

Technical note

COVARIS g-TUBE DNA SHEARING FOR SMRTBELL® PREP KIT 3.0

Introduction

PacBio® recommends that DNA shearing for whole genome sequencing be performed on the Diagenode Megaruptor 3 system, which is the only system validated by PacBio for shearing, in order to produce consistent HiFi yield and quality.¹ When a Megaruptor 3 system is unavailable, the Covaris g-TUBE presents an alternative shearing method that does not require instrumentation beyond a standard microcentrifuge.

Shearing system specifications

	g-TUBE	Megaruptor 3 system
Sheared fragment size	6–20 kb	5–100 kb
Input volume	150 µL	65–500 µL
Maximum input DNA	30 µg	75 µg
Throughput	1 sample per tube	1–8 samples in parallel
Price per sample	~\$30 USD	~\$20 USD
Validated by PacBio for WGS		✓

Required materials and equipment

Material	Part number
g-TUBE	Covaris 520079
Microcentrifuge	Eppendorf 5424 R
0.2 mL 8-tube strips	USA Scientific TempAssure 1402-4708

Protocol

- Bring up input DNA volume to a final volume of 150 µL with Low TE buffer.
- Transfer the gDNA sample to a g-TUBE and shear by spinning in an Eppendorf 5424R centrifuge at the respective speed for the desired fragment length. For other fragment lengths, see the manufacturer's instructions.
- Check for any residual sample remaining in the upper chamber of the g-TUBE. If present, re-spin for 1 minute. Repeat spin until the entire gDNA sample has passed through the g-TUBE orifice.
- Invert and spin the g-TUBE at the same speed selected in step 2 until the entire gDNA sample has passed through the g-TUBE orifice.
- Repeat step 4 two times for a total of *four* passes through the g-TUBE orifice.
- Transfer the recovered sheared DNA to a new 0.2 mL 8-tube strip. Up to 10% volume loss is typical.
- Proceed to the post-shearing SMRTbell bead cleanup in *Procedure & checklist – Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0*.

Genome	Shear speed	Insert length
Large (e.g., human)	2,164 × g (4,800 RPM)	15–18 kb
Small (e.g., microbe)	3,287 × g (7,000 RPM)	7–10 kb

KEY REFERENCES

- Procedure & checklist – Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0 (102-166-600)**