Nanopore Community Meeting 2018

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100 GENOMES IN 100 DAYS: THE STRUCTURAL VARIANT LANDSCAPE OF TOMATO GENOMES

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TOMATO AGRICULTURE & ANALYSIS

Tomato is one of the most valuable crops in the world

- Originally from South America, transported to Europe by early explorers in the 17th century, and then back to North America in 18th century
- Annual production >175 million tons & $85B US
- Major ingredient in many common foods:
  - Sauces, salsa, ketchup, soups, salads, etc

Tomatoes are an important plant model system

- Extensive phenotypic variation: >15,000 known varieties
  - Model for studying fruiting, taste, branching, morphology
- Member of important Solanaceae family
  - Potato, pepper, eggplant, tobacco, petunia, etc

TOMATO GENOMICS AND GENETICS

Tomato Reference Genome published in May 2012

- International consortium from 14 countries requiring years of effort and millions of dollars
  - Sanger + 454 + fosmids + BAC-ends + genetic map + FISH
- ‘Heinz 1706’ cultivar (v3)
  - 12 chromosomes, 950 Mbp genome, diploid
  - 22,707 contigs, 133kbp contig N50, 80M ’Ns’
  - 20Mb on “chromosome 0”
- Resource for thousands of studies
  - Candidate SNPs for many traits identified through GWAS
  - Candidate genes and pathways through RNAseq
  - Extensive investment into agricultural traits: ripening, fruit size, color, morphology

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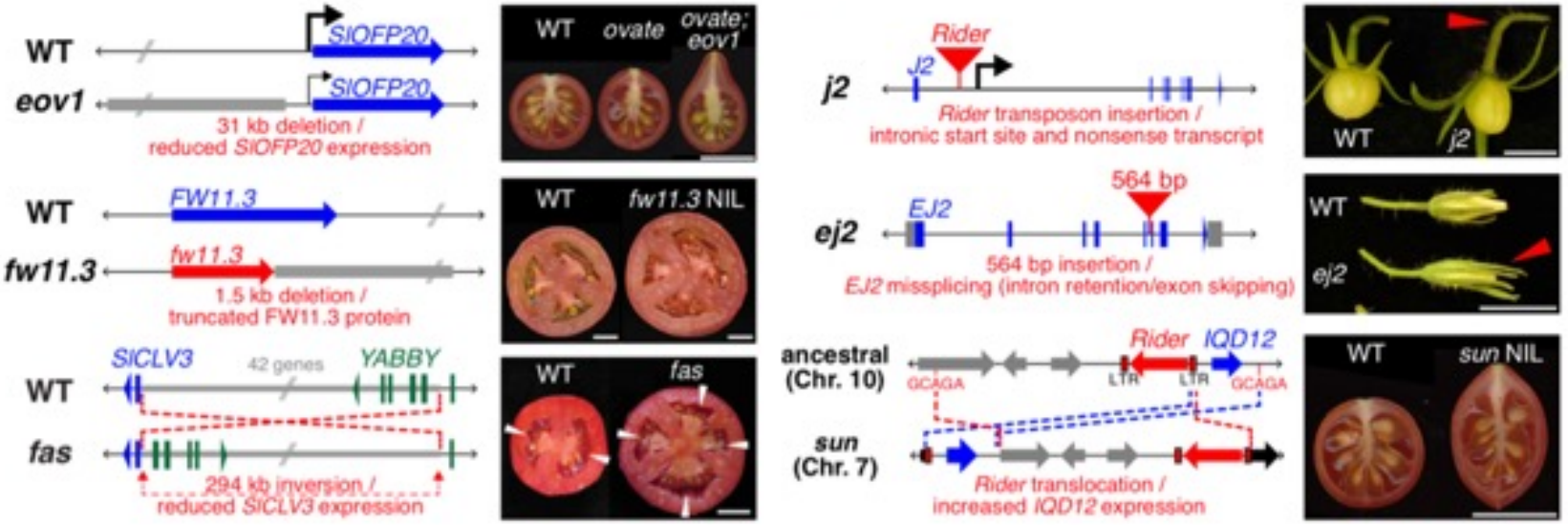
STRUCTURAL VARIATIONS ARE DRIVERS OF QUANTITATIVE VARIATIONS



Recent results highlight structural variations to play a major role in phenotypic differences

- SV are any variants >50bp: insertions, deletions, inversions, duplications, translocations, etc
- Adds, removes, and moves exons, binding sites, and other regulatory sequences
- Notoriously difficult to identify using short reads: high false positive & false negative rate

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STRUCTURAL VARIANT LANDSCAPES IN TOMATO GENOMES AND THEIR ROLE IN NATURAL VARIATION, DOMESTICATION AND CROP IMPROVEMENT

Zach Lippman
CSHL / HHMI

Joyce Van Eck
Boyce Thompson

Esther van der Knaap
Univ. of Georgia

Fritz Sedlazeck
Baylor

Sara Goodwin
CSHL

Project overview

1. Select diverse samples
2. Long read sequencing
3. Per-sample SV identification
4. Pan-tomato SV landscape
5. Identify and validate SVs associated with agricultural and phenotypic traits

