SVbyEye: A visual tool to characterize structural variation among whole genome assemblies

David Porubsky[1], Xavi Guitart[1], DongAhn Yoo[1], Philip C. Dishuck[1], William T. Harvey[1], Evan E. Eichler[1,2]

Affiliations:

1. Department of Genome Sciences, University of Washington School of Medicine, Seattle, WA, USA
2. Howard Hughes Medical Institute, University of Washington, Seattle, WA, USA

Correspondence to: porubsky@uw.edu

Abstract:

Motivation
We are now in the era of being able to routinely generate highly contiguous (near telomere-to-telomere) genome assemblies of human and nonhuman species. Complex structural variation and regions of rapid evolutionary turnover are being discovered for the first time. Thus, efficient and informative visualization tools are needed to evaluate and directly observe structural differences between two or more genomes.
Results
We developed SVbyEye, an open-source R package to visualize and annotate sequence-to-sequence alignments along with various functionalities to process alignments in PAF format. The tool facilitates the characterization of complex SVs in the context of sequence homology helping resolve the mechanisms underlying their formation.
Availability and implementation
SVbyEye is available at https://github.com/daewoooo/SVbyEye.

Introduction:

Informative and efficient visualization of genomic structural variation (SV) is an important step to evaluate the validity of the most complex regions of the genome, helping us to develop new hypotheses and draw biological conclusions. With advances in long-read sequencing technologies such as HiFi (high-fidelity) PacBio (Wenger et al. 2019) and ONT (Oxford Nanopore) (Deamer and Branton 2002) we are now able to fully assemble even the most complex regions of the genome such as segmental duplications (SDs) (Vollger et al. 2022), acrocentric regions (Guarracino et al. 2023), and centromeres (Logsdon et al. 2024) into continuous, highly accurate linear assemblies–also known as telomere-to-telomere (T2T) assemblies (Jarvis et al. 2022; Nurk et al. 2022). A large part of our understanding of the evolution of complex biological systems comes from comparative analyses including direct visual observations (Paparella et al. 2023; Yoo et al. 2024).

The challenge with these analyses is that many large-scale structural changes between genomes are mediated by large highly identical repeat sequences that are not readily annotated by existing software. This necessitated the development of visualization tools to complement T2T comparative studies. We have developed SVbyEye for three purposes; 1) to directly characterize structurally complex regions including insertions, duplications, deletions and inversions by comparison to a linear genome reference, 2) to place these changes in the context of sequence homology by characterizing associated sequence identity, and 3) to define the breakpoints including the length and orientation of homologous sequence mediating the rearrangement. SVbyEye is inspired by the previously developed tool called Miropeats (Parsons 1995) and brings its visuals to a popular scripting language R and visualization paradigm using ggplot2 (Wickham 2009).

Materials and Methods:

SVbyEye uses as input DNA sequence alignments in PAF format which can be easily generated with minimap2 (Li 2018). In principle, however, any sequence-to-sequence aligner that can export alignments in standard PAF format should be sufficient. We note, however, we have tested our tool only using minimap2 alignments. Such alignments can be read using the 'readPaf' function. Subsequently, imported alignments can be filtered and flipped into the desired orientation using 'filterPaf' and 'flipPaf' functions, respectively,

Visualization modes

There are four visualization modes offered by SVbyEye, visualization of pairwise alignments, alignments between more than two sequences, alignments within a single sequence and whole genome alignments.

The main function of this package is called 'plotMiro' and serves to visualize pairwise sequence alignments in a horizontal layout with the target (reference) sequence being at the top and query at the bottom (**Fig. 1A**). The user can control over a number of visual and alignment processing features. For instance, users can color sequence alignments by their orientation or percentage of matched bases (**Supplementary Notes**).

