WP 4 - General recommendations for bulk metabarcoding

Interreg
North Sea Region
GEANS
European Regional Development Fund

EUROPEAN UNION

Recommendations based on

1) Questionnaire

(based on 9 GEANS partner institutes)

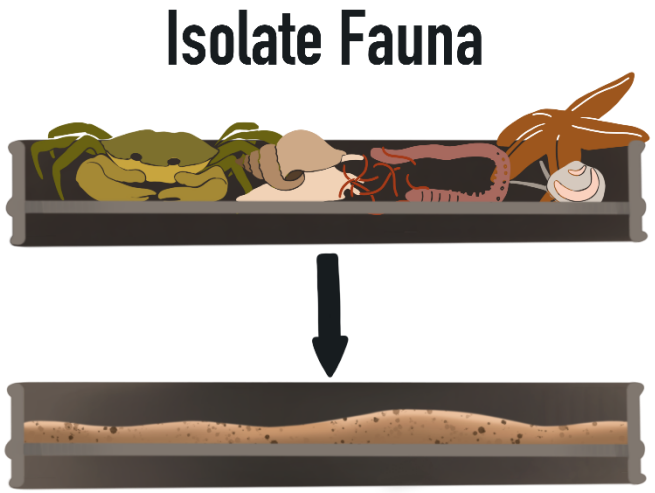
2) Literature study

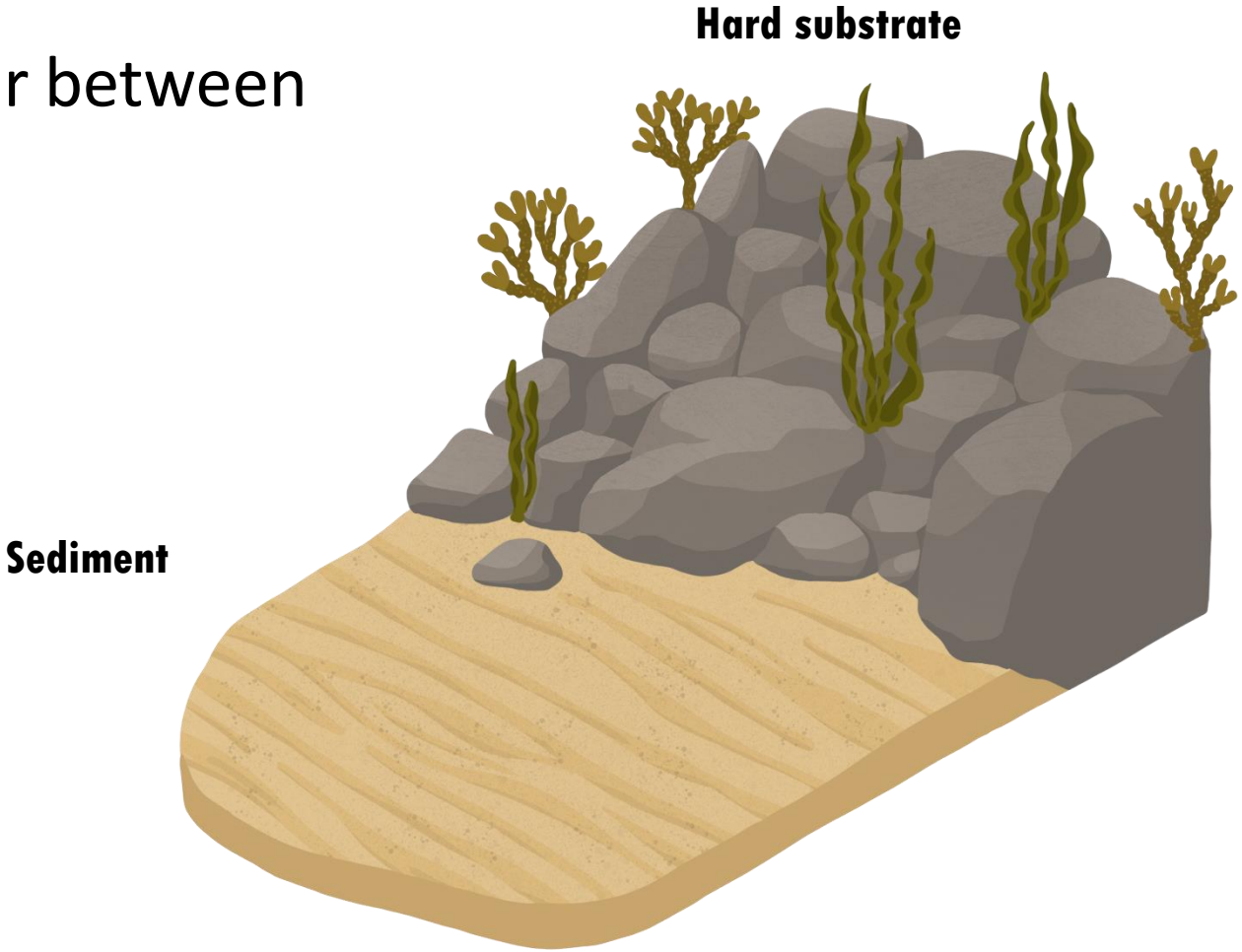
(based on 64 published articles)

