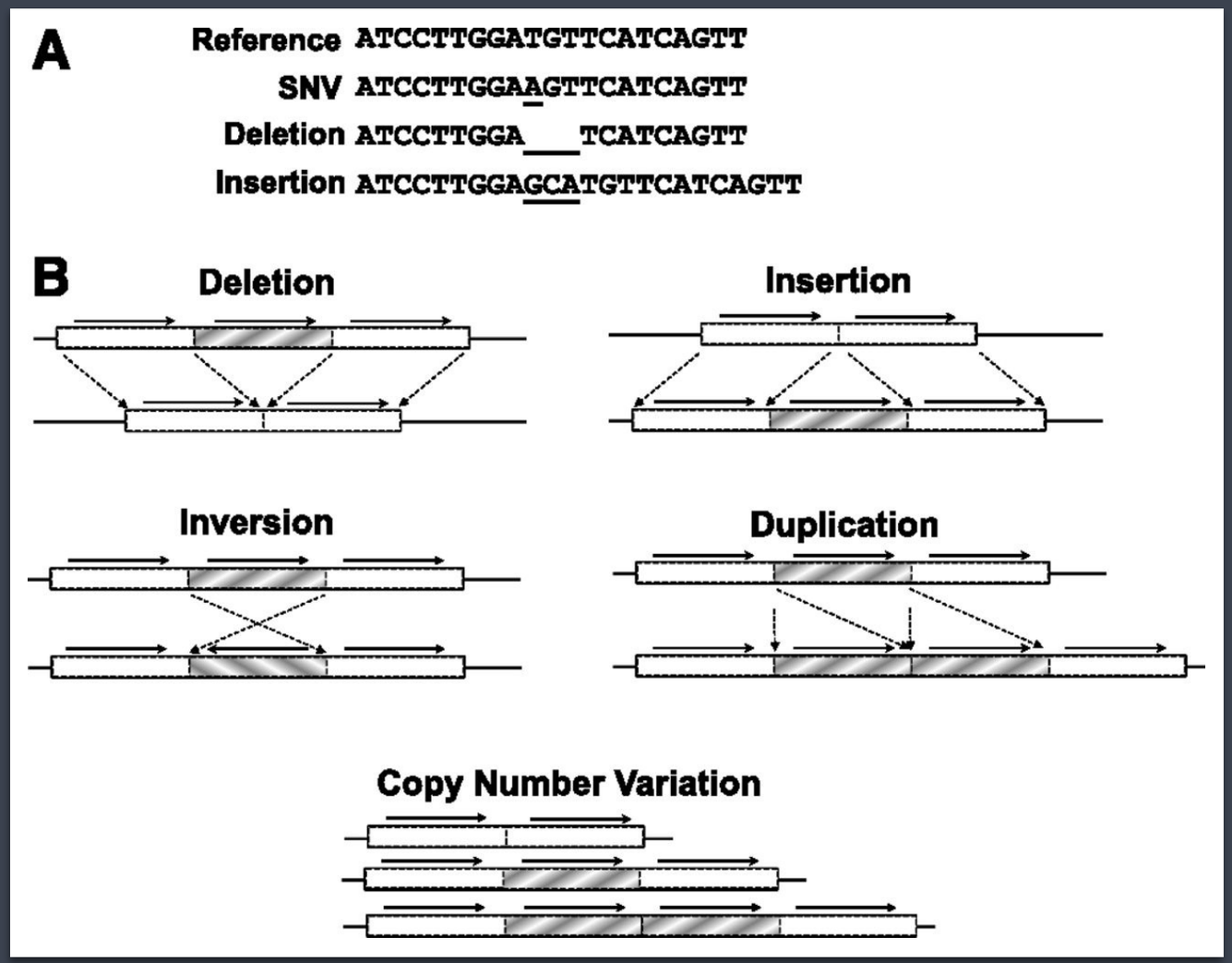
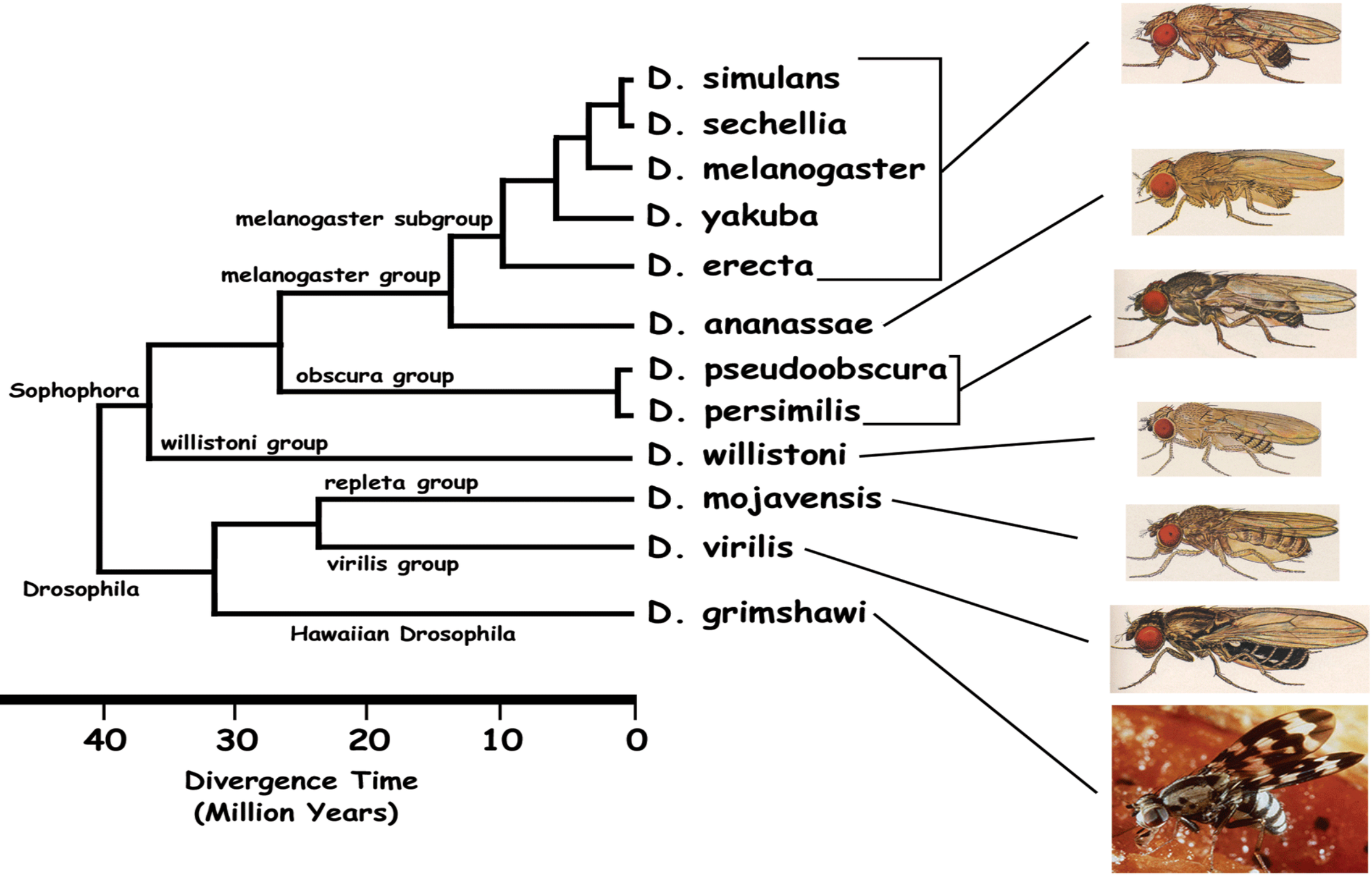
Introduction to Variant Detection

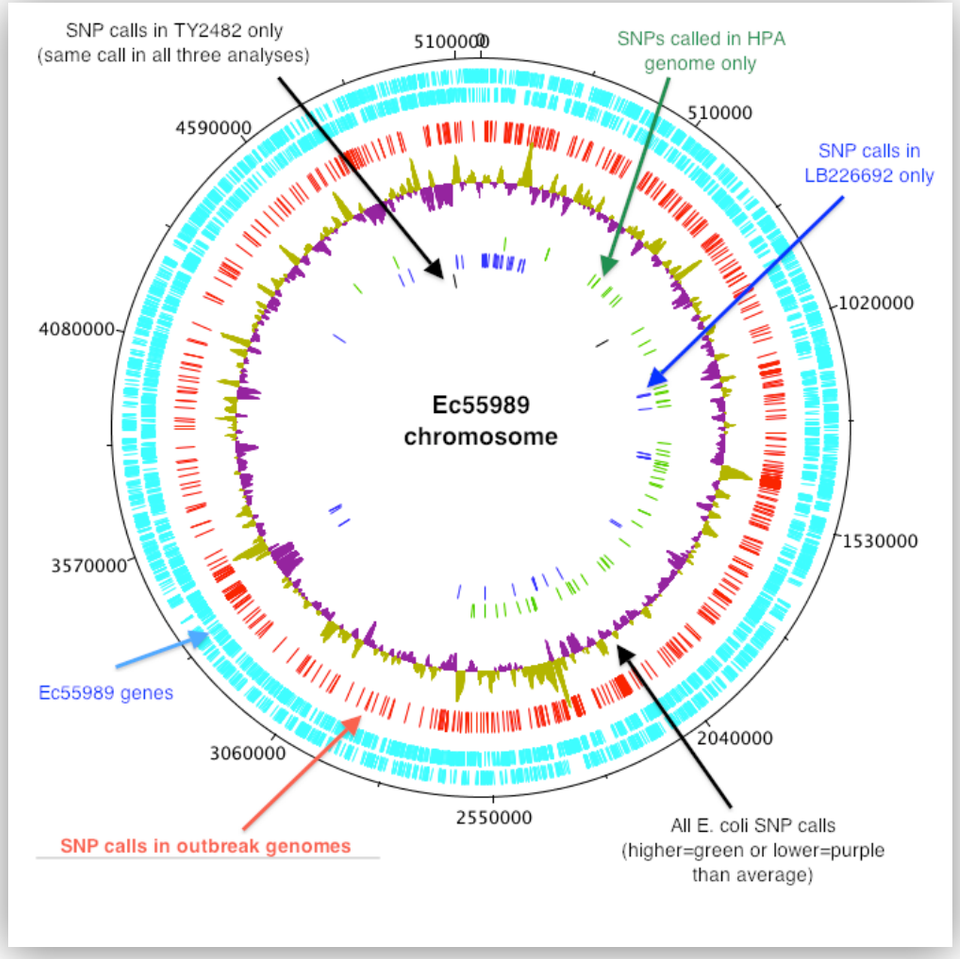


Johnson et al., Blood, 2013, 122(19)

