PCR-Free Libraries from 10 ng Input with the Accel-NGS® 2S PCR-Free DNA Library Kit

Modifications to the Accel-NGS 2S PCR-free workflow may be made to produce PCR-free libraries from as little as 10 ng DNA input. For example, the following sequencing applications and needs may benefit from these modifications:

- Low-pass whole genome sequencing (such as cell-free DNA for copy number variation analysis)
- Small genome sequencing (such as microbial)
- When maximizing uniquely-mapped reads and eliminating PCR duplicates is desired
- When maximizing coverage of AT-/GC-rich regions whose coverage is reduced by PCR is desired

The Accel-NGS 2S PCR-free standard protocol supports 100 ng of input gDNA for producing high quality PCR-free libraries. By preparing and sequencing multiple libraries simultaneously, PCR-free data can be obtained from inputs down to 10 ng per library. This Technical Note describes modifications to the standard Accel-NGS 2S PCR-free protocol and expected results when preparing PCR-free libraries from 10-100 ng of DNA. Some sample types may enable a lower input of 5 ng input DNA (see below).

Requirements for Preparing PCR-Free Libraries from Low Input

- Each sample must be prepared from the same input quantity in order to normalize the libraries and achieve even representation of each library in the sequencing results.
- The multiple libraries must be pooled following the Ligation II incubation and co-sequenced. The table below the workflow diagram provides guidelines for the number of libraries that must be prepared depending on the input amount, as well as the expected distribution of sequencing reads.

Input DNA (200 bp)	Approx. Yield per Library (in combined 20 µl final eluate)	Min. No. of Libraries Required for Co-Sequencing as PCR-free Preps	Combined Library Yield for Sequencing (in combined 20 µl final eluate)	Approx. No. of Reads per Library (sequenced together on HiSeq® 2500 in Rapid Run Mode)	Indexing Kit Options
100 ng	2.0 nM	1	≥ 2 nM	600 M	SI-ILM2S-48A or SI-ILM2S-48B
50 ng	1.0 nM	2	≥ 2 nM	300 M	
25 ng	0.5 nM	4	≥ 2 nM	150 M	
10 ng	0.2 nM	10	≥ 2 nM	60 M	
5 ng	0.1 nM	20	≥ 2 nM	30 M	SI-ILM2S-96

Low Input PCR-Free Library Preparation

To prepare PCR-free libraries from low input samples, process each sample individually and follow the directions in the Accel-NGS 2S PCR-free Instruction Manual until you reach the Ligation II step.

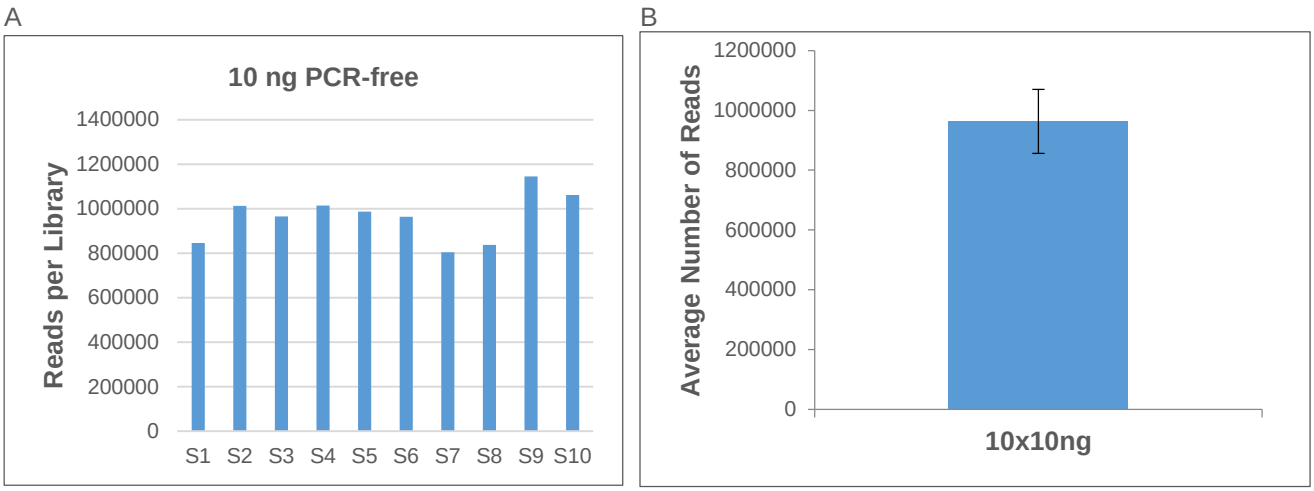
1. After the Ligation II incubation, add the specified PEG NaCl volume to each tube.
2. Pool all the reactions together to make a single SPRI incubation.
   Note: this may require a larger tube (e.g., use a 1.5 ml tube for four 25 ng libraries or ten 10 ng libraries; use a 2 ml tube for twenty 5 ng libraries, etc.). Mix by pipetting 10 times.

