Newly designed PCR-based method for the detection of jellyfish in aquaculture system

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ABSTRACT

Scyphozoa, commonly referred to as jellyfish within the Cnidaria, can lead mass mortality in aquaculture and fisheries through a direct harmful impact with their toxins derived from their nematocysts or indirect effects such as gill malfunctions. In addition, their adhesive polyps can directly attach to aquaculture systems and produce ephyra and medusae. Jellyfish blooms are becoming more frequent due to global warming and increased anthropogenic activity, which means their impact on an aquaculture and fisheries will become increasingly significant. Efficient and precise monitoring of jellyfish occurrences and blooms is important to prevent subsequent problems and find solutions. Therefore, we designed new COI target primer sets with higher resolution for the detection of jellyfish and conducted an *in silico* evaluation. Scyphozoa COI sequences were assembled from NCBI search results for "Scyphozoa, complete" and aligned with MEGA-X software. Primers were designed to cover highly variable regions, meeting criteria such as GC%, amplicon size, and primer length. Consequently, primer sets were developed to generate approximately 350 bp amplicons. The accuracy of the newly designed primers was evaluated using BLASTn and PrimerMiner in R package. With these detection methods, we expect rapid detection of jellyfish bloom both in and outside of aquaculture environments.

Method

1. Sequence collect → 2. Alignment → 3.Primer design → 4. *In silico* PCR → 5. *In vitro* validation

NCBI · MEGA Molecular Evolutionary Genetics Analysis · GENE RUNNER · PrimerMiner Version 0.3b

- Synthesized gBlock DNA
- PCR

Results

Fig 1 Location within COI sequence of newly designed primer set

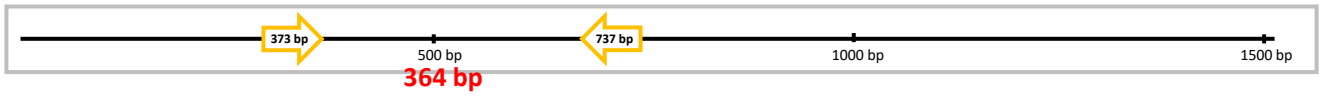
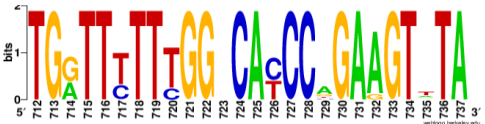


Fig 2 Sequence logos represent the base of Scyphozoa targeted primer regions and below show the designed primer sequences.

> Forward

5'-GGDACNGGNTGRACNGTNTAYCCNC-3'

> Reverse



5'-TGRTTYTTTGGKCAYCCWGAAGTTTA-3'

Fig 3 Comparison of the *in silico* PCR results of existing primers and newly designed primer set

Threshold value was specified as 200.

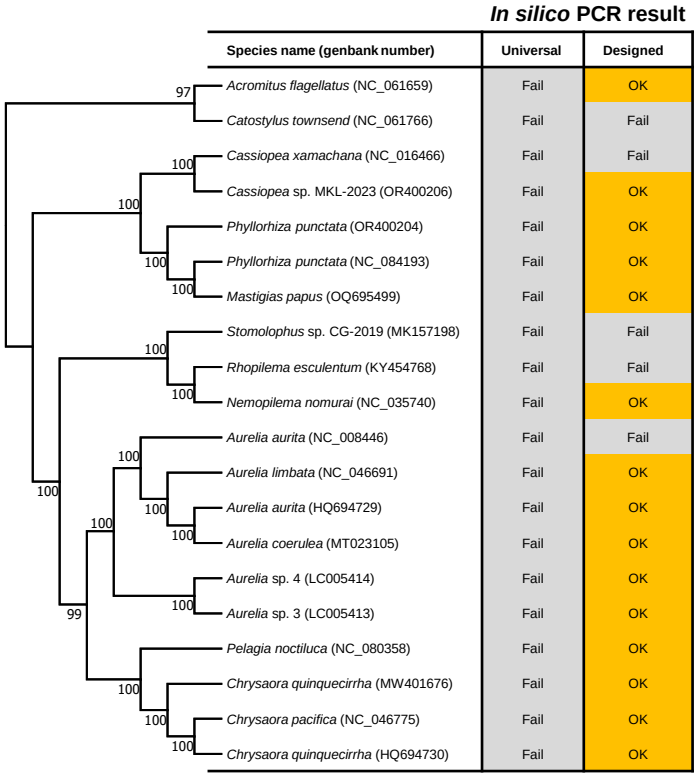
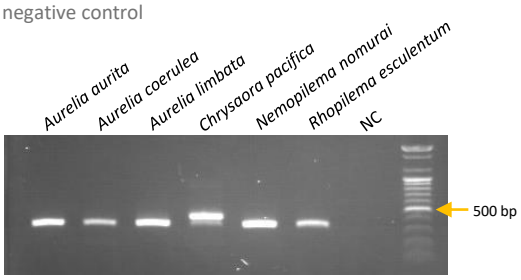


Fig 4 Electrophoresis result of gBlock DNA amplification through PCR method

NC; negative control



Conclusion

- ✓ The newly designed primer set is expected to be able to rapid detection of jellyfish bloom both in and outside of aquaculture environments.
- ✓ Easily detecting and responding quickly to polyp stages that are difficult to identify will help solve problems such as increasing weight of nets, causing deformation, and reducing buoyancy of the structures that easily occur in aquaculture.
- ✓ Tests on actual DNA sample obtained from environmental are required to confirm applicability in the field.