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Development of One cell or One individual Direct PCR of Protozoan or Metazoan 18S rRNA Gene for Molecular Ecology

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Abstract: The new method for one cell or one individual direct PCR to build a local DNA database of protozoans and metazoans for the molecular ecological studies was developed. At first, we applied a glass capillary method for isolating a protozoan cell and a metazoan individual. The other sources were from wastewater treatment (activated sludge) systems in Nagasaki. The addition of BSA to a water droplet was very useful for the quick isolation of a protozoan cell due to reducing the protozoanmotion. In order to decompose dissolved DNA of other organisms, DNase-I was added to the PCR tube and incubated for 30 min. Then, 70 per cent EtOH of 100 µl was added to the PCR tube .It was sequentially treated by sonication for 30 sec and heated for 2min in a micro wave oven. We applied a nested PCR for 18S rRNA gene of the isolated rotifer individual and a protozoan cell. Finally, we determined the sequence of each PCR amp icon for the rotifer individual or the protozoa. As a result, using the developed new method, we could correctly determine the partial sequence of 18r RNA genes of 17 samples of ciliates and rotifers in 20 samples from natural ponds and activated sludge systems.

***Keywords*:** Protozoan and metazoan, 18S rRNA gene, Molecular ecology, One cell or one individual direct PCR