

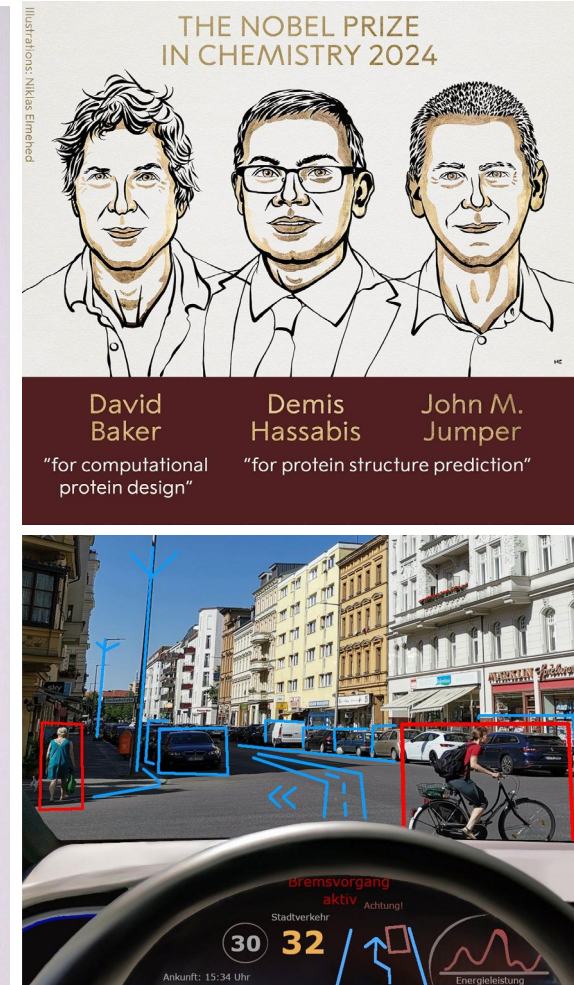
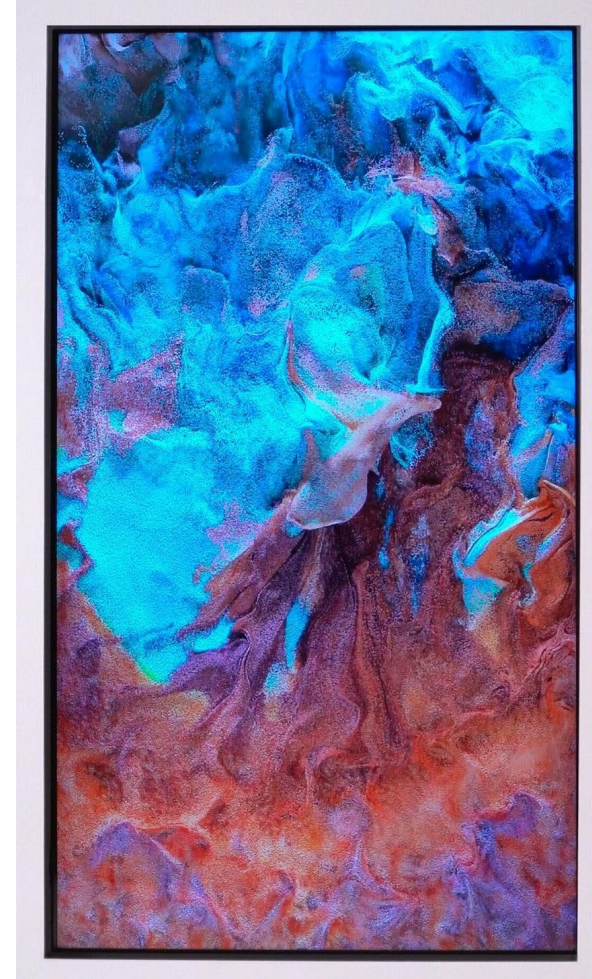
A machine-learning based alternative to phylogenetic bootstrap

Tal Pupko,
The Shmunis School of Biomedicine and Cancer
Research

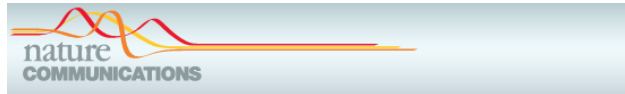
IMSI, Aug 12 2025
Chicago, USA

AI revolution is transforming the world

- Large Language Models, e.g., ChatGPT
- Autonomous systems (self-driving cars, drones)
- Creativity - first Christie's AI art sale
- Drug discovery and health care, AlphaFold2



Our research aims to harness AI to improve phylogenetic inference



ARTICLE

<https://doi.org/10.1038/s41467-021-22073-8> OPEN

Harnessing machine learning to guide phylogenetic-tree search algorithms

Dana Azouri^{1,2}, Shiran Abadi¹, Yishay Mansour³, Itay Mayrose^{1,5} & Tal Pupko^{1,2,5}

Bioinformatics, 38, 2022, i118–i124
<https://doi.org/10.1093/bioinformatics/btad252>
ISCB/ISMB 2022



A LASSO-based approach to sample sites for phylogenetic tree search

Noa Ecker¹, Dana Azouri^{1,2}, Ben Bettisworth^{3,4}, Alexandros Stamatakis^{3,4}, Yishay Mansour⁵, Itay Mayrose^{2,*} and Tal Pupko^{1,*}

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Abstract

Bioinformatics, 2025, 41(1), btaf009
<https://doi.org/10.1093/bioinformatics/btaf009>
Advance Access Publication Date: 8 January 2025
Original Paper



Phylogenetics

BetaAlign: a deep learning approach for multiple sequence alignment

Edo Dotan^{1,2}, Elya Wygoda¹, Noa Ecker¹, Michael Albuquerque¹, Oren Avram^{1,3}, Yonatan Belinkov^{2,*}, Tal Pupko^{1,*}

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³The Department of Computer Science, University of California Los Angeles, Los Angeles, CA 90095, United States

ModelTeller: Model Selection for Optimal Phylogenetic Reconstruction Using Machine Learning

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²School of Molecular Cell Biology & Biotechnology, Tel-Aviv University, Tel-Aviv, Israel
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Associate editor: Li Liu

Abstract

Statistical criteria have long been the standard for selecting the best model for phylogenetic reconstruction and downstream statistical inference. Although model selection is regarded as a fundamental step in phylogenetics, existing methods for this task consume computational resources for long processing time, they are not always feasible, and sometimes depend on preliminary assumptions which do not hold for sequence data. Moreover, although these methods are dedicated to revealing the processes that underlie the sequence data, they do not always produce the most accurate trees. Notably, phylogeny reconstruction consists of two related tasks, topology reconstruction and branch-length estimation. It was previously shown that in many cases the most complex model, GTR+I+G, leads to topologies that are as accurate as using existing model selection criteria, but overestimates branch lengths. Here, we present ModelTeller, a computational methodology for phylogenetic model selection, devised within the machine-learning framework, anti-

The Tree Reconstruction Game: Phylogenetic Reconstruction Using Reinforcement Learning

Dana Azouri^{1,2,†}, Oz Granit^{1,3,†}, Michael Albuquerque¹, Yishay Mansour^{1,3,*}, Tal Pupko^{1,2,*} and Itay Mayrose^{1,*}

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[†]These authors contributed equally.

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Associate editor: Andrey Rzhetsky

Abstract

The computational search for the maximum-likelihood phylogenetic tree is an NP-hard problem. As such, current tree search algorithms might result in a tree that is the local optima, not the global one. Here, we introduce a paradigm shift for predicting the maximum-likelihood tree, by approximating long-term gains of likelihood rather than

Bioinformatics, 2024, 40, 1095–1117
<https://doi.org/10.1093/bioinformatics/btad255>
ISMB 2024



A machine-learning-based alternative to phylogenetic bootstrap

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³The Shmuni School of Natural History and National Research Center, Tel Aviv University, Tel Aviv 6997801, Israel
⁴The Blavatnik School of Computer Science, Raymond & Beverly Sackler Faculty of Exact Sciences, Tel Aviv University, Tel Aviv 6997801, Israel
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Abstract

Motivation: Currently used methods for estimating branch support in phylogenetic analyses often rely on the classic Felsenstein's bootstrap, parametric tests, or their approximations. As these branch support scores are widely used in phylogenetic analyses, having accurate, fast, and interpretable scores is of high importance.
Results: Here, we employed a data-driven approach to estimate branch support values with a probabilistic interpretation. To this end, we simulated thousands of realistic phylogenetic trees and the corresponding multiple sequence alignments. Each of the obtained alignments was used to infer the phylogeny using state-of-the-art phylogenetic inference software, which was then compared to the true tree. Using these extensive data, we trained machine-learning algorithms to estimate branch support values for each bipartition within the maximum-likelihood trees.

Part 1:

Bioinformatics, 2024, 40, i208–i217
<https://doi.org/10.1093/bioinformatics/btae255>
ISMB 2024

OXFORD

A machine-learning-based alternative to phylogenetic bootstrap

Noa Ecker¹, Dorothee Huchon ^{2,3}, Yishay Mansour ⁴, Itay Mayrose ⁵, Tal Pupko ^{1,*}

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Abstract

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Phylogenetic-based tools and algorithms



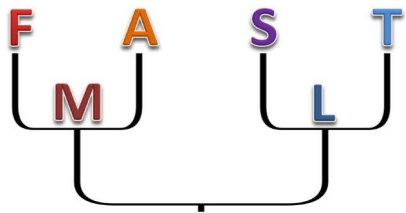
The GUIDANCE2 Server

Server for alignment confidence score

[HOME](#) [OVERVIEW](#) [GALLERY](#) [SOURCE CODE](#) [CITING & CREDITS](#) [CONTACT US](#)

M1CR0B1AL1Z3R 

A web server for analyzing bacterial genomics data. Easily.

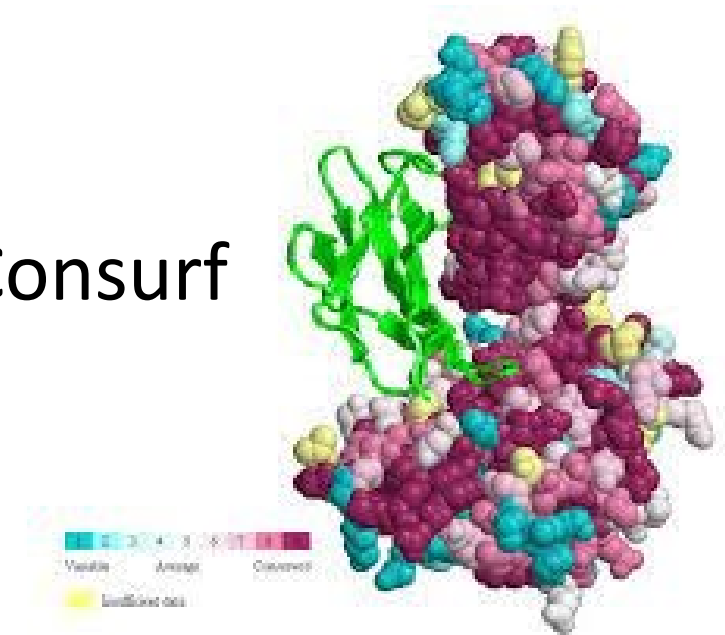


The FASTML Server

Server for computing Maximum Likelihood
ancestral sequence reconstruction

[HOME](#) [OVERVIEW](#) [GALLERY](#) [SOURCE CODE](#) [CITING & CREDITS](#)

Consurf

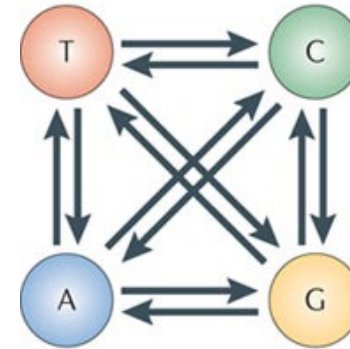


The tree score = log-likelihood

T = Tree



M = Evolutionary model



Tree likelihood

$P(D | M, T)$: The conditional probability of the data, given the model, M , and the tree, T

