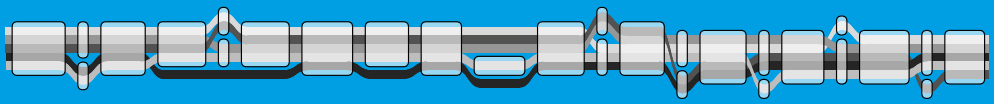


Intérêt d'une référence du pangénome humain pour l'étude des variants structuraux

Jean Monlong

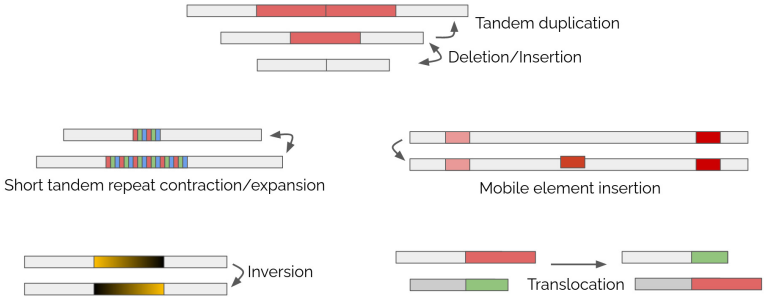
COLLOQUE ACLF
01/10/2025

irsd
Institut de recherche en santé digestive

Inserm
La science pour la santé
From science to health

Structural variants (SVs) come in diverse shapes and sizes

Variant size: from 50 bases to megabases.



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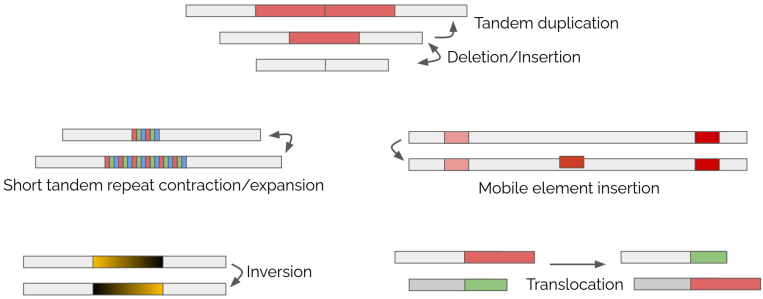
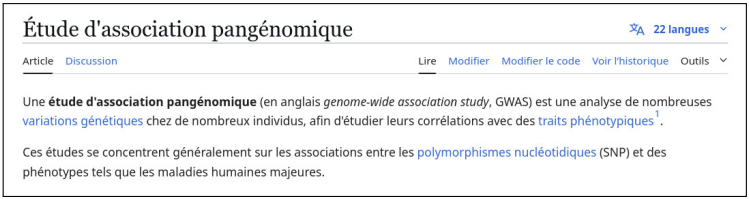


- High functional impact
- Involved in rare and common diseases, and cancers.
- **Hard to detect**

Pangenomics?

Pangenomics?

Not “Genome-wide” in French.



Étude d'association pangénomique

22 langues

Article Discussion Lire Modifier Modifier le code Voir l'historique Outils

Une **étude d'association pangénomique** (en anglais *genome-wide association study*, GWAS) est une analyse de nombreuses variations génétiques chez de nombreux individus, afin d'étudier leurs corrélations avec des traits phénotypiques[1].

Ces études se concentrent généralement sur les associations entre les polymorphismes nucléotidiques (SNP) et des phénotypes tels que les maladies humaines majeures.

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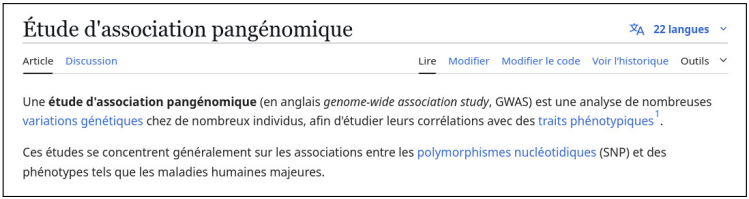
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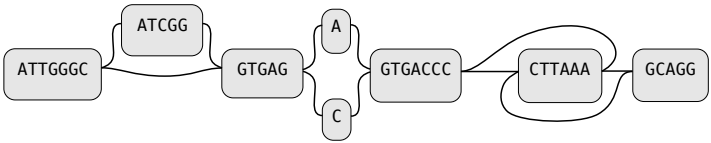
Ces études se concentrent généralement sur les associations entre les polymorphismes nucléotidiques (SNP) et des phénotypes tels que les maladies humaines majeures.

Also not exactly the set of **genes** from all strains within a clade, like in microbial pangenome.

Pangenomes represent genetic diversity succinctly

A pangenome represents a **collection of genomes** and the genetic variants among them.

