Clinical Interpretation of Sequence and Copy Number Variants (Cancer NGS)

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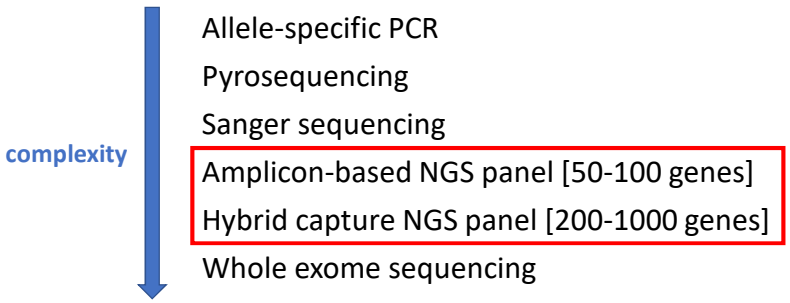
Cancer Genotyping Methods



Outline

- Part I: Sequence Variants
- Part II: Copy Number Variants
- Part III: Integrated Analysis (bonus)

Sequence Alteration Interpretation

- Types
  - Substitutions (single nucleotide variants)
  - Insertions and deletions

  } Different algorithms: MuTect, GATK
- Technical Interpretation
  - Is this variant real?
- Clinical Interpretation
  - What does this variant mean for the patient?

Clinical Interpretation

- Define interpretable elements
  - Gene function
  - Variant function
  - Biological significance
  - Clinical significance
- Issue report according to lab protocol

Example: *EGFR* p.L858R in Lung Adenocarcinoma

- Gene function
  - *EGFR* is a receptor tyrosine kinase.
- Variant function
  - *EGFR* p.L858R leads to constitutive kinase activation.
- Biological significance
  - *EGFR* mutations are observed in ~20% of lung adenocarcinomas.
- Clinical significance
  - *EGFR* p.L858R is associated with response to *EGFR* kinase inhibitors as first line monotherapy for advanced lung cancers.

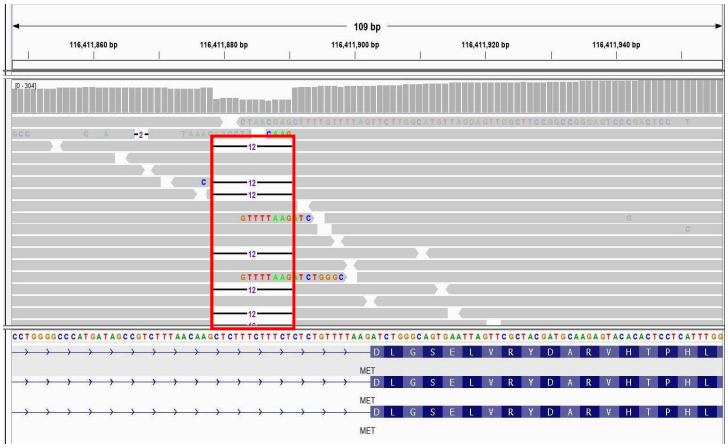
Example: *BRAF* p.V600E in Colorectal Adenocarcinoma

- Gene function
  - *BRAF* encodes a serine/threonine kinase involved in signal transduction in the RAS-MAPK pathway.
- Variant function
  - *BRAF* p.V600E leads to constitutive kinase activation.
- Biological significance
  - *BRAF* p.V600E is observed in ~50% of CRCs with microsatellite instability.
  - *BRAF* p.V600E is observed in ~5% of CRCs with stable microsatellites.
- Clinical significance
  - *BRAF* p.V600E is associated with response to dual BRAF and EGFR inhibitors (encorafenib and cetuximab) for advanced colorectal cancers.
  - *BRAF* p.V600E is associated with sporadic cancer and not Lynch syndrome in CRCs with microsatellite instability.
  - *BRAF* p.V600E is associated with aggressive behavior for microsatellite stable CRCs.

