NxSeq UltraLow DNA Library Kit* - 12 reactions

Build high quality, Illumina-compatible DNA fragment libraries from 50 pg to 75 ng of DNA in only 3 hours

The NxSeq™ UltraLow DNA Library Kit and NxSeq Single 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 75 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).

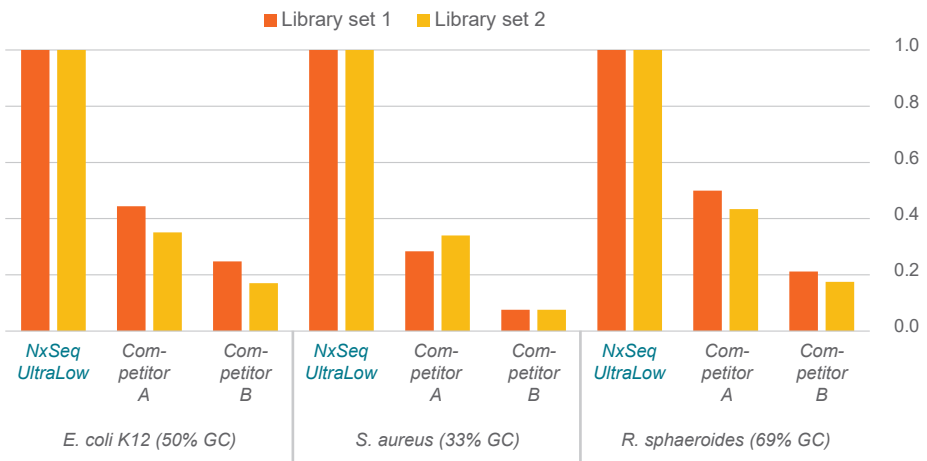


Figure 1. Efficiency of adaptor ligation to DNA library fragments. Two independent sets of libraries were prepped per kit/organism using manufacturer's recommended protocols and 1 ng of the same sheared genomic DNA input per library. Briefly, DNA fragments with adaptors ligated to both ends were measured using triplicate qPCR assays and an universal qPCR primer set, designed by LGC, Biosearch Technologies, 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 Ultra-Low 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 75 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

* and NxSeq single indexing kits

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

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

NxSeq UltraLow DNA Library Kit, 12 reactions and NxSeq Single Indexing Kits specification

Special instructions/ requirements	The NxSeq UltraLow DNA Library Kit and NxSeq Single Indexing Kits are only compatible with Illumina sequencers. *Please note that at least one NxSeq Single Indexing Kit is required to complete library prep and must be purchased along with a library kit.*
Components	The NxSeq UltraLow DNA Library Kit contains Enzyme Mix (EM), 2X Buffer (2XB), Ligase (LIG), 2X PCR Master Mix (MM) and Elution Buffer (EB). Each NxSeq Single Indexing Kit contains a Universal Adaptor and (12) different Primer Indexing Mixes with enough Universal Adaptor for 48 libraries and enough of each Primer Indexing Mix for 4 library amplification reactions (48 total reaction for all primer sets). Set A contains Primer Indexing Mixes 1-12 and Set B contains Primer Indexing Mixes 13-16, 18-23, 25, and 27. Each NxSeq Single Index equals the TruSeq® LT Index with the same number.

Performance information

Sheared gDNA input	50 pg	250 pg	500 pg	75 ng
PCR cycles used	16	15	13	4
Reads sampled per library	312,500	312,500	312,500	312,500
No. mapped reads	303,442	304,135	304,894	304,935
Percent mapped reads	97.10%	97.32%	97.57%	97.58%
CLC mapped duplicates	0.84%	0.15%	0.08%	0.02%
Ave. coverage depth	9.62	9.72	9.74	9.84
Coverage depth standard dev.	3.75	3.71	3.71	3.6
No. of zero locations	109	91	91	56
Ave. zero region length (bp)	24	24	25	27
Total zero length (bp)	2651	2214	2175	1491

Table 1. Generation and sequencing of DNA fragment libraries from 50 pg to 75 ng of E. coli gDNA. Mechanically sheared (300 bp peak) E. coli K12 genomic DNA was diluted and used to make duplicate libraries starting with the indicated input amounts and number of PCR amplification cycles using the NxSeq UltraLow DNA Library Kit and a NxSeq Single Indexing Kit. The final libraries were quantitated, diluted to 2 nM, pooled and sequenced on a MiSeq using 2×150 bp chemistry. The average data from the duplicate libraries are presented.

High quality whole genome sequencing data from ≥50 pg of input DNA

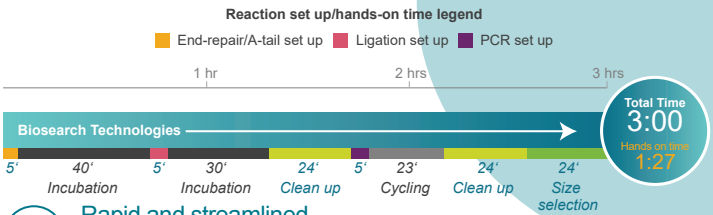
	NxSeq UltraLow Kit	Competitor A	Competitor B
Total PF sampled reads	200,000,000	200,000,000	200,000,000
R1 mapped reads	96.52%	96.02%	96.10%
R2 mapped reads	95.51%	95.36%	94.97%
Fragment length (bp)	289 +/- 110	191 +/- 61	225 +/- 109
Duplicates (proper read pairs)	2.15%	3.73%	3.54%
Coverage depth	8.32	6.15	6.96
Autosomal coverage	98.64%	98.40%	98.73%

Table 2. HiSeq X Ten sequencing results with fragment libraries made with 10 ng of human genomic DNA. DNA fragment libraries were constructed using the indicated kits according to each manufacturer's recommended protocol. Briefly, human genomic DNA (NA15510, Coriell) was sheared (median size 300 bp), and 10 ng aliquots were used to make duplicate libraries with each kit. The libraries were PCR amplified for the following number of cycles: NxSeq UltraLow Kit, 8 cycles; Competitor A, 8 cycles; and Competitor B, 10 cycles. The amplified libraries were cleaned/size selected as recommended. The final libraries were sent to Hudson Alpha for sequencing, and each set of duplicates was pooled and sequenced on its own HiSeq X Ten lane using 2×150 bp chemistry. During analysis, the optical duplicates generated by the HiSeq X Ten were removed using the Clumpify program, and then the indicated number of reads were analysed. The averaged data from the replicate libraries are shown.

Improved sequencing performance on the HiSeq X Ten Instrument

Ordering information

VWR cat no.	Size	Description
76180-800	**12 rxn**	NxSeq UltraLow DNA Library Kit, 12 reactions
76180-802	**48 rxn (12x4 rxn)**	NxSeq Single Indexing Kit, Set A
76180-804	**48 rxn (12x4 rxn)**	NxSeq Single Indexing Kit, Set B



Rapid and streamlined 3 hour protocol

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Integrated tools. Accelerated science.

ISO 13485 Medical Devices Quality Management
FM 598408

For Research Use Only. Not for use in diagnostic procedures.

0320 Lit. No. 120079WREV