ATTGGGC**ATCGG**GTGAG**A**GTGACCC**TTTAAG**GCAGG
ATTGGGC**-----**GTGAG**C**GTGACCC**CTTAAA**GCAGG



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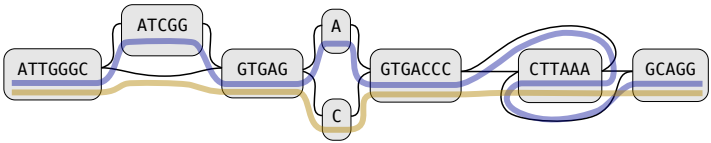
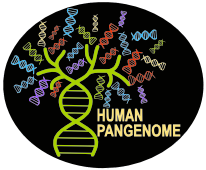


Building a Human pangenome reference



- Human Pangenome Reference Consortium (**HPRC**)
- Latest sequencing technologies for **350 diverse individuals**

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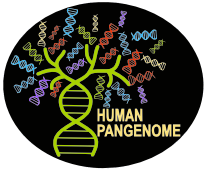
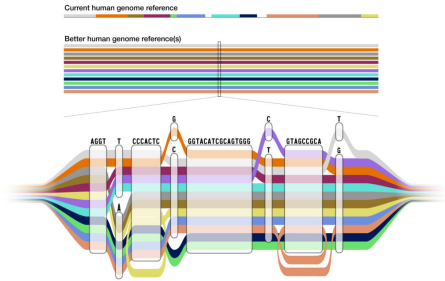
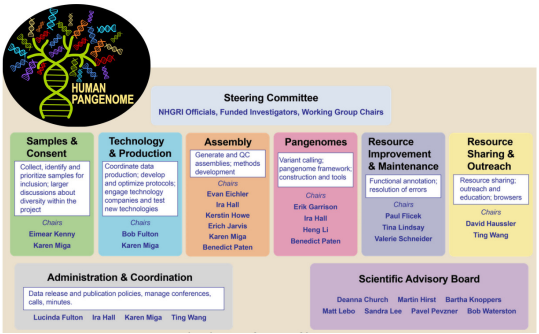


- Human Pangenome Reference Consortium (**HPRC**)
- Latest sequencing technologies for **350 diverse individuals**
- Generate **telomere-to-telomere phased assemblies**
- Pangenome containing a comprehensive catalog of (structural) variants



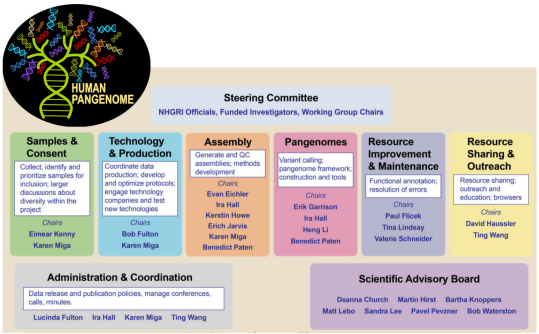
Hickey*, Monlong*, et al. Nat. Biotechnol. 2023

Building a Human pangenome reference



Liao*, Asri*, Ebler*, et al. Nature 2023

Building a Human pangenome reference

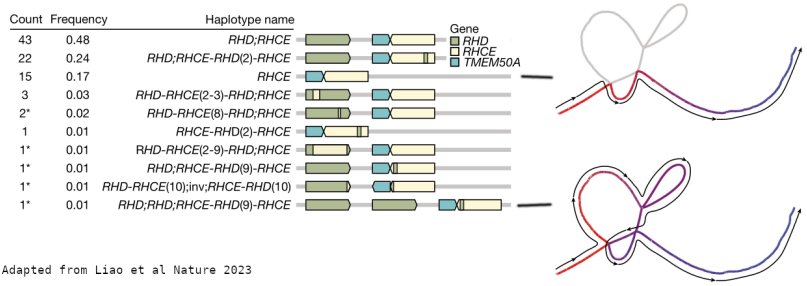


Liao*, Asri*, Ebler*, et al. Nature 2023

Check out the latest data at: `https://data.humanpangenome.org`

(Ongoing) Release 2: **466 haplotypes** of near-T2T quality.

Complex structural variants in the HPRC pangenome

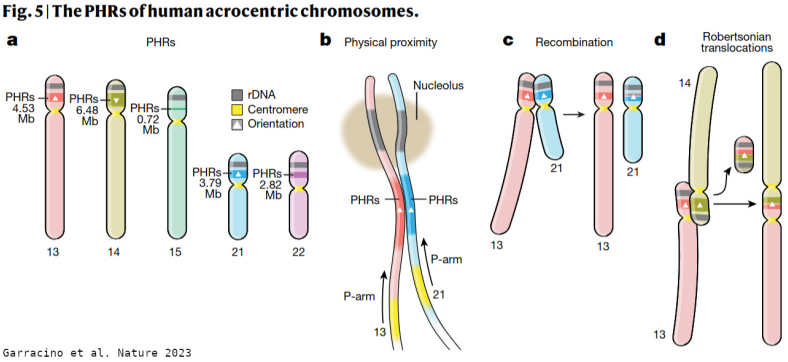


Adapted from Liao et al Nature 2023

Liao*, Asri*, Ebler*, et al. Nature 2023

Recombination between heterologous human acrocentric chromosomes

"we show that acrocentric chromosomes contain pseudo-homologous regions (PHRs) indicative of recombination between non-homologous sequences. "



