What's new in the human (pan)genome

Adam M. Phillippy

CSHL Advanced Sequencing Technologies & Bioinformatics Analysis Course
November 22, 2024

@aphillippy.bsky.social

National Human Genome Research Institute

The Forefront of Genomics®

A 20-year anniversary

Finishing the euchromatic sequence of the human genome

International Human Genome Sequencing Consortium*

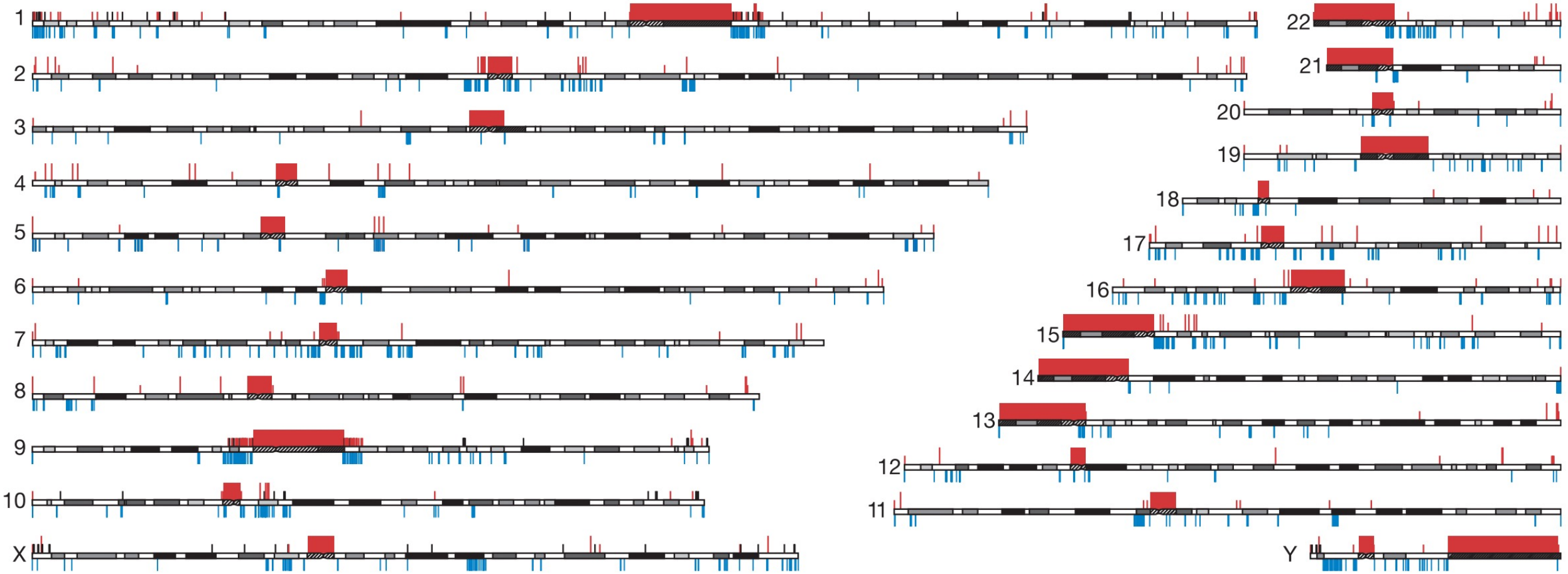
**A list of authors and their affiliations appears in the* Supplementary Information

The sequence of the human genome encodes the genetic instructions for human physiology, as well as rich information about human evolution. In 2001, the International Human Genome Sequencing Consortium reported a draft sequence of the euchromatic portion of the human genome. Since then, the international collaboration has worked to convert this draft into a genome sequence with high accuracy and nearly complete coverage. Here, we report the result of this finishing process. The current genome sequence (Build 35) contains 2.85 billion nucleotides interrupted by only 341 gaps. It covers ~99% of the euchromatic genome and is accurate to an error rate of ~1 event per 100,000 bases. Many of the remaining euchromatic gaps are associated with segmental duplications and will require focused work with new methods. The near-complete sequence, the first for a vertebrate, greatly improves the precision of biological analyses of the human genome including studies of gene number, birth and death. Notably, the human genome seems to encode only 20,000–25,000 protein-coding genes. The genome sequence reported here should serve as a firm foundation for biomedical research in the decades ahead.

NIH NHGRI

Where were the gaps?

Satellites
Segmental duplications
Gene arrays (e.g. rDNA)



NIH NHGRI

Finishing the euchromatic sequence of a human genome. IHGSC. *Nature* (2004)

Finishing the human genome

2003 (hg35)

- 2.85 Gbp
- 341 gaps
- 1 error per 100,000 bases?
- $5,000,000,000

2023 (T2T-CHM13)

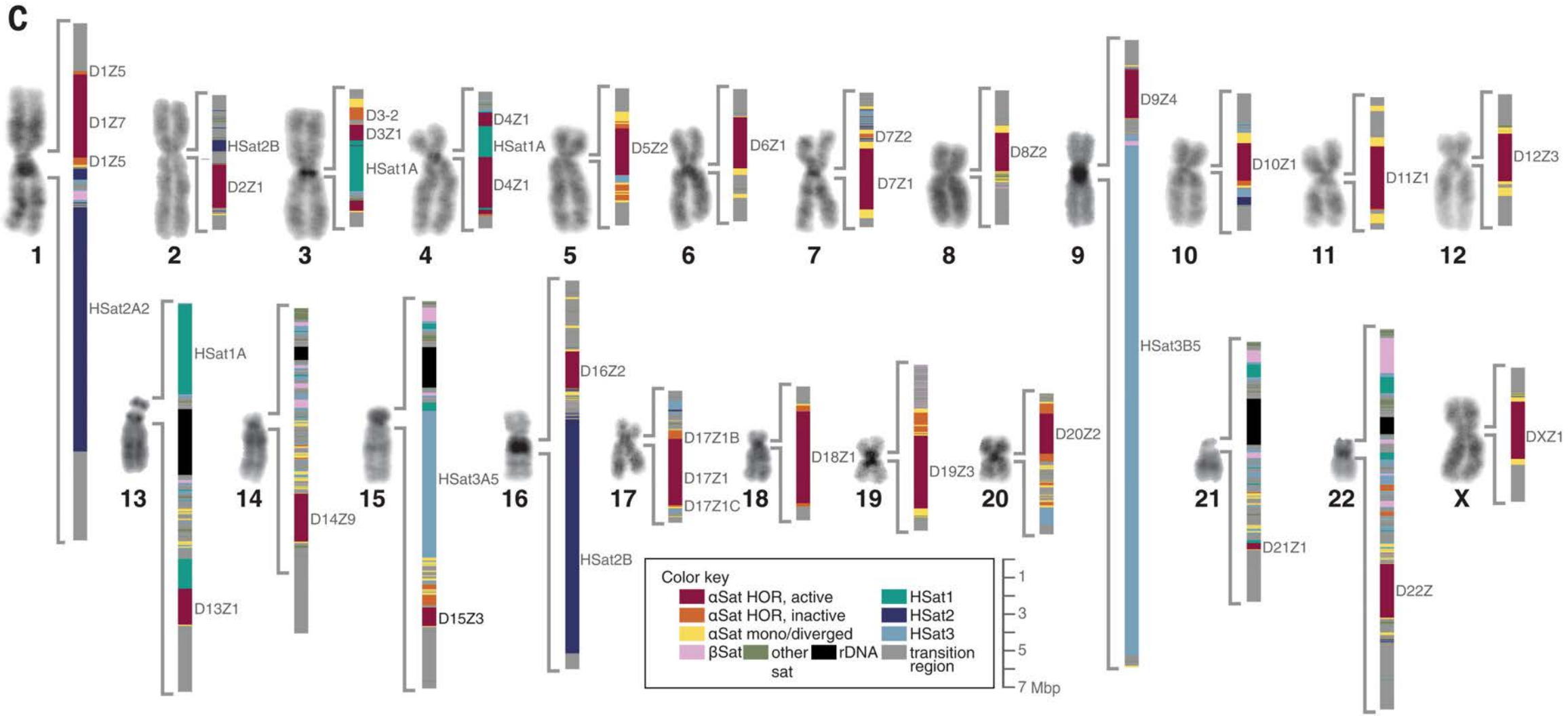
- 3.12 Gbp
- 0 gaps
- 1 error per 10,000,000 bases
- $5,000

MILLION-fold reduction in cost!
Driven by decades of investment and technology development

NIH NHGRI

The complete sequence of a human genome.
Nurk, Koren, Rhie, Rautiainen, et al. *Science* (2022)

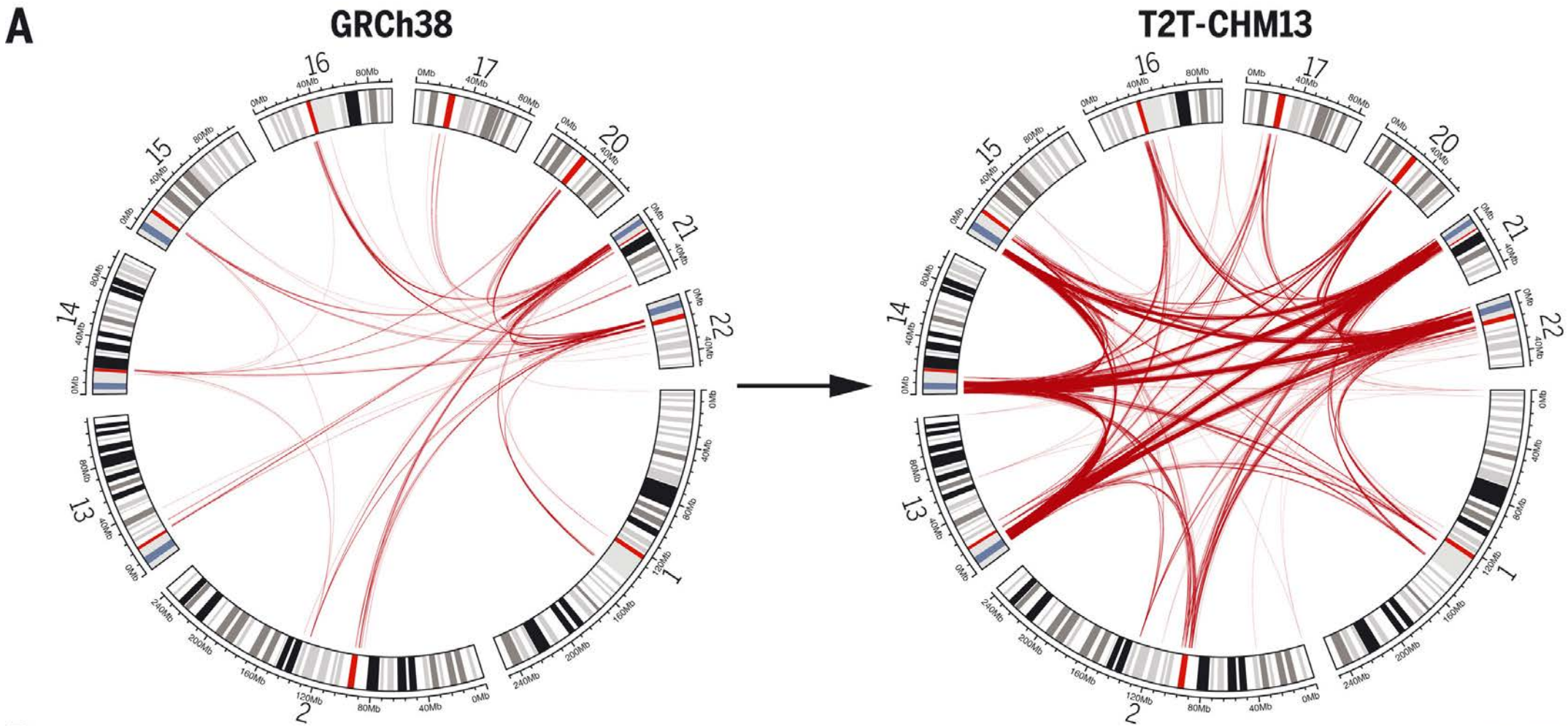
New satellites in CHM13



Complete genomic and epigenetic maps of human centromeres.
Altemose, et al. *Science* (2022)

NIH NHGRI

New segdups in CHM13



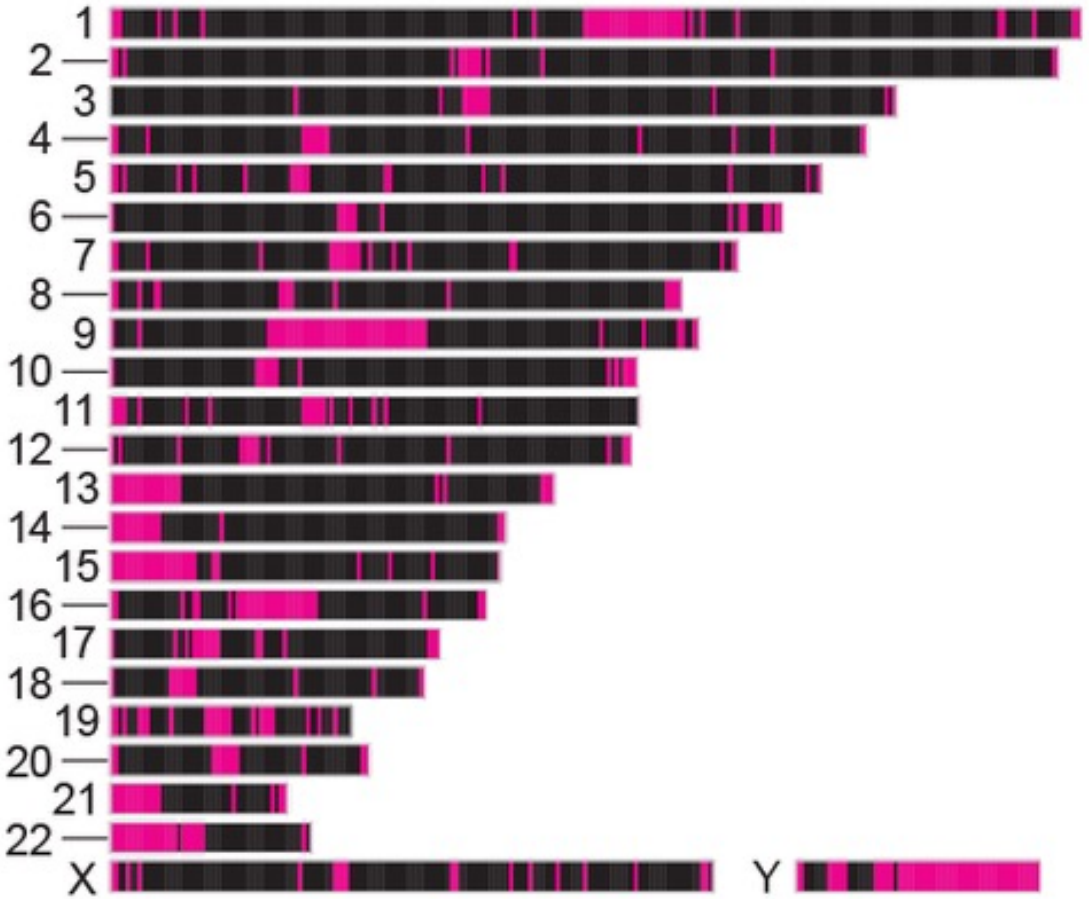
Segmental duplications and their variation in a complete human genome.
Vollger, et al. *Science* (2022)

