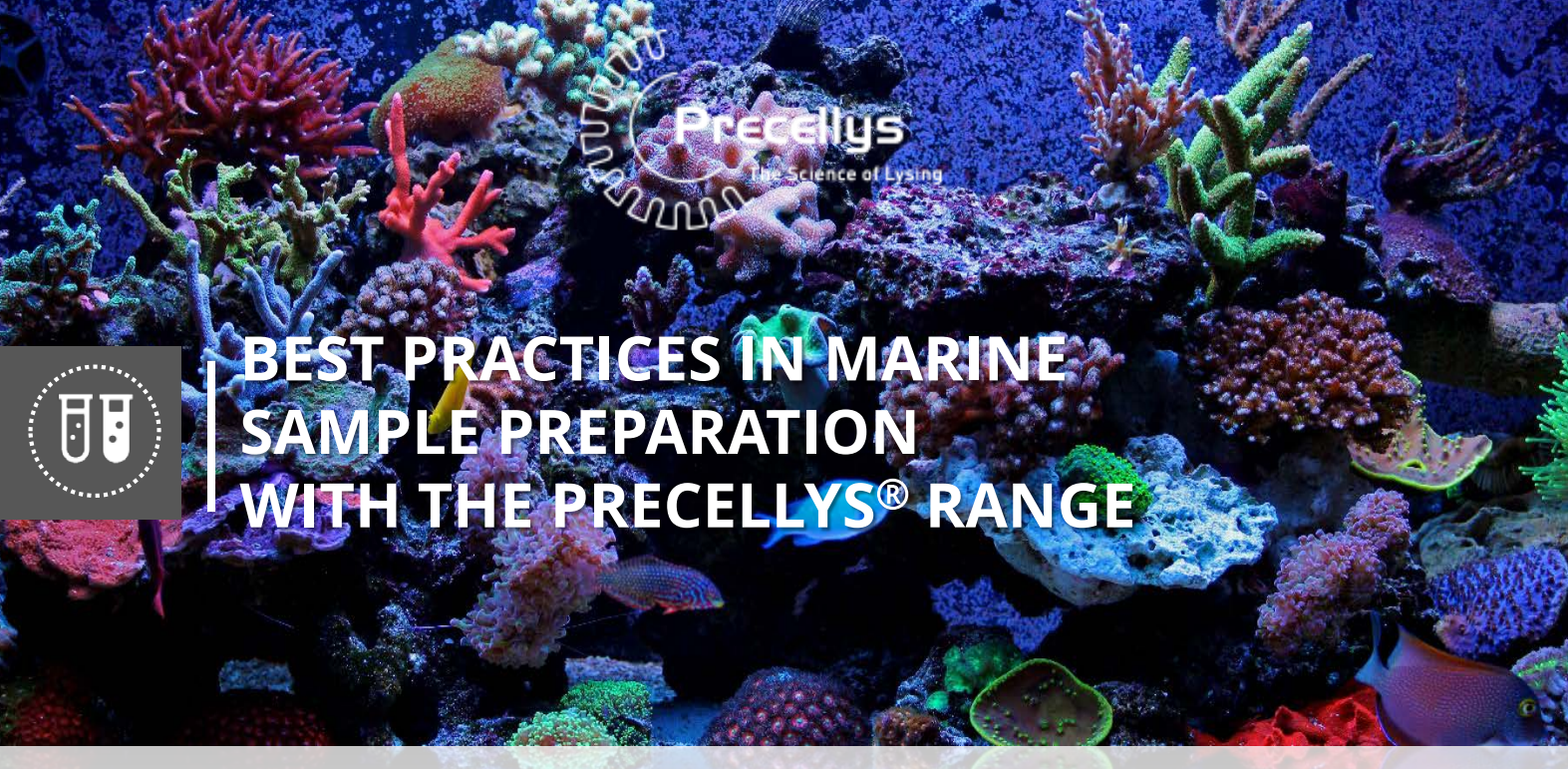


BEST PRACTICES IN MARINE SAMPLE PREPARATION WITH THE PRECELLYS® RANGE

When the ocean is dying, the planet is too... Coral reefs are already heavily affected, but the entire marine flora and fauna is threatened by ocean acidification and global warming. According to Australian researchers, greenhouse gas emissions might lead to a "collapse" of marine species and thus of the food chain. 10 to 12% of the species might disappear if the temperature increases by 2 °C compared to the pre-industrial era.

As an example the loss of corals will be a disaster for 500 million people who depend on this ecosystem to live and feed themselves. Advances in research regarding the behavior of the marine world (seeds, eggs, larvae, corals reef, algae...) using protein markers, genetics and direct observations allow researchers to better apprehend the biodiversity and ecological balance. As these researches are characterized by a large variety of life forms, researchers need a flexible tool to prepare their sample before proceeding to molecular downstream analysis.

Bertin Instruments offers a broad range of robust, efficient and flexible homogenizers based on the bead beating technology, to grind any biological sample while maintaining reproducibility amongst biological replicates and eliminating cross-contamination. This instrument is frequently used on research protocols ensuring a fast and efficient sample disruption process.

