Harnessing conserved secondary structure to computationally identify RNA homologs

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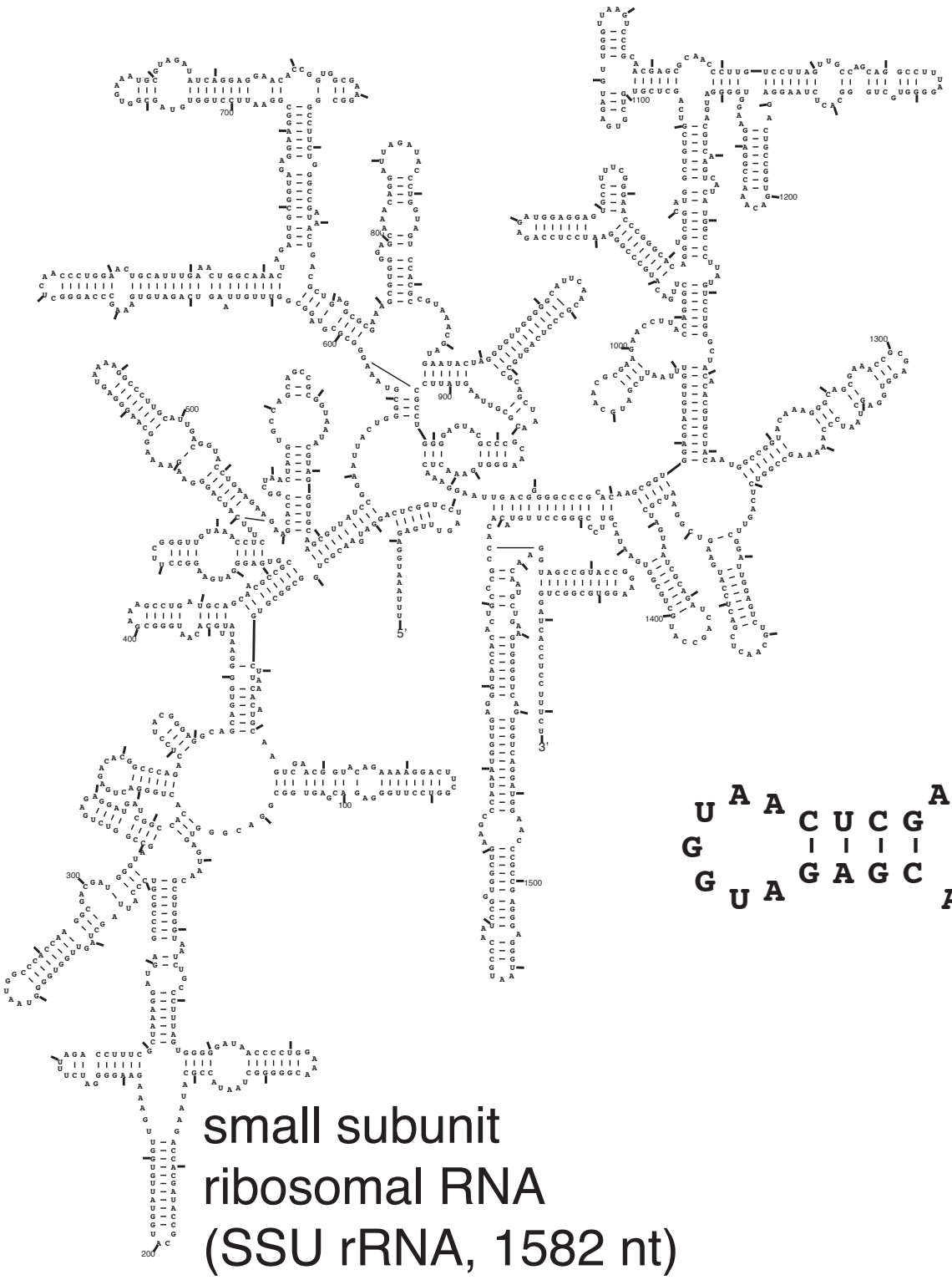
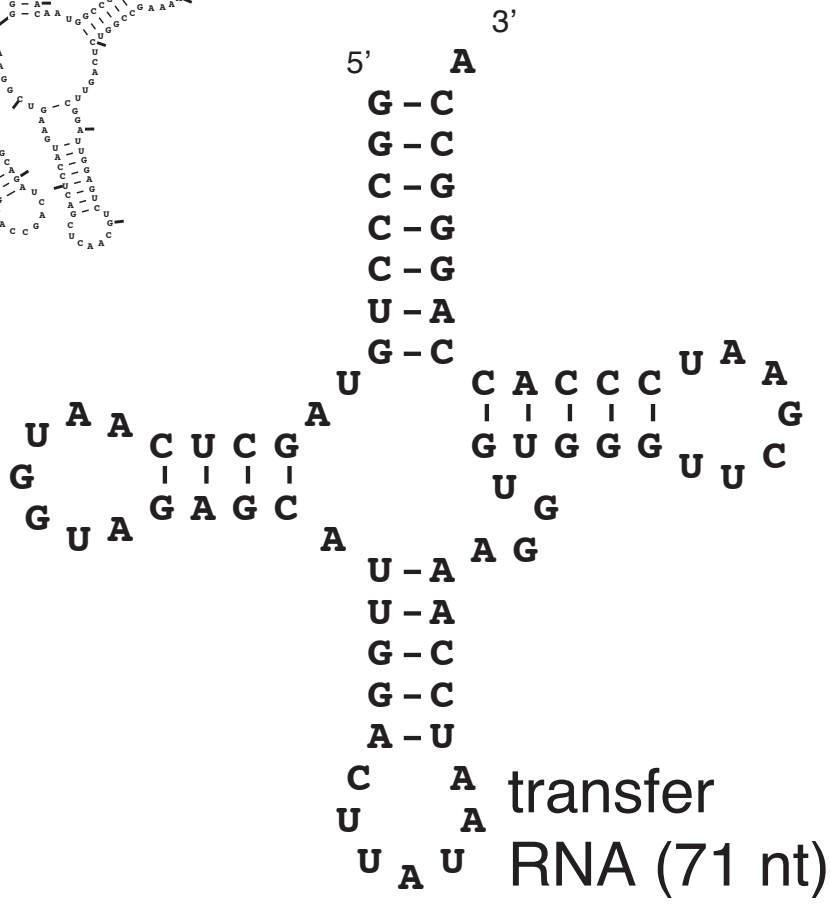
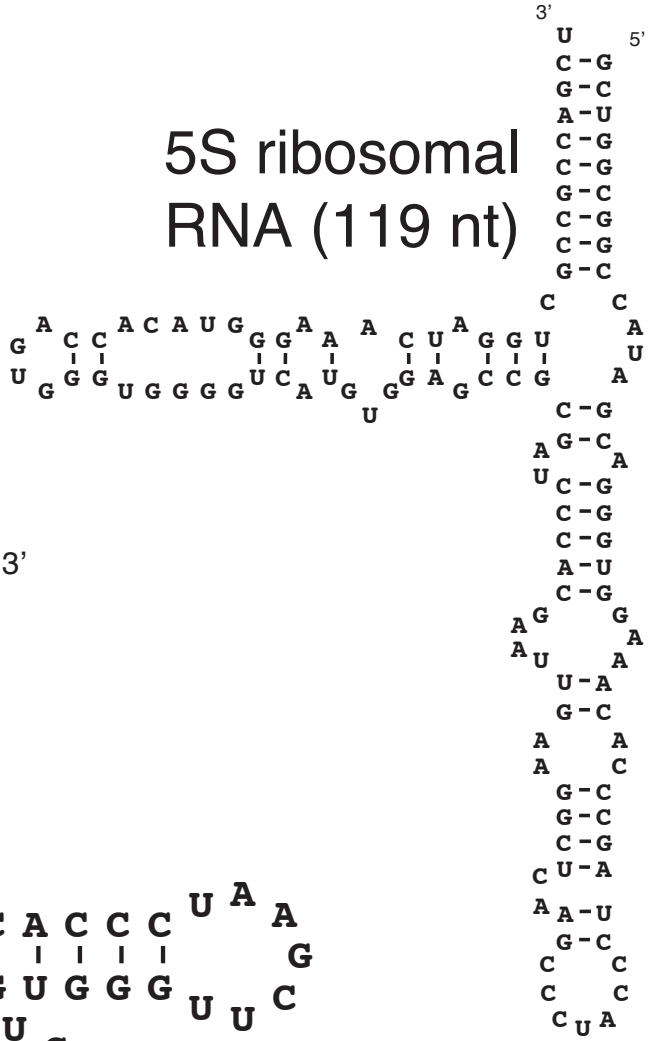


small subunit ribosomal RNA (SSU rRNA, 1582 nt)



transfer RNA (71 nt)



5S ribosomal RNA (119 nt)

Functional RNAs play many vital roles in the cell

	key RNAs involved	archaea	bacteria	eukarya
translation	ribosomal RNAs	x	x	x
	transfer RNAs	x	x	x
	RNase P RNA	x	x	x
	snoRNAs	x		x
	SRP RNA	x	x	x
	tmRNA		x	
	RNaseMRP			x
gene expression	riboswitches	?	x	?
	microRNAs			x
	6S RNA			x
splicing	U1, U2, U4, U5, U6			x
other	telomerase RNA			x
	Y RNA			x
	Vault RNA			x
	many more...			

Functional RNAs play many vital roles in the cell

	key RNAs involved	archaea	bacteria	eukarya
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	transfer RNAs	x	x	x
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	snoRNAs	x		x
	SRP RNA	x	x	x
	tmRNA		x	
	RNaseMRP			x
gene expression	riboswitches	?	x	?
	microRNAs			x
	6S RNA			x
splicing	U1, U2, U4, U5, U6			x
other	telomerase RNA			x
	Y RNA			x
	Vault RNA			x
	many more...			

database of more than 2400 non-coding RNA families
each represented by a secondary structure, alignment, and covariance model.

Outline of talk

1. Motivation: collecting homologs facilitates comparative sequence analysis.
1965: Secondary structure determination of transfer RNA.

2. Sequence and sequence+structure profiles

3. Accelerating RNA homology search

4. Implications for Rfam

5. New features in latest version of Infernal

Structure of a Ribonucleic Acid

ROBERT W. HOLLEY, JEAN APGAR
GEORGE A. EVERETT
JAMES T. MADISON
MARK MARQUISEE, SUSAN H. MERRILL
JOHN ROBERT PENSWICK, ADA ZAMIR
U.S. Plant, Soil, and Nutrition Laboratory, U.S. Department of Agriculture, and
Department of Biochemistry, Cornell University, Ithaca, New York

Abstract. *The complete nucleotide sequence of an alanine transfer RNA, isolated from yeast, has been determined. This is the first nucleic acid for which the structure is known.*

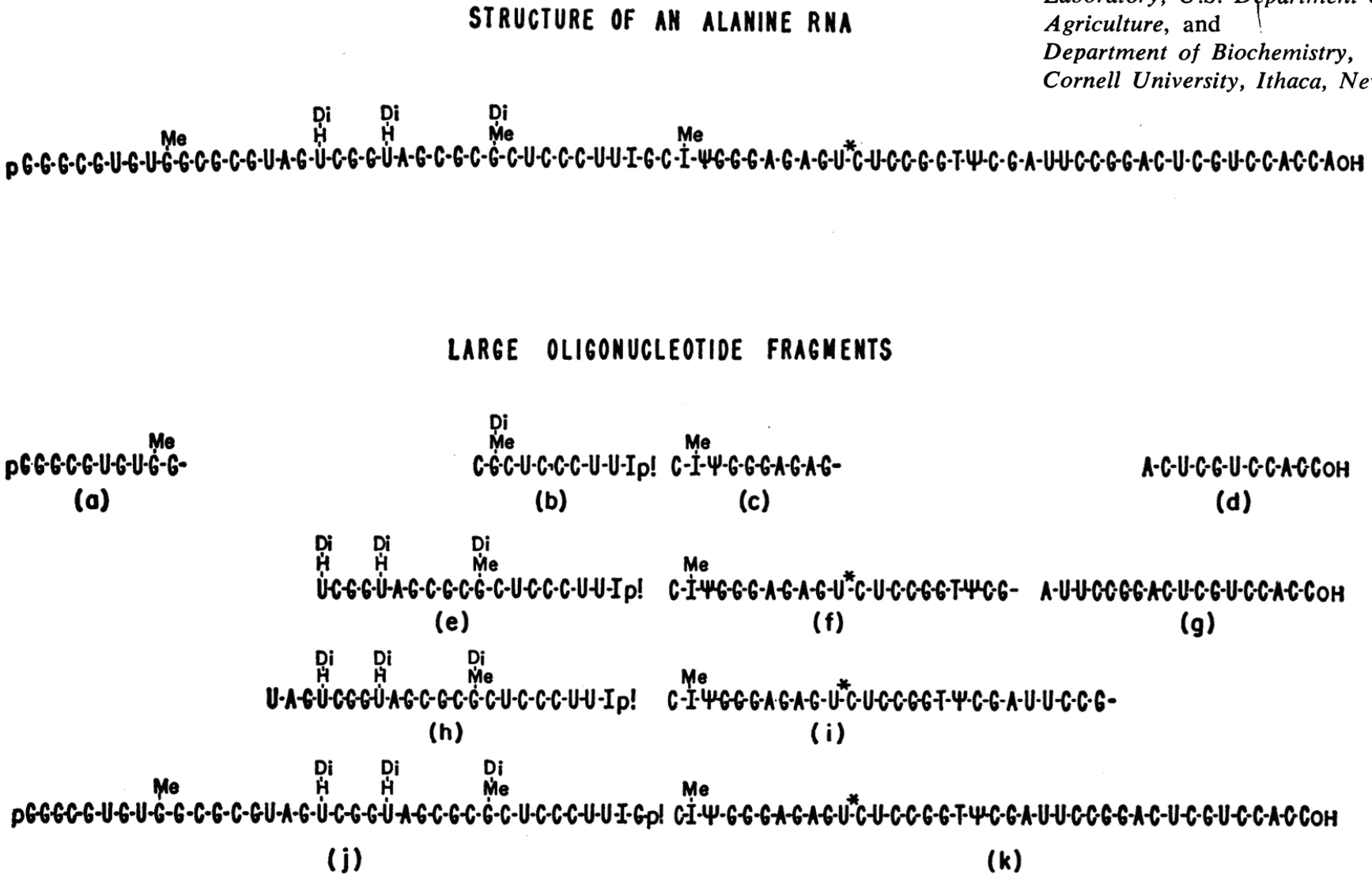


Fig. 1. The structure of an alanine transfer RNA, isolated from yeast, is shown at the top. Large oligonucleotide fragments that were crucial in the proof of structure are shown below.

Holley, Robert W., et al. "Structure of a ribonucleic acid." Science 147.3664 (1965): 1462-1465.

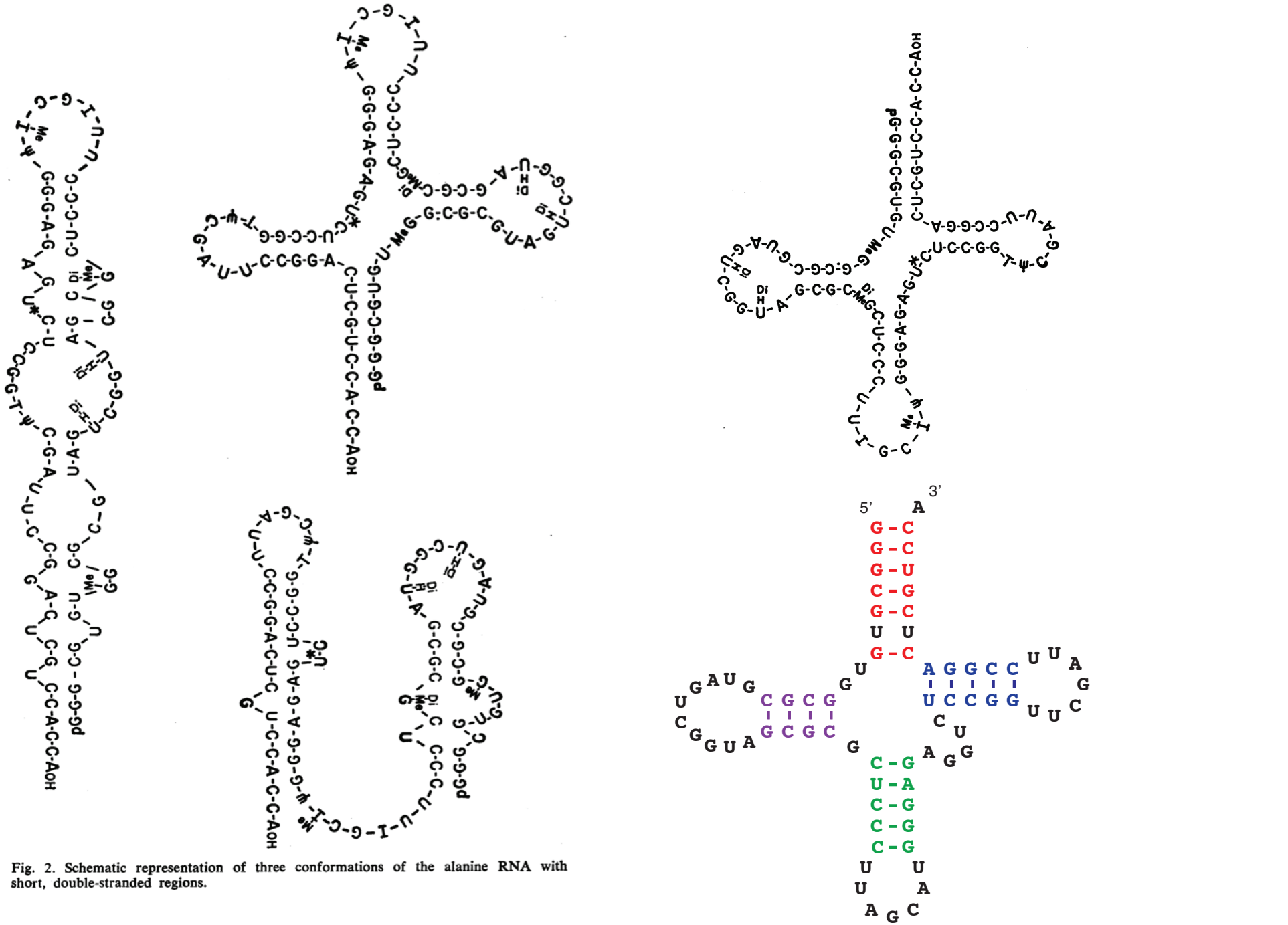
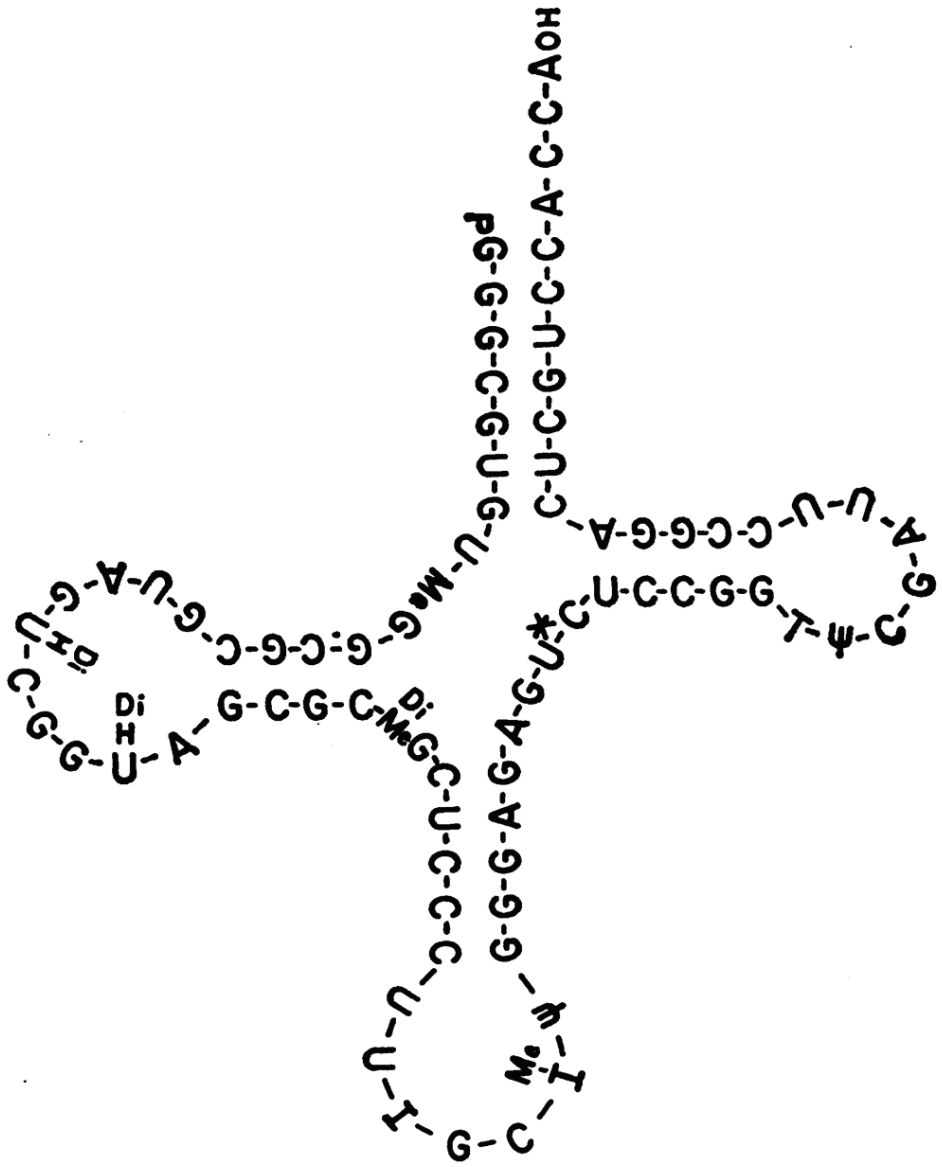


Fig. 2. Schematic representation of three conformations of the alanine RNA with short, double-stranded regions.

