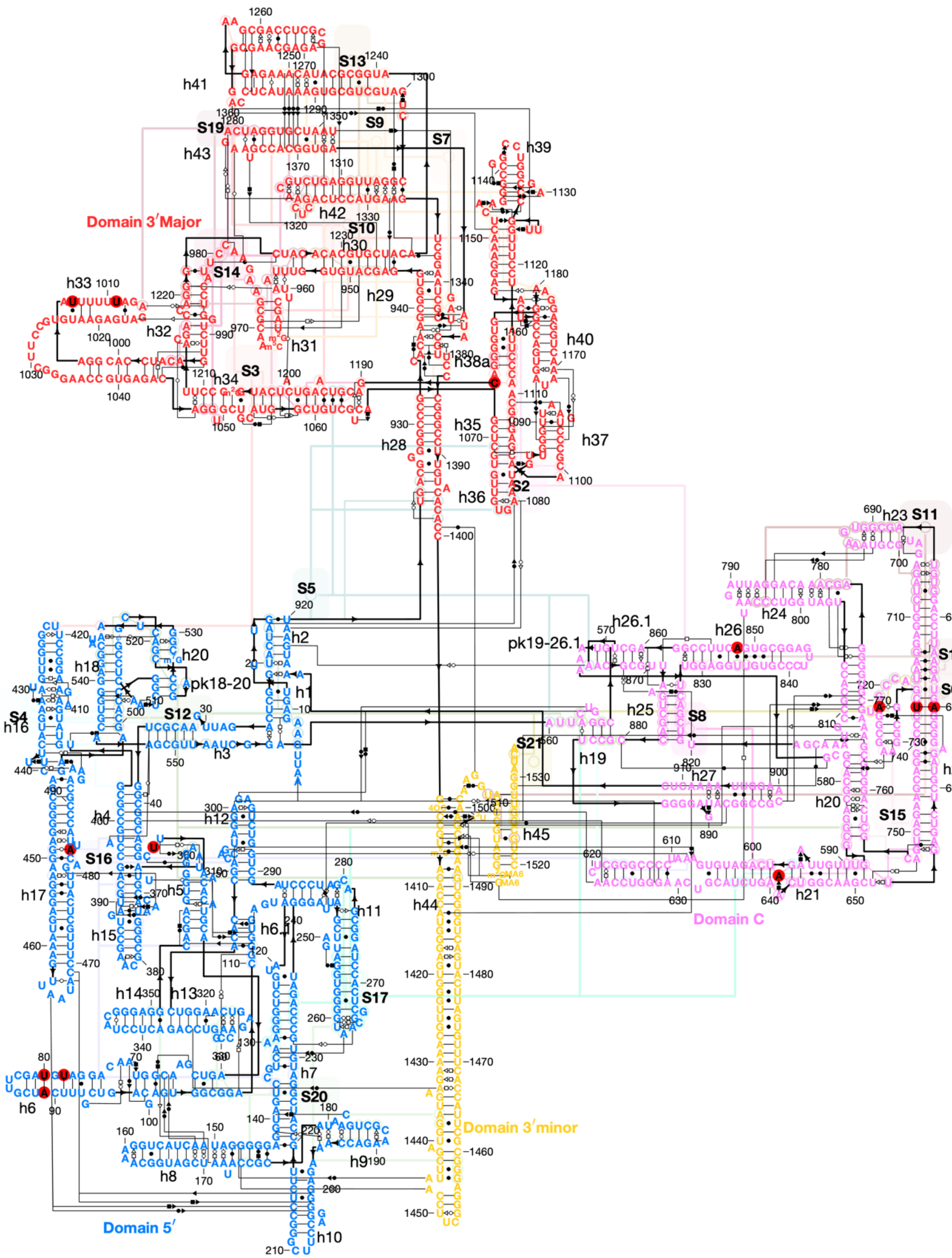

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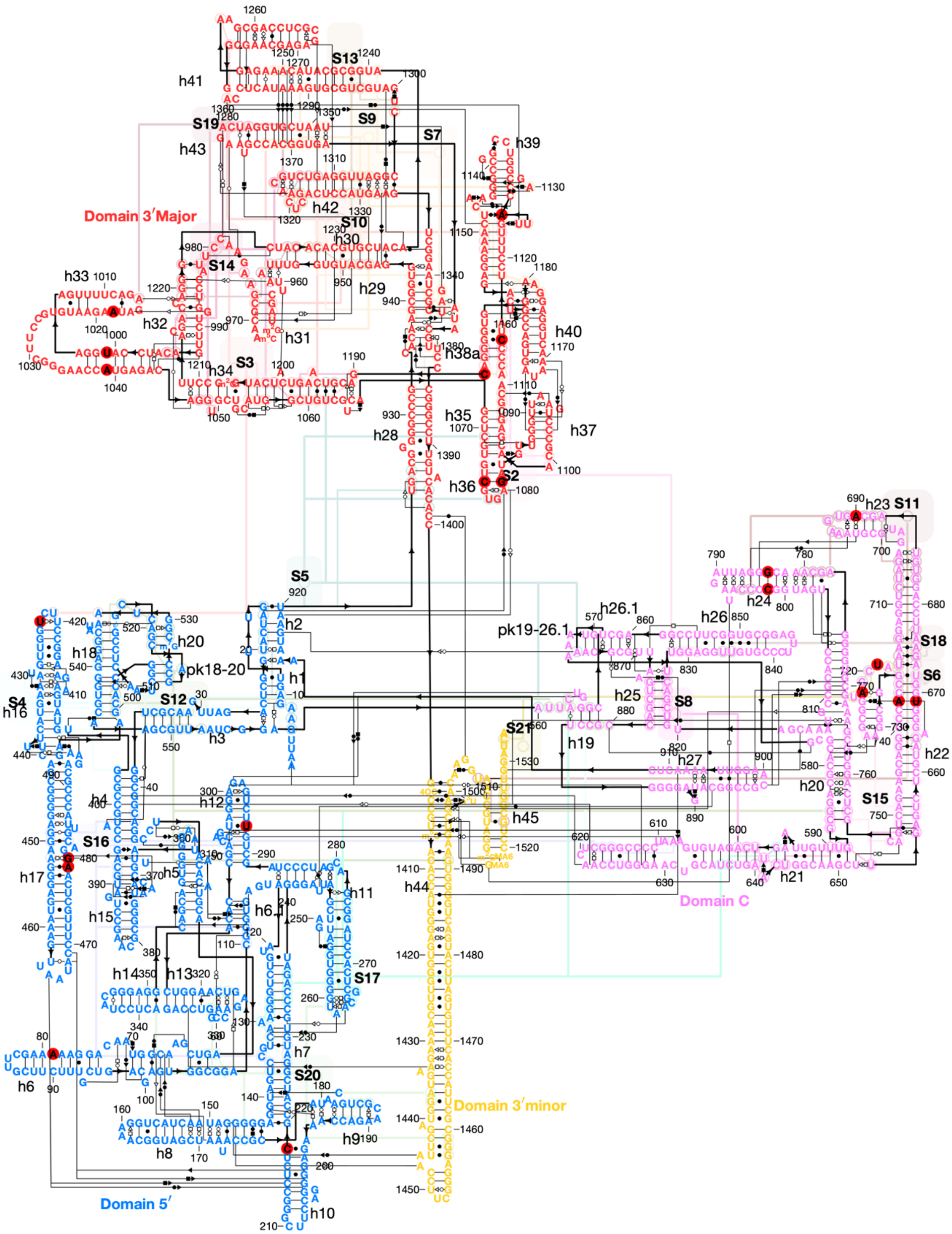

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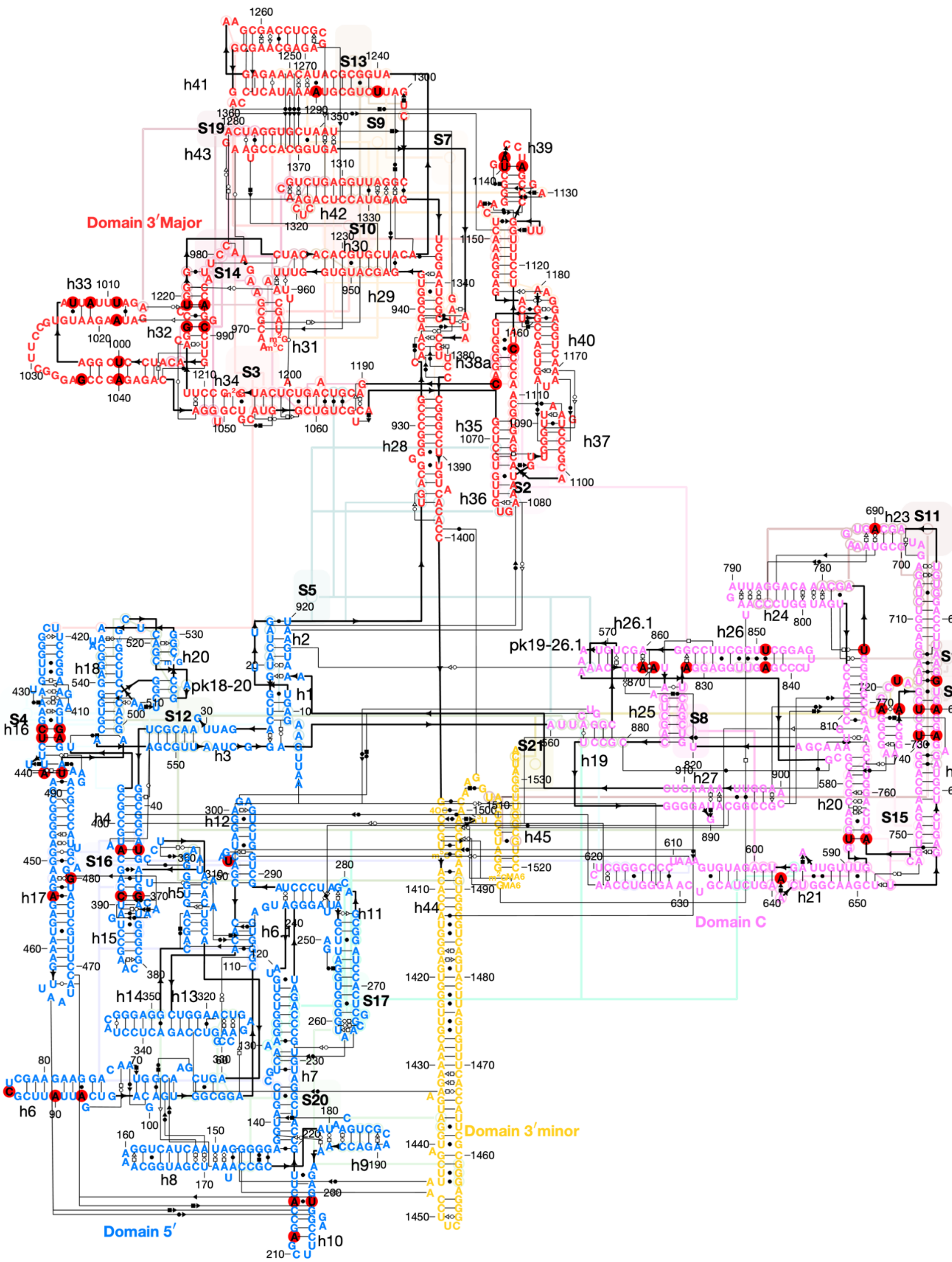

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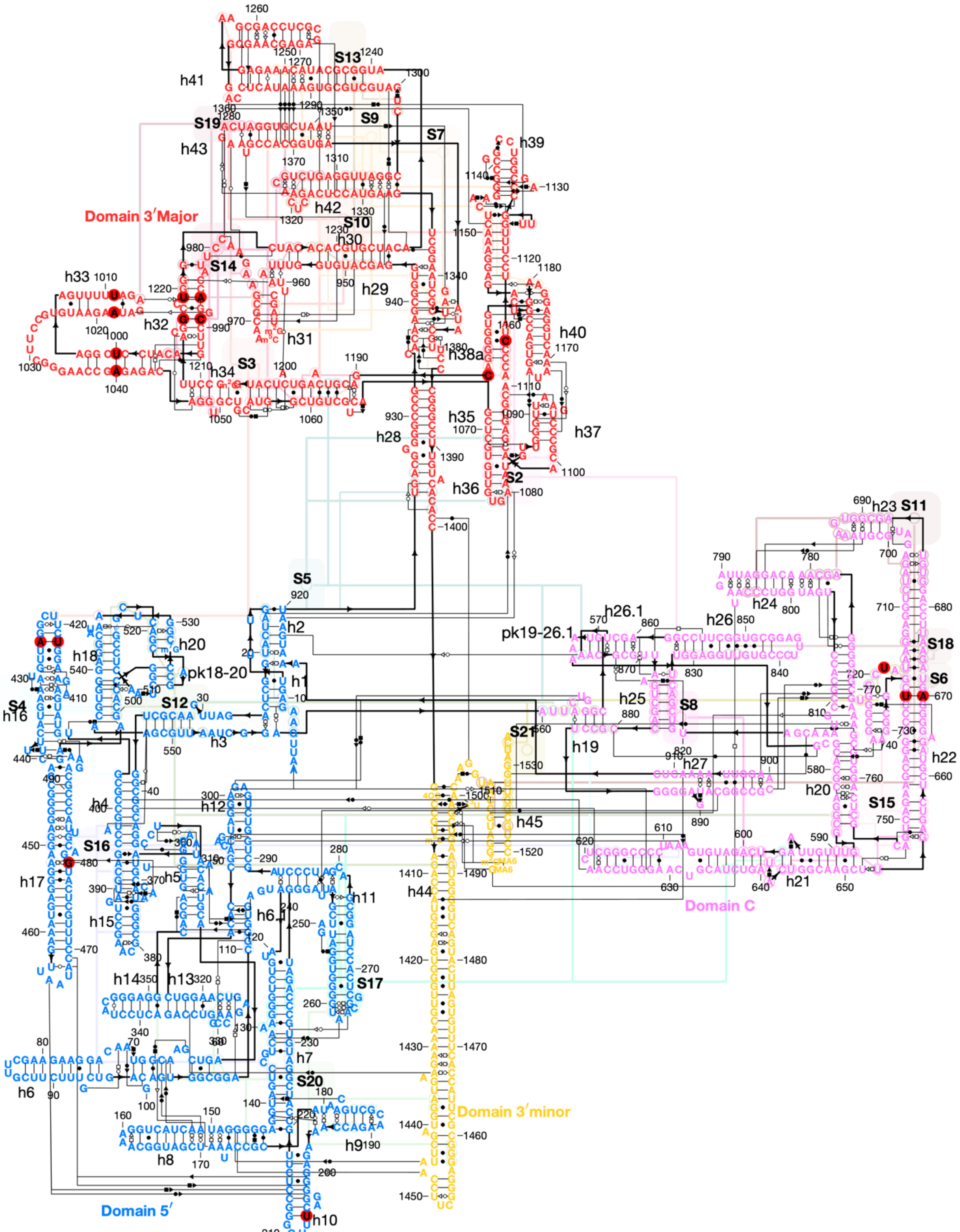

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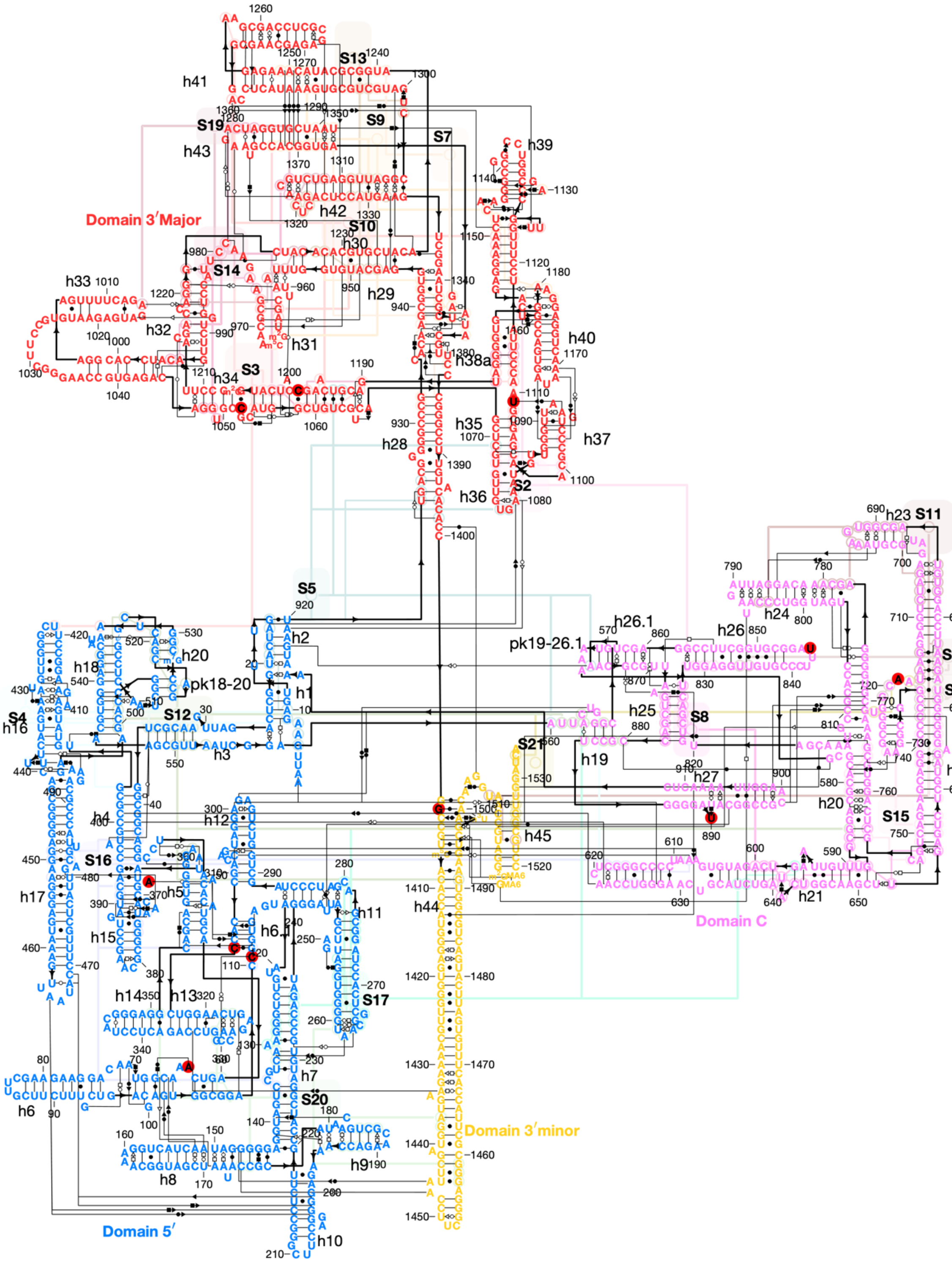

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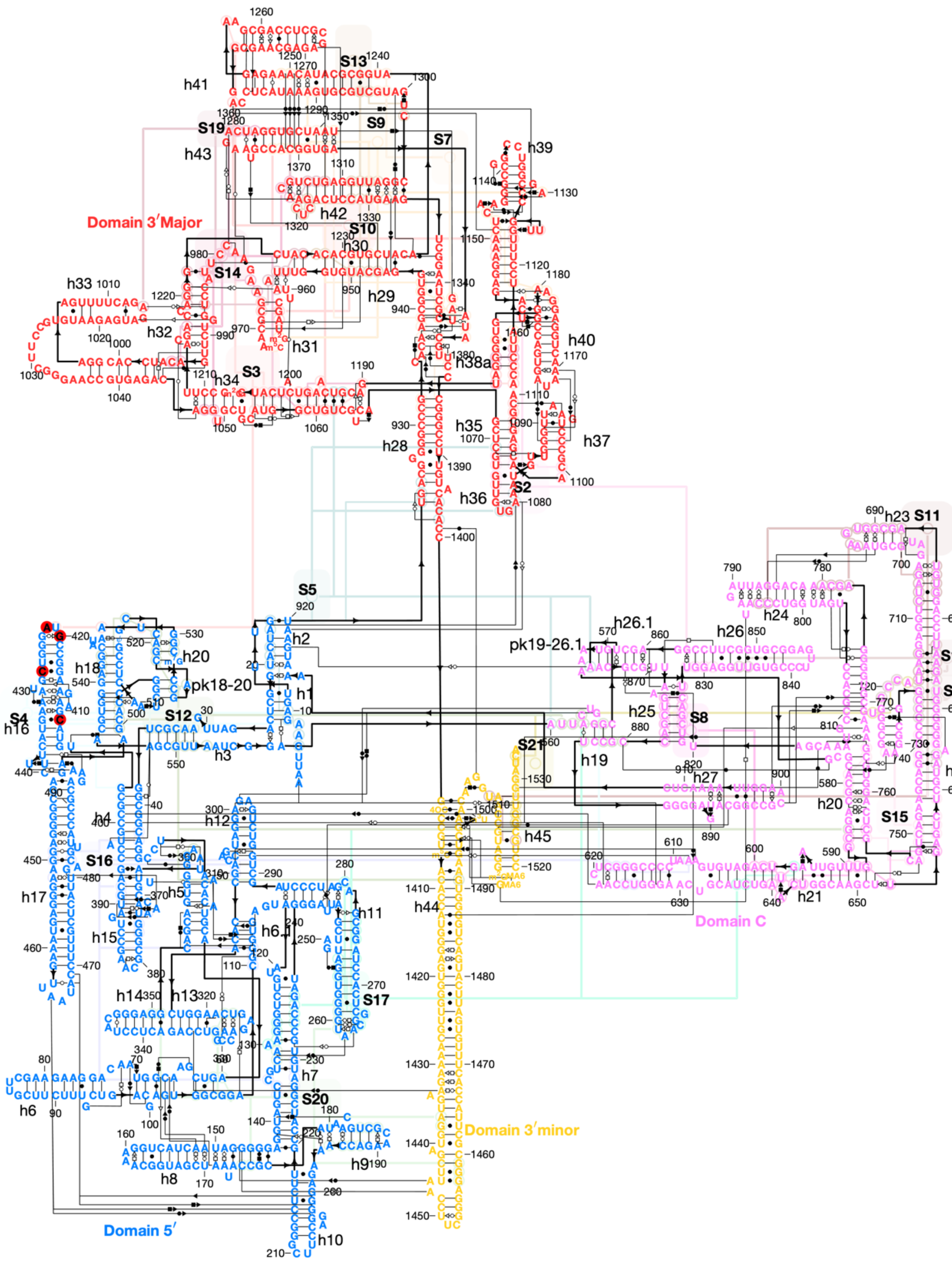

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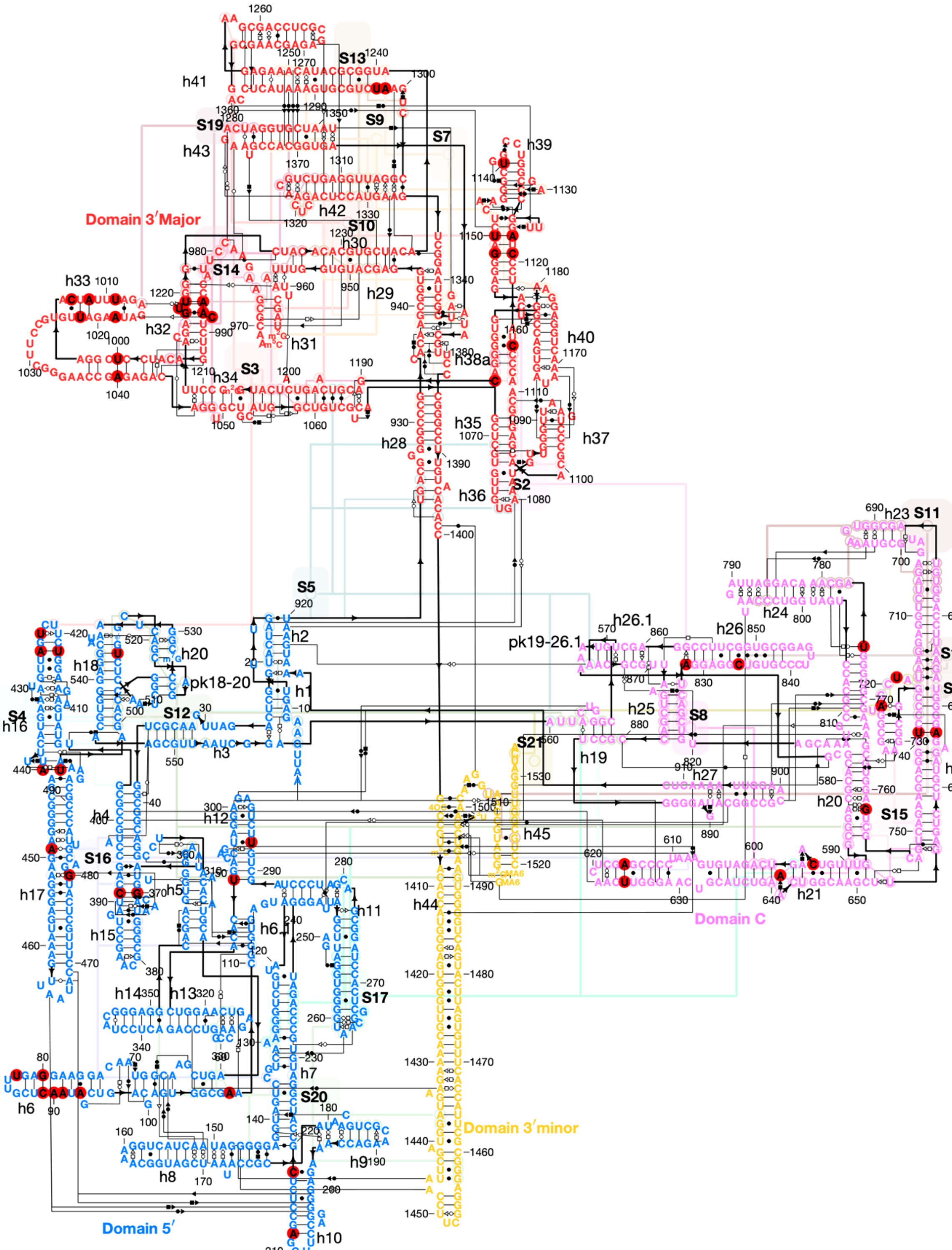

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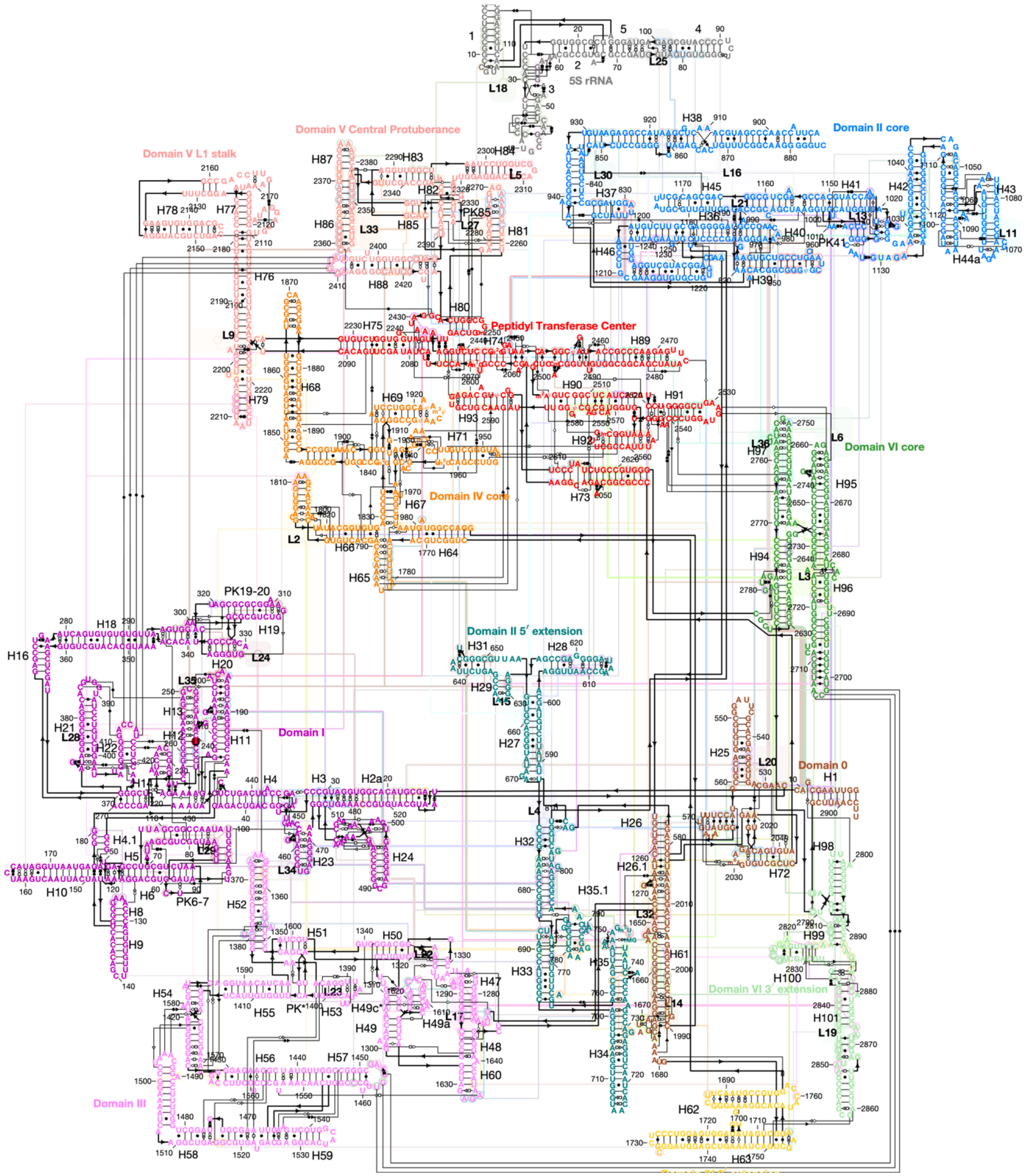

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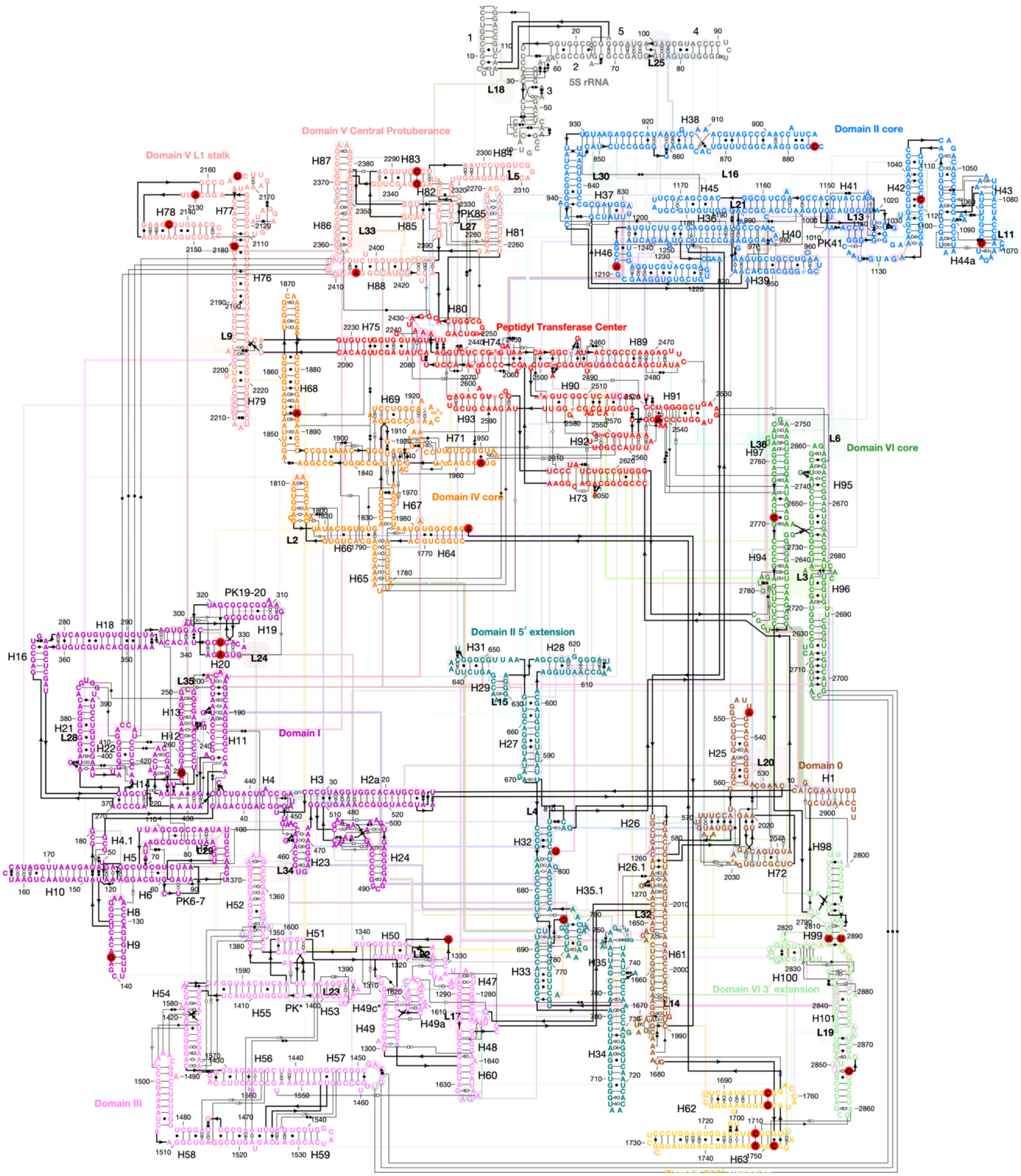

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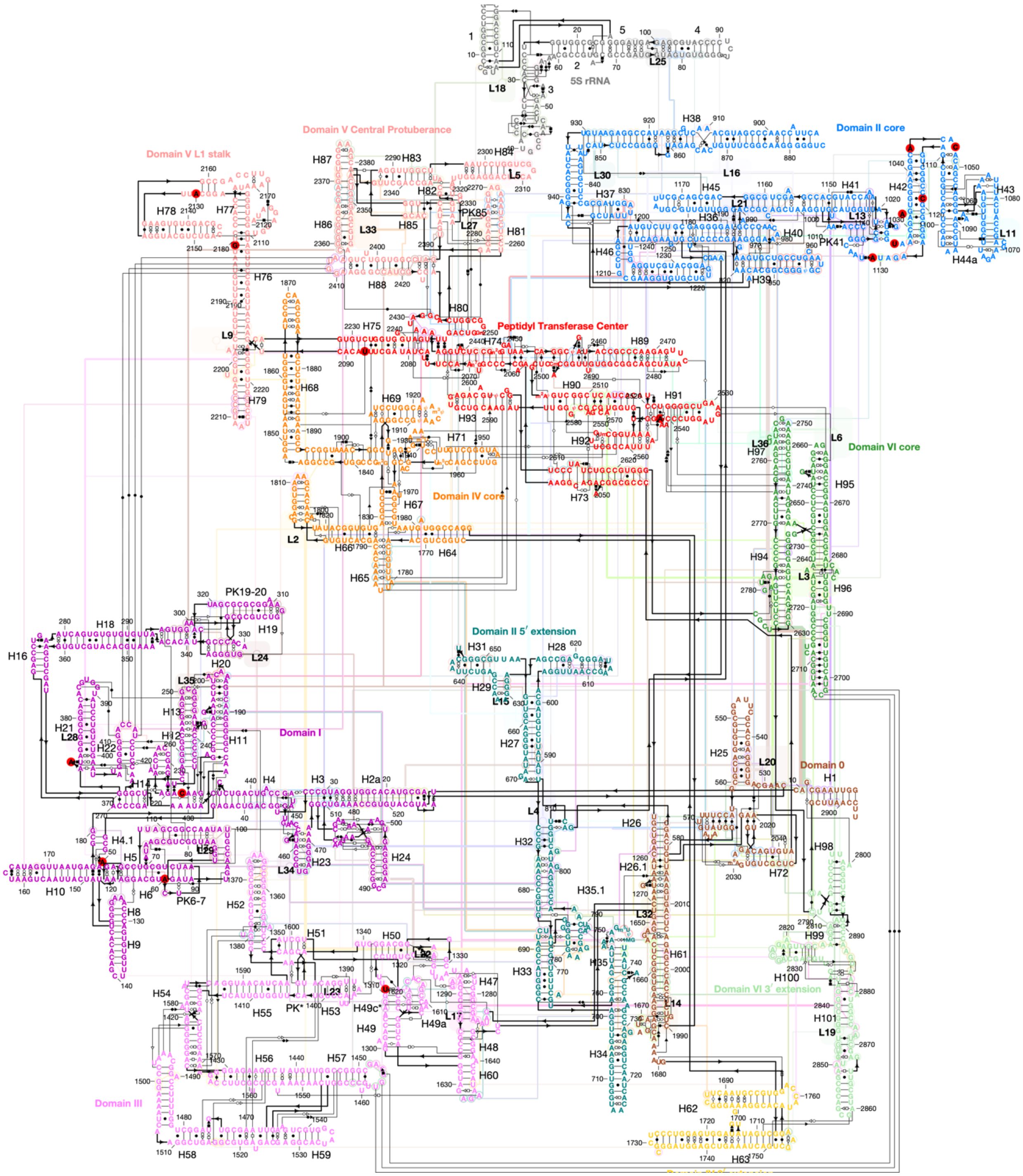

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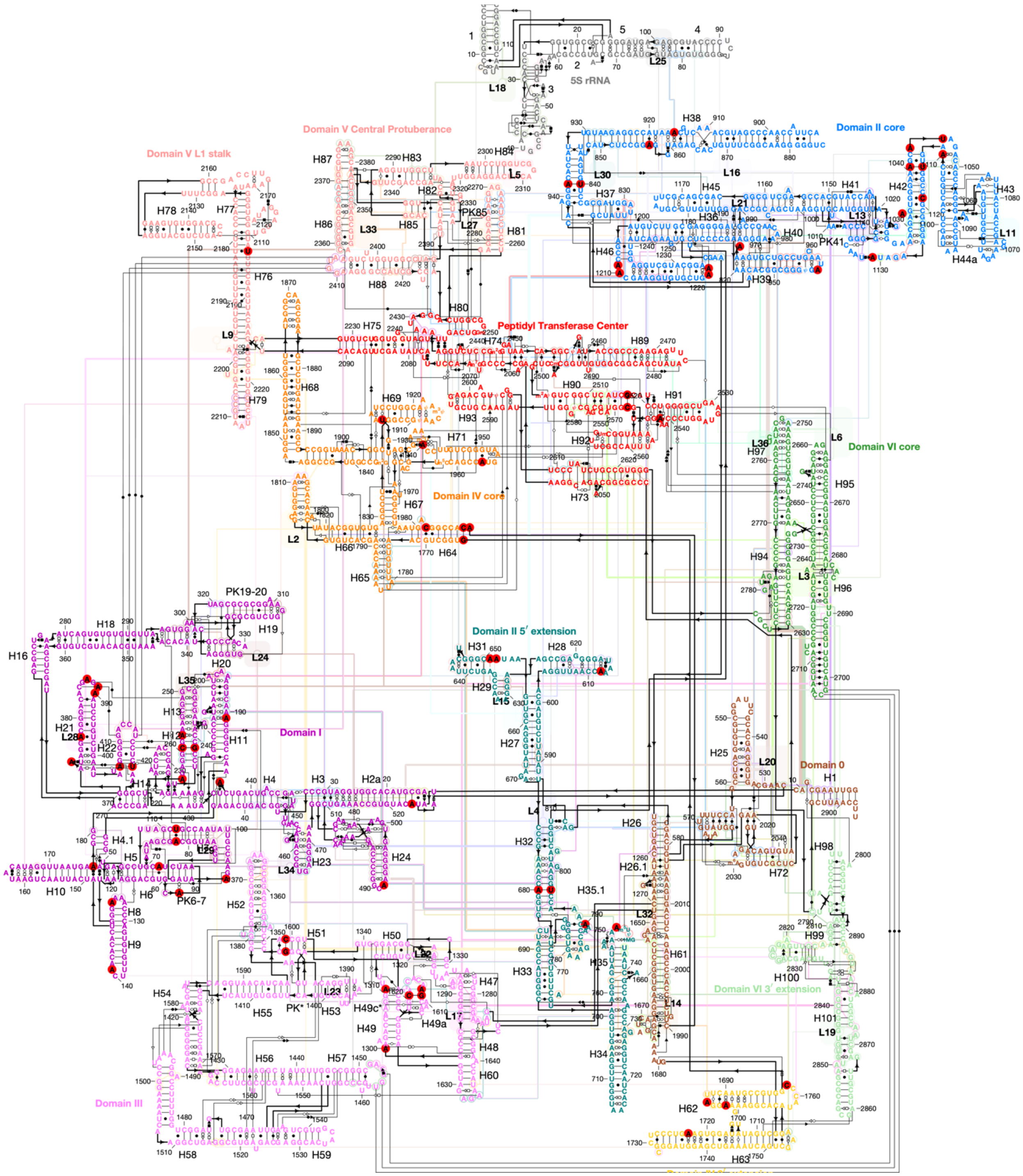

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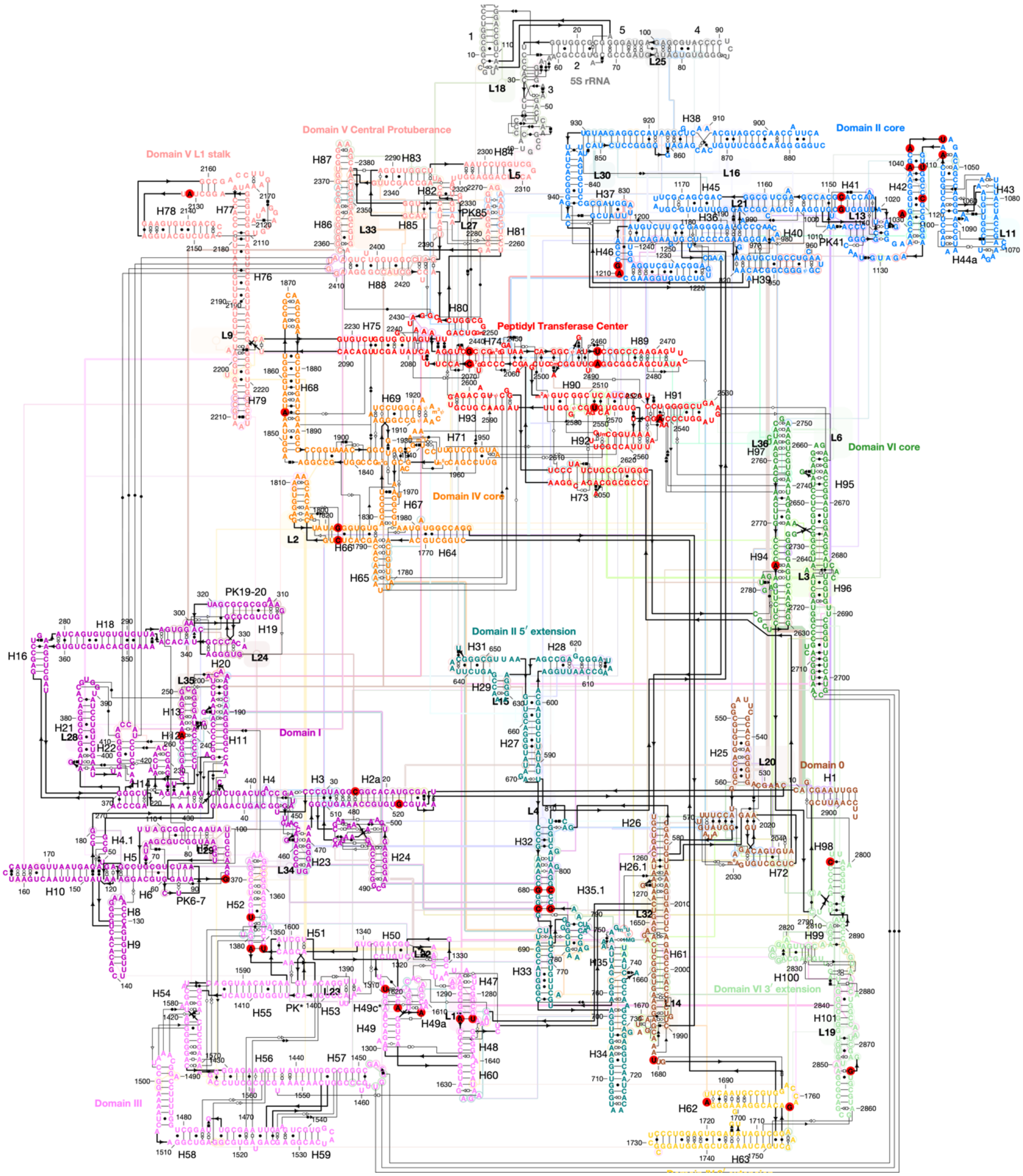

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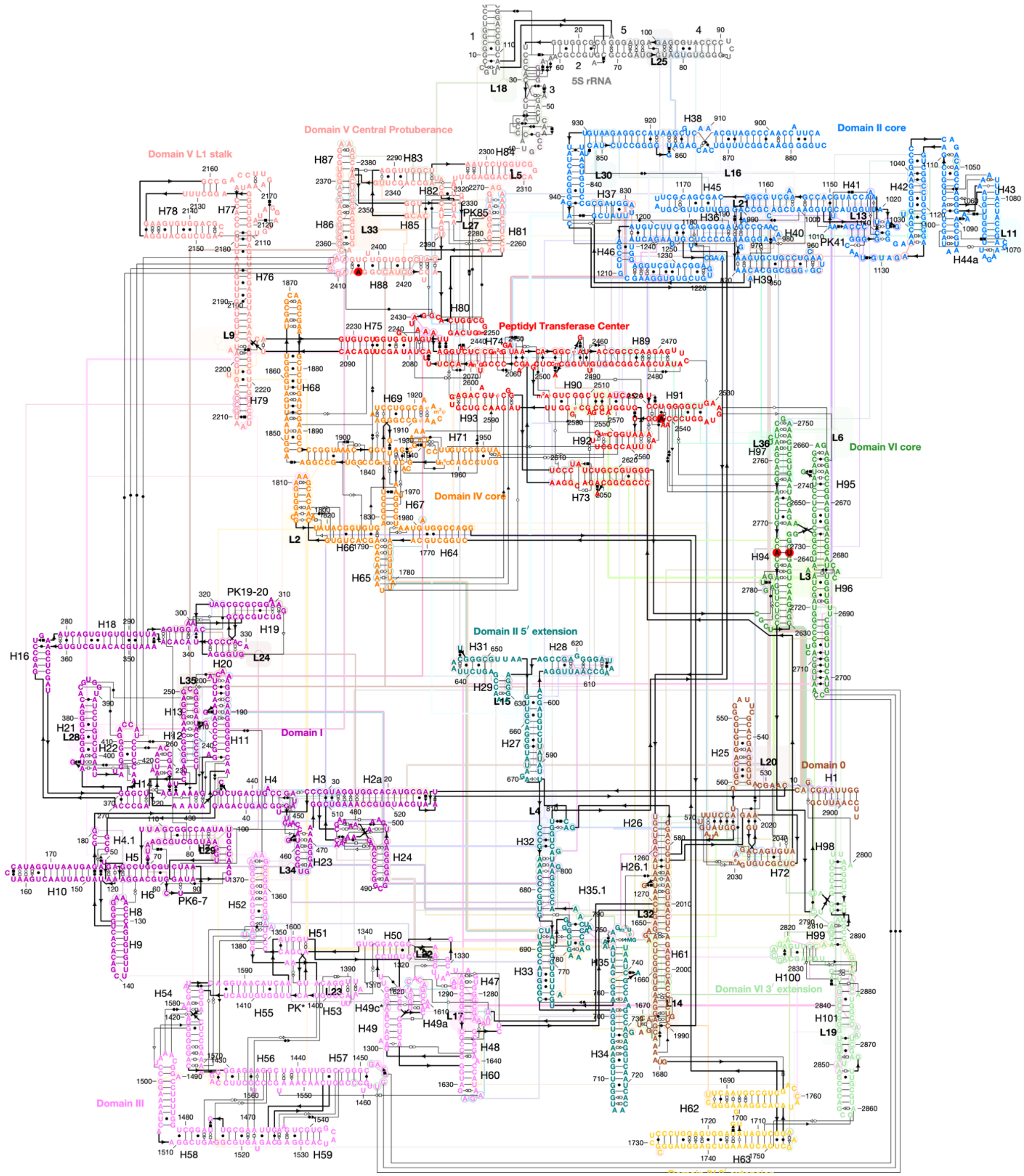

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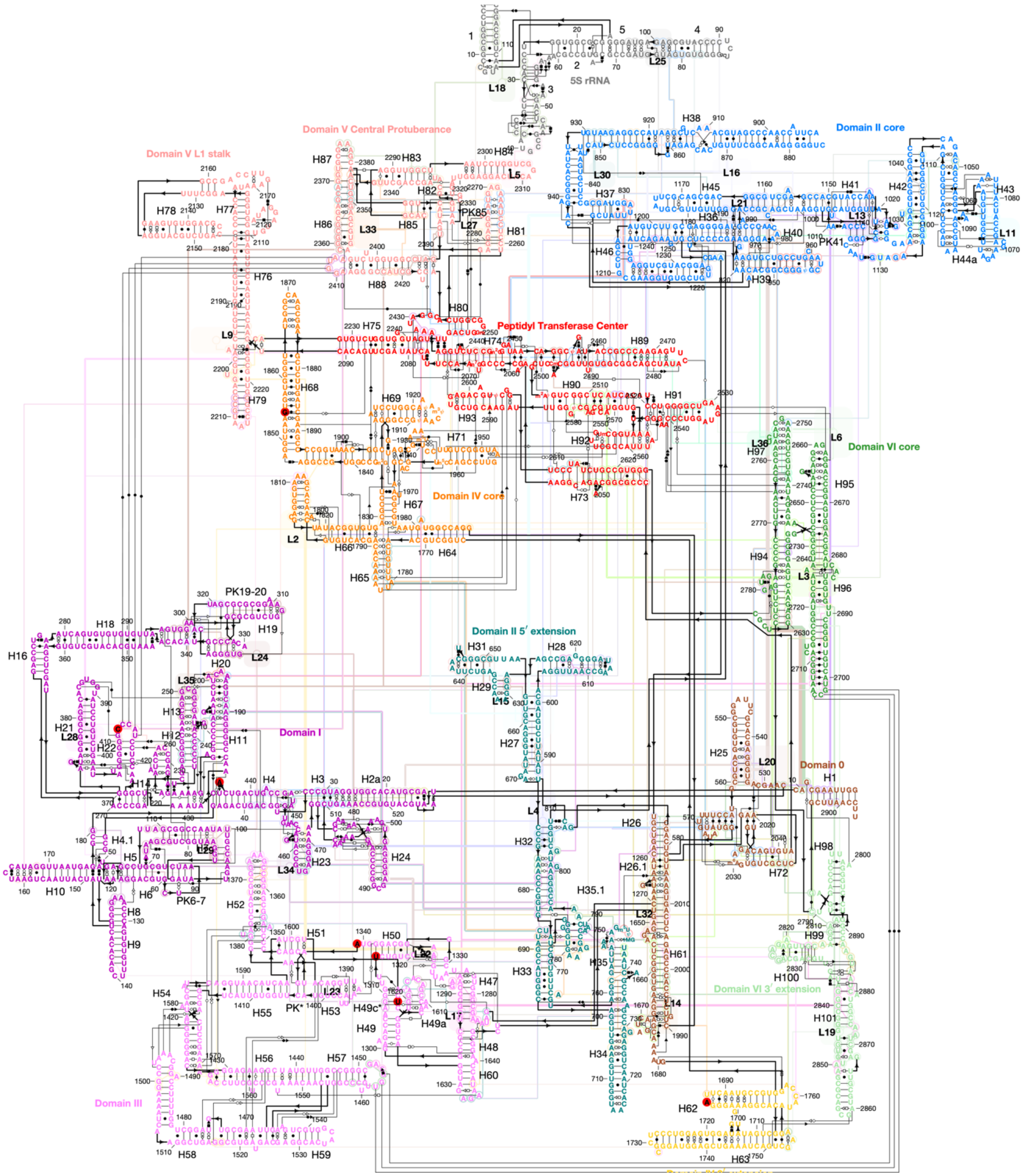

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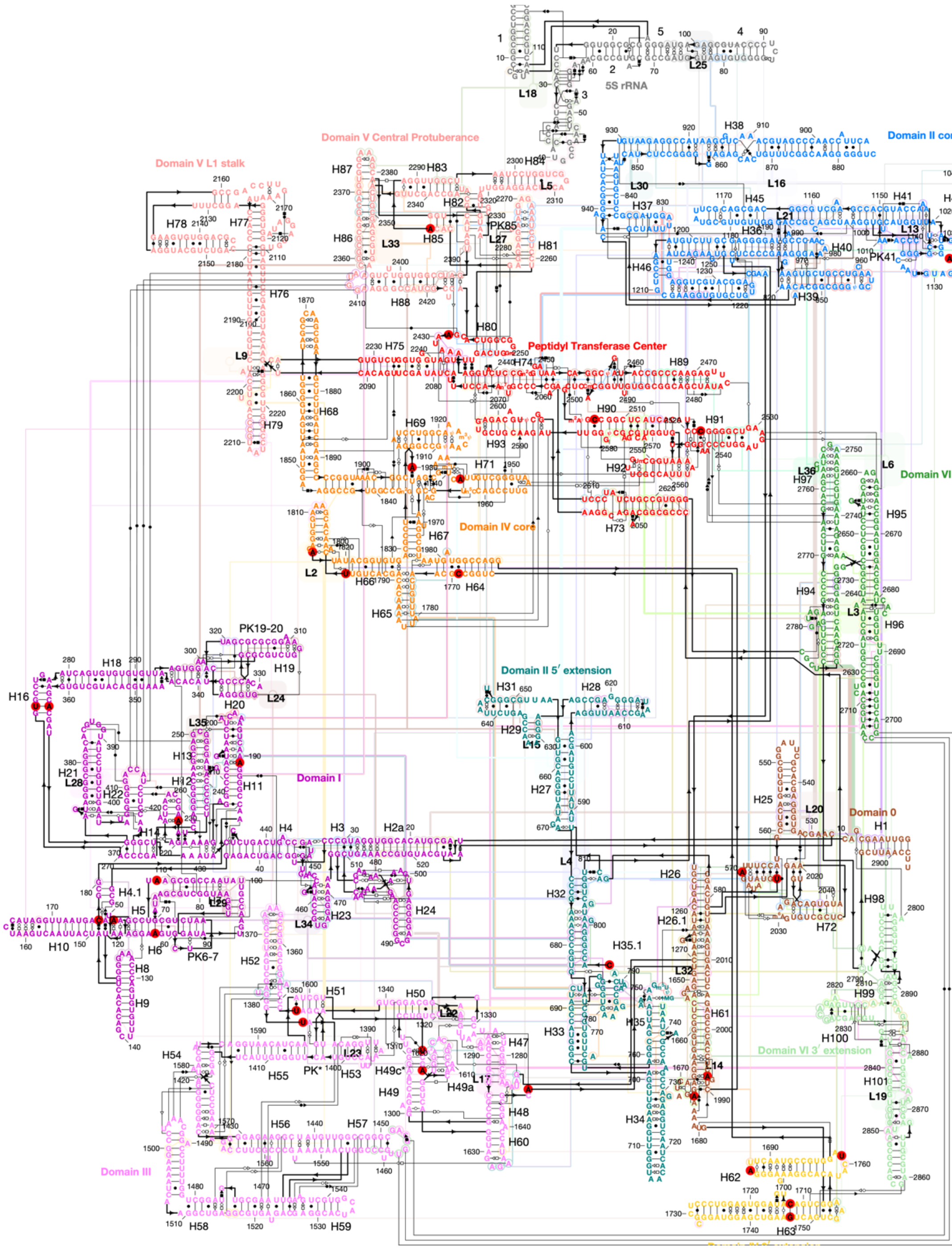

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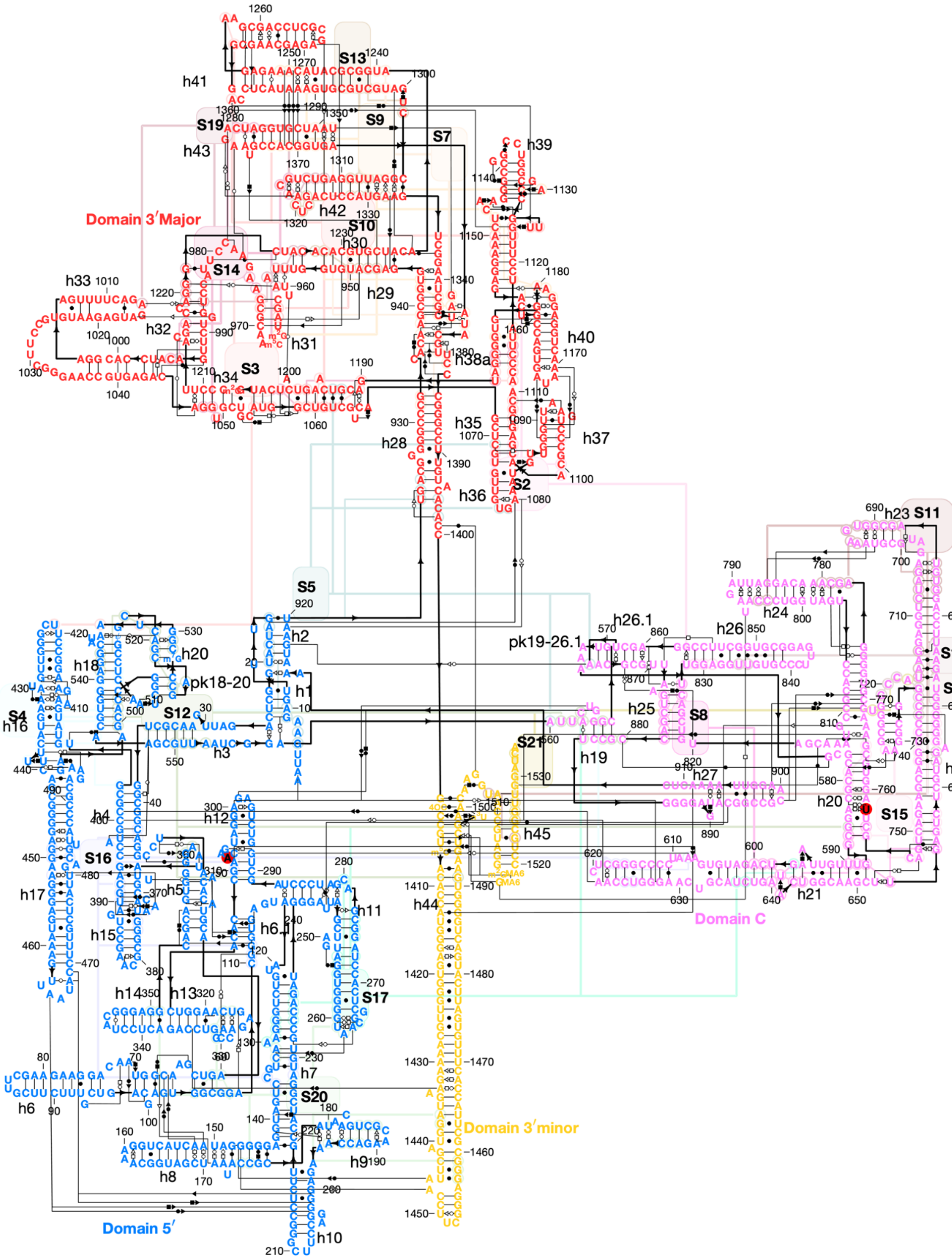

