Structural RNA and viral sequence analysis

Eric Nawrocki

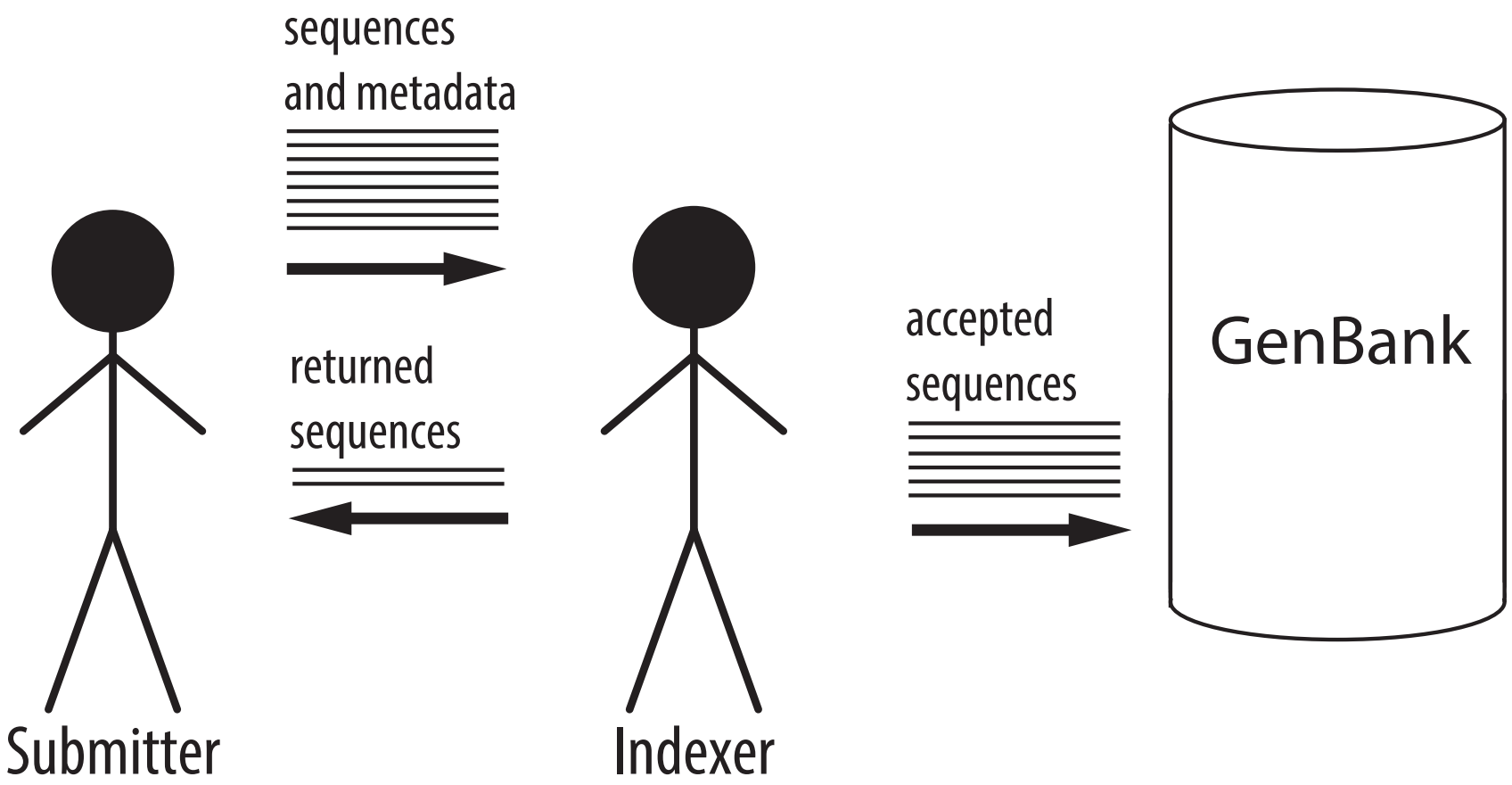
Intramural Research Program
National Library of Medicine
National Institutes of Health

Two main areas of my research:

1. Viral sequence analysis tools, since 2015

2. Structural RNA analysis tools, since 2004

GenBank indexers handle incoming sequence submissions



Schäffer *et al. BMC Bioinformatics* (2020) 21:211
https://doi.org/10.1186/s12859-020-3537-3

BMC Bioinformatics

SOFTWARE Open Access

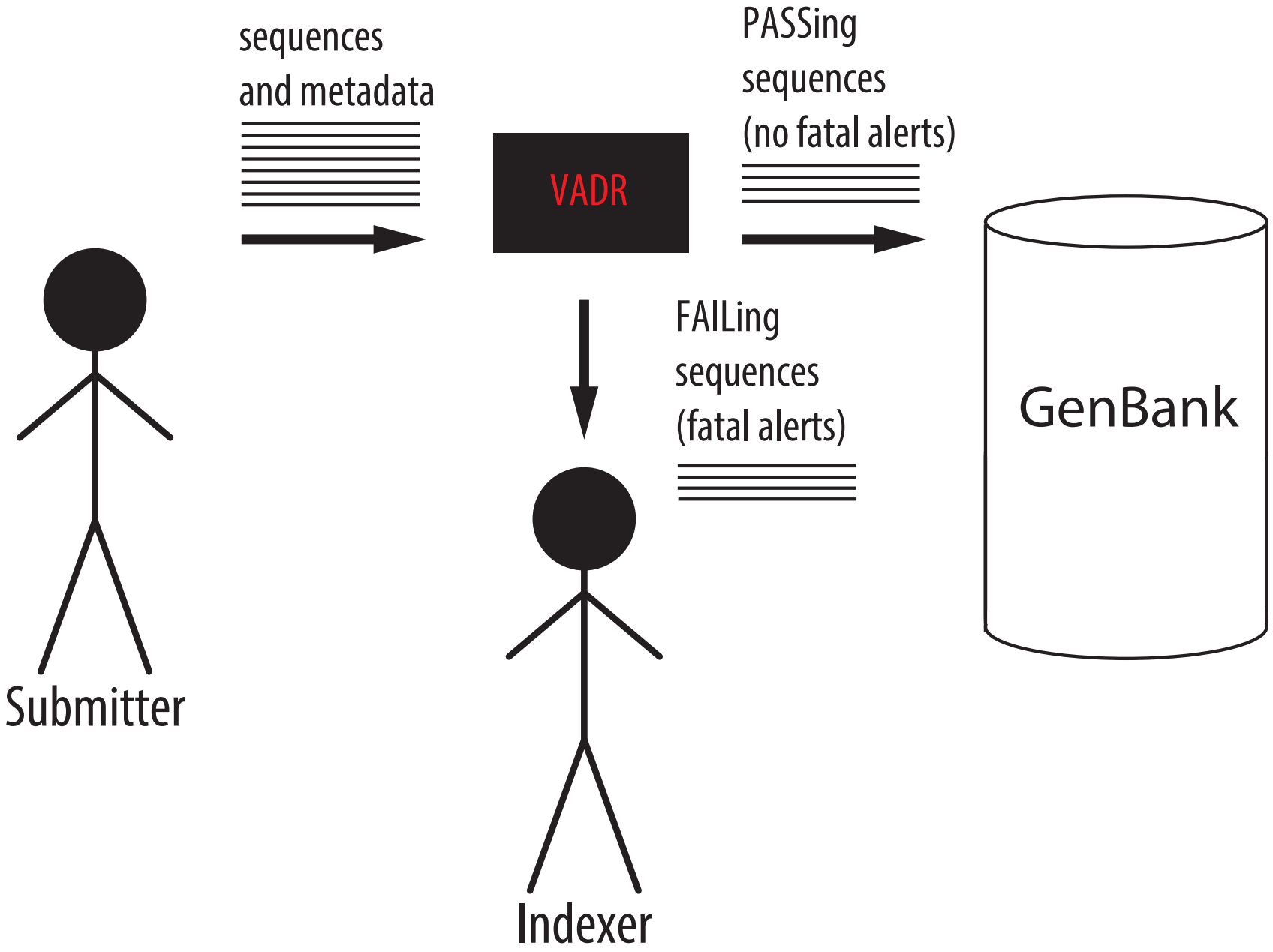
VADR: validation and annotation of virus sequence submissions to GenBank

Alejandro A. Schäffer[1,2], Eneida L. Hatcher[2], Linda Yankie[2], Lara Shonkwiler[2,3], J. Rodney Brister[2], Ilene Karsch-Mizrachi[2] and Eric P. Nawrocki[2*]

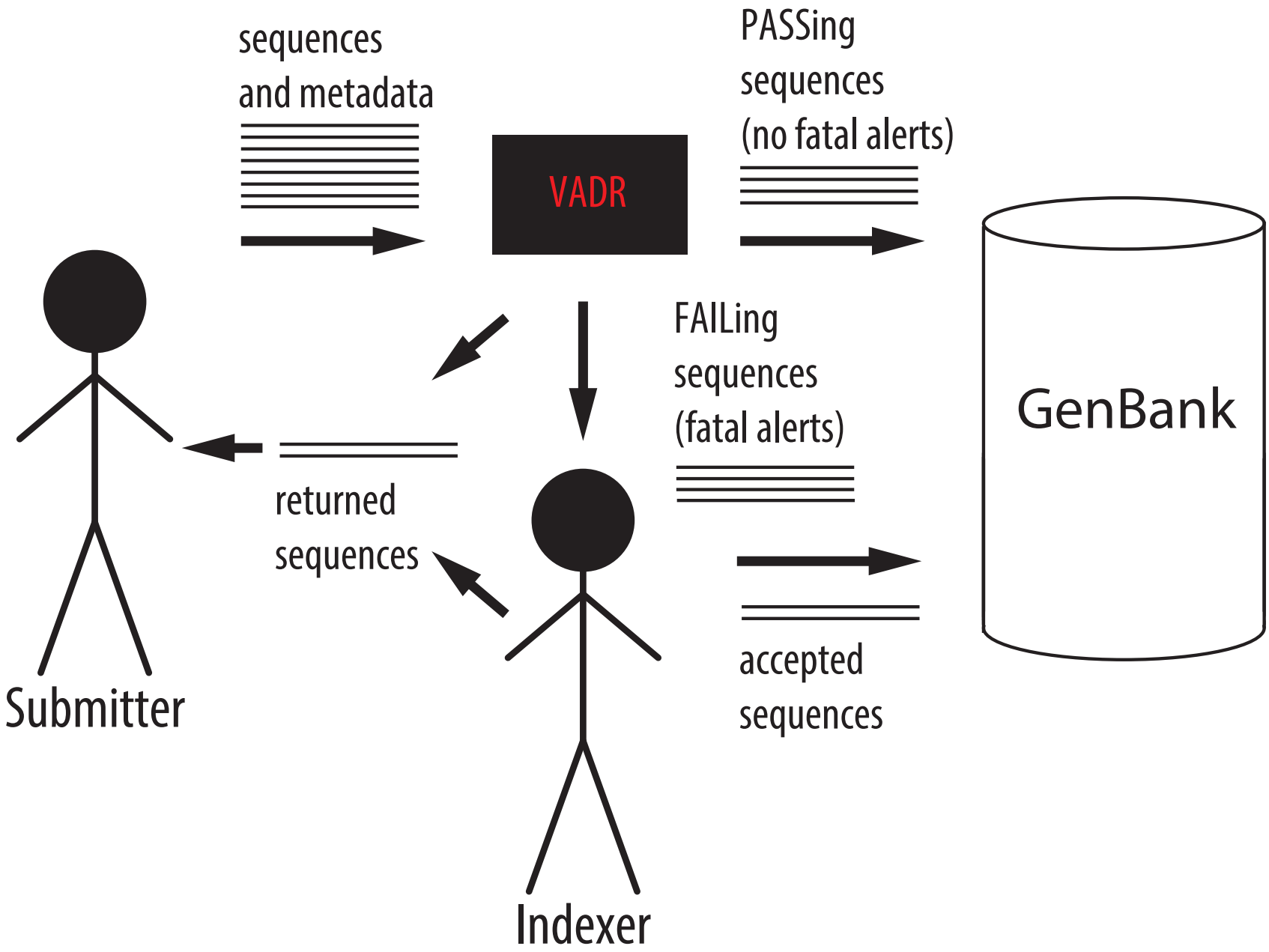
- general tool for reference-based annotation of viral sequences
- used for Norovirus and Dengue virus submissions since 2018
- used for SARS-CoV-2 submissions since March 2020
- also used manually for RSV, Mpox, and some Influenza submissions

VADR assists GenBank indexers:

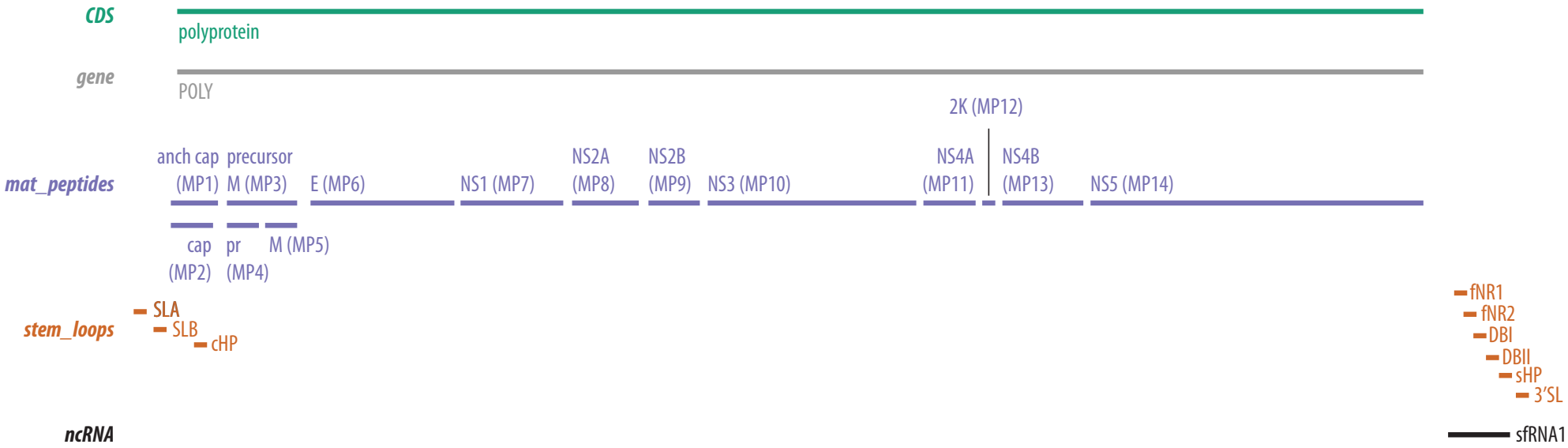
Each sequence PASSes or FAILs

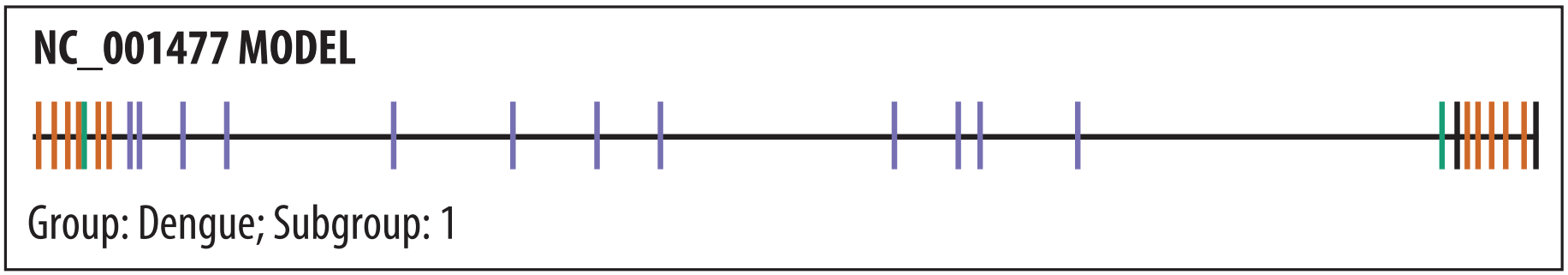


Indexers decide fate of some FAILing sequences
but some are sent directly back to submitter with error reports



VADR builds a reference model of a RefSeq and its features





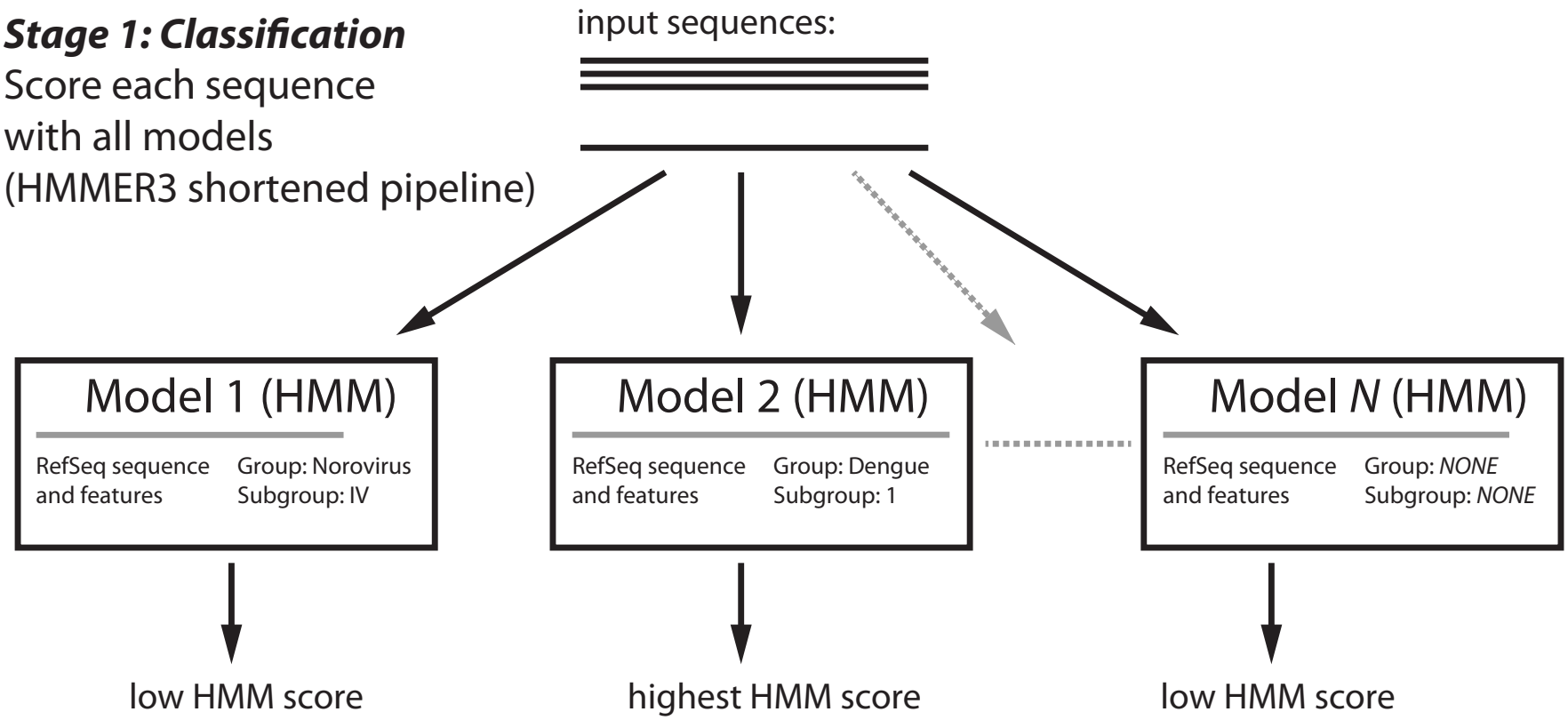
VADR validates and annotates each input sequence using its best-matching model

- Each sequence S proceeds through 4 stages:
  1. **Classification**
  2. **Coverage determination**
  3. **Alignment**
  4. **Protein validation**

Different types of alerts are identified and reported at each stage

Stage 1: Classification

Score each sequence
with all models
(HMMER3 shortened pipeline)