Holley, Robert W., et al. "Structure of a ribonucleic acid." Science 147.3664 (1965): 1462-1465.

```
struct     (((((((..<<<<............>>>>.<<<<<.......>>>>>.....<<<<<.......>>>>>)))))))
Ala        GGGCGUGUGGCGCGUAGUCGGU..AGCGCGCUCCCUUAGCAUGGGAGAG.UCUCCGGUUCGAUUCCGGACUCGUCCA
Tyr        CUCUCGGUAGCCA..AGUUGGUUUAAGGCGCAAGACUGUA..UCUUGAGAUCGGGCGUUCGACUCGCCCCCGGGAGA
Val        GGUUUCGUGGUCU..AGUCGGU.UAUGGCAUCUGCUUAACACGCAGAACGUCCCCAGUUCGAUCCUGGGCGAAAUCA
Iln        GGUCUCUUGGCCC..AGUUGGU.UAAGGCACCGUGCUAAUAACGCGGGGAUCAGCGGUUCGAUCCCGCUAGAGACCA
Glu        UCCGAUAUAGUGU..AAC.GGC.UAUCACAUCACGCUUUCACCGUGGAGA.CGGGGUUCGACUCCCCGUAUCGGAG
identical          * *     * * ** ***   *       *   *          **     ******  *
>0 non-WC  ((((((..<<<<............>>>>.<<<<<.......>>>>>.....<<<<<.......>>>>>))))))
```

Alignment color legend:
Black: Watson-Crick or GU/UG basepair
Red: non-Watson-Crick and non-GU/UG basepair
Grey: not basepaired



```
struct      <<<<<.<<..<<..<<<.......<<..<.<<<<<.......>>>>>.>..>>......>>>...>>.>>.>>.>>>
Ala         GGGCGUGUGGCGCGUAGUCGGU..AGCGCGCUCCCUUAGCAUGGGAGAG.UCUCCGGUUCGAUUCCGGACUCGUCCA
Tyr         CUCUCGGUAGCCA..AGUUGGUUUAAGGCGCAAGACUGUA..UCUUGAGAUCGGGCGUUCGACUCGCCCCGGGAGA
Val         GGUUUCGUGGUCU..AGUCGGU.UAUGGCAUCUGCUUAACACGCAGAACGUCCCCAGUUCGAUCCUGGGCGAAAUCA
Iln         GGUCUCUUGGCCC..AGUUGGU.UAAGGCACCGUGCUAAUAACGCGGGGAUCAGCGGUUCGAUCCCGCUAGAGACCA
Glu         UCCGAUAUAGUGU..AAC.GGC.UAUCACAUCACGCUUUCACCGUGGAGA.CCGGGGUUCGAUCUCCCGUAUCGGAG
identical           * *     * * ** ***   *        *   *          **    *******  *
>0 non-WC   <<<<<.<<..<<..<<<.......<<..<.<<<<<.......>>>>>.>..>>......>>>...>>.>>.>>.>>>
```

Alignment color legend:
Black: Watson-Crick or GU/UG basepair
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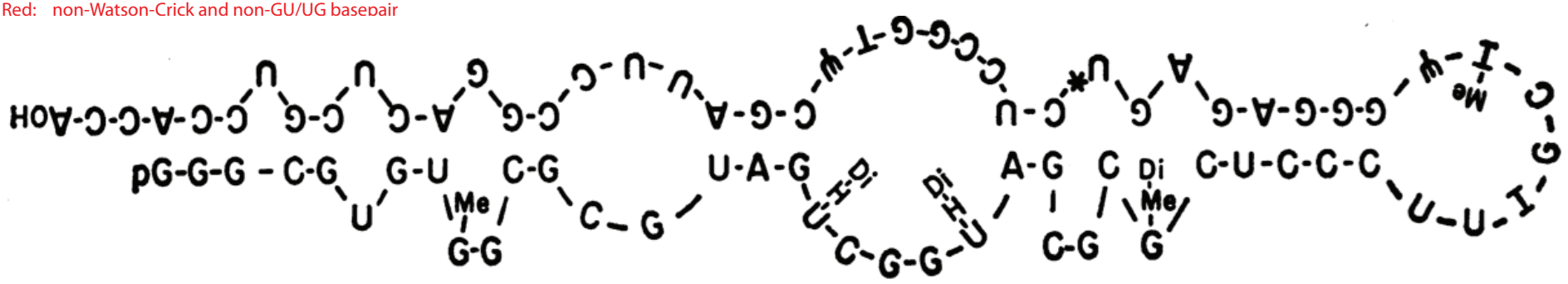

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struct      <<<<<.<<..<<..<<<.......<<..<.<<<<<.......>>>>>.>..>>......>>>...>>.>>.>>.>>>
Ala         GGGCGUGUGGCGCGUAGUCGGU..AGCGCGCUCCCUUAGCAUGGGAGAG.UCUCCGGUUCGAUUCCGGACUCGUCCA
Tyr         CUCUCGGUAGCCA..AGUUGGUUUAAGGCGCAAGACUGUA..UCUUGAGAUCGGGCGUUCGACUCGCCCCGGGAGA
Val         GGUUUCGUGGUCU..AGUCGGU.UAUGGCAUCUGCUUAACACGCAGAACGUCCCCAGUUCGAUCCUGGGCGAAAUCA
Iln         GGUCUCUUGGCCC..AGUUGGU.UAAGGCACCGUGCUAAUAACGCGGGGAUCAGCGGUUCGAUCCCGCUAGAGACCA
Glu         UCCGAUAUAGUGU..AAC.GGC.UAUCACAUCACGCUUUCACCGUGGAGA.CCGGGGUUCGAUCUCCCGUAUCGGAG
identical         * *      * * ** ***   *        *   *           **     *******   *
>0 non-WC   <<<<<.<<..<<..<<<.......<<..<.<<<<<.......>>>>>.>..>>......>>>...>>.>>.>>.>>>
clover      << <                      <<<<<.......>>>>>                        > >>
overlap
```

Alignment color legend:
Black: Watson-Crick or GU/UG basepair
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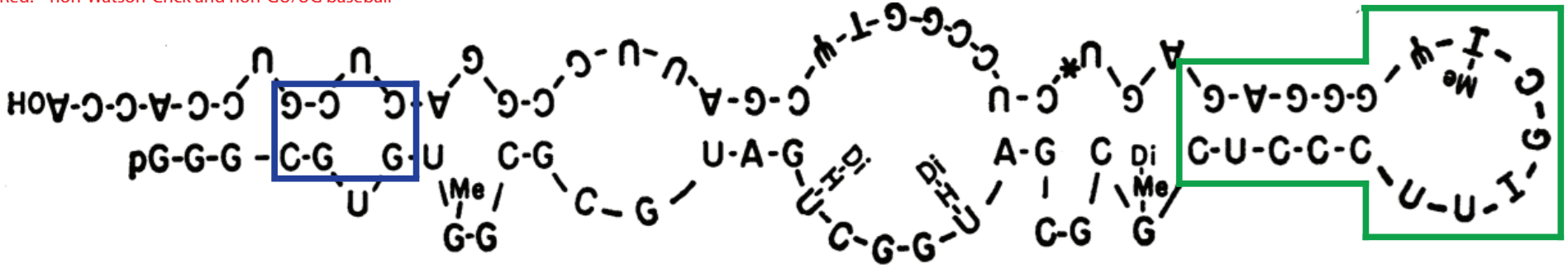

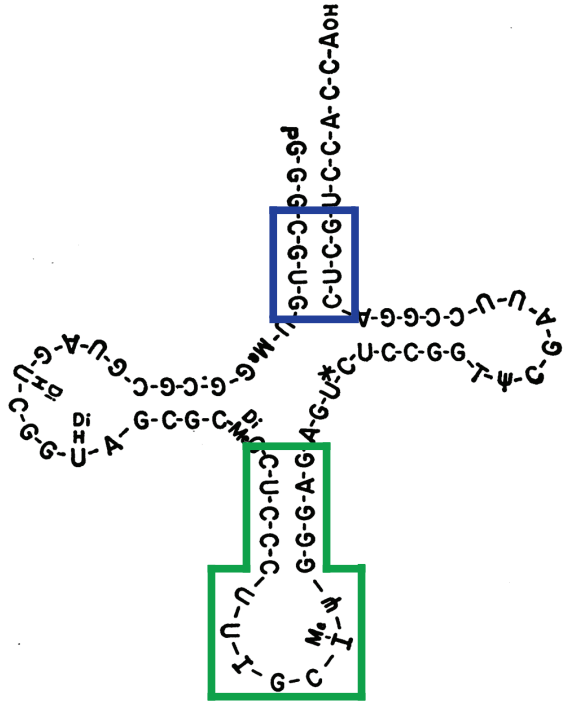

```
struct          <<<.<....<<<<............>>>>.>.>>>........<<<<<<...<<<<<.......>>>>>>>>.>>>.
Ala             GGGCGUGUGGCGCGUAGUCGGU..AGCGCGCUCCCUUAGCAUGGGAGAG.UCUCCGGUUCGAUUCCGGACUCGUCCA
Tyr             CUCUCGGUAGCCA..AGUUGGUUUAAGGCGCAAGACUGUA..UCUUGAGAUCGGGCGUUCGACUCGCCCCCGGGAGA
Val             GGUUUCGUGGUCU..AGUCGGU.UAUGGCAUCUGCUUAACACGCAGAACGUCCCCAGUUCGAUCCUGGGCGAAAUCA
Iln             GGUCUCUUGGCCC..AGUUGGU.UAAGGCACCGUGCUAAUAACGCGGGGAUCAGCGGUUCGAUCCCGCUAGAGACCA
Glu             UCCGAUAUAGUGU..AAC.GGC.UAUCACAUCACGCUUUCACCGUGGAGA.CCGGGGUUCGACUCCCCGUAUCGGAG
identical              * *     * * ** ***   *        *   *         **    ******  *