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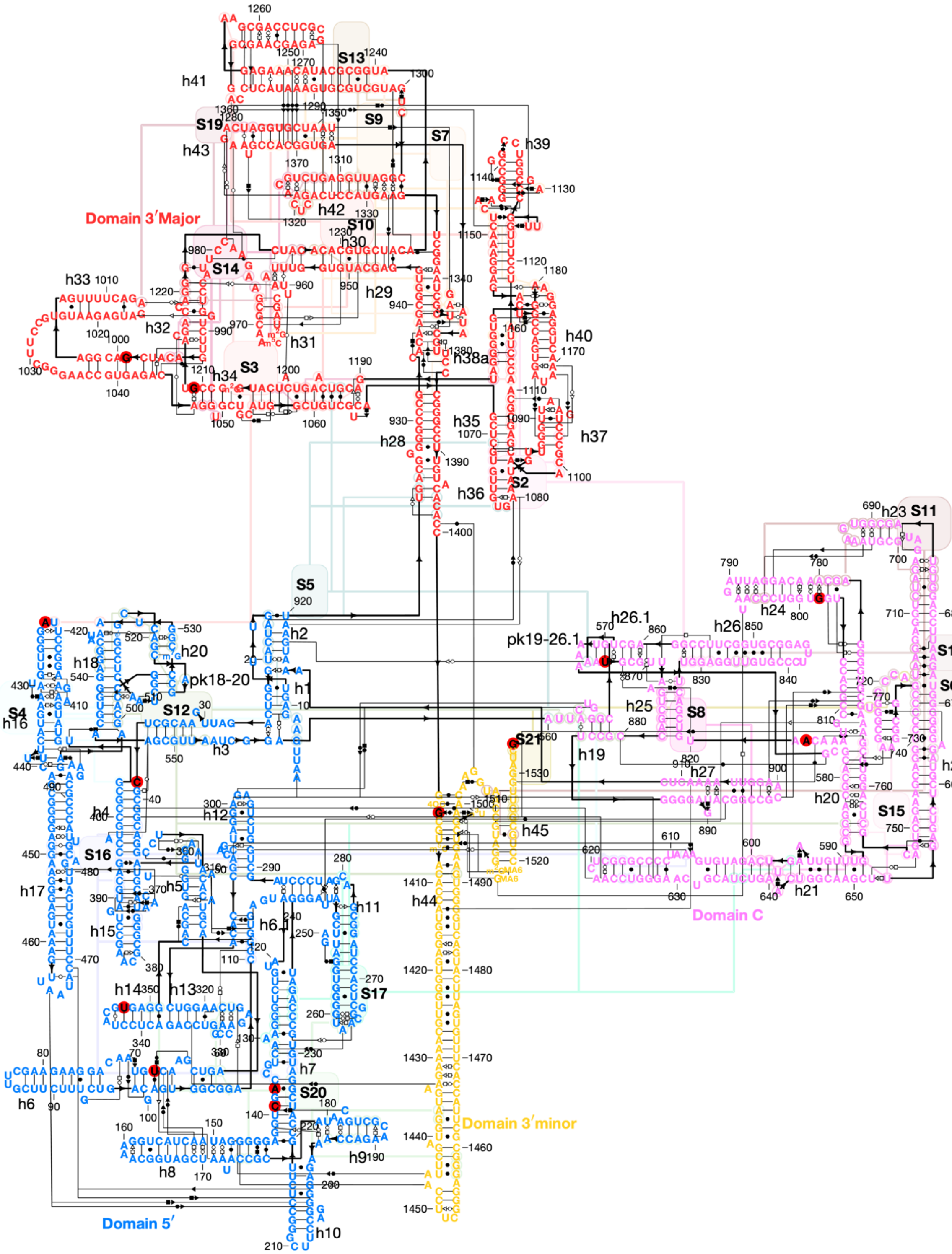

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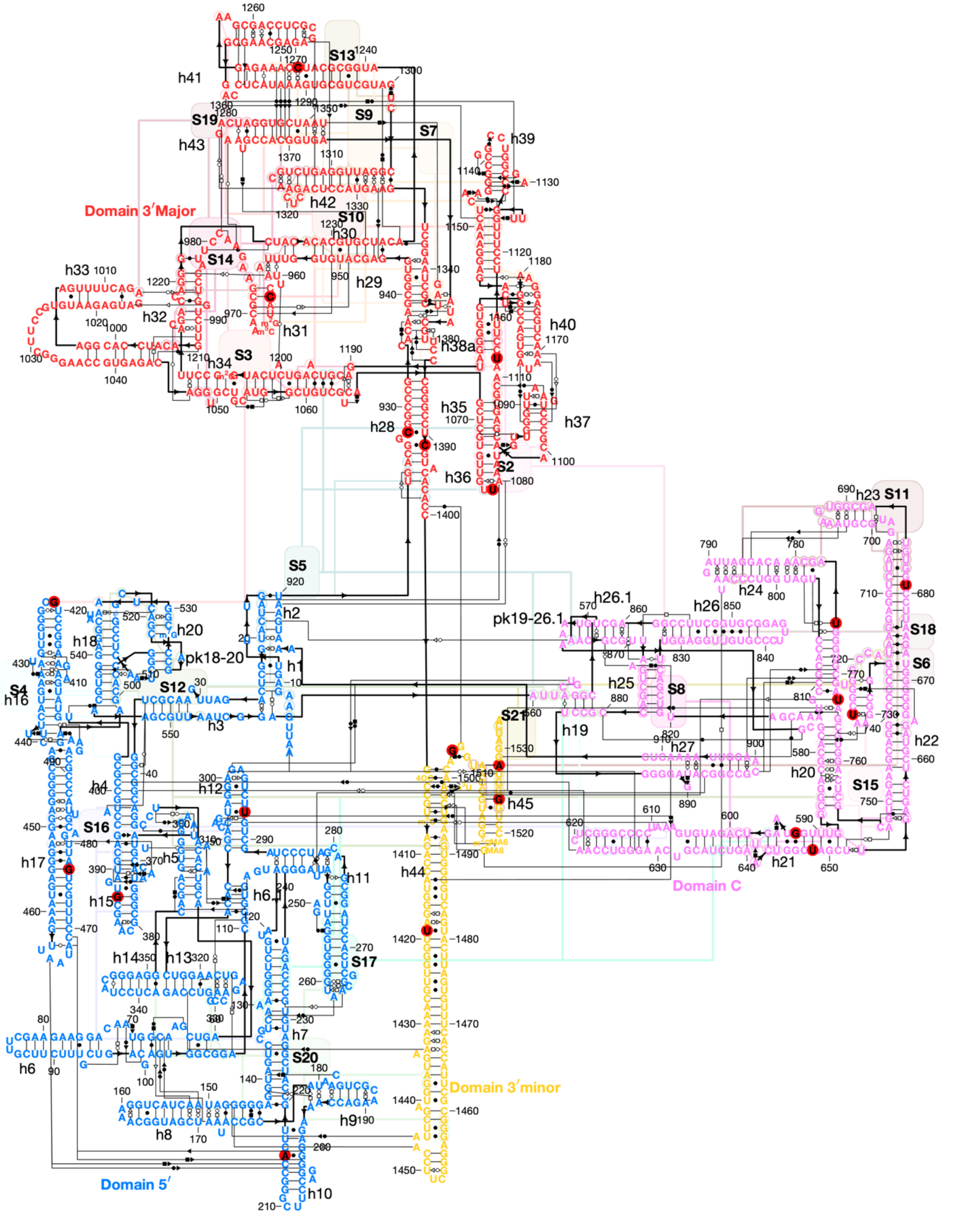

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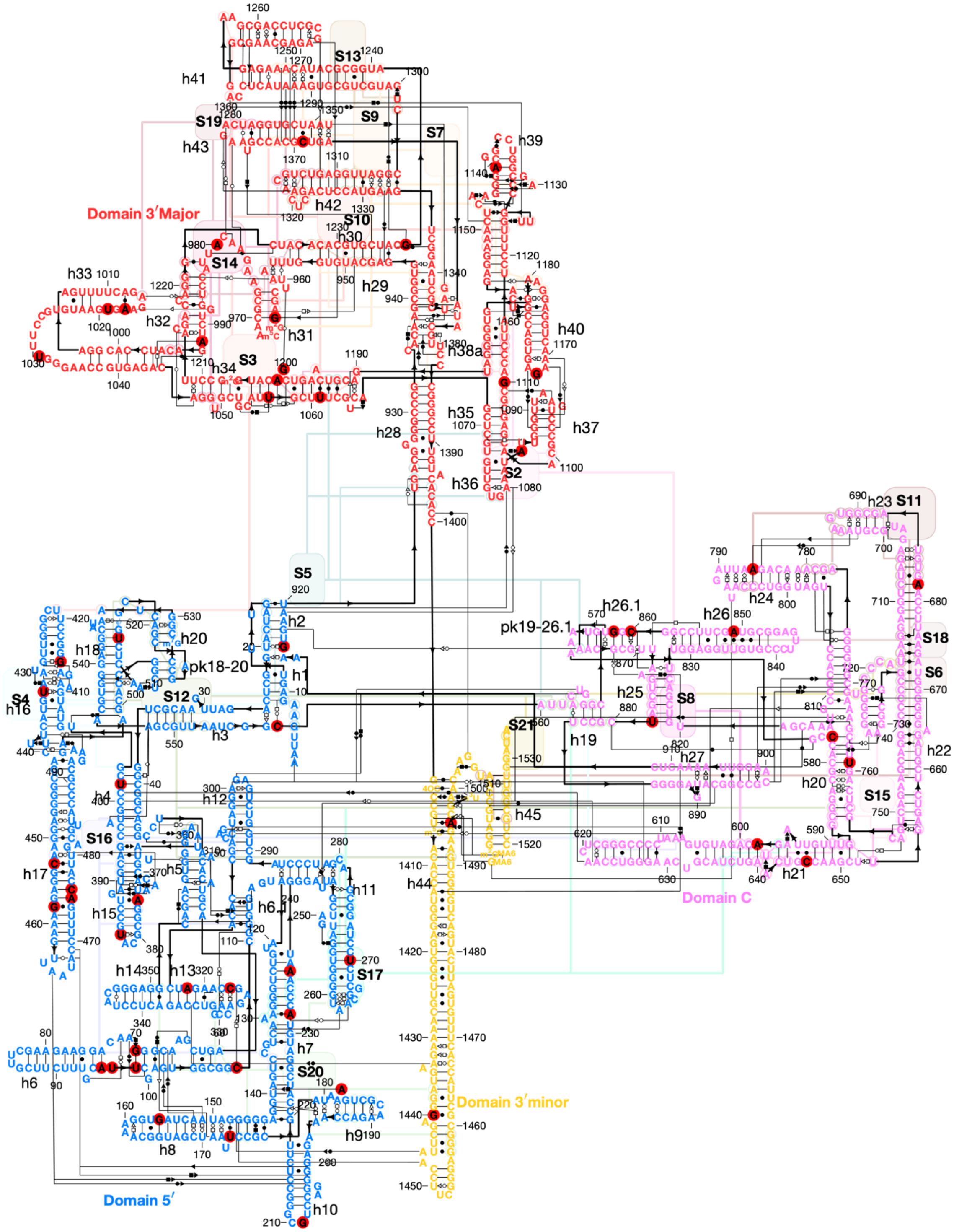

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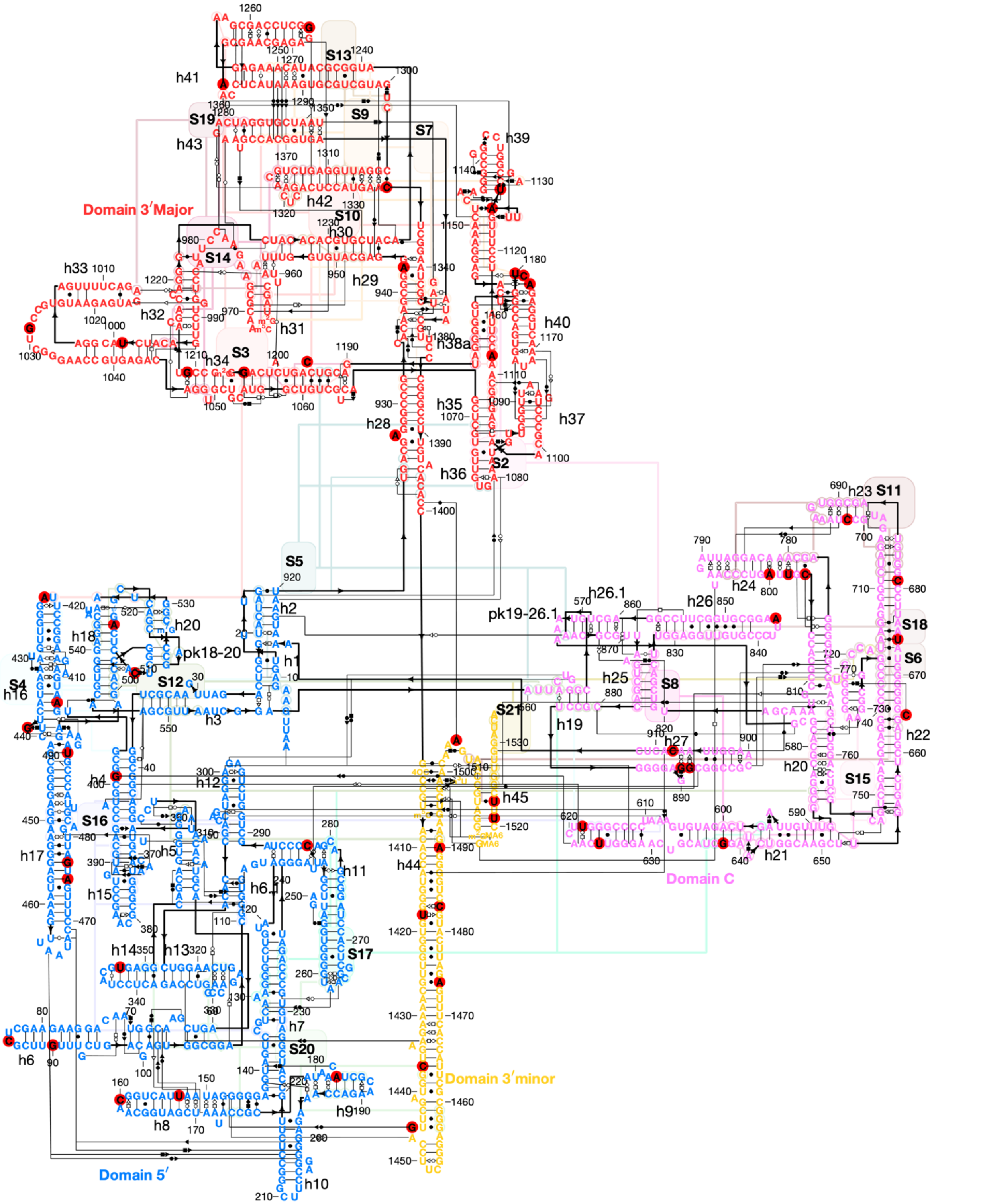

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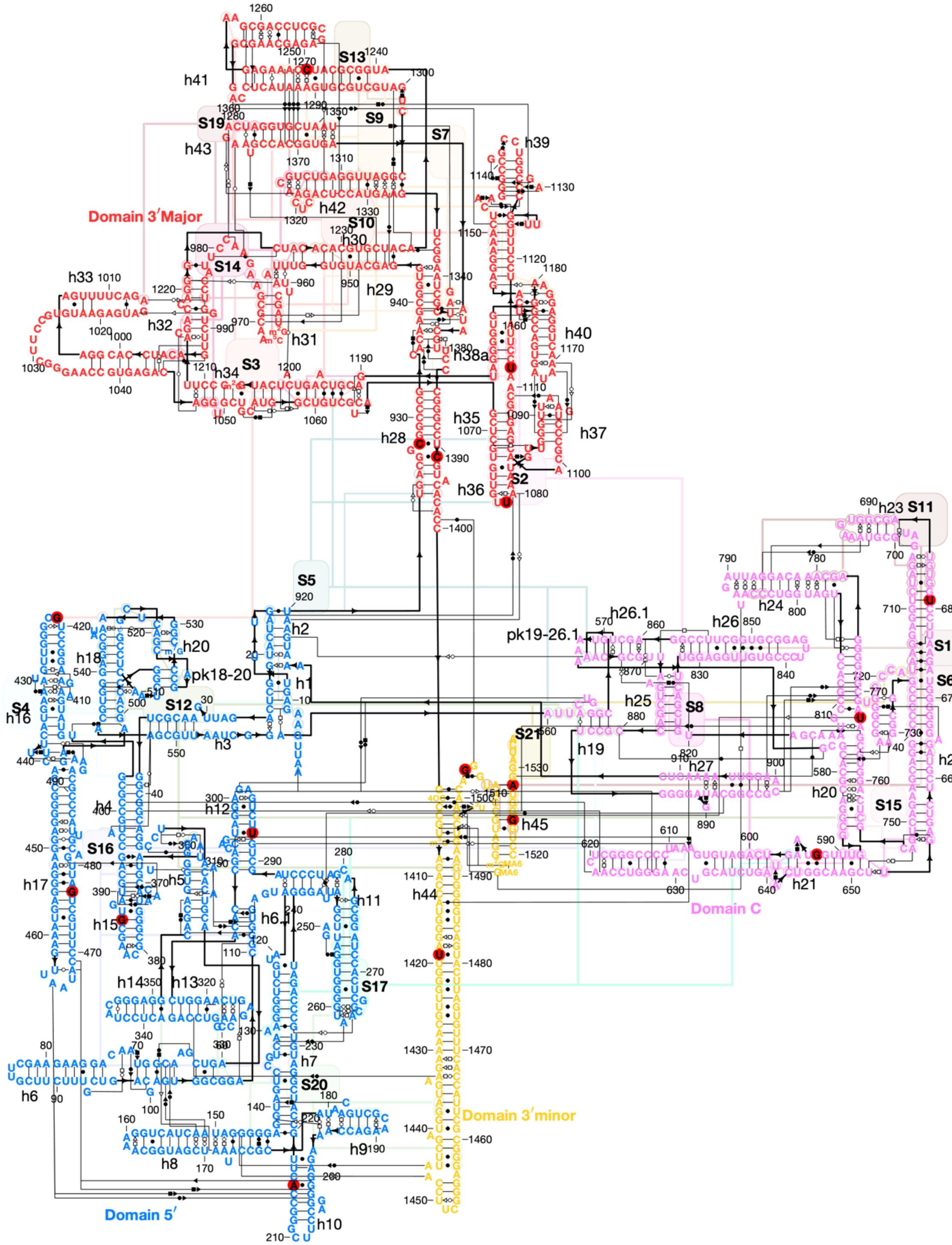

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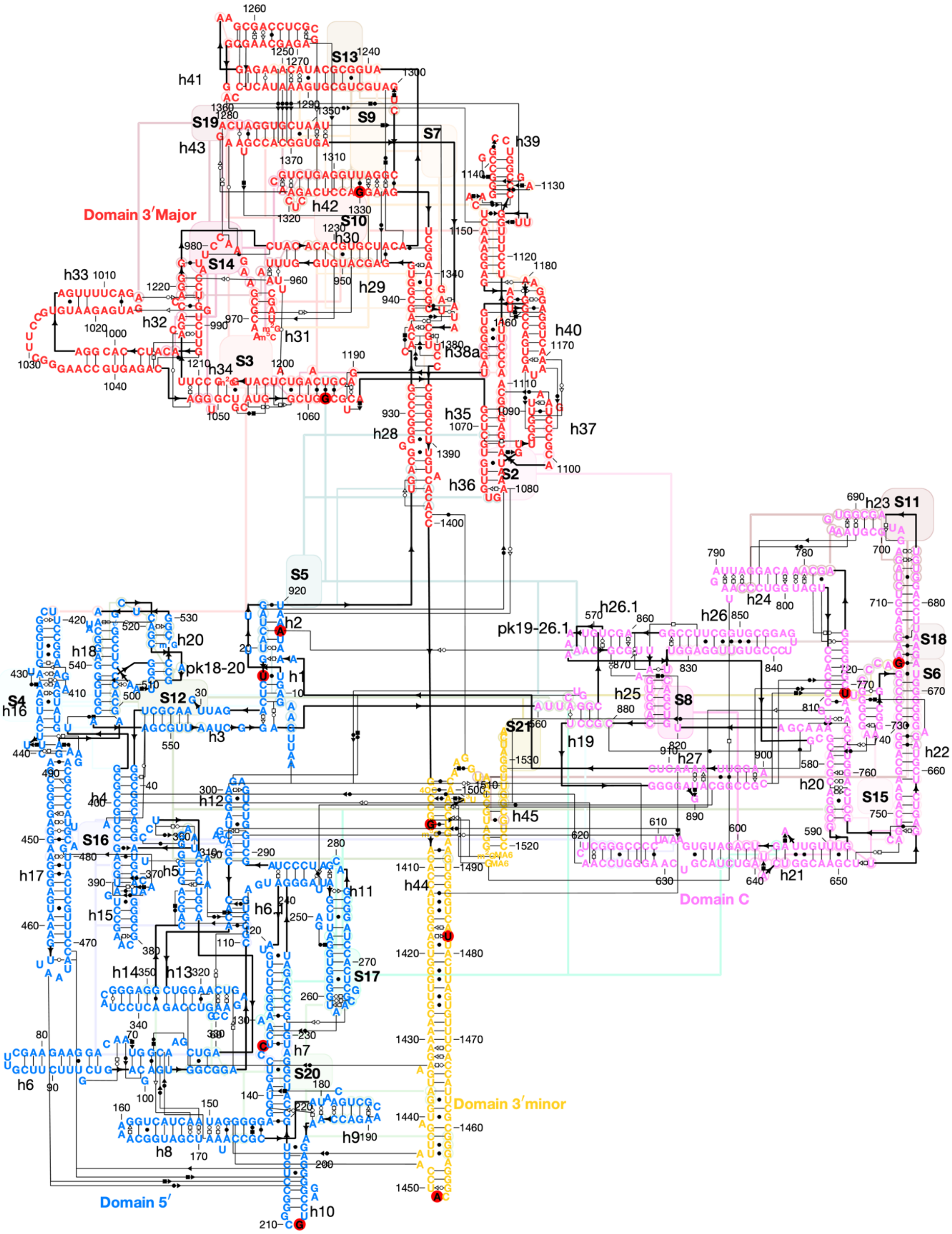

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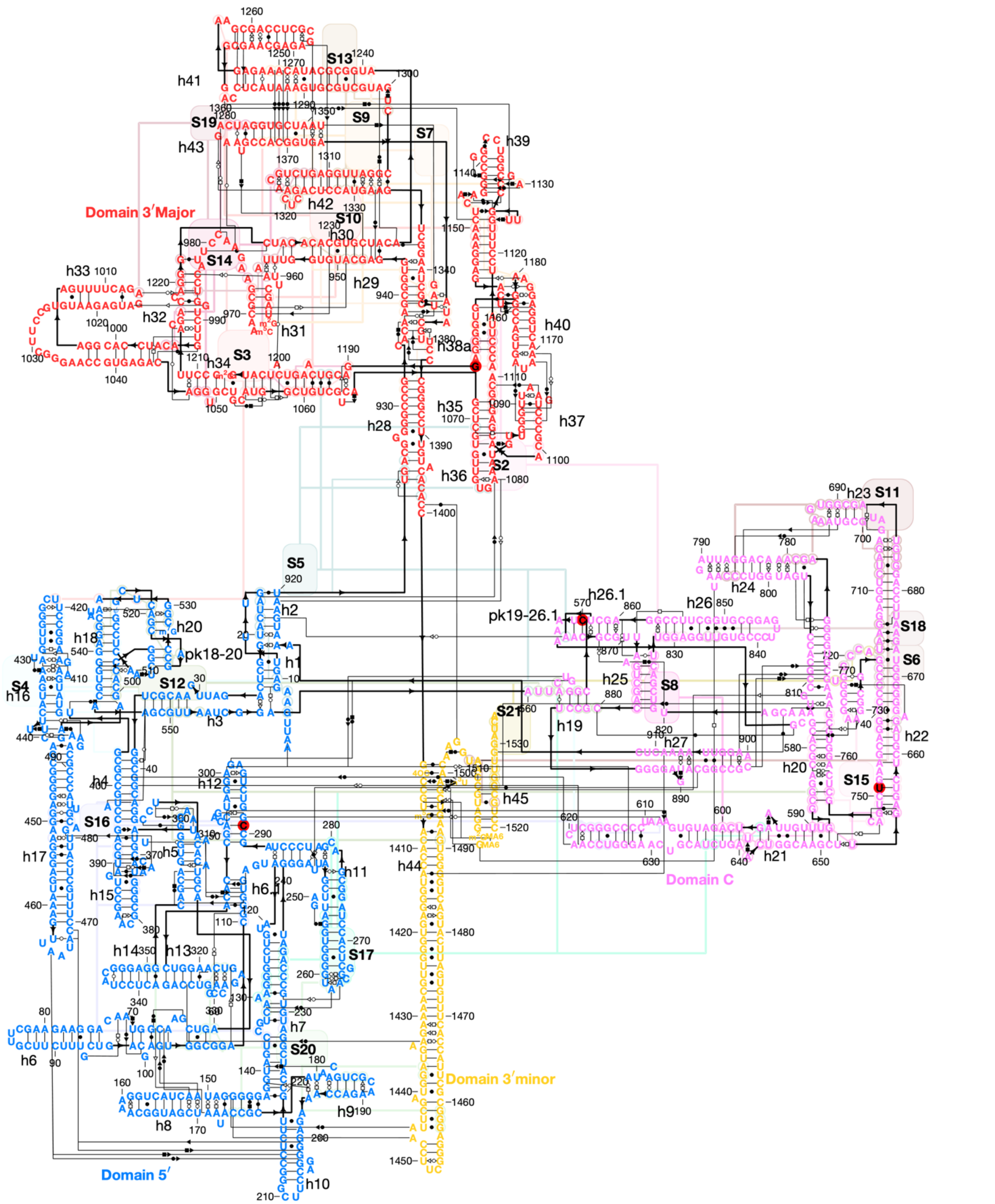

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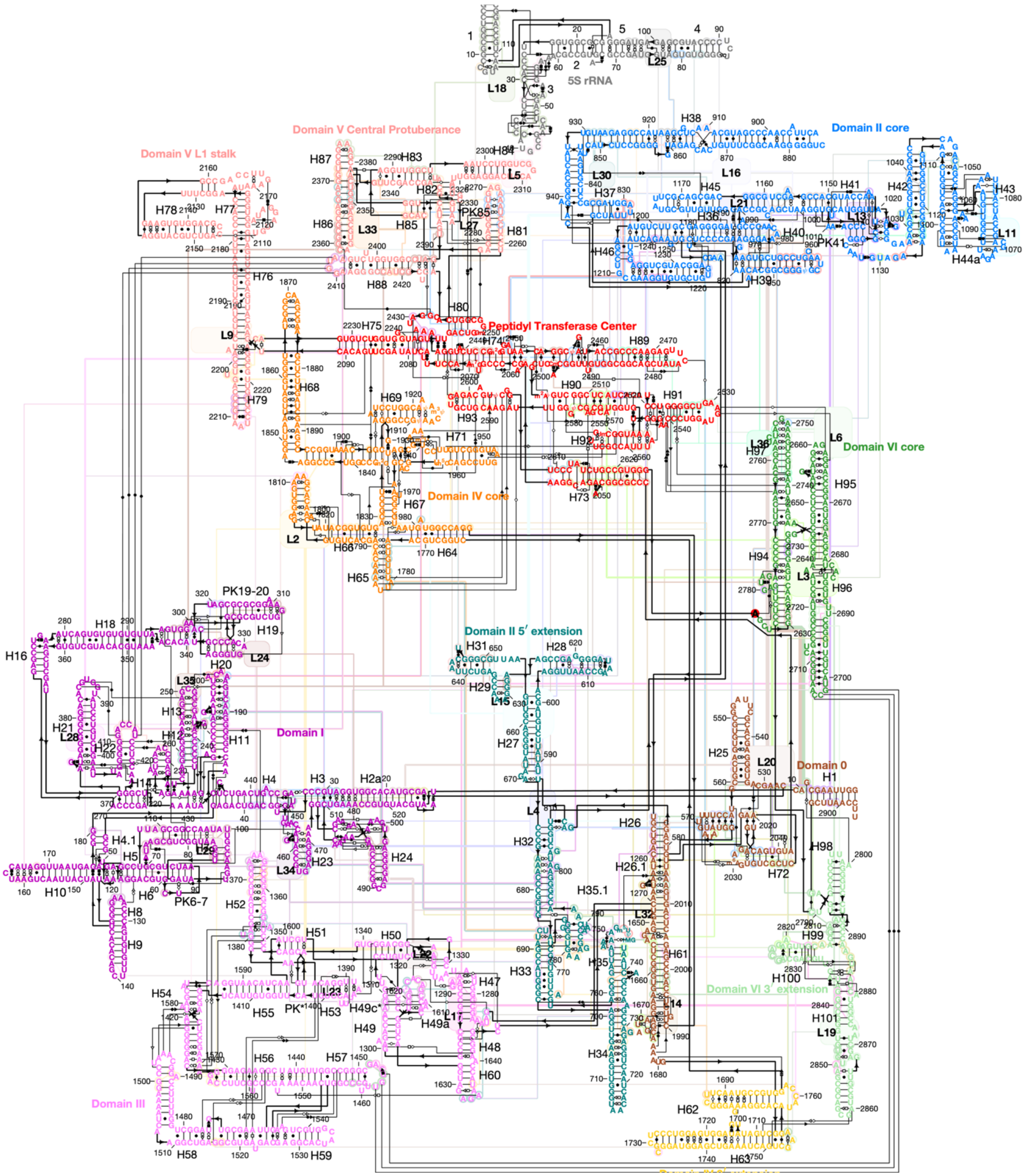

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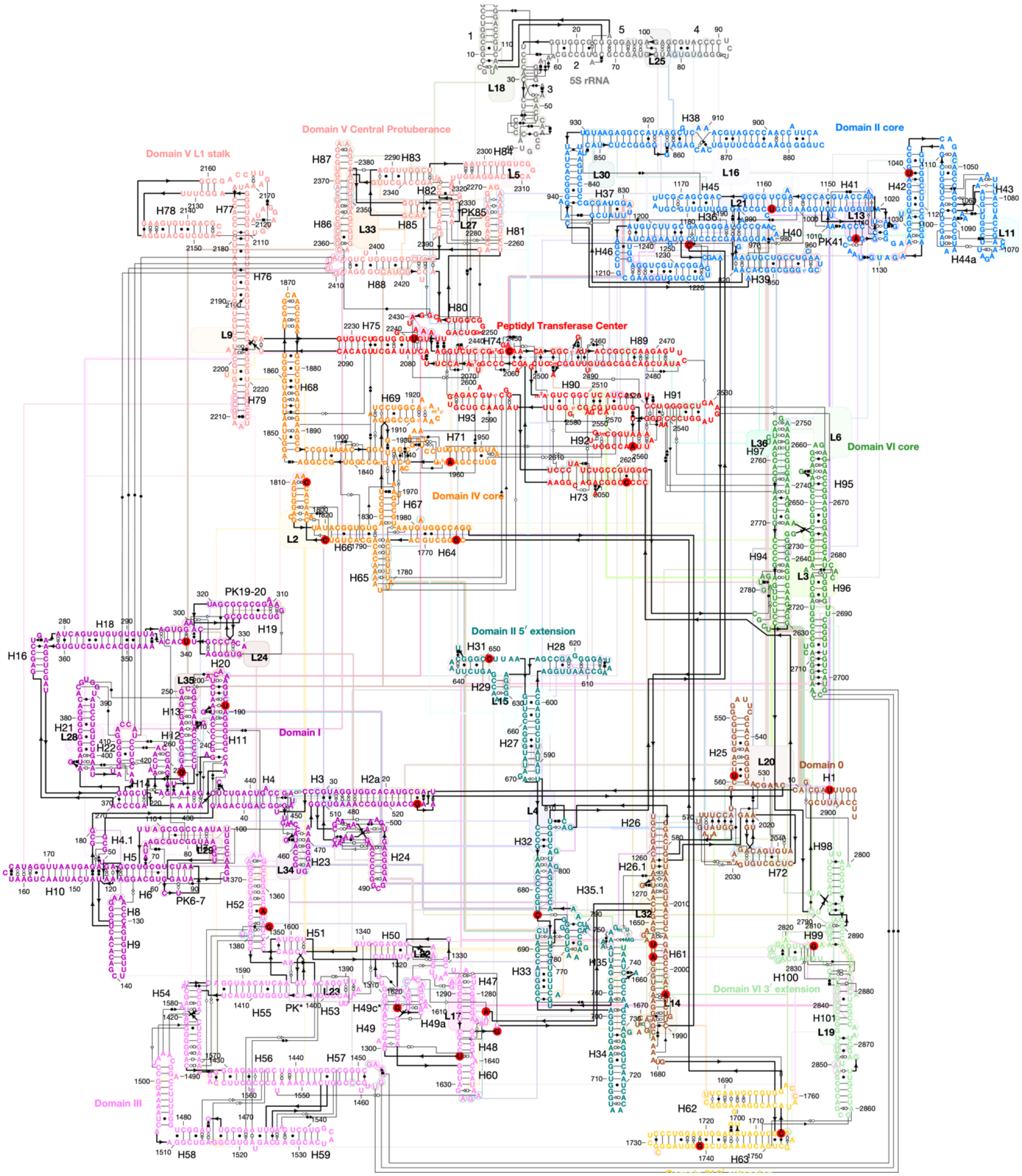

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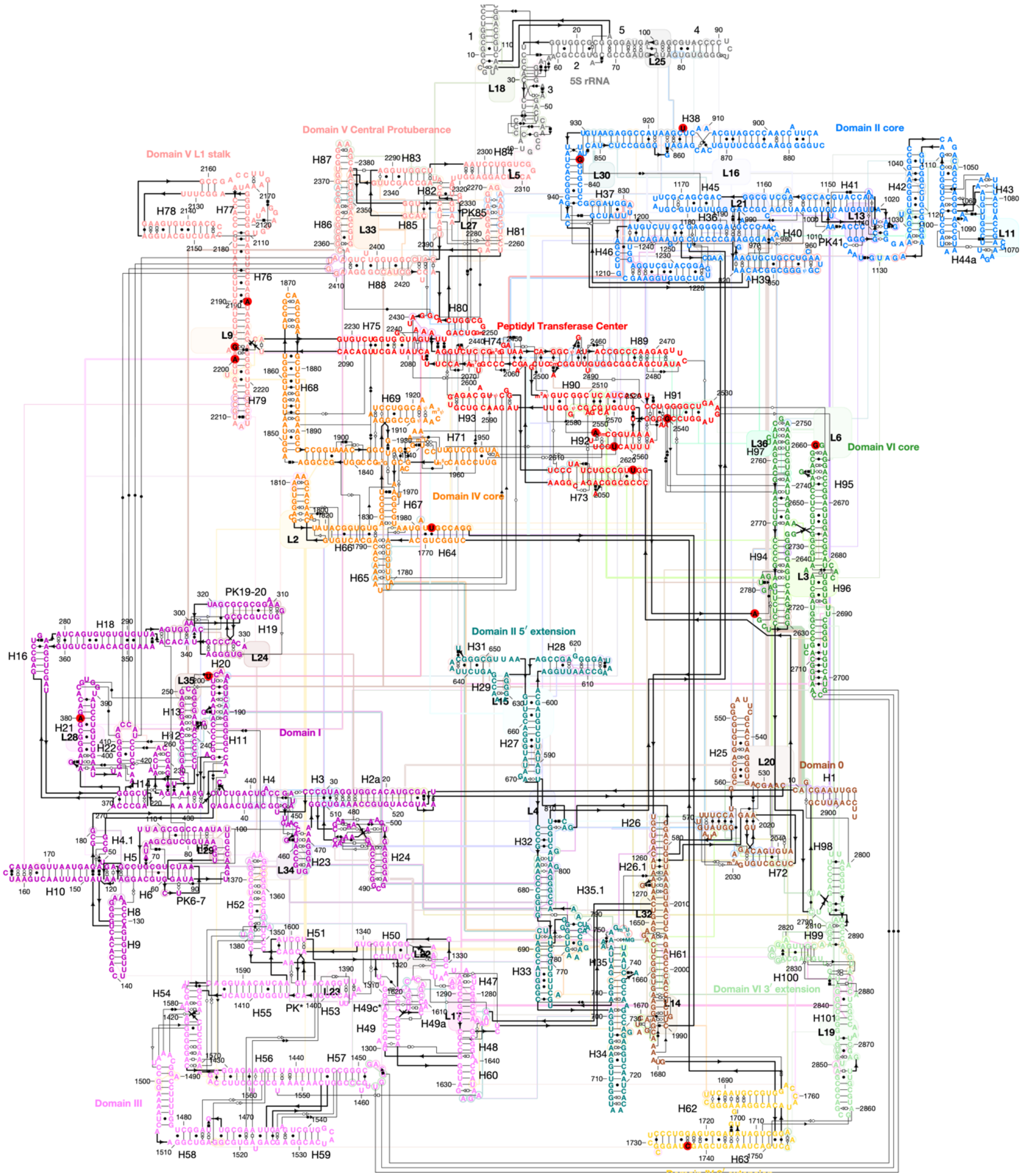

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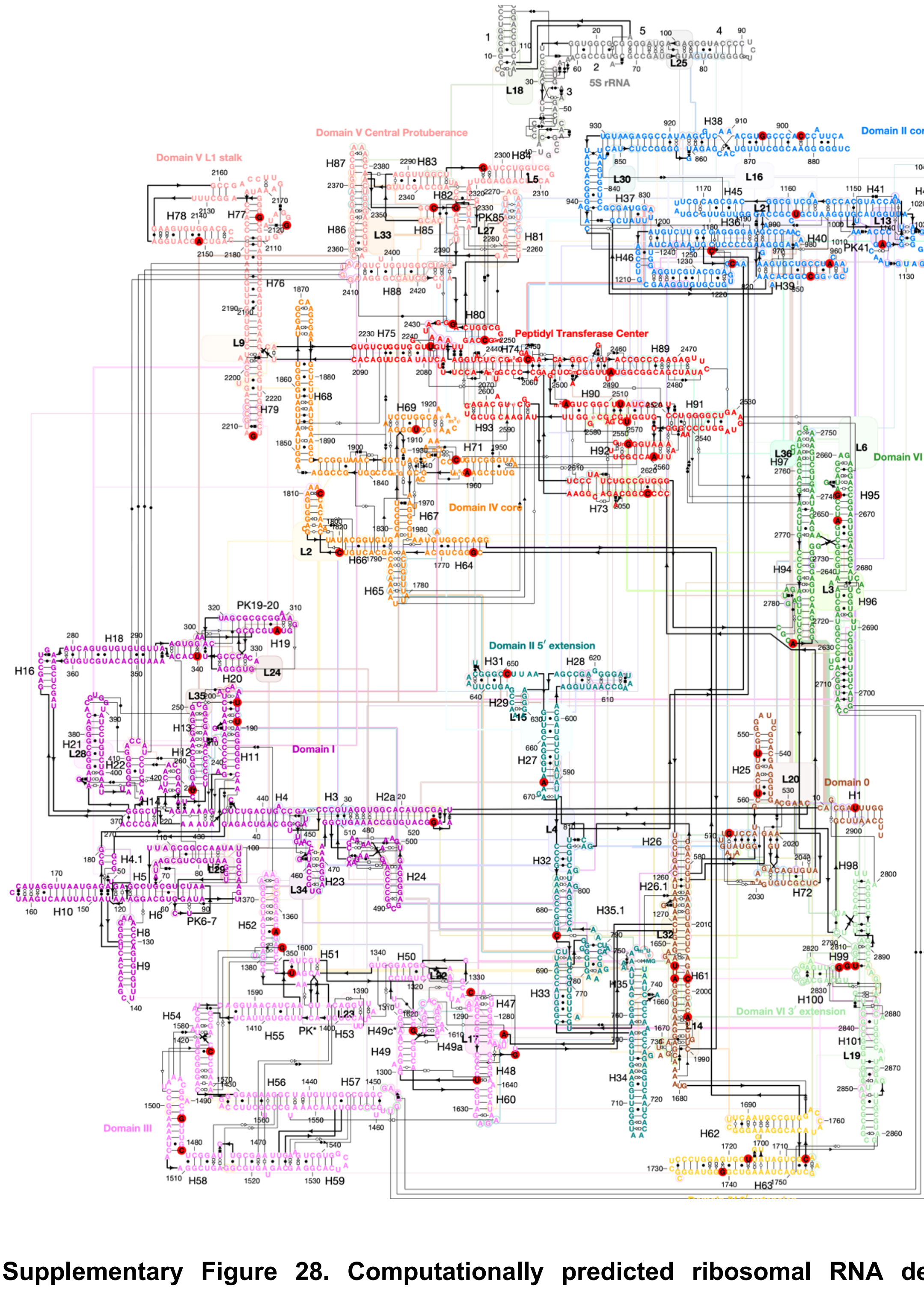

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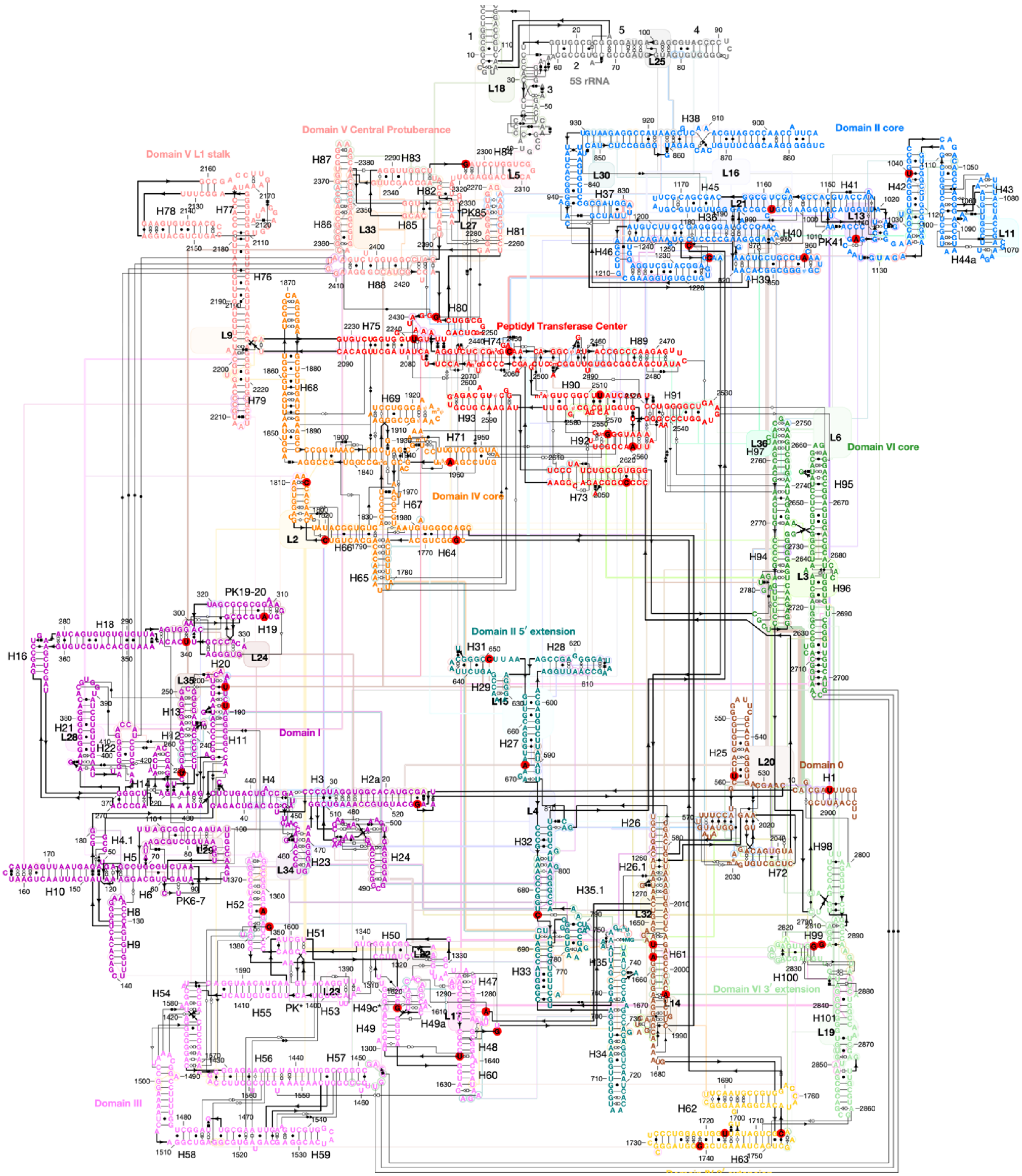

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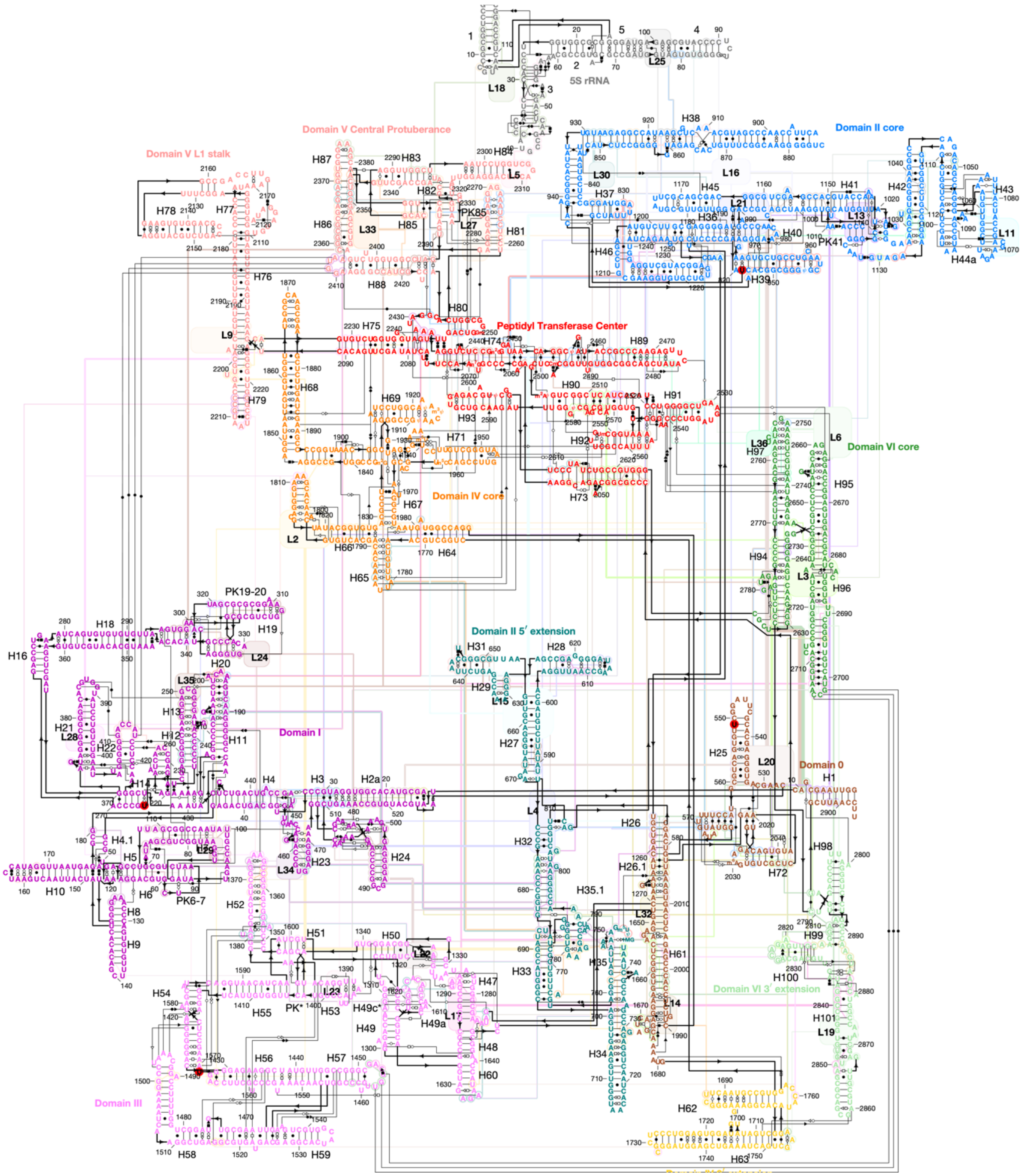

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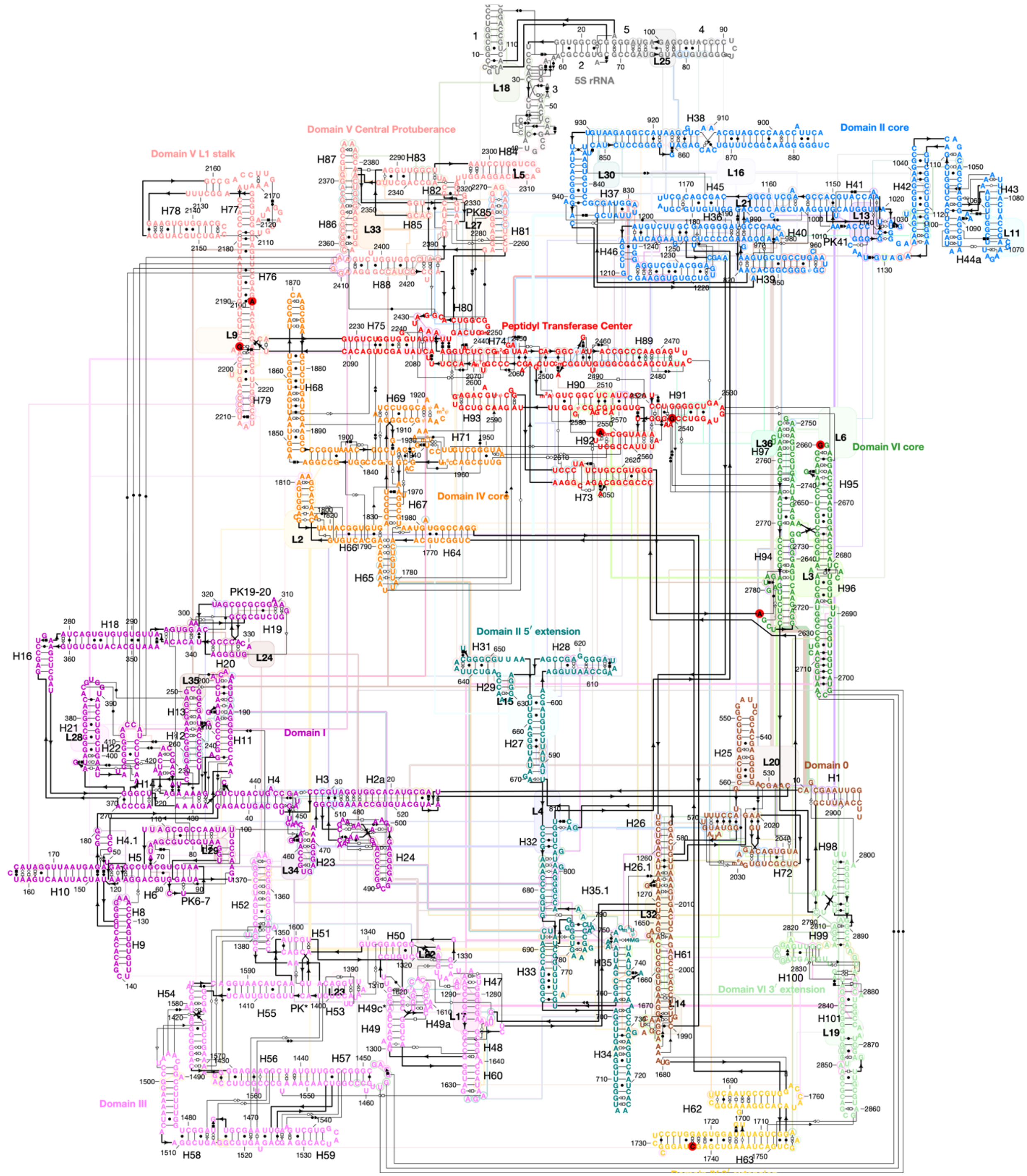

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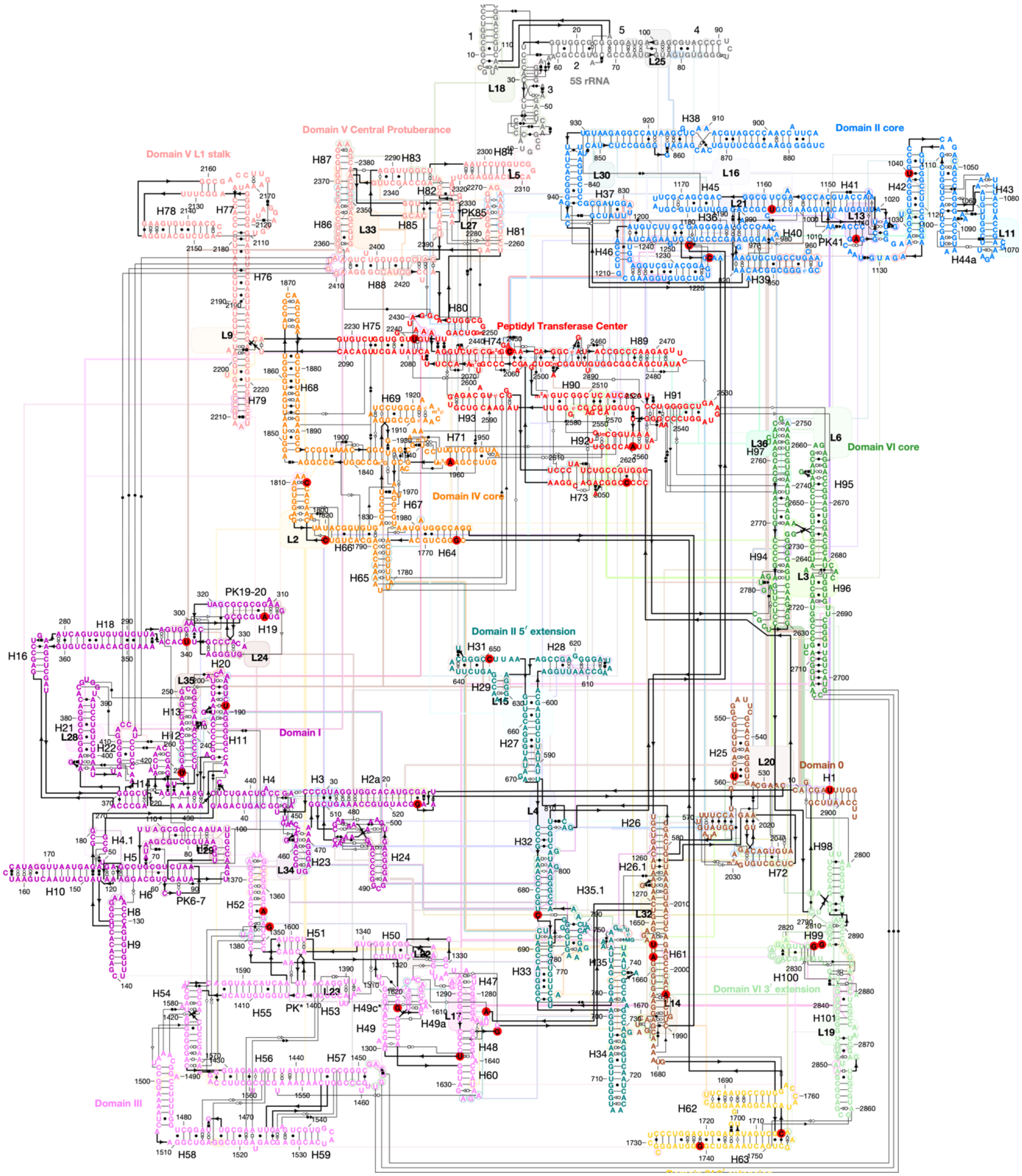

