

Amplicon Sequencing with Accel-NGS® 2S DNA Library Kits

Prior to Using the Library Prep Kit

1. A magnetic bead-based clean-up (SPRIselect™ or AMPure® XP) is required to remove primer dimers and other sample contaminants. See table below and Bioanalyzer traces on the next page to determine the magnetic bead ratio appropriate for your amplicon size. For a mixture of amplicons of differing lengths, the magnetic bead ratio should accommodate the smallest amplicon, while still excluding smaller primer dimers.
2. Quantify your sample using Qubit® or NanoDrop®. For inputs ≥ 100 ng, library amplification by PCR is optional. For inputs < 100 ng, library amplification by PCR is required. See the Accel-NGS 2S Instruction Manuals for the recommended number of PCR cycles.
3. For large amplicons exceeding sequencing read length, you may wish to perform fragmentation to obtain sequence reads from interior regions of the amplicon. Follow the standard Accel-NGS 2S Plus and 2S PCR-free protocols for amplicons fragmented to 200 bp or 350 bp.

For Unfragmented Amplicons

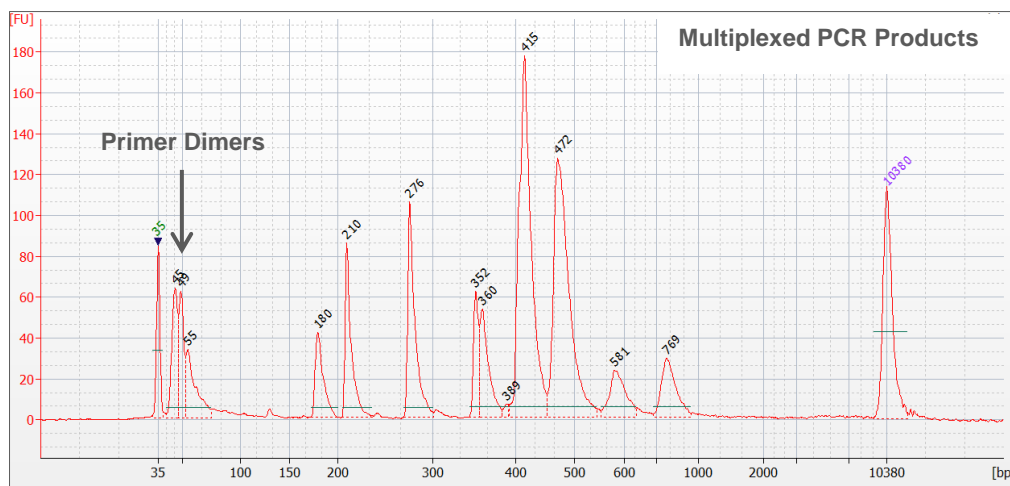
- Omit Repair I step.
- Repair II step is not required, but can increase library yield up to 2-fold. If it is performed, use the table below to determine the magnetic bead ratio for the Post-Repair II SPRI™.
- Refer to the standard Accel-NGS 2S protocols to determine the amount of PEG NaCl solution appropriate for your amplicons for the Post-Ligation I SPRI, Post-Ligation II SPRI, and the Post-PCR SPRI steps. For smaller amplicons, contact Technical Support at TechSupport@swiftbiosci.com or by calling 734.330.2568 and pressing 2 when prompted.

Amplicon Size Retained	Magnetic Bead Ratio
≥ 200 bp	1.5X
≥ 350 bp	1.0X

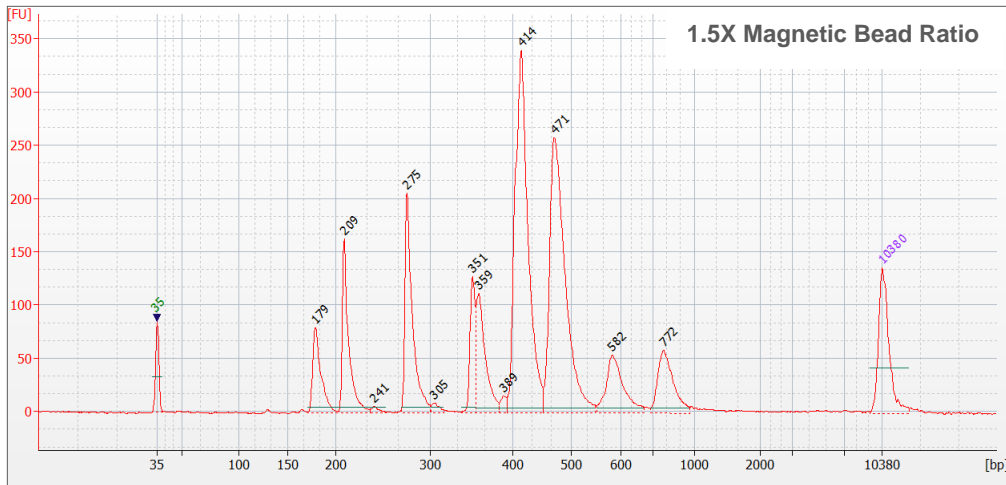
Bioanalyzer Traces

Bioanalyzer traces from a sample containing amplicons ranging in size from approximately 200 bp to 800 bp.

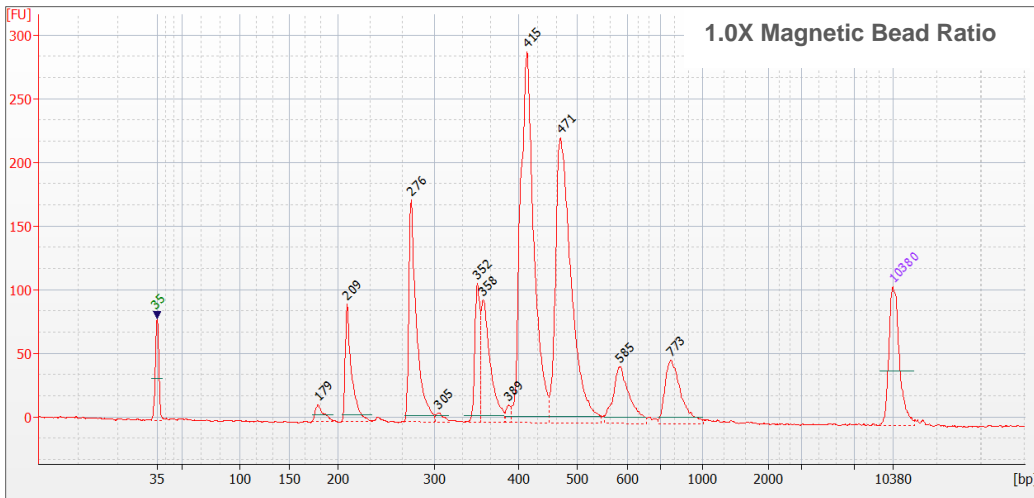
- A. Prior to clean-up, multiplexed PCR products contain small primer dimers.



- B. A clean-up with a magnetic bead ratio of 1.5X excludes small primer dimers, but includes amplicons ≥ 200 bp in length.



- C. A clean-up with a magnetic bead ratio of 1.0X excludes small primer dimers, but includes amplicons ≥ 350 bp in length.



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