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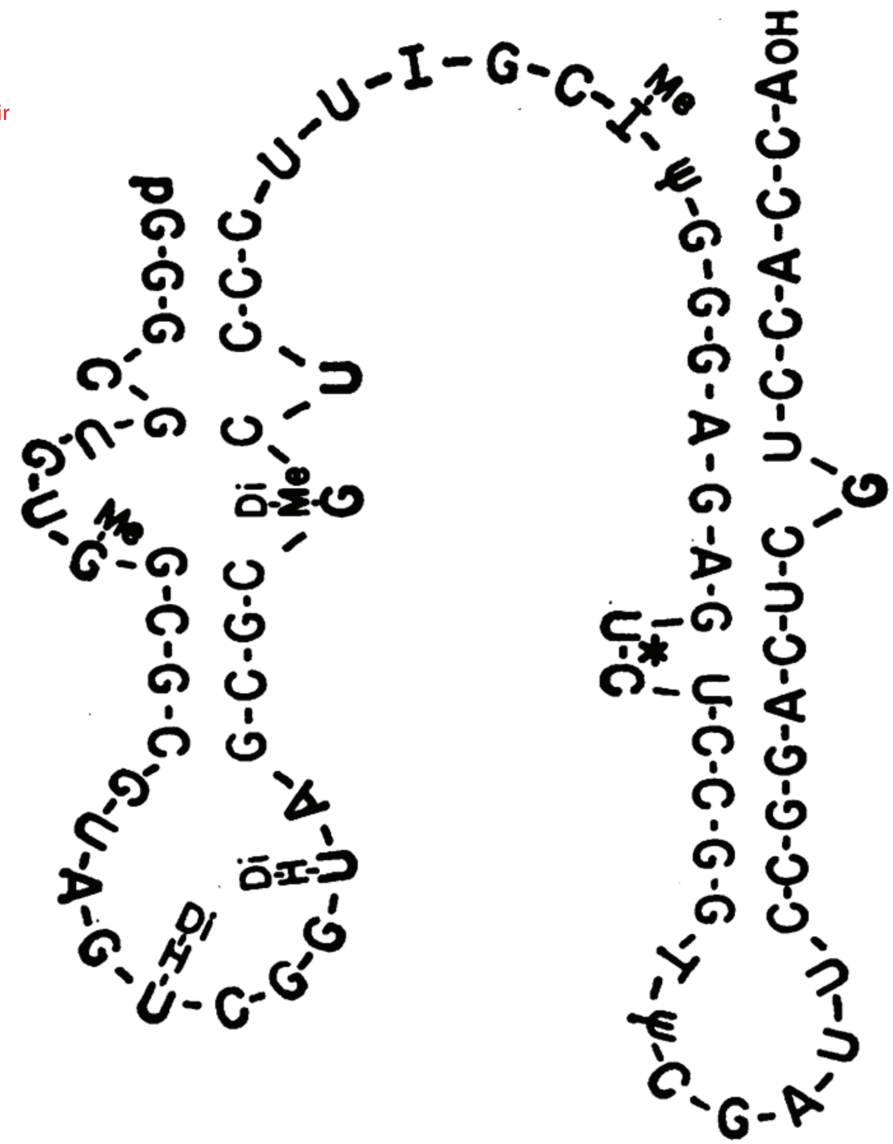

```
struct      <<<.<....<<<<.............>>>>.>.>>>........<<<<<...<<<<.......>>>>>>>>.>>>.
Ala         GGGCGUGUGGCGCGUAGUCGGU..AGCGCGCUCCCUUAGCAUGGGAGAG.UCUCCGGUUCGAUUCCGGACUCGUCCA
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Iln         GGUCUCUUGGCCC..AGUUGGU.UAAGGCACCGUGCUAAUAACGCGGGGAUCAGCGGUUCGAUCCCGCUAGAGACCA
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identical           * *     * * ** ***   *       *   *           **     ******   *
>0 non-WC   <<<.<....<<<<.............>>>>.>.>>>........<<<<<...<<<<.......>>>>>>>>.>>>.
clover                <<<<............>>>>                    <<<<.......>>>>>
overlap
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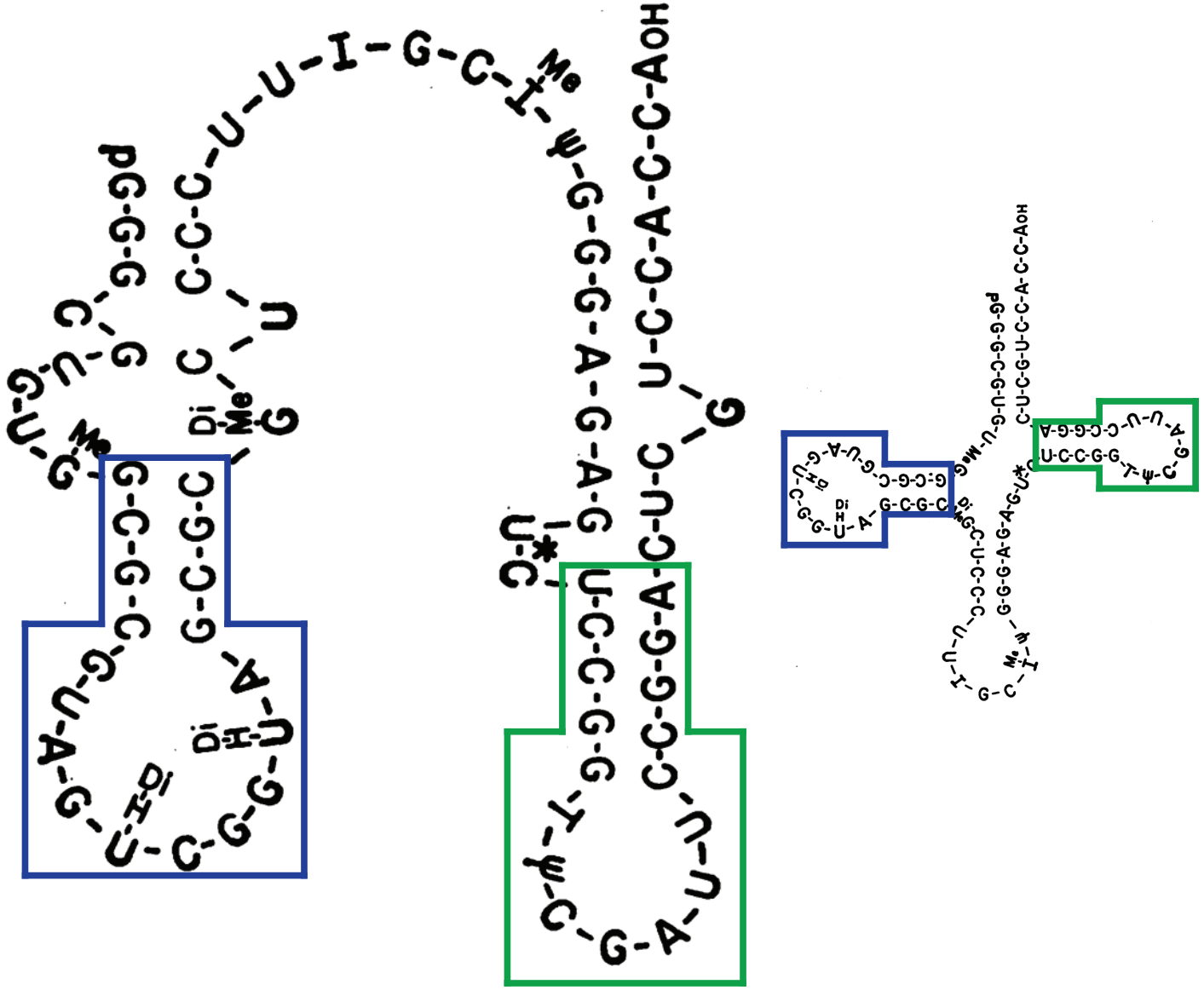

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struct     (((((((..<<<<............>>>>.<<<<<.......>>>>>.....<<<<<.......>>>>>)))))))
Ala        GGGCGUGUGGCGCGUAGUCGGU..AGCGCGCUCCCUUAGCAUGGGAGAG.UCUCCGGUUCGAUUCCGGACUCGUCCA
Tyr        CUCUCGGUAGCCA..AGUUGGUUUAAGGCGCAAGACUGUA..UCUUGAGAUCGGGCGUUCGACUCGCCCCCGGGAGA
Val        GGUUUCGUGGUCU..AGUCGGU.UAUGGCAUCUGCUUAACACGCAGAACGUCCCCAGUUCGAUCCUGGGCGAAAUCA
Iln        GGUCUCUUGGCCC..AGUUGGU.UAAGGCACCGUGCUAAUAACGCGGGGAUCAGCGGUUCGAUCCCGCUAGAGACCA
Glu        UCCGAUAUAGUGU..AAC.GGC.UAUCACAUCACGCUUUCACCGUGGAGA.CGGGGUUCGACUCCCCGUAUCGGAG
identical          * *     * * ** ***   *       *   *          **     ******  *
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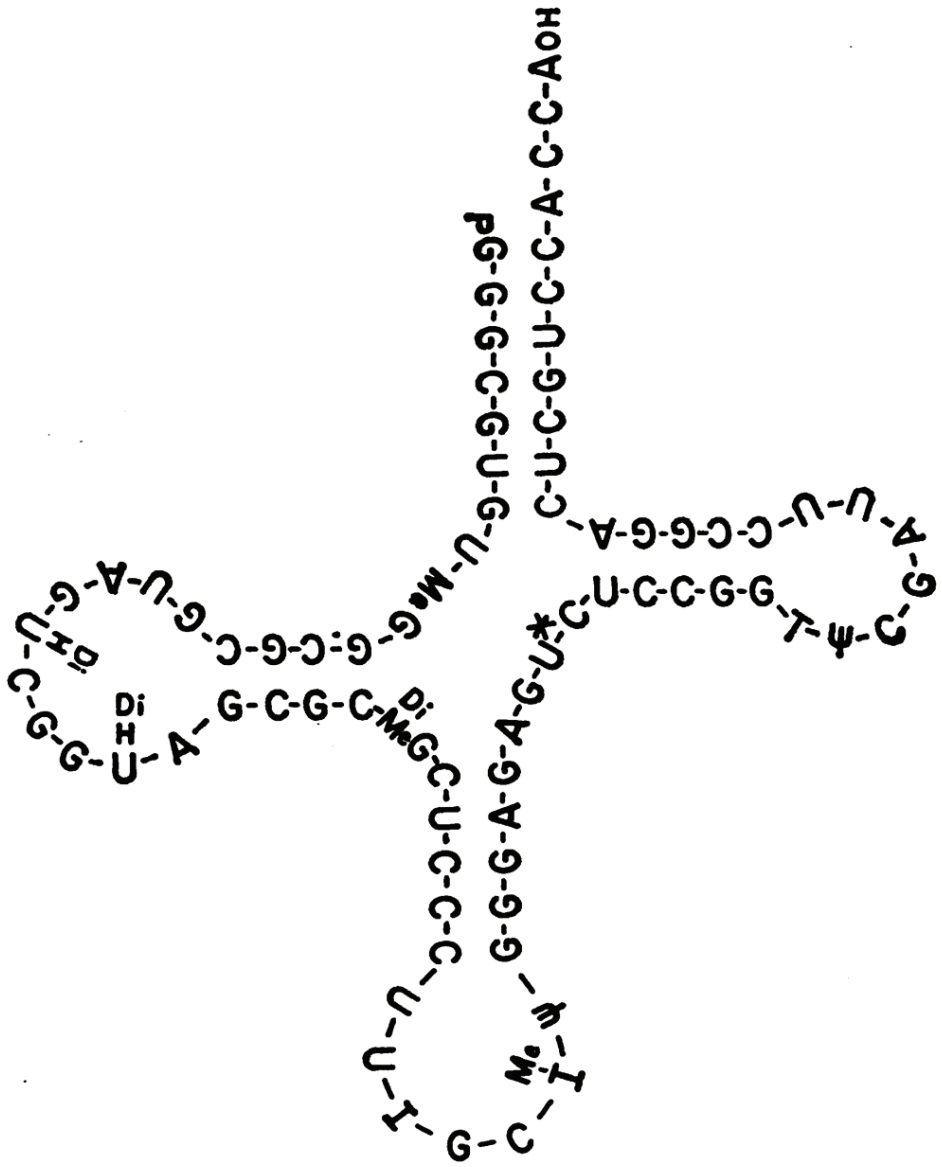
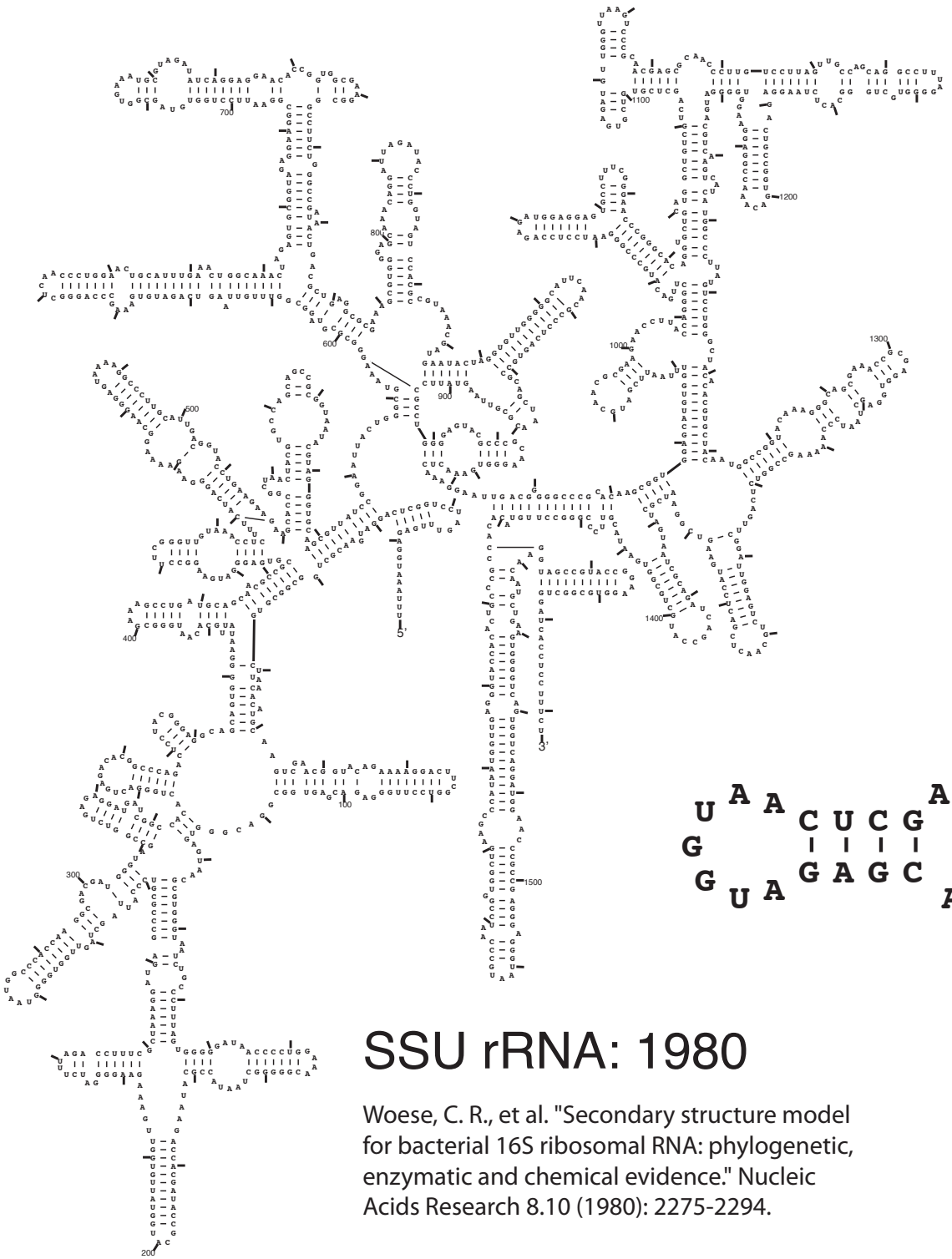
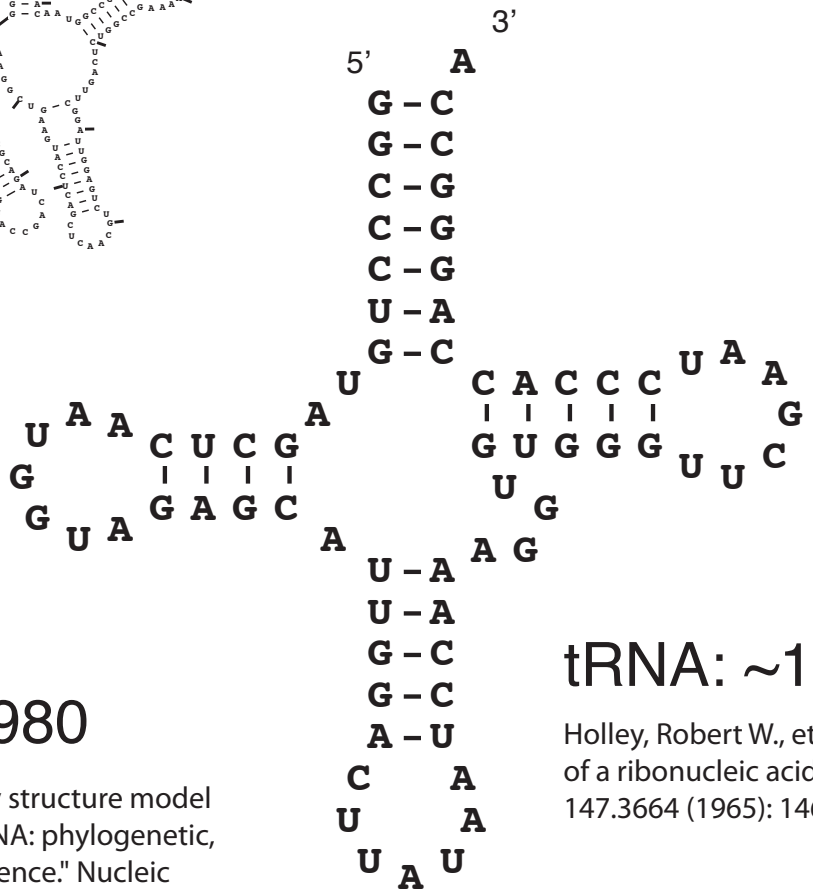




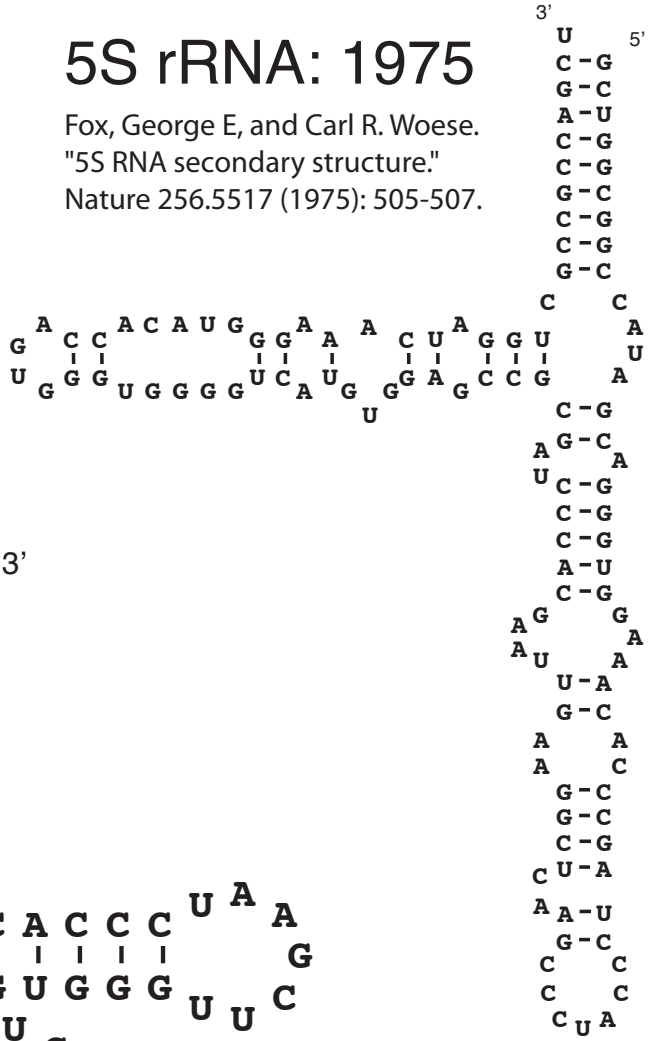
SSU rRNA: 1980

Woese, C. R., et al. "Secondary structure model for bacterial 16S ribosomal RNA: phylogenetic, enzymatic and chemical evidence." Nucleic Acids Research 8.10 (1980): 2275-2294.



tRNA: ~1966

Holley, Robert W., et al. "Structure of a ribonucleic acid." Science 147.3664 (1965): 1462-1465.



5S rRNA: 1975

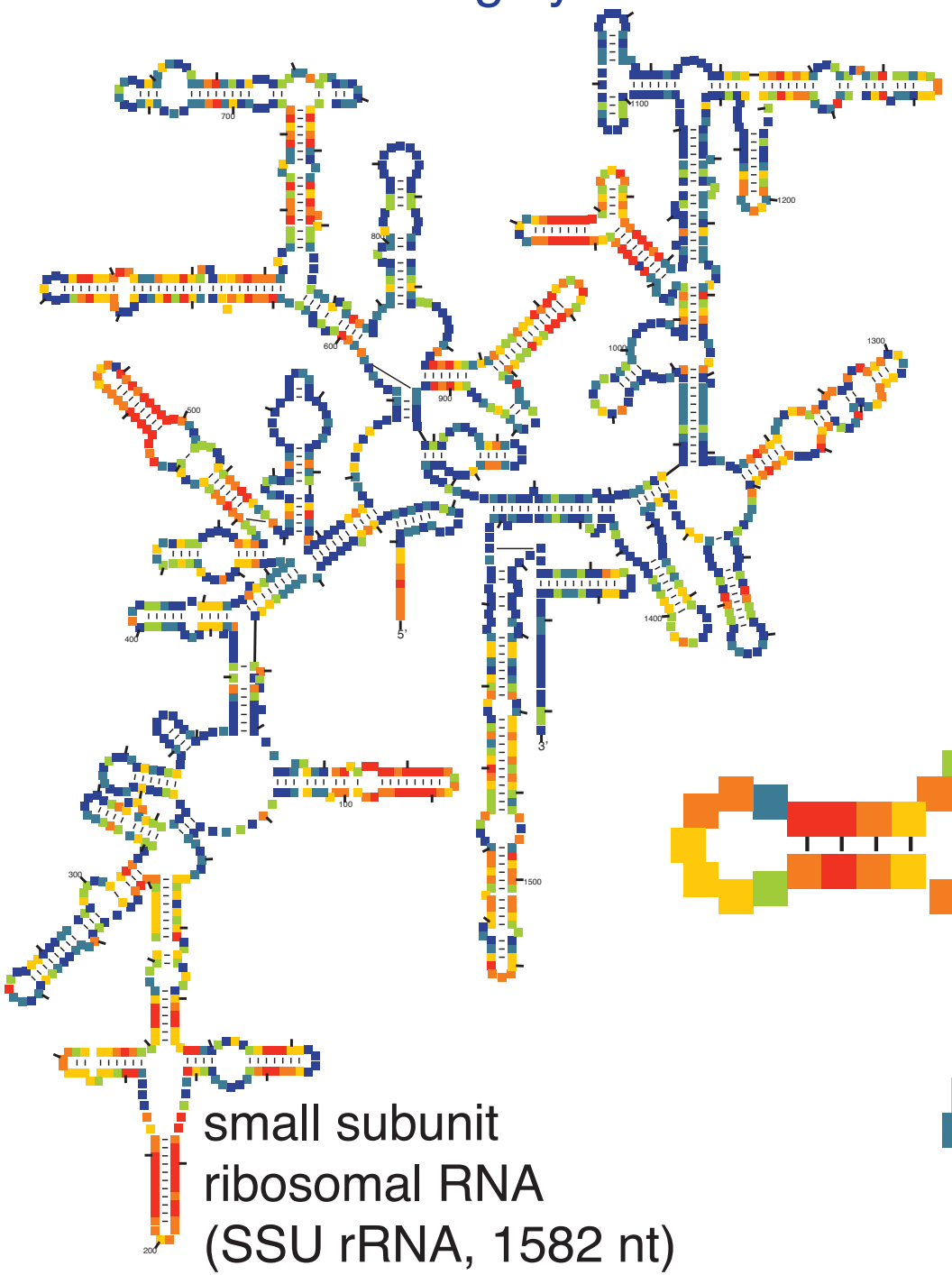
Fox, George E, and Carl R. Woese. "5S RNA secondary structure." Nature 256.5517 (1975): 505-507.

Comparative sequence analysis of homologs informs biologists

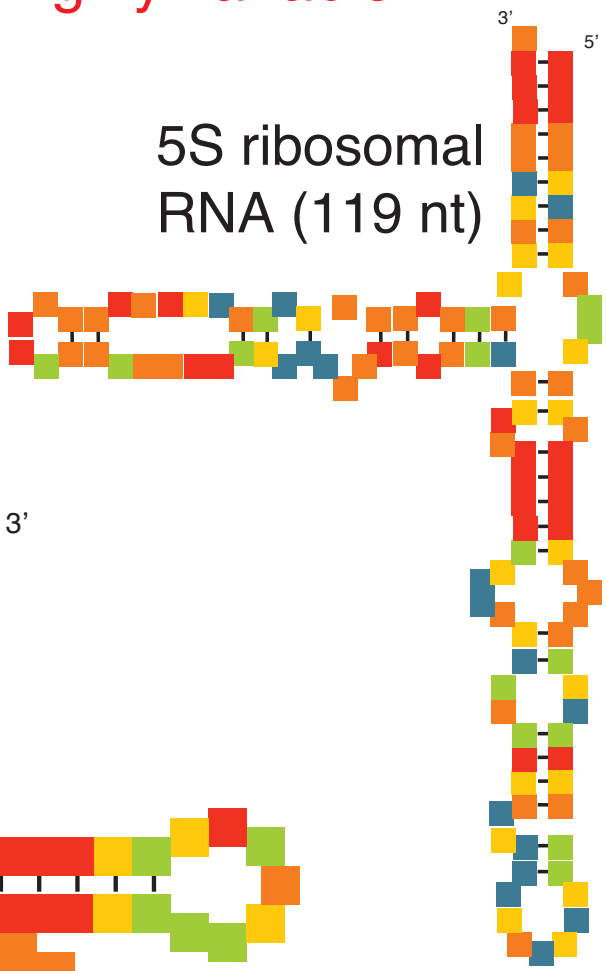
- Inference of structure
