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Next-Generation Sequencing Resources for Pathologists

Introduction

Next-generation sequencing (NGS) has revolutionized biomarker analyses delivering unprecedented multiplexing capabilities. Unlike PCR testing or capillary electrophoresis (CE)–based sequencing (also known as Sanger sequencing), NGS can multiplex to assay many genes simultaneously, replacing the need to order multiple tests. Compared to traditional methods, which are often inconclusive and require iterative testing on limited tissue, NGS has the potential to reduce costs and improve patient care, realizing the vision of precision medicine. Increasingly, NGS is being used to understand the genetic changes within cancers, identify the causative variants of rare and inherited disorders, advance prenatal and reproductive care, and anticipate potential adverse reactions to a chosen therapy. The speed and accuracy of NGS continues to enrich our understanding of disease, with the potential to enable earlier detection and reduce the time to diagnosis for high-quality, cost-effective health care.

Immunohistochemistry and *in situ* hybridization interrogate samples for protein or nucleic acid expression, respectively, to detect copy changes and fusion events. NGS multiplexes these qualitative assays into multimodal and quantitative assessments. NGS can identify all classes of clinically meaningful alterations including single nucleotide variants, insertions/deletions, copy number alterations, and structural rearrangements. Moreover, advances in sequencing technology and changes to medical guidelines are removing many of the barriers that traditionally made sequencing-based testing challenging. NGS is increasingly becoming the preferred method for identifying clinically relevant variants, as demonstrated by a growing number of publications and method recommendations. With the emergence of FDA-cleared sequencers and assays, NGS technology is becoming more accessible to the clinical community. Together, the MiSeqDx® Platform—the first FDA-cleared *in vitro* diagnostic NGS instrument—and the MiSeqDx Universal Kit 1.0 enable molecular pathology laboratories to develop custom assays and bring NGS into their facilities.

Resources

Guidelines and Recommendations for Implementing NGS

1. Aziz N, Zhao Q, Bry L, et al. College of American Pathologists' laboratory standards for next-generation sequencing clinical tests. *Arch Pathol Lab Med.* 2015;139:481-493.
   Checklist for clinical testing using NGS that sets standards for the analytic wet bench process and for downstream bioinformatics and reporting.
   http://www.archivesofpathology.org/doi/pdf/10.5858/arpa.2014-0250-CP
2. Rehm HL, Bale SJ, Bayrak-Toydemir P, et al. ACMG clinical laboratory standards for next-generation sequencing. *Genetics Med.* 2013;15:733-747.
   Professional standards and guidelines set forth by the American College of Medical Genetics and Genomics to assist clinical laboratories with validation of NGS approaches, monitoring for quality, interpreting and reporting of variants found.
   http://www.ncbi.nlm.nih.gov/pubmed/23887774
3. *Next Generation Sequencing (NGS) Guidelines for Somatic Genetic Variant Detection*. Albany, NY: New York State Department of Health: 2015
   Articulates requirements for the development of standard operating procedures and validation of NGS assays for the detection of somatic genetic variants in a molecular pathology laboratory.
   www.wadsworth.org/labcert/TestApproval/forms/NextGenSeq_ONCO_Guidelines.pdf

4. Gargis AS, Kalman L, Berry MW, et al. Assuring the quality of next-generation sequencing in clinical laboratory practice. *Nat Biotechnol*. 2012;30:1033-1036.
An expert workgroup of clinical laboratory directors, clinicians, US government agency representatives, software developers, and bioinformaticians offer NGS guidelines for molecular diagnostic laboratories. They address test validation, quality control, and proficiency testing for independent assessment of NGS performance with additional reference materials.
http://www.ncbi.nlm.nih.gov/pubmed/23138292

5. Korf BR, Rehm HL. New approaches to molecular diagnosis. *J Am Med Assoc*. 2013 Apr 10;309(14):1511-1521. PMID: 23571590.
American Medical Association viewpoint piece on how physicians may recognize where new approaches to genetic and genomic testing may be applied clinically and with context-dependent interpretation of test results..
http://www.ncbi.nlm.nih.gov/pubmed/23571590

6. *Establishing Molecular Testing in Clinical Laboratory Environments; Approved Guideline*. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
Guideline on implementing molecular diagnostic testing with quality control and regulatory requirements with additional consideration for subspecialties including infectious disease, pharmacogenetics, and oncology.
http://shop.clsi.org/c.1253739/site/Sample_pdf/MM19A_sample.pdf

7. Santoro S, Arnaout R, Burton MP, et al. *Molecular Pathology Resource Guide. Version 5.0(1)*. Northfield, IL: College of American Pathologists; 2014.
CAP resource guide curates recent application-specific journal articles as well as CAP resources that includes learning opportunities, proficiency testing, and accreditation related to molecular diagnostics utilizing NGS.
http://www.cap.org/apps/docs/membership/md_resource_guide.pdf

8. Cree IA, Deans Z, Ligtenberg MJL, et al. Guidance for laboratories performing molecular pathology for cancer patients. *J Clin Pathol*. doi:10.1136/jclinpath-2014-202404.
European Society of Pathology Task Force on Quality Assurance in Molecular Pathology and the Royal College of Pathologists provide minimum requirements for the management of molecular pathology laboratories utilizing NGS.
http://jcp.bmj.com/content/early/2014/07/10/jclinpath-2014-202404

