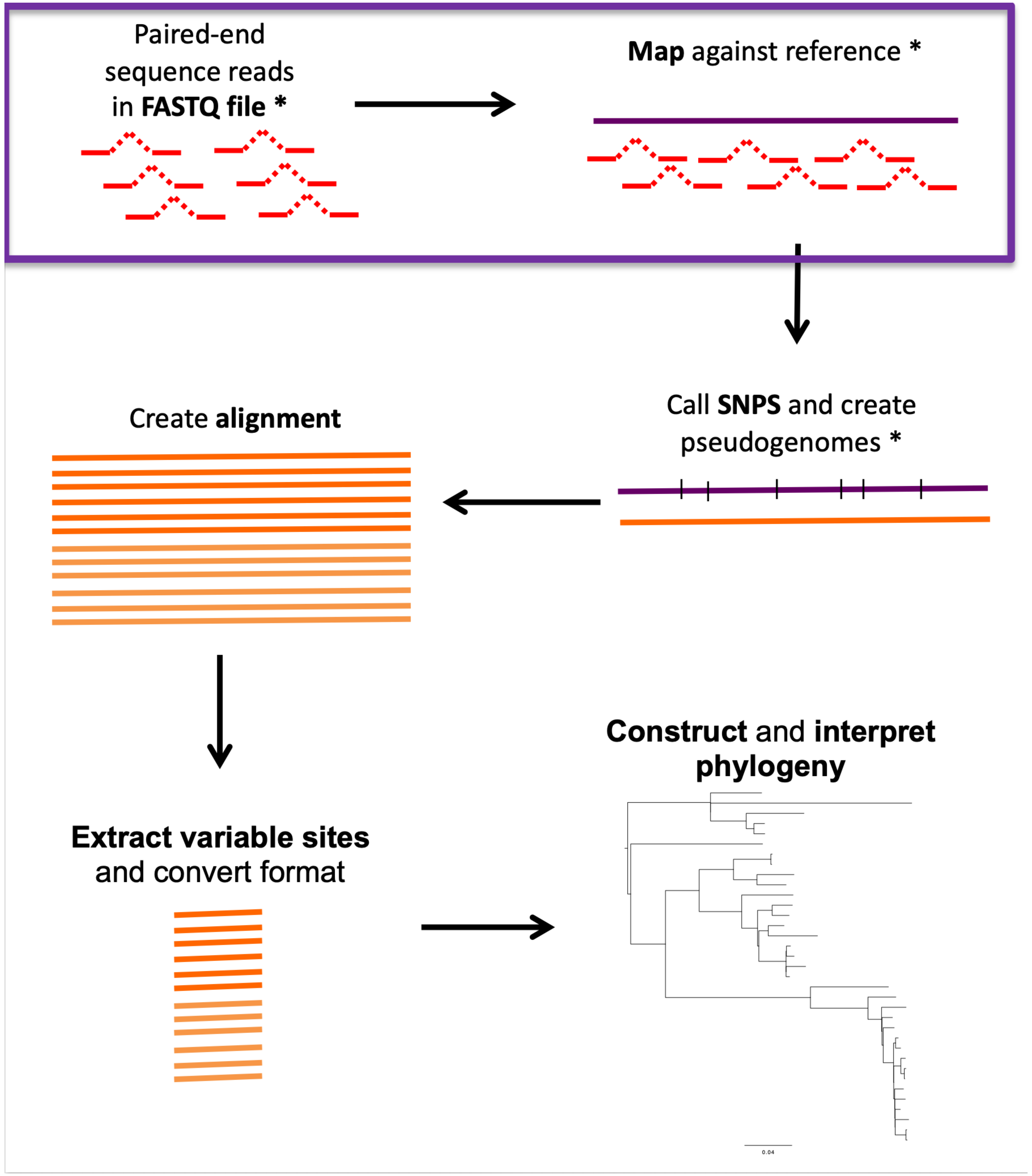
LINEAGE ASSIGNMENT AND PHYLOGENETICS

Today’s Agenda

- ✓ Lecture
- ✓ SARS-CoV-2 lineage calling with Pango and NextClade
- ✓ Align sequences with Mafft
- ✓ Phylogeny



Paired-end sequence reads in **FASTQ file** *

Map against reference *

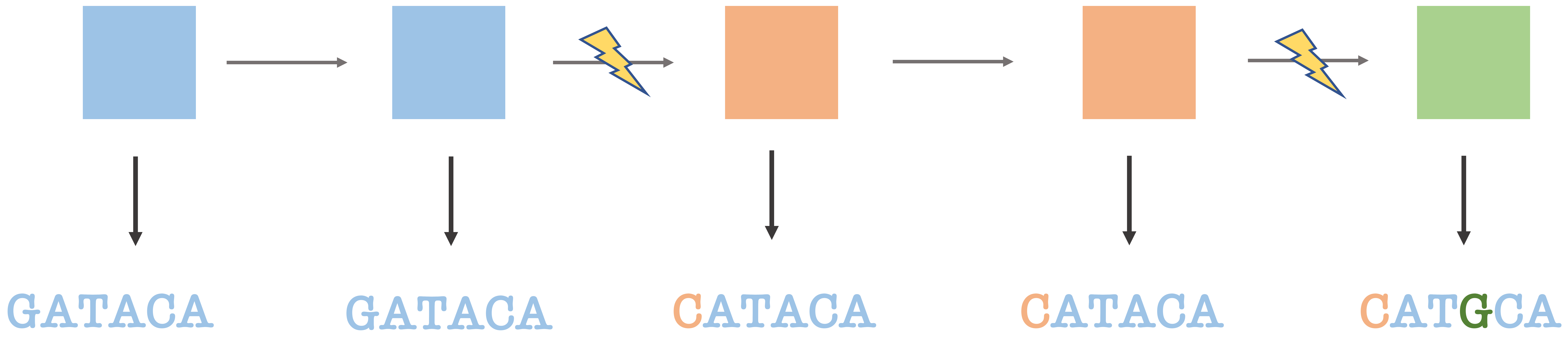
Call **SNPS** and create pseudogenomes *