- Inference of phylogeny of organisms
- Inference of functional regions based on conservation levels

Sequence conservation per position

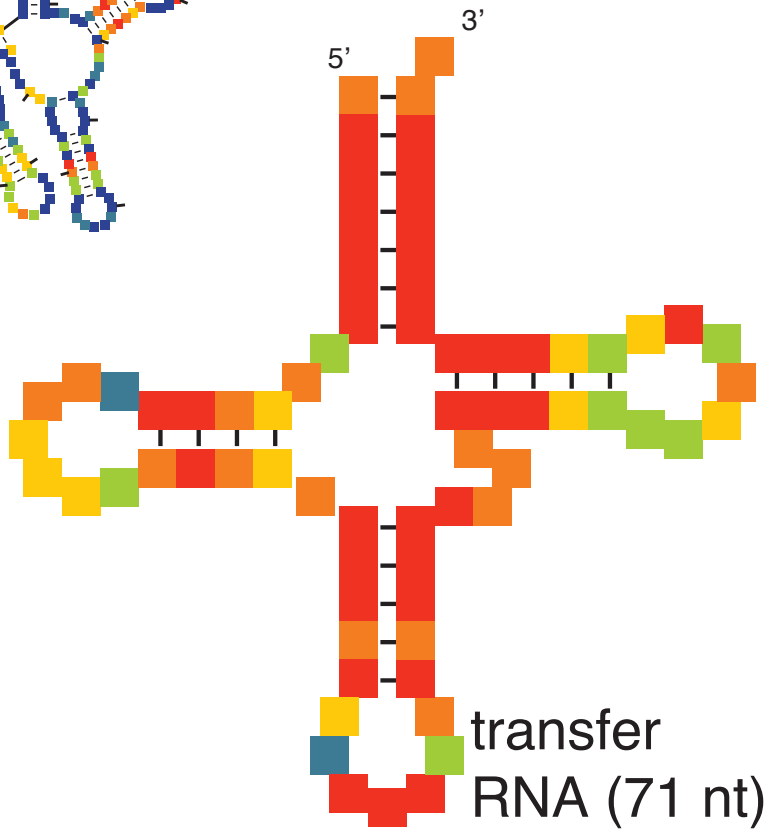
blue:highly conserved ..... red: highly variable



small subunit ribosomal RNA (SSU rRNA, 1582 nt)



5S ribosomal RNA (119 nt)



transfer RNA (71 nt)

Comparative sequence analysis of homologs informs biologists

- Inference of structure
- Inference of phylogeny of organisms
- Inference of functional regions based on conservation levels

Computational homology search methods use one or more known family members to find additional homologs.

Outline of talk

1. Motivation: collecting homologs facilitates comparative sequence analysis.
1965: Secondary structure determination of transfer RNA.

2. Sequence and sequence+structure profiles

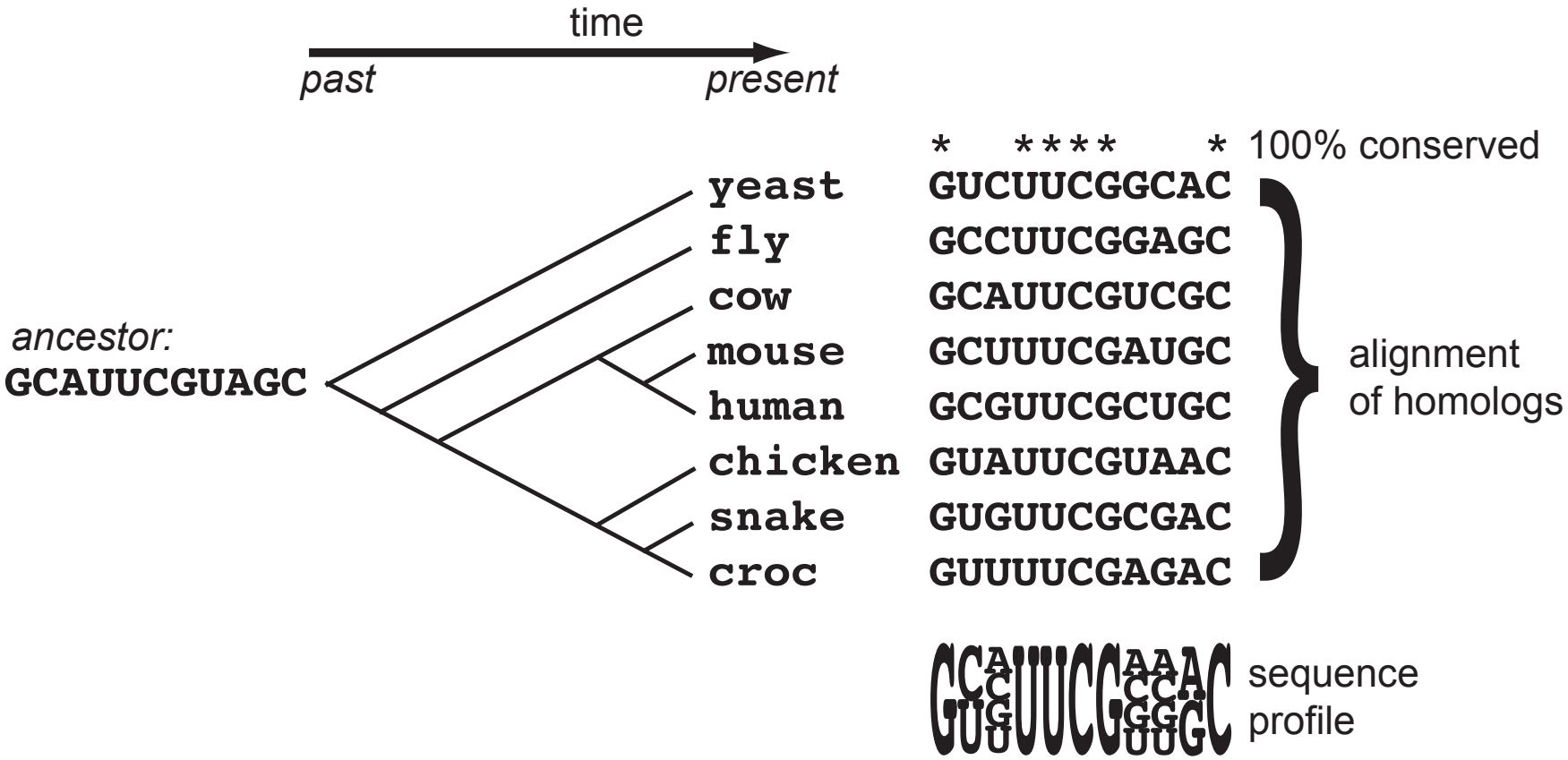
3. Accelerating RNA homology search

4. Implications for Rfam

5. New features in latest version of Infernal

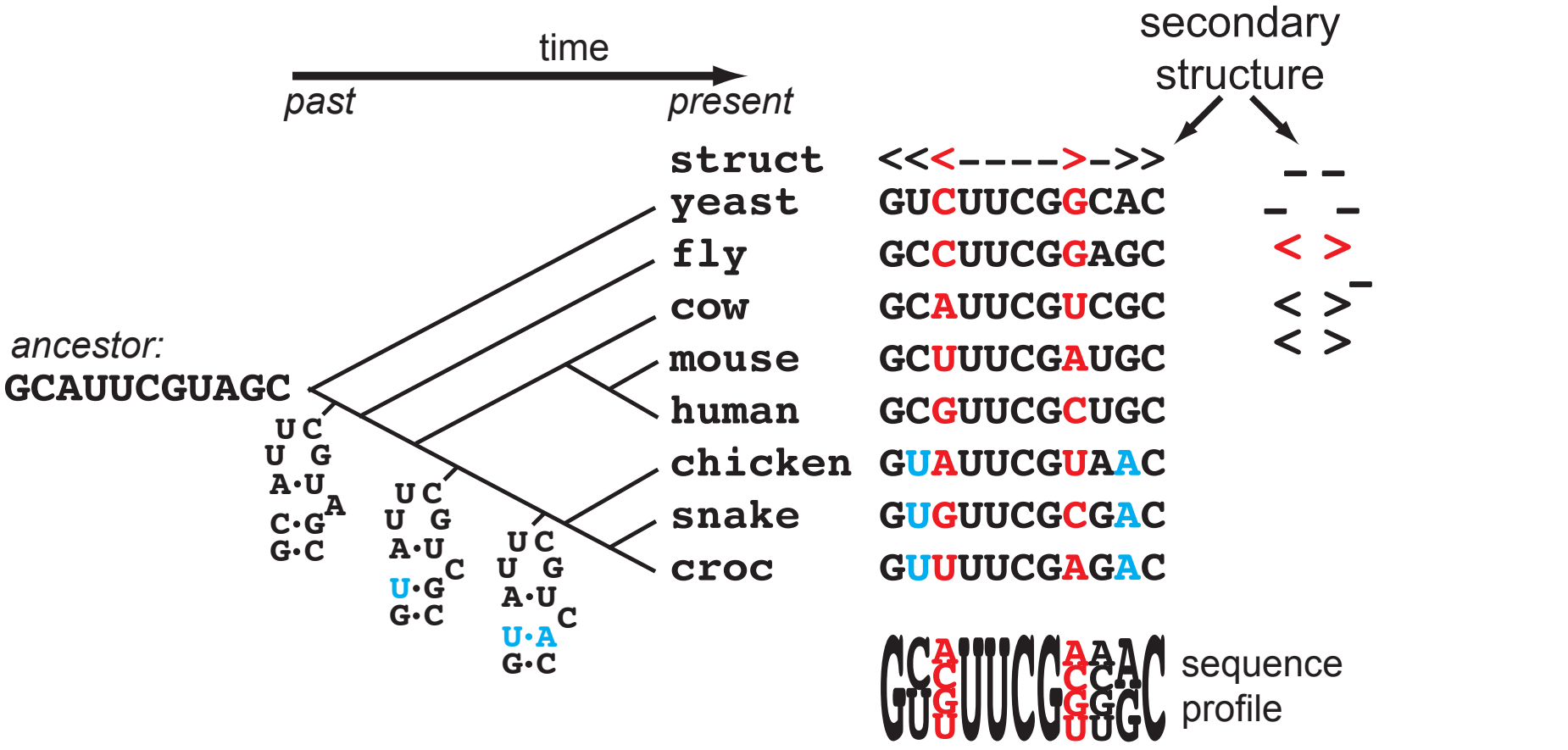
Sequence conservation provides information for homology searches

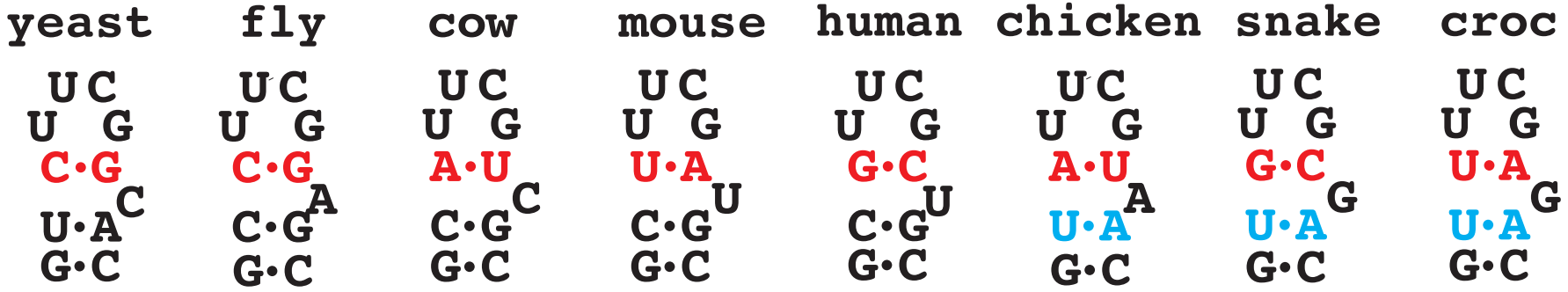
Conservation levels vary across alignment columns.



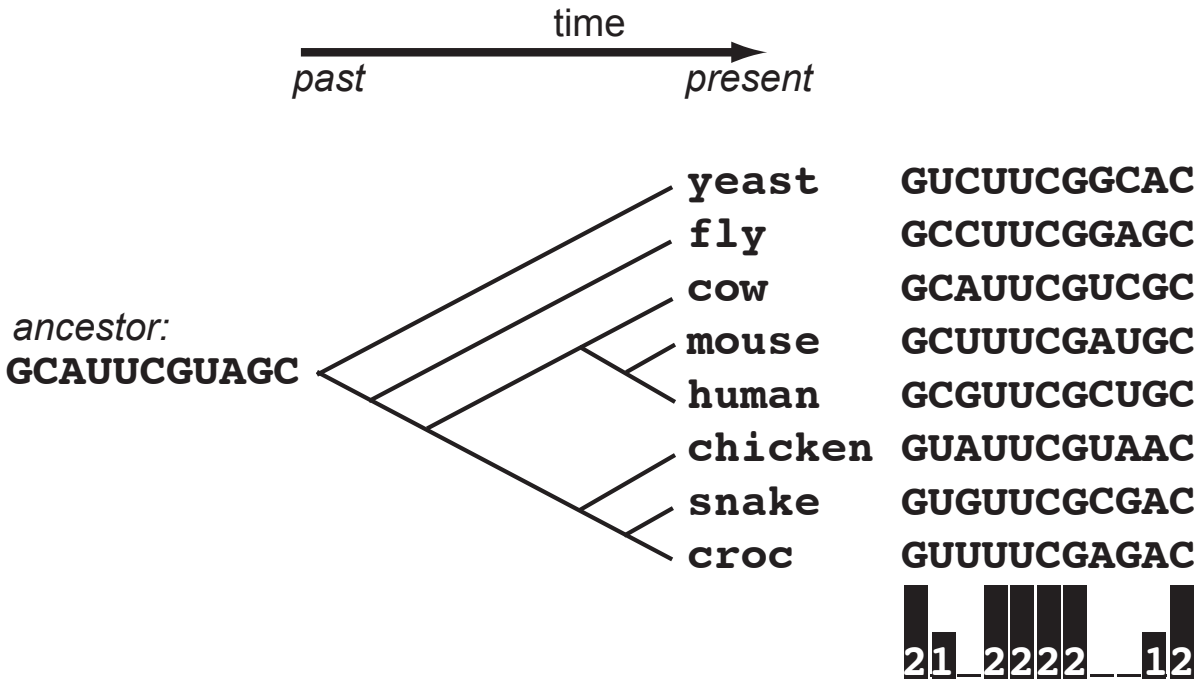
Structure conservation provides additional information

Base-paired positions covary
to maintain Watson-Crick complementarity.





Amount of information in a profile can be measured in bits



Each column is independent

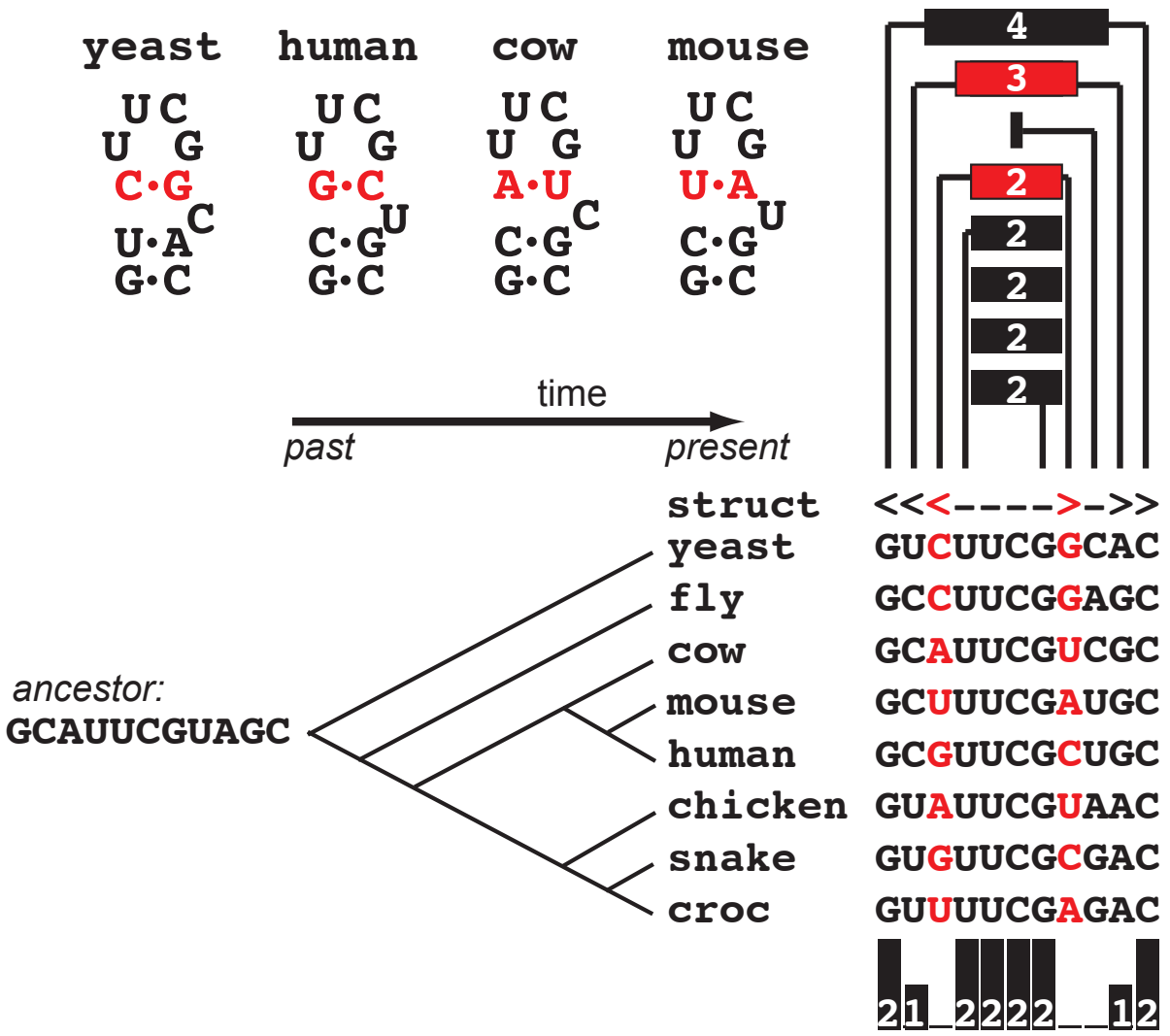
Maximum information is 2 bits per position, if column is 100% conserved.

$\log_2 (4/1) = 2$ bits

sequence profile: 14 bits

expect a match by chance: 1 in 2^{14} nt =~ 16 Kb

Structure contributes additional information from covariation



sequence + structure profile: 17 bits

Base-paired columns are not independent

Maximum ***extra*** info: 2 bits per base pair

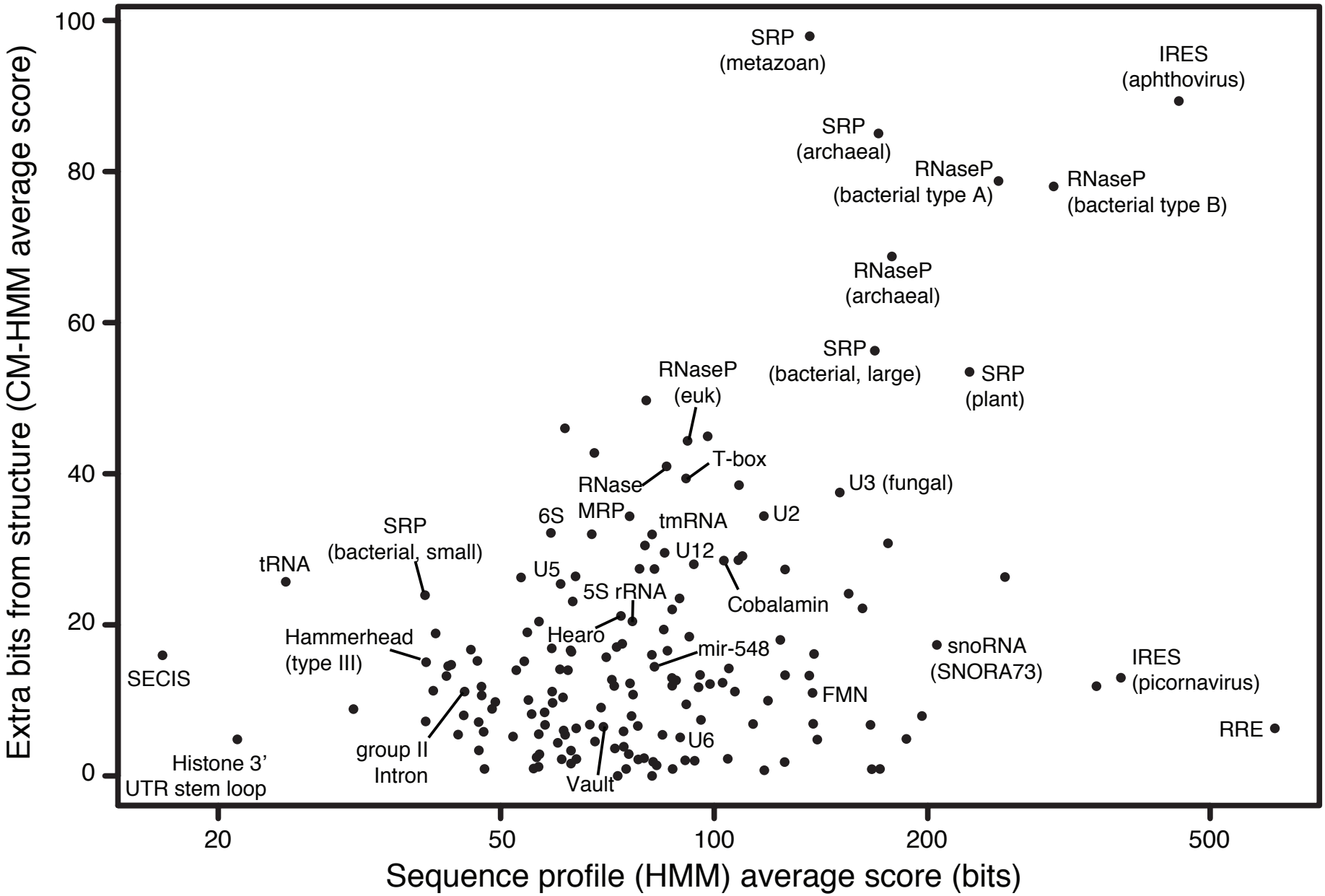
$\log_2 (16/4) = 2$ bits

sequence profile: 14 bits

expect a match by chance: 1 in 2^{17} nt =~ 130 Kb

reducing expected false positives by $2^3 = 8$-fold

Levels of sequence and structure conservation in RNA families



Eddy lab software for profile probabilistic models (since 1994)

	sequence profiles	sequence and structure profiles
models	profile HMMs	covariance models (CMs)
software	HMMER	Infernal
