HARVARD MEDICAL SCHOOL

Specimen Handling for Molecular Success

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Disclosures

Consultant- GV20 Therapeutics
Consulting fees (to my institution) – Genentech, Lilly
Research funding (to my institution) –Genentech

Specimen Challenges

QUANTITY

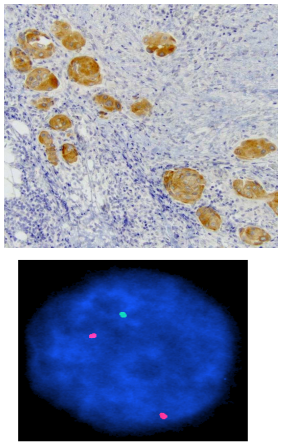
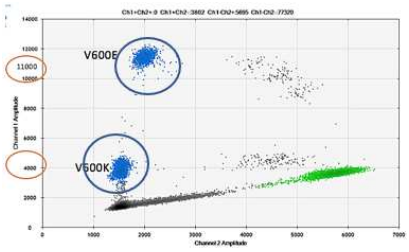
QUALITY

QUANTITY

How much does your assay require?

How much tissue (total DNA)?

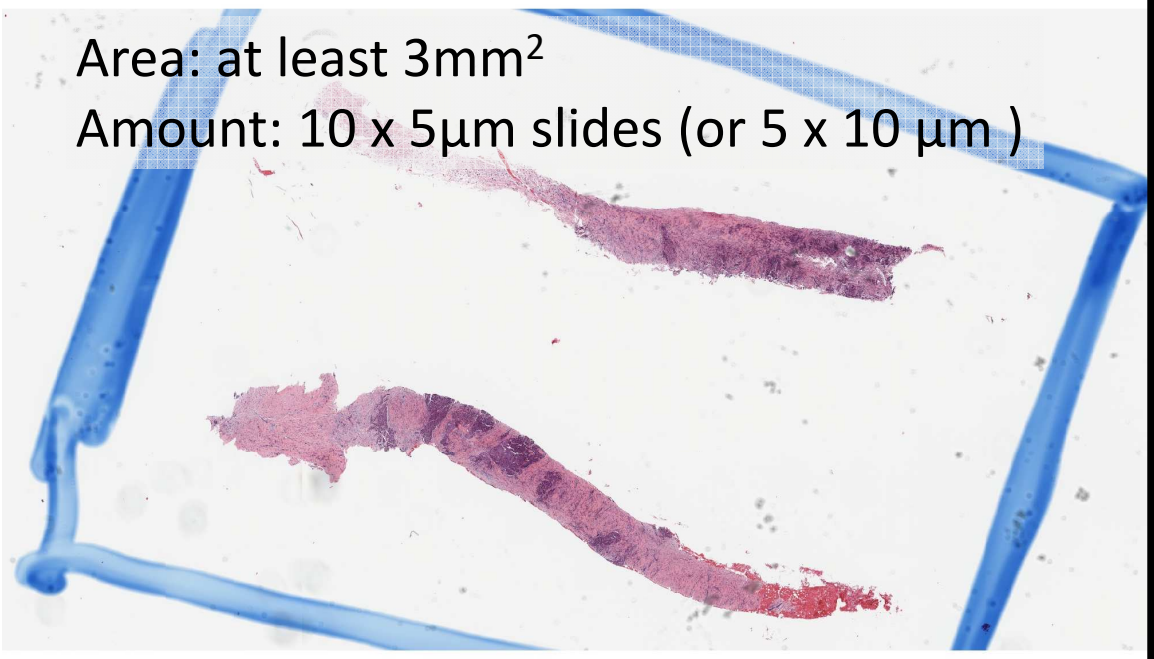
How much tumor (percent tumor content)?

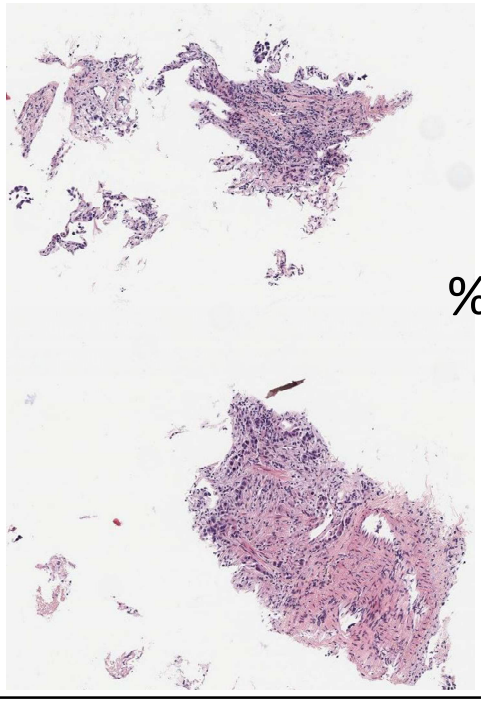
In situ assays	High sensitivity single gene tests	Panel NGS
		
50-100 *cells*	5-15 ng nucleic acids	50-100s ng nucleic acids

For high-complexity, high-cost tests, establishing clear tissue size criteria will increase likelihood of success.

