

An Express Workflow to Generate SMRTbell® Libraries for Genomic DNA in as little as 4 Hours

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 supporting *de novo* genome sequencing and structural variant detection projects. Our large-insert gDNA protocol has been streamlined to support SMRTbell library generation in only 4 hours, making complete construction in less than a day. This significantly reduces time to results for generating high-quality genome assemblies to fully characterize SNPs and structural variants. Additional key benefits of this template preparation kit and updated protocol include library generation with as little as 2 to 3 µg input gDNA and flexibility to accommodate and adjust input amount in accordance with the extracted gDNA quality.

Workflow

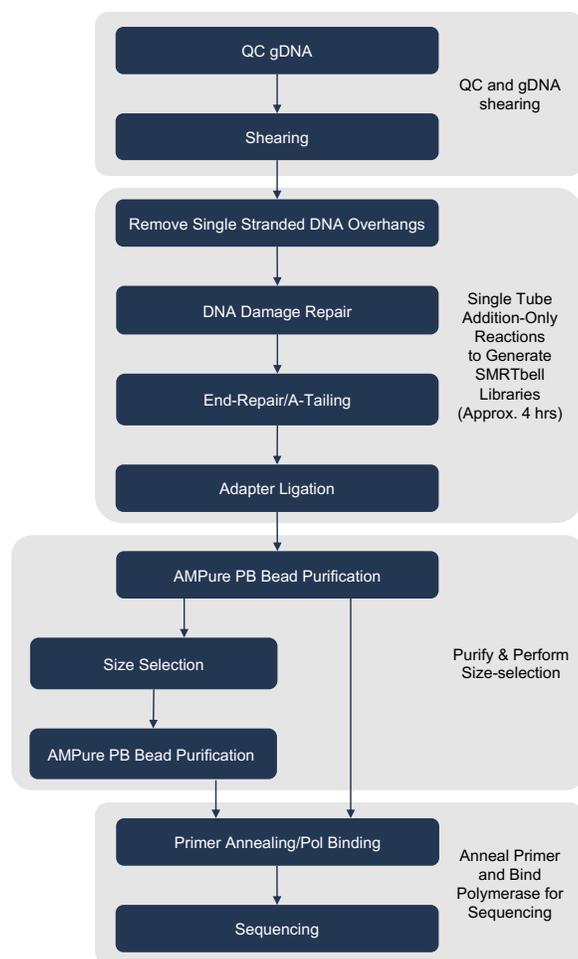


Figure 1. SMRTbell Express Template Prep Kit 2.0 workflow for generating large-insert libraries in ~ 4 hours. Does not include QC and size-selection time.



Figure 2. SMRTbell Express Template Prep Kit 2.0 (PN 100-938-900)

Supported Applications Discussed in this Product Note are:

- *De novo* genome assemblies
- Low-fold coverage structural variant detection

Key Benefits:

- Reduced gDNA input requirement
- Fast library template preparation
- Minimal handling-induced gDNA damage

Protocol

- Procedure & Checklist - Preparing gDNA Libraries Using the SMRTbell® Express Template Preparation Kit 2.0

Library Generation Time

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

Libraries Supported

- # Rxns: 18 large-insert gDNA libraries
- Insert Sizes: 10 kb to >50 kb

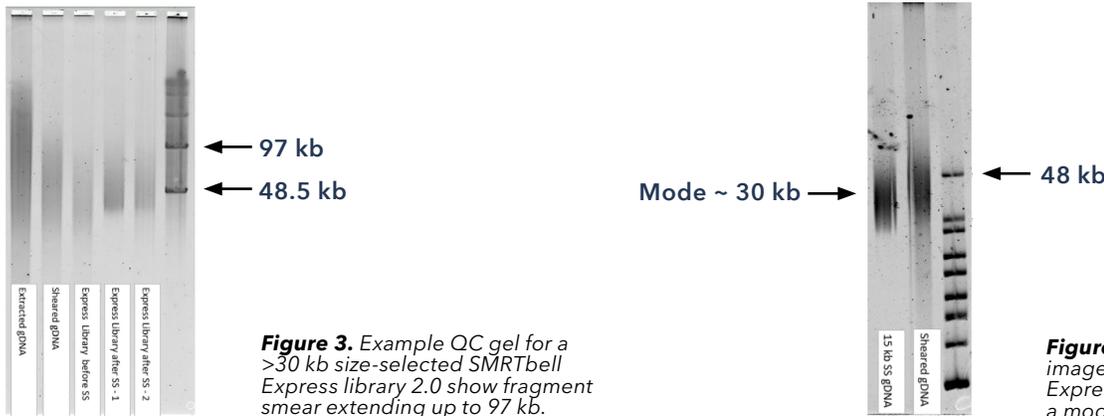
Expected Performance*

Library Type SMRTbell insert library size supported	SMRTbell Library Yield Yield of SMRTbell libraries for sheared gDNA input	Sequencing Yield Mapped bases per input ug of sheared gDNA	SMRT Cells Minimum number of SMRT Cells supported by library
15 kb Size-selected Libraries	up to 27%	up to 250 Gb	>20
30 kb Size-selected Libraries	up to 20%	up to 35 Gb	>4
10 kb Non-size-selected Libraries	up to 75%	up to 450 Gb	>40

Table 1. Estimated sequencing yield for respective library types.

Generating Large-insert Libraries:

Example gel images for >30 kb and >15 kb size-selected libraries



Sequencing Performance*

Library Type with Sequencing 3.0	Polymerase Read Length (Average)	Polymerase Read Length (N50)	Longest Subread (Average)	Longest Subread (N50)
>30 kb size-selected libraries	up to 30 kb	up to 45 kb	up to 35 kb	up to 40 kb
10 kb libraries with no size selection	up to 24 kb	up to 50 kb	up to 6.5 kb	up to 8.9 kb

Table 2. Estimated sequencing read length performance for given library

Input Requirements

Recommended shearing methods and input gDNA qualities for supported library types:

Insert Size Target	Size Selection	gDNA Quality Required	Shearing Method	Range of Sheared and Concentrated Input gDNA (µg)	Recommended Sheared and Concentrated DNA Amount
~10 kb	No	Mode >20 kb	g-TUBE	1.0 to 2.0 µg	1.0 µg
>15 kb (size-selected using BluePippin system)	Yes	Mode >40 kb	g-TUBE or Megaruptor®	2.0 to 5.0 µg	3.0 µg
>30 kb (size-selected using BluePippin system)	Yes	Mode >50 kb	26G needle or Megaruptor	3.0 to 5.0 µg	5.0 µg

Resources

1. Applications Website: Whole Genome Sequencing Application
2. Application Brief: Plant and Animal Whole Genome Sequencing Best Practices
3. Application Brief: Low-coverage, Long-read Whole Genome Sequencing for Structural Variation
4. Infographic: Gold-Standard Reference Genomes Accelerate Science

*Sequencing performance, reads/data per SMRT Cell and other expected results vary based on sample quality/type and insert size.