Automation of NuGEN Ovation Ultralow Library Systems on an Agilent NGS Bravo

Application Note

Authors

Lisa Knapp

Agilent Technologies, Inc.

1362 Kirklees Dr

Carmel, IN 46032

Lin Z Pham

NuGEN Technologies, Inc.

201 Industrial Road, Suite 310

San Carlos, CA 94070

Introduction

With the reduction in cost and increase in throughput, genetic sequencing is going beyond traditional total genome sequencing to multiple new and exciting life science applications in basic science and clinical diagnostics. As such, laboratories have to face the challenge of consistently running a large number of samples through the entire NGS process, starting with sample preparation. For large volume studies, automation of the next-generation sequencing (NGS) sample preparation process including DNA/RNA isolation, cDNA preparation, library construction, multiple purification, quality control steps, and potentially target enrichment steps, offers significant advantages in terms of manual labor reduction and elimination of operator induced errors and variability. Agilent NGS automation solutions streamline sample preparation for a variety of applications to provide excellent reliability and reproducibility. The solution consists of the industry's most compact and versatile Bravo Automated Liquid Handling Platform (Figure 1), predeveloped protocols and optional solutions for labware management.

The Ovation Ultralow Library Systems from NuGEN Technologies provide a simple, fast and scalable solution for producing libraries that can be used in a broad range of NGS applications starting with 1 to 100 ng of DNA. The methods used for fragmentation and adaptor ligation provide for low bias libraries suitable for RNA-Seq, Digital Gene Expression (DGE), genomic DNA sequencing, target capture, amplicon sequencing, ChIP-Seq, and more.

Agilent Technologies

As shown in Figure 2, the streamlined workflow consists of four main steps:

1. Fragmentation of either genomic DNA or double-stranded cDNA
2. End repair to generate blunt ends
3. Adaptor ligation for multiplexing or no multiplexing
4. PCR amplification to produce the final library

The entire workflow, including fragmentation, can be completed in as little as four hours and yields DNA libraries ready for cluster formation and either single read or paired-end sequencing.

Figure 1. Agilent Bravo platform for NGS.

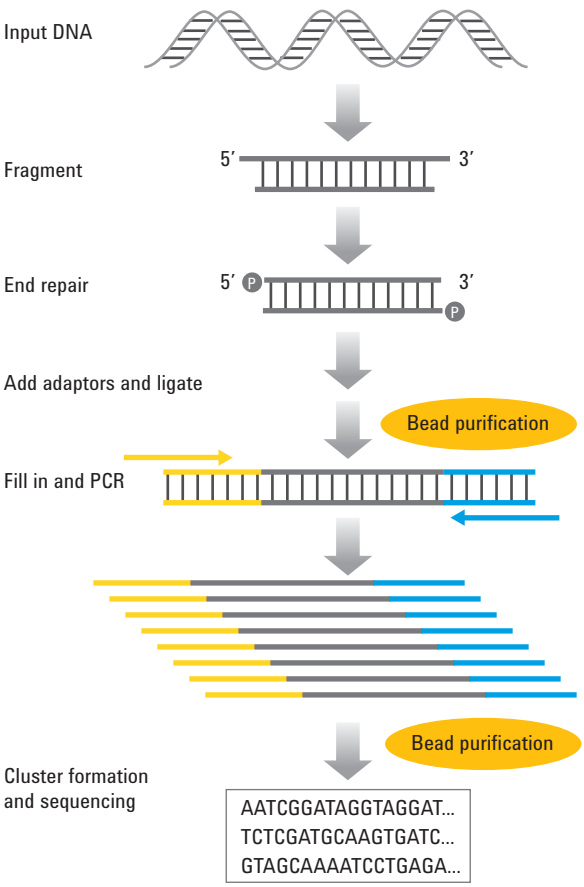

Figure 2. The Ovation Ultralow Library Systems Workflow.

