

Alignment

The main goal is to compare some multiple alignment algorithms.

1. Materials and methods

I work with nucleotide sequences of gene of translation termination factor (SUP35) from different species of yeasts

In this project I use the following programmes:

- CLUSTAL 2.1
- MUSCLE v3.8.1551
- MAFFT v7.475
- Kalign (3.3)
- T-COFFEE Version_13.41.0.28bdc39
- PRANK v.170427
- transeq (Version: EMBOSS:6.6.0.0)
- getorf (Version: EMBOSS:6.6.0.0)

2. Using 6 various alignment algorithms on 10 DNA sequences

clustalw

By default we generate the alignment in clustalw format

```
clustalw -INFILE=./data/raw_data/SUP35_10seqs.fa
```

time:

```
real    0m3,747s
user    0m3,732s
sys     0m0,015s
```

Determining the output file:

```
clustalw -INFILE=./data/raw_data/SUP35_10seqs.fa -OUTFILE=./data/processed_data/SUP35_10seqs.clustalw
```

Generating the output in fasta format:

```
clustalw -INFILE=./data/raw_data/SUP35_10seqs.fa -OUTPUT=FASTA -OUTFILE=./data/processed_data/SUP35_10seqs.clustalw.fa
```

muscle

```
muscle -in ./data/raw_data/SUP35_10seqs.fa -out ./data/processed_data/SUP35_10seqs_muscle.fa
```

time:

```
real    0m4,742s
user    0m4,712s
sys     0m0,022s
```

mafft

```
mafft --auto ./data/raw_data/SUP35_10seqs.fa >./data/raw_data/SUP35_10seqs_mafft.fa
```

time:

```
real    0m4,486s
user    0m4,351s
sys     0m0,156s
```

kalign

```
kalign <./data/raw_data/SUP35_10seqs.fa >./data/processed_data/SUP35_10seqs_kalign.fa
```

time:

```
real    0m0,242s
user    0m0,357s
sys     0m0,008s
```

t_coffee

```
t_coffee -infile=./data/raw_data/SUP35_10seqs.fa -outfile=./data/processed_data/SUP35_10seqs_tcoffee.fa
```

time:

```
real    1m31,287s
user    1m29,863s
sys     0m1,477s
```

prank

```
prank -d=./data/raw_data/SUP35_10seqs.fa -o=./data/processed_data/SUP35_10seqs_prank.fa
```

time:

```
real    0m12,179s
user    0m12,006s
sys     0m0,163s
```

3. Comparison of the results on 10 DNA sequences

The table containing real times and graphical representation of the results can be found in Supplementary (Table 1, Figures 1-5)
I find mafft and t-coffee the best ones

4. Reverse complement problem

While opening the file ./data/raw_data/SUP35_10seqs_strange_aln.fa we can see that alignment for 1 sequence is not satisfactory (Supplementary, fig. 6). But if we BLAST it, we see that it is the same gene of the same organism. The answer is to find the reverse complement sequence and realign it

5. Using 6 various alignment algorithms on 250 DNA sequences

clustalw

```
clustalw -INFILE=./data/raw_data/SUP35_250seqs.fa OUTPUT=FASTA -OUTFILE=./data/processed_data/SUP35_250seqs.clustalw.fa
```

time:

```
real    29m3,728s
user    29m2,200s
sys     0m1,483s
```

muscle

```
muscle -in ./data/raw_data/SUP35_250seqs.fa -out ./data/processed_data/SUP35_250seqs_muscle.fa
```

time

```
real    1m20,447s
user    1m20,169s
sys     0m0,276s
```

mafft

```
mafft --auto ./data/raw_data/SUP35_250seqs.fa >./data/processed_data/SUP35_250seqs_mafft.fa
```

time:

```
real    0m39,807s
user    0m39,104s
sys     0m0,744s
```

kalign

```
kalign <./data/raw_data/SUP35_250seqs.fa >./data/processed_data/SUP35_250seqs_kalign.fa
```

time:

```
real    0m3,912s
user    0m7,910s
sys     0m0,105s
```

t_coffee

```
t_coffee -infile=./data/raw_data/SUP35_250seqs.fa -outfile=./data/processed_data/SUP35_250seqs_tcoffee.fa
```

I decided to interrupt the process after:

```
real    39m1,756s
user    0m0,761s
sys     0m0,275s
```

prank

```
prank -d=./data/raw_data/SUP35_250seqs.fa -o=./data/processed_data/SUP35_250seqs_prank.fa
```

time:

```
real    2m39,690s
user    2m35,851s
sys     0m1,994s
```

6. Comparison of the results on 250 DNA sequences

The table containing real times and graphical representation of the results can be found in Supplementary (Table 1, Figures 7-10)
I find CLUSTAL to be the best when you can wait or muscle when you want to make it faster.

