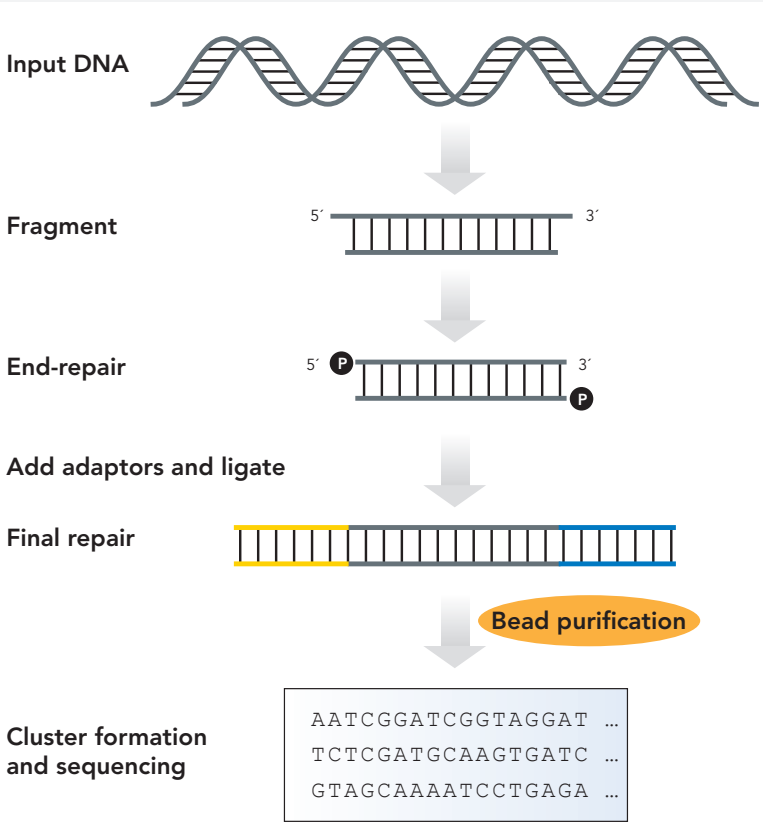
Encore® Rapid Library Systems

Prepare next-generation sequencing libraries in fewer than 2 hours

Highlights of the Encore Rapid Library Systems

- **Simple, rapid and automatable**—Produce DNA libraries for sequencing from as little as 100 ng genomic DNA
- **Amplification-free workflow**—Enable PCR-free library construction that can be successfully used for genomes that are susceptible to PCR artifacts, such as those with high or low GC content
- **Cost-effective and scalable**—Offer optional barcoding for multiplex sequencing to improve sample throughput and reduce sequencing costs

FIGURE 1 The Encore Rapid Library System Workflow



Introduction

The Encore® Rapid Library Systems provide simple, quick and scalable solutions for producing libraries for next-generation sequencing applications. Starting with as little as 100 ng of fragmented genomic DNA, these systems produce high quality libraries in fewer than 2 hours. Additionally, the automatable workflow requires no DNA amplification, making it a valuable tool for sequencing genomes, such as those with high or low GC content, where PCR artifacts often complicate sequencing reads.

The Encore Rapid Library Systems are suitable for a variety of sequencing applications including RNA-Seq, Digital Gene Expression (DGE), genomic DNA sequencing, amplicon sequencing, ChIP-Seq and more.

These systems are compatible with NuGEN's Ovation® pre-amplification systems and are available for singleplex sequencing or multiplex sequencing using NuGEN inline (IL) and dedicated read (DR) barcodes.

Streamlined Library Construction Workflow

As shown in **Figure 1**, the streamlined workflow consists of four main steps: fragmentation of either genomic DNA or double-stranded cDNA, end repair to generate blunt ends, adaptor ligation, and final repair. Unlike many other NGS protocols, the library construction does not require PCR amplification. The entire workflow includes only one bead purification step and no gel purifica-

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tion. Starting with as little as 100 ng of fragmented double-stranded DNA (ds-DNA), the protocol can be completed in fewer than 2 hours and yields libraries ready for cluster formation and either single read or paired-end sequencing.

