A streamlined workflow for high-throughput, multiplexed HiFi sequencing of microbial genomes

Meredith Ashby[1], Harsharan Dhillon[1], Aaron Wenger[1], Joan Wilson[1], Shreyasee Chakraborty[1], Davy Lee[1], Christopher Dunn[1], Ivan Sovic[1], Zev Kronenberg[1], Heather Ferrao[1], Primo Baybayan[1]
[1]PacBio, 1305 O'Brien Drive, Menlo Park, CA 94025

Introduction

Highly accurate, long HiFi reads have brought new levels of contiguity, completeness, and accuracy to large genome assembly. However, obstacles in efficiently scaling library prep have limited adoption for microbe sequencing.

Here, we introduce a highly parallelizable shearing method, a streamlined library prep workflow, and an assembly algorithm based on HiFi reads that together enable a high throughput, end-to-end solution for HiFi microbial genome sequencing.

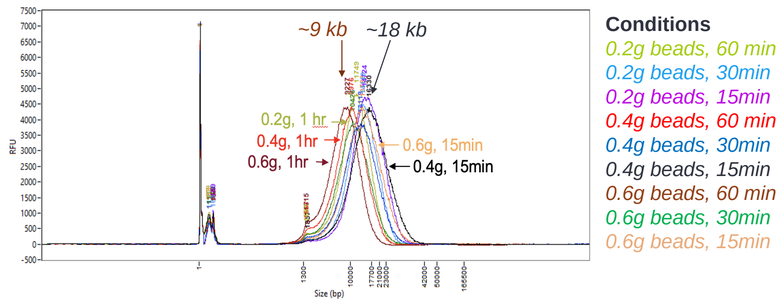
HiFi reads enable workflow upgrades

- HiFi accuracy means shorter libraries can produce the same or better assembly results for microbial WGS.
- Tolerance for shorter input DNA loosens shearing requirements and makes closed genomes accessible for more samples.
- Higher raw accuracy reduces coverage requirements, enabling higher multiplexing and lowering per sample costs.

Scalable, low-cost DNA shearing

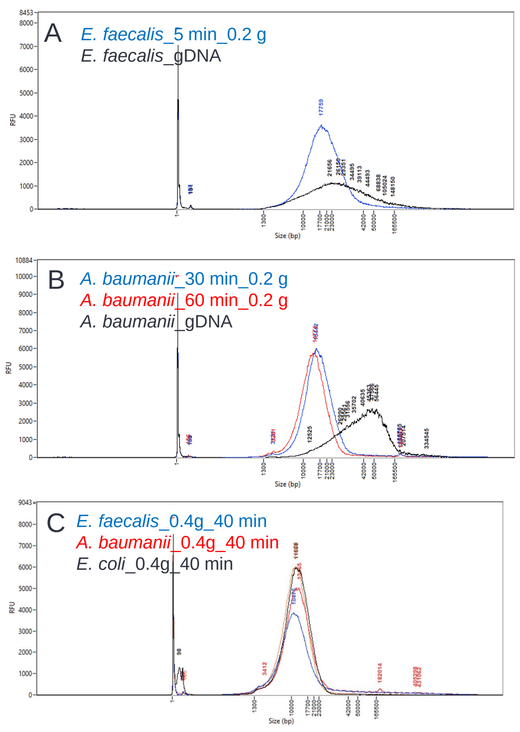
- Current options for shearing high molecular DNA add complexity, cost and time to long read library preparation when scaled.
- We present an inexpensive, scalable DNA shearing method compatible with HiFi sequencing using acid washed glass beads and agitation on a Vortex Genie2.

Figure 1. Glass bead shearing yields a tight fragment distribution at an adjustable peak



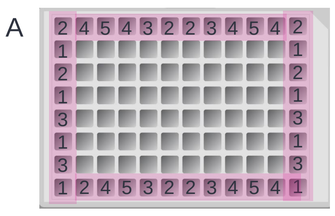
- 300 ng of E. coli gDNA in 50 µL of water was added to 0.2–0.6 g of glass beads in 300 µL of water and vortexed for 15–60 min at 3,000 rpm.

Figure 2. Glass bead shearing produces consistent shears even with variable quality input DNA



- Degraded (2A), partially degraded (2B), and high molecular weight (not shown, *E. coli*) DNA all produce near identical fragment size distributions when sheared with the same conditions (2C).

Figure 3. Up to 36 samples can be sheared in parallel per vortex device



- ~0.2 g of beads were aliquoted into the 36 outer wells of a 96 deep-well plate.
- The plate was agitated for 1 hr.

