0) Files description

Target file

The group you want to find candidate regions

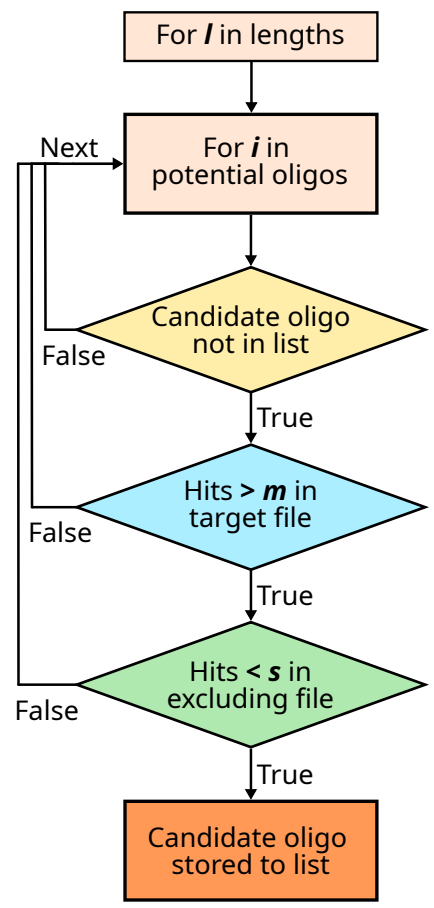
Excluding file

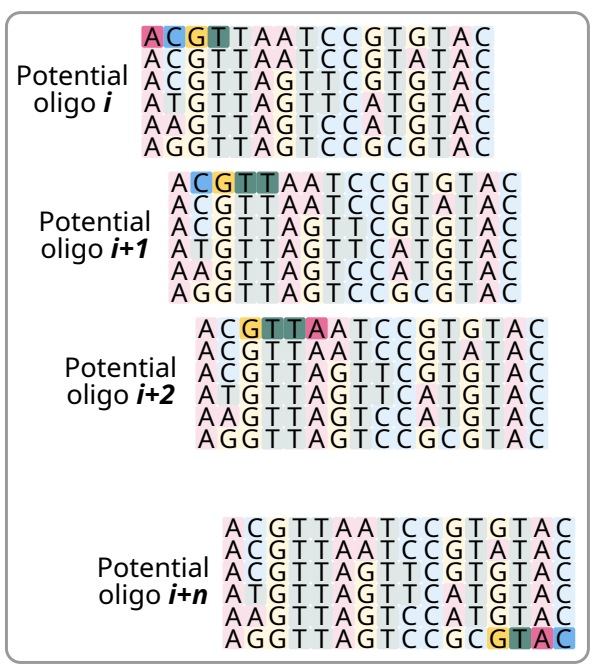
The sequences you want to exclude (or the rest of the diversity)

1) Find oligos

Searches candidate oligonucleotides by sliding window

findOligo -t target **-e** excluding **-l** length **-s** specificity **-m** minMatch





- Sequence
- Reverse complement sequence
- GC content
- Basic melting temperature
- Hits in target file
- Hits in excluding file

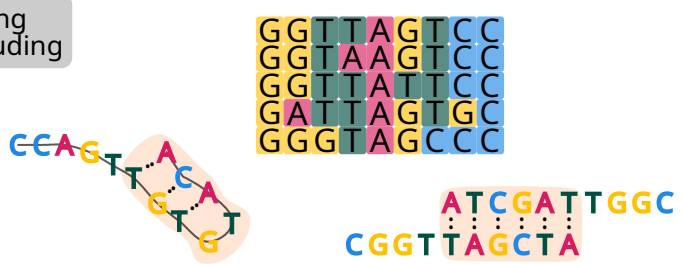
...

2) Test oligos

Test oligonucleotides for self-dimers, hairpin, mismatches, ...

testOligo -f primers **-e** excluding
testThorough -f oligos **-e** excluding

For ***p*** in candidate oligo
Self-dimers
Hairpins
Max consecutive bases
For ***n*** in mismatches
Hits against excluding file allowing ***n*** mismatches



- Self-dimer count
- Hairpin count
- Max consecutive bases
- Hits allowing n mismatches
  - Position of the mismatch
  - Identity of the mismatch

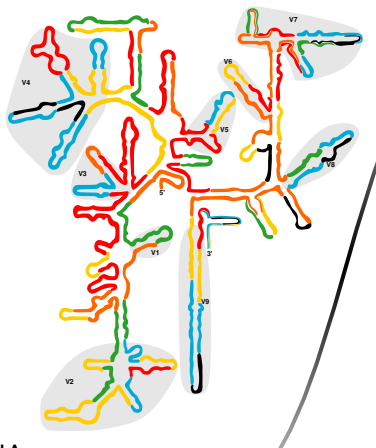
...