The Encore Rapid Library System (Part No. 0316) contains reagents for production of non-barcoded libraries. The Encore Rapid IL Multiplex Systems 1-8 (Part No. 0317) and 9-16 (Part No. 0318) each provide eight unique barcoded inline (IL) adaptors for multiplex sequencing. The Encore Rapid DR Multiplex Systems 1–8, 9–16, and 1–96 provide extensive flexibility in barcoding options. Use the Encore Rapid DR Multiplex Systems 1–8 and 9–16 separately or together for multiplexing up to 8 or 16 samples, respectively. Use the Encore Rapid DR Multiplex System 1–96 to achieve even higher levels of multiplexing, any number up to 96.

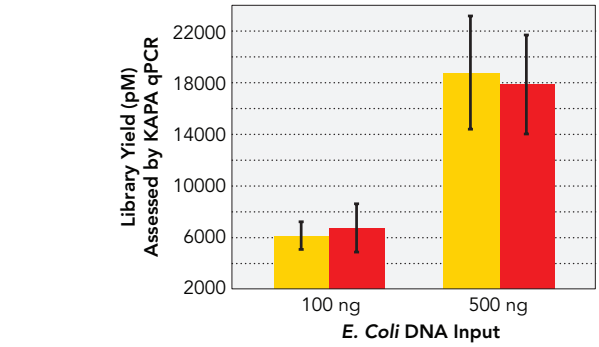
Fast Production of Libraries Ready for Cluster Formation and Sequencing

The Encore Rapid Library System protocol quickly yields DNA libraries ready for cluster formation and either single read or paired-end sequencing. To demonstrate the robust library yield from this system, differing amounts of fragmented *E. coli* genomic DNA (gDNA) were used as input to create two replicate libraries using the Encore Rapid Library System. After final elution, the library product was quantified using the Library Quantification Kit for Illumina platforms from KAPA Biosystems (PN KK4835), according to the KAPA Biosystems protocol.

As shown in **Figure 2**, the Encore Rapid Library System produced libraries with greater than 2000 pM yield, starting with just 100 ng of *E. coli* gDNA. Library yield increased with the amount of input gDNA. Average fragment length for these libraries was about 350 base pairs.

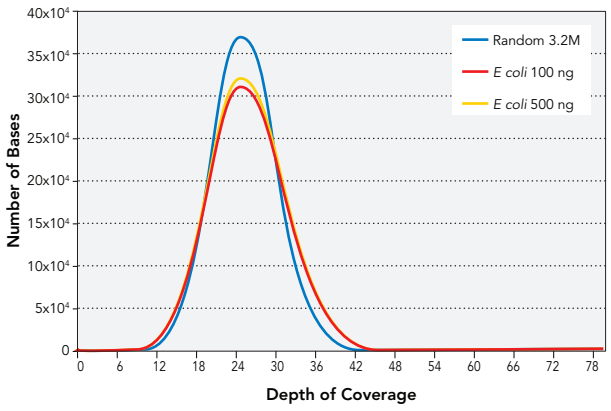
These libraries were sequenced on the Illumina Genome Analyzer IIx using 40 base-pair single end reads. To determine depth of coverage across the *E. coli* genome the distribution of reads was plotted, as shown in **Figure 3**. The distribution of reads for each library was mapped to the reference genome and to a Gaussian distribution of the equivalent number of reads generated randomly from the genome sequence. No coverage bias was observed and mapping these 3.2 million reads provided an average 24X genomic coverage with high reproducibility.

FIGURE 2 Library Yield with Differing Inputs of ***E. coli*** gDNA



Samples 1 and 2 represent two independent replicate libraries prepare with Encore® Rapid Library System. After final elution, the library product was quantified using the Library Quantification Kit for Illumina platforms from KAPA Biosystems (Part No. KK4835), according to the KAPA Biosystems protocol. Each of the two libraries was quantified four times. The average picomolar quantification is shown with the standard deviation for each sample. There is sufficient library yield at all input amounts for cluster formation.

FIGURE 3 Coverage of the ***E. coli*** Genome in Libraries Constructed with Varying Amounts of Input gDNA



Using the same libraries shown in Figure 2, uniform distribution of sequences is observed across the *E. coli* genome.

