Genomics Reimagined in a Reference Laboratory Setting

Hunter Best, Ph.D.

Medical Director, Molecular Genetics & Genomics

Associate Professor, University of Utah Department of Pathology

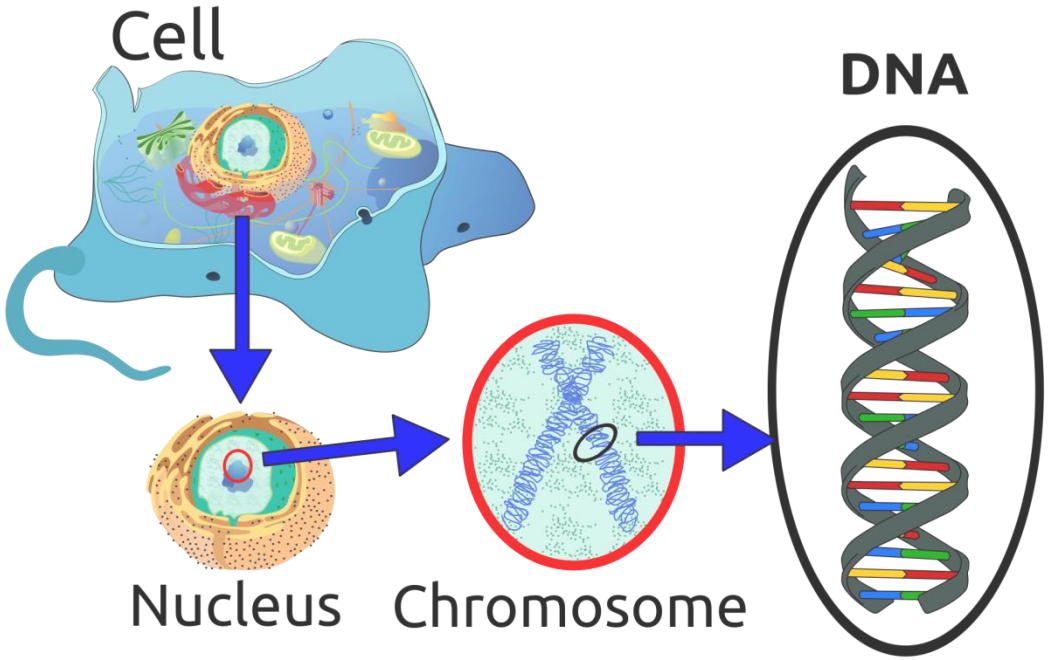
Learning Objectives

1. Understand massively parallel sequencing (MPS) and the role that it plays in clinical diagnostics
2. Describe problems commonly encountered by clinical laboratories during implementation of MPS-based testing
3. Describe the steps that can be taken to streamline MPS clinical workflows

Learning Objectives

1. **Understand massively parallel sequencing (MPS) and the role that it plays in clinical diagnostics**
2. Describe problems commonly encountered by clinical laboratories during implementation of MPS-based testing
3. Describe the steps that can be taken to streamline MPS clinical workflows

The Role of DNA in Diseases



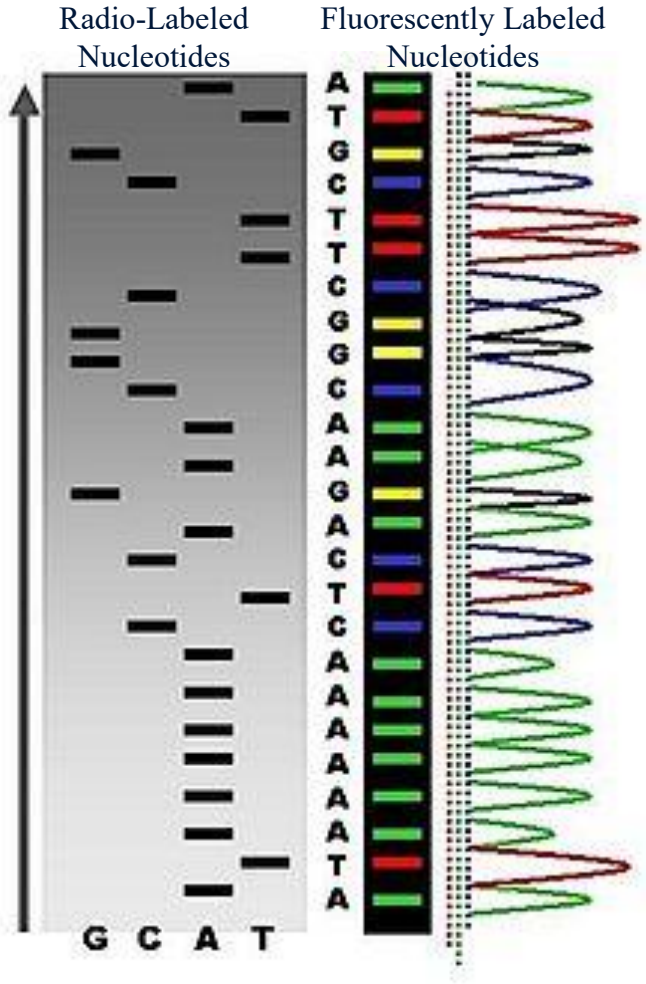
Disease-causing Variants: Variation in DNA sequence that cause aberrant activation or loss of different genes

Germline: Inherited variations obtained from parents that increase risk of developing cancer, developmental diseases, etc. (*CFTR*, *BRCA*, *TP53*, *APC*)

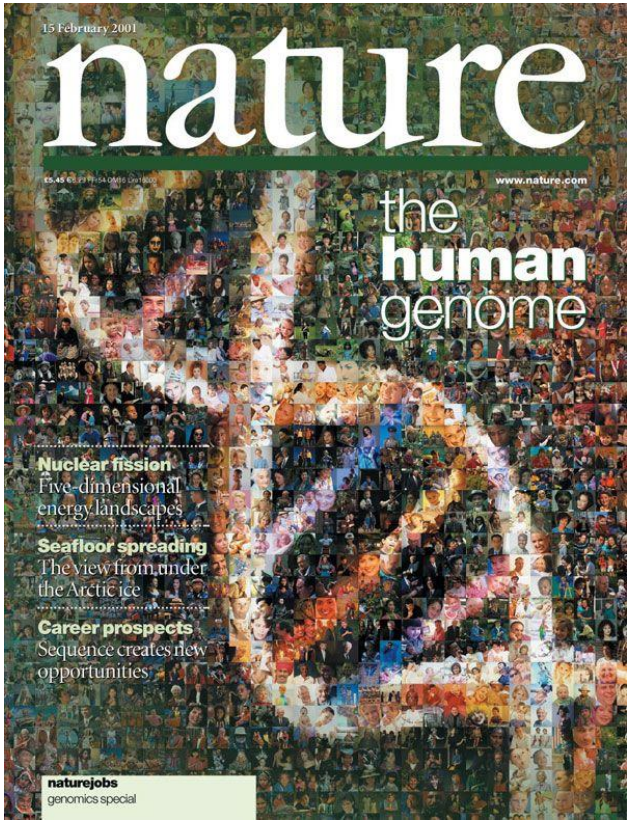
Somatic: Variations that sporadically occur caused by mistakes during cell division & carcinogen exposure (smoking, chemicals, alcohol, radiation, etc)

Basics of DNA Sequencing

- **Sanger Sequencing**
  - Developed in the 1970's
  - Originally used radio-labeled chain-terminating dideoxynucleotides
    - Cytosine (C)
    - Guanine (G)
    - Adenine (A)
    - Thymine (T)
  - sequencing gels
- **Automated capillary electrophoresis**
  - Fluorescently labeled dideoxynucleotides
  - Largely used for sequencing of fragments between 300-1000 bp
  - ABI 3730 capable of generating 1-2 Mb of sequence per day



Human Genome Project

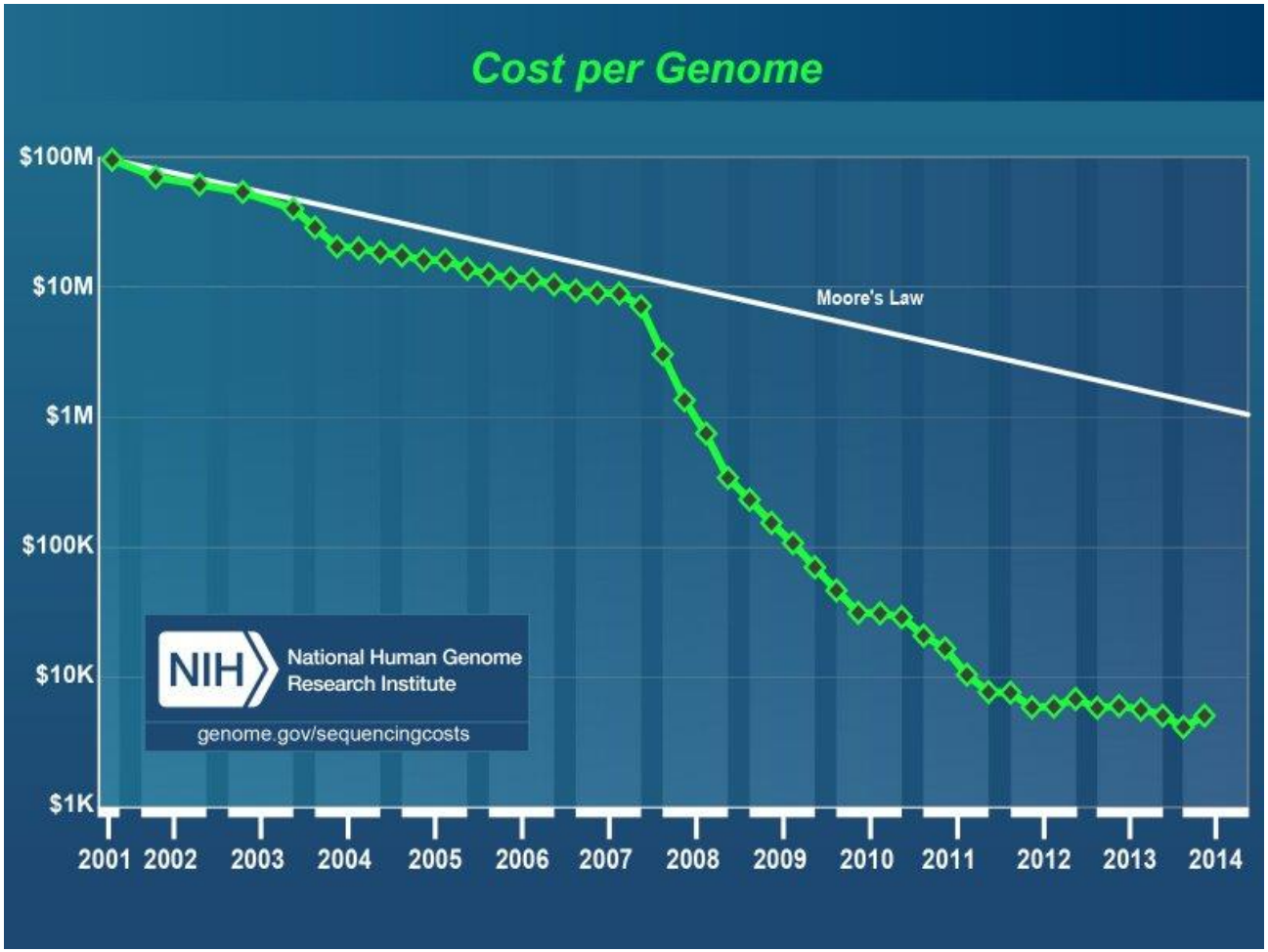


- **Started in 1990**
  - Goal to sequence the ~3,000,000,000 bp human genome
  - 20 institutions across 6 countries
  - Sanger sequencing methodology
  - Cost ~3 billion dollars
- **Completed in 2003**
  - 22,300 protein coding regions in the genome
- **Demonstrated that there was a need to develop high throughput, cheaper and faster DNA sequencing technologies**

