TIPP tutorial

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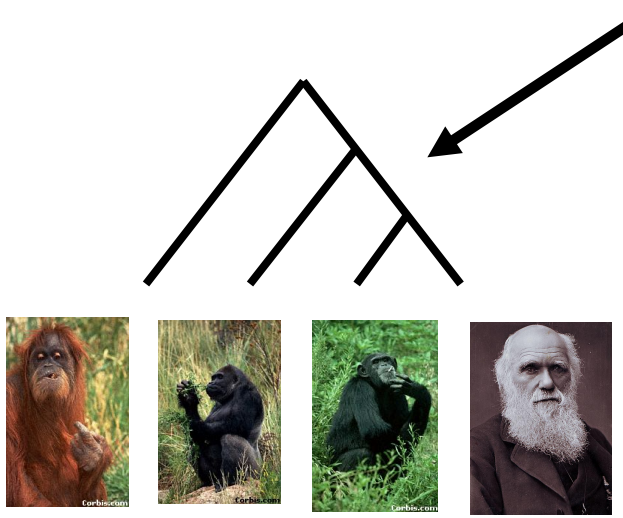
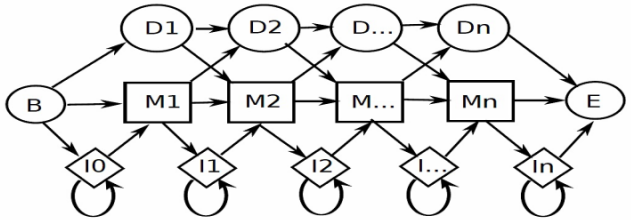
Multiple Sequence Alignment (MSA)

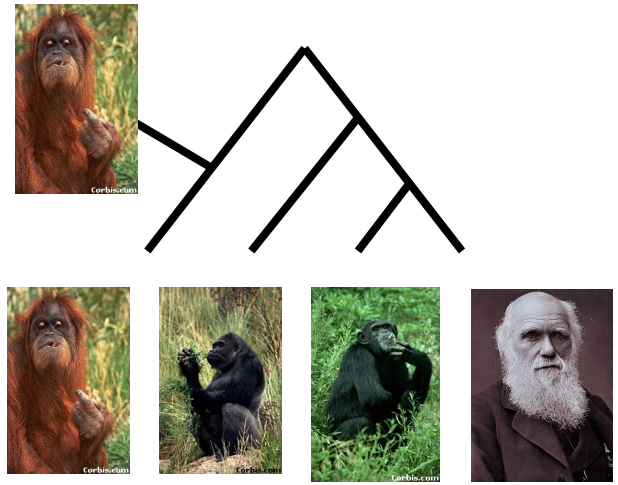
```
Hu = AGGCTATCACCGACTCCA          Hu = -AGGCTATCACGACCTCCA
Ch = TAGCTATCACGACCGC      →     Ch = TAG-CTATCACGACCGC--
Go = TAGCTGACCGC                 Go = TAG-CT-----GACCGC--
Or = TCACGACCGACA                Or = -------TCACGACCGACA
```



Using the MSA and tree to identify reads

Hu =
Ch =
Go =
Or =
Qu = TCACCCCCTCACC-CC----





Q

Represent MSA using a
profile Hidden Markov Model (HMM)

Outline

- Start a profiling job
- Inputs/outputs
- Parameter options

Before we begin....

- cd /path/to/sepp/ (on the VM, the path is ~/tools/sepp/)
- chmod u+x run_tipp_tool.py
- export PATH=$PATH:/path/to/sepp/

Running an initial profiling job

- First move to the test directory located within the sepp directory
  - On the VM type
    - cd ~/tools/sepp/test/unittest/data/mock/pyrg
  - All others
    - cd /path/to/sepp/test/unittest/data/mock/pyrg

- Run
  - run_tipp.py -R pyrg -f pyrg.even.fas -o output

Output

- Alignment files
  - output_alignment.fasta
  - output_alignment_masked.fasta
- Placement file
  - output_placement.json

Output (continued)

- Classification file

output_classification.txt

```
EAS25_26_1_15_381_1761_0_1,2157,Archaea,superkingdom,1.0000
EAS25_26_1_15_381_1761_0_1,1,root,root,1.0000
EAS25_26_1_15_381_1761_0_1,183925,Methanobacteria,class,1.0000
EAS25_26_1_15_381_1761_0_1,2172,Methanobrevibacter,genus,1.0000
EAS25_26_1_15_381_1761_0_1,28890,Euryarchaeota,phylum,1.0000
EAS25_26_1_15_381_1761_0_1,2158,Methanobacteriales,order,1.0000
EAS25_26_1_15_381_1761_0_1,2173,Methanobrevibacter smithii,species,1.0000
```