SVbyEye also allows to visualize alignments between more than two sequences. This can be done by aligning multiple sequences to each other using so-called all-versus-all (AVA) or stacked alignments and submitting them to the 'plotAVA' function. In such a way alignments are visualized in subsequent order where the alignment of the first sequence is shown with respect to the second and then second sequence to the third and so on (**Fig. 1B**). Many of the same parameters from 'plotMiro' also apply to 'plotAVA' as well. We highlight a use of binning PAF alignments into a defined bins (by setting a parameter 'binsize') and coloring them by percentage of matched based; a useful feature to reflect regional or pairwise differences in sequence identity (**Fig. 1C**).

To accommodate a use case of visualizing regions that are homologous to each other within a single sequence we implemented the 'plotSelf' function. This function takes PAF alignments of a sequence to itself and visualizes them in a so-called horizontal dotplot (**Fig. 1D**). Such visualization can tell us a relative orientation, identity and size of intrachromosomally aligned regions, an important feature of segmental duplications that predispose intervening sequence to recurrent rearrangements (Itsara et al. 2012; Coe et al. 2014; Porubsky et al. 2022). We note

that self-alignments can also be visualized as arcs or arrowed rectangles connecting aligned regions (**Supplementary Notes**).

To allow for a full overview of whole genome assembly with respect to a reference SVbyEye offers 'plotGenome' function. With this function whole genome alignments can be visualized to observe large structural rearrangements such as large para- and peri-centromeric inversions between chimpanzee and human genome (**Fig. 1E**).

