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A Genomic Portrait of Tumor Progression Using Second Generation Sequencing

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Pediatric Oncology Branch
National Cancer Institute, NIH

Pediatric
ONCOLOGY BRANCH

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Neuroblastoma

- Most common extracranial solid tumor of childhood.
  - ~ 8 % of all childhood cancers
  - ~ 15% of deaths from childhood cancers
  - ~ 600 new cases in the U.S. annually

- Arises in Sympathetic Nervous System

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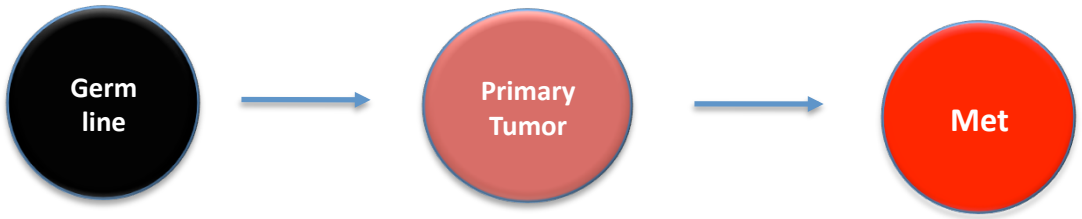
Objective



- **To portray progression of a neuroblastoma genome**
- **Single Nucleotide Variants**
- **Chromosomal Aberrations**

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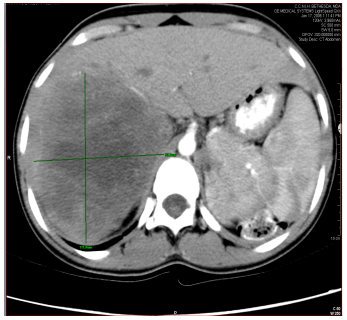
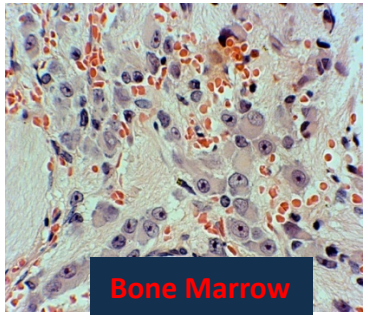
Patient with Metastatic Neuroblastoma

Diagnosis

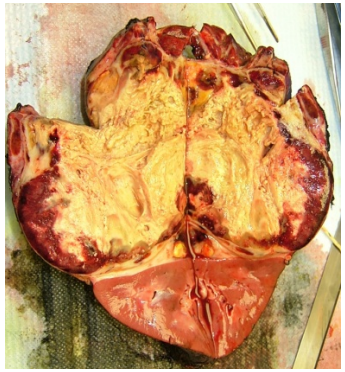


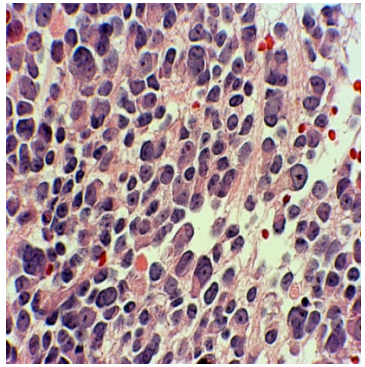


1. Met1-BM: Bone marrow biopsy at diagnosis

~4 Months

Surgery

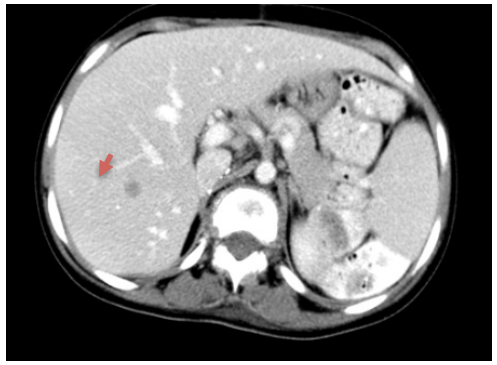




2. Primary: Primary tumor removed by surgery

3 years

Death



3. Met2-Liver: Liver metastasis at autopsy

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Outline

- Second Generation Sequencer
- Identify Somatic Mutations

  Two Somatic Mutations in Expressed Genes
- Chromosomal Changes

  Stable Pattern of Allelic Imbalance

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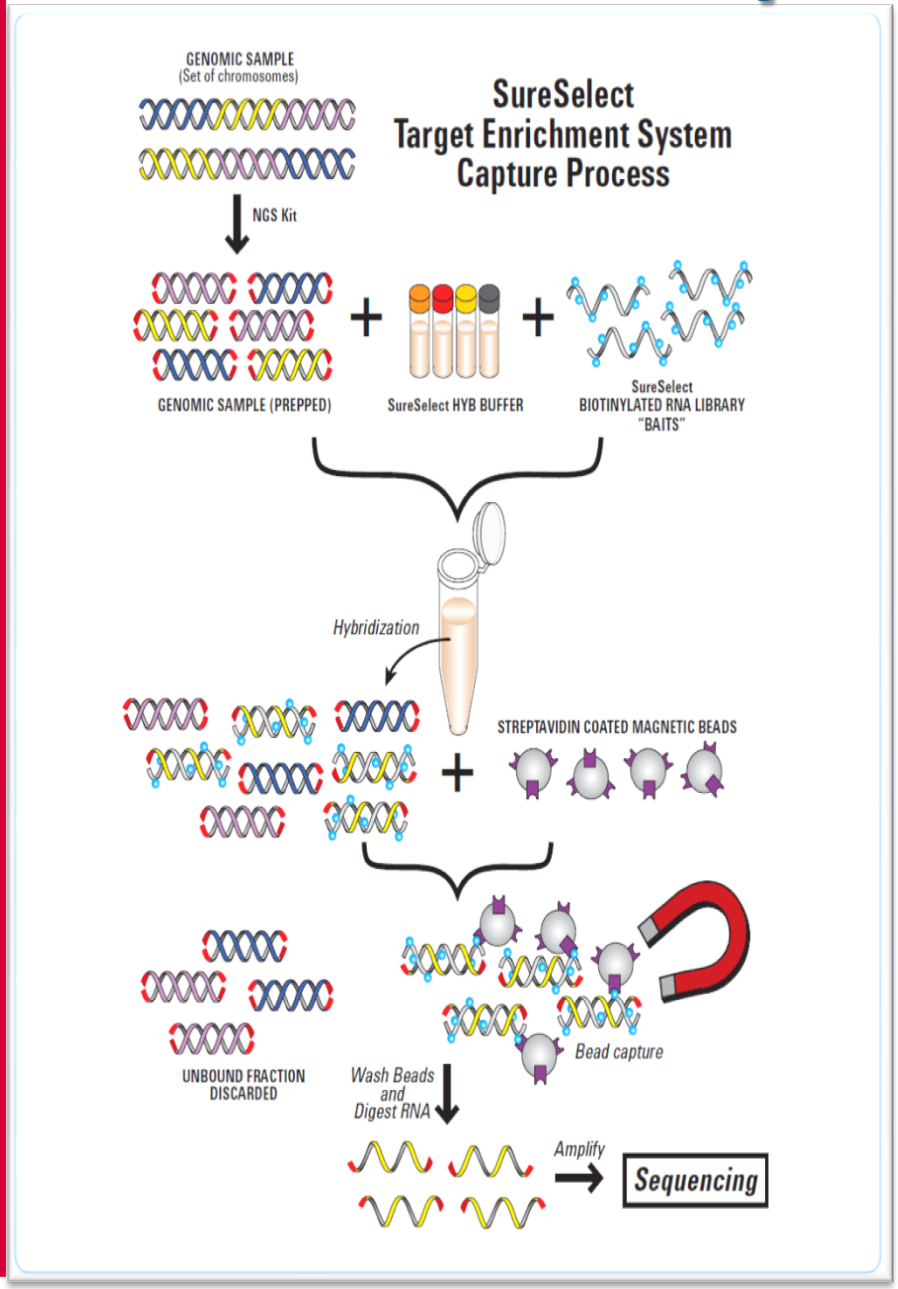
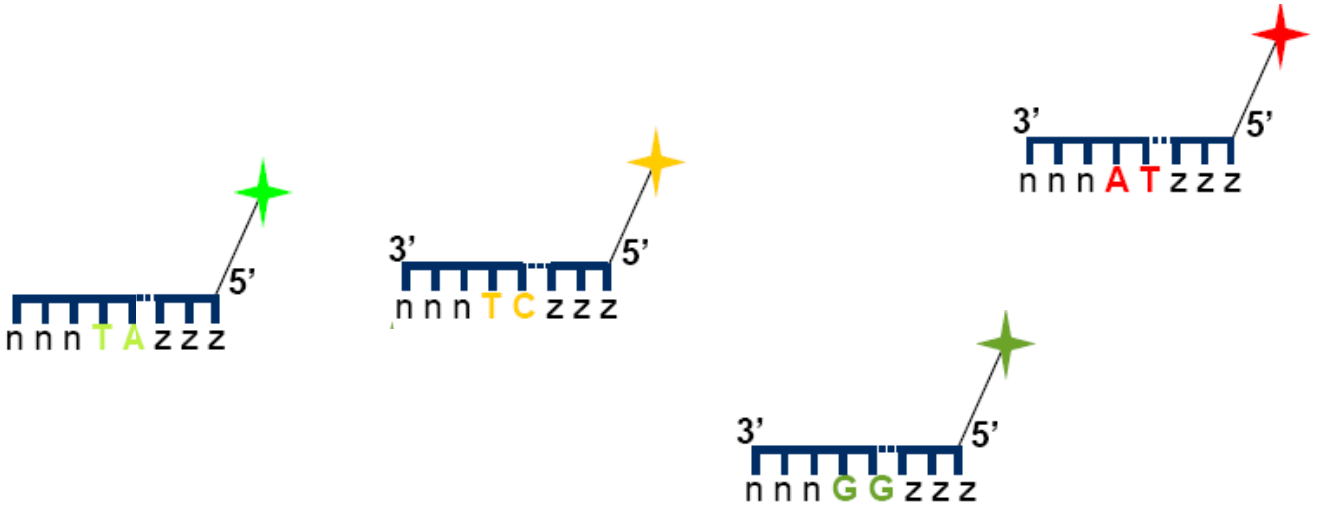
Exome Sequencing

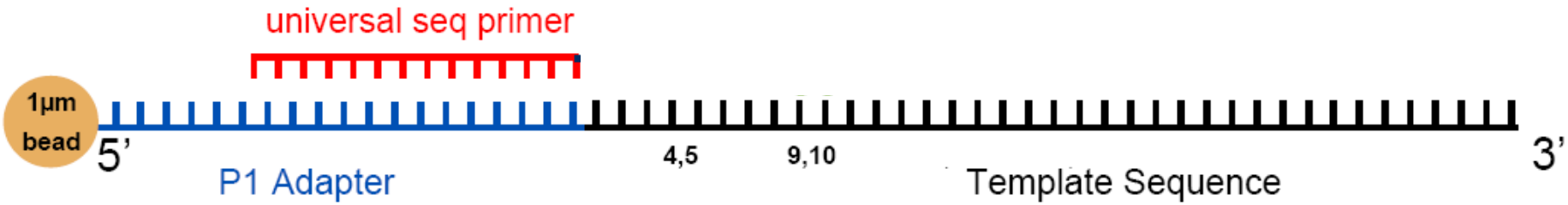


- Targeted 37.8Mb

SOLiD 4-color ligation chemistry system

Sequencing by **O**ligonucleotide **Li**gation and **D**etection





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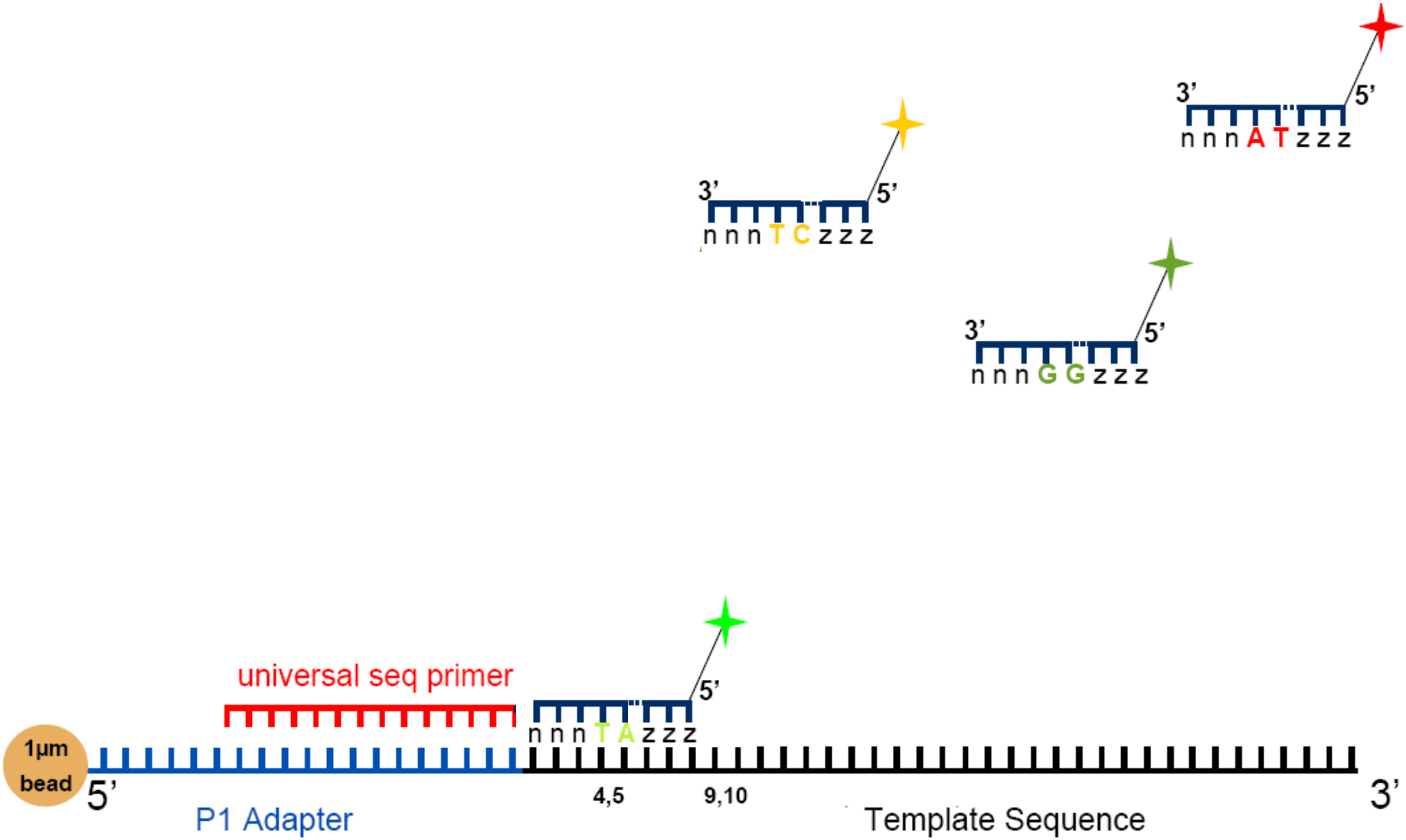

