Modeling Structural RNA Families with Infernal

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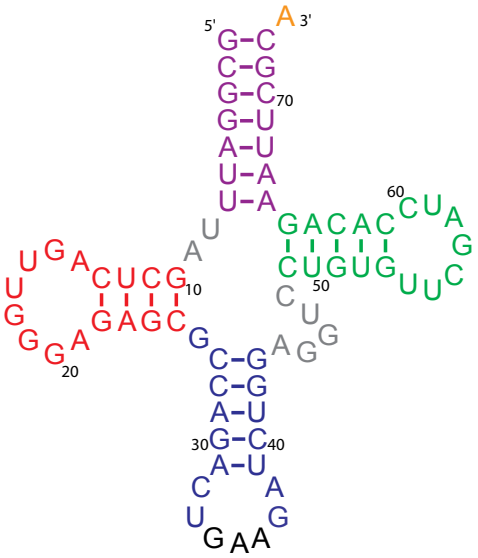
Many functional RNAs adopt a conserved 3-dimensional structure

Three representations of a transfer RNA:

Primary sequence

GCGGAUUUAGCUCAGUUGGG
AGAGCGCCAGACUGAAGAUC
UGGAGGUCCUGUGUUCGAUC
CACAGAAUUCGCA

Secondary structure



3-dimensional structure

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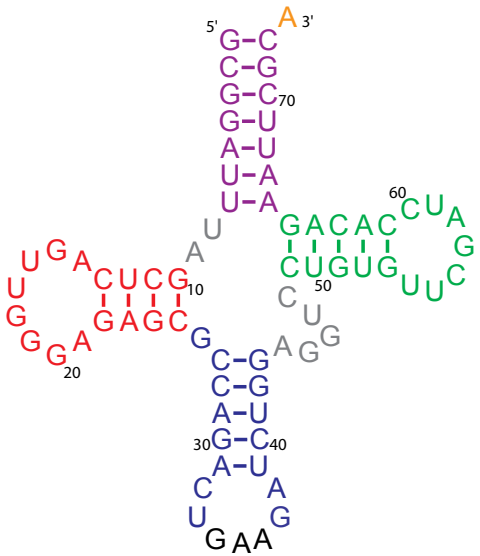
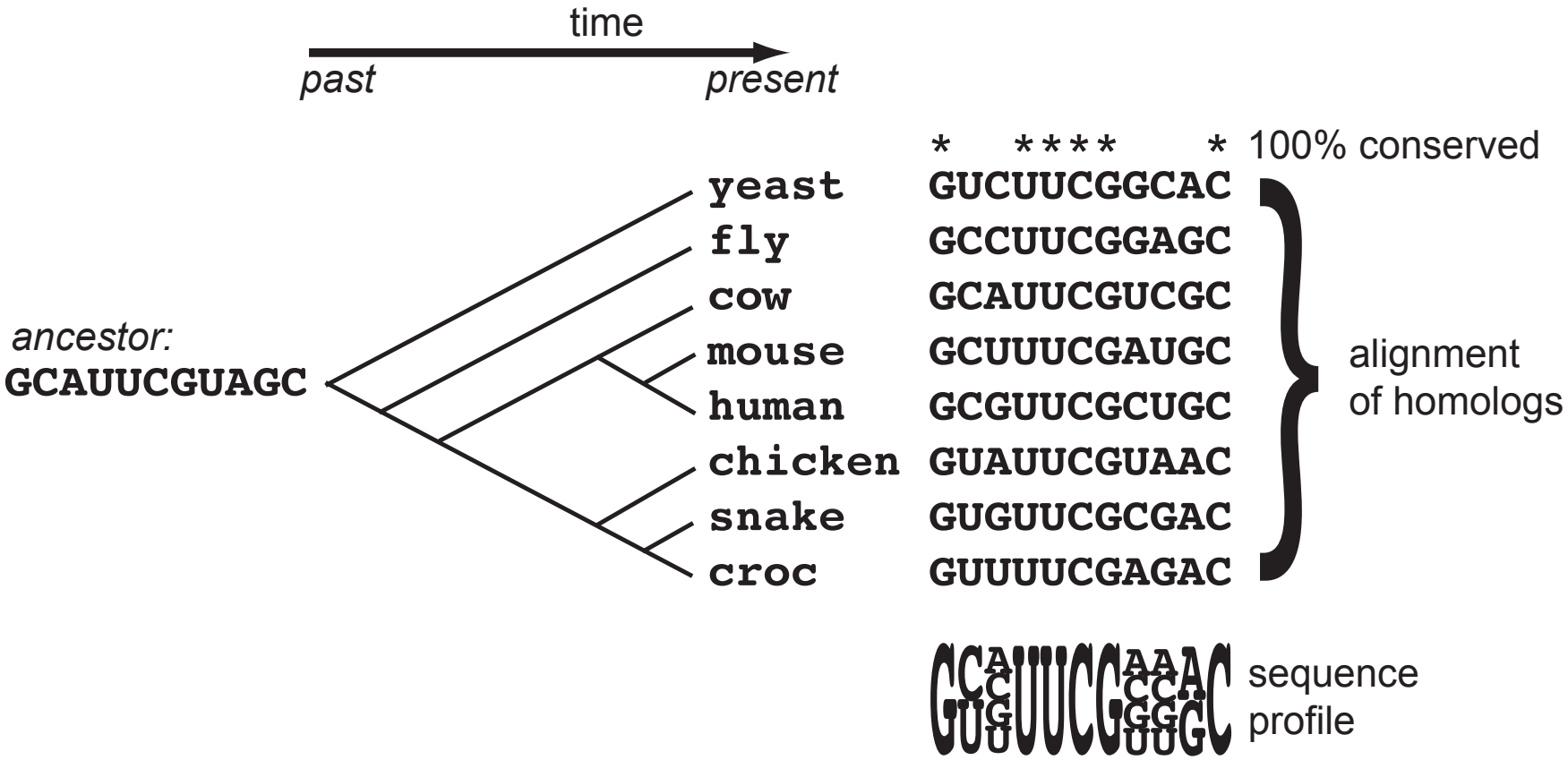


3-dimensional structure

- BLAST: given a single sequence, search genomes for similar sequences.
- BLAST cannot take advantage of:
  - secondary structure
  - sequence conservation levels, which vary across the gene

