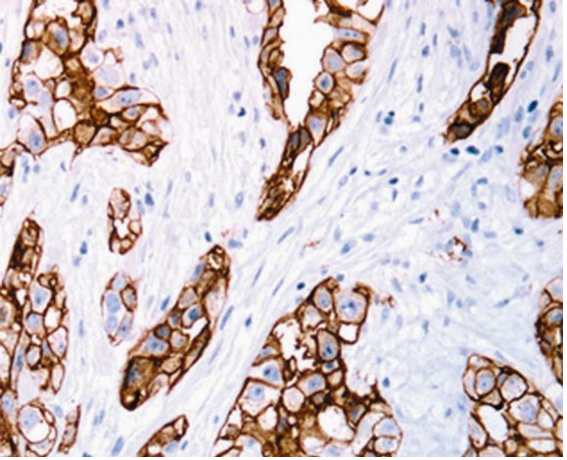
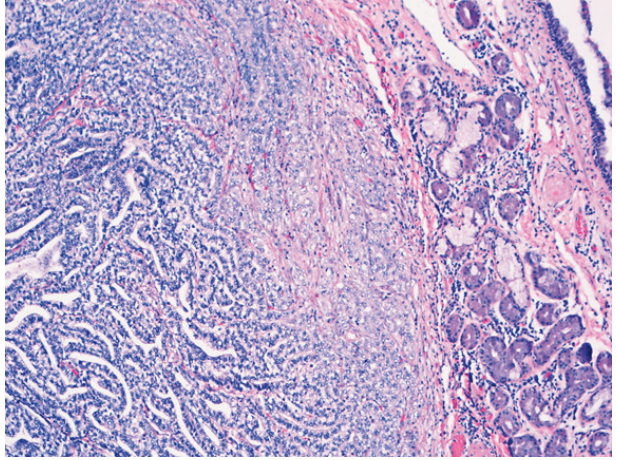
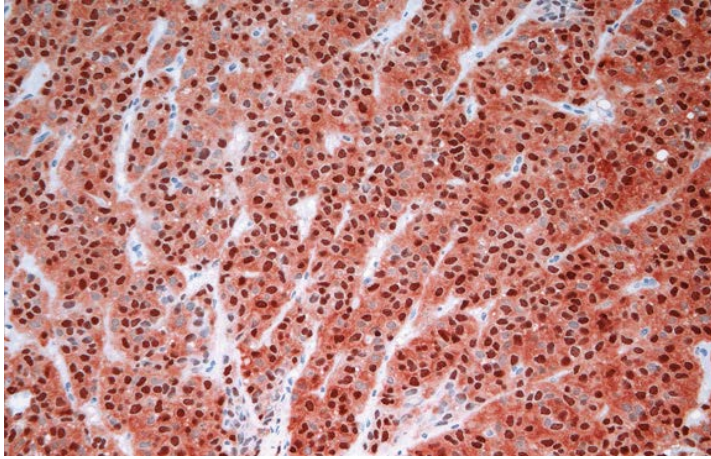
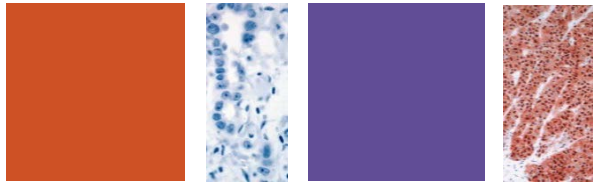




Next Generation Sequencing for Solid Tumors Diagnostics: Current Practice and New Developments

Larissa V. Furtado, MD FASCP

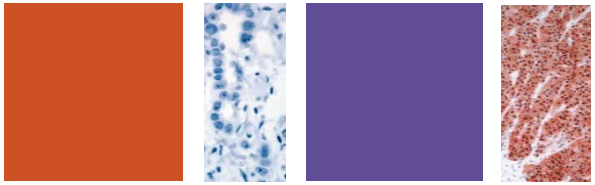


Speaker Disclosure

In the past 12 months, we have not had a significant financial interest or other relationship with the manufacturer(s) of the product(s) or provider(s) of the service(s) that will be discussed in our presentation.

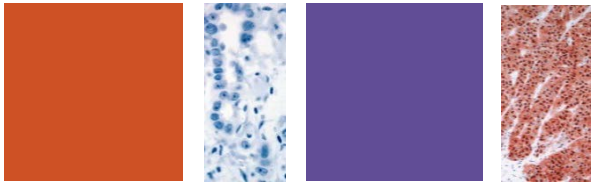
Larissa V. Furtado, MD FASCP

Program Objectives



1. Demonstrate familiarity with next-generation sequencing (NGS) and the various applications for which it can be used in the oncology setting.
2. Recognize the indications, specimen requirements, assay design considerations and limitations of NGS-based testing for solid tumors.
3. Understand interpretive principles for review and reporting of clinically relevant findings within the proper solid tumor contexts.
4. Become familiar with future trends in personalized tumor management

Sections



1. Introduction to Personalized Oncology Diagnostics
2. Technology, Test Selection and Test Capabilities
3. Future Trends in Solid Tumor Genomic Diagnostics

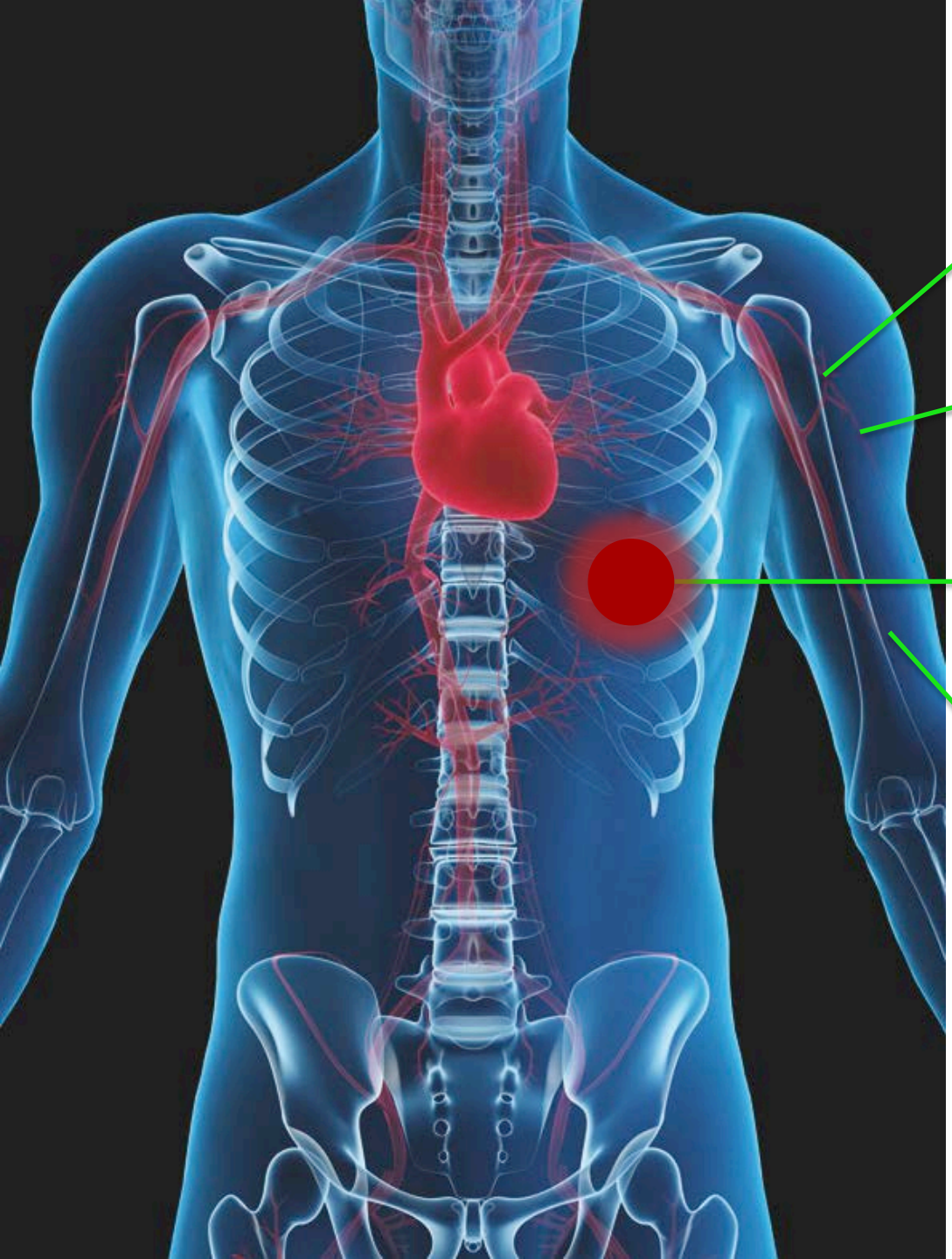
Personalized Medicine in Oncology

Prediction

Diagnosis

Prognosis

Therapy



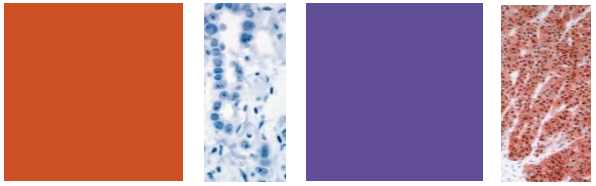
Inborn genetics:
- Genetic disease
- Risk factors

Disease Genetics:
- Early screening

Disease Genetics:
- Diagnosis
- Prognosis
- Therapy

Disease Genetics:
- Residual disease testing
- Resistance mutation surveillance

Cancer Genomics Targets



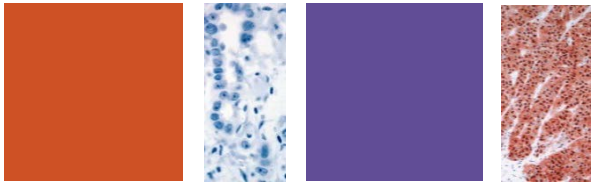
Mutations (TS and OG)
Point mutations
Insertions and deletions (indels)

Structural Variations
Large scale deletions/duplications
Fusions/rearrangements
Aneuploidy
Chromothripsis

Epigenetics
Altered DNA methylation
Altered histone methylation
Altered DNA-protein interactions
Altered chromatin structure

Gene Expression
OG or TS dysregulation
Pathway activation
MicroRNAs
LncRNAs
Alternative Splicing
Allele-specific expression
RNA binding protein interactions

Cancer Genomics Targets



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Gene Expression
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Pathway activation
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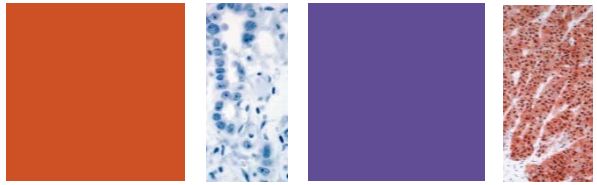
Alternative Splicing
Allele-specific expression
RNA binding protein interactions

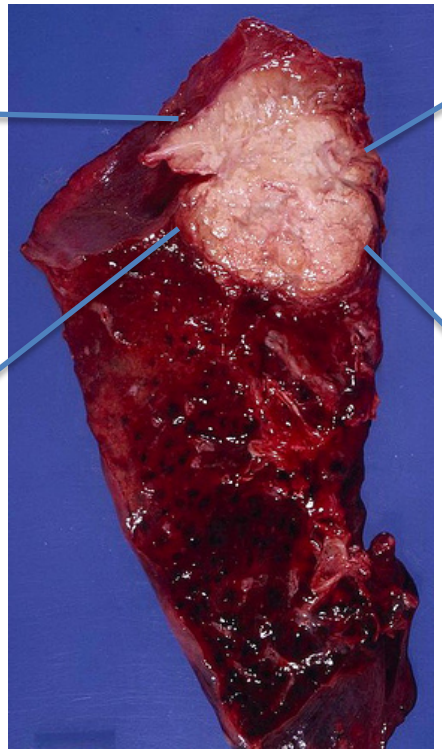
NGS – Effective at All Size Scales

Lung Cancer Targets



Point Mutations
EGFR L858R, G719S, etc.
KRAS
PIK3CA
BRAF

Copy Number Alterations
MET amplification
EGFR amplification

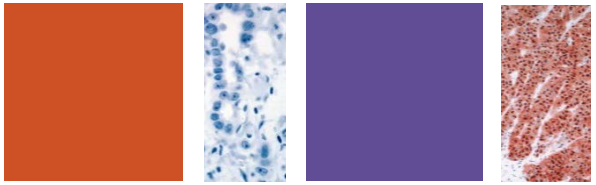


Lung Adenocarcinoma

Small Deletions
EGFR exon 19

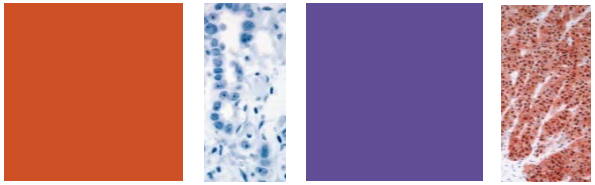
Gene Fusions
ALK (e.g. EML4-ALK)
RET
ROS1
NTRK1

NGS vs. Traditional Methods



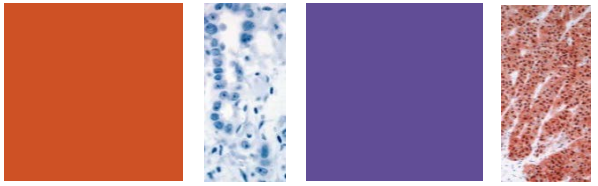
- Multiple anomalies at different genomic scales can be assayed simultaneously.
- More sensitive than Sanger sequencing.
- Single extraction and single test instead of multiple tests.
  - Cost effective
  - Improved turn-around time by avoiding sequential testing
  - Tissue preservation – many genes simultaneously assessed from single extraction
- Potential for discovery of novel actionable targets.
- Extreme flexibility of analysis types.

NGS Oncology Challenges



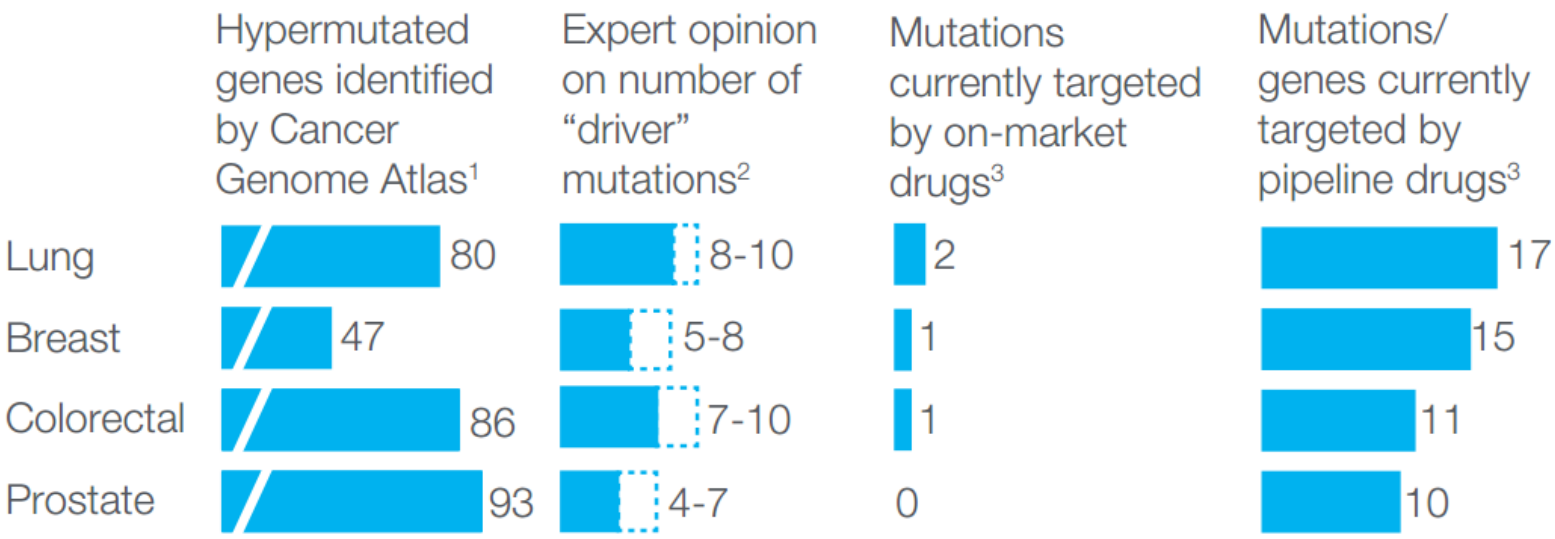
- Cost of implementation
- Significant requirement for informatics infrastructure and expertise
- Rapidly changing nature of technologies
- No standardized guidelines available for data analysis, interpretation and reporting
- Uncertainty of reimbursement
- Uncertainty of clinical utility
- Intense market competition

Commercial Testing Landscape



- Assay types
  - Cancer profiling panels (small to large):
    - Mutations
    - Copy number changes
    - Translocations
  - Circulating tumor DNA assays
  - Immune clonality profiling
    - Lymphoma (including residual disease testing)
    - Tumor-associated lymphocytes
- Hype vs. reality?

Personalized Therapeutics in Oncology



1 Based on q-value analysis using MutSig software from the Broad Institute
2 Based on expert interviews
3 Based on Evaluate Pharmaceuticals database; for pipeline, includes Phase 1 and above only

- A significant number of mutated genes have been identified in the four major tumor types, although only a limited set have been shown to be "driver" mutations.

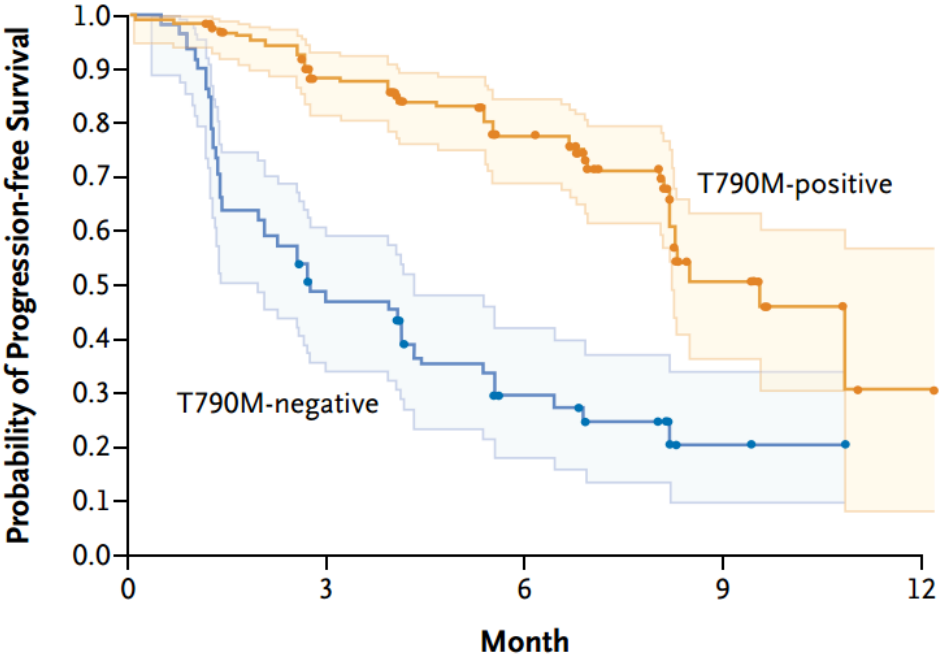
- The number of actionable mutations remains limited
  - Pharma companies are developing drugs against a number of other gene targets as well as 2nd- or 3rd-line treatments

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 30, 2015 VOL. 372 NO. 18

AZD9291 in EGFR Inhibitor–Resistant Non–Small-Cell Lung Cancer

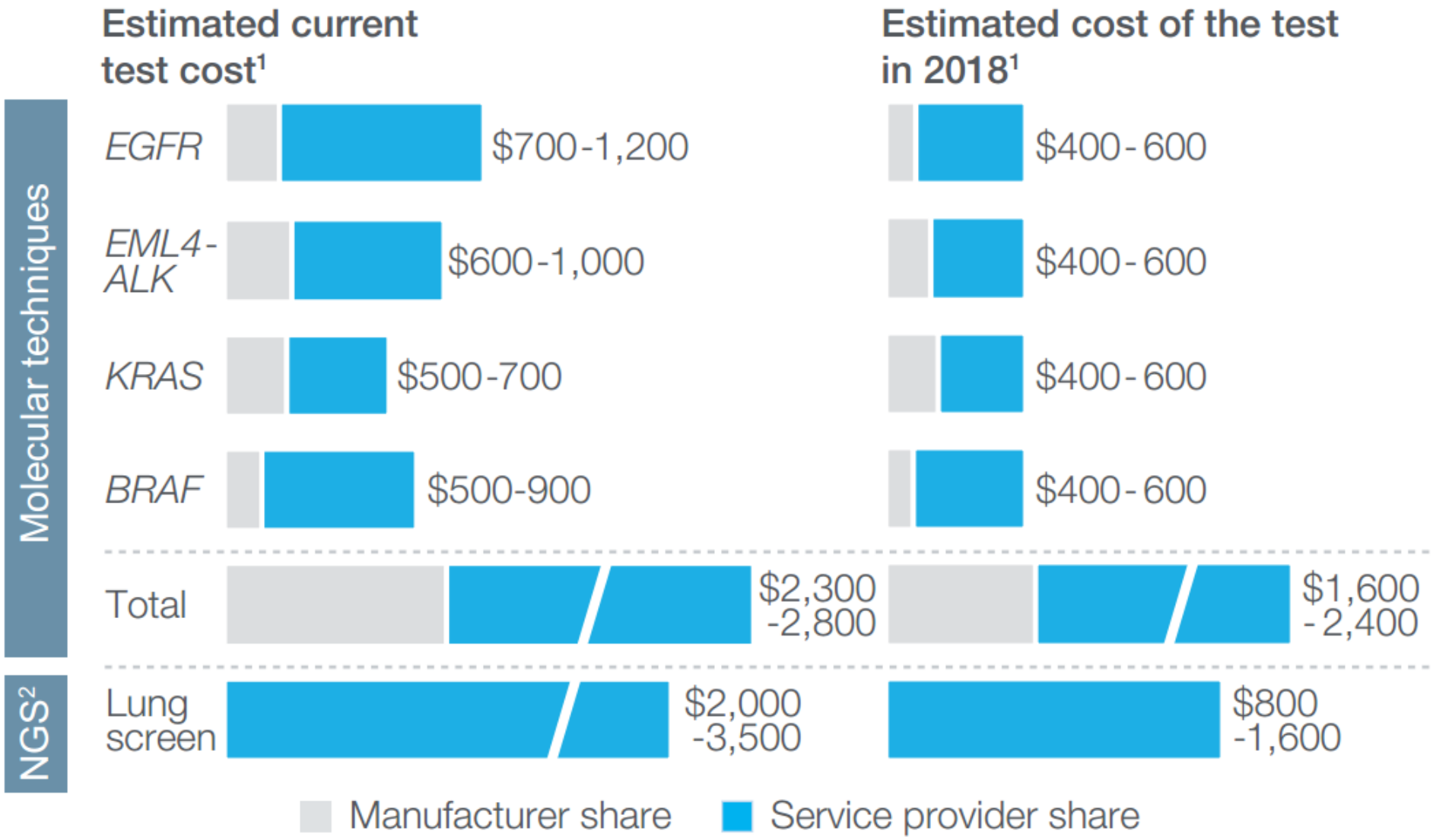
TAGRISSO™ (AZD9291) approved by the US FDA for patients with *EGFR* T790M mutation-positive metastatic non-small cell lung cancer



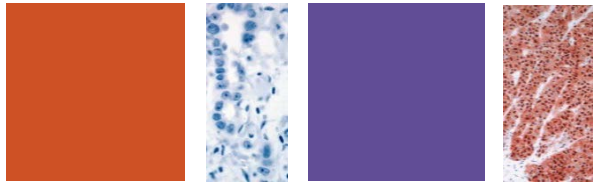
Objective response rate of 59% and duration of response of 12.4 months.

