AACC Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

Pearl Title Molecular Testing of Solid Tumors

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Introduction

- Molecular testing of solid tumors
  - Complementary to other tests
  - Diagnostic
  - Prognostic and Predictive
  - Therapeutic Targets

Specimen Type

- Formalin fixed paraffin embedded (FFPE): Most common
  - DNA degraded over time to fragments less than 500 base pair in length
  - In vitro mutations (fixation and processing artefacts)
  - Fixation time < 48-72 hours
- Cytology specimens
- Fresh/ snap frozen

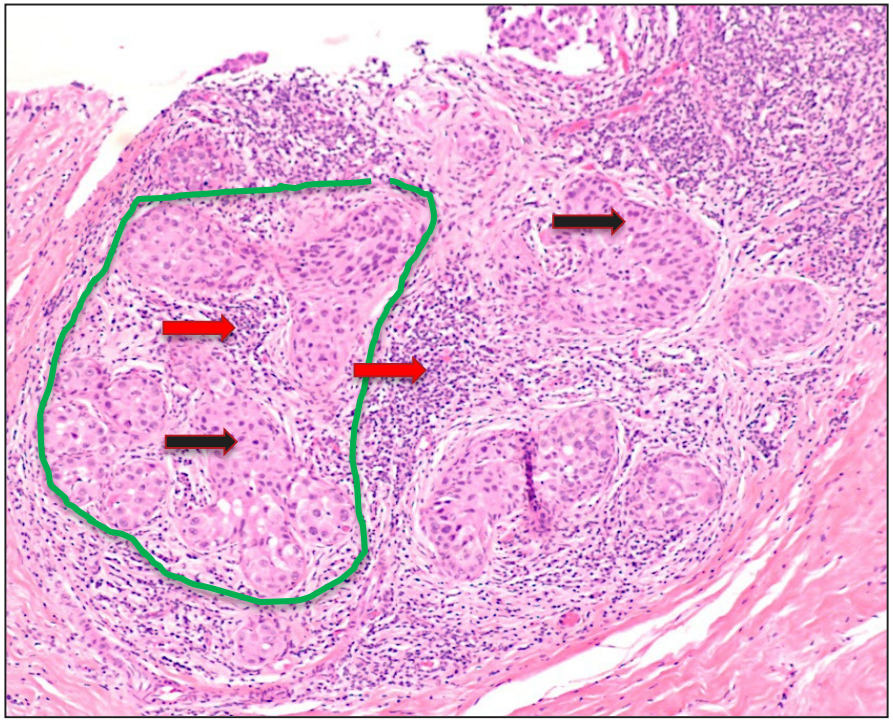
Fixatives to Avoid

- Acids in decalcifying solutions damage nucleic acids
- Heavy metals mercuric chloride inhibit enzymes used in amplification

Specimen Selection

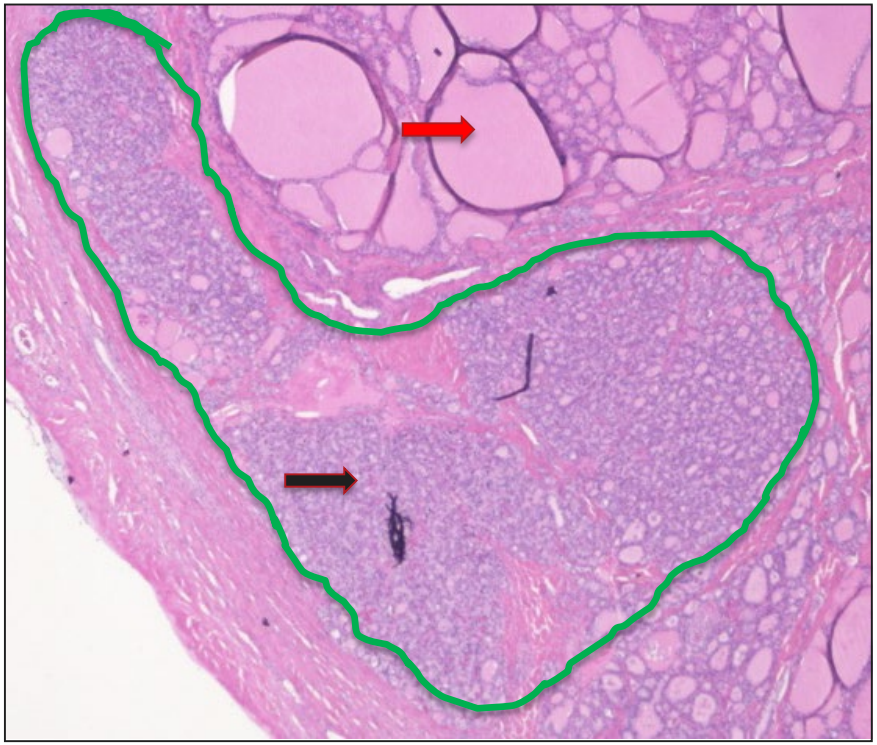
- Mark an area enriched for neoplastic cells
  - Cellularity: Preferable to have minimum 30-40% enrichment for neoplastic cells
  - Macro and micro dissection
  - Select best block with highest tumor concentration
  - Inflammatory cells: dilute out the neoplastic cells, careful evaluation
  - Avoid crushed, cauterized cells, necrotic areas

