The genome of reniform nematode, *Rotylenchulus reniformis* is a pest that causes considerable damage to cotton. For example, in 2011 yield losses of approximately 279,000 bales (total estimated value > $90 million) were attributed to RN damage. Ostensibly, sequencing the genome of *R. reniformis* represents a key step in identifying genes underlying RN’s ability to infect host plants. Ultimately, knowledge of the genome may suggest means of minimizing targeted disruption of RN-specific gene pathways. Towards this end, we determined the RN genome size and initiated whole genome sequencing of the nematode.

**Materials & Methods**

A pooled population of *R. reniformis* mixed-stage vermiform tissue was collected from host plant cotton cultures and surface-sterilized with 0.01% HgCl₂.

**Flow Cytometry**

To estimate the genome size of *R. reniformis* by flow cytometry, nuclei were isolated from vermiform tissue. The tissue was homogenized in 2 mL Dounce tissue grinder with ice cold Galfraith buffer (45 mM MgCl₂, 30 mM sodium citrate, 20 mM MOPS, 1mg/mL Triton X-100, 1mg/L boiled RNAse, pH 7.2). The homogenate was passed through 10 µm nylon membrane and 1mg/L boiled RNase, pH 7.2. The homogenate was used as known control with calculated genome size of 100 Mb. N2 strain of *C. elegans* was used as known control with genome size of ~50 Mb. Roche 454 SE 462594 161287004 Roche 454 PE (Bib insert)* 7 lanes of 1x75 bp reads with 350 bp inserts

**Genome Sequencing**

Genomic DNA for sequencing was isolated using a QIAGEN DNeasy Blood & Tissue Kit. The pooled sample contained a number of nematodes, resulting in increased SNP sampling.

**Illumina Sequencing:**

Single reads – 2 lanes of 1x75 bp reads
Pair End reads w/ inserts – 1 lane of 2x100 bp reads with 250 bp inserts 1 lane of 2x100 bp reads with 350 bp inserts

Assembly was performed using both the ABySS de novo assembly algorithm (version 1.3.2) and the Roche 454 GS De Novo Assembler (version 2.6). Separate assemblies were generated using the illumina sequences with ABySS and the Roche 454 sequences with the Roche 454 GS De Novo Assembler. A combined assembly was generated using the Roche 454 assembly and ABySS.

**Annotatiom Progress**

The 1,571 contigs > 2,000 bp in length (N50 = 2,812) covering a total of 4.7 Mb of genomic sequence.

**Microsatellite Analysis**

Microsatellite analysis was performed using PHOBOS 3.3.11 and only examined perfect microsatellites from 1 to 6 bp in length, with detection thresholds of 12 repeats (for 1 bp microsatellites), 8 repeats (for 2 bp microsatellites), and 5 repeats (for 3,4,5, and 6 bp microsatellites).

The frequencies of the 10 most abundant microsatellites were then counted and compared to previously identified microsatellites from an SSR-enriched library of *R. reniformis* (Arias, et al. *J. of Nematology*. 41(2):146-156. 2009).

**Future Work**

- Cross-check the genome size estimate with other known control organisms
- More sequencing of *R. reniformis* DNA extracted and amplified from single ehp
- Further refinement of the assembly using alternative assembly algorithms
- Further structural and functional annotation - nRNAs, repeat elements, GO annotation
- Incorporation of transcriptome sequences to help further refine our predicted gene models
- Using our *R. reniformis* BAC-end library to further improve the assembly
- Proteomics (Proteogenomic Mapping) to help refine structural genome annotation

**Acknowledgement**