IMPROVE EXTRACTION YIELDS OF RNA & DNA FROM BIOLOGICAL SAMPLE USING THE PRECELLYS® RANGE

SUMMARY

DETERMINATION OF NUTRIENT HOT SPOTS ON CORAL REEFS USING A COMMON MACROALGAE

Burkepile Community Ecology Lab, Moorea Coral Reef Long Term Ecological Research Lab, University of California, Santa Barbara

/ CONTEXT

Coral reefs are diverse underwater ecosystems held together by calcium carbonate structures secreted by corals. Coral reefs are built by colonies of tiny animals found in marine water that contain few nutrients. This research focuses on nutrient dynamics on coral reefs. The Burkepile lab in collaboration with the Moorea Coral Reef Long Term Ecological Research Lab collected a common macroalgae, *Turbinaria ornata,* at 190 points around the island of Moorea, French Polynesia. Nutrient hot spots were evaluated by analyzing the tissue content of dried *Turbinaria* for carbon and nitrogen as well as stable isotopes.

/ MATERIALS

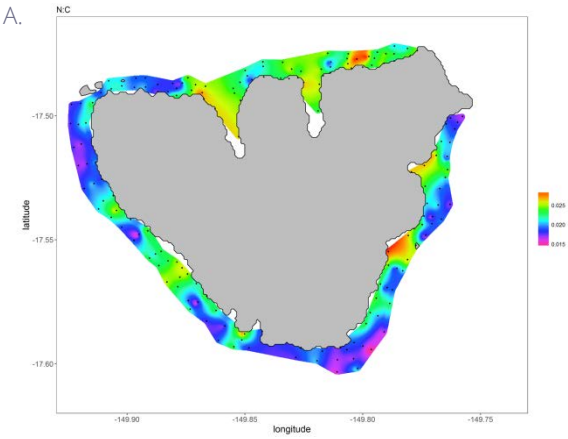
- Bead beating Homogenizer: Precellys® Evolution
- Lysing Kit: 7mL metal tubes (KT03961-1-602.M), 2 x 6.8mm Zirconium oxide beads (KT03961-1-107.BK)
- Tissue Samples: 10 dried *Turbinaria ornata* blades

/ PROTOCOL

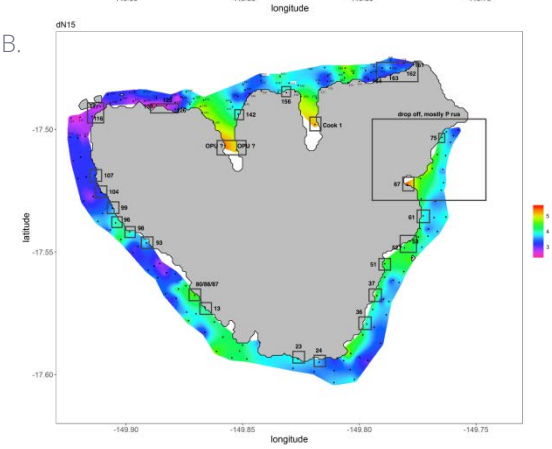
1) Collect 10 individuals of *Turbinaria ornata,* remove one blade 5 cm from apical tip on thallus
2) Scrub algae to remove epifauna, dry at 60°C for 72 hours
3) Load each sample (about 10 blades) in 7mL metal tube with two 6.8mm ceramic beads
4) Place tubes in Precellys® Evolution homogenizer with blocking plate, and set grinding protocols to 5,500 RPM 2 cycles of 20 sec with 20 sec pause
5) Repeat cycle if sample is not completely homogenized
6) Send homogenized samples to analytical lab for CHN, Stable Isotope Analysis

/ RESULTS

A.



B.



A. Nitrogen : Carbon ratio B. Delta N15 stable isotopic signatures. Warm colors on maps indicate increased nitrogen enrichment

/ CUSTOMER

Moorea Coral Reef LTER
member of the NSF Long Term Ecological Research Network

PREC-026-DU132-A

Using a Carbon, Hydrogen, Nitrogen analyzer and continuous-flow isotope ratio mass spectrometer with macroalgae, we have mapped nutrient-enriched hot spots around an inhabited island in the South Pacific. This allows us to better understand the impact of anthropogenic nutrients on coral reefs and the areas of concern, including the populated north shore, two bays, and passes on the reef. The Precellys® Evolution with the 7ml metal tubes gives us confidence that our samples are uniformly homogenized and free of contamination for these analyses.

DNA EXTRACTION FROM OYSTER LARVAE

IFREMER-Laboratoire de Génétique et Pathologie des Mollusques Marins-FRANCE

/ CONTEXT

This laboratory is working on the genetic diversity of cupped oysters at different scales. This study is focusing on hybridization and introgressive hybridization between the species C. gigas and C. angulata. Both, coming from Asia, are present in South Portugal in Europe. To characterize their relationship, F2 experimental crosses of biparental families were performed in our experimental hatchery.

