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This lecture on **"Next-Generation Sequencing for the Clinical Laboratory"** is given by Karl V. Voelkerding, MD, FCAP

Your host is Jill Kaufman, PhD.
For comments about this webinar or suggestions for upcoming webinars, please contact Jill Kaufman at jkaufma@cap.org

THE WEBINAR WILL BEGIN MOMENTARILY. ENJOY!

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Karl Voelkerding, MD, FCAP

- Professor of Pathology at the University of Utah
- Medical Director for Genomics and Bioinformatics at the ARUP Laboratories
- Past President of the Association for Molecular Pathology
- Board certified in Clinical and Molecular Genetic Pathology
- His research interests include translation of nucleic acid based technologies into diagnostics with a current focus on complex genetic analyses by next generation sequencing

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Next-Generation Sequencing for the Clinical Laboratory

Karl V. Voelkerding, MD, FCAP

July 20, 2011

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Disclosure

- I have nothing to disclose.

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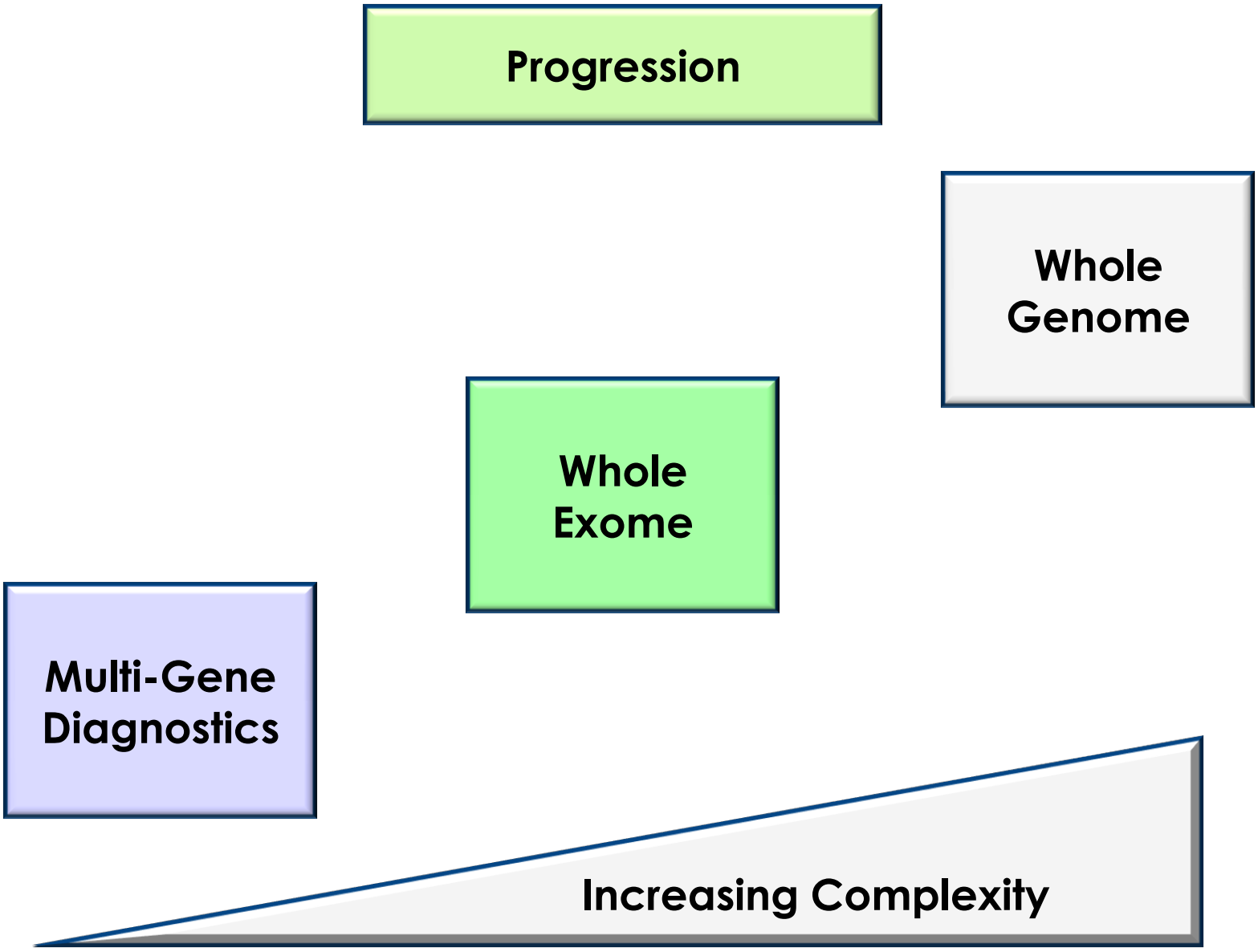
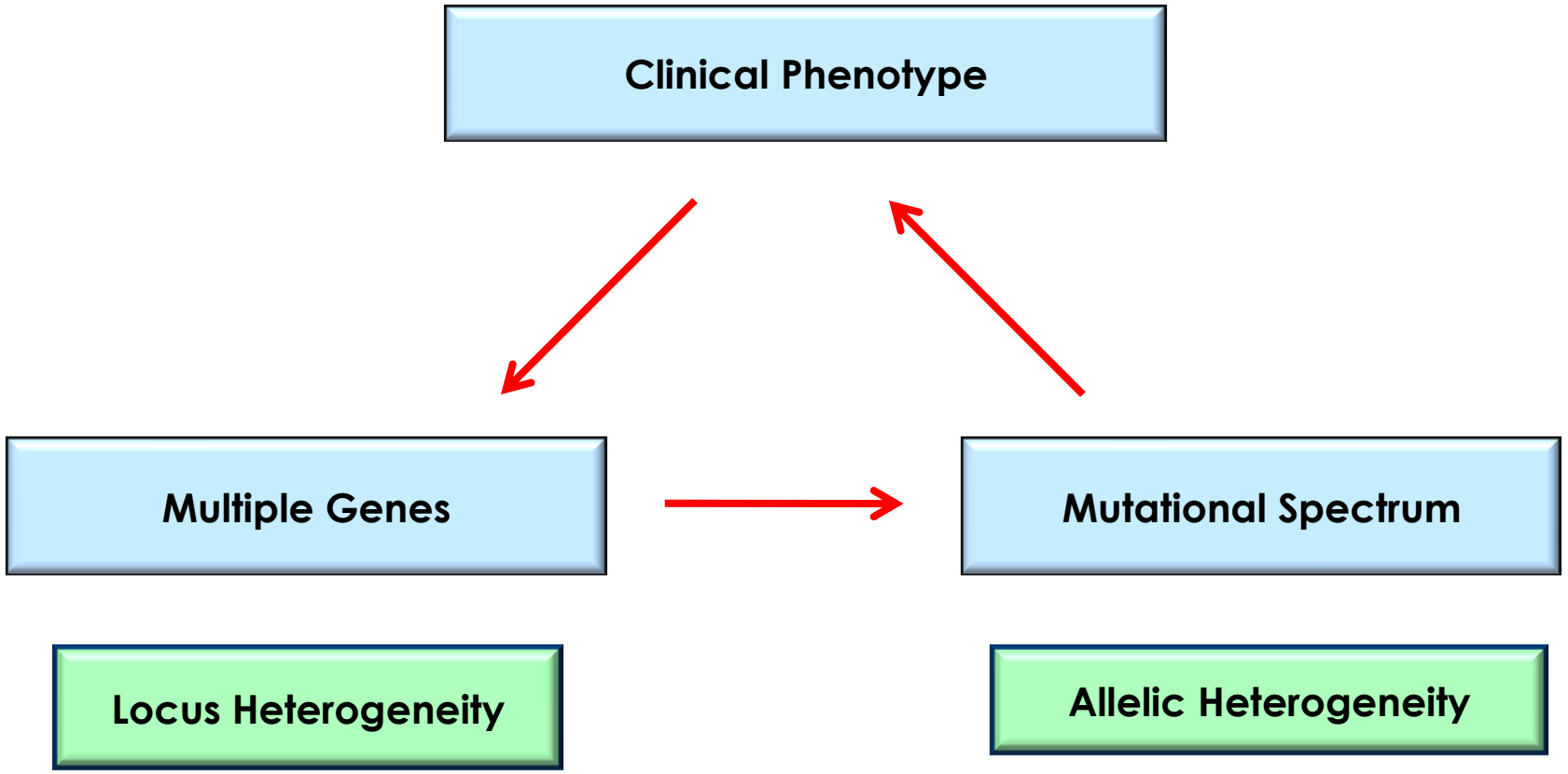
Outline

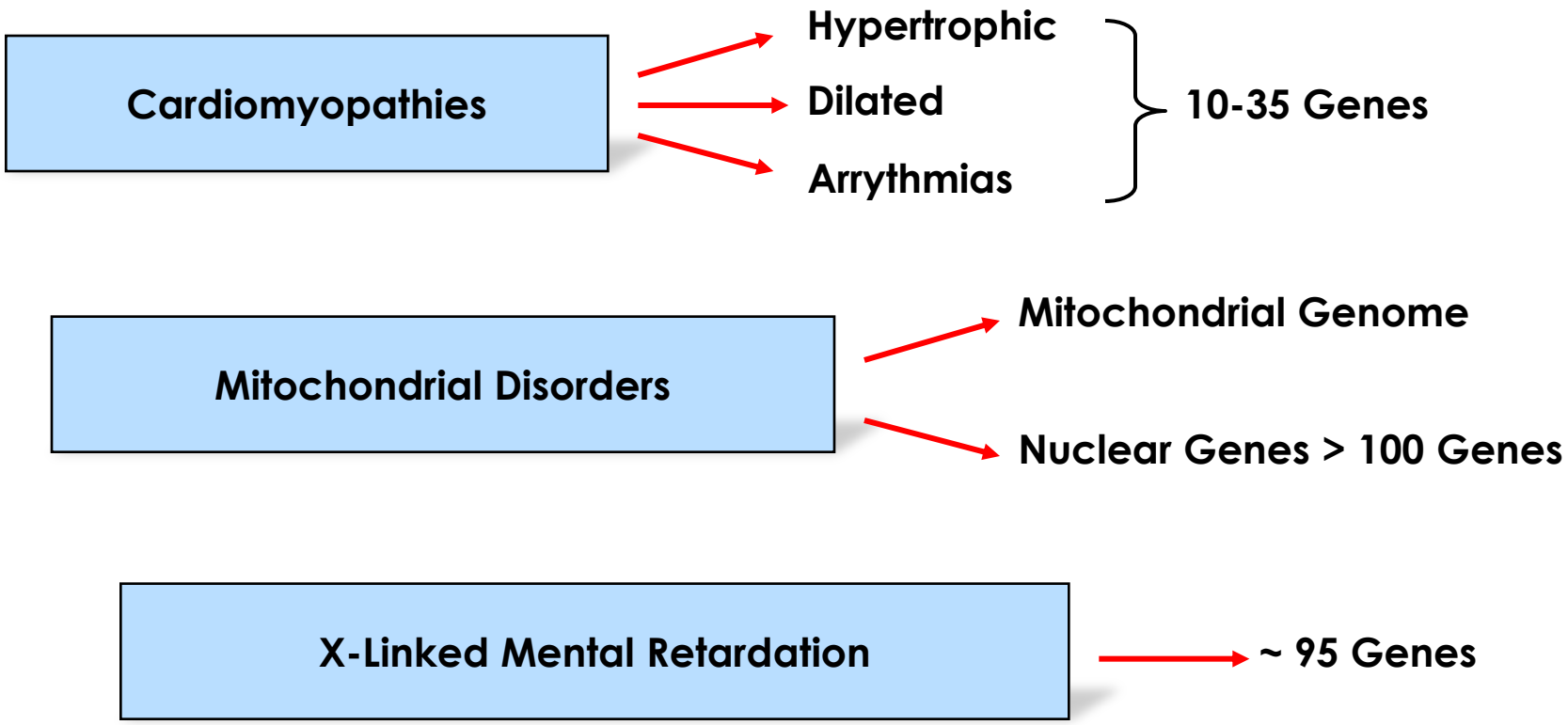
- **Progression: Gene Panels to Genomes**
- **Next Generation Sequencing Technology**
- **Bioinformatics**

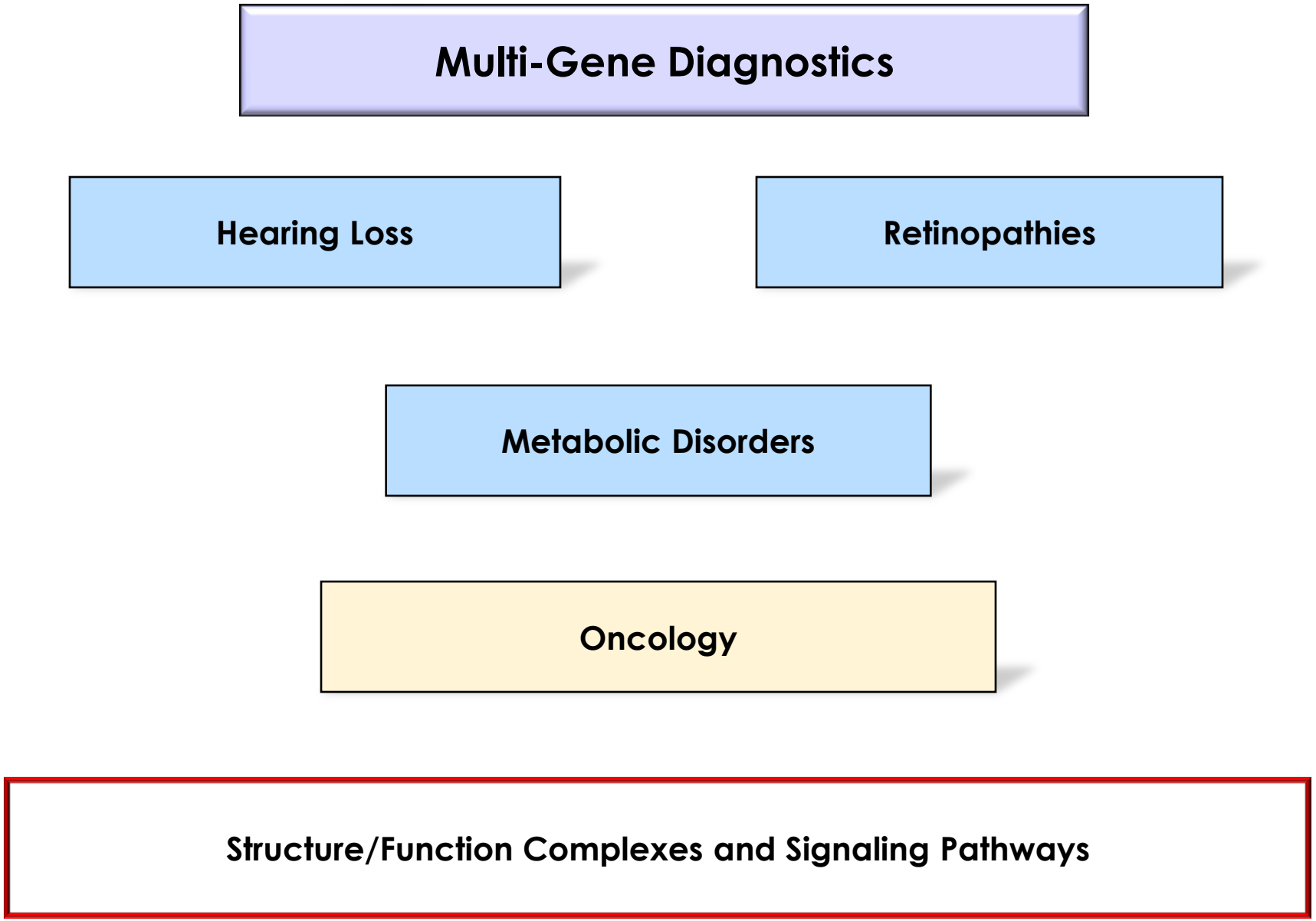

Progression
Whole Genome
Whole Exome
Multi-Gene Diagnostics
Increasing Complexity

Multi-Gene Diagnostics

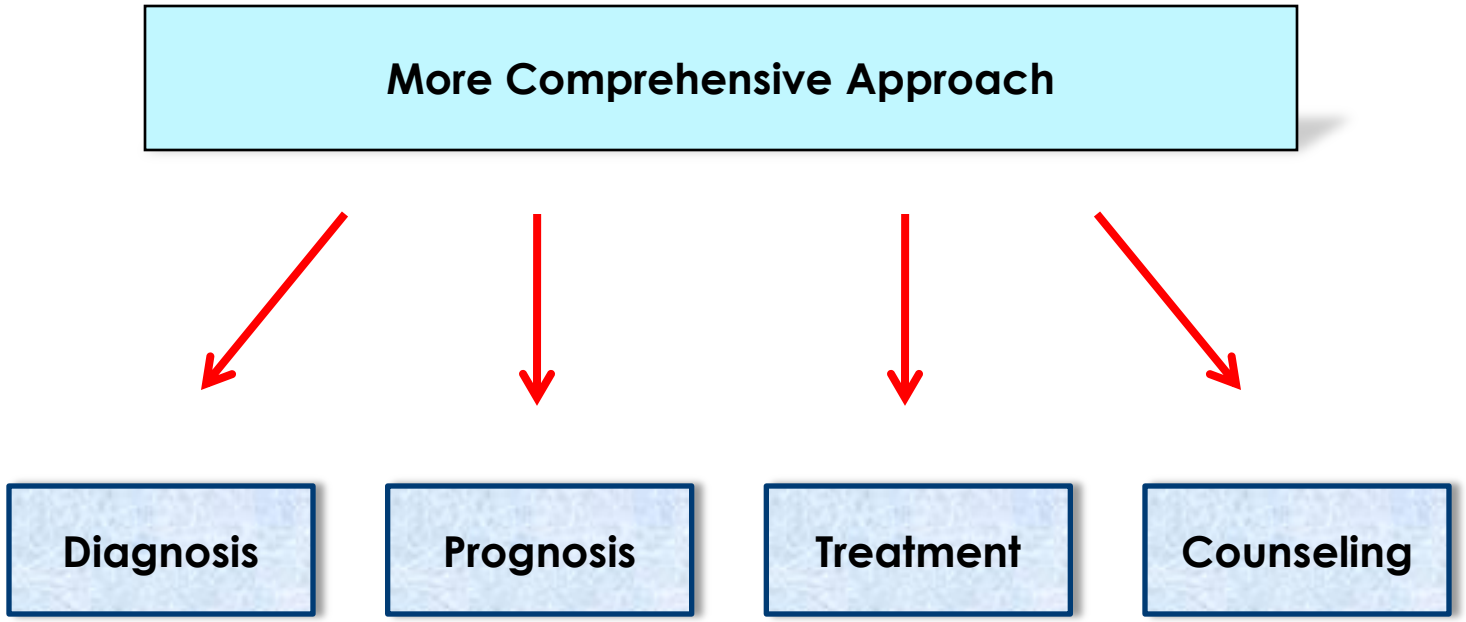


Multi-Gene Diagnostics




Multi-Gene Diagnostics
Hearing Loss
Retinopathies
Metabolic Disorders
Oncology
Structure/Function Complexes and Signaling Pathways