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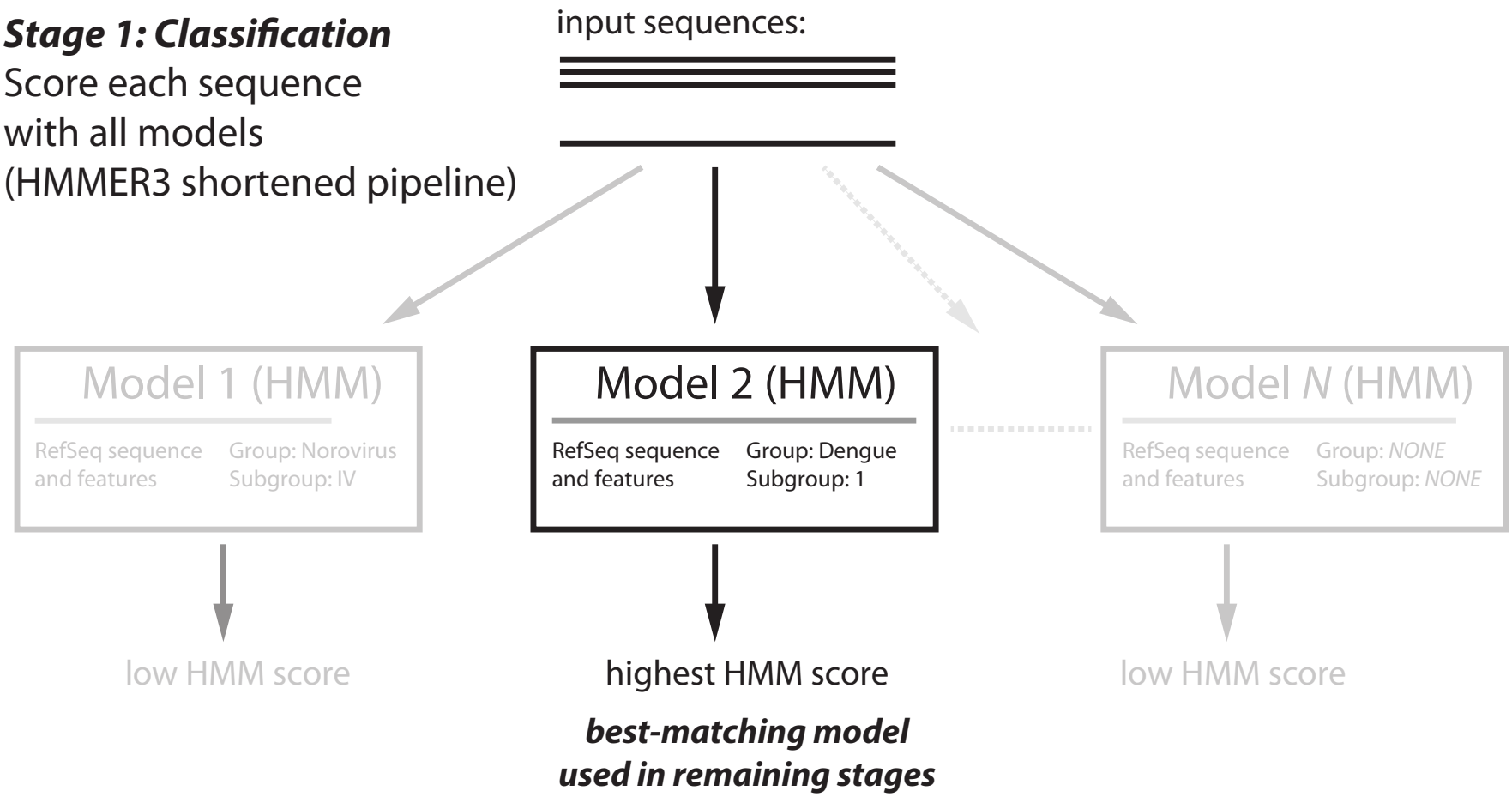
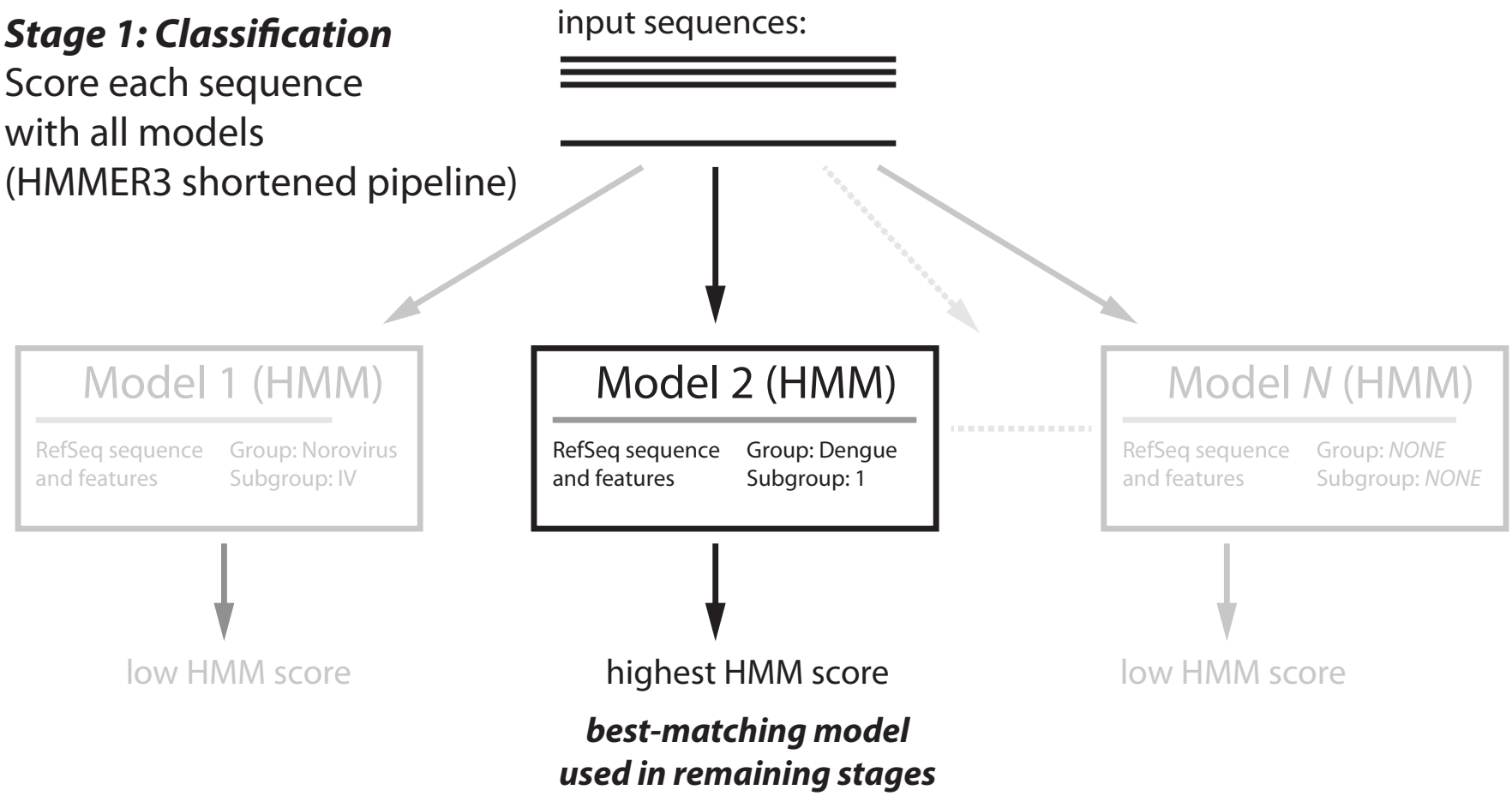
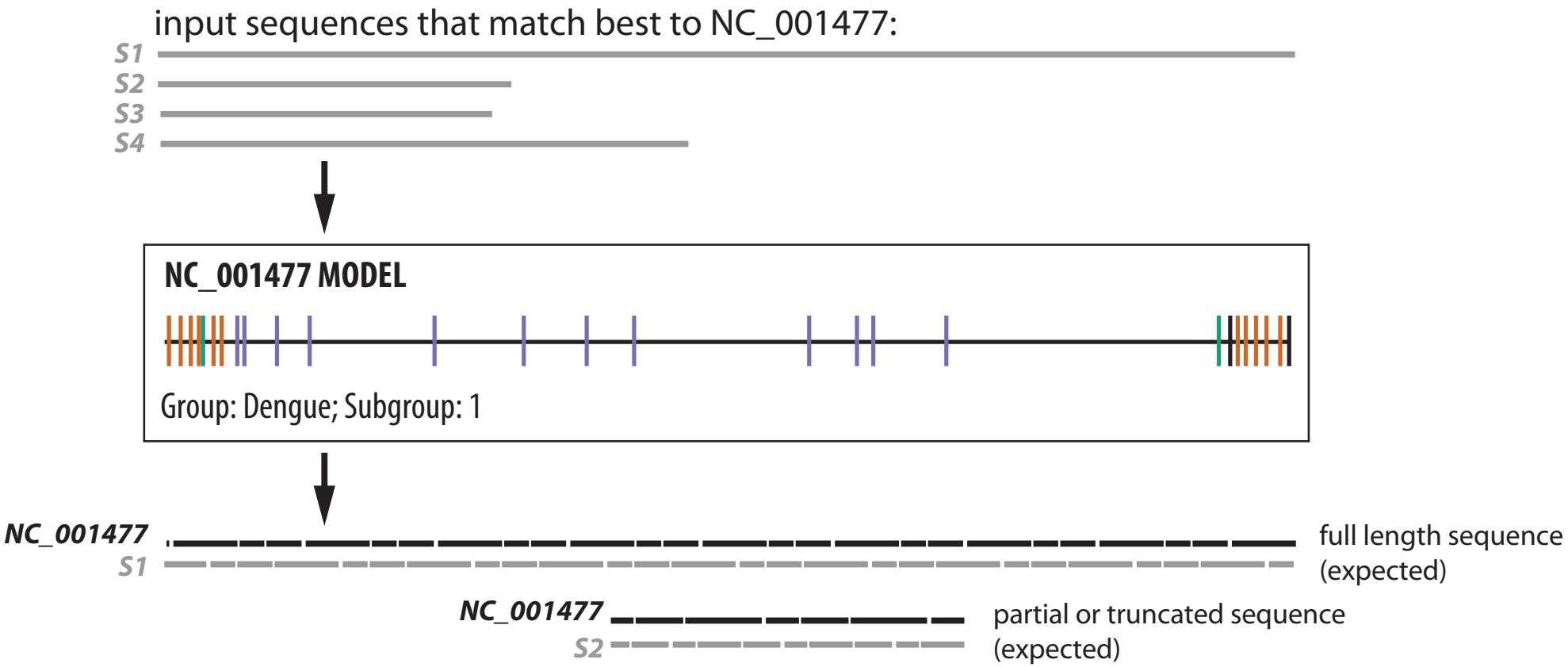




code	S/F	error message	description
Fatal alerts detected in the classification stage			
noannotn*	S	NO_ANNOTATION	no significant similarity detected
revcompl*	S	REVCOMPLEM	sequence appears to be reverse complemented
incsbgrp	S	INCORRECT_SPECIFIED_SUBGROUP	score difference too large between best overall model and best specified subgroup model
incgroup	S	INCORRECT_SPECIFIED_GROUP	score difference too large between best overall model and best specified group model
Non-fatal alerts detected in the classification stage			
qstsbgrp	S	QUESTIONABLE_SPECIFIED_SUBGROUP	best overall model is not from specified subgroup
qstgroup	S	QUESTIONABLE_SPECIFIED_GROUP	best overall model is not from specified group
indfclas	S	INDEFINITE_CLASSIFICATION	low score difference between best overall model and second best model (not in best model's subgroup)
lowscore	S	LOW_SCORE	score to homology model below low threshold

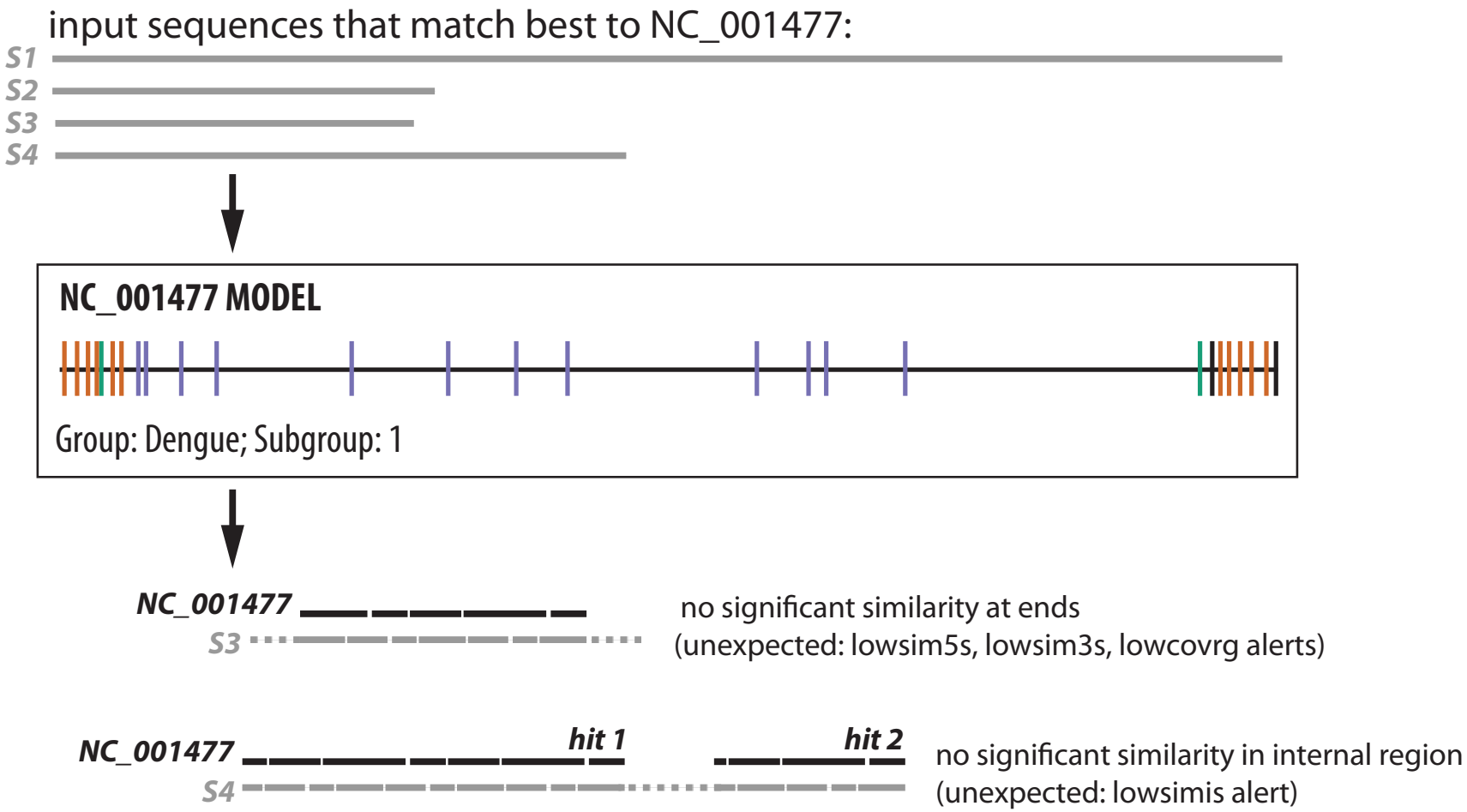
Stage 2: Coverage determination

Search each sequence with best-matching model (HMMER3 full pipeline)



Stage 2: Coverage determination

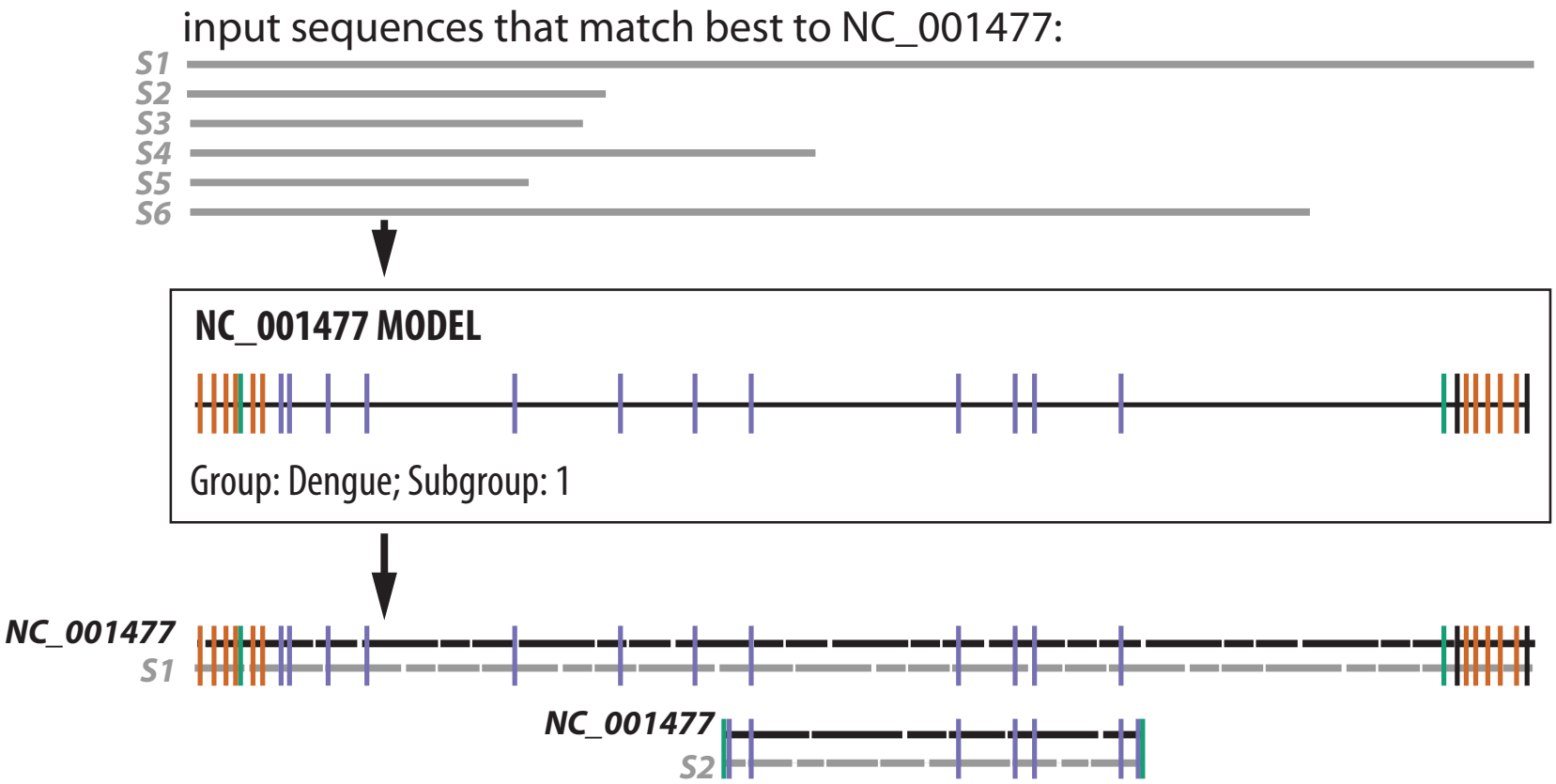
Search each sequence with best-matching model (HMMER3 full pipeline)



code	S/F	error message	description
Fatal alerts detected in the coverage stage			
lowcovrg	S	LOW_COVERAGE	low sequence fraction with significant similarity to homology model
dupregin	S	DUPLICATE_REGIONS	similarity to a model region occurs more than once
discontn	S	DISCONTINUOUS_SIMILARITY	not all hits are in the same order in the sequence and the homology model
indfstrn	S	INDEFINITE_STRAND	significant similarity detected on both strands
lowsim5s	S	LOW_SIMILARITY_START	significant similarity not detected at 5' end of the sequence
lowsim3s	S	LOW_SIMILARITY_END	significant similarity not detected at 3' end of the sequence
lowsimis	S	LOW_SIMILARITY	internal region without significant similarity
Non-fatal alerts detected in the coverage stage			
biasdseq	S	BIASED_SEQUENCE	high fraction of score attributed to biased sequence composition

Stage 3: Alignment and feature mapping

Align each sequence to its best-matching model (Infernal's cmalign)



Stage 3: Alignment and feature mapping

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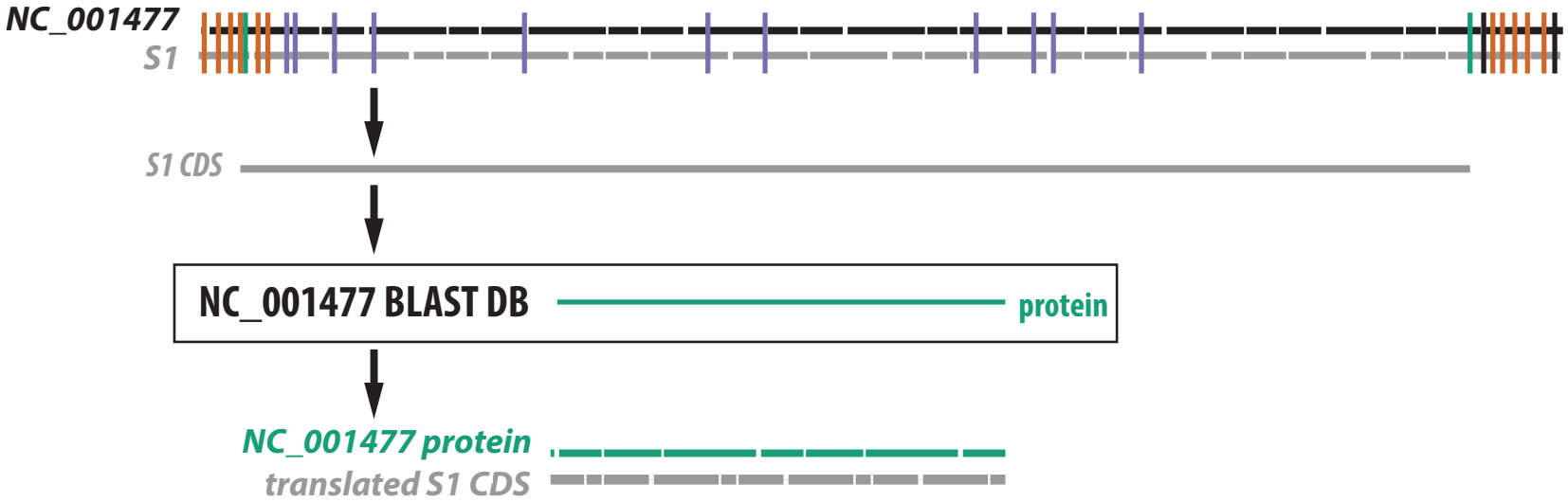
gap or low confidence at feature boundary
(indf5gap, indf3gap, indf5loc, indf3loc alert)

early stop codon in CDS
(cdsstopn alert)

code	S/F	error message	description
Fatal alerts detected in the annotation stage			
unexdivg*	S	UNEXPECTED_DIVERGENCE	sequence is too divergent to confidently assign nucleotide-based annotation
noftrann*	S	NO_FEATURES_ANNOTATED	sequence similarity to homology model does not overlap with any features
mutstart	F	MUTATION_AT_START	expected start codon could not be identified
mutendcd	F	MUTATION_AT_END	expected stop codon could not be identified, predicted CDS stop by homology is invalid
mutendns	F	MUTATION_AT_END	expected stop codon could not be identified, no in-frame stop codon exists 3' of predicted valid start codon
mutendex	F	MUTATION_AT_END	expected stop codon could not be identified, first in-frame stop codon exists 3' of predicted stop position
unexleng	F	UNEXPECTED_LENGTH	length of complete coding (CDS or mat_peptide) feature is not a multiple of 3
cdsstopn	F	CDS_HAS_STOP_CODON	in-frame stop codon exists 5' of stop position predicted by homology to reference
peptrans	F	PEPTIDE_TRANSLATION_PROBLEM	mat_peptide may not be translated because its parent CDS has a problem
pepadjcy	F	PEPTIDE_ADJACENCY_PROBLEM	predictions of two mat_peptides expected to be adjacent are not adjacent
indfantn	F	INDEFINITE_ANNOTATION	nucleotide-based search identifies CDS not identified in protein-based search
indf5gap	F	INDEFINITE_ANNOTATION_START	alignment to homology model is a gap at 5' boundary
indf5loc	F	INDEFINITE_ANNOTATION_START	alignment to homology model has low confidence at 5' boundary
indf3gap	F	INDEFINITE_ANNOTATION_END	alignment to homology model is a gap at 3' boundary
indf3loc	F	INDEFINITE_ANNOTATION_END	alignment to homology model has low confidence at 3' boundary
lowsim5f	F	LOW_FEATURE_SIMILARITY_START	region within annotated feature at 5' end of sequence lacks significant similarity
lowsim3f	F	LOW_FEATURE_SIMILARITY_END	region within annotated feature at 3' end of sequence lacks significant similarity
lowsimif	F	LOW_FEATURE_SIMILARITY	region within annotated feature lacks significant similarity

Stage 4: Protein validation (Alejandro Schäffer)

Compare each predicted CDS to model (RefSeq) proteins with BLASTX



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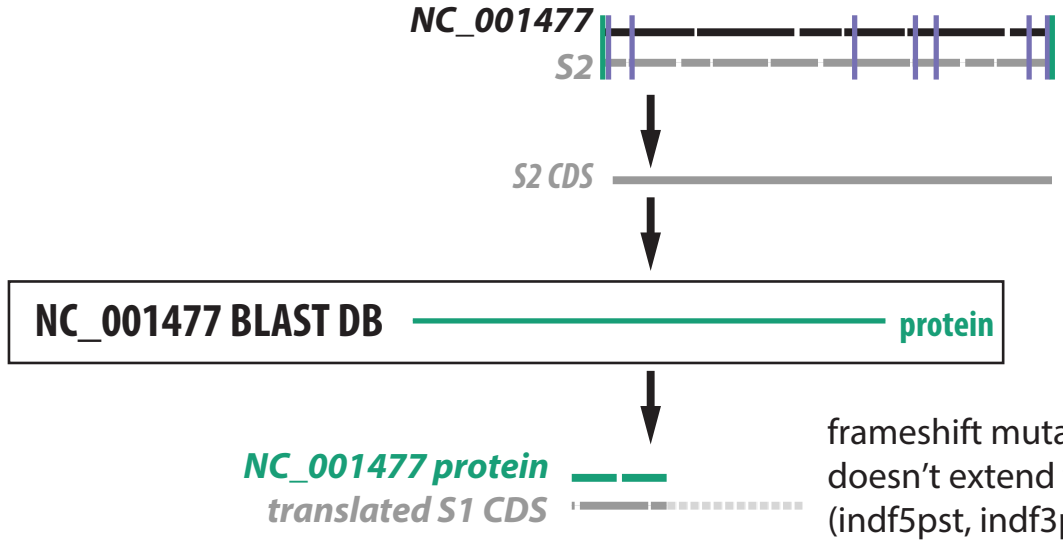


frameshift mutation; BLASTX alignment doesn't extend full length of CDS prediction (indf5pst, indf3pst alerts)

code	S/F	error message	description
Fatal alerts detected in the protein validation stage			
cdsstopp	F	CDS_HAS_STOP_CODON	stop codon in protein-based alignment
indfantp	F	INDEFINITE_ANNOTATION	protein-based search identifies CDS not identified in nucleotide-based search
indf5plg	F	INDEFINITE_ANNOTATION_START	protein-based alignment extends past nucleotide-based alignment at 5' end
indf5pst	F	INDEFINITE_ANNOTATION_START	protein-based alignment does not extend close enough to nucleotide -based alignment 5' endpoint
indf3plg	F	INDEFINITE_ANNOTATION_END	protein-based alignment extends past nucleotide-based alignment at 3' end
indf3pst	F	INDEFINITE_ANNOTATION_END	protein-based alignment does not extend close enough to nucleotide -based alignment 3' endpoint
indfstrp	F	INDEFINITE_STRAND	strand mismatch between protein-based and nucleotide-based predictions
insertnp	F	INSERTION_OF_NT	too large of an insertion in protein-based alignment
deletinp	F	DELETION_OF_NT	too large of a deletion in protein-based alignment

VADR used for Norovirus and Dengue virus sequences since 2018

	Norovirus	Dengue virus
length	7.6Kb	10.7Kb
# seqs	44,936	113,211
% seqs full length	5.1%	8.4%
% Ns	0.5%	0.2%
% seqs with stretch of >= 50 Ns	1.0%	0.4%
average % identity	81.6%	94.4%

VADR v1.0 performance		
seconds per sequence	42.4	92.6
required RAM	8Gb	8Gb
total running time, CPU days	1.1	10.2