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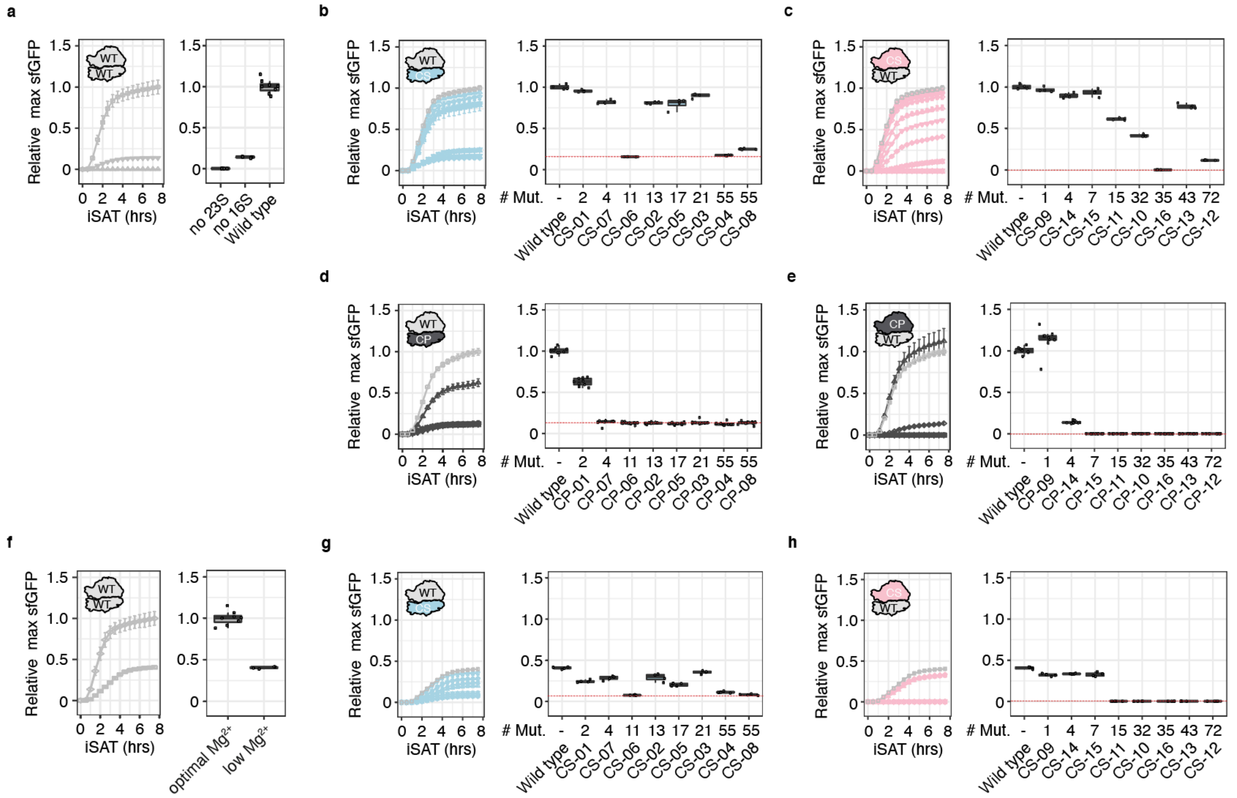

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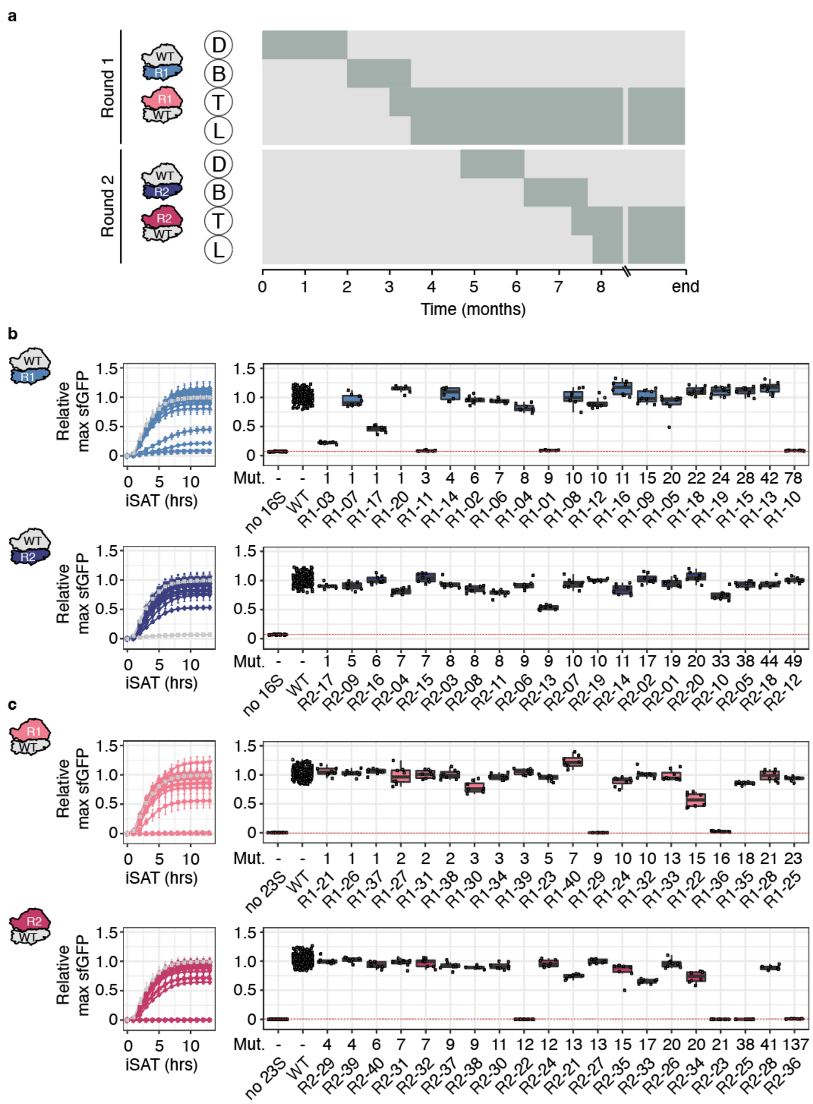

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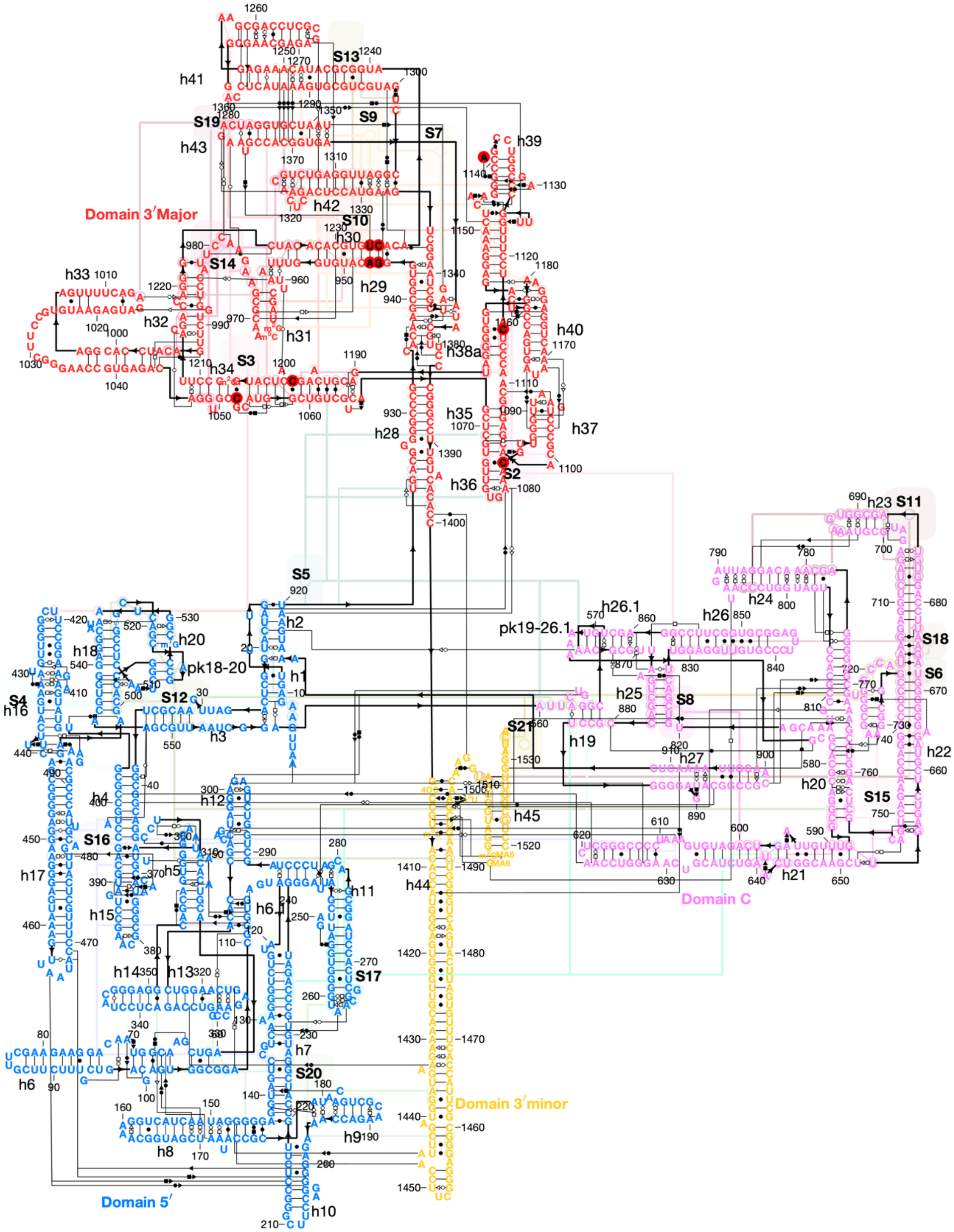

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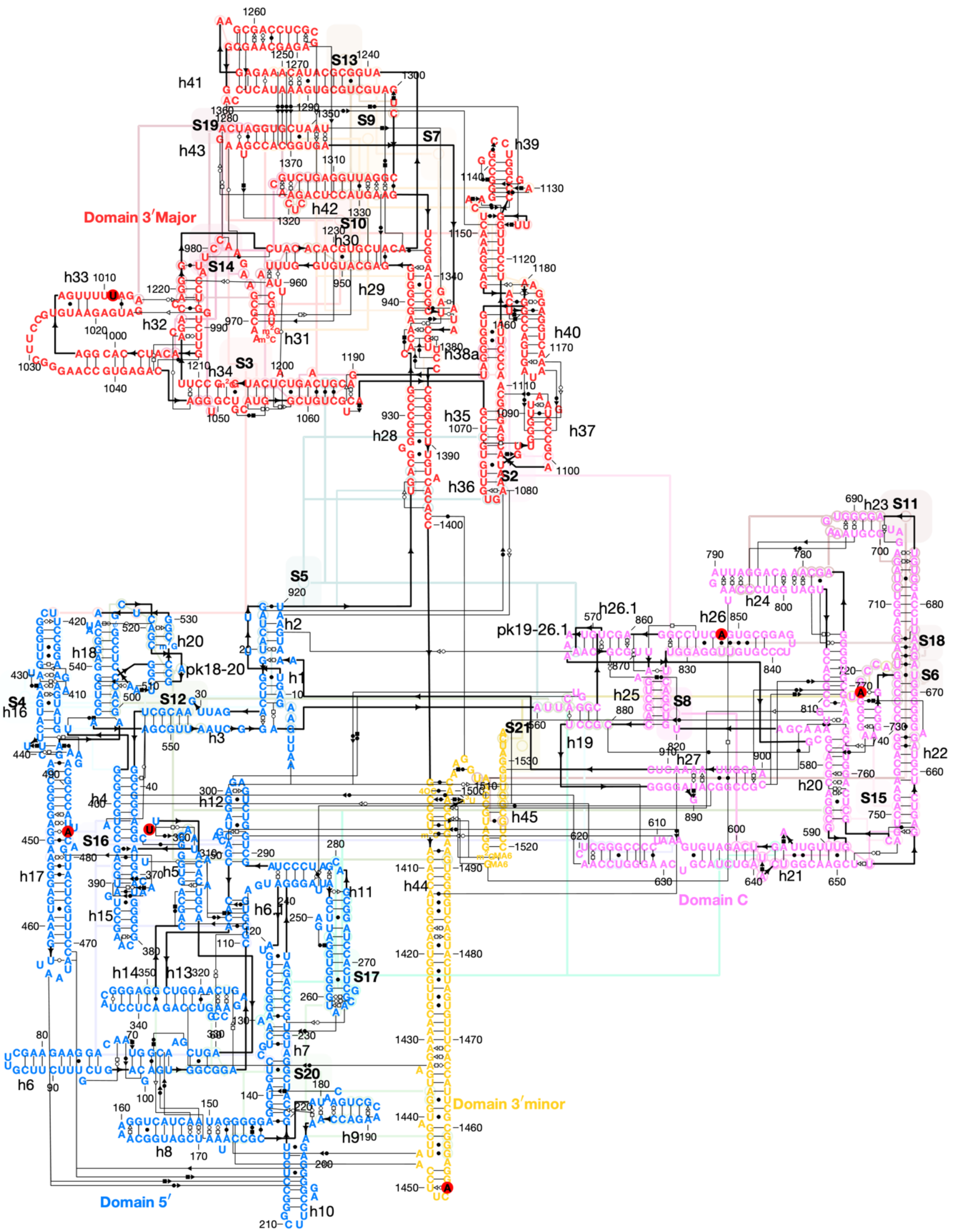

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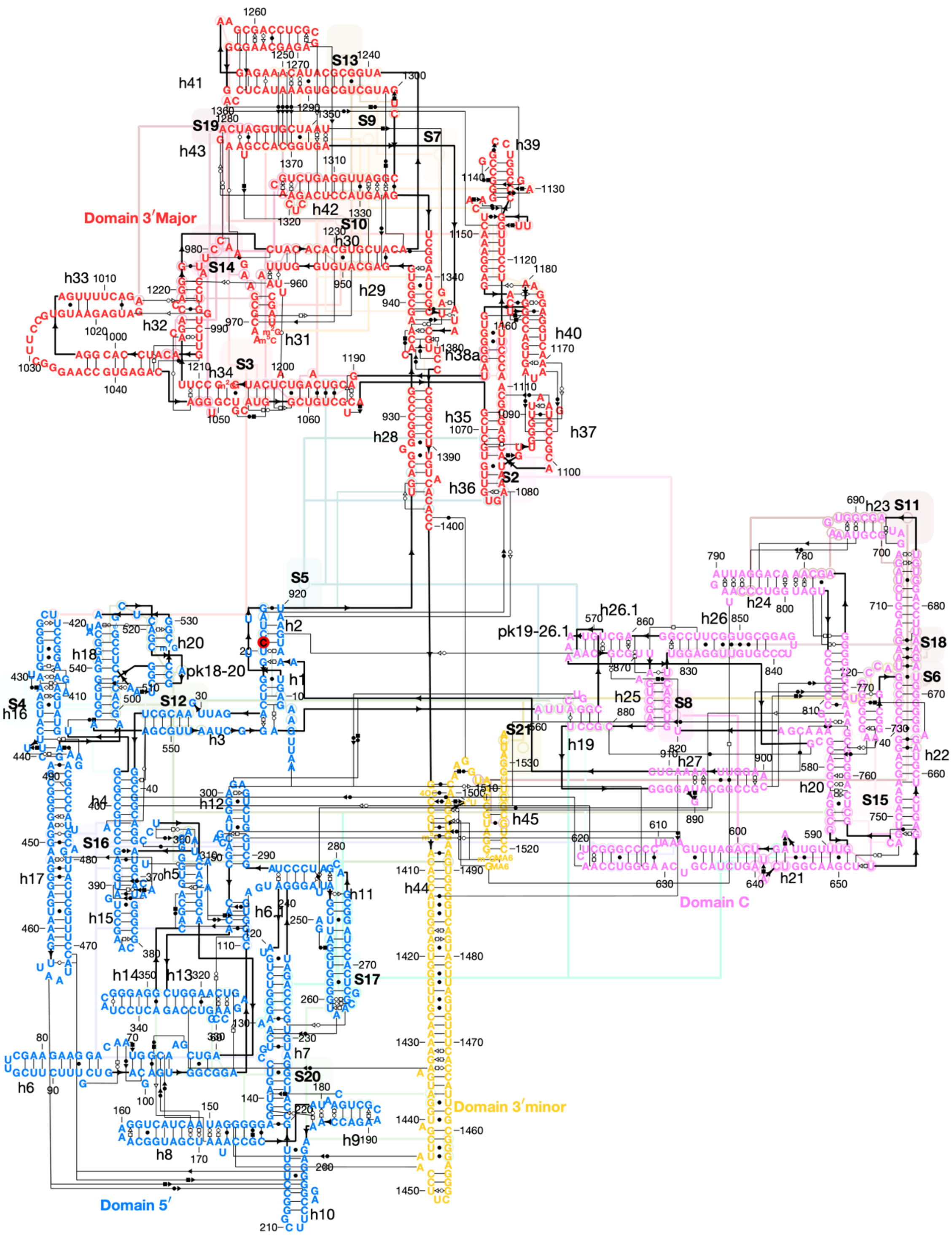

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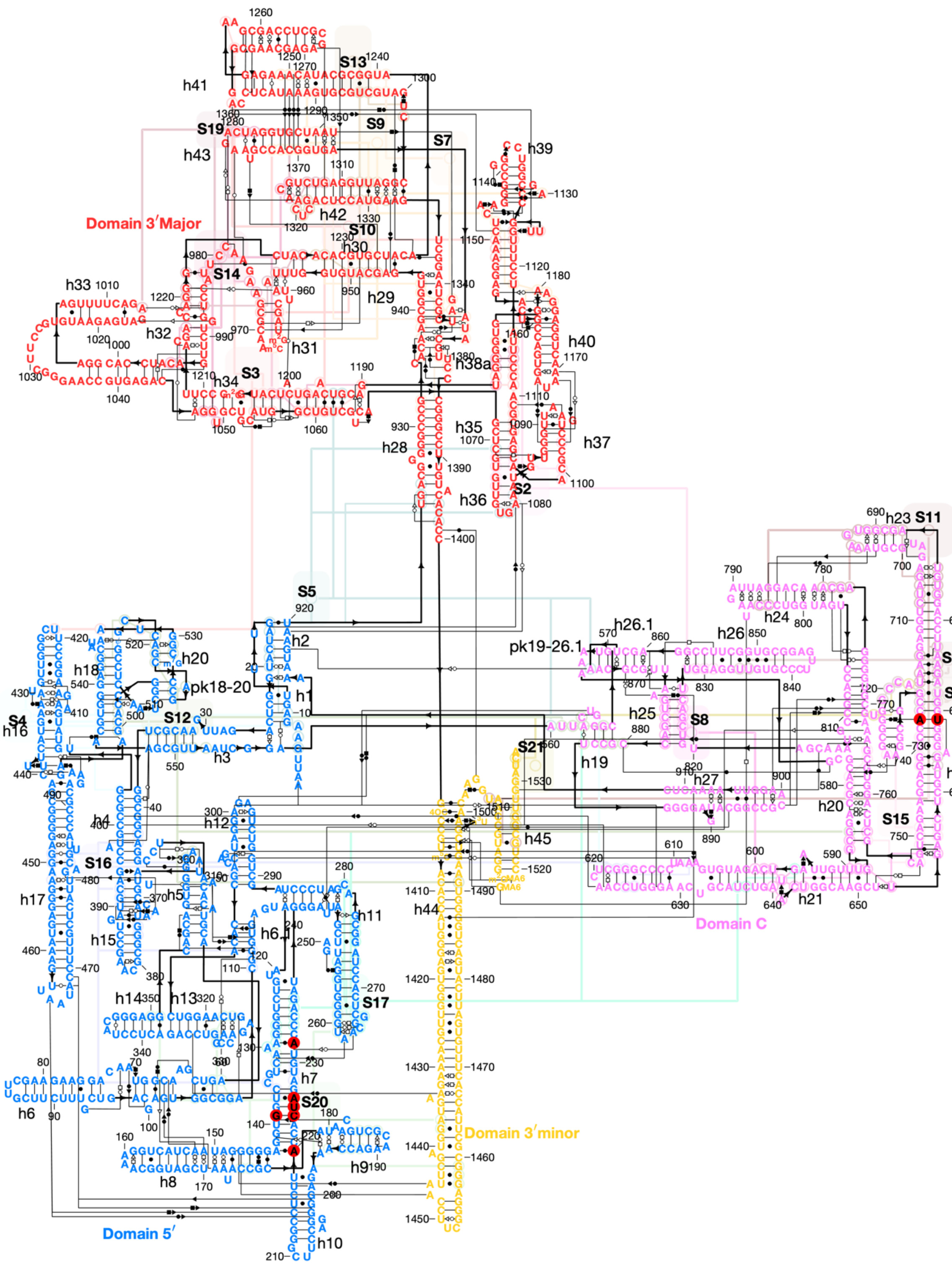

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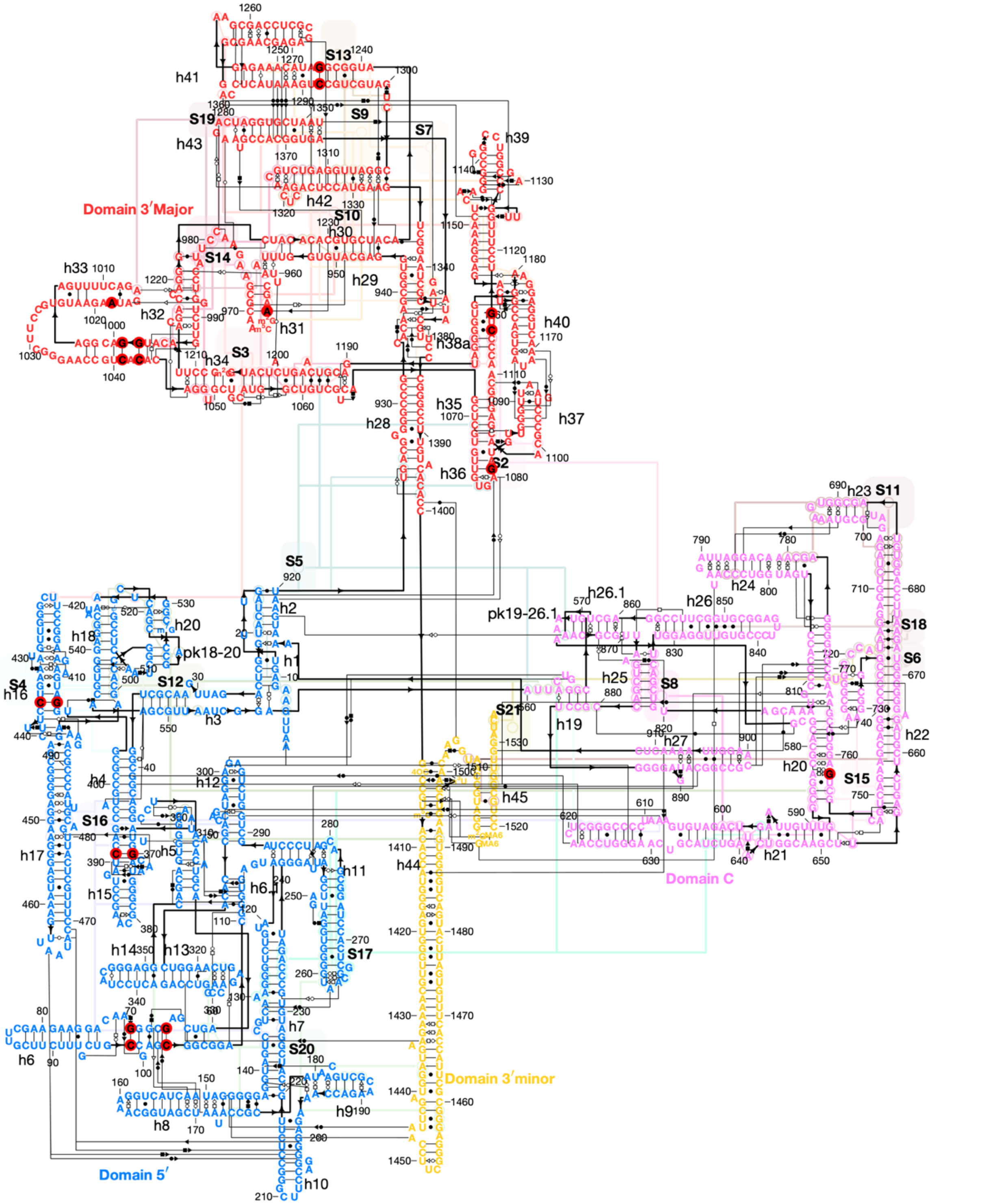

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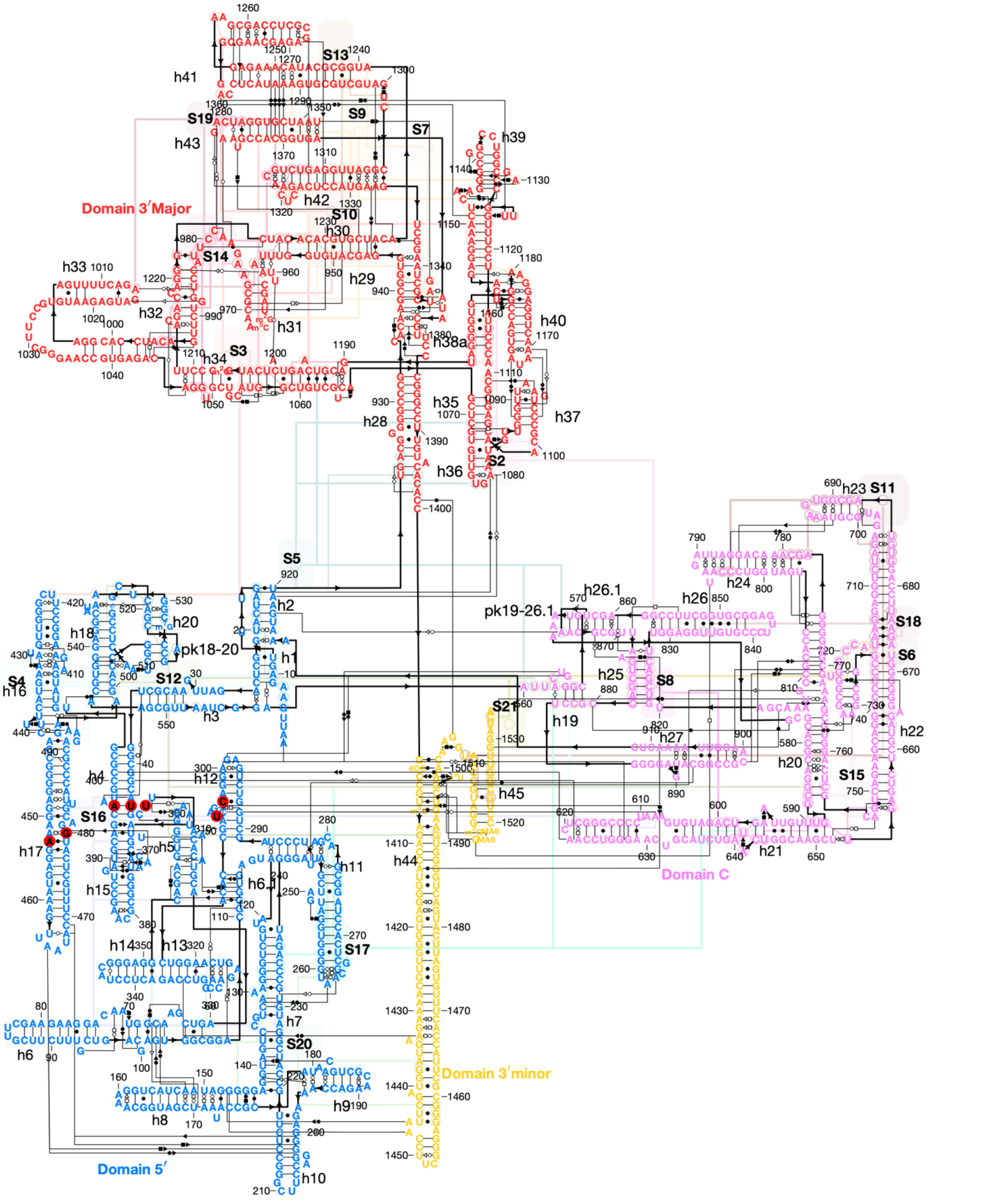

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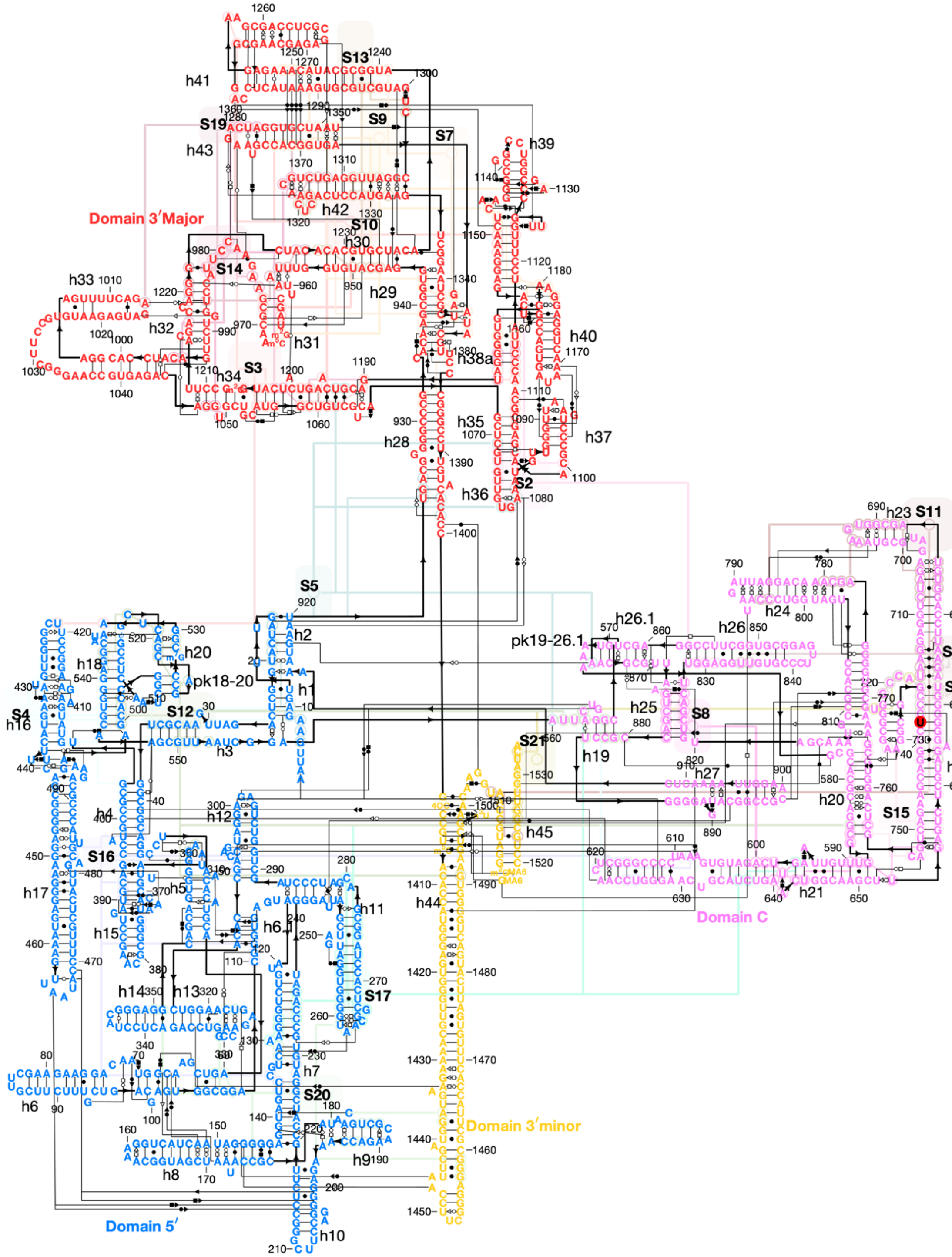

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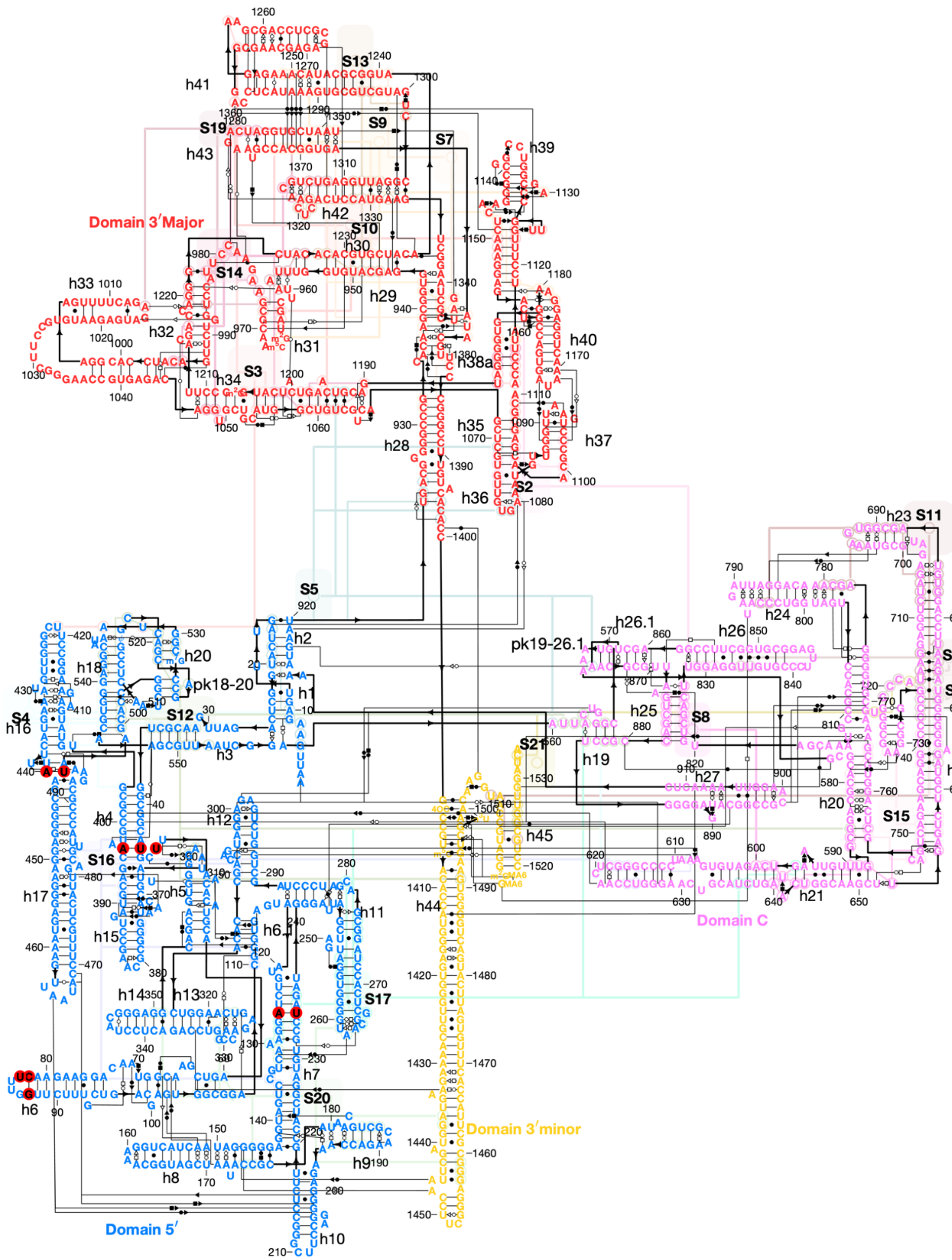

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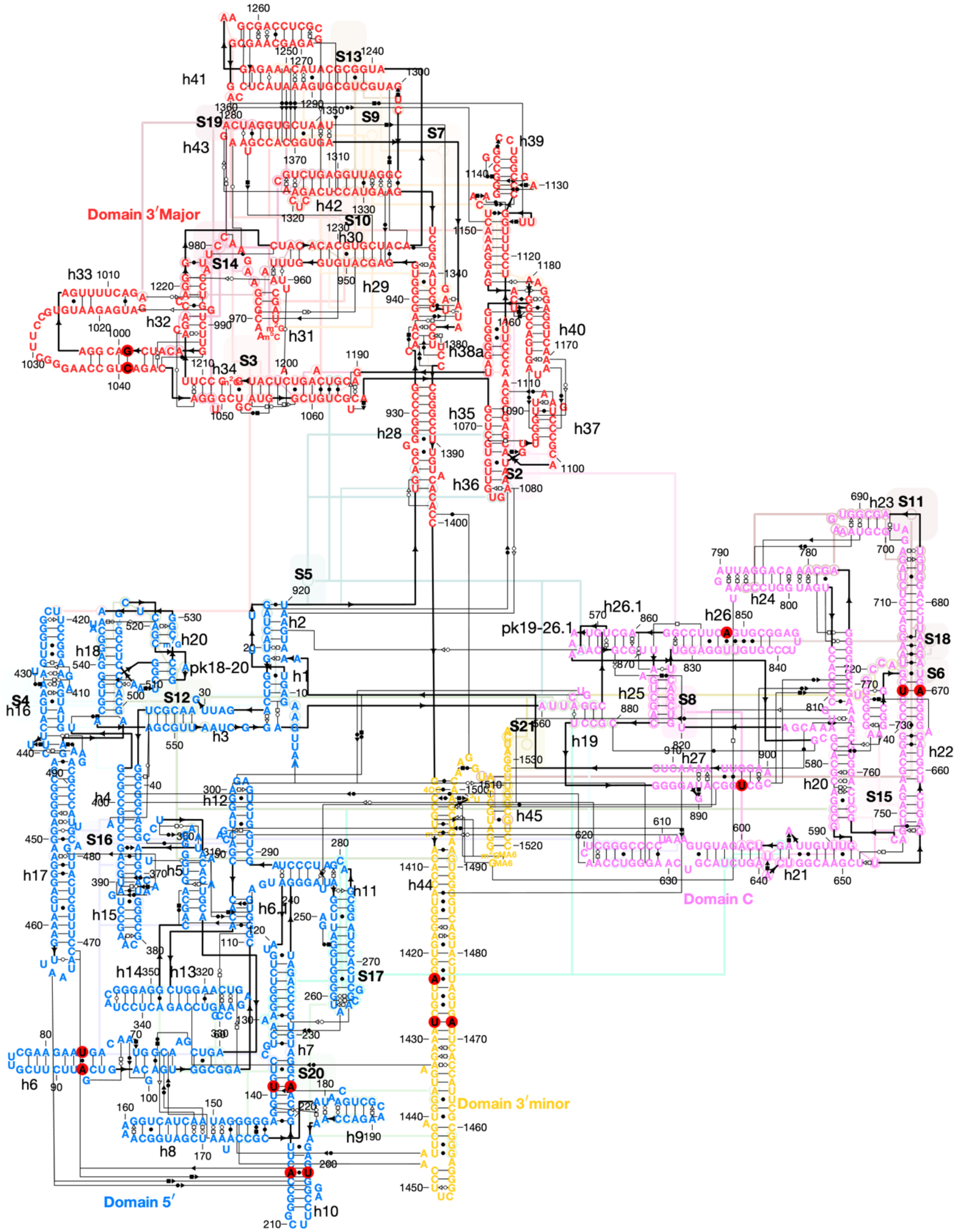

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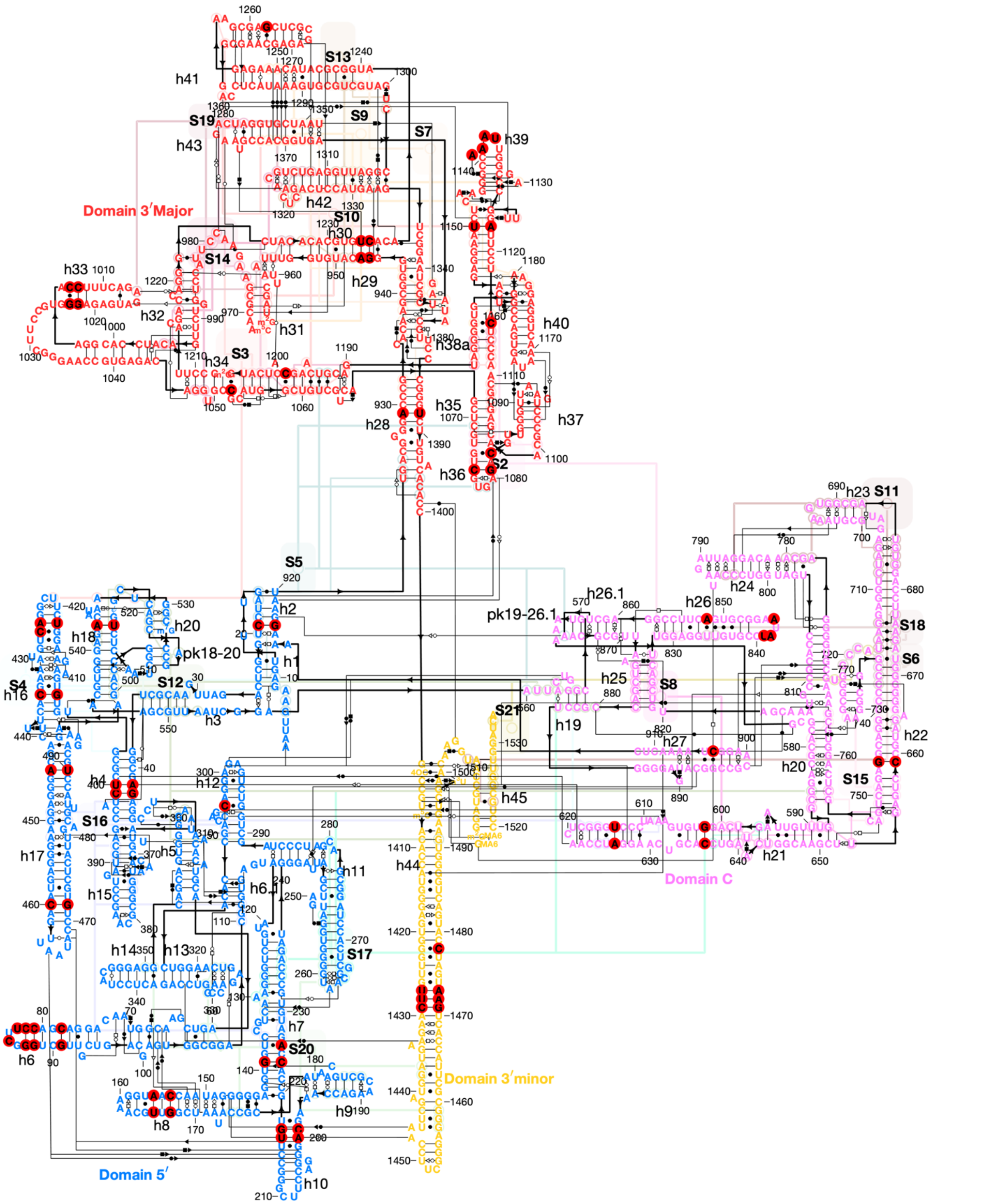

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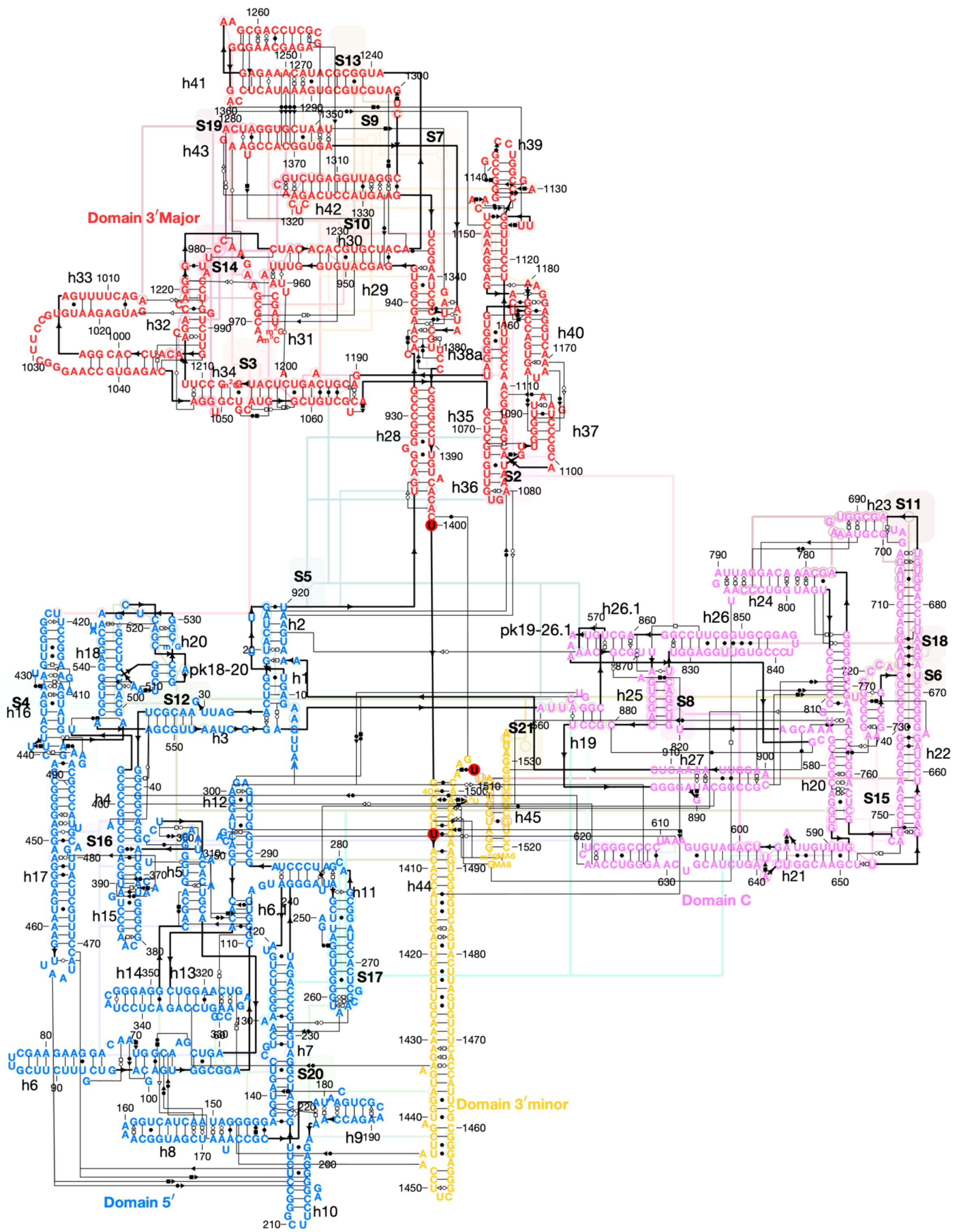

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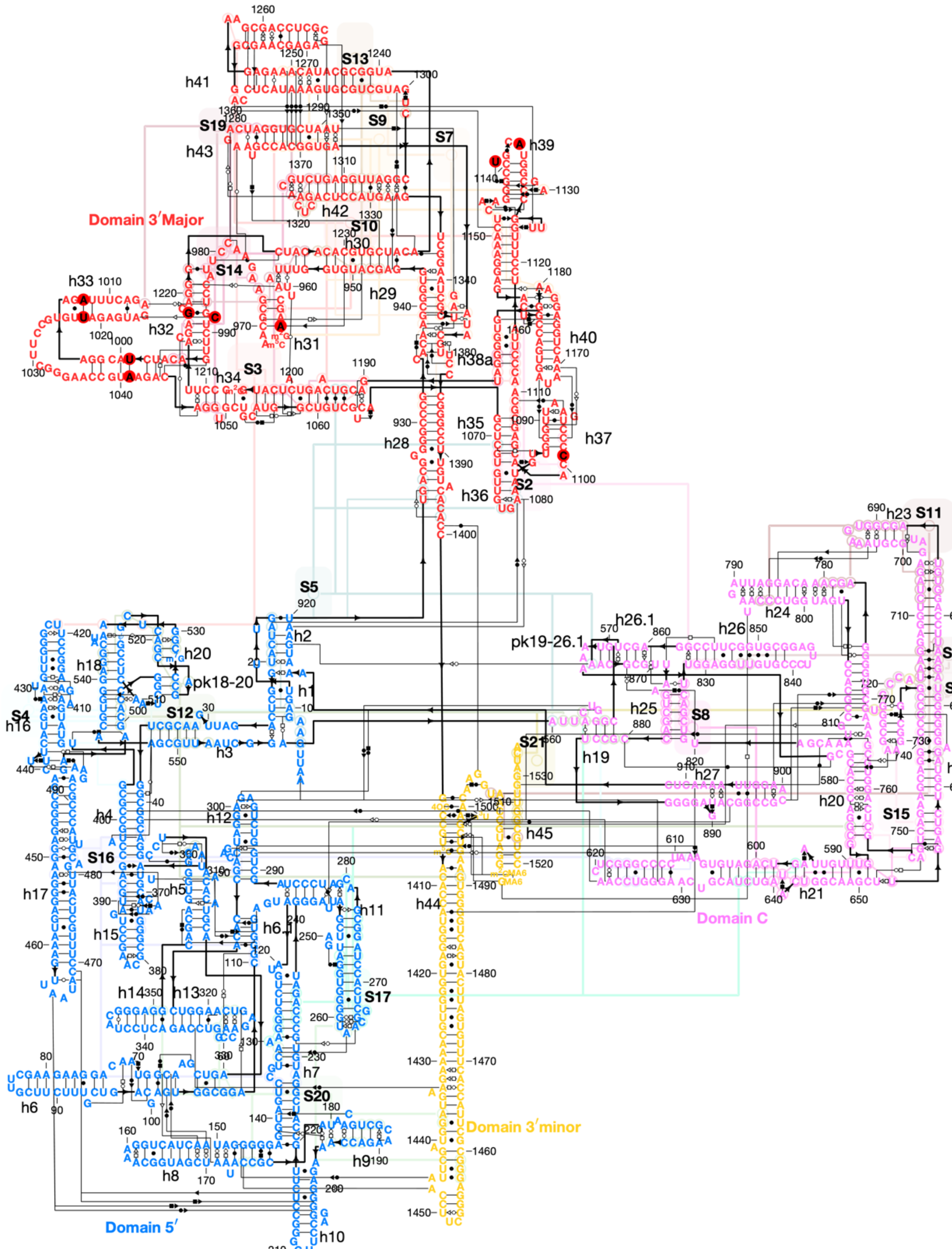

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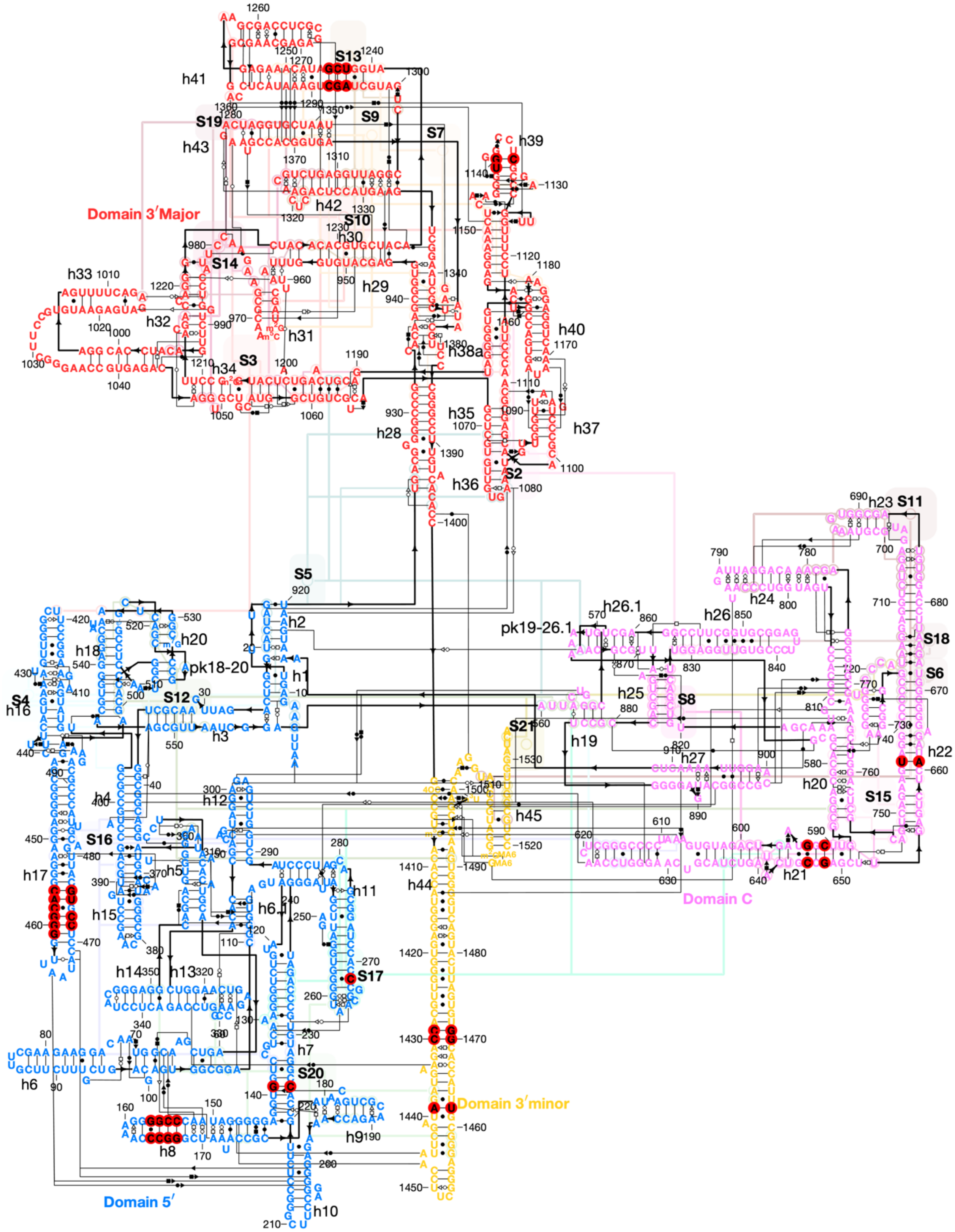

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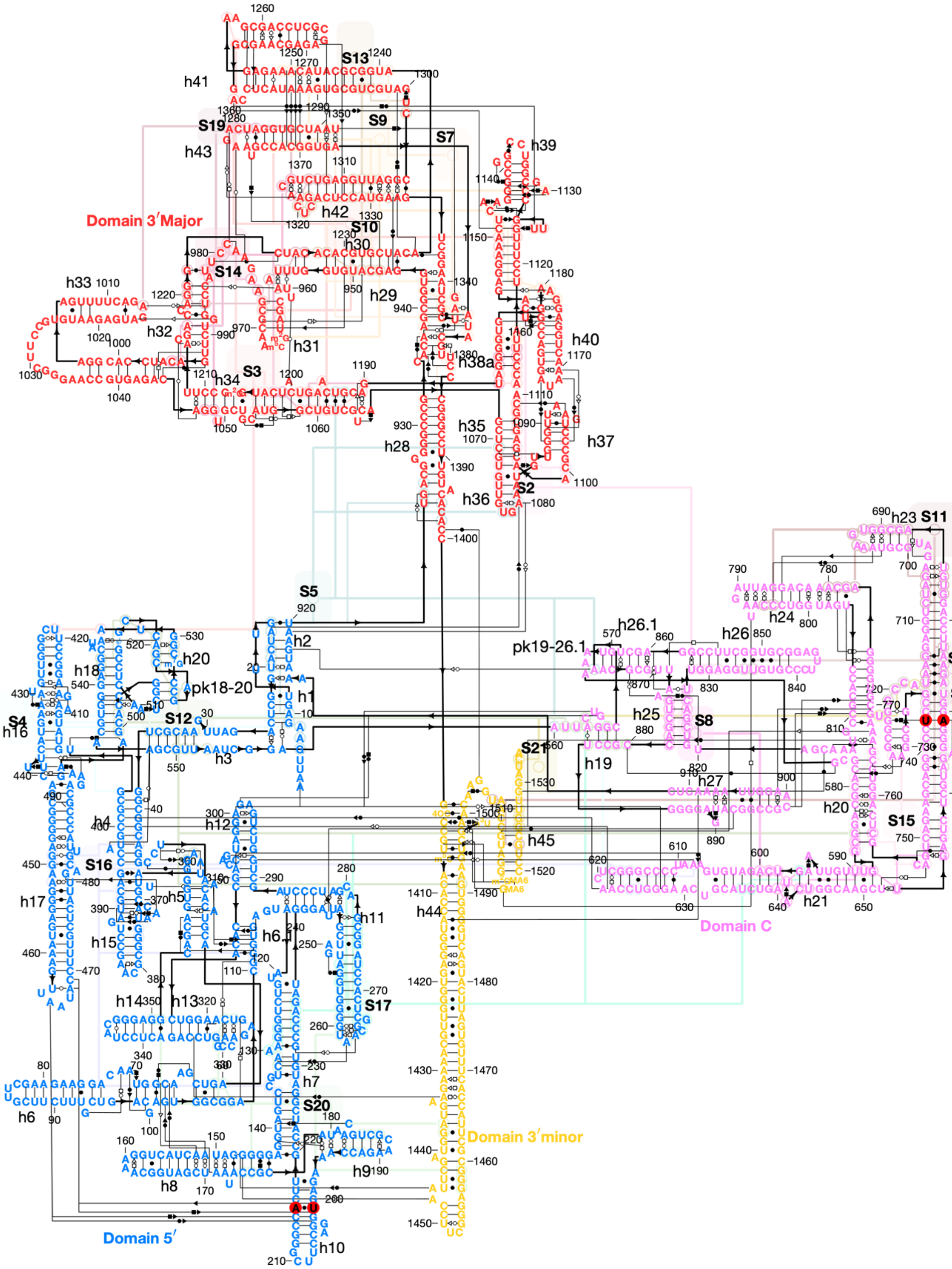

