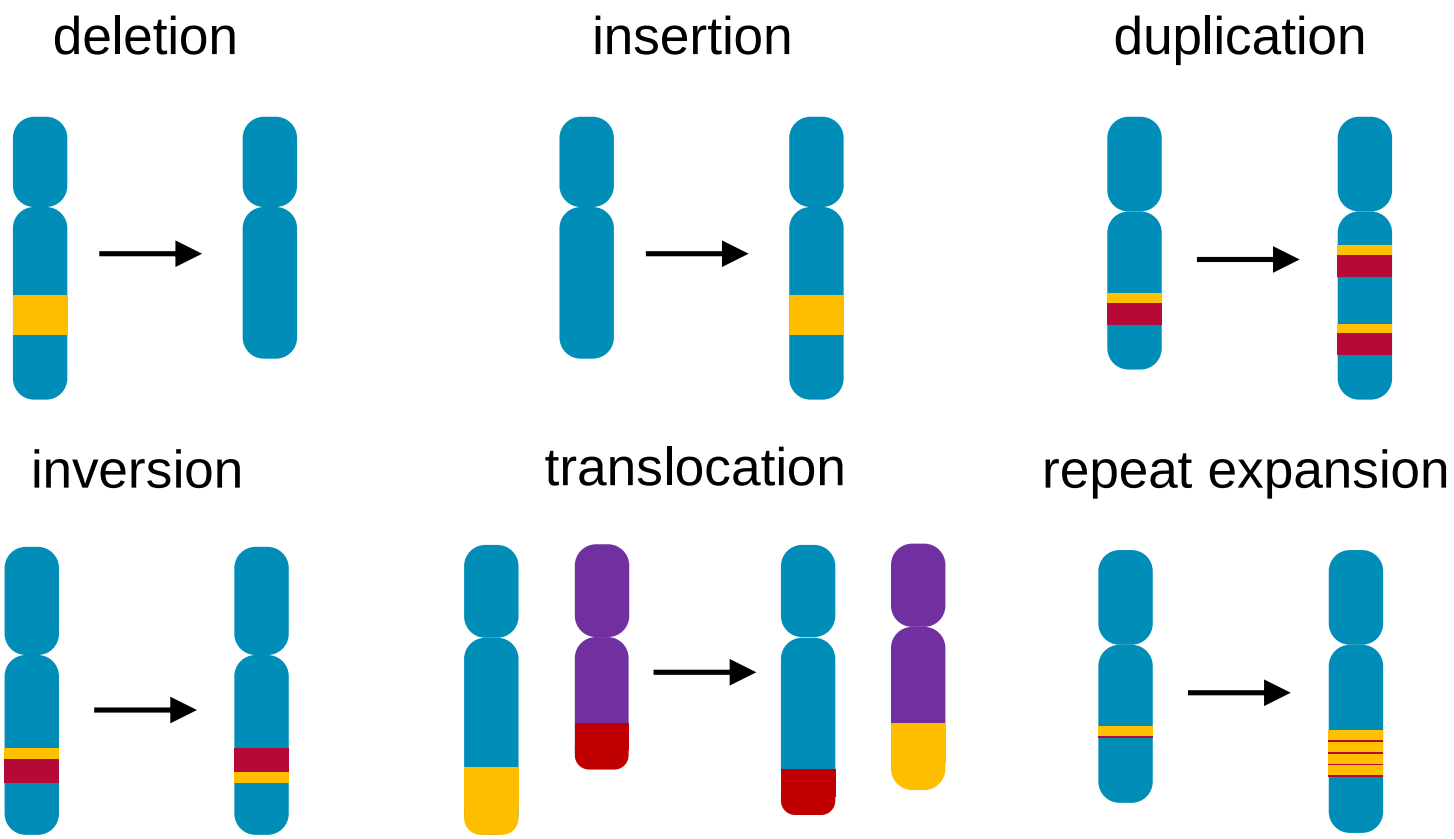
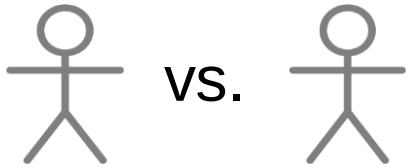
PACIFIC BIOSCIENCES®

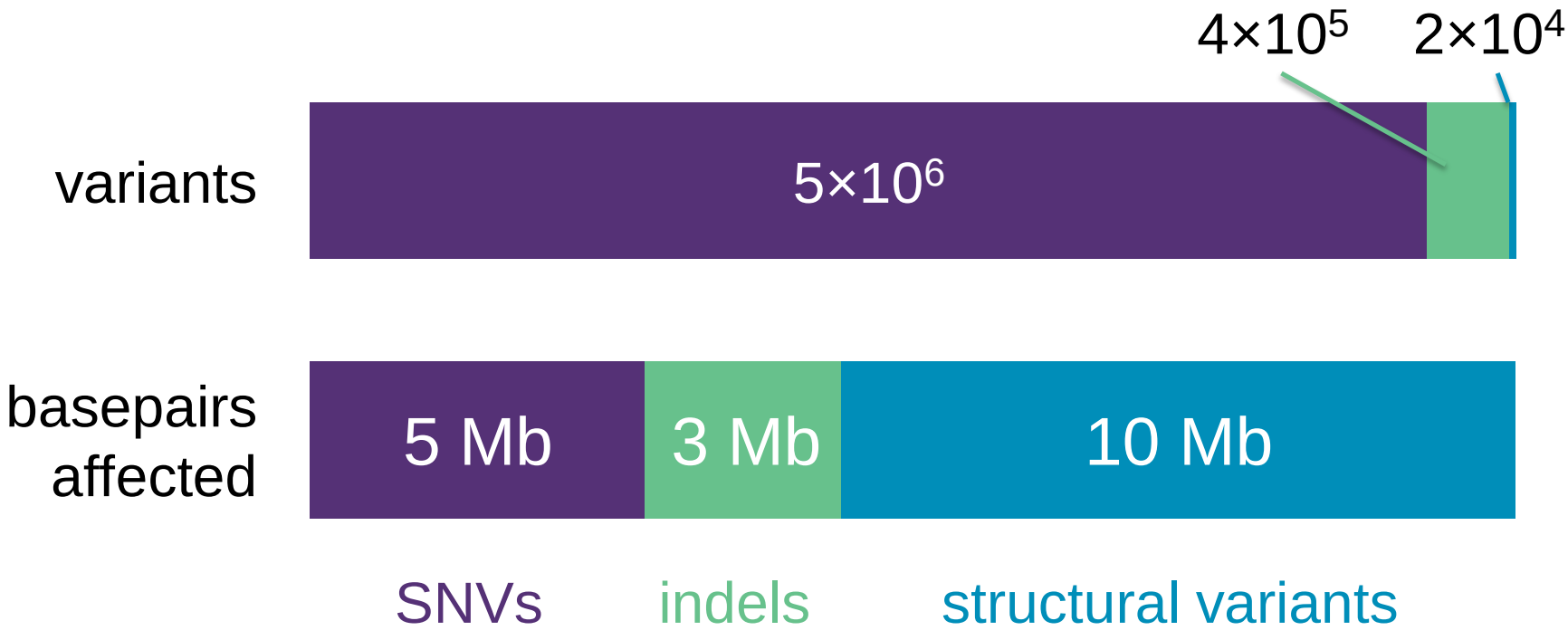
Structural Variant Detection in SMRT Link powered by pbsv