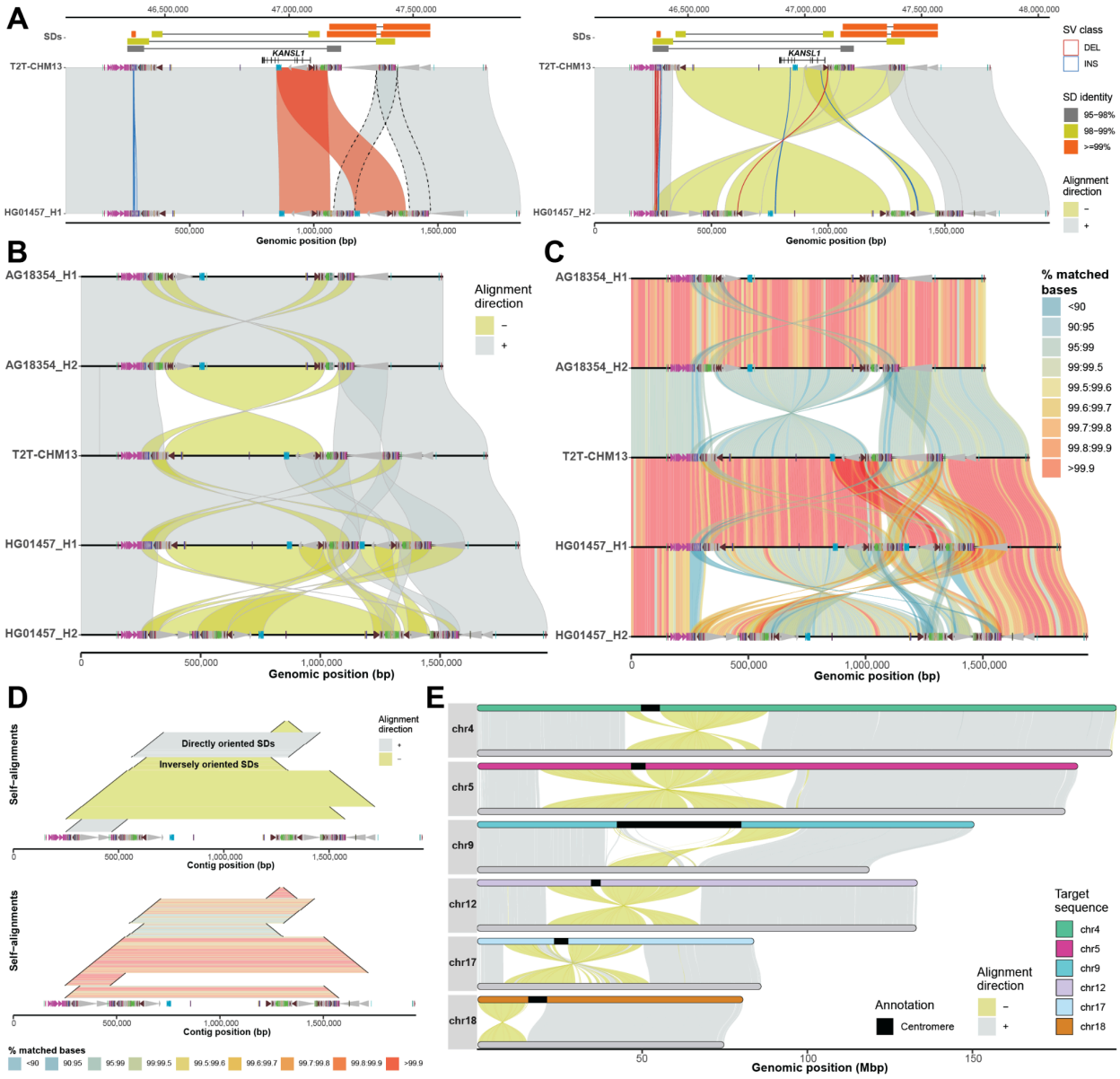


Figure 1: Example of SVbEye visualization modes.

A) The plot depicts a minimap alignment of a 1.7 Mbp region from chromosome 17q21.31 of two human sequences: HG01457 haplotypes (query) vs. T2T-CHM13 reference (target). SD pairwise alignments are shown (top) (connected by horizontal line) colored by their sequence identity with gene annotation (*KANSL1* exons) depicted below as annotated in the UCSC Genome Browser. Minimap2 alignments are shown as gray (forward'+' orientation) and yellow

(reverse '-' orientation) polygons between query (bottom) and target (top) sequences. Duplicon annotations as defined by DupMasker (Jiang et al. 2008) are indicated for both query and target sequence by colored arrowheads pointing forward or backward based on their orientation. A SV embedded within the SDs between query and target sequence (>= 1 kbp) is highlighted as blue (insertion) and red (deletion) outlines facilitating breakpoint definition. **B**) A "stacked" SVbyEye plot depicting 17q21.31 region for two chimpanzee haplotypes followed by three human haplotypes from T2T-CHM13 and HG01457. Each sequence is compared to the sequence immediately above and clearly defines a 750 kbp inversion between chimpanzee and human flanked by inverted repeats. A larger 900 kbp inversion polymorphism is also identified in human mediated by inverted SDs. **C**) Shows the same alignments as in B but with a "% identity grid" colored by percentage of matched bases per 10 kbp long sequence bin. Human inversion shows significant divergence indicating a deeper coalescence of the 17q21.31 region (Zody et al. 2008). **D**) A 'horizontal dotplot' visualization which shows self-alignments of HG01457 (haplotype 2) indicating the size (black line), the orientation (inverted=yellow and gray=direct; top panel) and the pairwise identity (colored grid; bottom panel). The largest and most identical segments are preferred sites for NAHR breakpoints. **E**) A telomere-to-telomere view of six chimpanzee chromosomes (query, bottom) aligned to human syntenic chromosomes (T2T-CHM13, target, top). This view readily defines the extent of paracentric and pericentric inversions.

Alignment processing and annotation functionalities

SVbyEye has the ability to break PAF alignments at the positions of insertions and deletions and thereby delineate their breakpoints. This is done by parsing alignment CIGAR strings if reported in the PAF file. Thus by setting the minimum size of insertions and deletions to be reported one can visualize SVs as red (deletions) and blue (insertions) outlines (**Fig. 1A**). For further interrogation users can also opt to report alignment embedded insertions and/or deletions in a data table format using the 'breakPaf' function.

