# RNA Search and Motif Discovery

## Lecture 9

CSEP 590A

Autumn 2008

# Outline

- Whirlwind tour of ncRNA search & discovery
  - RNA motif description (Covariance Model Review)
  - Algorithms for searching
    - Rigorous & heuristic filtering
  - Motif discovery
  - Applications

# Motif Description

# RNA Motif Models

"Covariance Models" (Eddy & Durbin 1994)

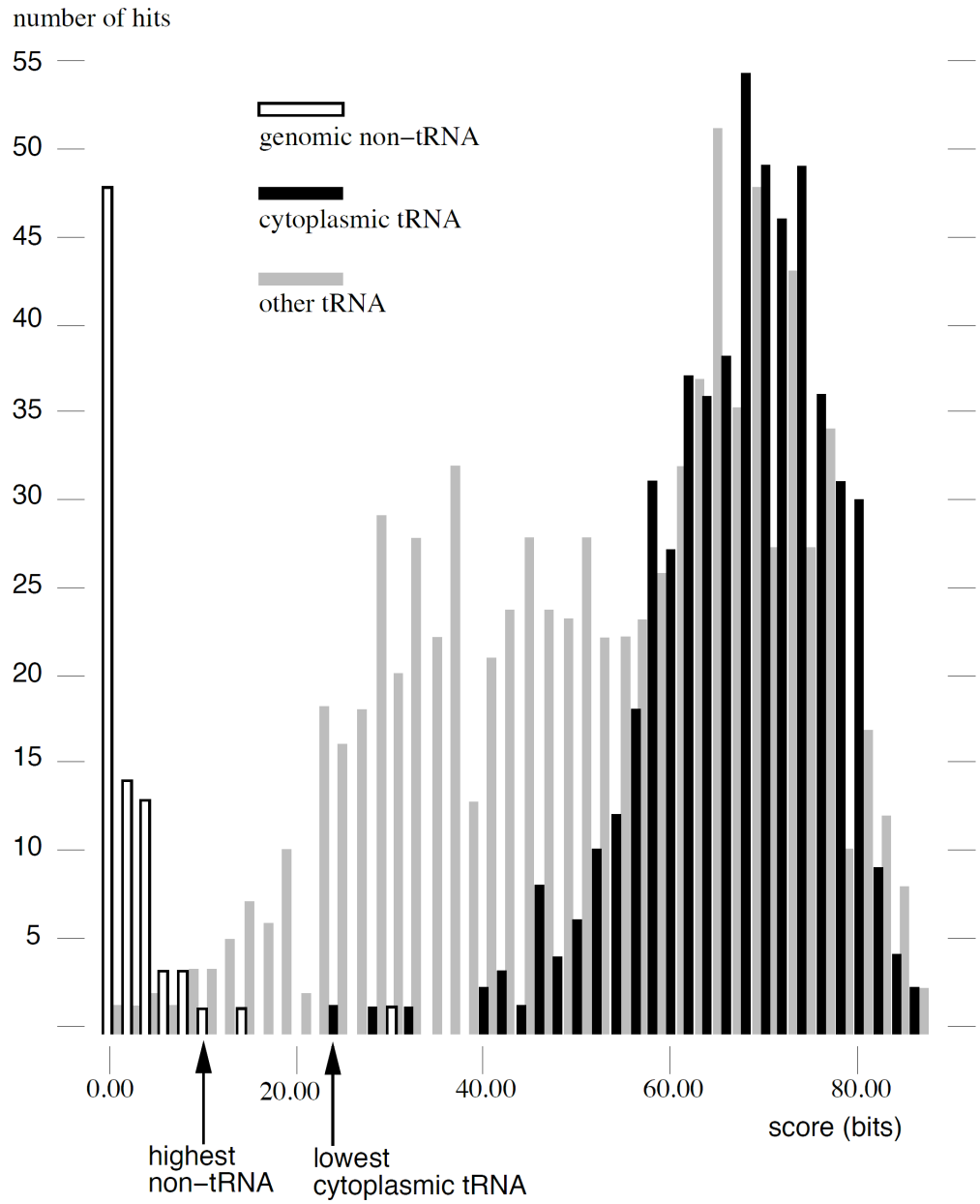
- aka profile stochastic context-free grammars
- aka hidden Markov models on steroids

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

Pro: accurate

Con: model building hard, search slooooow

# Example: searching for tRNAs



# Profile Hmm Structure

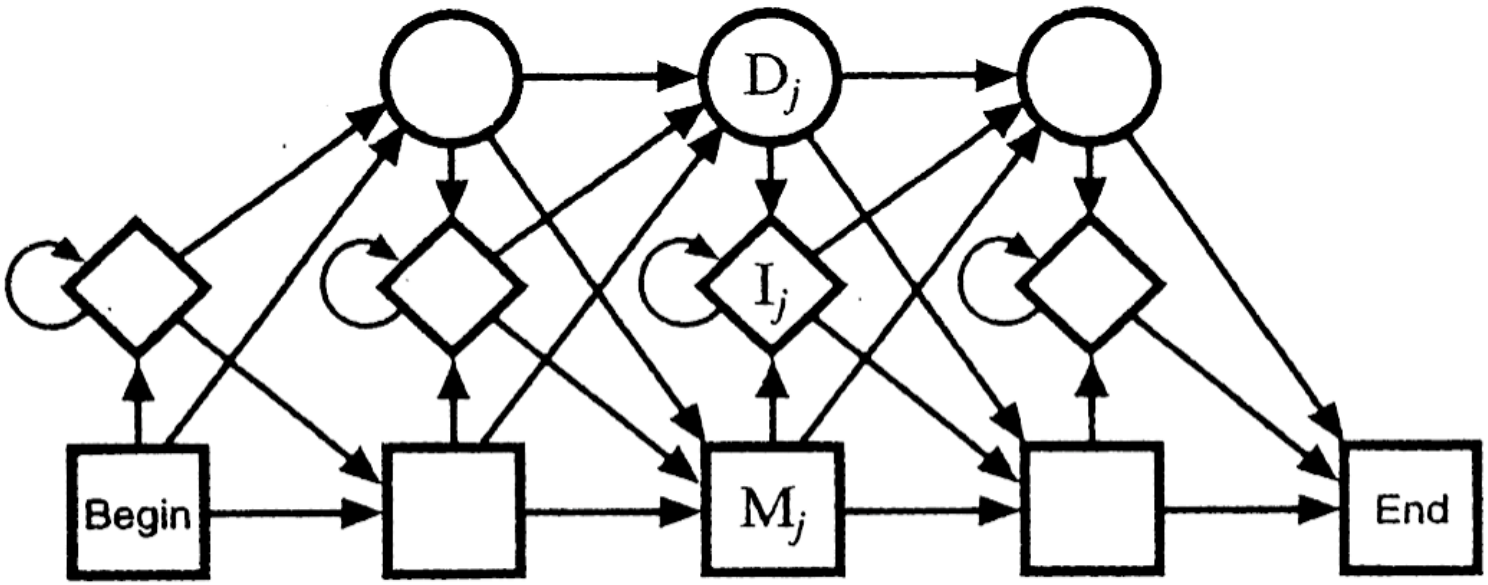



**Figure 5.2** *The transition structure of a profile HMM.*

$M_j$: Match states (20 emission probabilities)

$I_j$: Insert states (Background emission probabilities)

$D_j$: Delete states (silent - no emission)

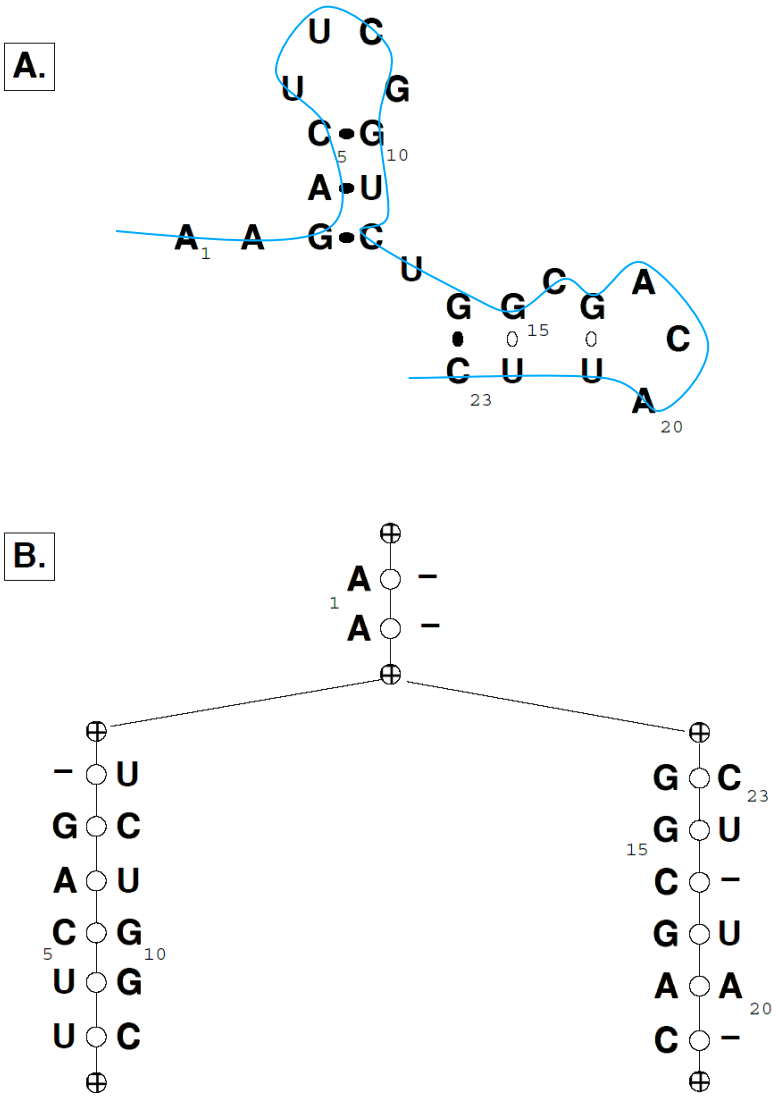
# CM Structure

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)



# CM Viterbi Alignment

$x_i \quad = i^{th}$ letter of input

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

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

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

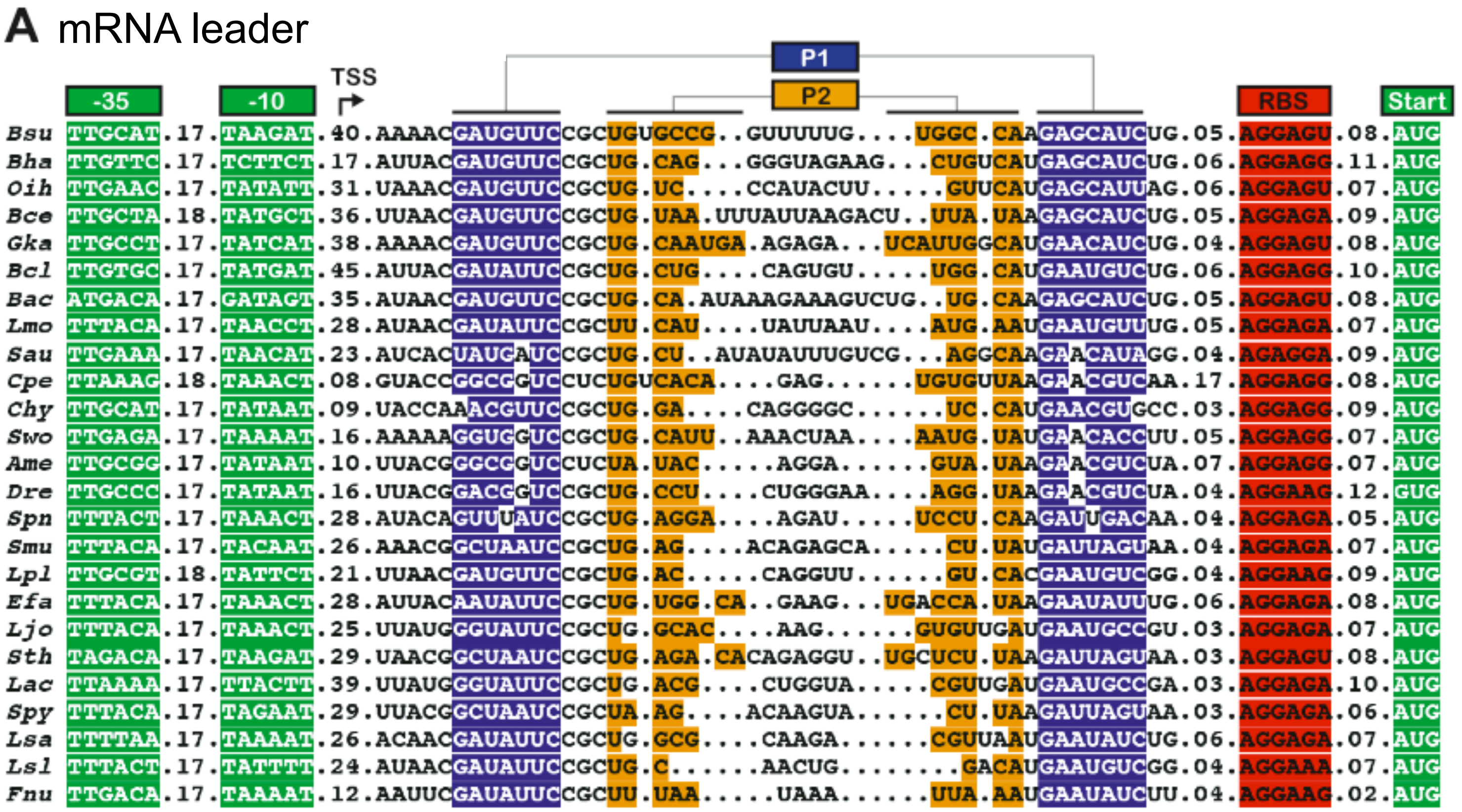
$S^{y}_{ij} \quad = \max_{\pi} \log P(x_{ij} \text{ gen'd starting in state } y \text{ via path } \pi)$

