JetSeq™ DNA Library Preparation Kits

Powering NGS

- **Low input:** end-repair, A-tailing and ligation combined in the same tube, thereby eliminating cleanup steps and improving sample yield

- **Improved confidence:** simpler protocol with fewer steps for reduced risk of sample loss and offering greater peace-of-mind

- **Increased speed:** fast library preparation for reduced time to results and increased sample throughput

- **Highly efficient:** reaction buffer pre-optimized to provide maximum reaction efficiency and highest conversion rates

- **High-yield:** PCR polymerase and buffer, developed specifically for library preparation, giving maximum yield of sequence-ready library DNA

- **Improved quality:** optimized, high quality reagents result in reliable library preparation from even very challenging samples, providing maximum coverage

- **Flexibility:** a PCR and PCR-free kit for use with Illumina adapters and indexes

The JetSeq™ NGS Library Preparation Kits are designed to generate high-quality next generation sequencing (NGS) libraries suitable for sequencing on Illumina® instruments.

The success of next-generation sequencing is partly dependent upon the precise and accurate processing of the input DNA. High-quality library preparation from sheared DNA requires efficient processing during a series of molecular biology reactions and good recovery during the intermediate purification steps.

FASTER LIBRARY CONSTRUCTION

By combining end repair, A-tailing and ligation in a single tube, the JetSeq™ NGS Library Preparation Kits offer a faster turnaround time (Fig. 1), with minimal manual effort, thereby enabling complete library construction in as little as 3 hours. The elimination of purification steps minimizes sample loss, while optimization of the JetSeq buffer system ensures high-yield of sequence-ready libraries.
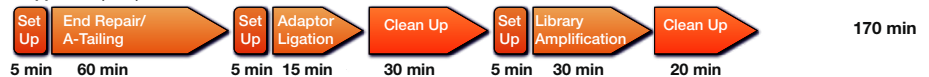

Fig. 1 DNA Library Preparation Workflow
The JetSeq NGS Library Preparation Kits incorporates fewer steps. The simpler, shorter protocols reduce both hands-on time and the total time required for preparation of library DNA.

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HIGH LIBRARY QUALITY AND YIELD

The PCR-free JetSeq ER & Ligation Kit contains all of the enzymes and buffers necessary for single tube end repair, A-tailing and ligation in a convenient, optimized master mix formulation and is recommended for NGS applications that do not require PCR amplification of the library prior to sequencing. In addition to the enzymes and buffers for end repair A-tailing and ligation, the JetSeq Flex DNA Library Preparation Kit also contains the polymerase and buffer required for PCR amplification, for preparation of libraries from lower input material. These kits do not contain adapters, but have been validated using standard, indexed Illumina adapters, and are also compatible with other adapters of similar design from other reputable oligonucleotide suppliers.
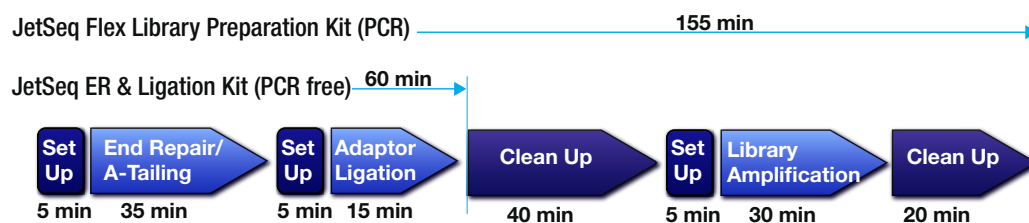
JetSeq Flex Library Preparation Kit (PCR) ——————————————— 155 min

JetSeq ER & Ligation Kit (PCR free) — 60 min

| Set Up | End Repair/ A-Tailing | Set Up | Adaptor Ligation | Clean Up | Set Up | Library Amplification | Clean Up |
| 5 min | 35 min | 5 min | 15 min | 40 min | 5 min | 30 min | 20 min |

Fig. 2 JetSeq Workflow
Single-tube end-repair, A-tailing and ligation steps, plus reduction in cleanup steps offers significant improvement in library preparation time and convenience, as well as improving yield and quality of sequence-ready libraries.

