NEXT GENERATION SEQUENCING
for Clinical Genomic Medicine
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Cancer testing technologies

Pathology: Identify morphologic changes to tissues and cells

Cytogenetics: Identify changes to chromosomes (number, gains, losses, etc.)

Sequencing: Identify changes to DNA sequence of genes

Breast carcinoma histology

HER2 positive breast cancer

Single gene tests - common

Multiple gene tests – emerging (Next-generation sequencing, NGS)
Types of DNA sequence variants

- **Exon**
- **Intron**
- **Hotspot**

Types of genetic variation:

- **SNV**
- **Del**
- Expanded repeat
- **CNV (dup)**
- **Structural Variant (translocation)**
- **Aneuploidy, trisomy 21**

Clinical NGS—Full service testing or customized components & consultation

Clinical NGS Testing Workflow:

- **Specimen**
- **Custom Assay Testing**
- **DNA/RNA & library prep**
- **Clinical-grade Sequence**
- **Clinical-grade variants**
- **Pathology Intake & Extraction**
- **Sequencing**
- **Custom Panel Analysis**
- **Clinical report**

- **Repository**
- **FASTQ sequence**
- **Clinical variants ± Custom report**

- **FISH**
- **CMA**
- **IHC**
- **Flow**
- **Assay Design & Optimization**
- **Data Analysis & Support**
### Comprehensive Cancer Gene set—version 3.1: how did we get here?

**SOLID TUMOR** (65 genes)

- AKT1
- BRCA2
- ERBB2
- FGFR1*
- IDH2
- MET
- NTRK1*
- RET*
- VHL

- AKT2
- BRIP1
- ERBB4
- FLT3
- KIT
- NF1
- PKC3A
- STK11

- AKT3
- CDKN2A
- ERBB2
- FGFR4
- JAK1
- MLH1
- PALB2
- RIT1

- AKT1
- CDH1
- ERBB3
- FLT1
- JAK2
- MTOR
- PDGFRB
- ROS1*

- ALK*
- CDKN2A
- ERBB4
- FLT3
- KIT
- NF1
- PDGFRB
- ROS1*

- BRAF
- CUTF/N1
- FGFR1
- FLT3
- KIT
- NF1
- PKC3A
- STK11

- BRAF
- DDR1
- FGFR2*
- IDH2
- NOTCH2
- RAAS4B
- TSC1

**MELANOMA** (13 genes)

- AKT1
- ERBB4
- MAP2K1
- PTEN

- AKT1
- AKT2
- RH2
- SMO

- BRAF
- IDH2
- SMAD4

- AKT1
- AKT2
- PKC3A

**CNS TUMOR** (24 genes)

- AKT1
- ERBB2
- RET*

- AKT1
- Ankyrin
- Kras
- Slu1

- BRAF
- IDH2
- SMAD4

- AKT1
- AKT2
- PKC3A

**SOMA** (16 genes)

- AKT1
- CDKN2A
- FGFR2*
- GNAS

- AKT1
- AKT2
- PKC3A

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**HEMATOPOIETIC DISORDERS** (54 genes)

- ABL1
- CEBPA
- FBXW7
- IL7R

- ABL1
- CEBPA
- GATA1
- KDM6A

- ABL1
- CEBPA
- GATA2
- KDM6A

- ABL1
- CEBPA
- KDM6A
- NPM1

- ABL1
- CEBPA
- KDM6A
- SF3B1

- ABL1
- CEBPA
- KDM6A
- SF3B1

- ABL1
- CEBPA
- KDM6A
- SF3B1

- ABL1
- CEBPA
- KDM6A
- SF3B1

- ABL1
- CEBPA
- KDM6A
- SF3B1

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**LYMPHOMA** (10 genes)

- ABL1
- CEBPA
- FGFR3

- ABL1
- CEBPA
- GATA1

- ABL1
- CEBPA
- GATA1

- ABL1
- CEBPA
- GATA1

- ABL1
- CEBPA
- GATA1

- ABL1
- CEBPA
- GATA1

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*Selected introns also to be sequenced for rearrangement detection

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**Clinical Test Validation and Performance—highly regulated**

