RNA Search and Motif Discovery

Lectures 17-19

CSE 527

Autumn 2006

The Human Parts List, circa 2001

3 billion nucleotides, containing:

- 25,000 protein-coding genes (only ~1% of the DNA)
- Messenger RNAs made from each
- Plus a double-handful of other RNA genes

Noncoding RNAs

Dramatic discoveries in last 5 years

- *100s* of new families
- Many roles: Regulation, transport, stability, catalysis, …

1% of DNA codes for protein, but 30% of it is copied into RNA, i.e. ncRNA >> mRNA

Outline

Task 1: RNA 2^{ary} Structure Prediction (last time)

Task 2: RNA Motif Models

- Covariance Models
- Training & "Mutual Information"

Task 3: Search

- Rigorous & heuristic filtering

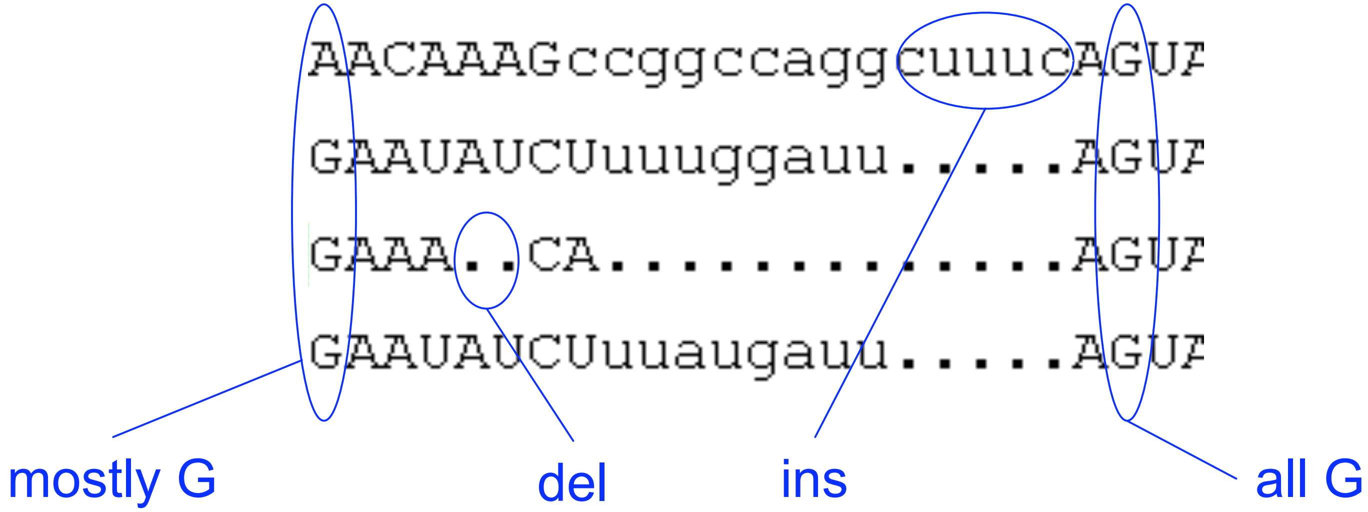
Task 4: Motif discovery

Task 2: Motif Description

How to model an RNA "Motif"?

Conceptually, start with a profile HMM:

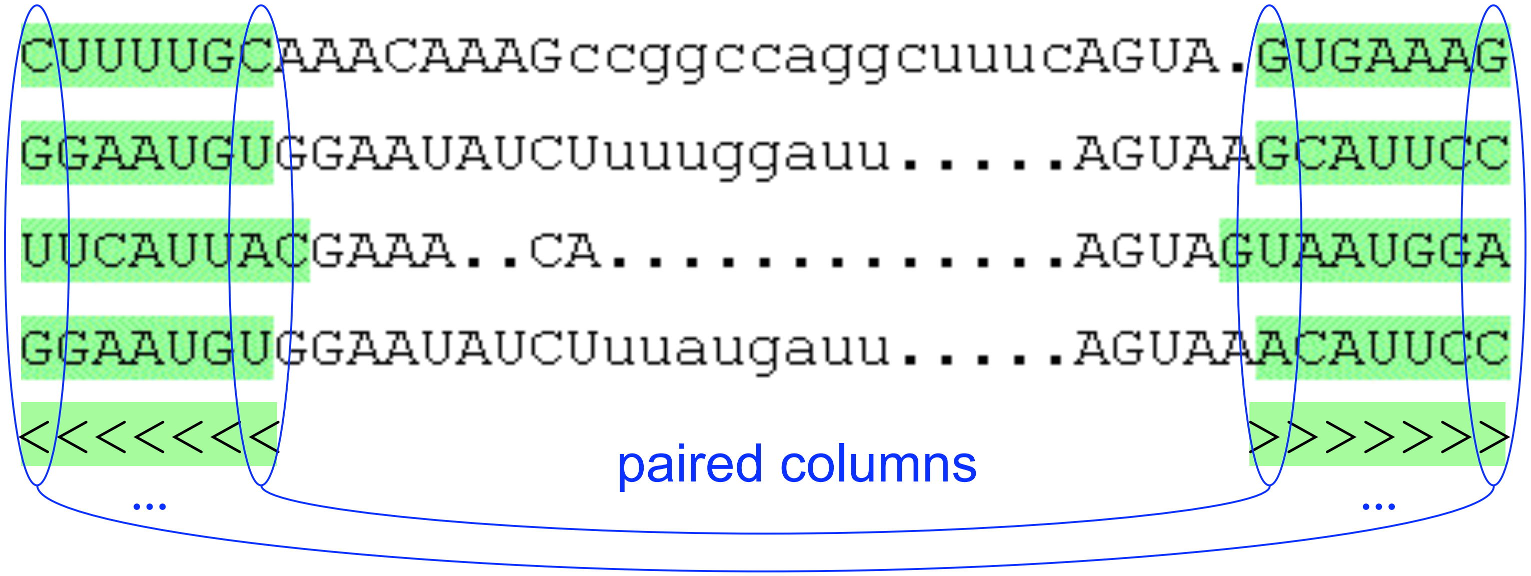
- from a multiple alignment, estimate nucleotide/ insert/delete preferences for each position
- given a new seq, estimate likelihood that it could be generated by the model, & align it to the model



```
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
<<<<<<<                          >>>>>>>
```

RNA Motif Models

“Covariance Models” (Eddy & Durbin 1994)

aka profile stochastic context-free grammars

aka hidden Markov models on steroids

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

Pro: accurate

Con: model building hard, search sloooow

“RNA sequence analysis using covariance models”

Eddy & Durbin

Nucleic Acids Research, 1994
vol 22 #11, 2079-2088

(see also, Ch 10 of Durbin *et al.*)

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 - current favorite)

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

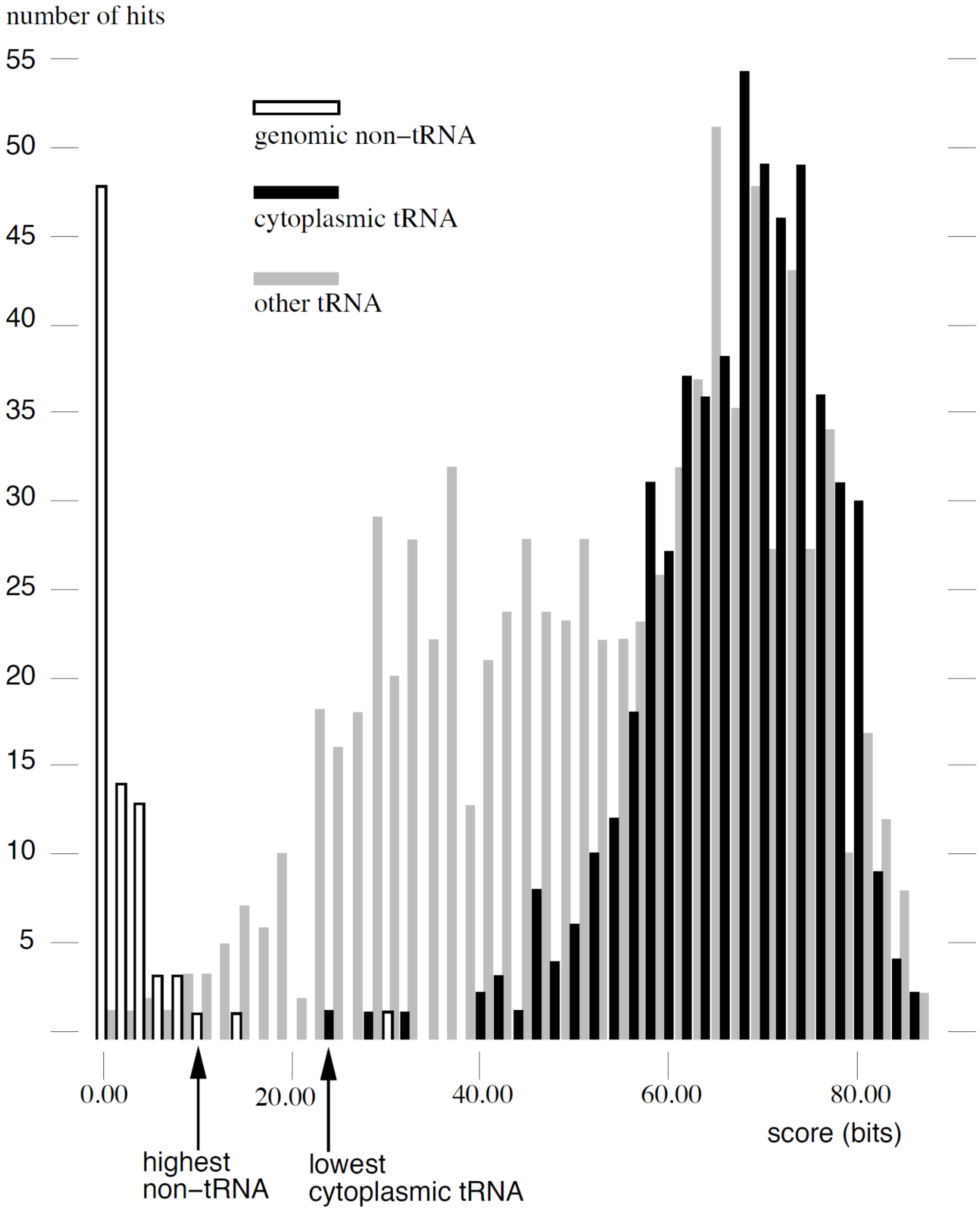
You set a score threshold

Anything above threshold → a "hit"

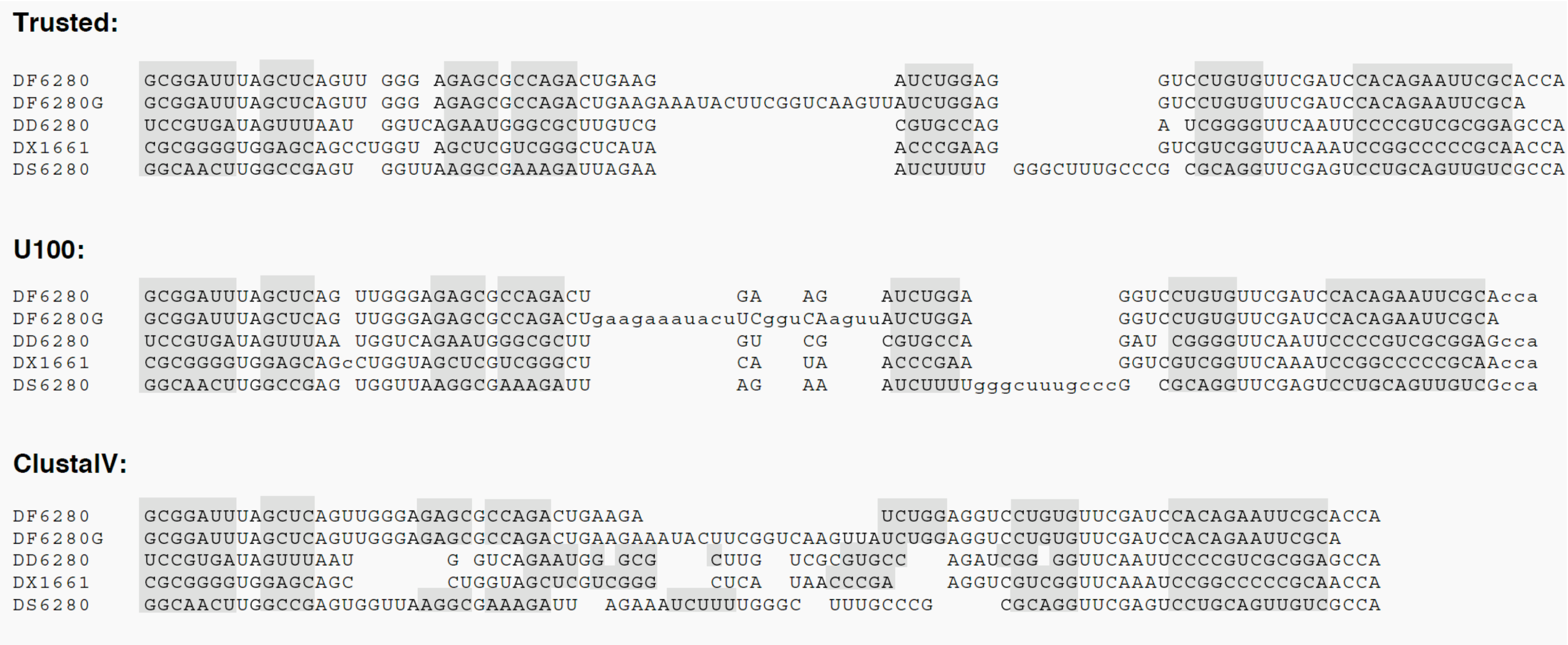
Scoring:

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

Example: searching for tRNAs



Alignment Quality



Trusted:

```
DF6280    GCGGAUUUAGCUCAGUU GGG AGAGCGCCAGACUGAAG                  AUCUGGAG            GUCCUGUGUUCGAUCCACAGAAUUCGCACCA
DF6280G   GCGGAUUUAGCUCAGUU GGG AGAGCGCCAGACUGAAGAAAUACUUCGGUCAAGUUAUCUGGAG            GUCCUGUGUUCGAUCCACAGAAUUCGCA
DD6280    UCCGUGAUAGUUUAAU  GGUCAGAAUGGGCGCUUGUCG                  CGUGCCAG            A UCGGGGUUCAAUUCCCCGUCGCGGAGCCA
DX1661    CGCGGGGUGGAGCAGCCUGGU AGCUCGUCGGGCUCAUA                  ACCCGAAG            GUCGUCGGUUCAAAUCCGGCCCCCGCAACCA
DS6280    GGCAACUUGGCCGAGU  GGUUAAGGCGAAAGAUUAGAA                  AUCUUUU  GGGCUUUGCCCG CGCAGGUUCGAGUCCUGCAGUUGUCGCCA
```

U100:

```
DF6280    GCGGAUUUAGCUCAG UUGGGAGAGCGCCAGACU           GA   AG    AUCUGGA           GGUCCUGUGUUCGAUCCACAGAAUUCGCAcca
DF6280G   GCGGAUUUAGCUCAG UUGGGAGAGCGCCAGACUgaagaaauacuUCgguCAaguuAUCUGGA           GGUCCUGUGUUCGAUCCACAGAAUUCGCA
DD6280    UCCGUGAUAGUUUAA UGGUCAGAAUGGGCGCUU           GU   CG    CGUGCCA           GAU CGGGGUUCAAUUCCCCGUCGCGGAGcca
DX1661    CGCGGGGUGGAGCAGcCUGGUAGCUCGUCGGGCU           CA   UA    ACCCGAA           GGUCGUCGGUUCAAAUCCGGCCCCCGCAAcca
DS6280    GGCAACUUGGCCGAG UGGUUAAGGCGAAAGAUU           AG   AA    AUCUUUUgggcuuugcccG  CGCAGGUUCGAGUCCUGCAGUUGUCGcca
```