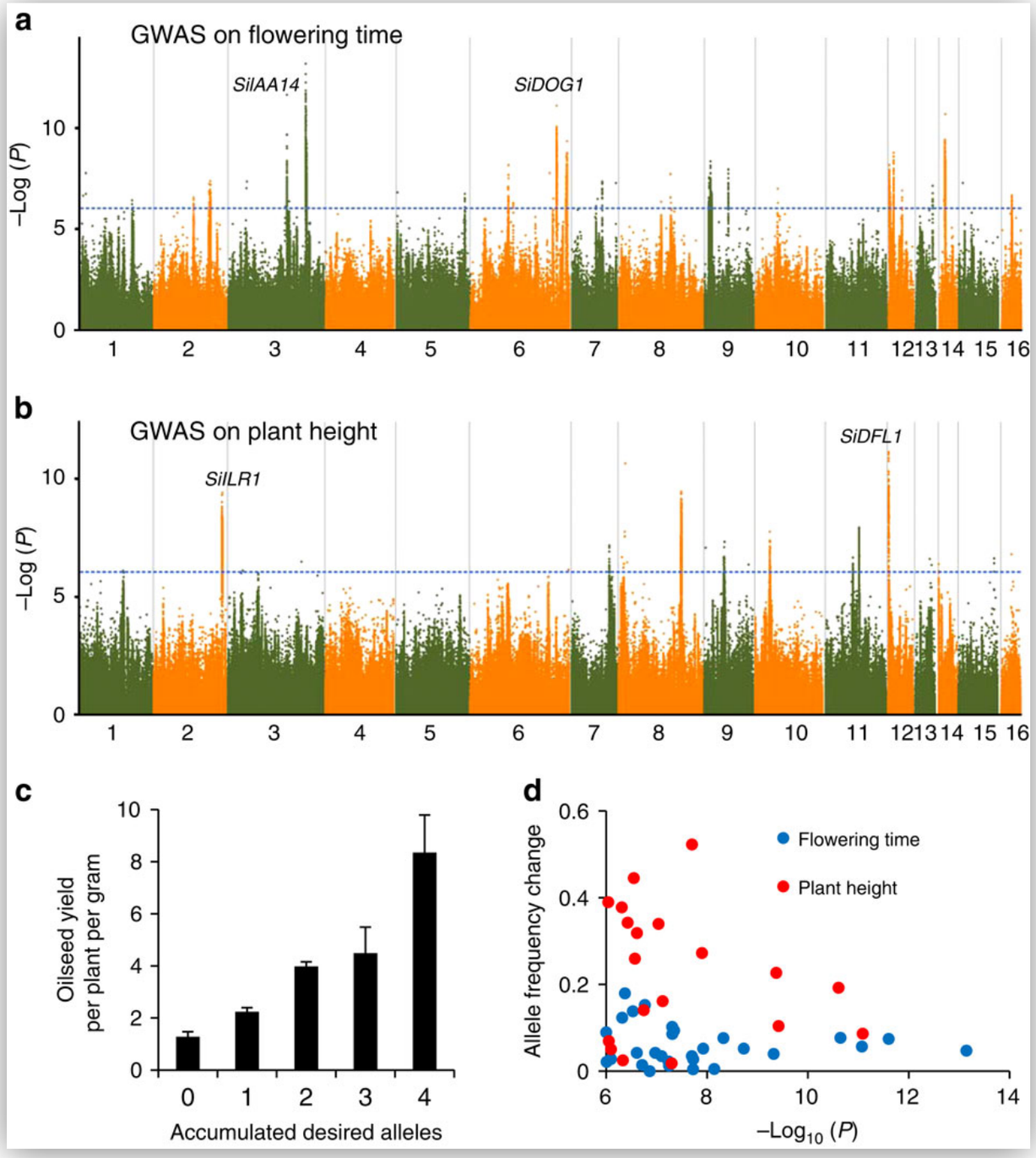


Evolutionary analysis

http://insects.eugenes.org/DroSpeGe/

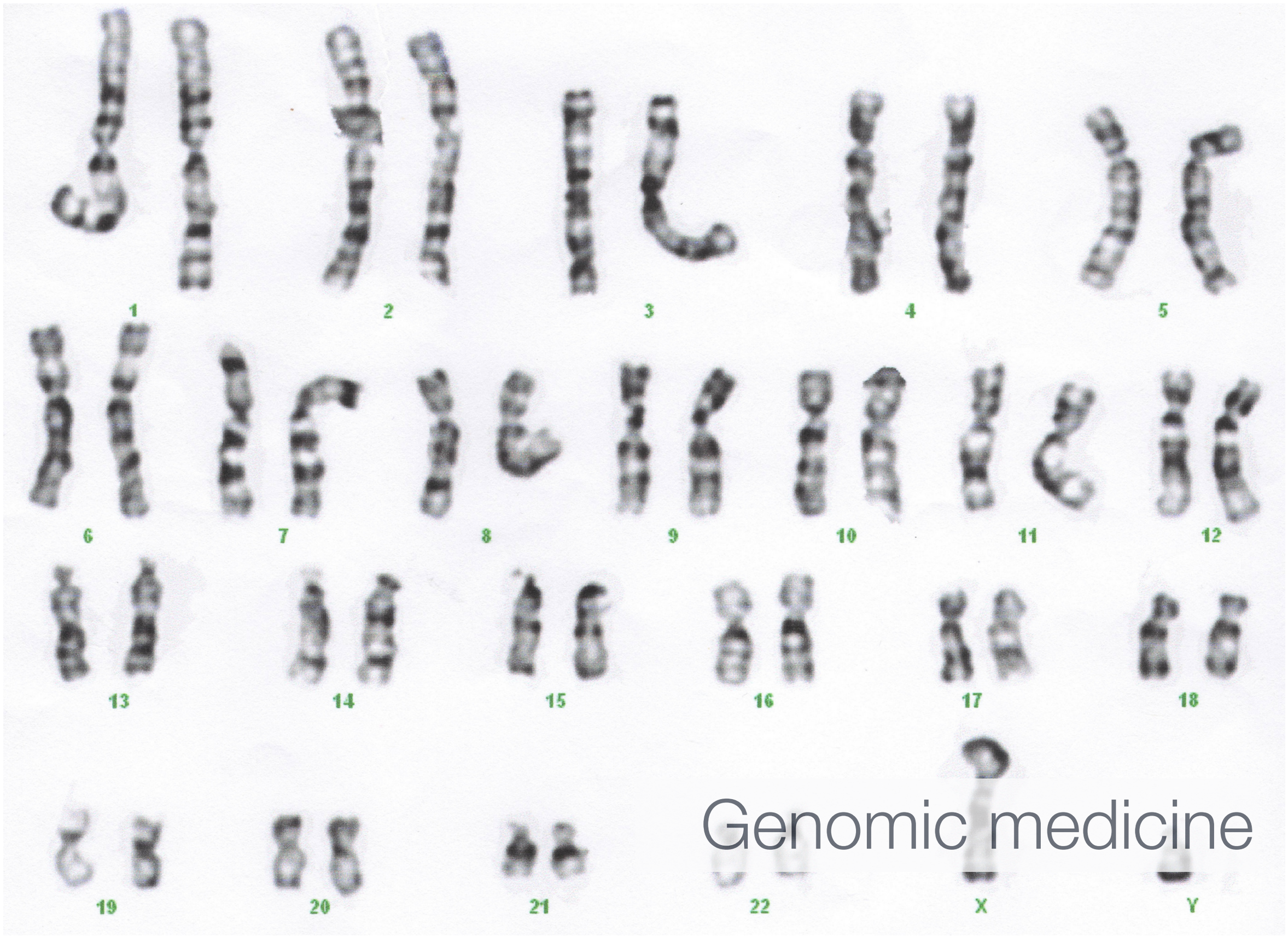


https://bacpathgenomics.wordpress.com/tag/snp/



http://www.nature.com/ncomms/2015/151019/ncomms9609/fig_tab/ncomms9609_F6.html

Medicine and Agriculture



Genomic medicine

Overview

- Human variations
  - Germline
  - Somatic
- Types of Variations
- Sequencing strategies to identify variants
- Generalized analysis workflow (GATK best practice guidelines)

Any heritable “mutation” is considered a germline variant.

- found in populations, discovered by large-scale population analyses, and contained in databases like dbSNP, HapMap
- most are not deleterious

Germline vs Somatic mutations

Nature 491, 56–65 (01 November 2012)

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- most are not deleterious

A somatic variant is any mutation that arises in a single cell of an individual and is only present in the descendants of that cell, not all the cells of that individual.

- found in rapidly growing cancer cells
- can be silent or pathogenic

Germline vs Somatic mutations

Nature 491, 56–65 (01 November 2012)

Most human genomic variants have no phenotypic impacts

- Ones that have an impact are either positively selected, i.e. they confer a reproductive advantage
- Or they are neutral. These are often associated with ethnic origin, typically affecting traits like height, facial features, hair or skin color

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Some genomic variants have deleterious effects

- Most of these are recessive: their effect is observed only if both alleles are affected
- Those that are dominant will either be selected against and disappear, or have effects that minimally impact reproductive fitness

Types of variations

Single Nucleotide Polymorphisms (SNPs)

Small Insertions/Deletions (Indels)

Copy Number Variations (CNVs)

Structural Variations (SVs)

For SNPs, many different methods have been used:

- Hybridization based, primarily SNP arrays
- Enzyme-based methods, primarily oligonucleotide ligation and RFLP
- Methods measuring physical properties of DNA

How to assess genomic diversity?

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NGS can detect all types of variants

(Paired-end data preferred!)

How to assess genomic diversity?

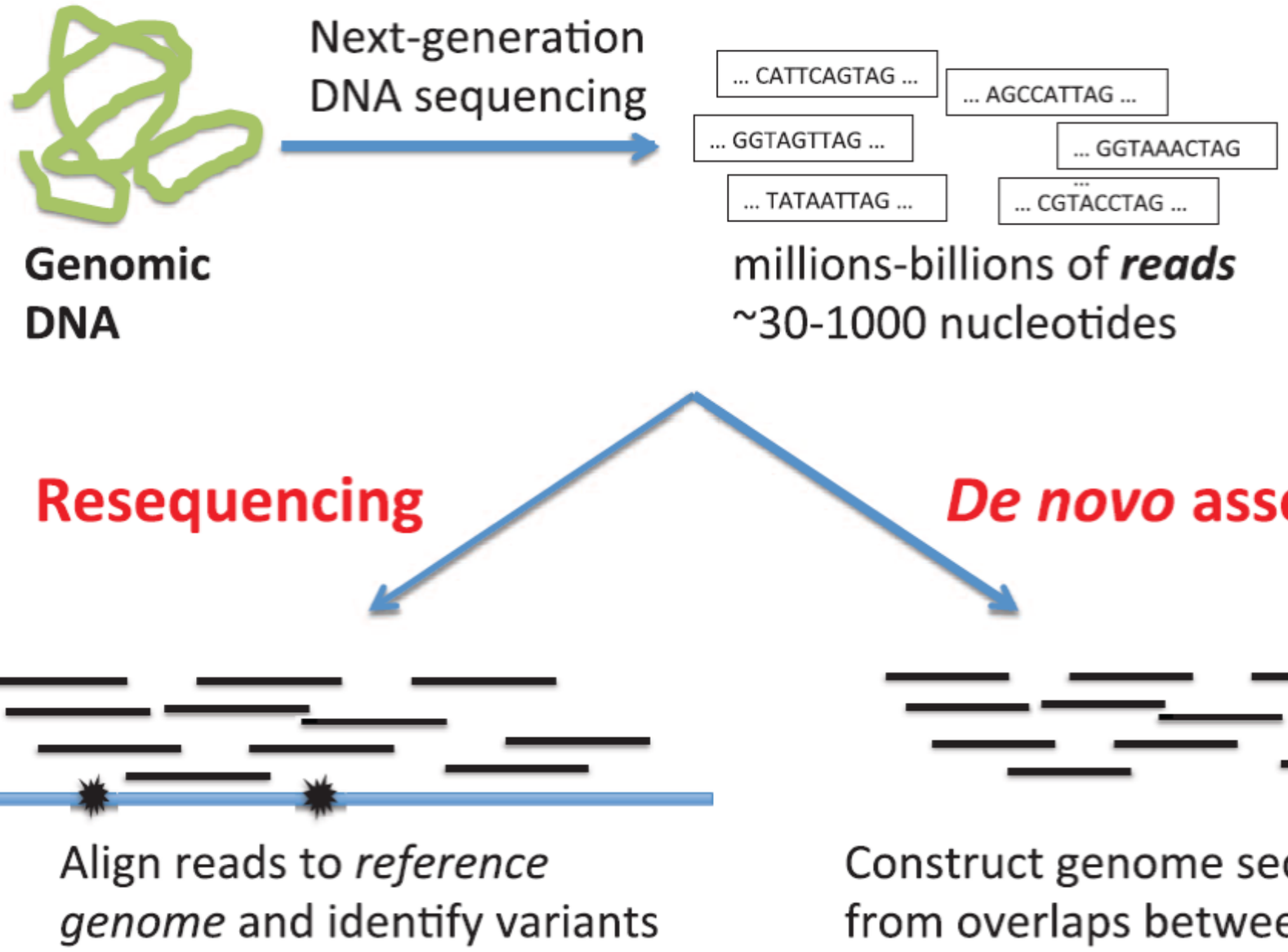
Sequencing strategies

Whole Genome Sequencing (WGS)

(for SNPs/Indels, CNVs and SVs)

Exome Sequencing (for SNPs/Indels)

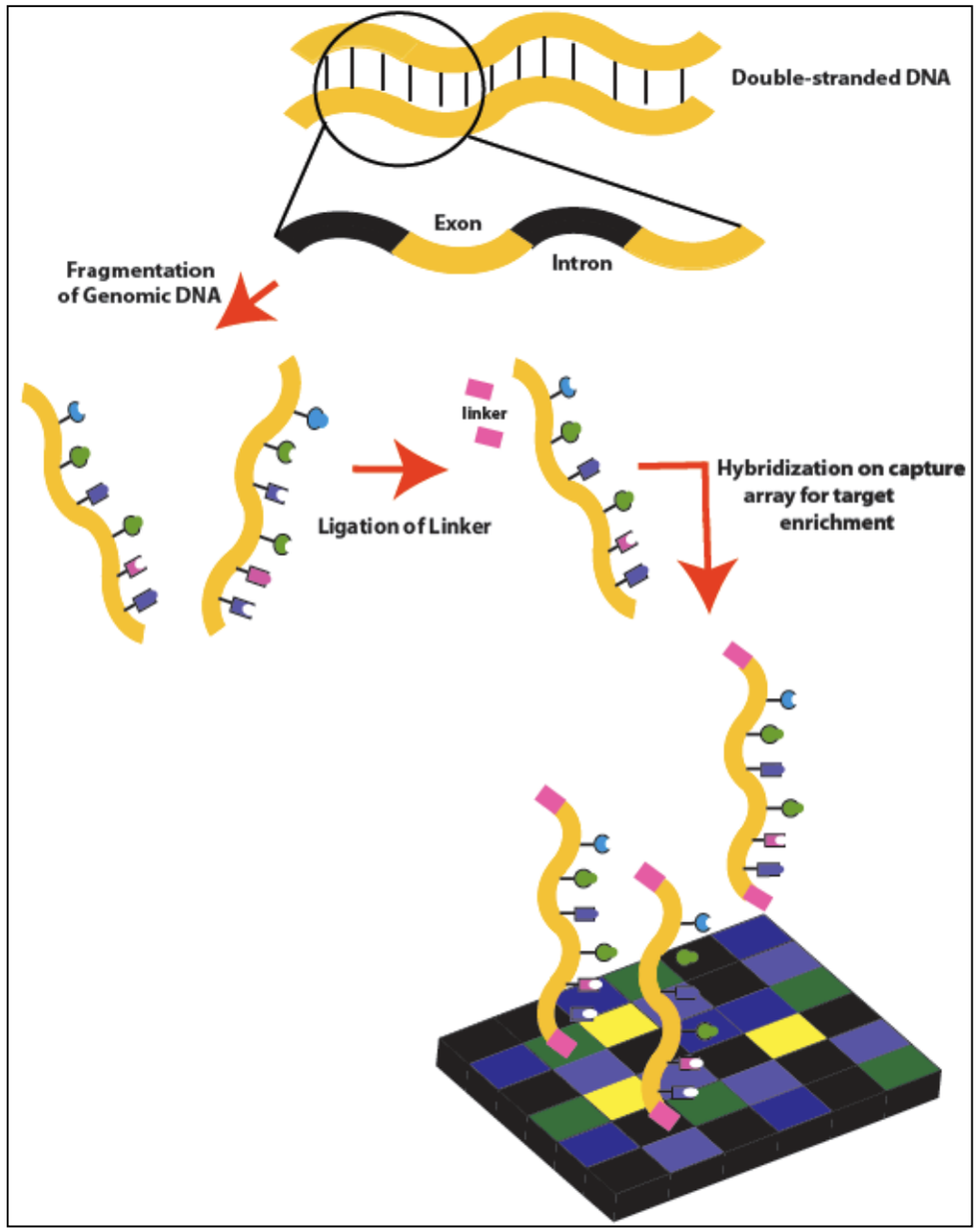
Gene Panels (for SNPs/Indels)



