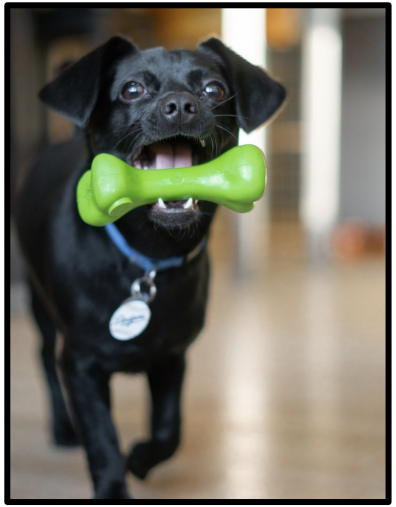
Variation of Genome Structure

Michael Alonge

Applied Comparative Genomics: EN.601.749

2-24-20


THIS IS BIRDLAND



Outline

- **Introduction to genome “structure”**
- **Functional importance of genome structure**
- **The Bioinformatics of SV calling**
  - Assembly
    - Whole genome alignment to a reference
    - (Whole genome) Alignment free analysis
  - Read Mapping
    - Short-read mapping
    - Long-read mapping
- **Applications in Tomato**

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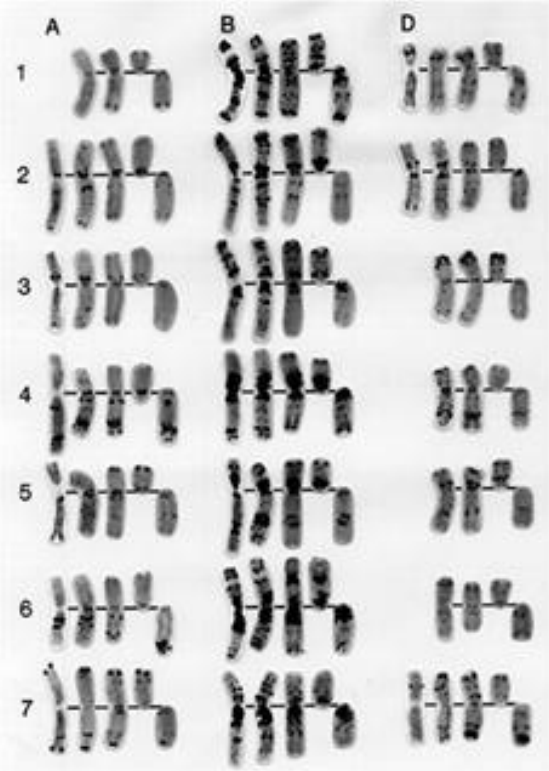
What is Genome Structure?



Bread wheat (*Triticum aestivum*)

https://ebrary.net/26390/environment/laying_foundation_cytogenetic_genome_mapping_wheat_aneuploid_stocks

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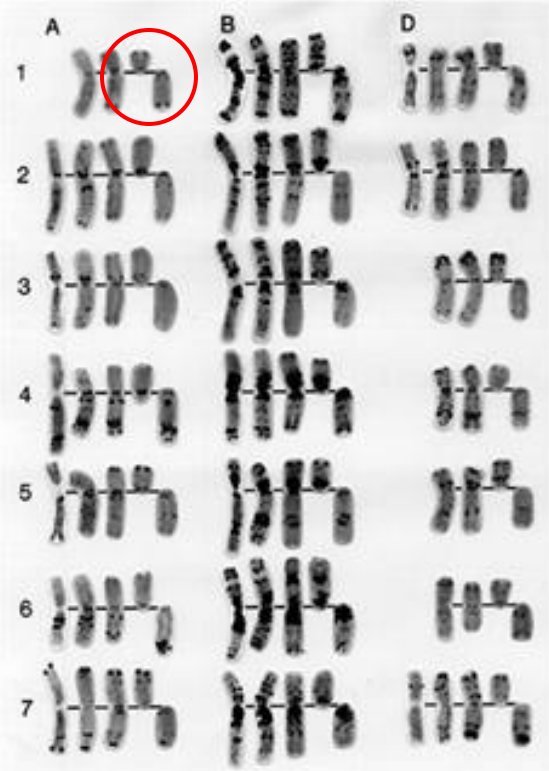

Bread wheat (*Triticum aestivum*)

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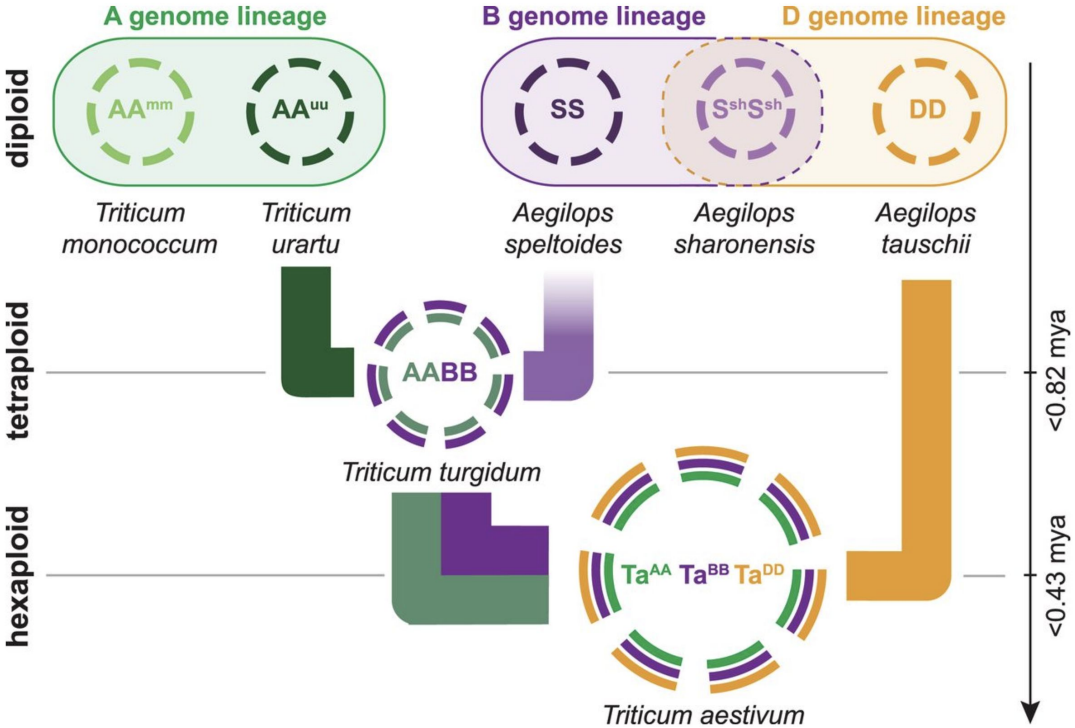
What is Genome Structure?

- **My definition**: Genome structure refers to large-scale genomic sequence composition.

- **Example**:
  - A genome assembly contig that accurately represents a whole chromosome would be considered “structurally accurate”.
  - If that contig was missing a large chunk of the chromosome, it would be considered “structurally inaccurate”.

* Sometimes, genome “structure” refers to 3D organization/characteristics of the genome. That is **not** what we will be discussing today.



Plant genomes have dynamic structure:

- Polyploidization
- TE activity
- Gene loss/duplication

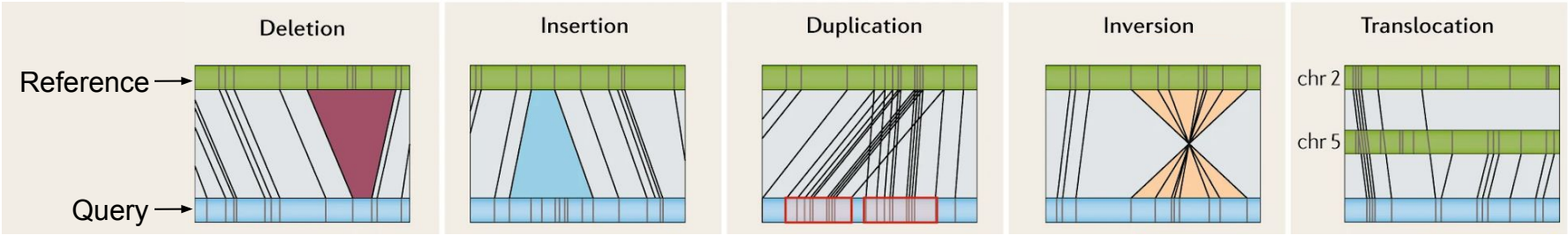
International Wheat Genome Sequencing Consortium. "A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome." *Science* 345.6194 (2014): 1251788.

What is Structural Variation?

- **My definition**: Variation of genome structure in a population.
- A **Structural Variant** (SV) is a particular variable locus.

- **Examples**:
  - Mike has two copies of the *DODGERSFAN* gene, while Bob only has 1.
  - “Brandywine” (an heirloom tomato variety) has a rare transposable element insertion in the *FLAVOR* gene.

How is Structural Variation Classified?



- Just as with small variant calling, we typically classify structural variation by comparing two individuals to each other.
  - There are many ways to do this “comparison” which will be covered later.
- One individual is designated as the “reference” and the other the “query”.
- We then define query structural variants “with respect to” the reference
  - Mike has an insertion with respect to the reference
  - Bob has a deletion with respect to the reference
- SVs are usually defined as being longer than 50 bp.

Ho, Steve S., Alexander E. Urban, and Ryan E. Mills. "Structural variation in the sequencing era." *Nature Reviews Genetics* (2019): 1-19.

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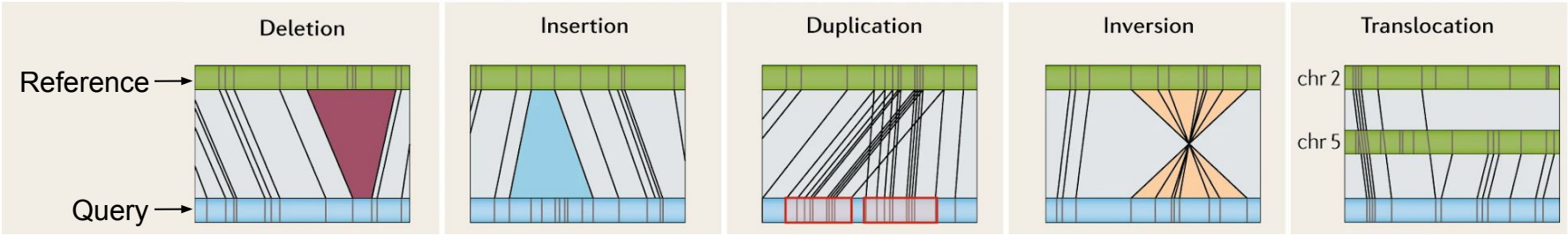

Deletion	Insertion	Duplication	Inversion	Translocation
A reference locus is not found in the query	The query locus is not found in the reference	The reference has 1 copy of a locus and the query has >1 copy of that locus.	Loci in the reference and query are reverse complements of one another.	A locus in the query moved to a different part of the genome

Ho, Steve S., Alexander E. Urban, and Ryan E. Mills. "Structural variation in the sequencing era." *Nature Reviews Genetics* (2019): 1-19.

How is Structural Variation Classified?

Copy Number Variants (CNVs)

- A distinct but related SV classification
- Refers to variation in the copy number of a locus
- Example: The *CUTE* gene is copy number variable in dogs
  - The reference genome has 1 copy of the *CUTE* gene.
  - Rover has 0 copies of the *CUTE* gene (A.K.A a “deletion”).
  - Baxter has 2 copies of the *CUTE* gene (A.K.A a “duplication”).
  - Tupper has 15 copies of the *CUTE* gene.

How Is Structural Variation Created?

- **Faulty repair of DNA damage**
- Transposable element activity
- Non-disjunction
- DNA replication errors
- Unequal crossing-over

* SVs are usually created or mediated by repeats

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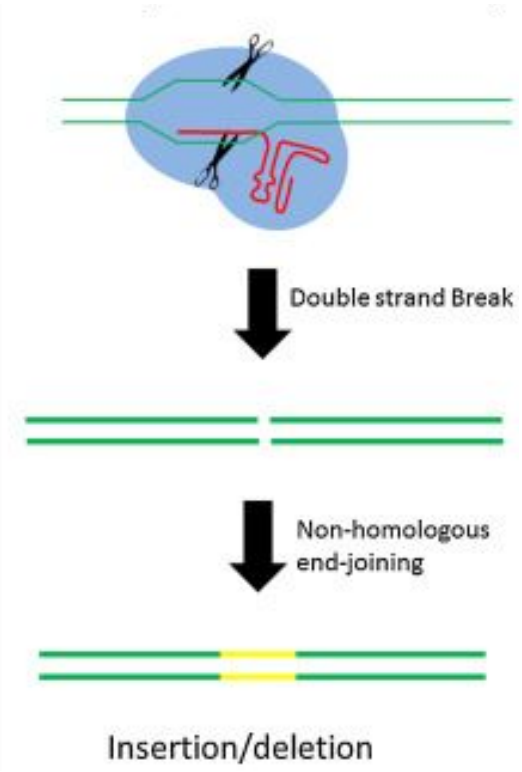
CRISPR/Cas9 Gene Editing



Schaeffer, Scott M., and Paul A. Nakata. "CRISPR/Cas9-mediated genome editing and gene replacement in plants: transitioning from lab to field." *Plant Science* 240 (2015): 130-142.

