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Tree Lab: Portable genomics for early detection of plant viruses and pests in Sub-Saharan Africa

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Abstract

In this case study we successfully teamed the PDQeX DNA purification technology developed by MicroGEM, New Zealand, with the MinION and MinIT mobile sequencing devices developed by Oxford Nanopore Technologies to produce an effective point-of-need field diagnostic system. The PDQeX extracts DNA using a cocktail of thermophilic proteinases and cell wall degrading enzymes, thermo-responsive extractor cartridges and a temperature control unit. This single-step closed system delivers purified DNA with no cross contamination. The MinIT is a newly released data processing unit that converts MinION raw signal output into base called data locally in real time, removing the need for high specification computers and large file transfers from the field. All three devices are battery powered with an exceptionally small footprint that facilitates transport and set up.

To evaluate and validate capability of the system for unbiased pathogen identification by real-time sequencing in a farmer's field setting, we analysed samples collected from cassava plants grown by subsistence farmers in three sub-Sahara African countries (Tanzania, Uganda and Kenya). A range of viral pathogens, all with similar symptoms, greatly reduce yield or completely destroy cassava crops. 800 million people worldwide depend on cassava for food and yearly income, and viral diseases are a significant constraint on its production (https://cassavavirusactionproject.com). Early pathogen detection at a molecular level has great potential to rescue crops within a single growing season by providing results that inform decisions on disease management, use of appropriate virus resistant or replacement planting.

This case study presented conditions of working in-field with limited or no access to mains power, laboratory infrastructure, internet connectivity and highly variable ambient temperature. An additional challenge is that, generally, plant material contains inhibitors of downstream molecular processes

55 making effective DNA purification critical. We successfully undertook real-time on-farm genome
56 sequencing of samples collected from cassava plants on three farms, one in each country. Cassava
57 mosaic begomoviruses were detected by sequencing leaf, stem, tuber and insect samples. The entire
58 process, from arrival on farm to diagnosis including sample collection, processing and provisional
59 sequencing results was complete in under 4 hours. The need for accurate, rapid and on-site diagnosis
60 grows as globalized human activity accelerates. This technical breakthrough has applications that are
61 relevant to human and animal health, environmental management and conservation.

62

63 **Keywords:** cassava, cassava mosaic begomovirus, cassava mosaic disease, *Bemisia tabaci*, whitefly,
64 MinION, MinIT, PDQeX, Tanzania, Uganda, Kenya.

65

66 **Introduction**

67 Crop losses due to viral diseases and pests are major constraints on food security and income for millions
68 of households in sub-Saharan Africa (SSA). Such losses can be reduced if plant diseases and pests are
69 correctly diagnosed and identified early. To date, researchers have utilized conventional methods for
70 definitive identification of plant viruses and their vectors in SSA including PCR, qPCR, Next Generation
71 and Sanger sequencing, but these require laboratory infrastructure, are costly and time consuming, and
72 can delay time-sensitive corrective actions that could be taken. Direct rapid DNA/RNA sequencing of
73 infected material on-the-spot or near sample collection sites turns this conventional paradigm on its
74 head by taking the laboratory closer to farmers' fields. This reduces overall costs and gives crop
75 protection officers and farmers in rural communities' information that is critical for sustainable crop
76 production and management of pests and diseases, ensuring food and income security for millions of

77 Africans. Currently, provision of data on viruses which is essential for developing virus resistant varieties,
78 sharing virus-indexed germplasm between regions and deployment of virus-free certified planting
79 materials is hampered by the long time taken to receive results generated using the aforementioned
80 conventional diagnostic methods. Our innovation will simplify information flow and fast track the
81 deployments of virus resistant or tolerant cassava varieties directly to the farmers field. The emergence
82 of new tools for real-time diagnostics, such as the Oxford Nanopore MinION, has proved useful for the
83 early detection of Ebola (Quick et al. 2016) and Zika viruses (Faria et al. 2016, Quick et al. 2017). MinION
84 consensus sequence accuracy of 99% is sufficient to identify pathogen and strain type (Calus et al. 2018).
85 However, it can take months before results generated using other high throughput sequencing
86 approaches (e.g. Illumina, PacBio) are available, particularly when local scientists are reliant on third-
87 party service providers, who are often located in other countries. The delay in detecting or identifying
88 viruses impedes quick in-situ decision making necessary for early action, crop protection advice and
89 disease management strategies by farmers. This ultimately compounds the magnitude of crop losses and
90 food shortages suffered by farmers. We have decreased the time to precisely detect and identify
91 pathogens, vectors or pests, and increased resolution and reliability of results by utilizing the power of
92 low-cost portable DNA extraction, sequencing and data analysis devices, coupled with our innovative
93 data analysis pipelines. This real-time diagnosis in the field or located in regional laboratories quickly
94 provides high quality and reliable diagnostics data to help farmers, seed certification agencies, scientists,
95 crop protection and extension officers make timely and informed decisions. The immediate data
96 accessibility makes possible dissemination of results downstream to extension officers and farmers for
97 early disease control action via Information and Communication Technologies (ICT) applications. The

