0) Files description Target file

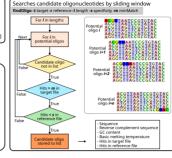
1) findOligo



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

2) testOligo Test oligonucleotides by allowing mismatches





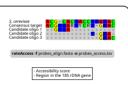
3) rateAccess

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

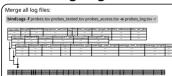
- Create consensus sequence of target group
- Alian to the S. cerevisae 18S rDNA sequence
- Add candidate oligos to the alignment

alignPrimers -t target.fasta -p probes.fasta -o probes_align.fasta





4) Selecting oligonucleotides



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 reference file allowing mismatches - highly accessible
- For example:

filterLog -I probes_log.tsv -s "0.4" -m "0.001" -M "0.0001" -c "III"

selectLog -I probes_log_filtered.tsv -n "4"

5) Concluding remarks

Complementary softwares:

- ARB (SILVA; arb-home.de/)
- BLAST (blast.ncbi.nlm.nih.gov/Blast.cgi) PR2-primers (app.pr2-primers.org/)

Future implementations: - R2DT (rnacentral.org/r2dt):

Automatic visualization of 2D structure with the probes -Check for self-binding: (e.g.) ACGTnnnnACGT

Remember that probe design is a tedious work that requires a final empirical test for its completion.

Therefore, bioinformatic pipelines will only provide theoretical candidate probes, that have to be tested in the laboratory