How Pooled Oligo Synthesis is Fostering Innovation in Drug Target Discovery, Protein Engineering, and Synthetic Biology

Introduction

Oligos, short for oligonucleotides, are fundamental tools in molecular biology, genetics, and biotechnology that play a key role in research and development. They are generally chemically synthesized, short nucleic acid sequences made up of a variety of nucleotides and can be either DNA or RNA-based. By nature, they are highly specific and have unique compositions suited for precise molecular and genetic applications. Today, they are essential components in various workflows, such as PCR, DNA sequencing, gene expression analysis, and gene synthesis.

Pooled oligo synthesis refers to the process of chemically synthesizing thousands of different oligo sequences in massively parallel fashion on a silicon-chip based substrate using high resolution DNA writing instrumentation. After synthesis is complete, the single stranded oligos are cleaved from the substrate and consolidated as a single library or pool of thousands of custom oligo sequences. As a research tool, oligo pools have emerged as a low-cost source of synthetic DNA for the creation of the enormous sequence diversity required for use in high-throughput genomics screening and next-generation protein and pathway engineering strategies. The significant cost savings relative to other commercial sources of custom sequences also enables lower cost gene assembly and more cost-effective screening when performing massively parallel reporter assays (MPRA) or even loss of function or gain of function research in the form of pooled CRISPR screening. In this context, the oligo pools produced on high resolution DNA writing platforms present researchers with a powerful technology built on high-throughput, parallel synthesis of large numbers of unique oligo sequences on a single chip for custom, high-precision synthetic DNA produced with speed and at scale.

This Expert Insights eBook begins with a study on splicing assays in precision medicine. The research study from Rhine *et al*. [1] represents significant advancements in high throughput splicing assays, specifically the Massively Parallel Splicing Assay (MaPSy), for comprehending and categorizing the effects of genetic variants in precision medicine. The advancement of these assays and predictive modeling of splicing (MMSplice) has the potential to improve the understanding of genetic mutations, especially those that impact pre-mRNA splicing, and their relevance in clinical genetics and disease management.

Our second paper by Kuiper *et al*. [2] is a research review that examines the utility of oligo pools in gene assembly and high-throughput oligonucleotide library design for protein and metabolic pathway engineering. Utilizing custom oligo pools is essential for protein engineering and provides a low-cost source of synthetic DNA, greatly reducing expenses compared to other methods. The authors point out that although oligo pools are an affordable alternative, there are challenges associated with their use in advanced engineering strategies, such as low concentrations and high error rates. Despite the challenges, the review highlights the importance of oligo pools in performing many applications and techniques that should be more accessible to the broader bioengineering community. The researchers summarized currently available methods that use affordable, array-synthesized oligo pools as a source of synthetic DNA for next-generation protein engineering libraries.

Our last research paper is a protocol published by Eroshenko *et al*. [3]. It presents an exciting technique for constructing lengthy double-stranded DNA structures using oligonucleotides that are synthesized on DNA chips with high density. This method overcomes the high costs and technical

challenges of traditional synthesis. Long double-stranded DNA synthesis is critical in many biological and bioengineering applications. However, high cost and complexity have kept it from being widely used, especially in academic settings. The researchers in this study developed a protocol for assembling 500- to 800-base pair gene fragments from commercially available DNA chips.

Overall, oligo pools have become indispensable tools in applications as diverse as synthetic biology, pooled CRISPR screening, and high-throughput gene assembly. Furthermore, utilization has driven advances in data driven computational modeling and predictive algorithm development. These developments have had far-reaching consequences in accelerating the research supported by pooled oligo synthesis platforms. This accelerated understanding of functional genomics and protein engineering will undoubtedly improve research outcomes in precision medicine and variant discovery as well as in novel enzyme and therapeutic protein development.

Through this Expert Insights eBook, we hope to educate researchers on oligo pools and potential applications in functional genomics pooled screening and synthetic biology. For more information, we encourage you to visit Agilent's Pooled Oligo Synthesis page to gain a deeper understanding of available options for accelerating your research.

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References

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