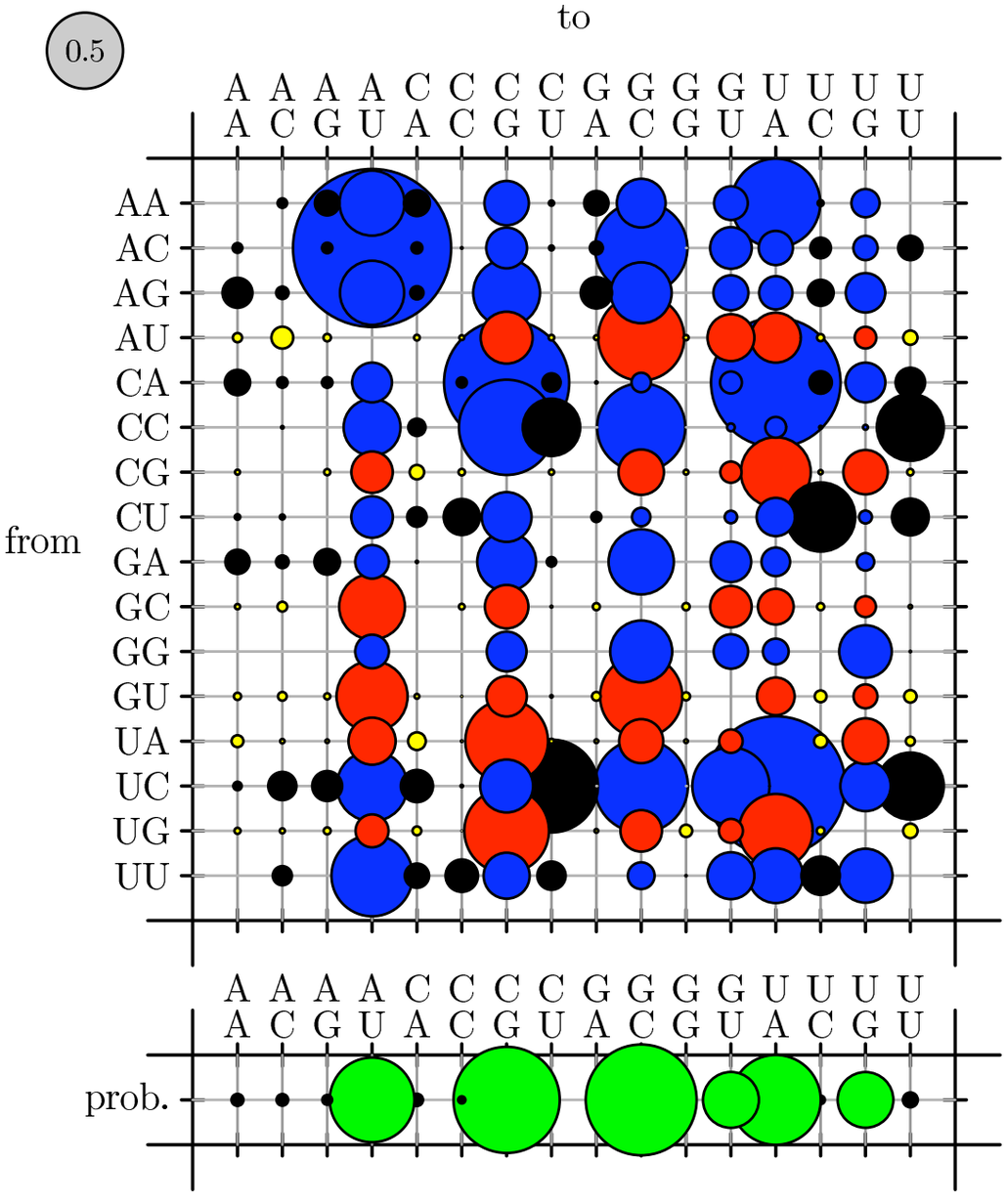
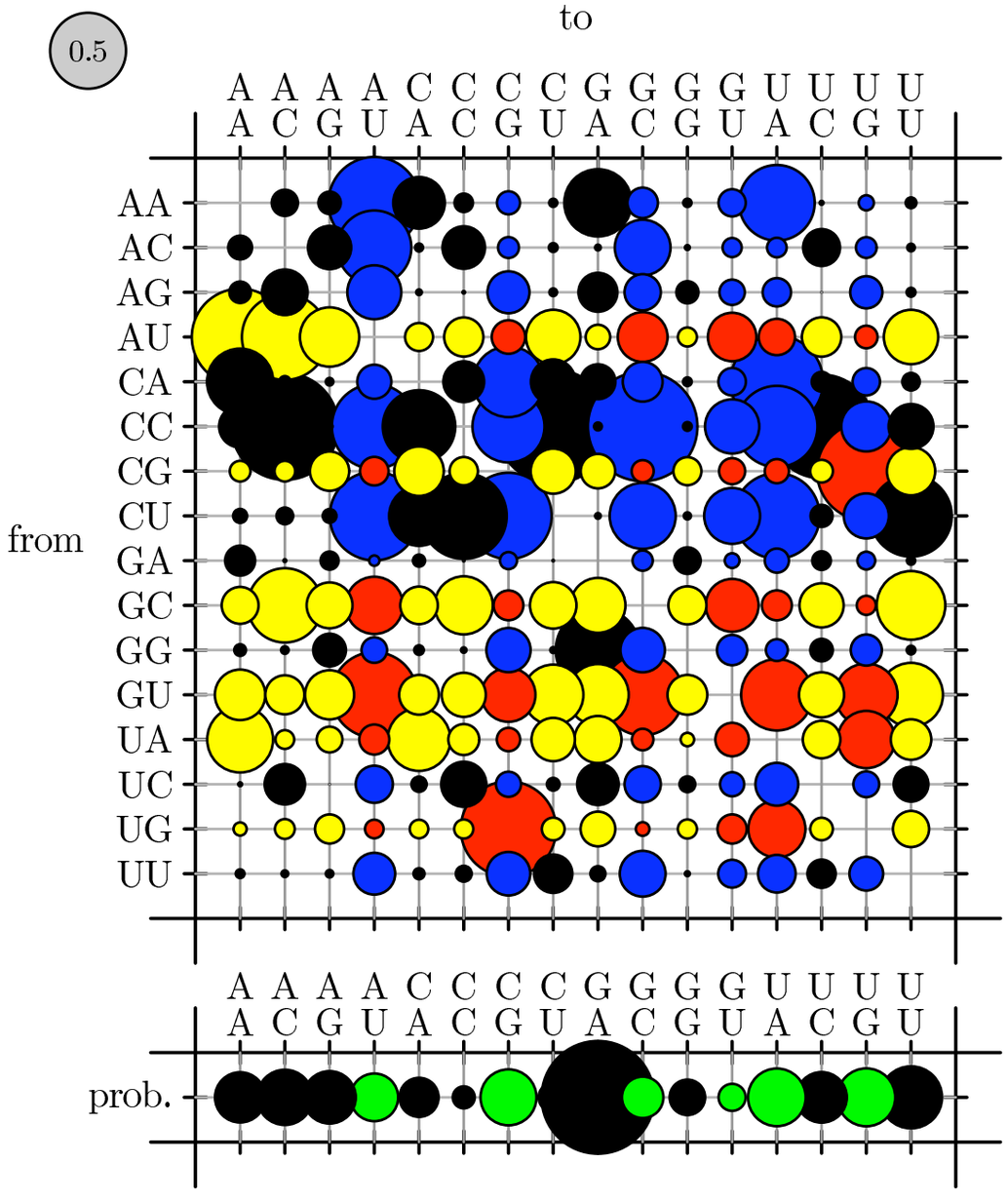
Discovery, alignment & reconstruction of ncRNAs

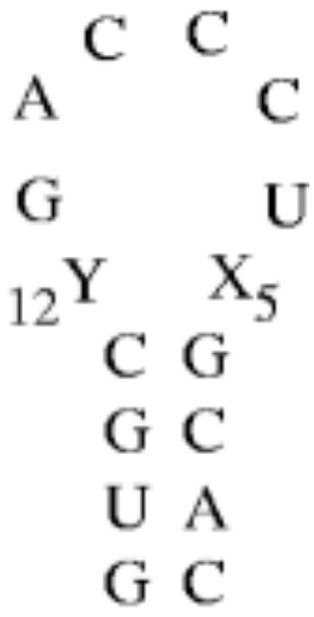
Ian Holmes



Base pairs in stems

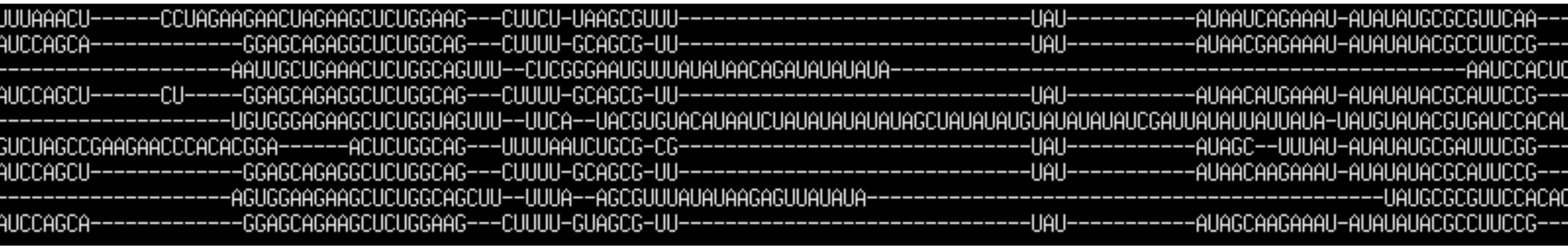


Loop-closing base pairs



False positives

Features of real genome alignments: low-complexity DNA, repetitive sequence, microsatellite expansions, big indels...



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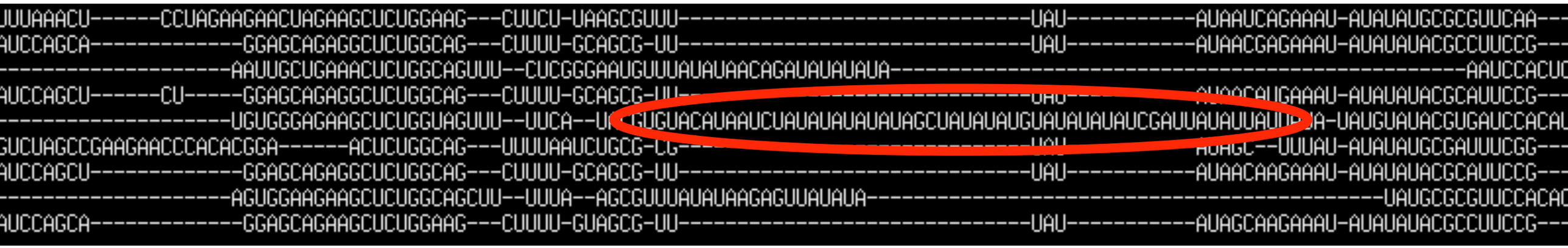

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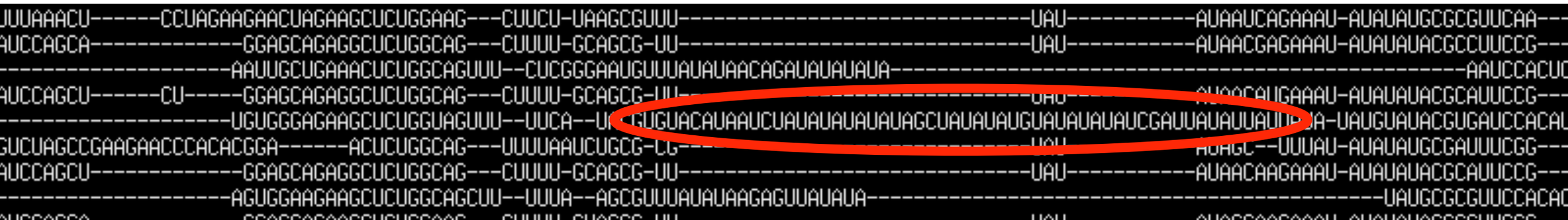

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Dmel_CG_RFAM_INF_49002367 UGGAGCAGAGGCUCUGGCAGCUUUUGCAGCGUUUAUAUAACAUGAAAUAUAUAUACGCAUUCCG
Dsec_GM_RFAM_INF_49004331 UGGAGCAGAGGCUCUGGCAGCUUUUGCAGCGUUUAUAUAACAAGAAAUAUAUAUACGCAUUCCG
Dsim_GD_RFAM_INF_49005002 UGGAGCAGAGGCUCUGGCAGCUUUUGCAGCGUUUAUAUAACAAGAAAUAUAUAUACGCAUUCCG
Dvir_GJ_RFAM_INF_49005646 UGGA__AGAAGCUCUGGCAGCUUUUUAAGCGUUUAUAUAAGA_GUUAUAUAUAUGCGCGUUCCA
#=GC SS_cons              <<<<<<<<<<<<<_____>>>>>>>>>_<<<<_<<<<<<_______>>>>>>_>>>>__>>>>
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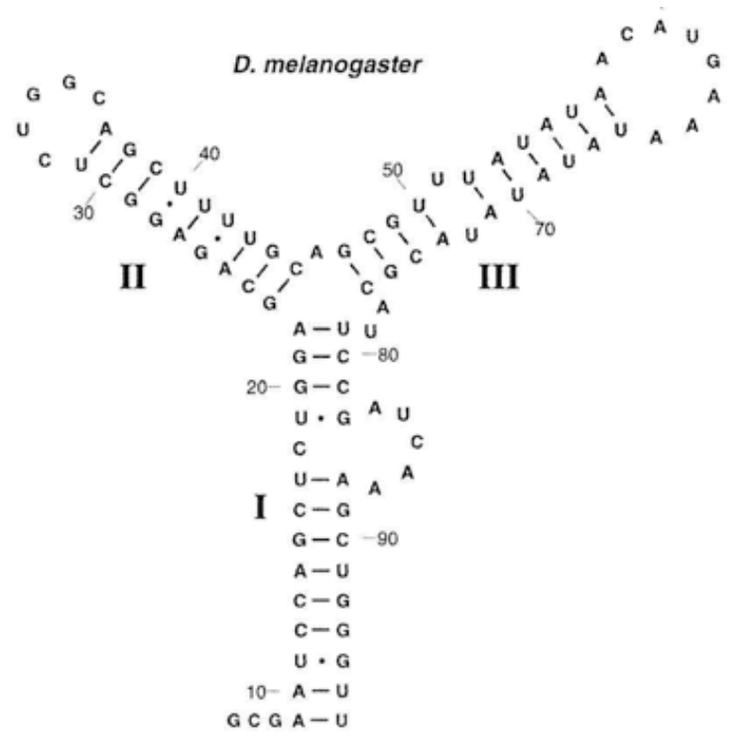

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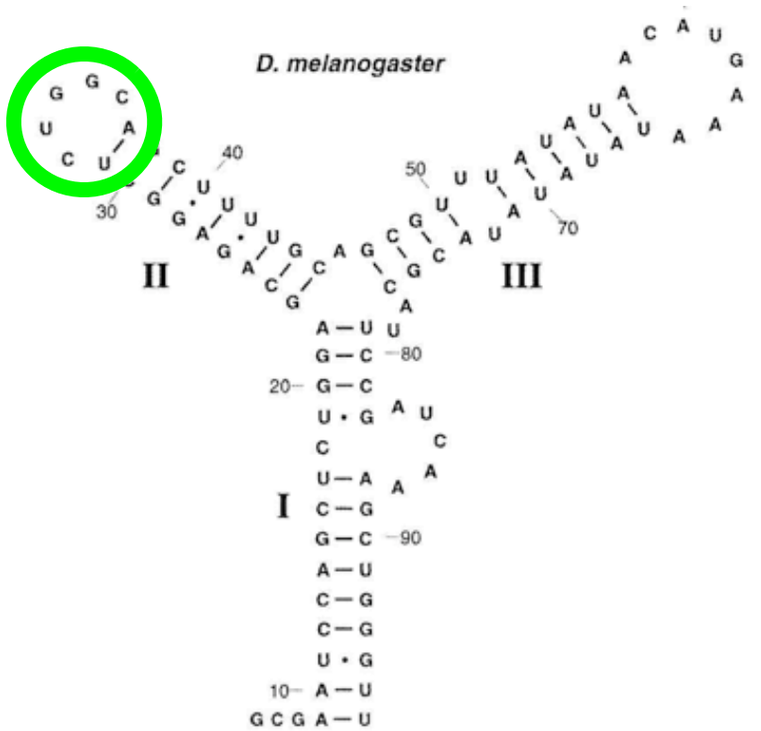