Create **alignment**

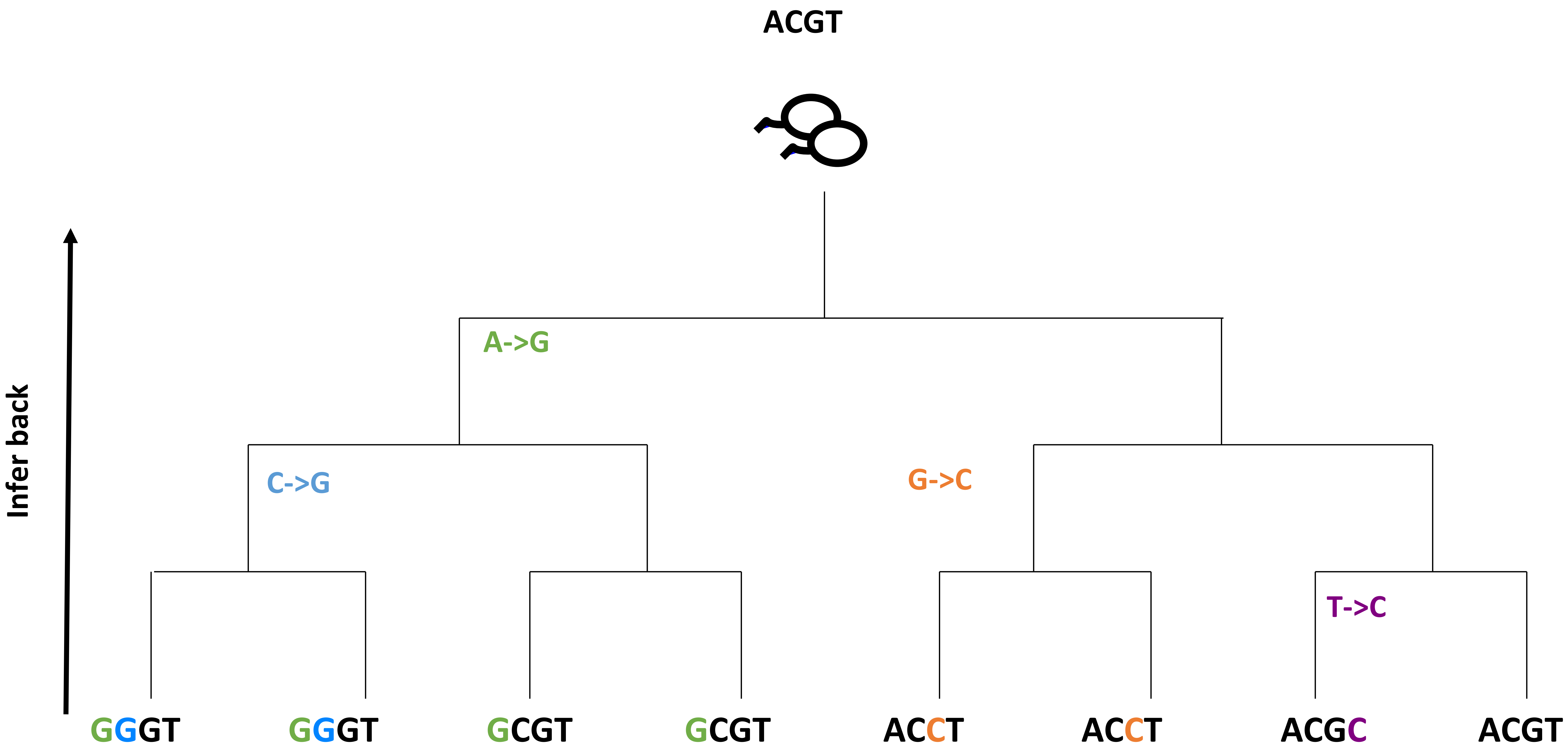
Extract variable sites and convert format

