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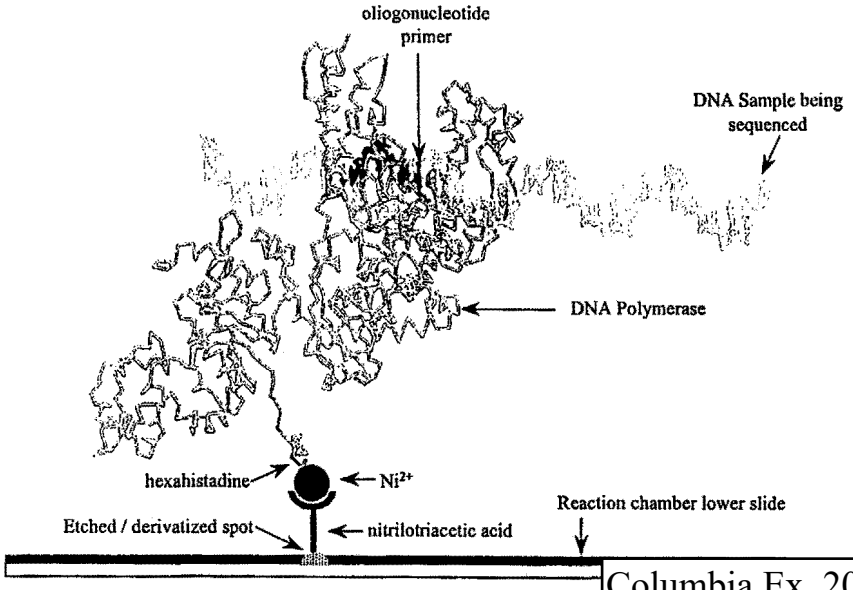
(54) Title: A METHOD FOR DIRECT NUCLEIC ACID SEQUENCING



Example of a DNAS Reaction

(57) Abstract

The present invention provides a novel sequencing apparatus and the methods many single nucleic acid molecules simultaneously, in parallel. The methods and ap effective, high through–put method by which nucleic acid molecules from any source can be readily sequenced without the need for prior amplification of the sample or prior knowledge of any sequence information.

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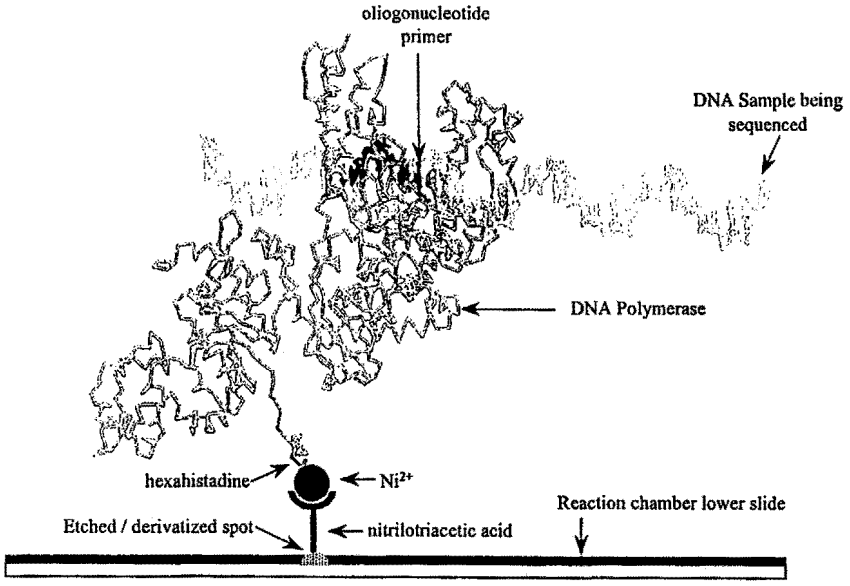

Example of a DNAS Reaction Center

(57) Abstract

The present invention provides a novel sequencing apparatus and the methods employed to determine the nucleotide sequence of many single nucleic acid molecules simultaneously, in parallel. The methods and apparatus of the present invention offer a rapid, cost effective, high through–put method by which nucleic acid molecules from any source can be readily sequenced without the need for prior amplification of the sample or prior knowledge of any sequence information.

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A METHOD FOR DIRECT NUCLEIC ACID SEQUENCING

FIELD OF THE INVENTION

The present invention relates to methods for sequencing nucleic acid samples. More specifically, the present invention relates to methods for sequencing without the need for amplification; prior knowledge of some of the nucleotide sequence to generate the sequencing primers; and the labor-intensive electrophoresis techniques.

