# Encore™ NGS Library System I and Multiplex System I

**Simple and fast preparation of DNA libraries for a range of Next-Generation Sequencing applications**

## Highlights of the Encore NGS Library System I and Multiplex System I

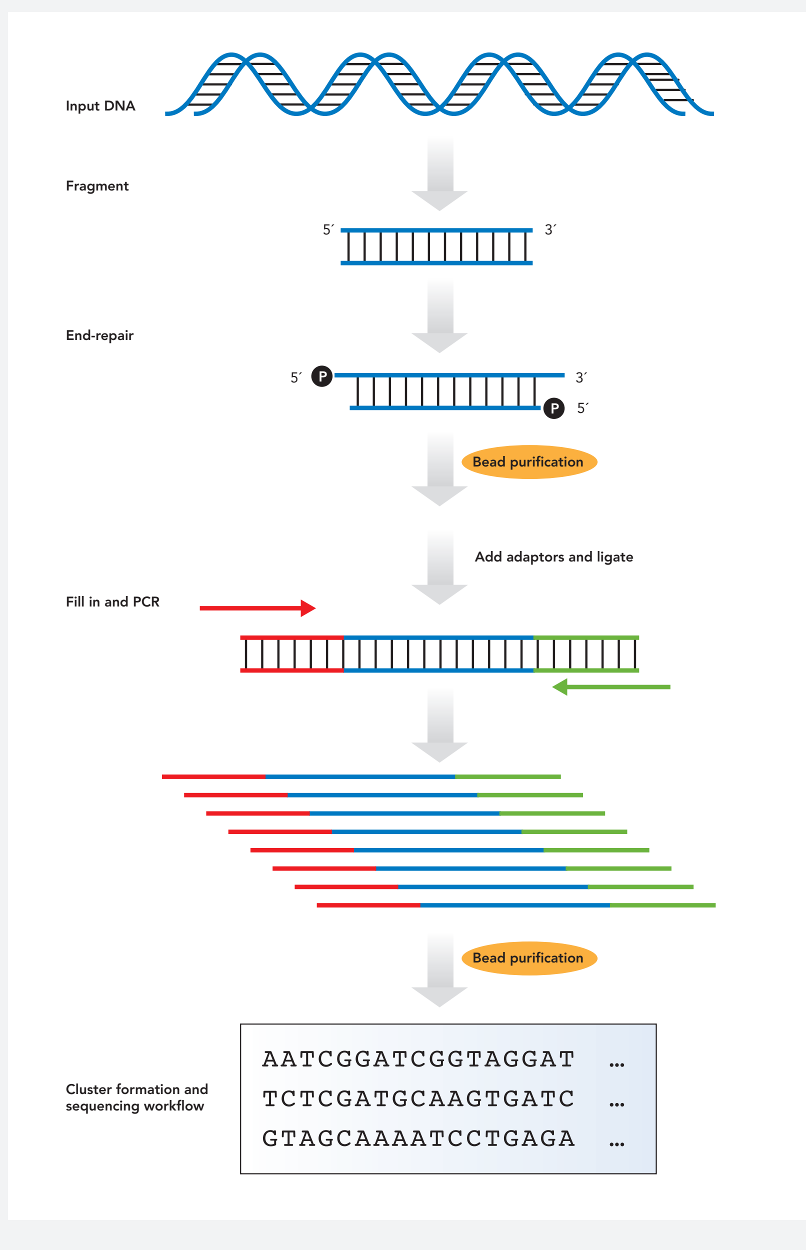
- **Simple, fast, automatable workflow:** Library construction in as little as three hours, with only two purification steps and no gel purification required
- **A complete solution for a range of NGS applications:** All required components included to make libraries for RNA-Seq, genomic DNA sequencing, ChIP-Seq or Digital Gene Expression applications on Illumina NGS platforms
- **Cost-effective and scalable:** Optional indexing capability for multiplex sequencing to increase sample throughput and reduce sequencing costs

## Introduction

Recent advances in Next-Generation Sequencing (NGS) technology have increased both the throughput and capacity of sequencing platforms, calling for increased efficiency in sample preparation. The Encore NGS Library System I and Multiplex System I address this need by providing a simple, rapid and affordable means to construct DNA libraries for all major NGS applications.