HIGHER LIBRARY ADAPTER CONVERSION EFFICIENCY

Ligation efficiency strongly influences library diversity and quality. The JetSeq NGS Library Preparation Kits are highly efficient at converting DNA into an adapter-ligated library, resulting in higher sequence coverage. The kits contain all of the enzymes and buffers necessary for highly-efficient conversion of the input DNA to a sequenceable, adapter-ligated library.

The efficiency of the end repair and ligation steps can be measured by qPCR quantitation of adapter-ligated fragments prior to library amplification. By using adapter specific primers, the rate of conversion of input DNA to adapter-ligated fragments, (i.e. sequenceable molecules) can be determined. JetSeq enables higher rates of conversion as compared to other commercially available kits (Fig. 3).
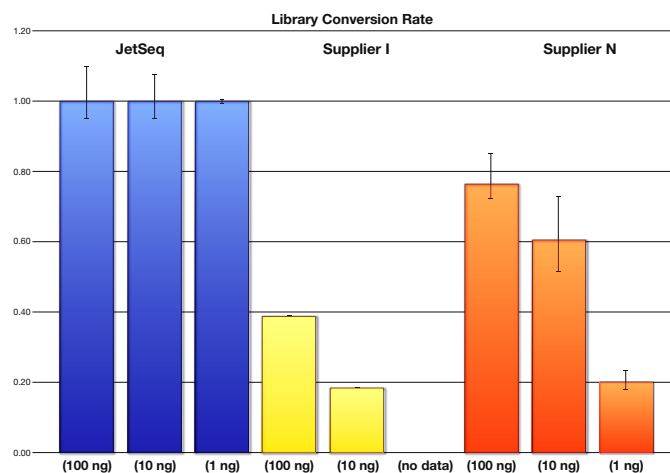
Fig. 3 Library Conversion Rates
The libraries were prepared starting from 1 ng, 10 ng or 100 ng of Covaris-sheared human genomic DNA quantified with Qubit, using Y-shaped universal sequences compatible with Illumina sequencers. The manufacturers' recommendations were followed, excluding the PCR amplification step which was not performed. The library conversion rates were quantified in qPCR using JetSeq Library Quantification Kit and the obtained values were normalized to JetSeq Kit. The results demonstrate that JetSeq has a higher library conversion rate than suppliers I and N.

REDUCED AMPLIFICATION BIAS

The GC content plays an important role in the efficiency of fragments passing through each step of the sample preparation and sequencing workflow. Differences in the percentage of GC in fragments can result in these fragments becoming relatively enriched or depleted, leading to misleading sequence data and NGS results. This means that more sequencing per sample is needed, so that a minimum level of coverage in a region of interest is achieved.