7. Translation

Let's translate the 10 DNA sequences:

```
transeq -sequence ./data/raw_data/SUP35_10seqs.fa -outseq ./data/processed_data/SUP35_10seqs.t.faa
```

Another way is to use getorf. getorf gives you the ORF and its coordinates in nucleotides. We should give the minsize near the protein size (in nucleotides) to get its sequence without other small peptides.

```
getorf -sequence ./data/raw_data/SUP35_10seqs.fa -outseq ./data/processed_data/SUP35_10seqs.g.faa -noreverse -minsize 500
```

8. Using 6 various alignment algorithms on 10 protein sequences

We use protein sequences of the same gene from the same organisms

clustalw

```
clustalw -INFILE=./data/processed_data/SUP35_10seqs.g.faa -OUTFILE=./data/raw_data/SUP35_10seqs.clustalw.faa -OUTPUT=FASTA -TYPE=pro
```

time:

```
real    0m0,715s
user    0m0,715s
sys     0m0,000s
```

```
clustalo --infile=./data/processed_data/SUP35_10seqs.g.faa --outfile=./data/processed_data/SUP35_10seqs.clustalo.faa --verbose
```

time:

```
real    0m0,625s
user    0m1,072s
sys     0m0,109s
```

muscle

```
muscle -in ./data/processed_data/SUP35_10seqs.g.faa -out ./data/processed_data/SUP35_10seqs_muscle.faa
```

time:

```
real    0m0,358s
user    0m0,342s
sys     0m0,016s
```

mafft

```
mafft --auto ./data/processed_data/SUP35_10seqs.g.faa >./data/processed_data/SUP35_10seqs_mafft.faa
```

time:

```
real    0m0,618s
user    0m0,576s
sys     0m0,094s
```

kalign

```
kalign <./data/processed_data/SUP35_10seqs.g.faa >./data/processed_data/SUP35_10seqs_kalign.faa
```

time:

```
real    0m0,076s
user    0m0,176s
sys     0m0,016s
```

t_coffee

```
t_coffee -infile=./data/processed_data/SUP35_10seqs.g.faa -outfile=./data/processed_data/SUP35_10seqs_tcoffee.faa
```

time:

```
real    0m15,731s
user    0m15,329s
sys     0m0,402s
```

prank

```
prank -d=./data/processed_data/SUP35_10seqs.g.faa -o=./data/processed_data/SUP35_10seqs_prank.faa
```

time:

```
real    0m12,729s
user    0m12,461s
sys     0m0,317s
```

9. Comparison of the results on 10 protein sequences

The table containing real times and graphical representation of the results can be found in Supplementary (Table 1, Figures 11-15)
I think muscle is the best for this goal.

10. Add more alignments using muscle/mafft:

Here I align 2 more DNA sequences and add them to the files with 250 aligned DNA sequences using muscle or mafft.

```
muscle -in ./data/raw_data/SUP35_2addseqs.fa -out ./data/processed_data/SUP35_2addseqs_muscle.fa
muscle -profile -in1 ./data/processed_data/SUP35_250seqs_muscle.fa -in2 ./data/processed_data/SUP35_2addseqs_muscle.fa -out ./data/p
```

```
mafft --auto ./data/raw_data/SUP35_2addseqs.fa > ./data/processed_data/SUP35_2addseqs_mafft.fa
mafft --add ./data/processed_data/SUP35_2addseqs_mafft.fa ./data/processed_data/SUP35_250seqs_mafft.fa > ./data/processed_data/SUP35
```

Supplementary

	CLUSTAL	MUSCLE	MAFFT	KALIGN	T-COFFEE	PRANK
10 DNA SEQUENCES	3,747s	4,742s	4,486s	0,242s	1m 31,287s	12,179s
250 DNA SEQUENCES	29m 3,728s	1m 20,447s	39,807s	3,912s	> 39m	2m 39,690s
10 PROTEIN SEQUENCES	0,715s	0,358s	0,618s	0,076s	15,731s	12,729s