Result: recommendations for SOPs

Recommendations

1) Sampling procedures will differ between institutes and sample types





Recommendations

2) If staining is necessary for morphological analyses, it is recommended to split the samples before staining and use an unstained sample for DNA analyses.

Jones et al 2014

Recommendations

3) It is important to homogenize the samples well before subsamples are taken for DNA extraction. Homogenization is preferably done with a blender for larger samples and an Ultra Turrax or bead beater for small samples. The use of a lysis buffer step is also important.

→ Mortar and pestle requires sterilization between samples and therefore prone to contaminations.

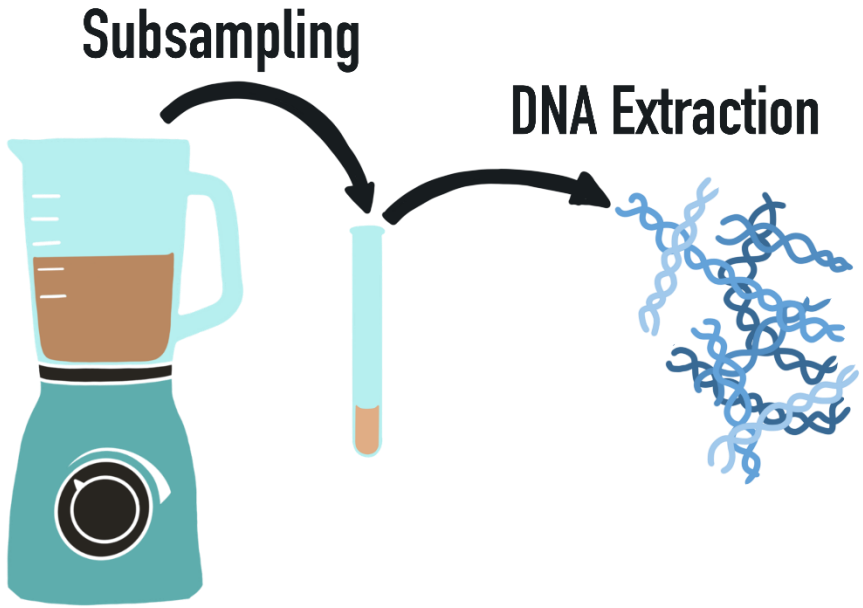
Recommendations

4) Whether to use ethanol or DMSO/DESS as standard fixative is currently being tested in a pilot study by SeaAnalytics and Senckenberg.

→ ARMS protocol will use DMSO/DESS
→ Naturalis will likely use freeze-dried samples

Recommendations

5) For sediment samples and hard substrate samples, the DNeasy PowerSoil kit (Qiagen/MO-BIO) is recommended, as previous studies showed that this kit is highly effective to obtain high purity DNA and to remove the general inhibitors that sediments usually contain.