ClustalV:

```
DF6280    GCGGAUUUAGCUCAGUUGGGAGAGCGCCAGACUGAAGA                  UCUGGAGGUCCUGUGUUCGAUCCACAGAAUUCGCACCA
DF6280G   GCGGAUUUAGCUCAGUUGGGAGAGCGCCAGACUGAAGAAAUACUUCGGUCAAGUUAUCUGGAGGUCCUGUGUUCGAUCCACAGAAUUCGCA
DD6280    UCCGUGAUAGUUUAAU       G GUCAGAAUGG GCG    CUUG  UCGCGUGCC   AGAUCGG GGUUCAAUUCCCCGUCGCGGAGCCA
DX1661    CGCGGGGUGGAGCAGC       CUGGUAGCUCGUCGGG    CUCA  UAACCCGA    AGGUCGUCGGUUCAAAUCCGGCCCCCGCAACCA
DS6280    GGCAACUUGGCCGAGUGGUUAAGGCGAAAGAUU  AGAAAUCUUUUGGGC  UUUGCCCG     CGCAGGUUCGAGUCCUGCAGUUGUCGCCA
```

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

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

Slightly different
evaluation criteria

Profile Hmm Structure

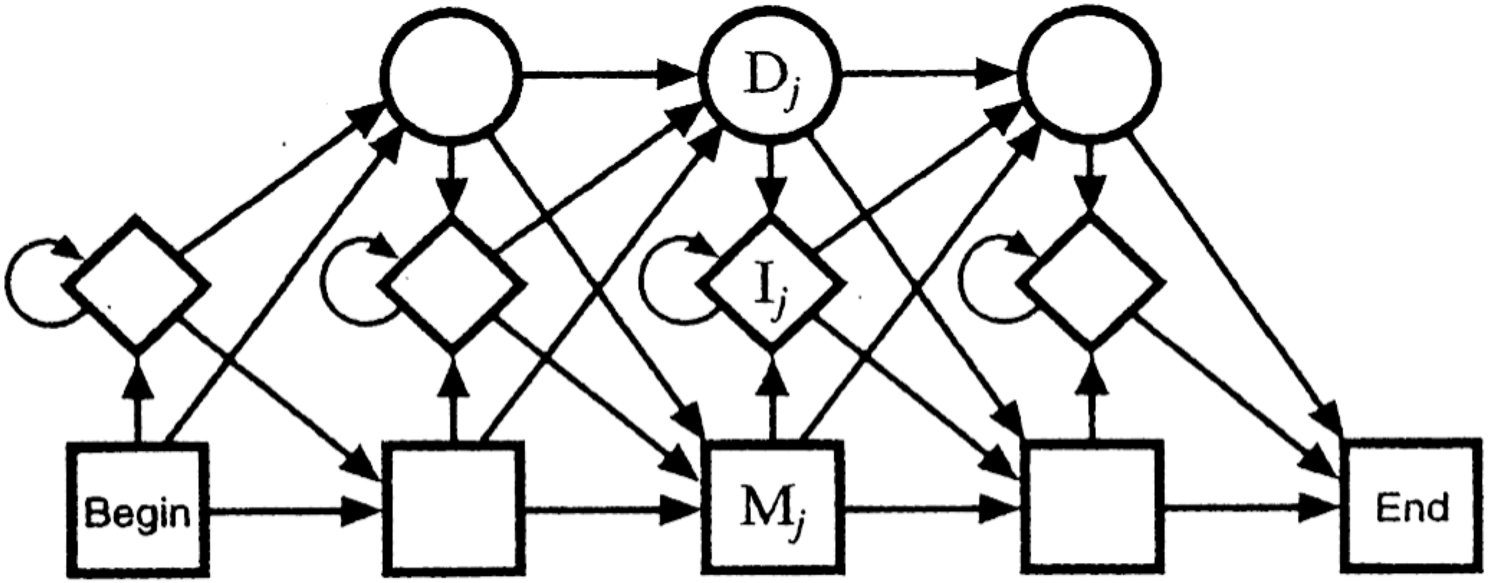


Figure 5.2 *The transition structure of a profile HMM.*

M_j: Match states (20 emission probabilities)

I_j: Insert states (Background emission probabilities)

D_j: Delete states (silent - no emission)

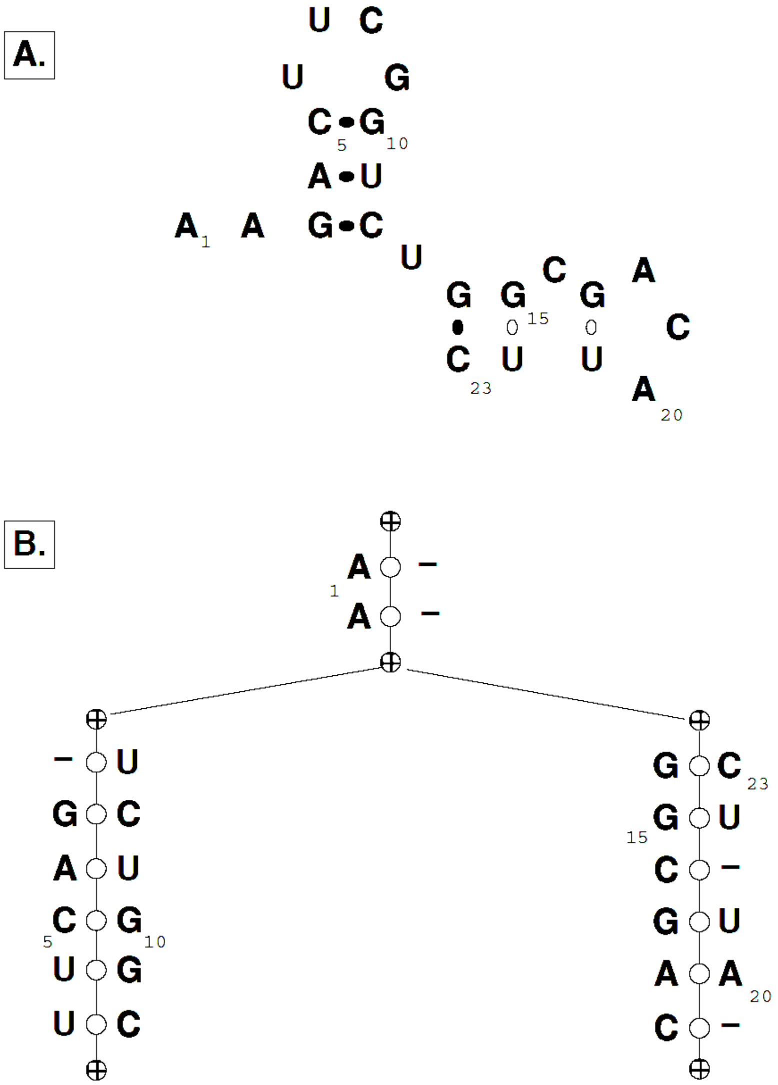
CM Structure

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)

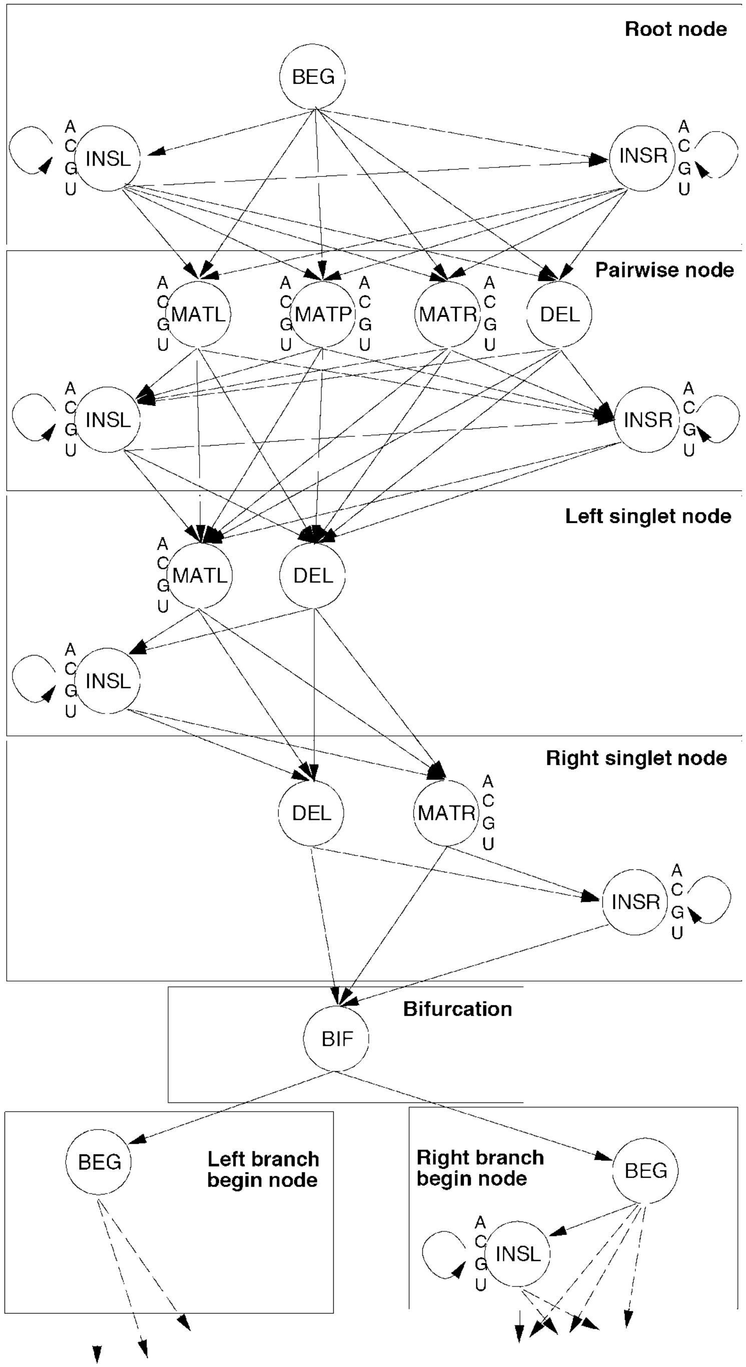


Overall CM Architecture

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

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

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

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

CM Viterbi Alignment

(the “inside” algorithm)

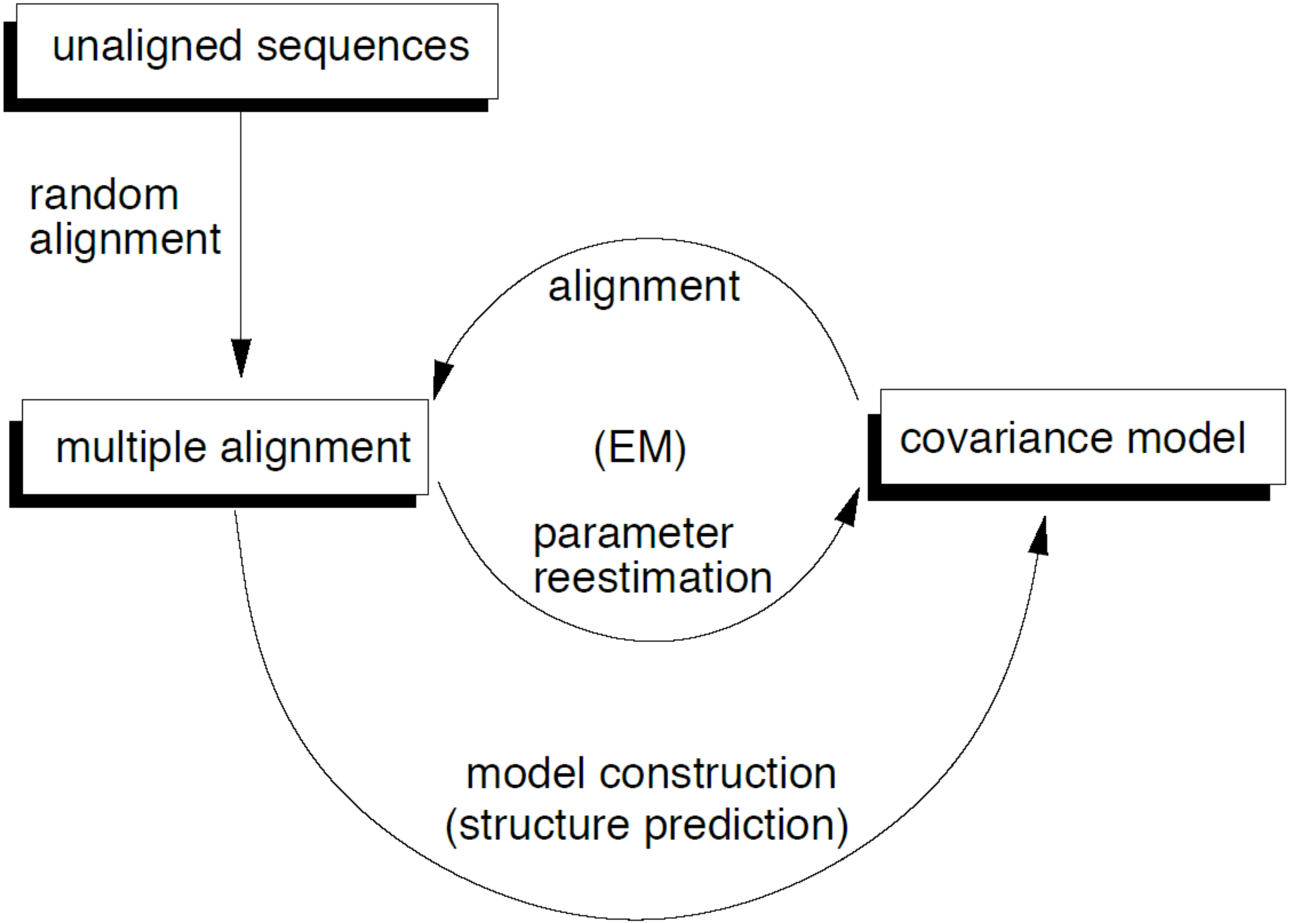
$$S_{ij}^{y} = \max_{\pi} \log P(x_{ij} \text{ generated starting in state } y \text{ via path } \pi)$$

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

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

compare: O(qn) for profile HMM

Model Training



unaligned sequences

random alignment

multiple alignment

alignment

(EM)

parameter reestimation

covariance model

model construction (structure prediction)

Mutual Information

$$M_{ij} = \sum_{xi,xj} f_{xi,xj} \log_2 \frac{f_{xi,xj}}{f_{xi} f_{xj}}; \quad 0 \le M_{ij} \le 2$$

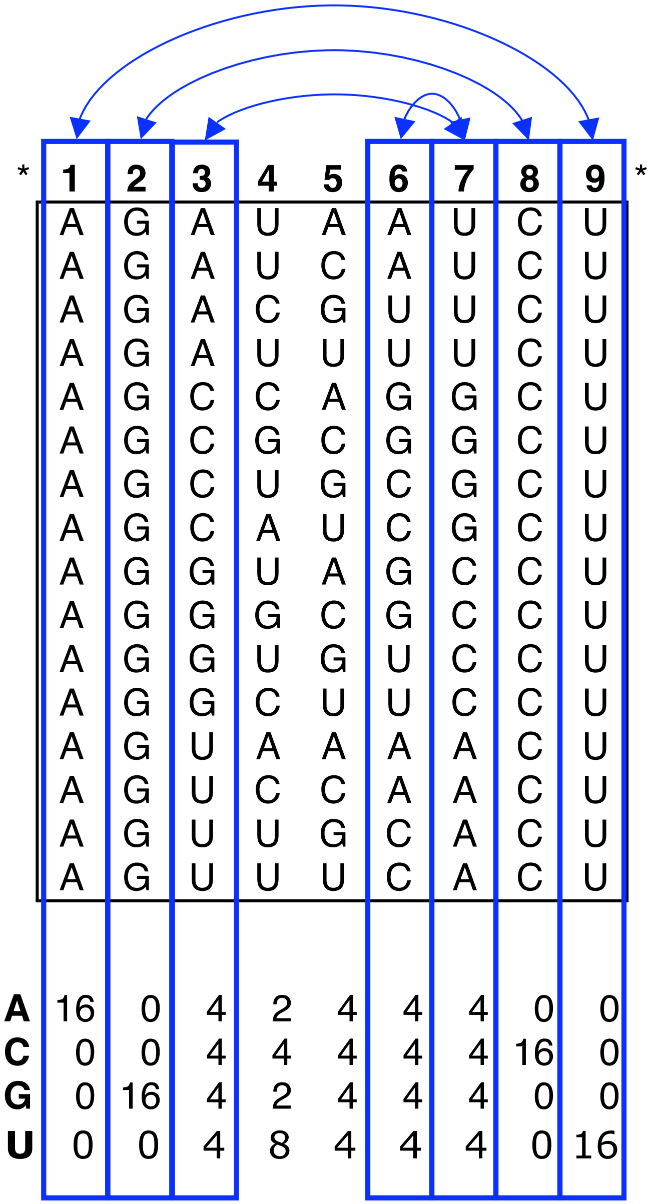
Max when *no* seq conservation but perfect pairing