N Engl J Med. 2015 Apr 30;372(18):1689-99

Opportunity for Panel Testing – lung cancer example



Sections



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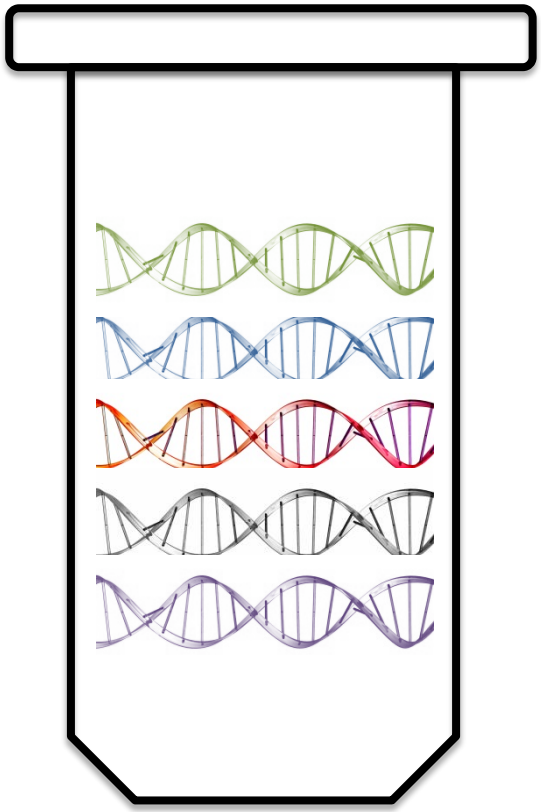
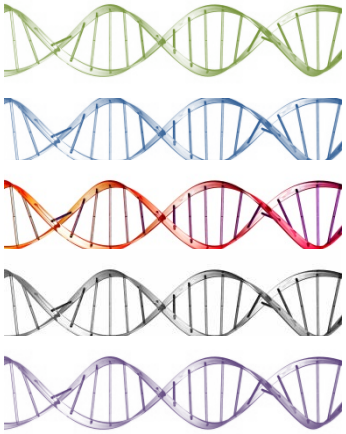
ABCs of NGS

Next Generation Sequencing

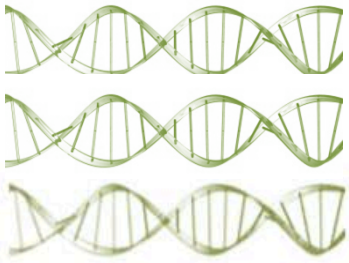


Random DNA

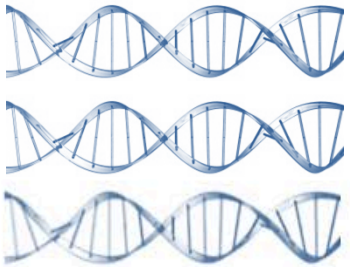
Next Generation Sequencing



Next Generation Sequencing

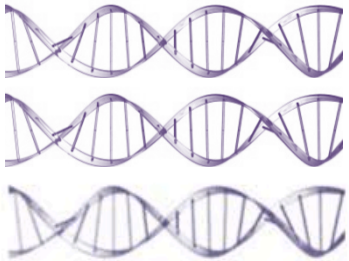


ACTGGTCAGCT

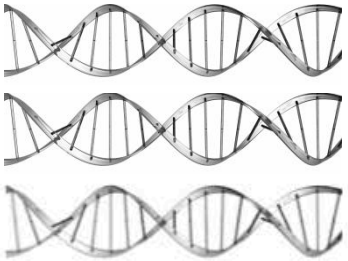


CCCCCATTATAA

TCTCTCTCATAT



GCTAAAATAAA

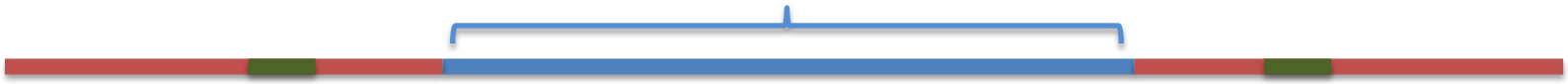


TTCAATATCGGG

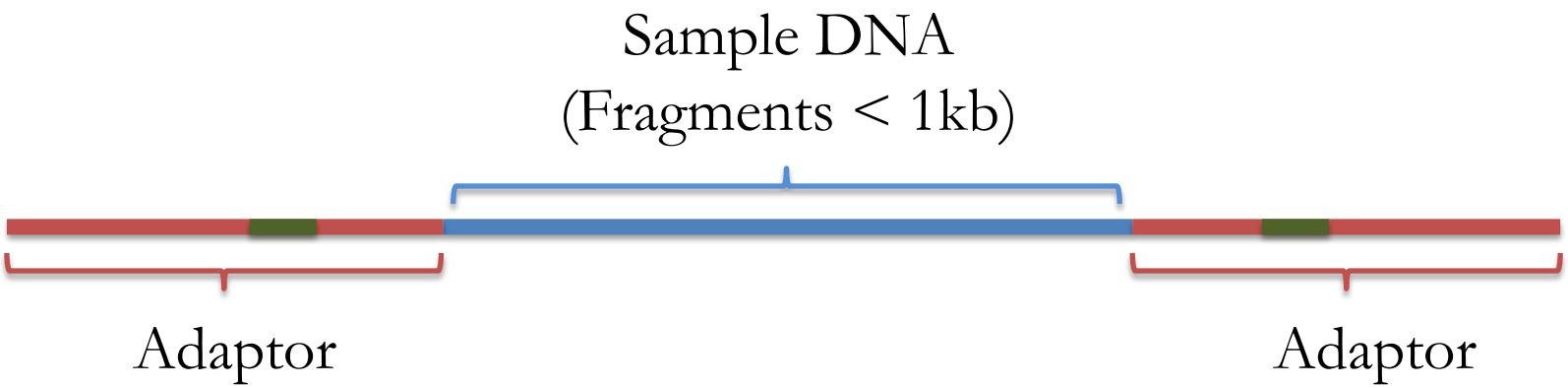
Library Preparation

Library Preparation

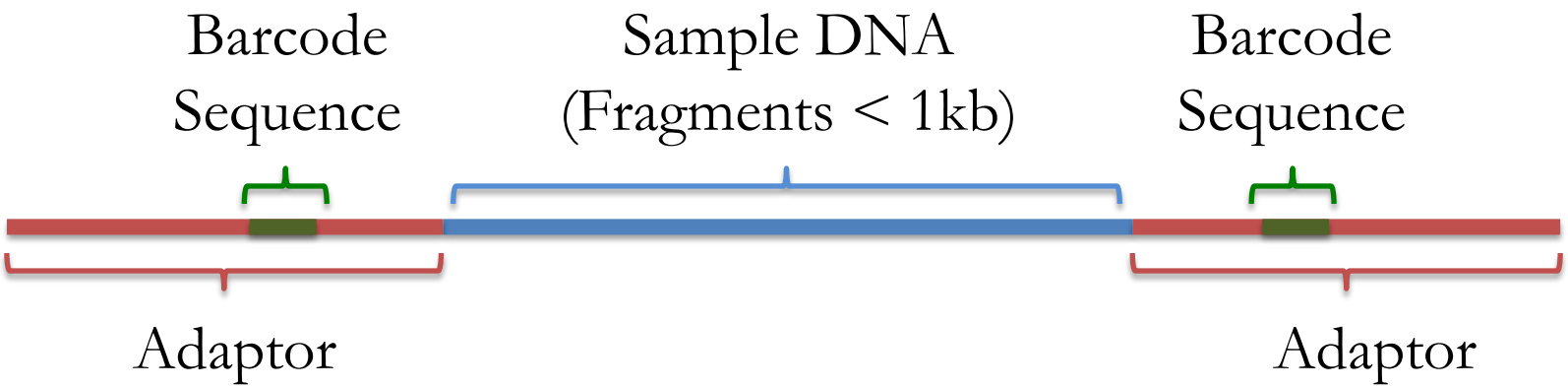
Sample DNA
(Fragments < 1kb)



Library Preparation

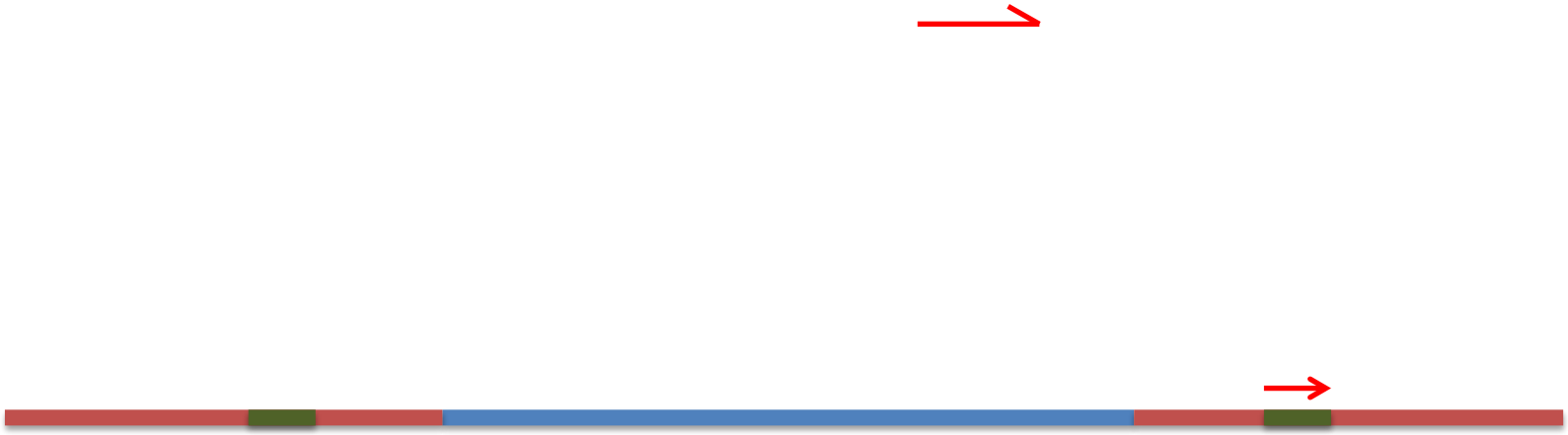


Library Preparation



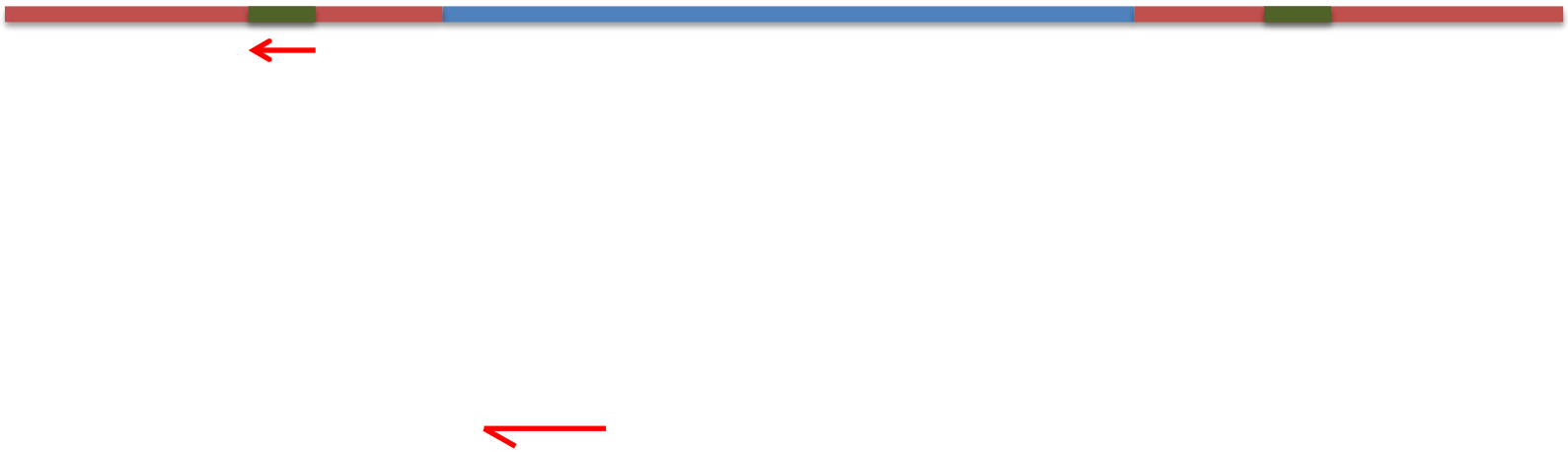
Sequencing

Sequencing



Sequencing

Sequencing

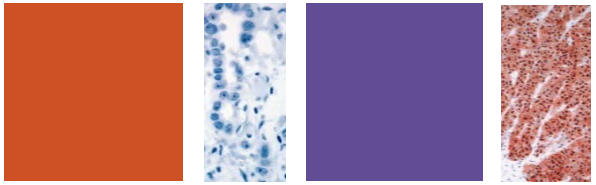


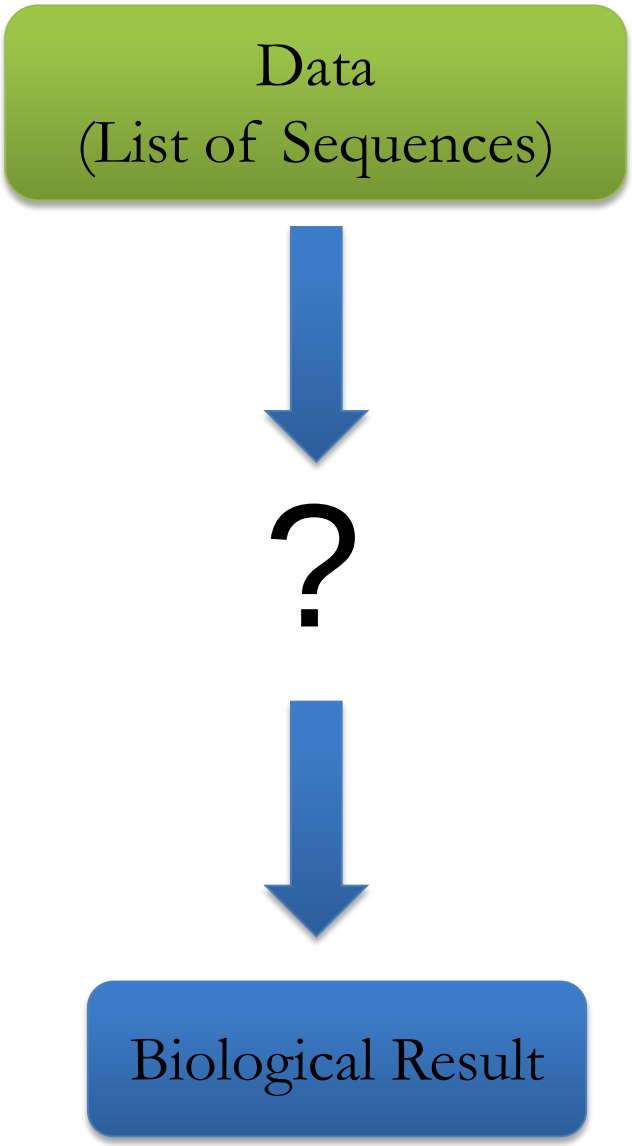

Welcome
HiSeq 2000
illumina

Data: a list of sequences of DNA molecules sampled from the input library

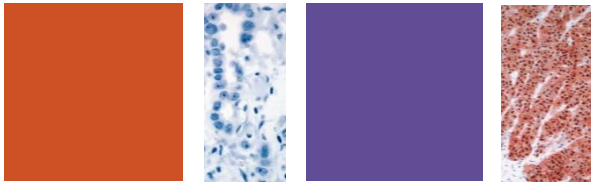
ACTGTCAGCTGACTAGCTACGATCG
TTTTCCCATATCGGCGTTGGGAGTG
AATTTTGGGCGTTCTGCTACGCTGAT
GGGGCCCCTTTCCGGCTCTGAGCTC
CTTTACGGGACTCTCGAGTAATATCA
CCCCTCTGAGGCGCATTTAGAGCTCC
ATCTCTCATCTATACTTTTATTTATTTT
GTGTGCCCACACACTCTTTGAAATCC
TTCATACCCTCCGAGAGAACTCTCGG
AATATATATACGCGCGCTCTCTAGCC
TGTCCCGAAACCTTCTCCCTCAGAGA

Informatics





Informatics (Alignment)



READ SEQUENCE

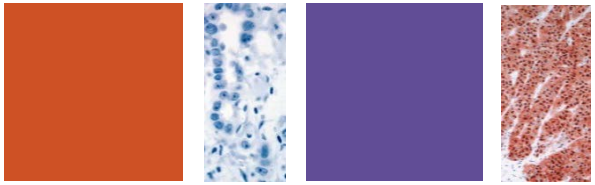
GACTTGACCGCAGTAGTATACGCGATCTGG

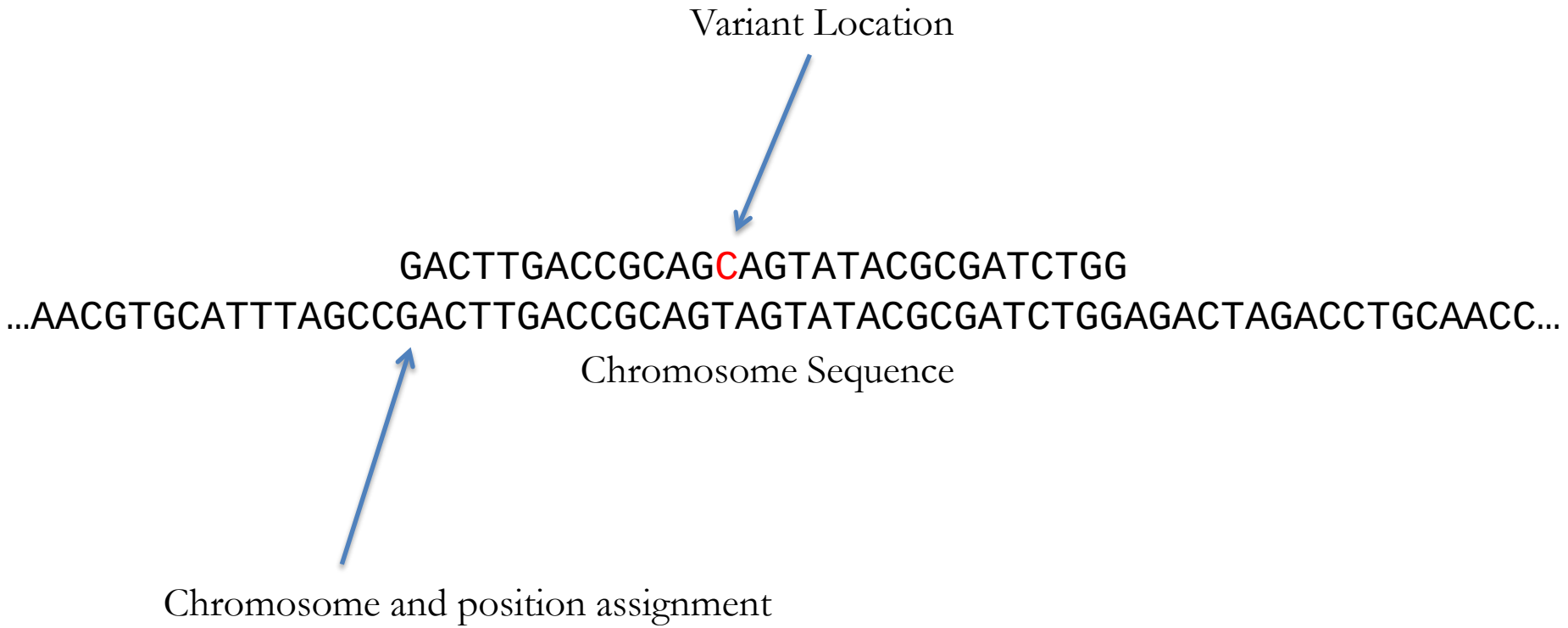
...AACGTGCATTTAGCCGACTTGACCGCAGTAGTATACGCGATCTGGAGACTAGACCTGCAACC...