Construct and **interpret phylogeny**

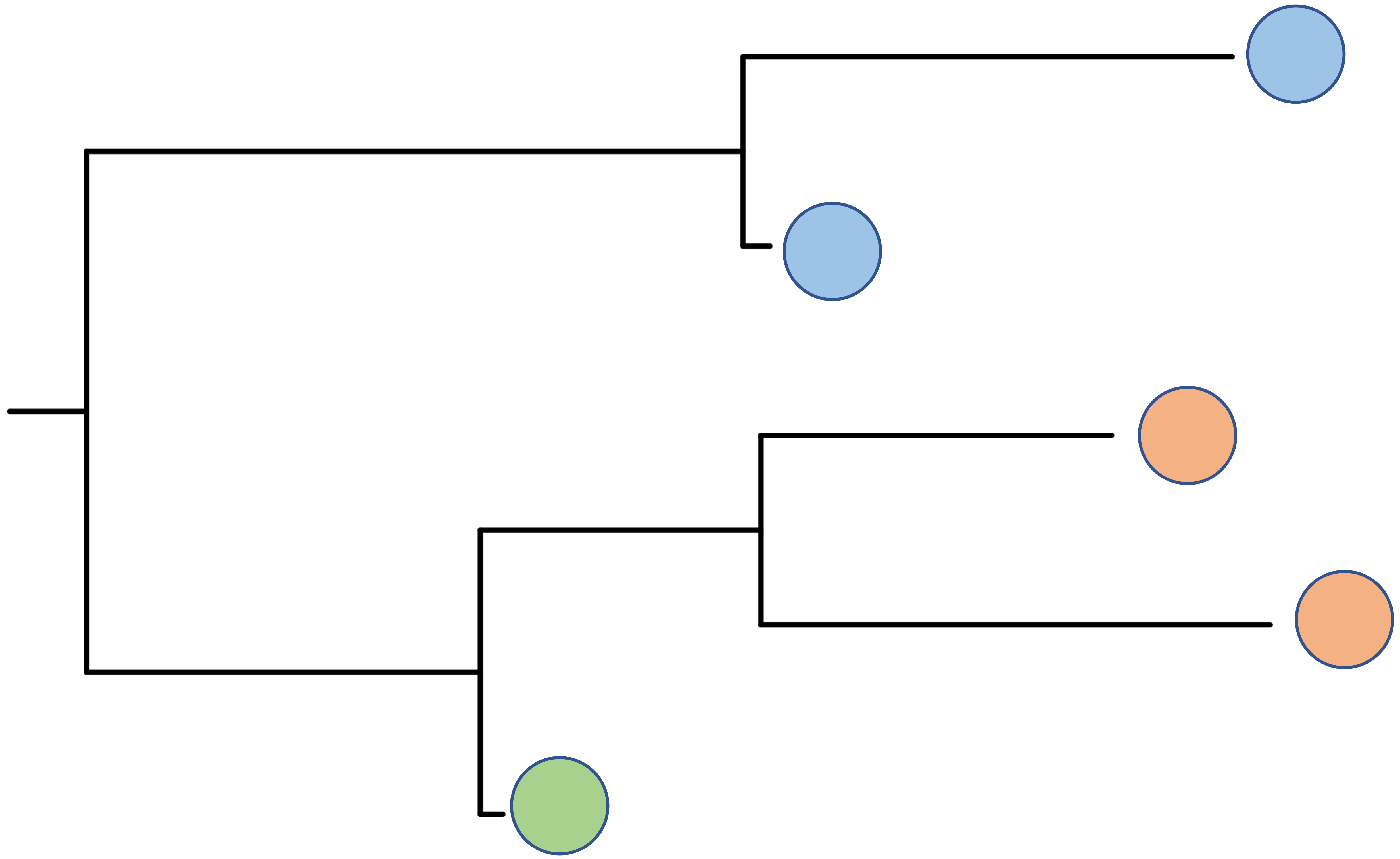
Organisms acquire mutations



Mutations tell us about relationships

