Reference-quality diploid genomes without \textit{de novo} assembly

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Why NOT de novo?

De novo is necessary for the first genome of a species, but is it really necessary for genomes 2 through N?

1. De novo is slow: Large inputs and intermediate files. Algorithms are complex to compare all the reads to each other. Mammalian genomes need thousands of core hours and terabytes of space.

2. De novo is demanding: Make the libraries just right, sequence the reads just right, set the parameters just right, launch and relaunch to optimize.

3. De novo is unpredictable: Add a little more (or a little less) data, and your contig N50 drops in half. Errors creep in ranging from SNPs to SVs. Heuristics break when the data or genome structure are unexpected.

4. De novo is just the beginning. Annotation from scratch is really hard, and to use it (variant analysis/selection analysis/regulatory analysis) you will probably need to align to a reference anyways.
Why **NOT** *de novo*?

De novo is necessary for the first genome of a species, but is it really necessary for genomes 2 through N?

1. **De novo is slow:** Large inputs and intermediate files. Algorithms are complex to compare all the reads needed for genomes. Wouldn't it be great if we could assemble high quality genome sequences and capture all the genetic diversity in a species without this complexity?

2. **De novo is demanding:** Make the libraries just right, sequence the reads just right, set the parameters just right, launch and relaunch to optimize.

3. **De novo is unpredictable:** Add a little more (or a little less) data, and your contig N50 drops in half. Errors creep in ranging from SNPs to SVs. Heuristics break when the data or genome structure are unexpected.

4. Annotation from scratch is really hard, and to use it (variant analysis/selection analysis/regulatory analysis) you will probably need to align to a reference anyways.
Reference-Guided Assembly

1. **High quality reference**
   - Contig N50 over 1Mbp
   - Scaffold N50 over 10Mbp
   - High Quality Gene Annotation (See VGP definition)
   - Your sample is sufficiently similar (~99% identity)

2. **Sample specific data**
   - **SNPs and Indels**: Illumina-based (Illumina PE or 10X)
   - **Structural Variants**: Long Reads (PacBio or ONT)
   - **Phasing Data**: 10X and/or HiC; trios when available

*Data requirements similar to de novo, but less demanding, more accurate, and more predictable*

*Comparative Genome Assembly (“AMOScmp”)*
CrossStitch

https://github.com/schatzlab/crossstitch

HQ Reference

my.mat.fa

my.pat.fa

In collaboration with Sedlazeck, Gingeras, Guido, Ring, & Gerstein labs
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Call SNPs + Indels
Phase SNPs + Indels

A -> C
A/C -> A|C

Call SVs
Phase SVs & Local Assembly

Phased SNPs, Indels & SVs.vcf
Assemble Diploid Sequence

HQ Reference
GAT -> XXX
GAT/XXX -> GAT|XXX

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Phasing Results

NA12878 Optimal phase block length increases with read length

Short Reads: ~1kbp
Long Reads: ~500kbp
Linked Reads: ~10Mbp
Hi-C: 100Mbp+
10X + Hi-C: 145Mbp N50 😊 (Healthy human, varies significantly by sample)

HapCUT2: robust and accurate haplotype assembly for diverse sequencing technologies
CrossStitch
https://github.com/schatzlab/crossstitch

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SVs using Short, Long and Linked Reads

Main Diagonal
- Calls per tool

Outer triplets
- Concordance by Technology

Inner triplets
- Concordance by Assembly
- Concordance by Mappers

Overall:
- Long reads give the most variants with the best concordance 😊
NGMLR + Sniffles

NGMLR: Convex gap penalty to balance frequent small sequencing errors with larger SVs
Sniffles: Scan within and between split reads to accurately find SVs (Ins, Del, Dup, Inv, Trans)
Mendelian concordance >95%, experimental validation also very high

Accurate detection of complex structural variations using single molecule sequencing
No more false positives!

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my.mat.fa

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Phase SVs & Local Assembly

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Local Assembly and SV Phasing

Transfer the phasing of the short read variants to the long reads
The phased long reads allow the SVs to be phased

**Phase SVs:** Make sure SVs are associated with the correct haplotype

**Local Assembly:** Refine sequence of insertions, resolve complex nested variants
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Assemble Diploid Sequence

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Assembling a “Perfect” Personalized Diploid Genome

Carefully “stitch” the phased variants into the reference genome at the right position to create a pair of phased chromosome fasta files

Phased SNPs, Indels & SVs.vcf

G A T T A C A

G A T T A C A
Assembling a “Perfect” Personalized Diploid Genome

Carefully “stitch” the phased variants into the reference genome at the right position to create a pair of phased chromosome fasta files

Phased SNPs, Indels & SVs.vcf

- sub
- inversion

G C T G T A A

G C T T T A C A A

- sub
- insertion
- del

A - A
Assembling a “Perfect” Personalized Diploid Genome

Carefully “stitch” the phased variants into the reference genome at the right position to create a pair of phased chromosome fasta files

Phased SNPs, Indels & SVs.vcf

Stitching based on AlleleSeq pipeline enhanced for SVs (Rozowsky et al, 2011)
• Maintains a mapping from reference to personal genome coordinates to make lift over of annotation straightforward to compute

Using 10X + HiC + PacBio, assemble essentially perfect diploid human genomes with haplotypes spanning entire chromosomes
• Phased diploid genome can be aligned or aligned against just like a de novo genome assembly
Applications

**Expression & Regulation**
- **Foundation for mapping functional data**
  - Discover novel genes and gene fusions
  - Analyze differential expression in CNVs
  - Discover new regulatory regions
  - Analyze allele-specific expression

**Population Genetics**
- **Framework for GWAS of Structural Variations**
  - Identified SVs in >900 accessions using short reads
  - Assembling the top 50 lines using long & linked reads
  - Perform GWAS of breeding traits

**Polyplody**
- **Studying heterozygosity in sugarcane**
  - Have a high quality PacBio-based assembly of POJ2878 using FALCON (140kbp N50)
  - Developing new methods for phasing (9-14 copies of each chromosome)
Reference-quality Genomes without *de novo* assembly

*De novo assembly is essential for exploring new species*
- Reference-free or mapping to a distant reference is difficult to impossible

*But once the first genome of a species has been assembled, shouldn’t the second genome be a little easier?*
- Use the right combination of data to capture and phase all types of variants
- Overnight analysis to create a high quality personalized genome that are more accurate, more predictable, and easier to use than a de novo assembly
- The personalized diploid genome will be a platform for functional and evolutionary analysis in many species

http://schatz-lab.org

CrossStitch  FALCON  SURVIVOR  NGM+Sniffles  Ribbon  Assemblytics
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