# Improved Chemistries for NGS Library Cleanup and Size Selection

**Charles Cowles, PhD, Douglas Horejsh, PhD, Mark Denhart, Curtis Knox, Adam Blatter, Marjeta Urh, PhD**
**Promega Corporation, 2800 Woods Hollow Rd. Madison, WI**

## 1. Introduction

Next Generation Sequencing (NGS) libraries require high quality nucleic acid inputs of varying quantities, concentration, and size depending on the library preparation methods and sequencing platforms used. Regardless of these variations, in most instances a magnetic bead-based chemistry is utilized as a portion of the overall protocol. The steps using magnetic bead chemistries fall into two categories of function:

1. Sample cleanup: Removes sequencing adaptors or PCR primers, dNTP's, enzymes or unwanted buffer formulations.

2. Size Selection: Removes unwanted nucleic acid fragment or library molecules that are above or below a specified size range optimal for the downstream sequencing platform.

By varying the ratio of bead chemistry added to the original volume of DNA in solution, the user can alter the size of DNA captured by the beads or left behind in solution.

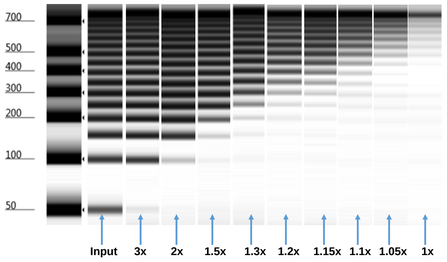



Fig. 1: Gel images demonstrating retention of varying sizes of DNA based on altering the ratio of bead volumes added to original sample volumes. Ratios and size retention shown are the result of sample cleanup of a 50bp ladder with decreasing ratios of Promega's ProNex® Size-Selective Purification System.[1]

## 2. Areas for Concern with Current Methods and Goals for New Product Improvements:

Traditionally, both functions described above involve the use of a magnetic bead with a carboxylated polymer-coated surface combined with a buffer containing a crowding agent (PEG) and salt. These are commercially known as AMPure® XP or SPRISelect® beads. Although AMPure XP beads and similar products are used extensively in NGS library prep methods, there are several areas where improvements could be made in the overall performance. These include:

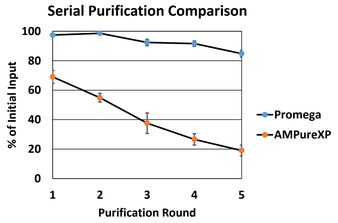
- Significant loss of up to 50% of DNA with each wash or size selection step
- Poor reproducibility between samples for both recovery % and size selection
- High viscosity of the chemistry, leading to difficulty in accurate pipetting and automation
- Retention of high molecular weight above the desired size range of the user

In order to improve overall performance in NGS library preparation, **Promega developed a new chemistry solution with the following goal**s:

- **Increased DNA recovery**, with an average of 80%+ DNA above target cutoff size retained after each wash or size selection step
- **Reduced viscosity and faster magnetic response** of the chemistry, providing more accurate pipetting and better reproducibility
- **More accurate size cutoffs**, with less high molecular weight DNA retained outside of desired range
- **Compatibility with commonly used NGS kits and workflows and improved performance relative to existing technologies**

## 3. Performance Improvements

Fig. 2: Serial buffer exchange/cleanup purification comparison between ProNex® Size-Selective Purification System and AMPure XP beads. Five sequential cleanup rounds (AMPure XP = 1.8x ratio, ProNex = 3x ratio) were performed on a 200bp DNA Step ladder following the manufacturers' instructions. Ratios designed to retain all fragments. N=8 replicates per sample.



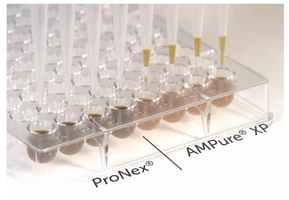
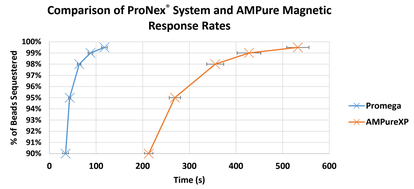




Fig 3: Comparison of retention in tips between chemistries due to viscosity of the solutions. An 8-channel pipette was used to simultaneously manually pipette both ProNex® System and AMPure XP products for one aspirate/dispense cycle of the chemistries. The volume of chemistry retained in tips is variable and noticeably higher for AMPure XP chemistry.