01

SAMPLE SELECTION

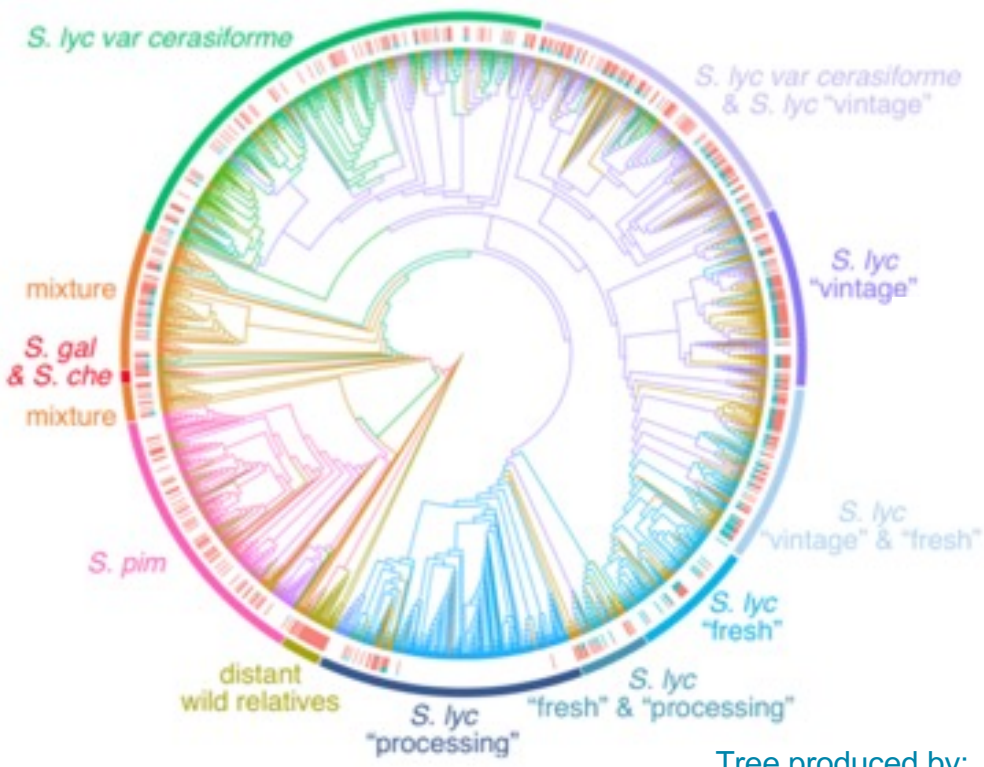
TOMATO POPULATION GENETICS

More than 900 varieties of tomato and related species have been sequenced

- Most are between 20x and 40x coverage
- SNP-based phylogenetic tree shows 10 major clades

Initial examination of SVs

- Identify variants using an aggregation of three individual methods to improve sensitivity
  - Lumpy, Manta & Delly
- Consensus calls using SURVIVOR (Jeffares *et al*, *Nature Communications*, 2017)
  - Retain calls supported by ≥ 2 callers
  - Reduces false-positives while not worsening false-negatives



Tree produced by:
Jose Jimenez-Gomez

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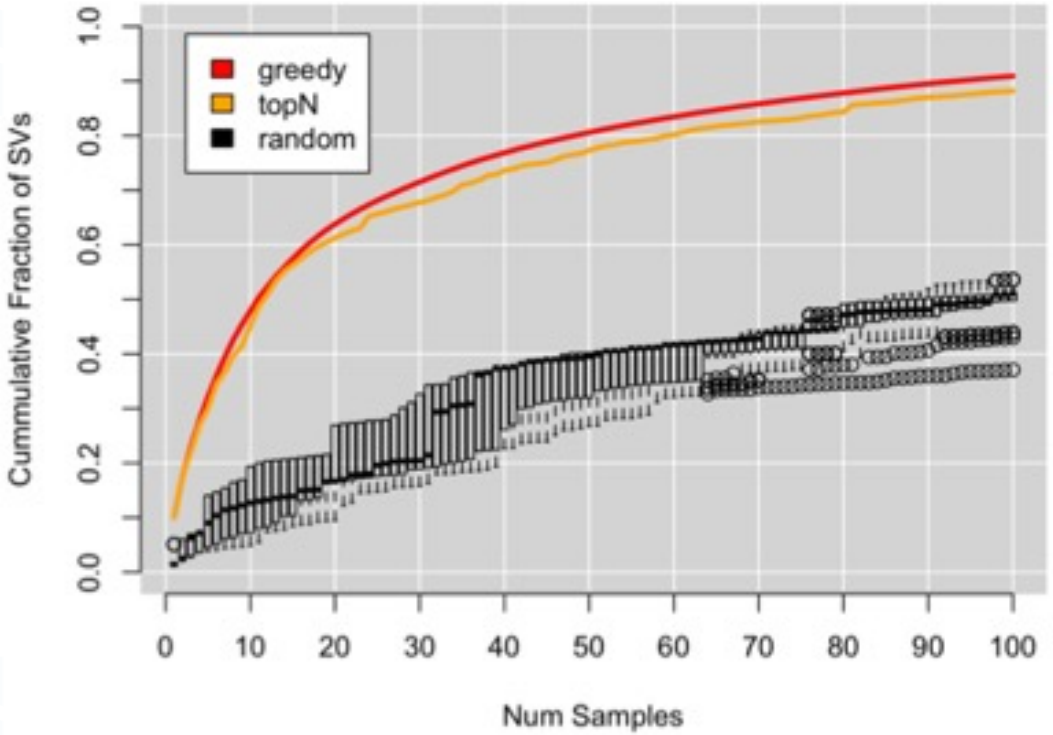
OPTIMIZED SAMPLE SELECTION



Our goal is to select the 100 samples that *collectively* capture the most diversity

- Short-read based SVs will under-sample variants but still represents relative diversity
- Selecting 100 at ***random only recovers about 40%*** of the total known diversity
- Ranking samples by number of variants picks diverse samples, although tends to pick siblings (nearly duplicate samples)
- ***Optimal strategy is NP-hard*** using a set-cover algorithm, we ***approximate using a greedy approach***

SVCollector: Optimized sample selection for validating and long-read resequencing of structural variants
Sedlazeck *et al* (2018) bioRxiv doi: https://doi.org/10.1101/342386

