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A new member in the real-time PCR menagerie: the Snake assay

Real-time PCR (RT-PCR) is a powerful diagnostic tool allowing rapid detection and quantitation of as little as a few copies of specific nucleic acids, for instance, pathogenic viral or bacterial DNA and RNA, and low-level rare mutations [1,2]. Several kinds of signal-generating systems have been developed for RT-PCR detection employing target amplicon-specific fluorescent probes. Typically, these detector probes contain both a reporter fluorophore and a quencher-type ligand that are in Förster resonance energy transfer (FRET) interaction, extinguishing the emission of a reporter fluorophore [3].

During the detection event, fluorophore is separated by some means from a quencher to disrupt FRET, and to yield accordingly a fluorescent signal.

Currently available FRET probes work either as the hybridization-triggered FRET probes, such as the hairpin-shaped molecular beacons [4] and Scorpion primers [5], or, alternatively, as cleavable FRET probes, like TaqMan probes [6]. Notwithstanding the value of these assorted probes in RT-PCR detection, none of them is perfect in terms of fluorescence background, signal productivity and detection of single-base sequence variations known as SNPs.

Now, Igor V. Kutyavin from Perpetual Genomics, the US-based biotech start-up, has reported an improved design of the RT-PCR FRET probes [7]. The novel promising method, he called Snake, employs *Taq* DNA polymerase having structure -specific 5′-nuclease activity [8], and PCR primers that carry a special 5′-overhang over the amplifiable sequence. In the complementary amplicon, the overhang's replica folds back making it possible for the PCR-running polymerase 'to bite' the 5′-flap of a FRET probe hybridized to an amplicon (Figure 1).

It was found in the study that this innovative design does not affect the PCR efficiency but substantially improves the cleavage of FRET probes by 5′-nuclease activity of *Taq* DNA polymerase, therefore considerably increasing a signal response. When directly compared to other conventional RT-PCR techniques, including molecular beacon, TaqMan and Scorpion assays, the Snake method also supersedes them in the sensitivity of SNPs detection [7]. The inventor optimistically anticipates that the new diagnostic technology will quickly replace all current approaches used for real-time nucleic acid detection, owing to the Snake's superior performance, cost-effectiveness and simplicity of design.

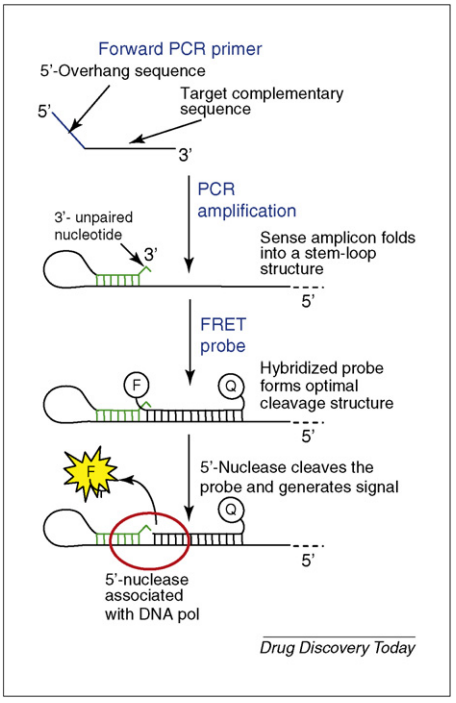

FIGURE 1

The key stages of the real-time Snake assay (courtesy of Igor V. Kutyavin, Perpetual Genomics, Woodinville, WA, USA).

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