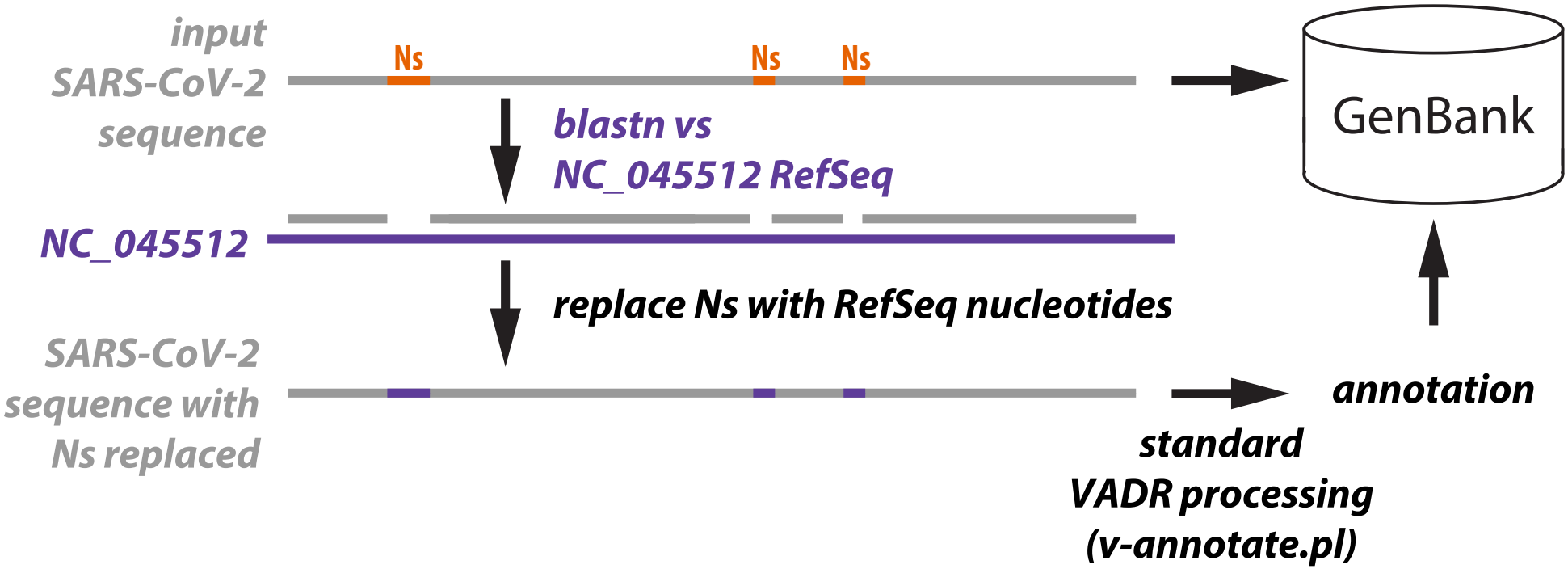
SARS-CoV-2 sequence submissions have increased since early 2020

month	year	#new seqs	#cumulative seqs
Jan	2020	32	32
Feb	2020	58	90
Mar	2020	332	422
Apr	2020	1541	1963
May	2020	2974	4937
Jun	2020	3394	8331
Jul	2020	3604	11,935
Aug	2020	3818	15,753
Sep	2020	6731	22,484
Oct	2020	11,939	34,423
Nov	2020	4274	38,697
Dec	2020	4530	43,227
Jan	2021	8775	52,002
Feb	2021	26,078	78,080
Mar	2021	42,607	120,687
Apr	2021	97,095	217,782
May	2021	104,729	322,511
Jun	2021	46,187	368,698
Jul	2021	43,336	412,034
Aug	2021	141,958	553,992
Sep	2021	267,562	821,554
Oct	2021	239,296	1,060,850
Nov	2021	267,270	1,328,120
Dec	2021	288,771	1,616,891
Jan	2022	258,522	1,875,413
Feb	2022	230,185	2,105,598

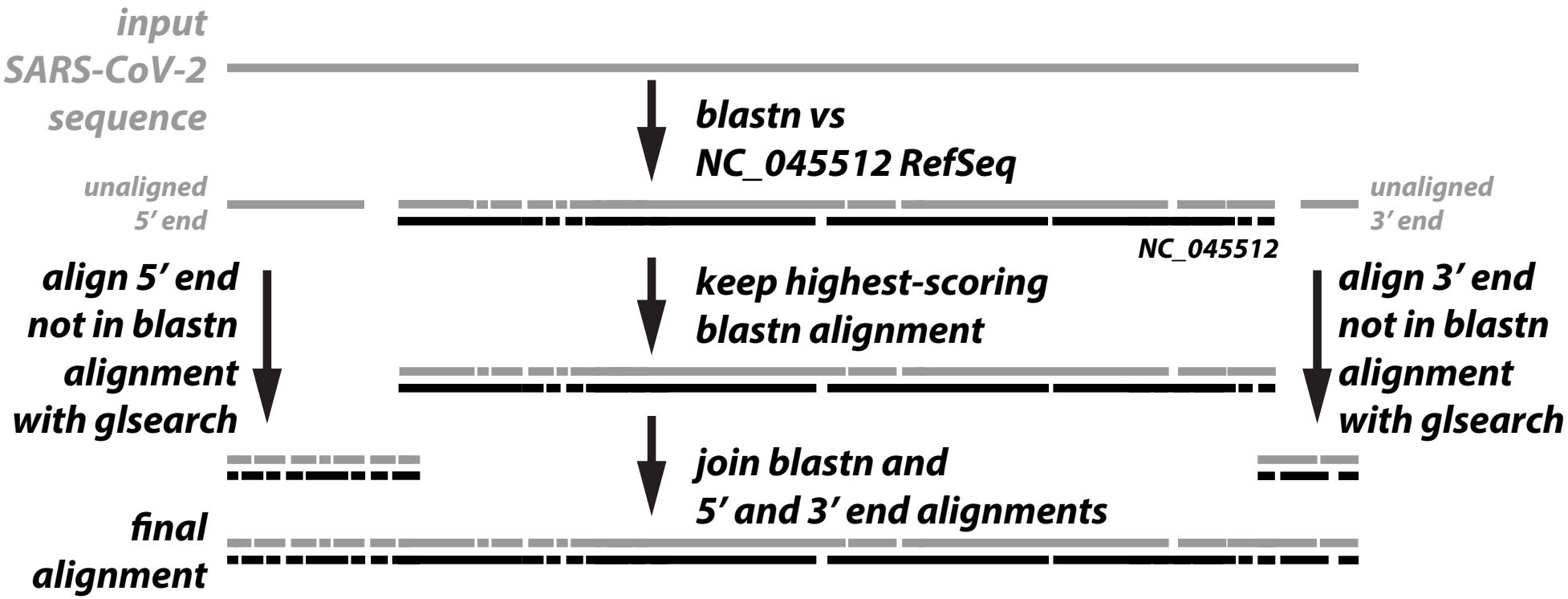
SARS-CoV-2 sequences differ from Norovirus and Dengue virus in several ways that impact VADR processing

	Norovirus	Dengue virus	SARS-CoV-2
length	7.6Kb	10.7Kb	29.9Kb
# seqs	44,936	113,211	1,616,891
% seqs full length	5.1%	8.4%	99.7%
% Ns	0.5%	0.2%	1.4%
% seqs with stretch of >= 50 Ns	1.0%	0.4%	38.7%
average % identity	81.6%	94.4%	99.4%
VADR v1.0 performance			
seconds per sequence	42.4	92.6	331.8
required RAM	8Gb	8Gb	64Gb
total running time, CPU days	1.1	10.2	6187.6

Replacing Ns with expected nucleotides allows many 'good' sequences to pass

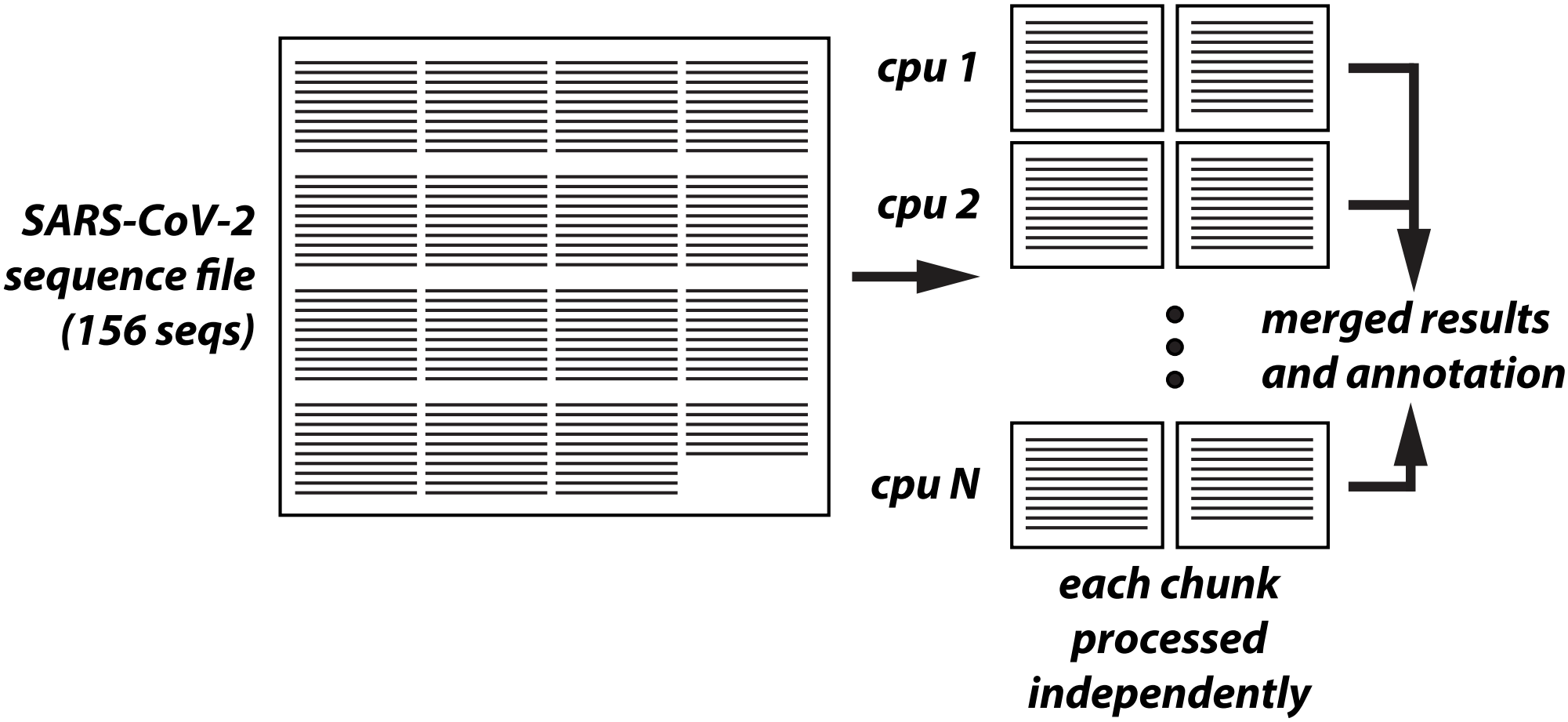


Seeded alignment using blastn makes alignment stage faster



Using glsearch instead of cmalign reduces memory requirement

- lower memory requirement (2Gb max) allows for multi-threading



VADR is now 1000-fold faster in practice for SARS-CoV-2 processing

VADR version	seeded align-ment?	N replace-ment?	glsearch?	# cpus	required RAM	secs per seq	hours per 100K seqs	speedup vs v1.0
v1.0	–	–	–	1	64 Gb	329.91	9164.3	-
v1.4.1	+	+	+	**8**	**16 Gb**	**0.33**	**9.3**	**986.8**

VADR is now fast enough to handle hundreds of thousands of sequences per month

month	year	#new seqs	#cumulative seqs
Jan	2020	32	32
Feb	2020	58	90
Mar	2020	332	422
Apr	2020	1541	1963
May	2020	2974	4937
Jun	2020	3394	8331
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Besides getting faster, VADR has changed in other ways (work with Linda Yankie and Vince Calhoun and GenBank team)

- 13 releases between March 2020 and January 2022
- 3 additional models (all eventually dropped):
  - B.1.1.7 (alpha)
  - B.1.525
  - 28254-deletion
- allow some alerts for non-essential ORFs without failing sequence (they become a `misc_feature` instead)

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https://doi.org/10.1093/nargab/lqad002

Faster SARS-CoV-2 sequence validation and annotation for GenBank using VADR

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Database, 2022, **2022(2022)**, 1–7
DOI: https://doi.org/10.1093/database/baac006
Original article

Rapid automated validation, annotation and publication of SARS-CoV-2 sequences to GenBank

Beverly A. Underwood, Linda Yankie, Eric P. Nawrocki, Vasuki Palanigobu, Sergiy Gotvyanskyy, Vincent C. Calhoun, Michael Kornbluh, Thomas G. Smith, Lydia Fleischmann, Denis Sinyakov, Colleen J. Bollin and Ilene Karsch-Mizrachi*

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OXFORD

Briefings in Bioinformatics, 22(2), 2021, 642–663

doi: 10.1093/bib/bbaa232
Advance Access Publication Date: 4 November 2020
Method Review

Computational strategies to combat COVID-19: useful tools to accelerate SARS-CoV-2 and coronavirus research

Franziska Hufsky, Kevin Lamkiewicz, Alexandre Almeida, Abdel Aouacheria, Cecilia Arighi, Alex Bateman, Jan Baumbach, Niko Beerenwinkel, Christian Brandt, Marco Cacciabue, Sara Chuguransky, Oliver Drechsel, Robert D. Finn, Adrian Fritz, Stephan Fuchs, Georges Hattab, Anne-Christin Hauschild, Dominik Heider, Marie Hoffmann, Martin Hölzer, Stefan Hoops, Lars Kaderali, Ioanna Kalvari, Max von Kleist, Renó Kmiecinski, Denise Kühnert, Gorka Lasso, Pieter Libin, Markus List, Hannah F. Löchel, Maria J. Martin, Roman Martin, Julian Matschinske, Alice C. McHardy, Pedro Mendes, Jaina Mistry, Vincent Navratil, Eric P. Nawrocki, Áine Niamh O'Toole, Nancy Ontiveros-Palacios, Anton I. Petrov, Guillermo Rangel-Pineros, Nicole Redaschi, Susanne Reimering, Knut Reinert, Alejandro Reyes, Lorna Richardson, David L. Robertson, Sepideh Sadegh, Joshua B. Singer, Kristof Theys, Chris Upton, Marius Welzel, Lowri Williams and Manja Marz

Additional VADR models and development

	length	num models	new feature(s)	author
RSV	15Kb	2	alignment-based models	Eric Nawrocki
COX-1	1.5Kb	86	protein-coding gene	Eric Nawrocki
Mpox	197Kb	1	minimap alignment	Eric Nawrocki
Influenza	1-2Kb	70	segmented virus	Eric Nawrocki
Zika	11Kb	?	?	EB Dickinson

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Database, 2024, baae091
DOI: https://doi.org/10.1093/database/baae091
Original article

Influenza sequence validation and annotation using VADR

Vincent C. Calhoun, Eneida L. Hatcher, Linda Yankie, Eric P. Nawrocki *

National Center for Biotechnology Information, U.S. National Library of Medicine, National Center for Biotechnology Information, 8600 Rockville Pike, Bethesda, MD 20894, United States

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Citation details: Calhoun, V., Hatcher, E., Yankie, L. *et al.* Influenza sequence validation and annotation using VADR. *Database* (2024) Vol. 2024: article ID baae091; DOI: https://doi.org/10.1093/database/baae091

Additional VADR models and development

- Alex Greninger's lab at Univ of Washington:
  - sequences a wide variety of human pathogenic viruses
  - previously developed the VAPiD software tool for validating and annotating viral sequences
  - now a collaborator that builds VADR models

Additional VADR models and development

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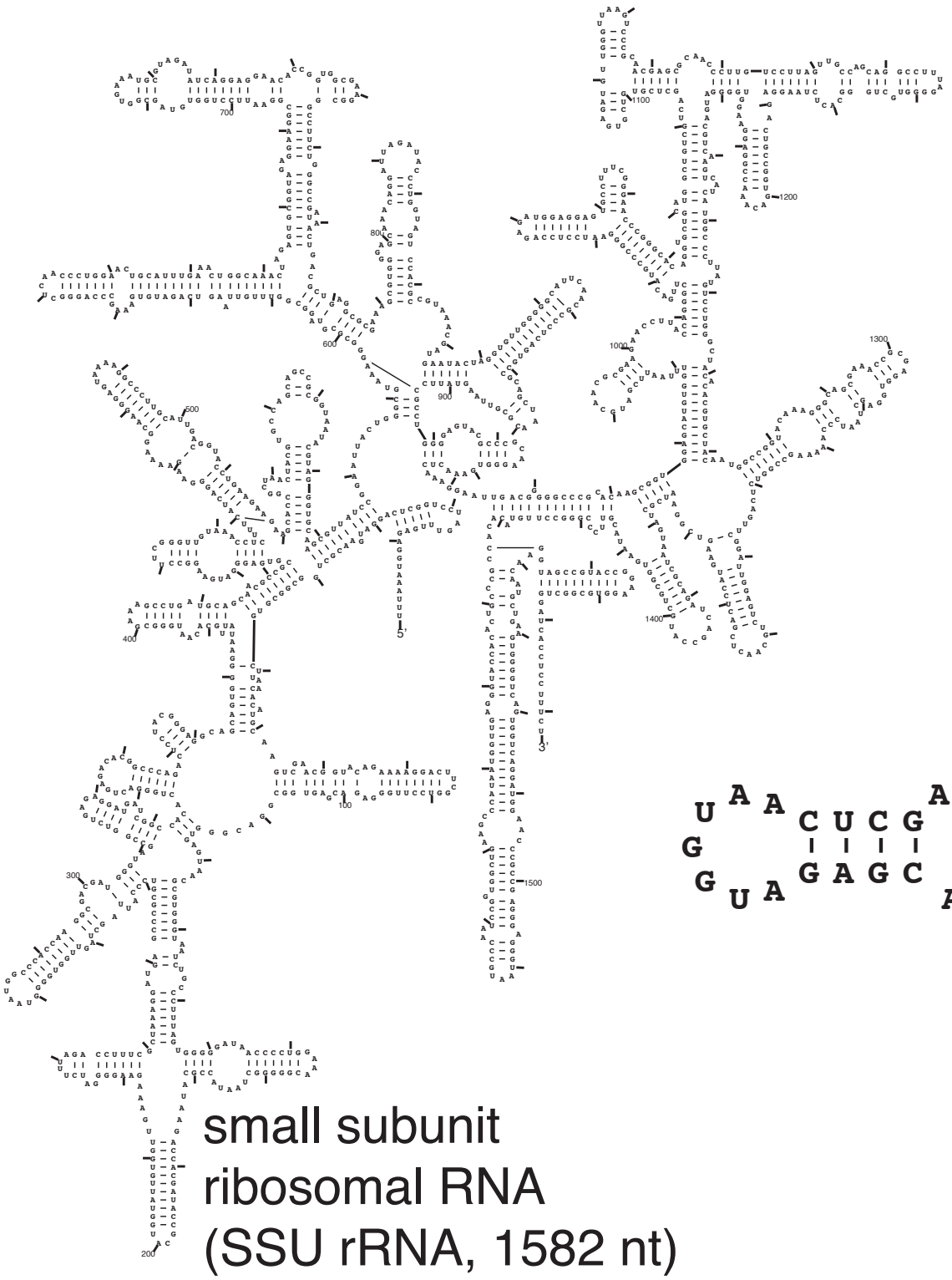
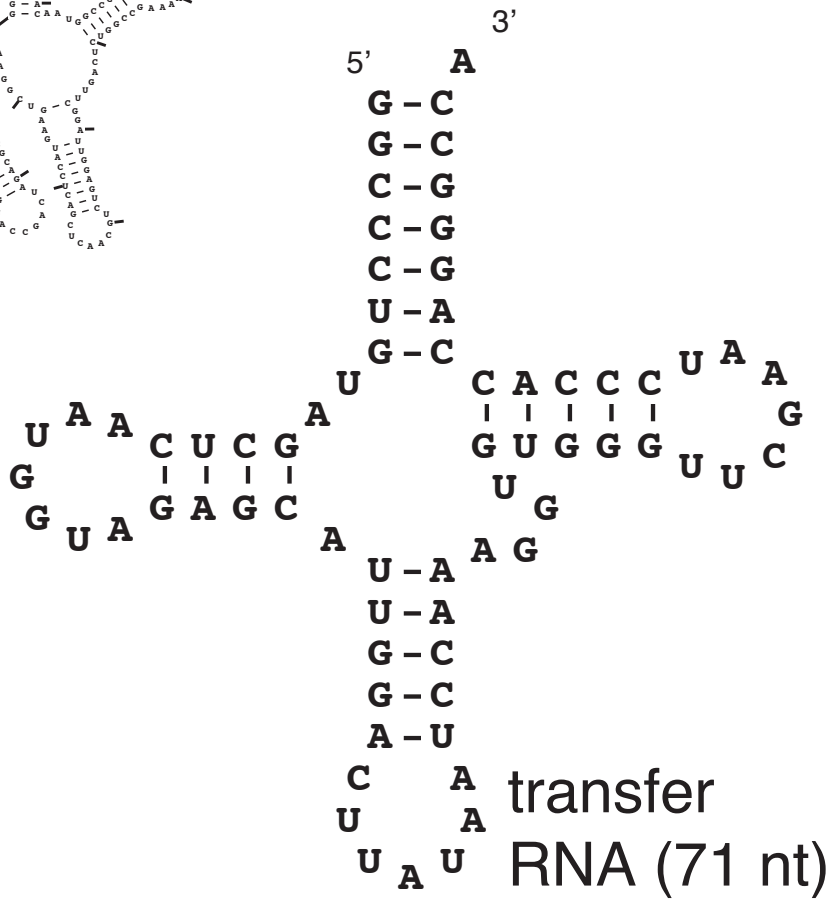
Influenza	1-2Kb	70	segmented virus	Eric Nawrocki
Zika	11Kb	?	?	EB Dickinson
Herpes Simplex Virus (HSV)	150Kb	2	-	Jaydee Sereewit (Greninger Lab)
Human meta-pneumovirus (HMPV)	13Kb	6	-	Jeffrey Furlong (Greninger Lab)
Human para-influenza virus (HPIV)	15Kb	5	-	Jeffrey Furlong (Greninger Lab)

Future directions for VADR

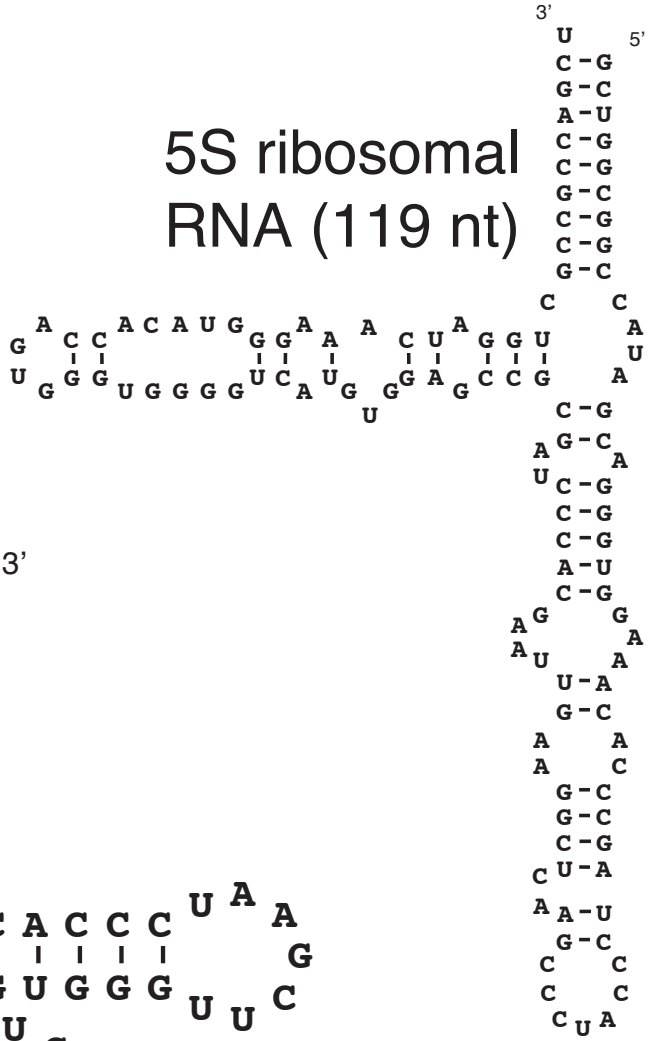
- NCBI Virus FY2025 goals:
  - VADR web server
  - Replacement of FLAN with VADR
- Models for more viruses (our group and Greninger lab)



small subunit ribosomal RNA (SSU rRNA, 1582 nt)



transfer RNA (71 nt)



5S ribosomal RNA (119 nt)

Functional RNAs play many vital roles in the cell

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

Functional RNAs play many vital roles in the cell

	key RNAs involved	archaea	bacteria	eukarya	viruses
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	snoRNAs	x		x	
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	RNaseMRP			x	
gene expression	riboswitches	?	x	?	
	microRNAs			x	x
	6S RNA		x	x	
splicing	U1, U2, U4, U5, U6			x	
other	tracrRNA	x	x		
	telomerase RNA			x	
	group I introns	x	x	x	x
	sfRNAs				x
	many more...				