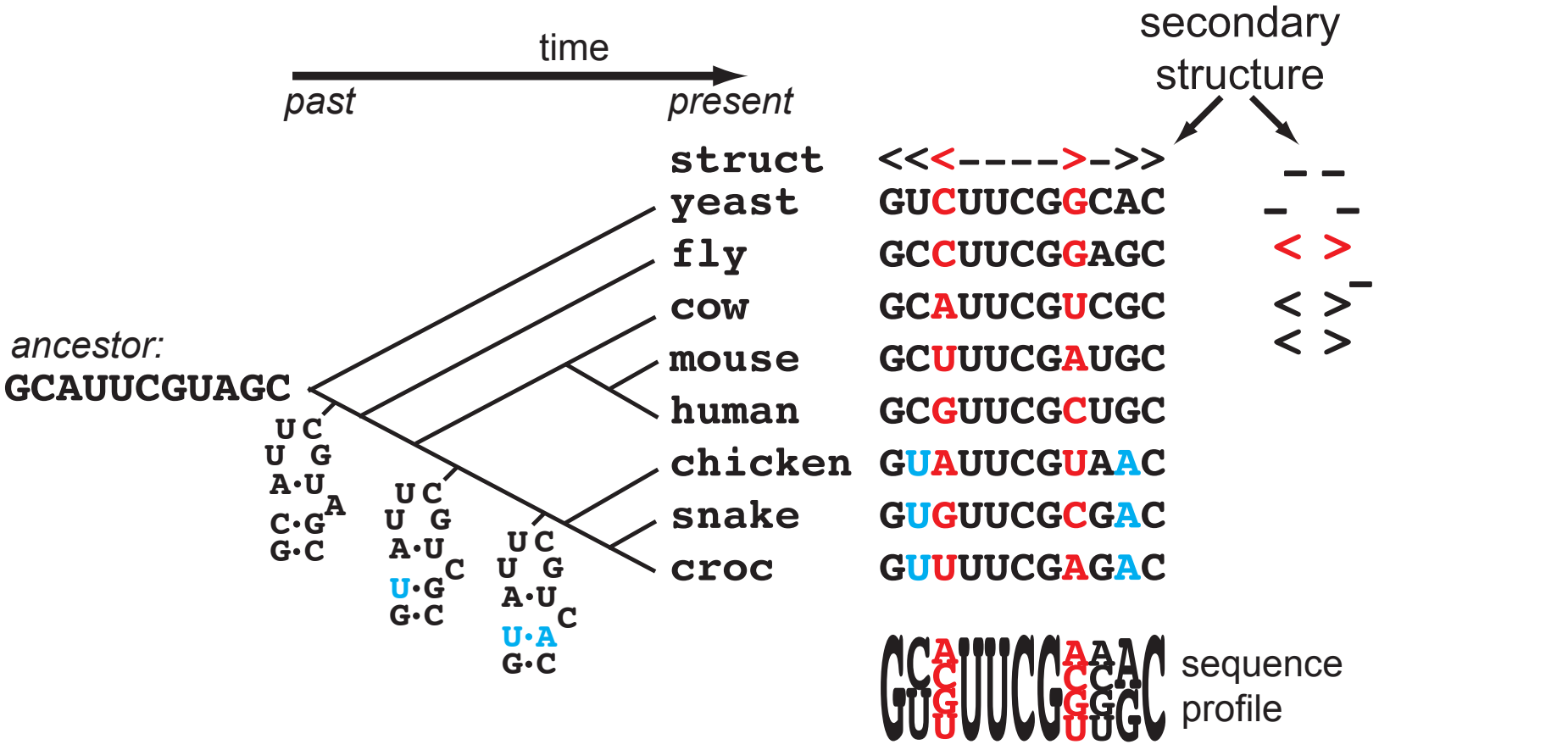
Sequence conservation provides information for homology searches

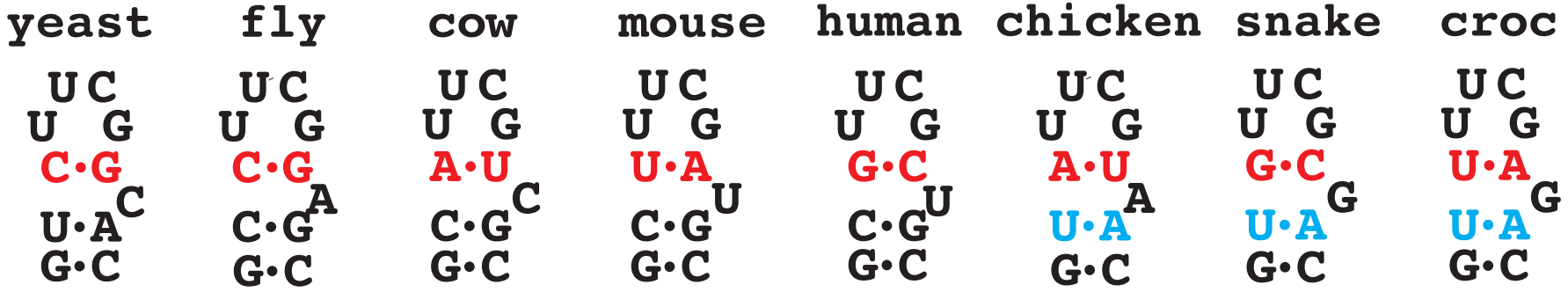
Conservation levels vary across alignment columns.



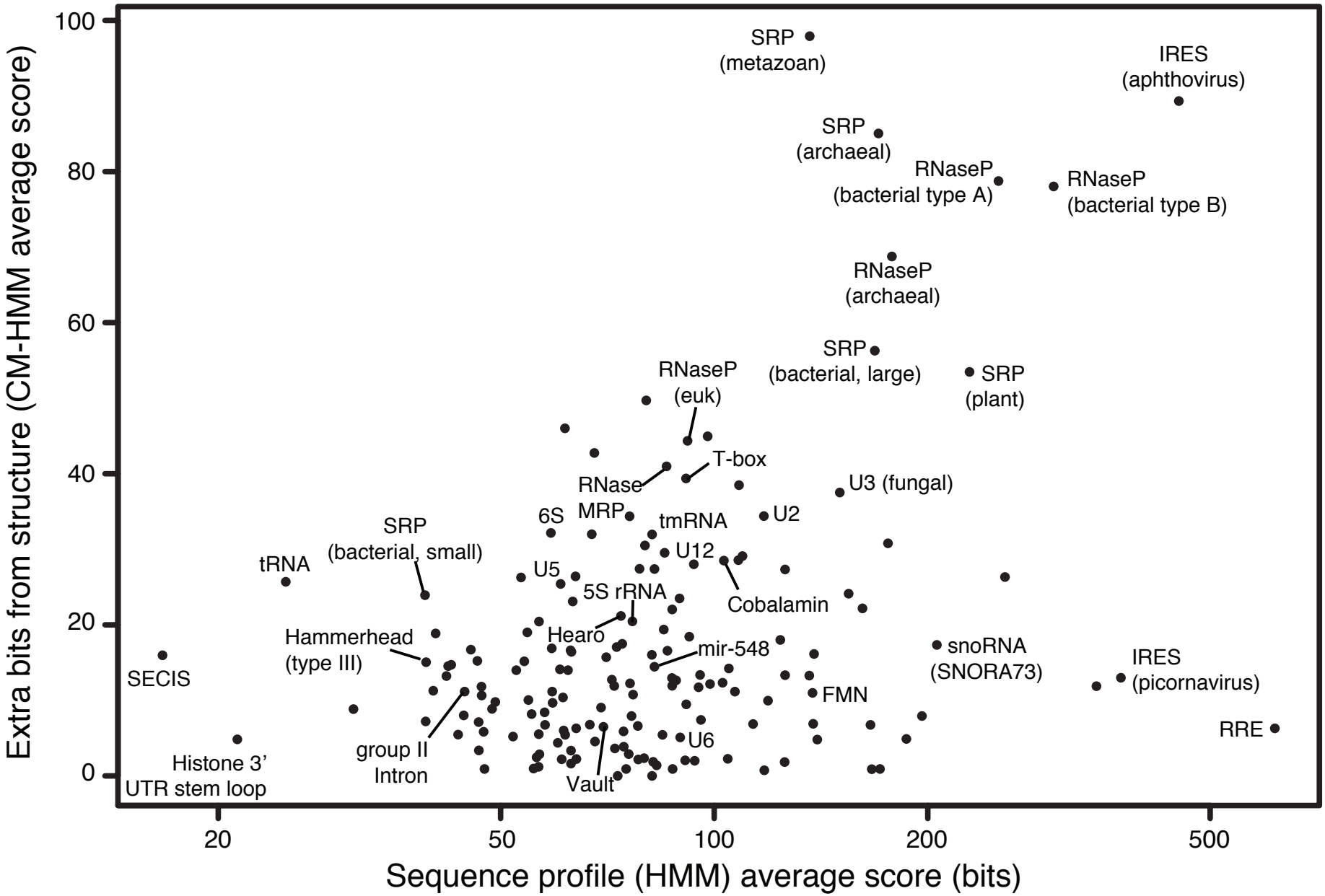
Structure conservation provides additional information

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





Levels of sequence and structure conservation in RNA families



Eddy lab software for profile probabilistic models (since 1994)

	sequence profiles	sequence and structure profiles
models	profile HMMs	covariance models (CMs)
software	HMMER	Infernal
main use	proteins, repetitive DNA elements	structural RNAs
databases	Pfam and Dfam (14831 and 1132 entries)	Rfam (2450 families)
performance for RNAs	faster but less accurate	slower but more accurate

http://hmmer.janelia.org

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

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

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

http://infernal.janelia.org

Nawrocki EP, Eddy SR Bioinformatics, 29:2933-2935, 2013.