BACKGROUND OF THE INVENTION

The sequencing of nucleic acid samples is an important analytical technique in modern molecular biology. The development of reliable methods for DNA sequencing has been crucial for understanding the function and control of genes and for applying many of the basic techniques of molecular biology. These methods have also become increasingly important as tools in genomic analysis and many non-research applications, such as genetic identification, forensic analysis, genetic counseling, medical diagnostics and many others. In these latter applications, both techniques providing partial sequence information, such as fingerprinting and sequence comparisons, and techniques providing full sequence determination have been employed. See, *e.g.*, Gibbs *et al.*, *Proc. Natl. Acad. Sci USA* **86**: 1919-1923 (1989); Gyllensten *et al.*, *Proc. Natl. Acad. Sci USA* **85**: 7652-7656 (1988); Carrano *et al.*, *Genomics* **4**: 129-136 (1989); Caetano-Annoles *et al.*, *Mol. Gen. Genet.* **235**: 157-165 (1992); Brenner and Livak, *Proc. Natl. Acad. Sci USA* **86**: 8902-8906 (1989); Green *et al.*, *PCR Methods and Applications* **1**: 77-90 (1991); and Versalovic *et al.*, *Nucleic Acid Res.* **19**: 6823-6831 (1991).

Most currently available DNA sequencing methods require the generation of a set of DNA fragments that are ordered by length according to nucleotide composition. The generation of this set of ordered fragments occurs in one of two ways: (1) chemical degradation at specific nucleotides using the Maxam-Gilbert method or (2) dideoxy nucleotide incorporation using the Sanger method. See Maxam and Gilbert, *Proc Natl Acad Sci USA* **74**: 560-564 (1977); Sanger *et al.*, *Proc Natl Acad Sci USA* **74**: 5463-5467 (1977). The type and number of required steps inherently limits both the number of DNA segments that can be sequenced in parallel, and the amount of sequence that can be determined from a given site. Furthermore, both methods are prone to error due to the anomalous migration of DNA fragments in denaturing gels. Time and

space limitations inherent in these gel-based methods have fueled the search for alternative methods.

In an effort to satisfy the current large-scale sequencing demands, improvements have been made to the Sanger method. For example, the use of fluorescent chain terminators simplifies detection of the nucleotides. The synthesis of longer DNA fragments and improved fragment resolution produces more sequence information from each experiment. Automated analysis of fragments in gels or capillaries has significantly reduced the labor involved in collecting and processing sequence information. See, *e.g.*, Prober *et al.*, *Science* **238**: 336-341 (1987); Smith *et al.*, *Nature* **321**: 674-679 (1986); Luckey *et al.*, *Nucleic Acids Res* **18**: 4417-4421(1990); Dovichi, *Electrophoresis* **18**: 2393-2399 (1997).

However, current DNA sequencing technologies still suffer three major limitations. First, they require a large amount of identical DNA molecules, which are generally obtained either by molecular cloning or by polymerase chain reaction (PCR) amplification of DNA sequences. Current methods of detection are insensitive and thus require a minimum critical number of labeled oligonucleotides. Also, many identical copies of the oligonucleotide are needed to generate a sequence ladder. A second limitation is that current sequencing techniques depend on priming from sequence-specific oligodeoxynucleotides that must be synthesized prior to initiating the sequencing procedure. Sanger and Coulson, *J. Mol. Biol.* **94**: 441-448 (1975). The need for multiple identical templates necessitates the synchronous priming of each copy from the same predetermined site. Third, current sequencing techniques depend on lengthy, labor-intensive electrophoresis techniques that are limited by the rate at which the fragments may be separated and are also limited by the number of bases that can be sequenced in a given experiment by the resolution obtainable on the gel.

In an effort to dispense with the need for electrophoresis techniques, a sequencing method was developed which uses chain terminators that can be uncaged, or deprotected, for further extension. See, U.S. Patent No. 5,302,509; Metzker *et al.*, *Nucleic Acids Res.* **22**: 4259-4267 (1994). This method involves repetitive cycles of base incorporation, detection of incorporation, and re-activation of the chain terminator to allow the next cycle of DNA synthesis. Thus, by detecting each added base while the DNA chain is growing, the need for size-fractionation is eliminated. This method is nevertheless still highly dependent on large amounts of nucleic acid to be sequenced and the use of known sequences for priming the initiation of chain growth. Moreover, this technique is plagued by any inefficiencies of

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