Unbiased Library Construction for Extreme Genomes

Genomes with high or low GC content can be particularly challenging for

sequencing applications because they are prone to PCR artifacts. The Encore Rapid Library System protocols do not require an amplification step, helping to minimize biases due to GC content. To demonstrate the ability of the Encore Rapid Library System to circumvent this bias, libraries were created from two microbial genomes with extreme GC content—*S. aureus* (33% GC) and *R. sphaeroides* (69% GC).

In **Figure 4**, the top pair of graphs show the expected genome coverage of these two extreme genomes based on a theoretical *in silico* generated library. The bottom row depicts the coverage observed with libraries produced using the Encore Rapid Library System. These graphs show very little GC bias, with some bias in the *R. sphaeroides* genome that may be attributed to the platform sequencing chemistry.

Uniform Distribution of Multiplex Sequencing Reads

With the introduction of the Encore Rapid Library System, NuGEN has created two new sets of barcoded adaptors for dedicated read (DR) multiplex sequencing. This barcode placement utilizes a dedicated sequencing primer to read the barcode and is compatible with the standard Illumina data analysis pipeline. Distribution of these barcodes during multiplex sequencing was investigated by sequencing 16 libraries constructed using the Encore Rapid DR Multiplex Systems 1–8 and 9–16.

As shown in **Figure 5**, the barcodes are evenly distributed among sequence reads.

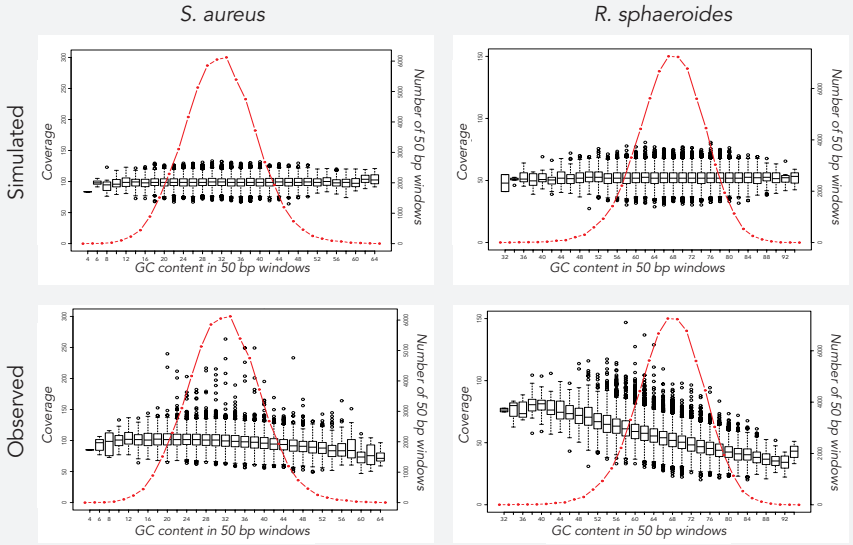
Recommended for Use with Ovation® RNA-Seq System V2

The Encore Rapid Library Systems are ideal for use with the Ovation RNA-Seq System V2 from NuGEN. When using the Ovation RNA-Seq System V2, output is generally 2–3 µg of ds-cDNA, significantly more than the recommended 500 ng used as starting material for library construction using the Encore Rapid Library Systems.

As illustrated in **Table 1**, approximately 93% of all sequencing reads can be attributed to aligned reads when using 500 ng of Ovation RNA-Seq System V2 ds-cDNA product as input into the library construction workflow. Additionally, approximately 60% of the reads mapping to the human reference genome are derived from non-rRNA sequences which is consistent with past performance of the Ovation RNA-Seq System V2 using other NuGEN library systems.