Automated protocols are now available for the NuGEN Ovation Ultralow Library Systems on Agilent NGS Bravo (part number G5541A). An easy to use graphic user interface (Figure 3) enables a quick scale up of the protocols for any lab.

Experimental Setup

The NuGEN Ovation Ultralow Library Systems form-based protocols provide an interactive, visual layout for the end user. The underlying VWorks protocols have been designed to allow laboratory personnel to use the same protocol to run one to 12 columns of samples. Library construction for 96 samples takes approximately 4 hours. Each protocol has a unique deck layout which is shown in Figure 3. The labware used in the protocol was selected for optimal performance while being cognizant of minimizing dead volumes when reagent conservation is critical, and ease of setup for those reagents where cost is negligible. The Current Tip State selection allows partial boxes of tips to be used to minimize tip waste.

Links are provided to a spreadsheet which calculates volumes for all master mixes (Figure 4) and has space provided to enter lot number information or any other information which may be required for documentation within the lab.

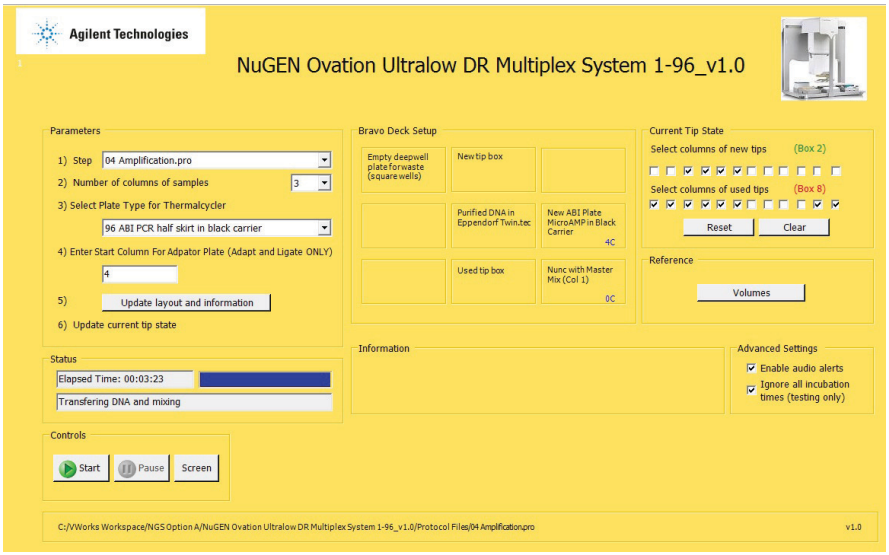

Figure 3. VWorks protocol form.