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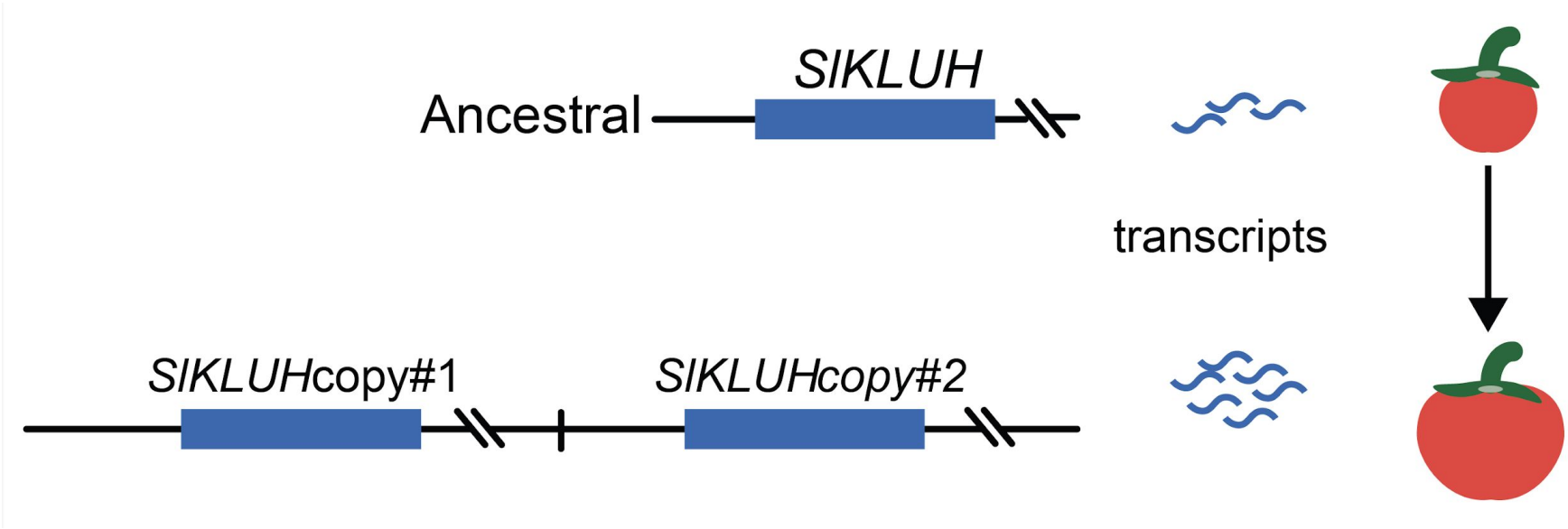
SVs Impact Function

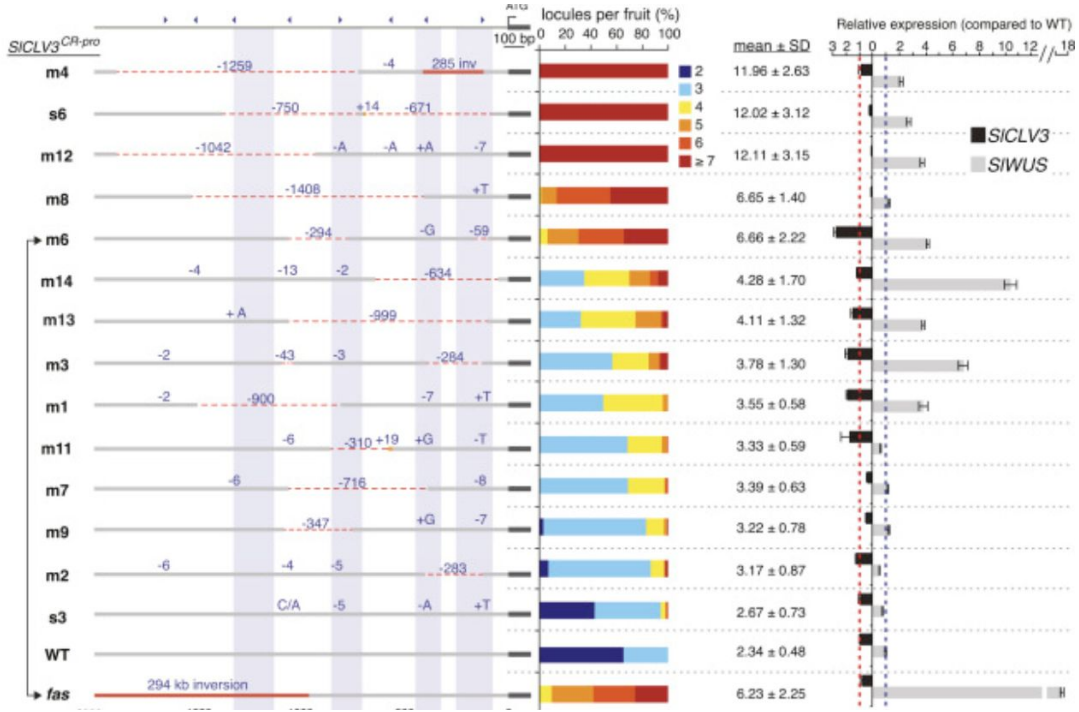
1. Protein coding genes



Alonge and Wang *et al*., unpublished

SVs Impact Function

2. Gene regulatory elements

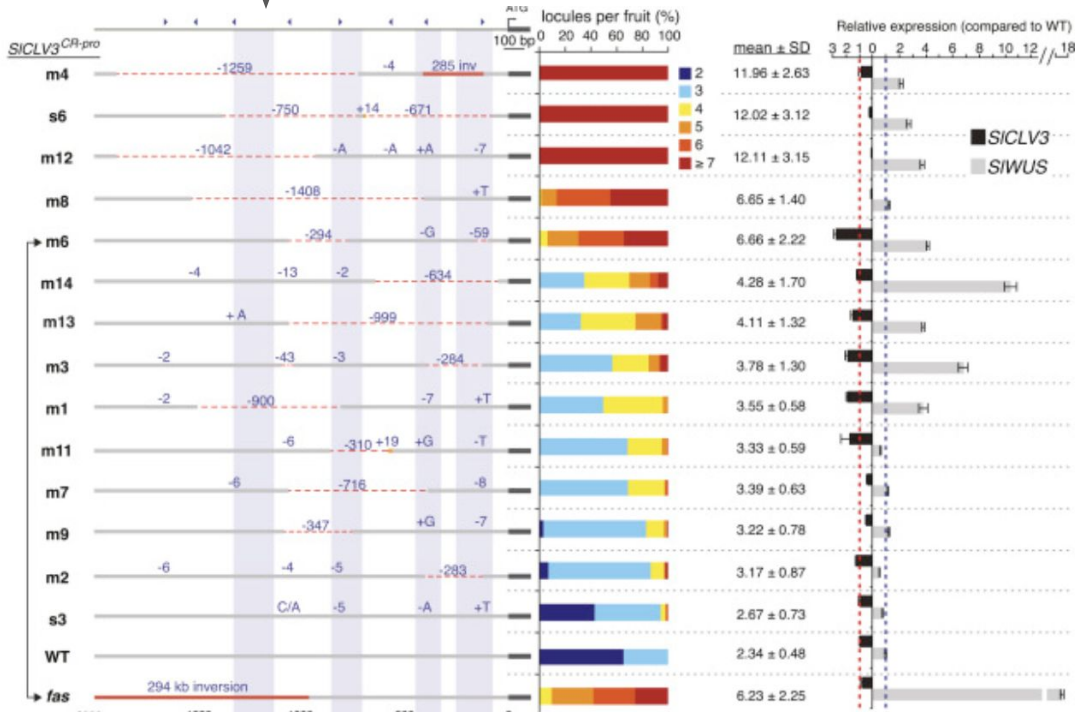


Rodríguez-Leal, Daniel, et al. "Engineering quantitative trait variation for crop improvement by genome editing." *Cell* 171.2 (2017): 470-480.

SVs Impact Function

2. Gene regulatory elements

Different SVs in the promoter of SlCLV3



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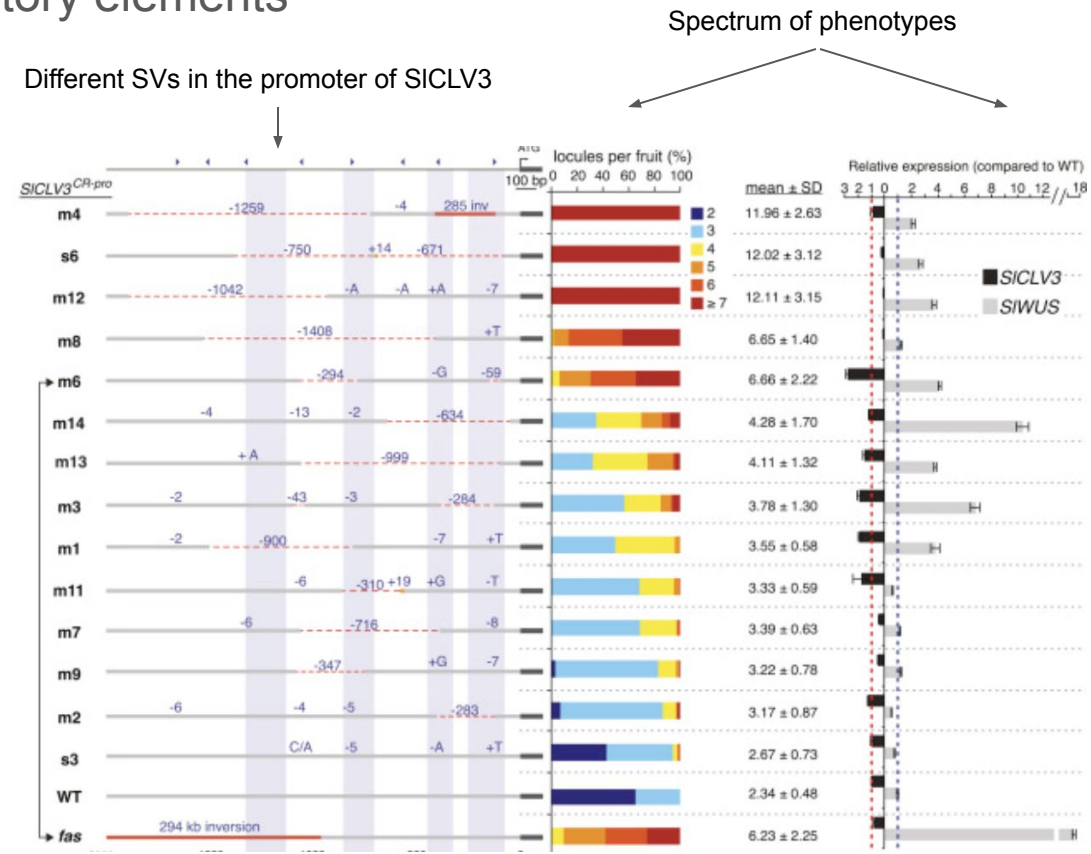

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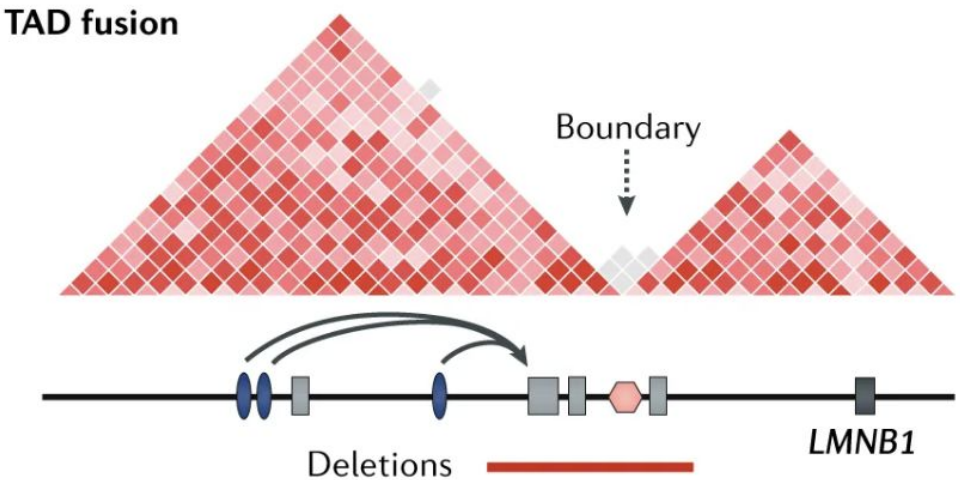
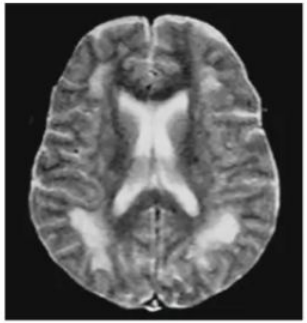
SVs Impact Function

3. 3D structure





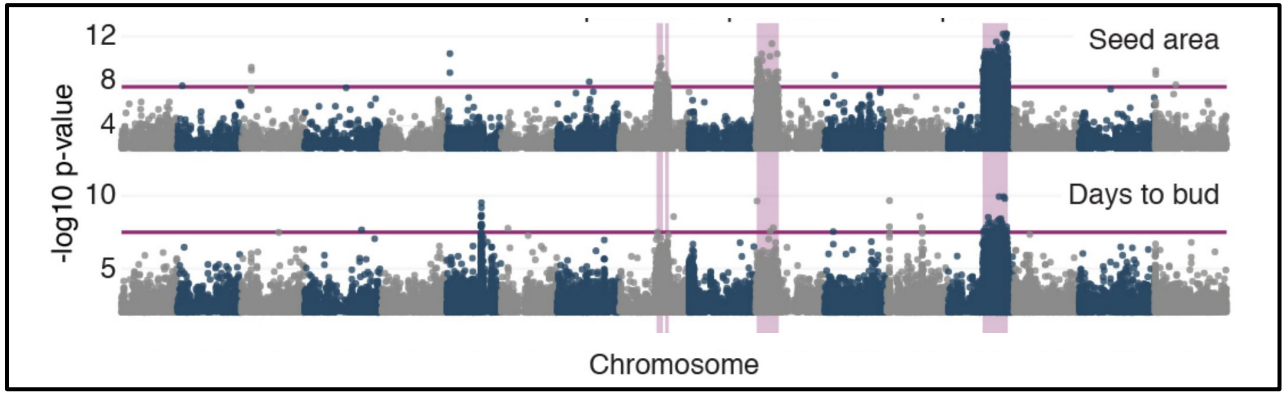
Adult-onset demyelinating leukodystrophy

Spielmann, Malte, Darío G. Lupiáñez, and Stefan Mundlos. "Structural variation in the 3D genome." *Nature Reviews Genetics* 19.7 (2018): 453-467.