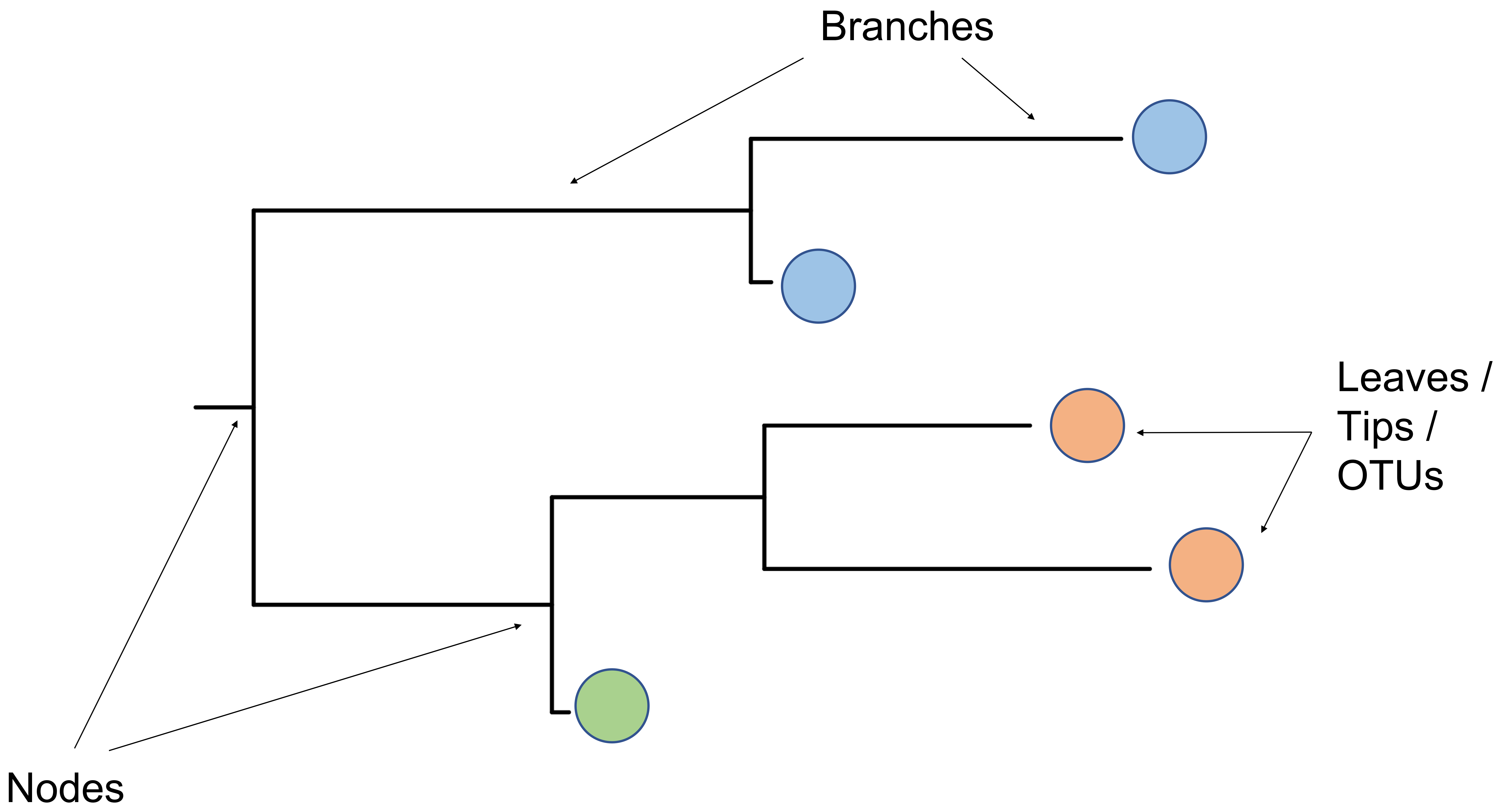


Phylogenetic trees reveal relationships

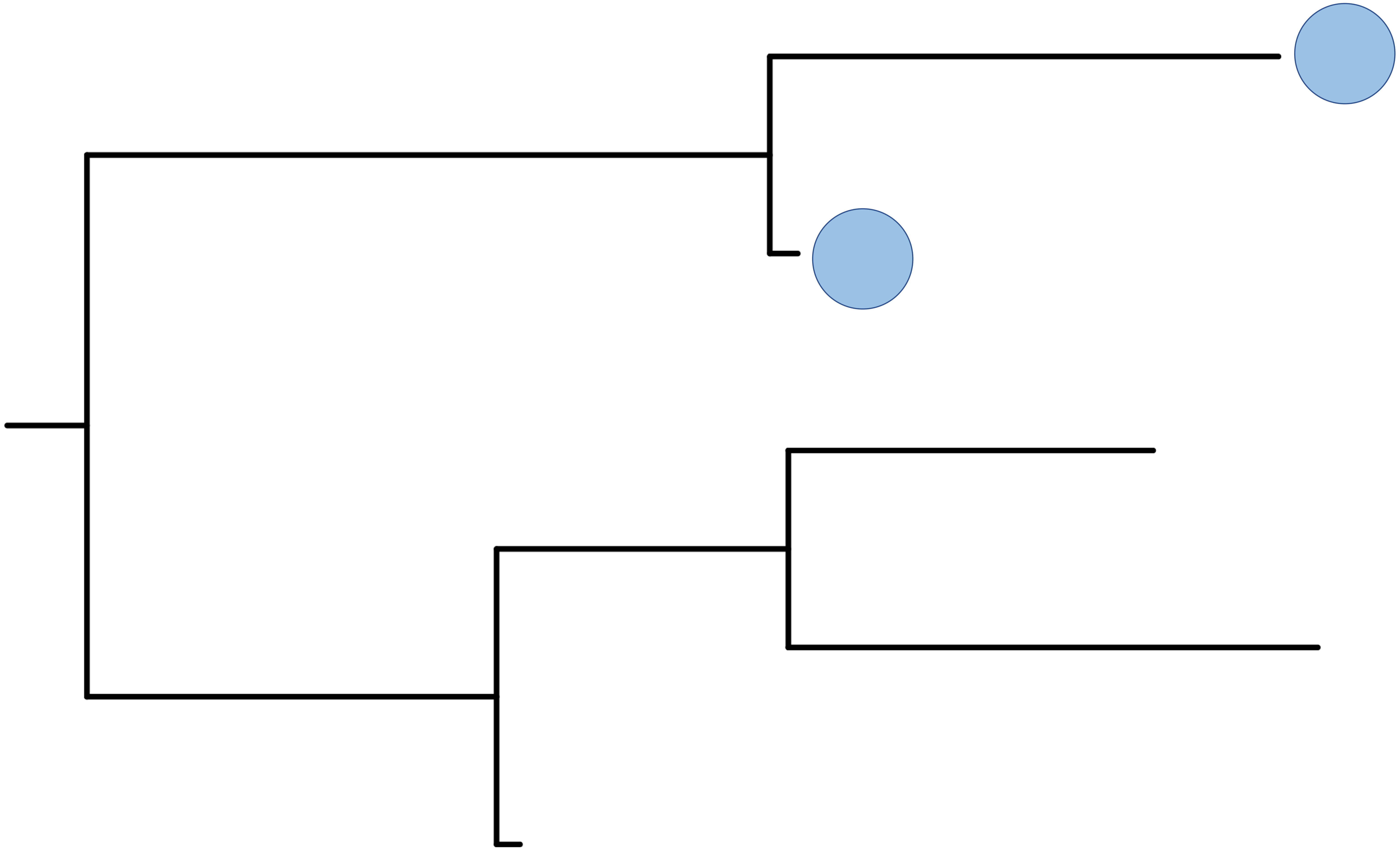


Genetic similarity

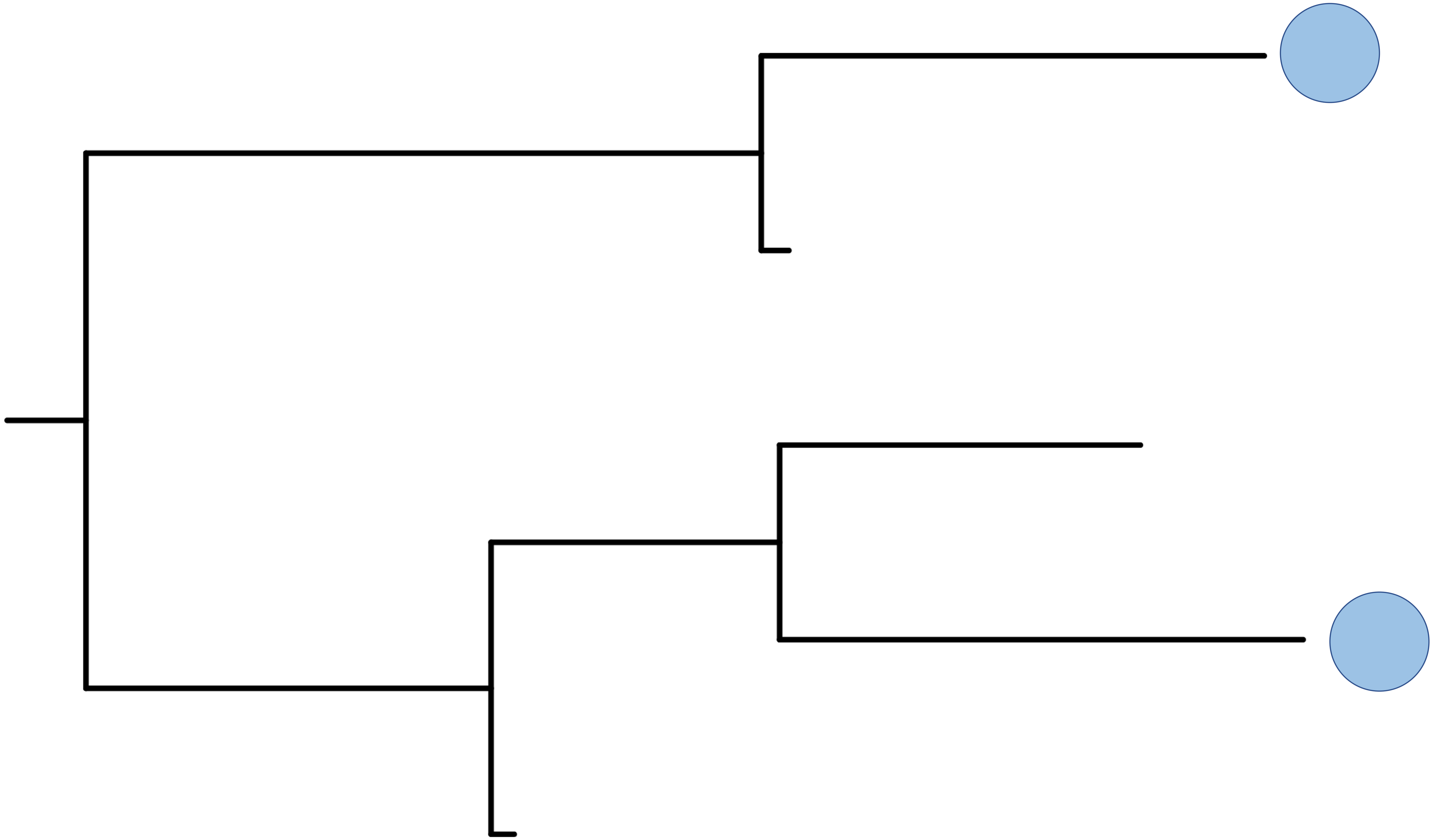