3. Incubate the samples for 5 minutes at room temperature.
4. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (~5 minutes).
5. Remove and discard the supernatant without disturbing the pellet. It is acceptable to leave 10 µl behind as this will be removed during the wash steps.
6. Add 200 µl (if working in a 0.2 ml tube) or 500 µl (if in a 1.5 or 2 ml tube) of freshly prepared 80% ethanol solution 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.
7. Repeat step 6 once for a second wash with the ethanol solution.
8. Pulse-spin the samples in a microfuge, place back onto the magnet and remove any residual ethanol solution from the bottom of the tube.
9. Air-dry the pellet, watching the pellet to avoid cracking or over-drying.
10. Add 20 µl of Low EDTA TE to resuspend the pellet, mixing well by pipetting up and down until homogenous. After at least 2 minutes, place the tube on the magnet for 3 minutes. Transfer the entire eluate to a new tube.

NOTE: Store freshly prepared libraries at 4 °C (or long term at -20 °C). The libraries are now ready for quantification. PCR-free libraries cannot be accurately quantified or assessed for library size on the Bioanalyzer (see Appendix in the Accel-NGS 2S PCR-free Instruction Manual).

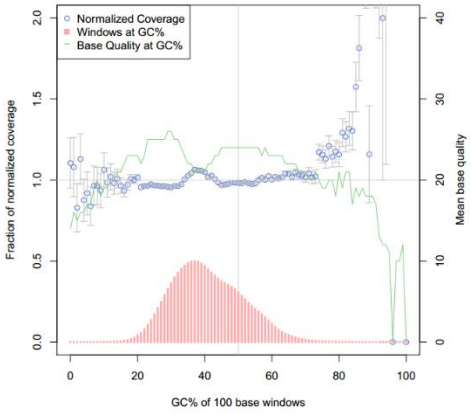
Expected Results

Figure 1: Even Distribution of Reads Across Multiplexed Low Input PCR-free Libraries



Ten uniquely-indexed PCR-free libraries were prepared from 10 ng each of Coriell HapMap NA12878 DNA, using the modified version of the Accel-NGS 2S PCR-free Library Kit presented in this Technical Note and sequenced using Illumina® MiSeq® v2 reagents. The number of reads per library obtained individually (A) and when averaged together (B, error bar representing standard deviation) showed even distribution of reads across the multiplexed low input PCR-free libraries.

Figure 2: Consistent Base Composition Coverage



Evenness of coverage across varying AT/GC composition sequences, a characteristic of Accel-NGS 2S PCR-free libraries, is preserved when using the modified version of the Accel-NGS 2S PCR-free Library Kit presented in this Technical Note with Coriell HapMap NA12878 DNA. The normalized coverage plot showed even coverage across the genome for a 10 ng PCR-free library that was co-sequenced with nine other libraries. Sequencing was performed using Illumina MiSeq v2 reagents and data was analyzed using Bowtie2 and Picard CollectGcBiasMetrics (picard.sourceforge.net).

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