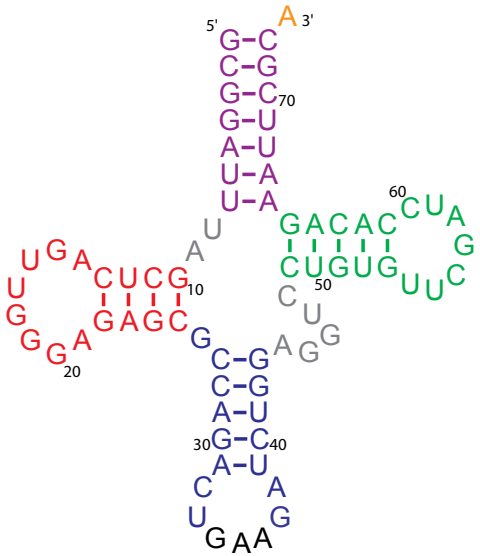
database of more than 4100 non-coding RNA families
each represented by a secondary structure, alignment, and covariance model.

Many functional RNAs adopt a conserved 3-dimensional structure

Primary sequence

GCGGAUUUAGCUCAGUUGGG
AGAGCGCCAGACUGAAGAUC
UGGAGGUCCUGUGUUCGAUC
CACAGAAUUCGCA

Secondary structure

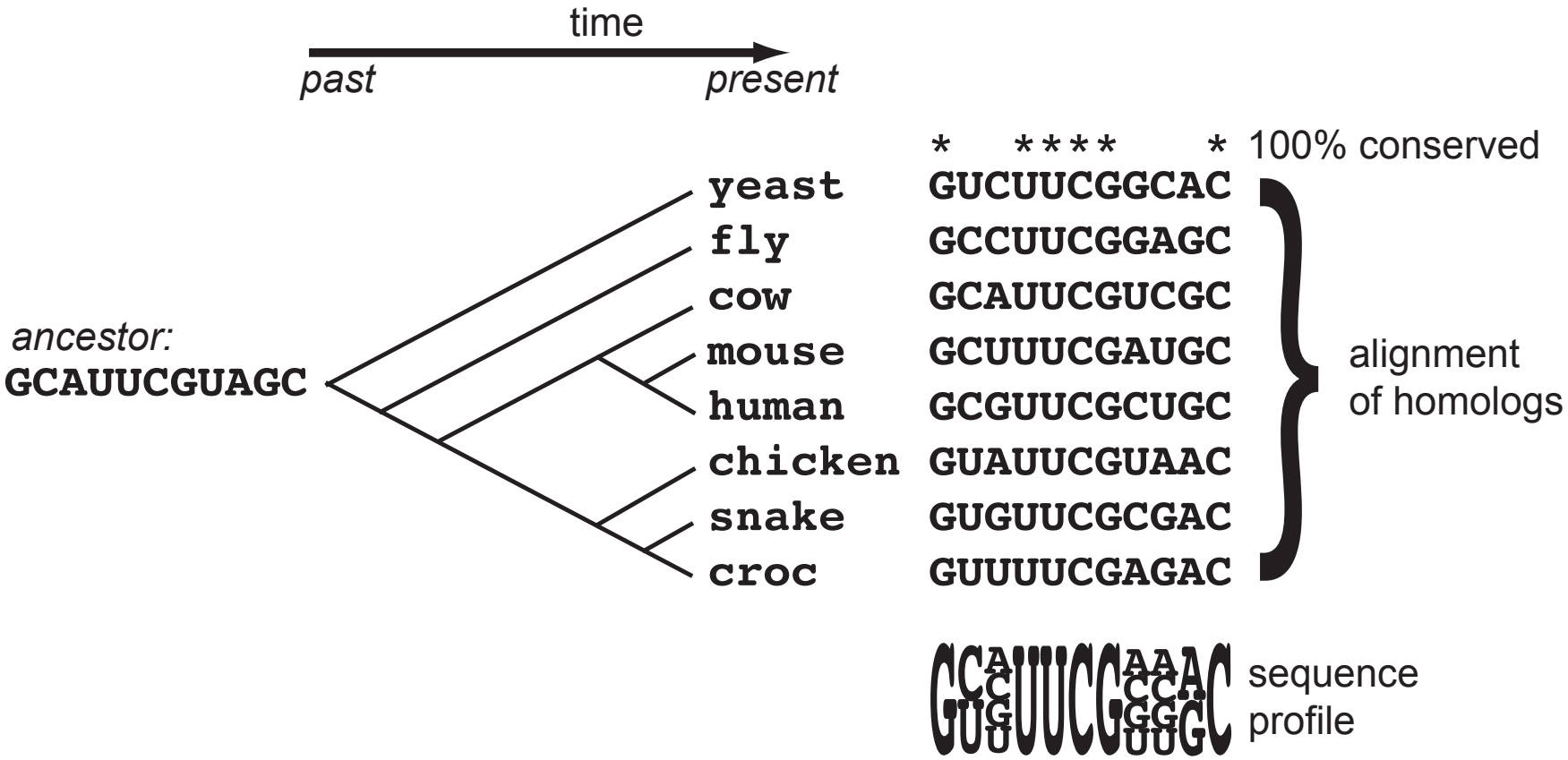


3-dimensional structure

- BLAST: given a single sequence, search genomes for similar sequences.
- Structural RNAs are difficult to find
  - short ($\sim$ 100 nt) and evolve rapidly at sequence level
  - lack open reading frames
  - small, 4 letter alphabet
- BLAST cannot take advantage of:
  - sequence conservation, which varies across the gene
  - secondary structure

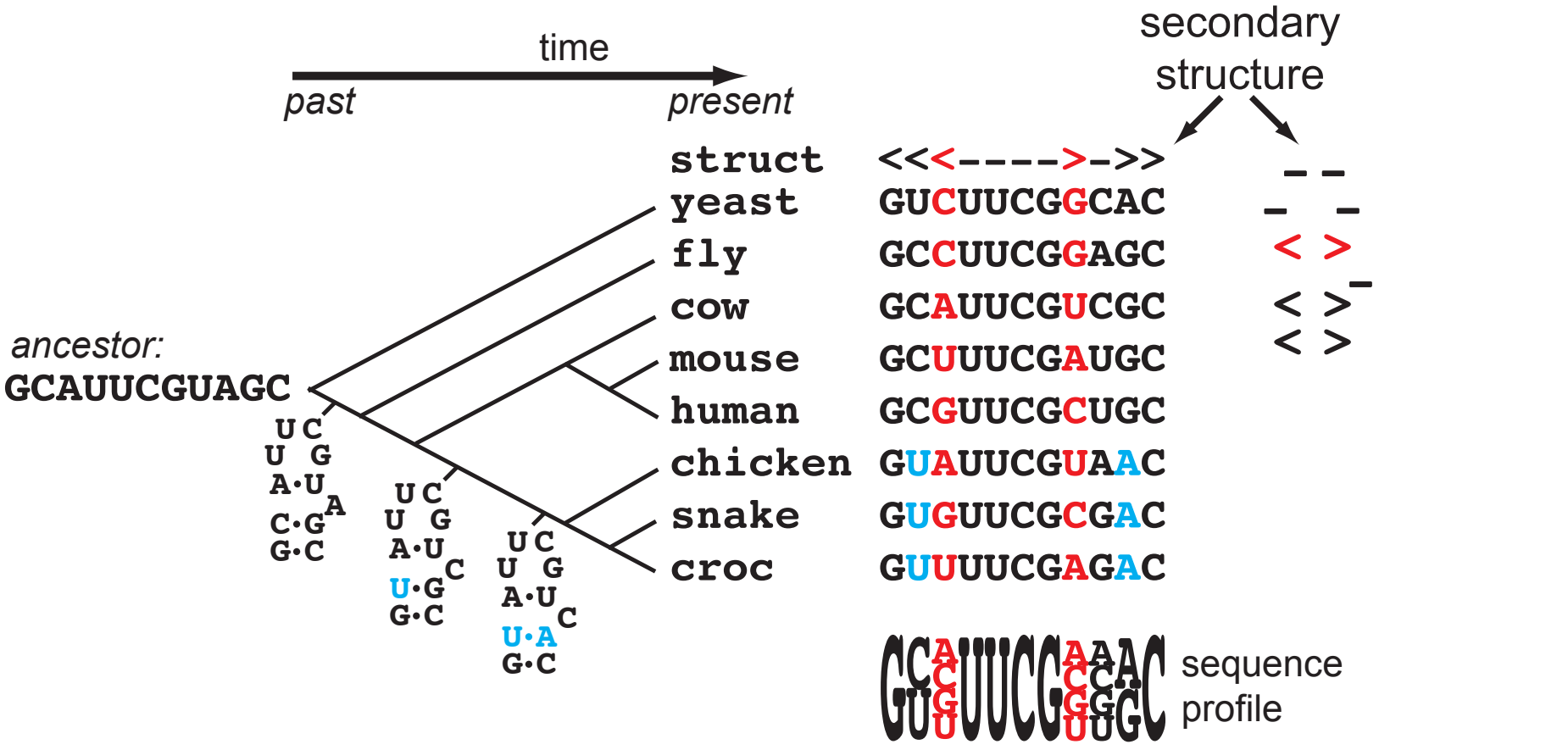
Sequence conservation provides information for homology searches

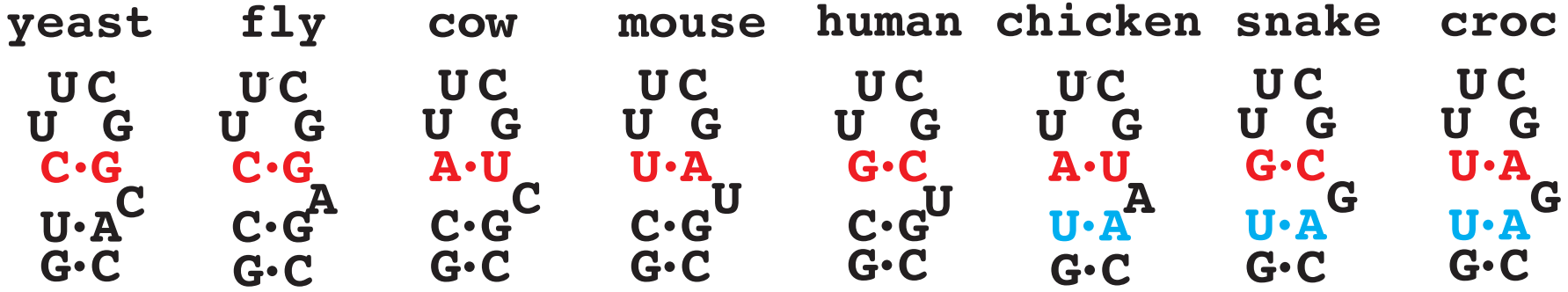
Conservation levels vary across alignment columns.



Structure conservation provides additional information

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





profile HMMs and covariance models

	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 (23794 and 4150 entries)	Rfam (4178 families)
performance for RNAs	faster but less accurate	slower but more accurate

http://hmmer.org

Potter et al. NAR 46:W200-204

Wheeler, TJ, Eddy SR. Bioinformatics, 29:2487-89, 2013.

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

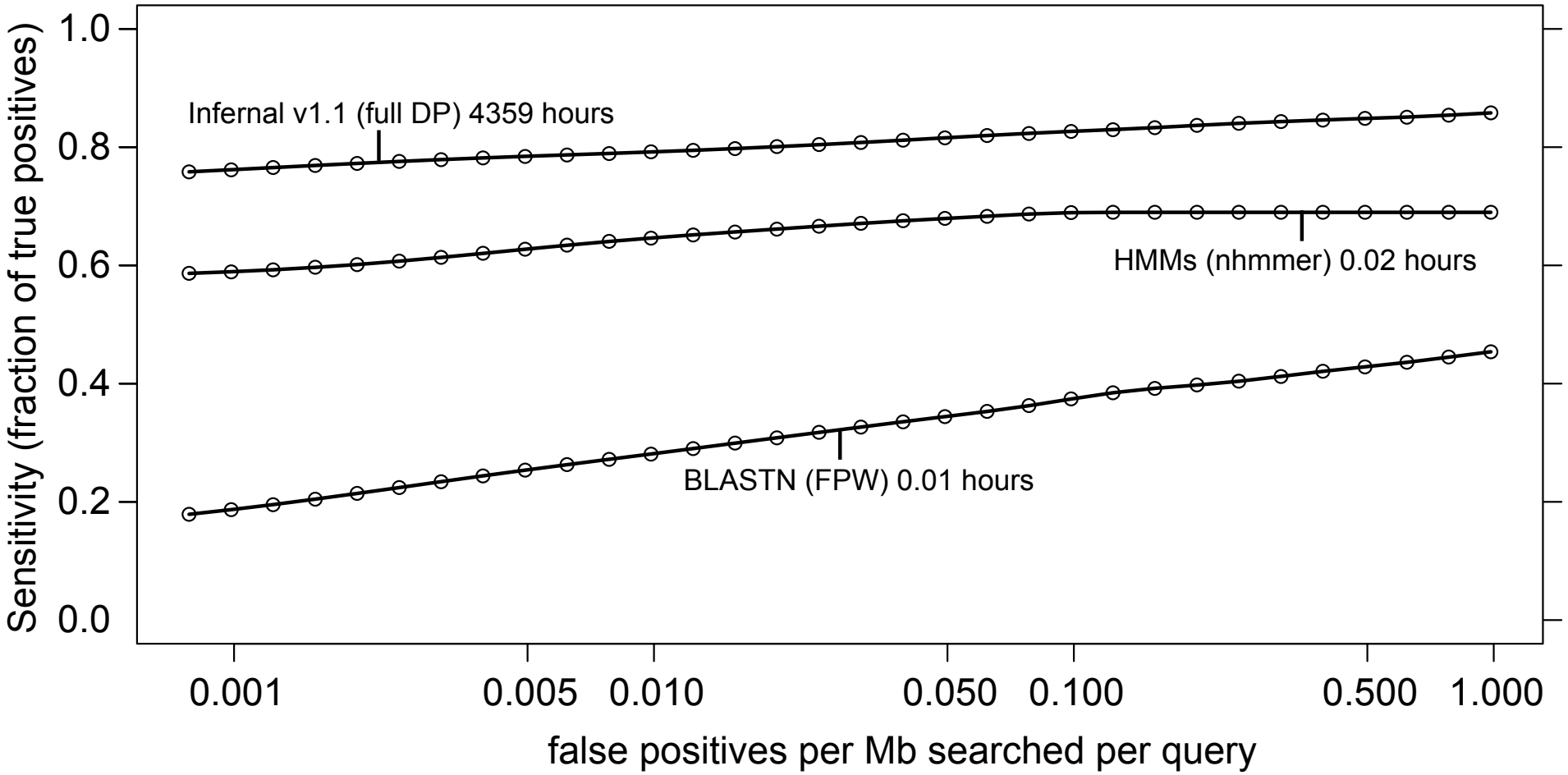
Eddy. SR. Bioinformatics, 14:755-763, 1998.

http://eddylab.org/infernal/

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

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

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

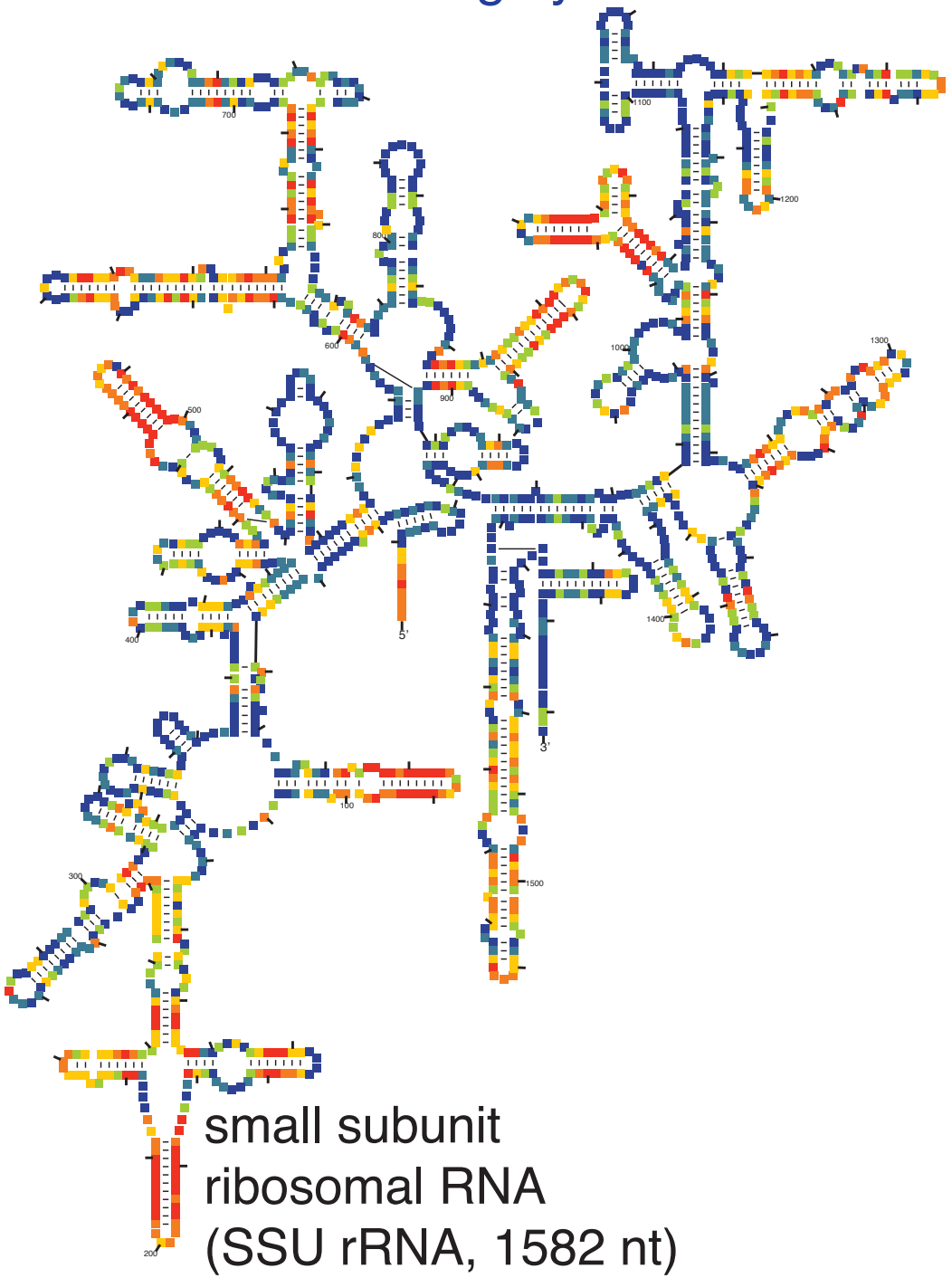


Nawrocki EP, Eddy SR. Bioinformatics, 29:2487-2489, 2013.

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

Sequence conservation per position

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



small subunit
ribosomal RNA
(SSU rRNA, 1582 nt)

Sequence conservation per position

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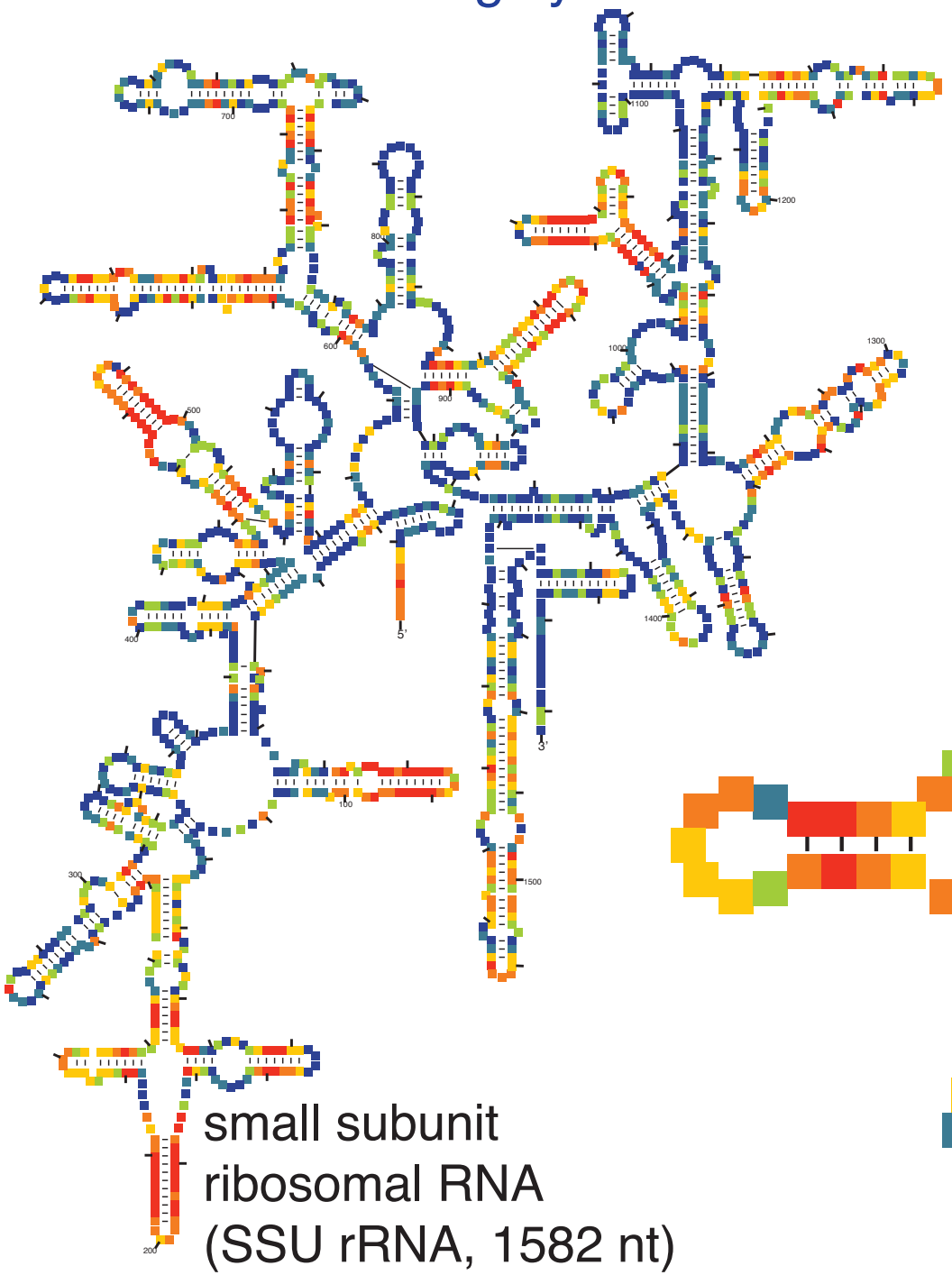
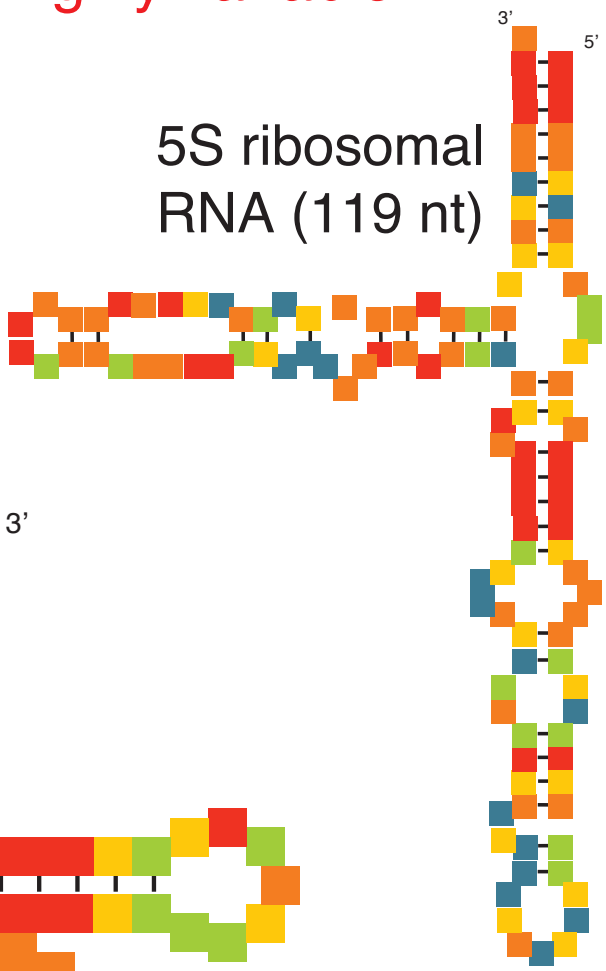
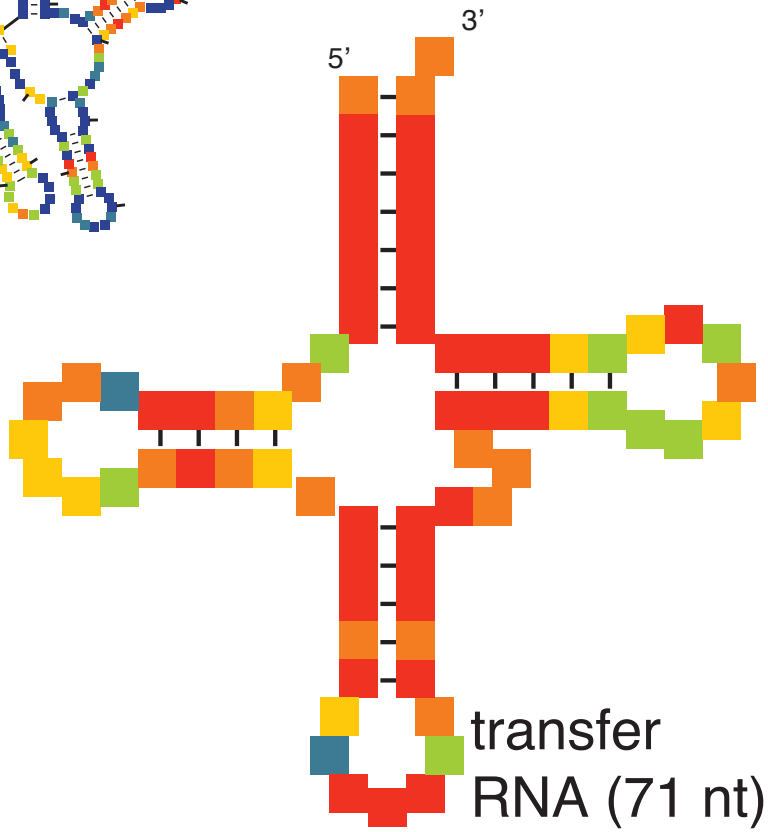


small subunit ribosomal RNA (SSU rRNA, 1582 nt)