MI = expected score gain from using a pair state

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	

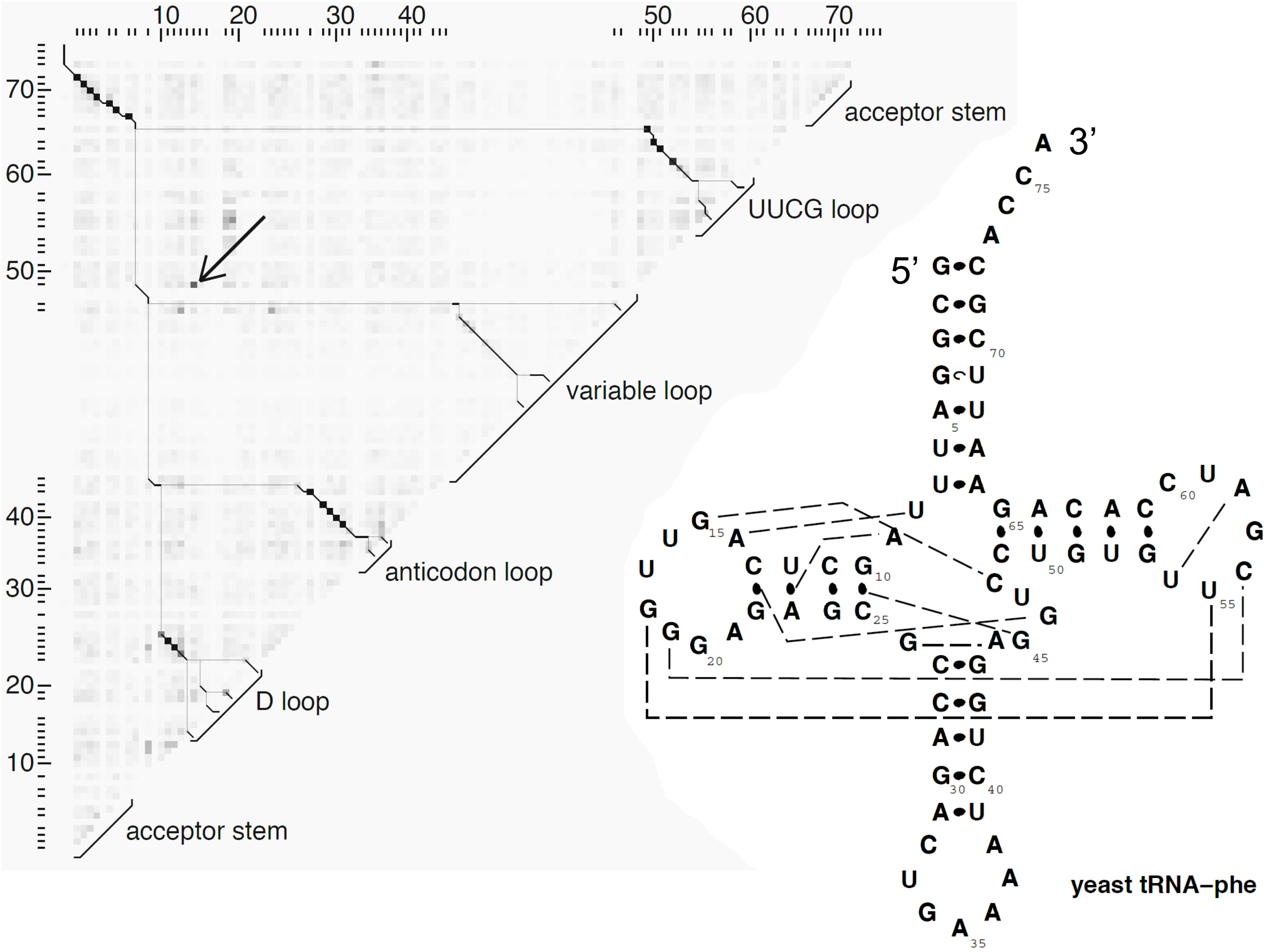
A	16	0	4	2	4	4	4	0	0
C	0	0	4	4	4	4	4	16	0
G	0	16	4	2	4	4	4	0	0
U	0	0	4	8	4	4	4	0	16

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.


10
20
30
40
50
60
70
acceptor stem
UUCG loop
variable loop
anticodon loop
D loop
acceptor stem
5’
3’
yeast tRNA–phe

MI-Based Structure-Learning

Find best (max total MI) subset of column pairs among i…j, subject to absence of pseudo-knots

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

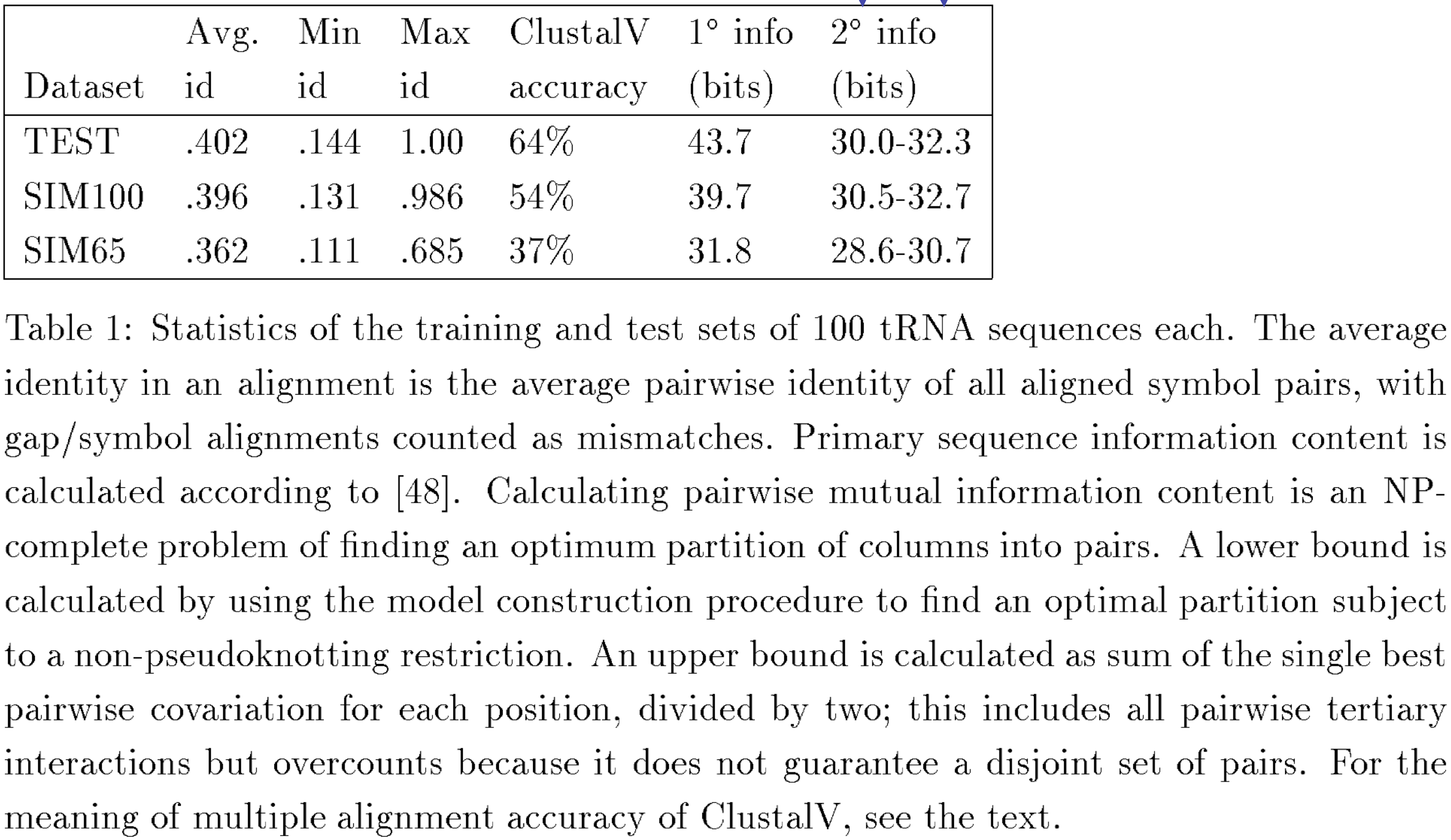
“Just like Nussinov/Zucker folding”

BUT, need enough data---enough sequences at right phylogenetic distance

Pseudoknots

disallowed allowed

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



	Avg.	Min	Max	ClustalV	1° info	2° info
Dataset	id	id	id	accuracy	(bits)	(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

Table 1: Statistics of the training and test sets of 100 tRNA sequences each. The average identity in an alignment is the average pairwise identity of all aligned symbol pairs, with gap/symbol alignments counted as mismatches. Primary sequence information content is calculated according to [48]. Calculating pairwise mutual information content is an NP-complete problem of finding an optimum partition of columns into pairs. A lower bound is calculated by using the model construction procedure to find an optimal partition subject to a non-pseudoknotting restriction. An upper bound is calculated as sum of the single best pairwise covariation for each position, divided by two; this includes all pairwise tertiary interactions but overcounts because it does not guarantee a disjoint set of pairs. For the meaning of multiple alignment accuracy of ClustalV, see the text.

Model	training set	iterations	score (bits)	alignment accuracy
A1415	all sequences (aligned)	3	58.7	95%
A100	SIM100 (aligned)	3	57.3	94%
A65	SIM65 (aligned)	3	46.7	93%
U100	SIM100 (degapped)	23	56.7	90%
U65	SIM65 (degapped)	29	47.2	91%

Table 2: Training and multiple alignment results from models trained from the trusted alignments (A models) and models trained from no prior knowledge of tRNA (U models).

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

Rfam – an RNA family DB

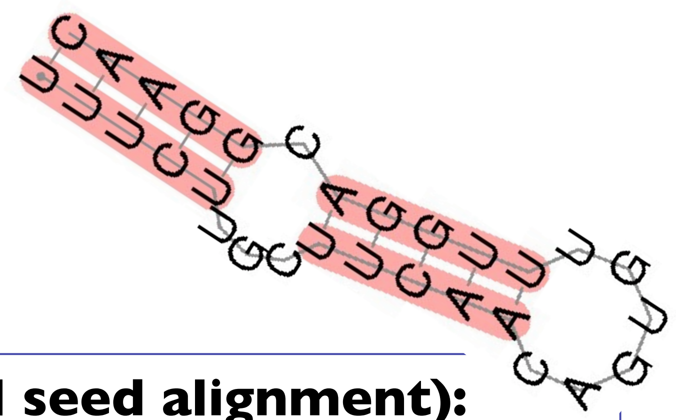
Griffiths-Jones, et al., NAR ‘03, ’05

Biggest scientific computing user in Europe - 1000 cpu cluster for a month per release

Rapidly growing:

- Rel 1.0, 1/03: 25 families, 55k instances
- Rel 7.0, 3/05: 503 families, >300k instances

Rfam



Input (hand-curated):

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

Output:

- CM
- scan results & "full alignment"