Multi-Gene Diagnostics



Multi-Gene Diagnostics

Technical Options

Sanger Sequencing of Individual Genes

Scanning and Sequencing

Multi-Gene Resequencing Microarrays

Next Generation Sequencing

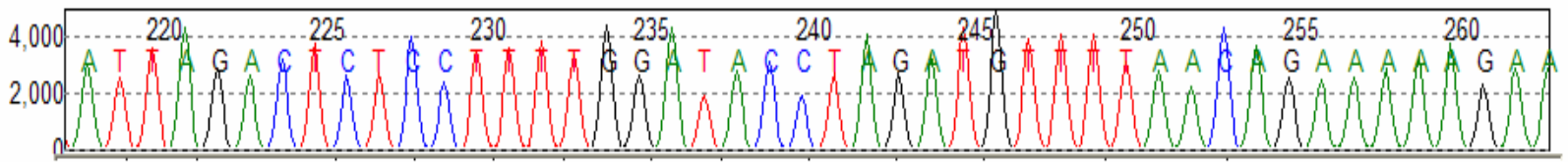
Outline

- Progression: Gene Panels to Genomes
- **Next Generation Sequencing Technology**
- Bioinformatics

Sanger Sequencing

PCR followed by Cycle Sequencing with dNTPs/ddNTPs

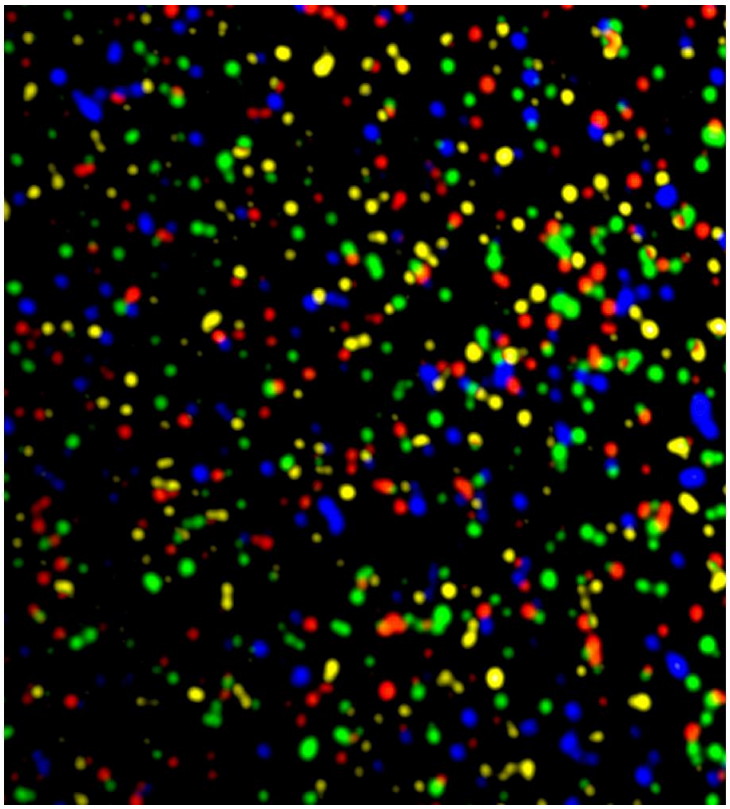
Electrophoretic separation of chain termination products

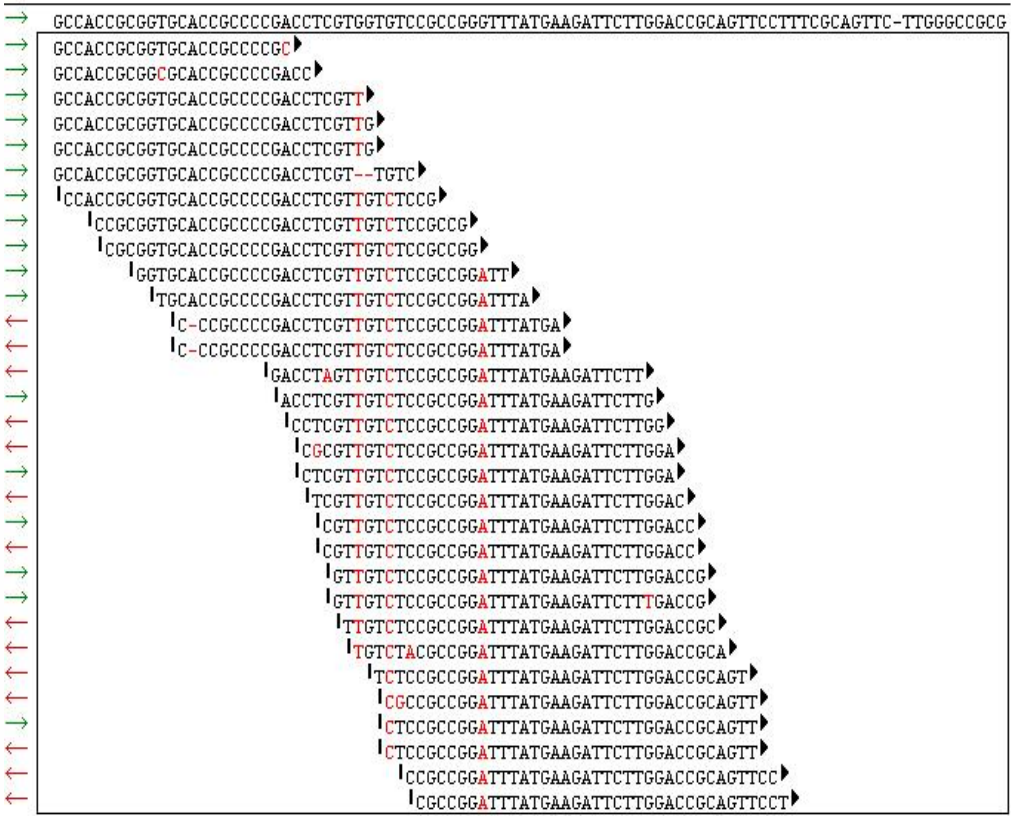


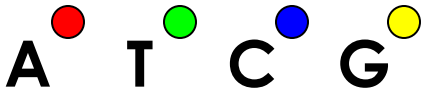
Next Generation Sequencing

Sequence DNA fragment library *in situ* in a flow cell

Massively parallel configuration







Process

Genomic DNA or Enriched Target Genes

Fragmentation

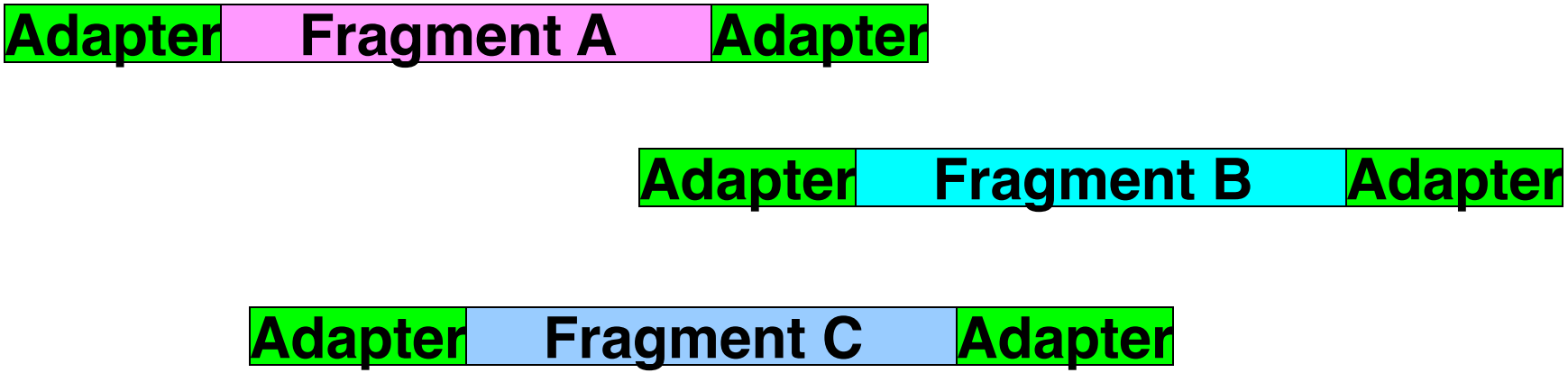
- Nebulization
- Sonication
- Acoustic Wave

150-500 bp

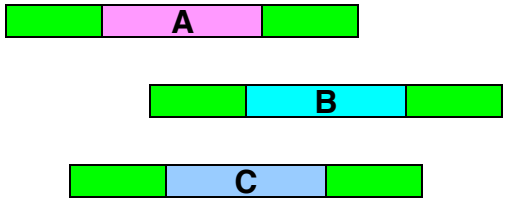
End Repair and Adapter Ligation

+/- PCR

"Fragment Library"



Process

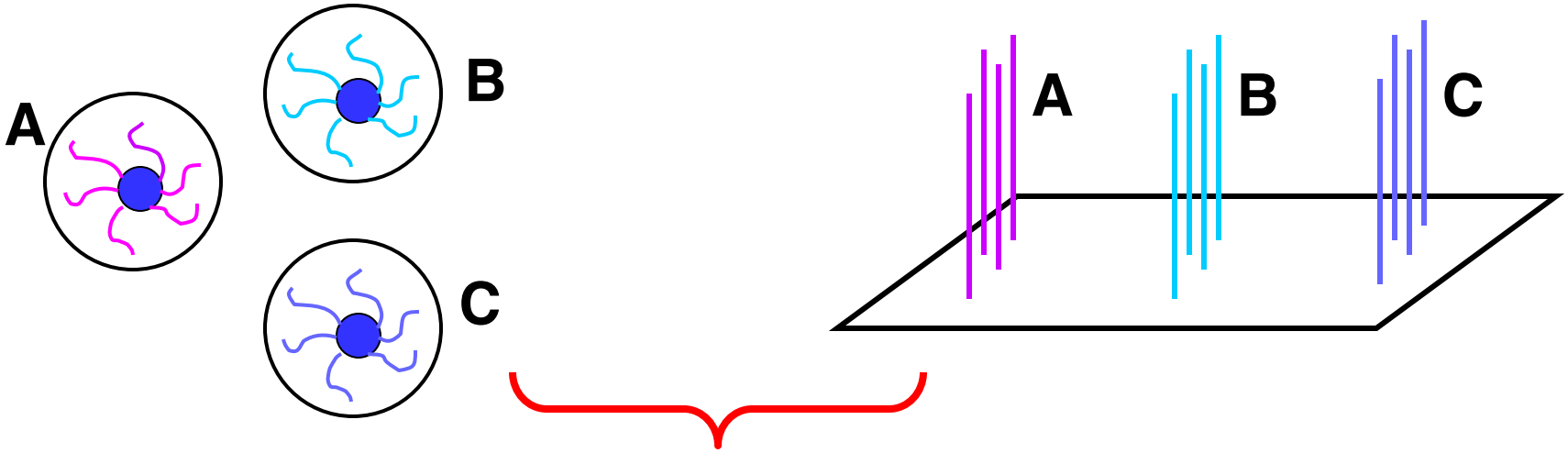


"Fragment Library"

Clonal Amplification of Each Fragment

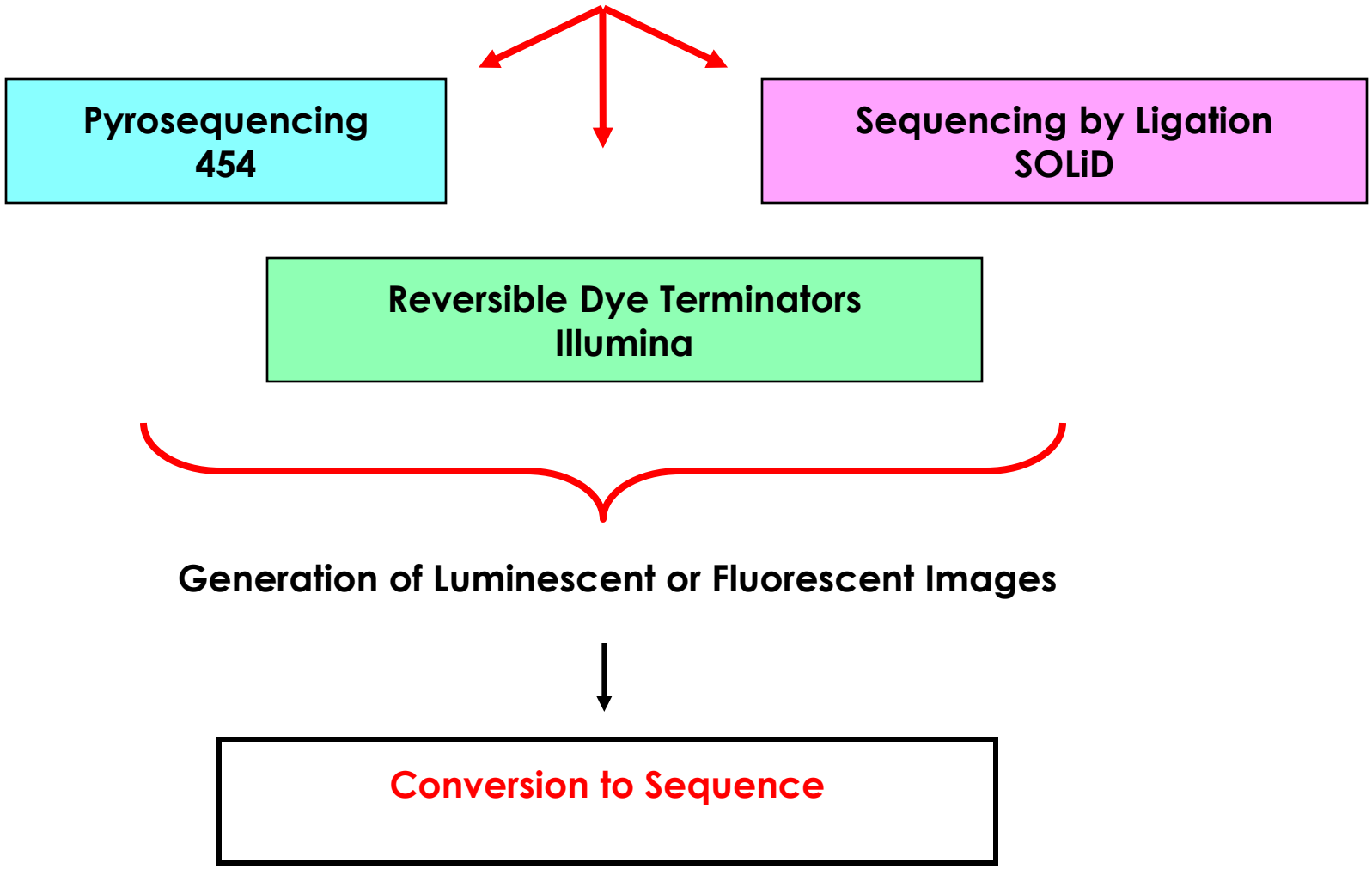
Emulsion Bead PCR

Surface Clusters

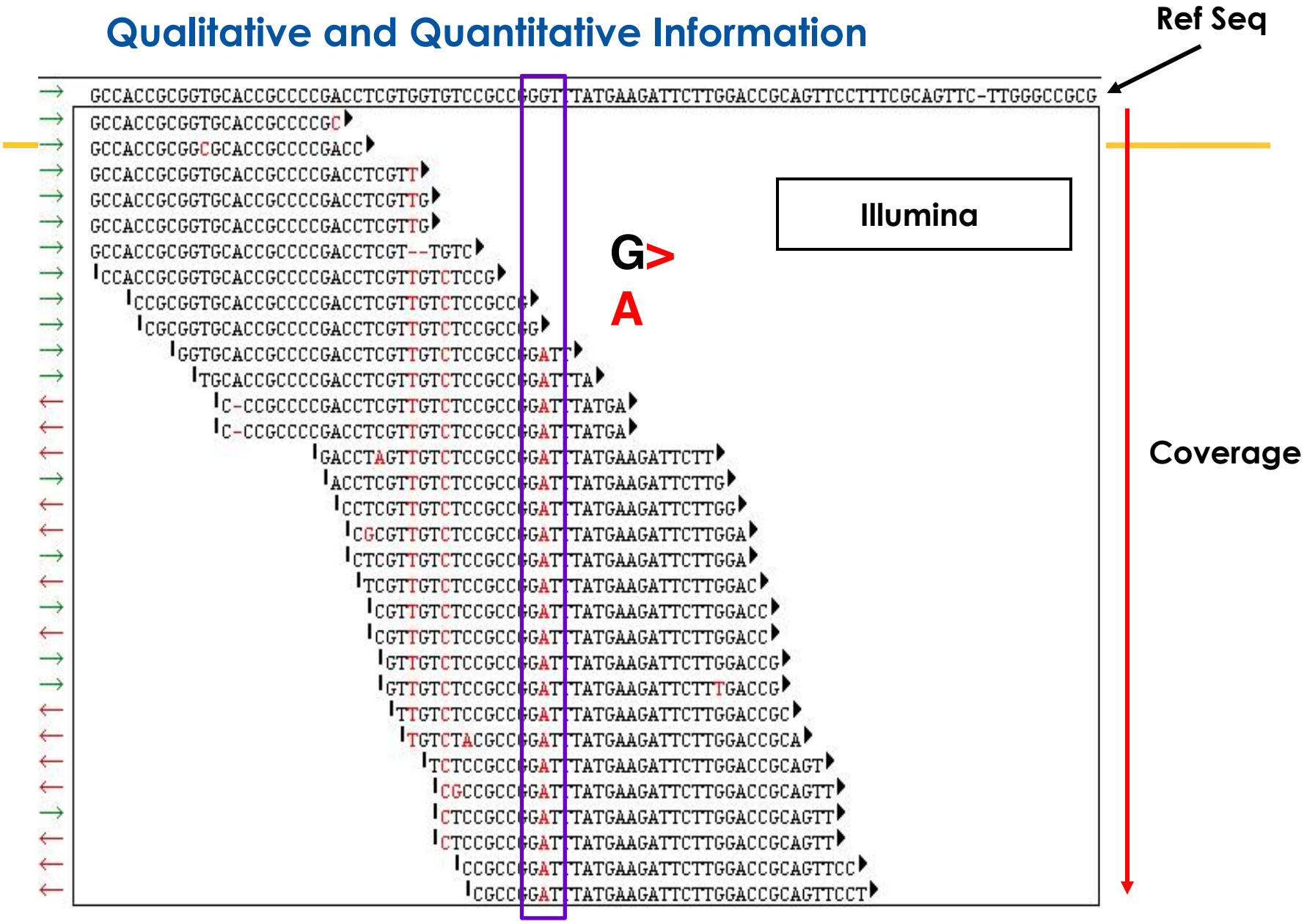


Sequencing of Clonal Amplicons in a Flow Cell

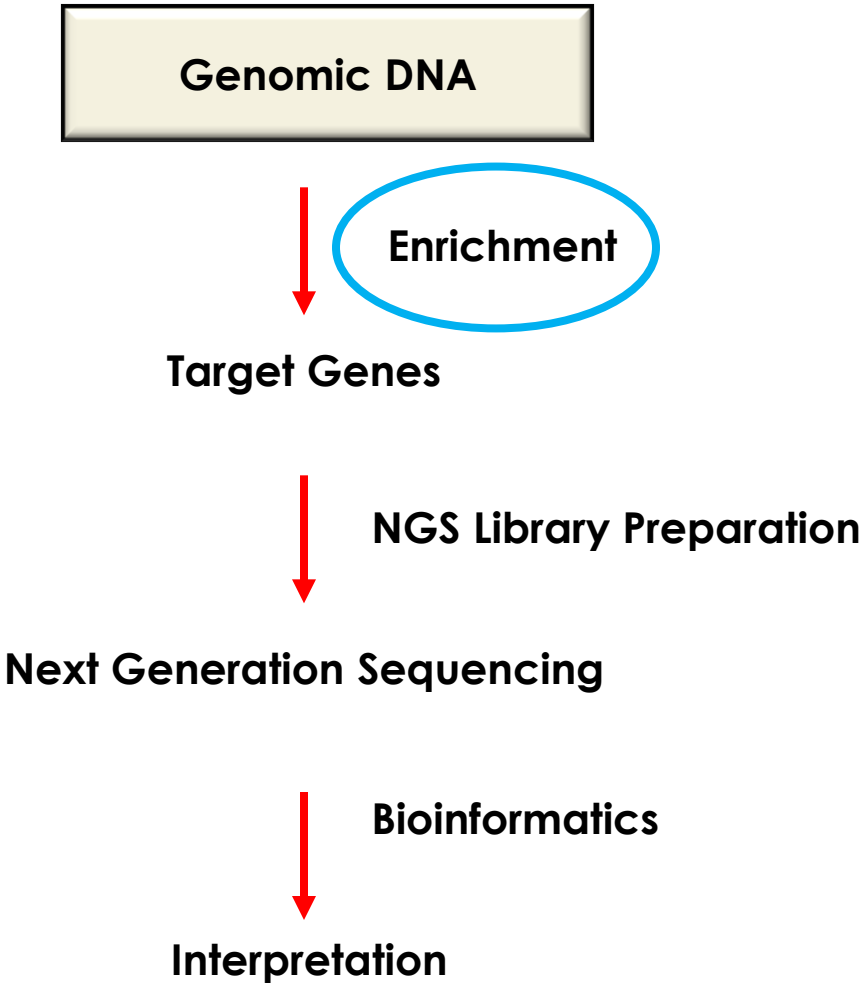
Process


Sequencing of Clonal Amplicons in a Flow Cell
Pyrosequencing
454
Sequencing by Ligation
SOLiD
Reversible Dye Terminators
Illumina
Generation of Luminescent or Fluorescent Images
Conversion to Sequence

