SENTARA®

NGS Implementation in a Clinical Laboratory

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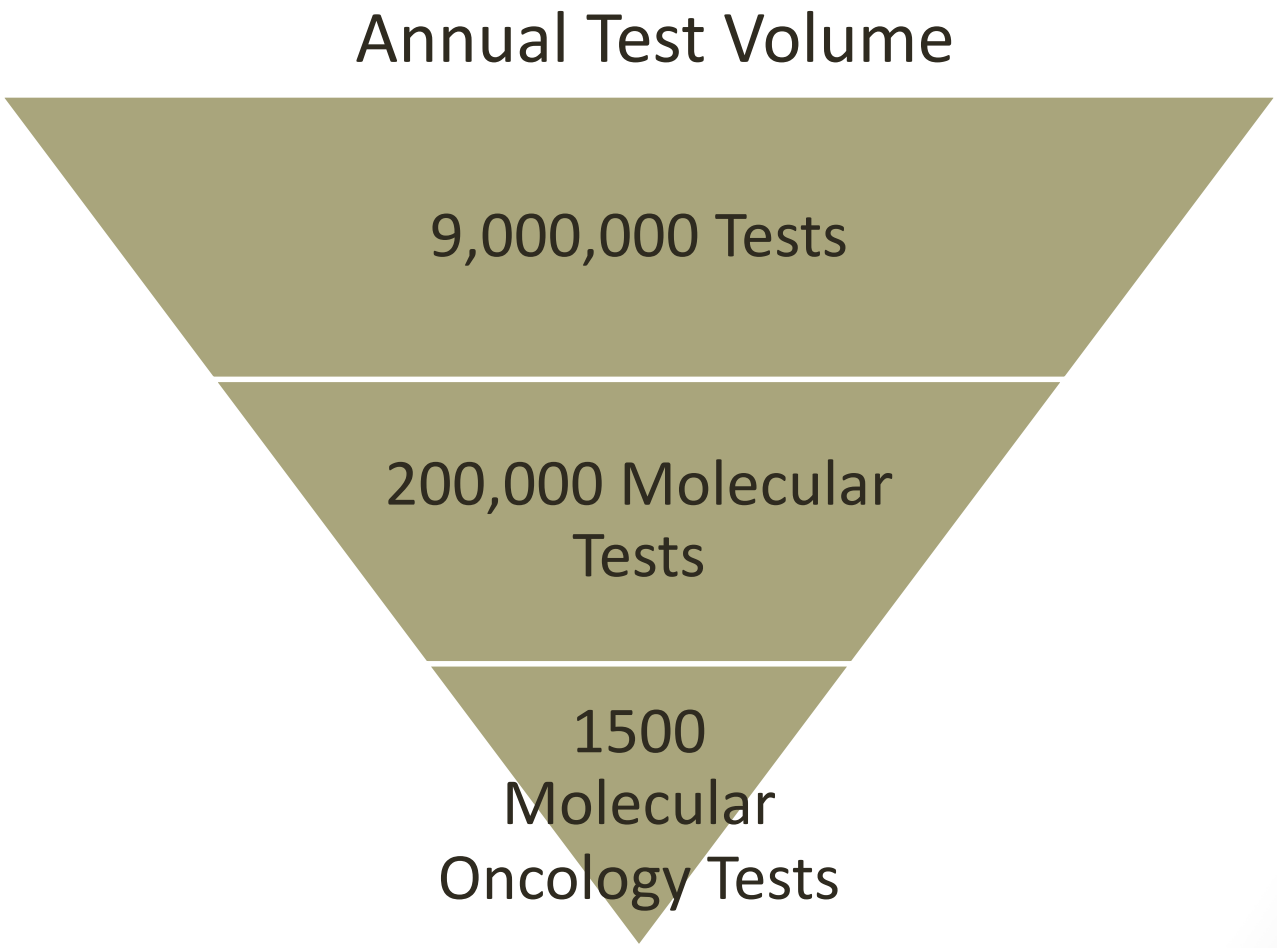
Overview

- Background
- Rational
- Test Menu Development
- Business Case
  - Alternate funding source
- NGS Utilization
  - Cystic Fibrosis (CF)
  - Cancer Hotspot v2 (CHPV2)
  - Oncomine Focus Assay (OFA)
  - Oncomine BRCA 1/2 Research Assay
  - Oncomine Myeloid Research Assay