Massively Parallel Sequencing (aka Next generation sequencing (NGS))

- Rapid and cost effective method for determining the sequence of millions of DNA molecules simultaneously
- Instruments can fit on a desktop
    - Illumina
    - IonTorrent
- Requires complex and powerful computing processes for data analysis
    - Anyone can generate data; analysis and interpretation are typically the bottlenecks

Impact of Massively Parallel Sequencing



2005: Roche 454

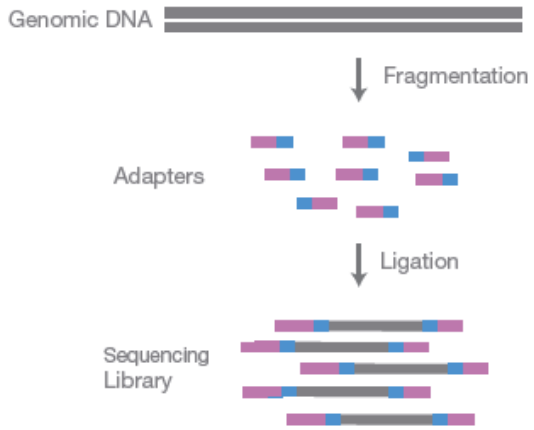
2006: Genome Analyzer

2010: Illumina HiSeq

2011: Ion Torrent PGM

MPS (Illumina) sequencing overview

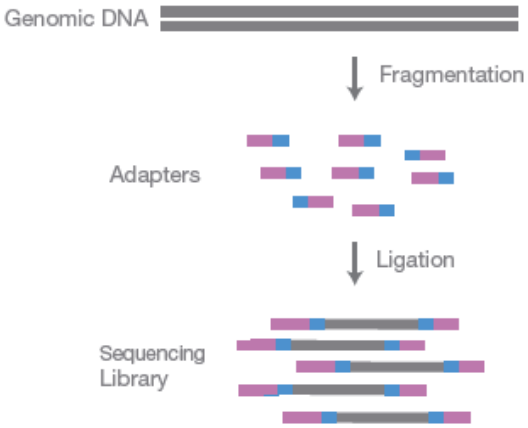
A. Library preparation



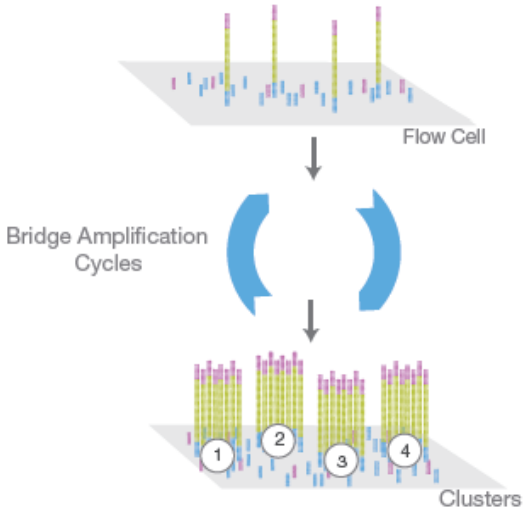
www.illumina.com

MPS (Illumina) sequencing overview

A. Library preparation



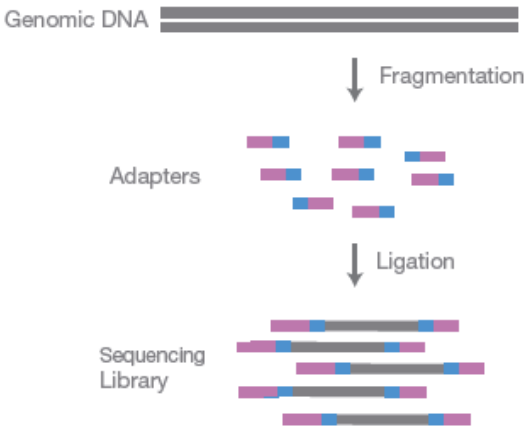
B. Cluster amplification



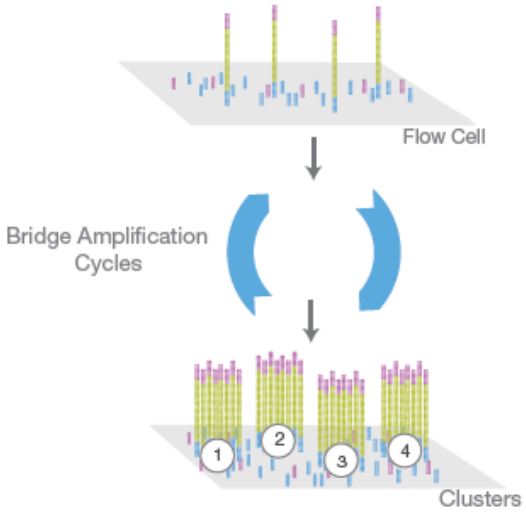
www.illumina.com

MPS (Illumina) sequencing overview

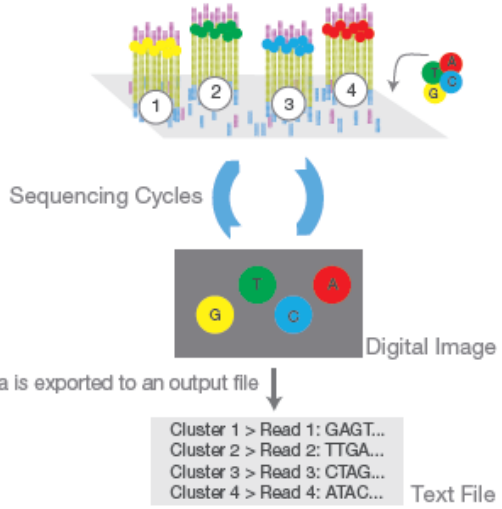
A. Library preparation

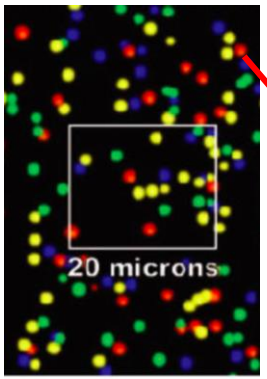


B. Cluster amplification



C. Sequencing





50-150 cycles (bases)

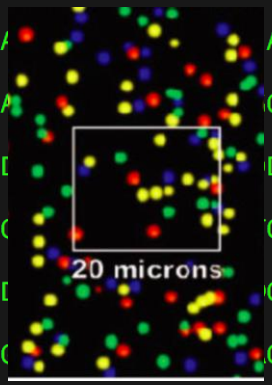
```
@JDQNNM1:207:c6ckfacxx:7:1101:1457:2221 1:N:0:TGACCA
AATTCCGCAAAAATTGATCATTTGCAAAGTCAAAACTATAGCCATATCCAAATCTTTTCCCCCTCCCAAGAGTTCTCAGTGTCTACATGTAGACTATTCCT
+
@@CDFFFFHHGHHJJIGJIIIIJIGIJHJEHIGJJJJJIJIIIJJEGHIEHJJIJJIIJJJJIIGJIJGEFHECDADDDDFEEEEEEDDCCDEDDDDEEED
```