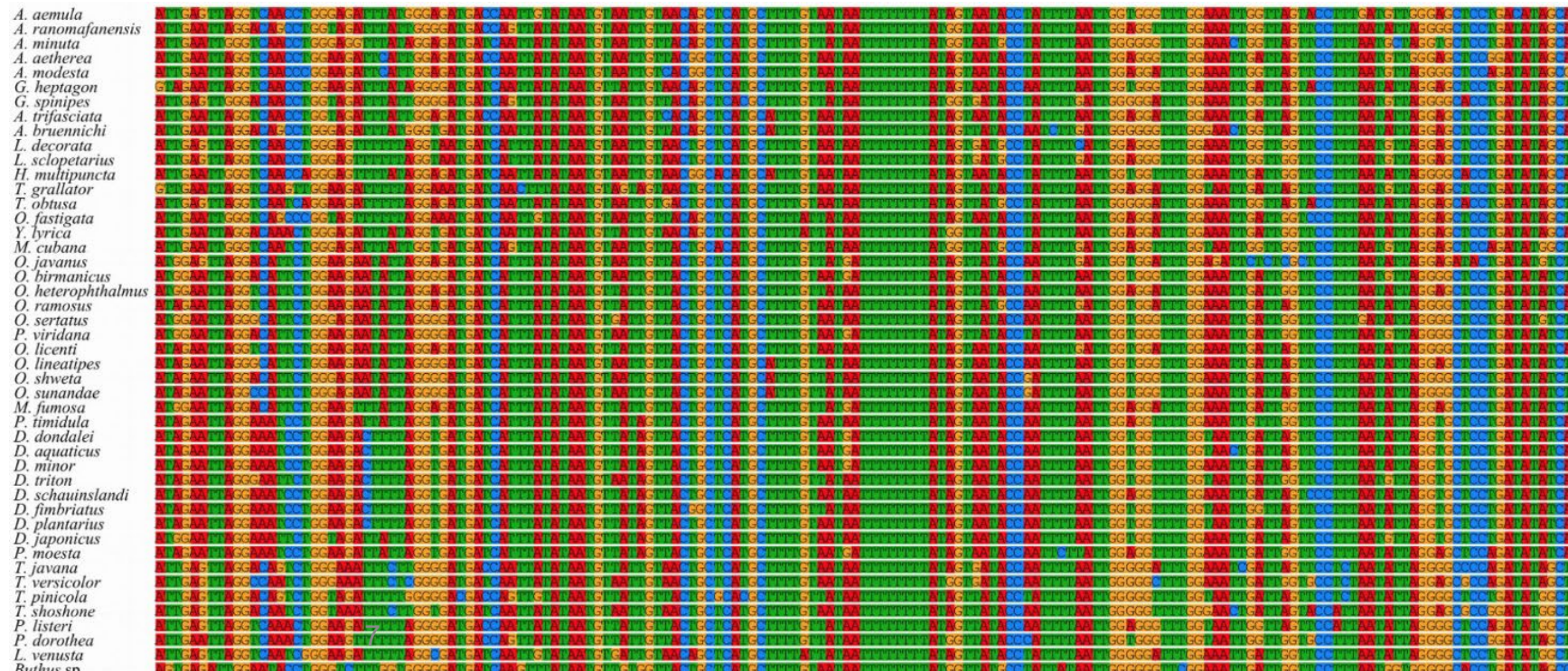
D = Data = Alignment

Acanthocalyx_albus	tcgaaacttg	cccagcagag	cgaccagcga	acacctccgt
Abelia_spathulata	tcgaaacctg	cacagcagaa	cgacccgcga	acacgttcgt
Abelia_engleriana	tcgaaacctg	cacagcagaa	cgacccgcga	acacgttcgt
Abelia_chinensis	tcgaaacctg	cacagcagaa	cgacccgcga	acacgttcgt
Abelia_parvifolia	-----g	cacagcagaa	cgacccgcga	acacgttcgt
Abelia_Agrandiflora	tcgaaacctg	cacagcagaa	cgacccgcga	acacgttcgt
Abelia_mexicana	-tcgaaactg	cacagcagaa	cgacccgcga	acacgttcgt
Abelia_occidentalis	tcgaaacctg	cacagcagaa	cgacccgcga	acacgttcgt

Finding trees keeps becoming more difficult

Today:

- Data: Aligned genomic sequences
- Size: Up to GB of DNA sites, thousands of species

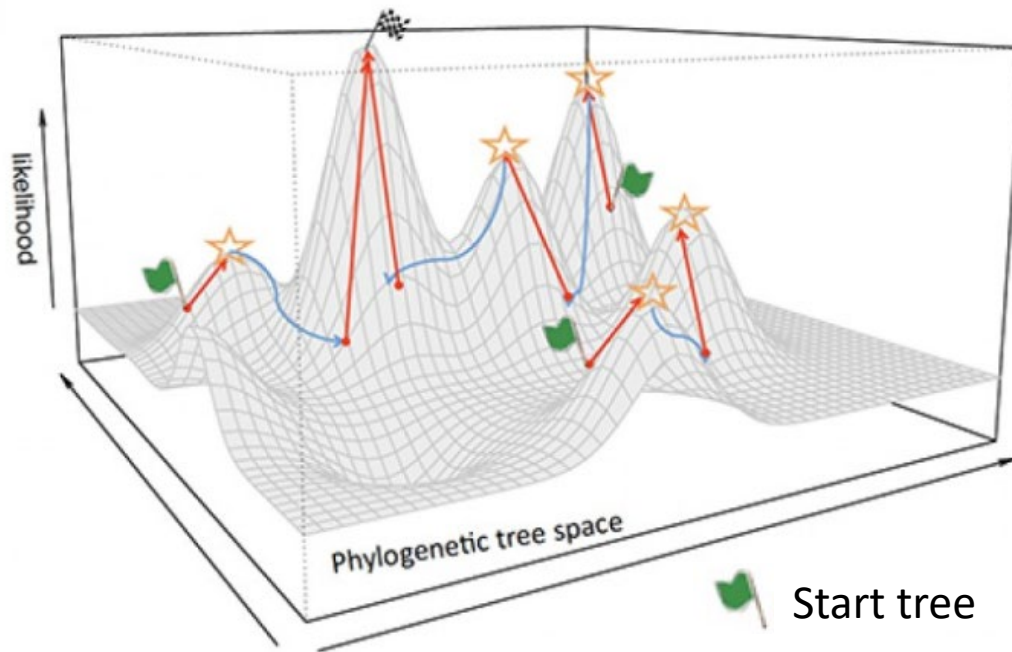


The tree space is huge

Number of Taxa	Number of rooted trees
3	3
4	15
5	105
6	945
7	10,395
8	135,135
9	2,027,025
10	34,459,425
20	8.20×10^{21}
30	4.95×10^{38}
40	1.01×10^{57}
50	2.75×10^{76}

Heuristic search

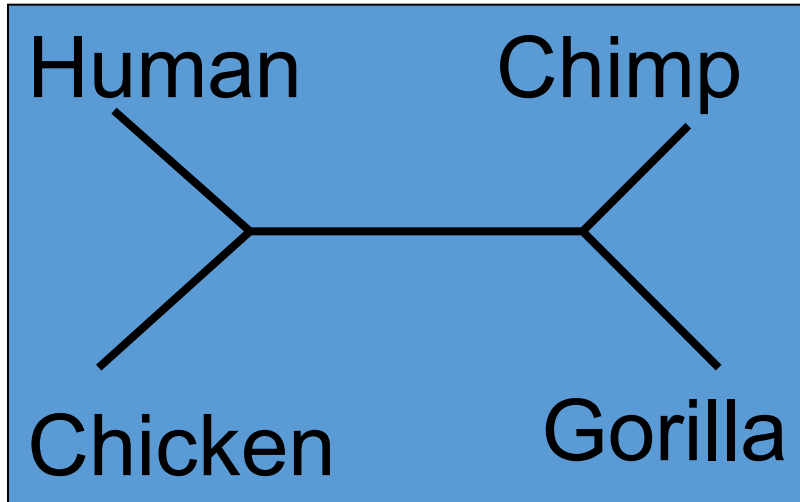
- Start the search with a good guess for a starting tree
- Examine all “neighboring” trees by making small modifications to the current tree
- Move to the neighbor with the highest (likelihood) score



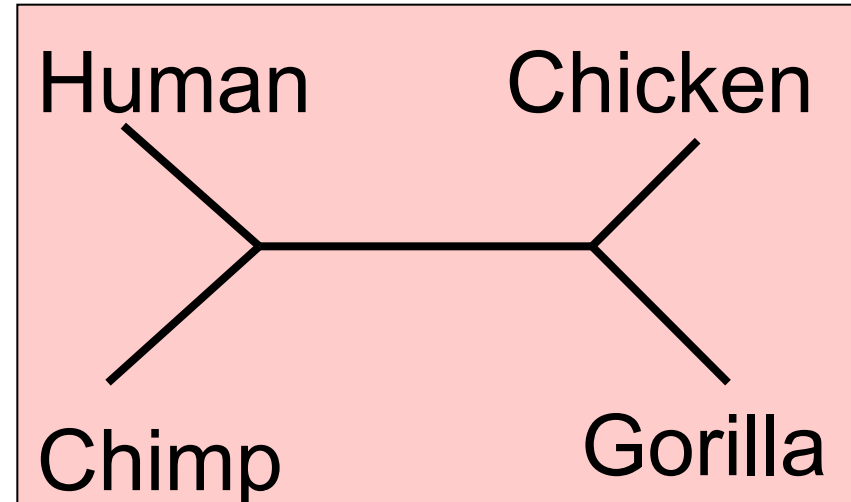
- To avoid local maxima, we start from multiple starting points

[illegible]

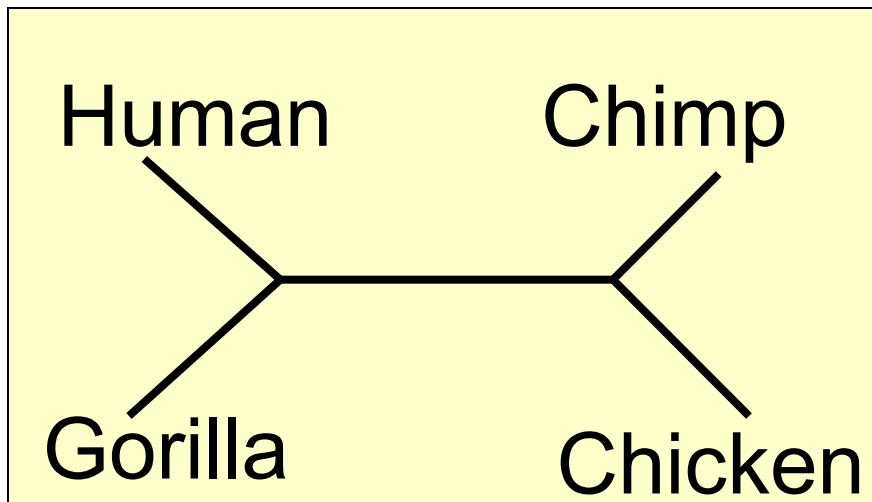
Different (unrooted) trees



LL score = -110



LL score = -107



LL score = -117

How confidence we
are in the best tree?

The phylogenetic jackknife



Jackknife

- We create new data sets by sampling randomly half of the characters without replacement.
- We generate 100 pseudo-data sets.
- We do not change the number of sequences, just the number of positions!

