RNA Search and Motif Discovery

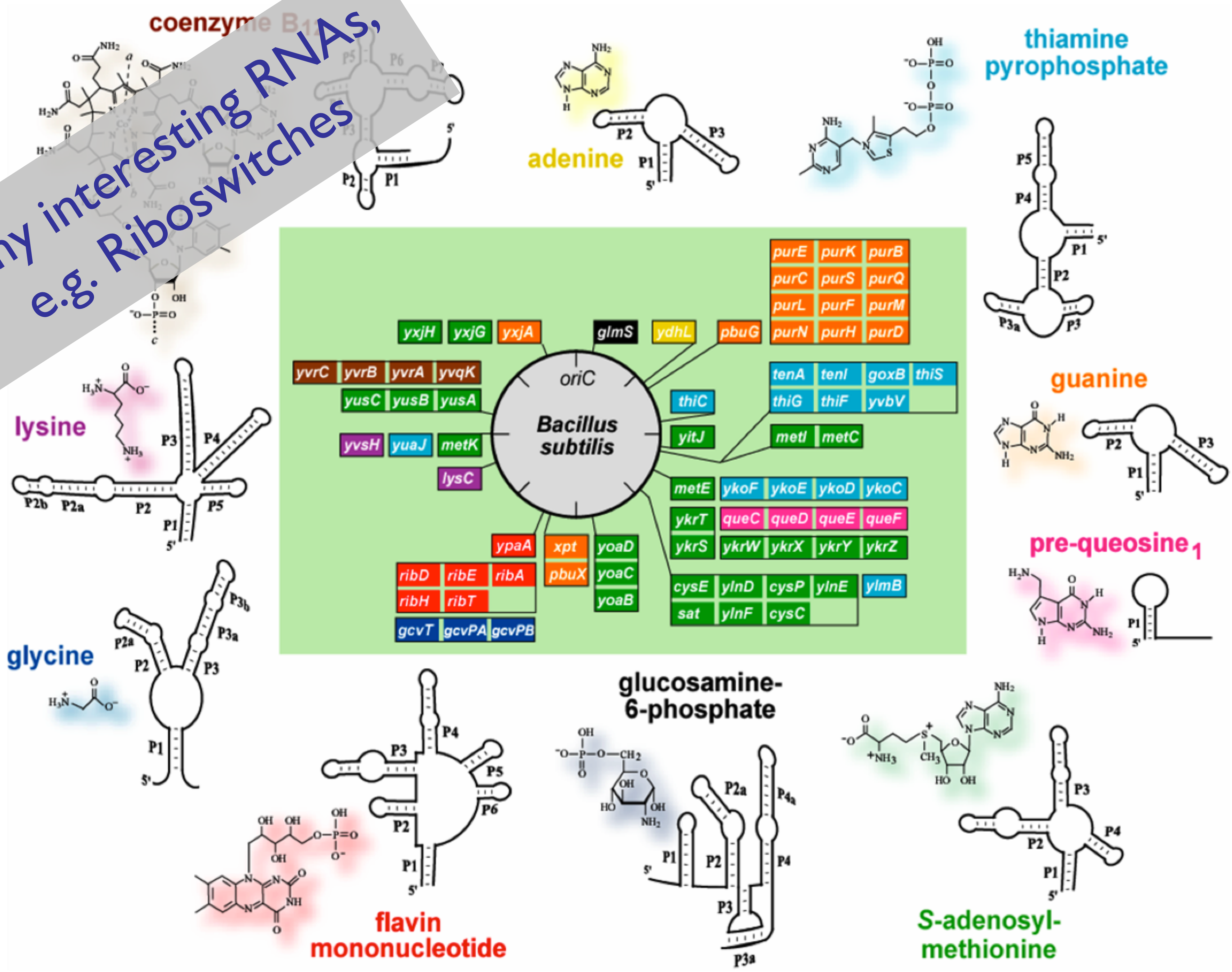
CSEP 527
Computational Biology

Previous Lecture

Many biologically interesting roles for RNA

RNA secondary structure prediction

Many interesting RNAs,
e.g. Riboswitches



Approaches to Structure Prediction

Maximum Pairing

+ works on single sequences
+ simple
- too inaccurate

Minimum Energy

+ works on single sequences
- ignores pseudoknots
- only finds “optimal” fold

Partition Function

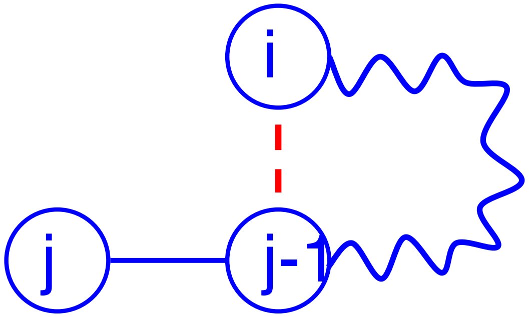
+ finds all folds
- ignores pseudoknots

“Optimal pairing of r_i … r_j”

Two possibilities

j Unpaired:

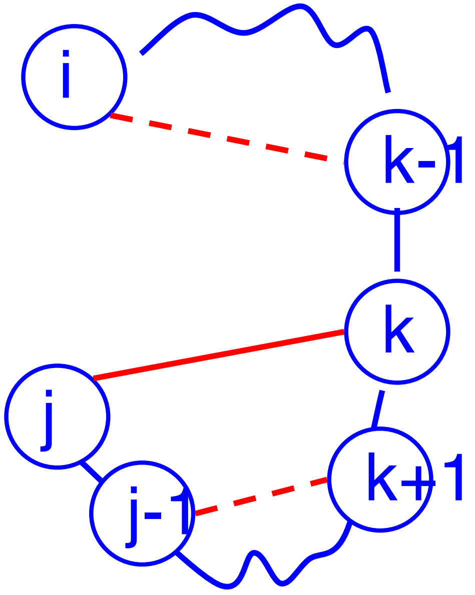
Find best pairing of r_i … r_{j-1}



j Paired (with some k):

Find best r_i … r_{k-1} +

best r_{k+1} … r_{j-1} plus 1

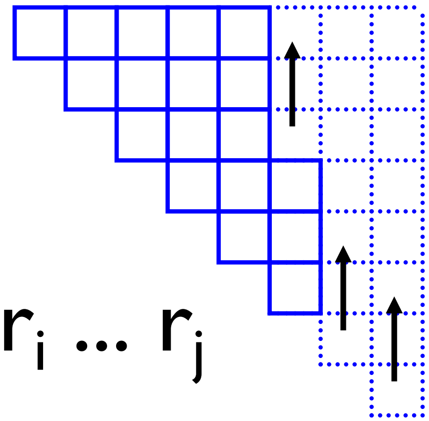


Why is it slow?

Why do pseudoknots matter?

Structure Prediction

Nussinov: A Computation Order



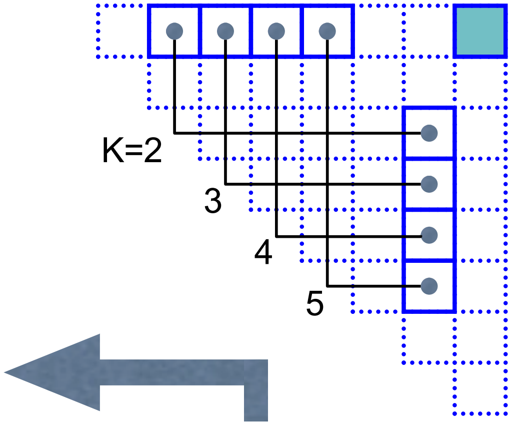
Or -energy

$B(i,j) = \boxed{\text{\# pairs}}$ in optimal pairing of $r_i \ldots r_j$

$B(i,j) = 0$ for all i, j with $i \geq j-4$; otherwise

$B(i,j) =$ max of:

$$\begin{cases} B(i,j-1) \\ \max\{\, B(i,k-1)+1+B(k+1,j-1) \mid \\ \quad i \leq k < j-4 \text{ and } r_k\text{-}r_j \text{ may pair}\} \end{cases}$$



Time: $O(n^3)$

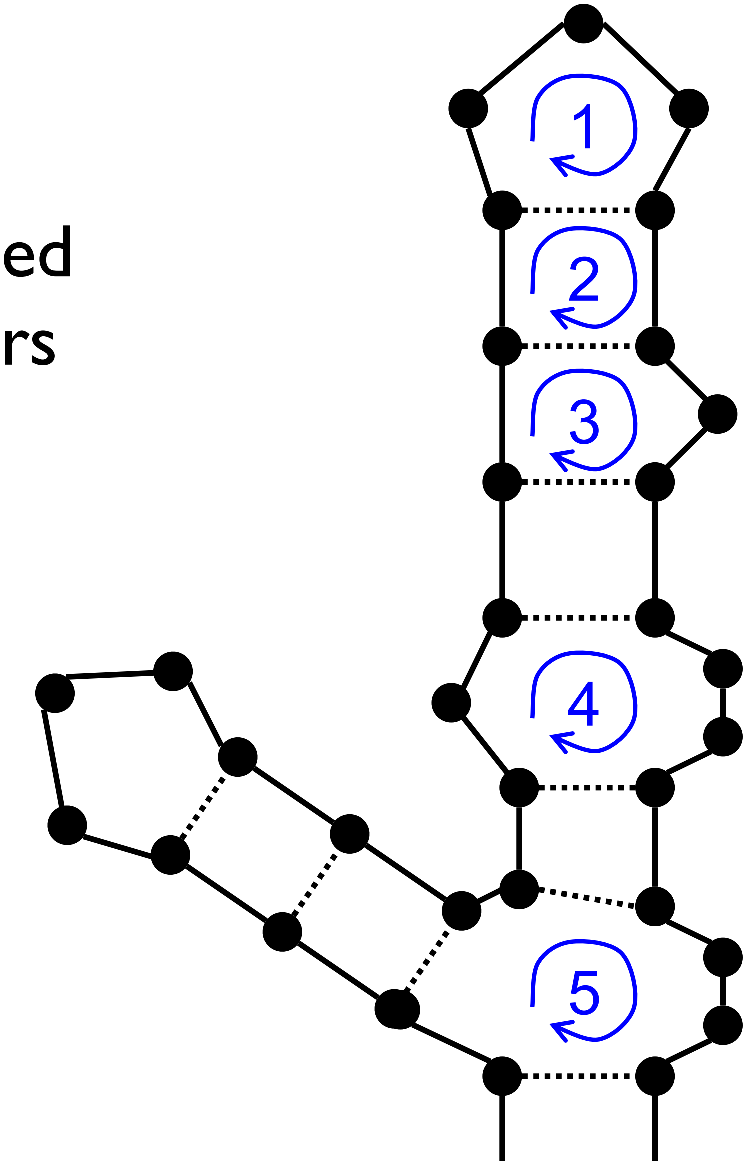
Loop-based energy version is better; recurrences similar, slightly messier

Loop Based Energy Minimization

Detailed experiments show it's more accurate to model based on *loops*, rather than just pairs

Loop types

1. Hairpin loop
2. Stack
3. Bulge
4. Interior loop
5. Multiloop



Single Seq Prediction Accuracy

Mfold, Vienna,... [Nussinov, Zuker, Hofacker, McCaskill]

Estimates suggest ~50-75% of base pairs predicted correctly in sequences of up to ~300nt

Definitely useful, but obviously imperfect

Today

Structure prediction via comparative analysis

Covariance Models (CMs) represent
RNA sequence/structure motifs

Fast CM search

Motif Discovery

Applications in prokaryotes & vertebrates

Structure Prediction

Approaches, II

Comparative sequence analysis

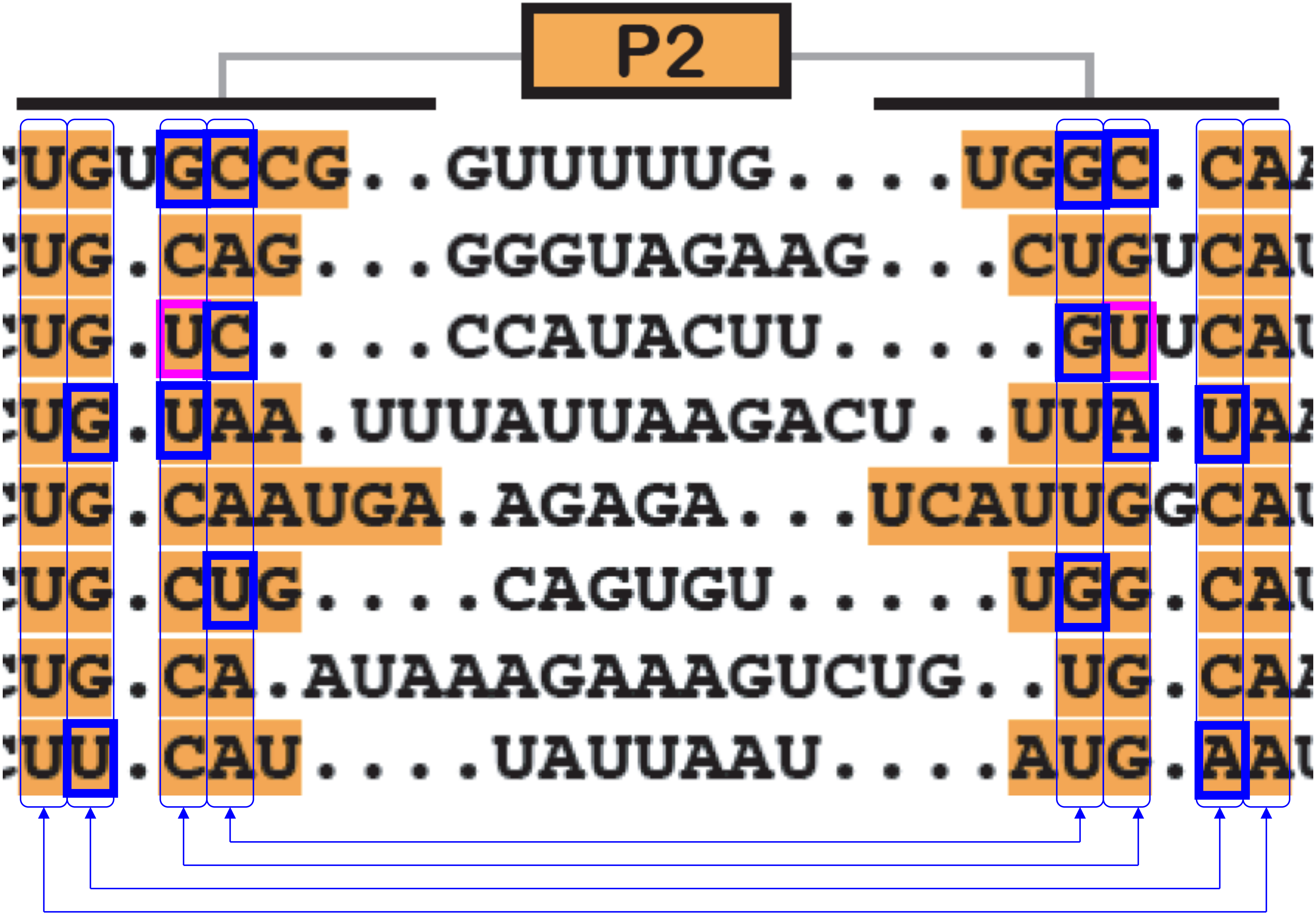
+ handles all pairings (potentially incl. pseudoknots)
- requires several (many?) aligned, appropriately diverged sequences

Stochastic Context-free Grammars

Roughly combines min energy & comparative, but no pseudoknots

Physical experiments (x-ray crystalography, NMR)

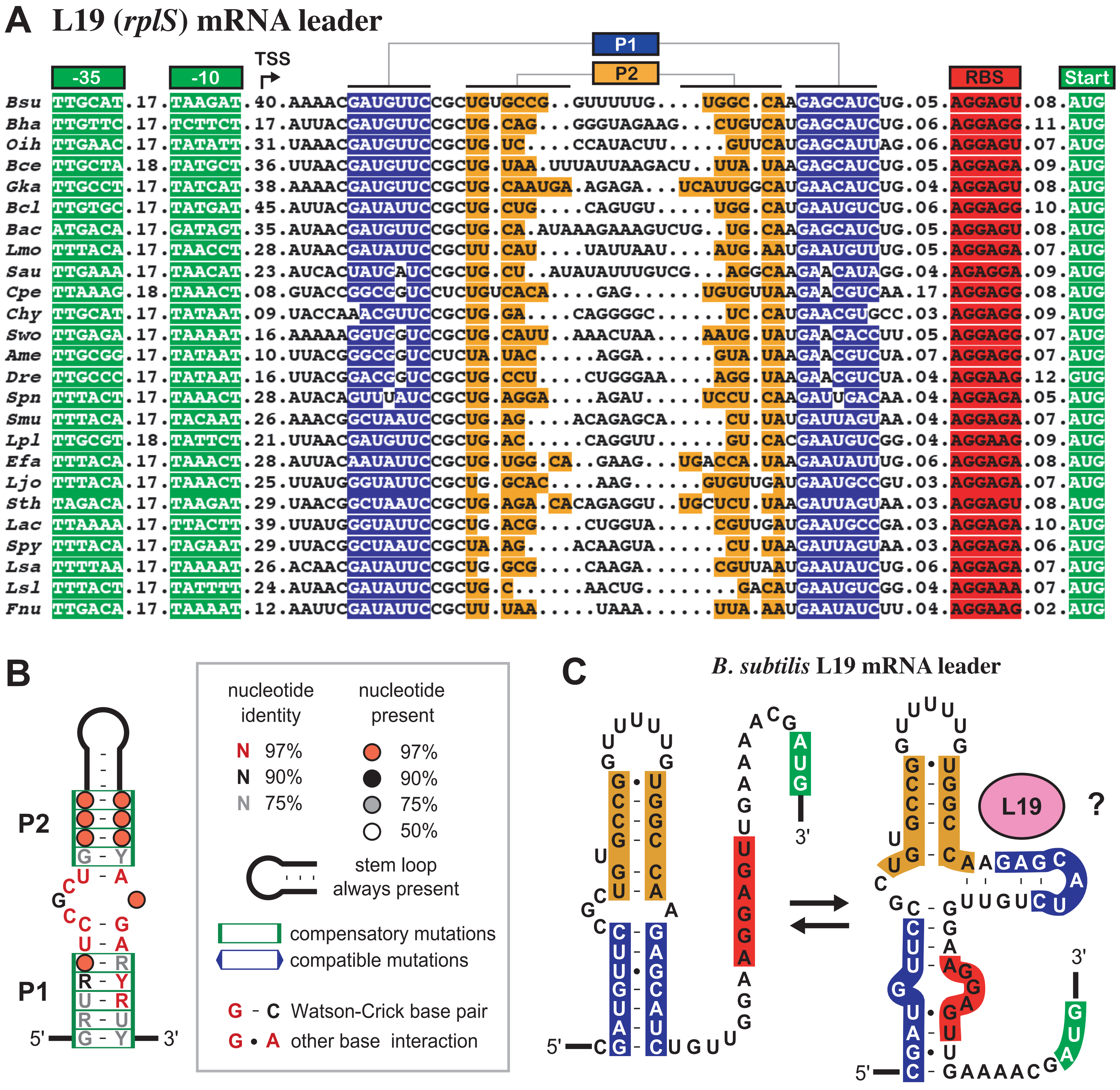
Today


P2
UGUGCCG..GUUUUUG....UGGC.CA
UG.CAG...GGGUAGAAG...CUGUCA
UG.UC....CCAUACUU.....GUUCA
UG.UAA.UUUAUUAAGACU..UUA.UA
UG.CAAUGA.AGAGA...UCAUUGGCA
UG.CUG....CAGUGU.....UGG.CA
UG.CA.AUAAAGAAAGUCUG..UG.CA
UU.CAU....UAUUAAU....AUG.AA

Covariation is strong evidence for base pairing

Example: Ribosomal Autoregulation:

Excess L19 represses L19 (RF00556; 555-559 similar)



A L19 (*rplS*) mRNA leader

```
Bsu TTGCAT.17.TAAGAT.40.AAAACGAUGUUCCGCUGUGCCG..GUUUUUG....UGGC.CAAGAGCAUCUG.05.AGGAGU.08.AUG
Bha TTGTTC.17.TCTTCT.17.AUUACGAUGUUCCGCUG.CAG...GGGUAGAAG...CUGUCAUGAGCAUCUG.06.AGGAGG.11.AUG
Oih TTGAAC.17.TATATT.31.UAAACGAUGUUCCGCUG.UC....CCAUACUU.....GUUCAUGAGCAUUAG.06.AGGAGU.07.AUG
Bce TTGCTA.18.TATGCT.36.UUAACGAUGUUCCGCUG.UAA.UUUAUUAAGACU..UUA.UAAGAGCAUCUG.05.AGGAGA.09.AUG
Gka TTGCCT.17.TATCAT.38.AAAACGAUGUUCCGCUG.CAAUGA.AGAGA...UCAUUGGCAUGAACAUCUG.04.AGGAGU.08.AUG
Bcl TTGTGC.17.TATGAT.45.AUUACGAUAUUCCGCUG.CUG....CAGUGU.....UGG.CAUGAAUGUCUG.06.AGGAGG.10.AUG
Bac ATGACA.17.GATAGT.35.AUAACGAUGUUCCGCUG.CA.AUAAAGAAAGUCUG..UG.CAAGAGCAUCUG.05.AGGAGU.08.AUG
Lmo TTTACA.17.TAACCT.28.AUAACGAUAUUCCGCUU.CAU....UAUUAAU....AUG.AAUGAAUGUUUG.05.AGGAGA.07.AUG
Sau TTGAAA.17.TAACAT.23.AUCACUAUGAUCCGCUG.CU..AUAUAUUUGUCG...AGGCAAGAACAUAGG.04.AGAGGA.09.AUG
Cpe TTAAAG.18.TAAACT.08.GUACCGGCGGUCCUCUGUCACA....GAG......UGUGUUAAGAACGUCAA.17.AGGAGG.08.AUG
Chy TTGCAT.17.TATAAT.09.UACCAAACGUUCCGCUG.GA....CAGGGGC......UC.CAUGAACGUGCC.03.AGGAGG.09.AUG
Swo TTGAGA.17.TAAAAT.16.AAAAAGGUGGUCCGCUG.CAUU..AAACUAA....AAUG.UAUGAACACCUU.05.AGGAGG.07.AUG
Ame TTGCGG.17.TATAAT.10.UUACGGGCGGUCCUCUA.UAC.....AGGA......GUA.UAAGAACGUCUA.07.AGGAGG.07.AUG
Dre TTGCCC.17.TATAAT.16.UUACGGACGGUCCGCUG.CCU....CUGGGAA....AGG.UAAGAACGUCUA.04.AGGAAG.12.GUG
Spn TTTACT.17.TAAACT.28.AUACAGUUUAUCCGCUG.AGGA....AGAU.....UCCU.CAAGAUUGACAA.04.AGGAGA.05.AUG
Smu TTTACA.17.TACAAT.26.AAACGGCUAAUCCGCUG.AG....ACAGAGCA.....CU.UAUGAUUAGUAA.04.AGGAGA.07.AUG
Lpl TTGCGT.18.TATTCT.21.UUAACGAUGUUCCGCUG.AC.....CAGGUU......GU.CACGAAUGUCGG.04.AGGAAG.09.AUG
Efa TTTACA.17.TAAACT.28.AUUACAAUAUUCCGCUG.UGG.CA..GAAG...UGACCA.UAAGAAUAUUUG.06.AGGAGA.08.AUG
Ljo TTTACA.17.TAAACT.25.UUAUGGGUAUUCCGCUG.GCAC....AAG......GUGUUGAUGAAUGCCGU.03.AGGAGA.07.AUG
Sth TAGACA.17.TAAGAT.29.UAACGGCUAAUCCGCUG.AGA.CACAGAGGU..UGCUCU.UAAGAUUAGUAA.03.AGGAGU.08.AUG
Lac TTAAAA.17.TTACTT.39.UUAUGGGUAUUCCGCUG.ACG....CUGGUA.....CGUUGAUGAAUGCCGA.03.AGGAGA.10.AUG
Spy TTTACA.17.TAGAAT.29.UUACGGCUAAUCCGCUA.AG....ACAAGUA......CU.UAAGAUUAGUAA.03.AGGAGA.06.AUG
Lsa TTTTAA.17.TAAAAT.26.ACAACGAUAUUCCGCUG.GCG....CAAGA......CGUUAAUGAAUAUCUG.06.AGGAGA.07.AUG
Lsl TTTACT.17.TATTTT.24.AUAACGAUAUUCCGCUG.C......AACUG........GACAUGAAUGUCGG.04.AGGAAA.07.AUG
Fnu TTGACA.17.TAAAAT.12.AAUUCGAUAUUCCGCUU.UAA.....UAAA......UUA.AAUGAAUAUCUU.04.AGGAAG.02.AUG
```