application of cutting-edge sequencing technology, genomics and bioinformatics for pest and disease control has great potential to improve food security and agricultural development at large.

We propose using this technology to rapidly diagnose plant viruses and pests affecting smallholder farmers' crops in SSA. Our case study has identified cassava DNA viruses on the farm allowing farmers, researchers and development actors to take early, positive corrective action based on rapid diagnosis of plants. This proof-of-concept shows that portable DNA sequencer technology has great potential to reduce the risk of community crop failure. We have previously conducted pilot projects in Tanzania, Uganda and Kenya testing symptomatic and asymptomatic cassava plants and already have shown that sample collection to diagnosis and results delivered to the farmer or crop protection officer can be completed within 48 hours (Boykin et al. 2018). This technology will put the power of genome sequencing directly in the hands of agriculturalists and, in the work presented here, for the first time has enabled pest and disease diagnosis within one day on-the-spot. This has significant implications for new pest and disease outbreaks, monitoring of existing disease outbreaks and biosecurity monitoring at borders between countries.

Materials and Methods:

Tree Lab locations: Three small scale family farms growing cassava were selected, one in each of the following counties: Tanzania, Uganda and Kenya. In Tanzania (Kisamwene, Mara Region. GPS: 315N, 1°40'5"S, 33°55'55"E, 4380ft) on 1 August 2018, in Uganda (Wakiso. GPS: 255W 0°30'29"N, 32°37'19"E, 3730 ft) on 8 August 2018, and in Kenya (Kiambu. GPS: 87E 1°5'33" S, 37°19'33"E, 4570 ft) on 14 August 2018. A video of the Tanzanian Tree Lab is found here: https://vimeo.com/329068227.

Sample selection: All samples collected are documented in Table 1. For each sequencing run we barcoded either 11, including DNA extractions from cassava leaves, stems and also whiteflies (*Bemisia tabaci*) that were found feeding on cassava mosaic disease symptomatic cassava leaves.

DNA extraction: The PDQeX DNA extraction system from MicroGEM NZ Ltd (Stanton et al. 2019) was used to prepare DNA from samples. Briefly, a Harris punch was used to collect four discs, 2 mm in diameter, from each leaf, stem or root sample. Homogenization was performed by hand in 1x GREEN plus buffer using a Dounce homogenizer made from sealing the end of a 1 ml pipette tip and a 1.5 ml microfuge tube. Ninety microlitres of each homogenate and 10 µl of Enhancer (MicroGEM Ltd) was added to a 200 µl tube containing a lyophilized 1x mix of the enzyme cocktail (Holmes et al. 2018); (*phyto*GEM kit, MicroGEM Ltd, New Zealand). The reaction was re-suspended by gently flicking the 200 µl tube until all reagents were well mixed. All of the reaction mix was transferred to a PDQeX extraction cartridge (Stanton et al. 2019) which was placed into the PDQeX1600 thermal incubation unit. PDQeX extraction was performed by a series of heating steps. First, incubation at 52°C for five minutes to promote cell lysis by activating cell wall degrading enzymes. Second, incubation at 75°C for five minutes to activate thermophilic proteinases to degrade sample proteins and enzymes from the previous step. Finally, heating to 95°C for 2 minutes to shrink the thermal responsive inner layer of the PDQeX extraction cartridge forcing the digested sample through a burst valve and a cleanup column into a collection tube (Stanton et al. 2019).