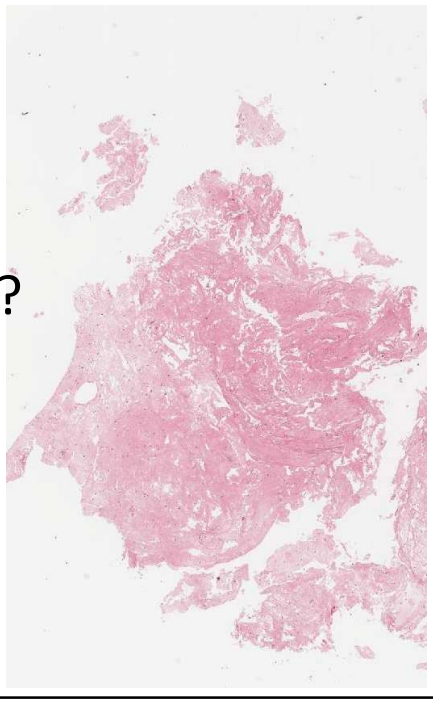
Area: at least 3mm^2

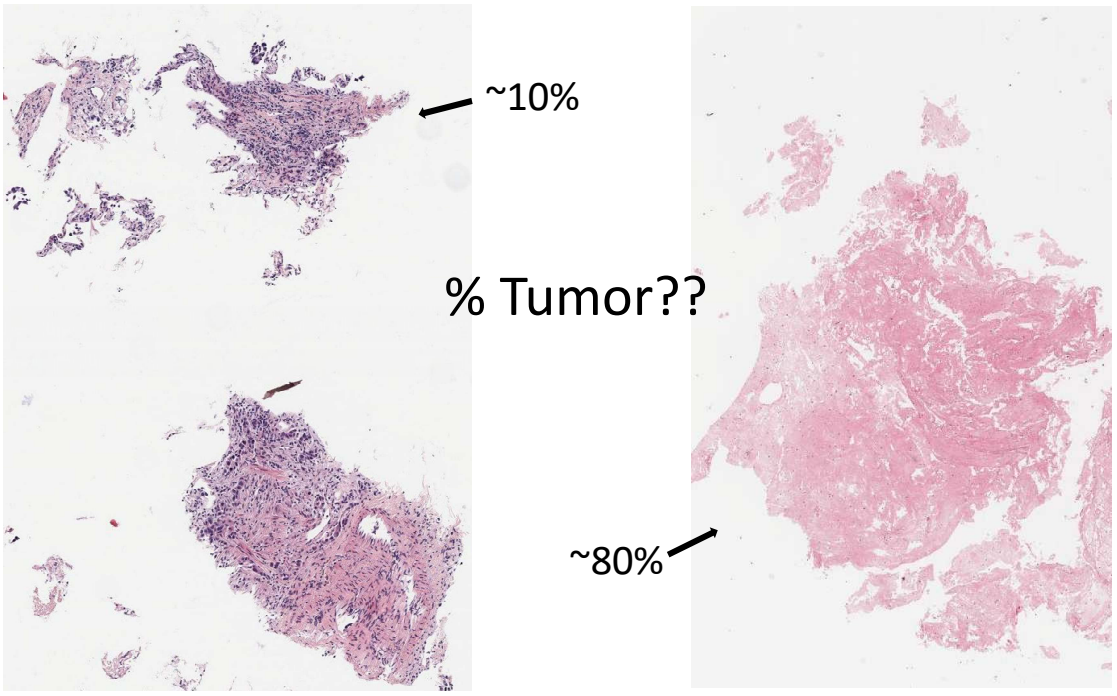
Amount: 10 x 5µm slides (or 5 x 10 µm)





% Tumor??





Published: 26 July 2013

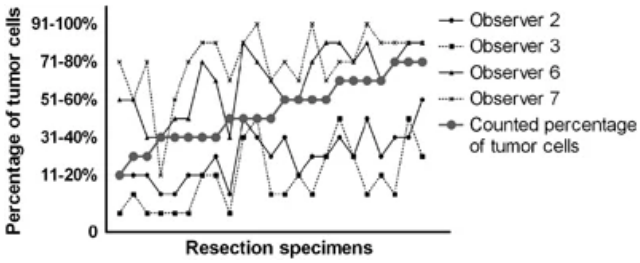
The estimation of tumor cell percentage for molecular testing by pathologists is not accurate

Alexander J J Smits, J Alain Kummer, Peter C de Bruin, Mijke Bol, Jan G van den Tweel, Kees A Seldenrijk, Stefan M Willems, G Johan A Offerhaus, Roel A de Weger, Paul J van Diest & Aryan Vink

Modern Pathology **27**, 168–174 (2014) | Cite this article

What is the problem?

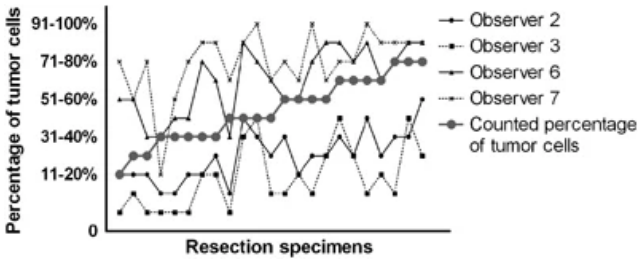
- Significant interobserver variability
  - Systematic overestimation
  - Systematic underestimation



Smits et al. Mod Pathol. 2014 Feb;27(2):168-74

What is the problem?

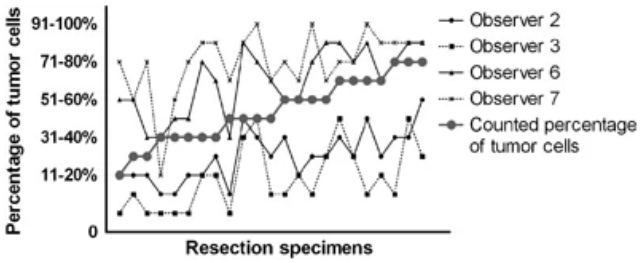
- Significant interobserver variability
  - Systematic overestimation
  - Systematic underestimation
- Random error
  - Misinterpretation
  - Benign/reactive vs malignant glands