Mutual Information

x_k: letter from col *k*; f_{xk}: its freq in col *k*; $f_{xi,xj}$: pair freq

$$M_{ij} = \sum_{xi,xj} f_{xi,xj} \log_2 \frac{f_{xi,xj}}{f_{xi} f_{xj}}; \quad 0 \le M_{ij} \le 2 \quad \text{(4 letters} \Rightarrow \text{2 bits)}$$

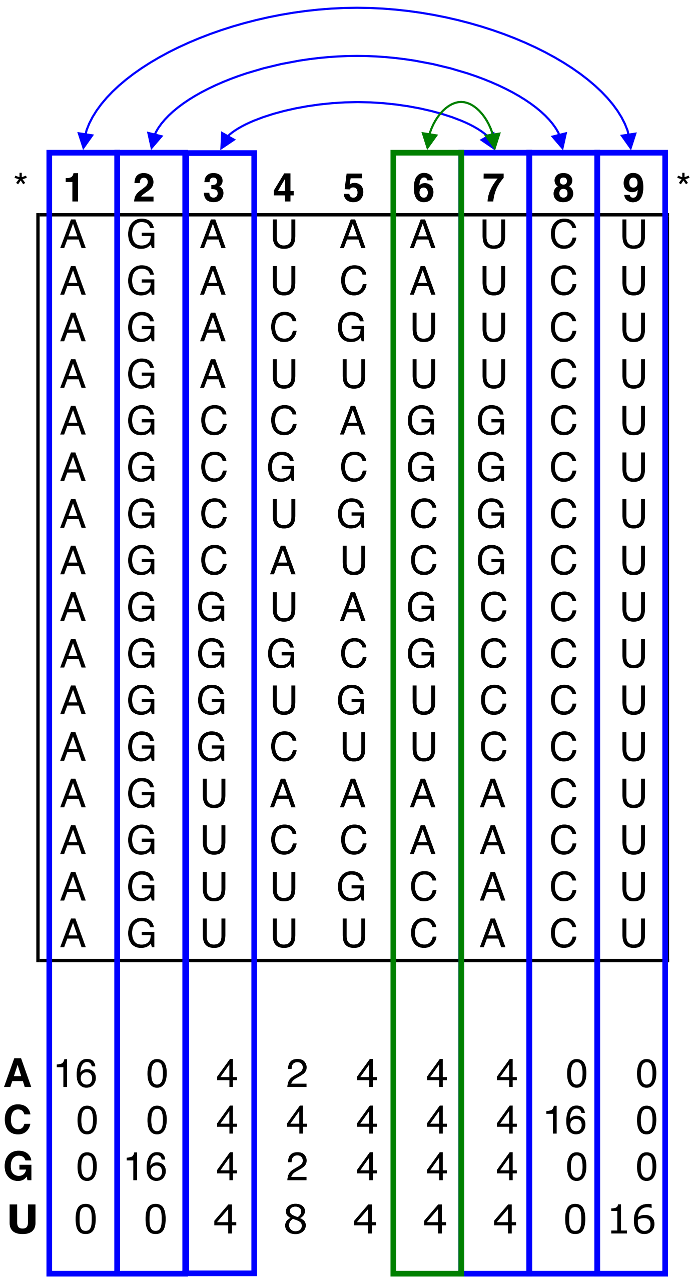
Max when *no* seq conservation but perfect pairing

$$\text{MI}=\begin{cases}\text{given letter in col } i\text{, what is mate in col } j\text{?}\\ \text{expected score gain from using a pair state (below)}\end{cases}$$

Finding optimal MI, (i.e., opt pairing of cols) is hard(?)

Finding optimal MI *without pseudoknots* can be done by dynamic programming

M.I. Example (Artificial)



*	1	2	3	4	5	6	7	8	9	*
	A	G	A	U	A	A	U	C	U	
	A	G	A	U	C	A	U	C	U	
	A	G	A	C	G	U	U	C	U	
	A	G	A	U	U	U	U	C	U	
	A	G	C	C	A	G	G	C	U	
	A	G	C	G	C	G	G	C	U	
	A	G	C	U	G	C	G	C	U	
	A	G	C	A	U	C	G	C	U	
	A	G	G	U	A	G	C	C	U	
	A	G	G	G	C	G	C	C	U	
	A	G	G	U	G	U	C	C	U	
	A	G	G	C	U	U	C	C	U	
	A	G	U	A	A	A	A	C	U	
	A	G	U	C	C	A	A	C	U	
	A	G	U	U	G	C	A	C	U	
	A	G	U	U	U	C	A	C	U	

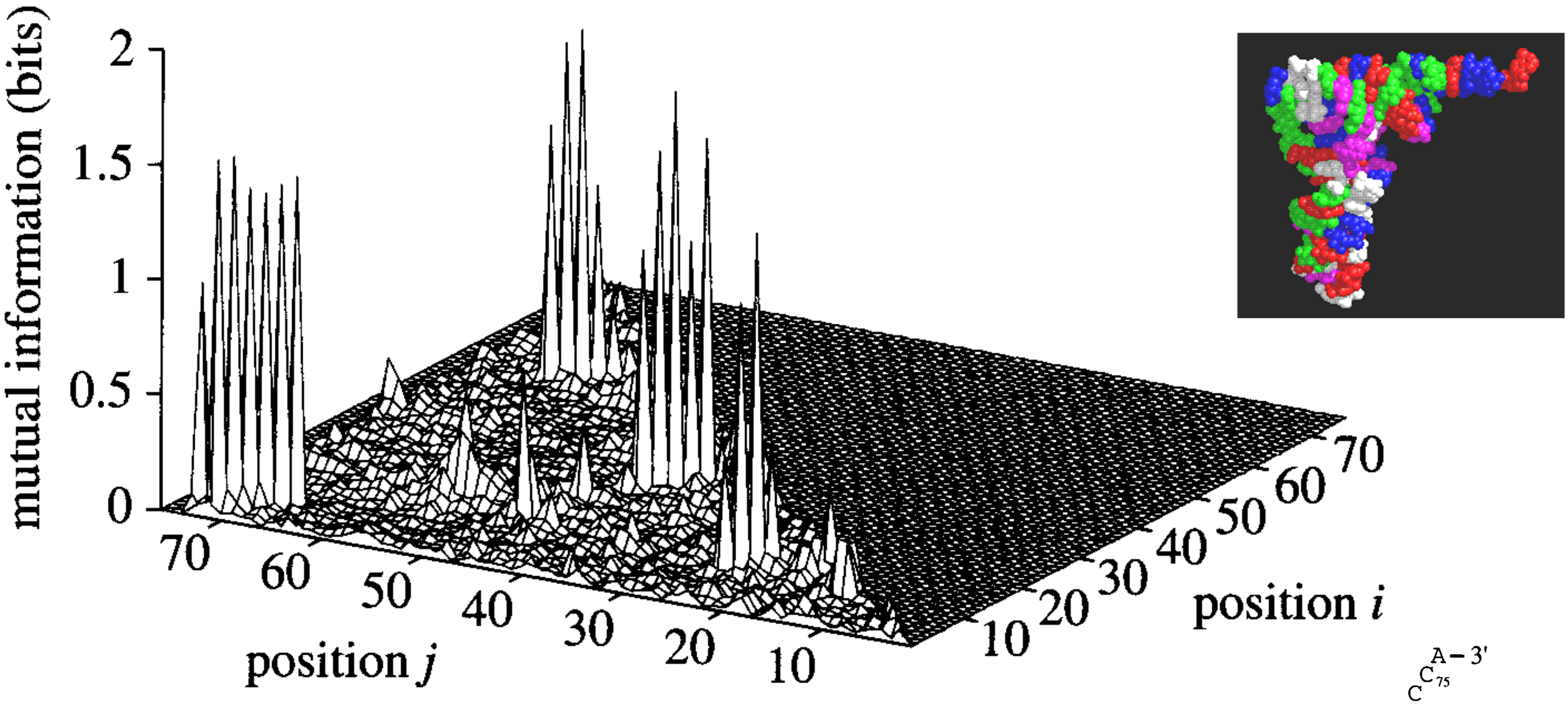
	1	2	3	4	5	6	7	8	9
A	16	0	4	2	4	4	4	0	0
C	0	0	4	4	4	4	4	16	0
G	0	16	4	2	4	4	4	0	0
U	0	0	4	8	4	4	4	0	16

MI:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
9	0	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0		
7	0	0	**2**	**0.30**	**0**	**1**			
6	0	0	**1**	**0.55**	**1**				
5	0	0	**0**	**0.42**					
4	0	0	**0.30**						
3	0	0							
2	0								
1									

Cols 1 & 9, 2 & 8: perfect conservation & *might* be base-paired, but unclear whether they are. M.I. = 0

Cols 3 & 7: *No* conservation, but always W-C pairs, so seems likely they do base-pair. M.I. = 2 bits.

Cols 7->6: unconserved, but each letter in 7 has only 2 possible mates in 6. M.I. = 1 bit.



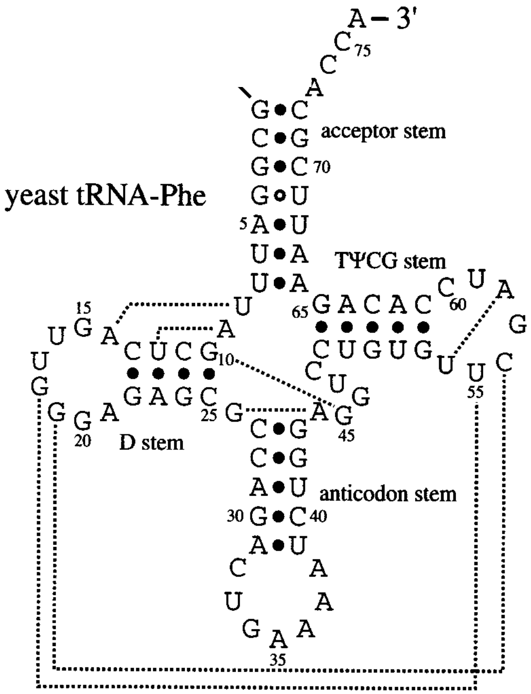


Figure 10.6 *A mutual information plot of a tRNA alignment (top) shows four strong diagonals of covarying positions, corresponding to the four stems of the tRNA cloverleaf structure (bottom; the secondary structure of yeast phenylalanine tRNA is shown). Dashed lines indicate some of the additional tertiary contacts observed in the yeast tRNA-Phe crystal structure. Some of these tertiary contacts produce correlated pairs which can be seen weakly in the mutual information plot.*

MI-Based Structure-Learning

Problem: Find best (max total MI) pseudo-knot-free subset of column pairs among i…j.

Solution: "Just like Nussinov/Zucker folding"

$$S_{i,j} = \max \begin{cases} S_{i,j-1} & \text{j unpaired} \\ \max_{i \le k < j-4} S_{i,k-1} + M_{k,j} + S_{k+1,j-1} & \text{j paired} \end{cases}$$

BUT, need the right data—enough sequences at the right phylogenetic distance

Computational Problems

~~How to predict secondary structure~~

How to model an RNA “motif”
(I.e., sequence/structure pattern)

Given a motif, how to search for instances

Given (unaligned) sequences, find motifs

How to score discovered motifs

How to leverage prior knowledge

Motif Description

RNA Motif Models

"Covariance Models" (Eddy & Durbin 1994)

aka profile stochastic context-free grammars (Sakakibara 94)

aka hidden Markov models on steroids

Model position-specific nucleotide preferences *and* base-pair preferences

Pro: accurate

Con: model building hard, search slow

Eddy & Durbin 1994: What

A probabilistic model for RNA families

- The “Covariance Model”
- ≈ A Stochastic Context-Free Grammar
- A generalization of a profile HMM

Algorithms for Training

- From aligned or unaligned sequences
- Automates “comparative analysis”
- Complements Nusinov/Zucker RNA folding

Algorithms for searching

Main Results

Very accurate search for tRNA

(Precursor to tRNAscanSE – a very good tRNA-finder)

Given sufficient data, model construction comparable to, but not quite as good as, human experts

Some quantitative info on importance of pseudoknots and other tertiary features

Probabilistic Model Search

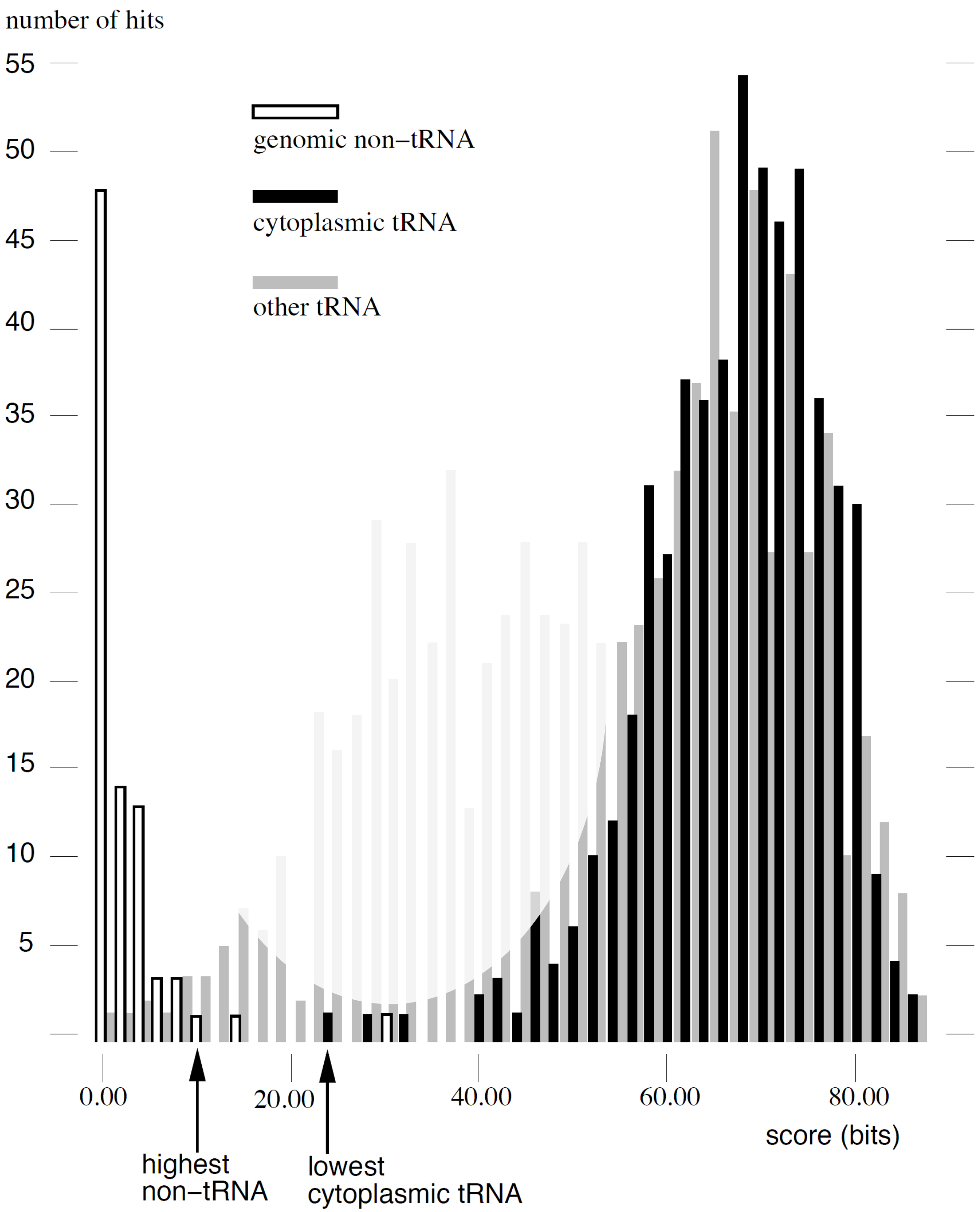
As with HMMs, given a sequence:

- You calculate likelihood ratio that the model could generate the sequence, vs a background model
- You set a score threshold
- Anything above threshold → a "hit"

Scoring:

- "Forward" / "Inside" algorithm - sum over all paths
- Viterbi approximation - find single best path
  (Bonus: alignment & structure prediction)

Example: searching for tRNAs



Recall

Profile Hmm Structure

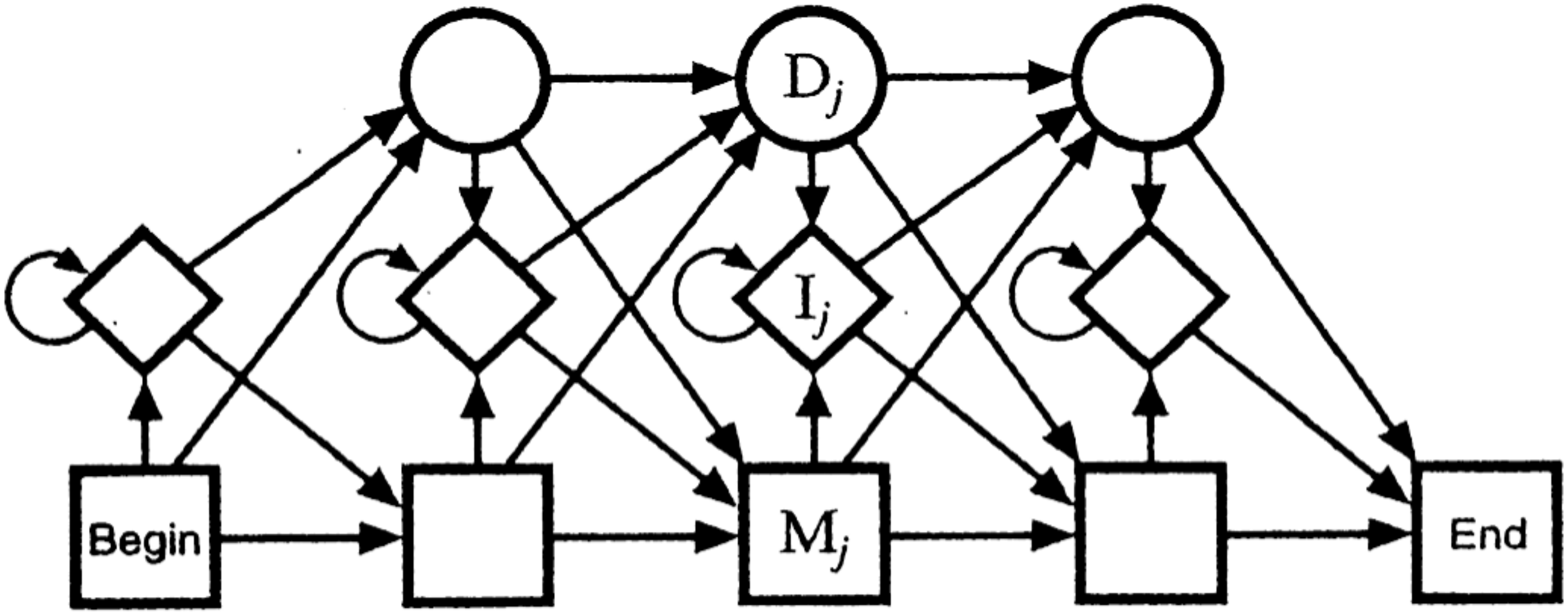


Figure 5.2 *The transition structure of a profile HMM.*

Mj: Match states (20 emission probabilities)

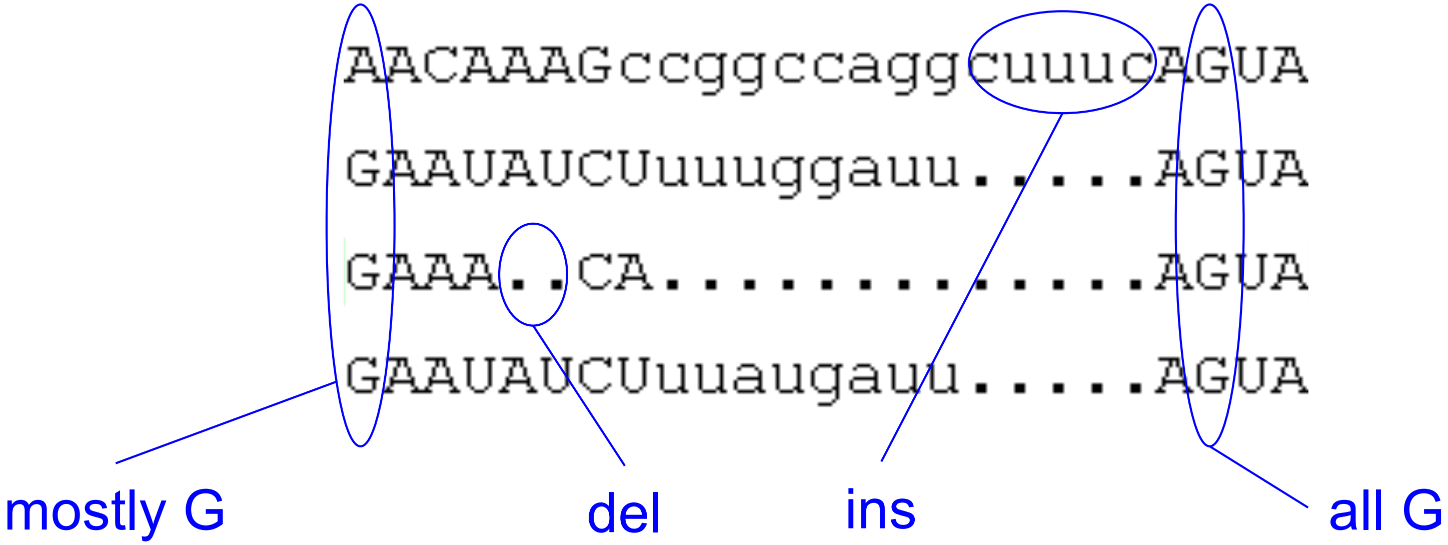
Ij: Insert states (Background emission probabilities)

Dj: Delete states (silent - no emission)

How to model an RNA “Motif”?