SVs Impact Function

4. Recombination and cellular processes

SVs suppress recombination

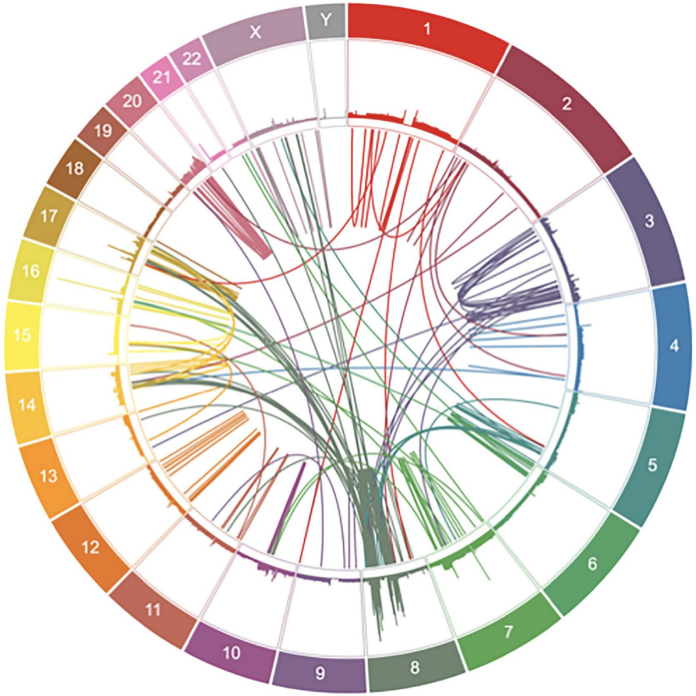


Todesco, Marco, et al. "Massive haplotypes underlie ecotypic differentiation in sunflowers." *bioRxiv* (2019): 790279.

SVs Impact Function

4. Recombination and cellular processes

Translocations in and SK-BR-3 breast cancer cell line



*SVs are prevalent in cancer cells

Nattestad, Maria, et al. "Complex rearrangements and oncogene amplifications revealed by long-read DNA and RNA sequencing of a breast cancer cell line." *Genome research* 28.8 (2018): 1126-1135.

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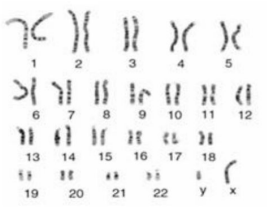
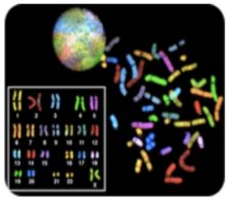
How to Find SVs

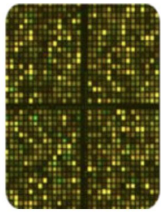
Our understanding of structural variation is driven by technology



1940s - 1980s
Cytogenetics / Karyotyping



1990s
CGH / FISH /
SKY / COBRA



2000s
Genomic microarrays
BAC-aCGH / oligo-aCGH

Today
High throughput
DNA sequencing

Long Read
DNA sequencing

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Whole genome alignment

1. Assemble a “query genome”

Nattestad, Maria, and Michael C. Schatz. "Assemblytics: a web analytics tool for the detection of variants from an assembly." *Bioinformatics* 32.19 (2016): 3021-3023.

Whole genome alignment

1. Assemble a “query genome”

2. Align the query to a reference genome with Nucmer or Minimap2

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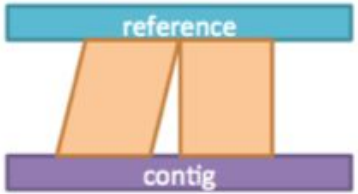

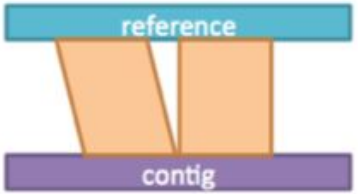

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Whole genome alignment

1. Assemble a “query genome”

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3. Infer SVs directly from the alignments

- **Tools**
  - **Assemblytics**
  - **Paftools.js**
  - **SyRI**

* Orange parallelograms represent alignments

Nattestad, Maria, and Michael C. Schatz. "Assemblytics: a web analytics tool for the detection of variants from an assembly." *Bioinformatics* 32.19 (2016): 3021-3023.

Whole genome alignment

Downsides

Nattestad, Maria, and Michael C. Schatz. "Assemblytics: a web analytics tool for the detection of variants from an assembly." *Bioinformatics* 32.19 (2016): 3021-3023.

Whole genome alignment

Downsides

- Assembly-to-assembly alignment is fallible
  - Sensitivity vs. specificity is hard to get right
  - Confounded by repeats
  - Alignment heuristics don’t always produce the best results (especially for plant genomes)
- Blind to some heterozygous SVs in unphased assemblies
- Genome assembly is hard!
  - Imperfections in assemblies lead to imperfections in the SV calls

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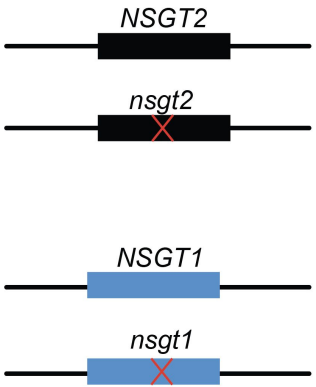
Alignment Free SV Calling

1. Instead of aligning whole genomes, just align smaller genomic elements, like genes. (e.g. with BLAST)

Various NSGT alleles



Alonge and Wang *et al*., unpublished

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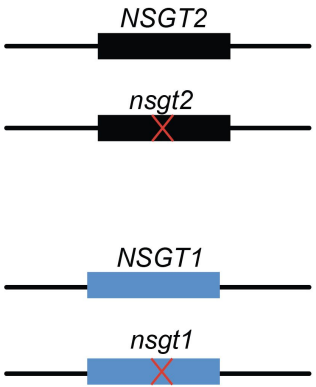

BLAST

5 different tomato assemblies

PAS014479

M82

BGV006775

Fla.8924

PI129033

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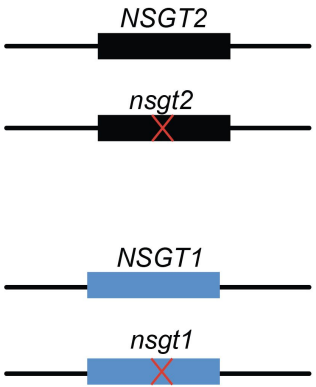


BLAST

5 different tomato assemblies

PAS014479

M82

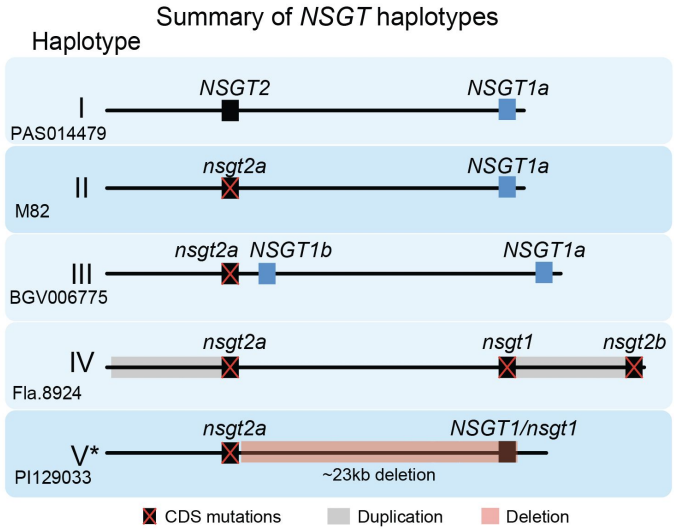
BGV006775

Fla.8924

PI129033

Manually Infer Haplotypes

NSGT CNVs



Alonge and Wang *et al*., unpublished

Alignment Free SV Calling

1. Instead of aligning w … (e.g. with BLAST)

Various NSGT alleles

NVs

SGT haplotypes

*** Aligning individual elements is time-consuming.**

This is usually done when you have a reason to suspect that a particular element may be structurally variable.

Alonge and Wang *et al.*, unpublished

Alignment Free SV Calling

2. Count k-mers to find duplications

1. Go through each k-mer

AACCGATTACAATCGGATTACATGTC

Alignment Free SV Calling

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AAC

Alignment Free SV Calling

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AACCGATTACAATCGGATTACATGTC
AAC
 ACC

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1. Go through each k-mer

AACCGATTACAATCGGATTACATGTC
AAC GAT ACA TCG ATT CAT
ACC ATT CAA CGG TTA ATG
CCG TTA AAT GGA TAC TGT
CGA TAC ATC GAT ACA GTC

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2. Store k-mer counts

AAC, 1	AAT, 1
ACC, 1	ATC, 1
CCG, 1	TCG, 1
CGA, 1	CGG, 1
GAT, 2	GGA, 1
ATT, 2	CAT, 1
TTA, 2	ATG, 1
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ACA, 2	GTC, 1
CAA, 1	

Alignment Free SV Calling

2. Count k-mers to find duplications

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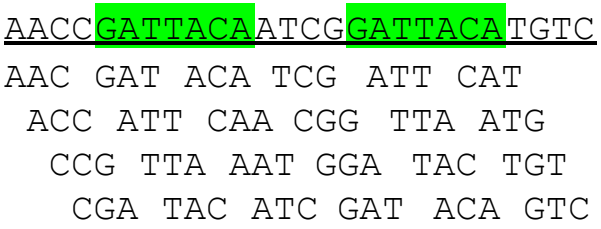
3. Assign a count to the k-mer starting at every offset



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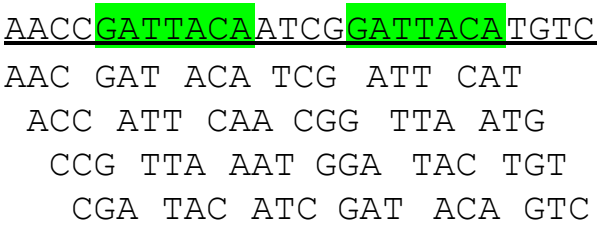

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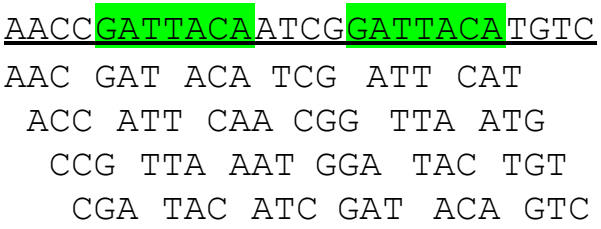

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CAA, 1	

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Alignment Free SV Calling

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AAC GAT ACA TCG ATT CAT

ACC ATT CAA CGG TTA ATG

CCG TTA AAT GGA TAC TGT

CGA TAC ATC GAT ACA GTC

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AAC, 1	AAT, 1
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GAT, 2	GGA, 1
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TTA, 2	ATG, 1
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ACA, 2	GTC, 1
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3. Assign a count to the k-mer starting at every offset

111122222111111222221111

AACCGATTACAATCGGATTACATGTC

Alignment Free SV Calling

2. Count k-mers to find duplications

1. Go through each k-mer

AACCGATTACAATCGGATTACATGTC

AAC GAT ACA TCG ATT CAT

ACC ATT CAA CGG TTA ATG

CCG TTA AAT GGA TAC TGT

CGA TAC ATC GAT ACA GTC

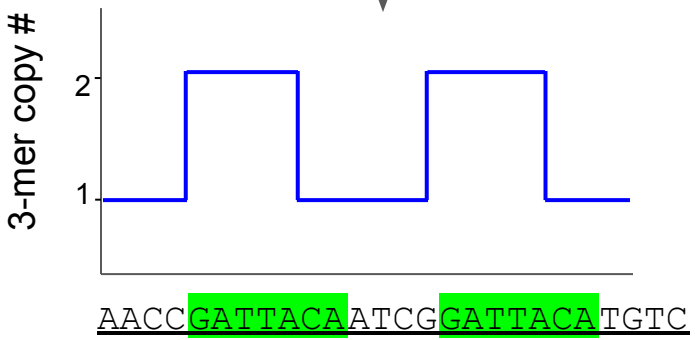
2. Store k-mer counts

AAC, 1	AAT, 1
ACC, 1	ATC, 1
CCG, 1	TCG, 1
CGA, 1	CGG, 1
GAT, 2	GGA, 1
ATT, 2	CAT, 1
TTA, 2	ATG, 1
TAC, 2	TGT, 1
ACA, 2	GTC, 1
CAA, 1	

