A Workflow for Generating and Polishing Nanopore Reads from Low Biomass Samples

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Project Goals: Microorganisms play key roles in soil carbon turnover and stabilization of persistent organic matter via their metabolic activities, cellular biochemistry, and extracellular products. Microbial residues are the primary ingredients in soil organic matter (SOM), a pool critical to Earth's soil health and climate. We hypothesize that microbial cellular-chemistry, functional potential, and ecophysiology fundamentally shape soil carbon persistence, and we are characterizing this via stable isotope probing (SIP) of genome-resolved metagenomes and viromes. We focus on soil moisture as a 'master controller' of microbial activity and mortality, since altered precipitation regimes are predicted across the temperate U.S. *Our SFA's ultimate goal is to determine how microbial soil ecophysiology, population dynamics, and microbe-mineral-organic matter interactions regulate the persistence of microbial residues under changing moisture regimes.*

Numerous technological and analytical advances have caused a revolution in the life sciences and revealed that microbes and their viruses represent hidden drivers of the nutrient and energy currencies that fuel our planet and our bodies. Arguably, the most impactful advance in the past decade has been the ability to view these micro- and nano-scale entities via sequencing rather than cultivation-based approaches—giving use a significantly broader window into the functional capabilities and interactions of microbe-based communities. Though powerful, current metagenomic sequencing approaches are limited in that short-read assemblies likely miss microdiverse populations and niche-defining hypervariable genomic islands, and routine long-read sequencing requires high-biomass inputs and has high error rates.

Here we optimized laboratory and informatics protocol of our VirION[1] approach to generate, analyze and polish Oxford Nanopore long reads (>10kb) from low (1ng) biomass samples. For method optimization, we used a three phage mock community of known genomes (size range: 38-130kb) and evaluated DNA extraction and sequencing library preparation options including: choice of DNA polymerase, DNA shearing size, number of PCR amplification cycles, input DNA amount and DNA cleanup strategies. This revealed that sheared DNA (15kb size) that was amplified by LA Takara (long-range) polymerase yielded the most consistent and significant increase in read lengths compared to other treatments. The optimized protocol achieved a median read length of 7095bp (up to 67kb), which represents a ~5,000 bp increase over that documented in the official Nanopore kit. The number of amplification cycles tested (15, 18, 20 and 22) did not significantly alter the number of chimeric reads produced (<1% in all treatments), which enables much lower input biomass than in the official Nanopore kit. Together these optimized protocols are now being applied to Hopland soils for this SFA project to better document short-read recalcitrant viral OTUs and their niche-defining hypervariable genomic islands.

References

1. Warwick-Dugdale, J. *et al.* Long-read viral metagenomics captures abundant and microdiverse viral populations and their niche-defining genomic islands. *PeerJ* **7**, e6800 (2019).

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