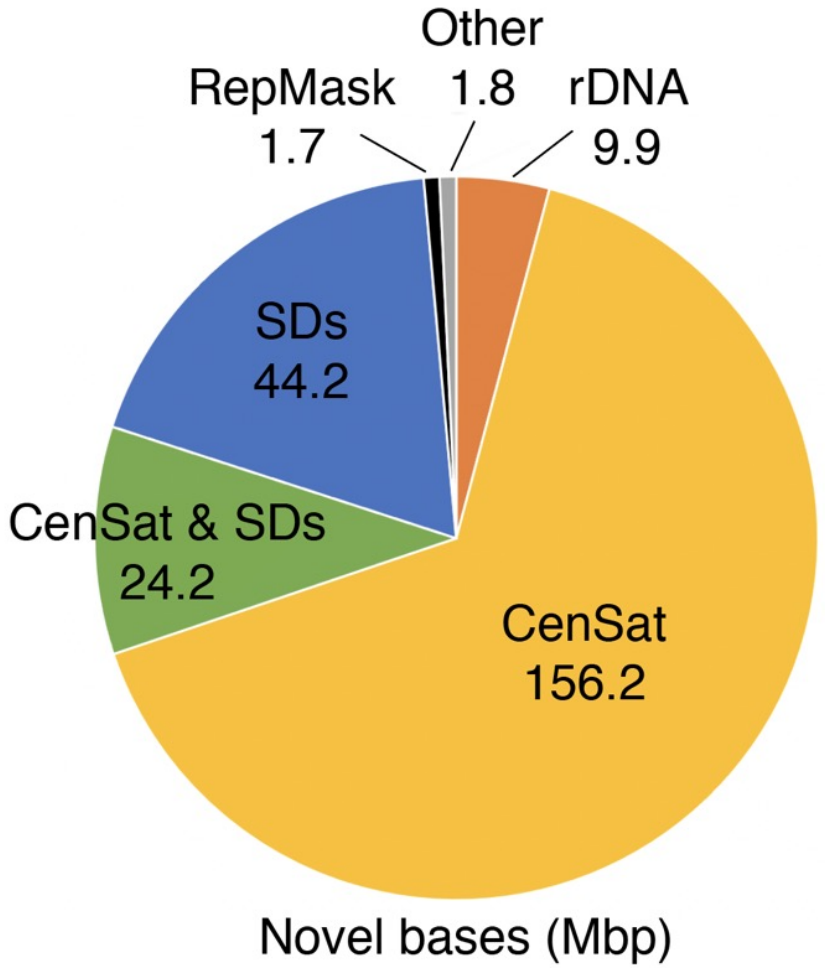
NIH NHGRI

Illuminating the dark regions of the human genome

NIH
NHGRI

What's new? Repeats!





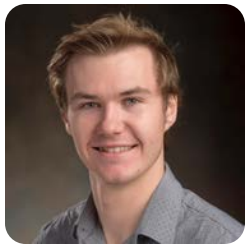
The complete sequence of a human genome.
Nurk, Koren, Rhie, Rautiainen, et al. *Science* (2022)

The complete sequence of a human Y chromosome.
Rhie, Nurk, et al. *Nature* (2023)

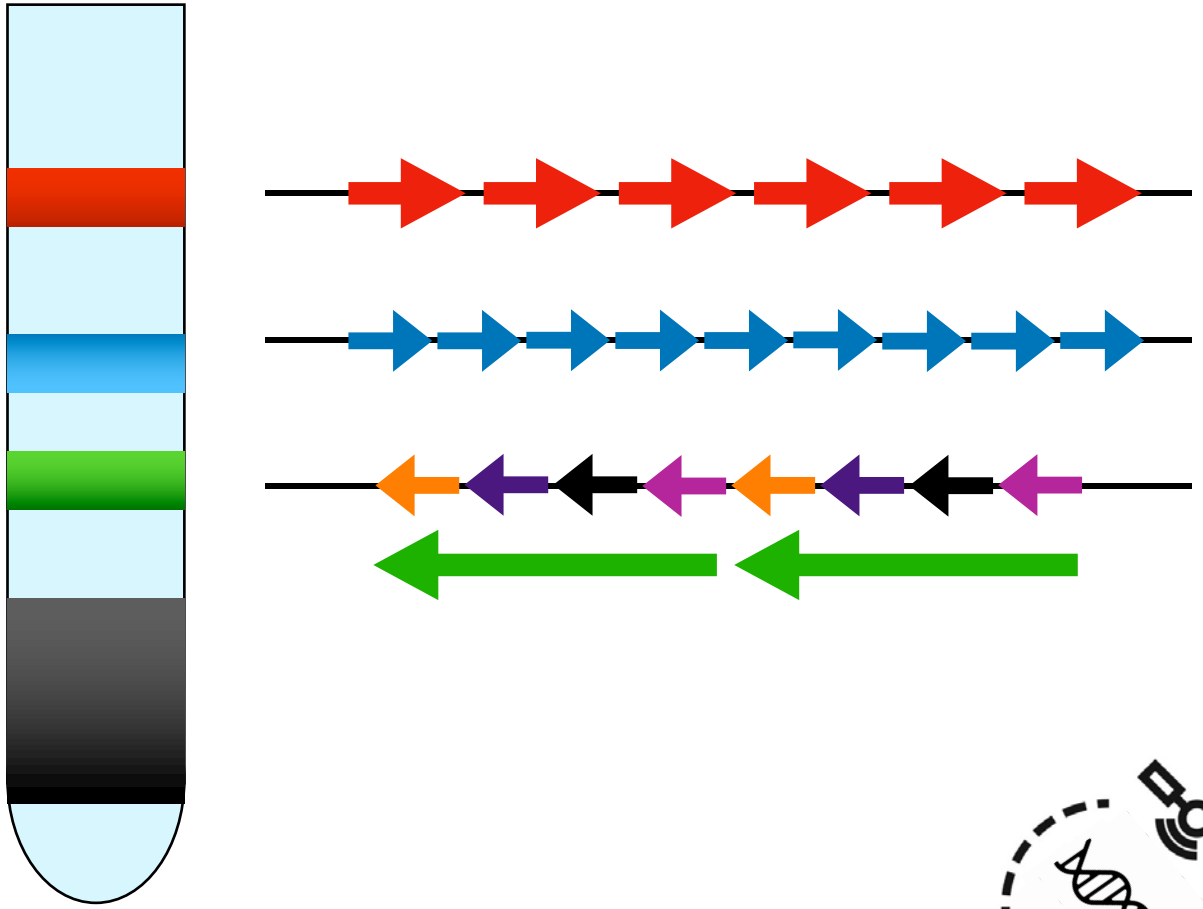
- >250 Mbp of new sequence
- ~2,000 new genes predicted

NIH NHGRI

Satellite DNA



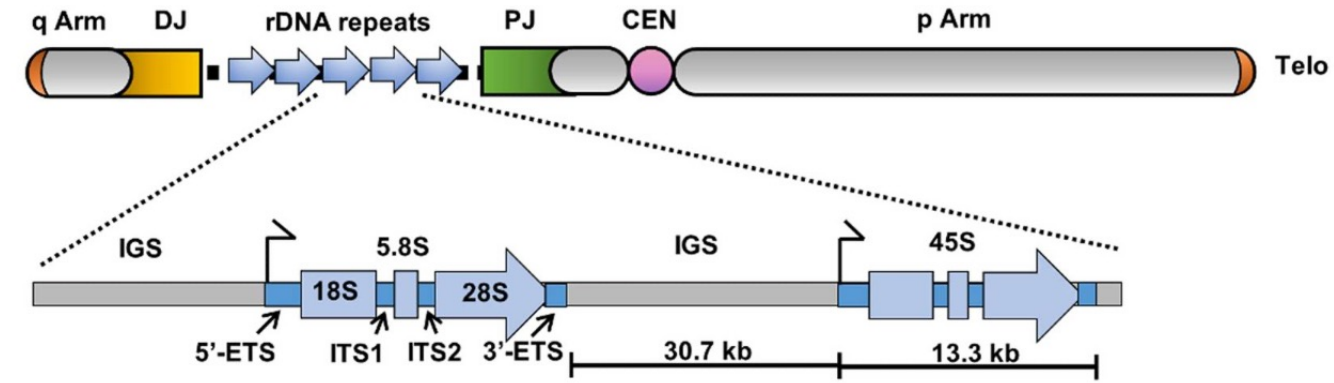
- Consists of **long arrays** of **tandemly repeating** sequences
- Primarily found in **centromeres** and **acrocentric** short arms
- Uniform repeats result in **unique properties** compared to bulk DNA

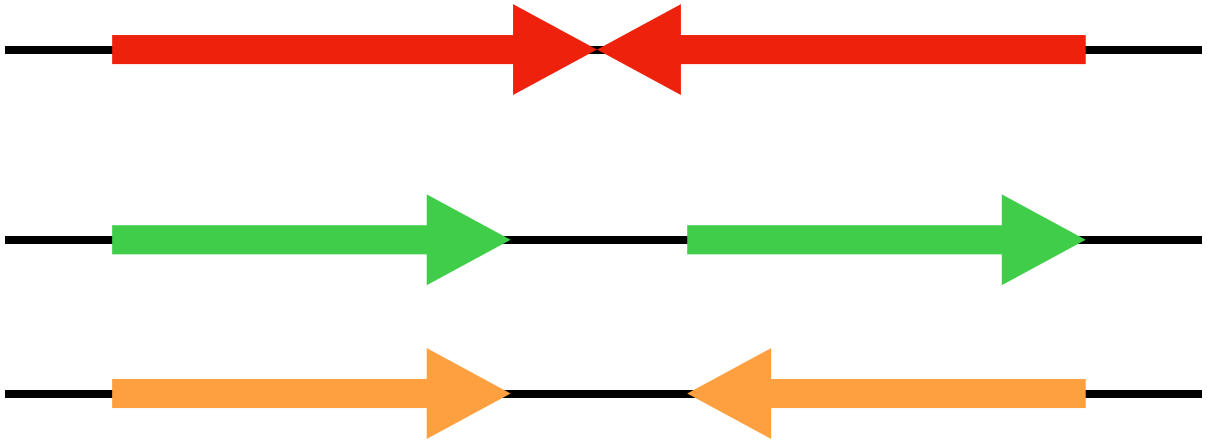


NIH
NHGRI

Segmental duplications

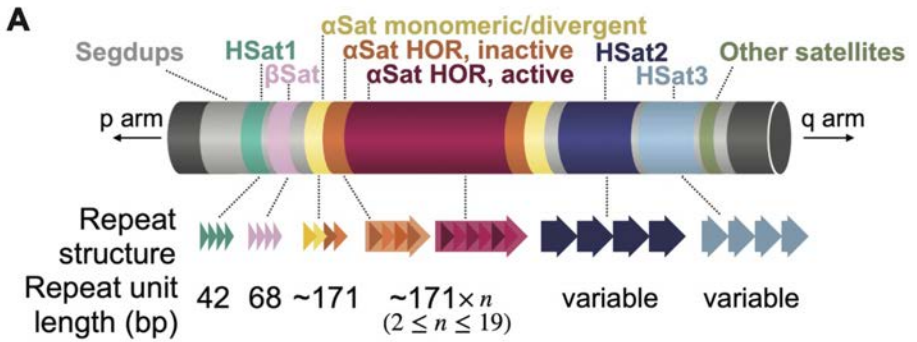
- Gene arrays
- Palindromes
- Directed repeats
- Inverted repeats