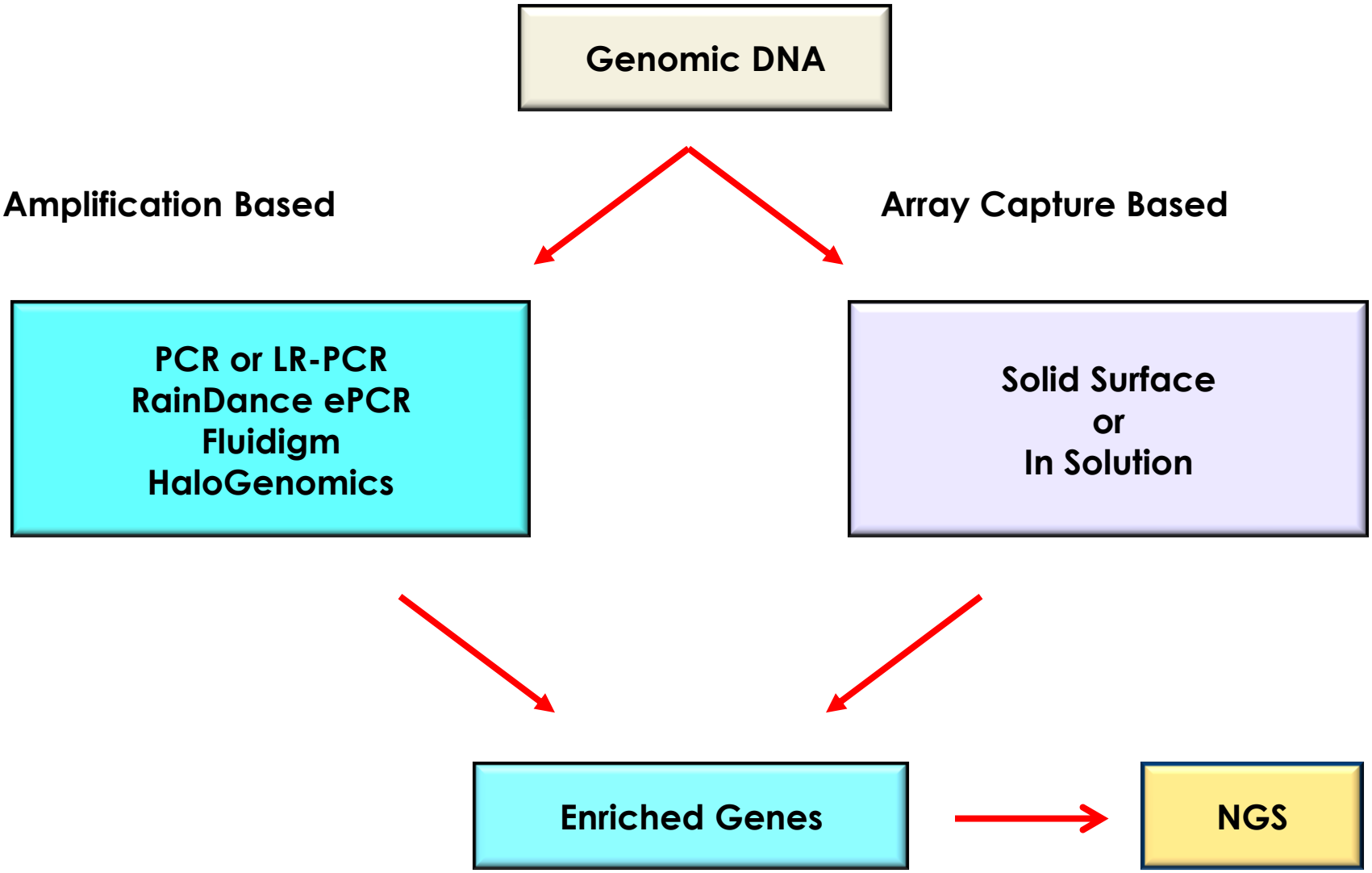
Qualitative and Quantitative Information



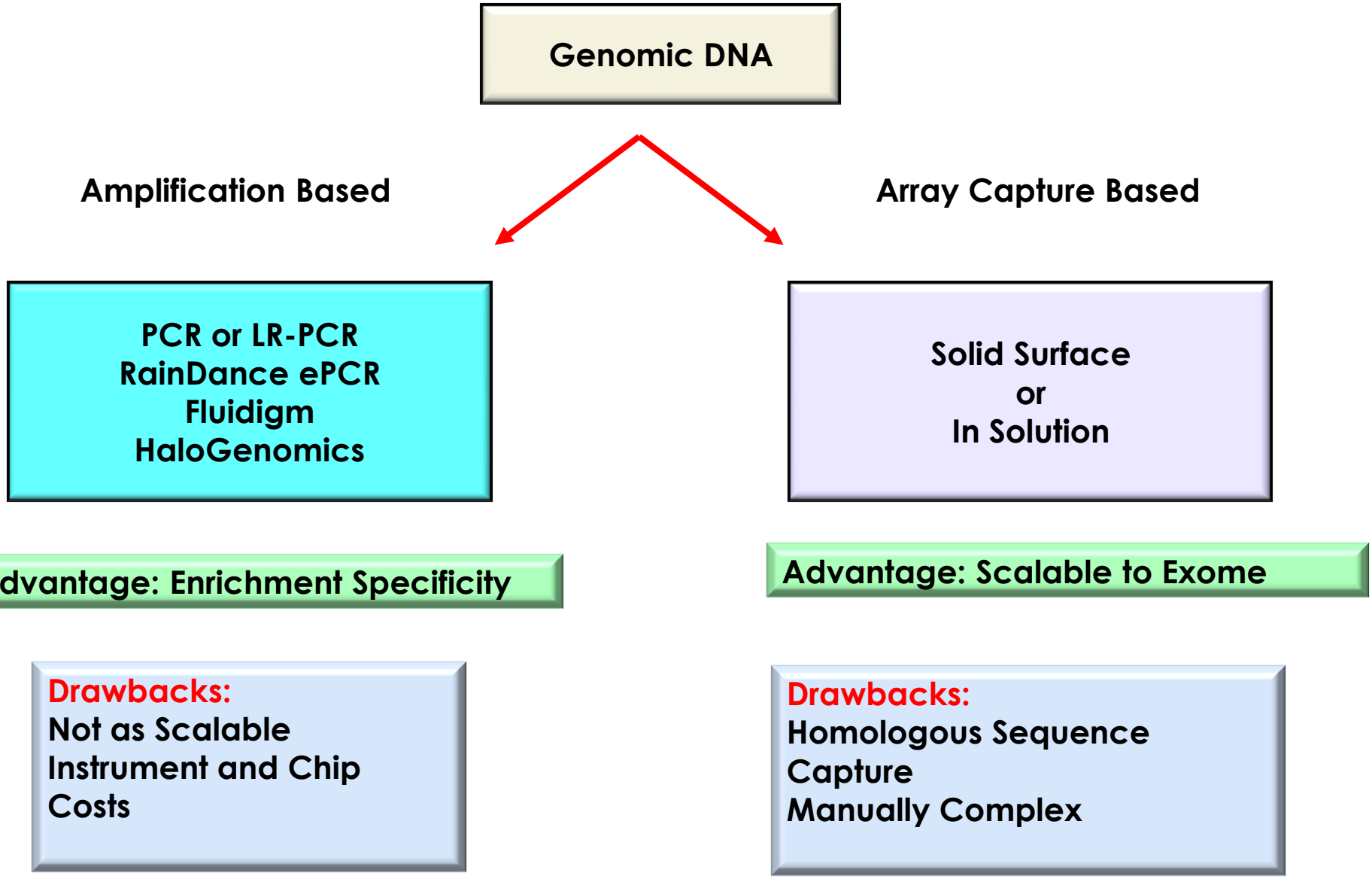
Multi-Gene Diagnostics



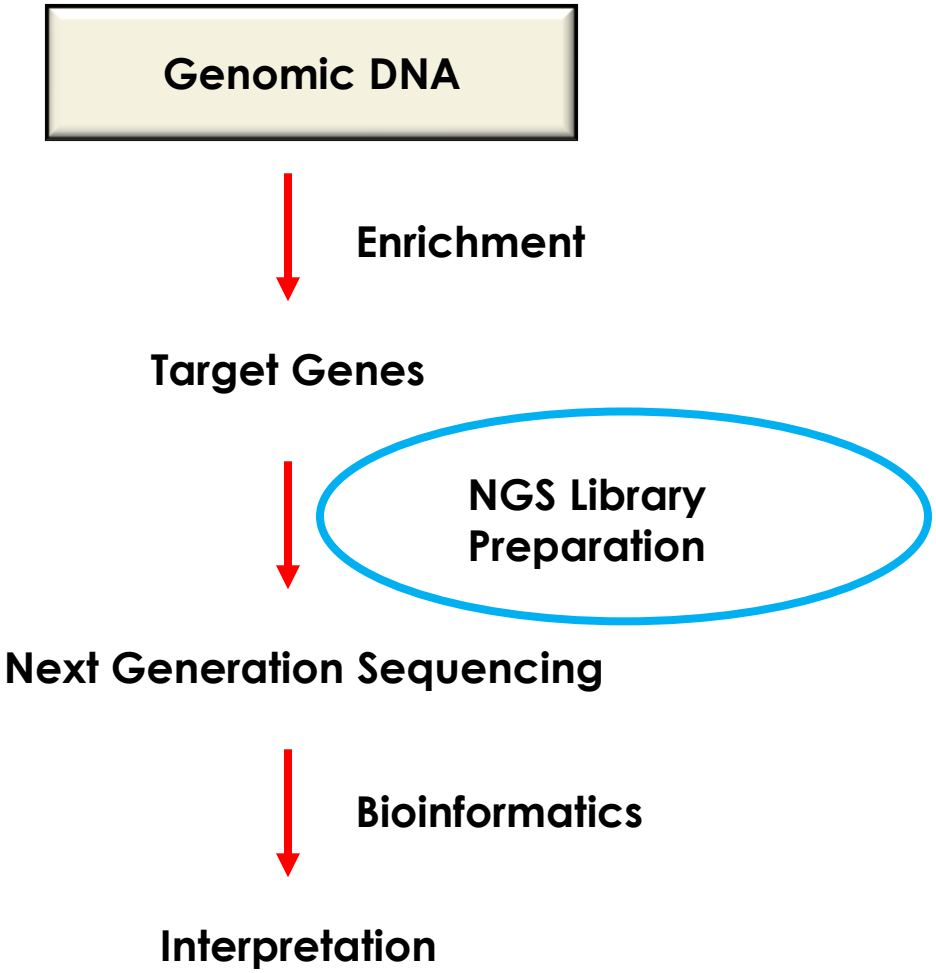
Gene Enrichment Approaches



Gene Enrichment Approaches



Multi-Gene Diagnostics



NGS Library Preparation

Genomic DNA

↓

Fragmentation

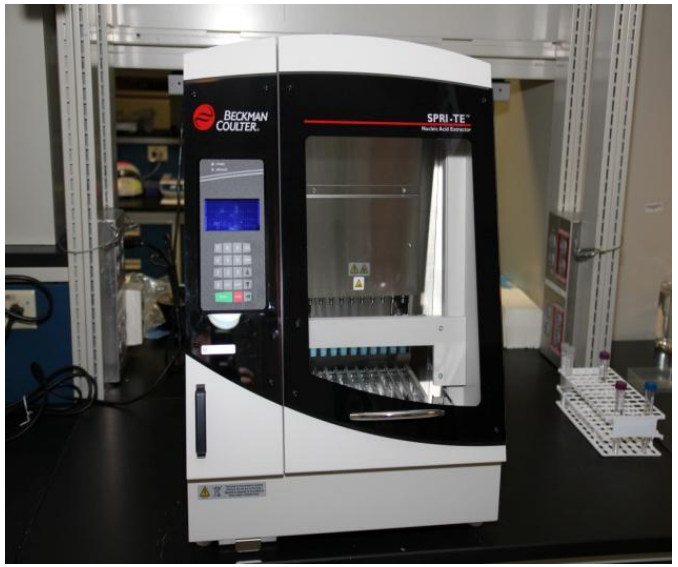
↓

Fragment End Repair

↓

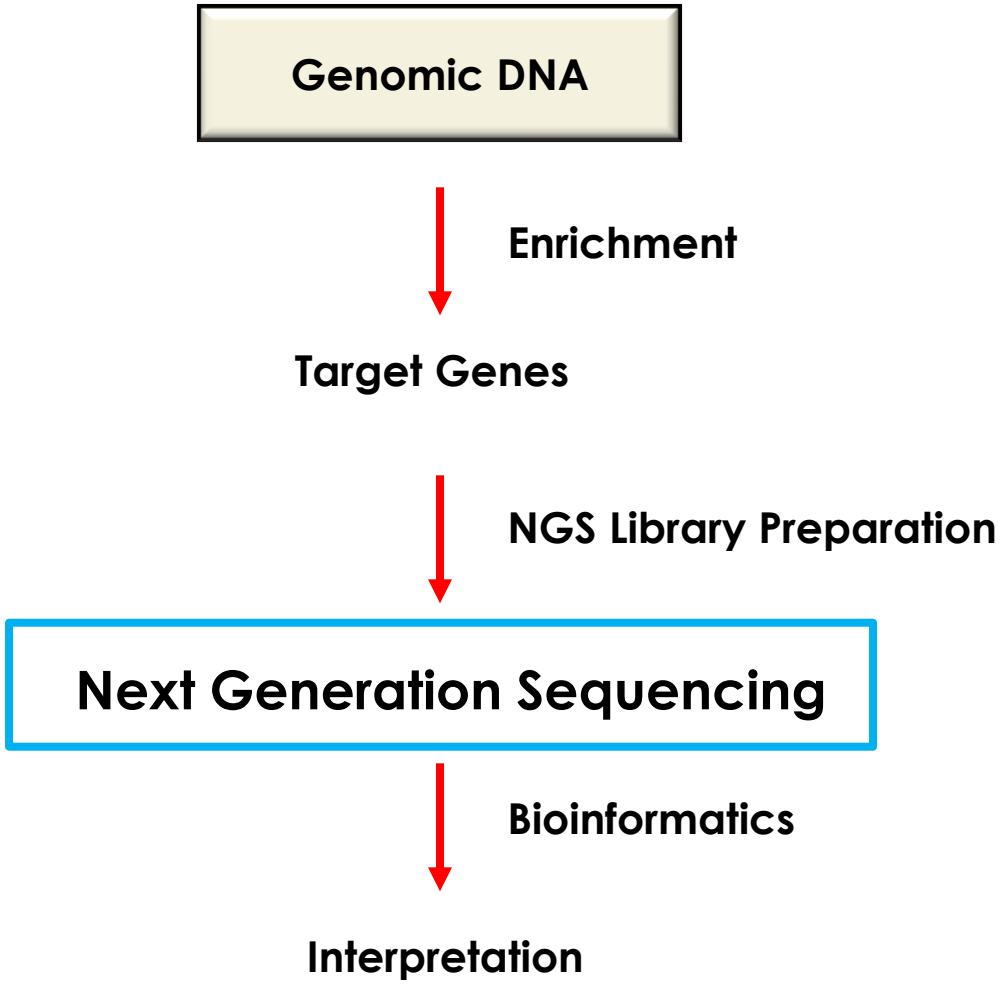
Adapter Ligation

Automation

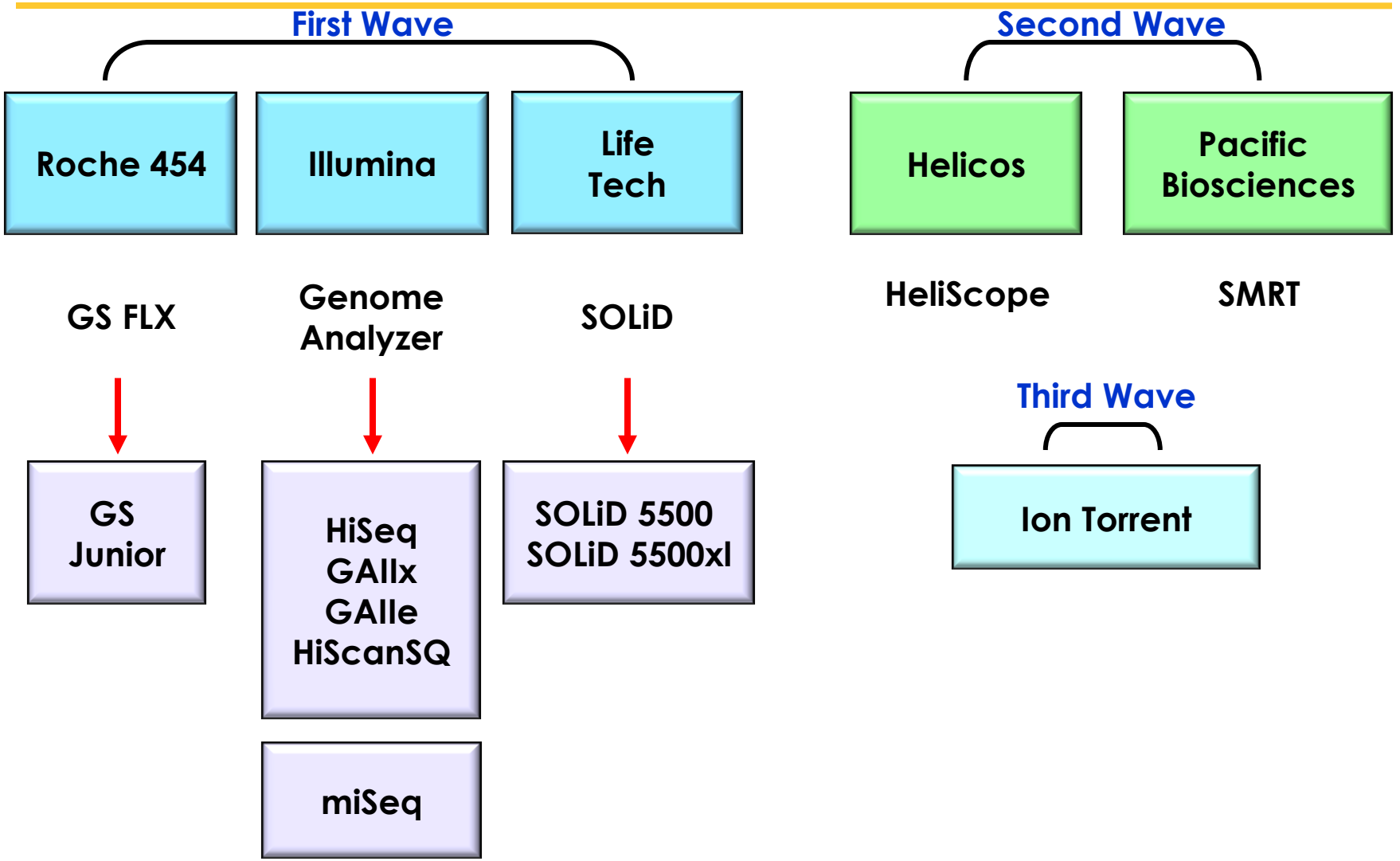


Beckman SPRI-TE
1-10 Samples

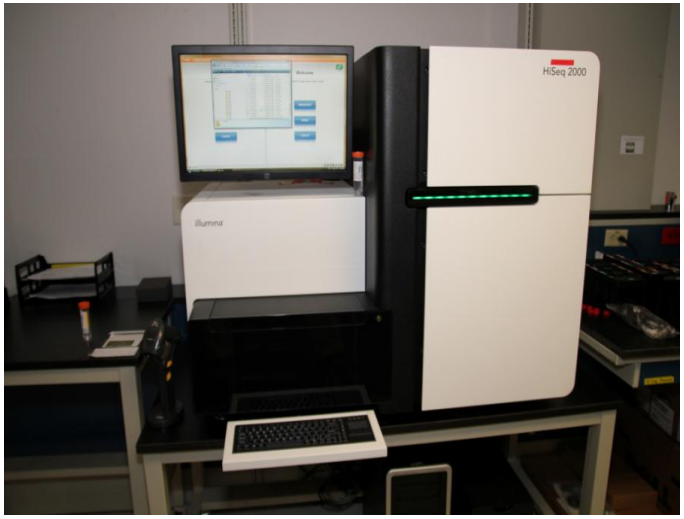
Multi-Gene Diagnostics



Next Generation Sequencing Technology



Illumina HiSeq 2000



Advantage: High Throughput

Independent Flow Cells
8 Lanes per Flow Cell

Drawbacks:
Batching
Sample Coordination

Multiple Panel Samples per Lane

1- 3 Exome(s) per Lane

1- 2 Genome per 8 Lanes

New Platforms
Lower Throughput - Faster TAT
"Random Access"