Table 1. Real times for the alignments



Figure 1. Alignment of 10 DNA sequences using CLUSTAL



Figure 2. Alignment of 10 DNA sequences using MUSCLE

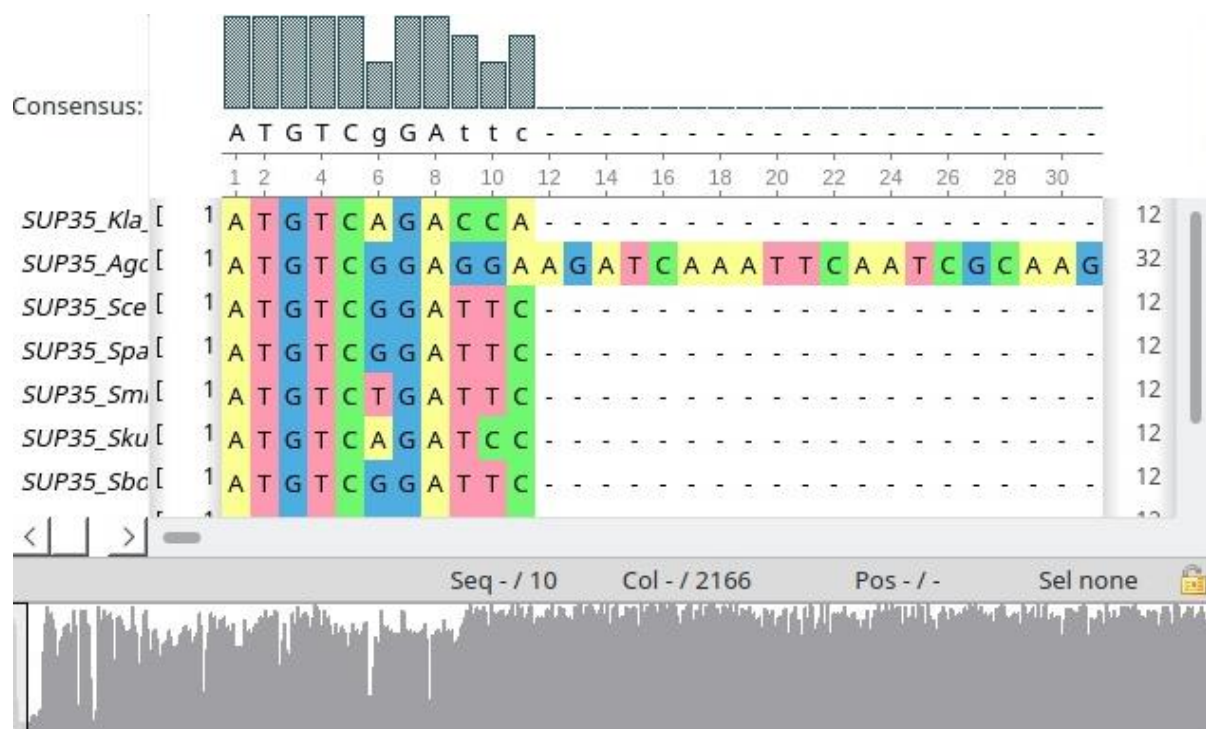


