APPLICATION NOTE

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Molecular Effects of Pollution in Cetacean Samples Using the Cytochrome P450, CYP 1A1, 1A2, and 1B1 as Biomarkers

With permission of Dr. Celine Godard-Codding, Ocean Alliance & The Whale Conservation Institute, Lincoln MA

Application Note SP015:
RNA Extraction

Apparatus:
Freezer/Mill® 6700

Application:
Molecular Effects of Pollution

Ocean Alliance is dedicated to the conservation of whales and the ocean environment through research and education. Ocean Alliance recently completed a five-year research voyage around the world known as the Voyage of the Odyssey (2000 to 2005). The Voyage of the Odyssey was launched to address the need for a globally integrated dataset allowing a consistent analysis of exposure to, and potential effects of, persistent organochlorines and other pollutants in marine life.

Many marine mammals harbor large fatty reserves in their body where high levels of organochlorines and other lipophilic contaminants can accumulate. Marine mammals are subject to bioaccumulation and biomagnification of those fat-soluble contaminants due to their relatively long life span and high trophic position within marine food chains. Therefore marine mammals can be considered environmentally relevant candidates for use as sentinel species when assessing marine pollution. The sperm whale *(Physeter macrocephalus)* was chosen as the indicator species for the Voyage of the Odyssey. A total of 960 sperm whale skin and blubber samples and over 100 samples from predatory fish were collected during the voyage.

Biomarker Project

The toxicology program of the Voyage of the Odyssey encompasses many projects. The biomarker project focuses on the use of the cytochrome P450 1 (CYP1) enzymes. CYP1 enzymes can metabolize and/or activate environmental pollutants, such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). In many animal species CYP1 induction is used as a biomarker of exposure and molecular effects to the above contaminants. We recently validated the CYP1 biomarker in cetaceans (Godard *et al.,* 2004).

The goal of our project is the investigation of the molecular effects of pollution in cetacean samples using the cytochrome P450 CYP 1A1, 1A2, and 1B1 as biomarkers. Our methods include isolating RNA sequencing, cloning and quantifying the expression of the 3 CYP genes in sperm whale skin biopsies. The sperm whale skin samples available for this particular biomarker project weigh only between 50-100ug. The amount of total RNA that can be extracted from such small samples is low; therefore we investigated the use of the SPEX 6700 Freezer/Mill in order to increase the quantity of total RNA that can be extracted from each sample and its quality.

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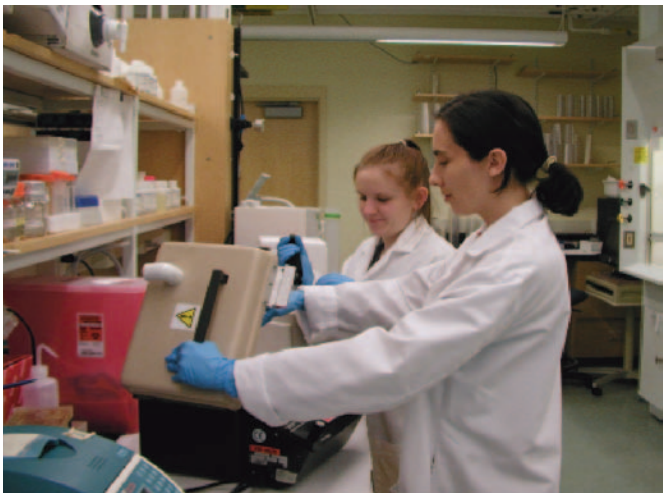
Materials and Methods

We have devised the following multi-step approach in preparing our samples and using the SPEX 6700 Freezer/Mill.

- All SPEX microvials and other utensils used to extract the sample from the 6700 Freezer/Mill are autoclaved before use to avoid sample contamination.
- The SPEX 6700 Freezer/Mill is pre-chilled before the grinding of the samples using liquid nitrogen.
- The tissue samples are sliced into smaller pieces to improve the grinding.
- Sliced tissue samples are pre-chilled in liquid nitrogen in order to prevent RNA degradation.
- The pre-chilled samples are placed into a SPEX microvial and kept in liquid nitrogen until placement into the SPEX 6700 Freezer/Mill.
- The SPEX 6700 Freezer/Mill is topped off with liquid nitrogen and the vials containing samples are put into the SPEX 6700 Freezer/Mill.
- The following grinding process has shown to be effective in preventing heating the samples. The SPEX 6700 Freezer/Mill is turned on for 2 minutes and then off for 1 minute and the cycle is repeated for a total of 10 to 15 minutes of grinding.
- Once the samples have been ground, they are kept in the SPEX microvials in liquid nitrogen until further analysis.
- Samples are then ready for total RNA isolation by phenol and guanidium thiocyanate extraction.



Dr. Celine Godard-Codding and Carolyne LaCerte

Conclusion

The SPEX 6700 Freezer/Mill has been a substantial asset to our research by allowing the isolation of total RNA from our small tissue samples with improved quality and less degradation than previous techniques.

References

Godard *et al.* (2004). Induction of Cetacean Cytochrome P4501A1 by -naphthoflavone

Exposure of Skin Biopsy Slices. *Toxicological Sciences.* **80,** 268-275.