As shown in **Figure 1**, the workflow consists of four main steps: (1) High molecular weight double-stranded DNA is fragmented using Adaptive Focused Acoustics™ (Covaris™) to produce fragments with a tight size distribution between 150–200 base pairs; (2) The ends of these fragments are repaired to generate blunt ends; (3) Adaptor molecules are ligated,

FIGURE 1 Schematic of Encore NGS Library System I Workflow

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placing specific adaptors on the 5´ and 3´ end of each fragment; and (4) Fragments with ligated adaptor sequences are generated by a fill-in reaction and simultaneous PCR amplification to produce the final library with the optional incorporation of index tags. The entire workflow including fragmentation can be completed in as little as three hours, and yields DNA libraries ready for cluster formation and either single read or paired-end sequencing on Illumina NGS platforms. The efficiency of NGS can be further enhanced with the Encore NGS Multiplex System I, which uses unique index tags to facilitate sequencing of up to eight samples in a single lane, thereby dramatically reducing the per sample cost and time required to obtain sequence data.

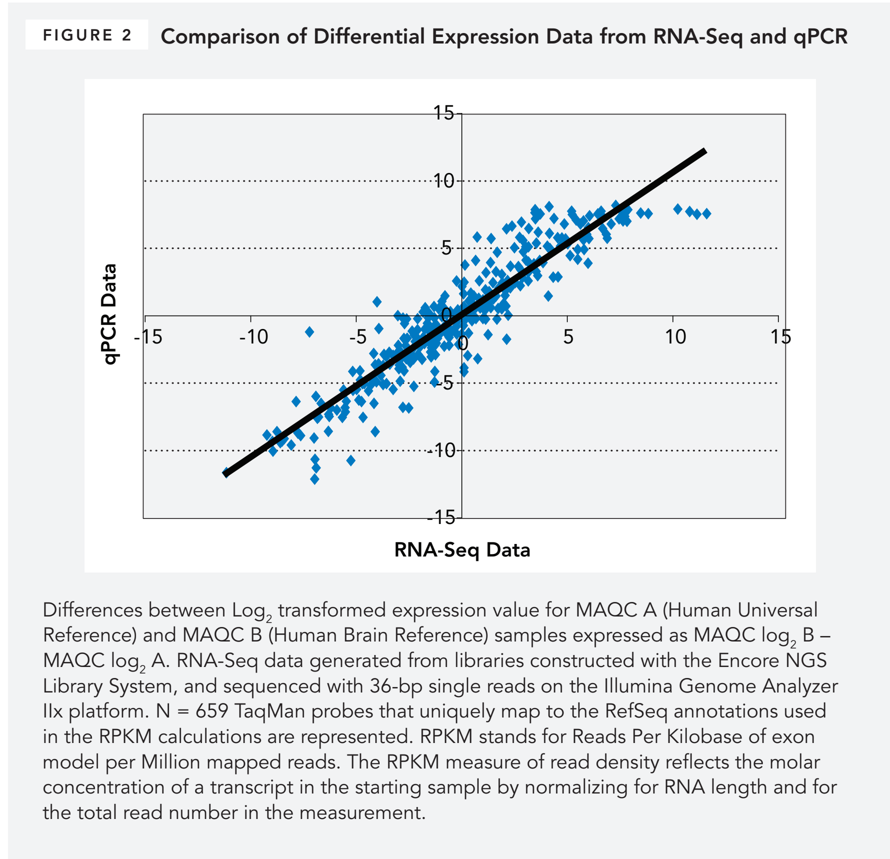
## Complete Solutions for RNA-Seq and Digital Gene Expression (DGE)

We currently offer two solutions for expression analysis by NGS, requiring as little as 500 pg of purified total RNA. The first solution, the Ovation® RNA-Seq System, provides essential reagents for the preparation of double-stranded cDNA directly from total RNA without the need for rRNA reduction or poly(A)+ selection and, therefore, ensures a more complete representation of the transcriptome. Amplification is initiated by primers at the 3´ end as well as randomly throughout the whole transcriptome using the proven NuGEN® Ribo-SPIA® (Single Primer Isothermal Amplification) technology.

