

## PROBE FOR NUCLEIC ACID SEQUENCING AND METHODS OF USE

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Nos. 60/749,729 and 60/749,858 both filed Dec. 12, 2005 and herein incorporated by reference.

### FIELD

[0002] This disclosure relates to probes and methods for sequencing nucleic acid molecules, such as DNA and RNA, which can be used for research and the diagnosis of disease in clinical applications.

### BACKGROUND

[0003] Numerous methods have been used to sequence nucleic acid molecules. The traditional Maxam-Gilbert chemical degradation method involves the chemical-specific cleavage of DNA (Maxam and Gilbert, *Proc. Natl. Acad. Sci., USA* 74:560, 1977). In this method, radio-labeled DNA molecules are incubated in four separate reaction mixtures, each of which partially cleaves the DNA at one or two nucleotides of a specific identity (G, A+G, C or C+T). The resulting DNA fragments are separated by polyacrylamide gel electrophoresis, with each of the four reactions fractionated in a separate lane of the gel. The DNA sequence is determined after autoradiography by observing the macromolecular separation of the fragments in the four lanes of the gel.

[0004] The Sanger dideoxy chain termination method involves generating DNA molecules of differing lengths by enzymatic extension of a synthetic primer, using DNA polymerase and a mixture of deoxy- and dideoxy-nucleoside triphosphates (Sanger et al., *Proc. Natl. Acad. Sci., USA* 74:5463, 1977). The reactions are separated in four parallel lanes on polyacrylamide gels and the sequence determined after autoradiography.

[0005] The use of fluorescent nucleotides has eliminated the need for radioactive nucleotides, and provides a means to automate DNA sequencing (for example see U.S. Pat. No. 5,124,247 to Anson, U.S. Pat. No. 5,242,796 to Prober et al., U.S. Pat. No. 5,306,618 to Prober et al., U.S. Pat. No. 5,360,523 to Middendorf et al., U.S. Pat. No. 5,556,790 to Pettit, and U.S. Pat. No. 5,821,058 to Smith et al.). However, methods that use fluorophores generally still require gels or capillary electrophoresis, and thus are slow and macroscopic.

[0006] Another potential obstacle with using fluorescently labeled dNTPs is that no one has been able to synthesize a fully fluorescently labeled DNA molecule. Therefore, sequencing methods that permit the synthesis of the complementary nucleic acid strand are still needed.

### SUMMARY

[0007] The present disclosure provides an improved probe that can be used in the sequencing of nucleic acid molecules, and methods for using the probe. In particular examples the probe can be used to determine the transcription levels of one or more genes. For example, the probe can be used to count individual RNA transcripts, thereby providing an estimate of the number produced in a cell. In particular examples, the probes and methods disclosed herein are used as an alternative to currently available microarray technologies.

[0008] In particular examples, the probe, named "Medusa", includes a polymerizing agent with one or more (such as a plurality of) molecular linkers attached to the polymerizing agent to link (and in some examples space) one or more chemical moieties (such as a nonhydrolyzable nucleotide) to the polymerizing agent. The chemical moieties are capable of reversibly binding to the template strand of a nucleic acid molecule, without being detached from the linker, by specifically binding with a complementary nucleotide in the target nucleic acid molecule. In disclosed examples the reversible incorporation occurs at the active site of the polymerizing agent. However, ideally the chemical moieties are not capable of being permanently incorporated into a growing nucleic acid strand. The specific binding of the chemical moiety on the linker with a complementary nucleotide in the target nucleic acid molecule is indicated by emission of a characteristic signal that indicates pairing of the chemical moiety on the linker with its complementary nucleotide.

[0009] The polymerizing agent includes an active site that is capable of binding to the target nucleic acid molecule to be sequenced, and in some examples is capable of promoting synthesis of a nucleic acid molecule complementary to the target nucleic acid molecule, wherein the complementary nucleic acid molecule elongates as complementary nucleotides are incorporated into the complementary nucleic acid molecule. Polymerizing agents include compounds capable of reacting monomer molecules (such as nucleotides) together in a chemical reaction to form linear chains (such as a complementary nucleic acid molecule). Exemplary polymerizing agents include but are not limited to, DNA polymerase, RNA polymerase, and reverse transcriptase. In particular examples, the polymerase is a GFP-polymerase. The choice of polymerizing agent can depend on the nucleic acid to be sequenced. For example, if the target nucleic acid molecule is DNA, the polymerizing agent can be a DNA or RNA polymerase, while if the target nucleic acid molecule is RNA, the polymerizing agent can be a reverse transcriptase.

[0010] The chemical moiety that is capable of reversibly binding to a complementary nucleotide in the template strand of the target nucleic acid molecule, without being detached from the linker, can include a nucleotide analog, such as a non-hydrolyzable nucleotide analog. Such analogs can pair with a complementary nucleotide in the target nucleic acid molecule, but are not permanently incorporated into the elongating complementary nucleic acid strand. Non-hydrolyzable nucleotide analogs are known in the art, and include non-hydrolyzable triphosphate nucleotide analogs, such as a non-hydrolyzable triphosphate nucleotide analog with an alpha-beta bond that is non-hydrolyzable.

[0011] In particular examples, the probe includes at least four independent linkers, each of which carries a different chemical moiety capable of specifically pairing with a different nucleotide in the target nucleic acid molecule, but not capable of being permanently incorporated into the elongating complementary nucleic acid molecule. In other examples, the probe includes a plurality of linkers that are joined to form a branched structure, wherein each branch carries a different chemical moiety capable of specifically pairing with a different nucleotide in the target nucleic acid molecule, but not capable of being permanently incorporated into the elongating complementary nucleic acid molecule. For example, the branched structure may only attach to the polymerizing agent at one point.