Hong Kong Offshore LNG Terminal Project
Marine Conservation Enhancement Fund

Executive Summary

Project Period: June 1, 2022 – May 31, 2025

1. Project Title:

Exploiting a Novel Digital Droplet PCR Platform for the Study of Marine Biodiversity: A Pilot Study on Targeted Fishes

2. Project Number: MCEF20108

3. Executive Summary

Hong Kong is rich in marine biodiversity, particularly in the areas of western waters and southern waters, which are natural habitats of many endangered species of high conservation values. Recognizing the importance of conserving local biodiversity conservation within Hong Kong, the government has formulated the city-level strategy, namely the Hong Kong Biodiversity Strategy and Action Plan 2016-2021, to implement actions such as improving our knowledge towards the priority marine species and habitats. Environmental DNA (eDNA), rich in marine environmental samples, has emerged as a promising biomarker for the studies of biodiversity or targeted detection across various taxa. Constrained by the currently available nucleic acid analytical tool, the analysis is either expensive or cannot identify targets of minute amount in the environmental samples.

Aiming at developing a platform for routine analysis of endangered rare species and habitats for sustainable management of marine ecosystems in Hong Kong, this project sets to exploit a novel beaded digital polymerase chain reaction (bdPCR) platform for the analysis of eDNA, as a biomarker for the study of marine biodiversity. Supported by the Marine Conservation Enhancement Fund, this key contributions of this project are detailed in the following:

1. **Develop the novel beaded digital polymerase chain reaction (bdPCR) platform for the analysis of eDNA:** The research team has established the backbone technology of bdPCR platform for the analysis of rare DNA. The procedures of sample collection from designated water bodies have also been validated by the research team. The protocol for the analysis of eDNA has also been validated. Moreover, the detection sensitivity and specificity based on the established protocol have also been identified.
2. **Analyze the eDNA presented in the environmental samples collected from some representative marine water bodies in Hong Kong:** Through this project, we have analyzed the eDNA sample collected from Lamma Island, including samples from full-depth water (9 L seawater × 3 replicates) and sediment (3 g × 3 replicates) at 3 sample sites.
3. **Identify the targeted fish species presented in the eDNA samples by bdPCR and compare with the results analyzed by DNA sequencing:** We have designed primers specific to for 5 targeted fish species: *Hippocampus* sp. (*H. kuda, H. kelloggi, H. reidi, H. trimaculatus*, very rare, protected species, ecologically important); *Pennahia anea* (abundant croakers in Hong Kong, prey species for dolphins, economically and/or ecologically important). Genus-specific primers have also been designed for genus *Hippocampus* and *Pennahia*, family-specific primers have been designed for family Syngnathidae and Sciaenidae. Three sets of universal fish primers have been selected for screening of the

whole fish community. These primers have been tested for their specificities by normal bulk PCR. The detection sensitivity has been established by traditional water-in-oil ddPCR and qPCR. Subsequently, the performance for the bdPCR has been contrasted. Through analysis of the metabarcoding result, we have identified more fish species by bdPCR, showcasing the potential of bdPCR.

The research results are published in 2 journal articles and 3 conference papers. 1 journal article is currently under review. With the developed bdPCR under this project, we may expedite the investigation of eDNA and marine diversity in general. We have shared our results and experiences through at least 7 seminar talks or presentations with researchers in the community, industrial partners and government sectors. In summary, the developed bdPCR represents a novel, facile and yet reliable strategy for routine eDNA analysis *via* metabarcoding, and ultimately for the investigations of rare and endangered marine species and the screening of marine biodiversity.