

Development of PCR-HRMA for detection and genetic epidemiological analysis in *Vibrio harveyi*

1. **Developing Staff Members** : Development of PCR-HRMA for detection and genetic epidemiological analysis in *Vibrio harveyi*.

2. Developing Staff Members

Department	Name	Position
General Research	Shun	Assistant professor
Service Center	Maekawa	rank research fellow

3. Development Idea

High-resolution melting analysis (HRMA) is a post-PCR analytical methodology used for identifying mutations or SNPs in nucleic acid sequences. In this project, we developed the molecular technic to specific clustering methodology of *Vibrio harveyi*, as an important pathogen in aquaculture, using PCR-HRMA analysis.

4. Technological Competition and

Industrial Application

There are many kits for detecting specific pathogens, which show high market value in research and veterinary diagnosis in the aquaculture area. The technology in this project will be premised on commercializing reagents and experimental methods as a detection kit. Moreover, the biological technic in this project allows us to analyze the epidemiology of *V. harveyi*. Therefore, the findings of this project and related products

will provide high market value in aquaculture field.

5. Merchandise Statement of Achievement

For PCR reaction condition, we found the better reaction conditions for this project, which were using 2 × EvaGreen® reagent (Bio-Rad) and CFX Connect Real-Time PCR Detection System (Bio-Rad) . PCR reactions were performed under the following conditions, 15 s at 95°C, 15 s at a 56°C for 40 cycles and 0.1°C/sec for DNA melting analysis. The amount of *Vibrio ahrveyi* were used per sample in 0.01 - 10 ng. In this study, we also have identified the specific 8 primer sets for epidemiological analysis of hierarchical clustering.