Sequence bias

The JetSeq Flex DNA Library Preparation Kit and JetSeq DNA Library Preparation Kit use a highly efficient polymerase, together with an optimized buffer formulation to ensure uniform amplification of all genomic regions including those that contain highly variable GC content (Fig. 4), there by reducing bias and ensuring even coverage in subsequent sequencing reactions (Fig. 5).
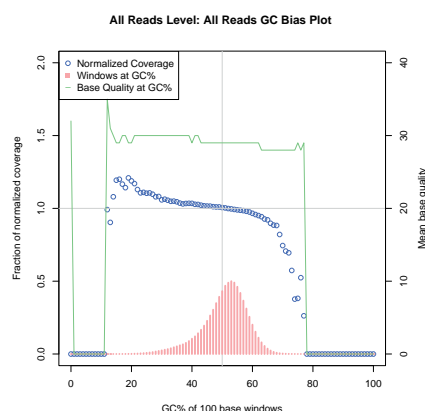
JetSeq
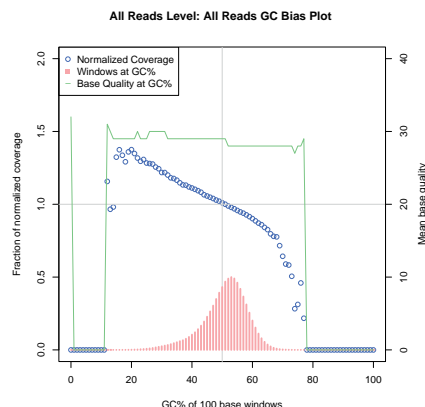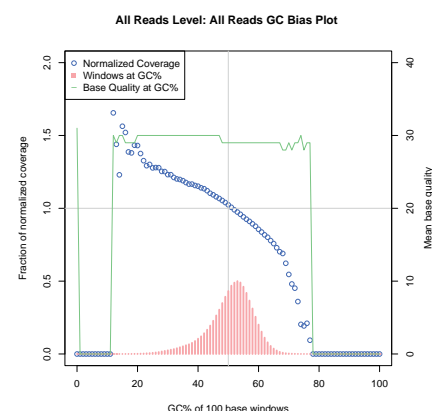
Supplier I
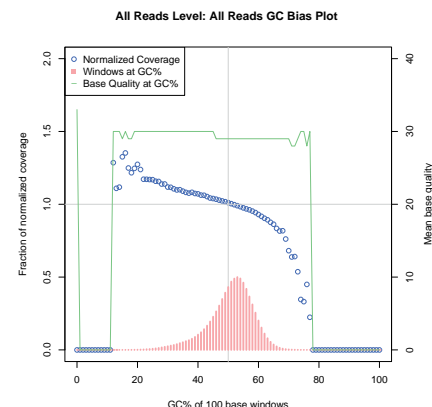
Supplier K

Supplier N

Fig. 4 Picard Plots
Libraries were constructed and amplified from 100 ng *E. coli* DNA using either the JetSeq Flex DNA Library Preparation Kit or the standard library preparation reagents and protocol from suppliers I, N and K. Individual libraries were sequenced on an Illumina HiSeq 2000. The results show normalized coverage (blue), %GC of the reference sequence (red) and base quality at GC% (green). Comparable results were achieved for the libraries prepared using the JetSeq and the other suppliers.
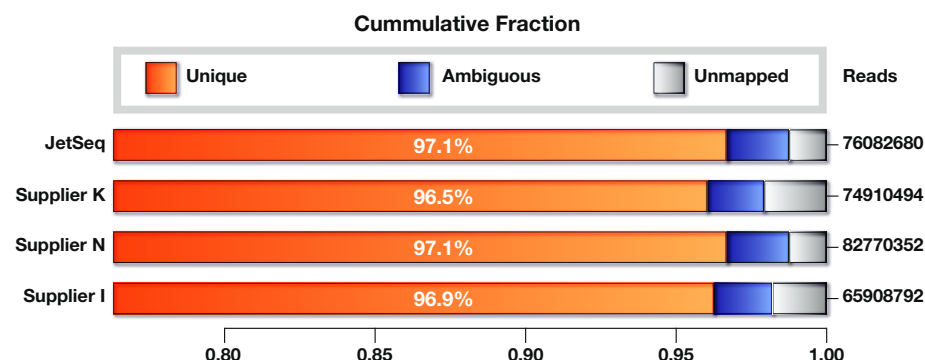
Cummulative Fraction

	Reads
JetSeq 97.1%	76082680
Supplier K 96.5%	74910494
Supplier N 97.1%	82770352
Supplier I 96.9%	65908792

Legend: Unique | Ambiguous | Unmapped

0.80 0.85 0.90 0.95 1.00

Fig. 5 Summary of Mapping Results
For each sample, the fraction of uniquely mapped (mapped to only one place on a reference sequence), ambiguously mapped and unmapped reads relative to the total number of reads per sample is shown. The results illustrate that JetSeq library preparation gives comparable results to other suppliers.