Similarly, the Ovation 3´-DGE System uses the same Ribo-SPIA technology with primers at the 3´ end of transcripts to produce cDNA products ideally suited to DGE (Tag profiling) applications starting with only 10 ng total RNA. For added flexibility, each of these solutions can be used in conjunction with the Prelude™ Direct Lysis Module to enable input of direct cell lysates from 50 to 10,000 cells, without the added work of isolating total RNA.

The Encore NGS Library System I and Multiplex System I have been designed for seamless integration with both the Ovation RNA-Seq and 3´-DGE Systems, providing complete RNA analysis sample preparation solutions for NGS that can be completed in less than two days.

**FIGURE 2** Comparison of Differential Expression Data from RNA-Seq and qPCR




Differences between $Log_2$ transformed expression value for MAQC A (Human Universal Reference) and MAQC B (Human Brain Reference) samples expressed as MAQC $log_2$ B – MAQC $log_2$ A. RNA-Seq data generated from libraries constructed with the Encore NGS Library System, and sequenced with 36-bp single reads on the Illumina Genome Analyzer IIx platform. N = 659 TaqMan probes that uniquely map to the RefSeq annotations used in the RPKM calculations are represented. RPKM stands for Reads Per Kilobase of exon model per Million mapped reads. The RPKM measure of read density reflects the molar concentration of a transcript in the starting sample by normalizing for RNA length and for the total read number in the measurement.

As illustrated in **Figure 2**, high-quality data were obtained by the combined use of the Ovation RNA-Seq System and the Encore NGS Library System I to prepare libraries for sequencing on the Illumina Genome Analyzer IIx platform. MAQC A and B samples were analyzed by RNA-Seq to compare with differential expression data generated by quantitative PCR (qPCR) using the TaqMan assay. Differential expression calls are concordant with this reference expression assay (R = 0.934) without significant data compression.

**Figure 3** shows that input of U937 direct cell lysates prepared with the Prelude Direct Lysis Module to the Ovation RNA-Seq System followed by library construction with the Encore NGS Library System I yields high-quality RNA-Seq results which are equivalent to results obtained with isolated total RNA (R = 0.937). Taken together, these results demonstrate that differentially expressed genes can be accurately quantified with RNA-Seq libraries prepared with the Encore NGS Library System I and the integrated workflow provided by NGS solutions from NuGEN.

## Deep Sequencing of Libraries Prepared with Genomic DNA

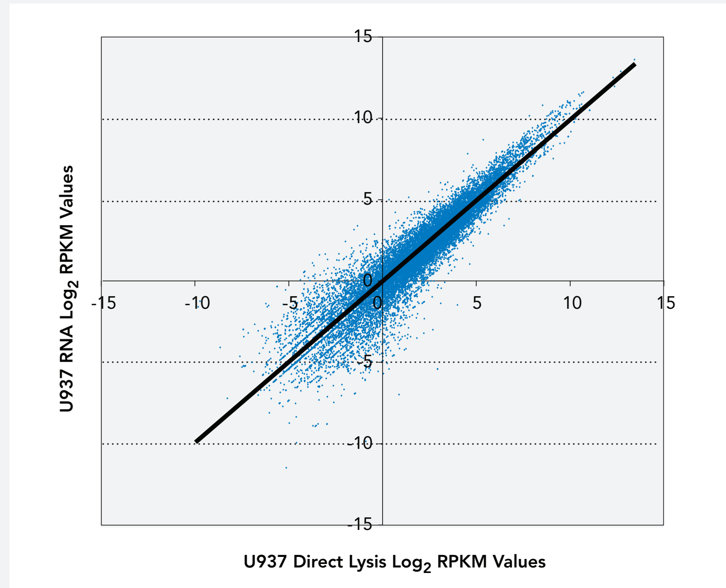
In order to evaluate the performance of the Encore NGS Library System I with genomic DNA, triplicate *E. coli* libraries were constructed and sequenced on the Illumina Genome Analyzer IIx in different flow cell lanes during the same run. The distribution of reads from each sample was plotted to determine the depth of coverage across the *E. coli* genome, as well as to assess the reproducibility of library construction and sequencing. An average of 30.1 million Total Reads was obtained for each sample and 4.0 mil-