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

Is the added complexity worth it?

RMARK: a challenging internal RNA homology search benchmark

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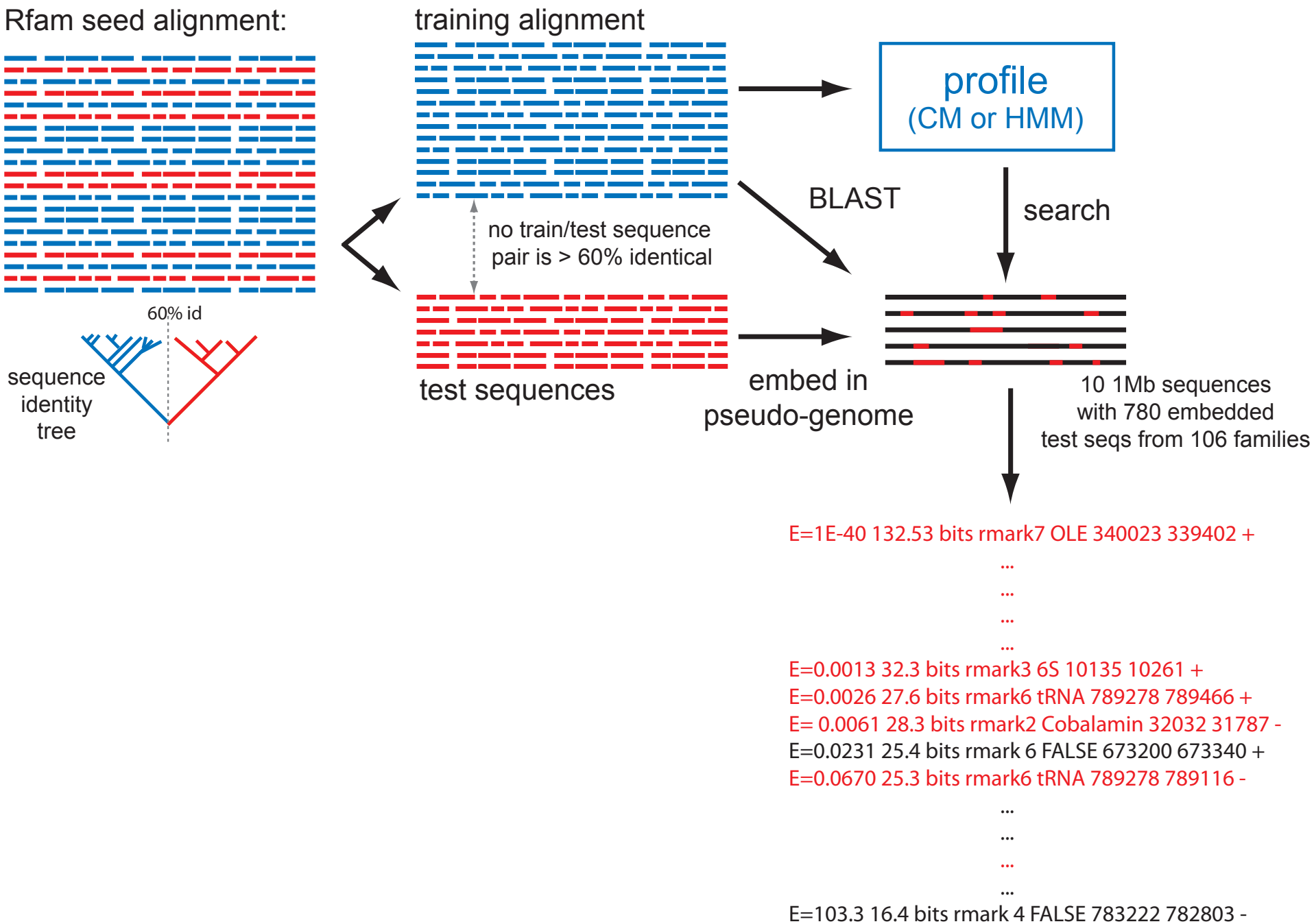
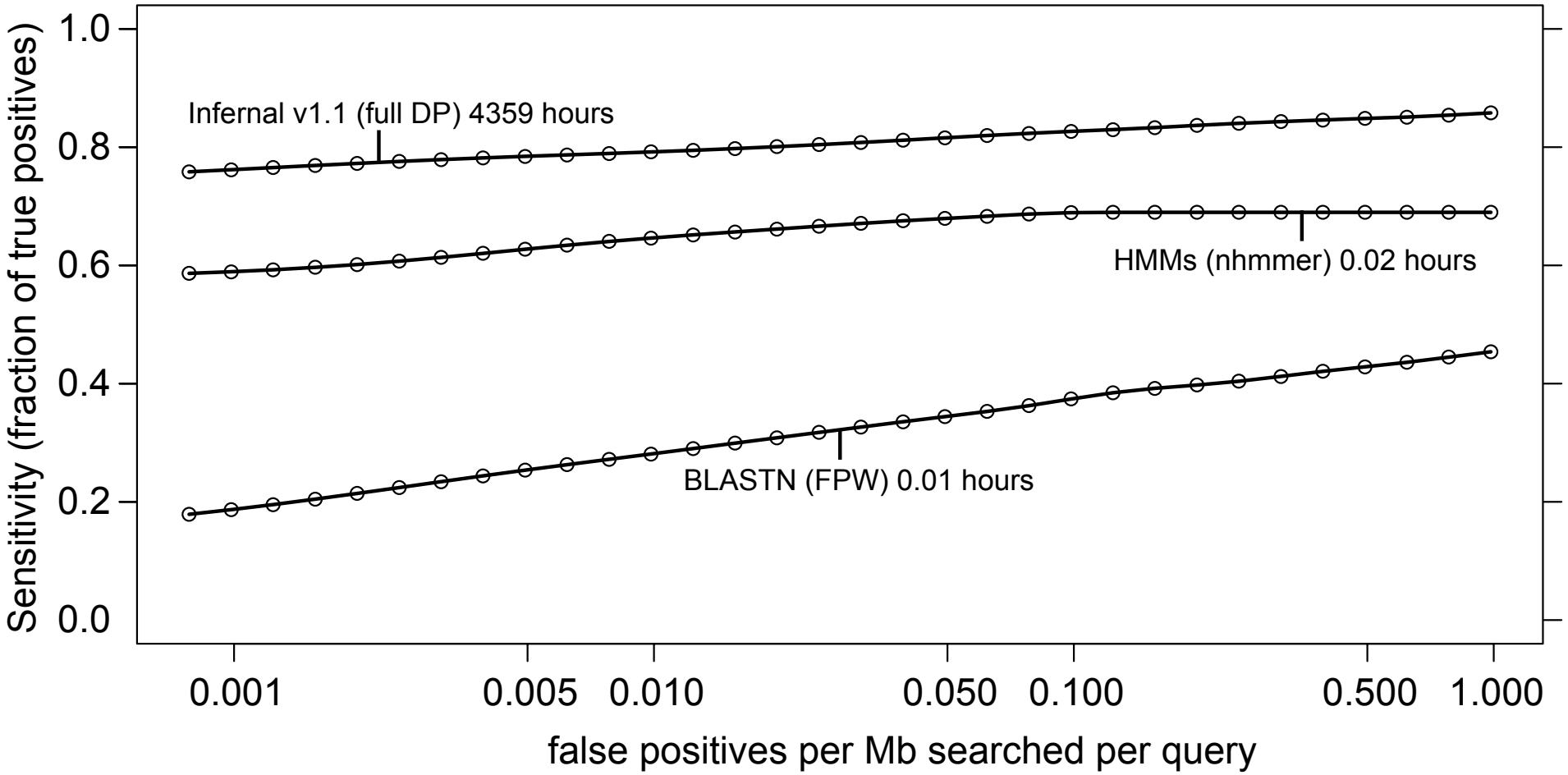


