

Triplex assay used to assay duplex genomic DNA

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Direct detection of base sequence in duplex nucleic acid has long been an unfulfilled objective. Ingeneus Research will publish "Heteropolymeric Triplex-Based Genomic Assay® to Detect Pathogens or Single-Nucleotide Polymorphisms in Human Genomic Samples" in the March 21st issue of the international open access online journal, *PLoS ONE*.

In the article they present a wealth of data relating to the assay of pathogens in samples also containing human genomic duplex DNA and to the assay of SNPs present in human genomic samples. The assays are carried out homogeneously and in solution at room temperature. Reactions can be monitored after as little as five minutes. The highly sensitive diagnostic assay allows for the direct detection of base sequence in human genomic duplex samples, thereby obviating the use of PCR which has inherent problems and is costly.

"We developed the heteropolymeric triplex assay step by step" says Jasmine Daksis, Senior Scientist with Ingeneus Research. "We started with synthetic 50-mer duplex targets and have developed our methods to the point where human genomic samples can be assayed." The assay uses YOYO-1, a bis-intercalator, to de-condense the duplex target, which renders the duplex nucleic acid readily reactive to oligo ssDNA probes. Any sequence present in the duplex may be specifically assayed. It is surmised that specific third strand binding creates additional grooves into which additional YOYO-1 molecules intercalate.

"We have decided not to focus on improving probe chemistry at this time, but rather to develop a flow injection based instrument which is matched to our chemistry," continued Daksis. Their Genome Flow® instrument, which employs hardware from FIALab Instruments of Bellevue, Washington, has one moving part, the syringe pump. It allows samples to be automatically quantitated, a necessary step in the Genomic Assay® because samples must be brought to a standard concentration, so they can be mixed with standard amounts of oligo probes for the purpose of automatic in solution assay. The instrument is easy to program, self-cleaning and inexpensive.

Daksis indicated that she expected to soon publish data on the use of the Genome Flow® instrument to carry out triplex assaying of genomic samples for pathogens or SNPs.

Source: Public Library of Science

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