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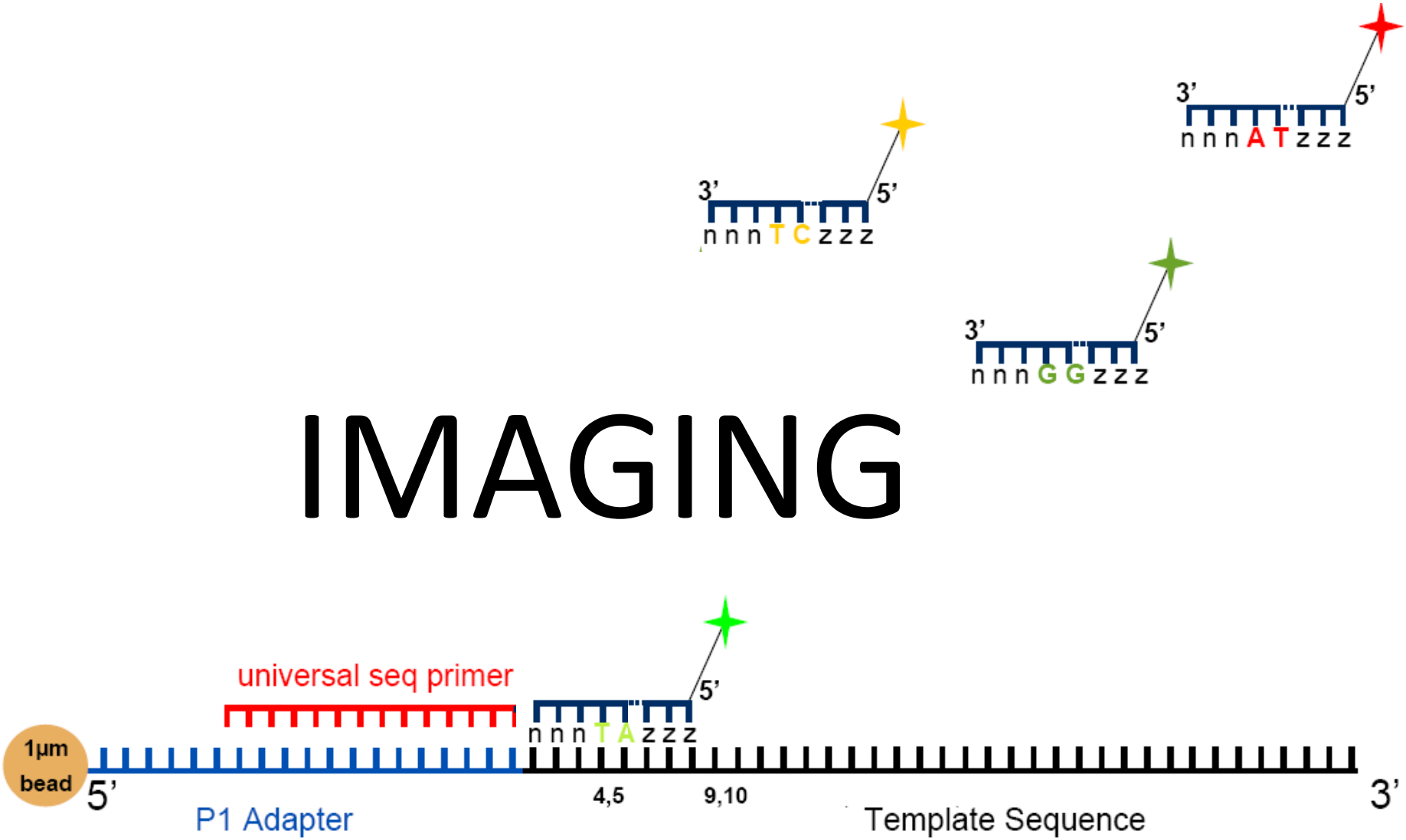

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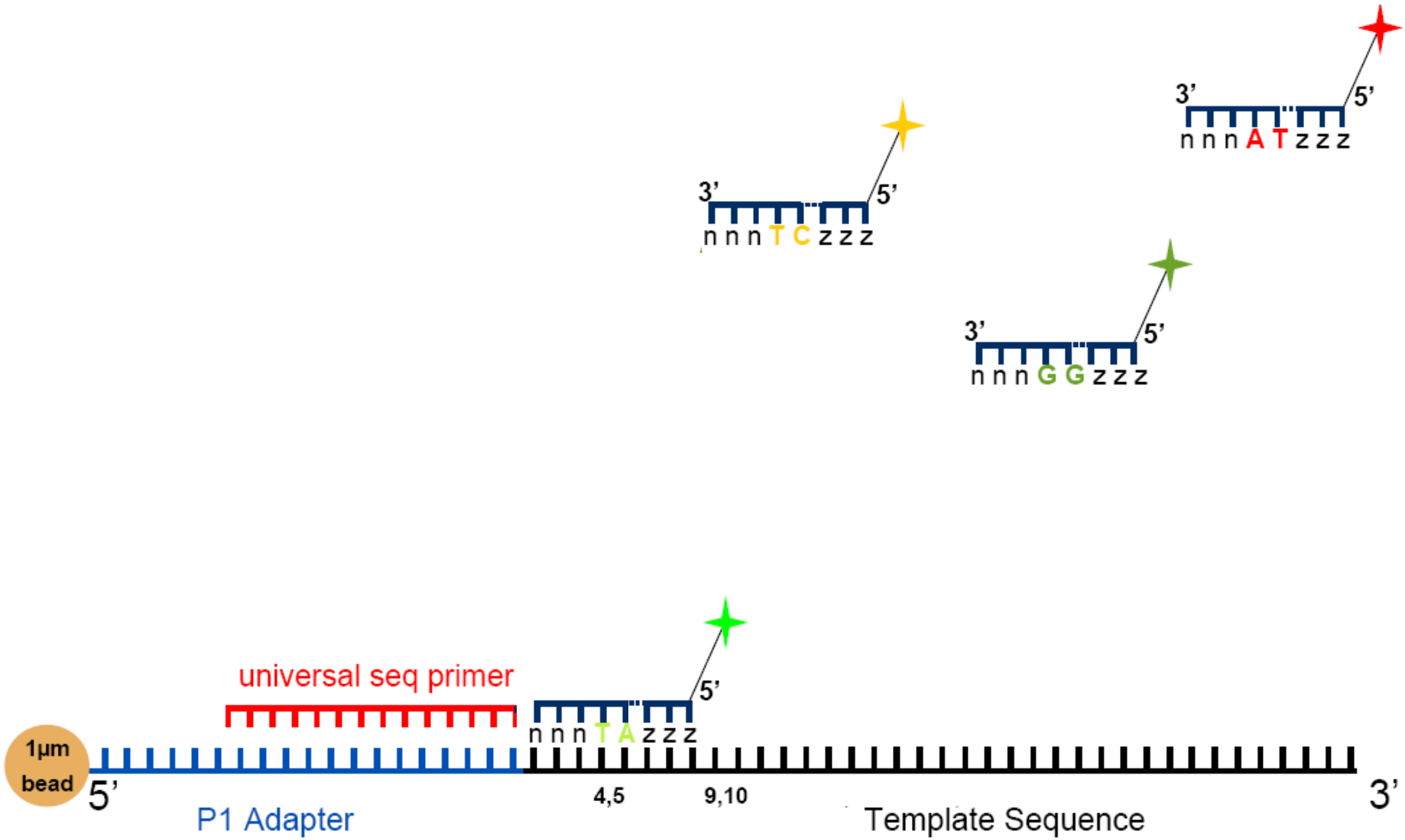

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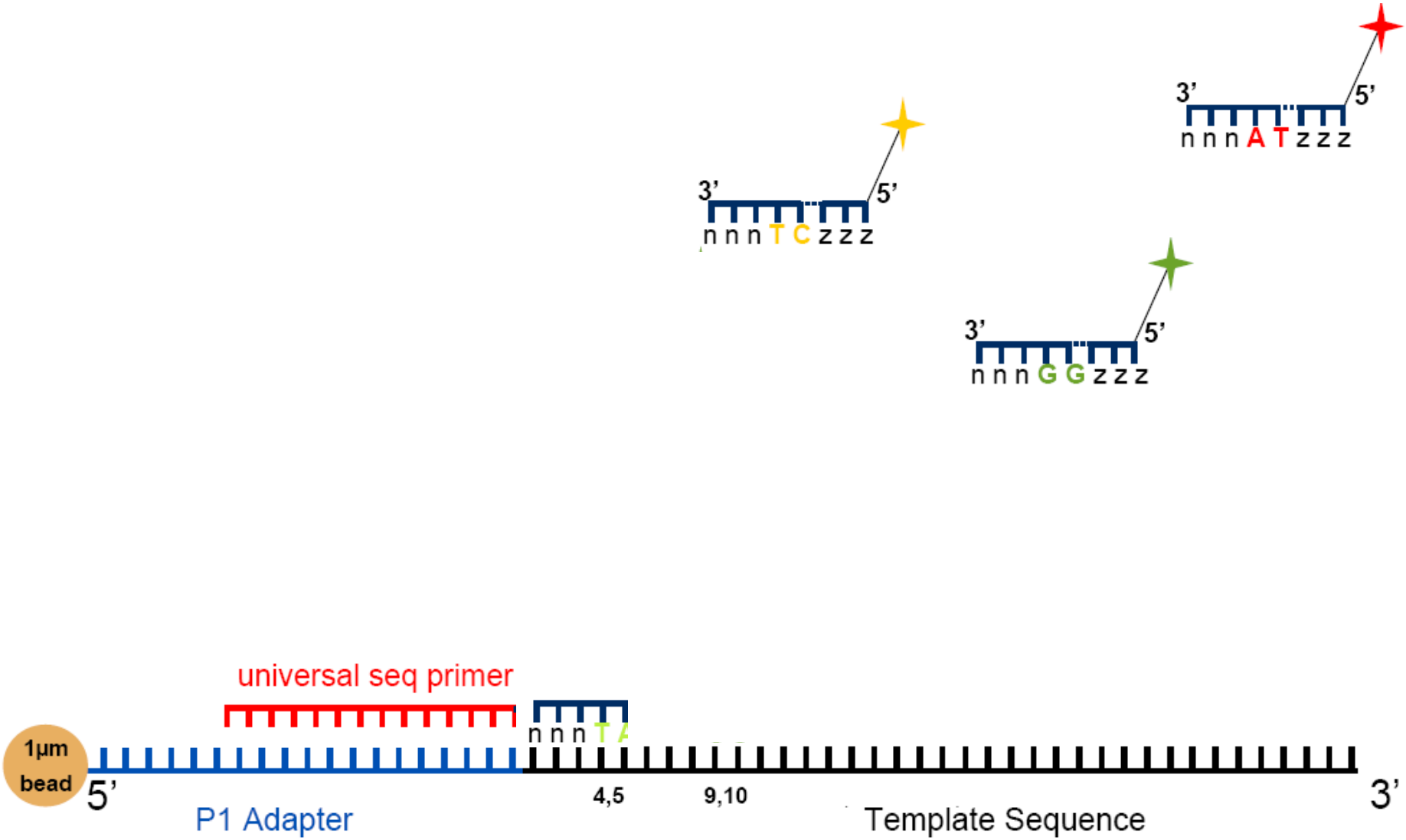