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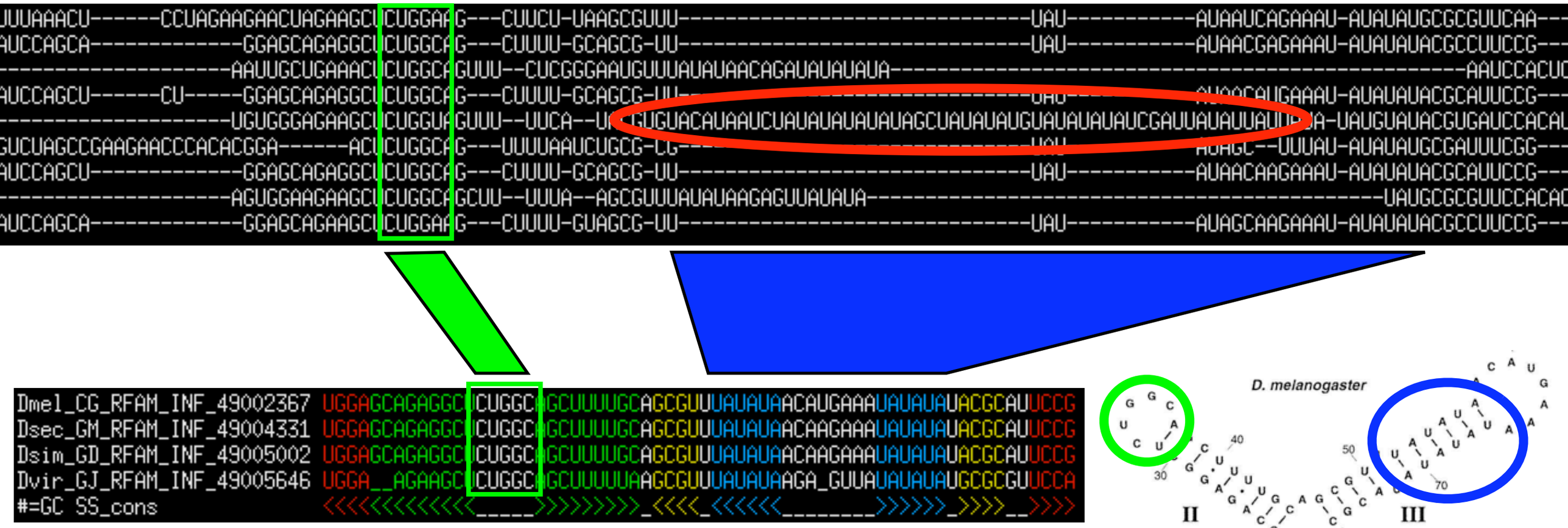

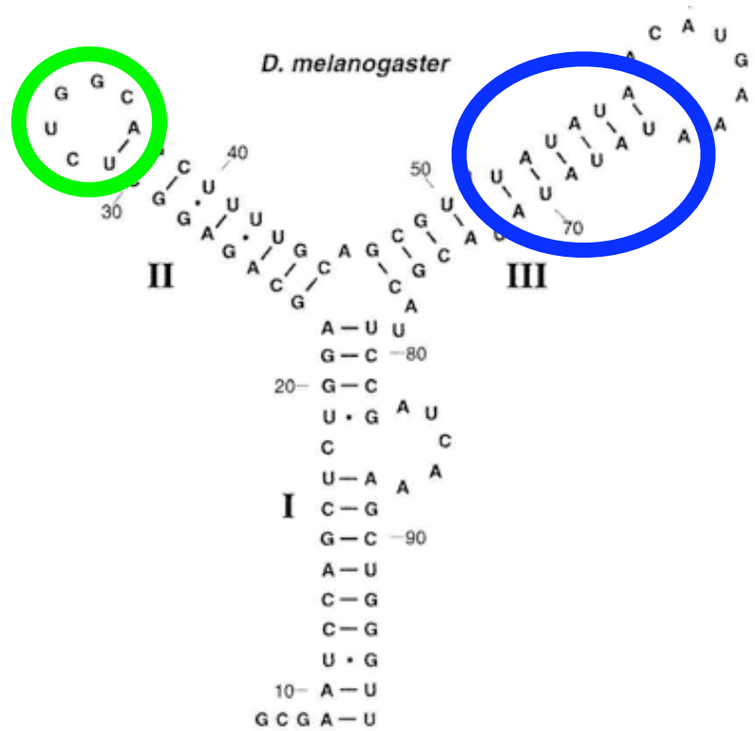

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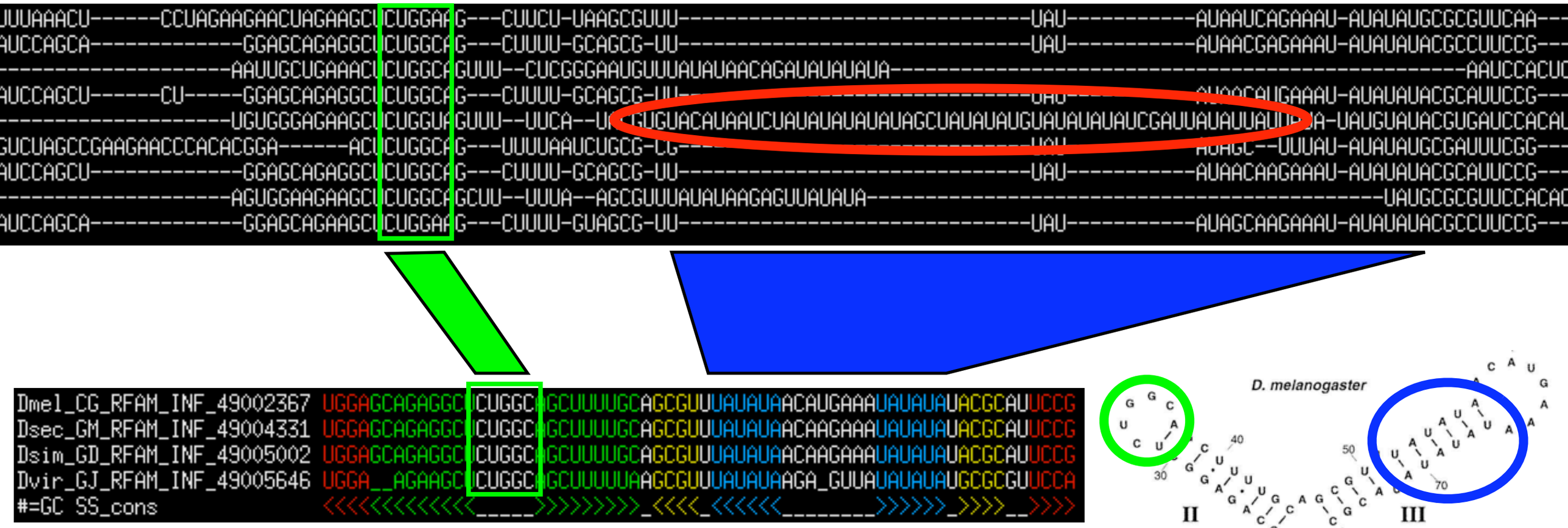
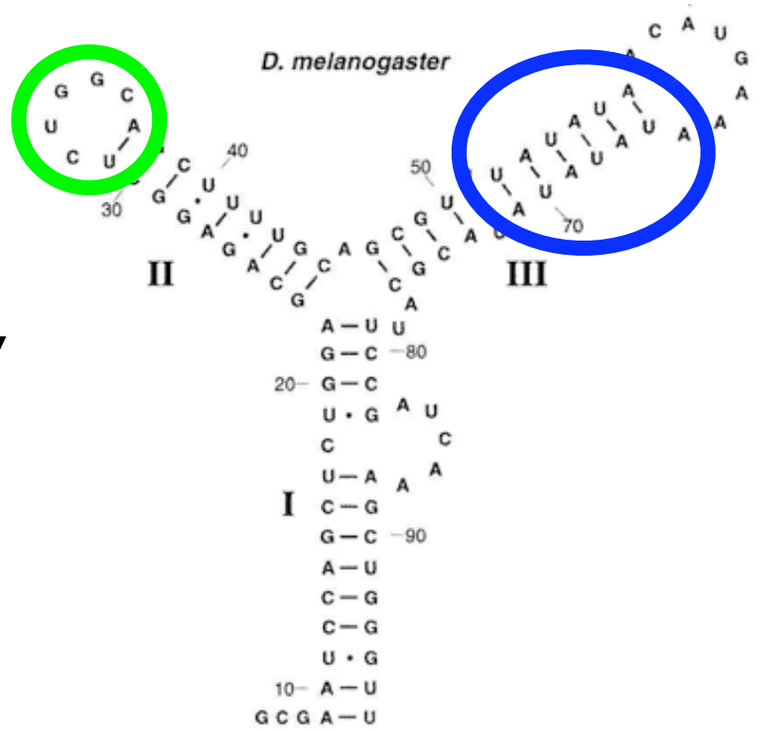
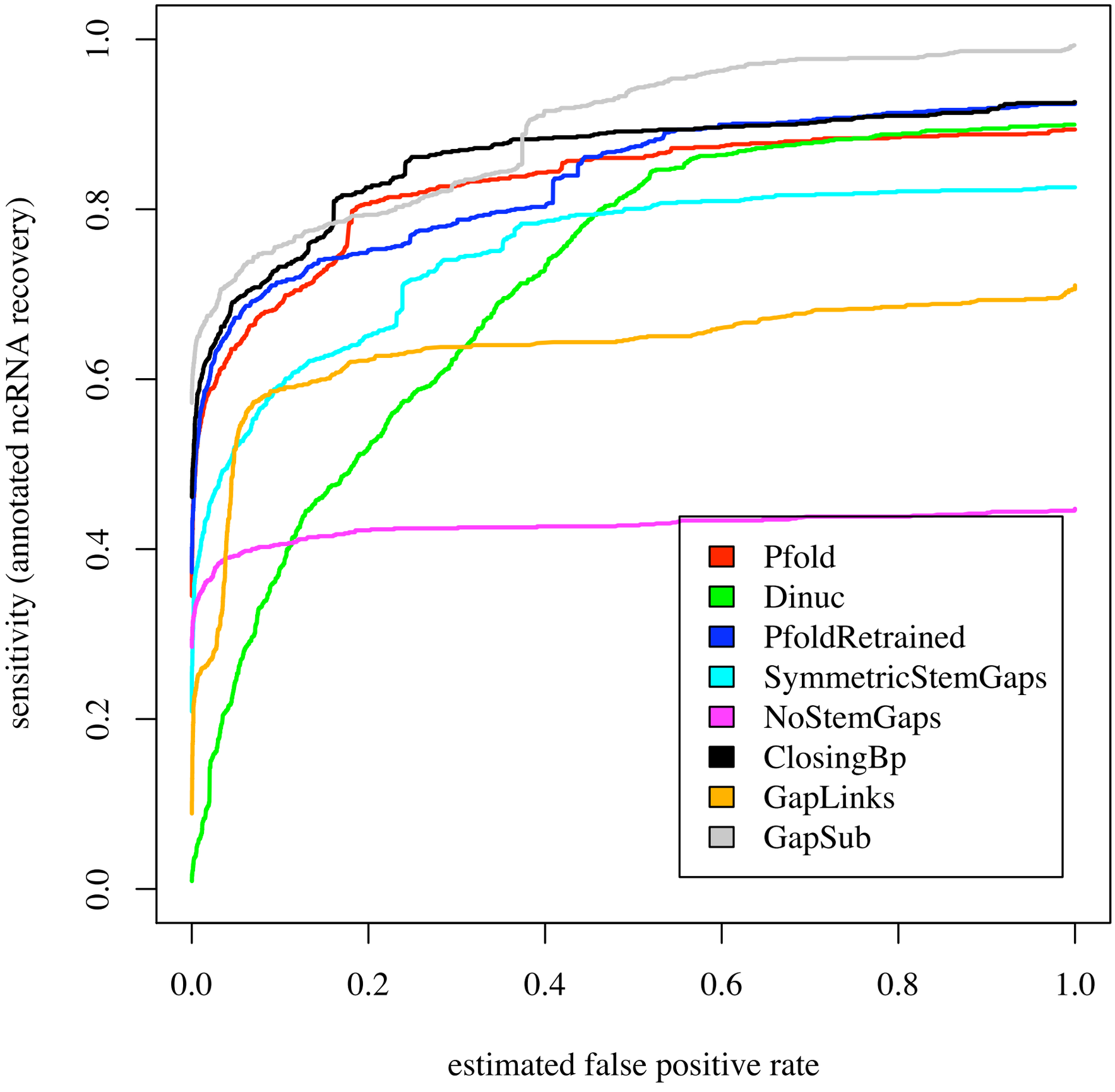
Features of real genome alignments: low-complexity DNA, repetitive sequence, microsatellite expansions, big indels...



We don't want to just mask these out; they could be real. Instead, we simulate such processes, to estimate their effect on our false positive rate.



