

Signal Amplification Technology

Rapid · Sensitive · Robust

Introduction

Tm Bioscience (TMB) has recognized the need for a technology that significantly improves the sensitivity of detection across a number of assay formats. As a result, Signal Amplification Technology (SAT) was developed. Application of SAT has resulted in at least a 1000 fold increase in the level of detectable analyte. SAT can be readily adapted to immunologic or nucleic acid detection tests. As such the analyte for detection can be either a nucleic acid or a protein that is captured on a solid phase support. Applications for SAT include genotyping or SNP analysis, clinical diagnostics and gene expression profiling where microarray technology is in current use.

SAT Overview

In the basic form of SAT (see Fig. 1) the first step in the signal amplification process involves the binding of an analyte specific reagent, which depending on the analyte can be either an oligomer probe or an antibody. The analyte specific reagent contains two regions for capture. The first region binds specifically to the analyte while the second portion hybridizes to the amplifying entity, a homopolynucleotide, which in the example depicted in Figure 1 is a synthetic polyA molecule. Methods for cost efficient preparation of homopolynucleotides that are used as amplifying entities have been developed and standardized. The polyA is subsequently hybridized with labeled signaling moieties that form multiple hybrids along the length of the homopolynucleotide. Labeling of the signaling moieties will depend on the assay requirements and can be any of the standard labels for detection (isotopic, chemiluminescent fluorescent etc.).

There are several possible adaptations of the basic protocol for implementing SAT that can be utilized in assay development. For example, Figure 2 illustrates a direct approach of the use of SAT in detecting a nucleic acid analyte. In this format the analyte is prepared with a polyT tail and captured by means of a hairpin capture probe that is bound to a solid support. This allows the polyA amplifying entity to bind directly to the analyte through its polyT tail. This approach was utilized in the model system that is described in generating the results of Figure 3.

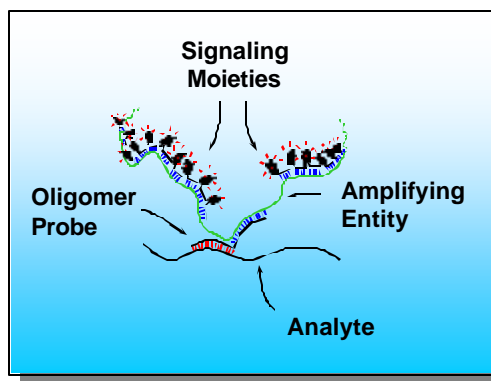


Figure 1. SAT Schematic

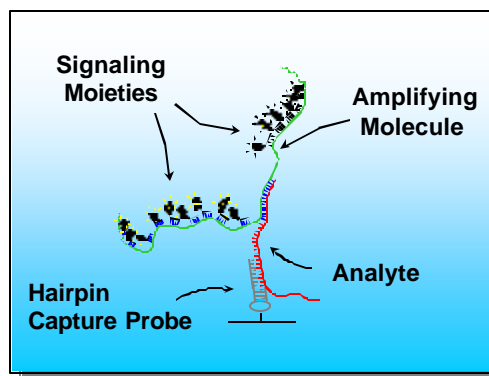


Figure 2. Alternate SAT Schematic

Research and development of SAT has been carried out on a genotyping test that detects a point mutation in a receptor gene (Lisle C. et al). Using this model system the SAT has been evaluated for enhancing sensitivity, signal-to-noise and linear dynamic range. Typical results for the genotyping assay with SAT have shown that the assay is capable of detecting greater than 1000-fold more target than what can be detected by direct labeling (Fig. 3). Utilizing SAT made possible the detection of sub-attomolar copies of the gene target. Also of note, the dynamic linear range for the assay when SAT is applied is at least 10^4 with a signal-to-noise ratio of greater than 2.

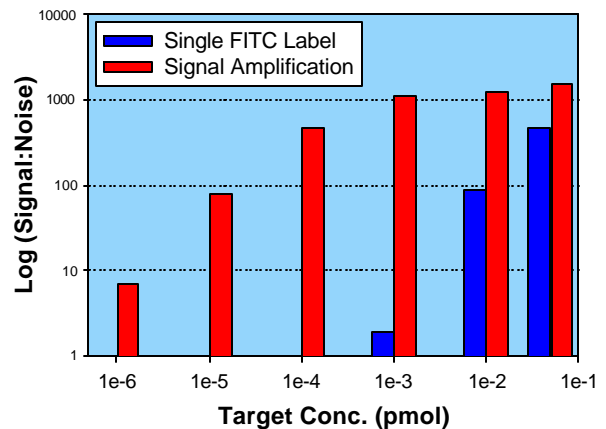


Figure 2. Comparison of Signal Amplified Target with Direct Target Labeling

Conclusions

Incorporation of TMB's SAT is an effective means of generating a substantial increase in sensitivity (greater than 10^3 fold) of detection. Tm Bioscience's SAT can be directly applied to existing assays in several areas including SNP analysis, clinical diagnostics and gene expression profiling. The adaptability of this technology to multiple, existing assay formats will provide a seamless transition to enhanced assay performance.

SAT Status

This technology has been patented in the United States (Patent No. 5,902,724). Additional US and International patent filings are pending. Companies interested in licensing this technology or discussing the development of custom ligand cocktails should contact:
Dr. Jeremy Bridge Cook, VP Business Development at (416)-593-4323 ext. 229 or by e-mail at bridgecook@tmbioscience.com.

References

Lisle C. M., Bortolin S., Benight A. S., Janeczko R. J., Zastawny R. L., A Novel Signal Amplification Technology With Applications in DNA and Protein Detection Systems. BioTechniques, 2001, In press.