Conceptually, start with a profile HMM:

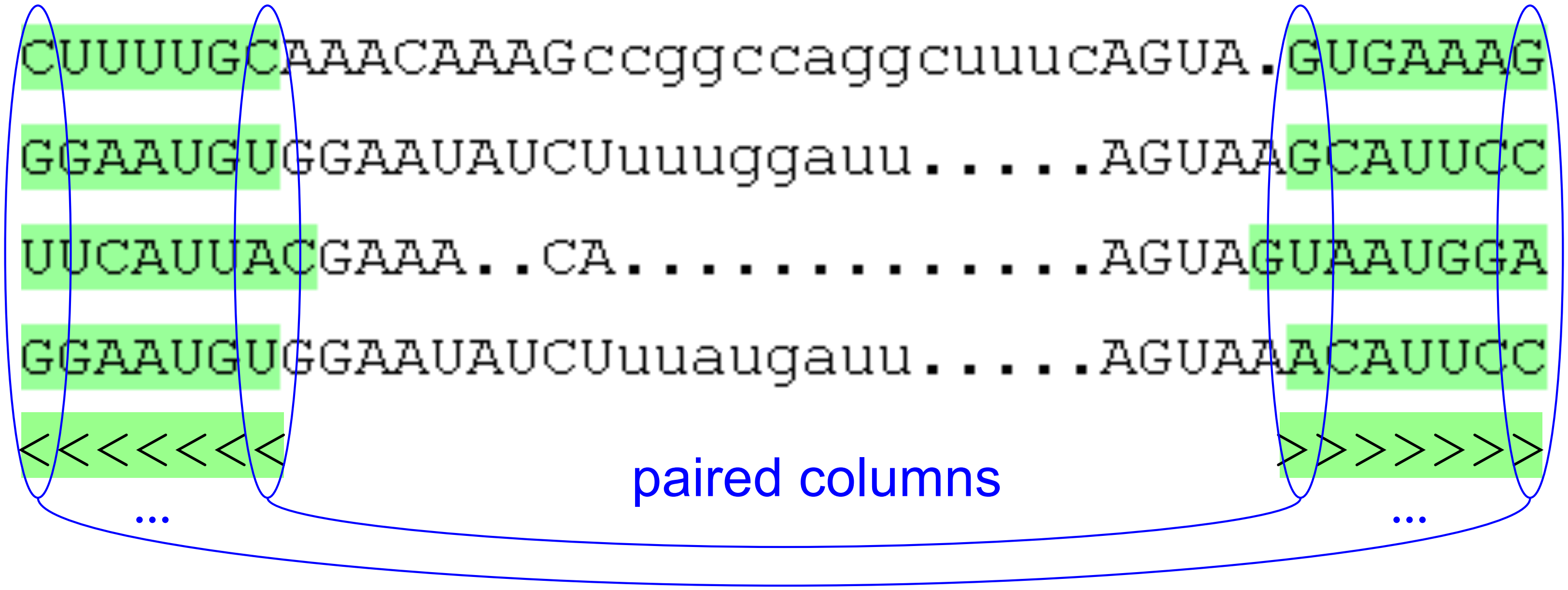
- from a multiple alignment, estimate nucleotide/ insert/delete preferences for each position
- given a new seq, estimate likelihood that it could be generated by the model, & align it to the model



```
AACAAAGccggccaggcuuucAGUA
GAAUAUCUuuuggauu.....AGUA
GAAA..CA.............AGUA
GAAUAUCUuuaugauu.....AGUA
```

How to model an RNA “Motif”?

Add “column pairs” and pair emission probabilities for base-paired regions



```
CUUUUGCAAACAAAGccggccaggcuuucAGUA.GUGAAAG
GGAAUGUGGAAUAUCUuuuggauu.....AGUAAGCAUUCC
UUCAUUACGAAA..CA.............AGUAGUAAUGGA
GGAAUGUGGAAUAUCUuuaugauu.....AGUAAACAUUCC
<<<<<<<                           >>>>>>>
```

Profile Hmm Structure

Does not handle “paired columns” above

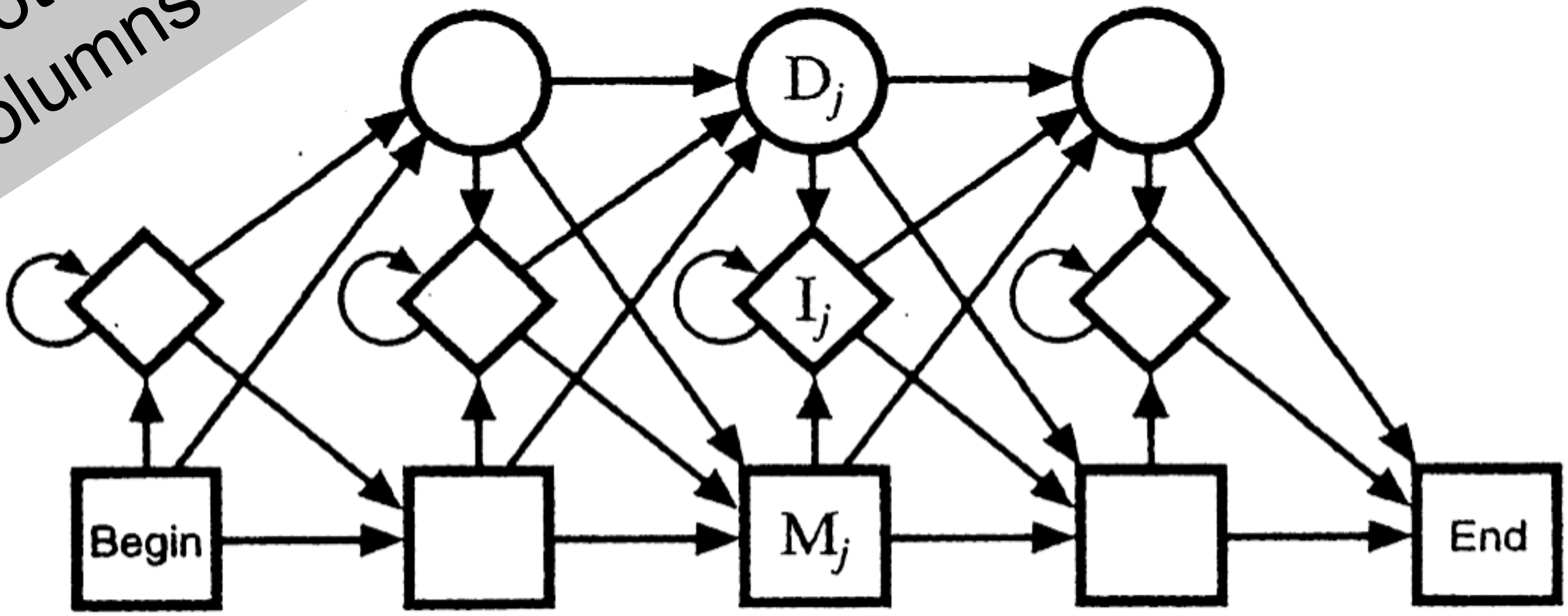


Figure 5.2 *The transition structure of a profile HMM.*

M$_j$: Match states (20 emission probabilities)

I$_j$: Insert states (Background emission probabilities)

D$_j$: Delete states (silent - no emission)

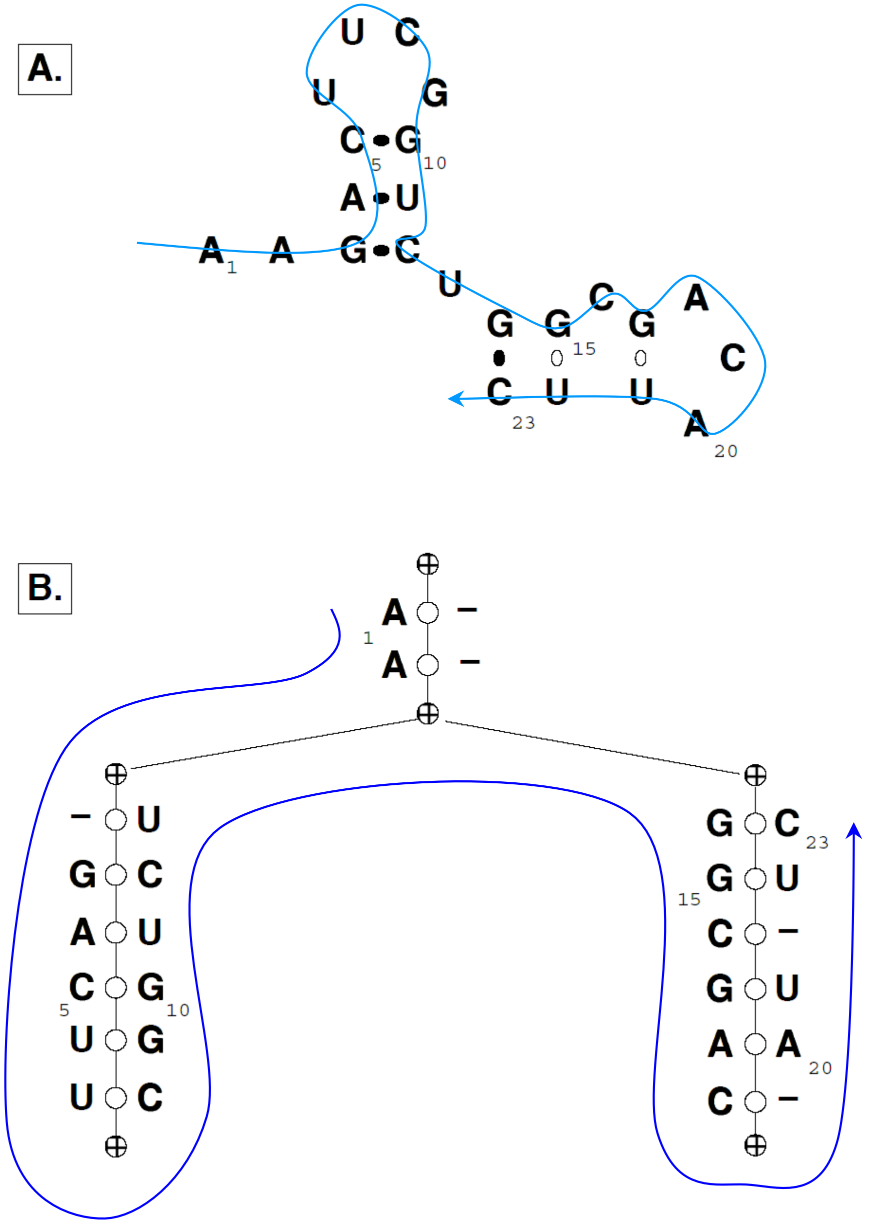
CM Structure

A: Sequence + structure

B: the CM “guide tree”

C: probabilities of letters/ pairs & of indels

Think of each branch being an HMM emitting *both* sides of a helix (but 3’ side emitted in reverse order)

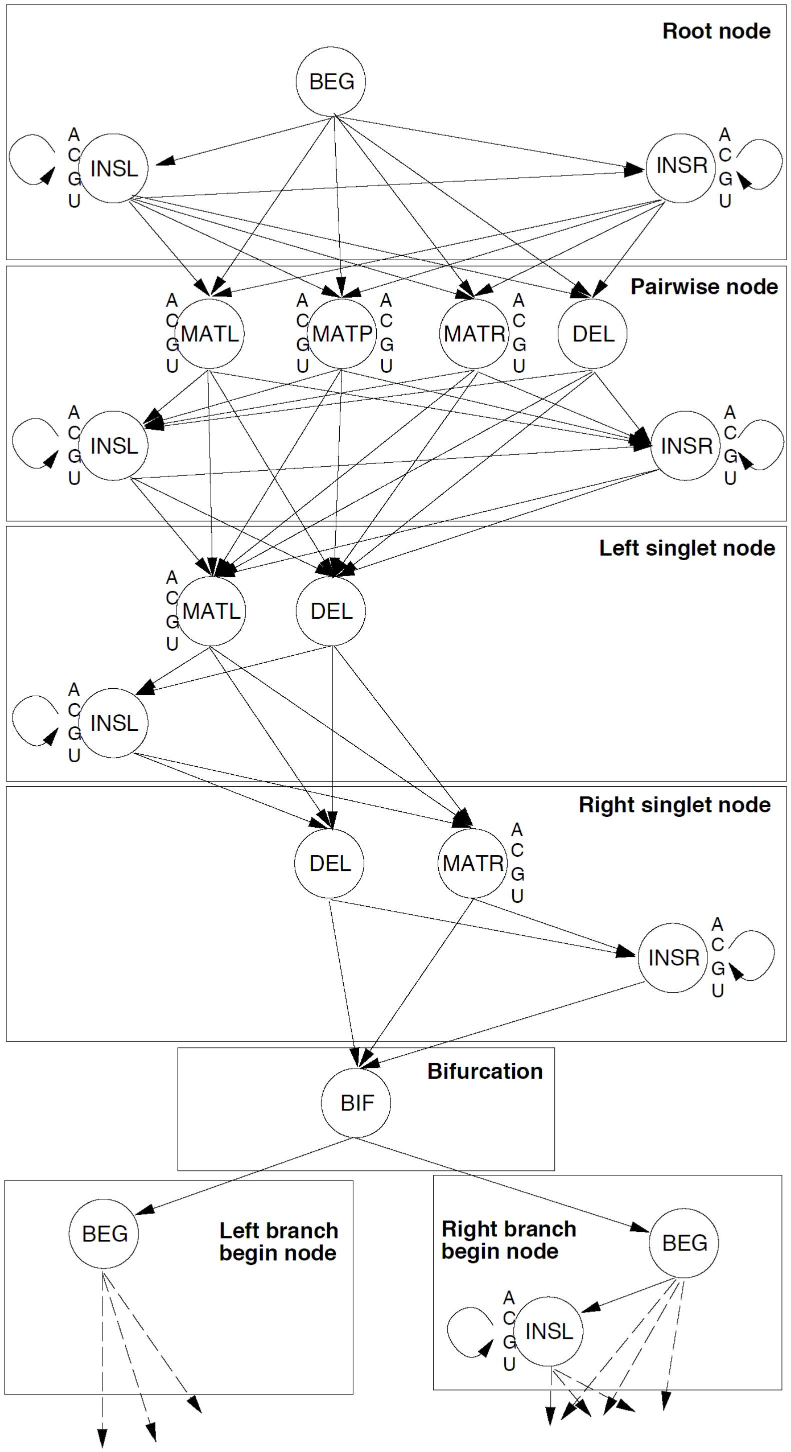


Overall CM Architecture

One box ("node") per node of guide tree

BEG/MATL/INS/DEL just like an HMM

MATP & BIF are the key additions: MATP emits *pairs* of symbols, modeling base-pairs; BIF allows multiple helices



CM Viterbi Alignment

(the “inside” algorithm)

x_i = i^{th} letter of input

x_{ij} = substring $i,\ldots,j$ of input

T_{yz} = $P(\text{transition } y \rightarrow z)$

$E^{y}_{x_i,x_j}$ = $P(\text{emission of } x_i, x_j \text{ from state } y)$

S^{y}_{ij} = $\max_{\pi} \log P(x_{ij} \text{ gen'd starting in state } y \text{ via path } \pi)$

CM Viterbi Alignment

(the “inside” algorithm)

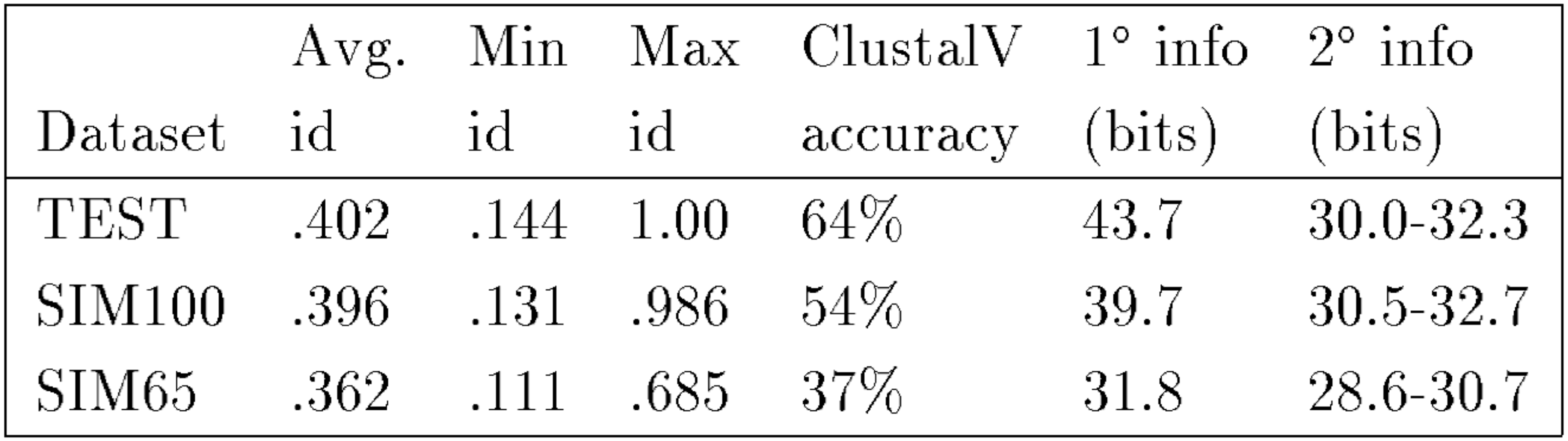
$$S_{ij}^{y} = \max\nolimits_{\pi} \log P(x_{ij} \text{ generated starting in state } y \text{ via path } \pi)$$

$$S_{ij}^{y} = \begin{cases} \max_z [S_{i+1,j-1}^{z} + \log T_{yz} + \log E_{x_i,x_j}^{y}] & \text{match pair} \\ \max_z [S_{i+1,j}^{z} + \log T_{yz} + \log E_{x_i}^{y}] & \text{match/insert left} \\ \max_z [S_{i,j-1}^{z} + \log T_{yz} + \log E_{x_j}^{y}] & \text{match/insert right} \\ \max_z [S_{i,j}^{z} + \log T_{yz}] & \text{delete} \\ \max_{i<k\le j} [S_{i,k}^{y_{left}} + S_{k+1,j}^{y_{right}}] & \text{bifurcation} \end{cases}$$

Time O(qn^3), q states, seq len n

compare: O(qn) for profile HMM

Primary vs Secondary Info



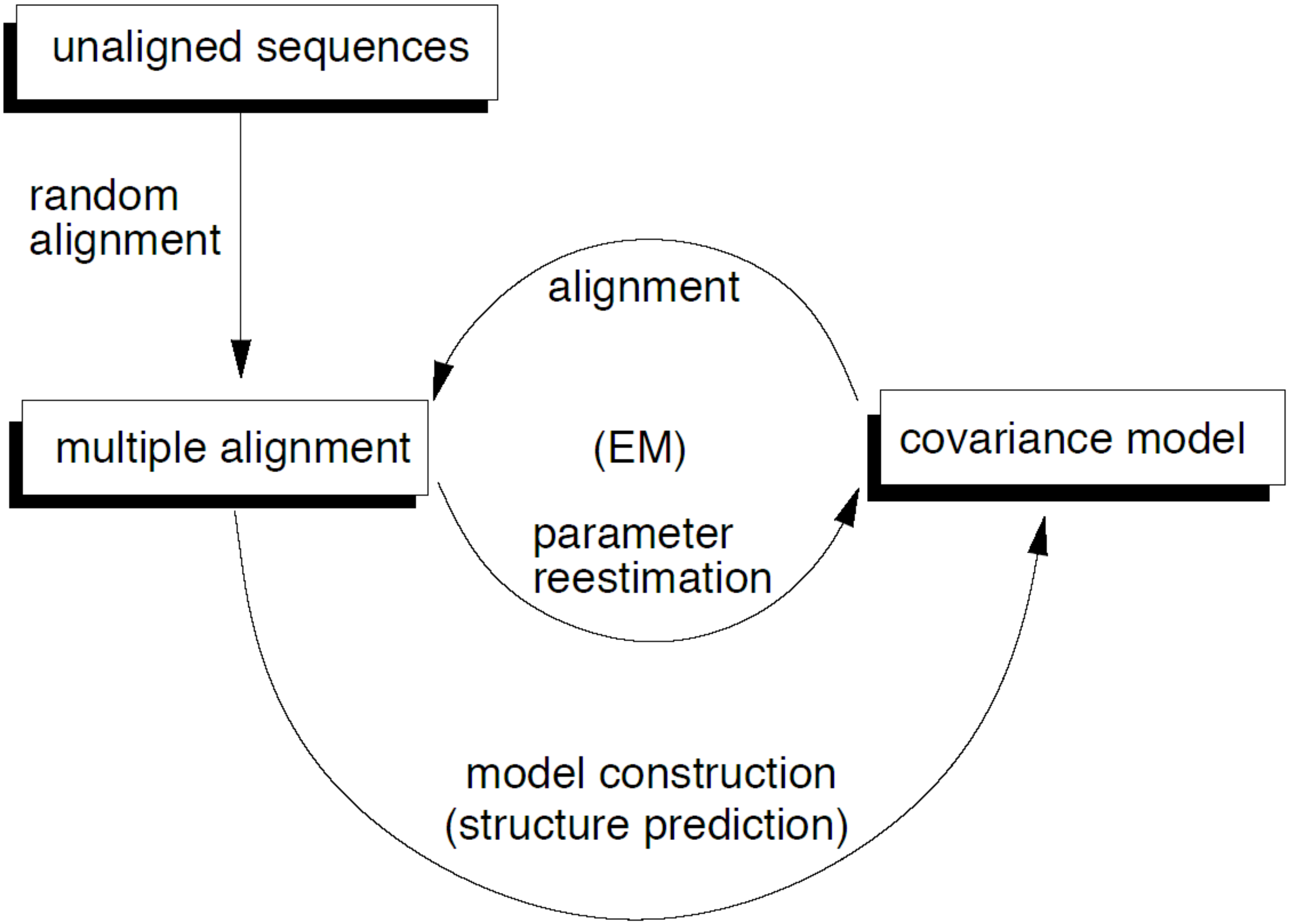
Dataset	Avg. id	Min id	Max id	ClustalV accuracy	1° info (bits)	2° info (bits)
TEST	.402	.144	1.00	64%	43.7	30.0-32.3
SIM100	.396	.131	.986	54%	39.7	30.5-32.7
SIM65	.362	.111	.685	37%	31.8	28.6-30.7

3 test sets from ED 94

disallowing / allowing pseudoknots

$$\left(\sum_{i=1}^{n} \max_j M_{i,j}\right) / 2$$

Model Training



unaligned sequences

random alignment

multiple alignment

alignment

(EM)

parameter reestimation

covariance model

model construction (structure prediction)

Comparison to TRNASCAN

Fichant & Burks - best heuristic then

- 97.5% true positive
- 0.37 false positives per MB

CM A1415 (trained on trusted alignment)

- > 99.98% true positives
- < 0.2 false positives per MB

Slightly different evaluation criteria

Current method-of-choice is “tRNAscanSE”, a CM-based scan with heuristic pre-filtering (including TRNASCAN?) for performance reasons.

tRNAScanSE

Uses 3 older heuristic tRNA finders as prefilter

Uses CM built as described for final scoring

Actually 3(?) different CMs

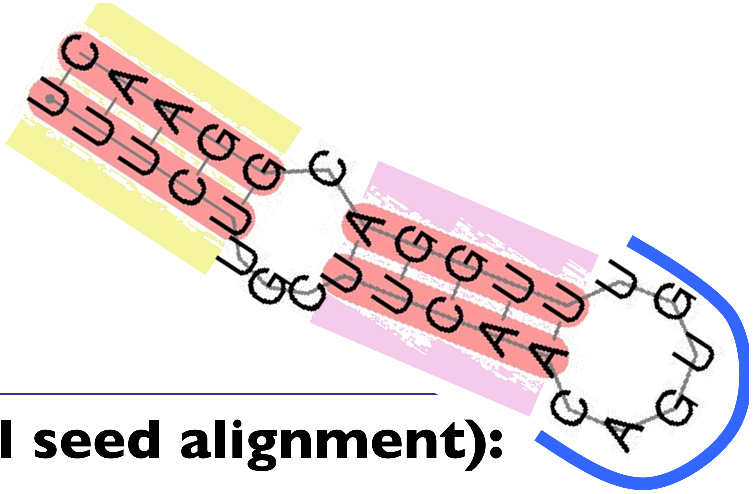
- eukaryotic nuclear
- prokaryotic
- organellar

Used in “all” genome annotation projects

An Important Application: Rfam

A Database of RNA Families

RF00037: Example Rfam Family



Input (hand-curated):

- MSA "seed alignment"
- SS_cons
- Score Thresh T
- Window Len W

Output:

- CM
- scan results & "full alignment"
- phylogeny, etc.

