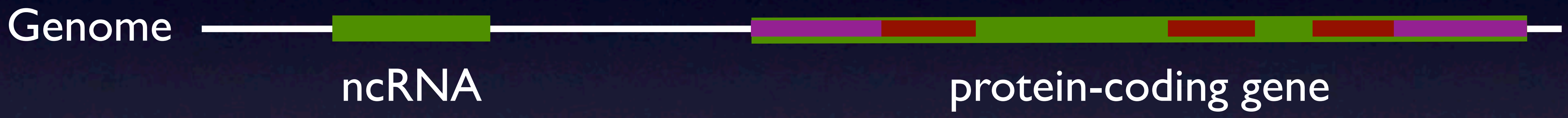
Comparative identification of novel human structural RNA families

Jakob Skou Pedersen
Department of Molecular Medicine
Aarhus University, Denmark

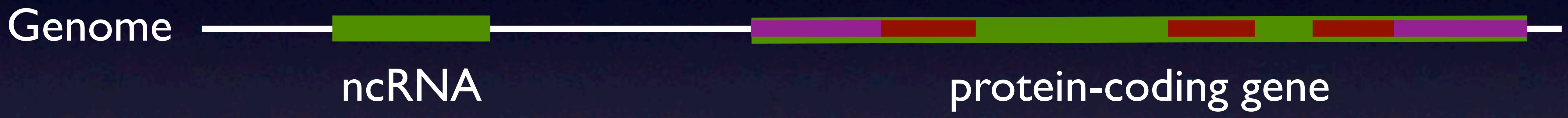
Benasque 2012

Structural RNA



Definition: Any RNA sequence that folds into a structure of functional importance

Structural RNA



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Such as:

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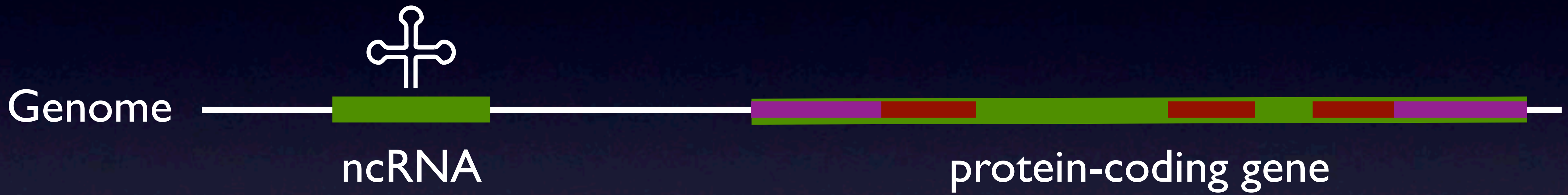

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Such as:

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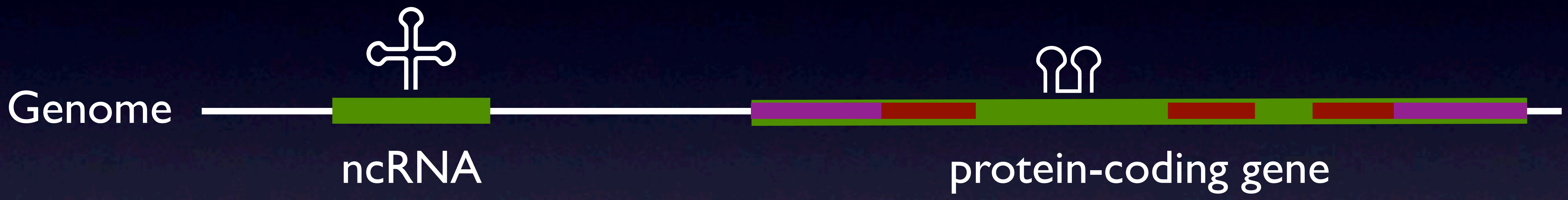

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- ncRNAs excised from longer transcripts

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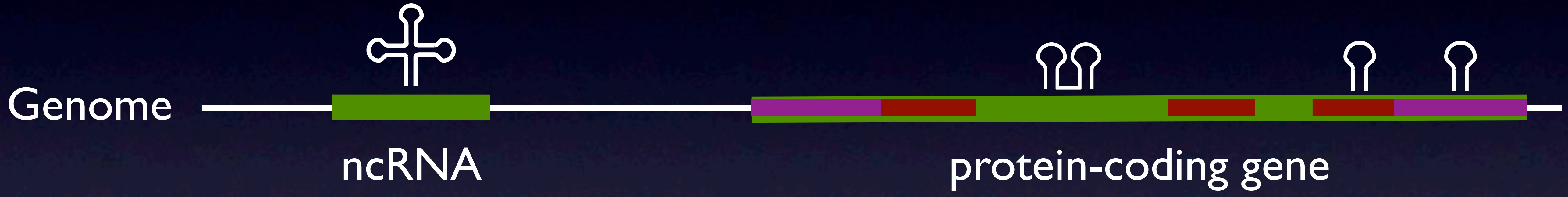
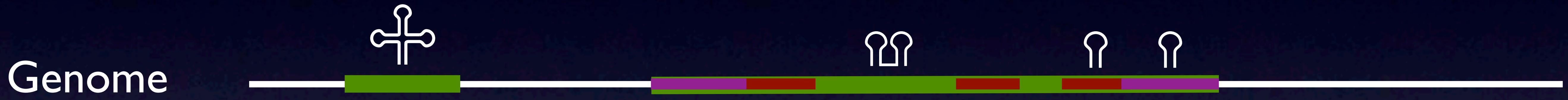


Definition: Any RNA sequence that folds into a structure of functional importance

Such as:

- independently transcribed ncRNAs
- ncRNAs excised from longer transcripts
- cis-regulatory elements within protein-coding genes and ncRNAs

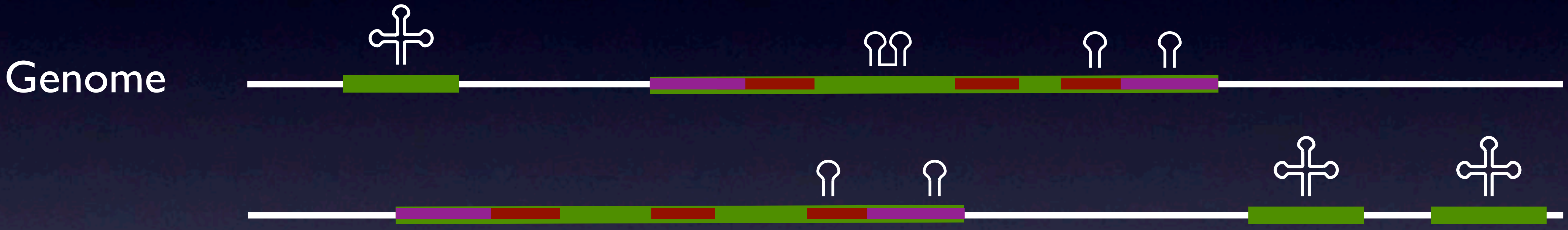
Families of structural RNA



Family members share ancestry

- Duplications may be local or far apart

Families of structural RNA



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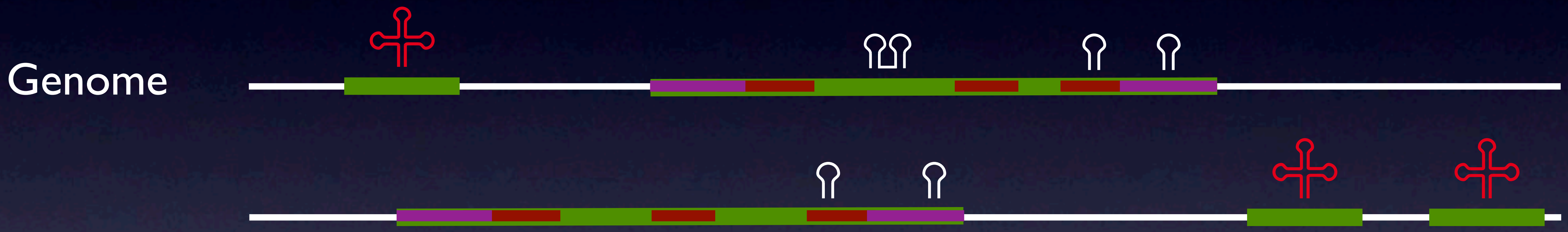

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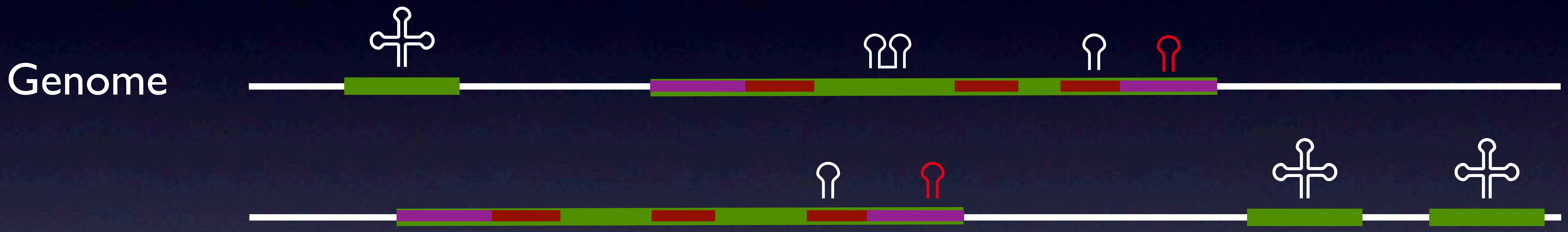

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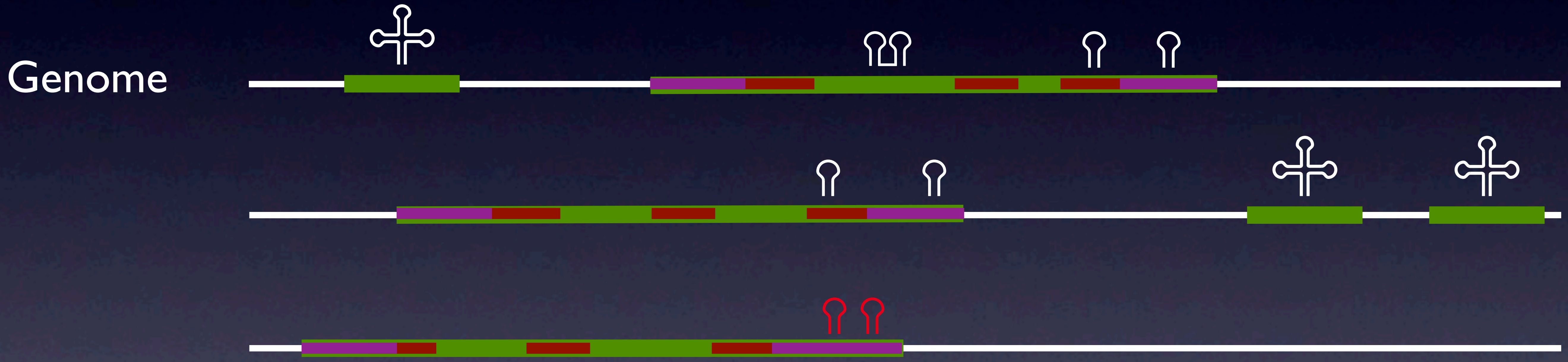

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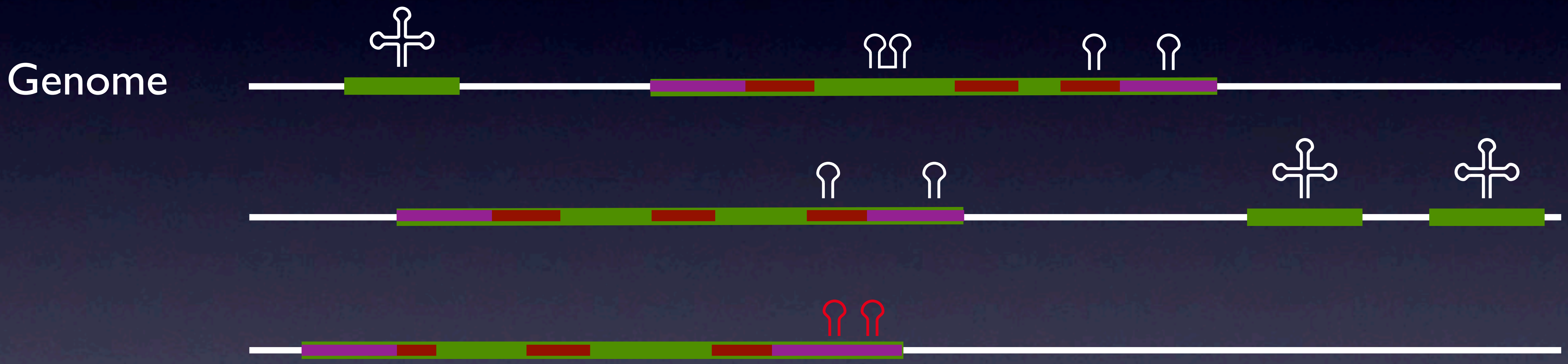

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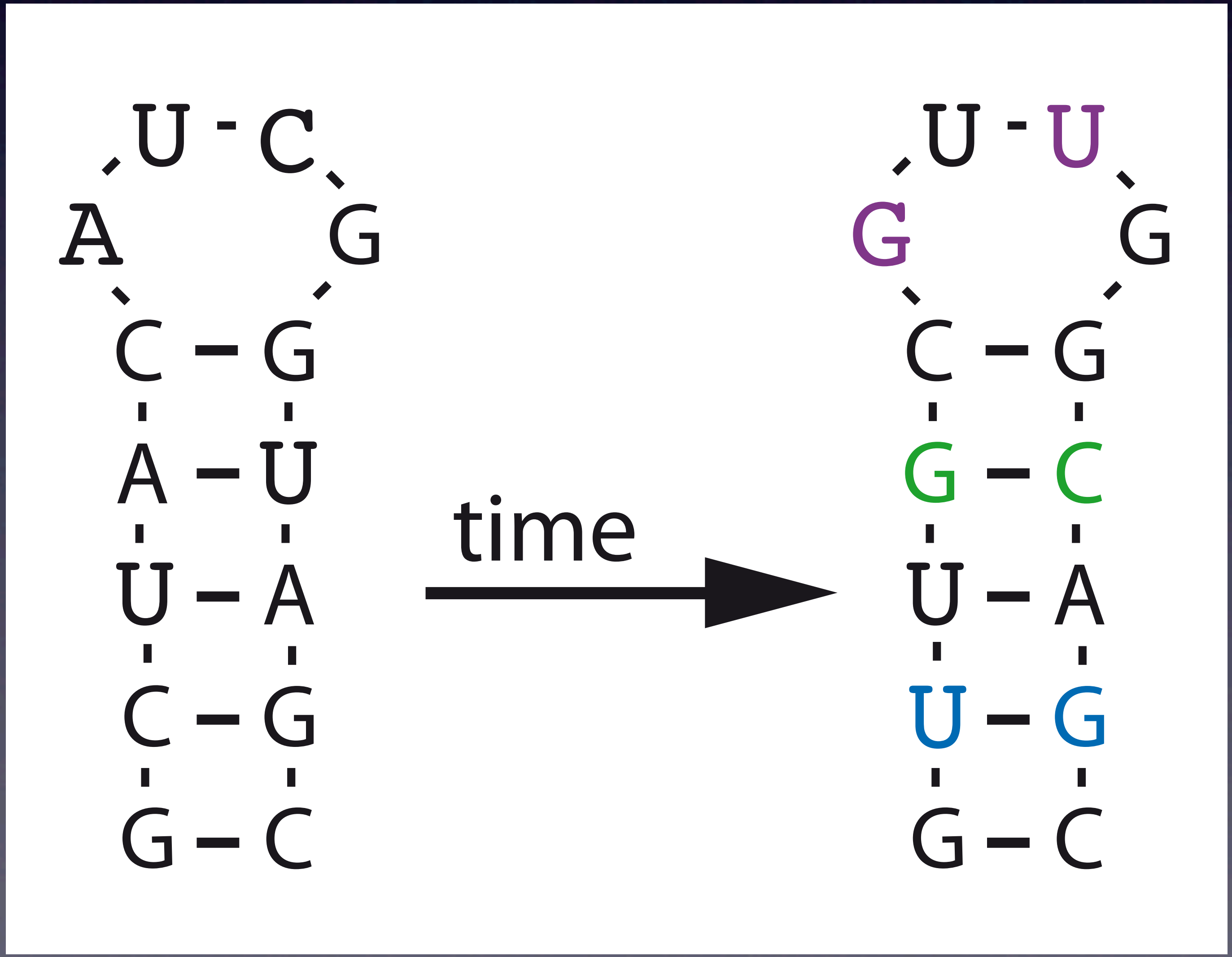
For simple structures convergent evolution may be possible

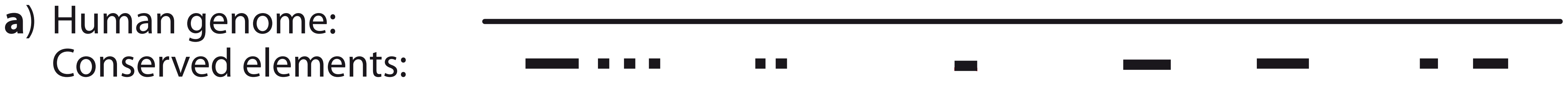
Genomic structure screen

Evolution constrained by structure

Characteristic substitution pattern



EvoFold structure prediction



a) Human genome:
Conserved elements:

Pedersen et al., 2006, PLoS Computational Biology.
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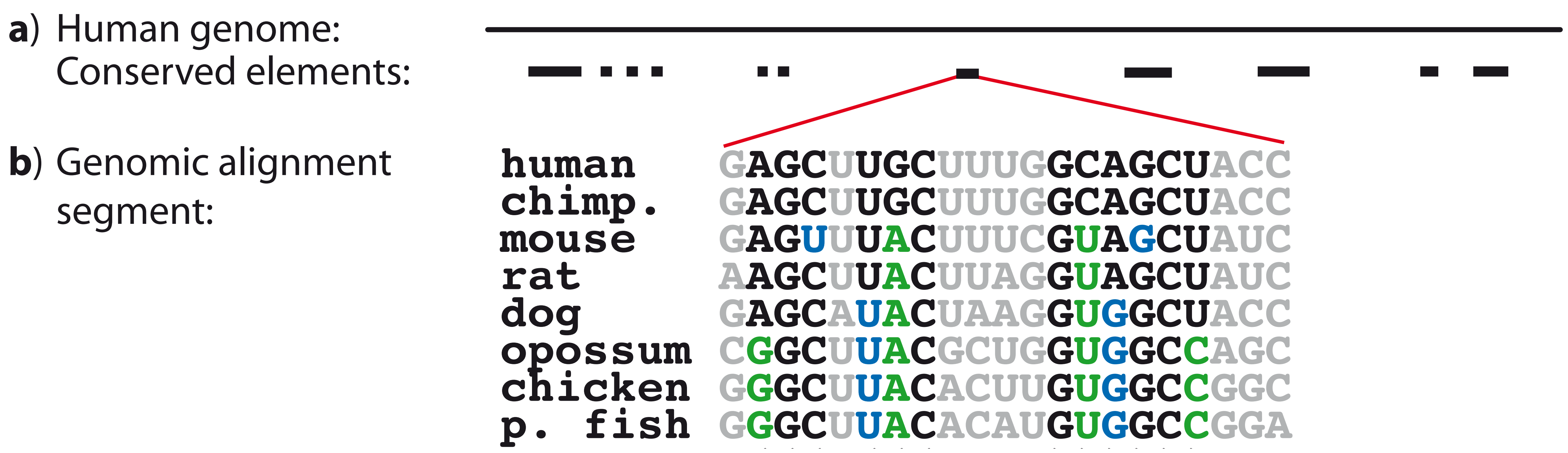

a) Human genome:
Conserved elements:

b) Genomic alignment segment:

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human    GAGCUUGCUUUGGCAGCUACC
chimp.   GAGCUUGCUUUGGCAGCUACC
mouse    GAGUUUACUUUCGUAGCUAUC
rat      AAGCUUACUUAGGUAGCUAUC
dog      GAGCAUACUAAGGUGGCUACC
opossum  CGGCUUACGCUGGUGGCCAGC
chicken  GGGCUUACACUUGUGGCCGGC
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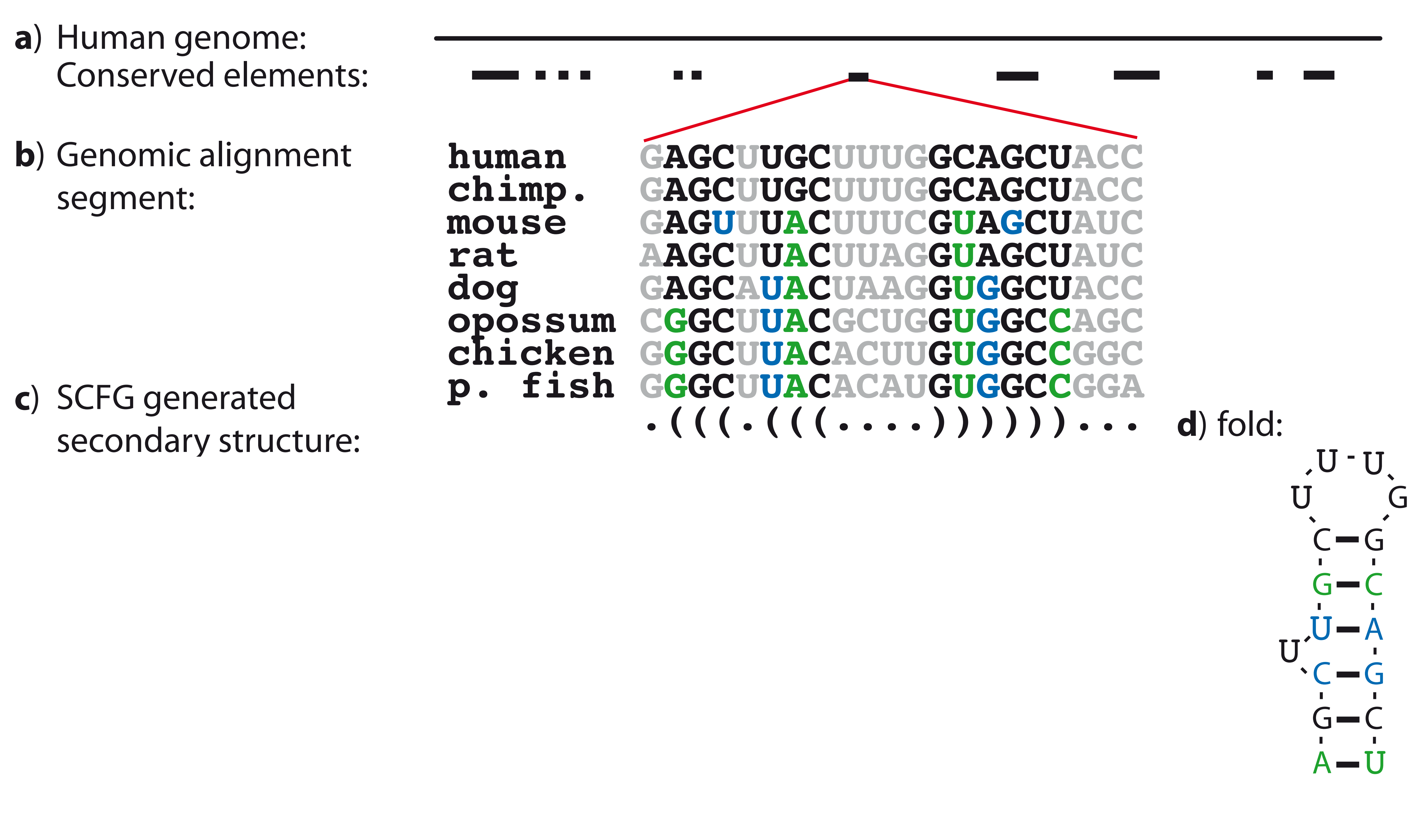

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         .(((.(((....))))))...
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c) SCFG generated secondary structure:

d) fold:

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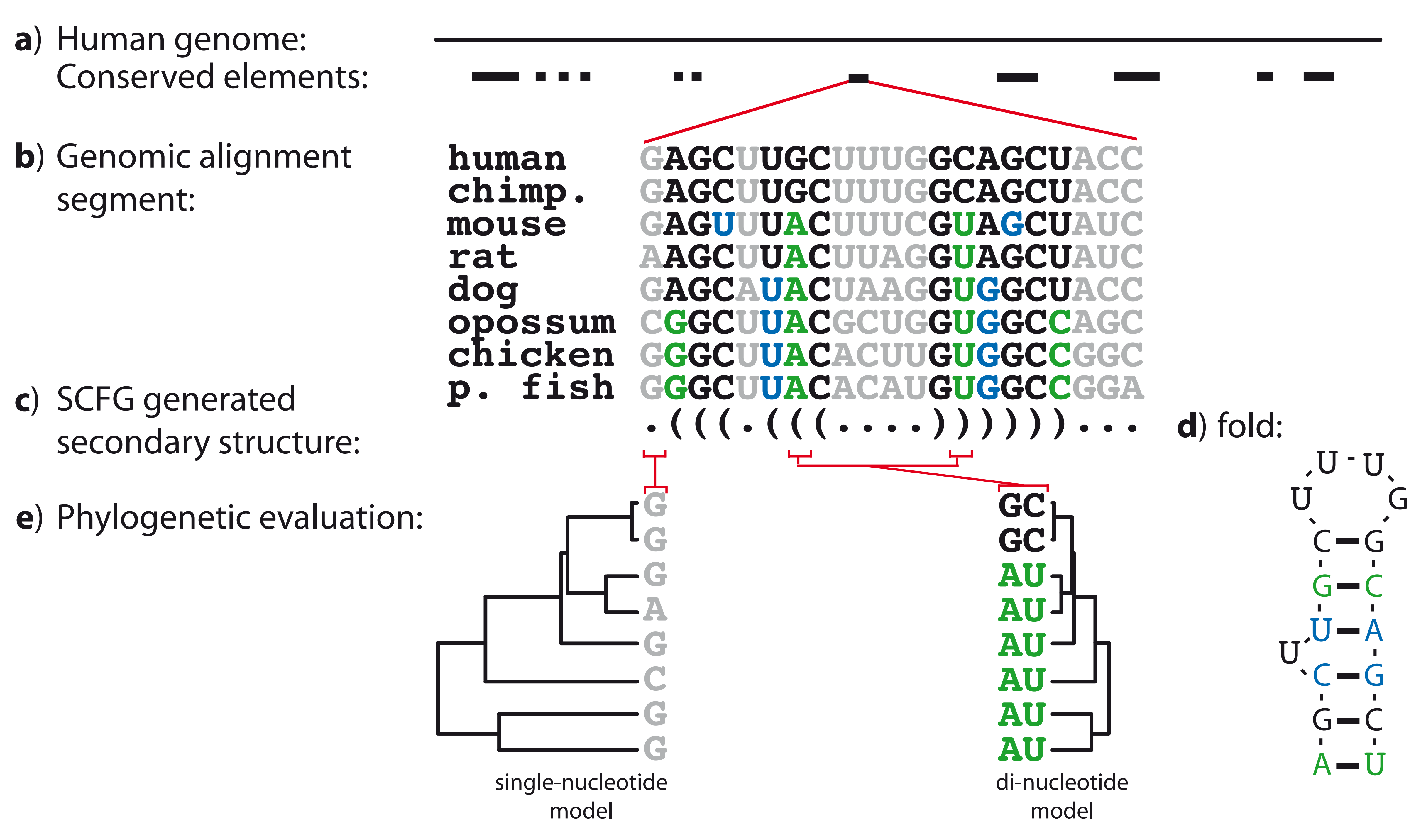

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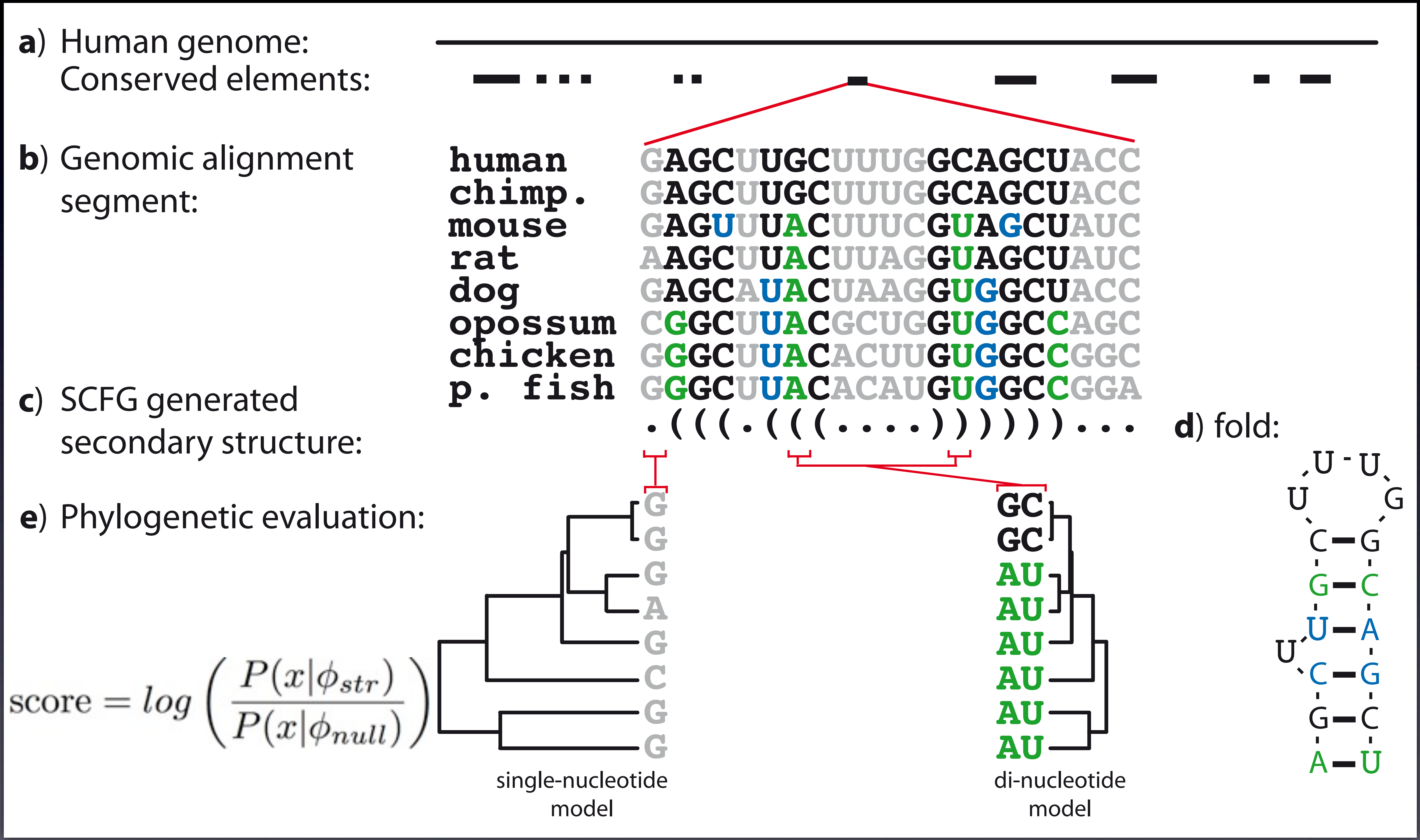

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$$\text{score} = log\left(\frac{P(x|\phi_{str})}{P(x|\phi_{null})}\right)$$

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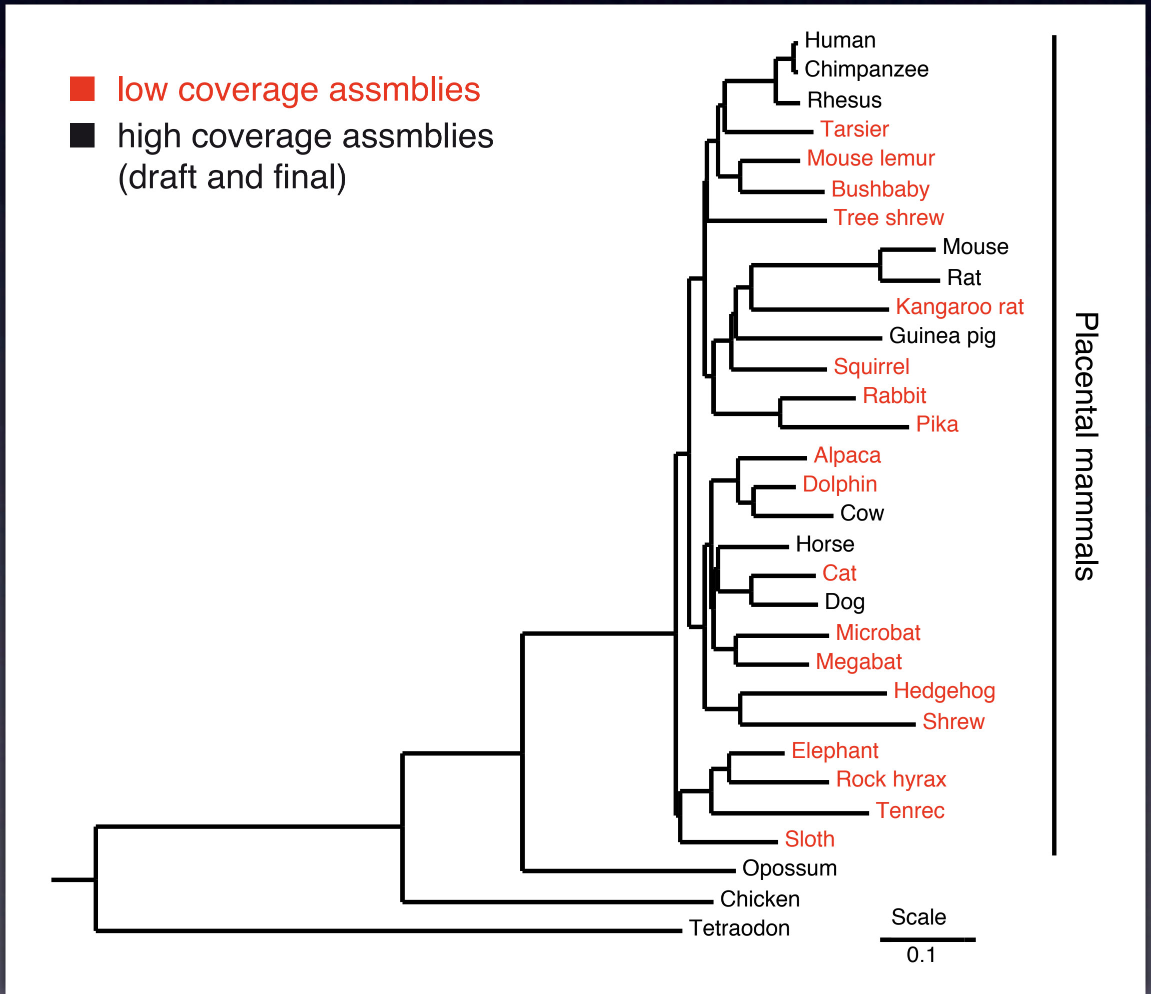
Screen of 31-way vertebrate alignments

Input: conserved alignment segments (5.6% of genome)

Phylogenetic tree of input species



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Output: 37,381 predictions (0.05% of genome)