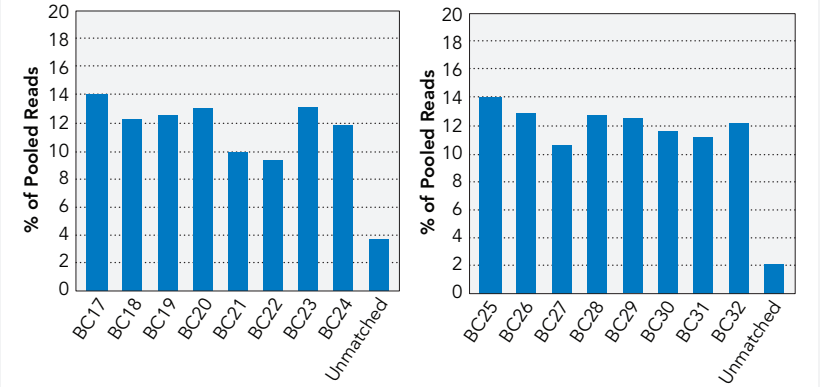
The combined workflow of the Ovation RNA-Seq System V2 and the Encore

FIGURE 4 GC Content for Extreme Genomes



Top graphs show expected genome coverage for *S. aureus* (33% GC) and *R. sphaeroides* (69% GC) based on a theoretical in silico generated library. Note the shift of the curve to the right with greater GC content. Observed genome coverage was based on 100 ng of input DNA into the Encore Rapid Library System. No significant GC bias was detected, with minimal bias observed in the *R. sphaeroides* reads. This bias may be attributable to the platform sequencing chemistry.

FIGURE 5 Distribution of Sequencing Reads, Pooled Based on KAPA Quantification (***E. coli*** DR Barcodes)



Libraries produced using the Encore Rapid DR Multiplex Systems 1–8 and 9–16 were pooled and sequenced on the Illumina Genome Analyzer IIx using 40 base-pair single end reads. The data demonstrate equal representation of sequences using each of the 16 DR barcodes.

TABLE 1 Ovation RNA-Seq System V2 Results with cDNA Inputs to the Library Workflow of 500 or 750 ng

Library Input	500 ng	750 ng
% of all reads		
Not aligned	6.9%	6.2%
Aligned	93.1%	93.8%
% of mapped reads		
non-rRNA	63.0%	57.9%
all mito rRNA	34.4%	32.8%
all cyto rRNA	2.6%	9.3%
non-rRNA single site	47.9%	42.9%
non-rRNA multiple site	15.1%	15.0%

2.0 ng of MAQC Brain reference total RNA was used to generate cDNA with the Ovation RNA-Seq System V2. Either 500 or 750 ng of the resulting cDNA was used as input to the Encore Rapid Library Systems workflow. All mito rRNA = 12S and 16S rRNA; All cyto rRNA = 5.8S, 18S and 28S rRNA; Non-rRNA single site = reads mapping to a single site in the reference genome; Non-rRNA multiple site = reads mapping at two or greater sites in the reference genome; non-rRNA is the sum of these latter two categories

ORDERING INFORMATION

Part No.	Product Name
0316	Encore® Rapid Library System
0317	Encore Rapid IL Multiplex System 1–8
0318	Encore Rapid IL Multiplex System 9–16
0319	Encore Rapid DR Multiplex System 1–8
0320	Encore Rapid DR Multiplex System 9–16
0328	Encore Rapid DR Multiplex System 1–96
Realted Products	
7102	Ovation® RNA-Seq System V2
7150	Ovation RNA-Seq FFPE System
Technical Documents	
Encore Rapid Library System User Guide	

Rapid Library System takes approximately 6.5 hours from total RNA sample to sequence-ready libraries.

Conclusions

The Encore Rapid Library Systems offer a simple, fast and automatable solution for quickly constructing DNA libraries for a variety of NGS applications. Using as little as 100 ng of fragmented genomic DNA, the protocol can be completed in fewer than 2 hours, with only one bead purification step and no gel purification required. The PCR-free workflow makes these systems well-suited for library construction with high or low GC-content genomes, which are often prone to PCR artifacts.

The Encore Rapid Library Systems are recommended for use NuGEN's Ovation RNA-Seq System V2 and other Ovation pre-amplification products. The Encore Rapid Library Systems include kits for both single- and multiplex sequence runs, with options for either inline or dedicated read barcoding. Starting from fragmented gDNA or ds-cDNA, all Encore Rapid Library System kits produce cDNA libraries ready for cluster formation and either single read or paired-end sequencing in fewer than 2 hours.

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