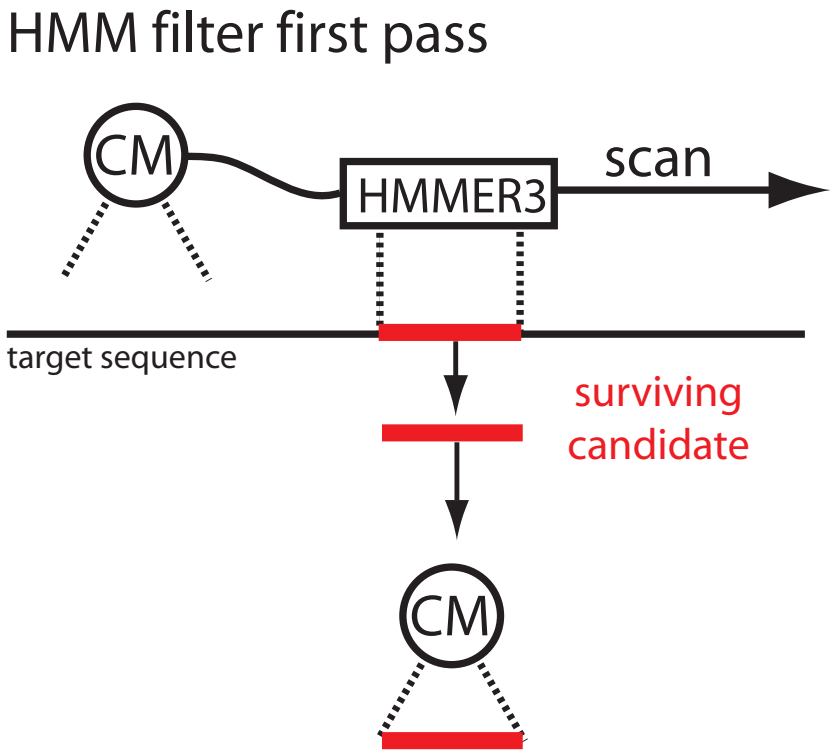


5S ribosomal RNA (119 nt)



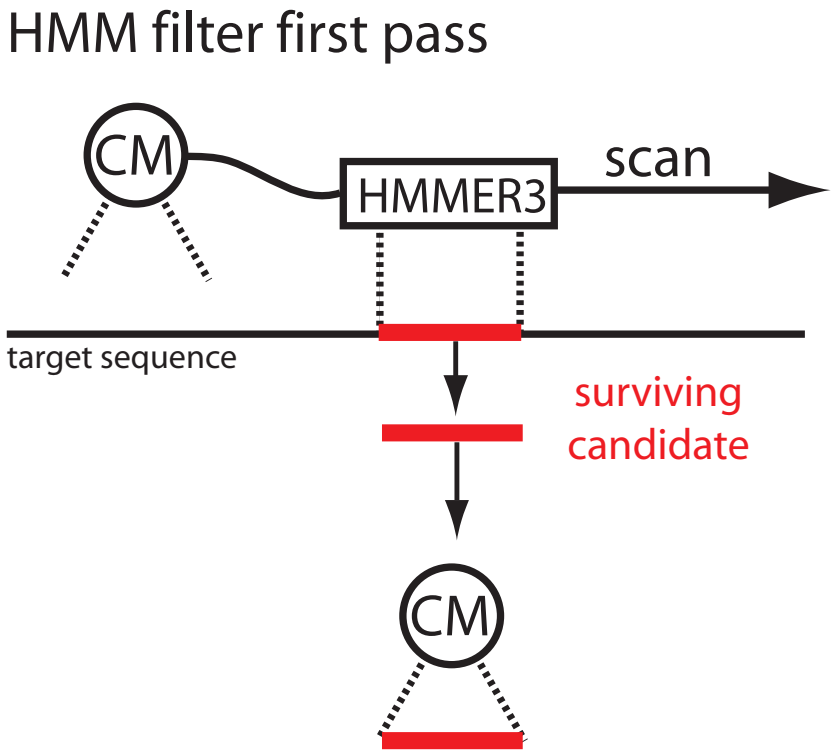
transfer RNA (71 nt)

Filter target database using profile HMMs*



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

Filter target database using profile HMMs*

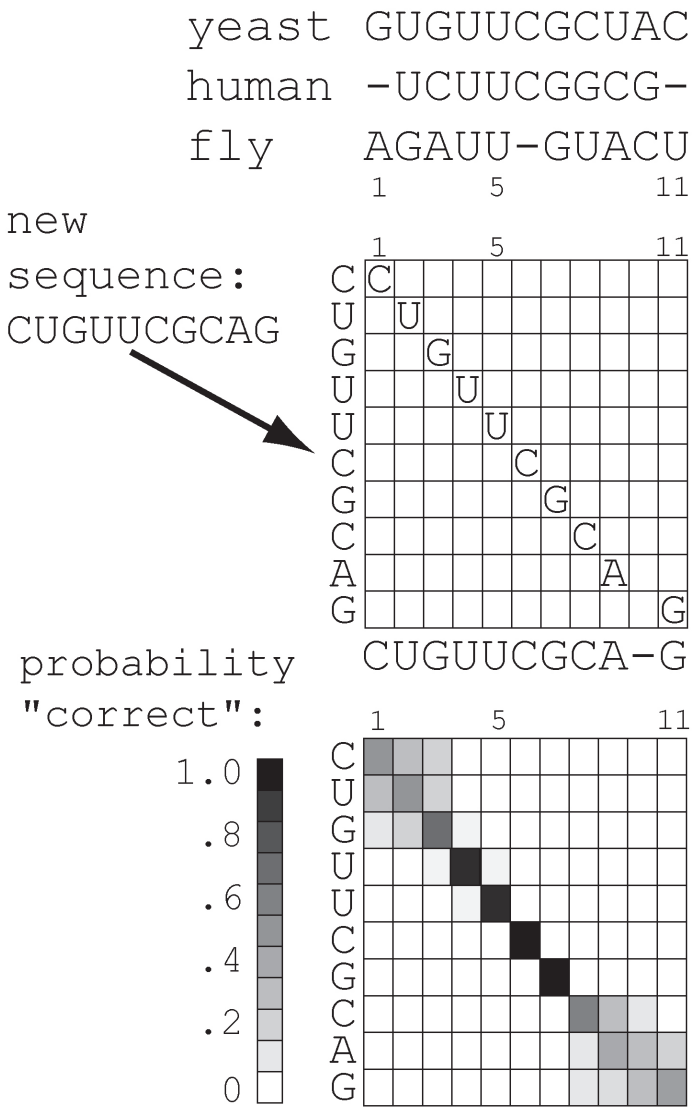


- Even if we filter out 99% of the database (for up to 100X acceleration), searches will still be too slow.

- CM step needs to be accelerated.

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

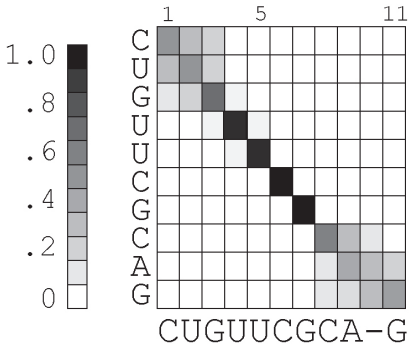
Accelerating CM alignment step 1: HMM posterior decoding to get confidence estimates

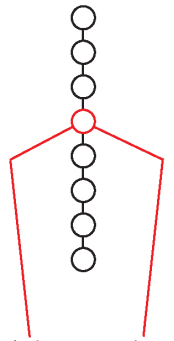


Accelerating CM alignment step 2: use HMM alignment confidence to constrain CM alignment

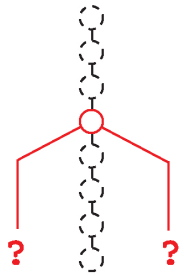
HMMs – Each column of seed alignment corresponds to a column of matrix.

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





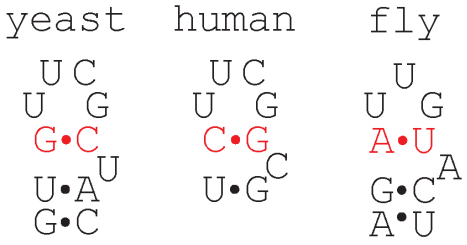
```
struct <<<<----->->>
yeast  GUGUUCGCUAC
human  -UCUUCGGCG-
fly    AGAUU-GUACU
```



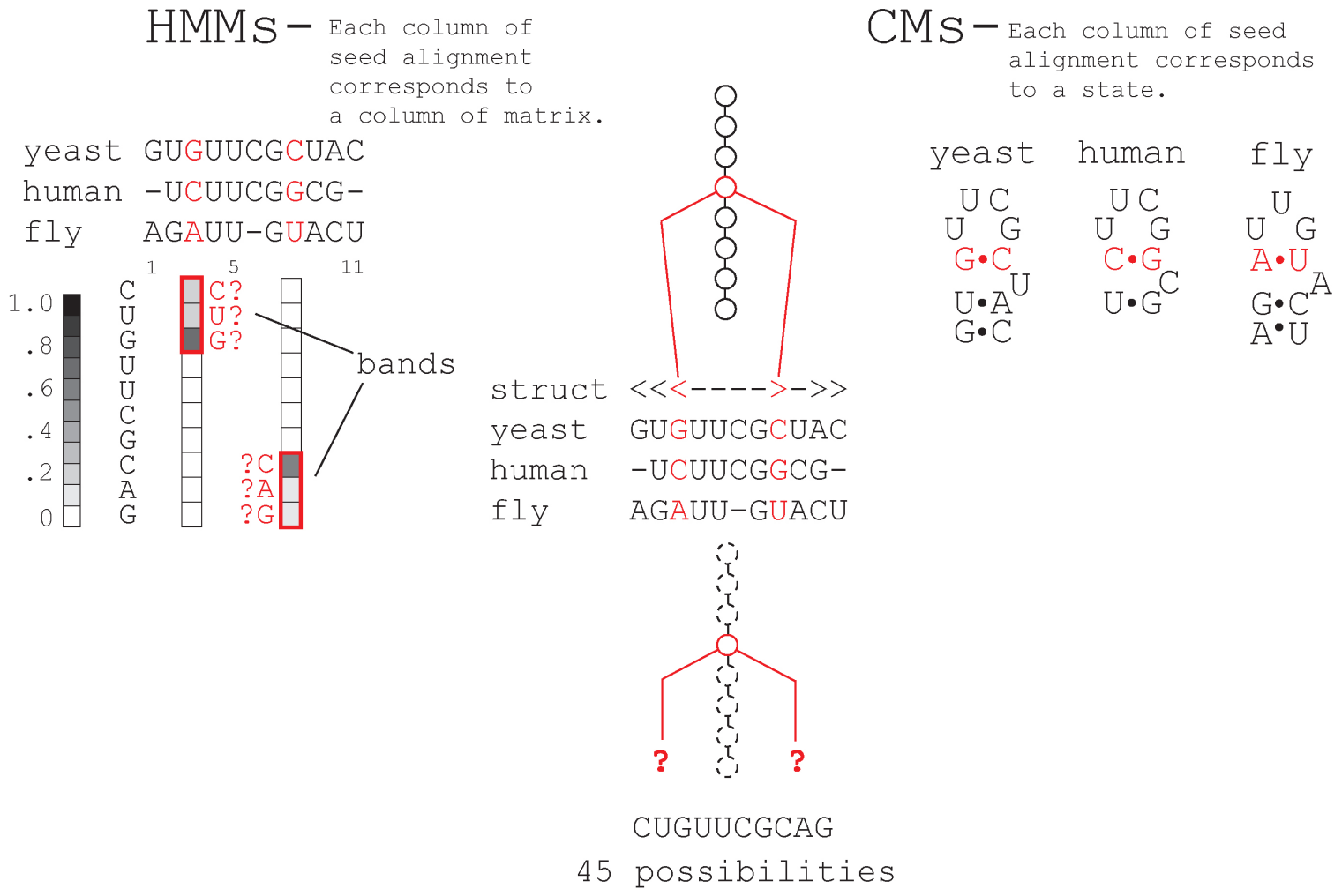
CUGUUCGCAG

45 possibilities

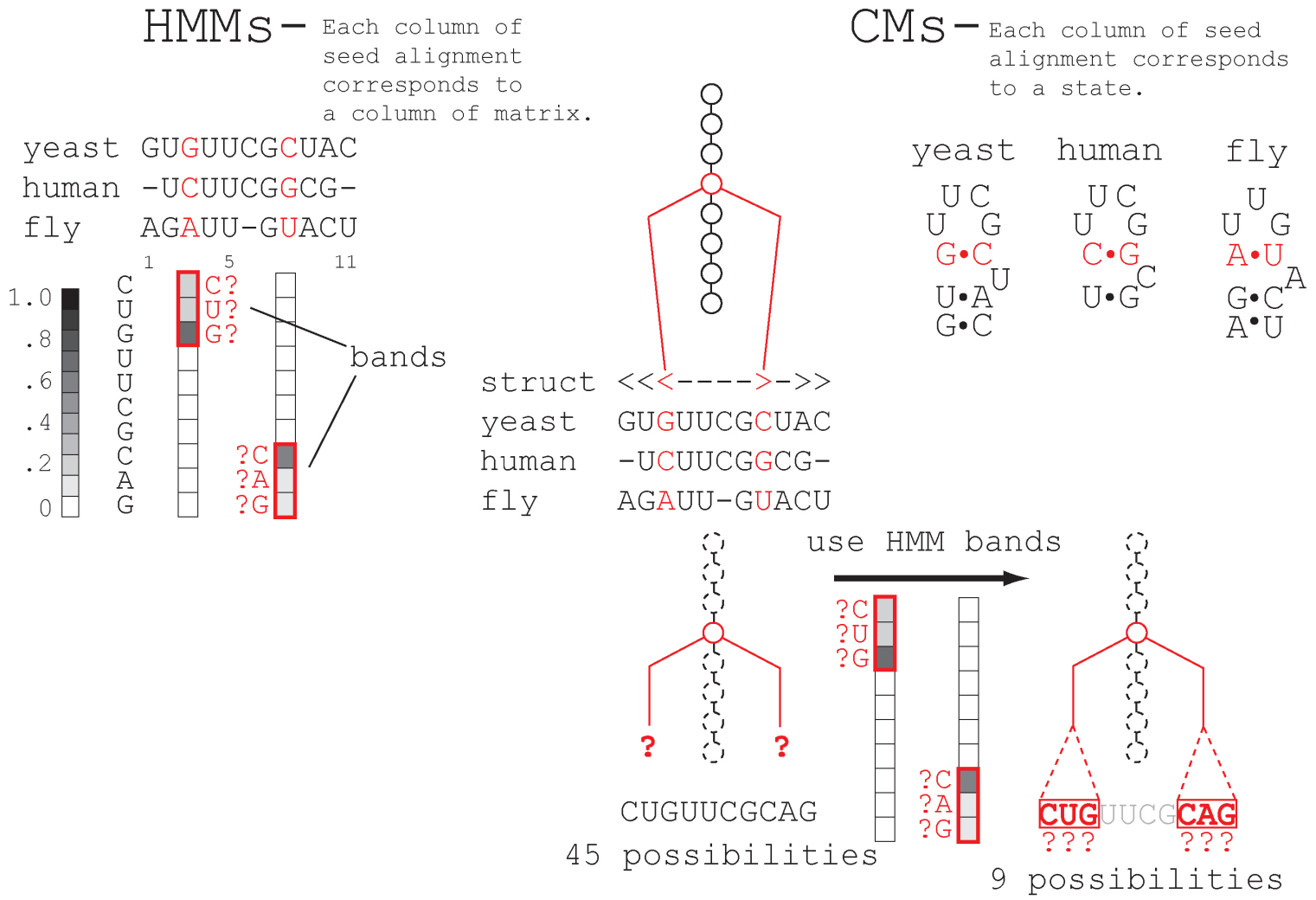
CMs – Each column of seed alignment corresponds to a state.



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

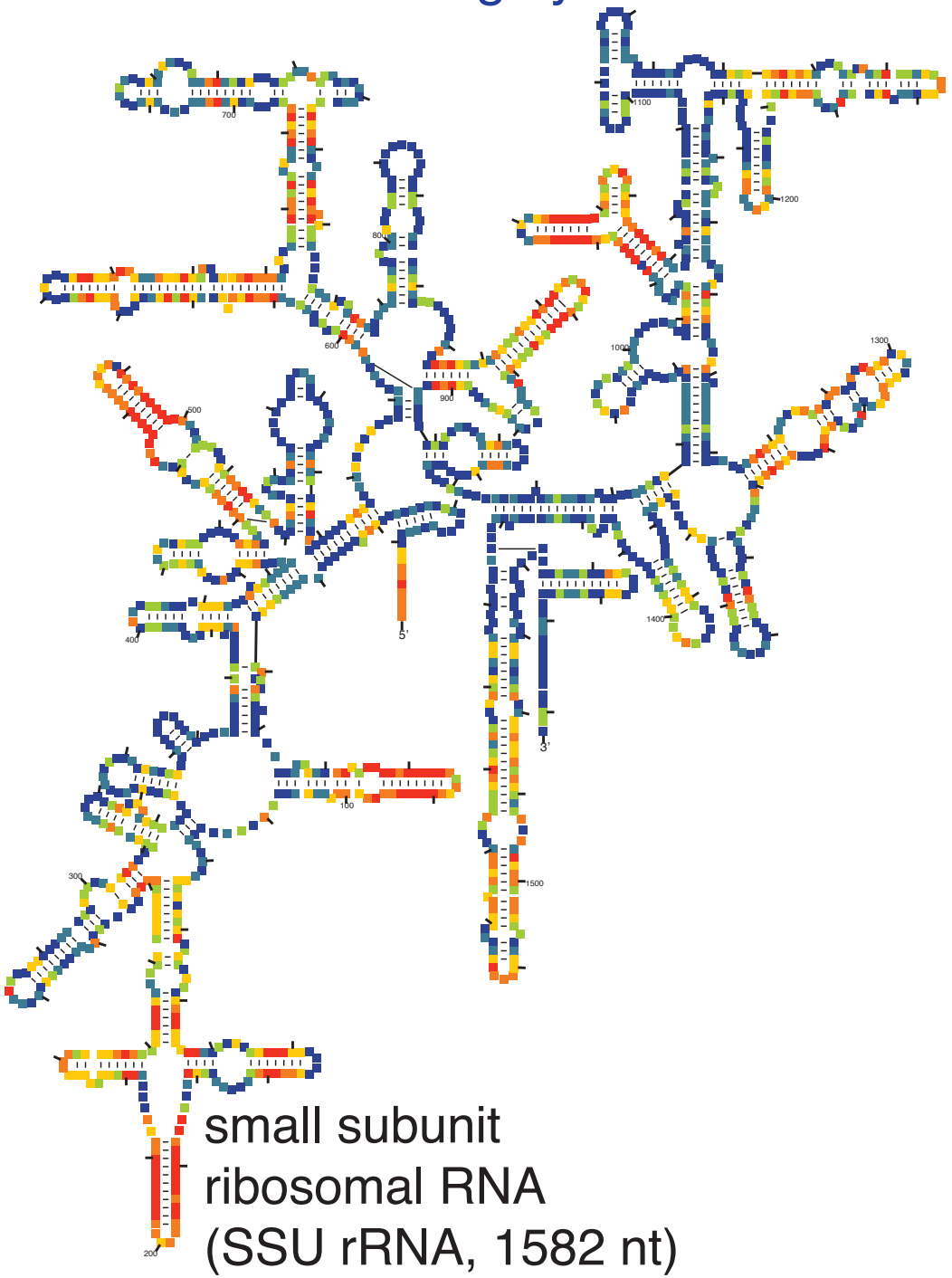


Accelerating CM alignment step 3: use HMM alignment confidence to constrain CM alignment



Sequence conservation per position

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



small subunit
ribosomal RNA
(SSU rRNA, 1582 nt)