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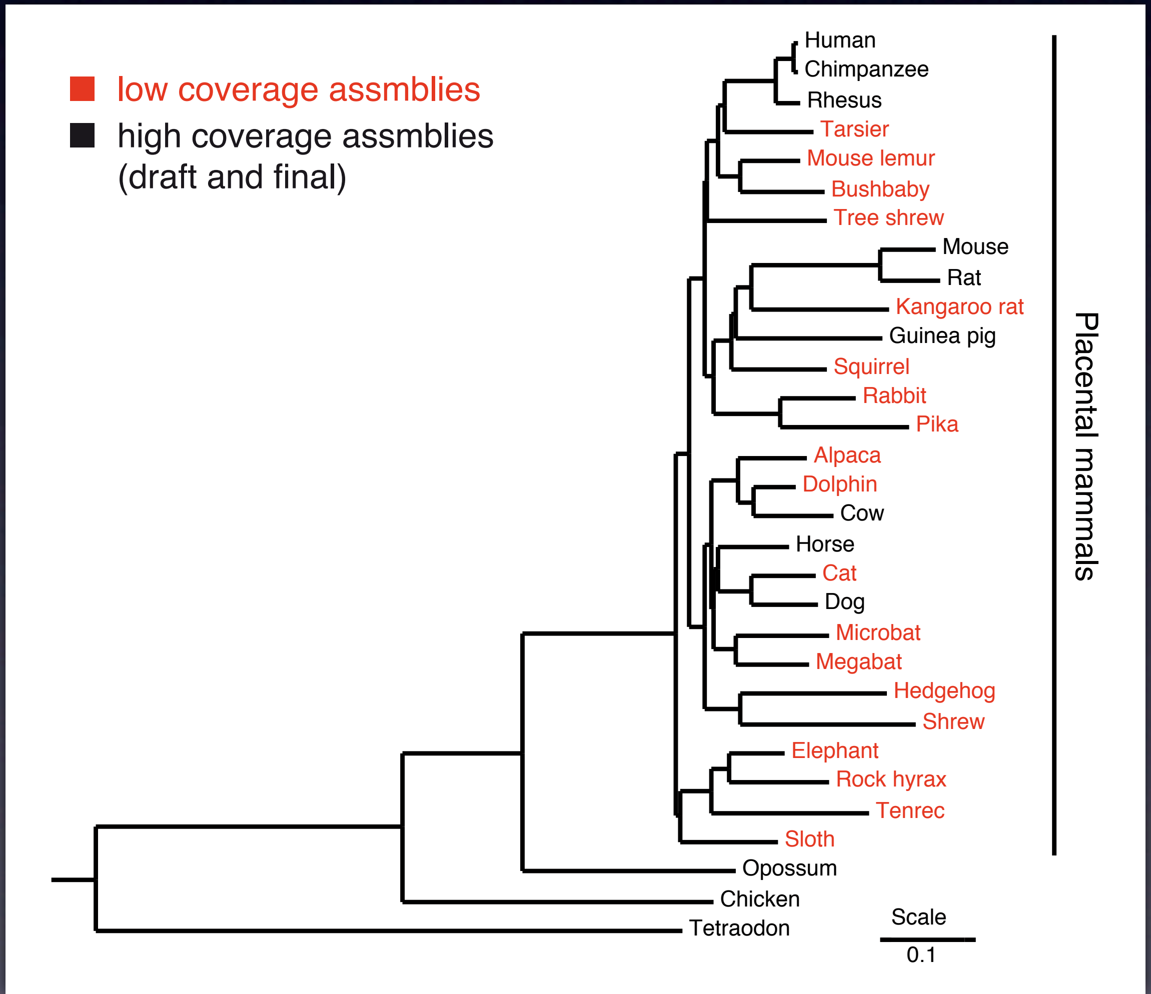

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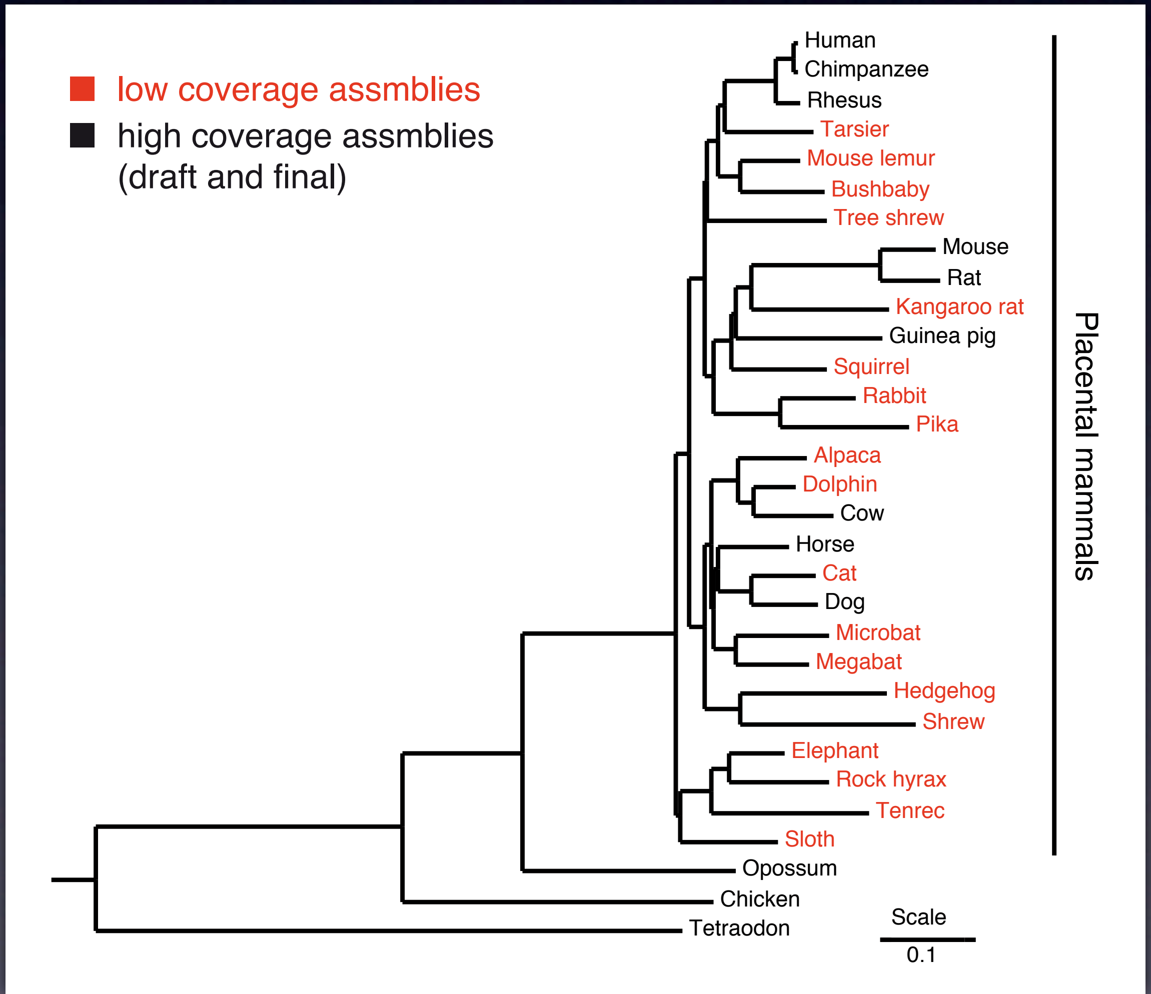
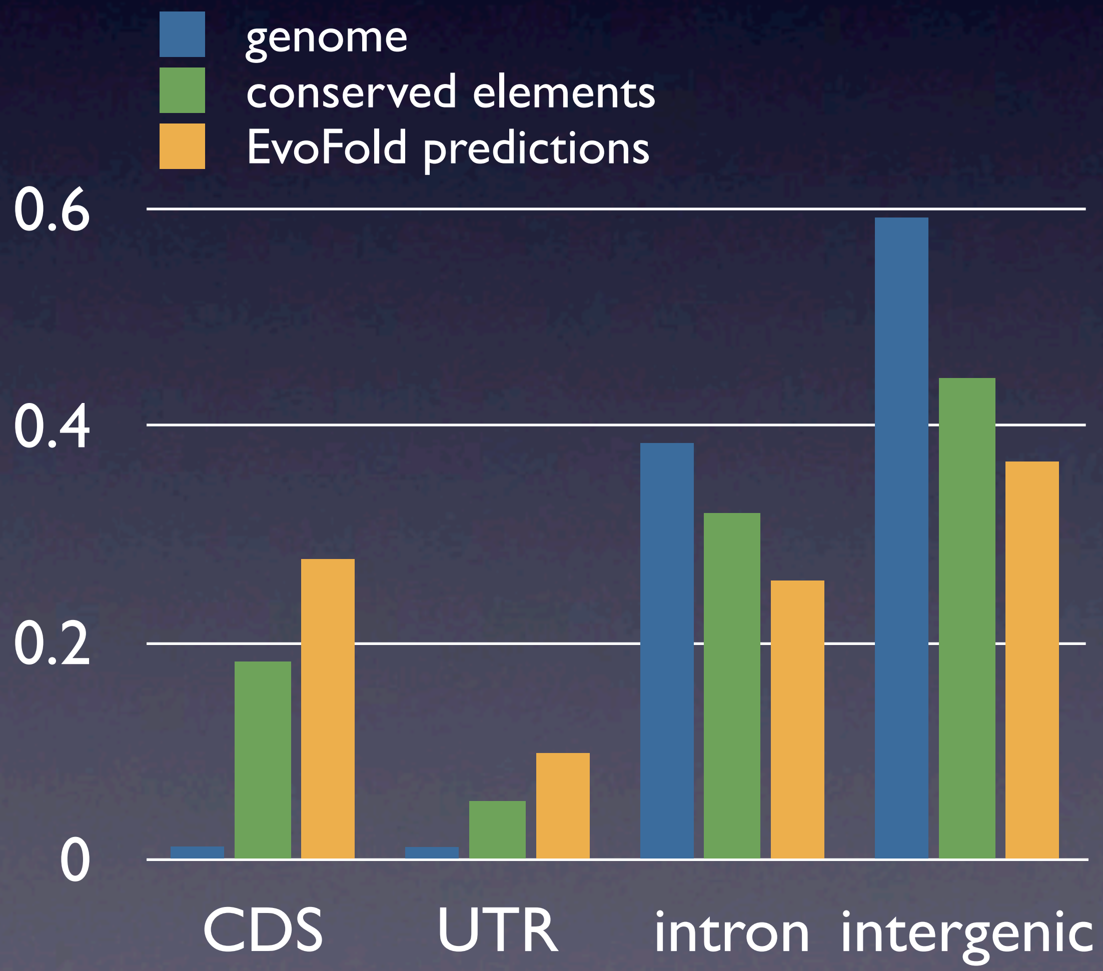


Genomic distribution



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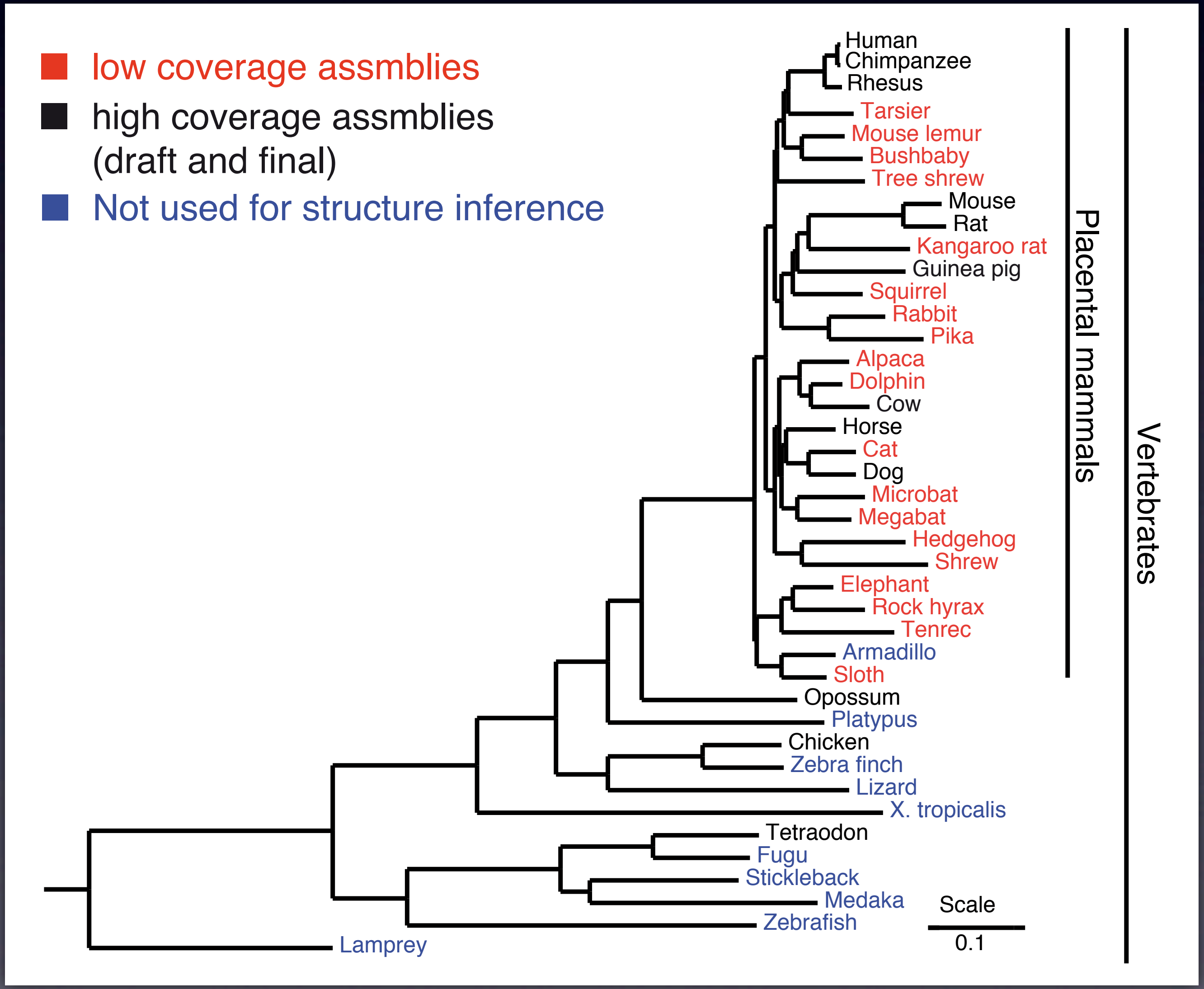
Significance evaluation using additional vertebrate genomes

EvoP method

Question: how surprising is the observed number of double substitutions?

- Monte Carlo approach:
  - Simulate iid substitutions across columns on phylogeny.
  - Count double substitutions given structure.
  - Estimate P-value as fraction simulations with at least as many double substitutions.

Ten vertebrates genomes not used for structure inference



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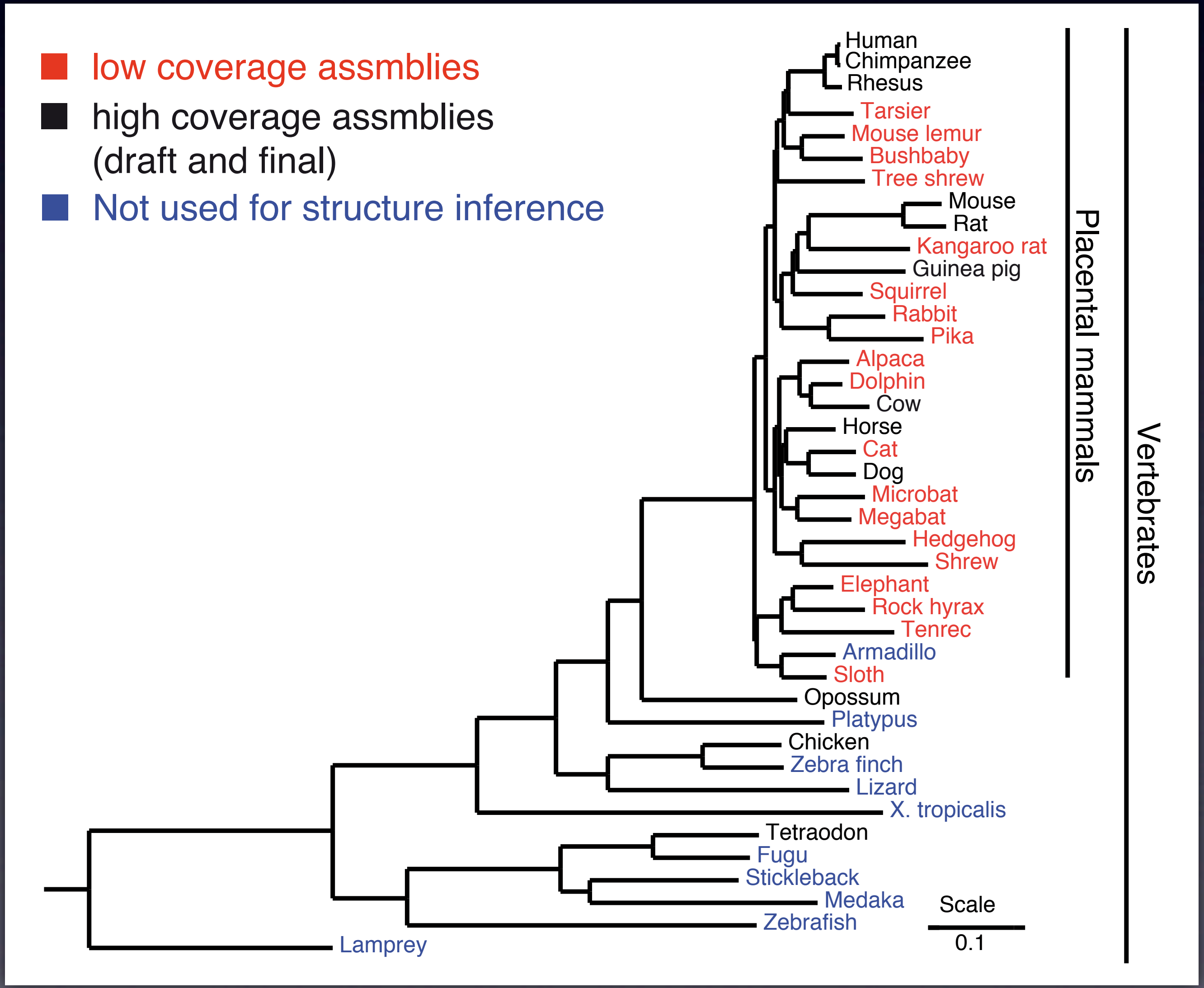

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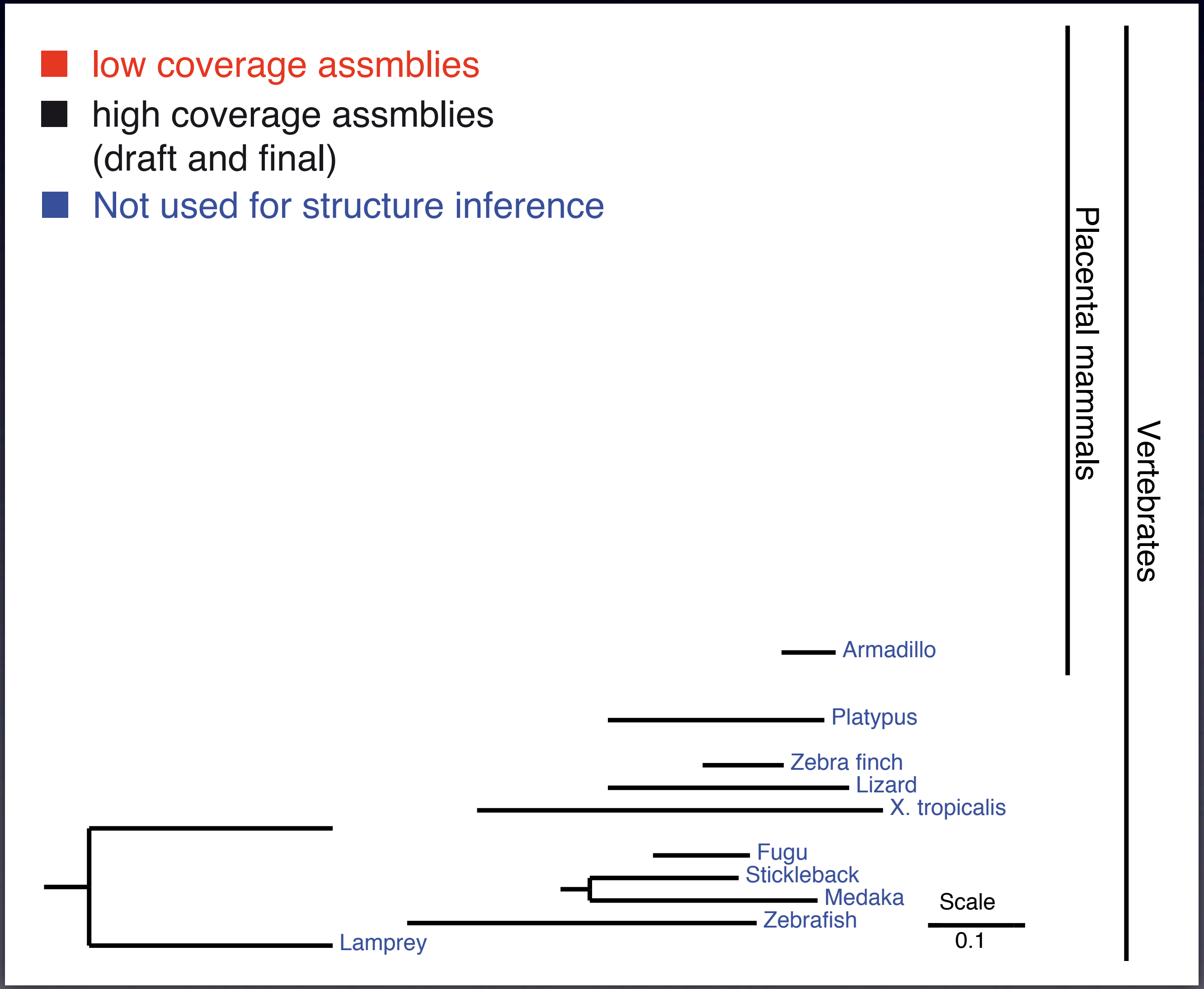

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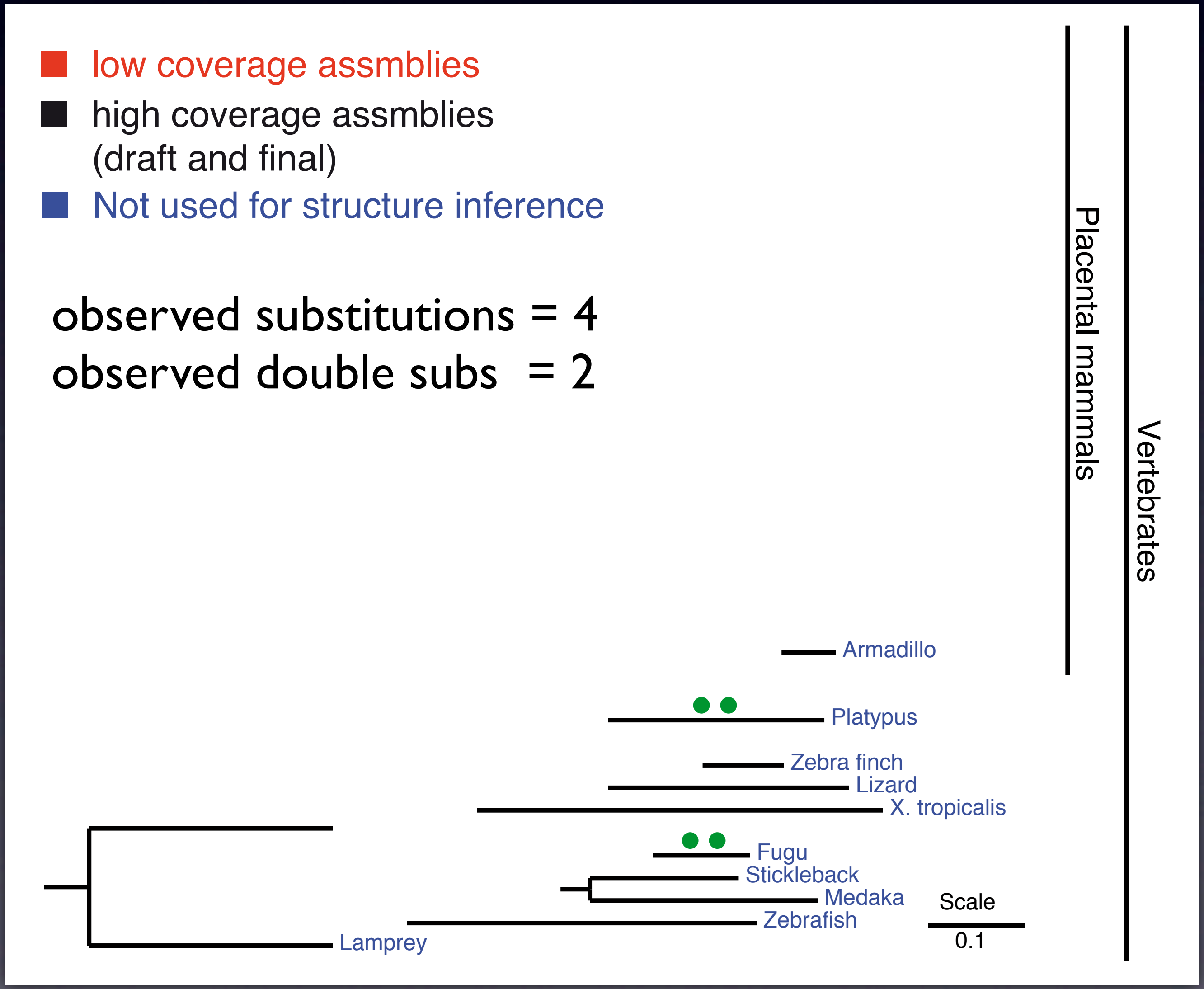

🟥 low coverage assmblies
⬛ high coverage assmblies (draft and final)
🟦 Not used for structure inference

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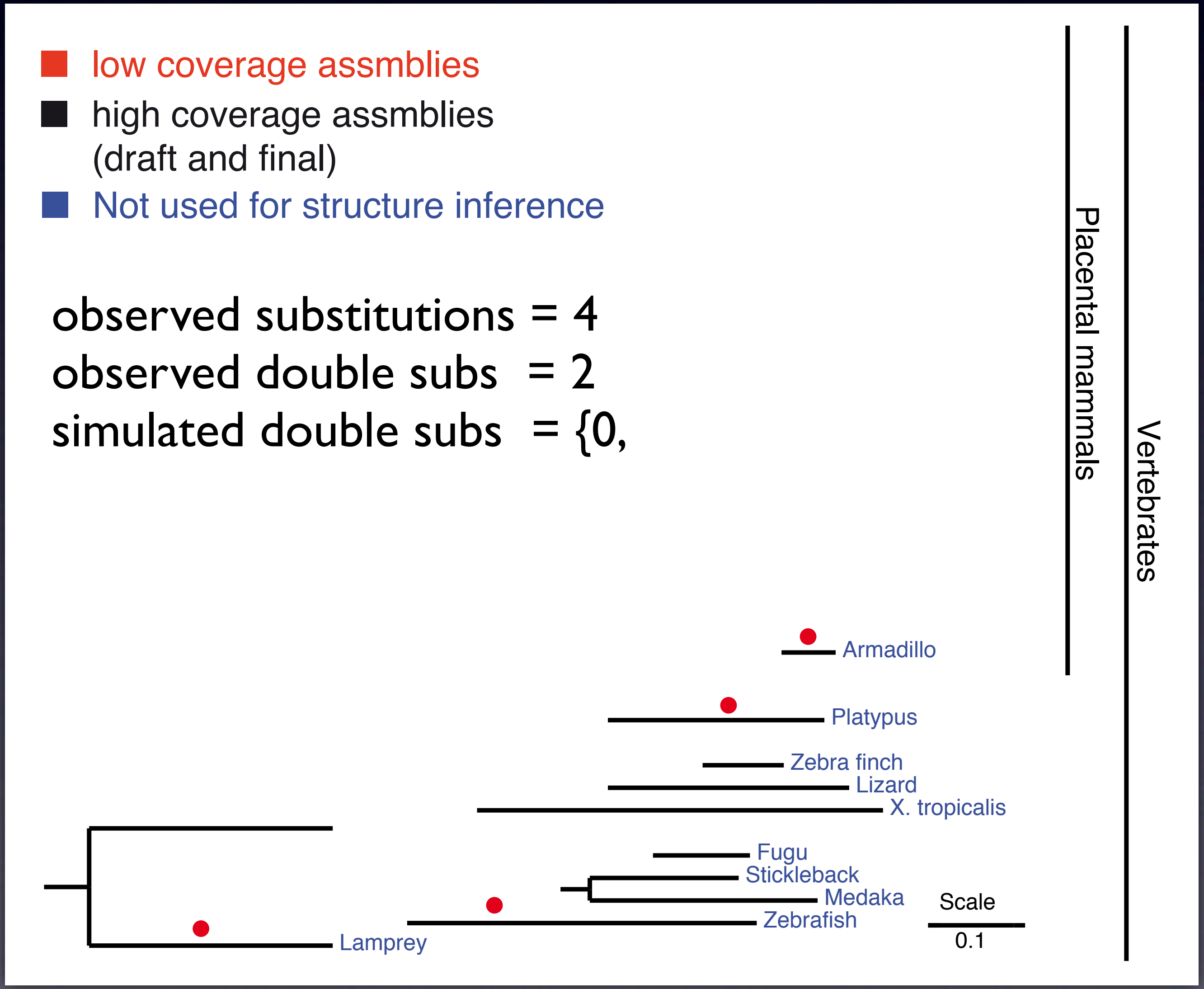

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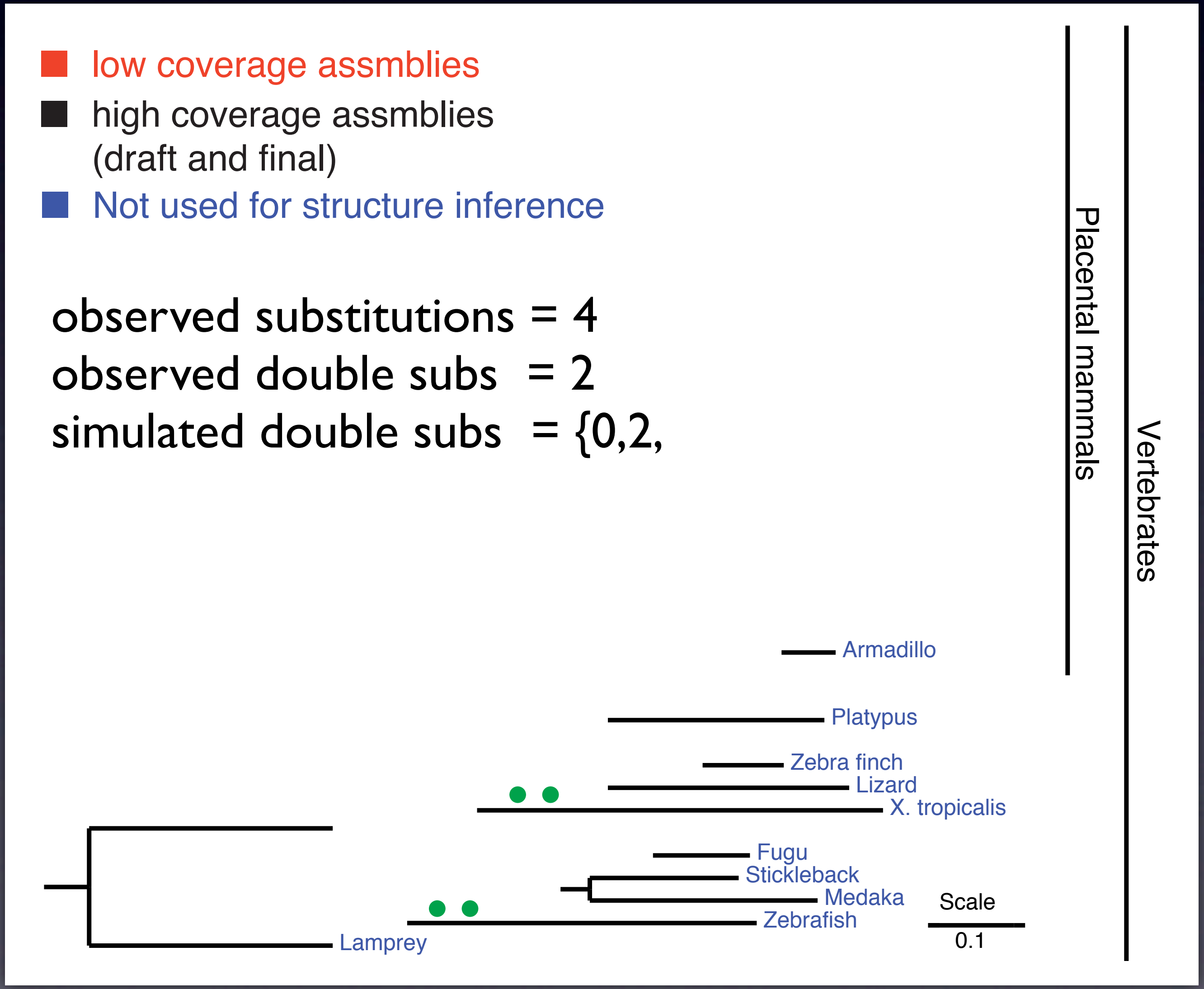

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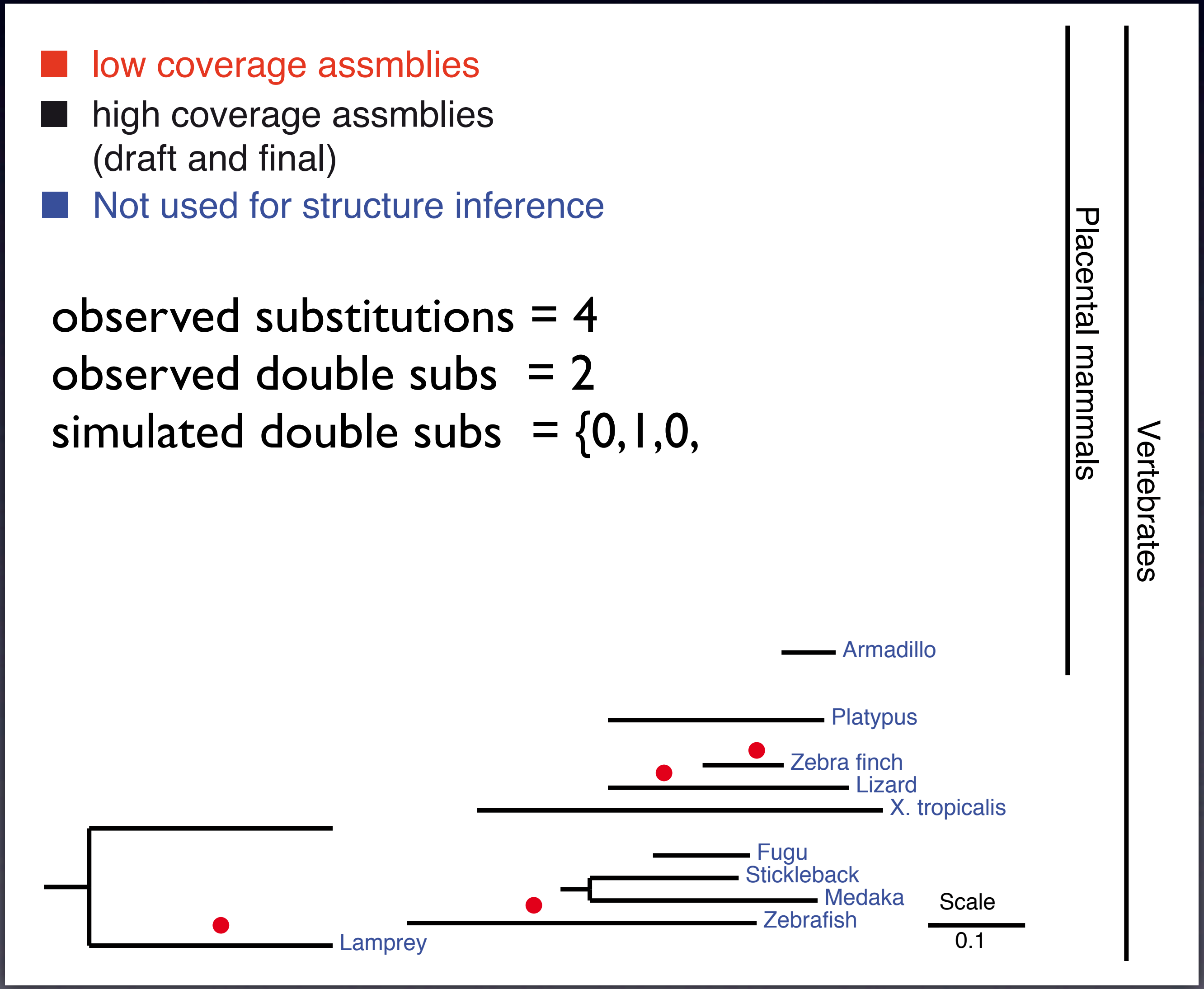