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

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

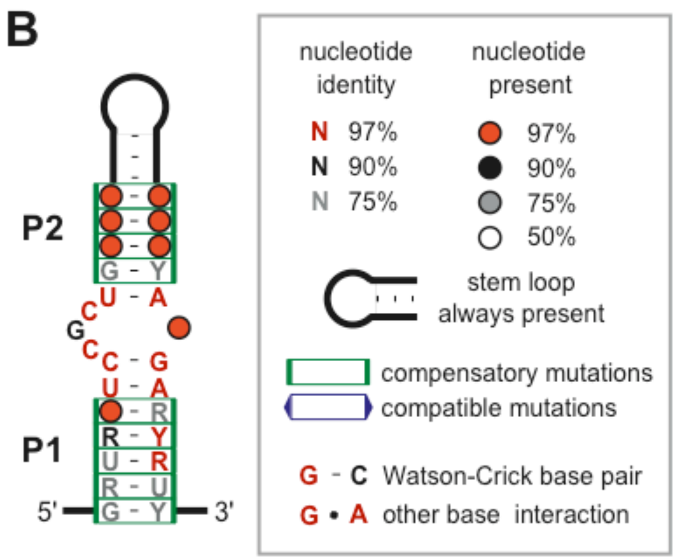
Time $O(qn^3)$, q states, seq len n

## A 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
```

## B



| nucleotide identity | | nucleotide present | |
|---|---|---|---|
| N | 97% | ● | 97% |
| N | 90% | ● | 90% |
| N | 75% | ● | 75% |
| | | ○ | 50% |

stem loop always present

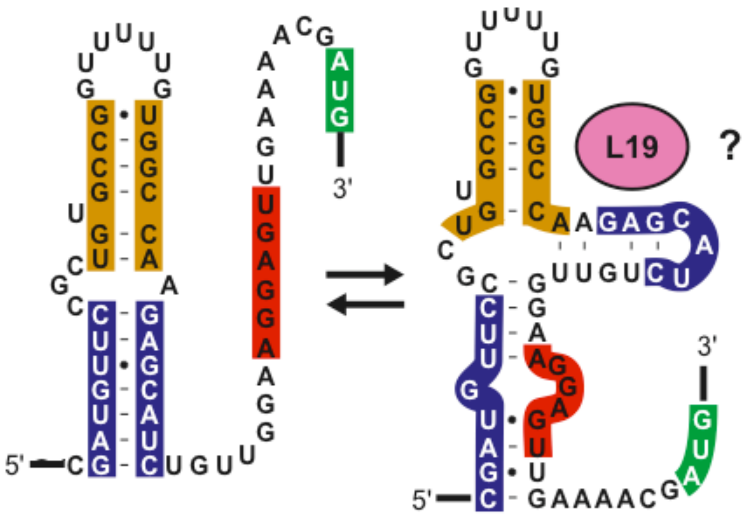
🟩 compensatory mutations

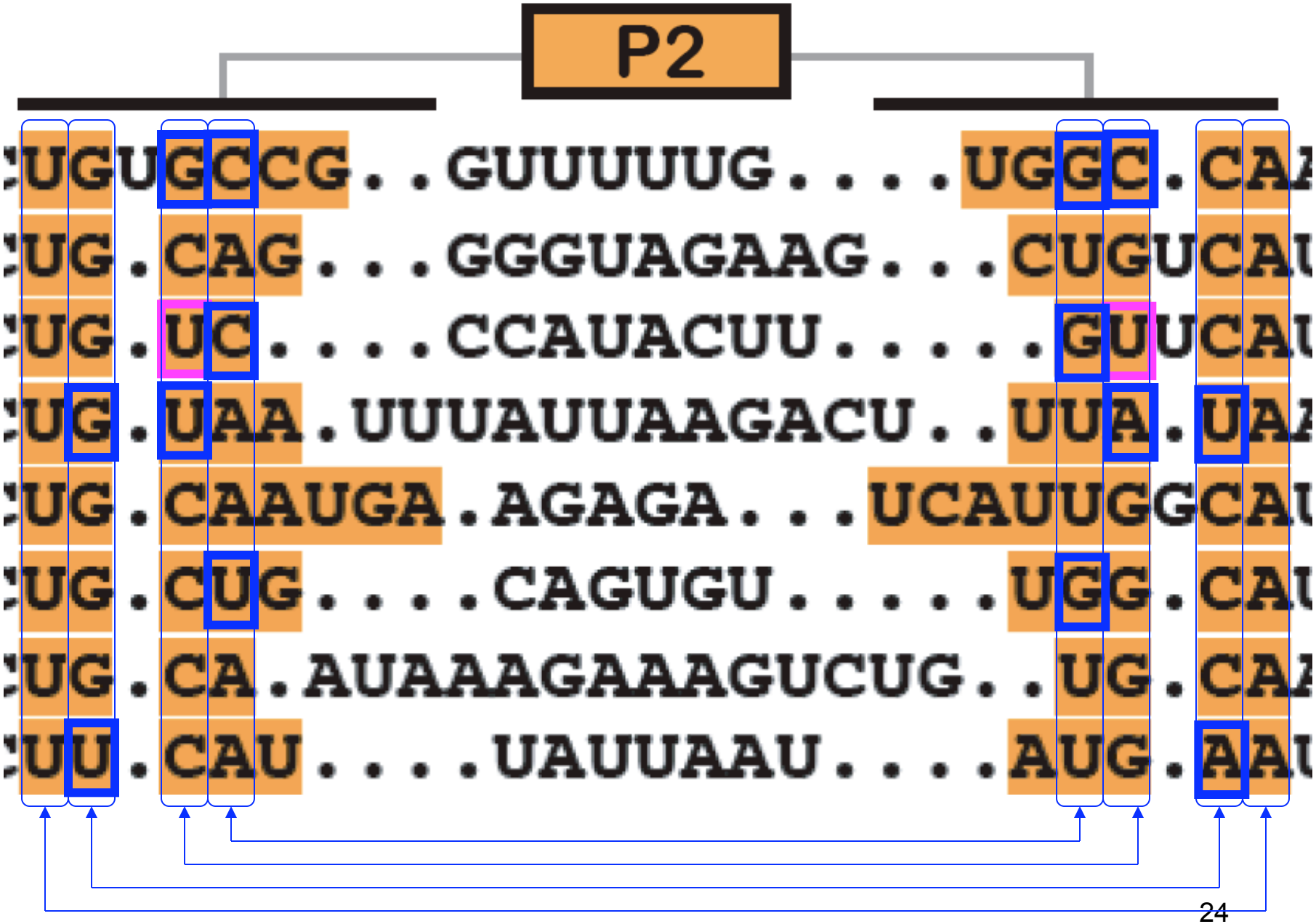
🟦 compatible mutations

G - C Watson-Crick base pair

G • A other base interaction

## C mRNA leader switch?




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

# Mutual Information

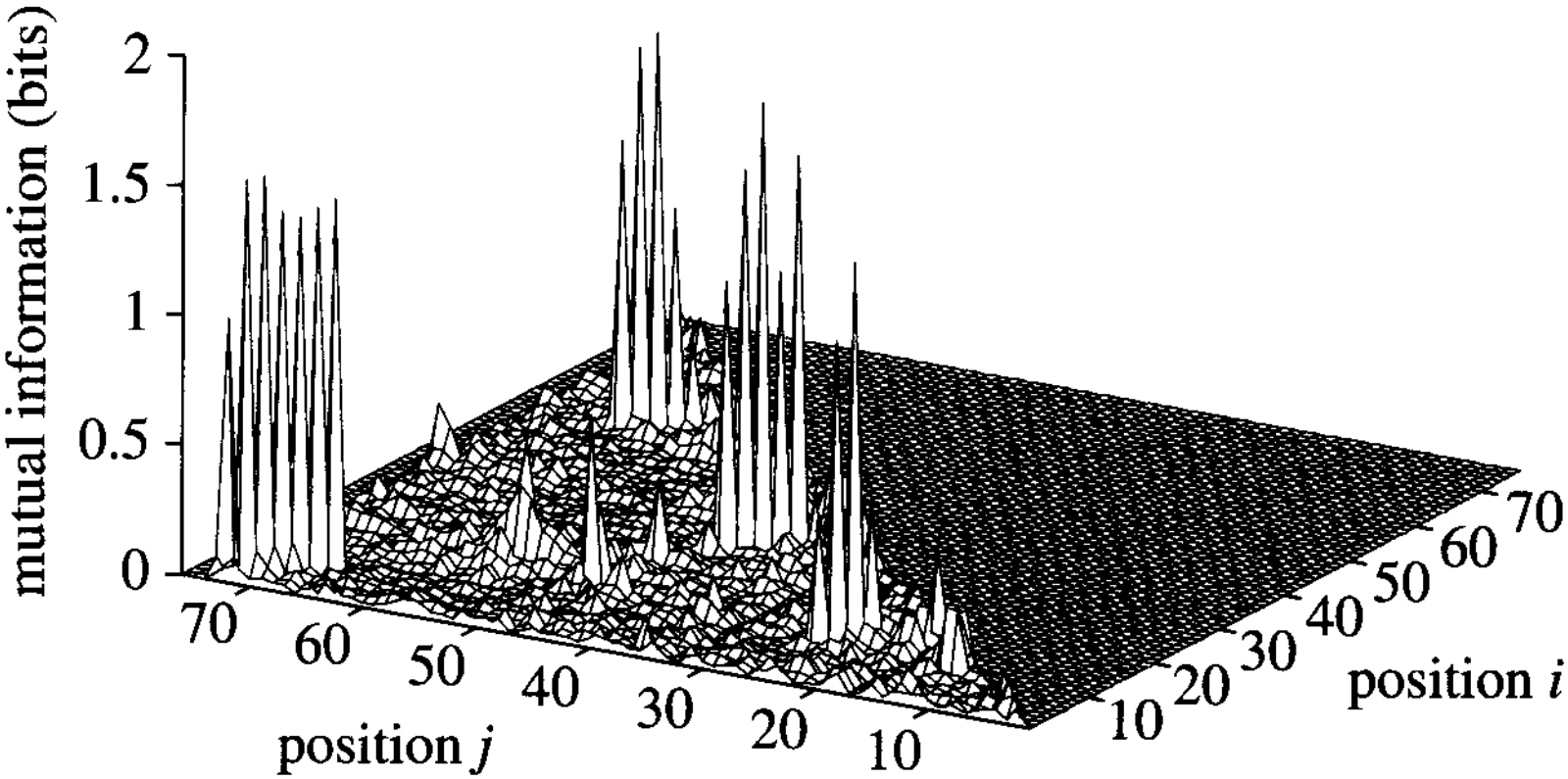
$$M_{ij} = \sum_{xi,xj} f_{xi,xj} \log_2 \frac{f_{xi,xj}}{f_{xi} f_{xj}}; \quad 0 \le M_{ij} \le 2$$

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



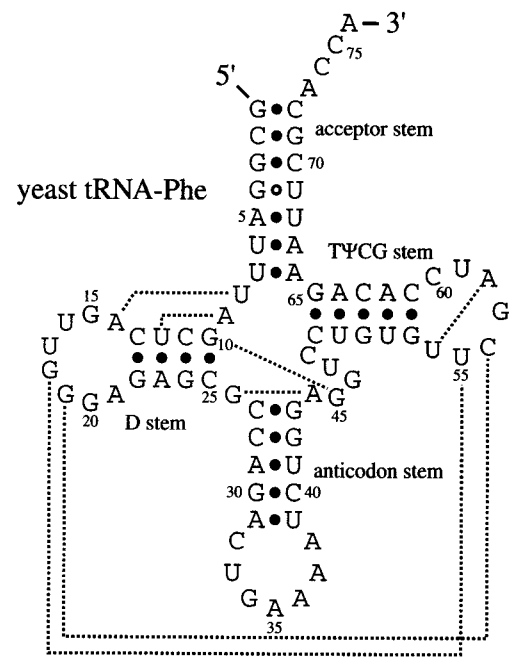




**Figure 10.6** *A mutual information plot of a tRNA alignment (top) shows four strong diagonals of covarying positions, corresponding to the four stems of the tRNA cloverleaf structure (bottom; the secondary structure of yeast phenylalanine tRNA is shown). Dashed lines indicate some of the additional 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.*

Pseudoknots

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

| 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 |

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.

# Rfam – an RNA family DB

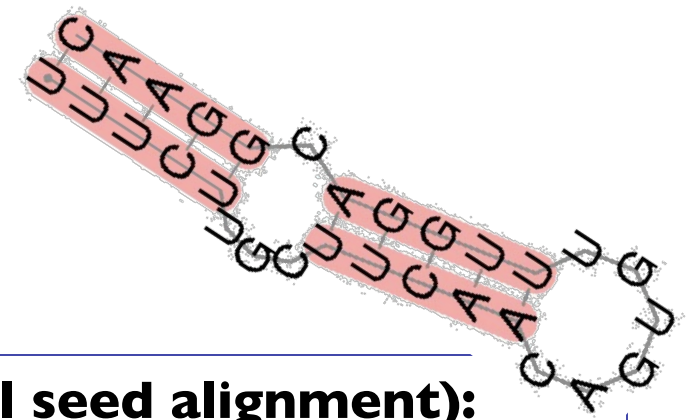
Griffiths-Jones, et al., NAR '03,'05

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

# 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 score < threshold;

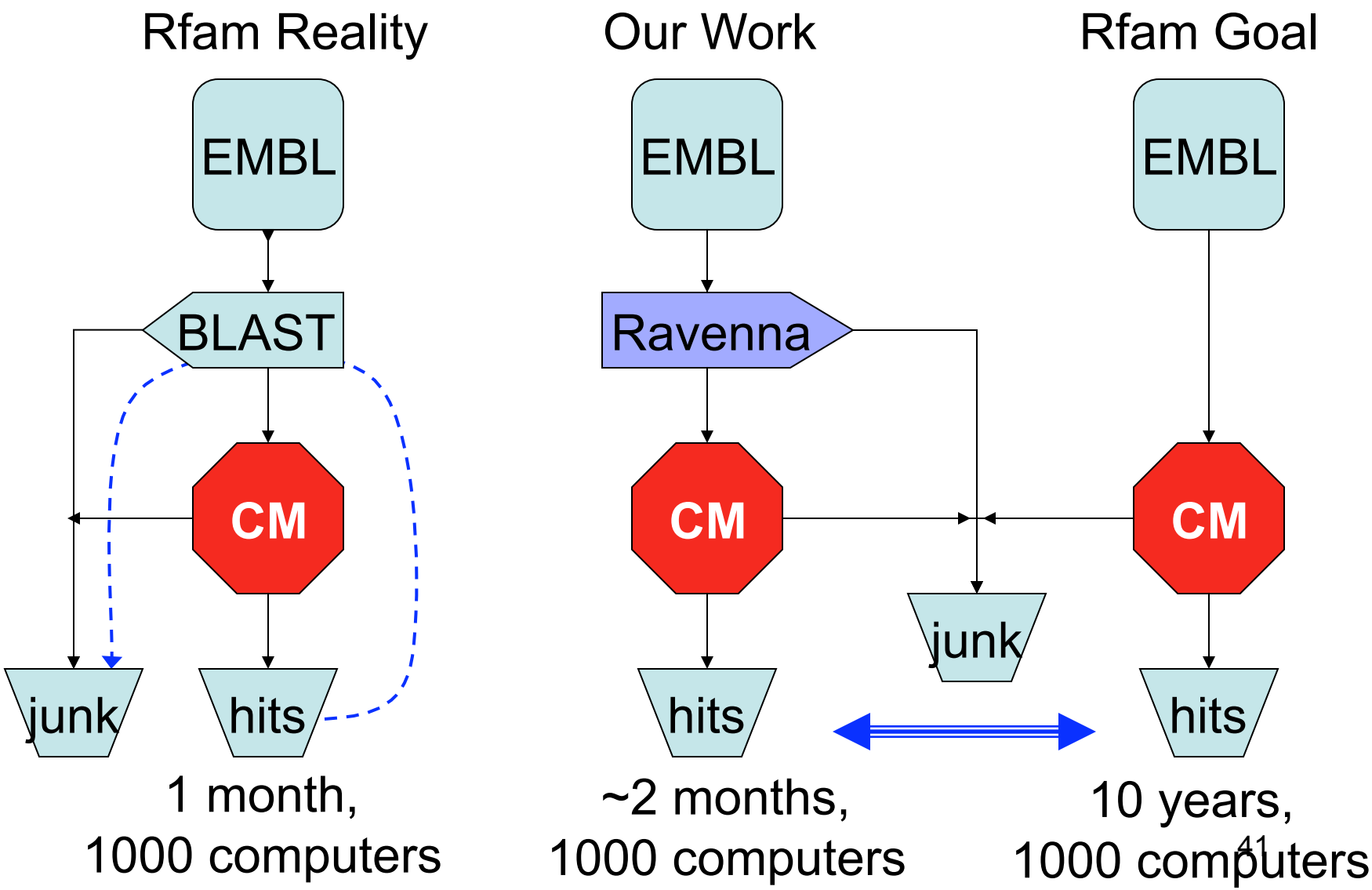
actually run CM on the rest (the promising parts)

assignment of HMM transition/emission scores is key

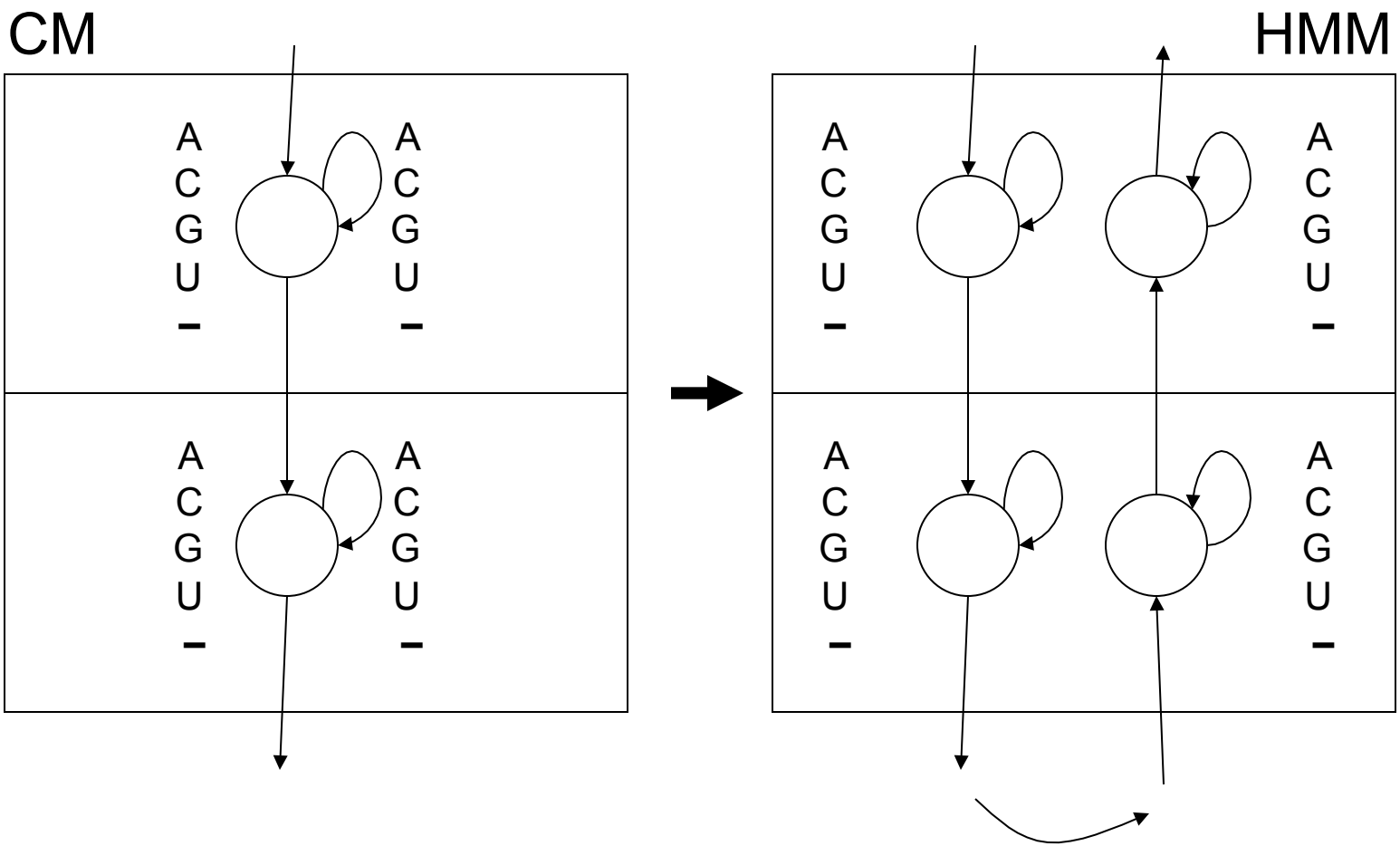
(large convex optimization problem)

Weinberg & Ruzzo, *Bioinformatics*, 2004, 2006

# CM's are good, but slow



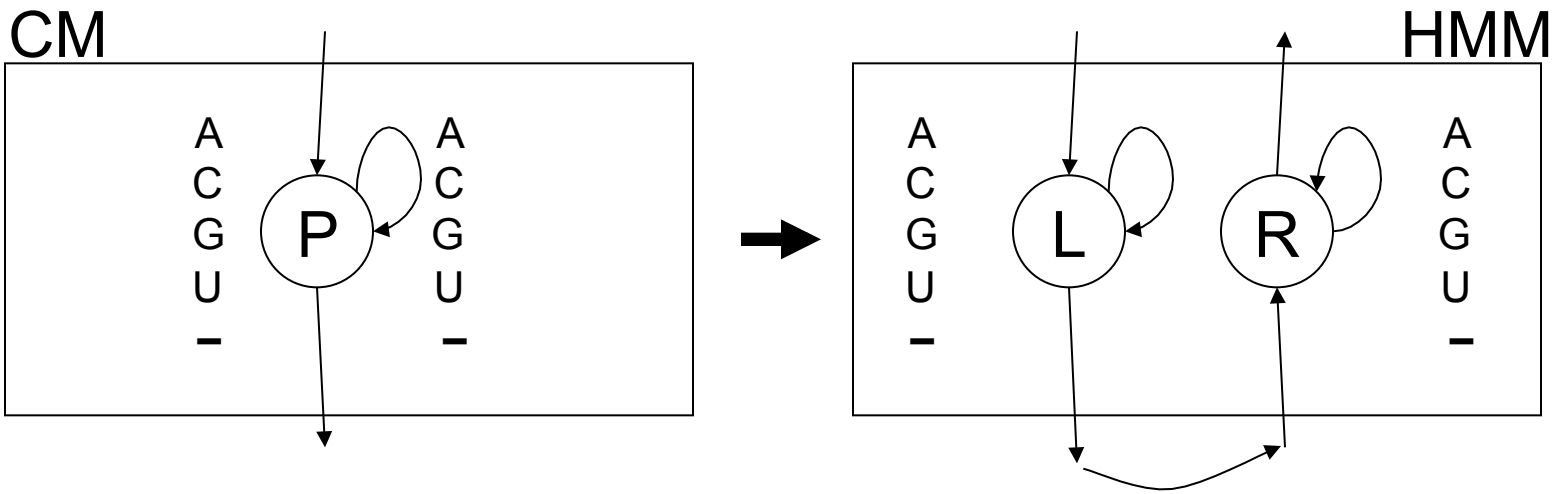
# CM to HMM




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 $\pi$ defines transitions/emissions

Score($\pi$) = product of "probabilities" on $\pi$

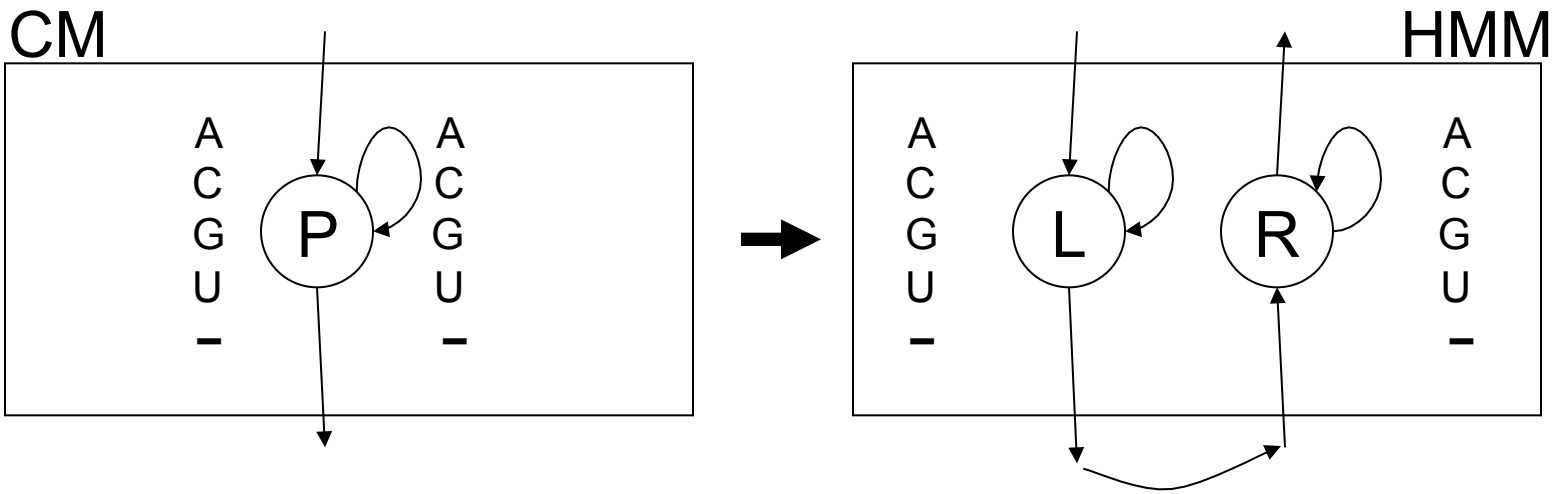
NB: ok if "probs" aren't, e.g. $\Sigma \neq 1$
(e.g. in CM, emissions are odds ratios vs
0th-order background)

For any nucleotide sequence x:

Viterbi-score(x) = max{ score($\pi$) | $\pi$ emits x}

Forward-score(x) = $\Sigma${ score($\pi$) | $\pi$ emits x}

# Key Issue: 25 scores → 10




Need: log Viterbi scores CM ≤ HMM

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

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

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

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

$P_{A–} \le L_A + R_–$ $\quad P_{C–} \le L_C + R_–$ $\quad \ldots$