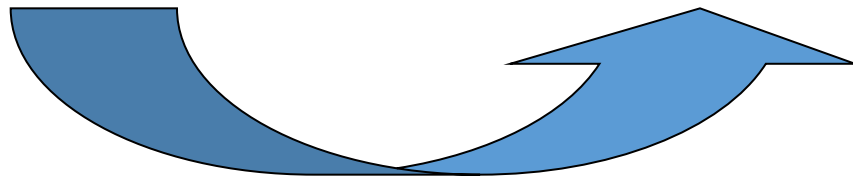
Jackknife

Original data

s1	A	A	G	T	A	A
s2	C	A	A	A	A	C
s3	C	A	G	G	A	A
s4	A	A	A	T	A	C

Pseudo-data 1

s1	A	G	A
s2	A	A	A
s3	A	G	A
s4	A	A	A

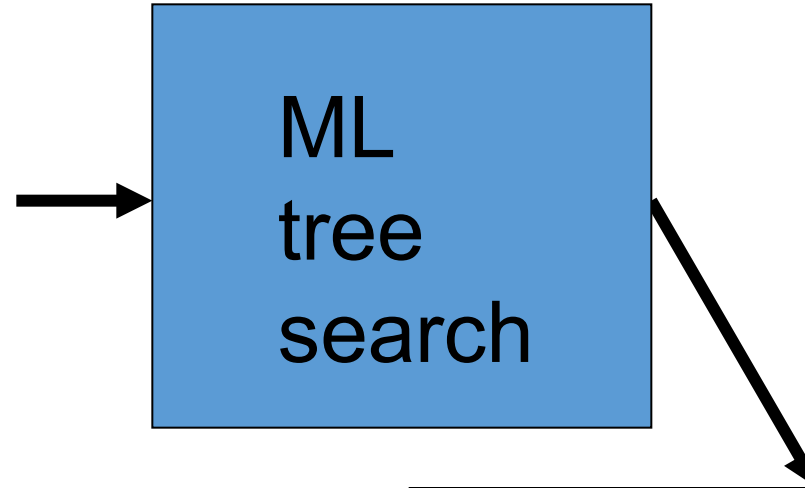


We removed positions 1, 4, and 6

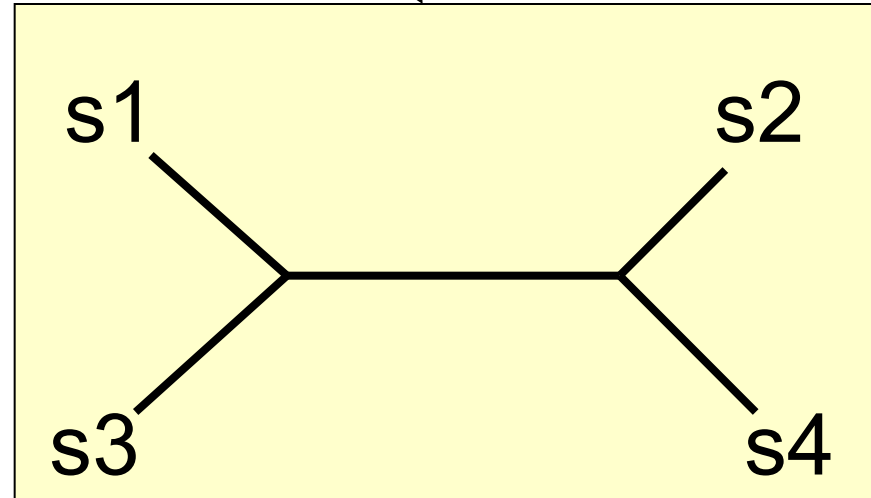
Jackknife

Pseudo-data 1

s1 AGA
s2 AAA
s3 AGA
s4 AAA

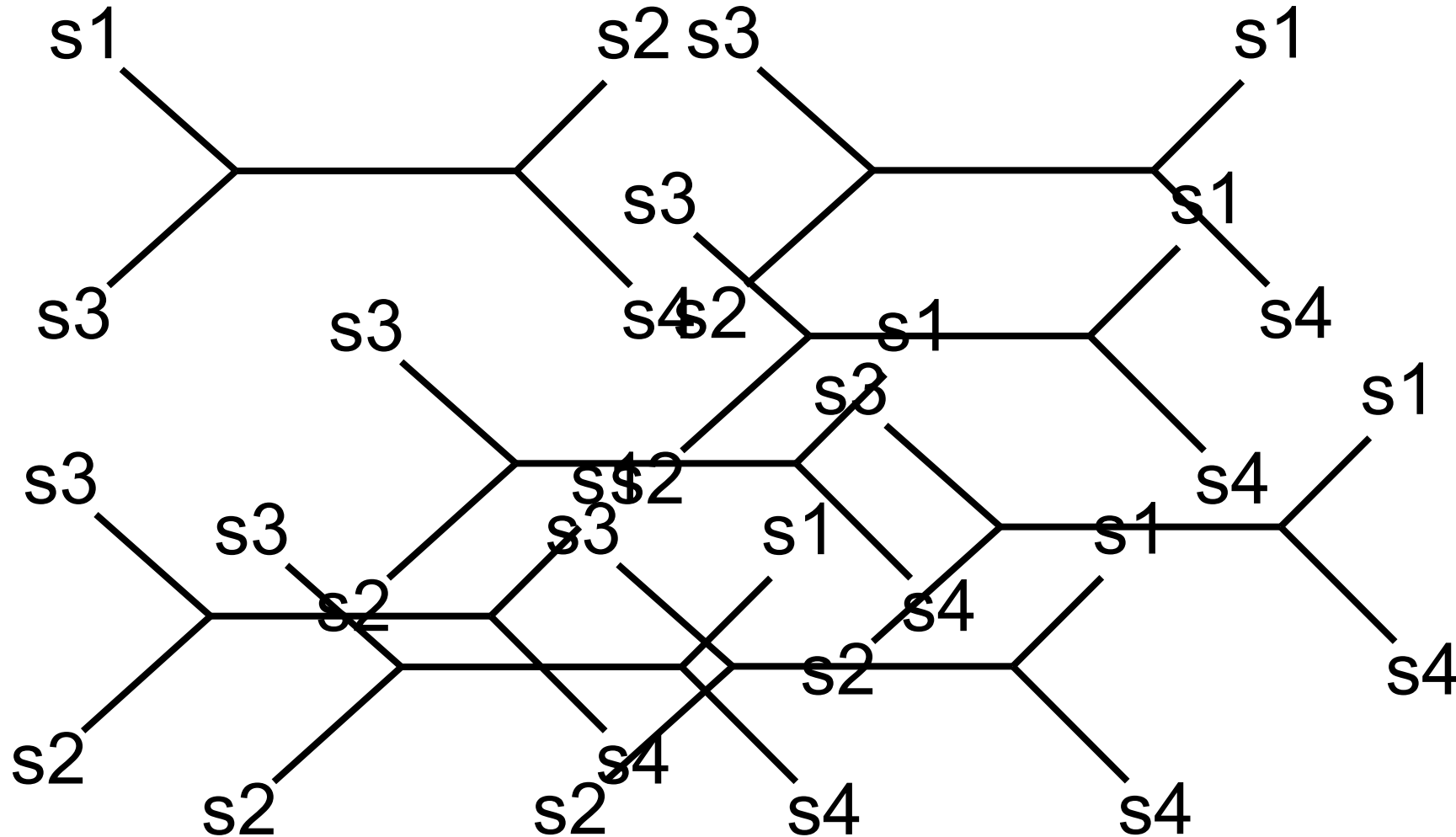


Best tree for
pseudo-data 1

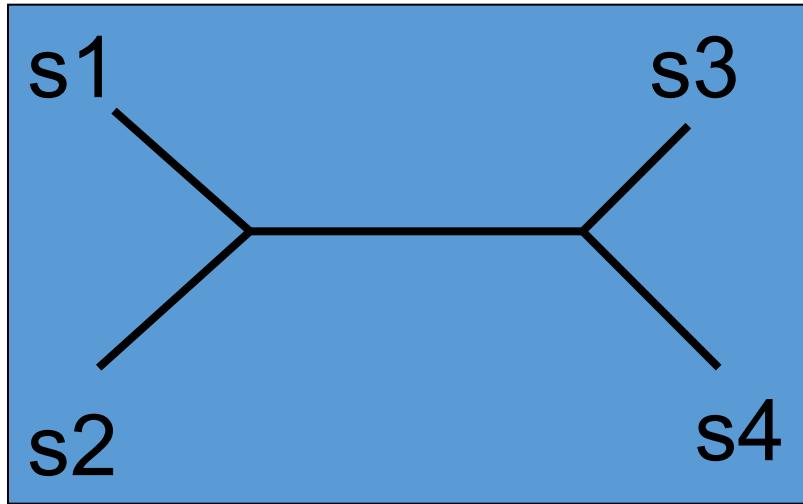


Jackknife

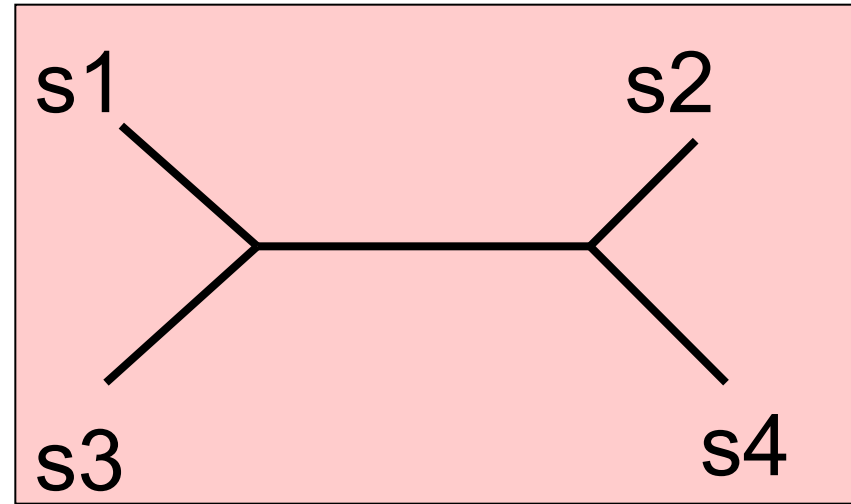
- We repeat the process 100 times and get 100 best trees



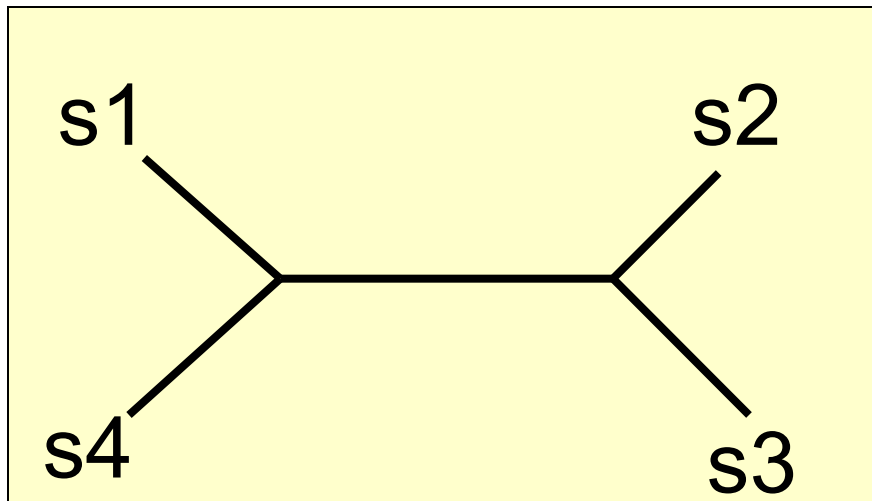
Jackknife percentage



30 times



50 times



20 times

Our confidence in the pink tree in terms of jackknife support is 50%.

The phylogenetic bootstrap



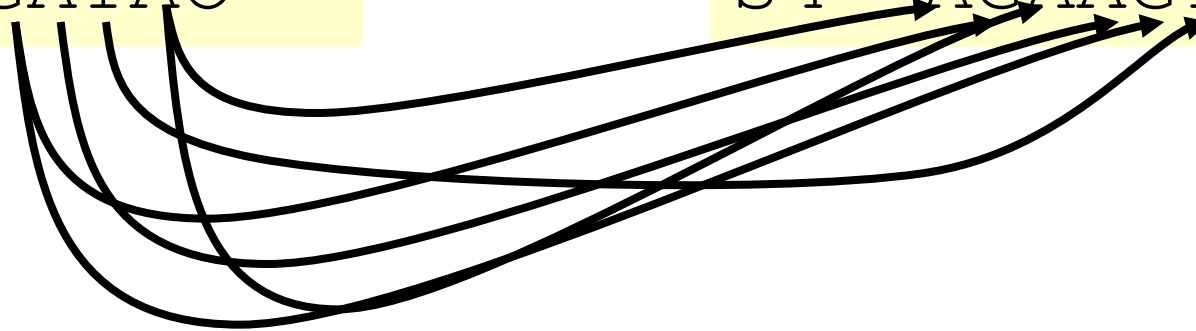
Bootstrap

Original data

s1	AAGTAA
s2	CAAAAC
s3	CAGGAA
s4	AGATAC

Pseudo-data 1

s1	AGAGAT
s2	AAAAAA
s3	AAAGAG
s4	AGAAGT



Same idea as jackknife but we sample with repetition. In this example, we sample positions 2 twice and position 5 twice and zero for position 1 and 6.

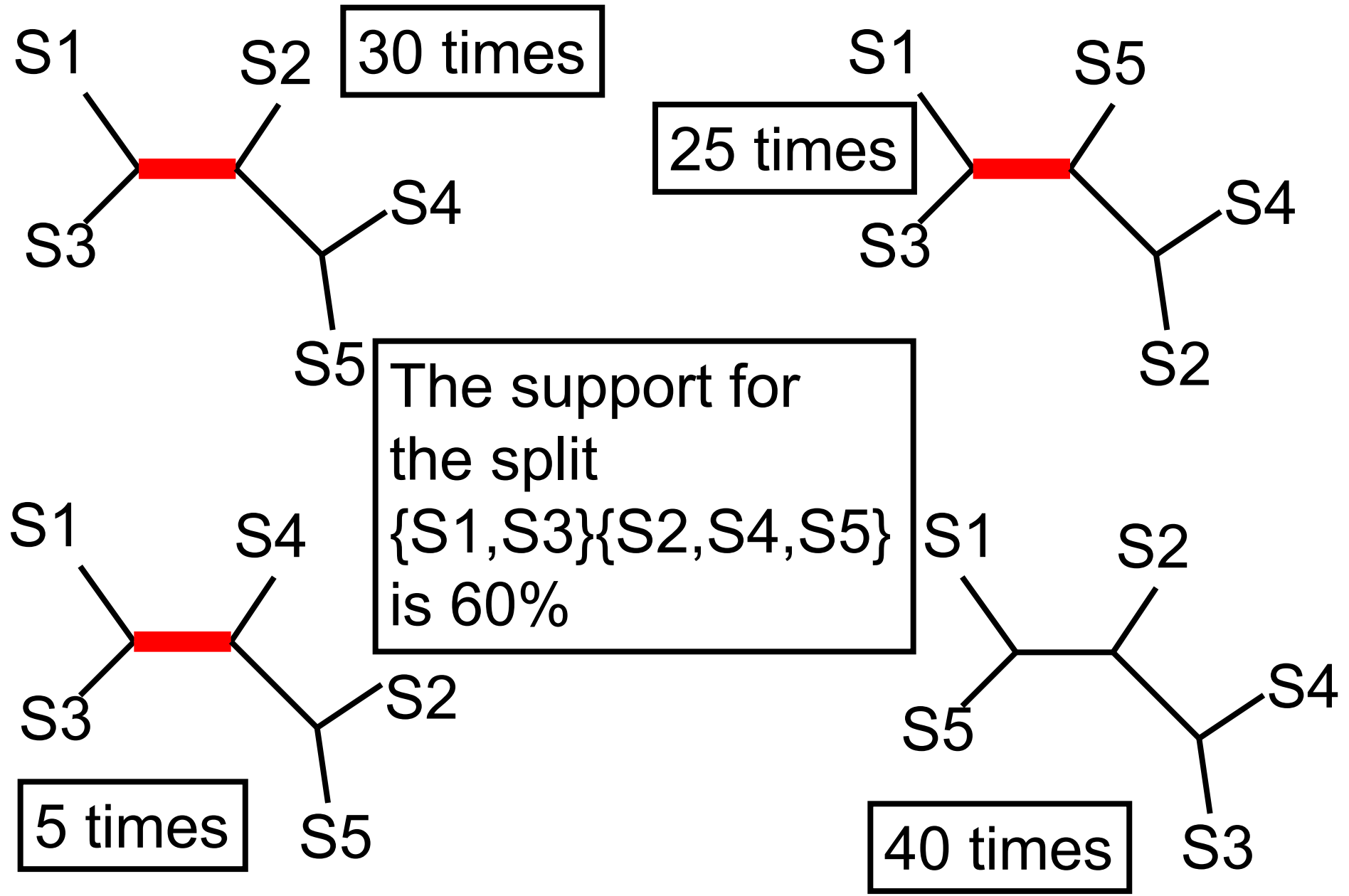
Bootstrap

- Bootstrap is used more than jackknife in phylogeny, because it has the same data-size as the original data.

Bootstrap for splits

- Instead of getting the support for each tree, we can compute the support for a given split.
- The support for a given split is the percentage of pseudo-tree in which this split appears.

Bootstrap for splits



Bootstrap is very slow



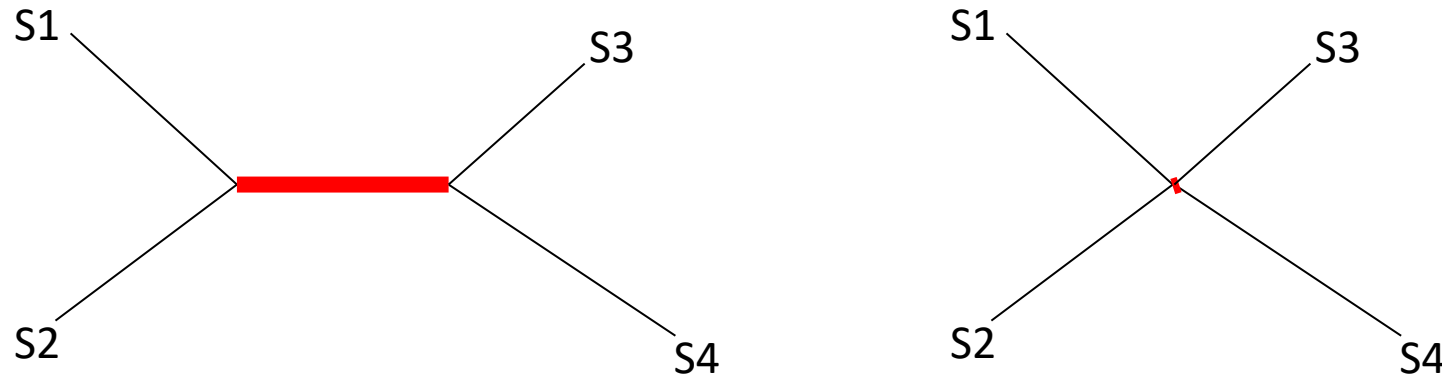
There's a need for faster estimates

Table 1. Several branch support methods implemented in current tree search software.