Chromosome Sequence

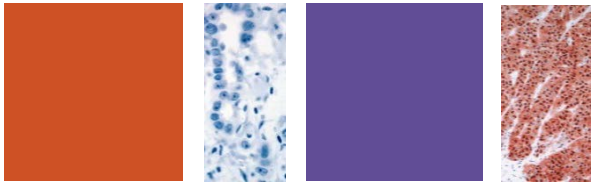
Chromosome and position assignment
(e.g. chr 2, position 123,224,414)

Informatics (Alignment)





Indel Alignment



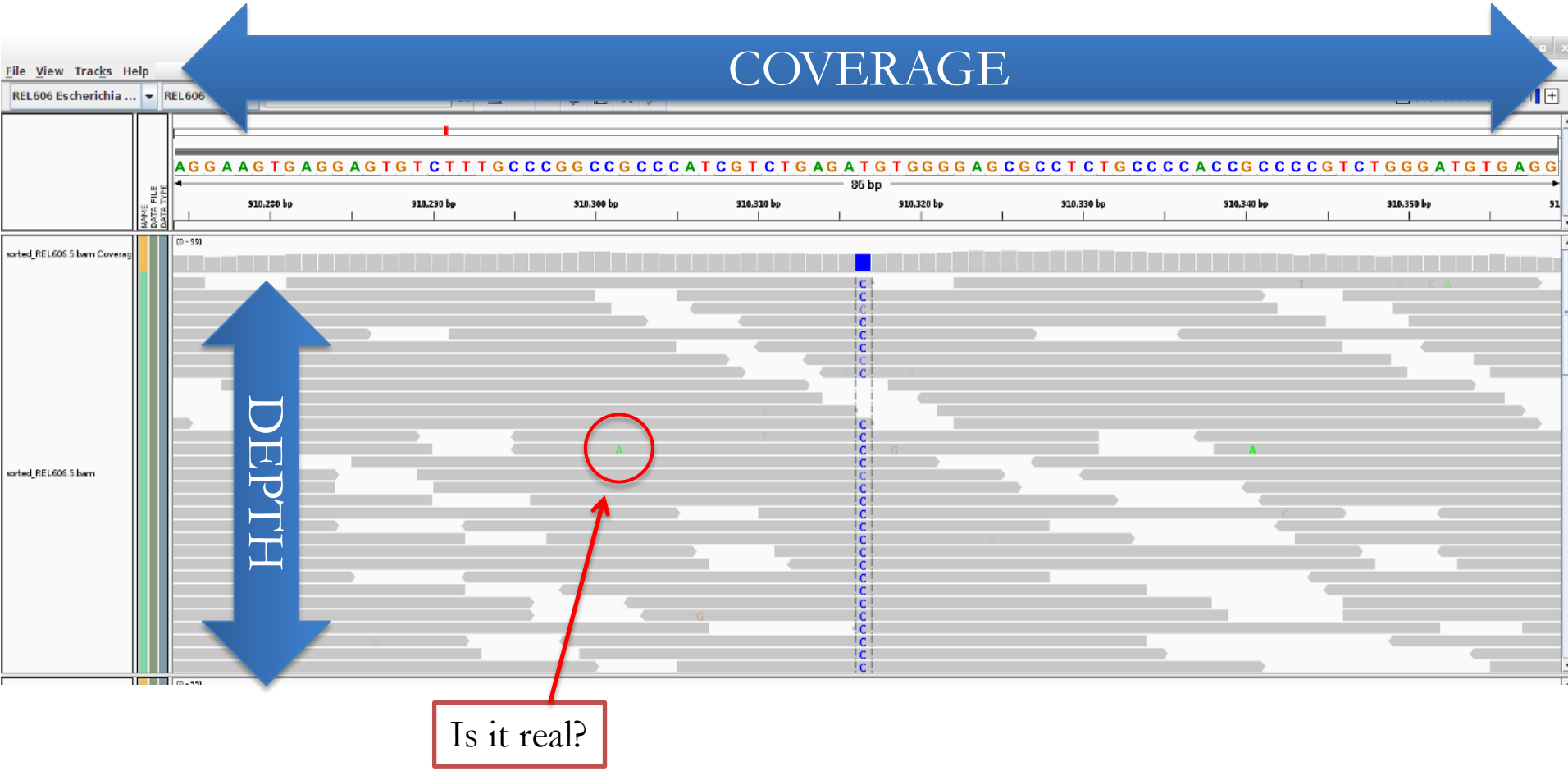
READ SEQUENCE

ACGTGCATTTAGC TGGAGACTAGACCTGC

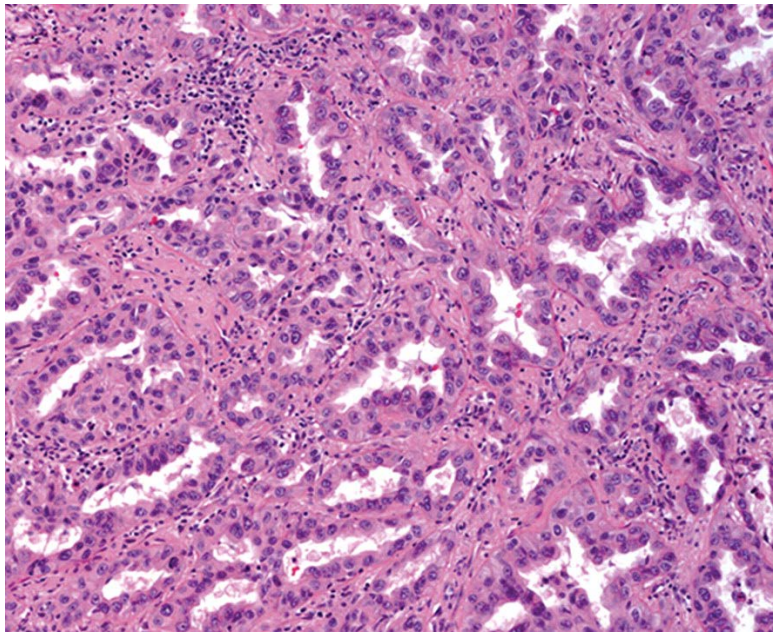
...AACGTGCATTTAGCCGACTTGACCGCAGTAGTATACGCGATCTGGAGACTAGACCTGCAACC...

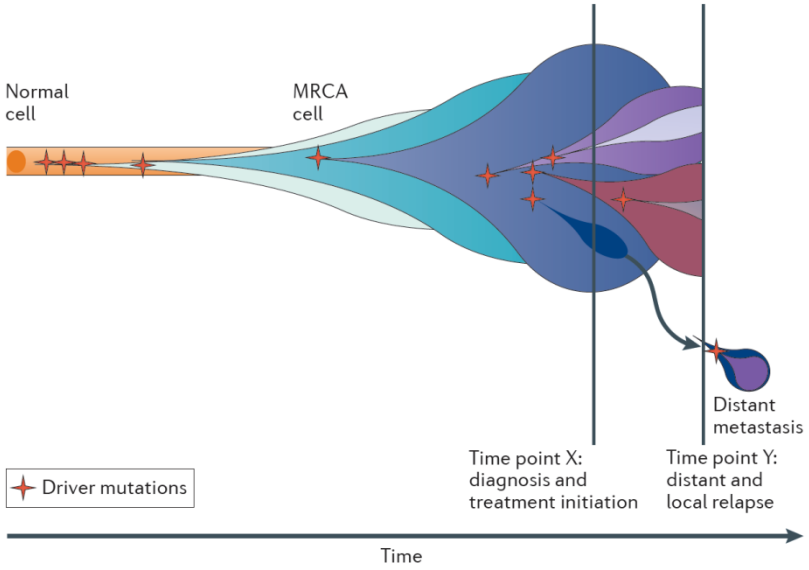
Chromosome Sequence

Variant Detection



Cancer – Low % Mutations





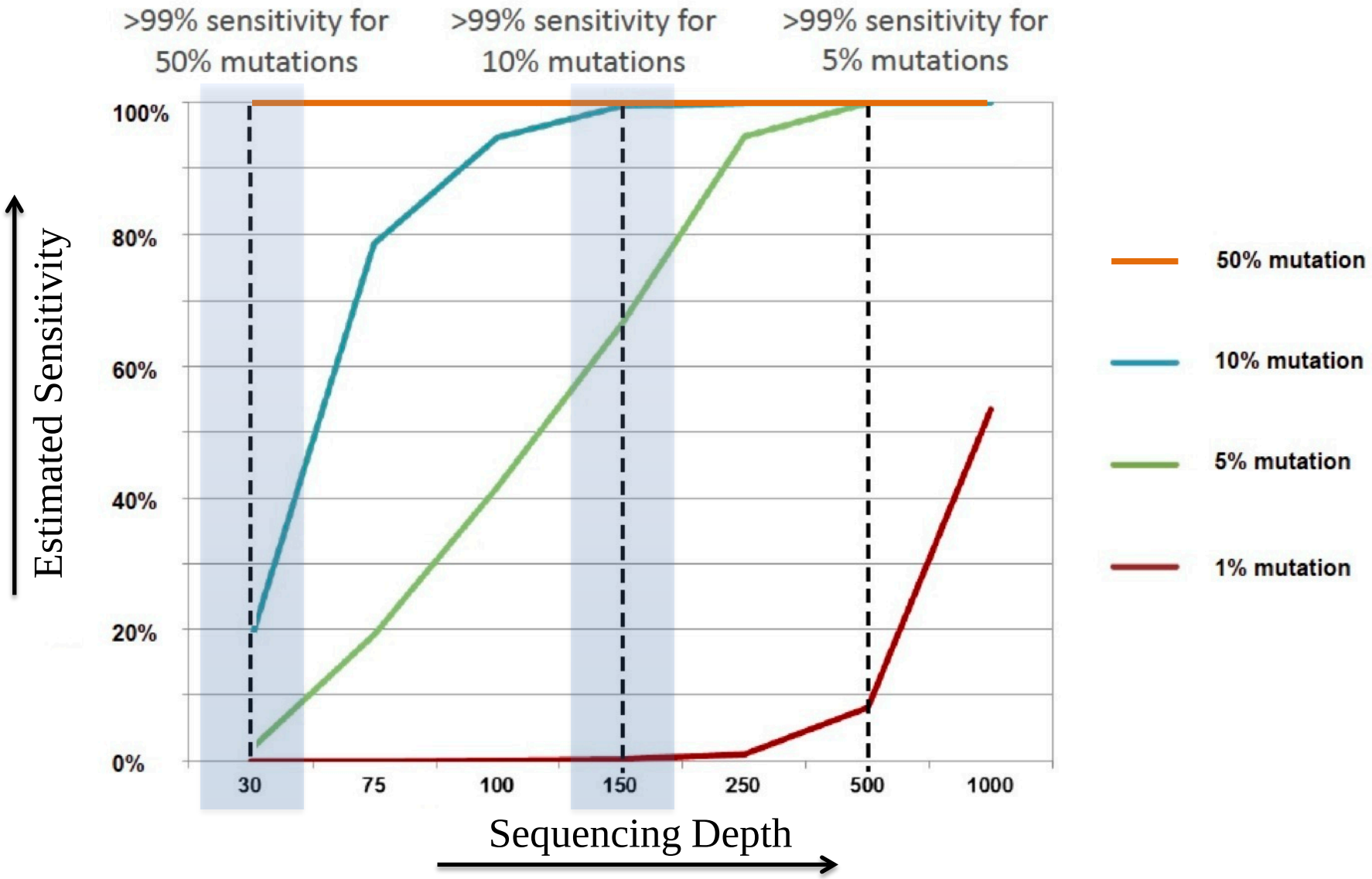
Tumor cell percentage

Tumor heterogeneity

Low mutation allelic percentage

Yates and Campbell, *Nat. Rev. Genetics.* 2012.

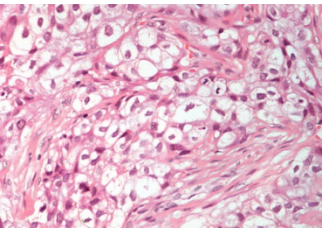
Increased Depth Improves Mutation Detection



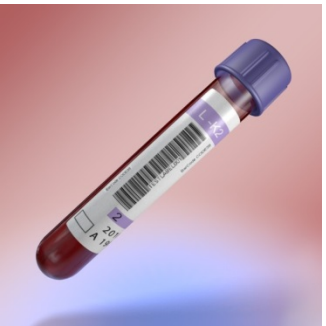
Foundation Medicine, 2011
Agilent Users Meeting, Boston, MA

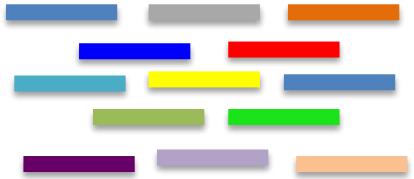
How to select targets for sequencing?

Tissue



Blood

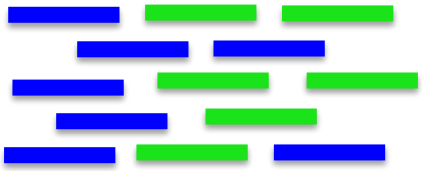




Prepare **Unselected** Fragments

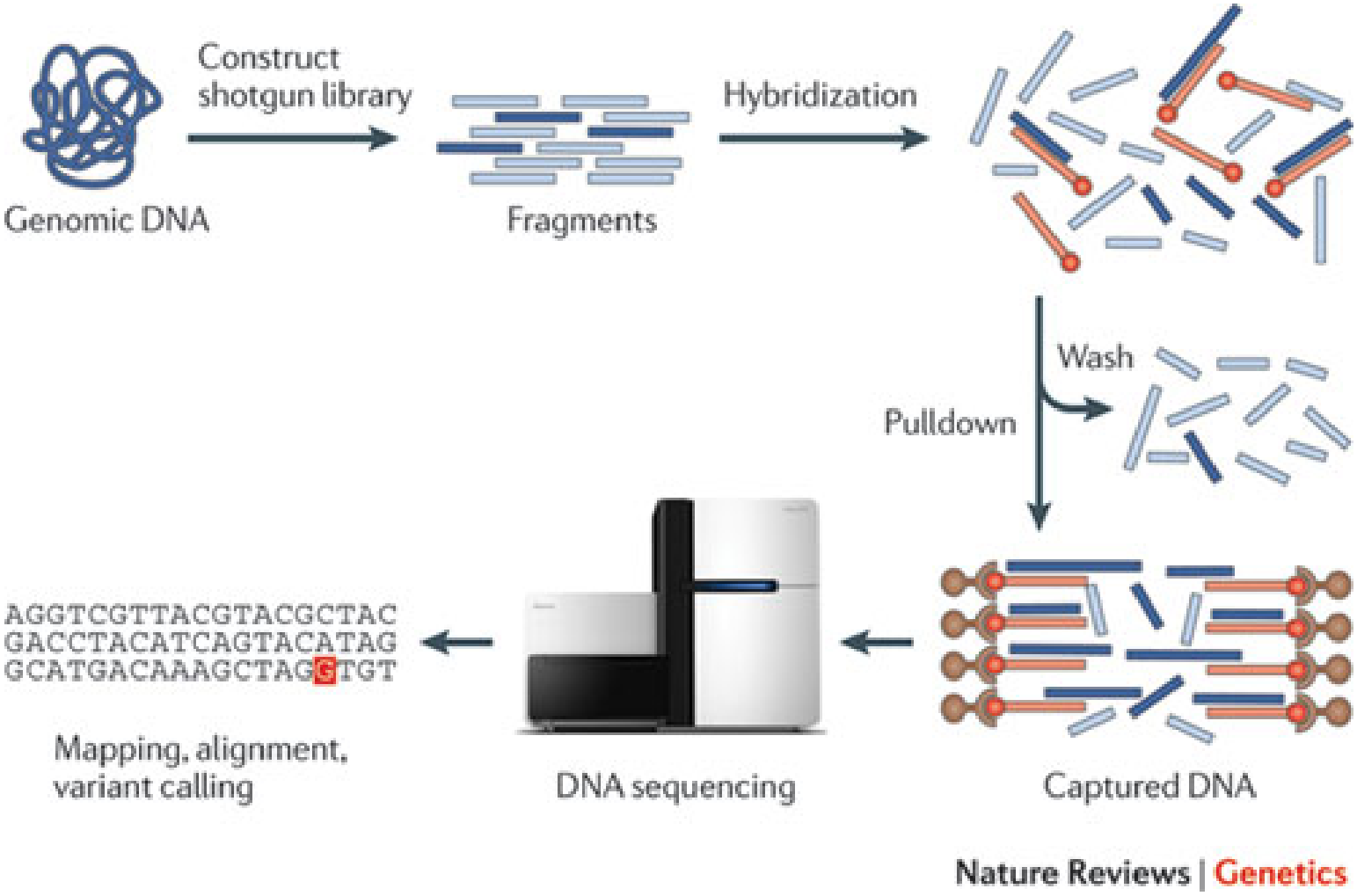
Whole Genome Sequencing

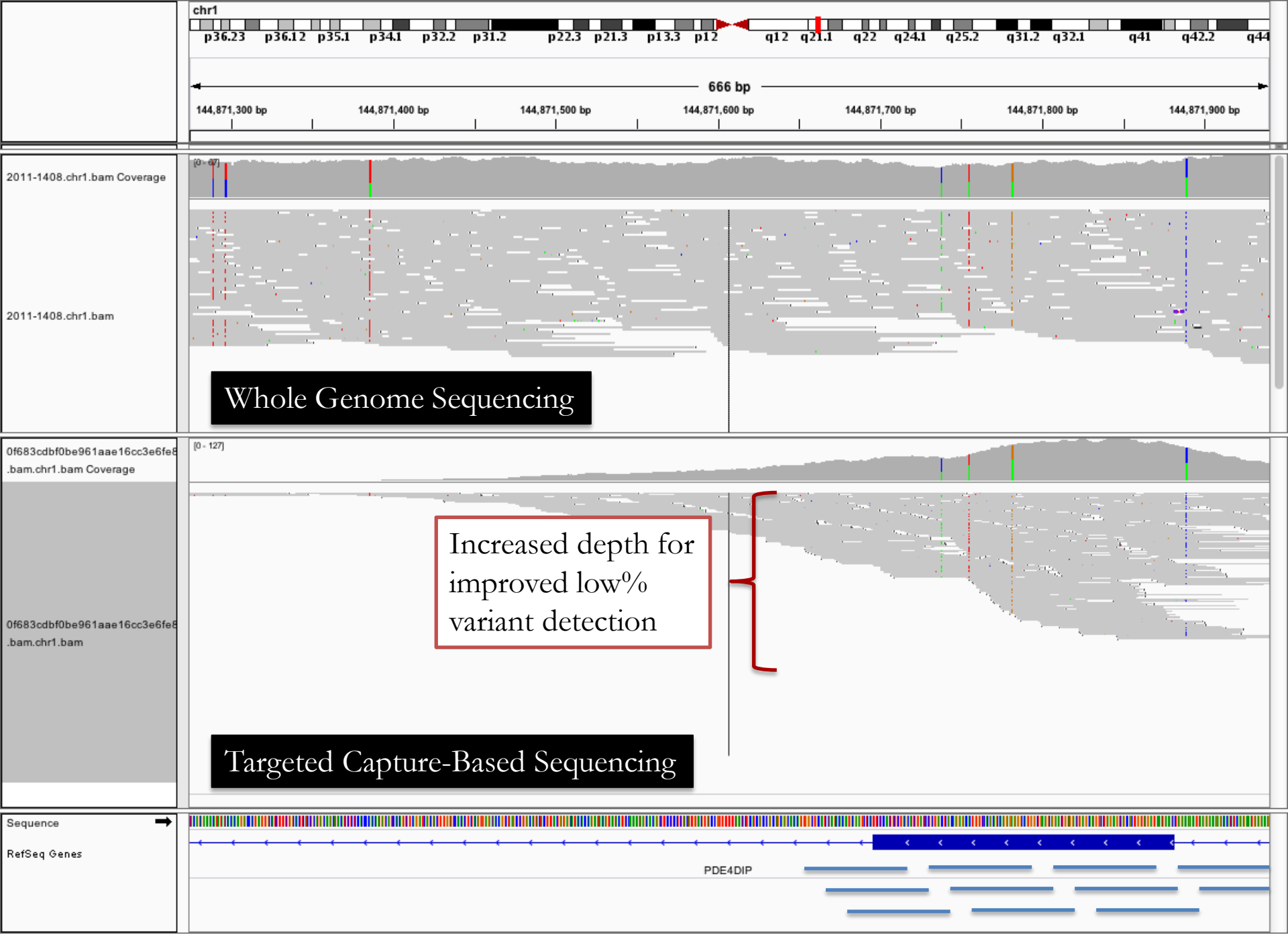
Prepare **Selected** Fragments



Targeted Sequencing

Targeted Sequencing– Hybrid Capture




chr1
p36.23 p36.12 p35.1 p34.1 p32.2 p31.2 p22.3 p21.3 p13.3 p12 q12 q21.1 q22 q24.1 q25.2 q31.2 q32.1 q41 q42.2 q44
666 bp
144,871,300 bp
144,871,400 bp
144,871,500 bp
144,871,600 bp
144,871,700 bp
144,871,800 bp
144,871,900 bp
2011-1408.chr1.bam Coverage
2011-1408.chr1.bam
Whole Genome Sequencing
0f683cdbf0be961aae16cc3e6fe8
.bam.chr1.bam Coverage
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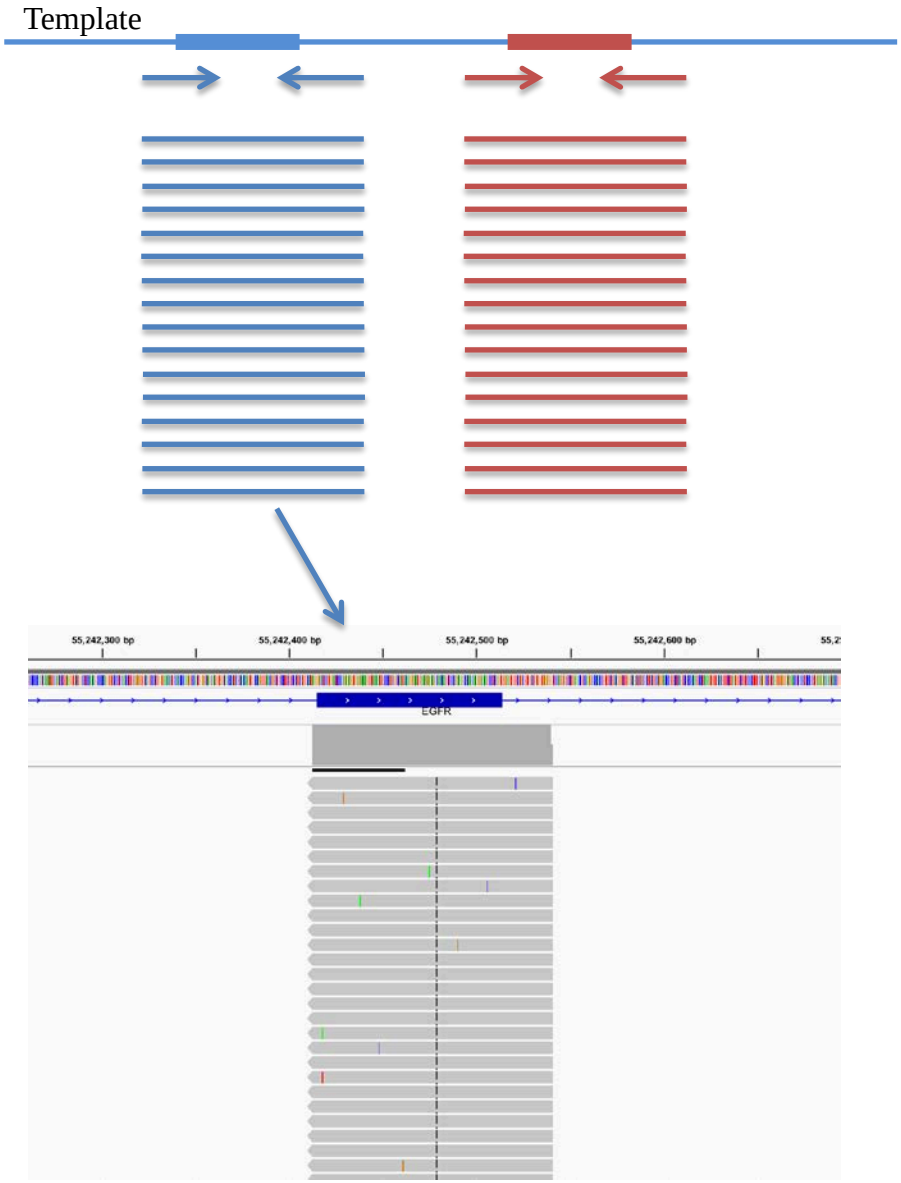
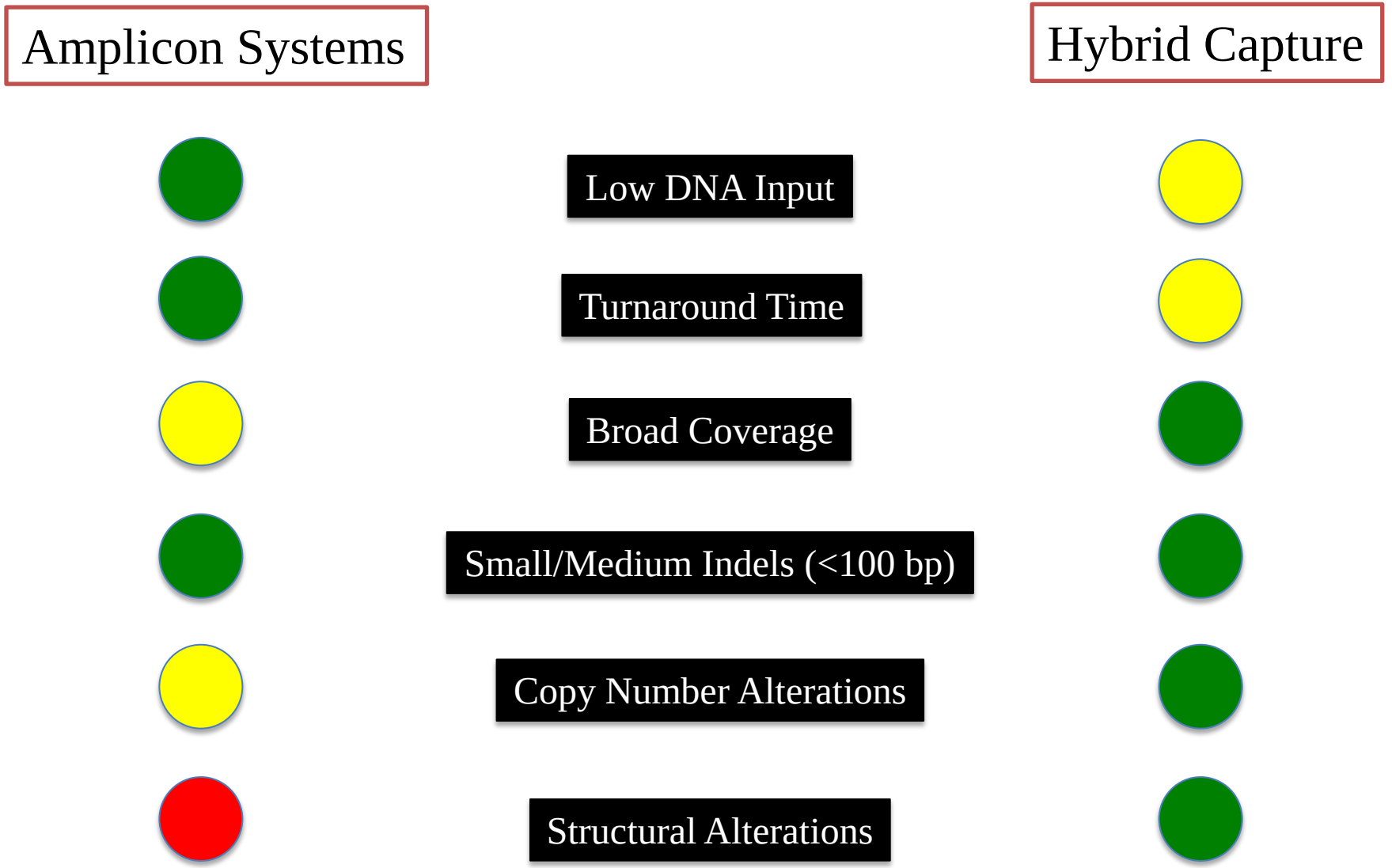
.bam.chr1.bam
Increased depth for improved low% variant detection
Targeted Capture-Based Sequencing
Sequence
RefSeq Genes
PDE4DIP

