Product note

WORKFLOW SOLUTIONS

Accelerated, simplified workflow for multiplexing microbial genomes with SMRTbell® express template prep kit 2.0

Figure 1. SMRTbell express template prep kit 2.0 (PN: 100-938-900)

The SMRTbell express template prep kit 2.0 provides a streamlined, single-tube reaction strategy to generate SMRTbell libraries from 500 bp to >50 kb insert size targets to support large-insert genomic libraries, multiplexed microbial genomes, and amplicon sequencing. With this new formulation, we have increased both the yield and efficiency of SMRTbell library preparation for SMRT® sequencing while further minimizing handling-induced DNA damage to retain the integrity of genomic DNA (gDNA).

This product note highlights the key benefits, performance, and resources available for obtaining complete microbial genome assemblies with multiplexed sequencing. By using a single-tube, addition-only strategy, the streamlined workflow reduces the number of AMPure PB cleanup steps. This provides an opportunity to explore automation solutions for high-volume projects, while using as little as 1 µg of input DNA.

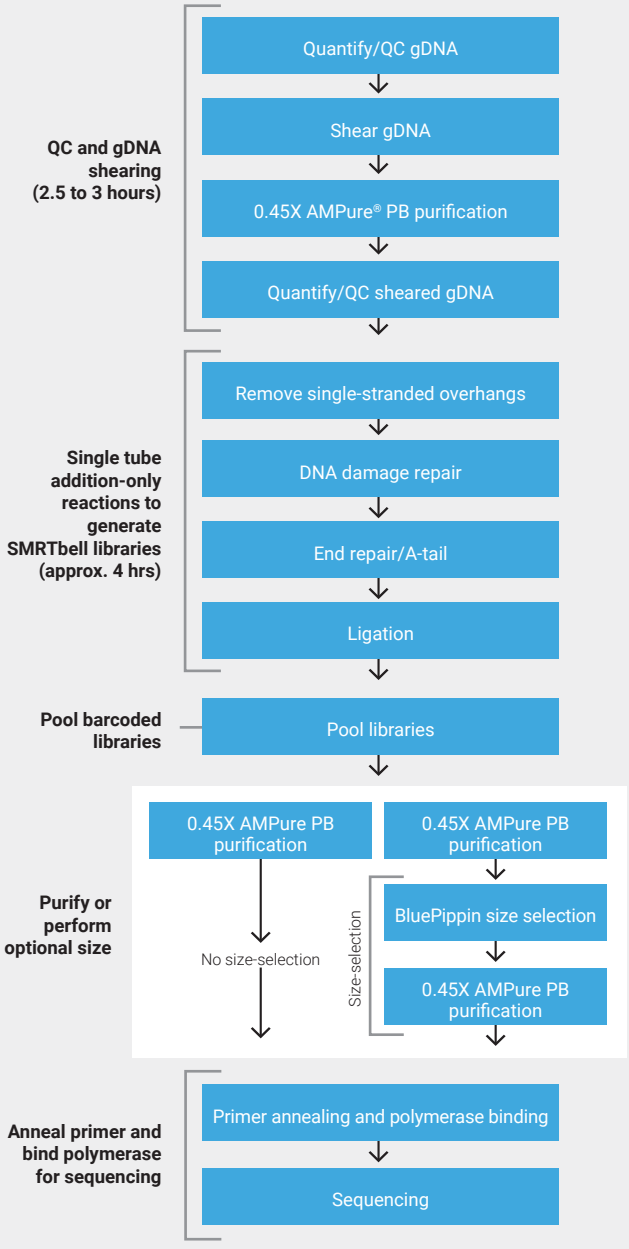

Figure 2. SMRTbell express template prep kit 2.0 workflow for generating SMRTbell libraries in ~ 4 hours. Does not include QC and size-selection time. Time to complete end-to-end workflow will also depend on number of samples being processed for multiplexed sequencing; we estimate approximately 8.5 hours.

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Supported applications discussed in this product note

- Multiplexed microbial genome assemblies

Key benefits

- 1 µg gDNA input requirement
- Fast library template preparation in 4 hours
- Minimal handling-induced gDNA damage

Product

- SMRTbell express template prep kit 2.0 (PN 100-938-900)
- Barcoded overhang adapter kit 8A (PN 101-628-400)
- Barcoded overhang adapter kit 8B (PN 101-628-500)
- Elution buffer (50 mL) (PN 101-633-500)

Protocol

- Procedure + checklist — preparing multiplexed microbial libraries using SMRTbell® express template prep kit 2.0
- Analysis procedure — Multiplexed microbial assembly with SMRT® Link v6.0.0 and express template prep kit 2.0
- Express microbial multiplexing calculator

Library generation time

Approx. 4 hours (excluding QC and size-selection)

Experimental workflow time

Approx. 8.5 hours (subject to multiplexing design)