Example: *BRAF* p.V600E in Lung Adenocarcinoma

- Gene function
  - *BRAF* encodes a serine/threonine kinase involved in signal transduction in the RAS-MAPK pathway.
- Variant function
  - *BRAF* p.V600E leads to constitutive kinase activation.
- Biological significance
  - *BRAF* p.V600E is observed in 1-2% of lung adenocarcinomas.
- Clinical significance
  - *BRAF* p.V600E is associated with response to dual BRAF and MEK inhibitors (dabrafenib and trametinib) in advanced lung cancers.

Example: *MET* intron 13 variant in LUAD



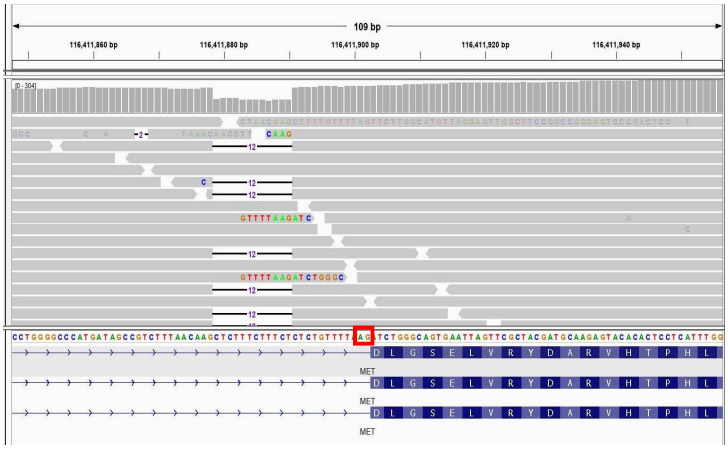
Donor site – 5’ of intron GU

Acceptor site – 3’ of intron AG

Branch site – near 3’ of intron, always includes Adenine, YNYYRAY (Y=pyrimidine, R=purine)

Polypyrimidine tract – 5-40 bp before 3’ end of intron, 15-20 bp long

Example: *MET* intron 13 variant in LUAD



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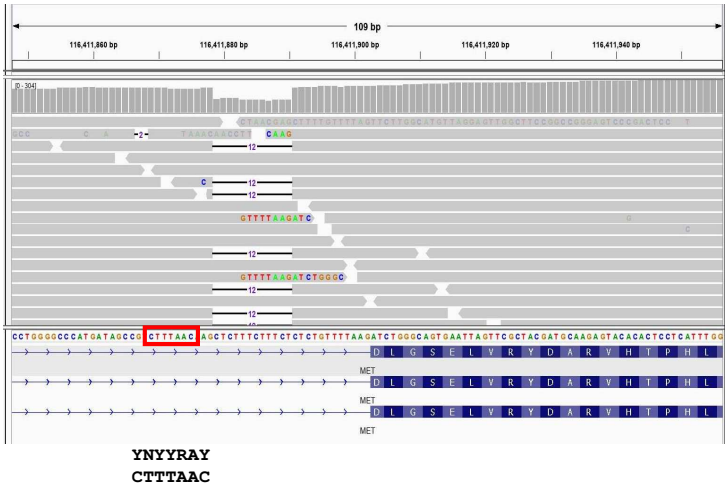

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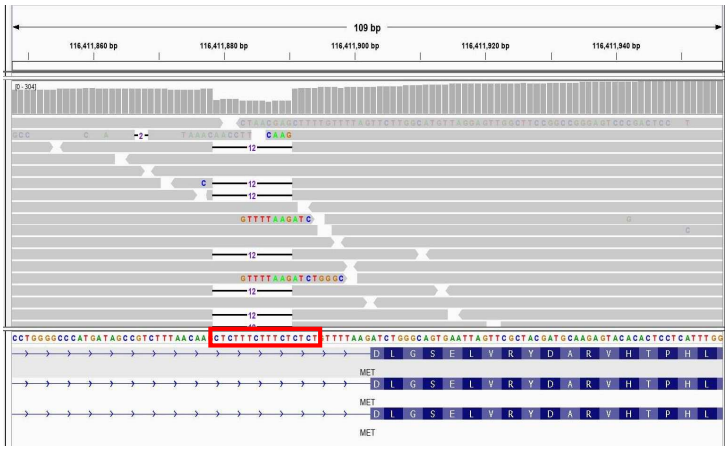

