Preparing Whole Genome Sequencing Libraries using SMRTbell® Prep Kit 3.0 Quick Reference Card

Additional Information

- Procedure & Checklist - Preparing Whole Genome Sequencing Libraries using SMRTbell Prep Kit 3.0
- PacBio Tech Support

Notes

- This Quick Reference Card requires gDNA inputs ranging from 0.3 µg – 5 µg
- SMRTbell libraries should be stored at 4°C for use within 7 days or -20°C for use after 7 days
- Refer to the Procedure and Checklist for additional protocol details

Required Materials
SMRTbell Prep Kit 3.0
SMRTbell Cleanup Beads
Low TE Buffer
TempAssure PCR 8-tube strips and compatible magnet
8-channel pipettes for processing multiple samples (200 µL and 20 µL)
Qubit and qubit reagents
8-tube PCR strip tube compatible thermal cycler

1. **Shear 1 µg** of gDNA on the Megaruptor 3 System at a shear speed of **31**.
   1.1. Sample should be in EDTA buffer with a volume of **100 – 140 µL** and a target concentration of **20 ng/µL** (acceptable range of 3 ng/µL – 36 ng/µL).
2. Perform bead purification using **SMRTbell Cleanup Beads**.
   2.1. **1.0x** volume SMRTbell Cleanup Beads.
   2.2. 10-minute incubation at room temperature.
   2.3. Wash **2x** with fresh **80% EtOH**.
   2.4. Resuspend in **47 µL** of **Low TE buffer** and elute by incubating for **5 minutes** at **room temperature**.
3. Using **1 µL** of sample, dilute and quant the DNA on the Qubit System and measure size on the Femto Pulse System.
4. Perform **Repair and A-tailing** by adding the following to 46 µL of sheared DNA:
   4.1. **8 µL** Repair buffer
   4.2. **4 µL** End Repair Mix
   4.3. **2 µL** DNA Repair Mix
5. Mix well by pipetting and incubate for **30 minutes** at **37°C**, **5 minutes** at **65°C** and **hold** at **4°C**.
6. Perform SMRTbell Adapter Ligation by adding the following reagents in this order to 60 µL of sample from step 5:
   6.1. **4 µL** SMRTbell Adapter
   6.2. **30 µL** Ligation Mix
   6.3. **1 µL** Ligation Enhancer
7. Mix well by pipetting and incubate for **30 minutes** at **20°C** and **hold** at **4°C**.
8. Perform bead purification using **SMRTbell Cleanup Beads**.
   8.1. **1x (95 µL)** volume SMRTbell Cleanup Beads.
   8.2. 10-minute incubation at room temperature.
   8.3. Wash **2x** with fresh **80% EtOH**.
   8.4. Resuspend in **40 µL** of EB and elute by incubating for **5 minutes** at **room temperature**.
9. Perform nuclease digestion of incomplete SMRTbell molecules by adding the following to 40 µL of library from step 8.4:
   9.1. **5 µL** Nuclease Buffer
   9.2. **5 µL** Nuclease Mix

10. **Mix** well by pipetting and incubate for **15 minutes** at **37°C** and **hold** at **4°C**.
11. Immediately proceed to AMPure® PB Beads Size-Selection.
    11.1. Make a **35%** dilution of AMPure PB beads by adding **1.75 mL** of resuspended beads to **3.25 mL** of Elution Buffer (can be stored at 4°C for 30 days).
    11.2. **3.1x (155 µL)** diluted beads from step 11.1.
    11.3. 20-minute incubation at room temperature.
    11.4. Wash **2x** with fresh **80% EtOH**.
    11.5. Resuspend in **15 µL** of EB and elute by incubating for **5 minutes** at **room temperature**.
12. Using **1 µL** of sample, dilute and quant the DNA on the Qubit System and measure size on the Femto Pulse System.
13. Samples can be stored at **4°C** for short term storage or **-20°C** for long term storage. When
14. ready, proceed to the Sample Setup module in SMRT® Link to prepare SMRTbell libraries for sequencing.

Revision History (Description)	Version	Date
Initial release.	01	Nov 2021