Figure 3. Alignment of 10 DNA sequences using MAFFT



Figure 4. Alignment of 10 DNA sequences using T-COFFEE

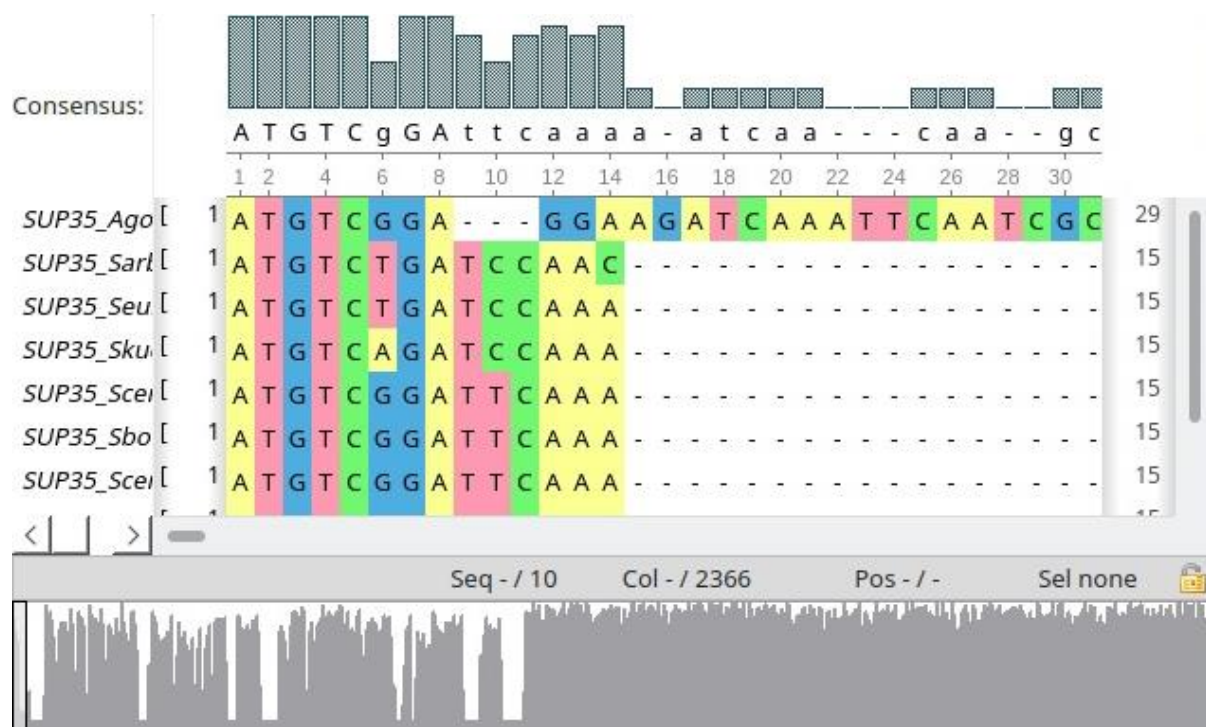


Figure 5. Alignment of 10 DNA sequences using PRANK