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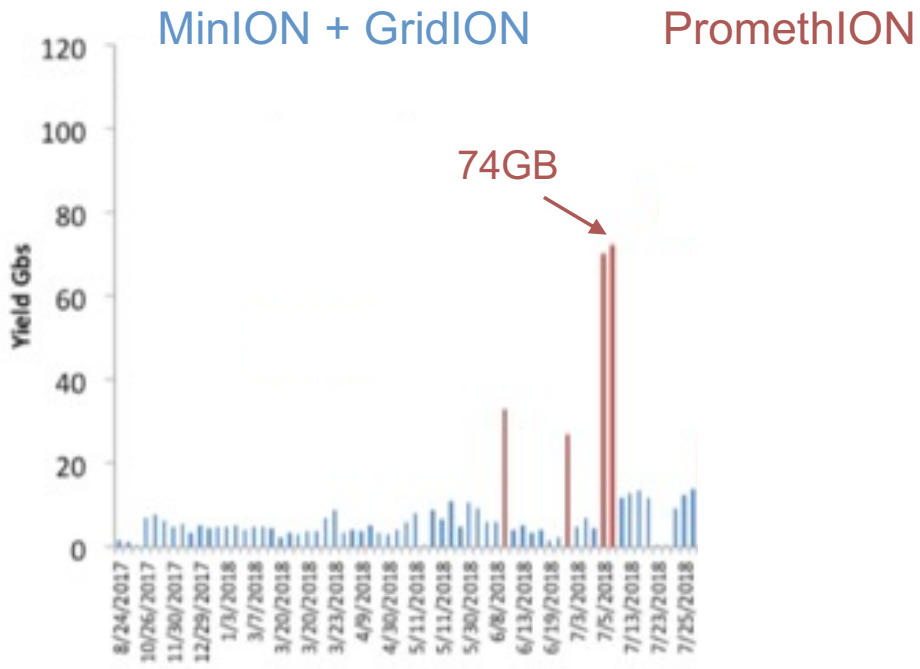
02

LONG READ SEQUENCING

NANOPORE PERFORMANCE AT CSHL

Sara Goodwin



Sequencing strategy

- Initial proposal called for mix of short, long, and linked read sequencing
- MinION and GridION became feasible spring/summer 2018
- Encouraging PromethION yields on test runs mid-summer 2018 motivated switch in strategy

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NANOPORE PERFORMANCE AT CSHL

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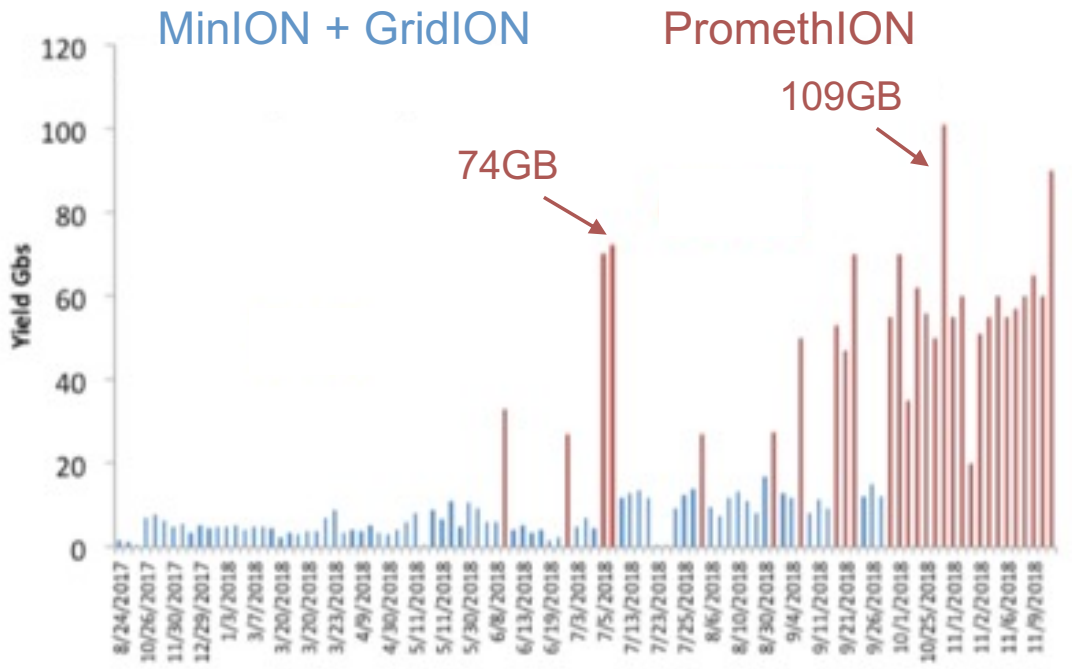

Sequencing strategy

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Currently running 6 to 8 PromethION flow cells in parallel twice a week

- First 34 samples have been sequenced to at least 40x coverage
- Now at 12 to 16 samples per week
  - 100 samples in <100 days