TYPES OF STRUCTURAL VARIATION



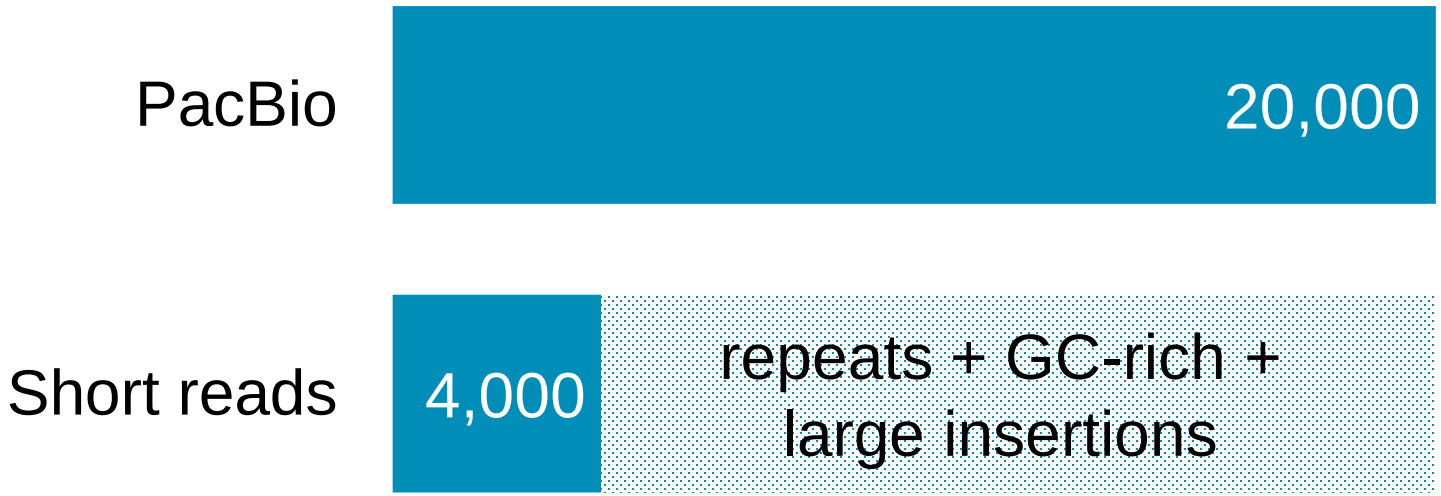
VARIATION BETWEEN TWO HUMAN GENOMES





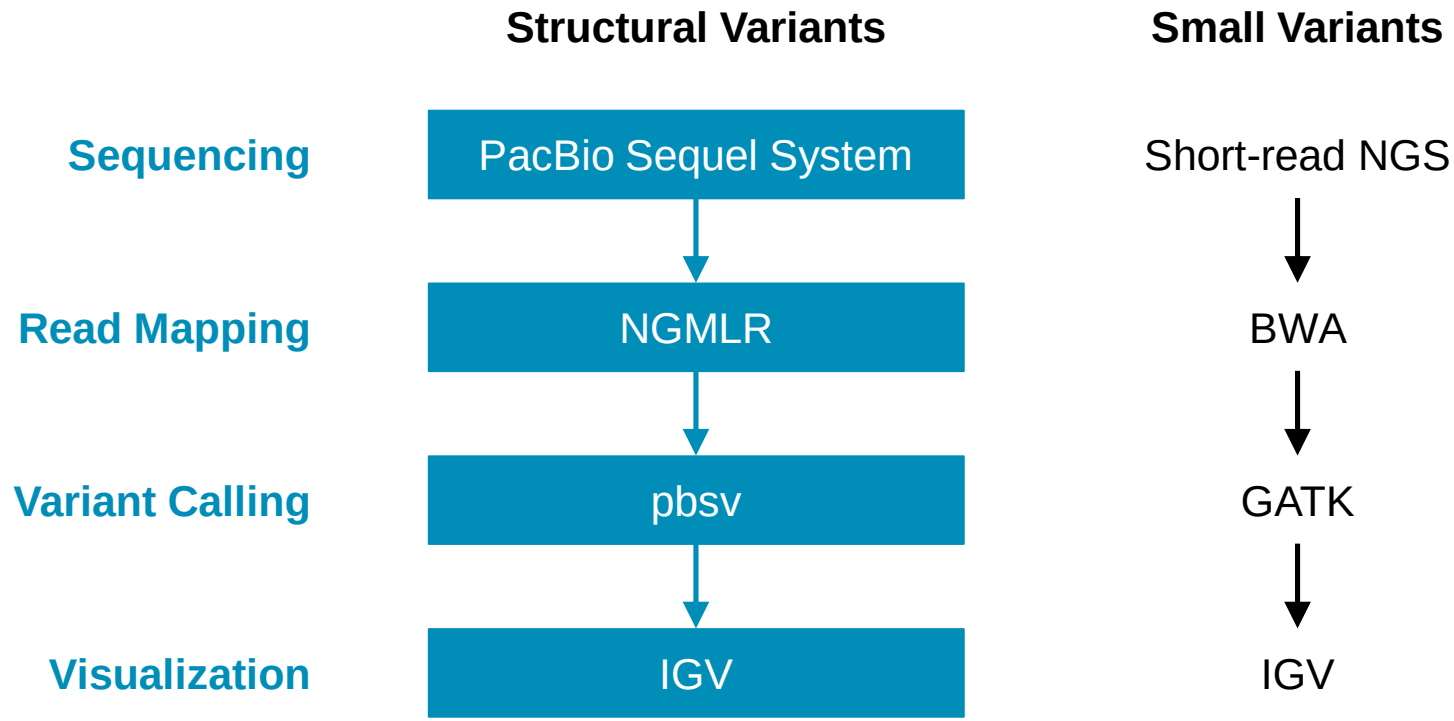
Huddleston et al. (2017) *Genome Research* 27(5):677-85.