www.illumina.com

Massively Parallel Sequencing

millions of individual sequences

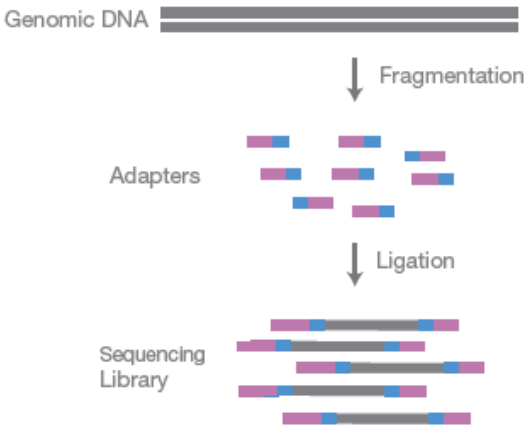
```
@JDQNNM1:207:c6ckfacxx:7:1101:1457:2221 1:N:0:TGACCA
AATTCCGCAAAAATTGATCATTTGCAAAGTCAAAACTATAGCCATATCCAAATCTTTTCCCCCTCCCAAGAGTTCTCAGTGTCTACATGTAGACTATTCCT
+
@@CDFFFFHHGHHJJIGJIIIIJIGIJHJEHIGJJJJJIJIIIJJEGHIEHJJIJJIIJJJJIIGJIJGEFHECDADDDDFEEEEEEDDCCDEDDDDEEED
@JDQNNM1:207:c6ckfacxx:7:1101:1517:2156 1:N:0:TGACCA
CCTGGTGAAAGTGGTGGGGCTGTATGGCTTCATGCTCAGCATATCGCCTCGACTCACCCTCCTTTCTCTGCTGCACATGCCCTTCACAATAGCAGCGGAGA
+
??@BDDD?FDF?DG2ABEHE<@?EFFHBABGFADGE9BBGGFDD4?DB:AAAE=ECCHH?DEC;@CCCCACC>AC@C>@CCCACCCCCCCCCC@ABB@B>B
@JDQNNM1:207:c6ckfacxx:7:1101:1893:2234 1:N:0:TGACCA
CATTTTGGTAAAGACAAATTATACAGACAGTAAAAAGTAAAAGGCAAAATTATAGAGACAGTAAAAAATCTGGAAAGGCTATATACTGTATGATTCCATAC
+
BBCFFFFFHHHHHIJJJJJJJJJJJJJJJJHIJJJJJGFHIJJIJJJJJJJJJJJJJJJJGIJIJJJIHHHHHFFFFFEEEEEEEEEEEEEDEEFFFFCD
@JDQNNM1:207:c6ckfacxx:7:1101:3454:2163 1:N:0:TGACCA
AGGGGCGATGCATTCGAACGCGTTGGGGTTCTTCTCTCGGGAGTAGATGATGGTCTCCGTCTTCTGCCTTCTGAGGAACTCATCTCTTGTGACGATATCTG
+
8?@4B<?A1<CBF?FGGGE:FBH8@FHI;;DECAEECH8BE>A(5;CDCC>@@,:A@:=@??BC>CAC>C:@:>@?:<<CCCAC>4>:4>@>@<<BBB>::
@JDQNNM1:207:c6ckfacxx:7:1101:3554:2157 1:N:0:TGACCA
AGTTGGAGGTATTGATGCATGTGTTGGTTTGCCAACATTATCTCTATGATATTGATCTCTATTTGCTTGACGGCTGCCCTTCTTAAGATCATGAATTACAA
+
1=8BDA?BB+2<<A4AFBHFIBBCACCGEFC<D?ED;1:B>??DF>?D>?BBD<DFFEI<F8BFD===3(8;B8=BBD.;;>A                    A>
@JDQNNM1:207:c6ckfacxx:7:1101:3932:2232 1:N:0:TGACCA
AGGTAGTGGCTTGACAGCTCAGGGATGTTGGTATGGGTCTCGTTAATCCAATTAACAAACCAGGCAATACTTTCACACGTGCA                    CG
+
@@CADEEFHHHHHHIGGGGHGIIJBFGEGEH?FCGIJDFFGEGHGHEBHGHIJIIGHHJCGGGIHFHFFFFFFFFCECDBBDD                    DB
@JDQNNM1:207:c6ckfacxx:7:1101:4020:2157 1:N:0:TGACCA
GTCTCACCCTCAGCACACTGGCAGACTTCAGCAGAACACAAGGTGGCCAAGAGTCTGCTCTTACTTGGTGCCCCGTAAAACAC                    GG
+
@BBDFFFFFHHHHJJJJJJJIJJJGIJJJIIJJIJJIJJIJGIDFFCHHIIIJCHGIIIIGGIJIIHFHEHHFFDDCDDDDDD                    CC
@JDQNNM1:207:c6ckfacxx:7:1101:4229:2203 1:N:0:TGACCA
TATAGAGGAACCCTAAAGTGGGGTCCATTGTTCCGGGGGCTGGAGCTGTCCCCGGTGCTGGAAGACTCAGCAGATGGGGGGTC                    GC
+
CCCFFFFFGHHHHJIJJJAFHHID@FAHIJGHIIJJJIJHEDFBCEDEDDDDDDD@BDDDCACDDDCDDDDDDDCCCDDD@09<BDDDD@BDDCDBDBDDD
```



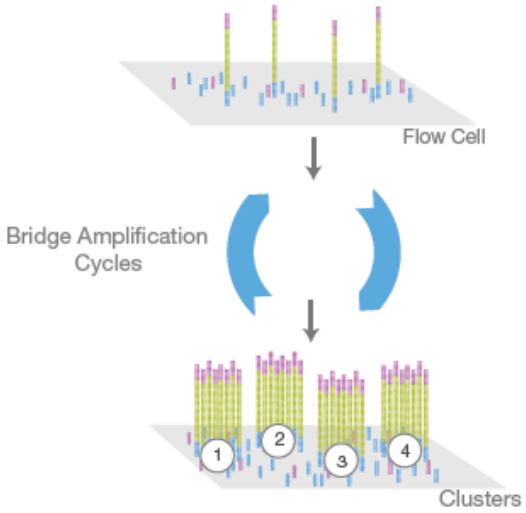
Millions of sequencers sequencing in parallel!

NGS (Illumina) Sequencing Overview

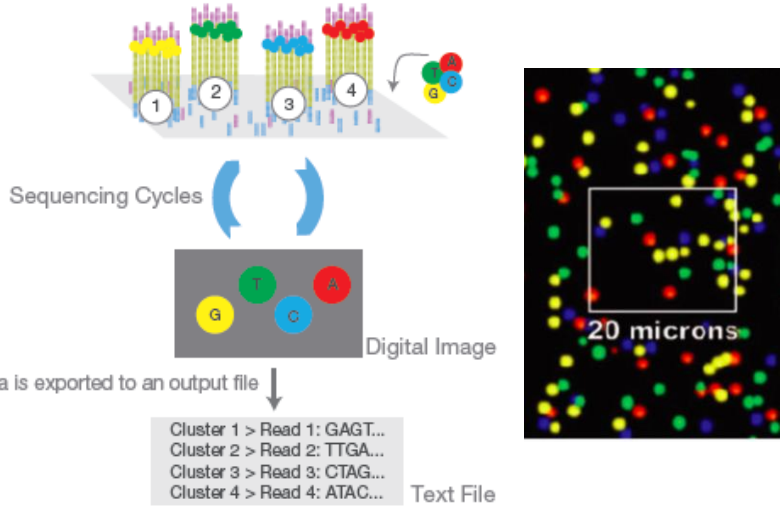
A. Library preparation



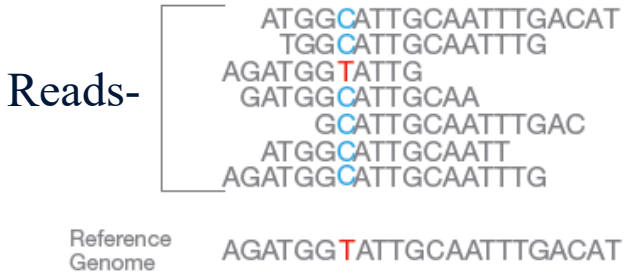
B. Cluster amplification



C. Sequencing



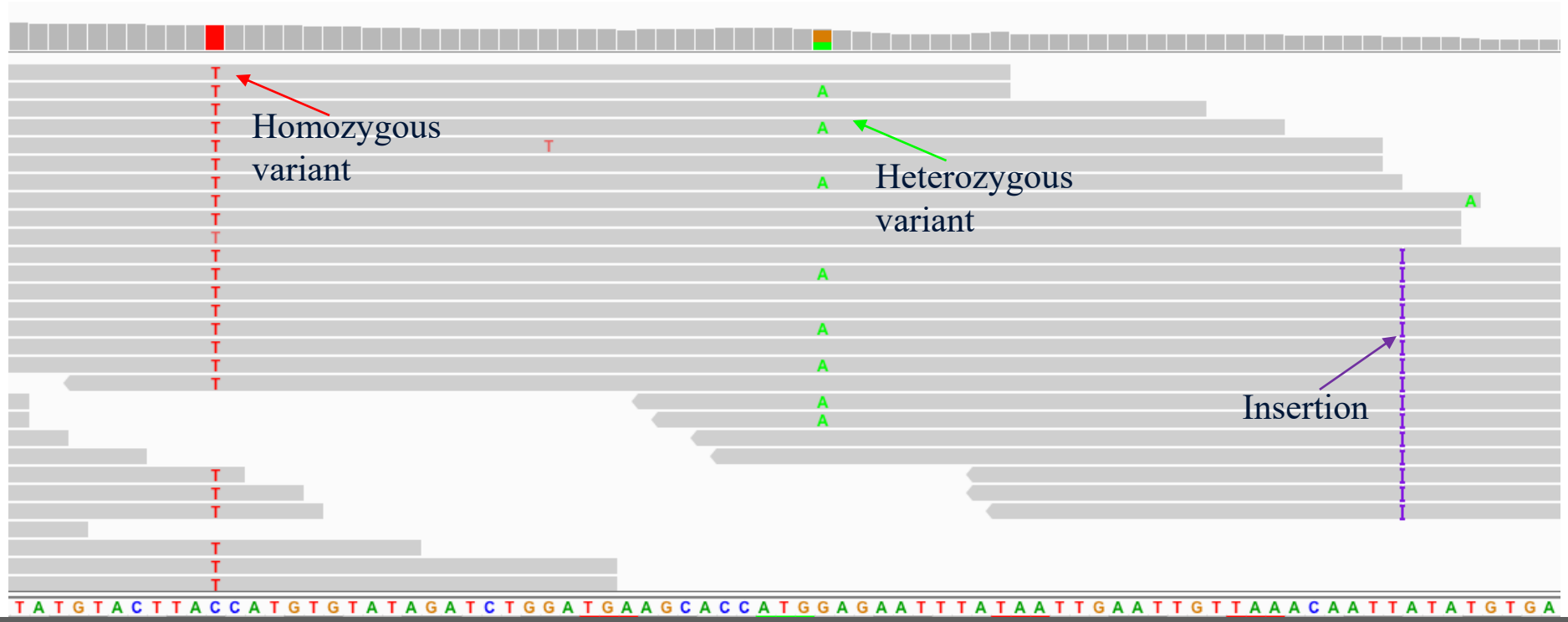
D. Bioinformatics:
Alignment, variant calling and analysis



www.illumina.com

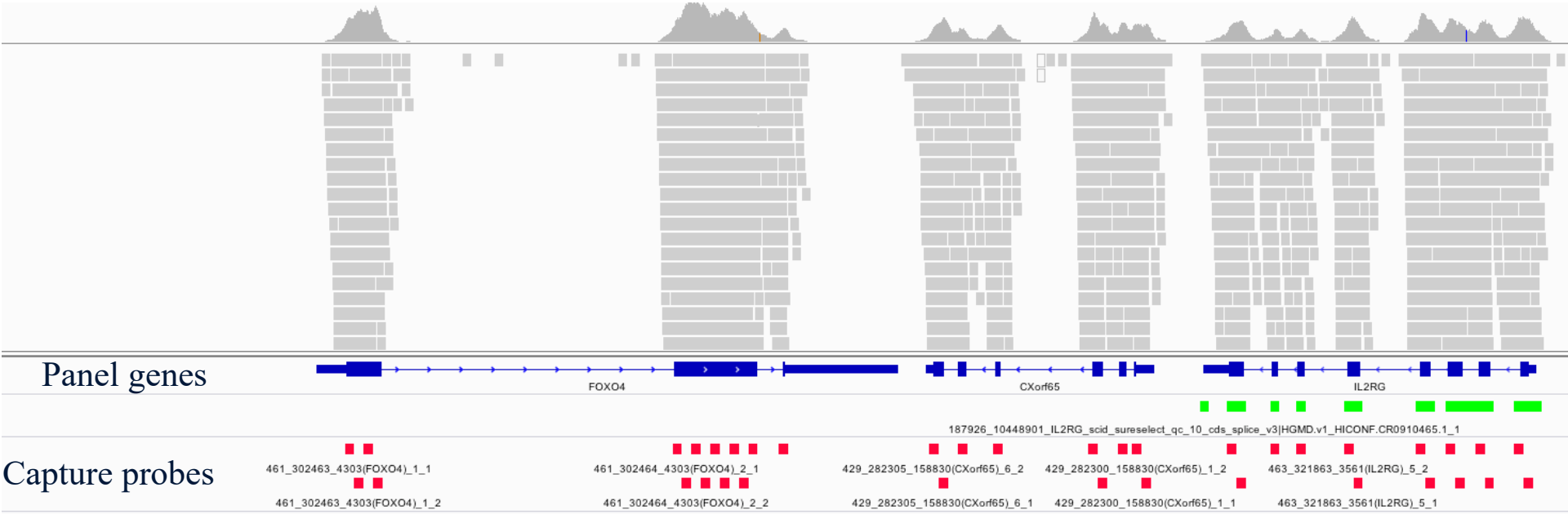
Bioinformatics Data Processing

- Bioinformatics ‘pipeline’ produces variant calls
- Aligns reads to a reference sequence, calls variants, then annotates the variants
-



