Advantages of using array-synthesized, double stranded DNA capture probes in NGS targeted sequencing

Application Note

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Advantages of using array-synthesized, double stranded DNA capture probes in NGS targeted sequencing

Introduction

The introduction of next-generation sequencing (NGS) technology has given scientists the ability to gather large amounts of genetic information about complex organisms in a short period of time and at a low cost. However, sequencing a whole genome is expensive and requires a significant amount of laboratory time. In addition, this method generates large data sets that can be cumbersome to analyze. To address these challenges, several methods have been developed. One such method, called target enrichment, uses capture probes to extract only the genes of interest from the sample DNA for sequencing. This provides a cost-effective way for high-throughput genetic studies. In addition, it improves sequence accuracy by reducing the complexity of the DNA and optimizing sequencing depth [1]. Multiple methods can be used for target enrichment of genomic DNA, including in-solution DNA hybridization to a capture oligonucleotide. In this method, DNA or RNA oligonucleotide probes physically capture and isolate the genomic regions of interest prior to sequencing [2].

The key to excellent target enrichment using DNA hybridization relies on the use of high quality capture probes. Creating optimized target enrichment probes improve the efficiency of targeted NGS and reduce the number of sequencing reads needed to obtain high-confidence data. Currently, there are multiple types of capture probes available, including single stranded or double stranded DNA. Single stranded DNA (ssDNA) capture probes are individually synthesized using column-based synthesis, whereas current double stranded DNA (dsDNA) capture probes are synthesized simultaneously using an array-based oligonucleotide synthesis platform.

GenScript's dsDNA capture probes are synthesized on an advanced semiconductor-based chip using an electrochemical synthesis process. In order to test the performance of our dsDNA capture probes in NGS target enrichment methods, our dsDNA probe panel was compared to a commercially available ssDNA probe panel. The quality and target capture efficiency of our array-synthesized, dsDNA capture probes was assessed.

Case Study

Sequences from a predesigned, commercially available panel of ~8,000 probes against 127 genes were synthesized using our advanced semiconductor-based chip oligo synthesis platform to create 120 mer, dsDNA capture probes. For comparison, column-synthesized ssDNA probes with the same sequences were obtained from Vendor T. A DNA library was created from a commercially available standard sample and enriched for an 800 kb target region using either capture panel of DNA probes. In order to evaluate target enrichment efficiency of dsDNA probes, two key parameters, coverage depth and the ratio of sequence reads to bases on target region (i.e., on-target bases or reads), were assessed for both panels of capture probes.

High depth of coverage using array-synthesized, double stranded DNA capture probes

Sequencing coverage depth is determined by the number of times a sequenced DNA fragment aligns with a genomic target. Deeper coverage of a targeted region implies this region of interest was sequenced more times than other regions, indicating higher reliability and sensitivity of the sequencing assay. In addition, a high uniformity of coverage reduces the amount of sequencing required to reach a sufficient depth of coverage of all regions of interest.

Side-by-side comparison of array-synthesized, dsDNA capture probes (GenScript-1 & 2) to column-synthesized, ssDNA probes (Vendor T) revealed no difference in sequence coverage depth, indicating comparable quality between both sets of capture probes . A second panel of dsDNA probes with the same sequences were synthesized using the chip-based platform and used to sequence the same targeted region. The total target coverage rate between the first dsDNA probe panel (GenScript-1) and second dsDNA probe panel (GenScript-2) was similar, indicating low variation and high consistency between batches (**Figure 1**).

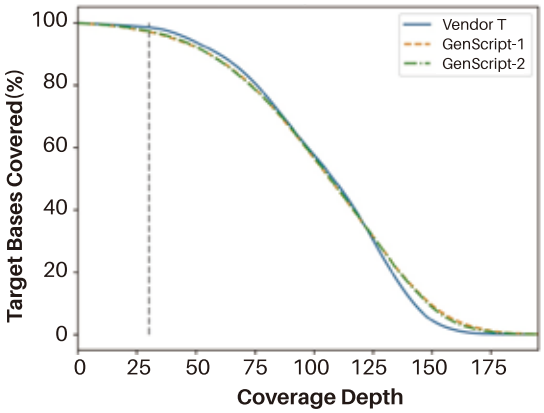

Figure 1: Deep, uniform sequence coverage of targeted regions using chip-based, dsDNA probes.

