Molecular Engineering of Novel Nucleotide Analogues for DNA Sequencing and Analysis

Jia Guo

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ABSTRACT

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DNA sequencing by synthesis (SBS) on a solid surface during the polymerase reaction can decipher multiple DNA sequences in parallel. The first part of this thesis presents the development of a DNA sequencing method that is a hybrid between the Sanger dideoxy chain terminating reaction and SBS. In this approach, four nucleotides, modified as reversible terminators by capping the 3'-OH group with a small reversible moiety so that they are still recognized by DNA polymerase as substrates to extend the DNA chain, are used in combination with a small percentage of four cleavable fluorescent dideoxynucleotides to perform SBS. Sequences are determined by the unique fluorescence emission of each fluorophore on the DNA products terminated by ddNTPs. Upon removing the 3'-OH capping group from the DNA products generated by incorporating the 3'-O-modified dNTPs and the fluorophore from the DNA products terminated with the ddNTPs, the polymerase reaction reinitiates to continue the sequence determination. Various DNA templates, including those with homopolymer regions were accurately sequenced with readlengths of over 30 bases using this hybrid SBS method on

a chip and a four-color fluorescent scanner. To further extend the read-length of this hybrid sequencing method, a consecutive DNA sequencing by primer reset approach is developed. Upon removing the sequenced DNA strand and reattaching the original primer to allow the extension of this primer with a combination of natural and modified nucleotide analogues to the end of the first round sequence, the hybrid SBS can be carried out from that point to decipher the adjacent cluster of bases on the template. The sequencing read-length of a DNA template immobilized on a chip is almost doubled using this primer reset approach.

Single nucleotide polymorphisms (SNPs) are important markers for disease gene identification and for pharmacogenetic studies. The second part of this thesis describes the design, synthesis and evaluation of a chemically cleavable biotinylated nucleotide analogue, ddATP-N_3-biotin, for multiplex SNP analysis by MALDI-TOF MS. This nucleotide analogue has a biotin moiety attached to the 7-position of 2',3'-dideoxyadenosine 5'-triphosphate through a chemically cleavable azide-based linker. We have demonstrated that this ddATP-N_3-biotin is faithfully incorporated by the DNA polymerase Thermo Sequenase. The generated DNA extension products can be efficiently isolated by a streptavidin-coated surface and recovered under a mild chemical cleavage conditions. Single and mutiple primer extension reactions were performed using ddATP-N_3-biotin to generate and isolate DNA extension products for MALDI-TOF MS analysis.

DNA microarray technology offers a paradigm for the study of genome-wide patterns of gene expression. The cDNA labeling step plays an important role in the accuracy and reproducibility of a microarray experiment. The third part of this thesis

focuses on the development of a click chemistry based cDNA labeling strategy for microarray analysis. In this approach, azide modified nucleotide analogues along with natural nucleotides are incorporated in reverse transcription reactions with RNA samples as templates. The azide groups on the generated cDNAs are coupled with alkyne functionalized fluorophores by click chemistry. Due to the high stability of the azide and alkyne groups in aqeous solution, the cDNAs are labeled efficiently with sufficient amount of the fluorescent molecules for microarray analysis using this approach.

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