lion of these were randomly sub-sampled to mimic the 4.6 million base pair *E. coli* reference genome. As shown in **Figure 4**, the reads from each library (red, gold, and green traces) mapped to the reference genome with a normal distribution centered on the theoretical read distribution of a random 40 base-pair sequence in blue. No positional bias or G+C bias was observed, and mapping of these 4.0 million reads provides an average depth of 35X. These results demonstrate that DNA libraries constructed with the Encore NGS Library System I provide a high depth of coverage in a reproducible fashion with no bias in the positional mapping of reads.

## Unique Indexing Tags to Enable Sample Multiplexing

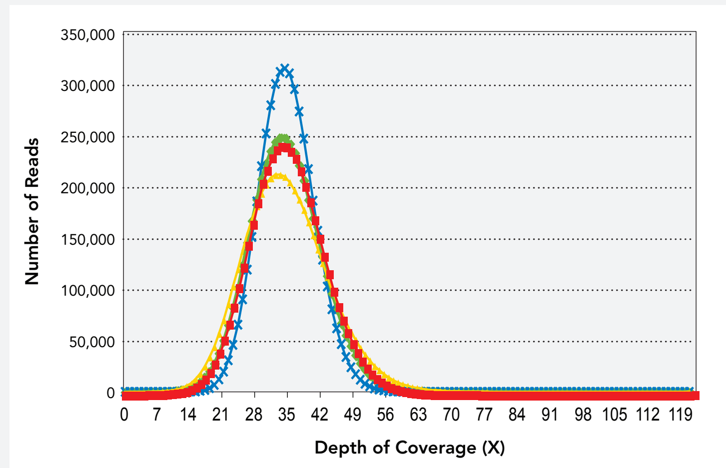
In order to further enhance the efficiency and cost-effectiveness of NGS, the Encore NGS Library System I is available with an optional indexing feature to increase the number of samples that can be sequenced in a given lane. The Encore NGS Multiplex System I is supplied with a set of eight indexed adaptors to facilitate multiplex sequencing. These four-base codes are intrinsic to the first sequencing read and are designed to provide equal representation across all nucleotide bases in the first several sequencing cycles. The index sequences are unambiguous, meaning that sequencing errors or deletion errors do not corrupt the code integrity. As shown in **Table 1**, use of the index tags in either an 8-plex or 4-plex sequencing experiment provides even read distribution, demonstrating there is no preferential cluster formation or sequencing from libraries constructed with each tag. Use of the Encore NGS Multiplex System I allows researchers to reliably segregate sequencing reads from multiple libraries to enhance throughput and reduce sequencing costs without sacrificing data quality.

**FIGURE 3** **Highly Concordant Results by RNA-Seq Using Cell Lysates or Isolated Total RNA**




2,000 U937 cells were processed using the Prelude Direct Lysis Module, or total RNA was isolated from U937 cells using the QIAGEN® RNeasy® Mini Kit. Cell lysates or 20 ng of isolated total RNA was amplified using the Ovation RNA-Seq System, and libraries constructed using the Encore NGS Library System I. Single-read sequencing was done with 40 base-pair reads on the Illumina Genome Analyzer IIx platform. Fastq reads were processed with Tophat v1.0.11 and Bowtie 0.12.3 to generate RPKM values (**R**eads **P**er **K**ilobase per **M**illion mapped reads) for 20,420 transcripts. $Log_2$ RPKM values are plotted.