An important feature of SVbyEye is its capability to annotate query and target sequences with genomic ranges such as gene position, position of segmental duplications (SDs) or other DNA functional elements. This is done by adding extra annotation layers on top of the target and/or query alignments using 'addAnnotation' function (**Supplementary Notes**). Annotation ranges are visualized as either a rectangle or an arrowhead. Arrowheads are especially useful for conveying an orientation of a genomic range. Similar to PAF alignments also annotation ranges can be colored by a user defined color scheme (**Fig. 1A**). If there is a need to highlight specific PAF alignments between query and a target one can do so with the 'addAlignments' function that adds selected alignment(s) over the original plot highlighted by unique outline and/or color (**Fig. 1A**).

There are a number of other useful functionalities that come with SVbyEye, for instance lifting coordinates from target to query and vice versa provided by 'liftRangesToAlignment' function. Users can also subset alignments from a desired region on a target sequence using the 'subsetPaf' function. Last, there is a possibility to disjoin PAF alignments at regions where two and more alignments overlap each other with 'disjoinPafAlignments' function to provide exact boundaries of duplicated regions (**Fig. 1A**).

Conclusion:

We developed SVbyEye, a data visualization R package, to facilitate direct observation of structural differences between two or more sequences. SVbyEye provides several visualization modes depending on application. It offers ample ways to annotate both query and target sequences along with a number of functionalities to process alignments in PAF format. A more detailed package documentation along with code examples can be found at:

:https://htmlpreview.github.io/?https://github.com/daewoooo/SVbyEye/blob/master/man/doc/SVbyEye.html

Acknowledgements:
This article is subject to HHMI's Open Access to Publications policy. HHMI lab heads have previously granted a non-exclusive CC BY 4.0 license to the public and a sublicensable license to HHMI in their research articles. Pursuant to those licenses, the author-accepted manuscript of this article can be made freely available under a CC BY 4.0 license immediately upon publication.

Funding:
This research was supported, in part, by funding from the National Institutes of Health (NIH) grants R01 HG002385 and R01 HG010169 (to E.E.E.). E.E.E. is an investigator of the Howard Hughes Medical Institute.

Conflict of Interest: E.E.E. is a scientific advisory board (SAB) member of Variant Bio, Inc.

References:

Coe, Bradley P., Kali Witherspoon, Jill A. Rosenfeld, Bregje W. M. van Bon, Anneke T. Vulto-van Silfhout, Paolo Bosco, Kathryn L. Friend, et al. 2014. "Refining Analyses of Copy Number Variation Identifies Specific Genes Associated with Developmental Delay." *Nature Genetics* 46 (10): 1063–71.

Deamer, David W., and Daniel Branton. 2002. "Characterization of Nucleic Acids by Nanopore Analysis." *Accounts of Chemical Research* 35 (10): 817–25.

Guarracino, Andrea, Silvia Buonaiuto, Leonardo Gomes de Lima, Tamara Potapova, Arang Rhie, Sergey Koren, Boris Rubinstein, et al. 2023. "Recombination between Heterologous Human Acrocentric Chromosomes." *Nature* 617 (7960): 335–43.

Itsara, Andy, Lisenka E. L. M. Vissers, Karyn Meltz Steinberg, Kevin J. Meyer, Michael C. Zody, David A. Koolen, Joep de Ligt, et al. 2012. "Resolving the Breakpoints of the 17q21.31 Microdeletion Syndrome with next-Generation Sequencing." *American Journal of Human Genetics* 90 (4): 599–613.

Jarvis, Erich D., Giulio Formenti, Arang Rhie, Andrea Guarracino, Chentao Yang, Jonathan Wood, Alan Tracey, et al. 2022. "Semi-Automated Assembly of High-Quality Diploid Human Reference Genomes." *Nature* 611 (7936): 519–31.

Jiang, Zhaoshi, Robert Hubley, Arian Smit, and Evan E. Eichler. 2008. "DupMasker: A Tool for Annotating Primate Segmental Duplications." *Genome Research* 18 (8): 1362–68.