Converting classification format

- Type:
  - mkdir profile
  - run_tipp_tool.py -g pyrg -a profile -o profile -p pyrg -i output_classification.txt -t 0.95

Tab separated classification file

pyrg.classification

fragment	species	genus	family	order	class	phylum
EAS25…	2173	2172	2159	2158	183925	28890
EAS25…	NA	NA	2159	2158	183925	28890

Abundance profile

abundance.species.csv

taxa abundance
Methanobrevibacter smithii 0.7969
Methanococcus maripaludis 0.0156
unclassified 0.1875

Profiling a metagenomic dataset

- Change to the mixed test directory
  - cd ../mixed

- Run the abundance profiler
  - run_abundance.py -f facs_simhc.short.fas -c ~/.sepp/tipp.config -d out

Phylogenetic Placement

- Align each query sequence to backbone alignment:
  - HMMALIGN (Eddy, Bioinformatics 1998)
  - PaPaRa (Berger and Stamatakis, Bioinformatics 2011)
- Place each query sequence into backbone tree, using extended alignment:
  - pplacer (Matsen et al., BMC Bioinformatics 2010)
  - EPA (Berger et al., Systematic Biology 2011)

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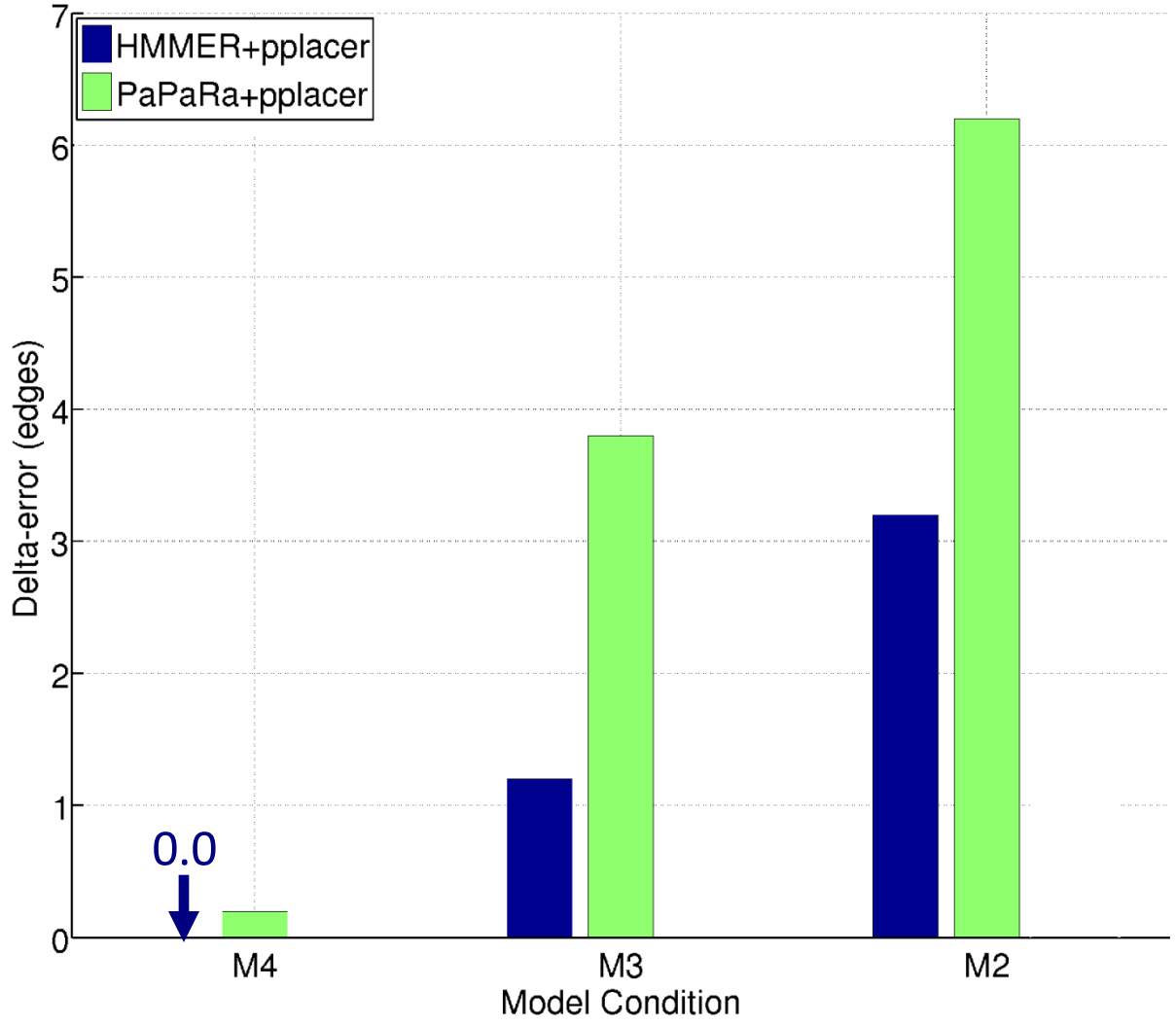
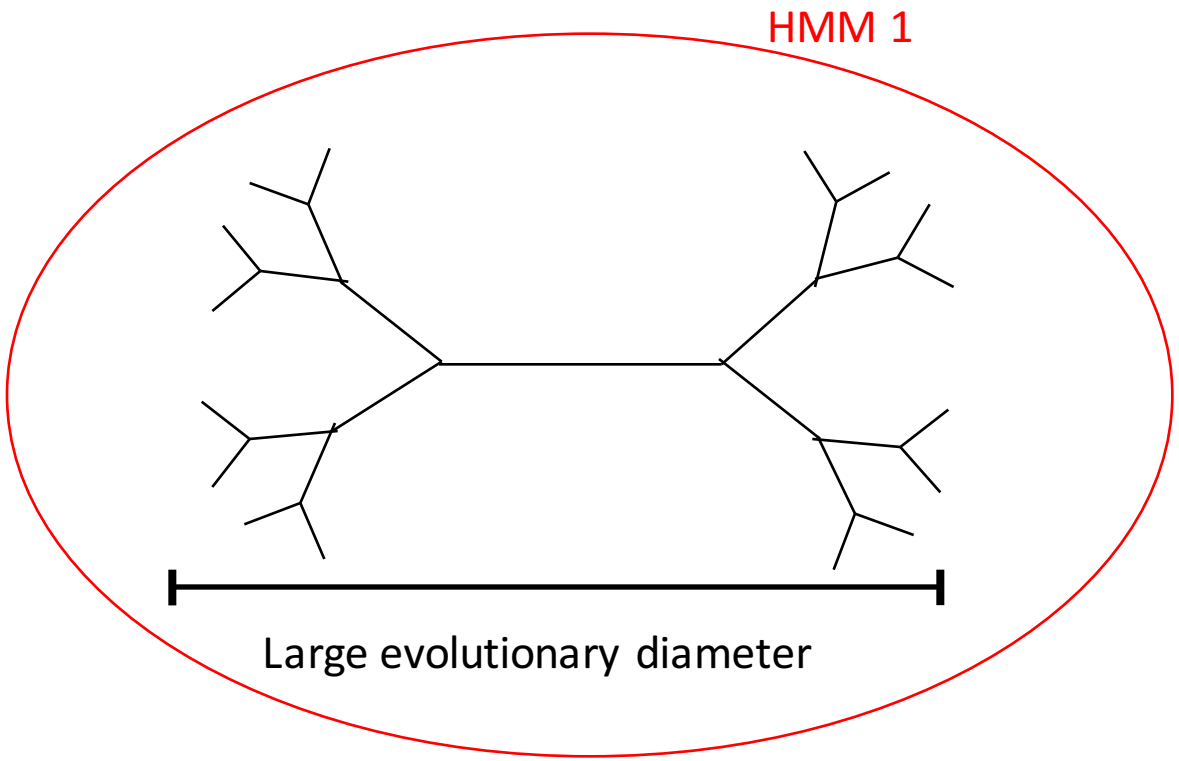
HMMER and PaPaRa results



