Four-Color DNA Sequencing by Synthesis on a Chip Using Cleavable Fluorescent Nucleotide Reversible Terminators

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Submitted in partial fulfillment of the
requirements for the degree
of Doctor of Philosophy
in the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2008

ABSTRACT

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DNA sequencing by synthesis (SBS) on a solid surface during the polymerase reaction offers a new paradigm for the deciphering of DNA sequences. This thesis focuses on the construction of such a novel DNA sequencing system using molecular engineering approaches. In this approach, four nucleotides (A, C, G, T) are modified as reversible terminators by attaching a cleavable fluorophore to the base and capping the 3'-OH with a small chemically reversible moiety so that they are still recognized by DNA polymerase as substrates. First, we used a 2-nitrobenzyl based photocleavable (PC) linker to attach a fluorophore to 3'-O-allyl-modified nucleotides, forming photocleavable fluorescent nucleotide reversible terminators, 3'-O-allyl-dNTPs-PC-fluorophore, for application in SBS. The fluorophore and the 3'-O-allyl group on a DNA extension product, which is generated by incorporating the 3'-O-allyl-dNTPs-PC-fluorophore in a polymerase reaction, are removed by photocleavage (irradiation at 355 nm) and Pd-catalyzed deallylation, respectively. This allows the re-initiation of the polymerase reaction and continuation of SBS. We then found that an allyl moiety can be used successfully as a linker to tether a fluorophore to 3'-O-allyl-modified nucleotides, forming chemically cleavable fluorescent nucleotide reversible terminators, 3'-O-allyl-dNTPs-allyl-fluorophore, for application in SBS. The fluorophore and the 3'-O-allyl group on a DNA

extension product were now able to be removed simultaneously in 30 seconds by the Pd-catalyzed deallylation reaction in an aqueous buffer solution. This one-step dual-deallylation reaction thus allowed the re-initiation of the polymerase reaction and increased the SBS efficiency. We also developed an alternative sequencing method that is a hybrid between the Sanger dideoxy chain terminating reaction and sequencing by synthesis (SBS) and delineate the advantages that come with this hybrid sequencing strategy. In this approach, four nucleotides, modified as reversible terminators (3'-O-PC-dNTPs) by capping the 3'-OH with a PC reversible 2-nitrobenzyl moiety so that they are still recognized by DNA polymerase as substrates, are used in combination with four PC fluorophore labeled dideoxynucleotides (ddNTPs-PC-fluorophore) to generate Sanger sequencing fragments during SBS. The DNA sequence was determined by the unique fluorescence emission of each fluorophore on the ddNTPs. Upon removing the 3'-OH blocking group on the dNTPs and the fluorophore from the ddNTPs, the polymerase reaction can reinitiate and the DNA sequences can be continuously determined. Four-color DNA sequencing was performed using these novel fluorescent nucleotide analogues and a four-color fluorescent scanner to identify sequences of DNA template immobilized on a chip. The DNA chip was constructed by covalently attaching alkyne-modified self-priming DNA template onto an azido-PEG functionalized glass slide by using 1,3-dipolar cycloaddition chemistry. DNA sequences were obtained for various DNA templates, including DNA templates containing homopolymeric regions in their sequence.

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