ABSTRACT
With the advent of more affordable “personal” next generation sequencing instruments from major platform providers, NGS applications are moving outside of core sequencing labs to enable a variety of projects. As the number of samples for sequencing increases, there is a need for library prep automation that matches throughput of the instruments while not requiring an investment equal to the sequencer itself. We are developing a combination of new NGS sample prep chemistry and a small automated platform that reduces time to final prepared library and increases efficiency and consistency. The DNA sample prep chemistry combines typical end-repair and A-tailing steps into one master mix step with buffers directly compatible with downstream ligation steps, eliminating the need for multiple cleanup steps throughout the process. The chemistry has also been optimized to drive higher A-tailing efficiencies, which reduces chimera formation from blunt fragments and loss of fragments tagged with nucleotides other than the necessary “A”. These chemistries were designed to be easily automatable, and subsequent development led to the design of a compact liquid handling platform to perform these tasks. The instrument described in this presentation allows numerous DNA libraries to be prepared simultaneously, including incorporation of barcoded adapters for multiplex PCR and sequencing. In contrast to other small, dedicated systems, the instrument is also open and programmable, meaning users can choose to utilize the platform for other applications. In this poster, we will discuss the feasibility of automating the chemistry on the instrument and the utility of an inexpensive NGS sample prep system for laboratories with a range of sample throughput needs.

NxSeq® Sample Prep Technology
The NxSeq DNA Sample Prep kit was developed to:
• Reduce time to complete NGS sample prep.
• Improve A-tailing and downstream ligation of fragments to adapters.

An optimized master mix of enzymes and buffers enables end-repair and A-tailing of DNA fragments in one tube without the need for buffer exchange or sample cleanup. The result is a manual system for NGS sample prep from sheared DNA that cuts traditional workflow by up to 50% (2 hours) from the most commonly used method. In addition, hands-on time is reduced by up to 75% (see figure 1).

NxSeq® DNA Prep Kit Validation
Previously, we validated the performance of the NxSeq DNA Sample Prep kits by shearing DH10B E. coli genomic DNA, and preparing identical samples with the TrueSeq® Sample prep kit v2, The Roche 454 Sample prep kit, and the NEBNext™ DNA Sample Prep Master Mix Set 1. All samples were verified for target size range on an Agilent Bioanalyzer and then run on an Illumina Genome Analyzer fx using barcoded adapters (see figures 2 and 3).

Fig. 2. Bioanalyzer trace comparison of DH10B Genomic DNA libraries made with NxSeq, NEB, and Roche prep kits. Equivalent performance was obtained for all kits.

Fig. 3. Coverage comparison of DH10B Genomic DNA libraries. Plots indicate depth of coverage achieved in a moving 100 base read window. Equivalent performance was obtained for all kits.

Table 1: Capacities of the NxSeq/PIPETMAX Method

<table>
<thead>
<tr>
<th>Capacity</th>
<th>Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to run 8 samples</td>
<td>~3 hours</td>
</tr>
<tr>
<td>Samples per run – single</td>
<td>1-96</td>
</tr>
<tr>
<td>adapter</td>
<td></td>
</tr>
<tr>
<td>Manual intervention steps</td>
<td>1</td>
</tr>
</tbody>
</table>

Automated System Optimization
To optimize performance of the automated method, we will prepare NGS samples from DH10B genomic DNA using NxSeq chemistry both manually and on the PIPETMAX.

For the manual preparation, all steps will be performed as described in the product manual. For the automated version, all reagents will be placed on the deck of the platform, with the method running without interruption except for manually moving a single plate to and from a thermal cycler for the heated incubation steps. Both ligation to a barcoded adapter, and size selection (200-500 bp target range) will be performed on-deck with size selection using a modified AMPure XP bead protocol.

Optimization experiments will include:
• Range of wash drying times to facilitate removal of alcohol.
• Pipetting speeds and volumes to eliminate bead carryover.
• Minimizing time to final sample and tip usage.

Conclusions
The PIPETMAX instrument, combined with NxSeq DNA Prep Kit chemistry, is capable of preparing DNA libraries for NGS applications with selectable size ranges specific to platform needs. Optimization experiments are ongoing and will result in a system that will be suitable for hands-free, barcoded library preparation on a compact, cost effective, liquid handling platform.

We anticipate the fully validated method to be available through Gilson as part of the PIPETMAX package in Spring, 2013.