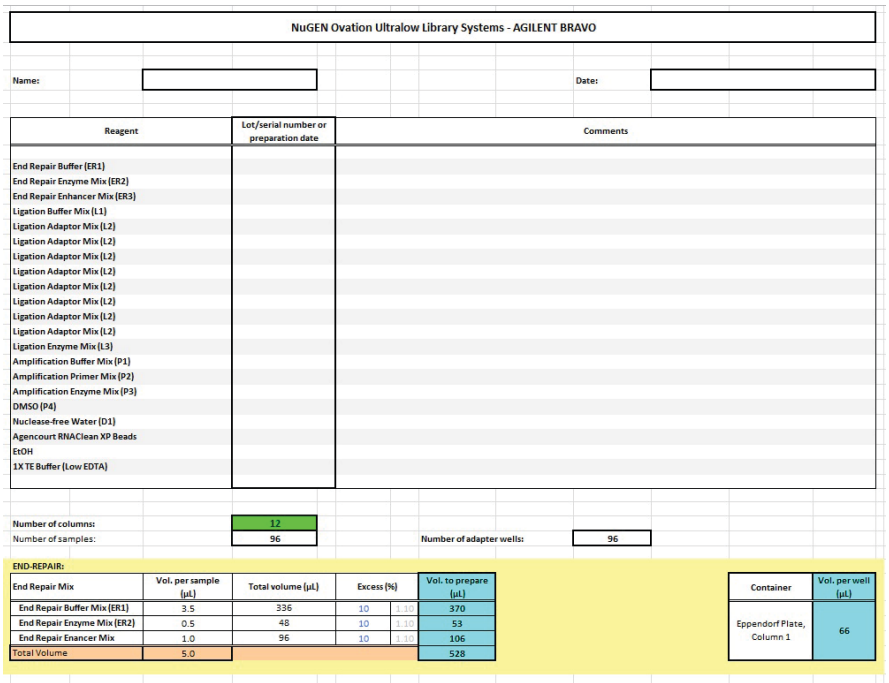

Figure 4. Volume tables and data sheet.

Results and Discussion

Multiple sequencing libraries were constructed using Ovation Ultralow Library System on a NGS Bravo with 1.0 ng of sheared genomic DNA from *E. Coli* (GC: 51 %), *Staphylococcus aureus* (low GC: 33 %), and *Rhodobacter sphaeroides* (high GC: 69 %), respectively. Triplicate sequencing libraries for each bacteria were sequenced on an Illumina sequencer. The distribution of reads from each sample is plotted to determine the depth of coverage across the genomes. Sequencing data from manual library prep were also plotted for comparison. Figures 5A, 5B, and 5C show the coverage plots for *E. coli*, *Staphylococcus aureus*, and *Rhodobacter sphaeroides*, respectively. The grey track represents the theoretical distribution, and the other tracks are from the experimental data generated from either the Bravo library prep or the manual prep.

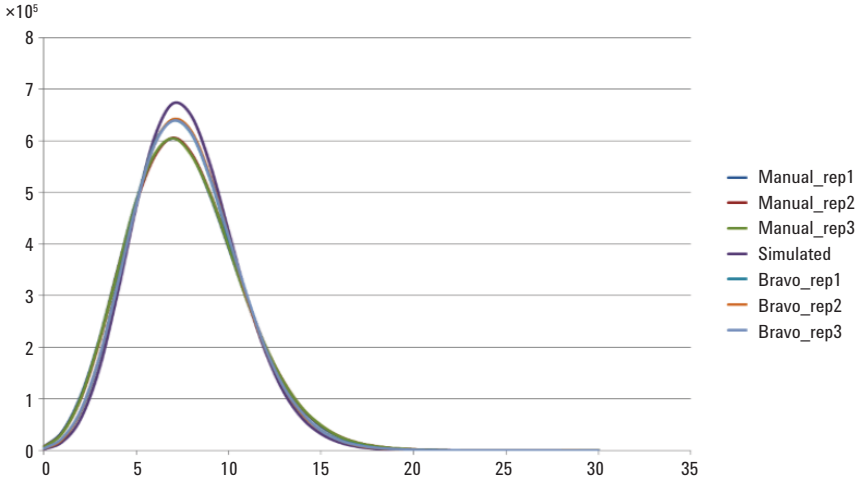

Figure 5A. Coverage plot from sequencing libraries with 1 ng of *E coli* genomic DNA.