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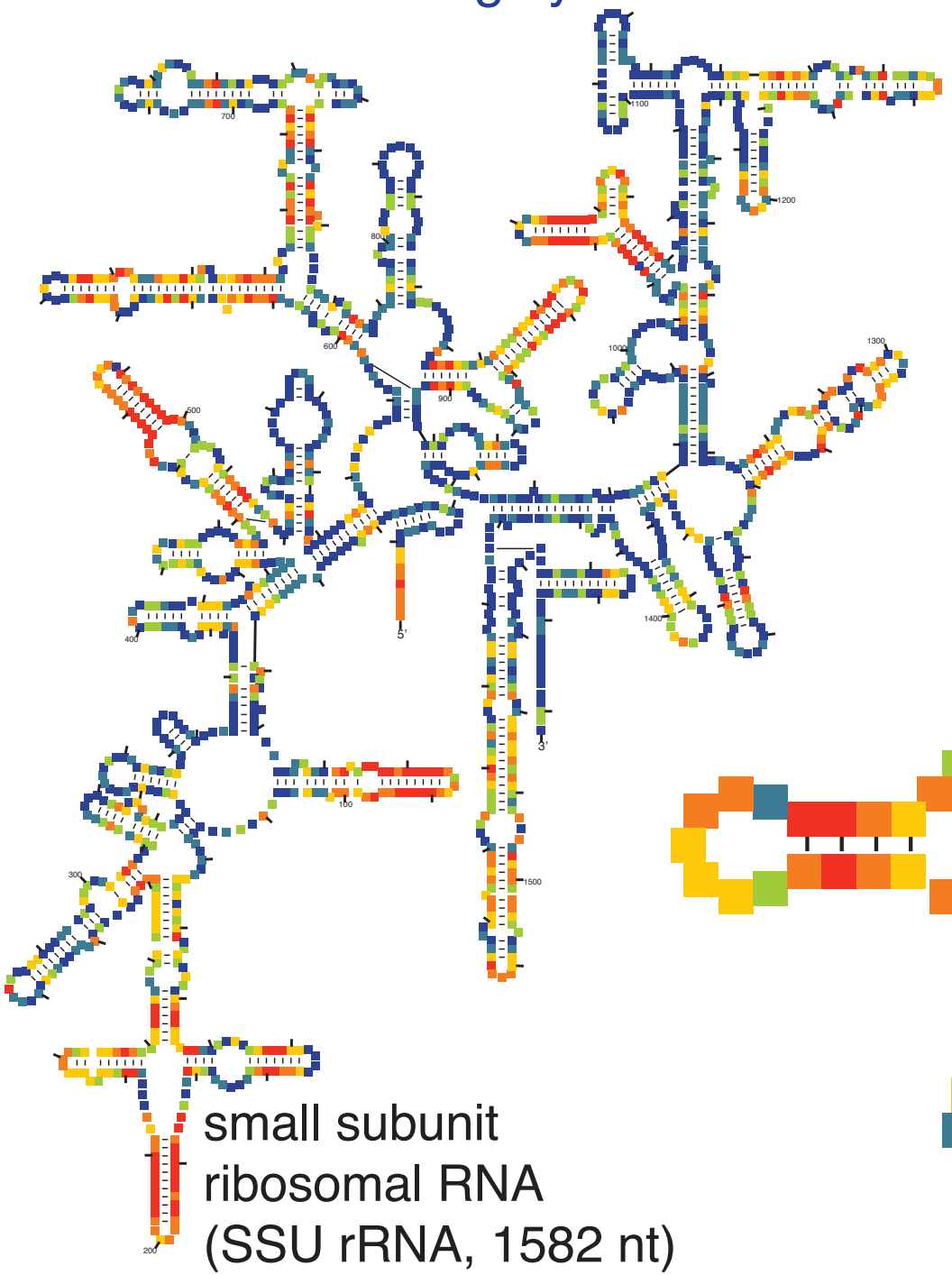
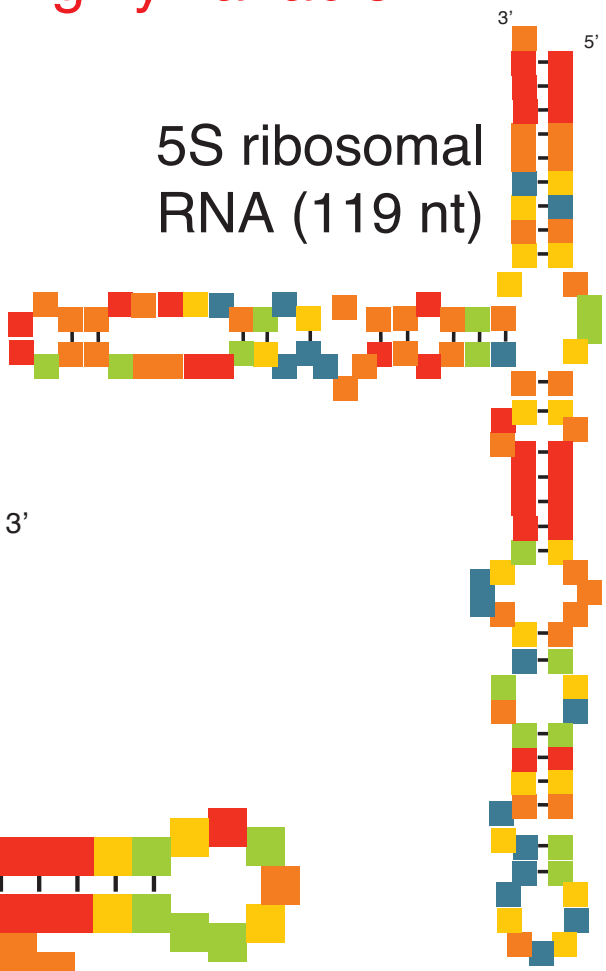


small subunit ribosomal RNA (SSU rRNA, 1582 nt)



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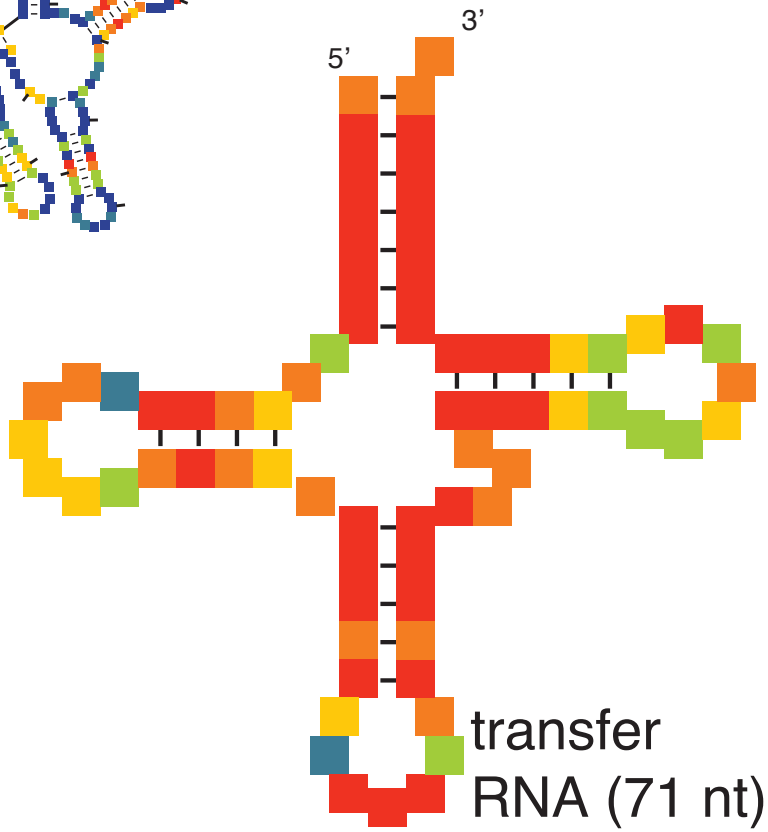
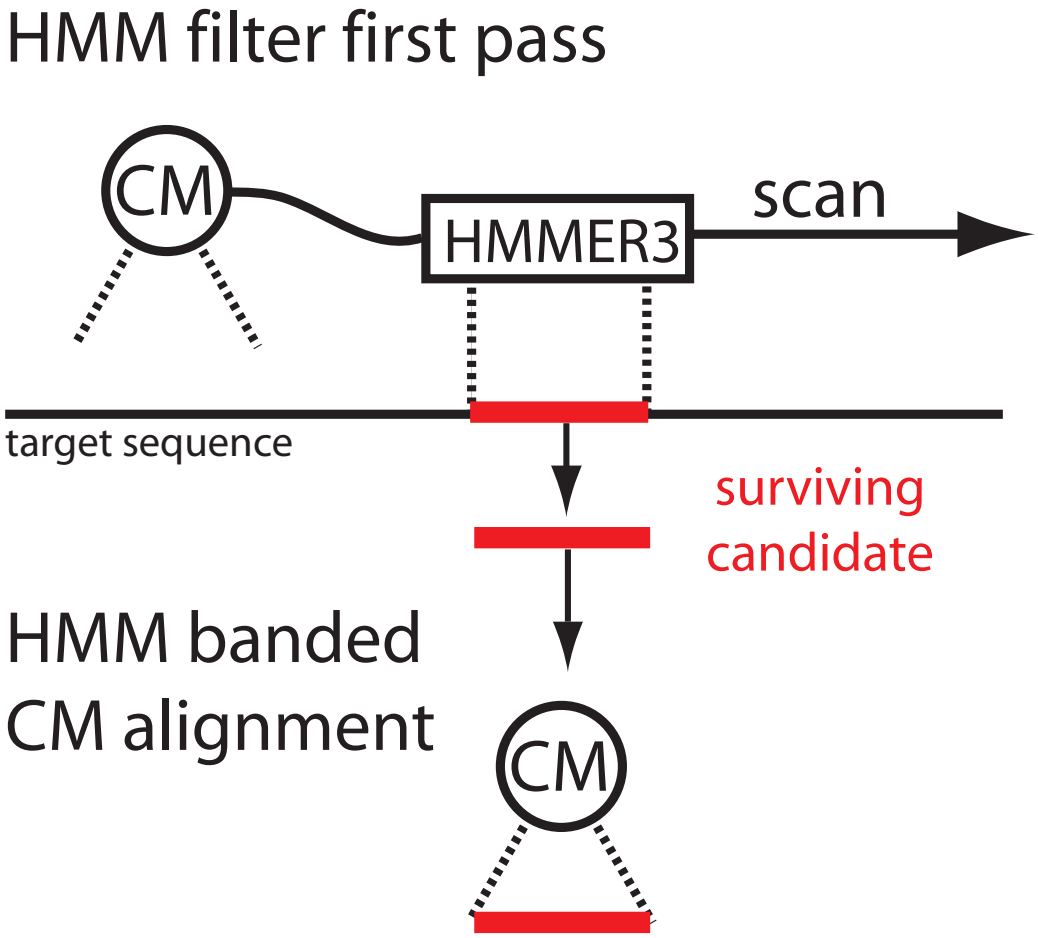
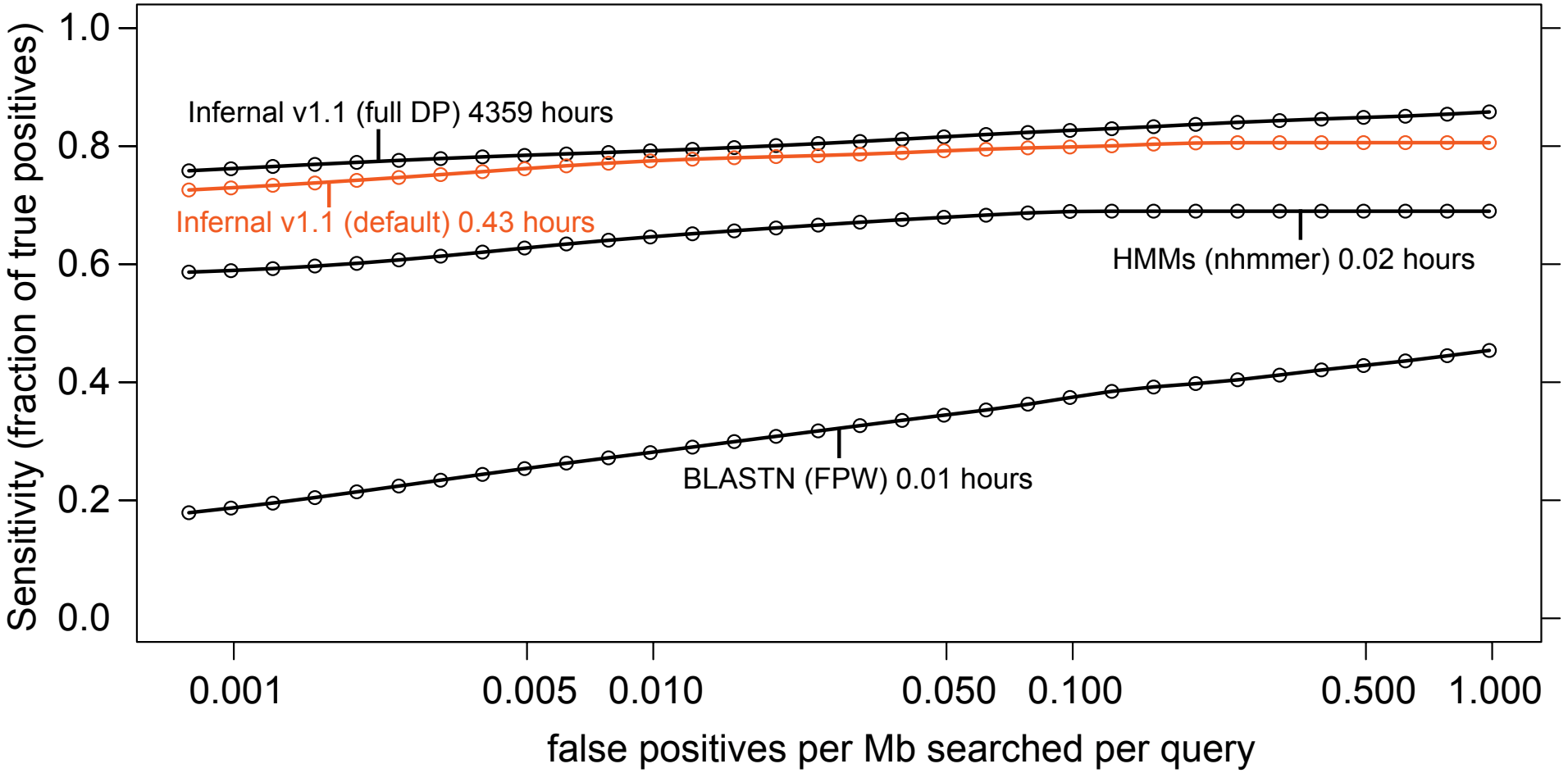


transfer RNA (71 nt)

Use HMMs as filters and to constrain CM alignment



HMM-based acceleration makes Infernal 10,000 times faster



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

Practical structural RNA genome annotation

- Faster Infernal integrated into:
  - NCBI prokaryotic genome annotation pipeline PGAP (Azat Badretdin)
  - NCBI eukaryotic genome annotation (Françoise Thibaud-Nissen)

6614–6624 *Nucleic Acids Research, 2016, Vol. 44, No. 14* *Published online 24 June 2016*
doi: 10.1093/nar/gkw569

NCBI prokaryotic genome annotation pipeline

Tatiana Tatusova[1,†], Michael DiCuccio[1,†], Azat Badretdin[1], Vyacheslav Chetvernin[1], Eric P. Nawrocki[1], Leonid Zaslavsky[1], Alexandre Lomsadze[2], Kim D. Pruitt[1], Mark Borodovsky[2,3,*,‡] and James Ostell[1,‡]

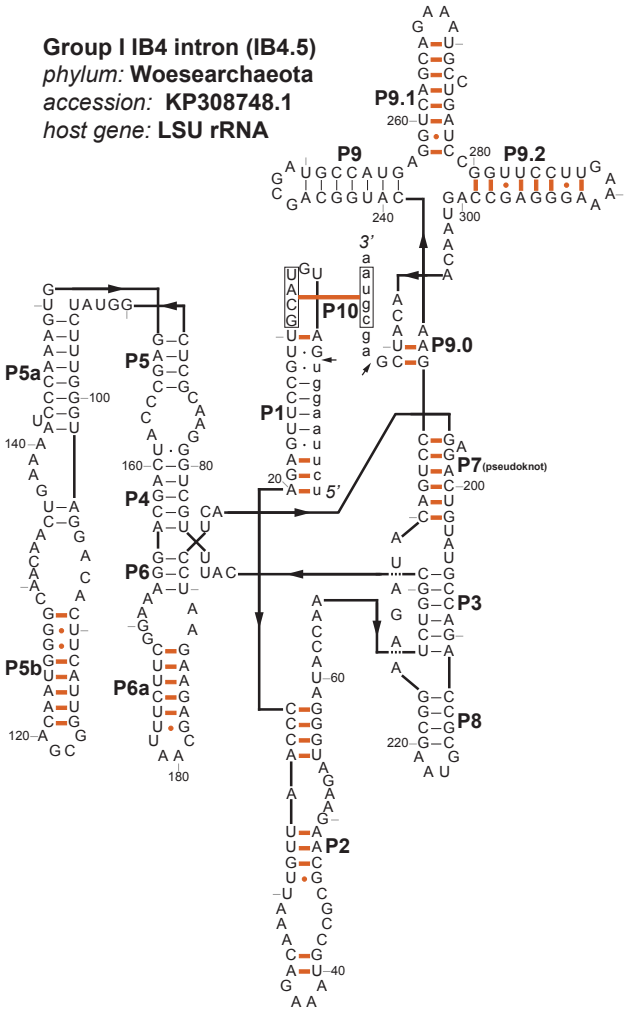
[1]National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, MD 20894, USA, [2]Wallace H. Coulter Department of Biomedical Engineering, Georgia Tech, Atlanta, GA 30332, USA and [3]School of Computational Science and Engineering, Georgia Tech, Atlanta, GA 30332, USA

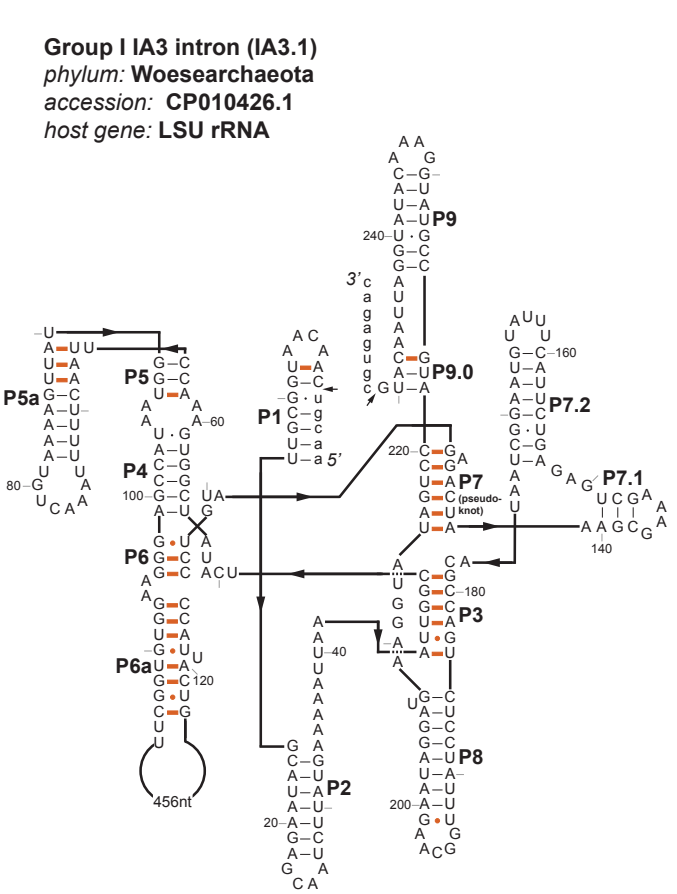
7970–7976 *Nucleic Acids Research, 2018, Vol. 46, No. 15* *Published online 18 May 2018*
doi: 10.1093/nar/gky414

Group I introns are widespread in archaea

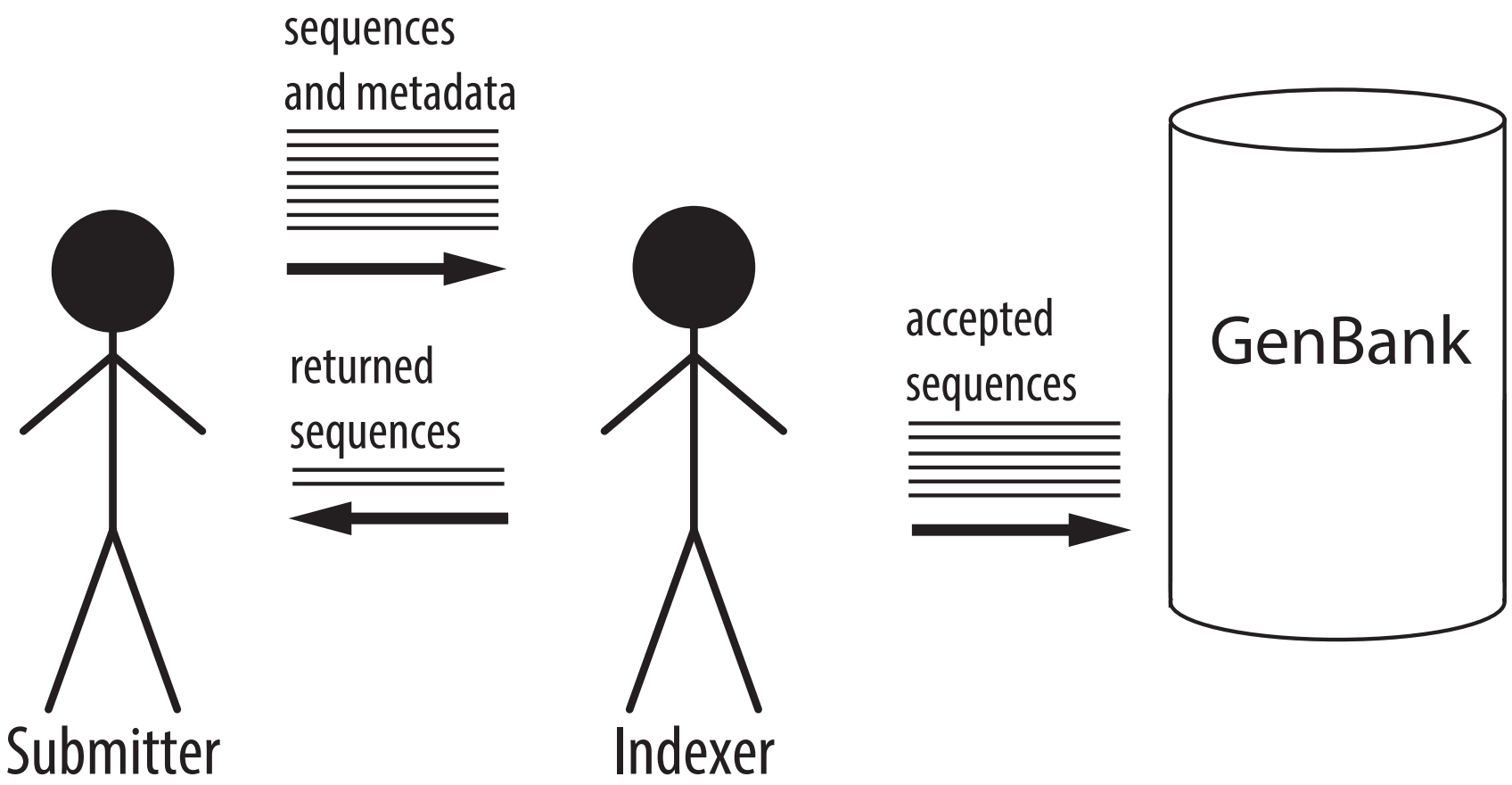
Eric P. Nawrocki[1,*], Thomas A. Jones[2,3] and Sean R. Eddy[2,3,4,*]

[1]National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, MD 20894, USA, [2]Howard Hughes Medical Institute, Harvard University, Cambridge, USA, [3]Department of Molecular and Cellular Biology, Harvard University, Cambridge, USA and [4]School of Engineering and Applied Sciences, Harvard University, Cambridge, USA

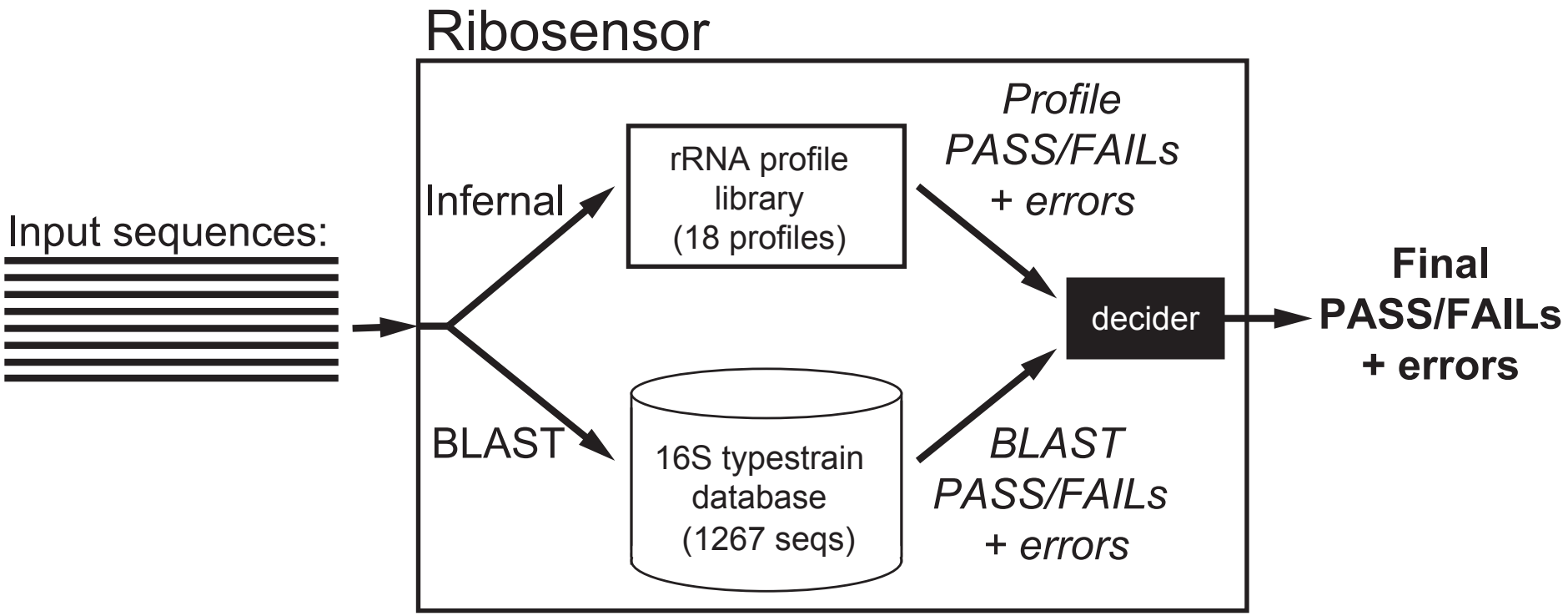




GenBank indexers handle incoming sequence submissions



Ribosensor: a tool for evaluating ribosomal RNA datasets using profiles and BLAST*