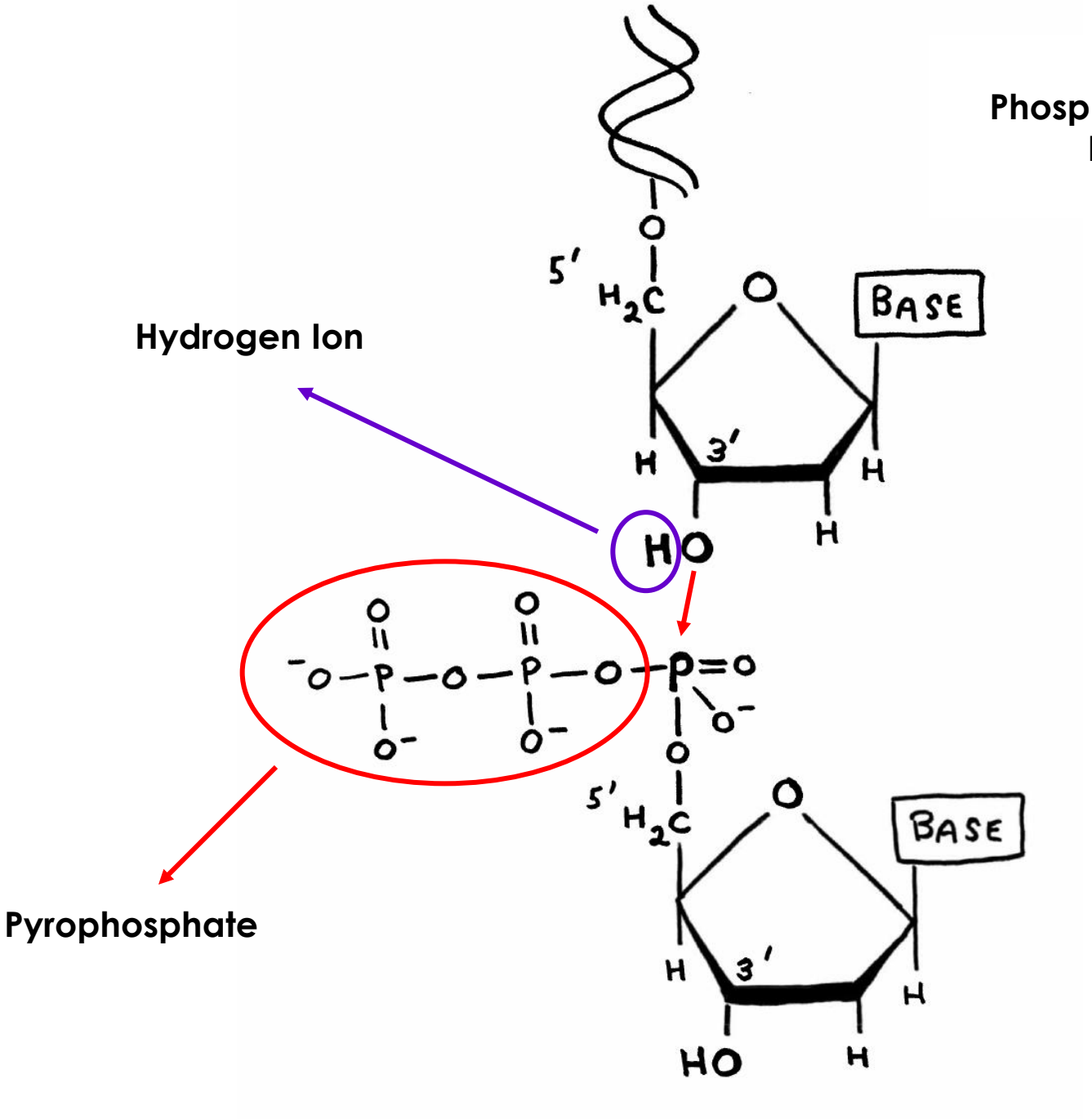
Illumina miSeq

Ion Torrent PGM

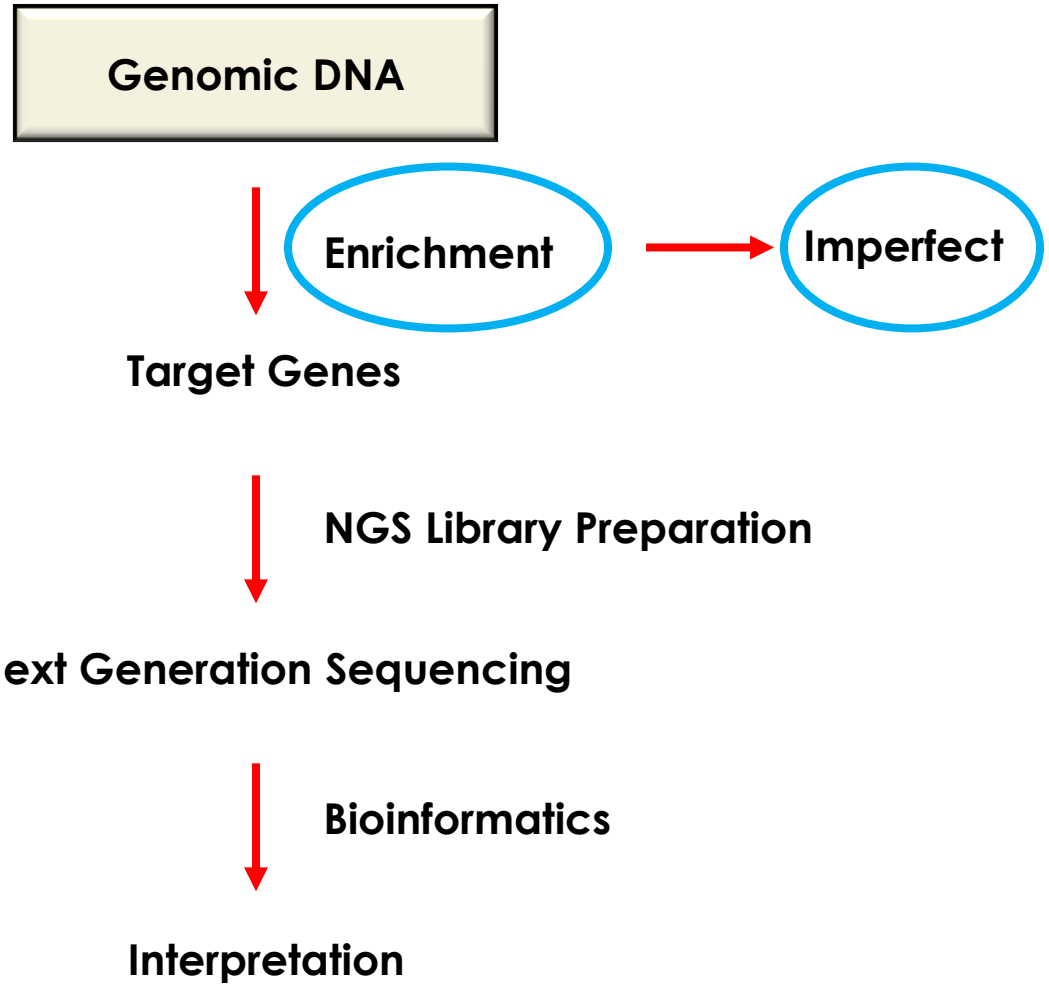
Reversible Dye Terminators

Monitors H+ Release

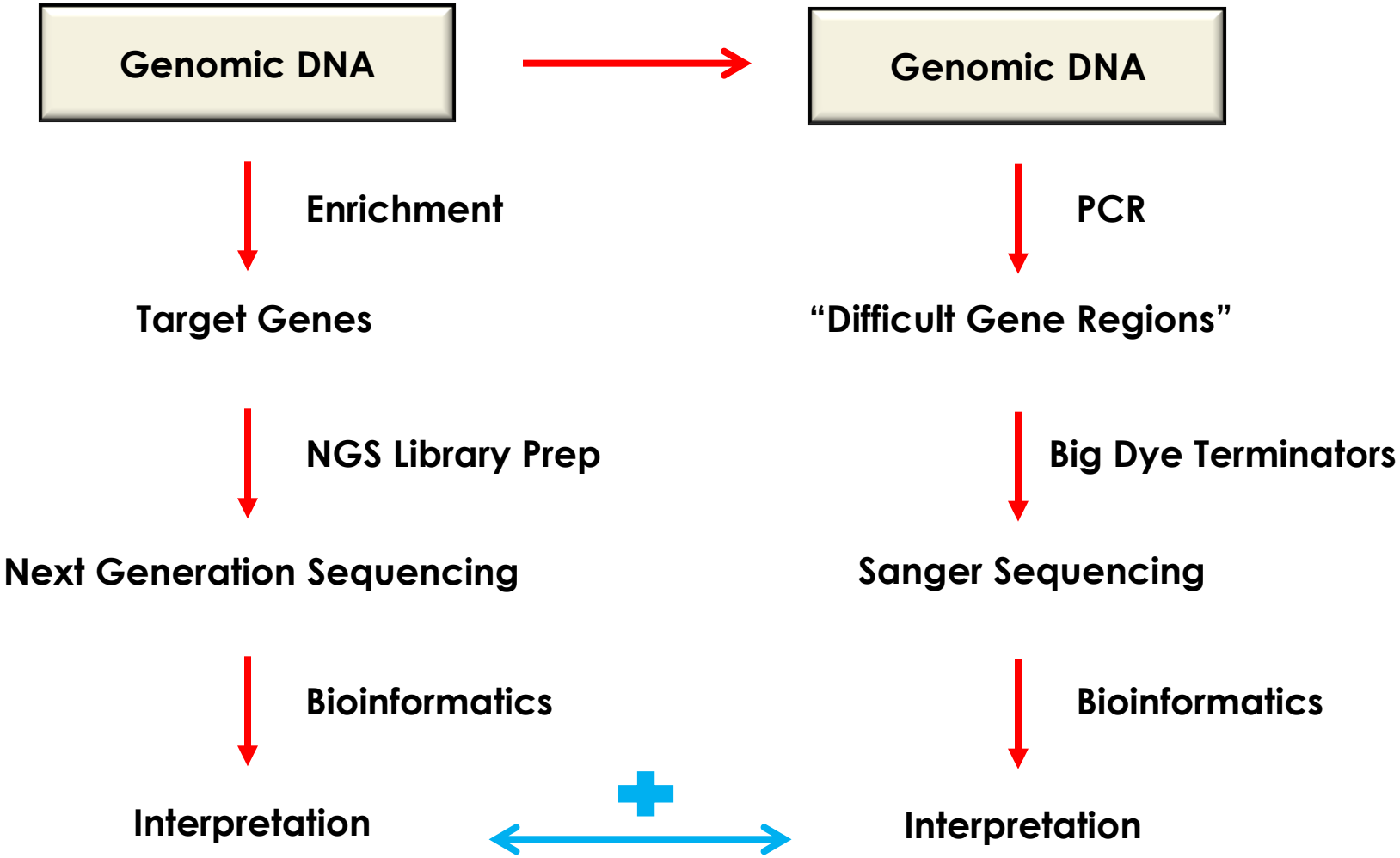
Phosphodiester Bond Formation



Multi-Gene Diagnostics

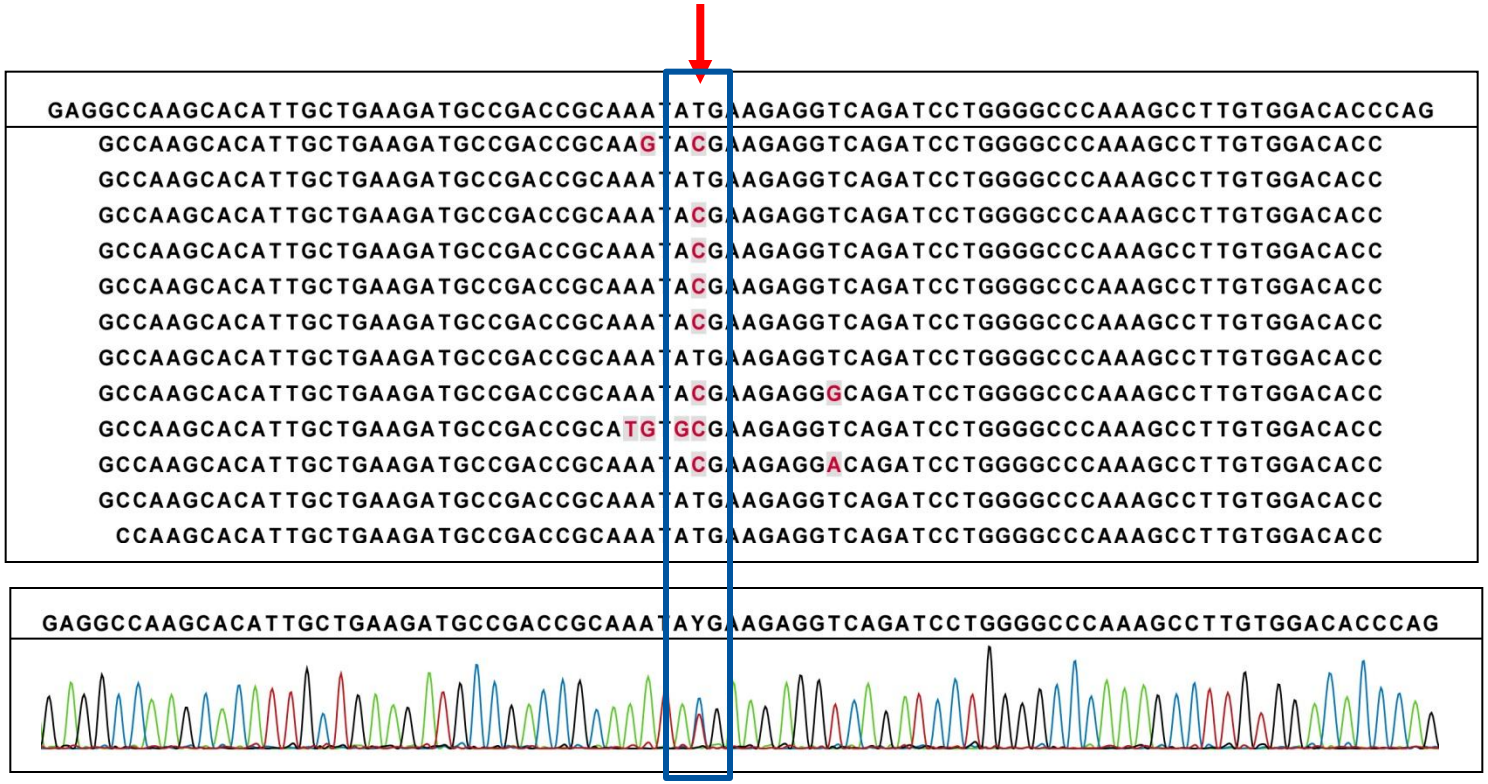


Multi-Gene Diagnostics – Parallel Testing



Re-Sequencing – Cardiomyopathy Genes

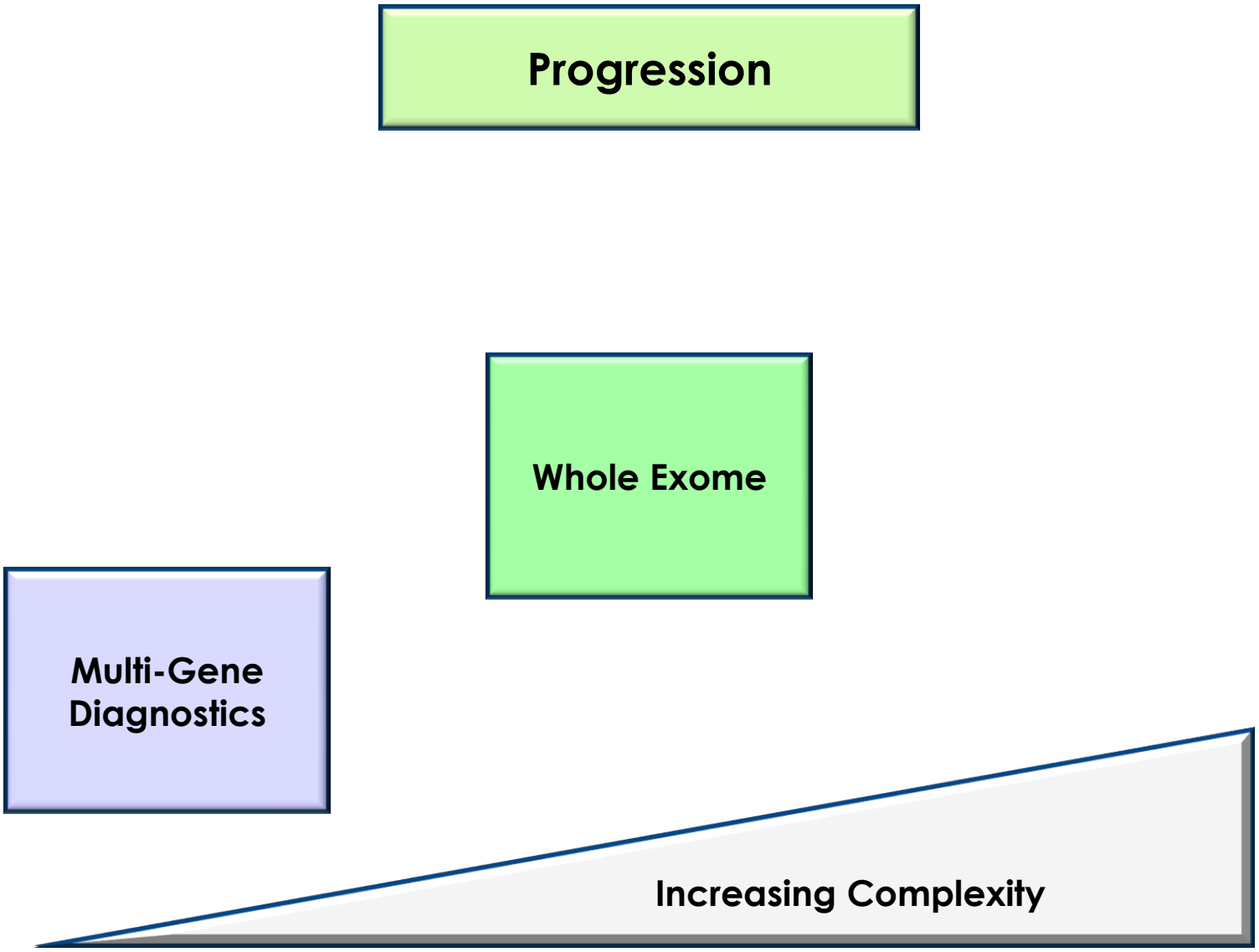
Variant g.34142190T>C in *TPM1*



Reference

LR-PCR 47%

Sanger


Progression
Whole Exome
Multi-Gene Diagnostics
Increasing Complexity

Human Exome

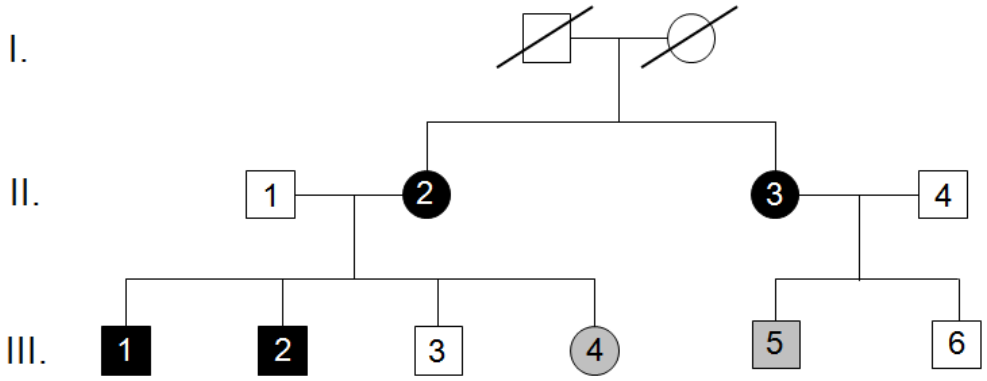
"Journey to the Center of the Genome"