Sentara Network

- 12 Hospital System
- >200 Physician Offices
- Own a private payer insurance
- Reference Lab is located in the flagship hospital

Sentara Reference Laboratory



Molecular Test Menu

Molecular Oncology

- Oncomine Focus Assay (NGS)
- *EGFR*
- *KRAS*
- *BRAF*
- *NRAS*
- *JAK2*

Molecular Genetics

- *CFTR*
- Fragile X
- *SMN1*
- *FVL, PT, MTHFR*

Molecular Infectious Disease

- HIV (viral load & genotype)
- HCV (viral load & genotype)
- HBV
- CMV
- BK
- HSV-1/-2
- BV
- Yeast
- RPP
- Bordetella

How we decide to insource a test?

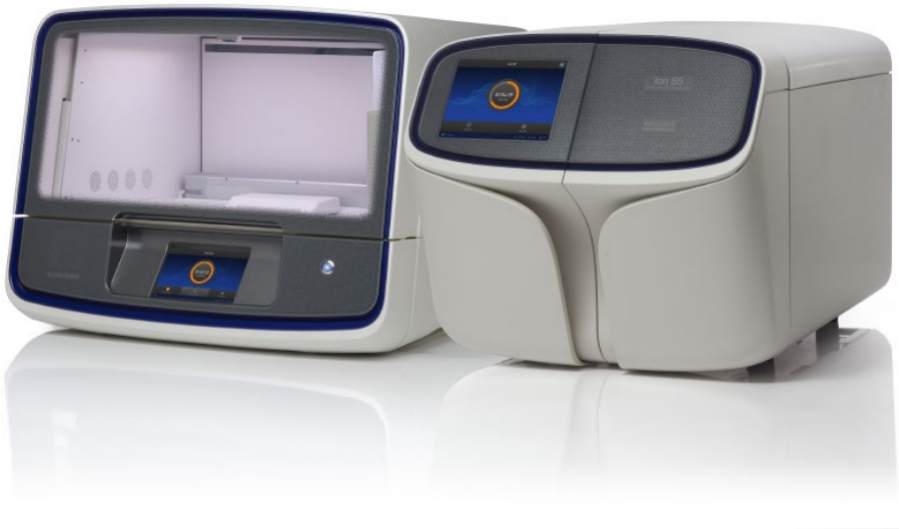
- Turnaround times sensitive?
- High enough volume?
  - Review Reference Lab Utilization
  - Top 20 tests by volume or spend
- Assay available on current instruments?
- Does my staff already have competency on a similar test?
- Can I perform an equivalent test for a lower cost?

In-house testing efficiencies:

- Expense avoidance
  - Most molecular tests cost hundreds of dollars to send to reference labs for testing
  - Buy vs own analysis
- Improved TAT
  - Many molecular tests take weeks to result from reference labs
  - We perform esoteric testing weekly
- Local physician input into test menu
  - Increased communication between providers and the laboratory allows us to develop our test menu in concert with physician ordering patterns.

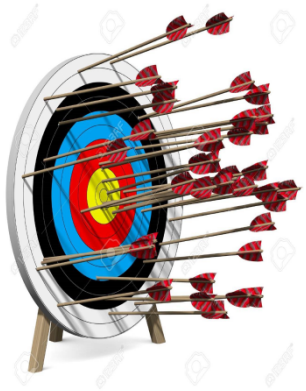
Next-Generation Sequencing

- Considerations
  - Cost of in-house NGS vs single gene assays and send-out testing
  - Throughput vs single gene assays
  - Provider needs
  - Guideline changes both current and future



