NxSeq UltraLow DNA Library Kits v2

Build high quality, Illumina-compatible DNA fragment libraries from 50 pg to 100 ng of DNA in only 3 hours

The NxSeq™ UltraLow DNA Library Kits v2 and Indexing Kits allow you to build complex, high quality DNA fragment libraries from extremely low DNA input amounts – as low as 50 pg (Table 1).
If you have more DNA, no problem; you can use as much as 100 ng of input DNA with this system.
In order to build high quality DNA fragment libraries from limiting DNA inputs, the entire library prep workflow must be optimised starting with adaptor ligation efficiency (Figure 1) followed by un-biased, robust PCR amplification of the library to produce enough sequenceable material. This system is designed and built to produce the highest quality libraries possible (Table 1).

High quality libraries and sequencing data start with high efficiency adaptor ligation

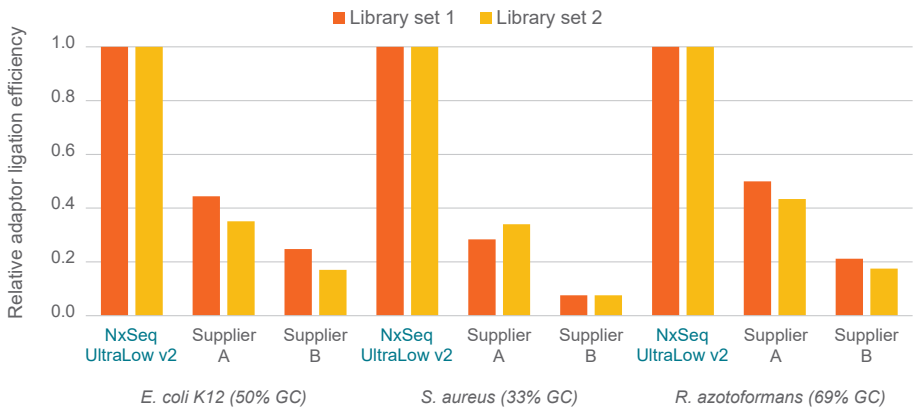

Figure 1. Efficiency of adaptor ligation to DNA library fragments. Two independent sets of libraries were prepped per kit/organism using manufacturer's recommended protocols and 1 ng of the same sheared genomic DNA input per library. Briefly, DNA fragments with adaptors ligated to both ends were measured using triplicate qPCR assays and a Lucigen-designed universal qPCR primer set that binds to and amplifies all adaptor-ligated DNA fragments independent of the kit used. Efficiency was determined by comparing the qPCR quantitation to fluorescence DNA quantitation. Efficiency data was averaged and then normalised to the corresponding NxSeq UltraLow Library Kit data (1.0) and plotted.

- **High quality data:** High efficiency adaptor ligation reactions produce complex libraries that yield improved sequencing depth, uniformity, and fewer "zero coverage" regions than other kits
- **Sensitive:** Construct DNA fragment libraries from as little as 50 pg to as much as 100 ng of sheared/ fragmented DNA
- **Minimal bias:** Robust, uniform PCR amplification improves coverage and sequencing depth uniformity
- **Flexible:** Extensively tested in whole genome sequencing and re-sequencing, and compatible with other applications, such as exome-seq, ChIP-seq and sequencing of difficult FFPE DNA samples
- **Fast and easy-to-use:** Simplified, 3 hour protocol gets your samples on the sequencer quickly while decreasing the risk of handling errors
- **High value:** Cost-effective, high performance library and indexing kits

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Fast, streamlined library preparation with minimal components required

