Title: Chem-NAT bead-based assays for quantifying microRNAs directly from biological sources

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Nucleic acid testing by dynamic chemistry (Chem-NAT) is unique and distinguishable from ALL existing enzymatic methods of nucleic acid analysis. This PCR-free technology can be used to identify any known target nucleic acid sequences. Chem-NAT technology commercilised by DestiNA Genomics has been used to genotype cystic fibrosis patients, distinguish high homologous DNA sequences from different parasite species and quantify, withh single base resolution accuracy, the expression of circulating miR-122 in drug-induced liver injury (DILI) patients.

Here, we present the integration of bead-based DestiNA chemistry with single molecule arrays (Simoa) and fluorescence plate readers for measuring miRNAs from serum/plasma and cell lines without requiring miRNA extraction. Moreover, Stabiltech DestiNA buffer allows storing serum samples at ambion temperature without RNA degradation, hence offering a PCR-free, sensitive and highly specific assay. The assay is based on the hybridization of a specific miRNA to an abasic peptide nucleic acid (PNA) probe attached to superparamagnetic beads, followed by the specific incorporation of a biotinylated SMART nucleobase. The biotin labels are then labeled with an enzyme, which catalyze the generation of fluorescent solution.

The miRNA single molecule assay had a limit of detection (LOD) of 500 fM, approximately 500 times more sensitive than a corresponding analog bead-based assay. The specificity of the assay to a single base mismatch in the microRNA sequence is $>10^7$-fold. These assays will have utility in drug development as well as in clinical practice as it has the potential to deliver the user-independent sensitivity and time-to-result that are needed to inform pre-clinical and clinical decision making.