STRUCTURAL VARIANTS DETECTED IN A HUMAN GENOME

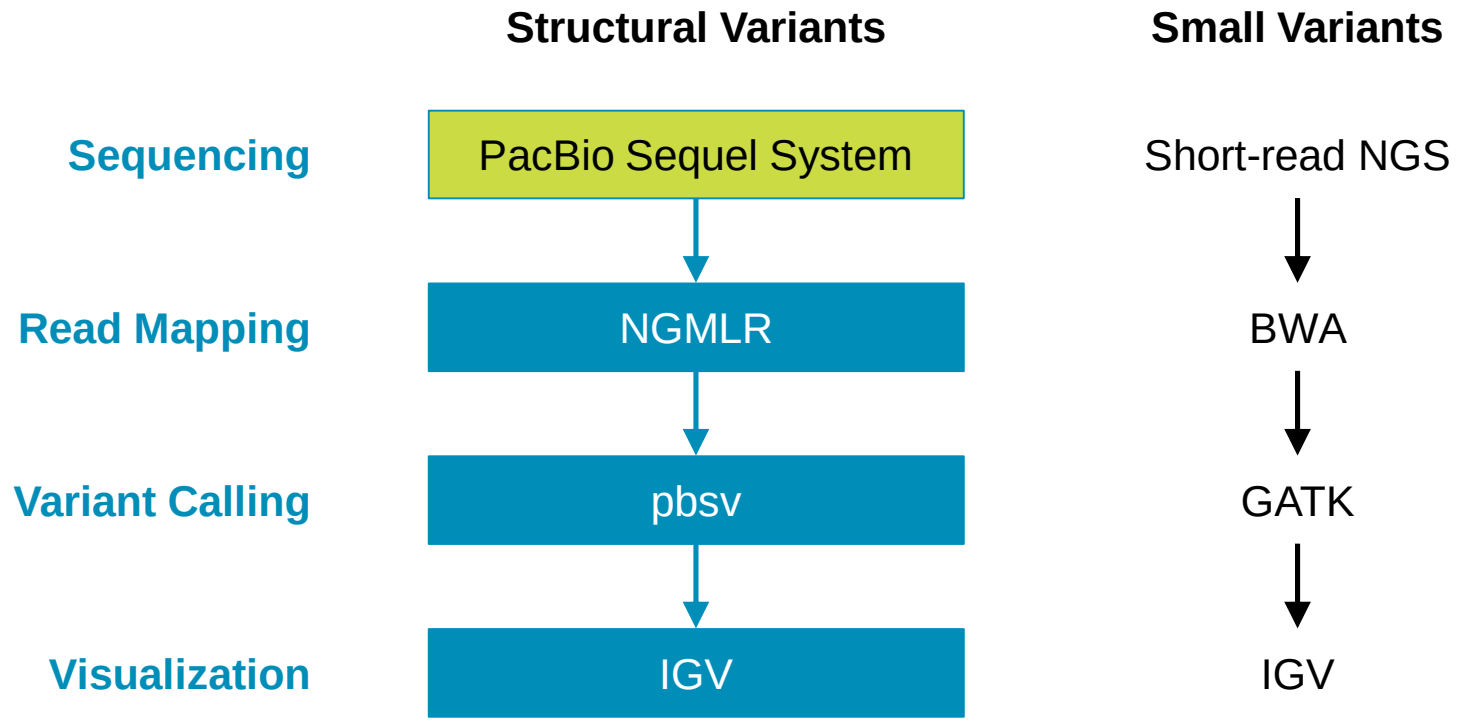


Huddleston et al. (2017) *Genome Research* 27(5):677-85.
Seo et al. (2016) *Nature* 538:243-7.
Sudmant et al. (2016) *Nature* 526:75-81.