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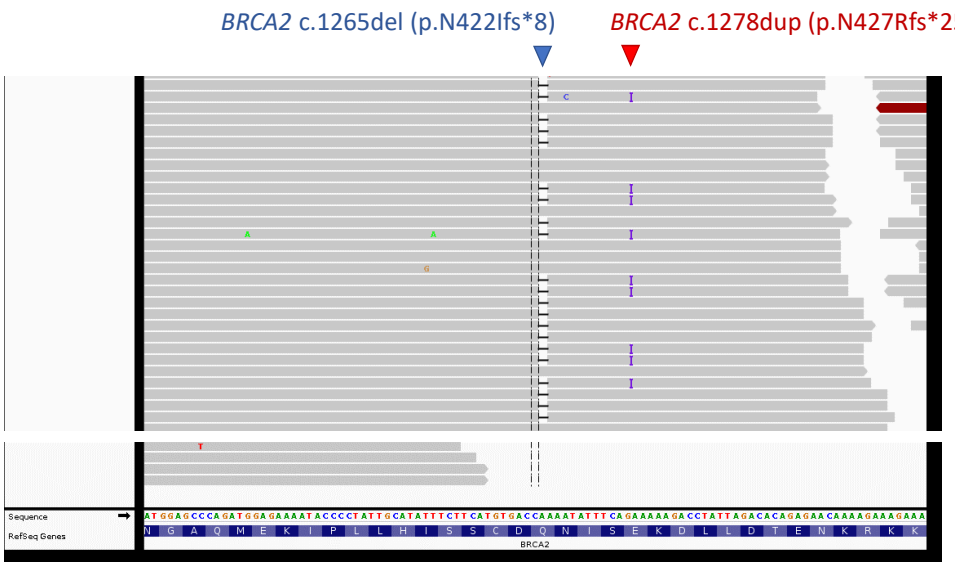
Example: *BRCA2* variants in breast cancer



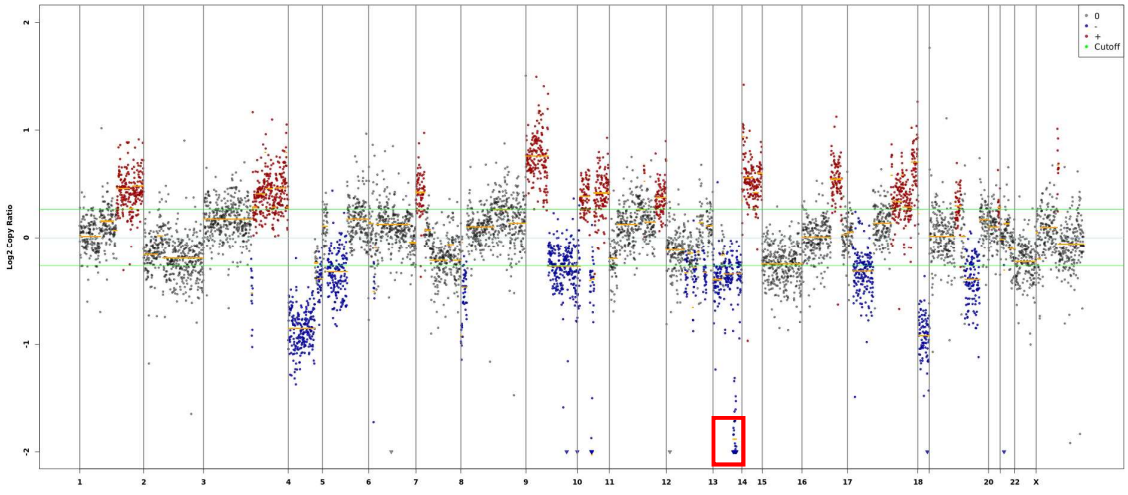
Guidelines for Sequence Variant Interpretation

- Li, et al. J Mol Diag 2017
- Tier I:
  - Variants with strong clinical significance
- Tier II:
  - Variants with potential clinical significance
- Tier III:
  - Variants of unknown significance
- Tier IV:
  - Variants deemed benign or likely benign