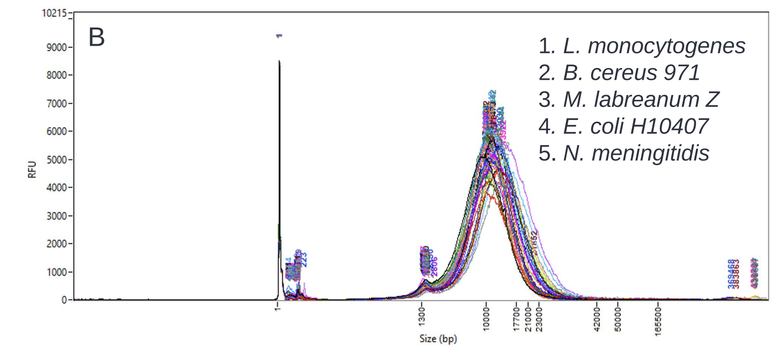
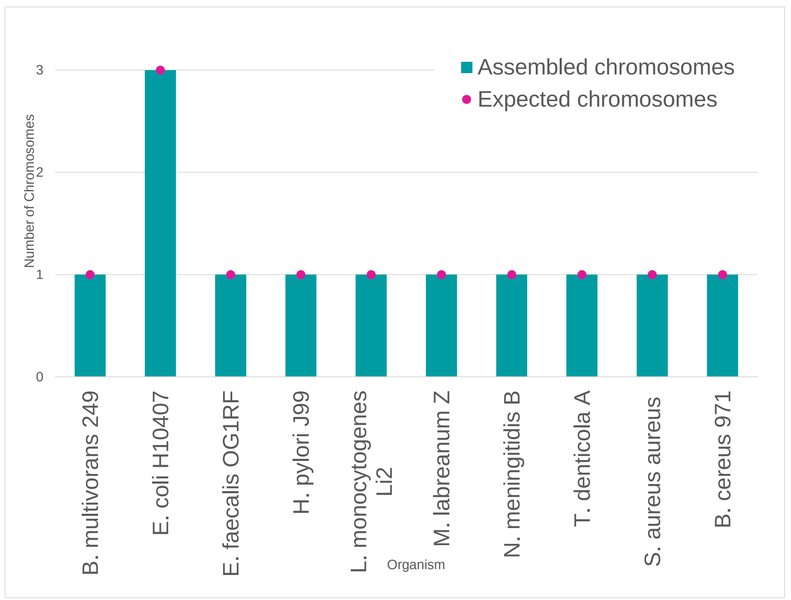


Figure 4. Sequencing of glass bead sheared microbial samples

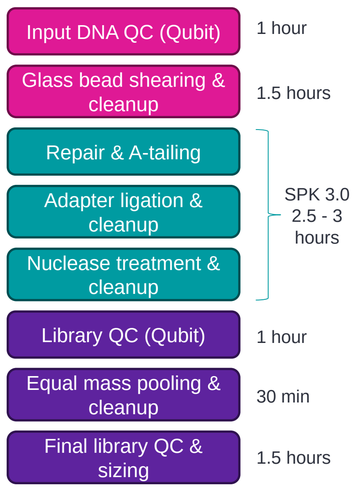


- 10 samples were sheared, prepped, and sequenced on 1 SMRT Cell 8M.
- The data was down-sampled to 30-fold coverage and assembled using the Microbial Assembly workflow in SMRT Link.
- All samples produced closed, circular genomes

Simpler, automatable SMRTbell library prep

- The new SMRTbell library prep kit (SPK3.0) has fewer steps, fewer reagents, and is automatable.
- Paired with glass bead shearing, SPK 3.0 creates an efficiently scalable high throughput workflow.

Figure 5. SMRTbell library prep workflow for up to 96 samples

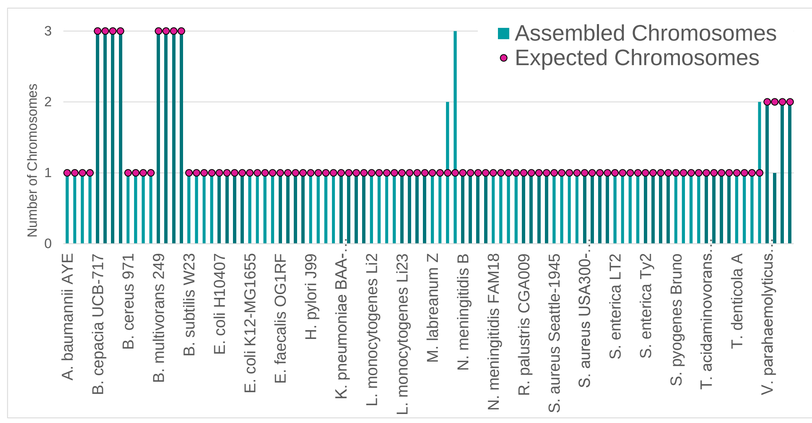


- The SMRTbell barcoded adapter plate 3.0 enables multiplex sequencing of up to 96 samples
- Bead shearing cleanup and SPK 3.0 steps can be automated
- Gel sizing of libraries can now be omitted, with samples pooled by equal mass
- With normalized input amounts, equal volume pooling can save an additional hour