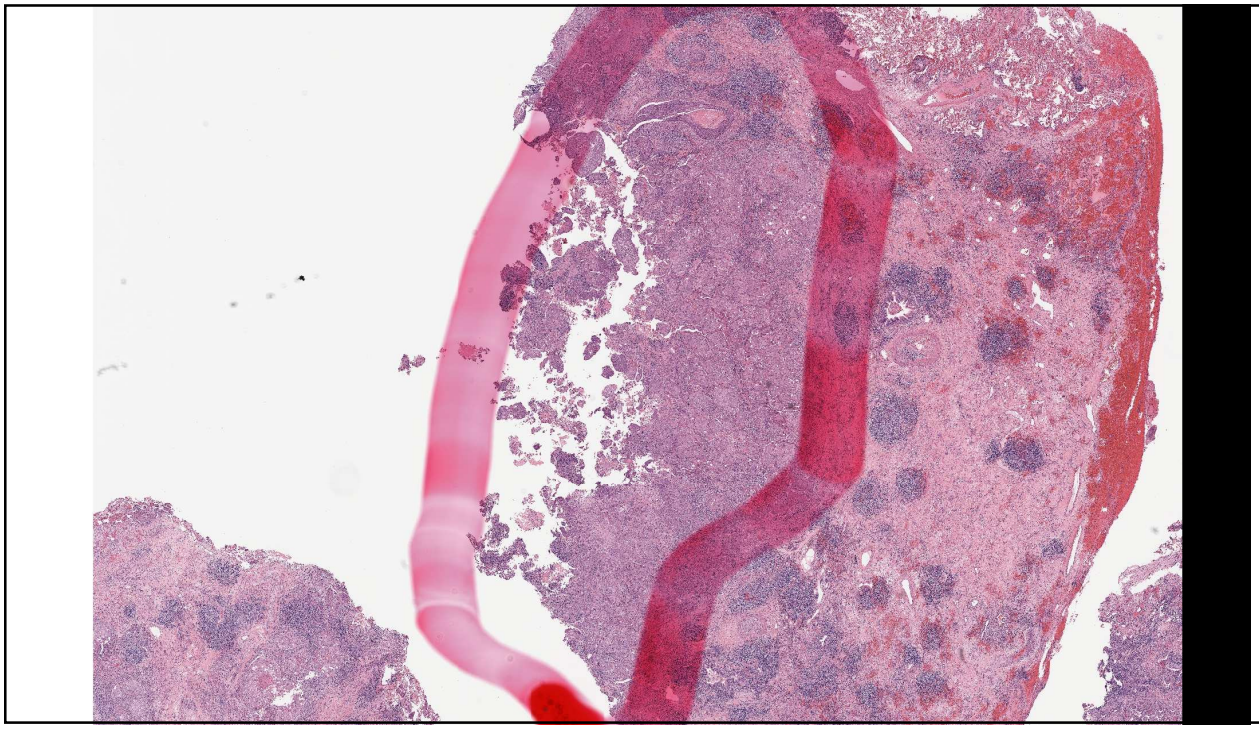
Smits et al. Mod Pathol. 2014 Feb;27(2):168-74

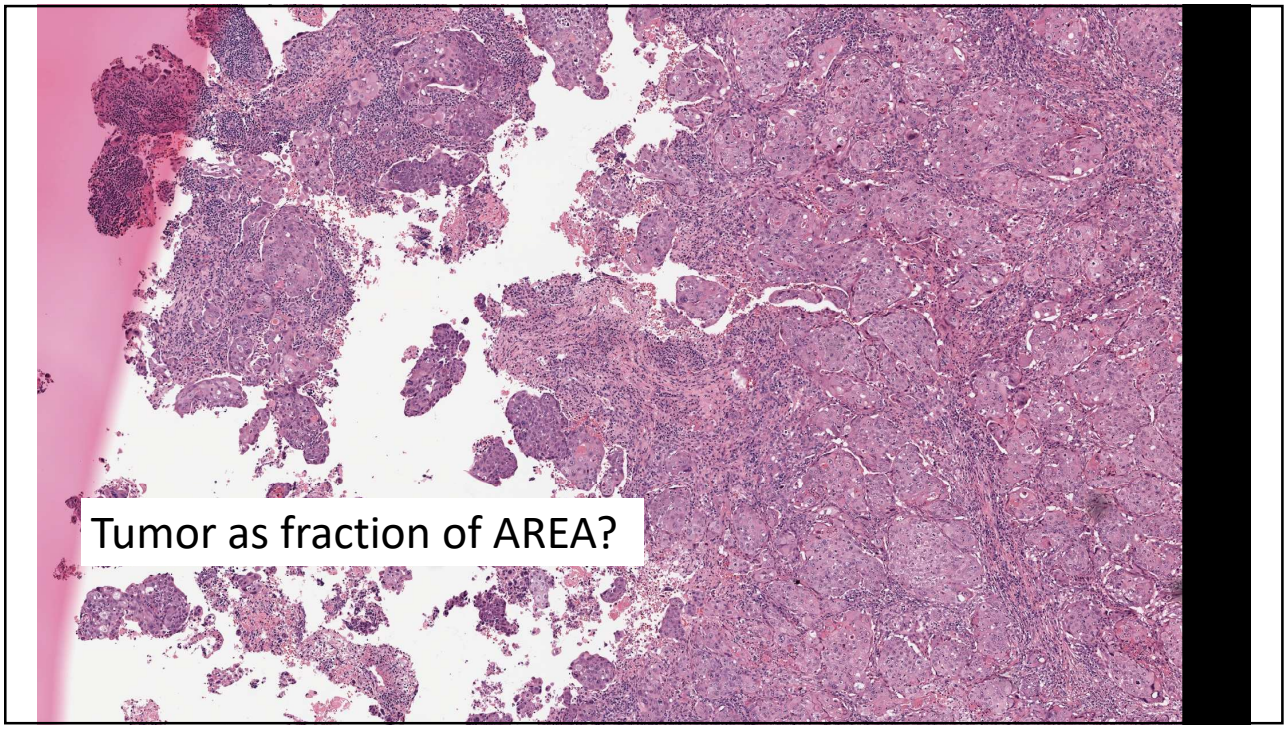
What is the problem?

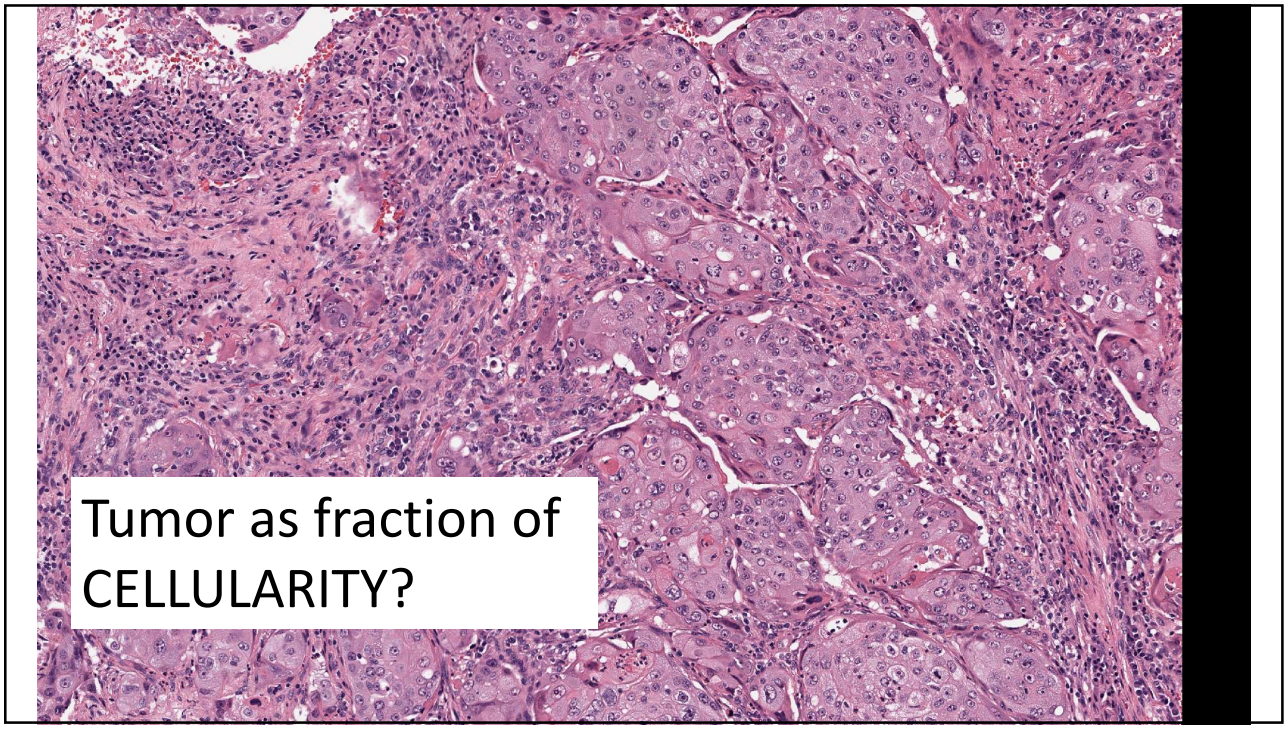
- Significant interobserver variability
  - Systematic overestimation
  - Systematic underestimation
- Random error
  - Misinterpretation
  - Benign/reactive vs malignant glands
- Failure to feedback test results to pathologists