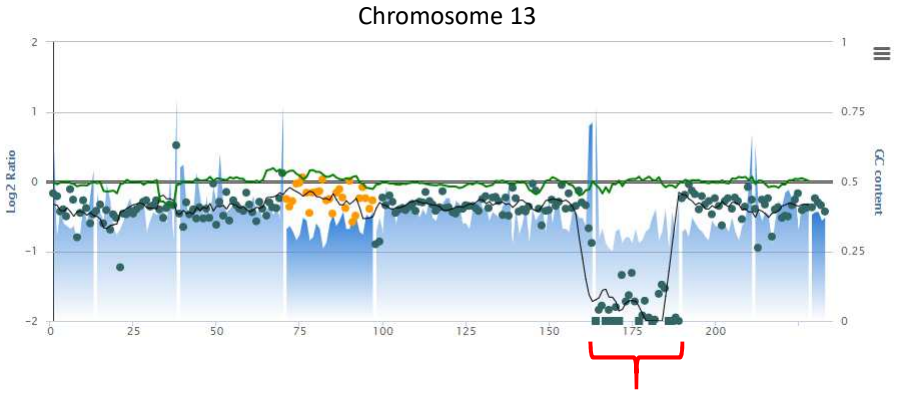
Summary: Sequence Alterations

- Two key components in interpretation
  - Technical
  - Clinical
- Key elements to consider for interpretation
  - Gene function
  - Variant function
  - Biological significance
  - Clinical significance

Copy Number Variants



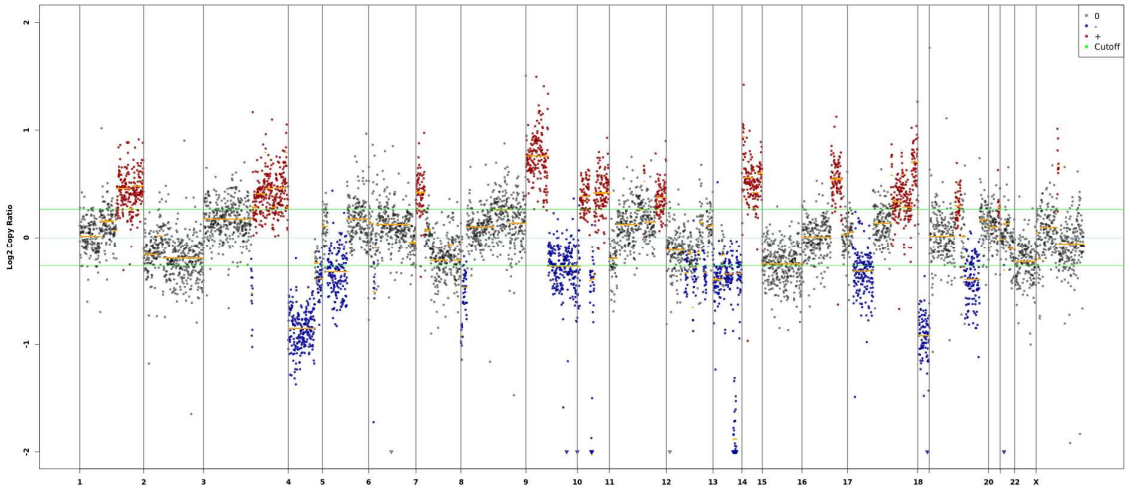
Copy Number Variants



Focal *RB1* Deletion

Copy Number Variants

What does a dot represent?

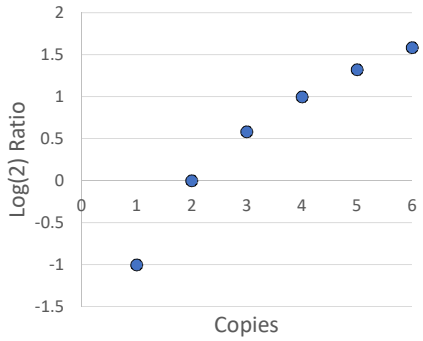


Copy Number Calculation

$$\text{Copy Number Ratio} = \log(2) \frac{\dfrac{\text{Reads from Genomic Region (tumor)}}{\text{All Reads (tumor)}}}{\dfrac{\text{Reads from Genomic Region (normal)}}{\text{All Reads (normal)}}}$$