Microbial Assembly with HiFi Reads

The PacBio Microbial Assembly tool is optimized for bacterial genome assembly, with distinct chromosomal and plasmid assembly stages, circular-aware polishing, and oriC rotation.

Figure 6. HiFi data consistently produces closed genomes with default settings



- 96 SMRTbell libraries were made from 24 distinct bacteria relevant to food safety, hospital outbreaks, and infectious disease, encompassing a wide range of GC content, genome size, and genome complexity.
- Nearly all samples yielded closed chromosomes without any manual curation. Those that did not could be closed with hifiasm using custom settings.

Figure 7. Microbes assembled with HiFi data have gold standard accuracy

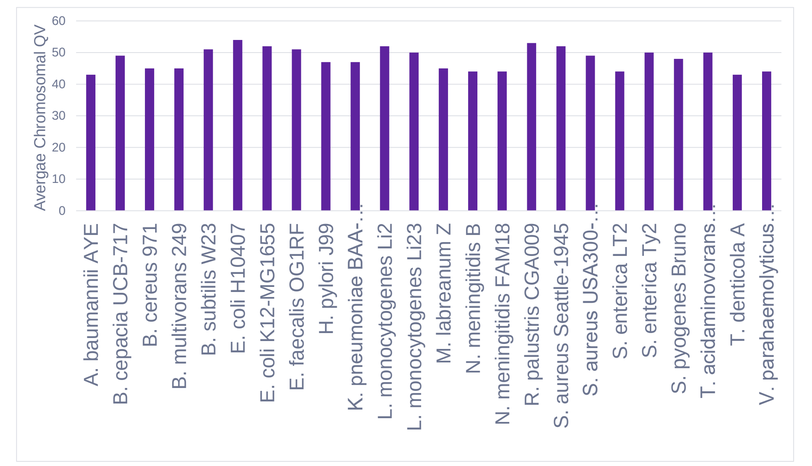
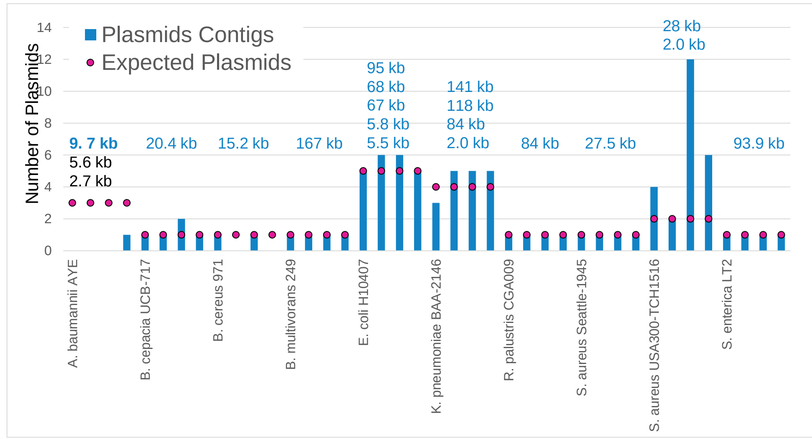


Figure 8. HiFi sequencing consistently recovers closed plasmids



- Expected plasmid sizes are noted above samples grouped by species. Plasmids in blue were recovered in at least one replicate.
- Plasmid recovery is shown using default parameters without curation. Manual review can improve contiguity (*S. aureus USA300-TCH1516; E. coli H10407*) and identify assembly artifacts.
- Recovery of smaller plasmids may require use of smaller insert sizes.

Conclusions

- Inexpensive, scalable shearing with glass beads removes a significant barrier to high multiplex SMRT sequencing of microbial genomes.
- SPK 3.0 shortens, simplifies, and enables the automation of SMRTbell library prep.
- The update of Microbial Assembly to accept HiFi data lets more microbes be sequenced from shorter libraries while preserving assembly quality, completing an end-to-end high throughput workflow for PacBio microbial WGS.