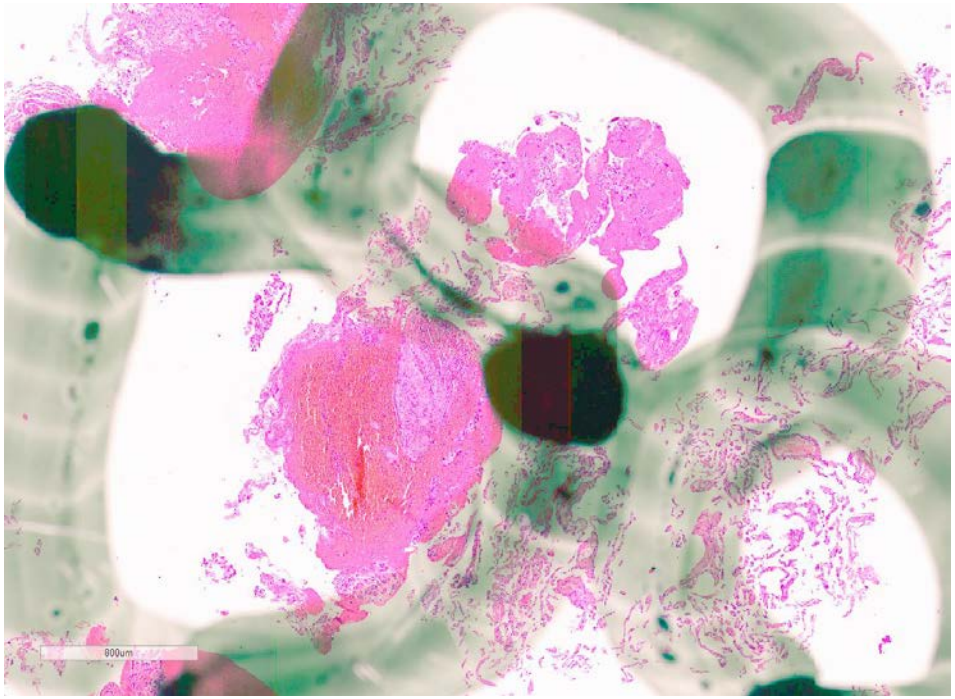
Targeted Sequencing – Amplicon Assays



Simplified Assay Type Comparisons



Amplicon Assays for Minute Specimens
ARUP – Solid Tumor Mutation Panel



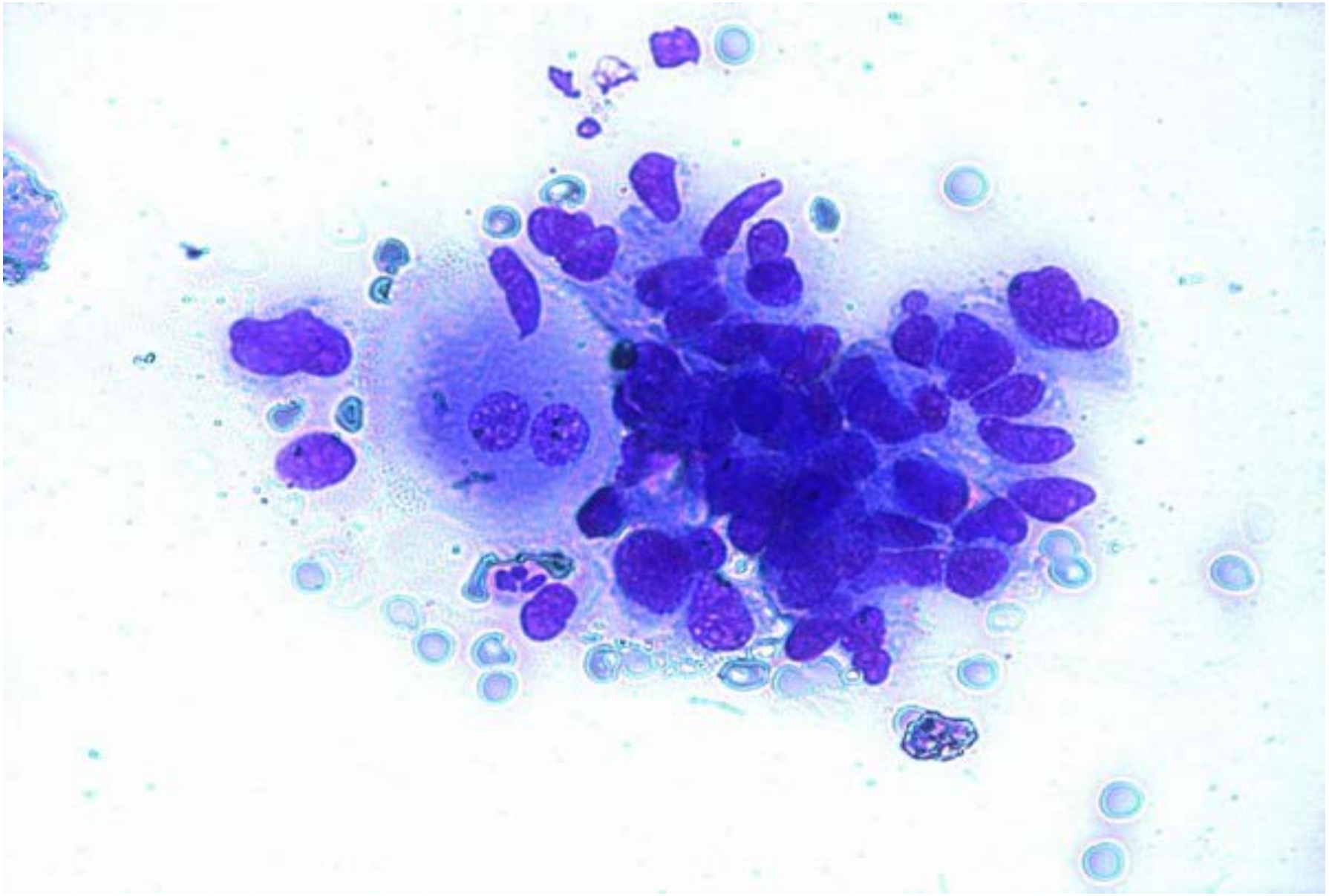
50 gene amplicon panel

ABL1	*EZH2*	*JAK3*	*PTEN*
AKT1	*FBXW7*	*IDH2*	*PTPN11*
ALK	*FGFR1*	*KDR*	*RB1*
APC	*FGFR2*	*KIT*	*RET*
ATM	*FGFR3*	*KRAS*	*SMAD4*
BRAF	*FLT3*	*MET*	*SMARCB1*
CDH1	*GNA11*	*MLH1*	*SMO*
CDKN2A	*GNAS*	*MPL*	*SRC*
CSF1R	*GNAQ*	*NOTCH1*	*STK11*
CTNNB1	*HNF1A*	*NPM1*	*TP53*
EGFR	*HRAS*	*NRAS*	*VHL*
ERBB2	*IDH1*	*PDGFRA*	
ERBB4	*JAK2*	*PIK3CA*	

~10 ng FFPE DNA, 10-15% tumor cells:
EGFR mutation negative
KRAS c.34G>T, p.G12C (NM_033360) – 5% MAF

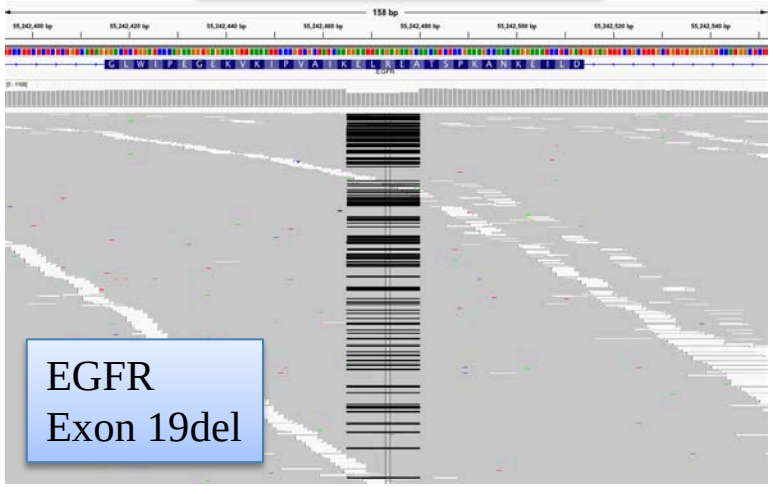
1 mm

Low Input Allows for Direct Testing of Cyto Smears

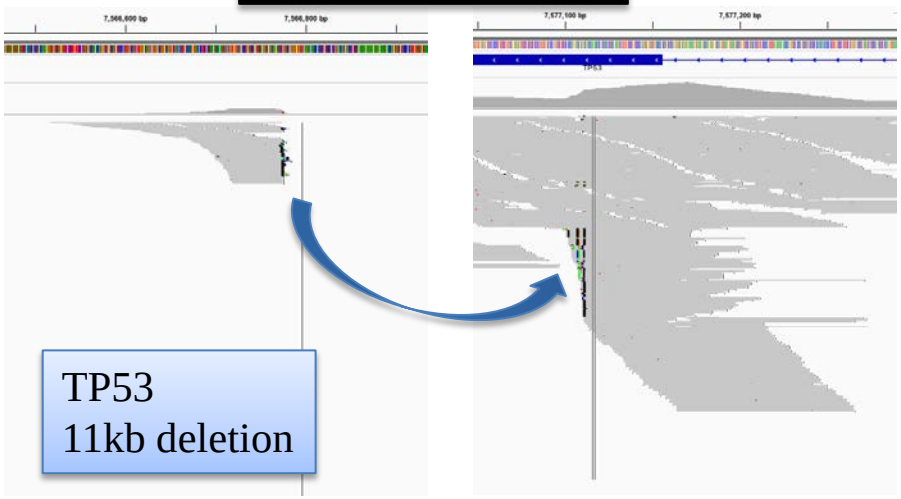


Capture Assay Flexibility

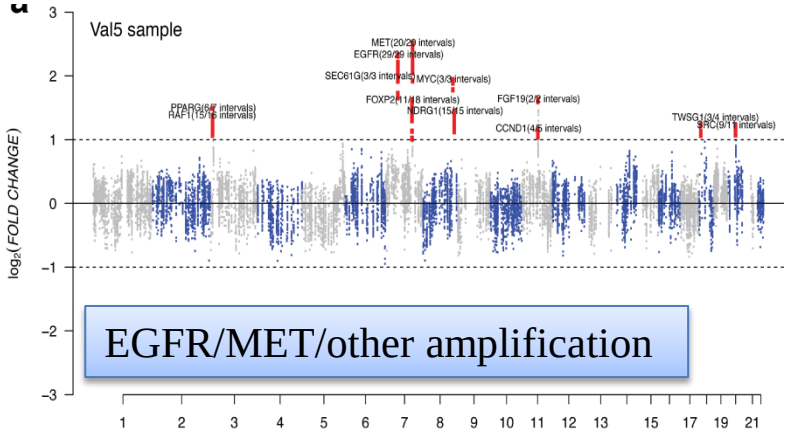
Mutations/Indels



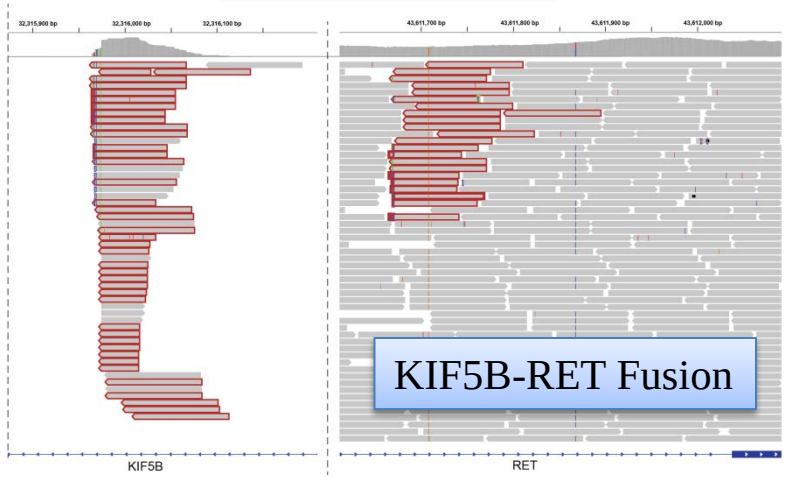
Rearrangements



Copy Number Events

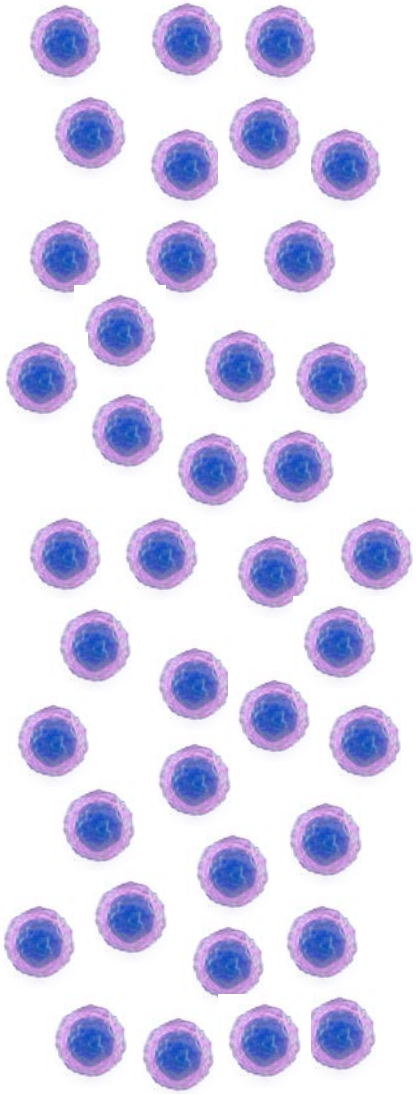


Gene Fusions

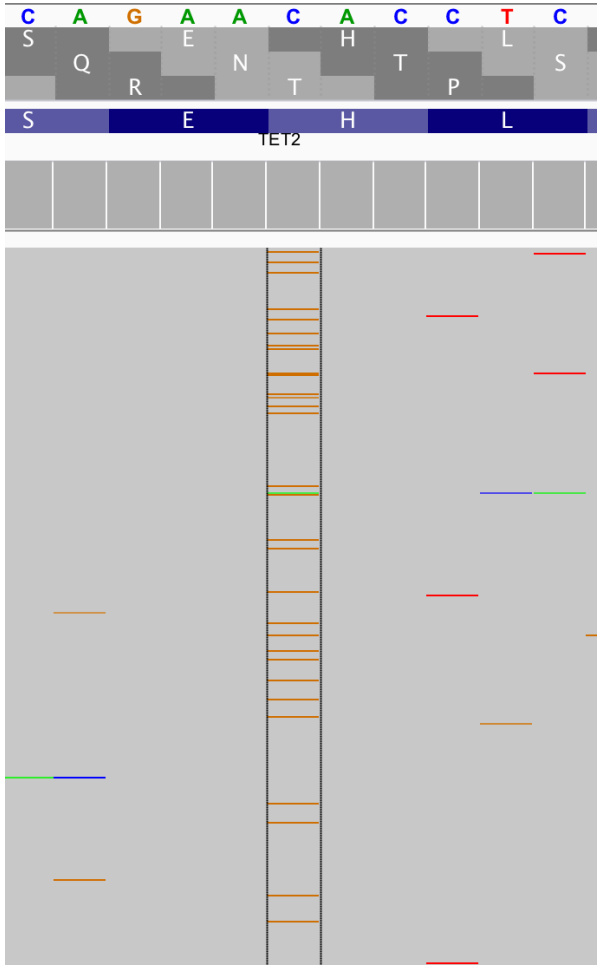


Need to ensure good sampling!

GOOD Sampling

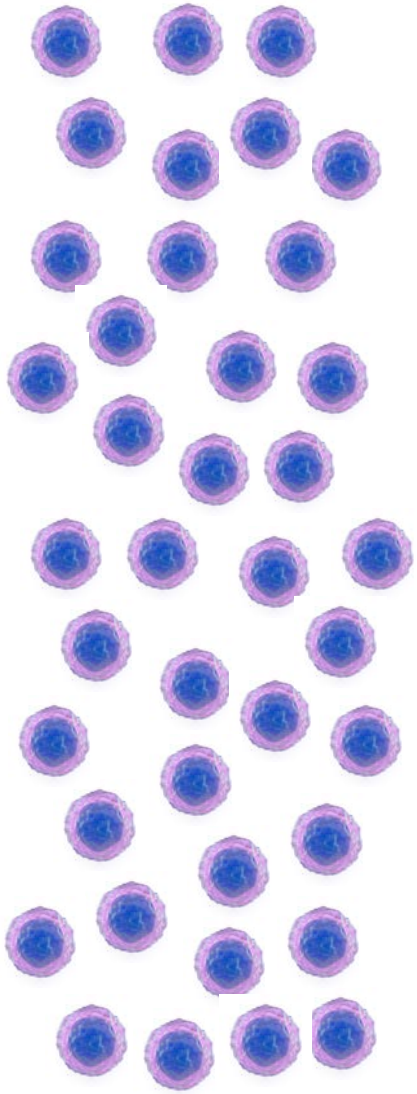


Library Prep
&
Sequencing

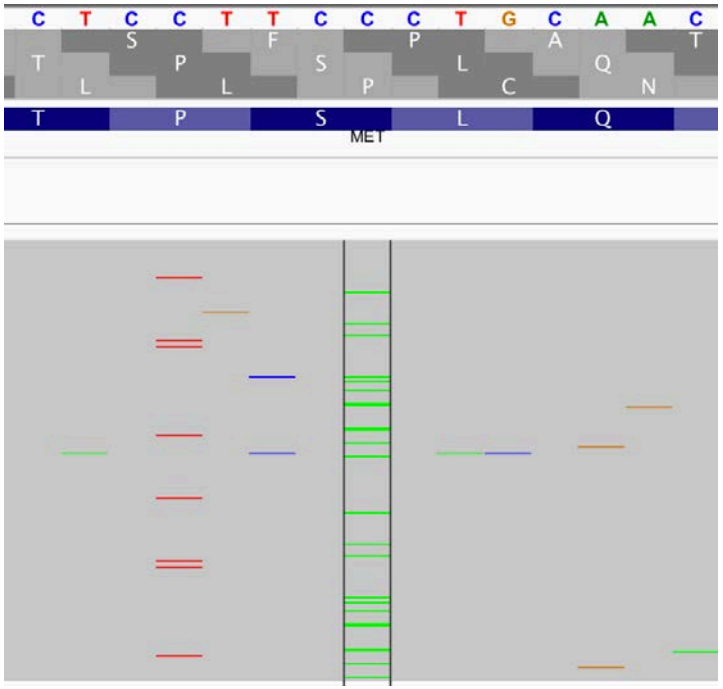


But….don't be fooled!

