**Background**: The software that Artandi and his team created and use in this paper is called Telometer. They are currently on the fourth release, and this is available to download via their GitHub repository. Telometer looks for telomeric repeats in the form of 5’-TTAGGG-3’ or 5’-AATCCC-3’.

The steps I outlined below are the key bioinformatic analysis details in applying this method at Rutgers. The first step is the data collection for DNA collection and telomere capture. I am not sure if there is already data collected that you would like to use or not. The second step is basecalling which allows us to get the raw data into DNA bases. The next step is alignment to a reference genome. Artandi and his team site the reference genome that they used in this step. Such files have to then be converted and sorted in preparation to run Telometer, which will produce measurements with summary statistics.

**First Step**: Benchtop Protocol for Telomere Capture Library

This can be found within the paper and also in downloadable files on the GitHub Repository in more detail.

**Second Step**: R10 High Accuracy Basecalling with Dorado

Basecalling in this step refers to converting the raw electrical signals from the nanopore sequencing into nucleotide sequences.

The information on how to download Dorado 0.9.0 can be found at this link: <https://dorado-docs.readthedocs.io/en/latest/#__tabbed_1_3>

The software does not run on my personal version of mac, but there looks like there could be some work-arounds.

**Third Step:** Alignment

The basecalled reads need to be aligned to the telomere-to-telomere (t2t) genome with minimap2. Minimap2 is a software that aligns long reads of sequencing data to a reference genome. In this paper, the team used a combined reference genome.

The lasted human t2t assembly can be downloaded from <https://github.com/marbl/CHM13?tab=readme-ov-file>

The team also appended subtelomere assemblies from a prior paper that can be found at <https://pubmed.ncbi.nlm.nih.gov/24676094/>

I think I could get access to the combined T2T v2.0 and Stong 2014 reference if we wanted to use this as the reference genome also.

**Fourth Step:** Convert and Sort Files:

BAM file processing is done using samtools v.1.16.0. This software sorts and indexes data for easier analysis.

**Fifth Step:** Run Telometer

Telometer can be installed with directions on the GitHub repository.

Telometer searches reads that align to the first or last several thousand bps of the reference chromosome and measures telomeres from reads that are more than 1000 bps (minimum recommended). For whole-genome sequencing, there should be a minimum of 4000 bps. After alignment, the program checks that the first or last 100 bps of a read are “telomere-rich” to make sure that telomere measurements are terminal and not interstitial telomere sequences.

Once installed and with the appropriate files downloaded, I think this could be run successfully.