NB: HMM not a prob. model

# Rigorous Filtering

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

*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)

NB: E is a function of $L_i$, $R_i$ only

Under 0th-order background model

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 $E(L_i, R_i)$

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

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 $E(L_i, R_i)$

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

Forward:

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

Viterbi:

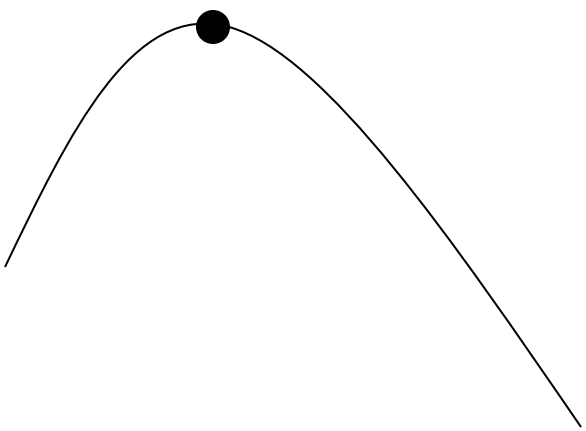
$$v_l(i+1) = e_l(x_{i+1}) \cdot \max_k (v_k(i)\, a_{k,l})$$

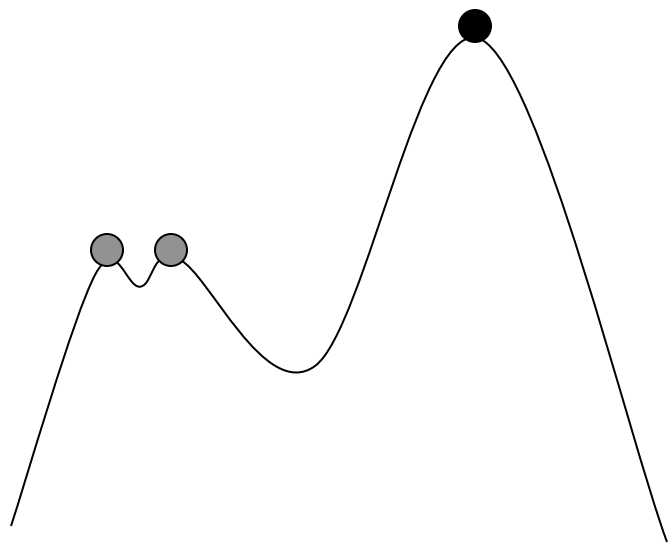
$$\frac{\partial E(L_1, L_2, \ldots)}{\partial L_i}$$

# “Convex” Optimization

Convex:
local max = global max;
simple “hill climbing” works

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 |

~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 |

# 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

# 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.

# 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

# Approaches

Align sequences, then look for common structure

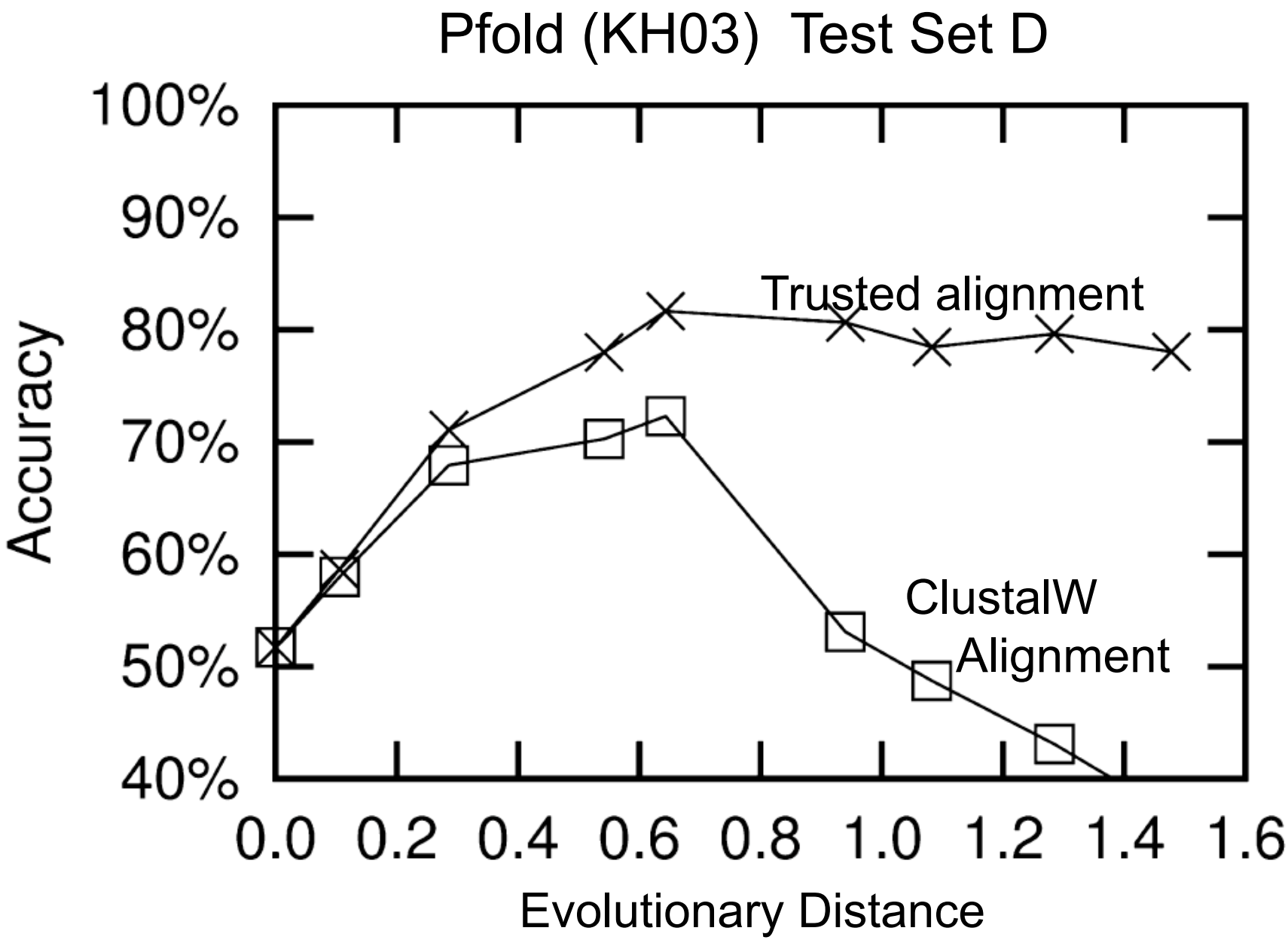
Predict structures, then try to align them

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)




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

# Our Approach: CMfinder

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

Yao, Weinberg & Ruzzo, *Bioinformatics*, 2006

# 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

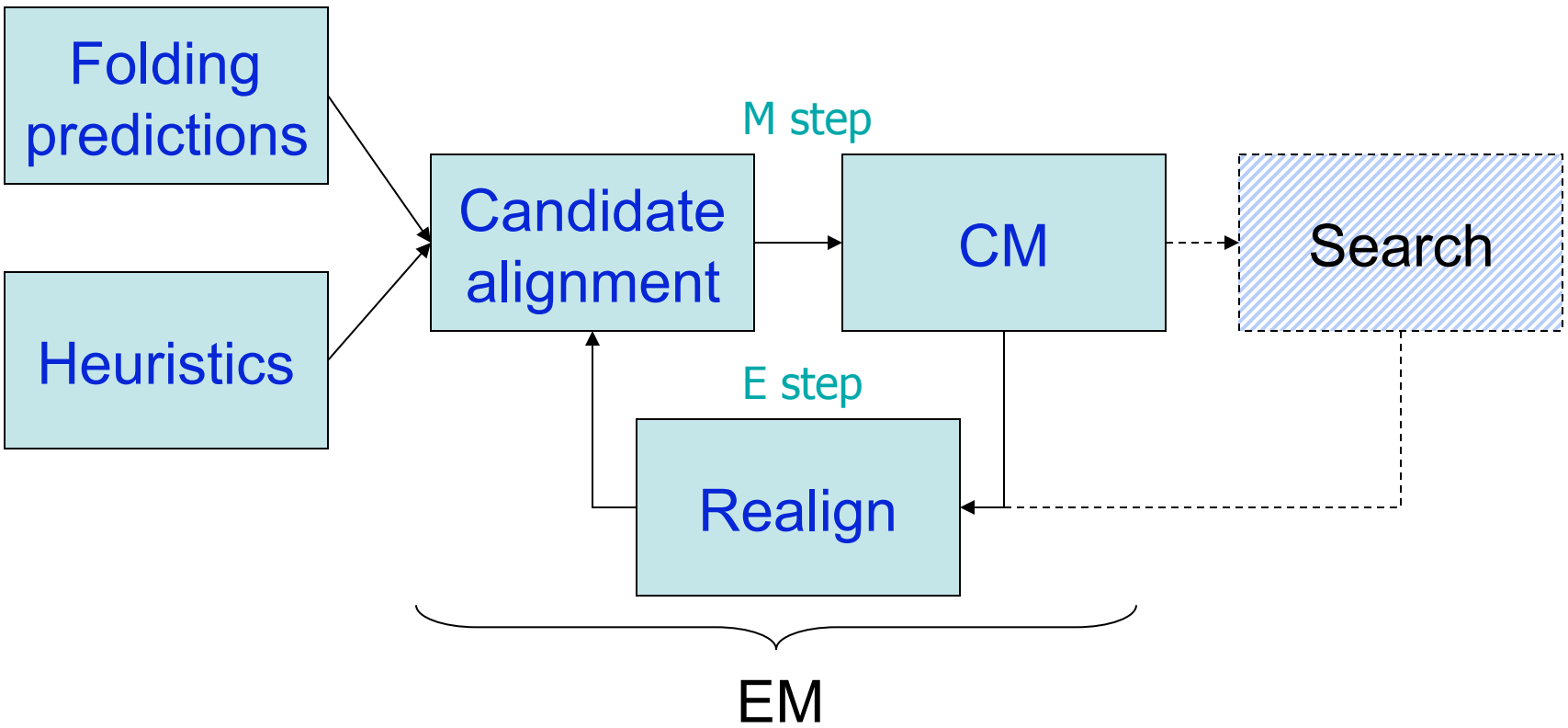
Similar to HMM, but slower

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

# Structure Inference

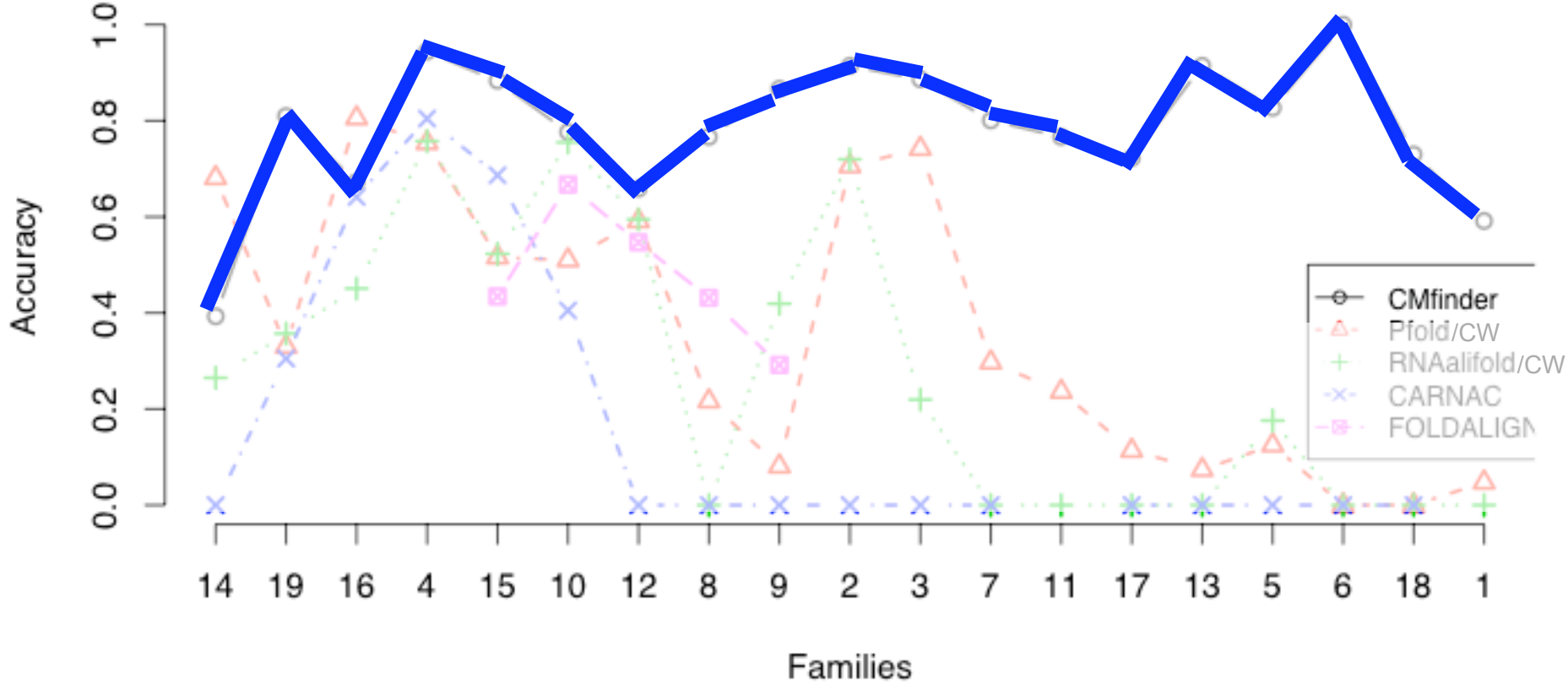
Part of M-step is to pick a structure that maximizes data likelihood

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

# CMfinder Accuracy

## (on Rfam families *with* flanking sequence)



# Application I

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

Yao, Barrick, Weinberg, Neph, Breaker, Tompa and Ruzzo.
PLoS Computational Biology. 3(7): e126, July 6, 2007.

# 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

# 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, find similar genes ("homologs"), look at adjacent DNA

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

# Approach

Get bacterial genomes

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

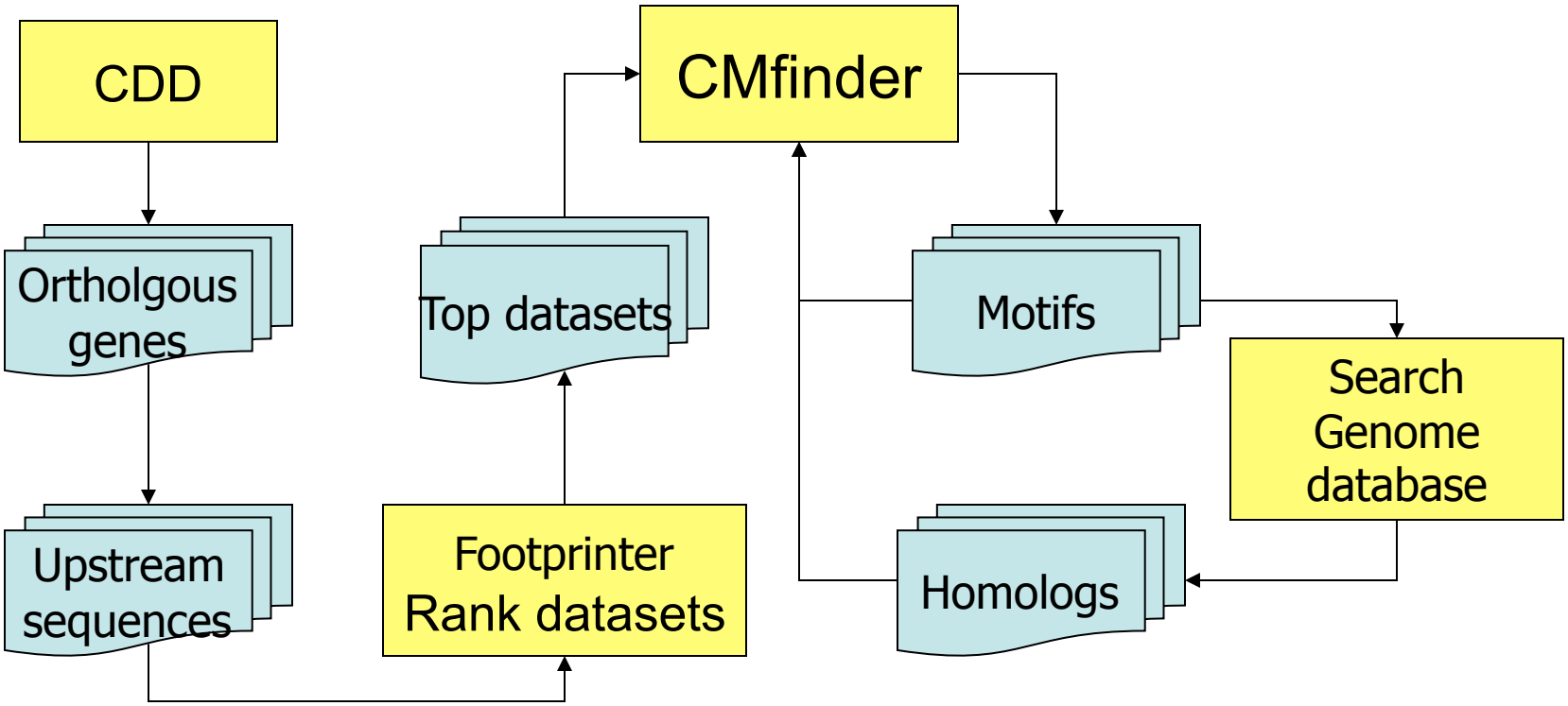
Find most promising genes, based on conserved sequence motifs (Footprinter)

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




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

# Genome Scale Search: Why

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

In most close relatives

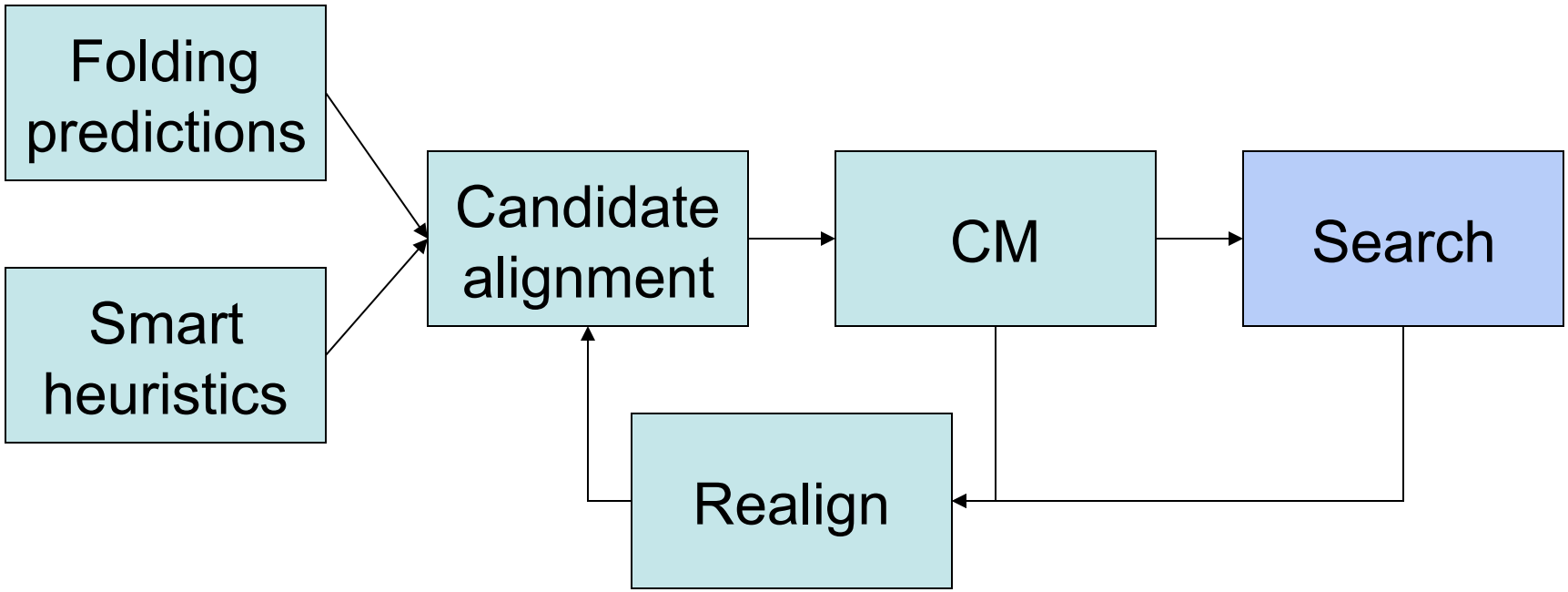
More examples give better model, hence even more examples, fewer errors

