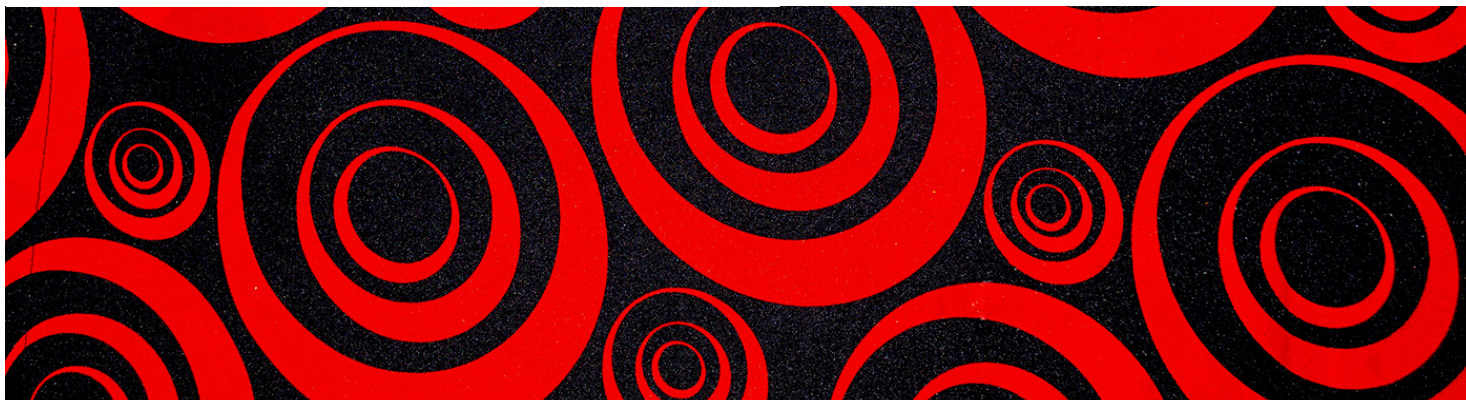




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Growing Options for Enriching Target DNA



June 17, 2021 | [Caitlin Smith](#)

Enriching sample DNA for targets of interest prior to sequencing has more than one practical advantage. It makes sequencing cheaper, and data analysis easier, and both faster. It can rid a sequencing library of unwanted or complicating DNA prior to sequencing. Two main methods of target enrichment (hybridization- and amplicon-based) are well-established; this article will also discuss strategies that have emerged from recent technological advances such as long-read sequencing and the CRISPR/Cas9 gene editing system.

Hybridization-based enrichment

Also known as the hybrid-capture method, this widespread approach uses complementary probes that hybridize to and capture DNA fragments containing the target sequences. Probes usually contain barcodes and/or unique molecular identifiers (UMIs). This method is especially suited for targeting larger regions in whole-exome sequencing, and for targets involving structural changes. The lower specificity of hybridization probes makes them less well-suited for smaller panels,

SNPs, or small indels (for which the amplicon-based method is more appropriate; see below).

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Jon Cash, Marketing Manager in Sequencing at Roche Diagnostics, says that the hybridization-based approach is their customers' most common method of target enrichment for targeted DNA

sequencing and whole-exome sequencing. "We provide custom probe pool design service at no cost (HyperDesign), custom probe pools for human (HyperChoice) and non-human (HyperExplore) designs, fixed probe pool designs that are available off the shelf (for example, SARS-CoV-2 panel), and a fixed design for whole-exome sequencing (HyperExome)," he says.

Agilent's hybridization-based SureSelect platform is used in applications ranging from genetic disease and cancer, to infectious disease and agrigenomics, according to Kevin Meldrum, VP and GM of Agilent's Genomics Solution Division. "SureSelect can be used for DNA-seq, RNA-seq, and Methyl-seq applications with nucleic acids derived from FFPE tissue biopsies, liquid biopsies, and other sample types." The SureSelect portfolio includes "panels for whole-exome sequencing and tumor profiling, along with our panel customization service powered by the SureDesign web application and our team of custom panel experts," adds Meldrum.

Amplicon-based enrichment

Also called amplification-, PCR-, or multiplex PCR-based enrichment, this approach uses pairs of primers to amplify specific regions of interest, which are then sequenced by adapter ligation. This method is sensitive, quantitative, and especially good for low DNA input levels, or for screening smaller panels of genes. In general, it can be faster, easier, and less expensive than the hybridization-based method for such uses.

Numerous vendors provide tools for amplicon-based target enrichment. For example, Agilent's amplicon-based HaloPlex HS platform offers custom kits for genomic DNA targets less than 5 Mb. "The kit contains all reagents, including

restriction enzymes for DNA fragmentation, needed to prepare NGS libraries from isolated DNA,” says Meldrum.

MIPs and single-primer enrichment

Other strategies rely on molecular inversion probes, single primers, adaptive sampling, and the CRISPR/Cas9 system to enrich for targeted DNA. In selective circularization, molecular inversion probes (MIPs) are used to create circular gene constructs containing target sequences, when are then amplified. MIPs were first developed for SNP detection, and the method is suitable for capturing and enriching a small number of targets. However, it can be too expensive for large targets.

Another method—similar to but distinct from amplicon-based enrichment—is target enrichment using single primers, supported by Tecan Genomics’ Allegro® Target Genotyping V2 with Single Primer Enrichment Technology (SPET). Enzymatically fragmented sample DNA is ligated to adaptors containing barcodes with their DimerFree® technology. The barcoded samples are pooled and then hybridized to probes/primers, which are extended and the results amplified with PCR to create a targeted library.