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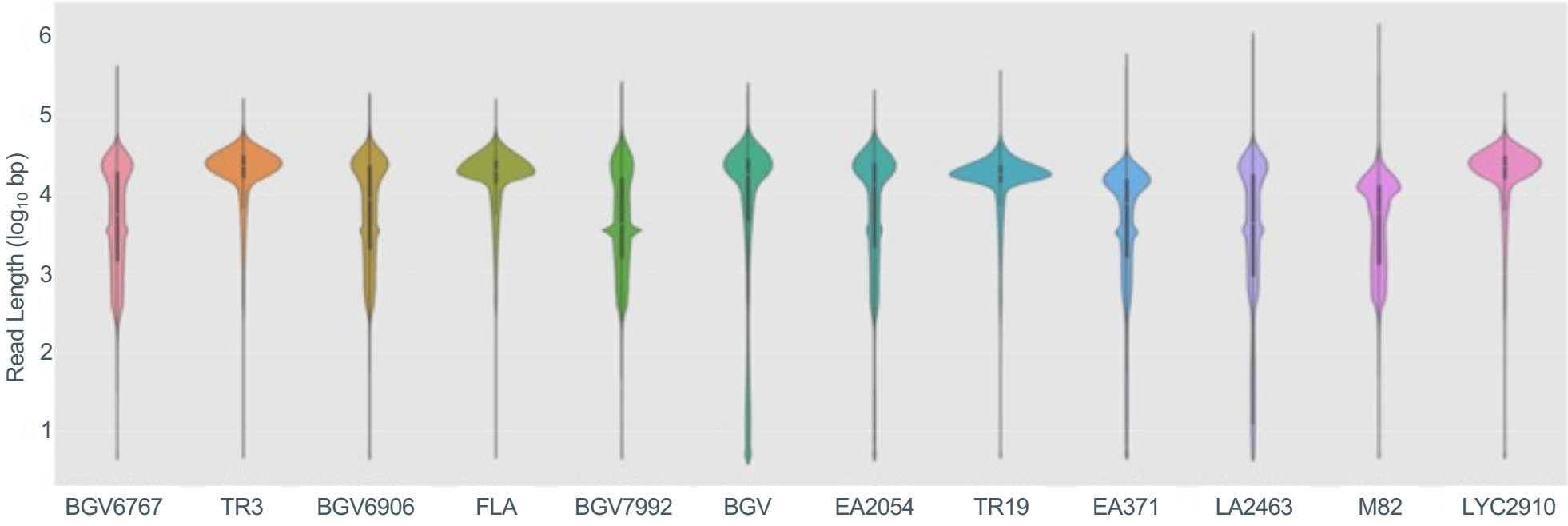
NANOPORE READ LENGTHS

Optimized Sequencing strategy

- Fragmentation at 30kbp using the Megarupter
- 109 Ligation Sequencing Kit yields both long reads and high yield

Very long reads with PromethION

- Mean read length: 10kbp – 20kbp
- Read length N50: 15kbp – 30kbp
- Over 20x coverage of reads over 20kbp



DATA MANAGEMENT

High throughput of PromethION has introduced some new IT challenges

- Upgraded the fiber connection between the sequencing lab and the data center
- Substantial storage requirements
- Substantial load on filesystem to manage hundreds of millions of fast5 files

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03

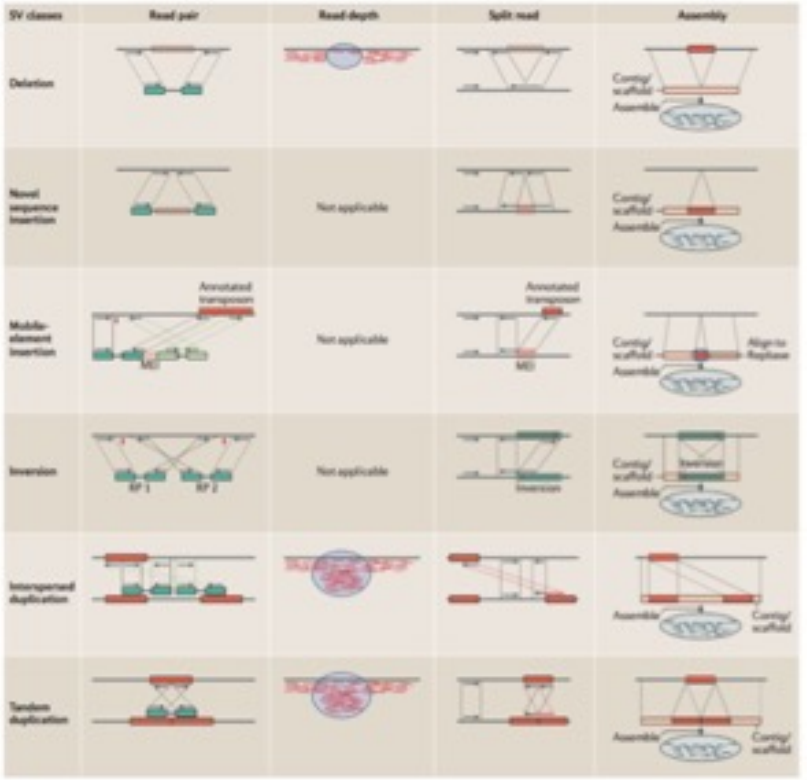
STRUCTURAL VARIATION ANALYSIS

STRUCTURAL VARIATION ANALYSIS

Two major strategies for detection

- ***Alignment-based detection***
  - Split-read alignment to detect the breakpoints of events
  - Fast, accurately identifies most variants, including heterozygous variants
  - Very long insertions may be incomplete

- ***Assembly-based detection***
  - De novo assembly followed by whole genome alignment
  - Can capture novel sequences and other complex variants
  - Slow, demanding analysis, limited by contig length, heterozygous variants challenging