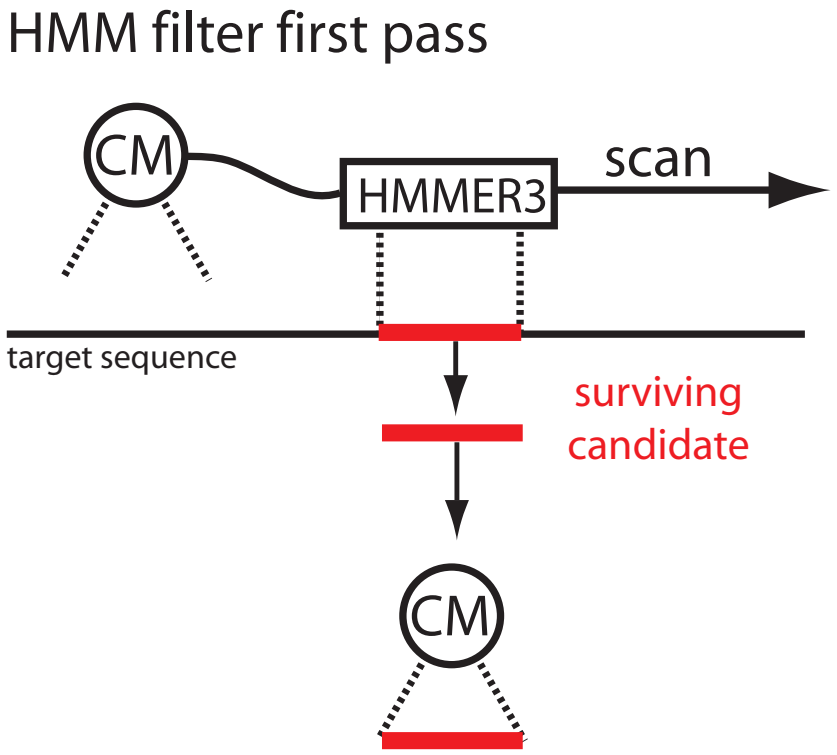
Infernal outperforms primary-sequence based methods on our benchmark (and others*, not shown)



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

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

Filter target database using profile HMMs*



*Weinberg, Ruzzo, RECOMB, 243-251, 2004

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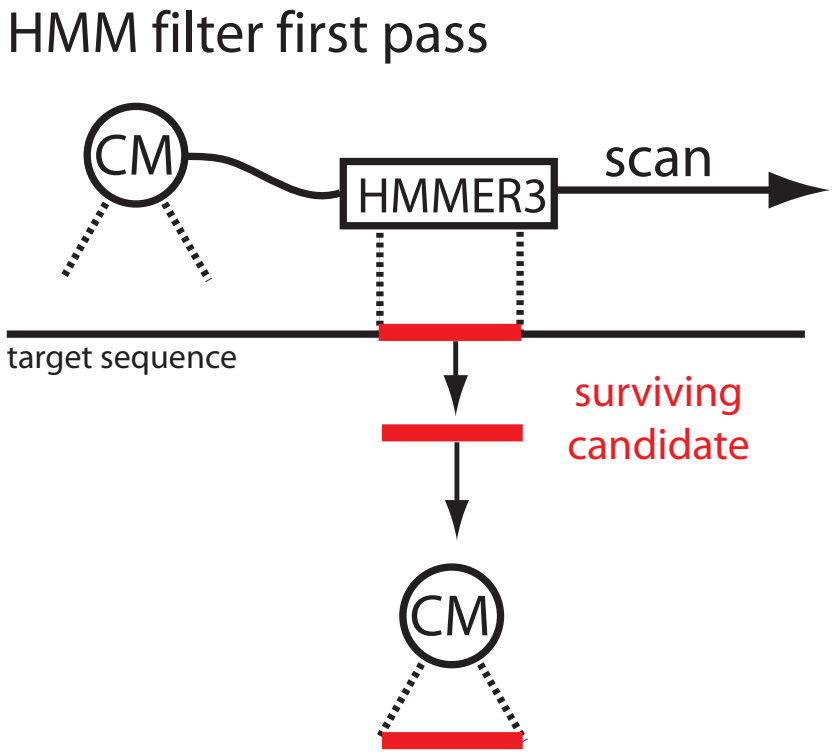
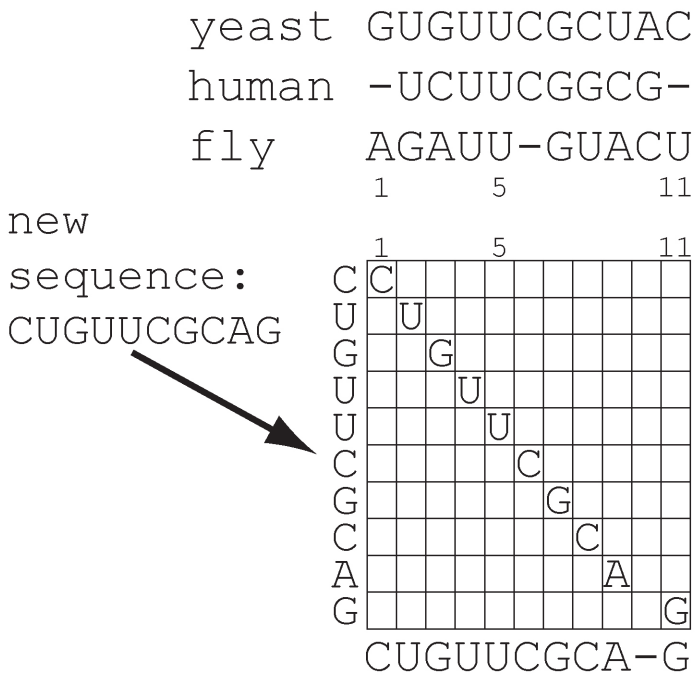


- Even if we filter out 99% of the database (for up to 100X acceleration), searches will still be too slow.