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

But inclusion of non-examples can degrade motif…

# Genome Scale Search

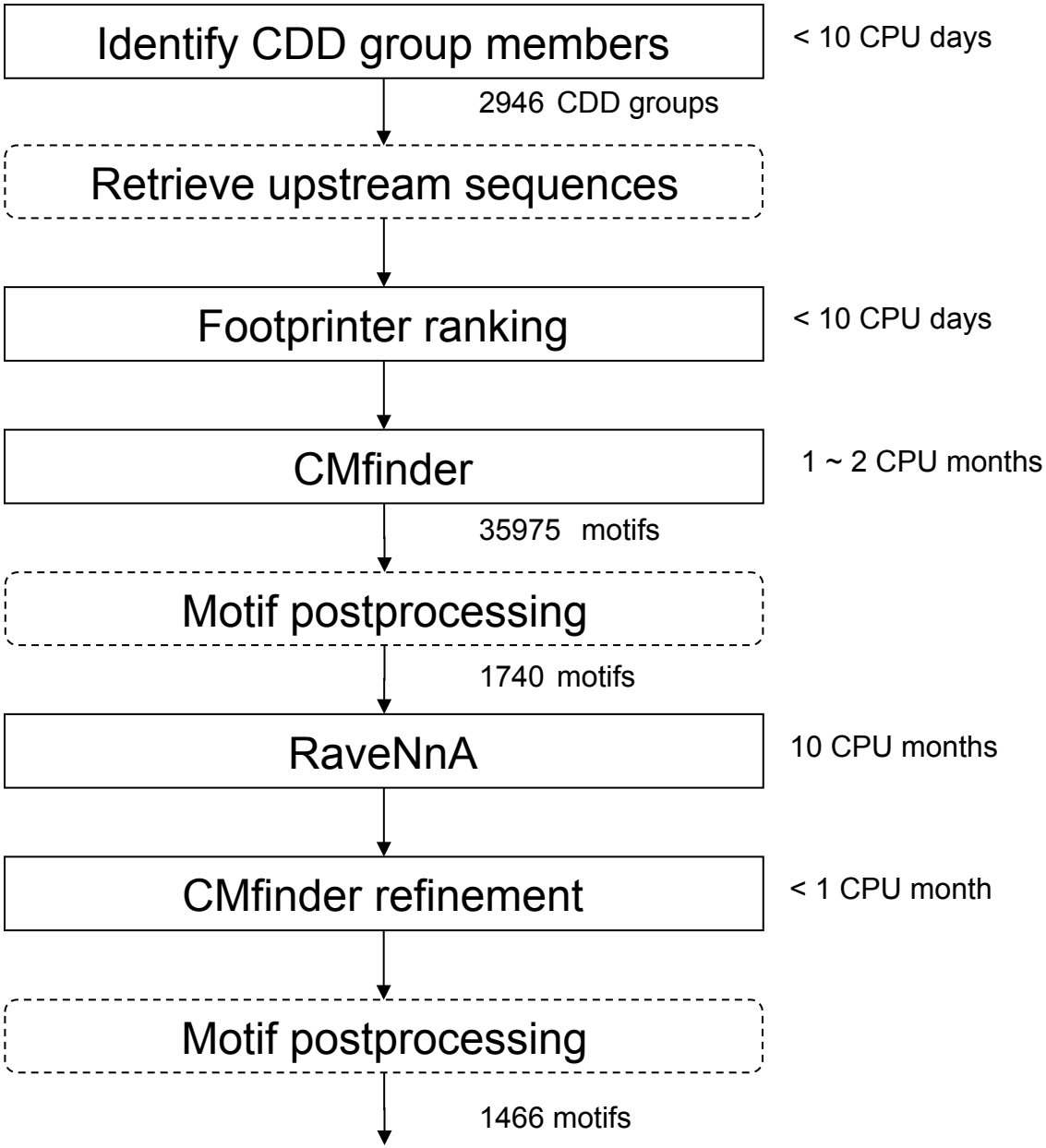
CMfinder is directly usable for/with search



# Results

Have analyzed most sequenced bacteria (~2005)

- bacillus/clostridia
- gamma proteobacteria
- cyanobacteria
- actinobacteria
- firmicutes


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

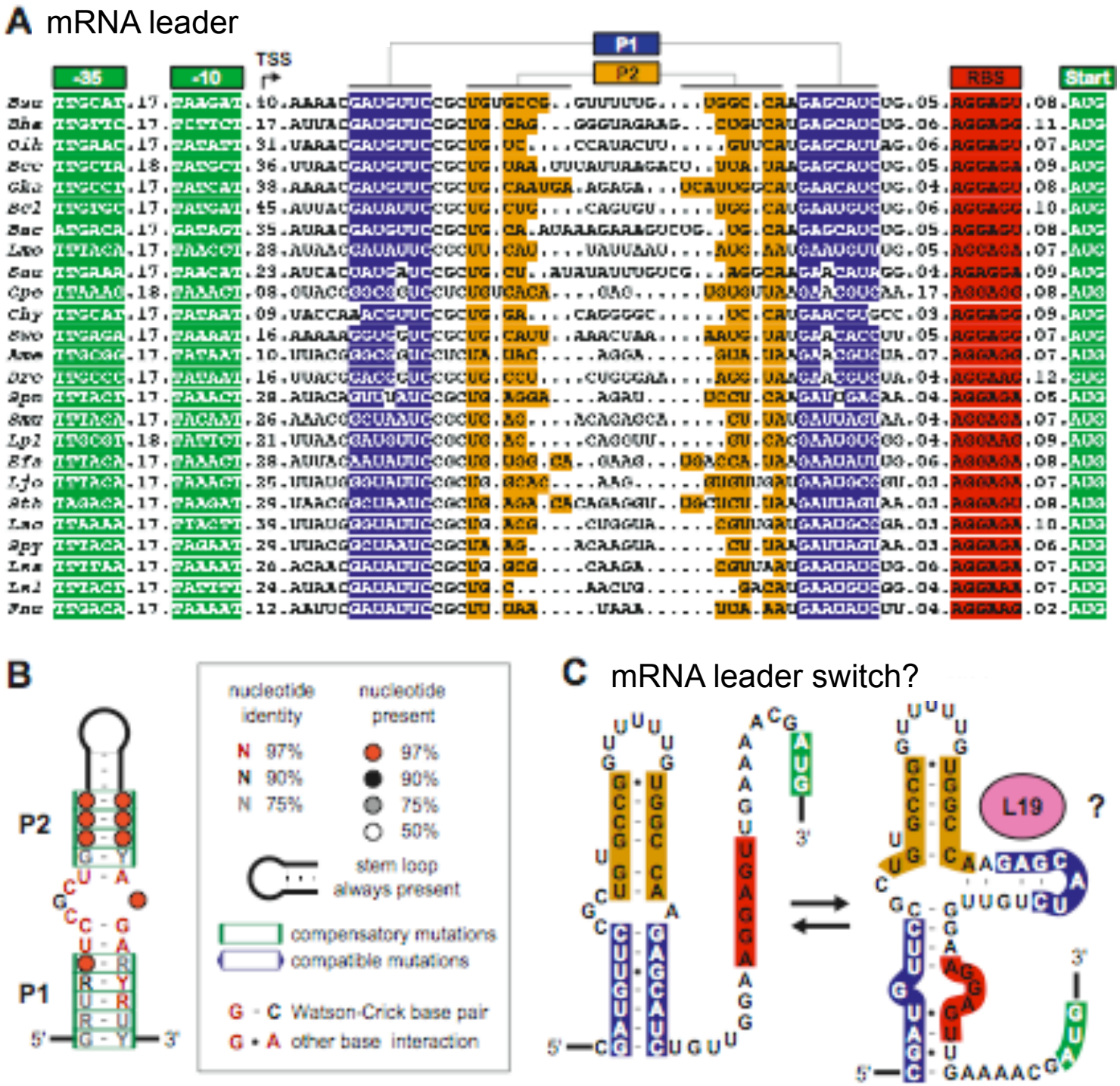
| Rank | | | Score | # | | CDD | | | Rfam |
|---|---|---|---|---|---|---|---|---|---|
| RAV | CMF | FP | | RAV | CMF | ID | Gene | Description | |
| 0 | 43 | 107 | 3400 | 367 | 11 | 9904 | IlvB | Thiamine pyrophosphate-requiring enzymes | RF00230 T-box |
| 1 | 10 | 344 | 3115 | 96 | 22 | 13174 | COG3859 | 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 |

Table 2: Motif prediction accuracy vs prokaryotic subset of Rfam full alignments. “Membership”: the number of sequences in the overlap between our predictions and Rfam’s (“#”), the sensitivity (“Sn”) and specificity (“Sp”) of our membership predictions. “Overlap”: avg length 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”: avg number of correctly predicted canonical base pairs (in overlapped regions) and the sensivity (“Sn”) and specificity (“Sp”) of our predictions. [1]After another iteration of RaveNnA scan and refinement, the membership sensitivities of Glycine and Cobalamin increased to 76% and 98% respectively, while the specificity of Glycine remained the same, and specificity of Cobalamin dropped to 84%.

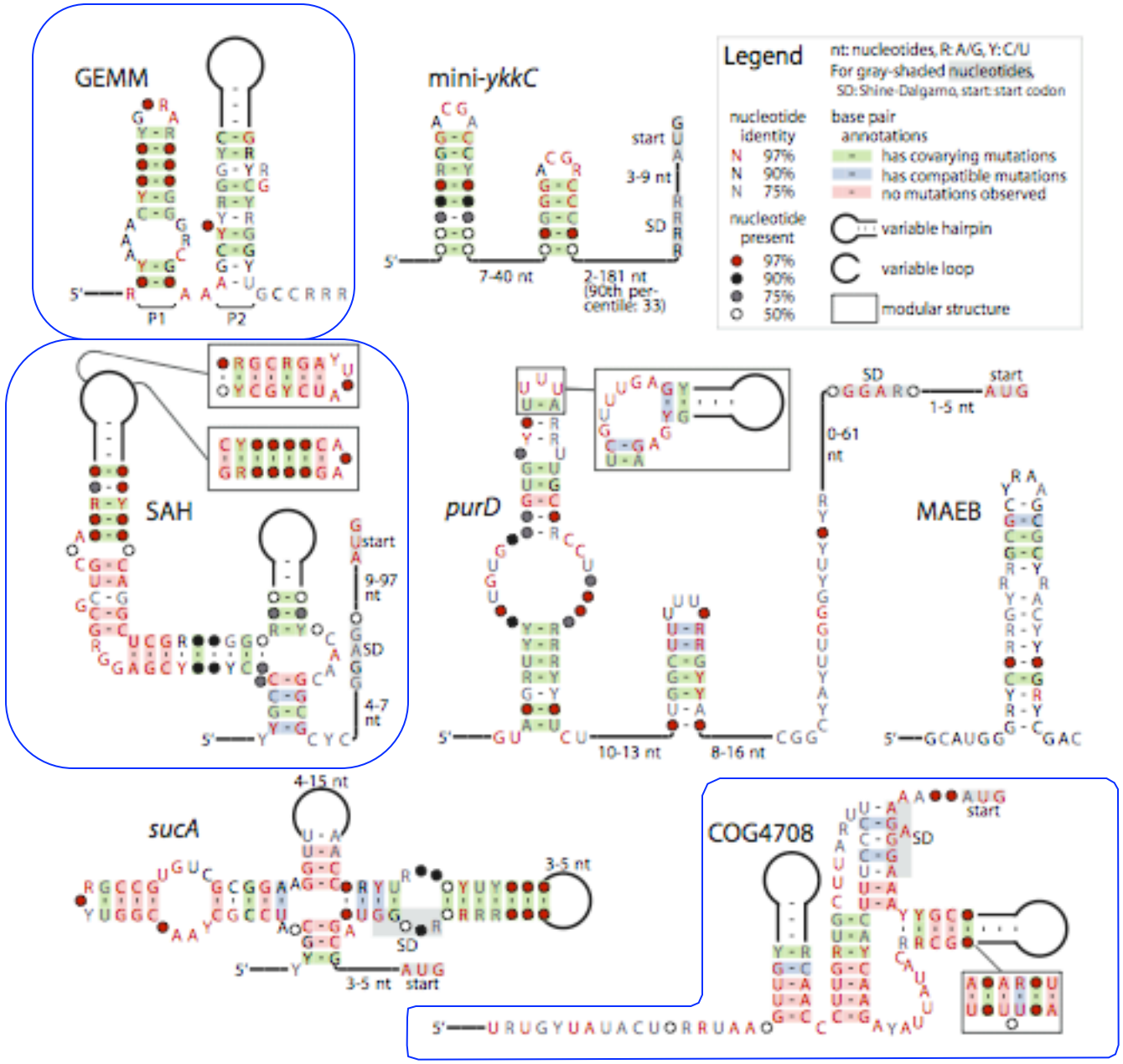
| Rank | # | CDD | Gene: Description | Annotation |
|---|---|---|---|---|
| 6 | 69 | 28178 | DHOase IIa: Dihydroorotase | PyrR attenuator [22] |
| 15 | 33 | 10097 | RplL: Ribosomal protein L7/L1 | L10 r-protein leader; see Supp |
| 19 | 36 | 10234 | RpsF: Ribosomal protein S6 | S6 r-protein leader |
| 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 |


A mRNA leader
TSS
-35
-10
P1
P2
RBS
Start
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'

# Application II

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

Weinberg, Barrick, Yao, Roth, Kim, Gore, Wang, Lee, Block, Sudarsan, Neph, Tompa, Ruzzo and Breaker. Nucl. Acids Res., July 2007 35: 4809-4819.




boxed = confirmed riboswitch (+2 more)



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

# New Riboswitches

| | |
|---|---|
| SAM – IV | (S-adenosyl methionine) |
| SAH | (S-adenosyl homocystein) |
| MOCO | (Molybdenum Cofactor) |
| PreQ1 – II | (queuosine precursor) |
| GEMM | (cyclic di-GMP) |

# GEMM regulated genes

Pili and flagella

Secretion

Chemotaxis

Signal transduction

Chitin

Membrane Peptide

Other - *tfoX*, cytochrome c

GEMM sense a metabolite (cyclic di-GMP) produced for signal transduction or for cell-cell communication.

# Utility?

Unknown

BUT