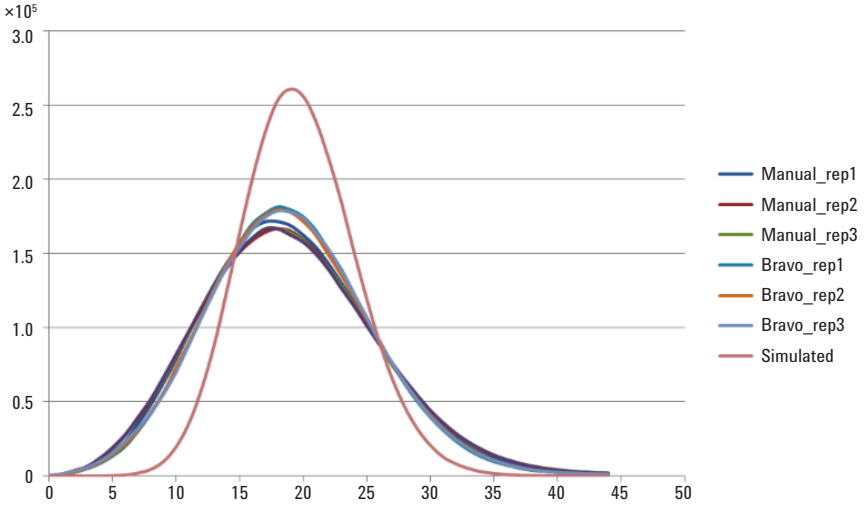

Figure 5B. Coverage plot from sequencing libraries with 1 ng of *Staphylococcus aureus* genomic DNA.

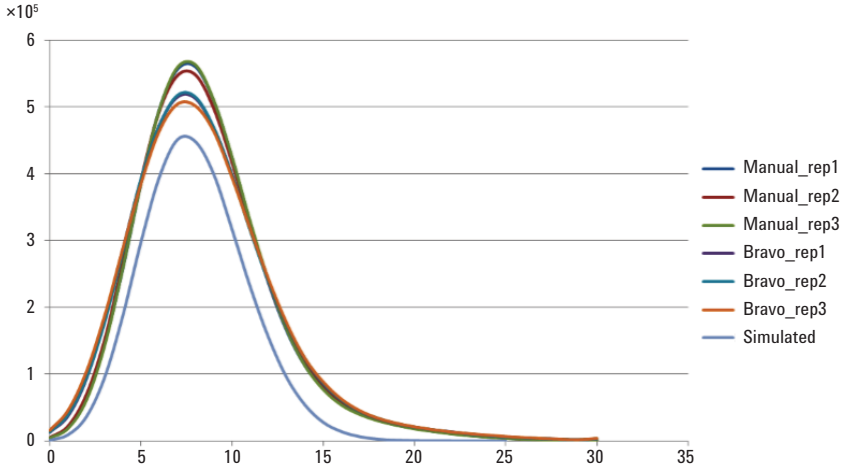

Figure 5C. Coverage plot from sequencing libraries with 1 ng of *Rhodobacter sphaeroides* genomic DNA.

Shown in Table 1, minimal bias was observed with microbial genomes having extreme GC content, 33 % (*S. aureus*), and 69 % (*R. sphaeroides*), as well as *E. coli* genome (~51 % GC) from libraries made both manually or on the NGS Bravo. The GC content observed by sequencing matches closely with the known GC content of each genome, and no significant difference was observed between the manual and Barvo library prep.

Taken together, these results demonstrate Ovation Ultralow Library Systems generate high complexity libraries with no significant coverage or GC bias in a reproducible fashion on Agilent NGS Bravo.

Table 1. Sequencing alignment metrics with a range of GC content.

Species	Library prep method	Total reads	Aligned reads (%)	Known GC content (%)	Observed GC content (%)
E. coli	Manual	1,000,000	98.8	50.8	50.6
E. coli	Bravo	1,000,000	99.4	50.8	50.4
S. aureus	Manual	1,000,000	99.2	32.8	36.4
S. aureus	Bravo	1,000,000	99.3	32.8	35.1
R. Sphaeroides	Manual	1,500,000	99.0	68.5	67.5
R. Sphaeroides	Bravo	1,500,000	99.1	68.5	67.1

Conclusion

Automated protocols of Ovation Ultralow DR Multiplex Systems generated similar technical performance as compared to the manual process. With automation and NuGEN barcode designs, up to 96 samples can be processed simultaneously. This is a marked improvement in throughput over manual method without comprising the integrity of the experiment. The Ovation Ultralow DR Mutiplex System is also available from NuGEN in a 96 reaction size (part number 0329-96)

www.agilent.com/chem

Published in the USA, November 7, 2013
5991-3599EN

Agilent Technologies