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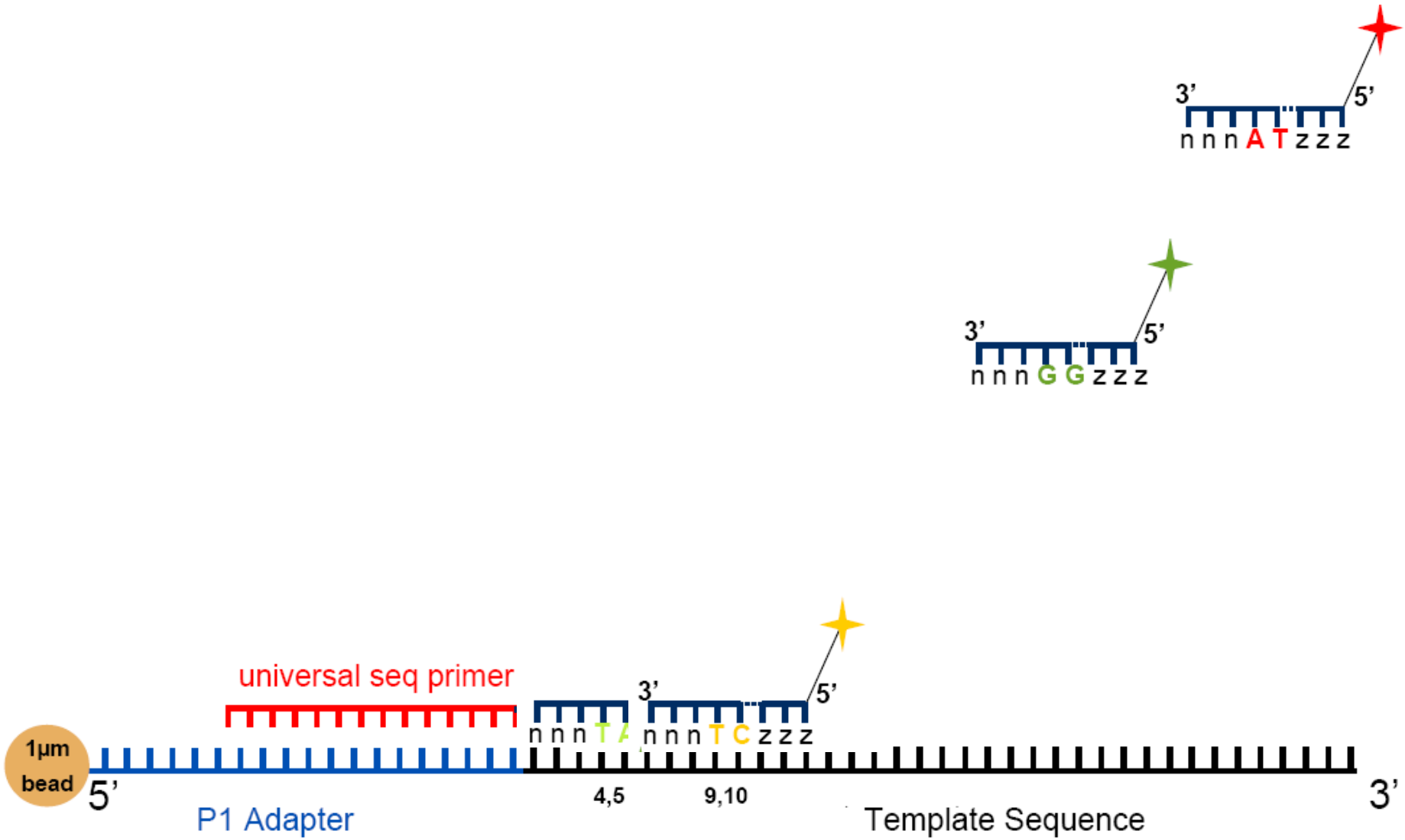

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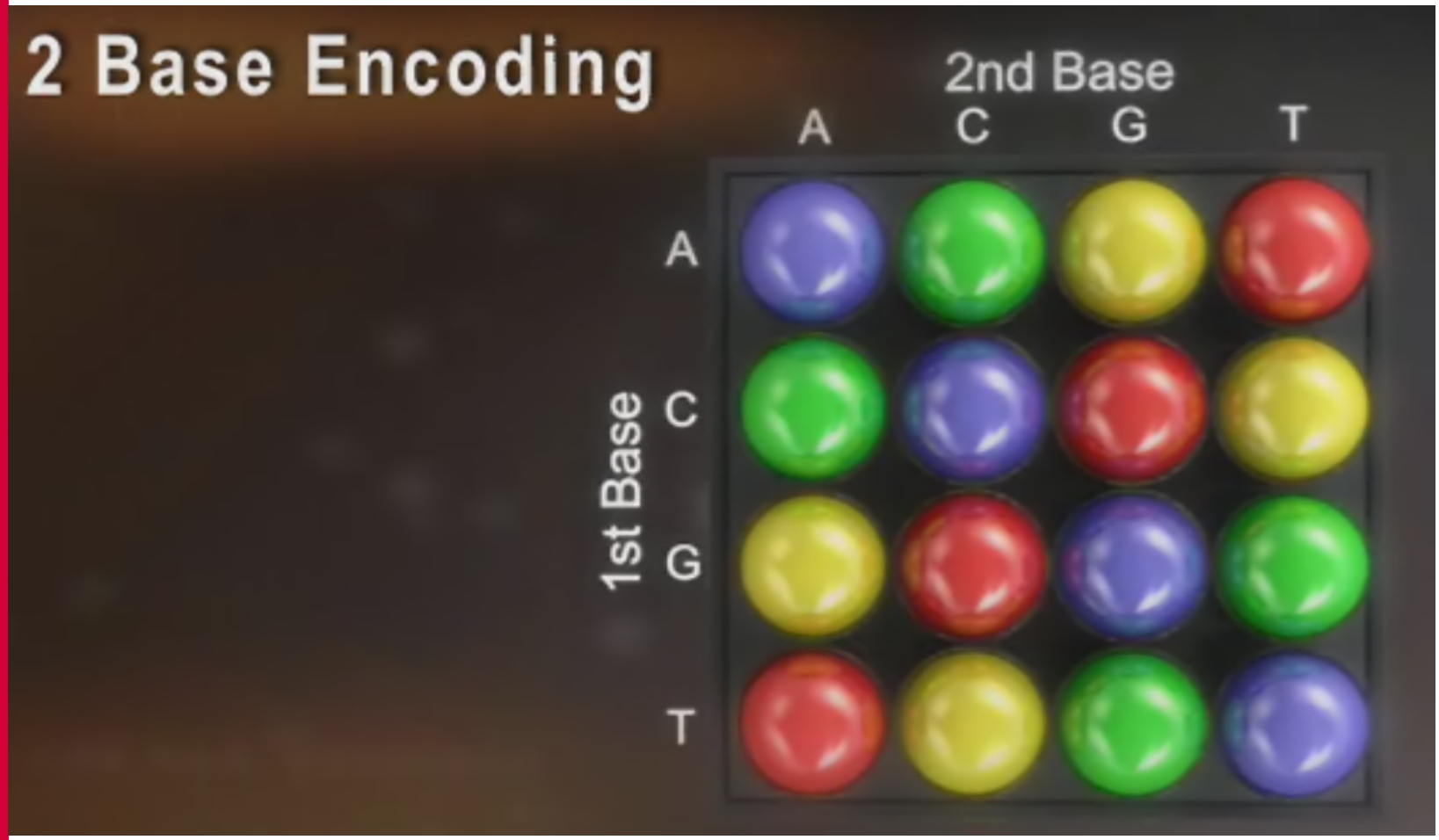
2 Base Encoding Improves Accuracy



Advantages of 2 base encoding

- Double base interrogation eases the discrimination between system ***errors*** and ***true*** polymorphism

True polymorphisms produce 2 color changes while system errors produce a single color change

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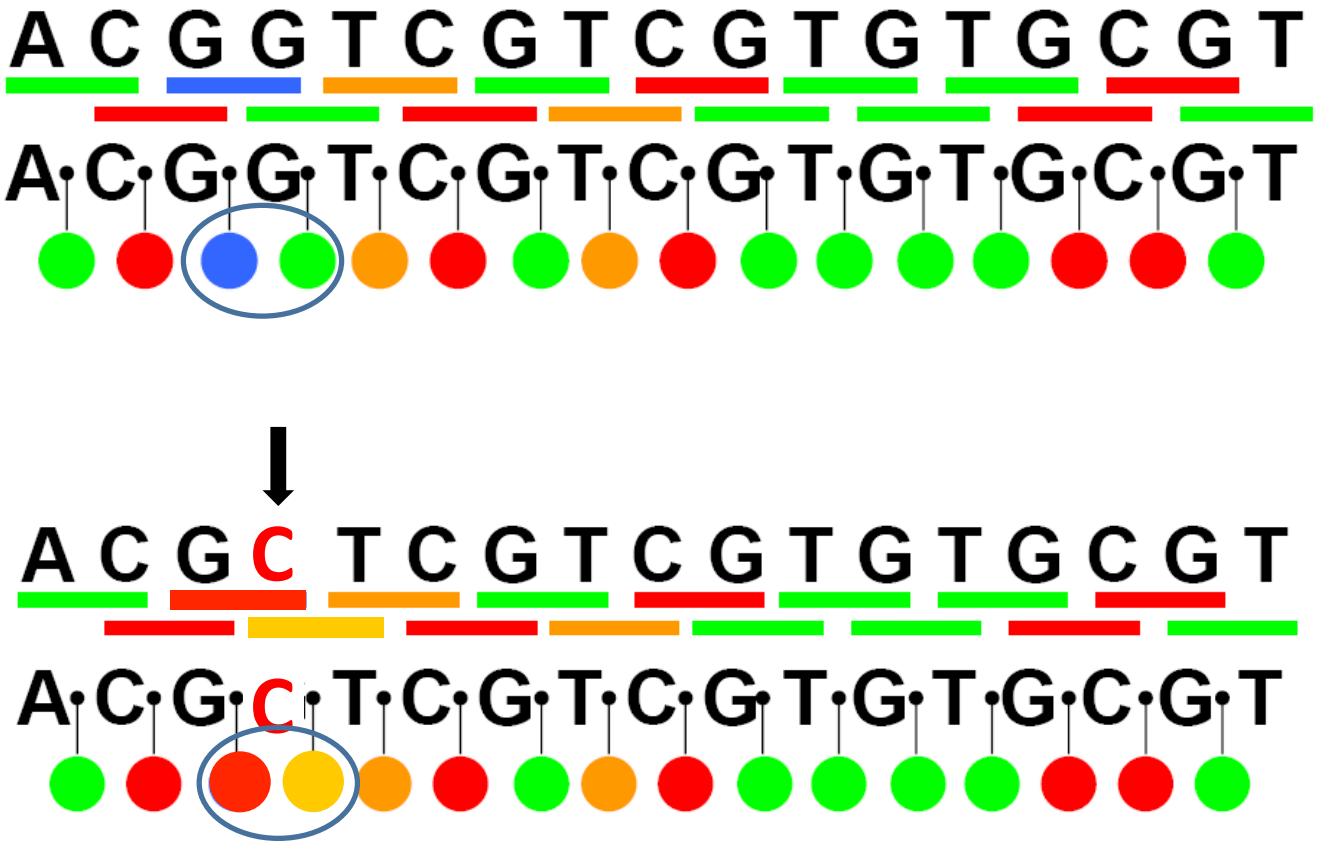

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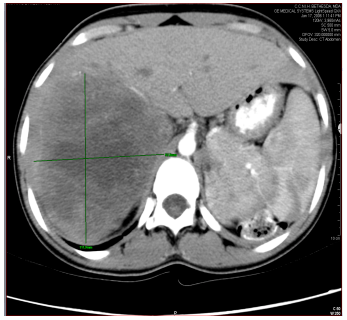
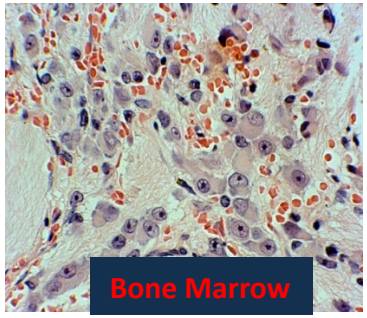
Patient with Metastatic Neuroblastoma

Diagnosis





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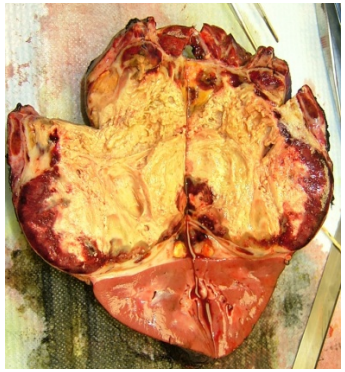

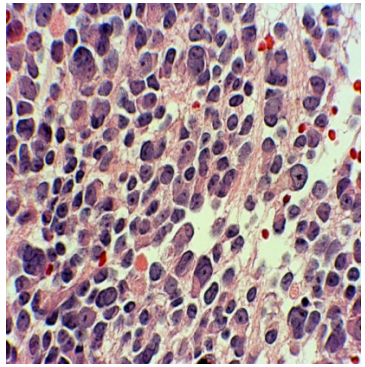

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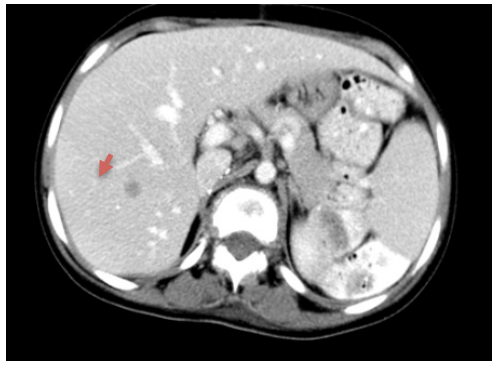