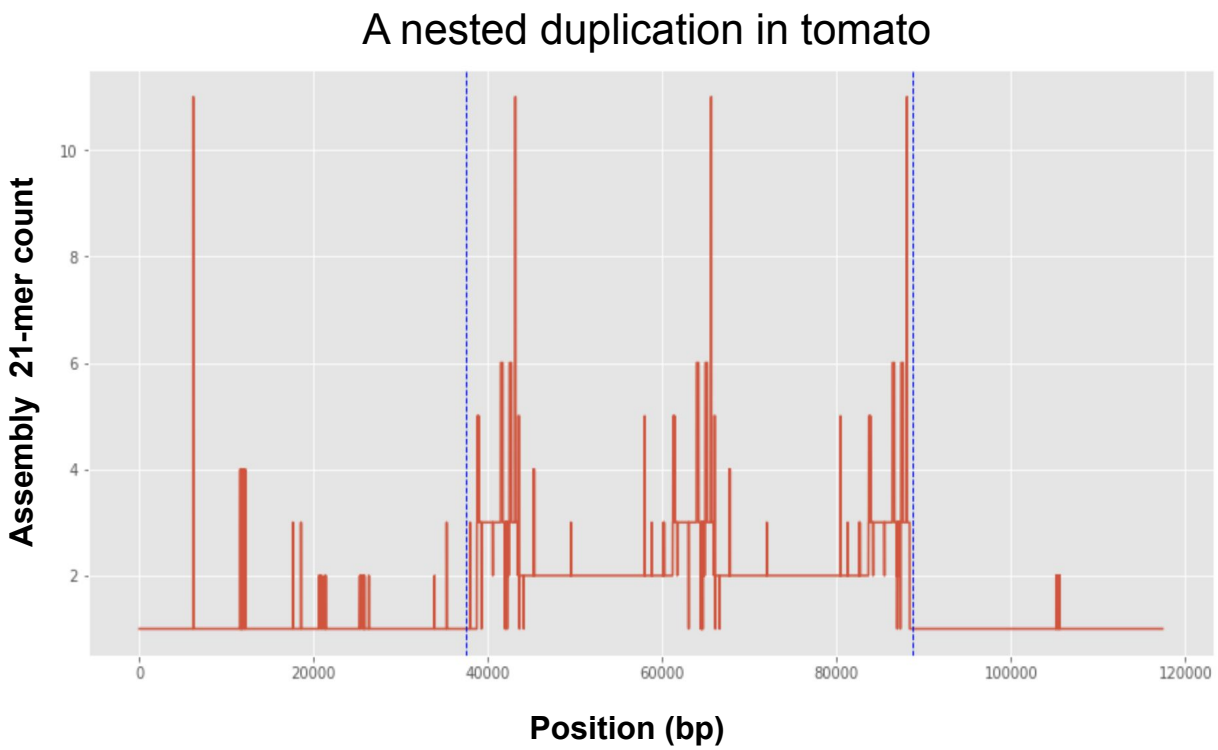
3. Assign a count to the k-mer starting at every offset

111122222111111222221111

AACCGATTACAATCGGATTACATGTC



Alignment Free SV Calling



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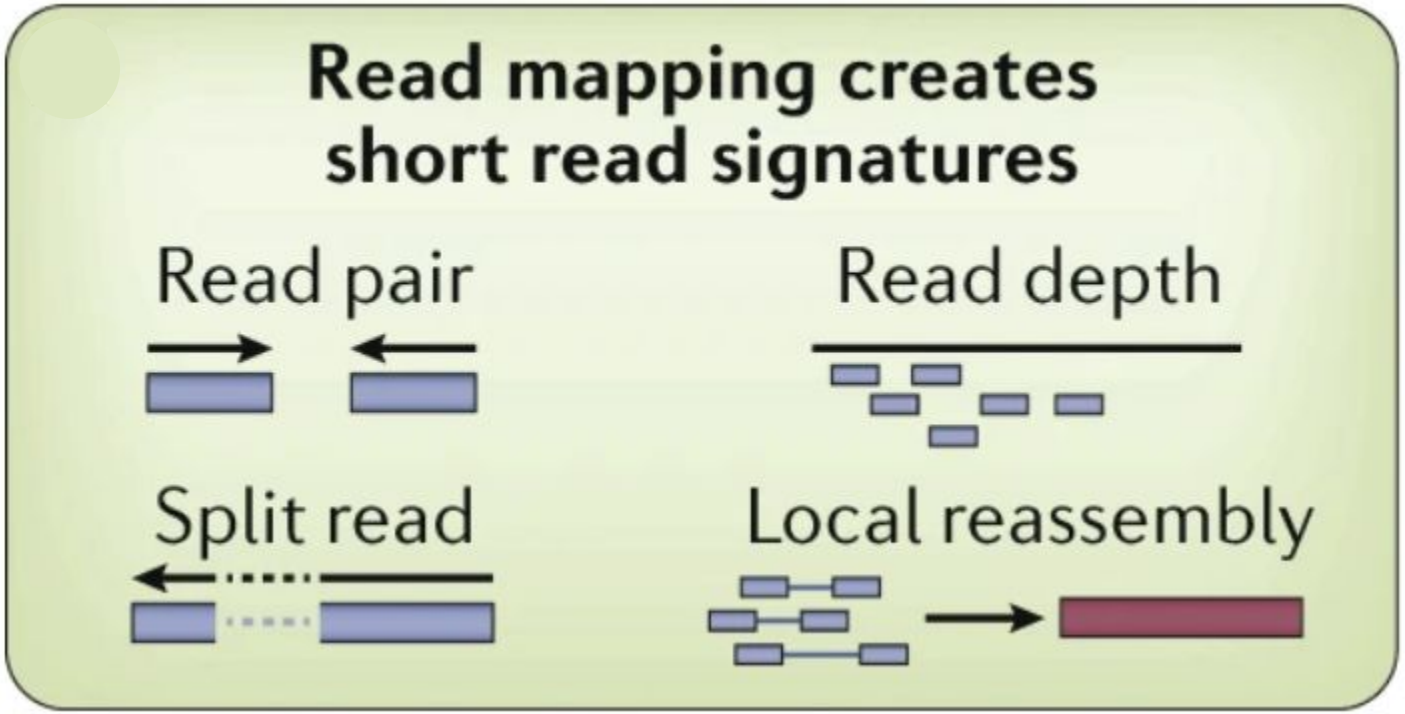
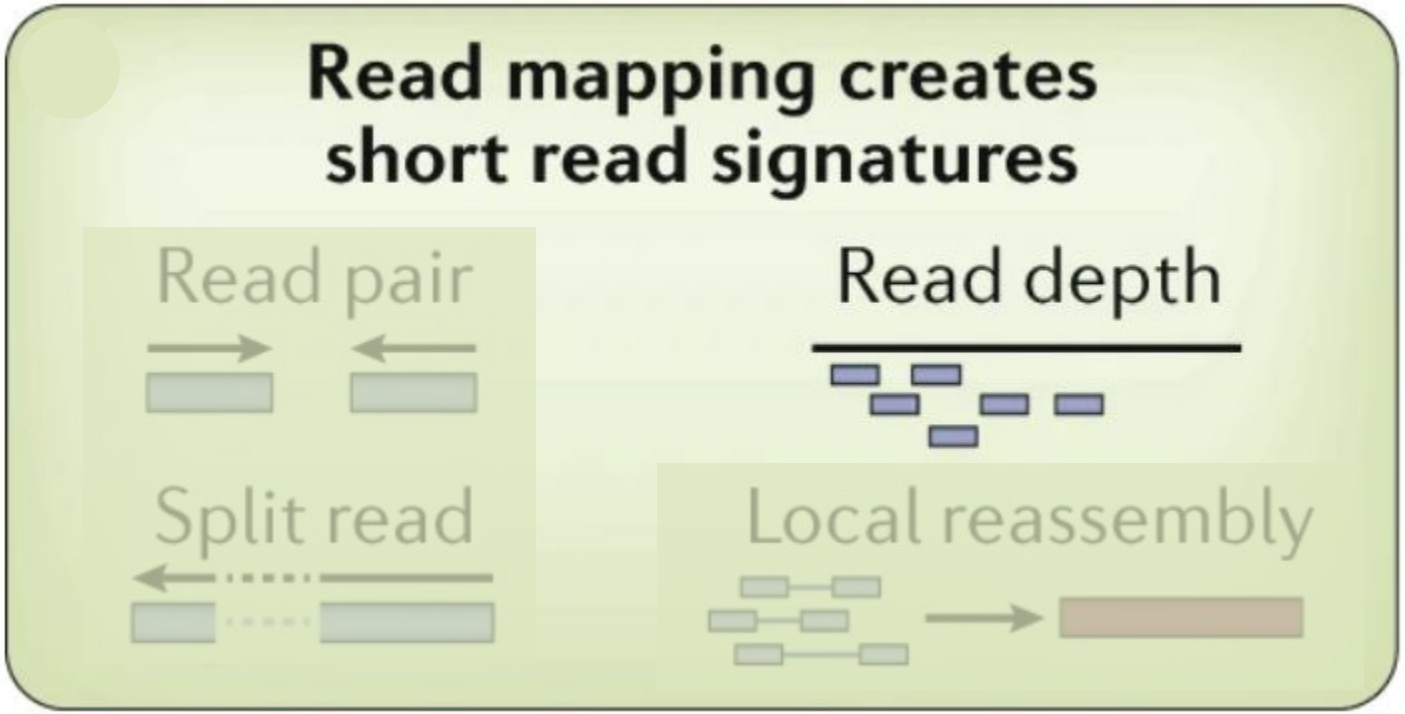
Short-read Mapping



Ho, Steve S., Alexander E. Urban, and Ryan E. Mills. "Structural variation in the sequencing era." *Nature Reviews Genetics* (2019): 1-19.

Short-read Mapping: coverage



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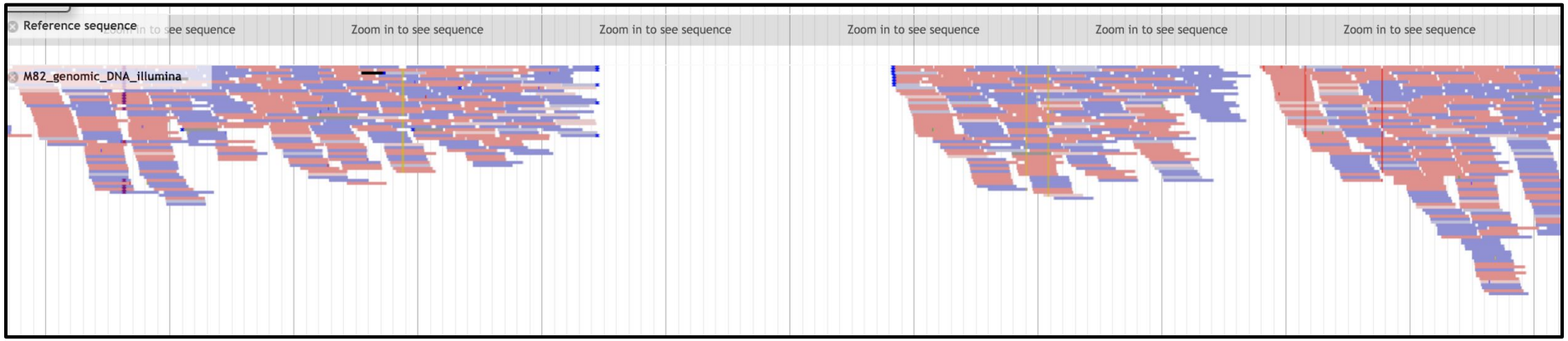
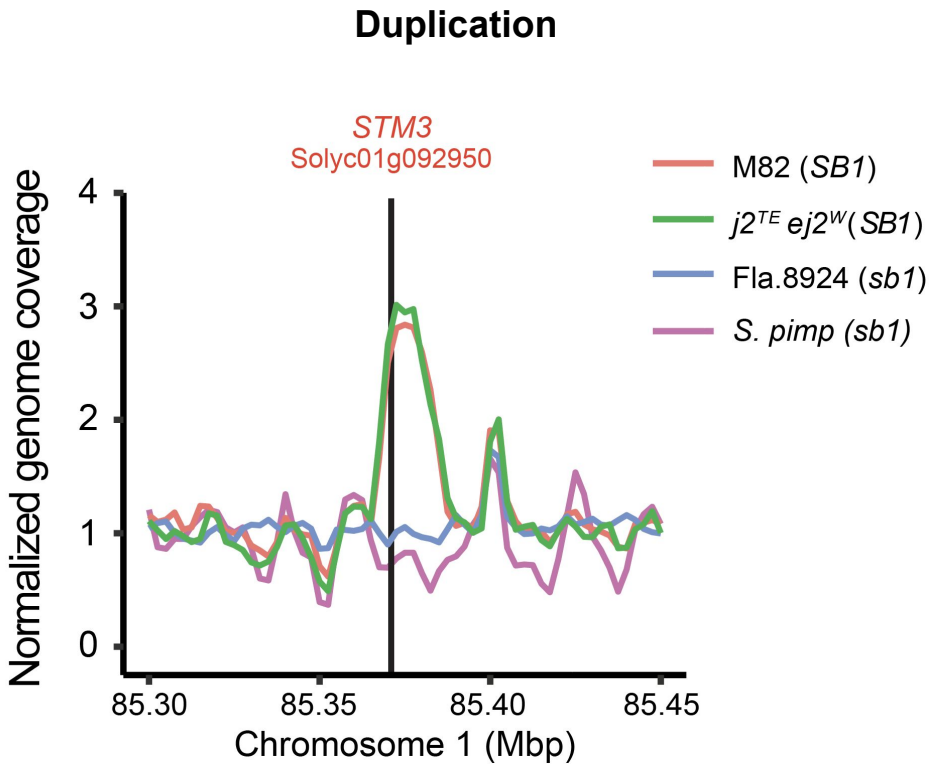
Short-read Mapping: Coverage

