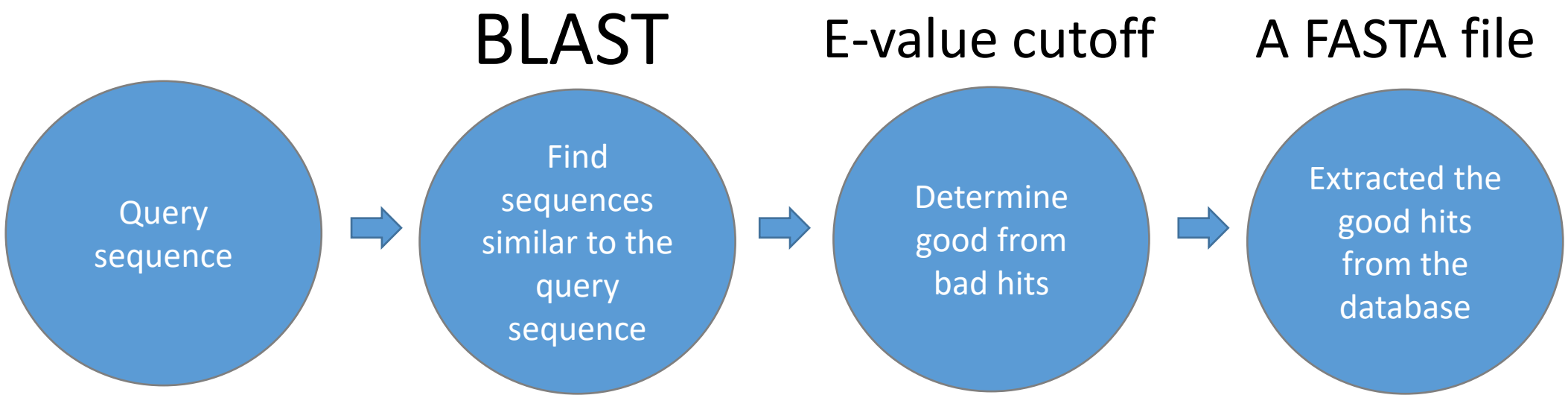
MULTIPLE SEQUENCE ALIGNMENTS

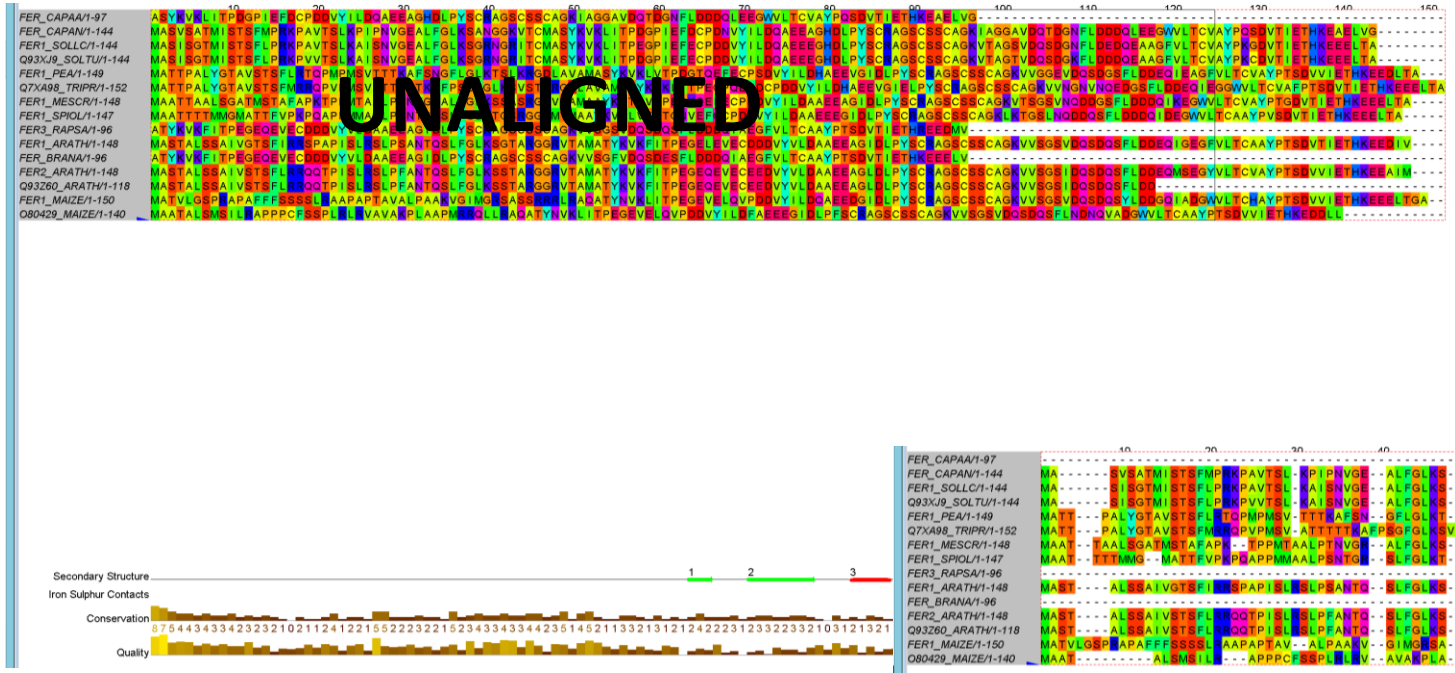
Line up of homologous positions

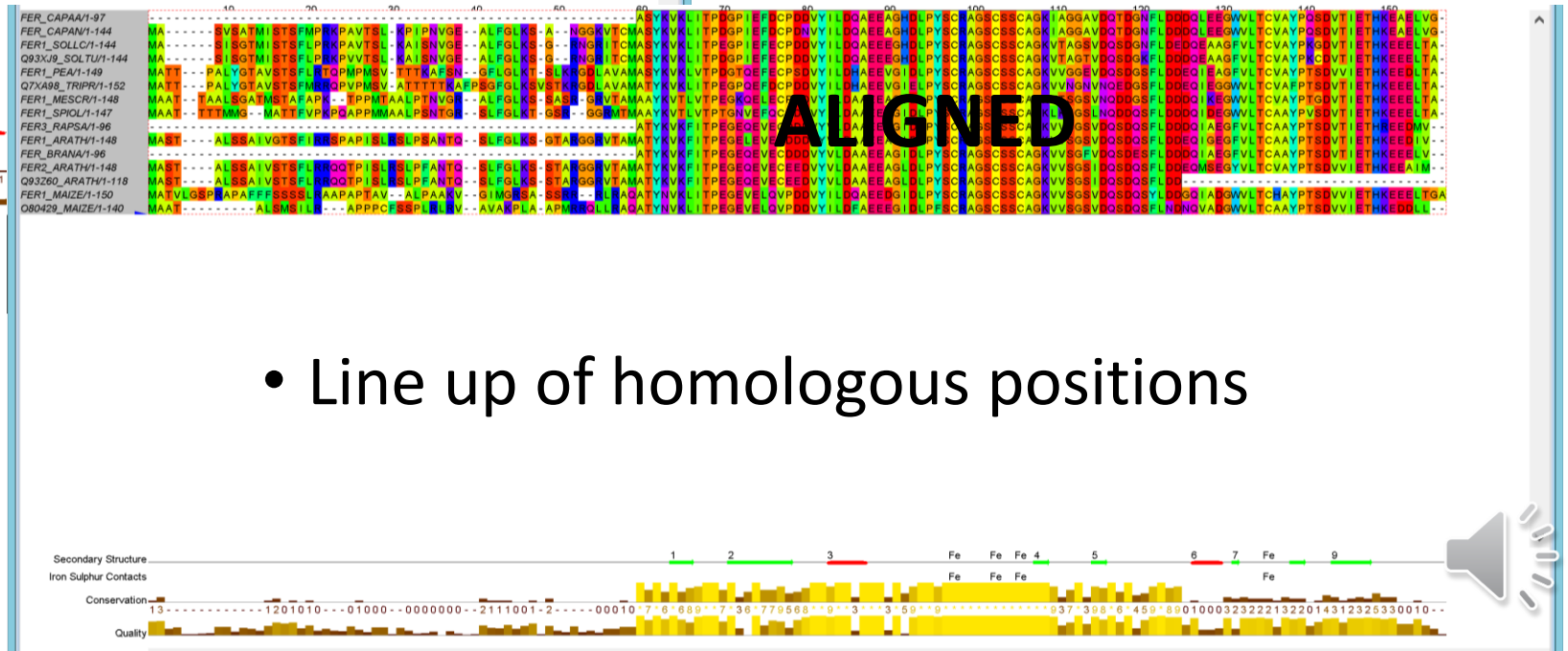

BLAST
E-value cutoff
A FASTA file
Query sequence
Find sequences similar to the query sequence
Determine good from bad hits
Extracted the good hits from the database

Why do we need so many sequences?

- Comparative studies, more information
  - Even if we are only interested in one particular sequence
- What other species has a similar sequence?
  - When did this gene appear?
  - Has it been lost in some species or taxonomic group?
  - When did potential gene duplications happen?

Why do we need to align our sequences?





- Line up of homologous positions

How to align multiple sequences?

Multiple Alignment Algorithms

- ClustalW
- Muscle
- Mafft
- T-coffee
- And more
- http://www.ebi.ac.uk/Tools/msa/ or Jalview or other

FASTA FILE

```
>SEQ_A
THIESRPSRL
>SEQ_B
TRIESRPSRL
>SEQ_C
HIESRRSR
>SEQ_D
TVIESKPSRL
>SEQ_E
SQHVESRQSR
```

Add the pairwise sequence identities to complete the table.

	SEQ_A	SEQ_B	SEQ_C	SEQ_D	SEQ_E
SEQ_B	90				
SEQ_C					
SEQ_D					
SEQ_E					

$$\text{Distance} = 1 - \frac{pairwise\ sequence\ identity\ \%}{100}$$

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	SEQ_A	SEQ_B	SEQ_C	SEQ_D	SEQ_E
SEQ_B	90				
SEQ_C	87.5	75			
SEQ_D	80	80	62.5		
SEQ_E	66.67	55.56	75	44.44	