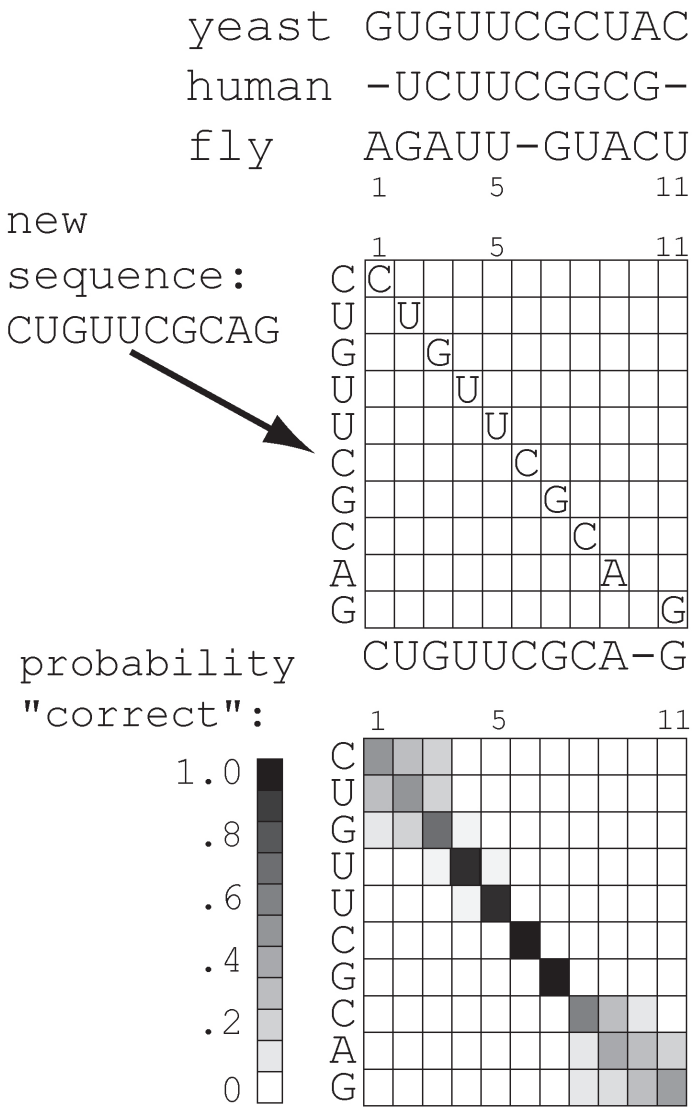
- CM step needs to be accelerated.

*Weinberg, Ruzzo, RECOMB, 243-251, 2004

Accelerating CM alignment step 1: align sequence with HMM



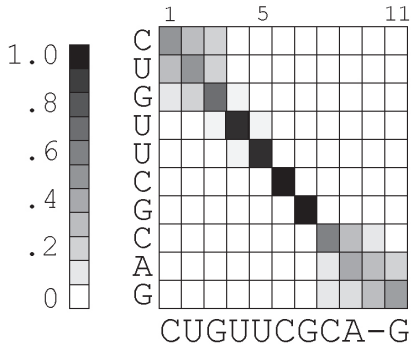
Accelerating CM alignment step 2: HMM posterior decoding to get confidence estimates

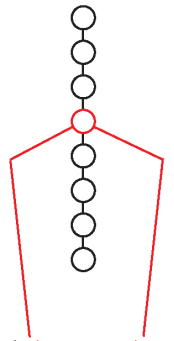


Accelerating CM alignment step 3: use HMM alignment confidence to constrain CM alignment*

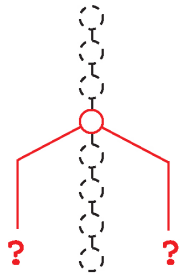
HMMs – Each column of seed alignment corresponds to a column of matrix.

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





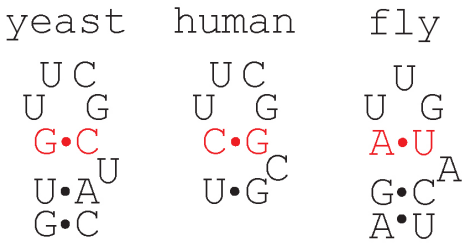
```
struct <<<<----->->>>
yeast  GUGUUCGCUAC
human  -UCUUCGGCG-
fly    AGAUU-GUACU
```



CUGUUCGCAG

45 possibilities

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



*M. P. Brown. Proc. Int. Conf. ISMB, 8:57–66, 2000.

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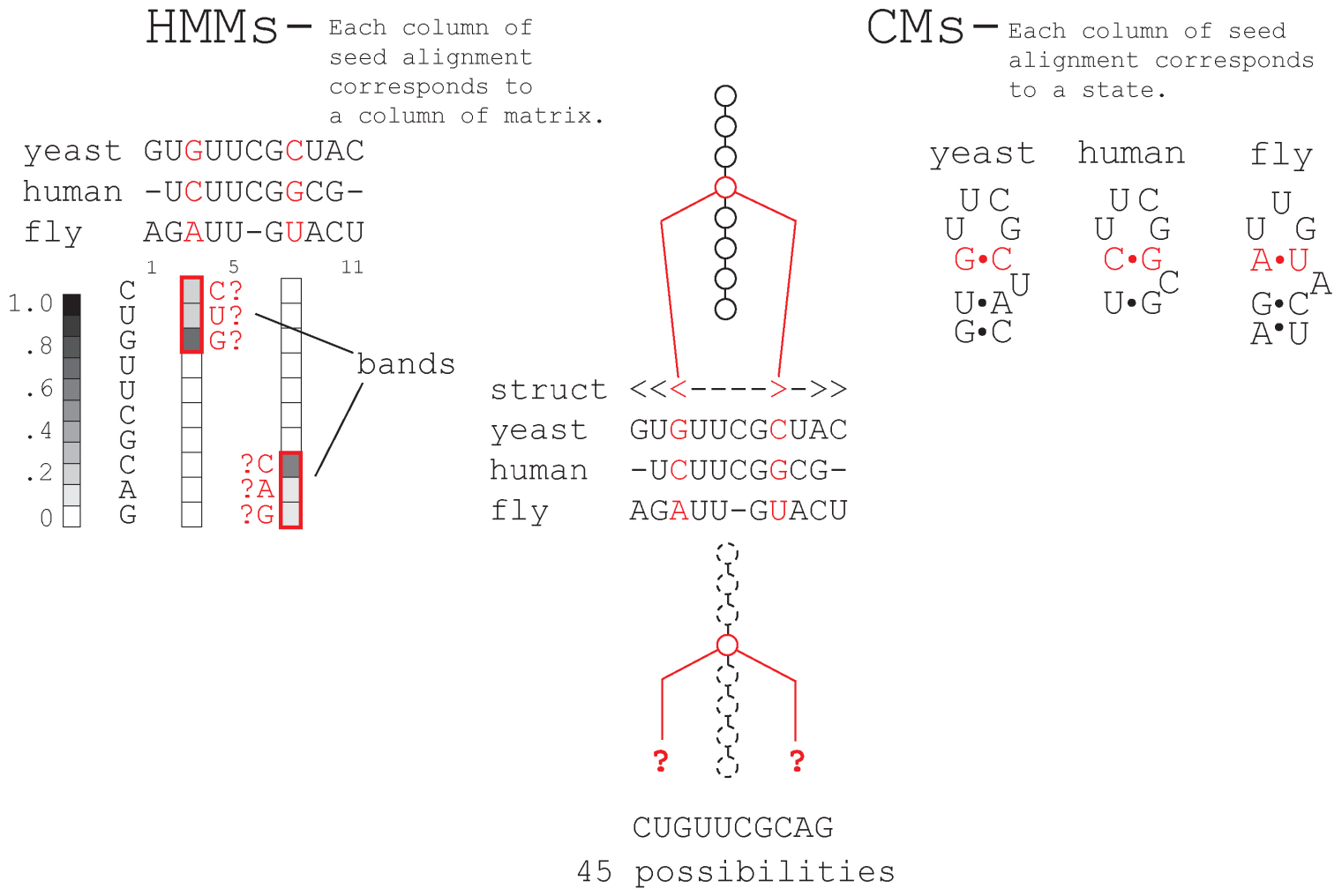