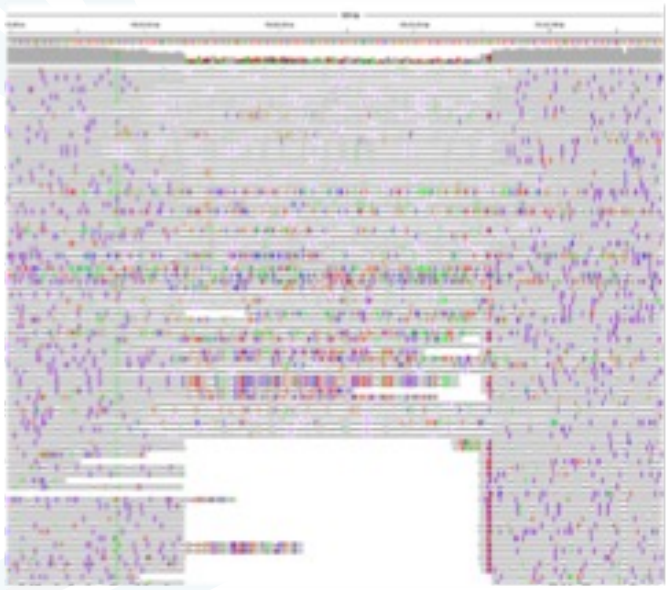


Genome structural variation discovery and genotyping

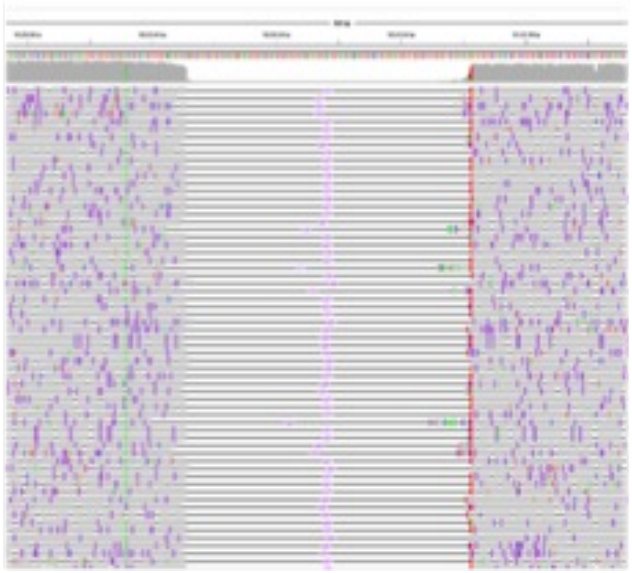
Alkan, C, Coe, BP, Eichler, EE (2011) *Nature Reviews Genetics.* May;12(5):363-76. doi: 10.1038/nrg2958.

ALIGNMENT BASED ANALYSIS

BWA-MEM



NGMLR



NGMLR: Dual mode scoring to accommodate many small gaps from sequencing errors along with less frequent but larger SVs

Accurate detection of complex structural variations using single molecule sequencing
Sedlazeck, Rescheneder, *et al* (2018) *Nature Methods*. doi:10.1038/s41592-018-0001-7

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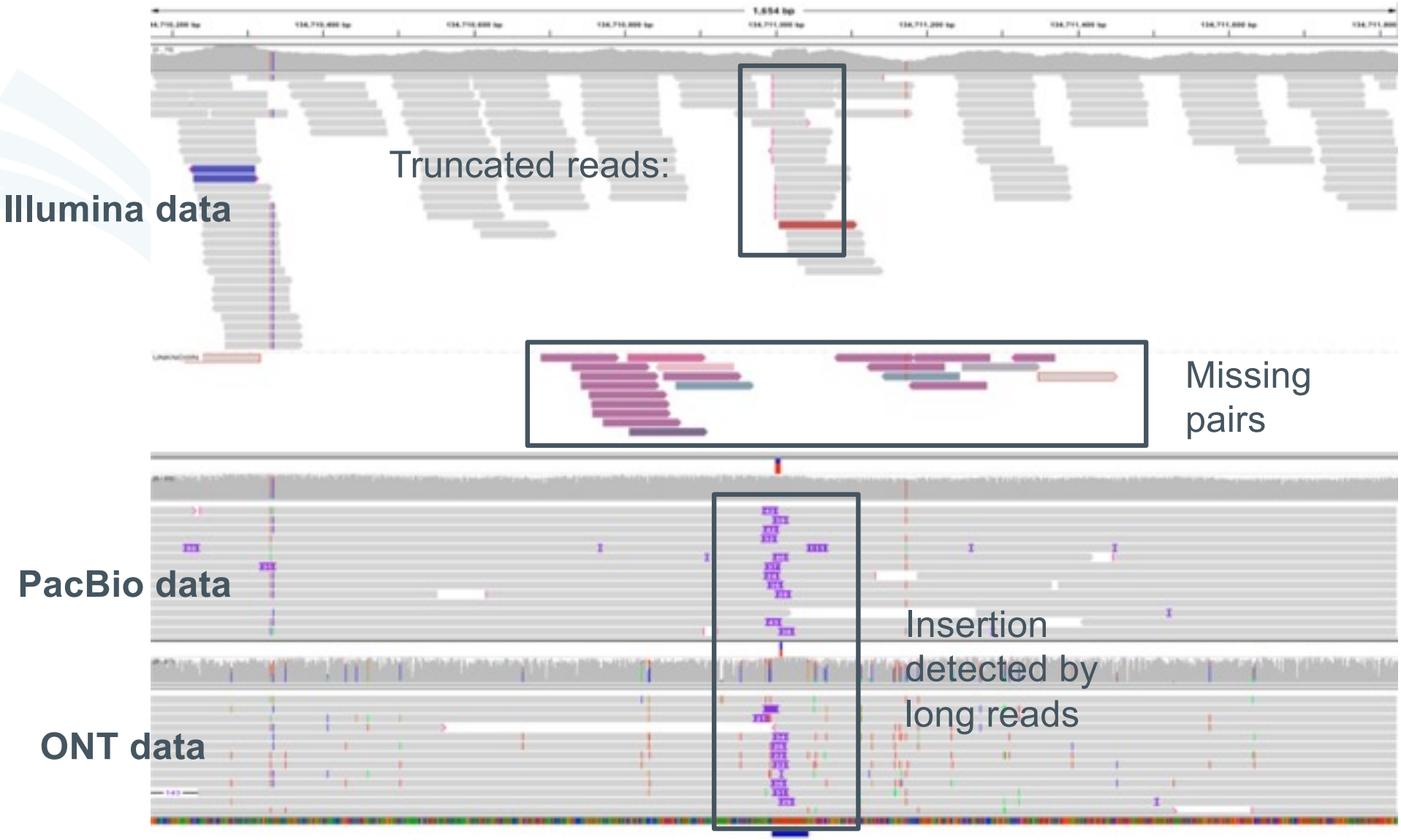
Illumina data


Illumina data
Truncated reads:
Missing pairs
PacBio data
Insertion detected by long reads
ONT data