3. Met2-Liver: Liver metastasis at autopsy

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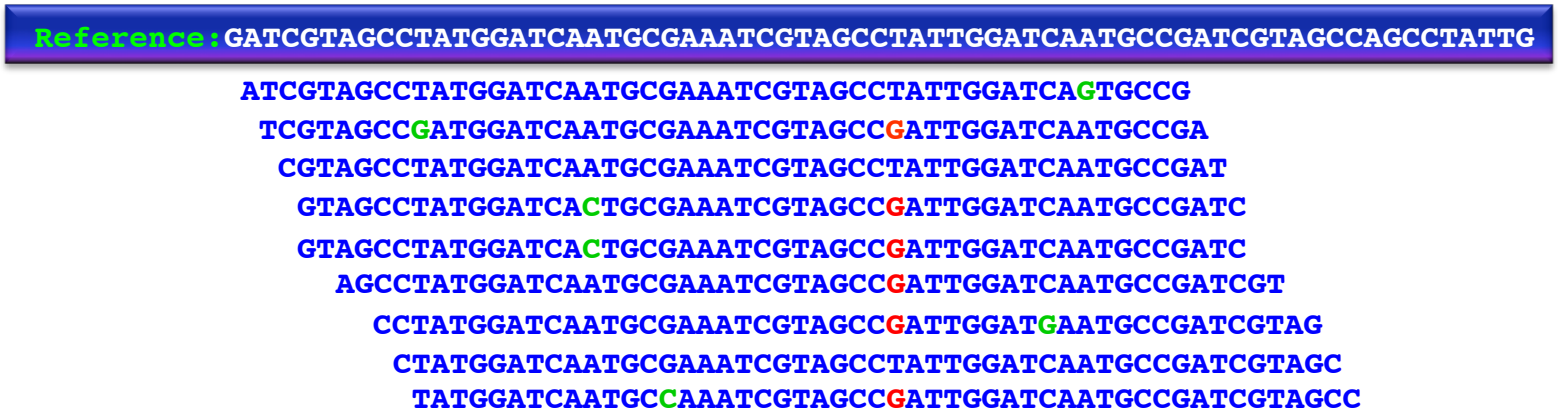
Align To Reference Genome



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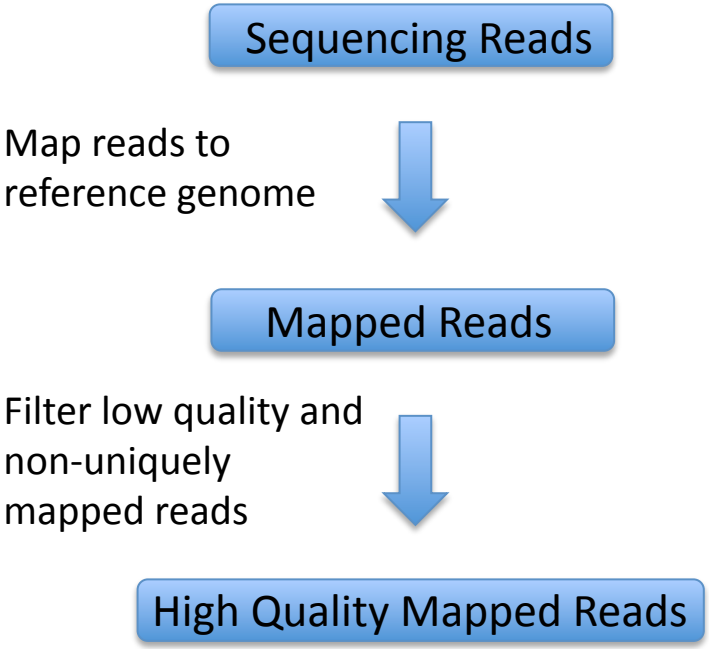
Align and Filter Reads



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High Coverage

Sample	Primary Tumor	Met1	Met2	Liver	Skin
Number of Mapped Reads	279M	216M	217M	259M	181M
High Quality Reads	231M	186M	180M	223M	153M
On Target Reads	165M	146M	143M	175M	134M
Coverage	187X	182X	184X	193X	205X

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Second Generation Sequencing

DNA

Mutations
- Insertions/Deletions
- Copy Number

Allelic Imbalance
- Translocations
- Inversions

RNA

Mutations

Gene Expression
- Splice Variants
- Fusion Genes
- Novel Transcripts

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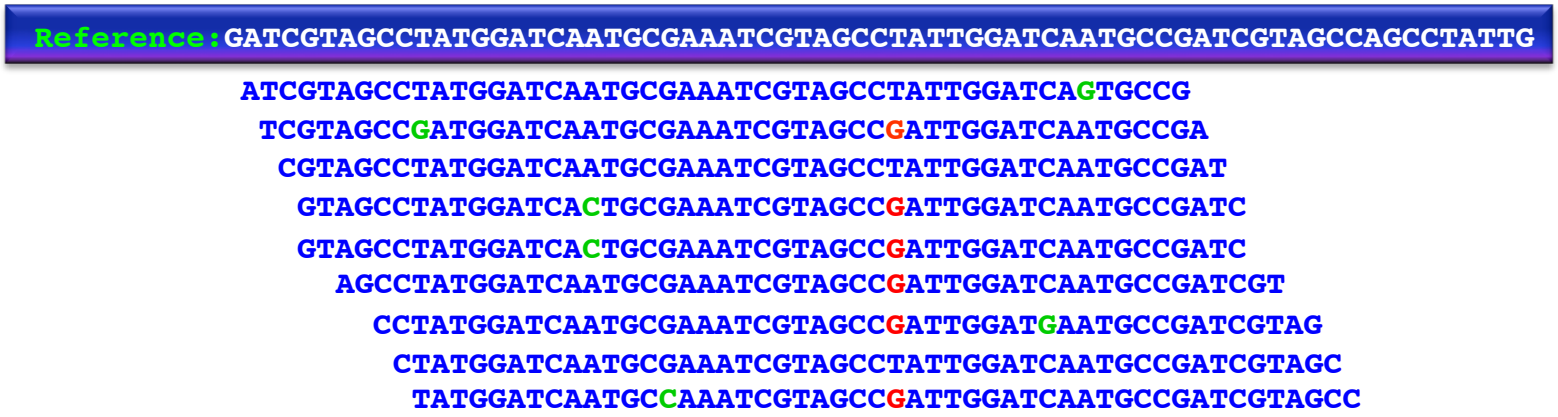
Identify a Mutation



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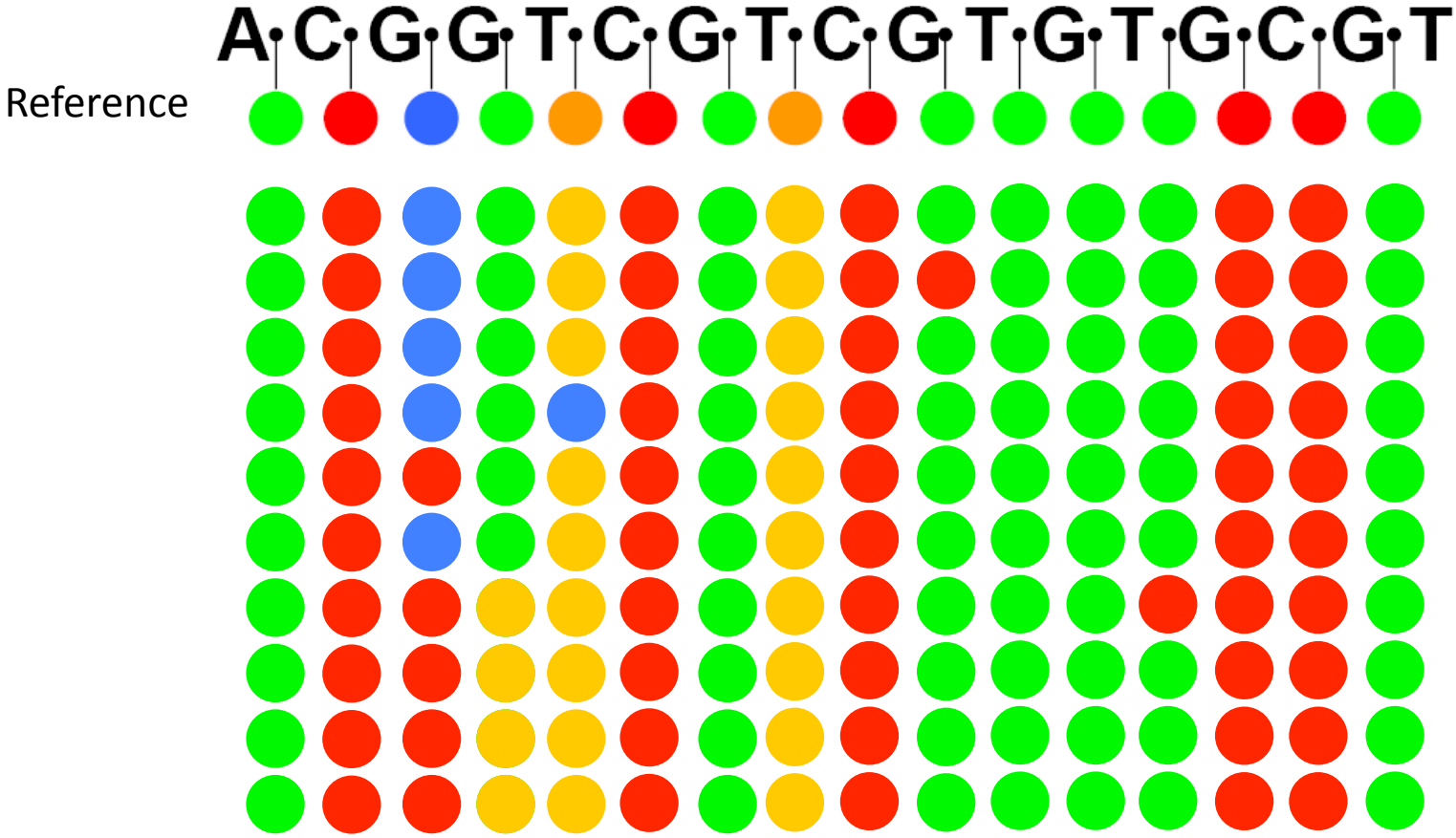
Identify a Mutation in Color Space



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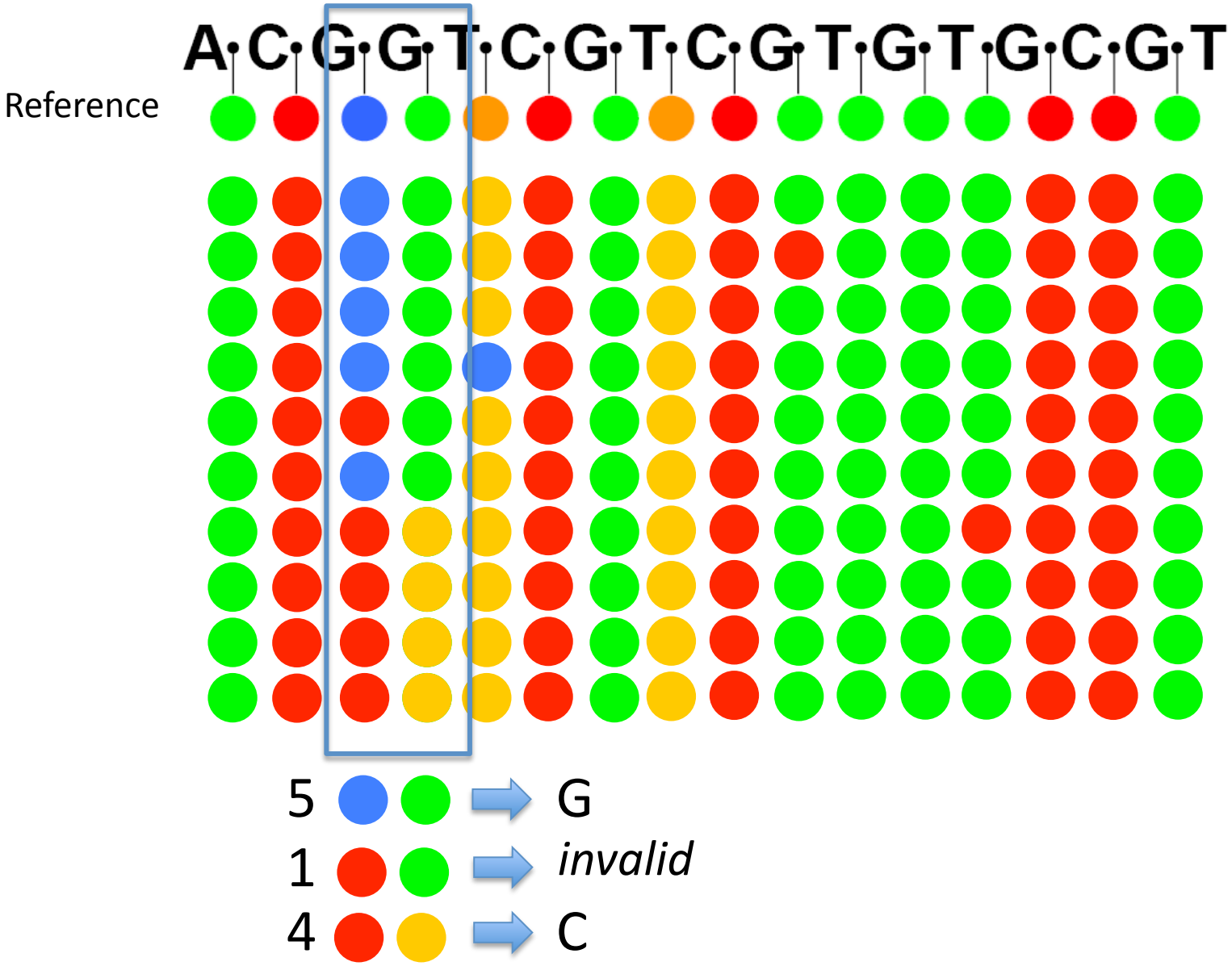

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Identify a Mutation - diBayes

Bayesian Algorithm:

$$P(\text{Genotype}|\text{Data}) = \frac{P(\text{Data}|\text{Genotype})\ P(\text{Genotype})}{P(\text{Data})}$$

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Identify a Mutation - diBayes

Bayesian Algorithm:

$$P(\text{Genotype}|\text{Data}) = \frac{P(\text{Data}|\text{Genotype})\ P(\text{Genotype})}{P(\text{Data})}$$