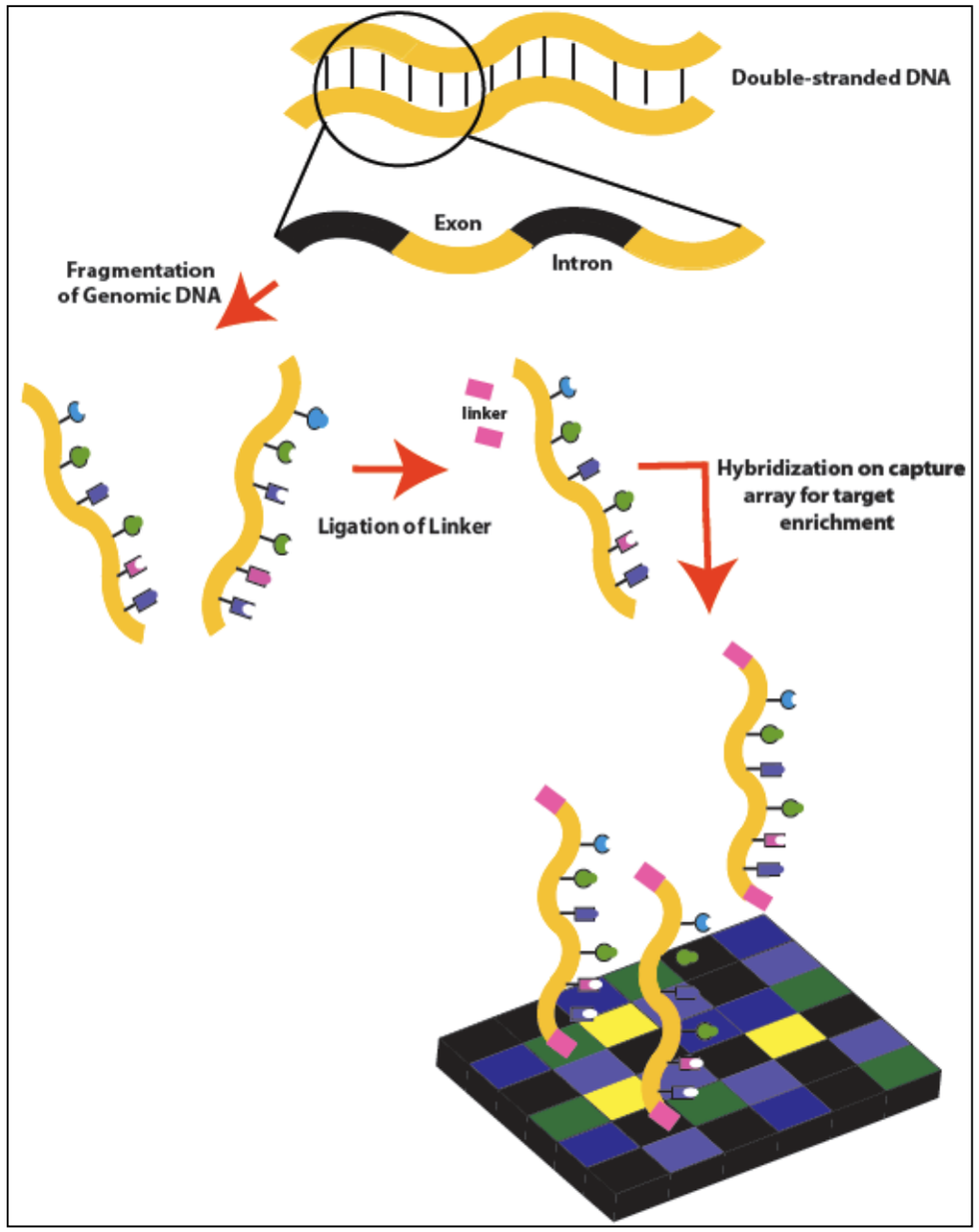
Benjamin J. Raphael*

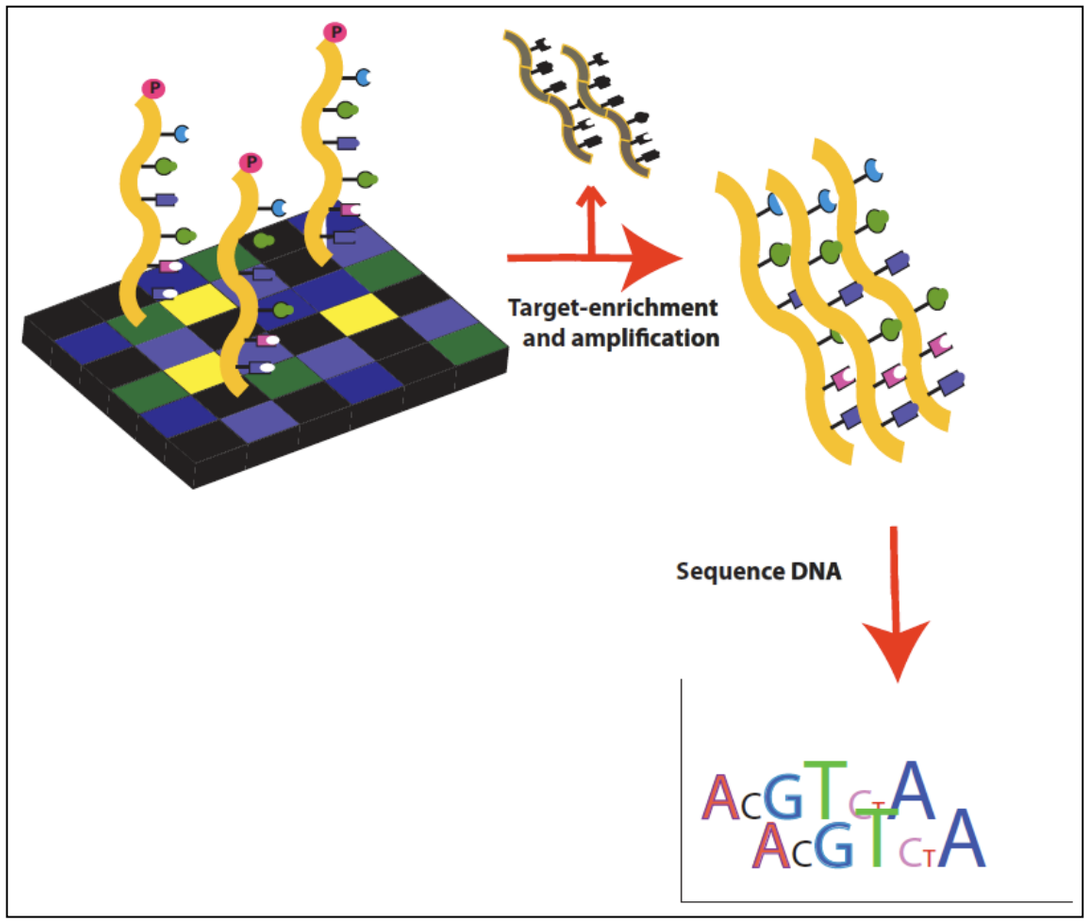
Department of Computer Science and Center for Computational Molecular Biology, Brown University, Providence, Rhode Island, United States of America

Whole genome sequencing

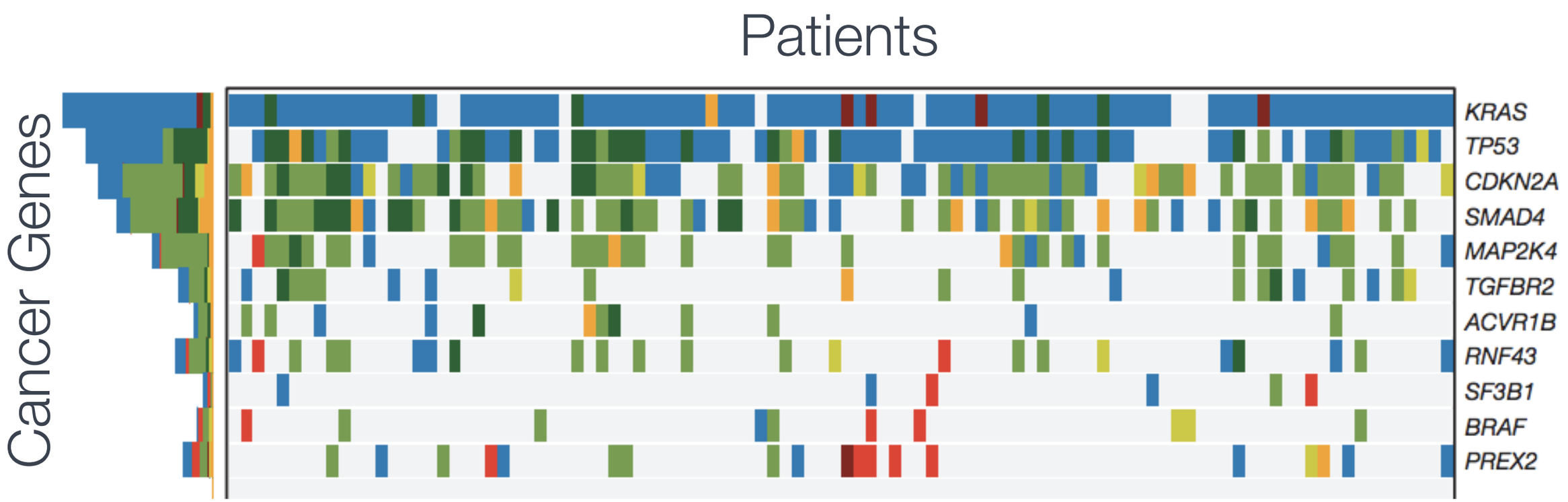


Exome sequencing





Exome sequencing

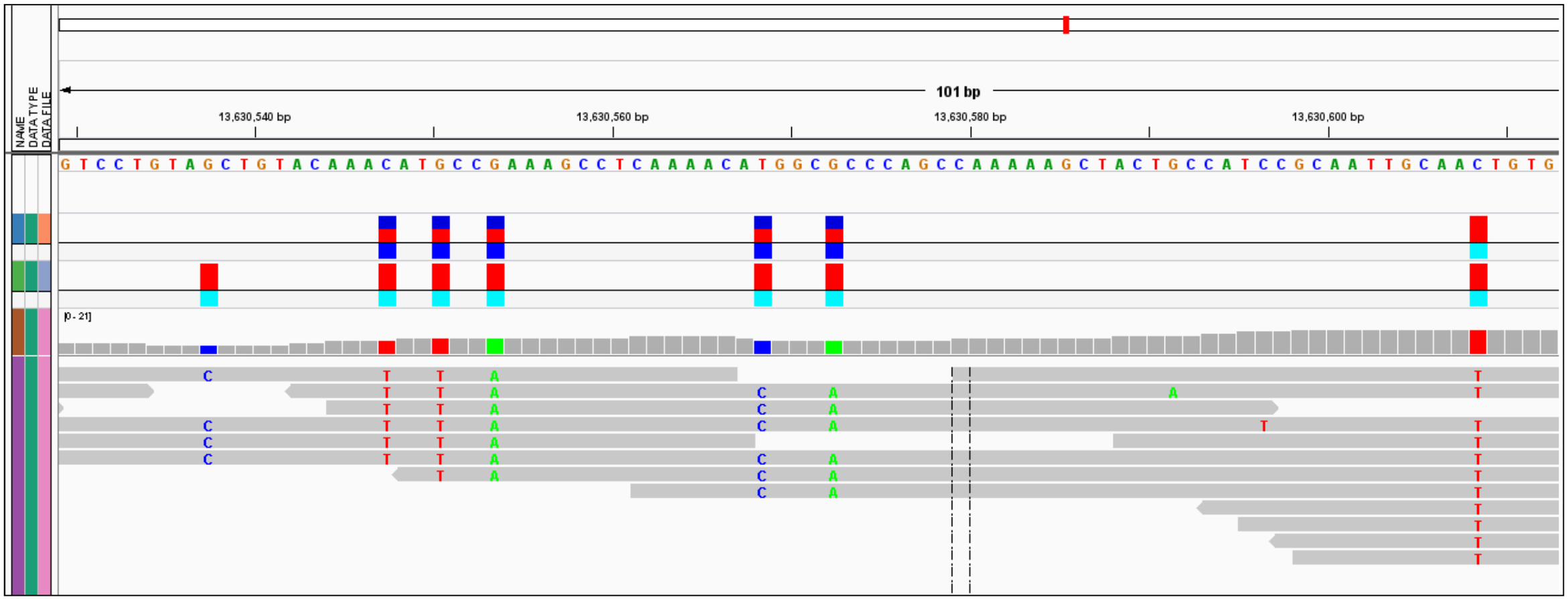


A visualization of an analysis using a panel of known cancer genes

Gene panel sequencing for diagnostics

- Targeted gene panels are most commonly used for diagnostics/clinical work
- Coverage: cost considerations for various methods, based on number of samples
- Variants in un-targeted or non-exonic regions will be missed

Gene panels or ES or WGS:
Which one is “better”?



Sequencing depth and cost

For WGS

- Haploid genome size => 3.2 Giga base pairs (3.2 billion)

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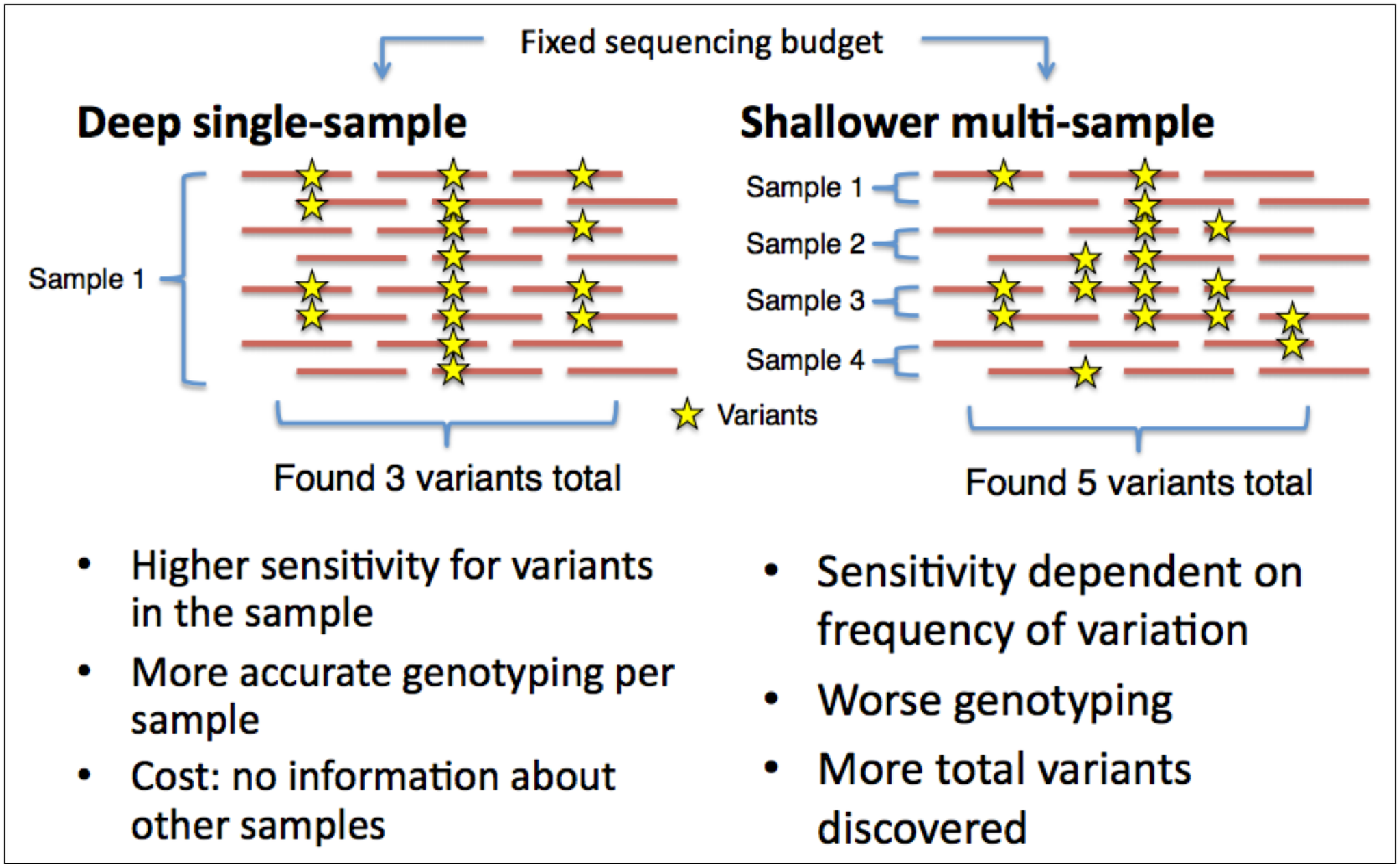
For Gene Panels