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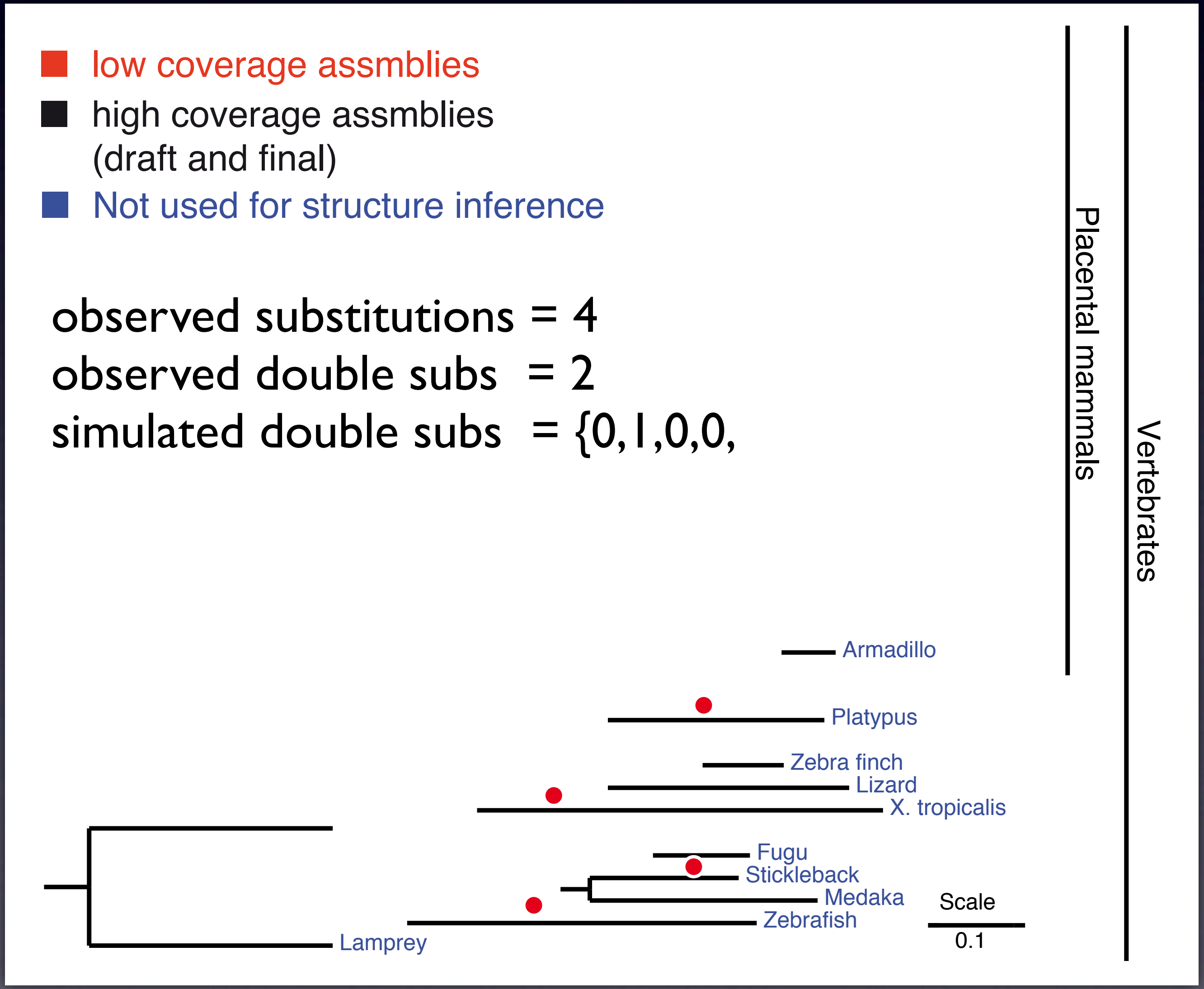

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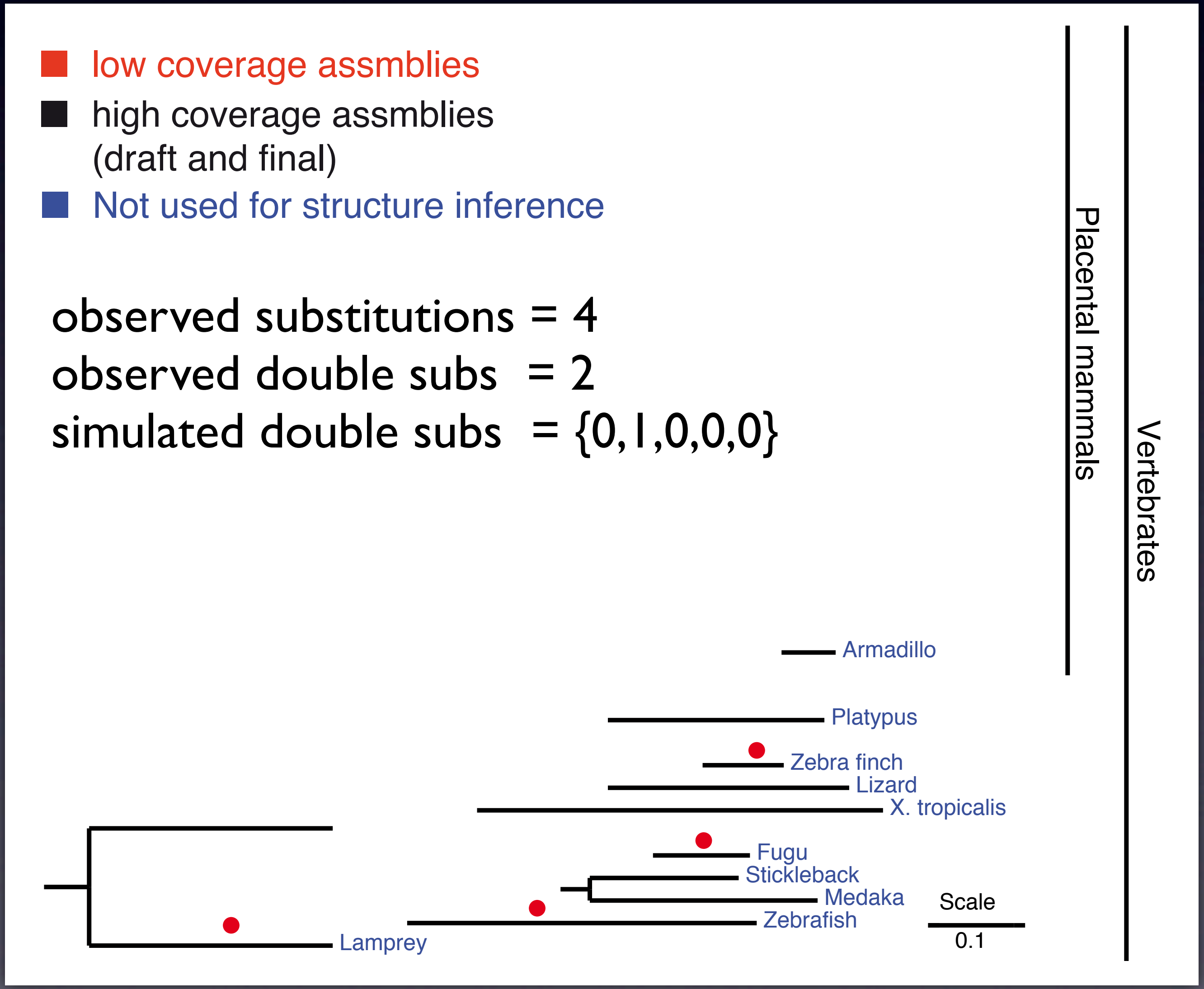

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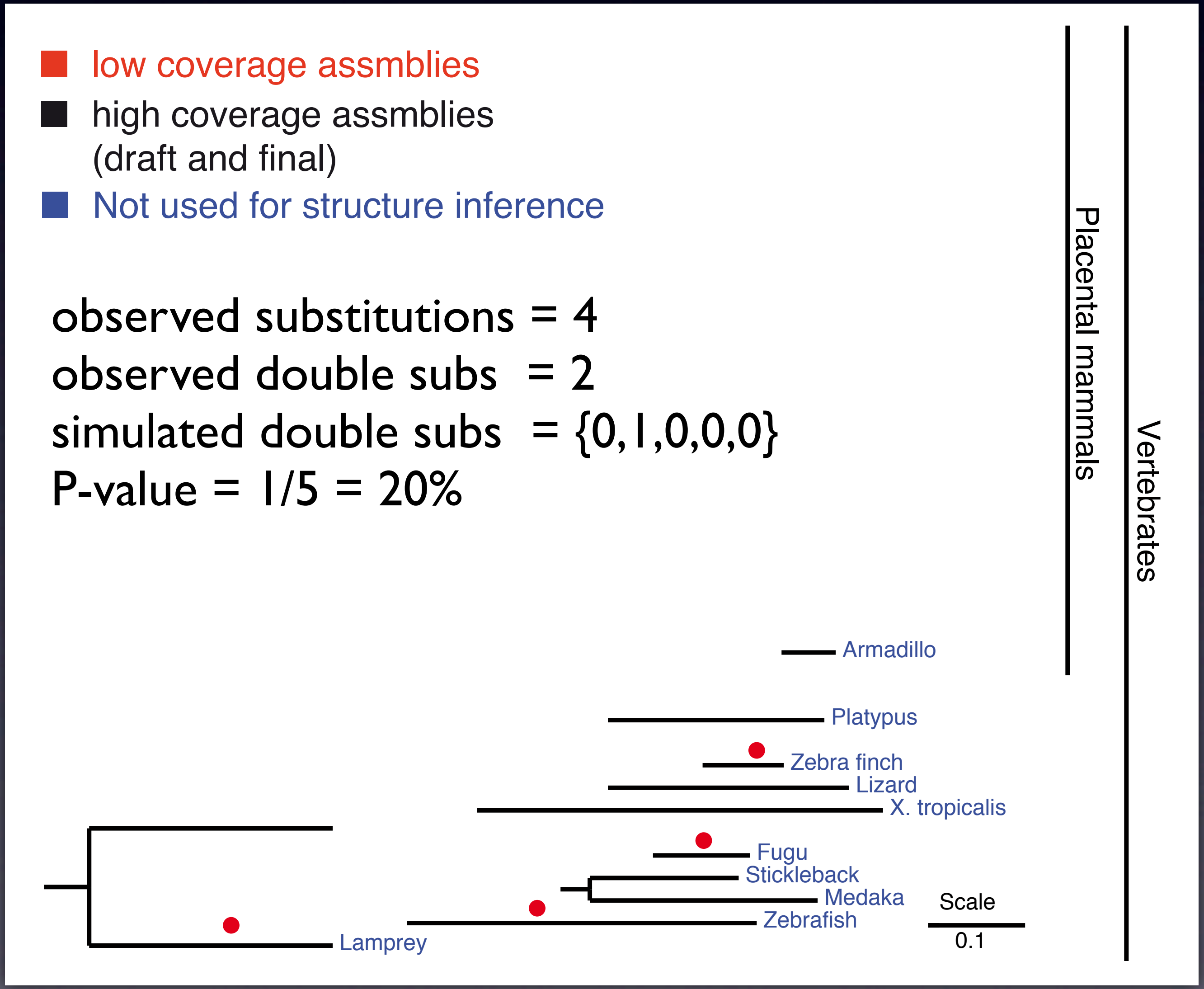

observed substitutions = 4
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simulated double subs = {0,1,0,0,0}
P-value = 1/5 = 20%

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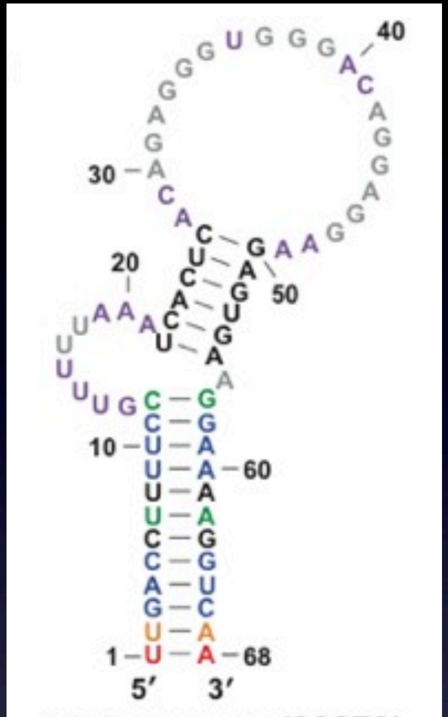
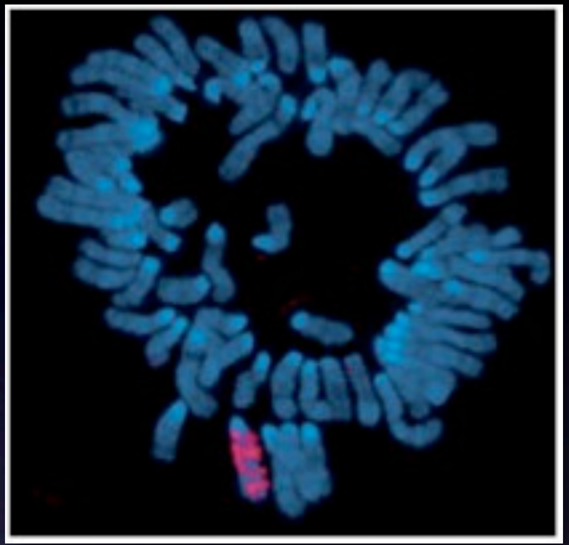
New structure in XIST



XIST structure (36870)

XIST Chromatin regulation



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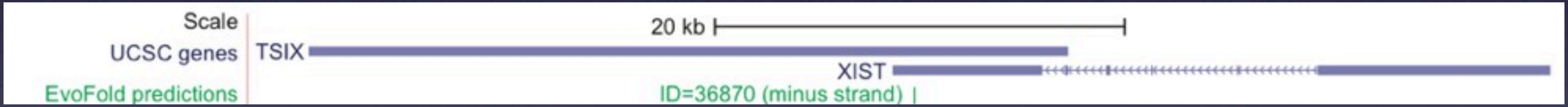

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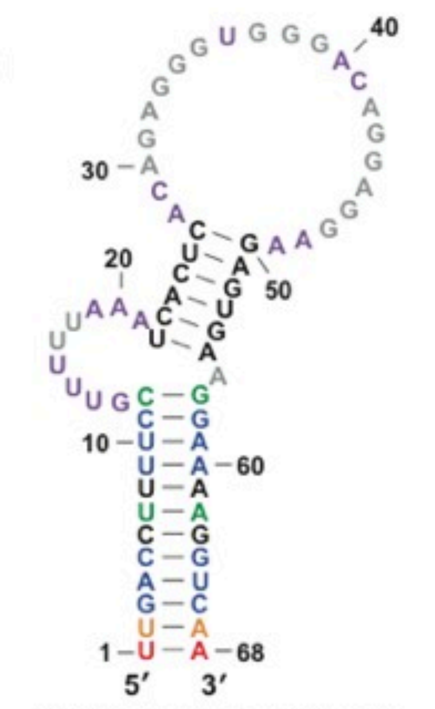

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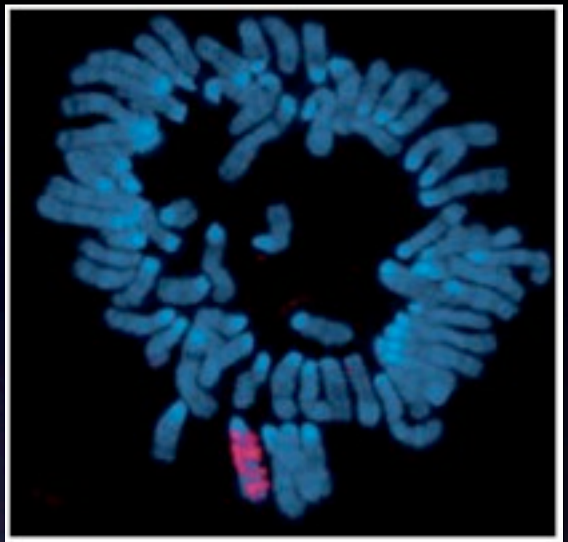

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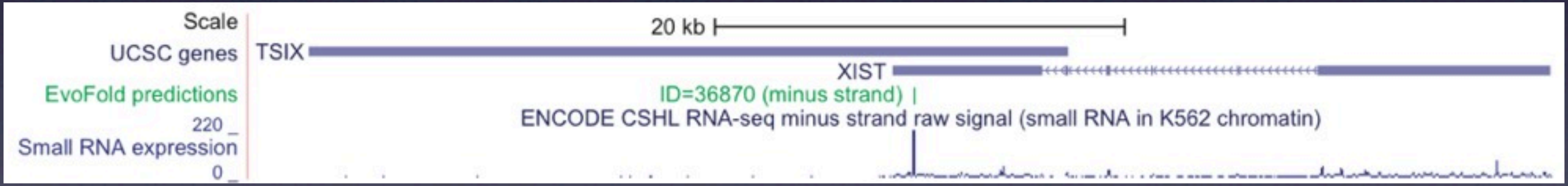

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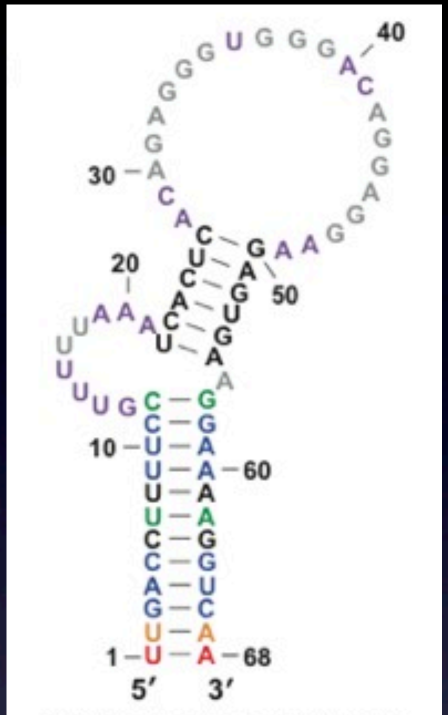

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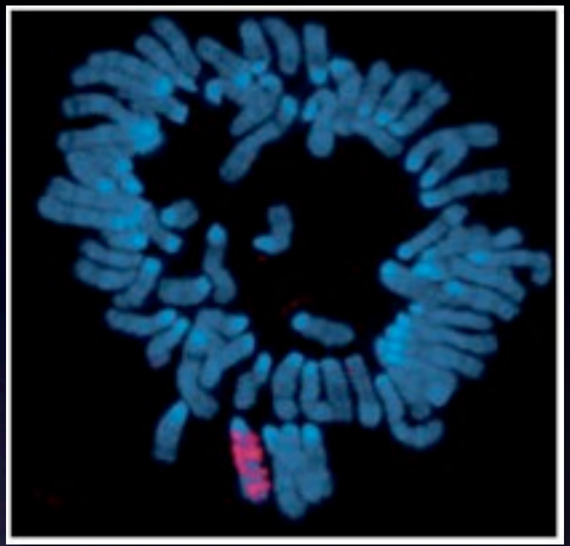

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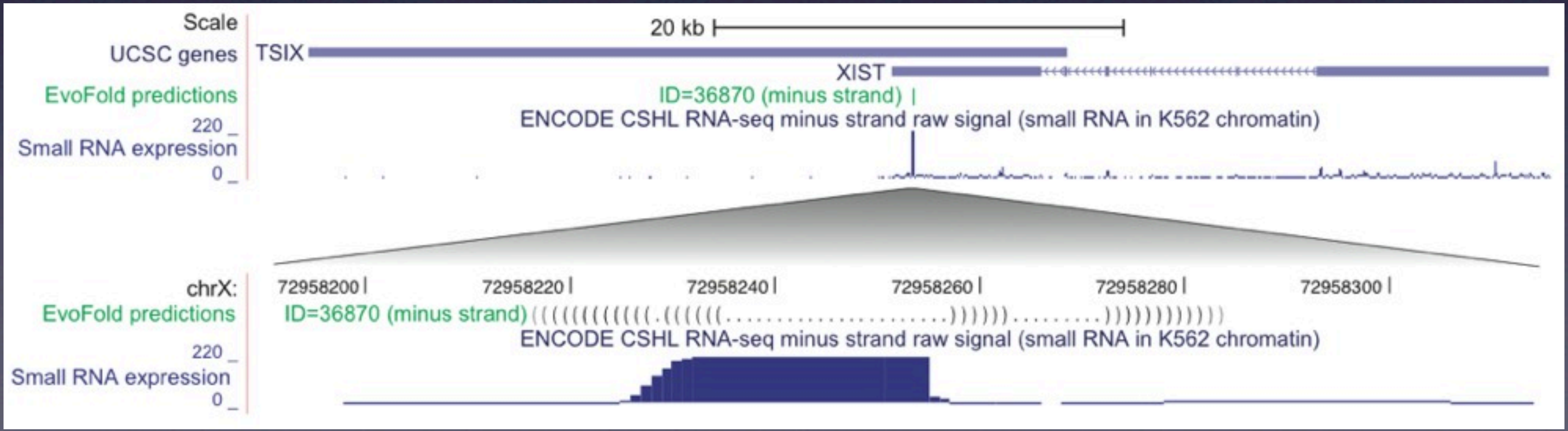
Scale
20 kb
UCSC genes
TSIX
XIST
EvoFold predictions
ID=36870 (minus strand)
ENCODE CSHL RNA-seq minus strand raw signal (small RNA in K562 chromatin)
220
Small RNA expression
0
chrX:
72958200
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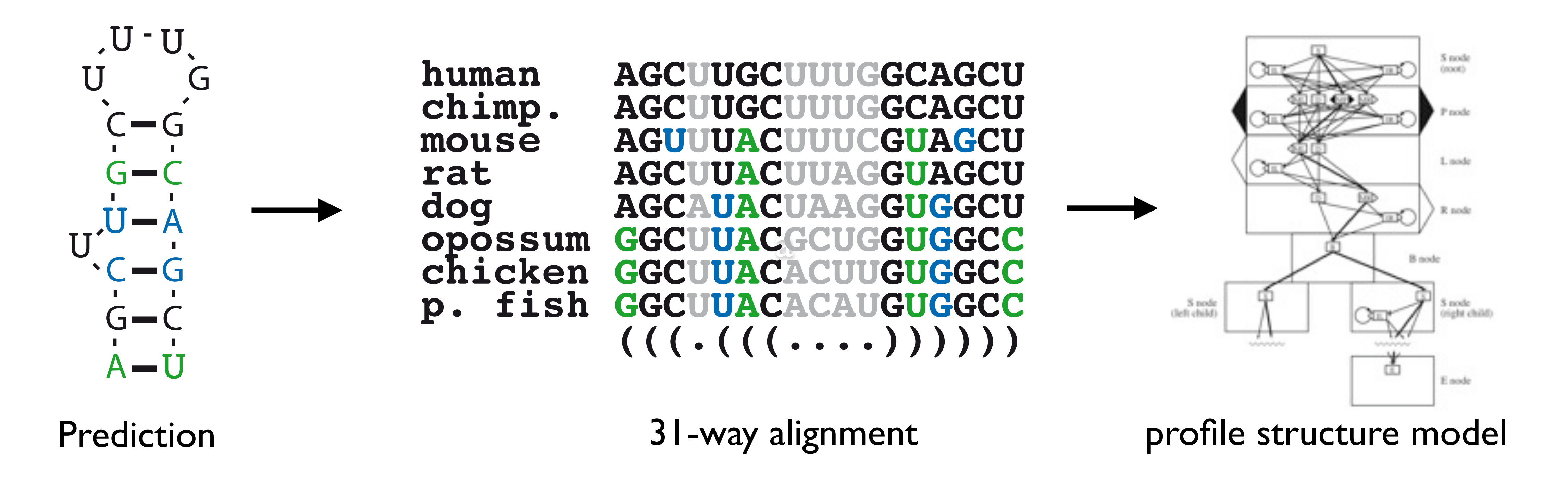
Family classification

Similarity measure

Build profile -SCFGs / co-variance models for every prediction



```
human    AGCUUGCUUUGGCAGCU
chimp.   AGCUUGCUUUGGCAGCU
mouse    AGUUUACUUUCGUAGCU
rat      AGCUUACUUAGGUAGCU
dog      AGCAUACUAAGGUGGCU
opossum  GGCUUACGCUGGUGGCC
chicken  GGCUUACACUUGUGGCC
p. fish  GGCUUACACAUGUGGCC
         (((.(((....))))))
```

Prediction → 31-way alignment → profile structure model

Models made with Infernal tools from Sean Eddy's group.

Structure model figure: Durbin et al, Biological Sequence Analysis, Cambridge University Press.
Nawrocki EP, Kolbe DL, Eddy SR. 2009. Infernal 1.0: inference of RNA alignments. Bioinformatics 25: 1335–1337.

(Dis)similarity measure

Initially, we wanted to use Kullback-Liebler divergence (KL):

$$D_{KL}\left(M_1 \parallel M_2\right) = \sum_i P_{M_1}(i) \log \frac{P_{M_1}(i)}{P_{M_2}(i)}$$

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We couldn’t compute and resorted to sampling:

$$\tilde{D}_{KL}\left(M_1 \parallel M_2\right) = 1/n \sum_{i=1}^{n} 1/l(s_{1,i}) \cdot \left(\log\left(P_{M_1}(s_{1,i})\right) - \log\left(P_{M_2}(s_{1,i})\right)\right)$$

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Still slow. Approximated by one sample only - human sequence from training alignment. Also replaced probabilities with (Infernal) normalized scores:

$$\tilde{D}_{KL,human}\left(M_1 \| M_2\right) = 1/l(s_{1,human}) \cdot \left(S(s_{1,human}, M_1) - S(s_{1,human}, M_2)\right).$$

$$S(s,M) = \log_2 \frac{P_M(s)}{P_{null}(s)}.$$

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Problem: Models of different complexities have different false positive rates. Hence replace score with E-score.

$$\tilde{D}(M_1 \| M_2) = E\left(S\left(seq_1^{human}, M_2\right)\right) - E\left(S\left(seq_1^{human}, M_1\right)\right)$$

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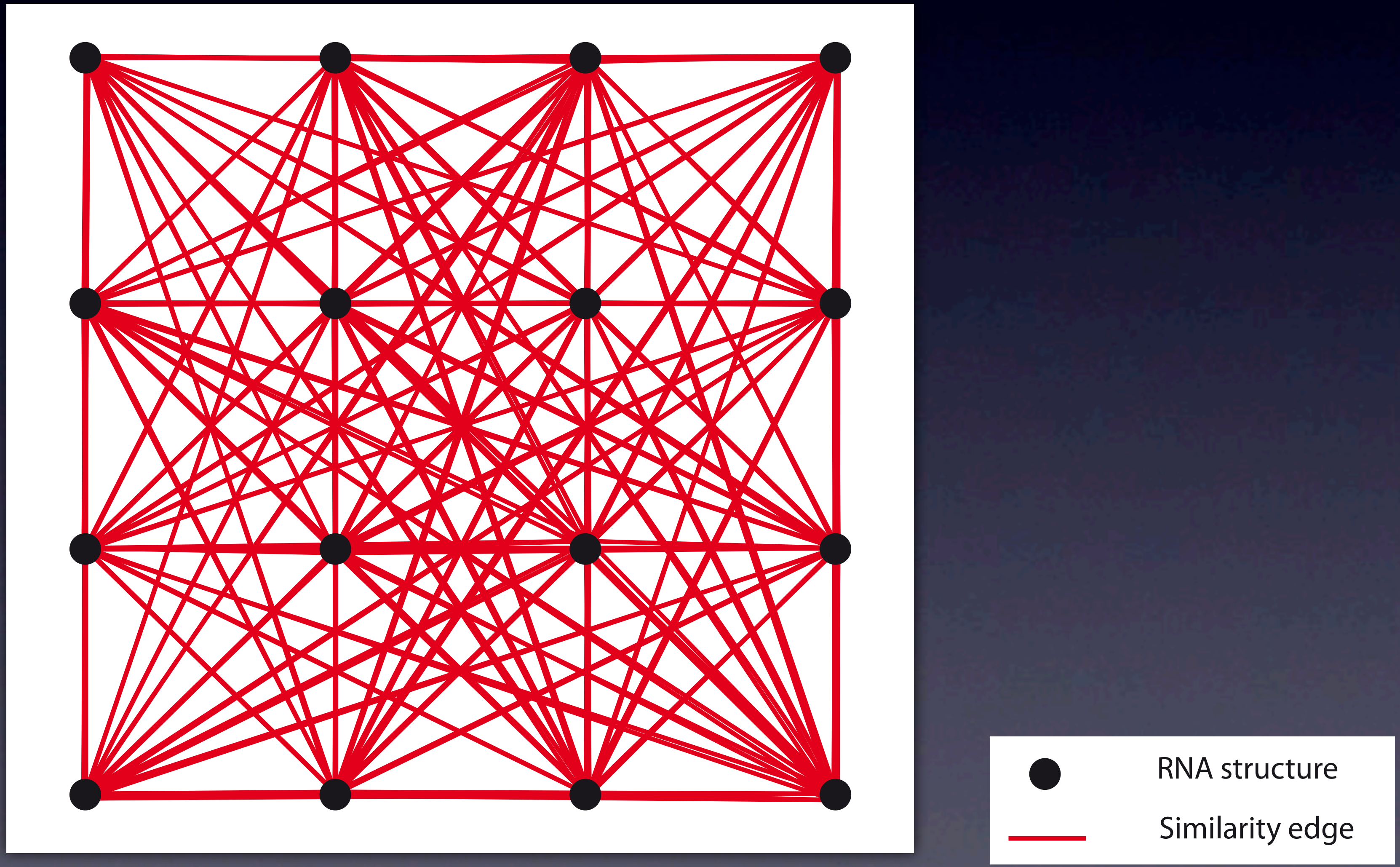
Finally, be conservative and symmetrize by max:

$$D(M_1 \| M_2) = \max\left(\tilde{D}_{E,human}(M_1 \| M_2), \tilde{D}_{E,human}(M_2 \| M_1)\right)$$

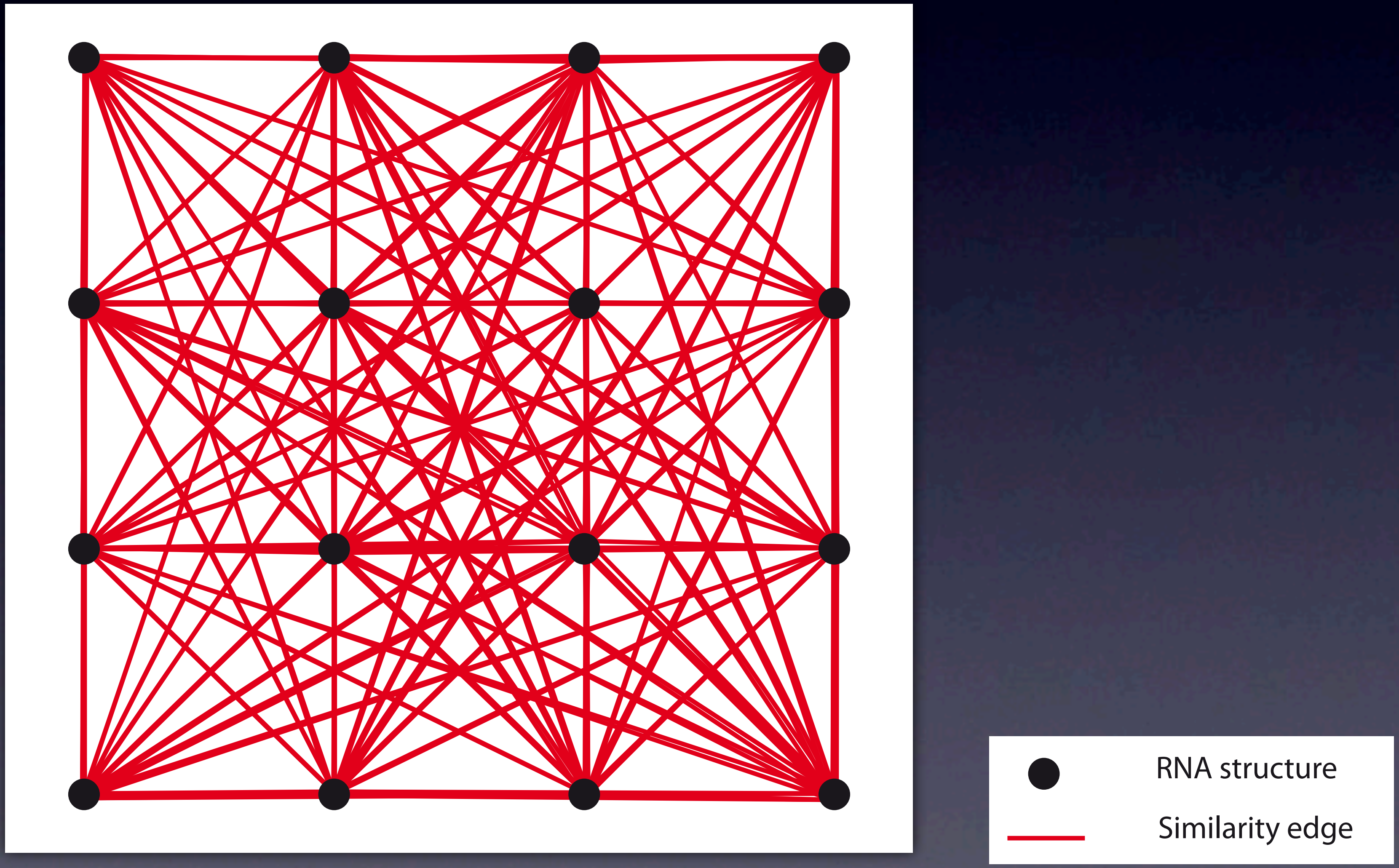
Graph-based clustering into families

All pairwise comparisons



Graph-based clustering into families

Threshold on similarity significance



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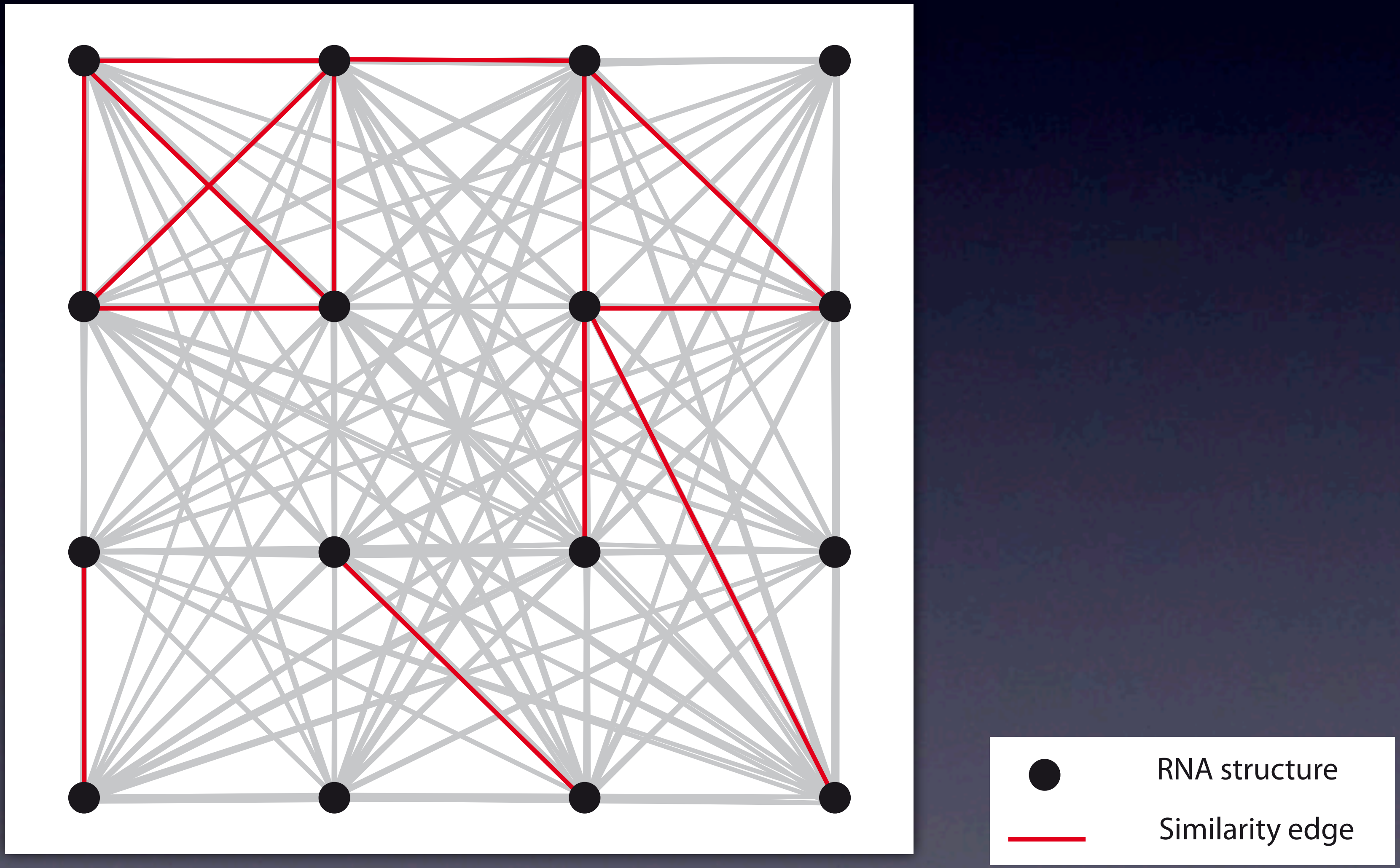

