

Tm-100 Universal Sequence Set

Flexible • Specific • Cost-Effective

Introduction

Genomics is a constantly changing field. Each day, novel genes are being discovered and the underlying biochemical mechanisms linking these genes to various disease states are continually being identified. Whether applied to diagnostics, drug discovery or pharmacogenomics, DNA-based tests must be flexible enough to change with the progressive and dynamic nature of the genomics field. Single tube assays measuring a single analyte in a single reaction vessel are slowly being replaced by array-based methods capable of analyzing multiple analytes in a single reaction. However, several drawbacks including high cost, lack of standardization, lack of reproducibility and lack of flexibility, have hampered the widespread acceptance of arrays as the method of choice for DNA-based testing. Arrays have undoubtedly played a significant role in advancing DNA-based testing methodologies by allowing for massive parallel analysis, however much room for improvement still exists. The Tm-100 Universal Sequence Set represents a major advance in addressing the issues of flexibility, standardization and cost.

Tm-100 Universal Sequence Set

The Tm-100 Universal Sequence Set comprises 100 unique 24mer DNA sequences designed to work in concert in a single reaction with minimal non-specific hybridization. The Tm-100 Universal Sequence Set was derived using Tm's proprietary Sequence Design Engine, an algorithm that combines the principles of DNA thermodynamics with pattern matching and similarity thresholds. The 100 sequences are unrelated to the genomic DNA being queried and are designed to be isothermal and exhibit uniform kinetics. When used on an array platform, whether two-dimensional or bead-based, these sequences provide a 'universal' starting point for any DNA-based test regardless of the application. In other words, the Tm-100 Universal Sequence Set remains constant regardless of the test in question and therefore optimization, in terms of hybridization, is performed only once. This is in stark contrast to the conventional 'custom' array which uses target-specific sequences such that each application (eg. HIV screening panel, SNP detection panel, etc.) requires a separate array and subsequently, multiple, individual optimizations. The Tm-100 Universal Sequence Set is an indispensable tool providing a cost-effective and flexible alternative to the 'custom' array.

Experimental Approach

To demonstrate the exquisite specificity of the Tm-100 Universal Sequence Set, the Luminex LabMAP™ platform was used. The Luminex platform derives from classical flow cytometry. Arrays consist of spectrally addressable polystyrene microspheres that may be used to multiplex up to 100 analytes, thus making this system an ideal candidate for application of the Tm-100 Universal Sequence Set. Validation experiments were carried out such that each of the 100 sequences making up the Universal Set was chemically immobilized onto a different bead population. The 100 spectrally distinct bead populations, now bearing the Tm-100 Universal Sequences, were mixed in a single multiplexed reaction and hybridized individually to synthetic, biotinylated targets representing the complements of each universal sequence. In other words, each target was examined individually for its specific hybridization with its complementary bead-immobilized universal sequence as well as for its non-specific hybridization with the other 99 bead-immobilized universal sequences present in the reaction. A streptavidin-phycoerythrin conjugate was the reporter used to measure signal generated by a hybridization event.

Results

The ability of each individual Universal Sequence to recognize only its specific complement is illustrated in **Figure 1**. Of the possible 9900 non-specific hybridization events that could have occurred when each of the 100 complementary sequences was hybridized individually to the pool of 100 bead-immobilized universal sequences, only 6 events were observed (**A**). Of these 6 events, the highest non-specific event generated a signal equivalent to 10.2% of the signal observed for the perfectly matched pair (ie. specific hybridization event). When 10 times less target DNA was used for hybridization, non-specific hybridization was not observed (**B**).

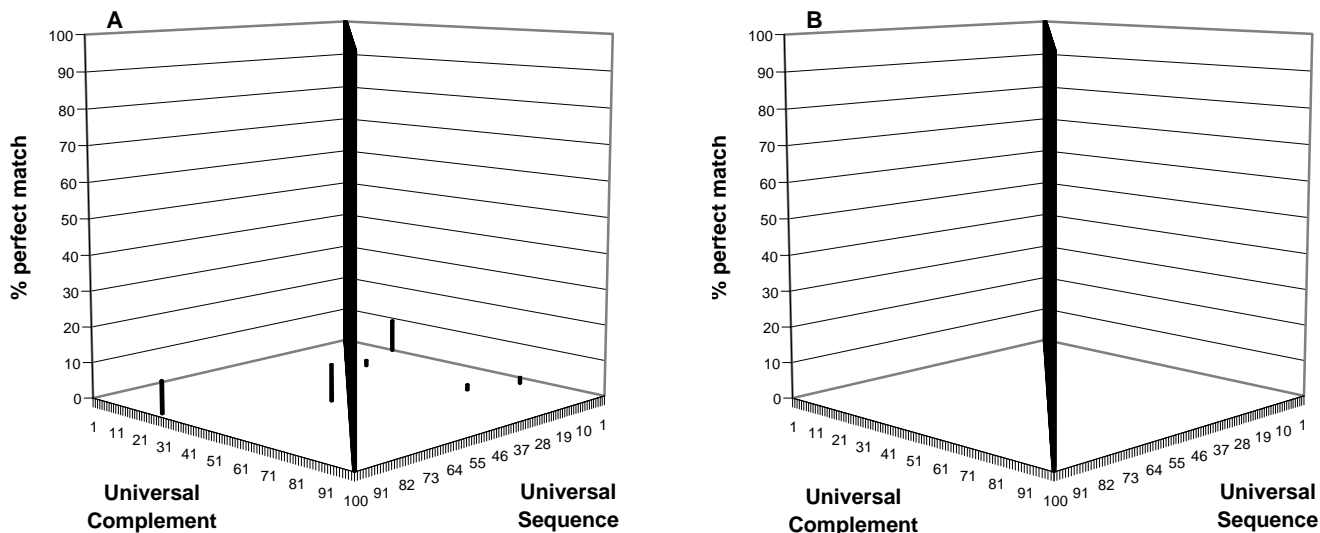


Figure 1: Comparison of the Tm-100 Universal Sequence Set hybridized to each of the 100 complements as described above. The randomly dispersed bars in (A) represent the 6 cross-talk events observed at high target concentration while (B) shows the absence of cross-talk when 10 times less target DNA was used. The centre wall represents the 100 perfectly matched pairs.

Conclusions

Our results clearly demonstrate the high level of specificity attainable in a multiplexed system that utilizes the Tm-100 Universal Sequence Set. While 100 Universal Sequences are currently available, any number of the 100 sequences may be used together depending on the requirements of each individual application. In other words, an HIV test panel may only require analysis of 50 sequences. Furthermore, an array currently measuring 60 analytes may require an additional 20 control analytes for meaningful interpretation of results. Using a bead-based array in combination with Tm's Universal Sequences, such a change requires only that the additional sequences be added to the multiplexed reaction. A 'custom' array would likely require additional optimization in addition to fabrication of a totally new array.

The Tm-100 Universal Sequence Set is an indispensable tool providing an optimized 'universal' starting point for the development of any multiplexed DNA-based test. The Sequence Set combines **high specificity** with **utmost flexibility**. The Tm-100 Universal Sequence Set will prove especially useful in this rapidly evolving genomics field where cost-effective, standardized methods are always in demand.

Tm-100 Universal Sequence Set Technology Status

Multiple patents describing this technology and its uses have been filed. We are seeking companies interested in licensing the IP rights to this technology. For additional information, please contact Dr. Jeremy Bridge Cook, Vice-President, Business Development at 416-593-4323 ext. 229.