WGS FOR STRUCTURAL VARIANT DISCOVERY



WGS FOR STRUCTURAL VARIANT DISCOVERY



SEQUENCING

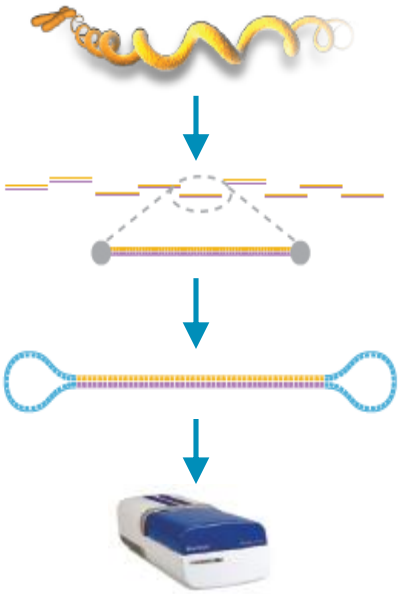
Library Preparation

5 µg DNA

20 kb shear
+ damage repair

SMRTbell adapter ligation

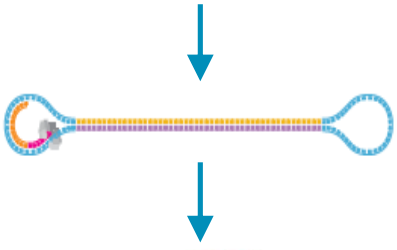
15 kb size selection



SEQUENCING

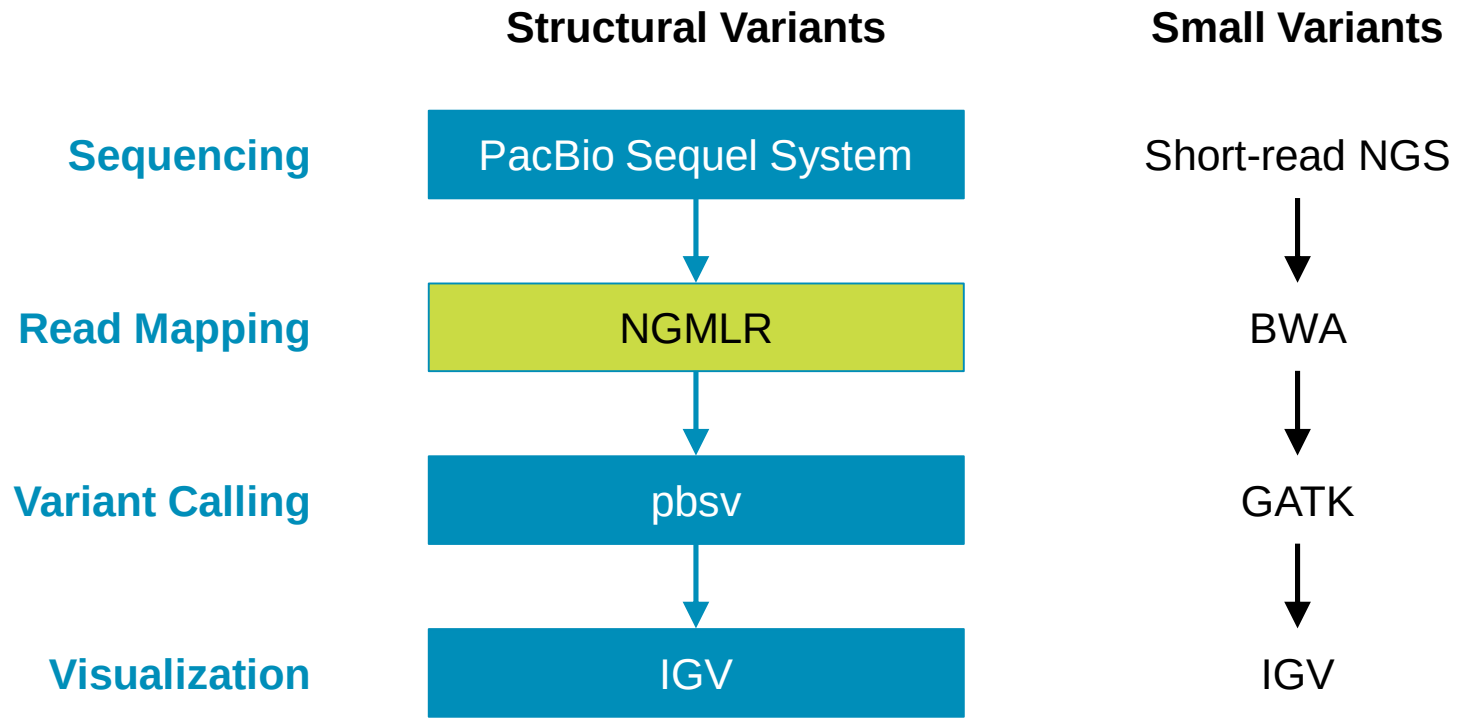
Sequencing

polymerase binding

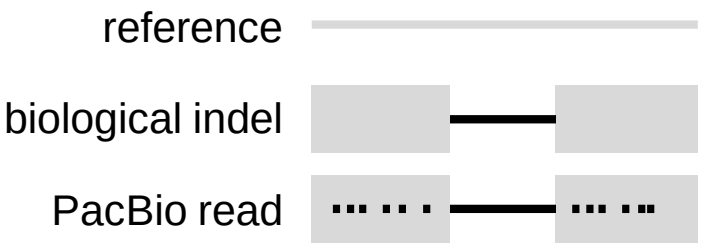


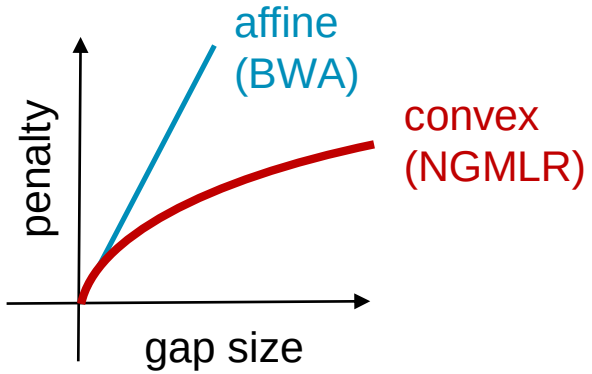
Sequel System
(5 Gb per SMRT Cell)

WGS FOR STRUCTURAL VARIANT DISCOVERY

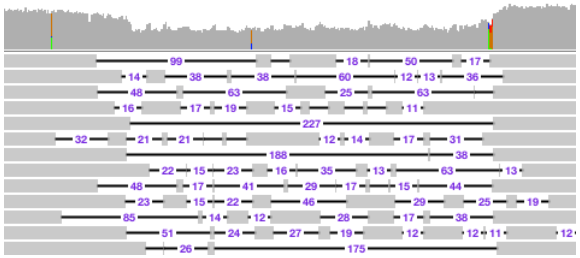


READ MAPPING

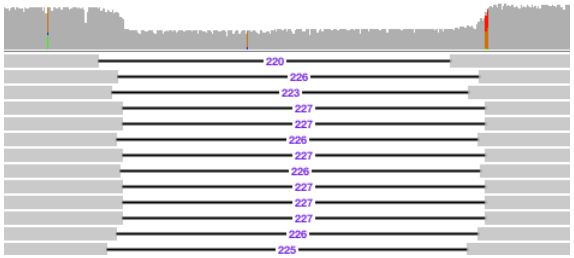




BWA

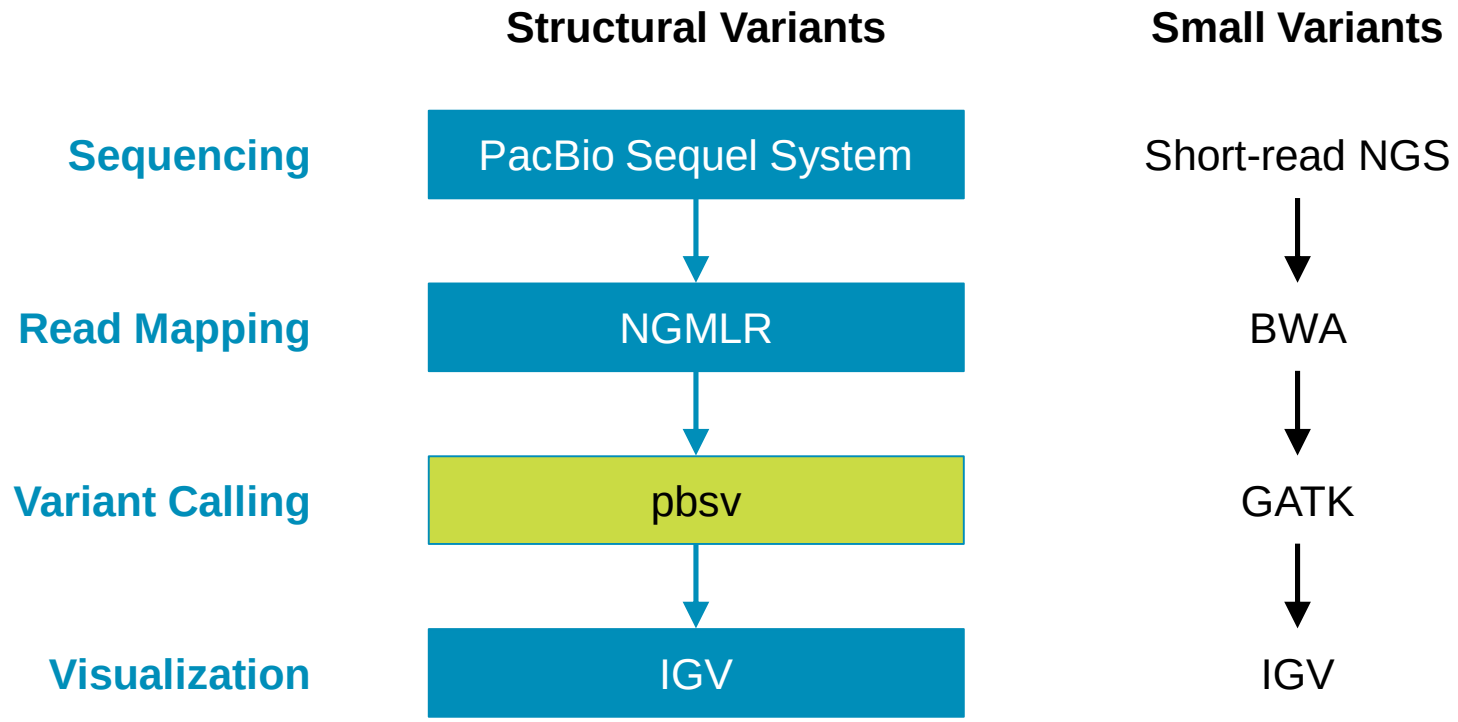


NGMLR



Sedlazeck et al. (2017) *bioRxiv*. doi:10.1101/169557.

WGS FOR STRUCTURAL VARIANT DISCOVERY



VARIANT CALLING

FIND SV SIGNATURES	CIGAR D & I ≥50 bp
CLUSTER SV SIGNATURES	nearby with similar sequence
FILTER	≥2 and ≥20% reads support
SUMMARIZE INTO SV	consensus of supporting reads
GENOTYPE	supporting reads / covering reads