DNA was also extracted from whitefly. A single insect was fished from a pool of whiteflies in ethanol collected from leaves using a Pooter. The whitefly was transferred by pipette, taking as little ethanol as possible, to 98 µl of 1x BLUE buffer (MicroGEM Ltd) and pipetted up and down several times. The whole mix was added to 1x lyophilized enzyme cocktail in a 200 µl tube (*prep*GEM, MicroGEM Ltd). Reagents were re-suspended by gentle flicking and the contents transferred to a PDQeX extraction cartridge. The cartridge was placed in the PDQeX1600 thermal unit and heated as follows: 35°C for five minutes; 52°C for five minutes; 75°C for five minutes; 95°C for 2 minutes. DNA extraction took approximately 20 minutes in total and 7.5 µl of the collected elute was used directly for Rapid DNA library construction for MinION Sequencing. The PDQeX1600 thermal unit was powered by a 12-volt Lithium Polymer battery. The PDQeX1600 was operated using a purpose-made App from a Smart Phone that permitted run initiation, temperature profile selection and editing, and monitoring of run progress.

Library preparation and sequencing: We utilized the Rapid Barcoding kit SQK-RBK004 with 9.4.1 flow cells (Oxford Nanopore Technologies). The SQK-RBK004 protocols were performed as described by the manufacturer (RBK_9054_v2_revB_23Jan2018). We completed the optional clean-up steps using AMPure XP beads. The 30°C and 80°C steps were performed using the PDQeX1600 thermal incubation unit. All libraries were loaded directly onto the MinION that was connected to a MinIT and live base calling was enabled. For each Tree Lab the MinION and the MinIT were plugged into a 20000mAh laptop powerbank (Comsol) set at 20V (Figure 1). The key to using a power bank for this purpose is to make sure it not only has USB inputs but also has a DC port. It ran on average 4.5 hours set on 16.5V. When the run stopped, we immediately plugged the devices into a second power bank and data generation continued.

Tree Lab data Analyses: As the data was being basecalled on the MinIT we made a test folder on the laptop called "treelab" and inside that folder we added a demultiplex folder, into which we then transferred the first two .fastq files from the MinIT into. Demultiplexing was run with Porechop (Wick 2019), preinstalled on the laptop, using the following commands >porechop -i /Users/lboykin/Desktop/treelab -b /Users/lboykin/Desktop/treelab/demultiplex. A cassava mosaic disease (CMD) reference data set was pre-curated and configured to work as a local database within Geneious vR11.1.2 (Kearse et al. 2012) (https://figshare.com/articles/Nanopore_sequencing_of_cassava_from_Tanzania_Uganda_and_Kenya/6667409). Twelve folders were created in Geneious, and the associated .fastq files from the "demultiplex" folder were drag and dropped into the relevant folder created within Geneious. BLASTn (Altschul et al. 1990) analysis was performed, ensuring the local CMD database was specified. The results from the search against the CMD database were visualized in-situ within Geneious and discussed with farmers and extension workers.

Post Tree Lab data analyses: Scripts from David Eccles' [Bioinformatics Scripts repository] (https://doi.org/10.5281/zenodo.596663) were used to carry out subsequent read QC and analysis. Sequenced read lengths were measured using [fastx-length.pl], and these lengths were used to generate length-based QC plots using [length_plot.r].

Assembly: To determine whether any barcoded read sets could be assembled, an initial assembly attempt was made on each subset using Canu v1.8, with a genome size of 400M, ignoring any warning

messages about coverage being too low. The large genome size ensured that no reads are discarded and suppressing the coverage warning ensured that Canu would attempt an assembly with all the available reads. Previous discussions with Canu developer Sergey Koren (pers. comm.) indicated that adjusting the target genome size had no other effect on the contig assemblies that Canu produces.

Blast: We confirmed these in-field results by performing a post diagnostic blast of reads on the Nimbus Cloud at the Pawsey Supercomputing Center with blast 2.2.31 against the full NCBI nucleotide database to confirm results. For reference the specific database used was {$ blastcmd –db nt/nt –info} Database: Nucleotide collection 49,266,009 sequences; 188,943,333,900 total bases Date: Aug 8, 2018 12:38 PM. The data were processed into a blast archive using a blast script with the following parameters (Script attached) {$blastn -query "$file" -db /mnt/nucdb/nt/nt -outfmt 11 -culling_limit 10 -out "out.$file.asn" -num_threads 17 } then converted into XML (for loading into Geneious) and HTML for viewing.