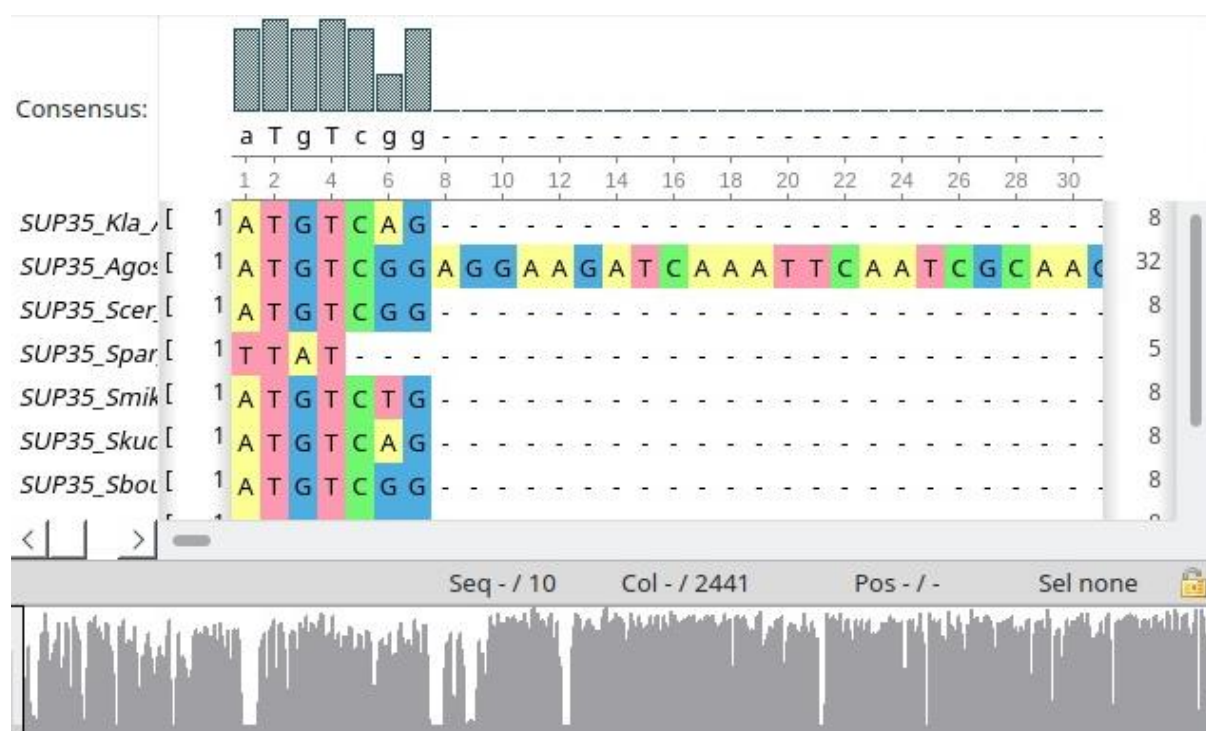


Figure 6. Strange alignment

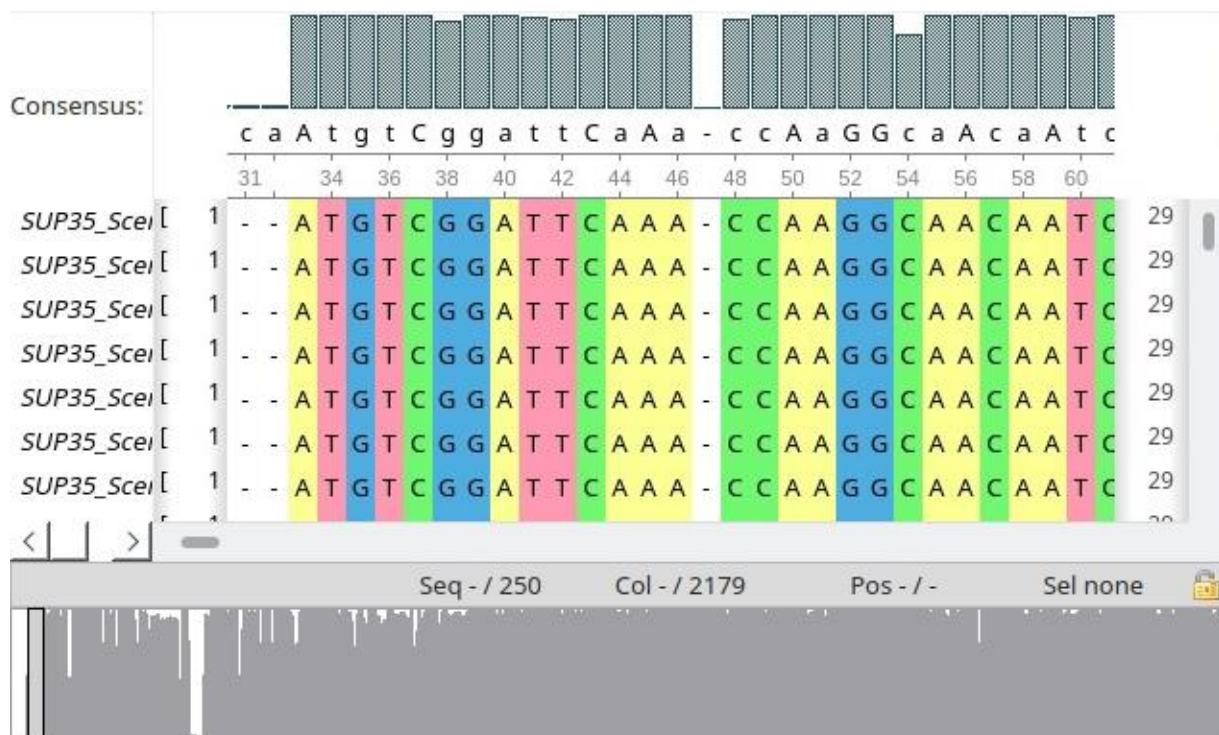


Figure 7. Alignment of 250 DNA sequences using CLUSTAL

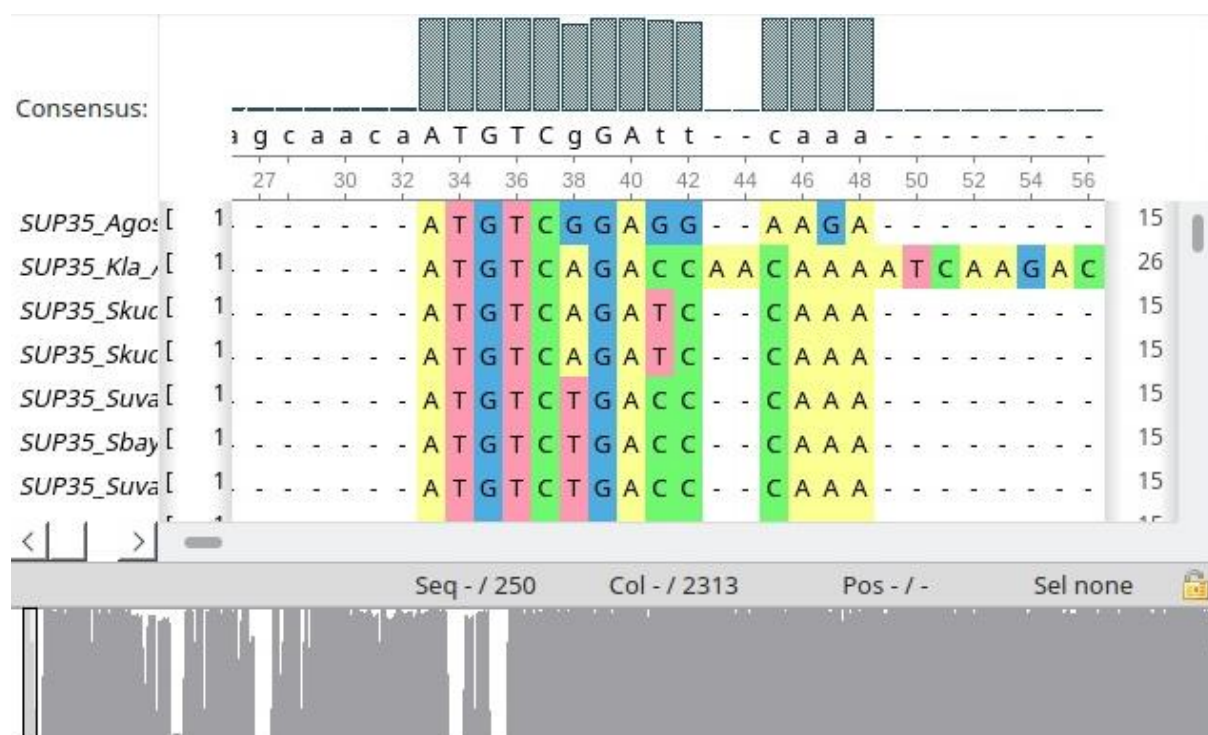