Program	Branch-support method	References
<u>RAXML-NG</u>	Standard <u>Felsenstein's</u> bootstrap	(Kozlov et al., 2019)
<u>RAXML-NG</u>	Transfer bootstrap expectation	(Kozlov et al., 2019; Lemoine et al., 2018)
IQTREE	Ultrafast bootstrap	(Hoang et al., 2018; Minh et al., 2013)
IQTREE	<u>aLRT</u> test	(Soang et al., 2018; Anisimova and Gascuel, 2006)
IQTREE	<u>aBayes</u> test	(Hoang et al., 2018; Anisimova et al., 2011)
<u>FastTree</u>	SH-like test	(Price et al., 2010)

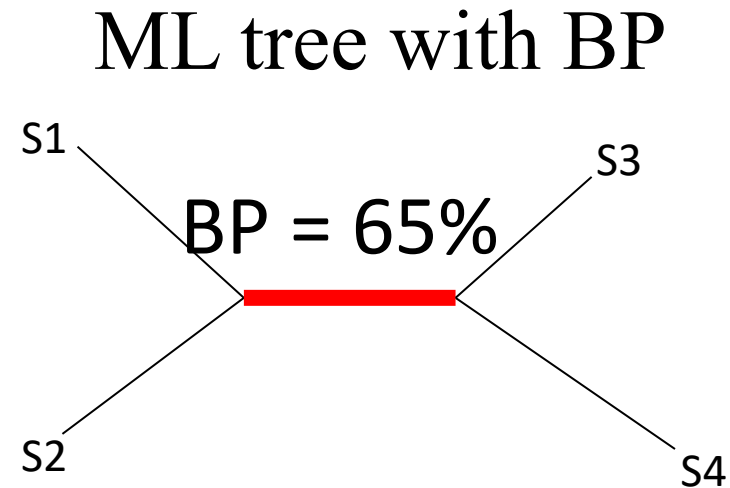
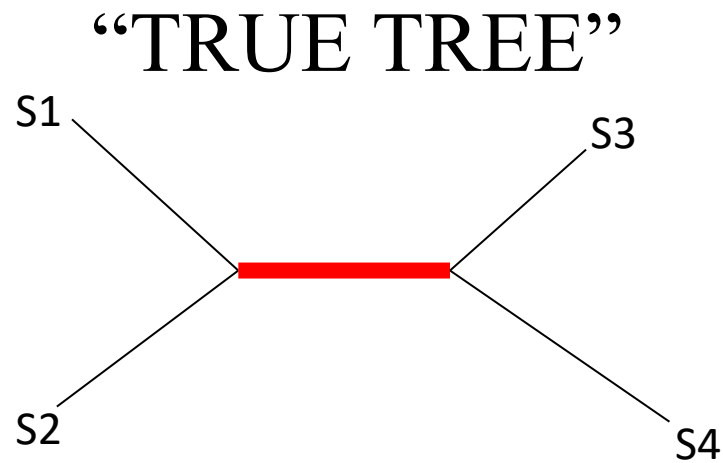
□

Likelihood based methods



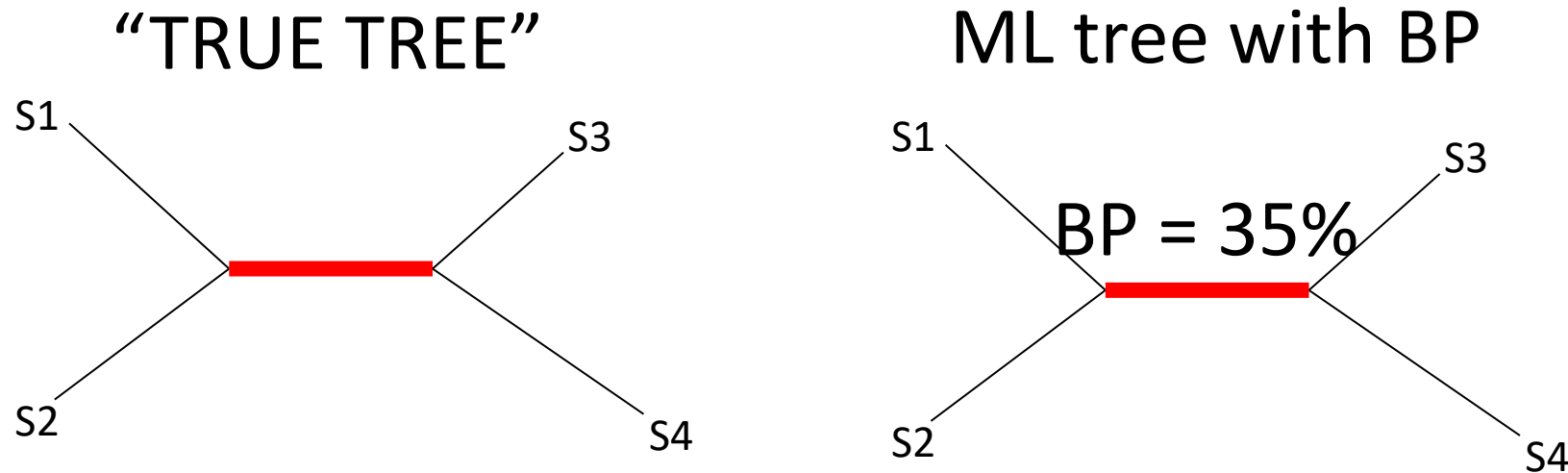
- Likelihood-based methods will estimate the support using differences in log-likelihood

How to evaluate different branch-support methods?



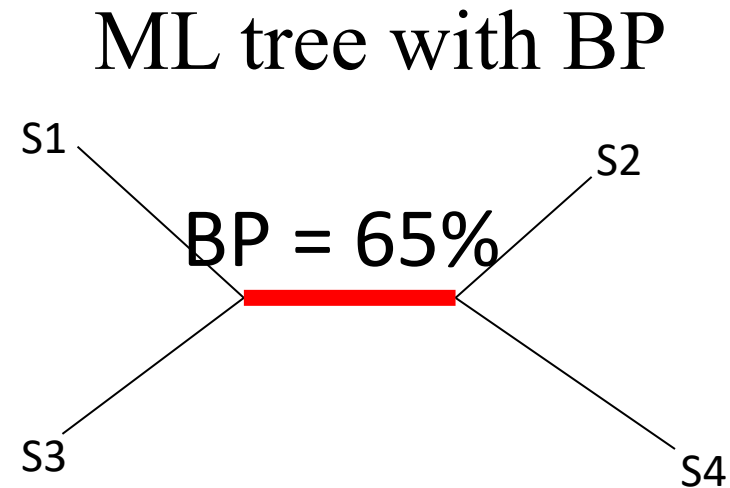
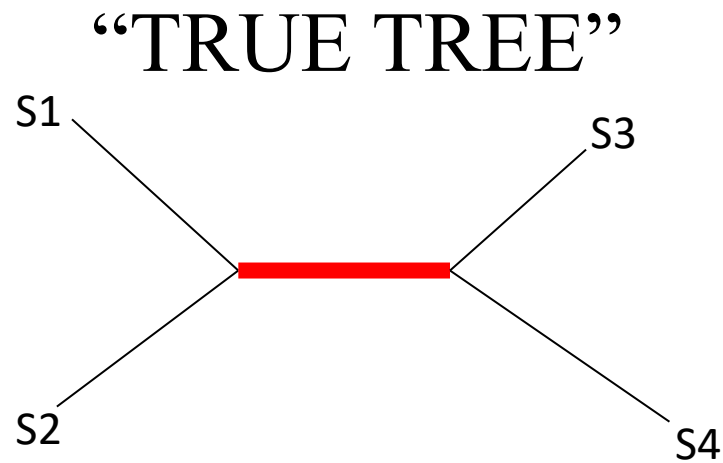
- In this case we have a true positive inference (TP)
- POSITIVE = the estimate is that the split exists
- TRUE = the estimate is correct

How to evaluate different branch-support methods?



- In this case we have a false negative inference (FN)
- NEGATIVE = the estimate is that the split does not exist
- FALSE = the estimate is wrong

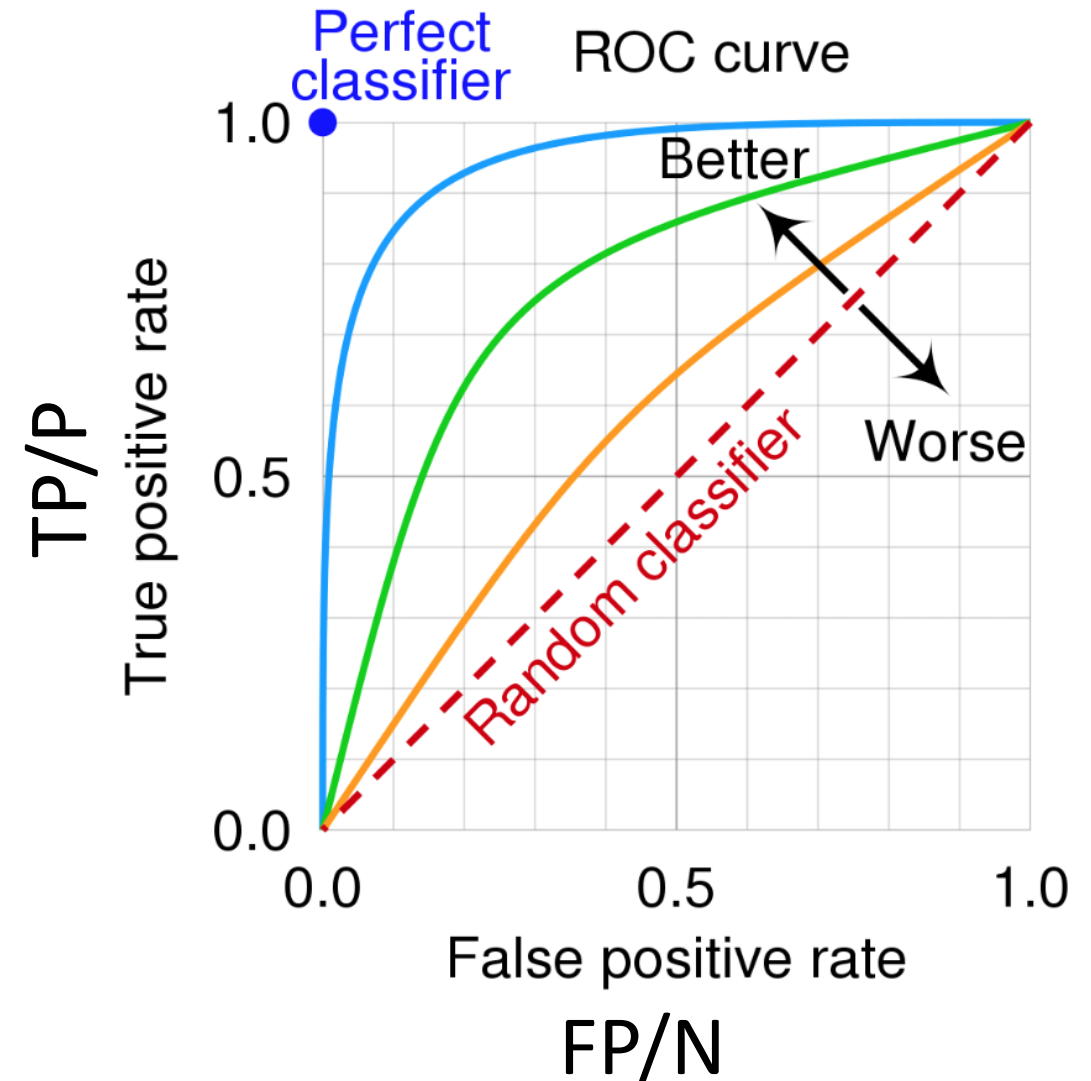
How to evaluate different branch-support methods?