- Profile-based analysis:
  - 18 ribosomal RNA models (15 SSU rRNA, 3 LSU rRNA); 8 from Rfam
  - Detects unexpected features ("UnacceptableModel", "DuplicatedRegions", etc.)
- Profile and BLAST results considered together to determine PASS/FAIL

*Alejandro Schäffer developed the BLAST-based scheme

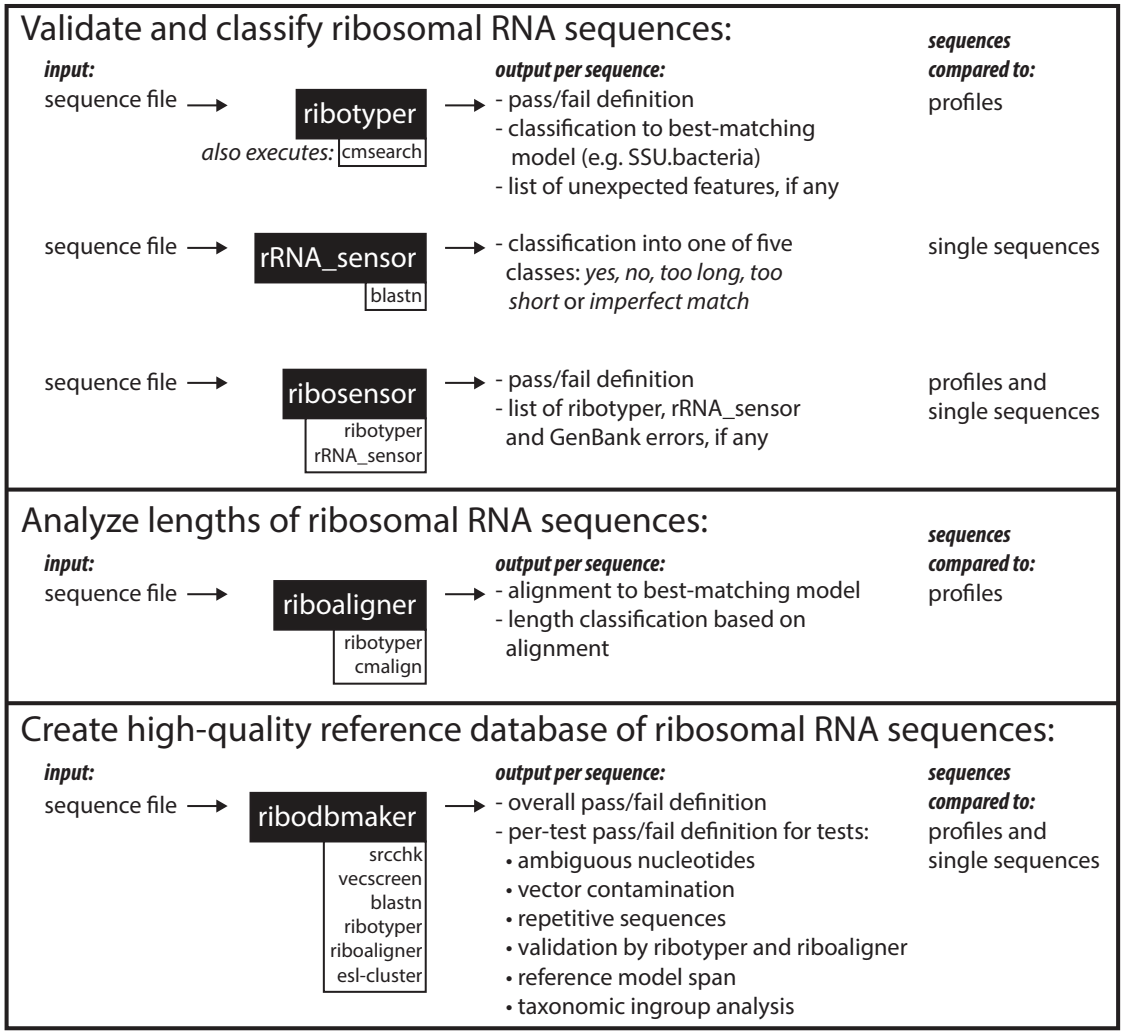
Schäffer *et al. BMC Bioinformatics* (2021) 22:400
https://doi.org/10.1186/s12859-021-04316-z

BMC Bioinformatics

SOFTWARE Open Access

Ribovore: ribosomal RNA sequence analysis for GenBank submissions and database curation

Alejandro A. Schäffer[1,2], Richard McVeigh[2], Barbara Robbertse[2], Conrad L. Schoch[2], Anjanette Johnston[2], Beverly A. Underwood[2], Ilene Karsch-Mizrachi[2] and Eric P. Nawrocki[2*]



Nucleic Acids Research, 2025, **53**, D258–D267
https://doi.org/10.1093/nar/gkae1023
Advance access publication date: 11 November 2024
Database issue

Rfam 15: RNA families database in 2025

Nancy Ontiveros-Palacios [1], Emma Cooke [2], Eric P. Nawrocki [3], Sandra Triebel [4,5], Manja Marz [4,5], Elena Rivas [6], Sam Griffiths-Jones [7], Anton I. Petrov [8], Alex Bateman [1] and Blake Sweeney [1,*]

[1]European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, Hinxton, Cambridge CB10 1SD, UK
[2]SciBite Limited, BioData Innovation Centre, Wellcome Genome Campus, Hinxton, Cambridge CB10 1DR, UK
[3]National Center for Biotechnology Information, U.S. National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA
[4]RNA Bioinformatics and High-Throughput Analysis, Friedrich Schiller University Jena, 07743 Jena, Germany
[5]European Virus Bioinformatics Center, Friedrich Schiller University Jena, 07743 Jena, Germany
[6]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA
[7]School of Biological Sciences, Faculty of Medicine, Biology and Health, Michael Smith Building, The University of Manchester, Dover St, Manchester M13 9NT, UK
[8]Riboscope Ltd, Cambridge CB1 1AH, UK

[*]To whole correspondence should be addressed. Tel: +44 1223 494359; Email: bsweeney@ebi.ac.uk

Published online 5 November 2018 *Nucleic Acids Research, 2019, Vol. 47, Database issue* ***D221–D229***
doi: 10.1093/nar/gky1034

RNAcentral: a hub of information for non-coding RNA sequences

The RNAcentral Consortium[1–38,*]

D212–D220 *Nucleic Acids Research, 2021, Vol. 49, Database issue* *Published online 27 October 2020*
doi: 10.1093/nar/gkaa921

RNAcentral 2021: secondary structure integration, improved sequence search and new member databases

RNAcentral Consortium[1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,*]

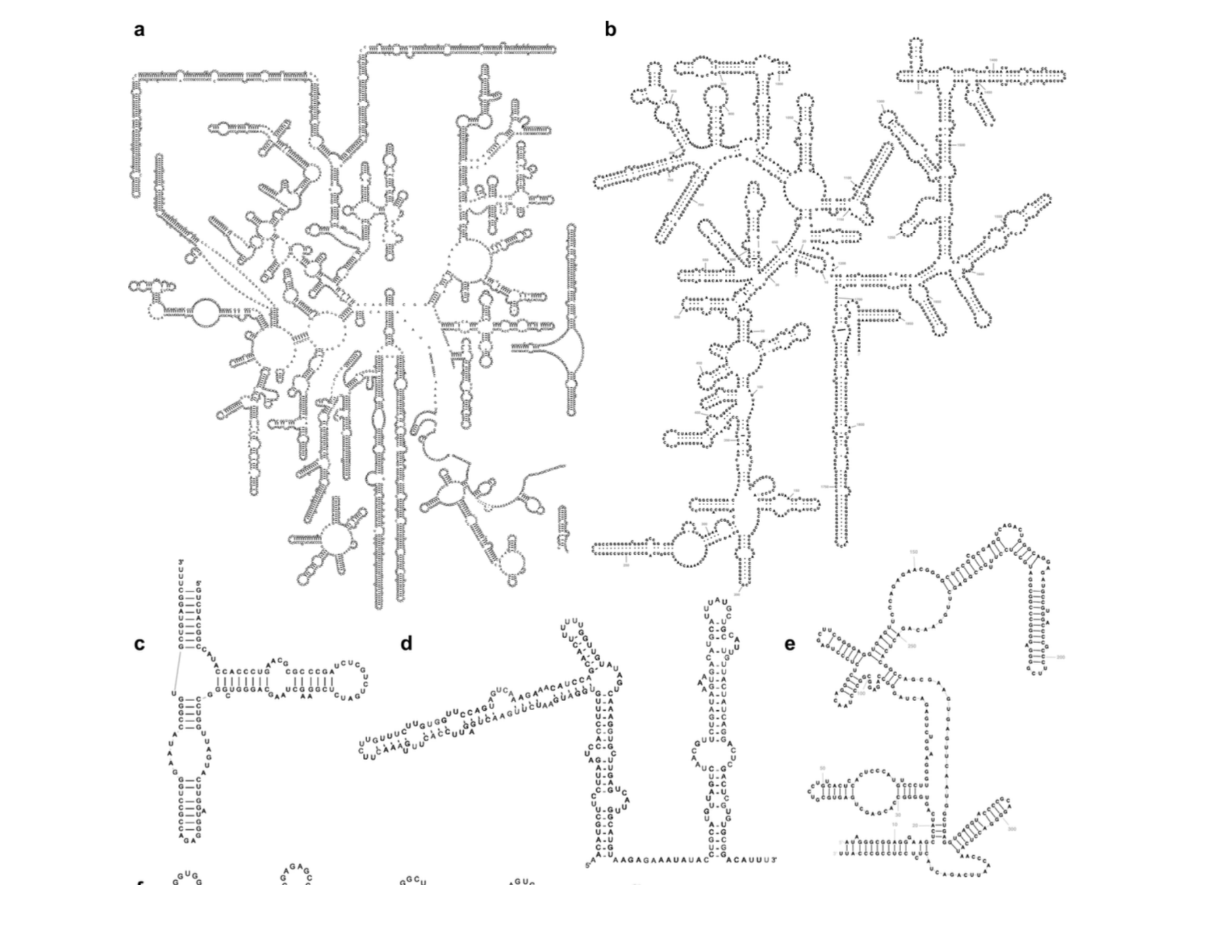
nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-23555-5 OPEN

R2DT is a framework for predicting and visualising RNA secondary structure using templates

Blake A. Sweeney[1,7], David Hoksza[2,7], Eric P. Nawrocki[3], Carlos Eduardo Ribas[1], Fábio Madeira[1], Jamie J. Cannone[4], Robin Gutell[4], Aparna Maddala[5], Caeden D. Meade[5], Loren Dean Williams[5], Anton S. Petrov[5], Patricia P. Chan[6], Todd M. Lowe[6], Robert D. Finn[1,8] & Anton I. Petrov[1,8]✉


a
b
c
d
e

Resource

The genome of the colonial hydroid *Hydractinia* reveals that their stem cells use a toolkit of evolutionarily shared genes with all animals

Christine E. Schnitzler,[1,2] E. Sally Chang,[3,4] Justin Waletich,[1,2] Gonzalo Quiroga-Artigas,[1,2,5] Wai Yee Wong,[6] Anh-Dao Nguyen,[3] Sofia N. Barreira,[3] Liam B. Doonan,[7] Paul Gonzalez,[3] Sergey Koren,[3] James M. Gahan,[7,8] Steven M. Sanders,[9,10] Brian Bradshaw,[7] Timothy Q. DuBuc,[7,11] Febrimarsa,[7,12] Danielle de Jong,[1,2] Eric P. Nawrocki,[4] Alexandra Larson,[1] Samantha Klasfeld,[3] Sebastian G. Gornik,[7,13] R. Travis Moreland,[3] Tyra G. Wolfsberg,[3] Adam M. Phillippy,[3] James C. Mullikin,[3,14] Oleg Simakov,[6] Paulyn Cartwright,[15] Matthew Nicotra,[9,10] Uri Frank,[7] and Andreas D. Baxevanis[3]

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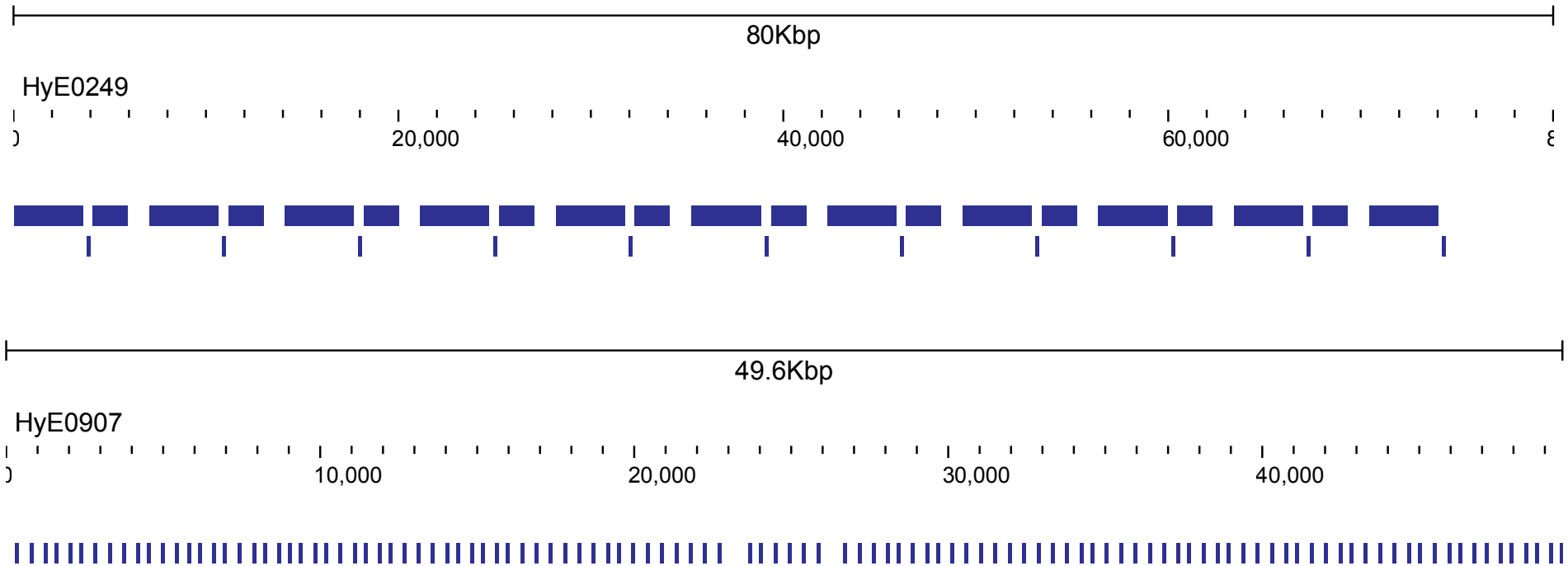

Expansion of the tmRNA sequence database and new tools for search and visualization

Authors

Eric P. Nawrocki[1], Anton I. Petrov[2], Kelly P. Williams[3]

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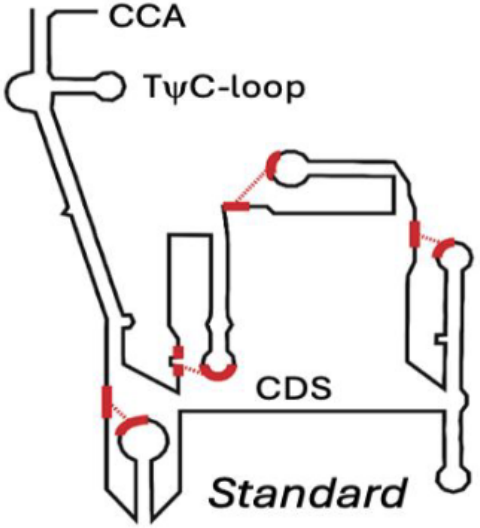
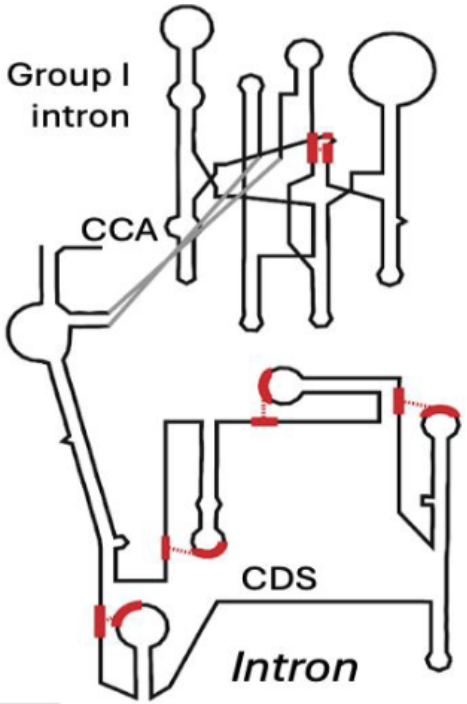
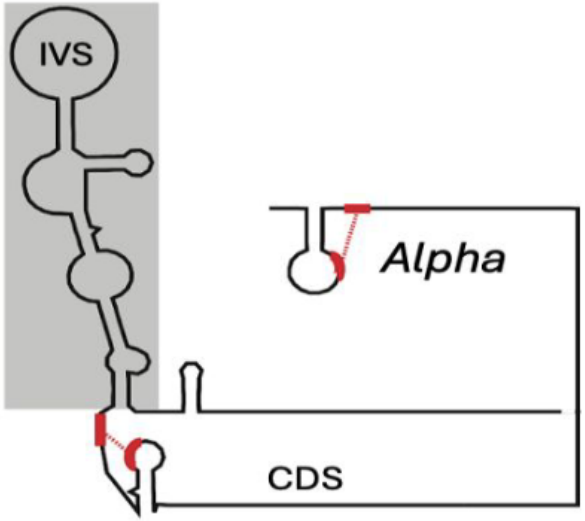
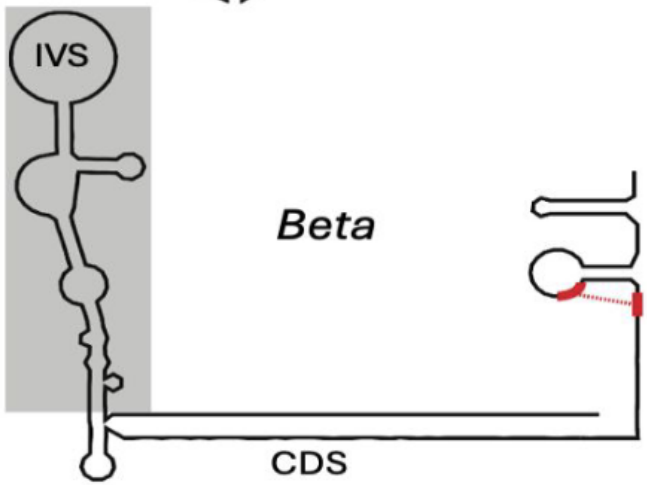


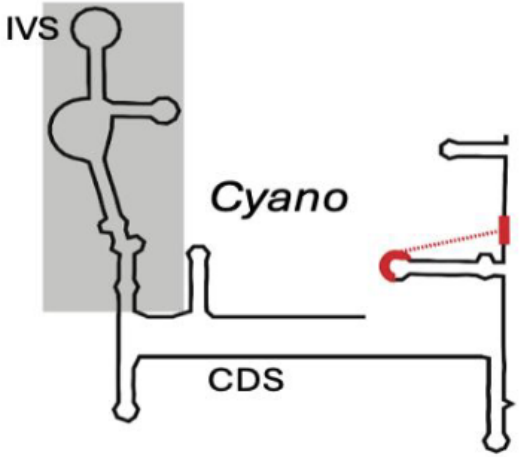


Template characteristics:

Model	Length	Bps	Stems	Pknots
Standard	358	117	12	4
Intron	616	180	22	5
Alpha	355	55	8	2
Beta	331	55	7	1
Cyano	288	72	9	1
Mito	77	19	3	0







Future directions for structural RNA research

- Further development of Infernal
  - iterative search
  - meta-models for clade-specific scoring
- Structural RNA annotation
  - group I introns: improved covariance models for Rfam
  - viral structural RNA annotations by VADR

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Conrad Schoch

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Tatiana Tatusova
Mark Borodovsky

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Diana Kolbe
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Elena Rivas
Michael Farrar

Rfam/RNAcentral
Anton Petrov
Blake Sweeney
Nancy Ontiveros
Kelly Williams
Sam Griffith-Jones
Paul Gardner

NLM - leadership
David Landsman
Richard Scheuermann
Steve Sherry
Kim Pruitt
Jim Ostell
David Lipman

Profile HMMs: sequence family models built from alignments

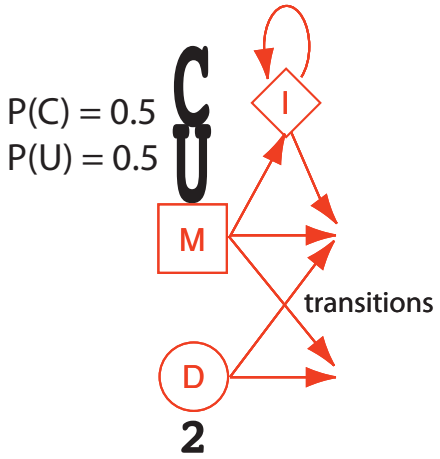
yeast	GUCUUCGGCAC
fly	GCCUUCGGAGC
cow	GCAUUCGUCGC
mouse	GCUUUCGAUGC
human	GCGUUCGCUGC
chicken	GUAUUCGUAAC
snake	GUGUUCGCGAC
croc	GUUUUCGAGAC

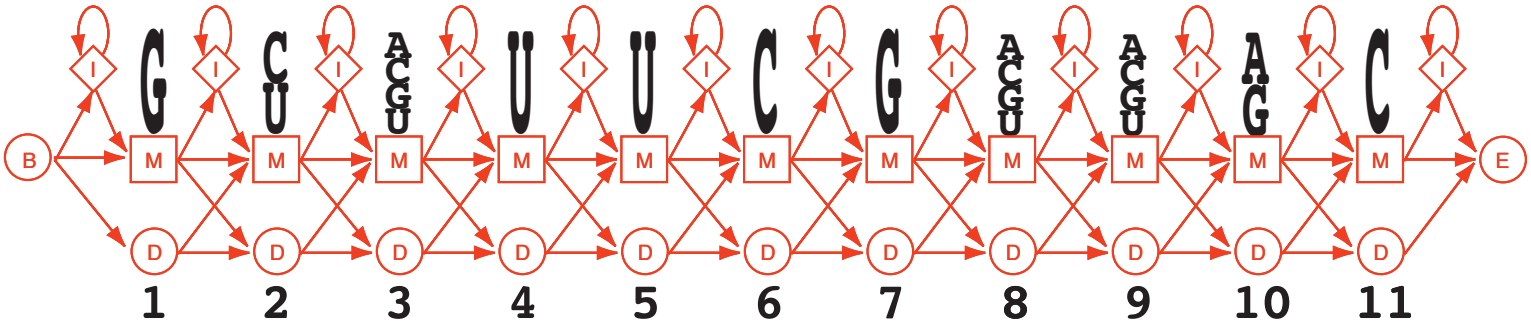
One HMM node per alignment column

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

HMMs generate homologous sequences.