ROC curves comparing ncRNA genefinding grammars



Simulations: GSIMULATOR

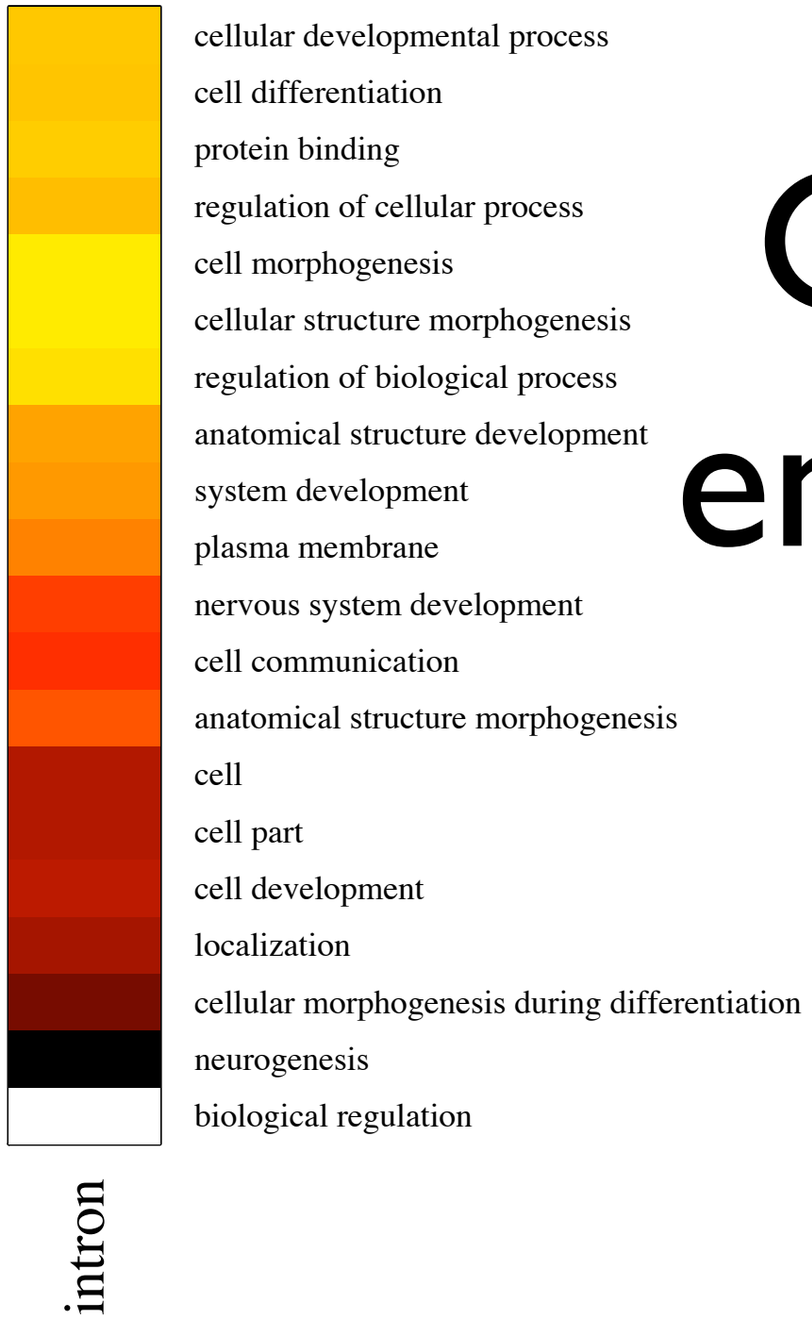
Genome-wide scan, ClosingBp

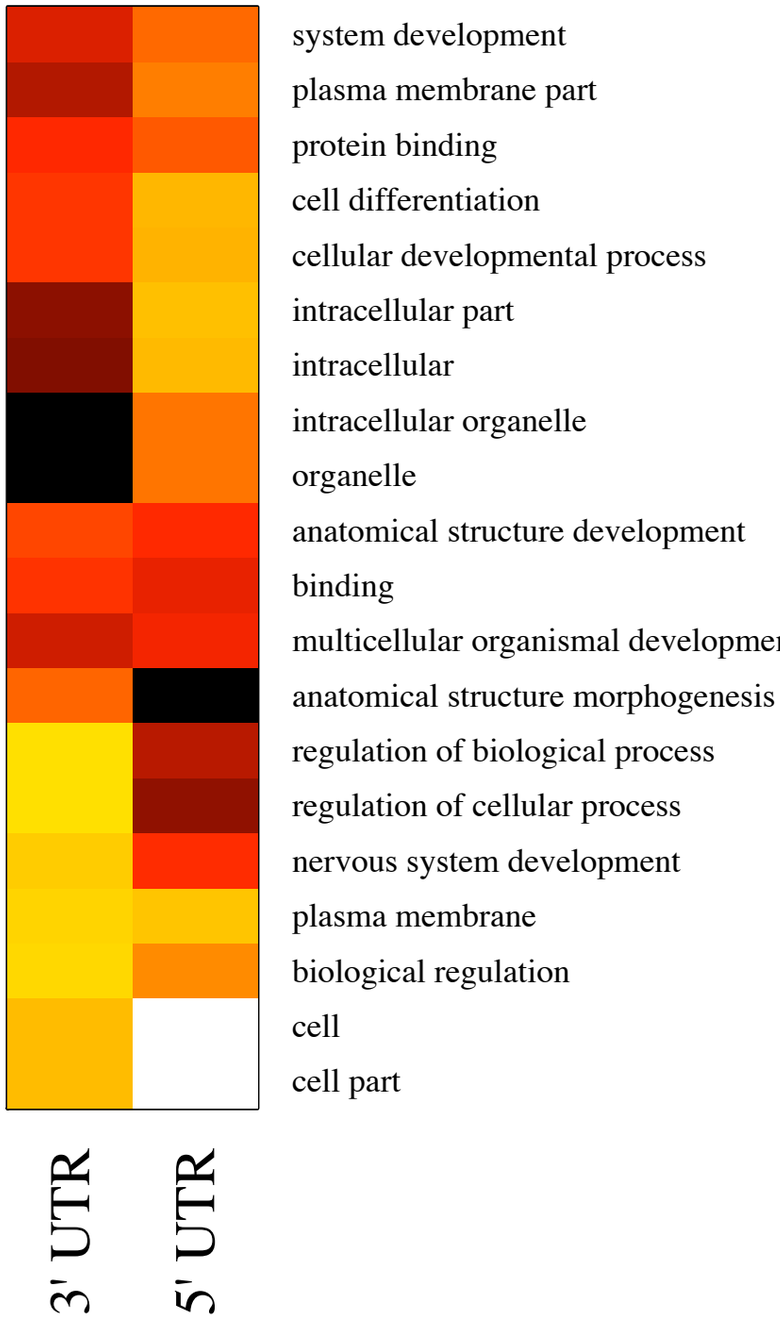
	Predicted	Estimated false +ves
Putative genes	**1,949**	1%
In 3' UTRs	1,788	2%
In 5' UTRs	2,252	1%
In CDS	37,758	n/a
In pseudogenes	13	3%

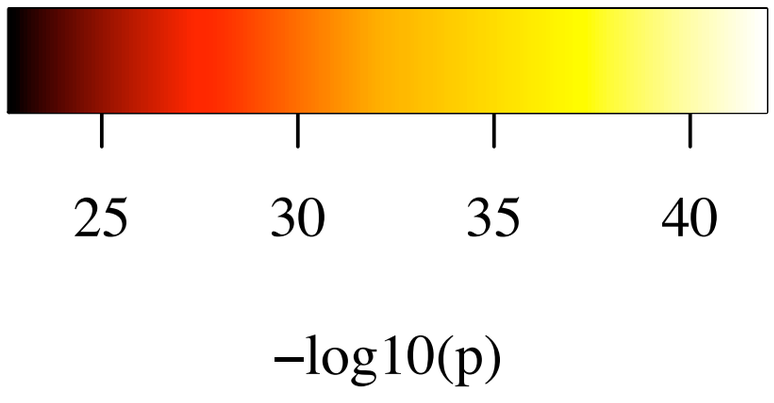
GROSS under-estimates!

Filtered by max gappiness, min number of species in alignment, min number of basepairs, min number of compensatory mutations; intergenic hits (predicted "genes") further restricted by intersection w/Affy transfrag

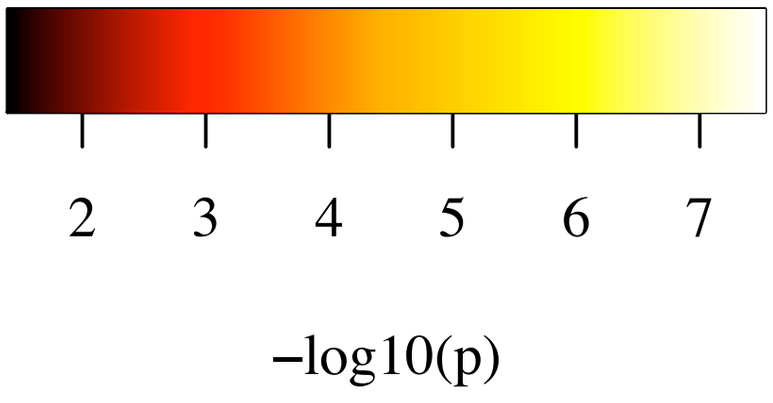
GO term enrichment



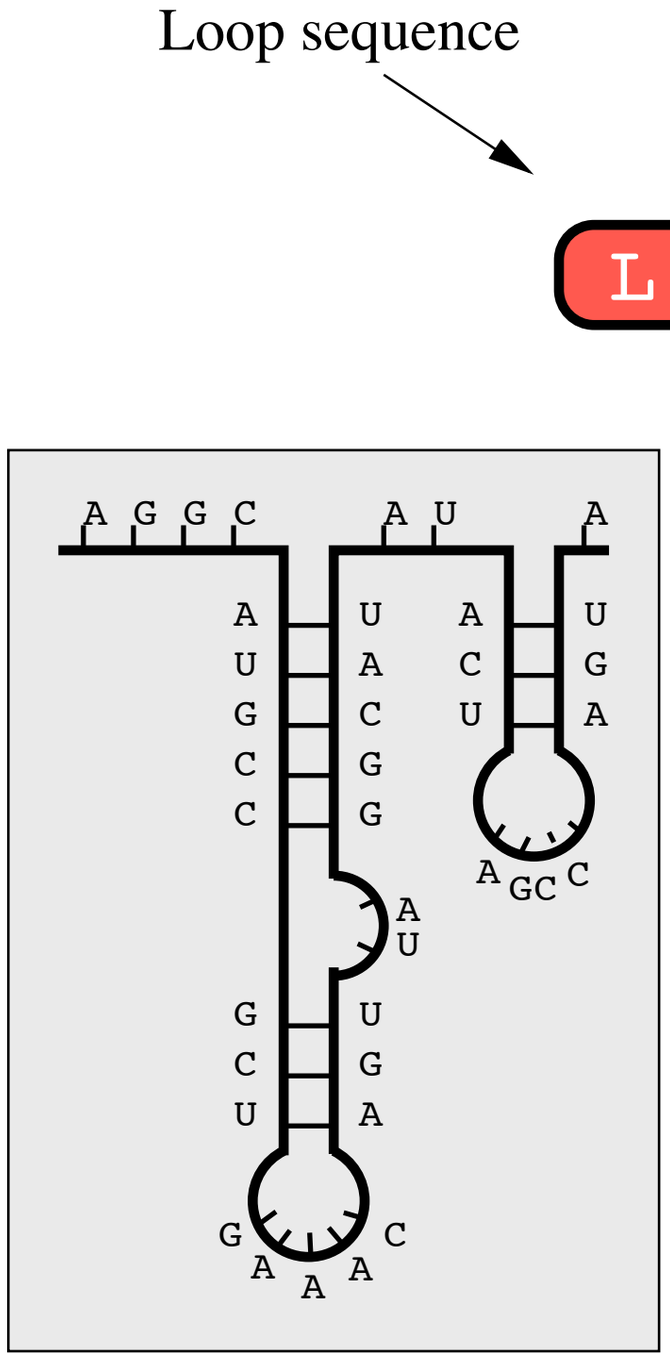


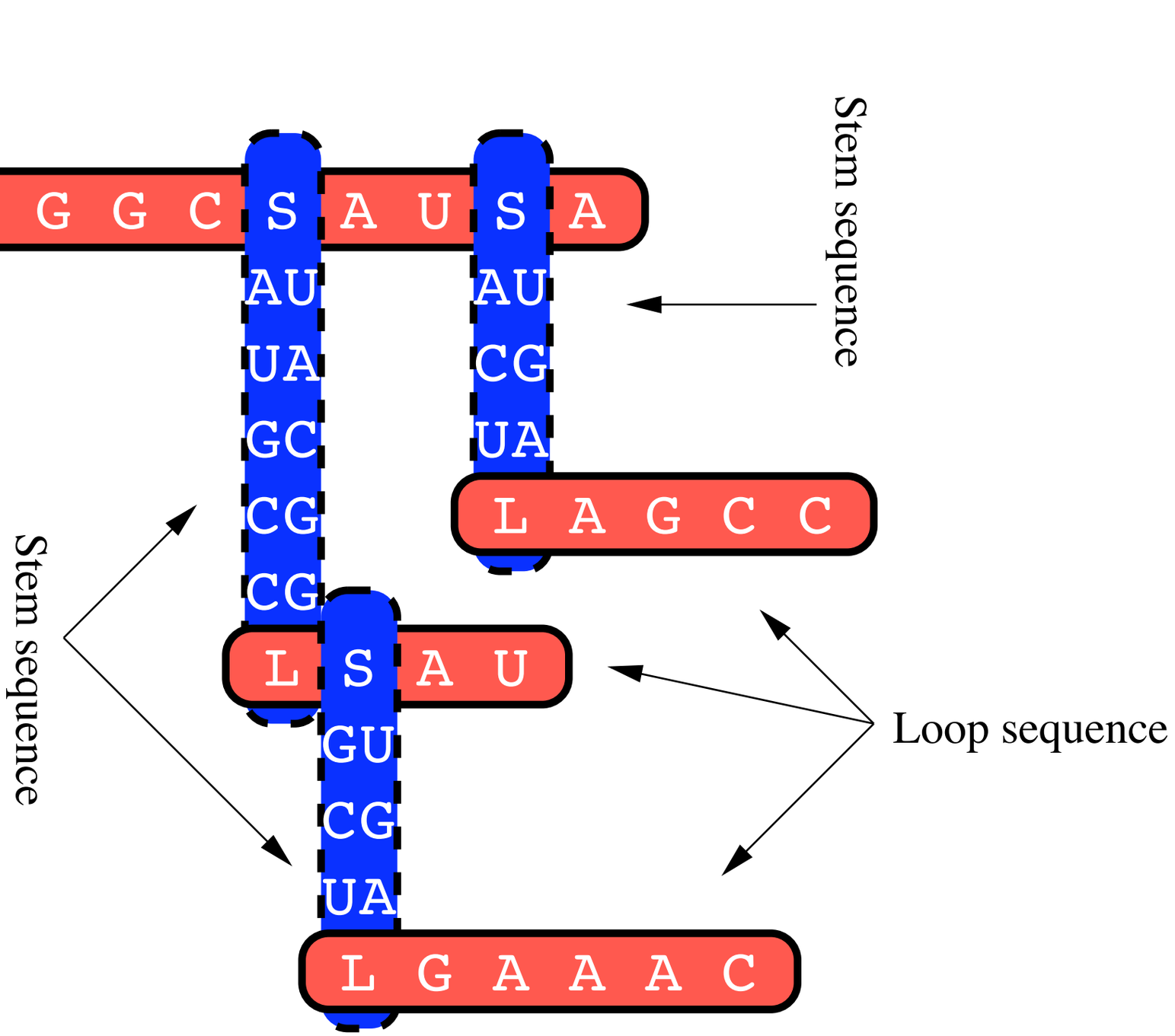


GO::TermFinder
(Gavin Sherlock)