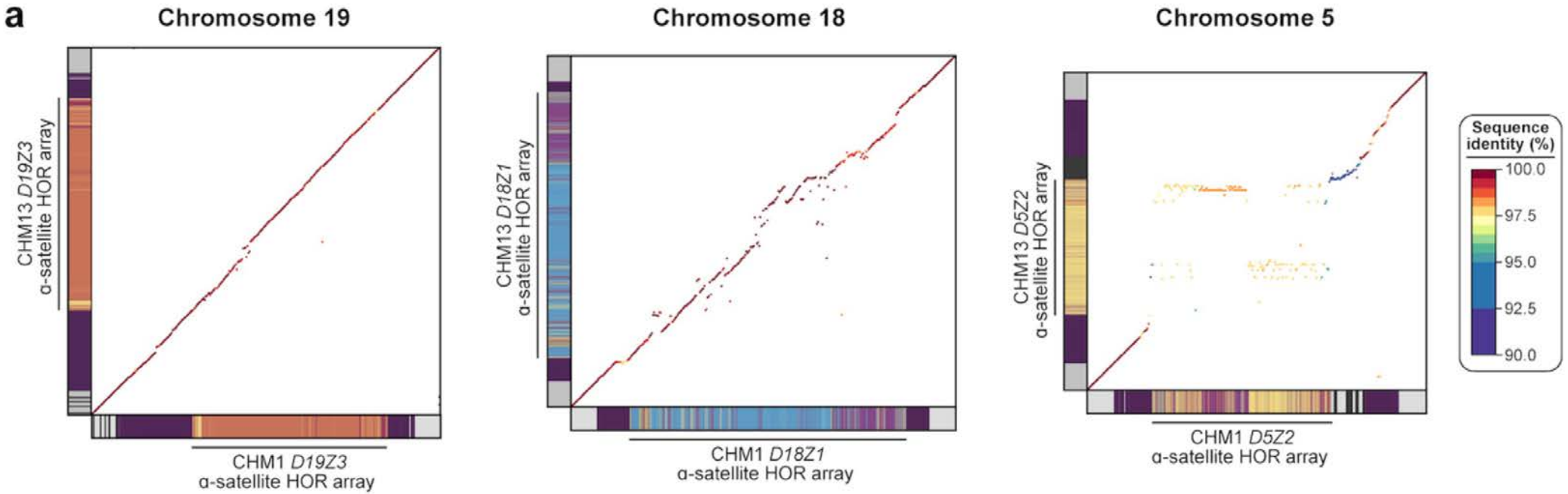




NIH
NHGRI

Satellites are often unalignable



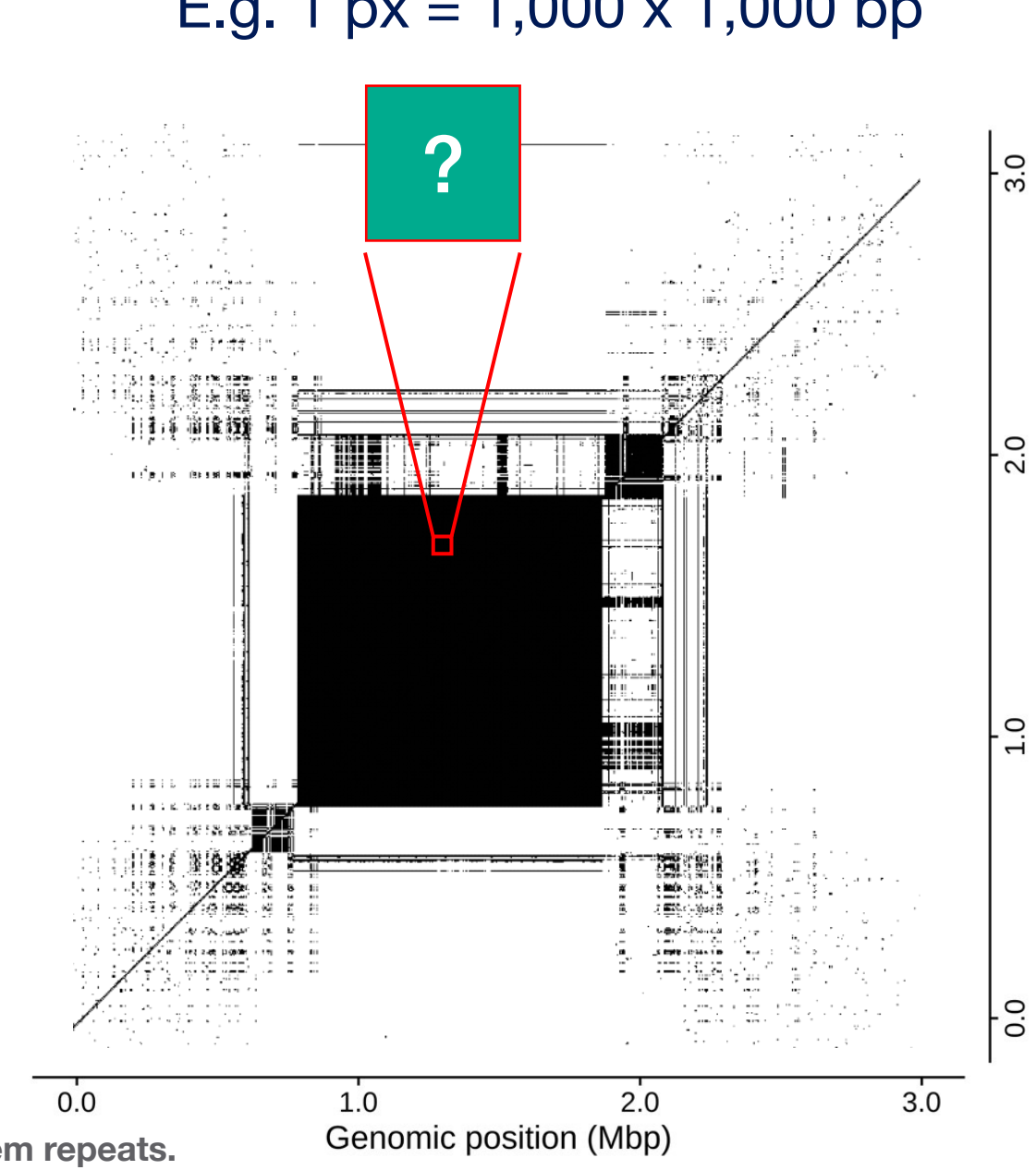


The variation and evolution of complete human centromeres.
Logsdon, et al. *Nature* (2024)

NIH NHGRI

A better dotplot

- Each pixel represents a pair of intervals of a fixed size
- Efficiently estimate the sequence identity for each pixel using min-hashing

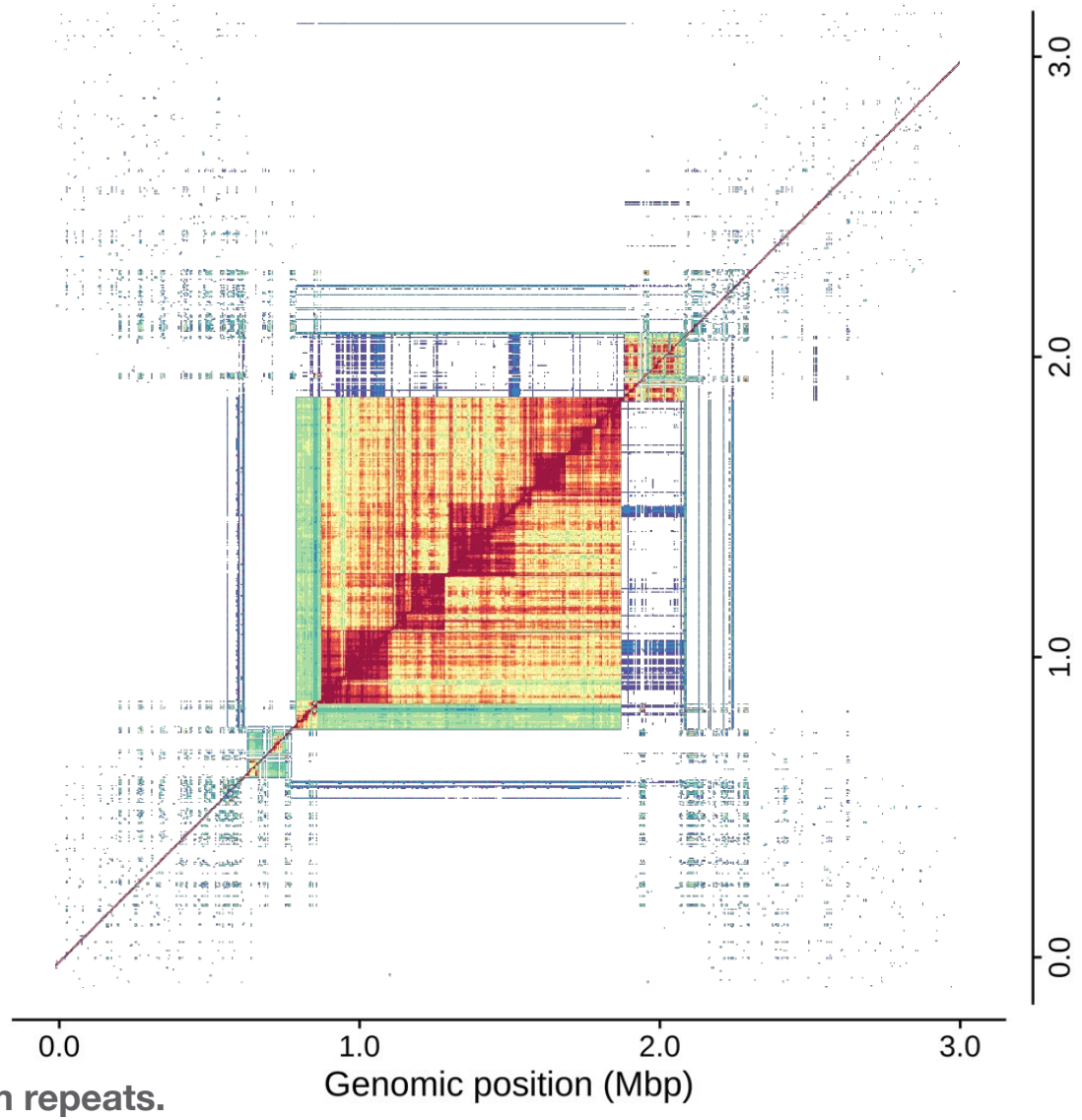


ModDotPlot—rapid and interactive visualization of tandem repeats.
Sweeten, et al. *Bioinformatics* (2014)

NIH NHGRI

A better dotplot

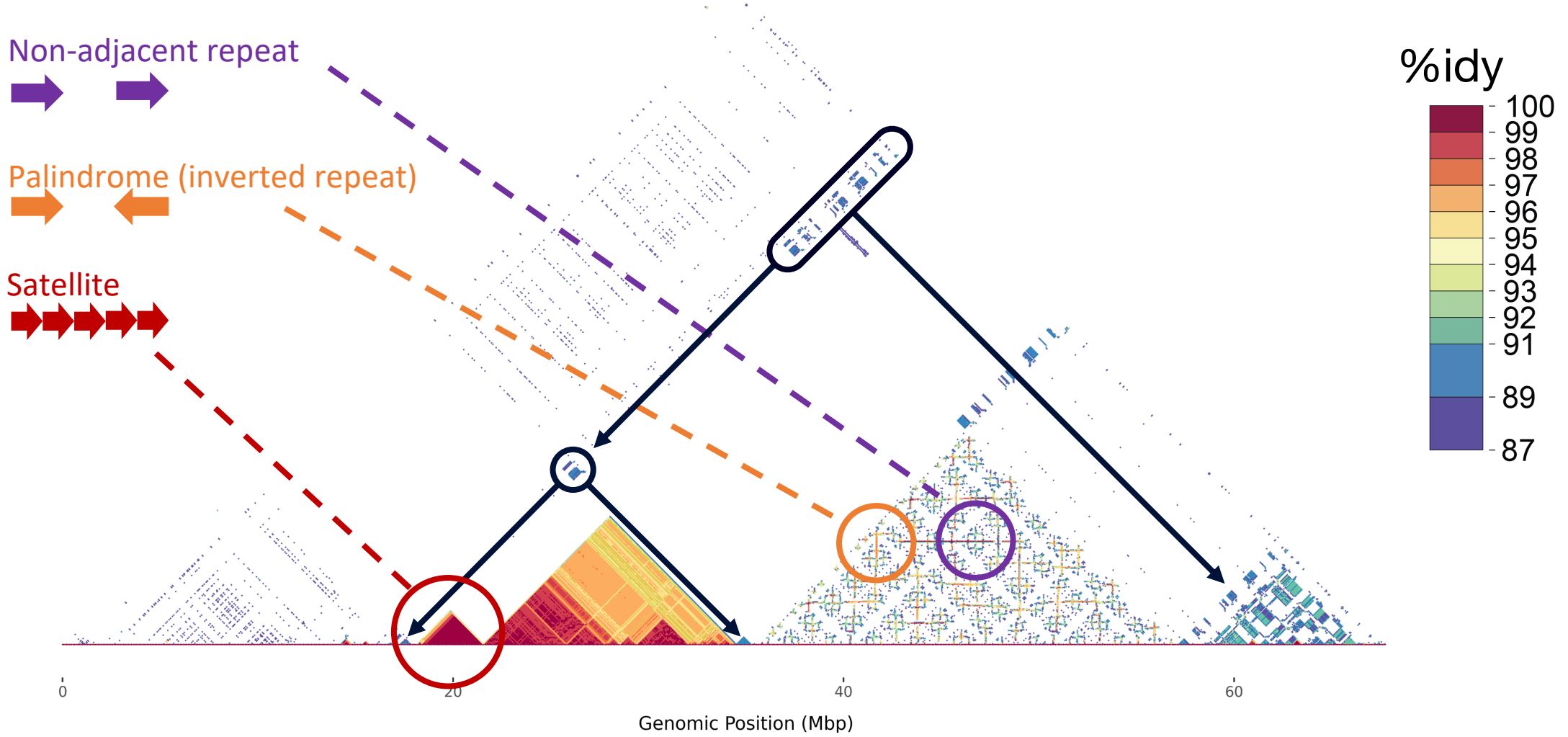
- Each pixel represents a pair of intervals of a fixed size
- Efficiently estimate the sequence identity for each pixel using min-hashing
- Reveals the structure and evolution of complex repeats



ModDotPlot—rapid and interactive visualization of tandem repeats.
Sweeten, et al. *Bioinformatics* (2014)

NIH NHGRI

StainedGlass & ModDotPlot



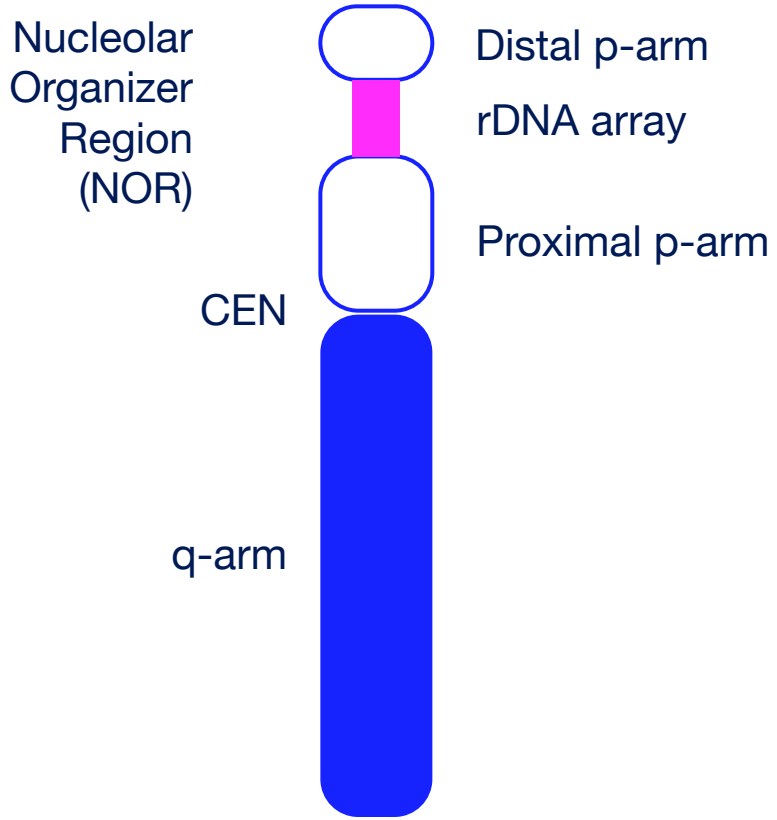
https://github.com/mrvollger/StainedGlass
https://github.com/marbl/ModDotPlot