Deletion



Short-read Mapping: Coverage



Alonge and Wang *et al*., unpublished

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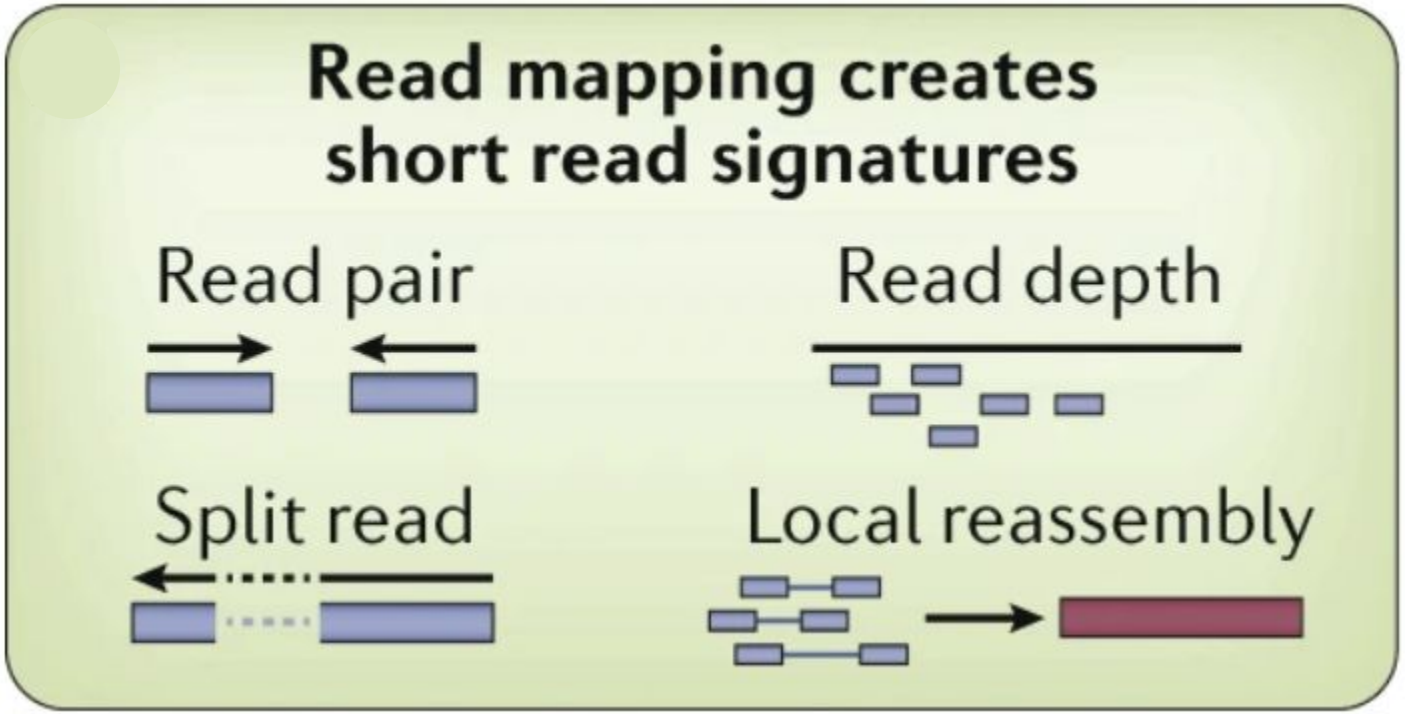

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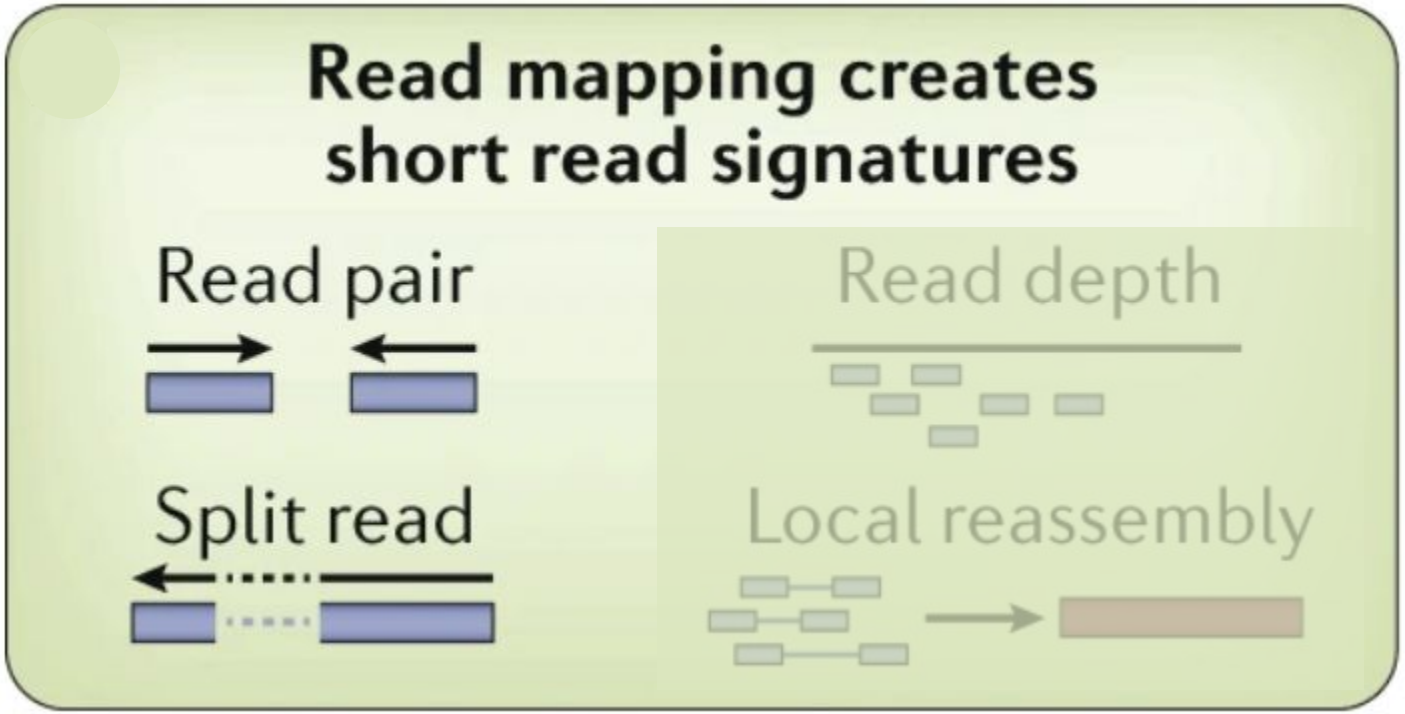
Short-read Mapping: Mates and Split-reads



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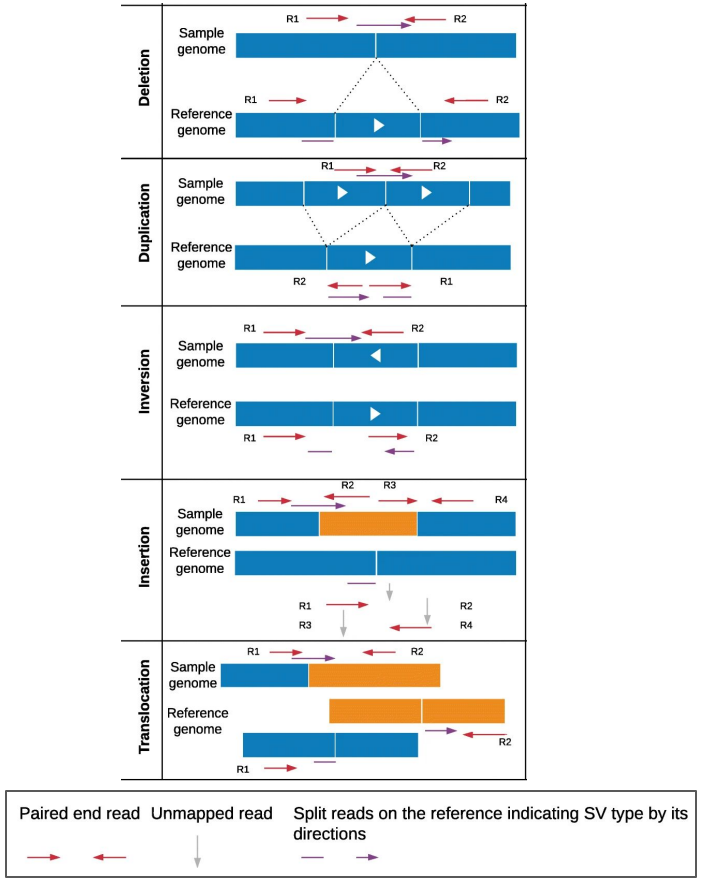

Mahmoud, Medhat, et al. "Structural variant calling: the long and the short of it." *Genome biology* 20.1 (2019): 246.

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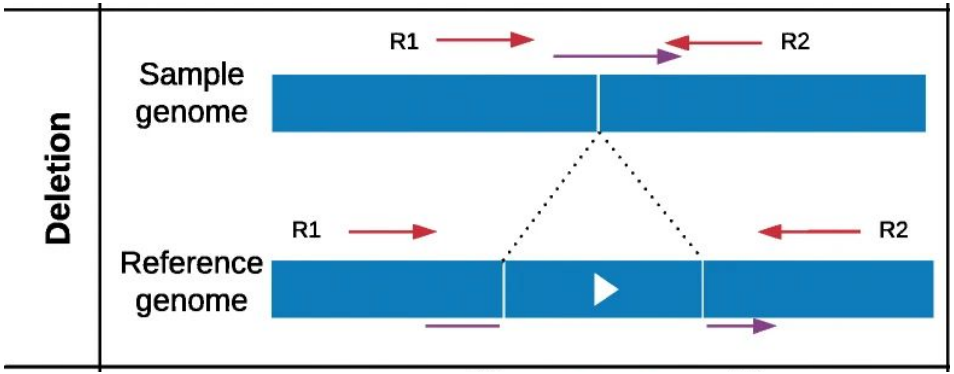

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Short-read Mapping: Downfalls

Coverage

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- Coverage can be affected by other factors aside from SVs
  - Reference bias
  - Repeats

Short-read Mapping: Downfalls

Coverage

- Coverage can be affected by other factors aside from SVs
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  - Repeats

Split-read/paired-end

- Reads are already short, so they must be split into very short fragments to produce split-read alignments.
  - These short fragments can produce unreliable alignments
- Discordant mate-pair alignments are often misleading

Short-read Mapping: Downfalls

Insertions

- Reads are usually too short to contain an insertion and anchor it to flanking sequence
- Supporting insertion reads (if you can find them) are hard to assemble into a proper insertion sequence
- Many short-read SV callers don’t even bother trying to call insertions

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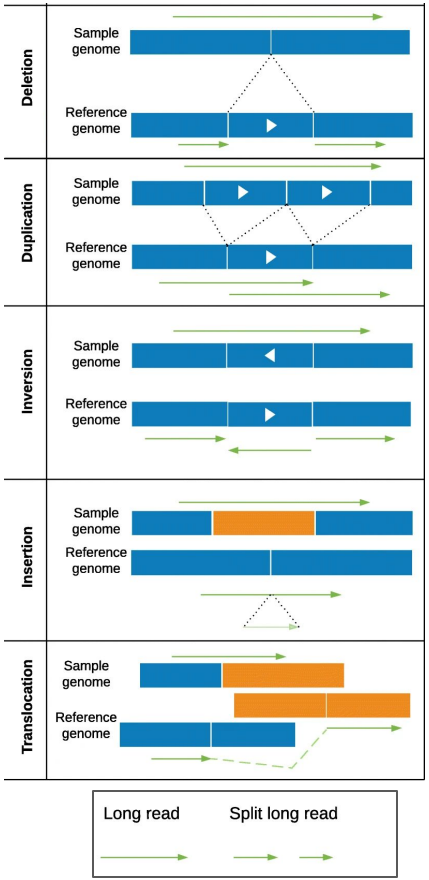
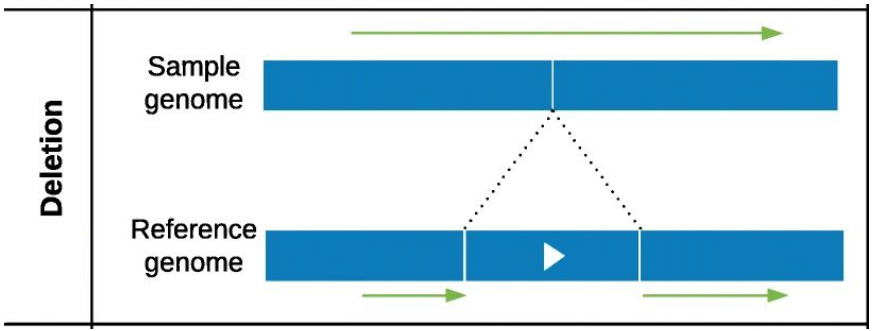
Long-read Mapping



* Each green arrow is the same long read (or a portion of that long read)

Mahmoud, Medhat, et al. "Structural variant calling: the long and the short of it." *Genome biology* 20.1 (2019): 246.