Guarracino, et al. Nature 2023

The variation and evolution of complete human centromeres

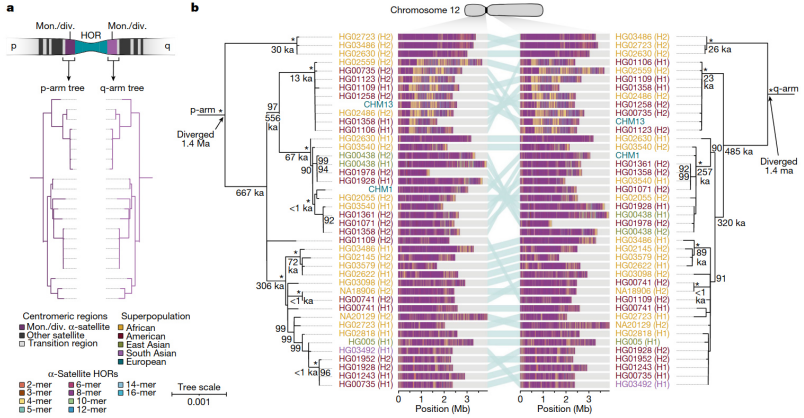


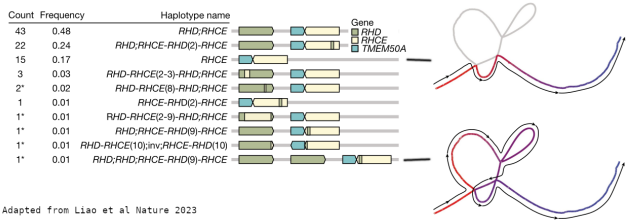
Fig. 7 | Phylogenetic reconstruction of human centromeric haplotypes and the saltatory amplification of new α-satellite HORs.

Logsdon et al. Nature 2024

Logsdon, et al. Nature 2024

Using the human pangenome resources

- Explore **high-quality assembled haplotypes** across diverse individuals



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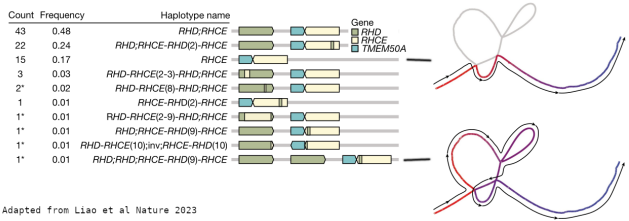
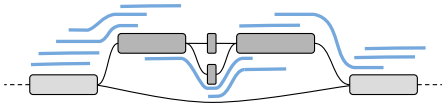


Adapted from Liao et al Nature 2023

- **As a reference** to study new samples with sequencing data.
  1. Genotype SVs with short-read data
  2. Characterize complex SVs with long-read data

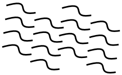


A pangenome reference to genotype SVs with short-read data

Genome sequencing

DNA

DNA fragments



Sequencing machines

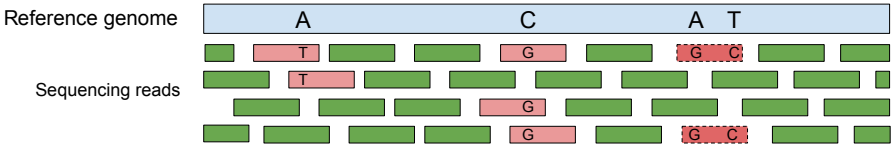
File (~100-300 Gb)

```
@ERR903030.219 HWI-D00574:82:C6L01ANXX:3:1101:3953:1913/1
AGCTCTTATTATTTTGAAATATGTCCCATCAATACCTAATTTATTGAGAGTTTTTAGCATGAAGGGTTGTTGAAT
+
<<A0?FGGGGGGGGGGGEGGGGGGGGGGGGGGGGG:CFCGDFGG>FG1F@0:FGGGGGF@>FG1=F@FFGGGGFGGC
@ERR903030.220 HWI-D00574:82:C6L01ANXX:3:1101:3863:1914/1
ACCATGAAAGACAGGTGTTAGAATCAGTACAAGAAGCAACAGGGAGCCATTGCATTTTGAGCATTTGTTGTATCA
+
<ABBGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGEGGG
@ERR903030.221 HWI-D00574:82:C6L01ANXX:3:1101:3906:1914/1
GATGGGGTTTCACATTGGCCAGGCTGGTCTCAAACTCCTAACCTCAAGGGATCCACCCACCTCGGCCTCCCAAAG
+
<<AAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGFGGGGGGGGGGGGGGGGGGGGGGGGGGGGG>
@ERR903030.222 HWI-D00574:82:C6L01ANXX:3:1101:3833:1922/1
TACTGAAGAATCAGTGTCAGTTTTGTTAGTTGTGATAATGACATTCTGCTAAGCTAAAGTATAGAGGGGAGAAAT
+
=<BBGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGEGGGFGFGEGEGGGGFGGG
@ERR903030.223 HWI-D00574:82:C6L01ANXX:3:1101:3942:1927/1
AAAAAATGAACTAAAAATGCATTAAAGACCAAATGTAATACCTAAAAATGTAAAACTTTTAGAAGGAAACATAGG
+
=ABBGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
@ERR903030.224 HWI-D00574:82:C6L01ANXX:3:1101:3920:1929/1
ATTCCATTCGATTCCATTCGATGATTCCATTGGATTCCATTCGATGATGATTCCATTCAAATCCATTCGATAATT
+
=ABBGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGC>FFGGGEF@EDFGCEGFGGGGGGGGEGGGGCFB9EG
...
...
```

Sequencing reads

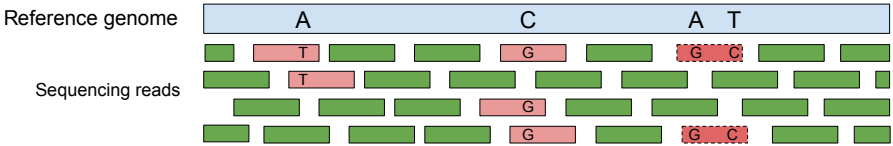
- **Short:** 150-250 bp (current tech)
- **Long:** 10,000s-100,000s bp (new tech. $$$)

Aligning reads to a reference genome



Assuming the reads are correctly placed, small variants are identified as recurrent differences between reads and the reference genome.