Specimen Selection: Tumor Enrichment



Tumor

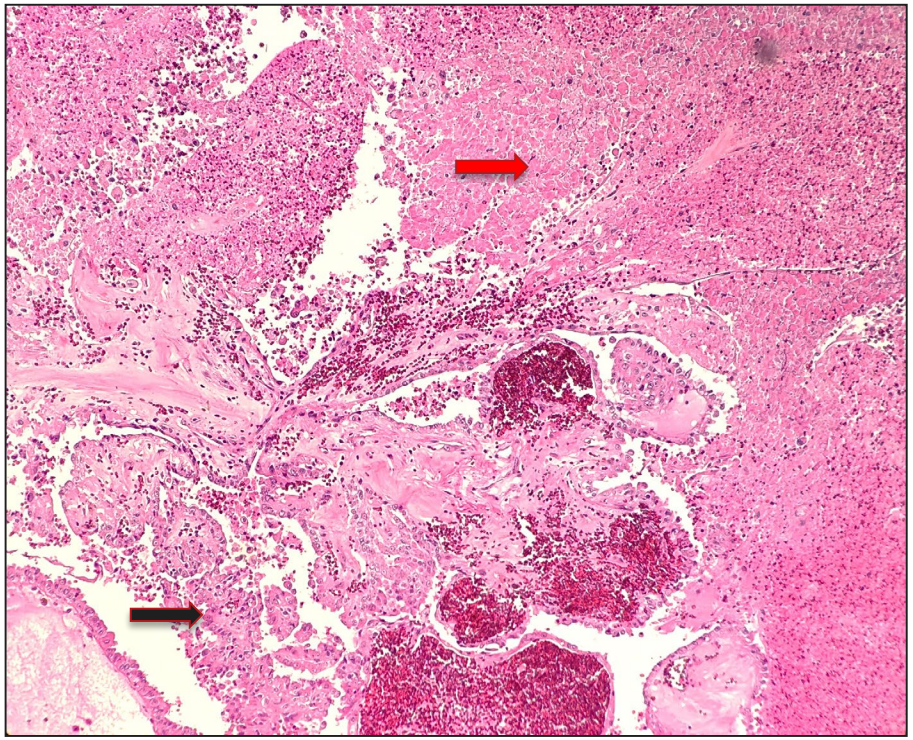
Inflammatory cells

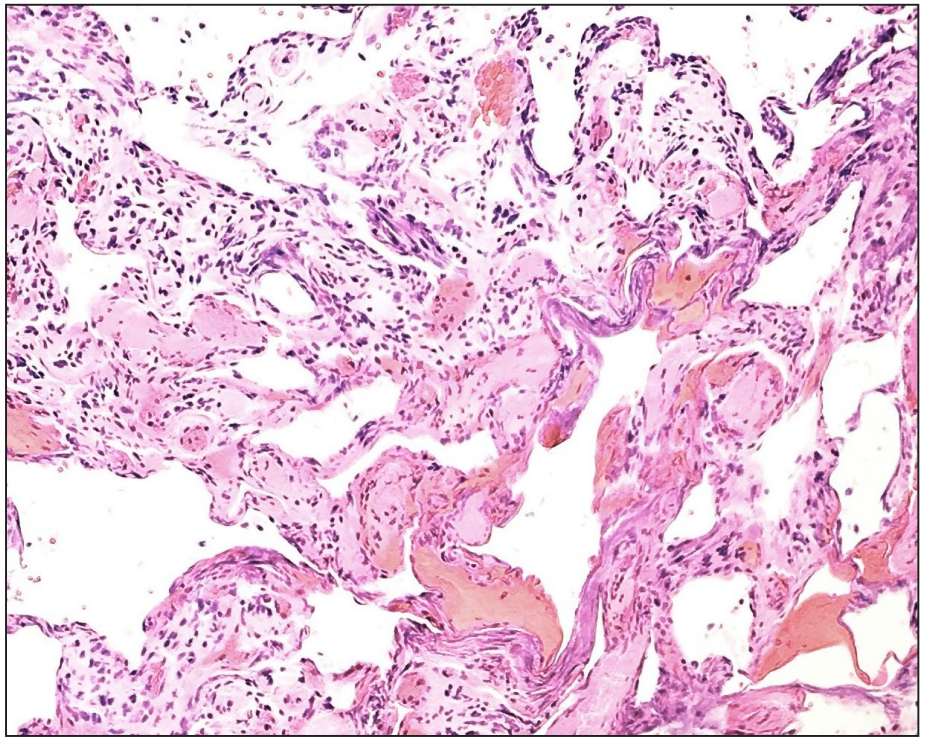


Tumor

Normal

Specimen Selection: Necrosis and Artefacts





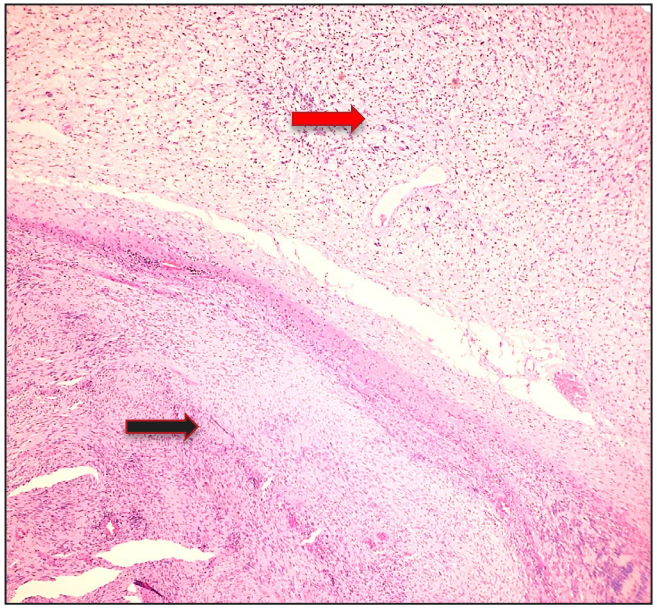
Tumor

Necrotic Tissue

Cauterized Tissue

Specimen Selection: Tumor Heterogeneity

- Tumor heterogeneity
  - Variation in morphology and grade:
    - Selection of higher grade
    - May require testing on different morphologic patterns



Tumor with areas of 2 distinctive morphologic patterns

Specimen Selection: Other Considerations

- Choice of tissue: primary/ metastatic
- Tumor and normal tissue
  - Comparative analysis
  - Germline alterations

- Having selected a specimen, the choice of assay is guided by the clinically expected underlying molecular alteration

Clinically Significant Alterations in Solid tumors

- Single base change
- Insertions, deletions
- Duplications, inversions, translocations
- Copy number changes
- Methylation
- Oncogenic viruses

Testing Methodologies to Detect Clinically Significant Alterations

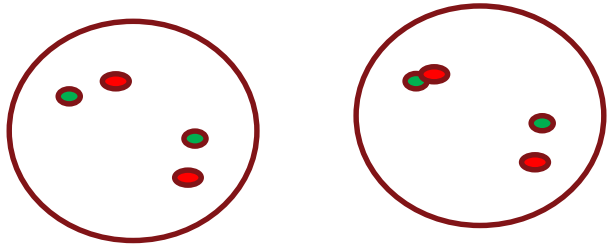
- FISH
- Sanger sequencing
- Single gene tests
- Next generation sequencing
- Methylation testing

Fluorescence in Situ Hybridization (FISH)