Long-read Mapping: Deletions



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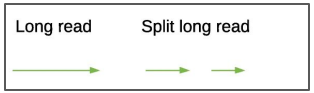

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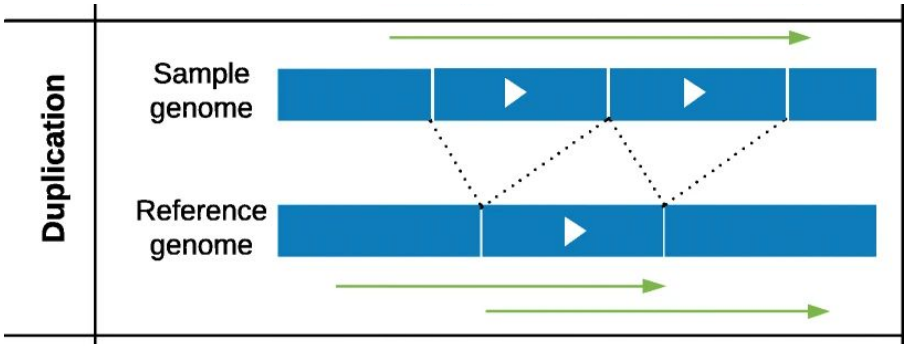
Long-read Mapping: Duplications



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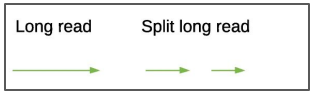

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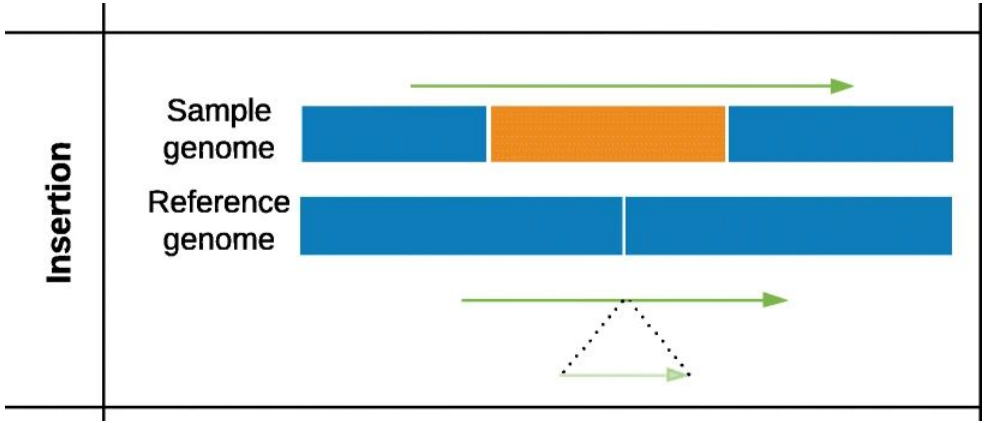
Long-read Mapping: Insertions



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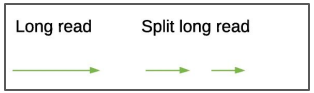

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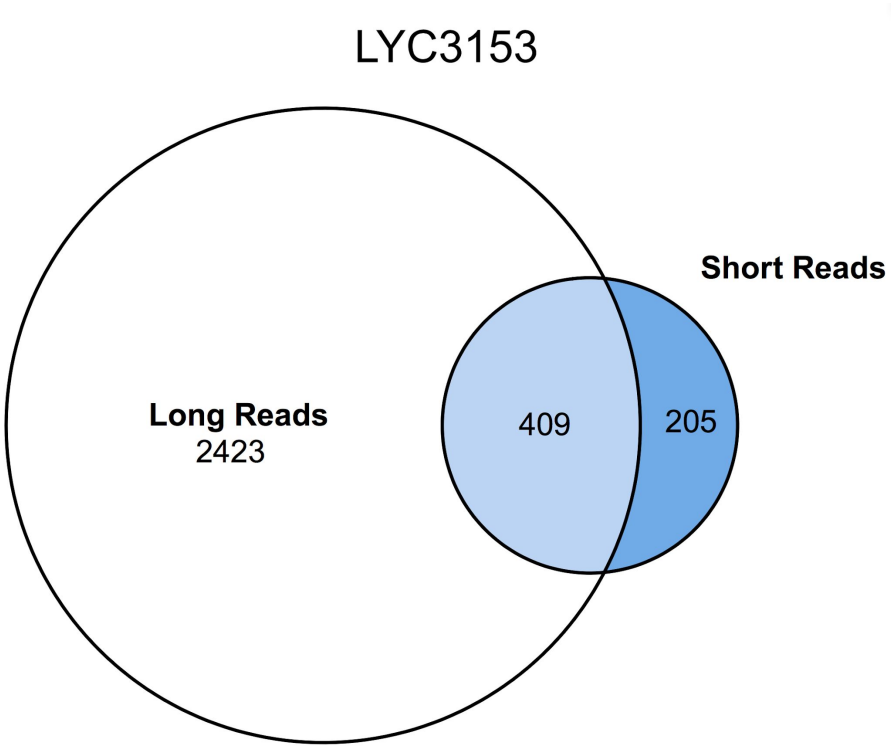
Long-Read Mapping > Short-Read Mapping

- Long-reads provide more sensitive SV calls

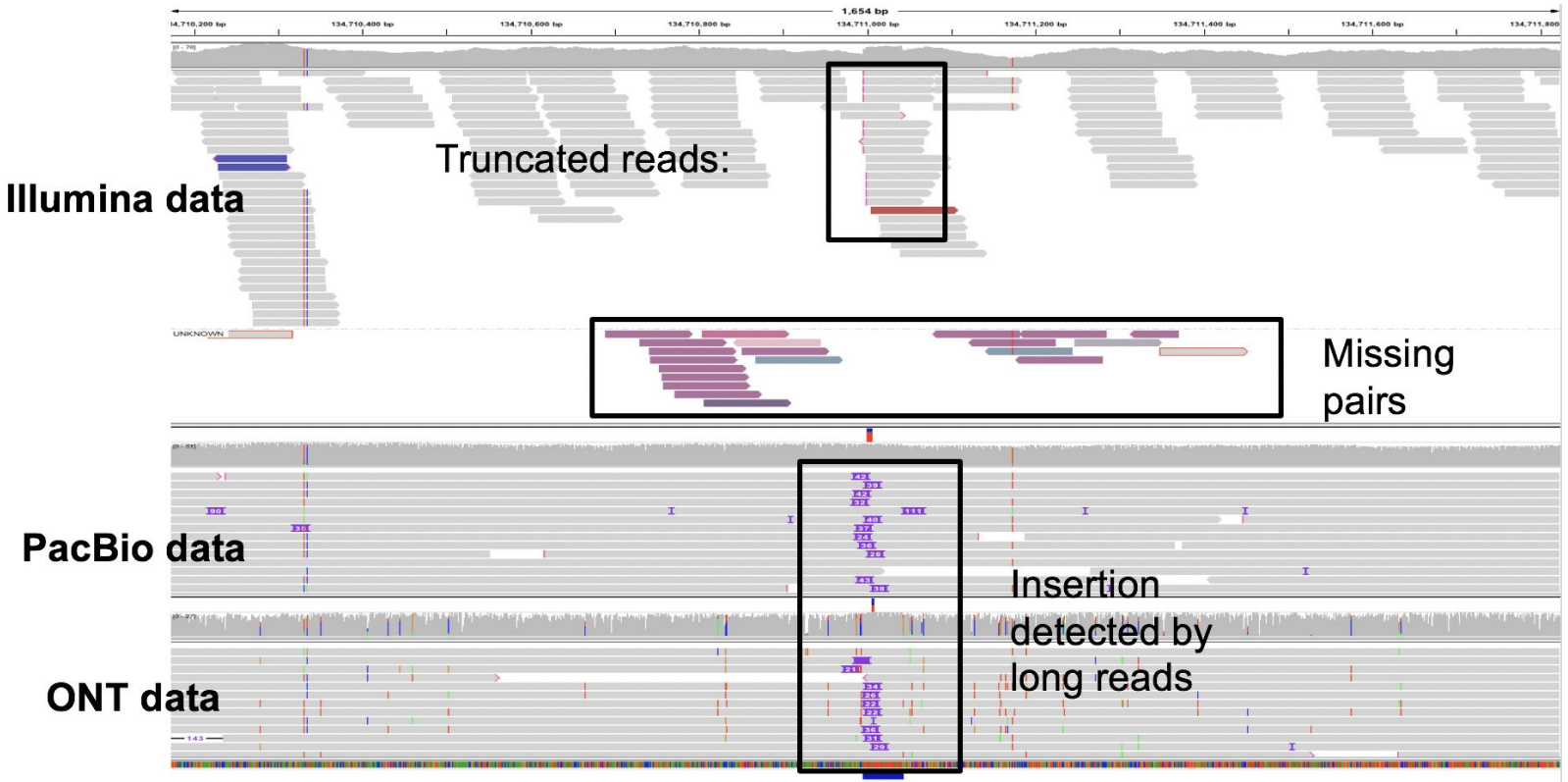


Alonge and Wang *et al*., unpublished

Long-Read Mapping > Short-Read Mapping

Accurate detection of complex structural variations using single molecule sequencing
Sedlazeck, Rescheneder et al (2017) *bioRxiv https://doi.org/10.1101/169557*

Long-Read Mapping > Short-Read Mapping



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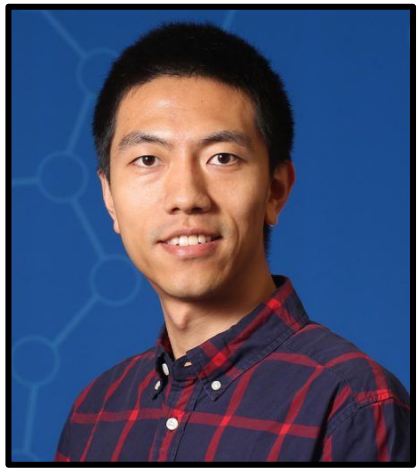
Applications: Tomato

Zach Lippman
CSHL/HHMI

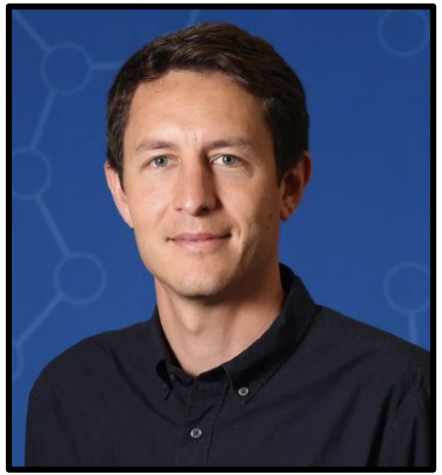
Matthias Benoit
Postdoc, CSHL

CSH Cold Spring Harbor Laboratory



Xingang Wang
Postdoc, CSHL

Sebastian Soyk
Asst. Professor, UNIL
(formerly postdoc, CSHL)



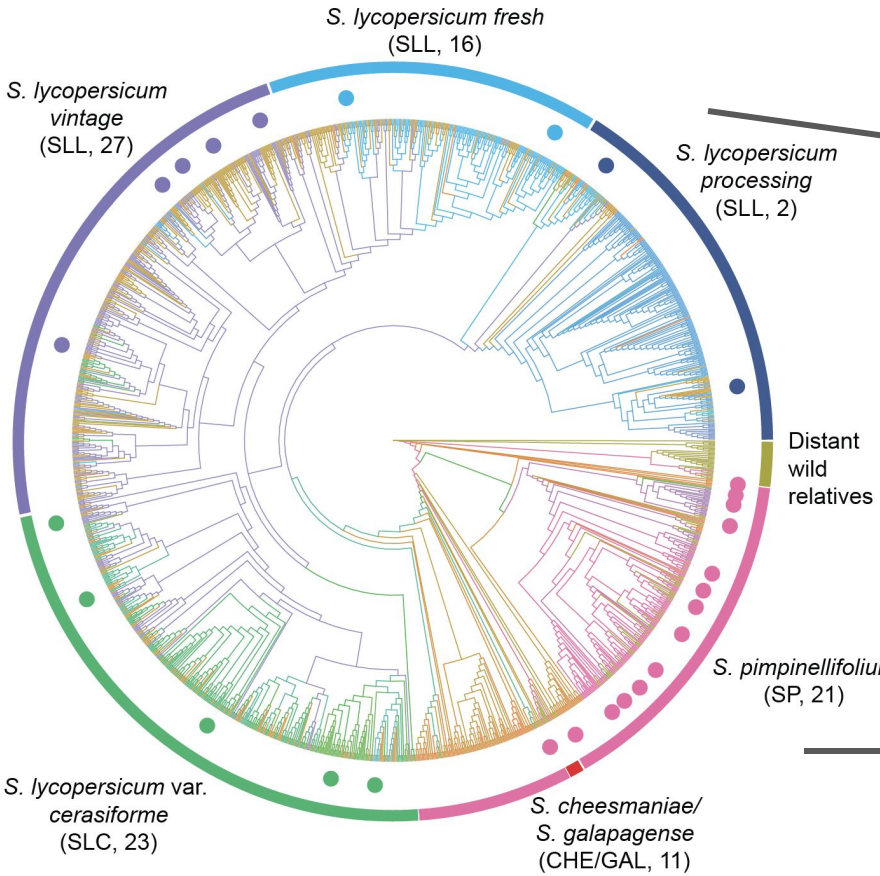
Tomato is an Important Model and Application