Aligning reads to a reference genome

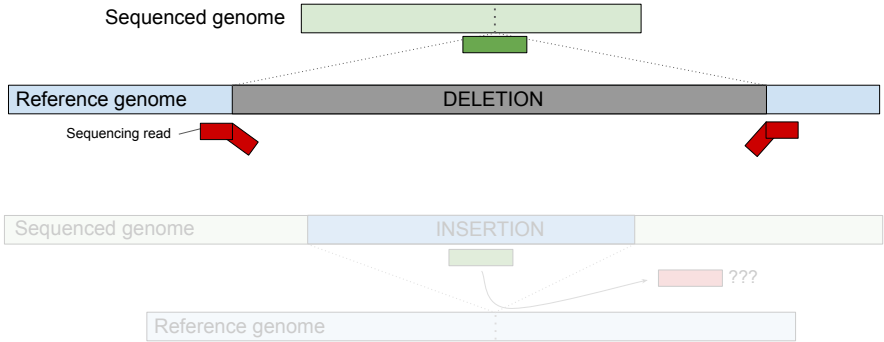


Assuming the reads are correctly placed, small variants are identified as recurrent differences between reads and the reference genome.

Variants can be missed, resulting in **reference bias**.

The challenges of structural variant detection

Around breakpoints, short sequencing reads are hard to map on the reference genome.



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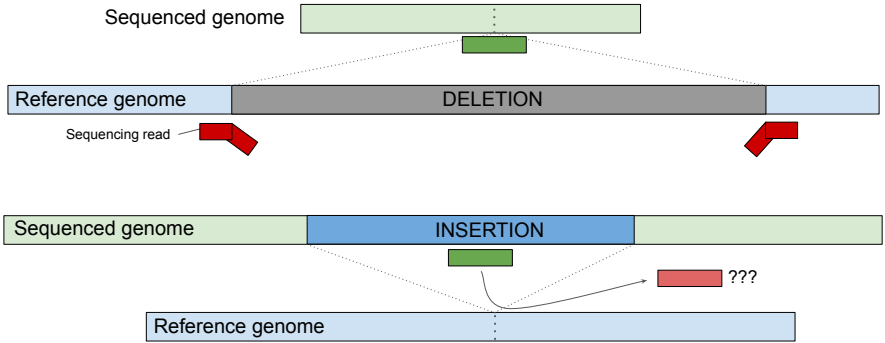
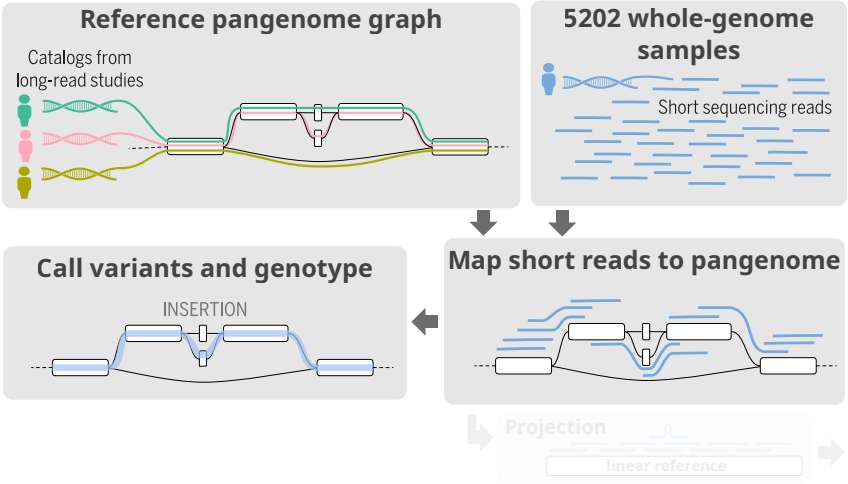
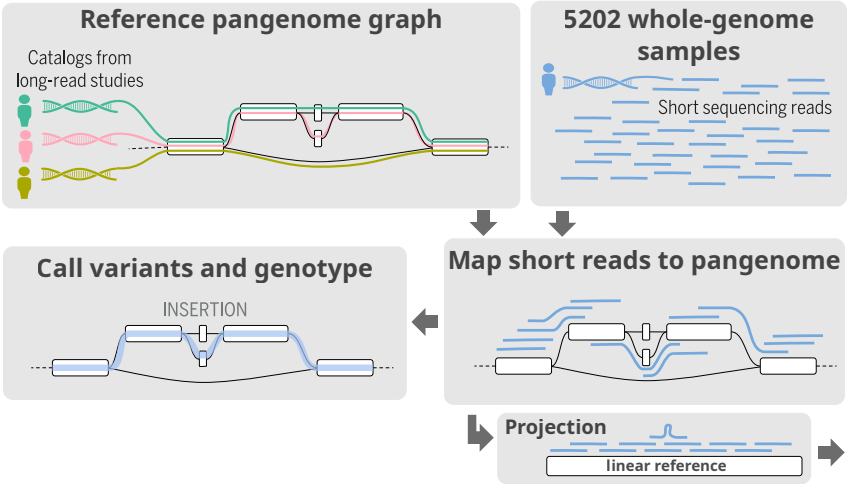