NGS

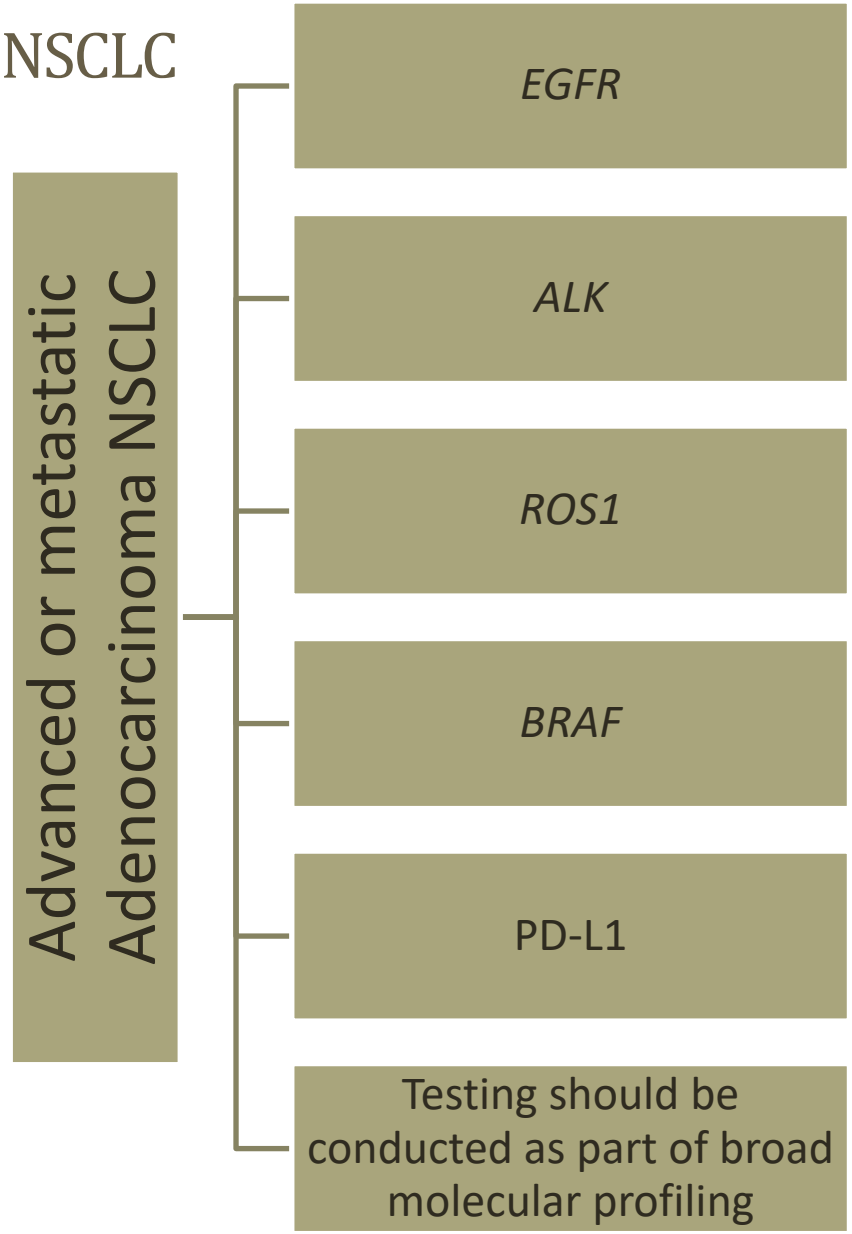
- Broad
- High Throughput
- Highly multiplexed
- Expensive, but low cost per gene
- DATA
- Bioinformatics experience needed
- Long workflow, although shorter than serially testing genes
- Analytical and clinical interpretation required