Blastn analysis – MEGAN:

Blastn results produced from the Nimbus cloud analysis pipeline were also visualized using MEGAN Community Edition version 6.12.6 (Huson et al. 2016) on the Zeus computing resource located at the Pawsey Supercomputing Center.

Blastn analysis - Kraken:

We used kraken2 [https://github.com/DerrickWood/kraken2] to classify demultiplexed reads using the Loman Lab "maxikraken2" database

206 [https://lomanlab.github.io/mockcommunity/mc_databases.html#maxikraken2_1903_140gb-march-
207 2019-140gb], on the Zeus computing resource located at the Pawsey Supercomputing Center.
208
209 **Results**
210
211 **Tree Lab**
212 All essential equipment that were used in the Tree Labs in Kenya, Uganda and Tanzania are listed in Table
213 1 and shown in Figure 1. Summary statistics for our three Tree Labs are shown in Table 2 and Figure 2.
214 Table 2 summarizes DNA sequencing metrics from all three Tree Lab experiments. Each MinION run
215 contained 11 barcoded libraries representing 11 individual samples. All DNA samples, except the ACMV
216 and EACMV controls (Tanzania) were extracted using the PDQeX system with 11 samples prepared in lab
217 from exemplar material collected from scientific plots and 20 DNA samples extracted on farm. A total of
218 1,442,599 sequences were produced across all the experiments. Of these, barcodes could only be
219 resolved for 550,938 sequences using Porechop to demultiplex the samples. Mean sequence length
220 across all sequencing runs ranged from 355bp to 948bp with the longest read being 276,793 bp.
221
222 Raw reads of Cassava mosaic begomoviruses (CMBs) sequences were detected in 21 samples with the
223 longest CMB read reaching 2808 bp, close to the full genome size. A total of 18 leaf samples were
224 sequenced of which 15 were found to contain CMBs. Two of the 5 stem samples sequenced were found
225 to contain CMBs whereas neither of the two root samples sequenced presented CMB sequences. Six
226 single whiteflies were tested with 2 being positive for CMBs. All libraries, regardless of CMB content
227 produced DNA reads indicating that sequencing was successful for all samples. CMBs were detected in

plants with symptoms and there was a suggestion that the number of CMB sequences detected possibly correlated with symptom severity scores, but more data will be required to prove this. Interestingly, a known healthy plant taken from the scientific plot at JKUAT did not yield CMB sequences (Table 2). Following assembly with Canu 8 of the 21 samples gave complete assembled virus genomes, however, gave less than 10 fold coverage.

Post Tree Lab data analyses

We investigated whether there was any effect of sample type on read length. The cumulative density curves (Figure 2) show the proportion of sequenced bases with length greater than a particular length (with L10/L50/L90 highlighted). Additional length-based QC plots can be found in the supplemental information (Supplemental File 1).

MEGAN results

The primary targets of this analysis were known cassava viruses, as well as the host, either cassava plant (*Manihot esculenta*) or the whitefly (*Bemisia tabaci*) and its endosymbionts. Results are summarized in Table 3, and in general the desired result of virus (EACMV or ACMV) and host DNA were recovered from all symptomatic samples.

Kraken2 results

The analysis using Kraken2 had an approximately 50% classification success rate (IQR 45-52% unclassified reads). This database is for human + microbial and viral sequence, so any eukaryote reads (e.g. from cassava or whitefly) would probably be unclassified by Kraken2 or assigned to the human taxa. The

sample with the highest classification success was the ACMV positive control from Tanzania (mr_BC11, 5.6% unclassified), and the lowest classification success was the leaf tissue sample #2 from Kenya (mm_BC02, 61% unclassified).

Results were aggregated into a table using Pavian (Supplemental File 2) to identify common elements of each sample. Begomovirus reads were detected in 15 samples, with very high proportions of Begomovirus (8.6%) in the ACMV Positive control from Tanzania (mr_BC11), and above-average proportions (0.25%) in Kwatempale sample #5 from Sarah's Farm in Uganda (ut_BC05). ACMV and EACMV were detected in 11 samples.