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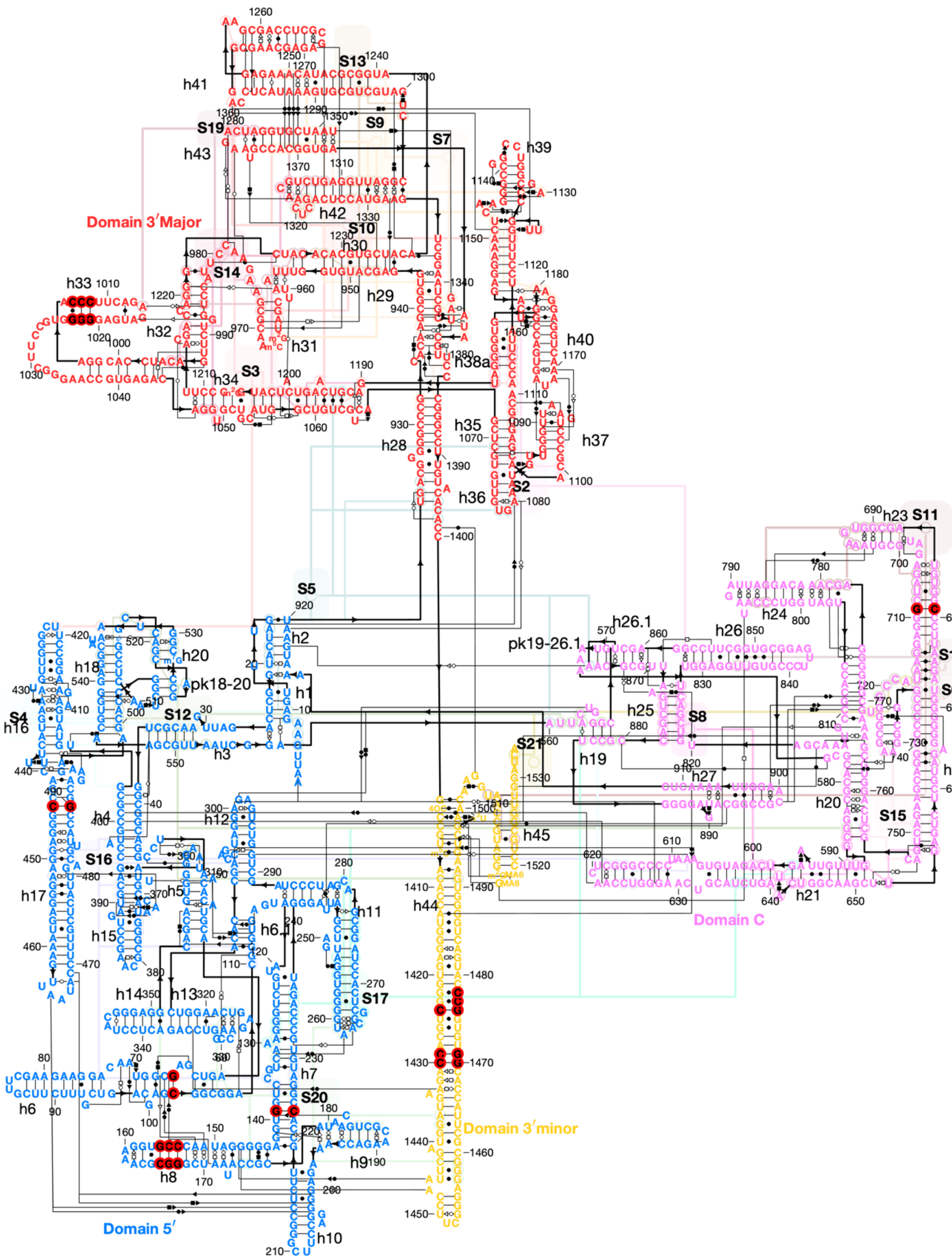

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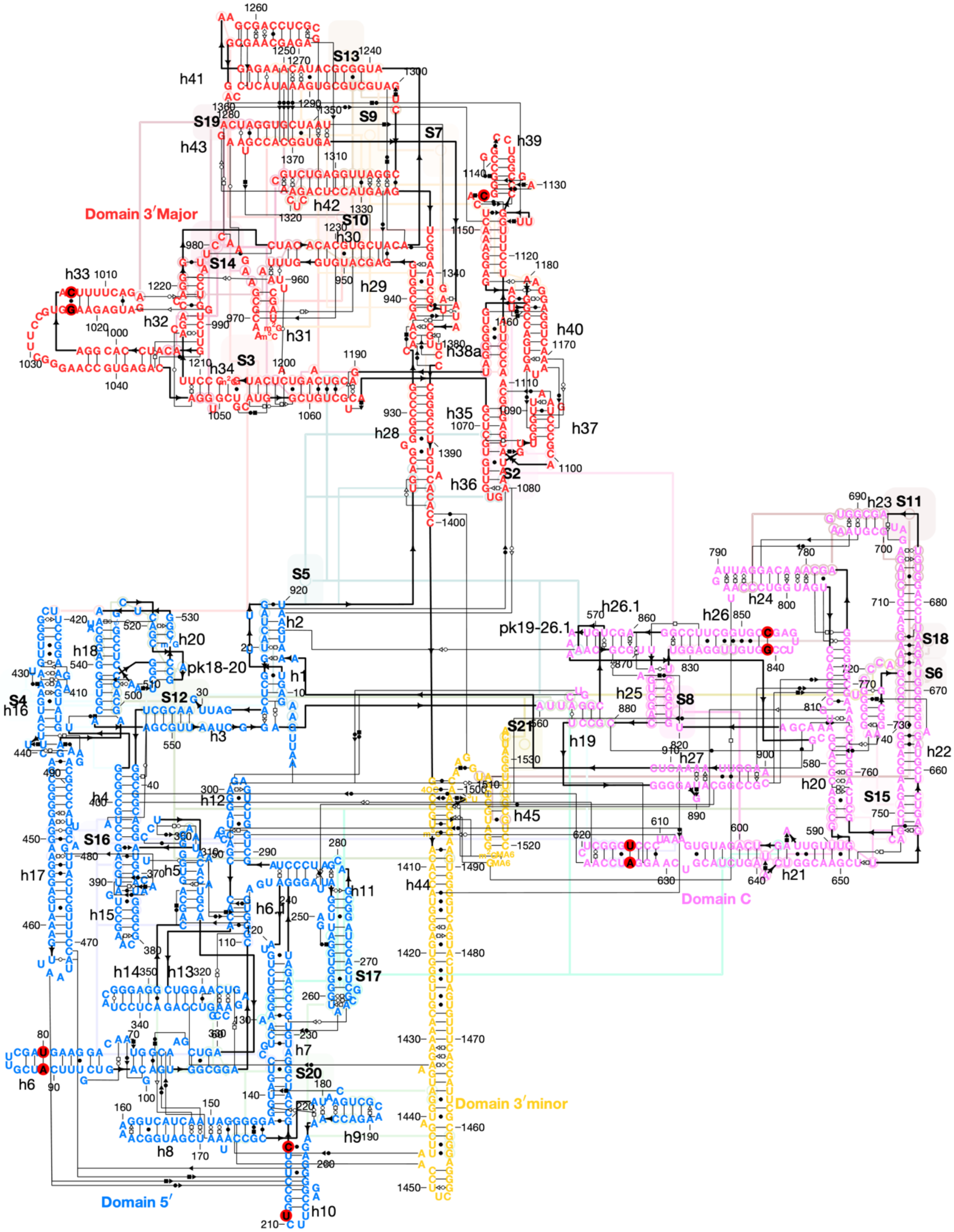

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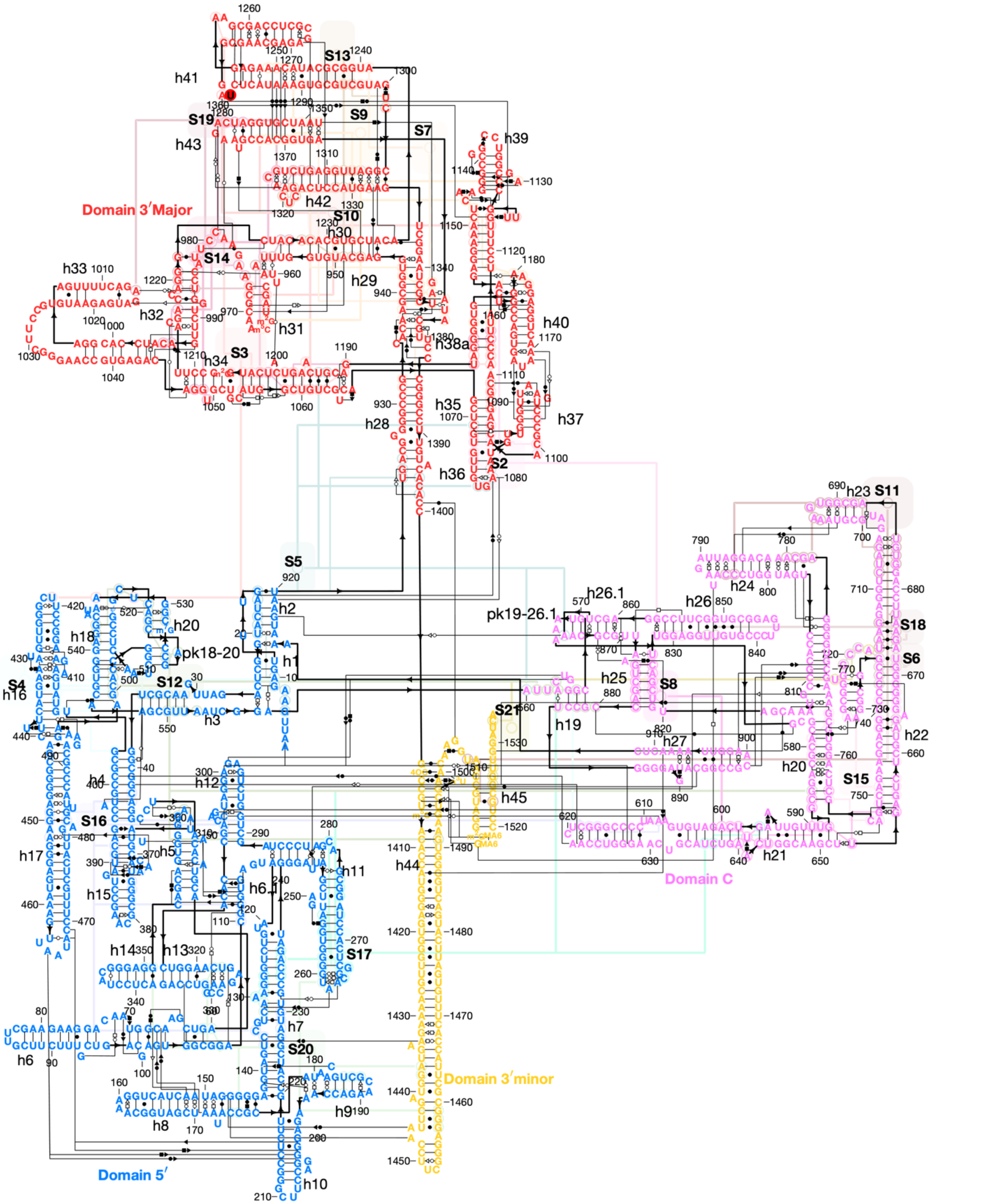

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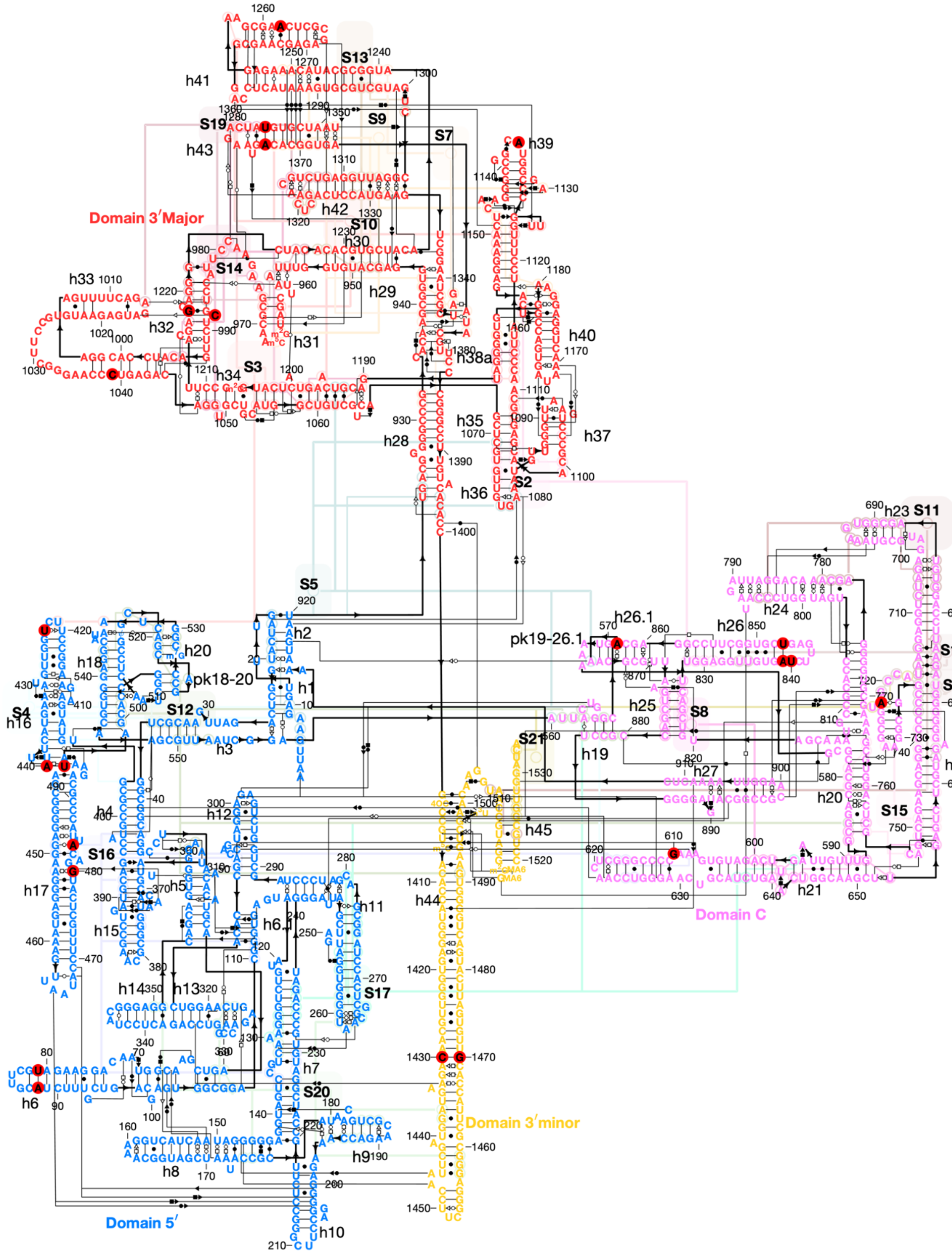

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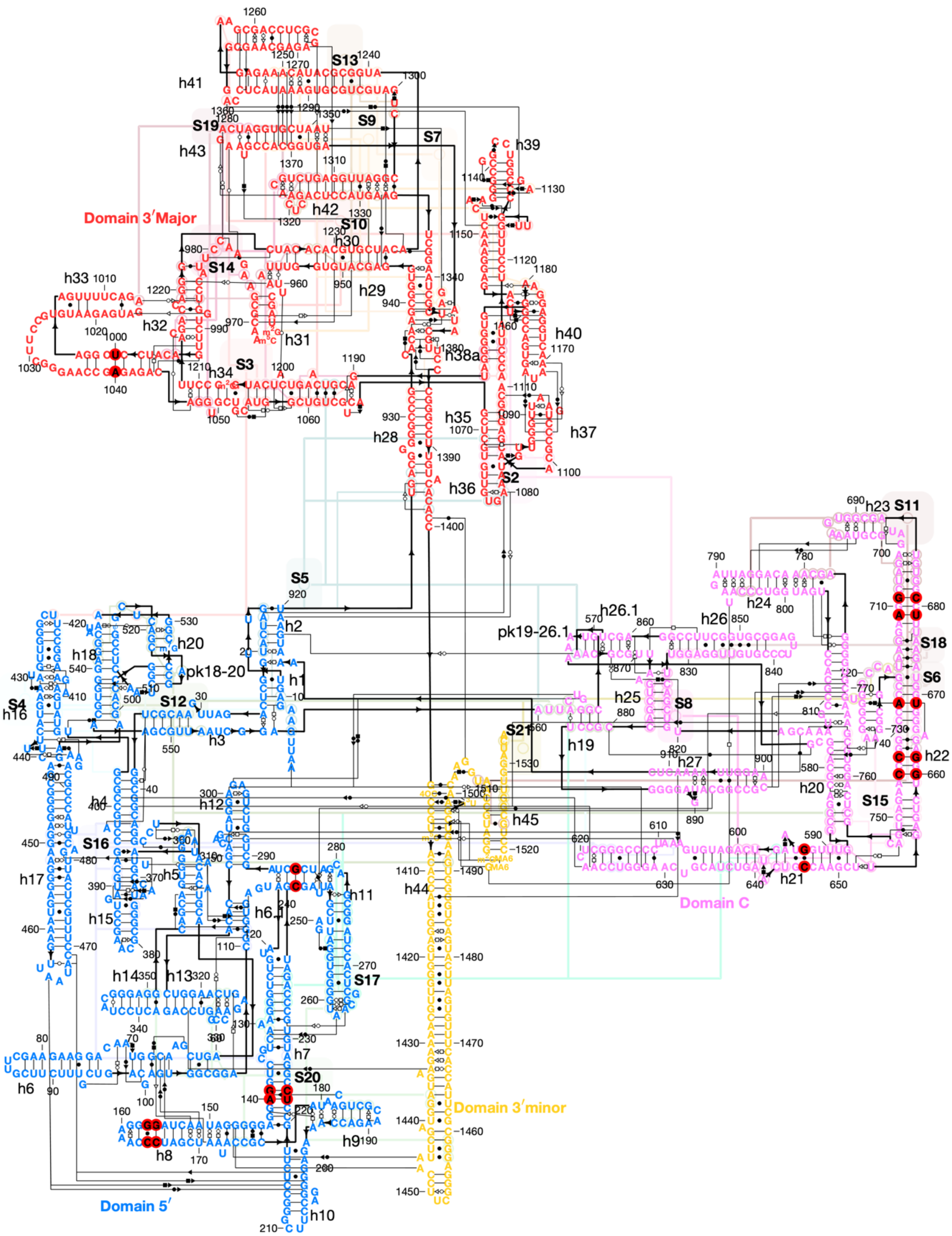

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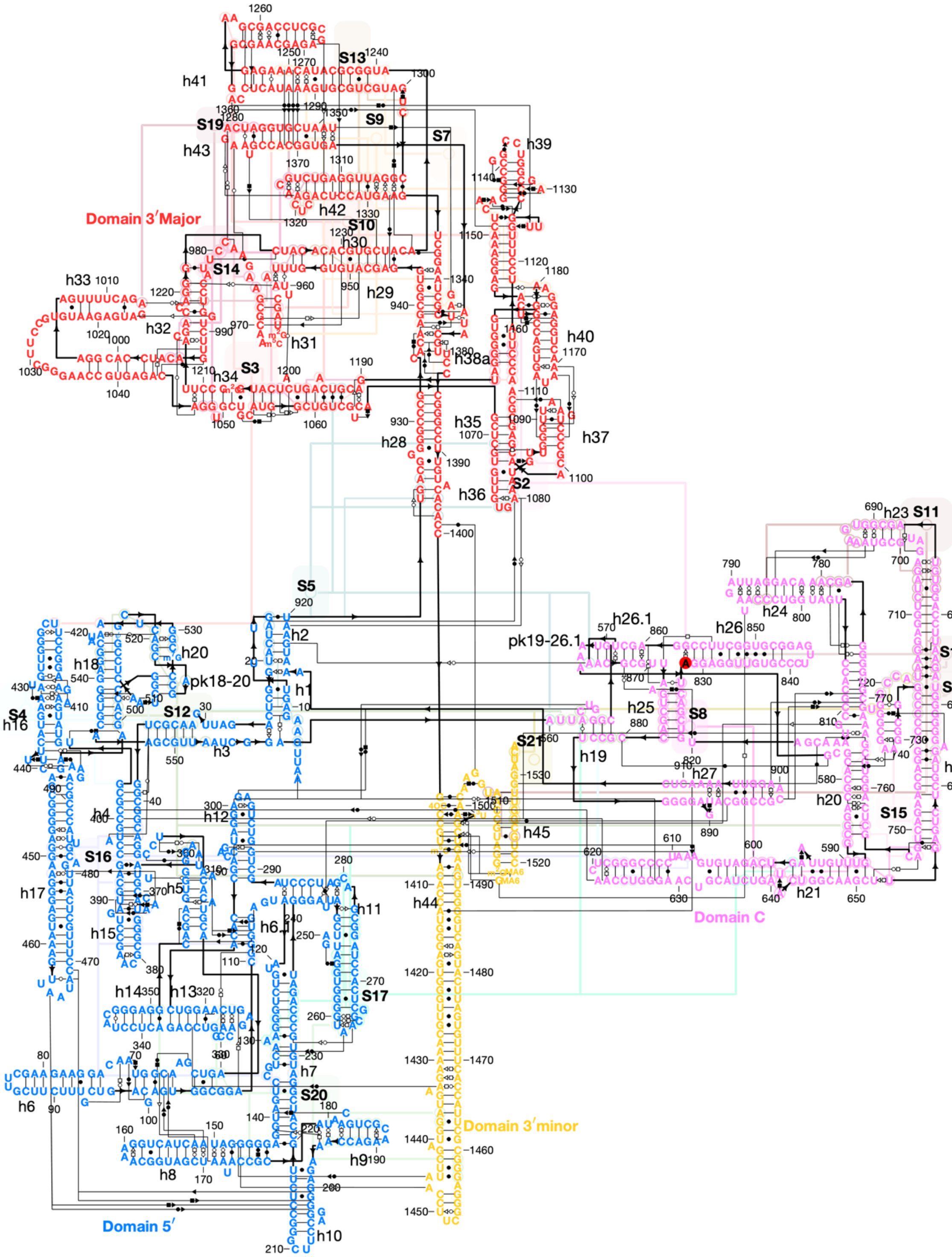

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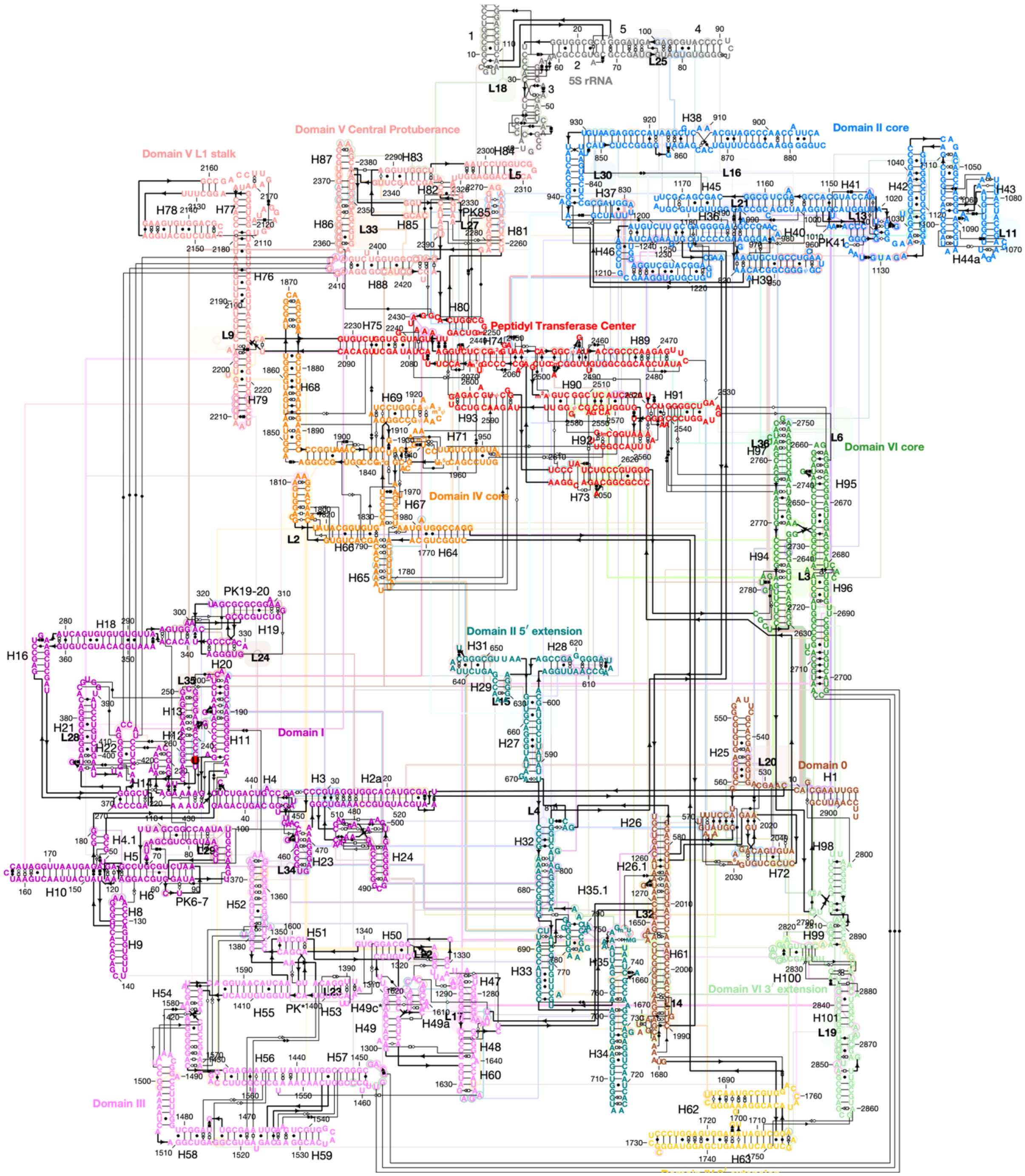
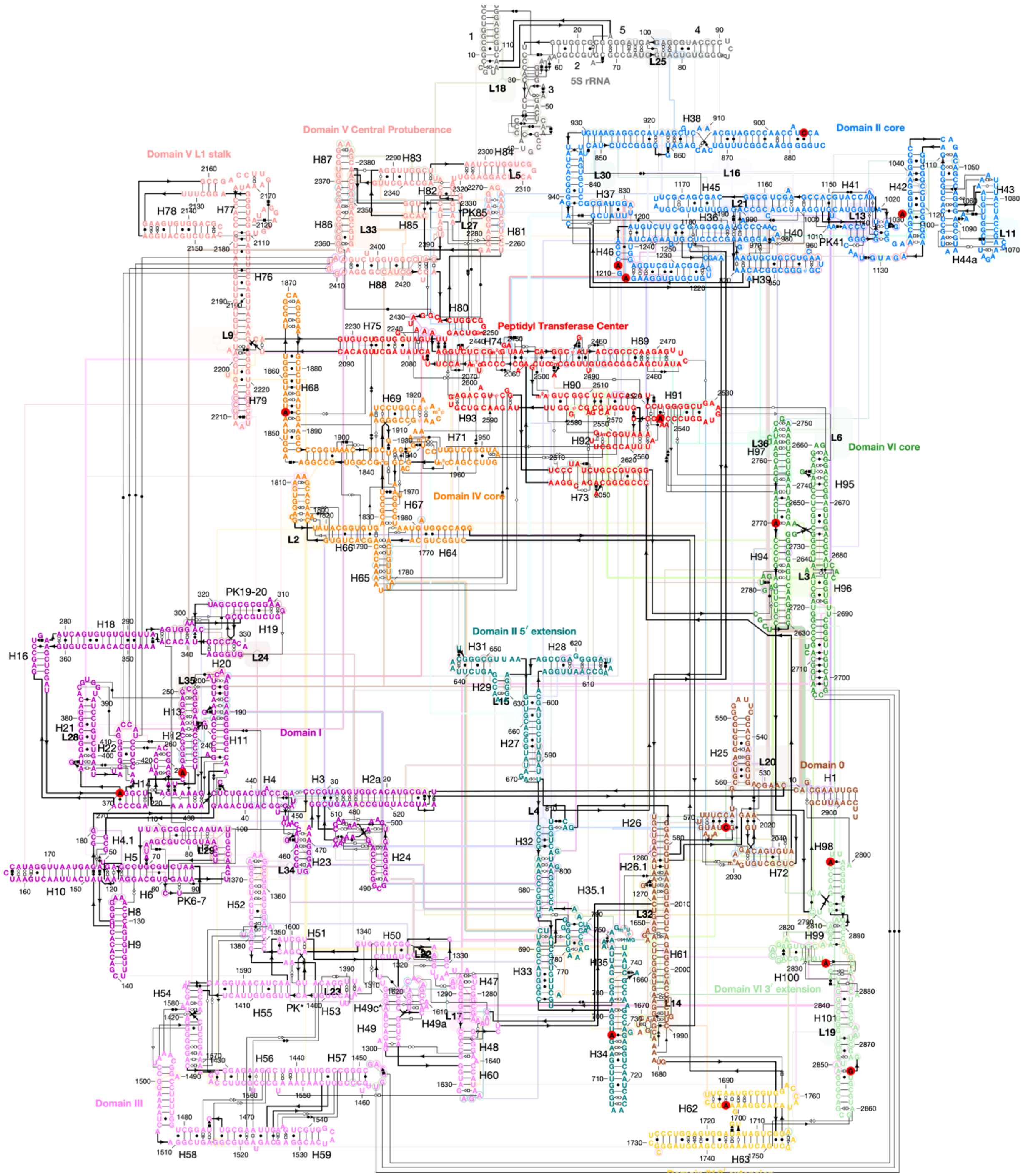
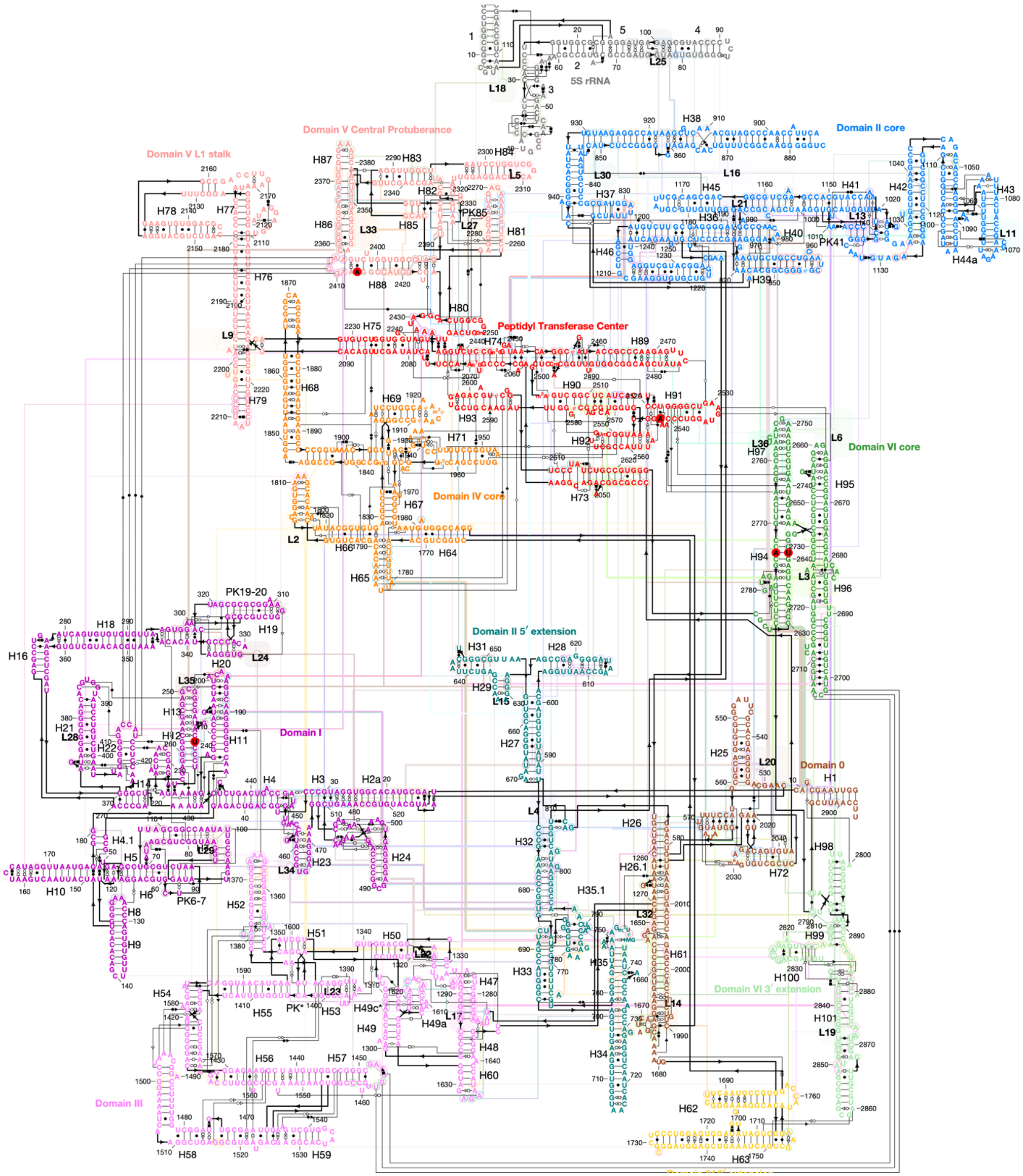


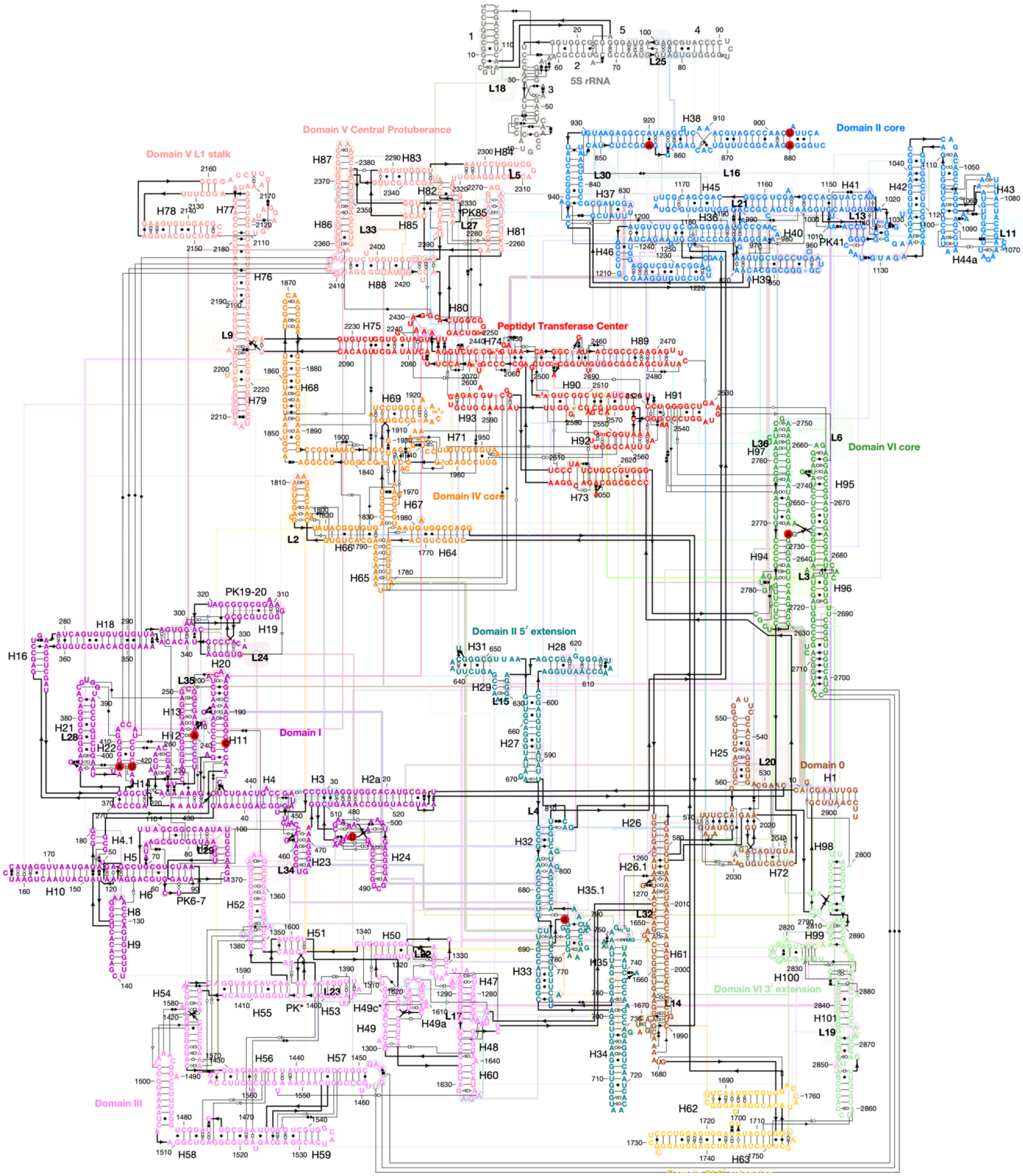
Supplementary Figure 55. Eterna participants'-designed ribosomal RNA design R1-21 prepared with RiboDraw[1].



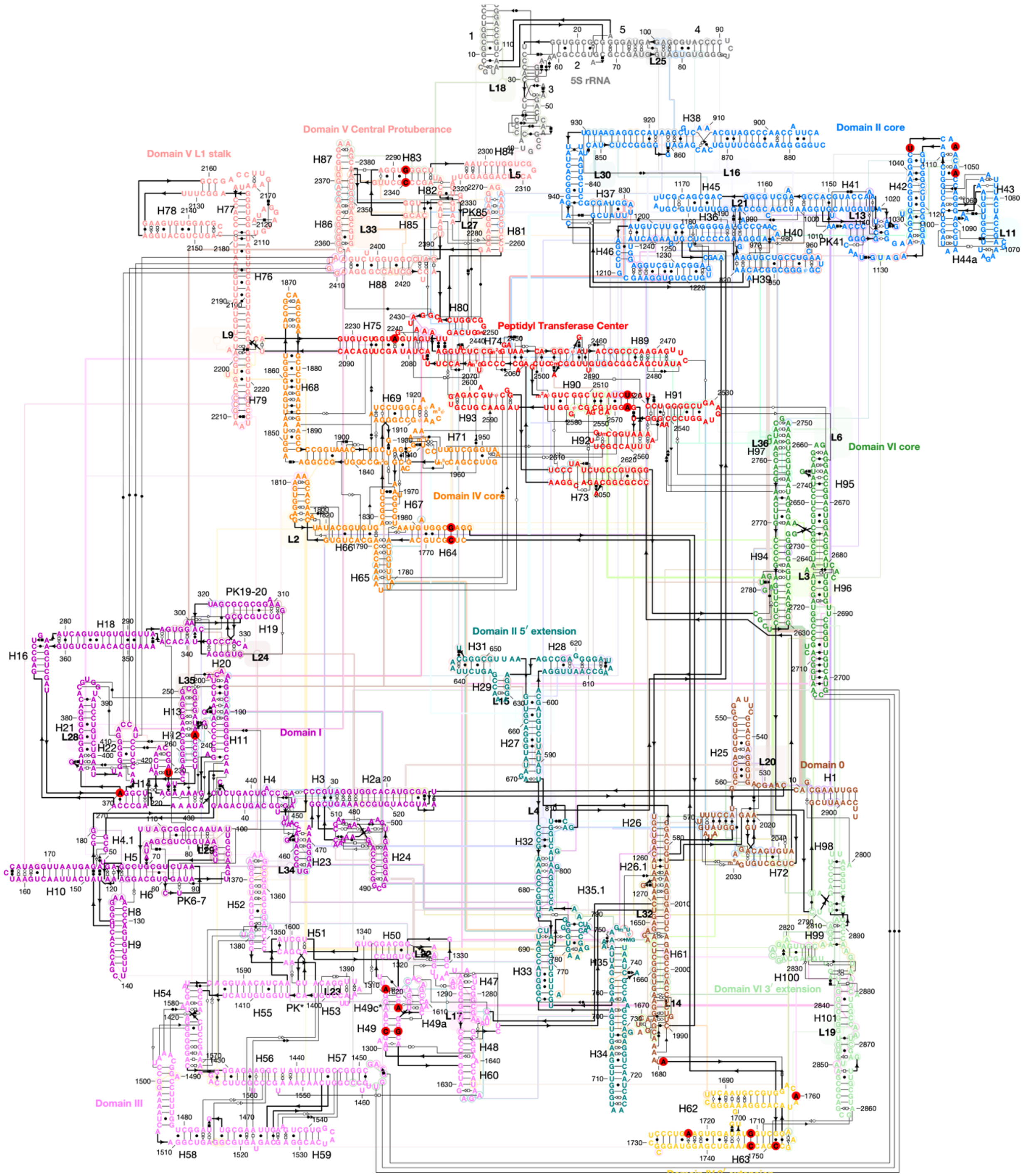
Supplementary Figure 56. Eterna participants'-designed ribosomal RNA design R1-22 prepared with RiboDraw[1].



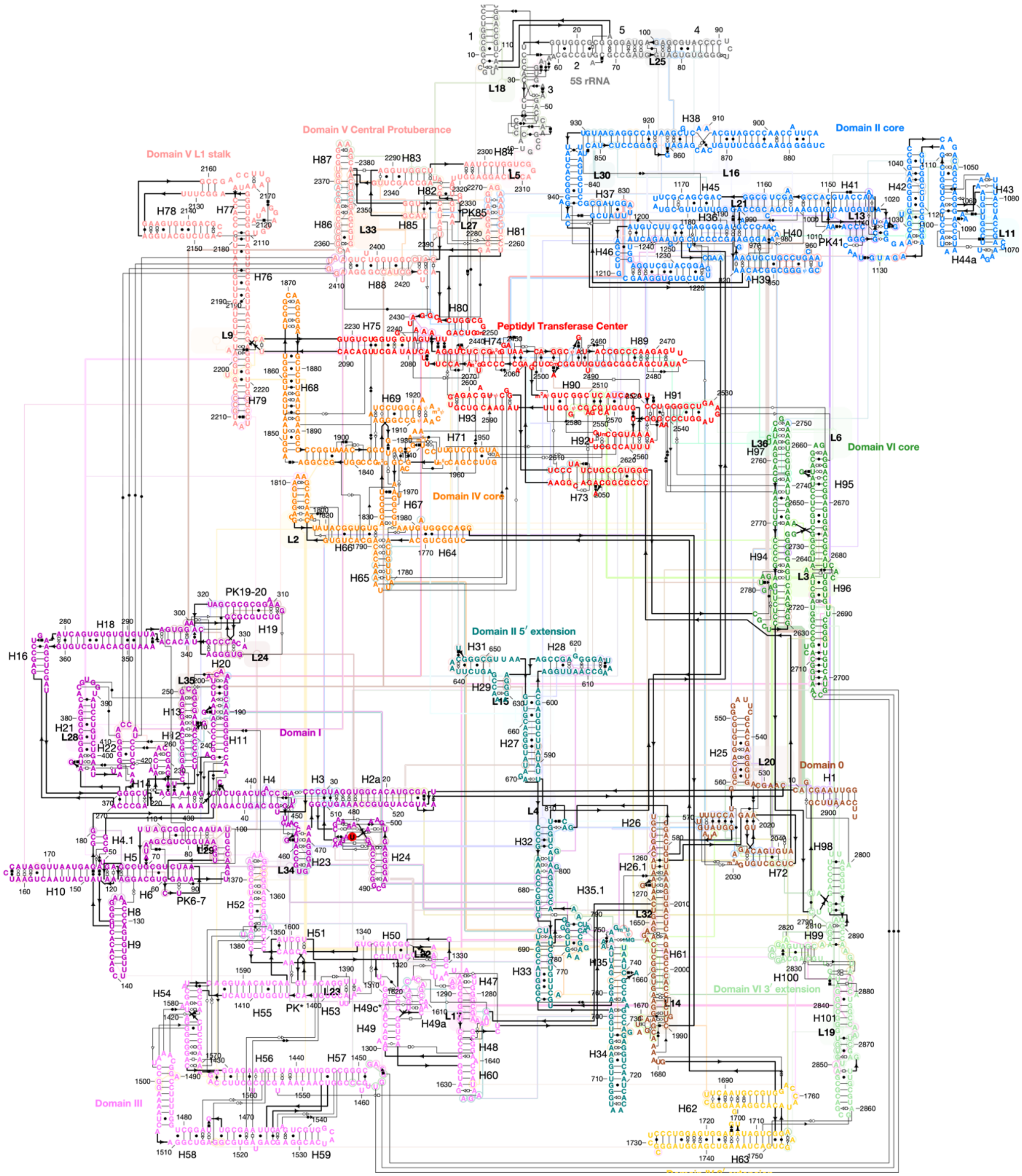
Supplementary Figure 57. Eterna participants'-designed ribosomal RNA design R1-23 prepared with RiboDraw[1].



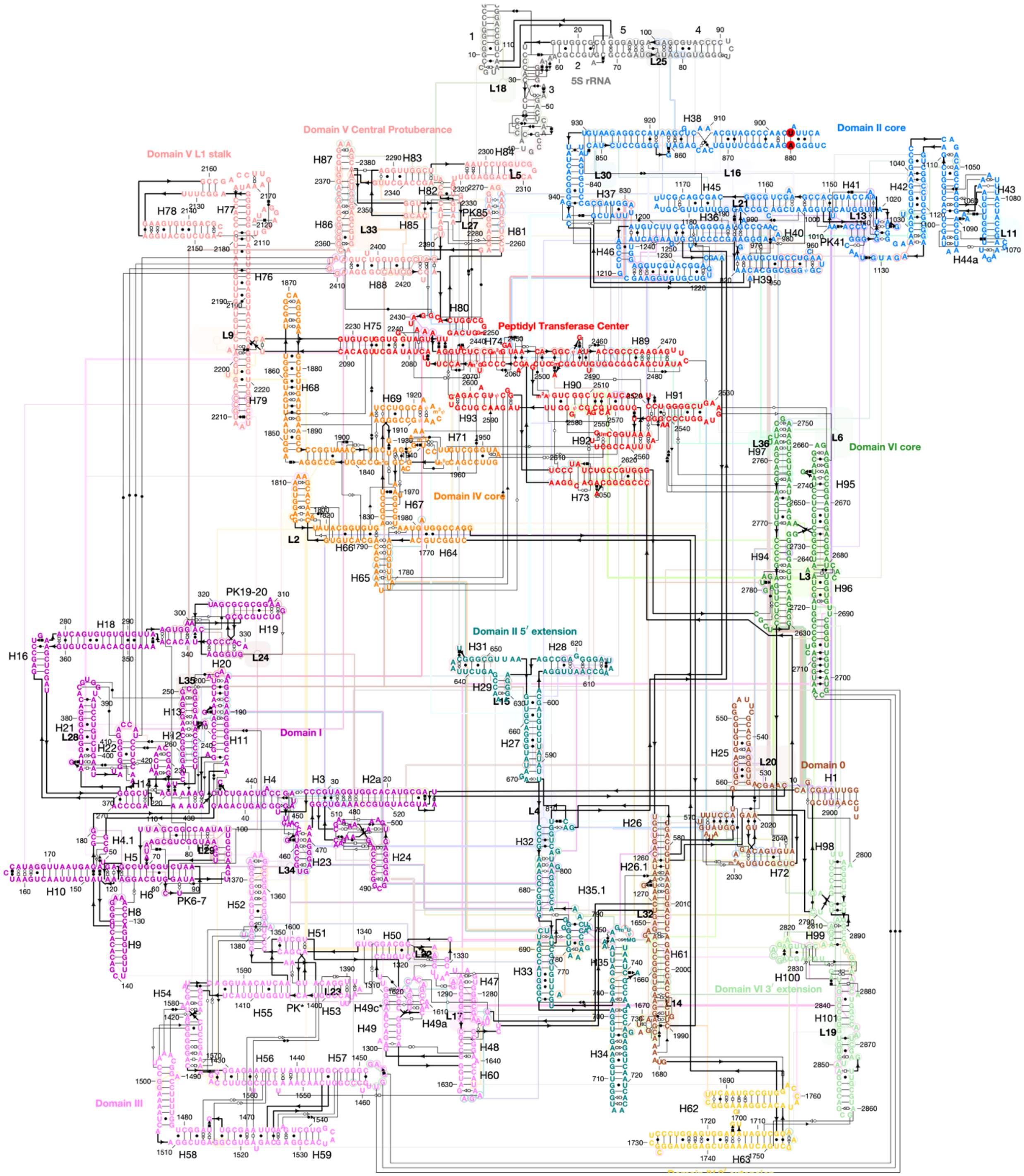
Supplementary Figure 58. Eterna participants'-designed ribosomal RNA design R1-24 prepared with RiboDraw[1].



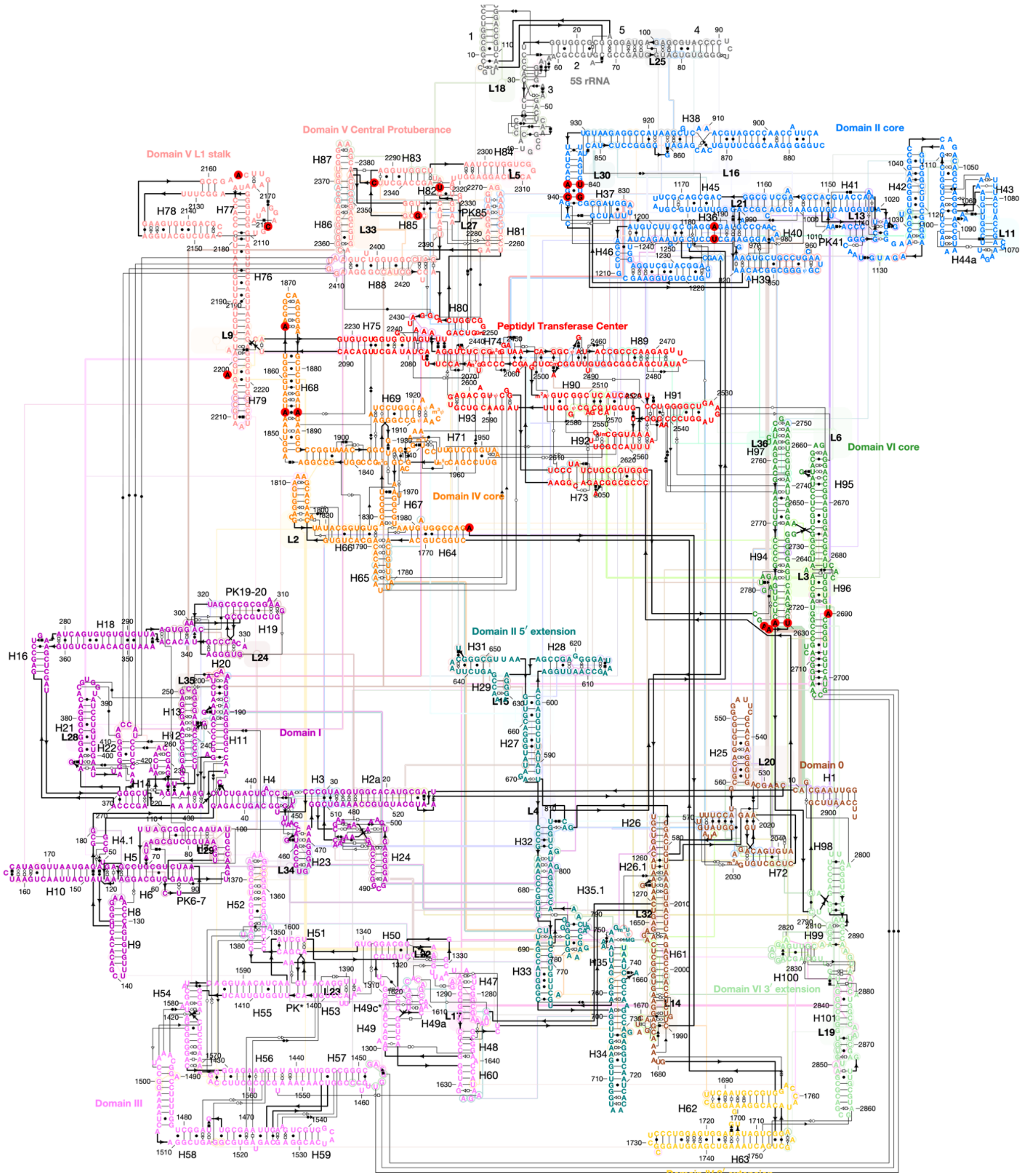
Supplementary Figure 59. Eterna participants'-designed ribosomal RNA design R1-25 prepared with RiboDraw[1].



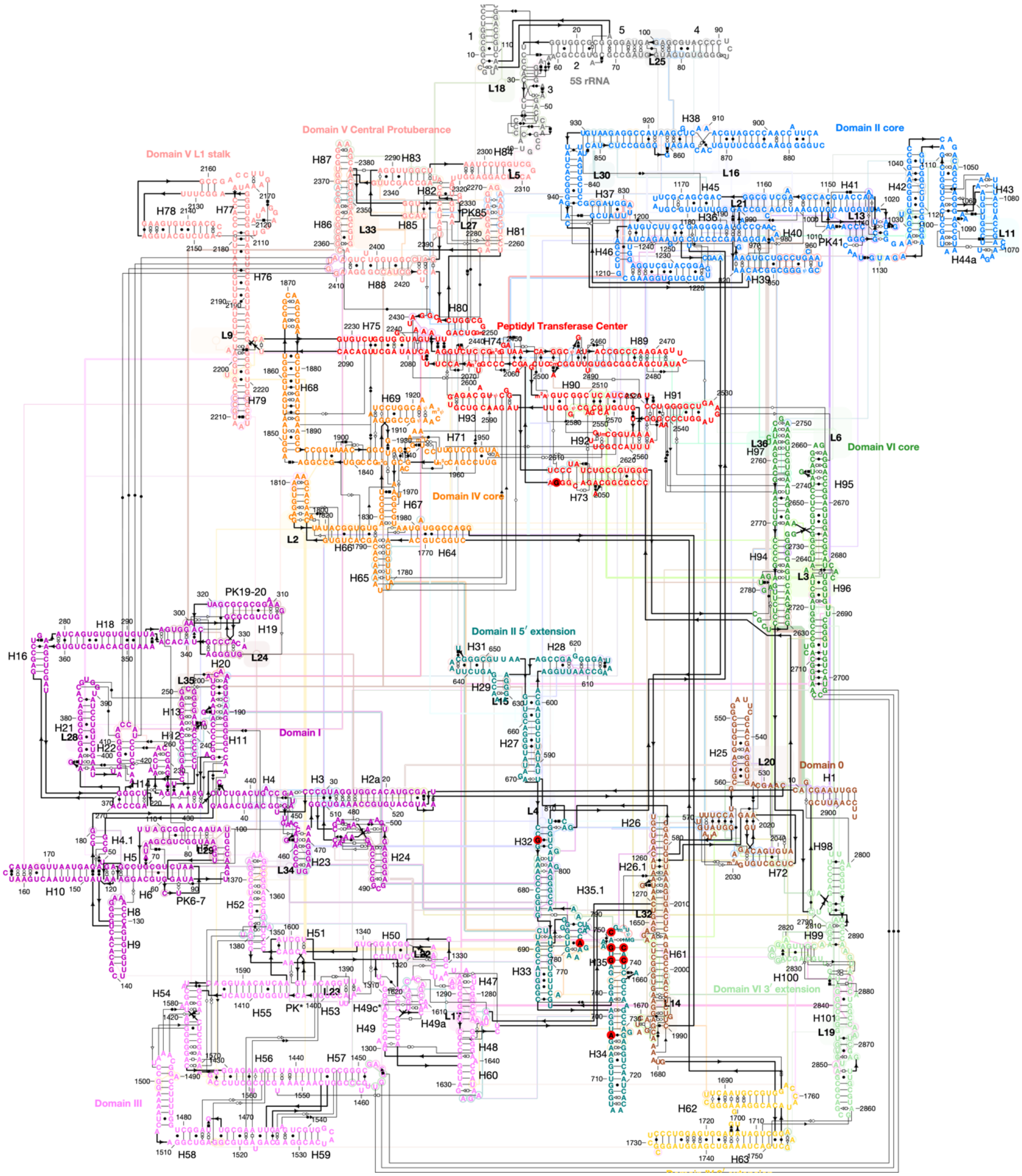
Supplementary Figure 60. Eterna participants'-designed ribosomal RNA design R1-26 prepared with RiboDraw[1].



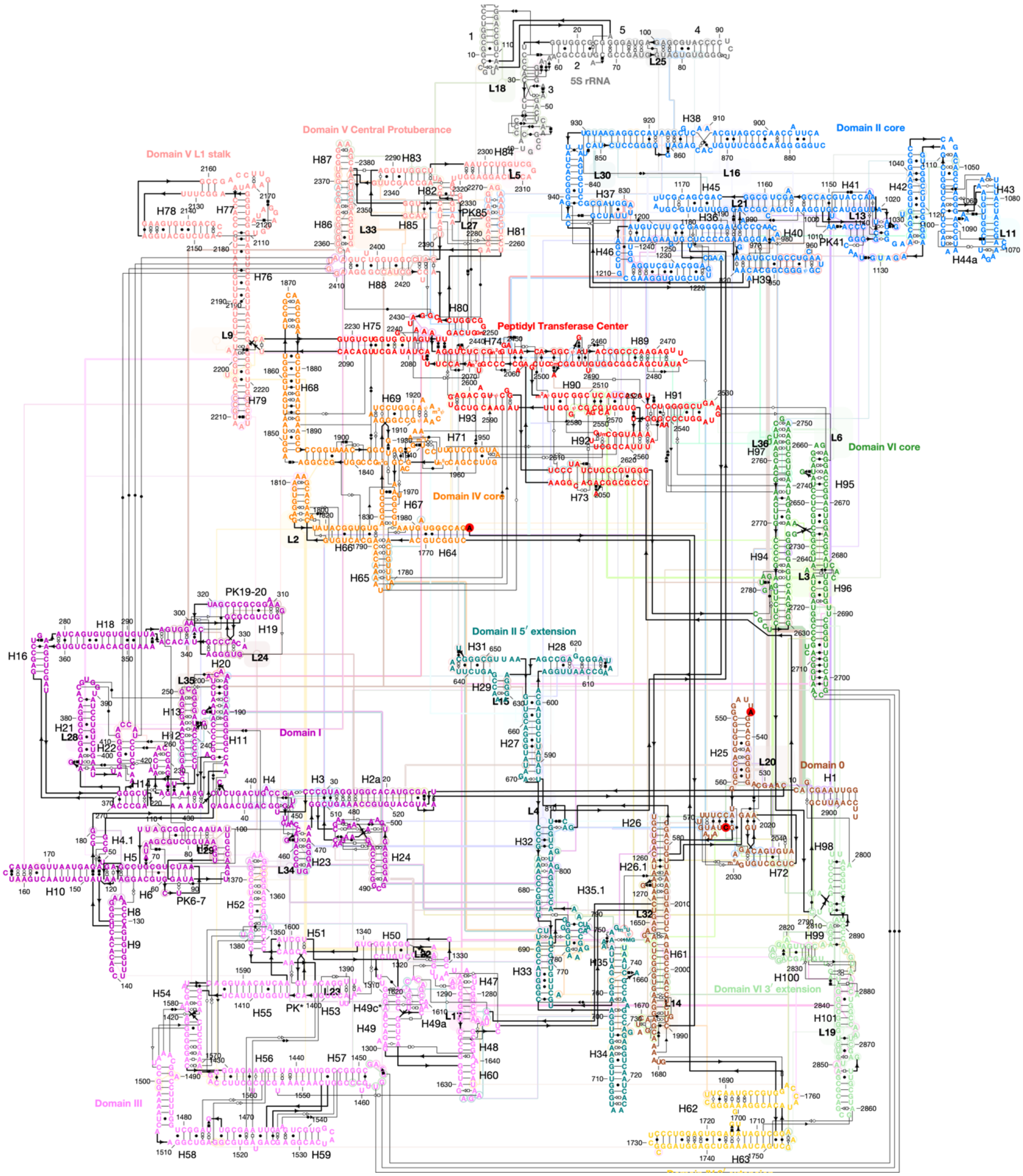
Supplementary Figure 61. Eterna participants'-designed ribosomal RNA design R1-27 prepared with RiboDraw[1].