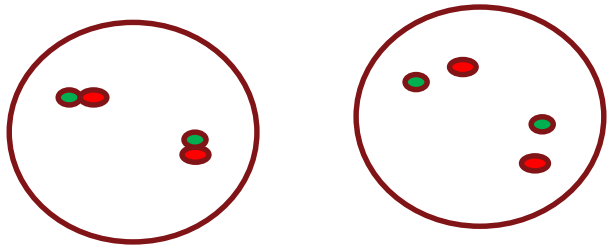
- Fluorescently tagged DNA or RNA probes are used to identify region of interest
  - Gene amplification
  - Gene deletion
  - Structural rearrangements
- Can be done on tissue sections
- Allows for correlation with morphology
- Rapid
- Limited number of probes
- Example:
  - ERBB2 amplification in breast carcinoma
  - ALK rearrangement in Non small cell lung carcinoma

FISH: Fusion Gene Detection

- Dual color FISH probes
  - 2 probes with different wavelengths hybridize to 2 different regions generating 2 different colors
  - When fused, they generate a 3rd color

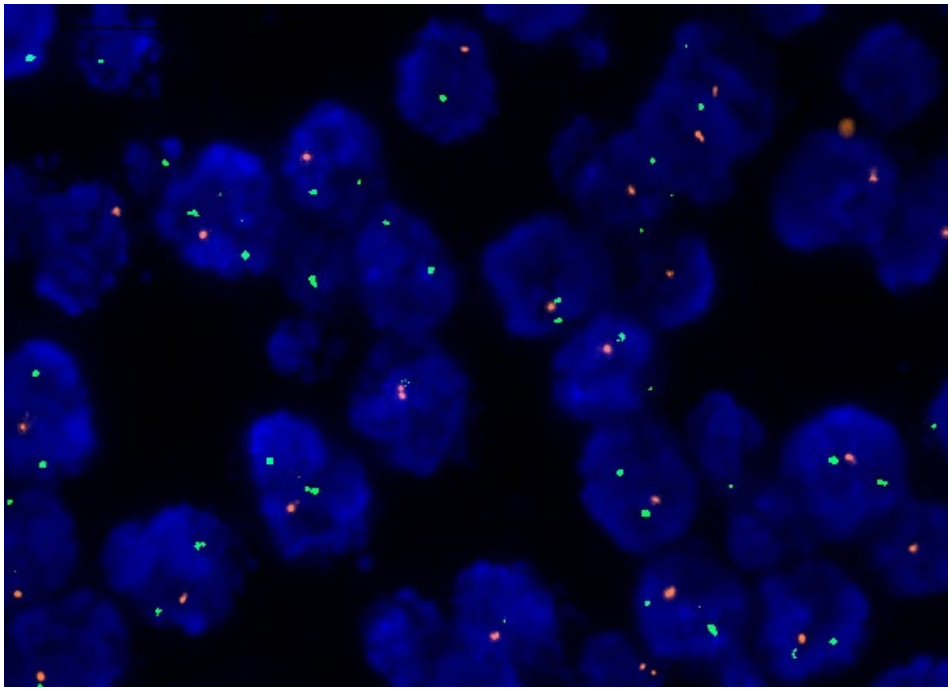


Fusion probes