IRE (partial seed alignment):

```
Hom.sap.    GUUCCUGCUUCAACAGUGUUUGGAUGGAAC
Hom.sap.    UUUCUUC.UUCAACAGUGUUUGGAUGGAAC
Hom.sap.    UUUCCUGUUUCAACAGUGCUUGGA.GGAAC
Hom.sap.    UUUAUC..AGUGACAGAGUUCACU.AUAAA
Hom.sap.    UCUCUUGCUUCAACAGUGUUUGGAUGGAAC
Hom.sap.    AUUAUC..GGGAACAGUGUUUCCC.AUAAU
Hom.sap.    UCUUGC..UUCAACAGUGUUUGGACGGAAG
Hom.sap.    UGUAUC..GGAGACAGUGAUCUCC.AUAUG
Hom.sap.    AUUAUC..GGAAGCAGUGCCUUCC.AUAAU
Cav.por.    UCUCCUGCUUCAACAGUGCUUGGACGGAGC
Mus.mus.    UAUAUC..GGAGACAGUGAUCUCC.AUAUG
Mus.mus.    UUUCCUGCUUCAACAGUGCUUGAACGGAAC
Mus.mus.    GUACUUGCUUCAACAGUGUUUGAACGGAAC
Rat.nor.    UAUAUC..GGAGACAGUGACCUCC.AUAUG
Rat.nor.    UAUCUUGCUUCAACAGUGUUUGGACGGAAC
SS_cons     <<<<<...<<<<<......>>>>>.>>>>>
```

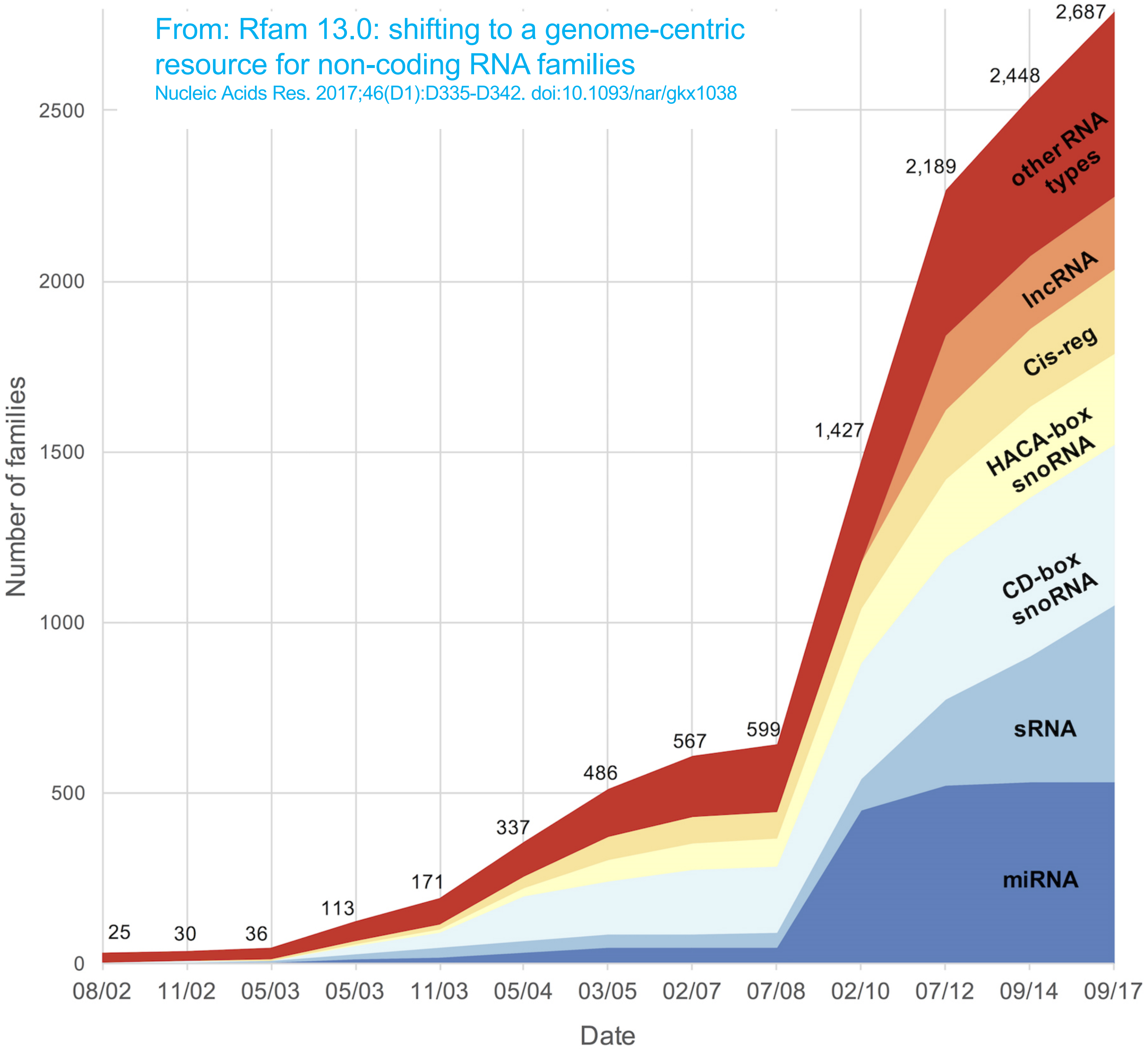
Rfam – an RNA family DB

Griffiths-Jones, et al., NAR ’03, ’05, ’08, ’11, ’12

Was biggest scientific comp user in Europe - 1000 cpu cluster for a month per release

Rapidly growing:

			DB size:
Rel 1.0, 1/03:	25 families,	55k instances	
Rel 7.0, 3/05:	503 families,	363k instances	~8GB
Rel 9.0, 7/08:	603 families,	636k instances	
Rel 10.0, 1/10:	1446 families,	3193k instances	~160GB
Rel 11.0, 8/12:	2208 families,	6125k instances	~320GB
Rel 12.0, 9/14:	2450 families,	19623k instances	
Rel 12.1, 4/16:	2474 families,	9m instances	
Rel 13.0, 9/17:	2686 families		
Rel 14.3, 9/20:	3446 families		


From: Rfam 13.0: shifting to a genome-centric resource for non-coding RNA families

Nucleic Acids Res. 2017;46(D1):D335-D342. doi:10.1093/nar/gkx1038

Rfam – key issues

Overly narrow families

Variant structures/unstructured RNAs

Spliced RNAs

RNA pseudogenes

- Human ALU is SRP-related w/ 1.1m copies
- Mouse B2 repeat (350k copies) tRNA related

Speed & sensitivity

Motif discovery/hand-made models

CM Summary

Covariance Models (CMs) represent conserved RNA sequence/structure motifs

They allow accurate search

But

a) search is slow

b) model construction is laborious

An Important Need:
Faster Search

Homology search

“Homolog” – similar by descent from common ancestor

Sequence-based

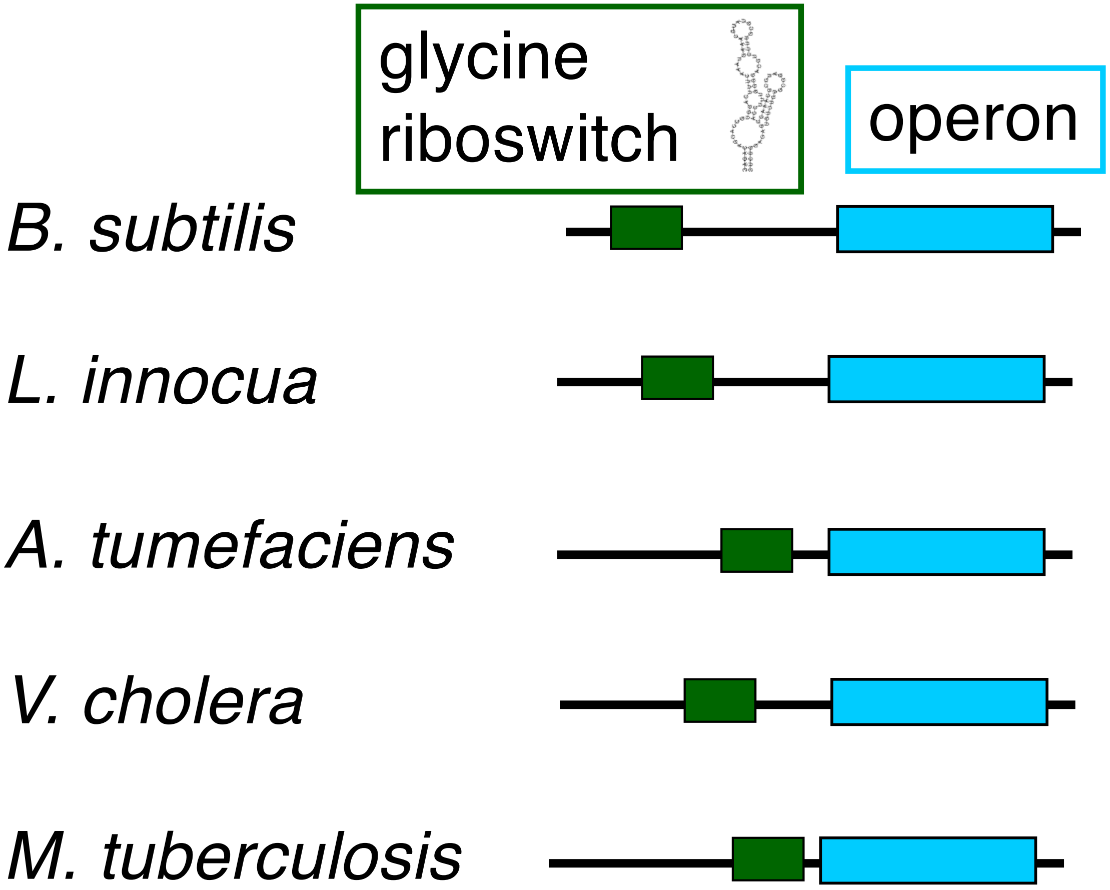
- Smith-Waterman
- FASTA
- BLAST

For RNA, sharp decline in sensitivity at ~60-70% identity

So, use structure, too

Impact of RNA homology search

(Barrick, *et al.*, 2004)



glycine riboswitch

operon

B. subtilis

L. innocua

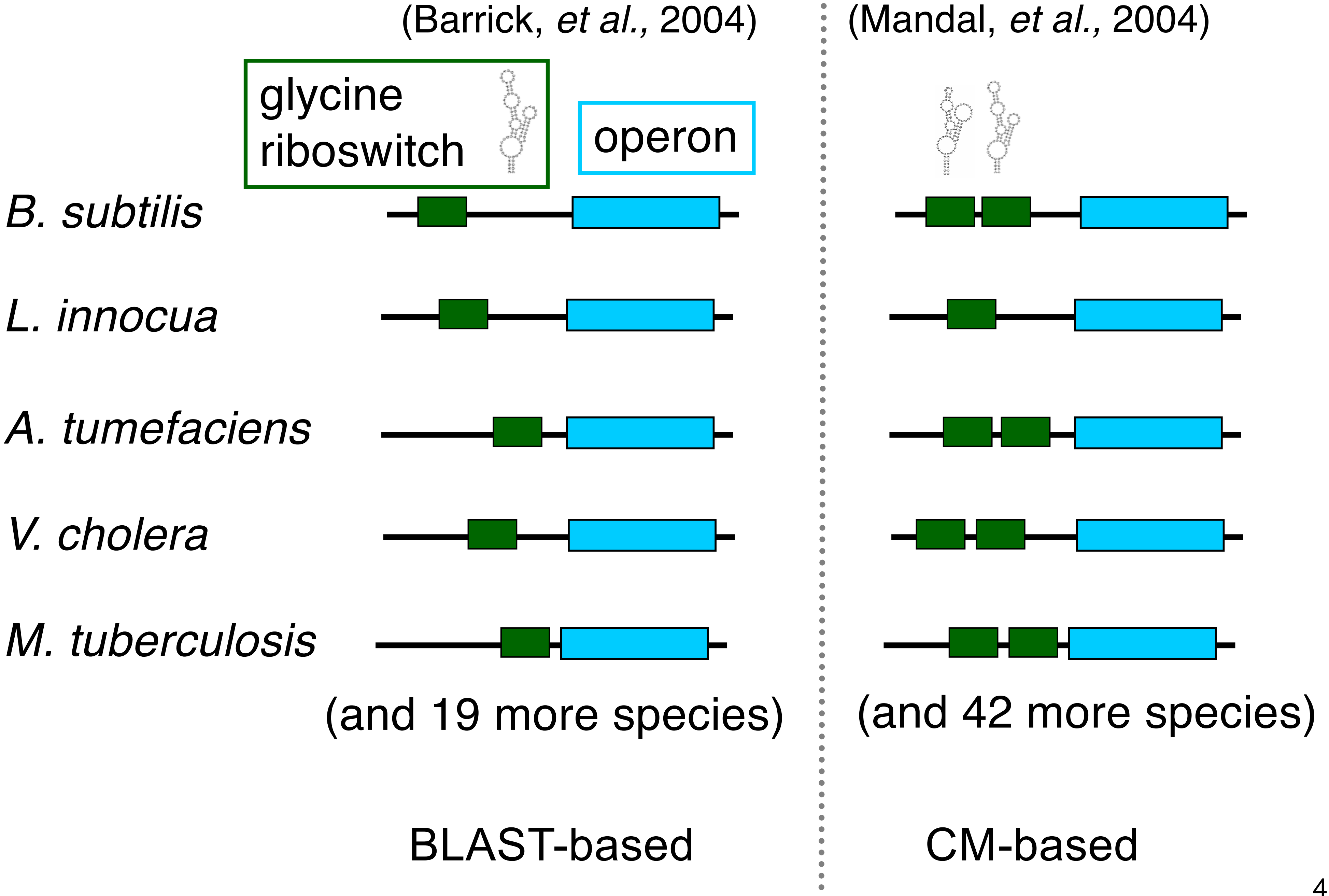
A. tumefaciens

V. cholera

M. tuberculosis

(and 19 more species)

Impact of RNA homology search



(Barrick, *et al.*, 2004)

(Mandal, *et al.*, 2004)

glycine riboswitch

operon

B. subtilis

L. innocua

A. tumefaciens

V. cholera

M. tuberculosis

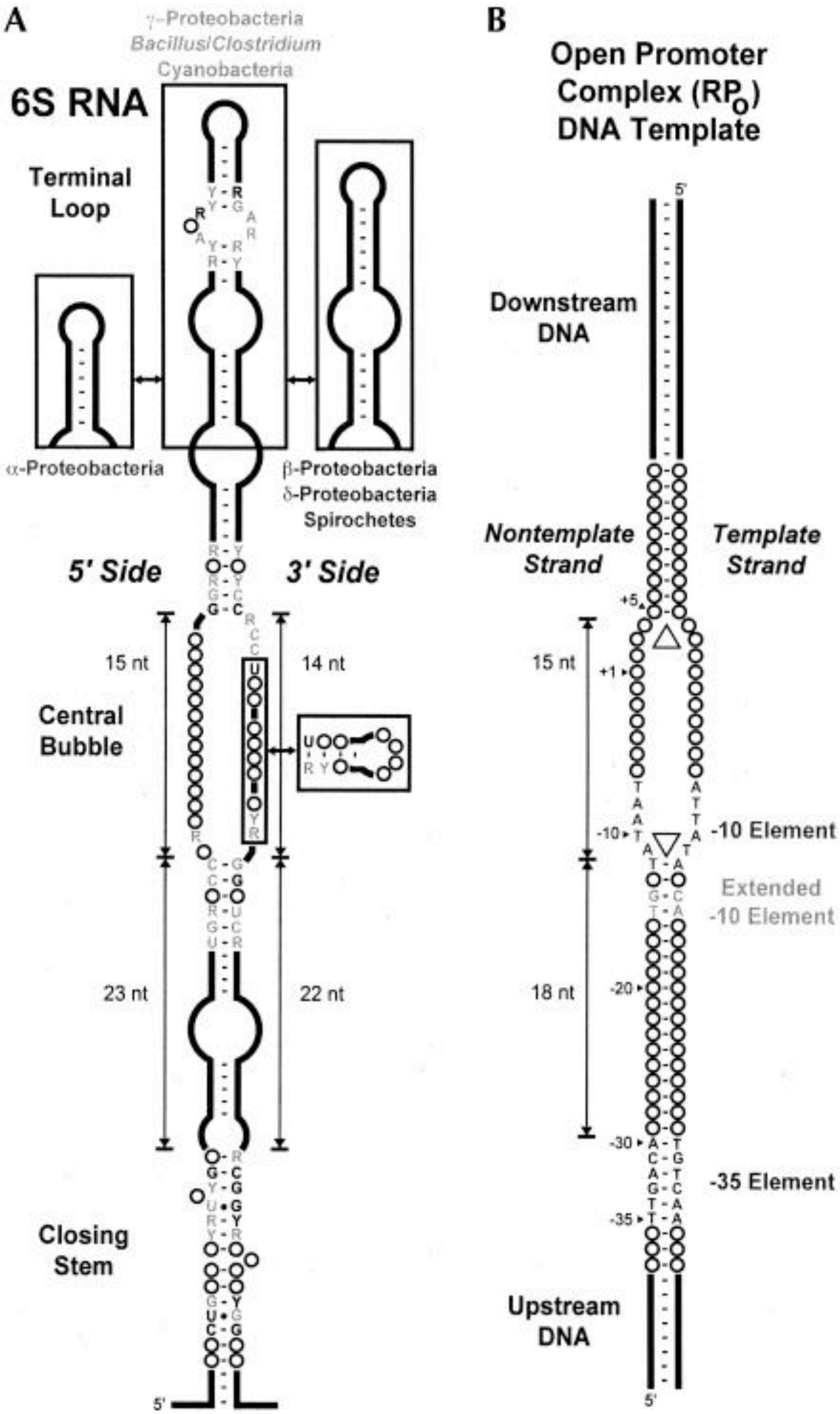
(and 19 more species)

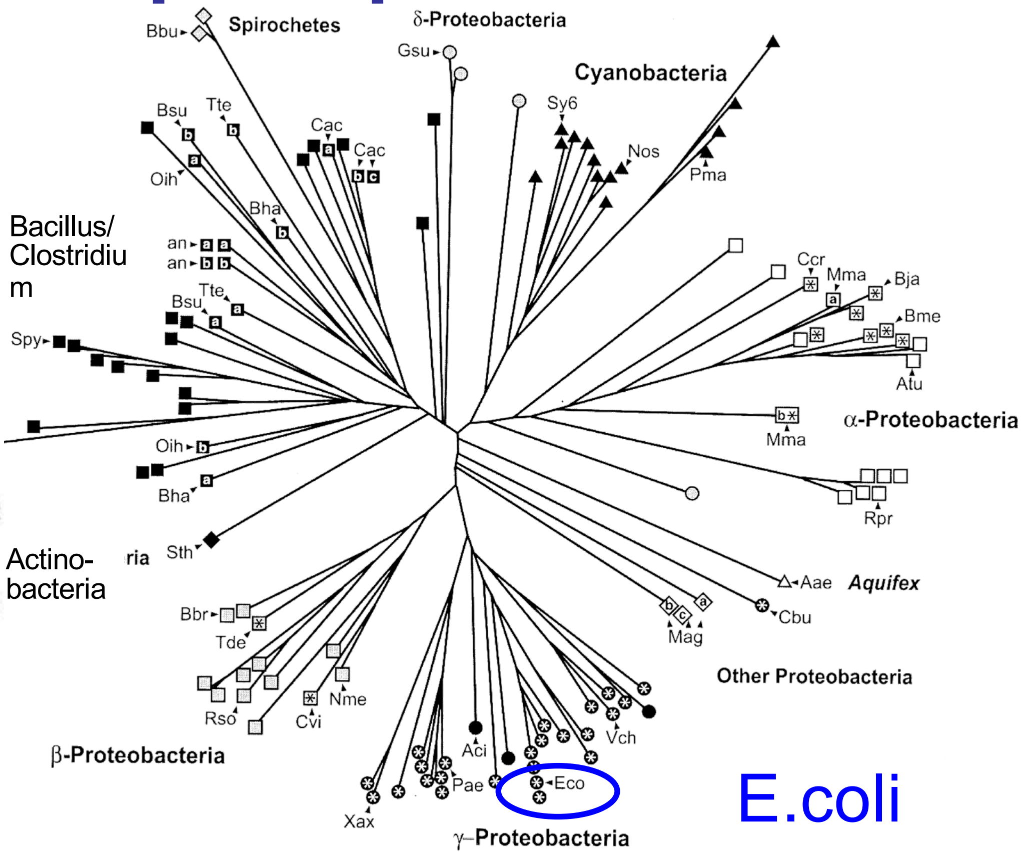
(and 42 more species)