Discussion

This case study was designed to show the possibility to go from sample to diagnosis, in a regional setting, on farm in three hours versus the normal 6 months with conventional methods. The results of this research show that it is indeed possible, and that it is possible to use a range of battery powered devices to achieve DNA extraction, long read sequencing and analysis all under a tree on the farm while the farmers wait for results.

Access to next generation sequencing technology, or to services that offer access has been a major barrier to their use in diagnostics for scientists, and particularly many agricultural scientists in SSA. The advent of the Oxford Nanopore Technologies MinION has brought this technology to their door in recent years, and with access to training through various institutions and especially the Oxford Nanopore Technologies run “Pore Safari” there are more and more users in the region. Previous studies that have

used the technology for real time analysis of pathogen outbreaks, such as the Ebola and Zika studies (Faria et al. 2016, Quick et al. 2016, Quick et al. 2017) have still relied heavily on the transport of bulky laboratory equipment, or local acquisition of it to perform their work. Previous work by our team of scientists (Boykin et al. 2018) showed that the turnaround time to result could be 48 hours, and now with the addition of the PDQeX and MinIT to the system we have been able to reduce the time to 4 hours and perform the entire process in the field and under a tree.

One of the major barriers to producing these outputs in the field has until now been the lack of a simple, quick and effective methods to extract DNA from a sample without the need for laboratory equipment requiring mains power and space, items such as benchtop centrifuges, fridges, freezers and temperature sensitive extraction kits which can be bulky and rely on traditional laboratory infrastructure. The use of the PDQeX in the system described in this case study was the real game changer: compact and able to operate from a battery, it made nucleic acid extraction possible.

This study also highlighted where the next gains for in-field sequencing are to be made, as improvements are required in rapid data analysis. The MinIT eliminated obstacles to base calling, by converting the raw reads into .fast5 and .fastq reads in real time. This moves the data analysis bottleneck in the pipeline to the Blast analysis. Blast is not fast analysis, and so for now we must rely on a pre-curated database of known or expected pathogens and host genomes. This poses risks, in that new and emerging pathogens or vectors could be missed in the first instance, and not seen until subsequent data analysis when the scientist has returned to the lab or is within range of a good internet connection capable of uploading large amounts of data to the cloud. In our case, we can predict what sorts of genomes should be in our

294 custom database, but for use in biosecurity, and at borders between countries a better solution is
295 required.
296
297 Read length distributions were generally quite similar for all samples. The Tanzania samples showed the
298 greatest difference in read length distribution (L50 range 400 - 2000 bp). The ACMV Positive control from
299 Tanzania (mr_BC11) showed a very pronounced spike of reads at around 4kb (presumably near full-
300 length ACMV sequence). Apart from that sample, there was no obvious association between read length
301 distribution and tissue type or variety. The Uganda samples had a moderate read length distribution
302 spread (L50 range 500-1000 bp), which split into two clusters of slightly shorter and longer reads (BC02,
303 BC04, BC05, BC06, BC07; BC01, BC03, BC08, BC12). These clusters did not appear to have any relationship
304 with tissue type or variety. The Kenya samples had a similarly moderate read length distribution spread
305 (L50 range 500-1000 bp), with no obvious clustering, or association of distribution with tissue type or
306 variety.
307
308 MinION Rapid libraries use transposase to fix sequencing adaptors to DNA fragments. The ratio of DNA
309 to transposase complex for the MinION Rapid kit has been optimized for 400 ng DNA and at lower
310 amounts DNA is susceptible to over fragmentation. This may account for DNA fragment length falling
311 around 900 bp, however, the control DNA also gave similar read length characteristics. Though there
312 was not enough data collected on farm for a thorough statistical analysis these results did show both
313 yield and integrity of the DNA extracted using the PDQeX was of sufficient quality for diagnostic
314 sequencing. We successfully retrieved enough data from each sample to establish whether the virus in
315 the plant was EACMV or ACMV. Assembly with Canu suggested that in this case, while there was enough

316 data to assemble whole genomes, the average coverage meant the quality was not sufficient for
317 downstream applications such as recombination detection and other evolutionary analyses. We
318 anticipate that as the DNA extraction methods improve, and in field library preparation becomes easier
319 this will be possible. An alternative would be to investigate the use of a panel-like targeted amplicon
320 approach or CRISPR/Cas9 enrichment, but again this removes the likelihood of detecting unknowns in
321 the samples, and could lead to samples giving negative results not being followed up, or the time to
322 result being blown out to days or weeks if they need to return to a laboratory to complete a different
323 type of library preparation.

