Fluorescence turn-on molecular probes for rapid detection of DNA/RNA sequences

Background
Modern DNA and RNA sequence identification methods offer remarkable capabilities for reading the base sequence of biological samples. These can record up to the entire genetic content of a sample or employ discrete molecular probes that operate on a one probe–one target principle. Some sequence identification methods are widely used for microscopic study of DNA/RNA in cells.

The problem
While powerful, the current DNA/RNA sequence detection solutions suffer from several drawbacks. Next-gen sequencing can be expensive, slow, and need sophisticated instruments. Nanopore sequencing can have higher error rates than next-gen sequencing. G:C base pairs are intrinsically more stable than A:T base pairs, so GC-rich sequences may form stable duplexes even in the presence of one or two mismatches. Accordingly, DNA microarrays, fluorescence in situ hybridization (FISH), molecular beacons, isothermal amplification, and PCR struggle with specificity for sequences that differ by only one base aka single-nucleotide polymorphisms (SNPs).

SDSU Solution
The Purse Lab is developing novel methods to build fluorescence reporting capabilities directly into DNA/RNA sequences. These involve modification of cytidine such that it can be placed internally within DNA/RNA strands and can distinguish matched from mismatched sequences by reading a complementary genetic letter directly to induce a fluorescence change (Figure 1). The modified cytidine (DEAtC) probe with built-in G-selective fluorophore is prepared using a sequence of chemical reactions. DEAtC provides direct fluorescent read-out capability of complementary sequences and allows for rapid detection of specific DNA/RNA sequences.

Figure 1. The fluorescent probe DEAtC distinguishes matched from mismatched DNA or RNA by a fluorescence turn-on when incorporated into a probe strand of desired sequence.
Value proposition

In the conventional fluorescent readout methods, the fluorescent reporter is carried along with the probe, but does not interact directly with the target sequence. This indirect fluorescent reporting based on probe binding limits the ability to distinguish SNPs (Figure 2A). Purse Lab’s technology incorporates unique fluorescence turn-on that requires both, binding and correct sequence (Figure 2B). This new technology’s unique direct fluorescence readout can offer an improved ability to distinguish SNPs and other difficult targets with high accuracy, simplicity, and rapidity. DEAtC is prepared using synthetic organic chemistry reactions with the final product being a nucleoside phosphonamidite. The latter are standard chemical building blocks used to make DNA and RNA synthetically. Accordingly, DEAtC can be used in any DNA or RNA sequence and is customizable to any customer’s targets. It can provide instantaneous readout that can be visual, quantitative, nearly instantaneous, and spatially resolved.

**Existing Technology** (tethered fluorophore)

![Existing Technology Diagram]

**Figure 2A:** Classical fluorescence hybridization probes have challenges distinguishing nearly identical sequences because the fluorophore responds indirectly to sequence, driving by binding alone.

**New Technology**

<table>
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<tr>
<th>Synthetic DNA or PNA probe with built-in G-selective fluorophores</th>
<th>Our DEAtC probe molecule provides this G-selective fluorescence turn-on</th>
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</table>

![New Technology Diagram]

**Figure 2B:** Fluorescence turn-on molecular probes by Purse Lab

Applications

The technology can be useful for DNA/RNA detection where only a small number of targets are relevant. It can be adopted for a spectrofluorometer or a fluorescence microscope. Applications include:

- Drug screening assays, biochemical studies on drug metabolism and on- and off-target effects
- Molecular diagnostics: Point-of-care detection/rapid identification of specific pathogens; detection of SNPs in human diseases
- Use in fine chemicals for research purposes
Stage of Development

Working prototype. DEAtC has been synthesized and placed into molecular probe strands of DNA, and works for differentiation of matched from mismatched targets. DEAtC is being further tested and optimized with respect to its performance in complex mixtures, in longer probe sequences, and its sensitivity of detection, in addition to the improvement of efficiency of its synthesis.

Intellectual Property

United States Patent No. 11,447,519 – “COMPOUNDS FOR FLUORESCENCE SENSING OF DUPLEX FORMATION”

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