Clinical Laboratory Improvement Amendments—1988 (2003 revision): CLIA

- Federal law empowering Centers for Medicare and Medicaid Services (CMS) to regulate clinical labs
- CMS delegates enforcement through deemed organizations, who then provide framework for accreditation (e.g., College of American Pathologists, CAP)
- Rigorous understanding of instruments and assay performance through defined ‘SOPs’ (Standard Operating Procedures)
- What are your error rates & sources of error?
- What controls and monitors do you need to reproducibly generate the best data possible?
- How can you improve your processes and performance?
Clinical Test Validation and Performance—highly regulated

Clinical Laboratory Improvement Amendments—1988 (2003 revision): CLIA

- Platform validation AND Assay validation
- All components in a complex system must be independently evaluated as potential sources for error
  - "wet" validation = technical; specimen processing thru raw data generation
  - "dry" validation = data analysis components, i.e., the ‘pipeline’ to include variant annotation, interpretation and reporting
- Quality control throughout both components

U.S. Food and Drug Administration (FDA)

- Laboratory tests are ‘Medical Devices’
  - IVD: in vitro diagnostic device (approved assay)
  - IUO: investigational use only
  - RUO: research use only; optimized version of IUO, on path to IVD
  - LDT: CLIA laboratory-developed test; minimal FDA oversight
- FDA Approval—2 major pathways
  - Premarket Assessment—Full Monty, clinical trials (time & $)
  - Premarket Notification ("510k submissions)—requires an existing predicate
  - Depends on test classification (I, II, III)—potential risk to patient
    - Is is safe? Is it effective?
- Analyte-specific reagents (ASRs) are the active ingredient(s) used in IVDs & LDTs
  - Who can order
  - Who can perform
  - Strict labeling guidelines
Clinical Test Validation and Performance—published guidelines

- College of American Pathologists’ Molecular Pathology, Laboratory General and All Common Checklists. 04.21.2014;
- S Richards et al. (ACMG Laboratory Quality Assurance Committee); *Genet in Med.*; May 2015; 17(5): 405-424.

Validation Considerations for Clinical NGS tests

- **New Assay or existing assay modification—determines** whether you must perform full validation, or partial revalidation/demonstration of equivalence
- What is the clinical indication and who are the tested subjects (disease)?
- What is the method (e.g., amplicon, targeted hyb capture, etc.) and what is the platform (instruments and associated reagent kits)?
- What are the desired specimen types (fresh/frozen tissue, FFPE, CSF…)?
- What are the variant types to be detected and at what analytical sensitivity (Limit of Detection, LOD)?
  - SNVs
  - CNVs (aneuploidies?)
  - Indels
  - SVs (translocations)
- LOD mixing studies
- Identification of gold std specimens/samples for concordance
- Cancer: Tumor-normal pairs to reduce # of annotated variants (increased cost)
Validation Considerations for Clinical NGS tests

- Are there new bioinformatics tools that will need to be included in the pipeline?
- Are there consistently inadequately covered regions (how to address this)?
- Are there pseudogenes that affect which variants you cannot ‘see’ (ambiguous mapping)
- Quality Control (QC) monitors?
- Bioinformatic transformations (carving out disease-specific gene panels)
- Confirmatory testing—under what conditions (which variant types) and by what orthogonal method? (reference lab selection criteria)
- Variant interpretation and Reporting rules
- Results archiving (→ variant database) and report/data transfers
- Proficiency Testing

NGS testing for Constitutional Diseases

**Strategy**: Exome backbone

- 69 genes
- 11 genes
- 25 genes
- ? genes
- >> other genes

**CardioGene Set**
- Arrhythmias
  - BrS
  - CPVT
  - LQTS
  - SQTS

**Cardiomyopathies**
- ARVC
- LVNC
- DCM
- HCM

**Renal Gene Set**
- Alport Syndrome
- Nephrotic Syndrome
- Metabolic disorders
- Complement defects

**Endocrine Gene Set**
- In development

**Other Disease Gene Sets**
- Noonan (11)
- aHUS (7)

Validated exome backbone provides flexibility in adding genes/tests