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     <<<<<...<<<<<......>>>>>.>>>>>
```

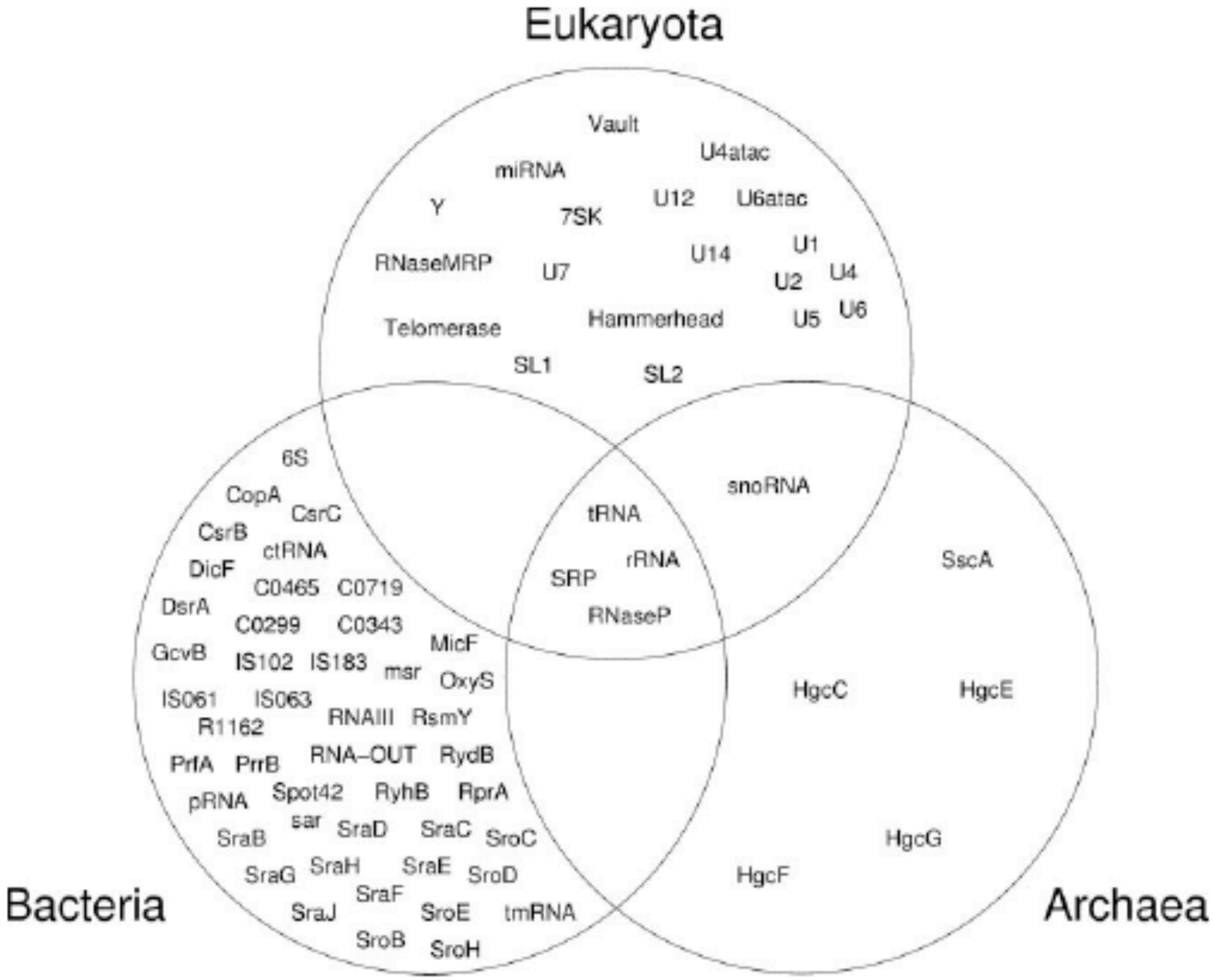


Figure 2. Taxonomic distribution of Rfam family members in the three kingdoms of life.

Rfam – key issues

Overly narrow families

Variant structures/unstructured RNAs

Spliced RNAs

RNA pseudogenes

- Human ALU is SRP-related, with 1.1×10^6 copies
- Mouse B2 repeat (350k copies) tRNA related

Speed & sensitivity

Motif discovery

Task 3: Faster Search

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

Zasha Weinberg

& W.L. Ruzzo

Recomb ‘04, ISMB ‘04, Bioinfo ‘06

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 will score < threshold;

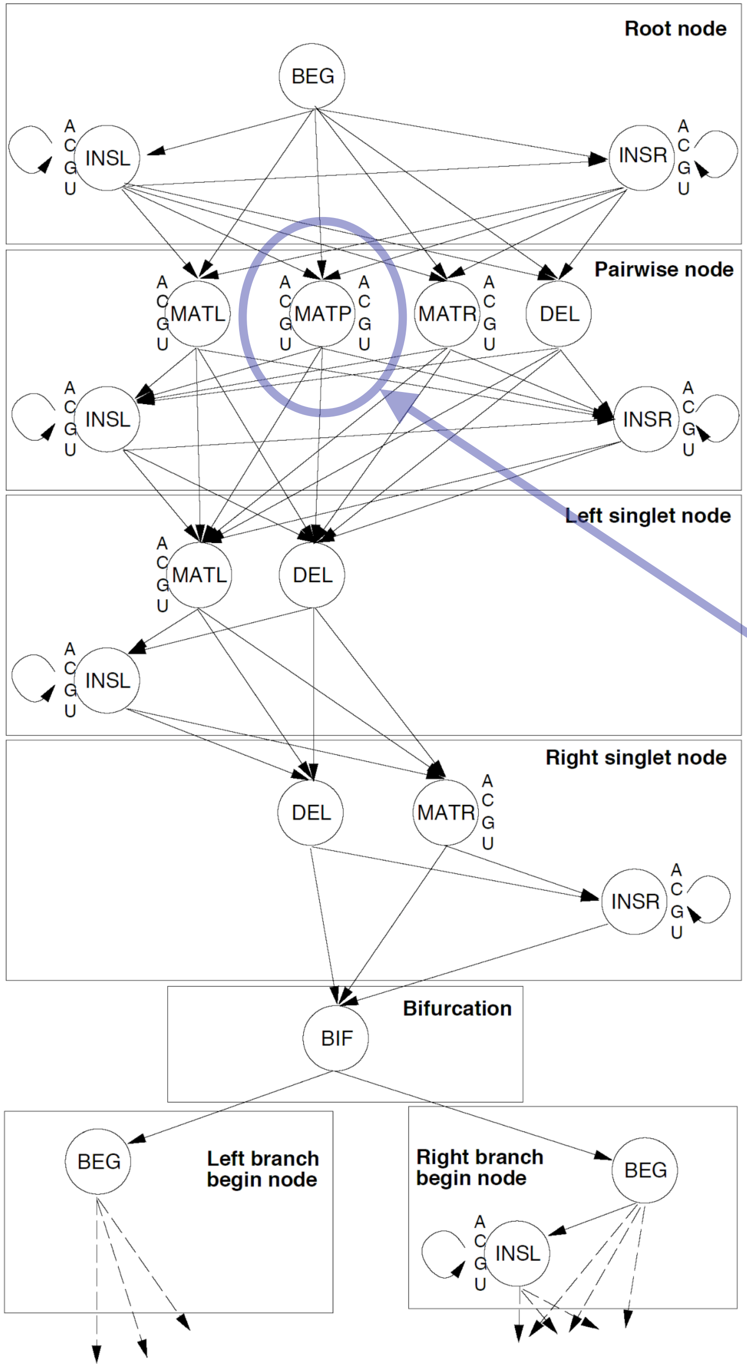
actually run CM on the rest (the promising parts)

assignment of HMM transition/emission scores is key

(large convex optimization problem)

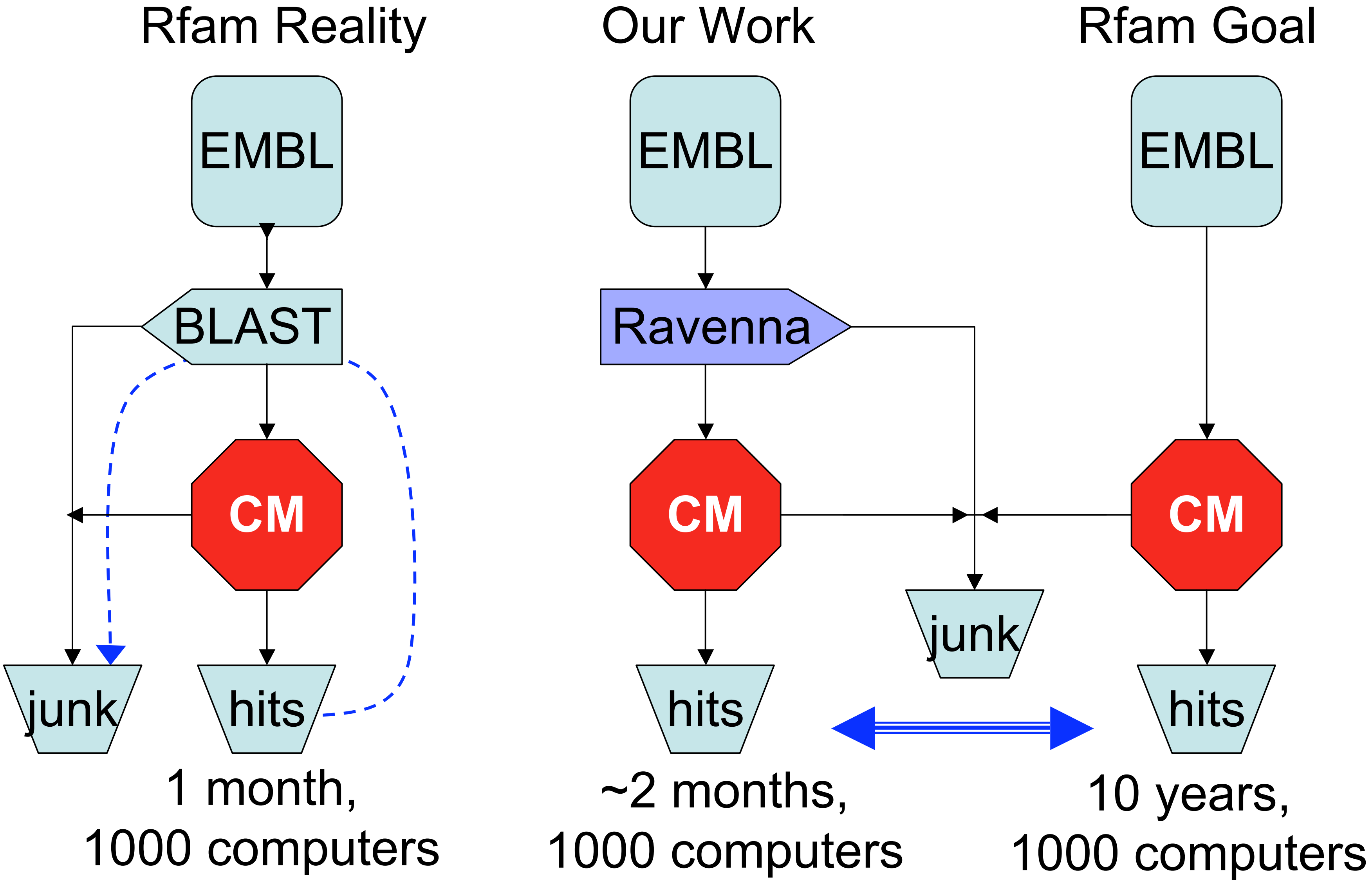
Weinberg & Ruzzo, *Bioinformatics*, 2004, 2006

Covariance Model



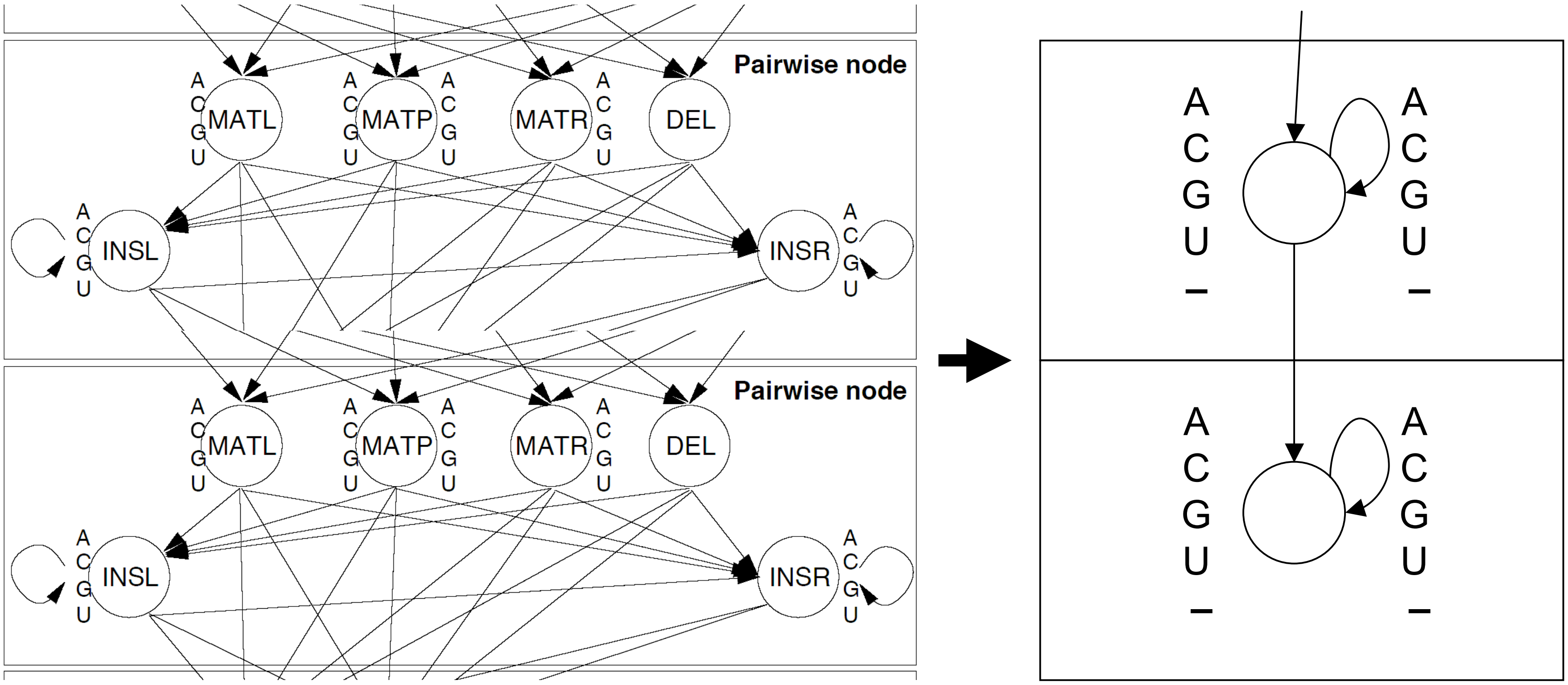
Key difference of CM vs HMM: Pair states emit paired symbols, corresponding to base-paired nucleotides; 16 emission probabilities here.

CM's are good, but slow

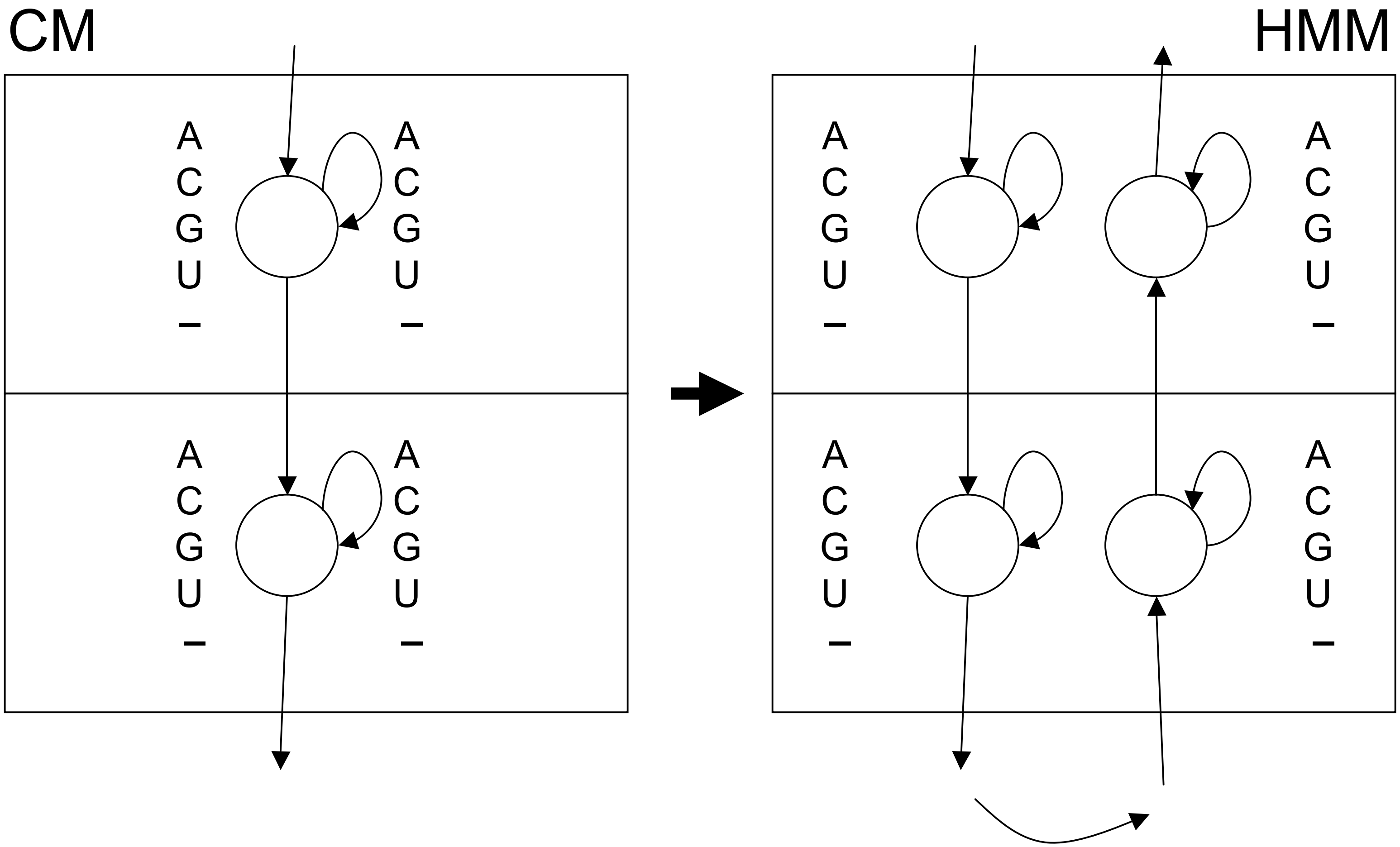


Simplified CM

(for pedagogical purposes only)



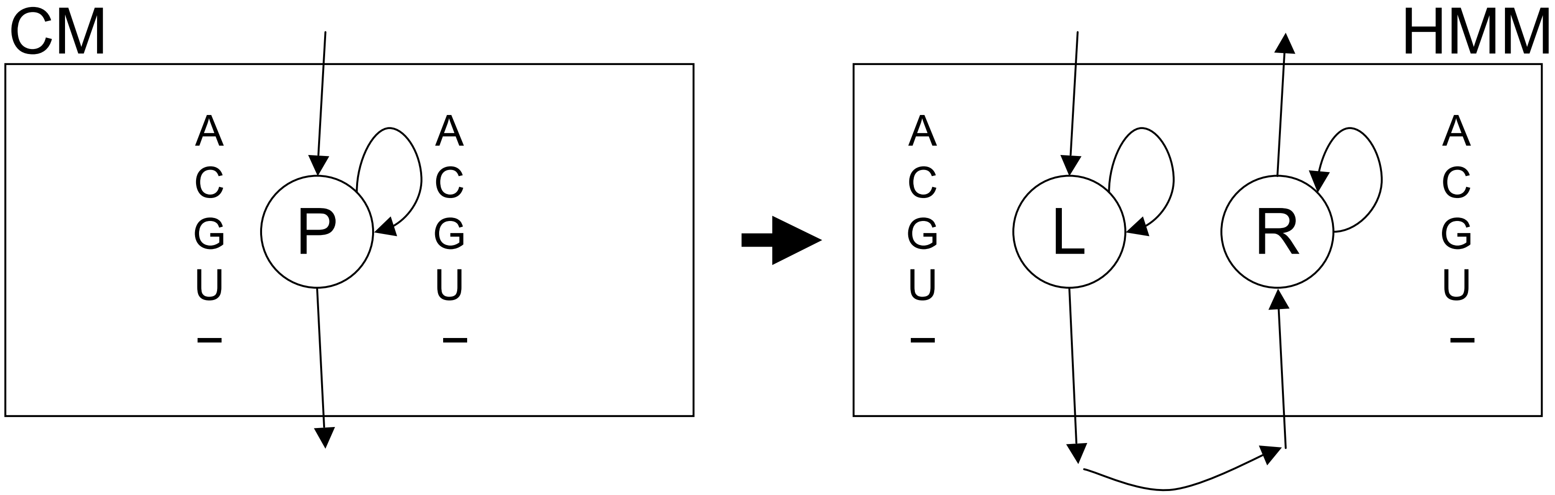
CM to HMM



25 emisions per state

5 emissions per state, 2x states

Key Issue: 25 scores → 10



Need: log Viterbi scores CM ≤ HMM

Viterbi/Forward Scoring

Path π defines transitions/emissions

Score(π) = product of "probabilities" on π

NB: ok if "probs" aren't, e.g. $\Sigma \neq 1$

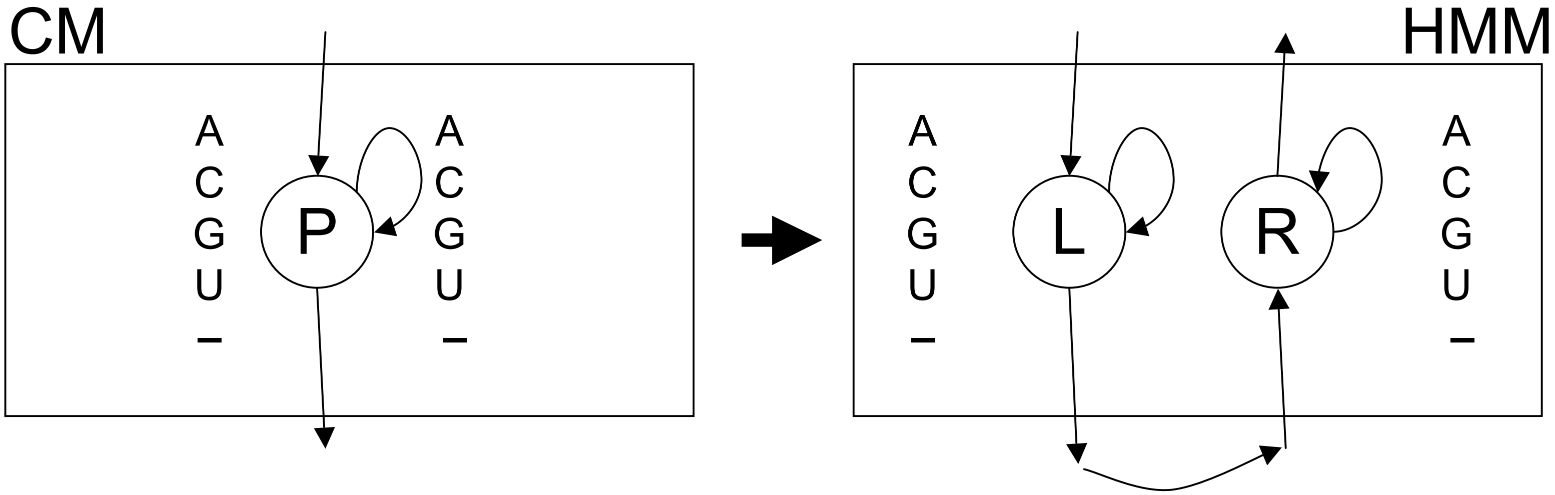
(e.g. in CM, emissions are odds ratios vs 0th-order background)

For any nucleotide sequence x:

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

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

Key Issue: 25 scores → 10



Need: log Viterbi scores CM ≤ HMM

$P_{AA} \leq L_A + R_A$	$P_{CA} \leq L_C + R_A$	…
$P_{AC} \leq L_A + R_C$	$P_{CC} \leq L_C + R_C$	…
$P_{AG} \leq L_A + R_G$	$P_{CG} \leq L_C + R_G$	…
$P_{AU} \leq L_A + R_U$	$P_{CU} \leq L_C + R_U$	…
$P_{A–} \leq L_A + R_–$	$P_{C–} \leq L_C + R_–$	…

NB: HMM not a prob. model

Rigorous Filtering

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

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) = Σ{ score(π) | π emits x }

Expected Forward Score

$E(L_i, R_i) = \Sigma_{\text{all sequences x}}$ 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 $E(L_i, R_i)$

$E(L_i, R_i) = \Sigma_x$ 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$_i$)

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:

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

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

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

~100x speedup

Results: New ncRNA's?

Name	# found BLAST + CM	# found rigorous filter + CM	# new
Pyrococcus snoRNA	57	180	123
Iron response element	201	322	121
Histone 3' element	1004	1106	102
Purine riboswitch	69	123	54
Retron msr	11	59	48
Hammerhead I	167	193	26
Hammerhead III	251	264	13
U4 snRNA	283	290	7
S-box	128	131	3
U6 snRNA	1462	1464	2
U5 snRNA	199	200	1
U7 snRNA	312	313	1

