An Extendable Method to Produce Specific Base Pair Lengths of DNA

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Short DNA oligonucleotides have been often exploited by DNA nanotechnology to construct a variety of nanostructures.[1, 2] However, oligonucleotides produced by automated DNA synthesis are limited in length, sequence and modification. Therefore, DNA of specific lengths and composition over 100 base pairs would be useful to improve the range of possible nanoarchitectures that could be assembled.

A PCR-based approach has been developed to provide an enzymatic method for the synthesis of long DNA consisting of multiple repeating sequences.[3] This method is capable of annealing DNA duplexes to form 'sticky ends' which results in extension of the DNA using a *Thermococcus gorgonarius* Family B polymerase exonuclease minus variant, Z3.[4] Any repeat sequence of DNA can be extended and produce products which range in length from 200 to 20,000+ base pairs.

In order to produce multiple DNA samples, each of a specific base pair length, we demonstrate here the PCR-based method in conjunction with a size recovery gel technique. The previously extended DNA sequences can be separated to obtain narrow ranges of base pair lengths, for example 200 to 400 base pairs, 500-700 base pairs and so on. The Lonza flash gel system™ can be exploited to aid simple recovery of user defined DNA lengths; however relatively low yields are obtained. Once separated, amplification of the recovered DNA sequences by PCR affords generous amounts of the target sequence.

Applications of this method to obtain DNA of specific base pair lengths are expected to be in the development of nanostructures and nanodevices.[1]

Scheme 1. Heat-cool cycles of ds-DNA followed by size recovery and amplification of the specific base pair length DNA.

[1] A. V. Pinheiro et al., Nature Nanotechnology 6 (2011) 763-772.
[2] M. Zahid et al., Nanoscale Research Letters 8 (2013) 1-13.
[3] C. J. Whitfield et al., Angewandte Chemie International Edition 54 (2015) 8971-8974.
[4] S. K. Jozwiakowski et al., ChemBioChem 12 (2011) 35-37.