PCR-based assays

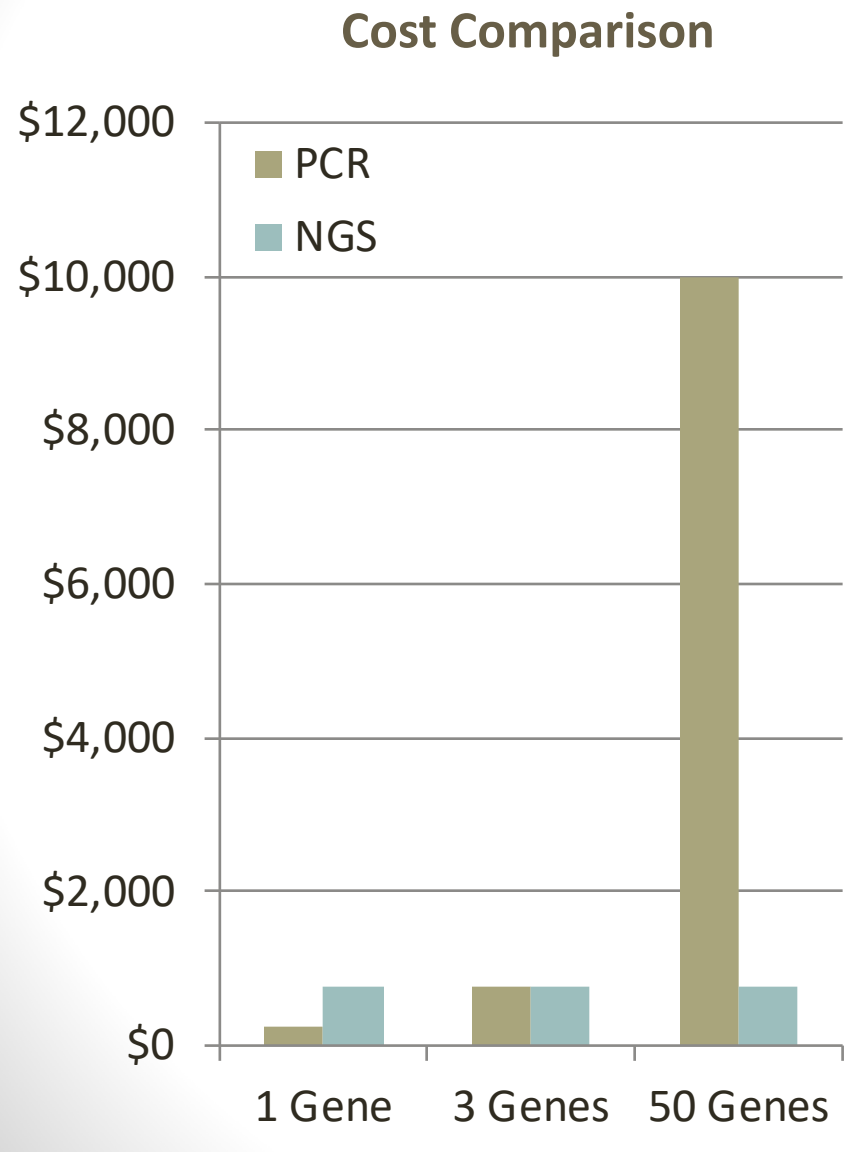
- Very targeted
- Quick
- Inexpensive
- Less experience required
- Ideal for single gene hotspot analysis
- Data interpretation is clear