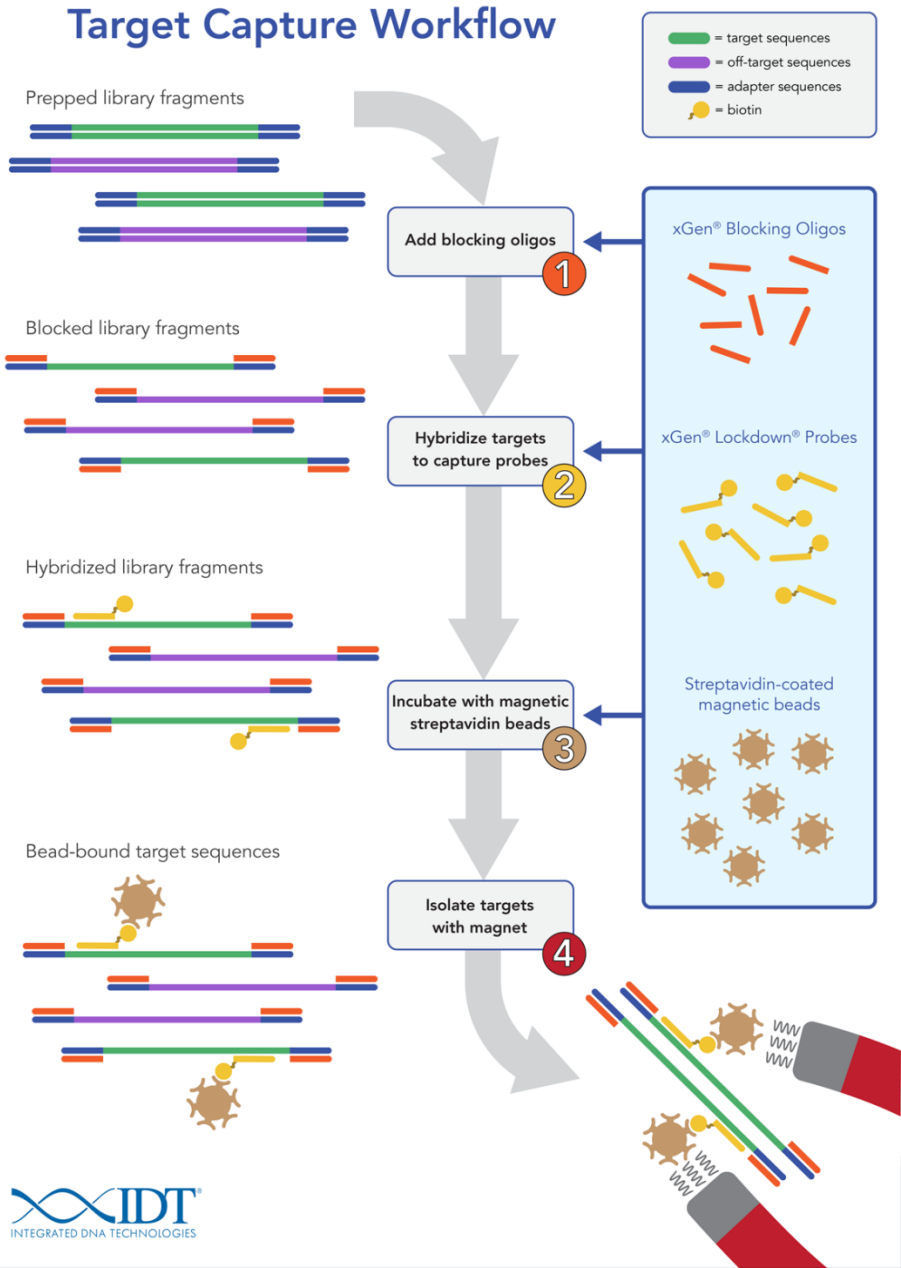
Common MPS Testing Terminology

- ***Capture*** = Probes targeting genes of interest
  - Typically targets only the coding regions (exons) of genes or noncoding regions with known clinical significance
- ***Panels*** = Subset of genes within a capture typically clustered by clinical features (disease, phenotype, etc.)



Targeted Hybrid Captures

- Biotinylated ‘baits’ designed to cover regions of interest only
- Target regions hybridized to baits in liquid phase
- Regions of interest are eluted using streptavidin magnetic beads
- Only the DNA sequence that is eluted with baits is sequenced
- From 1 to >20,000 genes can be targeted



Massively Parallel Sequencing Workflow in Three Basic Steps

Clinical laboratory "wet work" → Bioinformatics analysis → "Variant" annotation and interpretation

Common Challenges
-Physical space
-Large capital investment
-Standardization of workflow

Common Challenges
-Large capital investment
-Recruitment of talent
-Standardization of workflow

Common Challenges
-Difficult to automate
-Difficult to scale

Why use MPS in a clinical lab?

- Traditional testing modalities (e.g. Sanger sequencing) require you to look at a single gene at a time
  - For disorders with numerous causative genes (such as Retinitis Pigmentosa) the cost of determining the underlying genetic cause by traditional molecular testing routinely exceeds $10k
  - Time to diagnosis is usually prolonged and, in some cases, can take several years
- MPS allows for the simultaneous testing of many targets in a single test
  - All known causes of a disorder with allelic heterogeneity can be tested in a single assay
  - Also useful in targeting hotspot regions for somatic changes in cancer patients
- For inherited disease testing the introduction of MPS into the clinical laboratory has dramatically reduced the cost of testing and time to diagnosis for a number of disorders
- For somatic disorders the use of MPS has significantly decreased molecular testing costs and helps to tailor care of cancer patients

Common Types of MPS Tests

- Single gene tests
  - Ex. BCR-ABL1 mutation testing
- Panels
  - Typically a group of genes associated with a common phenotype (i.e. Inherited Breast Cancer, Myeloid Malignancies)
- Exome Sequencing
  - Coding sequences of genes (exons) only
  - Does include intron/exon boundaries
- Whole Genome sequencing
  - Entire genome

Distinctions Between Germline and Somatic MPS Testing

Germline

- Tests for variants in DNA common to all diploid cells
- Allelic ratios = 50% or 100% (heterozygous or homozygous)
- Sample type is typically blood
- Rare variants, SNVs and small insertion/deletions
- Expansive number of genes
- Extremely large number of probes to analyze

Somatic

- Tests for variants in a subset of cells
- Any allelic ratio is possible ranging from < 1 – 100%
- Challenging sample types (FFPE)
- Known/common variants, amplifications/translocations
- More limited gene set
- Moderate numbers of probes

ARUP Genomics Overview

- Massively parallel sequencing-based clinical testing has been available at ARUP for 5+ years
- ARUP was one of the first reference labs to offer clinical MPS
- We offer testing for germline disorders, solid tumor oncology and hematological malignancies
  - Current offerings include ~25 germline tests (including exome), 1 Solid Tumor hotspot test and 2 heme malignancy tests
  - All of our current tests are internally developed

Learning Objectives

1. Understand massively parallel sequencing (MPS) and the role that it plays in clinical diagnostics
2. **Describe problems commonly encountered by clinical laboratories during implementation of MPS-based testing**
3. Describe the steps that can be taken to streamline MPS clinical workflows

MPS Common Hurdles – Lab Standardization

- Consolidation of workflows to a single chemistry and sequencing platform is often difficult
  - Many labs implement kit based assays in order to quickly get into genomic testing
  - This often works for one area (e.g. solid tumor testing) but may be difficult to implement in others (e.g. germline gene panels)
  - This sometimes requires that multiple sequencing platforms be introduced
  - MPS workflows are highly complex and it is very difficult to automate processes that are not standardized

MPS Common Hurdles – Informatics and IT

- MPS generates an ***enormous amount of data*** and few labs already have infrastructure in place to handle the increased computing demands
  - In most cases implementation of MPS requires improved networking capability – **a single sequencing run can generate anywhere from several gigabytes to several terabytes of data**
  - Demultiplexing, alignment and annotation of data usually requires individuals with specialized training in bioinformatics
- It is possible to use sequencing platforms with onboard informatics but these typically still require significant networking and/or server maintenance infrastructure
  - Use of these systems also requires constant tracking of software updates. Lack of notification from vendor can result in testing discrepancies

MPS Common Hurdles – Analysis and Interpretation

- MPS generates much more data than traditional testing modalities requiring a significant time investment for interpretation
  - A large gene panel (>20 genes) routinely generates more than 100 variants that need to assessed for clinical significance
  - Exome sequencing generates ***thousands*** of variants
  - Interpretation of somatic and germline testing is very different and requires individuals with specialized training

ARUP's Experience With Common MPS Hurdles – Lab Standardization

- In order to initially enter the genomic testing space quickly, multiple technical approaches were taken by individual specialty areas
  - At one point our genomics laboratory was running tests using five different chemistries across several sequencing platforms (Illumina, IonTorrent)
- We were also using multiple sequencing systems within individual manufacturers
  - At one point our laboratory was running the Illumina NextSeq 500, the Illumina MiSeq, the Illumina HiSeq 2500 and the IonTorrent PGM
- This resulted in prolonged turnaround times, a challenging work environment for the genomics clinical lab staff, and concerns about maintaining quality for the multiple workflows

ARUP's Experience With Common MPS Hurdles – Informatics and IT

- Lack of standardization of laboratory workflows also resulted in a lack of standardization of our informatics processing
  - We essentially had individual bioinformatics processing pipelines for every test
  - This clearly was not scalable or sustainable in the long term
- Lack of cloud-based computing solutions meant that onsite computing requirements were substantial
  - Resulted in long processing times for individuals samples and that samples were serially analyzed
  - No real capability to do parallel sample processing