Short-read mapping and structural variant genotyping



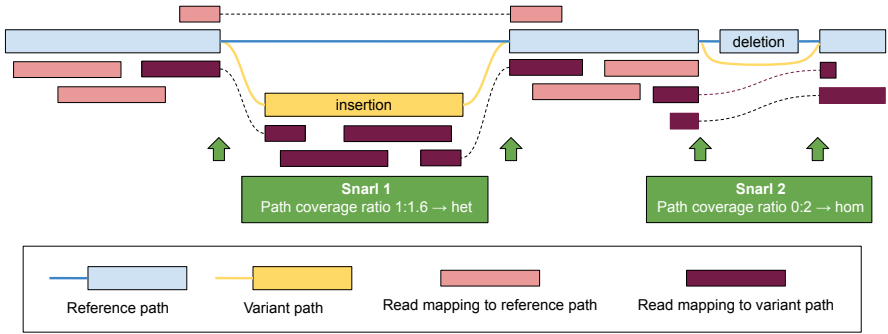
Siren*, Monlong*, Chang*, Novak*, Eizenga*, et al. Science 2021

Short-read mapping and structural variant genotyping



Siren*, Monlong*, Chang*, Novak*, Eizenga*, et al. Science 2021

Genotyping structural variation from pangenomic mapping

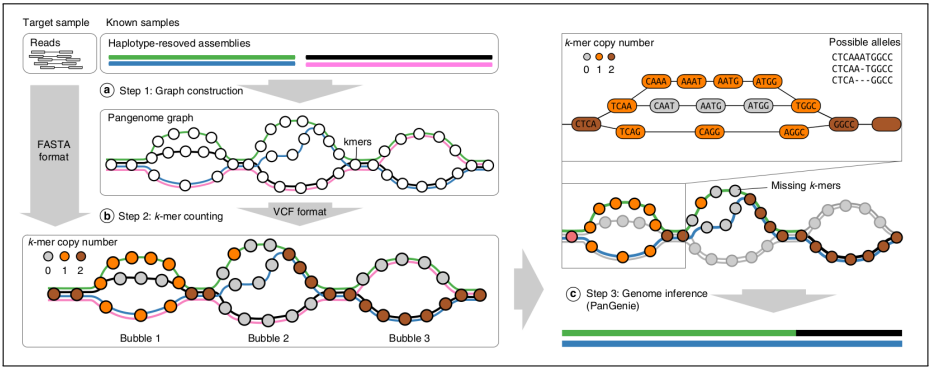


`https://github.com/vgteam/vg`

Hickey*, Heller*, Monlong*, et al. Genome Biology 2020

Genotyping structural variation from phased variants

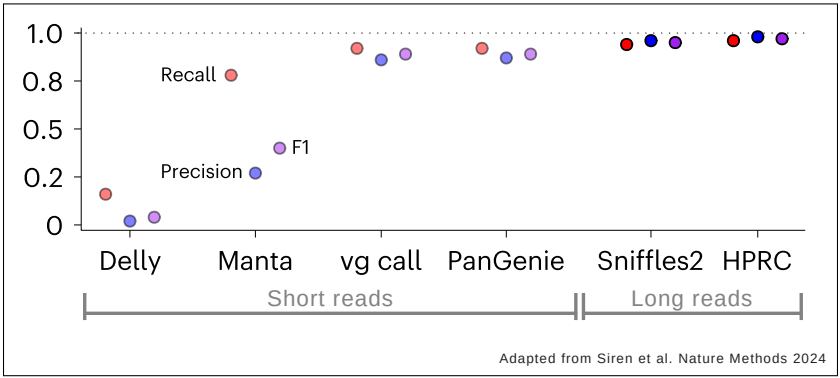
PanGenie uses k-mer and haplotype information to genotype SVs.



`https://github.com/eblerjana/PanGenie`

Ebler et al. Nature Genetics 2022

Structural variant genotyping performance

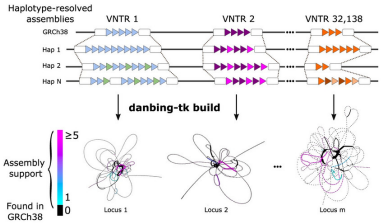


*vg call and PanGenie using the "personalized pangenome" approach (Sirén et al. Nature Methods 2024).

Other specialized methods

Variable Number Tandem Repeats (VNTR) characterization

danbing-tk genotypes them or predict their length from short read data.



Lu et al. Nature communications 2021

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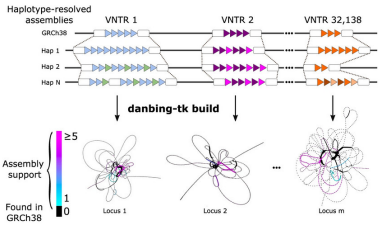
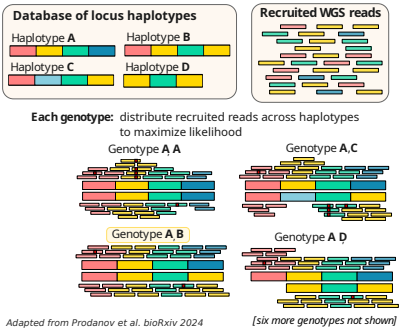


Lu et al. Nature communications 2021

Targeted genotyping of complex polymorphic genes

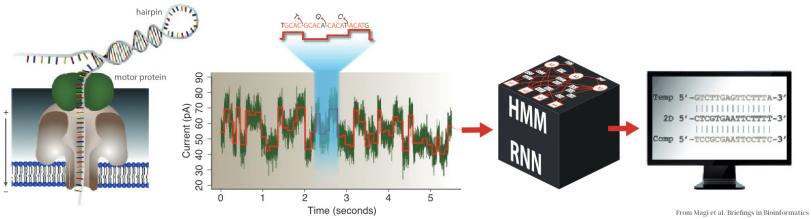
Locityper finds the best pair of known haplotypes from short reads.