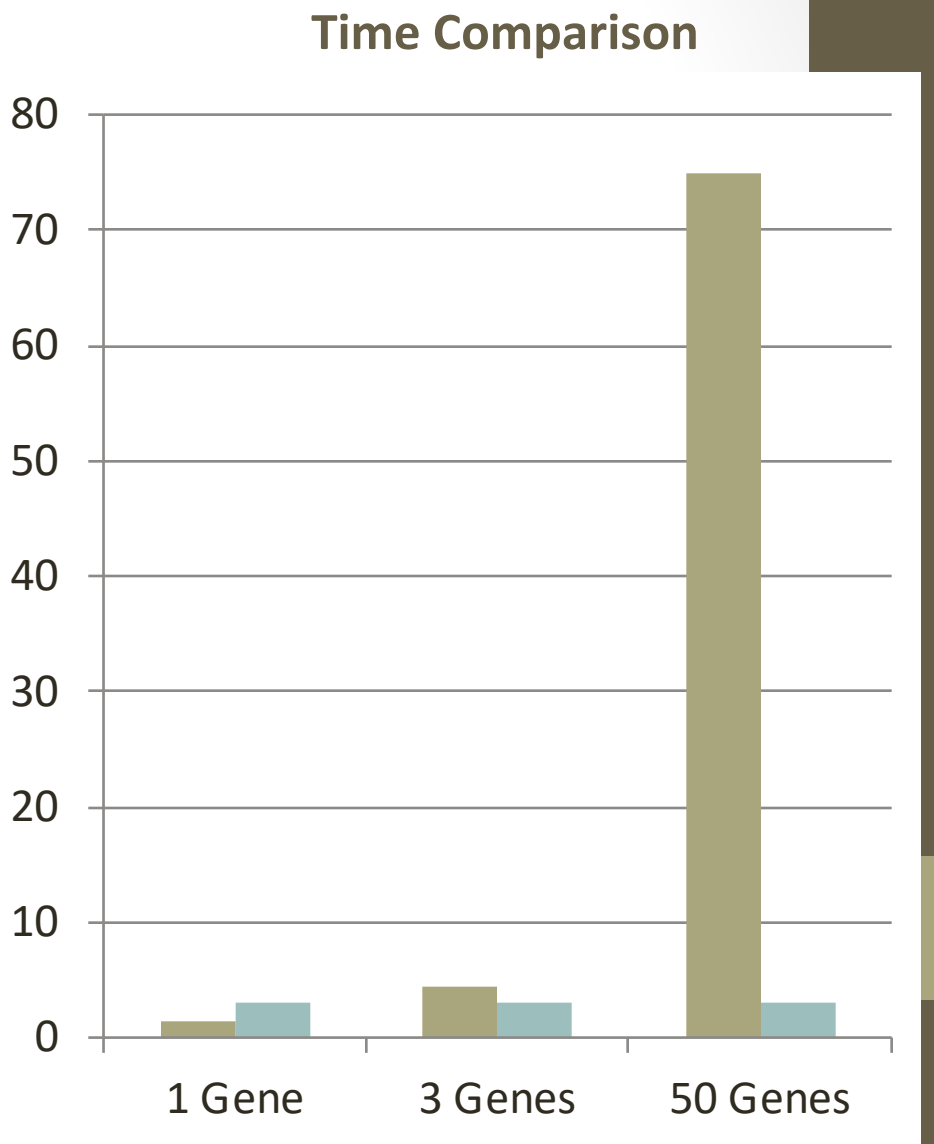
NCCN v2.2019 NSCLC



Version 2.2019, 11/21/18. National Comprehensive Cancer Network, Inc.

NGS Efficiencies





We decided insourcing NGS was the right thing to do for our health system.