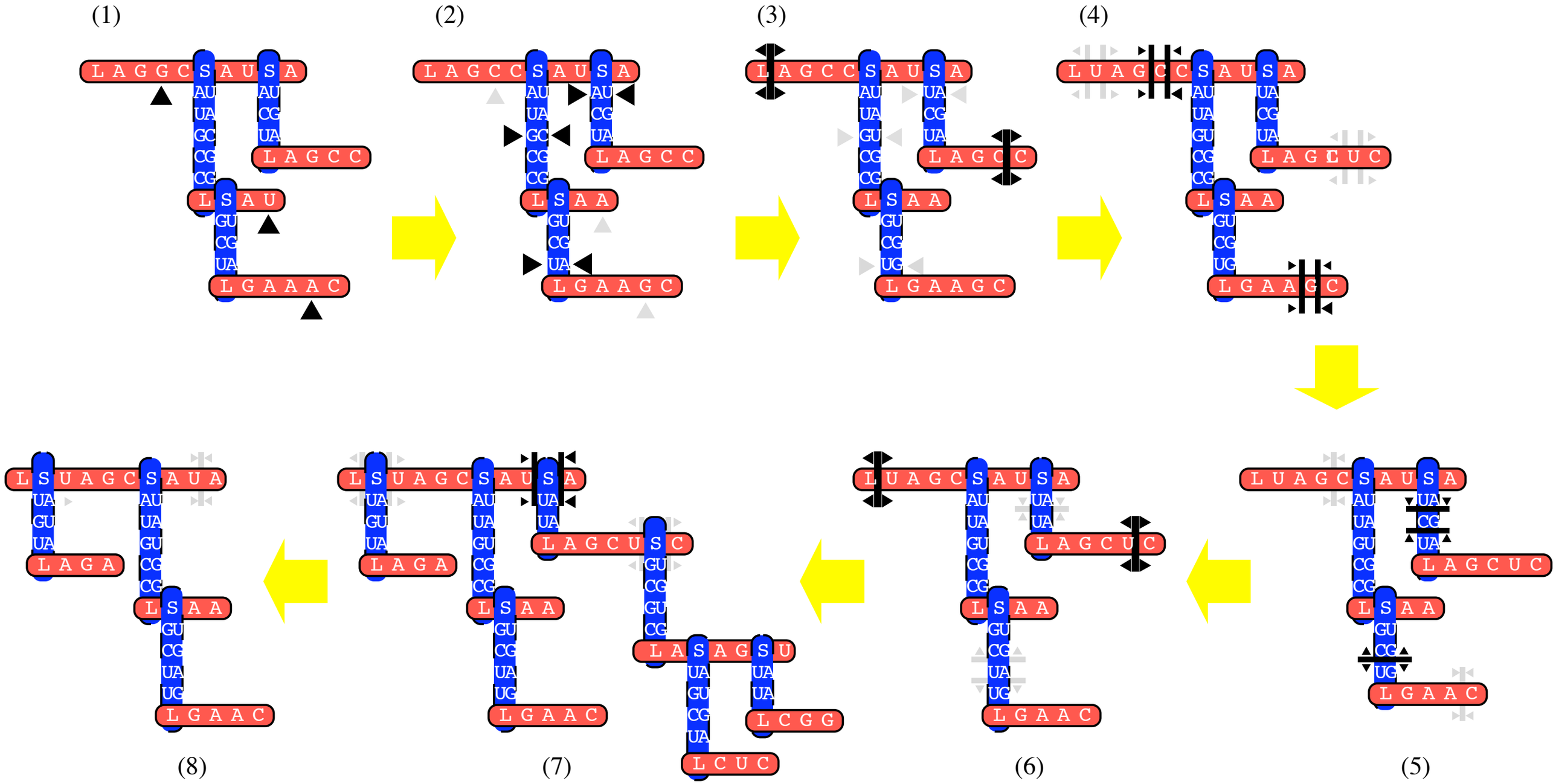
Evolution of RNA structure





Holmes, 2004

Allowed mutations



Structure Tree pair rules

Rule	lhs	→	rhs	$P(a)$	$P(d\|a)$
1.	L_1	→	$X_a Y_d L_1$	$\kappa_1 p_1(X)$	$(1-\beta_1)\alpha_1 M_1(X,Y)$
2.		|	$Y_d L_1$	1	$\beta_1 p_1(Y)$
3.		|	$X_a L_2$	$\kappa_1 p_1(X)$	$(1-\beta_1)(1-\alpha_1)$
4.		|	$S_1 L_1$	$\kappa_1 p_1(S)$	$(1-\beta_1)\alpha_1$
5.		|	$S_4 L_1$	1	$\beta_1 p_1(S)$
6.		|	$S_3 L_2$	$\kappa_1 p_1(S)$	$(1-\beta_1)(1-\alpha_1)$
7.		|	ϵ	$1-\kappa_1$	$1-\beta_1$
8.	S_1	→	$W_a Y_d S_1 Z_d X_a$	$\kappa_2 p_2(WX)$	$(1-\beta_2)\alpha_2 M_2(WX,YZ)$
9.		|	$Y_d S_1 Z_d$	1	$\beta_2 p_2(YZ)$
10.		|	$W_a S_2 X_a$	$\kappa_2 p_2(WX)$	$(1-\beta_2)(1-\alpha_2)$
11.		|	L_1	$1-\kappa_2$	$1-\beta_2$
12.	L_2	→	$X_a Y_d L_1$	$\kappa_1 p_1(X)$	$(1-\gamma_1)\alpha_1 M_1(X,Y)$
13.		|	$Y_d L_1$	1	$\gamma_1 p_1(Y)$
14.		|	$X_a L_2$	$\kappa_1 p_1(X)$	$(1-\gamma_1)(1-\alpha_1)$
15.		|	$S_1 L_1$	$\kappa_1 p_1(S)$	$(1-\gamma_1)\alpha_1$
16.		|	$S_4 L_1$	1	$\gamma_1 p_1(S)$
17.		|	$S_3 L_2$	$\kappa_1 p_1(S)$	$(1-\gamma_1)(1-\alpha_1)$
18.		|	ϵ	$1-\kappa_1$	$1-\gamma_1$
19.	S_2	→	$W_a Y_d S_1 Z_d X_a$	$\kappa_2 p_2(WX)$	$(1-\gamma_2)\alpha_2 M_2(WX,YZ)$
20.		|	$Y_d S_1 Z_d$	1	$\gamma_2 p_2(YZ)$
21.		|	$W_a S_2 X_a$	$\kappa_2 p_2(WX)$	$(1-\gamma_2)(1-\alpha_2)$
22.		|	L_1	$1-\kappa_2$	$1-\gamma_2$
23.	L_3	→	$X_a L_3$	$\kappa_1 p_1(X)$	1
24.		|	$S_3 L_3$	$\kappa_1 p_1(S)$	1
25.		|	ϵ	$1-\kappa_1$	1
26.	S_3	→	$W_a S_3 X_a$	$\kappa_2 p_2(WX)$	1
27.		|	L_3	$1-\kappa_2$	1
28.	L_4	→	$Y_d L_4$	1	$\kappa_1 p_1(Y)$
29.		|	$S_4 L_4$	1	$\kappa_1 p_1(S)$
30.		|	ϵ	1	$1-\kappa_1$
31.	S_4	→	$Y_d S_4 Z_d$	1	$\kappa_2 p_2(YZ)$
32.		|	L_4	1	$1-\kappa_2$

Pair SCFG

$$\alpha_n = \exp(-\mu_n t)$$

$$\beta_n = \frac{\lambda_n(1-\exp((\lambda_n-\mu_n)t))}{\mu_n - \lambda_n \exp((\lambda_n-\mu_n)t)}$$

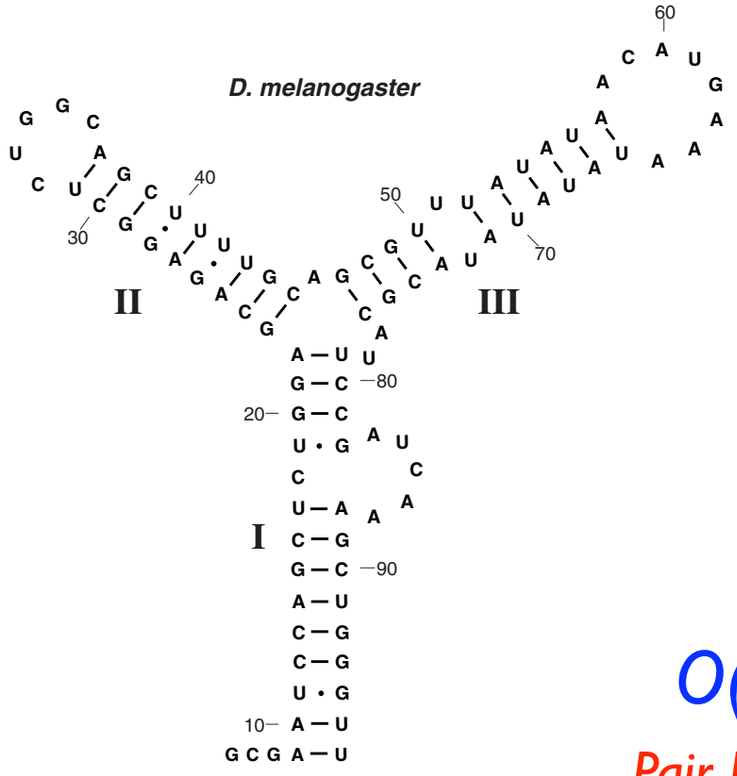
$$\gamma_n = 1 - \frac{\mu_n(1-\exp((\lambda_n-\mu_n)t))}{(1-\exp(-\mu_n t))(\mu_n - \lambda_n \exp((\lambda_n-\mu_n)t))}$$