- 10x-20x coverage for gene panels for heterozygous germline variants

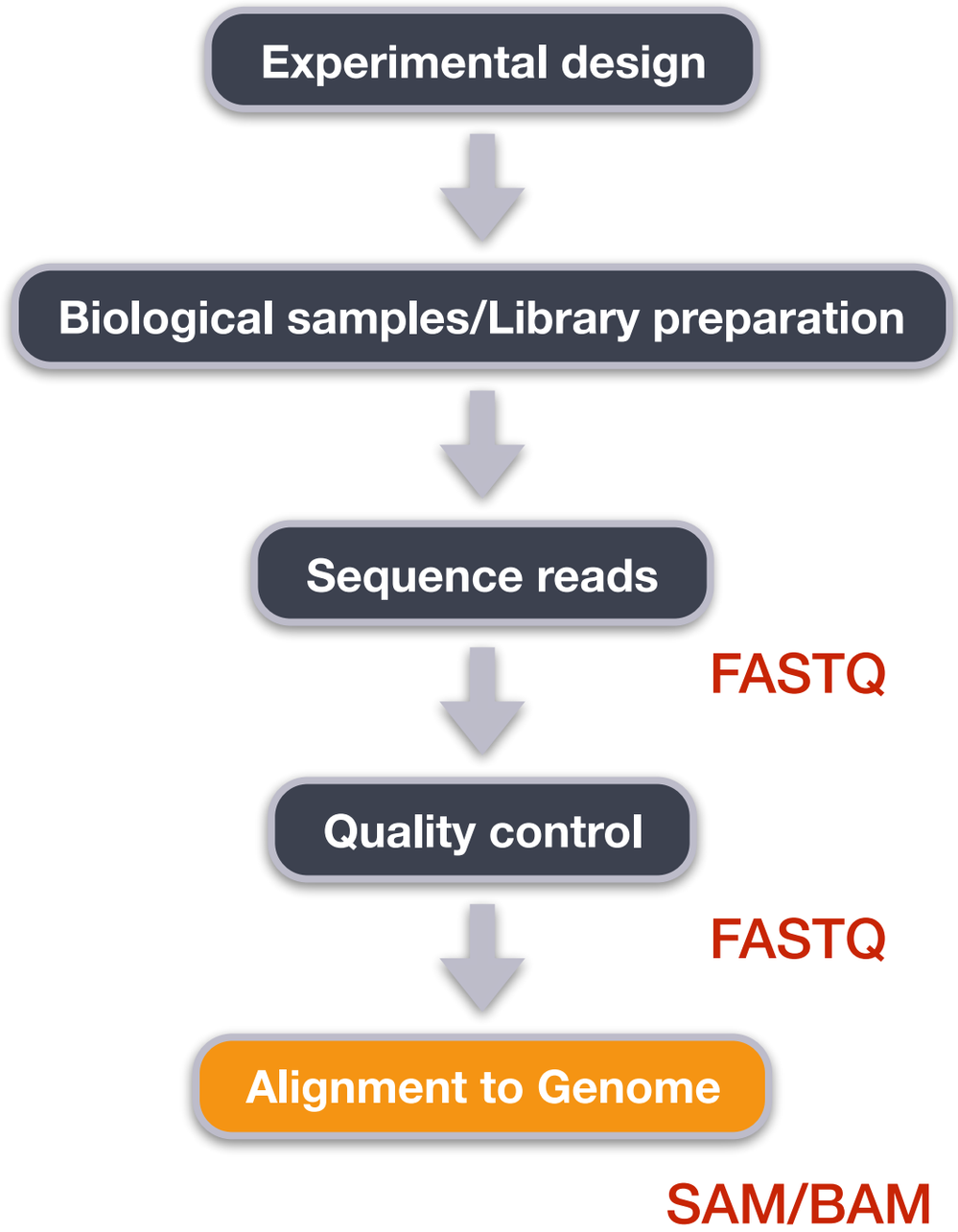
Sequencing depth?

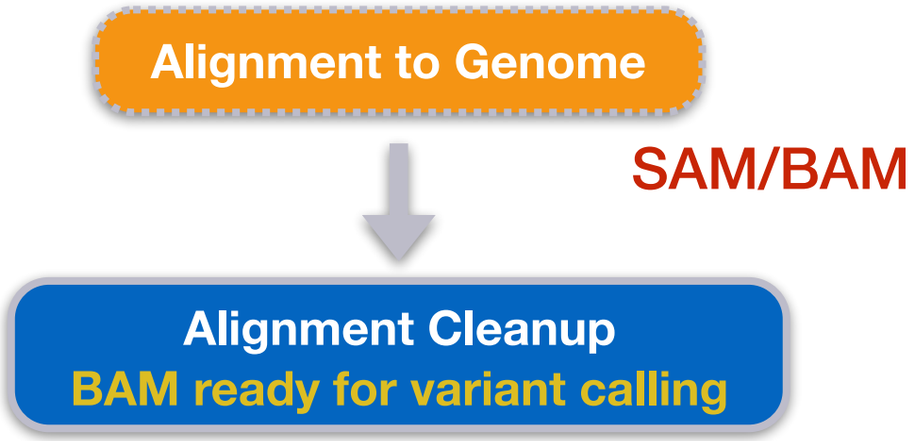


Sequencing depth and cost

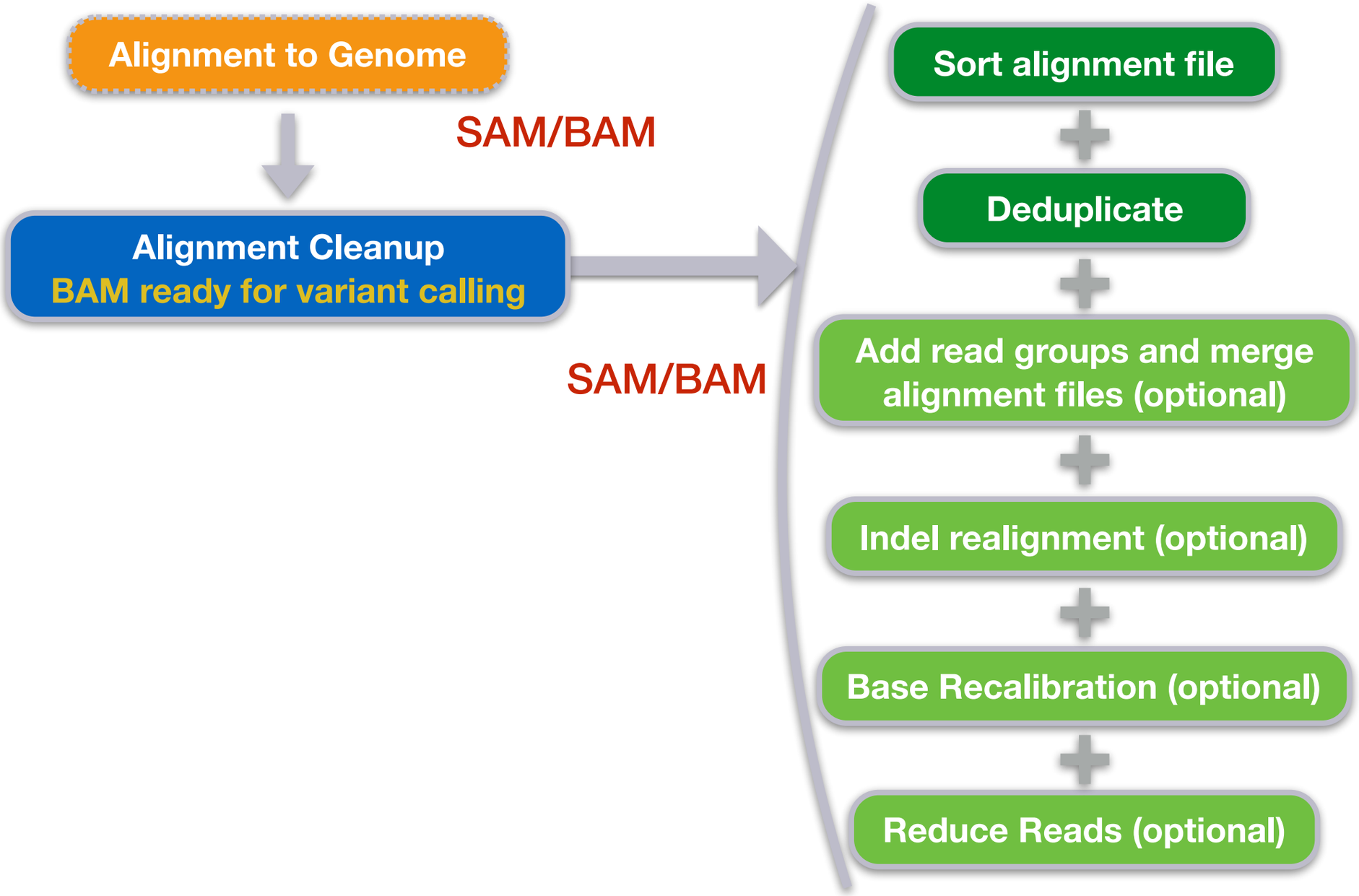
Adapted from GATK best practices guidelines (2012)