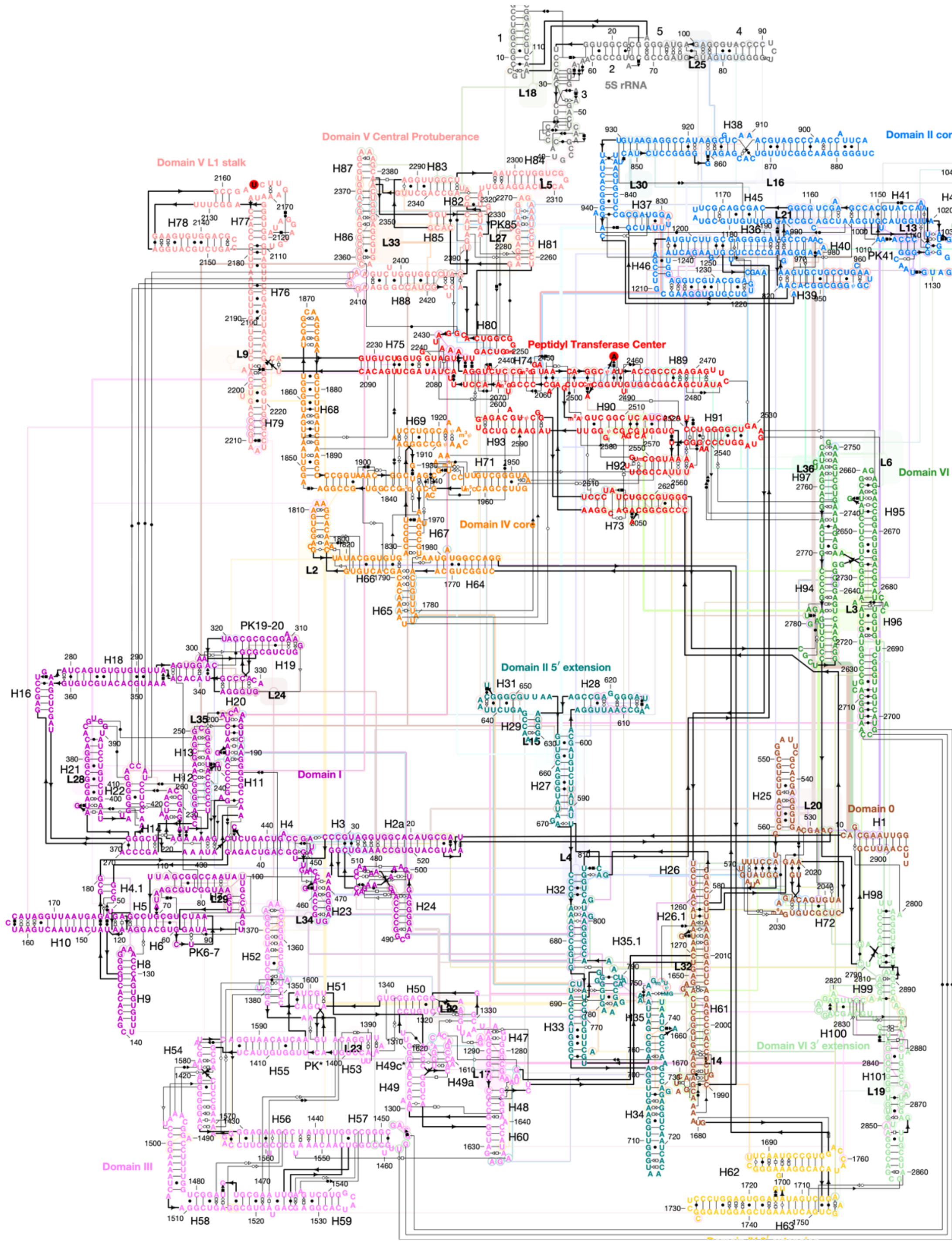
Supplementary Figure 62. Eterna participants'-designed ribosomal RNA design R1-28 prepared with RiboDraw[1].



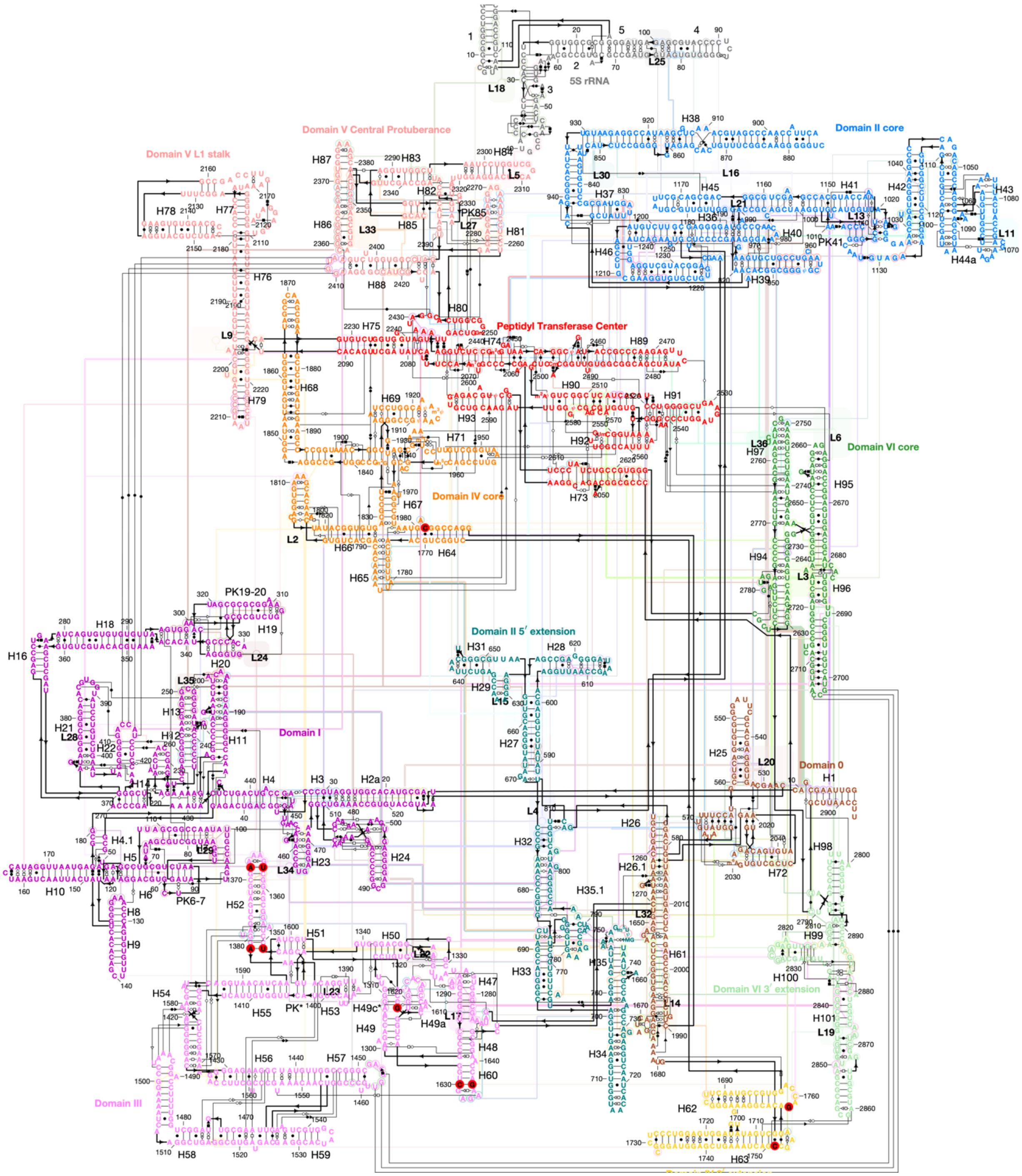
Supplementary Figure 63. Eterna participants'-designed ribosomal RNA design R1-29 prepared with RiboDraw[1].



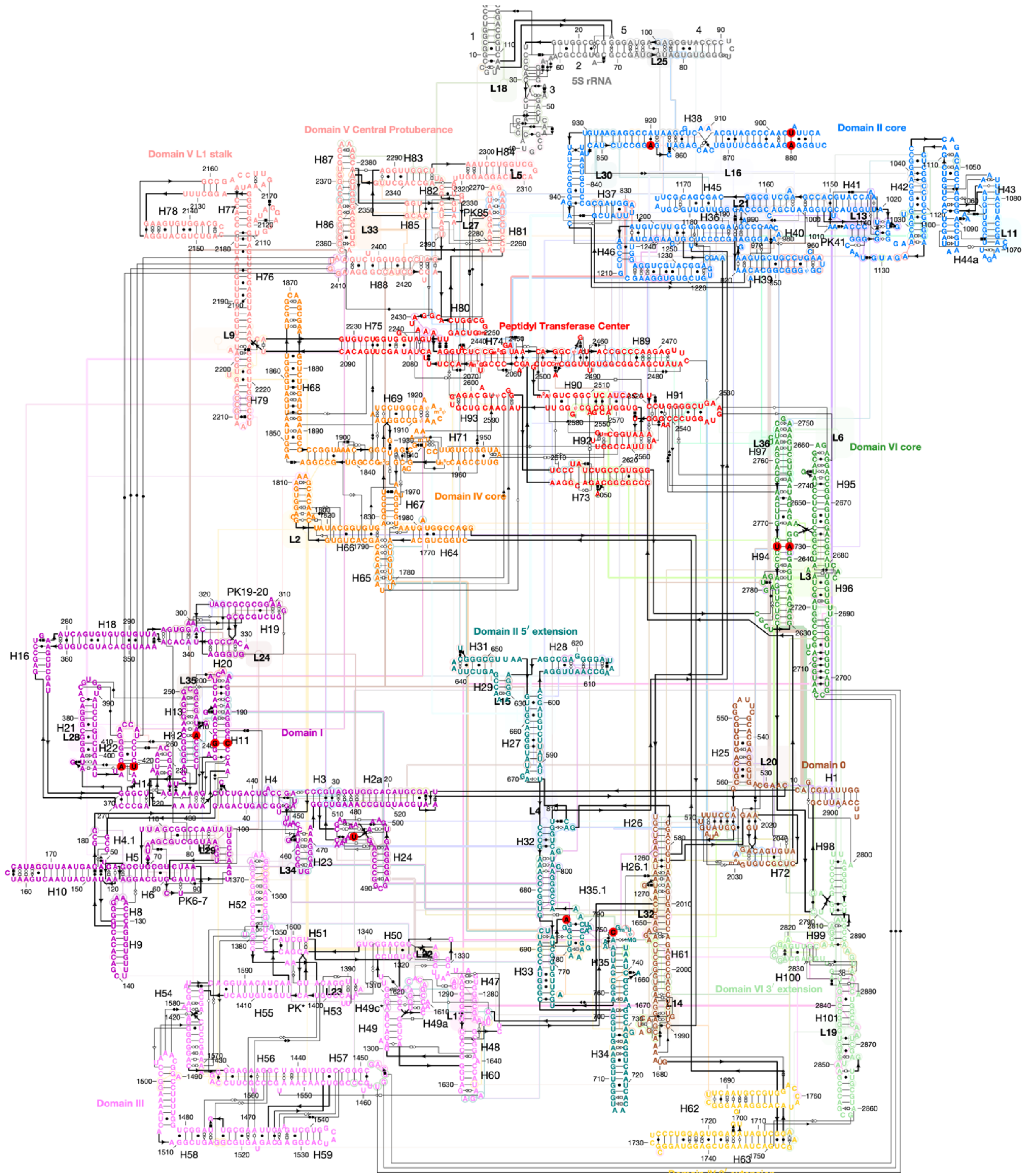
Supplementary Figure 64. Eterna participants'-designed ribosomal RNA design R1-30 prepared with RiboDraw[1]**.**



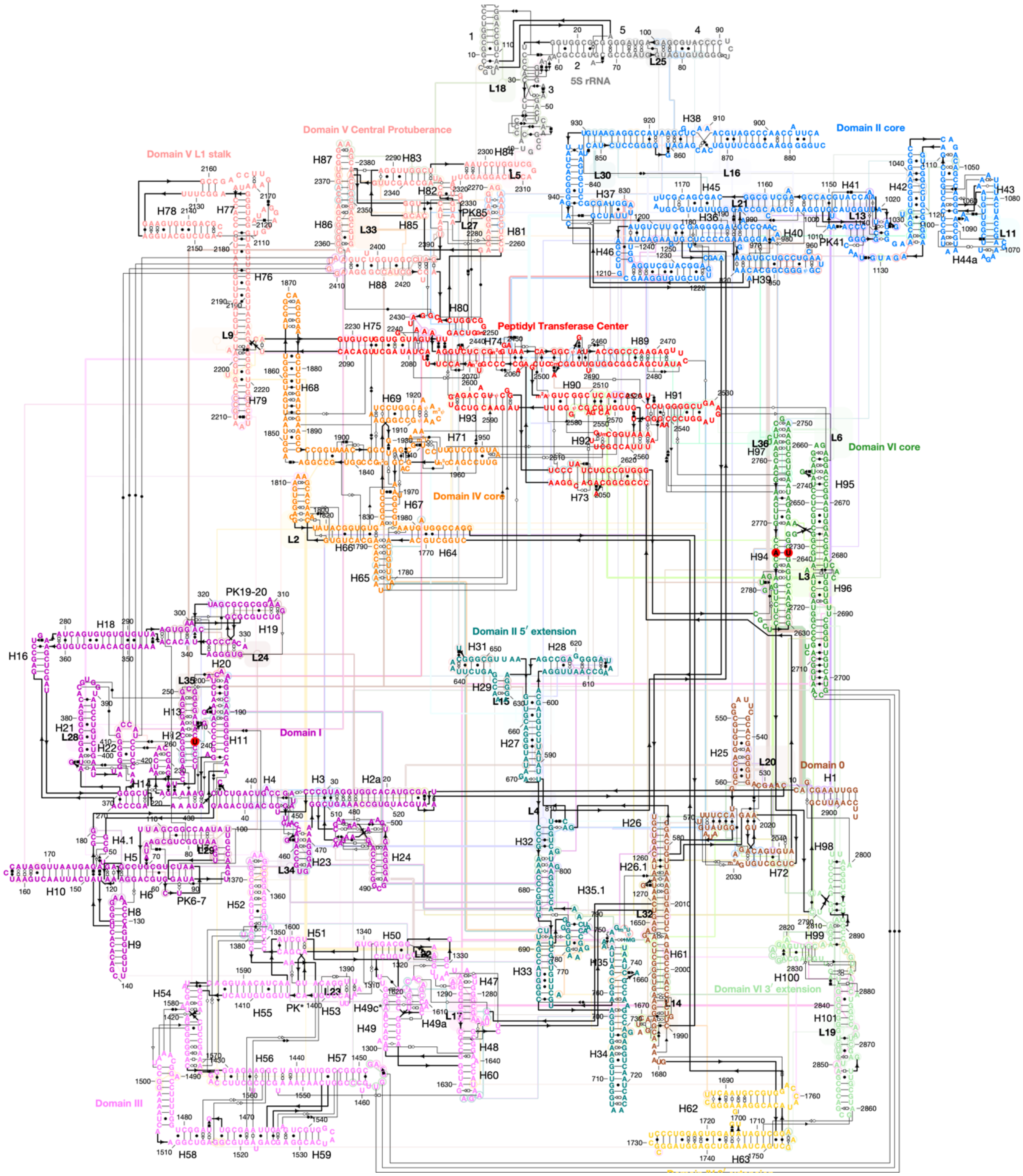
Supplementary Figure 65. Eterna participants'-designed ribosomal RNA design R1-31 prepared with RiboDraw[1].



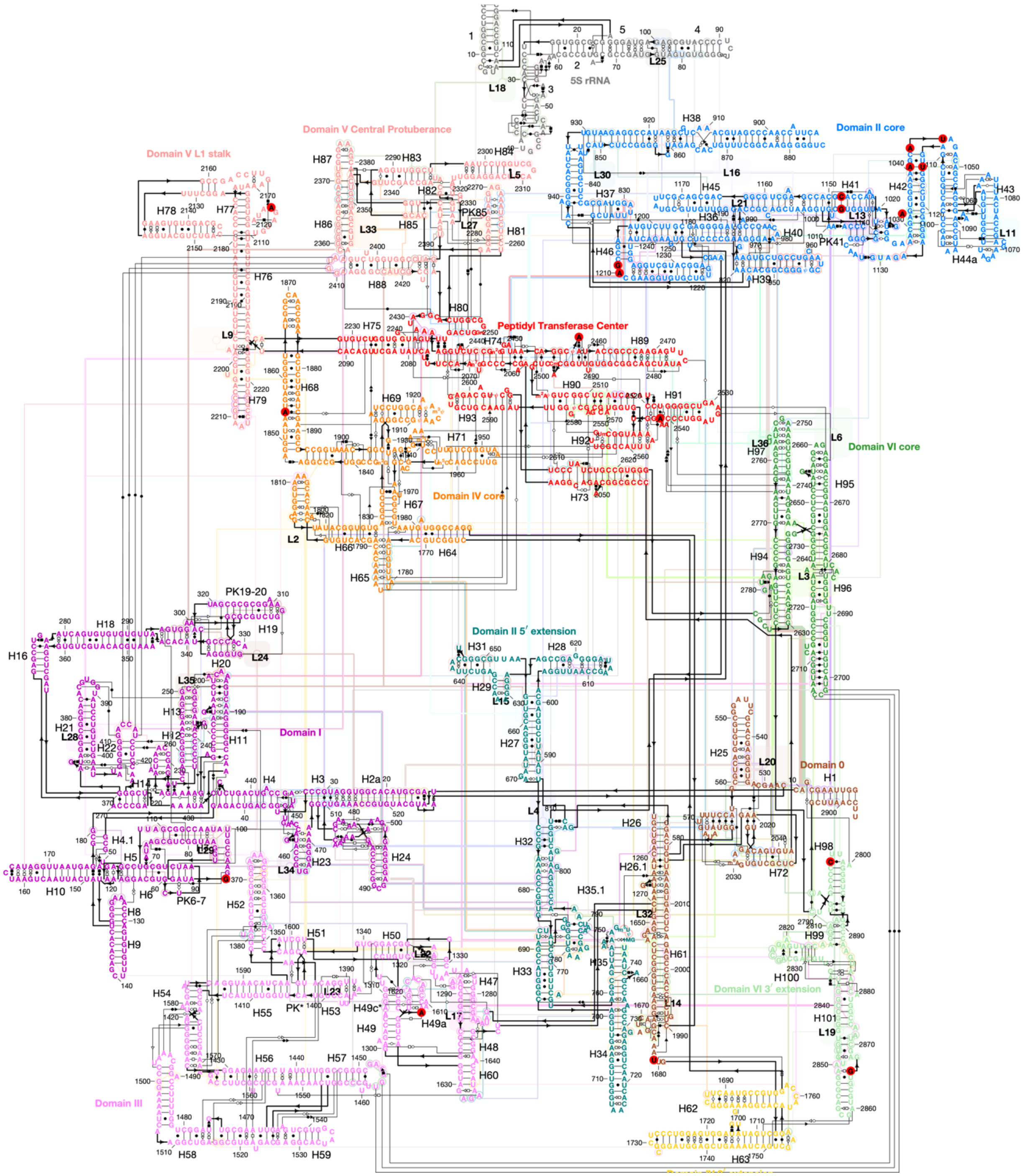
Supplementary Figure 66. Eterna participants'-designed ribosomal RNA design R1-32 prepared with RiboDraw[1].



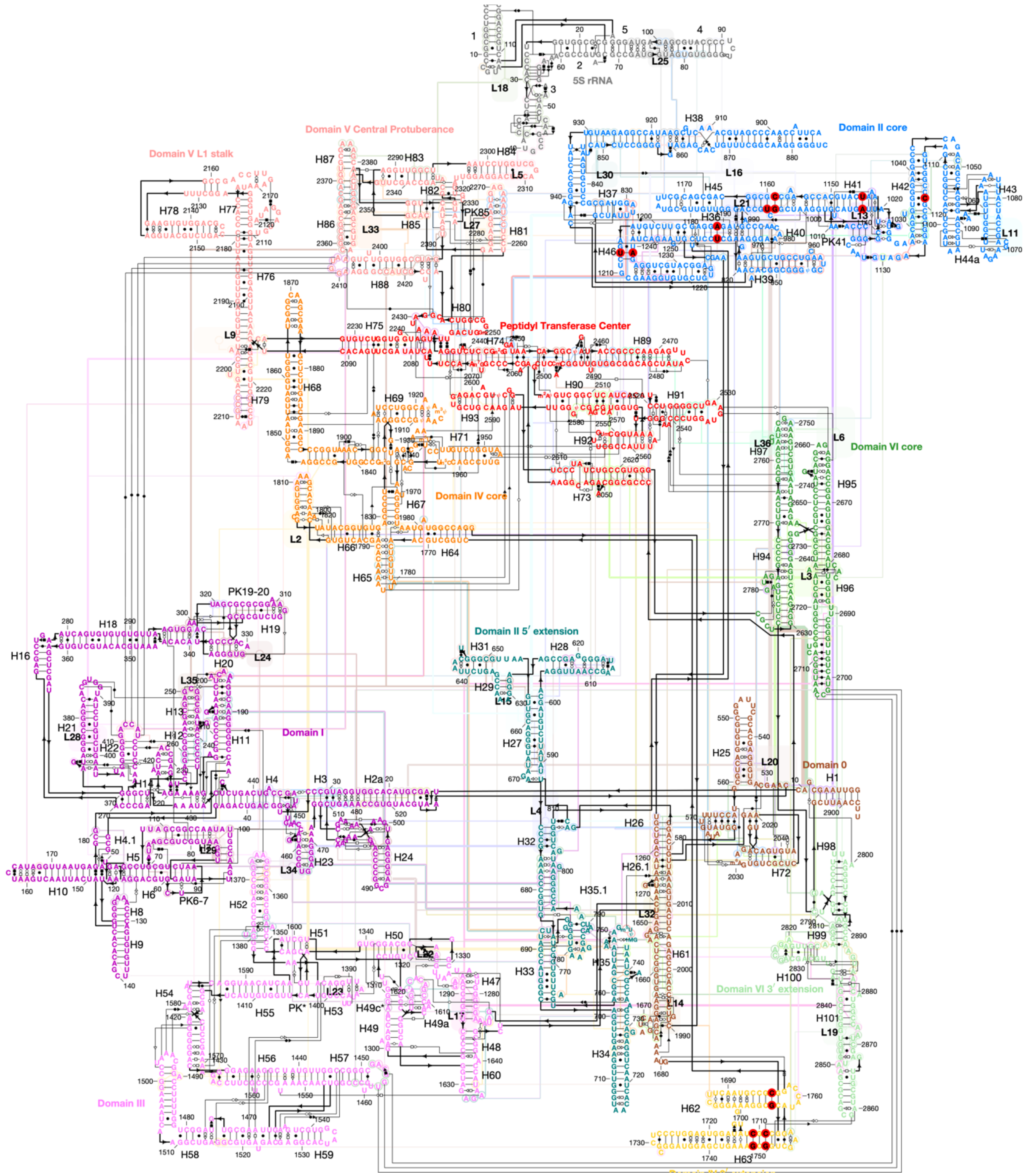
Supplementary Figure 67. Eterna participants'-designed ribosomal RNA design R1-33 prepared with RiboDraw[1].



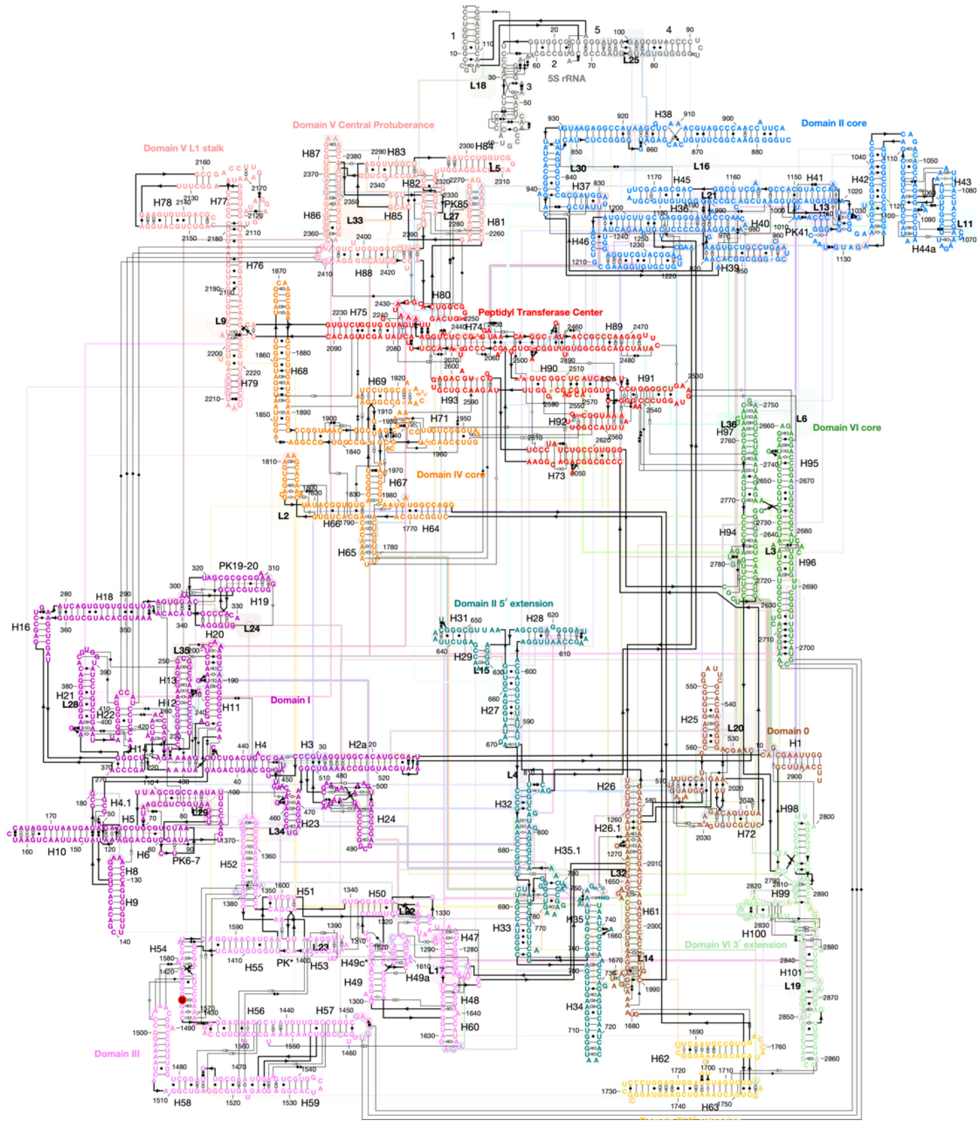
Supplementary Figure 68. Eterna participants'-designed ribosomal RNA design R1-34 prepared with RiboDraw[1].



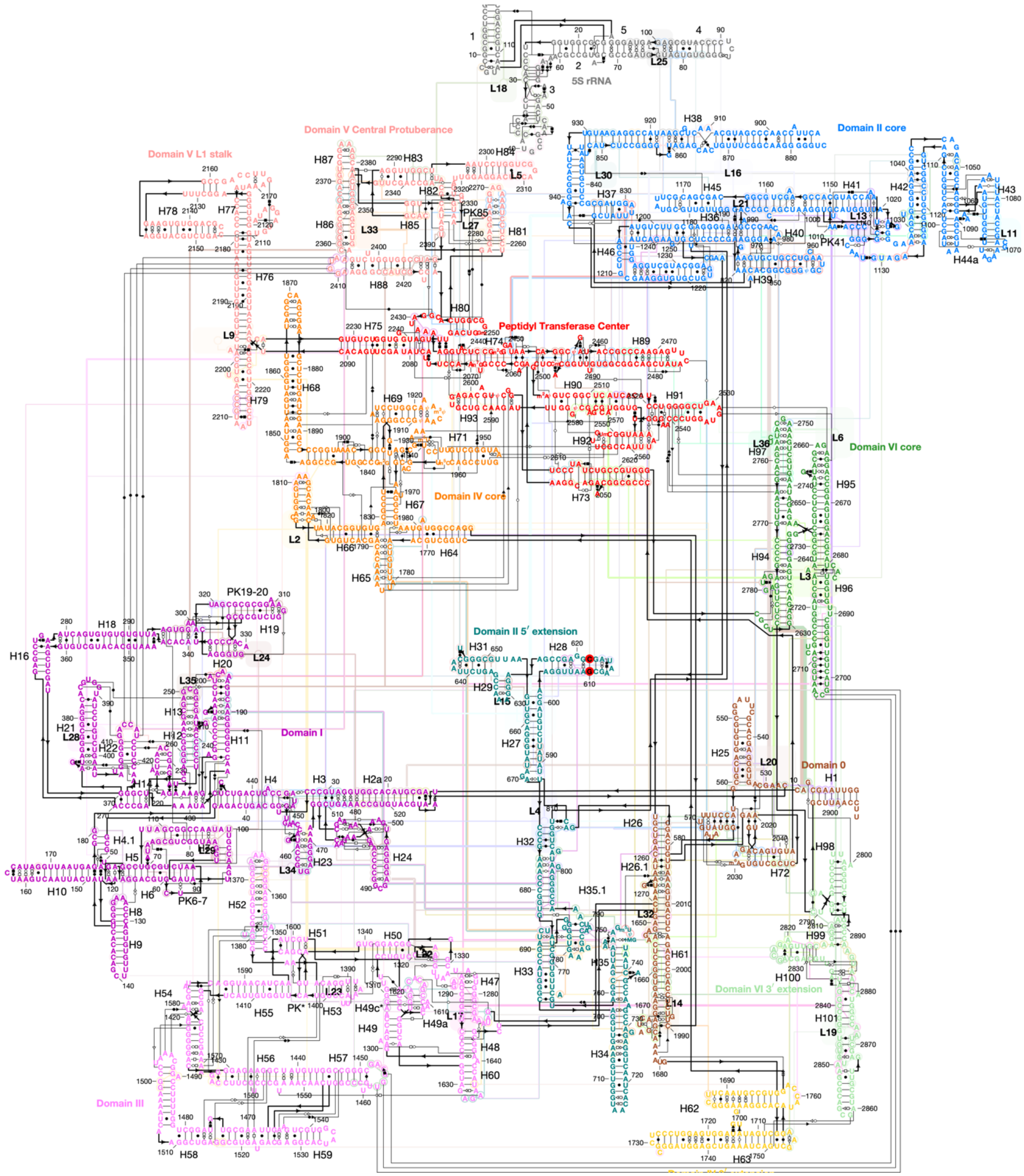
Supplementary Figure 69. Eterna participants'-designed ribosomal RNA design R1-35 prepared with RiboDraw[1].



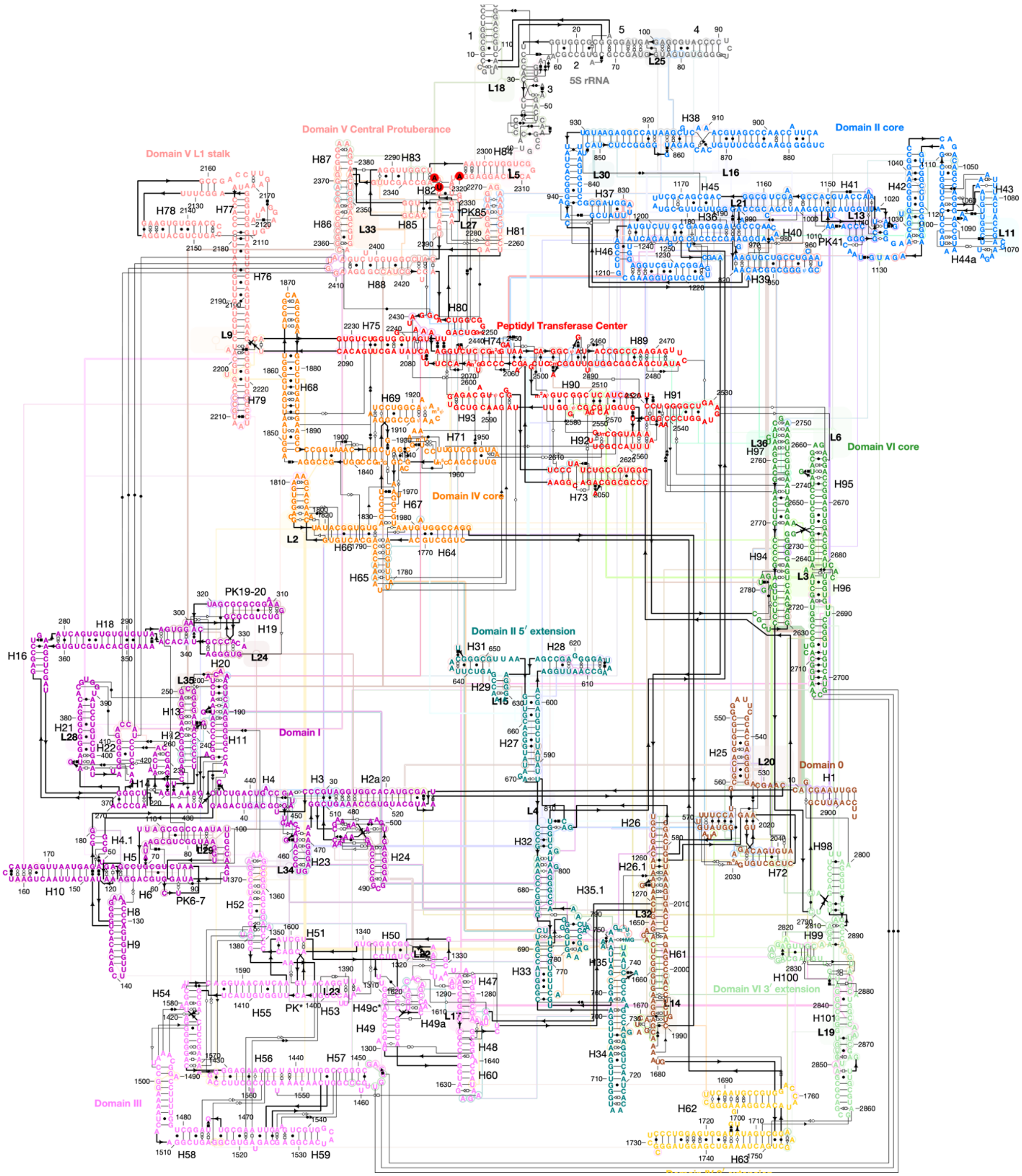
Supplementary Figure 70. Eterna participants'-designed ribosomal RNA design R1-36 prepared with RiboDraw[1]**.**



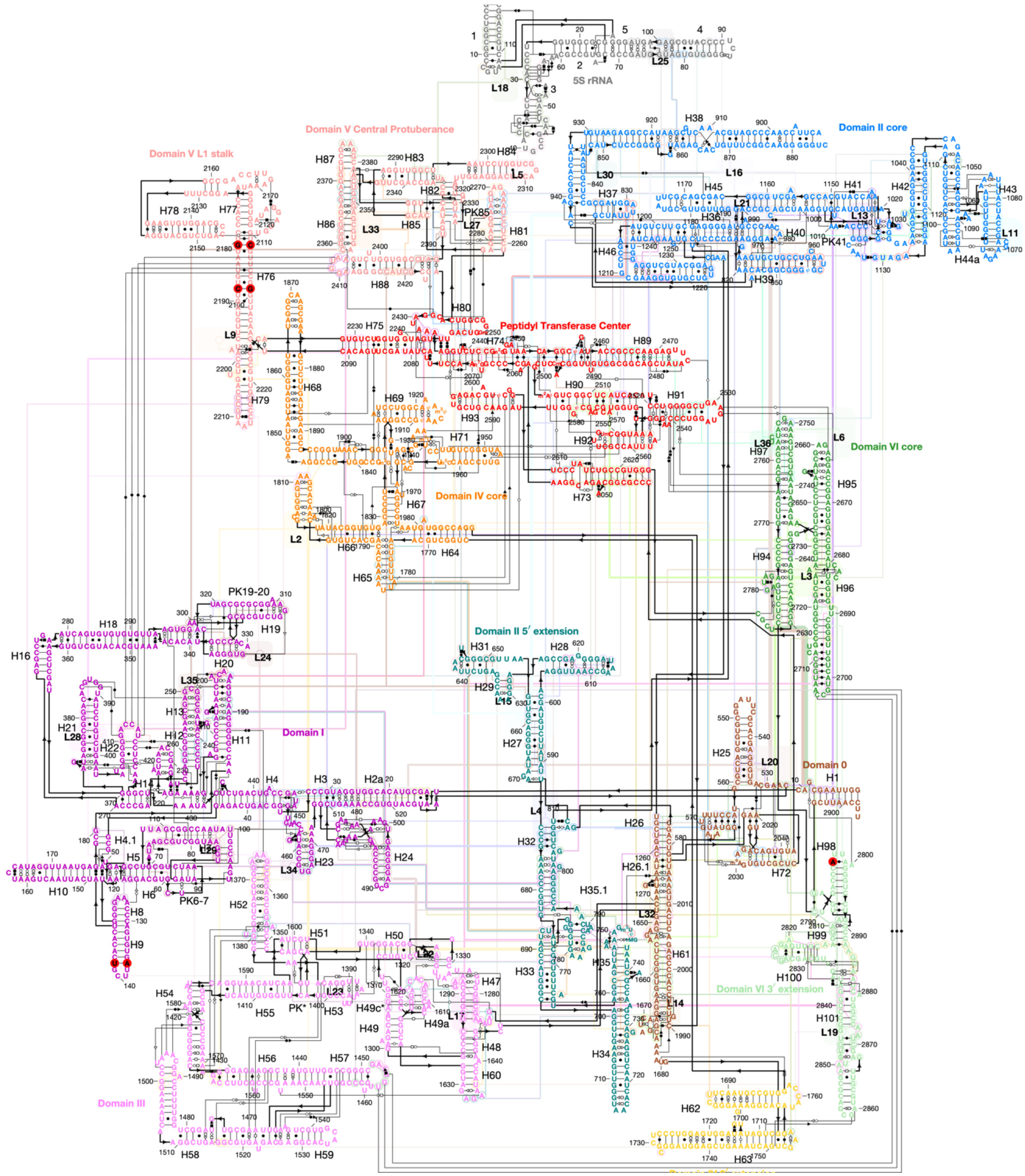
Supplementary Figure 71. Eterna participants'-designed ribosomal RNA design R1-37 prepared with RiboDraw[1].



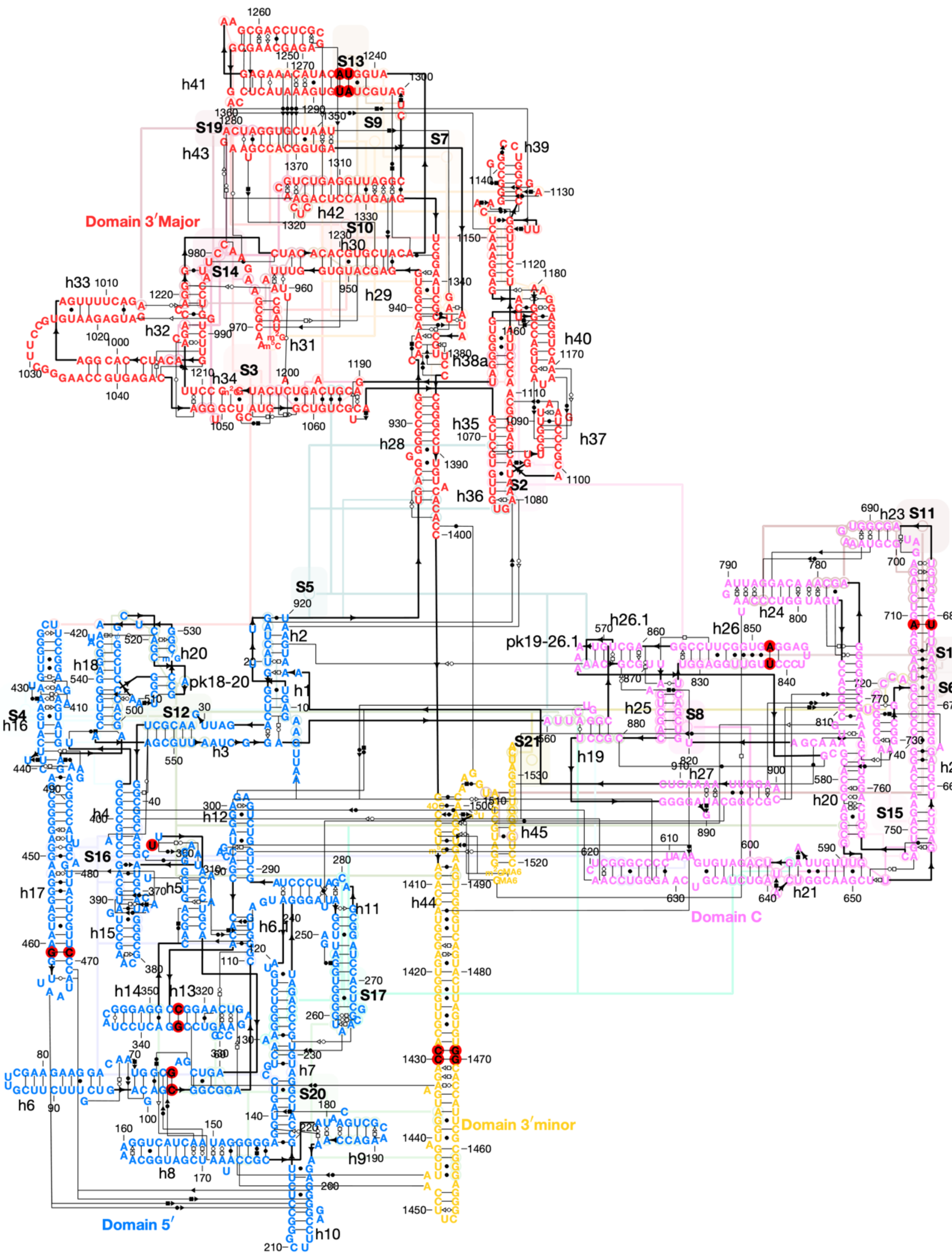
Supplementary Figure 72. Eterna participants'-designed ribosomal RNA design R1-38 prepared with RiboDraw[1].



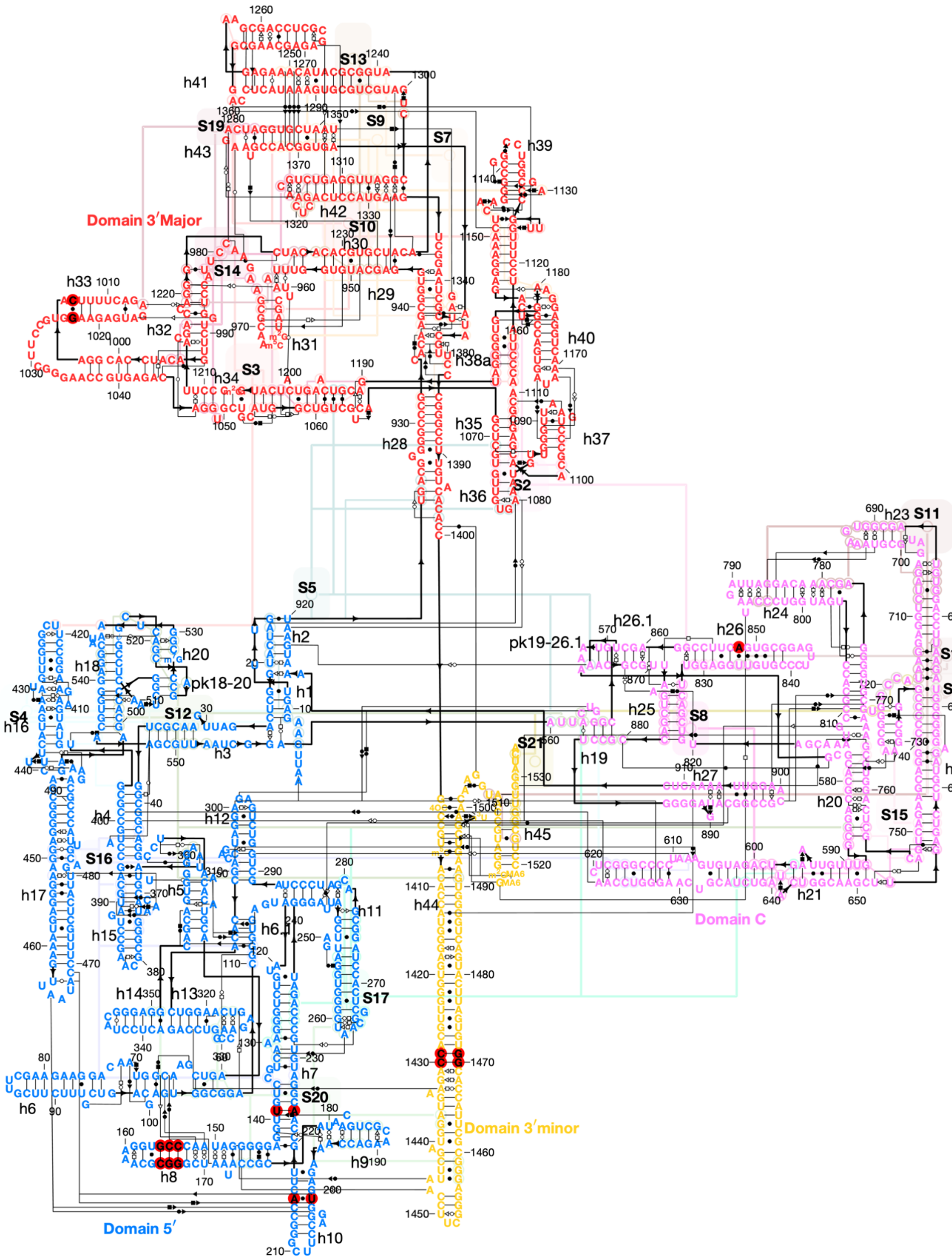
Supplementary Figure 73. Eterna participants'-designed ribosomal RNA design R1-39 prepared with RiboDraw[1].



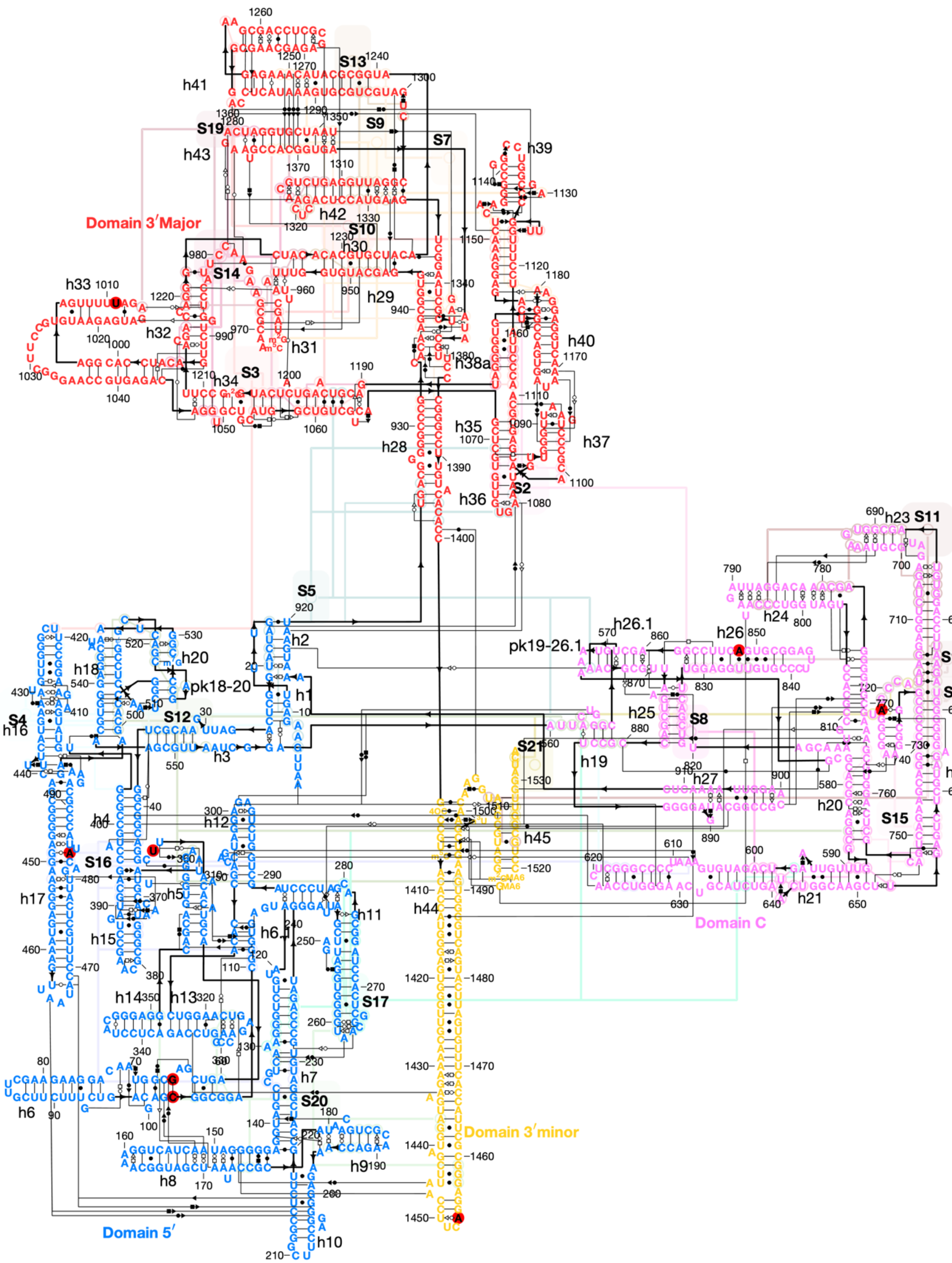
Supplementary Figure 74. Eterna participants'-designed ribosomal RNA design R1-40 prepared with RiboDraw[1]**.**



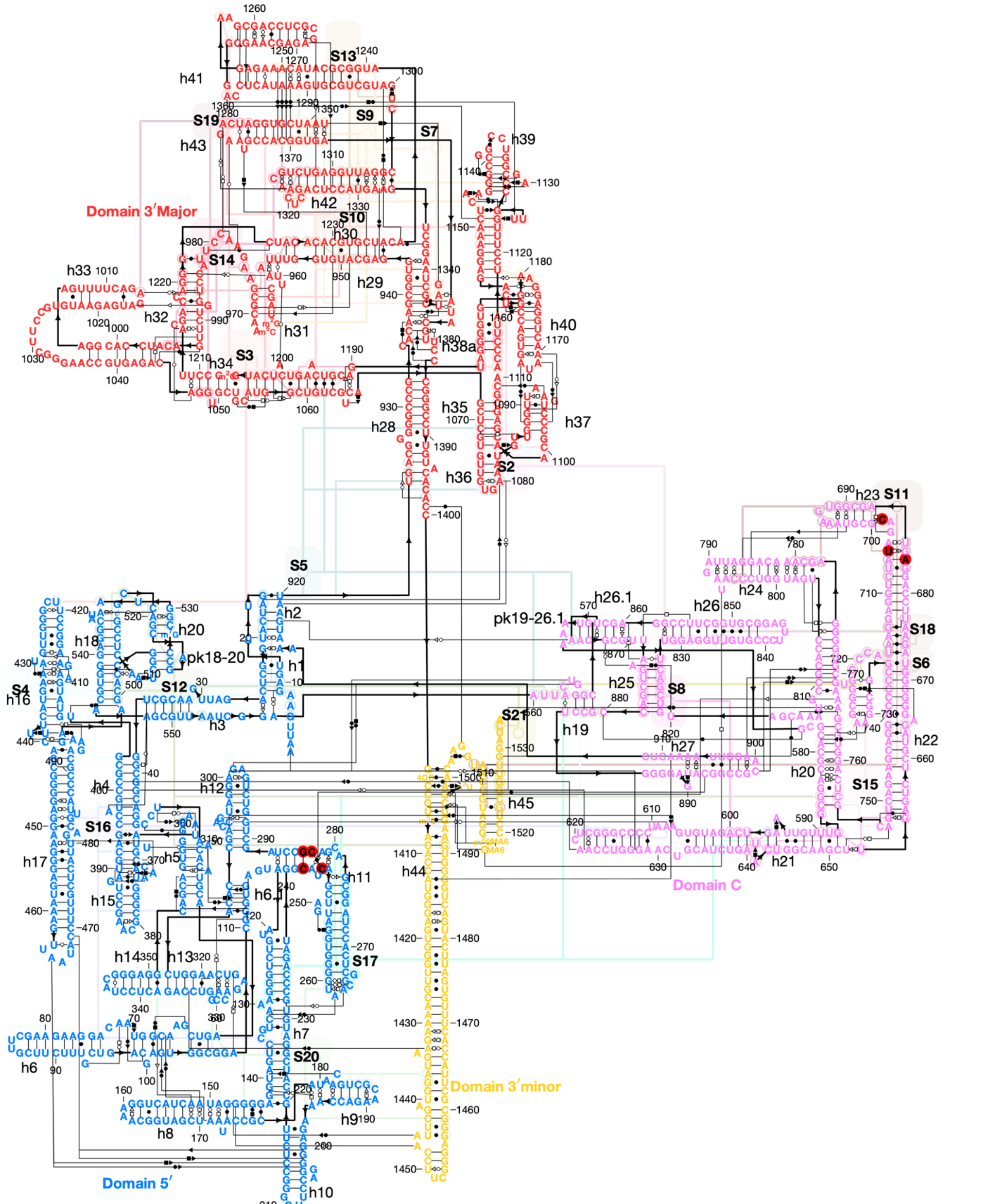
Supplementary Figure 75. Eterna participants'-designed ribosomal RNA design R2-01 prepared with RiboDraw[1].



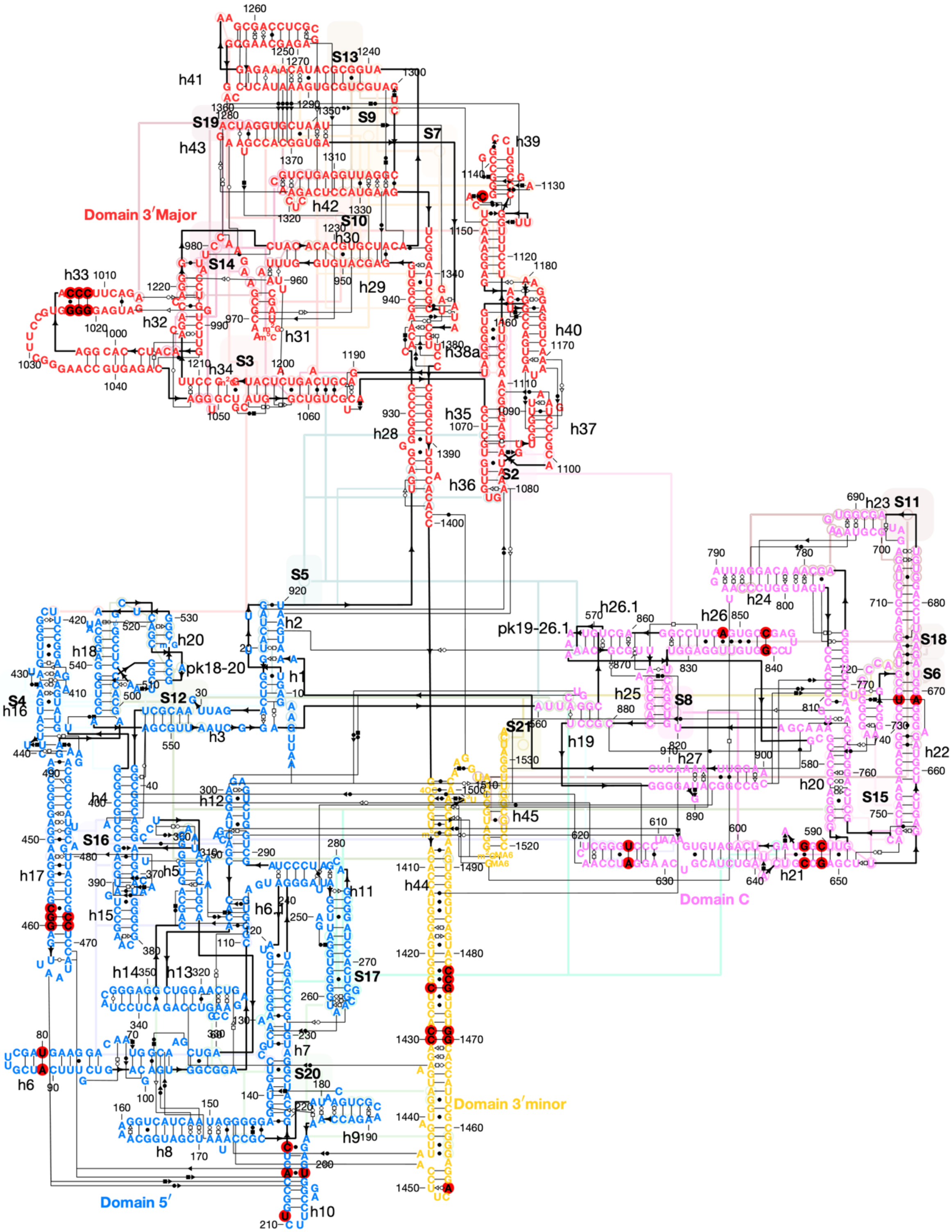
Supplementary Figure 76. Eterna participants'-designed ribosomal RNA design R2-02 prepared with RiboDraw[1].