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…			

“Additional work”

Profile HMM filters use *no* 2^{ary} structure info

- They work well because, tho structure can be critical to function, there is (usually) enough primary sequence conservation to exclude most of DB
- But not on all families (and may get worse?)

Can we exploit *some* structure (quickly)?

- Idea 1: “sub-CM”
- Idea 2: extra HMM states remember mate

} for some hairpins

- Idea 3: try lots of combinations of “some hairpins”
- Idea 4: chain together several filters (select via Dijkstra)

Filter Chains

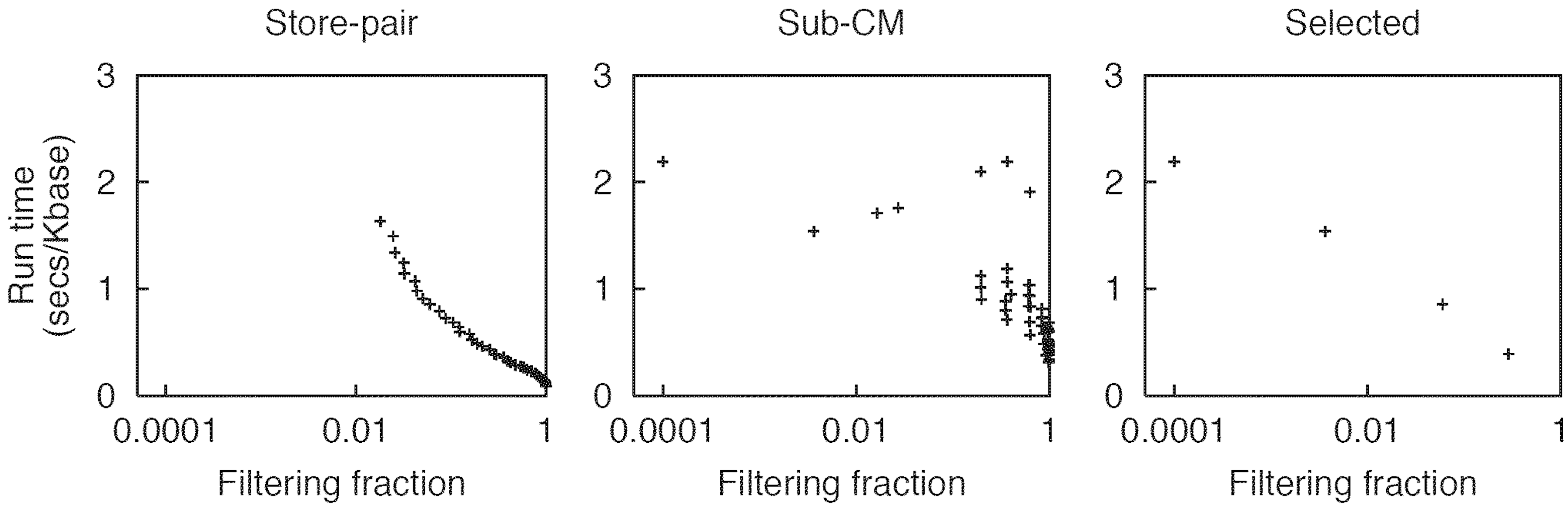


Fig. 2. Filter creation and selection. Filters for Rfam tRNA (RF00005) generated by the store-pair and sub-CM techniques and those selected for actual filtering are plotted by filtering fraction and run time. The CM runs at 3.5 secs/kbase. The four selected filters are run one after another, from highest to lowest fraction.

Heuristic Filters

Rigorous filters optimized for worst case

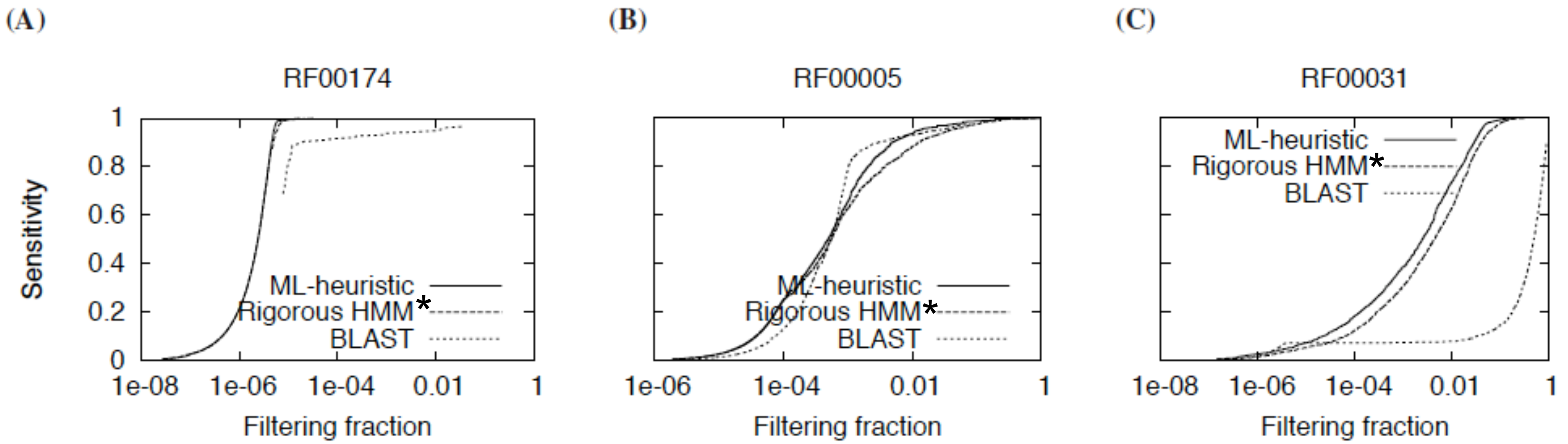
Possible to trade improved speed for small loss in sensitivity?

Yes – profile HMMs as before, but optimized for average case

"ML heuristic": train HMM from the infinite alignment generated by the CM

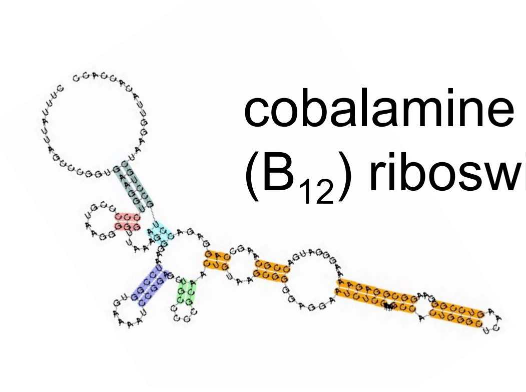
Often 10x faster, modest loss in sensitivity

Heuristic Filters

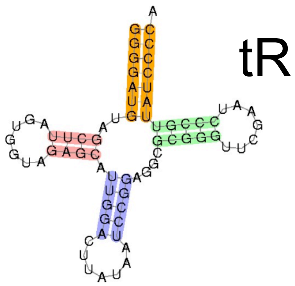


* rigorous HMM, not rigorous threshold

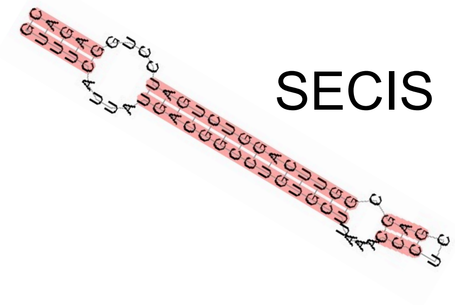
Fig. 1. Selected ROC-like curves. All plot sensitivity against filtering fraction, with filtering fraction in log scale. (A) RF00174 is typical of the other families; the ML-heuristic is slightly better than the rigorous profile HMM, and both often dramatically exceed BLAST. (B) Atypically, in RF00005, BLAST is superior, although only in one region. (C) BLAST performs especially poorly for RF00031. (Recall that rigorous scans were not possible for RF00031, so only ∼90% of hits are known; see text.) The supplement includes all ROC-like curves, and the inferior ignore-SS.



cobalamine (B_{12}) riboswitch



tRNA



SECIS

Task 4: Motif Discovery

RNA Motif Discovery

Typical problem: given a ~10-20 unaligned sequences of ~1kb, most of which contain instances of one RNA motif of, say, 150bp -- find it.

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

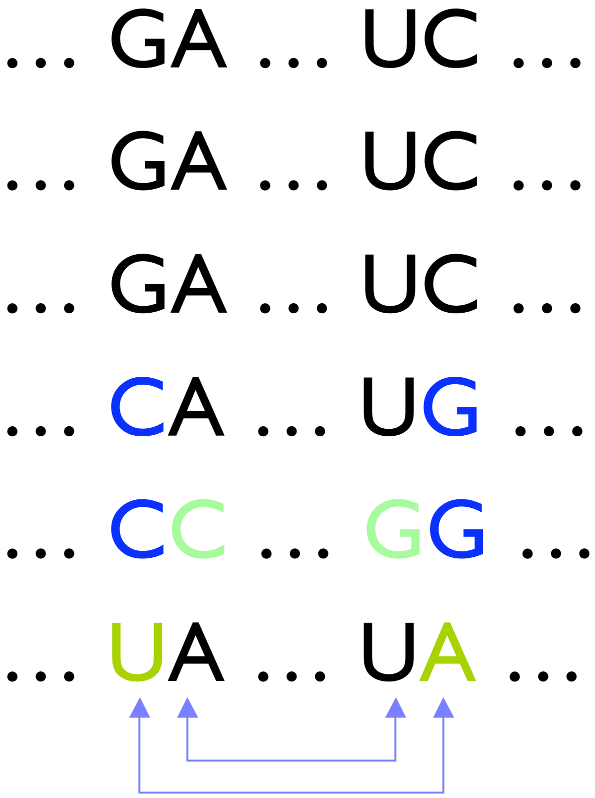
Approaches

Align sequences, then look for common structure

Predict structures, then try to align them

Do both together

“Obvious” Approach I: Align First, Predict from Multiple Sequence Alignment



… GA … UC …

… GA … UC …

… GA … UC …

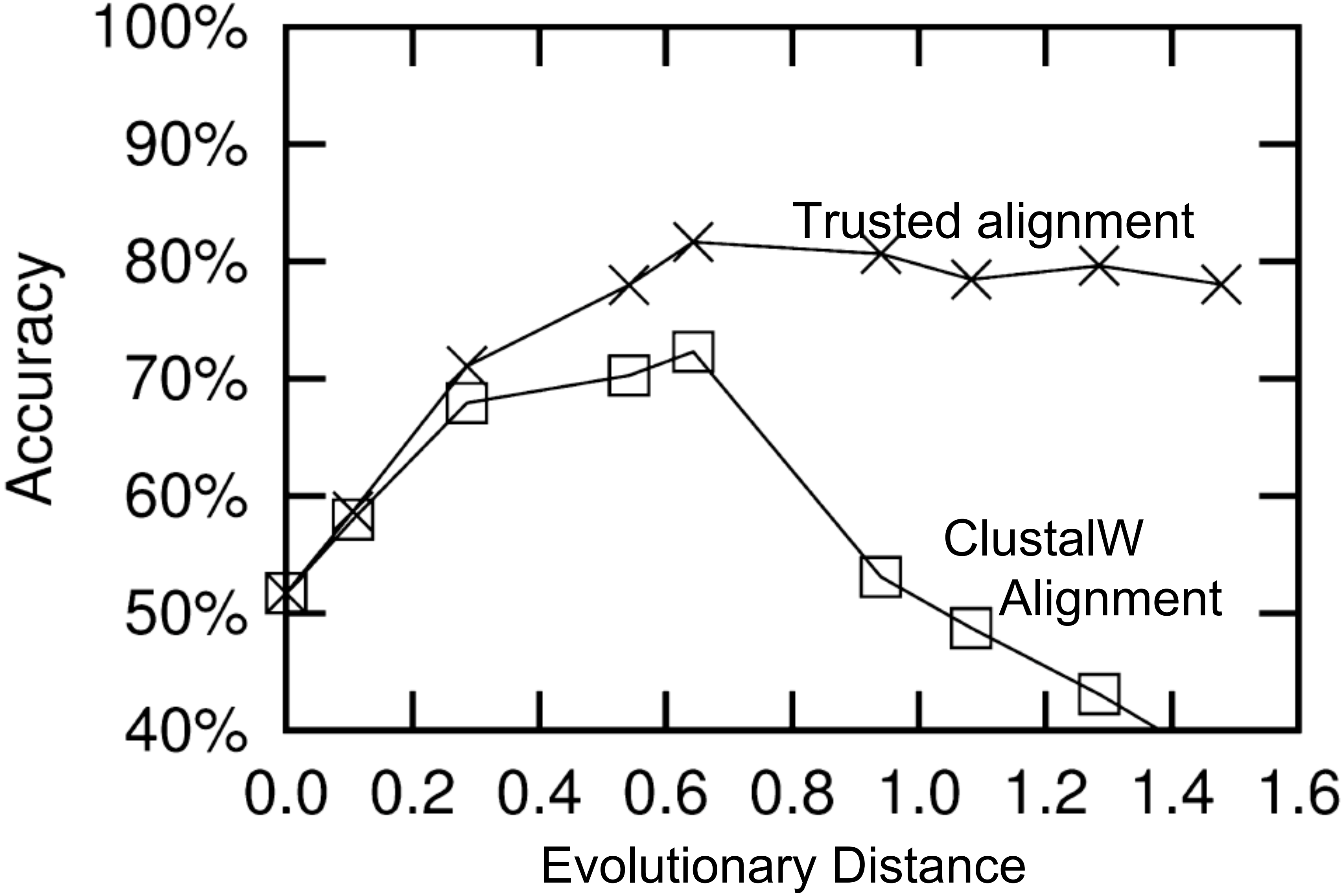
… CA … UG …

… CC … GG …

… UA … UA …

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

Pfold (KH03) Test Set D



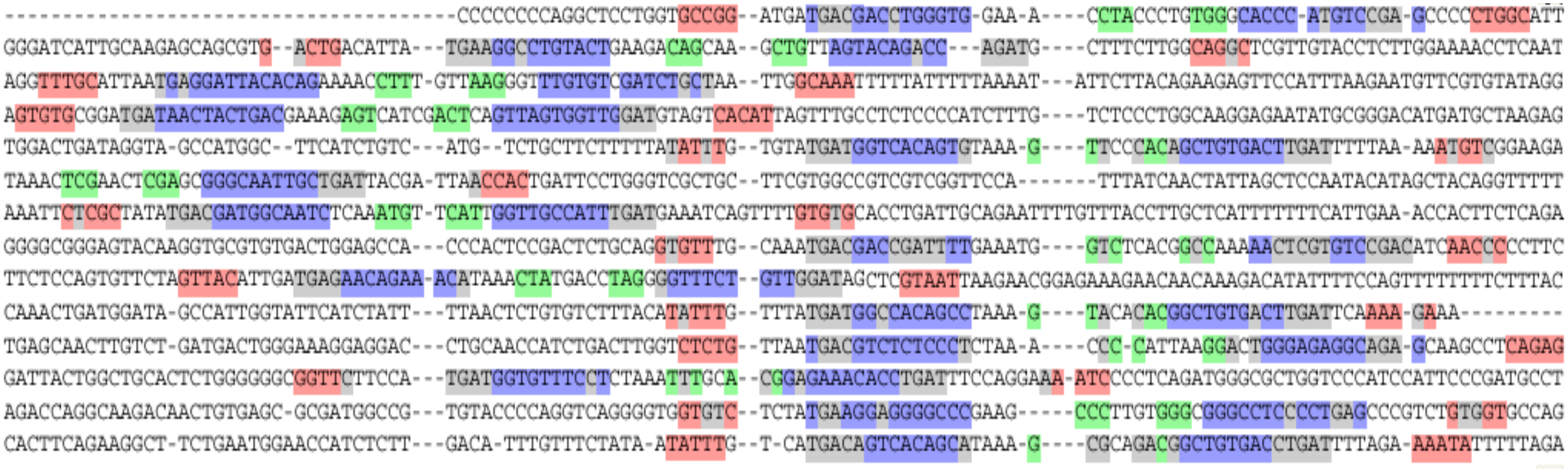
Knudsen & Hein, Pfold: RNA secondary structure prediction using stochastic context-free grammars, Nucleic Acids Research, 2003, v 31,3423–3428

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-ACATAAACTATGACCTAGGGGTTTCT--GTTGGATAGCTCGTAATTAAGAACGGAGAAAGAACAACAAAGACATATTTTCCAGTTTTTTTTCTTTAC
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----CGCAGACGGCTGTGACCTGATTTTAGA-AAATATTTTTAGA
```