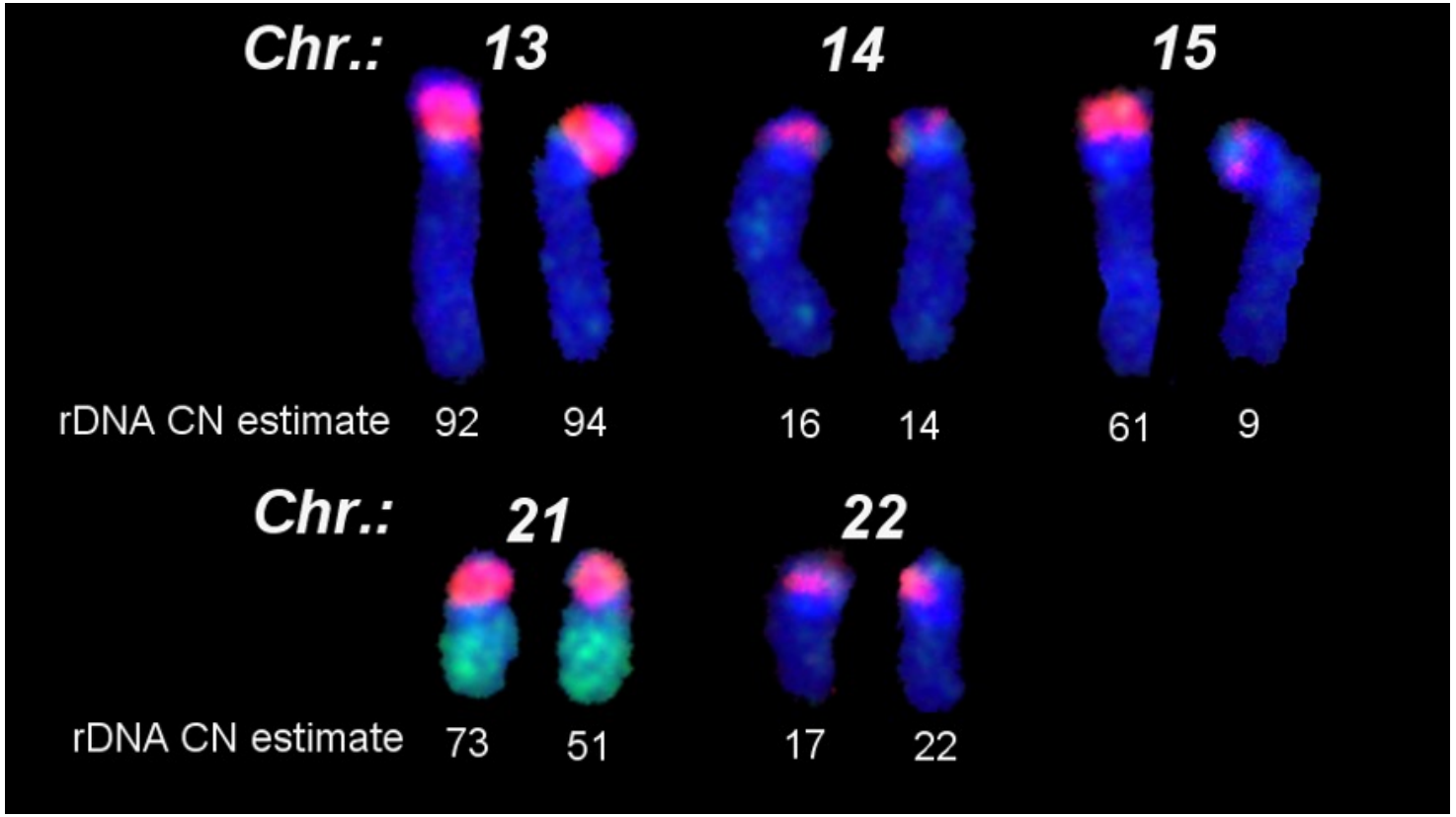
NIH
NHGRI

The acrocentrics: Satellites and segdups everywhere

NIH
NHGRI

The human acrocentrics

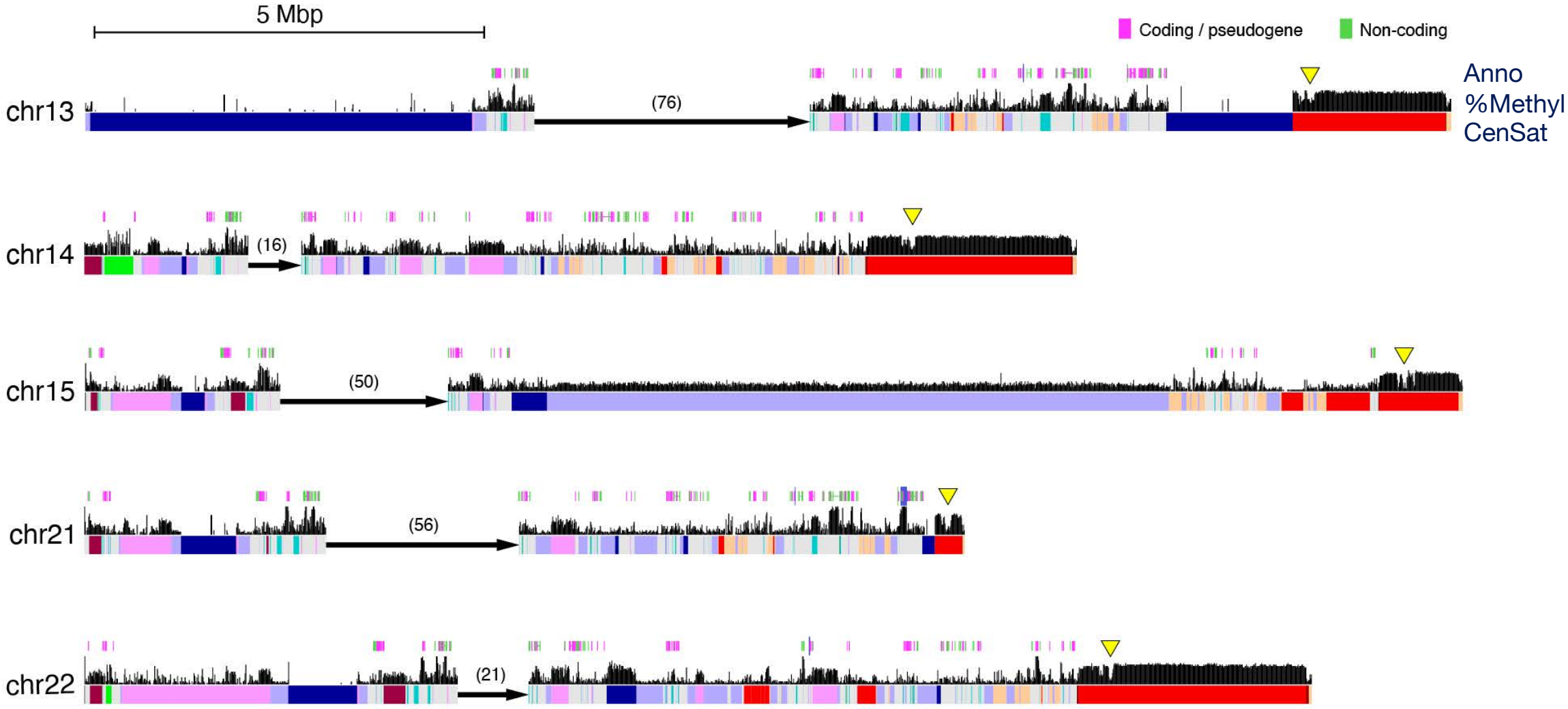




NIH NHGRI

CHM13 SKY by Tamara Potapova, Stowers Institute

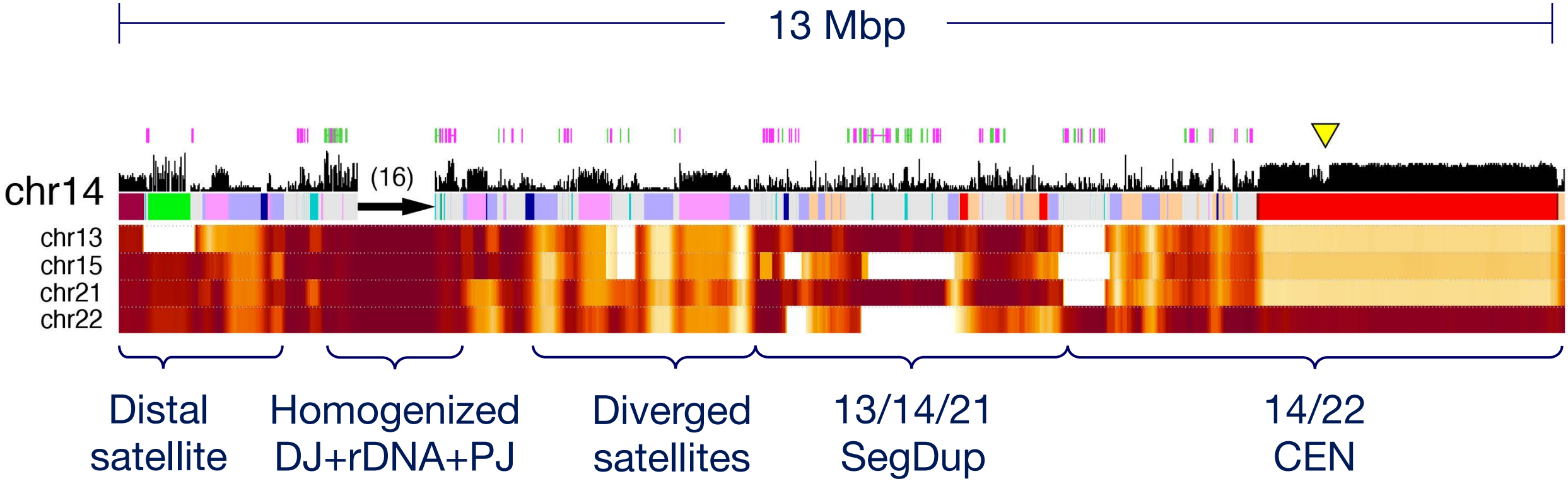
Short arms of the acrocentrics



The complete sequence of a human genome.
Nurk, Koren, Rhie, Rautiainen, et al. *Science* (2022)

NIH NHGRI

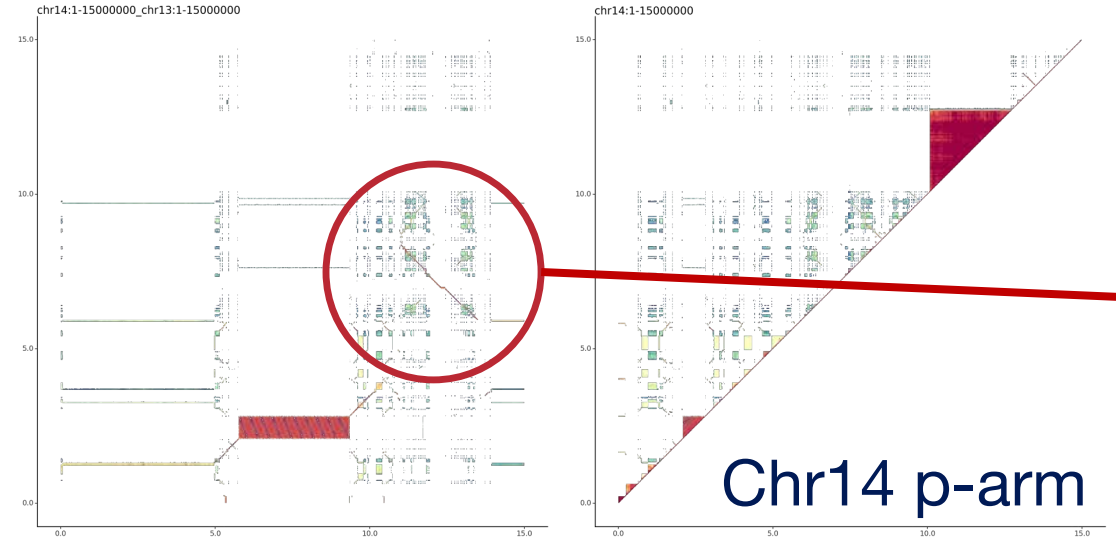
High sequence similarity between acros

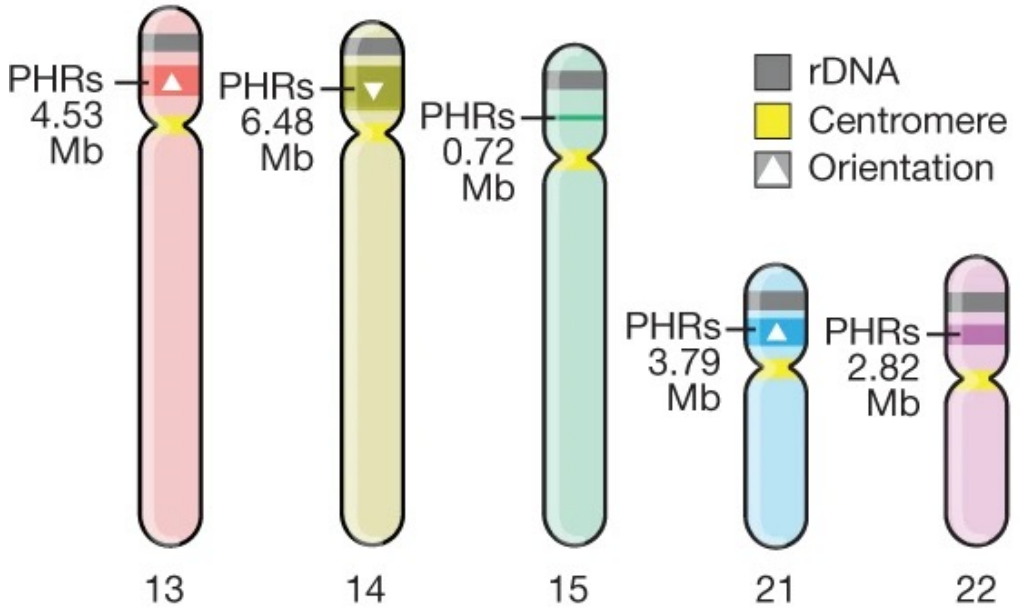


The complete sequence of a human genome.
Nurk, Koren, Rhie, Rautiainen, et al. *Science* (2022)