Figure 8. Alignment of 250 DNA sequences using MUSCLE



Figure 9. Alignment of 250 DNA sequences using MAFFT



Figure 10. Alignment of 250 DNA sequences using PRANK

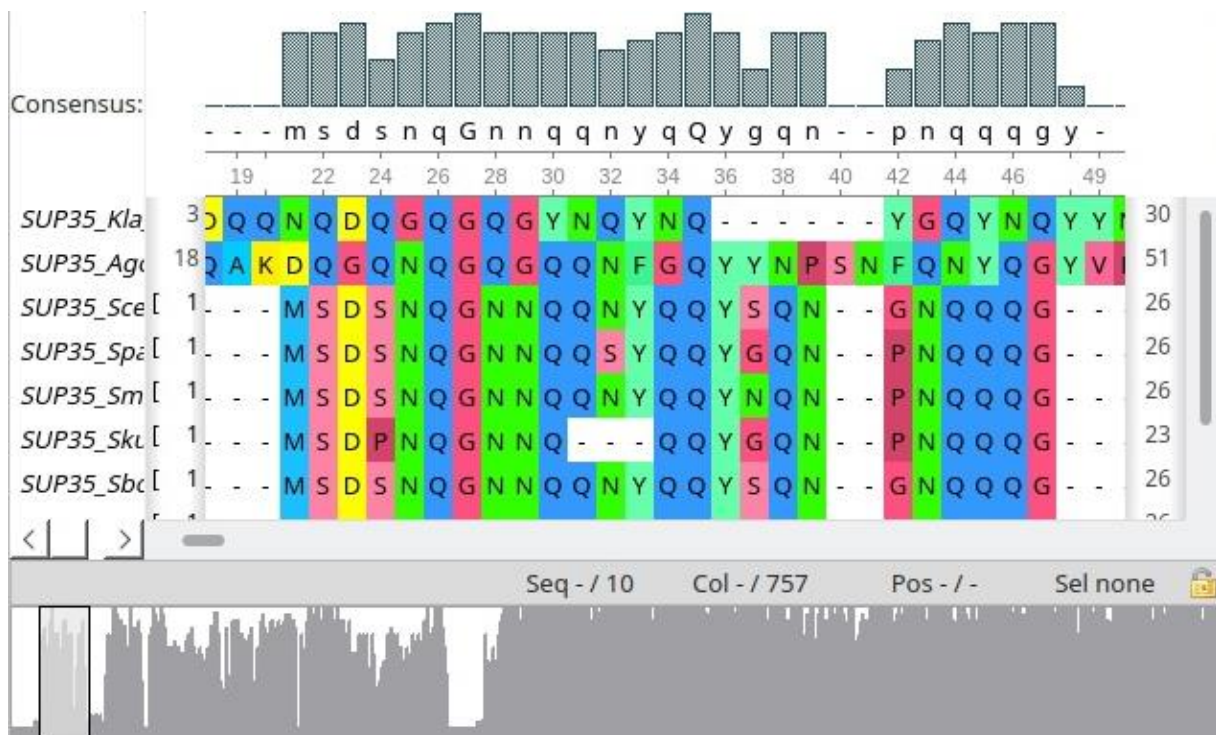