same-colored boxes *should* be aligned

Approaches

Align sequences, then look for common structure

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

Do both together

- Sankoff – good but slow
- Heuristic

“Obvious” Approach II: Fold First

Predict secondary RNA structure using MFOLD or Vienna

Problems

- false folding predictions
- comparing structures

Our Approach: CMfinder

Simultaneous alignment, folding and CM-based motif description using an EM-style learning procedure

Yao, Weinberg & Ruzzo, *Bioinformatics*, 2006

Cmfinder--A Covariance Model Based RNA Motif Finding Algorithm

Bioinformatics, 2006, 22(4): 445-452

Zizhen Yao

Zasha Weinberg

Walter L. Ruzzo

University of Washington, Seattle

Design Goals

Find RNA motifs in unaligned sequences

Seq conservation exploited, but not required

Robust to inclusion of unrelated sequences

Robust to inclusion of flanking sequence

Reasonably fast and scalable

Produce a probabilistic model of the motif that can be directly used for homolog search

Alignment → CM → Alignment

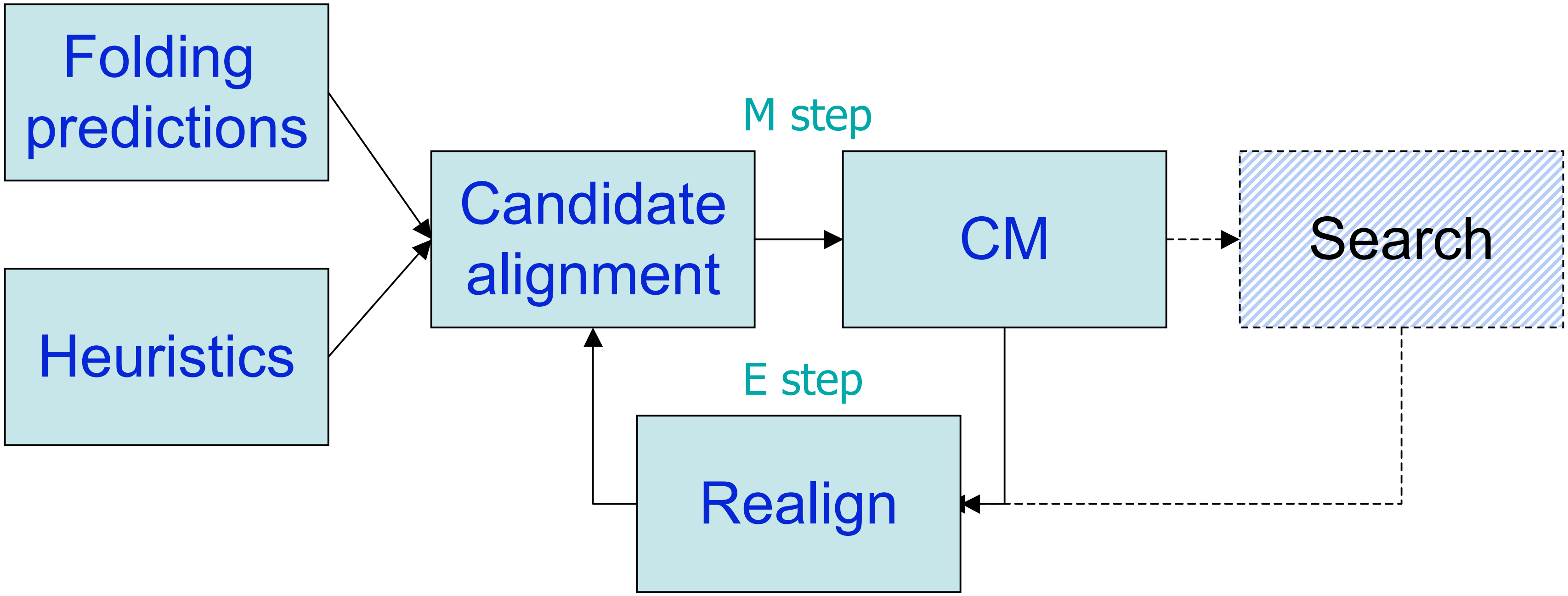
Similar to HMM, but much slower

Builds on Eddy & Durbin, ‘94

But new way to infer which columns to pair, via a principled combination of mutual information and predicted folding energy

And, it’s local, not global, alignment (harder)

CMfinder Outline



M-step uses M.I. + folding energy for structure prediction

Initial Alignment Heuristics

fold sequences separately

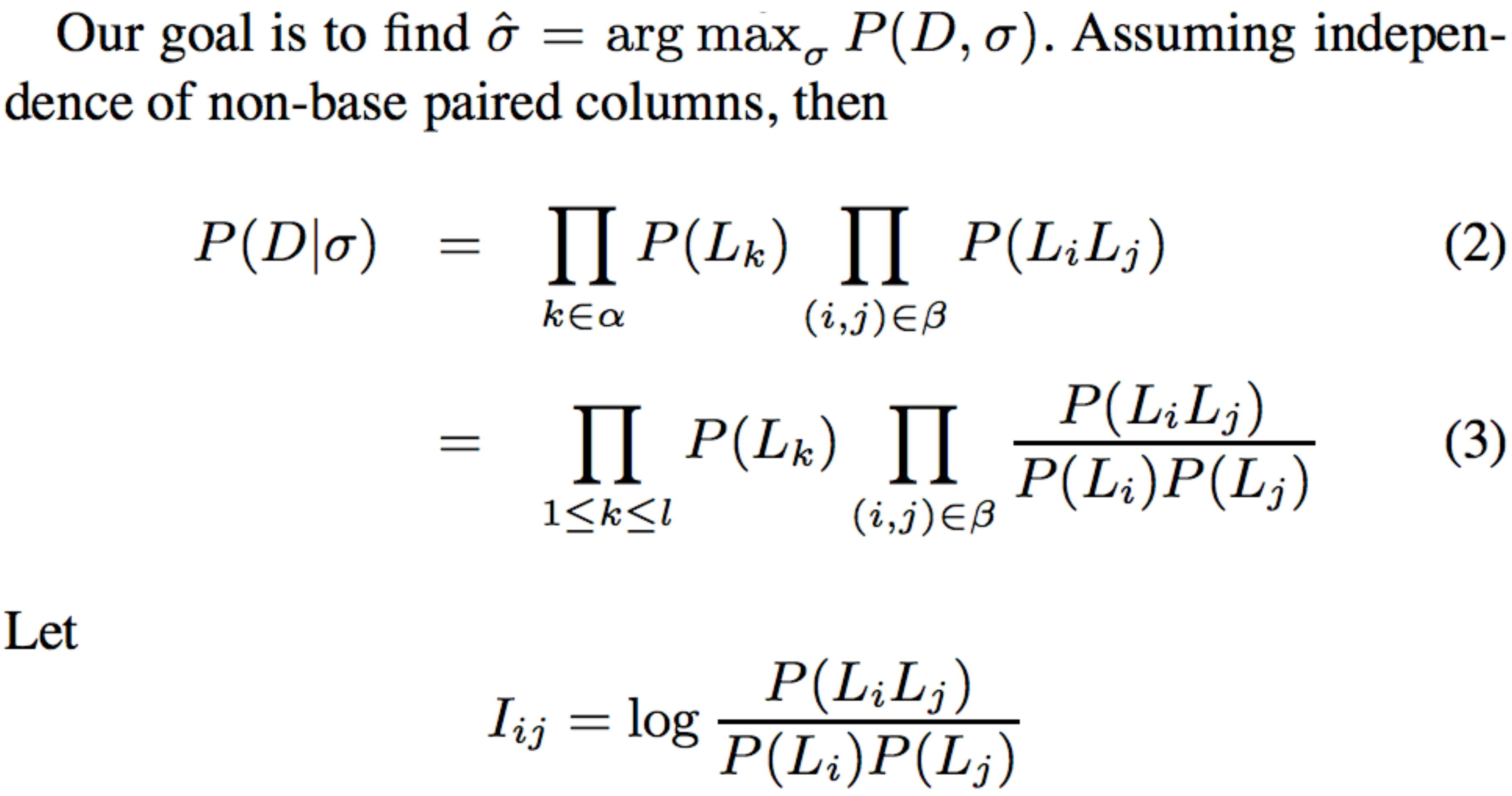
candidates: regions with low folding energy

compare candidates via “tree edit” algorithm

find best “central” candidates & align to them

BLAST anchors

L_i = column i; $\sigma = (\alpha, \beta)$ the 2^{ary} struct, α = unpaired, β = paired cols



Our goal is to find $\hat{\sigma} = \arg\max_\sigma P(D, \sigma)$. Assuming independence of non-base paired columns, then

$$P(D|\sigma) = \prod_{k \in \alpha} P(L_k) \prod_{(i,j) \in \beta} P(L_i L_j) \tag{2}$$

$$= \prod_{1 \leq k \leq l} P(L_k) \prod_{(i,j) \in \beta} \frac{P(L_i L_j)}{P(L_i) P(L_j)} \tag{3}$$

Let

$$I_{ij} = \log \frac{P(L_i L_j)}{P(L_i) P(L_j)}$$

With MLE params, I_{ij} is the *mutual information* between cols i and j

Let s_i be the prior for column i to be single stranded, and p_{ij} the prior for columns i, j to be base paired, then $P(\sigma) = \prod_{k\in\alpha} s_k \prod_{(i,j)\in\beta} p_{ij}$, and $P(D,\sigma)$ can be rewritten as

$$\begin{aligned} P(D,\sigma) &= P(D|\sigma)P(\sigma) \\ &= \prod_{1\leq k\leq l} P(L_k)s_k \prod_{(i,j)\in\beta} \frac{P(L_iL_j)}{P(L_i)P(L_j)} \frac{p_{ij}}{s_is_j} \qquad (4) \end{aligned}$$

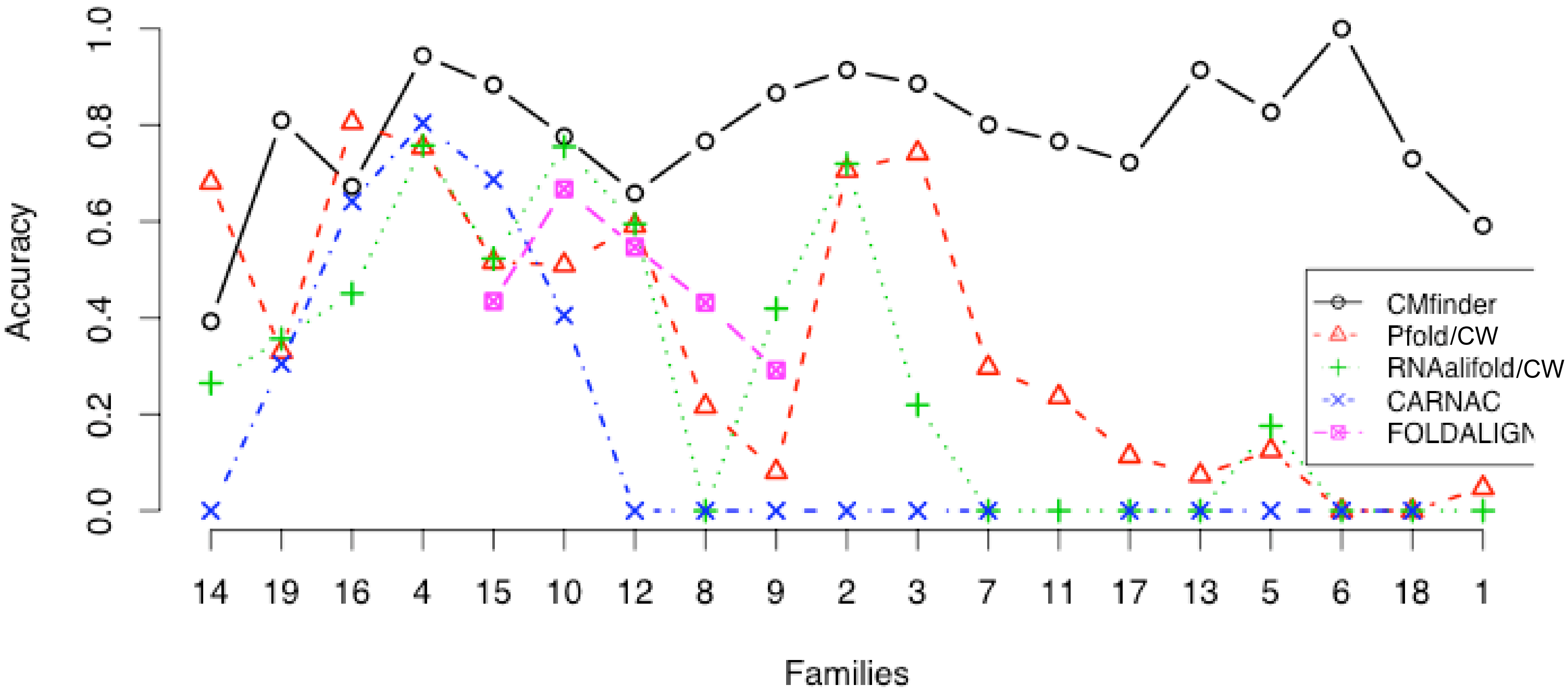
Let

$$K_{ij} = \log\left(\frac{P(L_iL_j)}{P(L_i)P(L_j)}\frac{p_{ij}}{s_is_j}\right) = I_{ij} + \log\frac{p_{ij}}{s_is_j},$$

