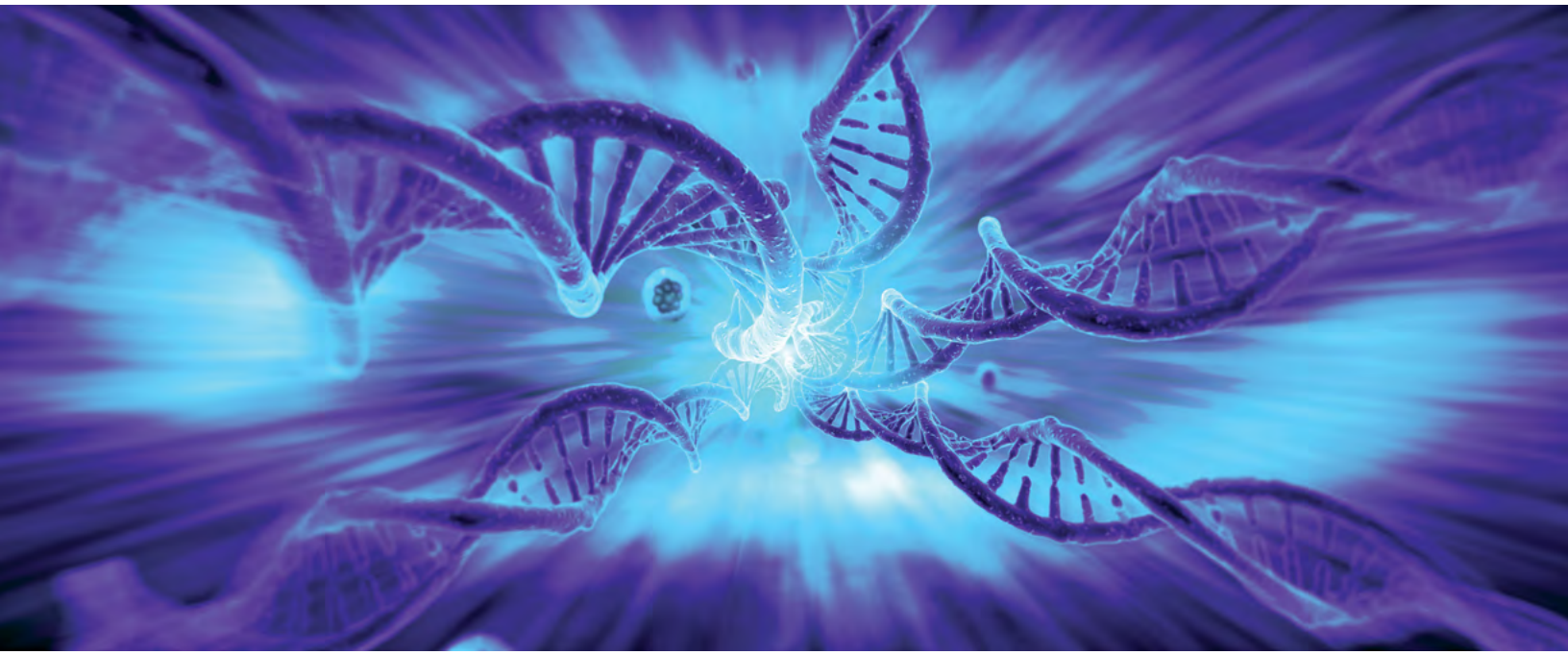
White Paper

Analytical Validation of a WES NGS Assay for Somatic Alterations of Solid Tumors

NEO GENOMICS



Whole exome sequencing (WES) is a next-generation sequencing (NGS) application that generates genomic information for all protein-coding genes across the human genome. Although targeted sequencing of a preselected gene set is an important aspect of the current testing strategy, WES provides an unbiased view of the exome.[1]

This comprehensive genomic analysis is considered the consensus gold standard approach for the detection of somatic variants required for emerging applications such as tumor mutation burden and neo-antigen discovery.[2–5] For many years, clinical and research studies have focused on the use of targeted panels due to several limiting factors commonly associated with exome sequencing, such as increased cost, increased sample requirements, and decreased analytical performance.[6,7] In many circumstances where targeted panels are used, there must be a compromise made to sacrifice potentially valuable genetic information to compensate for one or more of the aforementioned reasons.[8] With recent advances in NGS technology, many obstacles traditionally associated with WES have been resolved, making it an attractive alternative to a targeted panel.[9] Whole exome next-generation sequencing empowers clinicians and researchers to evaluate every gene in the human genome with the same level of analytical performance available from most targeted NGS panels.

Whole exome sequencing workflow

Tissue sections are cut and mounted on slides. One section is stained and used for pathology review while the remaining slides are processed for NGS. Tumor regions of interest identified by a board-certified pathologist are selectively removed from the slide to enrich the tumor cell population and minimize the presence of DNA from adjacent non-tumor cells. Nucleic acids are isolated from the tumor tissue and from nucleated cells collected from patient-matched whole blood samples. The purified DNA samples are prepared for next-generation sequencing through a series of reactions that have been highly optimized for DNA from FFPE samples. The DNA from the non-tumor cells and tumor cells are sequenced using a highly optimized hybrid capture probe-based methodology to generate a minimum mean target coverage of 100X and 250X, respectively. Bioinformatics tools are used to subtract germline variants identified in the patient-matched blood DNA from the tumor sequence data to identify somatic alterations.