Smits et al. Mod Pathol. 2014 Feb;27(2):168-74




Tumor as fraction of AREA?

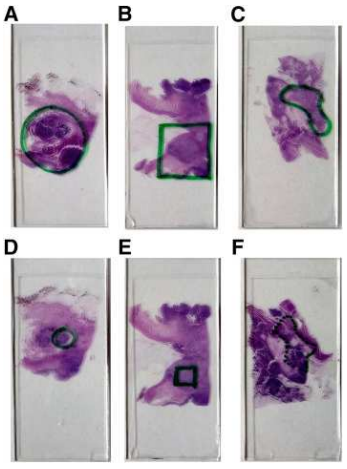

Tumor as fraction of
CELLULARITY?

What does the molecular testing suggest?

- EGFR L858R mutation at 28% of reads
- TP53 Q331* mutation at 16% of reads
- Polysomy of chromosome 7 (including EGFR, 4-5 copies)
- Loss of heterozygosity on 17p (including TP53)

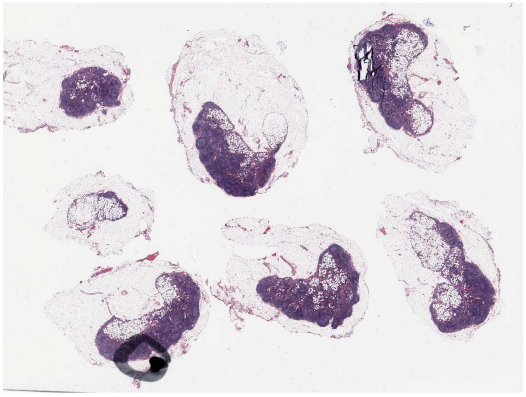
- Estimated 20% tumor content

“Oncocircling”

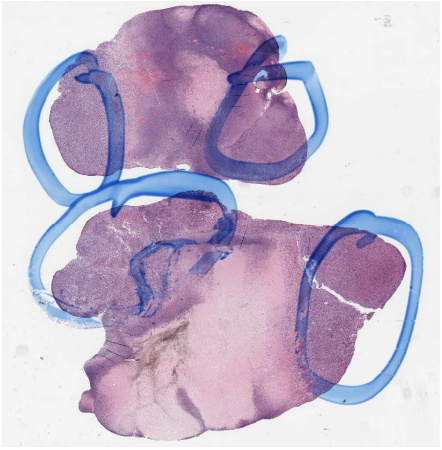


Dufraing et al. J Mol Diagn. 2018 Jul;20(4):455-464

Risks



Tiny tumor focus, complex slide profile.



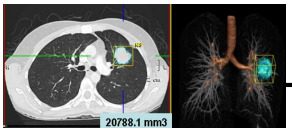
Large, pure tumor. Extensive necrosis.

Sample adequacy is a major challenge for tissue biomarker testing.

- Meric-Bernstam et al. J Clin Oncol 2015: <50 gene panel:
- ***77%*** of advanced stage cancer patients (all types) have adequate tissue

- Sholl et al. JCI Insight 2016: 270 gene panel:
- ***72%*** of cancer patients (all types, all stages) have adequate tissue

- Aggarwal et al. JAMA Oncol 2019: 20-153 gene panels:
- ***62%*** of advanced stage/relapsed NSCLC have adequate tissue



Lung Mass or suspected Lung Cancer referred for biopsy

Images (CT, PETCT, MR)

