Coverage Anomaly Polishing Instructions, February 7, 2023

This document is a guide to curating read coverage anomalies in HG002 diploid assemblies (currently the v0.7 verkko/rukki assembly), and describes the methods that we use to determine whether these anomalies are due to technical issues like sequencing bias (e.g., in HiFi regions with low coverage due to high GA/CT content) or actual errors in the assembly (e.g., duplicated or collapsed regions in the consensus on one or both alleles).

This document is a work in progress, and available to edit at https://docs.google.com/document/d/1lef3T5wdFdw4_m8oxCNkvRicckC8qZfoCbrM6Hjz9z8/edit so please feel free to suggest changes or additions as they occur to you.

HOW ARE READ COVERAGE ANOMALIES DETECTED?

Our current set of coverage "issues" (as of February, 2023) was created by running the T2T-Polish workflow (https://github.com/arangrhie/T2T-Polish/tree/master/coverage) on Winnowmap2 primary alignments of ONT and HiFi reads to the v0.7 HG002 assembly. This run tagged 352 regions, 10 of which heavily overlap the rDNA regions of the acrocentric p-arms. The 352 T2T-Polish regions have been added to the issue section of our github repository (https://github.com/marbl/HG002-issues/issues), and various labels have been added to annotate them. For each issue, the status (open/closed), the people assigned for curation, and the labels are mirrored in a Google spreadsheet visible at: https://docs.google.com/spreadsheets/d/1eRpT0fXYmODoA2A9YYK4Z3CR_o6NTJBJnYHOem5LRjo. *Note: this google spreadsheet will be updated regularly FROM the github site, so any changes made to columns that mirror github will be overwritten!*

In addition to the T2T-Polish calls, Mobin Asri has also provided us with Flagger (Liao et al., 2022) calls for the v0.7 assembly chromosomes, which mark regions that appear to be duplications, collapses, or errors. Nancy Hansen has compared these calls to the T2T-Polish regions with bedtools. Since Flagger predicts a large number of regions/bases to be erroneous, the polishing group's first round of curation will focus only on the intersection of Flagger's "ALT-removed" calls on the HiFi reads with ALT-removed calls from the ONT data (a.k.a., "flagger intersect" calls). On github, "flagger_intersect" labels have been added to all T2T-Polish coverage issues which intersect with these Flagger calls, but once we have a better handle on which Flagger calls are likely to be correct, we'll likely import regions that are called only by Flagger to github as well.

VIEWING THE DATA

Most of the data that we will use for validation is hosted on the human-pangenomics aws server at:

https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=T2T/HG002/assemblies/polishing/HG002/. A list of the various track descriptions will be maintained with their aws URLs at https://github.com/marbl/HG002-issues/tree/main/documentation/datatracks, so tracks can be loaded to IGV using their URLs, or using any of the IGV session files maintained at https://github.com/marbl/HG002-issues/tree/main/igv_sessions. These directories, session files, and the tracks on aws will be updated frequently as the project progresses, so they should be checked now and then to see what's new.

COVERAGE LABELS

The labels (in colored bubbles) attached to issues on the github site help to give context to why the particular region was reported as an issue. For coverage issues, here is a table of labels and their meanings:

Label	Meaning	Source
coverage_pri	Anomaly in long read coverage for reads aligned to both haplotypes	T2T-Polish pipeline
low_cov_hifi, low_cov_ont	Lower than expected coverage of HiFi or ONT reads, respectively	T2T-Polish pipeline
high_cov_hifi, high_cov_ont	Higher than expected coverage of HiFi or ONT reads, respectively	T2T-Polish pipeline
hsat2/hsat3	Region has been annotated as containing HSat sequence	Julian Lucas
alpha_sat	Region has been annotated as containing alpha satellite sequence	Julian Lucas
ga_tc, gc, at	Indicates high content of the label's nucleotides	T2T-Polish pipeline
error_kmer	Consensus contains kmers that are not observed in the read data	T2T-Polish pipeline
clipped	Region where read alignments show a high level of clipping	T2T-Polish pipeline
flagger_intersect	A T2T-Polish issue which overlaps with Flagger's "intersected" calls	Flagger v0.2

SCREENSHOTS

It may help to take screenshots of data for each issue region. Navigate to the region in IGV, then zoom out until at least one single-copy marker k-mer is within view, up until ~100kbp. Then take a screenshot, save in .png or .pdf with the issue ID as file name (e.g. Issue249.png).