- In this case we have a false positive inference (FP)
- POSITIVE = the estimate is that the split exists
- FALSE = the estimate is wrong

We can compute confusion matrices and AUC scores

		Predicted Class	
		Yes	No
Actual Class	Yes	TP	FN
	No	FP	TN



ML (machine learning) for branch support values



Noa Ecker

ML (machine learning) for branch support values



Prof. Yishay
Mansour



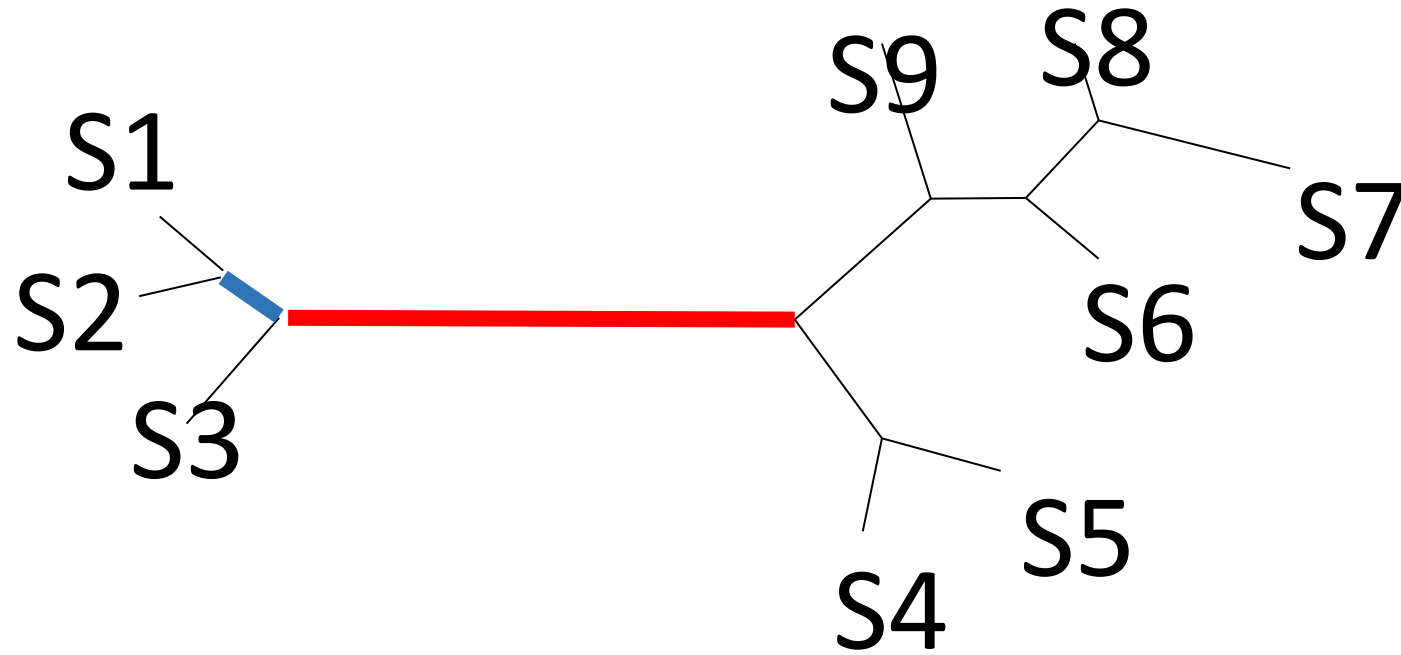
Prof. Itay
Mayrose



Prof. Dorothee
Huchon

Intuition

- I would trust the red branch more than the blue one

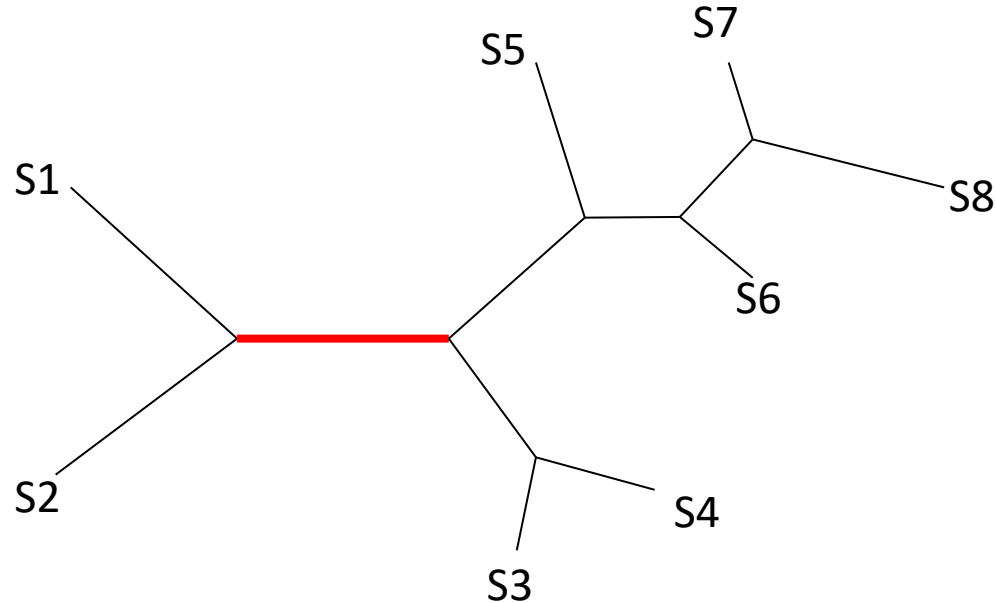


Features (out of 39)

- Branch length at the partition site
- Branch length divided by the mean branch length across the tree
- Branch length divided by the mean branch length among the four neighboring branches
- Number of MSA columns
- Number of unique MSA columns
- Percentage of constant sites
- The LL of NNI neighbors around the branch

Features (out of a 39)

- The count and proportion of taxa on the smaller or equal side of the bipartition



Proportion of taxa on the smaller side of the bipartition = 0.25

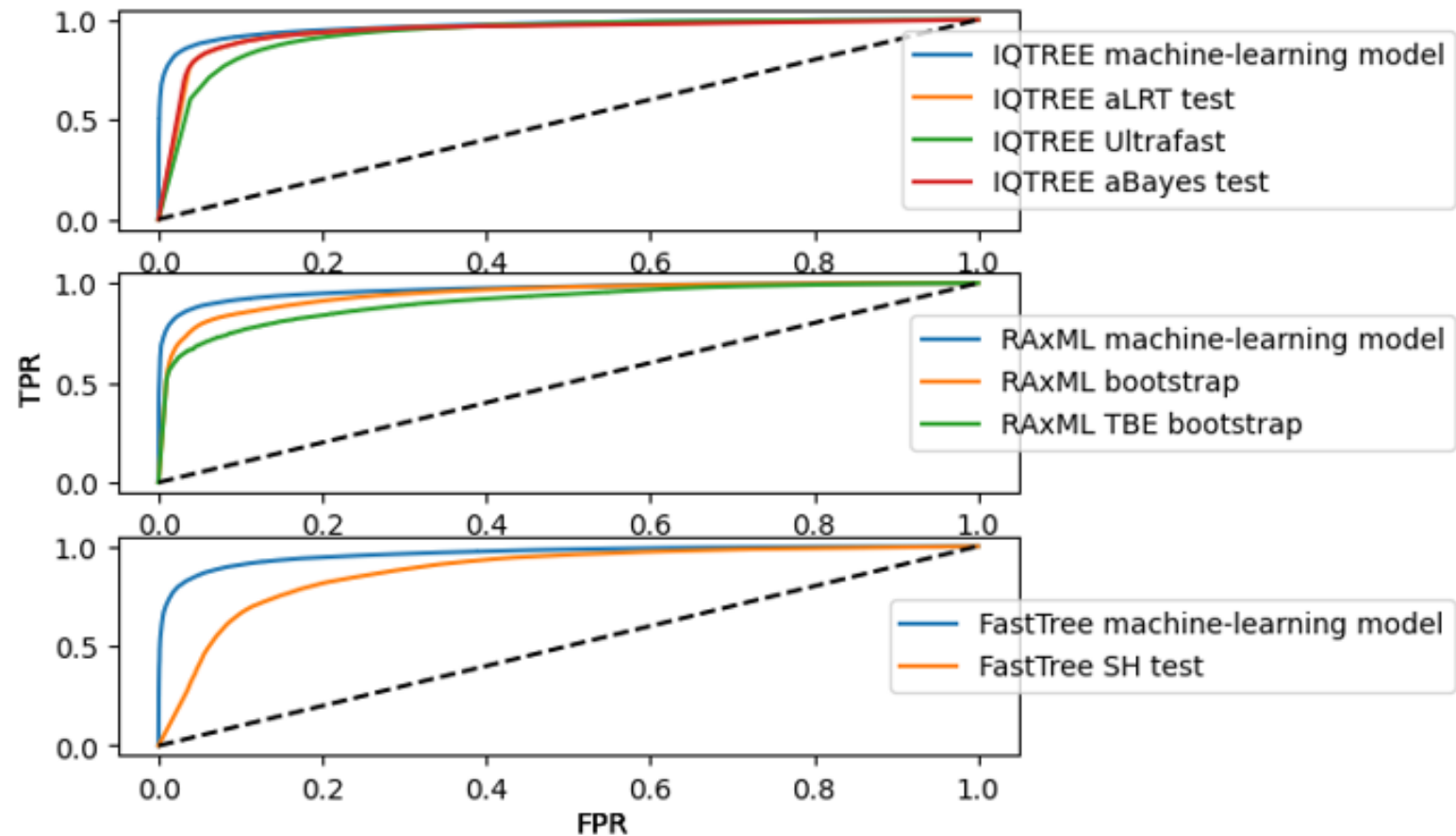
Number of taxa on the smaller side of the bipartition = 2

Train and test datasets

- 6,000 simulated MSAs with 100 to 10,000 sites and between 30 to 1,000 taxa.
- Each MSA was simulated along a different tree topology using Alisim (Ly-Trong et al., 2022), based on the script provided in the Github repository of RAxML-grove (Höhler et al., 2022).
- Each MSA was simulated using the DNA model associated with that tree in RAxML-Grove.
- Train set: 70% of the data; test set: the remaining 30%

Results, performance

- RAxML BP = 0.946 Machine-learning = 0.968
- RAxML transfer BP = 0.907

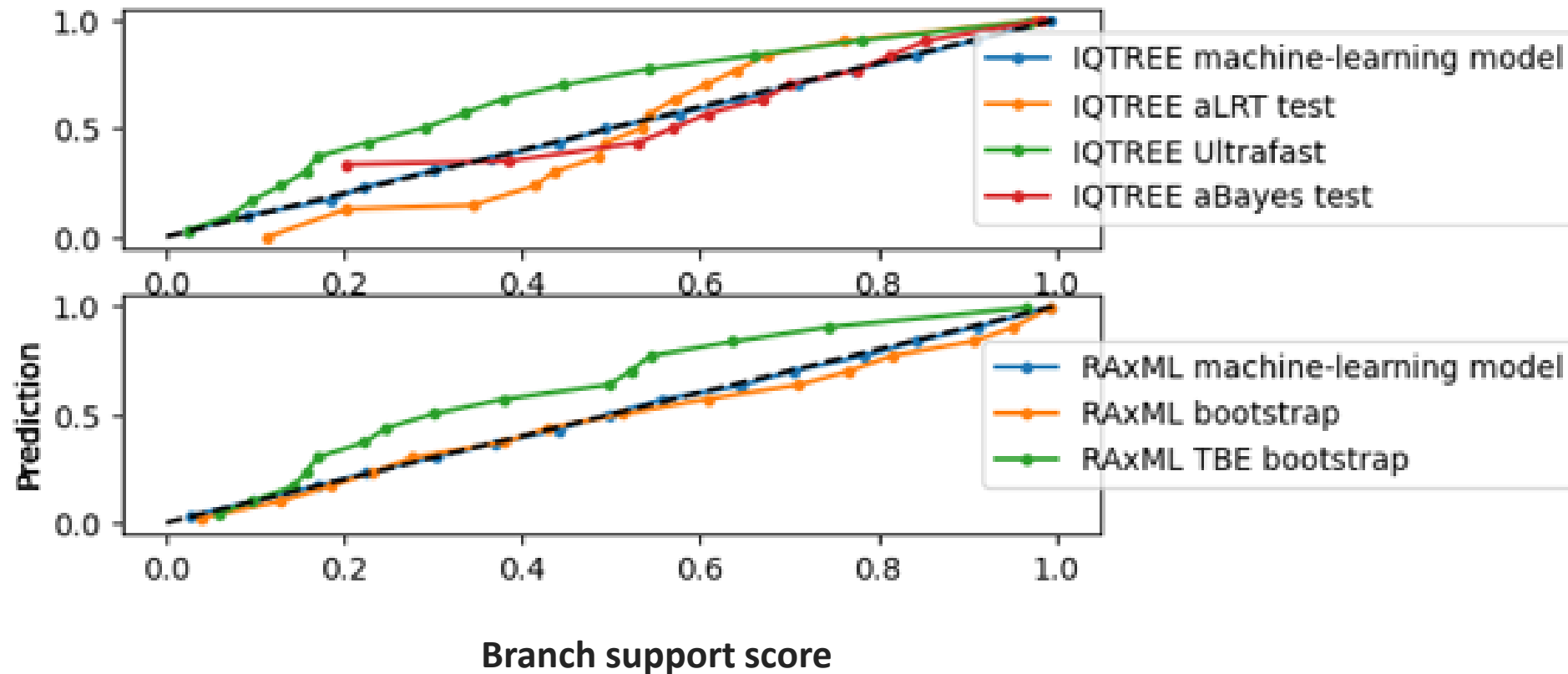


Results, running time

- **21 times faster** without optimizing the feature extraction algorithms:

The computation time of RAxML-NG standard bootstrap exhibited a median running time of 138 min on a single CPU. On the same data, our machine-learning model had a median running time of 6.5 min.