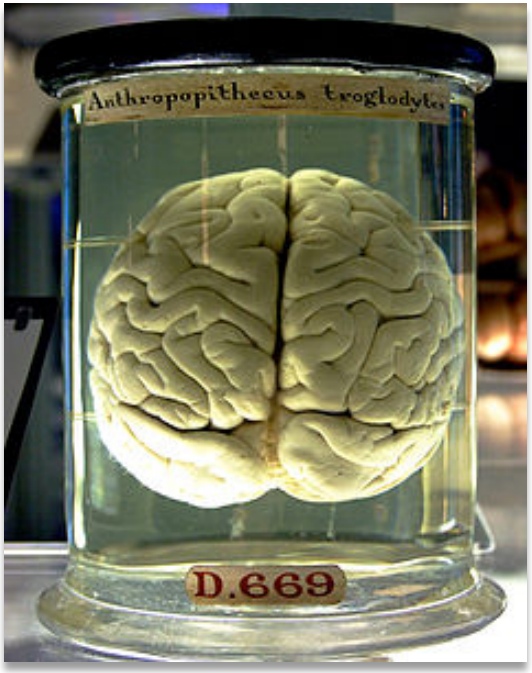
BAD Sampling

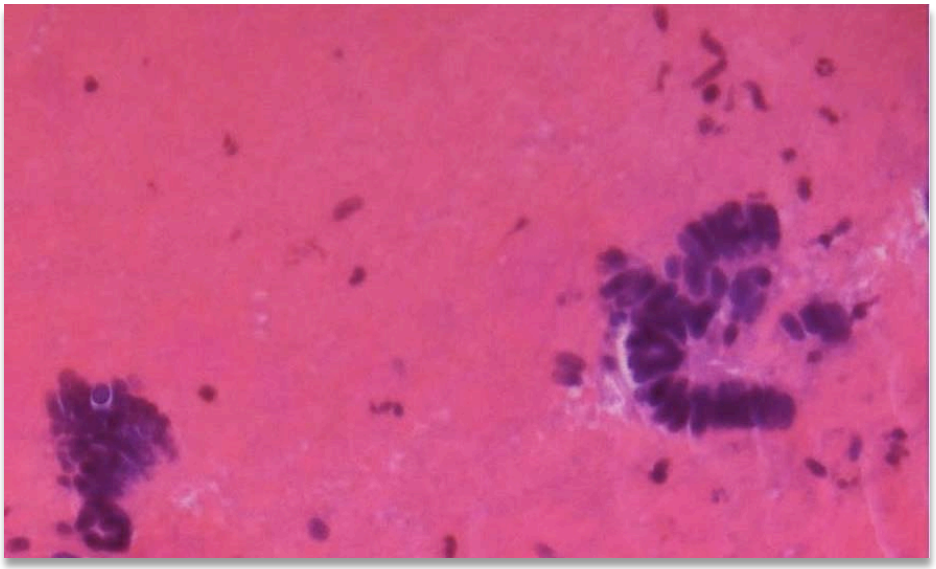


Library Prep
&
Sequencing



Cancer – Difficult Specimens





Formalin Fixation

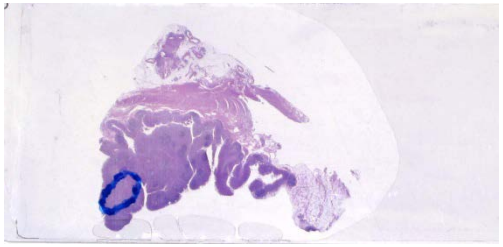
Fragmented, nicked, crosslinked, end-damaged DNA

Scant Tissue

Low yield of DNA

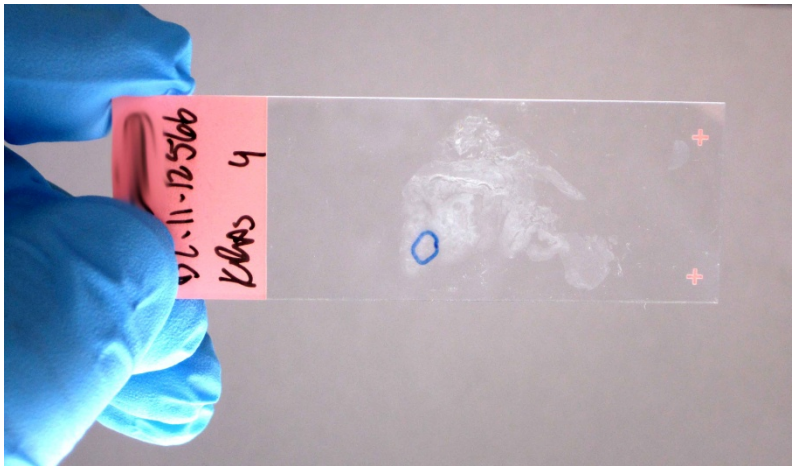
Brain photo: Gaetan Lee

Macrodissection – Laboratory Method to Enrich Tumor Content

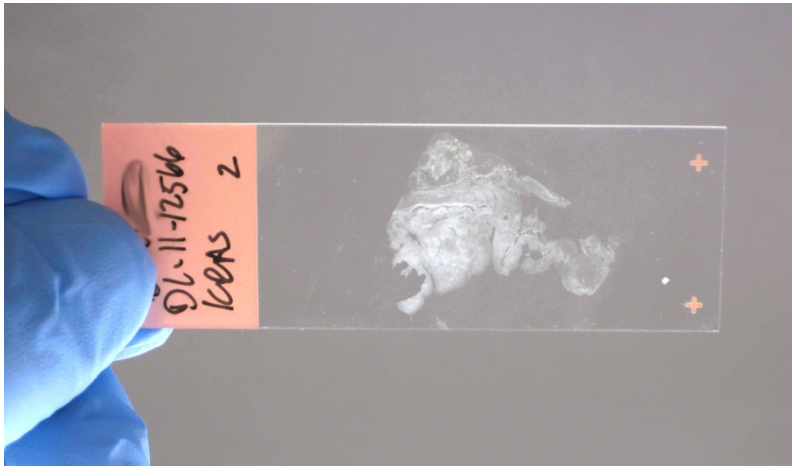


Pathologist reviews H&E for adequate tumor cell content

Selects and marks best tumor area



Corresponding area marked on serial unstained slide



Tumor area lifted from slide for DNA/RNA extraction

Key Considerations:
- Total yield
- Tumor cell %

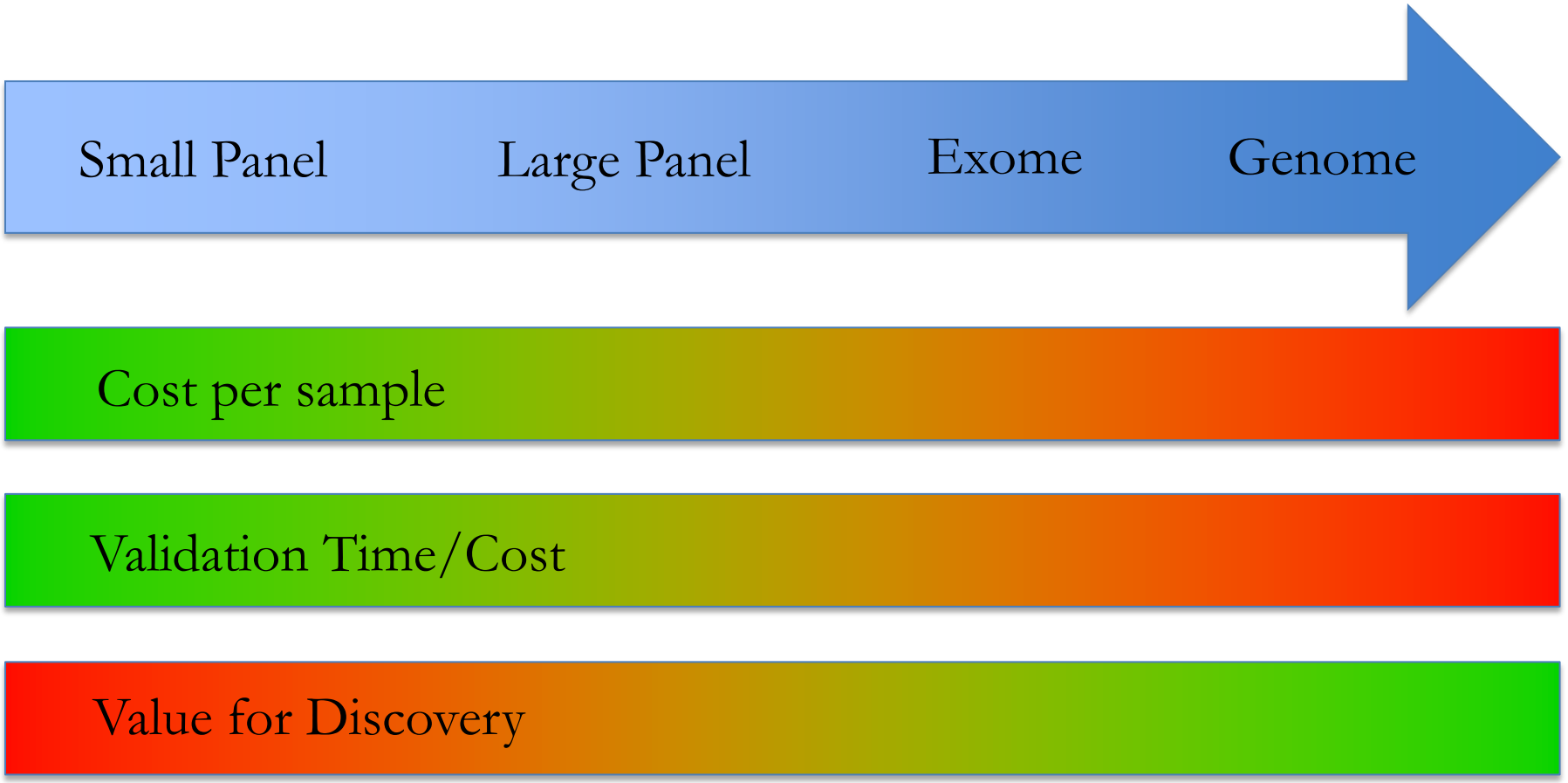
Slide courtesy from Bryan Betz, PhD

As a lab, how do you think about planning an assay?

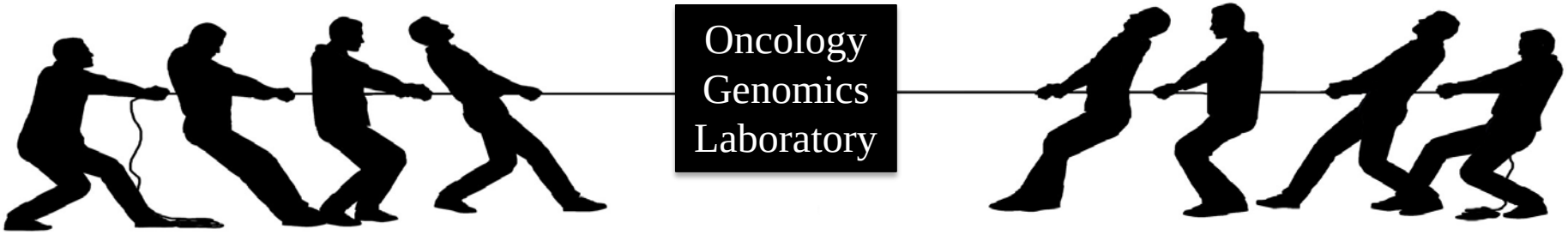
- What size (# genes)?
- What type of preparation?

- There is no clear consensus in this field about what is the ideal test.

Assay Design Considerations



What's the right size assay?



Smaller Targeted Assays

- Some clinicians
- Cancer specimens
- Validation effort
- Cost
- Reimbursement

Larger Comprehensive Assays

- Most clinicians
- Clinical requirements
- Translational research
- Lab Competition
- Technology

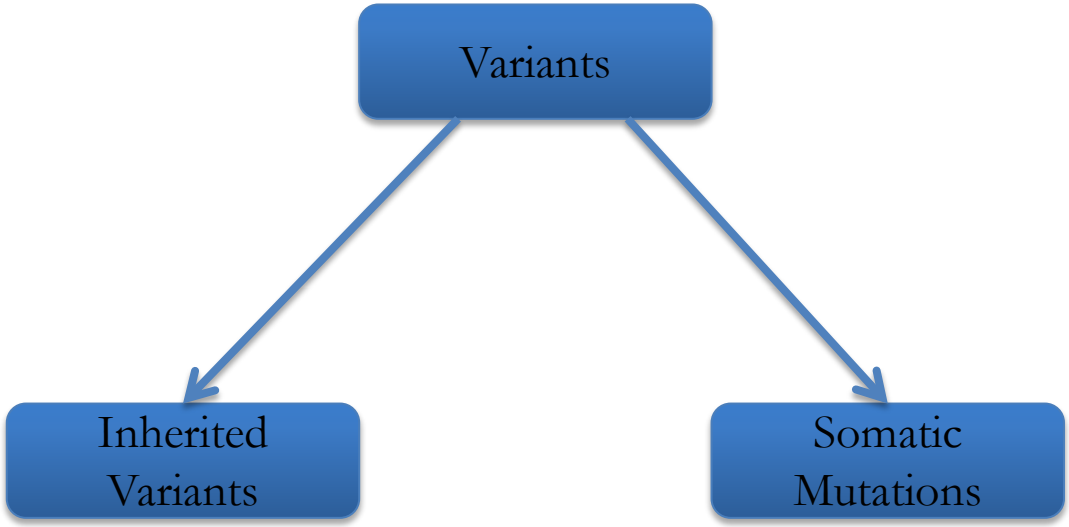
Summary: NGS Assay Development

- NGS allows many types of anomalies in many genes simultaneously.
- Design and strategy decisions are complex.
  - Many contributing factors and influences.
  - Many assay type choices with different pros and cons.

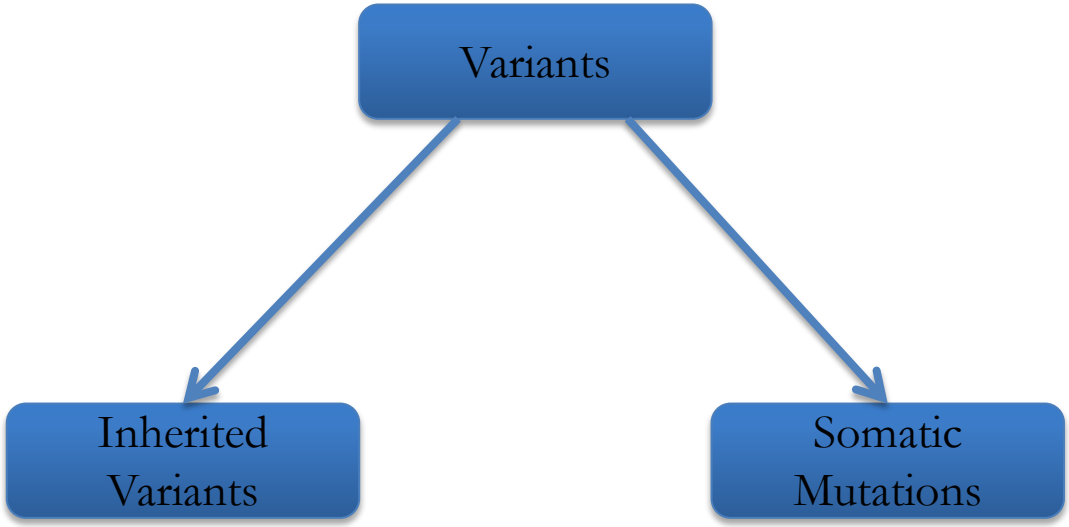
After Data Analysis…

- The back-end challenges of clinical NGS implementation can be daunting!
  - Proper databasing of clinical variants.
  - Workflow for analyzing individual cancer cases:
    - How many people involved?
    - Handing off responsibility and ensuring proper review.
    - Confirmatory assays for variants as necessary.
  - Generation of appropriate reports for clinicians.
  - Integration with electronic medical records and hospital information systems.

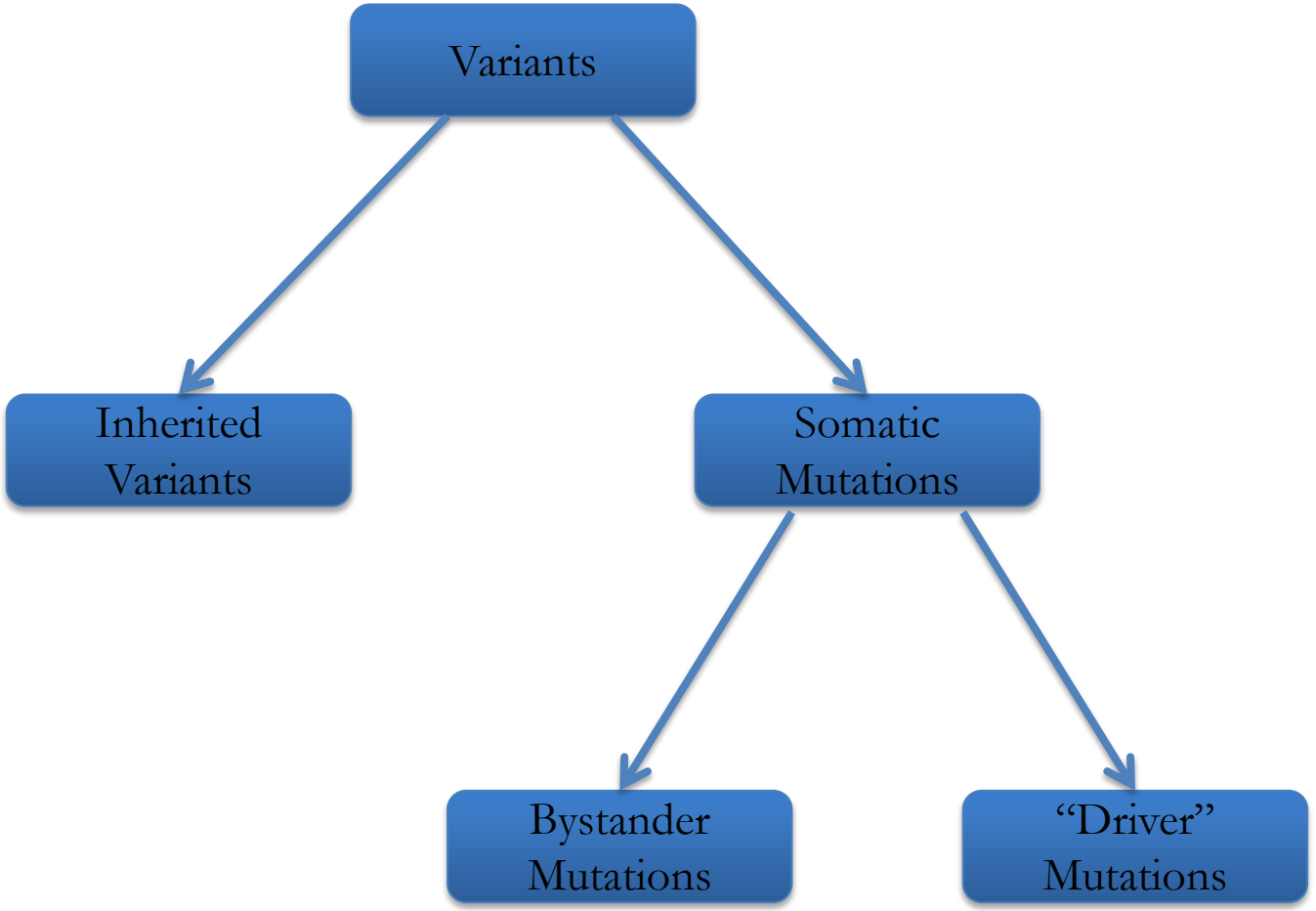
Cancer NGS Interpretation



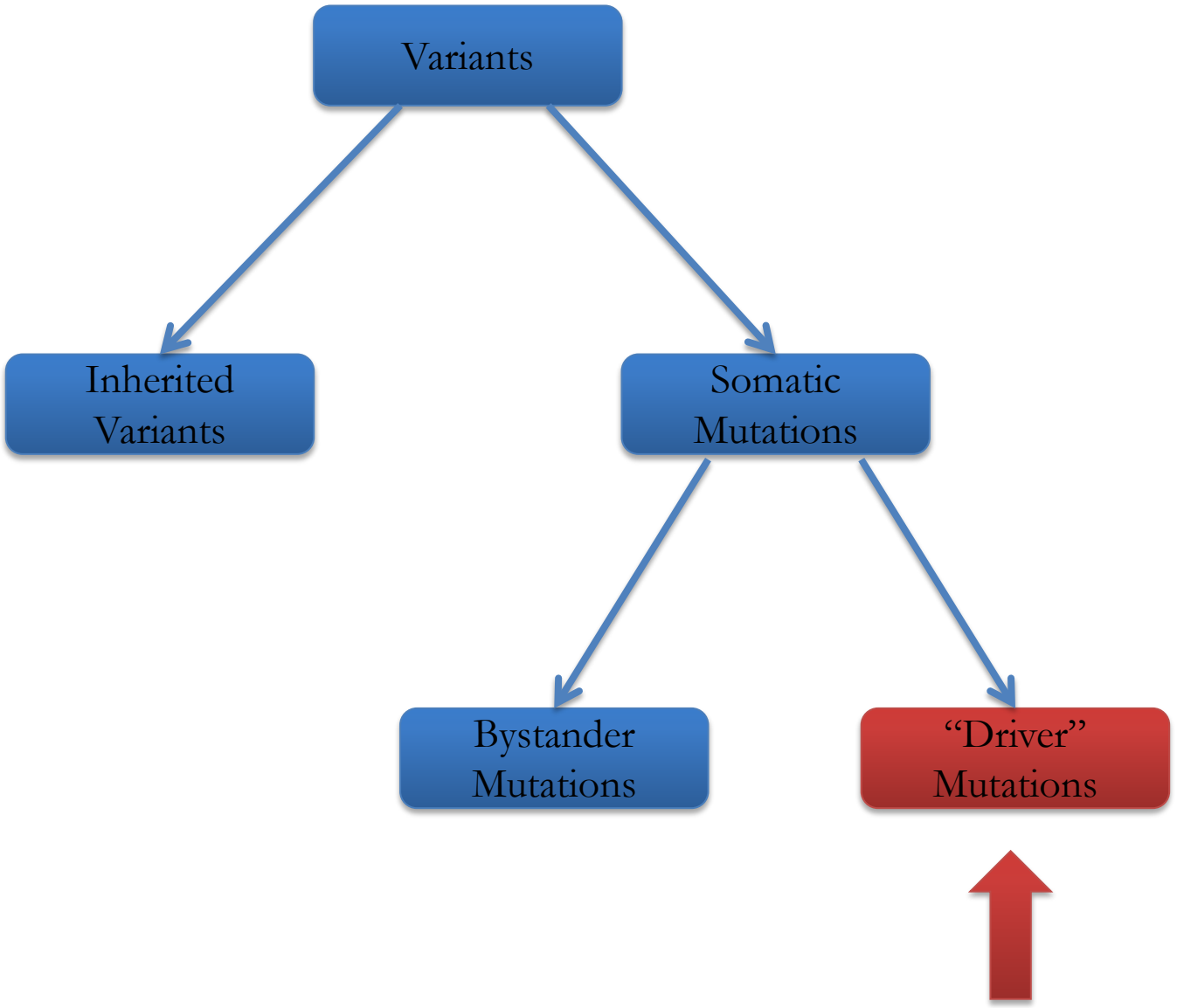
Cancer NGS Interpretation



Cancer NGS Interpretation



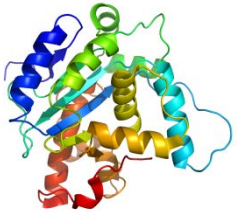
Cancer NGS Interpretation



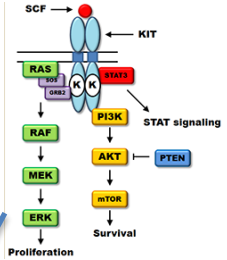
VARIANT:
chr4 55593464 A>C

Annotation Pipeline

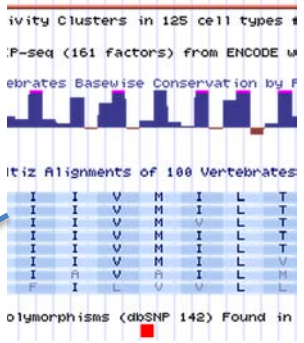
Protein Effects Prediction



Pathway Analysis



Evolutionary Conservation



Gene: KIT
Exonic missense
c.1621A>C, p.M541L

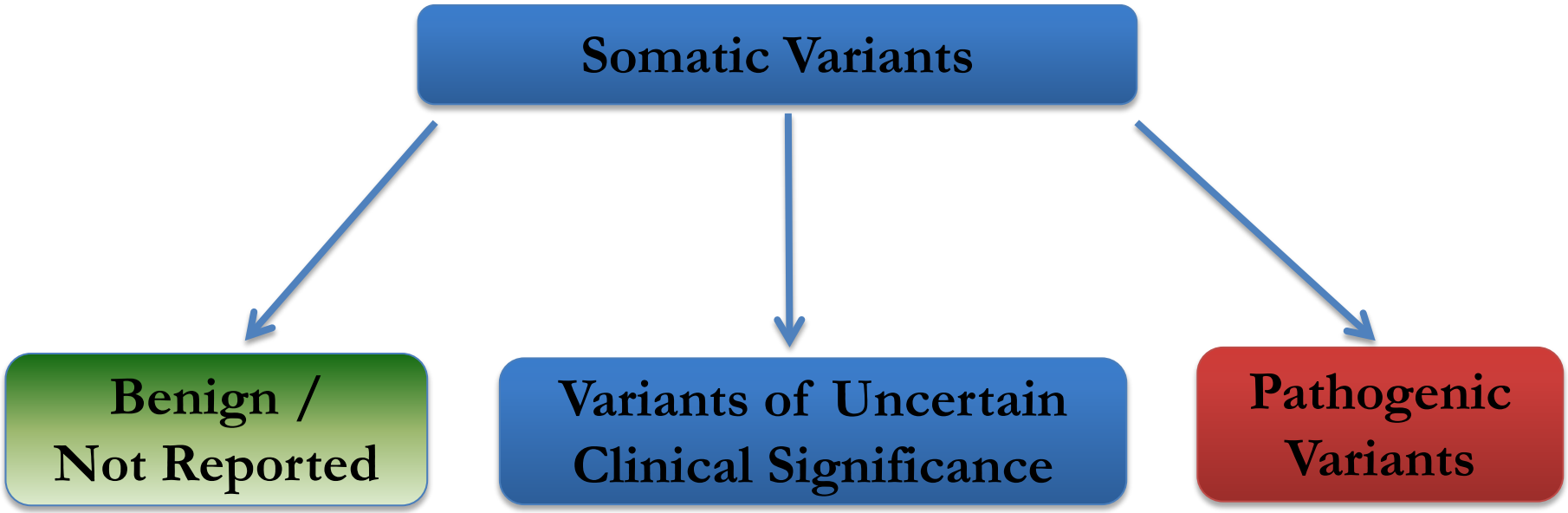
PubMed

Inherited Variants
1000 Genomes, dbSNP
Exome Variant Server

Cancer Variants
COSMIC db
TCGA db

Lab Variants
Internal db

Variant Classification



ARUP Tiers
Tier 1 – Actionable (FDA Approved Therapies in Patient Tumor Type, Established Diagnostic or Prognostic Significance)
Tier 2 – Potentially Actionable (FDA Approved Therapies in another Tumor Type, Potential Diagnostic or Prognostic Significance)
Tier 3 – Variants of Unknown Significance (VUS)