VARIANT CALLING

FIND SV SIGNATURES

CIGAR D & I
≥50 bp

CLUSTER SV SIGNATURES

nearby with similar sequence

FILTER

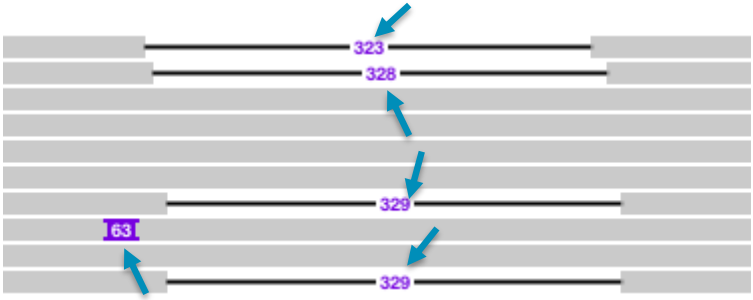
≥2 and ≥20% reads support

SUMMARIZE INTO SV

consensus of supporting reads

GENOTYPE

supporting reads / covering reads



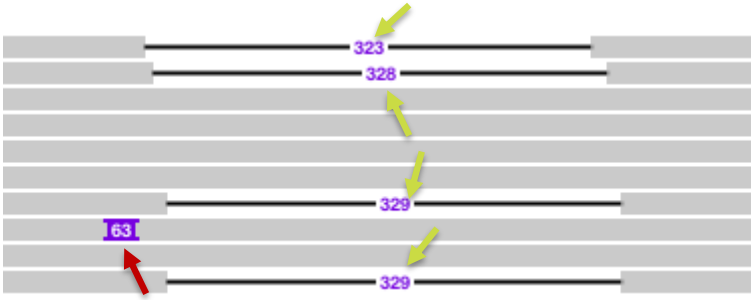
VARIANT CALLING

FIND SV SIGNATURES

CIGAR D & I ≥50 bp

CLUSTER SV SIGNATURES

nearby with similar sequence



FILTER

≥2 and ≥20% reads support

SUMMARIZE INTO SV

consensus of supporting reads

GENOTYPE

supporting reads / covering reads

VARIANT CALLING

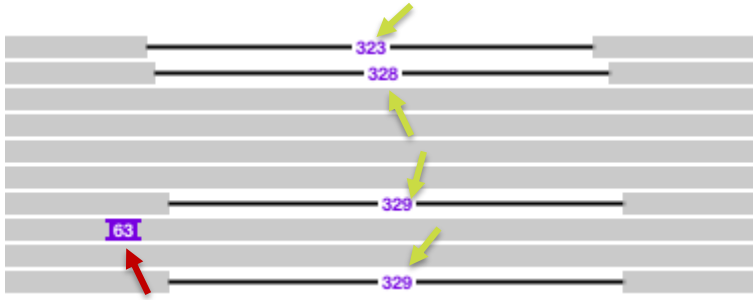
FIND SV SIGNATURES — CIGAR D & I ≥50 bp

CLUSTER SV SIGNATURES — nearby with similar sequence

FILTER — ≥2 and ≥20% reads support

SUMMARIZE INTO SV — consensus of supporting reads

GENOTYPE — supporting reads / covering reads



1 of 10

4 of 10

VARIANT CALLING

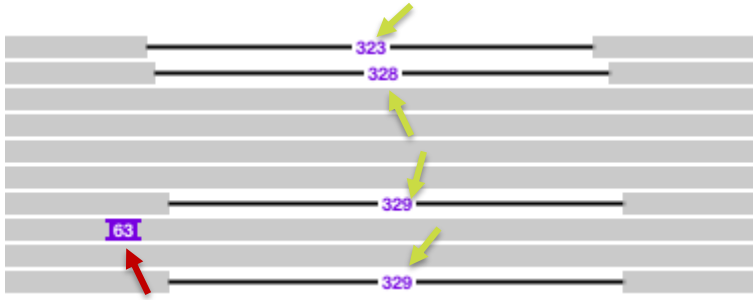
FIND SV SIGNATURES — CIGAR D & I ≥50 bp

CLUSTER SV SIGNATURES — nearby with similar sequence

FILTER — ≥2 and ≥20% reads support

SUMMARIZE INTO SV — consensus of supporting reads

GENOTYPE — supporting reads / covering reads



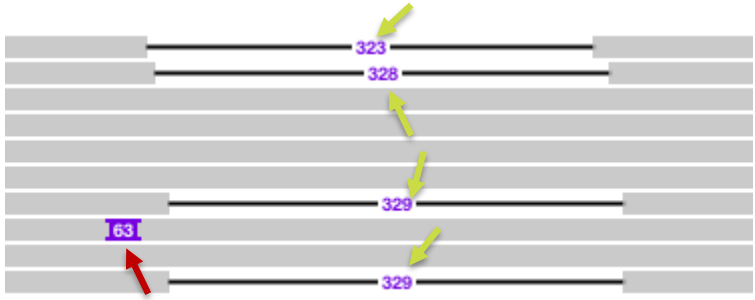
1 of 10 ✗

4 of 10 ✓

329 bp deletion

VARIANT CALLING

FIND SV SIGNATURES	CIGAR D & I ≥50 bp
CLUSTER SV SIGNATURES	nearby with similar sequence
FILTER	≥2 and ≥20% reads support
SUMMARIZE INTO SV	consensus of supporting reads
GENOTYPE	supporting reads / covering reads



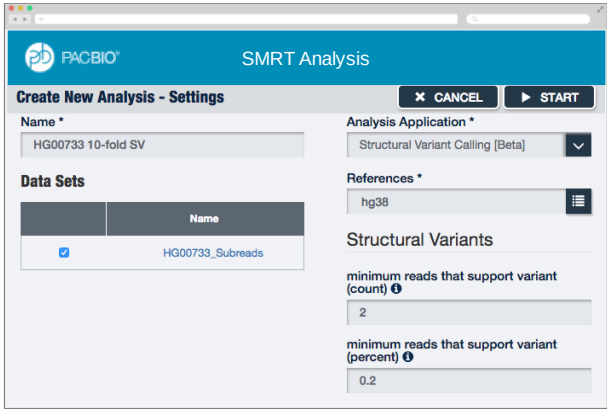
1 of 10

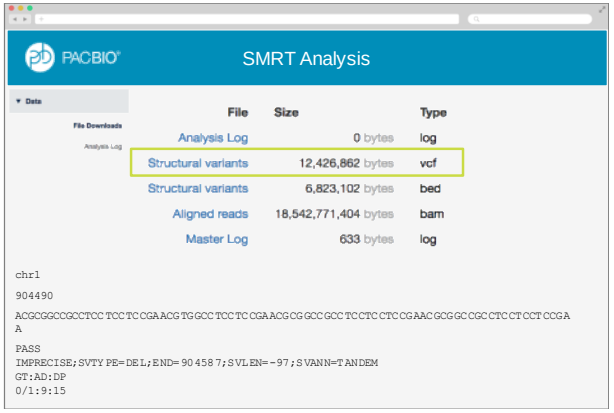
4 of 10

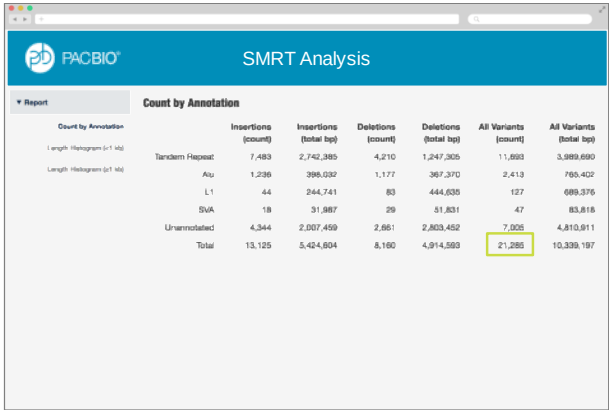
329 bp deletion

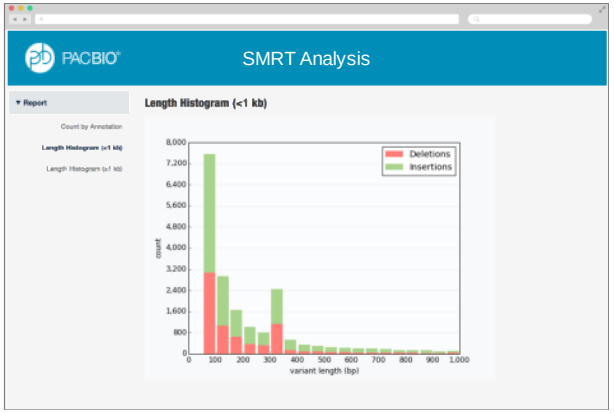
heterozygous (4 of 10)