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HIGH-QUALITY LIBRARY CONSTRUCTION

Sequence quality

Sequencing coverage describes the average number of reads that align to, or cover, known reference bases and determines whether variant discovery can be made with a certain degree of confidence at particular base positions. High-performance enzymes in the JetSeq Kits deliver exceptional sequence quality scores (Fig. 6), giving greater coverage and reducing the possibility of sequencing gaps.
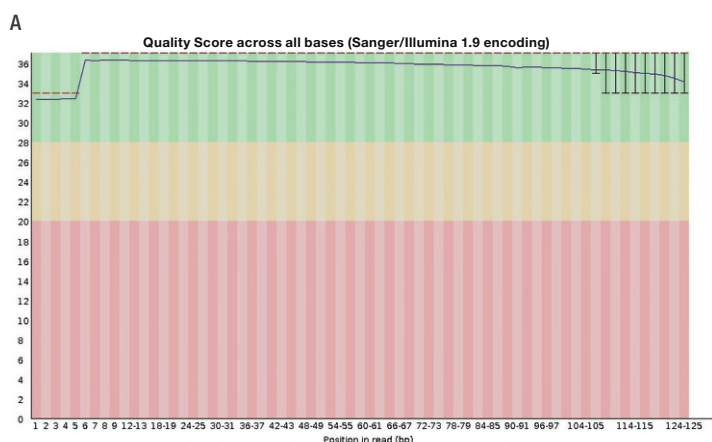
A

Quality Score across all bases (Sanger/Illumina 1.9 encoding)

B

Read Mappings

Fig. 6.A Quality Score Across all Bases
Libraries were constructed and amplified from 100 ng human genomic DNA from two different samples using the JetSeq Flex DNA Library Preparation Kit. The median per base quality score was high (greater than 30 giving a base call accuracy of 99.9%) for all bases from both samples, up to 125 bases into the read. This highly reproducible, high base quality score, reduces the requirement for post-acquisition bioinformatics (e.g. read size reduction to remove poor quality sequence), giving complete confidence in the data.
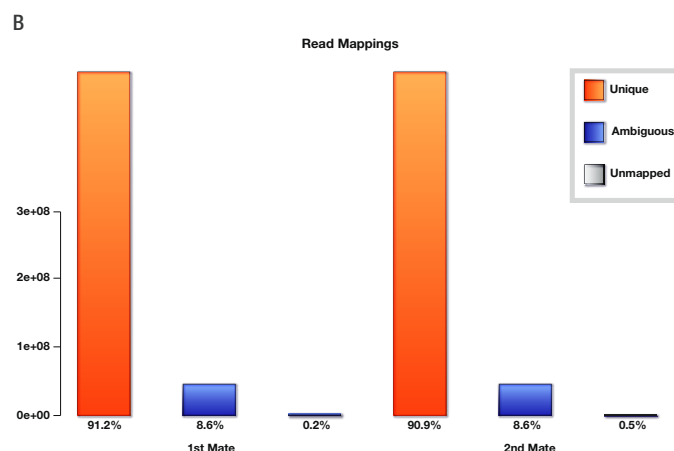
Fig. 6.B Quality Score Across all Bases
The fraction of uniquely mapped (mapped to only one place on a reference sequence), ambiguously mapped and unmapped reads relative to the total number of reads per sample is shown for the forward (1st Mate) and reverse (2nd Mate) paired end read primers. The results illustrate that JetSeq library preparation gives over 90% unique reads that could be aligned to the reference.

Ordering Information

JetSeq™ Kit	Size	Cat. #
JetSeq Flex DNA Library Preparation Kit (PCR)	96 Preparations	BIO-68027
JetSeq ER & Ligation Kit (PCR Free)	96 Preparations	BIO-68026
NGS ER Buffer, 5x	1000 Rxn	BIO-68027.C01
NGS ER Enzyme Mix	1000 Rxn	BIO-68027.C02
NGS Ligation Buffer, 5x	1000 Rxn	BIO-68027.C03
NGS Ligase Enzyme	1000 Rxn	BIO-68027.C04
NGS Accu Buffer, 10x	1000 Rxn	BIO-68027.C05
Accuzyme	1000 Rxn	BIO-21052.C01

Please contact us for institutional pricing, special price quotations and availability of bulk pack sizes.

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