Genotypes: GG, GC, GT, GA, CC, CT, CA, TT, TA, AA

Prior: P(Genotype) = 0.999 if same as reference (e.g. GG)
(1-0.999)/9 otherwise

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Identify a Mutation - diBayes

Bayesian Algorithm:

$$P(\text{Genotype}|\text{Data}) = \frac{P(\text{Data}|\text{Genotype})\ P(\text{Genotype})}{P(\text{Data})}$$

$$P(\text{Data}|\text{Genotype}) = P(\text{Read}_1|\text{Genotype})\ P(\text{Read}_2|\text{Genotype})\ldots$$

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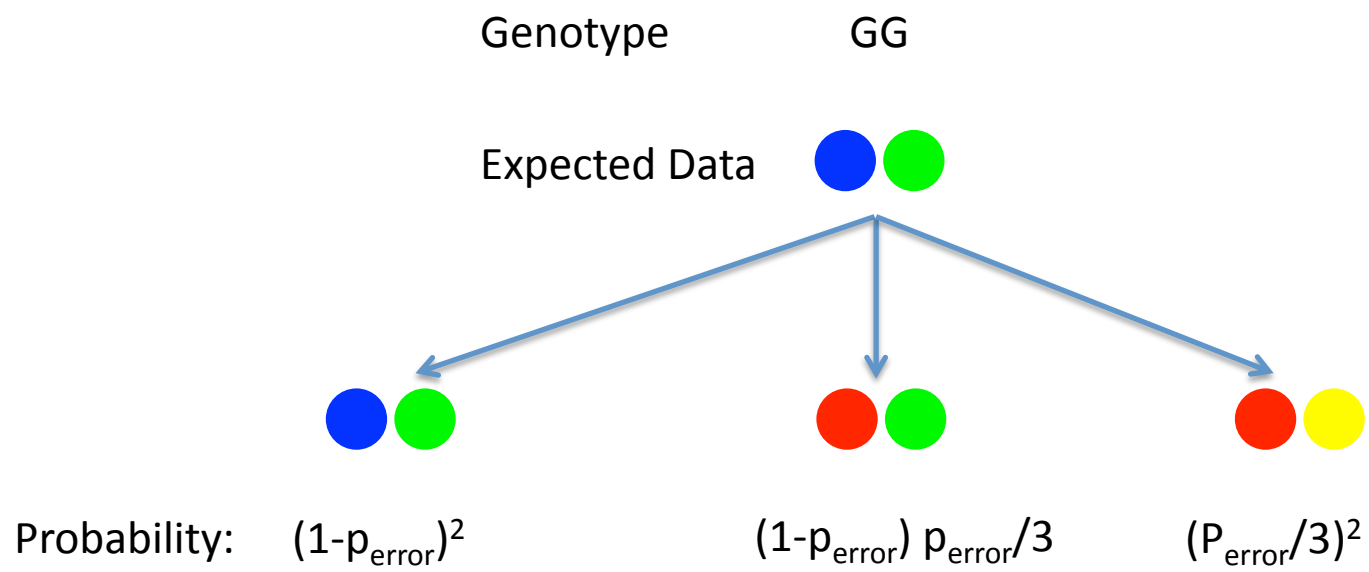
Identify a Mutation - diBayes

$P(Read_1 | Genotype)$



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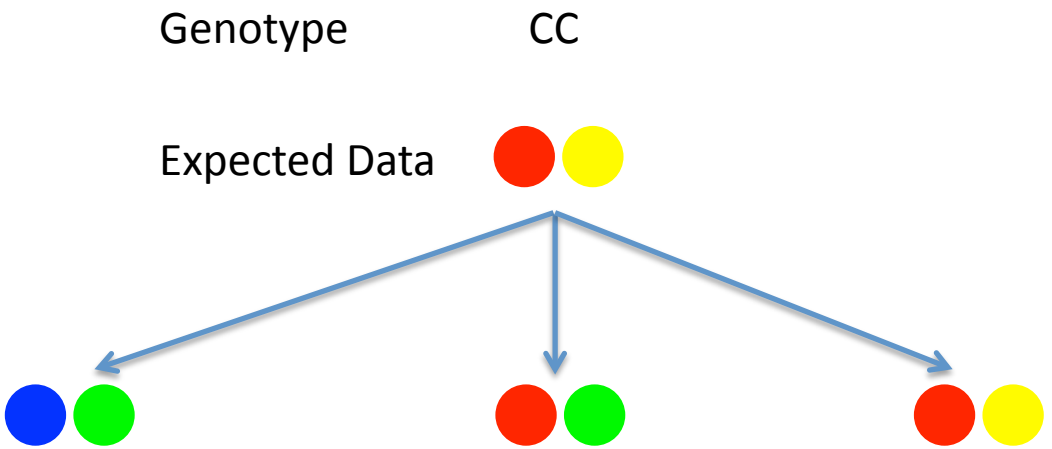

Probability: $(P_{error}/3)^2$ $(1-p_{error})\ p_{error}/3$ $(1-P_{error})^2$

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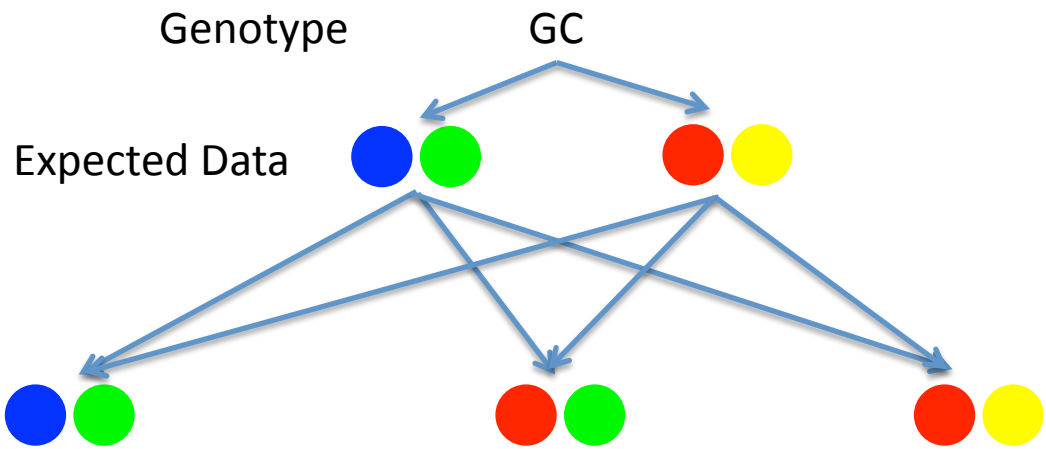
Identify a Mutation - diBayes

$P(Read_1 | Genotype)$



Probability: $0.5(1-P_{error})^2+0.5(P_{error}/3)^2$ $(1-p_{error})\ p_{error}/3$ $0.5(P_{error}/3)^2+0.5(1-P_{error})^2$

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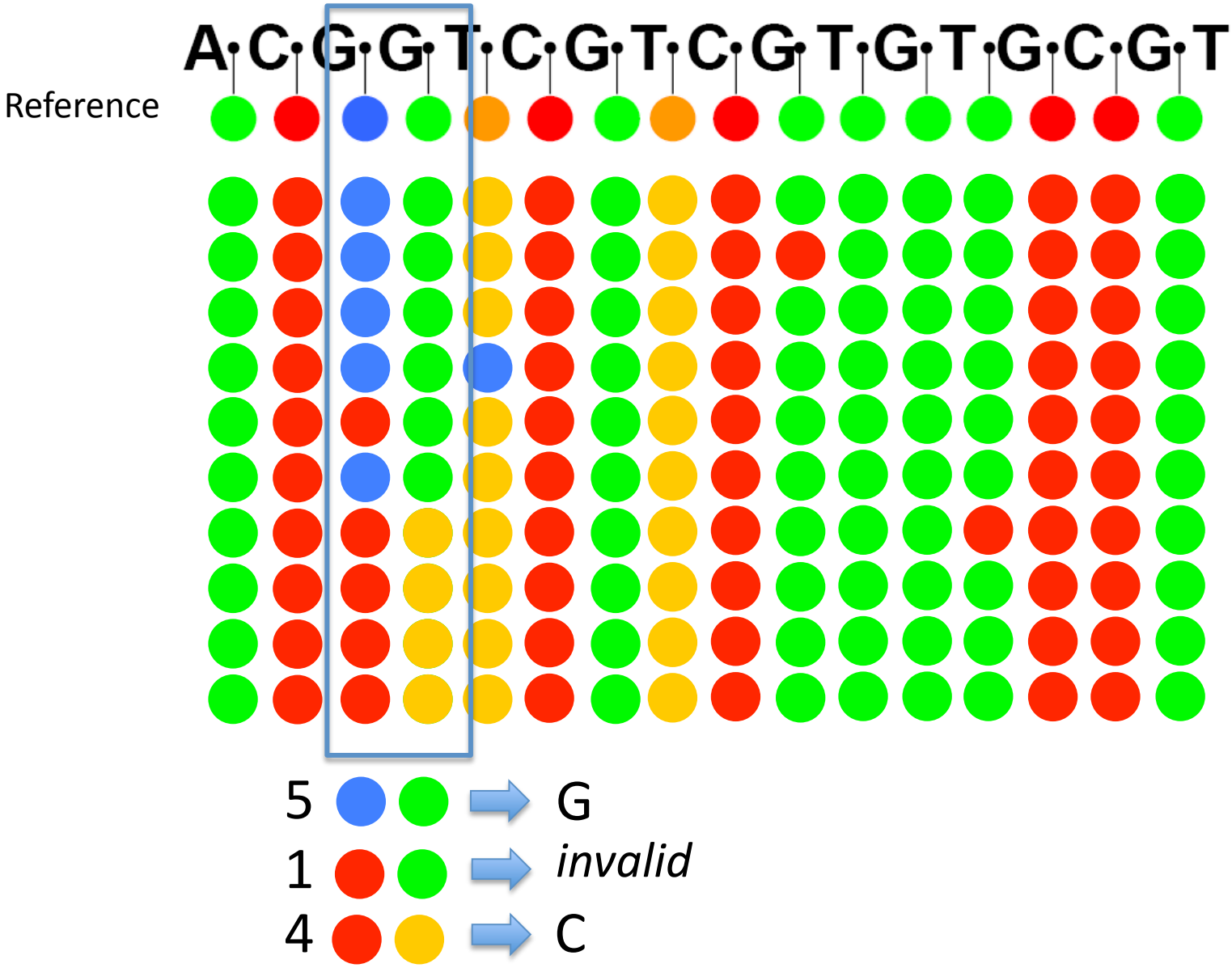
Identify a Mutation in Color Space



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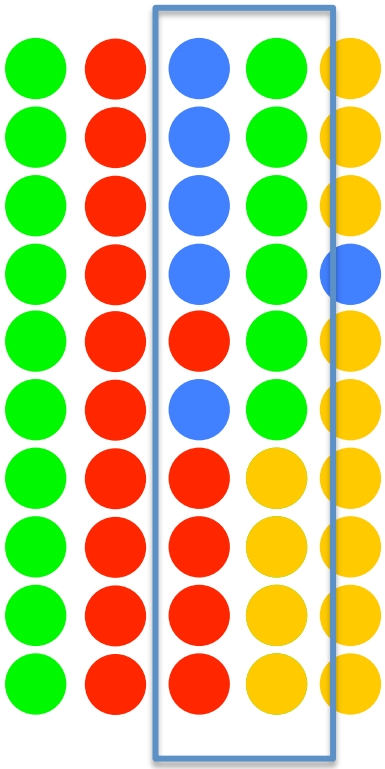

P(GG|Data) = P(GG) P(●●|GG)^{5}P(●●|GG)^{1}P(●●|GG)^4

P(GC|Data) = P(GC) P(●●|GC)^{5}P(●●|GC)^{1}P(●●|GC)^4

P(GG|Data) = P(CC) P(●●|CC)^{5}P(●●|CC)^{1}P(●●|CC)^4

5 ●● → G

1 ●● → *invalid*

4 ●● → C

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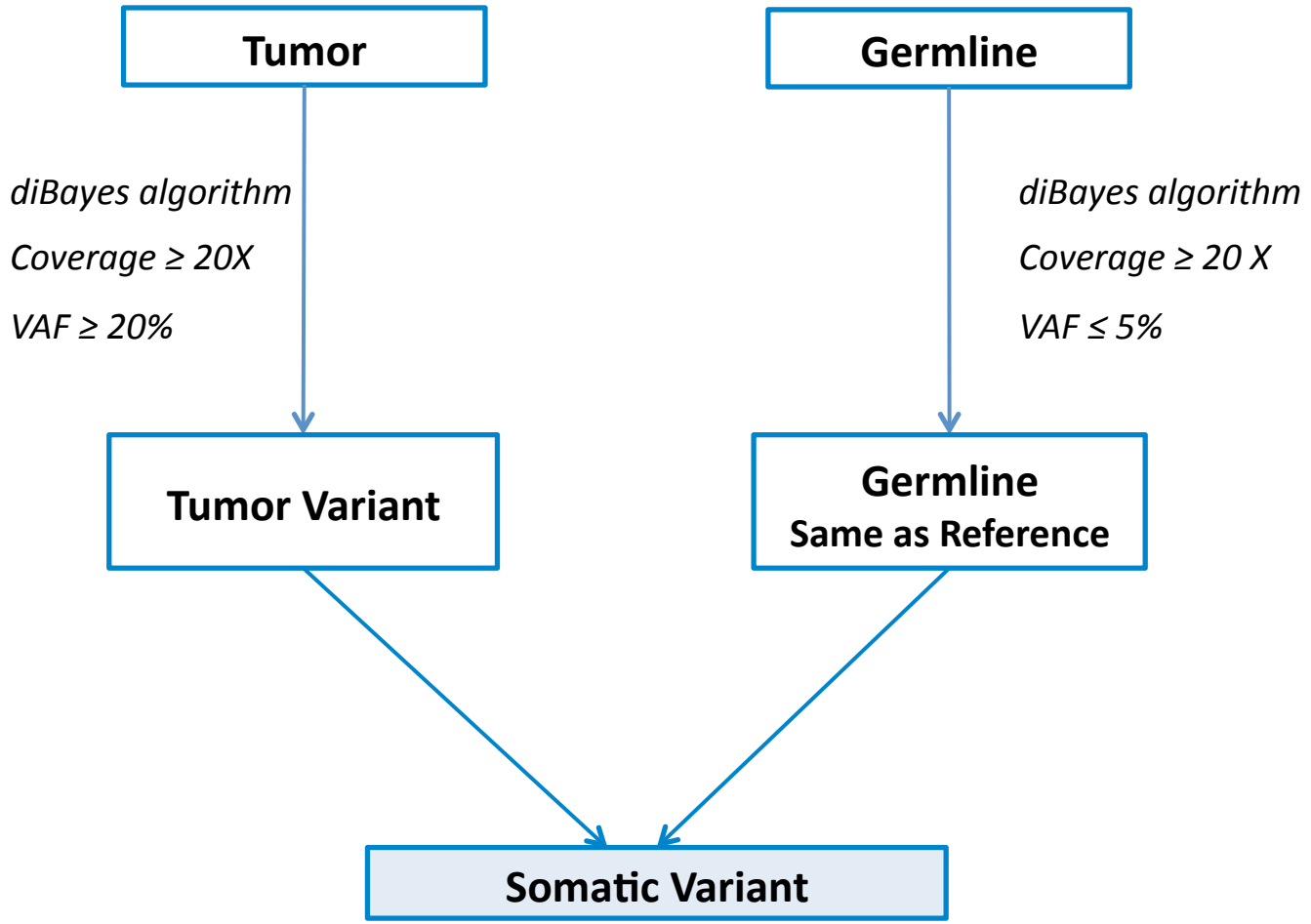
Somatic Mutation Detection