DISTANCE

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DISTANCE

	SEQ_A	SEQ_B	SEQ_C	SEQ_D	SEQ_E
SEQ_B	0.1				
SEQ_C	0.125	0.25			
SEQ_D	0.2	0.2	0.375		
SEQ_E	0.3333	0.4444	0.25	0.5556	

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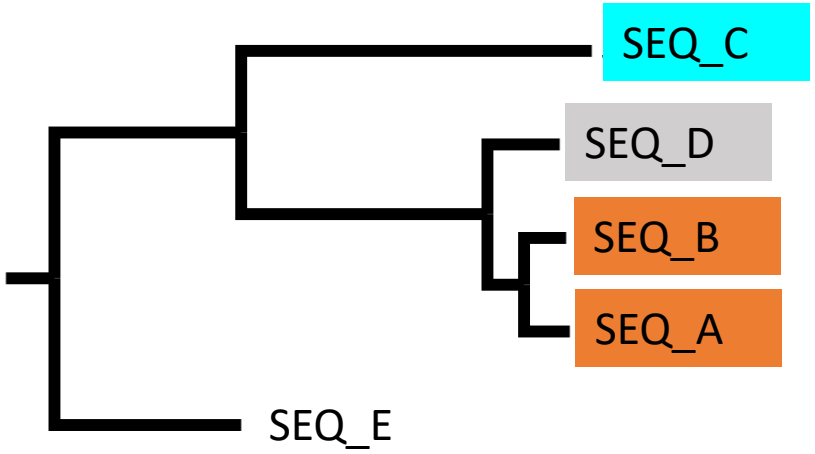
```

Based on the distance matrix, a distance tree is built.



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DISTANCE MATRIX

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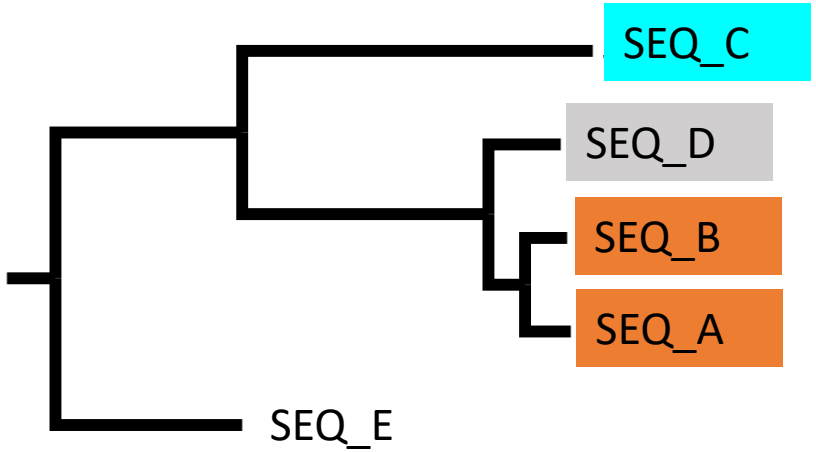