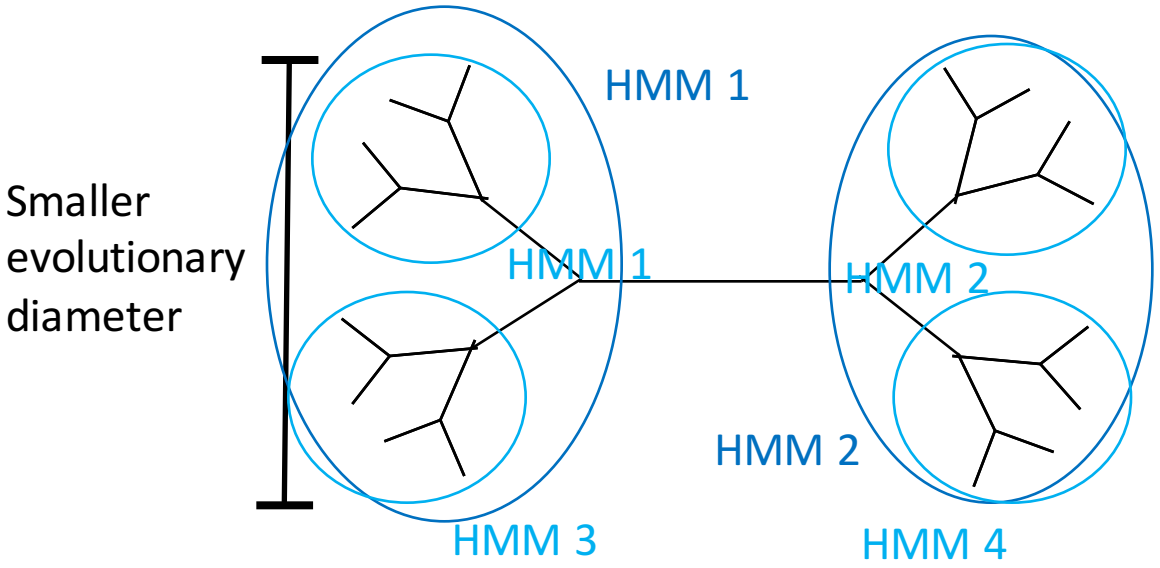
Increasing rate evolution

Backbone size: 500
5000 fragments
20 replicates

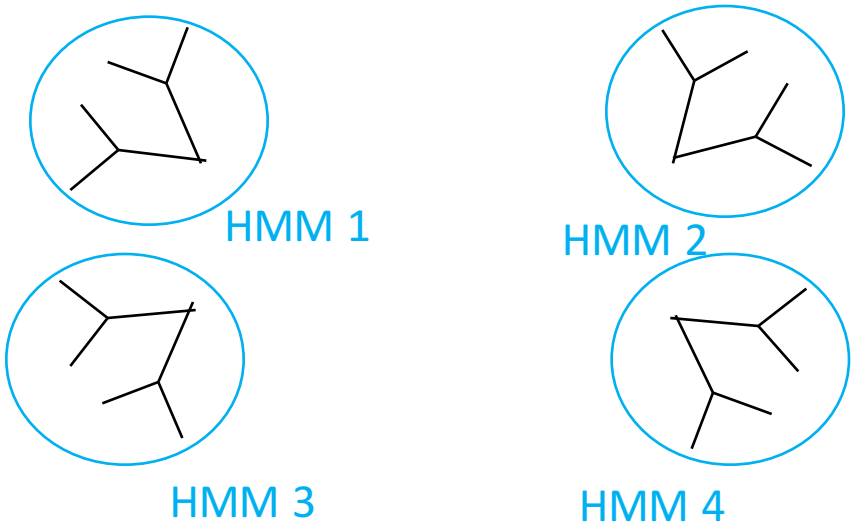
Standard approach (single HMM)