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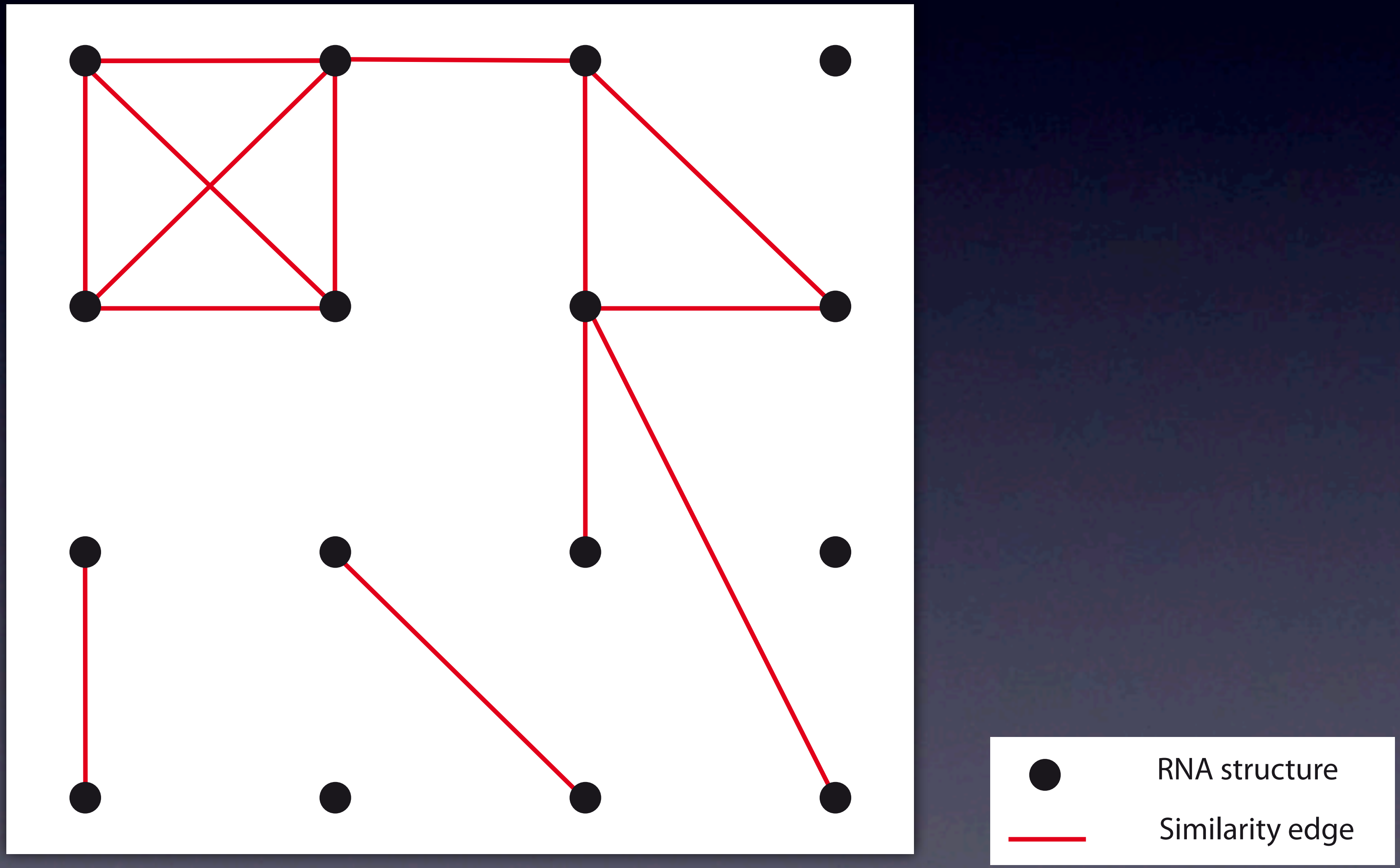
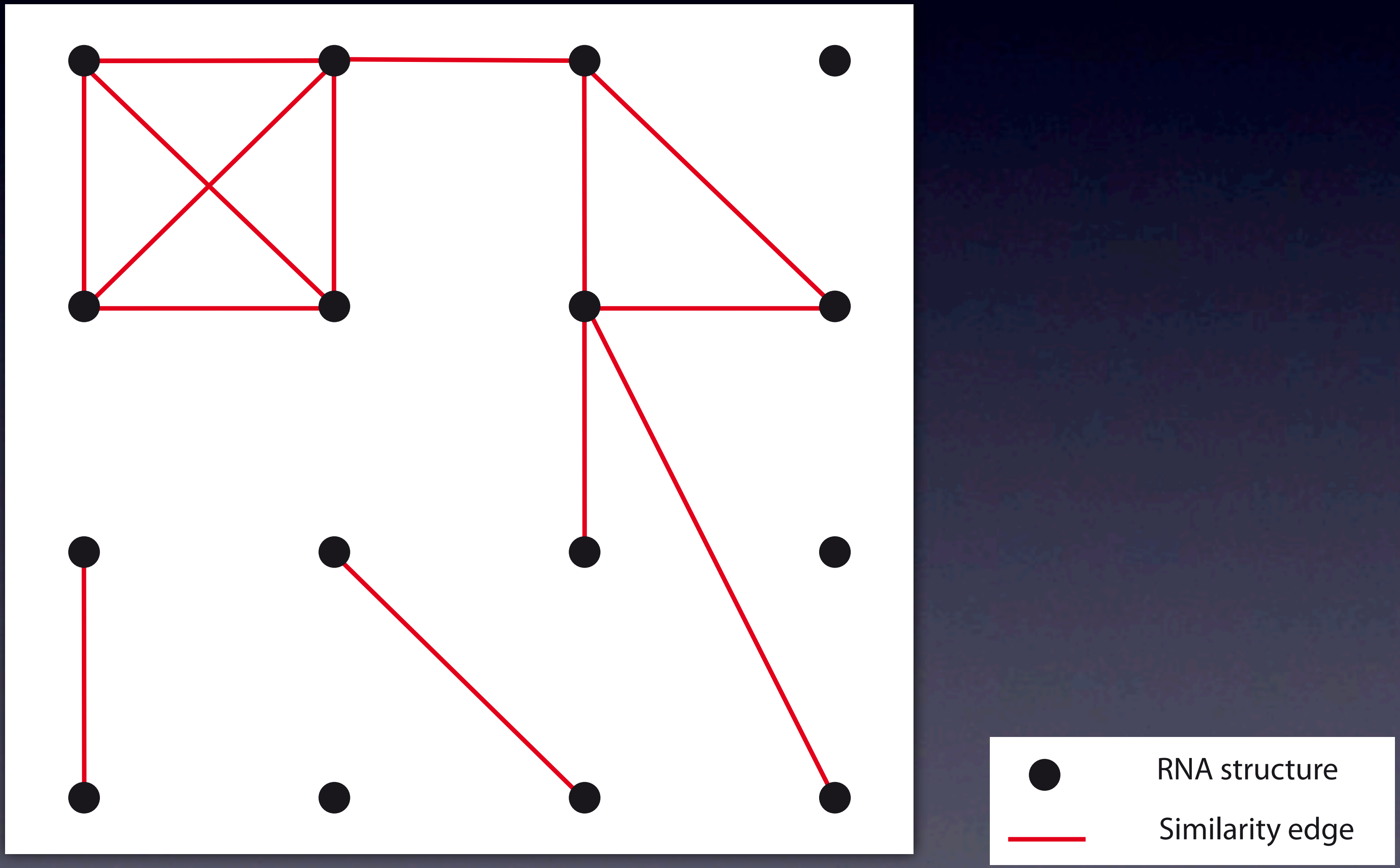


Hartuv and Shamir (2000)

Graph-based clustering into families

Identify highly connected subgraph (HCS)



Highly connected subgraph:

- edge connectivity < half number of vertices
- subgraph size two

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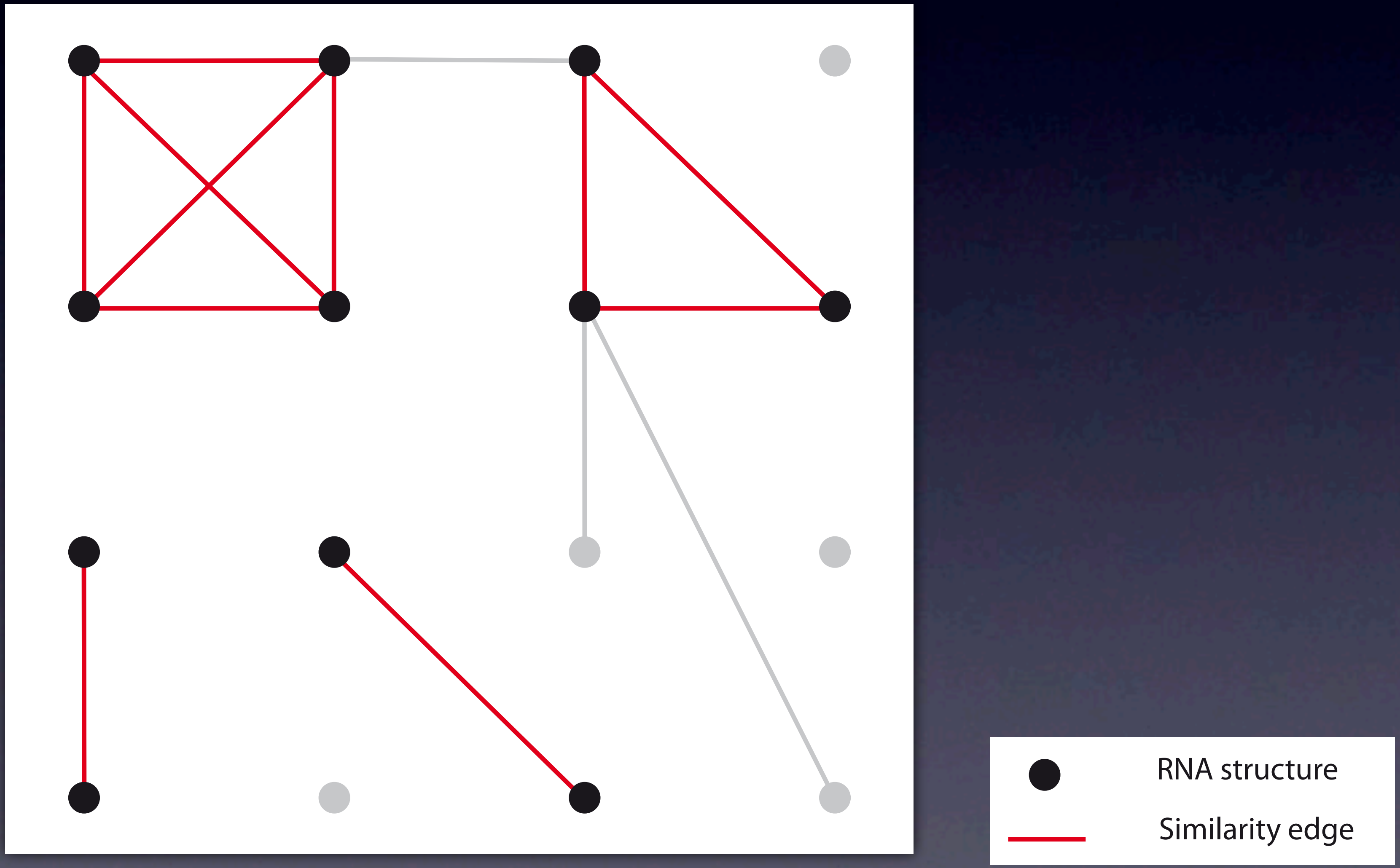

Highly connected subgraph:

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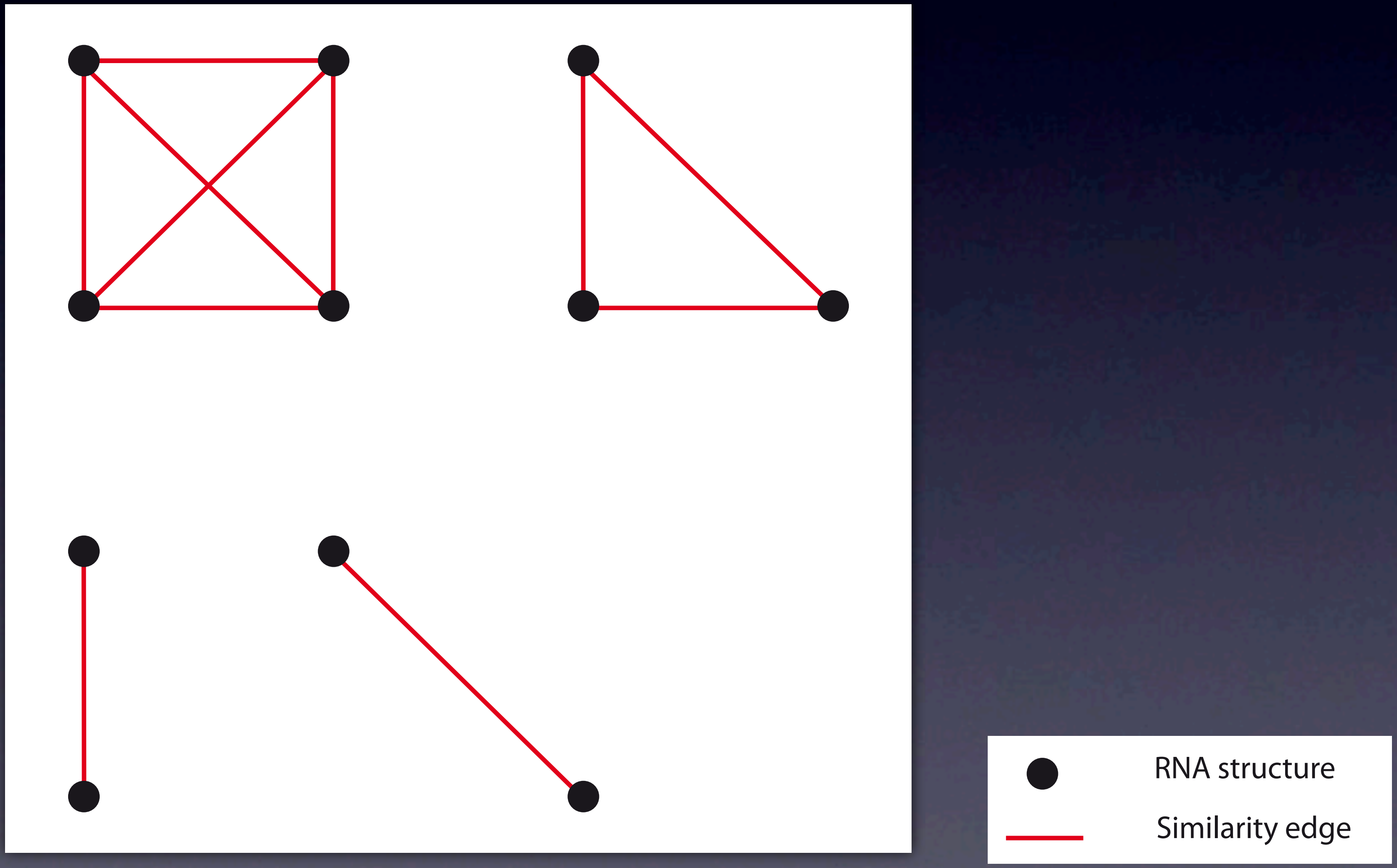
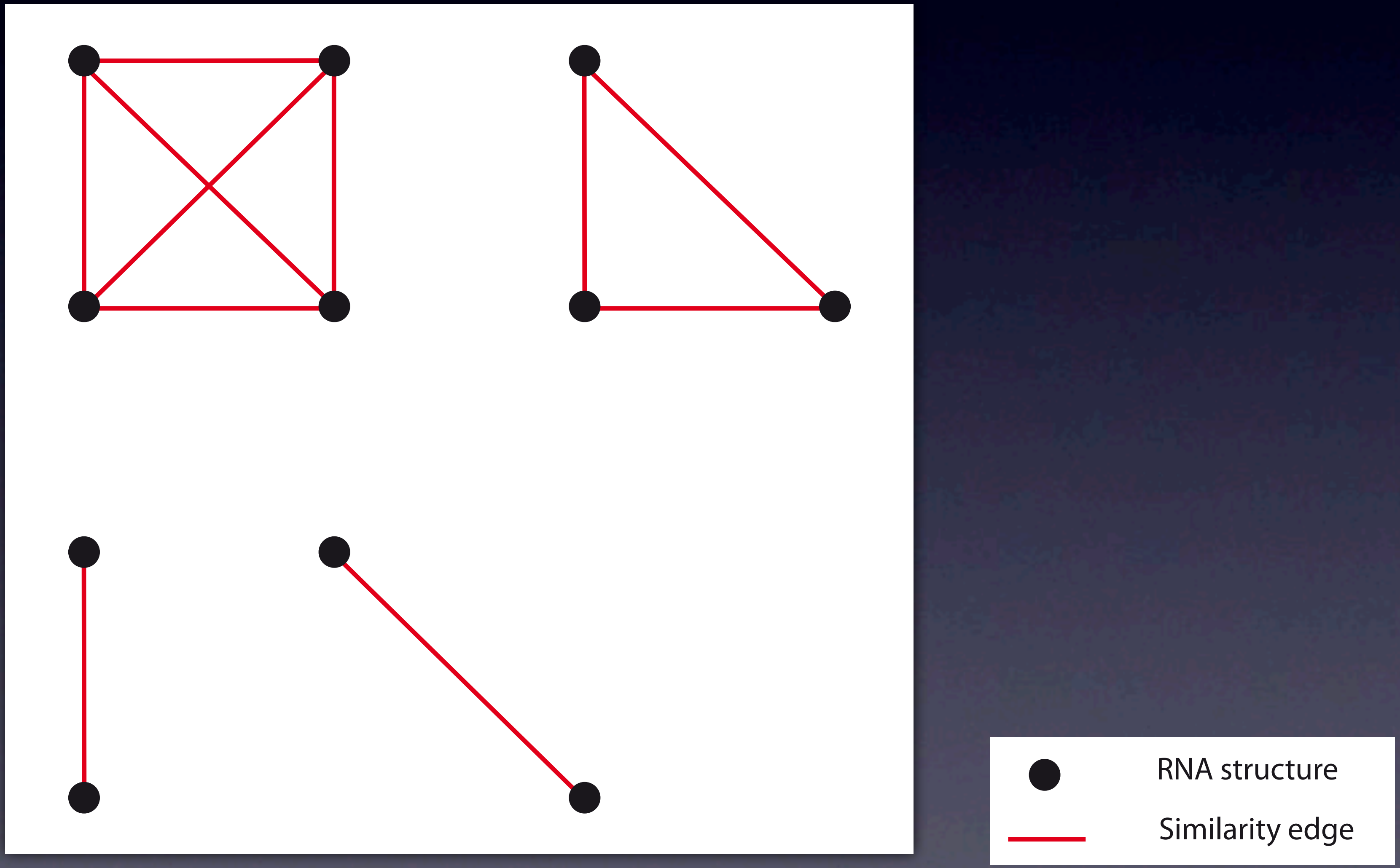
Graph-based clustering into families

Identify highly connected subsets (HCS)

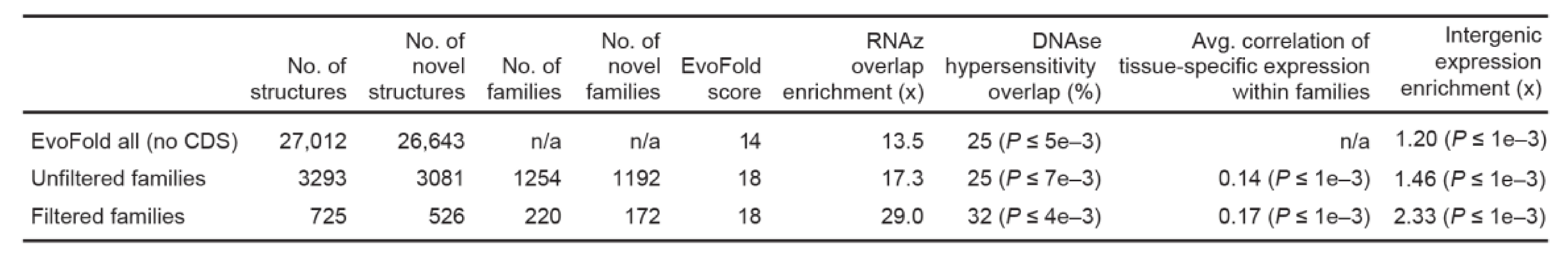


Graph-based clustering into families

Final family candidates



Family prediction overview



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Filtered families have either:

- EvoP test < 0.05
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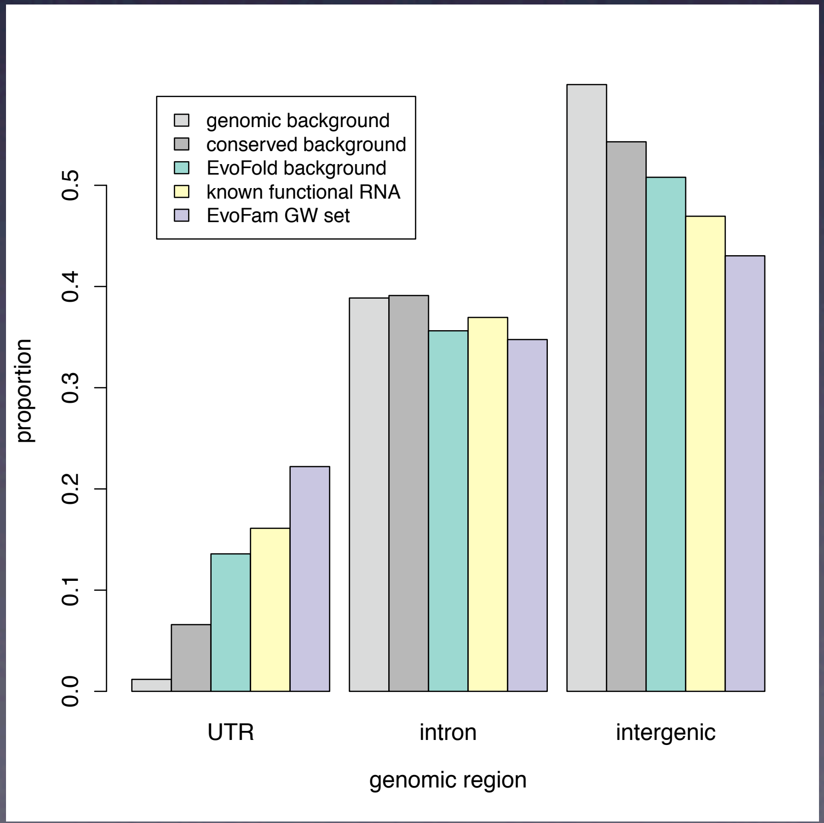
Genomic distribution



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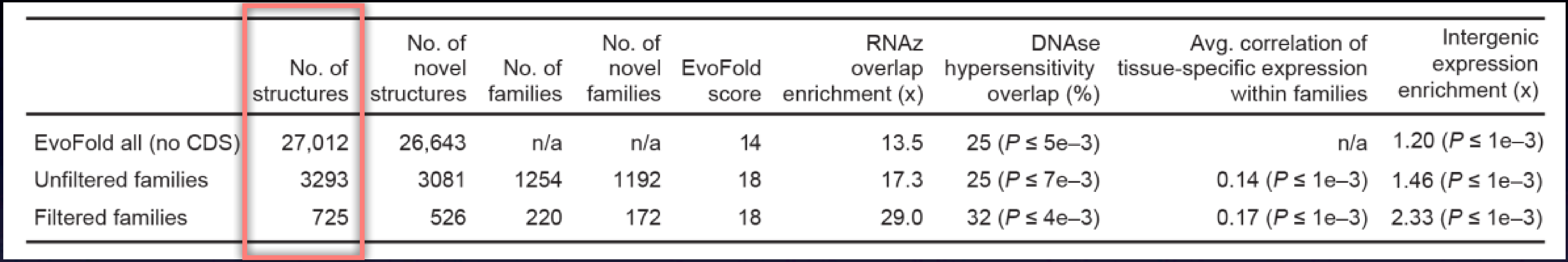

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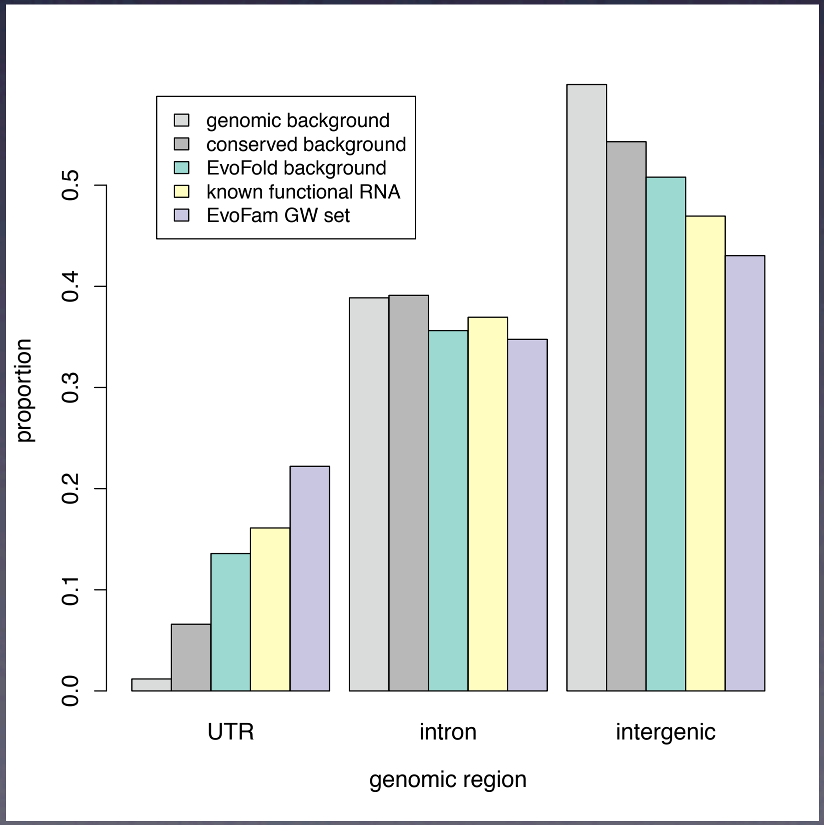

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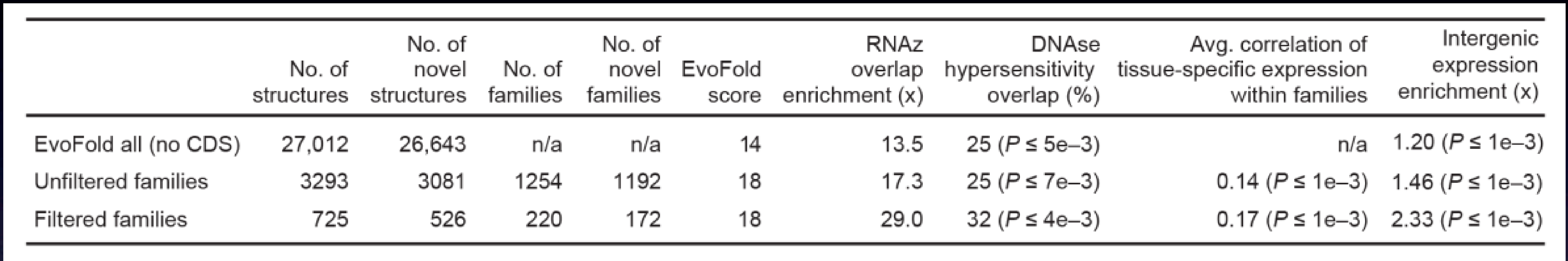

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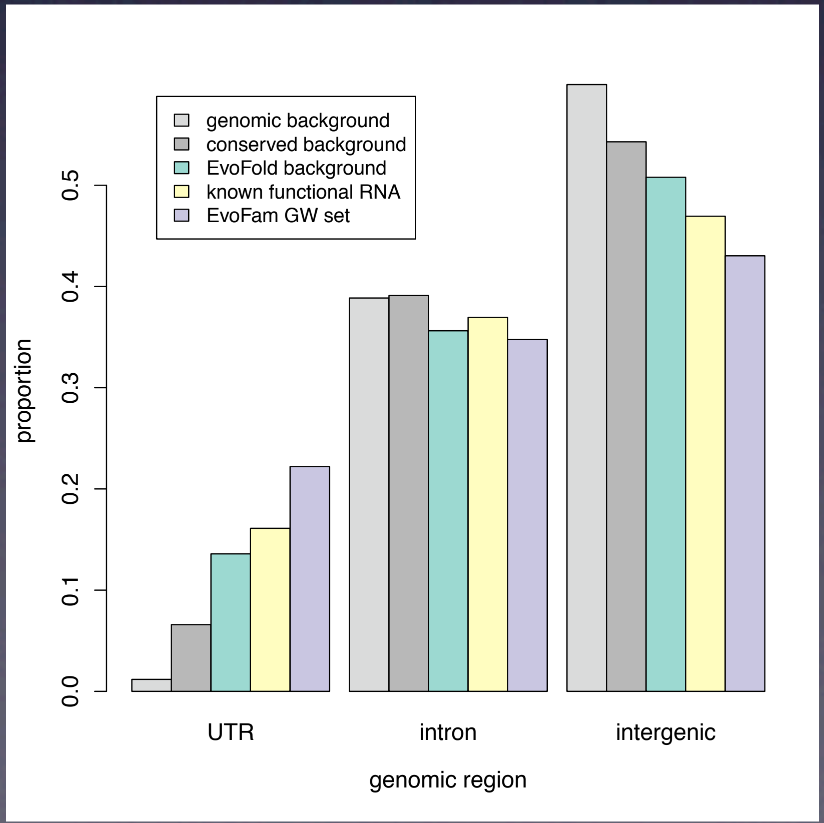

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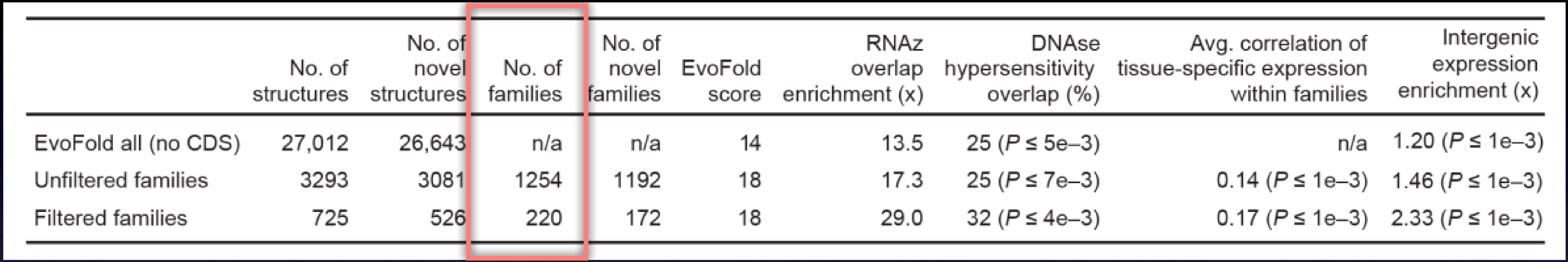

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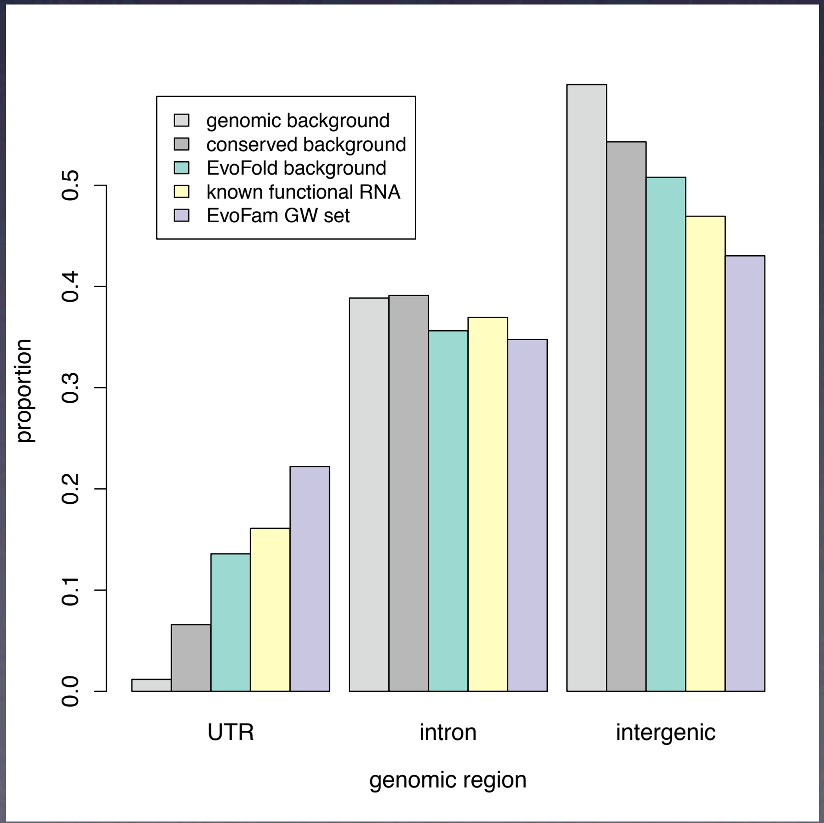

