Personalized Phased Diploid Genomes of the EN-TEx Samples

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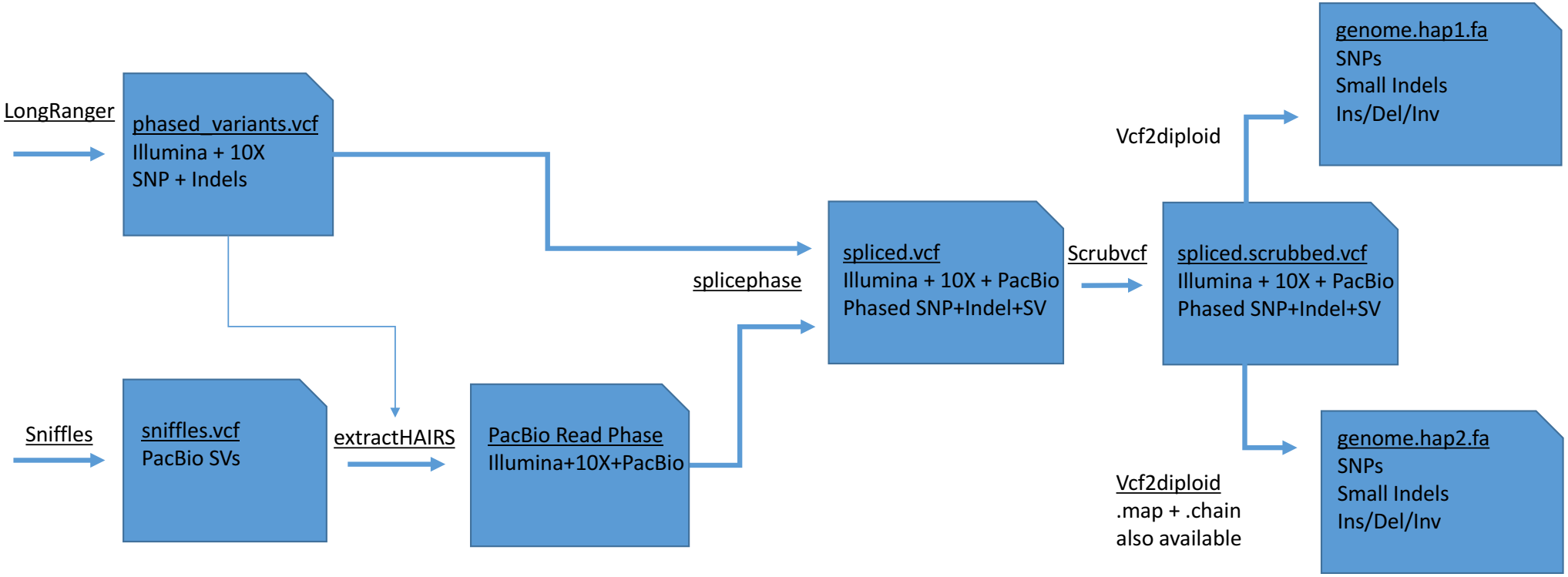
ENTex Update