main use	proteins, repetitive DNA elements	structural RNAs
databases	Pfam and Dfam (16306 and 4150 entries)	Rfam (2474 families)
performance for RNAs	faster but less accurate	slower but more accurate

http://hmmer.janelia.org

Eddy, SR. PLoS Comp. Biol., 7:e1002195, 2011.

Eddy, SR. PLoS Comp. Biol., 4:e1000069, 2008.

Eddy. SR. Bioinformatics, 14:755-763, 1998.

http://infernal.janelia.org

Nawrocki EP, Eddy SR. Bioinformatics, 29: 2487-2489, 2013.

Eddy SR, Durbin R. Nucleic Acids Research, 22:2079-2088, 1994.

Profile HMMs: sequence family models built from alignments

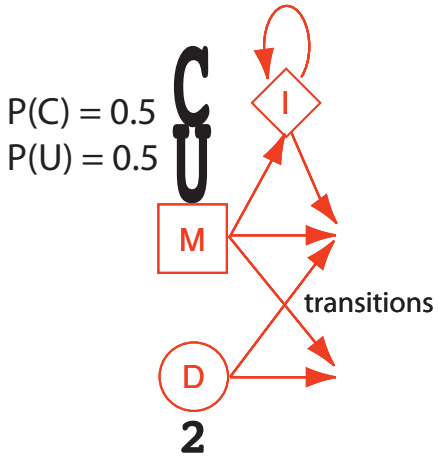
yeast	GUCUUCGGCAC
fly	GCCUUCGGAGC
cow	GCAUUCGUCGC
mouse	GCUUUCGAUGC
human	GCGUUCGCUGC
chicken	GUAUUCGUAAC
snake	GUGUUCGCGAC
croc	GUUUUCGAGAC

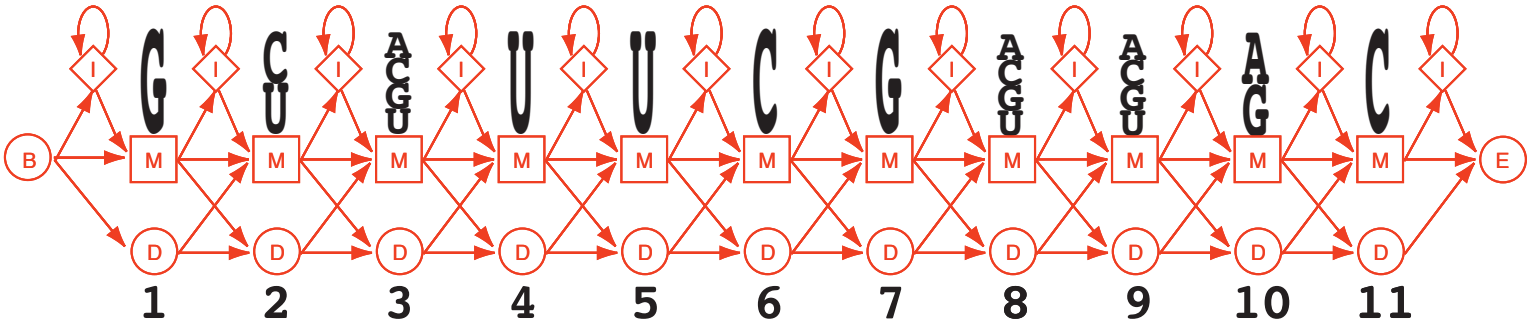
One HMM node per alignment column

3 states per node:
(M) Match: emits residues
(I) Insert: inserts extra residues
(D) Delete: deletes residues

HMMs generate homologous sequences.

Node for column 2:





Profile HMMs: sequence family models built from alignments

yeast	GUCUUCGGCAC
fly	GCCUUCGGAGC
cow	GCAUUCGUCGC
mouse	GCUUUCGAUGC
human	GCGUUCGCUGC
chicken	GUAUUCGUAAC
snake	GUGUUCGCGAC
croc	GUUUUCGAGAC

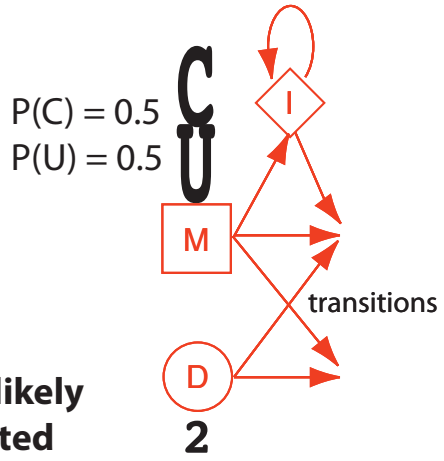
One HMM node per alignment column

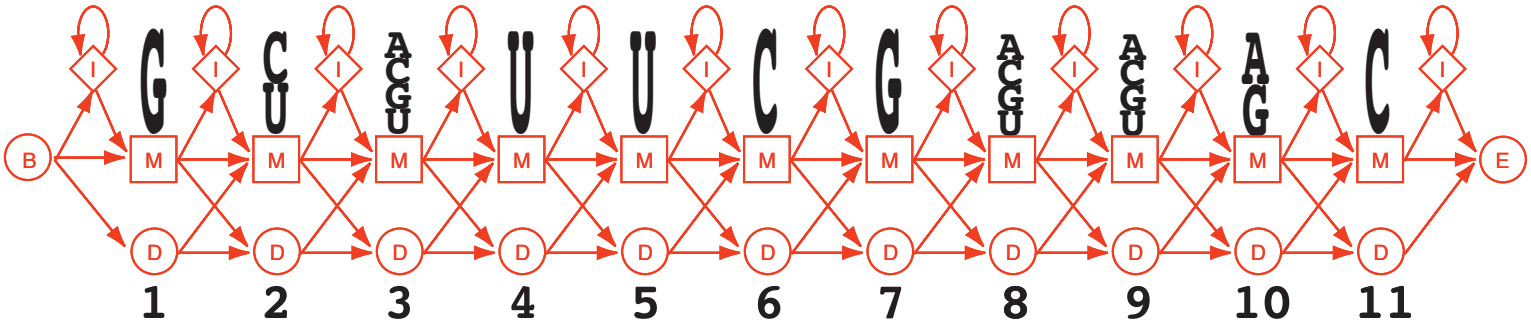
3 states per node:
(M) Match: emits residues
(I) Insert: inserts extra residues
(D) Delete: deletes residues

HMMs generate homologous sequences.

Given a sequence, the most likely path that could have generated that sequence can be computed.

Node for column 2:





Profile HMMs: sequence family models built from alignments

yeast	GUCUUCGGCAC
fly	GCCUUCGGAGC
cow	GCAUUCGUCGC
mouse	GCUUUCGAUGC
human	GCGUUCGCUGC
chicken	GUAUUCGUAAC
snake	GUGUUCGCGAC
croc	GUUUUCGAGAC
worm	GCGUUCGCGGC

One HMM node per alignment column

3 states per node:
(M) Match: emits residues
(I) Insert: inserts extra residues
(D) Delete: deletes residues

HMMs generate homologous sequences.

Given a sequence, the most likely path that could have generated that sequence can be computed.

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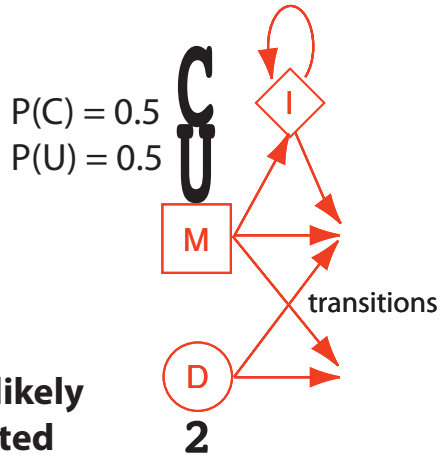
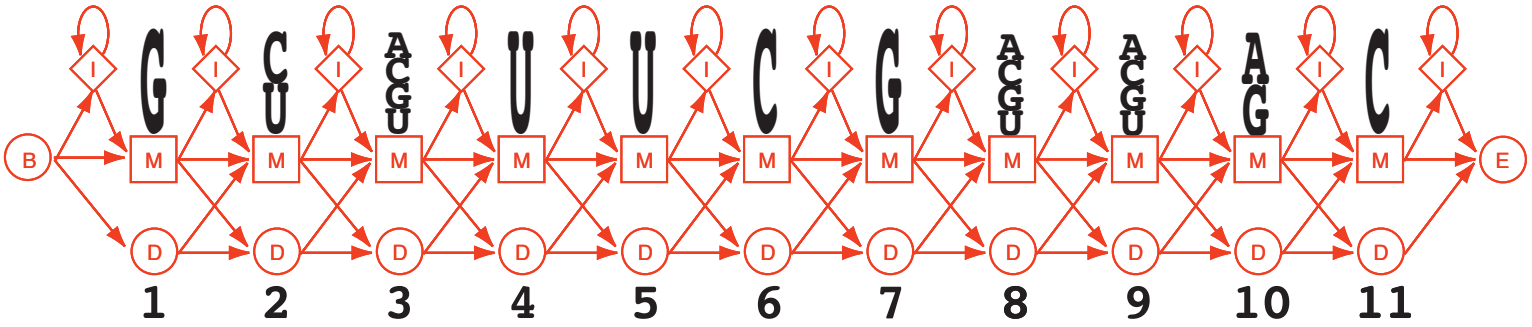
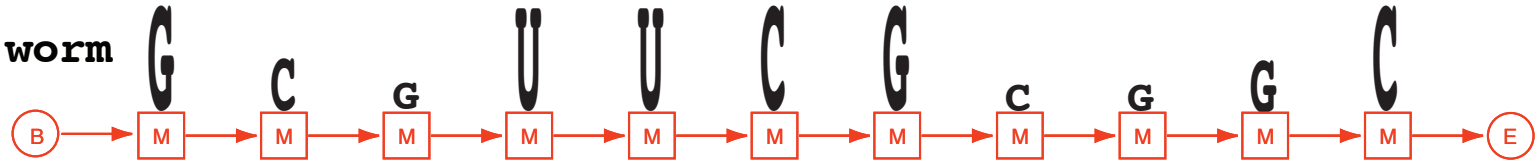






Profile HMMs: sequence family models built from alignments