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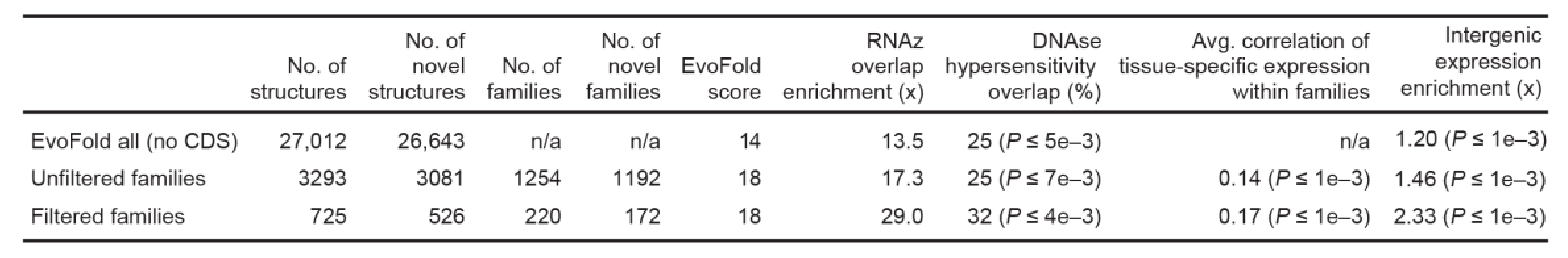

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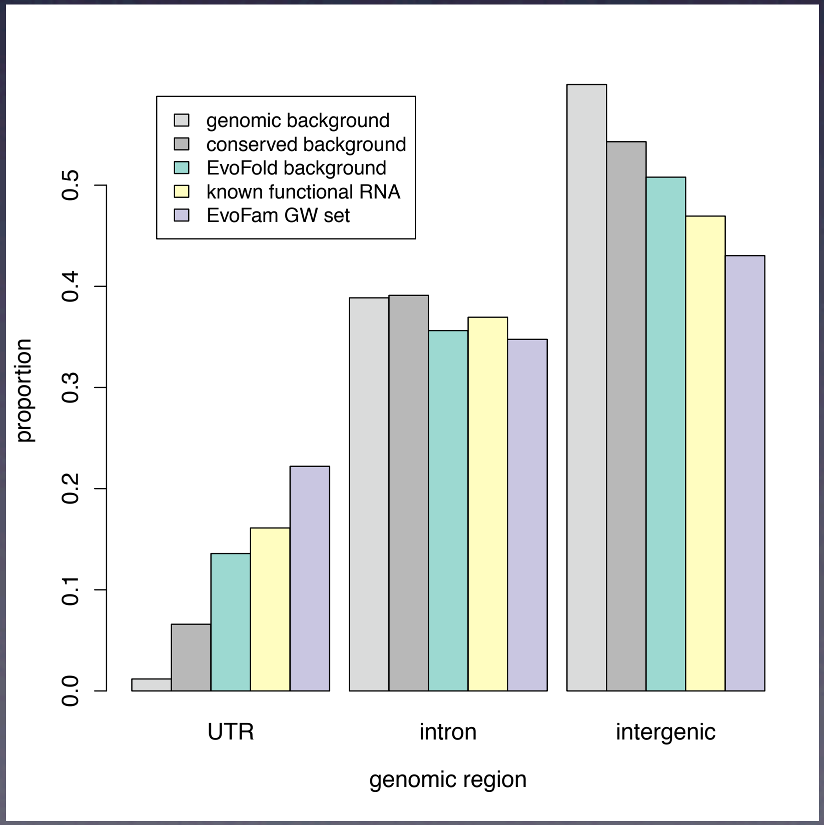

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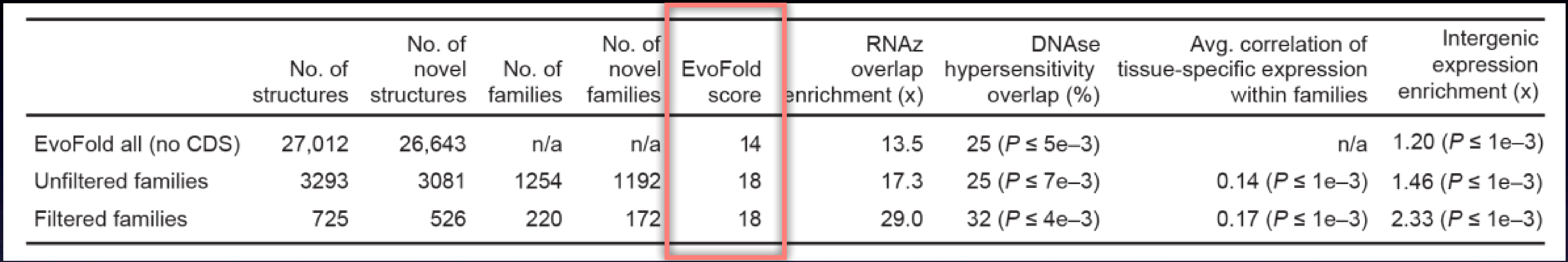

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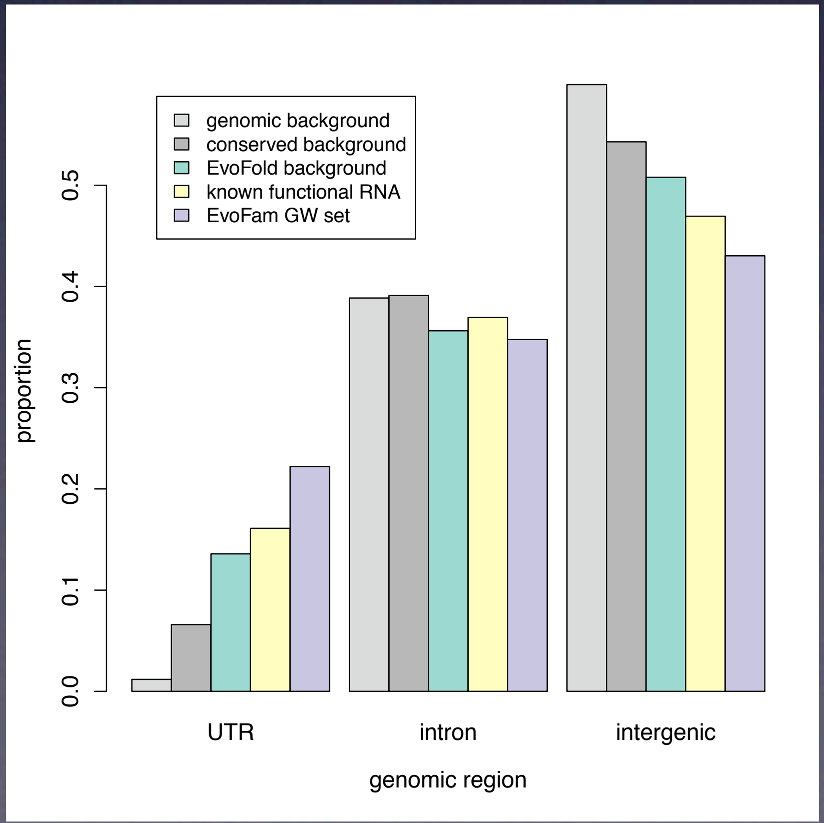

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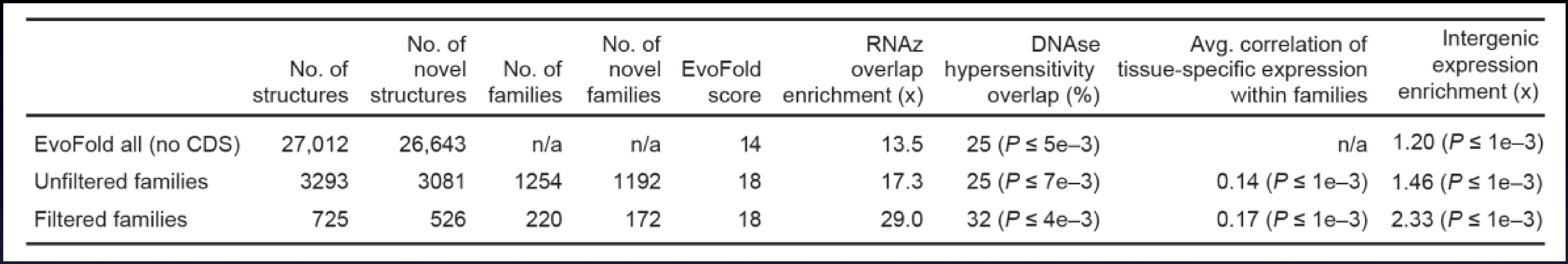

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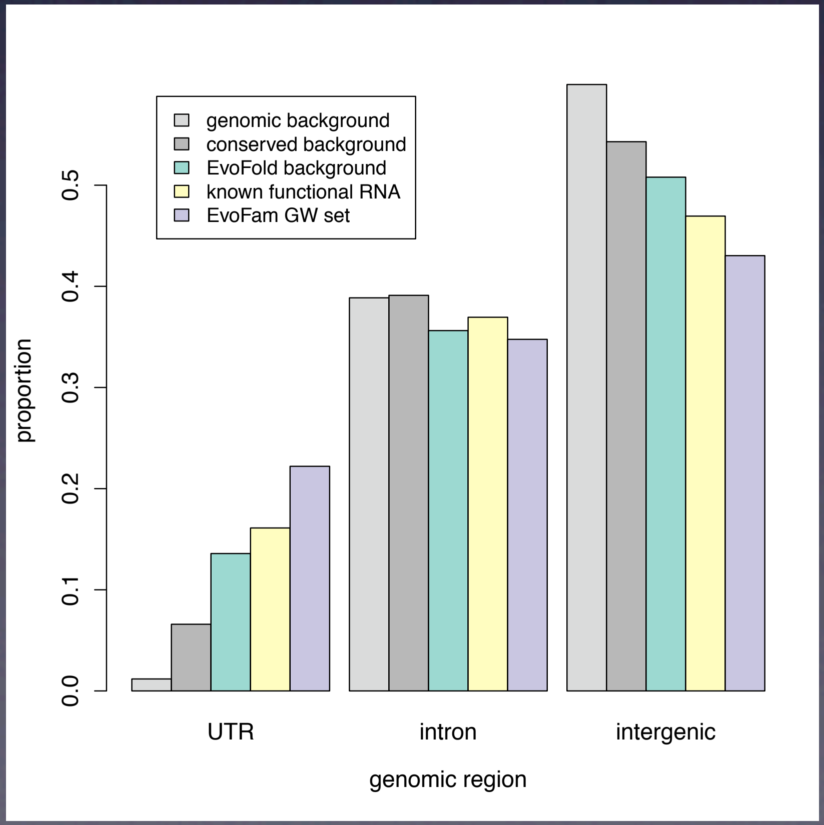

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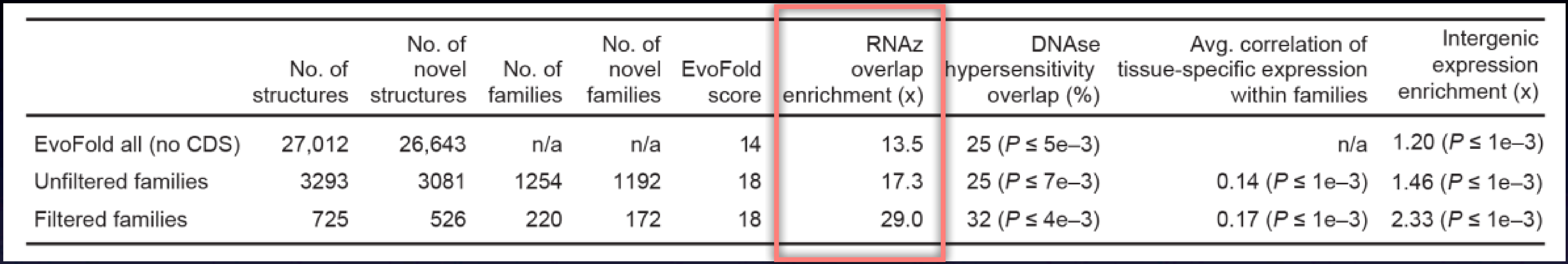

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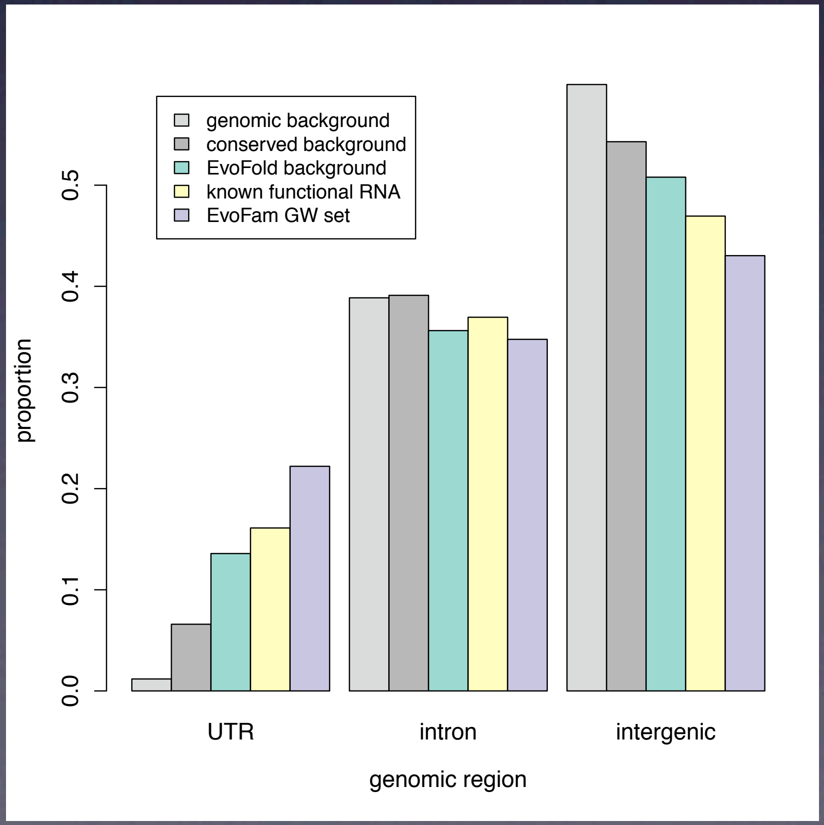

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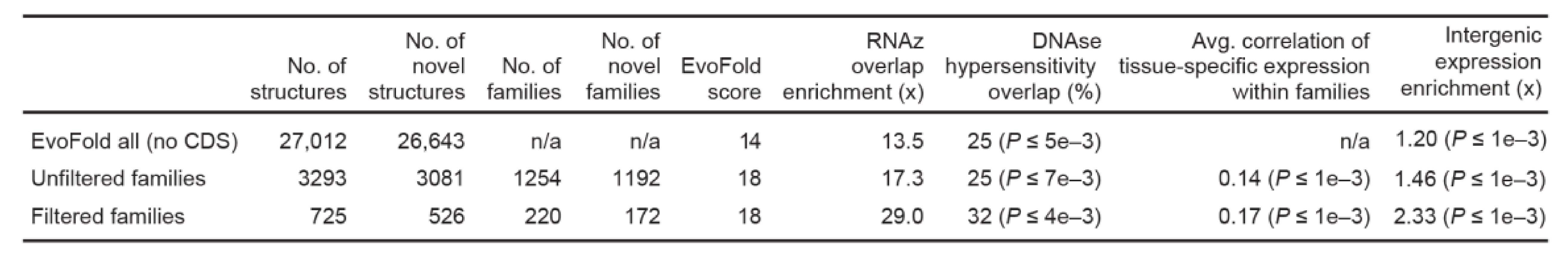

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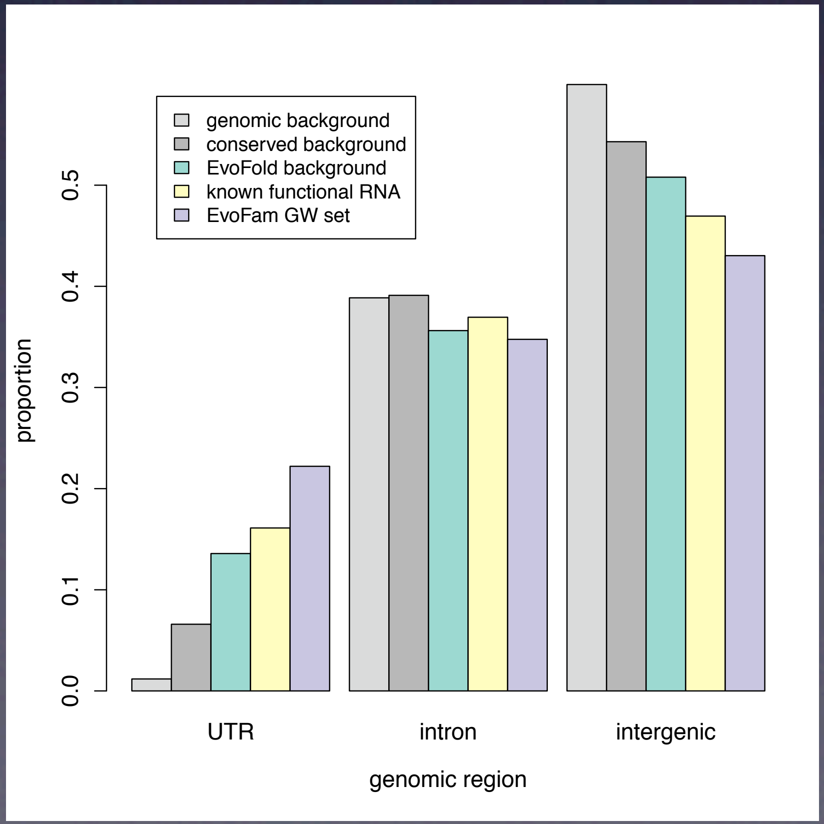

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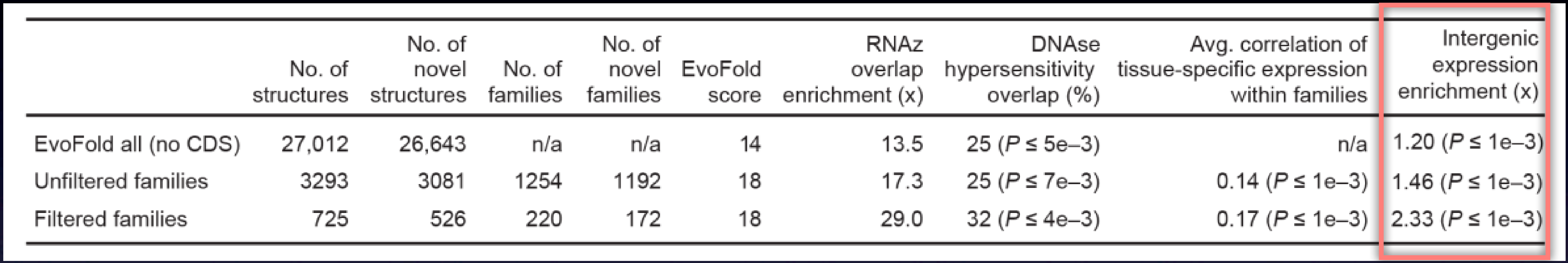

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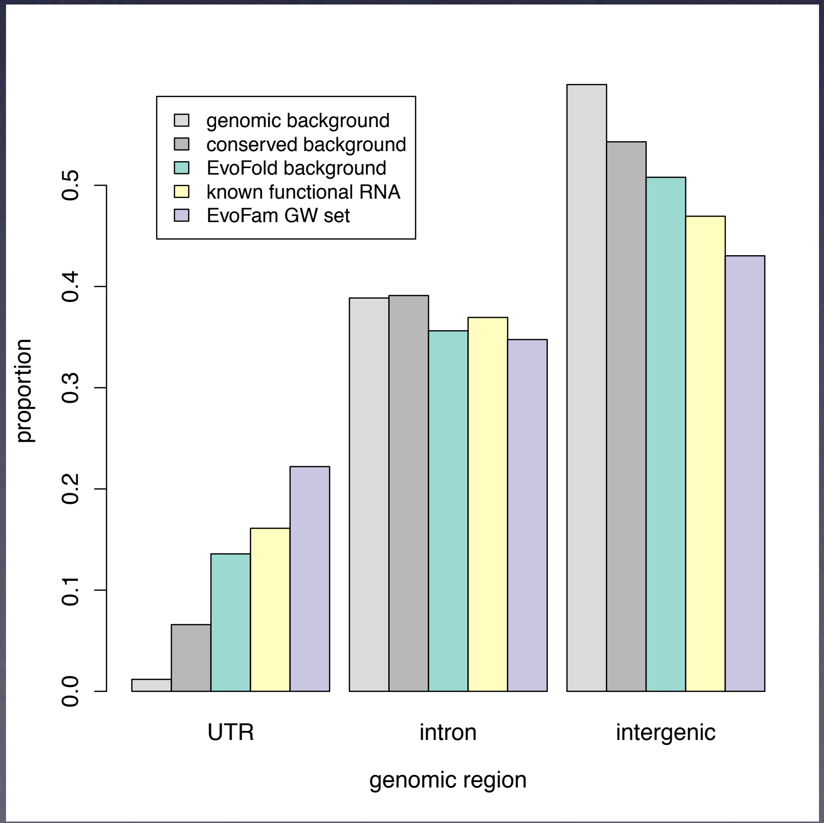

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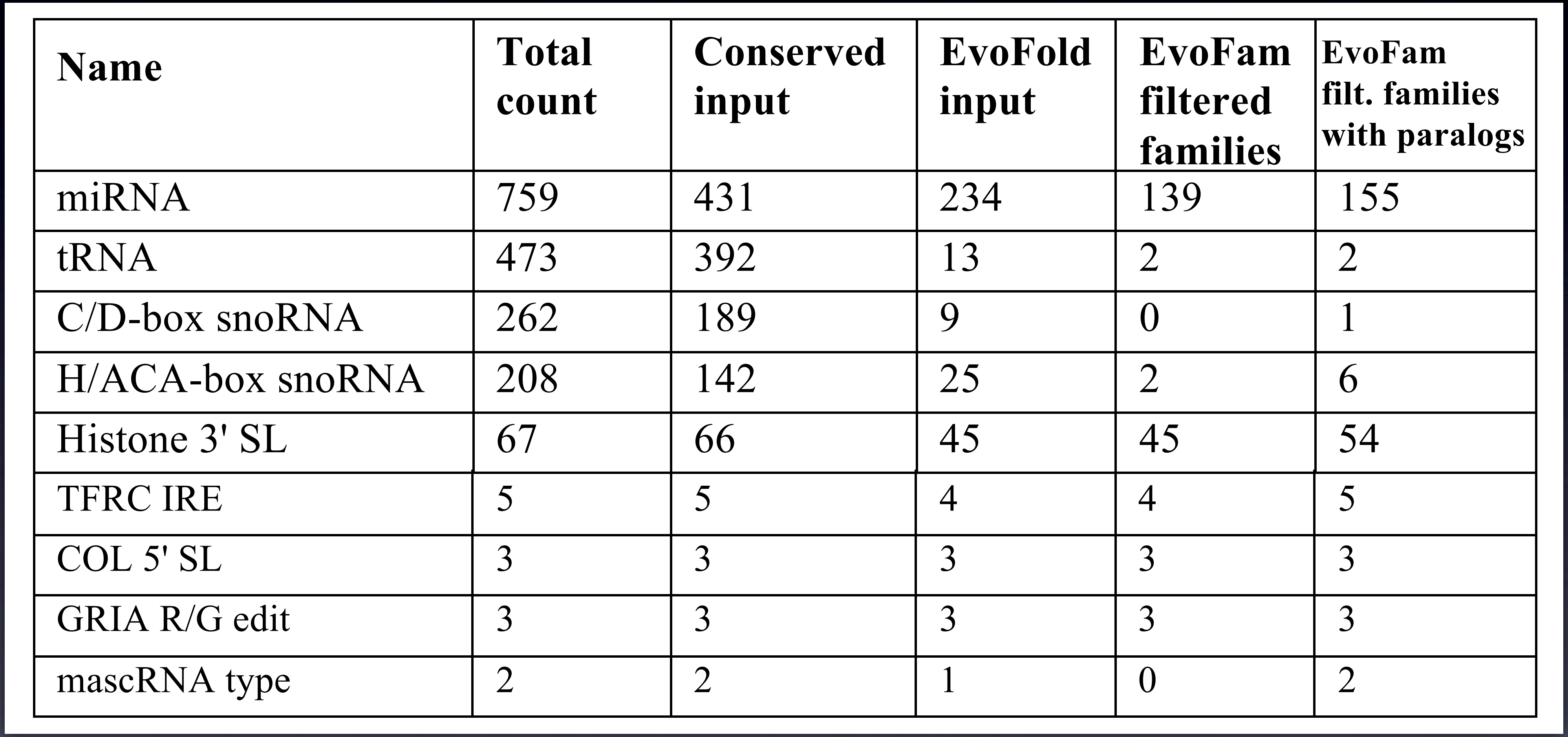

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COL 5' SL	3	3	3	3	3
GRIA R/G edit	3	3	3	3	3
mascRNA type	2	2	1	0	2

Performance

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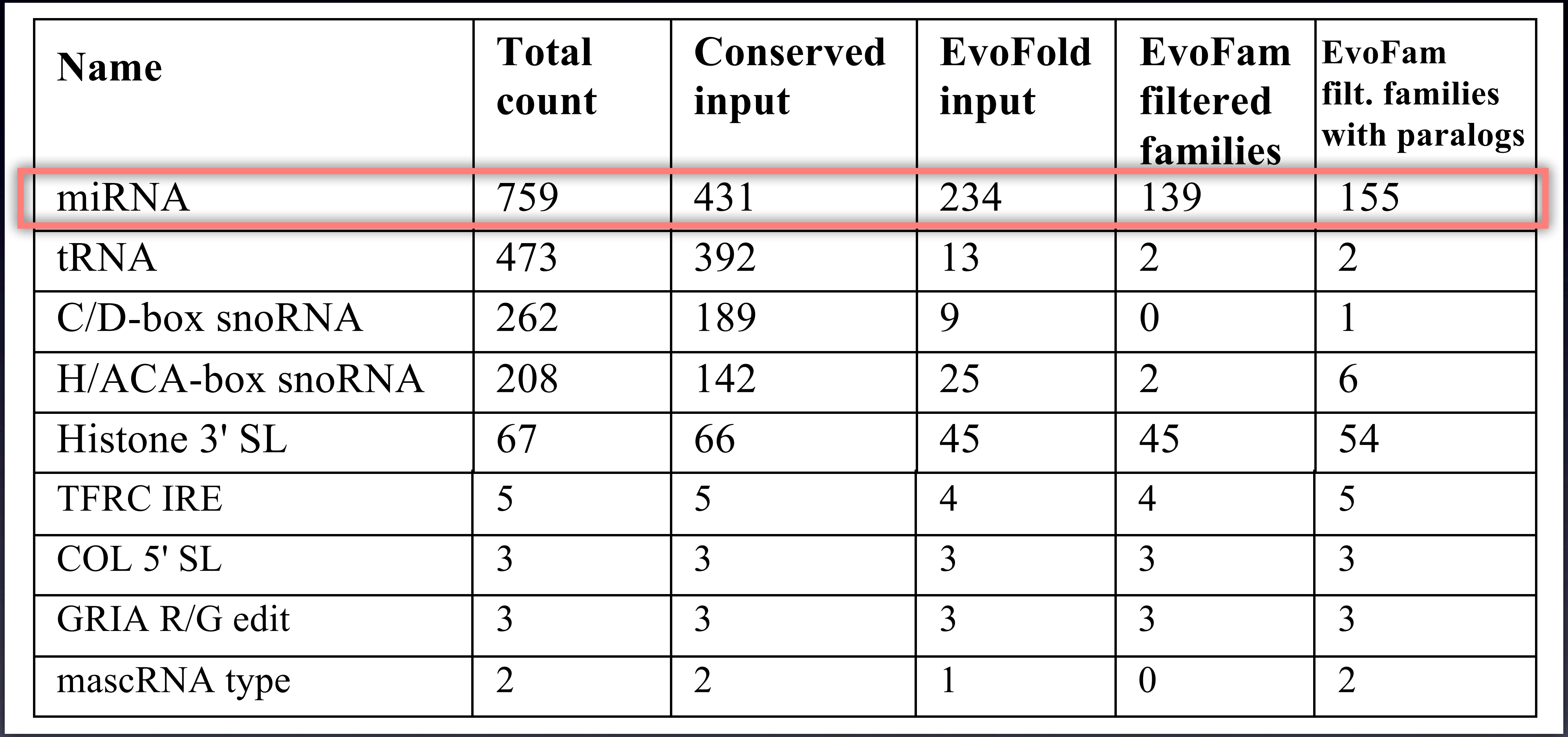

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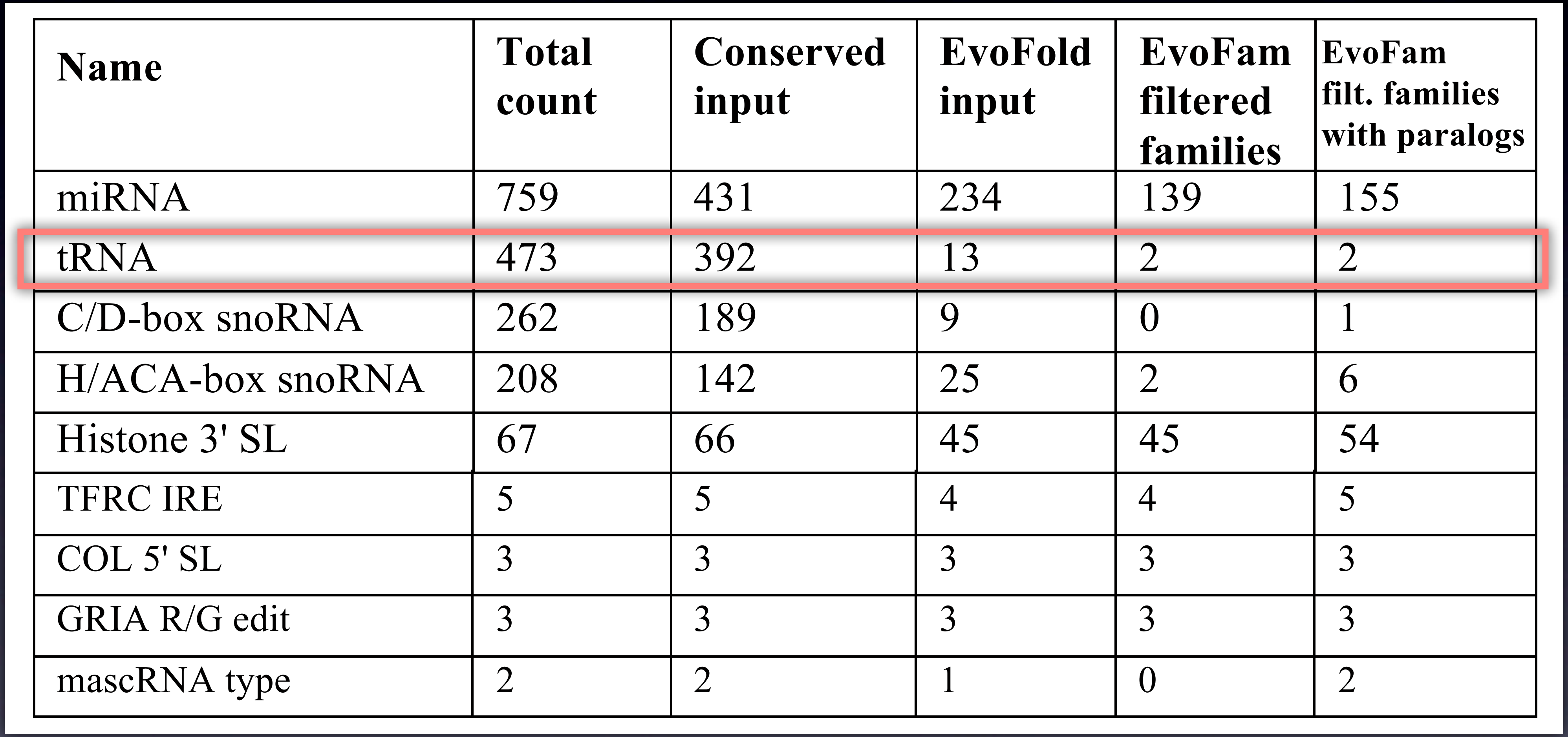

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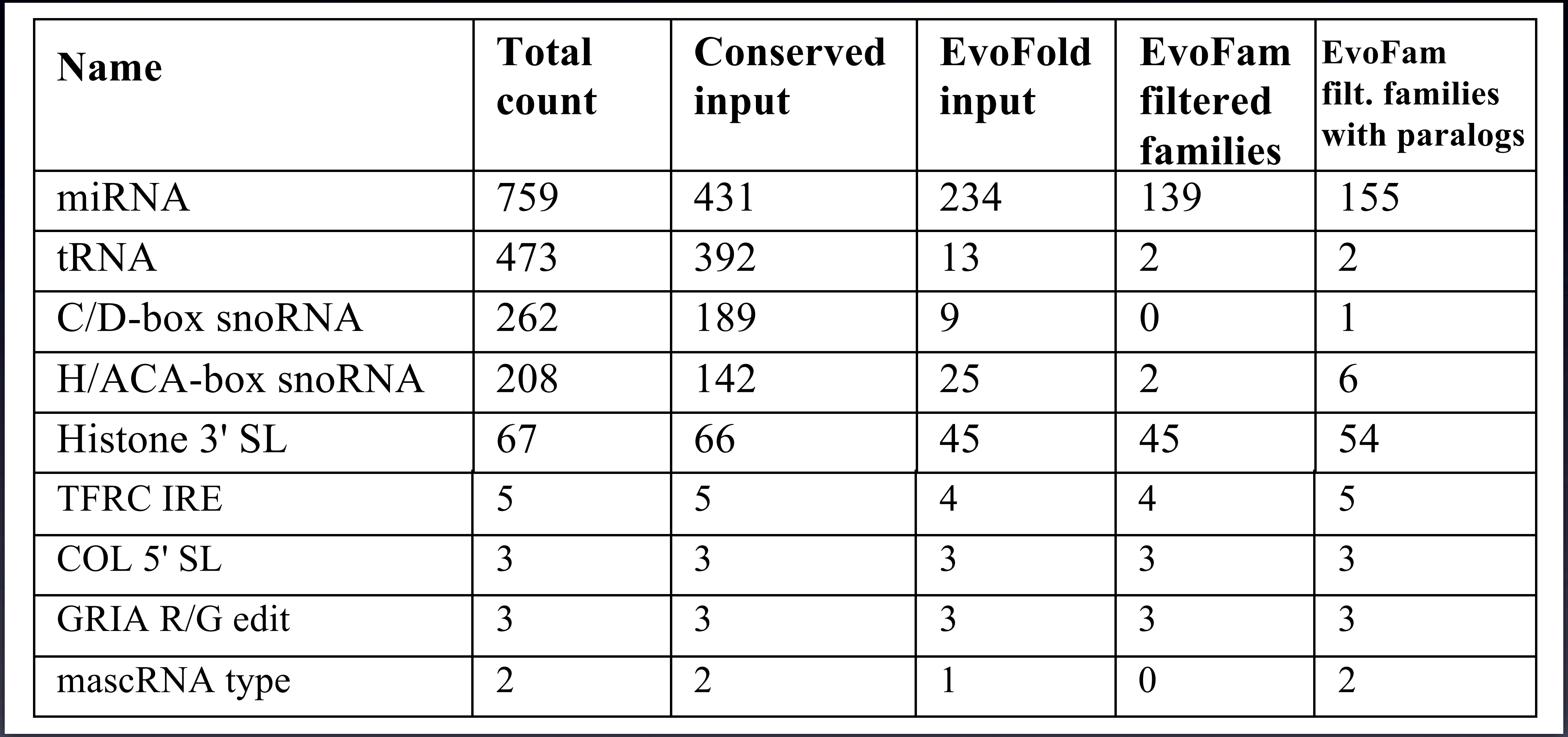

