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## **Sequencing at Sea – performing real-time DNA sequencing in the middle of the Pacific Ocean**

*Scientists overcame equipment failure, space constraints and shark-infested waters to do real-time DNA sequencing in a remote field location.*

SAN DIEGO, Calif. (August 19, 2014) — Daylight was breaking over the central Pacific and coffee brewing aboard the *MY Hanse Explorer*. Between sips, about a dozen scientists strategized for the day ahead. Some would don wetsuits and slip below the surface to collect water samples around the southern Line Islands' numerous coral reefs. Others would tinker with the whirring gizmos and delicate machinery strewn throughout the 158-foot research vessel. All shared a single goal: Be the first research group to bring a DNA sequencer out into the field to do remote sequencing in real time. Against an ocean of odds, they succeeded.

This three-week, five-island expedition took place last year with a research crew including San Diego State University computer scientist Rob Edwards, biologist Forest Rohwer, postdoctoral scholar Andreas Haas and graduate student Yan Wei Lim. They were accompanied by several other researchers from the San Diego region and around the world. The researchers published an account of their trip and methods [today in the journal PeerJ](#).

### **Line Island investigations**

Biologists and computer scientists at SDSU have been traveling to the Line Islands for the last decade, collecting and analyzing the coral habitat to better understand what organisms live there, how they compete for resources, and what effects their presence has on the reef's ecosystem. It always bothered Edwards that they had to wait until they were back home, on the other side of the world, before they could look at their data and develop new hypotheses.

"If only we had had that data out in the field, we could have asked those questions there and then," Edwards said.

That inkling grew into an ambitious plan to somehow, some way bring out to sea a cumbersome and expensive piece of equipment designed to analyze a sample's DNA makeup and spit out detailed information about its genome.

The project initially had its doubters.

"People are a little bit hesitant to take a half-million-dollar piece of equipment into the middle of the Pacific if you're not sure it's going to be coming back," Edwards said.

Undeterred, he and his colleagues devised a protocol for how to run a DNA sequencer on a ship. Finally, the team headed to Tahiti with a sequencer provided by San Diego-based biotech company Life Technologies. As the *Explorer* headed south en route to an eventual rendezvous with Antarctica, it picked up the researchers and their sequencer and set course for the Line Islands.

### **Problems and solutions**

The problems and hurdles were manifold.

First, the touch screen needed to operate the machine broke during transit. Edwards had to hack into the sequencer's software to make it operable with his laptop.

Next, they had to find room for all the various pieces of equipment. They set up the sequencer itself in the laundry room because it was the lowest point in the ship and it would sway the least as the *Explorer* rocked. The microbiology lab was set up on the upper aft deck. The DNA isolation station found its home in a cabin. The mess deck (or dining room, to landlubbers) hosted the PCR machine used to amplify the DNA samples into analyzable chunks.

Once everything was in place, Lim, a doctoral student, was tasked with calibrating the sequencer. Normally, this takes about 15 minutes in a lab. Due to the boat's sway, however, it took about five hours.

Then there were the sharks. Lim recalls that every time the researchers dove to the reef to collect samples, she counted between 20 and 30 curious sharks milling about.

"I wasn't afraid," she said. "They would just swim away if you got too close."

Despite the setbacks, the researchers managed to make it all work. They successfully collected samples, sequenced their DNA, and developed new research questions on the fly. In all, 26 bacterial genomes were sequenced, along with two metagenomes, which take into account all the DNA present in a given region.

Not a bad haul for a proof-of-concept voyage, but Edwards said next time they hope to collect even more data and develop and test more complex hypotheses out in the field.

"At the end of the day, we were able to come up with the data we needed," Edwards said. "But when we go back next time, we're going to be better prepared."

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**IMAGES:**



**Photographer:** Rob Edwards (CC BY)

**Caption:** Yan Wei Lim, SDSU graduate student and author on the paper, exploring corals in the southern Line Islands.



**Photographer:** Rob Edwards (CC BY)

**Caption:** SDSU Postdoctoral Researcher Andreas Haas takes a break from processing the water samples in the large tubes to survey the view of Vostock island from the aft deck of the Hanse Explorer.

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### Abstract (from the article):

Genomics and metagenomics have revolutionized our understanding of marine microbial ecology and the importance of microbes in global geochemical cycles. However, the process of DNA sequencing has always been an abstract extension of the research expedition, completed once the samples were returned to the laboratory. During the 2013 Southern Line Islands Research Expedition, we started the first effort to bring next generation sequencing to some of the most remote locations on our planet. We successfully sequenced twenty six marine microbial genomes, and two marine microbial metagenomes using the Ion Torrent PGM platform on the Merchant Yacht Hanse Explorer. Onboard sequence assembly, annotation, and analysis enabled us to investigate the role of the microbes in the coral reef ecology of these islands and atolls. This analysis identified phosphonate as an important phosphorous source for microbes growing in the Line Islands and reinforced the importance of L-serine in marine microbial ecosystems. Sequencing in the field allowed us to propose hypotheses and conduct experiments and further sampling based on the sequences generated. By eliminating the delay between sampling and sequencing, we enhanced the productivity of the research expedition. By overcoming the hurdles associated with sequencing on a boat in the middle of the Pacific Ocean we proved the flexibility of the sequencing, annotation, and analysis pipelines.