HSat1 BetaSat Alpha HOR
HSat3 Alpha MON Segdup

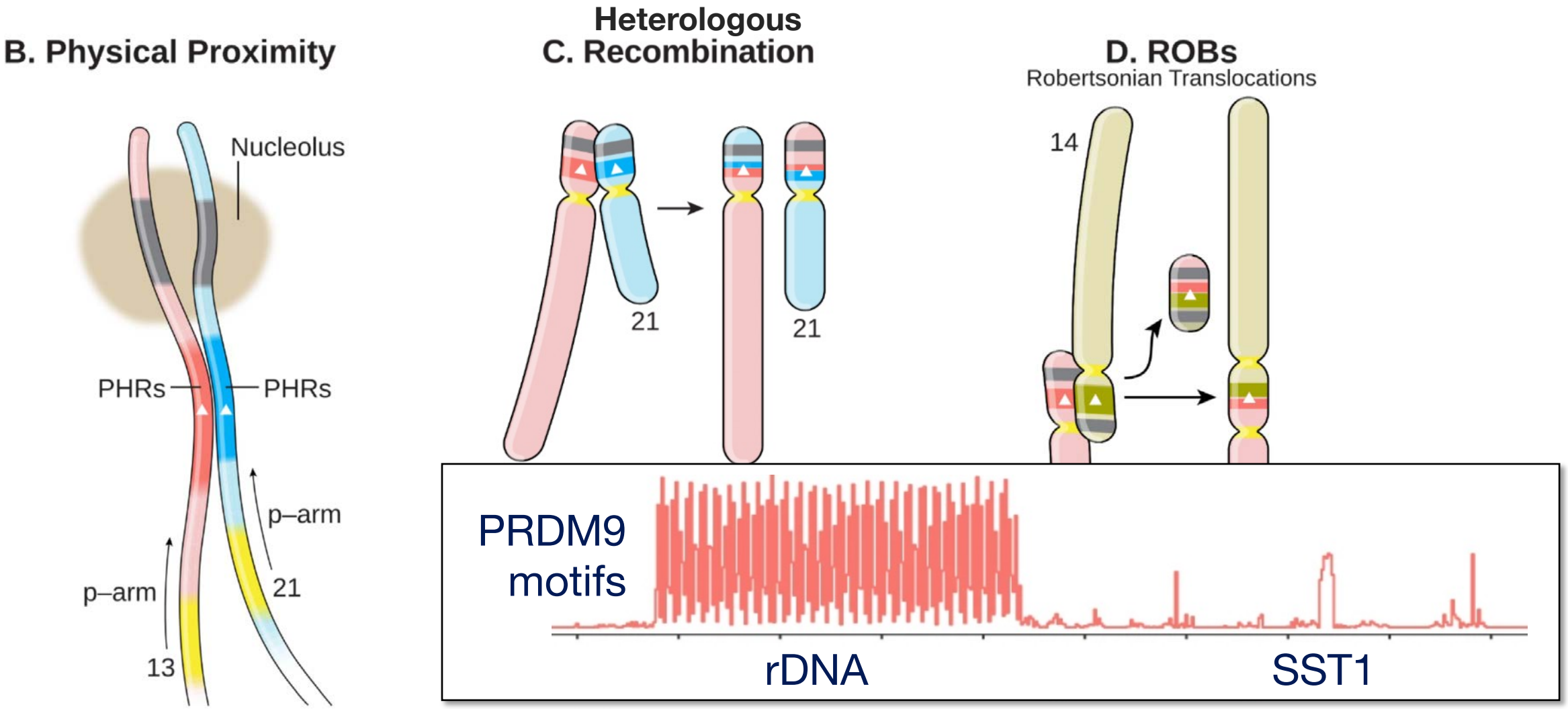
Pseudo-homologous regions





NIH
NHGRI

Short arm recombination

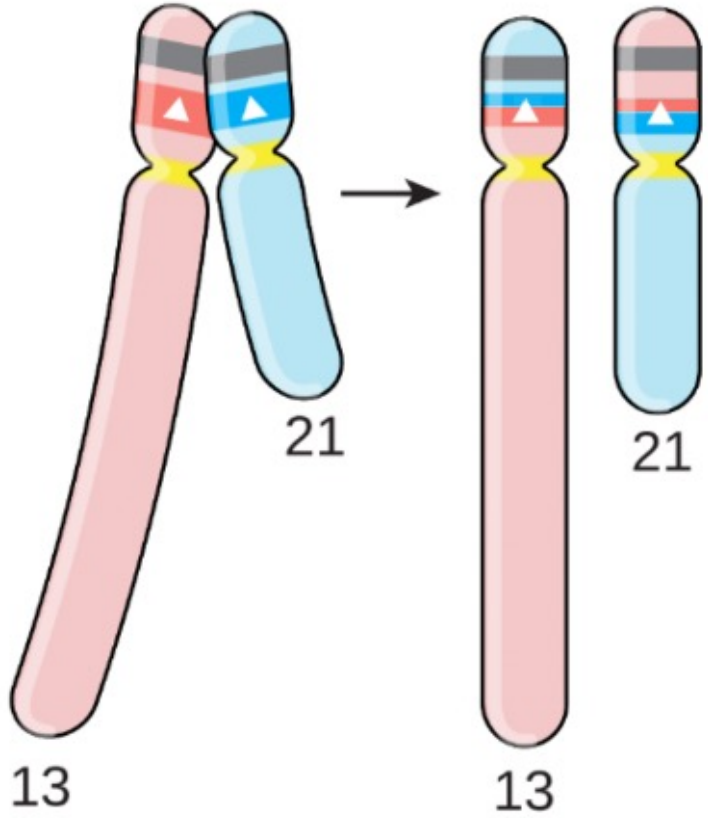


Recombination between heterologous human acrocentric chromosomes.
Guarracino, et al. *Nature* (2023)

NIH NHGRI

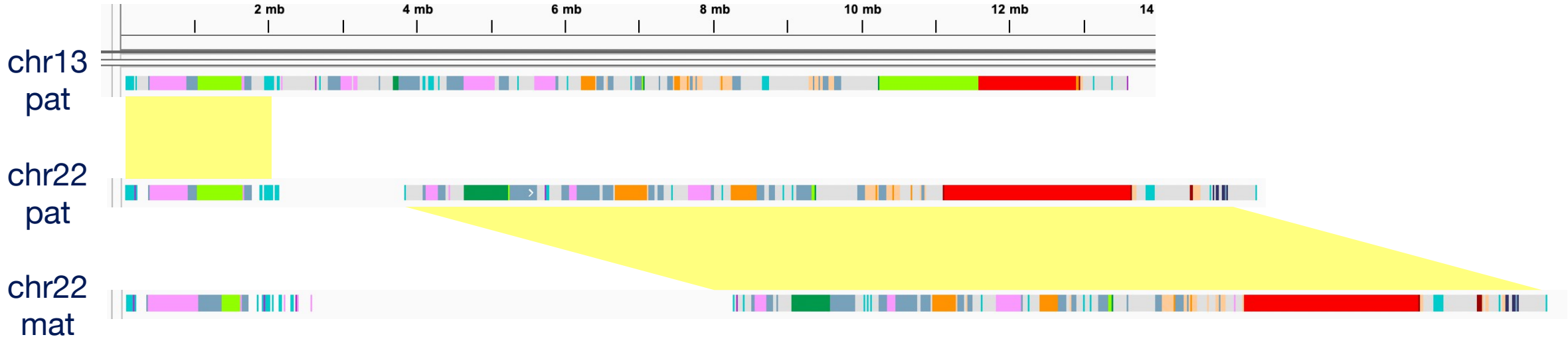
Why are rDNAs on the acros?

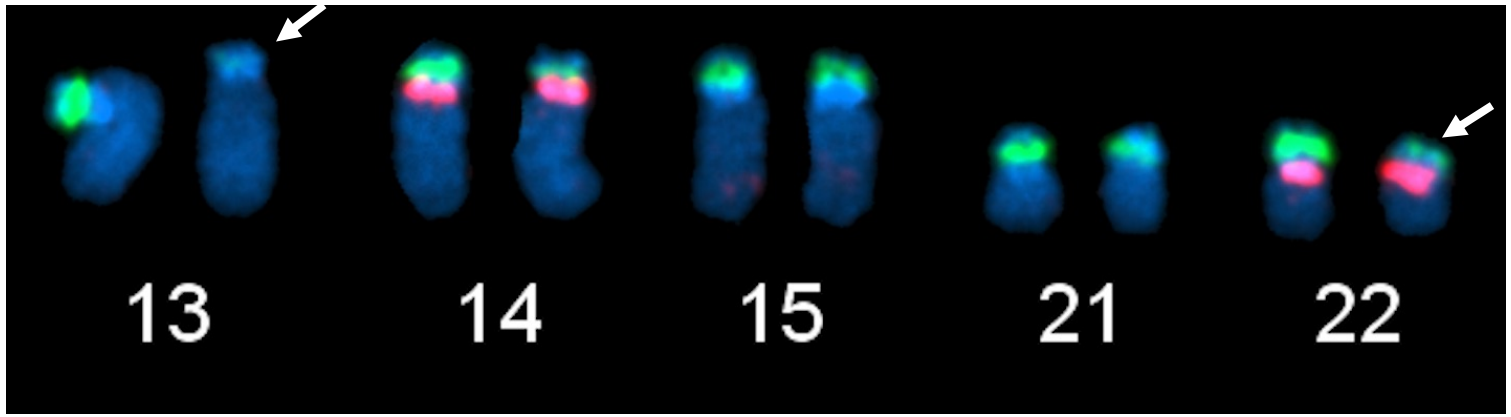
- **rDNAs seem to appear either:**
  - In one array on a metacentric chr
  - In multiple arrays on short arms
- **Why?**
  - Permissive of crossovers
  - Maintains rDNA concerted evolution
- **Conclusion**
  - Human NORs not likely chr-specific



NIH
NHGRI

HG002 distal copy between 13/22





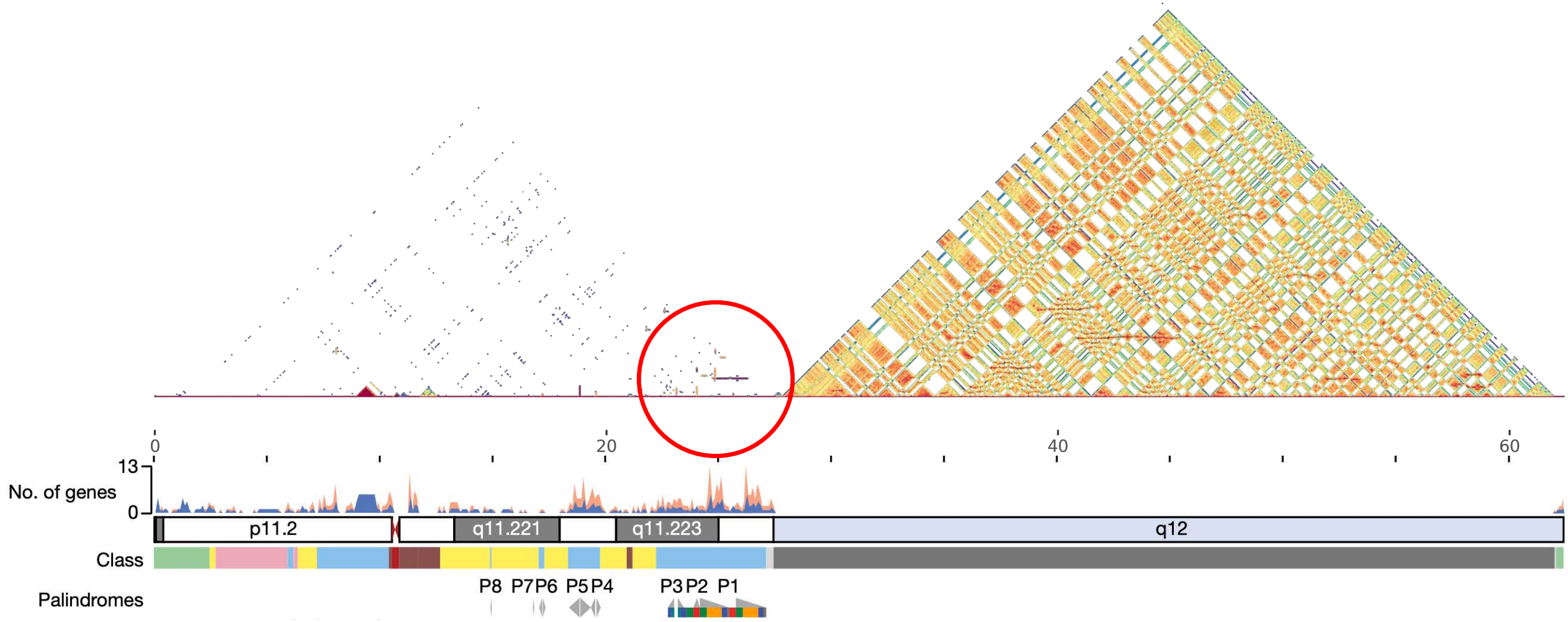
HG002 SKY by Tamara Potapova, Stowers Institute