E.g., there are no known human riboswitches, so potentially fewer side effects from drugs that might target them

Some such drugs (w/ previously unknown targets) have been known for decades!

# ncRNA discovery in Vertebrates

**Comparative genomics beyond sequence based alignments: RNA structures in the ENCODE regions**

E. Torarinsson, Z. Yao, E. D. Wiklund, J. B. Bramsen , C. Hansen, J. Kjems, N. Tommerup, W. L. Ruzzo and J. Gorodkin

Genome Research, Jan 2008

# ncRNA discovery in Vertebrates

Previous studies focus on highly conserved regions (Washietl, Pedersen et al. 2007)

- Evofold (Pedersen *et al.* 2006)
- RNAz (Washietl *et al.* 2005)

We explore regions with weak sequence conservation

# Approach

Extract ENCODE Multiz alignments

- Remove exons, most conserved elements.
- 56017 blocks, 8.7M bps.

Apply CMfinder to both strands.

10,106 predictions, 6,587 clusters.

- False positive rate: 50% based on a heuristic ranking function.

# Search in Vertebrates

Extract ENCODE Multiz alignments

- Remove exons, most conserved elements.
- 56017 blocks, 8.7M bps.

Apply CMfinder to both strands.

Trust 17-way alignment for orthology, not for detailed alignment

10,106 predictions, 6,587 clusters.

- High false positive rate, but still suggests 1000's of RNAs.

(We've applied CMfinder to whole human genome:
O(1000) CPU years. Analysis in progress.)

# Assoc w/ coding genes

Many known human ncRNAs lie in introns

Several of our candidates do, too, including some of the tested ones

- #6: *SYN3* (Synapsin 3)
- #10: *TIMP3*, antisense within *SYN3* intron
- #9: *GRM8* (glutamate receptor metabotropic 8)

# Overlap with known transcripts

Input regions include only one known ncRNA has-mir-483, and we found it.

40% intergenetic, 60% overlap with protein coding gene

| Sense | Antisense | Both | Intron | 5'UTR | 3'UTR |
|---|---|---|---|---|---|
| 1332 (33.8%) | 1721 (43.7%) | 884 (22.5%) | 3274 (83.1%) | 551 (14%) | 89 (2.3%) |

# Overlap w/ Indel Purified Segments

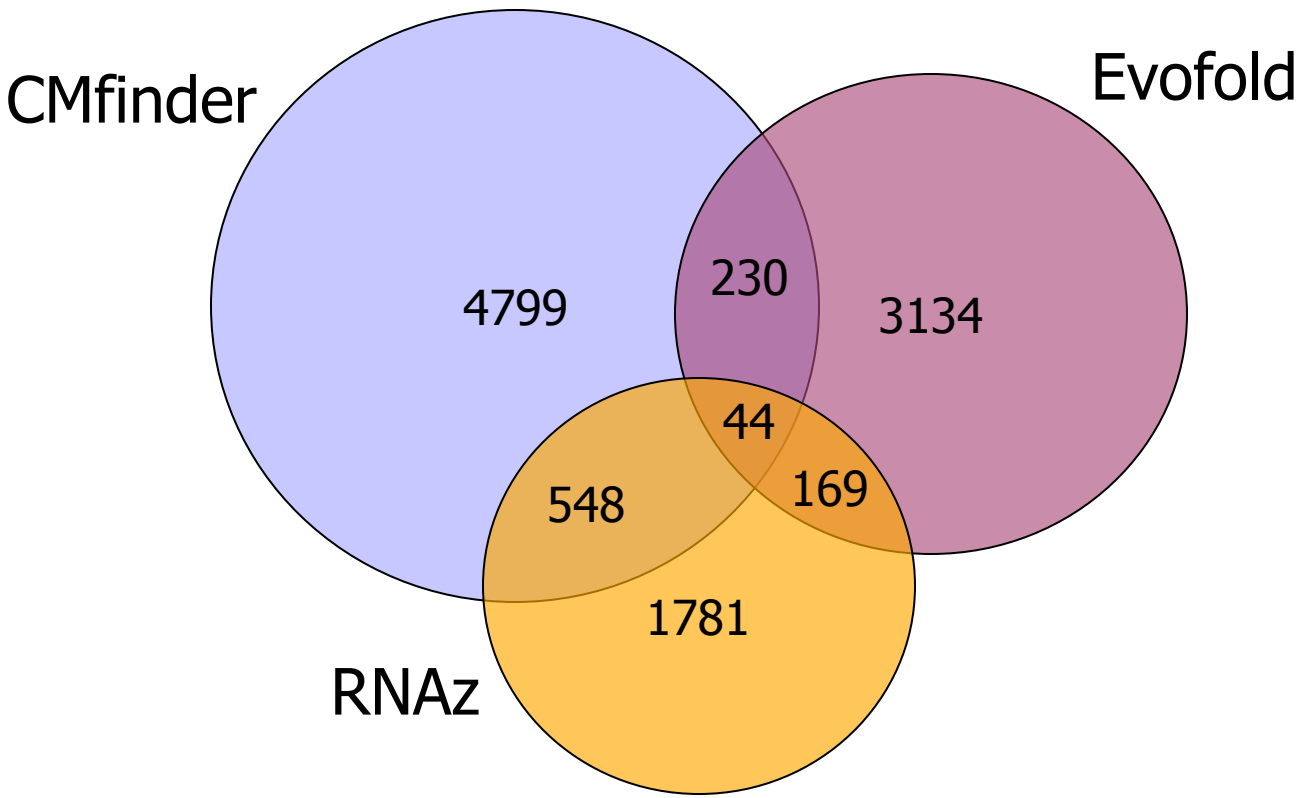
IPS presumed to signal purifying selection

Majority (64%) of candidates have >45% G+C

Strong P-value for their overlap w/ IPS

| G+C | data | P | N | Expected | Observed | P-value | % |
|---|---|---|---|---|---|---|---|
| 0-35 | igs | 0.062 | 380 | 23 | 24.5 | 0.430 | 5.8% |
| 35-40 | igs | 0.082 | 742 | 61 | 70.5 | 0.103 | 11.3% |
| 40-45 | igs | 0.082 | 1216 | 99 | 129.5 | 0.00079 | 18.5% |
| 45-50 | igs | 0.079 | 1377 | 109 | 162.5 | 5.16E-08 | 20.9% |
| 50-100 | igs | 0.070 | 2866 | 200 | 358.5 | 2.70E-31 | 43.5% |
| all | igs | 0.075 | 6581 | 491 | 747.5 | 1.54E-33 | 100.0% |

# Comparison with Evofold, RNAz




Small overlap (w/ highly significant p-values) emphasizes complementarity

# Alignment Matters

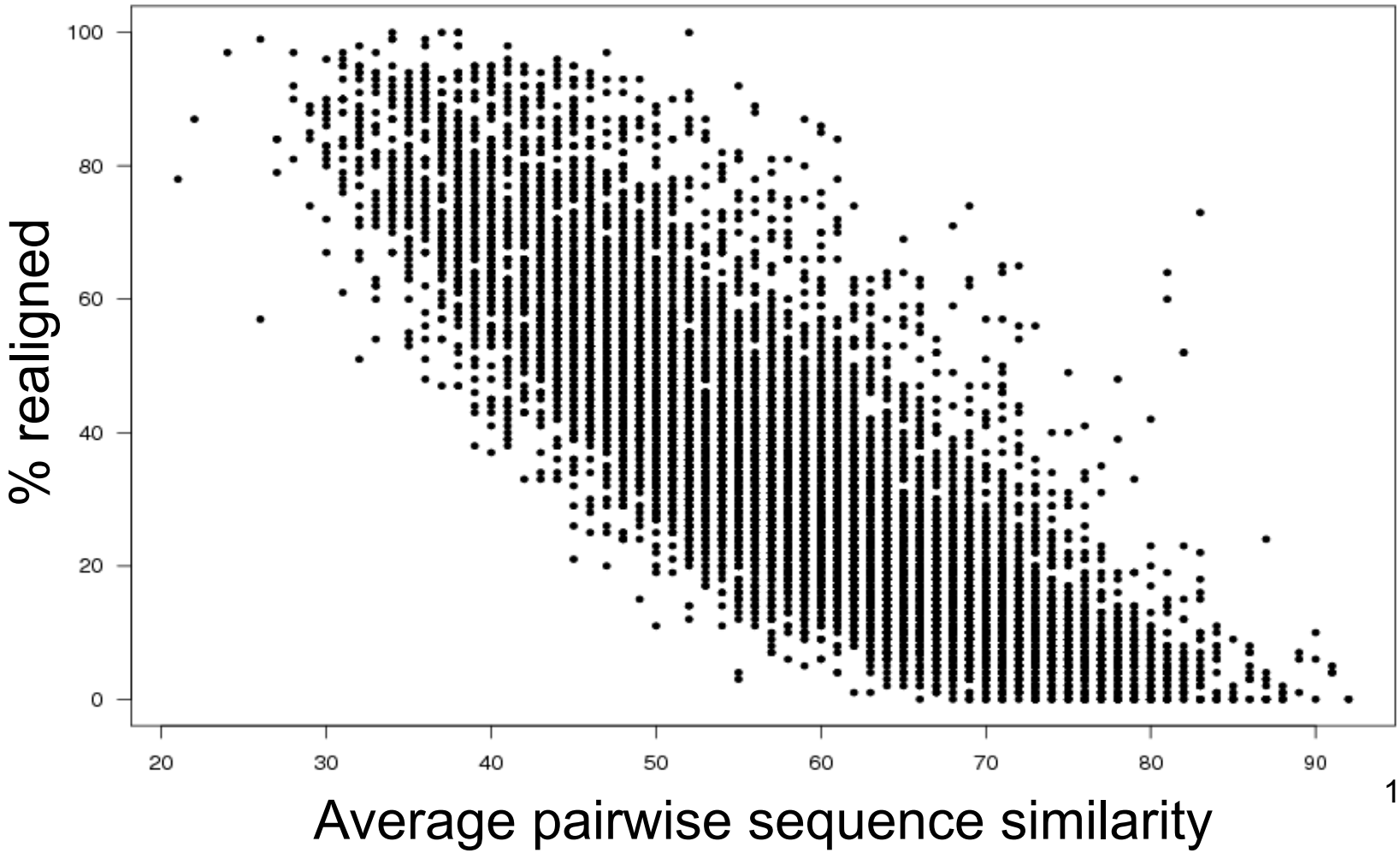
**B. The original MULTIZ alignment without the flanking regions – RNAz Score: 0.132 (no RNA)**