9. Olsen S, Berger AC. *Genome-Based Diagnostics: Clarifying Pathways to Clinical Use: Workshop Summary*. Washington, D.C.: The National Academies Press; 2012.
Standards in NGS delineate a framework of value creation for leveraging data assets to answer those contemporary questions asked by a broad ecosystem of pathologists, their physician colleagues, and the patients for whom they care.
http://www.nap.edu/openbook.php?record_id=13359&page=R1

10. Schilsky, RL. Implementing personalized cancer care. *Nat Rev Clin Oncol*. 2014;11:432–438.
Opinion article to facilitate delivery of personalized medicine to patients.
http://www.ncbi.nlm.nih.gov/pubmed/24687035

11. MacArthur DG, Manolio TA, Dimmock DP, et al. Guidelines for investigating causality of sequence variants in human disease. *Nature*. 2014 Apr 24;508(7497):469-476.
A working group of experts in genomic research, analysis and clinical diagnostic sequencing was convened by the US National Human Genome Research Institute. They discussed the key challenges of assessing sequence variants in human disease and integrating both gene-level and variant- level support for causality to propose guidelines for summarizing confidence in variant pathogenicity.
http://www.ncbi.nlm.nih.gov/pubmed/24759409

12. Easton DF, Pharoah PDP, Antoniou AC, et al. Gene-panel sequencing and the prediction of breast-cancer risk. *N Engl J Med*. 2015;372:2243-2257.
Utilization of NGS towards assigning risk to rare variants and review of NGS-based gene panels for which the evidence of association with breast cancer may be incorporated into personalized risk prediction.
http://www.nejm.org/doi/full/10.1056/NEJMsr1501341

13. Katsanis SH, Katsanis N. Molecular genetic testing and the future of clinical genomics. *Nat Rev Genetics*. 2013 Jun;14(6):415-426.
Review of the range of molecular diagnostic methods currently available in clinical settings with opportunities for NGS implementation in pathology workflows.
http://www.ncbi.nlm.nih.gov/pubmed/23681062

14. Dienstmann R, Dong F, Borgor D, et al. Standardized decision support in next generation sequencing reports of somatic cancer variants. *Mol Oncol*. 2014 Jul;8(5):859-873.
A systematic framework for variant annotation and prioritization is proposed with downstream structure for molecular pathology reporting using standardized terminology.
http://www.ncbi.nlm.nih.gov/pubmed/24768039

15. de Gramont A, Watson S, Ellis LM, et al. Pragmatic issues in biomarker evaluation for targeted therapies in cancer. *Nat Rev Clin Oncol*. 2015 Apr;12(4):197–212.
A review of the NGS biomarker validation processes in oncology, including preanalytical (sample-related), analytical, and postanalytical (data-related) aspects of assay development.
http://www.ncbi.nlm.nih.gov/pubmed/25421275

16. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics Med*. 2015;17:405-423.
The collective expert opinion of the workgroup with input from ACMG, AMP, and College of American Pathologists stakeholders. This opinion regards the breadth of genetic tests and their clinical reporting in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes.
http://www.nature.com/gim/journal/v17/n5/abs/gim201530a.html

Reimbursement Information

1. Oliver R. *CPT Code Changes for 2015 Pathology/Laboratory*. Chicago, IL: American Medical Association; 2015.
To provide details on the 2015 CPT® changes, McKesson (BPS) has prepared this summary of new, deleted and revised codes for pathology and laboratory issued by the American Medical Association (AMA).
http://mptrms.mckesson.com/rs/MckessonPT/images/2015CPTChangesPATH.pdf

2. Association for Molecular Pathology (AMP). Genomic Sequencing Procedure Cost and Value Models. http://www.amp.org/committees/economics/NGSPricingProject.cfm. Accessed September 16, 2015.
Models compiled by AMP represent a snapshot of current Genomic Sequencing Procedures (GSP) for laboratories to articulate the cost and value of the GSP services they provide to both Medicare and commercial payers.
https://www.amp.org/committees/economics/NGSPricingProject.cfm

3. Crawford JM, Bry L, Pfeifer J, et al. The business of genomic testing: a survey of early adopters. *Genetics Med*. 2014 Dec;16(12):954-961.
Laboratory medical directors and pathology department chairs of 13 different academic institutions viewed early adoption of genomic analyses as an imperative for developing expertise in the implementation of genomic precision medicine.
http://www.ncbi.nlm.nih.gov/pubmed/25010053

Regulatory Information

1. Collins FS, Hamburg MA. First FDA authorization for next-generation sequencer. *N Engl J Med.* 2013 Dec;369(25):2369-2371.
A shared perspective from the NIH Director and FDA Commissioner regarding the marketing authorization for the first next-generation genome sequencer. This represents a significant step forward in the ability to generate genomic information that increasingly impacts patient care.
http://www.ncbi.nlm.nih.gov/pubmed/24251383

2. US Food and Drug Administration. *Paving the Way for Personalized Medicine*. Silver Spring, MD: US Food and Drug Administration, US Dept of Health and Human Services; 2013.
The report describes the ways in which the FDA has worked to respond to, anticipate, and help drive scientific developments in personalized therapeutics and diagnostics as they pertain to standards, methods and tools.
www.fda.gov/downloads/scienceresearch/specialtopics/personalizedmedicine/ucm372421.pdf

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