```
yeast    GU.CUUCGGCAC
fly      GC.CUUCGGAGC
cow      GC.AUUCGUCGC
mouse    GC.UUUCGAUGC
human    GC.GUUCGCUGC
chicken  GU.AUUCGUAAC
snake    GU.GUUCGCGAC
croc     GU.UUUCGAGAC
worm     GC.GUUCGCGGC
corn     GUGAUUCGU.GC
```

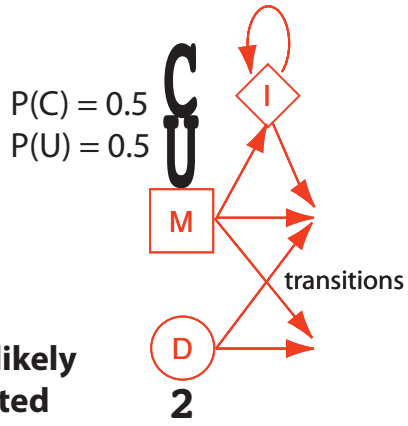
One HMM node per alignment column

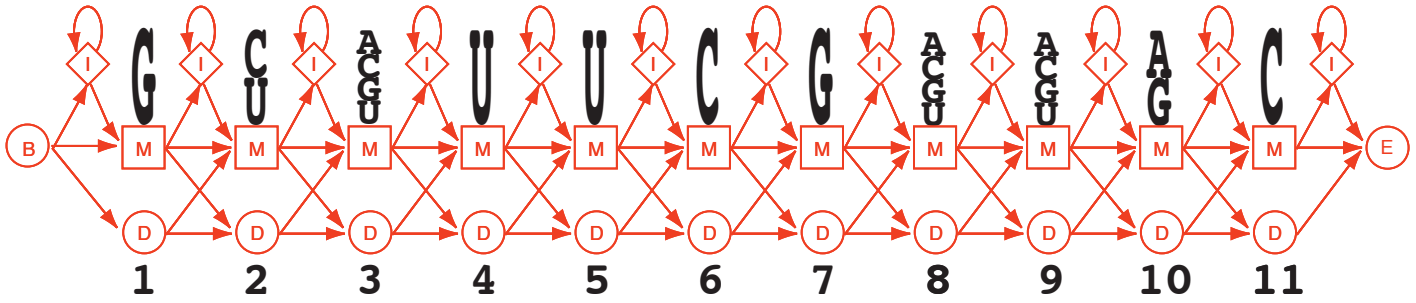
3 states per node:
(M) Match: emits residues
(I) Insert: inserts extra residues
(D) Delete: deletes residues

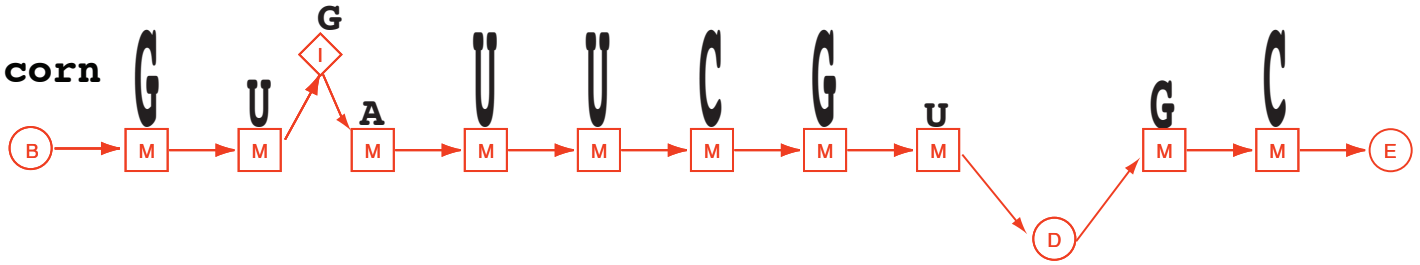
HMMs generate homologous sequences.

Given a sequence, the most likely path that could have generated that sequence can be computed.

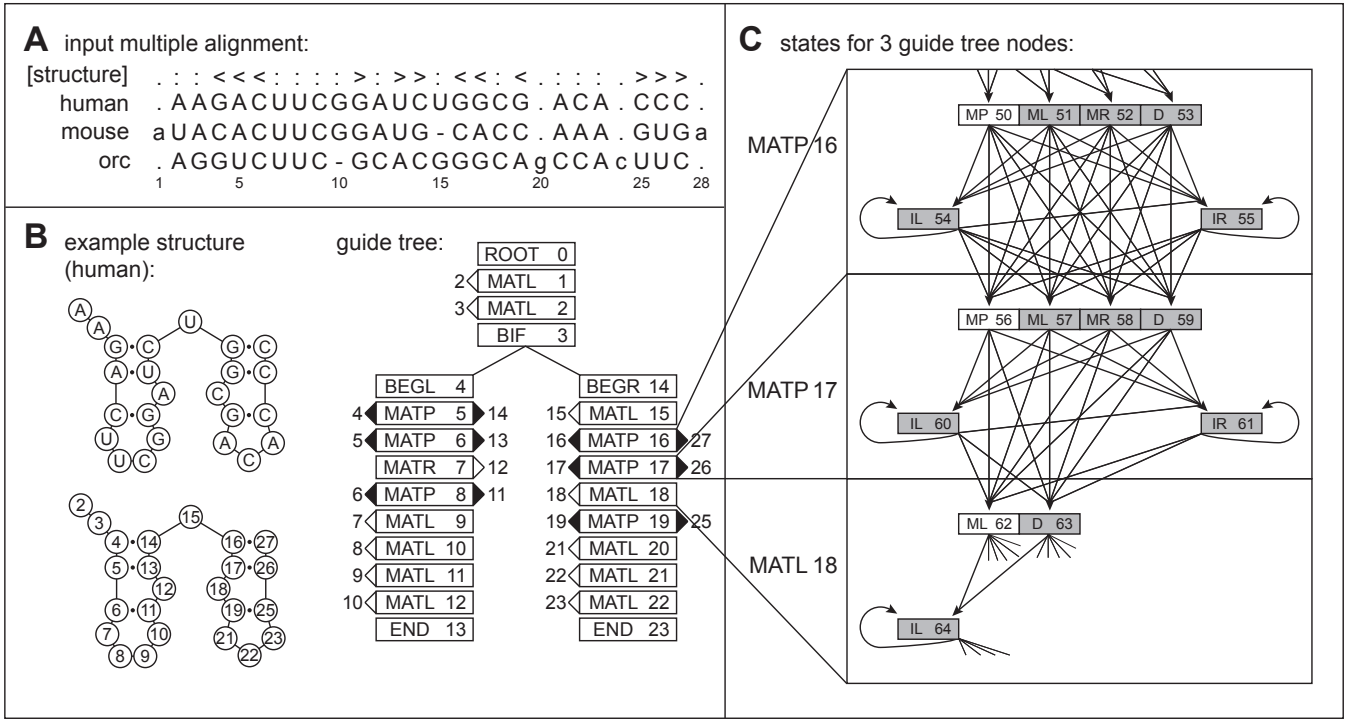
Node for column 2:

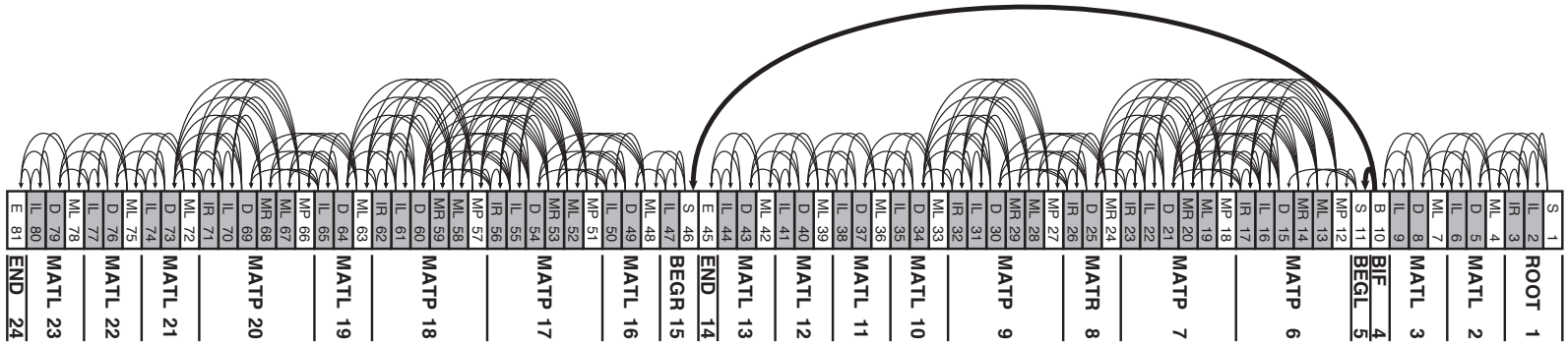






Covariance models (CMs) are built from structure-annotated alignments

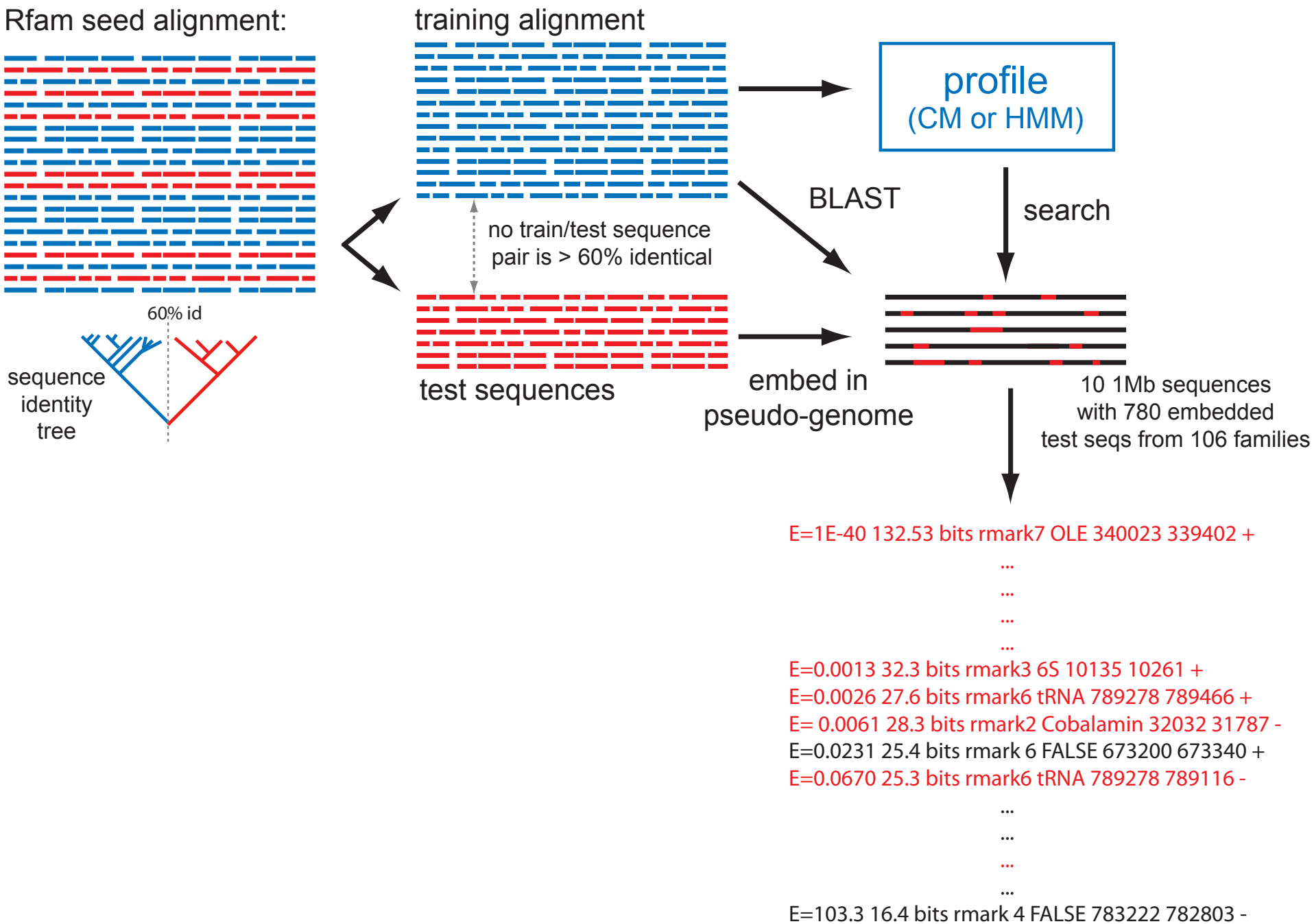




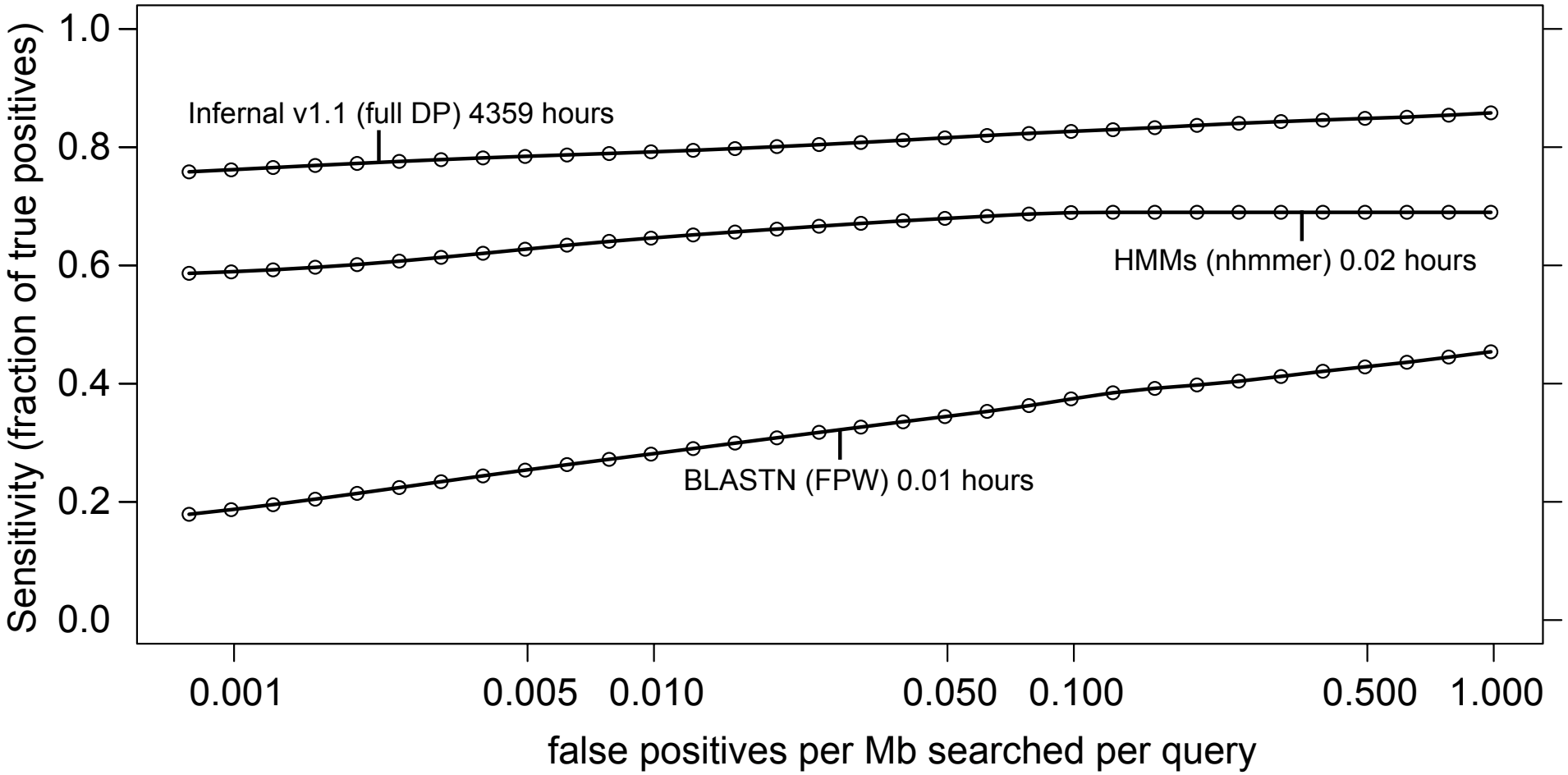
Is the added complexity worth it?

RMARK: a challenging internal RNA homology search benchmark

Is the added complexity worth it?
RMARK: a challenging internal RNA homology search benchmark



Infernal outperforms primary-sequence based methods on our benchmark (and others*, not shown)



Nawrocki EP, Eddy SR. Bioinformatics, 29:2487-2489, 2013.

*Freyhult EK, Bollback JP, Gardner PP. Genome Res. 2007 17: 117-125.

Outline of talk

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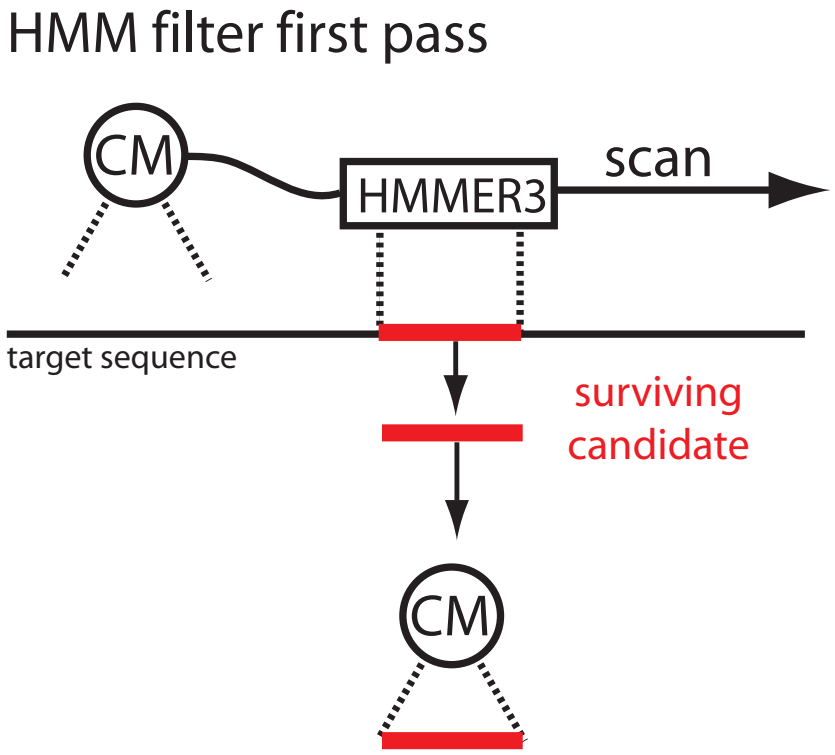
2. Sequence and sequence+structure profiles

3. Accelerating RNA homology search

4. Implications for Rfam

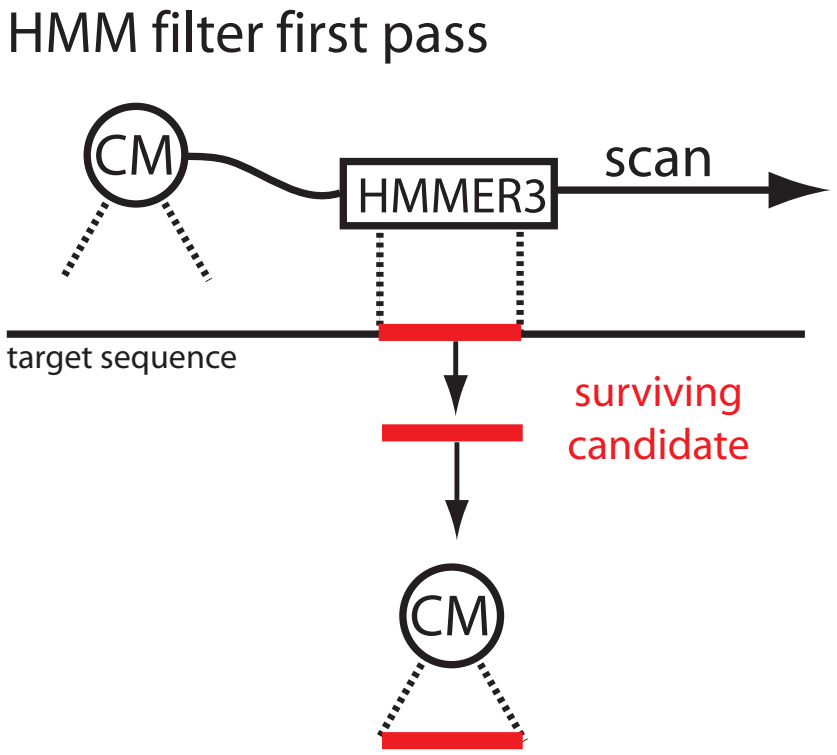
5. New features in latest version of Infernal

Filter target database using profile HMMs*



*Weinberg, Ruzzo, RECOMB, 243-251, 2004; Weinberg, Ruzzo, Bioinformatics, 22(1) 35-39 2006.

Filter target database using profile HMMs*

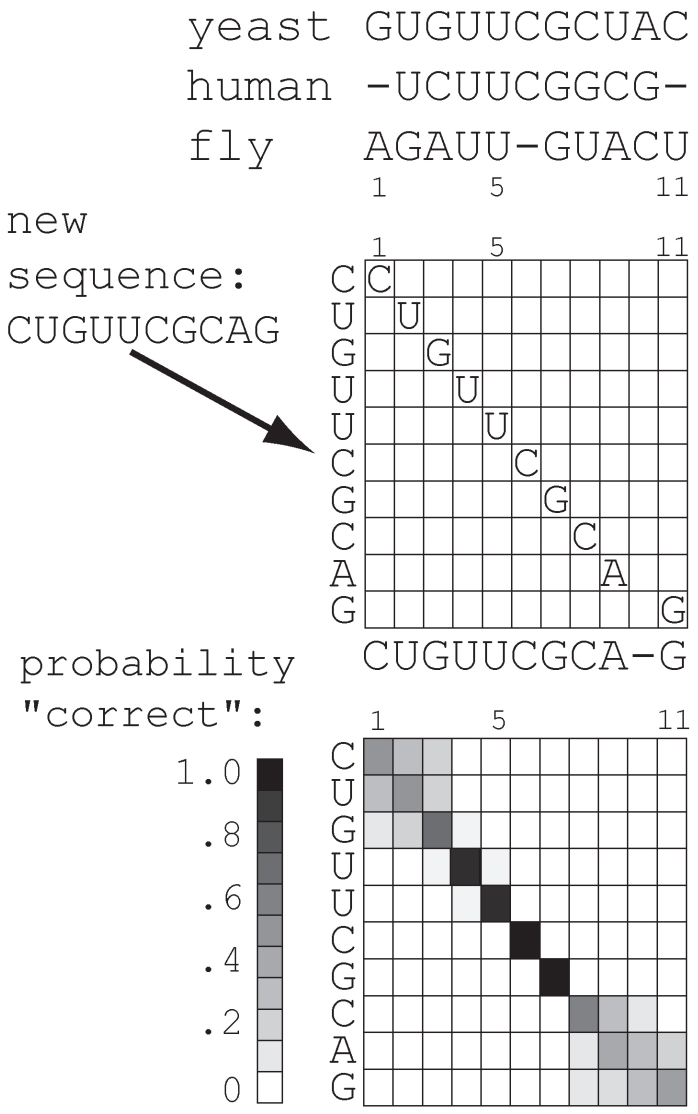


- Even if we filter out 99% of the database (for up to 100X acceleration), searches will still be too slow.

- CM step needs to be accelerated.

*Weinberg, Ruzzo, RECOMB, 243-251, 2004; Weinberg, Ruzzo, Bioinformatics, 22(1) 35-39 2006.

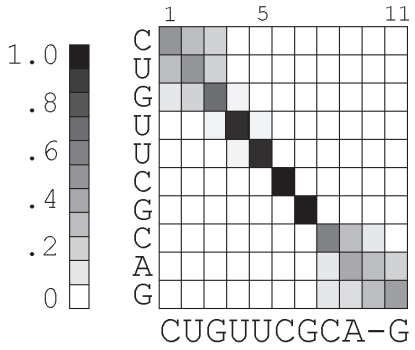
Accelerating CM alignment step 1: HMM posterior decoding to get confidence estimates

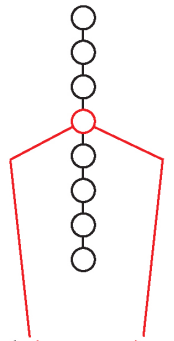


Accelerating CM alignment step 2: use HMM alignment confidence to constrain CM alignment*

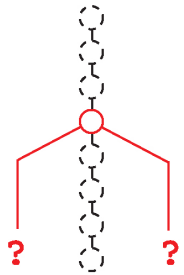
HMMs – Each column of seed alignment corresponds to a column of matrix.