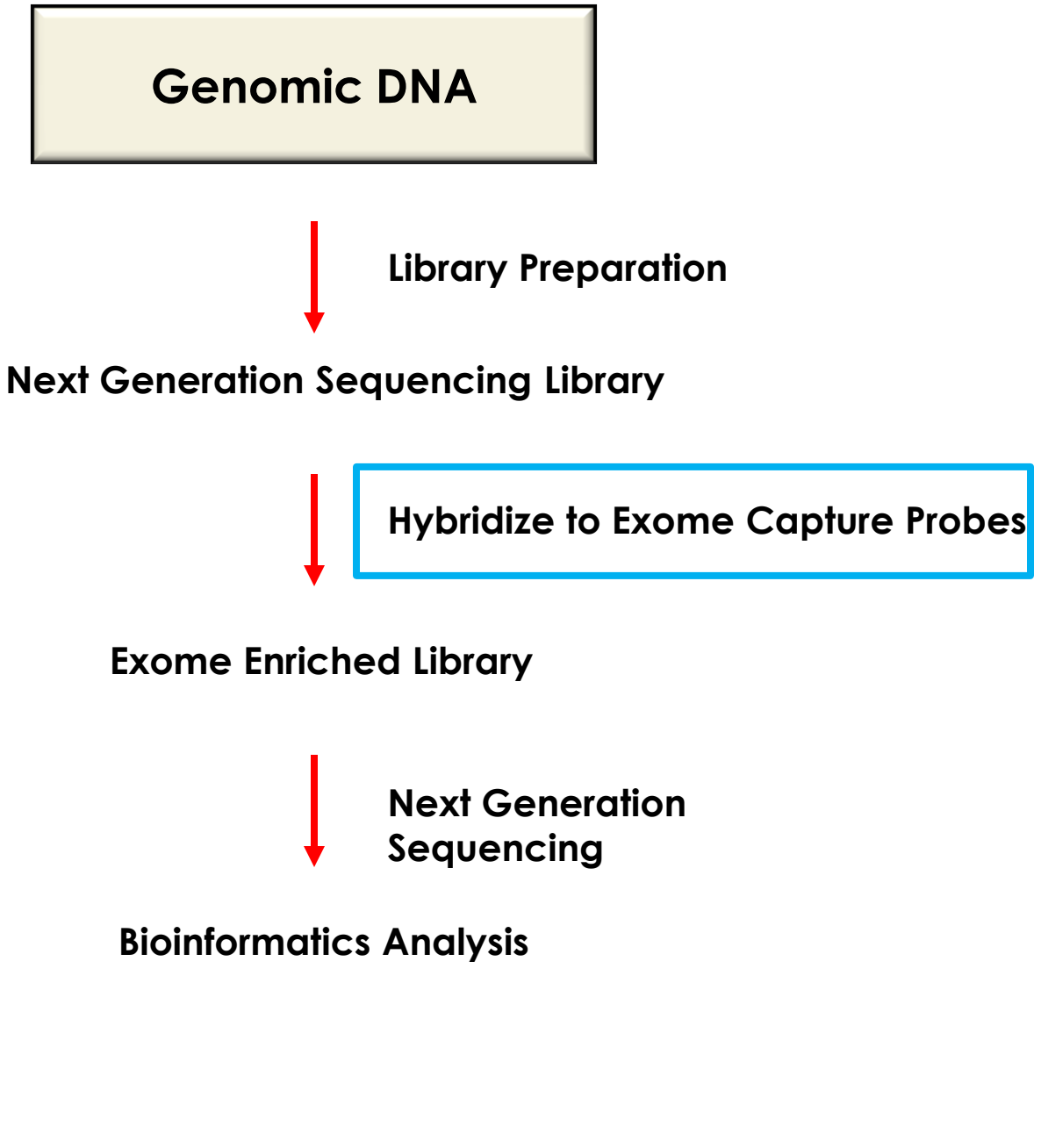
~ 30 Megabases (~ 1% of the genome)

~ 180,000 exons (~ 20,500 genes)

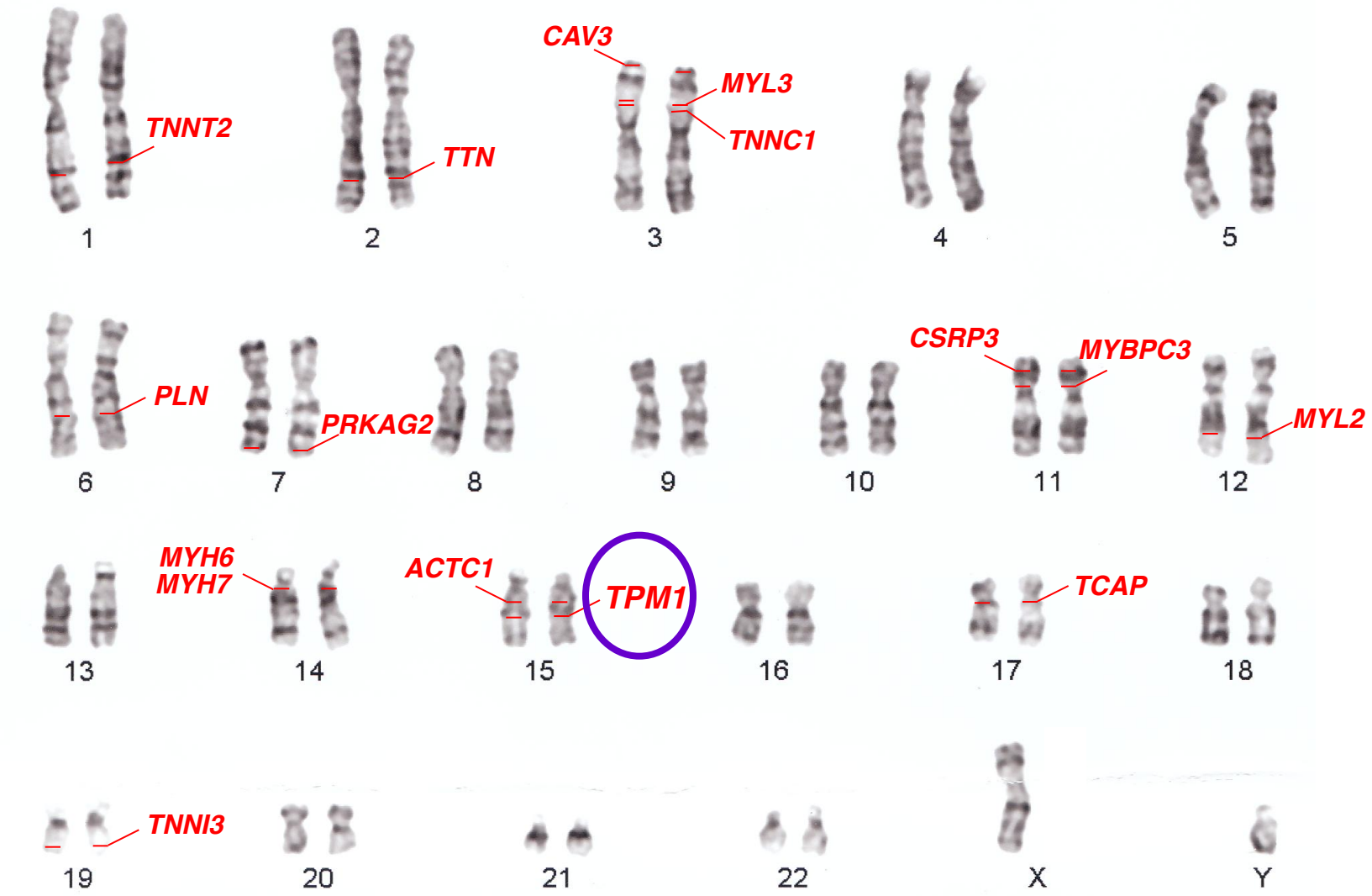
Harbors "Majority" of Mendelian Mutations

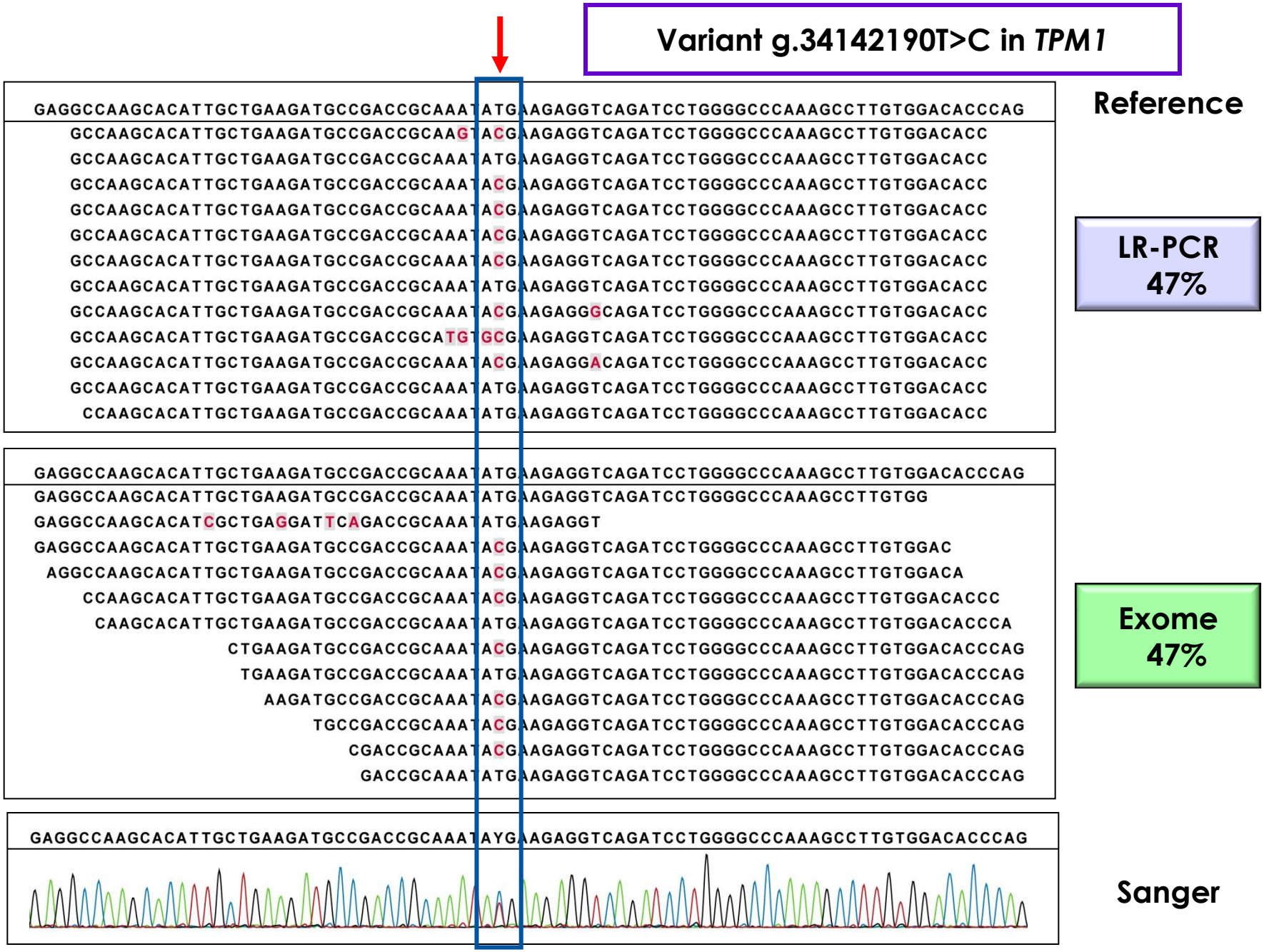
Gene Discovery
~ 40 Publications
July 2011

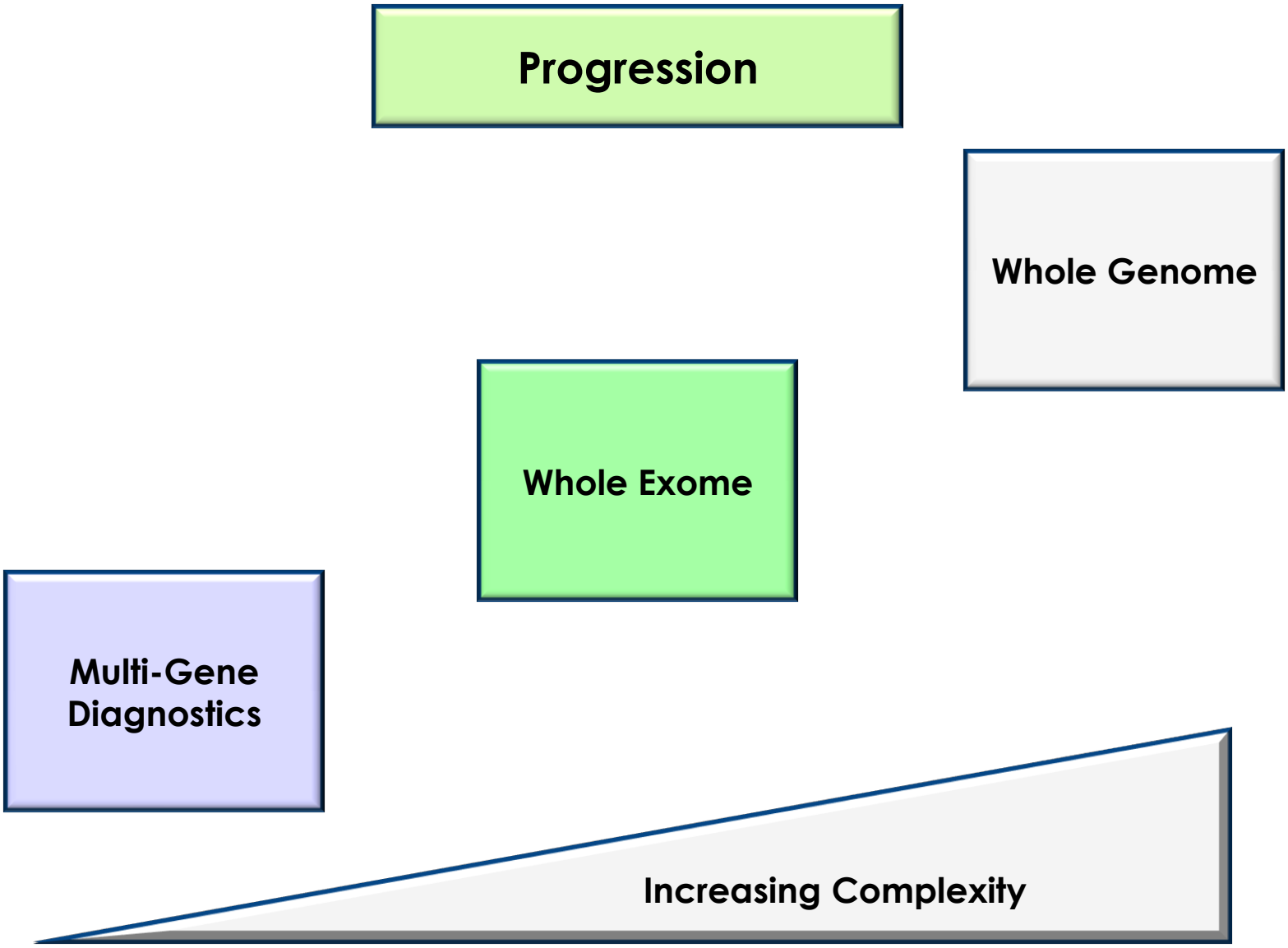



Genomic DNA
Library Preparation
Next Generation Sequencing Library
Hybridize to Exome Capture Probes
Exome Enriched Library
Next Generation Sequencing
Bioinformatics Analysis

Exome Sequencing – Cardiomyopathy Genes




Variant g.34142190T>C in *TPM1*
Reference
LR-PCR 47%
Exome 47%
Sanger


Progression
Whole Genome
Whole Exome
Multi-Gene Diagnostics
Increasing Complexity