ARUP's Experience With Common MPS Hurdles – Analysis and Interpretation

- Rapid growth in the area of genomics highlighted our lack of scalable infrastructure for interpretation and reporting
  - Existing processes used in the interpretation of our Sanger tests were not directly transferrable to genomic testing
  - Medical directors spent hours investigating the higher volume of variants identified by gene panels
  - Turnaround time was negatively impacted

Learning Objectives

1. Understand massively parallel sequencing (MPS) and the role that it plays in clinical diagnostics
2. Describe problems commonly encountered by clinical laboratories during implementation of MPS-based testing
3. **Describe the steps that can be taken to streamline MPS clinical workflows**

Focus on Standardization and Scalability

- For the last few years ARUP has been focused on reorganizing our genomics lab to focus on standardization and scalable processes
- This overhaul has been focused on:
  - Consolidating all of our testing to a single chemistry and sequencing platform
  - Reworking our bioinformatics pipeline such that it is state of the art and cloud based
  - Implementing full automation into the clinical lab
  - Standardizing analysis and interpretation

Laboratory Chemistry Standardization

Multiple Workflows in Germline and Oncology MPS Testing →

- One capture for germline testing
- One capture for somatic testing
- One capture for exome

Laboratory Chemistry Standardization

- 3 captures and one laboratory workflow
  - Each of our previous captures targeted < 300 genes

	Germline Captures		Somatic Capture
	Exome	Targeted	
# Genes	>18,000	~5000	~700 + intronic
Capture Size	39-57 Mb	14-18 Mb	6 Mb
# Probes	>400,000	>130,000	50,000

All genes, not all clinically relevant

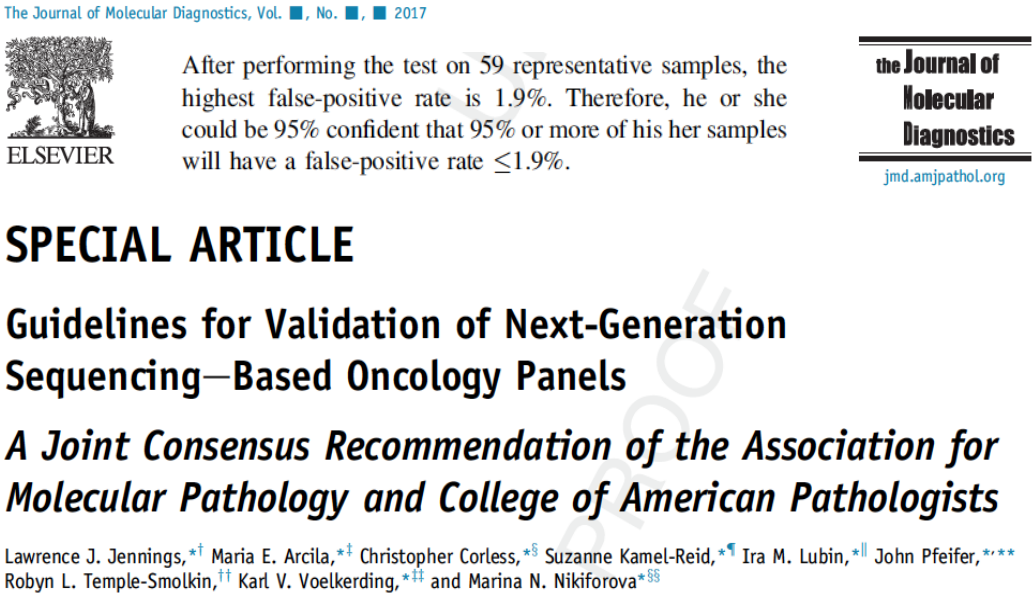
Disease associated genes

Benefits of Consolidating Capture Chemistry

- Significant efficiency gains in the laboratory by reducing workflow complexity
  - Regardless of the germline or somatic panel test order a single capture is performed by the laboratory
    - Data from genes not ordered is masked bioinformatically
  - Single chemistry allows for an easier automation build and therefore improves scalability
- Increased flexibility in test builds as a single validation is performed for the overall capture
  - New panel test additions are essentially ready at any time
  - Also allows for custom panel builds and single gene testing

Proper Validation of MPS-based Testing

- Variant types/classes
  - Single Nucleotide Variants (SNVs)
  - Small Deletions
  - Small Insertions
  - Large Deletions
  - Large Insertions
  - MNVs –multiple nucleotide variants (indels)
  - Translocations
  - Copy Number Variants (CNVs)
- Performance can (and usually does) vary for each variant class
- Variant classes need to be treated independently in validation
  - ***Hundreds of samples are required***



The Journal of Molecular Diagnostics, Vol. ■, No. ■, ■ 2017

ELSEVIER

After performing the test on 59 representative samples, the highest false-positive rate is 1.9%. Therefore, he or she could be 95% confident that 95% or more of his her samples will have a false-positive rate ≤1.9%.

the Journal of Molecular Diagnostics
jmd.amjpathol.org

SPECIAL ARTICLE

Guidelines for Validation of Next-Generation Sequencing–Based Oncology Panels

A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists

Lawrence J. Jennings,*† Maria E. Arcila,*‡ Christopher Corless,*§ Suzanne Kamel-Reid,*¶ Ira M. Lubin,*‖ John Pfeifer,*,** Robyn L. Temple-Smolkin,†† Karl V. Voelkerding,*‡‡ and Marina N. Nikiforova*§§

Why Not Just Run Exome on All Samples?

- Cost: 5x more sequencing for germline samples and at least 50x more for Oncology samples
  - Number of samples that you can pool goes down significantly due to the size of the exome capture
- Cost: Probe cost is significantly more for exome
- Very little flexibility in design
  - Careful probe design is required to cover all genes appropriately for targeted clinical panels

	Germline Captures		Oncology Capture
	Exome	Germline	Oncology
# Genes	>18,000	~5000	~700 + intronic
Mb	39-57 Mb	14-18 Mb	6 Mb
# probes	~450,000	~130,000	~35,000

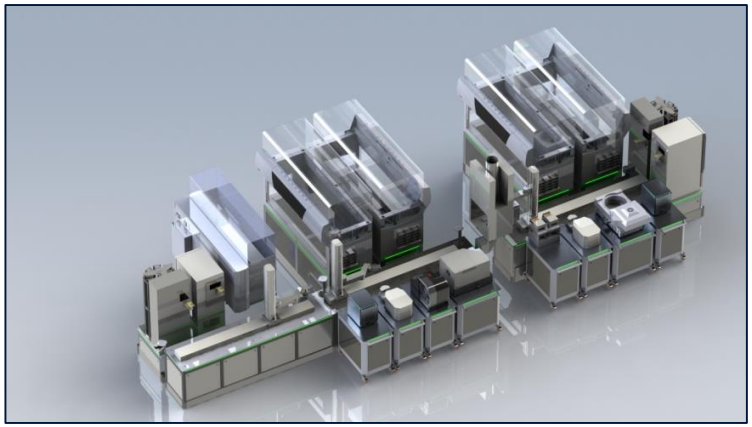
All genes, not all clinically relevant

Only disease associated genes tested

Full Lab Automation

Full Lab Automation: A Multi-Phase Process

- Automation is critical component of scalability and error reduction
- Our ultimate goal is to move to a fully automated workflow in our genomics lab
- As full automation is a large undertaking we have approached automation as a multi-phase process



Lab Automation
Phase 1: Manual to Semi-automation

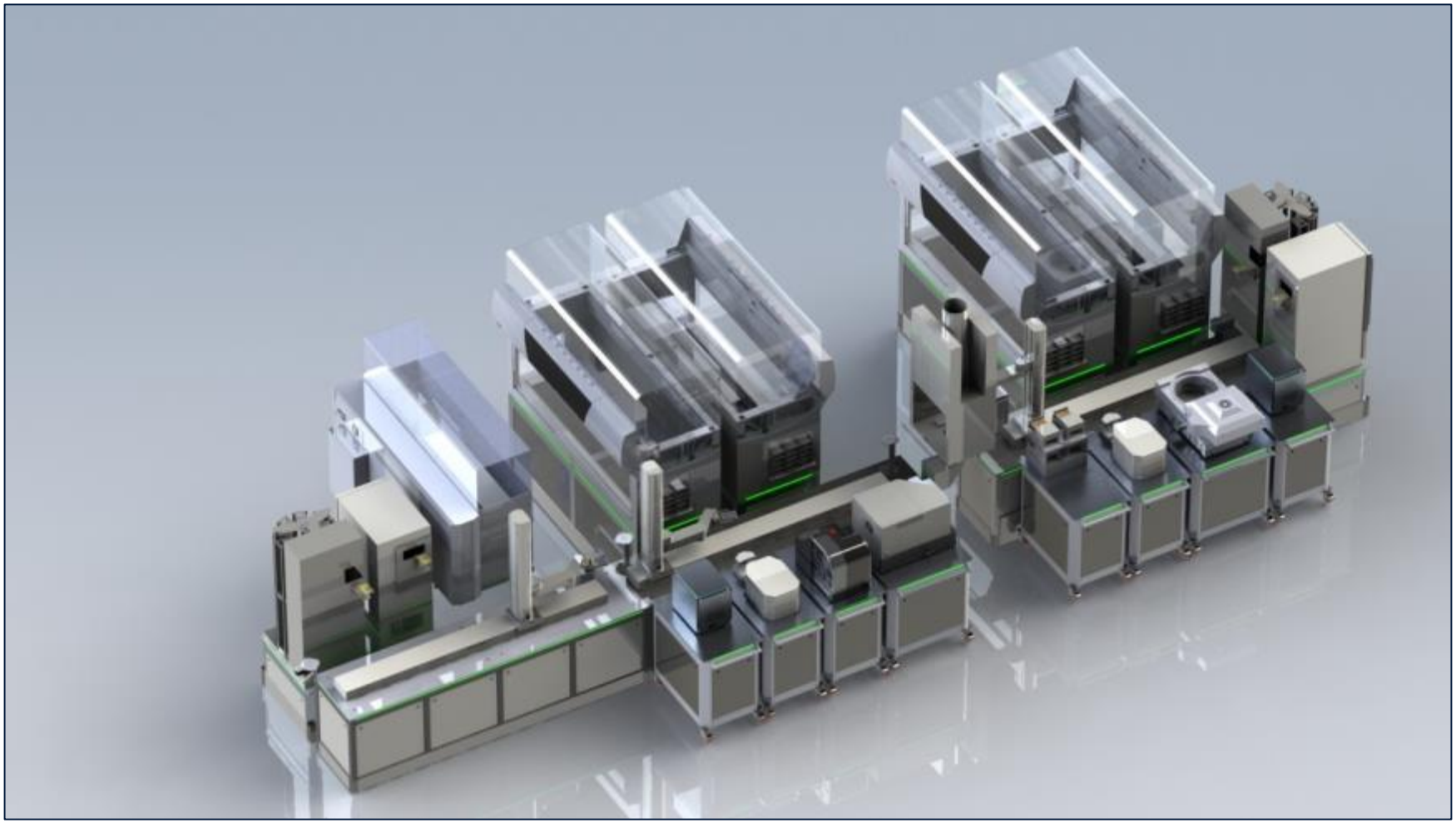
- Agilent Bravo B Liquid Handler
- Semi-Automated process
  - Library Preparation to Capture/Wash
  - Improves consistency between samples/preps
- Sample switching improvements
- Semi-scalable and robust

