Enzymatic Fragmentation of Input DNA

Mechanical fragmentation by sonication is widely recognized as the best practice for consistently producing fragments with a narrow size distribution. However, DNA fragmented by enzymatic means is also suitable for processing with the Accel-NGS® 2S DNA Library Kit. This Technical Note describes modifications to the standard Accel-NGS 2S methods and expected results when working with enzymatically-fragmented DNA. We recommend NEBNext® dsDNA Fragmentase® for enzymatic fragmentation of input DNA.

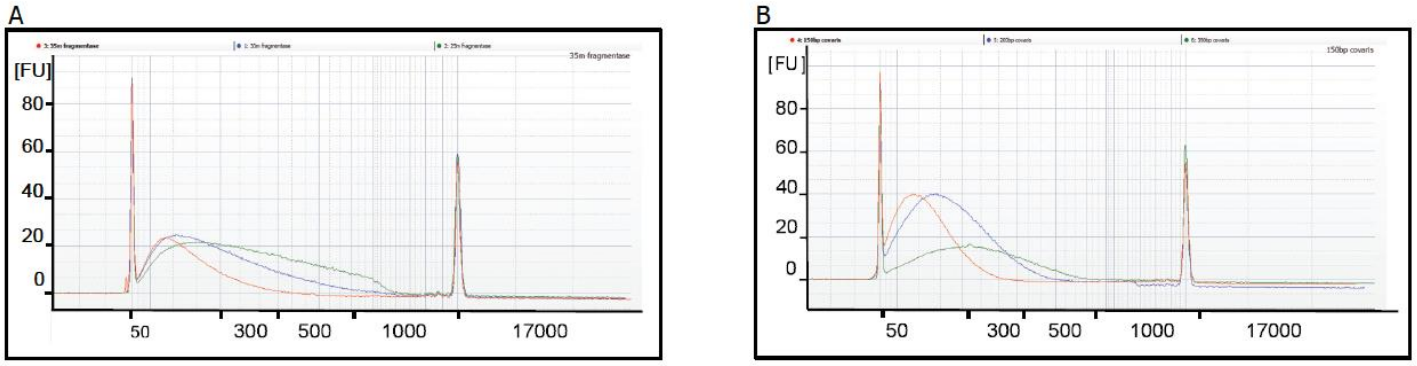

Figure 1: *High quality genomic DNA was fragmented by (A) NEBNext dsDNA Fragmentase for 25 minutes, 30 minutes or 35 minutes or (B) a Covaris® ultrasonicator with fragmentation settings of 350 bp, 200 bp and 150 bp. Fragmentase incubation times may need to be optimized for your specific sample type.*