**FIGURE 4** **Sequencing Coverage with *E. coli***




Genomic DNA was isolated from *E. coli* and libraries constructed using the Encore NGS Library System I. Single-read sequencing was done with 40 base-pair reads on the Illumina Genome Analyzer IIx platform using triplicate *E. coli* samples sequenced in different lanes during the same run. The distribution of Reads from each sample are plotted to determine the depth of coverage across the *E. coli* genome. The average number of Total Reads obtained for each sample was 30.1 million, and 4.0 million reads were randomly sub-sampled from each data set to generate the above plots. These experimental tracks are shown in red, gold, and green. The blue track represents the theoretical read distribution with randomly picked 40 base-pair sequences mapped to the *E. coli* genome.

**TABLE 1** Even Distribution of Indexed Library Reads for Optimal Multiplexing

| Adaptor | 8-Plex Sequencing | 4-Plex Sequencing Exp. #1 | 4-Plex Sequencing Exp. #2 |
|---|---|---|---|
| L2-BC1 | 11.1% | 21.6% | |
| L2-BC2 | 12.5% | | 26.7% |
| L2-BC3 | 11.6% | | 22.1% |
| L2-BC4 | 13.1% | 25.5% | |
| L2-BC5 | 13.0% | 26.1% | |
| L2-BC6 | 12.4% | | 19.7% |
| L2-BC7 | 12.0% | | 23.0% |
| L2-BC8 | 12.9% | 25.4% | |
| Unmatched | 1.4% | 1.4% | 8.5% |
| Total Reads | 29,348,362 | 27,660,506 | 26,443,830 |

Libraries were independently constructed with 200 ng fragmented *E. coli* genomic DNA using index-tagged adaptors in the Encore Multiplex System I. The libraries were then mixed prior to cluster formation in either an 8-plex or two different 4-plex formats (exp. #1 and #2 in table above). Single-read sequencing was done with 40 base-pair reads on the Illumina Genome Analyzer IIx platform. The results indicate an even distribution of reads derived from libraries containing each index tag, with no preferential cluster formation or sequencing from any of the indexed libraries. The theoretical distribution of indexed Reads for an 8-plex run is 12.5%, and 25% for 4-plex.

## Conclusion

The Encore NGS Library System I and Encore NGS Multiplex System I offer a number of advantages for researchers engaged in Next-Generation Sequencing:

- Simple, fast, automatable workflow — Library construction in as little as three hours, with only two purification steps and no gel purification required
- A wide range of applications — supports RNA-Seq, genomic DNA sequencing, ChIP-Seq or DGE
- Cost-effective and scalable — Optional indexing capability for multiplex sequencing up to eight samples to improve sample throughput and reduce sequencing costs
- Complete solution — seamless integration with NuGEN's Ovation RNA-Seq and 3´-DGE Systems
- Primers and adaptors included — compatible with the Illumina NGS sequencing platforms

## ORDERING INFORMATION

| Part No. | Product Name |
|---|---|
| **Next-Generation Sequencing Library Kit Products** | |
| 300 | Encore™ NGS Library System I (8 reactions) |
| 301 | Encore™ NGS Multiplex System I (32 reactions) |
| **3´-DGE Products** | |
| 7200 | Ovation 3´-DGE System (8 reactions) |
| 360 | 3´-DGE Complete Solution Without Indexing (8 reactions) |
| 361 | 3´-DGE Multiplex Complete Solution (32 reactions) |
| **RNA-Seq Products** | |
| 7100 | Ovation® RNA-Seq System |
| 350 | RNA-Seq Complete Solution Without Indexing (8 reactions) |
| 351 | RNA-Seq Multiplex Complete Solution (32 reactions) |
| **Technical Documents** | |
| Encore NGS Library System User Guide | |

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