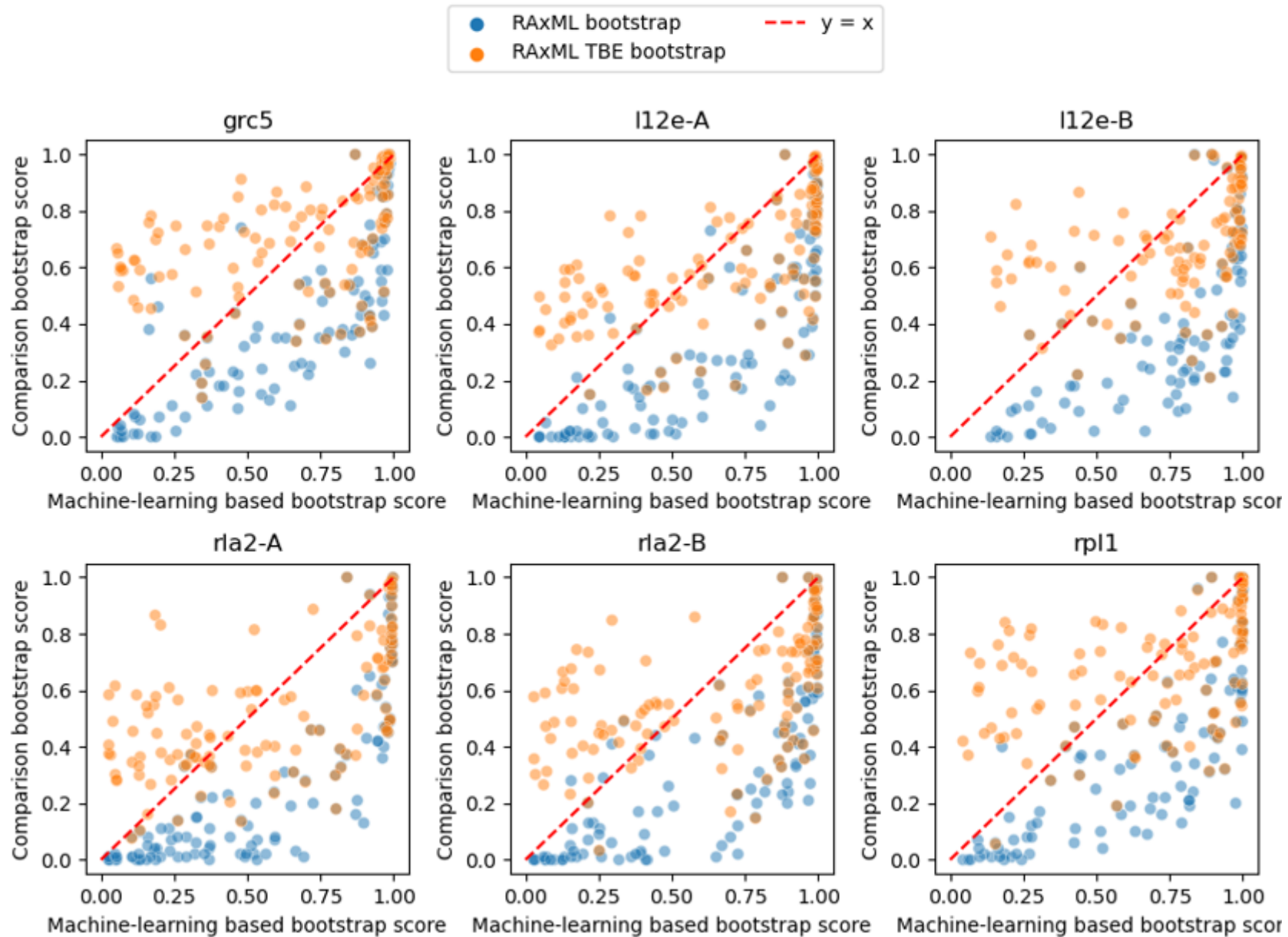
Results, calibration



Expected Calibration Error (ECE) of machine-learning method (IQTREE) = 0.002

ECE for ultrafast bootstrap (IQTREE) = 0.043

Results, empirical data






PART 2:

Bioinformatics, 2025, **41**(1), btaf009
<https://doi.org/10.1093/bioinformatics/btaf009>
Advance Access Publication Date: 8 January 2025
Original Paper

OXFORD

Phylogenetics

BetaAlign: a deep learning approach for multiple sequence alignment

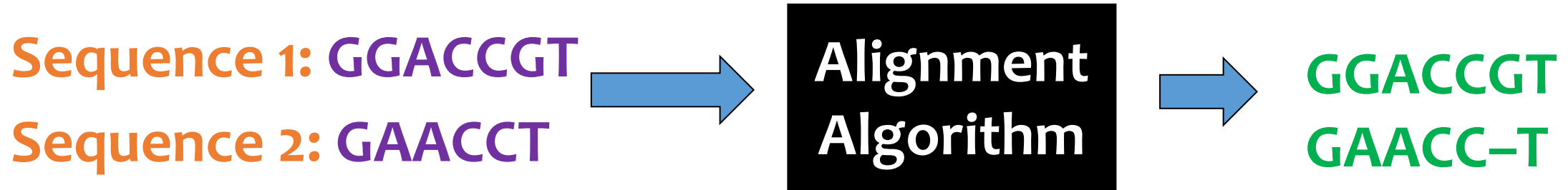
Edo Dotan ^{1,2}, Elya Wygoda¹, Noa Ecker¹, Michael Albuquerque¹, Oren Avram ³,
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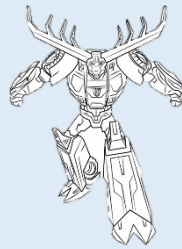
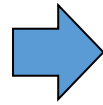
NLP-based sequence alignment



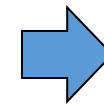
The transformer

INPUT

**L'ascension
des robots**



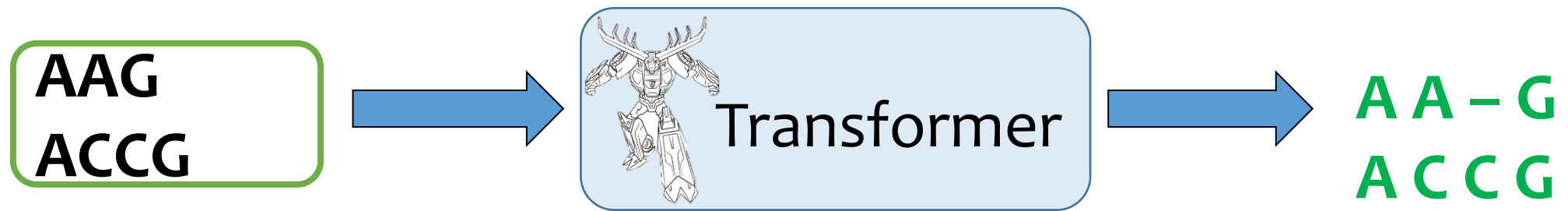
Transformer



OUTPUT

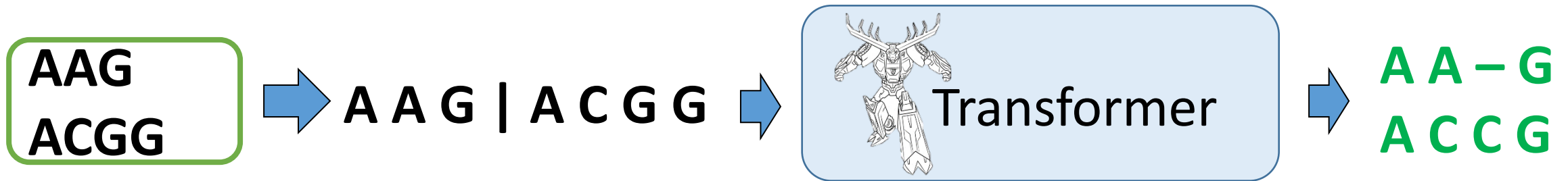
**Rise of the
robots**

BetaAlign



Encoding: the “concat” language

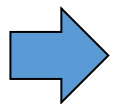
- Each letter in the “concat” language is a word, and the language has 5 different words (5 tokens)



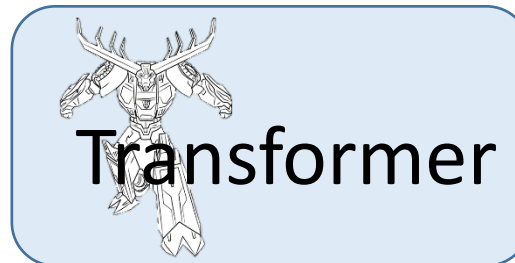
The output language

Upper letter	Lower letter	Symbol
A	A	A
A	C	B
A	G	C
A	T	D
A	-	E
...
-	T	X

AAG
ACGG



A A G | A C G G

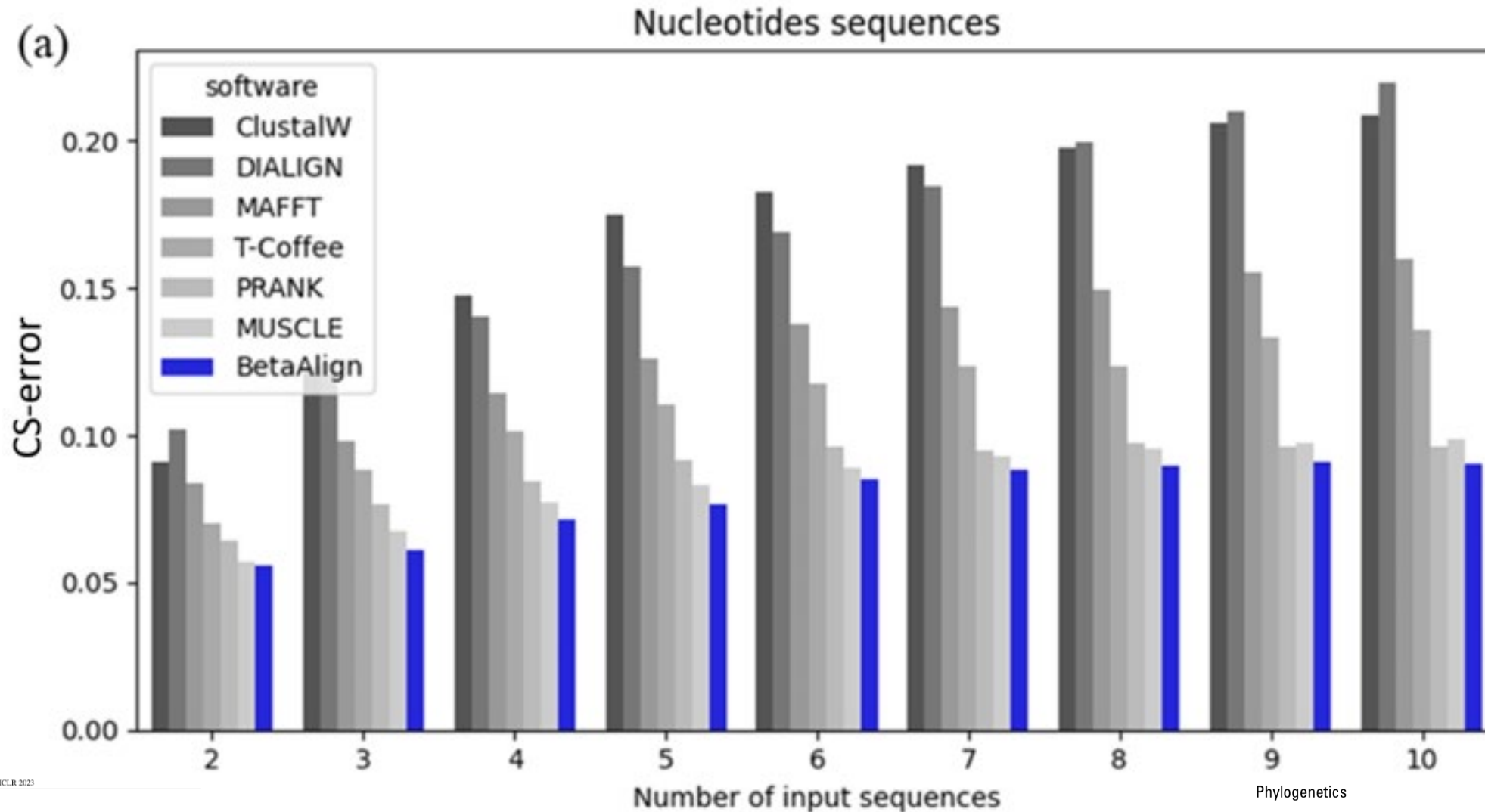


ABVM



A A - G
A C C G

Performance



Published as a conference paper at ICLR 2023

MULTIPLE SEQUENCE ALIGNMENT AS A SEQUENCE-TO-SEQUENCE LEARNING PROBLEM

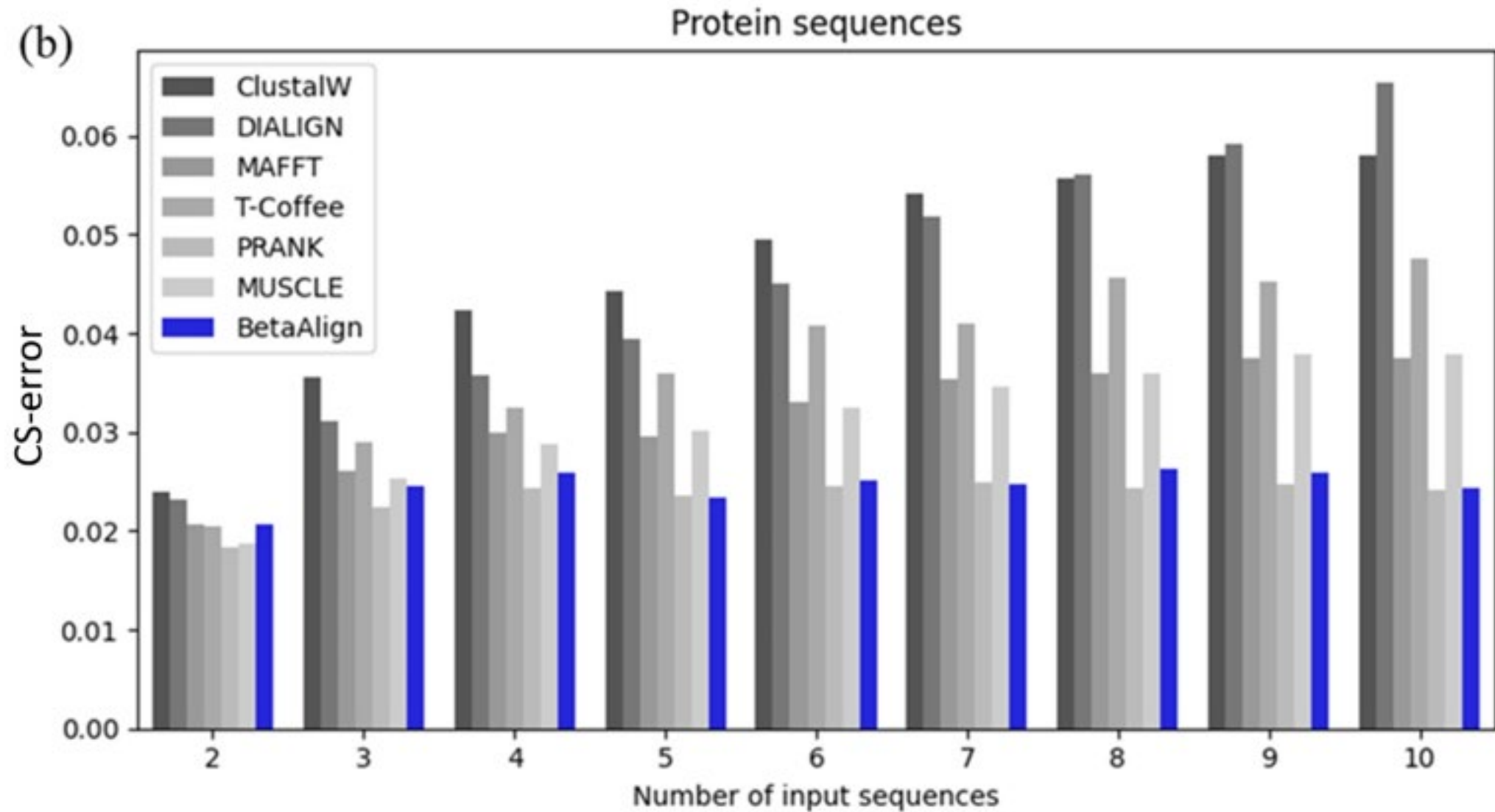
Edo Dotan^{1,2}, Yonatan Belinkov^{2,1*}, Oren Avram¹, Elya Wygoda¹, Noa Ecker¹, Michael Albuquerque¹, Omri Keren¹, Gil Loewenthal¹, and Tal Pupko^{1†}