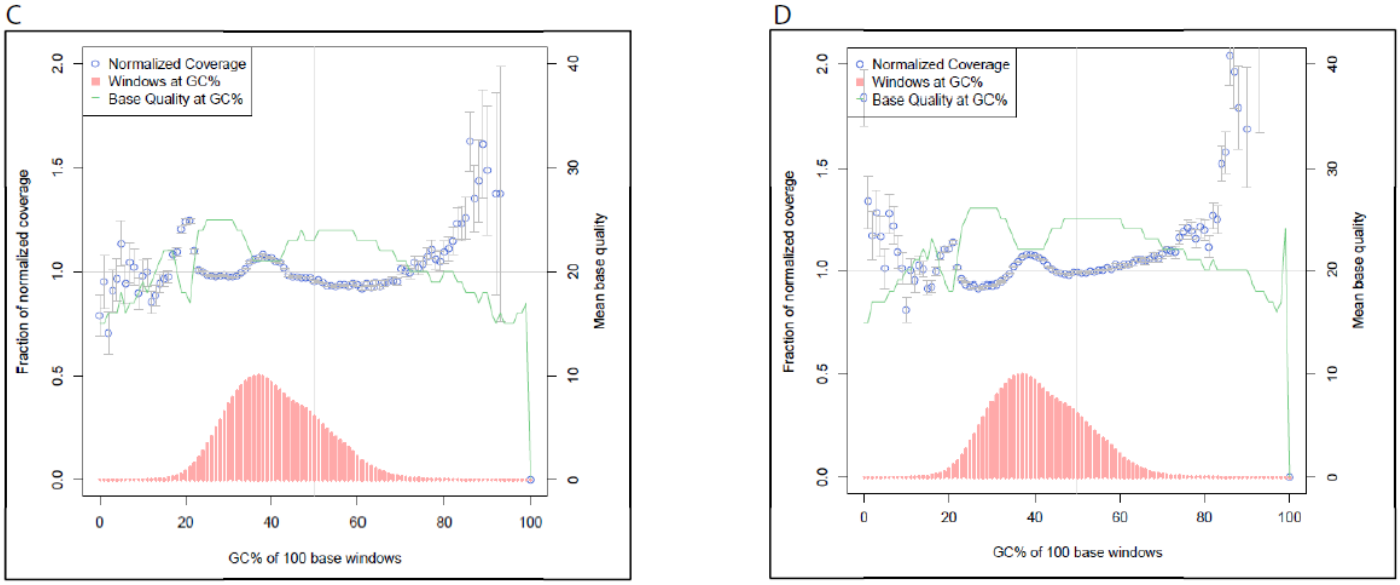

Figure 2: *Input DNA fragmented to 200 bp with (C) NEBNext dsDNA Fragmentase or (D) a Covaris ultrasonicator was converted into library with the Accel-NGS 2S DNA Library Kit and sequenced using Illumina® MiSeq® v2 reagents.*

Conclusion: There was no significant difference in base composition of libraries produced using mechanical fragmentation or enzymatic fragmentation.

Prior to starting the Accel-NGS 2S protocol, we recommend quantifying your DNA sample following the post-Fragmentase clean-up, as this clean-up step may result in loss of sample. Additionally, enzymatic fragmentation can result in broader fragment size distribution, as shown in the Bioanalyzer traces above. Accordingly, you may wish to perform size selection on this broad size distribution by a 2-sided SPRI™ (see next page), which could result in the elimination of more fragments. Therefore, larger input quantities may be required. In our hands, library yields from input DNA fragmented by

Fragmentase were decreased by approximately 2-fold (prior to PCR) compared to libraries using mechanically fragmented DNA.

2-Sided SPRI Recommendations

For input DNA fragmented with Fragmentase, follow the standard Accel-NGS 2S library prep methods for SPRI Steps 1 through 3, but substitute the following for SPRI Step 4. This clean-up protocol has been validated for 100 ng of input DNA (optimization may be required for other input quantities).

1. Invert or briefly vortex beads to homogenize the suspension before use.
2. Transfer each 50 µl sample to a 1.5 ml tube and add Right Side Selection Volume (see Table 1) beads to each sample and mix well.
3. Incubate the samples for 5 minutes at room temperature.
4. Pulse-spin the samples in a microfuge. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (≈ 5 minutes).
5. Transfer the clear supernatant, which contains the Right Side Size Selected sample to a new 1.5 ml tube. Discard the remaining beads.
6. Add Left Side Selection Volume (see Table 1) beads to each sample and mix well.
7. Incubate the samples for 5 minutes at room temperature.
8. Pulse-spin the samples in a microfuge. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (≈ 5 minutes).
9. Remove and discard the clear supernatant without disturbing the pellet (5-10 µl may be left behind).
10. Add 500 µl of freshly prepared 80% ethanol to the pellet while it is still on the magnet. Use care not to disturb the pellet. Incubate for 30 seconds, and then carefully remove the ethanol solution.
11. Repeat step 10 once for a second wash with the ethanol solution.
12. Pulse-spin the samples in a microfuge, place back onto the magnet and remove any residual ethanol solution from bottom of the tube.
13. Air-dry the pellet, watching the pellet to avoid cracking or over-drying.
14. Add 20 µl of Low EDTA TE to resuspend the pellet, mixing well by pipetting up and down until homogenous. If droplets of the resuspension are on the side of the tube, pulse-spin the tube in a microfuge to collect contents. After at least 2 minutes, place the tube on the magnet. Ensure that eluate does not contain magnetic beads (indicated by brown coloration in eluate). If magnetic beads are present, pipette eluate into a new tube, place on magnet and transfer eluate again.

Table 1: SPRI Bead Volumes

Insert Size	150 bp	200 bp	350 bp
Right Side Selection Volume	33 µl	28 µl	20 µl
Left Side Selection Volume	13 µl	13 µl	10 µl

For more information on SPRIselect™ size selection, please see Beckman Coulter's SPRIselect User Guide (B24965AA, October 2012).

Swift Biosciences, Inc.
58 Parkland Plaza, Suite 100 • Ann Arbor, MI 48103 • 734.330.2568 • www.swiftbiosci.com