The SPET primers are targeted to both strands of the target sequence for more robust detection in SNP analysis, CNV analysis, and re-sequencing. Using single primers instead of primer pairs greatly simplifies the design of primer panels, and allows higher levels of multiplexing compared to amplicon-based enrichment. “Unlike other primer-based solutions that run into issues of dimerization, probe interference, or PCR inefficiency when the number of targets exceed tens of thousands, SPET is highly scalable and can be used from 500 to 100k+ targets,” says Ashesh Saraiya, Senior Product Manager at Tecan Genomics. “It allows users a simple, scalable, and cost-effective approach for SNP and small INDEL genotyping starting with as little as 10 ng of DNA.”

Enriching while sequencing

Adaptive sampling is a software-based enrichment method that requires no additional sample preparation prior to preparing a library for sequencing. Oxford Nanopore Technologies’ adaptive sampling technique enriches for specified target

DNA during the sequencing process by selecting DNA molecules to sequence. Nanopore's platform passes single strands of DNA through narrow pores with an electrical current. The current varies depending upon the bases going through the pore, generating a real-time electrical readout of the DNA sequence. If the software determines that a DNA strand contains a target, then it sequences the strand to completion. If not, then the software stops sequencing the DNA, ejects it from the pore, and moves on to the next DNA strand.

Unlike other methods, there's no limit to target size in adaptive sampling, which can enrich for large, complex structural variants. "For a larger, more complex panel, designing an adaptive sampling assay may provide a simpler method than designing an extensive set of probes," says Dan Turner, VP of Applications at Oxford Nanopore Technologies.

CRISPR/Cas9-based enrichment

Recently the endonuclease activity of the CRISPR/Cas9 gene-editing system has been adapted for amplification-free enrichment of DNA prior to Nanopore sequencing, as well as [prior to next-generation sequencing](#) and Pacific Biosystems' Single Molecule Real Time (SMRT) [long-read sequencing](#). Its benefit is that it can enrich DNA with long repeats, which is difficult to do with hybridization- and amplicon-based methods. "I would recommend the CRISPR/Cas9-based method for targeted enrichment studies where the researcher needs to accurately detect rare variants or genomic regions from a small number of targets, where PCR amplification from the genome would introduce too much bias or errors," says Saraiya.

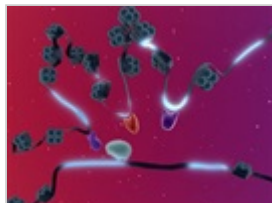
CRISPR/Cas9-based enrichment coupled with Nanopore sequencing can also enrich for structural variants, repeat expansions, and modified bases—regions that can be important diagnostically, yet difficult to access using amplification-based methods. Turner expects CRISPR/Cas9-based enrichment and adaptive sampling to become more widespread because both methods are PCR-free. "With these methods and Nanopore sequencing, it's possible to enrich and sequence previously inaccessible regions of the genome, and detect modifications in the same run," he says. "Both also allow the enrichment of much larger fragments than are possible to generate through PCR, allowing the sequencing of targets in

single long reads.” Going forward, the ongoing development of long read sequencing technologies may yet yield additional strategies for enrichment.

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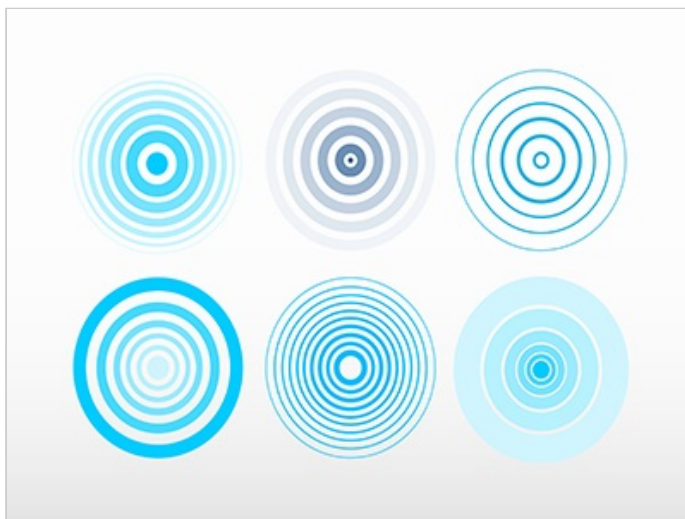
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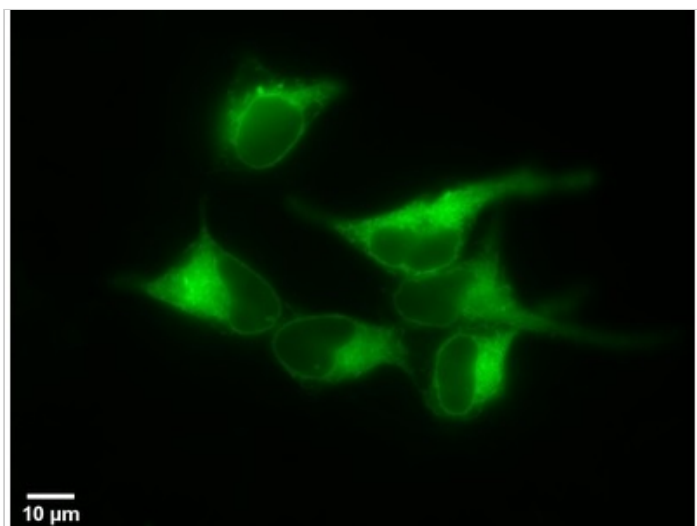
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