```
hg18.chr3          GGTCACTTCAAAGAGGGCTT-GTGGGGCTGTGAAACCAAGAGGT----CTTAACAGTATGACCAAAAACTGAAGT
panTro1.chr17      GGACATTTCAATGCGGGCTC-ATGGGGCTGTGAAGCCAAGAGCT----ATTAACACTATGACCAAGGACTGAAAT
bosTau2.chr18      GGTCATTTCAAAGAGGGCTT-ATGAGACCA--AAACCGGGAGCT----CTTAATGCTGTGACCAAAGATTGAAGT
canFam2.chr3       GGTCATTTCAAAGAGGGCTTTGTGGAACTA--AAACCAAGGGCT----CTTAACTCTGTGACCAAATATTAGAGT
oryCun1            GATCATTTCAAAGAGGGTTT-GTGGTGCTGTGAAGTCAAGAACT----CTTAACTGTATGCCCAAAGATTAAAGT
rheMac2.chr2       GGTCACTTCAAAGAGGGCTT-GTGGGGCTGTGAAACCAAGAGGTAGGTCTTAACAGTATAACCAAAGACTGAAGT
                   {{{{{{.....{{{{{{{...{{{.........}}}.}}}}....}}}.....}}}}}}............
```

**C. The local CMfinder re-alignment of the MULTIZ block – RNAz Score: 0.709 (RNA)**