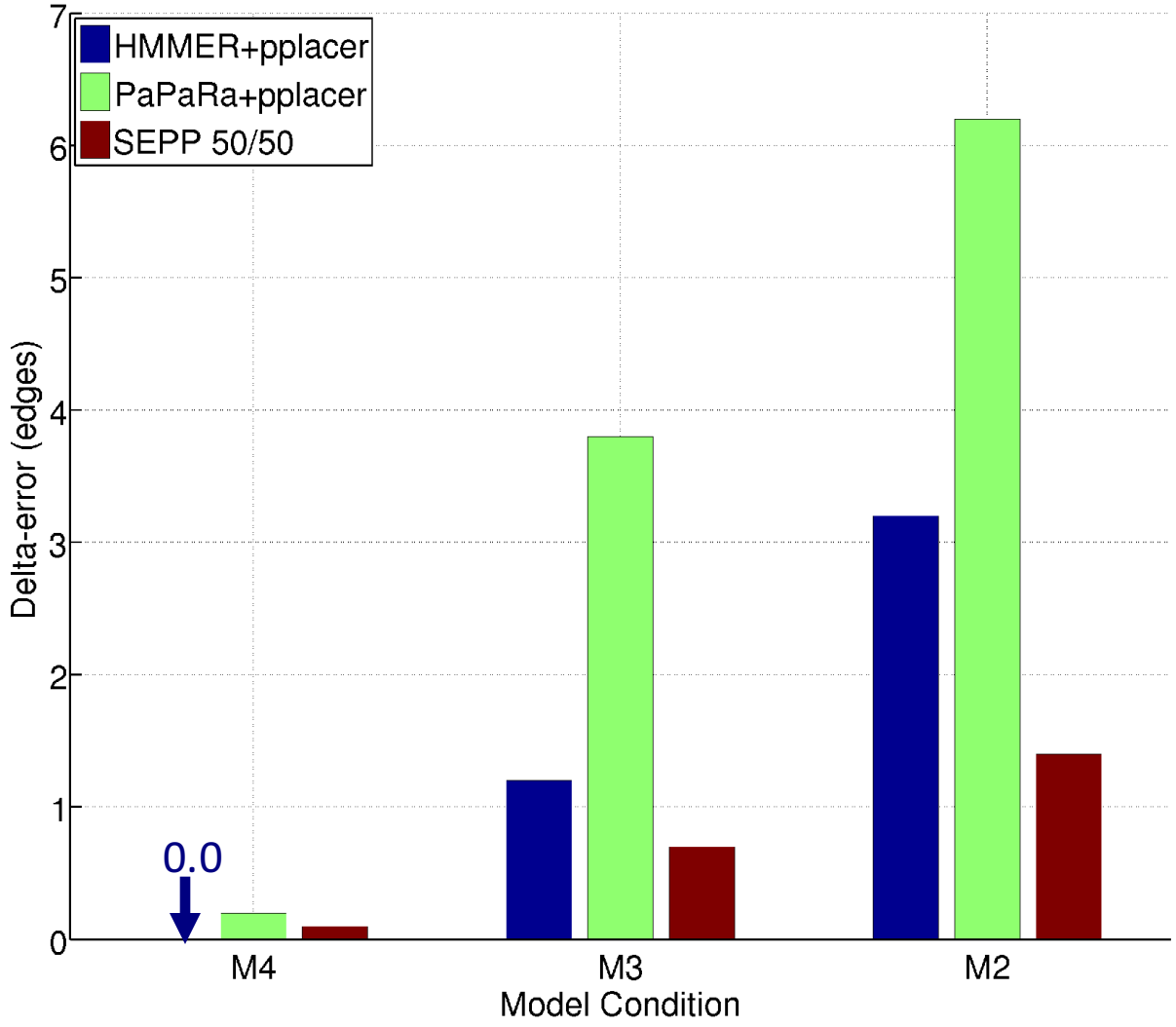
New approach



Ensemble of HHMs (eHMMs)



SEPP (10% rule) Simulated Results



Backbone size: 500
5000 fragments
20 replicates

Using SEPP

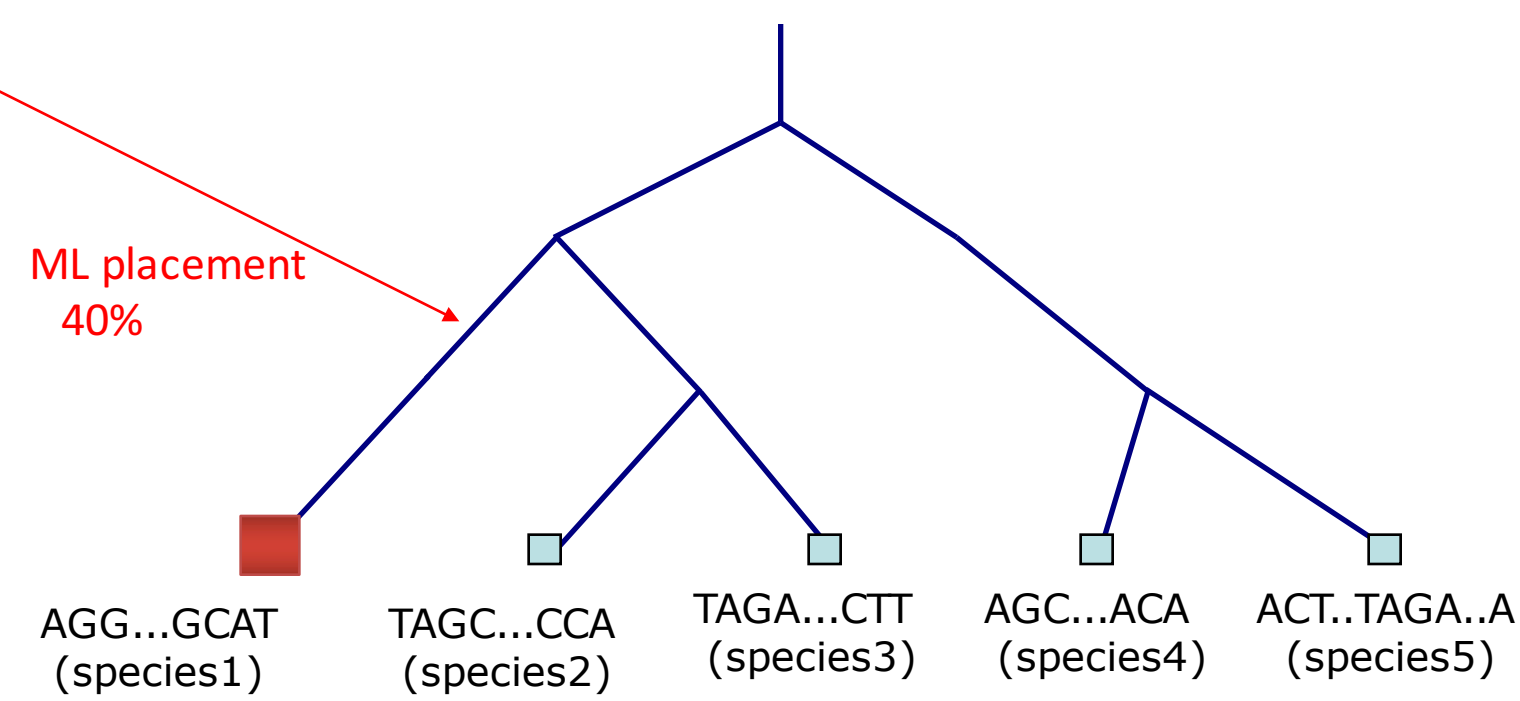
Fragmentary Unknown Reads:

(60-200 bp long)

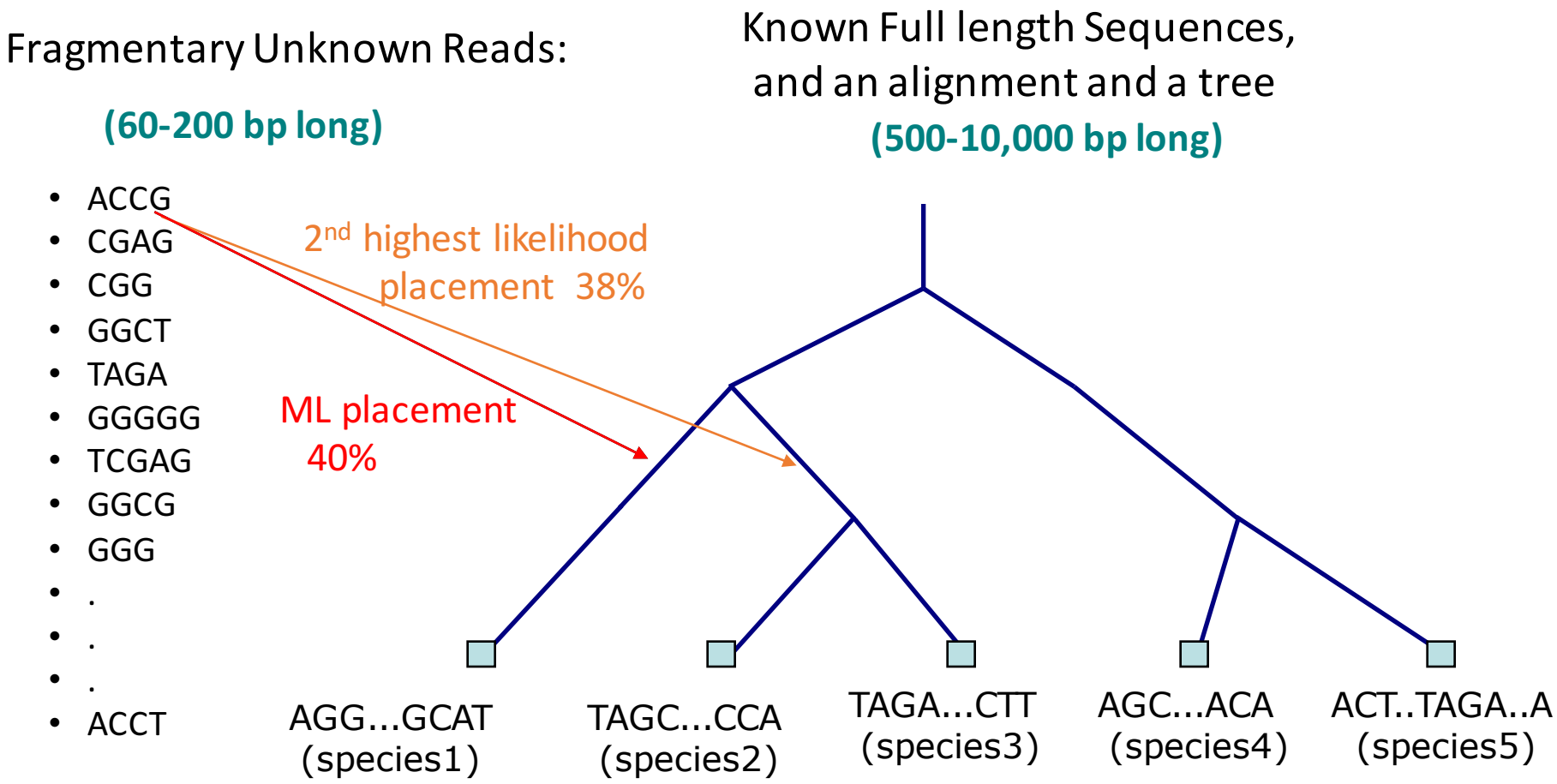
- ACCG
- CGAG
- CGG
- GGCT
- TAGA
- GGGGG
- TCGAG
- GGCG
- GGG
- .
- .
- .
- ACCT