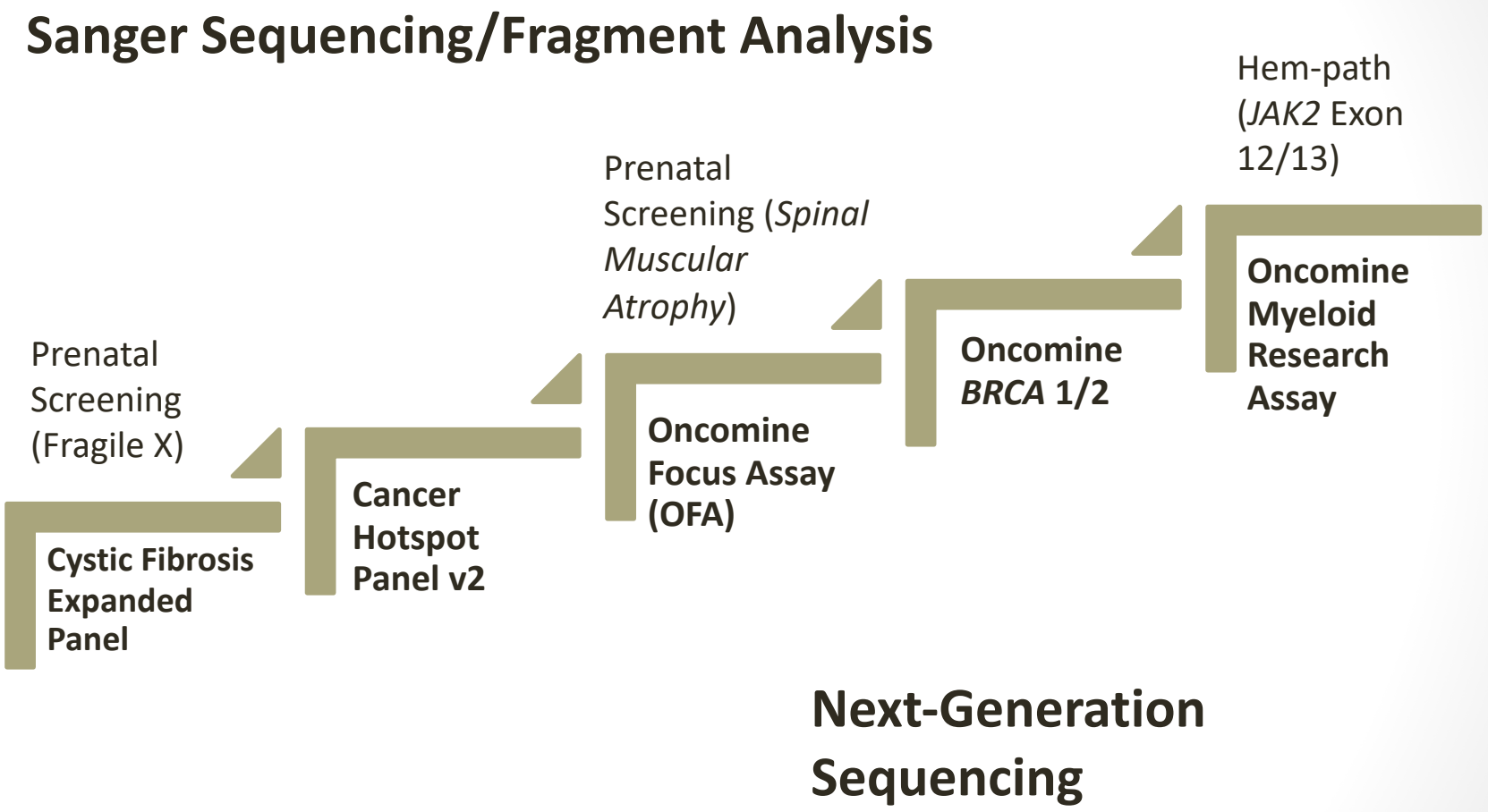
Choosing the Right Platform

- Vendor selection criteria
  - Accuracy
  - Throughput
  - Ease of workflow
  - Test menu alignment with our needs
  - Cost per sample
  - Cost of instrument
  - Reporting capabilities
  - Support after the sale
    - Instrument service
    - Bioinformatics
- Ultimately the Ion S5/Ion Chef workflow was the best fit for our organization.

Funding the Project

- We typically have one capital funding source for all laboratory equipment for our health system.
- We have an alternate funding source (strategic capital) outside of the laboratory funding source if the project meets certain criteria.
  - A minimum dollar amount
  - Must be cutting-edge and give our health system a strategic advantage
  - Has to be presented to the board for approval
- We created a project to increase the sequencing capabilities of our laboratory (NGS & Sanger sequencing) to meet the thresholds for strategic capital.

Test Menu Pipeline



Business Case

- The business case showed that it was favorable to insource this testing versus paying to send-out to a our reference laboratory (37.6% internal rate of return).
  - Cost per reportable (tech time, repeat rate, control cost, validation cost, QA cost)
  - Instrument Purchases (w/depreciation)
  - Instrument maintenance
  - Construction needed for instrument
  - Did not consider lease, electrical, etc.
- The favorable business case made it easy for us to get board approval for the project.