Laboratory data

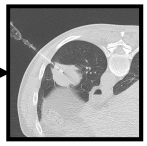
Review of Order and accepting procedure

Patient

Biopsy appointment Notification

Referring Physician

Biopsy scheduled, patient and referring physician informed



Biopsy performed and tissue sent to pathology

Creating PROCESSES to enhance the success of tissue molecular profiling

a.k.a. LUNGCOR

Formalin Fixed Biopsy

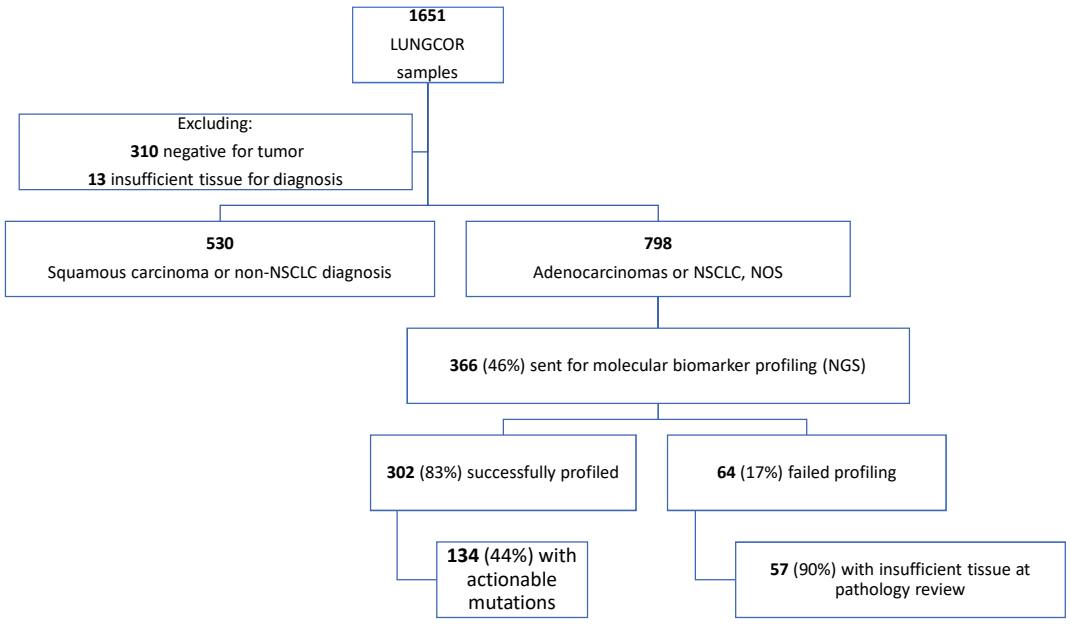
Paraffin Embedding

H&E + 18 FFPE + H&E

Pathologic Diagnosis Immunohistochemistry

Next Generation Sequencing Other Ancillary Studies

Ritu Gill, BI Deaconess Medical Center; Sarah Wu BWH Pathology

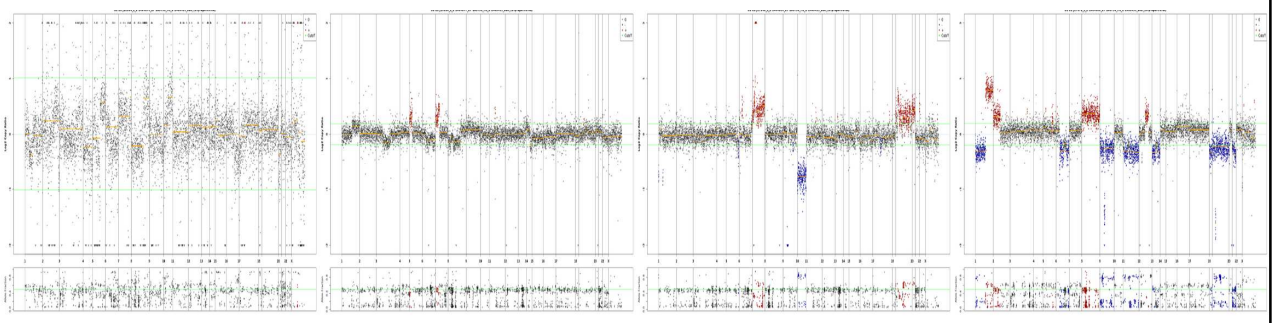


Sarah Wu. World Conference on Lung Pathology. 2020

QUALITY

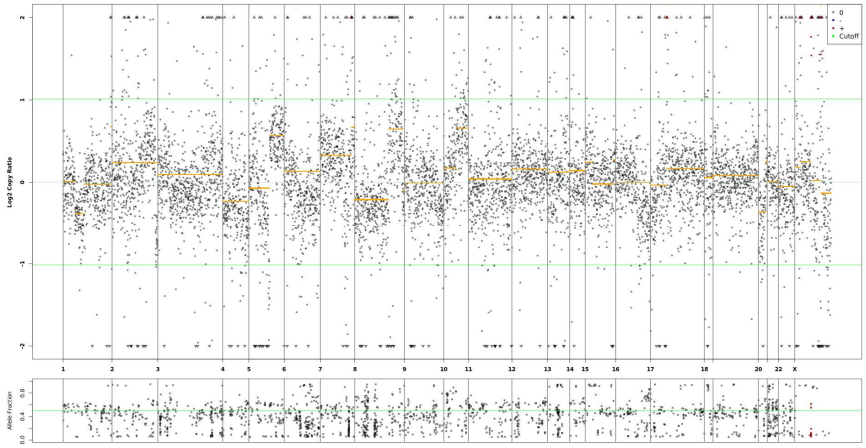
- What kind of specimen is it?
- How was it fixed?
- How was it stored/how old is it?
- How was it prepared?

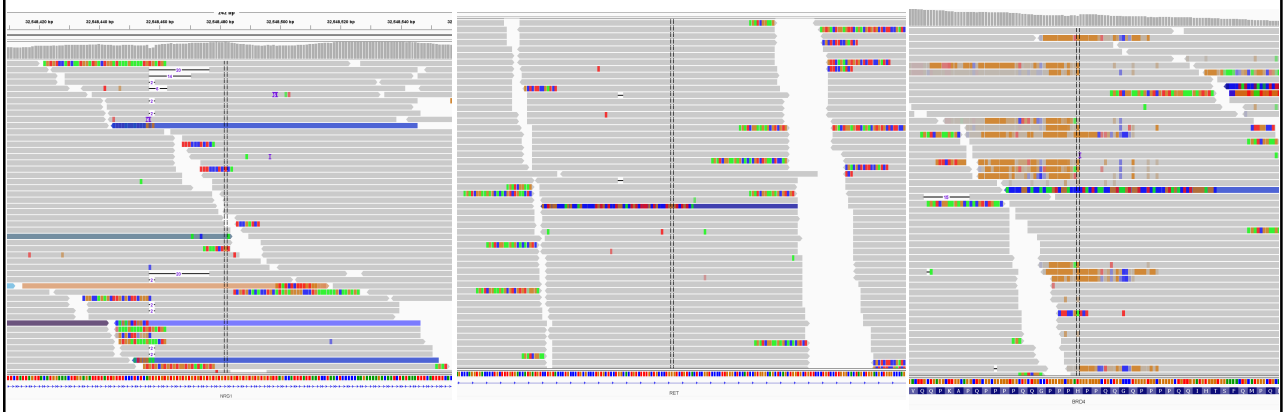
Which one of these is not like the others?



A **B** **C** **D**

Answer: A





What happened to this specimen?