Array-synthesized, double stranded DNA capture probes provide high targeting efficiency and targeted sequence coverage

To further investigate the targeting efficiency of the array-synthesized, dsDNA probes, the number of on-target reads using both dsDNA and ssDNA probe panels were determined. The on-target reads are those that aligned with the targeted genomic regions of interest. Both probe panels were used in a separate, but simultaneous sequencing experiment. After one round of sequencing, the number of on-target reads between the two panels were compared. Results showed there was a significantly high correlation for the number of on-target reads obtained between the probe panels, indicating similar targeting efficiency (**Figure 2**).

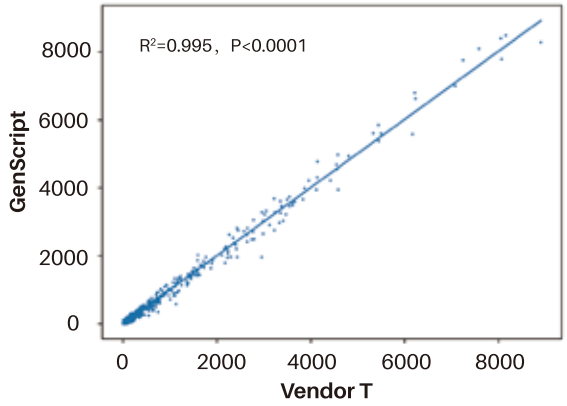

Figure 2: Correlation between the number of on-target reads captured between dsDNA and ssDNA probes.

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On average, both probe panels showed similar overall targeted sequence coverage, achieving a similar 'percentage of on-target bases' (i.e., on-target rate defined here) of around 60%. Thus, the targeted efficiency and overall quality of dsDNA probes is comparable to ssDNA probes.

Minimal variation and excellent targeting reproducibility using array-synthesized, double stranded DNA capture probes

In order to confirm the reproducibility of the targeting efficiency of dsDNA capture probes, the same probe sequences were re-synthesized and two replicate panels of dsDNA capture probes were used in simultaneous sequencing experiments. The number of on-target reads obtained from both dsDNA panels were compared. The comparison of on-target sequencing results between two technical replicates revealed low lot-to-lot variation and high reproducibility of capture (**Figure 3**). Therefore, the production of dsDNA capture probes using a semiconductor-based chip platform creates highly consistent NGS targeting probe panels.

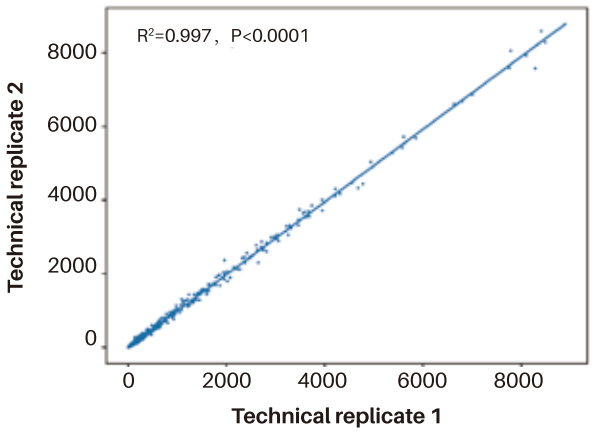

Figure 3: Using semiconductor-based chip for dsDNA capture probe synthesis provides high reproducibility of capture efficiency.

Summary

The dsDNA capture probes synthesized using GenScript's advanced semiconductor-based chip platform is of comparable quality and targeting efficiency to ssDNA capture probes and provides better performance at higher coverage depth. Minimal lot-to-lot variation was observed when two dsDNA probe panels were compared for targeting efficiency and the on-target capture rate was reproducible. Overall, utilizing dsDNA capture probe panel for target enrichment sequencing provides an improved target efficiency with reproducible results with subsequent dsDNA capture probe panels.

The use of NGS has moved from the pre-clinical to clinical setting with the development of NGS-based gene panels for detection of disease-causing genetic mutations and variants [3]. The development of NGS-based molecular diagnostics has giving healthcare providers the ability to utilize a patient's genetic make-up to personalize disease management and has laid a foundation for the development of precision medicine. These genetic panels require the use of capture probes to detect mutations and variations in genomic DNA. The use of dsDNA capture probes in the development of future NGS-based molecular diagnostics can be a cost-effective way to improve target capture and identification of disease-causing mutations in order to provide accurate diagnosis and personalized treatment options to improve the lives of patients.

References

1. Dapprich, J. et al. The next generation of target capture technologies – large DNA fragment enrichment and sequencing determines regional genomic variation of high complexity. BMC Genomics, 17:486 (2016).
2. Mertes, F. et al. Targeted enrichment of genomic DNA regions for next-generation sequencing. Brief Functional Genomics, 10(6):374-386 (2011).
3. Nagahashi, M. et al. Next generation sequencing-based gene panel tests for the management of solid tumors, 110(1):6-15 (2019).