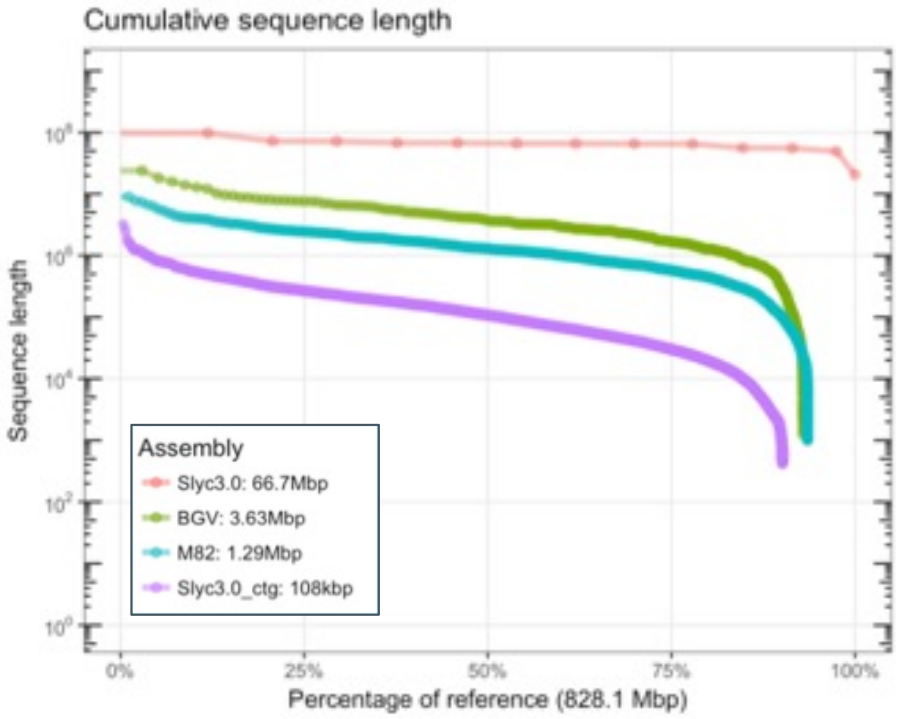
DE NOVO ASSEMBLY

Gold level assemblies with Canu

- Well-established, integrated correction & assembly
- Contig N50 sizes >10-fold better than reference
- Main challenge is speed
  - ~2 weeks per assembly on ~320 cores

Exploring faster options

- Miniasm (Li, *Bioinformatics*, 2016) runs in ~72 core hours (+1.5 days for consensus)
- Wtdbg2 (https://github.com/ruanjue/wtdbg2) runs in ~8 core hours (+1.5 days for consensus) although mixed results depending on sample
- Discussing cloud-enabled pipelines with DNAnexus

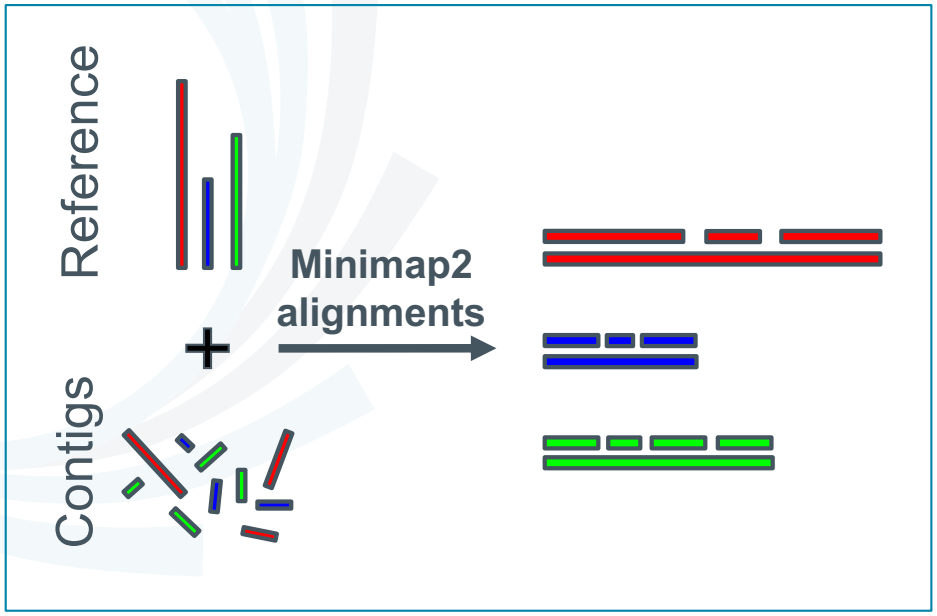


Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation
Koren *et al* (2018) *Genome Research. doi: 10.1101/gr.215087.116*