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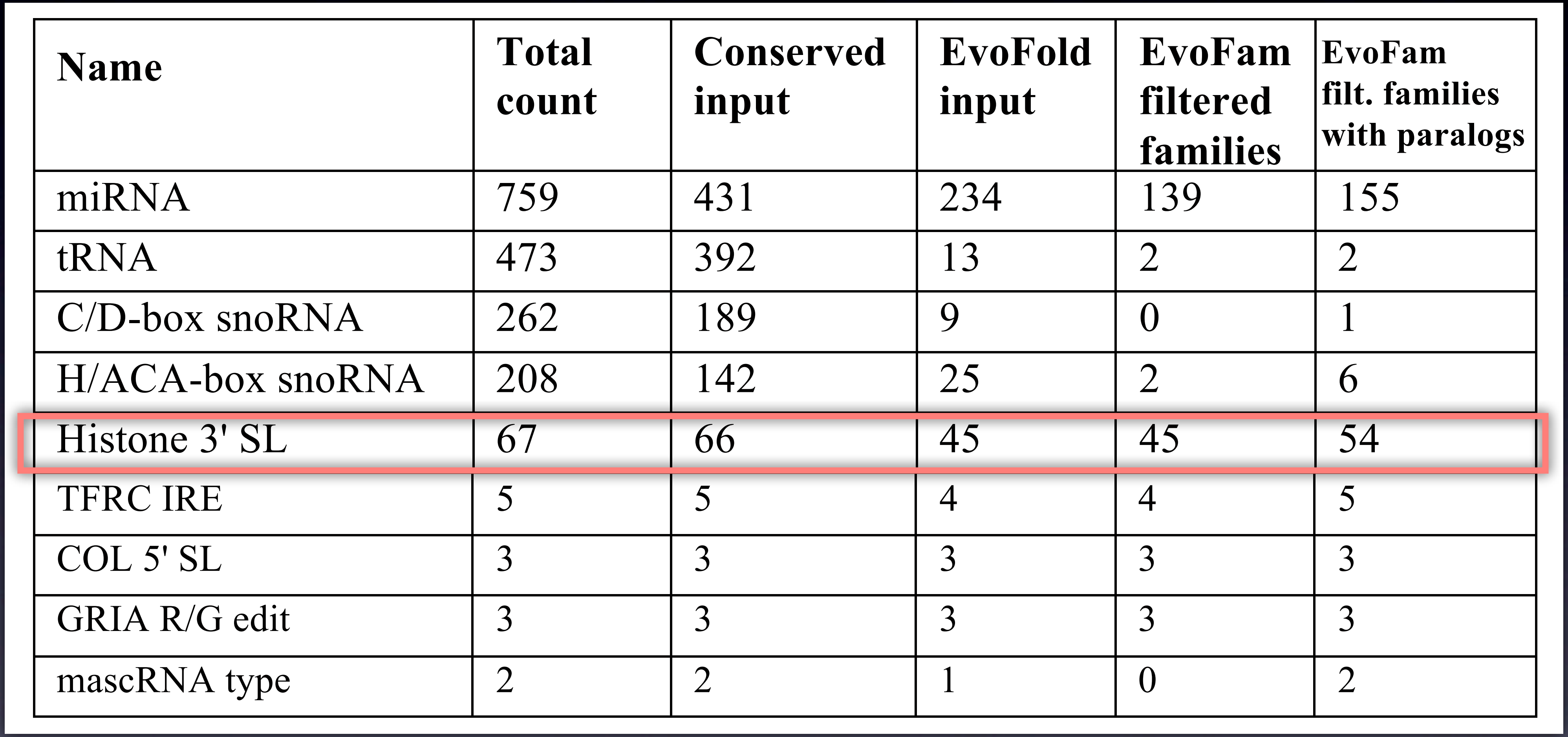

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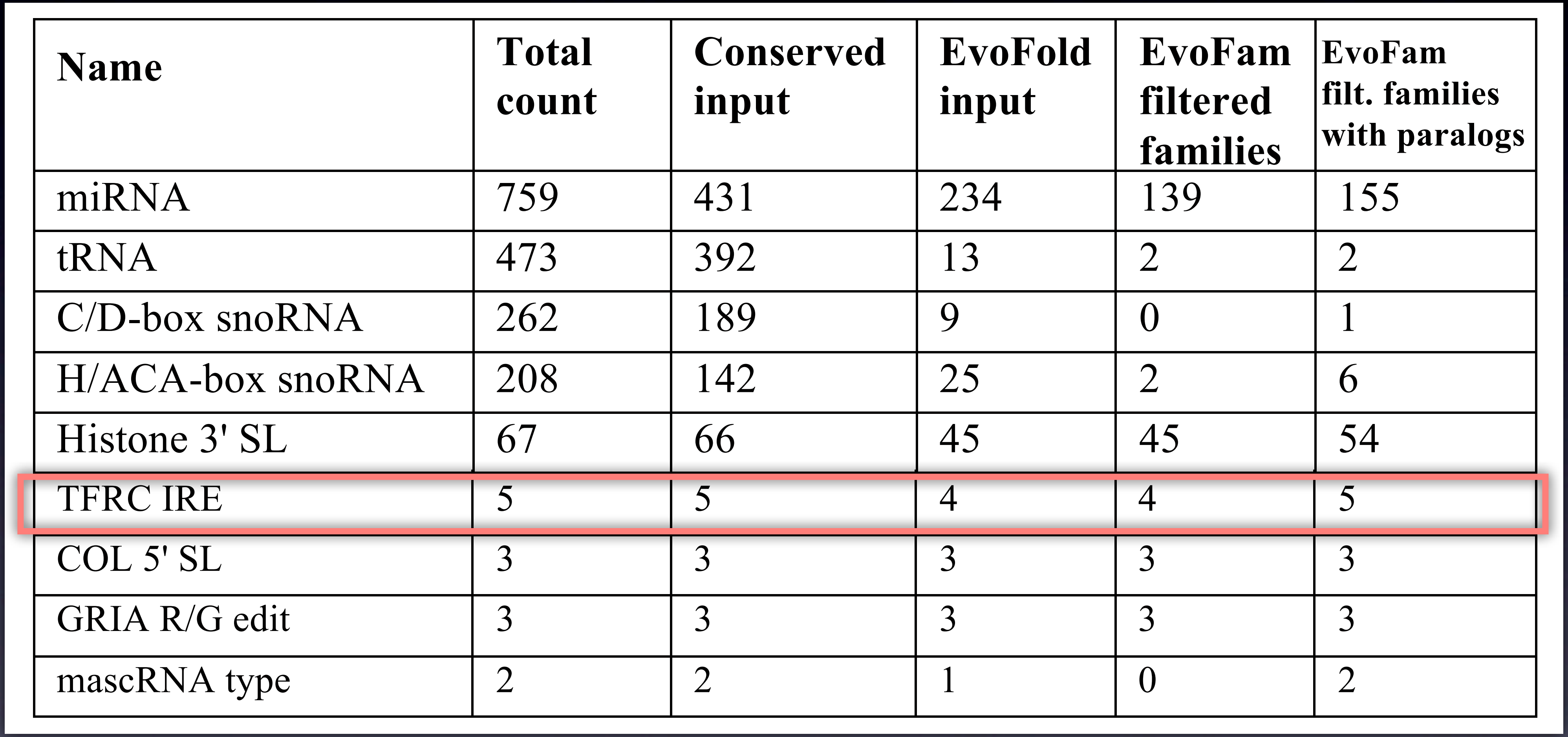

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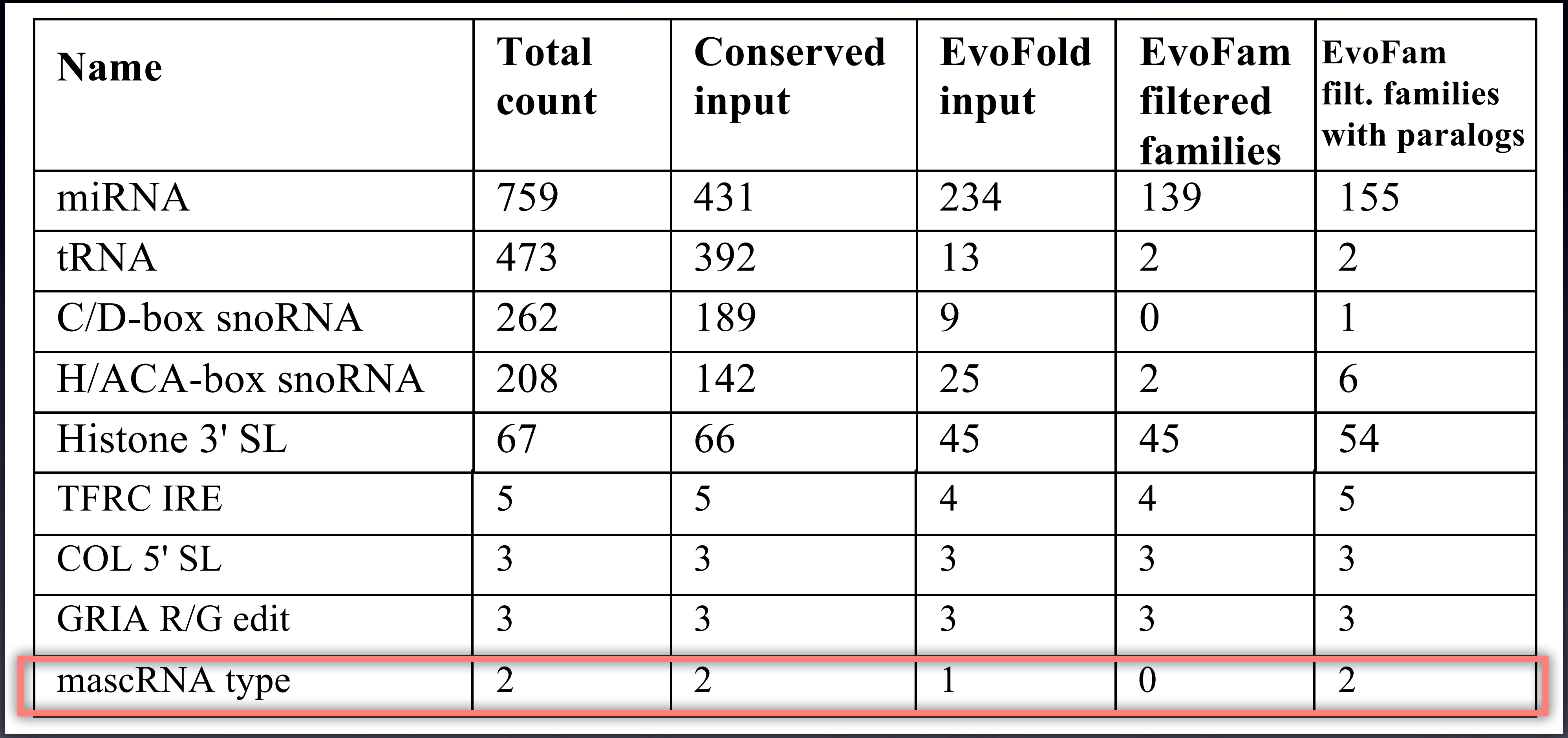

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GRIA R/G edit	3	3	3	3	3
mascRNA type	2	2	1	0	2

Performance

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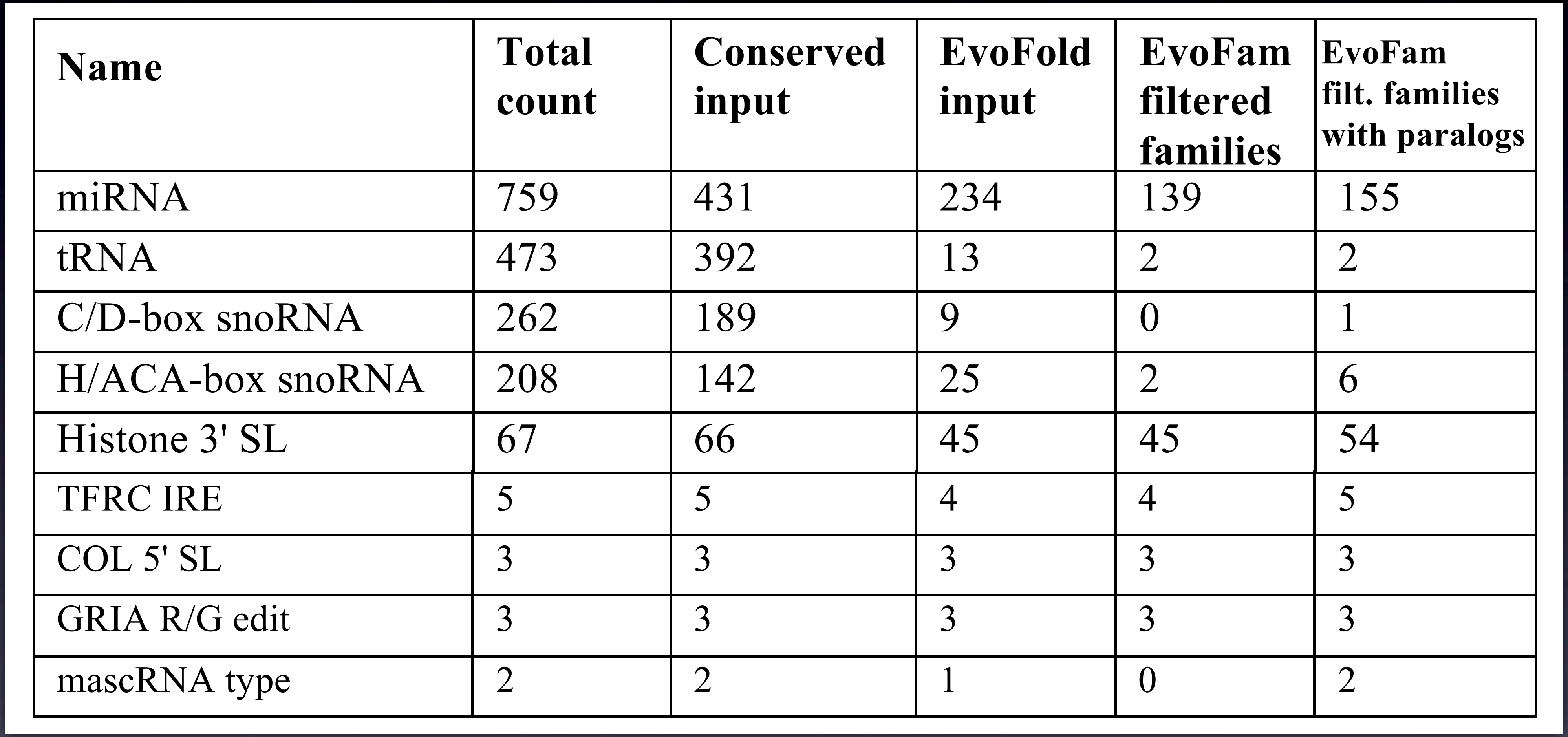

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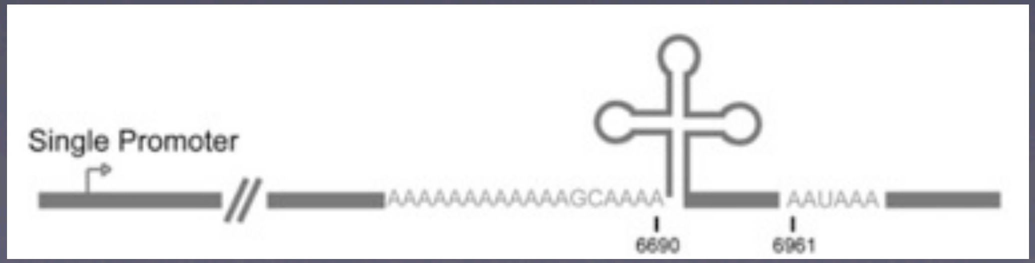
COL 5' SL	3	3	3	3	3
GRIA R/G edit	3	3	3	3	3
mascRNA type	2	2	1	0	2

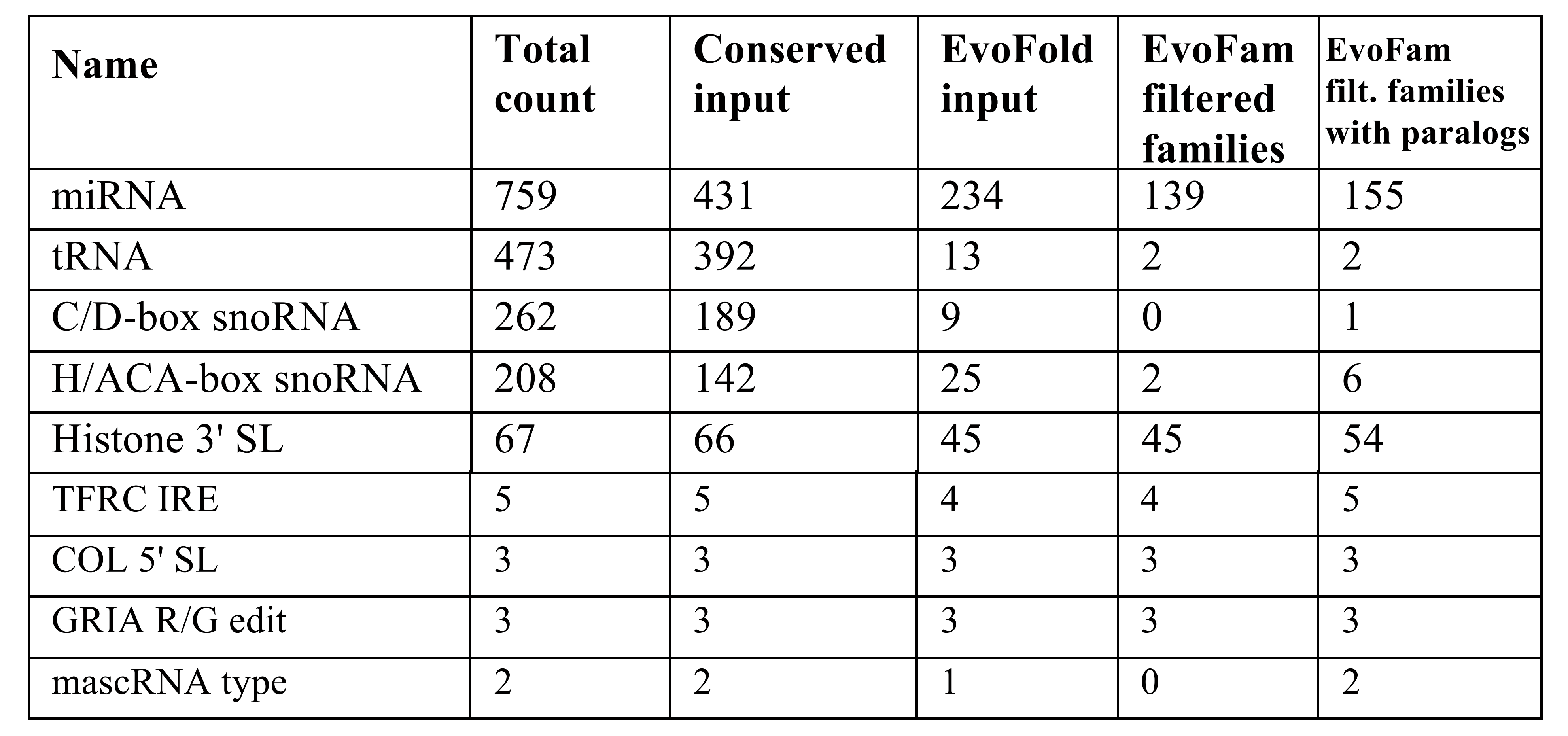
mascRNA family in MALAT 1 and Men β



Wilusz 2008; Sunwoo et al. 2009; Wilusz and Spector 2010

Performance

48 of 220 families contain known members (88% known)



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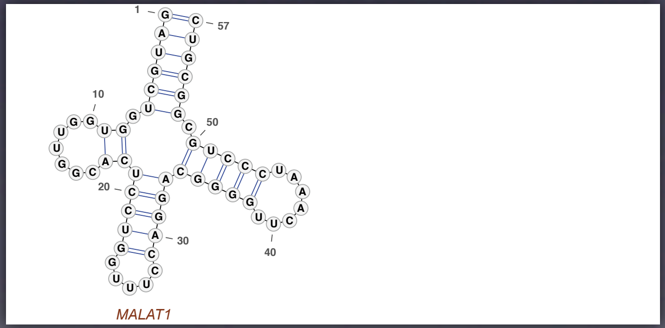
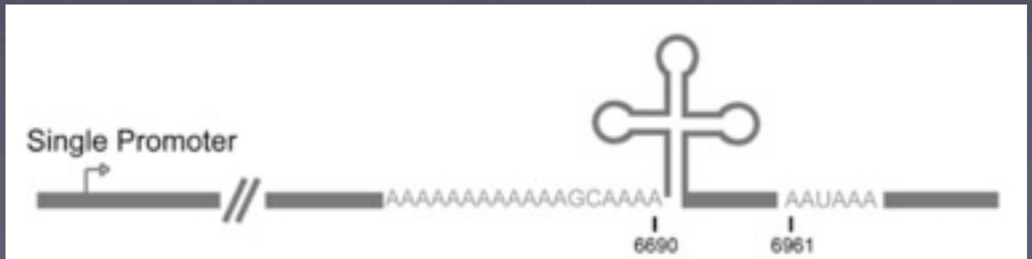
mascRNA type	2	2	1	0	2

mascRNA family in MALAT 1 and Men β





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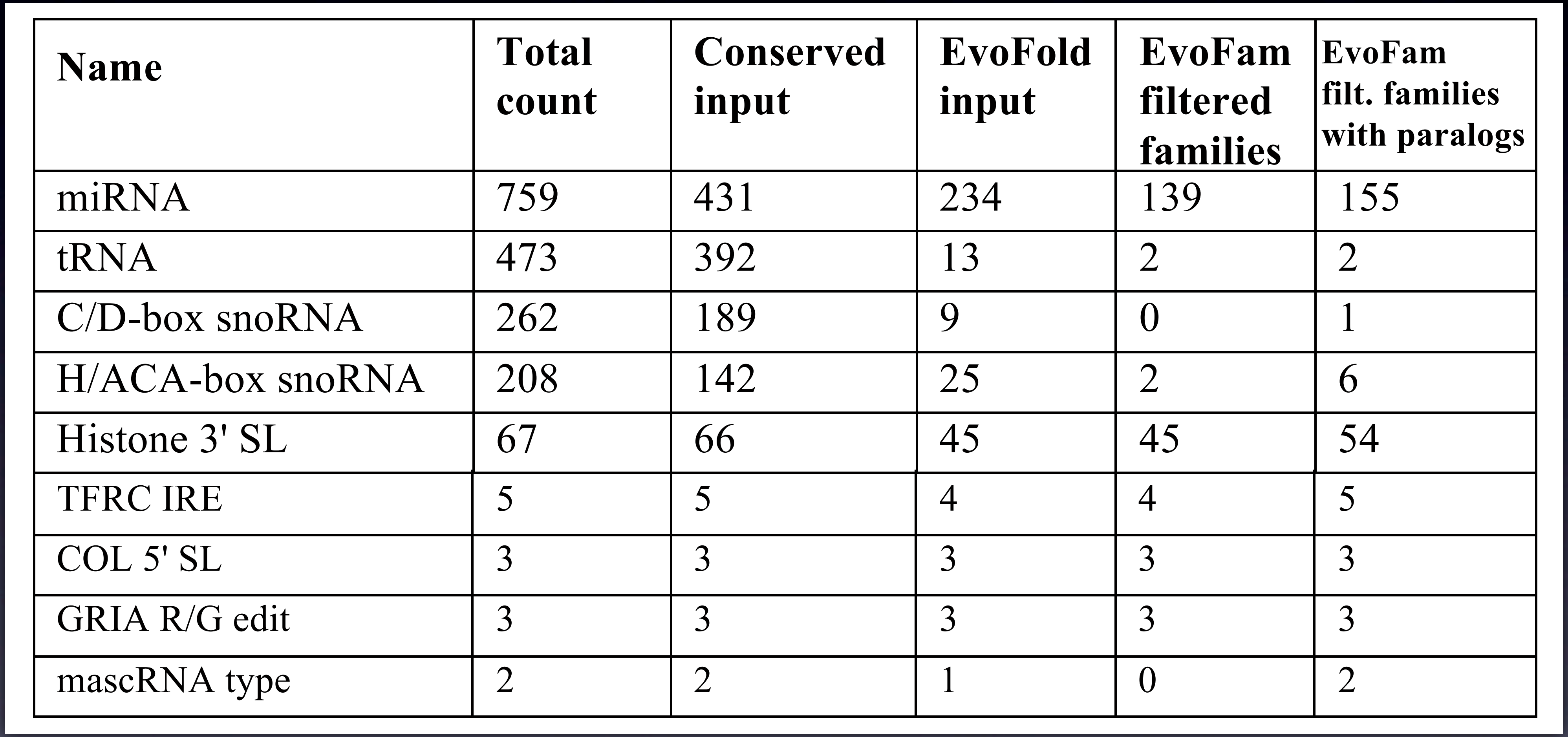

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GRIA R/G edit	3	3	3	3	3
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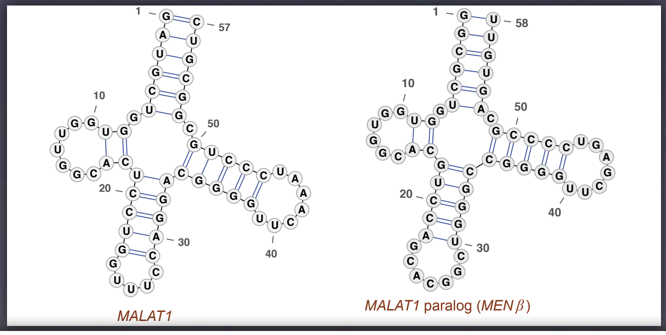
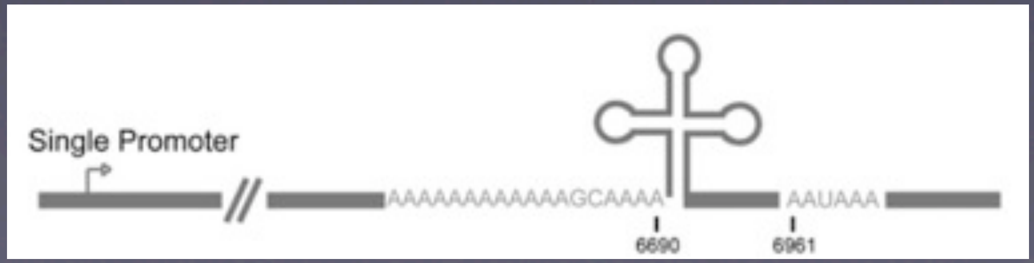
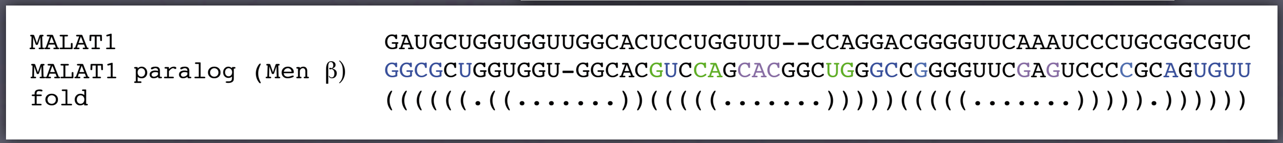
mascRNA family in MALAT 1 and Men β