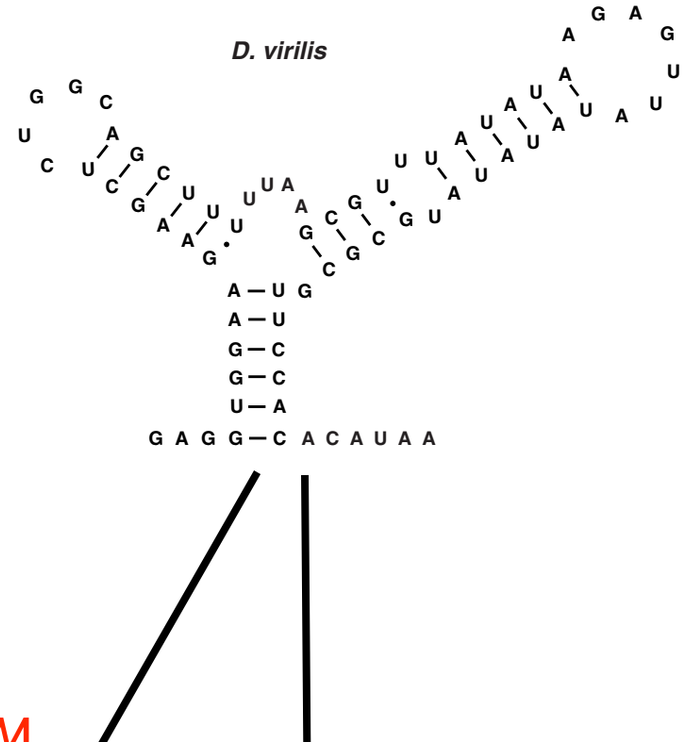
$$\kappa_n = \lambda_n / \mu_n$$

$$M_n(i,j) = \exp(\mathbf{R}_n t)_{ij}$$

Holmes, 2004

Constrained Sankoff





$O(L^2)$

Pair HMM

Single sequence folding

Pairwise primary alignment

Single sequence folding

$O(L^3)$

Single SCFG

$O(L^6)$

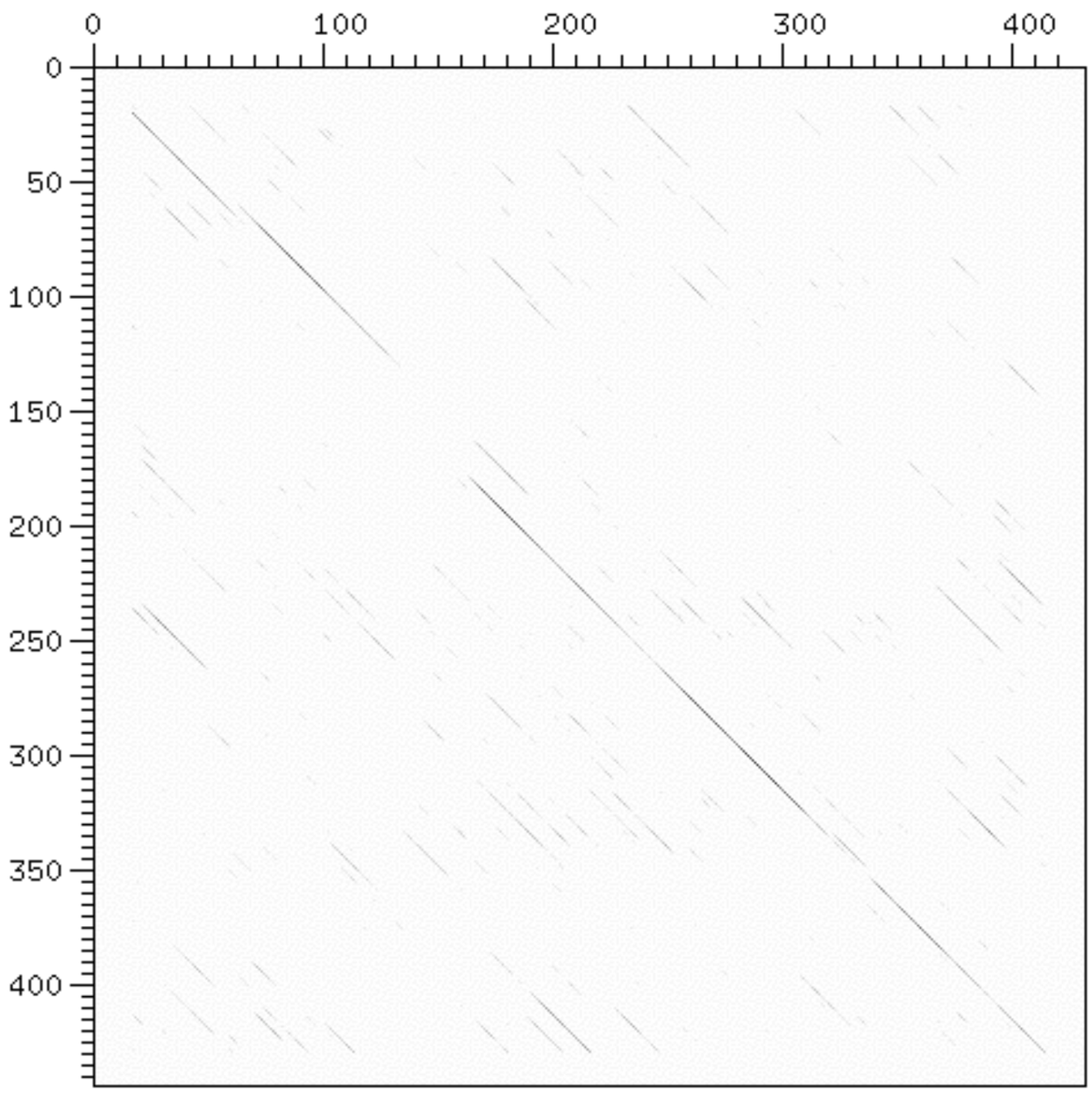
Pair SCFG

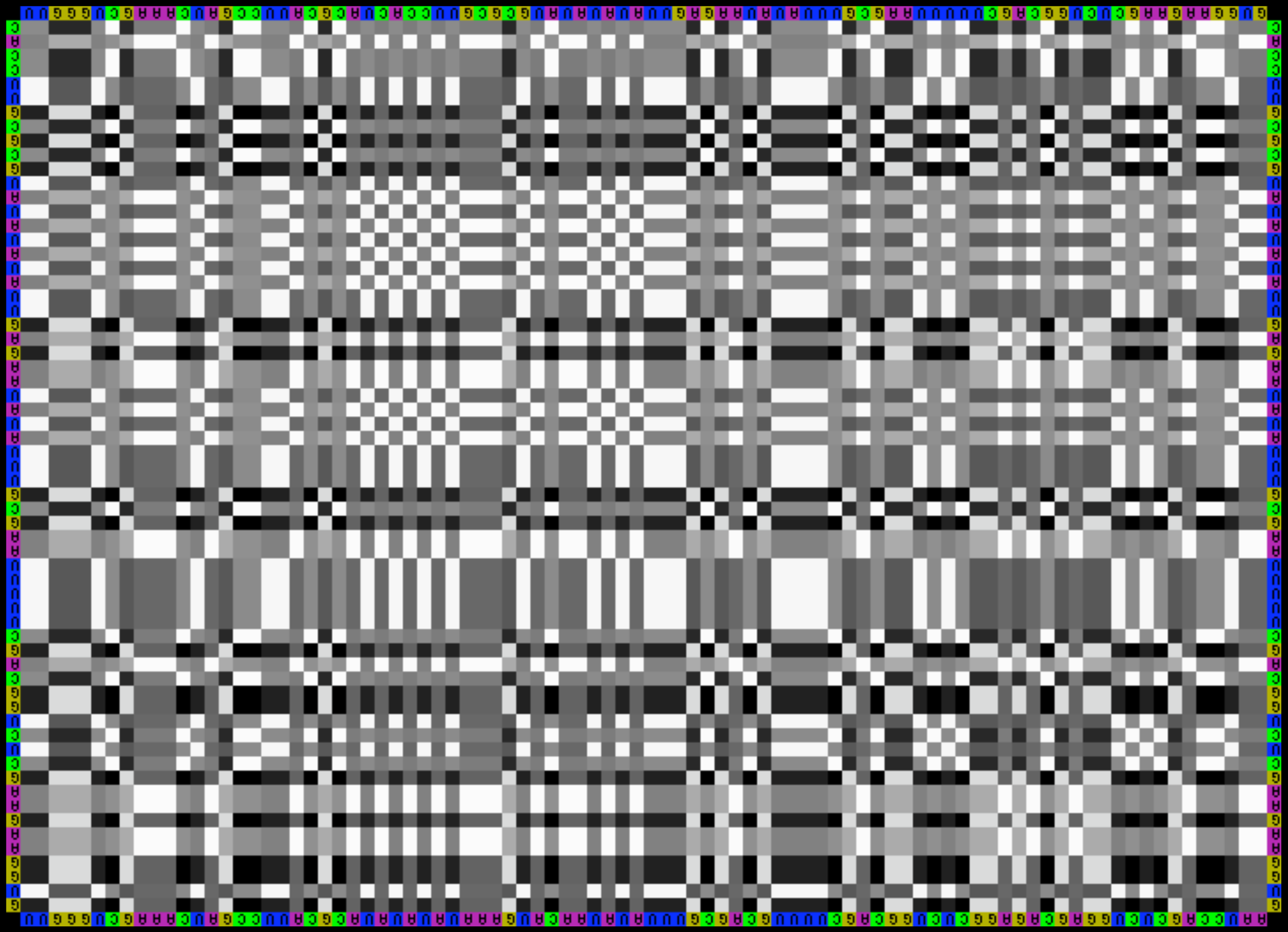
Pairwise alignment & folding

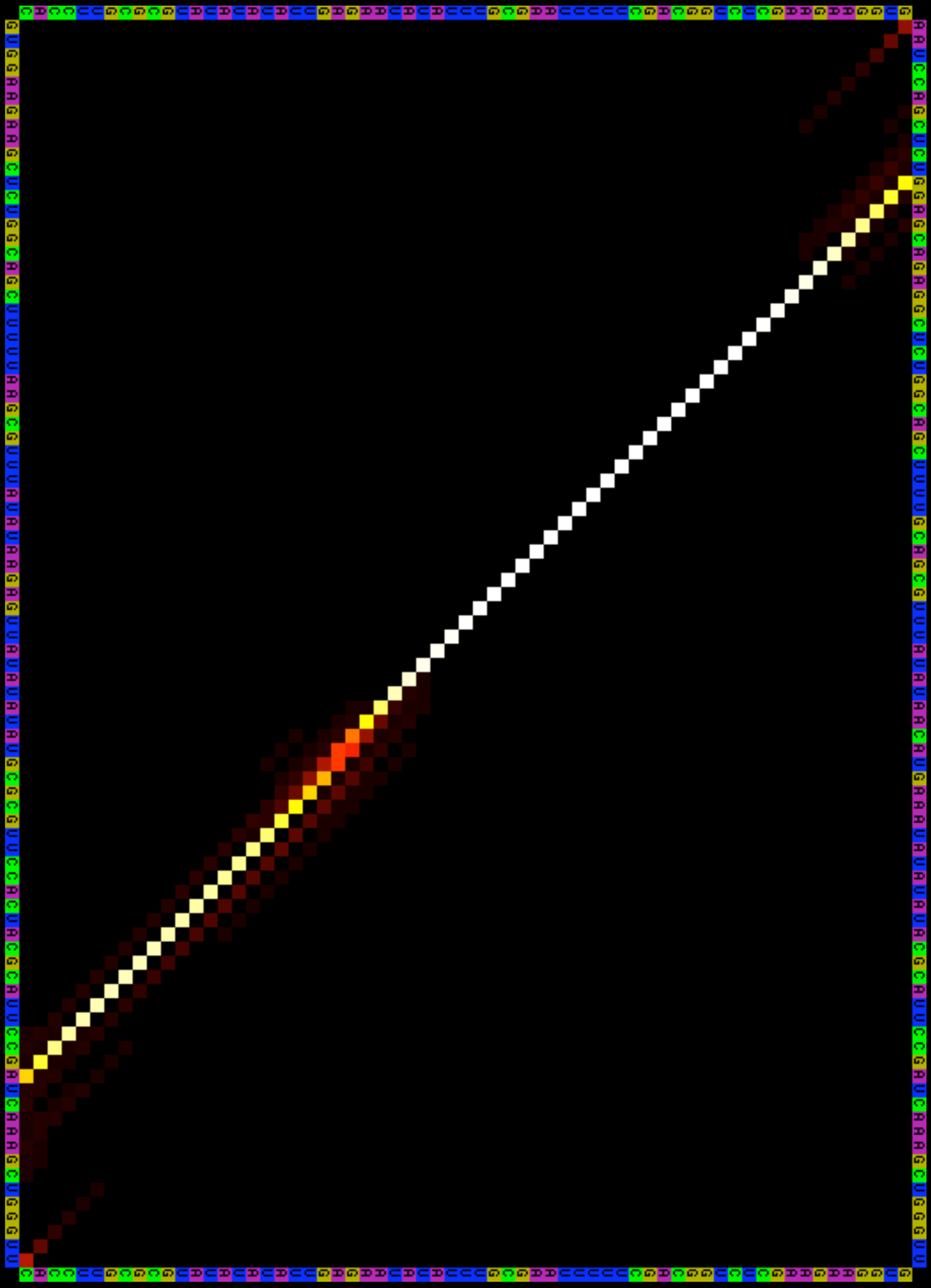
Likelihood, branch length

Multiple sequence alignment