NIH NHGRI

The Y chromosome: A wannabe acrocentric

NIH
NHGRI

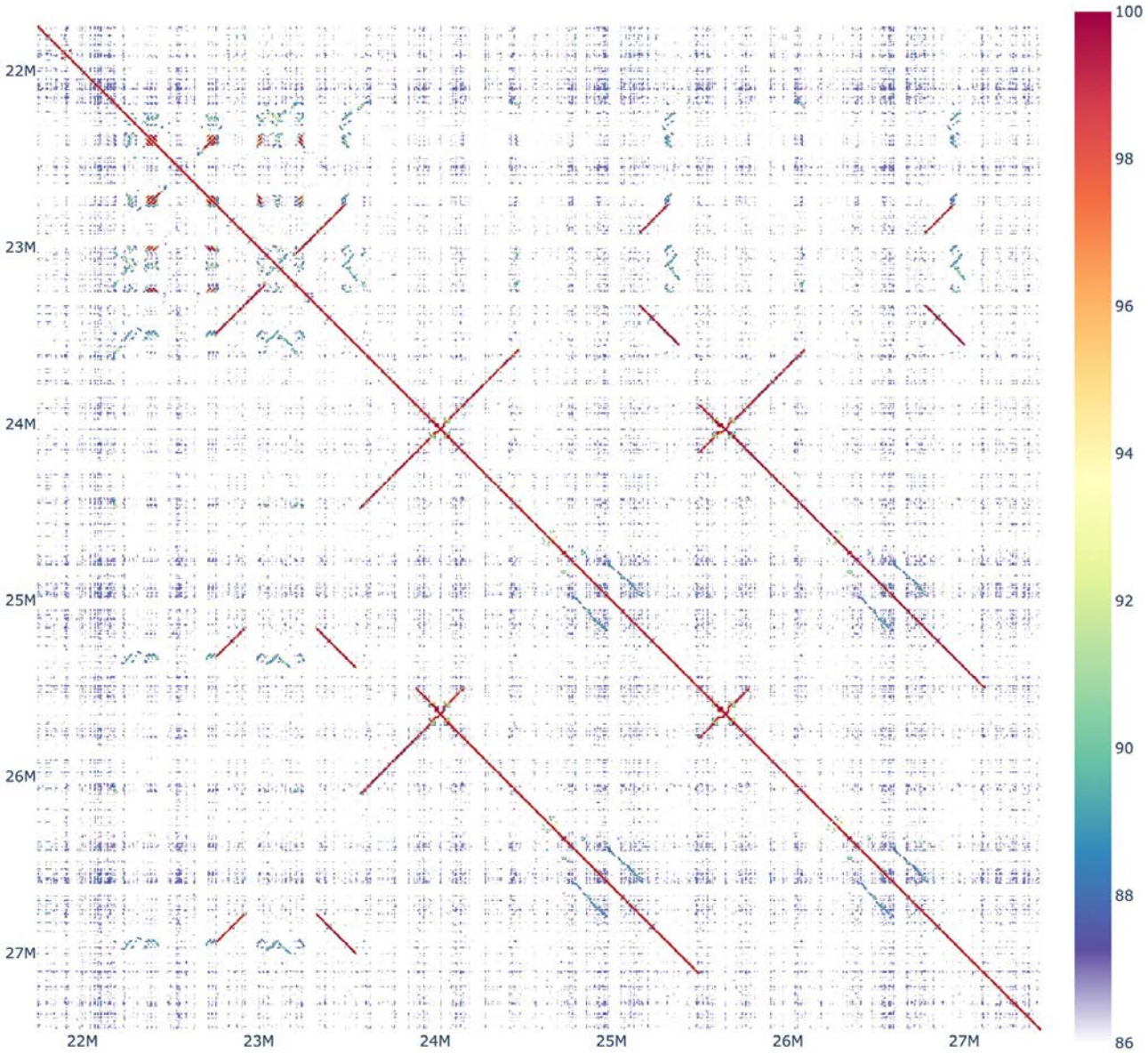
Human HG002 T2T-Y



The complete sequence of a human Y chromosome.
Rhie, Nurk, et al. *Nature* (2023)

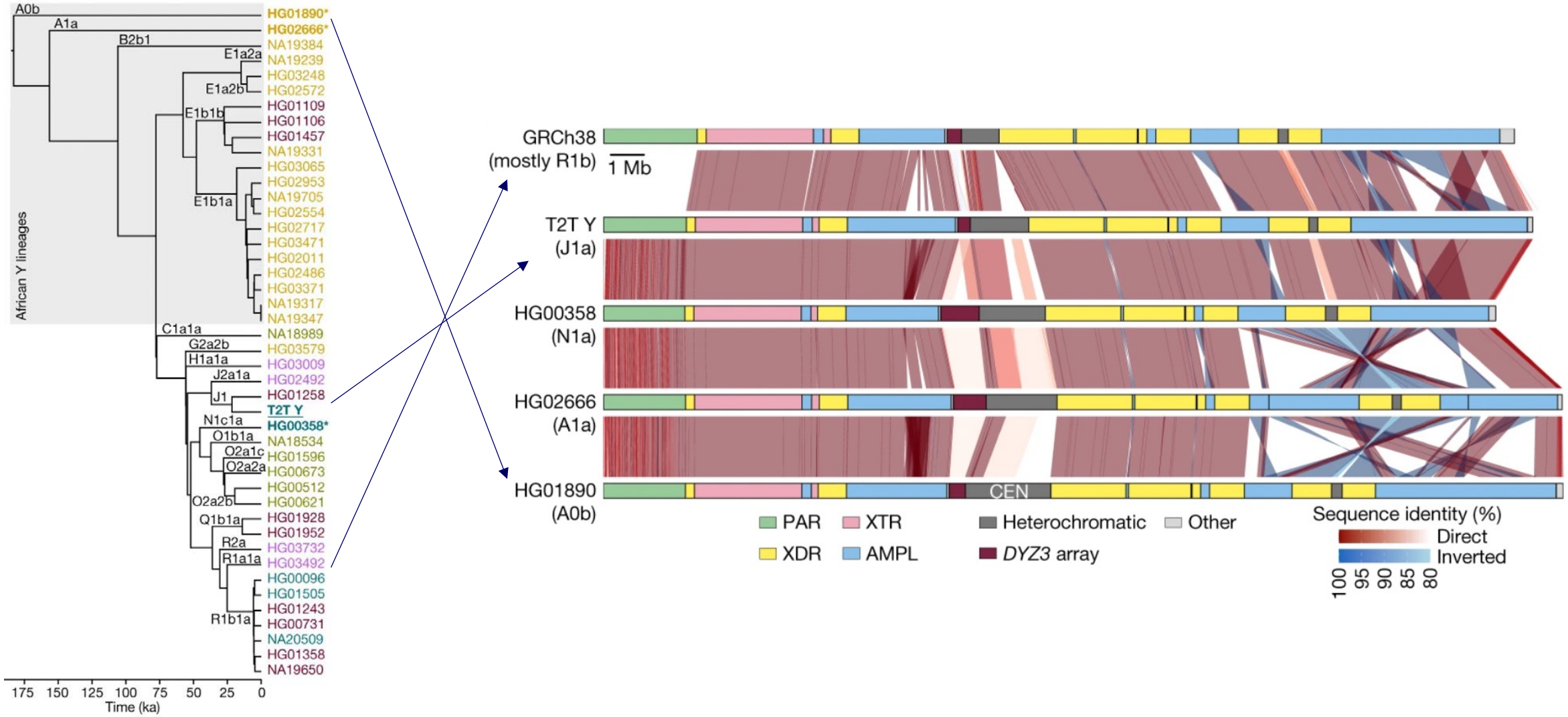
NIH NHGRI

T2T-Y palindromic region



NIH
NHGRI

Human chrY variation



Assembly of 43 human Y chromosomes reveals extensive complexity and variation.
Hallast, et al. *Nature* (2023)

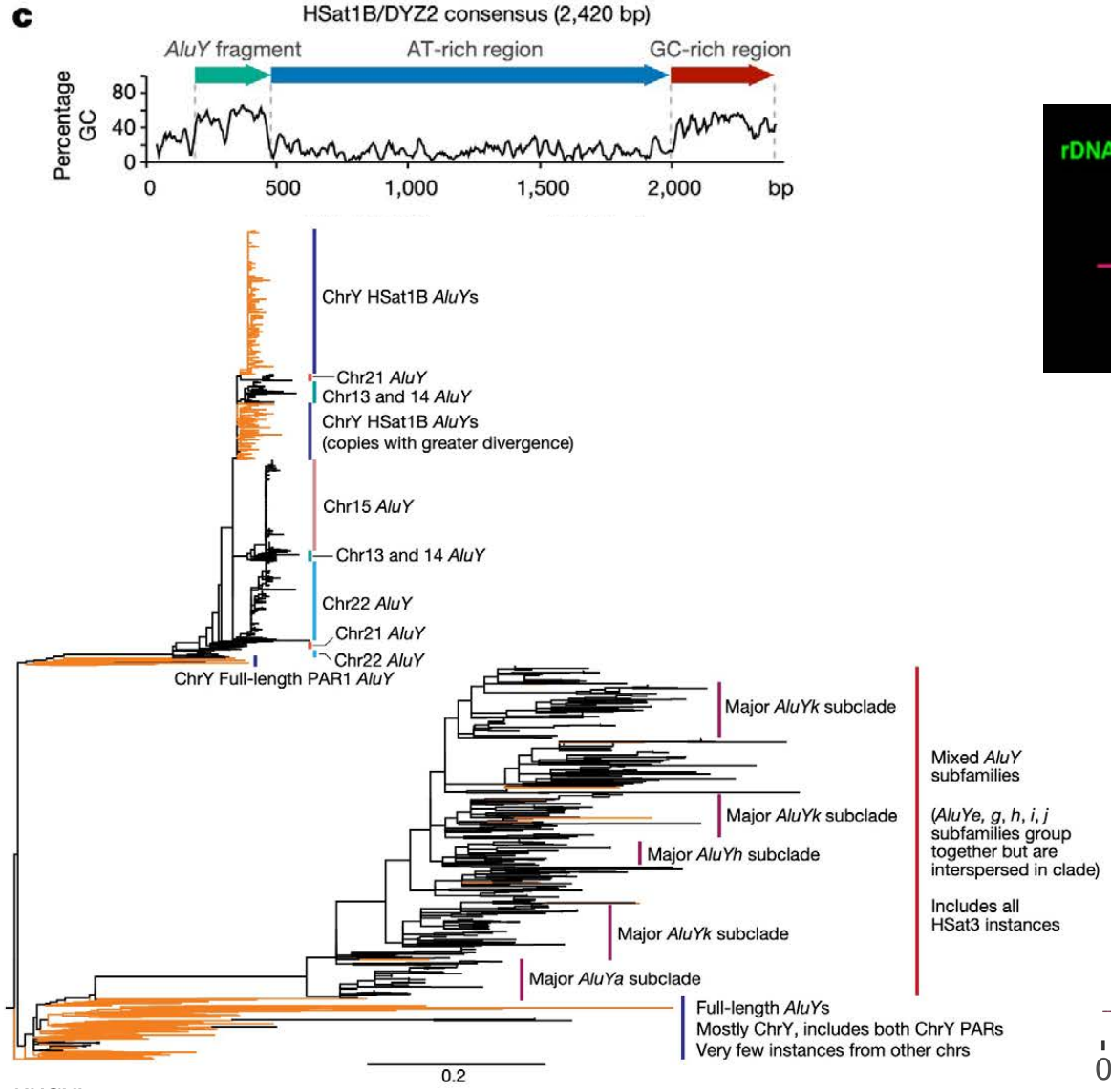
NIH NHGRI

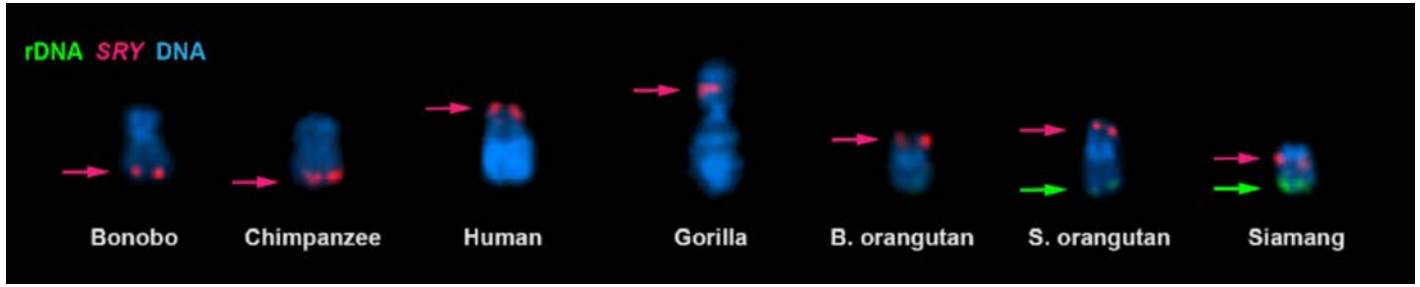
Ape chrY diversity

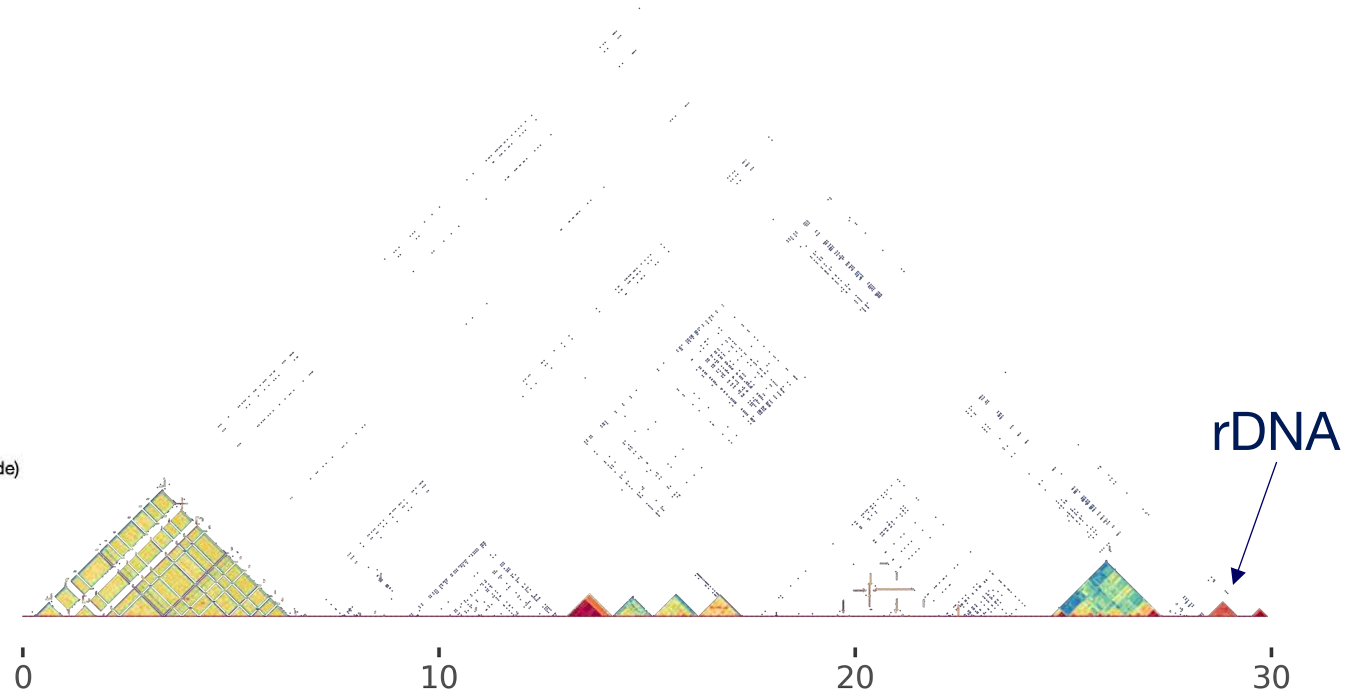
The complete sequence and comparative analysis of ape sex chromosomes.
Makova, Pickett, et al. *Nature* (2024)