ENTex Phased Diploid Genomes Version 1.0

http://labshare.cshl.edu/shares/schatzlab/www-data/encode/diploid/2017.10.26/

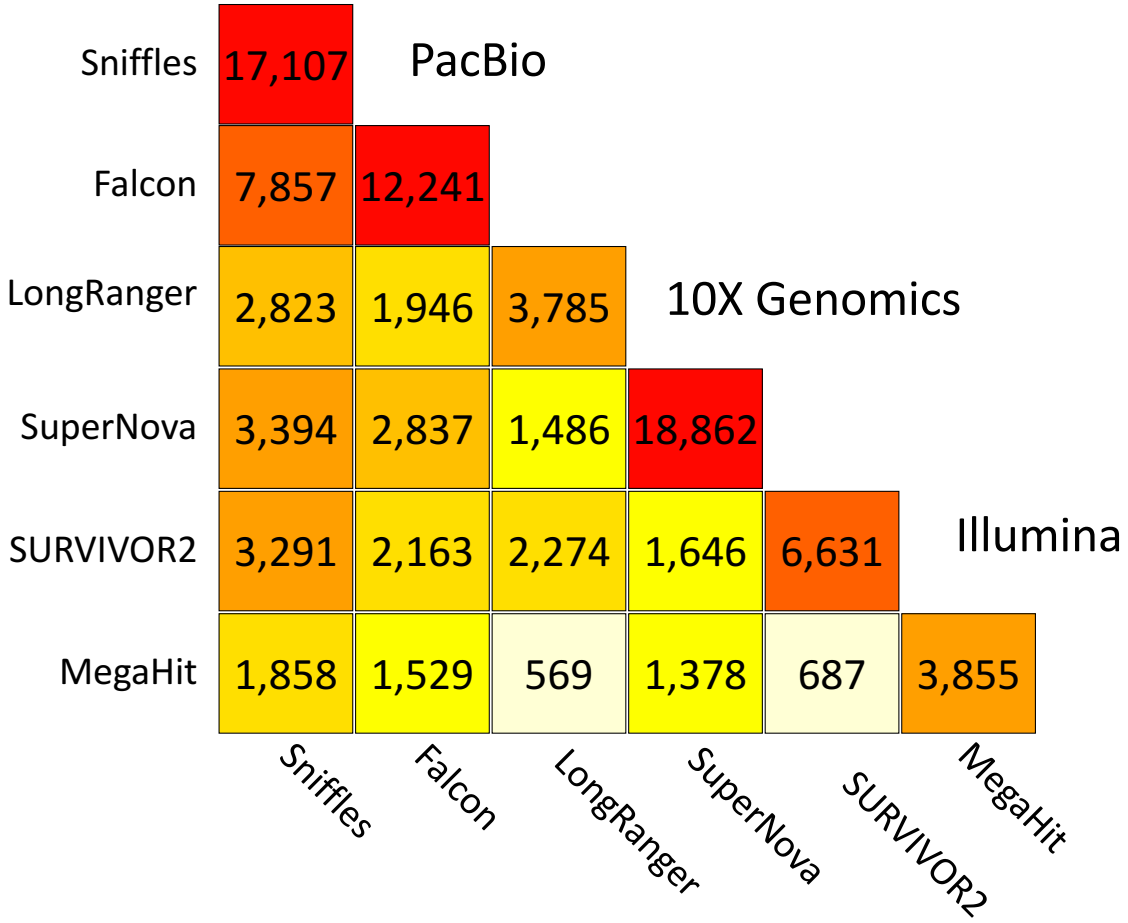


SV phasing software available at: https://github.com/schatzlab/phase_sniffles (name will change soon)

Why this approach?

- ***Major alternative is phased diploid de novo assembly followed by whole genome alignment***
  - Goal: Phase and find all variants directly from assembler, including very long insertions or other complex SVs!
  - aka FALCON + Assemblytics (Note I was corresponding author on both of these papers)
- ***Practical and Engineering Issues for assembly:***
  - Assembly takes days to weeks, mapping is done "overnight"
  - Sourcecode is specific to PacBio only, but we will use multiple technologies including ONT :)
  - After assembly, we still have to align to reference to find variants, annotate genes, etc
    - Repeats that are too long to reliably map reads, are also too long to reliably assemble contigs
- ***Potential sensitivity issues for assembly:***
  - Deeper coverage is needed over a region to successfully error correct and assemble, worse for het SVs
  - Hard to correctly align and find SVs near the end of contigs, while mapping has consistent performance
  - Variants flanked by repeats will probably fail to assemble, but could be captured by mapping
- ***Potential specificity issues for assembly:***
  - Ends of contigs are unreliable as they have lowest coverage and enriched for repeats
  - Hard to correctly characterize homo. versus het. since both alleles have to assemble and align well
  - Mis-assemblies hard to detect and will cause false variants; can have 3 or more contigs spanning some regions
- ***Empirical result:***
  - We benchmarked 6 SV calling approaches (3 mapping-based, 3 assembly-based), and found mapping (Sniffles) has the highest sensitivity and specificity

Structural Variations Concordance (ENC-002)



Main Diagonal

- Calls per tool

Outer triplets

- Concordance by Technology

Inner triplets

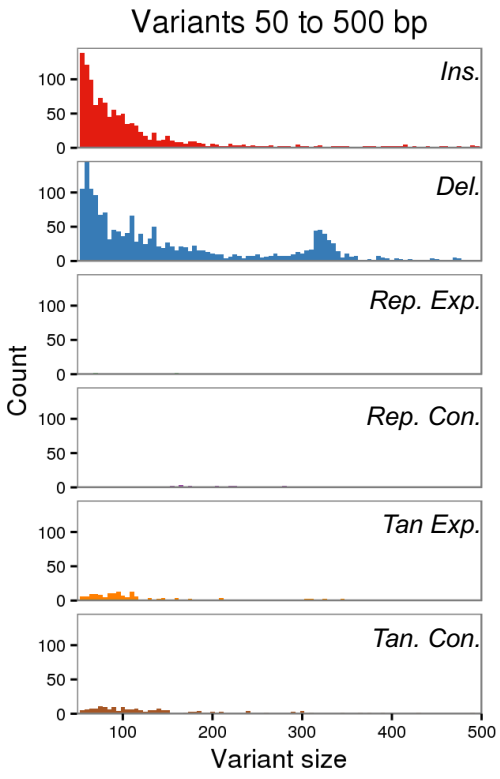
- Concordance by Assembly
- Concordance by Mappers