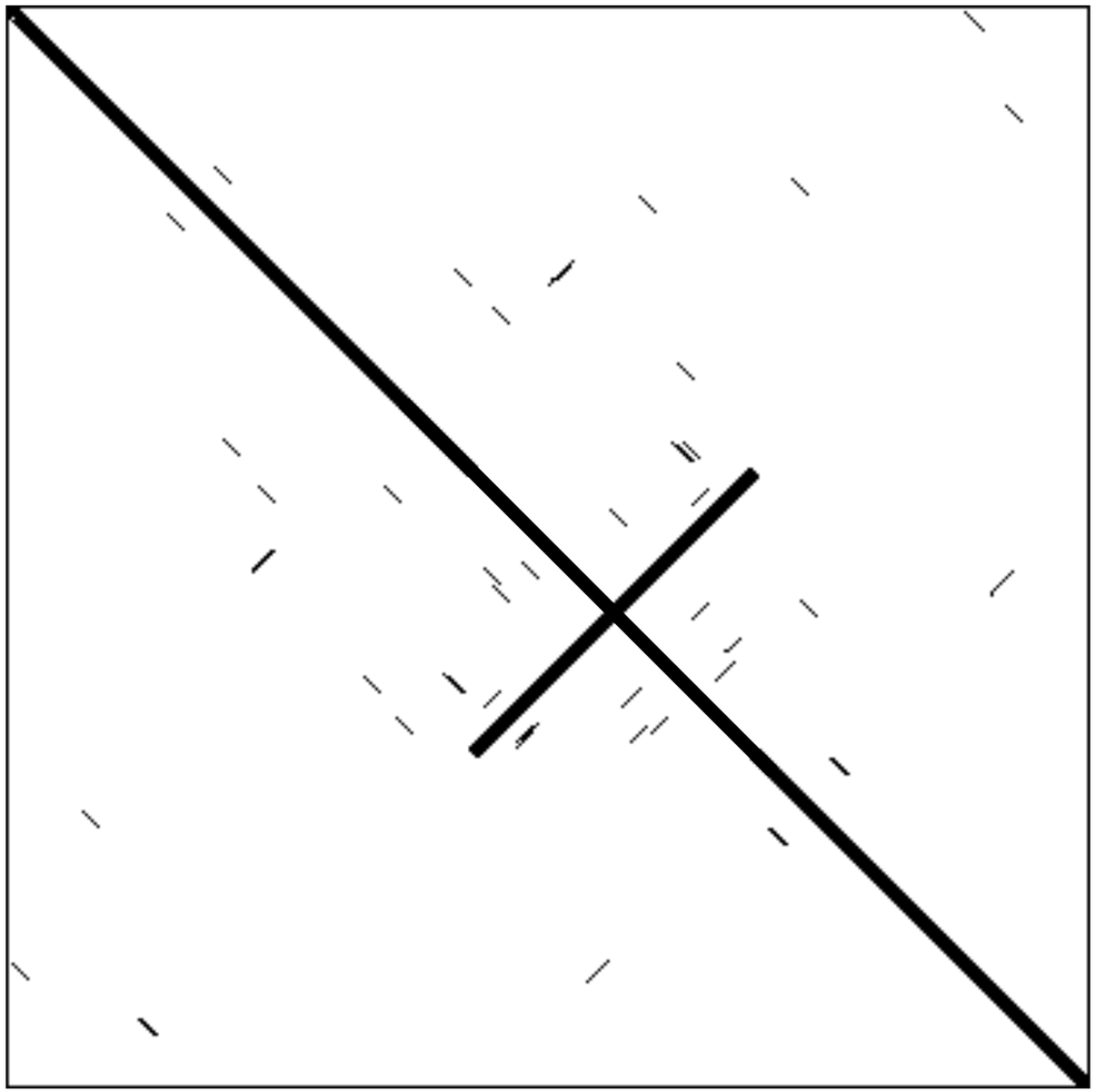
Dotplots

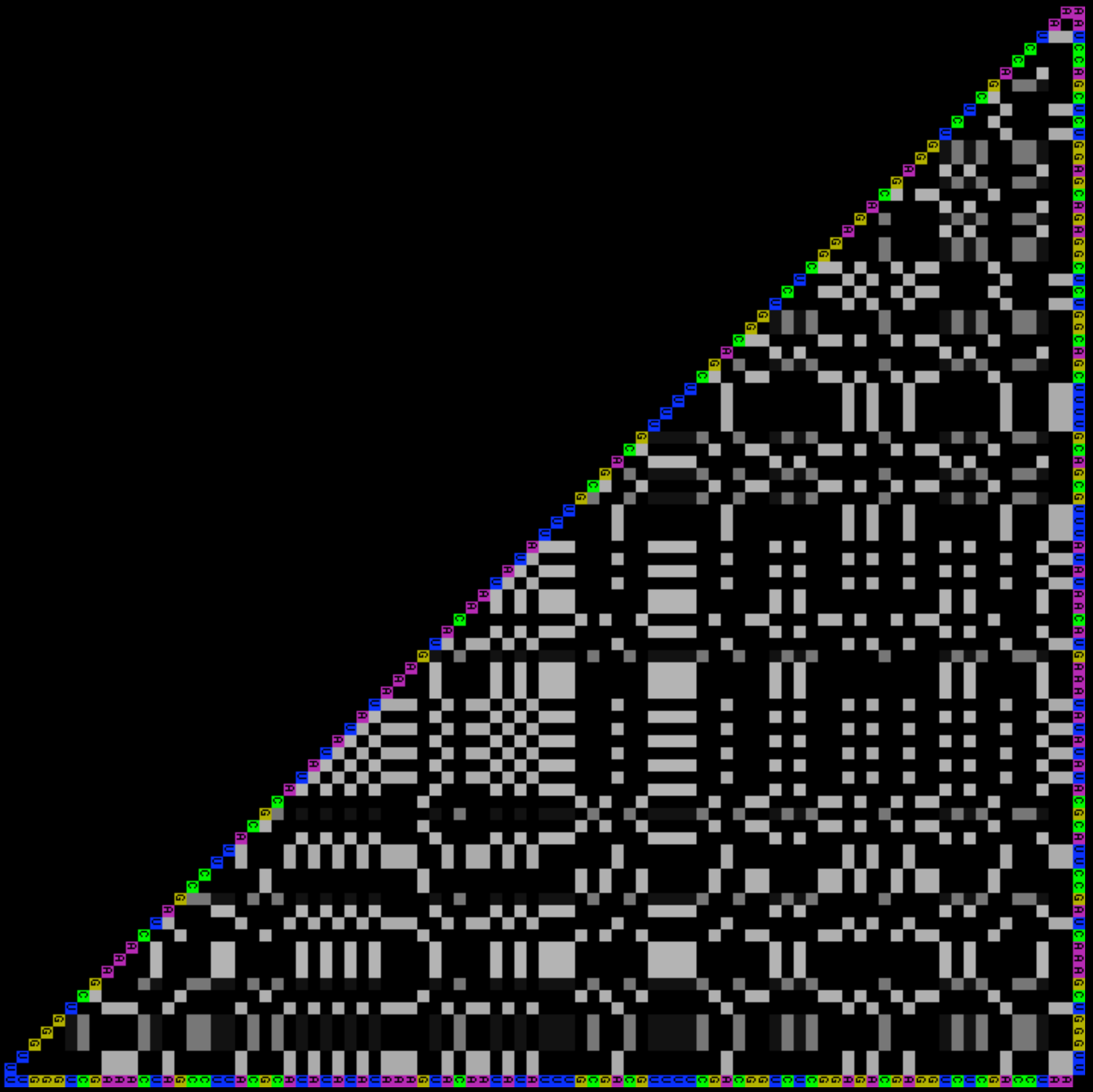




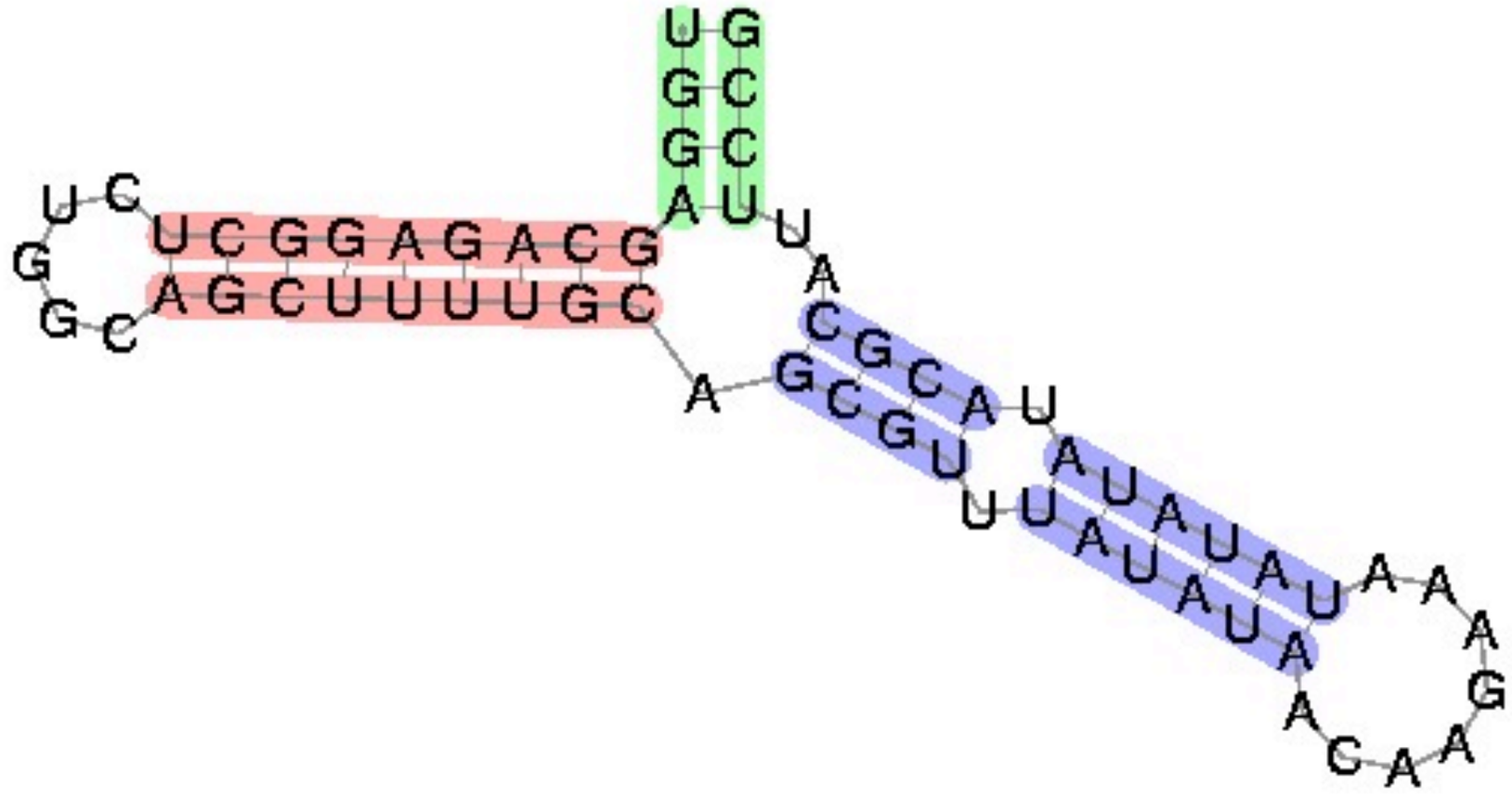


Dotplot vs. self: inverted repeat





nanos TCE


U G
G C
G C
A U
U C
U C G G A G A C G
G A G C U U U U G C
G C
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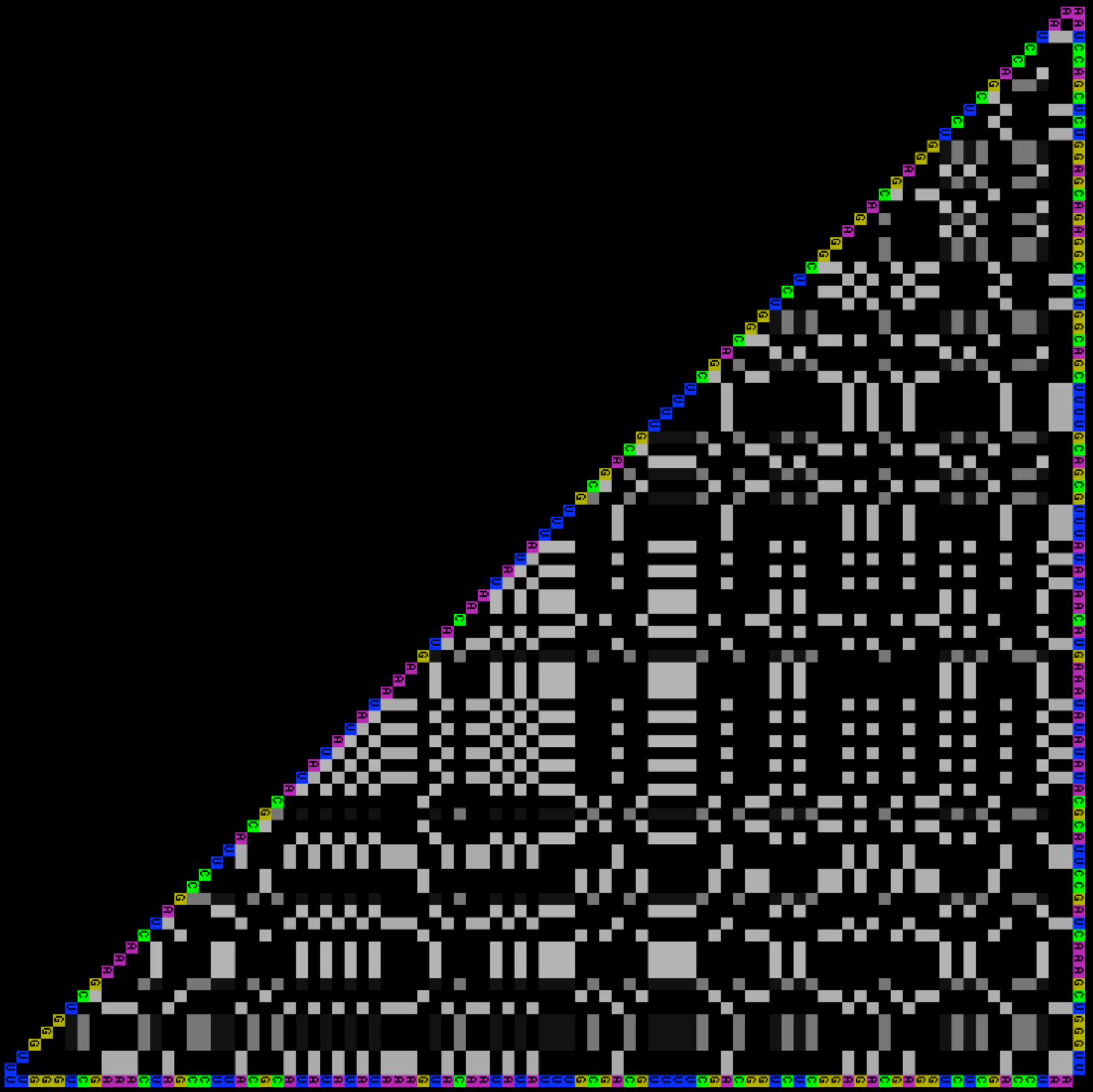
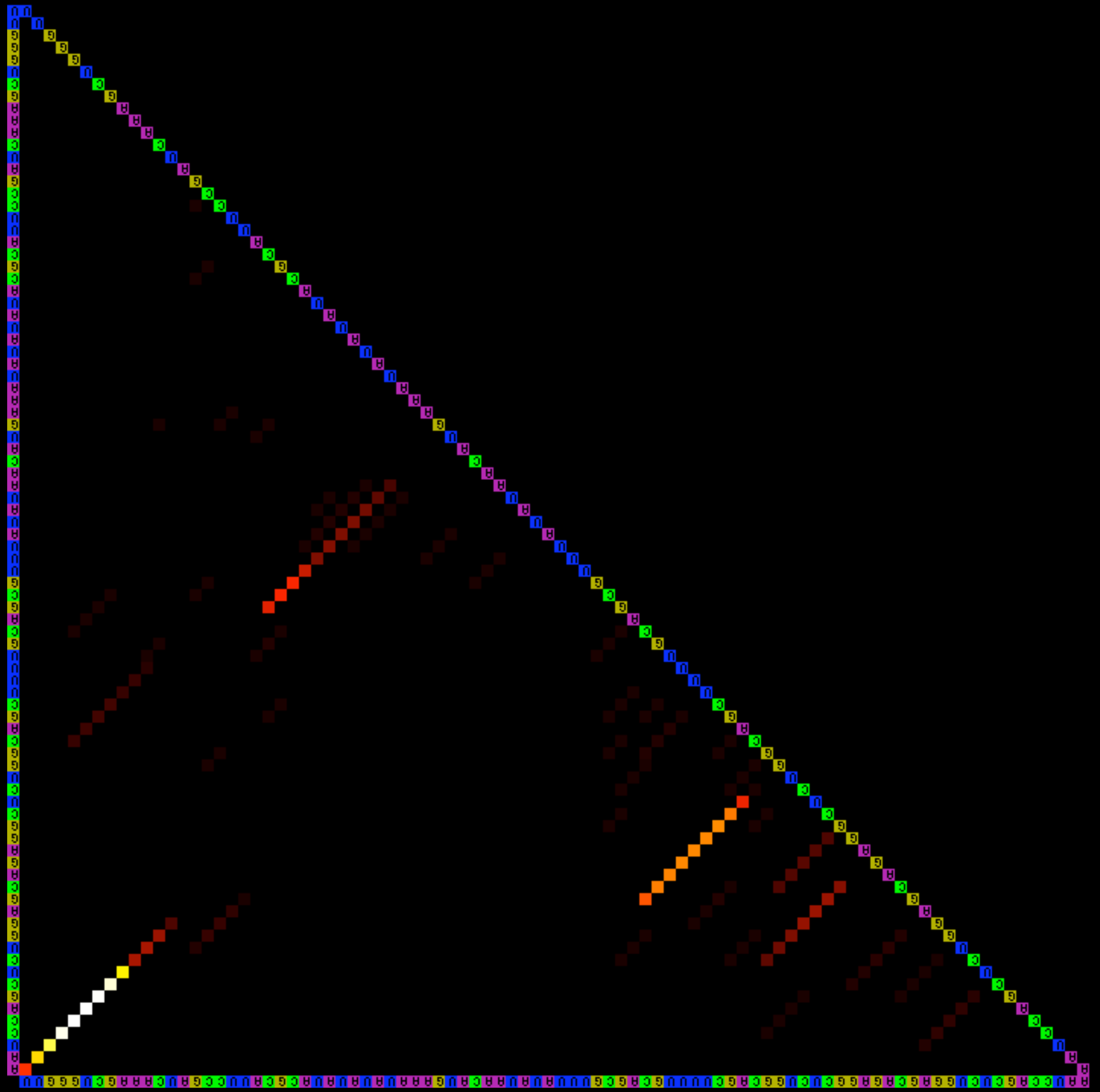
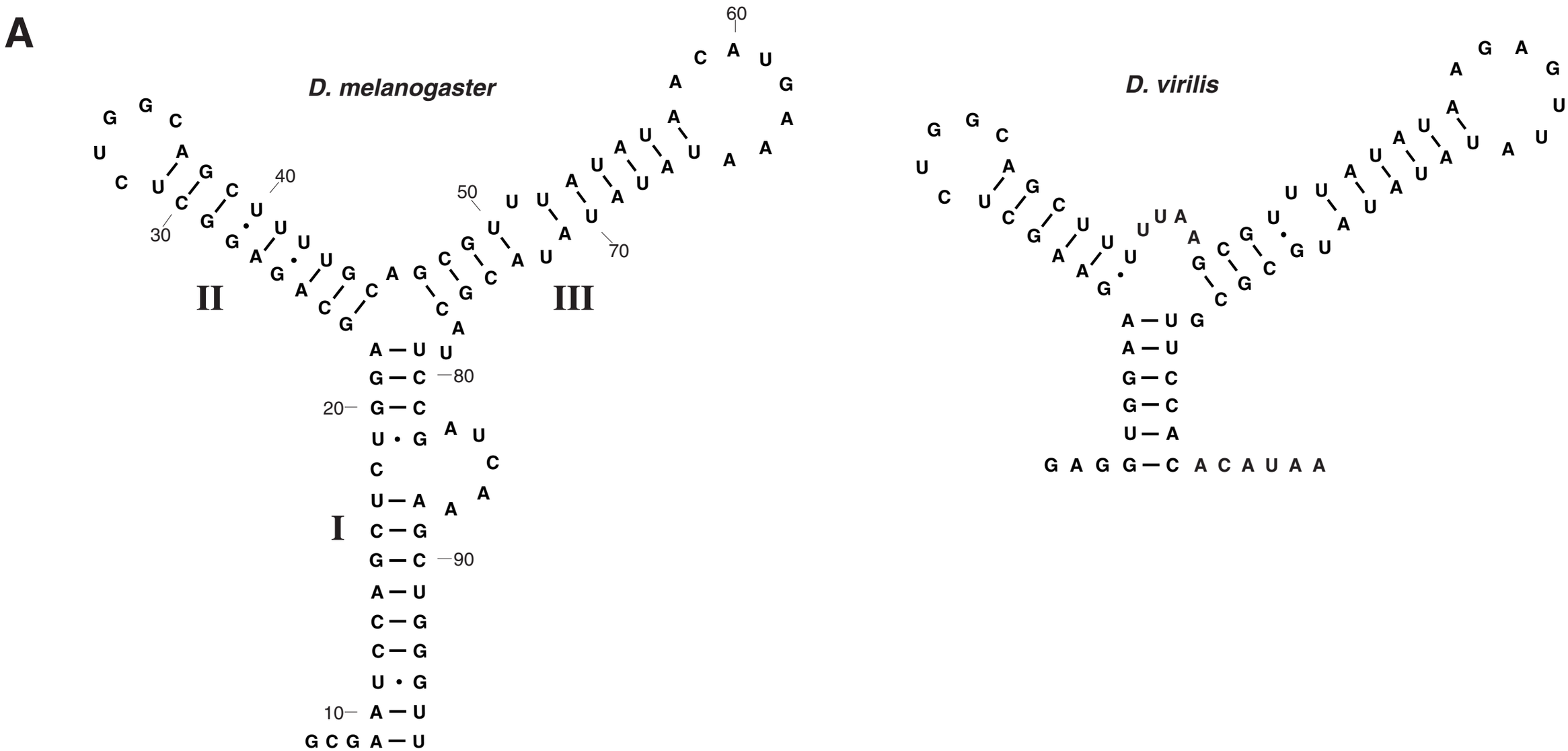
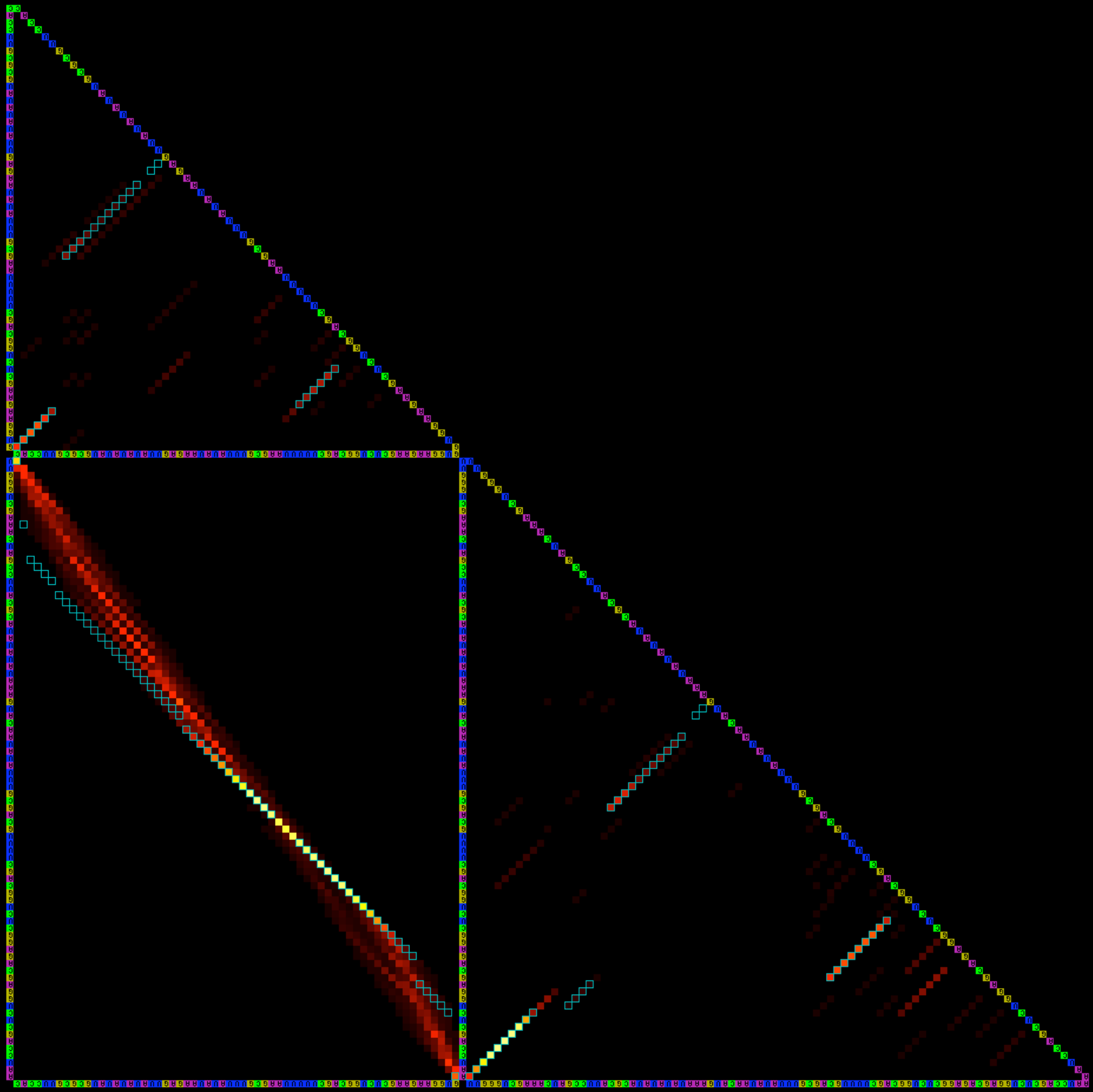
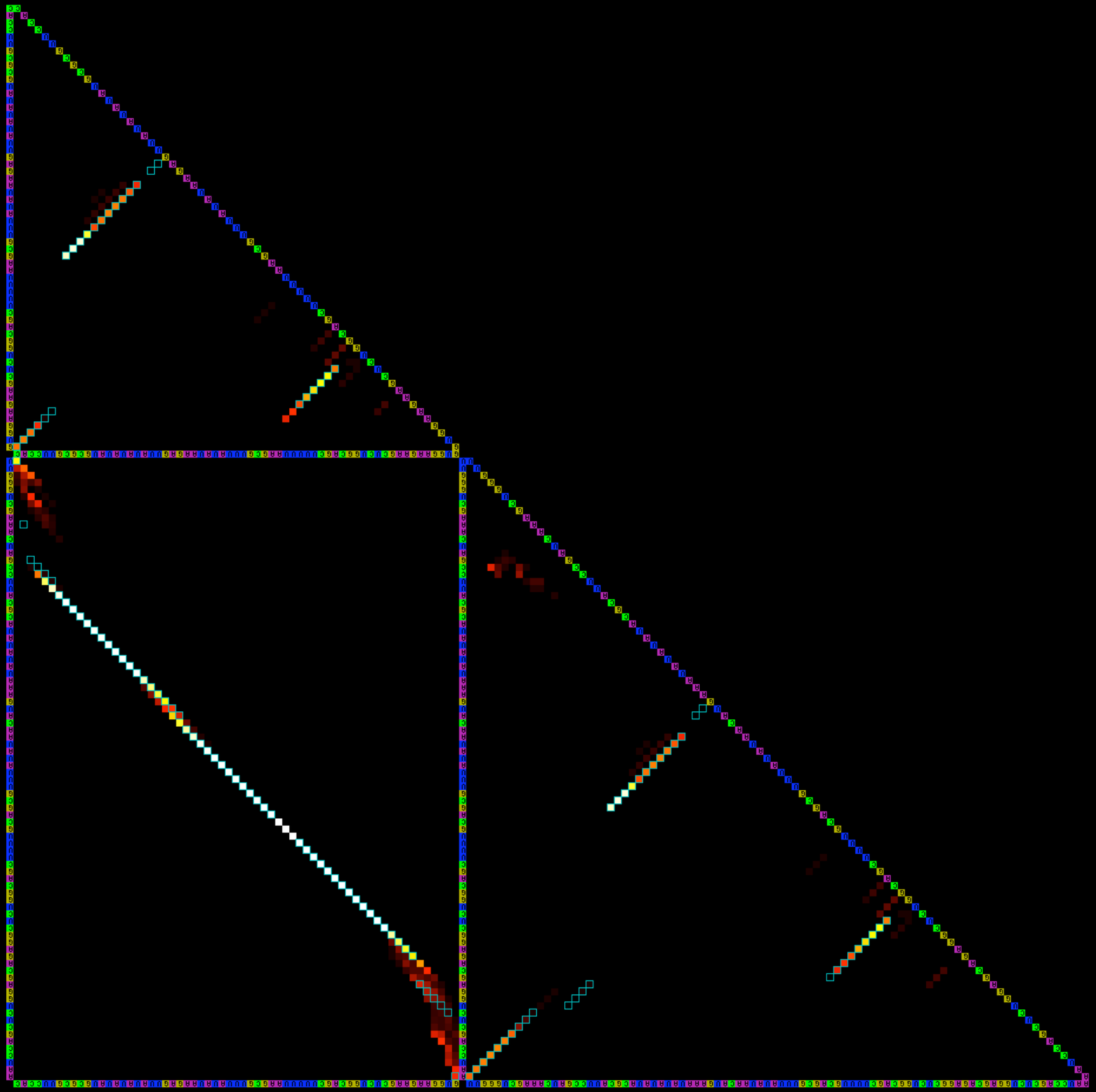