VARIANT CALLING

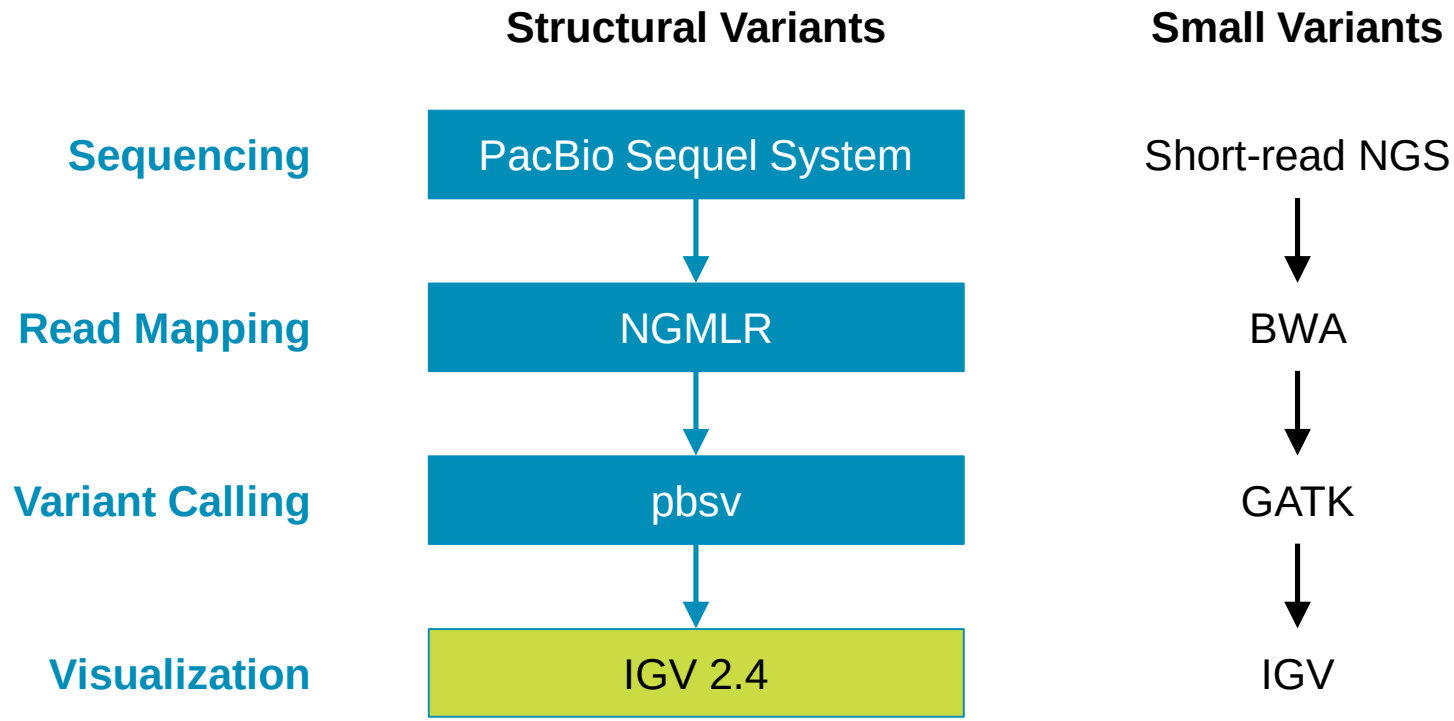




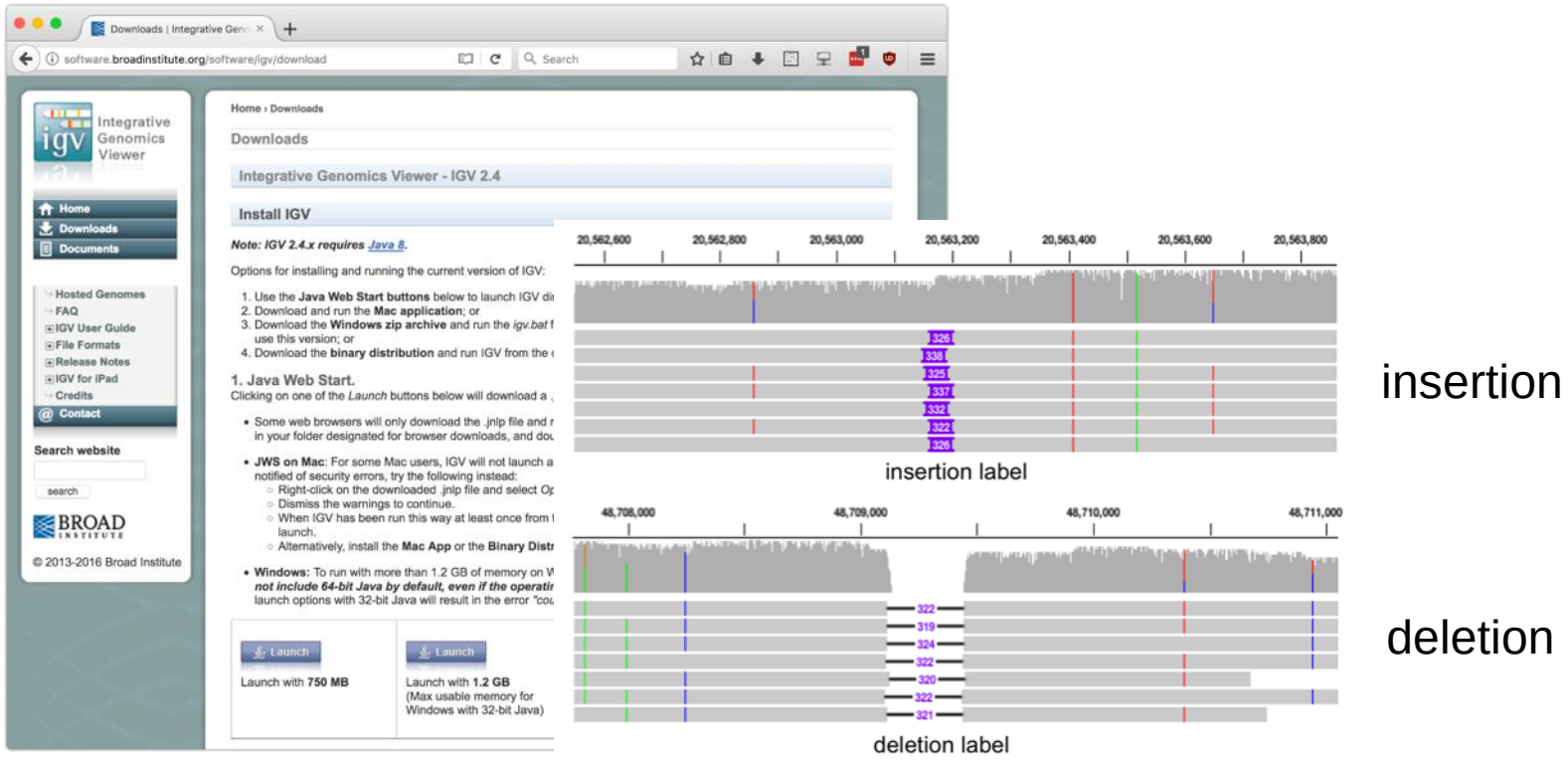




WGS FOR STRUCTURAL VARIANT DISCOVERY

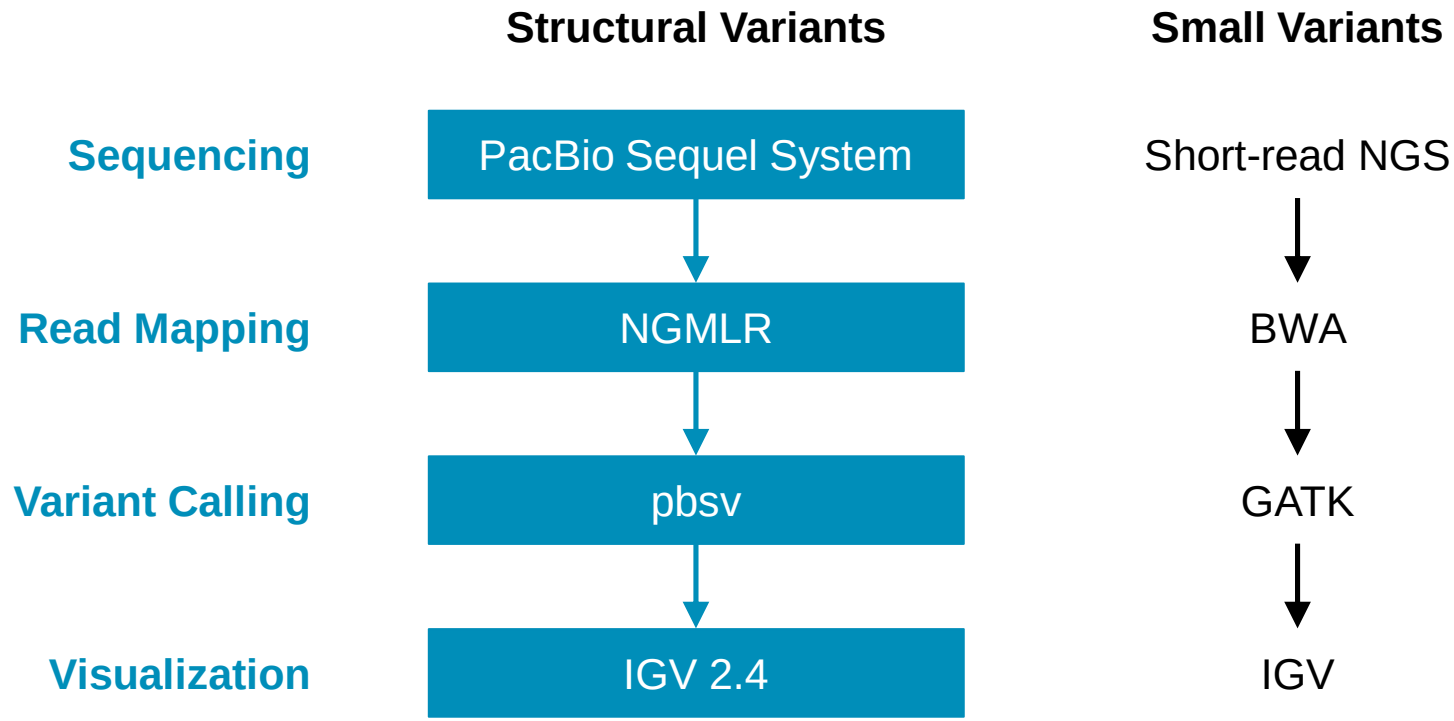


VISUALIZATION



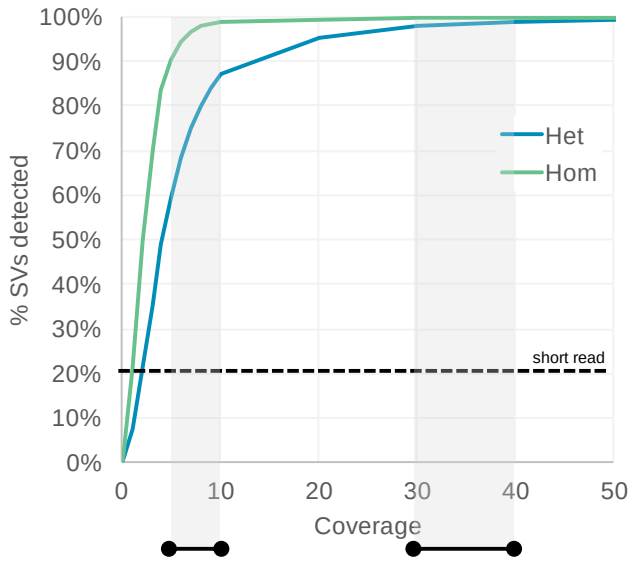
Robinson et al. (2011) *Nature Biotechnology*. doi:10.1038/nbt.1754.

WGS FOR STRUCTURAL VARIANT DISCOVERY



HOW MUCH TO SEQUENCE?

Human HG00733
Sequel System
211 Gb (70-fold)



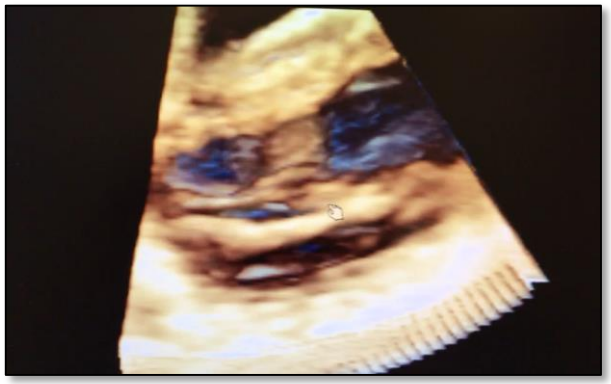
5- to 10-fold
optimal tradeoff of
cost vs. performance
disease gene discovery;
population characterization