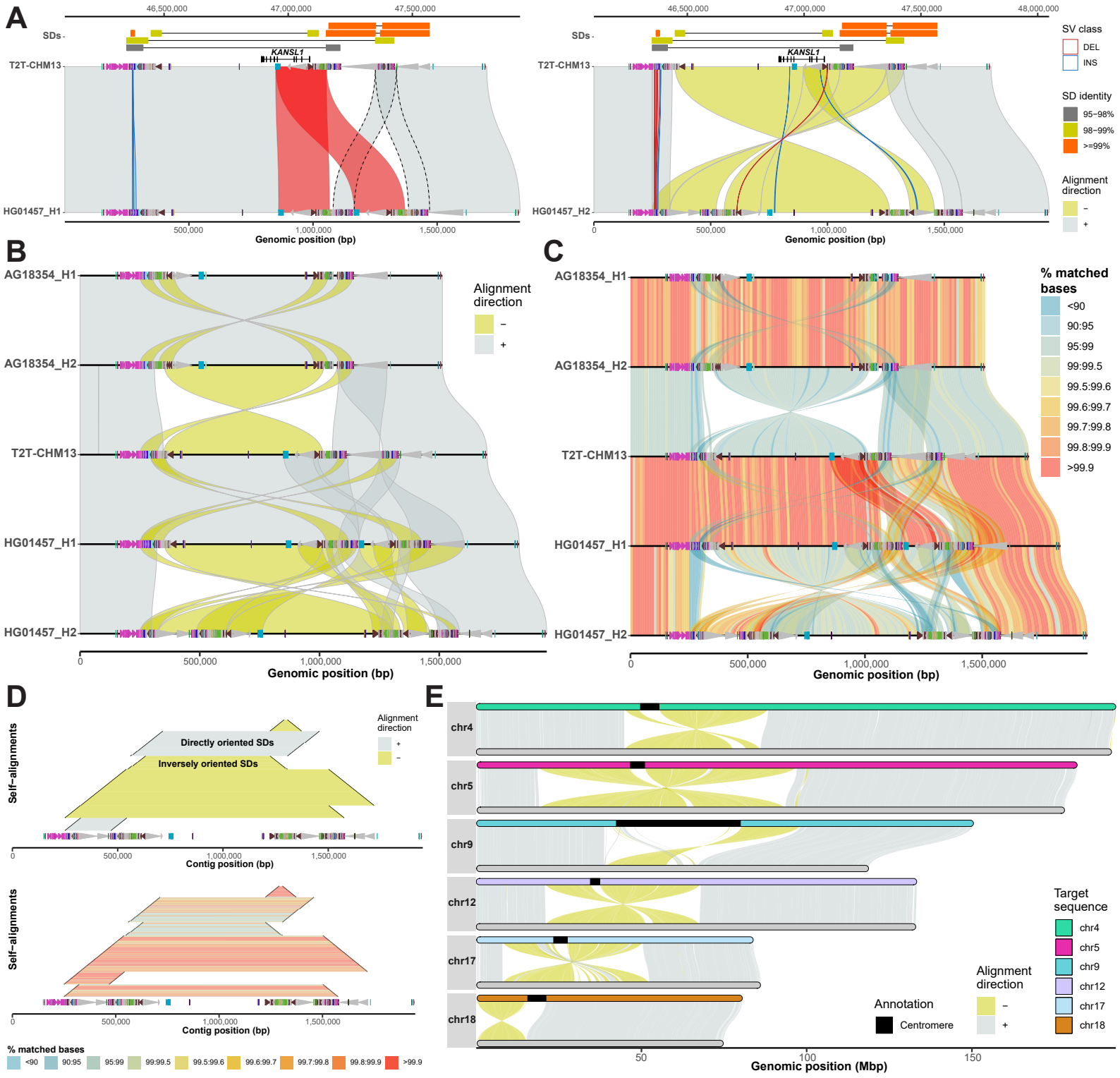
Li, Heng. 2018. "Minimap2: Pairwise Alignment for Nucleotide Sequences." *Bioinformatics* 34 (18): 3094–3100.