Figure 11. Alignment of 10 protein sequences using CLUSTAL

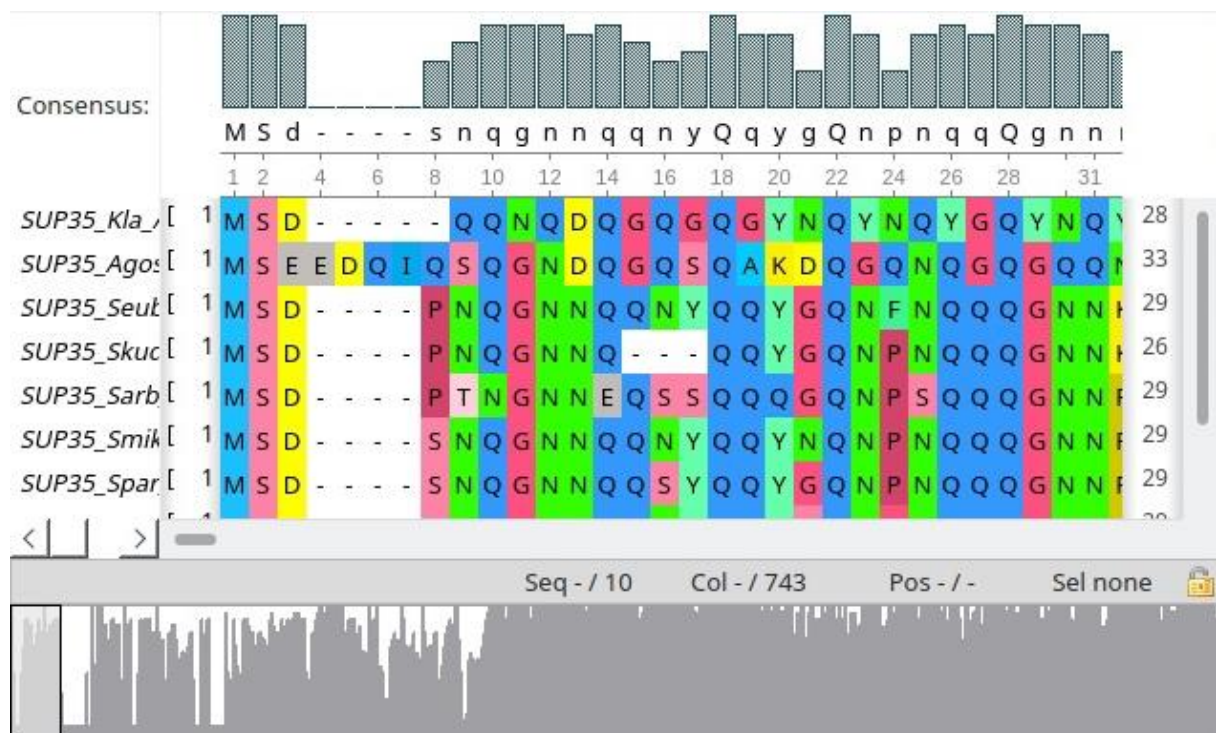


Figure 12. Alignment of 10 protein sequences using MUSCLE

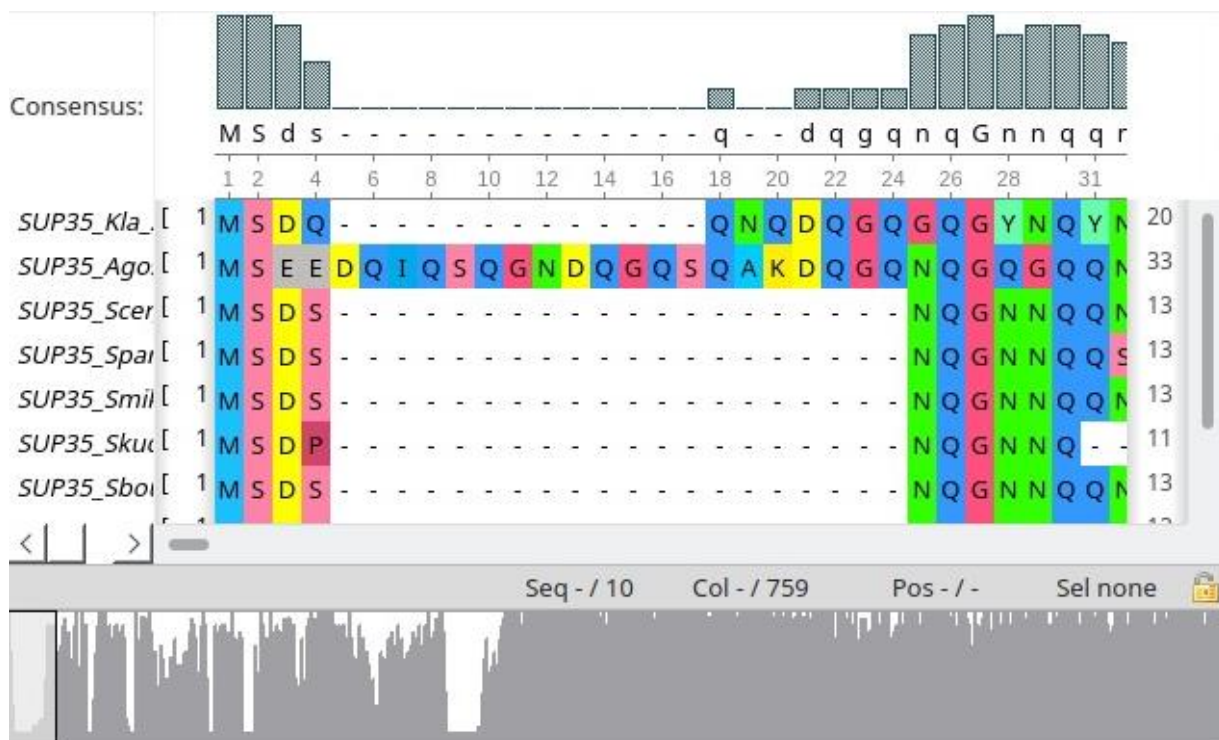