BLAST-based

CM-based

6S mimics an open promoter





Barrick et al. *RNA* 2005
Trotochaud et al. *NSMB* 2005
Willkomm et al. NAR 2005

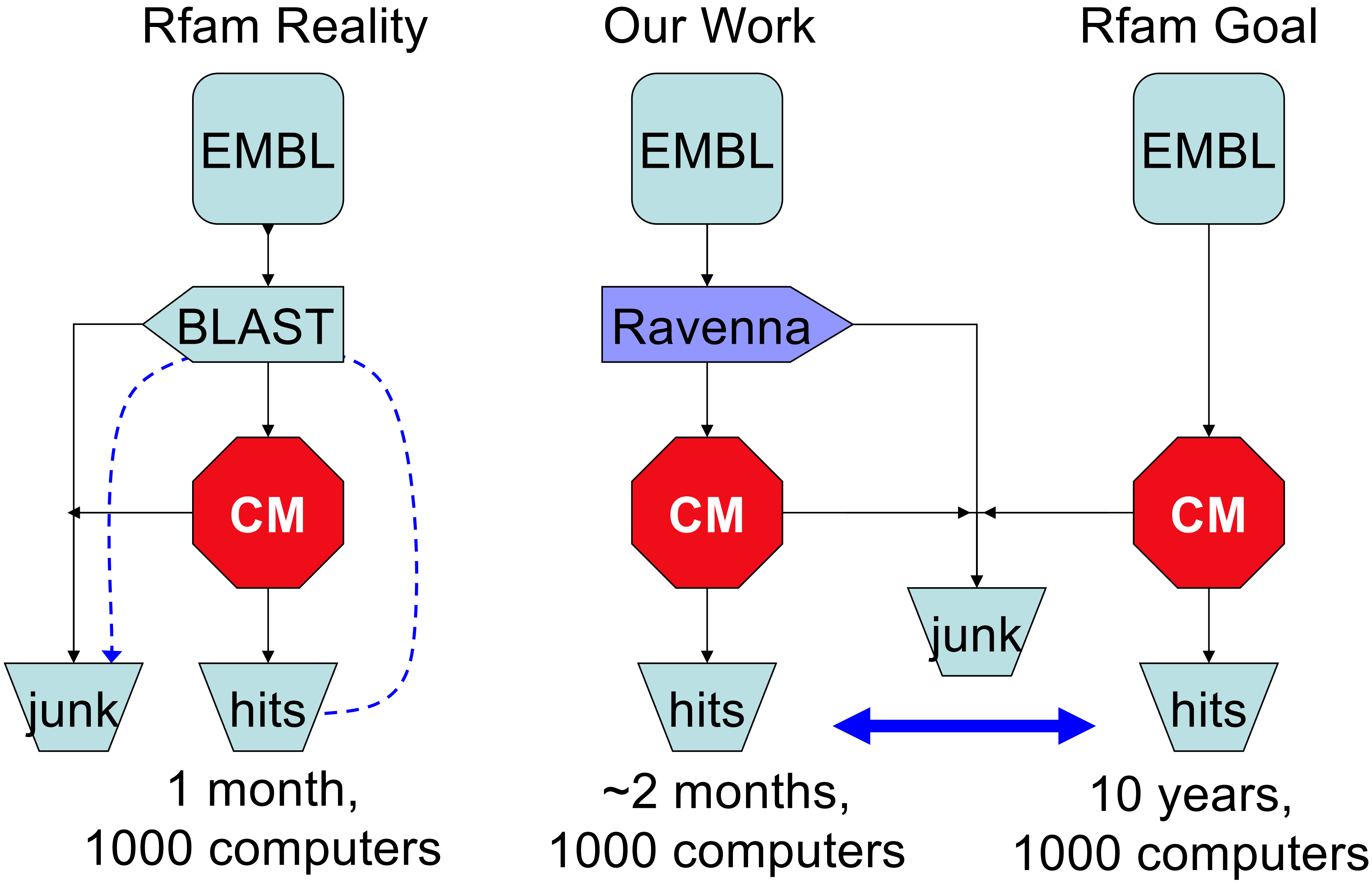
Faster Genome Annotation of Non-coding RNAs Without Loss of Accuracy

Zasha Weinberg

& W.L. Ruzzo

Recomb ‘04, ISMB ‘04, Bioinfo ‘06

CM's are good, but slow



RaveNnA: Genome Scale RNA Search

Typically 100x speedup over raw CM, w/ no loss in accuracy:

Drop structure from CM to create a (faster) HMM

Use that to pre-filter sequence;

Discard parts where, *provably*, CM score < threshold;

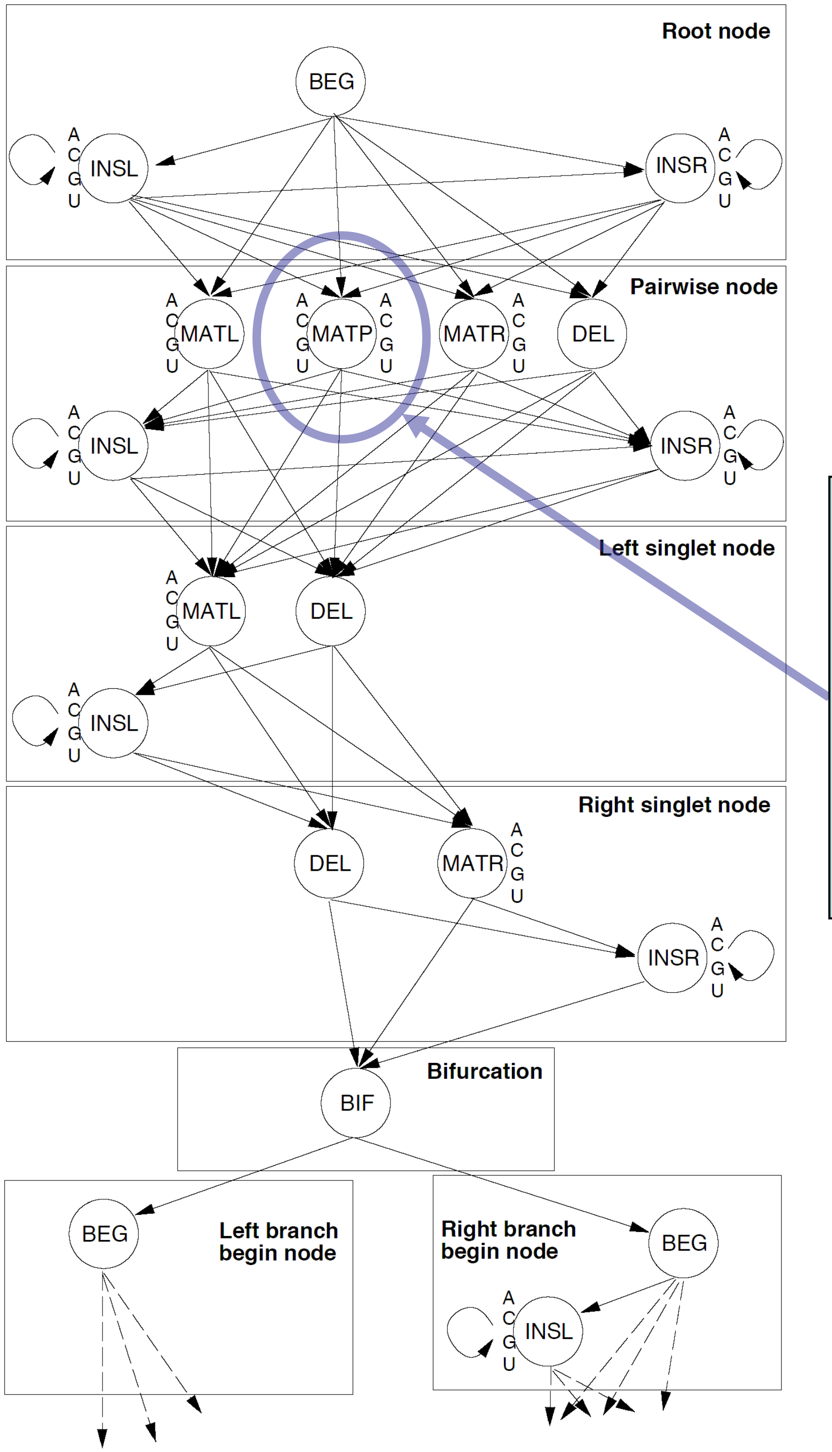
Actually run CM on the rest (the promising parts)

Assignment of HMM transition/emission scores is key

(a large convex optimization problem)

Weinberg & Ruzzo, *Bioinformatics*, 2004, 2006

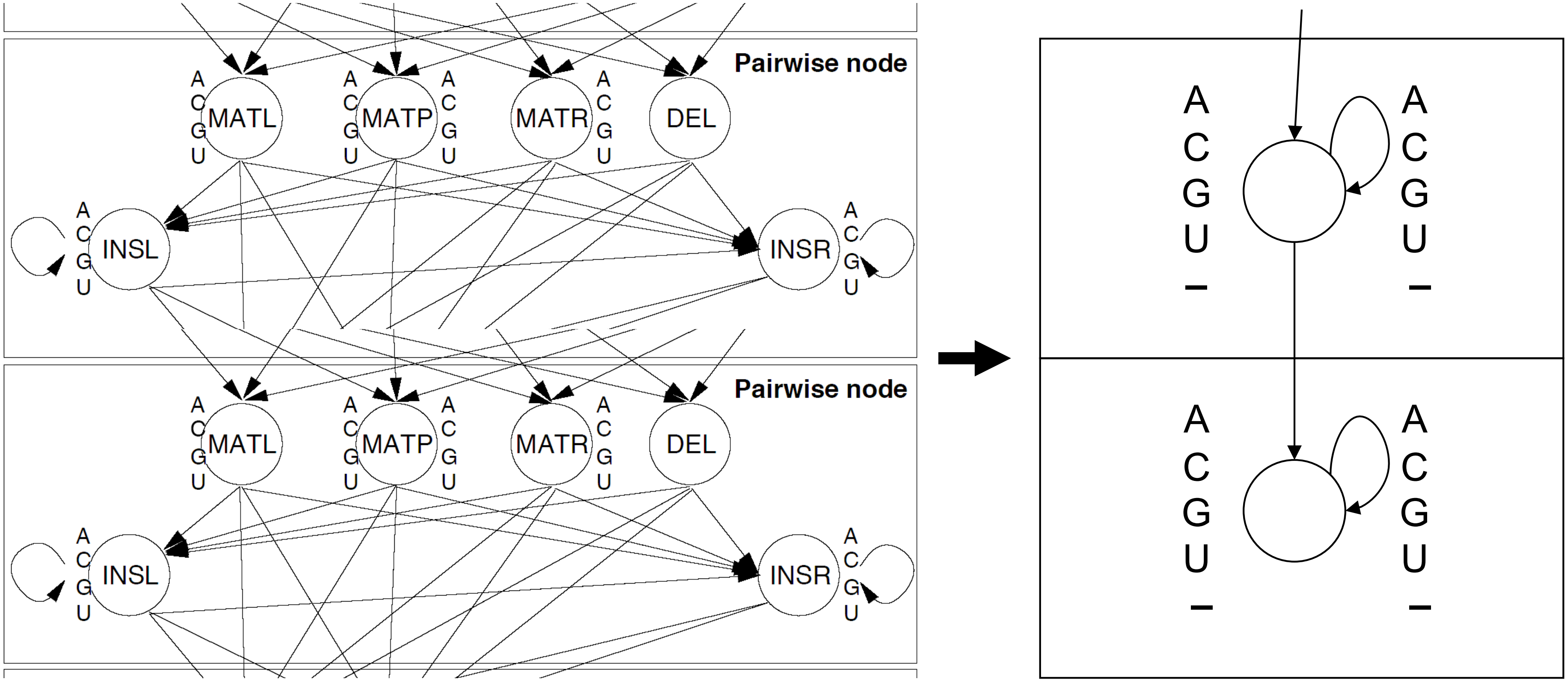
Covariance Model



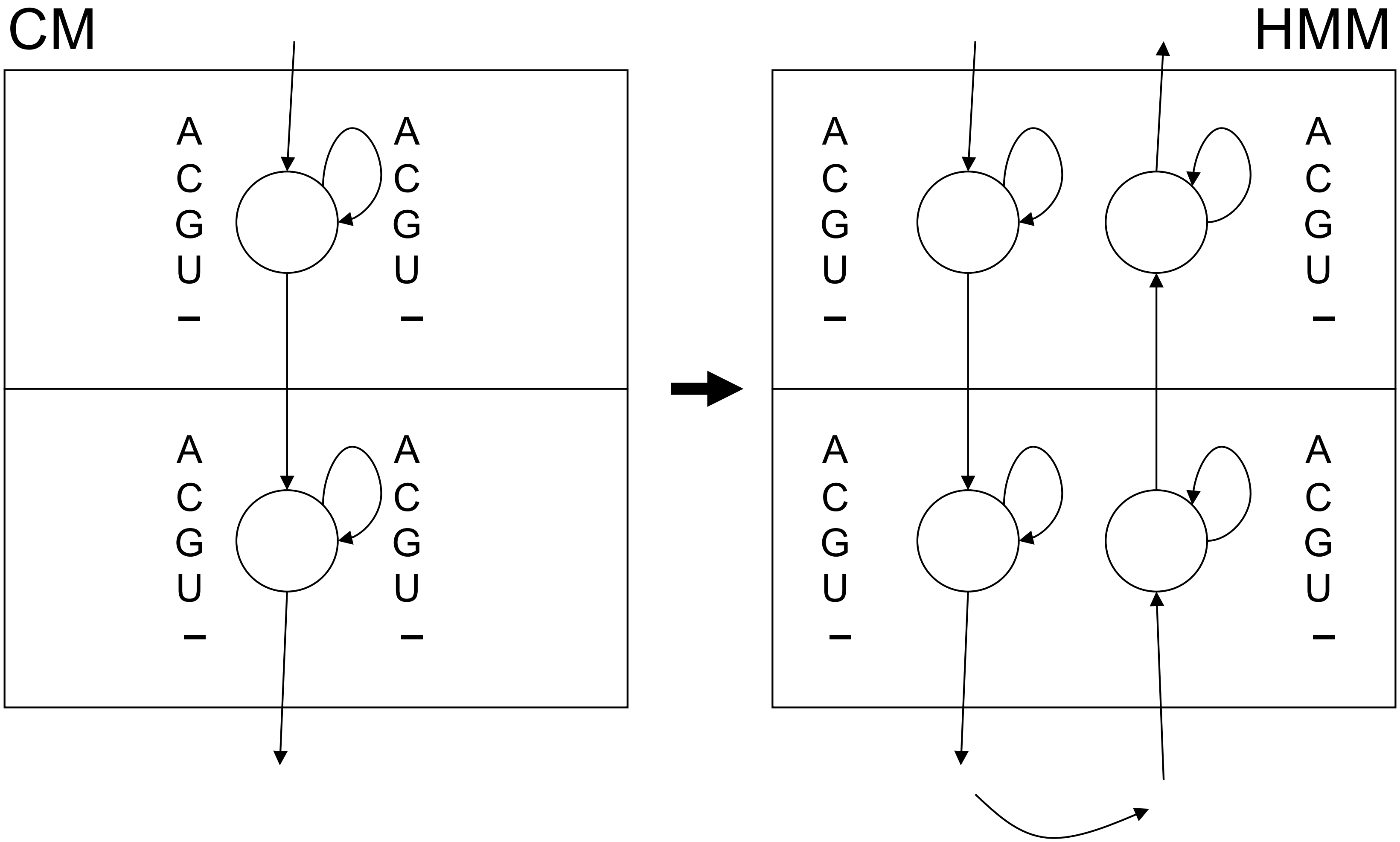
Key difference of CM vs HMM: Pair states emit paired symbols, corresponding to base-paired nucleotides; 16 emission probabilities here.

Oversimplified CM

(for pedagogical purposes only)



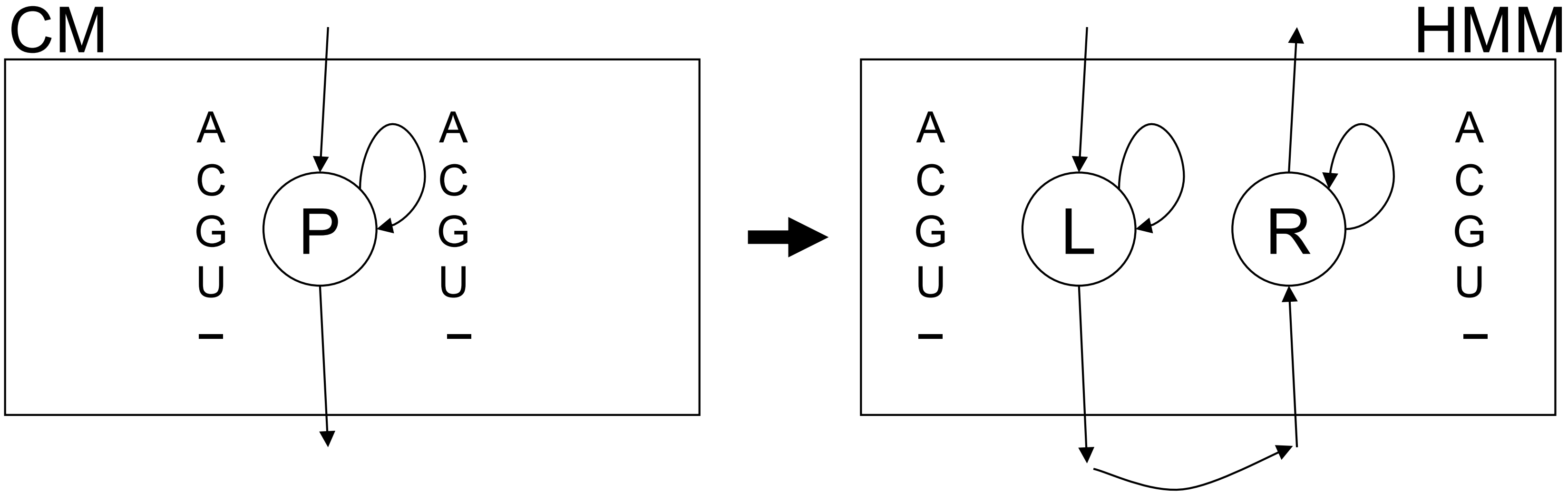
CM to HMM



25 emisions per state

5 emissions per state, 2x states

Key Issue: 25 scores → 10



Need: log Viterbi scores CM $\leq$ HMM

Viterbi/Forward Scoring

Path π defines transitions/emissions

Score(π) = product of “probabilities” on π

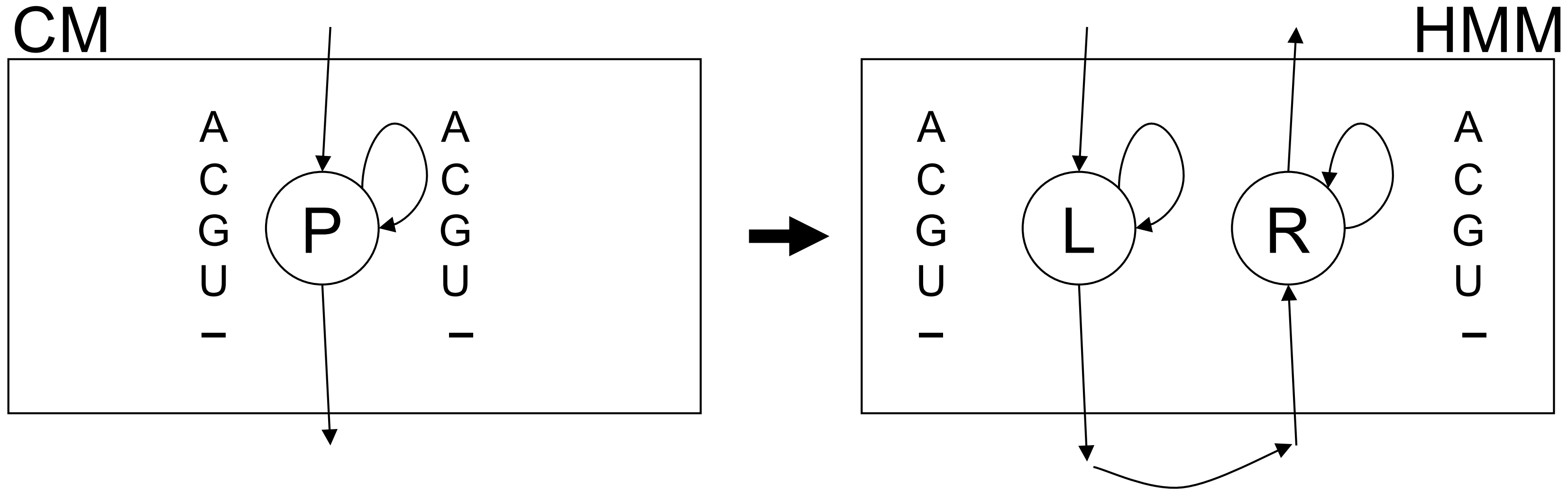
NB: ok if “probs” aren’t, e.g. $\sum \neq 1$
(e.g. in CM, emissions are odds ratios vs 0th-order background)

For any nucleotide sequence x:

Viterbi-score(x) = max{ score(π) | π emits x}

Forward-score(x) = $\sum${ score(π) | π emits x}

Key Issue: 25 scores → 10



Need: log Viterbi scores CM $\le$ HMM

$P_{AA} \le L_A + R_A$ $P_{CA} \le L_C + R_A$ …

$P_{AC} \le L_A + R_C$ $P_{CC} \le L_C + R_C$ …

$P_{AG} \le L_A + R_G$ $P_{CG} \le L_C + R_G$ …

$P_{AU} \le L_A + R_U$ $P_{CU} \le L_C + R_U$ …

$P_{A-} \le L_A + R_-$ $P_{C-} \le L_C + R_-$ …

NB: HMM not a prob. model

$P_{AA} \leq L_A + R_A$
$P_{AC} \leq L_A + R_C$
$P_{AG} \leq L_A + R_G$
$P_{AU} \leq L_A + R_U$
$P_{A-} \leq L_A + R_-$
…

Rigorous Filtering

Any scores satisfying the linear inequalities give rigorous filtering

Proof:

CM Viterbi path score
≤ "corresponding" HMM path score
≤ Viterbi HMM path score
(even if it does not correspond to *any* CM path)

Some scores filter better

$P_{UA} = 1 \leq L_U + R_A$

$P_{UG} = 4 \leq L_U + R_G$

Option 1:

$L_U = R_A = R_G = 2$

Option 2:

$L_U = 0, R_A = 1, R_G = 4$

Assuming ACGU ≈ 25%

Opt 1:

$L_U + (R_A + R_G)/2 = 4$

Opt 2:

$L_U + (R_A + R_G)/2 = 2.5$

Optimizing filtering

For any nucleotide sequence x:

Viterbi-score(x) = max{ score(π) | π emits x }

Forward-score(x) = ∑{ score(π) | π emits x }

Expected Forward Score

$E(L_i, R_i) = \sum_{\text{all sequences } x}$ Forward-score(x)*Pr(x)

Under 0th-order background model

NB: E is a function of L_i, R_i only

Optimization:

Minimize $E(L_i, R_i)$ subject to score Lin.Ineq.s

This is heuristic (“forward↓ ⇒ Viterbi↓ ⇒ filter↓”)

But still rigorous because “subject to score Lin.Ineq.s”

Calculating $E(L_i, R_i)$

$E(L_i, R_i) = \sum_x \text{Forward-score}(x)*\Pr(x)$

Forward-like: for every state, calculate expected score for all paths ending there; easily calculated from expected scores of predecessors & transition/emission probabilities/scores

Minimizing E(L$_i$, R$_i$)

(subject to linear constraints)

Calculate E(L$_i$, R$_i$) *symbolically*, in terms of emission scores, so we can do partial derivatives for numerical convex optimization algorithm

Forward:

$$\begin{aligned} f_k(i) &= P(x_1 \ldots x_i,\ \pi_i = k) \\ f_l(i+1) &= e_l(x_{i+1}) \textstyle\sum_k f_k(i) a_{k,l} \end{aligned}$$

Viterbi:

$$v_l(i+1) = e_l(x_{i+1}) \cdot \max_k (v_k(i)\, a_{k,l})$$

$$\frac{\partial E(L_1, L_2, \ldots)}{\partial L_i}$$

Assignment of scores/ “probabilities”

Convex optimization problem

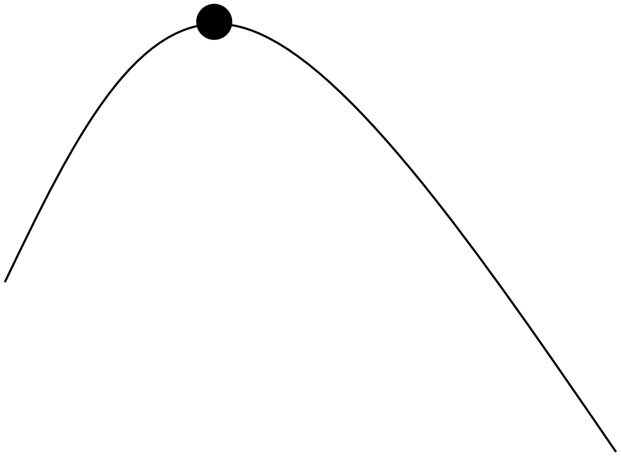
- Constraints: enforce rigorous property
- Objective function: filter as aggressively as possible

Problem sizes:

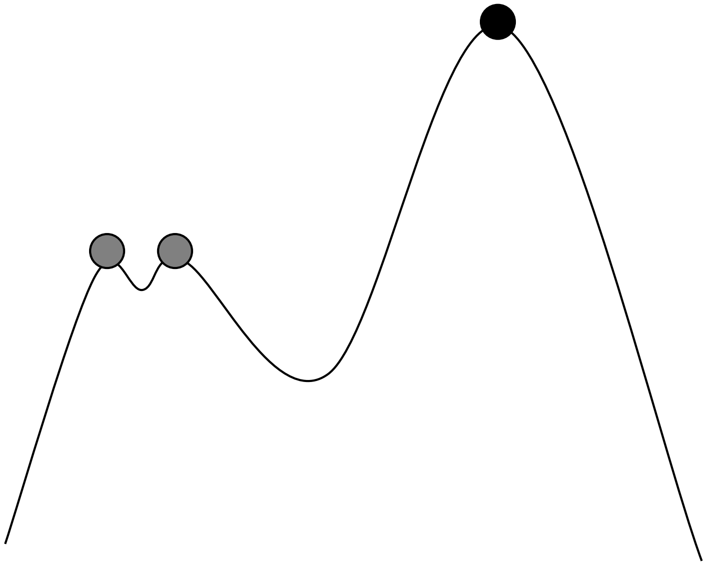
- 1000-10000 variables
- 10000-100000 inequality constraints

“Convex” Optimization

Convex:
local max = global max;
simple “hill climbing” works
(but better ways, often)



Nonconvex:
can be many local maxima,
≪ global max;
“hill-climbing” fails



Estimated Filtering Efficiency
(139 Rfam 4.0 families)

Filtering fraction	# families (compact)	# families (expanded)
< 10^{-4}	105	110
10^{-4} - 10^{-2}	8	17
.01 - .10	11	3
.10 - .25	2	2
.25 - .99	6	4
.99 - 1.0	7	3

≈ break even

~100x speedup

Averages 283 times faster than CM

Results: new ncRNAs (?)

Name	# Known (BLAST + CM)	# New (rigorous filter + CM)
Pyrococcus snoRNA	57	123
Iron response element	201	121
Histone 3' element	1004	102*
Retron msr	11	48
Hammerhead I	167	26
Hammerhead III	251	13
U6 snRNA	1462	2
U7 snRNA	312	1
cobalamin riboswitch	170	7

13 other families	5-1107	0

Results: With additional work

	# with BLAST+CM	# with rigorous filter series + CM	# new
Rfam tRNA	58609	63767	5158
Group II intron	5708	6039	331
tRNAscan-SE (human)	608	729	121
tmRNA	226	247	21
Lysine riboswitch	60	71	11
And more…			

Software

Ravenna implements both rigorous and heuristic filters

Infernal (engine behind Rfam) implements heuristic filters and some other (important)accelerations

- E,g., dynamic “banding” of dynamic programming matrix based on the insight that large deviations from consensus length must have low scores.

CM Search Summary

Still slower than we might like, but dramatic speedup over raw CM is possible with:

- No loss in sensitivity (provably), or
- Even faster with modest (and estimable) loss in sensitivity

Motif Discovery

RNA Motif Discovery

- CM's are great, but where do they come from?
- Key approach: comparative genomics
  - Search for motifs with common secondary structure in a set of functionally related sequences.
- Challenges
  - Three related tasks
    - Locate the motif regions.
    - Align the motif instances.
    - Predict the consensus secondary structure.
  - Motif search space is huge!
    - Motif location space, alignment space, structure space.

RNA Motif Discovery

Would be great if: given 100 complete genomes from diverse species, we could automatically find all the RNAs.

State of the art: that's hopeless

Hope: can we exploit biological knowledge to narrow the search space?

RNA Motif Discovery

More promising problem: given a 10-20 unaligned sequences of a few kb, most of which contain instances of one RNA motif of 100-200bp -- find it.

Example: 5' UTRs of orthologous glycine cleavage genes from γ-proteobacteria

Example: corresponding introns of orthogolous vertebrate genes

Orthologs = counterparts in different species

Approaches

Align-First: Align sequences, then look for common structure

Fold-First: Predict structures, then try to align them

Joint: Do both together

“Align First” Approach: Predict Struct from Multiple Alignment

… GA … UC …

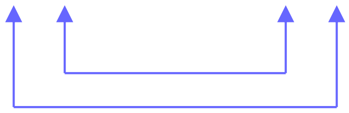
… GA … UC …

… GA … UC …

… CA … UG …

… CC … GG …

… UA … UA …



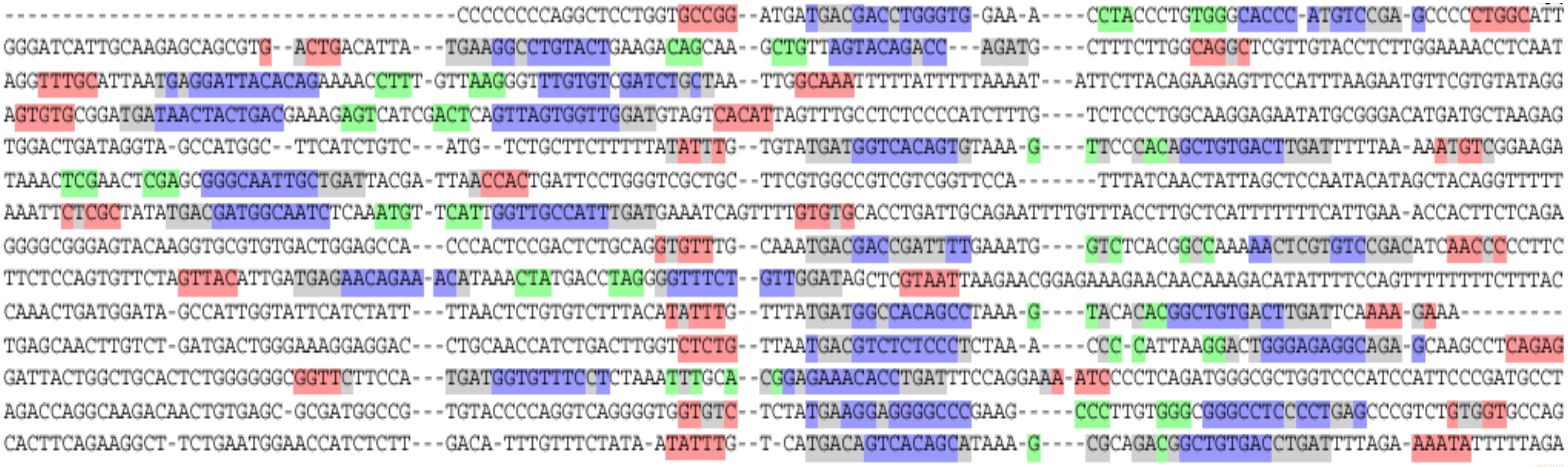
Compensatory mutations reveal structure (core of “comparative sequence analysis”) *but* usual alignment algorithms penalize them (twice)

Pitfall for sequence-alignment-first approach

Structural conservation ≠ Sequence conservation

Alignment without structure information is unreliable

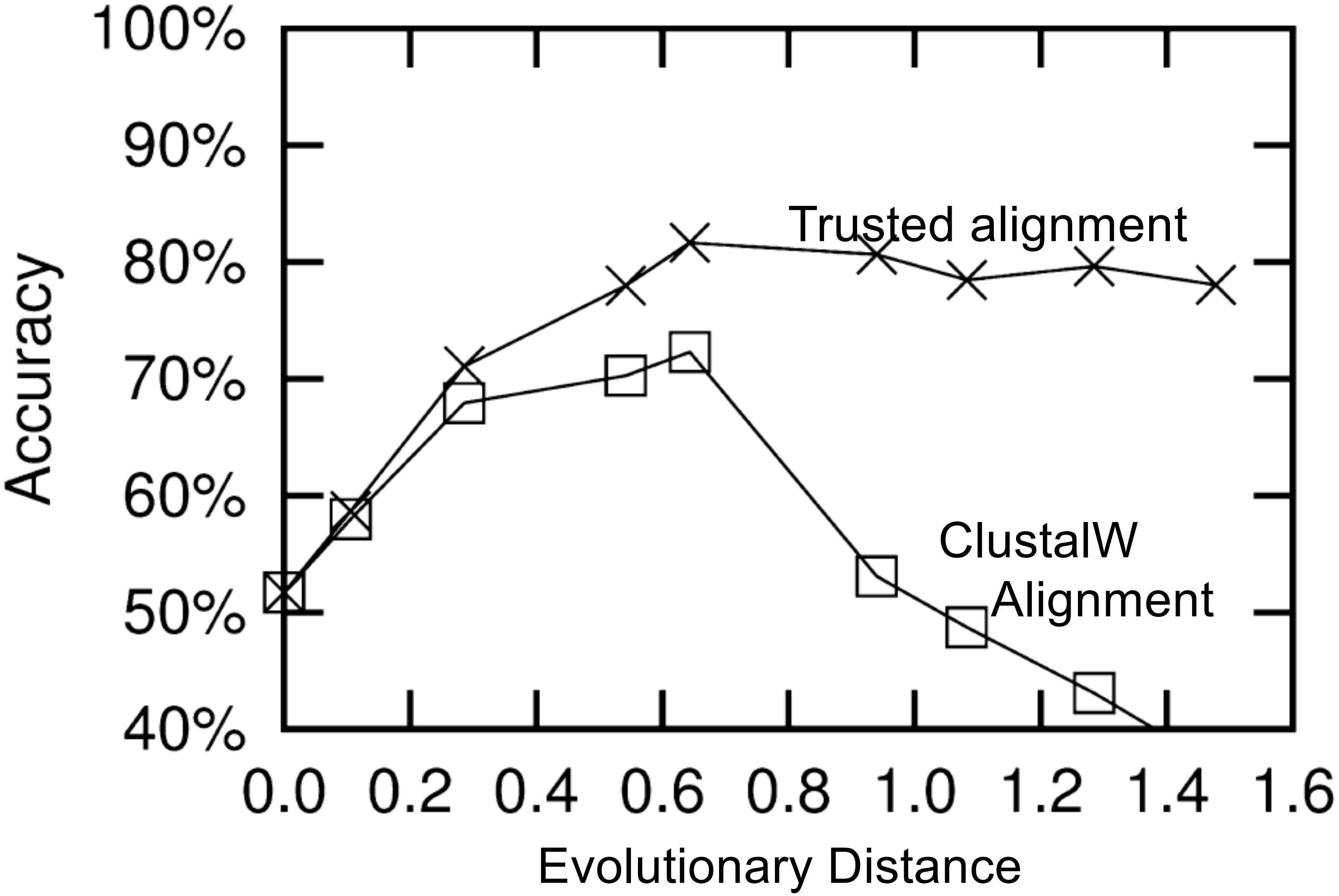
CLUSTALW alignment of SECIS elements with flanking regions



```
---------------------------------------CCCCCCCCAGGCTCCTGGTGCCGG--ATGATGACGACCTGGGTG-GAA-A----CCTACCCTGTGGGCACCC-ATGTCCGA-GCCCCCTGGCATT
GGGATCATTGCAAGAGCAGCGTG--ACTGACATTA---TGAAGGCCTGTACTGAAGACAGCAA--GCTGTTAGTACAGACC---AGATG----CTTTCTTGGCAGGCTCGTTGTACCTCTTGGAAAACCTCAAT
AGGTTTGCATTAATGAGGATTACACAGAAAACCTTT-GTTAAGGGTTTGTGTCGATCTGCTAA--TTGGCAAATTTTTATTTTTAAAAT---ATTCTTACAGAAGAGTTCCATTTAAGAATGTTCGTGTATAGG
AGTGTGCGGATGATAACTACTGACGAAAGAGTCATCGACTCAGTTAGTGGTTGGATGTAGTCACATTAGTTTGCCTCTCCCCATCTTTG---TCTCCCTGGCAAGGAGAATATGCGGGACATGATGCTAAGAG
TGGACTGATAGGTA-GCCATGGC--TTCATCTGTC---ATG--TCTGCTTCTTTTTATATTTG--TGTATGATGGTCACAGTGTAAA-G----TTCCCACAGCTGTGACTTGATTTTTAA-AAATGTCGGAAGA
TAAACTCGAACTCGAGCGGGCAATTGCTGATTACGA-TTAACCACTGATTCCTGGGTCGCTGC--TTCGTGGCCGTCGTCGGTTCCA-------TTTATCAACTATTAGCTCCAATACATAGCTACAGGTTTTT
AAATTCTCGCTATATGACGATGGCAATCTCAAATGT-TCATTGGTTGCCATTTGATGAAATCAGTTTTGTGTGCACCTGATTGCAGAATTTTGTTTACCTTGCTCATTTTTTTCATTGAA-ACCACTTCTCAGA
GGGGCGGGAGTACAAGGTGCGTGTGACTGGAGCCA---CCCACTCCGACTCTGCAGGTGTTTG--CAAATGACGACCGATTTTGAAATG----GTCTCACGGCCAAAAACTCGTGTCCGACATCAACCCCCTTC
TTCTCCAGTGTTCTAGTTACATTGATGAGAACAGAA-ACATAAACTATGACCTAGGGTTTCT--GTTGGATAGCTCGTAATTAAGAACGGAGAAAGAACAACAAAGACATATTTTCCAGTTTTTTTCTTTAC
CAAACTGATGGATA-GCCATTGGTATTCATCTATT---TTAACTCTGTGTCTTTACATATTTG--TTTATGATGGCCACAGCCTAAA-G----TACACACGGCTGTGACTTGATTCAAAA-GAAA---------
TGAGCAACTTGTCT-GATGACTGGGAAAGGAGGAC---CTGCAACCATCTGACTTGGTCTCTG--TTAATGACGTCTCTCCCTCTAA-A----CCC-CATTAAGGACTGGGAGAGGCAGA-GCAAGCCTCAGAG
GATTACTGGCTGCACTCTGGGGGGCGGTTCTTCCA---TGATGGTGTTTCCTCTAAATTTGCA--CGGAGAAACACCTGATTTCCAGGAAA-ATCCCCTCAGATGGGCGCTGGTCCCATCCATTCCCGATGCCT
AGACCAGGCAAGACAACTGTGAGC-GCGATGGCCG---TGTACCCCAGGTCAGGGGTGGTGTC--TCTATGAAGGAGGGGCCCGAAG-----CCCTTGTGGGCGGGCCTCCCCTGAGCCCGTCTGTGGTGCCAG
CACTTCAGAAGGCT-TCTGAATGGAACCATCTCTT---GACA-TTTGTTTCTATA-ATATTTG--T-CATGACAGTCACAGCATAAA-G----CGCAGACGGCTGTGACCTGATTTAGA-AAATATTTTTAGA
```

same-colored boxes *should* be aligned

Pfold (KH03) Test Set D



Knudsen & Hein, Pfold: RNA secondary structure prediction using stochastic context-free grammars, Nucleic Acids Research, 2003, v 31,3423–3428

Approaches

Align-first: align sequences, then look for common structure

Fold-first: Predict structures, then try to align them

- single-seq struct prediction only ~ 60% accurate; exacerbated by flanking seq; no biologically-validated model for structural alignment

Joint: Do both together

- Sankoff – good but slow
- Heuristic

Our Approach: CMfinder

RNA motifs from unaligned sequences

Simultaneous *local* alignment, folding and CM-based motif description via an EM-style learning procedure

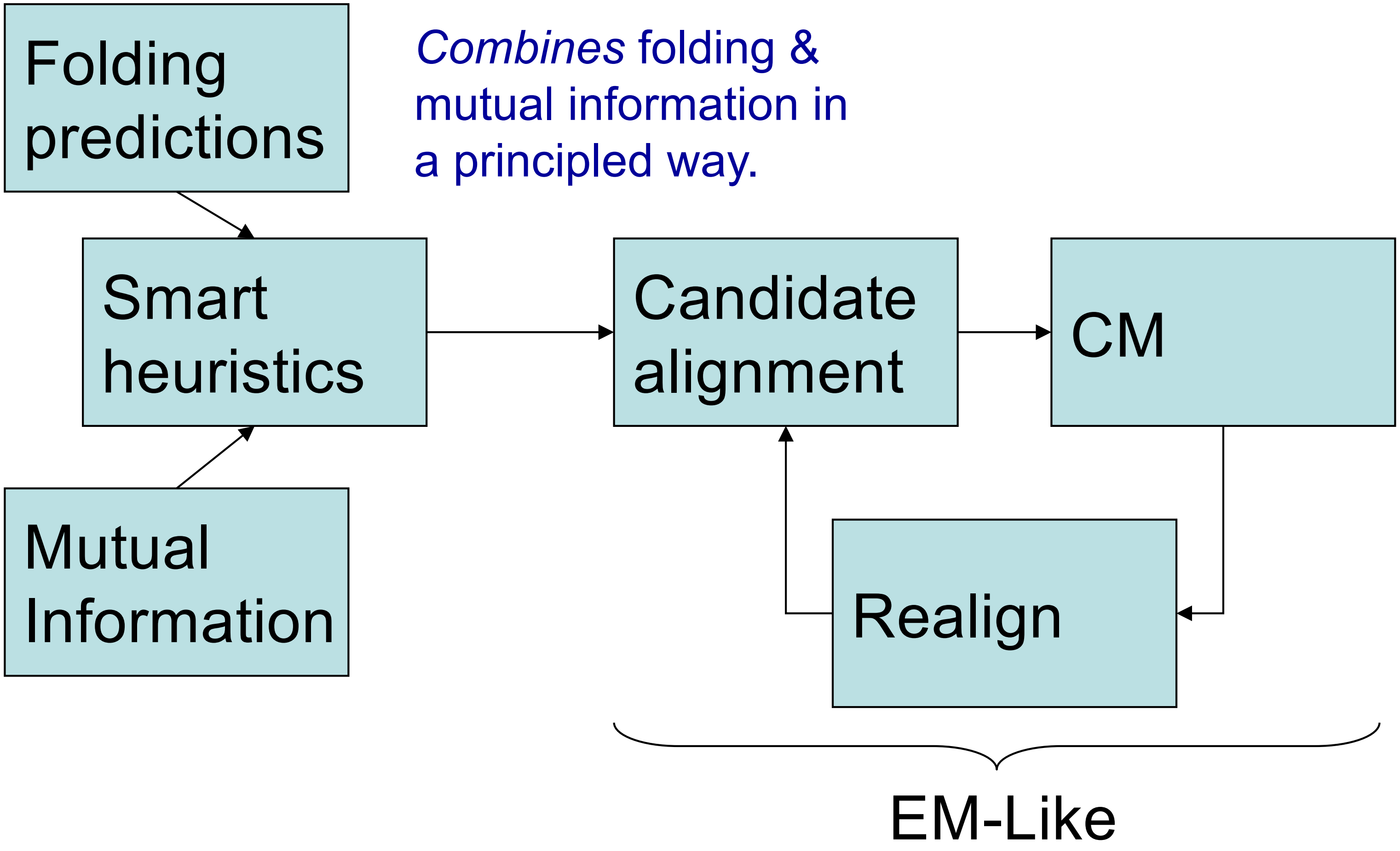
- Sequence conservation exploited, but not required
- Robust to inclusion of unrelated and/or flanking sequence
- Reasonably fast and scalable
- Produces a probabilistic model of the motif that can be directly used for homolog search

Yao, Weinberg & Ruzzo, *Bioinformatics*, 2006

CMFinder

Simultaneous alignment, folding & motif description

Yao, Weinberg & Ruzzo, *Bioinformatics*, 2006



Combines folding & mutual information in a principled way.