Prodanov et al. bioRxiv 2024

A pangenome reference to characterize complex SVs with long-read data

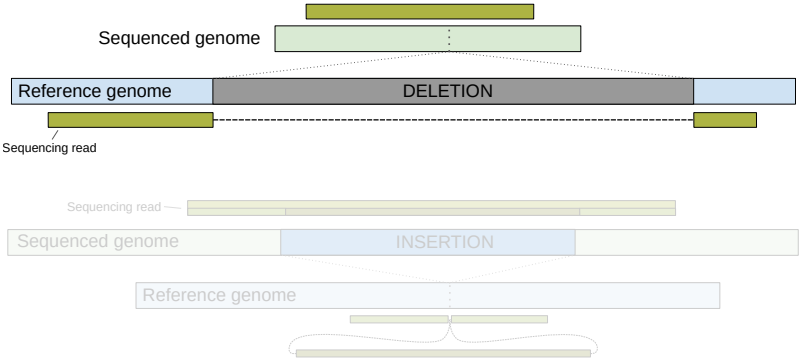
Long-read sequencing with Oxford Nanopore Technologies



As the DNA (or RNA) fragment passes through the pore, the current changes and is decoded to predict nucleotides.

Reads length of 1,000s-100,000s of nucleotides.

Longer reads improve structural variant detection



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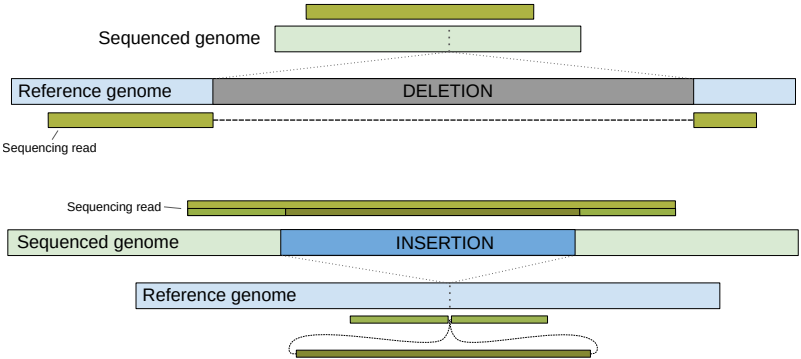


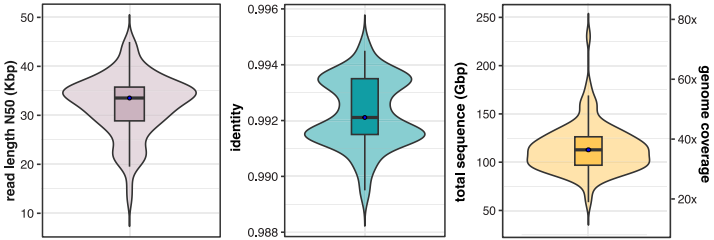
Application to a cohort of rare disease patients

Chan Zuckerberg Initiative

Children's National

GREGoR consortium

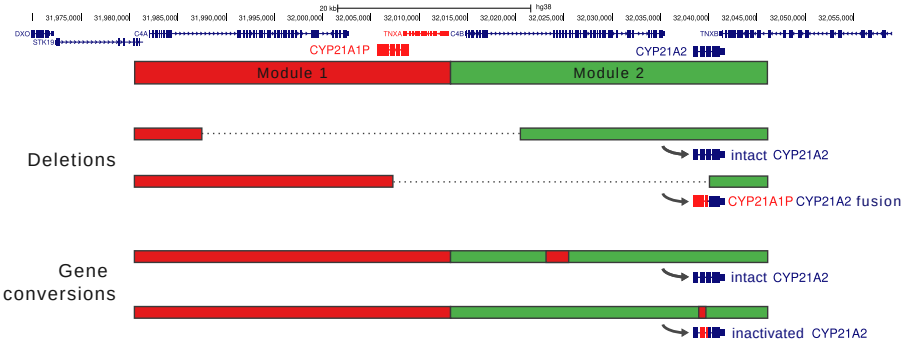
42 probands and 56 unaffected family members, sequenced with one-flowcell of ONT long-read sequencing (R10).



Negi et al. AJHG 2025

Challenging RCCX modules in the HLA region

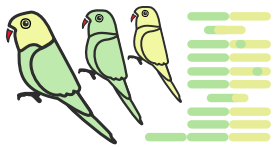
- Tandem-duplication of ∼30 Kbp genetic *module* (99% similar).
- CYP21A1P pseudogene and **CYP21A2 gene**.
- Variants cause congenital adrenal hyperplasia (recessive).

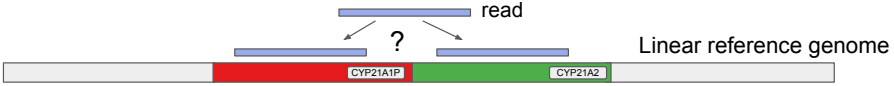


Parakit: paralog toolkit using collapsed pangenomes

Goal

Address multi-mapping confusion by mapping to a **collapsed pangenome** and by analyzing the alignment profile.