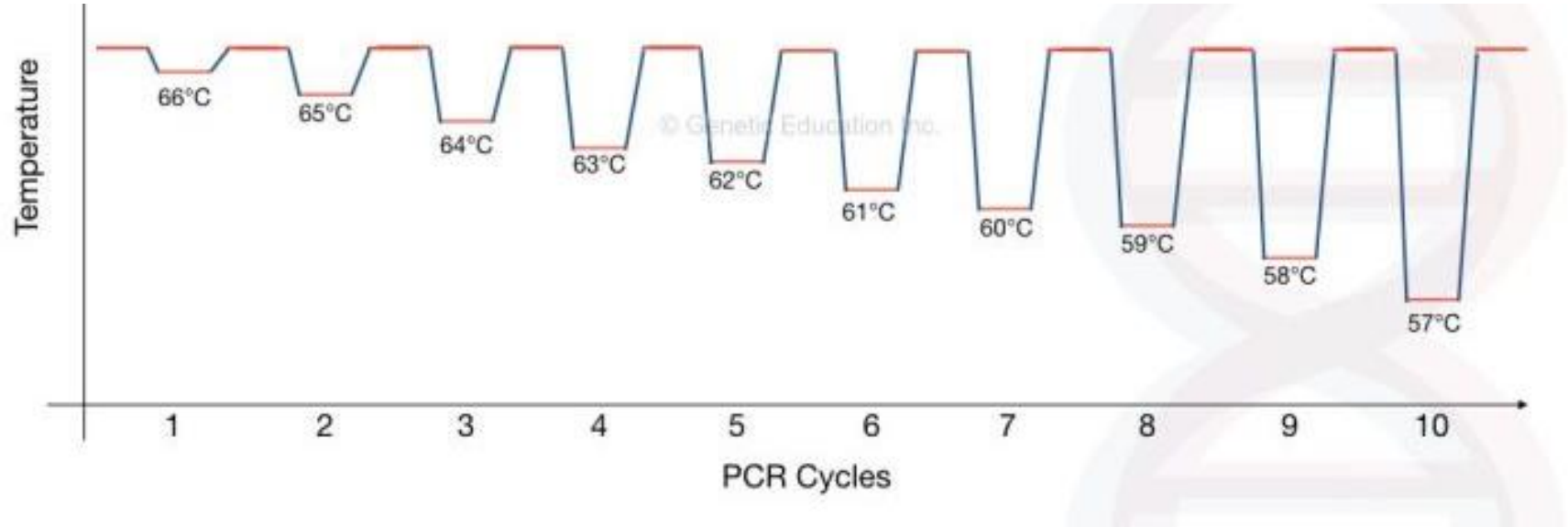
Recommendations

6) In metabarcoding studies, at least COI (313bp fragment) and 18S should be used, and if possible 12S or 16S in addition.

→ Primer sets will depend on the fragments we choose and goal of the study
→ We likely need to choose specific primers per pilot
→ It is important to use same primers within pilot study
→ Possible in the future: primer design per species group?

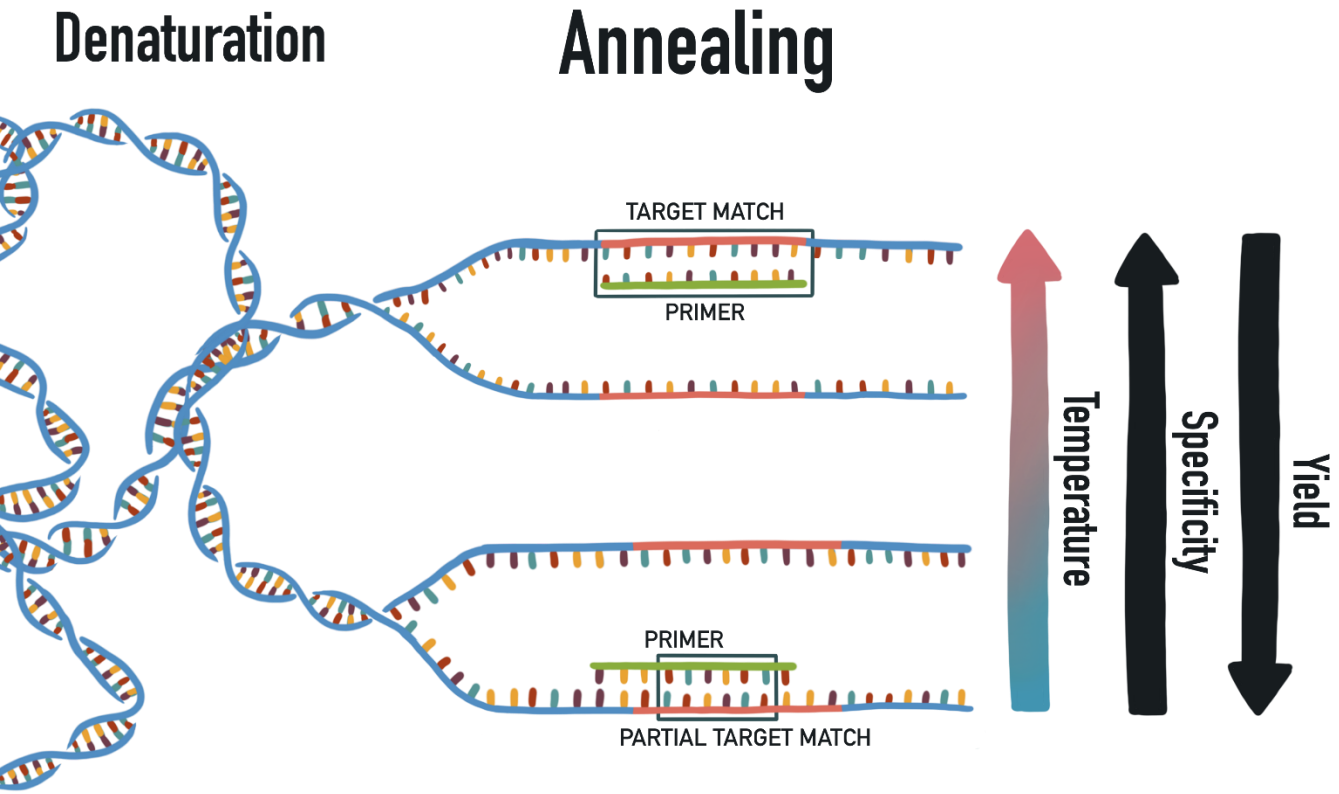
Recommendations

7) The PCR thermal profile should have a fixed annealing temperature. Touchdown protocols should be avoided.