```
hg18.chr3          GGTCACTTCAAAGAGGGCTT-GTGGGGCTGTGAAA-CCA-----AGAGGTCTTAACAGTATGACCAAAAACTGAA
panTro1.chr17      GGACATTTCAATGCGGGCTC-ATGGGGCTGT-GAAGCCA-----AGAGCTATTAACACTATGACCAAGGACTGAA
bosTau2.chr18      GGTCATTTCAAAGAGGGCTT-ATGAGACCA--AAA-CCG-----GGAGCTCTTAATGCTGTGACCAAAGATTGAA
CanFam2.chr3       GGTCATTTCAAAGAGGGCTTTGTGGAACTA--AAA-CCA-----AGGGCTCTTAACTCTGTGACCAAATATTAGA
oryCun1            GATCATTTCAAAGAGGGTTT-GTGGTGCTGT-GAAGTCA-----AGAACTCTTAACTGTATGCCCAAAGATTAAA
rheMac2.chr2       GGTCACTTCAAAGAGGGCTT-GTGGGGCTGTGAAA-CCAAGAGG-TAGGTCTTAACAGTATAACCAAAGACTGAA
                   {{{{{{.....{{{{{{{{..{{{.........}}}......}}}}}}}}.....}}}}}}..........
```

# Realignment

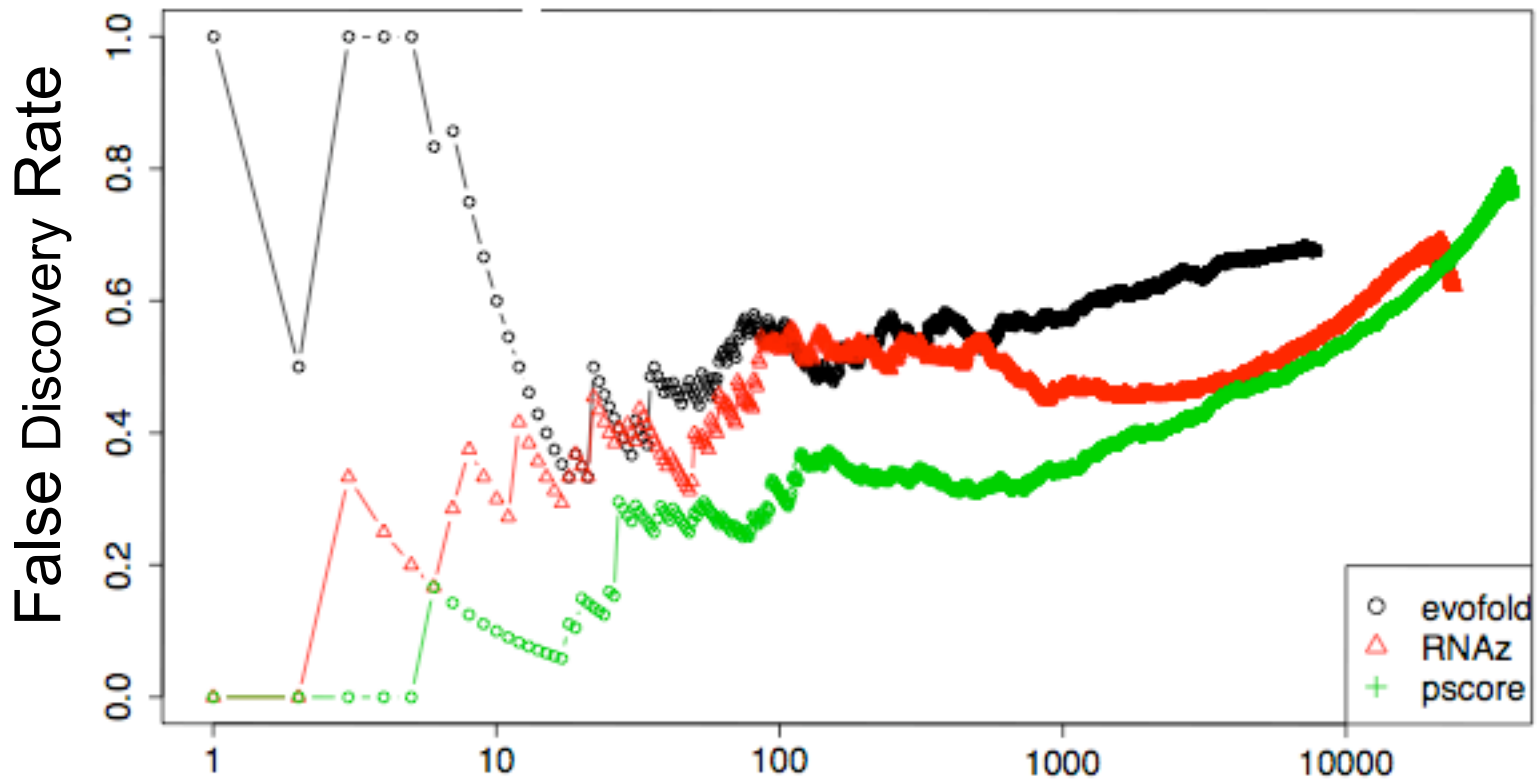


# New scoring scheme

Goal: improve false discovery rate for top ranking motifs

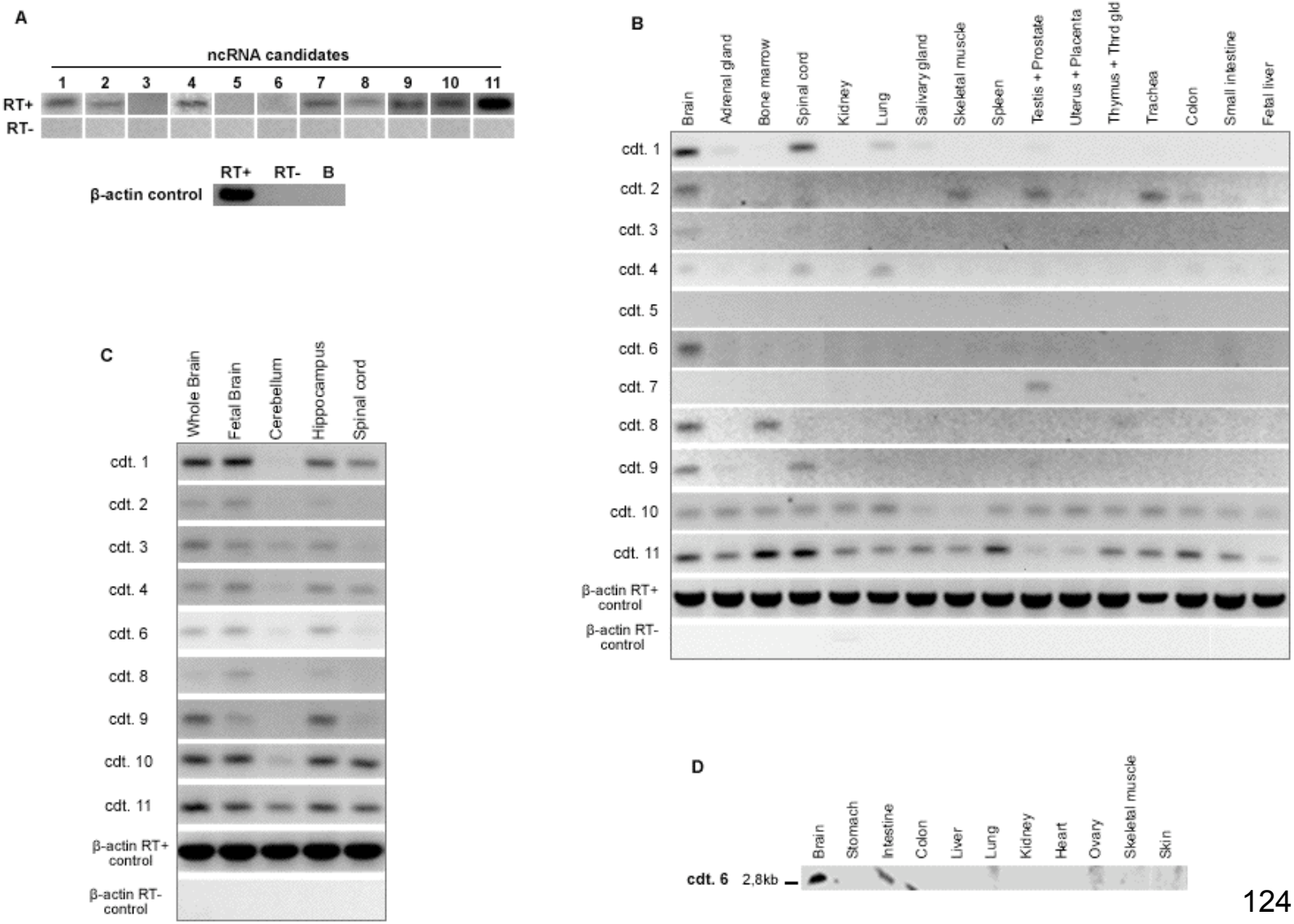
- Current methods can not improve beyond 50% FDR by using higher score threshold.
- Neither RNAz nor Evofold are robust on poorly conserved and gappy regions. (Of course, they weren't designed to be.)

# Test on CMfinder motifs in ENCODE regions




FDR vs score ranks in the original alignments

# 10 of 11 top (differentially) expressed



# 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 - good CM-based motif discovery in unaligned sequences

- Pipeline integrating comp and bio for ribowitch discovery
- Potentially many ncRNAs with weak sequence conservation in vertebrates.

# Summary

Lots of *structurally* conserved ncRNA

Functional significance often unclear

But high rate of confirmed tissue-specific expression in (small) set of top candidates in humans

BIG CPU demands…

Still need for further methods development & application

Thanks!

# Discovering ncRNAs in prokaryotes through genome-wide clustering

Elizabeth Tseng
UW CSE

# Our work

- Goal
  - Clustering for homologous ncRNA prediction
- Our Approach
  - Cluster genomic sequences by homology
  - Incorporate secondary structure information
- Challenges
  - Input: large search space
  - Homology inference: what tools to use?
  - How to evaluate?

# Overview

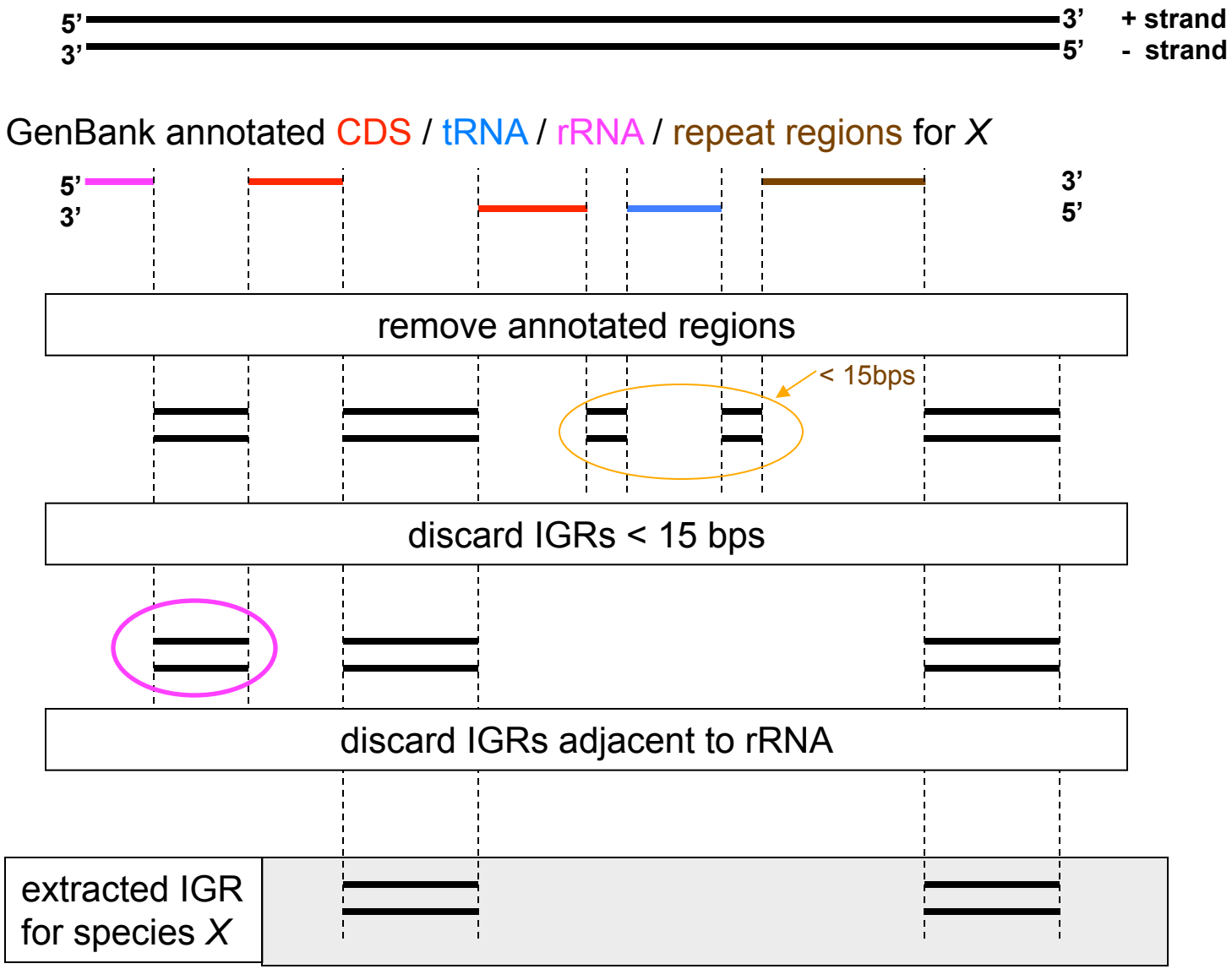
- Motivation
- Approach
  - Clustering based on homology
  - Incorporating secondary structure information
- Evaluation
- Conclusion

# Overview of approach

full genomic sequences

Intergenic Region (IGR) extraction

full genomic sequence for species *X*



# Overview of approach

full genomic sequences

Intergenic Region (IGR) extraction

pool of IGRs

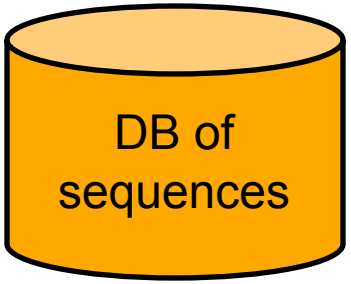
Homology search

# Homology search programs

Query

**GAGTAGTTGTAGCATTTAA**
**TATTTTGTCTGTAATTGAA**
**ATCAAC…..**

Subject
Database




Hits

Query segment : x1-x2
Subject segment: y1-y2
Matching score: S

# Homology search programs

- Popular homology search programs:
  - NCBI-BLAST
  - WU-BLAST
  - FASTA
  - SSEARCH

Uses dynamic programming to find matching regions between two sequences