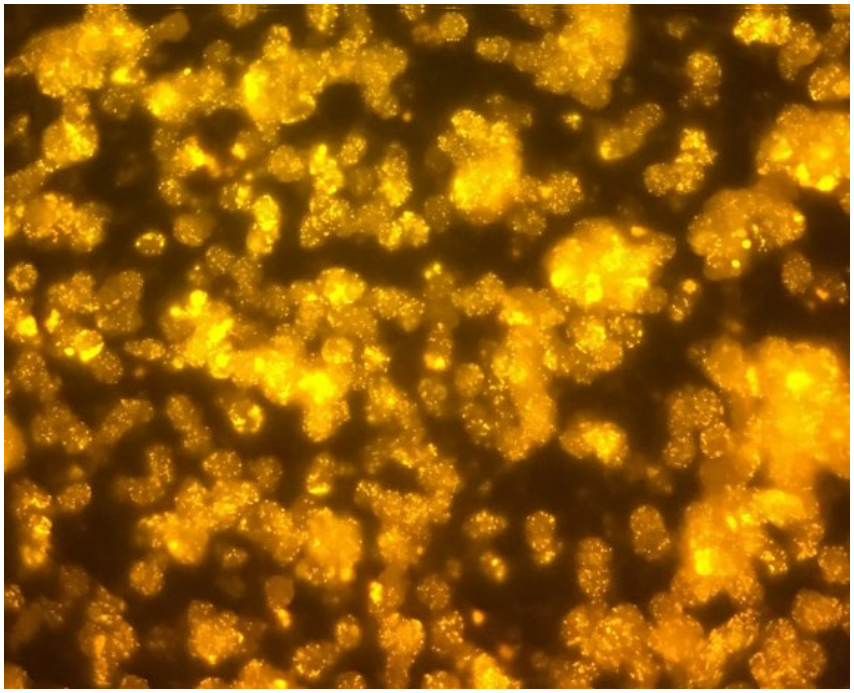


Break apart probes

FISH: Deletion and Amplification



1p deletion in gliomas. Seen here is 1 orange test probe directed to 1p and 2 reference green probes



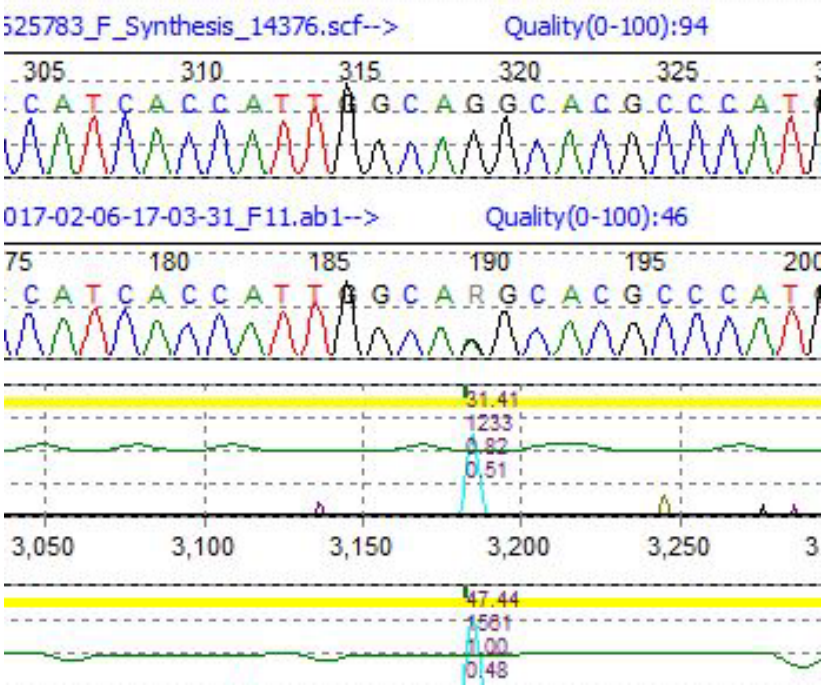
EGFR amplification in a glioma visualized by multiple orange signals in each cell

Chromogenic in situ hybridization (CISH)

- Colorimetric probes
- Do not fade with time
- No fluorescent microscope

Sanger Sequencing

- Variant detection
  - Single nucleotide variants (SNVs)
  - Small and medium size insertions/deletions
- Analytic sensitivity ~20%
- Considered the gold standard
- Requires 10ng DNA
- Example: EGFR, BRAF, c-KIT