A panel of specific SNP will be used on each individual larvae. Therefore, a grinding method is necessary to extract DNA. Our study was designed to see if the Precellys kit lysis permits to grind efficiently larvae to obtain homogenous lysis and good quality and quantity of DNA materials, with reproducibility, in order to perform PCR amplification.

/ MATERIALS

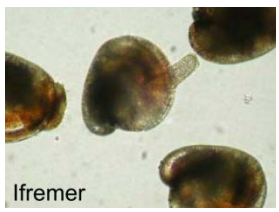
- Instrument: Precellys 24
- Precellys Lysing kit: Soil Grinding SK38, Cat n° KT03961-1-006.2: mix of glass and ceramic beads
- Sample: whole oyster larvae, 250 µm, ethanol fixed and conserved at -20°C.
- Buffer: Lysis buffer, 520µL

/ PROTOCOL

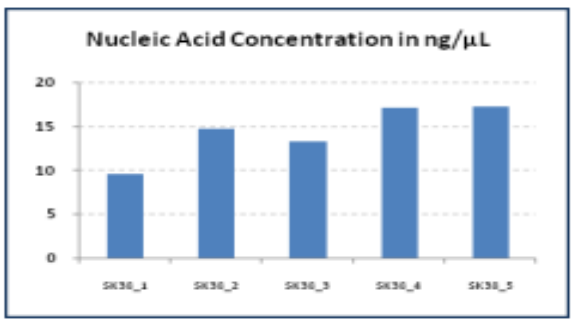
Grinding protocol:
Speed: 6000 rpm
Cycle: 3 x 20 sec
Break: 5 sec at RT

Analysis:
Extraction with Nucleospin Tissu XS Macherey ® adapted
Amplification PCR 16S and further with SNP panel.

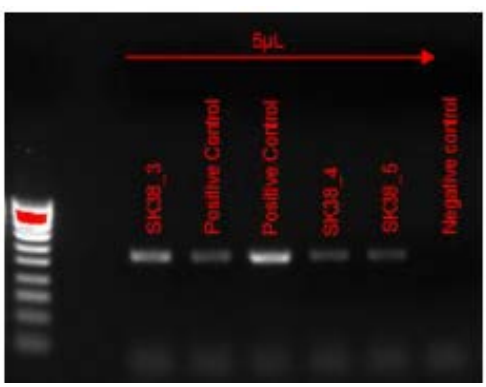
/ RESULTS



DNA extraction and quantification performed on 5 larvae.



Test with 16 S PCR amplification



/ CUSTOMER

Laboratoire de Génétique et Pathologie des Mollusques Marins

/ CONCLUSION

The combination of the Precellys 24 and the specific SK38 lysing kit permits to obtain homogeneous lysis of small whole fixed oyster larvae. The extracted DNA which presented a high quality and yield was amplified with success.

The Precellys associated with the lysing kit, offers a good alternative to standard method in order to perform genetic analysis on oyster larvae.

SPECIFIC PROTOCOL FOR MARINE SAMPLES

/ SPICULES – MARINE SPONGE – METABOLITES EXTRACTION

- SAMPLE TYPE: Marine Sponge
- TARGETED MOLECULE KITS: Bio silica
- KITS: MK28_R 2 mL
- QUANTITY: 200 mg
- BUFFER: Distilled Water
- PROTOCOL: 6800 rpm – 8 x 25 sec
- INSTRUMENT: Precellys® 24

/ METABOLOMIC STUDY OF THE RED ALGAE CHONDRUSCRISPUS

- SAMPLE TYPE: Sand
- TARGETED MOLECULE: Metabolites
- KITS: MK28_R 2 mL
- QUANTITY: 150 mg of sand
- PROTOCOL: 6800 rpm – 1 x 30 sec
- INSTRUMENT: Precellys® 24 + Cryolys

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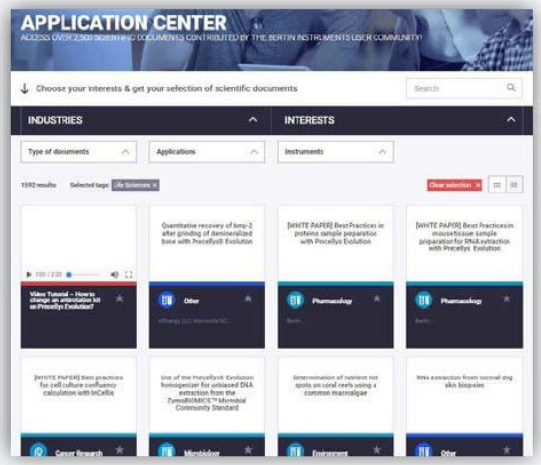

Precellys® Evolution is the most advanced homogenizer gathering high efficiency and versatility for all sample preparation needs:

- **Flexibility:** 24 x 2mL (or 0.5mL), 12 x 7mL, 6 x 15mL and 96 well-plate format
- **Efficiency:** up to 10 000 rpm speed to grind any type of sample
- **Integrity:** protect your molecules with the Cryolys® Evolution cooling unit

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