Fig 4: Magnetic response time comparison between Promega and AMPure XP. Promega chemistry clears approximately 5 times faster than AMPure XP, allowing quicker overall protocols for purification.



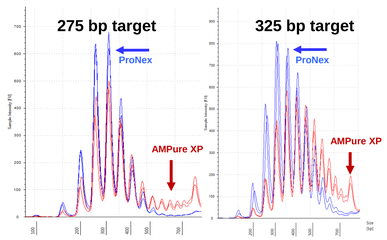
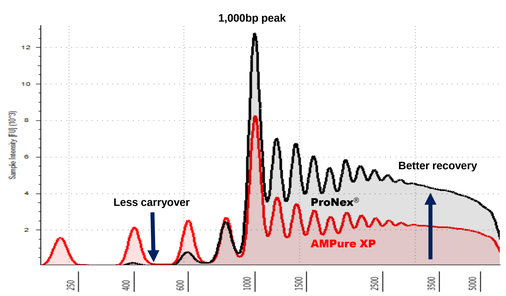




Fig 5: Comparison of ProNex® Chemistry vs. AMPure XP chemistries for size selection. A 50bp ladder was size-selected for two different library target sizes (275 and 325bp).

- Blue peaks = ProNex® chemistry
- Red peaks = AMPure XP
- Blue arrows indicate the higher recovery using Promega chemistry
- Red arrows indicate high molecular weight DNA retained by AMPure XP but removed by ProNex.

Fig. 6: Size-selective cleanup of a 200bp DNA step ladder, targeting retention of DNA >1kb and elimination of DNA <1kb. Promega ProNex® Size-Selective Purification System (black) was compared to AMPure XP beads (red). AMPure had greater carryover of the undesired small fragments, while ProNex reduced carryover of <1kb fragments and retained more of the desired high molecular weight DNA.



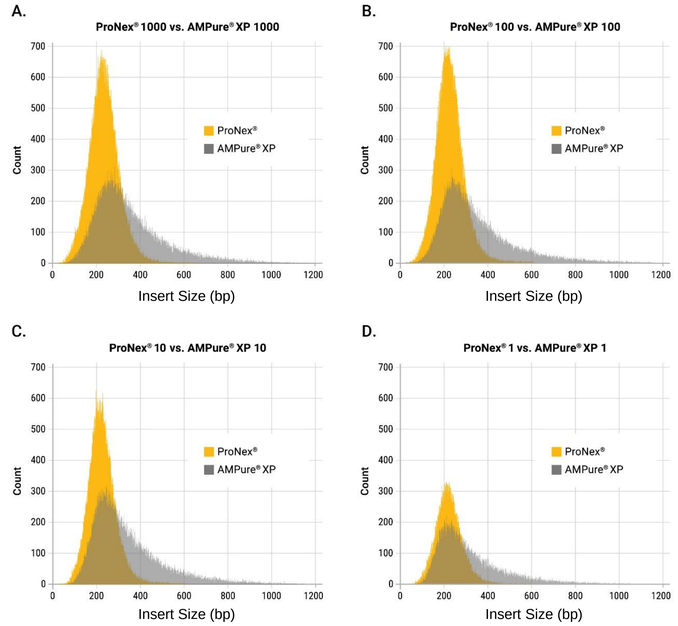




Fig 7: Comparison of the distribution of sequenced NGS library insert sizes. n=3 libraries were prepared with either ProNex® chemistry or AMPureXP using 1000 ng (panel A), 100 ng (panel B), 10 ng (panel C), or 1 ng (panel D) of input gDNA using the NEB Next Ultra II Kit for Illumina following the NGS Kit manufacturer's instructions for a total of 24 libraries. The ProNex-purified libraries show much sharper insert size distributions with peaks centered between 200-300bp, whereas the AMPureXP libraries have much broader distributions with significant amounts of contaminating high molecular weight (>450bp) DNA present.

Having higher final library yields and tighter insert size distributions is expected to enable deeper discovery of rare sequences and more complete NGS library sequencing results.

## 4. Conclusions

- Promega has developed a new chemistry for NGS library cleanup and size selection with significant improvements in performance:
  - Increased final library yields and reduced variance in total library obtained
  - Substantially reduced solution viscosity compared to standard products, resulting in faster bead response time, easier pipetting, and more reproducible results.
  - Enhanced size-selective sharpness and yield, with more complete removal of high molecular weight DNA from final libraries

[1] **For Research Use Only. Not for use in diagnostic procedures.**

November, 2017



Corresponding author: curtis.knox@promega.com