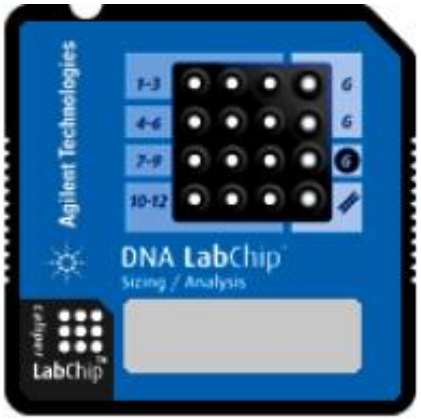
Genomic DNA

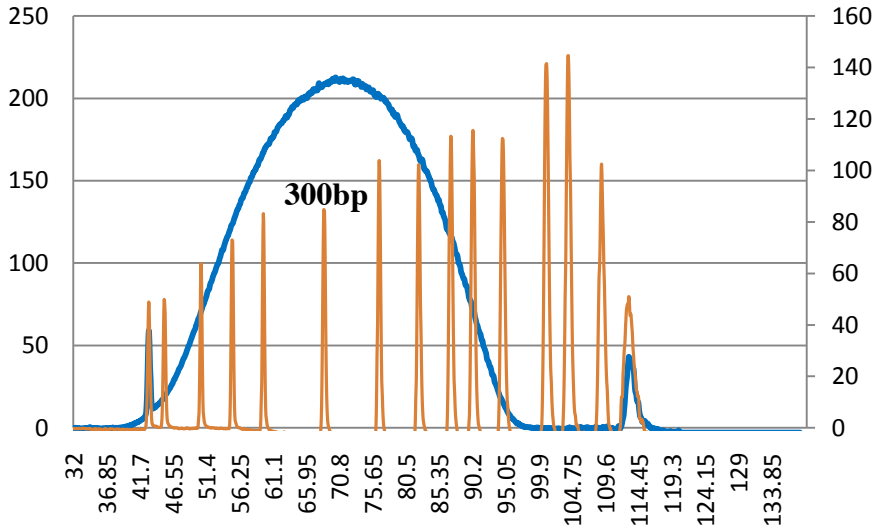
Fragmentation

QC

Process

Covaris
Acoustic Wave





Process

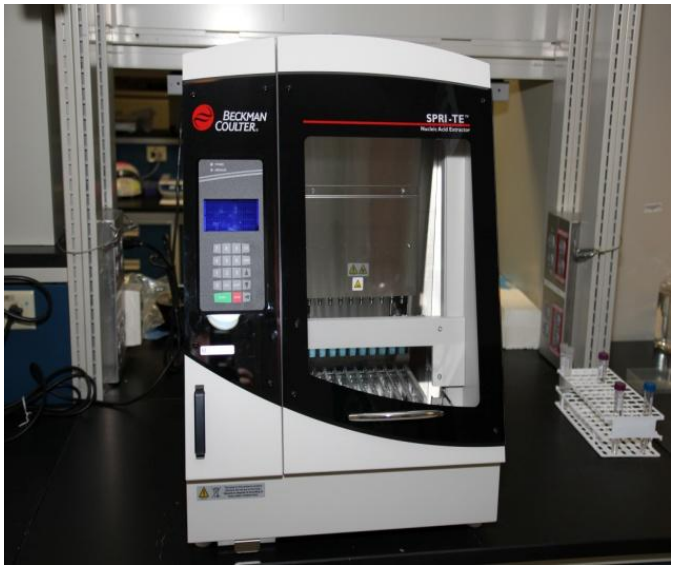
Library Preparation - Illumina

↓

Fragment End Repair

↓

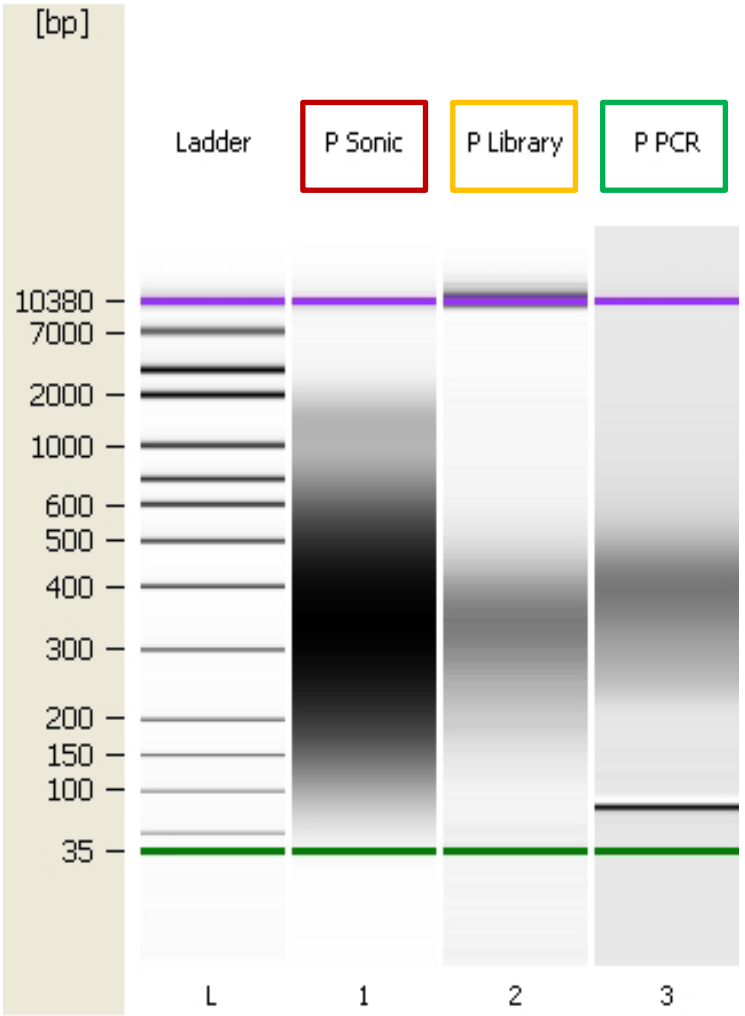
Adapter Ligation



Beckman SPRI-TE

PCR →

QC



BioAnalyzer

Process

PCR Amplified Library

↓

Gel Electrophoresis

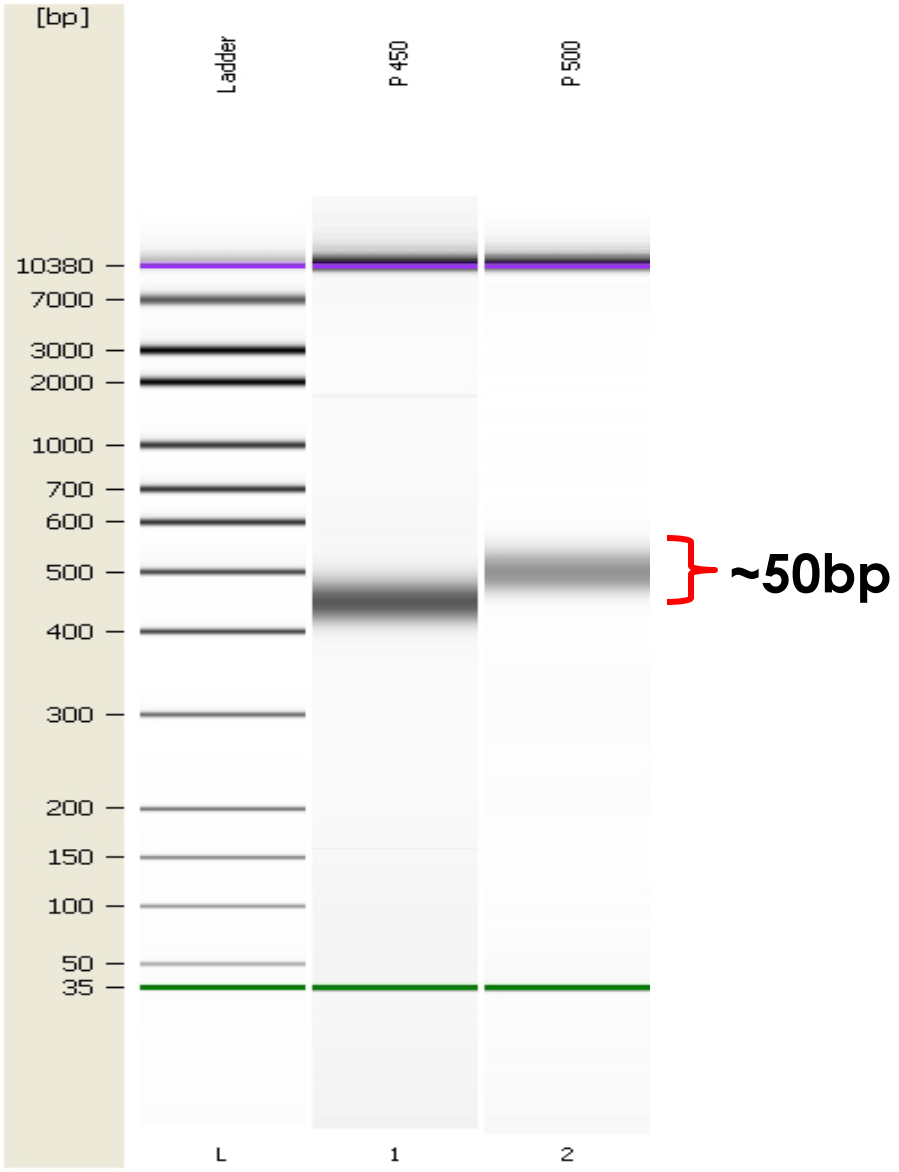
↓

Size Selection

↓

Gel Purification

↳ QC



Process

Gel Purified Library

↓

Quantitative PCR

↓ QC

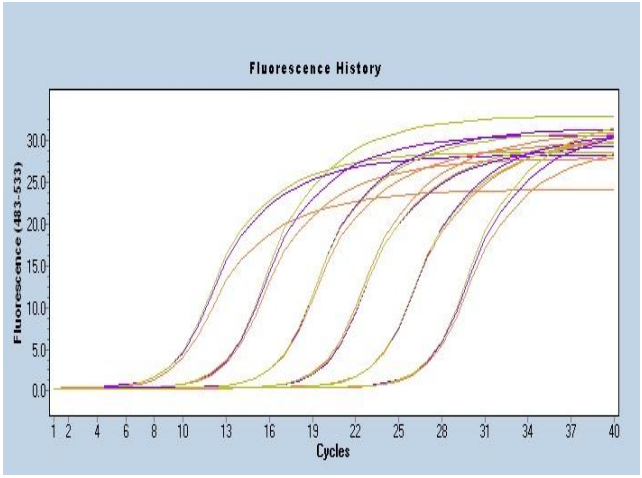
Dilution/Denaturation

↓ 5-7pM

Flow Cell Cluster Generation

↳

Illumina cBot



Process

Sequencing HiSeq 2000

↓

"First Base" Report

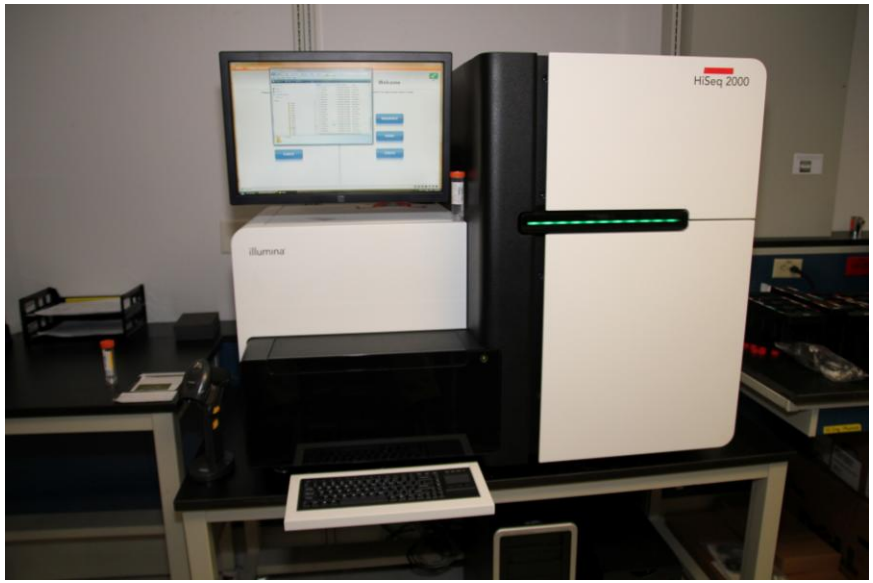
↓ QC

Cluster Densities/Intensities

↓

Real Time Analysis - Cycle 25+

↓ QC



2 X 100bp
Pair End

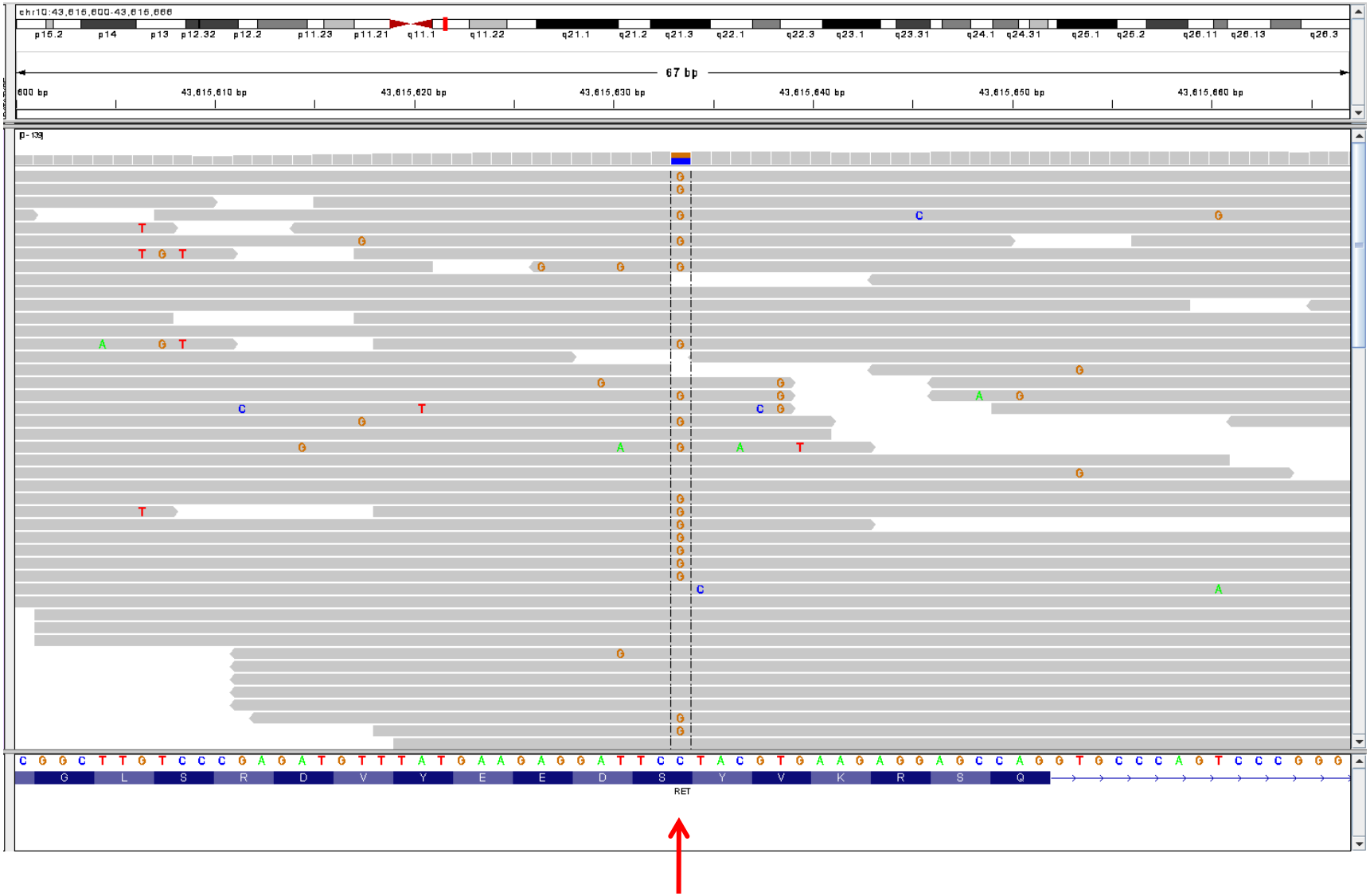
→

Target
~ 100+ Gb

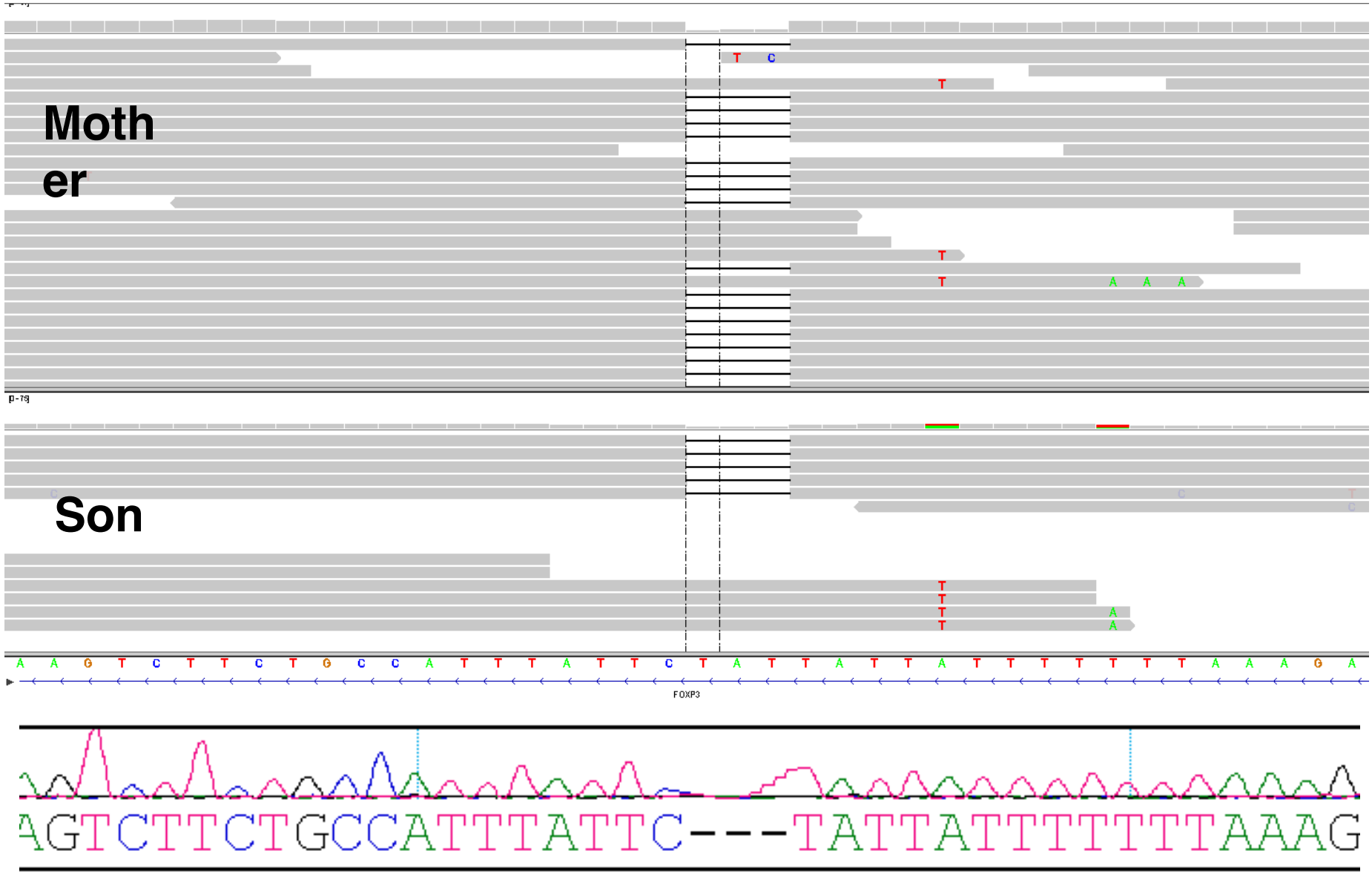
→

FastQ Files

Whole Genome Sequencing
Chr 10: g.43,615,633C>G in *RET*



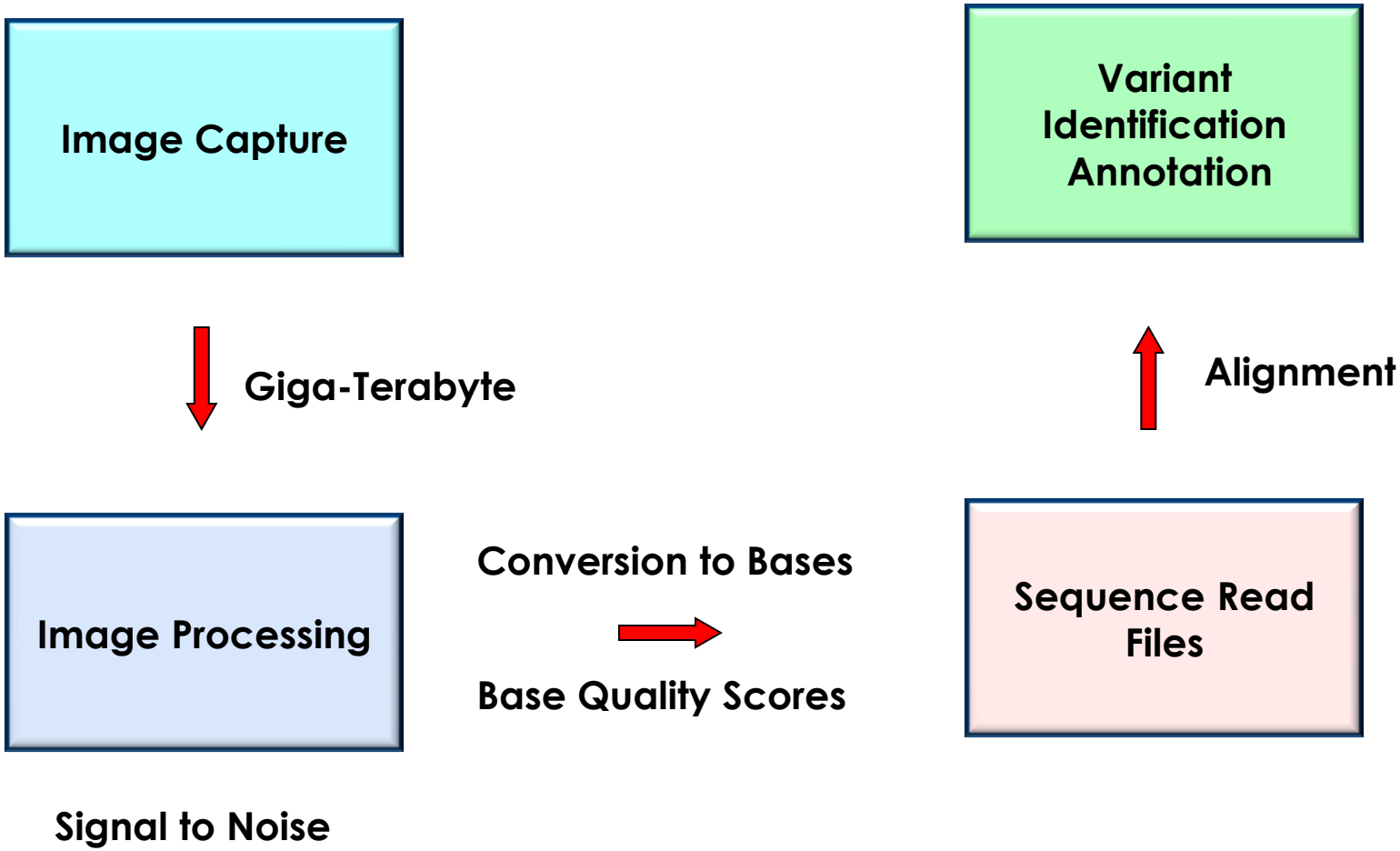