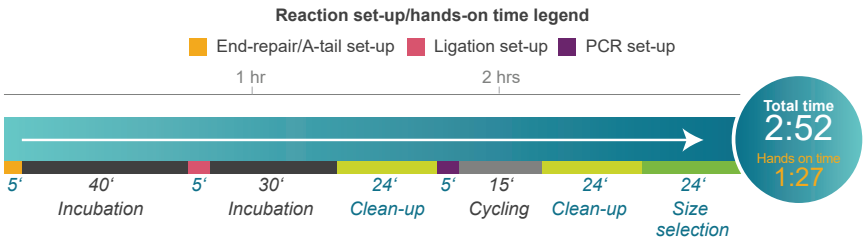

Figure 2. Time course for the NxSeq UltraLow DNA Library Kit v2 protocol. This figure illustrates the various steps and time required for each and total overall time. Please note that only three enzyme components are provided and required to complete library preparation, greatly simplifying library prep while reducing the risk of any errors.

Consistent, high quality sequencing data from 50 pg to 100 ng of genomic DNA

Sheared gDNA input	50 pg	1 pg	10 pg	100 ng
Mapped reads	309,272	309,283	309,307	309,303
PCR cycles used	15	10	8	5
Depth	9.82x	9.81x	9.87x	9.89x
Std dev of depth	3.87x	3.84x	3.80x	3.80x
Coverage	99.92%	99.93%	99.94%	99.95%
Duplicate reads	0.22%	0.043%	0.037%	0.037%
No. of zero coverage regions	135	130	98	93
Total zero coverage	3556 bp	3501 bp	2495 bp	2305 bp

Table 1. Sequencing data from DNA fragment libraries generated from 50 pg to 100 ng of sheared *E. coli* gDNA. Mechanically sheared (Covaris LE220, peak size 300 bp) *E. coli* genomic DNA was serially diluted and used to make triplicate libraries starting with the indicated gDNA input amounts and number of PCR amplification cycles using the NxSeq UltraLow DNA Library Kit v2 and a NxSeq Single Indexing Kit. The final libraries were quantitated and diluted to 2 nM based on Bioanalyzer (size) and fluorescence-based DNA quantitation. The diluted libraries were then pooled and sequenced on a MiSeq using 2 × 150 bp chemistry, and the average data from the triplicate libraries are shown.

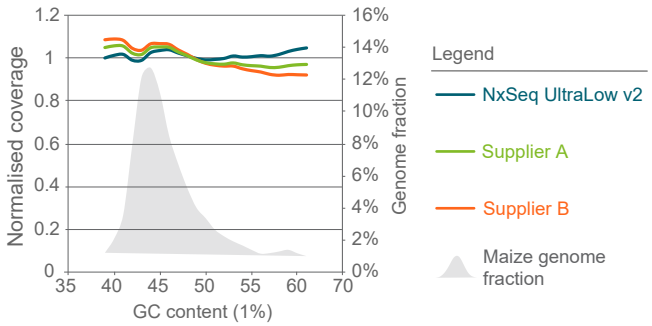

Figure 3. Sequence bias analysis of maize libraries constructed with three library preparation kits. Triplicate genomic DNA fragment libraries were generated from maize B73 gDNA using the NxSeq UltraLow DNA Library Kit v2, Supplier A Kit or Supplier B Kit according to the manufacturer's recommended protocols using 10 ng aliquots of the same mechanically sheared genomic DNA sample. The final libraries were pooled and sequenced on a NovaSeq using 2 × 150 chemistry. Normalised coverage was calculated as the (average coverage of all windows with x% GC content) divided by the (overall average coverage) and the data from each set of replicates was averaged and presented. The gray shaded area represents the percent of the maize genome with the indicated GC content.

Ordering information

Cat no.	Size	Description
15012-2	**12 rxn**	NxSeq UltraLow DNA Library Kits v2
15096-2	**96 rxn**	
15100-1	**48 rxn (12 x 4 rxn)**	NxSeq Single Indexing Kit, Set A
15200-1	**48 rxn (12 x 4 rxn)**	NxSeq Single Indexing Kit, Set B
15300-1	**96 rxn**	NxSeq HT Dual Indexing Kit

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