Lab Automation
Phase 1: Manual to Semi-automation

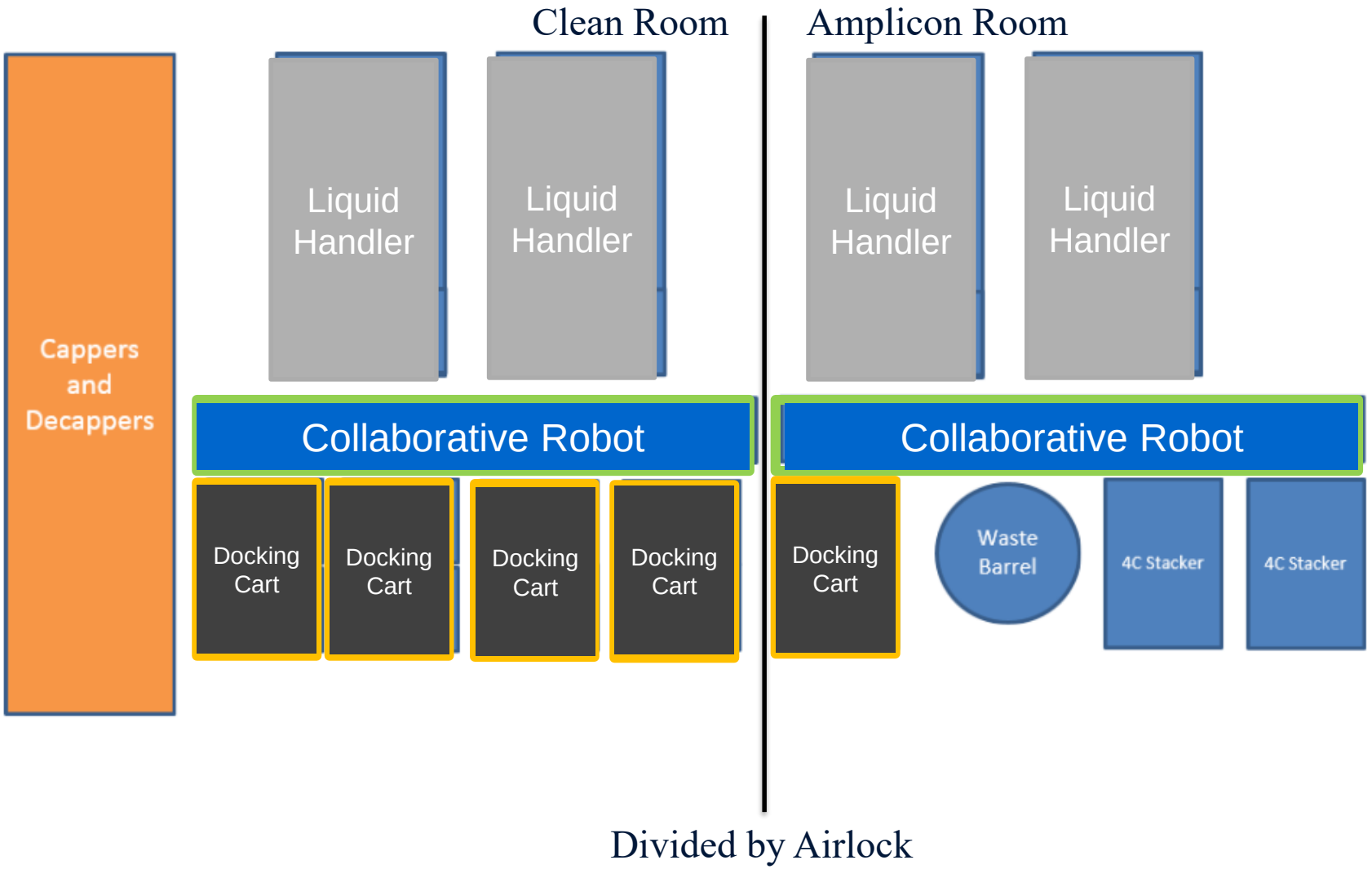
Disadvantages:

- Not true walk-away automation
- Limited scaling
- Poor LIMS/scheduling integration
- Limited workflow customizability/flexibility

The Next Phase: NGS Automation Work Cell

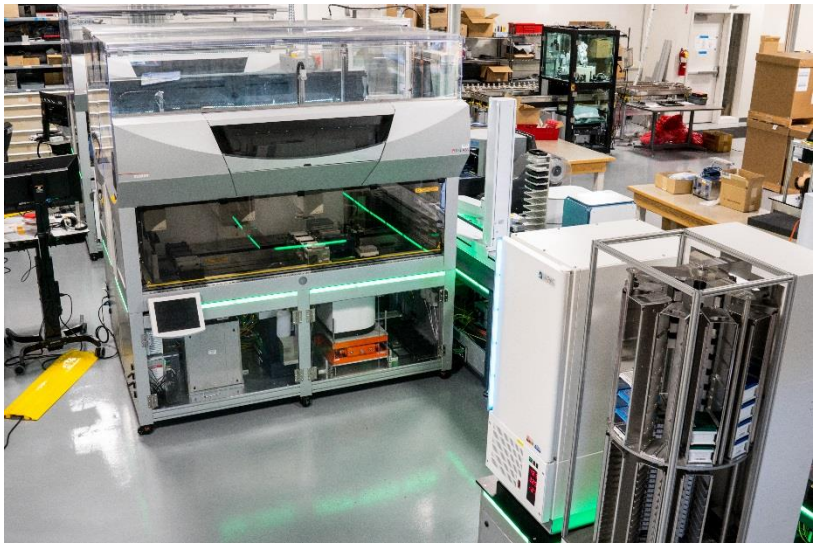


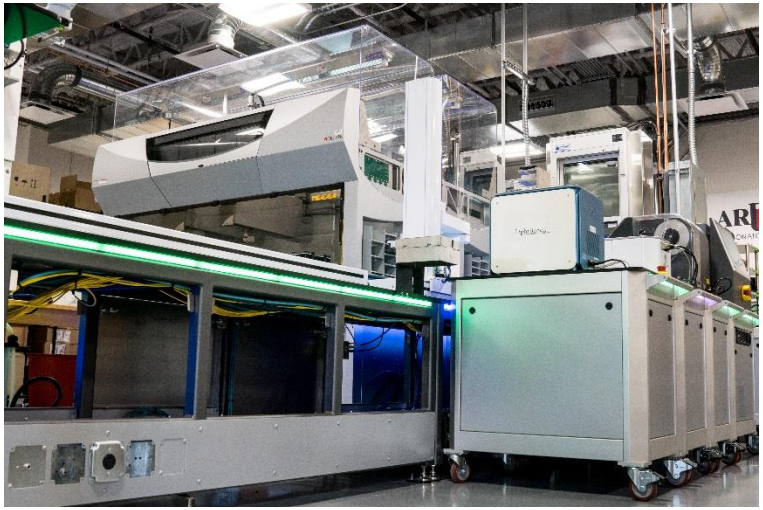
Automation Workcell Concept



Automation Workcell

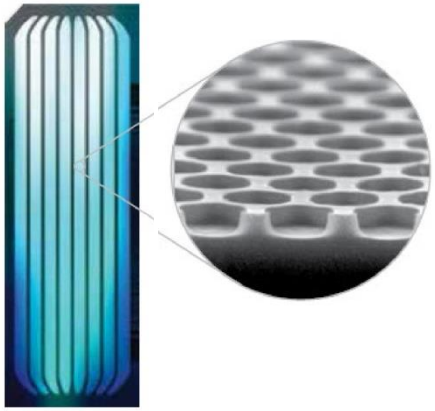
- Collaborative Robots
- Modular Carts
- Scheduling Software





Sequencing Consolidation and Scaling: HiSeq 4000 Sequencing

- Patterned Flowcell Technology
  - 2.5 billion pairs of reads (aka 150 bp sequences)
  - 750 Gb per flow cell in ~40 h
  - $20.71 per Gb or $0.00000002 per base
- Bioinformatics
  - Images converted to FASTQ Files



Bioinformatics Overhaul

Past Workflow

- Numerous bioinformatics pipelines
  - Essentially a different pipeline for each assay offered making it very difficult to properly maintain
  - Computing done on site using physical systems resulting in longer data processing times
  - Not scalable

Current Workflow

- Complete redesign of our informatics infrastructure
- One bioinformatics pipeline for germline/exome and one for somatic
- Cloud based data processing
  - Infinitely scalable (in theory)

Genomic Data Interpretation Issues

- Our original MPS data interpretation involved having the medical directors interpret all variants with no support
  - Time consuming: some variants would take hours to interpret
    - Turnaround time also negatively impacted as medical directors had to do the initial variant list review to determine required Sanger confirmations
  - Lack of standardization: Our genomics lab predates the ACMG guidelines on variant interpretation so criteria for variant classification weren't clearly defined
  - Very costly to have medical directors doing 100% of the initial interpretation and report building

