

【Workshop 5】 Perspectives of Metagenomics in Agricultural Research
Suppressed-Priming PCR, a Novel Concept of DNA Quantification
Based on PCR Kinetics

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PCR-DGGE with universal primers is useful in estimating the diversity of DNA templates in environmental samples or any other assemblage of small organisms. Despite the advantage of PCR-DGGE, biases in PCR amplification can lead to inaccurate estimation. To overcome this problem, we propose the concept of an unbiased DNA quantification method based on PCR kinetics, named suppressed-priming PCR. In this method, a set of PCRs was performed with a dilution series of primers, which differently limited DNA amplification. The difference in amplification efficiency between the DNA templates reflected the estimates of their relative amounts. The reliability of the concept was briefly tested by applying it to DNA quantification using PCR-DGGE as follows. Two nematode species, *Acrobeloides* sp. and *Tylencholaimus parvus*, maintained in laboratory culture were chosen for experimental use. *Acrobeloides* sp. was regarded as an internal standard against *T. parvus*, the target. DNA was extracted from the 2 species, purified and mixed in equal amount for subsequently use as DNA templates in PCR. After suppressed-priming PCR, the DNA amounts of the target and the internal standard in single PCR products were log-transformed, and regression analysis was done to estimate the initial concentration of the DNA template of the target. The log-transformed data sets were well regressed linearly with highly significant coefficients of determination. The average of estimates was comparable to the true value, although it was a slight overestimation. Hence, the model of suppressed-priming PCR appears to be a reasonable approximation of the fact. The estimates of target DNA amount varied widely across trials. This might be assigned to errors in post-PCR processes. The concept of suppressed-priming PCR is applicable to any PCR-based quantification method. We emphasize that the concept of suppressed-priming PCR might serve as a theoretical base for further development of accurate and robust PCR-based quantification methods, especially when combined with methods that sensitively detect traces of PCR products, e.g. PCR T-RFLP.