Generalized Variant Calling Workflow

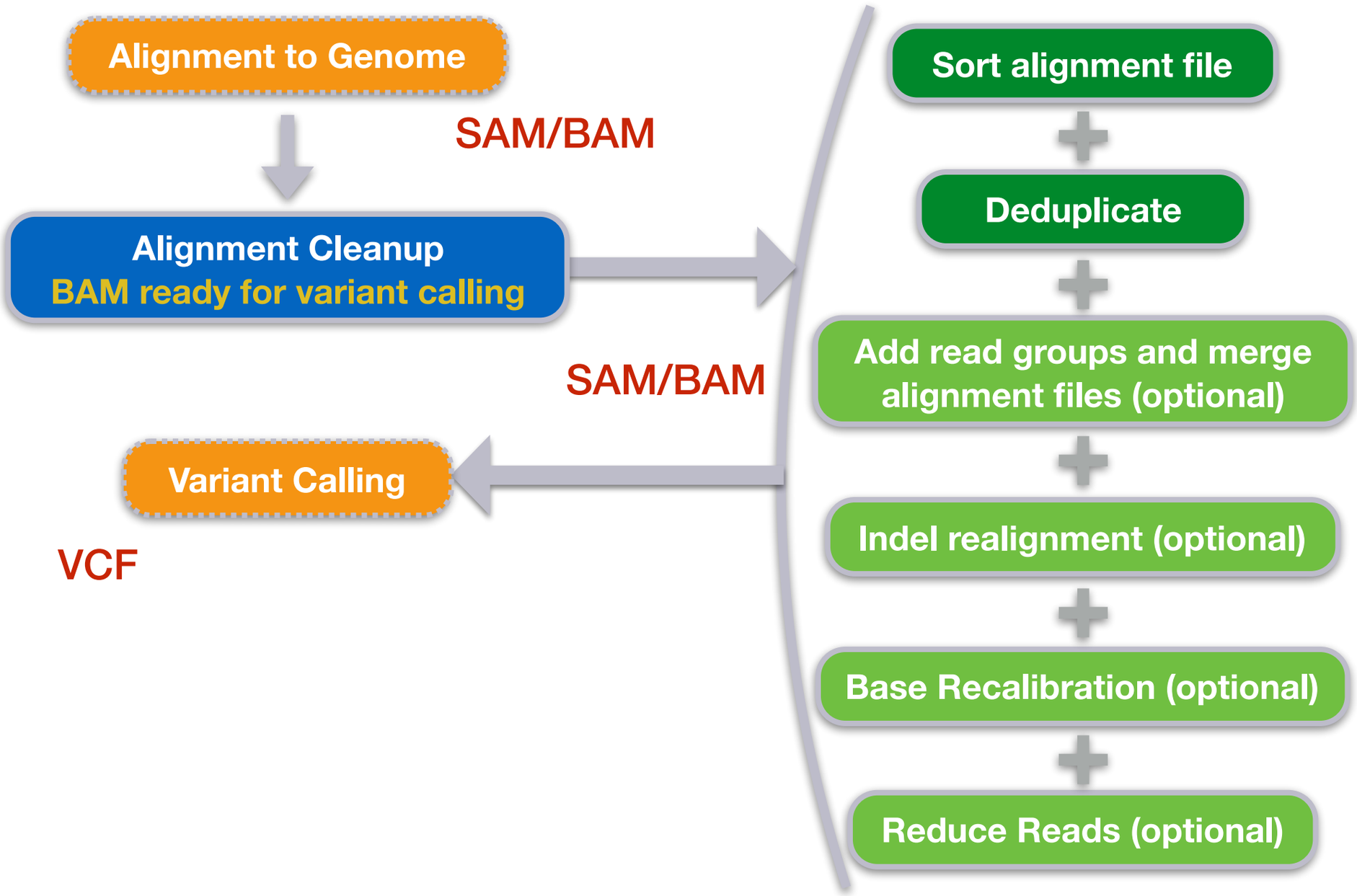




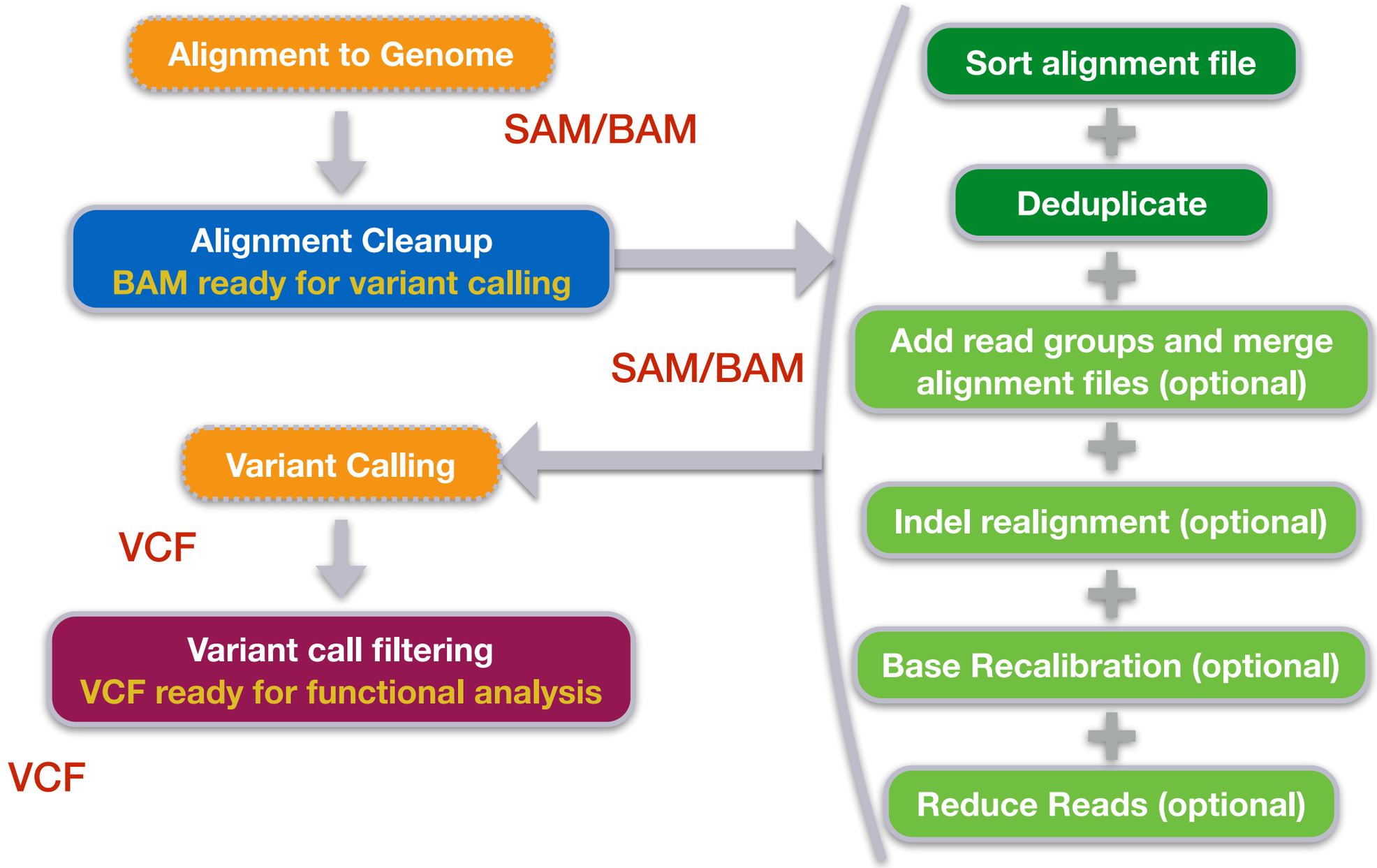
Generalized Variant Calling Workflow



Generalized Variant Calling Workflow



Generalized Variant Calling Workflow



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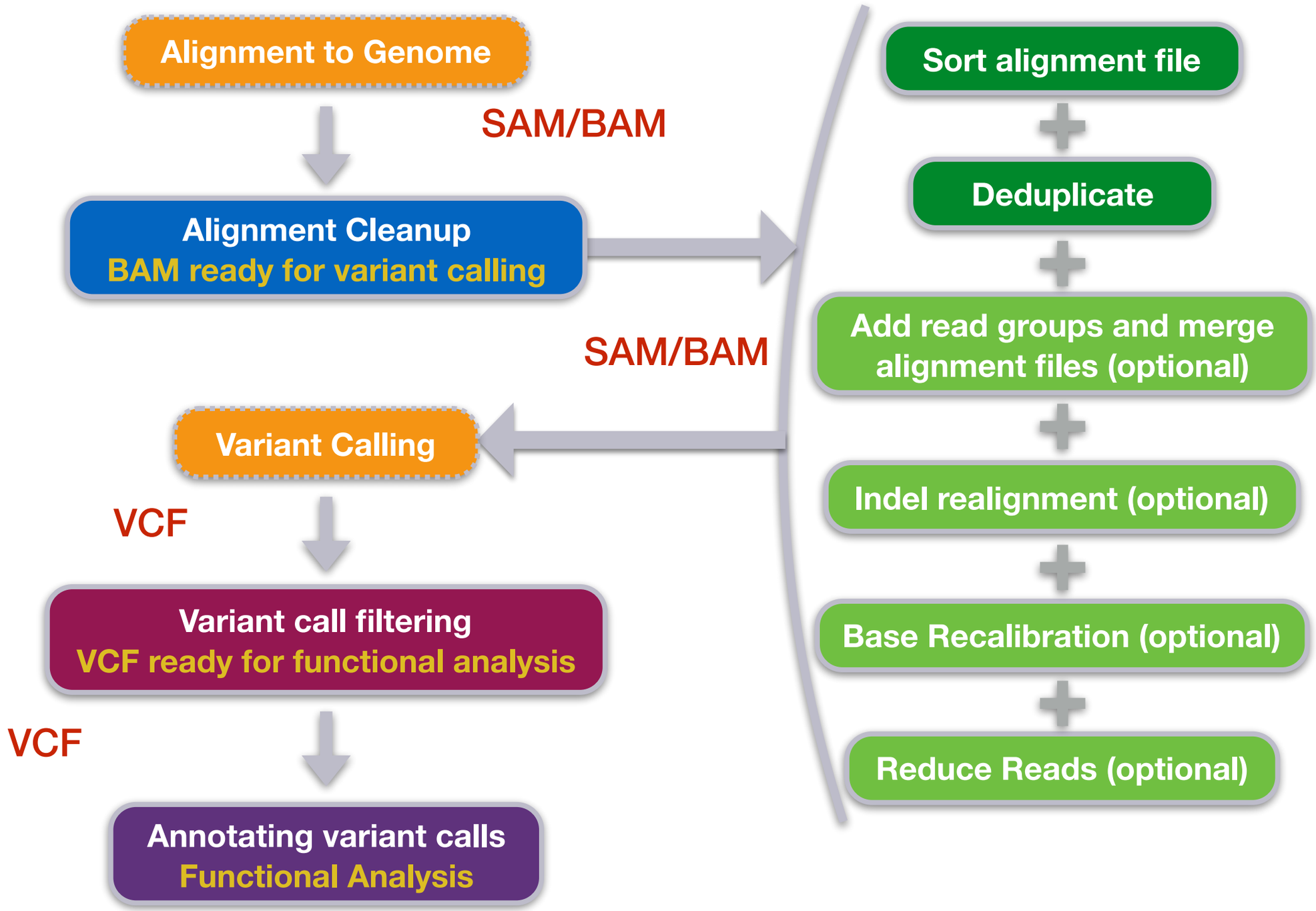

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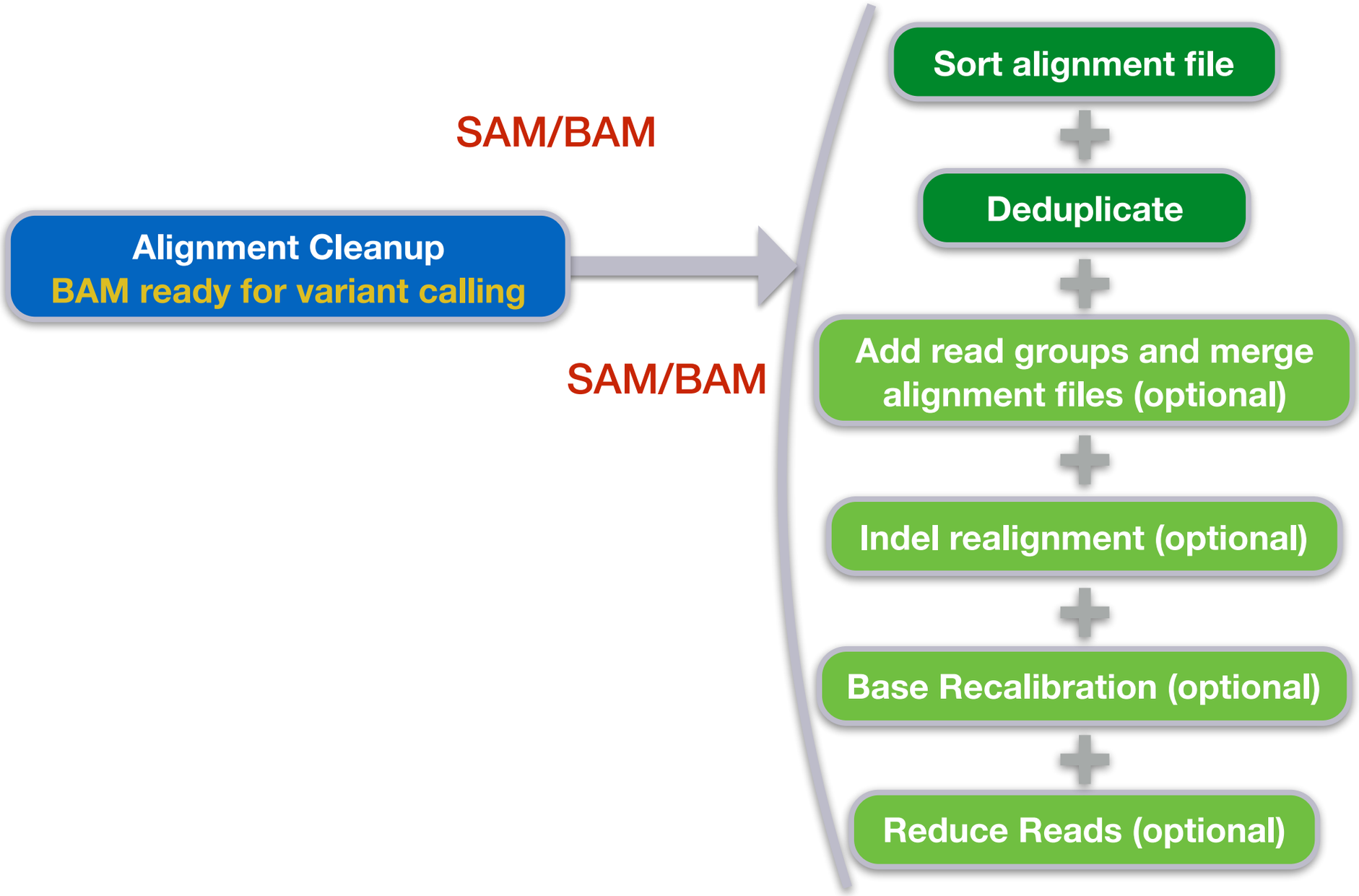
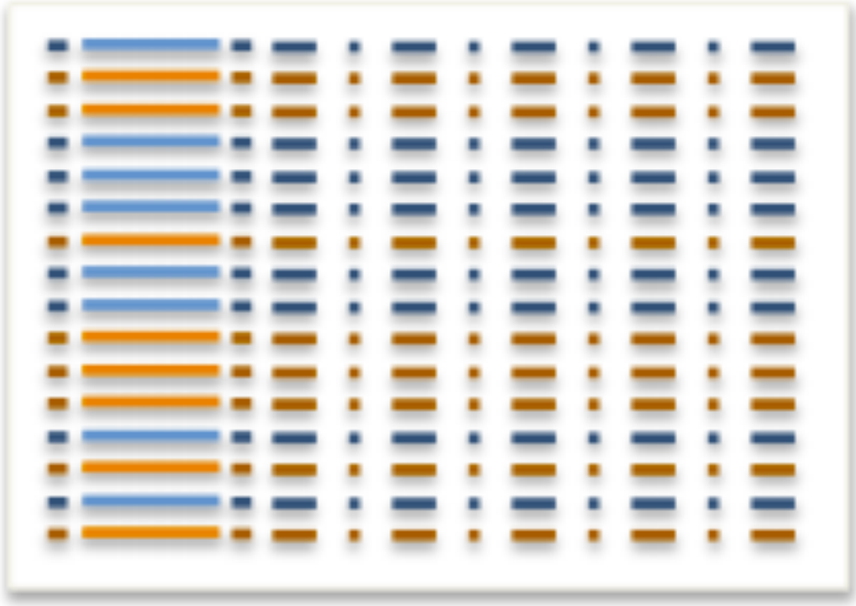
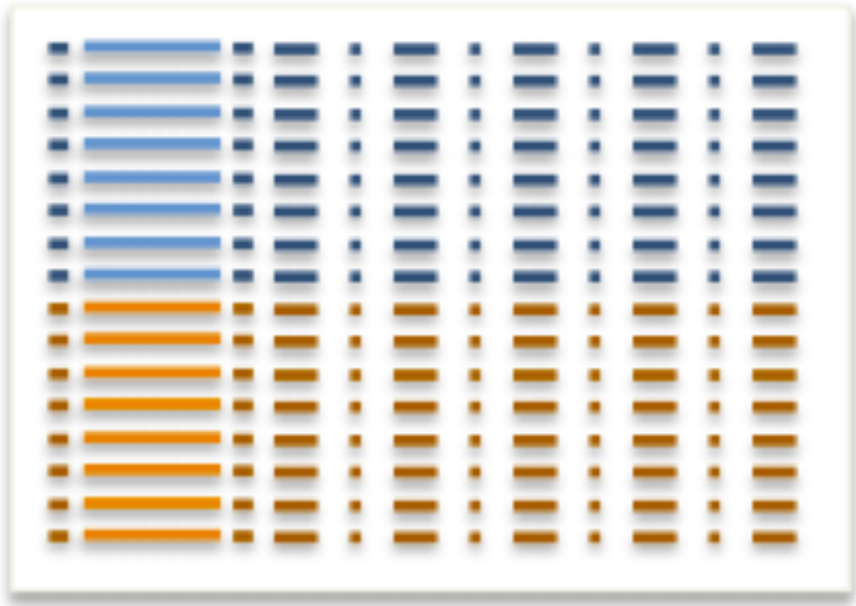