- **Naturally self-fertilizing**
- **Diploid**
- **Amenable to transformation**
  - **Gene editing with Cas9 well demonstrated.**
- Medium genome size (1 Gbp)
- Short life cycle (90 - 100 days)
- Amenable to cross-hybridization
  - Introgression Line (ILs) Populations
- Robust genetic/genomic resources
  - High-quality reference genome
  - Population-scale DNA and RNA seq databases.
  - Extensive mutant germplasm

- $50 billion industry
- Major source of nutrients

Achieving sustainable cultivation of tomatoes, Mattoo & Handa (2017)

Tomato Domestication

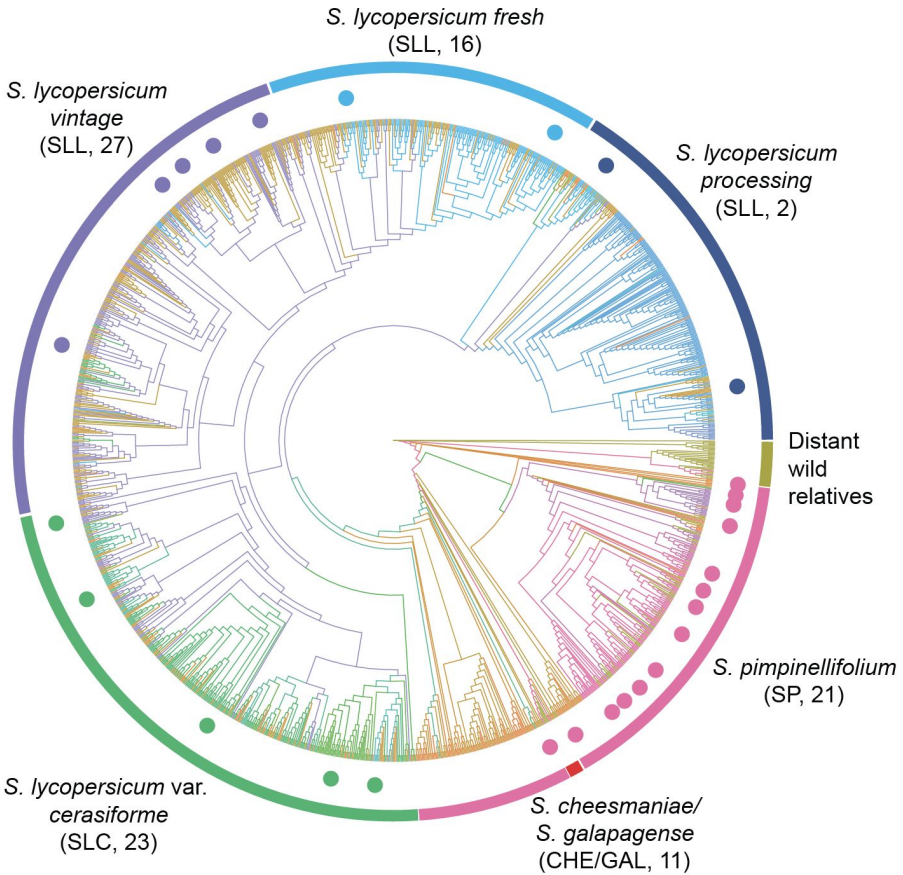


Alonge and Wang *et al*., unpublished

Modern

Wild Progenitor

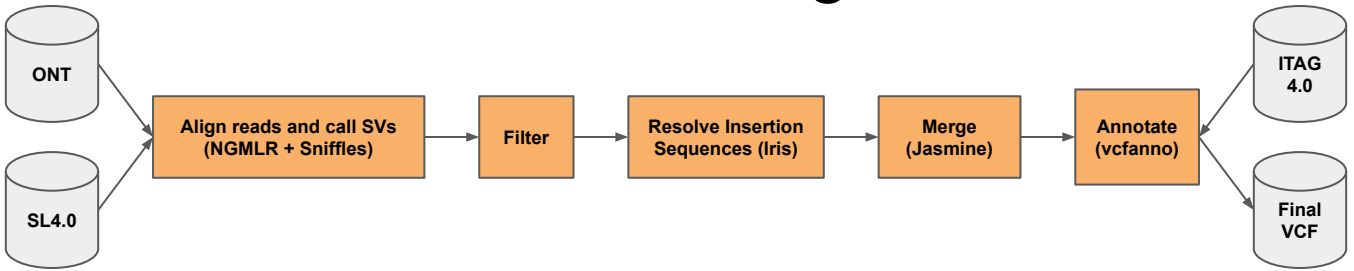
Sample Selection and Sequencing

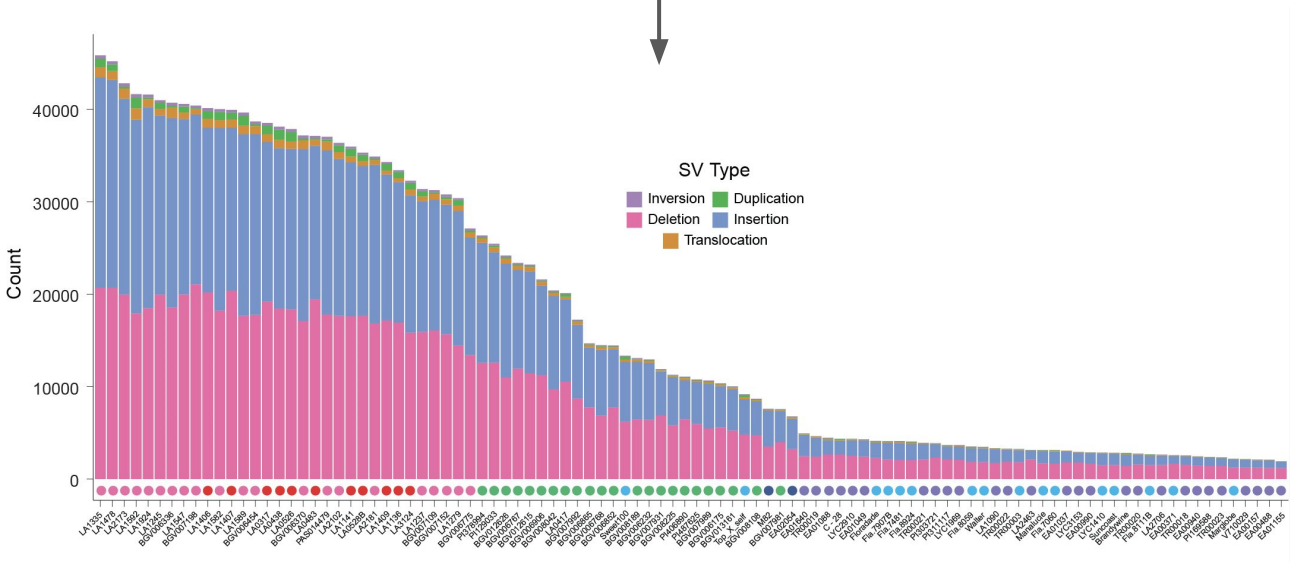


Alonge and Wang *et al*., unpublished

x 100

SV Calling

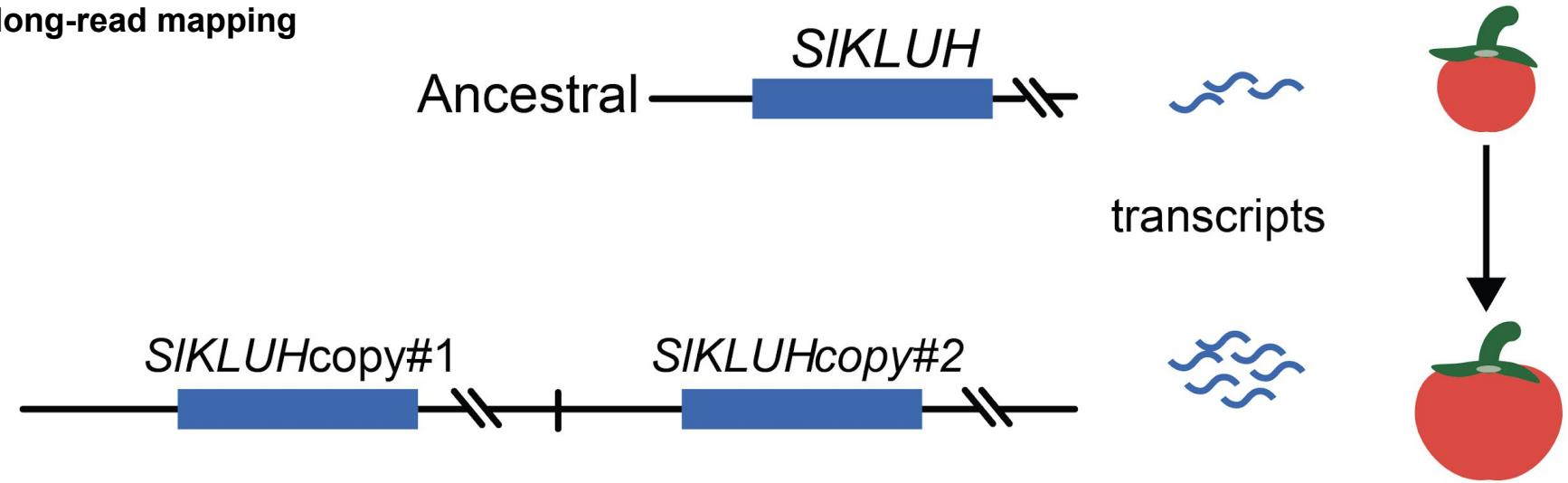




Alonge and Wang *et al*., unpublished

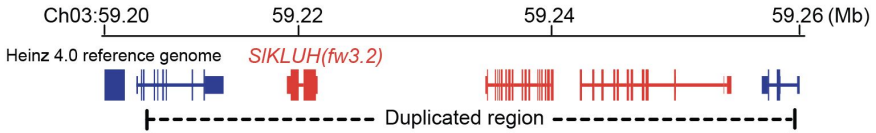
A Duplication Underlies a Fruit Weight QTL

*** Found with long-read mapping**



Alonge and Wang *et al*., unpublished

SVs Impact Gene Expression



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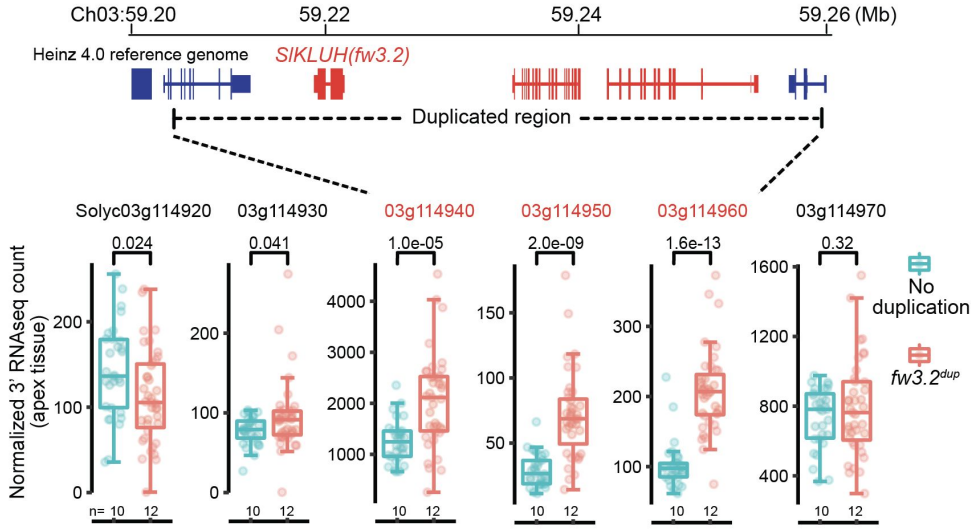

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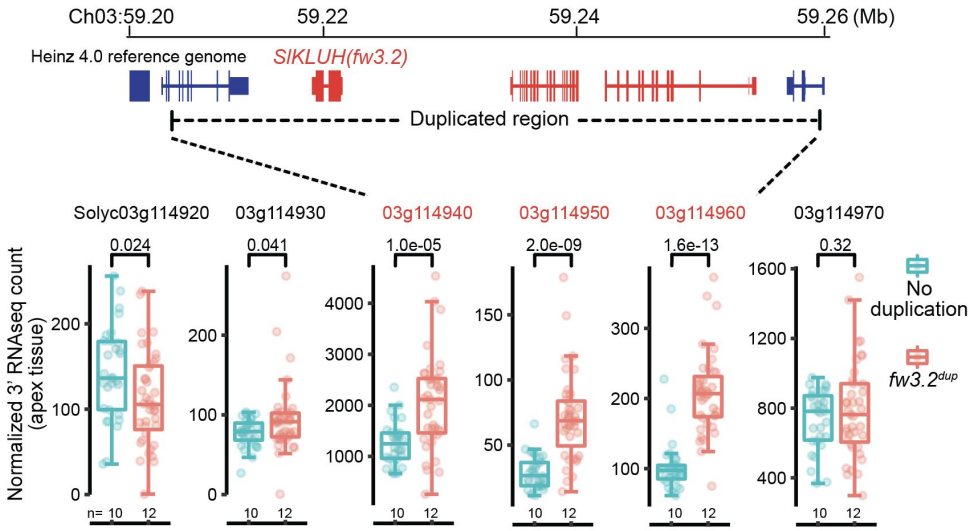