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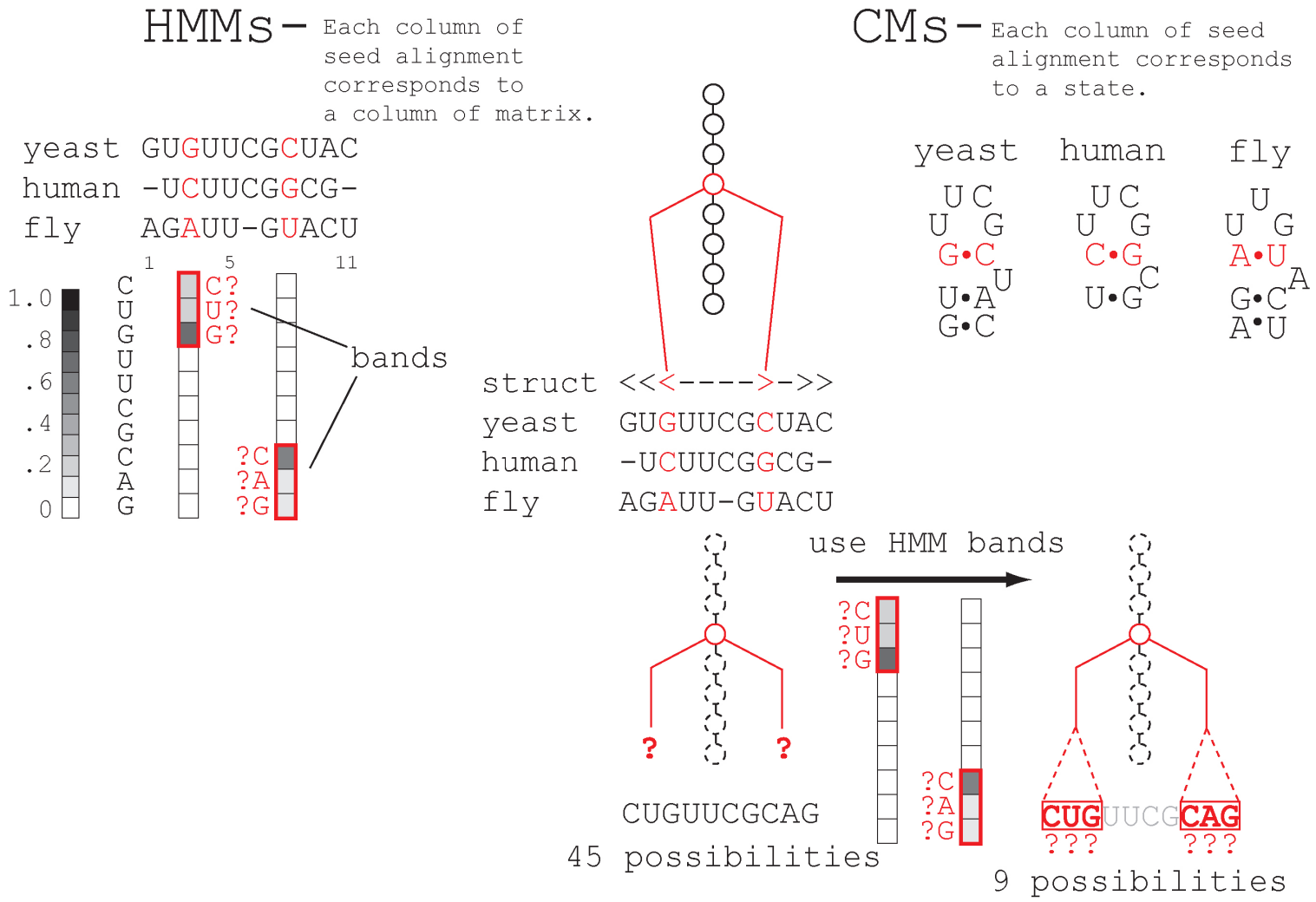
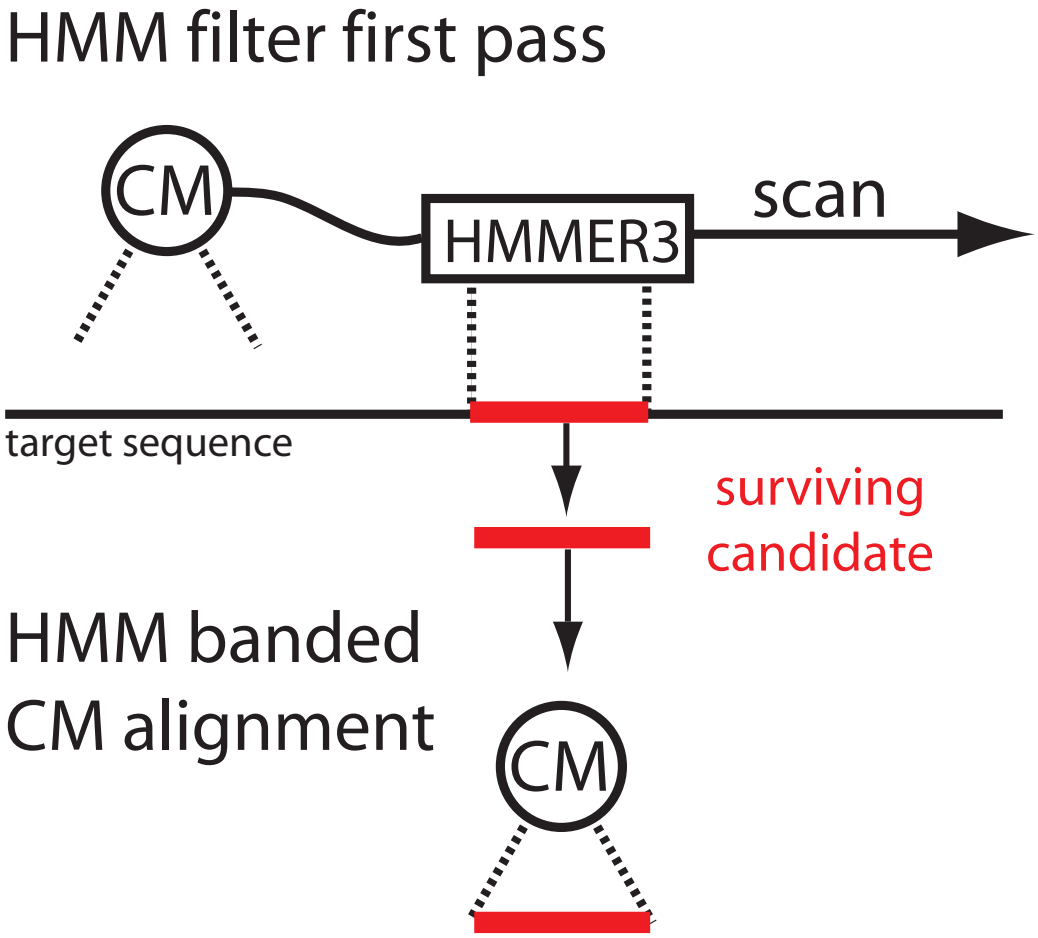
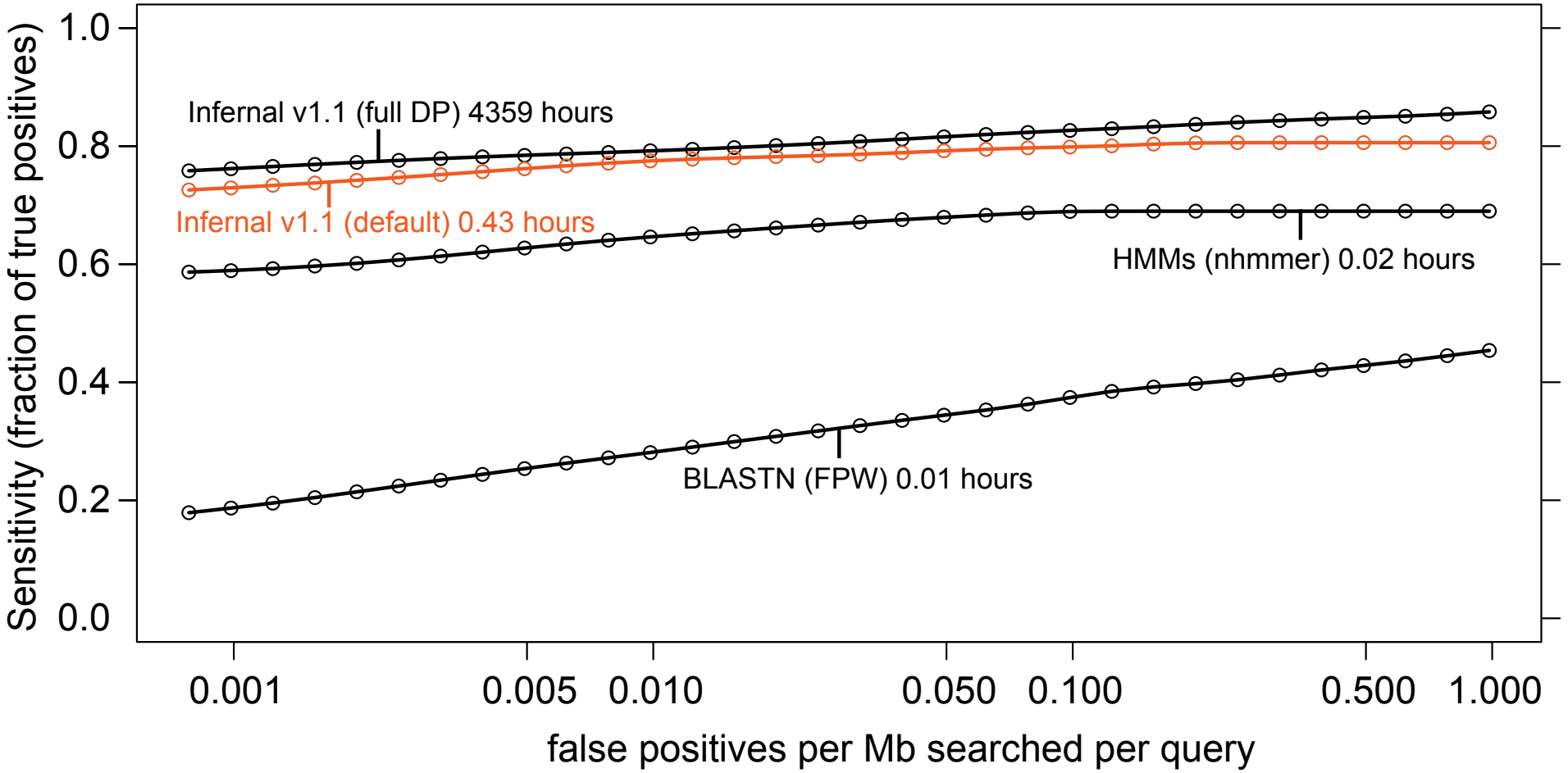