Phylogenetic trees



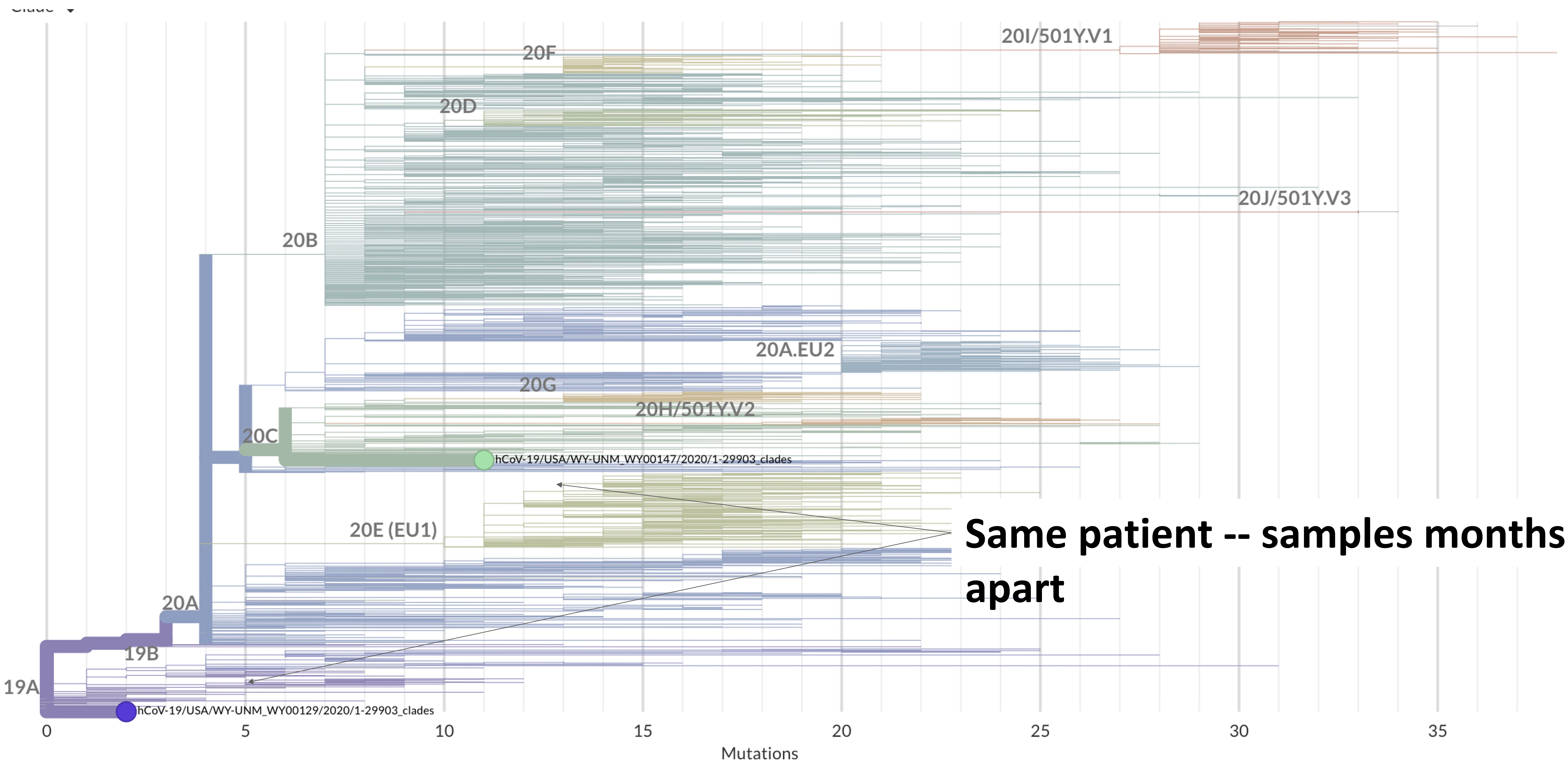
Links between positive cases



Or not



Reinfection or chronic infection?