```
yeast GUGUUCGCUAC
human -UCUUCGGCG-
fly   AGAUU-GUACU
```





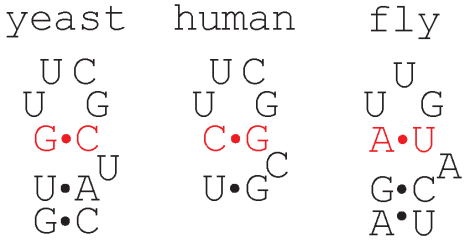
```
struct <<<<----->->>
yeast  GUGUUCGCUAC
human  -UCUUCGGCG-
fly    AGAUU-GUACU
```



CUGUUCGCAG

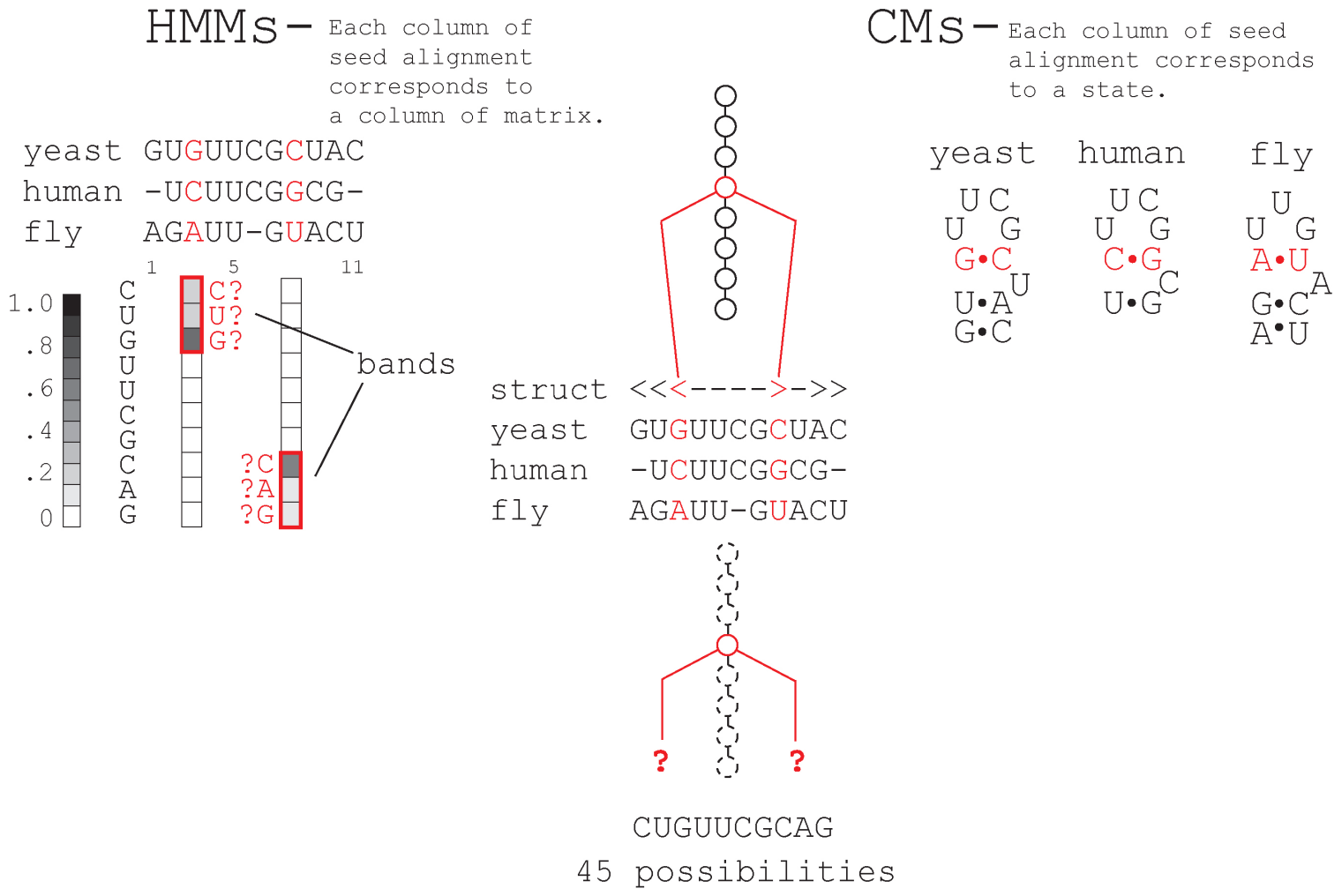
45 possibilities

CMs – Each column of seed alignment corresponds to a state.



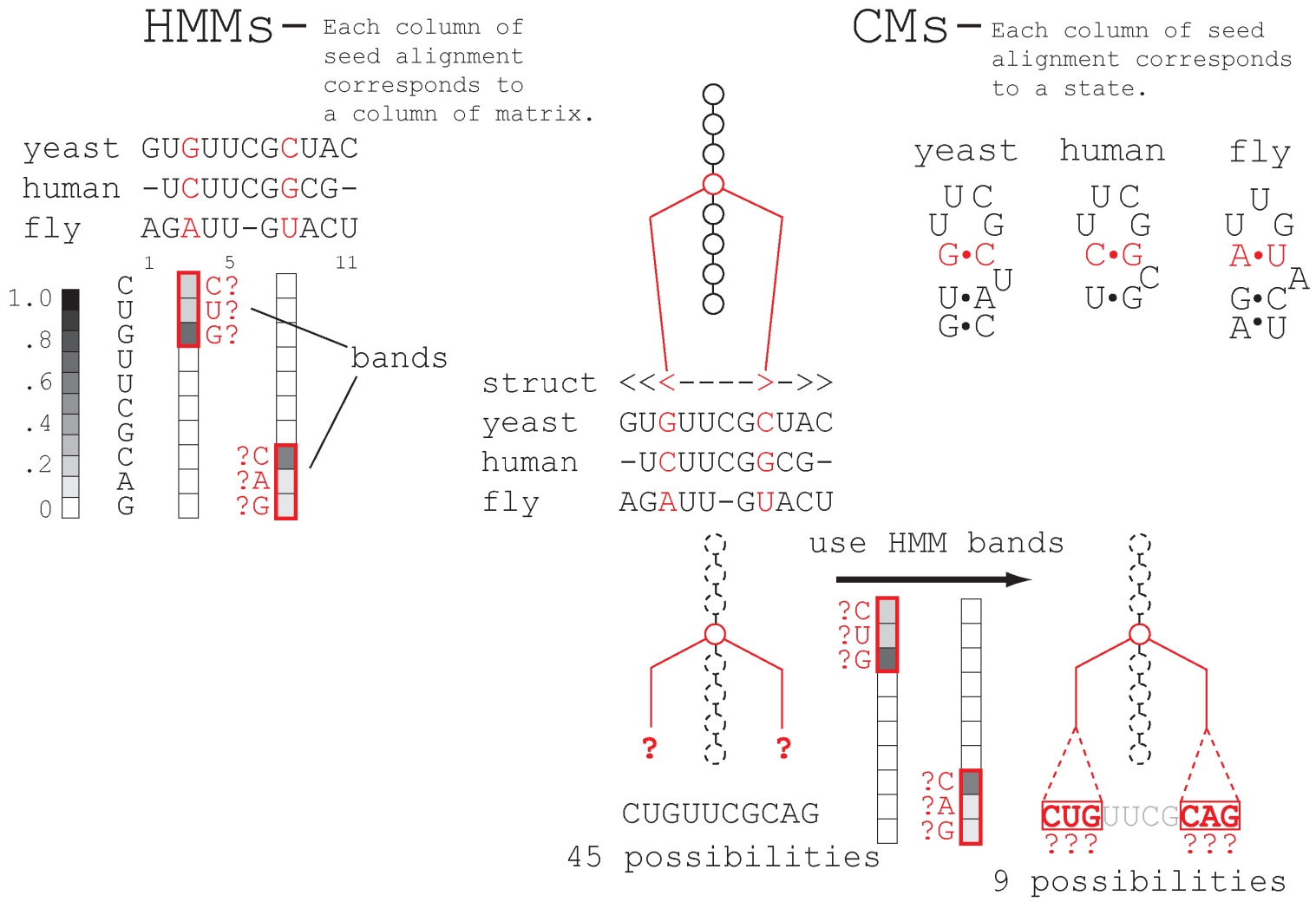
*M. P. Brown. Proc. Int. Conf. ISMB, 8:5766, 2000.

Accelerating CM alignment step 2: use HMM alignment confidence to constrain CM alignment*



*M. P. Brown. Proc. Int. Conf. ISMB, 8:5766, 2000.

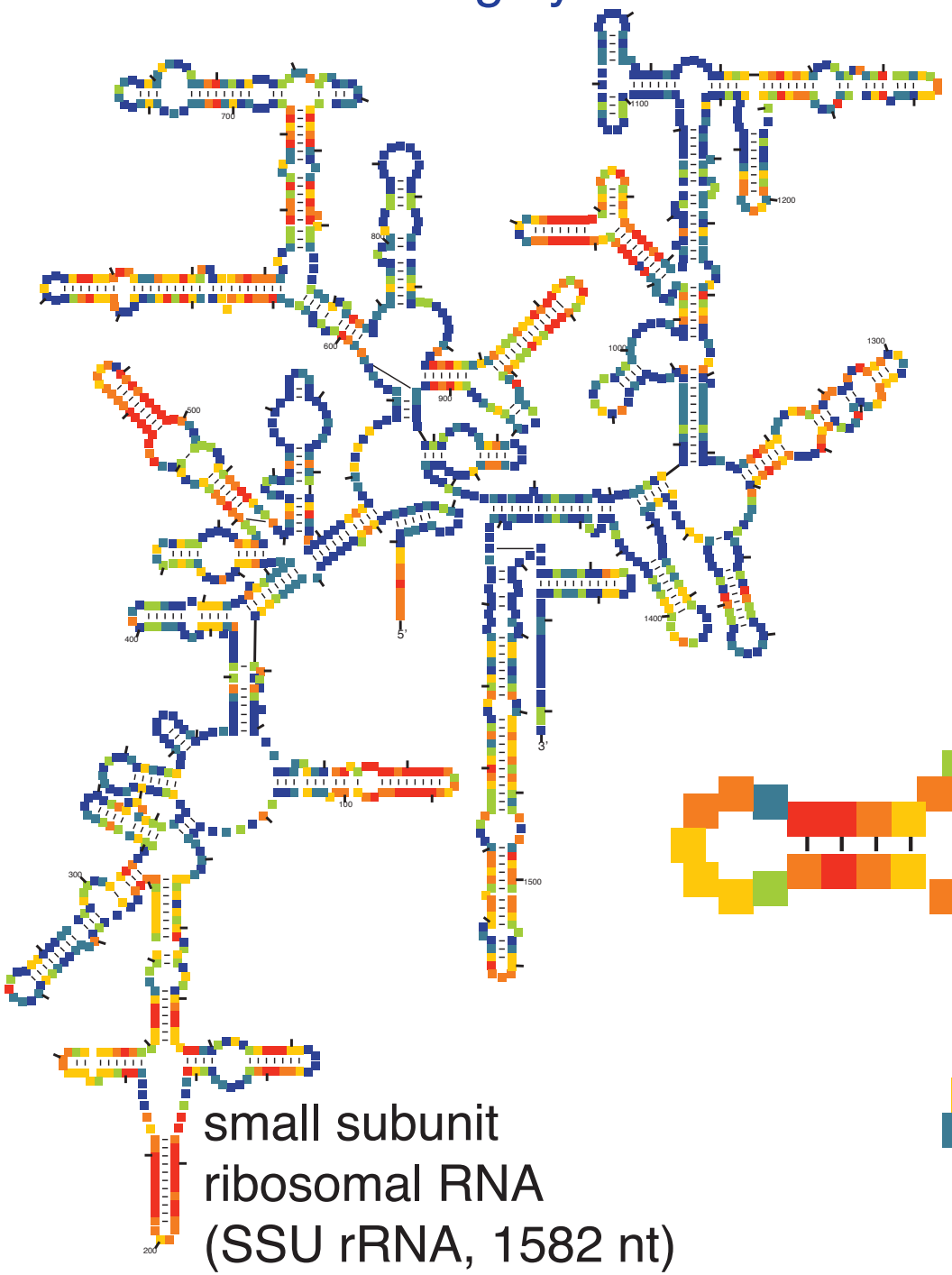
Accelerating CM alignment step 3: use HMM alignment confidence to constrain CM alignment*



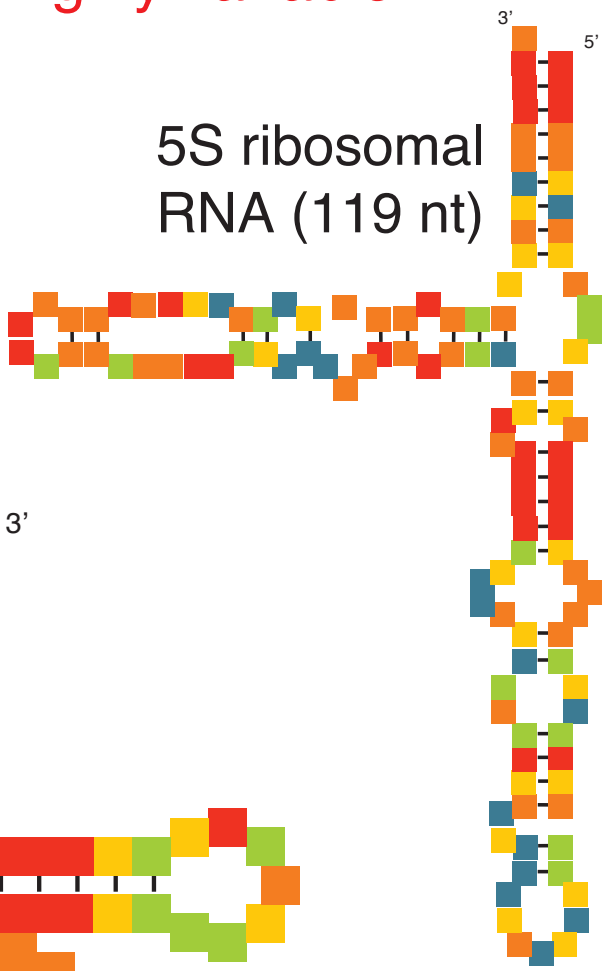
*M. P. Brown. Proc. Int. Conf. ISMB, 8:5766, 2000.

Sequence conservation per position

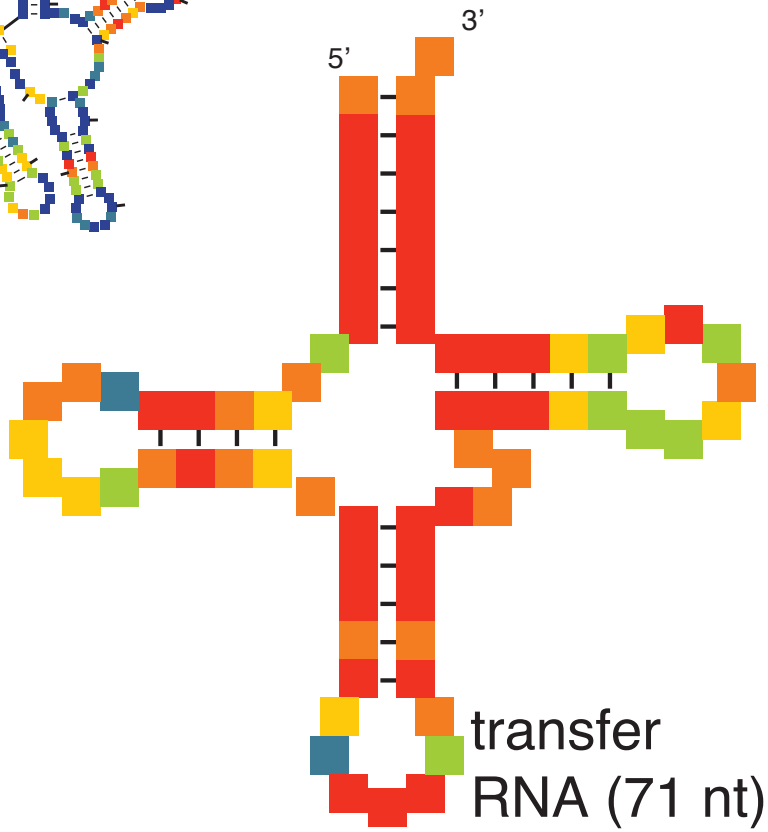
blue:highly conserved ..... red: highly variable



small subunit ribosomal RNA (SSU rRNA, 1582 nt)

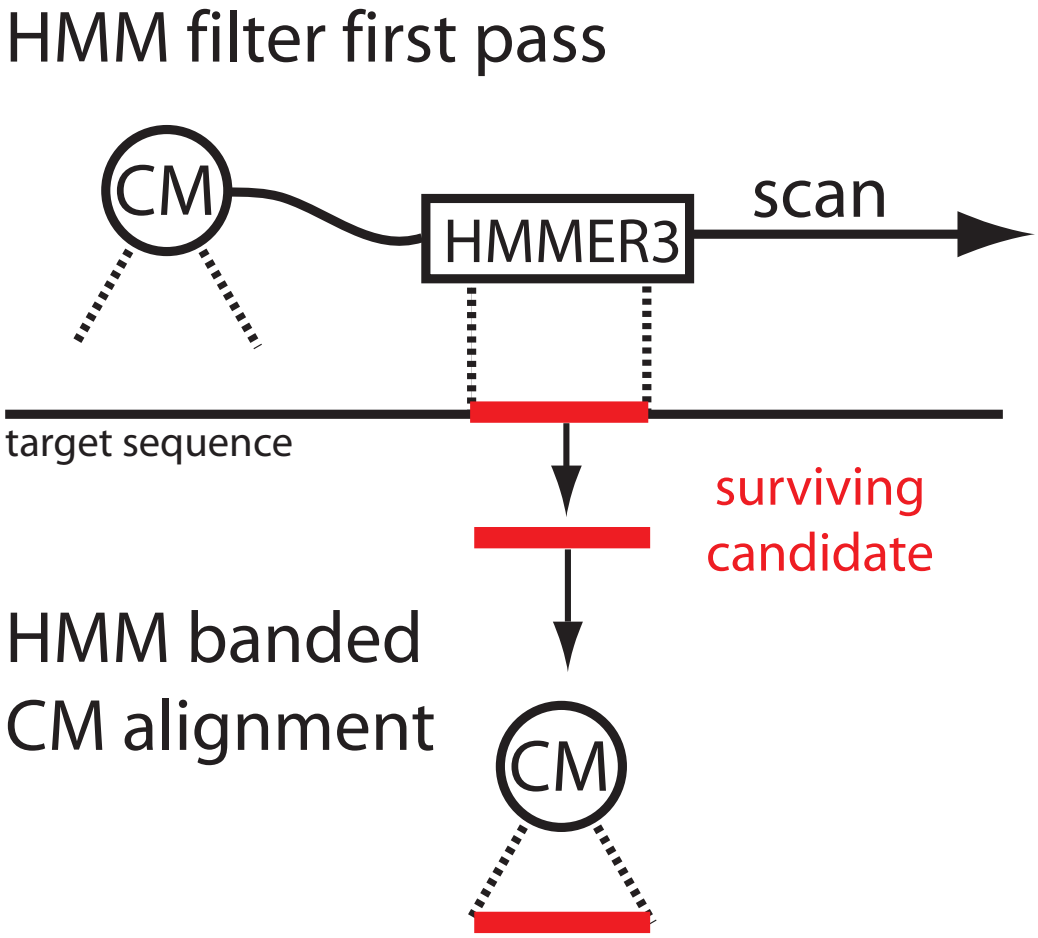


5S ribosomal RNA (119 nt)

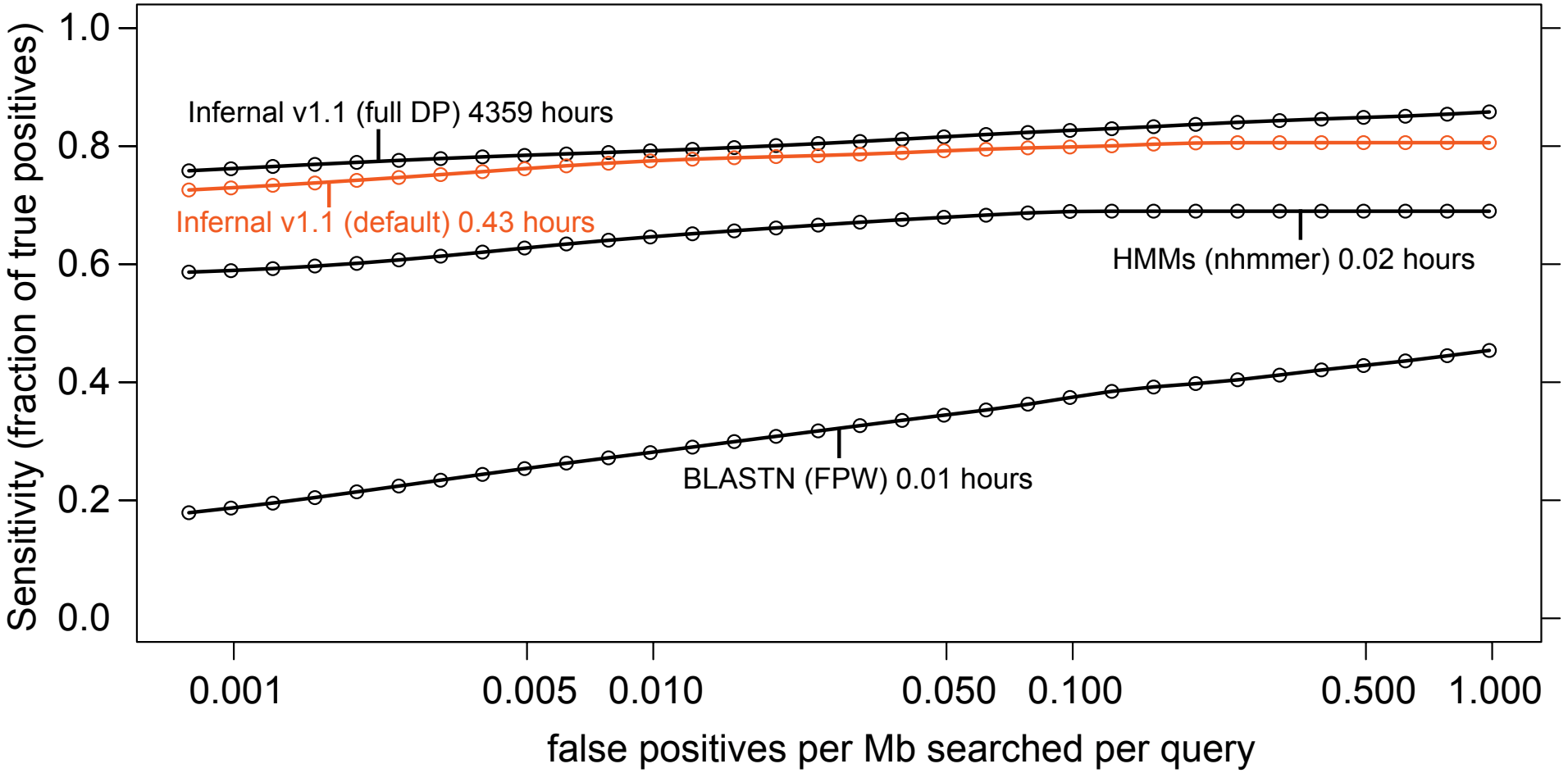


transfer RNA (71 nt)

Use HMMs as filters and to constrain CM alignment



HMM-based acceleration makes Infernal 10,000 times faster



Nawrocki EP, Eddy SR. Bioinformatics, 29:2487-2489, 2013.

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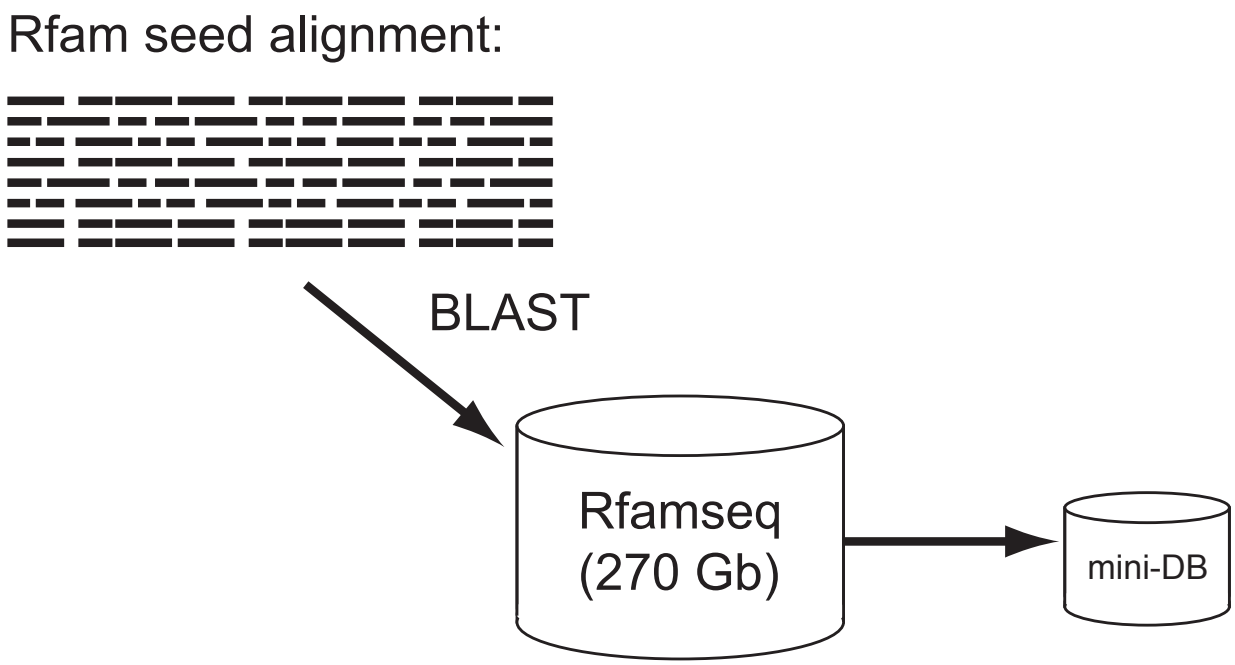
5. New features in latest version of Infernal

Rfam used BLAST filters from 2003 to 2012

- Rfam includes $>$ 2400 RNA families, each represented by an alignment, CM and set of predicted homologs in a large database (Rfamseq).



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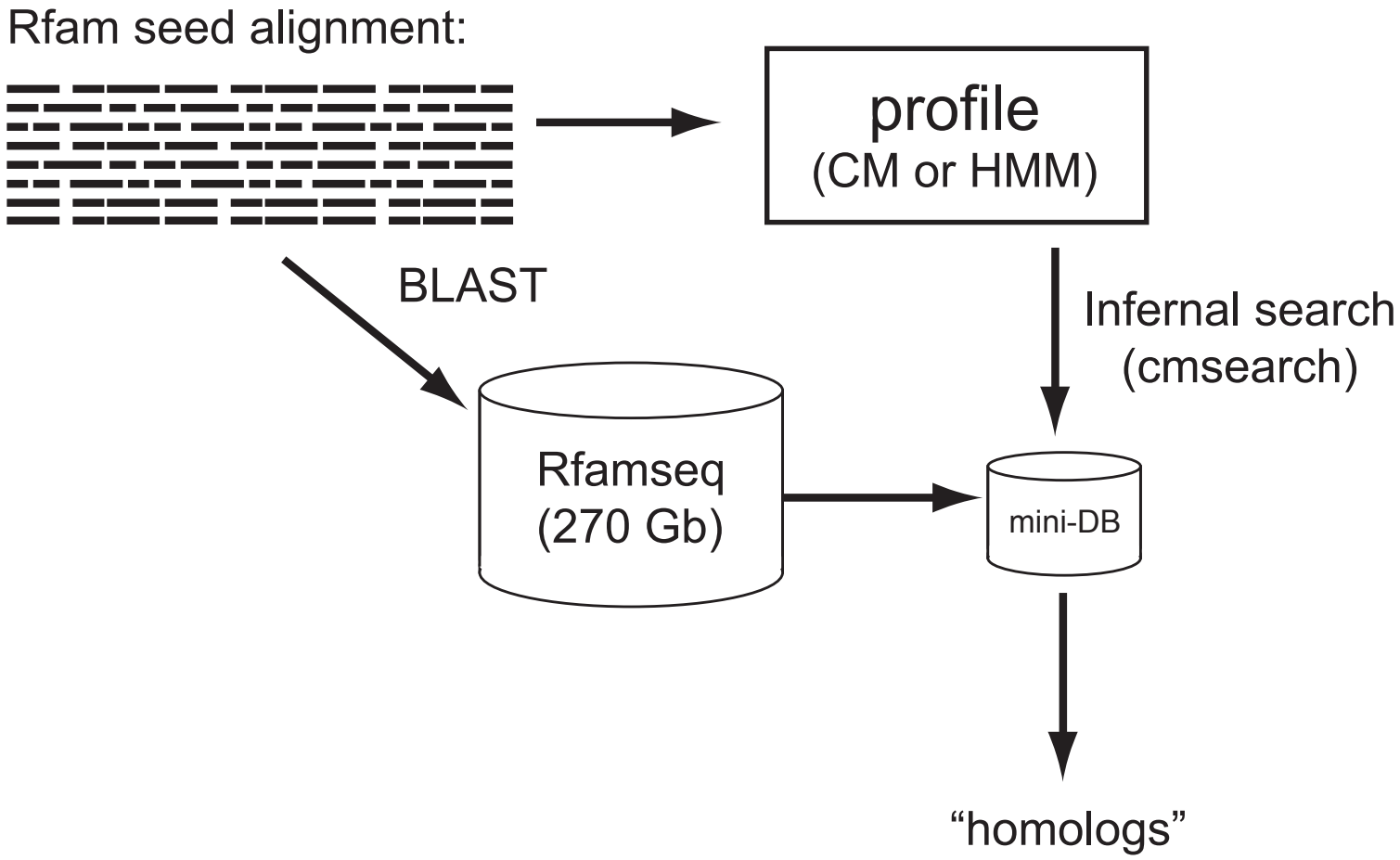
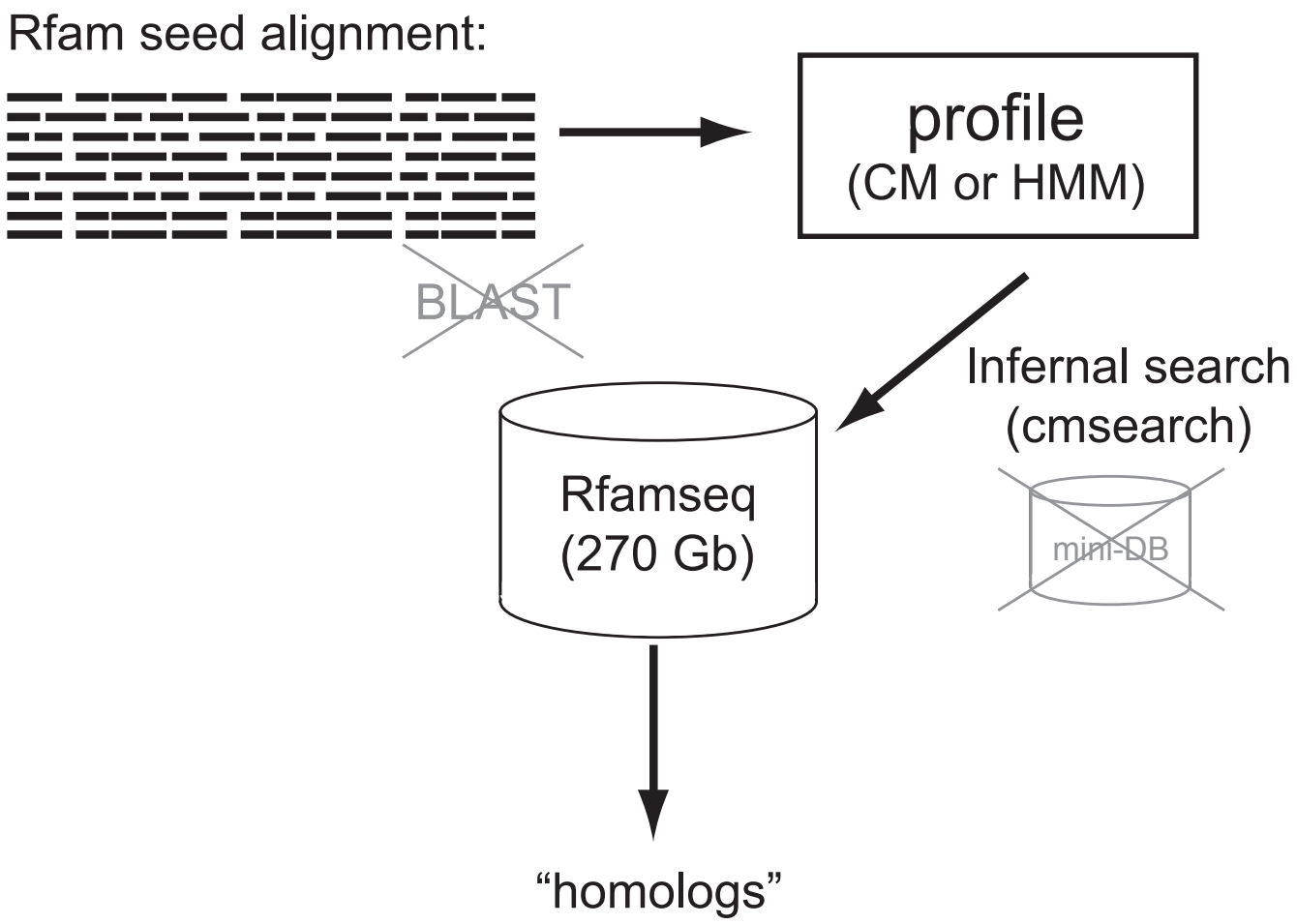


Rfam 12.0 (2014)*, first release without BLAST filtering



*Nawrocki, Burge et. al, NAR 43:D130-D137, 2015.

Rfam 12.0 (2014)*, first release without BLAST filtering

Search results against Rfamseq for 200 random families:

strategy	time (h)	# hits	# unique hits
Old (BLAST + Infernal 1.0)	4069.8	179,681	53
New (Infernal 1.1)	4222.2	201,814	22,312

*Nawrocki, Burge et. al, NAR 43:D130-D137, 2015.

Infernal 1.1 finds 11,000 new group I intron candidates

Table 1. Comparison of the old Rfam 11.0 BLAST and Infernal 1.0 search strategy versus the new Rfam 12.0 Infernal 1.1 search strategy for 15 of 200 randomly chosen families

Accession	Family ID	Length (nt)	#of seed seqs	Time new (h)	Time old (h)	Time (old/new)	New total hits	Old total hits	New unique hits	Old unique hits
Top five families										
RF00028	Intron_gpI	251	12	125.0	357.2	2.8	71 433	60 264	11 175	1
RF00026	U6	104	188	31.2	181.1	5.8	66 517	62 174	4367	14
RF00003	U1	166	100	11.6	64.0	5.5	15 770	14 867	904	1
RF00162	SAM	108	433	8.3	590.0	70.8	4905	4797	108	0
RF00050	FMN	140	144	17.1	169.9	23.9	4381	4306	76	1

Nawrocki, Burge et. al, NAR 43:D130-D137, 2015.

It is now easier to use Rfam/Infernal to annotate your own datasets.

- A bacterial genome takes about 30 minutes for all 2474 models.

Table 2. Summary statistics for Rfam-based annotation of RNAs in various genomes and metagenomics data sets

Genome/data set	Size (Mb)	# of hits	# of fams	CPU time (hours)	Mb/hour
Homo sapiens	3099.7	14 508	796	650	4.8
Sus scrofa (pig)	2808.5	6177	625	460	6.1
Drosophila melanogaster	168.7	4321	156	30	5.7
Caenorhabditis elegans	100.3	1022	175	20	5.2
Saccharomyces cerevisiae	12.2	376	96	1.7	7.3
Escherichia coli	4.6	256	112	0.46	10.2
Bacillus subtilis	4.1	211	52	0.57	7.2
Methanocaldococcus jannaschii	1.7	257	18	0.31	5.6
Aquifex aeolicus	1.6	52	7	0.22	7.3
Borrelia burgdorferi	0.9	44	7	0.22	4.1
Human immunodeficiency virus (HIV)	0.01	12	10	0.016	0.63
Human gut microbiome sample (sample ERS167139, 454 sequencing)	166.1	4342	54	22	7.7
Human gut microbiome sample (sample ERS235581, Illumina HiSeq sequencing) (28)	52.9	3159	47	8.5	6.2
Ocean metagenome (sample SRS580499, Illumina genome analyzer)	44.3	6692	59	13	3.5

Nawrocki, Burge et. al, NAR 43:D130-D137, 2015.

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Infernal version 1.1.2 (July 2016)

- First release after moving to NCBI (without Infernal as my primary project)

- Main difference is in the `cmscan` executable program
  - faster
  - annotates overlaps

cmsearch versus cmscan: a subtle but important difference

- cmsearch: hits reported by model
  - for each query CM
    - for each target sequence
      - search for high-scoring hits
- cmscan: hits reported by sequence
  - for each query sequence
    - for each target CM
      - search for high-scoring hits
- cmscan reads each CM for each sequence (all 2474 models)
- if sequences are short this makes reading CMs a bottleneck

cmsearch is faster than cmscan for datasets with many short sequences

Three sequence datasets:

1. *E. coli* genome: 4.6 Mb, 1 sequence
2. ERS167139 (human gut microbiome, 454): 166 Mb (avg 423nt) 393K sequences
3. ERS235581 (human gut microbiome, HiSeq): 53Mb (avg: 120nt), 440K sequences

	E.coli (4.6 mb, 1 seq)		ERS167139 (393K seqs, avg 423nt)		ERS235581 (440K seqs, avg 120nt)	
program	time	sec/seq	time	sec/seq	time	sec/seq