A. Delayed fixation

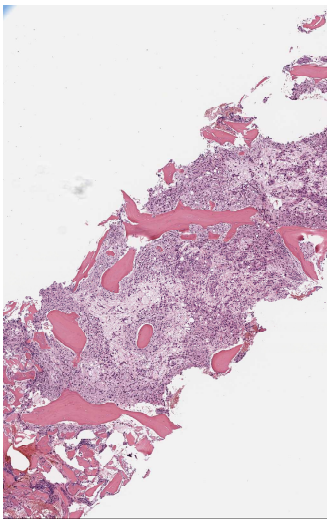
B. Formalin had the wrong pH

C. Decalcification

D. Extensive tissue necrosis

E. Any of the above

Answer: C. Decalcification



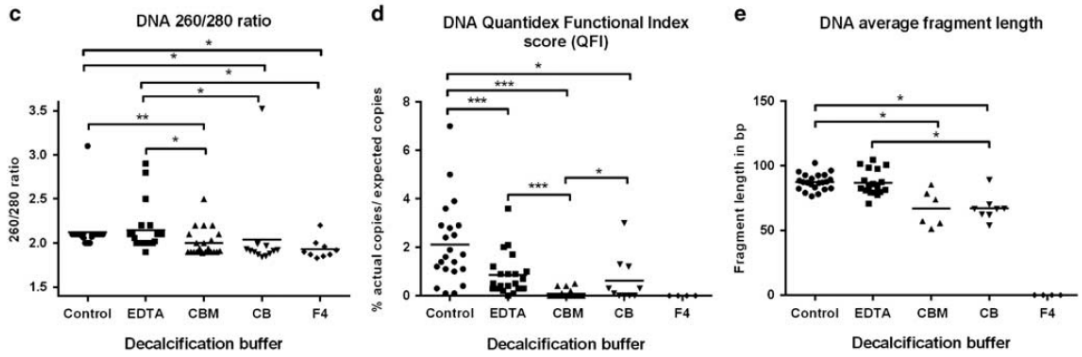
- Metastatic prostate carcinoma to the bone
- Required “gentle decal” prior to histologic processing
  - **proprietary formulation**
- Some interpretable molecular results
  - *TMPRSS2-ERG* fusion
  - *PTEN* c.955dup (p.T319Nfs*6) in 59% of 22 reads
  - *TP53* c.470T>C (p.V157A)in 47% of 392 reads
  - *AR* amplification (est. 15 copies)
- But “noisy” sequencing with many false positive structural variant and copy calls

CAP Preanalytics and Precision Medicine Project Team:
Top Preanalytical Factors for Tissue for the Maintenance of Nucleic Acid and Protein Quality and Integrity

- **Cold ischemia time:**
  - < 1 hour
- **Fixation:**
  - 10% phosphate-buffered formalin, pH 7.0
  - ≥6 hours, no more than 24-36 hours (longer for fatty specimens)
  - Avoid acid solutions
- **Processing:**
  - Specimen thickness less than 4-5mm; 10:1 formalin volume:mass ratio
  - Maintain processor and fluids per manufacturers instructions
  - Use low-melt paraffin
- **Storage:**
  - dry, pest free, 18-25°C
- **Documentation:**
  - processes that deviate from the above recommendations

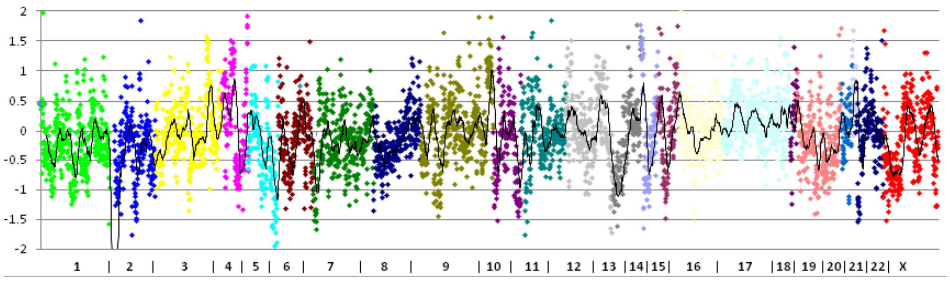
Compton CC et al. *Arch Pathol Lab Med*. 2019 Nov;143(11):1346-1363.