Optimization

A number of challenges are commonly encountered when using next-generation sequencing technology to generate exome coverage from DNA isolated from fixed tissue samples. Many of these challenges are inherent to the process by which tissues are collected and preserved for testing. Two major challenges for any assay to overcome are the negative impacts on the quality of sample DNA created during chemical fixation and the limited amount of suitable tissue available for testing. These two factors alone are the primary source of up to a 40% testing cancellation or failure rate experienced by some laboratories. This WES testing methodology was specifically optimized to tolerate and overcome a broad range of sample quality and size variability and deliver performance comparable to many panel based applications. Each step of the WES workflow was specifically optimized for challenging FFPE tissue samples to increase the chances of success for all samples. With the implementation of a holistically optimized workflow the NeoGenomics WES methodology has an overall success rate for all samples received of > 97%.

Validation

The NeoGenomics WES methodology has been extensively validated for the detection of single nucleotide variants and insertions/deletions across 39 Mb of the protein-coding region of the human genome. A comprehensive set of representative clinical samples — matched fresh and fixed tissue, pre-characterized cell lines, and controls from the National Institute of Standards and Technology, Platinum Genomes, and Genomes in a Bottle — were used to optimize and validate the assay performance. All samples included in the validation had exome wide variant calls provided by a CLIA certified orthogonal testing methodology via an outside laboratory or available in published literature. Additionally, clinically relevant alterations were confirmed with a CLIA certified targeted NGS panel and/or Sanger sequencing. Analytical performance parameters were validated at the optimal and lowest input range of the assay.

Analytical performance

To assess the system accuracy, variant calls were compared with orthogonal testing results across the exome and within clinically relevant targeted regions. All performance parameters were tested at the optimal and lowest input range of the assay.

Accuracy at lower detection limit

Accuracy results for FFPE tissue samples with matched normal controls. All samples were tested with a CLIA certified NGS targeted panel to determine allele fractions for each variant class. Only variants detected at 5 and 10% allele fractions from FFPE samples were included in the study. Variants across the exome as well as within genes that were clinically actionable were evaluated to establish the lower limit of detection.

Variant Class	Input (ng)	Allele Fraction	Sensitivity	Specificity	Accuracy
SNV	50ng	5%	>97%	>99%	>99%
		10%	>98%	>99%	>99%
Indel		5%	>93%	>99%	>99%
		10%	>99%	>99%	>99%
SNV	200ng	5%	>97%	>99%	>99%
		10%	>99%	>99%	>99%
Indel		5%	>96%	>99%	>99%
		10%	>99%	>99%	>99%

Limit of detection

The minor allele fraction (MAF) limit of detection was established from both fresh and FFPE cell line dilutions. Fresh and fixed cell lines harboring known variants were diluted at diminishing ratios into cell lines without the variant of interest. Contrived allele fractions were confirmed with a targeted NGS panel. Only variants within the targeted panel region were included in this study.

Variant Class	Sample Type	Input	LOD
SNV	Fresh	50ng	4.0%
	Fresh	200ng	2.1%
	FFPE	50ng	4.6%
	FFPE	200ng	4.6%
Indel	Fresh	50ng	3.3%
	Fresh	200ng	2.8%
	FFPE	50ng	6.1%
	FFPE	200ng	4.8%

Precision

Precision studies were performed with variants at the 5-10% allele fraction range from fresh and fixed cell line dilutions at the optimal and lower input amounts. The 50ng FFPE intra-assay studies were not performed (NP) due to limited sample availability. The inter-assay variability, which typically tends to be higher, may be considered in lieu of intra-assay data. Three or more replicates of each sample were processed within a single run as well as over three separate runs to establish the intra- and inter-assay precision.

Variant Class	Sample	Parameter	Input	Concordance
SNV	Fresh	Inter-assay	50ng	0.99
		Intra-assay	50ng	1.00
		Inter-assay	200ng	1.00
		Intra-assay	200ng	1.00
Indel		Inter-assay	50ng	0.97
		Intra-assay	50ng	0.97
		Inter-assay	200ng	0.96
		Intra-assay	200ng	0.96
SNV	FFPE	Inter-assay	50ng	0.99
		Intra-assay	50ng	NP
		Inter-assay	200ng	1.00
		Intra-assay	200ng	1.00
Indel		Inter-assay	50ng	0.94
		Intra-assay	50ng	NP
		Inter-assay	200ng	0.93
		Intra-assay	200ng	0.95

Conclusions

As the search for clinically relevant biomarkers expands beyond a preselected set of targeted genes, whole exome sequencing provides an alternative solution over target panel NGS. The methodology presented in this study demonstrates analytical performance comparable to several of panel based approaches currently available but without the data limitations associated with sequencing a preselected panel of genes. For every sample ever tested with a targeted panel all sequence information outside of the panel design has been lost, only recoverable by re-sequencing. Whole exome sequencing enables the ability to interrogate any sequence of interest from the protein-coding region of the genome without bias for an indefinite period of time. The genetic information outside of a defined gene set from each unique sample may contribute novel alterations to the ever-expanding search for actionable biomarkers. Whole exome sequencing provides an unbiased and indiscriminate genetic profile of the tumor, enabling the detection of pre-characterized actionable biomarkers and novel alterations which may have clinical and research significance.

Summary			
Target Region	39Mb	Input amount	50 ng minimum 200 ng maximum
Depth	100X Normal >250X. Tumor	TAT	14–21 days
Sample type	FFPE tumor and matched whole blood	QNS Rate	<3%

About NeoGenomics Pharma Services

NeoGenomics' Pharma Services unifies several innovative companies' scientific and medical leadership under one leading brand, offering one of the most comprehensive laboratory services menu available for biomarker testing supporting oncology clinical trials globally. We provide our clients with an unparalleled level of expertise, service, flexibility, and scalability. Additionally, we offer alternative business models and solutions across the continuum of development from pre-clinical research and development through commercialization.

To learn more about NeoGenomics Pharma Services visit us online at neogenomics.com/pharma-services, call us at 800.720.4363 or email us at pharmaservices@neogenomics.com.

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