`https://github.com/jmonlong/parakit` Monlong et al. medRxiv 2025

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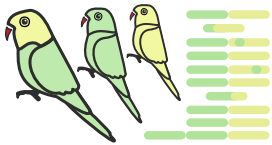
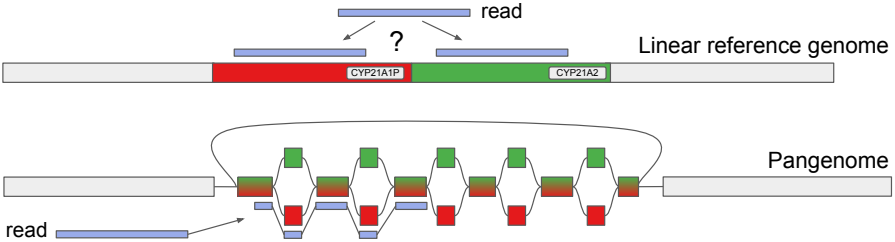
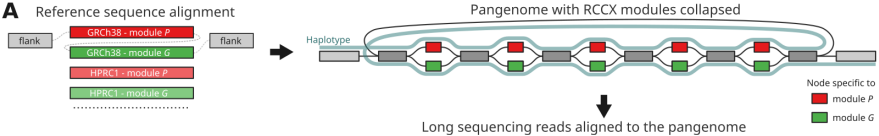


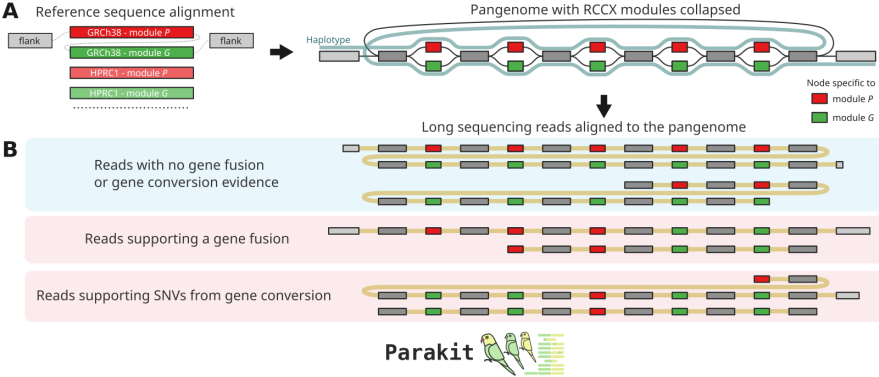


`https://github.com/jmonlong/parakit` Monlong et al. medRxiv 2025

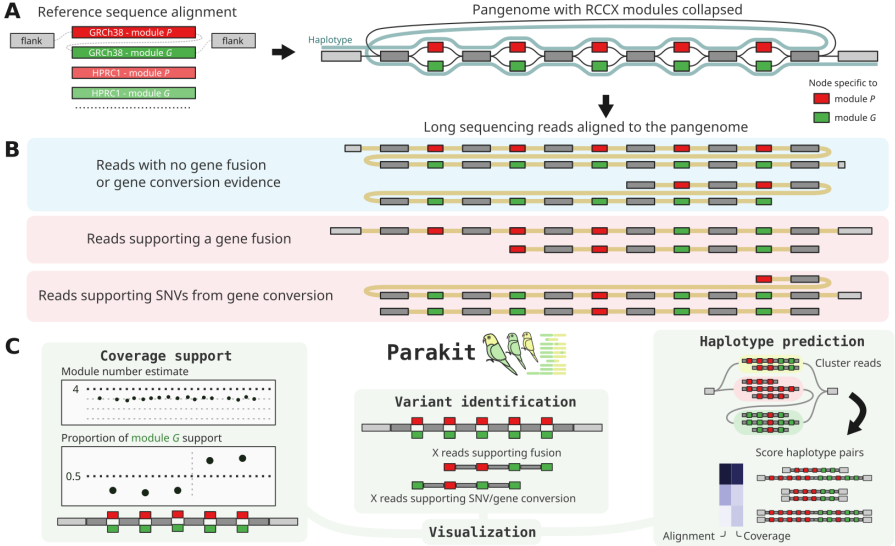


Parakit

`https://github.com/jmonlong/parakit` Monlong et al. medRxiv 2025

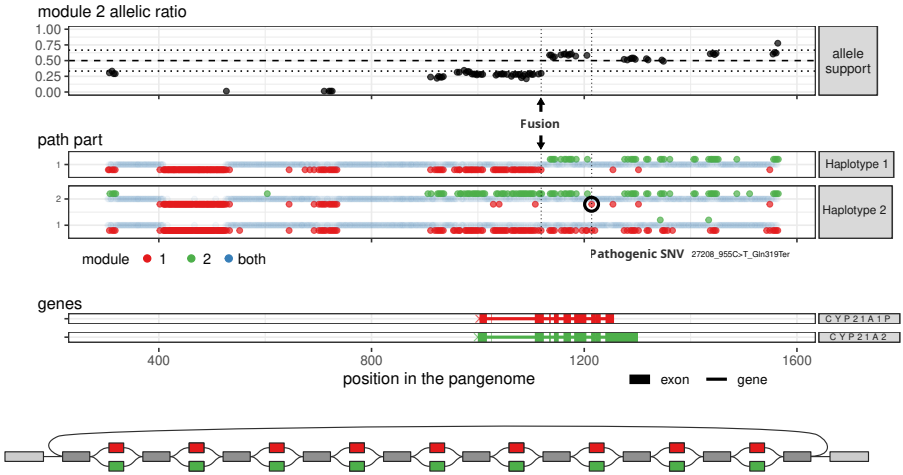


`https://github.com/jmonlong/parakit` Monlong et al. medRxiv 2025



https://github.com/jmonlong/parakit Monlong et al. medRxiv 2025

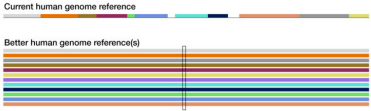
Example: patients with a gene fusion and pathogenic SNV