324

325 Compared with other in-field diagnostic tools, this system involving the MinION is unique in its ability to
326 detect anything that might be present in the sample. Other in-field diagnostic tools, including serological
327 based dipsticks, LAMP-PCR, in field qPCR and AI driven applications on smart phones all have one single
328 thing in common – they require a prior knowledge of the suspected pathogen, coupled with targeted
329 design of antibodies, primers or training for known positives to function effectively. The only decision
330 required to run the MinION is whether to prepare a DNA or an RNA library.

331 **Executive summary:**

332

333 **Can we go from sample to answer on the farm?** Yes
334 **DNA extraction to library prep to sequencing?** Yes
335 **Can we detect virus in leaves off the grid at the farm?** Yes
336 **Can we detect virus in whiteflies off the grid on the farm?** Yes
337 **Can we detect virus in stems off the grid on the farm?** Yes
338 **Do we get enough to coverage of the viral genomes to generate polished genomes to track the**
339 **evolution of the viruses real-time?** No

340

341

342 **Video of Tree Lab:** https://vimeo.com/329068227

343

344

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Table 1. Essential equipment for Tree Lab in Tanzania, Kenya and Uganda.

Sample collection plants
Envelopes
4 fine sharpies
Notebook
Scissors
Canvas sling bag to carry sample collection equipment
Gloves

Sample collection whiteflies
Pooter
70% ETOH
Eppendorf tubes
Transfer pipette to get the whitefly from the pooter to the Eppendorf tube

Lab under a tree/disease diagnostic camp
Blanket/floor covering
Hard flat surface- raised

Sample homogenization
1 microfuge tube/sample
1 ml tips with fine tip sealed to form pestle for crushing
Tip disposal/waste bags
1.5ml racks for holding
p10, p1000, p200 pipette (one set of pipettes)- tips for all 1 box

DNA extraction
2 MicroGEM kits (transported at ambient temperature)
    PDQeX phytoGEM kit for plants
    PDQex Universal kit for insects
Reagents lyophilized into 200µl tubes
Rack for 200µl microcentrifuge tubes
Nuclease free water
PDQeX thermal device
PDQeX Extraction tubes
Mobile phone being used as a remote controller- MicroGEM PDQeX app
Battery 2 12V batteries put together

Save the DNA for later quantification using fluorometry (e.g Qubit).

Library prep
Printed library prep protocol or iPad

Library kit
200µl tubes
1.5ml tubes
Ampure beads
Magnetic rack
Tube stands for the 0.2ml
Tris and sodium chloride
Nuclease free water
Syrafoam cooler and cold packs
Flow cell
MinIT
Laptop Power bank – one with a pin plug not just USB output- console
Laptop- with MinKnow installed

Table 2. Summary statistics and locality information for the three Tree Labs in Tanzania, Kenya and Uganda. * indicates DNA extraction carried out using PDQeX in the laboratory before sequencing under the tree.

