Quick reference card – Preparing SMRTbell® libraries using SMRTbell prep kit 3.0

Before you begin

- Use **300–1000 ng** of DNA per SMRT® Cell 8M.
- Divide **1000 ng** by the number of samples when multiplexing with SMRTbell barcoded adapters.
- Iso-Seq® samples require ≥160 ng of cDNA per SMRT Cell 8M.

DNA shearing	Human, plant, animal	Microbial or metagenomic
Megarupter 3	Speed 31	Speed 40
Modal size	15–18 kb	7–12 kb

- Resuspend samples in **47 µL** of low TE buffer using the SMRTbell cleanup bead steps prior to SMRTbell prep kit 3.0 steps.
- Adjust component volumes for the number of samples being prepared, plus 10% overage when preparing the reagent mixes.
- Add reagent components directly to sample(s) if not preparing reagent mixes. Pipette mix and spin down prior to incubation.

Step 1. Repair and A-tailing

1.1 Make reaction mix 1 (RM1) in new tube.

Reaction mix 1 (RM1) per sample volumes

Tube	Component	Volume
Purple	Repair buffer	8 µL
Blue	End repair mix	4 µL
Green	DNA repair mix	2 µL

1.2 Pipette mix and quick spin **RM1** to collect liquid.

1.3 Add **14 µL** of the **RM1** to each sample.

1.4 Incubate samples with the following program:

Temperature	Time
37°C	30 minutes
65°C	5 minutes
4°C	Hold

1.5 Proceed to next step.

Step 2. Adapter ligation and cleanup

2.1 Add **4 µL** of SMRTbell adapter (barcoded or non-barcoded) to each sample.

2.2 Make reaction mix 2 (RM2) in new tube.

Reaction mix 2 (RM2) per sample volumes

Tube	Component	Volume
Yellow	Ligation mix	30 µL
Red	Ligation enhancer	1 µL

2.3 Pipette mix and quick spin **RM2** to collect liquid.

2.4 Add **31 µL** of **RM2** to each sample.

2.5 Incubate samples using the following steps:

Temperature	Time
20°C	30 minutes
4°C	Hold

2.6 Clean up with SMRTbell cleanup beads and resuspend in **40 µL** of elution buffer.

Step 3. Nuclease treatment and cleanup

3.1 Make reaction mix 3 (RM3) in new tube.

Reaction mix 3 (RM3) per sample volumes

Tube	Component	Volume
Light purple	Nuclease buffer	5 µL
Light green	Nuclease mix	5 µL

3.2 Pipette mix **RM3** and quick spin to collect liquid.

3.3 Add **10 µL** of **RM3** to each sample.

3.4 Incubate samples using the following steps:

Temperature	Time
37°C	15 minutes
4°C	Hold

3.5 Clean up samples with SMRTbell cleanup or AMPure® PB beads depending on chosen size selection workflow.

SMRTbell cleanup bead steps

1. Add the appropriate volume of beads to each sample (e.g., 1X for WGS and 1.3X for amplicons).

2. Mix and bind for **10 minutes** at room temp.

3. Place samples on magnetic stand, allow beads to pellet, and remove supernatant.

4. Wash bead pellet with freshly prepared 80% EtOH. Repeat for a total of 2 washes.

5. Remove all residual EtOH.

6. Remove samples from magnet and immediately resuspend beads in the appropriate volume for the next step of the library prep.

7. Mix and leave at room temp for **5 minutes.**

8. Place samples back on magnet, allow beads to pellet, and transfer supernatant to new tube.

AMPure PB bead size selection steps

1. Prepare a **35%** (v/v) dilution of AMPure PB beads or use previously prepared dilution.

2. Add **3.1X** (v/v) of diluted beads to each sample.

3. Mix and leave at room temp for **20 minutes.**

4. Place samples on magnetic stand, allow beads to pellet, and remove supernatant.

5. Wash bead pellet with freshly prepared 80% EtOH. Repeat for a total of 2 washes.

6. Remove all residual EtOH.

7. Remove sample from magnet and immediately resuspend beads in **15 µL** of elution buffer.

8. Mix and leave at room temp. for **5 minutes**.

9. Place samples back on magnet, allow beads to pellet, and transfer supernatant to an Eppendorf DNA LoBind or PCR strip tube.

PN 102-443-000 REV01 MAY2022