Initial Alignment Heuristics

fold sequences separately

candidates: regions with low folding energy

compare candidates via “tree edit” algorithm

find best “central” candidates & align to them

BLAST anchors

Structure Inference

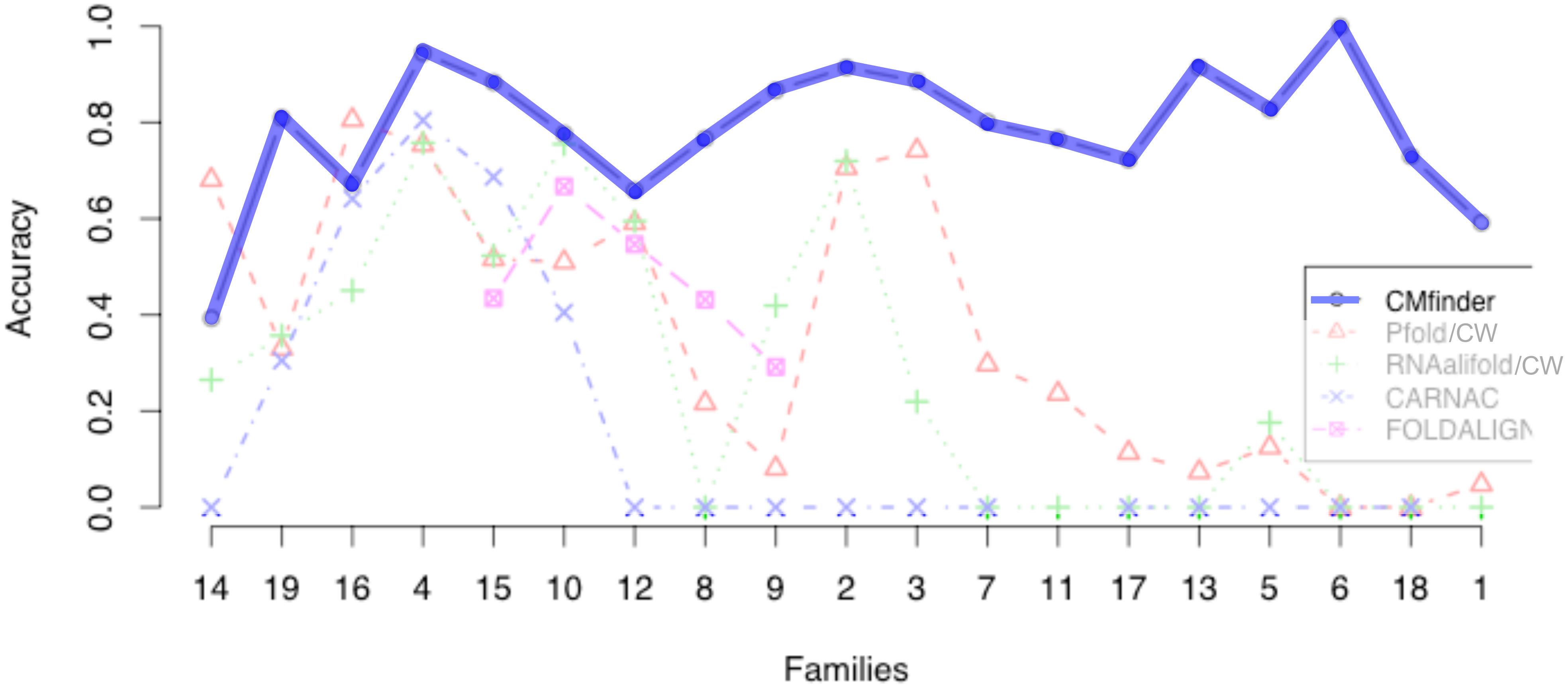
Part of M-step is to pick a structure that maximizes data likelihood

We combine:

- mutual information
- position-specific priors for paired/unpaired
  - (based on single sequence thermodynamic folding predictions)
- intuition: for similar seqs, little MI; fall back on single-sequence folding predictions
- data-dependent, so not strictly Bayesian
- Details: see paper

CMfinder Accuracy

(on Rfam families *with* flanking sequence)

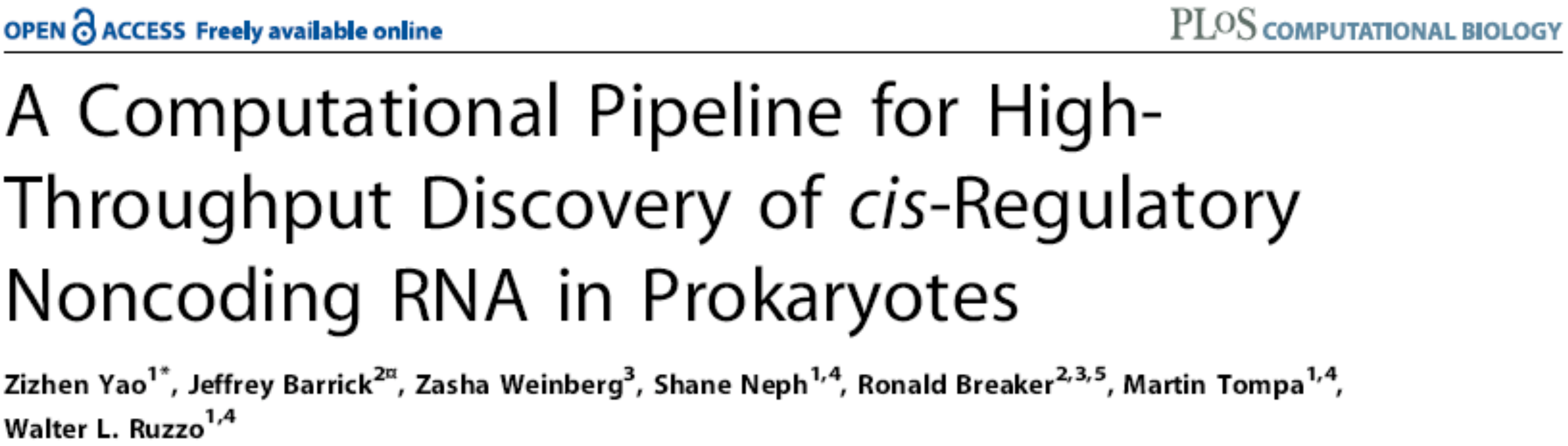


Summary of Rfam test families and results

ID	Family	Rfam ID	#seqs	%id	length	#hp	**CMfinder**	CW/Pfold	CW/RNAalifold	Carnac	Foldalign	ComRNA
1	Cobalamin	RF00174	71	49	216	4	**0.59**	0.05	0	X	-	0
2	ctRNA_pGA1	RF00236	17	74	83	2	**0.91**	0.70	0.72	0	0.86	0
3	Entero_CRE	RF00048	56	81	61	1	**0.89**	0.74	0.22	0	-	0
4	Entero_OriR	RF00041	35	77	73	2	**0.94**	0.75	0.76	0.80	0.52	0.52
5	glmS	RF00234	14	58	188	4	**0.83**	0.12	0.18	0	-	0.13
6	Histone3	RF00032	63	77	26	1	**1**	0	0	0	-	0
7	Intron_gpII	RF00029	75	55	92	2	**0.80**	0.30	0	0	-	0
8	IRE	RF00037	30	68	30	1	**0.77**	0.22	0	0	0.38	0
9	let-7	RF00027	9	69	84	1	**0.87**	0.08	0.42	0	0.71	0.78
10	lin-4	RF00052	9	69	72	1	**0.78**	0.51	0.75	0.41	0.65	0.24
11	Lysine	RF00168	48	48	183	4	**0.77**	0.24	0	X	-	0
12	mir-10	RF00104	11	66	75	1	**0.66**	0.59	0.60	0	0.48	0.33
13	Purine	RF00167	29	55	103	2	**0.91**	0.07	0	0	-	0.27
14	RFN	RF00050	47	66	139	4	0.39	**0.68**	0.26	0	-	0
15	Rhino_CRE	RF00220	12	71	86	1	**0.88**	0.52	0.52	0.69	0.41	0.61
16	s2m	RF00164	23	80	43	1	0.67	**0.80**	0.45	0.64	0.63	0.29
17	S_box	RF00162	64	66	112	3	**0.72**	0.11	0	0	-	0
18	SECIS	RF00031	43	43	68	1	**0.73**	0	0	0	-	0
19	Tymo_tRNA-like	RF00233	22	72	86	4	**0.81**	0.33	0.36	0.30	0.80	0.48
				Average Accuracy:			**0.79**	0.36	0.28	0.17	0.60	0.19
				Average Specificity:			0.81	0.42	0.57	**0.83**	0.60	0.65
				Average Sensitivity:			**0.77**	0.36	0.23	0.13	0.61	0.17

Min/Max in col **Bold** = best in row

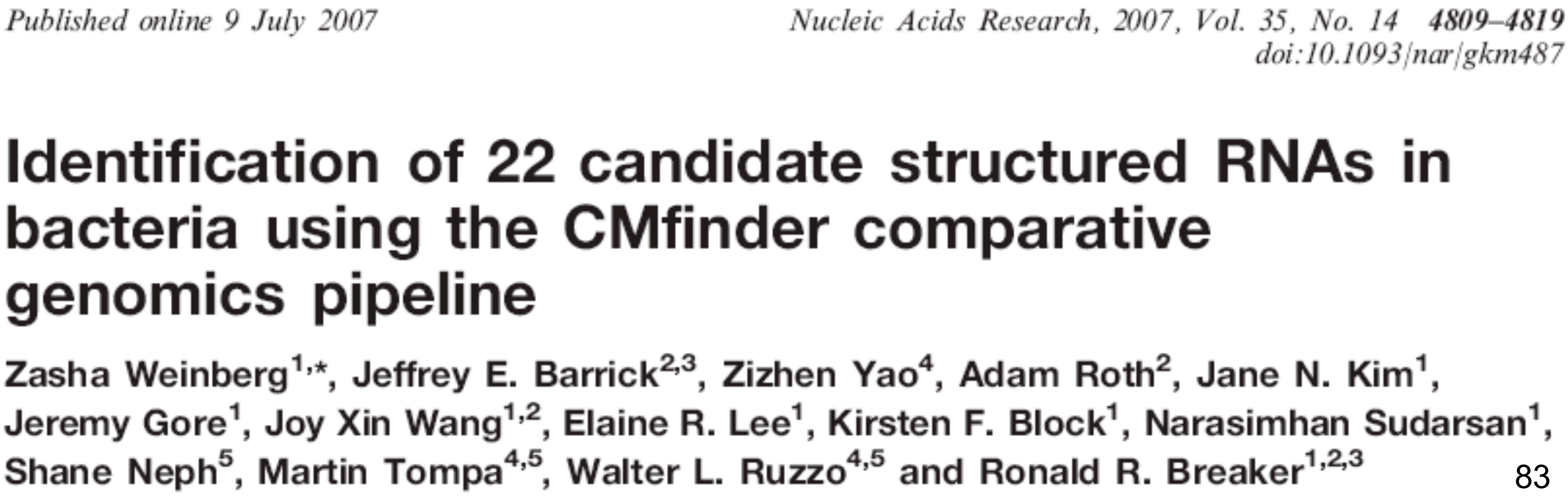
Discovery in Bacteria



OPEN ACCESS Freely available online PLoS COMPUTATIONAL BIOLOGY

A Computational Pipeline for High-Throughput Discovery of *cis*-Regulatory Noncoding RNA in Prokaryotes

Zizhen Yao[1*], Jeffrey Barrick[2¤], Zasha Weinberg[3], Shane Neph[1,4], Ronald Breaker[2,3,5], Martin Tompa[1,4], Walter L. Ruzzo[1,4]



Published online 9 July 2007 *Nucleic Acids Research, 2007, Vol. 35, No. 14* ***4809–4819***
doi:10.1093/nar/gkm487

Identification of 22 candidate structured RNAs in bacteria using the CMfinder comparative genomics pipeline

Zasha Weinberg[1,*], Jeffrey E. Barrick[2,3], Zizhen Yao[4], Adam Roth[2], Jane N. Kim[1], Jeremy Gore[1], Joy Xin Wang[1,2], Elaine R. Lee[1], Kirsten F. Block[1], Narasimhan Sudarsan[1], Shane Neph[5], Martin Tompa[4,5], Walter L. Ruzzo[4,5] and Ronald R. Breaker[1,2,3]

Predicting New *cis*-Regulatory RNA Elements

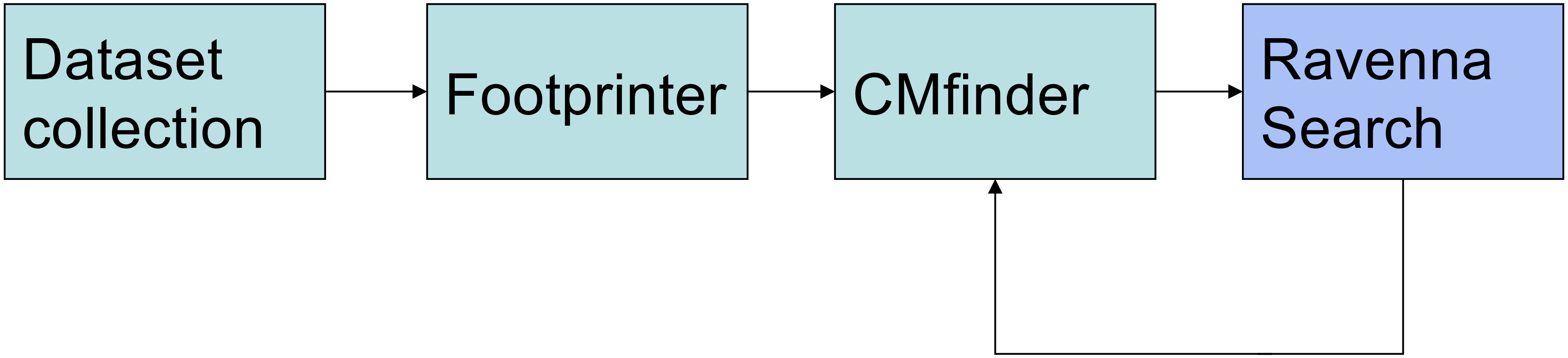
Goal:

- Given unaligned UTRs of coexpressed or orthologous genes, find common structural motifs

Difficulties:

- Low sequence similarity: alignment difficult
- Varying flanking sequence
- Motif missing from some input genes

Use the Right Data; Do Genome Scale Search



Right Data: Why/How

We can recognize, say, 5-10 good examples amidst 20 extraneous ones (but not 5 in 200 or 2000) of length 1k or 10k (but not 100k)

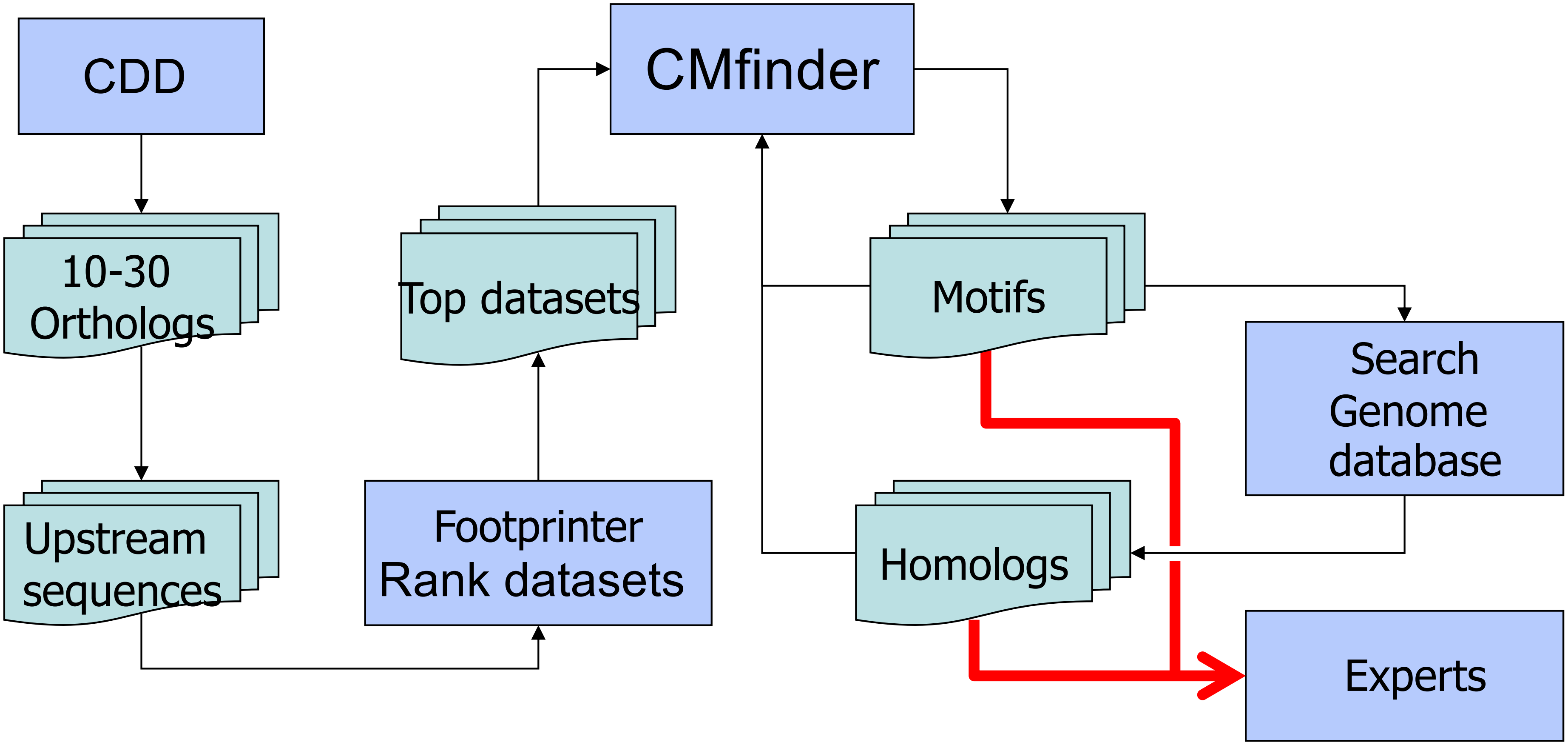
Regulators often near regulatees (protein coding genes), which are usually recognizable cross-species

So, look near similar genes ("homologs")

Many riboswitches, e.g., are present in ~5 copies per genome

(Not strategy used in vertebrates - 1000x larger genomes)

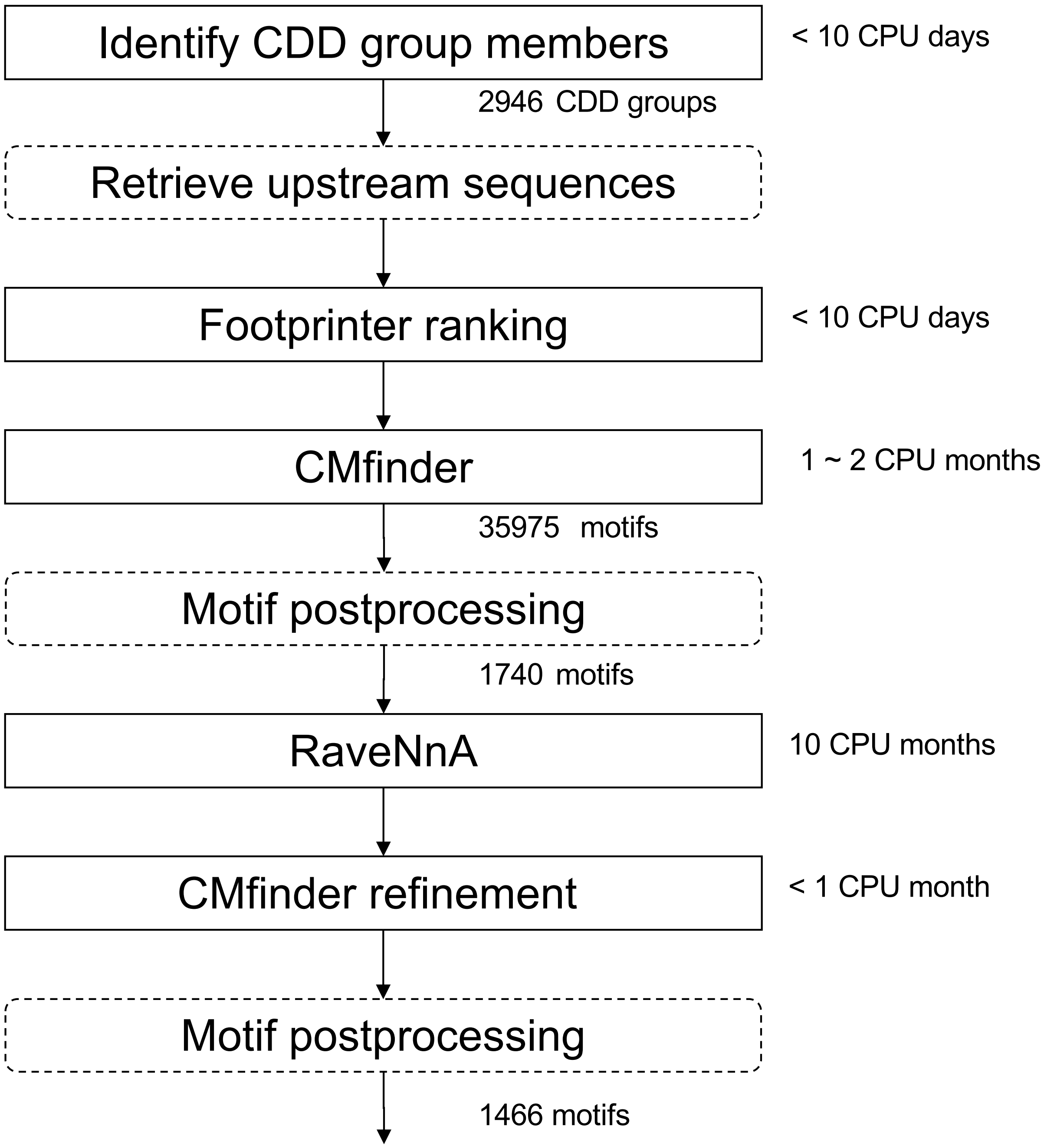
A pipeline for RNA motif genome scans



Yao, Barrick, Weinberg, Neph, Breaker, Tompa and Ruzzo. A Computational Pipeline for High Throughput Discovery of cis-Regulatory Noncoding RNA in Prokaryotes. PLoS Comput Biol. 3(7): e126, July 6, 2007.