Impact of EDTA- and formic acid-based decalcification solutions on DNA integrity

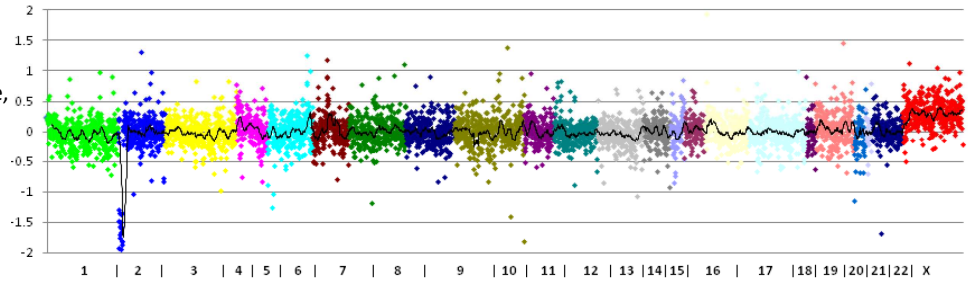


Schrijver et al. Mod Pathol (2016) 29, 1460–1470

Primary thymic carcinoma
9 year old archival FFPE block



Recurrent/metastatic disease,
1 month old FFPE block



Primary thymic carcinoma
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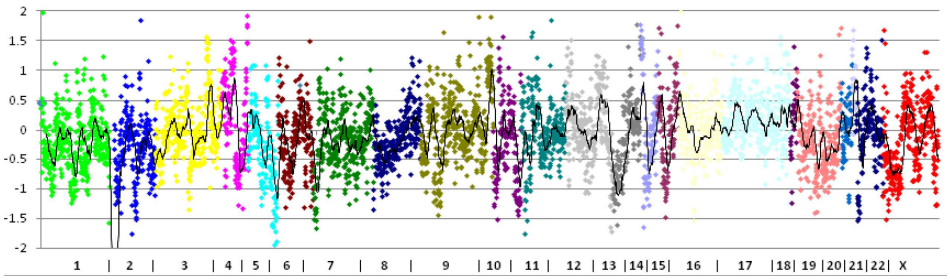
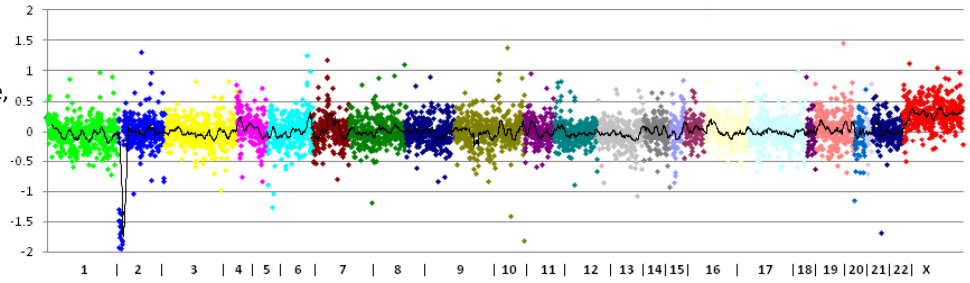
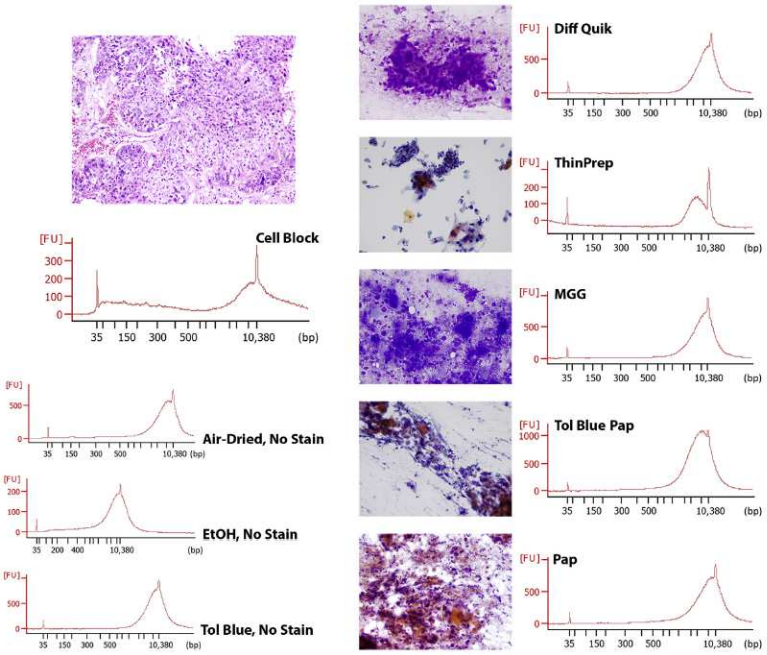


Impact of specimen age on DNA degradation and sequencing quality

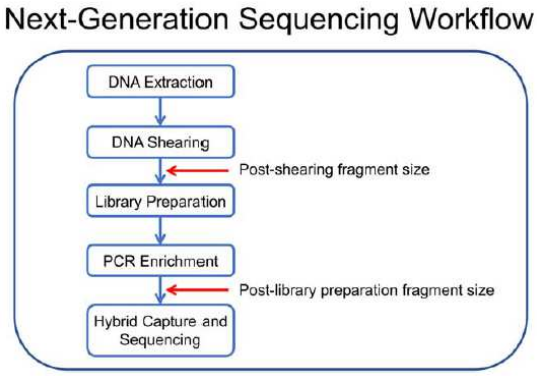
Recurrent/metastatic disease,
1 month old FFPE block

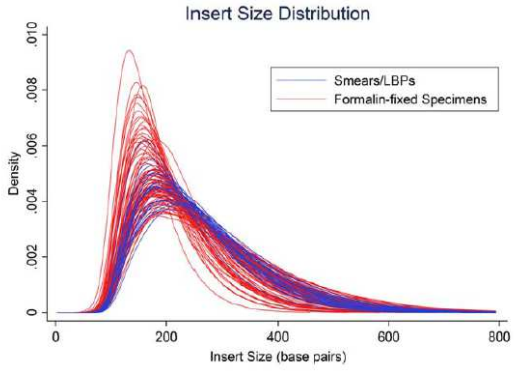




Balla A et al. *J Mol Diagn*. Vol. 20, No. 6, November 2018

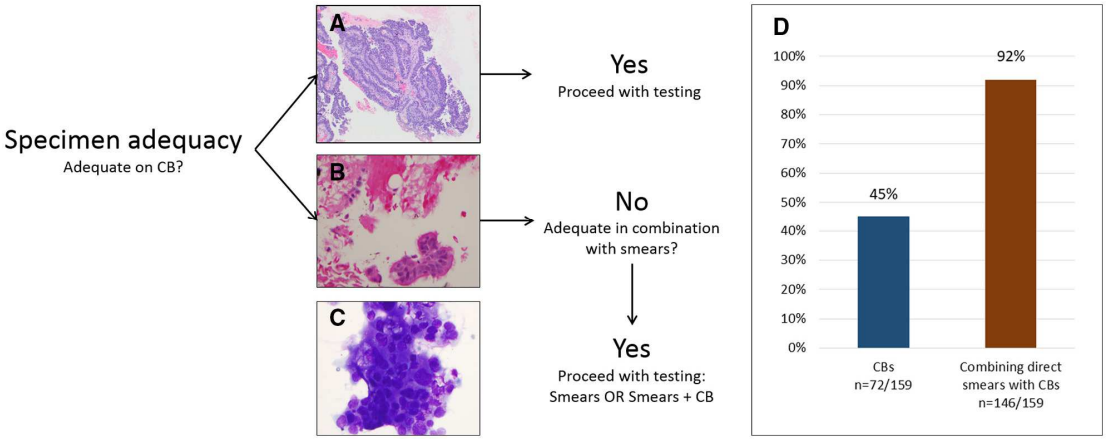
Larger target DNA fragments from smears/liquid based cytology preps





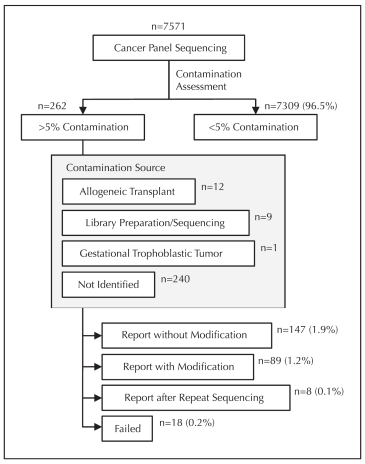
Hwang et al. Cancer Cytopathol. 2017 Oct;125(10):786-794.

Smear preps validated for RNAseq for fusion detection



Ramani et al. Cancer Cytopathology. 2021 May;129(5):374-382.