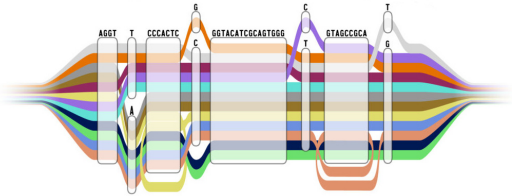


https://github.com/jmonlong/parakit Monlong et al. medRxiv 2025

The human pangenome

→ Complex structural variants at unprecedented resolution





→ Augmented (pan)genomic reference to:

- Genotype SVs from **short-read sequencing** data.
- Characterize complex SVs with **long-read sequencing**.

Acknowledgments

Univ. California, Santa Cruz

- **Benedict Paten**
- **Glenn Hickey**
- Jouni Sirén 
- Adam Novak 
- Xian Chang 
- Jordan Eizenga 
- **Shloka Negi**
- **Karen Miga**
- Brandy McNulty
- Melissa Meredith
- Paolo Carnevali
- Trevor Pesout
- Kishwar Shafin
- Mira Mastoras
- Mobin Asri

INSERM IRSD

- Sarah Djebali
- Matis Alias-Bagarre

NIH

- Mikhail Kolmogorov
- Cornelis Blauwendraat
- Kimberley Billingsley
- Pilar Alvarez Jerez

Univ. California, Irvine

- **Emmanuèle Délot**
- Eric Vilain

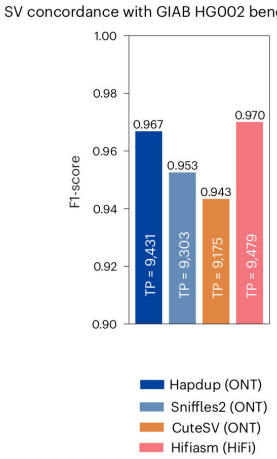
Children's National Research Institute

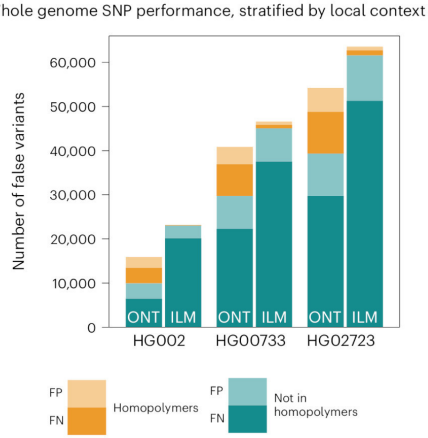
- Seth Berger
- Paolo Canigiula

INRAE

- Xian Chang
- Matthias Zytnicki

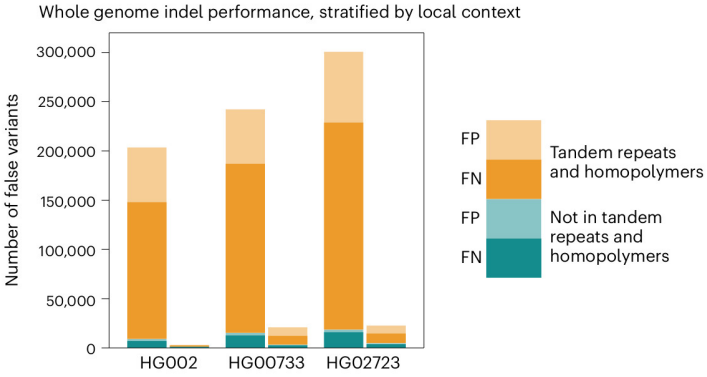
Better calls for both small and structural variants...





Kolmogorov*, Billingsley*, et al. Nature Methods 2023

...except for indels in homopolymers



Note: Results above are for the R9 chemistry. The new R10 chemistry has lower error rate and better (indel) calling performance.

Kolmogorov*, Billingsley*, et al. Nature Methods 2023

Small variants found by long-reads only

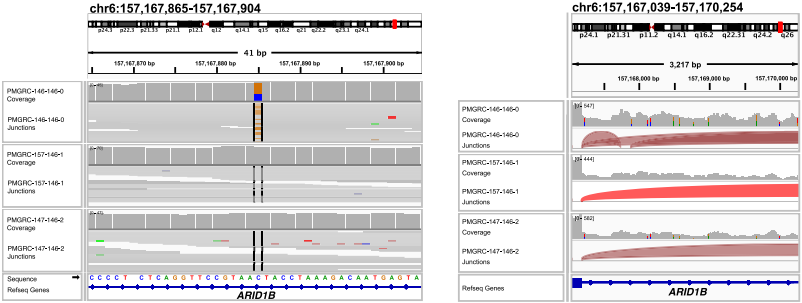
Missense mutation in *KRT86* disease gene (monilethrix) invisible with short reads.

Negi et al. AJHG 2025

Patient with complex neurodevelopmental phenotype

Variant of Uncertain Significance SNV in *ARID1B* gene (Coffin-Siris syndrome 1?).

- *De novo*, SRS and LRS, new splice site predicted *in silico* (SpliceAI).



Negi et al. AJHG 2025

Trimodular alleles also detected