Whole Genome Sequencing
Chr X: 3bp deletion in *FOXP3*



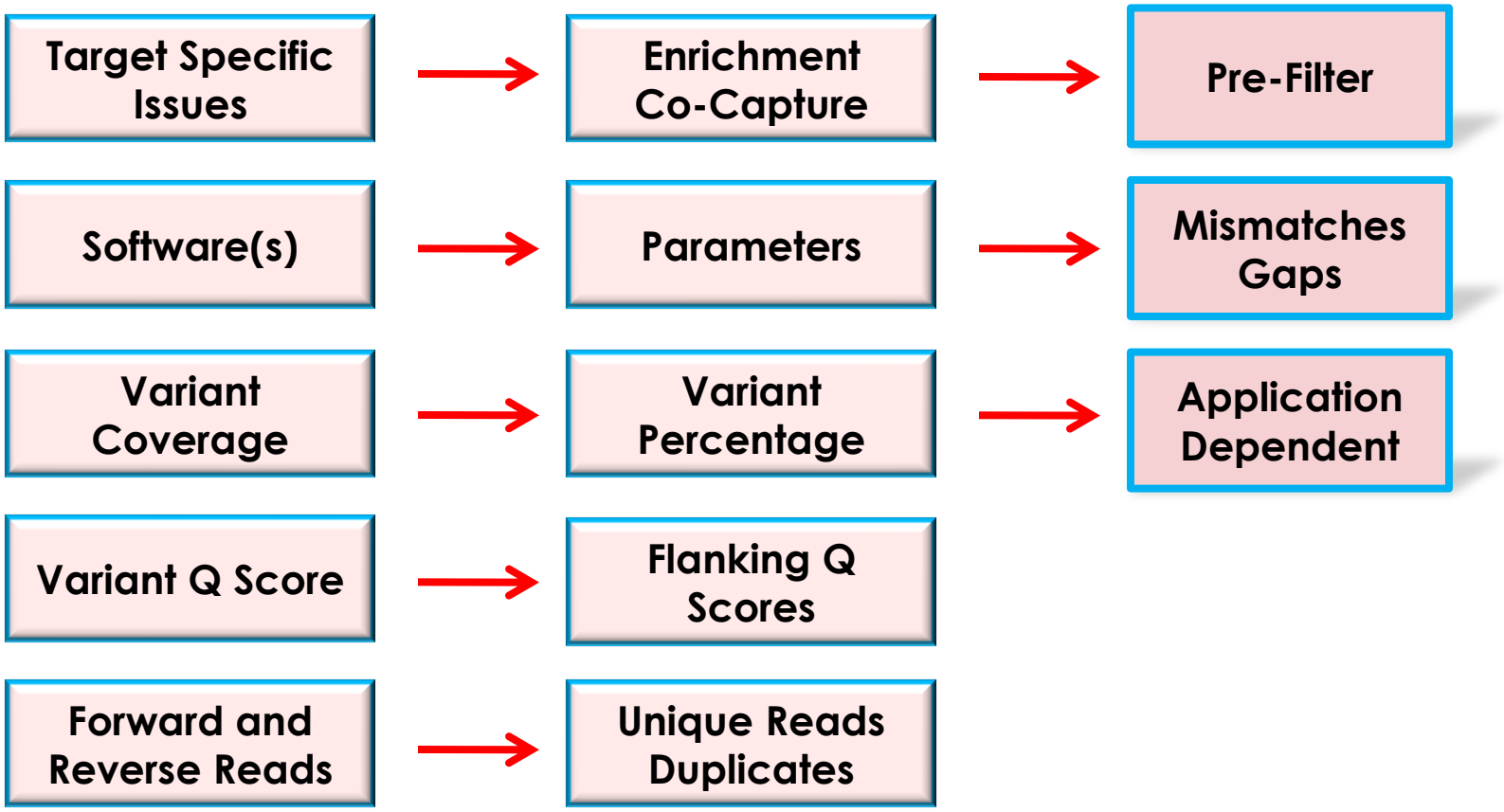
Outline

- Progression: Gene Panels to Genomes
- Next Generation Sequencing Technology
- **Bioinformatics**

Next Generation Sequencing Bioinformatics



Alignment Considerations



Alignment Softwares

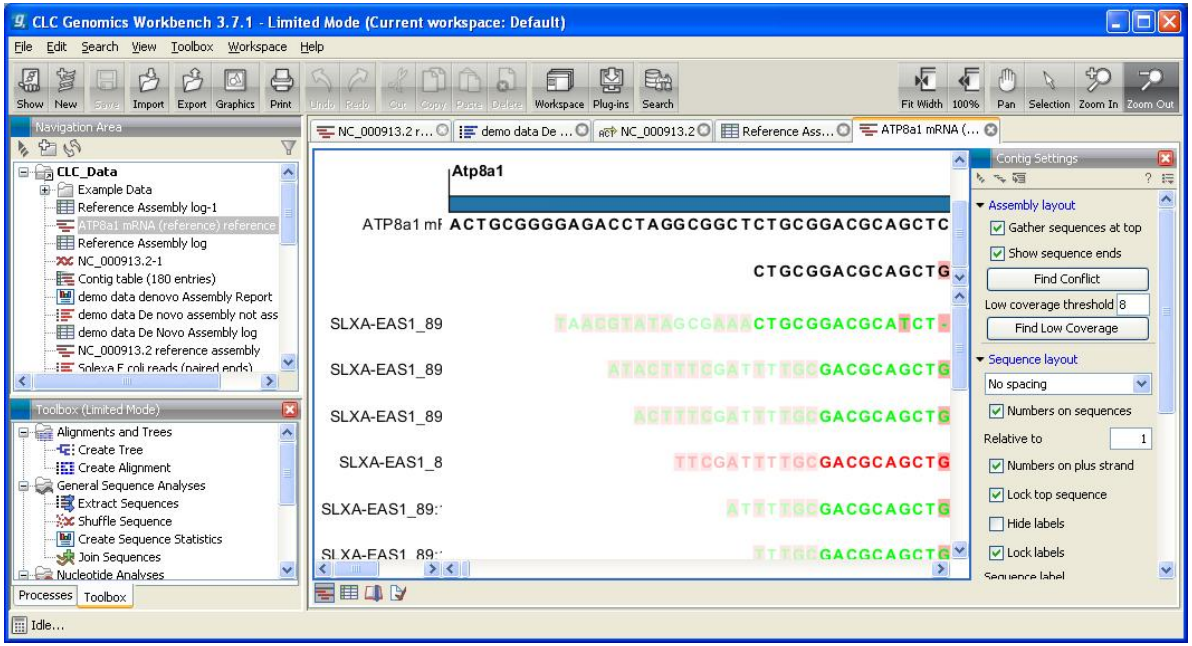
Academic Softwares

- Command Line
- Free
- Genome community support

```
maq assemble [-sp] [-m maxmis] [-Q maxerr] [-r
   hetrate] [-t coef] [-q minQ] [-N nHap]
   out.cns in.ref.bfa in.aln.map 2> out.cns.log
```

Commercial Softwares

- Feature rich user interface
- $$$
- Company support



Alignment Softwares

Commercial Softwares

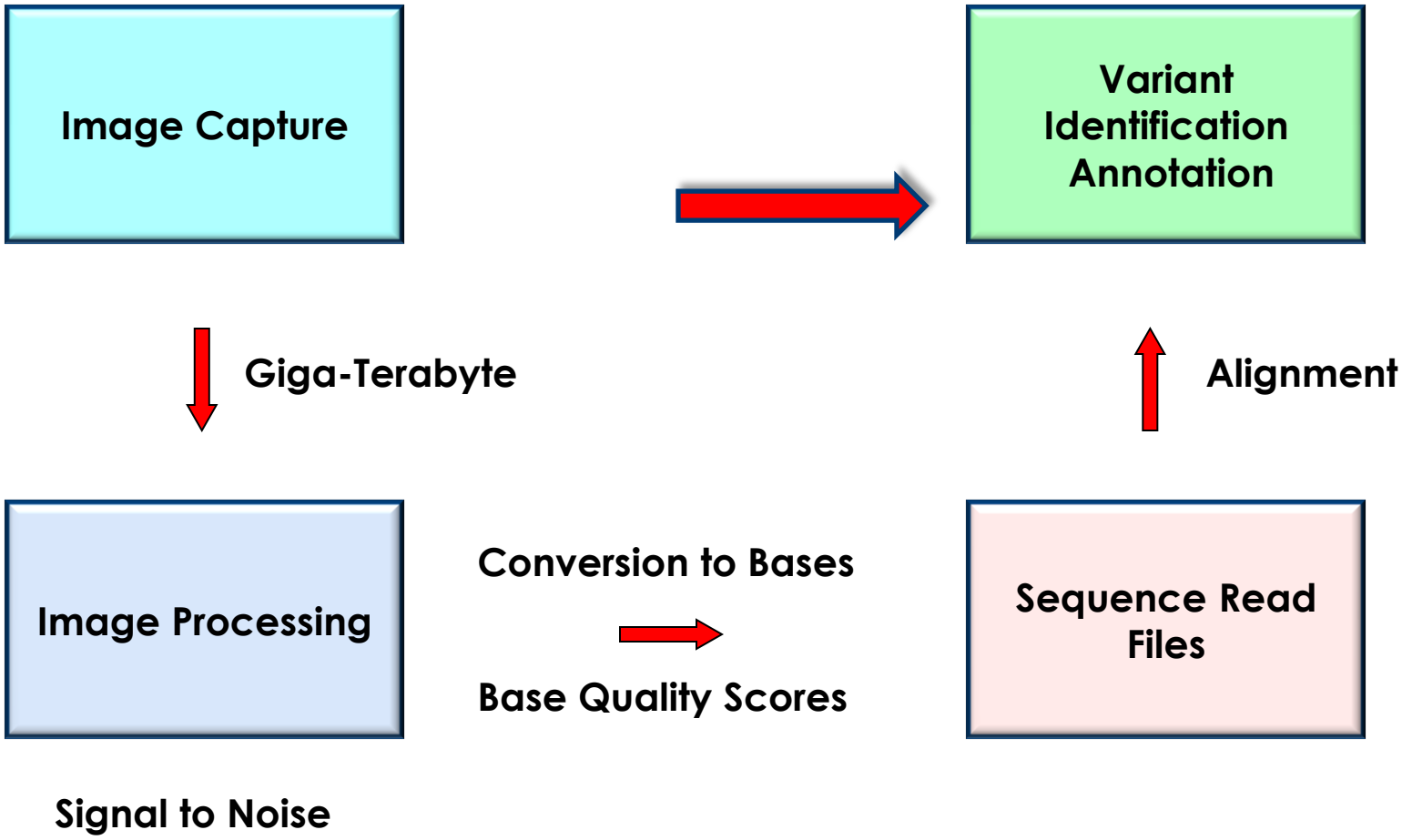
Academic Softwares

BFAST (UCLA)

BWA (Sanger Institute)

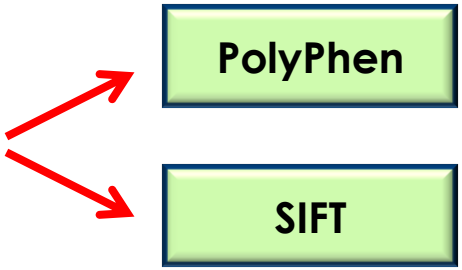
SAMtools (Sanger Institute)

Next Generation Sequencing Bioinformatics



Variant Annotation

- ✓ dbSNP ← 1,000 Genome Project
- ✓ OMIM
- ✓ Human Genome Mutation Database
- ✓ Locus Specific Databases
- ✓ Literature and Internet
- ✓ Functional Prediction Programs