Generalized Variant Calling Workflow

Sort alignment file



The reads are in no particular order…

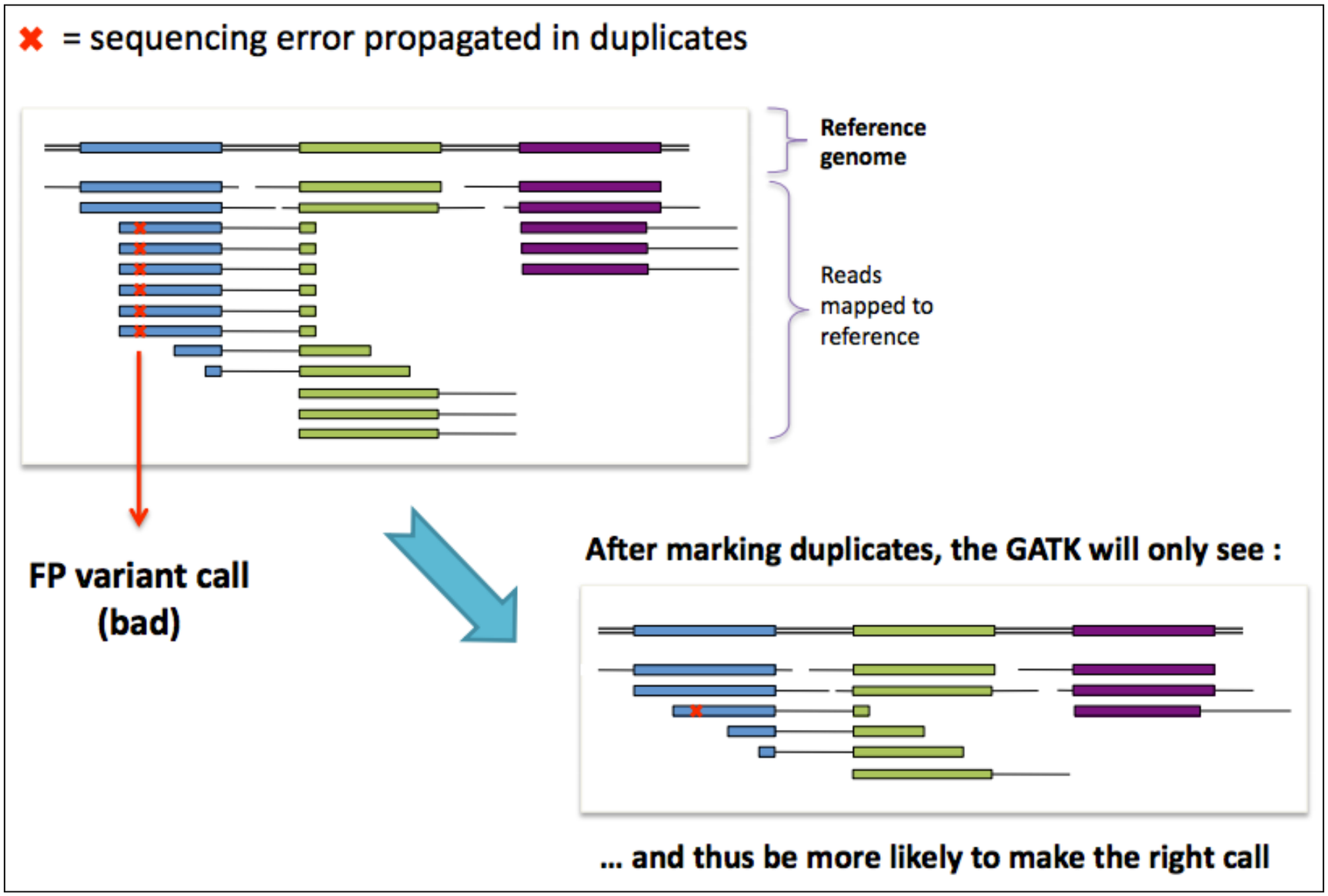


So we need to explicitly sort the SAM file…

Generalized Variant Calling Workflow

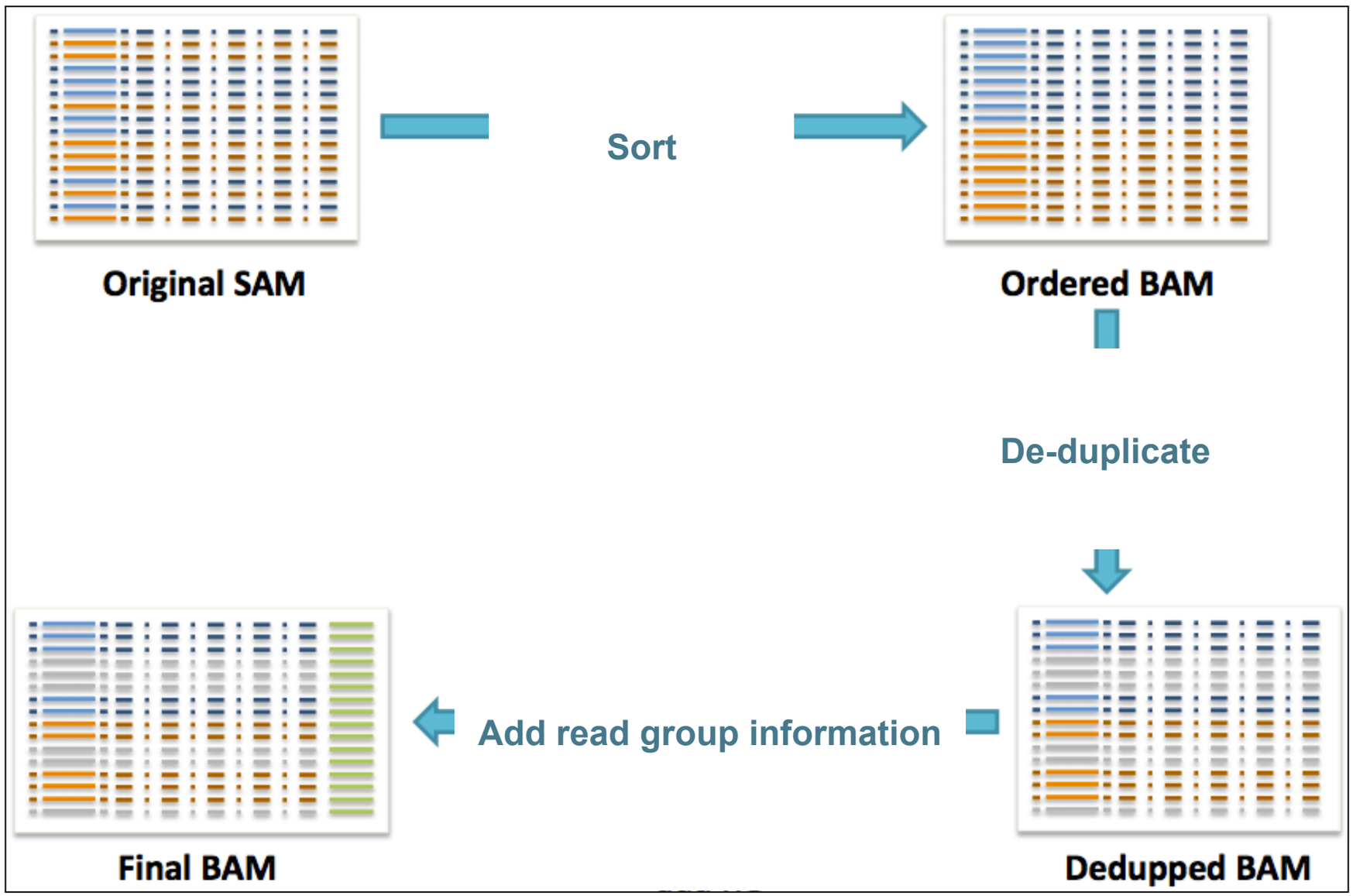
https://www.broadinstitute.org/gatk/guide/presentations?id=3391

Deduplicate



Generalized Variant Calling Workflow

https://www.broadinstitute.org/gatk/guide/presentations?id=3391



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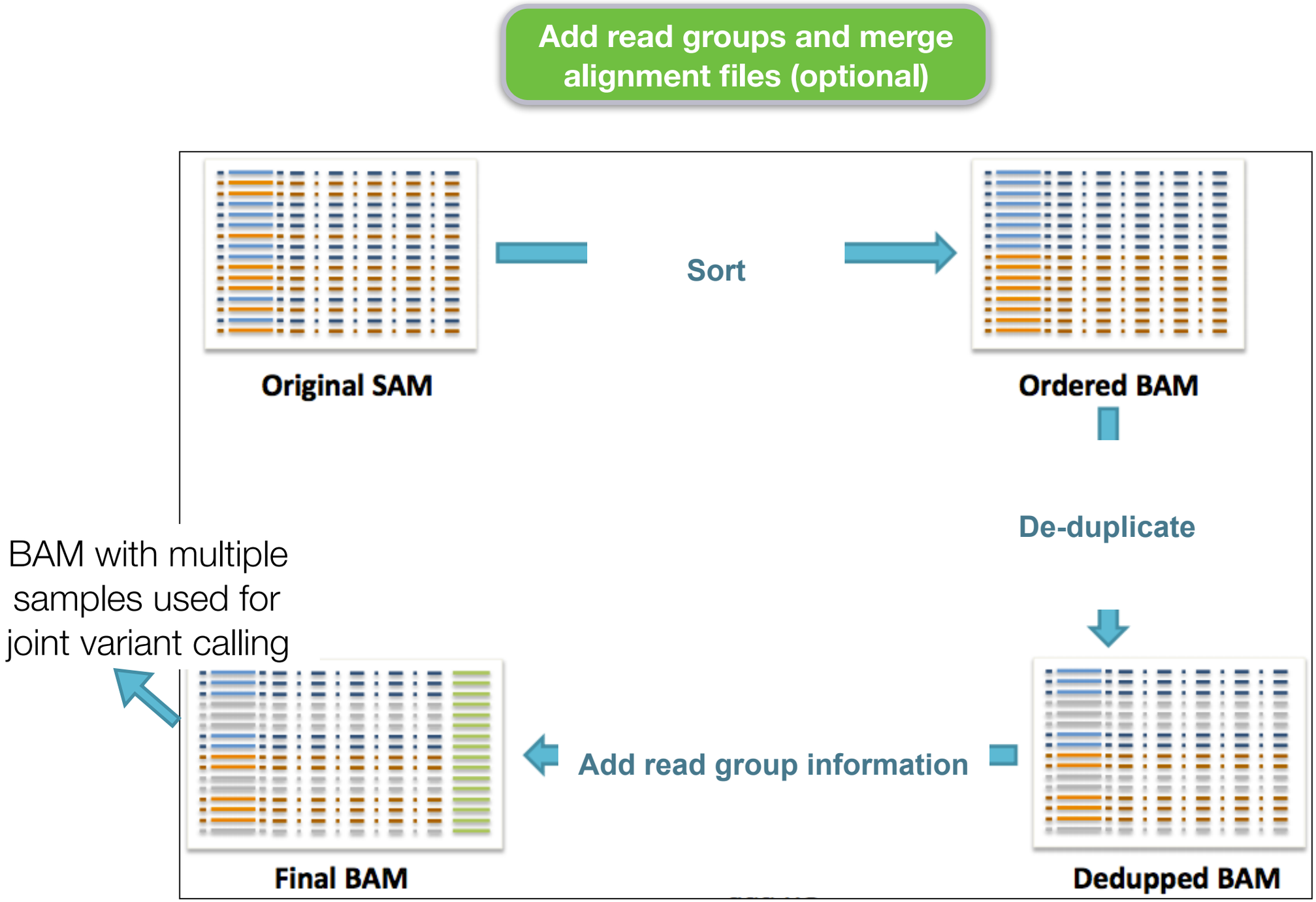
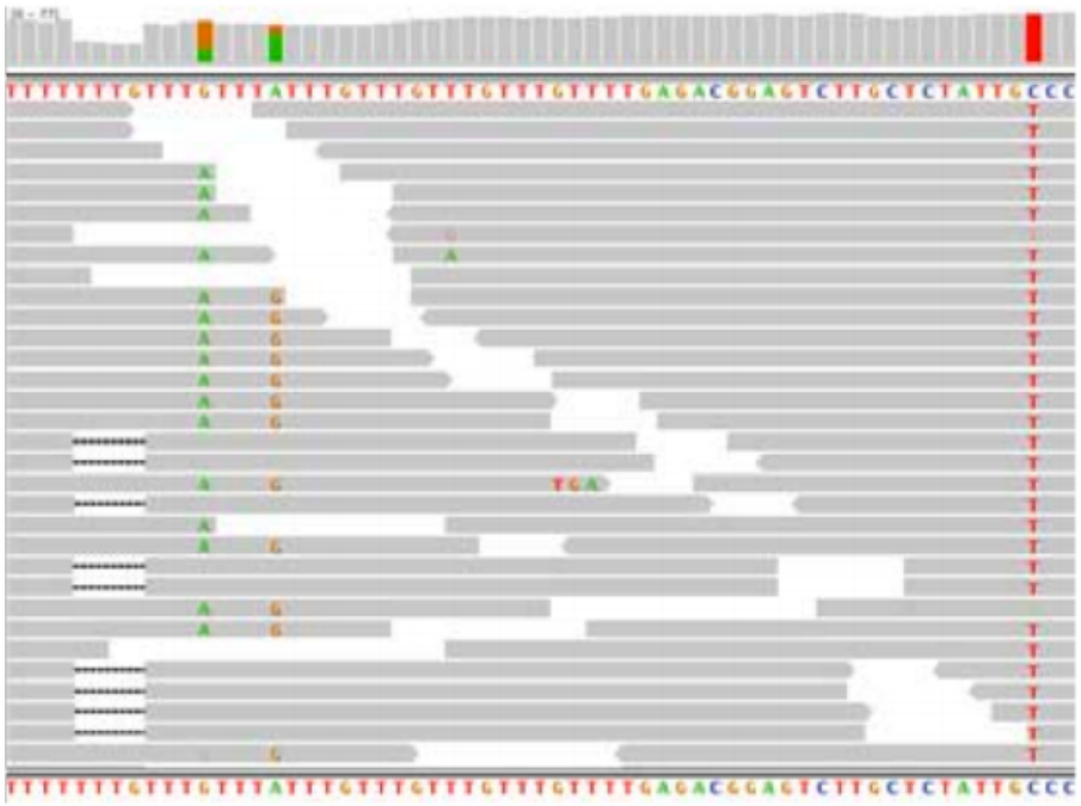


Generalized Variant Calling Workflow

https://www.broadinstitute.org/gatk/guide/presentations?id=3391

Indel realignment (optional)



Generalized Variant Calling Workflow

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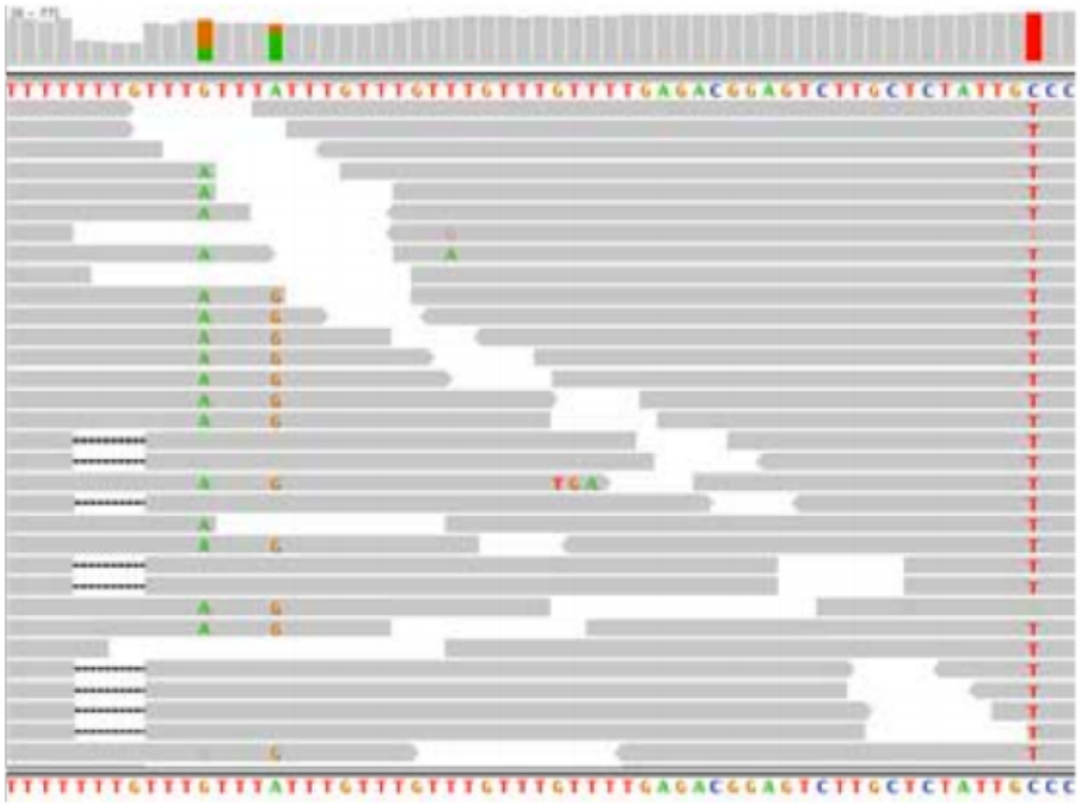
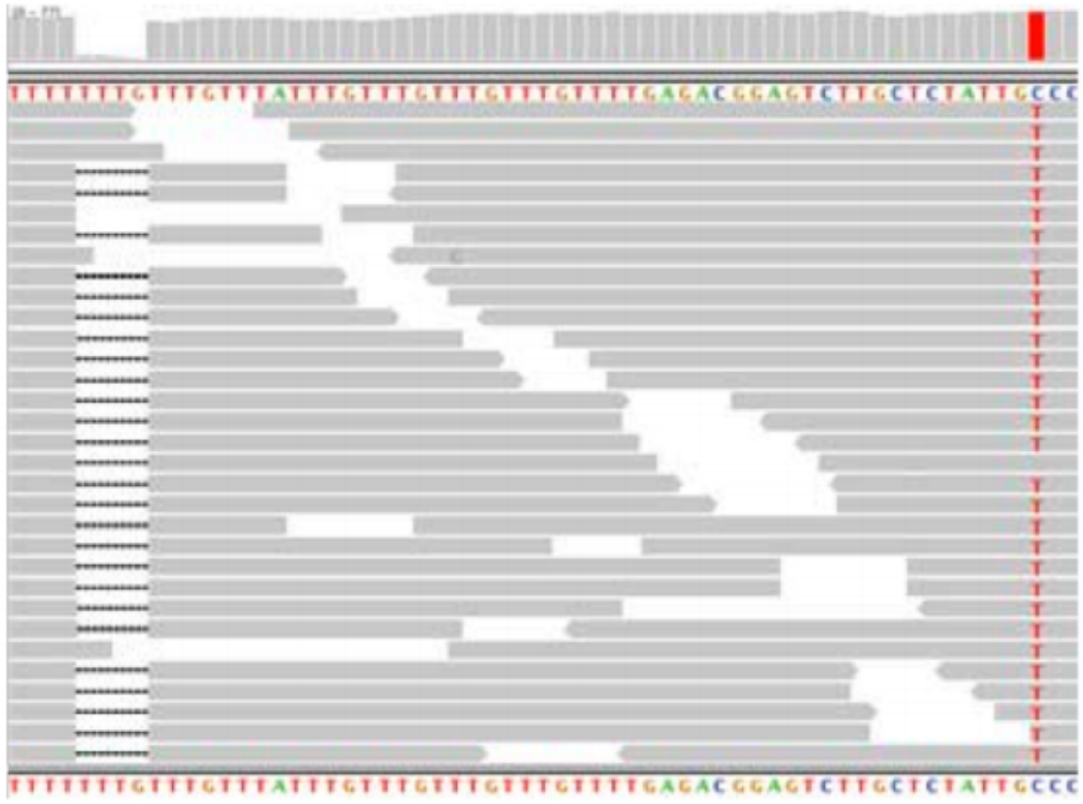
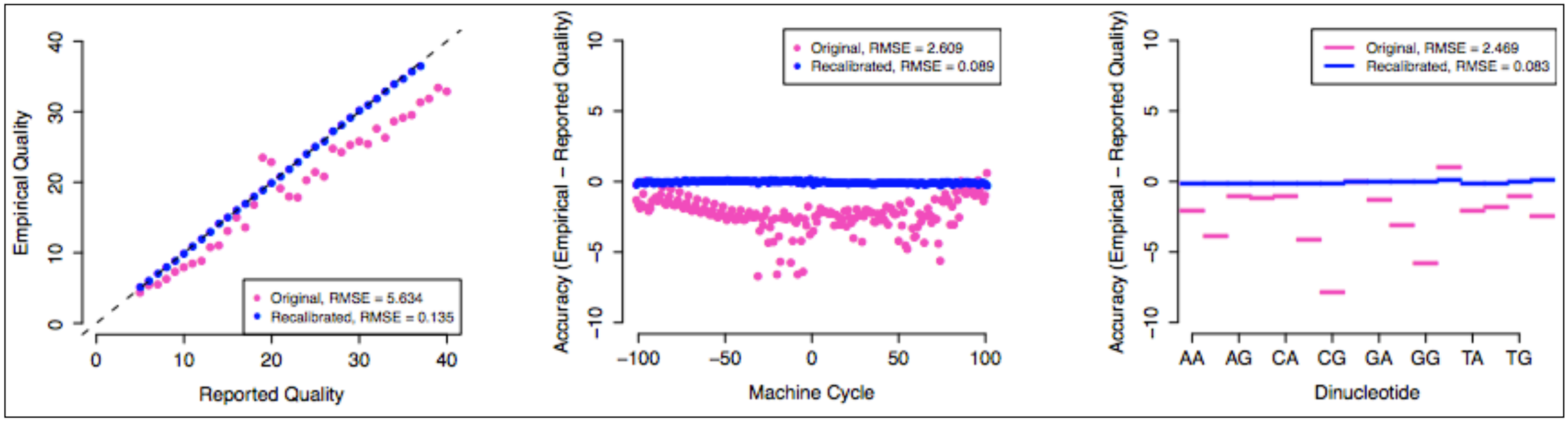