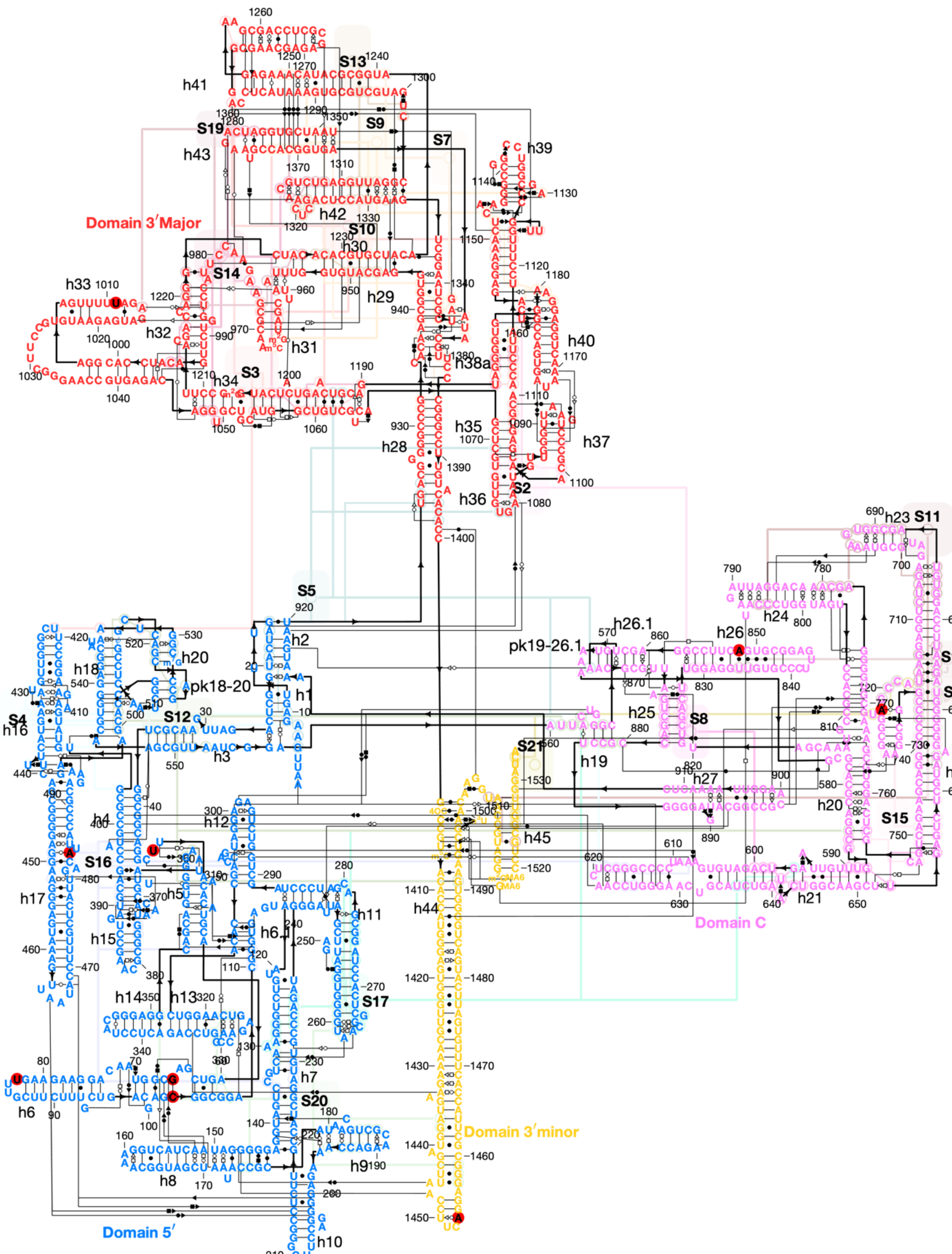
Supplementary Figure 77. Eterna participants'-designed ribosomal RNA design R2-03 prepared with RiboDraw[1].



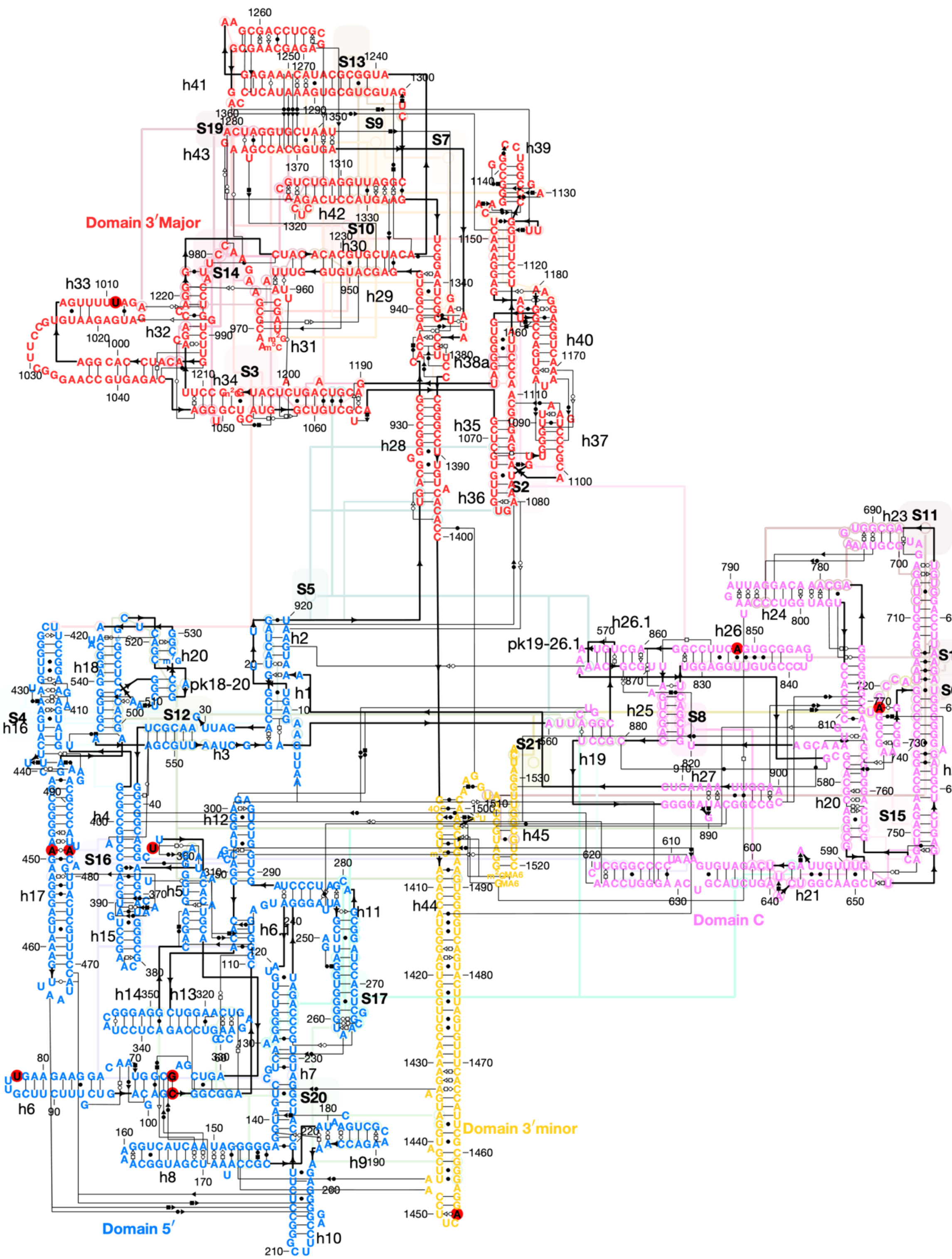
Supplementary Figure 78. Eterna participants'-designed ribosomal RNA design R2-04 prepared with RiboDraw[1].



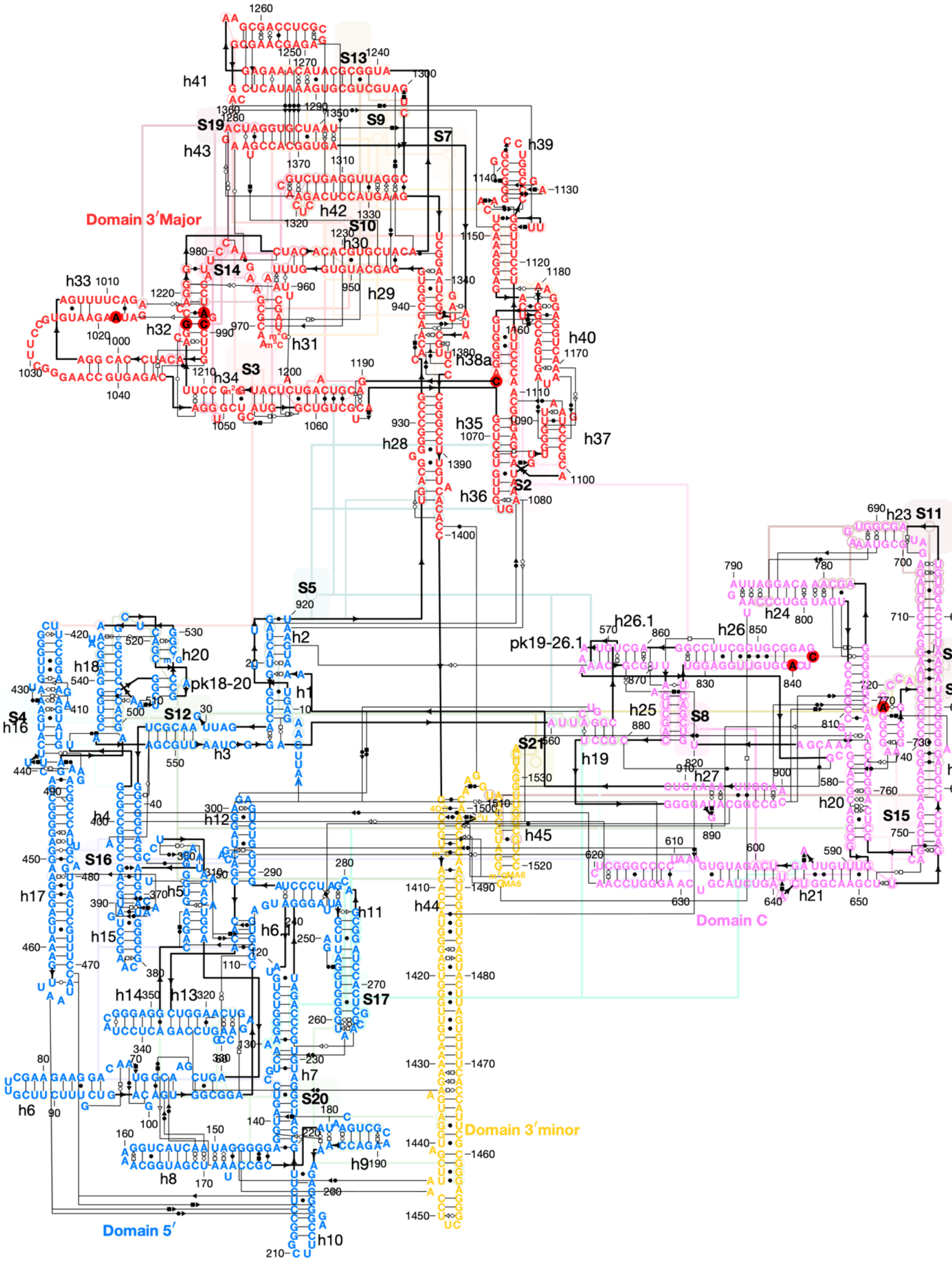
Supplementary Figure 79. Eterna participants'-designed ribosomal RNA design R2-05 prepared with RiboDraw[1].



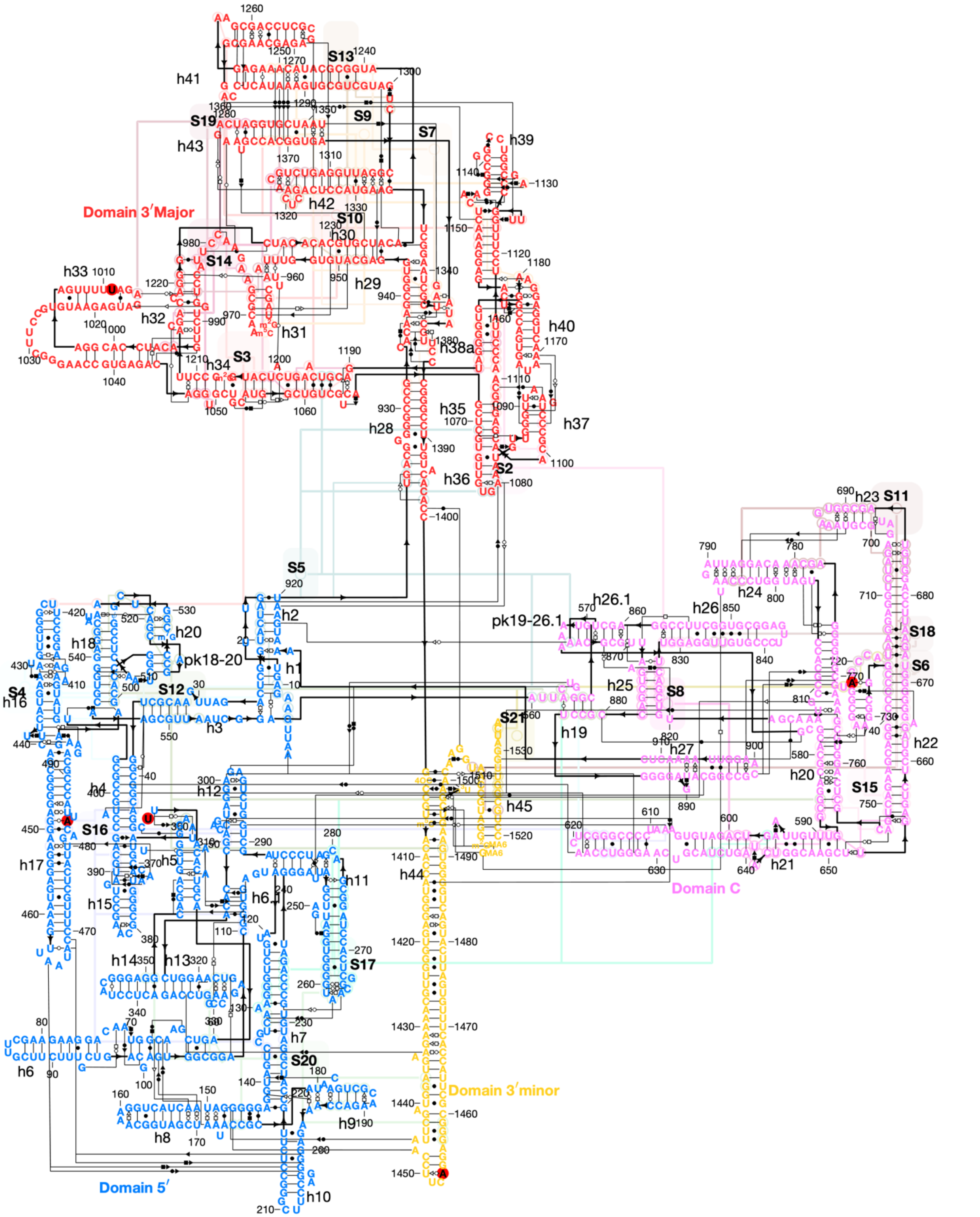
Supplementary Figure 80. Eterna participants'-designed ribosomal RNA design R2-06 prepared with RiboDraw[1].



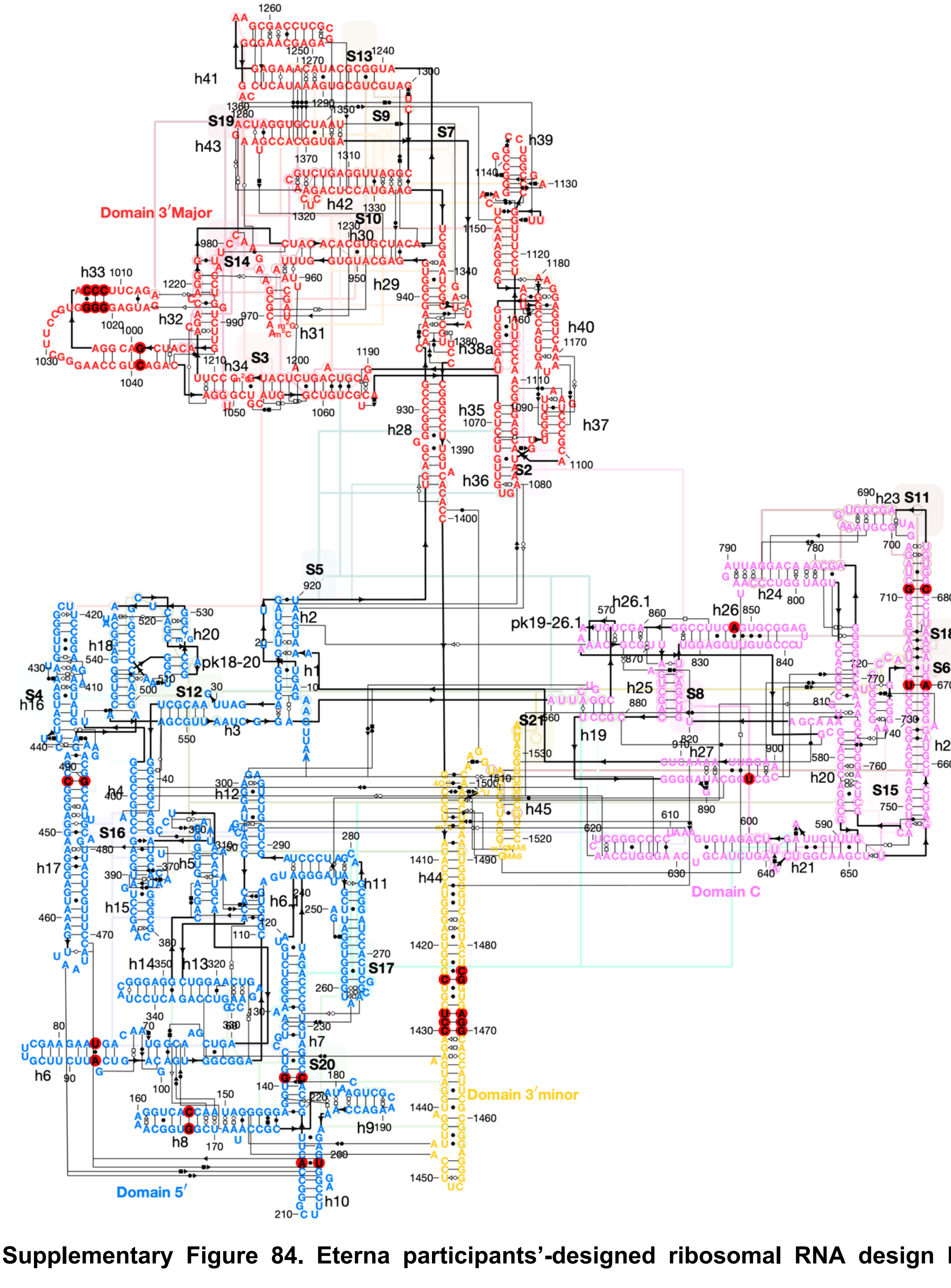
Supplementary Figure 81. Eterna participants'-designed ribosomal RNA design R2-07 prepared with RiboDraw[1].



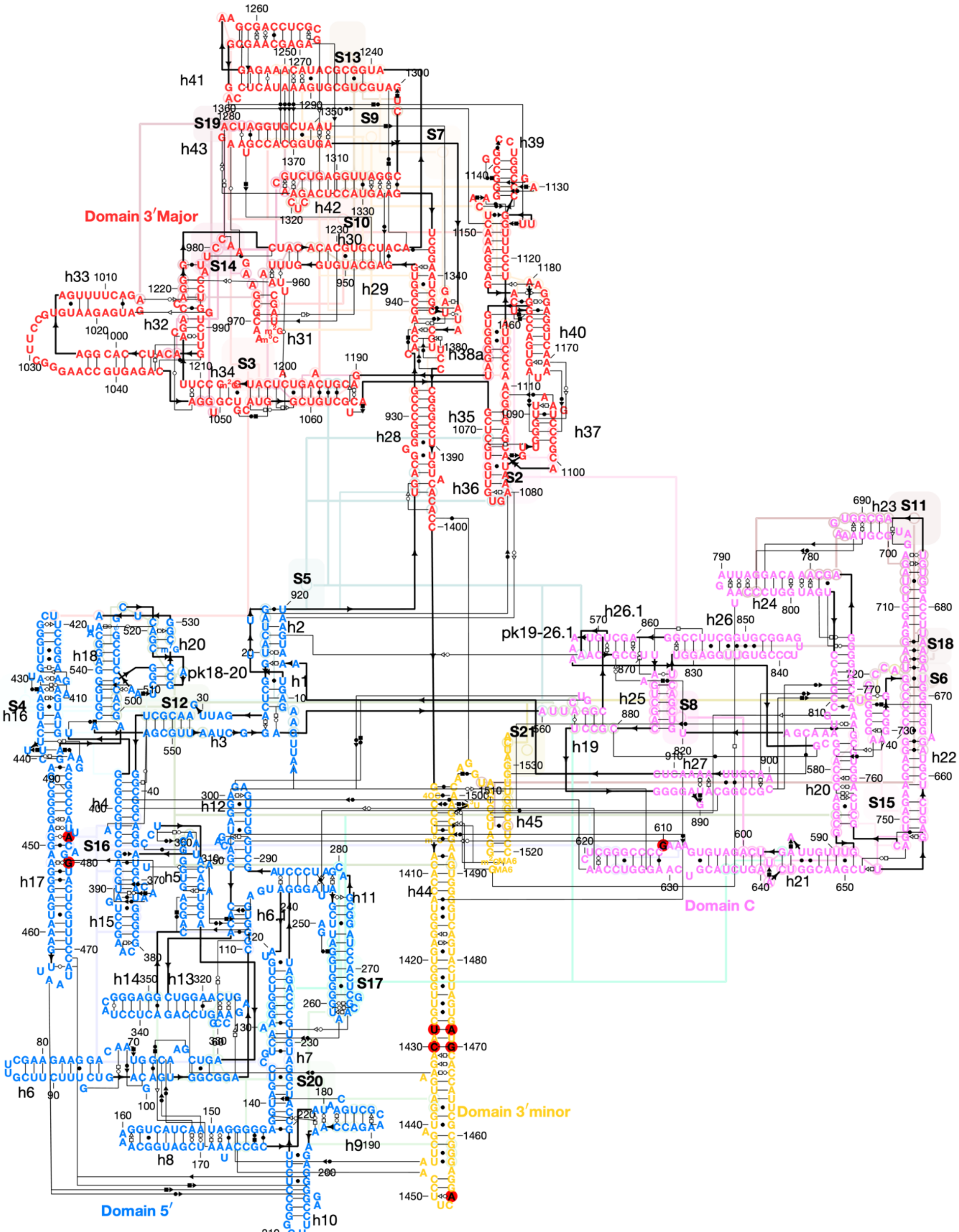
Supplementary Figure 82. Eterna participants'-designed ribosomal RNA design R2-08 prepared with RiboDraw[1].



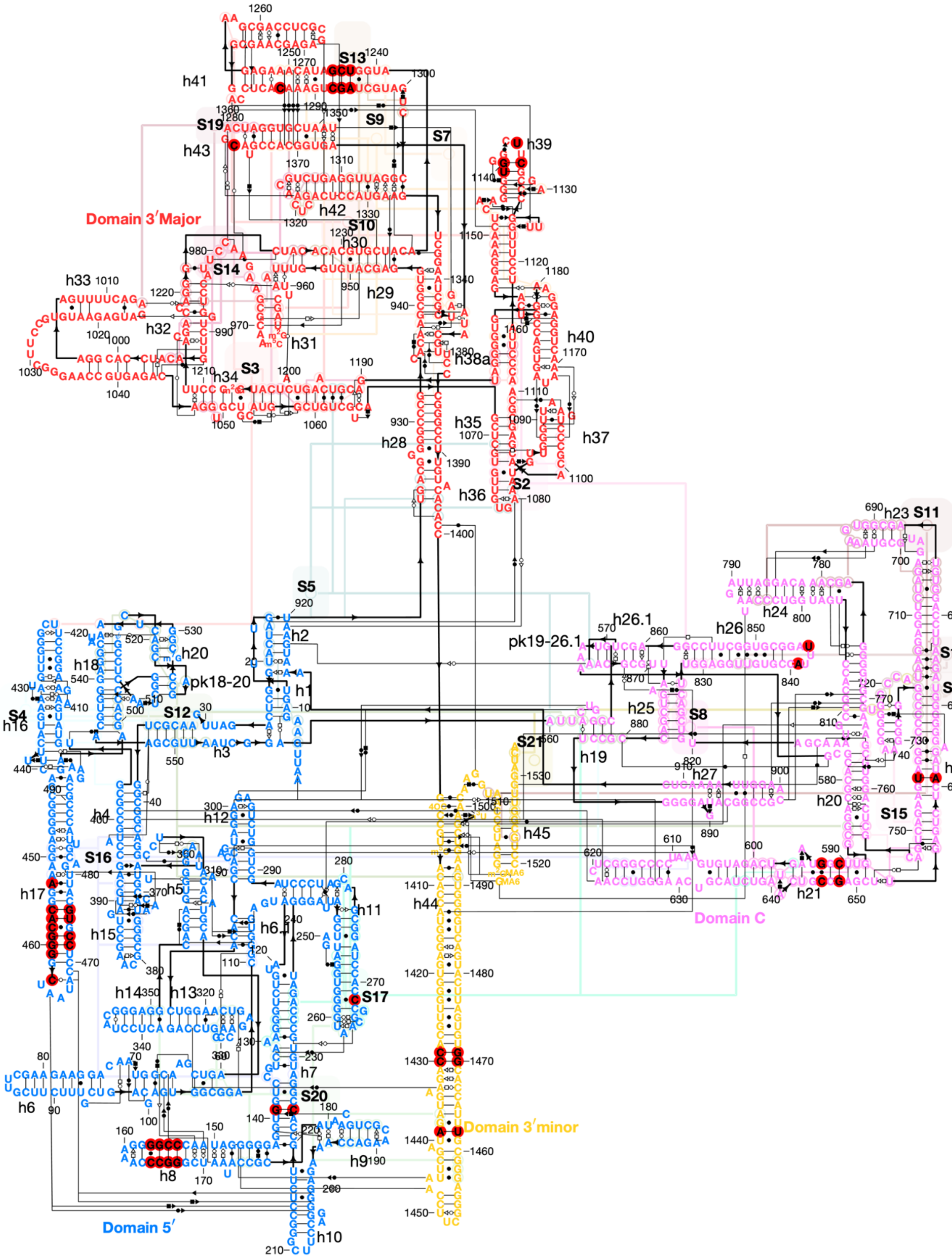
Supplementary Figure 83. Eterna participants'-designed ribosomal RNA design R2-09 prepared with RiboDraw[1].



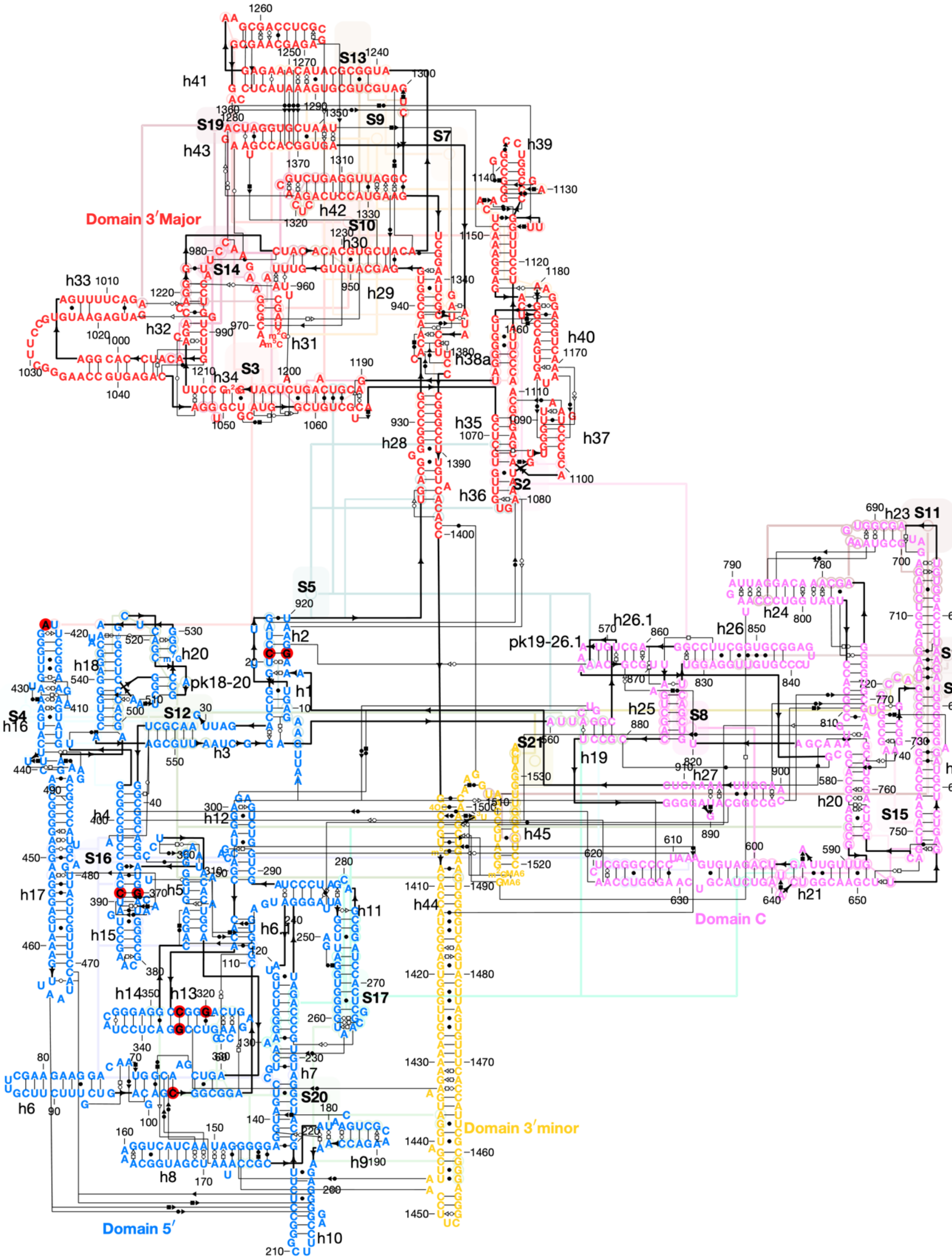
Supplementary Figure 84. Eterna participants'-designed ribosomal RNA design R2-10 prepared with RiboDraw[1].



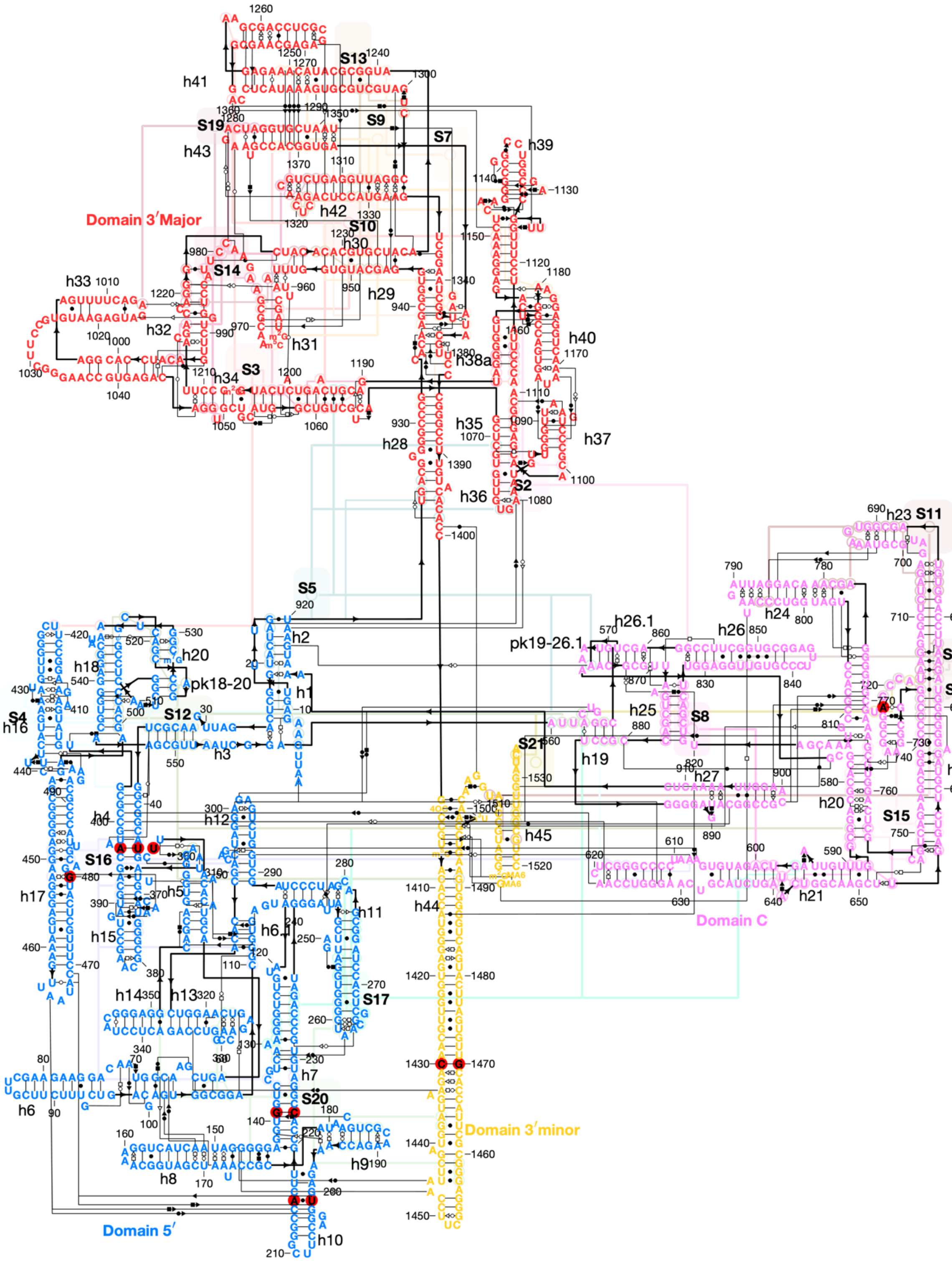
Supplementary Figure 85. Eterna participants'-designed ribosomal RNA design R2-11 prepared with RiboDraw[1].



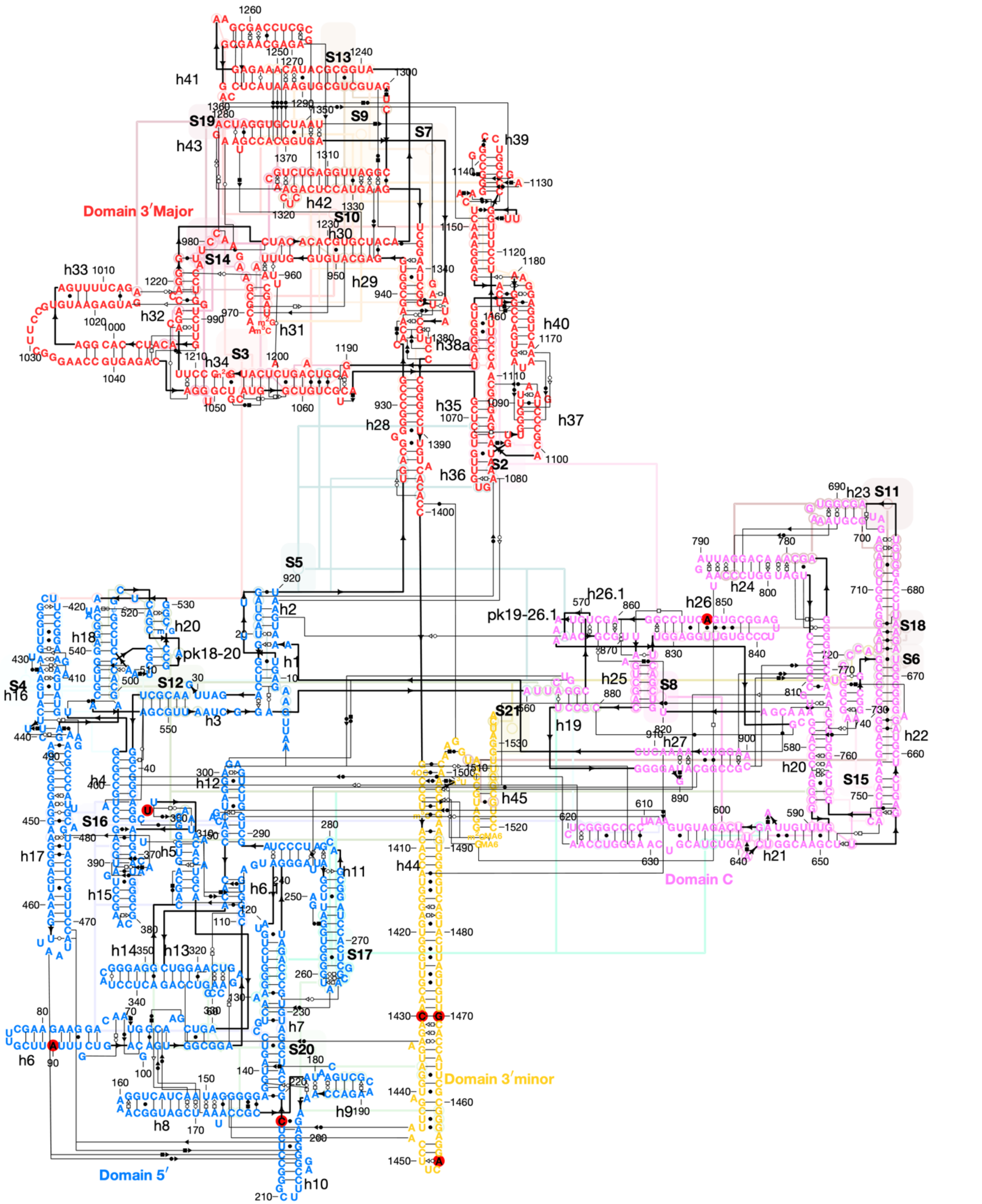
Supplementary Figure 86. Eterna participants'-designed ribosomal RNA design R2-12 prepared with RiboDraw[1].



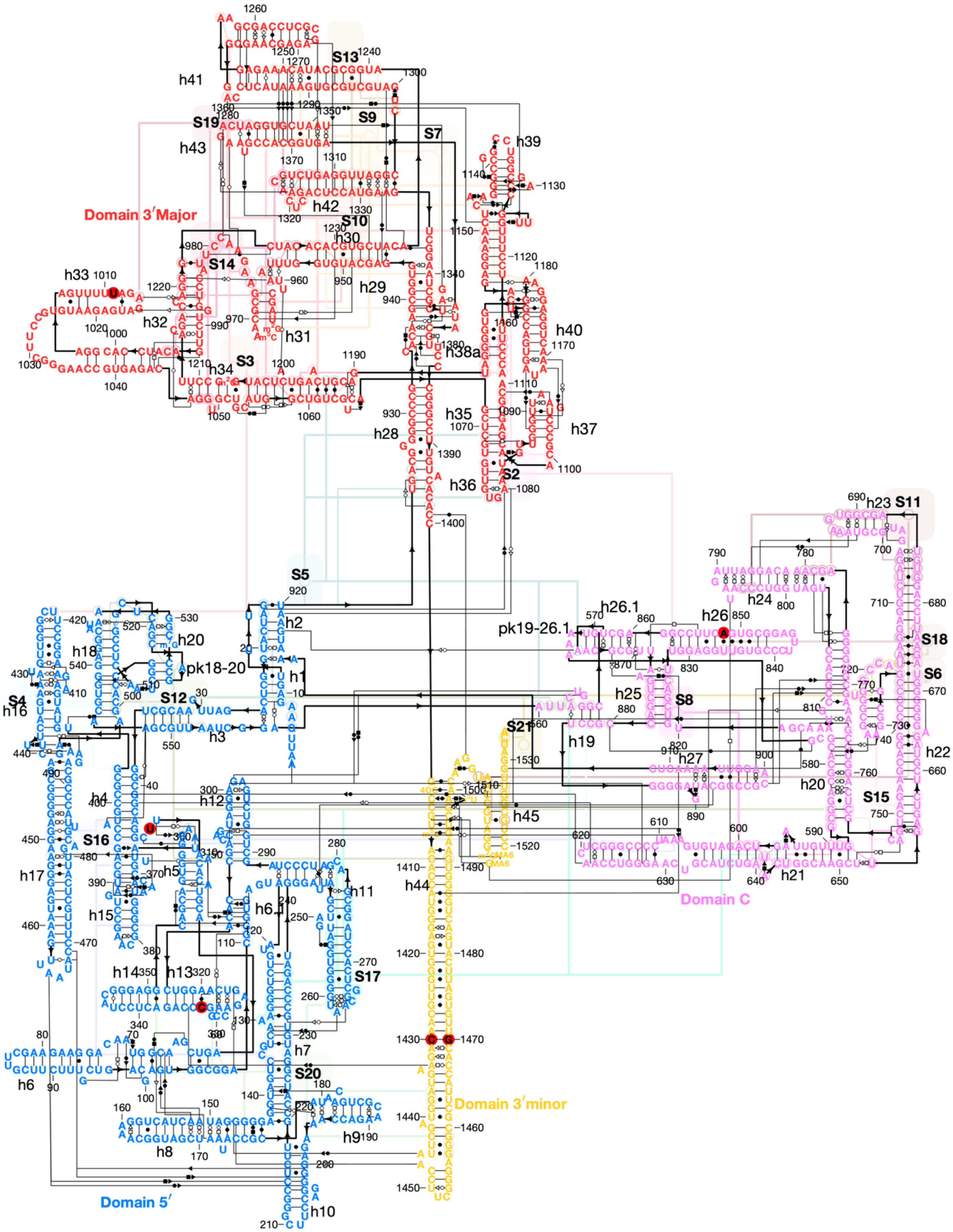
Supplementary Figure 87. Eterna participants'-designed ribosomal RNA design R2-13 prepared with RiboDraw[1].



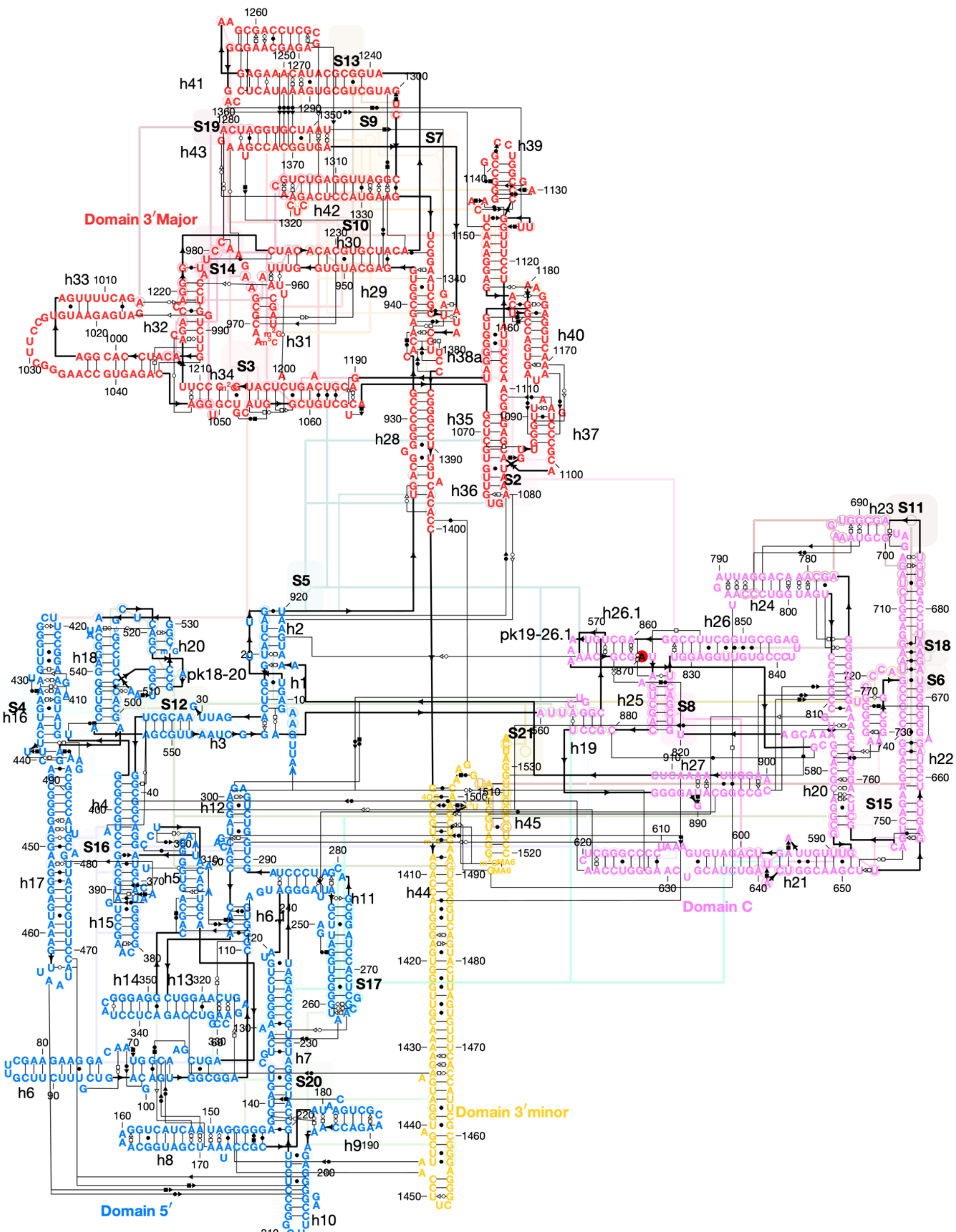
Supplementary Figure 88. Eterna participants'-designed ribosomal RNA design R2-14 prepared with RiboDraw[1].



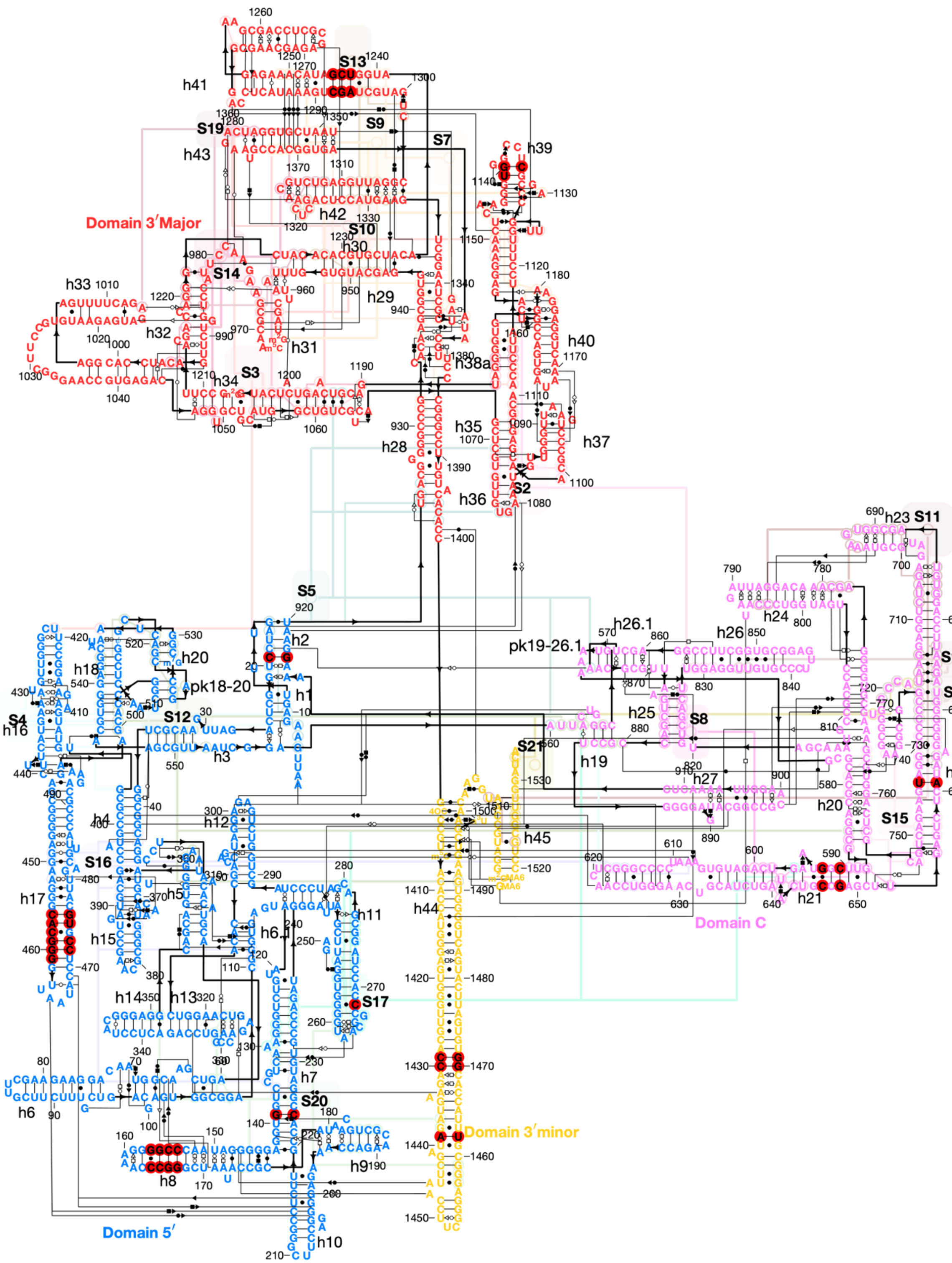
Supplementary Figure 89. Eterna participants'-designed ribosomal RNA design R2-15 prepared with RiboDraw[1].



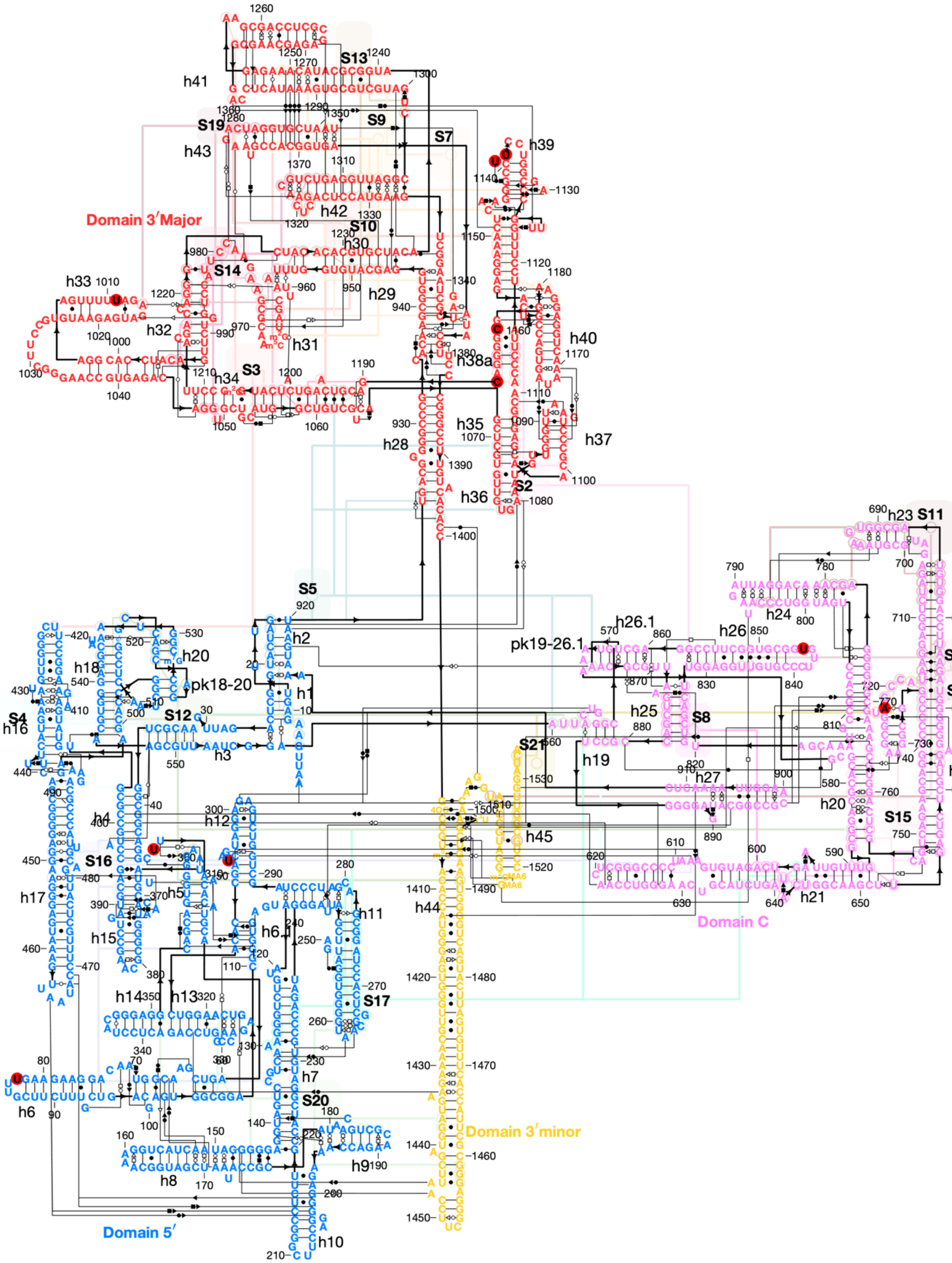
Supplementary Figure 90. Eterna participants'-designed ribosomal RNA design R2-16 prepared with RiboDraw[1].



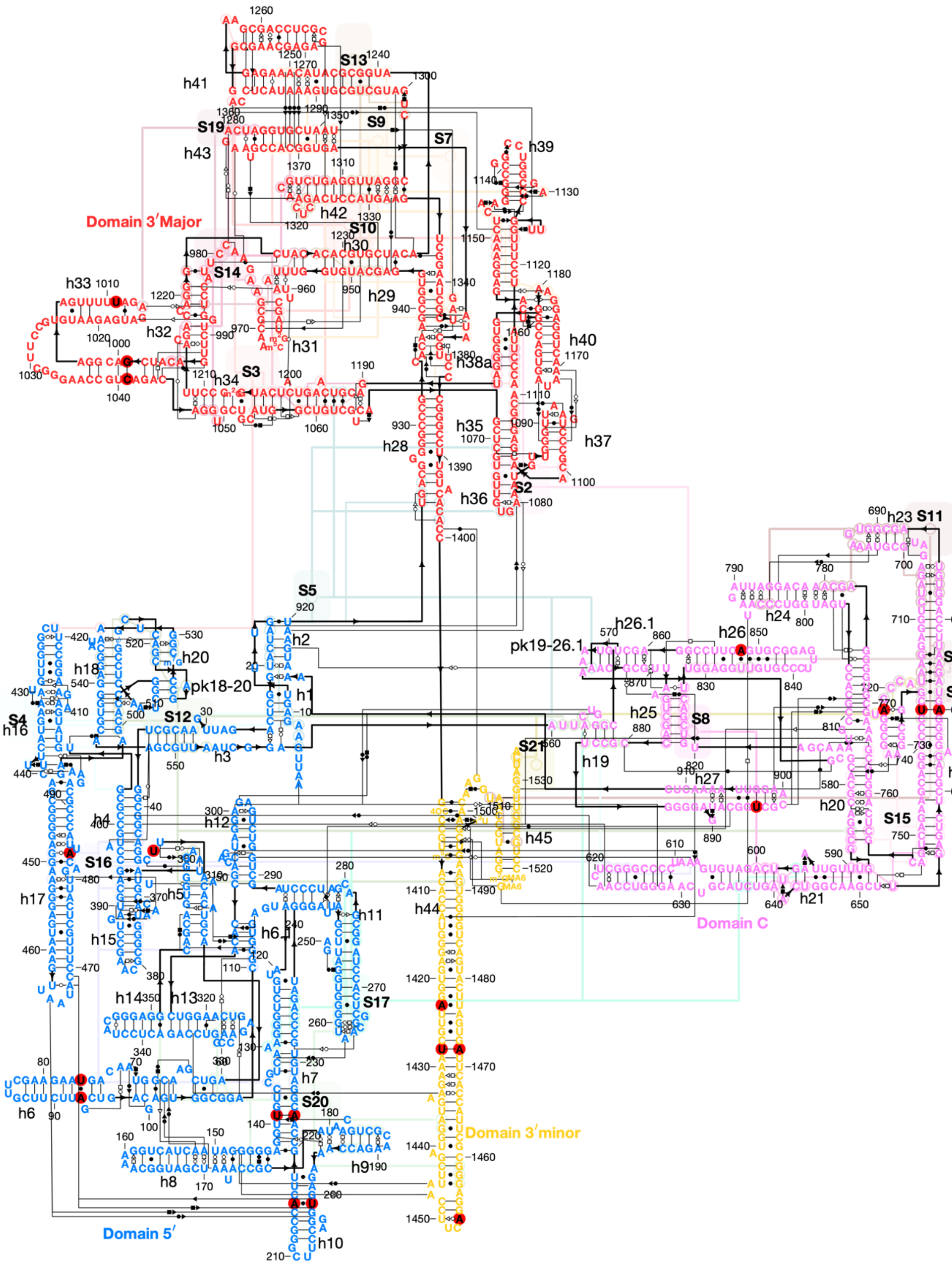
Supplementary Figure 91. Eterna participants'-designed ribosomal RNA design R2-17 prepared with RiboDraw[1].