Phylogenetics

BetaAlign: a deep learning approach for multiple sequence alignment

Edo Dotan^{1,2}, Elya Wygoda¹, Noa Ecker¹, Michael Albuquerque¹, Oren Avram¹, Yonatan Belinkov^{2,*}, Tal Pupko^{1,*}

Performance



PART 3 (work in progress):

| Better than sum-of-pairs: a machine-learning-based score to evaluate multiple sequence alignments

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An ML-based MSA objective function

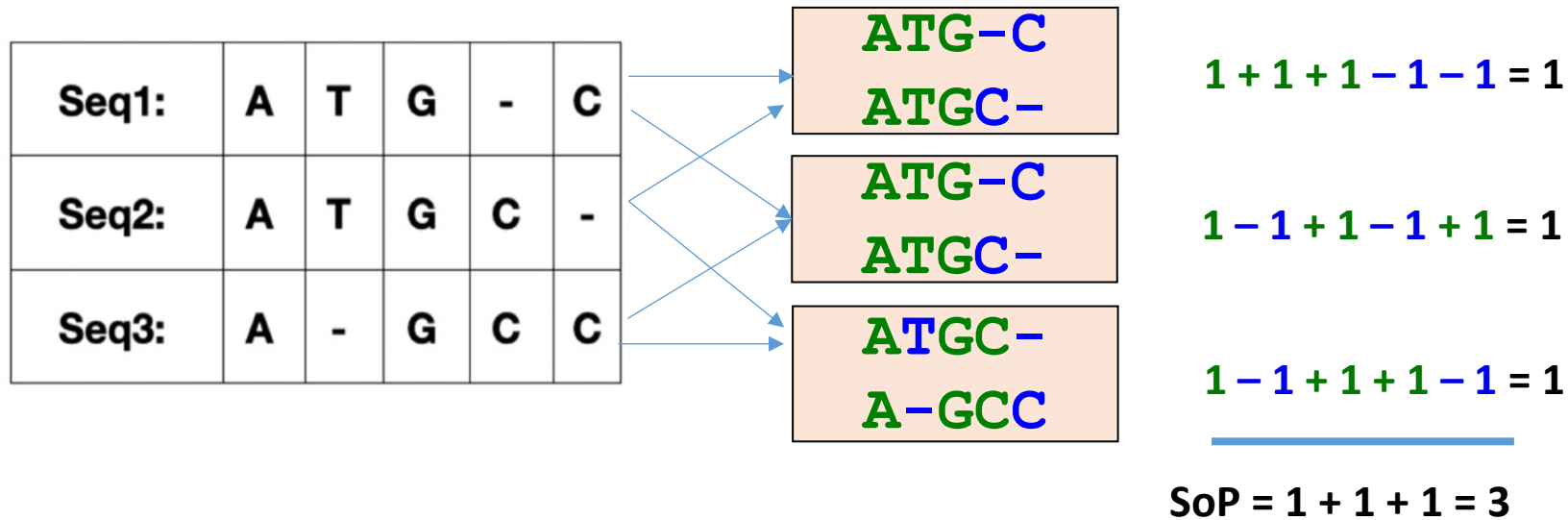
- Inference of MSAs is a very difficult problem
 - The implicitly assumed indel evolutionary models are oversimplified
 - Which objective function should be optimized (the likelihood are very difficult to compute)
 - Optimizing the objective function is difficult
 - The MSA depends on the tree and vice versa

An ML-based MSA objective function

- Inference of MSAs is a very difficult problem
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 - **Which objective function should be optimized (the likelihood are very difficult to compute)**
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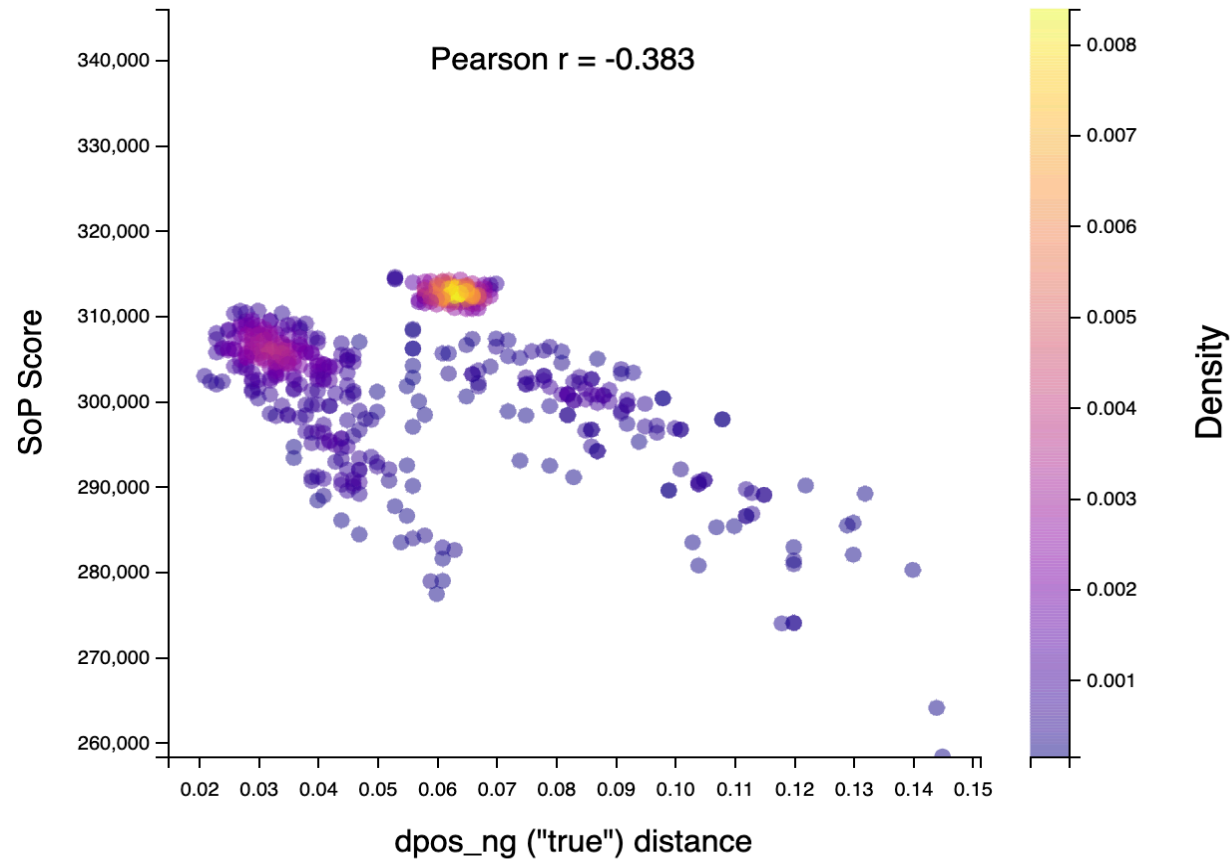
Sum of pairs

- The sum-of-pairs (SoP) score is widely used for MSA scoring
- Higher SoP is supposed to be an indicator of a better MSA



Sum of pairs does not correlate well with accuracy

- 500 alternative MSAs of a single dataset



Proposed solution

- Employing AI to develop novel scoring functions for MSAs that are better than the sum-of-pairs and to use our developed score to discriminate among MSAs

Features

70+

We tried to use many features related to MSA and their corresponding MLE trees; 26 features were used in the final version of the model

Alternative MSAs

>600K

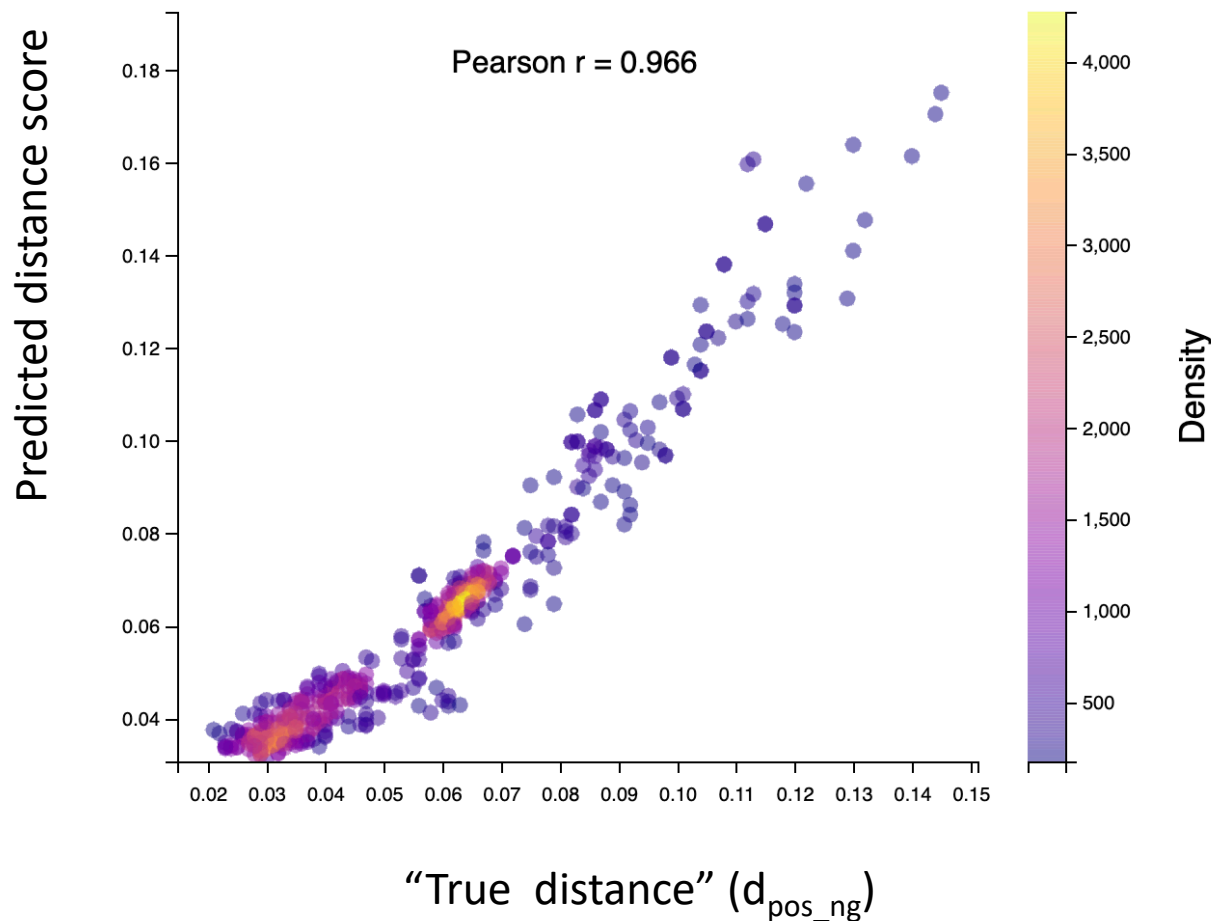
Alternative MSAs were created using four different aligning programs, GUIDANCE and by refinement

Deep Learning Network

Many model architectures, hyperparameters, scaling, and batching techniques were tested