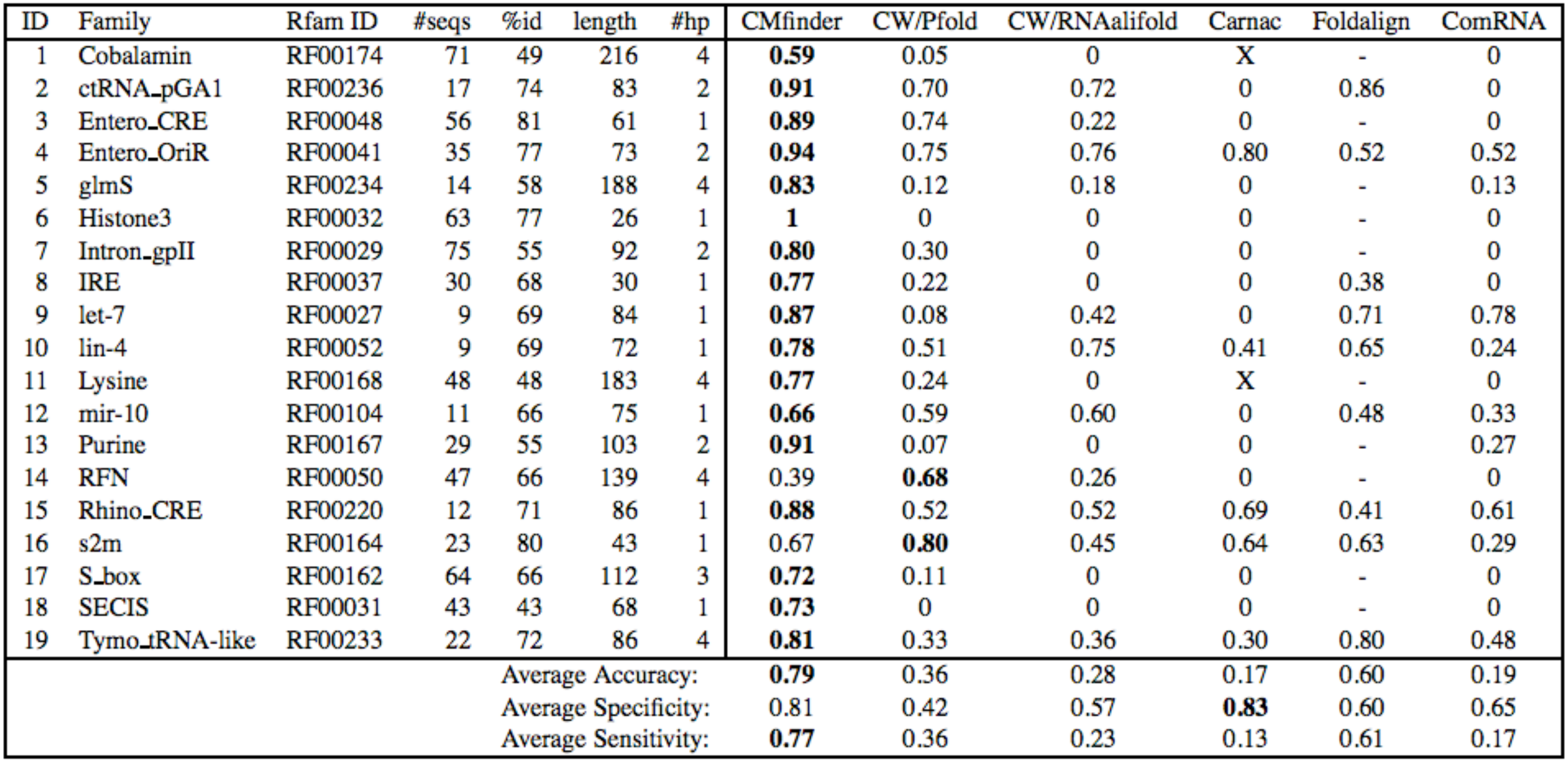
then the maximum likelihood structure σ maximizes $\sum_{(i,j)\in\beta} K_{ij}$.
Can find it via a simple dynamic programming alg.

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

Task 5: Application

Genome-wide search for
cis-regulatory RNA elements
(in prokaryotes, initially)

Searching for noncoding RNAs

CM's are great, but where do they come from?

An 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.

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

Approach

Choose a bacterial genome

For each gene, get 10-30 close orthologs (CDD)

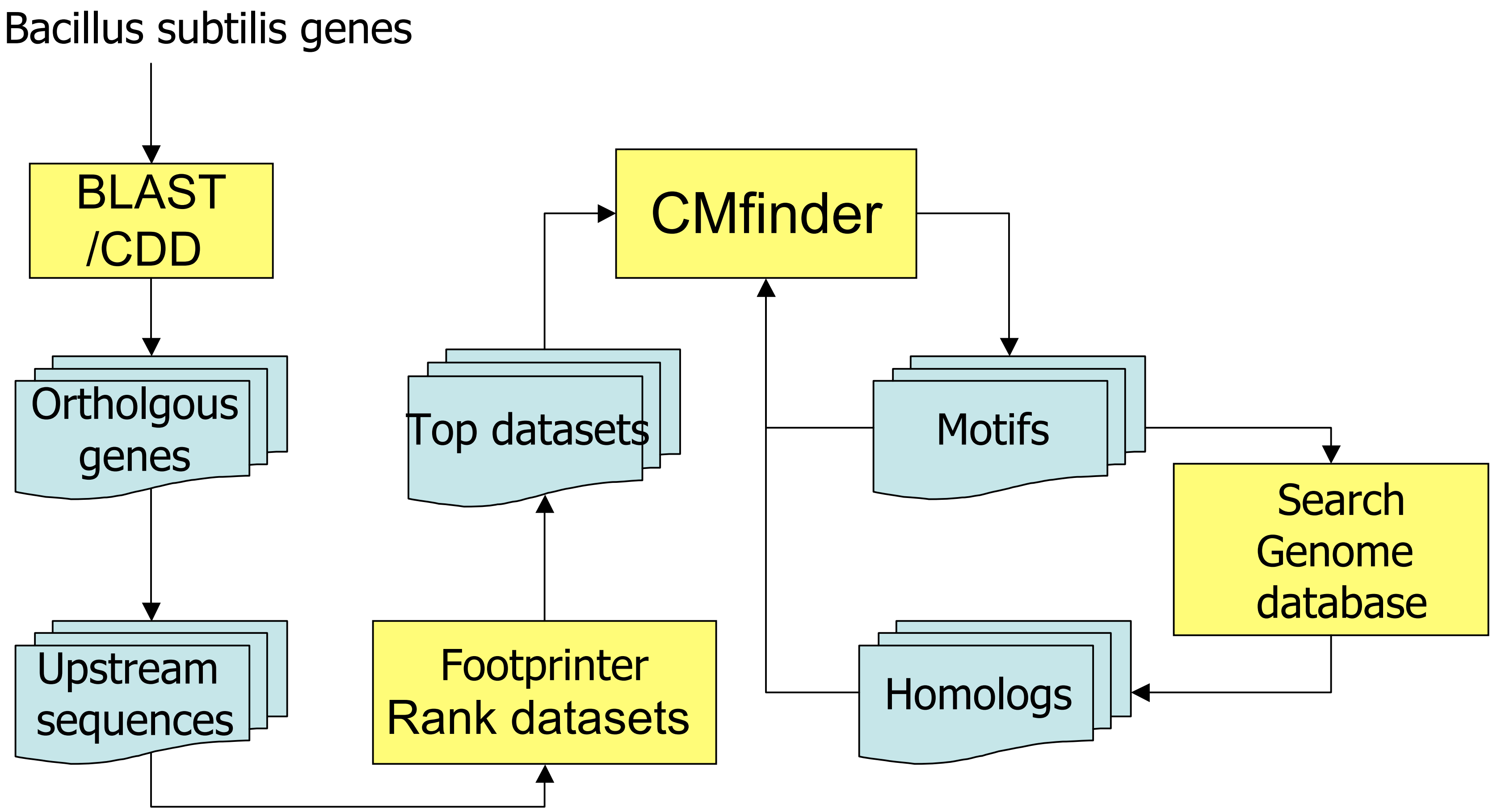
Find most promising genes, based on conserved sequence motifs (Footprinter)

From those, find structural motifs (CMfinder)

Genome-wide search for more instances (Ravenna)

Expert analyses (Breaker Lab, Yale)