RAGOO: FAST AND ACCURATE REFERENCE-GUIDED SCAFFOLDING

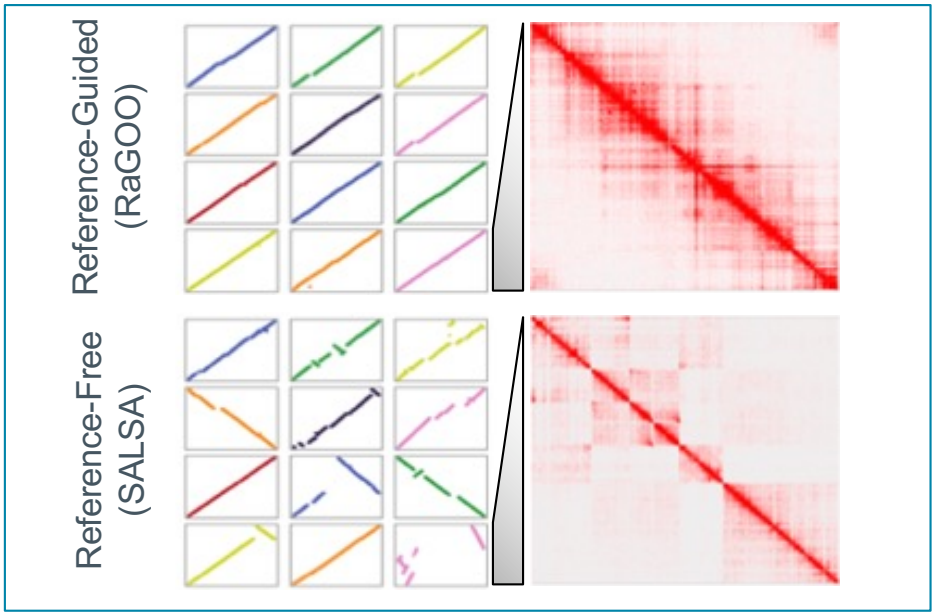
Reference guided scaffolding

- Use the reference genome as a “genetic map”
- Effective when sample is structurally similar to reference



Validation using Hi-C

- Reference-guided scaffolding leads to more complete and more accurate chromosomes



Alonge *et al* (2018) *In preparation*

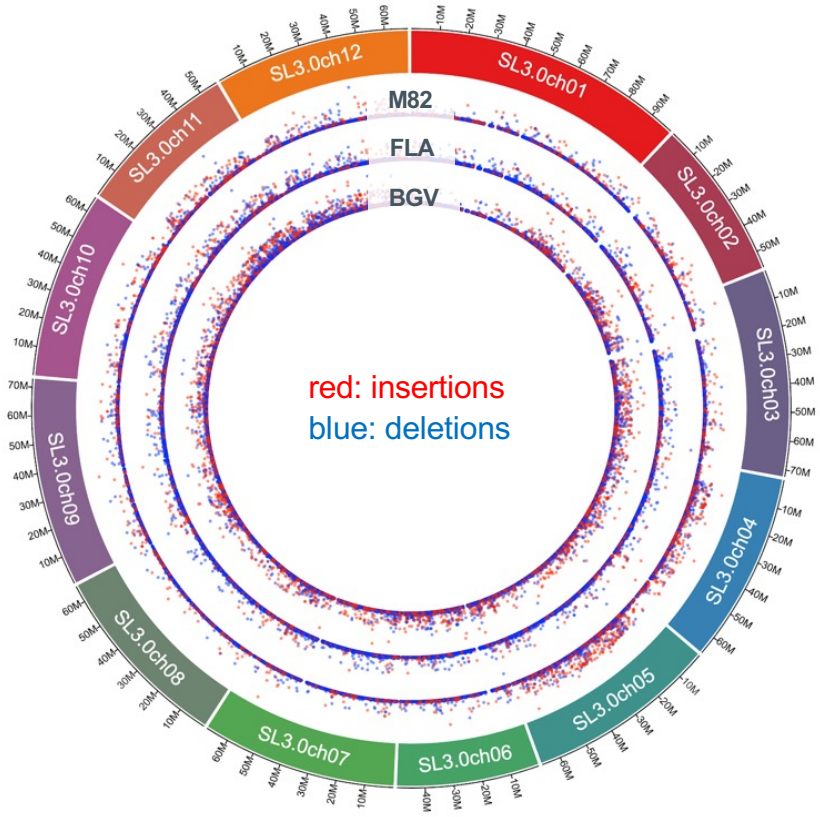
ASSEMBLY BASED ANALYSIS

RaGOO scaffolding yields essentially complete chromosomes

- Final polishing using Bowtie2 + Pilon
  - Substantially faster than Nanopolish, and modestly more accurate based on gene-analysis and alignment to reference
- Gene annotations using MAKER

Identify structural variants using Assemblytics

- Finds variants within and between alignments
- Especially important for large insertions of novel sequences
- Tens of thousands of SVs, widely distributed across chromosomes



Assemblytics: a web analytics tool for the detection of variants from an assembly
Nattestad, M, Schatz, MC (2016) *Bioinformatics*. doi: 10.1093/bioinformatics/btw369

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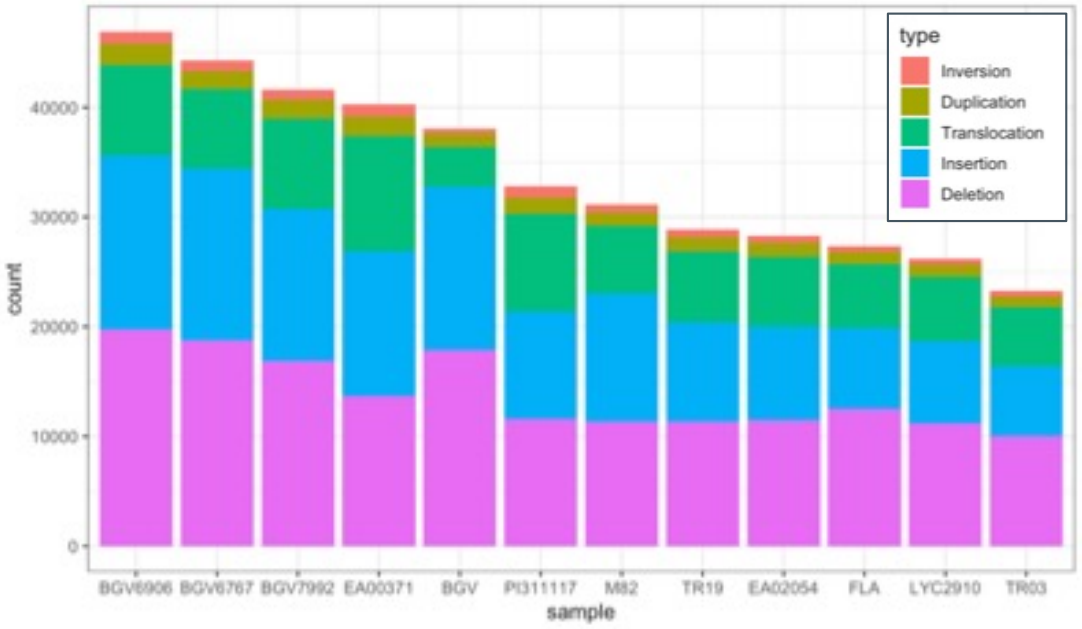
04