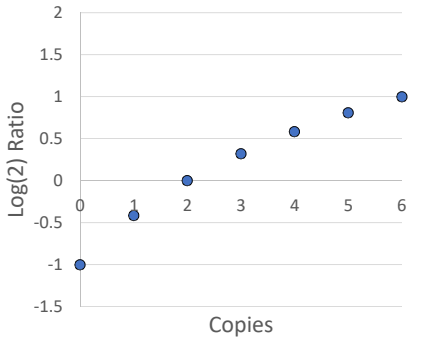
Copy Number Calculation

100% Tumor Purity



Copies	Log(2) Ratio
0	-∞
1	-1
2	0
3	0.58
4	1
5	1.32
6	1.58

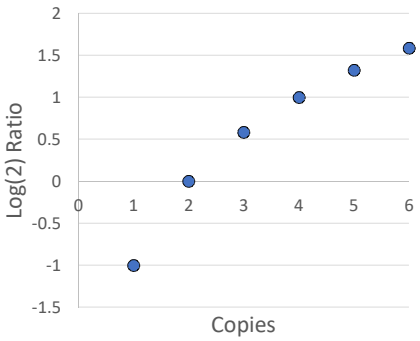
50% Tumor Purity



Copies	Log(2) Ratio
0	-1
1	-0.42
2	0
3	0.32
4	0.58
5	0.81
6	1

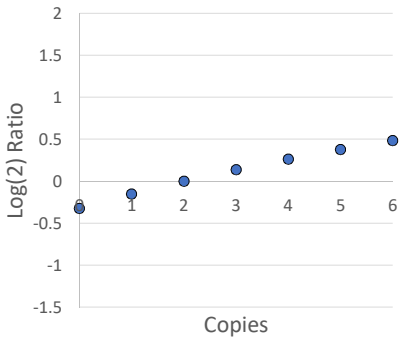
Copy Number Calculation

100% Tumor Purity



Copies	Log(2) Ratio
0	-∞
1	-1
2	0
3	0.58
4	1
5	1.32
6	1.58

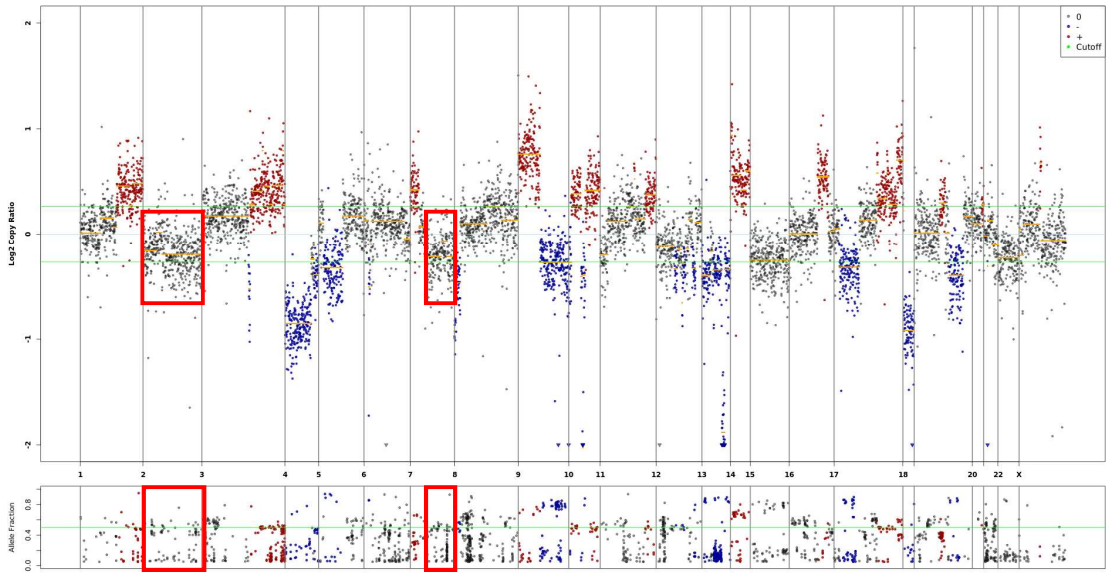
20% Tumor Purity



Copies	Log(2) Ratio
0	-0.32
1	-0.15
2	0
3	0.14
4	0.26
5	0.38
6	0.49

Copy Number Variants

Copy number changes relative, not absolute



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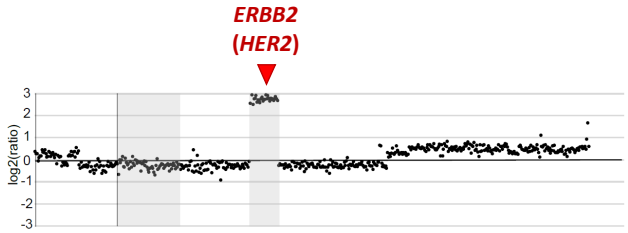


Focal *ERBB2* amplification

ERBB2 gain as part of 17q gain

ERBB2 gain as part of complex 17q copy number alterations

Clinical Interpretation

- Define interpretable elements
  - Gene function
  - Variant function
  - Biological significance
  - Clinical significance
- Issue report according to lab protocol

Summary: Copy Number Interpretation

- Principle of copy number analysis: ratio of read counts (tumor vs. normal control)
- Copy number analysis is affected by tumor purity
- Copy number analysis is relative, not absolute
- Focus on focal amplifications of oncogenes and focal deletions of tumor suppressor genes

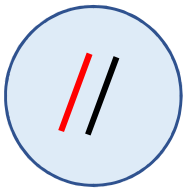
Bonus: Integrated Analysis