*M. P. Brown. Proc. Int. Conf. ISMB, 8:57–66, 2000.

Use HMMs as filters and to constrain CM alignment



HMM-based acceleration makes Infernal 10,000 times faster



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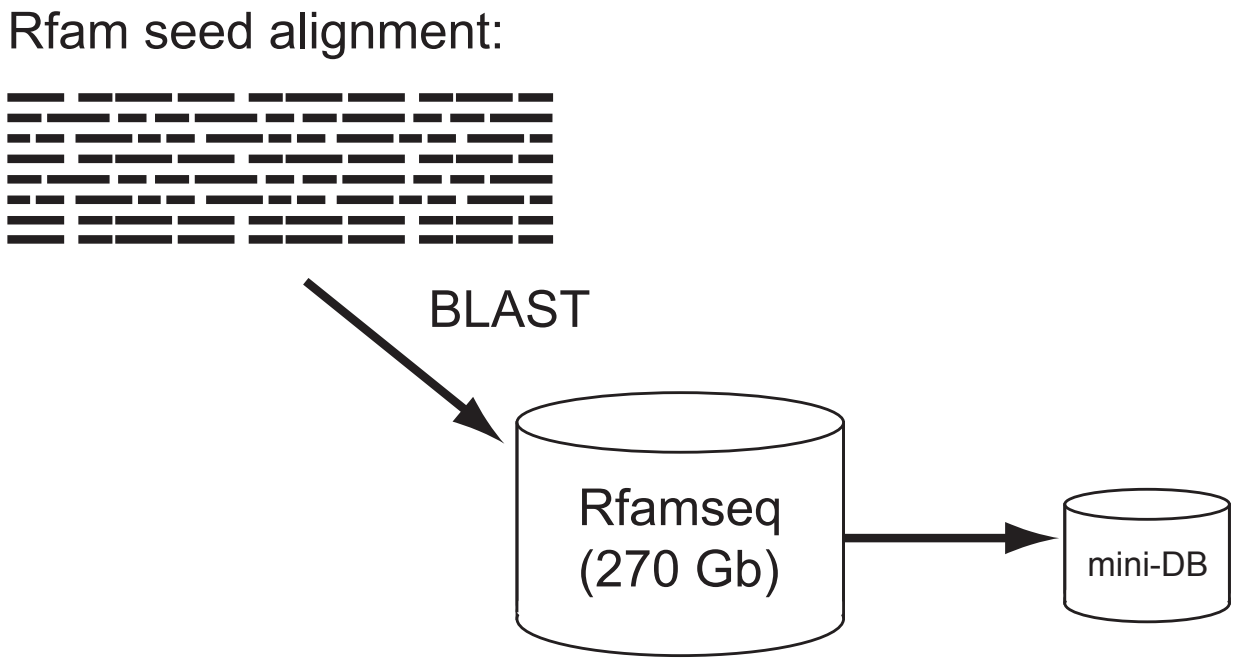
Rfam used BLAST filters from 2003 to 2012