NIH NHGRI

Crosstalk between acros and chrY

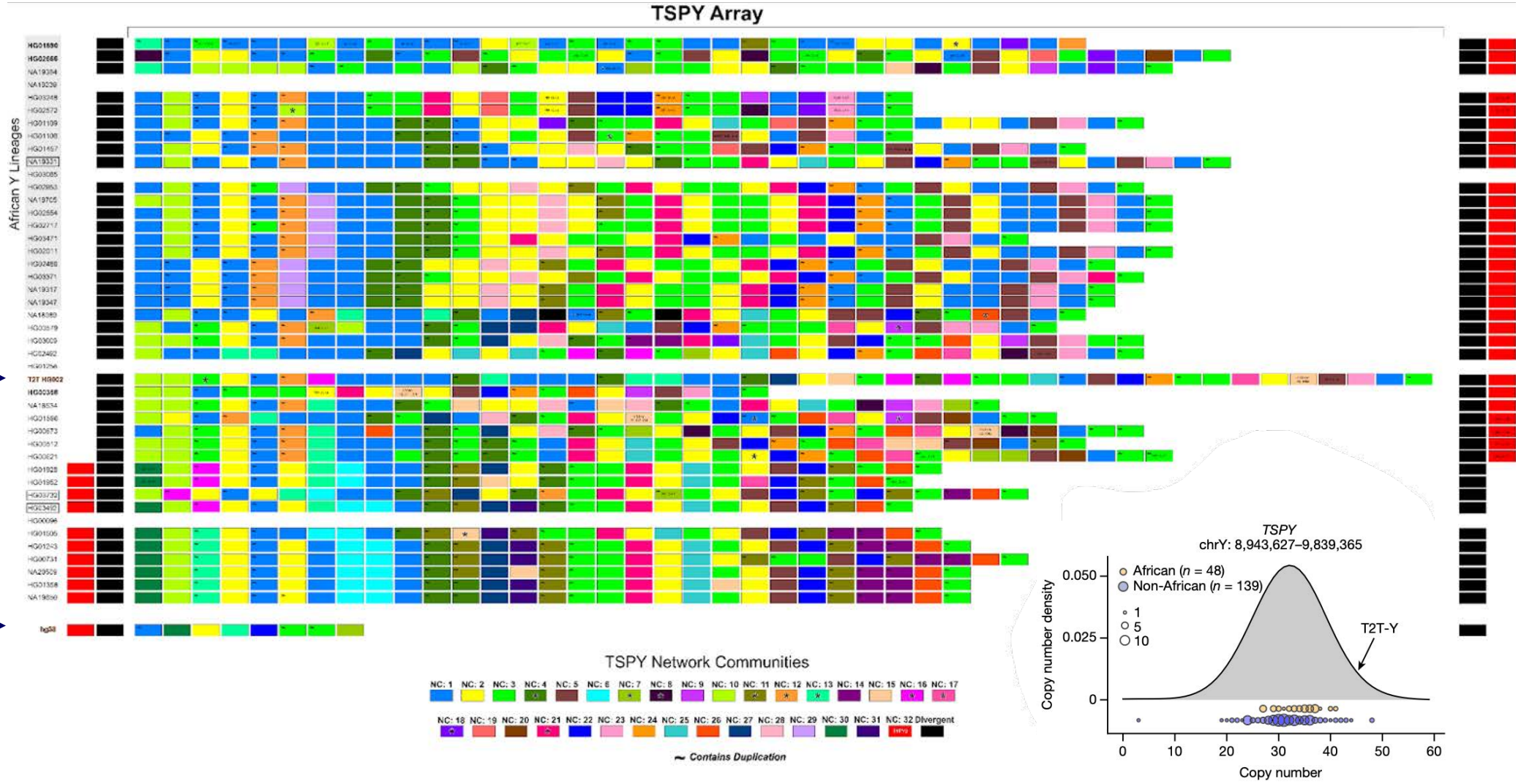






Mapping fails to characterize such dynamic regions properly

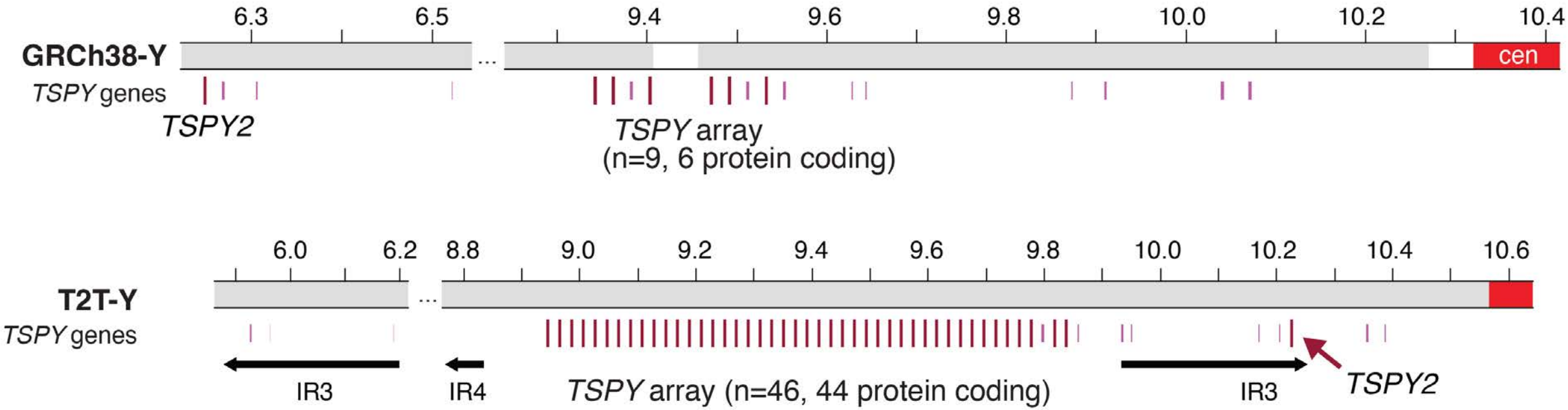
TSPY gene array



Assembly of 43 human Y chromosomes reveals extensive complexity and variation.
Hallast, et al. *Nature* (2023)

NIH NHGRI

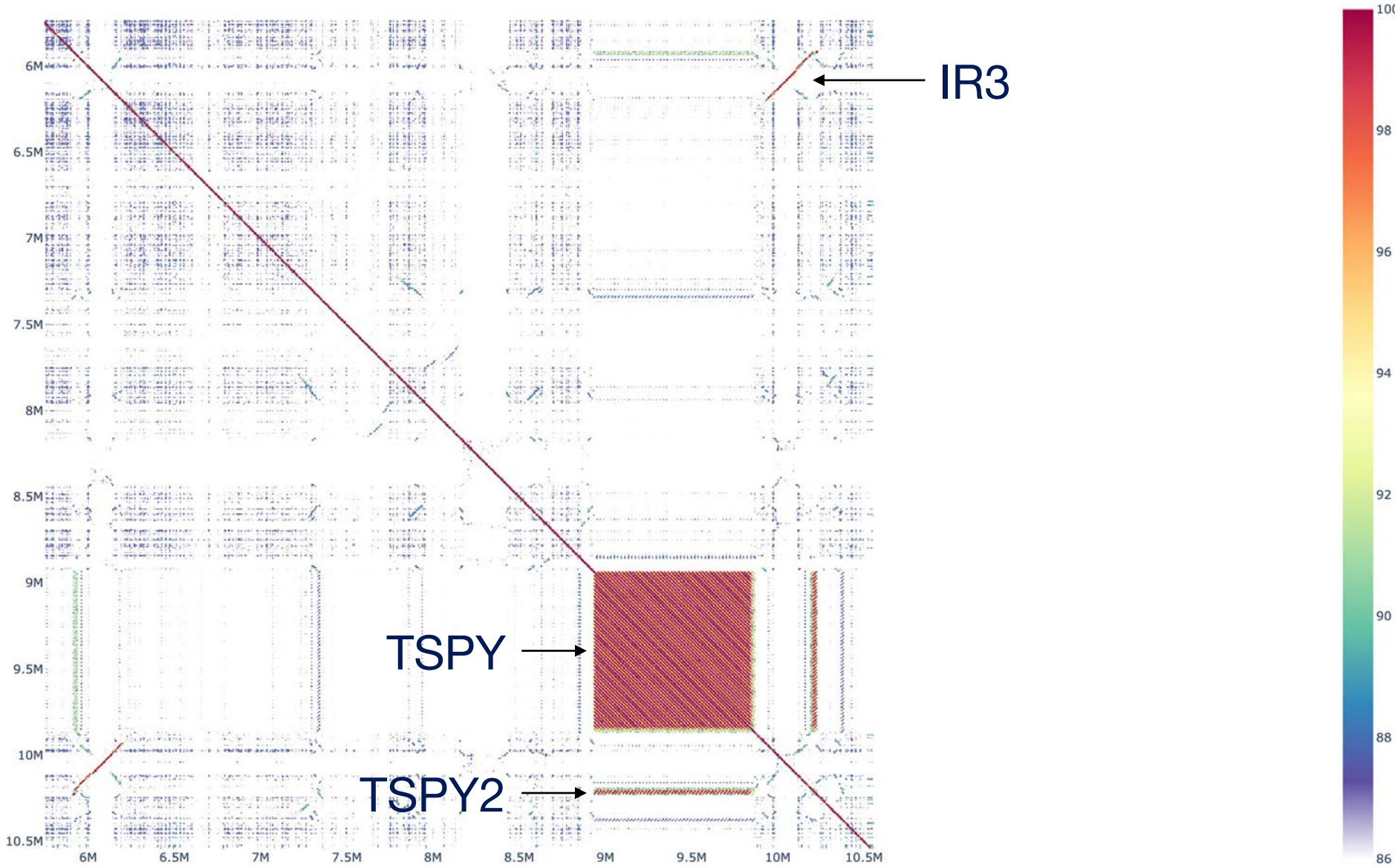
TSPY2 gene conversion



The complete sequence of a human Y chromosome.
Rhie, Nurk, et al. *Nature* (2023)

NIH NHGRI

TSPY region



NIH
NHGRI

Mapped ONT reads clip at TSPY2

GRCh38 ref

HG002 reads

Not just a problem on chrY

Lost in the WASH. The functional human WASH complex I gene is on chromosome 20

Daniel Cerdán-Vélez, Michael L. Tress

doi: https://doi.org/10.1101/2023.06.14.544951

This article is a preprint and has not been certified by peer review [what does this mean?].

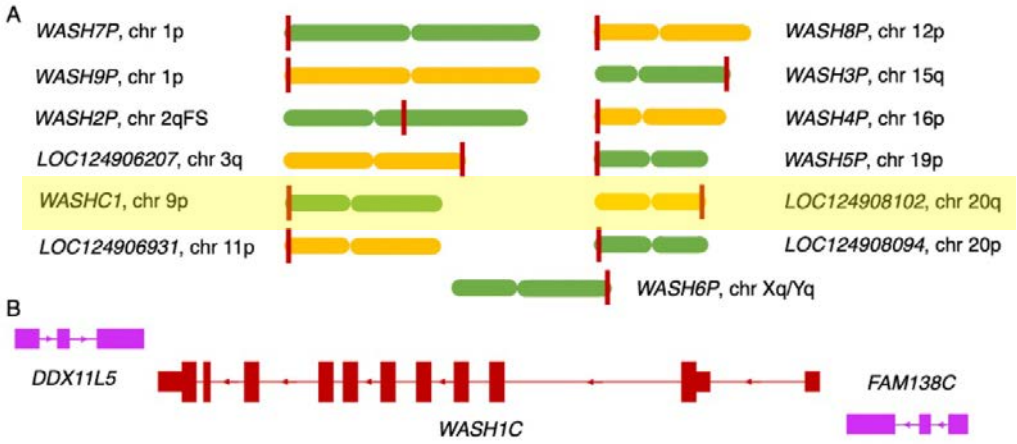
Abstract

The WASH1 gene produces a protein that forms part of the developmentally important WASH complex. The WASH complex activates the Arp2/3 complex to initiate branched actin networks at the surface of endosomes. As a curiosity, the human reference gene set includes nine WASH1 genes. How many of these are pseudogenes and how many are *bona fide* coding genes is not clear.

Eight of the nine WASH1 genes reside in rearrangement and duplication-prone subtelomeric regions. Many of these subtelomeric regions had gaps in the GRCh38 human genome assembly, but the recently published T2T-CHM13 assembly from the Telomere to Telomere (T2T) Consortium has filled in the gaps. As a result, the T2T Consortium has added four new WASH1 paralogues in previously unannotated subtelomeric regions.

Here we show that one of these four novel WASH1 genes, *LOC124908094*, is the gene most likely to produce the functional WASH1 protein. We also demonstrate that the other twelve WASH1 genes derived from a single *WASH8P* pseudogene on chromosome 12. These 12 genes include WASHC1, the gene currently annotated as the functional WASH1 gene.

We propose *LOC124908094* should be annotated as a coding gene and all functional information relating to the *WASHC1* gene on chromosome 9 should be transferred to *LOC124908094*. The remaining WASH1 genes, including *WASHC1*, should be annotated as pseudogenes. This work confirms that the T2T assembly has added at least one functionally relevant coding gene to the human reference set. It remains to be seen whether other important coding genes are missing from the GRCh38 reference assembly.



How frequent are subtelomere translocations and what phenotypes are the mediating?

NIH NHGRI

The issues with mapping

- **Reference bias**
  - Short reads, long repeats
  - A single, incomplete reference
  - Structural variants behave differently
- **Recombination between repeats**
  - Need *de novo* assemblies or specialized variant calling approaches for genotyping these atypical regions
- **Genes are not always where you think**
  - Gene conversion
  - Acrocentric and sex chromosome recombination
  - Subtelomere translocations

What’s the solution?