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(We'll get back to tree building. For now, this is a distance tree from Jalview.

The tree is rooted at mid-point.)

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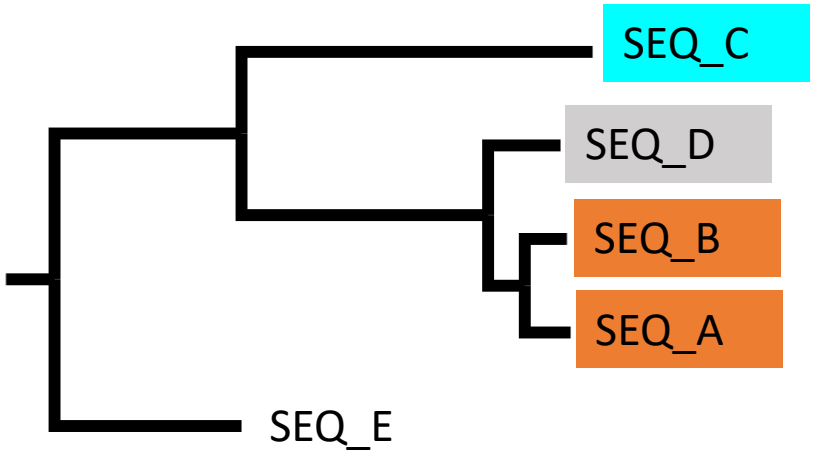

1. Start the progressive alignment with SEQ_A and SEQ B:

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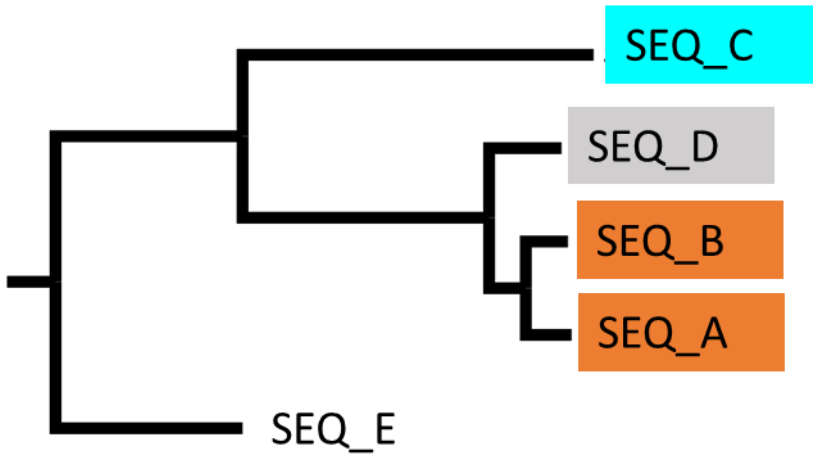
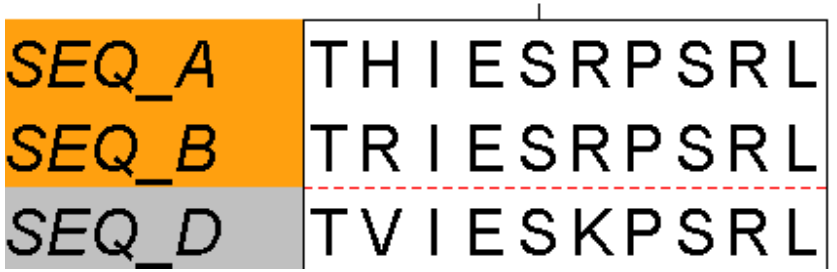


1. Start the progressive alignment with SEQ_A and SEQ_B:

2. Add sequence D:



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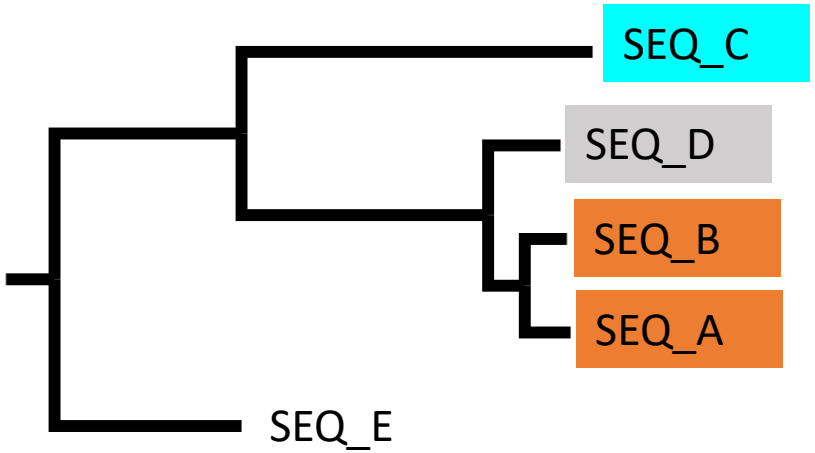
```