Node for column 2:





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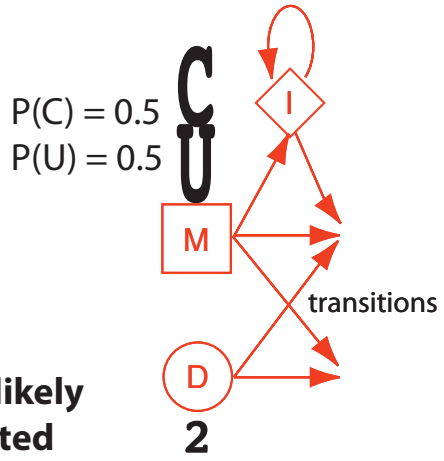

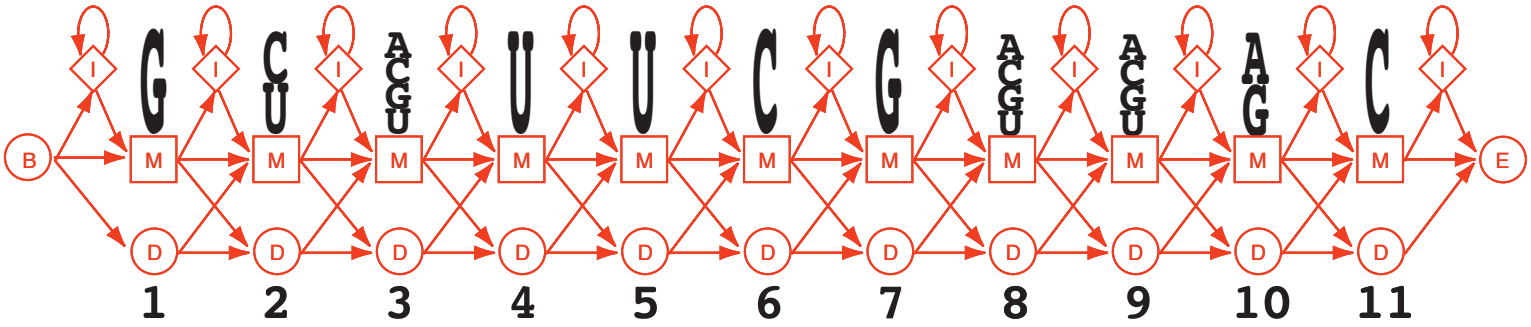

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croc	GUUUUCGAGAC
worm	GCGUUCGCGGC

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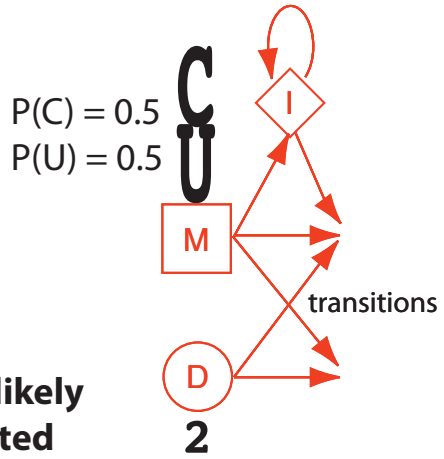

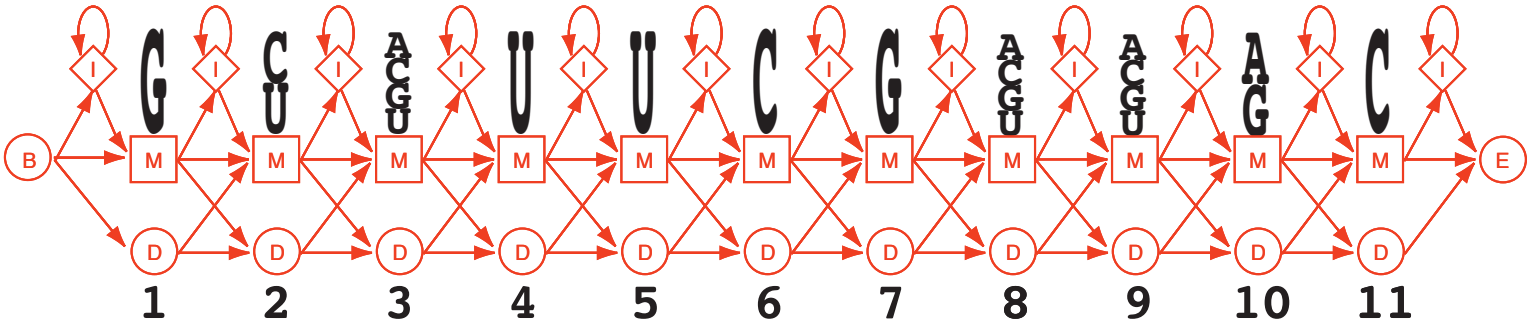

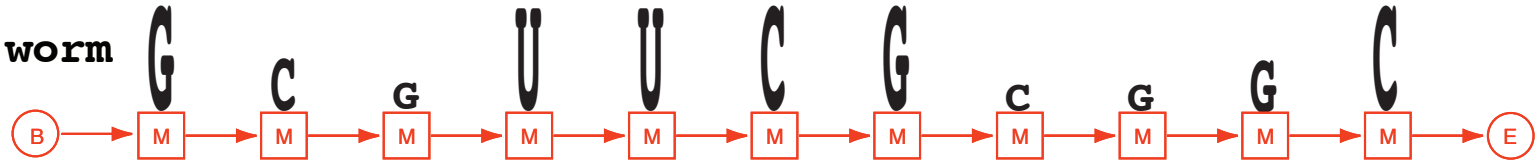

Profile HMMs: sequence family models built from alignments


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

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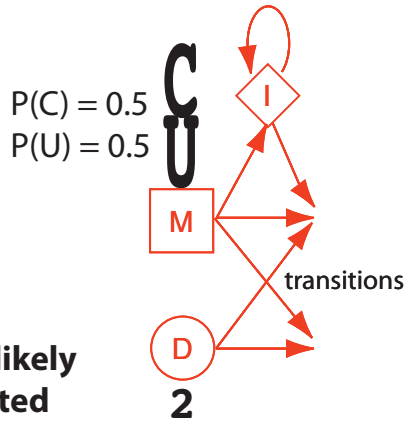
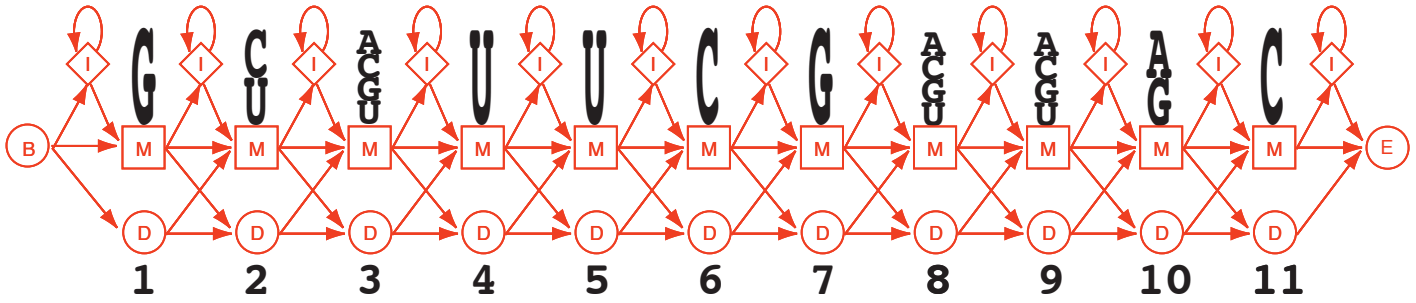
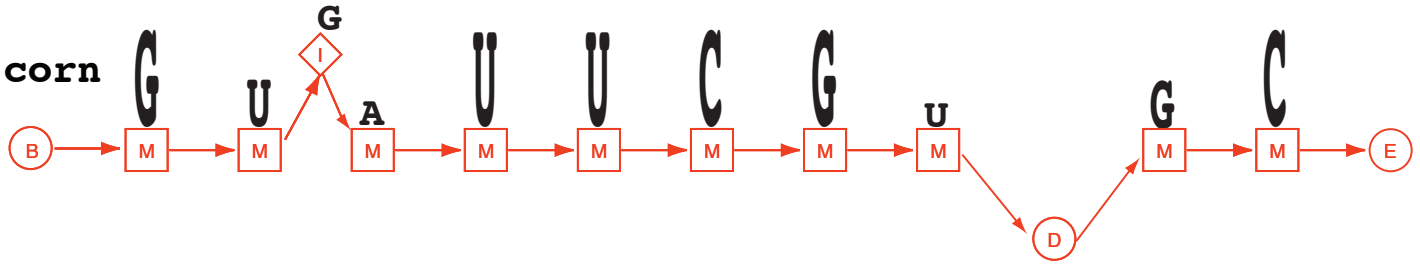
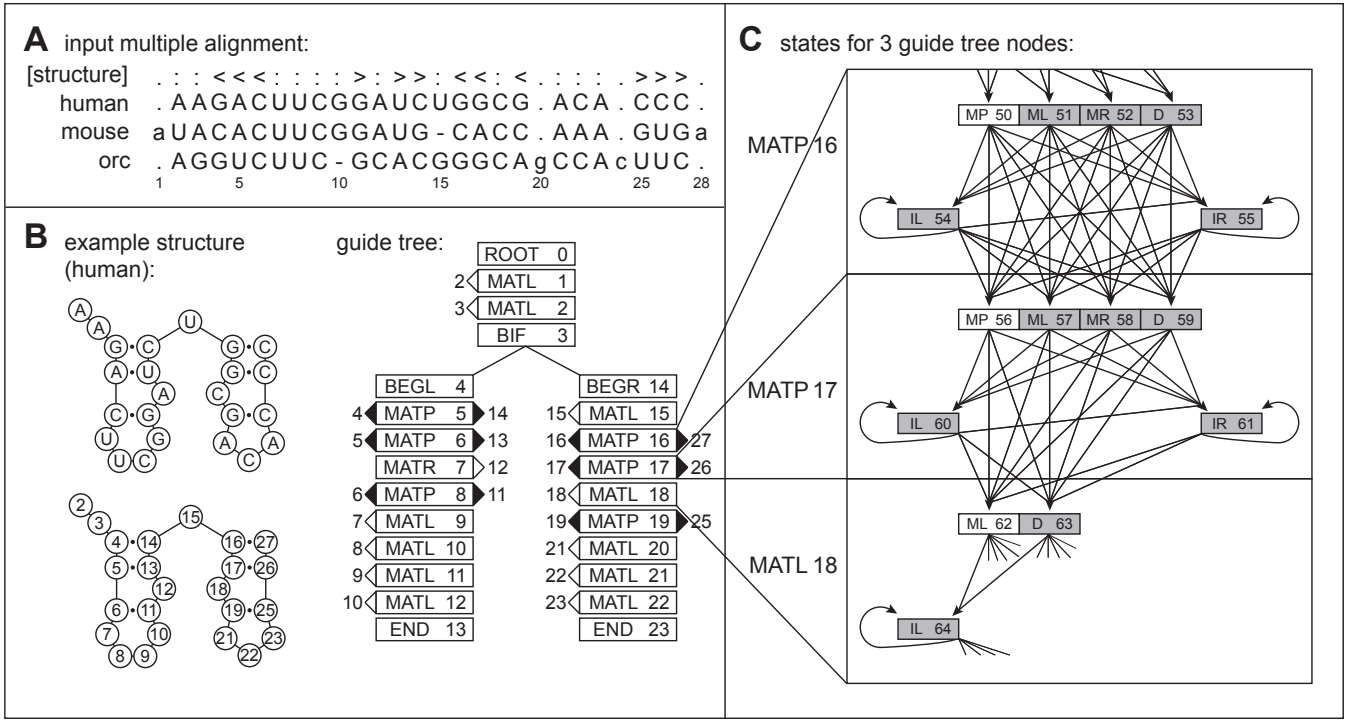
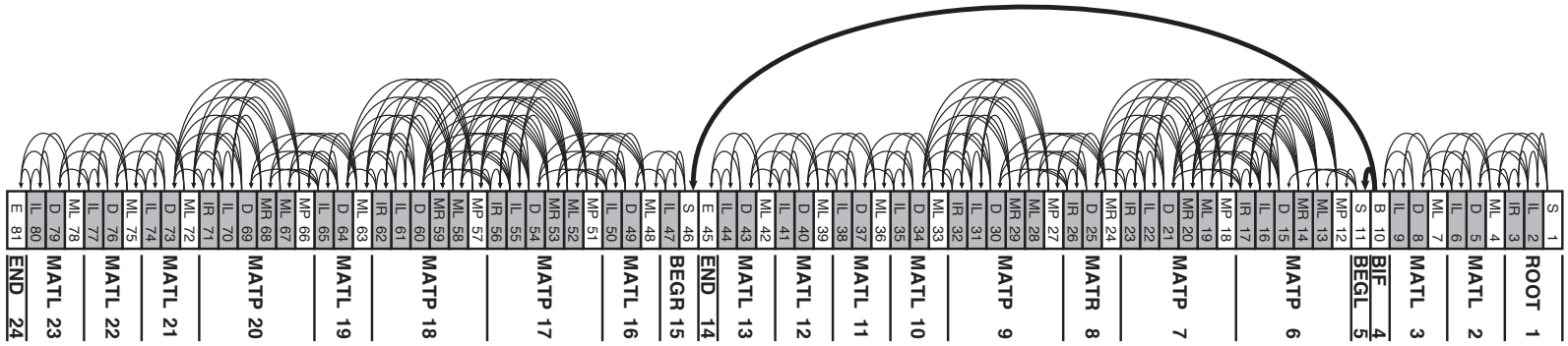






Covariance models (CMs) are built from structure-annotated alignments





Is the added complexity worth it?

RMARK: a challenging internal RNA homology search benchmark

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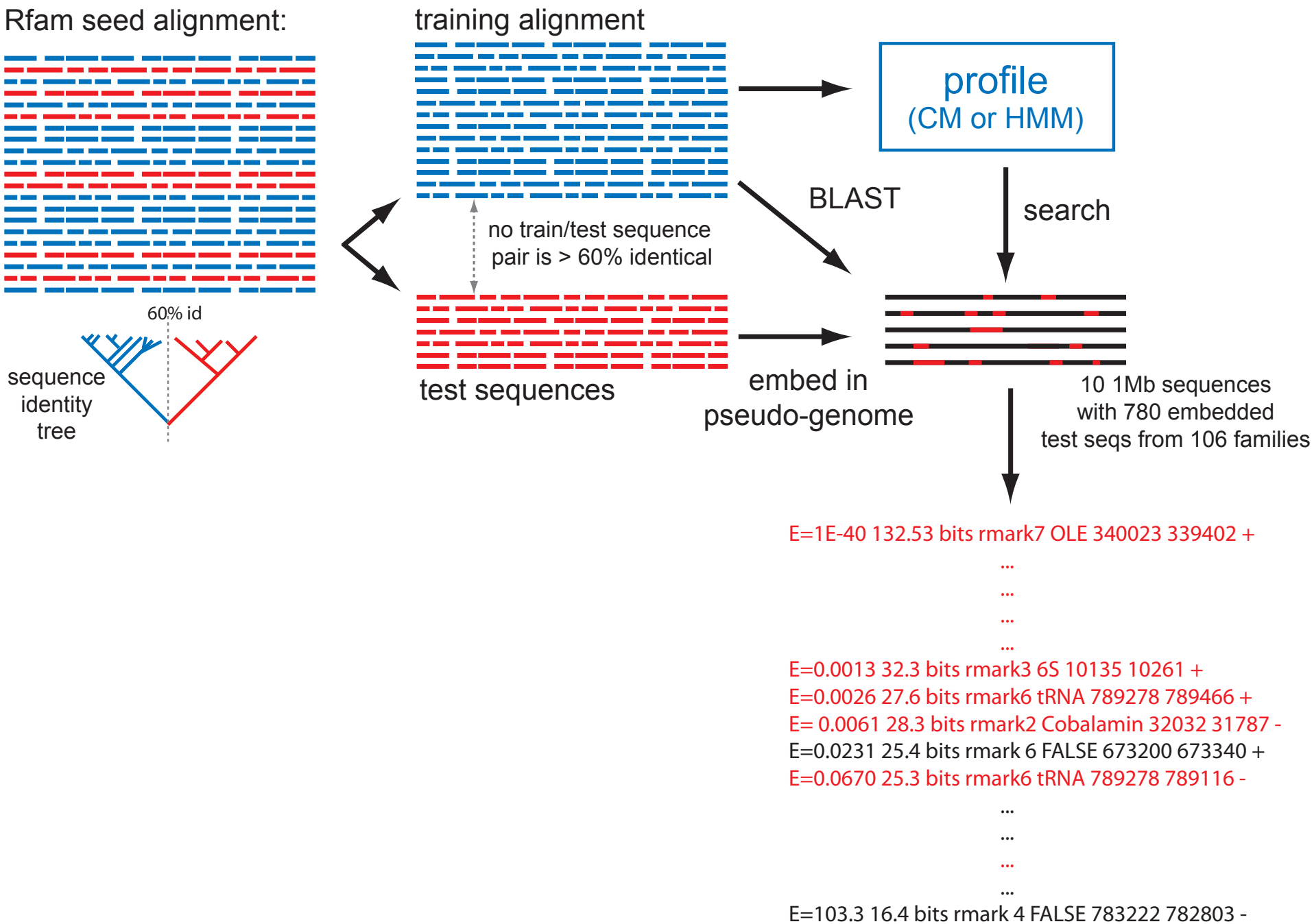
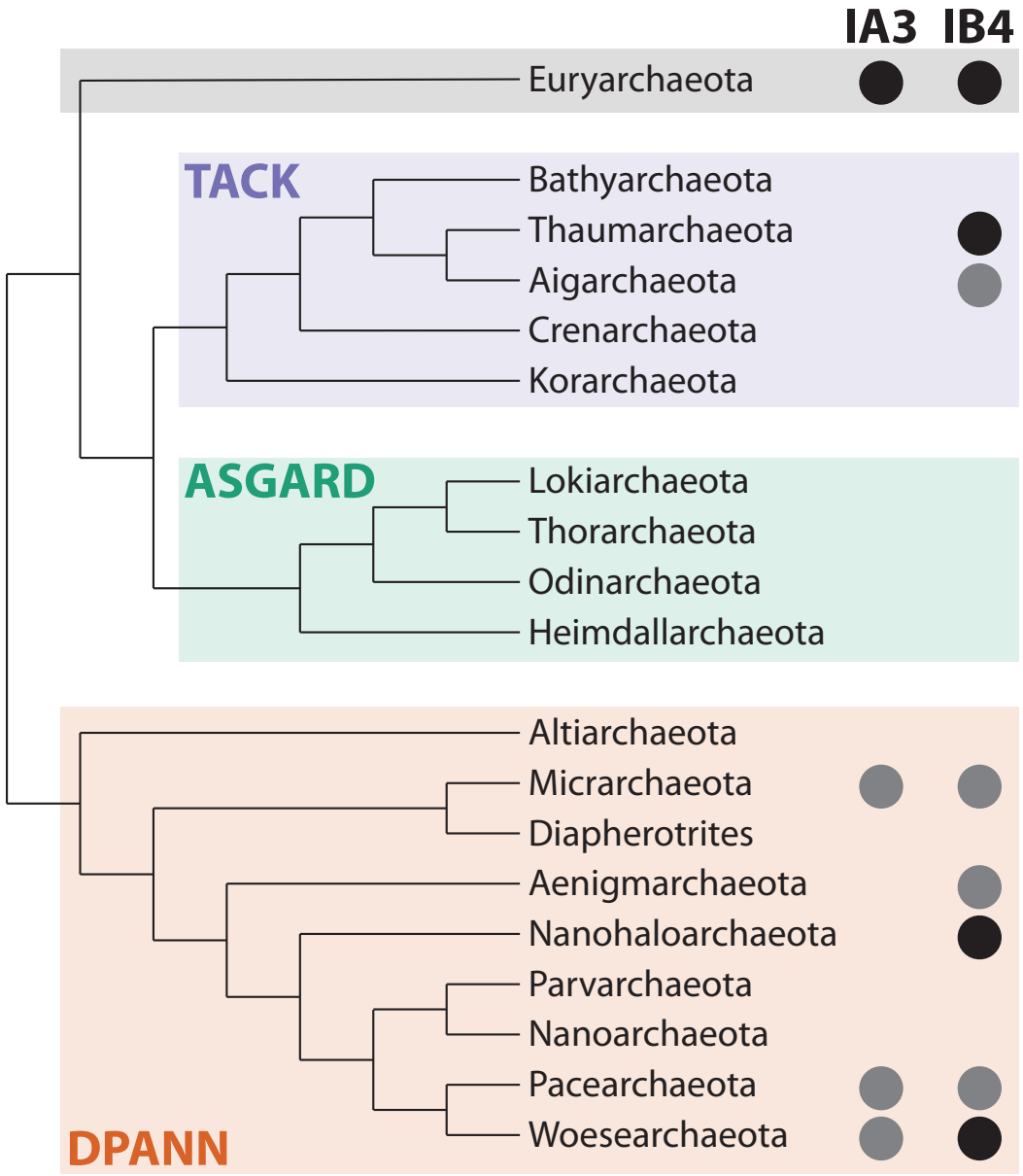


Group I introns are widespread in Archaea



Could archaeal group I introns have evolved into BHB introns?

Evolution of introns in the archaeal world

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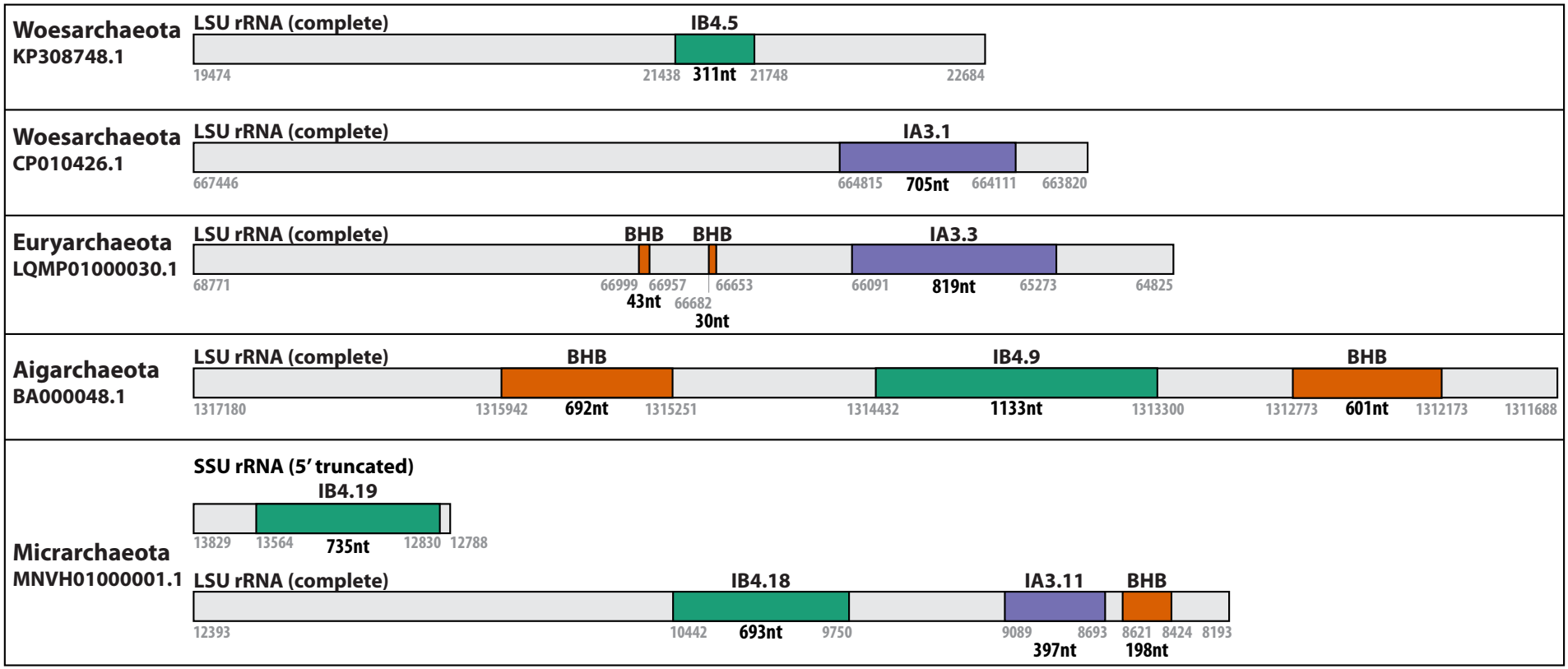
Contributed by Glauco P. Tocchini-Valentini, January 24, 2011 (sent for review December 1, 2010)

*

Archaeal group I introns can occur in same host gene as BHB introns



*PNAS March 22, 2011. 108 (12) 4782-4787;

Levels of sequence and structure conservation in RNA families