Known Full length Sequences,
and an alignment and a tree

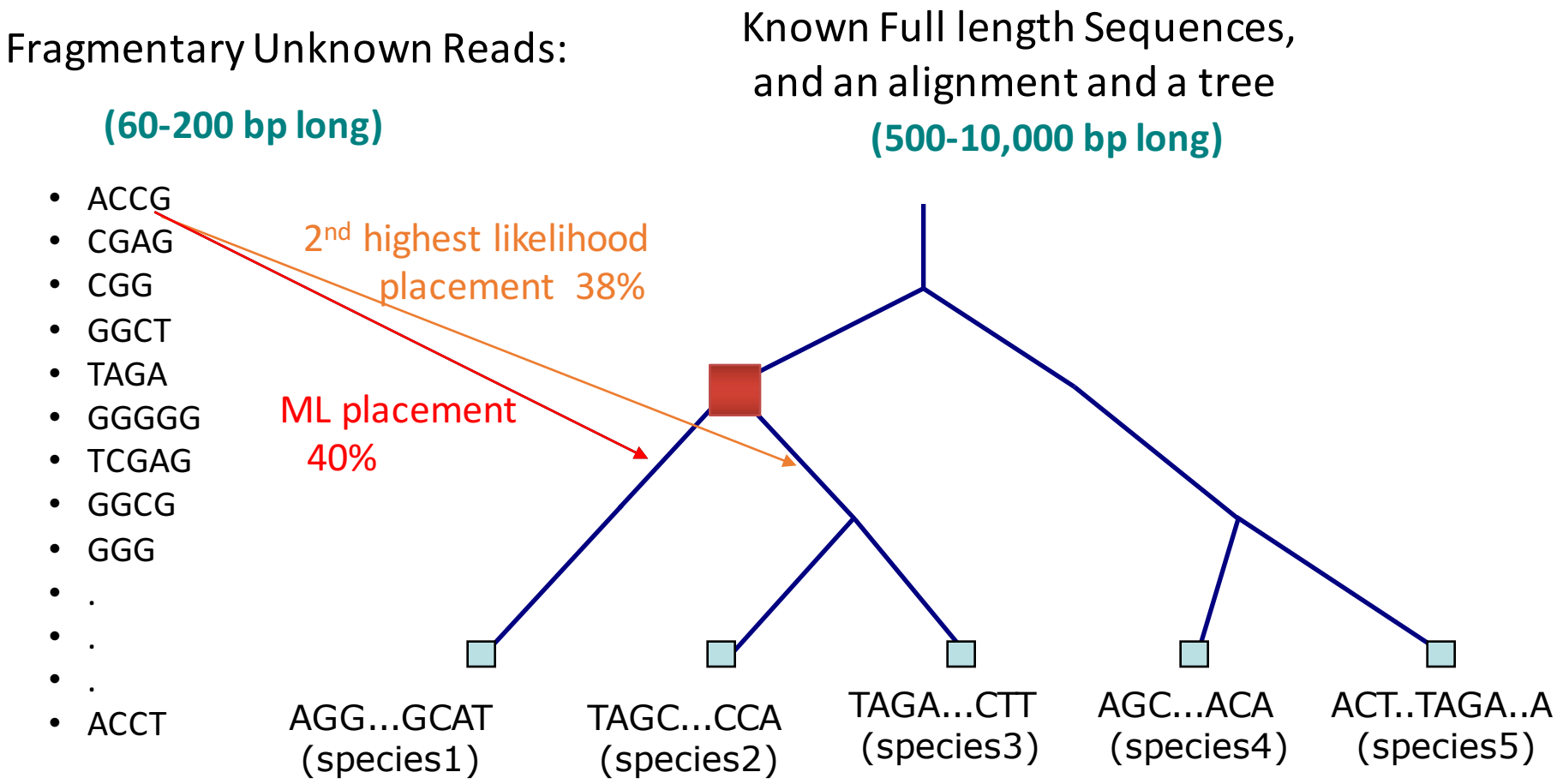
(500-10,000 bp long)