Logsdon, Glennis A., Allison N. Rozanski, Fedor Ryabov, Tamara Potapova, Valery A. Shepelev, Claudia R. Catacchio, David Porubsky, et al. 2024. "The Variation and Evolution of Complete Human Centromeres." *Nature* 629 (8010): 136–45.

Nurk, Sergey, Sergey Koren, Arang Rhie, Mikko Rautiainen, Andrey V. Bzikadze, Alla Mikheenko, Mitchell R. Vollger, et al. 2022. "The Complete Sequence of a Human Genome." *Science* 376 (6588): 44–53.

Paparella, Annalisa, Alberto L'Abbate, Donato Palmisano, Gerardina Chirico, David Porubsky, Claudia R. Catacchio, Mario Ventura, Evan E. Eichler, Flavia A. M. Maggiolini, and

Francesca Antonacci. 2023. “Structural Variation Evolution at the 15q11-q13 Disease-Associated Locus.” *International Journal of Molecular Sciences* 24 (21): 15818.
Parsons, J. D. 1995. “Miropeats: Graphical DNA Sequence Comparisons.” *Bioinformatics* 11 (6): 615–19.
Porubsky, David, Wolfram Höps, Hufsah Ashraf, Pinghsun Hsieh, Bernardo Rodriguez-Martin, Feyza Yilmaz, Jana Ebler, et al. 2022. “Recurrent Inversion Polymorphisms in Humans Associate with Genetic Instability and Genomic Disorders.” *Cell* 185 (11): 1986–2005.e26.
Vollger, Mitchell R., Xavi Guitart, Philip C. Dishuck, Ludovica Mercuri, William T. Harvey, Ariel Gershman, Mark Diekhans, et al. 2022. “Segmental Duplications and Their Variation in a Complete Human Genome.” *Science* 376 (6588): eabj6965.
Wenger, Aaron M., Paul Peluso, William J. Rowell, Pi-Chuan Chang, Richard J. Hall, Gregory T. Concepcion, Jana Ebler, et al. 2019. “Accurate Circular Consensus Long-Read Sequencing Improves Variant Detection and Assembly of a Human Genome.” *Nature Biotechnology* 37 (10): 1155–62.
Wickham, Hadley. 2009. *Ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer.
Yoo, Dongahn, Arang Rhie, Prajna Hebbar, Francesca Antonacci, Glennis A. Logsdon, Steven J. Solar, Dmitry Antipov, et al. 2024. “Complete Sequencing of Ape Genomes.” *bioRxiv.org: The Preprint Server for Biology*, July. https://doi.org/10.1101/2024.07.31.605654.
Zody, Michael C., Zhaoshi Jiang, Hon-Chung Fung, Francesca Antonacci, Ladeana W. Hillier, Maria Francesca Cardone, Tina A. Graves, et al. 2008. “Evolutionary Toggling of the MAPT 17q21.31 Inversion Region.” *Nature Genetics* 40 (9): 1076–83.


A
SDs
T2T-CHM13
HG01457_H1
HG01457_H2
KANSL1
Genomic position (bp)
SV class
DEL
INS
SD identity
95–98%
98–99%
>=99%
Alignment direction
B
AG18354_H1
AG18354_H2
T2T-CHM13
HG01457_H1
HG01457_H2
Alignment direction
C
% matched bases
<90
90:95
95:99
99:99.5
99.5:99.6
99.6:99.7
99.7:99.8
99.8:99.9
>99.9
D
Self–alignments
Directly oriented SDs
Inversely oriented SDs
Contig position (bp)
% matched bases
E
chr4
chr5
chr9
chr12
chr17
chr18
Genomic position (Mbp)
Target sequence
Annotation
Centromere