3) Rate accessibility

Compare candidate oligonucleotides to the accessibility map of *Saccharomyces cerevisae* 18S rDNA or *Escherichia coli* 16S (Behrens et al., 2003)

alignPrimers -t target.fasta **-p** oligos.fasta **-o** oligos_align.fasta
rateAccess -f oligos_align.fasta **-o** oligos_access.tsv

- Accessibility score
- Region in the 18S rDNA gene

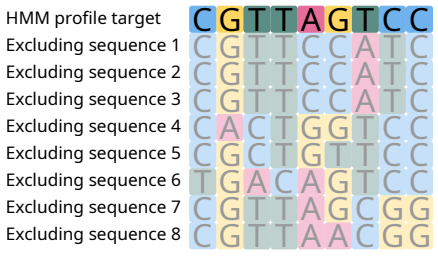


18S rDNA accessibility:

4) Get homologs

Get all aligned positions from the excluding file matching a HMM profile of a given region of the target file

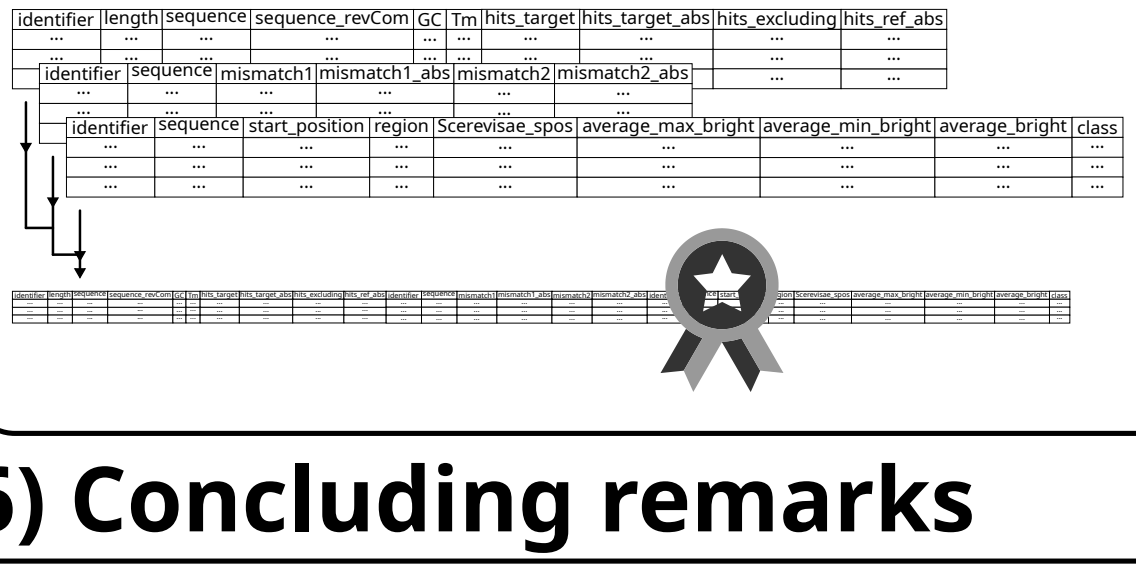
getHomolog -f region.fasta **-e** excluding.fasta



5) Selecting oligonucleotides

Merge all log files:

bindLogs -f oligos.tsv oligos_tested.tsv oligos_access.tsv **-o** oligos_log.tsv -r



Selecting the best oligonucleotide:

- covering most of the targeted diversity
- with a high GC content
- with similar theoretical melting temperature
- with low hits to the excluding file allowing mismatches
- highly accessible

For example:

filterLog -l oligos_log.tsv **-s** "0.4" **-m** "0.001" **-M** "0.0001" **-c** "III"

selectLog -l oligos_log_filtered.tsv **-n** "4"

6) Concluding remarks

Complementary softwares:

- **ARB** (Ludwig et al., 2004)
- **Decipher** (Wright et al., 2014)
- **primer3** (Untergasser et al., 2012)
- **oli2go** (Hendling et al., 2018)

Post-hoc test:

- **OligoCalc**
- **BLAST** (blast.ncbi.nlm.nih.gov/Blast.cgi)
- **PR2-primers** (app.pr2-primers.org/)

Remember that oligo design is a tedious work that requires a final empirical test for its completion.
Therefore, bioinformatic pipelines will only provide theoretical candidate oligos, that have to be tested in the laboratory