Oncology

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

A comparison of amplicon- 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. A library must be prepared before hybrid capture enrichment, with workflows that often require overnight ligation and larger sample pools to run efficiently. It can take several days to complete a full sequencing run and analysis using a hybrid capture workflow. Library preparation for amplicon-based enrichment does not require long adapter ligation and annealing reactions, enabling the delivery of final sequencing results much more quickly.

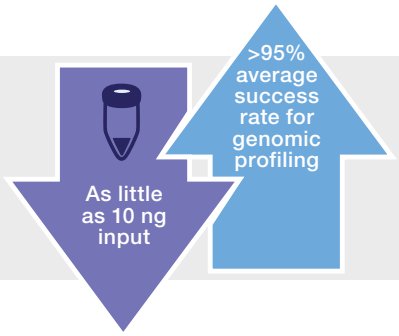
When quantity is not sufficient

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, full sequencing cannot be achieved. 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

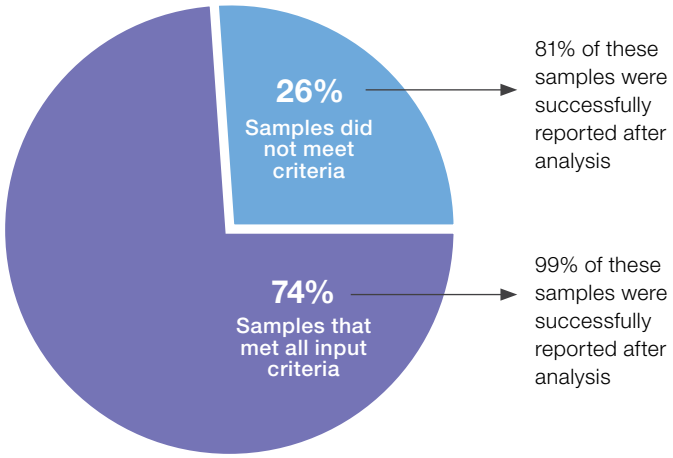
Each targeted NGS method has unique advantages, but a key benefit of using an amplicon-based sequencing method to interrogate solid tumor samples is that it requires less DNA and/or RNA than a hybrid capture–based method. The lower input requirement for amplicon-based sequencing is due to PCR-based amplification 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. Sectioned samples must often have surface areas of 25 mm^2 or more in hybrid capture–based NGS workflows, and the tumor content should be no less than 20%. Amplicon-based sequencing results can be successfully obtained with as little as 10 ng of nucleic acid, including nucleic acid from samples with low tumor 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 in the future [5].

Up to 22% of NSCLC samples failed to generate results when analyzed using hybrid capture–based NGS, because of QNS [1-3].



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

Study Results of Tumor Samples for NGS[6]



Oncomine™ Solutions for amplicon-based NGS are part of an end-to-end workflow for molecular characterization, and have high rates of sequencing success, as shown in several large-scale genomic testing studies (Table 1). In a 2021 study based in the United States, 31,165 FFPE samples representing a variety of different tumor types were consecutively analyzed using an amplicon-based NGS assay (PCR-CGP) [6].

Of the samples analyzed, 26% did not meet requirements set by leading commercial hybrid capture–based CGP tests, due to limited tumor content and/or surface area. However, 81% of these samples were successfully reported after analysis with the amplicon-based assay. Of the 74% of samples that satisfied all input criteria, 99% provided informative results. Tumor content was below 20% in 10.7% of all 31,165 samples tested, and 59.2% had a tumor surface area of less than 25 mm^2.

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 FFPETs	Lung FFPET	Lung FFPET
Success rate	94.2%	96.6%	94.5%
Reported QNS rate (1 – success rate)	<6%	<4%	<6%
Panel	Ion AmpliSeq™ (>400 genes)	Ion AmpliSeq (>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.

The amplicon-based NGS assay was even effective for tumor samples that had surface areas of 2 mm^2 or less. Overall, in this study the authors obtained a success rate of 94.2%. Of note, in a series of breast cancer samples that included QNS and other low-quality samples, the amplicon-based NGS assay obtained a 97% positive predictive value. In 2019, researchers in Germany [7] and Japan [8] who utilized amplicon-based NGS to test NSCLC samples reported similarly high success rates.

Maximizing molecular insights

Depending on the NGS method used for genomic profiling, QNS is a risk when tissue samples have limited tumor content. Targeted amplicon-based NGS can deliver informative results when tumor content is low, 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 in the future to more biomarker-based therapies and help guide treatment decisions for more patients with advanced solid tumors.

References

1. Davis W, Makar G, Mehta P et al. (2019) Next-generation sequencing in 305 consecutive patients: clinical outcomes and management changes. *JCO Oncol Pract* 15(12):595-610.
2. Hellmann MD, Paz-Ares L, Bernabe Caro R et al. (2019) Nivolumab plus Ipilimumab in advanced non-small cell lung cancer. *N Engl J Med* 381:2020-2031.
3. Heeke S, Benzaquen J, Long-Mira E et al. (2019) "Comparison of tumor mutational burden using the Ion Oncomine™ TML and FoundationOne™ assays with routine clinical FFPE tissue samples to predict durable clinical benefit in lung cancer and melanoma patients–a multivariate analysis integrating PD-L1 and $CD8^+$ evaluation." American Association for Cancer Research Annual Meeting 2019, March 29 to April 3, Atlanta, GA. **https://cancerres.aacrjournals.org/content/79/13_Supplement/4889**.
4. Zhu J, Tucker M, Marin D et al. (2019) Clinical utility of FoundationOne tissue molecular profiling in men with metastatic prostate cancer. *Urol Oncol* 37(11):813.e1-813.e9.
5. Rhodes D, Hovelson DH, Suga JM et al. (2020) PCR-based comprehensive genomic profiling (PCR-CGP): feasibility from >20,000 tissue specimens and predicted impact on actionable biomarker identification versus hybrid capture (H)-CGP and plasma (P)-CGP. *J Clin Oncol* 38(15)_suppl:3574.
6. Tomlins SA, Hovelson DH, Suga JM et al. (2021) Real-world performance of a comprehensive genomic profiling test optimized for small tumor samples. *JCO Precis Oncol* 5:1312-1324.
7. Volckmar A, Leichsenring J, Kirchner M et al. (2019) Combined targeted DNA and RNA sequencing of advanced NSCLC in routine molecular diagnostics: analysis of the first 3,000 Heidelberg cases. *Int J Cancer* 145(3):649-661.
8. Matsumoto S (2021) "Impact of rapid multigene assays with short turnaround time (TAT) on the development of precision medicine for non-small cell lung cancer (NSCLC)." American Society of Clinical Oncology 2021 Annual Meeting, Chicago, IL, June 3–7. **https://meetings.asco.org/abstracts-presentations/200654**.

Learn more at **oncomine.com/ruo**