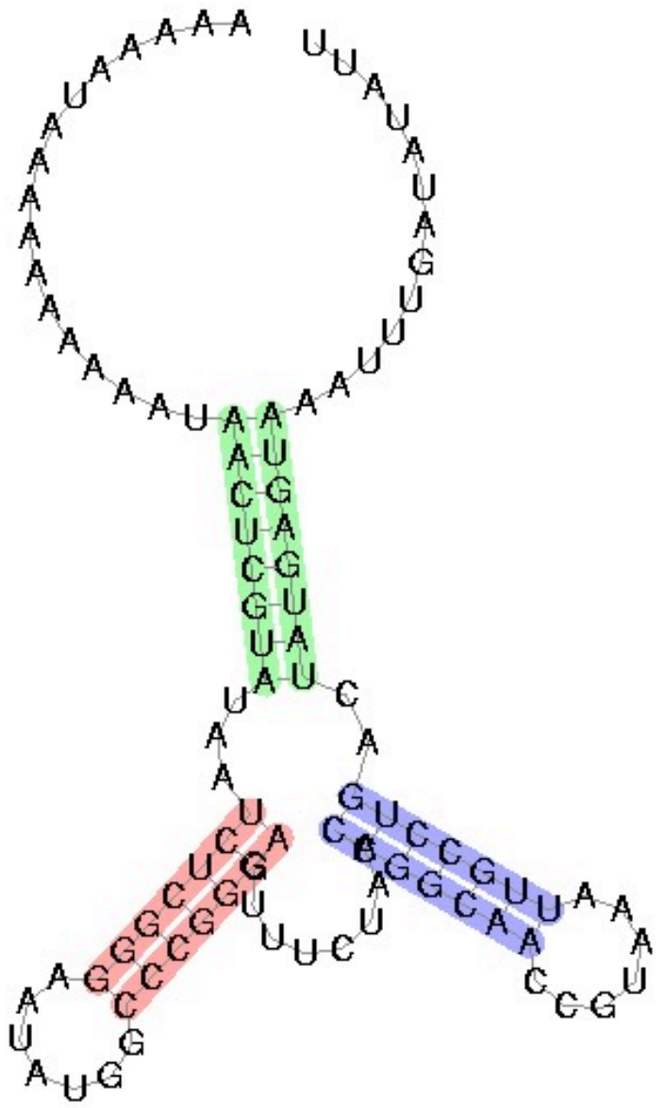
nanos TCE

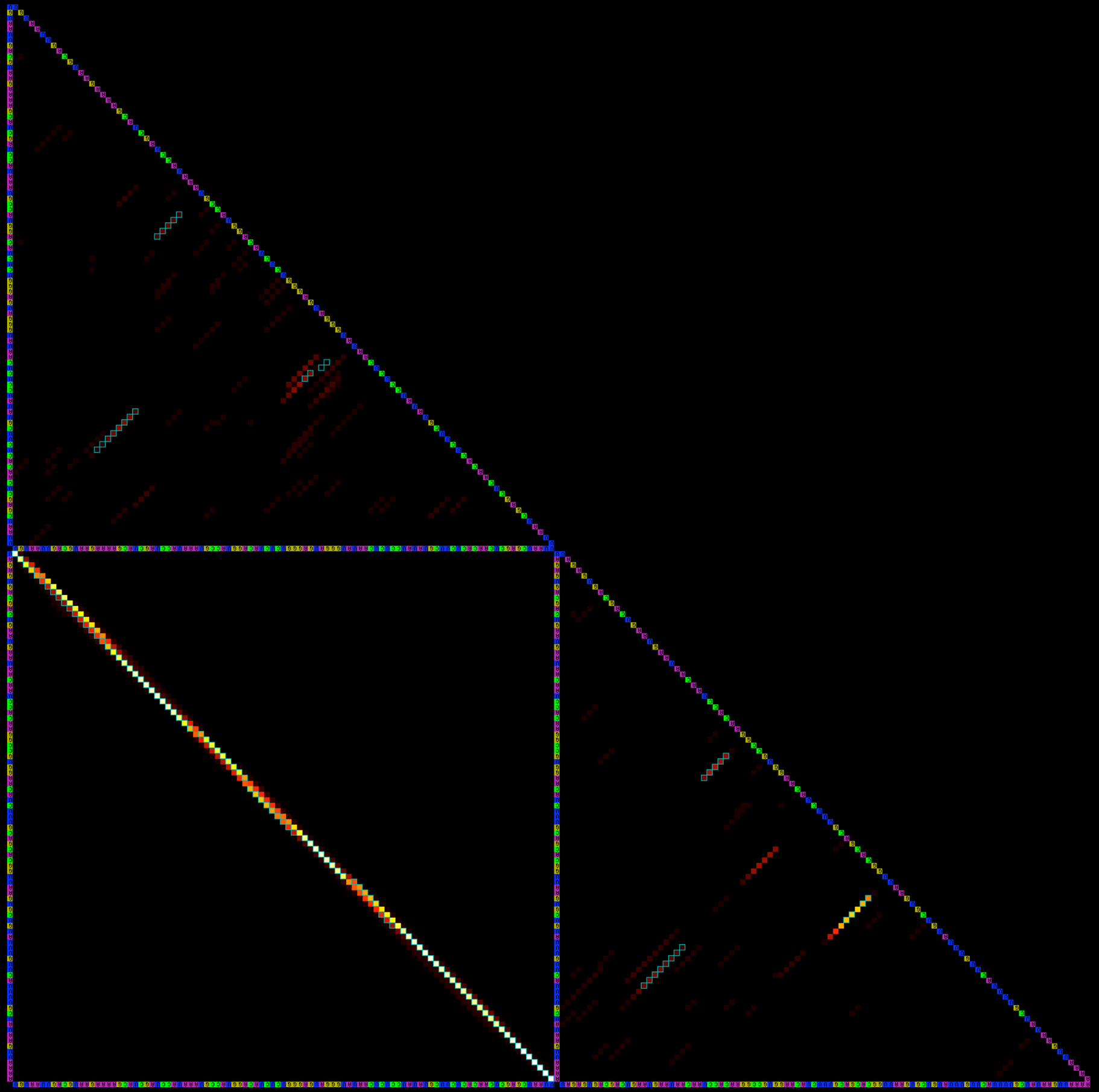


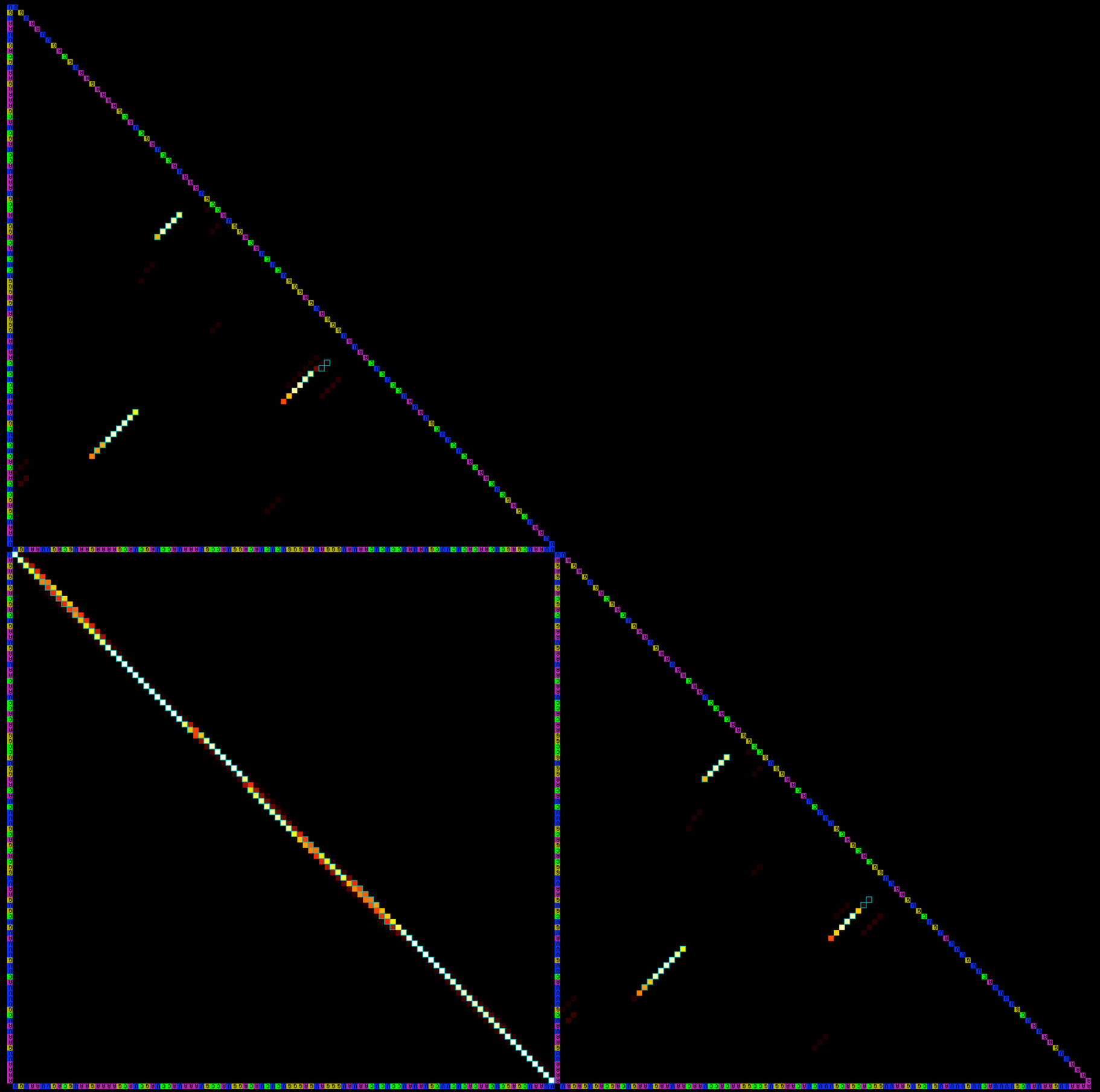




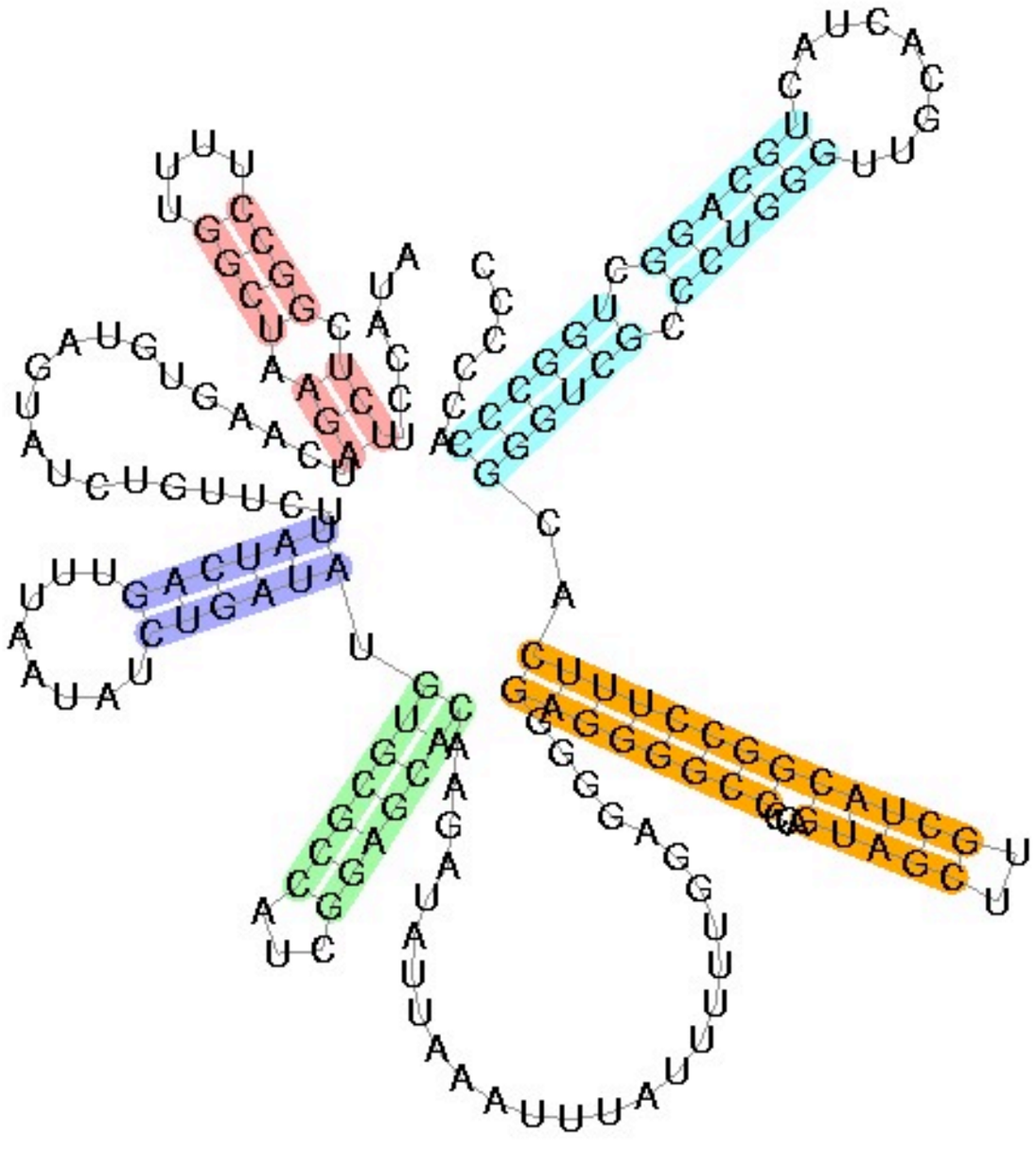
Purine riboswitch



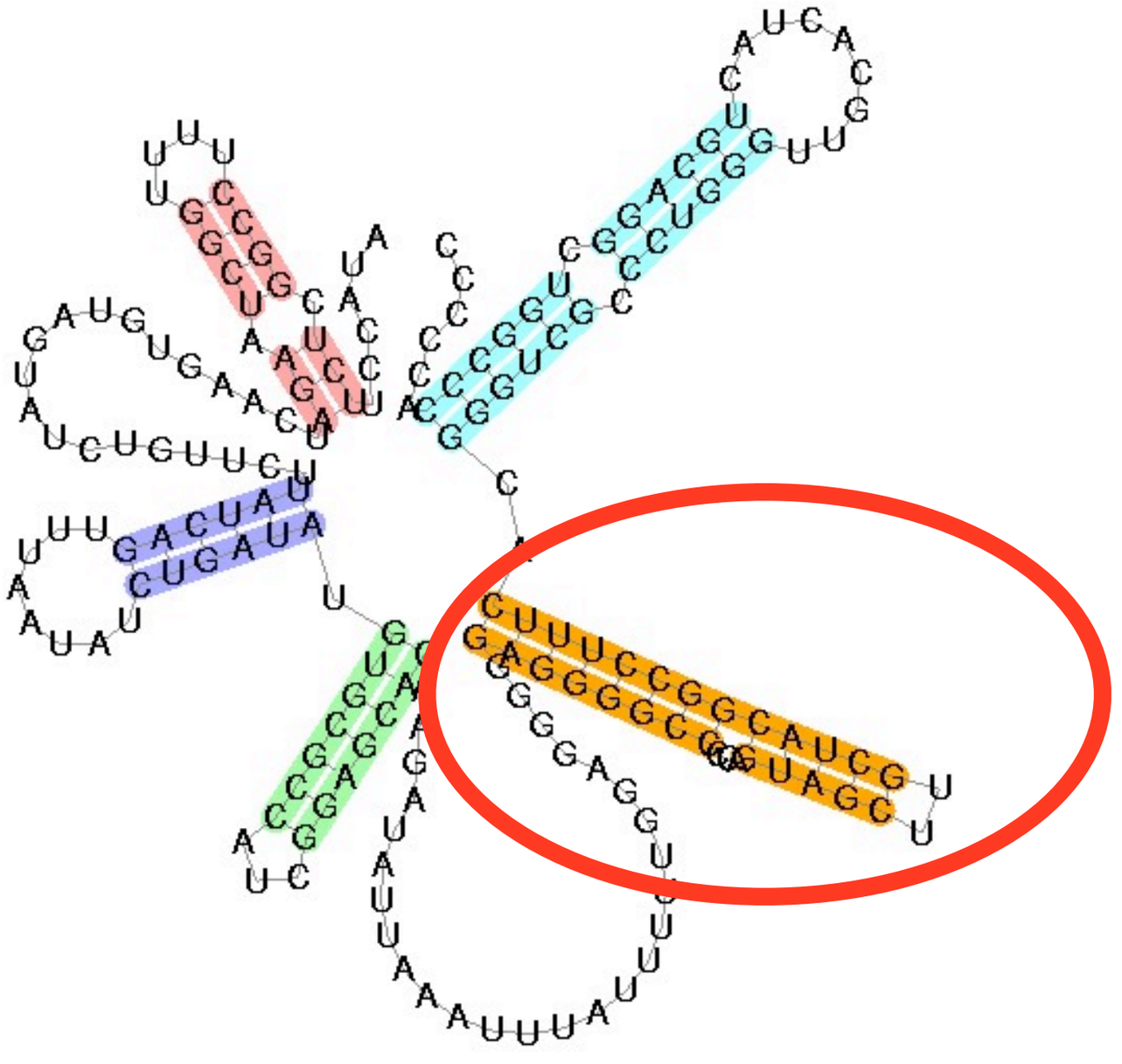