ARUP LABORATORIES | NGS.Web v 3.8.0.152

Home | Dispatcher | Search | Sanger | Reports | Research | QC | Admin

Dashboard: Onc-Med Director

Pending Samples

Last refresh: a few seconds ago

Show 25 entries

Search:

Accession	Test Mnemonic	Test DTA	Remaining TAT	Current Stage Task	In-Lab Status (Millennium)
16238113106	SOLID NGS	SOLID Interp	0 Days, 20Hours	Completed	In Lab
16238113193	SOLID NGS	SOLID Interp	0 Days, 20Hours	Completed	In Lab
16238116614	SOLID NGS	SOLID Interp	1 Days, 16Hours	Completed	In Lab

Showing 1 to 3 of 3 entries

Previous 1 Next

ARUP LABORATORIES | NGS.Web v 3.8.0.152

Larissa Furt

Search

Home | Dispatcher | Search | Sanger | Reports | Research | QC | Admin

16-238-113106 > SOLID Interp > Variants

Showing 1 to 12 of 12 entries

Search

Show 100 entries

Result File: 2016-08/16238113106_KDQ:

View/Modify Rules | Auto-Classify Al

Gene	Location	Nuc. Change	Protein Change	Variant Type	Depth	Allele Freq	1KG Freq	ESP Frequency	COSMIC Id	ARUP Obs.	dbSNP Id	Common Classifications	Interpretation	Classification
CSF1R	chr5: 149433596	c.*35CA>TC		3 prime UTR variant	1531	100	0		---			SNP (131/154 samples)		SNP
MET	chr7: 116411923	c.2908C>T	p.Arg970Cys	Nonsynonymous	4977	49.4	0	0.35	NOCOSMIC988		rs34589476	Uncertain Significance (2/3 samples)		Tier 3*
IDH1	chr2: 209113192	c.315C>T	p.Gly105Gly	Synonymous	4994	51.3	0.05	7.1	NOCOSMIC105		rs11554137	SNP (20/20 samples)		SNP
RET	chr10: 43615633	c.2712C>G	p.Ser904Ser	Synonymous	3226	51.4	0.16	16.09	---		rs1800863	SNP (59/59 samples)		SNP
PDGFRA	chr4: 55152040	c.2472C>T	p.Val824Val	Synonymous	4997	50.3	0.21	19.83	COSM22413		rs2228230	SNP (58/58 samples)		SNP
HRAS	chr11: 534242	c.81T>C	p.His27His	Synonymous	2296	50.8	0.3	35.45	COSM249860		rs12628	SNP (95/95 samples)		SNP
EGFR	chr7: 55249063	c.2361G>A	p.Gln787Gln	Synonymous	1181	100	0.42	45.76	---		rs1050171	SNP (316/316 samples)		SNP
TP53	chr17: 7579472	c.215C>G	p.Pro72Arg	Nonsynonymous	1395	99.9	0.6	37	---		rs1042522	SNP (154/154 samples)		SNP
APC	chr5: 112175770	c.4479G>A	p.Thr1493Thr	Synonymous	4974	49.2	0.66	41.38	---		rs41115	SNP (142/142 samples)		SNP
RET	chr10: 43613843	c.2307G>T	p.Leu769Leu	Synonymous	4993	100	0.72	19.74	---			SNP (152/152 samples)		SNP
PDGFRA	chr4: 55141055	c.1701A>G	p.Pro567Pro	Synonymous	4089	100	0.96	4.11	---		rs1873778	SNP (163/163 samples)		SNP
FGFR3	chr4: 1807894	c.1953G>A	p.Thr651Thr	Synonymous	2104	100	0.96	4.49	---		rs7688609	SNP (164/164 samples)		SNP

Patient Information

Patient Name:
Sex:
Date of Birth:
Ordering Physician:
Clinical History: Metastatic melanoma
Sample Source: Liver

Attachments

Generate Report: 16-238-113106 (SOLID NGS)

Overall Result: See Note

Choose References

Template: Solid Tumor 3 Tier

Use Global Template

Include the following variants:

*Notes shown here are not included in the report.

- IDH1 NM_005896.3: c.315C>T p.Gly10...
  SNP
- TP53 NM_000546.5: c.215C>G p.Pro72...
  SNP
- RET NM_020975.4: c.2712C>G p.Ser90...
  SNP
  common benign variant
  Revision auto-generated by 3.7.0 ...
- APC NM_000038.5: c.4479G>A p.Thr14...

Total Characters (including background): 3169

Update Draft | Save Draft | ✔Notify MD | Commit Report

SAMPLE SOURCE: Liver
CLINICAL HISTORY: Metastatic melanoma

I. TIER 1: Actionable (FDA Approved Therapies in Patient Tumor Type, Established Diagnostic or Prognostic Significance)

NONE DETECTED

II. TIER 2: Potentially Actionable (FDA Approved Therapies in another Tumor Type, Potential Diagnostic or Prognostic Significance)

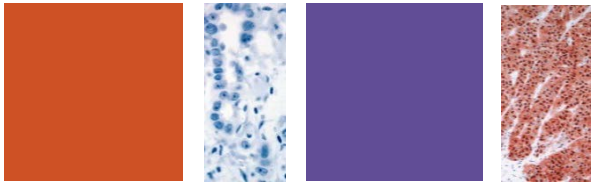
NONE DETECTED

III. TIER 3: Variants of Unknown Significance (VUS)

1. MET c.2908C>T, p.Arg970Cys (R970C) (NM_000245.2)

Interpretation: This variant occurs in the juxtamembrane domain and is recognized in the literature as either Arg970Cys or Arg988Cys. It has been observed infrequently in lung cancer (COSMIC database), colorectal cancer (Fumagalli et al., 2010), chronic myelomonocytic leukemia, endometrial cancer, thyroid cancer and melanoma (Tyner et al., 2010). The transformation ability of this variant is uncertain as some in vitro studies have shown a mild increase in cell proliferation and transformation (Ma et al., 2003), while others show no growth or transformative advantage (Tyner et al., 2010). In vivo studies in mice suggest this variant may increase susceptibility to lung cancer (Zaffaroni et al., 2005). This variant is listed in ClinVar as having conflicting interpretations of pathogenicity (benign, likely benign, and uncertain significance). This variant is listed as a germline variant in dbSNP (rs34589476) with a minor allele frequency (MAF) of 0.001, in the Exome Aggregation Consortium with a MAF of 0.0029, and in the NHLBI Exome Sequencing Project with a MAF of 0.0035. The functional consequence in this context is unknown. The clinical significance, if any, is uncertain.

Sections



1. Introduction to Personalized Oncology Diagnostics
2. Technology, Test Selection and Test Capabilities
3. Future Trends in Solid Tumor Genomic Diagnostics

Emerging Genomics Targets

Mutations (TS and OG)
Point mutations
Insertions and deletions (indels)

Structural Variations
Large scale deletions/duplications
Fusions/rearrangements
Aneuploidy
Chromothripsis

Epigenetics
Altered DNA methylation
Altered histone methylation
Altered DNA-protein interactions
Altered chromatin structure

Gene Expression
OG or TS dysregulation
Pathway activation
MicroRNAs
LncRNAs
Alternative Splicing
Allele-specific expression
RNA binding protein interactions

Other Applications
Circulating tumor DNA assays

Circulating Tumor DNA (ctDNA)

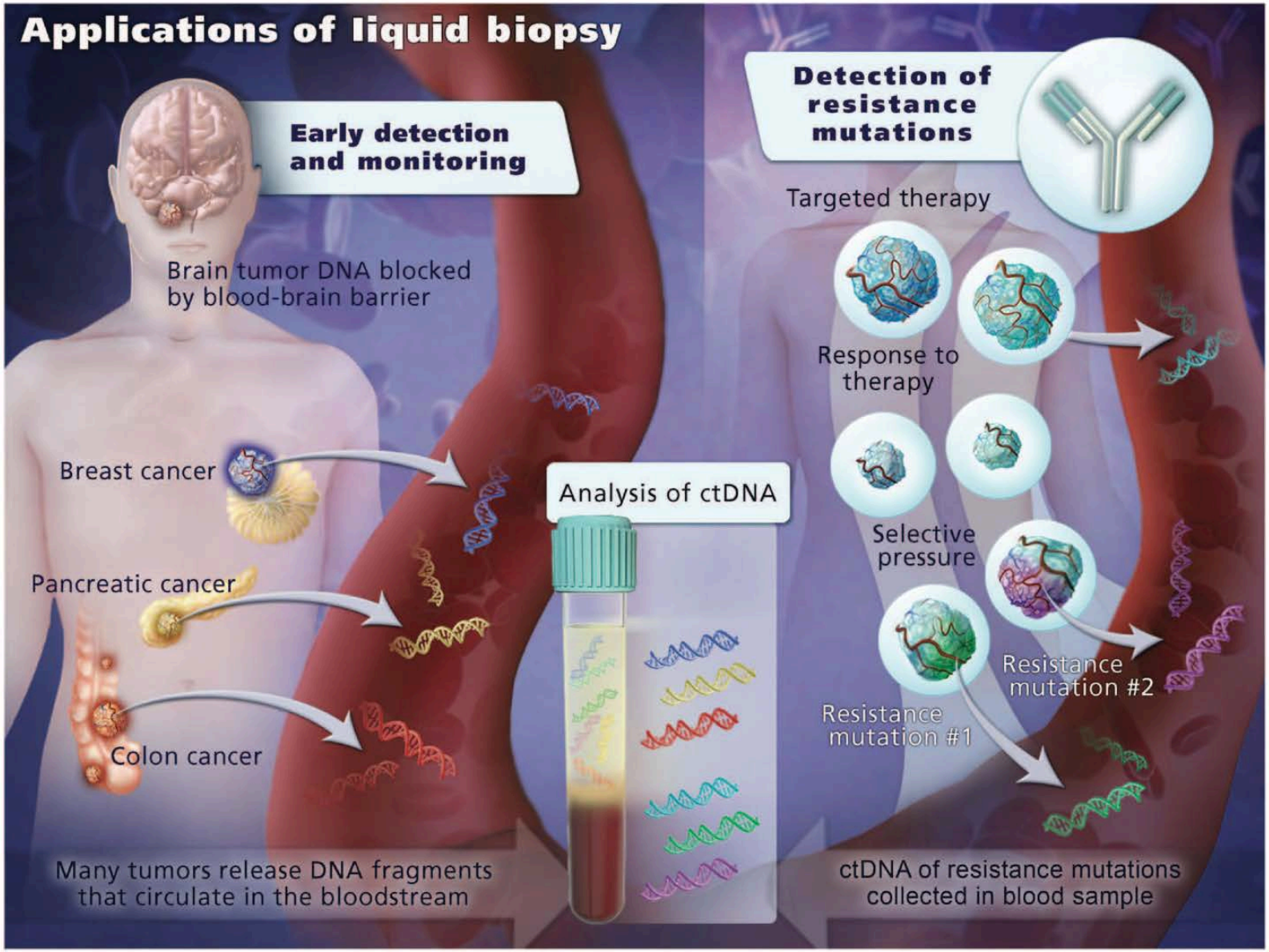
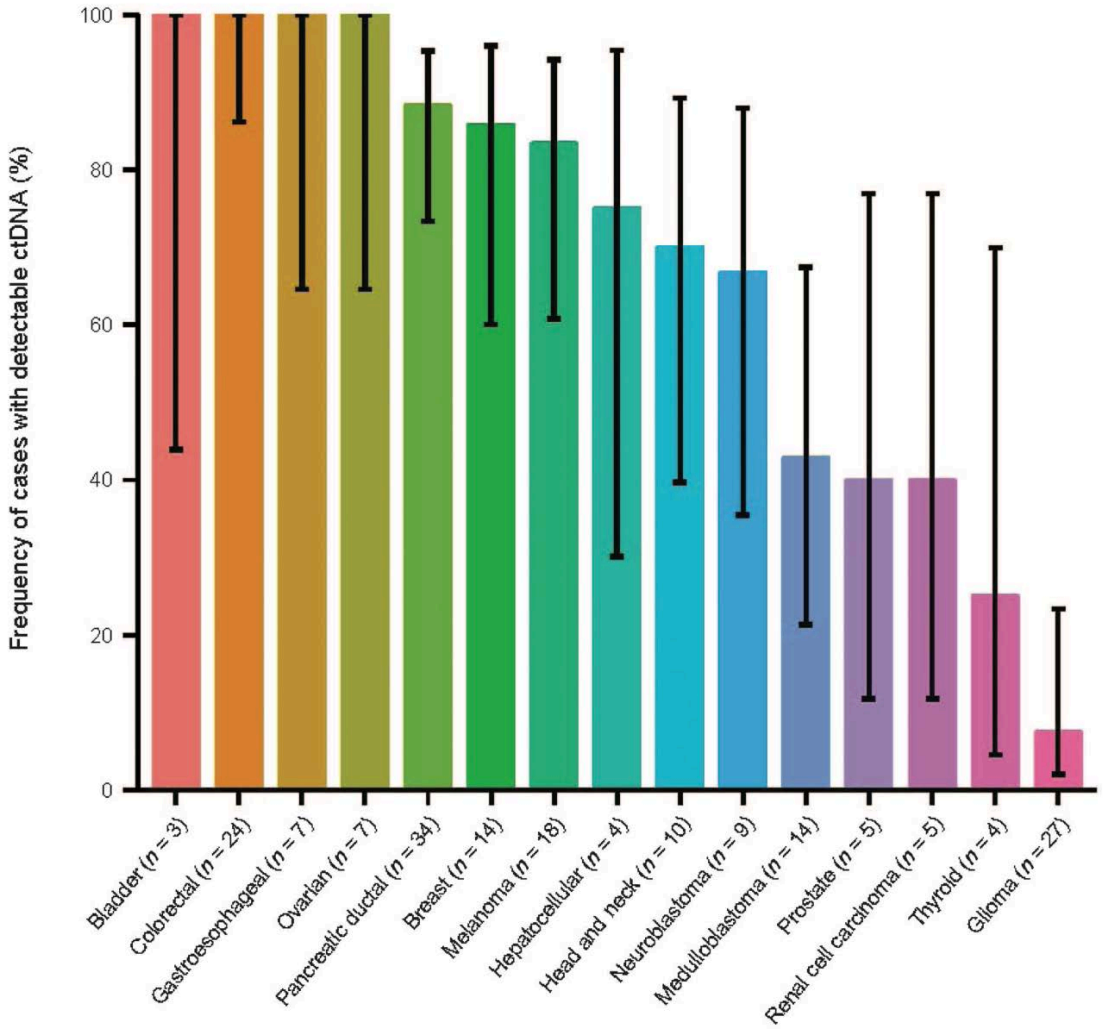


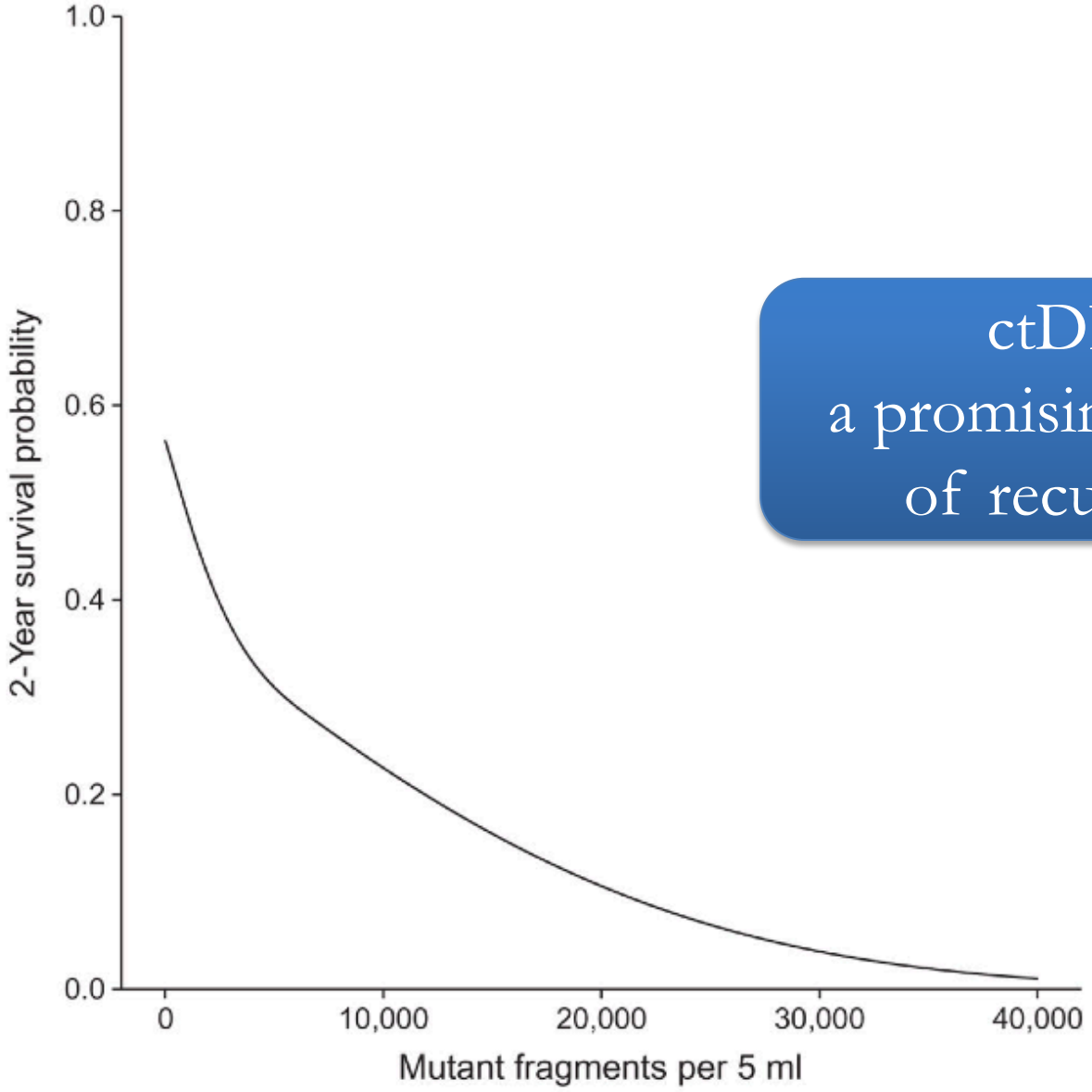
Fig. 1. Potential applications of ctDNA.

Sci Transl Med. 2014 Feb 19;6(224):224

Clinical Sensitivity Depends on Tumor Type



Sci Transl Med. 2014 Feb 19;6(224):224



ctDNA
a promising marker
of recurrence

Fig. 5. The relationship between ctDNA concentration (mutant fragments per milliliter) and 2-year survival

The association between survival and ctDNA concentration was assessed, holding known prognostic factors (age, ECOG PS, and CEA) constant. The 2-year survival was estimated on the basis of a multivariable Cox regression model, in which ctDNA concentration level was transformed with a natural spline function.