Same patient -- samples months apart

Building a Phylogenetic Tree

Identify protein, DNA or RNA sequences of interest

Fasta format file of concatenated sequences

Multiple sequence alignment

ClustalX, Muscle, Mafft

Construct phylogeny

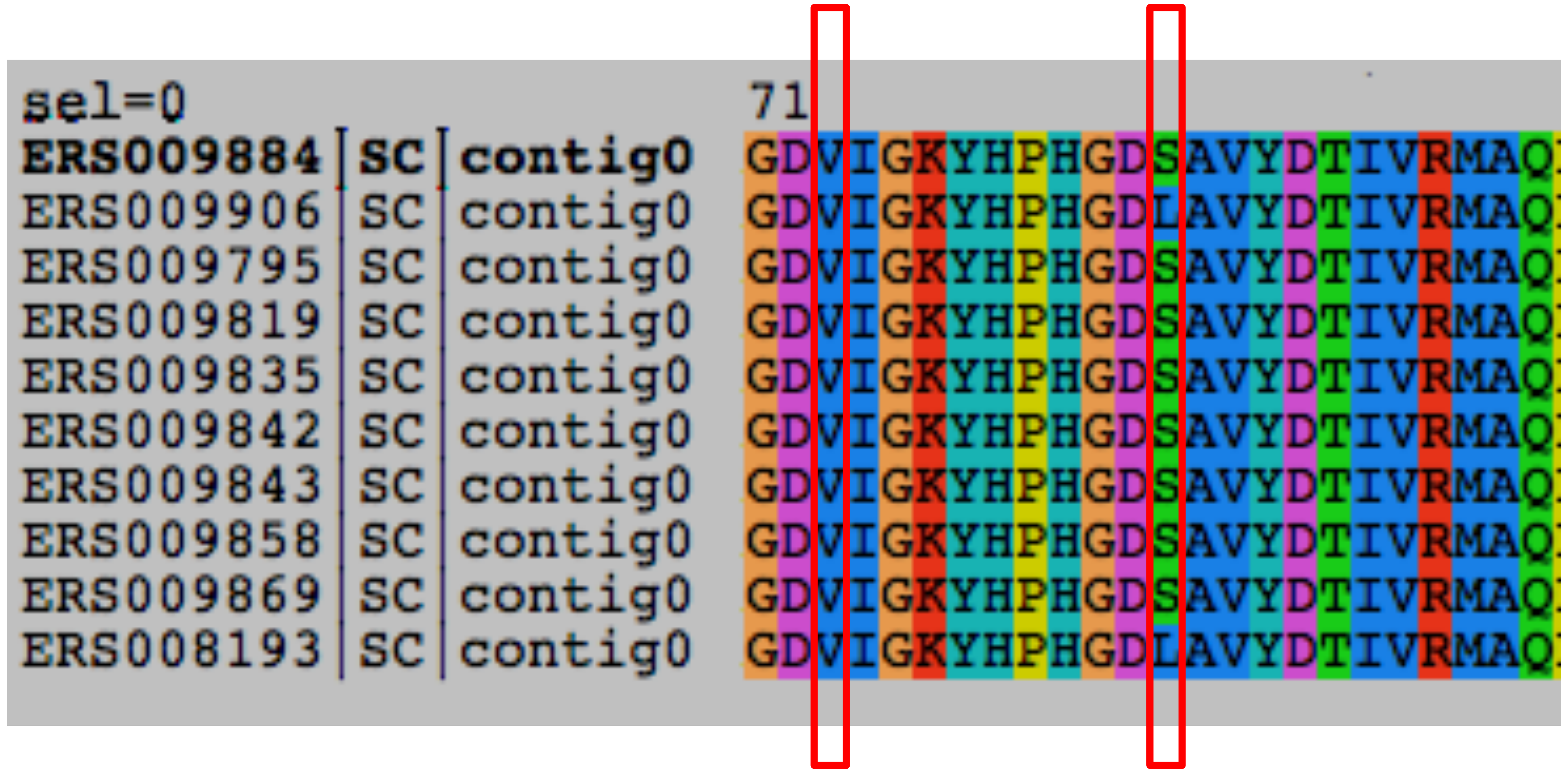
PHYML, RAxML, IQ-Tree, FastTree

View and edit tree

FigTree

Multiple sequence alignment (MSA)

MSA is best hypothesis of **positional homology** between bases/amino acids of different sequences



```
sel=0                        71
ERS009884|SC|contig0  GDVIGKYHPHGDSAVYDTIVRMAQ
ERS009906|SC|contig0  GDVIGKYHPHGDLAVYDTIVRMAQ
ERS009795|SC|contig0  GDVIGKYHPHGDSAVYDTIVRMAQ
ERS009819|SC|contig0  GDVIGKYHPHGDSAVYDTIVRMAQ
ERS009835|SC|contig0  GDVIGKYHPHGDSAVYDTIVRMAQ
ERS009842|SC|contig0  GDVIGKYHPHGDSAVYDTIVRMAQ
ERS009843|SC|contig0  GDVIGKYHPHGDSAVYDTIVRMAQ
ERS009858|SC|contig0  GDVIGKYHPHGDSAVYDTIVRMAQ
ERS009869|SC|contig0  GDVIGKYHPHGDSAVYDTIVRMAQ
ERS008193|SC|contig0  GDVIGKYHPHGDLAVYDTIVRMAQ
```

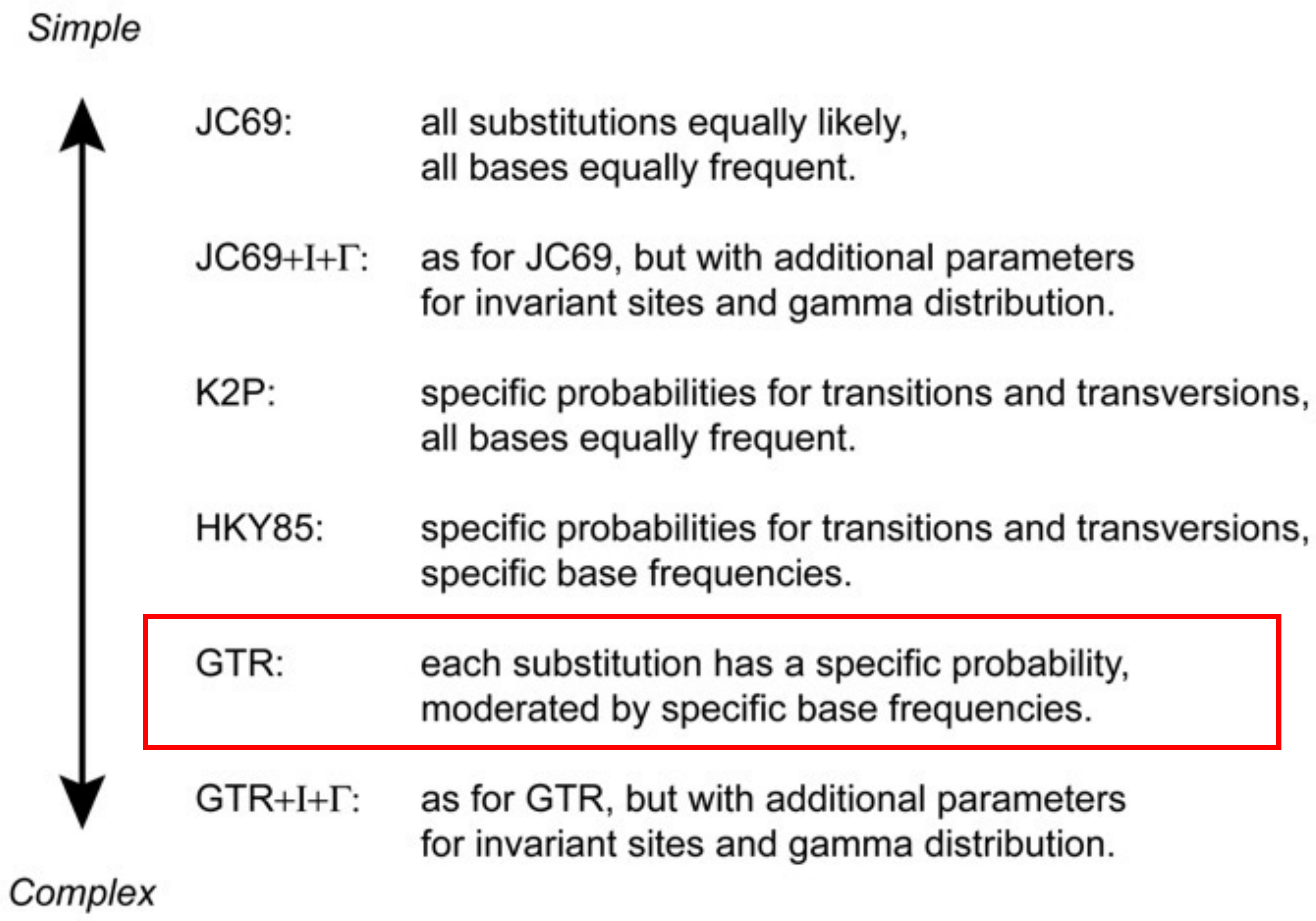
This is perhaps most important step!!

