Oncology

Minimizing the impact of quantity not sufficient (QNS) on next-generation sequencing (NGS)

A comparison of amplicon-based and hybrid capture–based NGS enrichment methods

Introduction

Next-generation sequencing (NGS) enables DNA and RNA sequence profiling and can generate a large amount of insightful data much faster than traditional Sanger sequencing. NGS is increasingly being applied to detect biomarkers using targeted panels, and it enables comprehensive genomic profiling (CGP) for oncology applications.

Common targeted NGS methods

Targeted NGS generally requires less input material than whole-genome sequencing and produces a manageable quantity of data. The two most commonly employed targeted NGS methods are amplicon-based and hybrid capture–based enrichment. Amplicon-based enrichment involves fewer steps, which means it is often faster and less costly than hybrid capture–based sequencing. Hybrid capture workflows often involve extended incubation steps, which may include overnight hybridization. It can thus take several days to generate and analyze results from hybrid capture–based NGS. In comparison, amplicon-based enrichment has a simpler workflow that enables delivery of final sequencing results much more quickly.

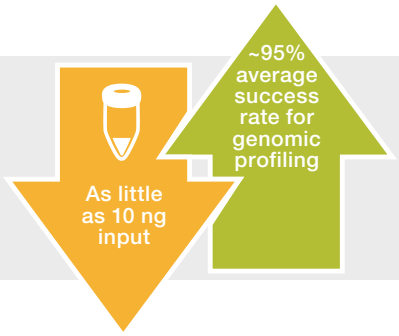
When quantity is not sufficient

Tissue biopsy is an invasive procedure that is not without potential complications. Collecting small tissue samples can reduce risk, but this limits the amount of nucleic acid that can be used for molecular profiling. When a tumor biopsy with limited surface area or tumor content does not contain enough nucleic acid for analysis, sequencing results cannot be generated reliably. In such cases, samples are considered “quantity not sufficient” (QNS). QNS can be a barrier to profiling tumor biopsies with NGS assays, as it makes it difficult to obtain meaningful, high-quality data. In three studies that employed hybrid capture–based NGS methods to analyze non-small cell lung cancer (NSCLC), 14–22% of all samples were QNS specimens and did not return valid results [1-3]. In another study that employed hybrid capture–based sequencing for the analysis of metastatic prostate cancer samples, 23% of all samples were classified as QNS [4].

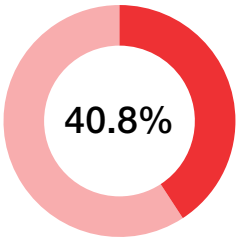
Advantages of amplicon-based NGS

Each targeted NGS method has unique advantages, but a key benefit of using an amplicon-based sequencing method to interrogate solid tumor samples is that it requires less DNA and/or RNA than a hybrid capture–based method. The lower input requirement for amplicon-based sequencing is due to PCR-based amplification of short targeted sequences, which affords greater depth of coverage and high accuracy. This is critical, particularly when a limited quantity of tumor biopsy is available. Hybrid capture methods often require 50–1,000 ng of nucleic acid. Samples must often have surface areas of 25 mm^2 or more in hybrid capture–based NGS workflows. Results from amplicon-based sequencing can be successfully obtained with as little as 10 ng of nucleic acid, including nucleic acid from samples with low tumor surface area and content. Amplicon-based NGS can thus deliver results for a significantly larger proportion of samples than hybrid capture–based sequencing and potentially benefit more patients [5].

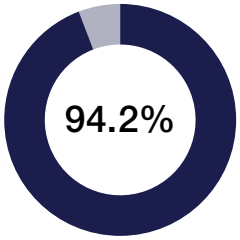
Up to 22% of NSCLC samples failed to generate results when analyzed using hybrid capture–based NGS, because of QNS.



Amplicon-based NGS requires significantly less input material, so a higher percentage of samples can be successfully profiled.



<50% of samples met the surface area requirement of >25 mm^2 for other NGS tests*



94.2% of samples were successfully reported with amplicon-based NGS

Real-world study of >30,000 consecutive samples [6]

Oncomine™ Solutions for amplicon-based NGS are part of an end-to-end workflow for molecular characterization of tumors, and they have enabled high rates of sequencing success in several large-scale genomic testing studies (Table 1). A 2021 study analyzed 31,165 real-world tumor samples consecutively received for genomic profiling across 25 US health care systems [6]. Less than 50% of the tumor samples received had tumor surface areas of at least 25 mm^2, the minimum surface area required for many leading commercial hybrid capture NGS tests. With amplicon-based NGS, 94.2% of the samples were successfully reported, including those with limited tumor content and/or surface area.

Table 1. Results of genomic profiling studies performed using amplicon-based NGS.

Study parameter	Strata Oncology [6]	Heidelberg University [7]	LC-SCRUM† [8]
Number of samples	31,165**	3,109	10,667
Sample type	Pan-cancer FFPE tissue	Lung FFPE tissue	Lung FFPE tissue
Success rate	94.2%	96.6%	94.5%
Panel	Ion AmpliSeq™ panel (>400 genes)	Ion AmpliSeq panel (>50 genes)	Oncomine™ Precision Assay (>50 genes)
System	Ion GeneStudio™ S5 System	Ion GeneStudio S5 System	Ion Torrent™ Genexus™ System

** Samples collected at 39 locations in the United States.
† LC-SCRUM is part of the Lung Cancer Genomic Screening Project for Individualized Medicine in Asia.

Notably, the amplicon-based NGS assay was even effective for tumor samples that had surface areas of 2 mm^2 or less. The amplicon-based NGS assay had a 97% positive predictive value for a series of breast cancer samples that included QNS and other low-quality samples. In 2019, researchers in Germany [7] and Japan [8] who utilized amplicon-based NGS to test NSCLC samples reported similarly high success rates.

In a study conducted at an academic medical institute [9], only 5 of 21 FFPE NSCLC samples were successfully tested at a commercial lab with hybrid-capture NGS for DNA + RNA, or RNA only. The samples were retested using amplicon-based NGS with an average of 2.6 x 5 µm slides, resulting in 20 of 21 samples successfully reported (Figure 1). Hence, amplicon-based NGS can also be considered a means to rescue QNS samples using minimal tissue input, in order to potentially identify targeted therapy treatment options.

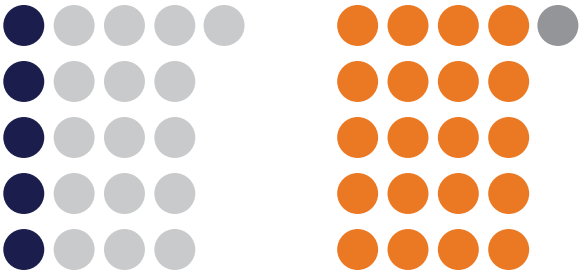


Figure 1. Rescue of QNS samples with amplicon-based NGS. Only 5 out of 21 FFPE NSCLC samples were successfully reported when tested with hybrid capture NGS. After retesting with an amplicon-based NGS assay, 20 of the 21 samples were successfully reported.

Maximizing molecular insights

Depending on the NGS method used for genomic profiling, QNS is a risk when tissue samples have limited tumor contents and surface areas. Targeted amplicon-based NGS can deliver informative results with less tissue, which can give molecular laboratories the confidence to achieve high success rates. Assays that employ amplicon-based NGS for genomic profiling could expand patient access to more biomarker-based therapies in the future and help guide treatment decisions for more patients with advanced solid tumors.

References

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