A pipeline for RNA motif genome scans



Genome Scale Search: Why

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

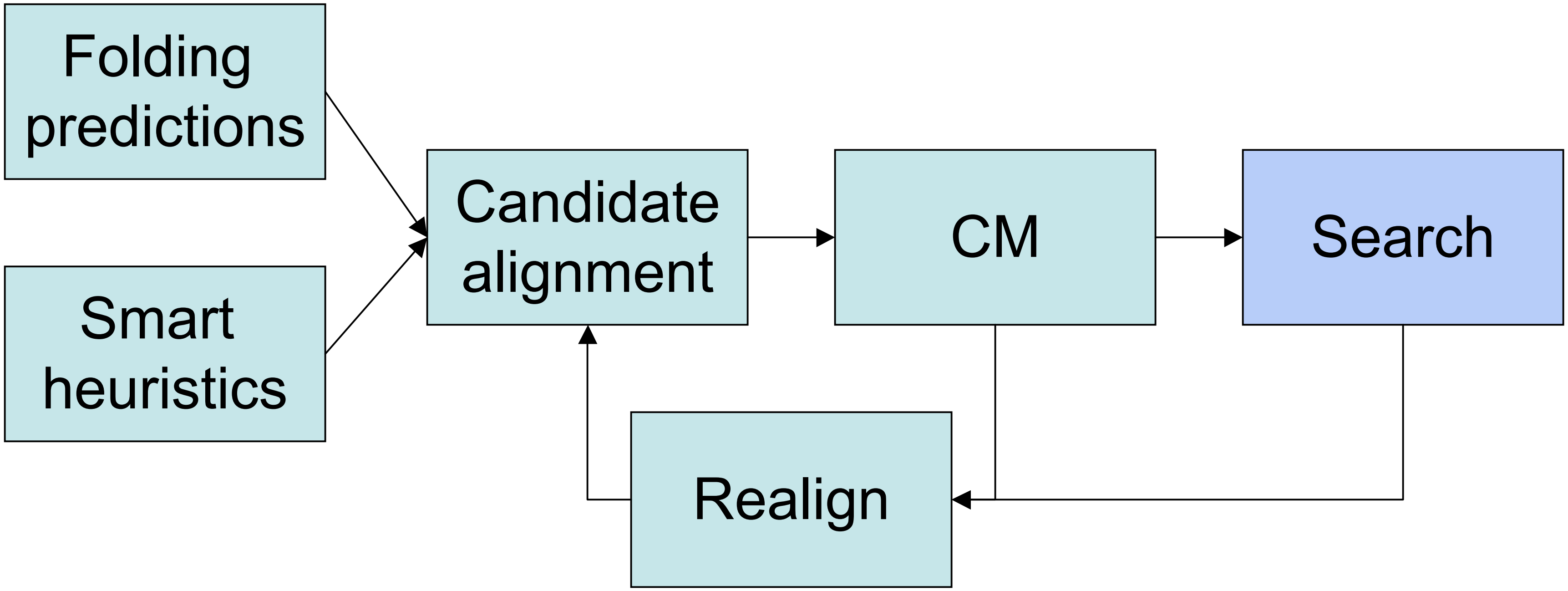
Throughout (most of) clade

More examples give better model, hence even more examples, fewer errors

More examples give more clues to function - critical for wet lab verification

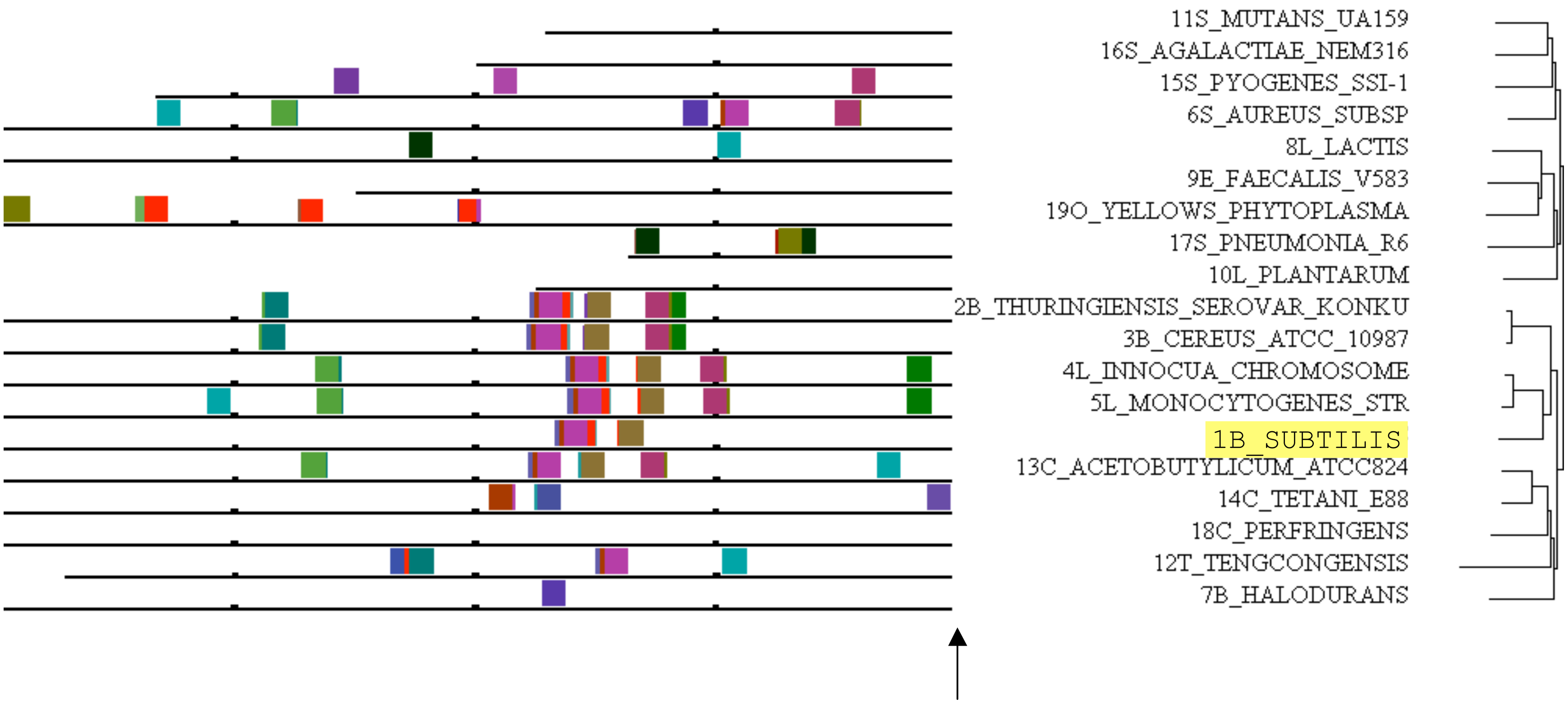
Genome Scale Search

CMfinder is directly usable for/with search



Footprinter finds patterns of conservation

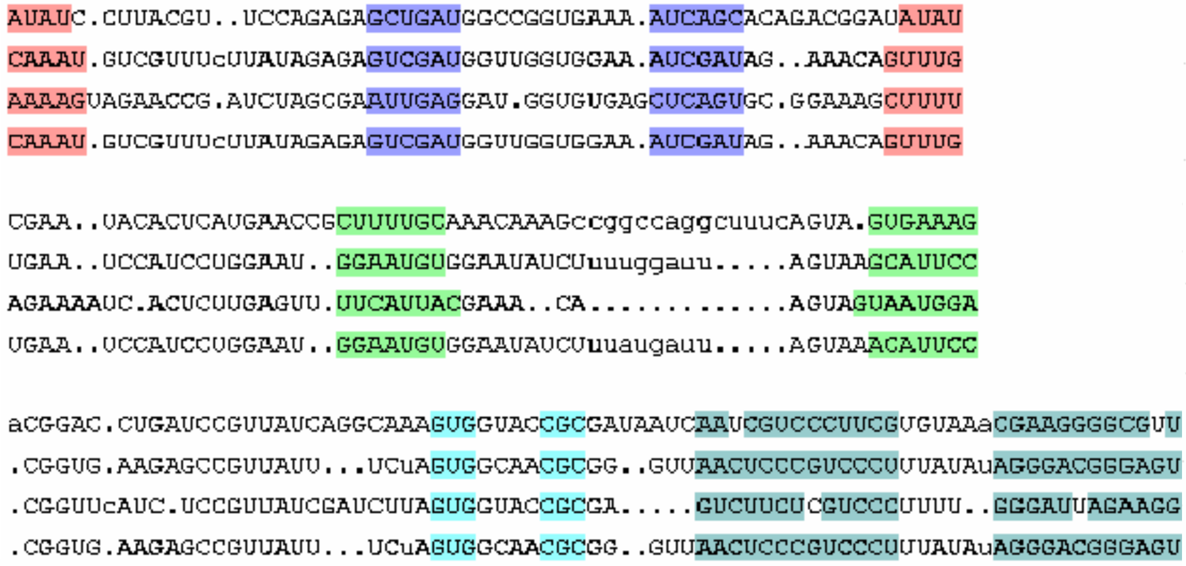
Upstream of folC



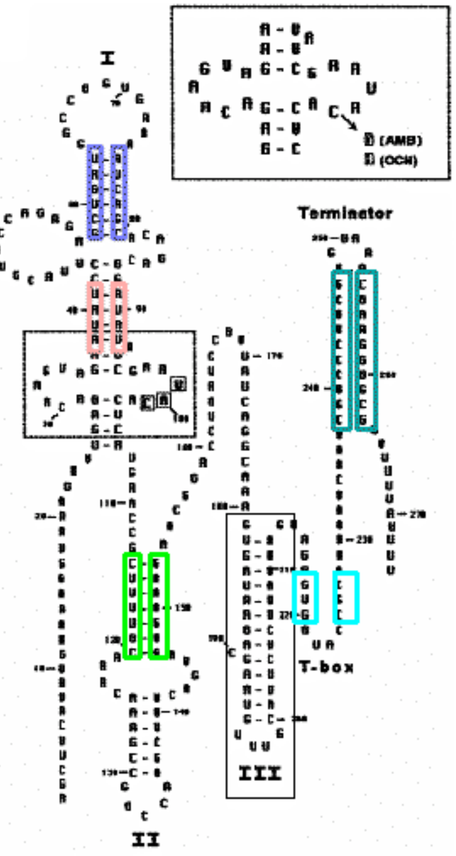
start codons

A blind test

1ST genome scan:	234 sequences
2ND genome scan:	447 sequences
The motif turned out to be T box	
Match to RFAM T box family:	299 OF 342
False Positives:	89/148 are probable (upstream of annotated tRNA-synthetase genes)



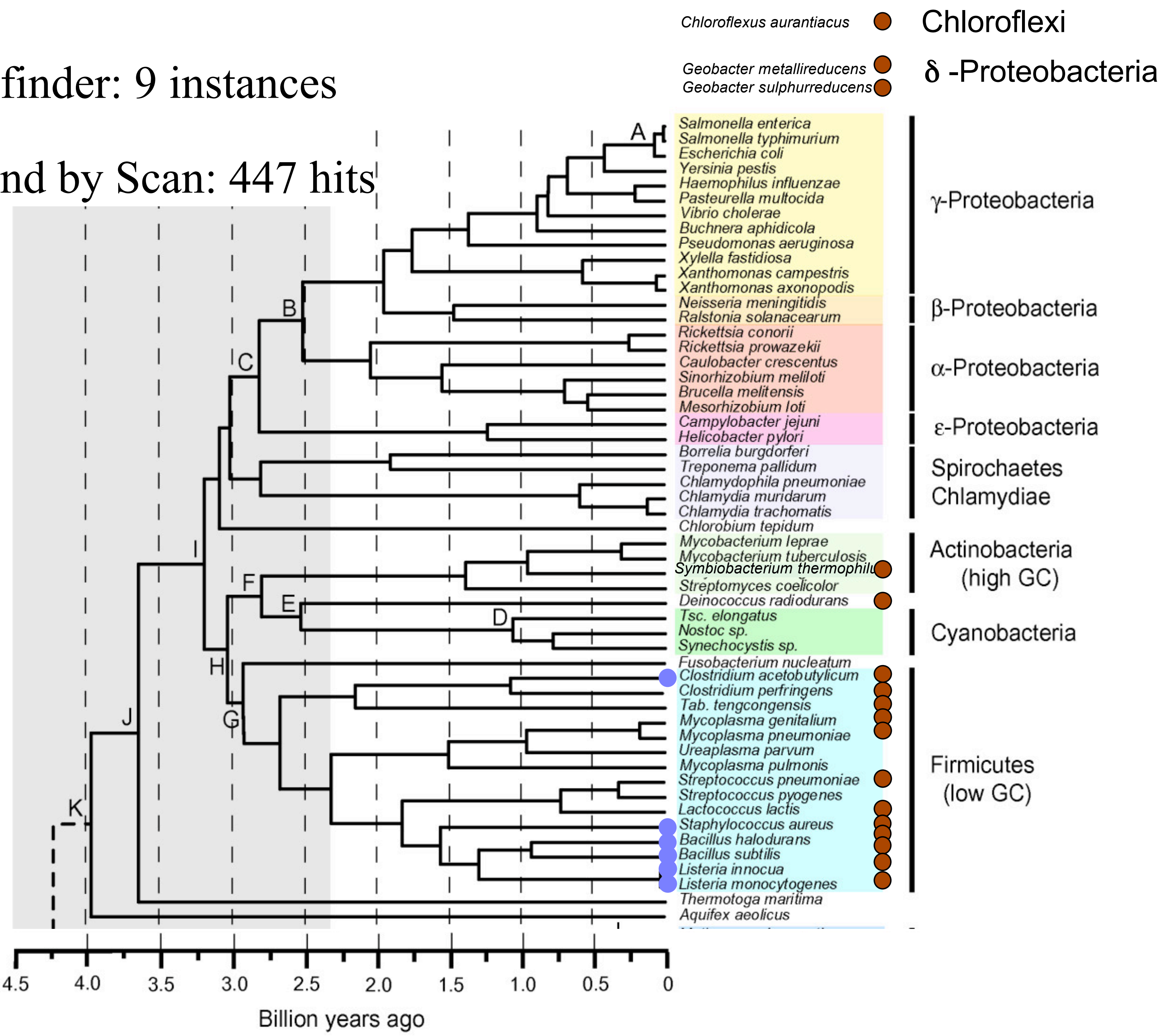
```
AUAUC.CUUACGU..UCCAGAGAGCUGAUGGCCGGUGAAA.AUCAGCACAGACGGAUAUAU
CAAAU.GUCGUUUcUUAUAGAGAGUCGAUGGUUGGUGGAA.AUCGAUAG..AAACAGUUUG
AAAAGUAGAACCG.AUCUAGCGAAUUGAGGAU.GGUGUGAGCUCAGUGC.GGAAAGCUUUU
CAAAU.GUCGUUUcUUAUAGAGAGUCGAUGGUUGGUGGAA.AUCGAUAG..AAACAGUUUG

CGAA..UACACUCAUGAACCGCUUUUGCAAACAAAGccggccaggcuuucAGUA.GUGAAAG
UGAA..UCCAUCCUGGAAU..GGAAUGUGGAAUAUCUuuuggauu.....AGUAAGCAUUCC
AGAAAAUC.ACUCUUGAGUU.UUCAUUACGAAA..CA.............AGUAGUAAUGGA
UGAA..UCCAUCCUGGAAU..GGAAUGUGGAAUAUCUuuaugauu.....AGUAAACAUUCC

aCGGAC.CUGAUCCGUUAUCAGGCAAAGUGGUACCGCGAUAAUCAAUCGUCCCUUCGUGUAAaCGAAGGGGCGUU
.CGGUG.AAGAGCCGUUAUU...UCuAGUGGCAACGCGG..GUUAACUCCCGUCCCUUUAUAuAGGGACGGGAGU
.CGGUUcAUC.UCCGUUAUCGAUCUUAGUGGUACCGCGA.....GUCUUCUCGUCCCUUUU..GGGAUUAGAAGG
.CGGUG.AAGAGCCGUUAUU...UCuAGUGGCAACGCGG..GUUAACUCCCGUCCCUUUAUAuAGGGACGGGAGU
```



tyrS T box structure

● CMfinder: 9 instances

● Found by Scan: 447 hits



Results

- Process largely complete in
  - bacillus/clostridia
  - gamma proteobacteria
  - cyanobacteria
  - actinobacteria
  - firmicutes
- Analysis ongoing

Some Preliminary Actino Results

8 of 10 Rfam families found

Rfam Family	Type (metabolite)	Rank
THI	riboswitch (thiamine)	4
ydaO-yuaA	riboswitch (unknown)	19
Cobalamin	riboswitch (cobalamin)	21
SRP_bact	gene	28
RFN	riboswitch (FMN)	39
yybP-ykoY	riboswitch (unknown)	48
gcvT	riboswitch (glycine)	53
S_box	riboswitch (SAM)	401
tmRNA	gene	Not found
RNaseP	gene	Not found

not cis-regulatory (got one anyway)

Preliminary results of genome scan

Top 115 datasets (some are redundant)
13 T box, 22 riboswitches, 30 ribosomal genes
RNase P, tRNA, CIRCE elements and other DNA binding sites

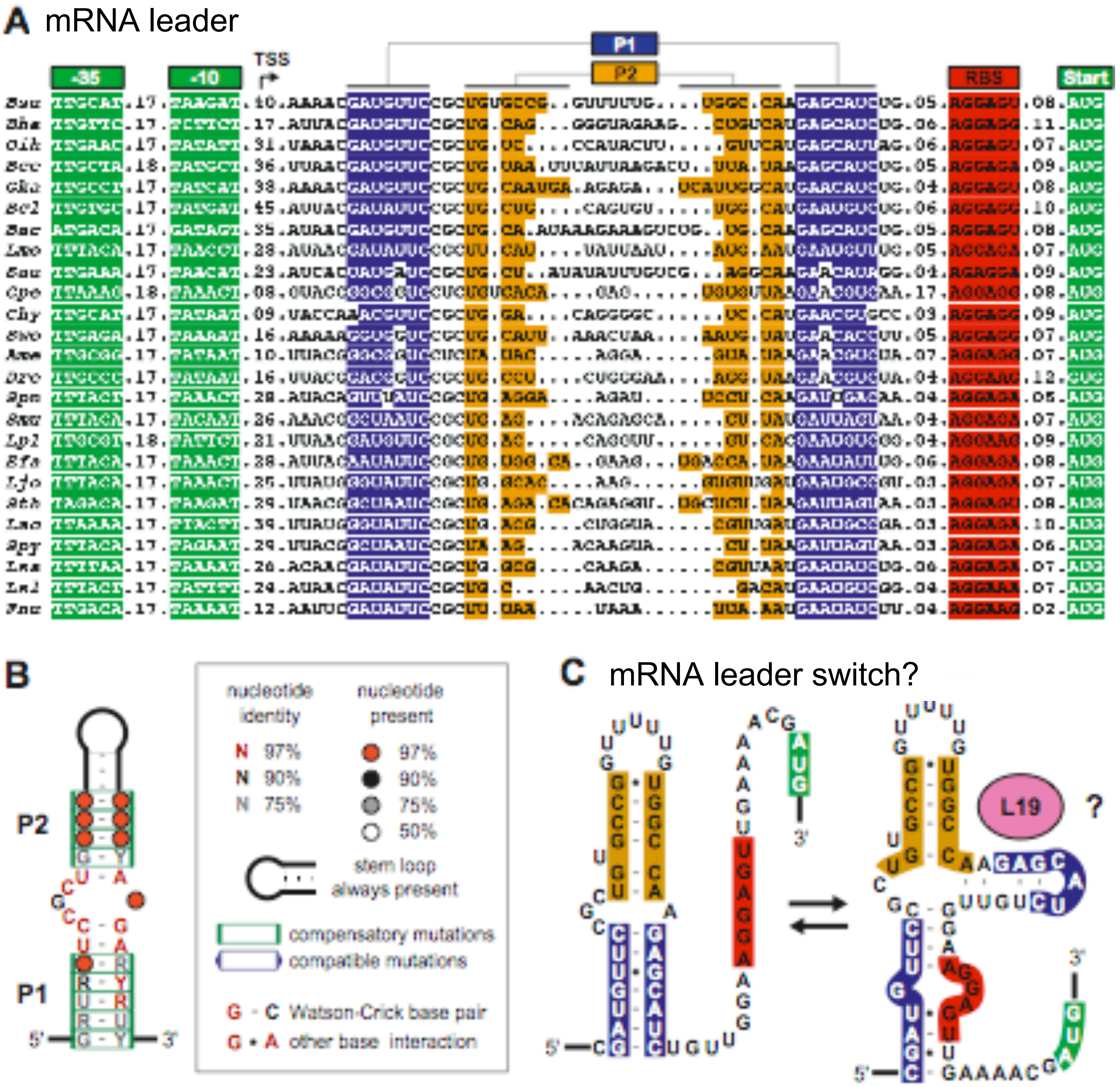
Gene	#motif	hits	RFAM	#seed	#full	#TP	specificity	sensitivity
metK	13	150	S_box	71	151	145	0.967	0.960
ribB	9	106	RFN	48	114	97	0.915	0.851
folC	9	447	T_box	67	342	299	0.669	0.874
xpt	14	106	Purine	37	100	97	0.915	0.970
glmS	16	33	glmS	14	37	33	1.000	0.892
thiA	16	305	THI	237	366	305	1.000	0.833
ykoY	10	34	yybP-ykoY	74	127	33	0.971	0.260

More Prelim Actino Results

Many others (not in Rfam) are likely real; of top 50:

known (Rfam, 23S)	10
probable (Tbox, CIRCE, LexA, parP, pyrR)	7
probable (ribosomal genes)	9
potentially interesting	12
unknown or poor	12

One bench-verified, 3-4 more in progress


A
mRNA leader
-35
-10
TSS
P1
P2
RBS
Start
Bsu
Bha
Oih
Bce
Gka
Bcl
Bsc
Lmo
Sau
Cpe
Chy
Swo
Ame
Dre
Spn
Ssu
Lpl
Efa
Ljo
Sth
Lac
Spy
Lsa
Lsl
Fnu
B
P2
P1
5'
3'
nucleotide identity
nucleotide present
N 97%
N 90%
N 75%
97%
90%
75%
50%
stem loop always present
compensatory mutations
compatible mutations
G - C Watson-Crick base pair
G • A other base interaction
C
mRNA leader switch?
L19
?
5'
3'

Ongoing & Future Work

Still automating a few steps, e.g. identifying duplicates

Improved ranking/motif significance stats

Better ortholog clustering

Performance & scale-up

Eukaryotic mRNAs, e.g. UTRs

Summary

ncRNA - apparently widespread, much interest

Covariance Models - powerful but expensive tool for ncRNA motif representation, search, discovery

Rigorous/Heuristic filtering - typically 100x speedup in search with no/little loss in accuracy

CMfinder - CM-based motif discovery in unaligned sequences