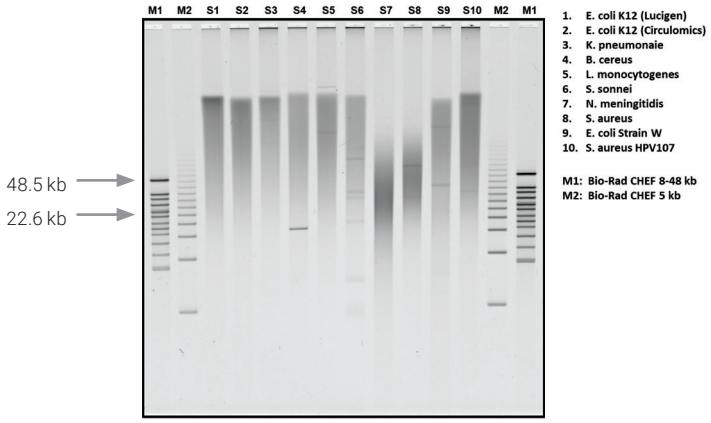
Libraries supported

Number of reactions:
48 microbial genomes

Insert size:
Approximately 12 kb

Recommended pooling strategy

- Up to 30 Mb total microbial genome size for general use
- Up to 40 Mb total microbial genome size for highly optimized workflows and advanced users



Generating microbial genome libraries

Example gel image for a 10-plex pool with a total genome size of 42 MB. Start with input genomic DNA predominantly greater than 20 kb.

Figure 3. Example QC gel for 10-plex microbial genome pool where all gDNA samples are predominantly >20 kb.

Assembly results

Barcode ID	Sample ID	Expected genome size (bp)	Polished contigs (#)	Max contig length (bp)	N50 contig length (bp)	Sum of contig lengths (bp)	Pre-assembled yield (%)	Filtered subread coverage (fold)	Assembly notes
BC1001	*E. coli* K12 (Lucigen)	4,653,240	1	4,642,499	4,642,499	4,642,499	93.10%	64	Complete chromosomal assembly
BC1002	*E. coli* K12 (Circulomics)	4,653,240	1	4,642,500	4,642,500	4,642,500	92.90%	55	Complete chromosomal assembly
BC1009	*K. pneumonaie*	5,781,501	5	5,435,746	5,435,746	5,746,850	92.50%	50	Complete chromosomal assembly, and 140 kb and 85 kb plasmid assemblies. 118 kb plasmid captured in 2 contigs; 2 kb plasmid was missed
BC1010	*B. cereus*	5,427,083	2	5,408,315	5,408,315	5,423,588	92.70%	59	Complete chromosomal and 16 kb plasmid assemblies
BC1012	*L. monocytogenes*	3,032,269	2	3,043,149	3,043,149	3,137,529	93.70%	66	Complete chromosomal assembly
BC1015	*S. sonnei*	5,062,953	1	4,813,454	4,813,454	4,813,454	93.10%	53	Complete chromosomal assembly. Missing eight expected plasmids
BC1016	*N. meningitidis*	2,194,961	1	2,213,947	2,213,947	2,213,947	92.20%	74	Complete chromosomal assembly
BC1018	*S. aureus*	2,806,345	2	2,778,860	2,778,860	2,806,350	92.60%	92	Complete chromosomal and 27 kb plasmid assemblies
BC1019	*E. coli* strain W	5,005,347	2	4,898,327	4,898,327	5,004,399	93.00%	68	Complete chromosomal and 103 kb plasmid assemblies. Missing 5 kb plasmid
BC1022	*S. aureus* HPV107	2,901,406	2	2,962,786	2,962,786	2,994,972	93.40%	82	Complete chromosomal and 24 kb plasmid assemblies

Table 1. Summary of HGAP genome assembly results for a 10-plex microbial pool run. We recommend a >60% minimum pre-assembly yield and >30-fold filtered subread coverage per genome for high-contiguity assemblies. Complete microbial chromosomal assemblies were captured for all microbes in the pooled run. In some instances, plasmid sequencing may also be included. We do not guarantee plasmid sequencing with this workflow.

Sequencing performance*

Library type with sequencing 3.0	Polymerase read length (average)	Polymerase read length (n50)	Longest subread (average)	Longest subread (n50)
Multiplexed microbial genome sequencing with ~12 kb insert	Up to 45 kb	Up to 85 kb	Up to 8 kb	Up to 11 kb

Table 2. Estimated sequencing read length performance for given library type.
* Sequencing performance, reads/data per SMRT® Cell and other expected results vary based on sample quality/type and insert size.

Input requirements

Genomic DNA	1 µg (predominantly >20 kb)

Learn about the SMRTbell express template prep kit 2.0: **pacb.com/tpk20**

KEY REFERENCES

1. **Application note: Microbial multiplexing workflow on the Sequel® system**
2. **Applications website: Microbial whole genome sequencing application**
3. **Application brief: Microbial whole genome sequencing — Best practices (2018)**

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