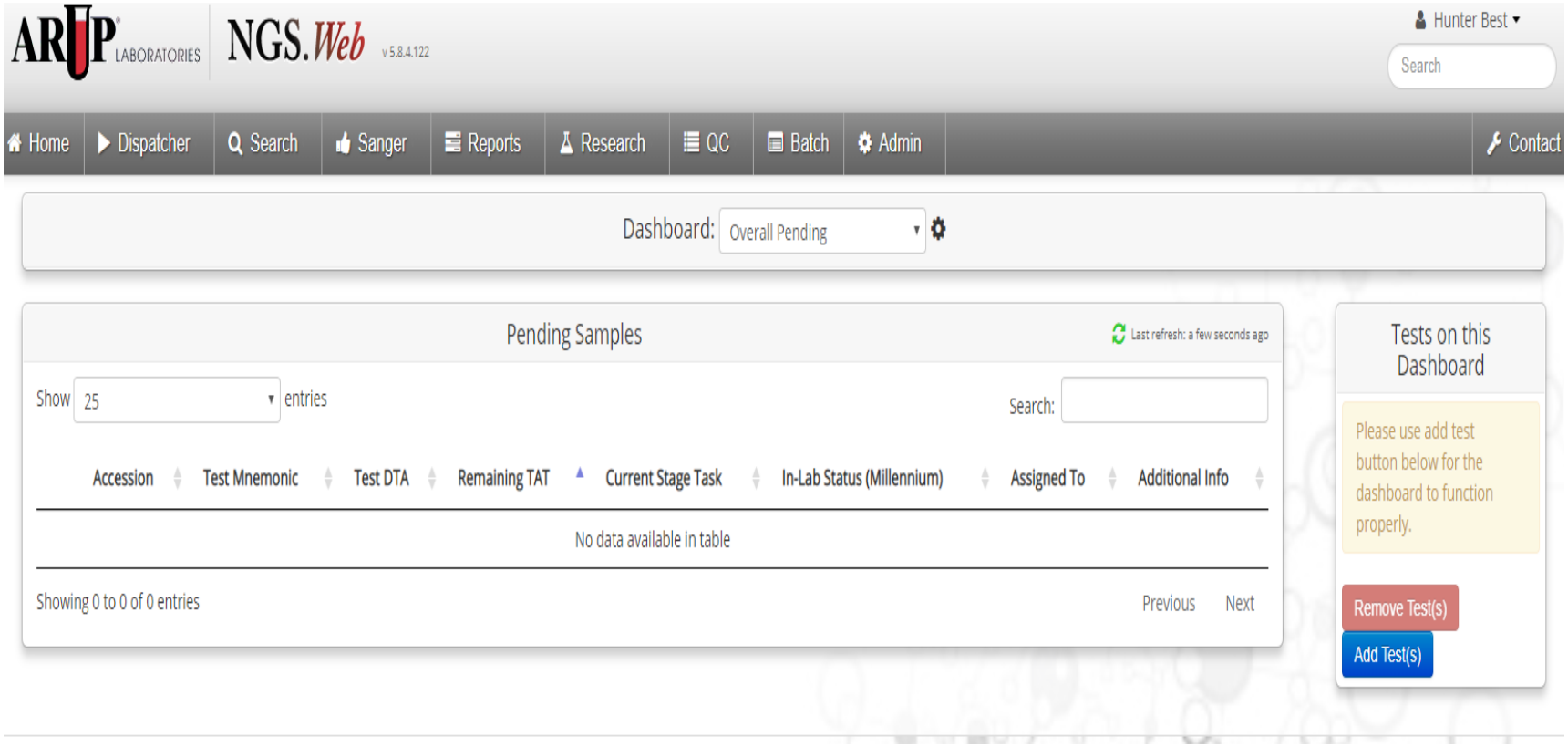
Genomic Data Interpretation Solutions

- Clinical Variant Scientist position created
  - Ph.D. level scientists hired specifically to do variant interpretation
  - These individual do all initial quality metric review, review of variant list, order any necessary Sanger confirmations and generate a draft report
  - ARUP now employs ~15 clinical variant scientists
    - Half focused on germline testing and half focused on somatic
  - Use of clinical variant scientists has been so successful with MPS-based testing that they are now being trained on other testing specialties (e.g. cytogenetics)

Genomics Data Interpretation Solutions – NGS.Web

- Easy visualization of data generated by MPS is important for streamlined interpretation
- When we first entered the MPS testing field there really weren't a lot of options for data visualization and clinical report generation
- After testing a few commercially available options we decided to build our own
  - Program is called NGS.Web
  - Created by our internal biocomputing group under the direction of our medical directors
  - Now used for all MPS based testing
  - Also used for Sanger testing

Genomics Data Interpretation Solutions – NGS.Web



Genomics Data Interpretation Solutions – NGS.Web

Remaining TAT:	2 days
Due Date:	12/16/2018
Creation Date:	11/19/2018 7:24:41 AM
Verified Date:	Not Verified
Workflow:	Genetics Default
Stage (Task):	Completed (Completed)
Medical Director:	Best, Hunter
Clincial Variant Scientist:	Not Assigned
Genetic Counselor:	Not Assigned

View Full Sample History View Medical Director History

17.8 KiB
Remove File

Patient Information

Patient Name: [redacted]
Sex:
Date of Birth:
Ordering Physician:
Client:

Previous Tests

Accession	Ordered Test	Collect Date	Result	Value
18-320-402698	DMD SEQ	Nov 05, 2018	-NO RESULTS-	(No Result)

Sample Notes

No sample notes

Add Note

Regions to Sanger

(1 requests)

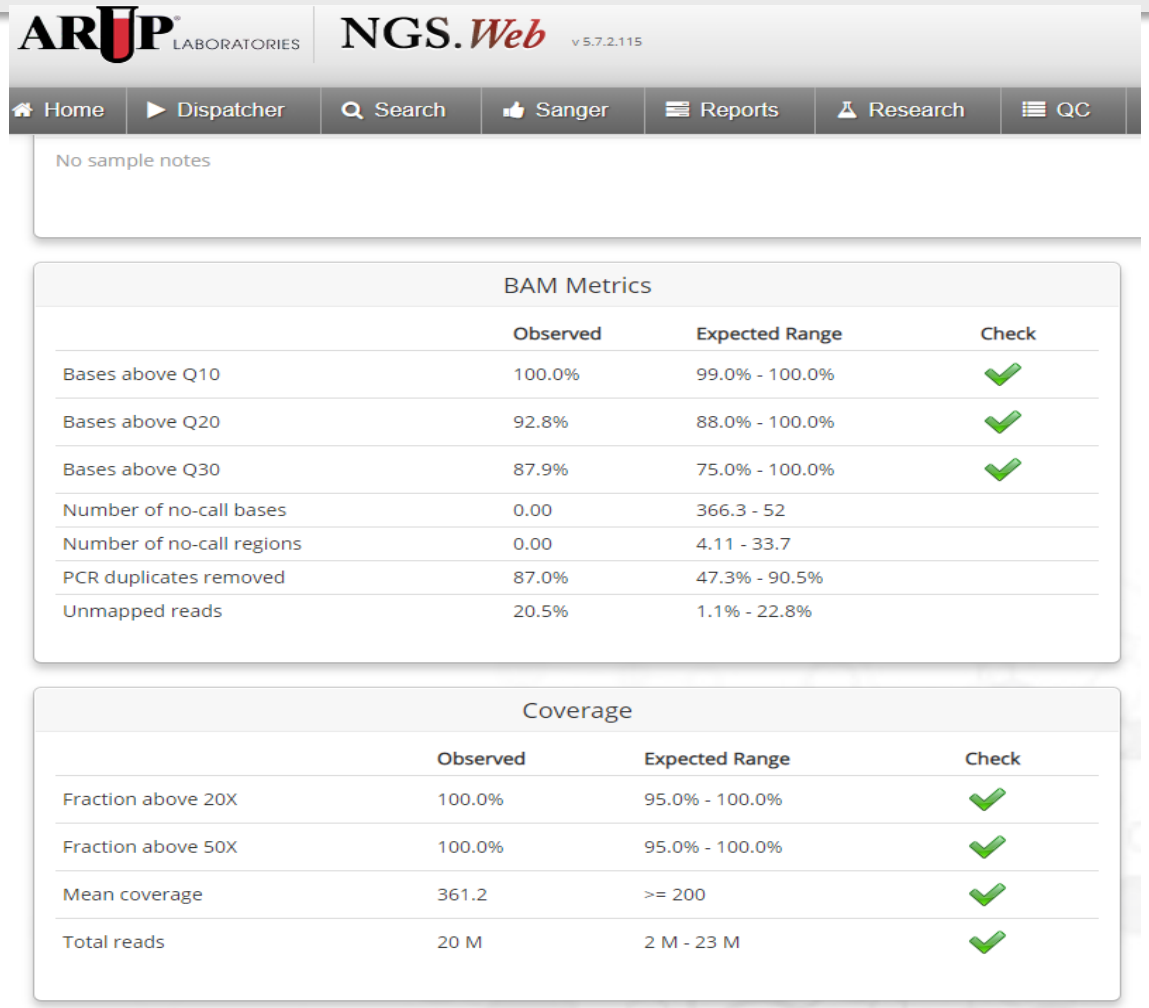
Gene/Exon	Location	Type	Approval	Result
* DMD c.5922+4A>G	chrX: 32360213	Variant Conf	Approved	Confirmed

Data Processing

Status:	Completed
Instrument:	Illumina NextSeq (NS500690)
Run ID:	0410
BED File:	DMD_1gene_qc_10_cds_v1.bed
Analysis Start Time:	11/30 6:53 AM
Total Variants:	2
Fraction Novel:	0.50
Mean Coverage:	842.729398838487
Fraction Bases > 20X:	1