1. Start the progressive alignment with SEQ_A and SEQ_B:

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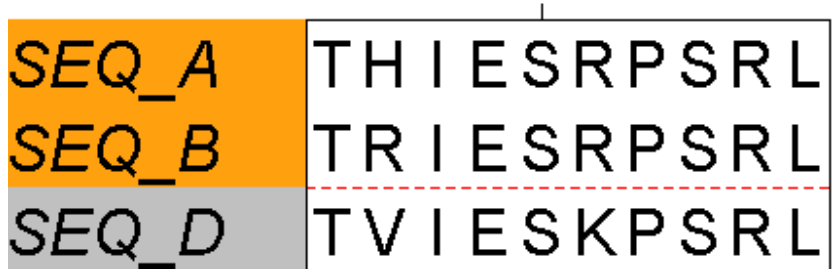

3. And so on…

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```
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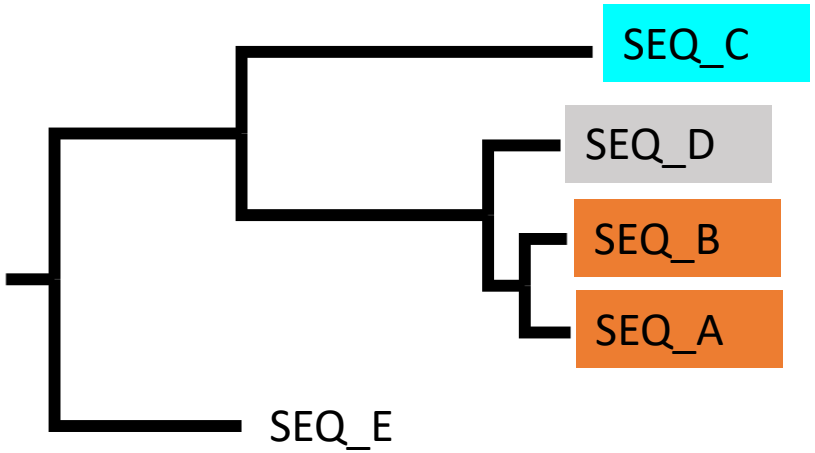