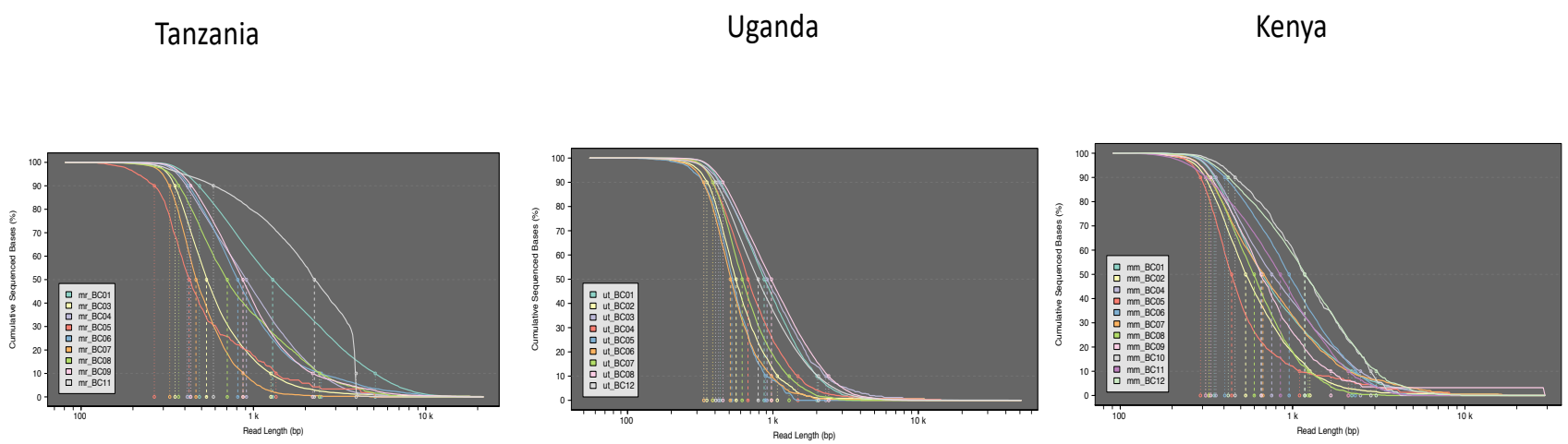
Sample	Barcode	Variety	Severity Score	Tissue type	Total reads	Max. Seq Length	CMV Blast hits	Max. CMV length	Min CMV length	% CMV reads	Canu Contigs
Tanzania											
1	1	Kilati - Local Variety	4	Leaf	61,317.0	26,107.0	125.0	2,808.0	196.0	0.204	0
3	3	Kilati - Local Variety	4	Leaf	21,468.0	16,098.0	14.0	815.0	288.0	0.065	0
4	4	Kasuxsali - Local Variety	3	Leaf	31,300.0	17,624.0	21.0	941.0	129.0	0.067	12
5	5	Mukombozi - Virus Resistant	1	Leaf	2,117.0	24,665.0	7.0	815.0	198.0	0.331	0
6	6	Mukombozi - Virus Resistant	1	Leaf	27,178.0	12,340.0	8.0	826.0	85.0	0.029	0
7	7	Mukombozi - Virus Resistant	1	Leaf	5,634.0	17,003.0	5.0	484.0	243.0	0.089	0
8	8	Whitefly close to Mukombozi - Virus Resistant	1	1x Whitefly, in EtOH	6,237.0	8,753.0	26.0	828.0	52.0	0.417	0
9	9	Whitefly close to Mukombozi - Virus Resistant	1	1x Whitefly, in EtOH	25,289.0	17,900.0	3.0	0.0	0.0	0	2
10	10	Whitefly close to Mukombozi - Virus Resistant	1	1x Whitefly, in EtOH	798.0	23,259.0	21.0	815.0	187.0	2.632	0
11*	11	ACMV - Positive Control DNA			10,966.0	28,541.0	4,311.0	1,598.0	31.0	39.312	0
12*	12	EACMV - Positive Control DNA			1,797.0	22,871.0	9.0	830.0	191.0	0.501	0
None	None	Porechop unable to match to Rapid Barcode			449,981.0	276,793.0	8,136.0	2,757.0	28.0	1.808	
Uganda											
1*	1	R39-B1-UG15F289P503	4	Leaf from stem 1	24,343.0	24,373.0	20.0	1,222.0	123.0	0.082	2
1.1*	2	R39-B1-UG15F289P503	4	Phloem, stem 1 - top	5,135.0	50,184.0	0.0	0.0	0.0	0	0
1.2*	3	R39-B1-UG15F289P503	4	Phloem, stem 1 - mid	4,010.0	9,768.0	0.0	0.0	0.0	0	0
1.3*	4	R39-B1-UG15F289P503	4	Phloem, stem 1 - bottom	10,314.0	48,265.0	1.0	402.0	402.0	0.010	0
5	5	Kwatempale from Sarah's Farm	4	Leaf	3,012.0	66,062.0	11.0	604.0	107.0	0.365	0
6	6	Kwatempale from Sarah's Farm	5	Leaf	5,074.0	5,243.0	1.0	235.0	235.0	0.020	0
7	7	Wild Plant from Naomi's Farm	3	Leaf	16,386.0	45,024.0	6.0	1,121.0	352.0	0.037	0
9	8	Sick branch from NAROCass1 from Naomi's Farm	3	Leaf	37,822.0	20,509.0	15.0	2,021.0	166.0	0.040	12
WF5	9	Whitefly from sample 5	4	1x Whitefly, no EtOH	347.0	41,599.0	0.0	0.0	0.0	0	0
WF7	10	Whitefly from sample 7	3	1x Whitefly, no EtOH	2,613.0	2,613.0	0.0	0.0	0.0	0	0
WF8	11	Whitefly from sample 8	1	1x Whitefly, no EtOH	705.0	15,596.0	0.0	0.0	0.0	0	0
None*	None	Porechop unable to match to Rapid Barcode			196,290.0	267,436.0	59.0	1,677.0	42.0	0.030	
Kenya											
1	1	Local	1	Leaf	43,049.0	39,851.0	1.0	123.0	123.0	0.002	1
2	2	Local	1	Leaf	15,890.0	66,540.0	0.0	0.0	0.0	0	0
4	4	Local	1	Leaf	38,291.0	53,843.0	3.0	890.0	251.0	0.008	1
5	5	Local	1	Leaf	9,213.0	44,230.0	0.0	0.0	0.0	0	0
L1*	6	Stem from CMV infected plant	4	Leaf	28,450.0	18,287.0	219.0	2,228.0	98.0	0.770	3
L2*	7	Stem from CMV infected plant	4	Leaf	17,320.0	42,191.0	76.0	2,127.0	78.0	0.439	0
S1*	8	Stem from CMV infected plant	4	Phloem, 22.5cm from tip	16,310.0	24,566.0	10.0	1,485.0	269.0	0.061	0
S2*	9	Stem from CMV infected plant	4	Phloem, 52.3cm from tip	5,346.0	54,245.0	0.0	0.0	0.0	0	0
R1*	10	Stem from CMV infected plant	4	Root 1, under outer bark	7,336.0	15,689.0	0.0	0.0	0.0	0	0
R2*	11	Stem from CMV infected plant	4	Root 2, under outer bark	21,576.0	24,853.0	0.0	0.0	0.0	0	0
H1*	12	Leaf from Healthy Plant	1	Leaf	44,295.0	33,307.0	0.0	0.0	0.0	0	3
None	None	Porechop unable to match to Rapid Barcode			245,390.0	265,898.0	75.0	2,404.0	28.0	0.031	
				TOTALS	**492,466.0**	**56,958.3**					