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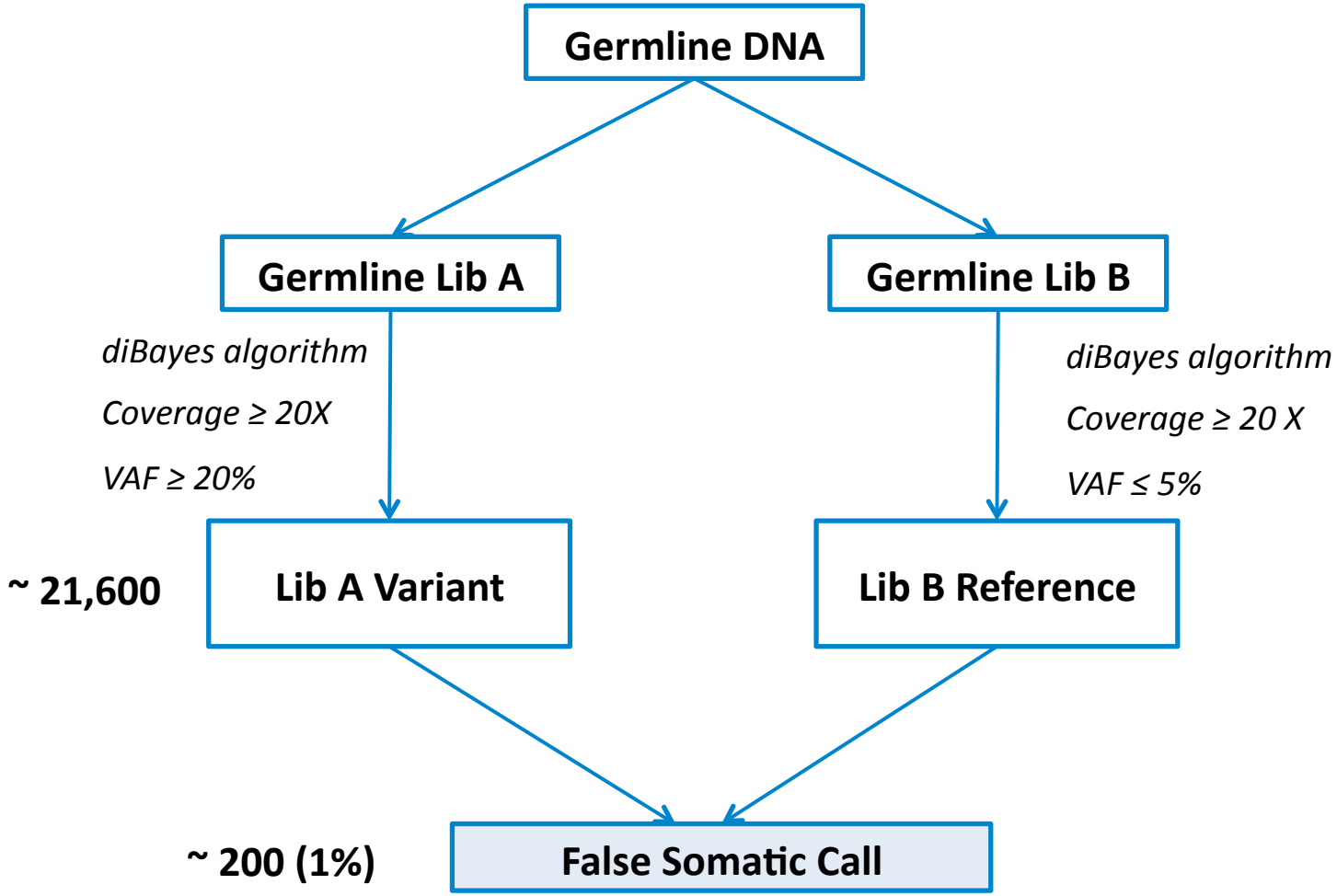
Estimation of False Call Rate



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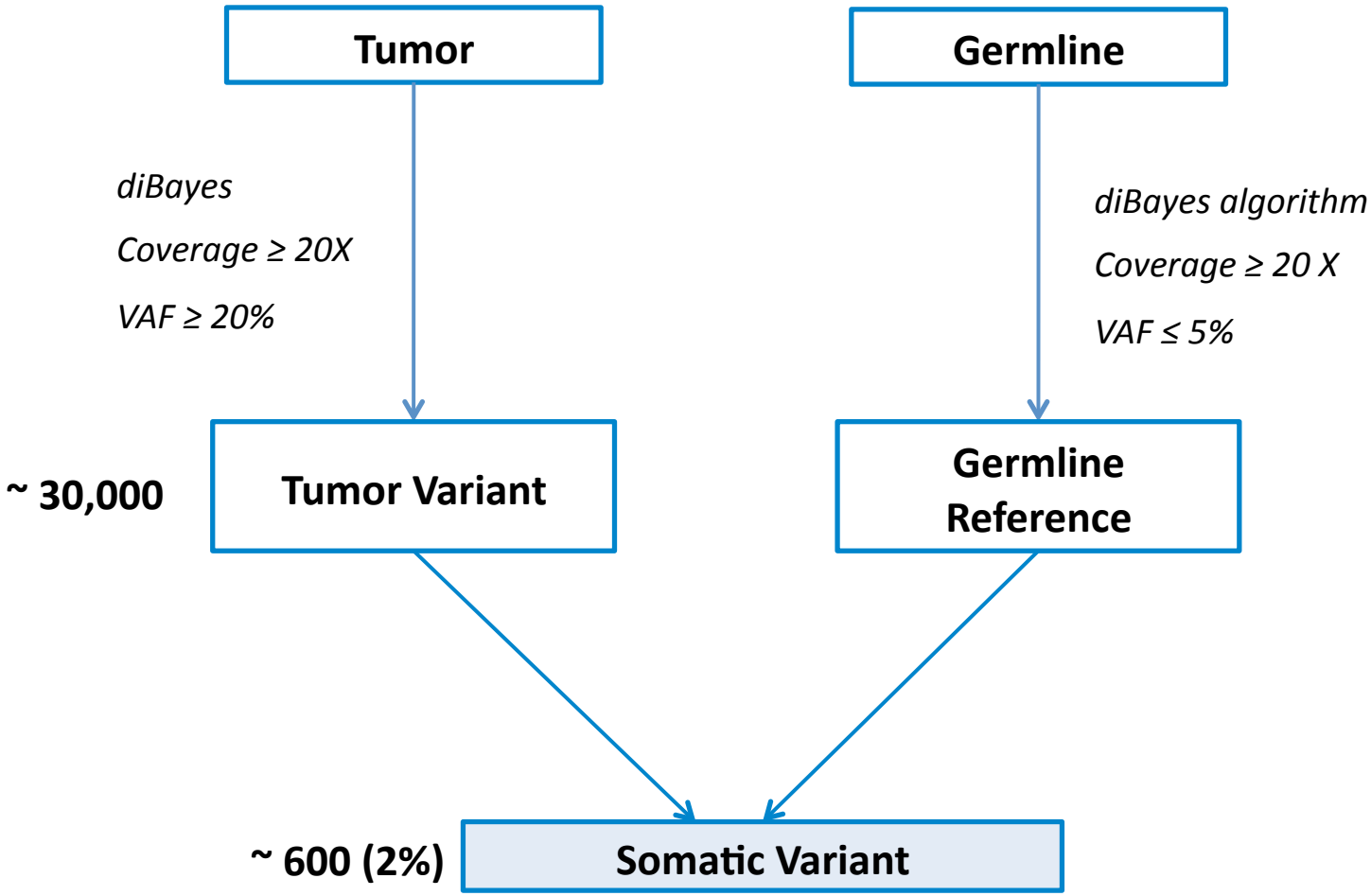
Somatic Mutation Detection



False Calls: ~300 (1% of 30,000)

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Somatic Mutations

	Primary Tumor	Met1 Bone Marrow	Met2 Liver
Variants	29,593	30,006	29,131
Somatic Variants	627	573	634
Protein Coding Region	239	243	287
Non-synonymous Variant	163	170	195

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Overlap with RNA data

- Reduce false positives by focusing on variants detected by both methods
- Identify therapeutic target – expressed by nature

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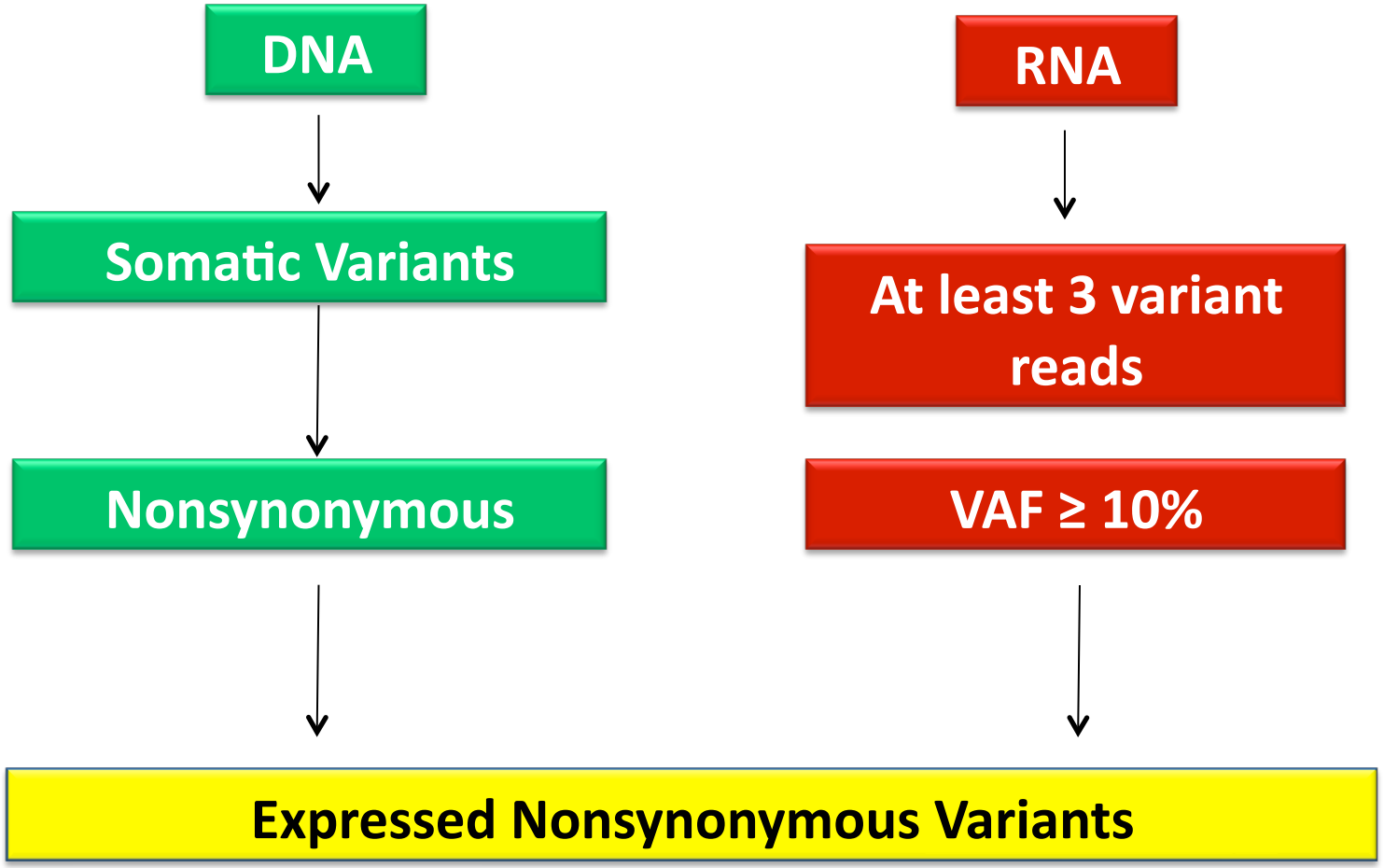
Expressed Nonsynonymous Variants