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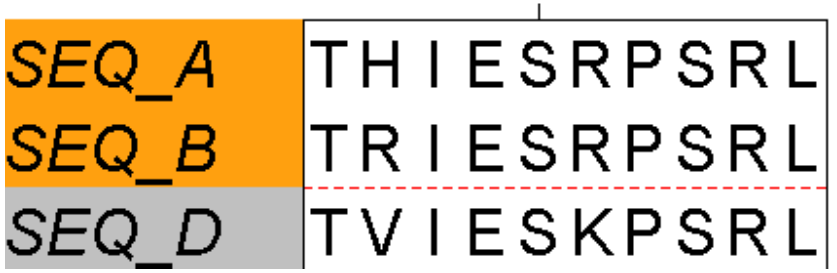


3. And so on…

A PROGRESSIVE MULTIPLE SEQUENCE ALIGNMENT!

Clustal W

1. Fast approximate alignments or full dynamic programming

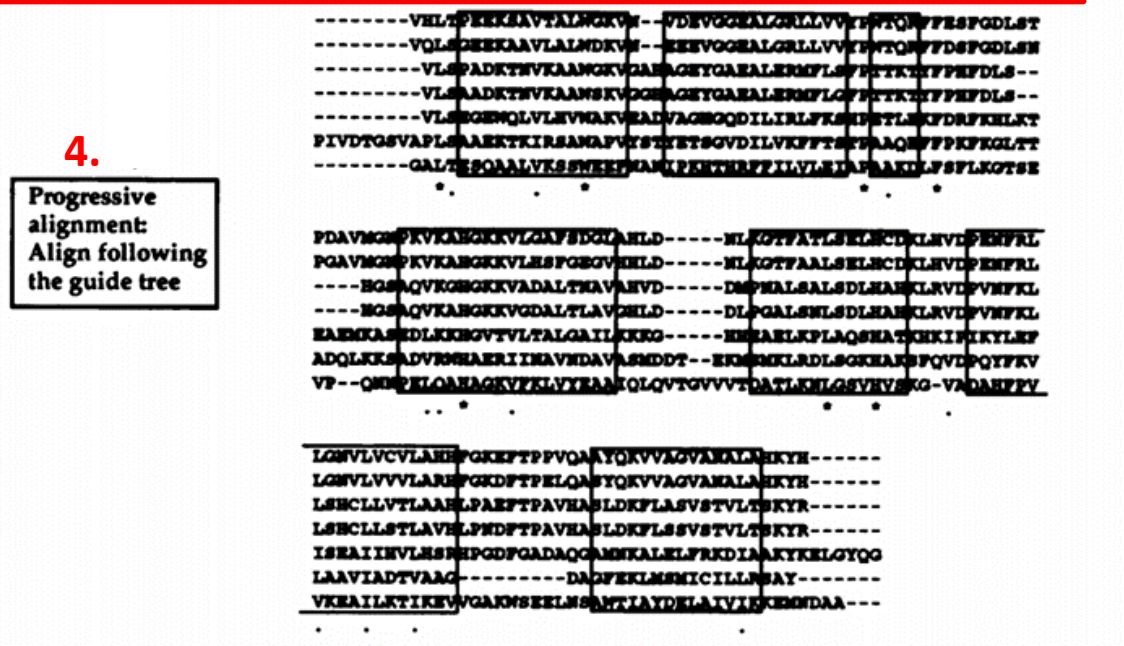
$$\text{Distance} = 1 - \frac{pairwise\ sequence\ identity\ \%}{100}$$