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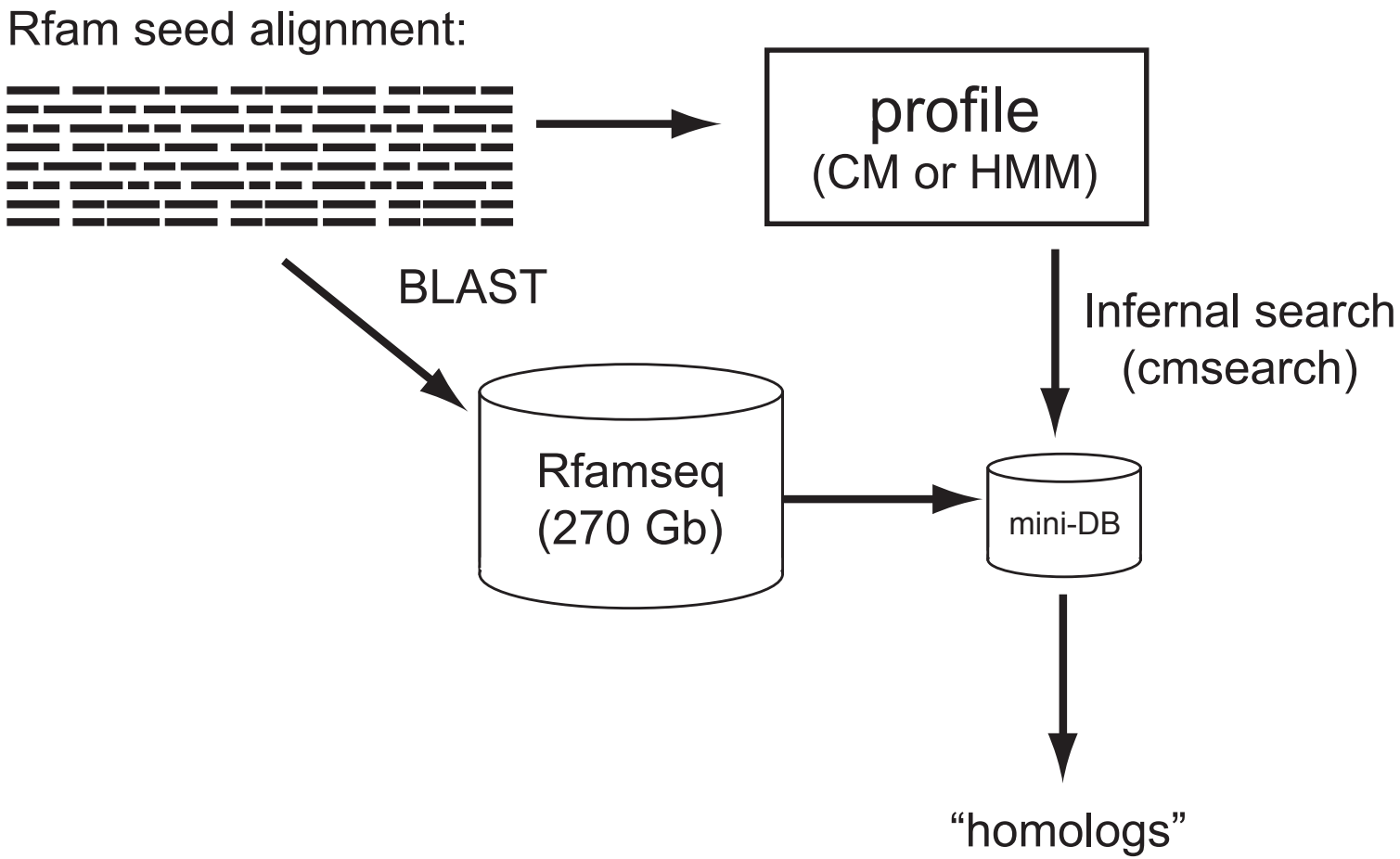
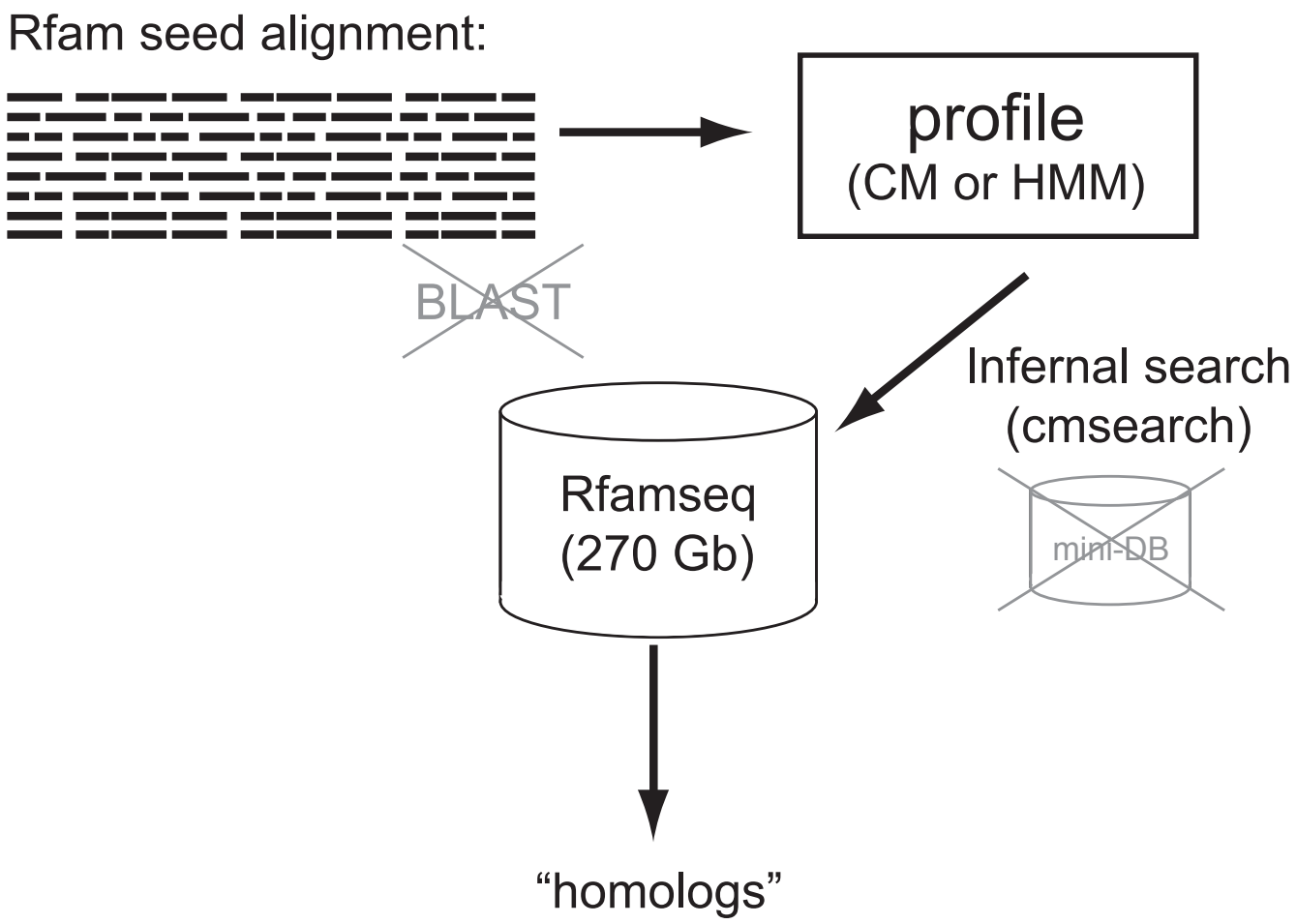


Rfam 12.0 (2014)* first release without BLAST filtering



*Nawrocki, Burge et. al, NAR 2014, in press

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Search results against Rfamseq for 200 random families:

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

*Nawrocki, Burge et. al, NAR 2014, in press

Acknowledgements

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Rob Finn	
Jody Clements	
Fred Davis	
Lee Henry	
Michael Farrar	