Figure 13. Alignment of 10 protein sequences using MAFFT

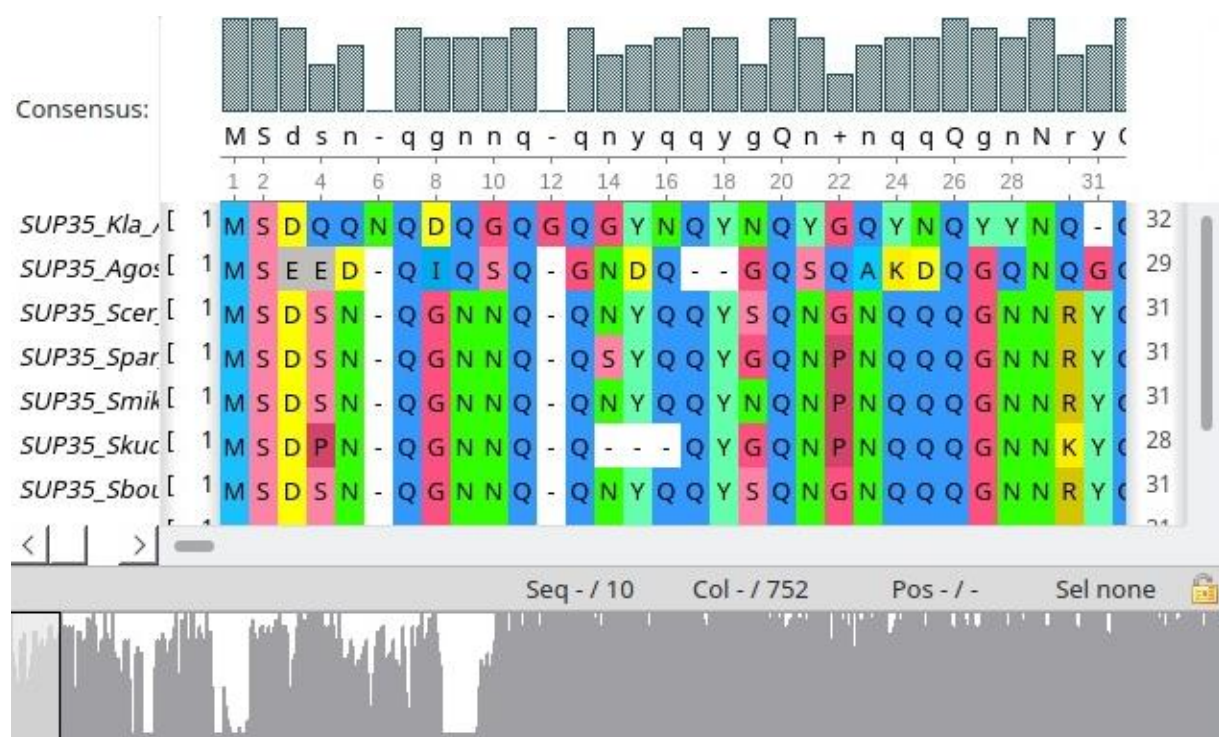


Figure 14. Alignment of 10 protein sequences using T-COFFEE



Figure 15. Alignment of 10 protein sequences using PRANK