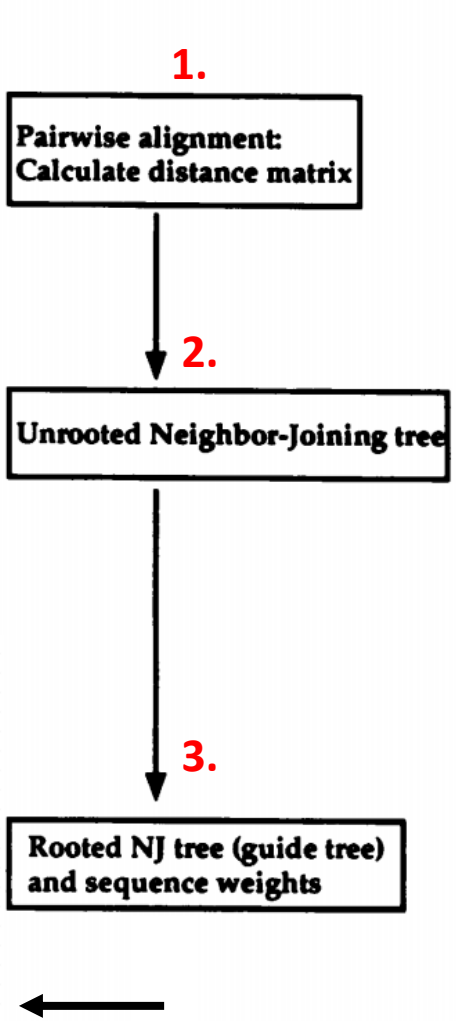
2. Unrooted NJ tree is rooted mid-point => guide tree

3. Sequence weights from branch lengths. Highly similar sequence have low weights.

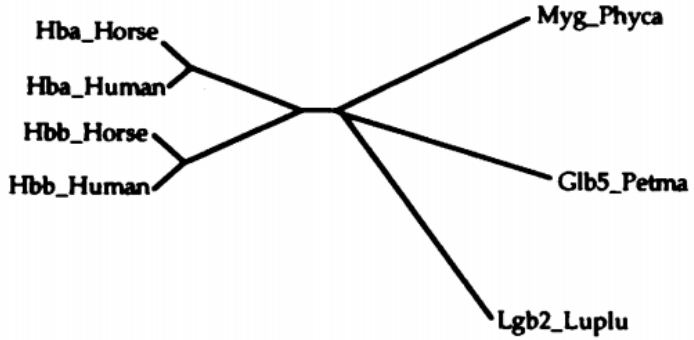
4. Aligning alignments…

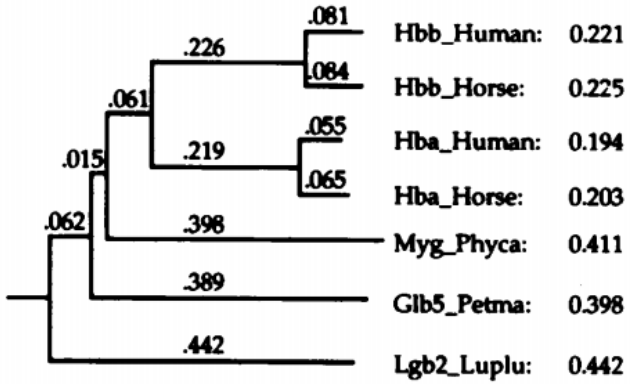
Early alignment errors have no way of being corrected.





		1	2	3	4	5	6
Hbb_Human	1	-					
Hbb_Horse	2	.17	-				
Hba_Human	3	.59	.60	-			
Hba_Horse	4	.59	.59	.13	-		
Myg_Phyca	5	.77	.77	.75	.75	-	
Glb5_Petma	6	.81	.82	.73	.74	.80	-
Lgb2_Luplu	7	.87	.86	.86	.88	.93	.90



