Evaluation of Metagenome Library Quality for Lipolytic Enzyme Discovery

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In this work, the influence of soil category, seasons of soil sampling and method of eDNA isolation on the quality of DNA was analyzed.

The soil samples were collected in the autumn, winter and spring and the DNA was isolated by the direct method. The greatest amount of DNA and highest efficiency was achieved in the samples collected in the spring. The selected soil sample was used for DNA isolation using various direct or indirect methods and eDNA quality analysis. The direct or indirect methods of eDNA separation differ from each other in the cell lysis process (physical, chemical or enzymatic) and DNA purification procedure. To compare the methods, three differentiators (the amount of DNA and its size and purity) were selected and an analysis for the presence of inhibitors of enzymatic reactions was conducted.

Among the indirect methods, the most favorable results were obtained with the classical direct extraction method. The calculated average eDNA size was 38 kb, with a yield of separation about 50 % lower than that obtained with the commercial kits, but over 95 % higher than that offered by other indirect methods. All the direct methods used in this study were based on bead-beating, and differed in the composition of buffers and methods of eDNA purification. The average size of eDNA isolated using direct methods was 20 kb and the yield was significantly higher (FastDNA® SPIN kit for soil – 46.6 µg DNA/ g soil) than that provided by the indirect methods (Hardeman & Sjoling method – 0.6 ng DNA/ g soil). Apart from the eDNA obtained using these two methods, the other methods applied in this study provided eDNA free of enzyme inhibitors.

The high purity of DNA was obtained using a GeneMatrix soil DNA isolation kit, *i.e.* $A_{260/280}$=1.83. The combination of the methods proposed by the FastDNA spin kit and GeneMatrix Soil DNA isolation kit, further optimization of the disintegration allowed to achieve high-quality DNA ideal for cloning into a plasmid vector, *i.e.* eDNA size of approximately 5 kb, purity 1.75 and isolation efficiency 24.8 µg DNA/ g soil.

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