30- to 40-fold
saturate discovery

de novo variant discovery

CLINICAL CASE HISTORY

7 yrs	left atrial myxoma resection, atrial repair
10 yrs	testicular mass, right orchiectomy
13 yrs	pituitary tumor
16 yrs	recurrence of myxomata, resection, adrenal microadenoma
18 yrs	recurrence of ventricular myxomata, resection, VT
19 yrs	ACTH-independent Cushing's disease, thyroid nodules
21 yrs	transphenoidal resection of pituitary
present (26 yrs)	recurrence of myxomata, consideration for heart transplant



genetics suggests Carney complex

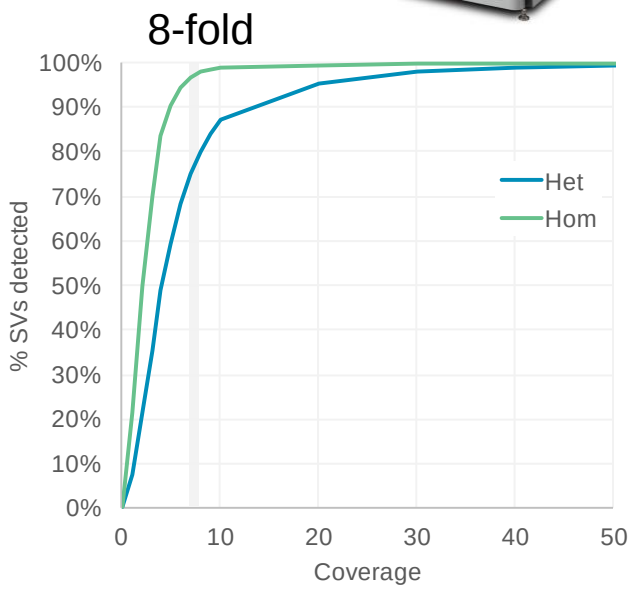
PRKAR1A testing negative

short-read whole genome sequencing negative

Merker et al. (2017) *Genetics in Medicine*. doi:10.1038/gim.2017.86.

EVALUATING STRUCTURAL VARIANTS

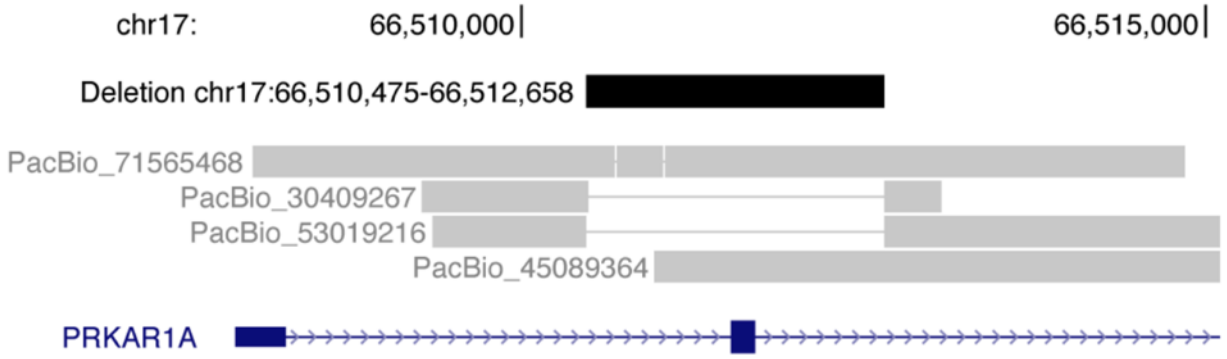
	Deletions	Insertions
Initial call set	6,971	6,821
Not in segdup	5,893	6,254
Not in NA12878 “healthy” control	2,476	3,171
Overlaps RefSeq coding exon	39	16
Gene linked to some disease in OMIM	3	3



Merker et al. (2017) *Genetics in Medicine*. doi:10.1038/gim.2017.86.

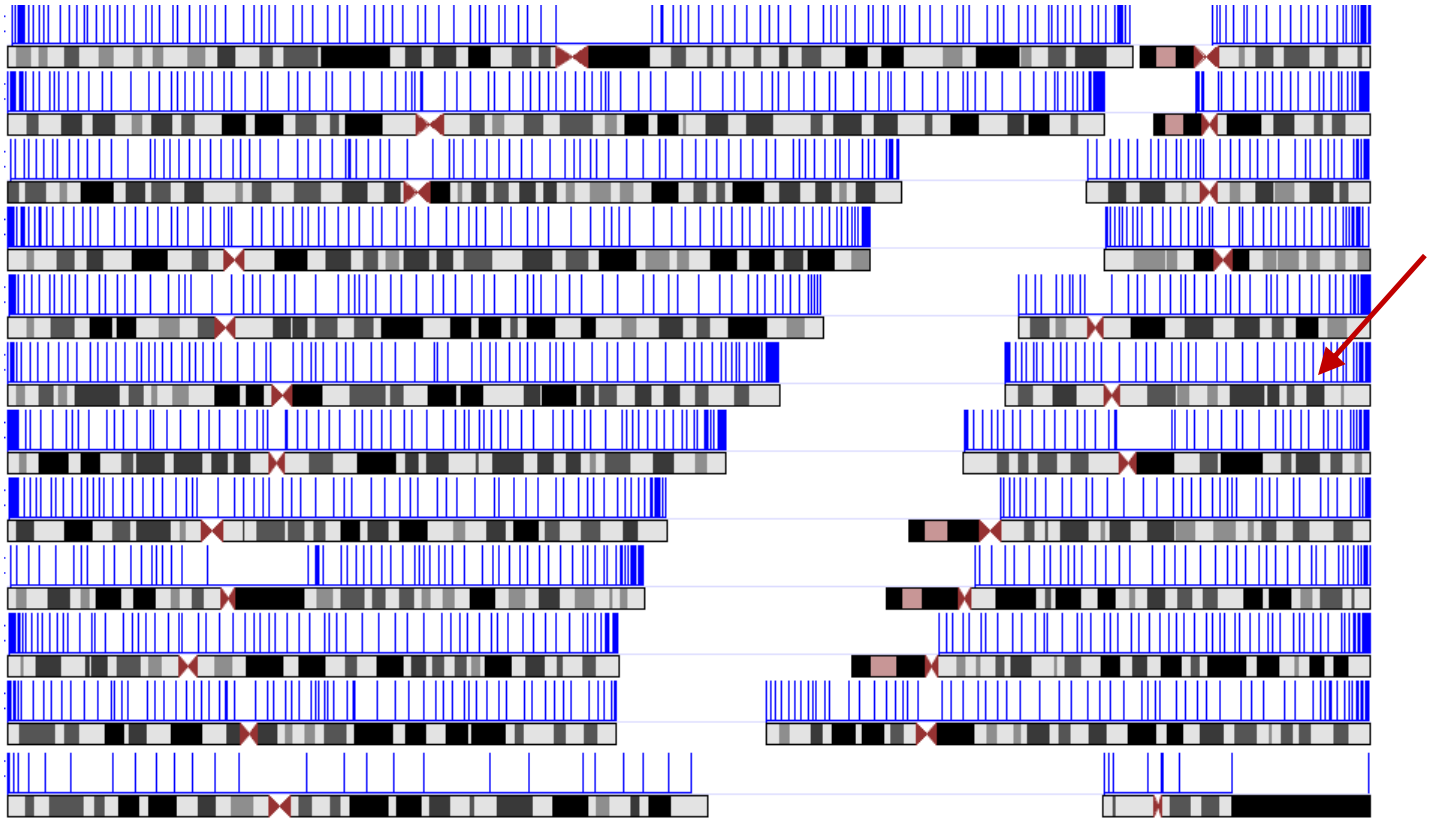
HETEROZYGOUS 2.2 KB DELETION IN *PRKAR1A*

PacBio discovery



Sanger confirmation

Merker et al. (2017) *Genetics in Medicine*. doi:10.1038/gim.2017.86.



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