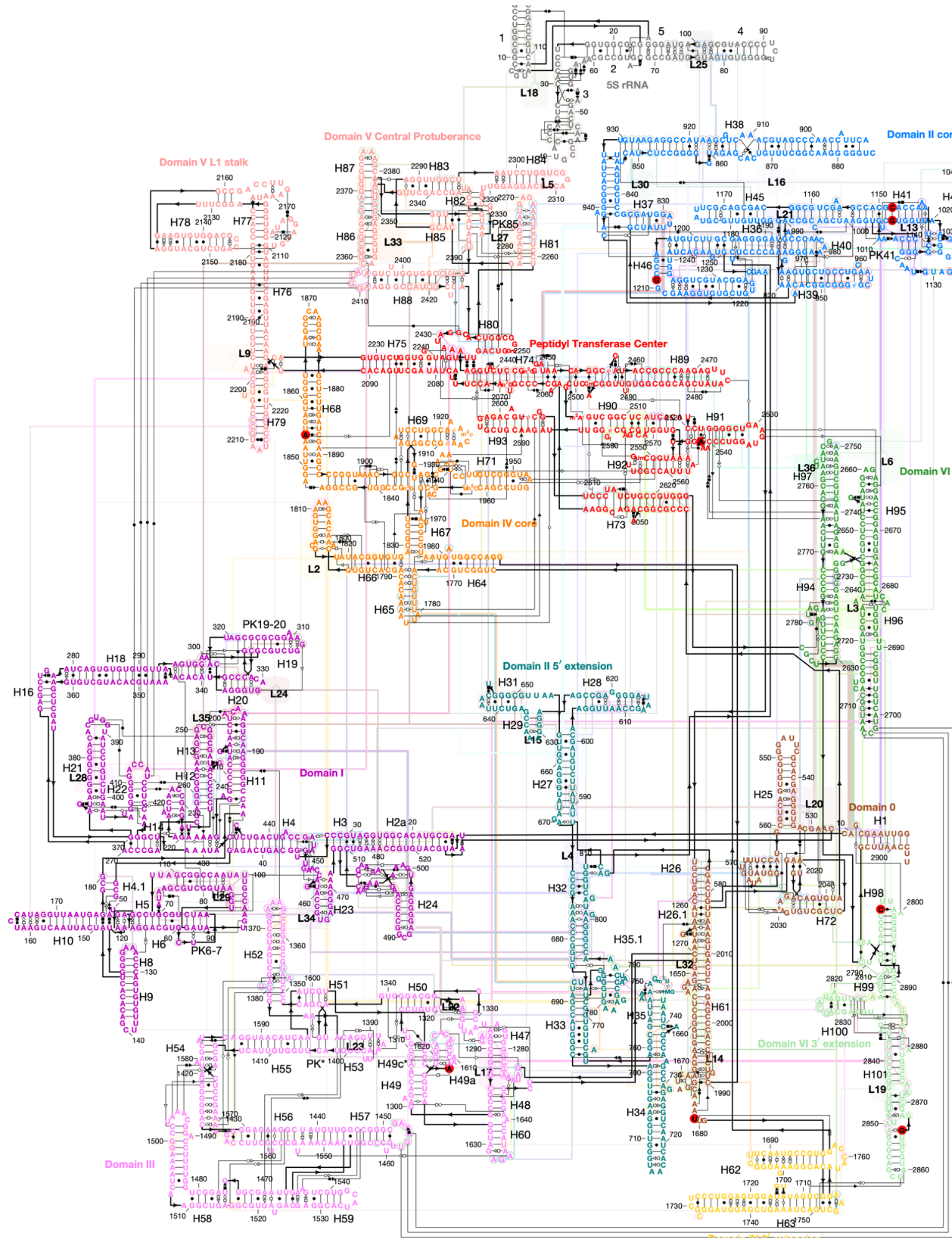
Supplementary Figure 92. Eterna participants'-designed ribosomal RNA design R2-18 prepared with RiboDraw[1].



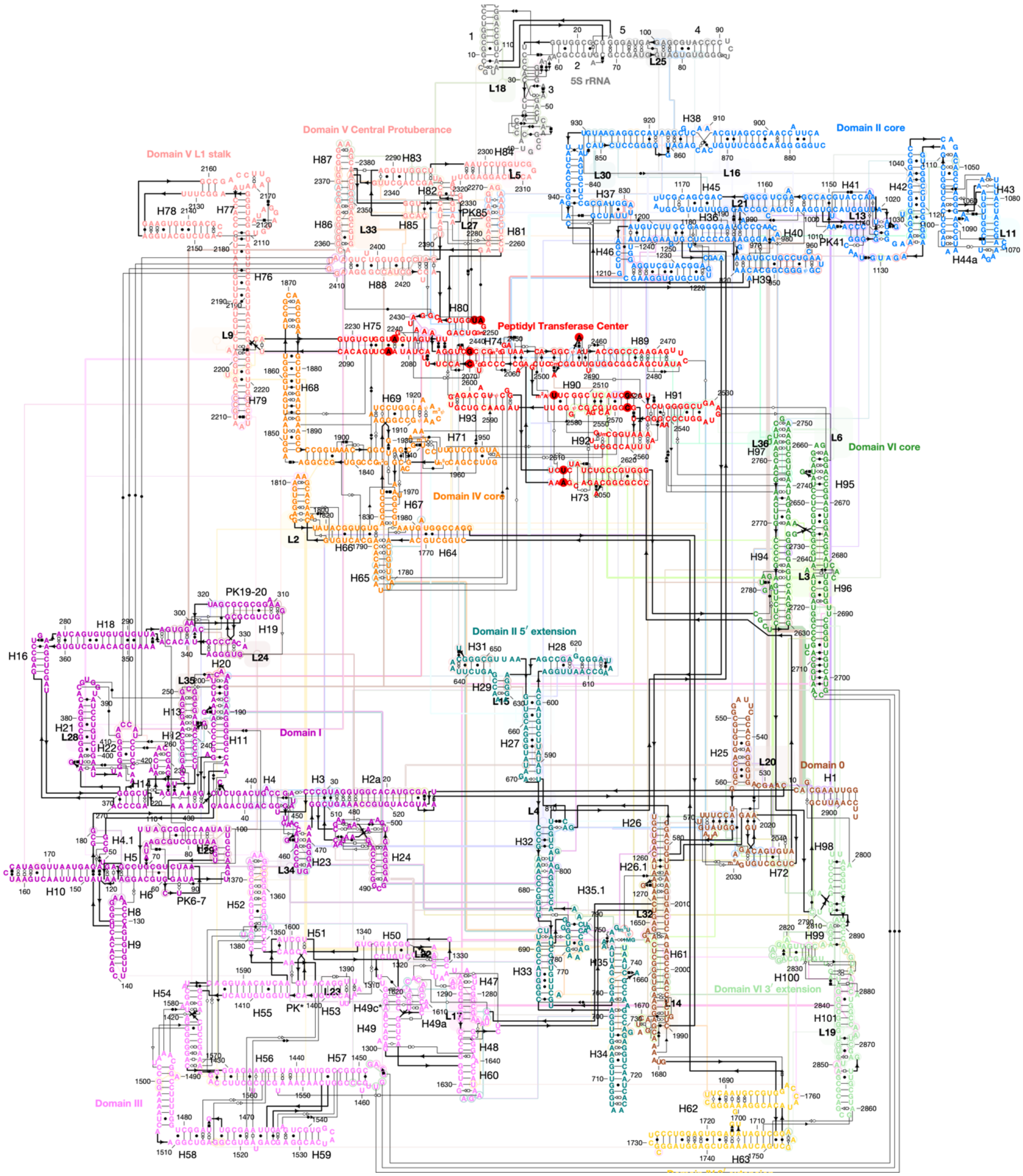
Supplementary Figure 93. Eterna participants'-designed ribosomal RNA design R2-19 prepared with RiboDraw[1].



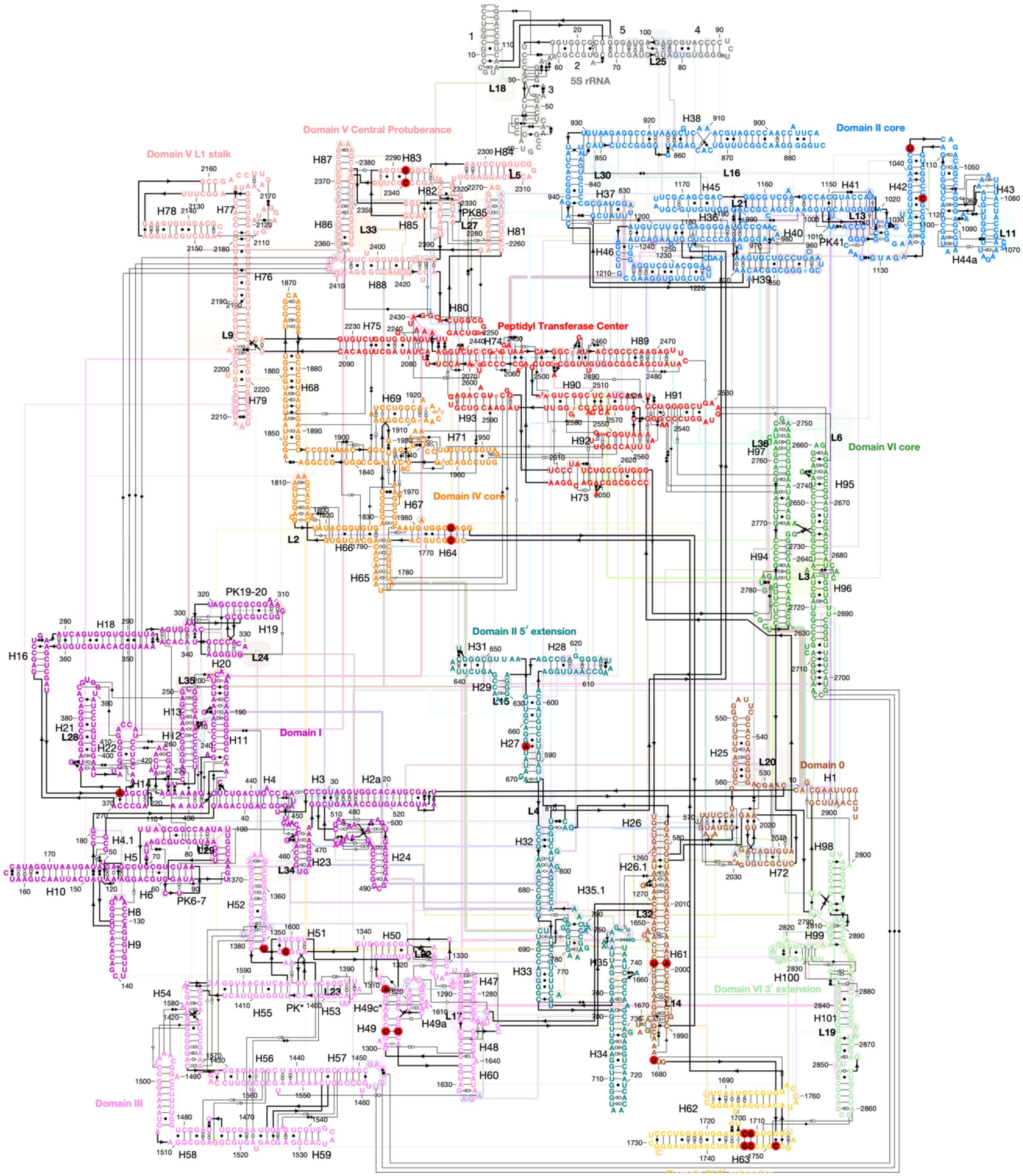
Supplementary Figure 94. Eterna participants'-designed ribosomal RNA design R2-20 prepared with RiboDraw[1].



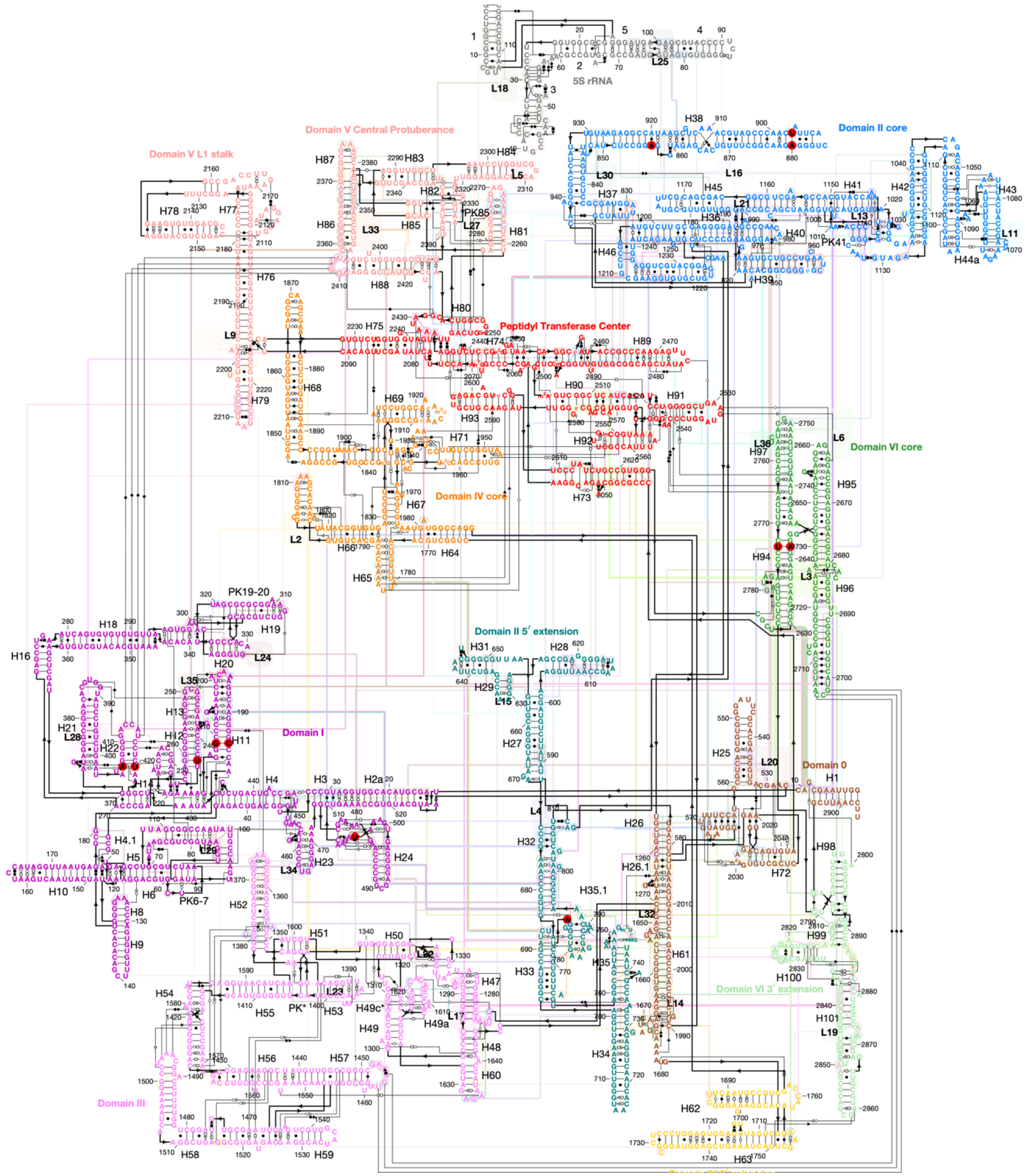
Supplementary Figure 95. Eterna participants'-designed ribosomal RNA design R2-21 prepared with RiboDraw[1].



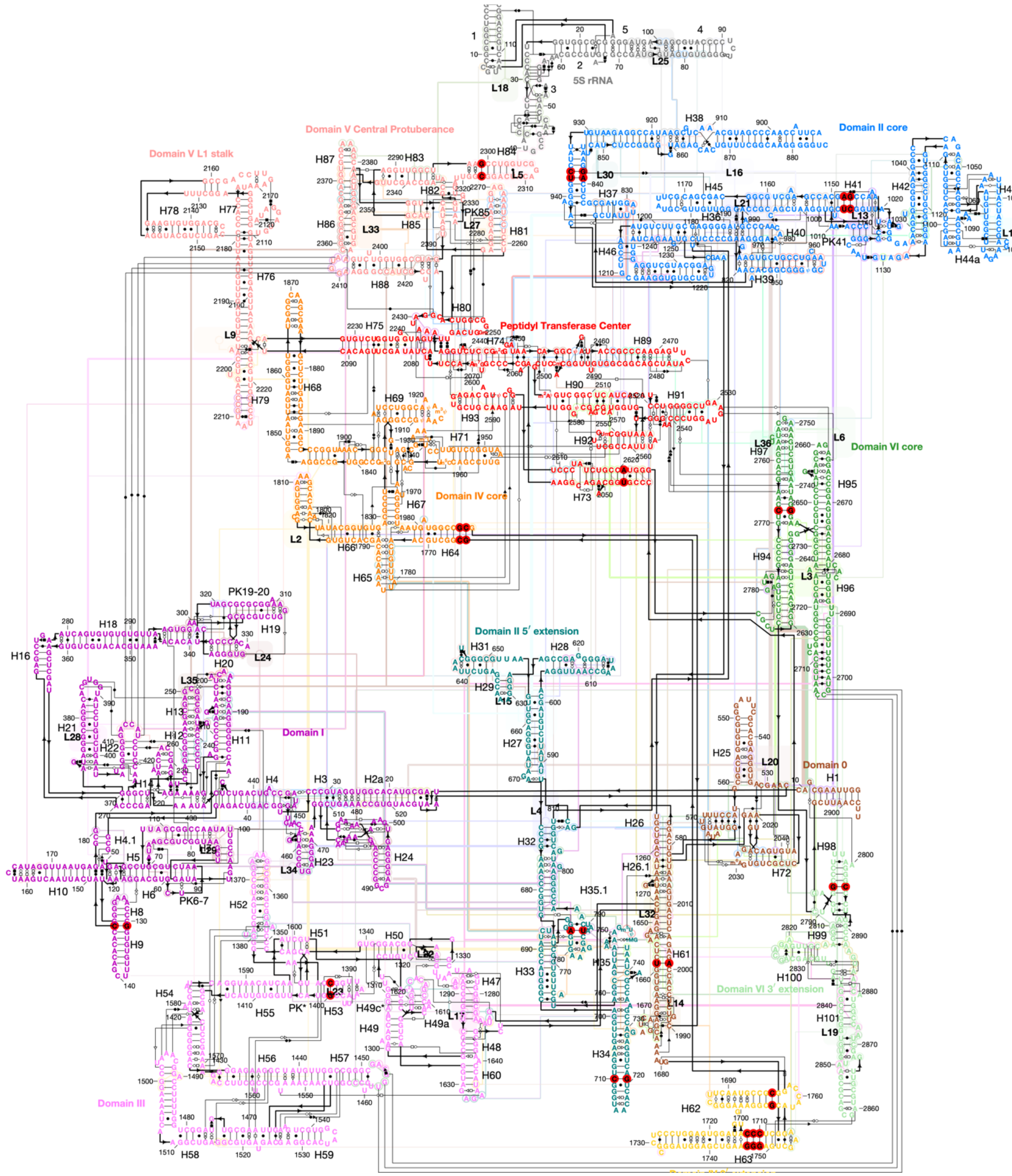
Supplementary Figure 96. Eterna participants'-designed ribosomal RNA design R2-22 prepared with RiboDraw[1].



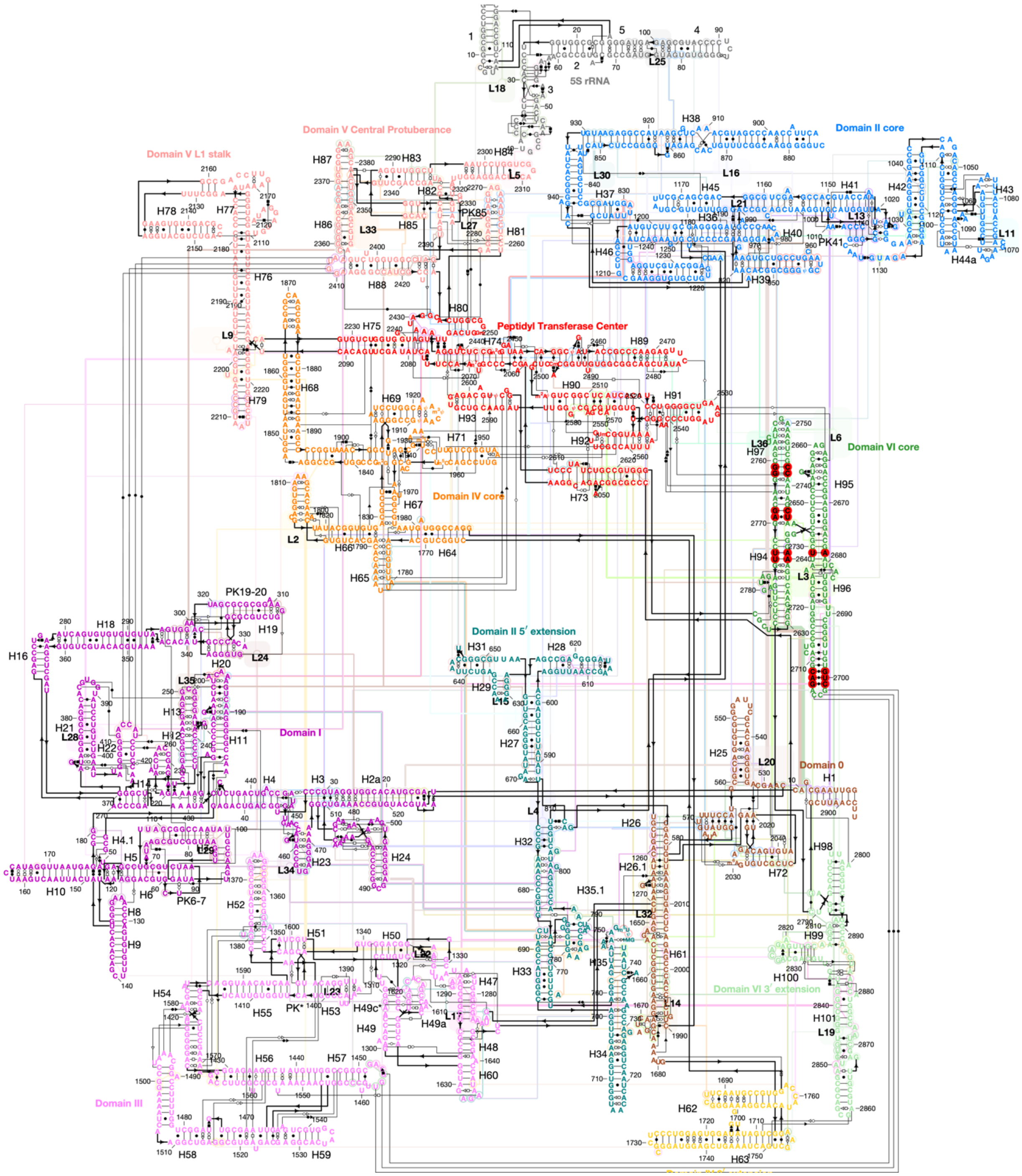
Supplementary Figure 97. Eterna participants'-designed ribosomal RNA design R2-23 prepared with RiboDraw[1].



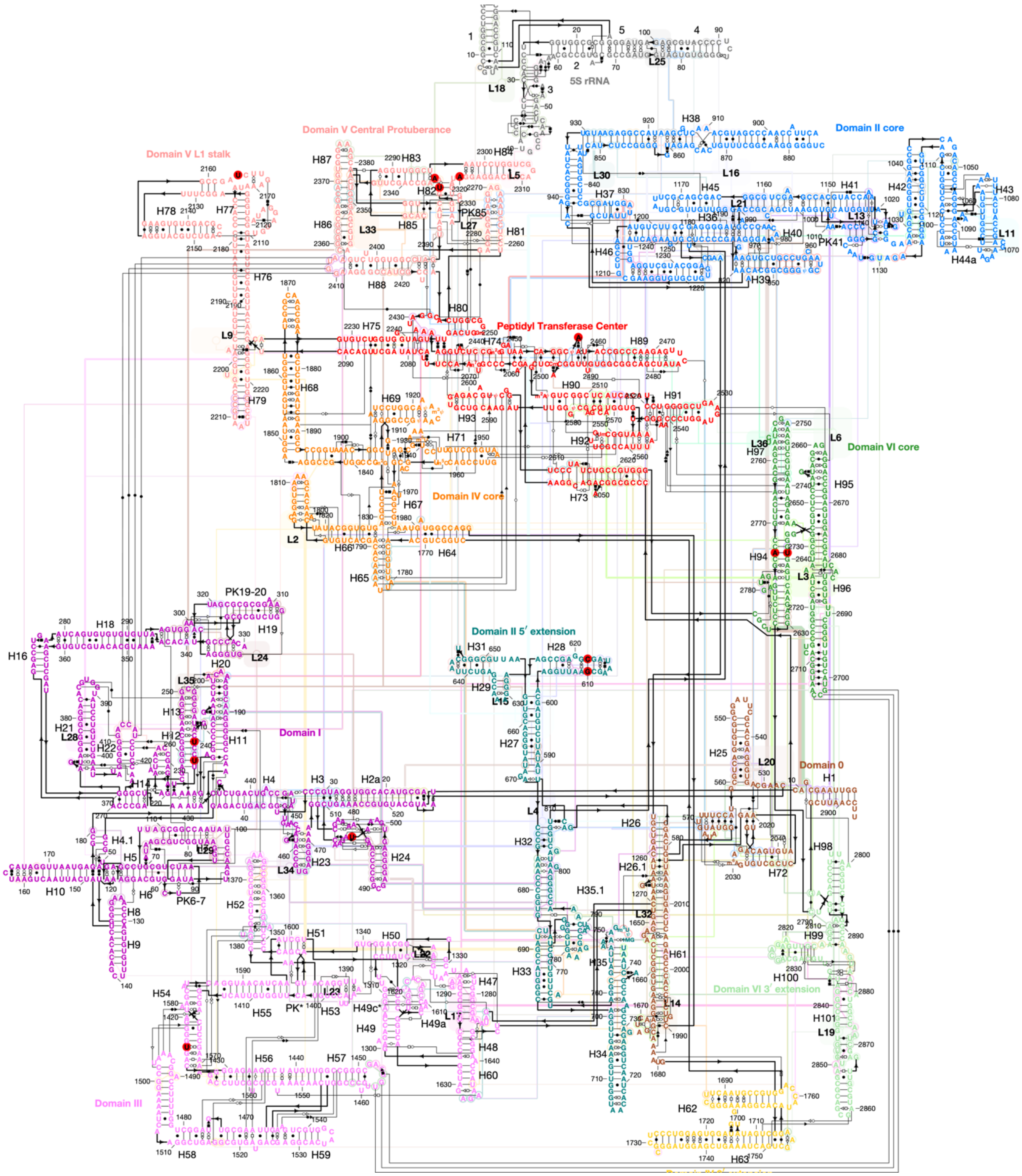
Supplementary Figure 98. Eterna participants'-designed ribosomal RNA design R2-24 prepared with RiboDraw[1].



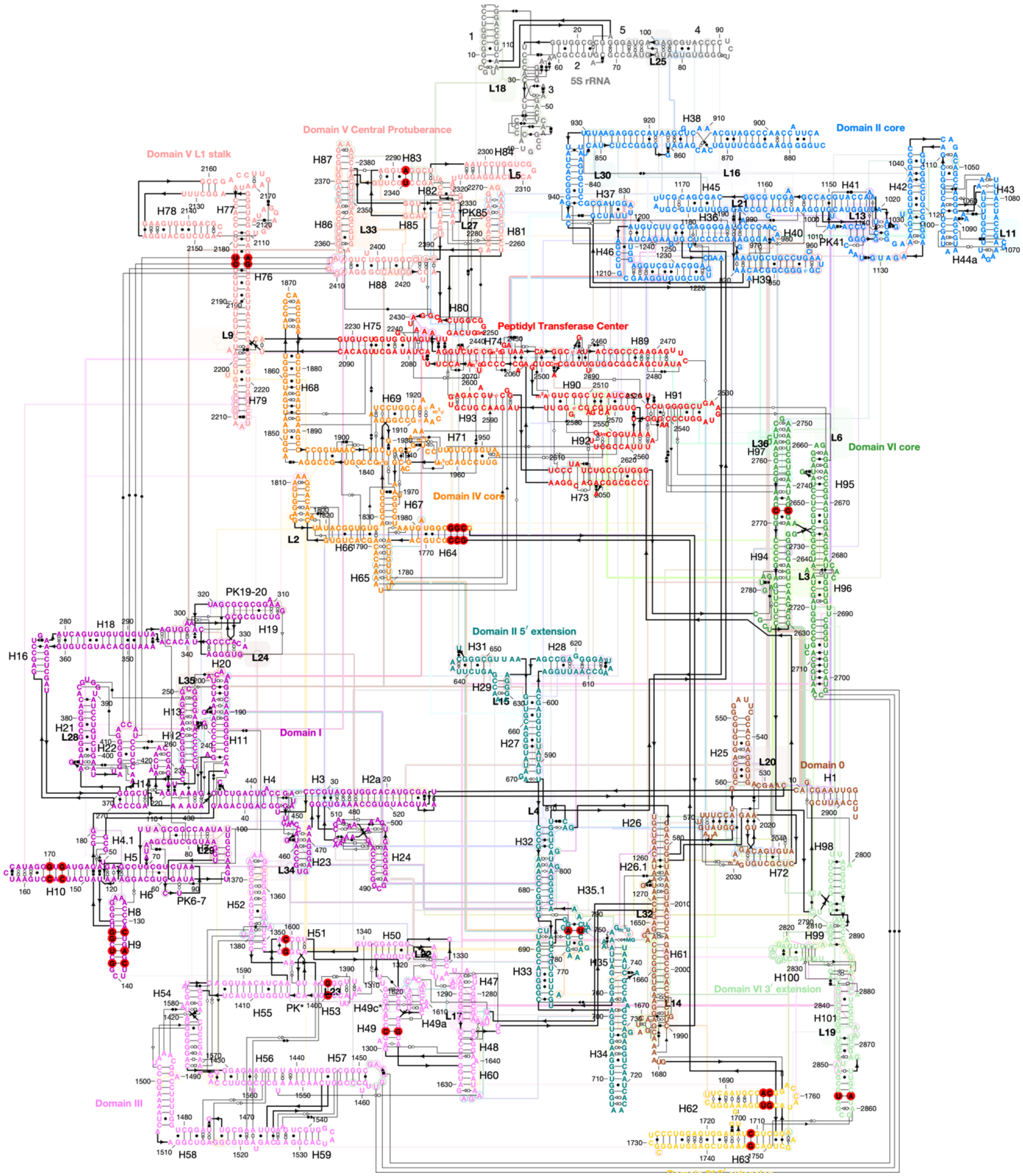
Supplementary Figure 99. Eterna participants'-designed ribosomal RNA design R2-25 prepared with RiboDraw[1].



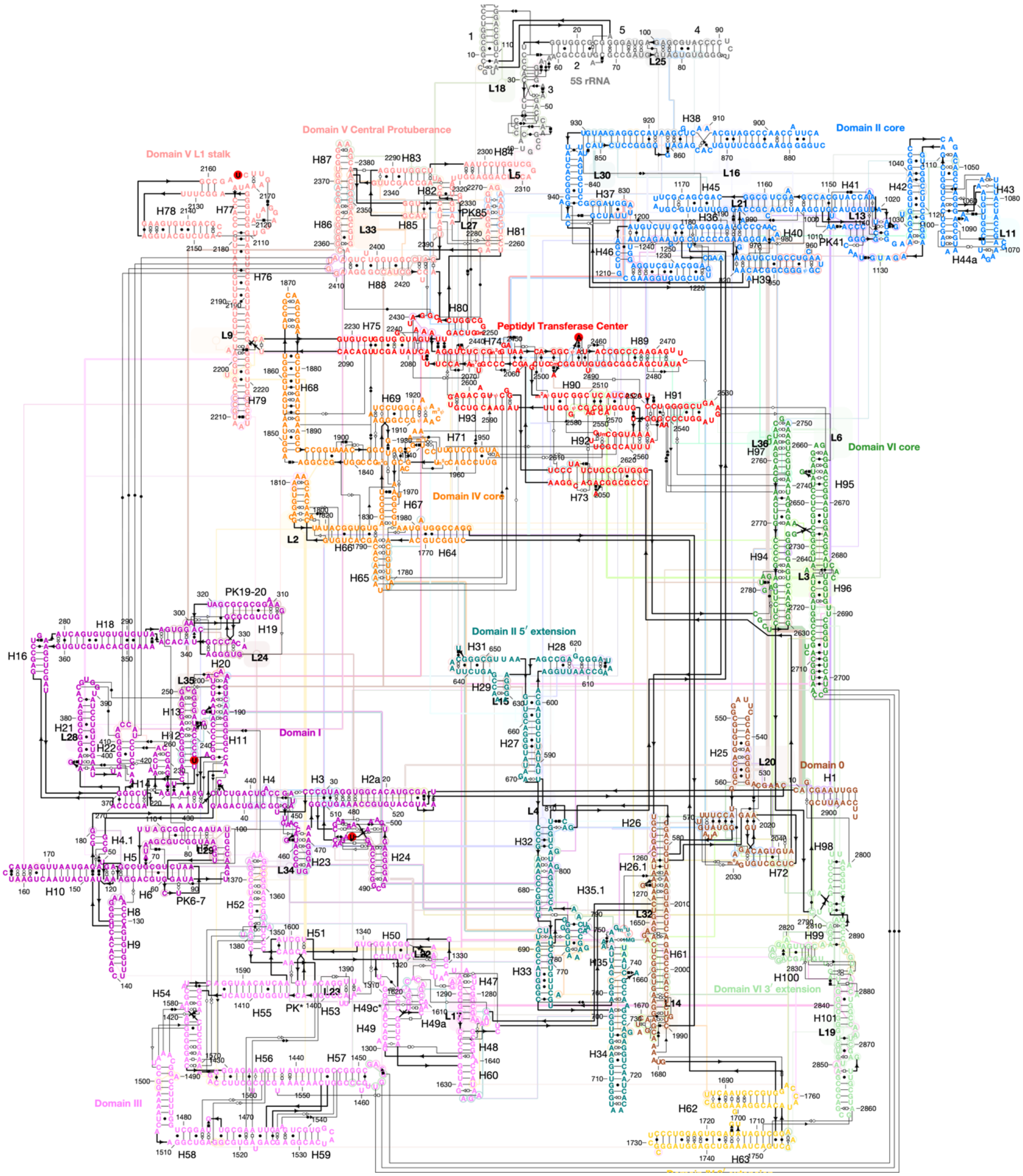
Supplementary Figure 100. Eterna participants'-designed ribosomal RNA design R2-26 prepared with RiboDraw[1].



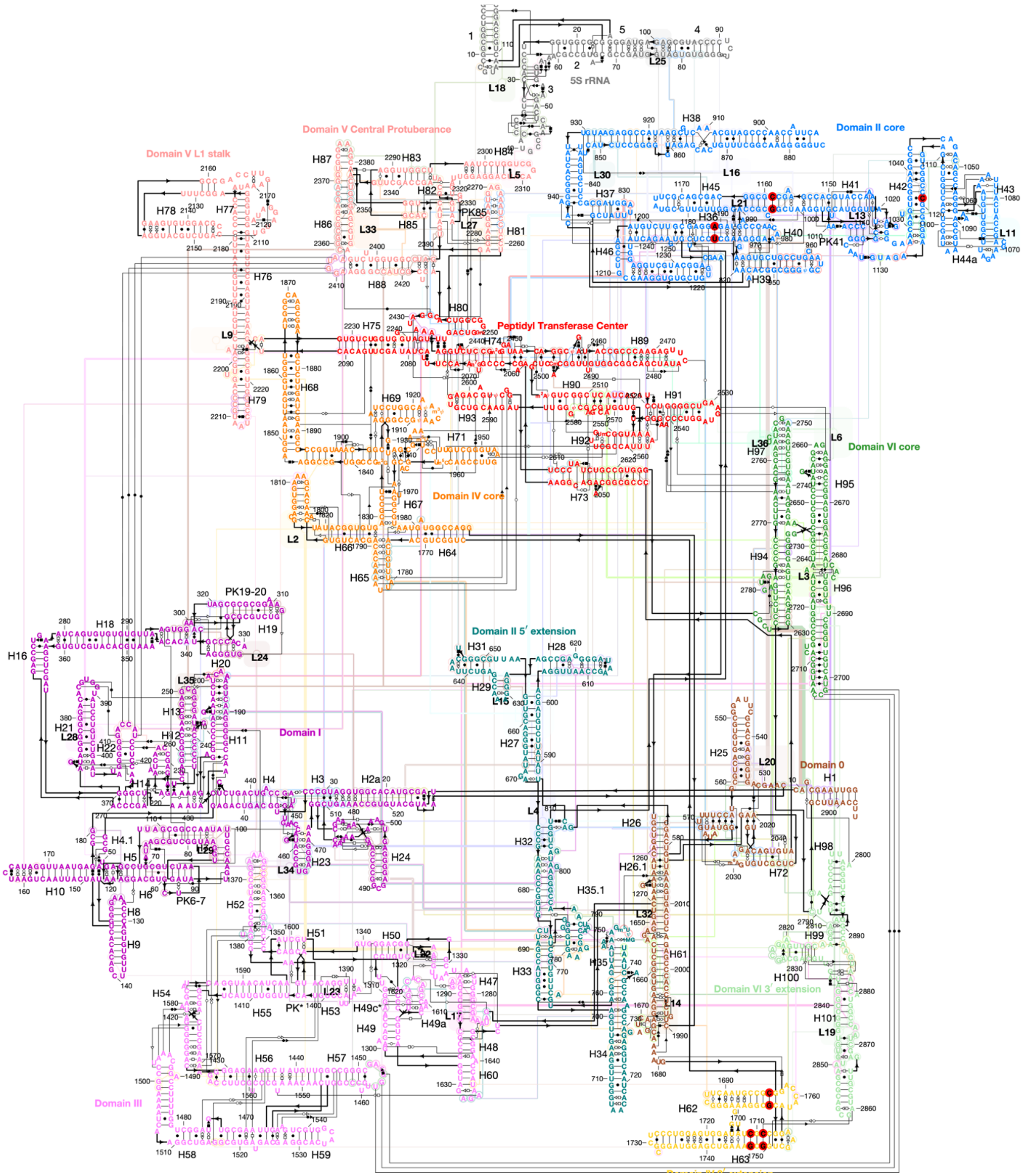
Supplementary Figure 101. Eterna participants'-designed ribosomal RNA design R2-27 prepared with RiboDraw[1].



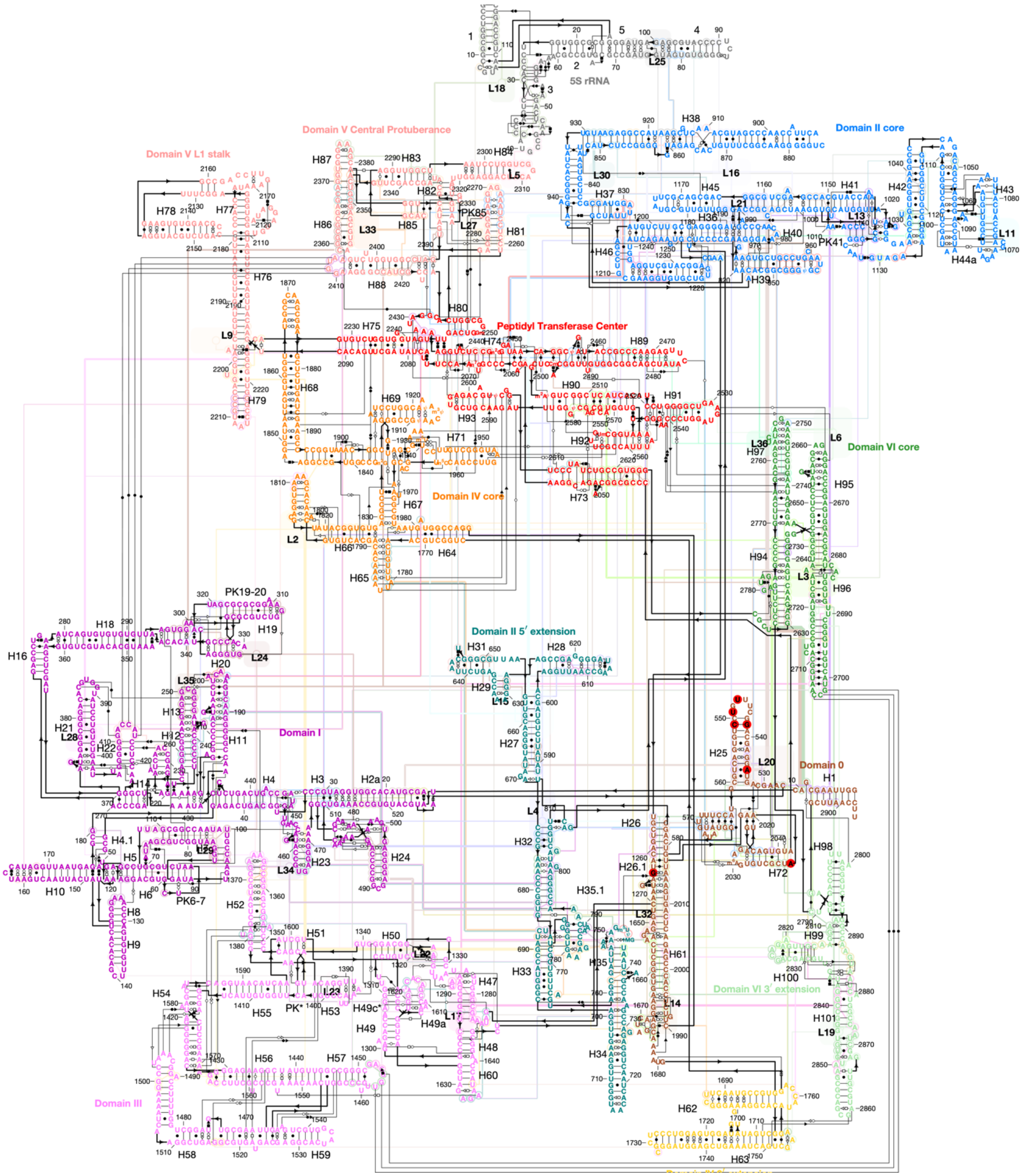
Supplementary Figure 102. Eterna participants'-designed ribosomal RNA design R2-28 prepared with RiboDraw[1]**.**



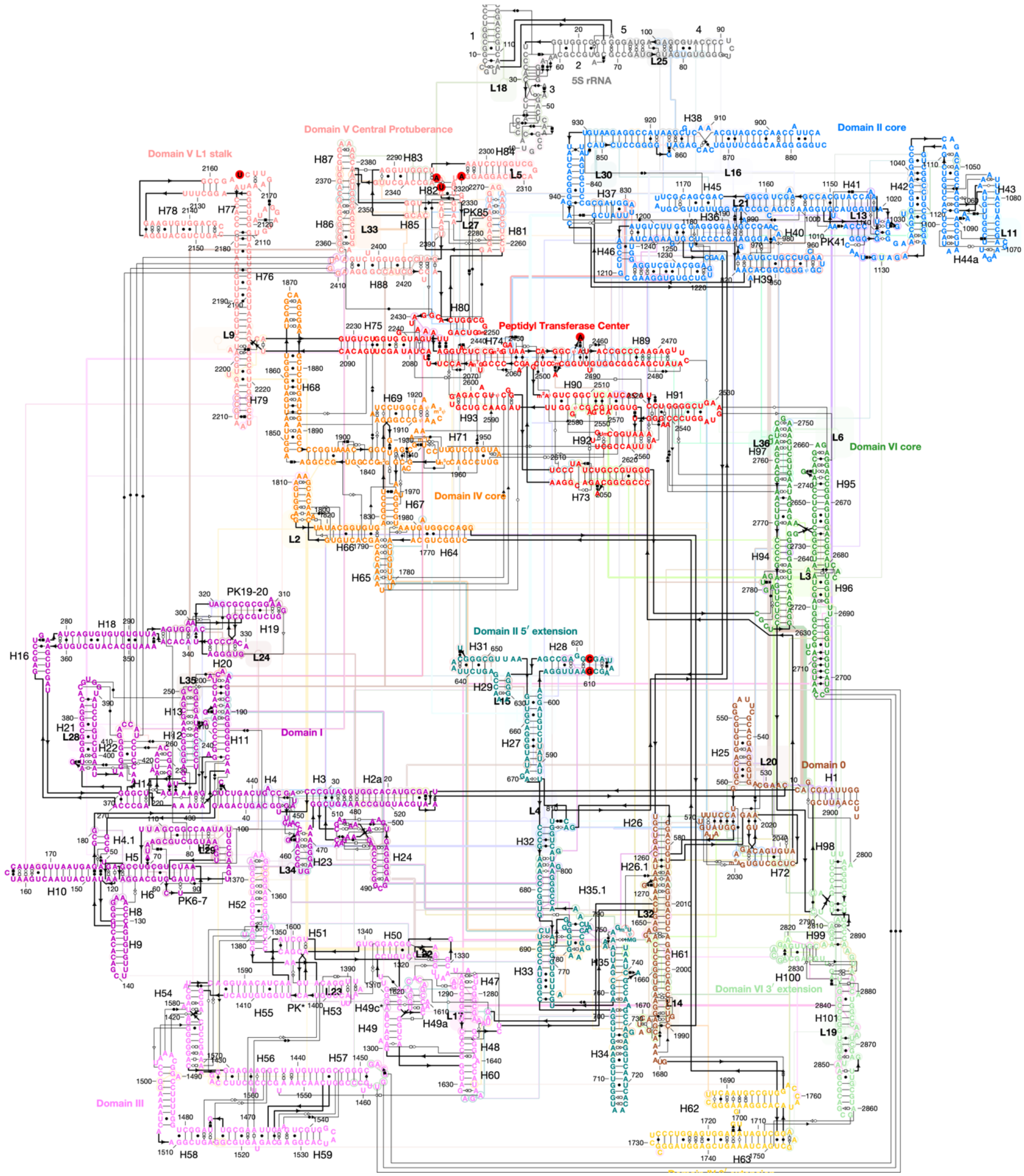
Supplementary Figure 103. Eterna participants'-designed ribosomal RNA design R2-29 prepared with RiboDraw[1].



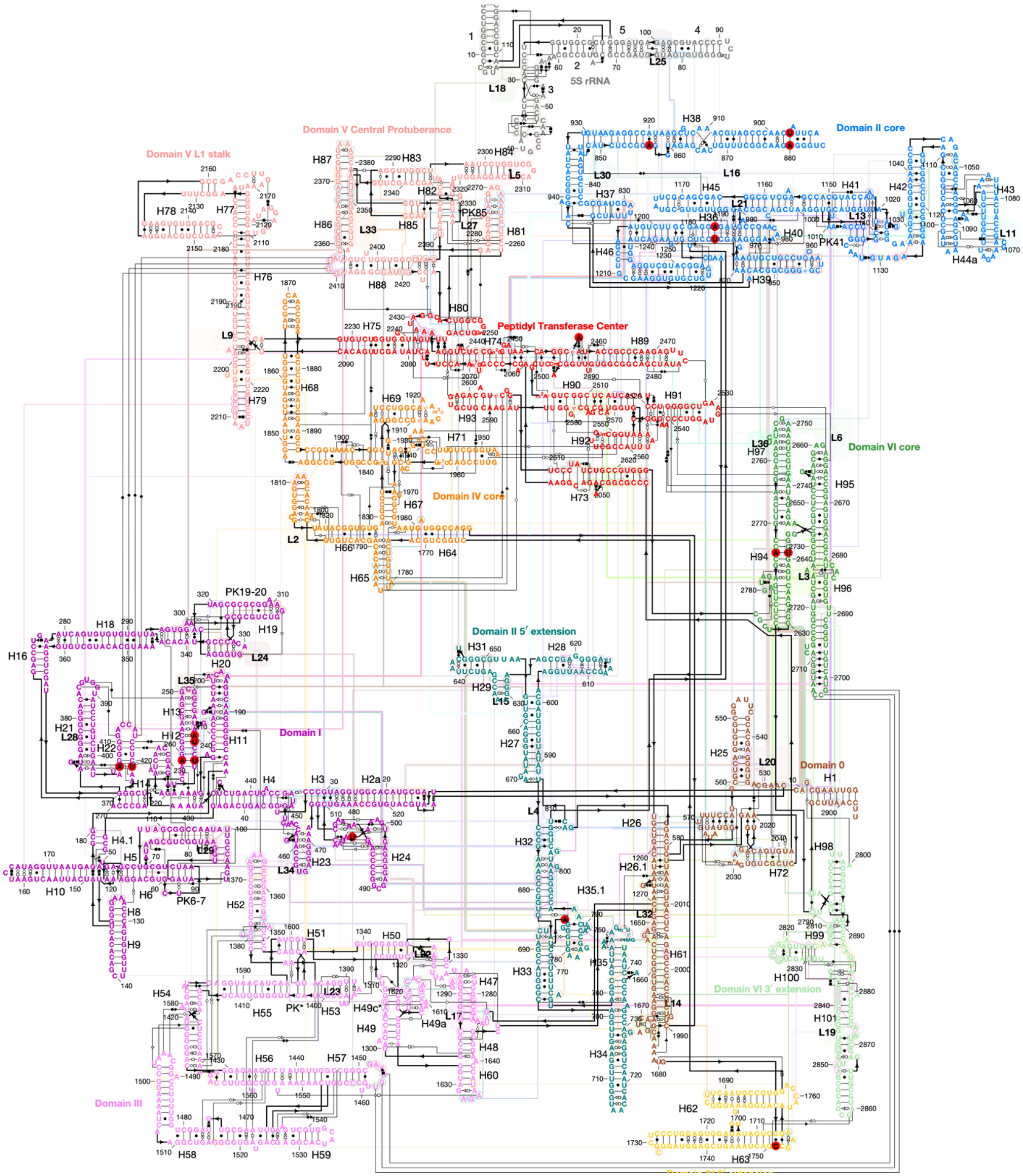
Supplementary Figure 104. Eterna participants'-designed ribosomal RNA design R2-30 prepared with RiboDraw[1].



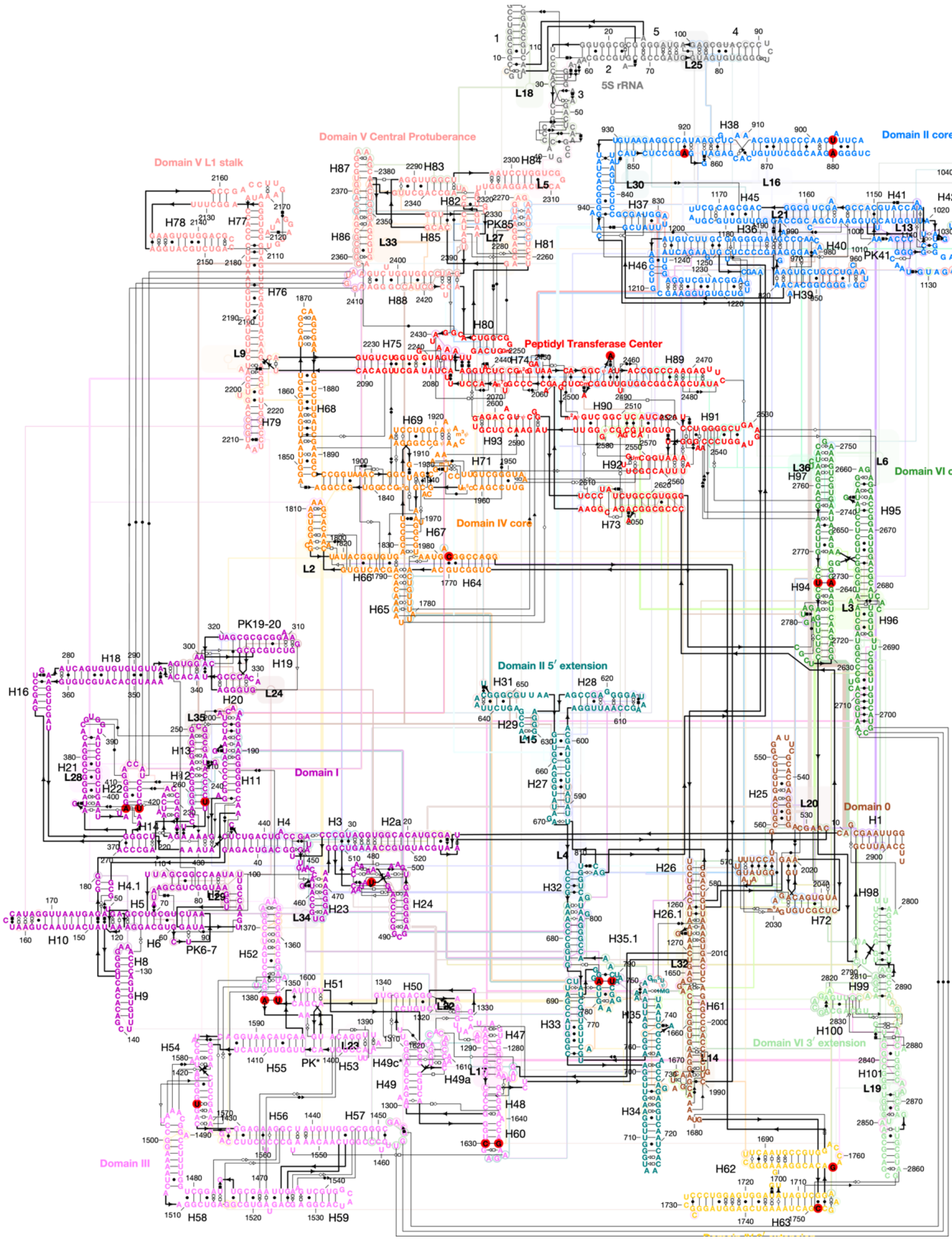
Supplementary Figure 105. Eterna participants'-designed ribosomal RNA design R2-31 prepared with RiboDraw[1]**.**



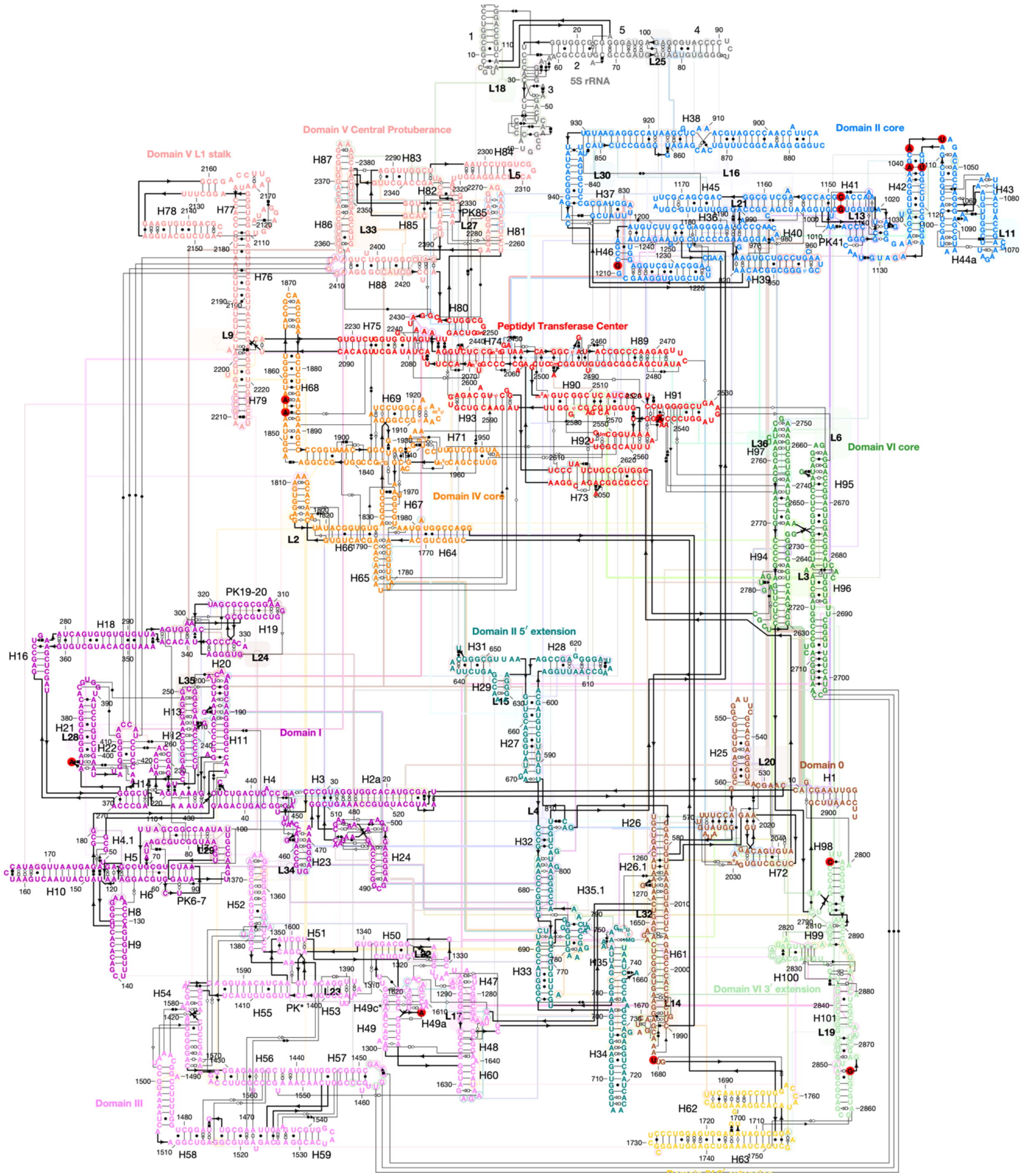
Supplementary Figure 106. Eterna participants'-designed ribosomal RNA design R2-32 prepared with RiboDraw[1].