Recommendations

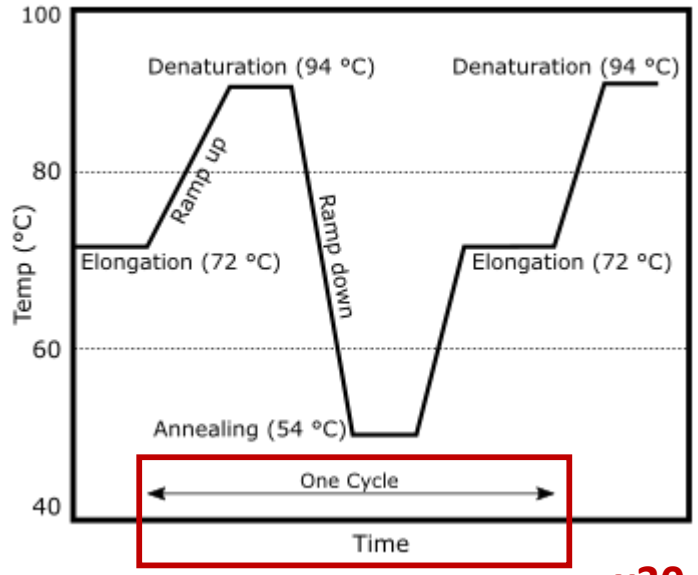
8) It is best to use a fixed annealing temperature for each specific primer pair across institutes.



→ The optimal temperature depends on the primer and fragment

Recommendations

9) The number of cycles in the PCR thermal profile should preferably be 35 cycles or less (excluding cycles needed for index PCR).

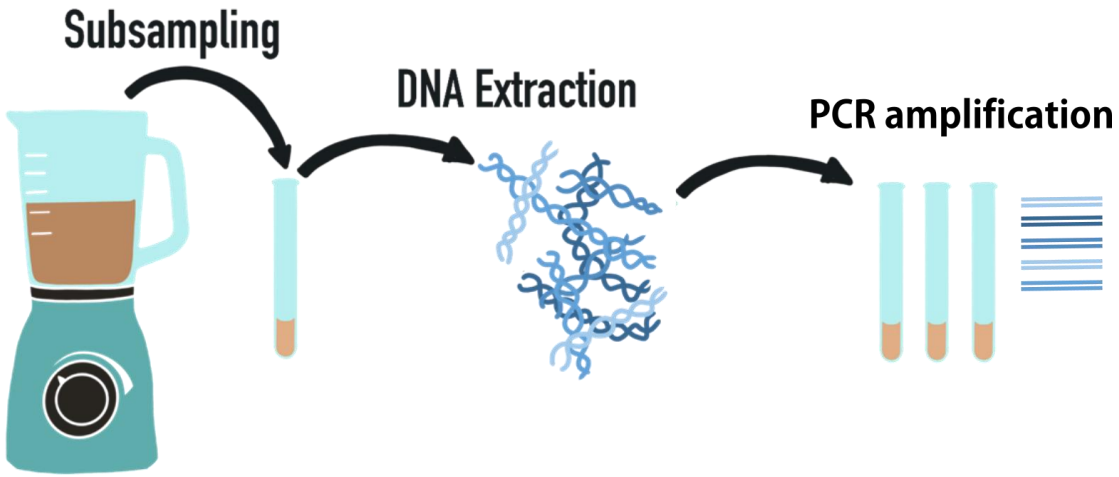


x30-35

Recommendations

10) For every sample of extracted DNA, 3 PCR replicates should be used. The amplified products can be pooled before sequencing.

→ Extracted DNA of subsamples from the same grab can be pooled before PCR replicates.
→ Possibly more PCR replicates for detection of NIS?



Recommendations

11) Include both positive and negative samples. Including a mock sample (positive sample) can help to test for optimal bioinformatics methods further down the pipeline and test for contamination.

→ The positive sample should contain something that you would not find in the real samples (like a freshwater snail)

Thanks

Interreg
North Sea Region
GEANS
European Regional Development Fund

EUROPEAN UNION