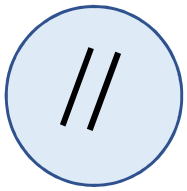
- Sequence alteration variant allele fraction
- Copy number state
- Tumor purity

Bonus: Integrated Analysis

Tumor Purity (T)

Normal (1-T)





Oncogene mutation

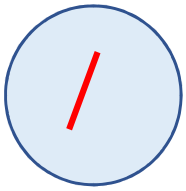
Heterozygous gain of function

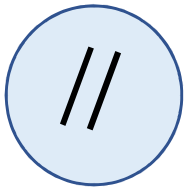
VAF = ½ T

Bonus: Integrated Analysis

Tumor Purity (T)

Normal (1-T)





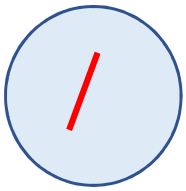
Tumor suppressor mutation

Loss of function + one copy deletion

VAF = T / (2-T)

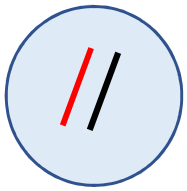
Bonus: Integrated Analysis

Tumor Purity (T)



Tumor suppressor mutation
Deletion of wild type allele

Normal (1-T)

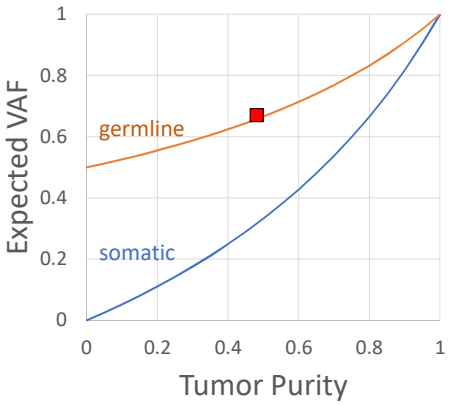


Loss of function mutation
Heterozygous germline

VAF = 1 / (2-T)

Bonus: Integrated Analysis Example

- *BRCA1* c.1165delA variant in tumor specimen at 67% VAF
- Concurrent one copy deletion of *BRCA1*
- Tumor purity is 48%, derived from NGS copy number changes
- Is this variant germline or somatic?



Summary

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- Two types of variant interpretation
  - Sequence alterations (substitutions and indels)
  - Copy number alterations
- Interpretation elements
  - Clinical
    - Gene function
    - Variant function
    - Biological significance
    - Clinical significance
- Advanced topic
  - Integration of complex data (SV, CNV) for clinical interpretation