SSEARCH 10 times slower than the rest

# Overview of approach

full genomic sequences

Intergenic Region (IGR) extraction

pool of IGRs

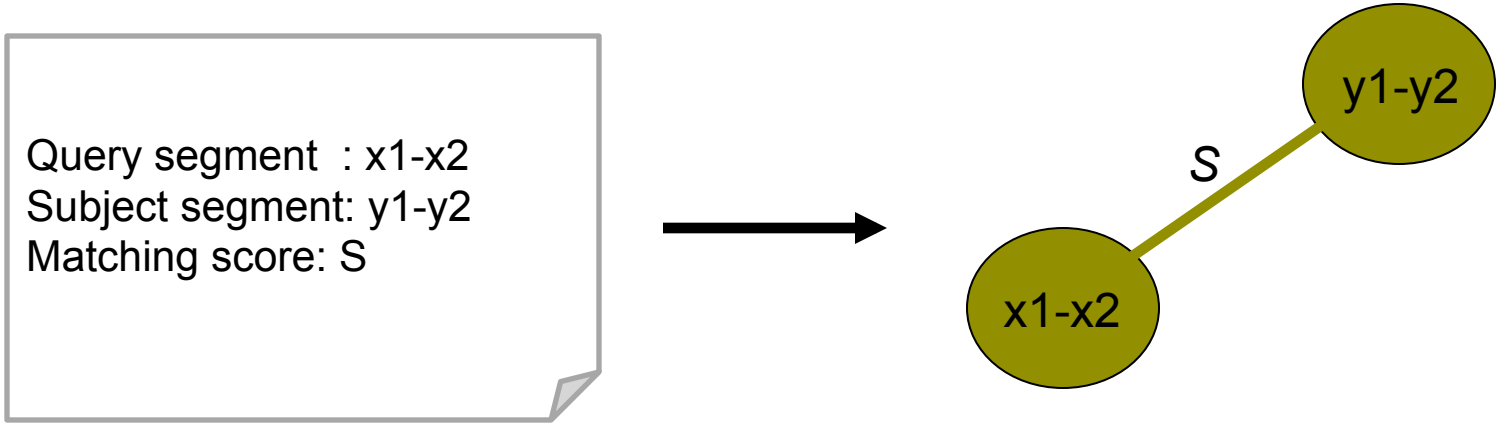
Homology search

homology hits

Hierarchical clustering

# Hierarchical clustering

- Homology program produces a list of hits between IGR *segments*
  - IGR segments ➔ *nodes*
  - A hit between two segments ➔ connecting *edge*
  - Similarity score ➔ *edge weight*

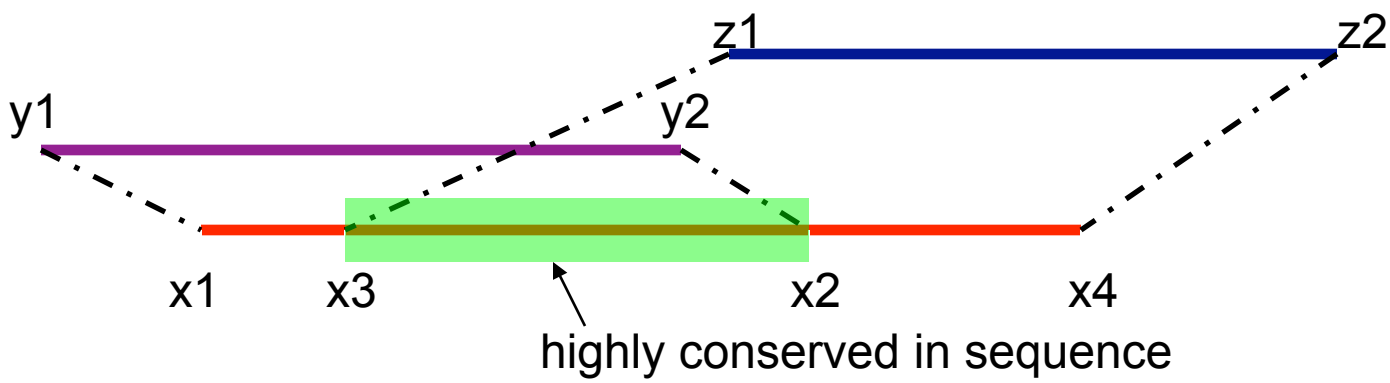


# What if segments overlap?

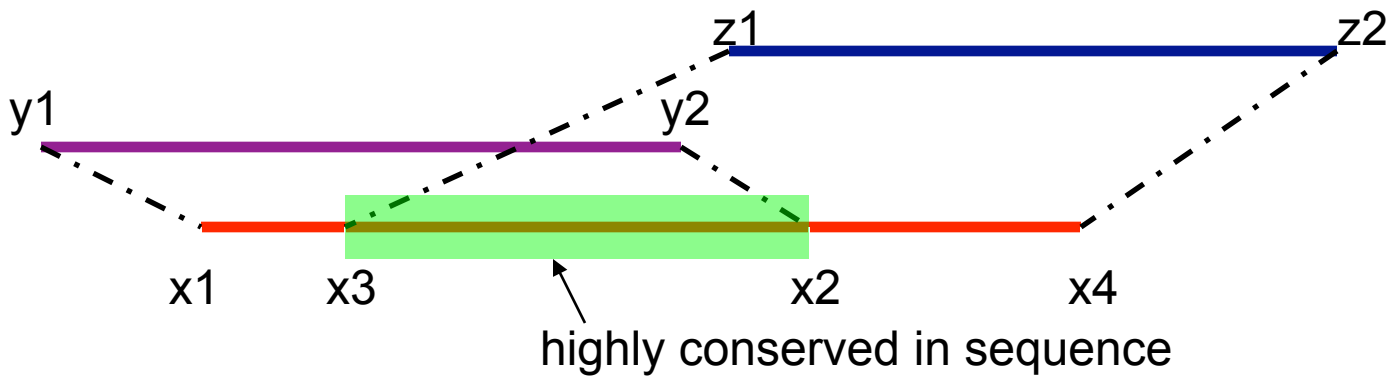
Query segment : x1-x2
Subject segment: y1-y2
Matching score: S1

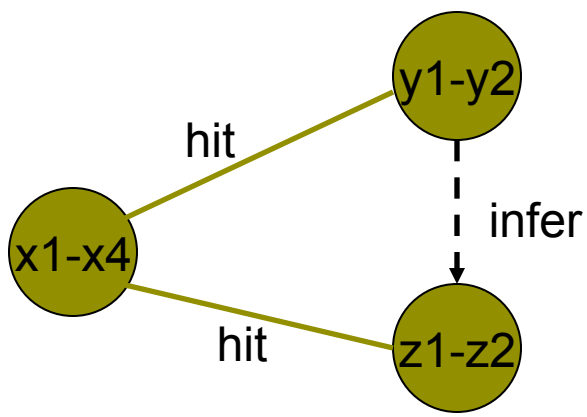
Query segment : x3-x4
Subject segment: z1-z2
Matching score: S2

What if (x1,x2) and (x3,x4) overlap by a significant portion?



# What if segments overlap?

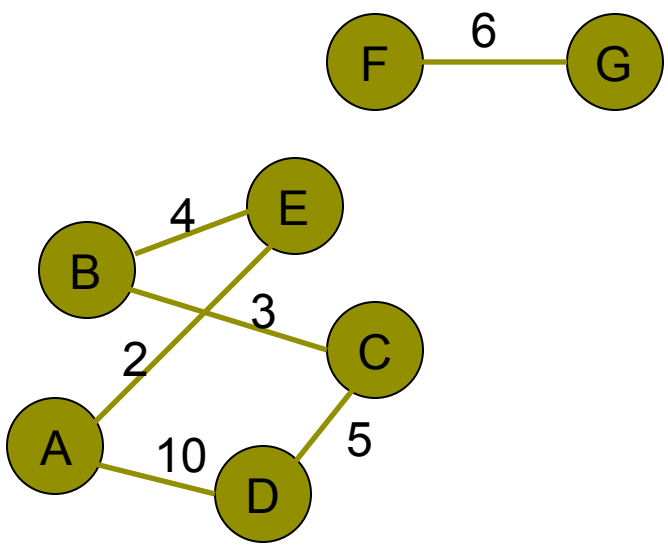


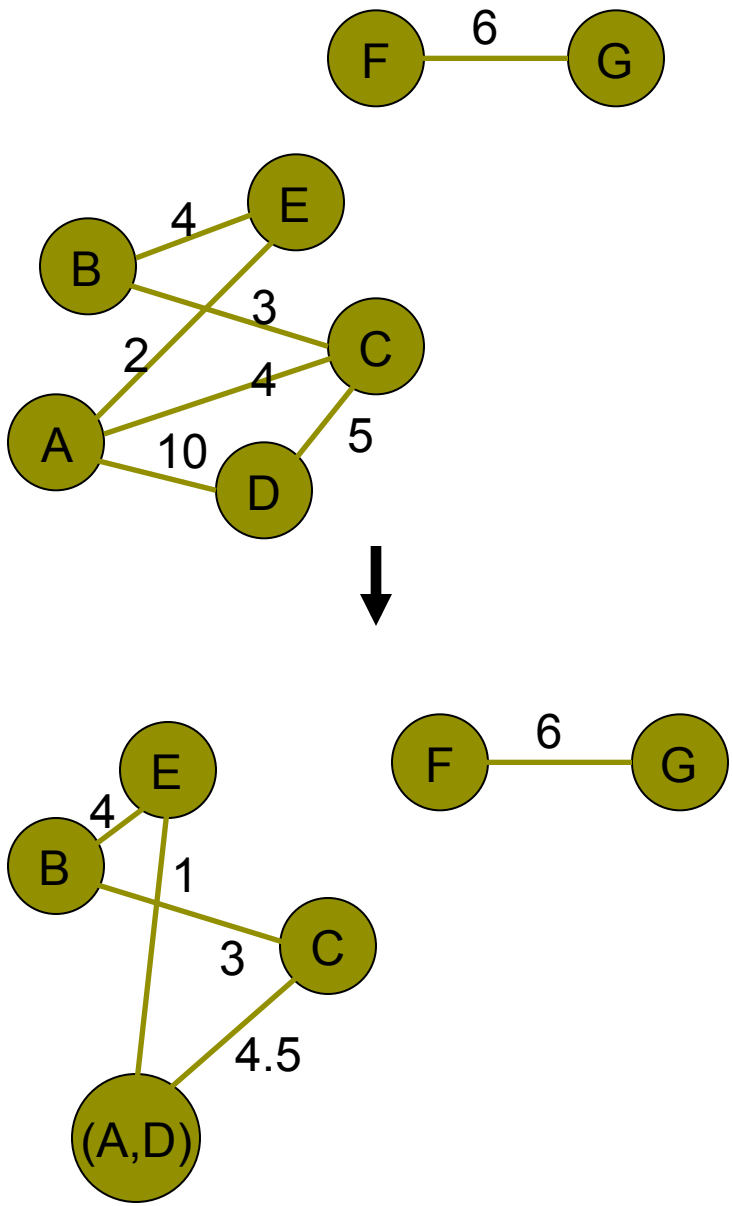



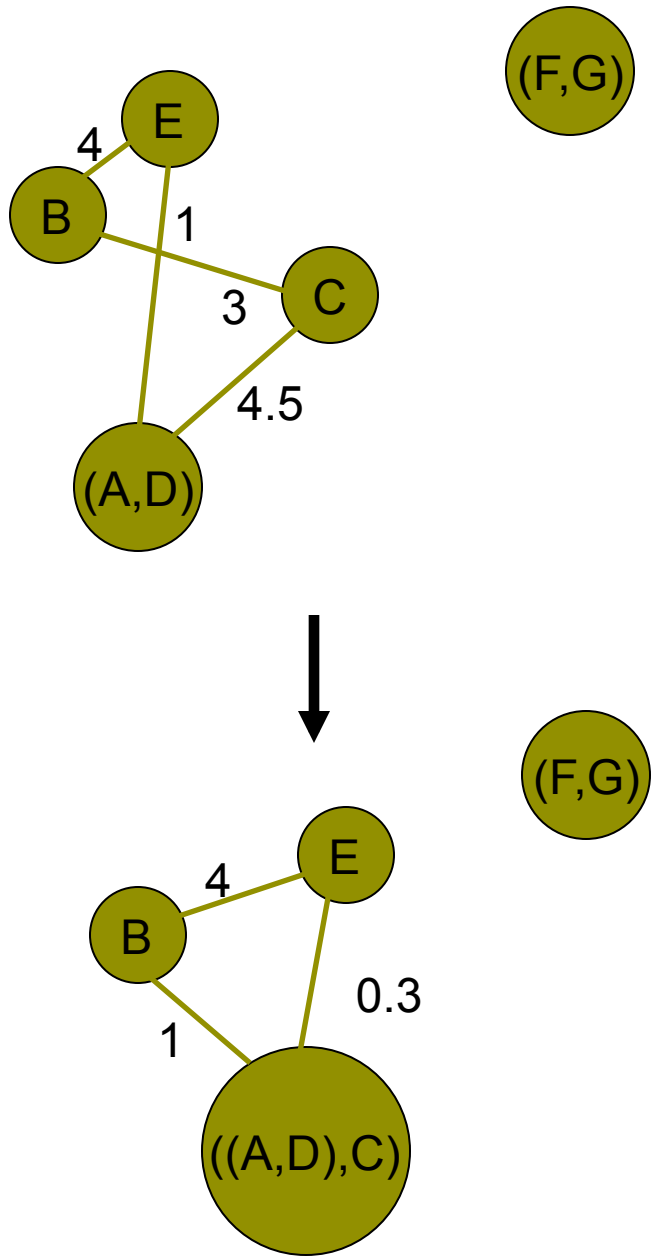
# WPGMA

(Weighted Pair Group Method using arithmetic Averaging)

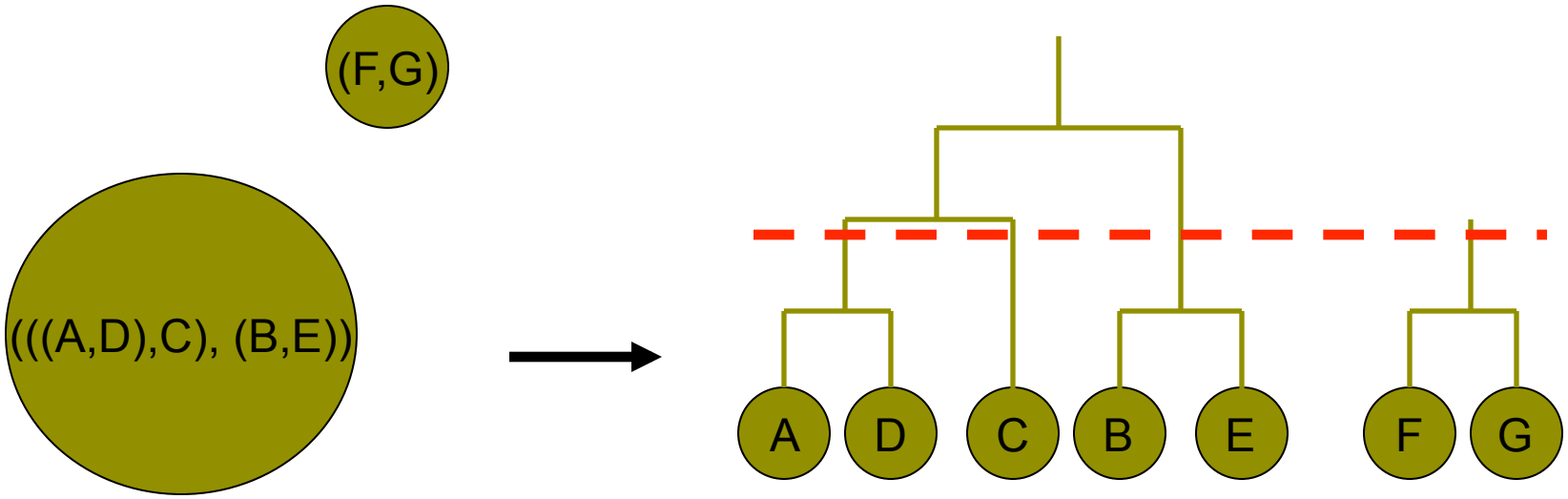
- While exists a connecting edge
  - Select the edge with highest weight
  - Replace the connected two nodes with an new *internal* node
  - Update edge associations




F
6
G
E
4
B
3
C
2
4
A
10
5
D
E
F
6
G
4
B
1
C
3
4.5
(A,D)


(F,G)
E
4
B
1
C
3
4.5
(A,D)
(F,G)
E
4
B
0.3
1
((A,D),C)

Use size threshold to cut down tree size




Cluster too big?

# Overview

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- Approach
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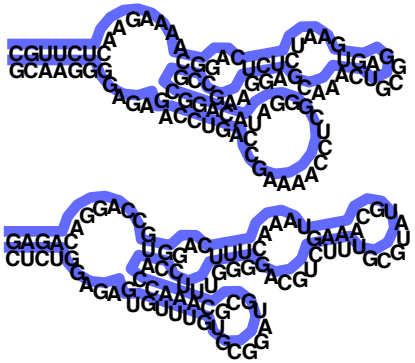
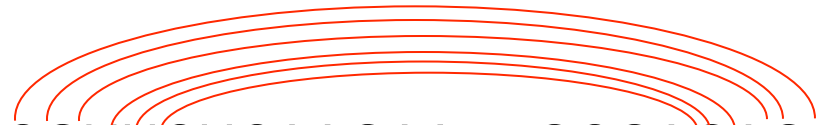
- Conclusion

# Secondary structure info





CGUUCUCAAGAA.....GCGAGAGGGGAACG
GAGACAGGACCG.....AC….AGAGGUCUC

- More conserved in structure than sequence
- Can we include secondary structure when searching for homologs?

# Secondary structure info

CGUUCUCAAGAA.....GCGAGAGGGGAACG
GAGACAGGACCG.....AC….AGAGGUCUC

<<<<<<_____....._____>>>>>>
<<<<_____.....__….____>>>>

- convert 4-alphabet (A,U,C,G) to 12-alphabet {<, >, _} x {A,U,C,G}
- allow for mismatches between alphabets that are from different nucleotides, but the same structure
- DIY scoring matrix

  (C< , C<) ➔ great

  (C< , G<) ➔ good

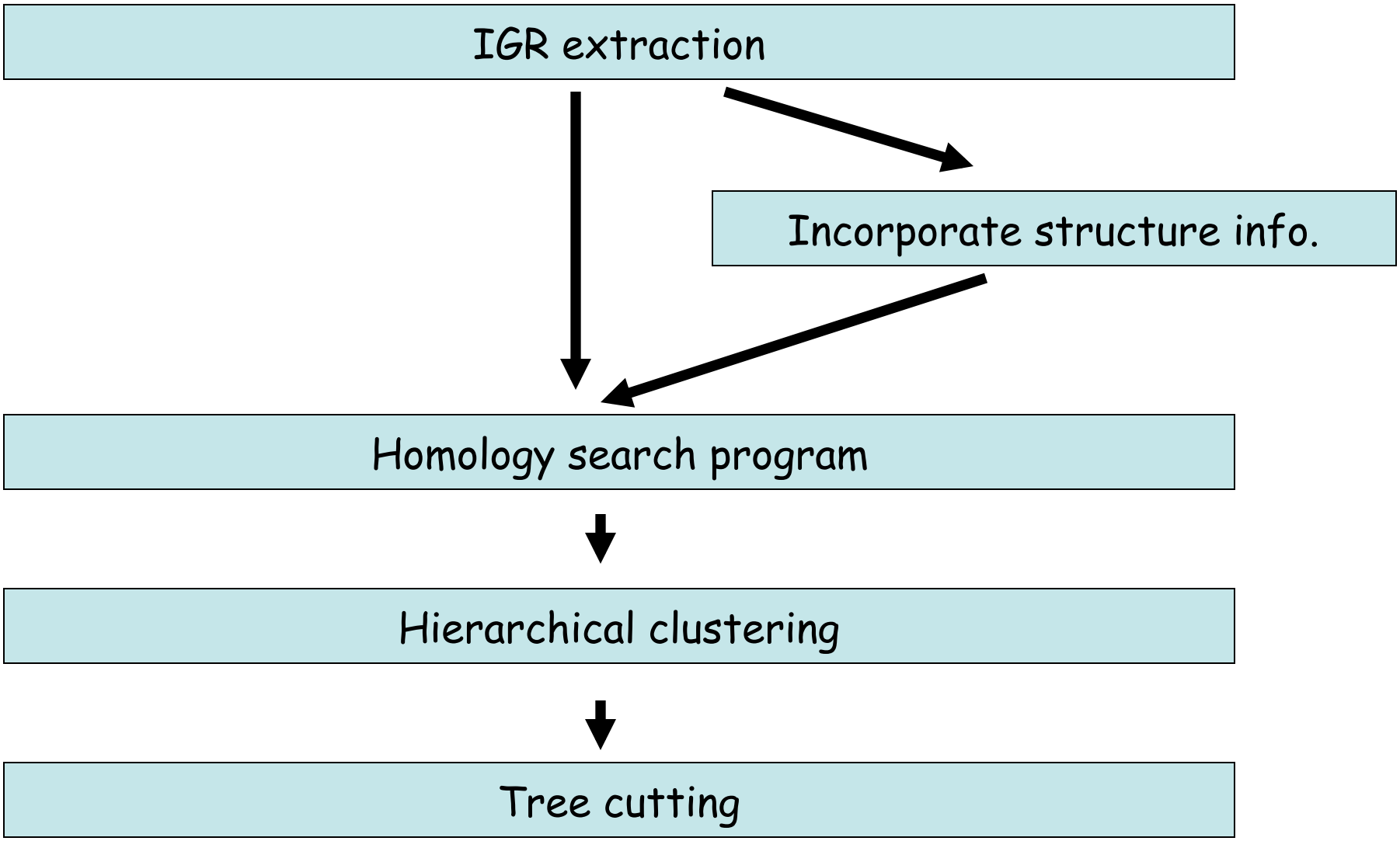
  (C< , U_) ➔ bad

# Predicting structures on IGR

Given an IGR:

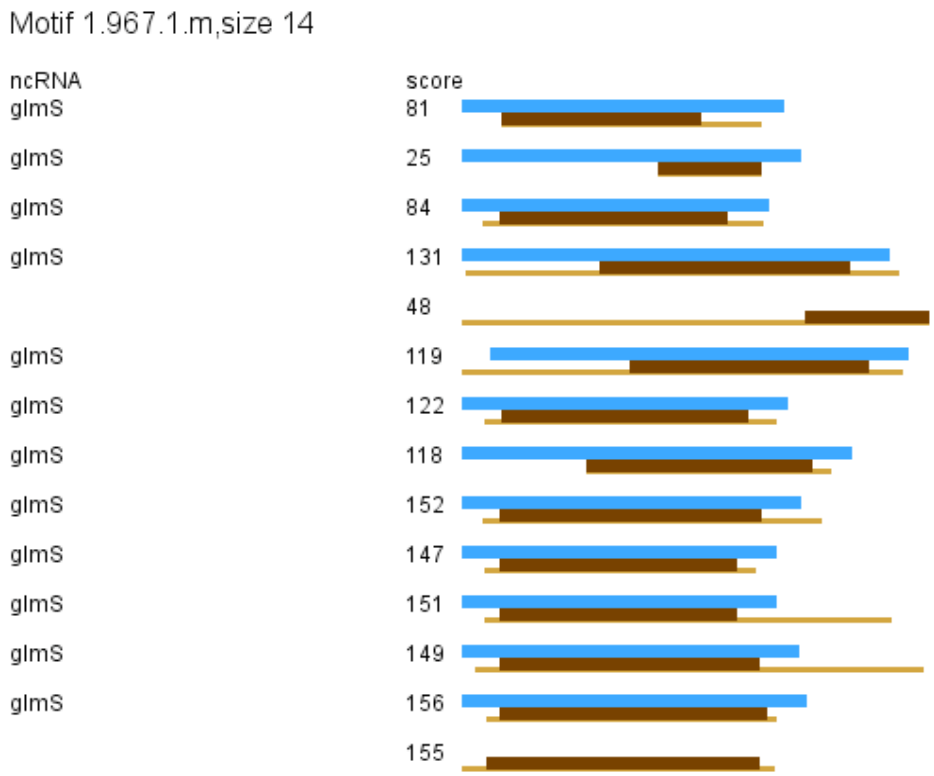
1. Break into overlapping pieces (prev. slide)
2. Feed each piece to RNAfold ➔ obtain structure
3. Convert pieces from 4-alphabet to 12-alphabet
4. Use homology program with DIY scoring matrix
5. Same clustering process…

INPUT: Genomic sequences

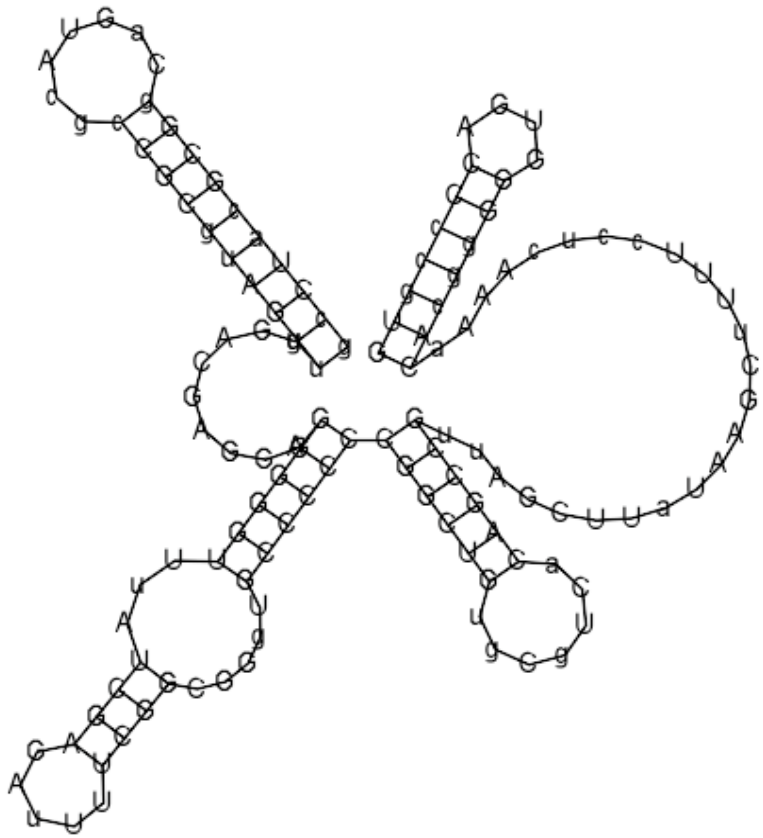



OUTPUT: Clusters of IGR segments

# Example of a good scan

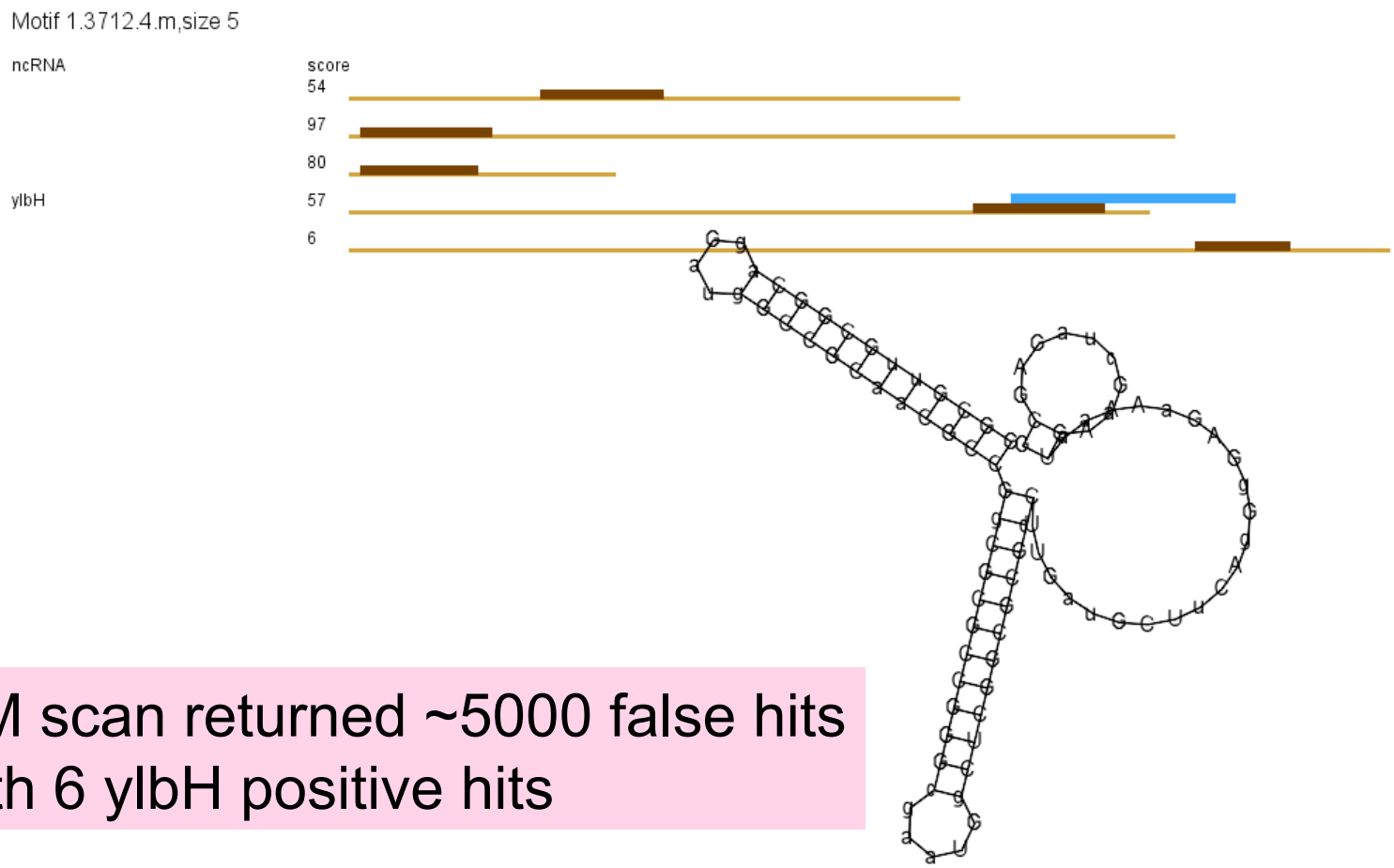



ncRNA
motif
IGR segment



CM scan recovered 95% of glmS with NO false hits!

# Example of a bad scan




CM scan returned ~5000 false hits with 6 ylbH positive hits