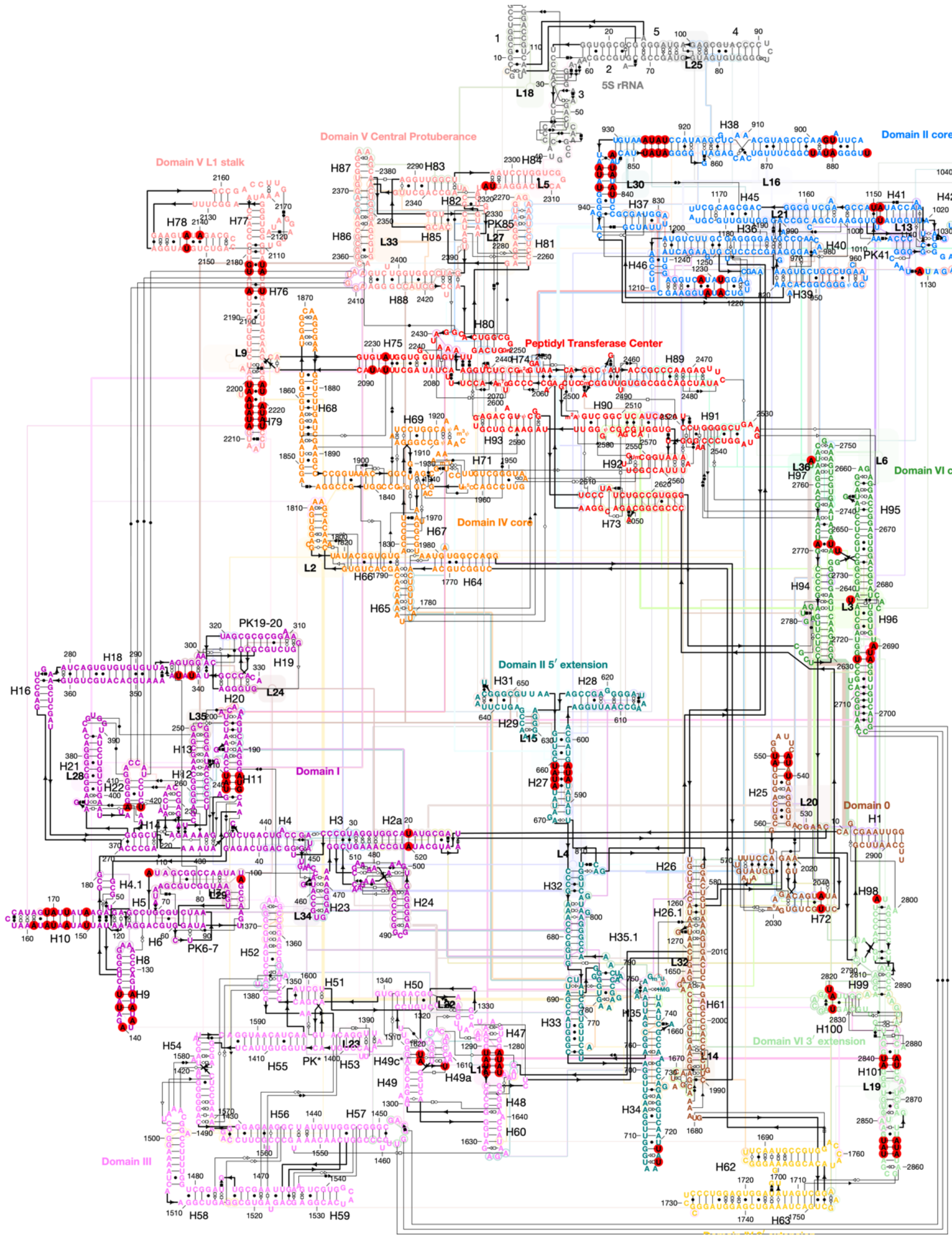
Supplementary Figure 107. Eterna participants'-designed ribosomal RNA design R2-33 prepared with RiboDraw[1].



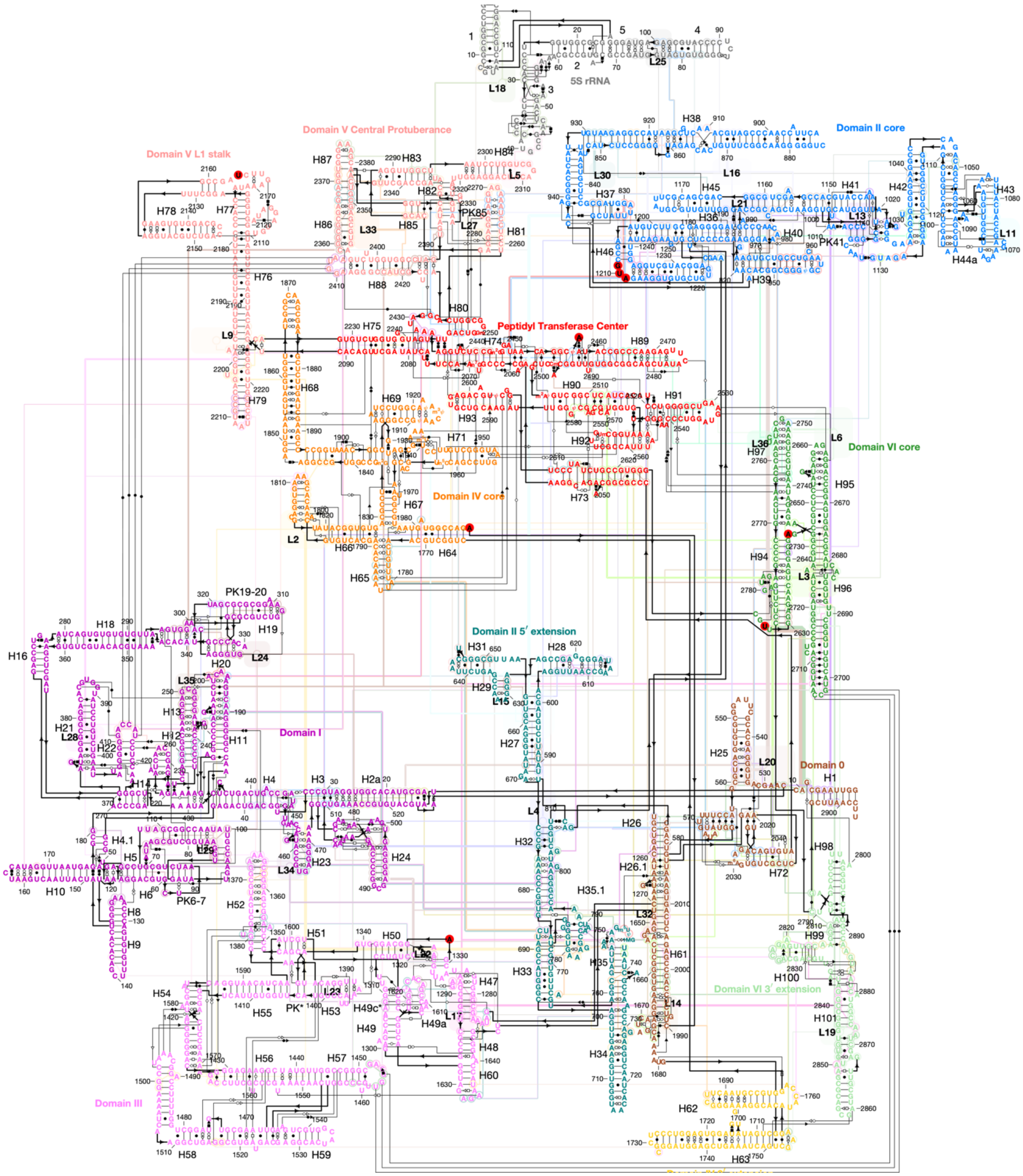
Supplementary Figure 108. Eterna participants'-designed ribosomal RNA design R2-34 prepared with RiboDraw[1].



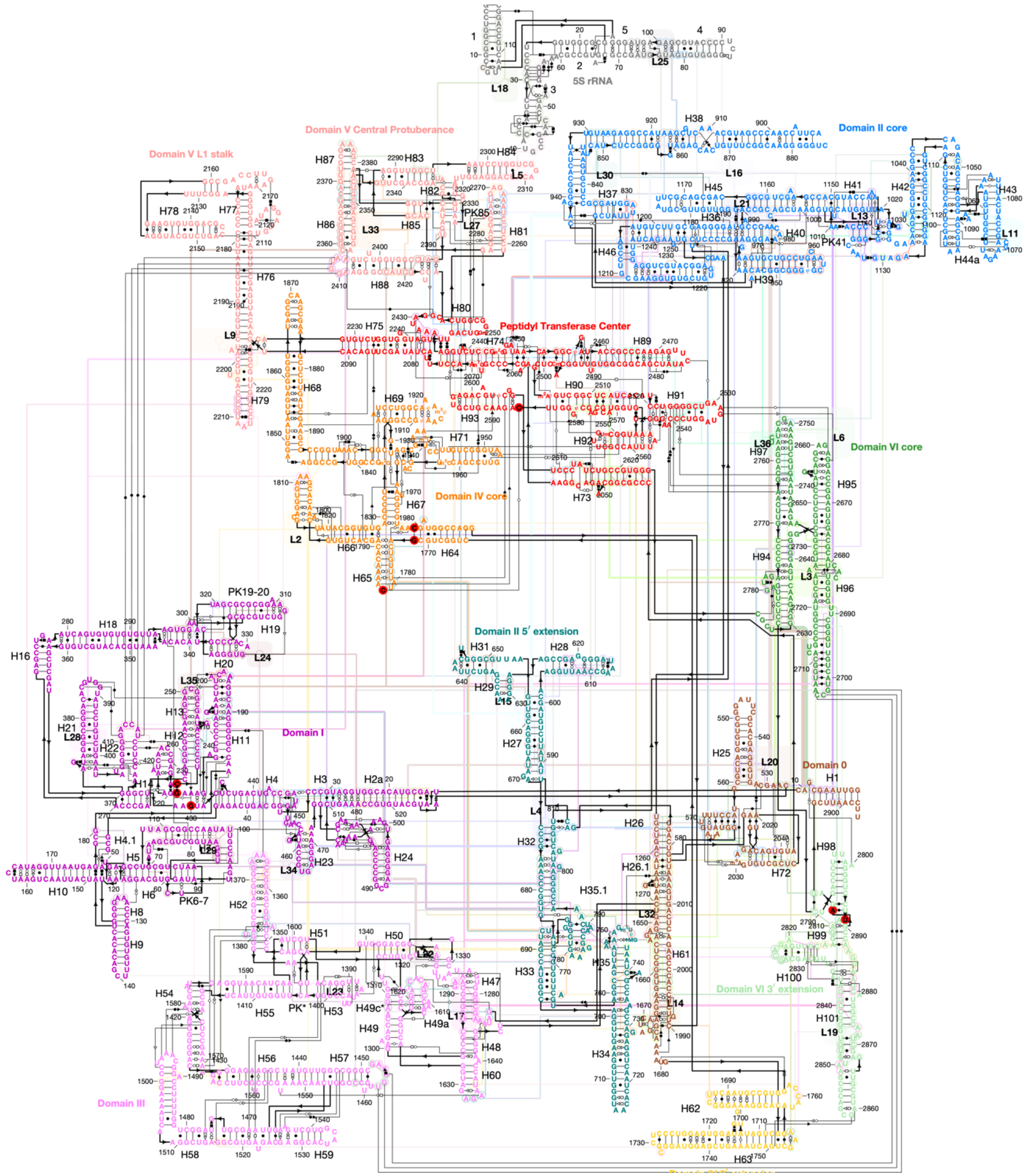
Supplementary Figure 109. Eterna participants'-designed ribosomal RNA design R2-35 prepared with RiboDraw[1].



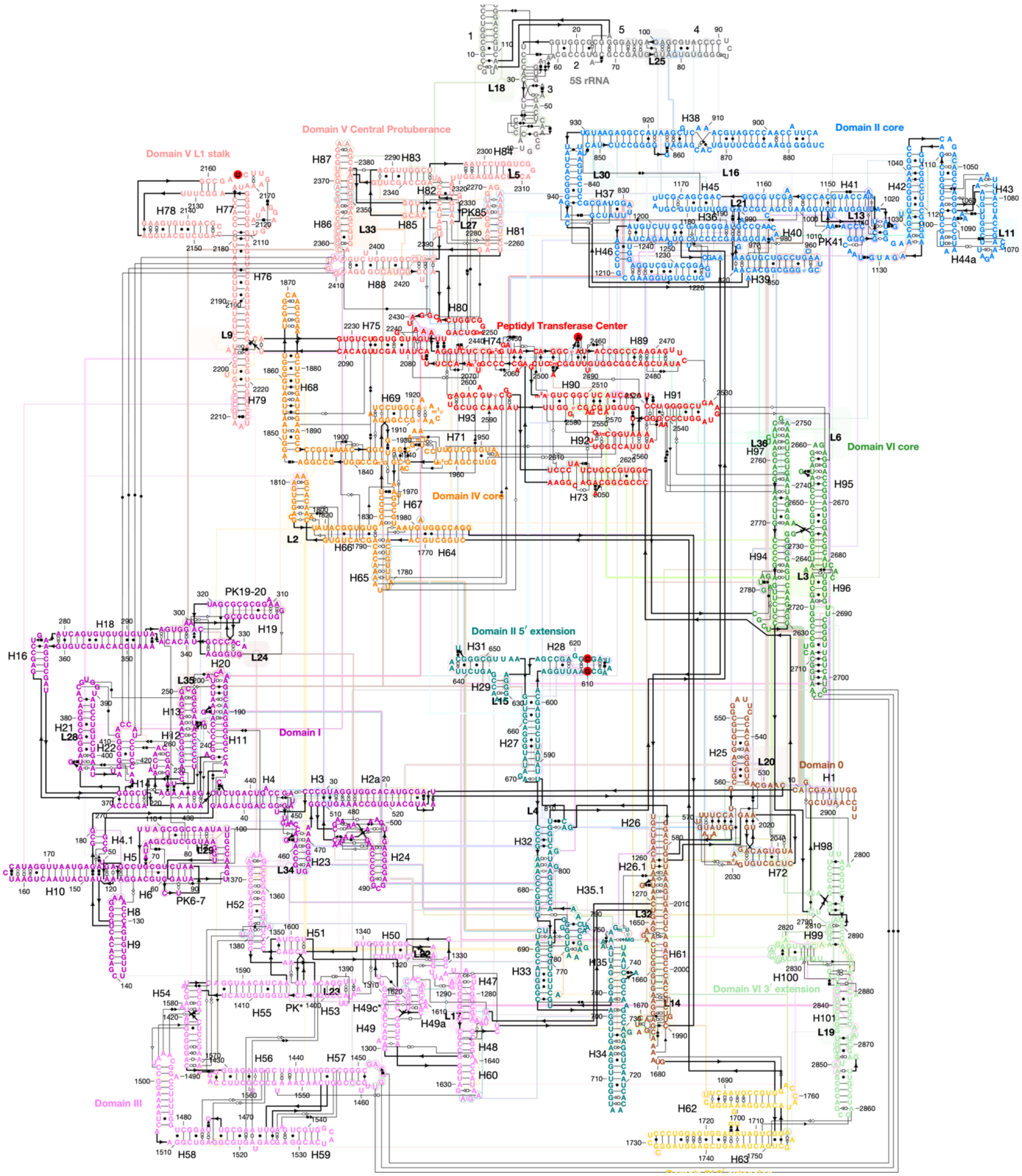
Supplementary Figure 110. Eterna participants'-designed ribosomal RNA design R2-36 prepared with RiboDraw[1].



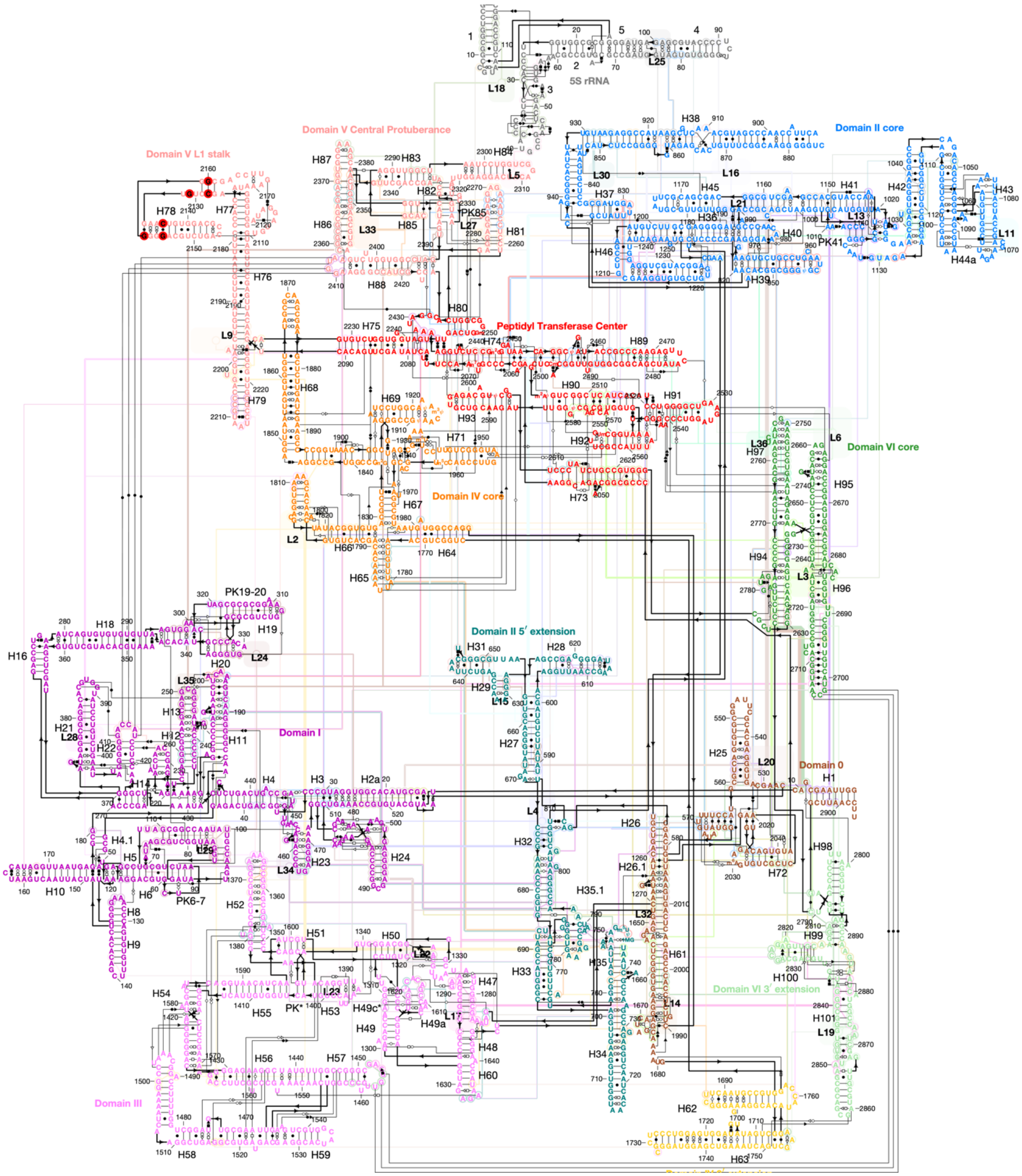
Supplementary Figure 111. Eterna participants'-designed ribosomal RNA design R2-37 prepared with RiboDraw[1].



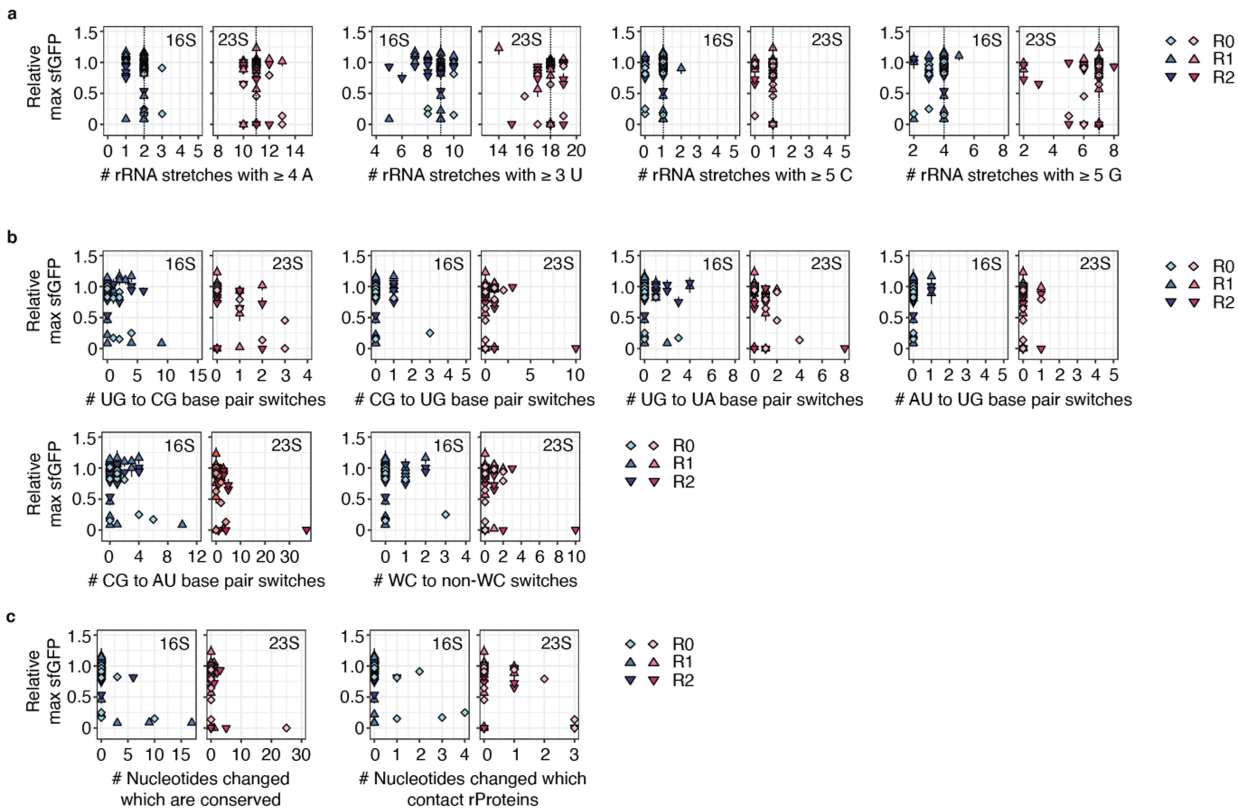
Supplementary Figure 112. Eterna participants'-designed ribosomal RNA design R2-38 prepared with RiboDraw[1].



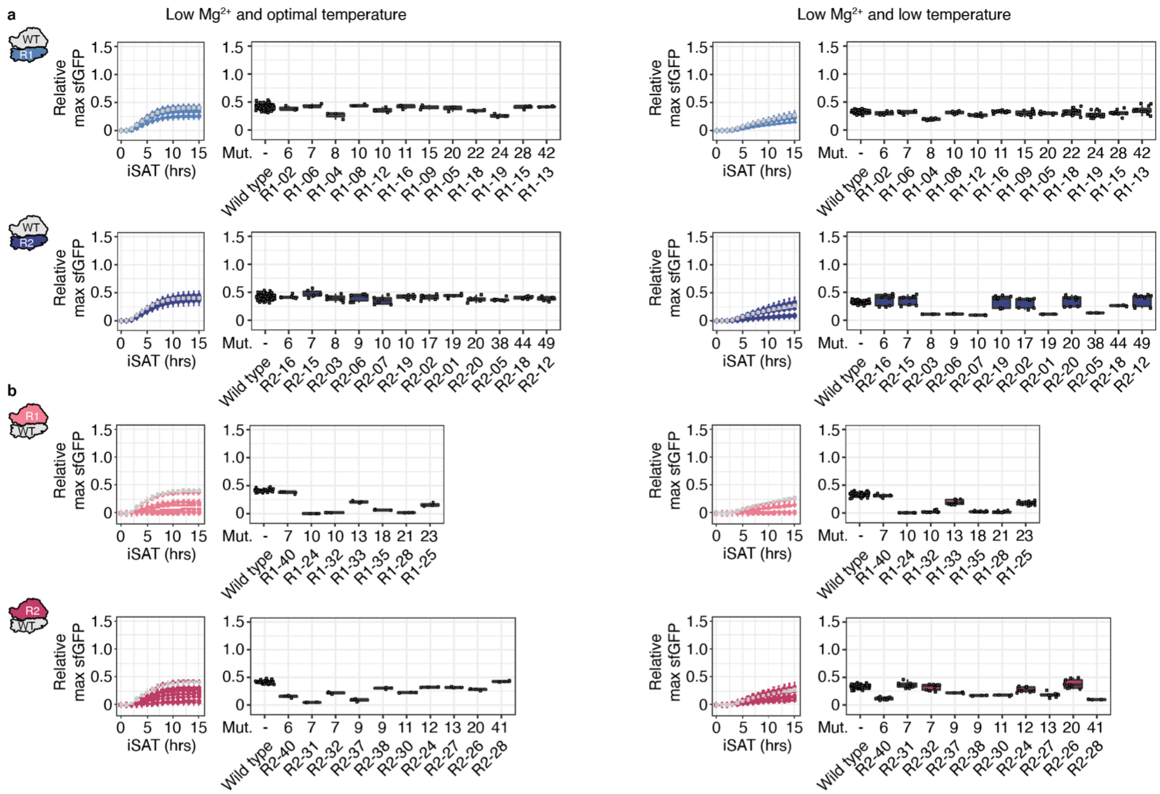
Supplementary Figure 113. Eterna participants'-designed ribosomal RNA design R2-39 prepared with RiboDraw[1].



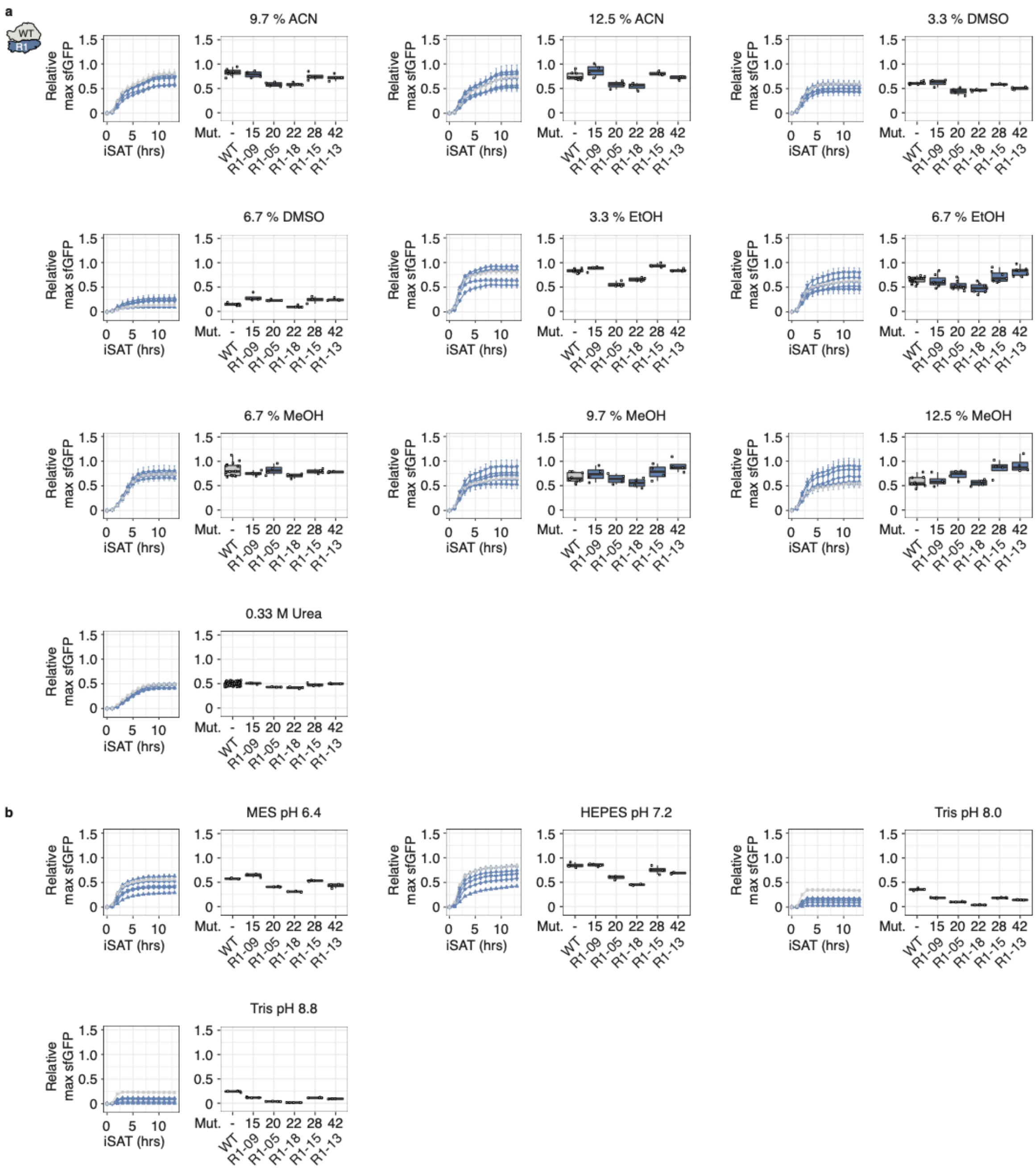
Supplementary Figure 114. Eterna participants'-designed ribosomal RNA design R2-40 prepared with RiboDraw[1].



Supplementary Figure 115. Community scientists followed and combined different strategies to improve rRNA performance. Relative maximum sfGFP expression made in iSAT reactions by each design was plotted against the instances of (**a**) stretches of consecutive identical nucleotides, (**b**) altered base pairing in rRNA secondary structures, or (**c**) changes in conserved nucleotides or nucleotides which contact rProteins. Data are shown from the “pilot round” (R0) and round 1 (R1) and round 2 (R2) as mean ± s.d.; n ≥ 3. Source data are provided as a Source Data file. Dotted line in (**a**) indicates wild type value. rProteins: ribosomal proteins; WC: Watson-Crick.

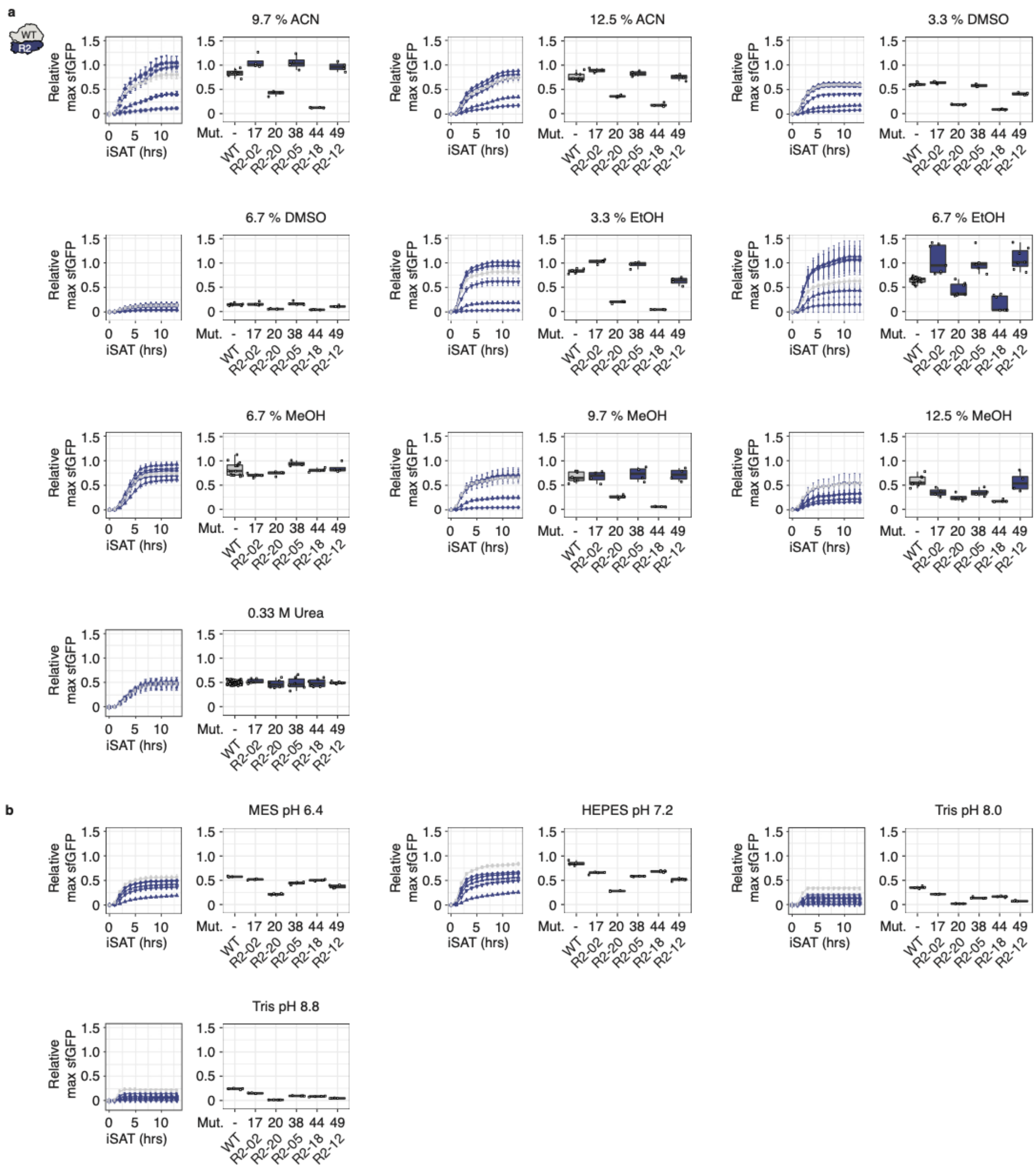


Supplementary Figure 116. iSAT kinetics and maximum yields for Eterna designed ribosomes under folding stress. sfGFP expression of (**a**) pT7-rrnB-16S R1 and R2 designs and (**b**) pT7-rrnB-23S R1 and R2 designs in iSAT at low Mg^{2+} concentration (3.75 mM) and optimal temperature (37º C) (left panels) and low Mg^{2+} concentration (3.75 mM) and low temperature (30º C) (right panels). sfGFP expression was determined in 15 hour iSAT reactions by fluorescence and normalized to max sfGFP of pT7-rrnB-WT at optimal iSAT conditions. Data are shown as mean. Error bars represent s.d.; n ≥ 3. Source data are provided as a Source Data file. These data were used to generate the heat maps in Main Figure 3. Mut: mutations; R1: round 1, R2: round 2, WT: wild type.

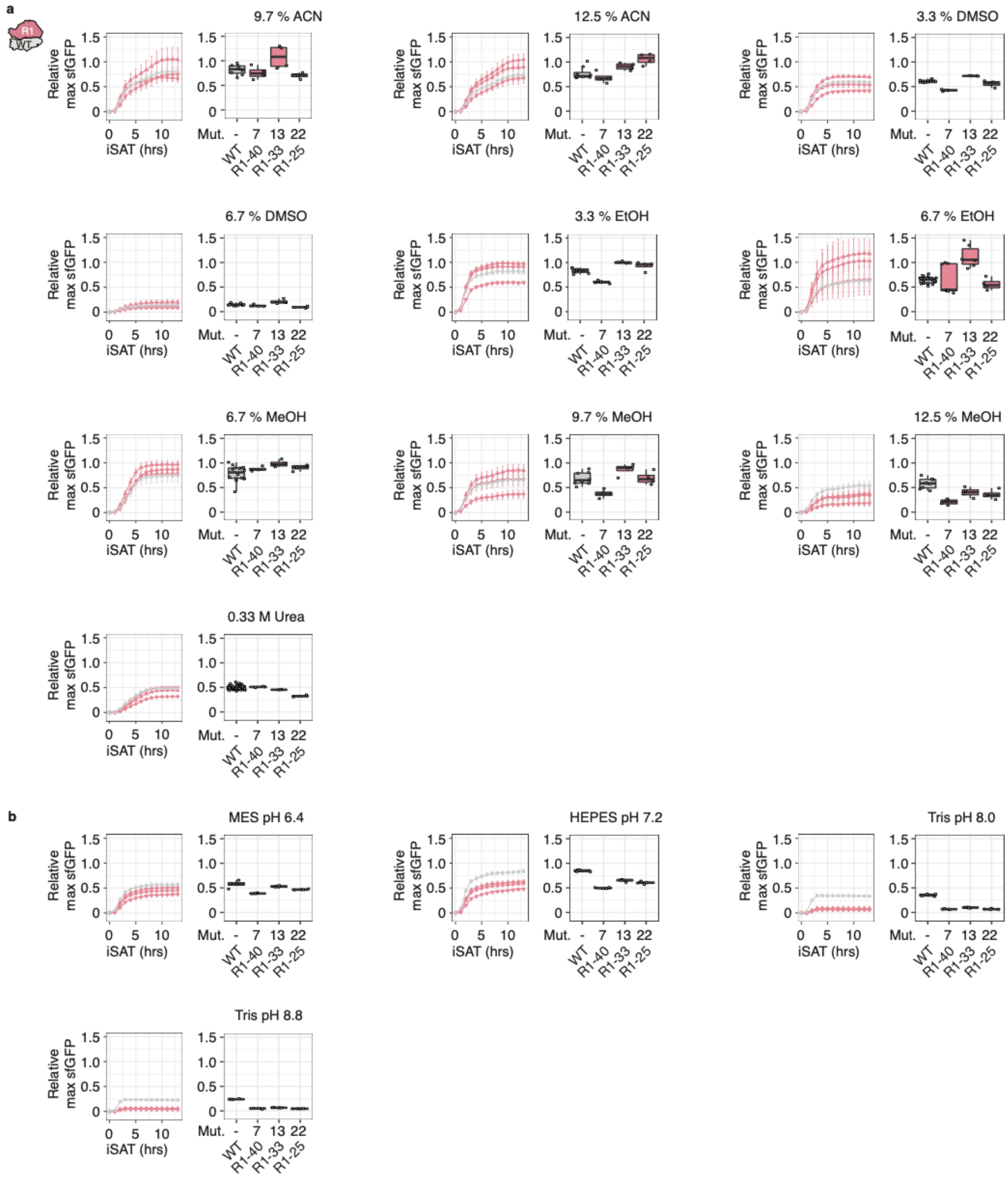


Supplementary Figure 117. Influence of solvents and pH on iSAT reactions of selected R1 16S rRNA designs. (**a**) Solvents (v/v %). (**b**) pH. Time course data are shown as mean ± s.d. Maximum sfGFP expression was determined in iSAT reactions by fluorescence and normalized to max sfGFP of pT7-rrnB-wild type at optimal iSAT conditions. Maximal sfGFP expression data are presented as boxplots. Error bars represent s.d.; n ≥ 3. Source data are provided as a Source

Data file. ACN: acetonitrile, DMSO: dimethylsulfoxide, EtOH: ethanol, MeOH: methanol, HEPES: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), MES: 2-(N-morpholino)ethanesulfonic acid, Tris: tris(hydroxymethyl)aminomethane, Mut: mutations, R1: round 1, WT: wild type.

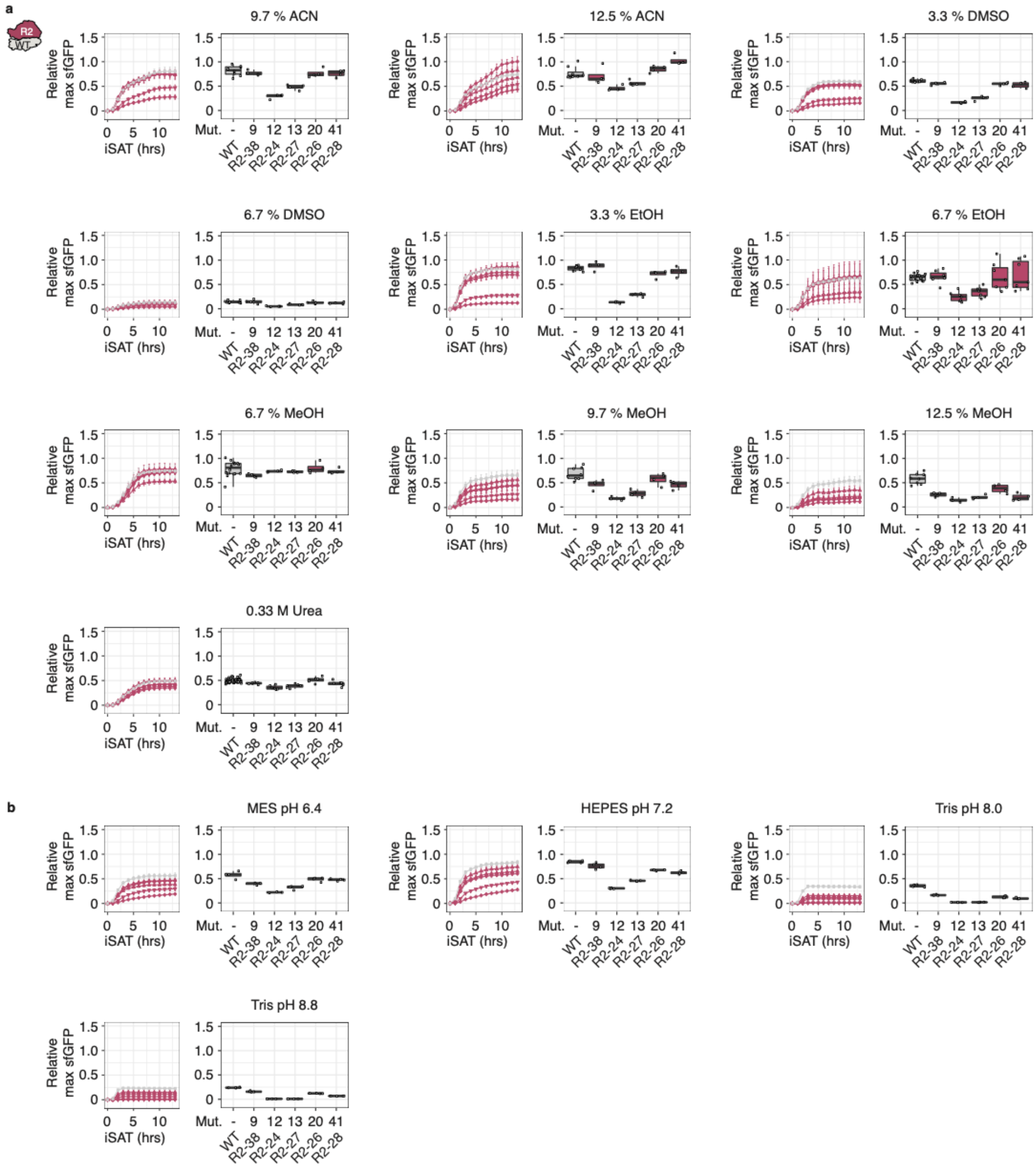


Supplementary Figure 118. Influence of solvents and pH on iSAT reactions of selected R2 16S rRNA designs. (**a**) Solvents (v/v %). (**b**) pH. Time course data are shown as mean ± s.d. Maximum sfGFP expression was determined in iSAT reactions by fluorescence and normalized to max sfGFP of pT7-rrnB-wild type at optimal iSAT conditions. Maximal sfGFP expression data are presented as boxplots. Error bars represent s.d.; $n \geq 3$. Source data are provided as a Source Data file. ACN: acetonitrile, DMSO: dimethylsulfoxide, EtOH: ethanol, MeOH: methanol, HEPES: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), MES: 2-(N-morpholino)ethanesulfonic acid, Tris: tris(hydroxymethyl)aminomethane, Mut: mutations, R2: round 2, WT: wild type



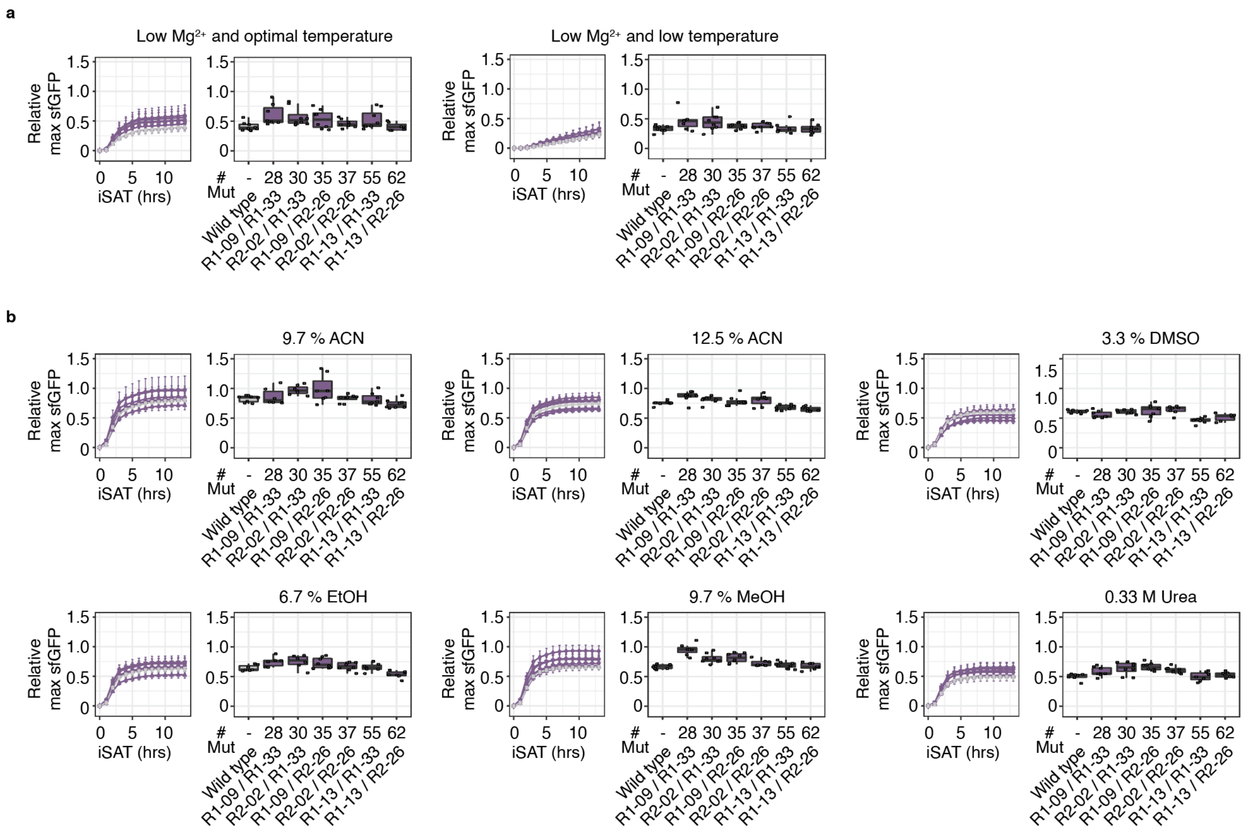
Supplementary Figure 119. Influence of solvents and pH on iSAT reactions of selected R1 23S rRNA designs. (**a**) Solvents (v/v %). (**b**) pH. Time course data are shown as mean ± s.d. Maximum sfGFP expression was determined in iSAT reactions by fluorescence and normalized to max sfGFP of pT7-rrnB-wild type at optimal iSAT conditions. Maximal sfGFP expression data are presented as boxplots. Error bars represent s.d.; n ≥ 3. Source data are provided as a Source

Data file. ACN: acetonitrile, DMSO: dimethylsulfoxide, EtOH: ethanol, MeOH: methanol, HEPES: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), MES: 2-(N-morpholino)ethanesulfonic acid, Tris: tris(hydroxymethyl)aminomethane, Mut: mutations, R1: round 1, WT: wild type.

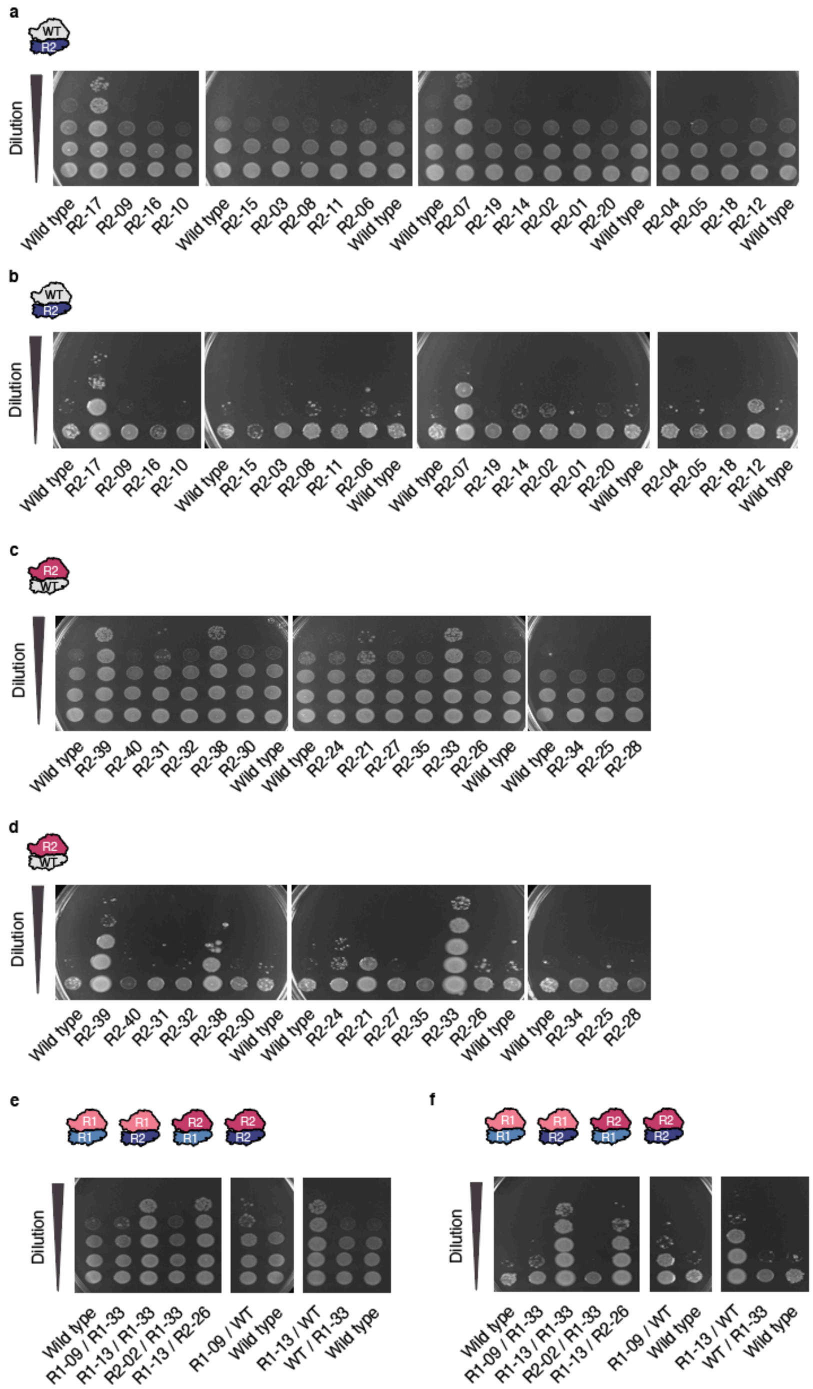


Supplementary Figure 120. Influence of solvents and pH on iSAT reactions of selected R2 23S designs. (**a**) Solvents (v/v %). (**b**) pH. Time course data are shown as mean ± sd. Maximum sfGFP expression was determined in iSAT reactions by fluorescence and normalized to max sfGFP of pT7-rrnB-wild type at optimal iSAT conditions. Maximal sfGFP expression data are presented as boxplots. Error bars represent s.d.; n ≥ 3. Source data are provided as a Source

Data file. ACN: acetonitrile, DMSO: dimethylsulfoxide, EtOH: ethanol, MeOH: methanol, HEPES: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), MES: 2-(N-morpholino)ethanesulfonic acid, Tris: tris(hydroxymethyl)aminomethane, Mut: mutations, R2: round 2, WT: wild type.



Supplementary Figure 121. Influence of *in vitro* solvent conditions on iSAT reactions of Eterna ribosomes. (**a**) Relative sfGFP expression of ribosomes with both 16S rRNA and 23S rRNA designs under iSAT conditions at low (3.75 mM)-magnesium (Mg^{2+}) and optimal or low (3.75 mM)-magnesium (Mg^{2+}) and low temperature. (**b**) Solvents (v/v %). Time course data are shown as mean ± s.d. Maximum sfGFP expression was determined in iSAT reactions by fluorescence and normalized to max sfGFP of pT7-rrnB-wild type at optimal iSAT conditions. Maximal sfGFP expression data are presented as boxplots. Error bars represent s.d.; n ≥ 3. Source data are provided as a Source Data file. ACN: acetonitrile, DMSO: dimethylsulfoxide, EtOH: ethanol, MeOH: methanol, Mut: mutations.



Supplementary Figure 122. R2 and combinatorial Eterna designs support life. (**a-d**) Un-cut images of spotted SQ171fg cells growing with pL-rrnB-wild type and pL-rrnB-R2 16S rRNA (**a, b**) and 23S rRNA (**c, d**) designs imaged after 24 hours at 37 °C (**a, c**) or 72 hours at 30 °C (**b, d**).

(**e-f**) Un-cut images of spotted SQ171fg cells growing with pL-rrnB-wild type and pL-rrnB-combinations imaged after 24 hours at 37 °C (**e**) or 72 hours at 30 °C (**f**). Stationary cells were diluted to an OD600 = 1, diluted stepwise 1:10, and spotted onto LB + $Carb_{100}$ plates. Data are representative of n ≥ 3. R1: round 1, R2: round 2, WT: wild type.

SUPPLEMENTARY TABLES

Supplementary Table 1. Wild type ribosome activity in iSAT in the presence of solvent concentrations.

Solvent Concentration	Concentration Unit	Solvent	Mean	Standard deviation
0	% (v/v)	Acetonitrile	1.004	0.070
0	% (v/v)	DMSO	1.004	0.070
0	% (v/v)	EtOH	1.004	0.070
0	% (v/v)	MeOH	1.004	0.070
0	% (v/v)	Urea	1.004	0.070
0.5	% (v/v)	Acetonitrile	1.033	0.021
1	% (v/v)	Acetonitrile	1.027	0.015
2.5	% (v/v)	Acetonitrile	0.997	0.014
5	% (v/v)	Acetonitrile	0.896	0.064
6.67	% (v/v)	Acetonitrile	0.889	0.111
9.38	% (v/v)	Acetonitrile	0.874	0.028
12.5	% (v/v)	Acetonitrile	0.708	0.046
0.5	% (v/v)	DMSO	0.994	0.027
1	% (v/v)	DMSO	0.933	0.027
2.5	% (v/v)	DMSO	0.779	0.028
5	% (v/v)	DMSO	0.376	0.012
6.67	% (v/v)	DMSO	0.113	0.002
9.38	% (v/v)	DMSO	0.057	0.003
12.5	% (v/v)	DMSO	0.015	0.001
0.5	% (v/v)	EtOH	0.939	0.084
1	% (v/v)	EtOH	0.994	0.007
2.5	% (v/v)	EtOH	0.914	0.038
5	% (v/v)	EtOH	0.700	0.009
6.67	% (v/v)	EtOH	0.619	0.104
9.38	% (v/v)	EtOH	0.504	0.013
12.5	% (v/v)	EtOH	0.302	0.040
0.5	% (v/v)	MeOH	0.985	0.032
1	% (v/v)	MeOH	0.929	0.076
2.5	% (v/v)	MeOH	0.971	0.051
5	% (v/v)	MeOH	0.735	0.009
6.67	% (v/v)	MeOH	0.817	0.033
9.38	% (v/v)	MeOH	0.652	0.035
12.5	% (v/v)	MeOH	0.574	0.063
0.05	M	Urea	0.983	0.058
0.1	M	Urea	0.941	0.027
0.25	M	Urea	0.703	0.014
0.5	M	Urea	0.152	0.002
0.6	M	Urea	0.029	0.002
0.94	M	Urea	0.020	0.001
1.13	M	Urea	0.017	0.000

Maximum sfGFP expression was determined in iSAT reactions by fluorescence and normalized to max sfGFP of pT7-rrnB-WT at optimal iSAT conditions. Maximal sfGFP expression data are presented as mean and s.d.; $n \geq 3$.

SUPPLEMENTARY NOTES

Supplementary Notes summarizing resources and online tools that players used for designing 16S and 23S rRNAs.

- Ribosome images: For example, from the Noller lab: http://rna.ucsc.edu/rnacenter/ribosome_images.html
- Online videos on ribosomes and related topics. For example, via Khan academy and YouTube: https://www.khanacademy.org; https://www.youtube.com
- Scientific literature on ribosomes and related topics. For example, papers about RNA motifs and r-protein binding sites[1–3]
- Comparative RNA web site and project[4]: https://crw-site.chemistry.gatech.edu/FAM/
- Protein Data Bank: https://www.rcsb.org[5] and https://www.ebi.ac.uk/pdbe/[6]
- The non-coding RNA sequence database[7,8]: https://rnacentral.org
- Molecular visualization software: Chimera[9,10] and PyMOL[11]
- Multiple sequence alignment tool from the EMBL-EBI web site: https://www.ebi.ac.uk/Tools/msa/muscle/[12]

SUPPLEMENTARY METHODS

Energetic rationale for base locks in ribosome puzzle definitions

Most base locks were chosen based on intra- or inter-subunit tertiary contacts, particularly when Watson-Crick, and protein-RNA contacts that would both enormously influence the folding energetics of the rRNA under investigation but could not be represented in a secondary structure folding model. Pseudoknotted residues were also locked; folding the ribosome with a pseudoknot-aware secondary structure model would be both physically unrealistic (ribosome folding is chaperoned; the only pseudoknots likely to form in designed ribosomes are those that form in the wild type) and computationally intractable.

Some "singlet" base pairs, however, were also locked. In large, folded RNAs, tertiary folding influences the secondary structure ensemble and can render stable features that otherwise might struggle to form. "Singlet" base pairs – those that do not form part of a secondary structure stem – are not favorable on their own, since they contribute no stabilizing stacking energy, and in energy models like Vienna will typically destabilize large loops.

Because this "secondary structure" constraint could not be satisfied in the energy model, we omitted it from the target secondary structure to make the objective more achievable for players, but we locked the nucleotides to ensure that these destabilized bases were not mutated.

Plasmid sequences

pL-rrnB- wild type:

GATCTCTCACCTACCAAACAATGCCCCCCTGCAAAAAATAAATTCATATAAAAAACATACAG
ATAACCATCTGCGGTGATAAATTATCTCTGGCGGTGTTGACATAAATACCACTGGCGGTTAT
ACTGAGCACGGGTACCGGCCGCTGAGAAAAAGCGAAGCGGCACTGCTCTTTAACAATTTA
TCAGACAATCTGTGTGGGCACTCGAAGATACGGATTCTTAACGTCGCAAGACGAAAAATGA
ATACCAAGTCTCAAGAGTGAACACGTAATTCATTACGAAGTTTAATTCTTTGAGCGTCAAAC
TTTTAAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAACGGTAACAGGAAGAAGCTTGCTTCTTTGCTGACGAGTGGCGGACGGGTGAG
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TGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACG
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pT7-rrnB- wild type:

TAATACGACTCACTATAGGGGCCGCTGAGAAAAAGCGAAGCGGCACTGCTCTTTAACAATT
TATCAGACAATCTGTGTGGGCACTCGAAGATACGGATTCTTAACGTCGCAAGACGAAAAAT
GAATACCAAGTCTCAAGAGTGAACACGTAATTCATTACGAAGTTTAATTCTTTGAGCGTCAA
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