Generalized Variant Calling Workflow

Base Recalibration (optional)

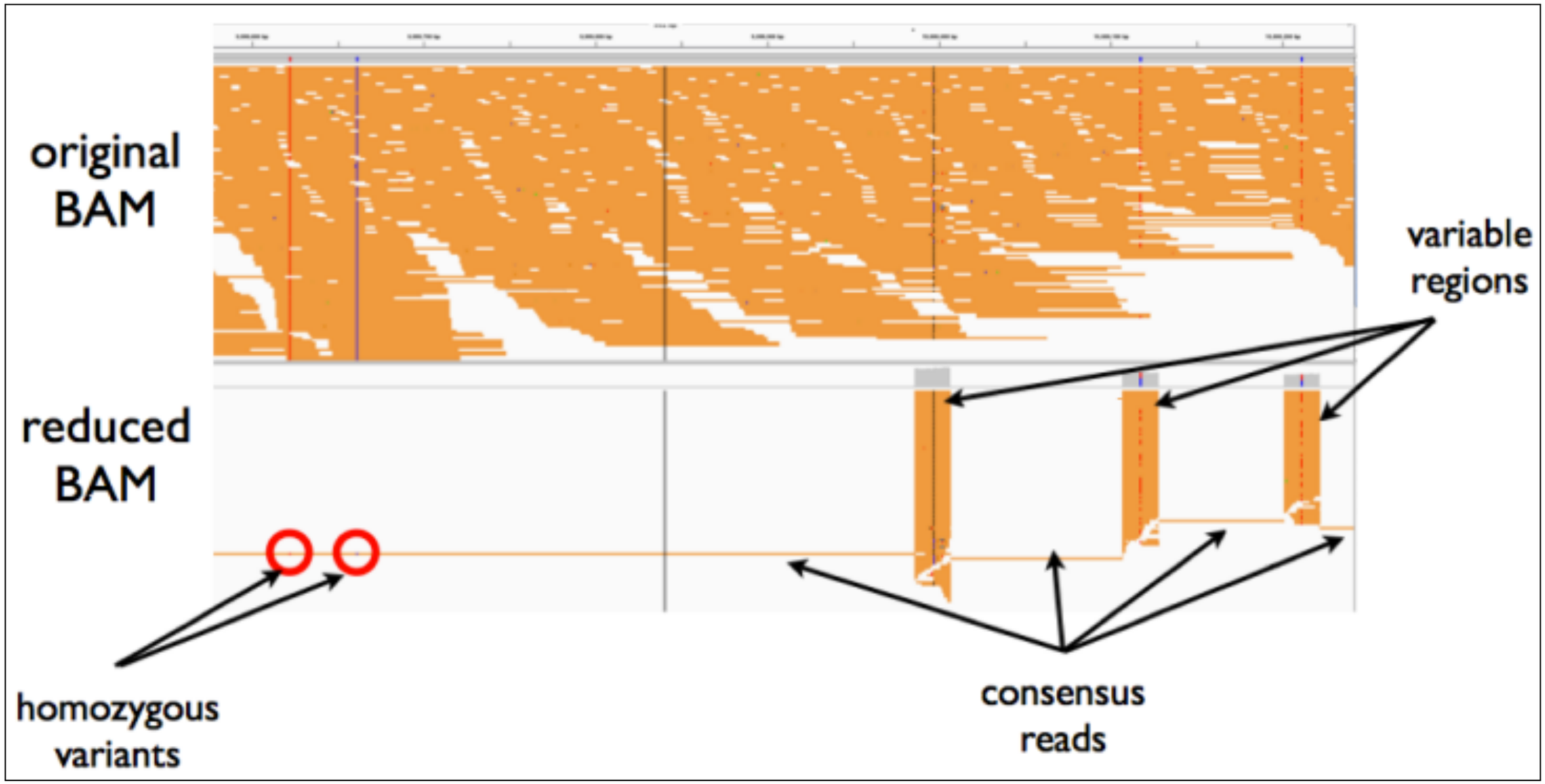
This step removes any systematic biases the creep in during sequencing



Generalized Variant Calling Workflow

https://www.broadinstitute.org/gatk/guide/presentations?id=3391

Reduce Reads (optional)



Generalized Variant Calling Workflow

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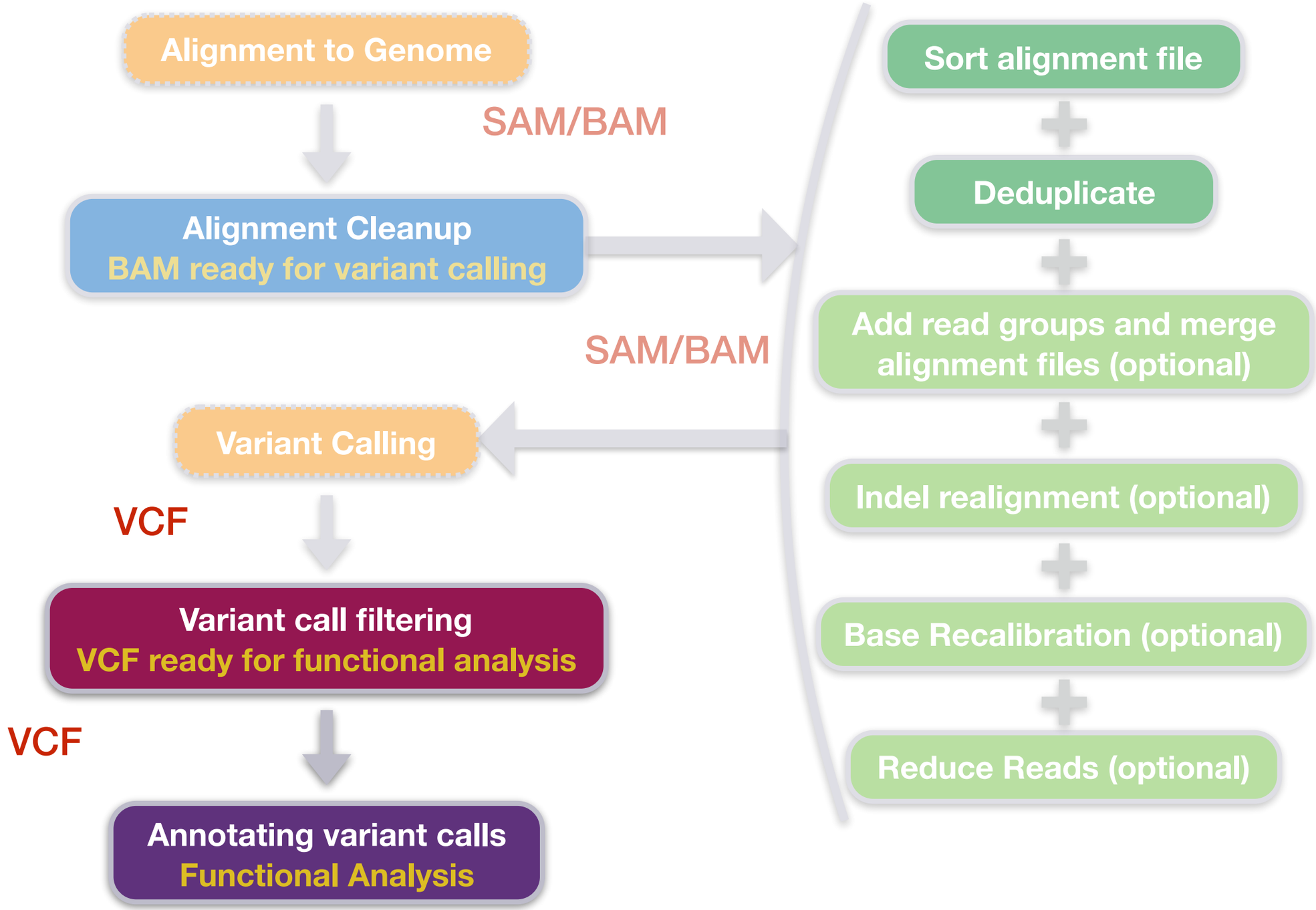
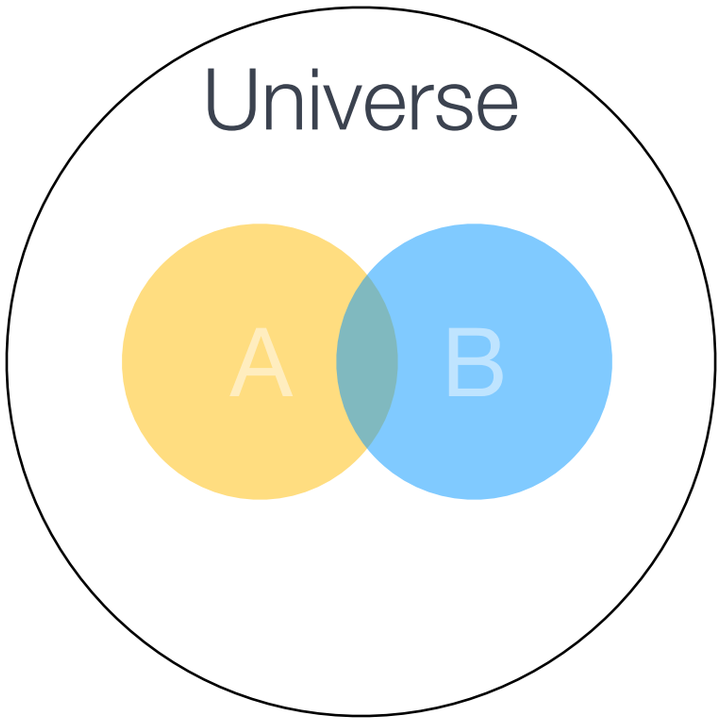


Generalized Variant Calling Workflow

Probability of real variant “A” given observed variants “B” in alignments



In theory

Variant detection

From Erik Garrison’s CSHL Advanced Sequencing Tutorial, http://clavius.bc.edu/~erik/CSHL-advanced-sequencing/freebayes-tutorial.html