Sci Transl Med. 2014 Feb 19;6(224):224

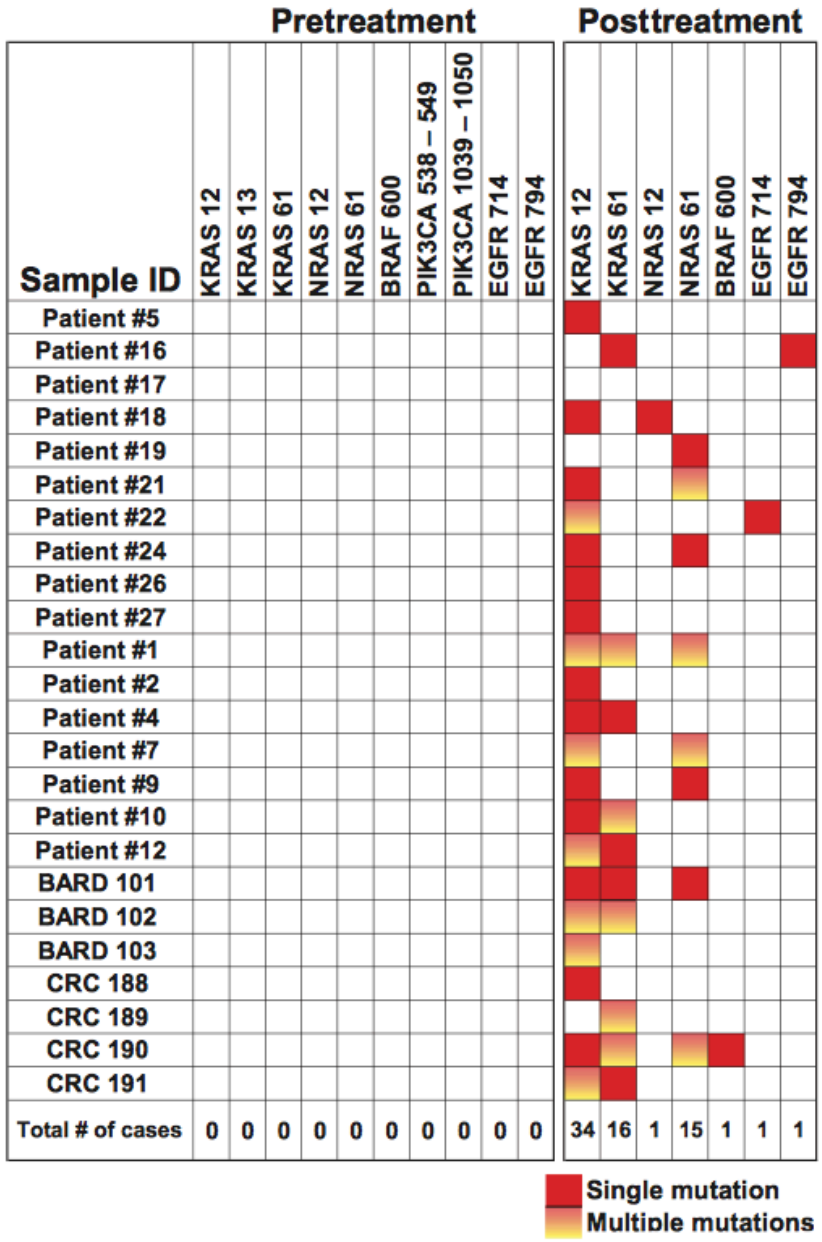
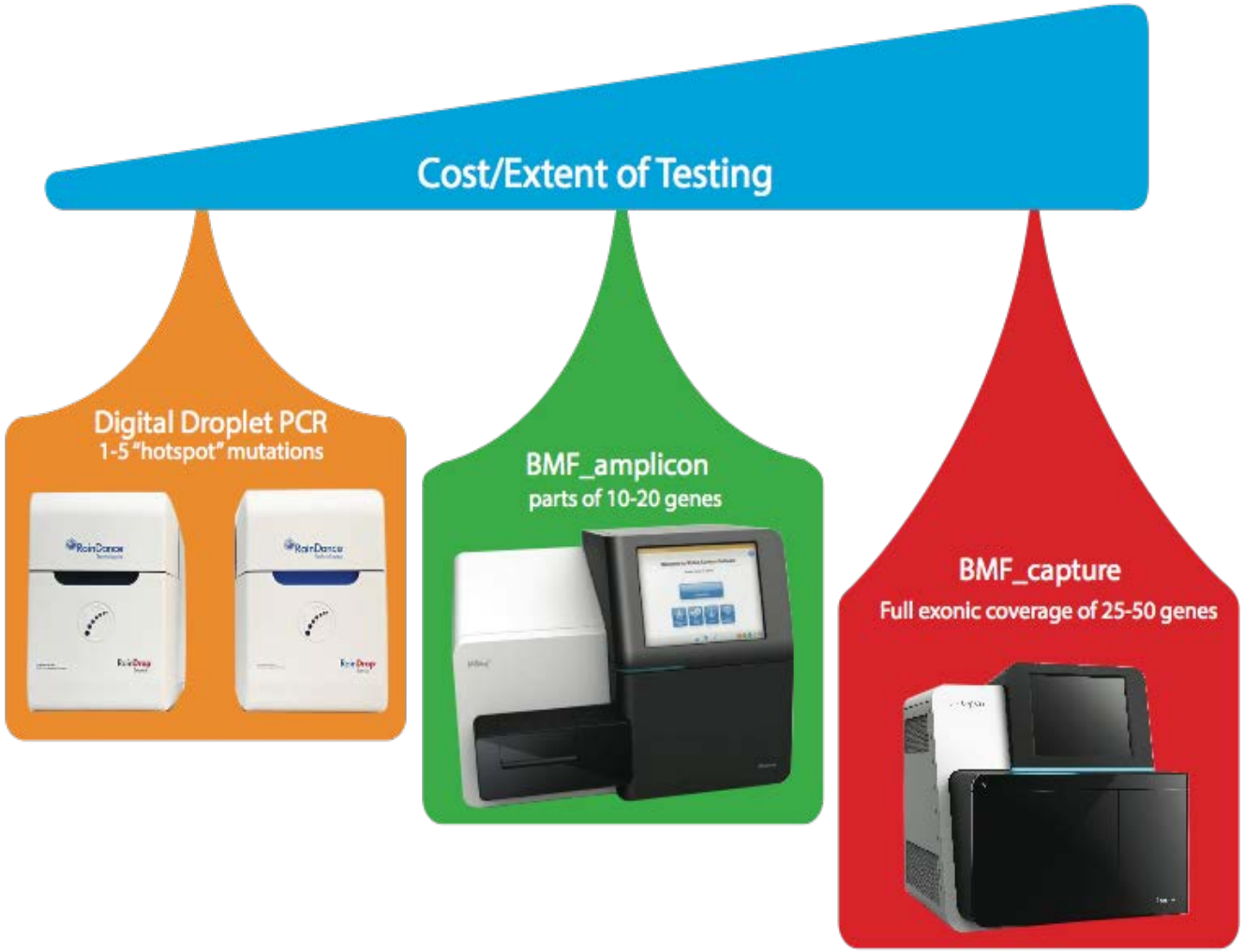


Fig. 6. Heat map of acquired resistance mutations to EGFR blockade in ctDNA from patients with metastatic CRC.

Also for looking at development of resistance

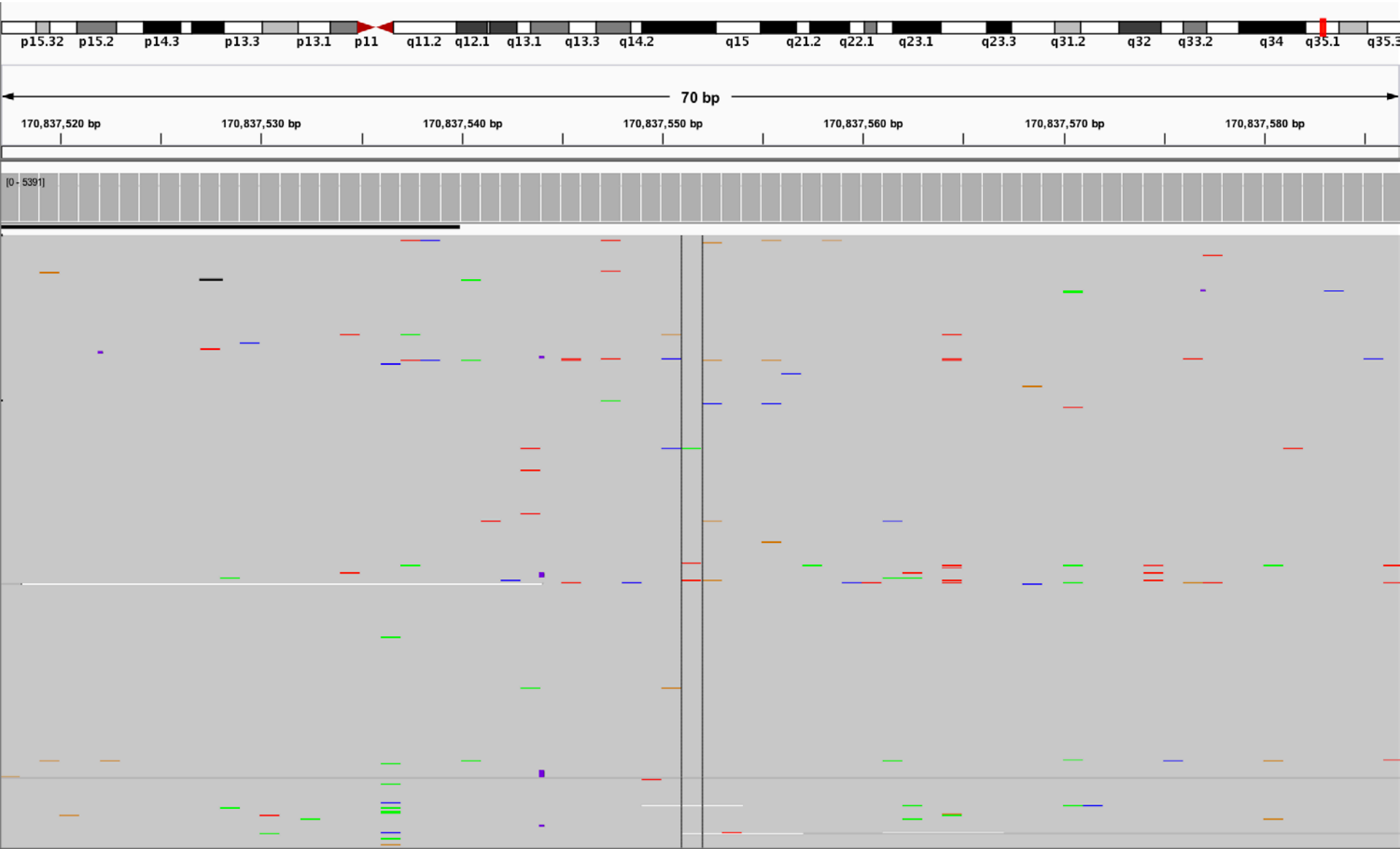
Sci Transl Med. 2014 Feb 19;6(224):224

Cell-free DNA technologies: Achieving high sensitivity

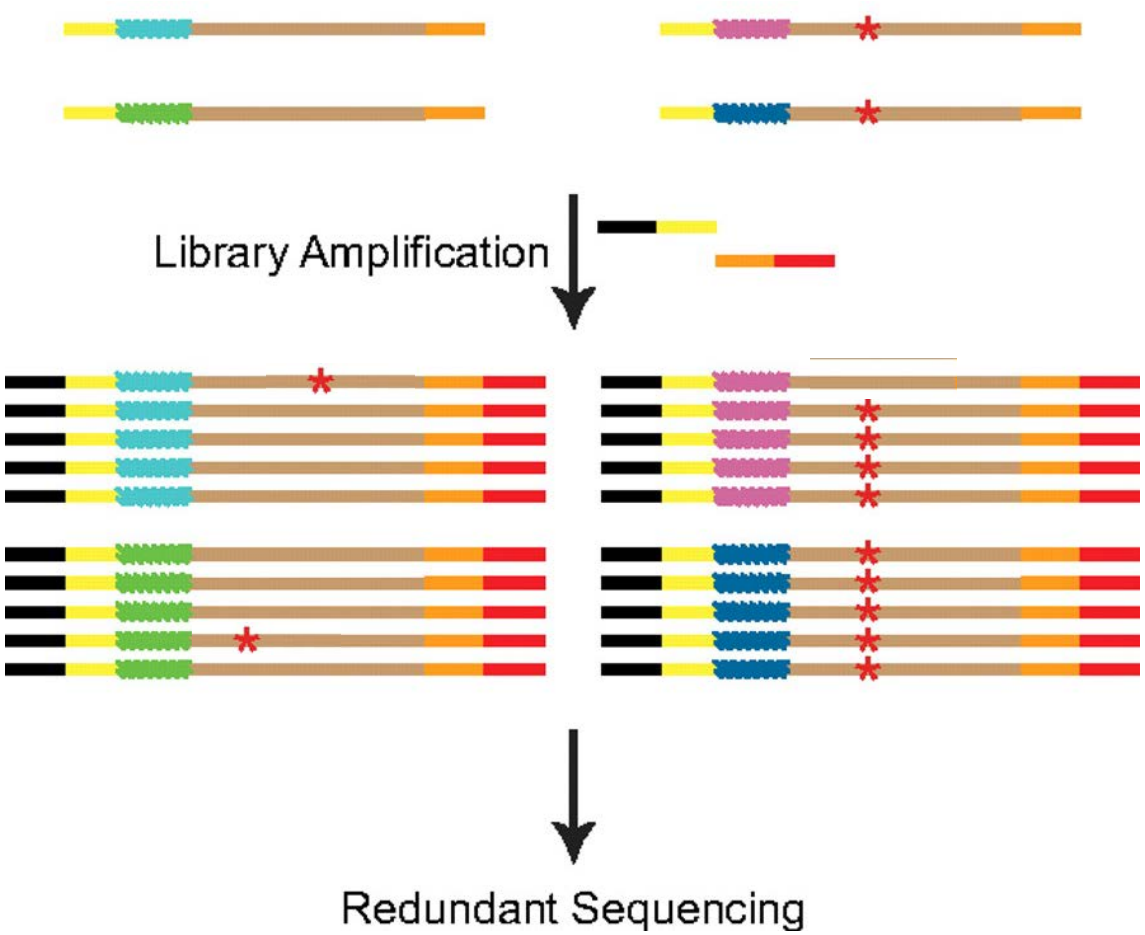


Slide courtesy from Sabine Hellwig, PhD

Challenge: Finding Low% Mutations in NGS Data

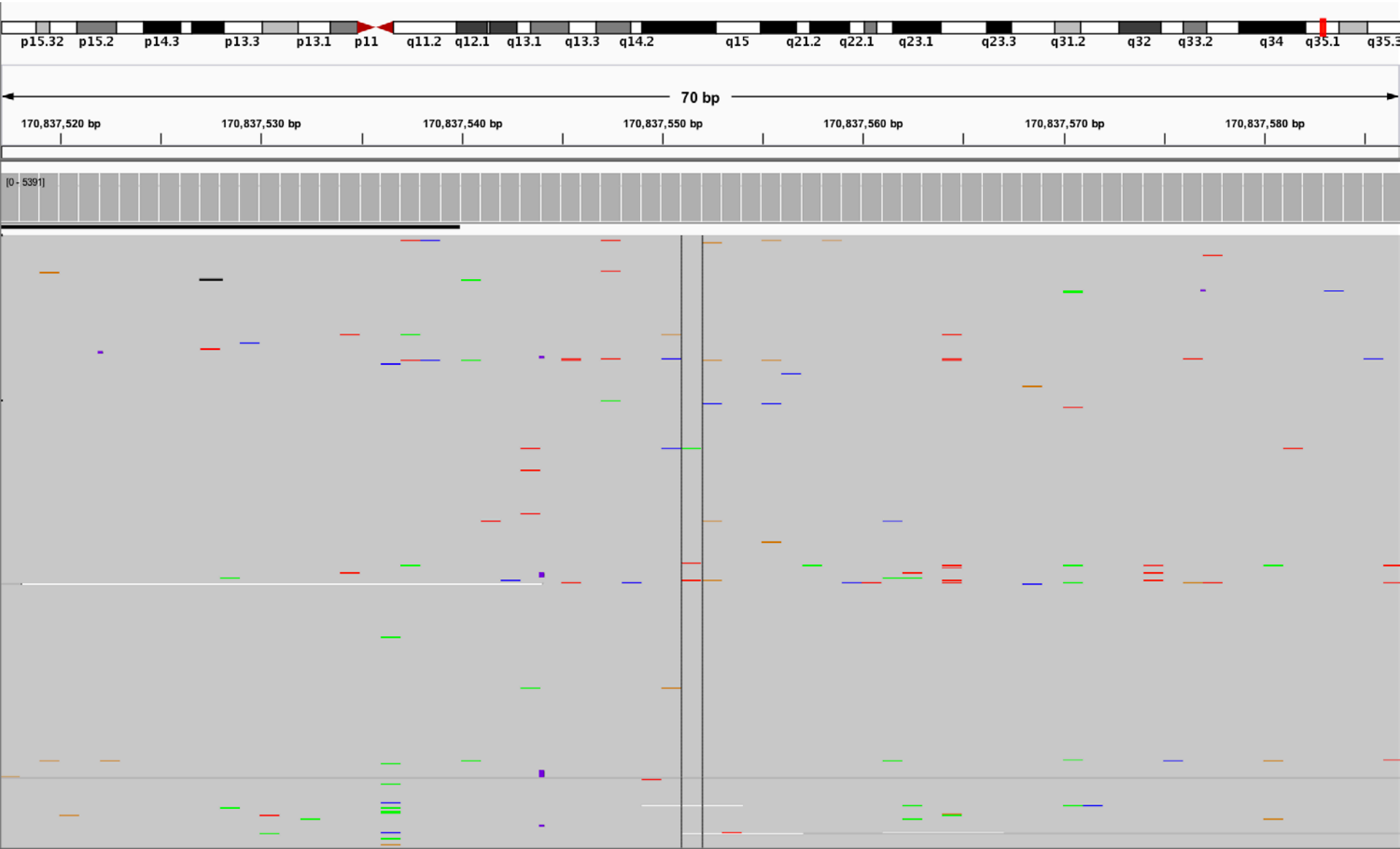


Molecular Barcode Proof-Reading

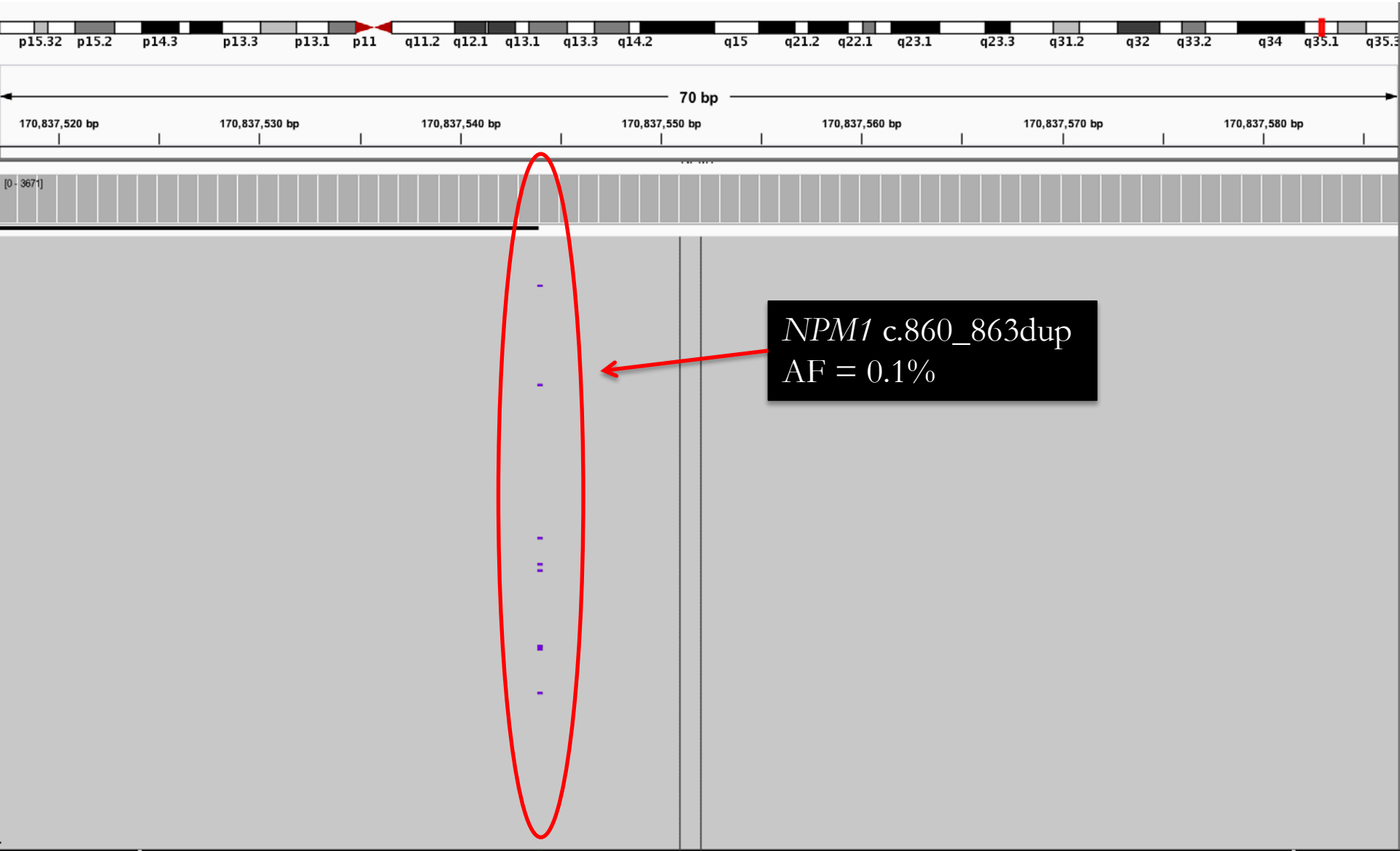


Kinde I et al. PNAS 2011;108:9530-9535

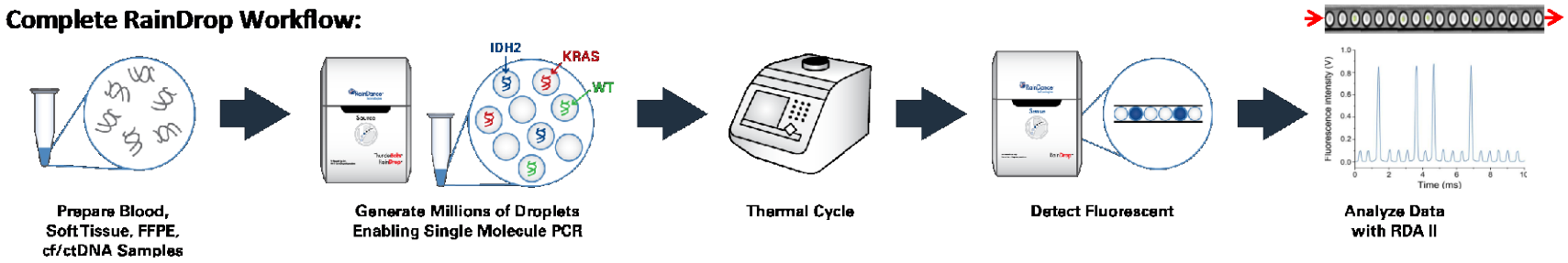
Molecular Barcode Proof-Reading

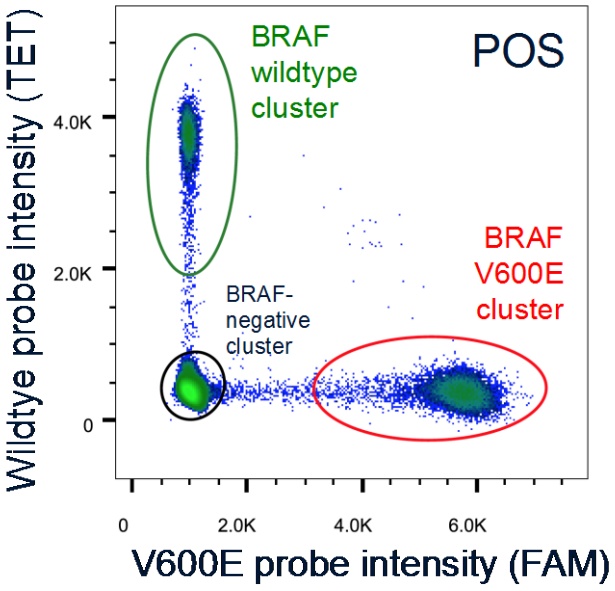


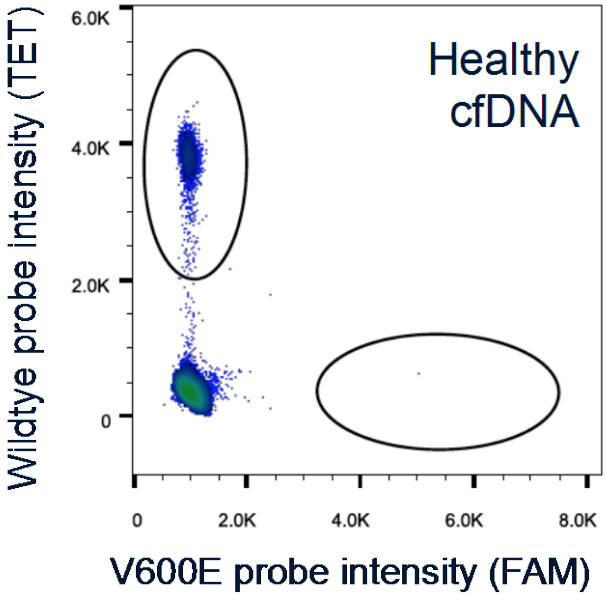
Molecular Barcode Proof-Reading



ddPCR Workflow and Analysis (RainDrop)