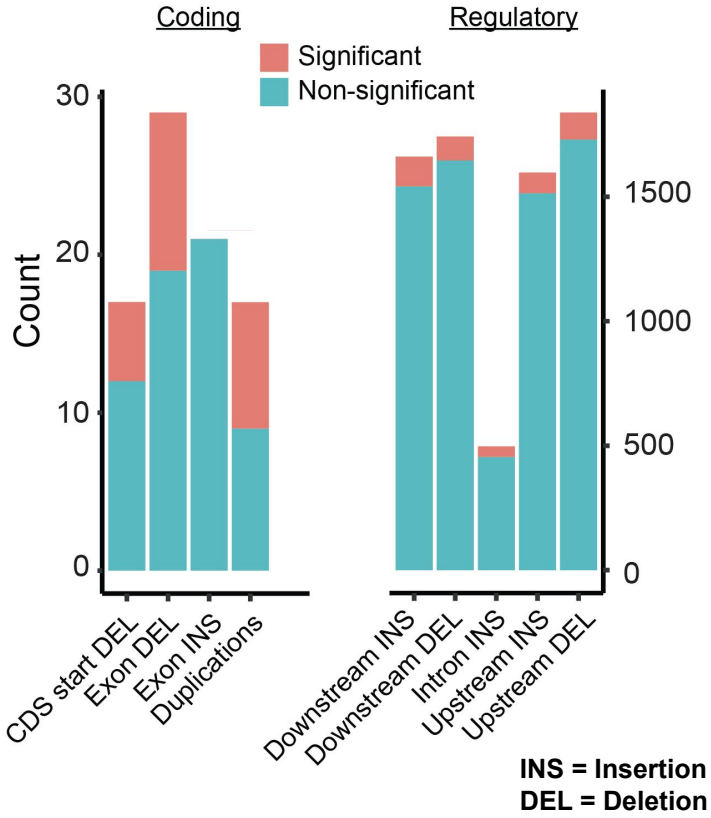

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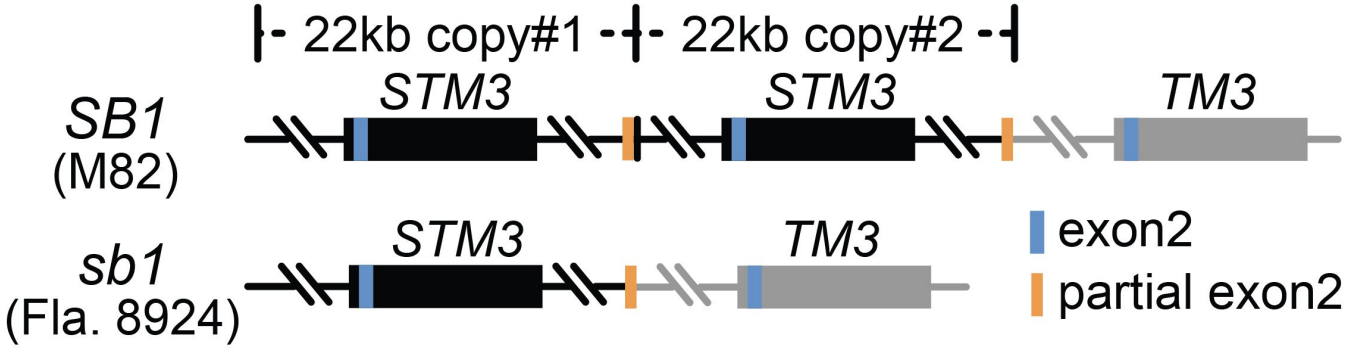
A Nested Duplication Influences Flowering

* Detected with
“alignment free”
assembly methods



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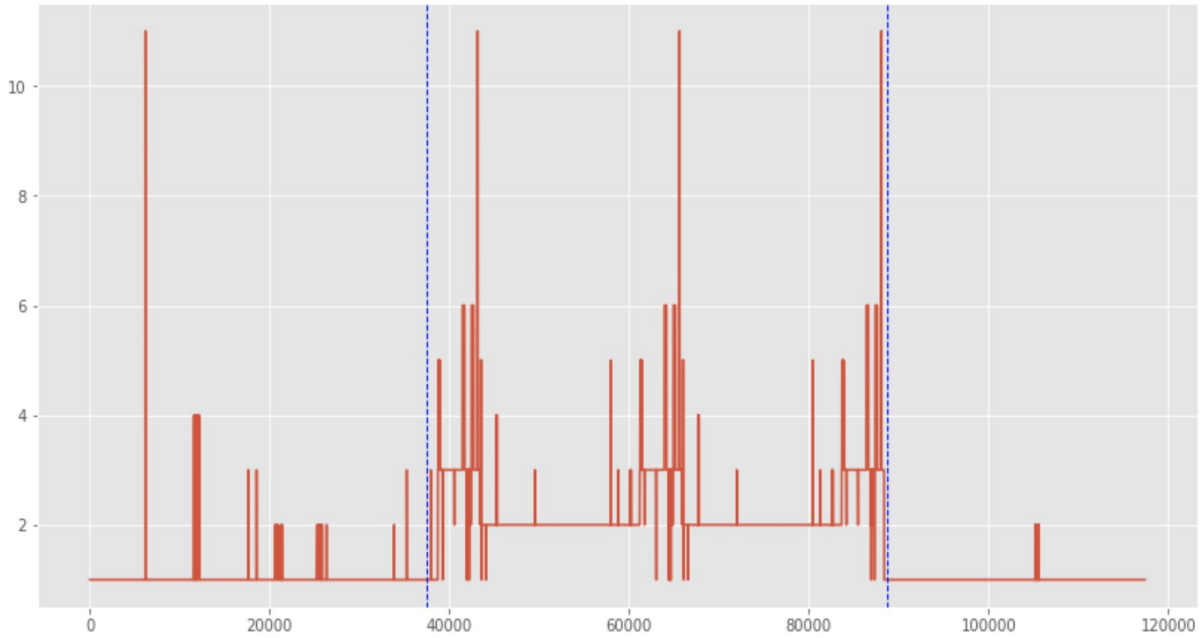

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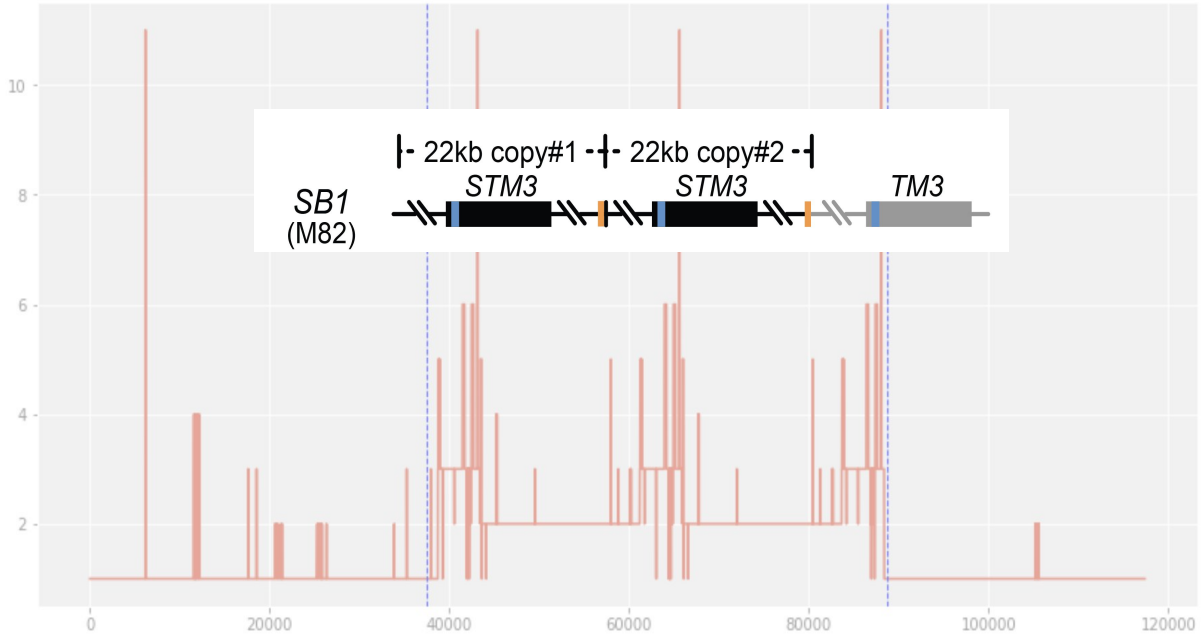

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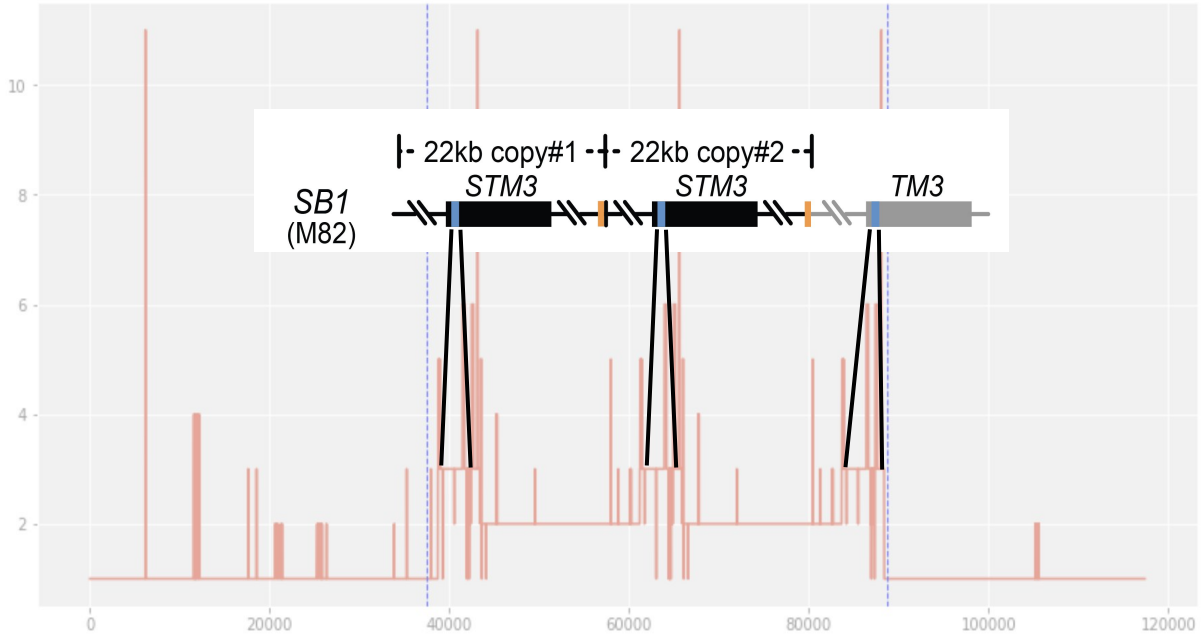

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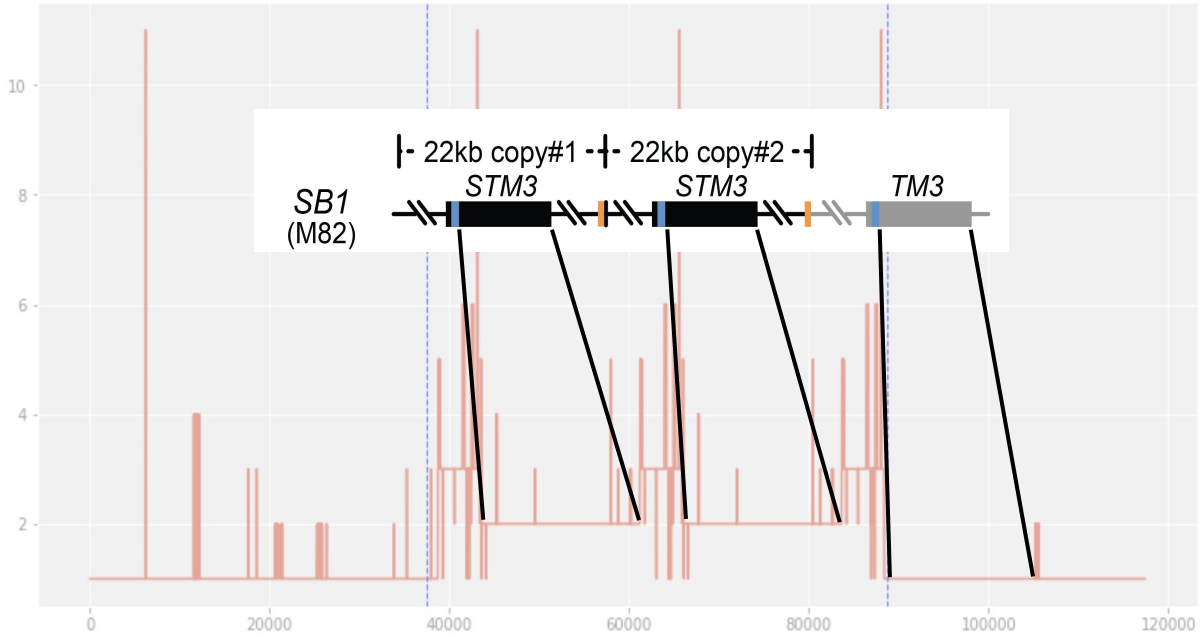

Conclusions: Why does this matter?

- SVs comprise a substantial portion of the natural genetic variation that we see in eukaryotes, both in terms of count and total bp.
- SVs underlie many of the traits that we care about:
  - Plant domestication and breeding QTL
  - Human diseases
- SVs impact cellular function and can shape evolution

Conclusions: Pan-genomics

- There is a lot of genomic “structure” in a population that is not captured by a single reference genome or a few reference genomes.
- Uncovering this structure has broad research impact. E.g.:
  - Helping the utility of resequencing experiments with a pan-genome graph-like datastructure.
    - Reduce reference bias
  - Discover more natural alleles!!!!
    - More assemblies reveal more potentially functional alleles
    - Assembly will probably replace WGS resequencing experiments for variant calling.