Crap in == Crap out!

Constructing a phylogenetic tree

Method	Data used	Tree search	Evolutionary Model
Distance	Pairwise distance	Simple algorithm	Can be complex
Parsimony	All sites	Mainly hill climbing	Simple
Maximum likelihood	All sites	Hill climbing	Can be complex
Bayesian Methods	All sites (+ other info)	MCMC	Can be very complex

Maximum likelihood phylogenetic models



Simple

JC69: all substitutions equally likely, all bases equally frequent.

JC69+I+Γ: as for JC69, but with additional parameters for invariant sites and gamma distribution.

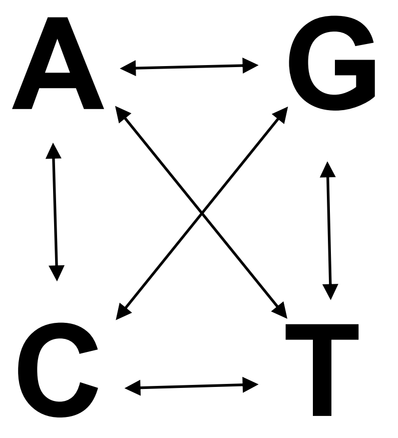
K2P: specific probabilities for transitions and transversions, all bases equally frequent.

HKY85: specific probabilities for transitions and transversions, specific base frequencies.

GTR: each substitution has a specific probability, moderated by specific base frequencies.

GTR+I+Γ: as for GTR, but with additional parameters for invariant sites and gamma distribution.