Overall Pipeline & Processing Times

Input from ~70 complete Firmicute genomes available in late 2005-early 2006, totaling ~200 megabases



Identify CDD group members — < 10 CPU days

2946 CDD groups

Retrieve upstream sequences

Footprinter ranking — < 10 CPU days

CMfinder — 1 ~ 2 CPU months

35975 motifs

Motif postprocessing

1740 motifs

RaveNnA — 10 CPU months

CMfinder refinement — < 1 CPU month

Motif postprocessing

1466 motifs

Table 1: Motifs that correspond to Rfam families

Rank			Score	#		CDD			Rfam
RAV	CMF	FP		RAV	CMF	ID	Gene	Description	
0	43	107	3400	367	11	9904	IlvB	Thiamine pyrophosphate-requiring enzymes	RF00230 T-box
1	10	344	3115	96	22	13174	COG3859	Predicted membrane protein	RF00059 THI
2	77	1284	2376	112	6	11125	MetH	Methionine synthase I specific DNA methylase	RF00162 S_box
3	0	5	2327	30	26	9991	COG0116	Predicted N6-adenine-specific DNA methylase	RF00011 RNaseP_bact_b
4	6	66	2228	49	18	4383	DHBP	3,4-dihydroxy-2-butanone 4-phosphate synthase	RF00050 RFN
7	145	952	1429	51	7	10390	GuaA	GMP synthase	RF00167 Purine
8	17	108	1322	29	13	10732	GcvP	Glycine cleavage system protein P	RF00504 Glycine
9	37	749	1235	28	7	24631	DUF149	Uncharacterised BCR, YbaB family COG0718	RF00169 SRP_bact
10	123	1358	1222	36	6	10986	CbiB	Cobalamin biosynthesis protein CobD/CbiB	RF00174 Cobalamin
20	137	1133	899	32	7	9895	LysA	Diaminopimelate decarboxylase	RF00168 Lysine
21	36	141	896	22	10	10727	TerC	Membrane protein TerC	RF00080 yybP-ykoY
39	202	684	664	25	5	11945	MgtE	Mg/Co/Ni transporter MgtE	RF00380 ykoK
40	26	74	645	19	18	10323	GlmS	Glucosamine 6-phosphate synthetase	RF00234 glmS
53	208	192	561	21	5	10892	OpuBB	ABC-type proline/glycine betaine transport systems	RF00005 tRNA[1]
122	99	239	413	10	7	11784	EmrE	Membrane transporters of cations and cationic drug	RF00442 ykkC-yxkD
255	392	281	268	8	6	10272	COG0398	Uncharacterized conserved protein	RF00023 tmRNA

Table 1: Motifs that correspond to Rfam families. “Rank”: the three columns show ranks for refined motif clusters after genome scans (“RAV”), CMfinder motifs before genome scans (“CMF”), and FootPrinter results (“FP”). We used the same ranking scheme for RAV and CMF. “Score”

Rfam		Membership			Overlap			Structure		
		#	Sn	Sp	nt	Sn	Sp	bp	Sn	Sp
RF00174	Cobalamin	183	0.74[1]	0.97	152	0.75	0.85	20	0.60	0.77
RF00504	Glycine	92	0.56[1]	0.96	94	0.94	0.68	17	0.84	0.82
RF00234	glmS	34	0.92	1.00	100	0.54	1.00	27	0.96	0.97
RF00168	Lysine	80	0.82	0.98	111	0.61	0.68	26	0.76	0.87
RF00167	Purine	86	0.86	0.93	83	0.83	0.55	17	0.90	0.95
RF00050	RFN	133	0.98	0.99	139	0.96	1.00	12	0.66	0.65
RF00011	RNaseP_bact_b	144	0.99	0.99	194	0.53	1.00	38	0.72	0.78
RF00162	S_box	208	0.95	0.97	110	1.00	0.69	23	0.91	0.78
RF00169	SRP_bact	177	0.92	0.95	99	1.00	0.65	25	0.89	0.81
RF00230	T-box	453	0.96	0.61	187	0.77	1.00	5	0.32	0.38
RF00059	THI	326	0.89	1.00	99	0.91	0.69	13	0.56	0.74
RF00442	ykkC-yxkD	19	0.90	0.53	99	0.94	0.81	18	0.94	0.68
RF00380	ykoK	49	0.92	1.00	125	0.75	1.00	27	0.80	0.95
RF00080	yybP-ykoY	41	0.32	0.89	100	0.78	0.90	18	0.63	0.66
mean		145	0.84	0.91	121	0.81	0.82	21	0.75	0.77
median		113	0.91	0.97	105	0.81	0.83	19	0.78	0.78

Tbl 2: Prediction accuracy compared to prokaryotic subset of Rfam full alignments.

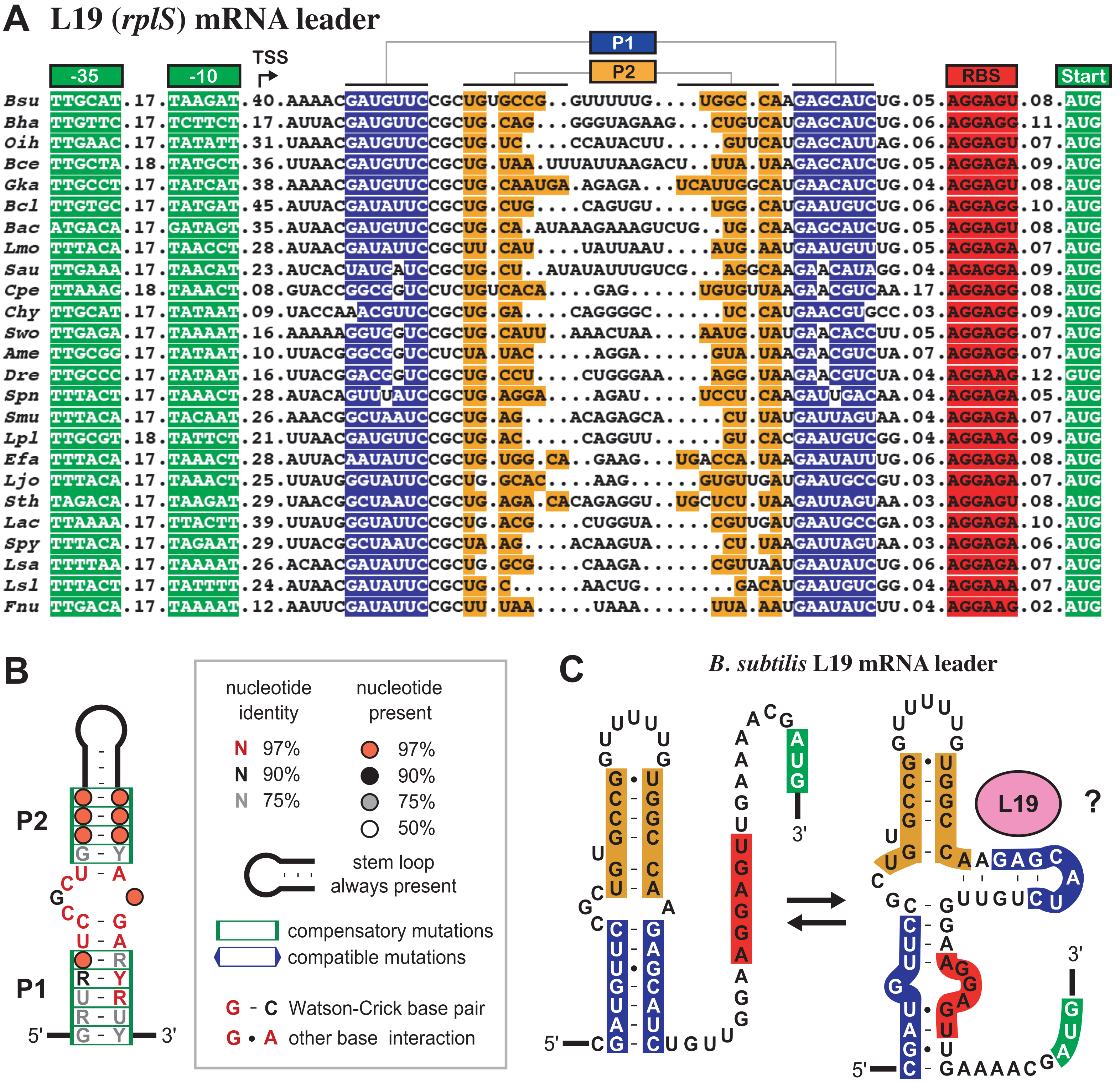
Membership: # of seqs in overlap between our predictions and Rfam's, the sensitivity (Sn) and specificity (Sp) of our membership predictions. Overlap: the avg len of overlap between our predictions and Rfam's (nt), the fractional lengths of the overlapped region in Rfam's predictions (Sn) and in ours (Sp). Structure: the avg # of correctly predicted canonical base pairs (in overlapped regions) in the secondary structure (bp), and sensitivity and specificity of our predictions. [1]After 2nd RaveNnA scan, membership Sn of Glycine, Cobalamin increased to 76% and 98% resp., Glycine Sp unchanged, but Cobalamin Sp dropped to 84%.

Table 3: High ranking motifs not found in Rfam

Rank	#	CDD	Gene: Description	Annotation
6	69	28178	DHOase IIa: Dihydroorotase	PyrR attenuator [22]
15	33	10097	RplL: Ribosomal protein L7/L1	L10 r-protein leader; see Supp
19	36	10234	RpsF: Ribosomal protein S6	S6 r-protein leader
22	32	10897	COG1179: Dinucleotide-utilizing enzymes	6S RNA [25]
27	27	9926	RpsJ: Ribosomal protein S10	S10 r-protein leader; see Supp
29	11	15150	Resolvase: N terminal domain	
31	31	10164	InfC: Translation initiation factor 3	IF-3 r-protein leader; see Supp
41	26	10393	RpsD: Ribosomal protein S4 and related proteins	S4 r-protein leader; see Supp [30]
44	30	10332	GroL: Chaperonin GroEL	HrcA DNA binding site [46]
46	33	25629	Ribosomal L21p: Ribosomal prokaryotic L21 protein	L21 r-protein leader; see Supp
50	11	5638	Cad: Cadmium resistance transporter	[47]
51	19	9965	RplB: Ribosomal protein L2	S10 r-protein leader
55	7	26270	RNA pol Rpb2 1: RNA polymerase beta subunit	
69	9	13148	COG3830: ACT domain-containing protein	
72	28	4174	Ribosomal S2: Ribosomal protein S2	S2 r-protein leader
74	9	9924	RpsG: Ribosomal protein S7	S12 r-protein leader
86	6	12328	COG2984: ABC-type uncharacterized transport system	
88	19	24072	CtsR: Firmicutes transcriptional repressor of class III	CtsR DNA binding site [48]
100	21	23019	Formyl trans N: Formyl transferase	
103	8	9916	PurE: Phosphoribosylcarboxyaminoimidazole	
117	5	13411	COG4129: Predicted membrane protein	
120	10	10075	RplO: Ribosomal protein L15	L15 r-protein leader
121	9	10132	RpmJ: Ribosomal protein L36	IF-1 r-protein leader
129	4	23962	Cna B: Cna protein B-type domain	
130	9	25424	Ribosomal S12: Ribosomal protein S12	S12 r-protein leader
131	9	16769	Ribosomal L4: Ribosomal protein L4/L1 family	L3 r-protein leader
136	7	10610	COG0742: N6-adenine-specific methylase	ylbH putative RNA motif [4]
140	12	8892	Pencillinase R: Penicillinase repressor	BlaI, MecI DNA binding site [49]
157	25	24415	Ribosomal S9: Ribosomal protein S9/S16	L13 r-protein leader; Fig 3
160	27	1790	Ribosomal L19: Ribosomal protein L19	L19 r-protein leader; Fig 2
164	6	9932	GapA: Glyceraldehyde-3-phosphate dehydrogenase/erythrose	
174	8	13849	COG4708: Predicted membrane protein	
176	7	10199	COG0325: Predicted enzyme with a TIM-barrel fold	
182	9	10207	RpmF: Ribosomal protein L32	L32 r-protein leader
187	11	27850	LDH: L-lactate dehydrogenases	
190	11	10094	CspR: Predicted rRNA methylase	
194	9	10353	FusA: Translation elongation factors	EF-G r-protein leader

Example: Ribosomal Autoregulation:

Excess L19 represses L19 (RF00556; 555-559 similar)



A L19 (*rplS*) mRNA leader

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    -35        -10      TSS                                   P1/P2                                           RBS         Start
Bsu TTGCAT.17.TAAGAT.40.AAAACGAUGUUCCGCUGUGCCG..GUUUUUG....UGGC.CAAGAGCAUCUG.05.AGGAGU.08.AUG
Bha TTGTTC.17.TCTTCT.17.AUUACGAUGUUCCGCUG.CAG...GGGUAGAAG...CUGUCAUGAGCAUCUG.06.AGGAGG.11.AUG
Oih TTGAAC.17.TATATT.31.UAAACGAUGUUCCGCUG.UC....CCAUACUU.....GUUCAUGAGCAUUAG.06.AGGAGU.07.AUG
Bce TTGCTA.18.TATGCT.36.UUAACGAUGUUCCGCUG.UAA.UUUAUUAAGACU..UUA.UAAGAGCAUCUG.05.AGGAGA.09.AUG
Gka TTGCCT.17.TATCAT.38.AAAACGAUGUUCCGCUG.CAAUGA.AGAGA...UCAUUGGCAUGAACAUCUG.04.AGGAGU.08.AUG
Bcl TTGTGC.17.TATGAT.45.AUUACGAUAUUCCGCUG.CUG....CAGUGU.....UGG.CAUGAAUGUCUG.06.AGGAGG.10.AUG
Bac ATGACA.17.GATAGT.35.AUAACGAUGUUCCGCUG.CA.AUAAAGAAAGUCUG..UG.CAAGAGCAUCUG.05.AGGAGU.08.AUG
Lmo TTTACA.17.TAACCT.28.AUAACGAUAUUCCGCUU.CAU....UAUUAAU....AUG.AAUGAAUGUUUG.05.AGGAGA.07.AUG
Sau TTGAAA.17.TAACAT.23.AUCACUAUGAUCCGCUG.CU..AUAUAUUUGUCG...AGGCAAGAACAUAGG.04.AGAGGA.09.AUG
Cpe TTAAAG.18.TAAACT.08.GUACCGGCGGUCCUCUGUCACA....GAG......UGUGUUAAGAACGUCAA.17.AGGAGG.08.AUG
Chy TTGCAT.17.TATAAT.09.UACCAAACGUUCCGCUG.GA....CAGGGGC......UC.CAUGAACGUGCC.03.AGGAGG.09.AUG
Swo TTGAGA.17.TAAAAT.16.AAAAAGGUGGUCCGCUG.CAUU..AAACUAA....AAUG.UAUGAACACCUU.05.AGGAGG.07.AUG
Ame TTGCGG.17.TATAAT.10.UUACGGGCGGUCCUCUA.UAC.....AGGA......GUA.UAAGAACGUCUA.07.AGGAGG.07.AUG
Dre TTGCCC.17.TATAAT.16.UUACGGACGGUCCGCUG.CCU....CUGGGAA....AGG.UAAGAACGUCUA.04.AGGAAG.12.GUG
Spn TTTACT.17.TAAACT.28.AUACAGUUUAUCCGCUG.AGGA....AGAU.....UCCU.CAAGAUUGACAA.04.AGGAGA.05.AUG
Smu TTTACA.17.TACAAT.26.AAACGGCUAAUCCGCUG.AG....ACAGAGCA.....CU.UAUGAUUAGUAA.04.AGGAGA.07.AUG
Lpl TTGCGT.18.TATTCT.21.UUAACGAUGUUCCGCUG.AC.....CAGGUU......GU.CACGAAUGUCGG.04.AGGAAG.09.AUG
Efa TTTACA.17.TAAACT.28.AUUACAAUAUUCCGCUG.UGG.CA..GAAG...UGACCA.UAAGAAUAUUUG.06.AGGAGA.08.AUG
Ljo TTTACA.17.TAAACT.25.UUAUGGGUAUUCCGCUG.GCAC....AAG......GUGUUGAUGAAUGCCGU.03.AGGAGA.07.AUG
Sth TAGACA.17.TAAGAT.29.UAACGGCUAAUCCGCUG.AGA.CACAGAGGU..UGCUCU.UAAGAUUAGUAA.03.AGGAGU.08.AUG
Lac TTAAAA.17.TTACTT.39.UUAUGGGUAUUCCGCUG.ACG....CUGGUA.....CGUUGAUGAAUGCCGA.03.AGGAGA.10.AUG
Spy TTTACA.17.TAGAAT.29.UUACGGCUAAUCCGCUA.AG....ACAAGUA......CU.UAAGAUUAGUAA.03.AGGAGA.06.AUG
Lsa TTTTAA.17.TAAAAT.26.ACAACGAUAUUCCGCUG.GCG....CAAGA......CGUUAAUGAAUAUCUG.06.AGGAGA.07.AUG
Lsl TTTACT.17.TATTTT.24.AUAACGAUAUUCCGCUG.C......AACUG........GACAUGAAUGUCGG.04.AGGAAA.07.AUG
Fnu TTGACA.17.TAAAAT.12.AAUUCGAUAUUCCGCUU.UAA....UAAA......UUA.AAUGAAUAUCUU.04.AGGAAG.02.AUG
```

B

nucleotide identity

N 97%
N 90%
N 75%

nucleotide present

● 97%
● 90%
● 75%
○ 50%

stem loop always present

compensatory mutations

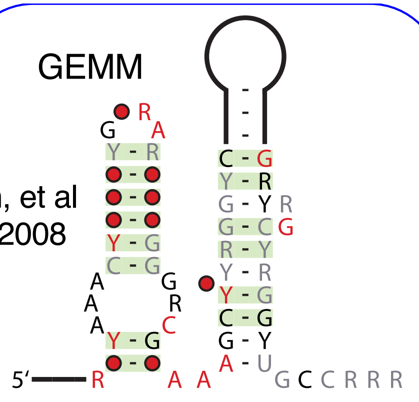
compatible mutations

G - C Watson-Crick base pair

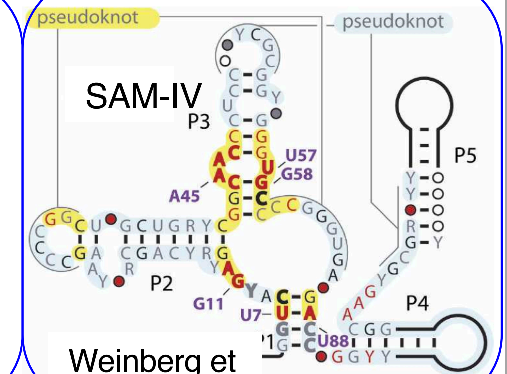
G • A other base interaction

C *B. subtilis* L19 mRNA leader

Examples: 6 (of 22) Representative motifs



Sudarsan, et al *Science*, 2008



Weinberg et al *RNA* '08

Legend

nt: nucleotides, SD: Shine-Dalgarno
start: start codon, R: A/G, Y: C/U

nucleotide identity	
N	97%
N	90%
N	75%

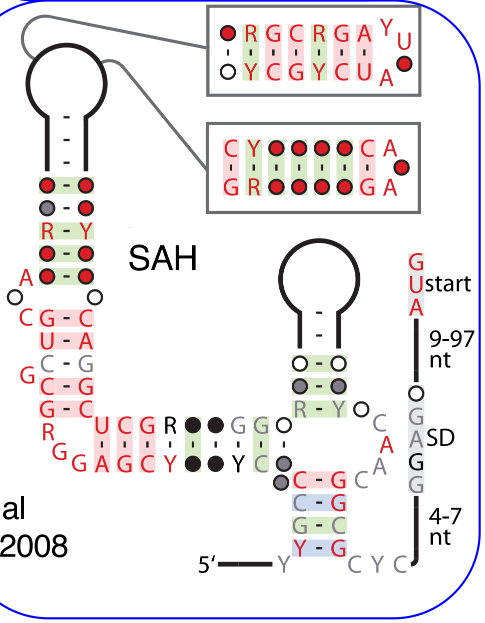
base pair annotations	
🟩	has covarying mutations
🟦	has compatible mutations
🟥	no mutations observed

nucleotide present	
●	97%
●	90%
●	75%
○	50%

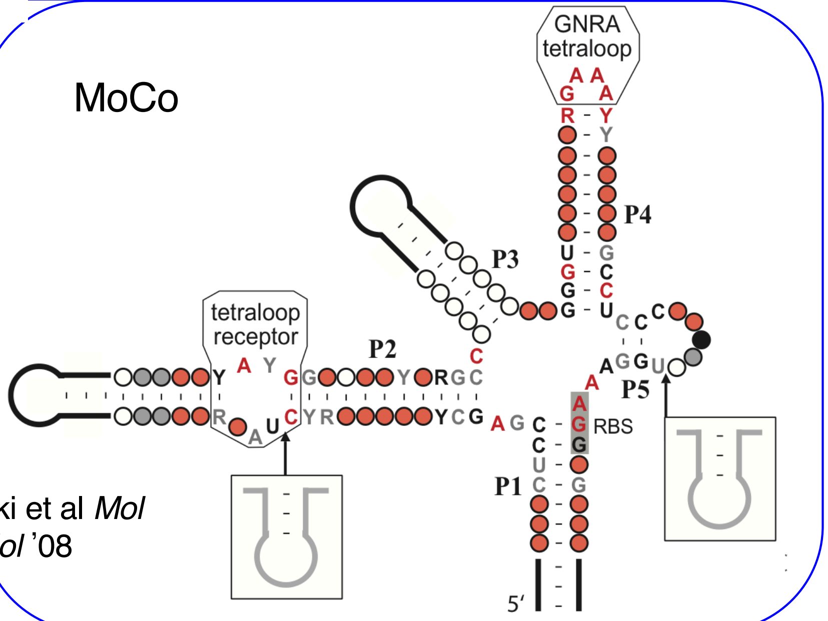
variable hairpin

variable loop

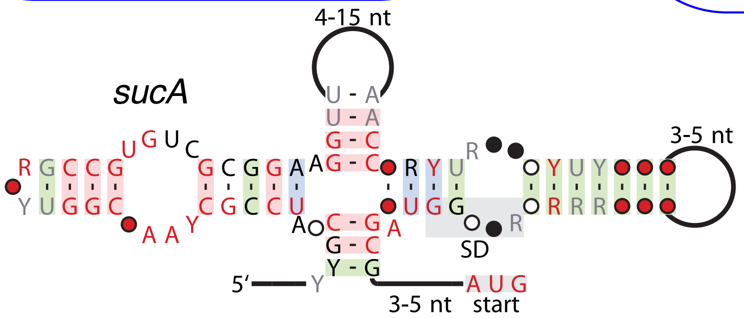
□ modular structure

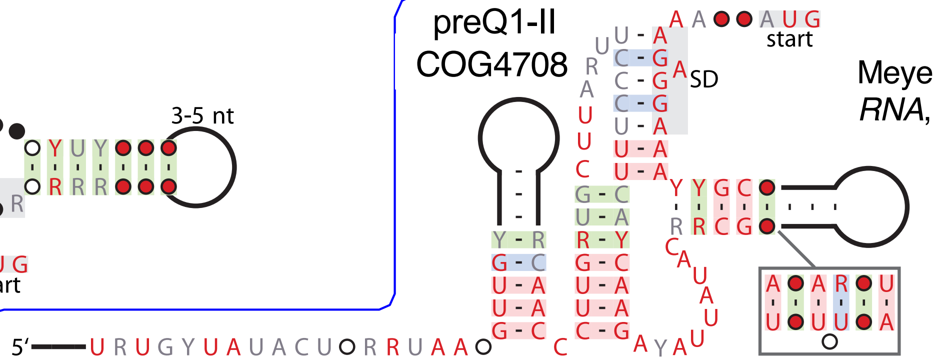


Wang, et al *Mol Cell*, 2008



Regulski et al *Mol Microbiol* '08





Meyer, et al *RNA*, 2008

boxed = confirmed riboswitch

Weinberg, et al. Nucl. Acids Res., July 2007 35: 4809-4819.

ncRNA Summary

ncRNA is a “hot” topic

For family homology modeling: CMs

Training & search like HMM (but slower)

Dramatic acceleration possible

Automated model construction possible

New computational methods yield new discoveries

Many open problems