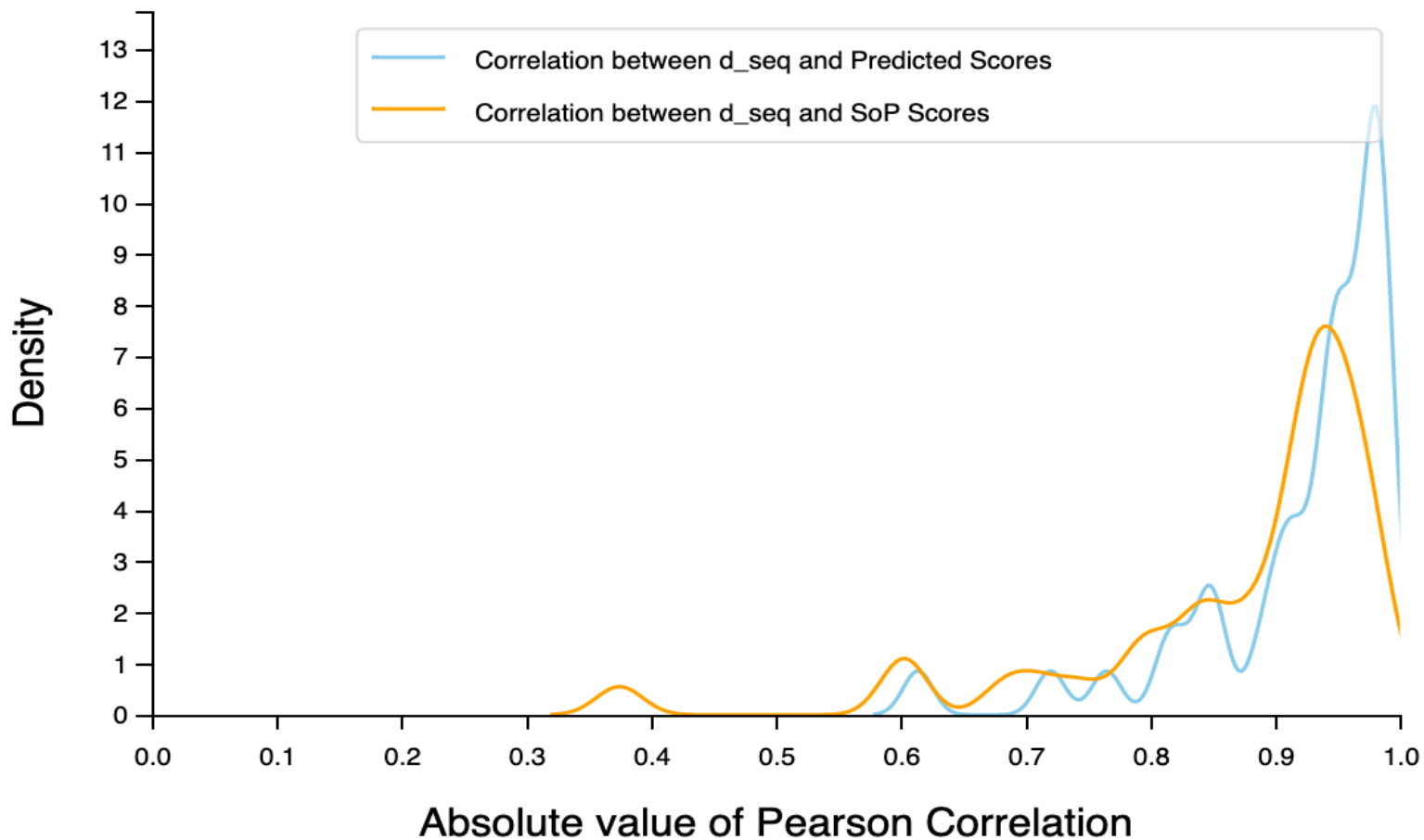
Our score well corelates with accuracy

- 500 alternative MSAs of a single dataset



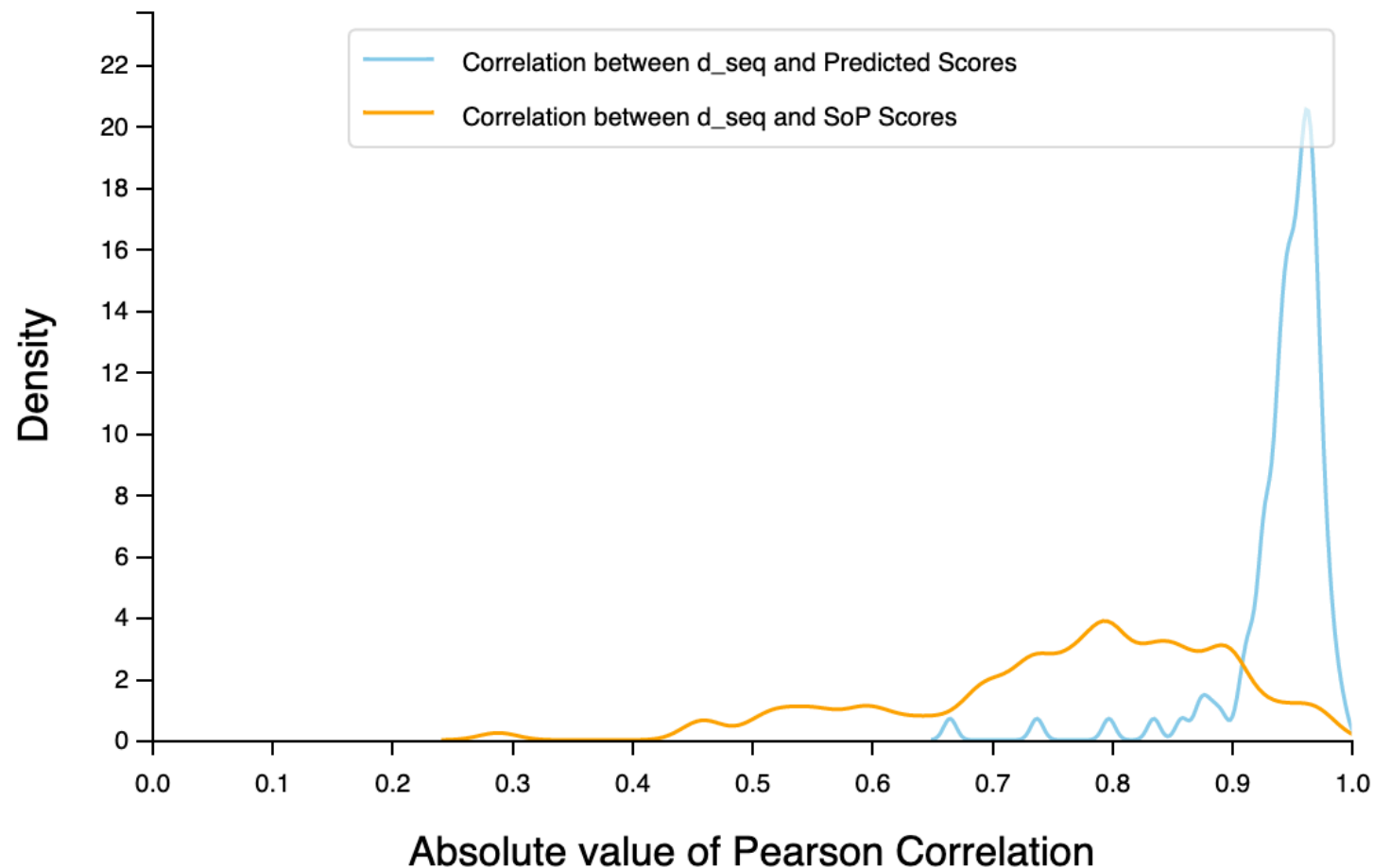
Our score well corelates with accuracy

- Simulated datasets



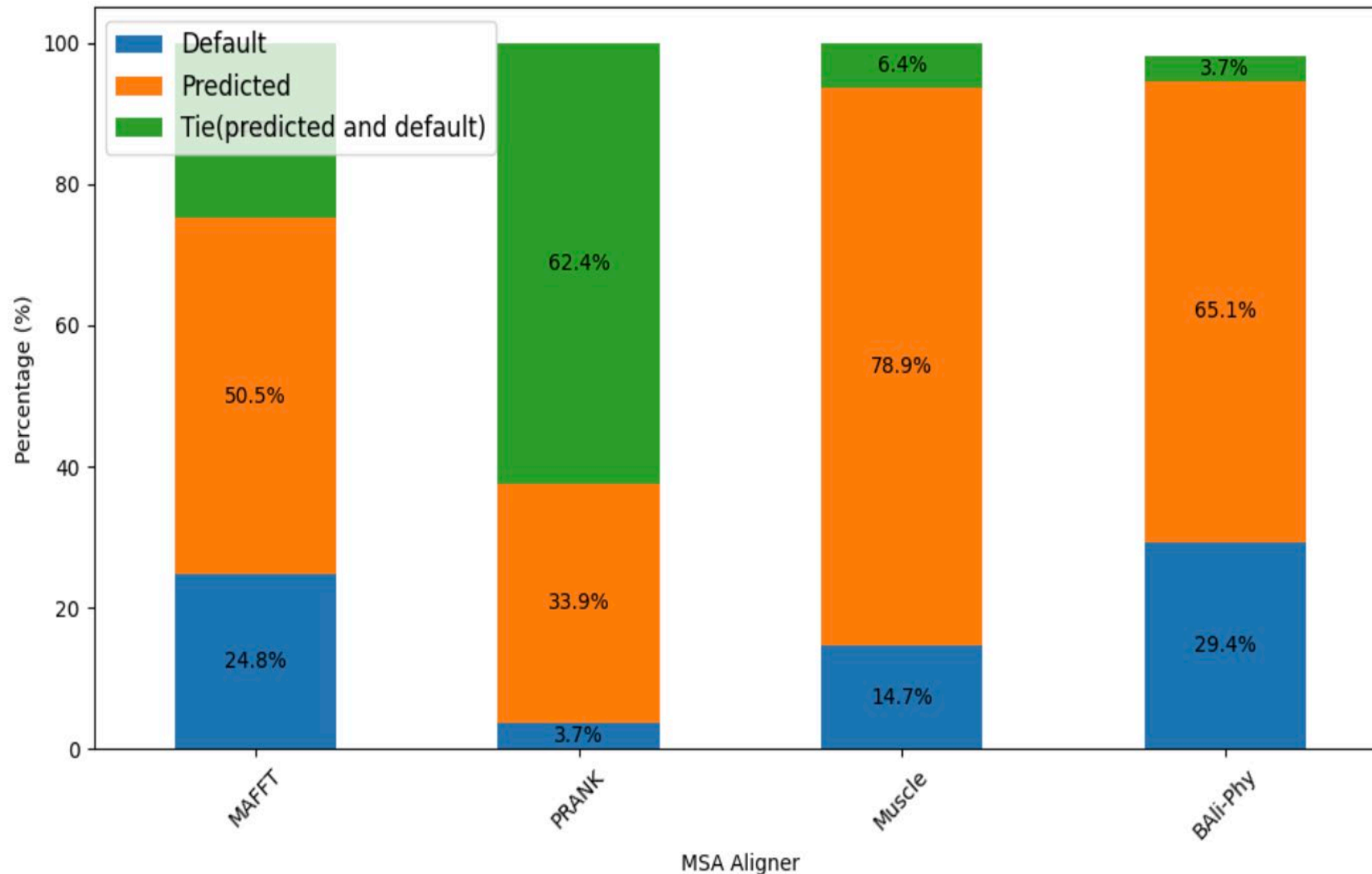
Our score well corelates with accuracy

- empirical datasets



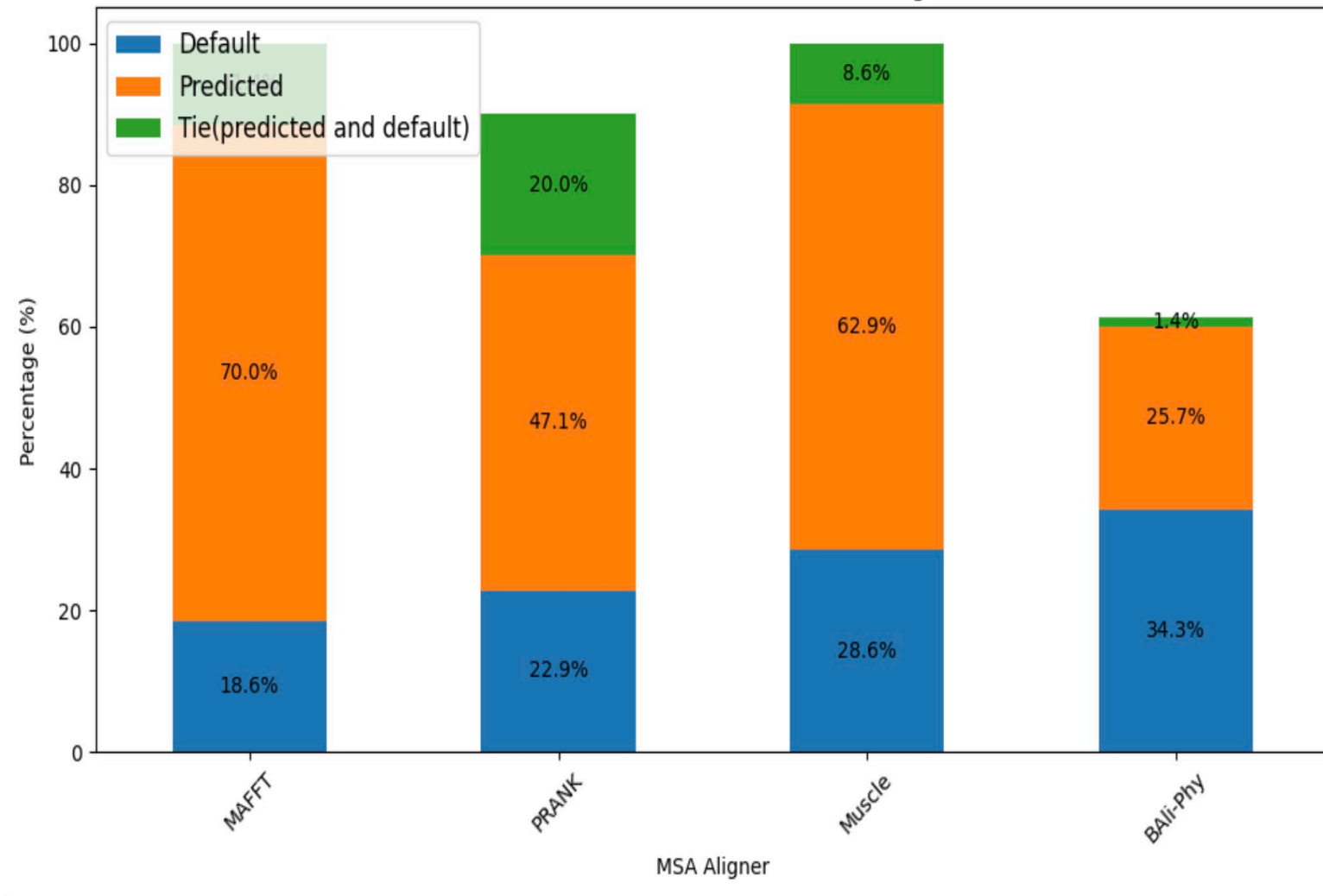
Our score well corelates with accuracy

- Pick me game (simulations)



Our score well corelates with accuracy

- Pick me game (empirical)



Joint work



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Mansour



Prof. Itay Mayrose



Dr. Yonathan
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