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Variants	29,593	30,006	29,131
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Translated Region	239	243	287
Non-synonymous	163	170	195
Expressed	3	3	3

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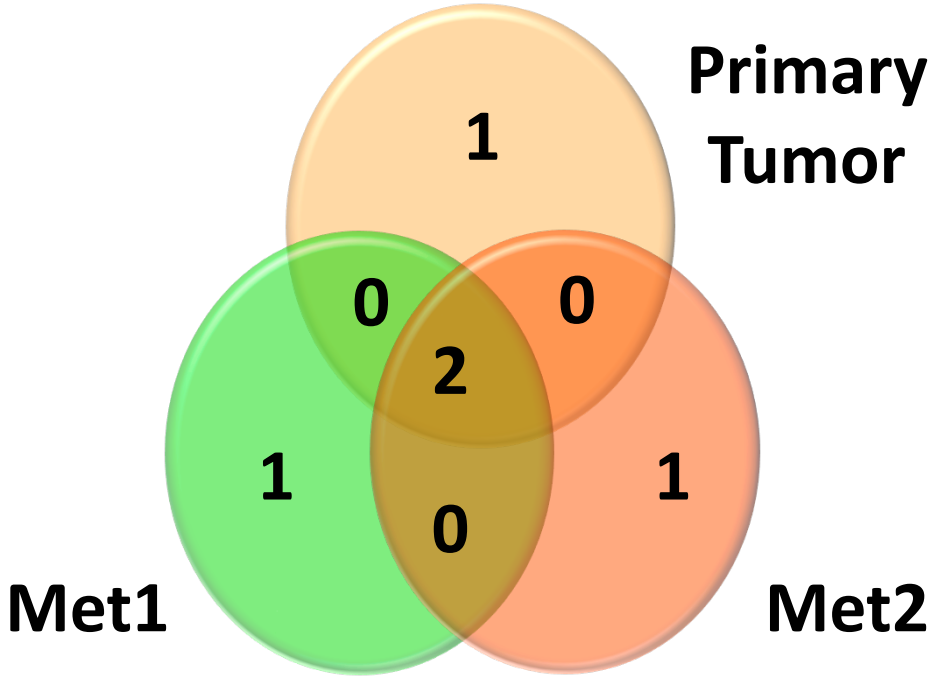
Expressed Nonsynonymous Variants



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Outline

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- Identify Somatic Mutations
  - Two Somatic Mutations in Expressed Genes
- Chromosomal Changes
  - Stable Pattern of Allelic Imbalance

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Allelic Imbalances

Maternal Copy

ACTGACTGGTACTGATTT

ACTCACTGGTACTGATTT

Paternal Copy

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Allelic Imbalances

Maternal Copy

ACTGACTGGTACTGATTT

ACTCACTGGTACTGATTT

Paternal Copy

ACTGACTGGTACTCATTT

Reference

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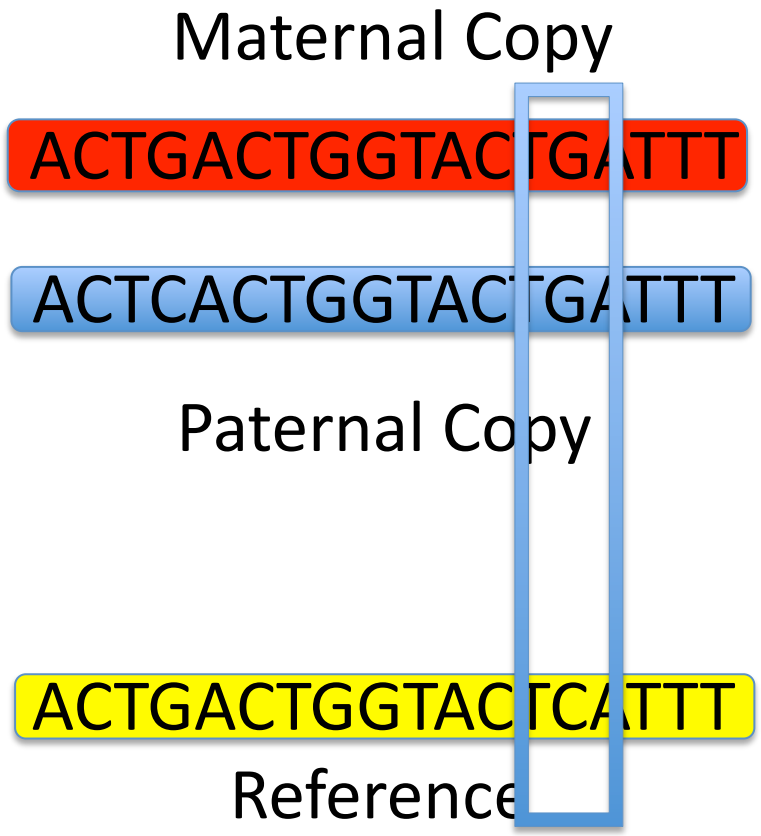
Allelic Imbalances



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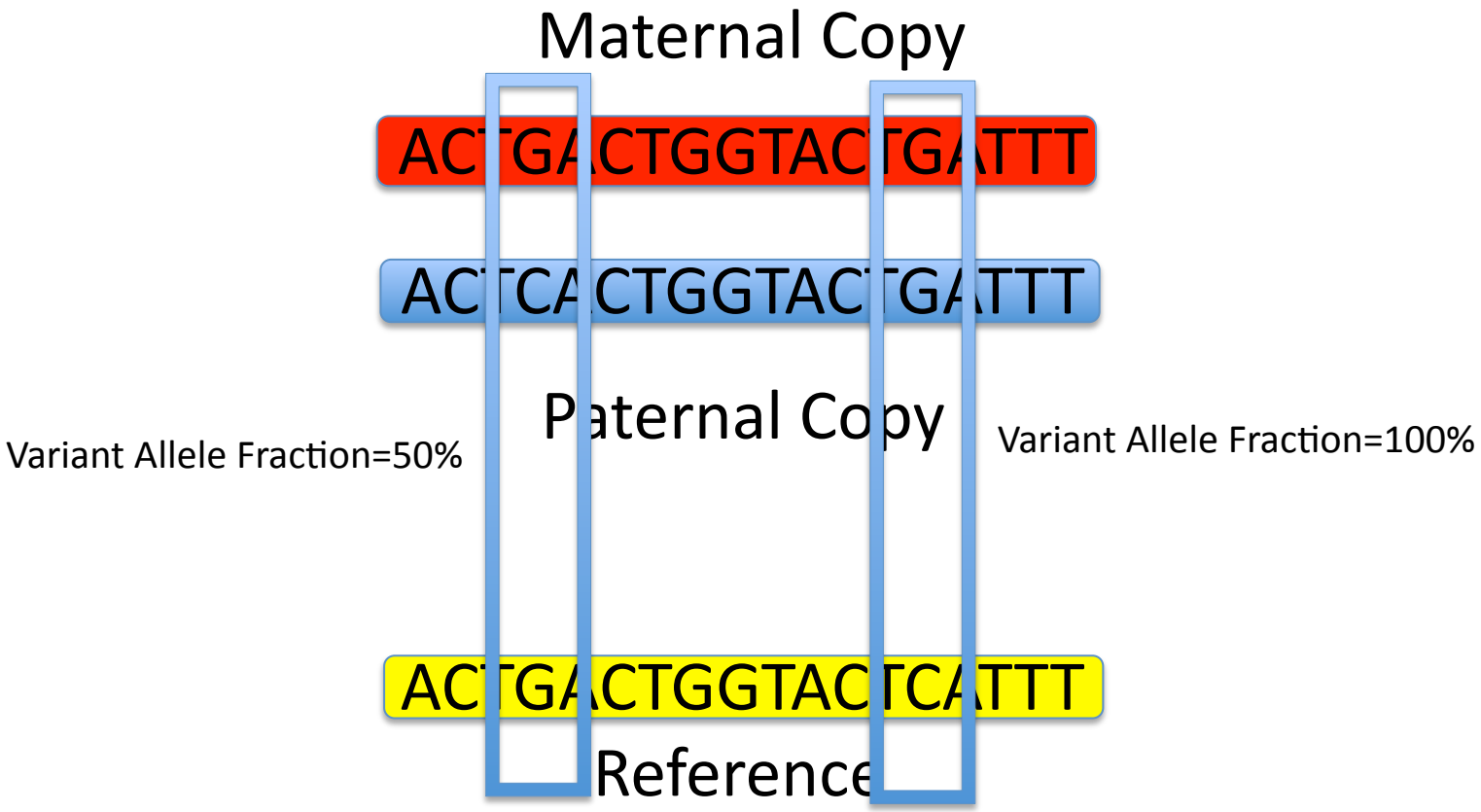

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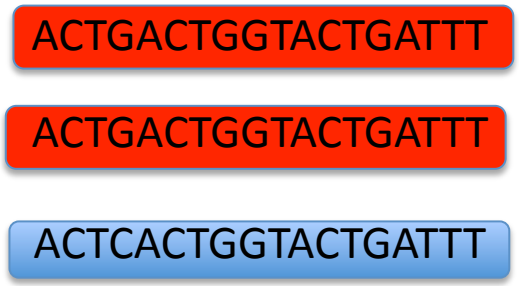
Allelic Imbalances

NORMAL
VAF: 0%, 50%, 100%

Loss of Heterozygosity (LOH)
VAF: 0%, 100%



Gain a Copy
VAF: 0%, 33%, 67%,100%

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Allelic Imbalances

ACTGACTGGTACTGATTT

ACTCACTGGTACTGATTT

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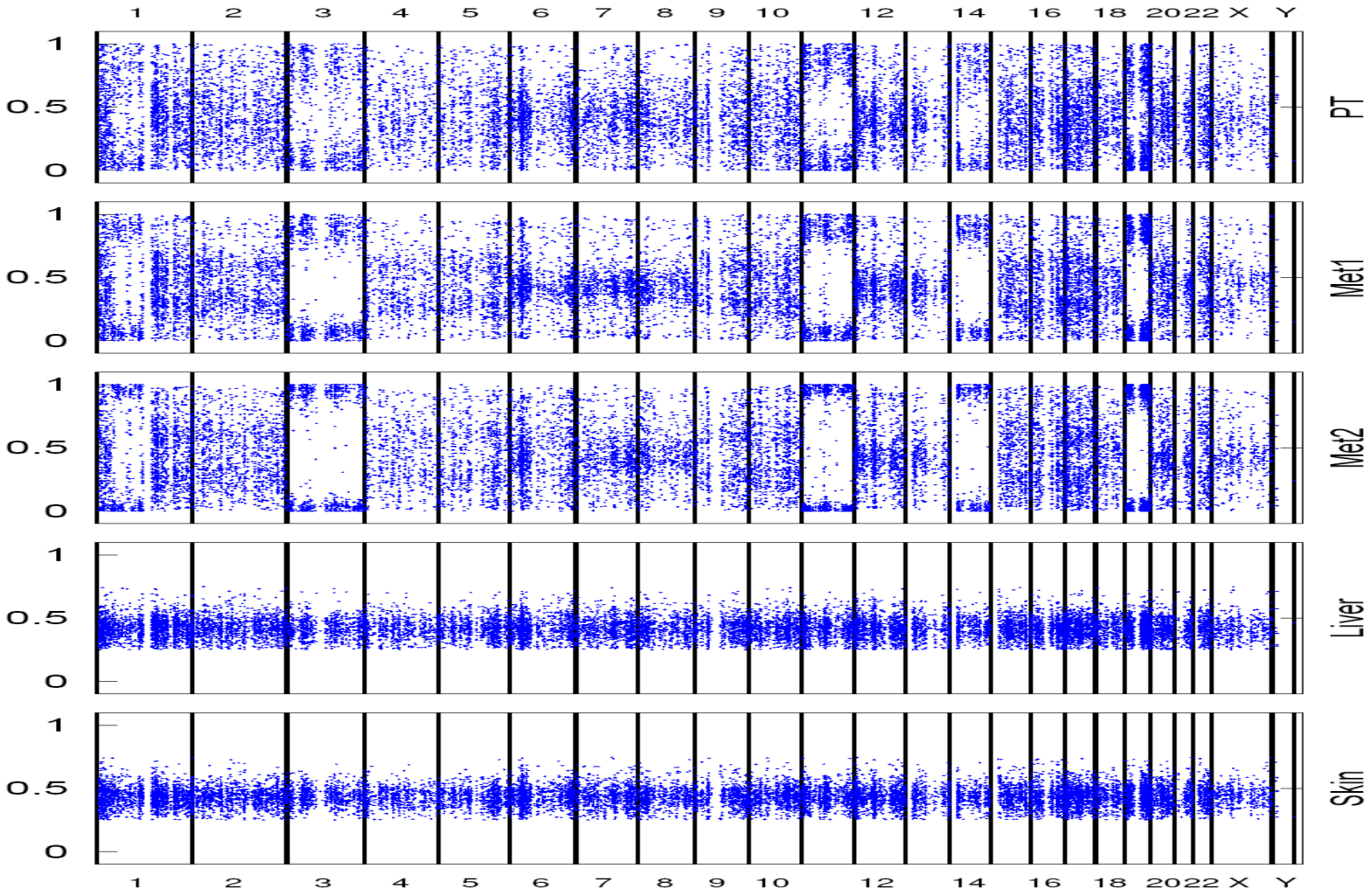