- Absolute count of amplified wildtype and mutant copies
- Detected copies/mL plasma or MAF can be calculated

Slide courtesy from Sabine Hellwig, PhD

ARUP Validated ddPCR assays

EGFR T790M

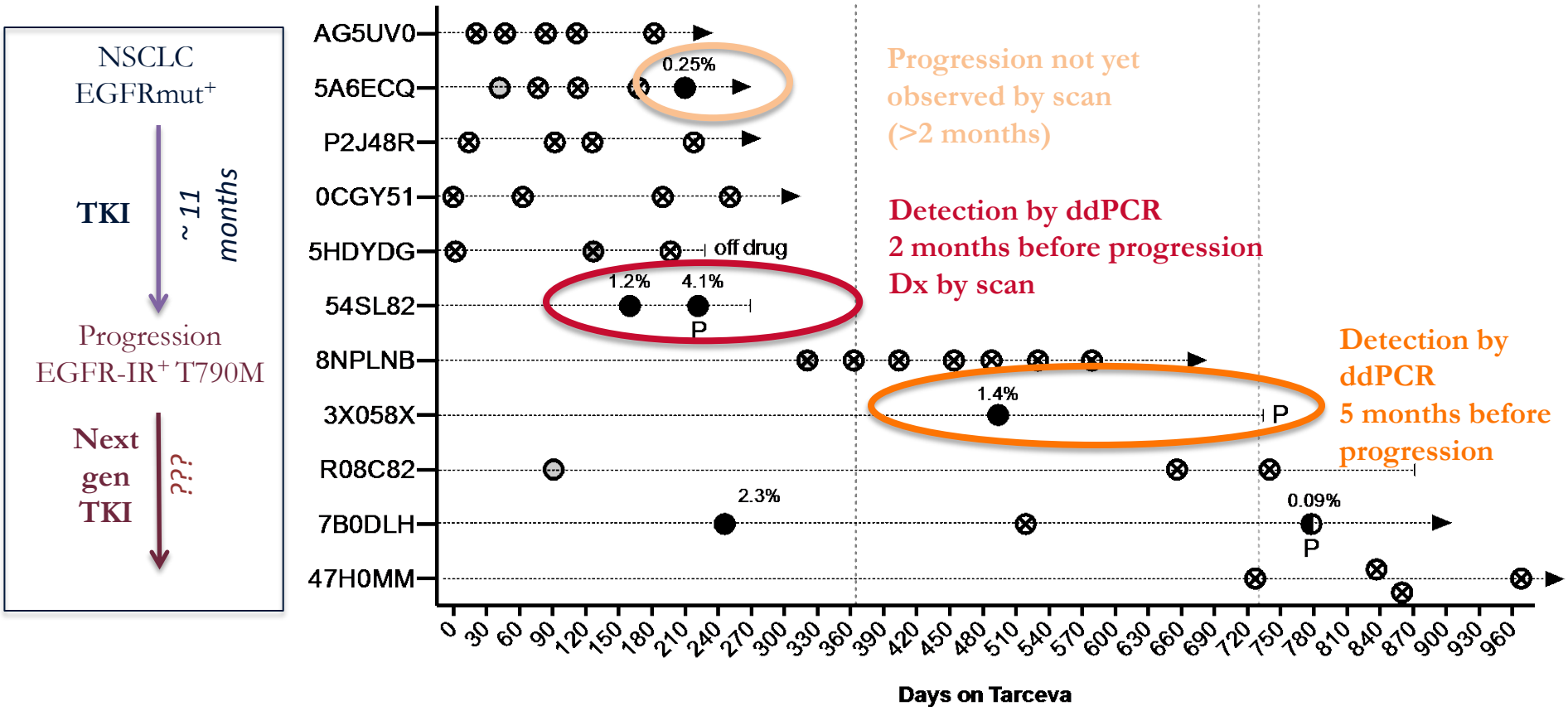
- Resistance mutation causing loss of sensitivity to EGFR-targeted primary TKI therapy (erlotinib, gefitinib) in non-small cell lung cancer (NSCLC)
  - Average progression on TKI after 11 months (100% progression rate)
  - T790M accounts for 2/3 of cases with acquired resistance
- Next generation TKI with (prospective) FDA approval:
  - Osimeritinib (*Tagrisso, Astra Zeneca*) – accelerated approval Nov 2015
  - Rocelitinib (*Clovis*) – delayed approval

BRAF V600E

- Activating point mutation in the BRAF kinase domain
  - 50% of melanoma, 20-40% of thyroid cancers, 8-15% of colorectal, 1-4% of NSCLC
  - Valine to glutamate accounts for ~90% of mutations at V600
- Associated with increased sensitivity to
  - Dabrafenib (BRAF inhibitor)
  - Vermurafenib (BRAF inhibitor)
  - Trametinib, cobimetinib (MEK inhibitors)

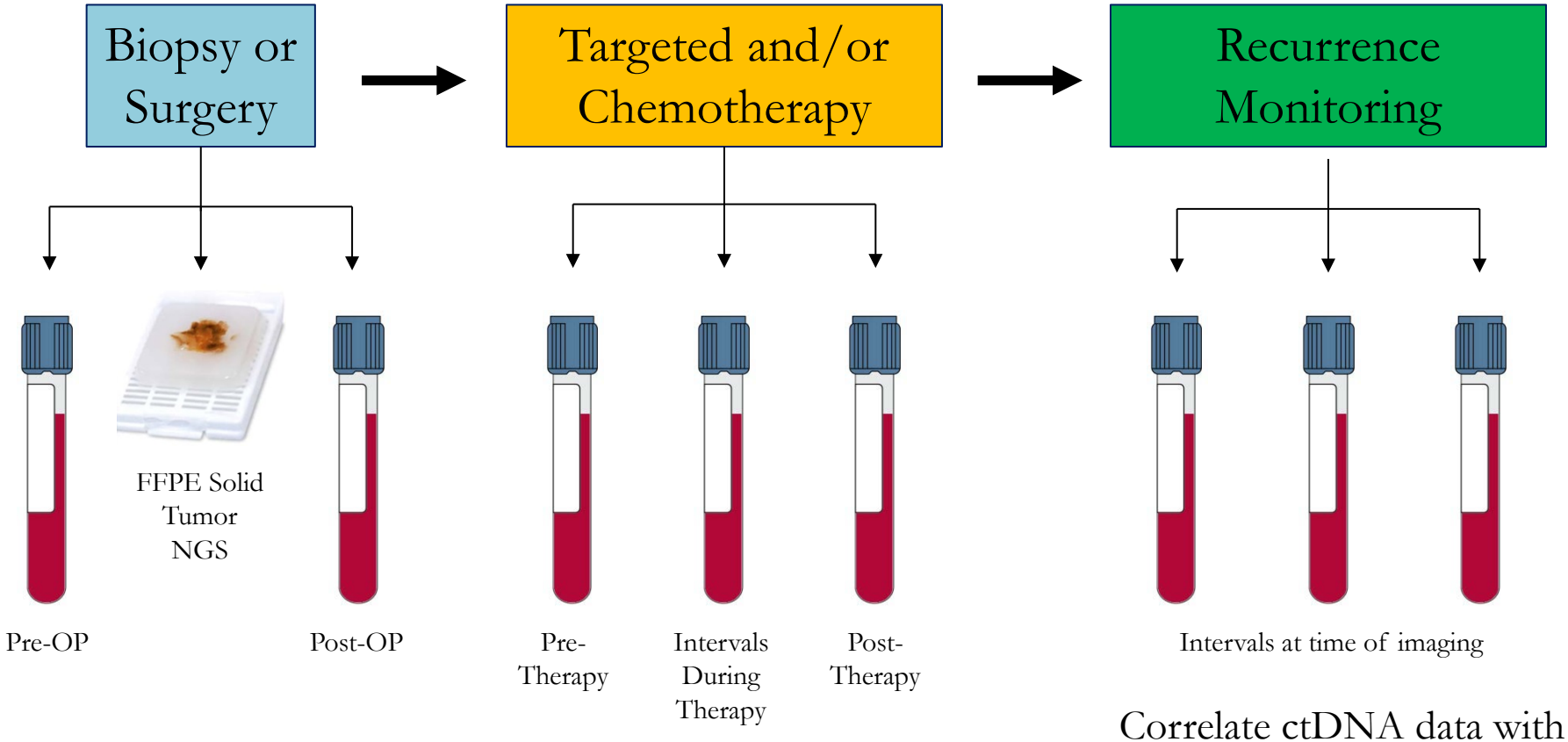
Slide courtesy from Sabine Hellwig, PhD

T790M Resistance monitoring: Tarceva cohort



Slide courtesy from Sabine Hellwig, PhD

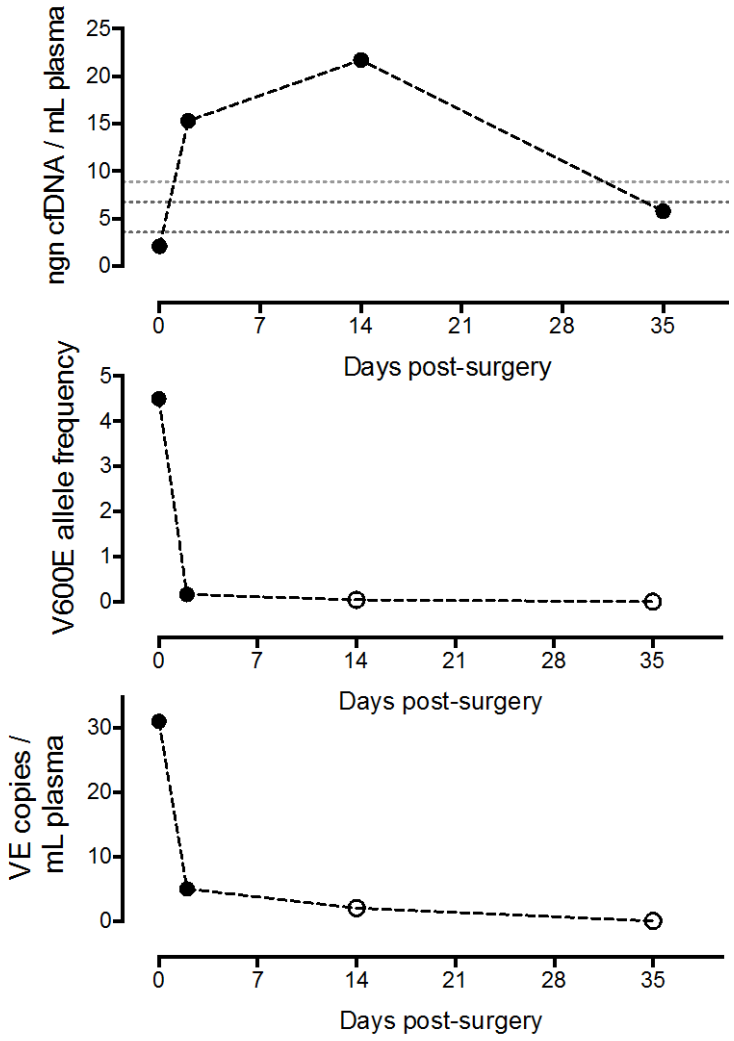
Molecular margins, treatment response, & early recurrence monitoring by ctDNA



Correlate ctDNA data with imaging, whole body and tumor perfusion sampling

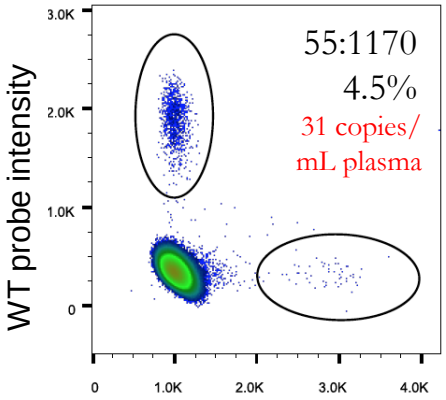
Slide courtesy from Sabine Hellwig, PhD

BRAF V600E - Surgical Margin monitoring (Melanoma case 1)



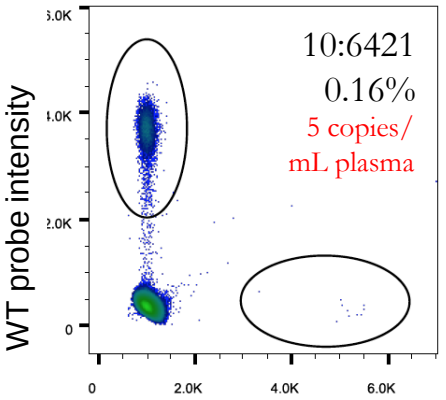
Pre-surgery

cfDNA: 2.1ng/mL plasma



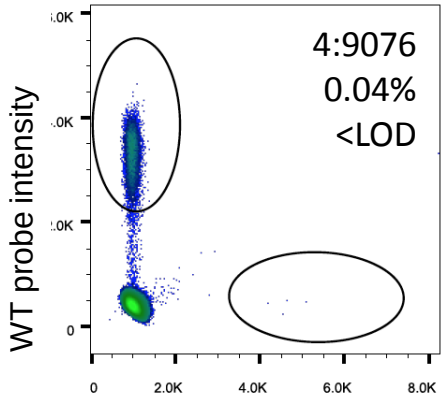
48h post surgery

cfDNA: 15.3ng/mL plasma



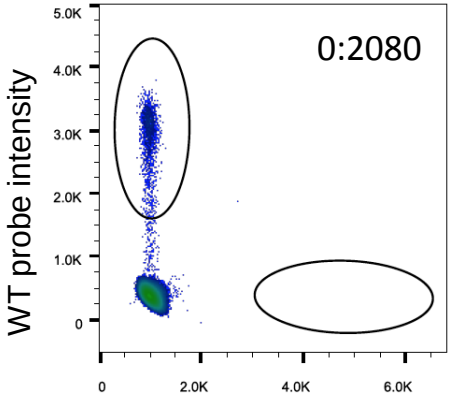
14d post surgery

cfDNA: 21.7ng/mL plasma



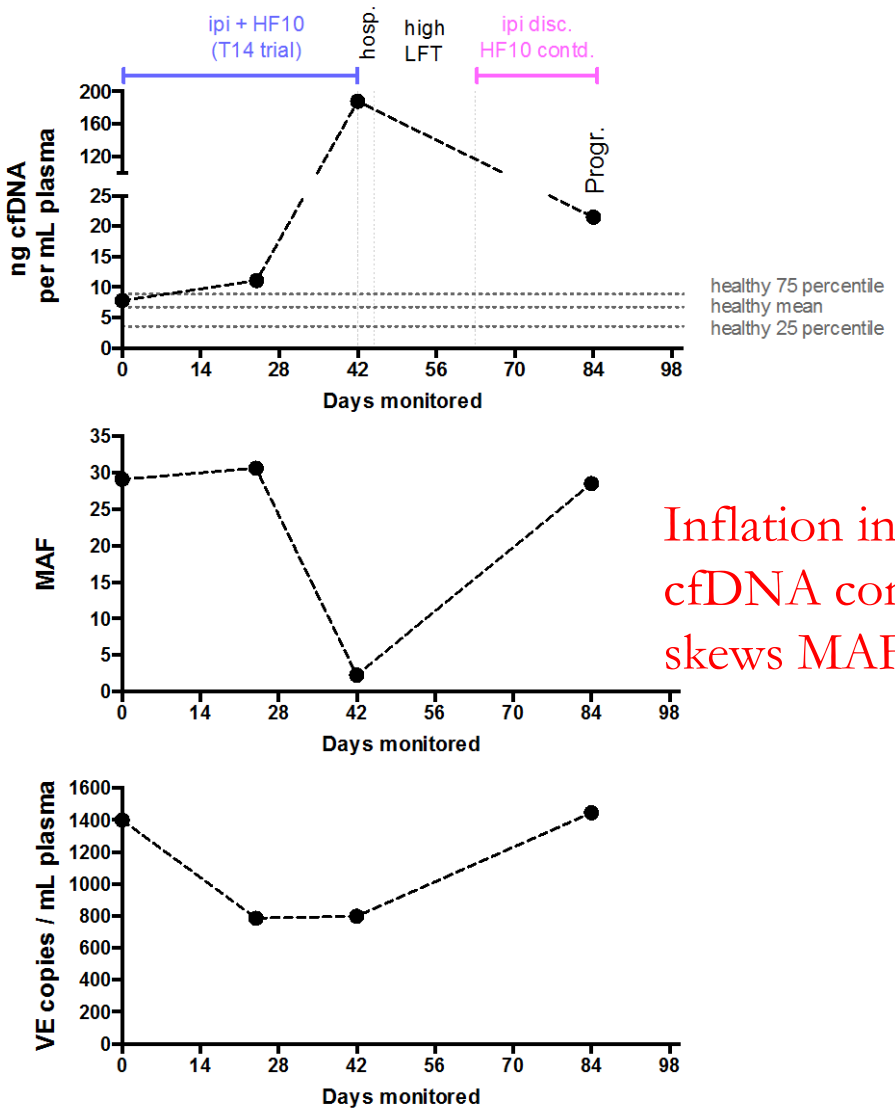
5w post surgery

cfDNA: 5.8ng/mL plasma

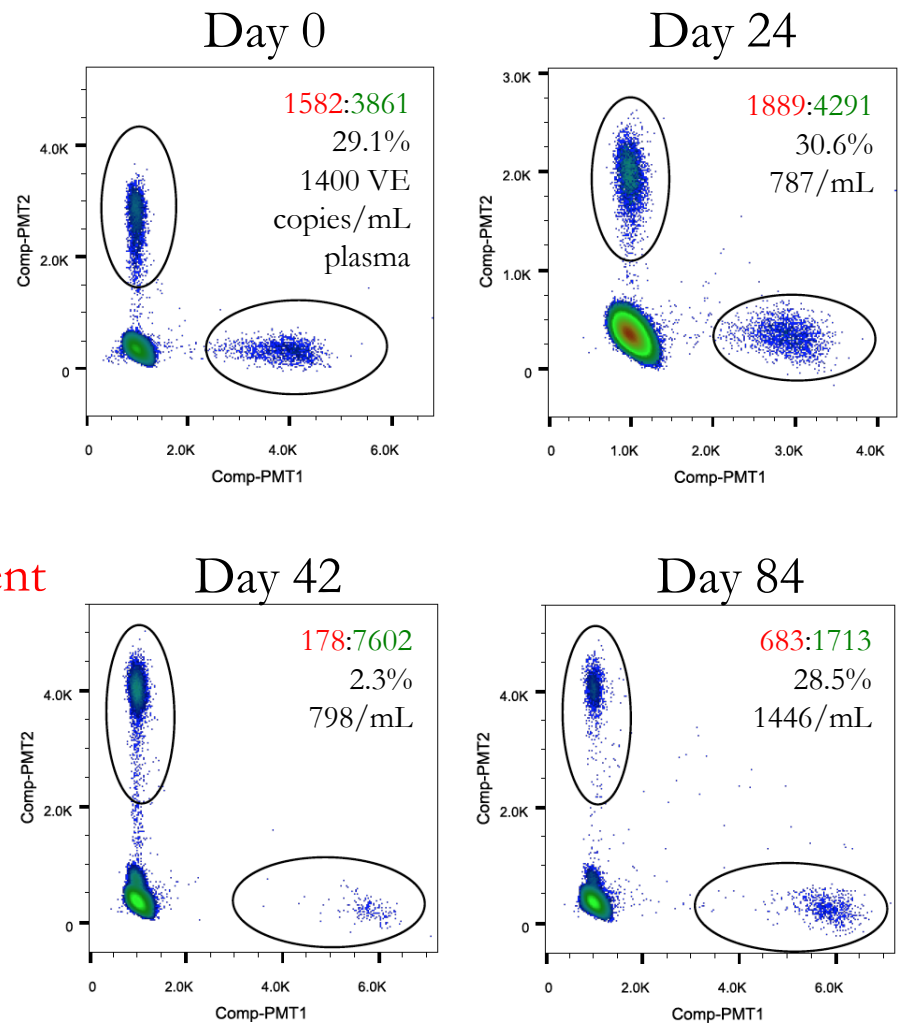


Slide courtesy from Sabine Hellwig, PhD

BRAF V600E - Melanoma case 2 – MAF v. copies/mL

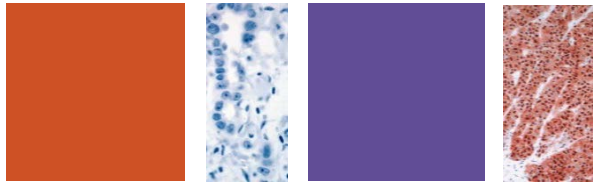


Inflation in cfDNA content skews MAF!



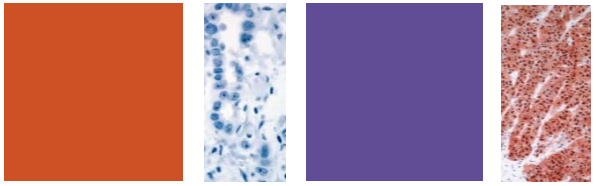
Slide courtesy from Sabine Hellwig, PhD

Liquid Biopsy: Replacing Tissue?



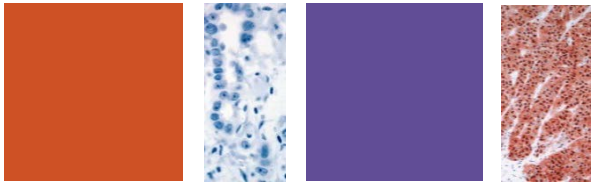
- Clearly not all tumors shed DNA into the blood in appreciable amounts.
- Discovered mutations do not necessarily come from the tumor of interest.
- Resistance mutations present in a subset of cells may not be discoverable by ctDNA, but may be detectable in tissue.
- Tissue testing seems likely to remain first-line, although there are great possibilities for liquid biopsy for surveillance.

Conclusions



- NGS continues to revolutionize personalized diagnostics in oncology.
- NGS is allowing for comprehensive analysis of difficult specimen types (small biopsies, cytology specimens and plasma and body fluid specimens).
- Many applications are emerging beyond simple sequence analysis (ctDNA, immune profiling, gene expression, epigenetics, etc.).
- Currently, the trends are towards increasing the breadth of analysis for each patient.
- Questions remain about optimal testing strategies.

Thanks! Questions?



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  – larissa.furtado@aruplab.com