Convergence of Chemistry + Bioinformatics

Pipelines

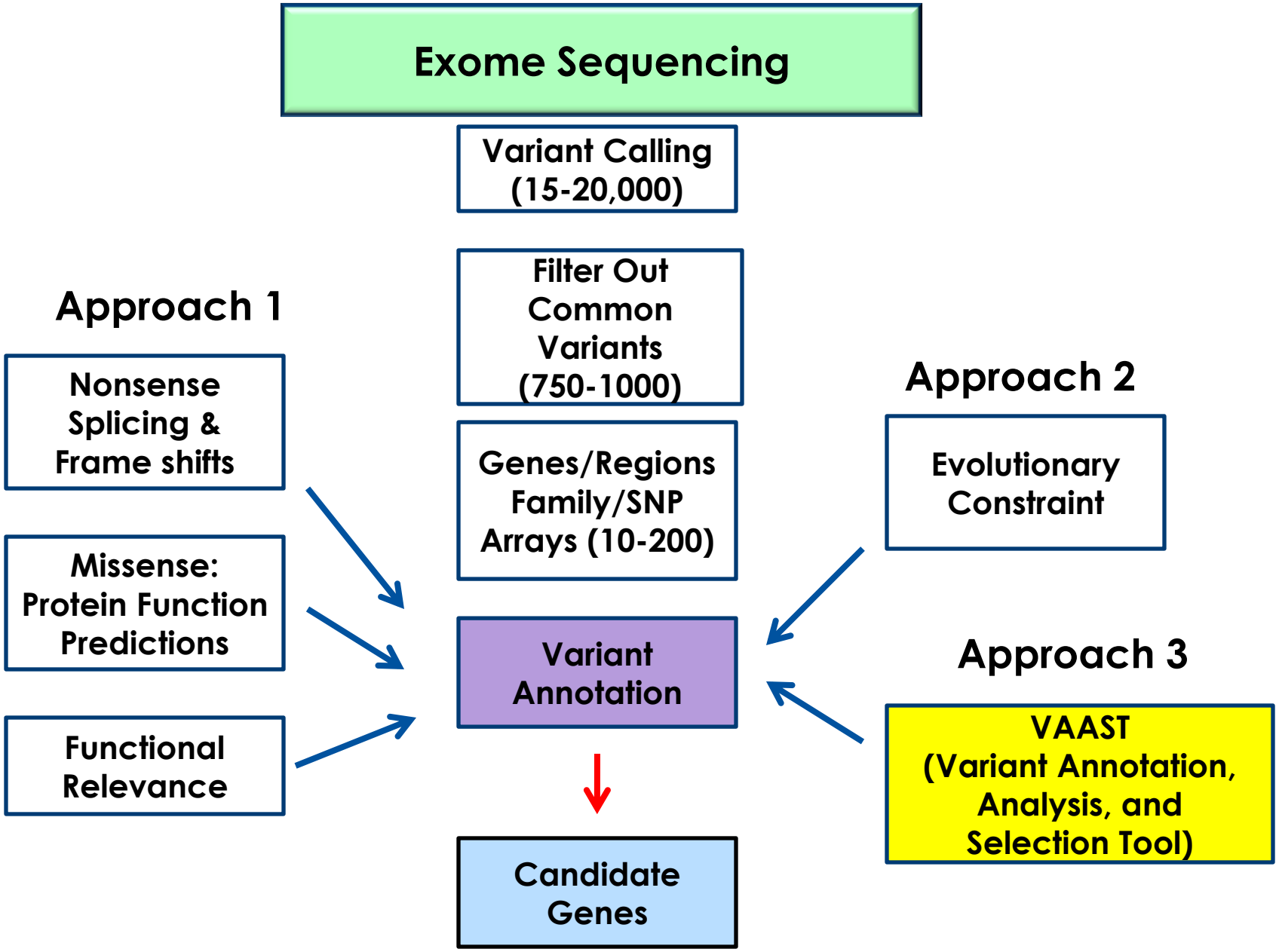
Manually Complex

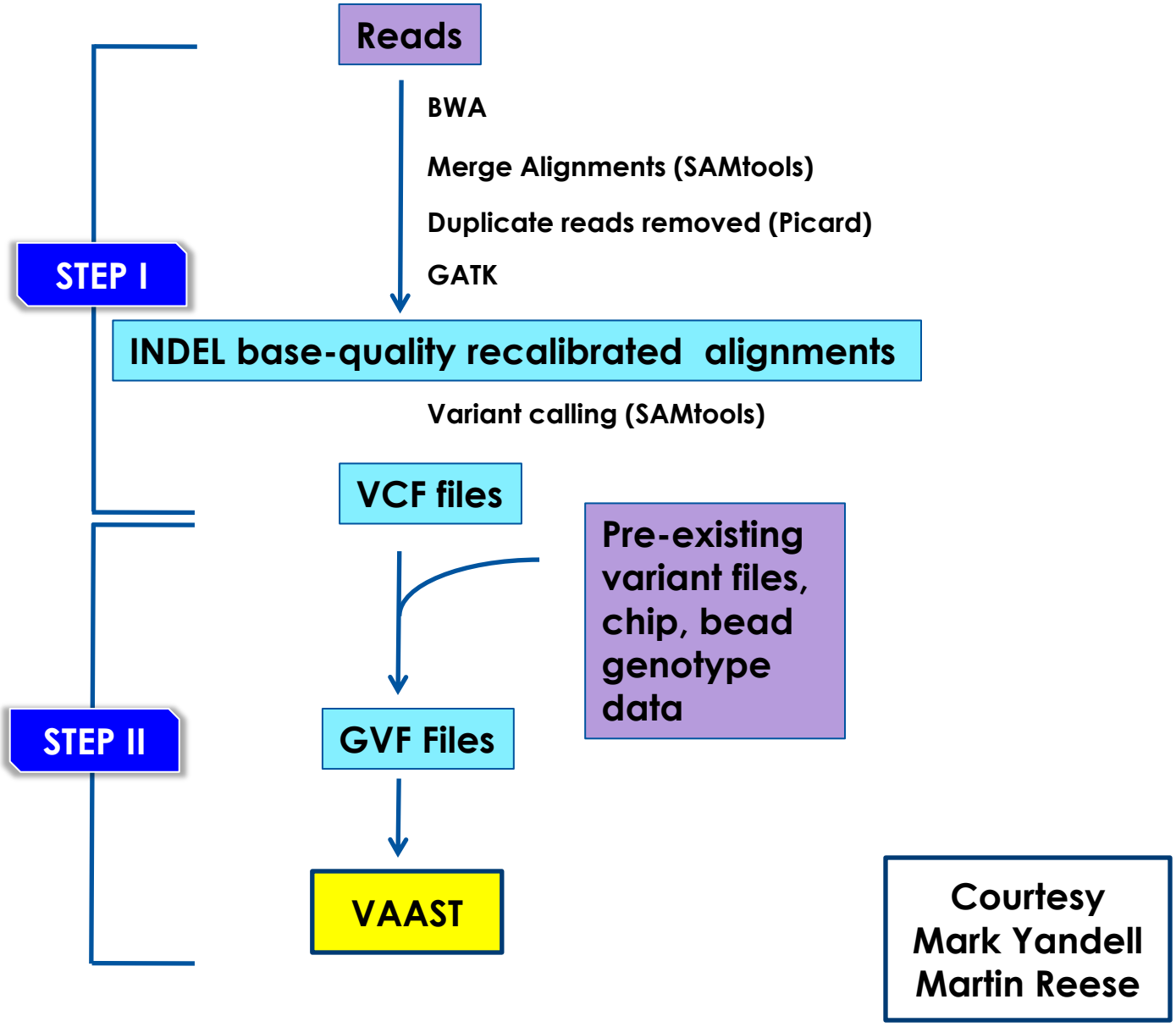
Large Cognitive Component

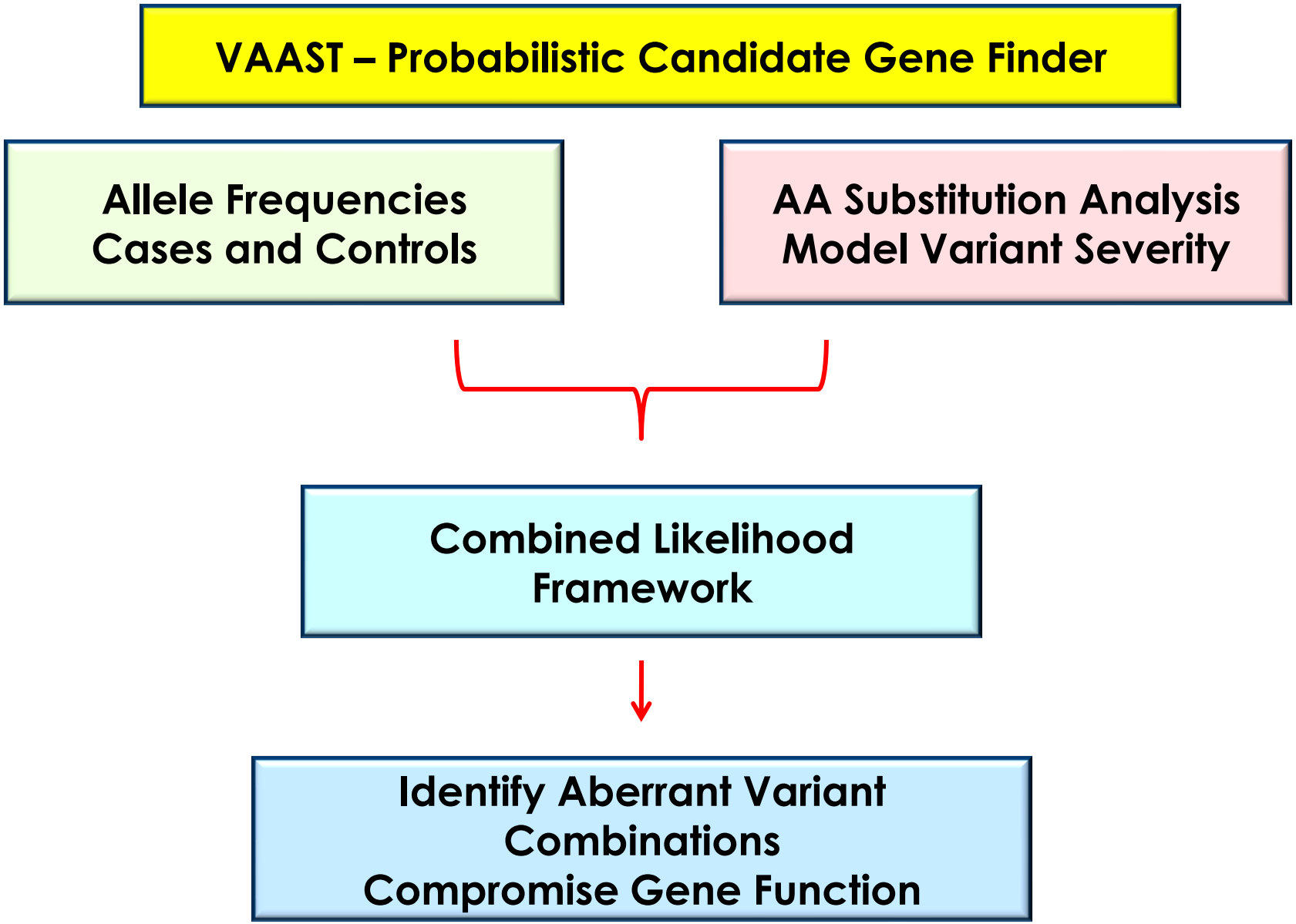
Automation

TCGAAGTCTGCCTAGCTGT

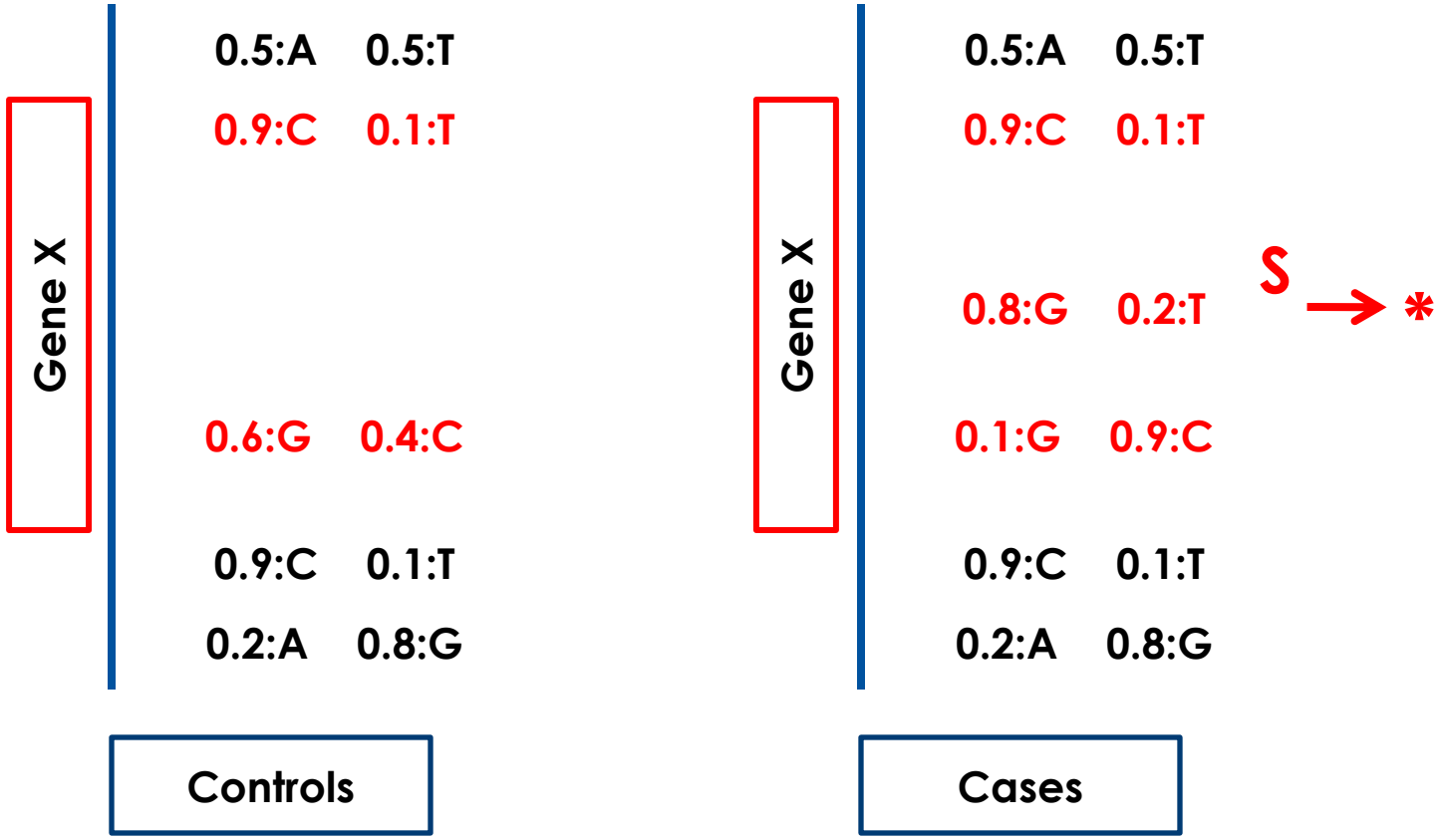
CCGTACGTCTGATGCGTA


Exome Sequencing
Variant Calling (15-20,000)
Filter Out Common Variants (750-1000)
Genes/Regions Family/SNP Arrays (10-200)
Approach 1
Nonsense Splicing & Frame shifts
Missense: Protein Function Predictions
Functional Relevance
Approach 2
Evolutionary Constraint
Approach 3
VAAST (Variant Annotation, Analysis, and Selection Tool)
Variant Annotation
Candidate Genes


Reads
BWA
Merge Alignments (SAMtools)
Duplicate reads removed (Picard)
GATK
STEP I
INDEL base-quality recalibrated alignments
Variant calling (SAMtools)
VCF files
Pre-existing variant files, chip, bead genotype data
STEP II
GVF Files
VAAST
Courtesy
Mark Yandell
Martin Reese


VAAST – Probabilistic Candidate Gene Finder
Allele Frequencies
Cases and Controls
AA Substitution Analysis
Model Variant Severity
Combined Likelihood
Framework
Identify Aberrant Variant
Combinations
Compromise Gene Function

VAAST – Probabilistic Candidate Gene Finder



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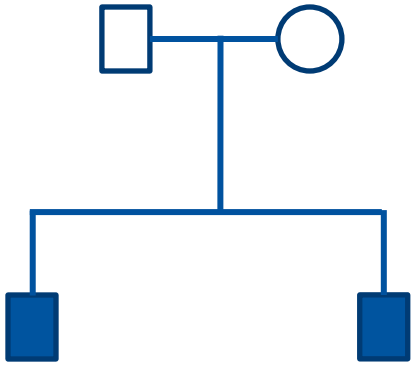
VAAST – Miller Kindred Quartet



Miller + PCD

Exome Sequence Data

Two Siblings

= Affected

VAAST

VAAST – Miller Kindred Quartet

Caucasian Only (65 genomes)				
Genome-wide Significant Genes	DHODH		DNAH5	
	Rank	P-Value	Rank	P-Value
17	14	9.93E-07	19	5.79E-05

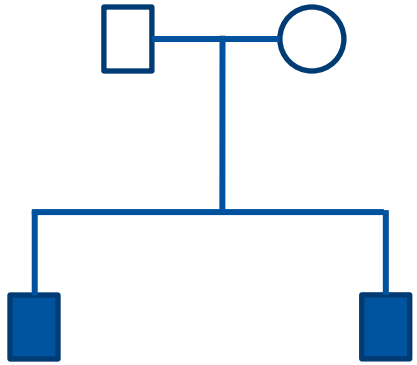
Mixed Ethnicities (189 Genomes)				
Genome-wide Significant Genes	DHODH		DNAH5	
	Rank	P-Value	Rank	P-Value
9	4	7.60E-09	5	1.18E-08

Miller : *DHOD*

PCD: *DNAH5*

GWS Alpha is 2.4 E-6

VAAST – Miller Kindred Quartet



Miller + PCD

Exome Sequence Data

Two Siblings

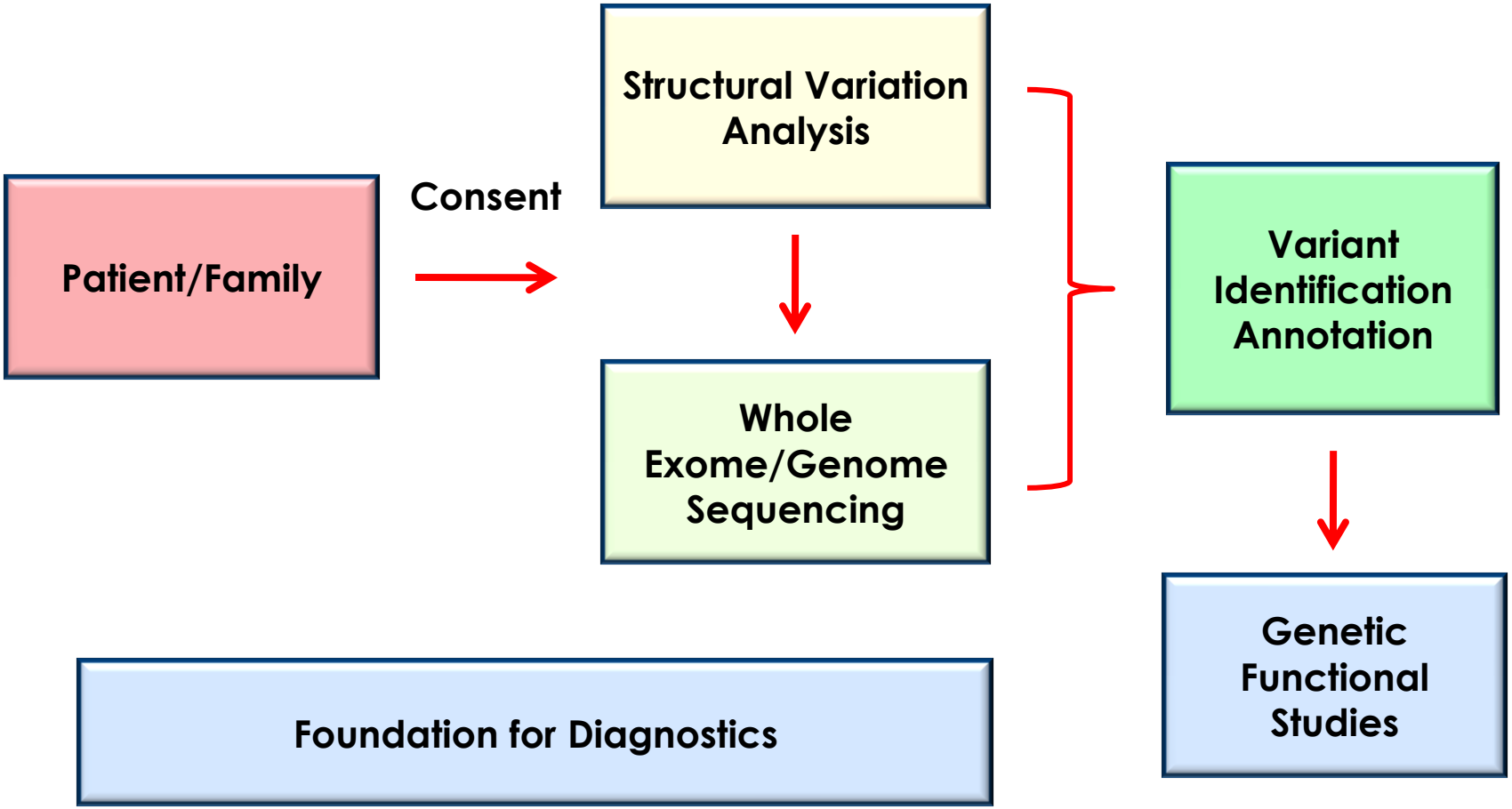
Parents

= Affected

VAAST

VAAST – Miller Kindred Quartet

Genomics Clinical Research Program



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Summary

- **Progression: Gene Panels to Genomes**
- **Next Generation Sequencing Technology**
- **Bioinformatics**

Acknowledgements

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Omicia
Martin Reese

ARUP Laboratories Institute
for
Clinical and Experimental Pathology

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Next in the Series of Free PHC Webinars

- **How to Have Successful Patient Interactions, Wednesday, August 17th, 11:00-12:00 pm CT**
  - Mary Ann Abrams, MD, MPH & Barbara Savage, MT(ASCP)
- Go to www.cap.org/institute For All Upcoming Webinars!

- Past Webinars Available Now Online at www.cap.org/institute
  - Accountable Care Organizations
  - Whole Genome Analysis as a Universal Diagnostic
  - How to Build and Fund a Financially Viable Molecular Lab
  - Cancer: The Critical Role of Pathology
  - Molecular Markers in Breast Cancer
  - Bethesda System: Integrating Cytology and HPV Molecular Testing
  - Molecular Diagnosis for Lung Cancer Patients
  - Molecular Diagnosis for Colorectal Cancer Patients

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CAP Events of Interest

- Don't Forget to Register for CAP'11 – THE Pathologists' Meeting – September 11 – 14, 2011 held at the Gaylord Texan in Grapevine, Texas!

  – Go to www.cap.org/CAP11 or call 1-800-967-4548. International attendees please call 1-847-996-5891.

For more information go to www.cap.org/CAP11

Tuesday, Sept 12th:

TP120 Breakfast Workshop – Hot Topics in Pathology: What Every Community Pathologist Should Know About Clinical Requests for Molecular Tests (6:30-7:45 am)
Faculty--Samuel K. Caughron, MD, FCAP
Frederick L. Kiechle, MD, PhD, FCAP
Michael S. Brown, MD, FCAP

ST109 Companion Diagnostics for Targeted Therapy in Cancer (2:00-5:30 pm)
Faculty--Sanja Dacic, MD, PhD, FCAP
David Hicks, MD, FCAP
Jeffrey Kant, MD, PhD, FCAP

Wednesday, Sept 13th:

ST110 Direct-to-Consumer Genetic Testing: Staying Ahead of Patients in This Current Trend (8:00-9:00 am)
Faculty--Nazneen Aziz, PhD
Elizabeth A. Mansfield, PhD

ST111 What's in It for Me? Using Technology to Become a Diagnostic Hero (8:00-11:30 am)
Faculty--Kenneth J. Bloom, MD, FCAP
John W. Turner, MD, FCAP

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