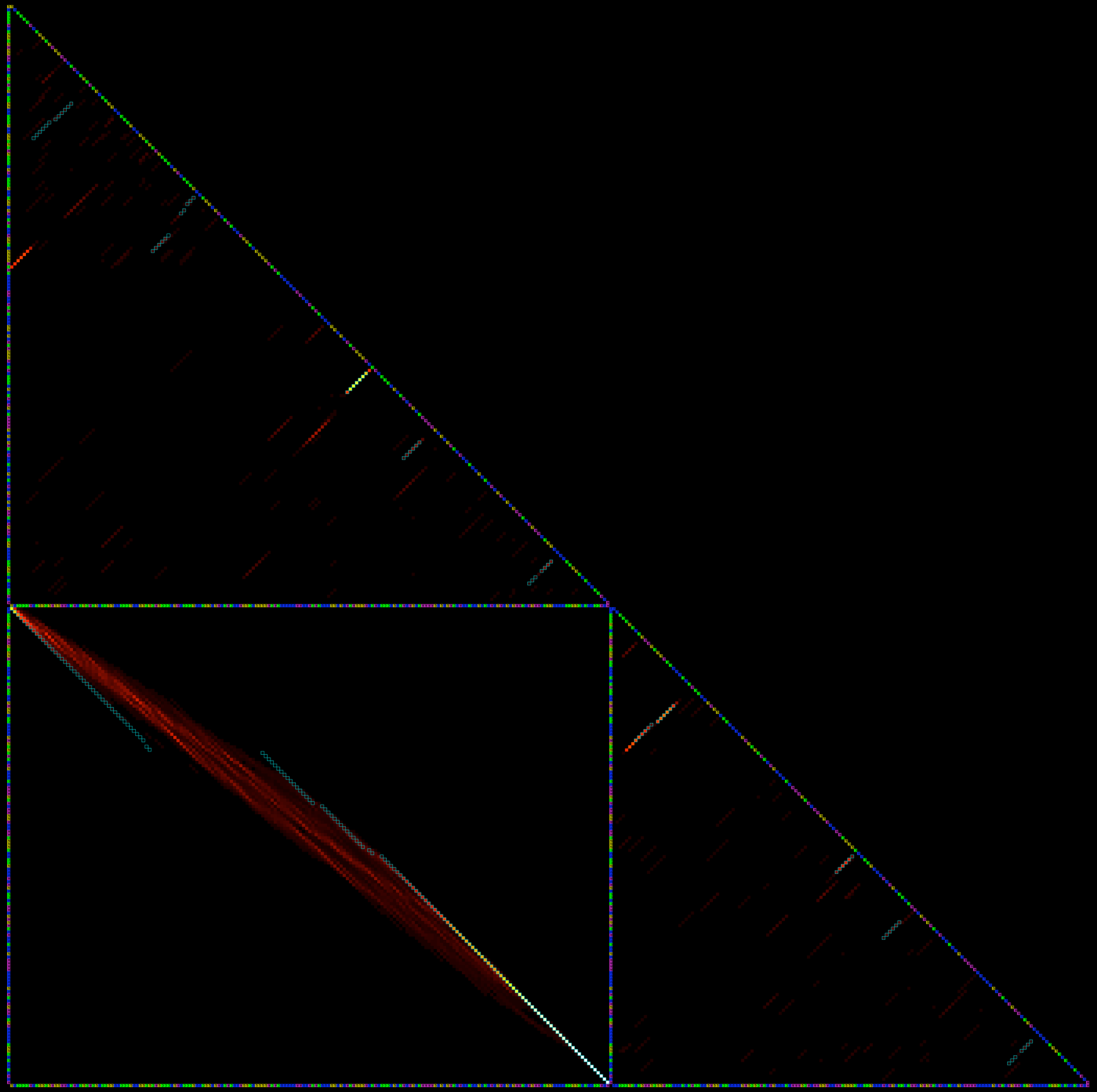


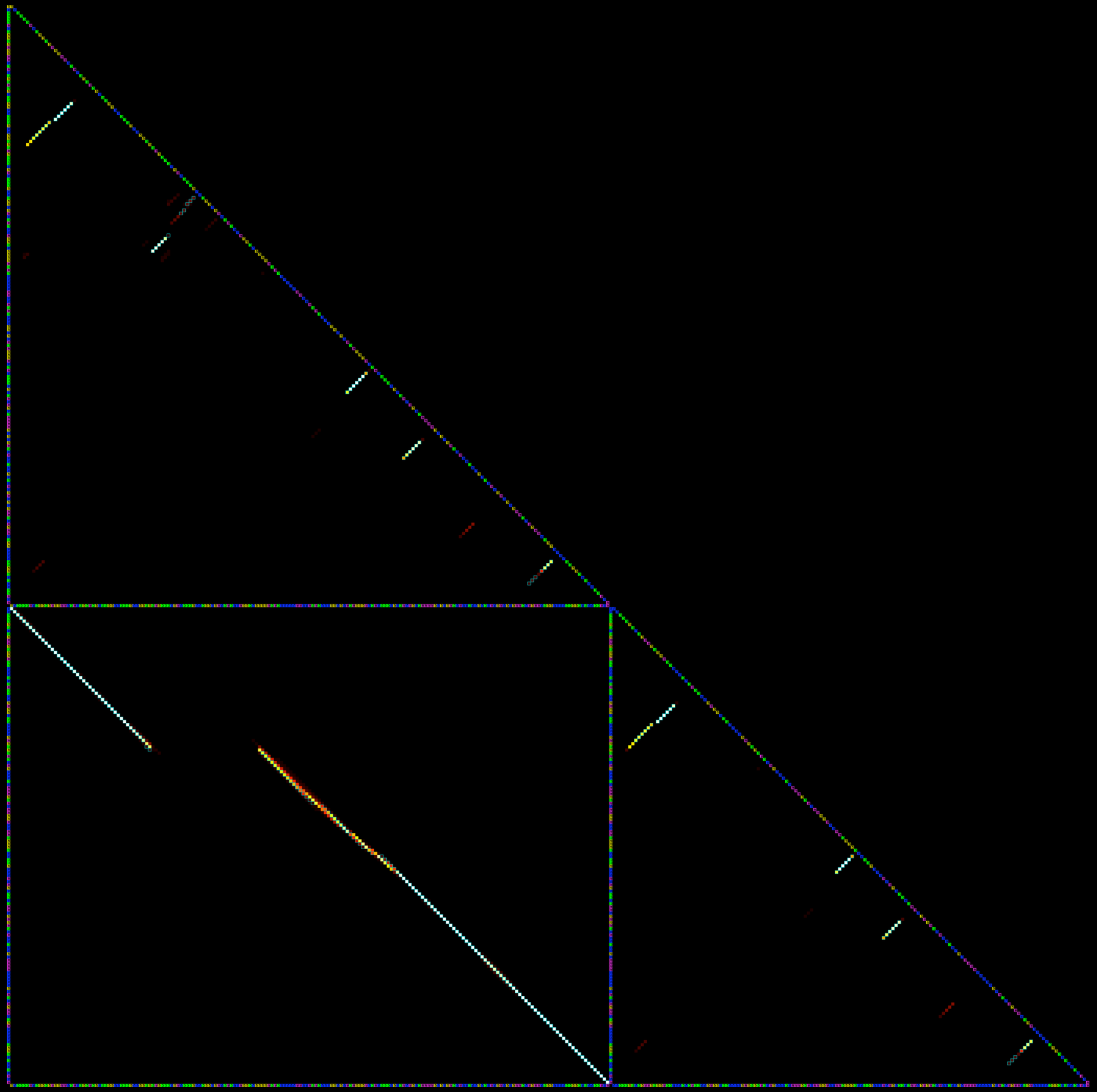
U2 spliceosomal RNA

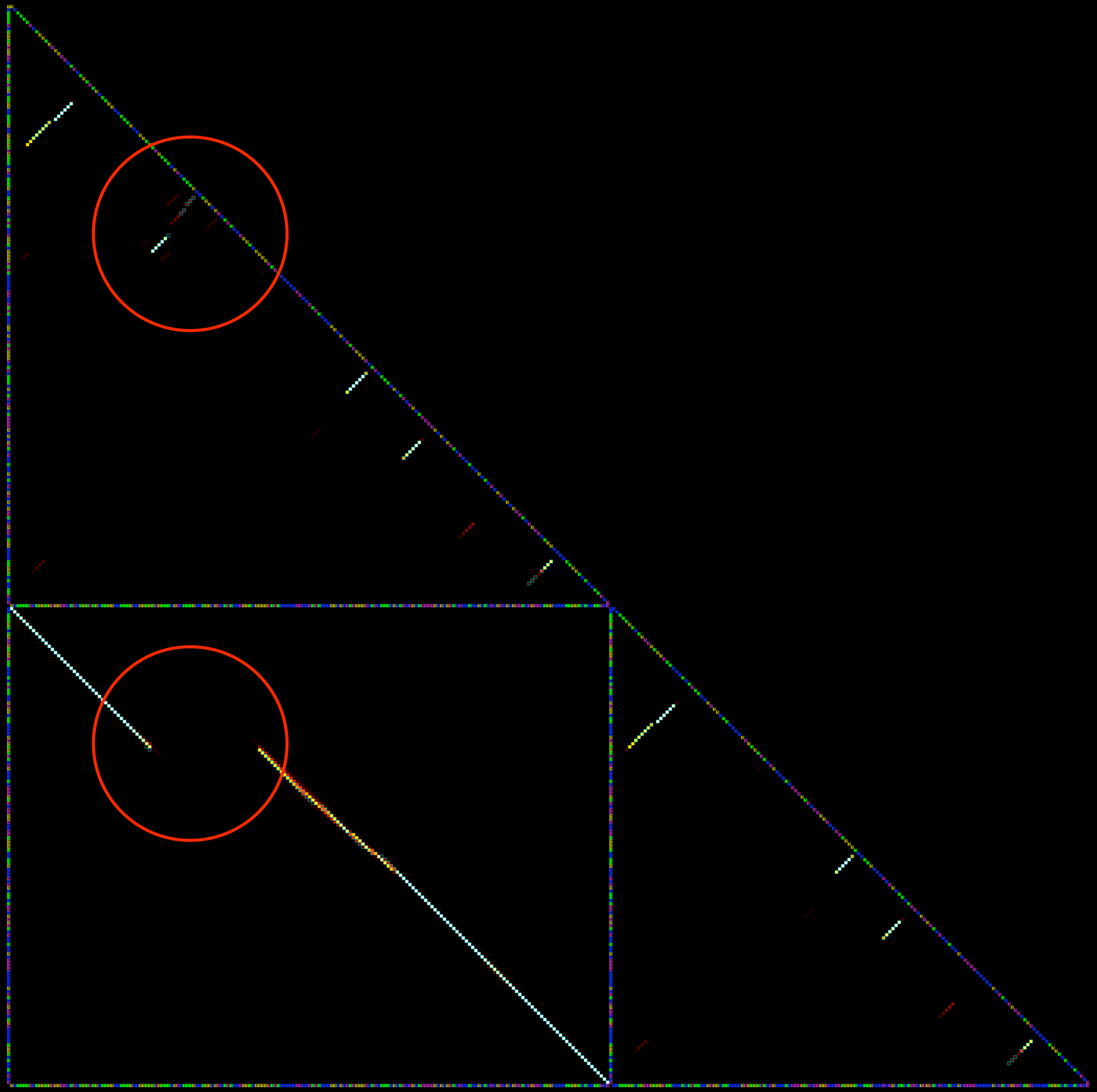


U2 spliceosomal RNA

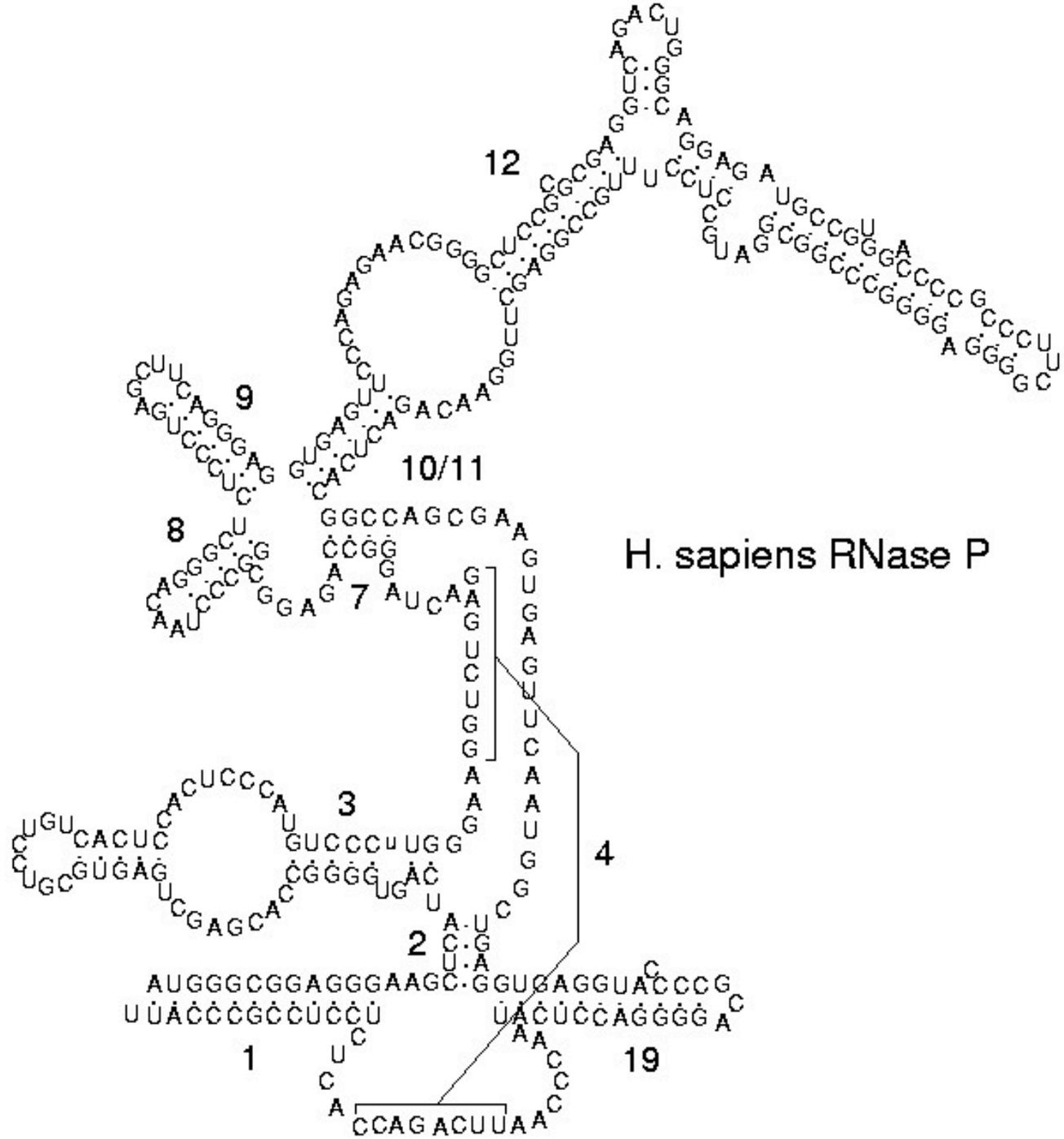








Nuclear RNase P



H. sapiens RNase P