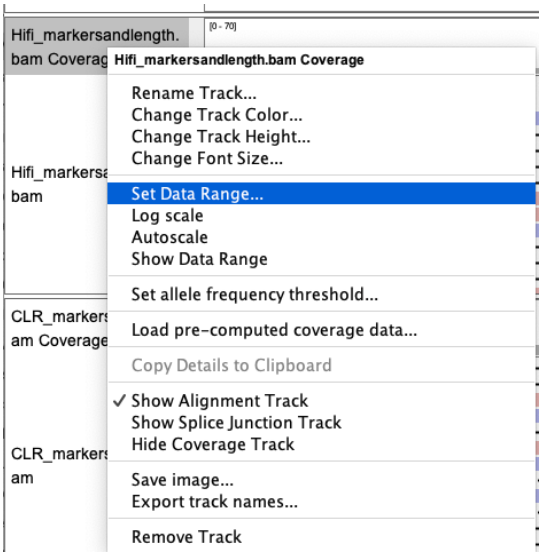
REQUIRED TRACKS

The following tracks will not be a part of the IGV session file, but you should download them and display them locally.

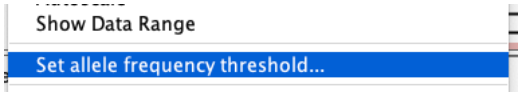
- t2t-chm13.20200904.fasta.gz
- single.tdf
- single.desert.bed
- t2t-chm13.20200904.lr.self.sv.vcf
- primary.bam
- markersandlength.bam
- patches_noHiFi.bed

You may download files outside the session file using wget on files below here.
https://dl.dnanex.us/F/D/pbx5K7zbzJ02BVY7PbZyBkJ88q9ZPqYv2Q1B1yF4/single.tdf
https://dl.dnanex.us/F/D/BVY0xVJP3x6kP2ypyZfG9Vb4gJQvGYVz944yk51V/single.desert.bed
https://dl.dnanex.us/F/D/Xf1fYBqbf6xQk21zF6J49F7F91xK2Gj0pB02Xzg6/patches_noHiFi.bed
https://dl.dnanex.us/F/D/0kxF2K5F57f65q1PgQ2yKp0qF0k57XKKPYbBVFyg/t2t-chm13.20200904.fasta.gz
https://dl.dnanex.us/F/D/5x0Jk3bbJF69ZJQ5xfzz21625Vyk3q3y64k8q978/t2t-chm13.20200904.lr.self.sv.vcf

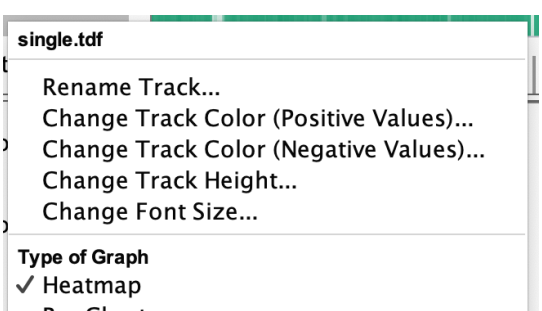
Format bam Coverage tracks

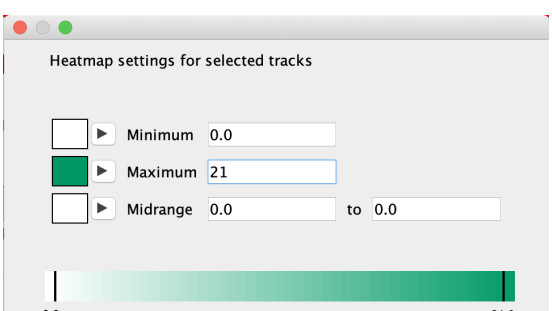


1) Set Data Range
    a) HiFi and CLR: Max to 70
    b) ONT: Max to 250
2) Set allele frequency threshold… to 0.3