Table 3. Megan Results of Blastn from Nimbus cloud.

	Total reads	Reads classified	Manihot esculenta	EACMV	ACMV	TLCV	Begomo-associated DNA-III	*Bemisia tabaci*	*Bemisia afer*	*Candidatus Portiera aleyrodidarum*	Other
Tanzania											
1	61,317	23,481	15,862	63	-	1					
3	21,468	5,407	3,509	5							
4	31,309	10,561	6,462	2	5	1					
5	2,117	325	144		2			3		3	
6	27,178	2,127	76		1			521	67	802	
7	5,634	1,141	669		1						
8	6,237	1,449	88		5			912		15	
9	25,289	2,303	126	2				506	57	905	
10	789	166	66		3			11		2	
11	10,966	7,843	69	3	616					2	
12	1,797	356	171		2			13		7	
Uganda											
1	18,853	5,662	3,073		11		1				
1.1	5,135	876	402								
1.2	4,010	1,034	591								
1.3	10,314	1,933	864		1						
5	3,012	556	255	1	7						
6	5,074	758	39		1						
7	16,386	4,608	2,666		4						
9	37,822	12,768	7,268	10							
WF5	347	51	20					10			
WF7	243	48	21								
WF8	705	128	35					9		1	
Kenya											
1	43,049	10,283	9,947		1	1	1				
2	15,890	2,968	2,854								
4	38,291	8,648	8,836	1	1						
5	8,213	1,959	1,887								
L1	28,450	9,968	9,580	45	81						SLCV (1)
L2	17,320	4,367	2,050	29	4						
S1	16,310	3,718	1,933	5		1	1				
R1	7,336	2,303	1,016								
R2	21,578	6,810	2,000								
H1	44,295	15,537	8,389				1				

Figure 1. Tree Lab in Kenya. Essential equipment is listed in Table 1.

Figure 2. Cumulative density curves showing the proportion of sequenced bases with length greater than a particular length (with L10/L50/L90 highlighted).



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