Adding Uncertainty

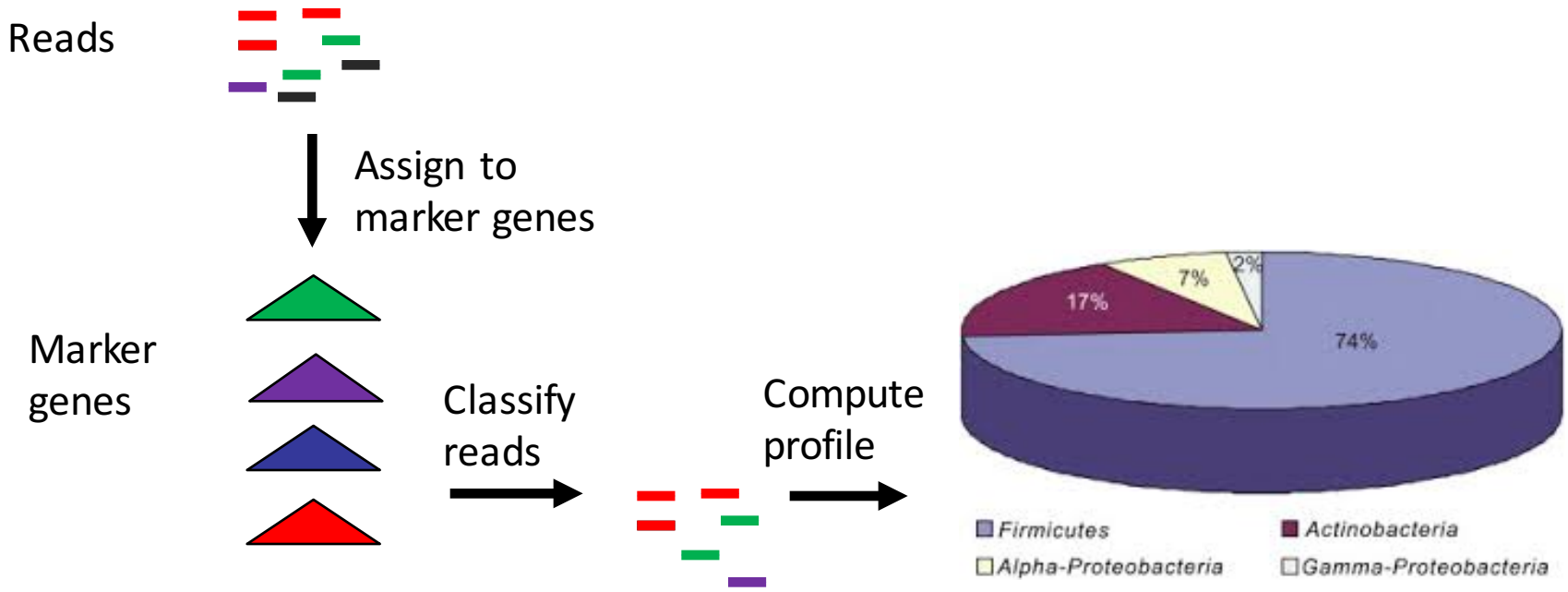


Adding Uncertainty



TIPP: **T**axonomic **I**dentification And **P**hylogenetic **P**rofiling

- Nguyen et al., Bioinformatics, 2014



Reference package

- Alignment (sate.fasta)
- Refined taxonomic tree (sate.taxonomy)
- Rate parameters (sate.taxonomy.RAxML_info)
- Taxonomy file (all_taxon.taxonomy)
- Sequence mapping (species.mapping)

Looking at results

- Pooled abundance profiles

- Results for each individual marker

16S amplicon analysis

- Change to the 16S_bacteria test directory
  - cd ../16S_bacteria

- Run TIPP on the gut data
  - run_tipp.py -R 16S_bacteria -f human_gut_16S.fas -o 16s -A 1000 -P 1000