Complex

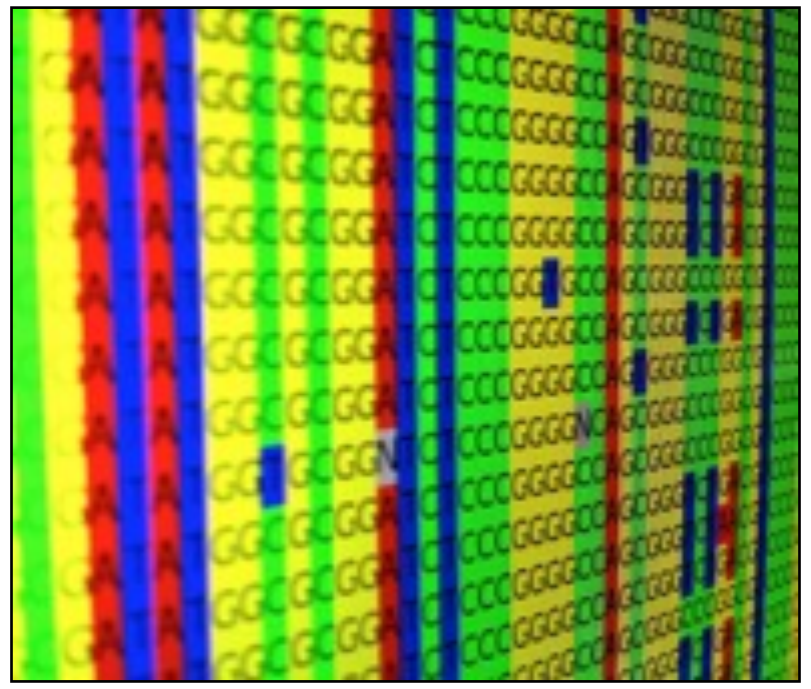


4 equilibrium base frequency parameters and 6 substitution rate parameters and

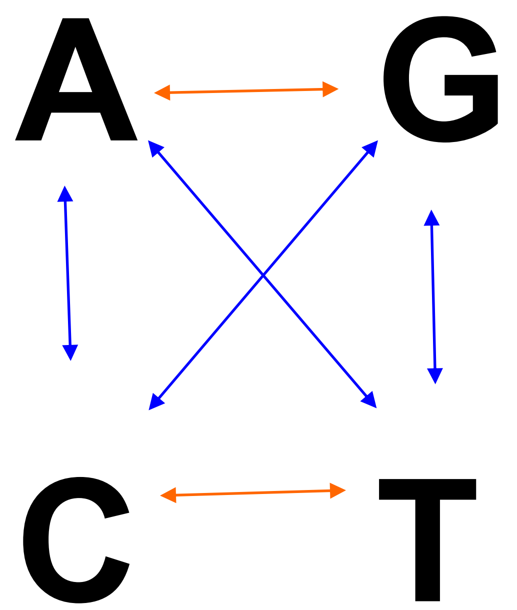
Putting it together

Maximum likelihood phylogenetic models maximize the probability of achieving …

these data…

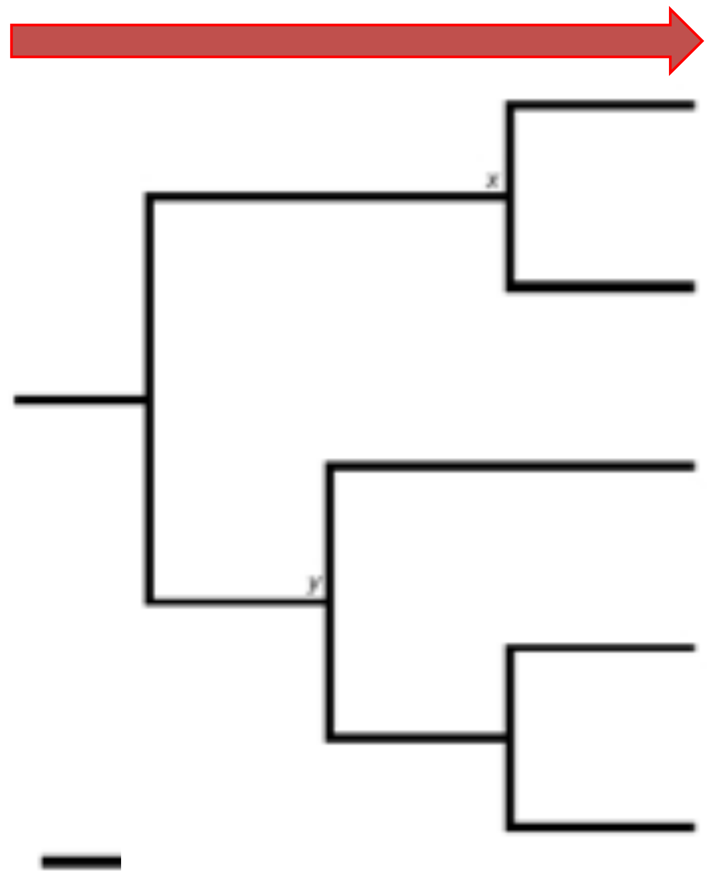


… if this happens…



+ composition

… over this tree



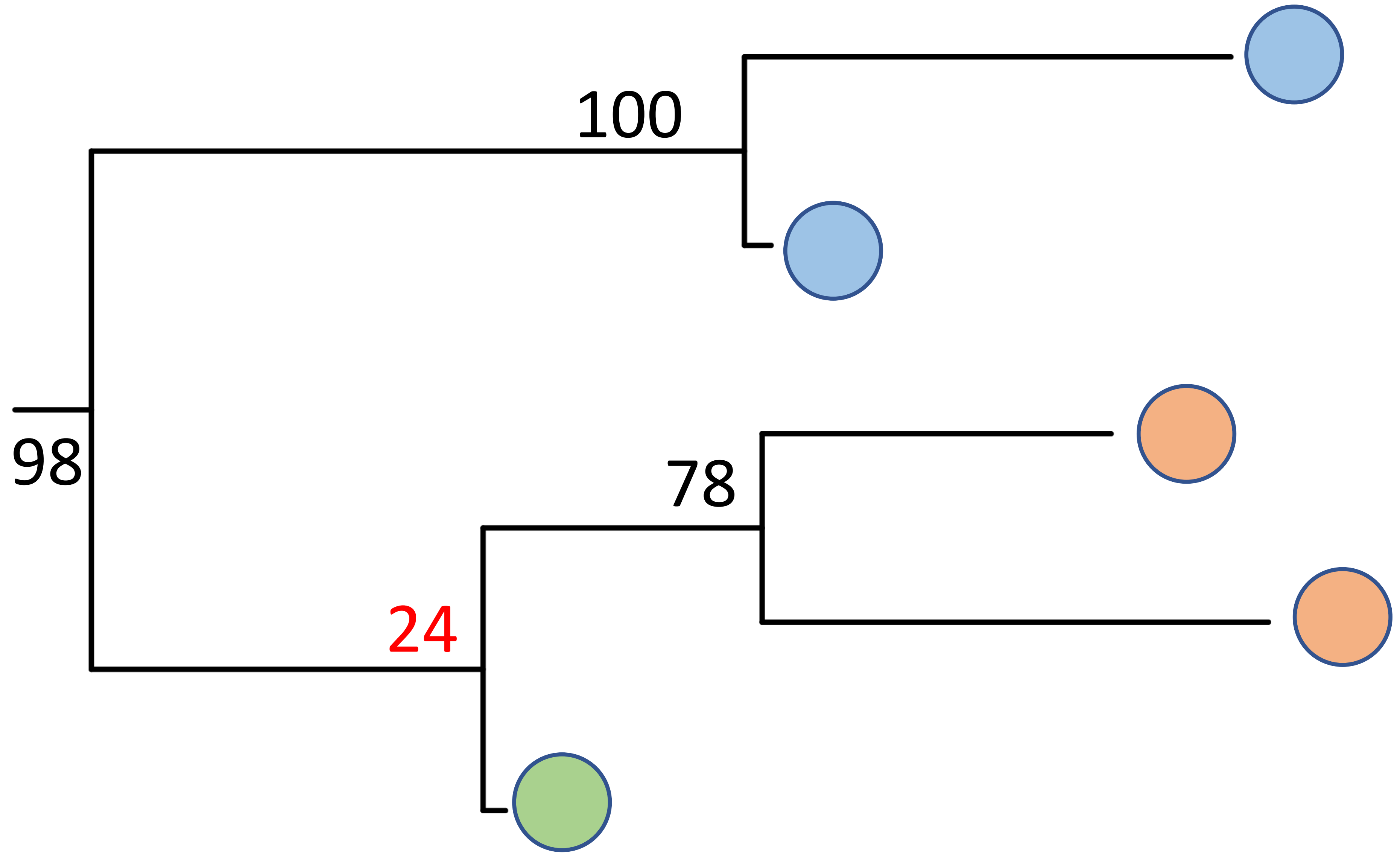
Gaining confidence : Bootstrapping

Bootstrapping is a way to produce a confidence measure in the topology relationships found in a phylogenetic analysis

X number of **bootstraps** (resampled replicates) are created of your input data (MSA)

Typically run 100 – 1,000 bootstraps for ML analysis

These are commonly used as a measure of support for these branches and are represented as a number on each tree branch



Questions?