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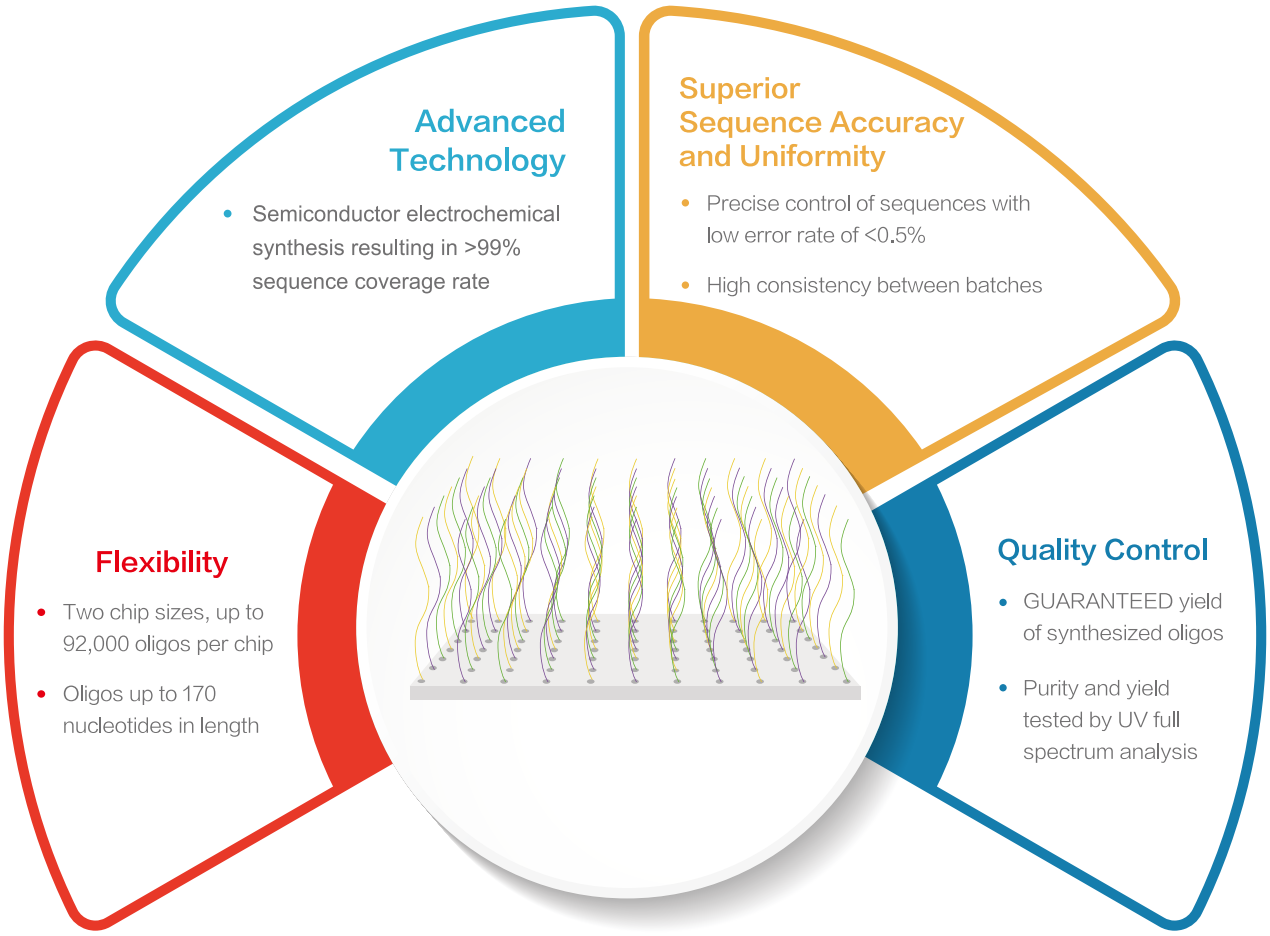

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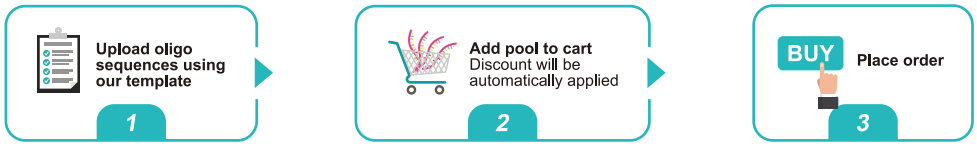

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