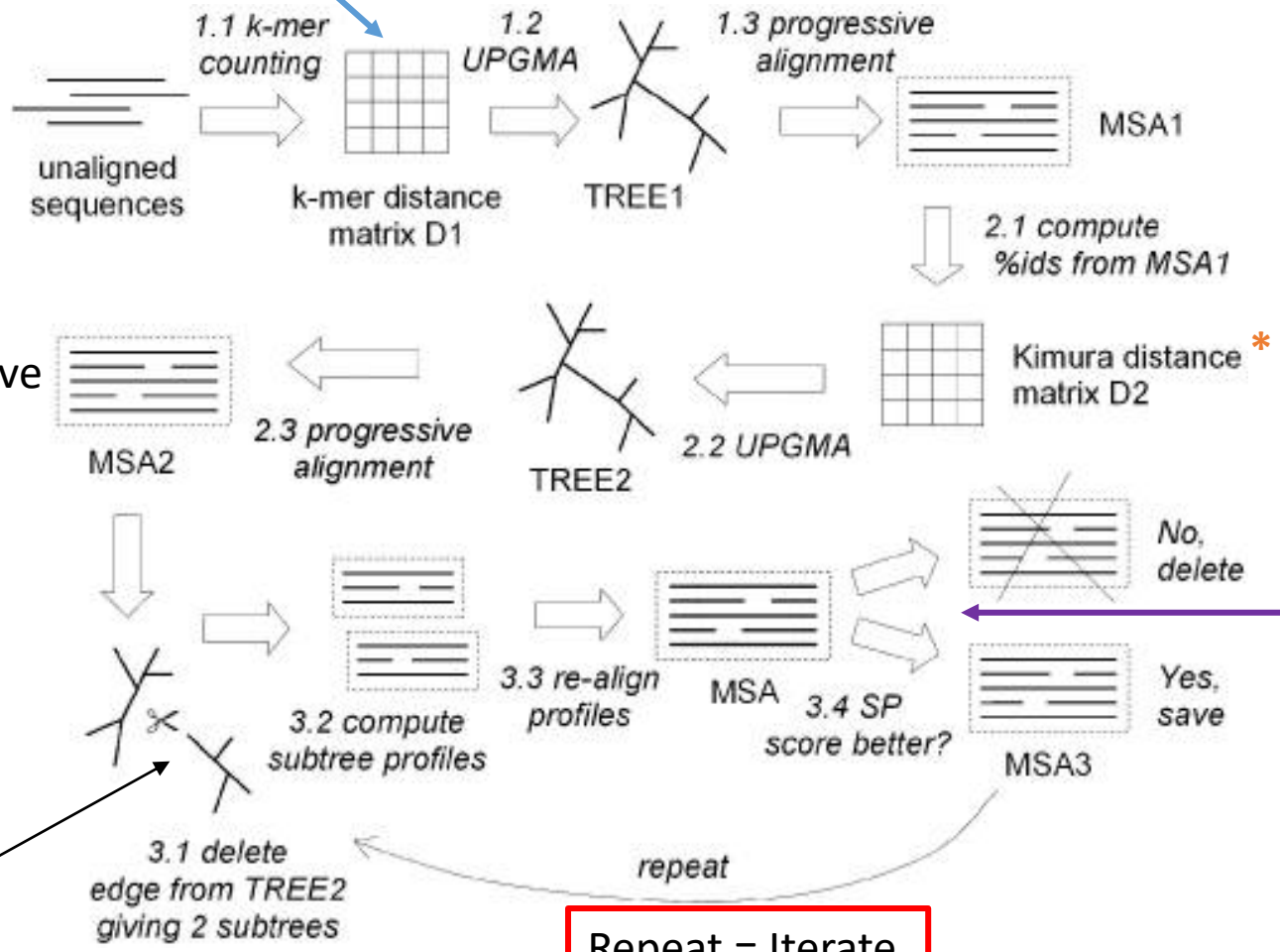


Thompson et al., Nucleic Acids Res. 1994; 22(22): 4673–4680.

Muscle

1. Draft progressive
2. Improved progressive
3. Refinement

How many k-mers match for each pair?



Iterate
For every iteration, each edge is cut once, starting from the leaves and traversing up to the root.

Iterations continue until it reaches convergence or a user-specified number of iterations.

Score = average sum of all pairwise scores

Repeat = Iterate

Amazon?

Adapted from Edgar RC. Nucleic Acids Res. 2004; 32(5): 1792–1797.

*Kimura's protein distance matrix

All gapped sites are ignored

$$S = \frac{\#\ exact\ matches}{Total\ \#\ of\ positions\ scored}$$

$$D = 1 - S \qquad \text{(uncorrected)}$$

$$d = -\ln(1 - D - 0.2\ D^2) \qquad \text{(corrected)}$$

Corrected here means corrected by the relationship between the number of observed amino acid substitutions and the actual amino acid substitutions.

We assume that residues in the same column are homologous!