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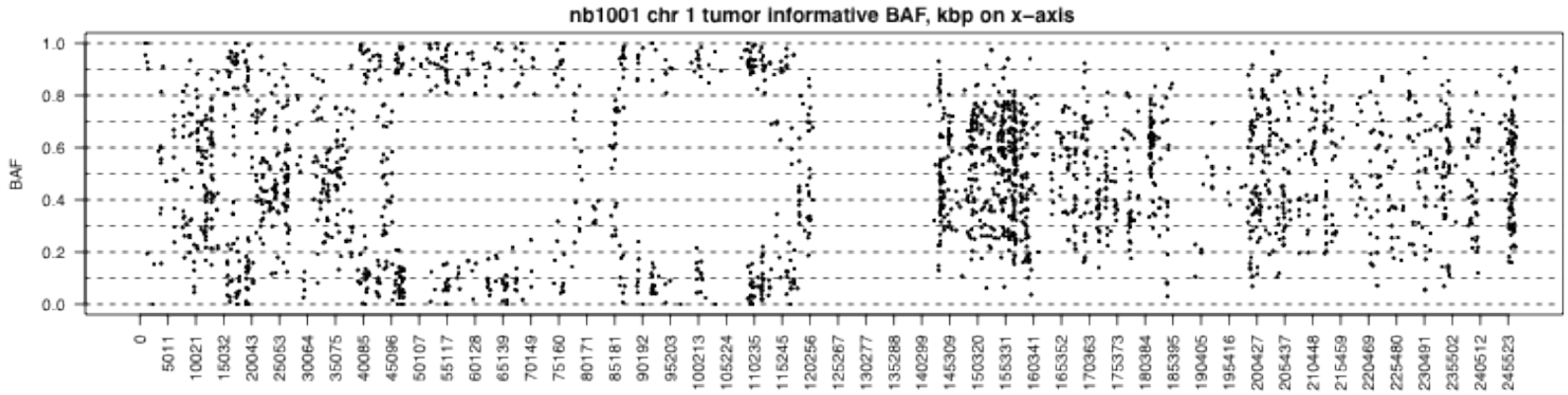
VAF Segmentation



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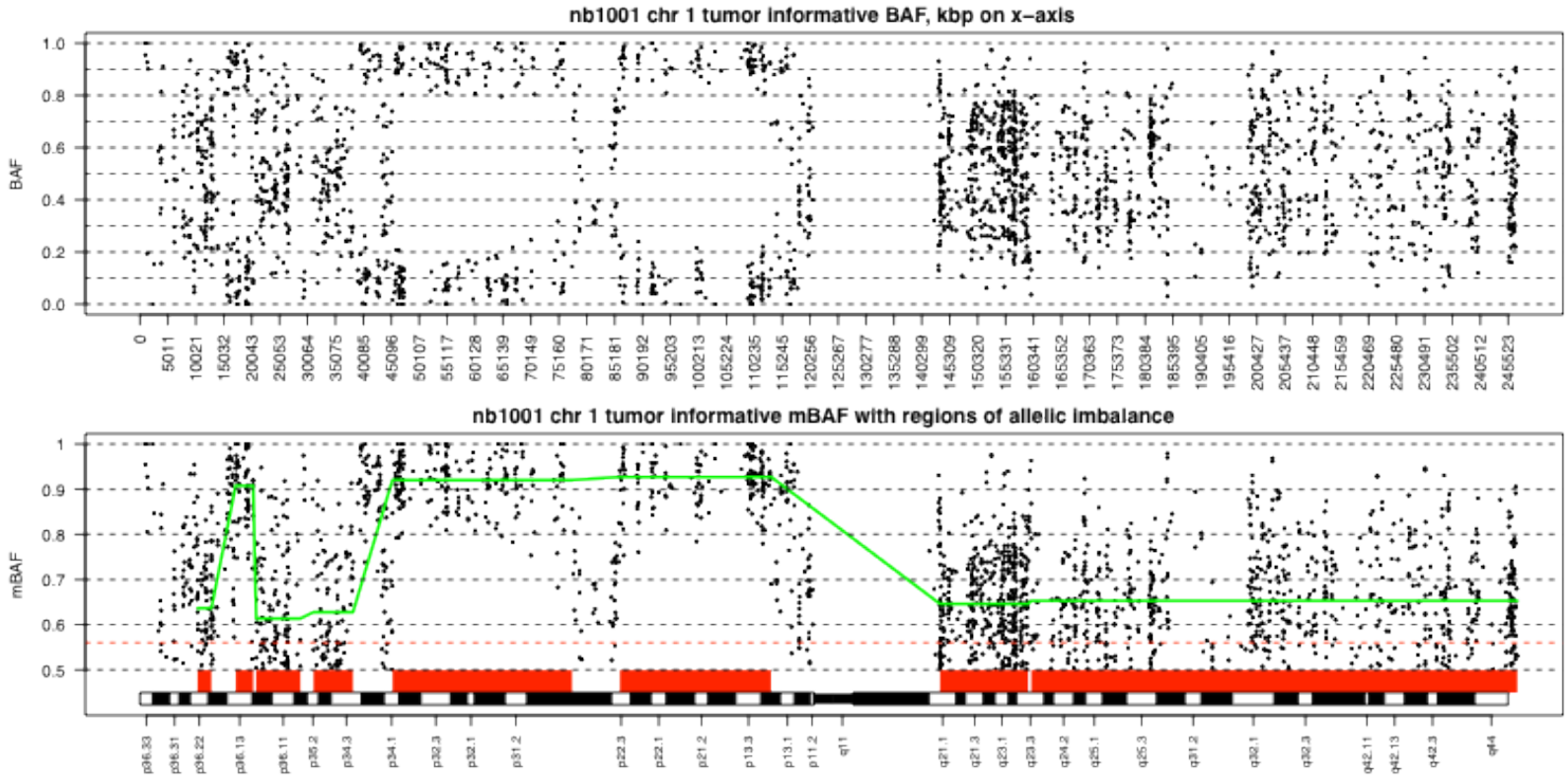
VAF Segmentation



Staaf *et al.*, *Genome Biology* **9**, R136 (2008)

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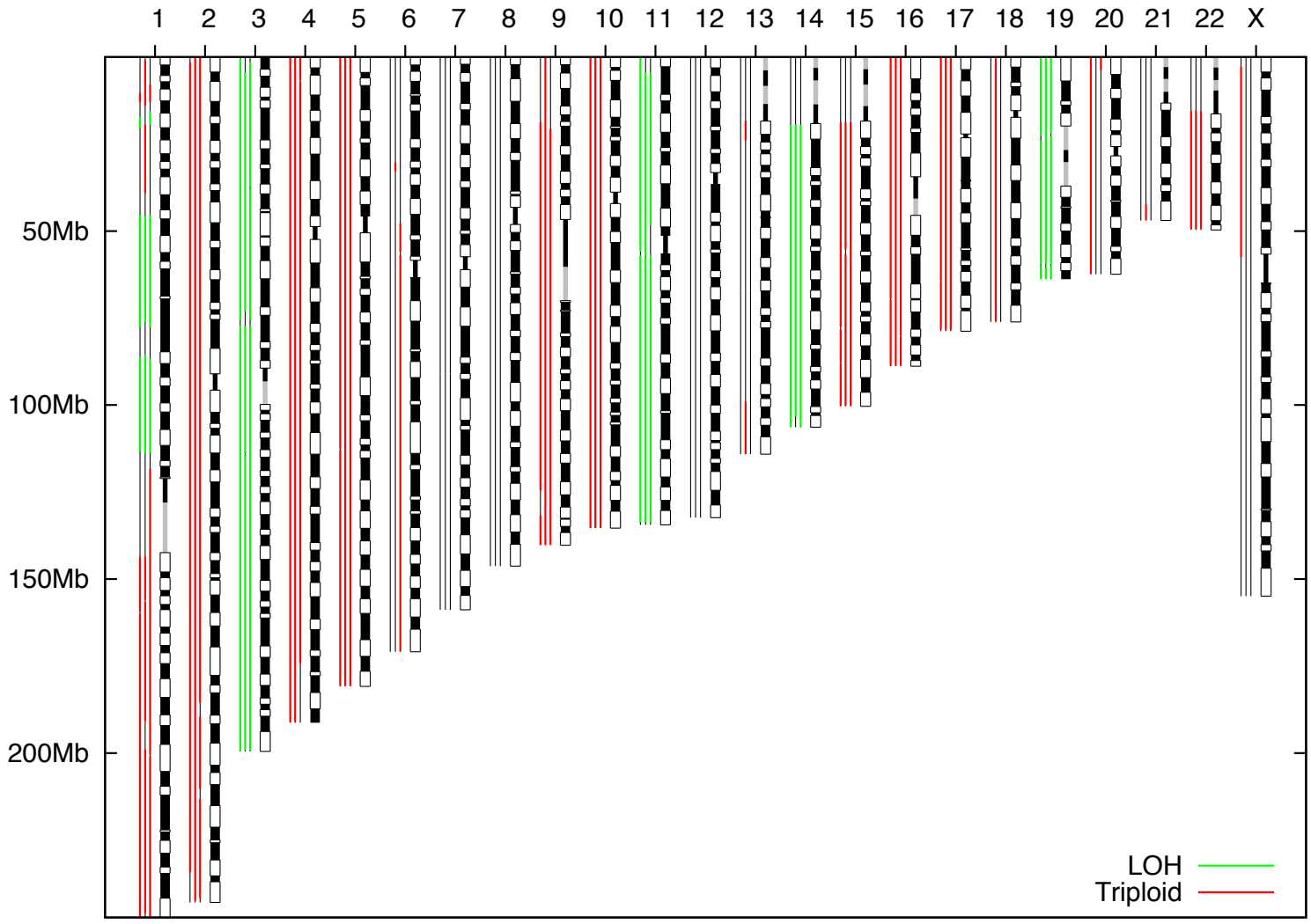
Similar Patterns of Allelic Imbalance



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Summary

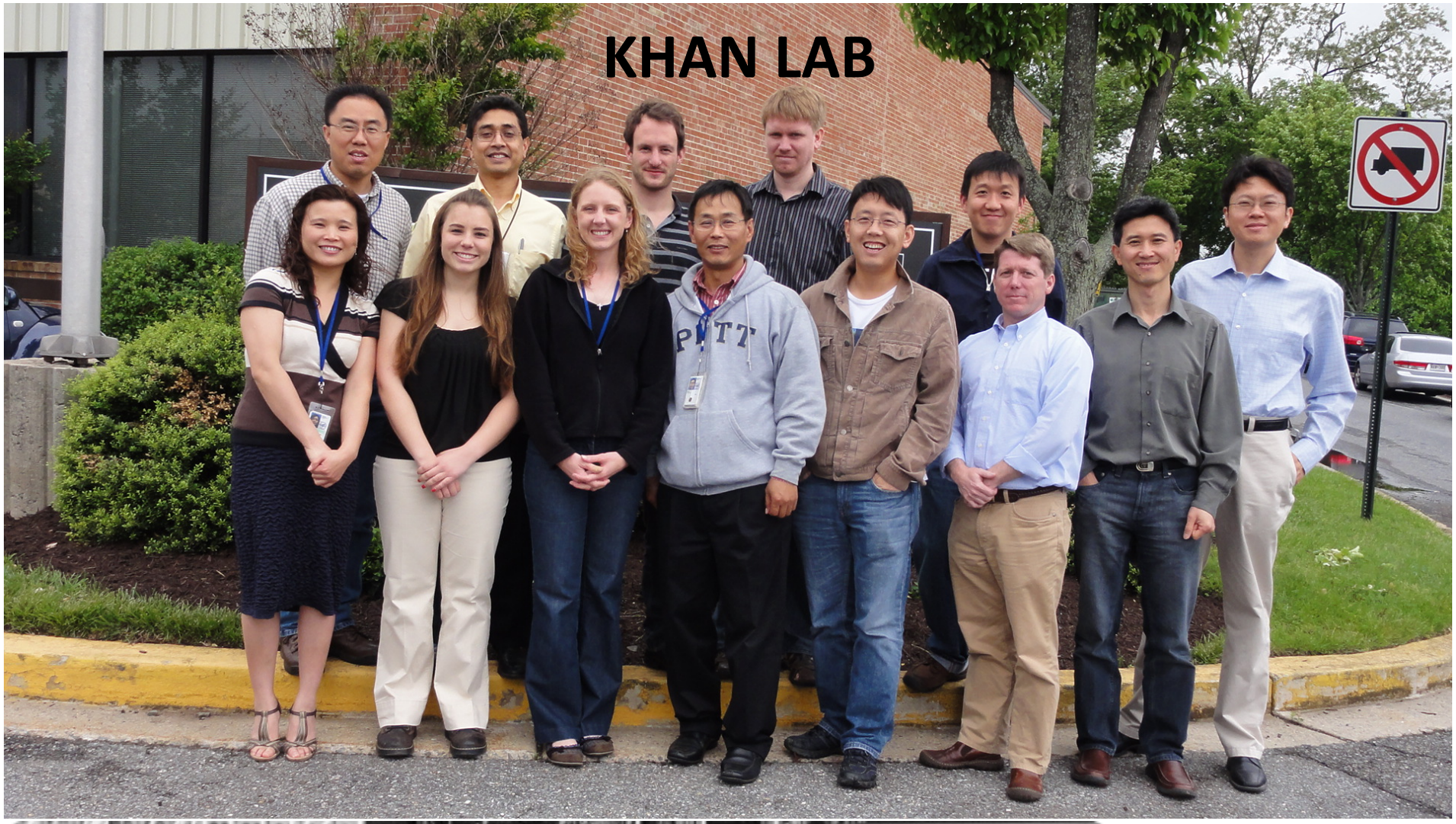
Somatic Mutations

- 2% of found variants in tumors are somatic
- We have identified two expressed somatic mutations present in all three samples

Allelic Imbalance

- Tumor showed a stable pattern of extensive allelic imbalances indicating a common origin

Acknowledgements



Biowulf Cluster