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MALAT1                  GAUGCUGGUGGUUGGCACUCCUGGUUU--CCAGGACGGGGUUCAAAUCCCUGCGGCGUC
MALAT1 paralog (Men β)  GGCGCUGGUGGU-GGCACGUCCAGCACGGCUGGCCGGGGUUCGAGUCCCCGCAGUGUU
fold                    ((((((.((.......))(((((.......)))))(((((.......))))).))))))
```

Wilusz 2008; Sunwoo et al. 2009; Wilusz and Spector 2010

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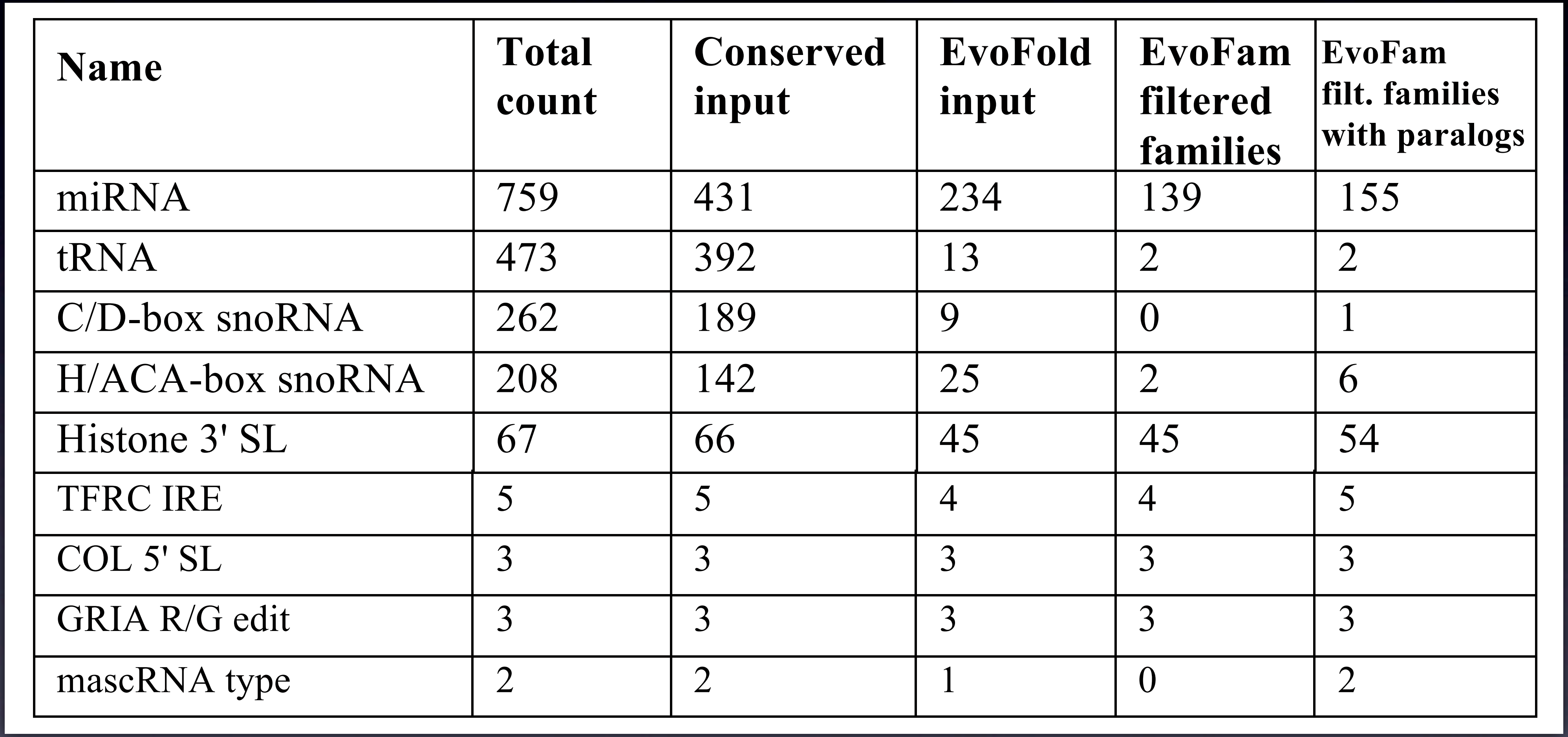

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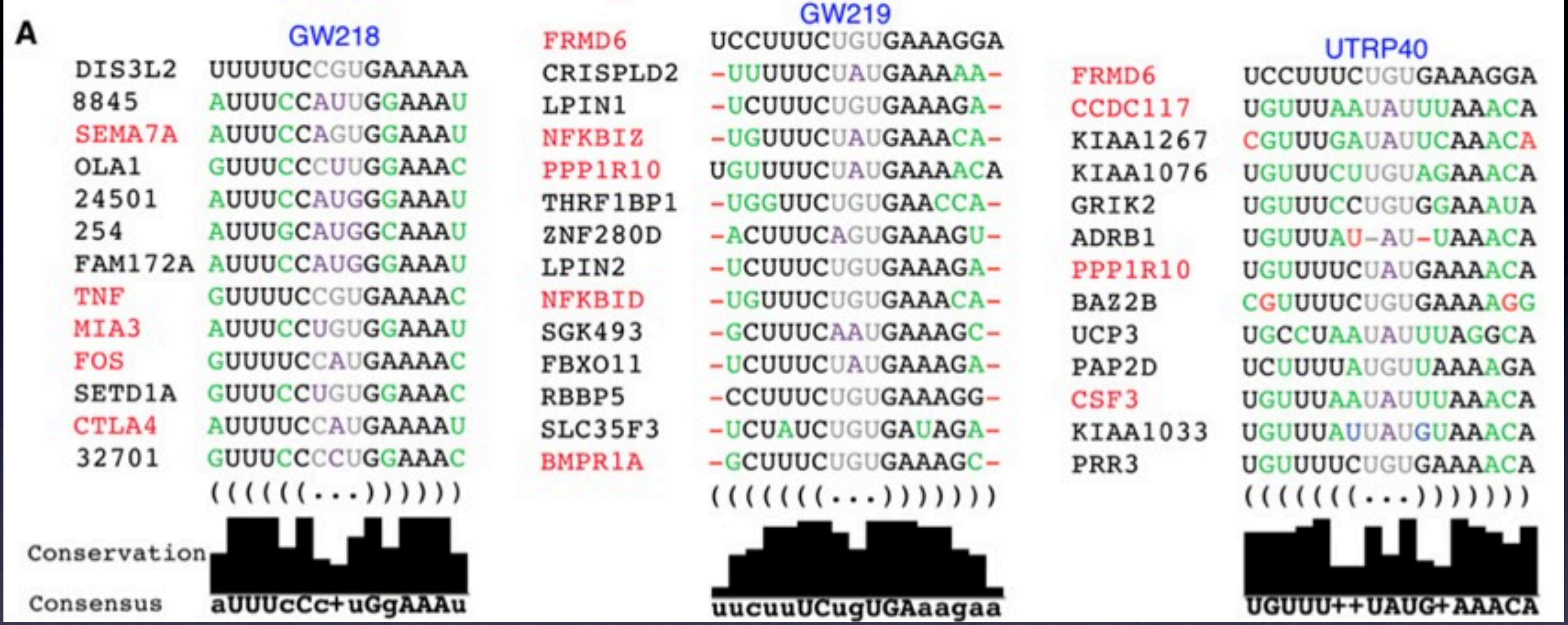
GRIA R/G edit	3	3	3	3	3
mascRNA type	2	2	1	0	2

Estimated false positive rate:

- 27 % for similarity edges
- 34 % for families of size three or larger

Immune related regulatory networks?

Families of short hairpins enriched in 3‘UTRs of immunity related genes



	GW218
DIS3L2	UUUUUCCGUGAAAAA
8845	AUUUCCAUUGGAAAU
SEMA7A	AUUUCCAGUGGAAAU
OLA1	GUUUCCCUUGGAAAC
24501	AUUUCCAUGGGAAAU
254	AUUUGCAUGGCAAAU
FAM172A	AUUUCCAUGGGAAAU
TNF	GUUUUCCGUGAAAAC
MIA3	AUUUCCUGUGGAAAU
FOS	GUUUUCCAUGAAAAC
SETD1A	GUUUCCUGUGGAAAC
CTLA4	AUUUUCCAUGAAAAU
32701	GUUUCCCCUGGAAAC
	((((((...))))))
Consensus	aUUUcCc+uGgAAAu

	GW219
FRMD6	UCCUUUCUGUGAAAGGA
CRISPLD2	-UUUUUCUAUGAAAAA-
LPIN1	-UCUUUCUGUGAAAGA-
NFKBIZ	-UGUUUCUAUGAAACA-
PPP1R10	UGUUUUCUAUGAAAACA
THRF1BP1	-UGGUUCUGUGAACCA-
ZNF280D	-ACUUUCAGUGAAAGU-
LPIN2	-UCUUUCUGUGAAAGA-
NFKBID	-UGUUUCUGUGAAACA-
SGK493	-GCUUUCAAUGAAAGC-
FBXO11	-UCUUUCUAUGAAAGA-
RBBP5	-CCUUUCUGUGAAAGG-
SLC35F3	-UCUAUCUGUGAUAGA-
BMPR1A	-GCUUUCUGUGAAAGC-
	(((((((...)))))))
	uucuuUCugUGAaagaa

	UTRP40
FRMD6	UCCUUUCUGUGAAAGGA
CCDC117	UGUUUAAUAUUUAAACA
KIAA1267	CGUUUGAUAUUCAAACA
KIAA1076	UGUUUCUUGUAGAAACA
GRIK2	UGUUUCCUGUGGAAAUA
ADRB1	UGUUUAU-AU-UAAACA
PPP1R10	UGUUUUCUAUGAAAACA
BAZ2B	CGUUUUCUGUGAAAAGG
UCP3	UGCCUAAUAUUUAGGCA
PAP2D	UCUUUUAUGUUAAAAGA
CSF3	UGUUUAAUAUUUAAACA
KIAA1033	UGUUUAUUAUGUAAACA
PRR3	UGUUUUCUGUGAAAACA
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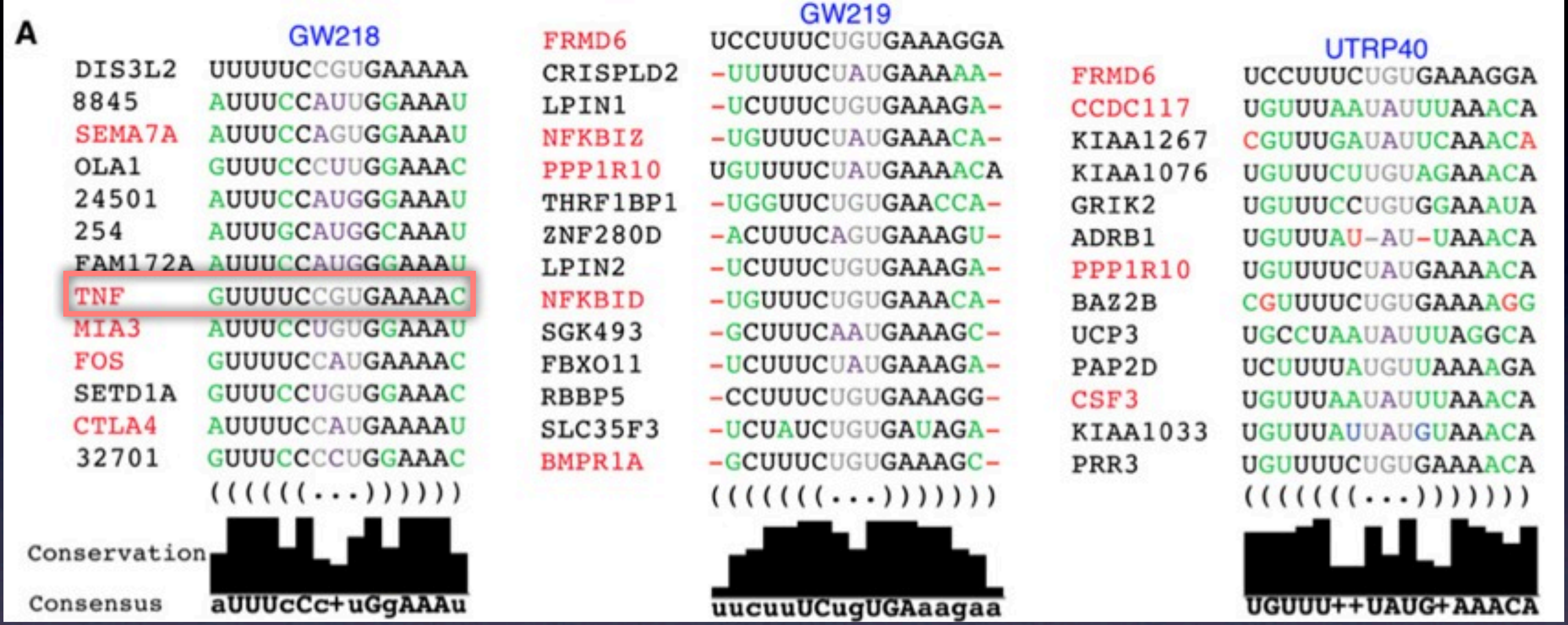
	UGUUU++UAUG+AAACA

Immune related regulatory networks?

Families of short hairpins enriched in 3‘UTRs of immunity related genes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Conservation

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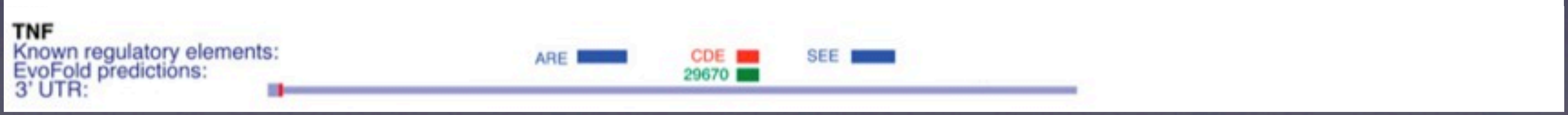
ADRB1	UGUUUAU-AU-UAAACA
PPP1R10	UGUUUUCUAUGAAAACA
BAZ2B	CGUUUUCUGUGAAAAGG
UCP3	UGCCUAAUAUUUAGGCA
PAP2D	UCUUUUAUGUUAAAAGA
CSF3	UGUUUAAUAUUUAAACA
KIAA1033	UGUUUAUUAUGUAAACA
PRR3	UGUUUUCUGUGAAAACA
	(((((((...)))))))
	UGUUU++UAUG+AAACA

Includes known destabilization hairpins

Constitutive Decay Element



Immune related regulatory networks?

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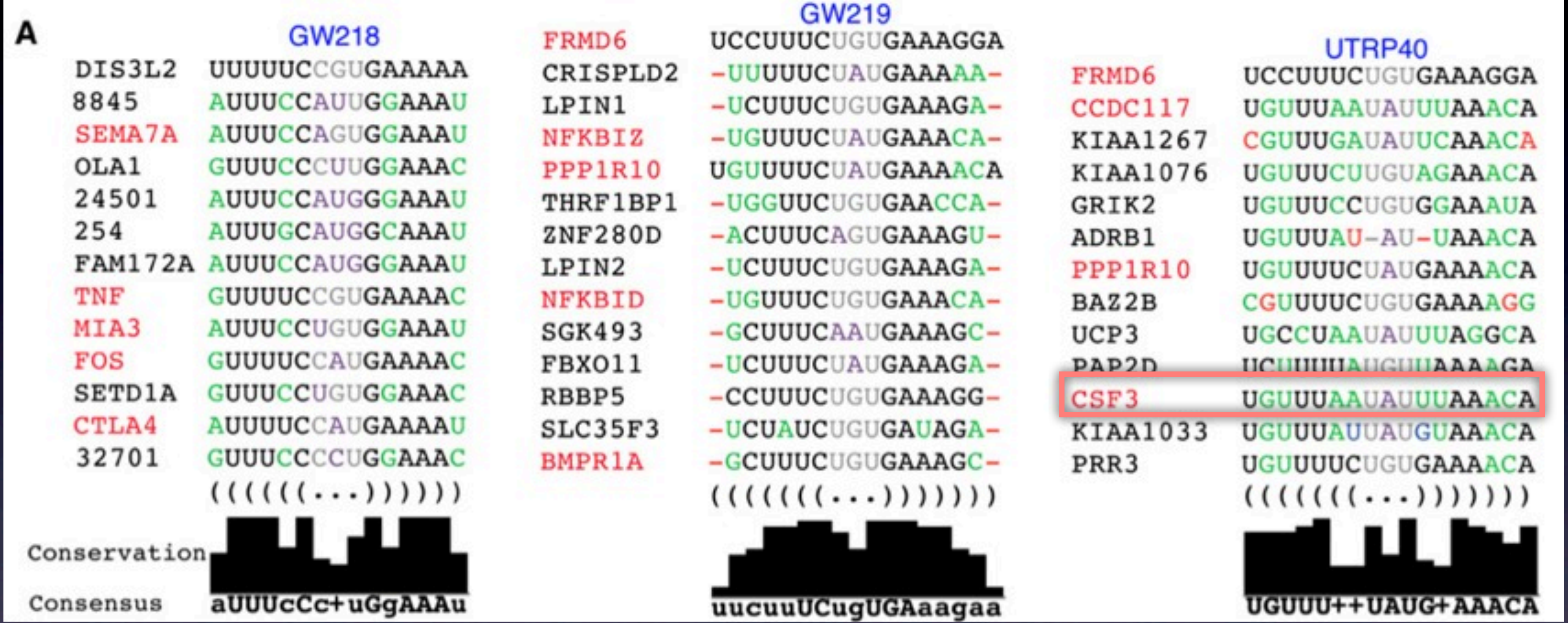

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FOS	GUUUUCCAUGAAAAC
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CTLA4	AUUUUCCAUGAAAAU
32701	GUUUCCCCUGGAAAC
	((((((...))))))
Conservation	
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LPIN1	-UCUUUCUGUGAAAGA-
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BMPR1A	-GCUUUCUGUGAAAGC-
	(((((((...)))))))
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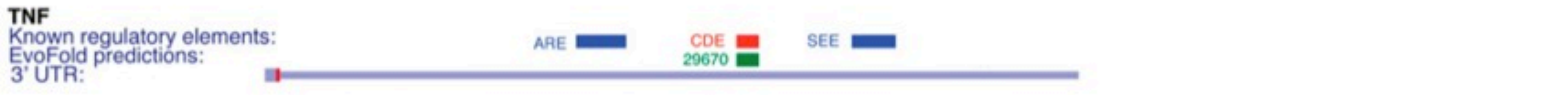
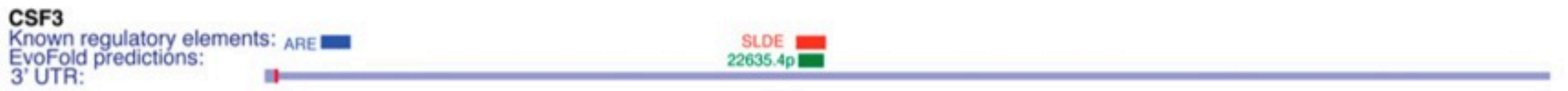
KIAA1076	UGUUUCUUGUAGAAACA
GRIK2	UGUUUCCUGUGGAAAUA
ADRB1	UGUUUAU-AU-UAAACA
PPP1R10	UGUUUUCUAUGAAAACA
BAZ2B	CGUUUUCUGUGAAAAGG
UCP3	UGCCUAAUAUUUAGGCA
PAP2D	UCUUUUAUGUUAAAAGA
CSF3	UGUUUAAUAUUUAAACA
KIAA1033	UGUUUAUUAUGUAAACA
PRR3	UGUUUUCUGUGAAAACA
	(((((((...)))))))
	UGUUU++UAUG+AAACA

Includes known destabilization hairpins

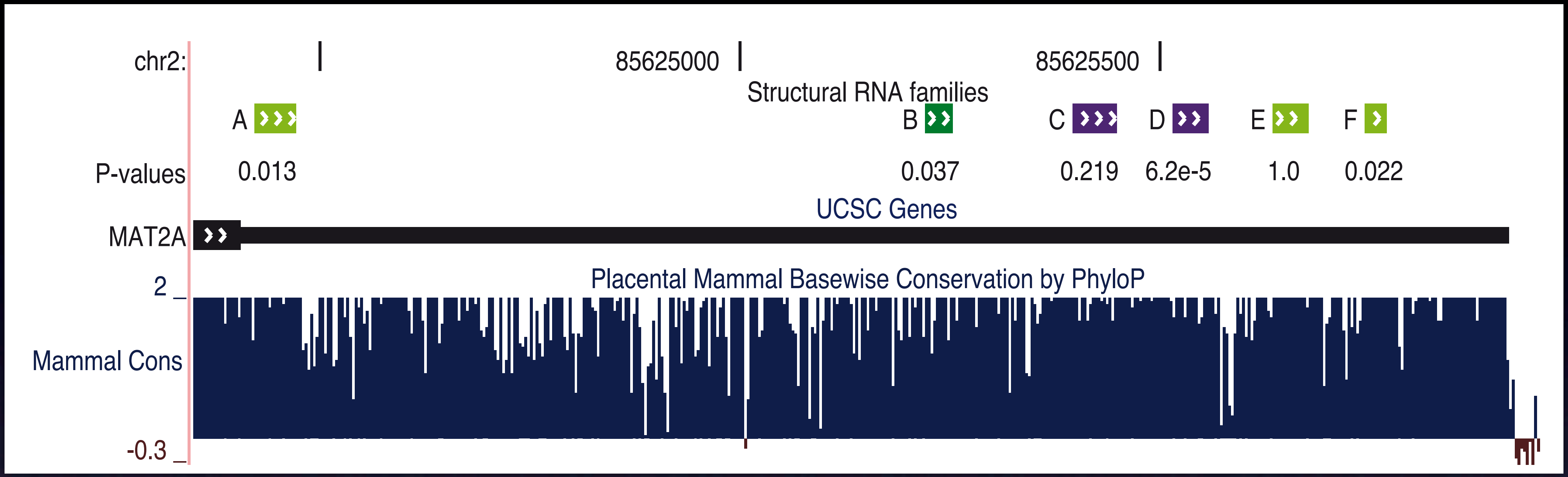
Constitutive Decay Element



Stem–Loop Destabilizing Element



Family of six hairpins all within 3‘UTR MAT2A



Brian J. Parker, et al. New families of human regulatory RNA structures identified by comparative analysis of vertebrate genomes. Genome Research (2011).

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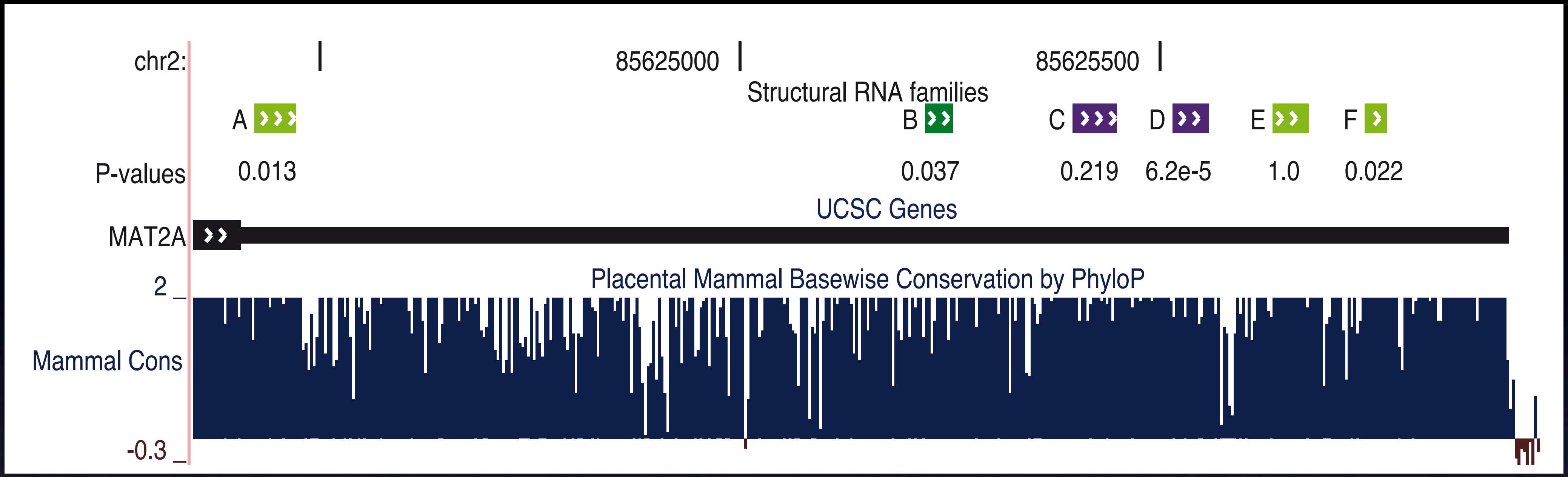
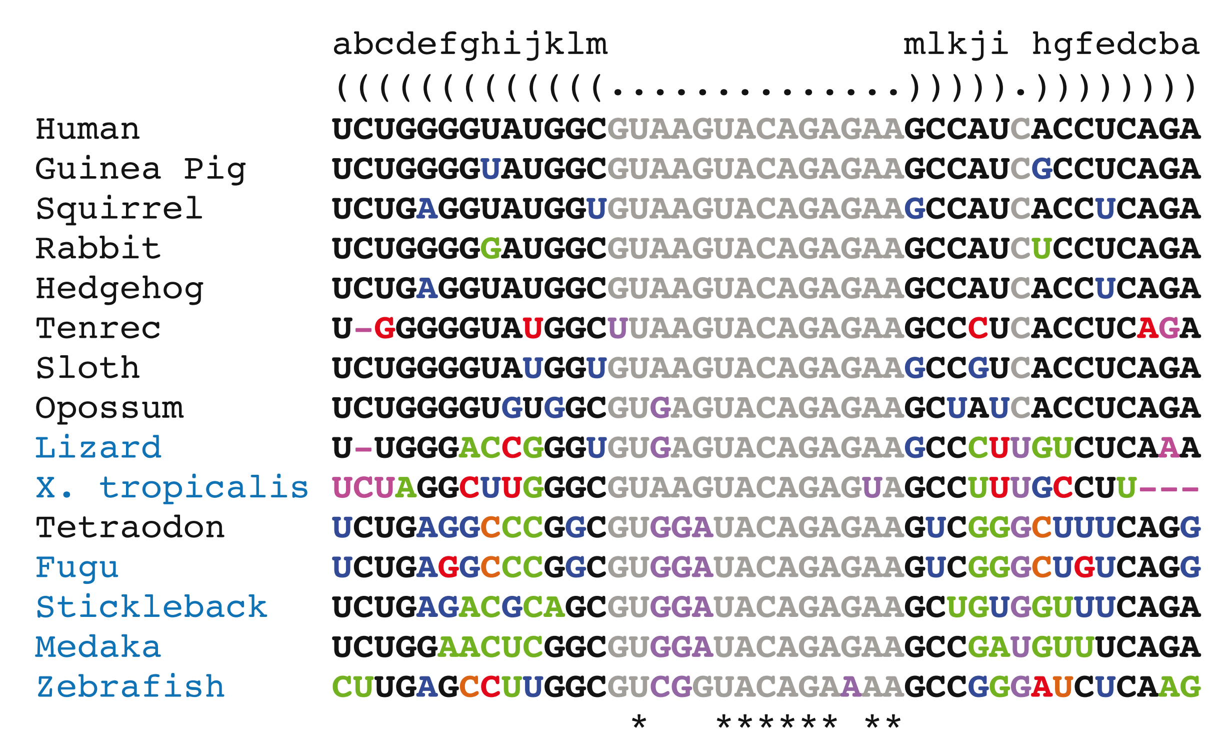


Vertebrate alignment for hairpin D



```
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Guinea Pig    UCUGGGGUAUGGCGUAAGUACAGAGAAGCCAUCGCCUCAGA
Squirrel      UCUGAGGUAUGGUGUAAGUACAGAGAAGCCAUCACCUCAGA
Rabbit        UCUGGGGGAUGGCGUAAGUACAGAGAAGCCAUCUCCUCAGA
Hedgehog      UCUGAGGUAUGGCGUAAGUACAGAGAAGCCAUCACCUCAGA
Tenrec        U-GGGGGUAUGGCUUAAGUACAGAGAAGCCCUCACCUCAGA
Sloth         UCUGGGGUAUGGUGUAAGUACAGAGAAGCCGUCACCUCAGA
Opossum       UCUGGGGUGUGGCGUGAGUACAGAGAAGCUAUCACCUCAGA
Lizard        U-UGGGACCGGGUGUGAGUACAGAGAAGCCCUUGUCUCAAA
X. tropicalis UCUAGGCUUGGGCGUAAGUACAGAGUAGCCUUUGCCUU---
Tetraodon     UCUGAGGCCCGGCGUGGAUACAGAGAAGUCGGGCUUUCAGG
Fugu          UCUGAGGCCCGGCGUGGAUACAGAGAAGUCGGGCUGUCAGG
Stickleback   UCUGAGACGCAGCGUGGAUACAGAGAAGCUGUGGUUUCAGA
Medaka        UCUGGAACUCGGCGUGGAUACAGAGAAGCCGAUGUUUCAGA
Zebrafish     CUUGAGCCUUGGCGUCGGUACAGAAAAGCCGGGAUCUCAAG
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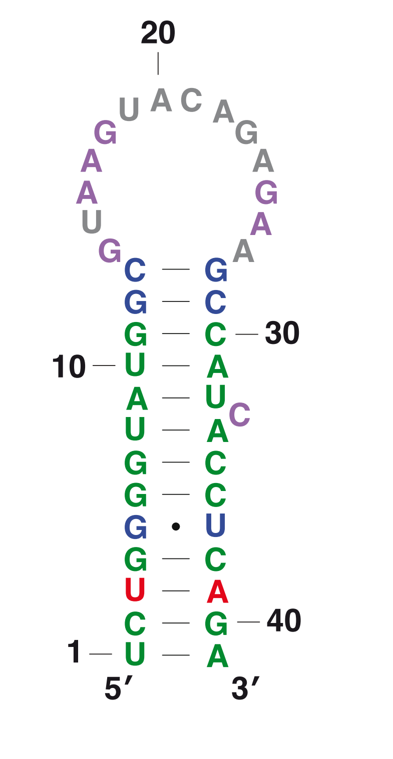
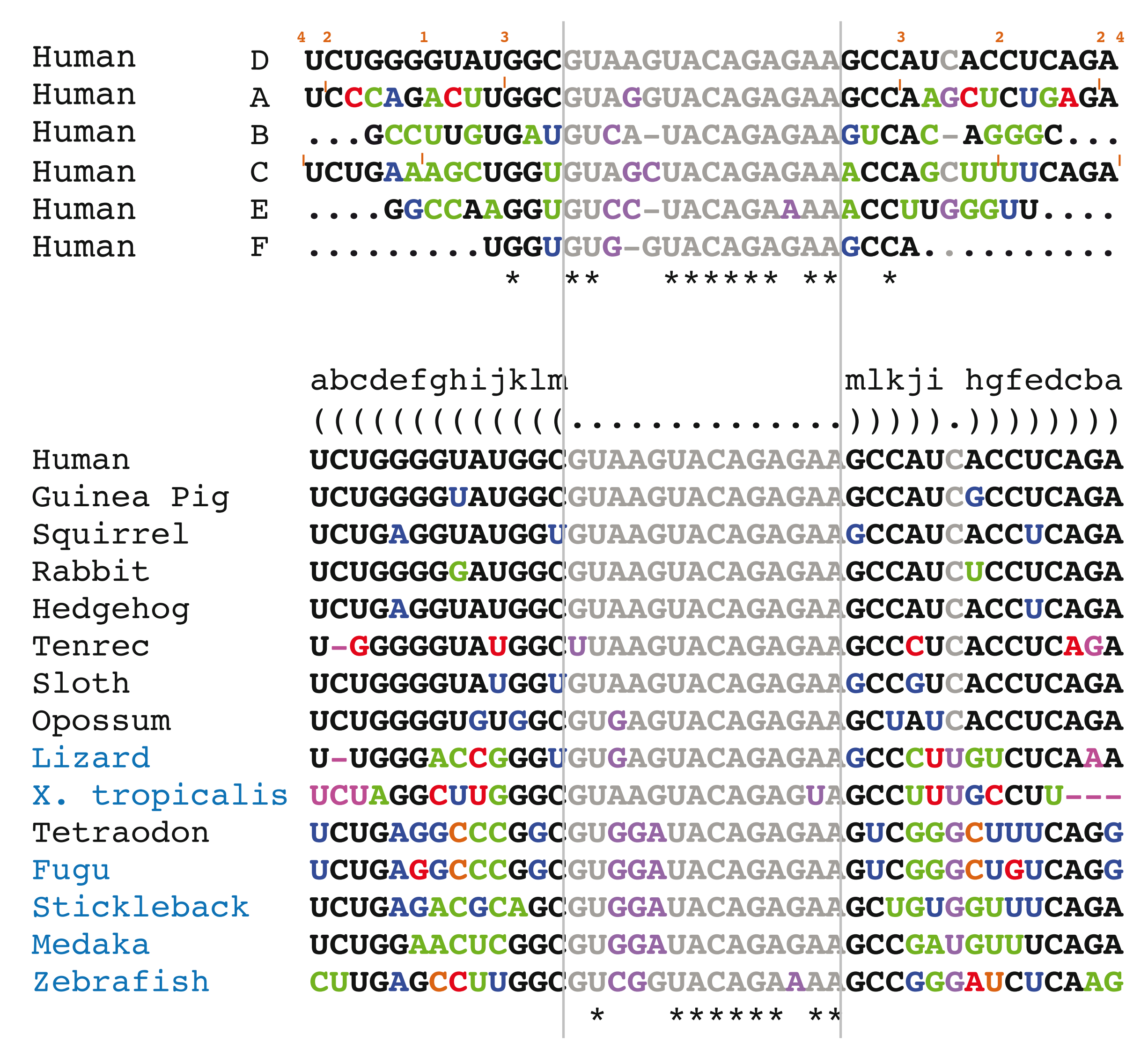
Hairpin D



Brian J. Parker, et al. New families of human regulatory RNA structures identified by comparative analysis of vertebrate genomes. Genome Research (2011).

Shared loop motif

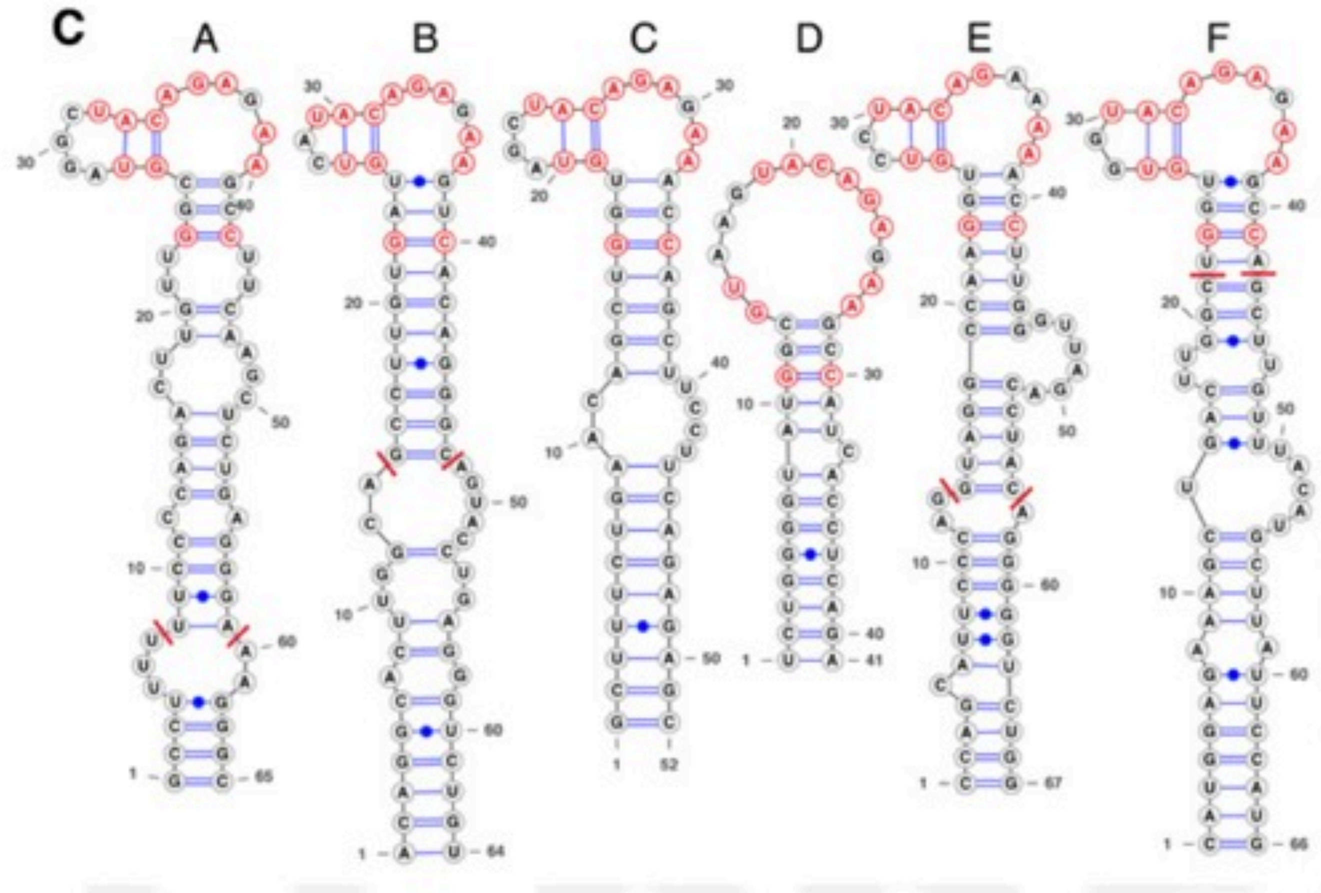
Loop motif shared between human members and down through vertebrates



```
                   42    1   3                   3    2    24
Human          D   UCUGGGGUAUGGCGUAAGUACAGAGAAGCCAUCACCUCAGA
Human          A   UCCCAGACUUGGCGUAGGUACAGAGAAGCCAAGCUCUGAGA
Human          B   ...GCCUUGUGAUGUCA-UACAGAGAAGUCAC-AGGGC...
Human          C   UCUGAAAGCUGGUGUAGCUACAGAGAAACCAGCUUUUCAGA
Human          E   ....GGCCAAGGUGUCC-UACAGAAAAACCUUGGGUU....
Human          F   .........UGGUGUG-GUACAGAGAAGCCA..........
                             *  **   ****** **  *


                   abcdefghijklm              mlkji hgfedcba
                   (((((((((((((..............))))).))))))))
Human              UCUGGGGUAUGGCGUAAGUACAGAGAAGCCAUCACCUCAGA
Guinea Pig         UCUGGGGUAUGGCGUAAGUACAGAGAAGCCAUCGCCUCAGA
Squirrel           UCUGAGGUAUGGUGUAAGUACAGAGAAGCCAUCACCUCAGA
Rabbit             UCUGGGGGAUGGCGUAAGUACAGAGAAGCCAUCUCCUCAGA
Hedgehog           UCUGAGGUAUGGCGUAAGUACAGAGAAGCCAUCACCUCAGA
Tenrec             U-GGGGUAUGGCUUAAGUACAGAGAAGCCCUCACCUCAGA
Sloth              UCUGGGGUAUGGUGUAAGUACAGAGAAGCCGUCACCUCAGA
Opossum            UCUGGGGUGUGGCGUGAGUACAGAGAAGCUAUCACCUCAGA
Lizard             U-UGGGACCGGGUGUGAGUACAGAGAAGCCCUUGUCUCAAA
X. tropicalis      UCUAGGCUUGGGCGUAAGUACAGAGUAGCCUUUGCCUU---
Tetraodon          UCUGAGGCCCGGCGUGGAUACAGAGAAGUCGGGCUUUCAGG
Fugu               UCUGAGGCCCGGCGUGGAUACAGAGAAGUCGGGCUGUCAGG
Stickleback        UCUGAGACGCAGCGUGGAUACAGAGAAGCUGUGGUUUCAGA
Medaka             UCUGGAACUCGGCGUGGAUACAGAGAAGCCGAUGUUUCAGA
Zebrafish          CUUGAGCCUUGGCGUCGGUACAGAAAAGCCGGGAUCUCAAG
                                 *   ****** **
```

Human structures with conserved motif



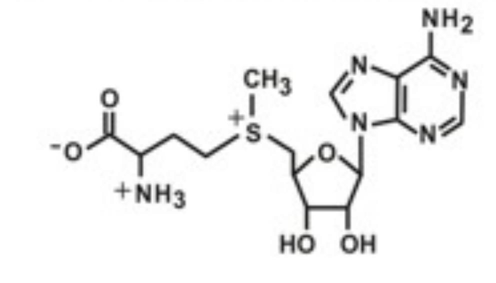
Brian J. Parker, et al. New families of human regulatory RNA structures identified by comparative analysis of vertebrate genomes. Genome Research (2011).

