OligoMix® Microarray Synthesized Oligos for Targeted Sequencing

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Recent major advances in DNA sequencing technologies have resulted in several new “next-generation” platforms capable of generating massive amounts of reads very quickly and relatively inexpensively. These new technologies hold the promise of one day, routinely sequencing entire complex eukaryotic genomes.

Next-generation DNA sequencing (NGS) has broad applications in both research and clinical diagnostics and has specifically become an important platform for identifying mutations and variants from clinical samples. Though NGS is capable of very high levels of coverage even on complex genomes, it is still advantageous to reduce the complexity of samples and sequence smaller targeted regions—in particular, when sample numbers are very high and the goal is detection of less prevalent or extremely rare mutations. Several academic and commercial groups have developed a variety of capture methods for enriching or selectively amplifying subsets of the genome for targeted sequencing.

The key performance parameters of these methods are capture specificity and sensitivity, the ability to multiplex many samples and capture large regions of interest, and of course cost. Recently developed methods include: Molecular Inversion Probes (MIP)\(^1\), Solution Hybrid Selection (SHS)\(^2\), Selective Genomic Circularization (SGC)\(^3,4\) and Oligo-Selective Sequencing (OS-Seq)\(^5\). All of these methods have been demonstrated as effective at selectively enriching desired regions of interest within a given genome.

In the MIP method, target-specific oligo probe sequences are hybridized to the target region of genomic DNA and the probe is circularized by DNA polymerase and ligase such that the target sequence is incorporated into the circular molecule. The genomic DNA is digested, and the target DNA is PCR-amplified and sequenced. In the SHS method, target-specific oligo probes are biotinylated, hybridized to modified genomic DNA libraries and captured with beads. Unbound DNA is washed away, and the target DNA is eluted and sequenced. In the SGC method, pools of target-specific oligo probes are incubated with a digest of genomic DNA and a general vector oligo. Annealing and ligation steps are performed to circularize the targets before library prep and sequencing. The SGC method differs from the MIP-based approach in that the genomic DNA target is directly incorporated into the circular molecules versus the oligo probe being circularized as in the MIP method. In the most advanced target capture method, OS-Seq, target-specific oligos modify primers which are immobilized on the sequencing flow cell and function as both a capture and sequencing substrate.

While each method has its own advantages and disadvantages that make it suitable for specific situations\(^6\), the common thread amongst these methods is the need for high quality synthesis of large numbers of target oligonucleotide sequences. This can prove expensive using conventional solid support column based synthesis methods as the number of sequences can reach into the tens of thousands. However, the use of microarray synthesized oligos produces the required numbers and quality of oligos quite effectively at a far lower cost\(^7\). LC Sciences OligoMix has been demonstrated as an effective method of oligo synthesis for targeted sequencing in MIP\(^8\), SGC\(^9\), and OS-Seq\(^5\) targeted sequencing methods.
OligoMix is produced via an advanced microarray synthesis technology. Termed µParaflo®, this technology represents a significant improvement over previous microarray synthesis methods such those utilizing specialized modified nucleotides or other inkjet-like printing technologies. The µParaflo® platform is unique in that it integrates a photo-generated acid (PGA) chemistry, digital photolithography (DLP), and advanced microfluidics to enable high throughput parallel synthesis of custom DNA microarrays10. The PGA chemistry enables the use of standard oligo building blocks, which have been widely used and optimized for high yield and fidelity, and eliminates the need for any specially modified nucleotides (which have been shown to exhibit lower coupling efficiency and lower sequence fidelity). DLP technology enables programmable synthesis of custom sequences and eliminates the need for expensive and impractical prefabricated masks. The µParaflo® microfluidic device contains the synthesis reactions each within a picoliter-scale reaction chamber (producing more uniform synthesis than reactions performed on the open surface of a slide).

For typical microarray applications such as gene expression profiling, issues with spot uniformity have been successfully moderated by image filtering methods or averaging of replicate spots to increase data confidence. However, applications that involve cleaving the probes from the microarray chip are much less forgiving, because any and all impurities from synthesis or spot non-uniformity end up in your capture probe mixture. There is no possibility to mitigate their effects through data manipulation. Ideally, you would use oligos of very high quality such as standard column synthesized oligos; however, as mentioned previously this is impractical when the need is for thousands of custom sequences. OligoMix microarray synthesized oligos have been compared to and demonstrated as effective as column synthesized oligos for use in targeted sequencing applications5 and have additionally been demonstrated for applications with even more stringent oligo quality requirements such as gene synthesis11,12,13, which demands extremely high oligo fidelity and purity.

The effect of NGS on biomedical research has been nothing short of revolutionary. Remarkable discoveries are being made even as you read this by innovative scientists using highly advanced techniques. Among these, target capture, as an efficient method of detecting disease causing mutations and variants, has great potential to translate into clinical applications. With the current rate of continued improvements to sequencing technologies, capture methods and oligo synthesis technologies, we should anticipate targeted sequencing in the clinic very shortly.


9. LC Sciences' customers – data not published.


