1996 Phase I

An Engineered RNA/DNA Polymerase to Increase Speed and Economy of DNA Sequencing

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DNA sequence information is the cornerstone for considerable experimental design and analysis in the biological sciences. The proposed studies will focus on advancing DNA sequencing by creating a new enzyme that eliminates the need for an oligonucleotide primer to initiate DNA synthesis at a defined site, and that can use dideoxy nucleotides for chain termination. The new method should reduce the time and cost required to obtain DNA sequences and enhance the speed and cost effectiveness of current DNA sequencing technologies. Phase I studies will focus on purifying mutant T7 RNA polymerases known to incorporate dNTPs into DNA chains, developing protocols for rapid small scale mutant enzyme purification, evaluating the purified mutants for properties relevant to DNA sequencing, developing facile mutagenesis schemes and producing mutant RNA/DNA polymerases with altered promoter recognition. The results from phase I will provide the foundation for Phase II research, which will focus on refining properties of the mutant by: (1) expanding the number of mutations examined using the purification protocols, assays, and mutagenesis screening methods developed in Phase I and (2) examining the effect of each mutation on enzymatic properties important to DNA sequencing applications, and (3) optimizing conditions for sequencing performance. In Phase III, Promega will commercialize the new mutant enzymes through its own extensive distribution network and by collaborating with major instrumentation firms to adapt the technology to automated DNA sequencing systems.

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Directed Multiple DNA Sequencing and Expression Analysis by Hybridization

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The overall goal of this project is to develop molecular resources with direct applications to either DNA sequence analysis or gene expression analysis in multiplexed formats using sequential hybridization of Peptide Nucleic Acid (PNA) oligomer probes. PNA oligomers hybridize more stably and specifically to cognate DNA targets than conventional DNA oligonucleotides. The Phase I project discussed here is concerned with development of PNA probe technology having direct application either to the directed sequencing process or to gene expression profiling. With regard to directed sequencing, we seek improvements in the three multiply repeated steps associated with this process, namely (1) probe assembly, (2) sequencing reactions, and (3) gel electrophoresis. In PNA hybridization sequencing, sequences are generated directly from the template by multiplex DNA sequencing using anchor primers known to have frequent annealing sites. Electrophoresis is performed en masse for each anchor primer reaction, blotted to nylon membranes and individual sequences are selectively exposed by iterative hybridization to specific 8-mer PNA probes derived from sequences statistically over-represented in expressed DNA and obtained from a pre-synthesized library. Additionally, the same PNA library can be used as a source of hybridization probes for querying expression patterns of specific genes in any cell line or tissue. Specific gene expression can be monitored by coupling gene-specific RT-PCR with hybridization when cDNA products are separated by gel electrophoresis and blotted to nylon membranes. Patterns of gene expression are then resolved by hybridization using PNA oligomers. Bands corresponding to specific genes can be deconvoluted using sequence information from RT-PCR primers and PNA probes. Higher throughput expression analysis can be achieved by multiplexed gel electrophoresis, blotting and iterative probing of RT-PCR reactions with individual PNA probes.

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