THE STRUCTURAL VARIATION LANDSCAPE OF TOMATO GENOMES

PRELIMINARY RESULTS

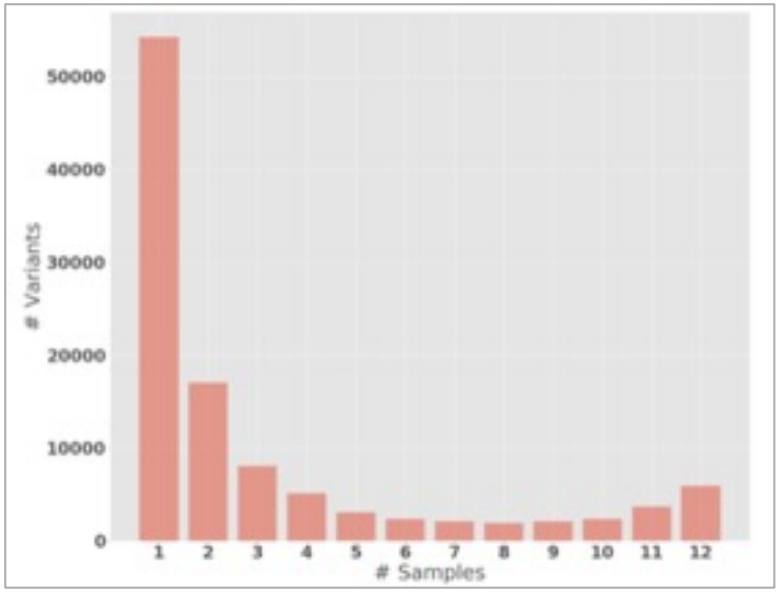
Landscape of the first 12 accessions

- Substantial variation between samples
  - 25 to 45 thousand structural variations each
  - Mostly insertions + deletions



Population Genetics

- Most variants specific to each sample
- Many variant shared by multiple samples, including some in all 12

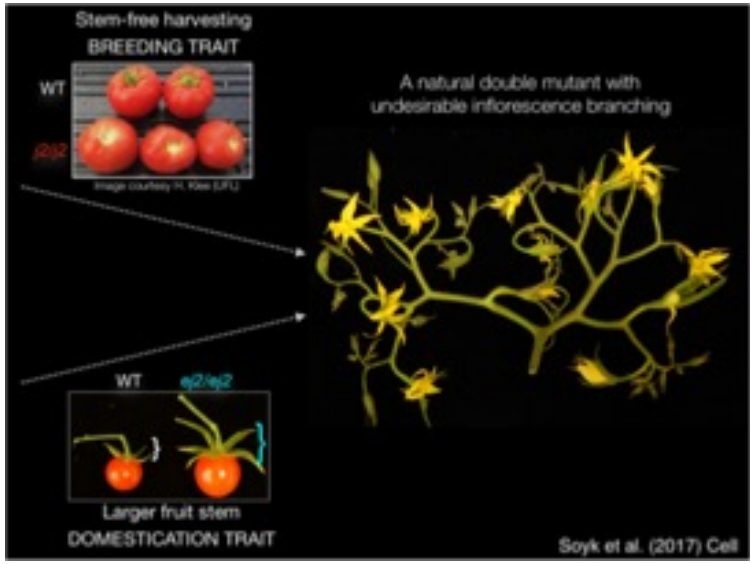


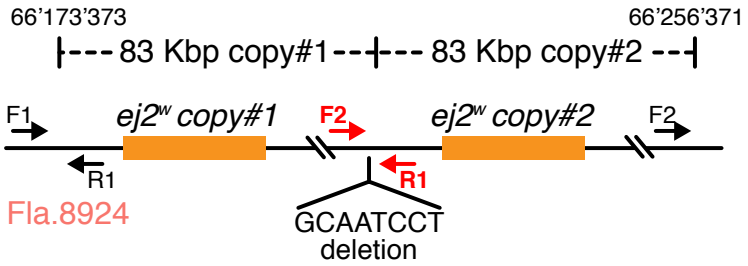
IDENTIFICATION OF THE EJ2 TANDEM DUPLICATION

Validation of our first SV association

- Crosses of tomato plants with a highly desirable breeding trait (j2: jointless2) and a desirable domestication trait (ej2: an enhancer for j2) are typically poorly producing plants – ***a negative epistatic interaction***
- However some breeding lines carry both alleles and yet have good yields through unknown means
  - One of our first samples was such a breeding line and revealed a 83kbp tandem duplication spanning ej2
  - Validated the duplication using PCR, RNA-seq and quantitative genetics to conclude the duplication of the locus causes stabilization of branching and flower production
- **Now able to use CRISPR/cas9 to overcome the negative epistatic interaction to improve fruit yields**

Soyk *et al* (2018) *In preparation*





SUMMARY

High throughput long read sequencing is unlocking the universe of structural variations

- Discovering tens of thousands of variants previously missed, as well as clarifying tens of thousands of false positives per sample
- Possible to rapidly characterize pan-genomes with >100 diverse samples

Beyond mere structural variation identification

..... towards “Rules of Life” interaction maps and beyond

- Identify the specific pathways for many important traits
- Discovery and dissection of cis-regulatory epistasis
- Analysis of epigenetic modifications
- Engineering domestication traits in “wild” plants

Expect to see similar results in all other plant and animal species

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Sam Kovaka

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Sara Goodwin

W. Richard McCombie

Baylor College of Medicine

Fritz Sedlazeck

Boyce Thompson

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Esther van der Knaap

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THANK YOU!

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