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Optimization of microbial community DNA isolation and purification from membrane bioreactor (MBR) biofilms treating petrochemical wastewater

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Characterization and understanding the dynamics in complex microbial communities in wastewater treatment systems such as membrane bioreactors (MBRs) is essential for design, optimal operation as well as efficiently control (Dabert et al., 2002). Molecular tools based on the analysis of 16S rRNA encoding genes have been successfully used to demonstrate the composition, structure and function, as well as succession within such complex communities (Dabert et al., 2002; Luxmy et al., 2000). Multi-factorial experimental design may be of value when evaluating and optimizing DNA extraction methods. This approach may drastically reduce the number of experiments required for optimizations (Boleda et al., 1996). In this study the influence of three parameters of a DNA extraction protocol, on the quality and yield of DNA isolated from petrochemical wastewater membrane bioreactor biofilm, was evaluated. The parameters included (i) necessity of freeze–thawing, (ii) phenol:chloroform:isoamyl alcohol incubation temperature and (iii) time of PVP addition (as part of the initial CTAB mix or after the freeze–thawing/initial incubation). The results show that DNA yields were generally high (3.0 ± 1.06 to 32.8 ± 18.89 μg/330 mg of biofilm wet weight) and the quality generally good (A_{260}/A_{280} above 1.70 ± 0.49). DNA yield, quality, PCR amplification and profiling by denaturing gradient gel electrophoresis data indicate that (i) addition of PVP after the initial CTAB incubation appears to be more effective than adding the PVP to the initial CTAB extraction buffer and (ii) hot (65 °C) phenol:chloroform:isoamyl alcohol extraction may be essential for extracting high quality DNA from petrochemical wastewater. Furthermore, a two-level, three-factor experimental design that was used in this study was useful for evaluating the DNA extraction protocol.

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Improvement on the two-step total gene synthesis method

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In the post-genomic era, the ability to synthesize any arbitrary DNA sequence is increasingly in demand. Conventionally, PCR-based gene synthesis uses a two-step approach (Lei, 2004). The major drawback of total gene synthesis is the high tendency of DNA sequence errors.

To minimize errors in the method of two-step synthesis, the products of two-synthesis step were treated with T7 endonuclease I, respectively. The full-length PCR products were denatured and rehybridized, so that all the mutations would end up in heteroduplexes. T7 endonuclease I recognizes and cleaves non-perfectly matched DNA. The cleavage site is at first, second or third phosphodiester bond that is 5′ to the mismatch (Liu et al., 2006). The full-length products were then separated from the cleaved products by agarose gel purification.

Using this improved two-step gene synthesis method, we synthesized an *Escherichia coli* codon optimized human growth hormone gene. The whole DNA fragment was 1040 bp. In the control group, there were total 33 base errors on whole 4158 nucleotides, none of the four clones was the accurate sequence. In four of the clones which were treated with T7 endonuclease I, two of them were confirmed to be the correct products. At the same time, only two base error on whole 4159 nucleotides.

Experiment demonstrates that the product of two-synthesis step is a significant improvement on the two-step total gene synthesis method.

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Reaction and complex(nanocrystal) formation of α-cyclodextrin and poly(ethylene glycol) and poly(vinyl alcohol)

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Cyclodextrin usually characterizes a doughnut or wreath-shaped truncated cones that have hydrophobic cavity of appropriate dimensions that can form inclusion complexes with a variety of organic compounds in aqueous solution. In these complexes, a guest molecule is held within the cavity of cyclodextrin host molecule. Complex formation is a dimensional fit between host cavity and guest molecule. The lipophilic cavity of cyclodextrin molecules provides a microenvironment into which appropriately sized non-polar moieties can enter to form inclusion complexes. In this research, we used this reaction for the formation of nanocrystals prepared by inclusion complex of α-cyclodextrin–poly(ethylene glycol). We tried to define the effect of temperature and time of reaction on crystal growth and their shapes and conversion and also the behavior of crystals varied in