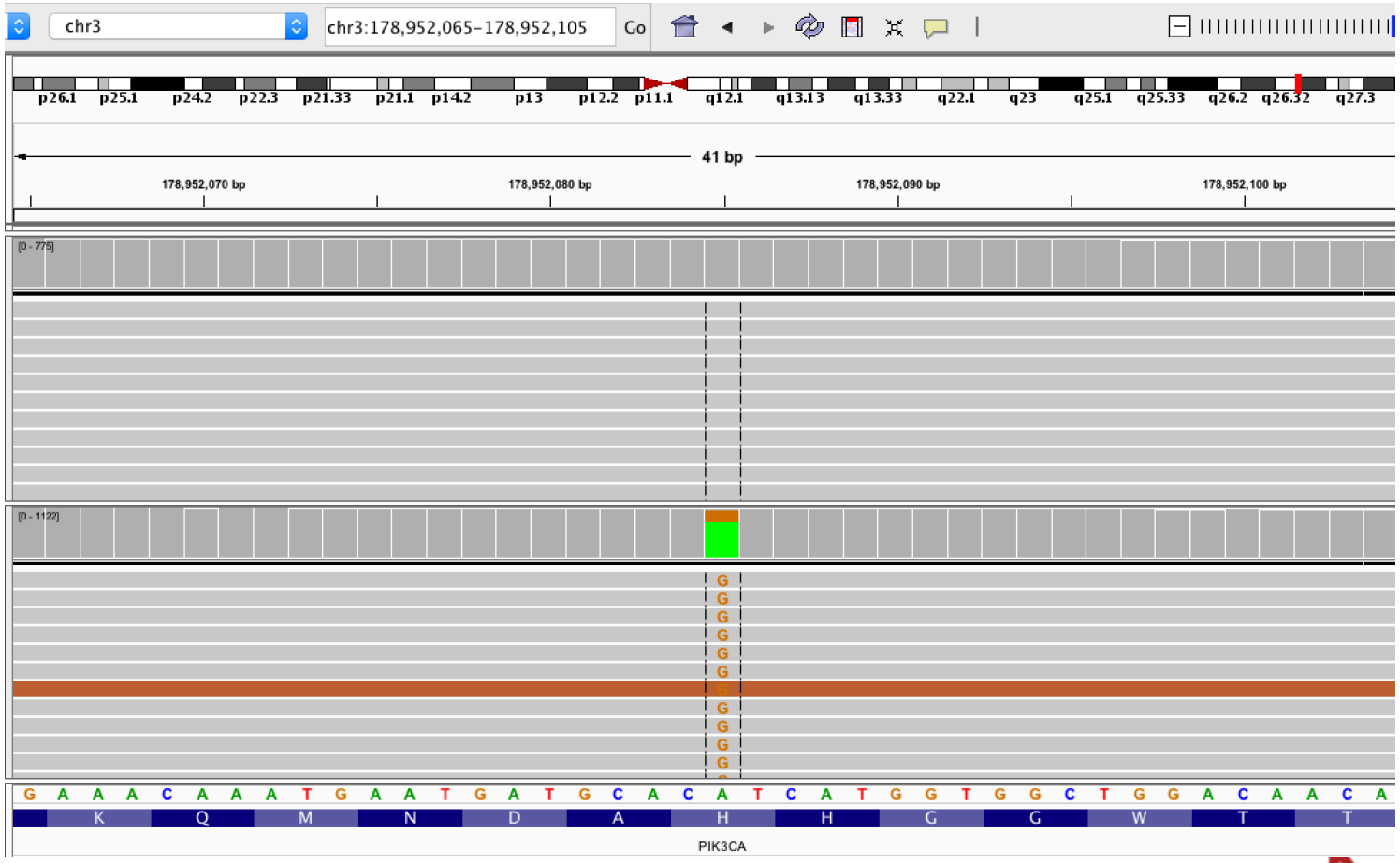
Next Generation Sequencing

- Cost effective way to detect many genetic alterations
  - Single base change
  - Insertions, deletions, duplications, inversions, translocations
  - Copy number changes
- Analytical sensitivity 5% allele frequency
- Requires 100 ng of DNA
- Shorter read lengths compared to Sanger