cmsearch v1.1.1	0.5h	1746.7	28.2h	0.26	9.8h	0.08
cmscan v1.1.1	0.5h	1678.6	37.3h	0.34	20.5h	0.17

cmscan v1.1.2 stores CM model parameters in memory instead of re-reading them for each sequence

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	E.coli (4.6 mb, 1 seq)		ERS167139 (393K seqs, avg 423nt)		ERS235581 (440K seqs, avg 120nt)	
program	time	sec/seq	time	sec/seq	time	sec/seq
cmsearch v1.1.1	0.5h	1746.7	28.2h	0.26	9.8h	0.08
cmscan v1.1.1	0.5h	1678.6	37.3h	0.34	20.5h	0.17
cmsearch v1.1.2	0.5h	1808.2	25.3h	0.23	8.3h	0.07
cmscan v1.1.2	0.5h	1735.0	26.7h	0.24	9.5h	0.08

Example of overlapping hits in cmscan v1.1.1 output

Infernal v1.1.1:

#target name	accession	query name	accession	mdl	mdl from	mdl to	seq from	seq to	strand	trunc	pass	gc	bias	score	E-value	inc	description of target
SSU_rRNA_bacteria	RF00177	G6NTHBW01DJ5GC	-	cm	963	1339	374	1	-	5'&3'	4	0.50	0.0	362.1	3.4e-114	!	-
SSU_rRNA_archaea	RF01959	G6NTHBW01DJ5GC	-	cm	919	1294	374	1	-	5'&3'	4	0.50	0.0	235.5	1.9e-67	!	-
SSU_rRNA_eukarya	RF01960	G6NTHBW01DJ5GC	-	cm	1218	1638	374	1	-	5'&3'	4	0.50	0.0	180.6	6.6e-52	!	-
SSU_rRNA_microsporidia	RF02542	G6NTHBW01DJ5GC	-	cm	841	1142	374	1	-	5'&3'	4	0.50	0.0	120.5	3.5e-34	!	-
LSU_rRNA_bacteria	RF02541	G6NTHBW01EMUQ5	-	cm	1	243	231	1	-	3'	3	0.49	0.0	189.0	2e-60	!	-
LSU_rRNA_archaea	RF02540	G6NTHBW01EMUQ5	-	cm	1	224	232	1	-	3'	3	0.49	0.0	155.5	2.7e-46	!	-
tRNA	RF00005	G6NTHBW01EMUQ5	-	cm	1	71	417	345	-	no	1	0.56	0.0	62.6	3.6e-13	!	-
5_8S_rRNA	RF00002	G6NTHBW01EMUQ5	-	cm	1	154	219	70	-	no	1	0.55	0.0	52.3	7.3e-12	!	-
LSU_rRNA_eukarya	RF02543	G6NTHBW01EMUQ5	-	cm	1	83	84	1	-	3'	3	0.42	0.0	49.3	2.2e-09	!	-
CRISPR-DR25	RF01338	G6NTHBW01DAB1L	-	cm	1	25	330	307	-	no	1	0.29	0.0	21.9	0.18	?	-
CRISPR-DR45	RF01354	G6NTHBW01DAB1L	-	cm	1	24	330	310	-	no	1	0.29	0.0	19.9	2	?	-
CRISPR-DR17	RF01328	G6NTHBW01DAB1L	-	cm	1	25	330	309	-	no	1	0.32	0.0	16.8	6.9	?	-

cmscan v1.1.2 marks up overlaps

Infernal v1.1.1:

#target name	accession	query name	accession	mdl	mdl from	mdl to	seq from	seq to	strand	trunc	pass	gc	bias	score	E-value	inc	description of target
SSU_rRNA_bacteria	RF00177	G6NTHBW01DJ5GC	-	cm	963	1339	374	1	-	5'&3'	4	0.50	0.0	362.1	3.4e-114	!	-
SSU_rRNA_archaea	RF01959	G6NTHBW01DJ5GC	-	cm	919	1294	374	1	-	5'&3'	4	0.50	0.0	235.5	1.9e-67	!	-
SSU_rRNA_eukarya	RF01960	G6NTHBW01DJ5GC	-	cm	1218	1638	374	1	-	5'&3'	4	0.50	0.0	180.6	6.6e-52	!	-
SSU_rRNA_microsporidia	RF02542	G6NTHBW01DJ5GC	-	cm	841	1142	374	1	-	5'&3'	4	0.50	0.0	120.5	3.5e-34	!	-
LSU_rRNA_bacteria	RF02541	G6NTHBW01EMUQ5	-	cm	1	243	231	1	-	3'	3	0.49	0.0	189.0	2e-60	!	-
LSU_rRNA_archaea	RF02540	G6NTHBW01EMUQ5	-	cm	1	224	232	1	-	3'	3	0.49	0.0	155.5	2.7e-46	!	-
tRNA	RF00005	G6NTHBW01EMUQ5	-	cm	1	71	417	345	-	no	1	0.56	0.0	62.6	3.6e-13	!	-
5_8S_rRNA	RF00002	G6NTHBW01EMUQ5	-	cm	1	154	219	70	-	no	1	0.55	0.0	52.3	7.3e-12	!	-
LSU_rRNA_eukarya	RF02543	G6NTHBW01EMUQ5	-	cm	1	83	84	1	-	3'	3	0.42	0.0	49.3	2.2e-09	!	-
CRISPR-DR25	RF01338	G6NTHBW01DAB1L	-	cm	1	25	330	307	-	no	1	0.29	0.0	21.9	0.18	?	-
CRISPR-DR45	RF01354	G6NTHBW01DAB1L	-	cm	1	24	330	310	-	no	1	0.29	0.0	19.9	2	?	-
CRISPR-DR17	RF01328	G6NTHBW01DAB1L	-	cm	1	25	330	309	-	no	1	0.32	0.0	16.8	6.9	?	-

Infernal v1.1.2:

#idx	target name	accession	query name	accession	clan name	mdl	mdl from	mdl to	seq from	seq to	strand	trunc	pass	gc	bias	score	E-value	inc	olp	anyidx	afrct1	afrct2
1	SSU_rRNA_bacteria	RF00177	G6NTHBW01DJ5GC	-	CL00111	cm	963	1339	374	1	-	5'&3'	4	0.50	0.0	362.1	3.4e-114	!	^	-	-	-
2	SSU_rRNA_archaea	RF01959	G6NTHBW01DJ5GC	-	CL00111	cm	919	1294	374	1	-	5'&3'	4	0.50	0.0	235.5	1.9e-67	!	=	1	1.000	1.000
3	SSU_rRNA_eukarya	RF01960	G6NTHBW01DJ5GC	-	CL00111	cm	1218	1638	374	1	-	5'&3'	4	0.50	0.0	180.6	6.6e-52	!	=	1	1.000	1.000
4	SSU_rRNA_microsporidia	RF02542	G6NTHBW01DJ5GC	-	CL00111	cm	841	1142	374	1	-	5'&3'	4	0.50	0.0	120.5	3.5e-34	!	=	1	1.000	1.000
1	LSU_rRNA_bacteria	RF02541	G6NTHBW01EMUQ5	-	CL00112	cm	1	243	231	1	-	3'	3	0.49	0.0	189.0	2e-60	!	^	-	-	-
2	LSU_rRNA_archaea	RF02540	G6NTHBW01EMUQ5	-	CL00112	cm	1	224	232	1	-	3'	3	0.49	0.0	155.5	2.7e-46	!	=	1	0.996	1.000
3	tRNA	RF00005	G6NTHBW01EMUQ5	-	CL00001	cm	1	71	417	345	-	no	1	0.56	0.0	62.6	3.6e-13	!	*	-	-	-
4	5_8S_rRNA	RF00002	G6NTHBW01EMUQ5	-	-	cm	1	154	219	70	-	no	1	0.55	0.0	52.3	7.3e-12	!	*	-	-	-
5	LSU_rRNA_eukarya	RF02543	G6NTHBW01EMUQ5	-	CL00112	cm	1	83	84	1	-	3'	3	0.42	0.0	49.3	2.2e-09	!	=	1	1.000	0.364
1	CRISPR-DR25	RF01338	G6NTHBW01DAB1L	-	CL00014	cm	1	25	330	307	-	no	1	0.29	0.0	21.9	0.18	?	^	-	-	-
2	CRISPR-DR45	RF01354	G6NTHBW01DAB1L	-	-	cm	1	24	330	310	-	no	1	0.29	0.0	19.9	2	?	*	-	-	-
3	CRISPR-DR17	RF01328	G6NTHBW01DAB1L	-	CL00014	cm	1	25	330	309	-	no	1	0.32	0.0	16.8	6.9	?	=	1	1.000	0.917

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Applications of CMs

- homology search/alignment: Infernal, COVE, Rfam[*], Alternal[†], RNATOPS[‡]
- RNA discovery: CMfinder[§], Zasha's pipeline(s)[¶]
- structure comparison: CMCompare[‖]
- family-specific programs:
  - tRNAscan-SE[**],
  - 16S/18S rRNA alignment: SSU-ALIGN[††]
  - bacterial terminator identification: RNIE[‡‡]

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