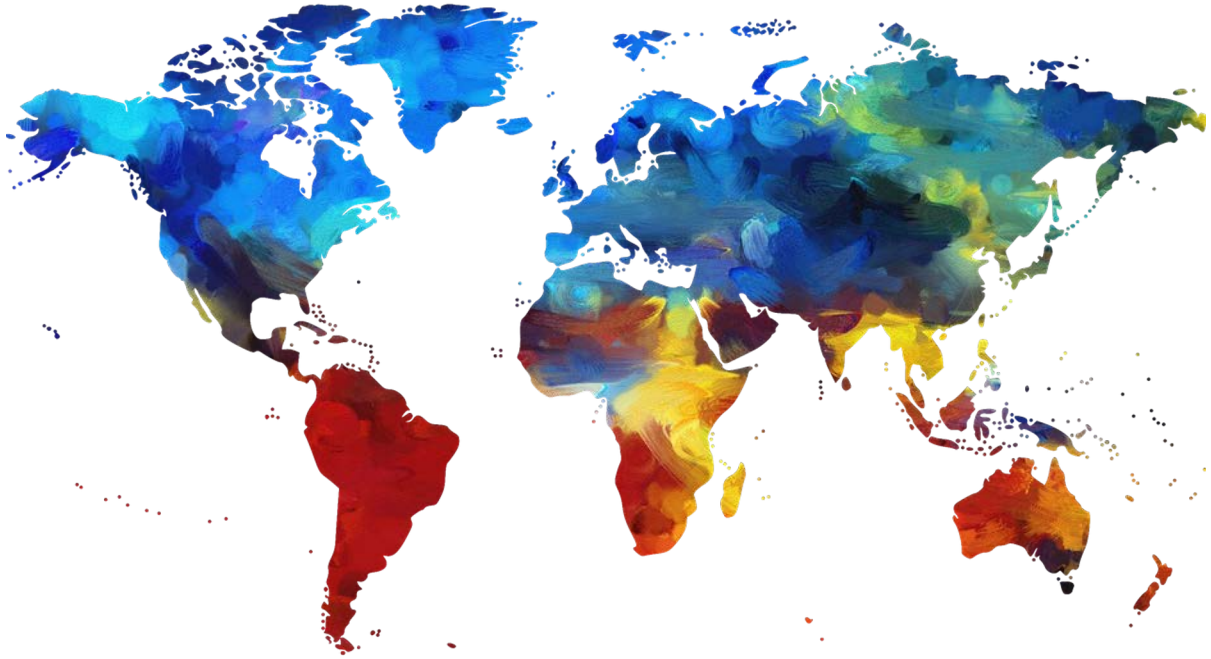
NIH
NHGRI

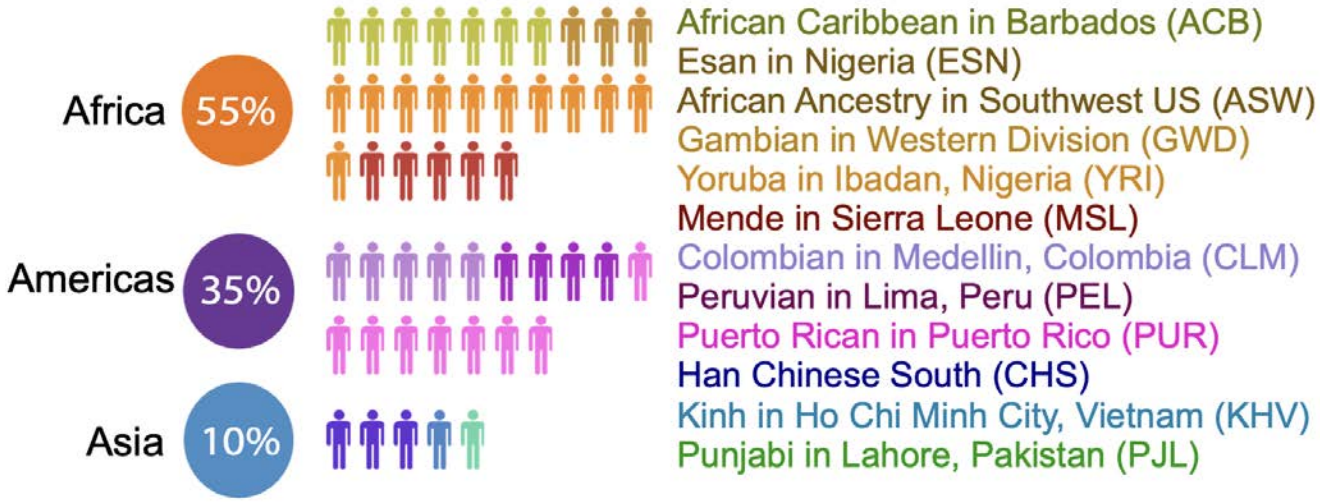
The human pangenome

- The pangenome is just a **set of haplotypes**
- Replacing one haplotype with a set of haplotypes **reduces reference bias**
- This yields **immediate improvements** to variant calling accuracy and comprehensiveness
- Will ultimately enable **predictive models** of variation

A draft human pangenome reference.
Liao, et al. *Nature* (2023)

A draft human pangenome reference





v1: HiFi assemblies (released)
v2: HiFi+ONT assemblies
v3: T2T assemblies

48 initial samples from 1KGP

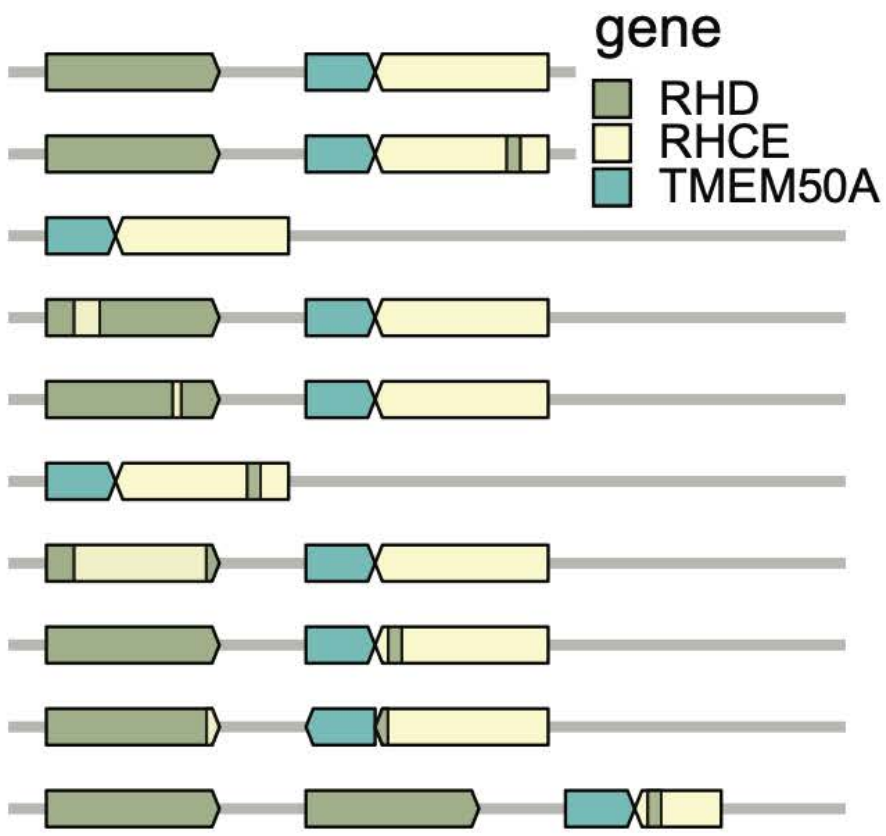
- Immortalized 1000G cell lines
- Cover allelic and geographic diversity
- Availability of low passage cell lines
- Availability of trios/parental data
- Aiming for 350+ diploid genomes

NIH NHGRI

A draft human pangenome reference.
Liao, et al. *Nature* (2023)

E.g. Rh blood group antigens

Count	Frequency	Haplotype name
43	0.48	RHD;RHCE
22	0.24	RHD;RHCE-RHD(2)-RHCE
15	0.17	RHCE
3	0.03	RHD-RHCE(2-3)-RHD;RHCE
2*	0.02	RHD-RHCE(8)-RHD;RHCE
1	0.01	RHCE-RHD(2)-RHCE
1*	0.01	RHD-RHCE(2-9)-RHD;RHCE
1*	0.01	RHD;RHCE-RHD(9)-RHCE
1*	0.01	RHD-RHCE(10);inv;RHCE-RHD(10)
1*	0.01	RHD;RHD;RHCE-RHD(9)-RHCE



A draft human pangenome reference.
Liao, et al. *Nature* (2023)

You are your own best reference

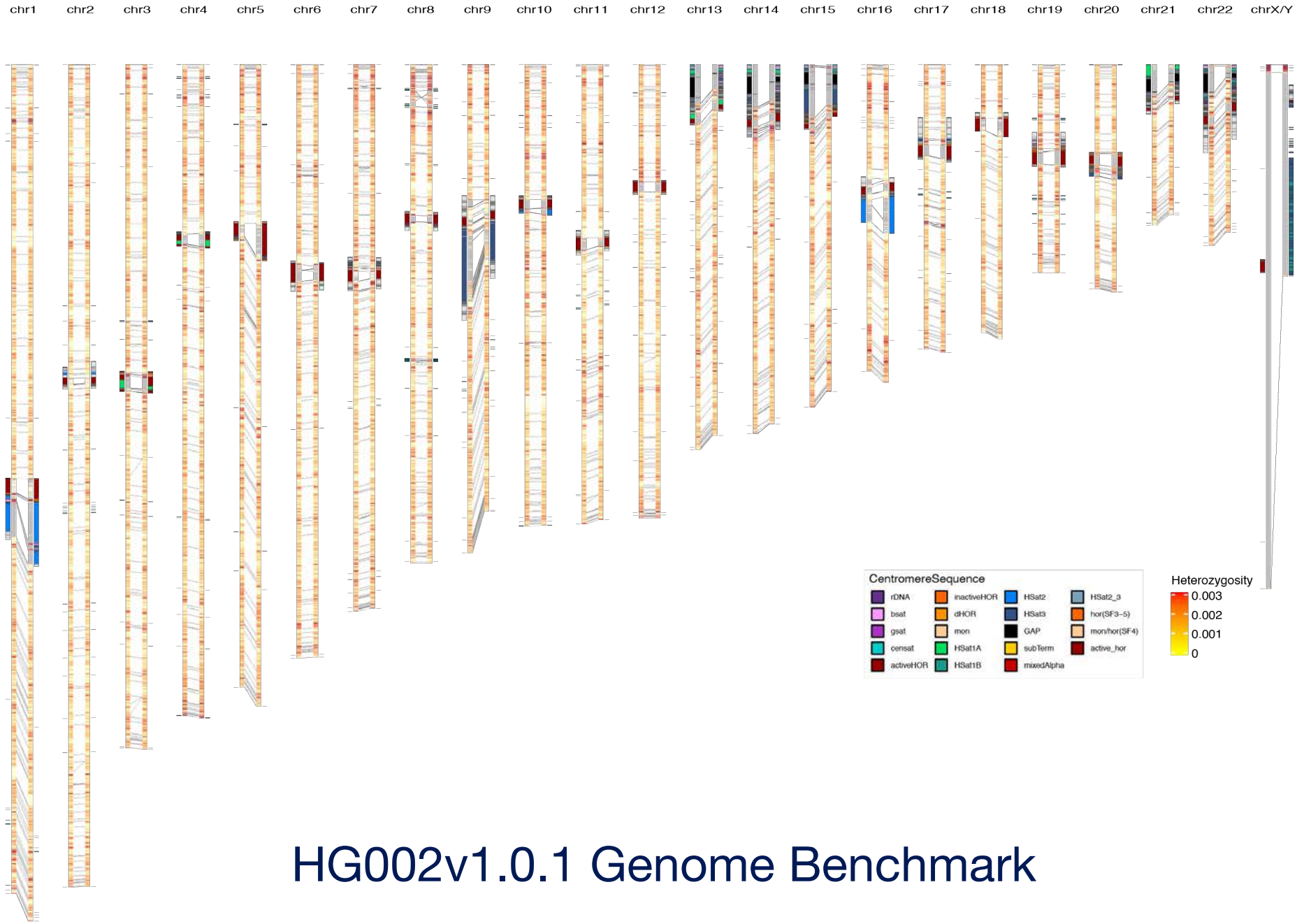
- **Goal: Complete, diploid genomes for all**
  - Variant calls are an incomplete genome assembly
  - *De novo* assembly is costly to scale

- **Working towards "genome inference"**
  - Use all information available (e.g. reads + pangenome)
  - Infer known alleles, assemble novel alleles
  - Genome assembly with priors

The “Q100” project

TELOMERE-TO-TELOMERE CONSORTIUM

- **NIST/GIAB standards are incomplete**
  - You are only told ~85% of what’s in the bottle
- **A genome benchmark**
  - Vs. a variant benchmark
  - Complete, diploid, no errors
  - Q100: 1 error per 10 billion bases
- **T2T-HG002v1.0 released!**
  - Reliable regions approach perfect quality and phasing
  - Unreliable regions (<1%) are labeled (e.g. rDNAs)
  - Exceeds quality of current NIST benchmarks



HG002v1.0.1 Genome Benchmark

NIH
NHGRI

2000: Draft genomes
2010: Reference genomes
2020: T2T genomes
2030: Genomes

NIH
NHGRI

The future is complete genomes

1. Routine *assembly* of T2T genomes
2. Comprehensive pangenome reference database
3. Cheap and routine *inference* of T2T genomes
4. ML-based annotation of personal, diploid genomes
5. Accurate functional/somatic view over lifespan

Flip the model: Don’t bring your genome to the annotation, bring the annotation to your genome!

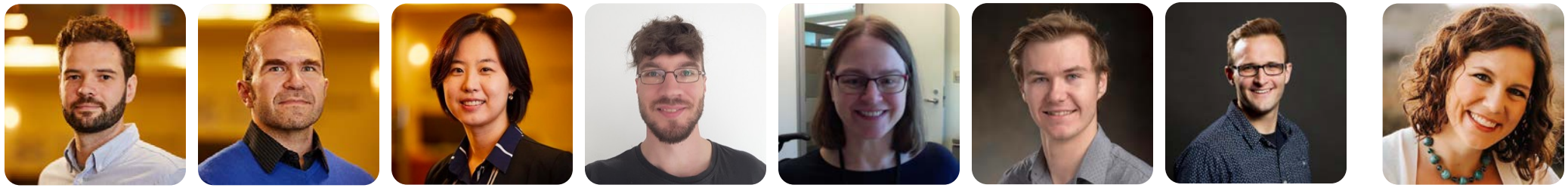
Resources

GitHub /MarBL

- Verkko assembler
  - https://github.com/marbl/verkko
- T2T-CHM13 reference
  - https://github.com/marbl/chm13
- HG002 diploid genome benchmark
  - https://github.com/marbl/hg002
- Human Pangenome Reference Consortium
  - https://humanpangenome.org
- ModT2T
  - https://www.genomeark.org/

NIH NHGRI

Team T2T, HPRC, (…and many more)



Contact me if interested in joining the T2T mailing list!

NIH NHGRI