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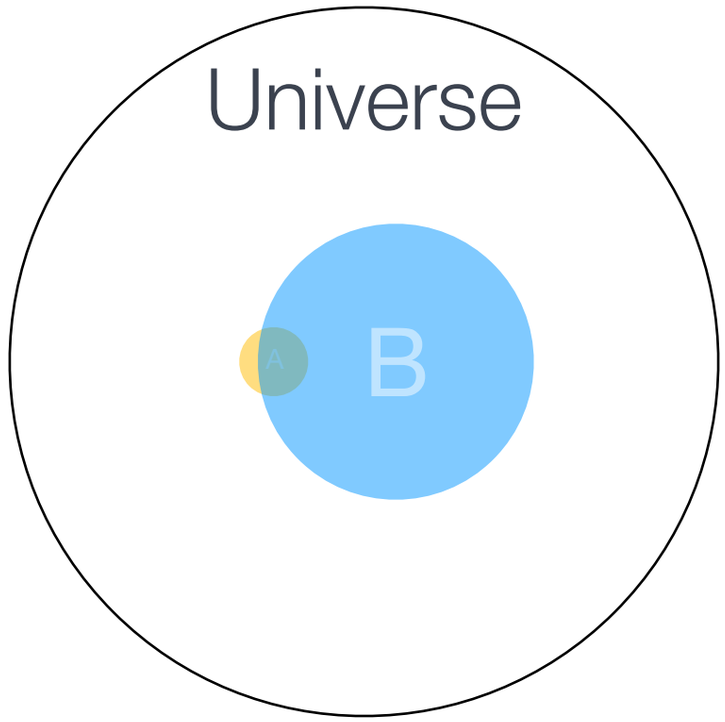
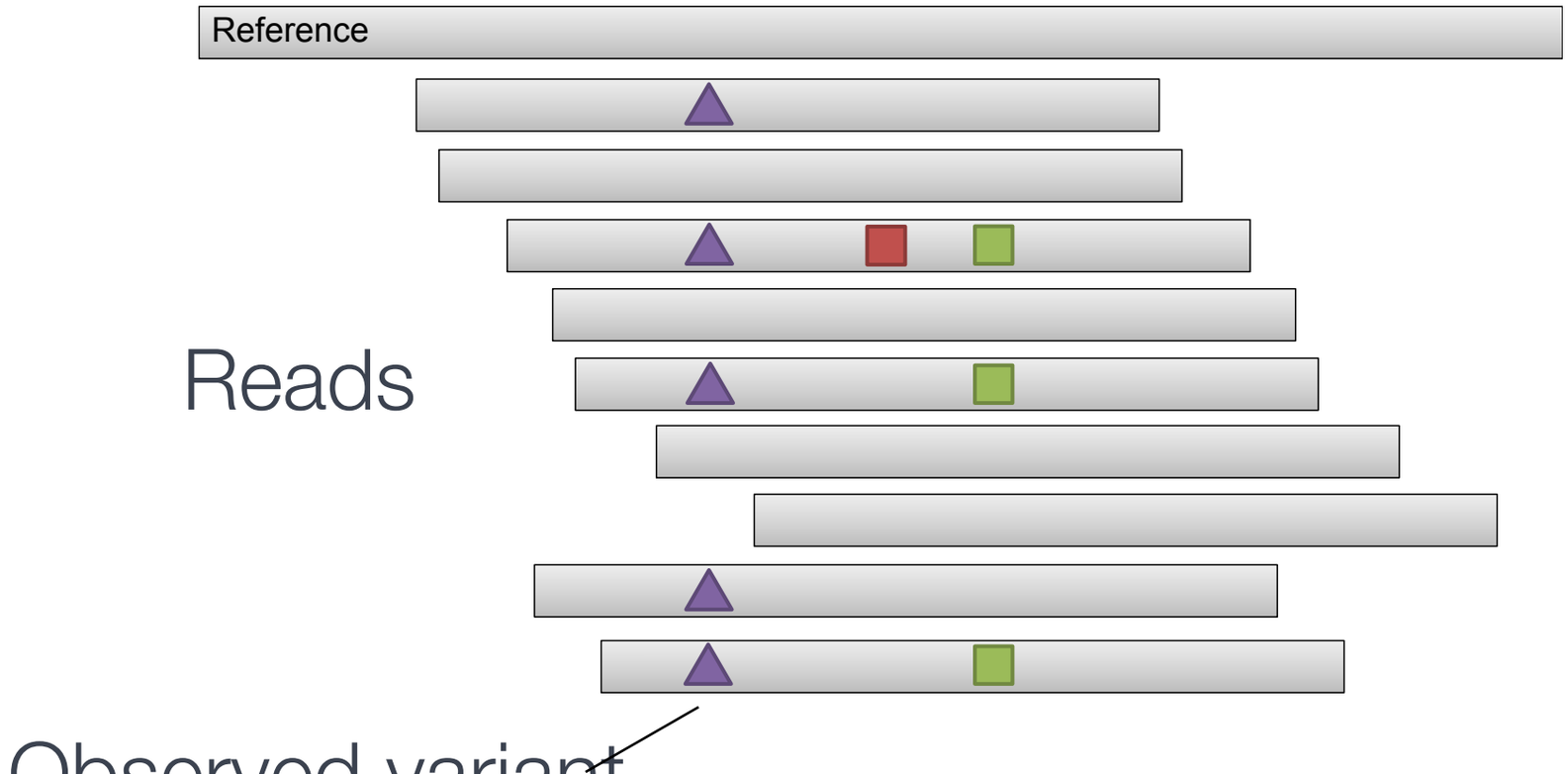


In practice

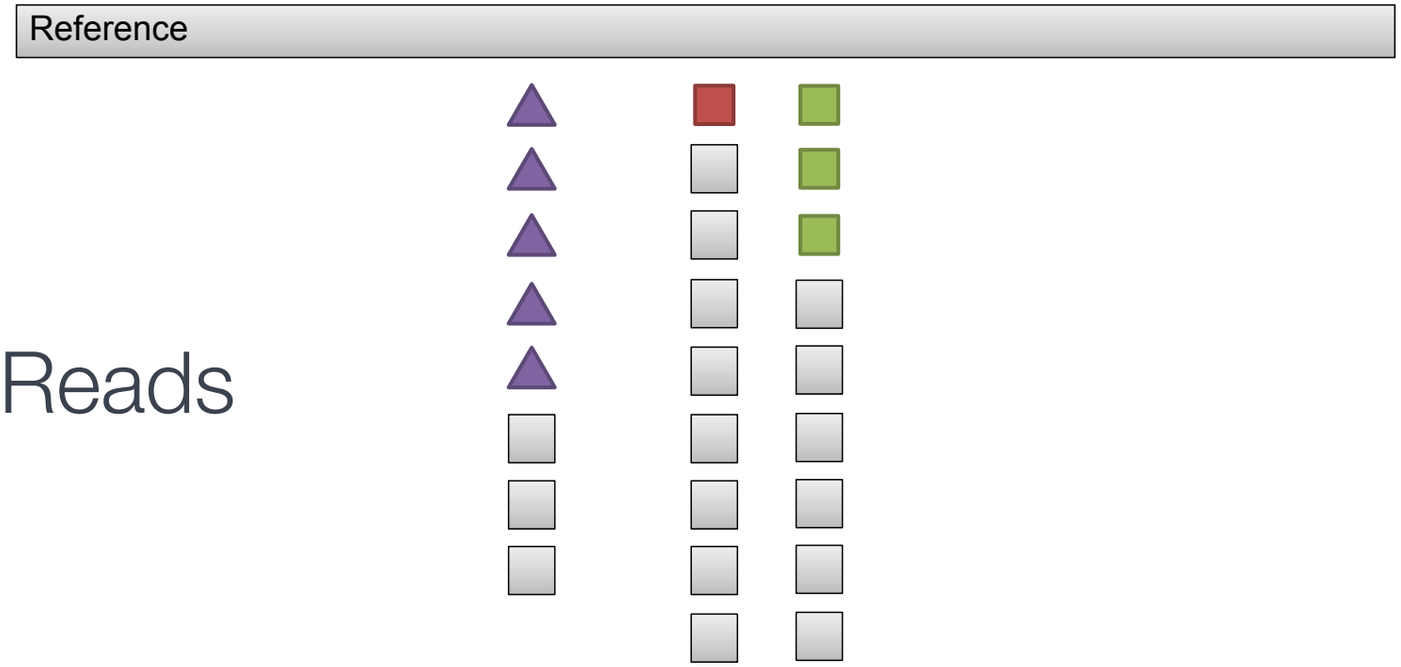
Variant detection

From Erik Garrison’s CSHL Advanced Sequencing Tutorial, http://clavius.bc.edu/~erik/CSHL-advanced-sequencing/freebayes-tutorial.html



Finding variants one at a time

From Erik Garrison's CSHL Advanced Sequencing Tutorial, http://clavius.bc.edu/~erik/CSHL-advanced-sequencing/freebayes-tutorial.html



Haplotype information is lost

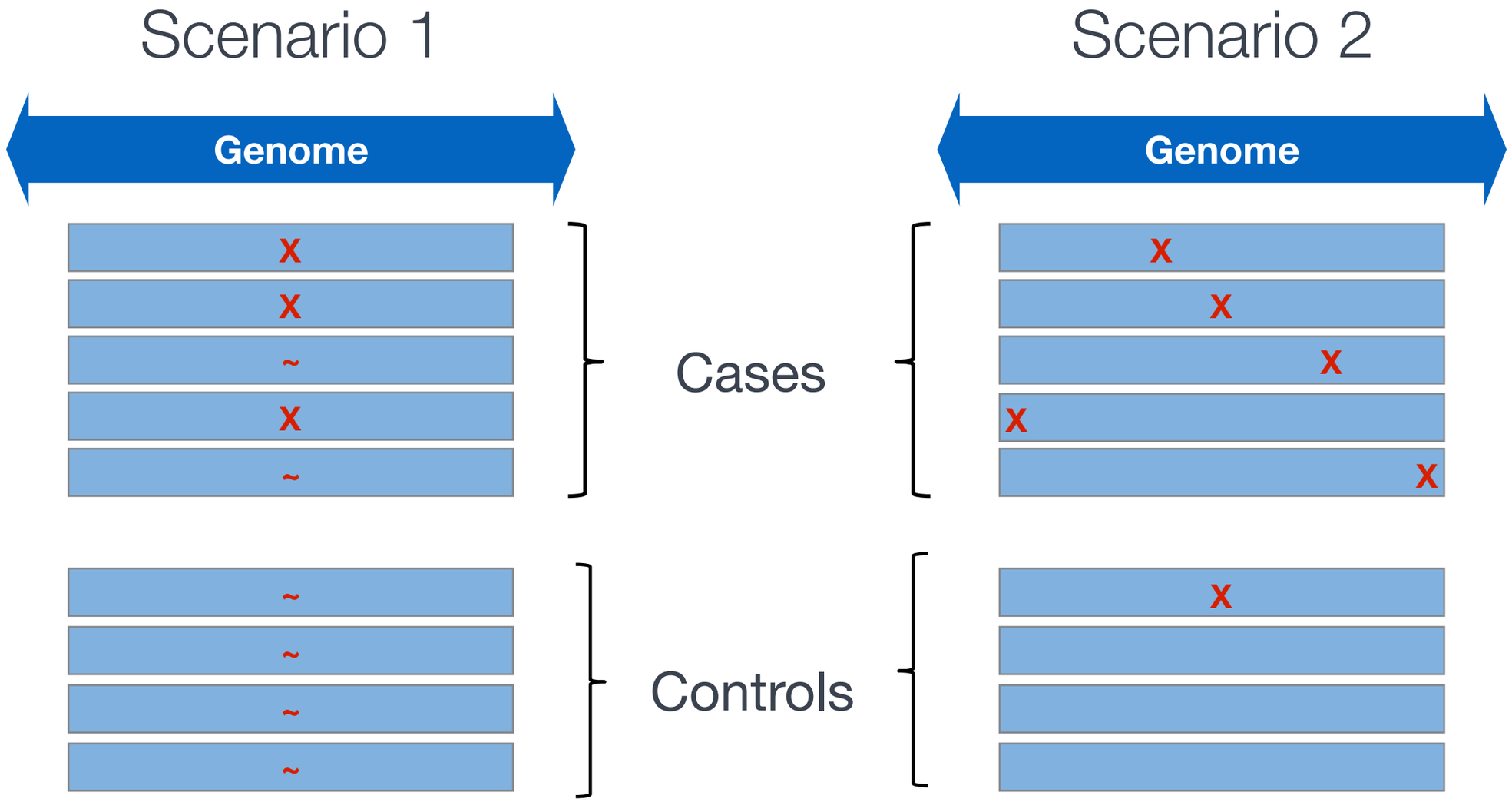
Finding variants one at a time

From Erik Garrison's CSHL Advanced Sequencing Tutorial, http://clavius.bc.edu/~erik/CSHL-advanced-sequencing/freebayes-tutorial.html

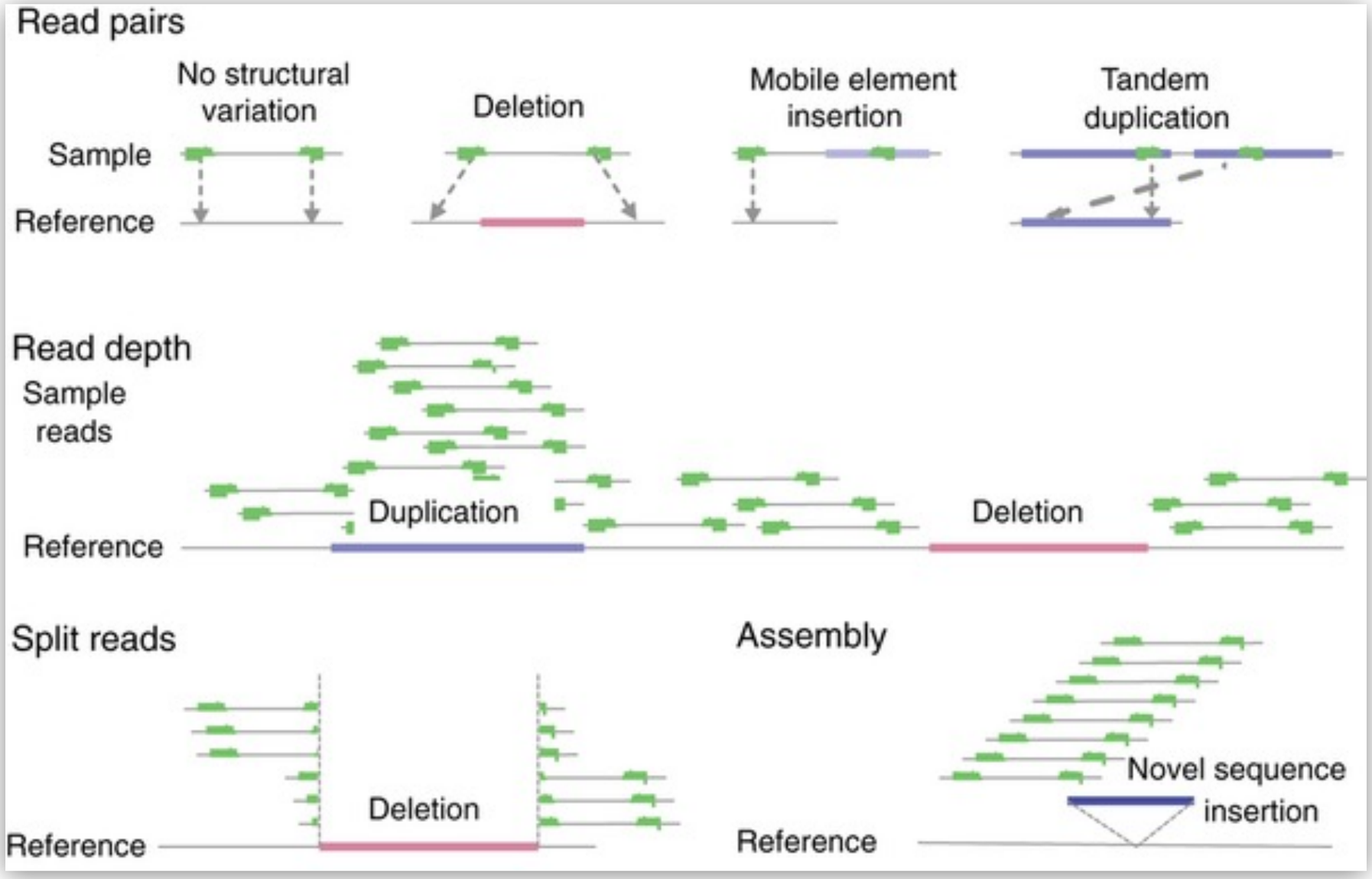
Finding variants with FreeBayes (or GATK)

From Erik Garrison's CSHL Advanced Sequencing Tutorial, http://clavius.bc.edu/~erik/CSHL-advanced-sequencing/freebayes-tutorial.html

How many samples does one need for a given population study?



CNVs and SVs



CNV and SV detection is more complex

Monya Baker, Nature Methods 9, 133–137 (2012)