Overall:

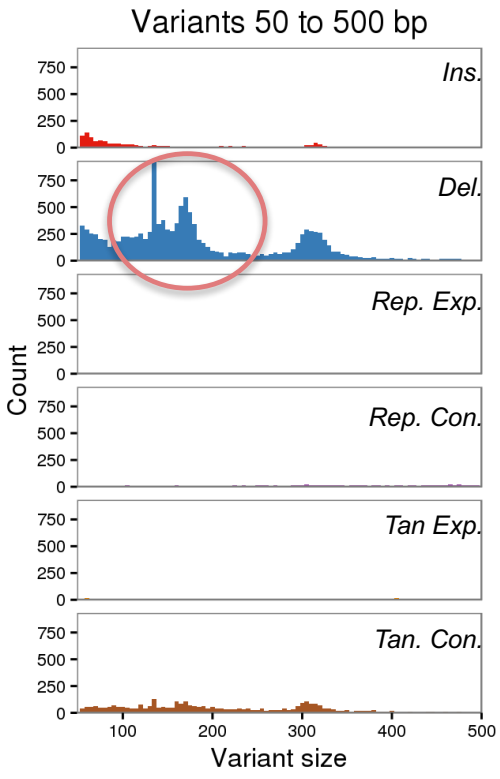
- We need multiple technologies and approaches

Missing Insertions from Short and Linked Read?

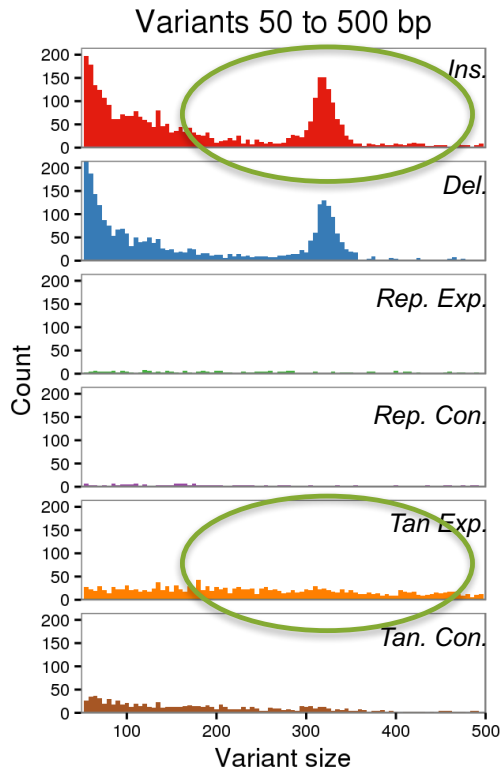
Illumina



10X Genomics



PacBio



Discussion and Future Work

- ***Phasing was performed using 10X genomics only, and the phase block N50 size is around 5Mbp.***
  - Only variants over the main chromosomes as 10X/LongRanger does not work well with alternative chromosomes
  - Not all variants (SNVs/Indels/SVs) are phased if there are not enough flanking SNVs to make a reliable call and will have a 0/1 or 1/0 genotype
  - The boundaries of the phase blocks can be determined by the PS field in vcf file
  - **TODO: Integrate in Hi-C once data quality is confirmed**

- ***For SVs, the vcf file only contains insertions, deletions and inversions that are at least 50bp***
  - Standard variants supported by at least 10 PacBio reads, or at least 5 reads for the sensitive version.
  - The sensitive version identifies a few thousand more SVs, although may have more false positives
  - **TODO: Bench validation to establish sensitivity limits on SVs and indels**

- ***The sequence fidelity of the SV insertions will only be around 90%***
  - The reported sequence is derived from a single raw PacBio read although multiple reads support the SV call
  - **TODO: Developing local assembler to improve the sequence accuracy, augment with additional variant types**

- ***Variant calls have been post-hoc filtered***
  - Remove any overlapping calls using simple left to right scan, preferring SVs to small variants
  - Only includes gender-appropriate chromosomes (male: 1X + 1Y; female: 2X)
  - **TODO: Better resolution of nested SVs and very long insertions**