View Variants View Analysis Log

Genomics Data Interpretation Solutions – NGS.Web



Genomics Data Interpretation Solutions – NGS.Web

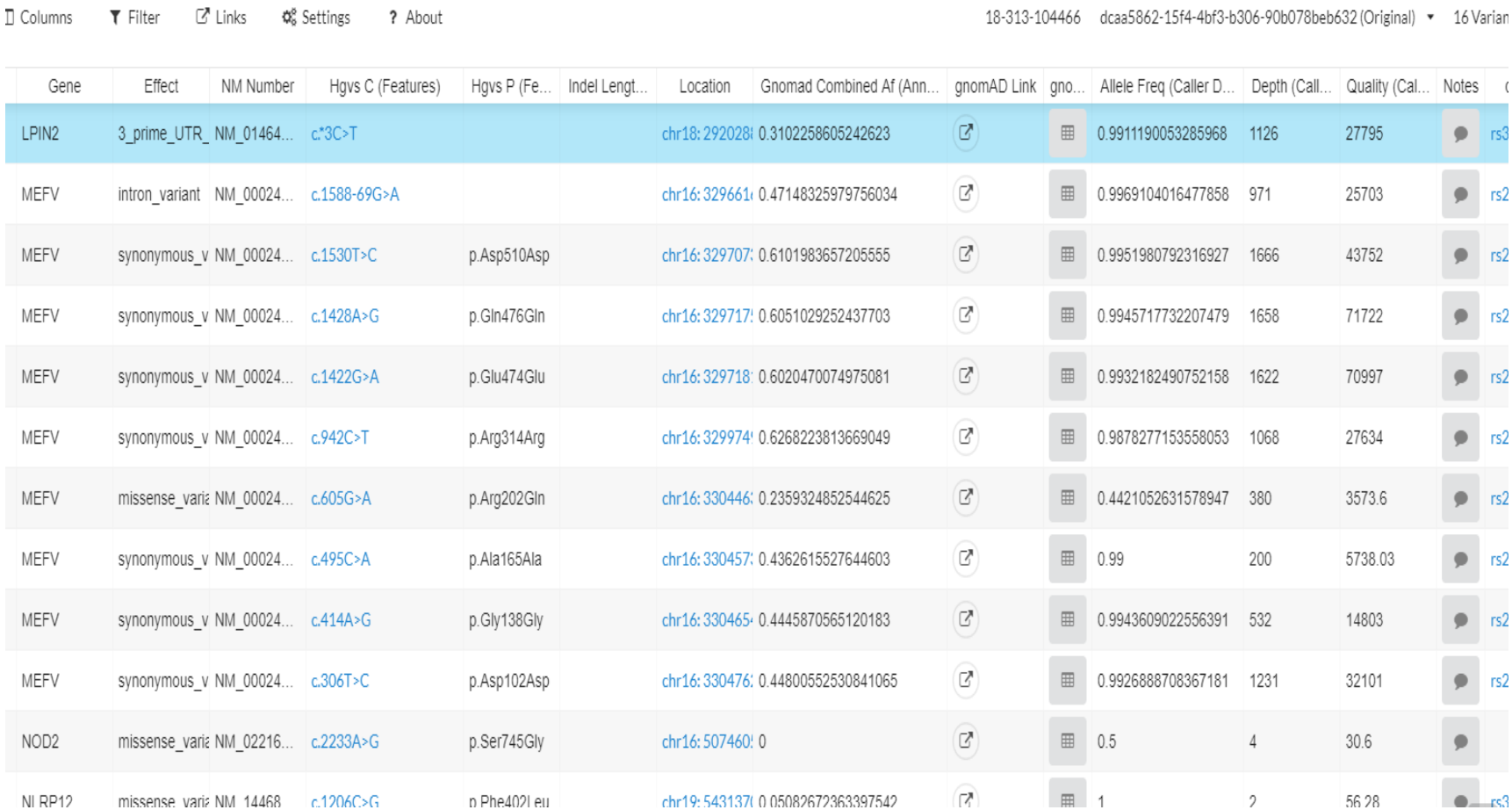
18-310-400940 > NC_TRK PF Seq > Variants

Result Set: Catalog Original (11/6/2018) | Search

Show 100 entries | Showing 1 to 28 of 28 entries

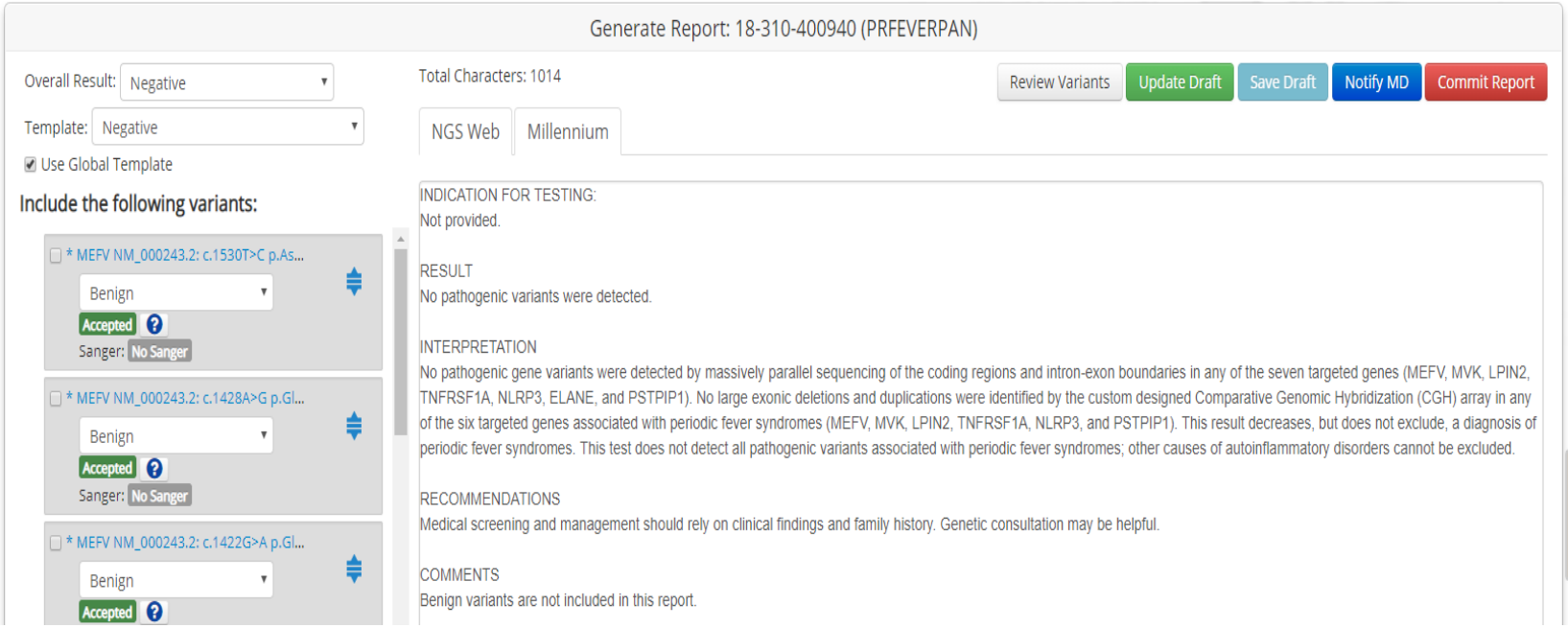
1KG Freq	ARUP Frequency	Gene	Variant Type	Location	Nuc. Change	Protein Change	dbSNP Id	HGMD & OMIM	Classification	Sanger	Note
0.043	0.003	LPIN2	3 prime UTR variant	chr18: 2917367	c.*2924delC		rs148191039	HG OM	Not Classified	No Request	
0.043	0.004	LPIN2	3 prime UTR variant	chr18: 2917368	c.*2923C>T		rs202079191	HG OM	Not Classified	No Request	
0.022	0.039	LPIN2	3 prime UTR variant	chr18: 2919790	c.*501T>C		rs35176958	HG OM	Not Classified	No Request	
0.078	0.015	LPIN2	Intronic	chr18: 2926479	c.1793+242G>A		rs73936863	HG OM	Not Classified	No Request	
0.036	0.01	LPIN2	Intronic	chr18: 2931225	c.1456+29A>G		rs16944068	HG OM	Benign	Confirmed	
0.0	0.0	LPIN2	Intronic	chr18: 2934196	c.1268+153G>A			HG OM	Not Classified	No Request	
0.061	0.005	LPIN2	Intronic	chr18: 2937400	c.1168+290C>T		rs7229067	HG OM	Not Classified	No Request	
0.0	0.0	LPIN2	Intronic	chr18: 2937543	c.1168+146_1168+147delTT		rs149050165	HG OM	Not Classified	No Request	
0.099	0.004	LPIN2	Intronic	chr18: 2950744	c.590+309T>C		rs7244259	HG OM	Not Classified	No Request	
0.0	0.038	LPIN2	Intronic	chr18: 2960513	c.192+134delT		rs376940122	HG OM	Not Classified	No Request	
0.0	0.021	MEFV	3 prime UTR variant	chr16: 3292392	c.*749C>T			HG OM	Not Classified	No Request	

Genomics Data Interpretation Solutions – NGS.Web



Gene	Effect	NM Number	Hgvs C (Features)	Hgvs P (Fe...	Indel Lengt...	Location	Gnomad Combined Af (Ann...	gnomAD Link	gno...	Allele Freq (Caller D...	Depth (Call...	Quality (Cal...	Notes	
LPIN2	3_prime_UTR_	NM_01464...	c.*3C>T			chr18: 292028	0.3102258605242623			0.9911190053285968	1126	27795		rs3
MEFV	intron_variant	NM_00024...	c.1588-69G>A			chr16: 329661	0.47148325979756034			0.9969104016477858	971	25703		rs2
MEFV	synonymous_v	NM_00024...	c.1530T>C	p.Asp510Asp		chr16: 329707	0.6101983657205555			0.9951980792316927	1666	43752		rs2
MEFV	synonymous_v	NM_00024...	c.1428A>G	p.Gln476Gln		chr16: 329717	0.6051029252437703			0.9945717732207479	1658	71722		rs2
MEFV	synonymous_v	NM_00024...	c.1422G>A	p.Glu474Glu		chr16: 329718	0.6020470074975081			0.9932182490752158	1622	70997		rs2
MEFV	synonymous_v	NM_00024...	c.942C>T	p.Arg314Arg		chr16: 329974	0.6268223813669049			0.9878277153558053	1068	27634		rs2
MEFV	missense_varia	NM_00024...	c.605G>A	p.Arg202Gln		chr16: 330446	0.2359324852544625			0.4421052631578947	380	3573.6		rs2
MEFV	synonymous_v	NM_00024...	c.495C>A	p.Ala165Ala		chr16: 330457	0.4362615527644603			0.99	200	5738.03		rs2
MEFV	synonymous_v	NM_00024...	c.414A>G	p.Gly138Gly		chr16: 330465	0.4445870565120183			0.9943609022556391	532	14803		rs2
MEFV	synonymous_v	NM_00024...	c.306T>C	p.Asp102Asp		chr16: 330476	0.44800552530841065			0.9926888708367181	1231	32101		rs2
NOD2	missense_varia	NM_02216...	c.2233A>G	p.Ser745Gly		chr16: 507460	0			0.5	4	30.6		
NLRP12	missense_varia	NM_14468...	c.1206C>G	p.Phe402Leu		chr19: 543137	0.05082672363397542			1	2	56.28		rs3

Genomics Data Interpretation Solutions – NGS.Web



ARUP Genomic Data Interpretation

- ARUP also employs more than 15 genetic counselors
  - GCs review all of our genomic test orders for appropriateness
  - GCs also review all germline reports after they are completed by the CVS (prior to medical director review)
- Final review of all reports is done by a board-certified medical director
  - ~30 medical directors at ARUP are involved in the sign out of MPS-based testing
  - Exome cases are discussed weekly at a focused case conference that includes multiple medical directors, GCs, CVSs and clinical geneticists

Summary

- Genomic testing using MPS has become the standard of care for the diagnosis and treatment determination in many disorders
- Clinical testing in genomics is complex and can be challenging to implement without the proper infrastructure
- Though many hurdles exist, the successful implementation of MPS-based testing can be achieved through strategic planning

Acknowledgements

Genomics Leadership Team
Devin Close, Ph.D.
Erica Cuttitta
Kim Frizzell, Ph.D.
Mike Graczyk

Genomics Project Managers
Sarah Teofilo
Devin Rhodenizer

Genomics Clinical Lab Supervisors
Scott Pew
Josh Raney

Automation Group

MPS R&D Group

Biocomputing Group

Genomics Clinical Lab

Medical Directorship

Too many others to list