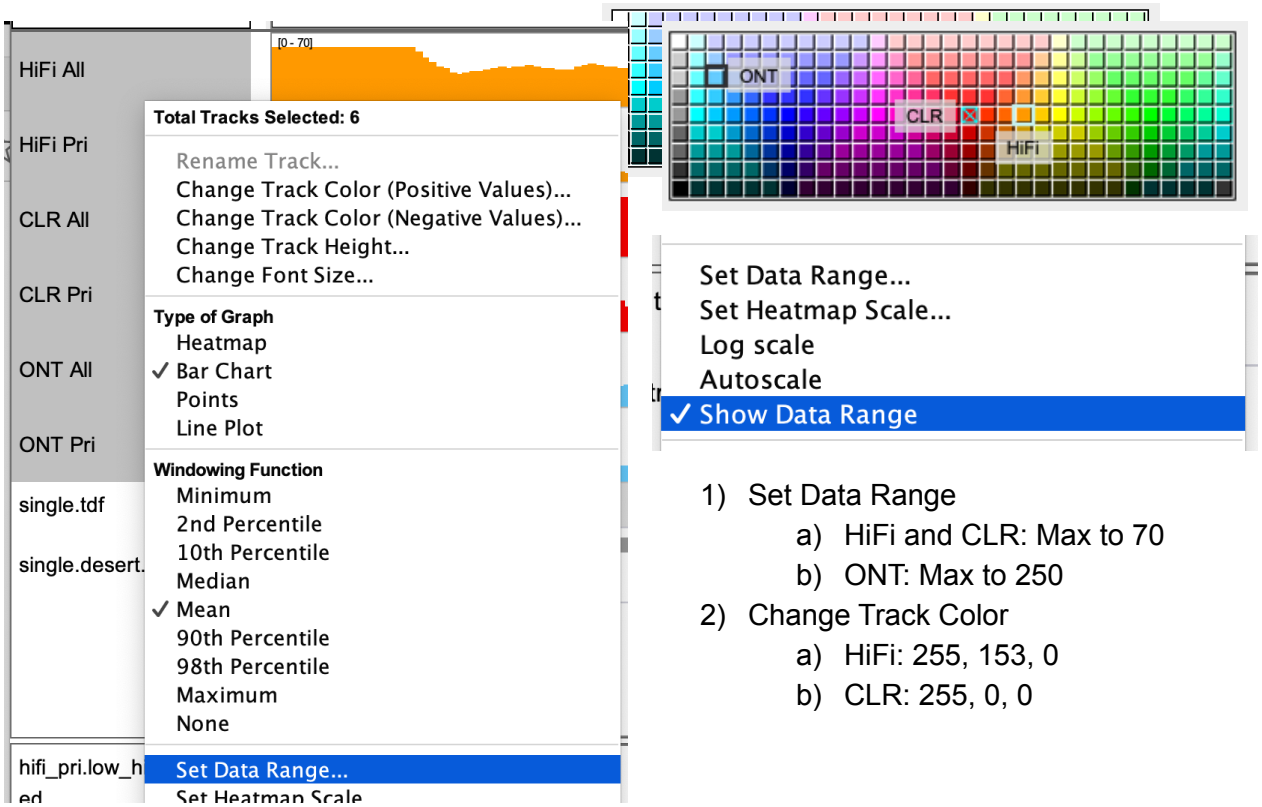
Format single.tdf track





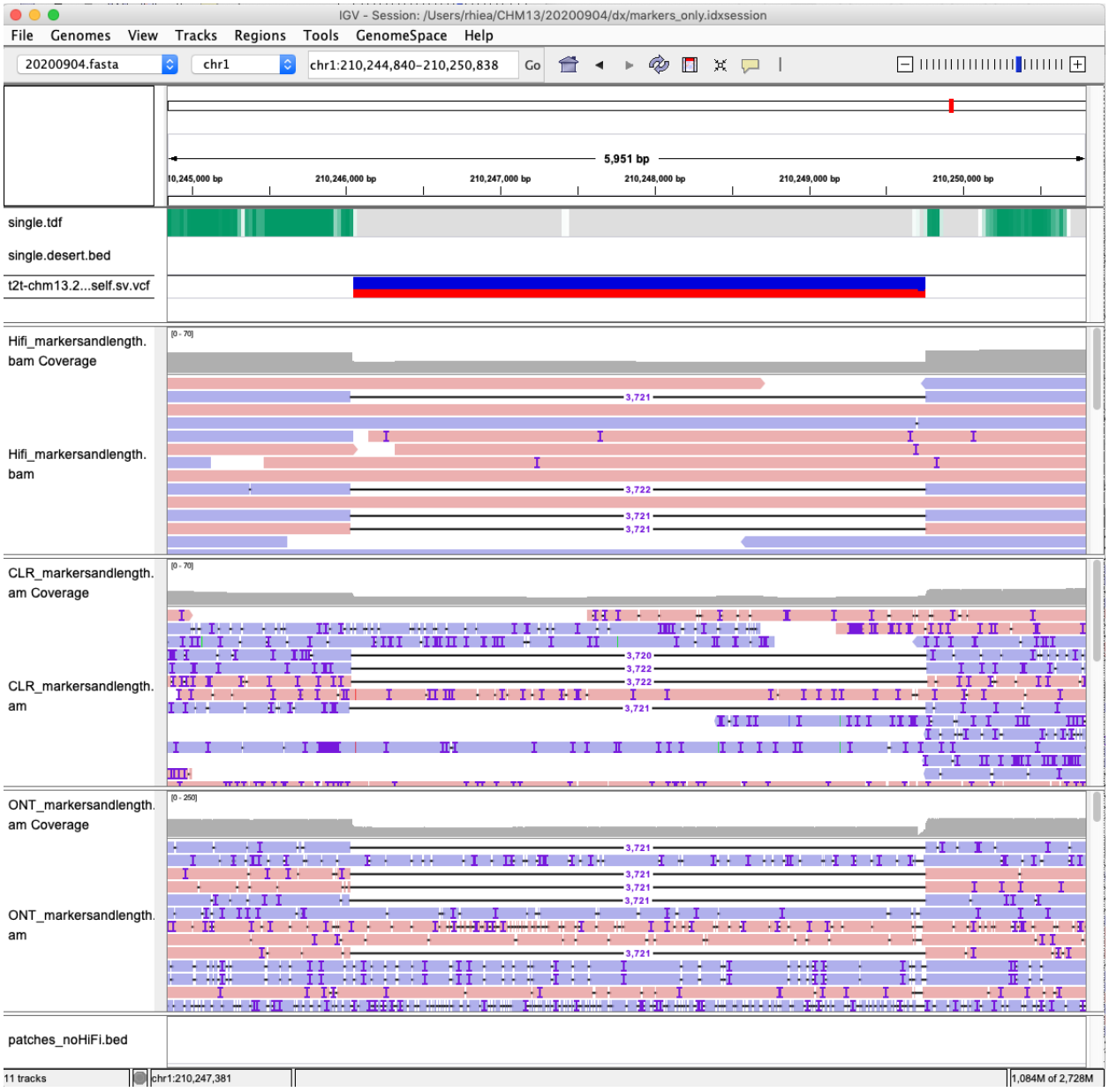
1) Select “Heatmap” as Type of Graph
2) Set Heatmap Scale
3) Set Maximum to 21
4) Select color and hit OK
5) Change Track Height to 30

Format .wig tracks



1) Set Data Range
    a) HiFi and CLR: Max to 70
    b) ONT: Max to 250
2) Change Track Color
    a) HiFi: 255, 153, 0
    b) CLR: 255, 0, 0

Example Screenshot



Checkpoints

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Screenshot tip on MAC

- Ctrl + Shift + 4, select IGV area. Open the preview, hit Done.
- Rename the .png file generated on the desktop