Post-transcriptional regulation of MAT2A

MAT2A: methionine adenosyltransferase II, alpha
MAT catalyzes the synthesis of SAM (adoMet)

Half-life of MAT2A transcript depends on SAM concetration
(Martínez-Chantar et al. J Biol Chem (2003))

SAM



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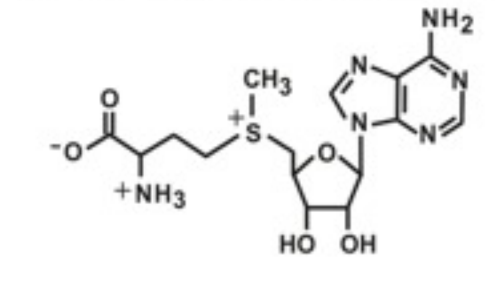
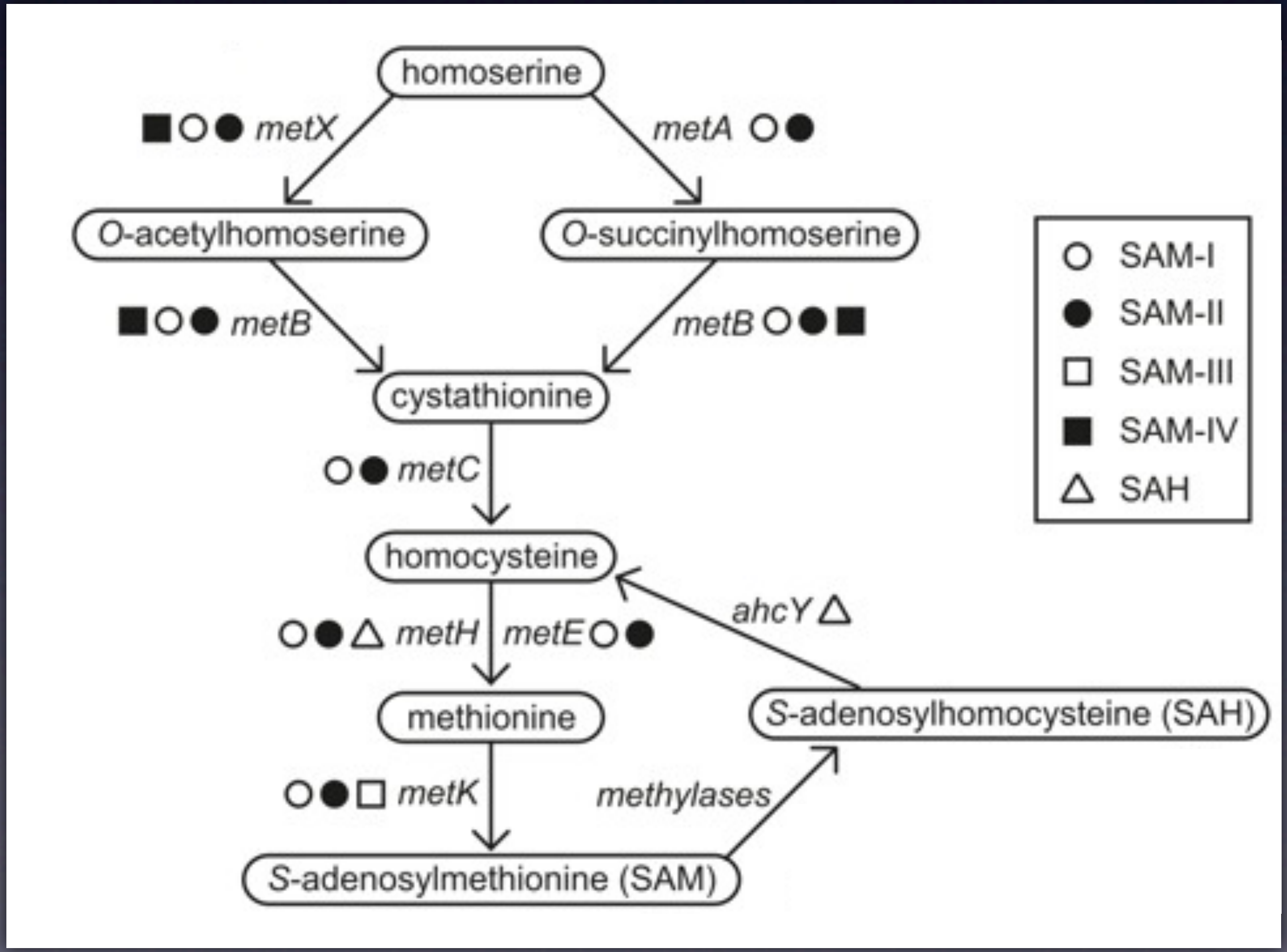
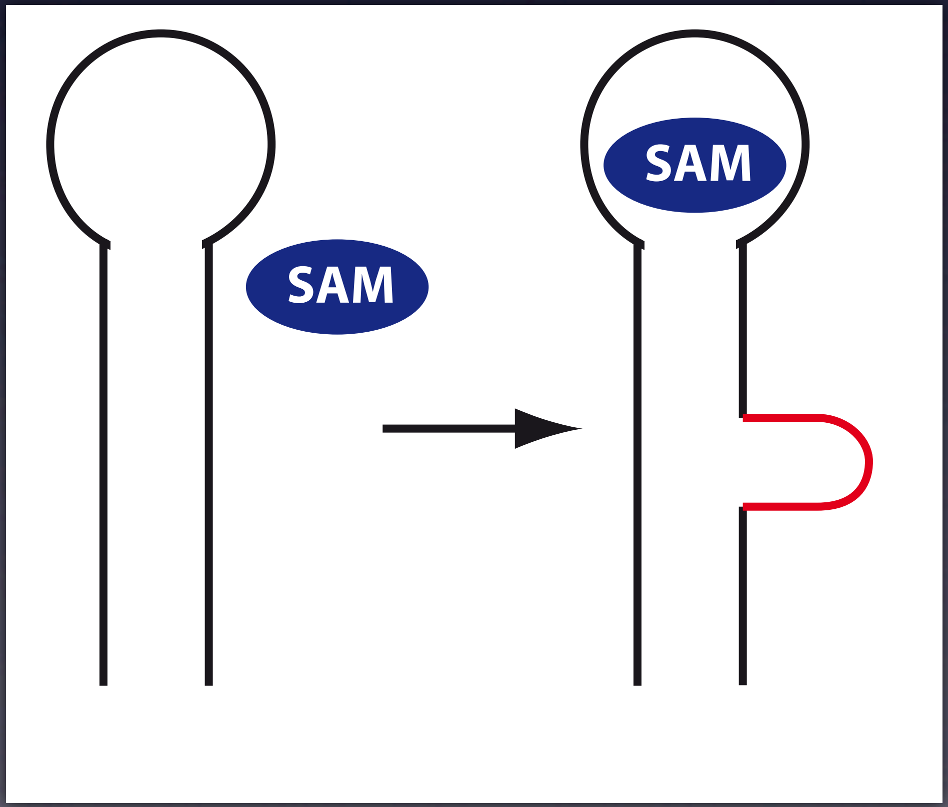


SAM riboswitches in bacteria



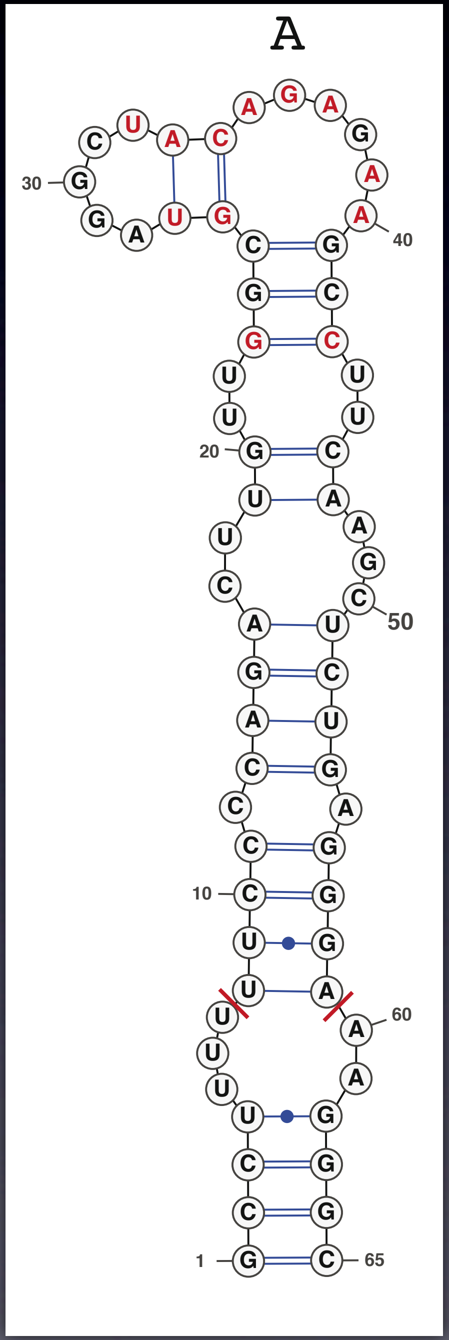
Wang and Breaker. Biochem Cell Biol (2008)

Riboswitches

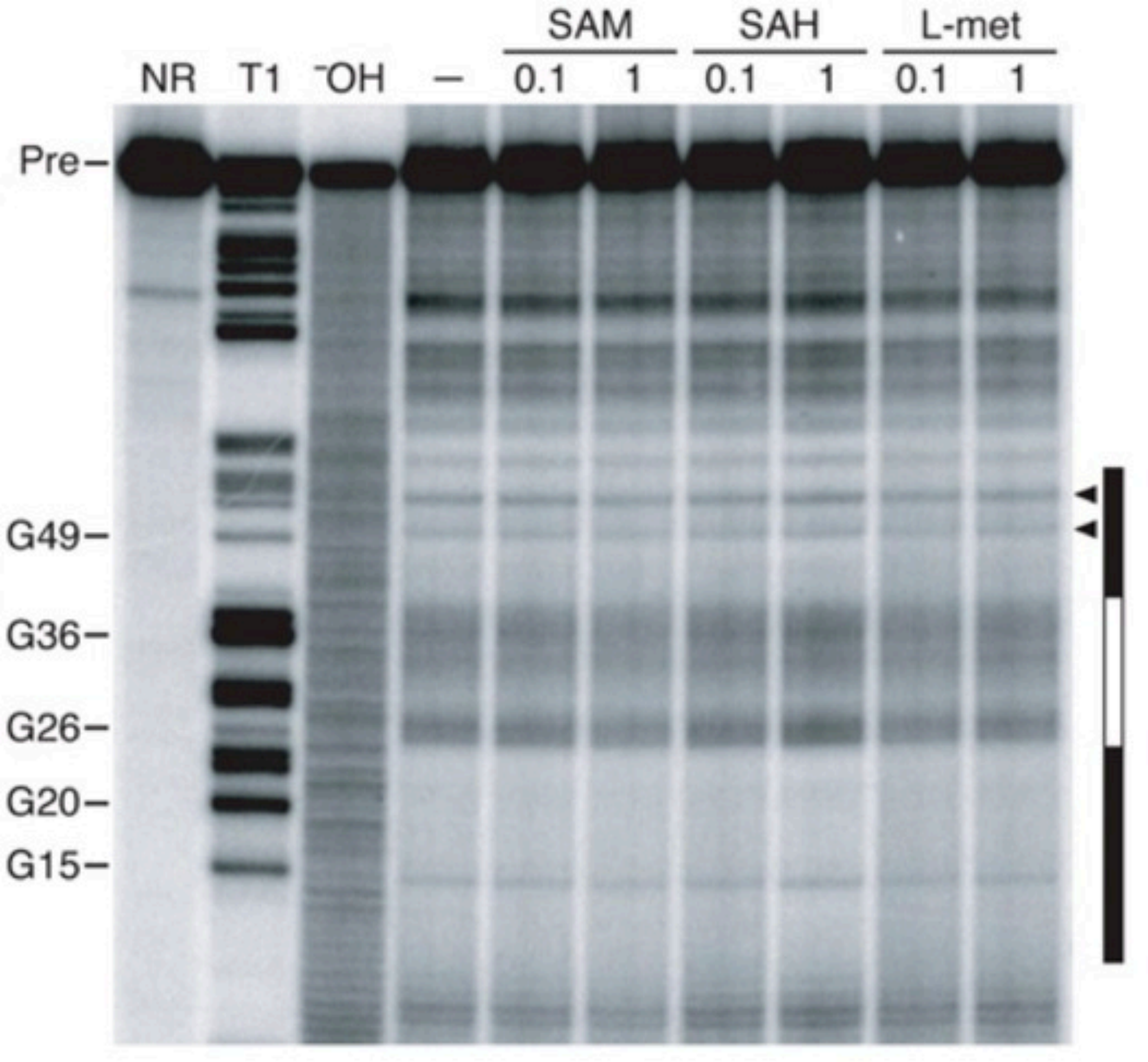


Human riboswitches?

Hairpin A



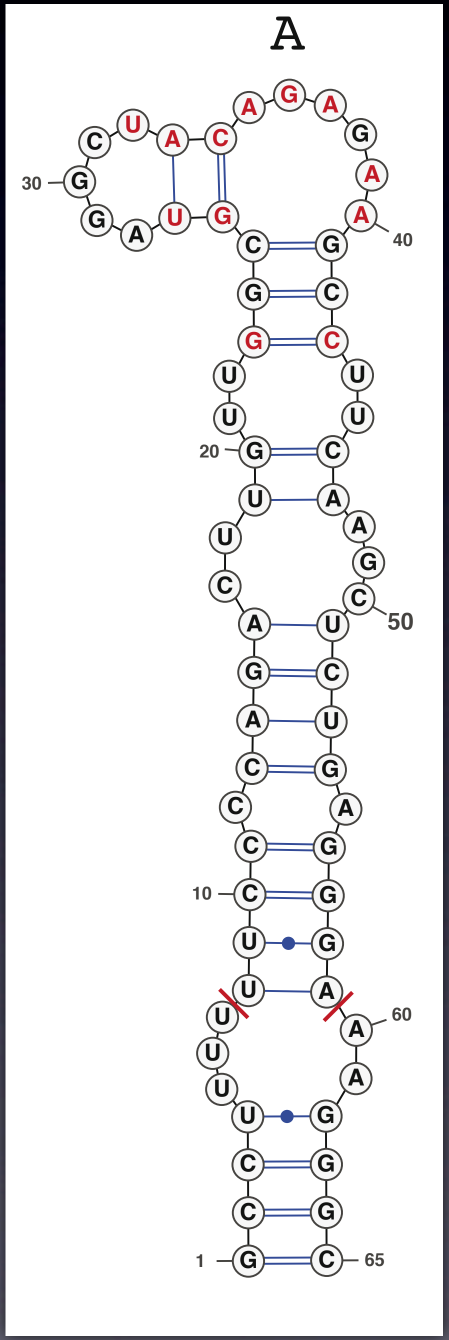
No structure change shown by in-line probing



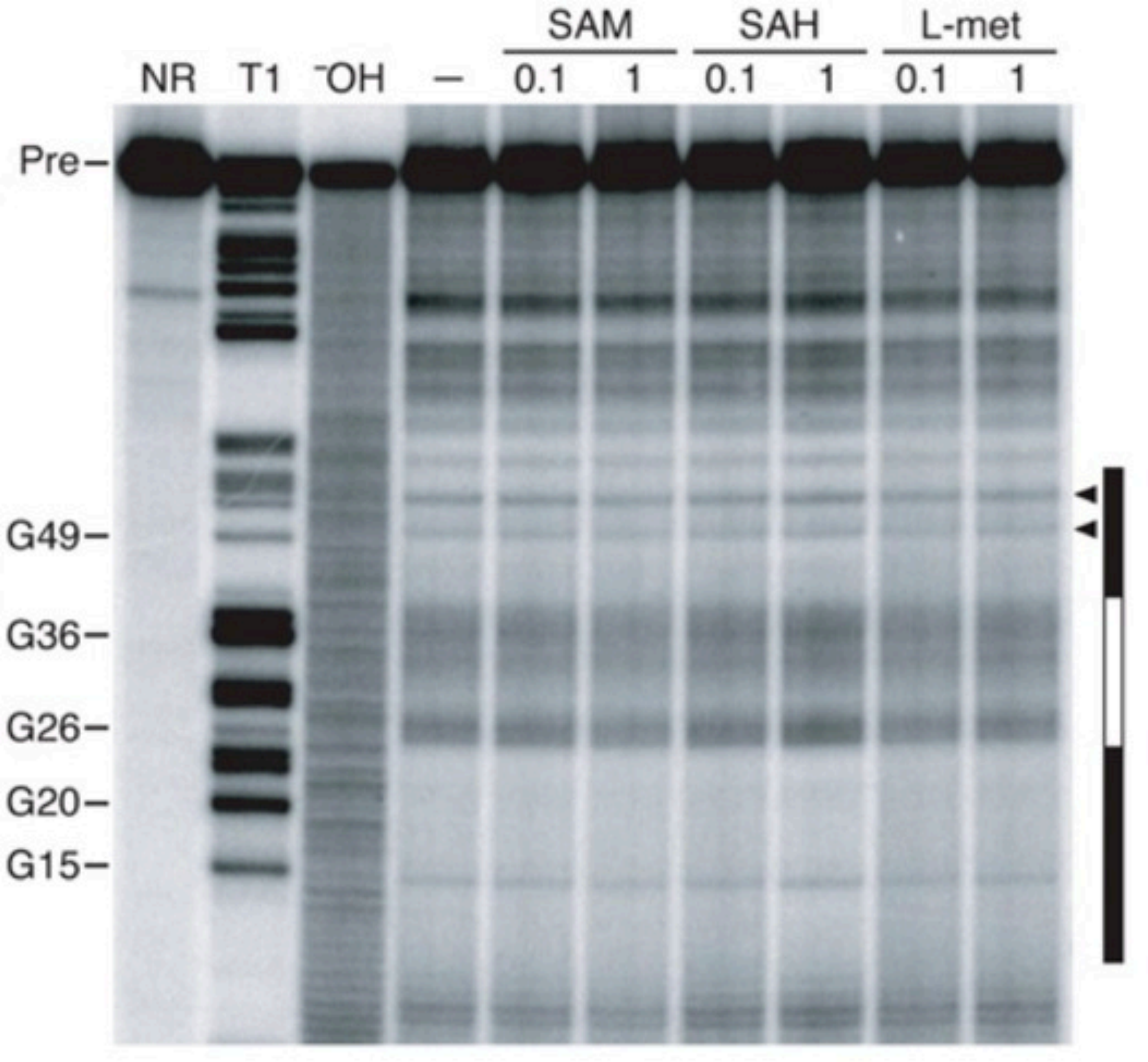
Experiments done by Adam Roth & Ronald Breaker (Yale).

Human riboswitches? Apparently not...

Hairpin A



No structure change shown by in-line probing

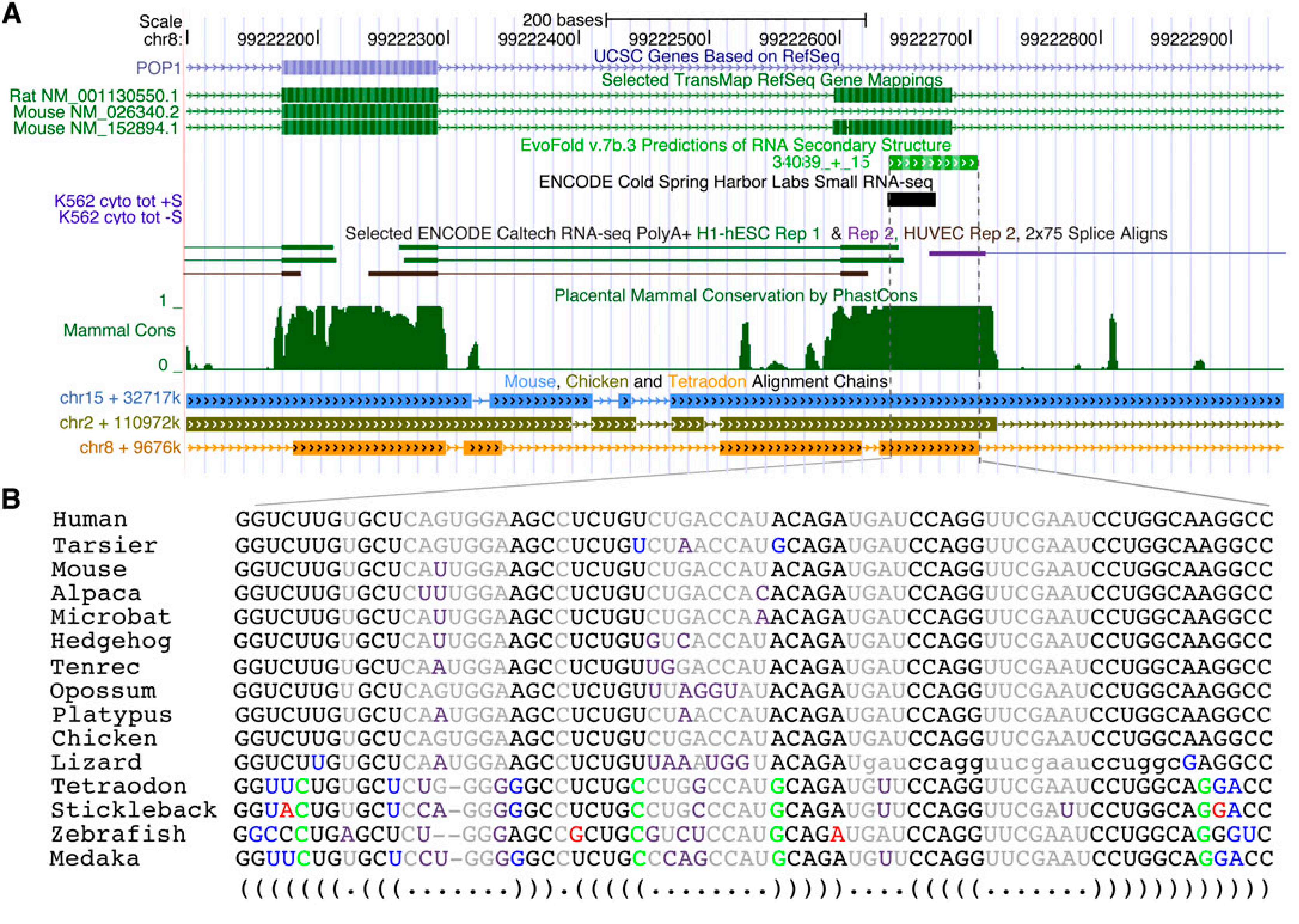


Experiments done by Adam Roth & Ronald Breaker (Yale).

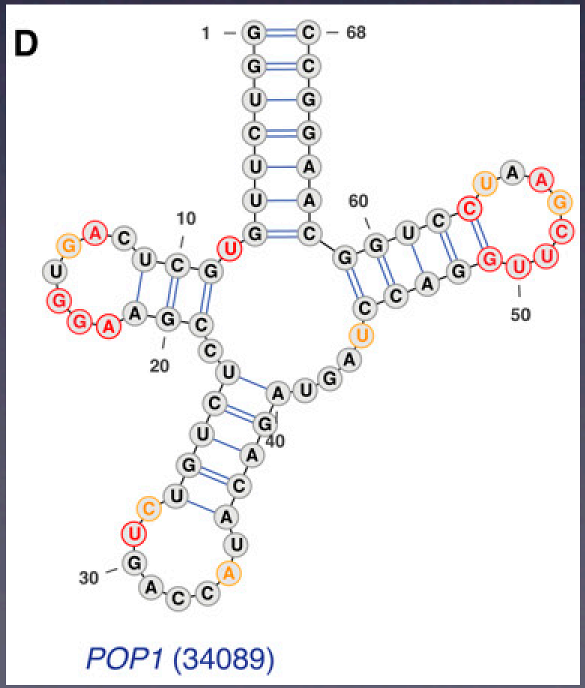
Example of auto-regulation?

POP1 is a ribonuclease, which is part of RNaseP that processes tRNAs.

tRNA-like structure in POP1 intron


A
Scale
chr8:
200 bases
99222200
99222300
99222400
99222500
99222600
99222700
99222800
99222900
POP1
UCSC Genes Based on RefSeq
Selected TransMap RefSeq Gene Mappings
Rat NM_001130550.1
Mouse NM_026340.2
Mouse NM_152894.1
EvoFold v.7b.3 Predictions of RNA Secondary Structure
34089_+_15
ENCODE Cold Spring Harbor Labs Small RNA-seq
K562 cyto tot +S
K562 cyto tot -S
Selected ENCODE Caltech RNA-seq PolyA+ H1-hESC Rep 1 & Rep 2, HUVEC Rep 2, 2x75 Splice Aligns
Placental Mammal Conservation by PhastCons
1
Mammal Cons
0
Mouse, Chicken and Tetraodon Alignment Chains
chr15 + 32717k
chr2 + 110972k
chr8 + 9676k
B

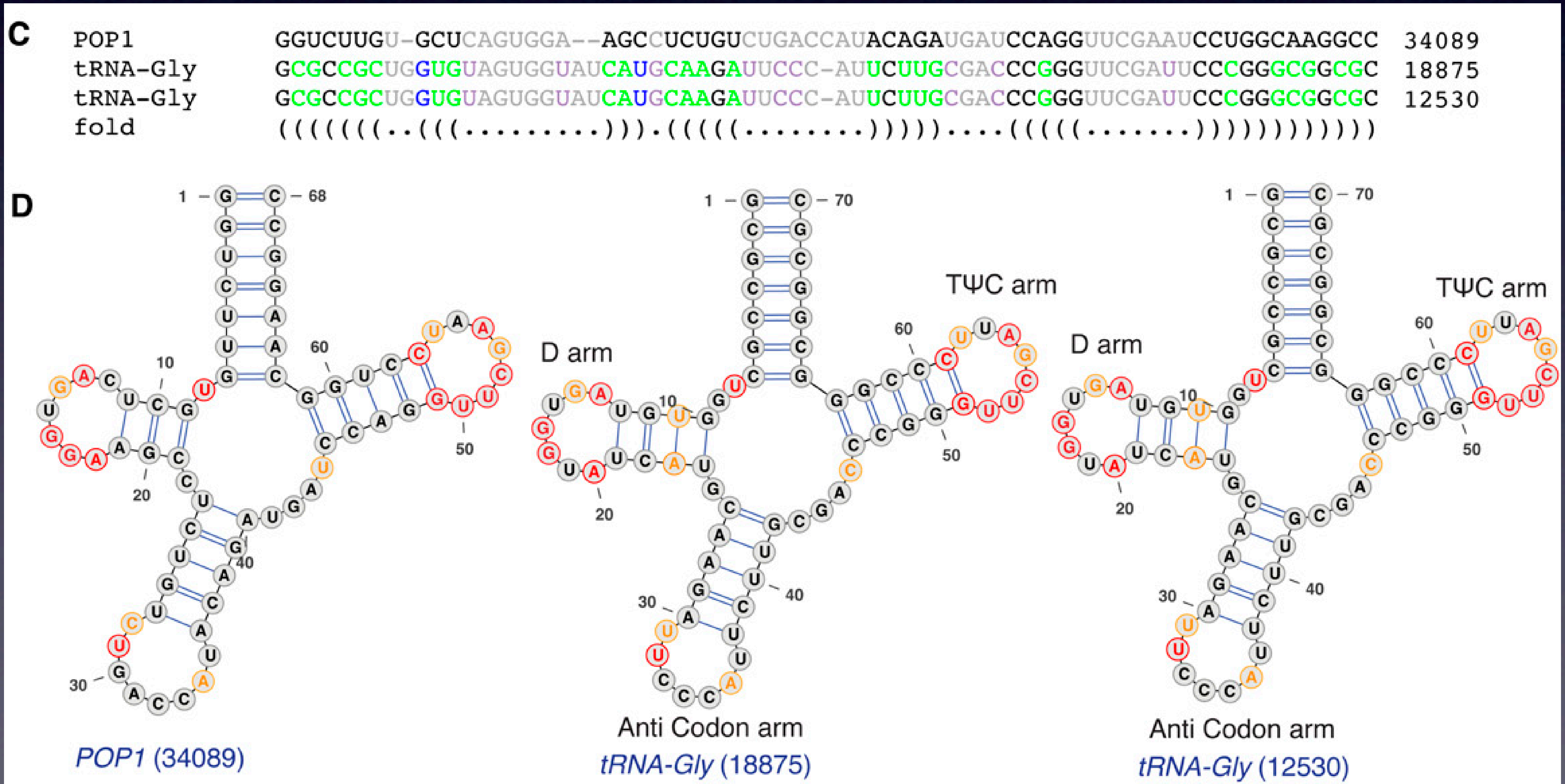
```
Human       GGUCUUGUGCUCAGUGGAAGCCUCUGUCUGACCAUACAGAUGAUCCAGGUUCGAAUCCUGGCAAGGCC
Tarsier     GGUCUUGUGCUCAGUGGAAGCCUCUGUCUAACCAUGCAGAUGAUCCAGGUUCGAAUCCUGGCAAGGCC
Mouse       GGUCUUGUGCUCAUUGGAAGCCUCUGUCUGACCAUACAGAUGAUCCAGGUUCGAAUCCUGGCAAGGCC
Alpaca      GGUCUUGUGCUCUUUGGAAGCCUCUGUCUGACCACACAGAUGAUCCAGGUUCGAAUCCUGGCAAGGCC
Microbat    GGUCUUGUGCUCAUUGGAAGCCUCUGUCUGACCAAACAGAUGAUCCAGGUUCGAAUCCUGGCAAGGCC
Hedgehog    GGUCUUGUGCUCAUUGGAAGCCUCUGUGUCACCAUACAGAUGAUCCAGGUUCGAAUCCUGGCAAGGCC
Tenrec      GGUCUUGUGCUCAAUGGAAGCCUCUGUUGGACCAUACAGAUGAUCCAGGUUCGAAUCCUGGCAAGGCC
Opossum     GGUCUUGU GCUCAGUGGAAGCCUCUGUUUAGGUAUACAGAUGAUCCAGGUUCGAAUCCUGGCAAGGCC
Platypus    GGUCUUGUGCUCAAUGGAAGCCUCUGUCUAACCAUACAGAUGAUCCAGGUUCGAAUCCUGGCAAGGCC
Chicken     GGUCUUGUGCUCAGUGGAAGCCUCUGUCUGACCAUACAGAUGAUCCAGGUUCGAAUCCUGGCAAGGCC
Lizard      GGUCUUGUGCUCAAUGGAAGCCUCUGUUAAAUGGUACAGAUgauccagguucgaauccuggcGAGGCC
Tetraodon   GGUUCUGUGCUCUG-GGGGCCUCUGCCUGGCCAUGCAGAUGUUCCAGGUUCGAAUCCUGGCAGGACC
Stickleback GGUACUGUGCUCCA-GGGGCCUCUGCCUGCCCAUGCAGAUGUUCCAGGUUCGAUUCCUGGCAGGACC
Zebrafish   GGCCCUGAGCUCU--GGGAGCCGCUGCGUCUCCAUGCAGAUGAUCCAGGUUCGAAUCCUGGCAGGGUC
Medaka      GGUUCUGUGCUCCU-GGGGGCCUCUGCCCAGCCAUGCAGAUGUUCCAGGUUCGAAUCCUGGCAGGACC
            (((((((.(((.......))).(((((........)))))....(((((.......))))))))))))
```



Parker et al. New families of human regulatory RNA structures identified by comparative analysis of vertebrate genomes. *in revision*.

Struture resembles tRNAs

POP1 structure groups together with tRNAs

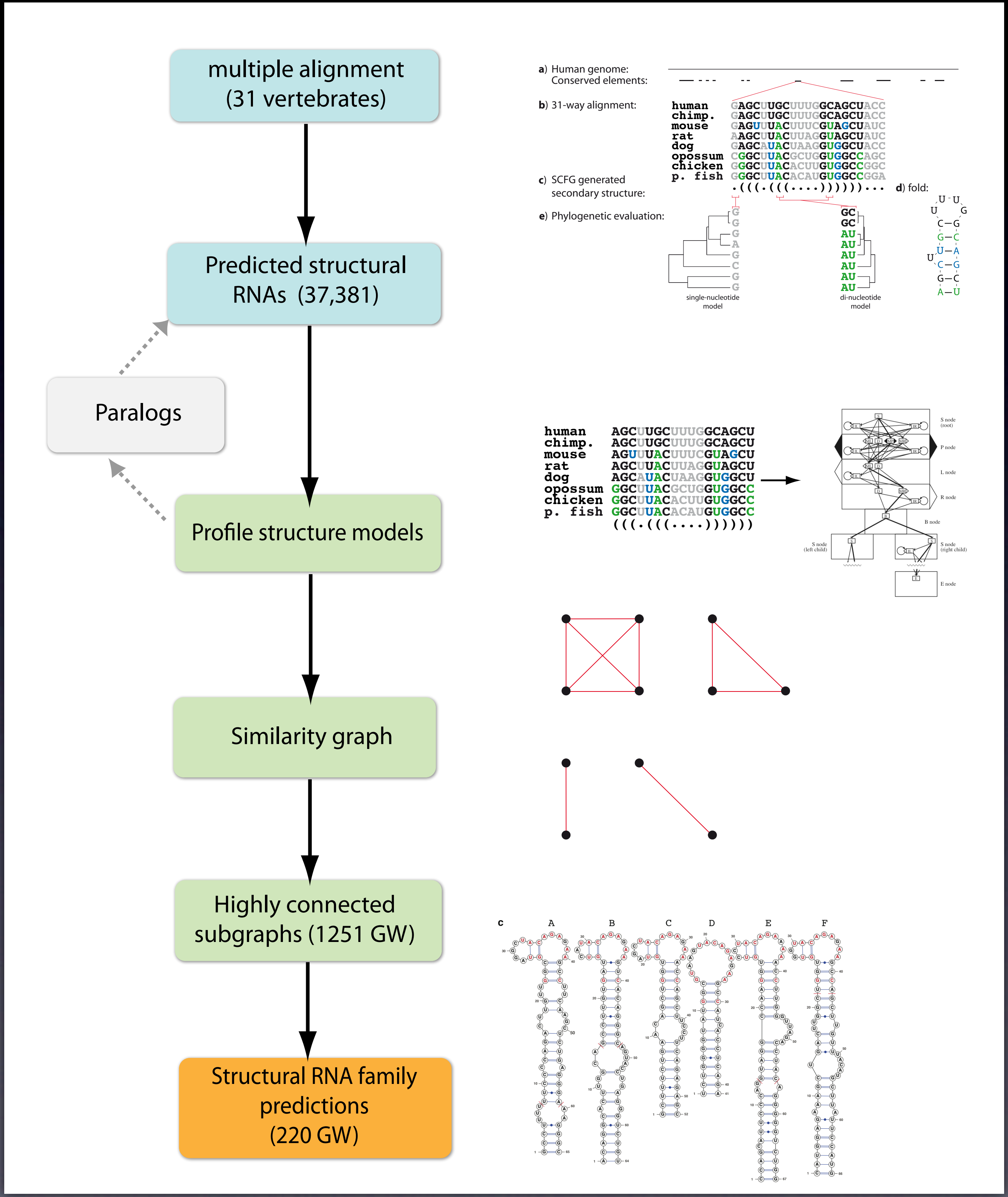


C

```
POP1       GGUCUUGU-GCUCAGUGGA--AGCCUCUGUCUGACCAUACAGAUGAUCCAGGUUCGAAUCCUGGCAAGGCC  34089
tRNA-Gly   GCGCCGCUGGUGUAGUGGUAUCAUGCAAGAUUCCC-AUUCUUGCGACCCGGGUUCGAUUCCCGGGCGGCGC  18875
tRNA-Gly   GCGCCGCUGGUGUAGUGGUAUCAUGCAAGAUUCCC-AUUCUUGCGACCCGGGUUCGAUUCCCGGGCGGCGC  12530
fold       (((((((..(((.........))).(((((........)))))....(((((.......))))))))))))
```

Parker et al. New families of human regulatory RNA structures identified by comparative analysis of vertebrate genomes. *in revision*.

EvoFam pipeline overview



multiple alignment (31 vertebrates)

Predicted structural RNAs (37,381)

Paralogs

Profile structure models

Similarity graph

Highly connected subgraphs (1251 GW)

Structural RNA family predictions (220 GW)

Future directions

- Make extensive use of deep genomic alignments (10K vertebrates project, etc)
- Exploit structure genome-wide structure probing data
- Integrate with expression data in cancer genomics settings
- Integrate with experimental evidence of binding sites of RNA binding proteins (HITS-CLIP, etc)

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Manolis Kellis (Broad)
Jakob Skou Pedersen (Aarhus University)

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Brian J. Parker et al. New families of human regulatory RNA structures identified by comparative analysis of vertebrate genomes. Genome Research (2011). http://moma.ki.au.dk/prj/mammals/
Kerstin Lindblad-Toh, Manuel Garber, Or Zuk, Michael F. Lin, Brian J. Parker, et al. A high-resolution map of human evolutionary constraint using 29 mammals. Nature (2011).