Specimen contamination headaches

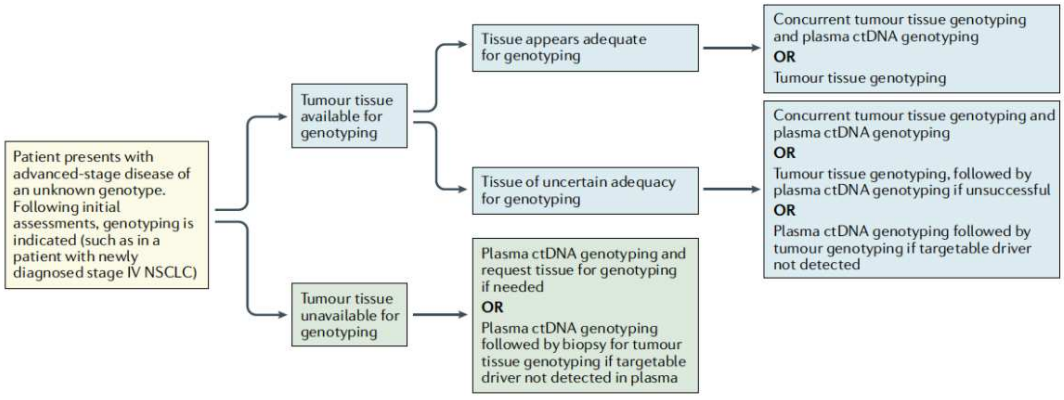


- ~4% of clinical cases have detectable contamination from another individual based on SNP profiling data
- Source of contamination is not identified in most
  - Histologic cross contamination (water/stain baths) is suspected
- Informatics tools are essential to detect and address this phenomenon

Li et al. Arch Pathol Lab Med. 2021 May 20. Online ahead of print
Platt et al. Arch Pathol Lab Med. 2009 Jun;133(6):973-8.

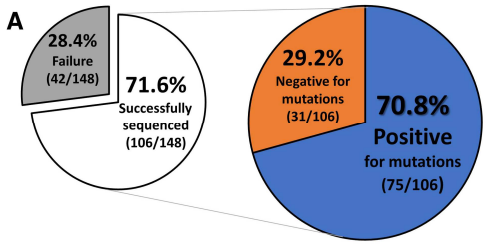
Beyond Tissue

Approaches to clinical implementation of circulating tumor DNA testing

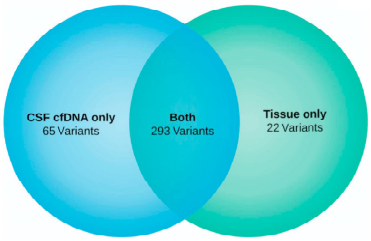


Aggarwal C et al. Nat Rev Clin Oncol. 2021 Jan;18(1):56-62.

Cell free DNA from cerebral spinal fluid in patients with leptomeningeal metastases



High sequencing success rates for cfDNA isolated from CSF in patients with leptomeningeal spread, including those with negative cytology.



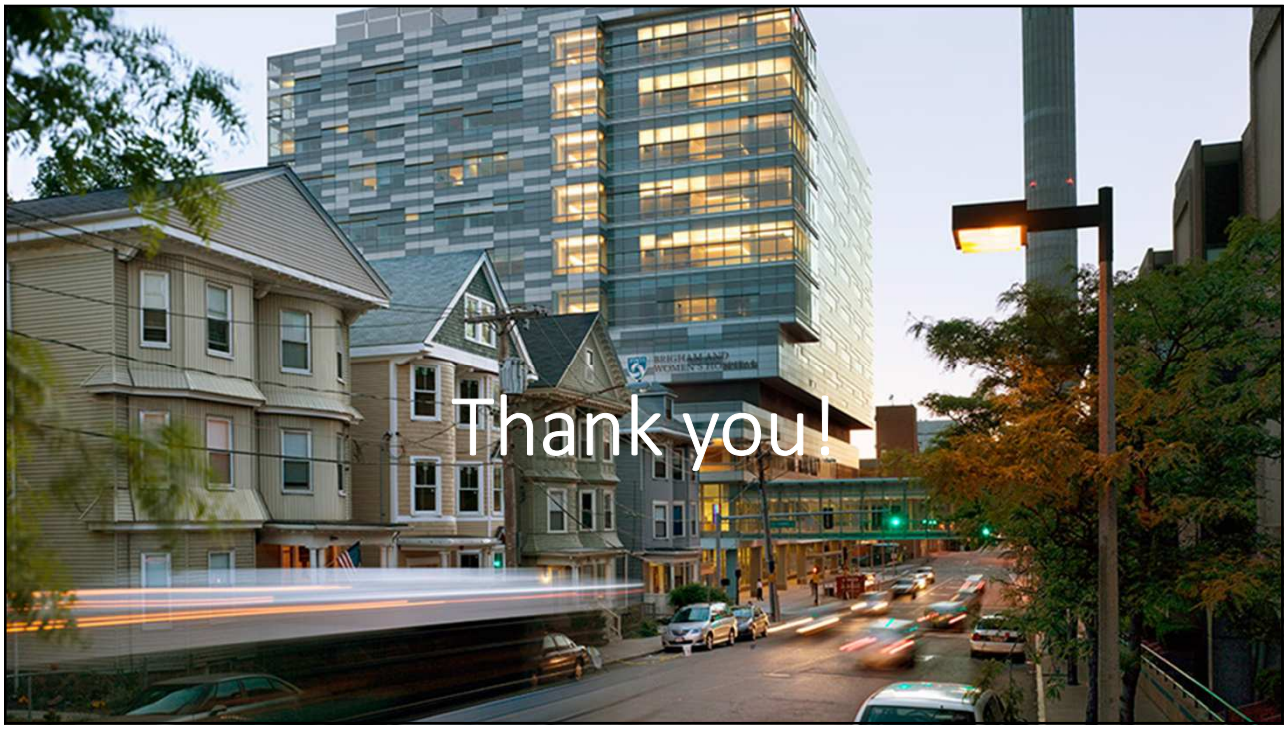
Comparison of CSF and tissue sequencing reveals tumoral heterogeneity.

Bale et al. J Mol Diagn. 2021 Jun;23(6):742-752.

Defining "Specimen Adequacy":

- No universal definition– this depends on the validated performance characteristics and limitations of the test being requested
- Interplay between nucleic acid quantity and quality
  - Lower input quantity may be acceptable if quality is high
  - Higher input quantity may be required if quality is low

Hadd AG et al. *J Mol Diagn*. 2013, 15:234-247.


Thank you!