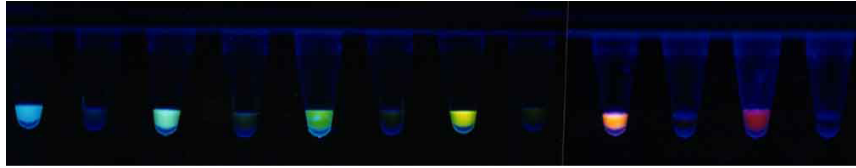
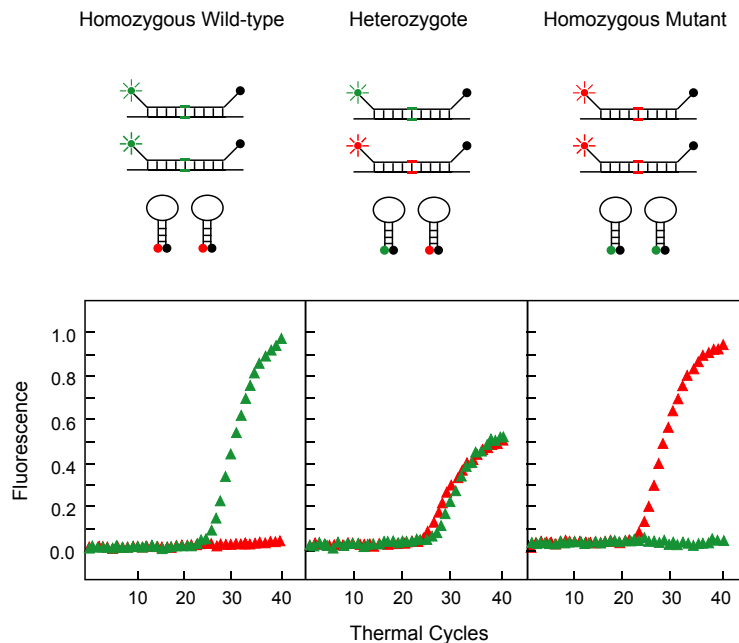


Molecular beacons can be synthesized that possess differently colored fluorophores, enabling assays to be carried out that simultaneously detect different targets in the same reaction. For example, multiplex assays can contain a number of different primer sets, each set enabling the amplification of a unique gene sequence from a different pathogenic agent, and a corresponding number of molecular beacons can be present, each containing a probe sequence specific for one of the amplicons, and each labeled with a fluorophore of a different color. The color of the resulting fluorescence, if any, identifies the pathogenic agent in the sample, and the number of amplification cycles required to generate detectable fluorescence provides a quantitative measure of the number of target organisms present. If more than one type of pathogen is present in the sample, the fluorescent colors that occur identify which are present. Moreover, due to the inherent design of gene amplification assays, the use of molecular beacons enables the abundance of a rare pathogen to be determined in the presence of a much more abundant pathogen.



Molecular beacons are extraordinarily specific. They easily discriminate target sequences that differ from one another by a single nucleotide substitution. The reason that molecular beacons are so "finicky" is that they can exist in two different stable physical states. In one state, the molecular beacons are hybridized to their targets, and energy is stored in the probe-target helix. In the second state, the molecular beacons are free in solution, and energy is stored in their stem helix. Molecular beacons are designed so that their probe sequence is just long enough for a perfectly complementary probe-target hybrid to be more stable than the stem hybrid. Consequently, the molecular beacons spontaneously form fluorescent probe-target hybrids. However, if as little as a single nucleotide in the target is not complementary to the probe sequence of the molecular beacon, the probe-target helix would be less stable. In this situation, the stem helix of the molecular beacon is more stable than the mismatched probe-target helix, and the molecular beacons remain unhybridized. Thus, molecular beacons can be thought of as "molecular switches" that are on their targets and brightly fluorescent when the targets are perfectly complementary to the probe, but remain off the targets and dark if the targets contain a mutation.

Molecular beacons are thus ideal probes for use in diagnostic assays designed for genetic screening, detection of single-nucleotide polymorphisms, and pharmacogenetic applications. For example, to determine the genotype of an individual at a particular locus, the genetic region of interest is amplified in the presence of two different molecular beacons, one perfectly complementary to the wild-type allele and labeled with a fluorophore of a particular color, and the other perfectly complementary to the mutant allele and labeled with a differently colored fluorophore. If the assay results in the generation of only the first fluorescent color, then the individual is homozygous wild type at that locus. If the assay results in the generation of only the other fluorescent color, then the individual is homozygous mutant. And finally, if both fluorescent colors are produced, then the individual is heterozygous.



In summary, molecular beacons have three key properties that enable the design of new and powerful diagnostic assays: 1) they only fluoresce when bound to their targets, 2) they can be labeled with a fluorophore of any desired color, and 3) they are so specific that they easily discriminate single-nucleotide polymorphisms. Now that a number of new and versatile spectrofluorometric thermal cyclers are available to clinical diagnostic and research laboratories, assays that simultaneously utilize as many as seven differently colored molecular beacons can be designed. This enables cost-efficient multiplex assays to be carried out that identify which member of a panel of potential infectious agents is present in a clinical sample.

Utilizing molecular beacons, assays can be developed that not only identify a causative infectious agent, they can simultaneously determine which antibiotics will be effective and which will be ineffective against the particular strain that is present. A panel of different genetic mutations responsible for the same disease can be identified in a single assay. And a single assay containing molecular beacons can screen an entire panel of single-nucleotide polymorphisms whose identity indicates whether a particular drug will be effective for a particular individual. Thus, molecular beacons provide a new tool for increasing the effectiveness, and lowering the cost, of clinical diagnostic assays.

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