

the nucleic acid sequence of a target nucleic acid molecule. In particular examples, the method is used to determine if a particular target molecule is present in a sample, and in some examples includes quantitating the amount of target nucleic acid molecule present. For example, methods are provided for using the probe to diagnose a subject having a disease that is associated with one or more nucleic acid mutations.

[0020] Sequencing can be done *in vitro* or *in situ* (for example on a microscope slide) and *in vivo* (for example by introducing the probe into a cell and observing the sequences of mRNA as they are produced). The method allows several nucleic acids to be sequenced simultaneously at the molecular level. For example, a plurality of sequencing reactions can be performed substantially simultaneously, and the signals from the plurality of sequencing reactions detected and converted into a nucleic acid sequence.

[0021] In particular examples, the method includes exposing the target nucleic acid molecule to the probes disclosed herein in the presence of an oligonucleotide primer and a mixture of hydrolyzable nucleotides (such as dATP, dCTP, dGTP, and dTTP or ATP, CTP, GTP and UTP) that are capable of being incorporated into an elongating nucleic acid molecule by base pairing with a complementary nucleotide in the target nucleic acid molecule, and replacing the chemical moiety carried by the linker that reversibly binds to the template strand of the nucleic acid molecule. The emission of a sequence of signals is detected, wherein the signals include the emission of a plurality of the characteristic signals that indicates pairing of the chemical moiety on the linker with its complementary nucleotide. In some examples, the emission of a sequence of signals is converted into a nucleic acid sequence.

[0022] In particular examples, the polymerizing agent is associated with a tag (such as a donor fluorophore), and each different type of chemical moiety (such as a non-hydrolyzable A, T/U, C or G nucleotide analog) is associated with a unique tag that identifies the particular chemical moiety carried by the linker, wherein interaction of the tag associated with the polymerizing agent with the tag associated with the chemical moiety induces emission of the characteristic signal that indicates pairing of the chemical moiety on the linker with its complementary nucleotide. In particular examples, the tag is directly attached to the polymerizing agent or the chemical moiety. However, the tag need not be directly attached, and instead can be found on a molecular linker in sufficient proximity to the polymerizing agent or the chemical moiety to produce an emission of the characteristic signal when the chemical moiety on the linker pairs with its complementary nucleotide.

[0023] For example, the tag associated with the polymerizing agent can be a donor fluorophore and the tag that identifies a particular chemical moiety can include one or more acceptor fluorophores, wherein interaction of the polymerizing agent and the chemical moiety that cannot be incorporated into a synthesized nucleic acid molecule brings the acceptor fluorophore into a proximity with a donor fluorophore that permits excitation of the acceptor fluorophore by the donor fluorophore. In such an example, detecting the signal can include detecting a fluorescent signal emitted from the acceptor fluorophore (or a decreased emission signal from the donor fluorophore). In particular examples, the method further includes exciting the donor fluorophore by a source of electromagnetic radiation (such as a laser) that specifically excites the donor fluorophore and not the acceptor fluoro-

phores. Alternatively, the donor fluorophore is a chemiluminescent molecule, for example aequorin. In this example, the donor fluorophore does not require excitation by a source of electromagnetic radiation, because the chemiluminescent donor fluorophore is naturally in an excited state. This excitation induces the donor to emit light at a wavelength that can transfer energy a distance only sufficient to excite the acceptor fluorophore(s) associated with the chemical moiety that is pairing with the target nucleic acid molecule.

[0024] In particular examples, the probe is attached or fixed to a substrate, for example in an addressable location via a linker molecule that attaches the polymerase component to the substrate. Exemplary linkers include streptavidin-biotin, histidine-Ni, S-tag-S-protein, and glutathione-glutathione-S-transferase (GST). In another example, the target nucleic acid molecule to be sequenced is attached or fixed to a substrate, for example in an addressable location. In particular examples the oligonucleotide primer is fixed to a substrate, for example at its 5' end. For example, a nucleic acid molecule can be attached to the substrate by its 5' end, 3' end or anywhere in between. In particular examples, the sequencing reaction is performed in a three dimensional polyacrylamide gel, wherein all of the reagents needed for sequencing are present in the gel.

[0025] In some examples, a plurality of probes, primers, or nucleic acid molecules are fixed directly or indirectly to the substrate in a predetermined pattern, for example in an addressable location. For example, the agents can be deposited into channels which have been etched in an orderly array or by micropipetting droplets containing the agent onto a slide, for example either by manually pipetting or with an automated arrayer. Such methods permit simultaneous (or substantially simultaneous) sequencing on a single substrate, in which case signals are detected from each of the sequencing reactions. The unique emission signals can be detected, for example with a charge-coupled device (CCD) camera, which can detect a sequence of signals from a predetermined position on the substrate and convert them into the nucleic acid sequence. The unique emission signals can be stored in a computer-readable medium.

[0026] The foregoing and other features and advantages of the disclosure will become more apparent from the following detailed description of several examples which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

[0027] FIG. 1A is a schematic drawing showing a nanoprobe that includes a polymerizing agent with four molecular linkers to attach the chemical moieties to the polymerizing agent.

[0028] FIG. 1B is a schematic drawing showing the nanoprobe attached to a target nucleic acid strand and a complementary primer, and the pairing of one of the chemical moieties with its complementary nucleotide in the target nucleic acid strand.

[0029] FIG. 1C is a schematic drawing showing a nanoprobe that includes a polymerizing agent with chemical moieties linked to a "hub" created by a molecular linker attached at a single point on the polymerase via a tether.

[0030] FIGS. 2A-D are schematic drawings of nanoprobes having the molecular linkers attached to the polymerizing agent at a single location in a variety of different configurations.