Single Base Changes/ Small- Medium Indels

Normal

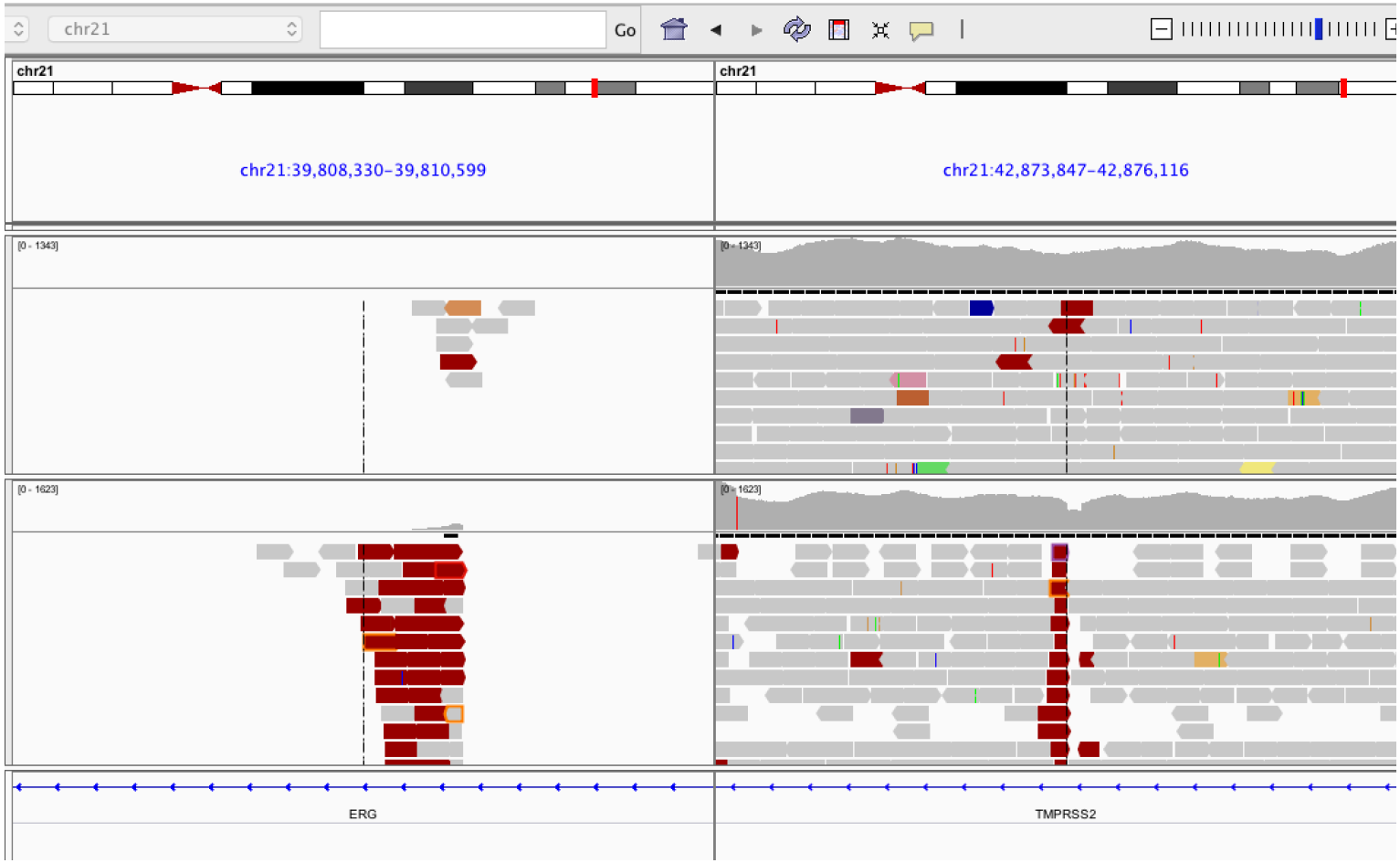
Tumor



Structural Variants

Normal

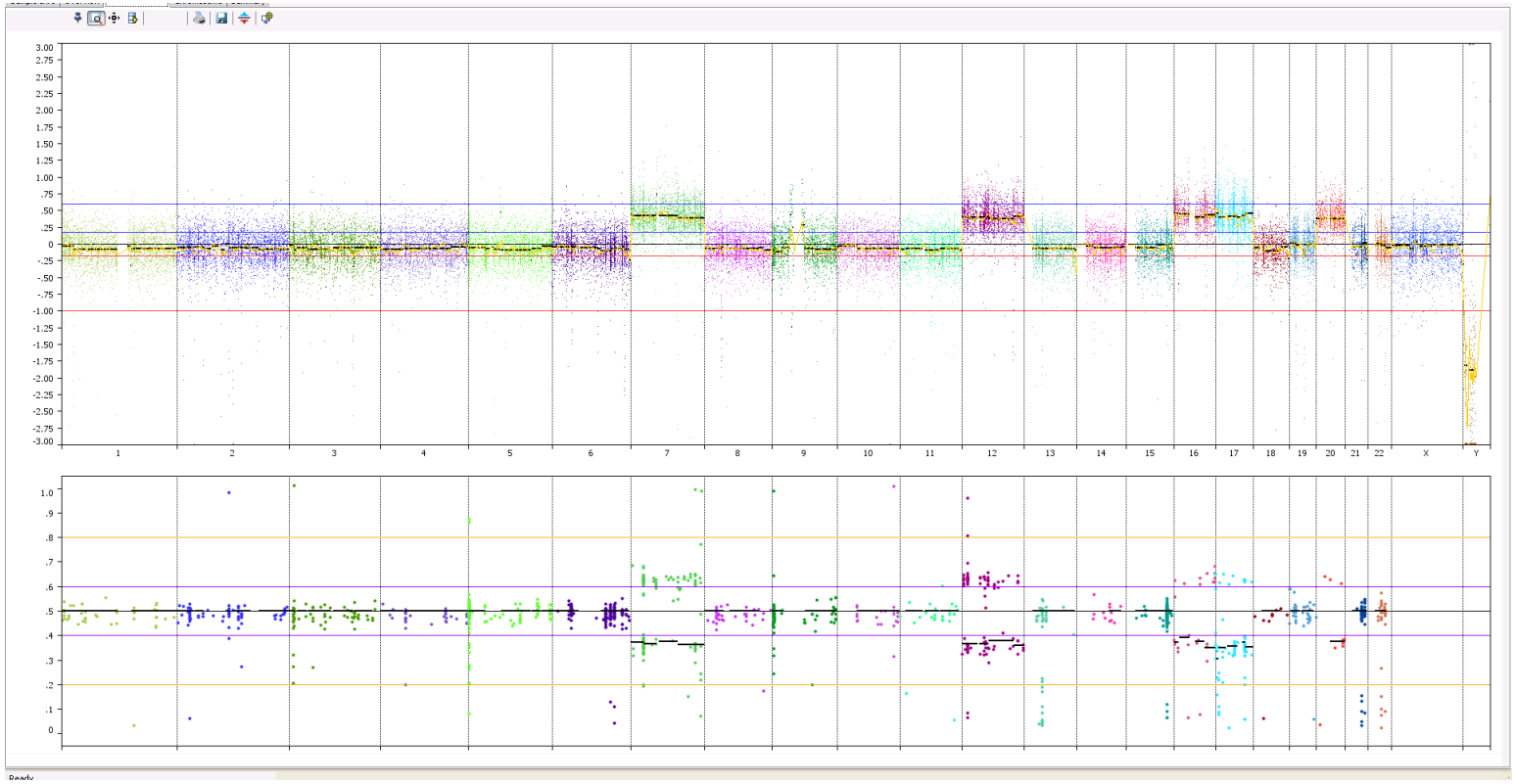
Tumor



Copy Number Changes

Log 2 ratio

B Allele frequency



DNA Methylation

- CpG dinucleotides present in 5' promoter regions of genes
- Prone to spontaneous methylation of Cytosine that results in silencing of the gene
- Methylation of tumor suppressor genes is a frequent mechanism of inactivation in cancers

DNA Methylation

- Treatment with sodium metabisulfite converts unmethylated cytosine to uracil, leaving methylated cytosine intact
- Cytosine and uracil can then be distinguished by different methods
  - Sequencing
  - Methylation specific PCR
  - Restriction endonuclease digestion with methylation-sensitive enzymes
- MLH1 hypermethylation
- MGMT methylation

Summary

- Major advancements in molecular testing of solid tumors
- Correct sample selection and assessment is crucial to solid tumor testing
- Assay selection based on specific molecular alteration in consideration

References

1. Varella-Garcia M. Molecular cytogenetics in solid tumors: laboratorial tool for diagnosis, prognosis, and therapy. Oncologist 2003;8(1):45-58.
2. Aisner DL, Sams SB. The role of cytology specimens in molecular testing of solid tumors: techniques, limitations, and opportunities. Diagn Cytopathol 2012;40(6):511-24.
3. Frayling IM. Methods of molecular analysis: mutation detection in solid tumours. Mol Pathol 2002;55(2):73-9.
4. Igbokwe A, Lopez-Terrada D. H. Molecular testing of solid tumors. Arch Pathol Lab Med 2011;135:67–82

Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**: No disclosures
- **Consultant or Advisory Role:** No disclosures
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