Automated NGS Workflow

Library Prep

15 minutes hands-on

7 hours walk-away

8 samples

Templating

15 minutes hands-on

10 hours walk-away

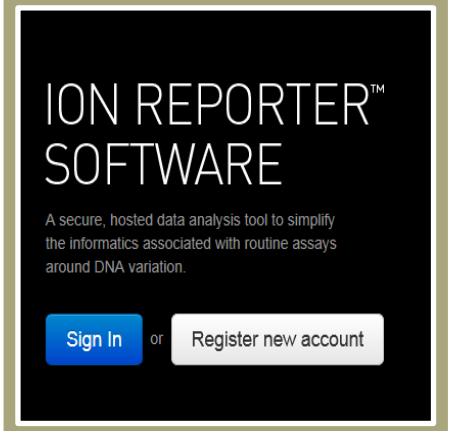
24-32 samples

Sequencing

15 minutes hands-on

3 hours walk-away

24-32 samples



Analysis

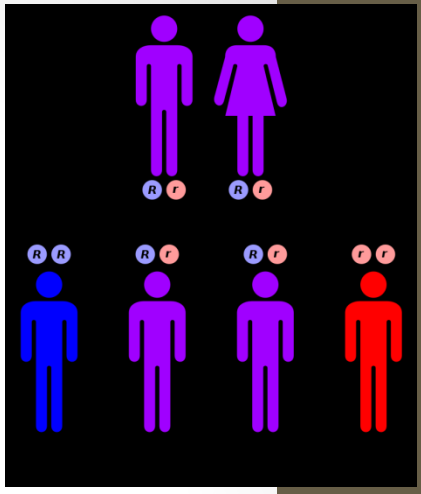
1.5 hours hands-on time

2.5 hours walk-away

24-32 samples

Day 1 → **Overnight** → **Day 2** → **Day 2**

NGS Testing



- Cystic Fibrosis Carrier Screening
  - Chosen first because:
    - High volume (30-40 per week)
    - Single gene with SNPs and Indels (least complex)
    - Needed a larger panel to match our clinicians ordering patterns.
  - CF assay design was completely customized using information on *CFTR* from CFTR2.org.
  - Use Ion Reporter for variant calling
  - Validation was complete in 3 months using our previously tested patients from Luminex and Coriell specimens.
  - All samples correlated well.
  - Based on the validation we confirm poly-T calls by Luminex in R117H positive patients.

Validations

- Cancer Hotspot Panel v2 (CHPv2)
  - Still only SNPs and Indels, 50 genes
  - Took more time optimizing the bioinformatics piece of the assay due to the somatic nature of the mutations (need better sensitivity than germline mutations).
  - Also had to chose a vendor for reporting.
    - Variant reporting, clinical trials, treatment/resistance information

Validations

- Oncomine Focus Assay (OFA)
- More Comprehensive
  - In addition to SNPs, MNVs, and INDEL mutations we had to validate RNA fusions and DNA copy number variants (CNVs)
    - More complex with RNA and DNA
    - Harder to source standards/positive patients due to low prevalence
  - Reevaluated reporting software to choose optimal platform that was capable of analyzing the addition of CNVs and Fusions.

New Panel: Oncomine Focus Assay

Hotspot genes, n=35

AKT1	IDH2
ALK	JAK1
AR	JAK2
BRAF	JAK3
CDK4	KIT
CTNNB1	KRAS
DDR2	MAP2K1
EGFR	MAP2K2
ERBB2	MET
ERBB3	MTOR
ERBB4	NRAS
ESR1	PDGFRA
FGFR2	PIK3CA
FGFR3	RAF1
GNA11	RET
GNAQ	ROS1
HRAS	SMO
IDH1	

Copy Number Variants, n=19

ALK	FGFR3
AR	FGFR4
BRAF	KIT
CCND1	KRAS
CDK4	MET
CDK6	MYC
EGFR	MYCN
ERBB2	PDGFRA
FGFR1	PIK3CA
FGFR2	

DNA Panel

Fusion drivers, n=23

ALK
RET
ROS1
NTRK1
NTRK2
NTRK3
FGFR1
FGFR2
FGFR3
MET
BRAF
RAF1
ERG
ETV1
ETV4
ETV5
ABL1
AKT3
AXL
EGFR
ERBB2
PDGFRA
PPARG

RNA Panel

52 unique genes

269 amplicons in DNA panel, 272 amplicons in RNA panel

Oncomine Knowledge Reporter (OKR)

- Best-in-class interpretation
- Performed with a cloud-based software
- Much faster to generate a report
  - Reduced data analysis time from 20 minutes per case to 5 minutes per case.
  - Saves 5 hours per week of tech time!
- Clear and concise report
- Flexible to meet Oncologist's needs
- Affordable

Current Validations

- Oncomine BRCA 1/2 Research Assay – 3 to 6 months from go-live
  - Two gene, two pool DNA panel
  - SNPs, INDELS, AND Large Genomic Rearrangements (LGRs)
    - LGRs span exon deletion/duplications, large INDELS, etc.
    - Samples sourced within one week by data mining our hospital networks EMR.
    - Commercial reference standards and patient DNA readily available.
    - Workflow optimized for automation from nucleic acid recovery to data analysis.
    - Reporting platform already selected.

Current Validations

- Oncomine Myeloid Research Assay
  - Have begun the validation on this assay.
  - Larger panel with fusions.
  - Panel optimized for nucleic acid extracted from fresh peripheral blood and bone marrow samples. FFPE embedded samples not recommended.
  - Commercial reference standards available.

Questions