Materials and Methods

For microbial genomes, recommend shearing genomic DNA to 10 kb and using adapters for post shearing and purification.

Sample input DNA for Exo VII depends on the genome size and complexity. For microbial WGS, recommend targeting 50-100 ng per genome and may require 20 to 30 µl library preparation with size selection.

To demonstrate performance, 12 unique barcodes assigned to 1.0 µg each of 12 strains of E.coli, each was sheared to 10 kb with Covaris g-TUBEs. The sheared DNA was then input into a single SMRTbell 5.0 reagent. Each SMRTbell was sequenced as an independent library for the highest scoring for each molecule. The highest scoring library was chosen for each molecule. This protocol scores and aligns barcodes against all potential barcodes and returns the highest scoring library for each molecule.

Mean Coverage 12 plex of 4 Mb pool

Table 1. Multiplexing DNA input recommendations

Table 2. Comparison of Exo VII treated and non treated DNA samples.

1. Samples were sequenced on the Sequel System with 2.0 chemistry and 600 minute movies.
2. To score and align each read with their barcodes, SMRT Cells were processed using Barcoding protocol in SMRT Link (GUI). This protocol scores and aligns barcodes against all potential barcodes and returns the highest scoring library for each molecule.
3. One subreadset was input into the barcoding dataset split – barcodes with (CL) and -100 input into each SMRT Link.
4. De novo assembly was run in SMRT Link (GUI) using KPAE for each individual subreadset from step 3.

Results

Multiplexing maximizes throughput and efficiency of microbial whole genome sequencing, while reducing cost and improving assembly performance.

Microbes

E.coli

B. subtilis

H. pylori

H.pylori

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Fig. 8. Kinetic detection distribution comparison of singleplex and multiplexed libraries.

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