igNext™

igNext™ NGS DNA Library Prep Kit for Illumina

Intact Genomics®

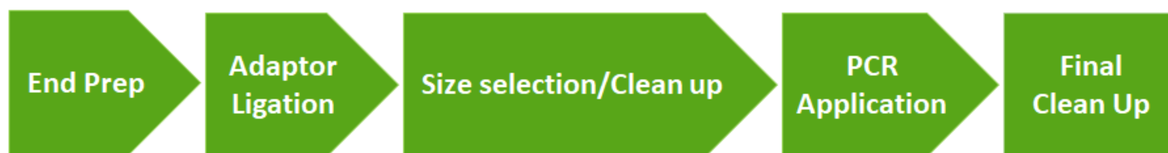
Why choose igNext™ NGS DNA Library Prep Kit?

- **Reliable -** Utilizes the classic protocol for DNA library preparation on the Illumina platform, enabling steady and proven performance for various sample types.
- **Simple -** End repair, dA-tailing and Adaptor ligation finished in the same tube, reducing clean up steps to minimize sample loss, and shortening process time to increase throughput.
- **Low input -** Designed for low input DNA amount (1 – 100 ng).
- **Flexible -** Unique multiplex index primer sets provide a large number of indices (barcodes).
    - Up to 48 unique dual-index and 2,304 dual index combinations - enables a multitude of options for library multiplexing
- **Good performance in large-scale multiplexing -** Superb coverage, without individual NGS library QC.
    - 98% of ~1,500 individual samples (averaging up to 100kb for each sample) can have greater than 100X depth of coverage by a Hiseq Xten lane (2x150 bp)
    - 94% of ~1,500 individual samples (averaging up to 100kb for each sample) can have greater than 5X depth of coverage by a Miseq run (2x75 bp).
- **Affordable –** Competitive prices for all reagents needed to prep NGS library (sample purification beads and unique indexes included!)

Workflow

- **Our igNext™ NGS kit workflow is simple and user-friendly. The process can be done within 3 hours.**

Ultrasonic fragmentation of Genomic DNA
Input 1-100 ng fragmented DNA



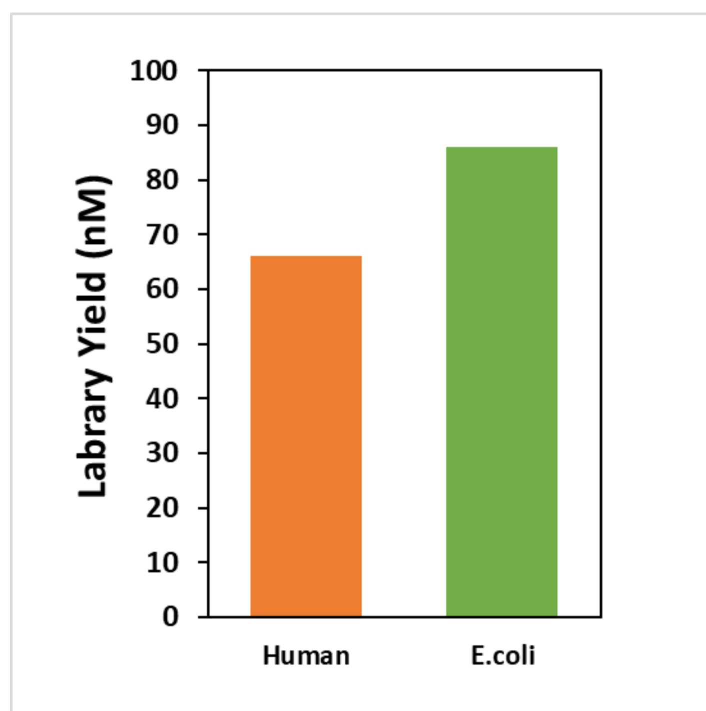
STEP	HANDS-ON	TOTAL
End Prep	2 min	50 min
Adaptor Ligation	2 min	15 min
Size selection / Clean up	4/2 min	50/30 min
PCR Application	2 min	30 min
Final Clean Up	2 min	30 min
Total	12 min	2.5 - 3 hours

Library Yield

- **igNext™ NGS Library Prep Kit Produces High Yield Libraries from Various Organisms.**

Intact Genomics igNext™ NGS Library Prep Kit has been tested extensively using different genomic DNA samples with various genome size and GC content. These sample types include (but not limited to) Human (large genome), *Escherichia coli* (small genome, medium GC content), *Plasmodium falciparum* (low GC content), and *Mycobacterium tuberculosis* (High GC content). As shown in Figure 2 below, the yield of the final purified, size selected library from various genomic DNA sources is high when utilizing the IG NGS Library Prep Kit. Final library yield data demonstrates high overall library preparation performance.

Figure 2. Library yield from genomic DNA of various organisms†



†Libraries were prepared from 100 ng genomic DNA of different organisms. The manufacturers' recommendation was followed for all processes. Library yield was assessed using Agilent 2100 Bioanalyzer with DNA 7500 kit.

Library Quality

In addition to sufficient and successful library yield, the quality of the library is imperative to overall sequencing success. Intact Genomics igNext™ NGS Library Prep Kit was used to obtain high quality sequencing data with a sampling of the results shown below.

IG i7 High-Fidelity DNA Polymerase is a key component in the igNext™ NGS Library Prep Kit. In our tests, the performance of IG i7 High-Fidelity DNA Polymerase was compared with NEBNext® Ultra™ II Q5® Master Mix. During the library preparation process, two equal amounts of input DNA (100 ng) were prepared simultaneously. After the size selection step, the two samples were combined and mixed well, then equally divided into two halves. One sample was amplified using i7 master mix and the other was

amplified using NEBNext® Ultra™ II Q5® Master Mix. Final libraries were sequenced using Illumina MiSeq®.

- **igNext™ NGS Library Prep Kit provides high quality sequencing data**

Table 1. Mapping information of libraries prepared from human genomic DNA

DNA Input	Module	Total Reads	Total Mapped	Duplication	Chimeras
100 ng	IG i7	9,568,162	97.65%	17.04%	0.58%
	NEB Q5	9,083,881	97.90%	18.34%	0.60%

Libraries were prepared from Human genomic DNA using the IG NGS Library Prep Kit and substituting two different High Fidelity DNA polymerases (NEB Q5 and IG i7). Libraries were sequenced on the Illumina MiSeq instrument. Sequencing reads were mapped to the Human GRCh37 reference by Bowtie 2.4.2.

Module: The DNA polymerase master mix used in amplification step

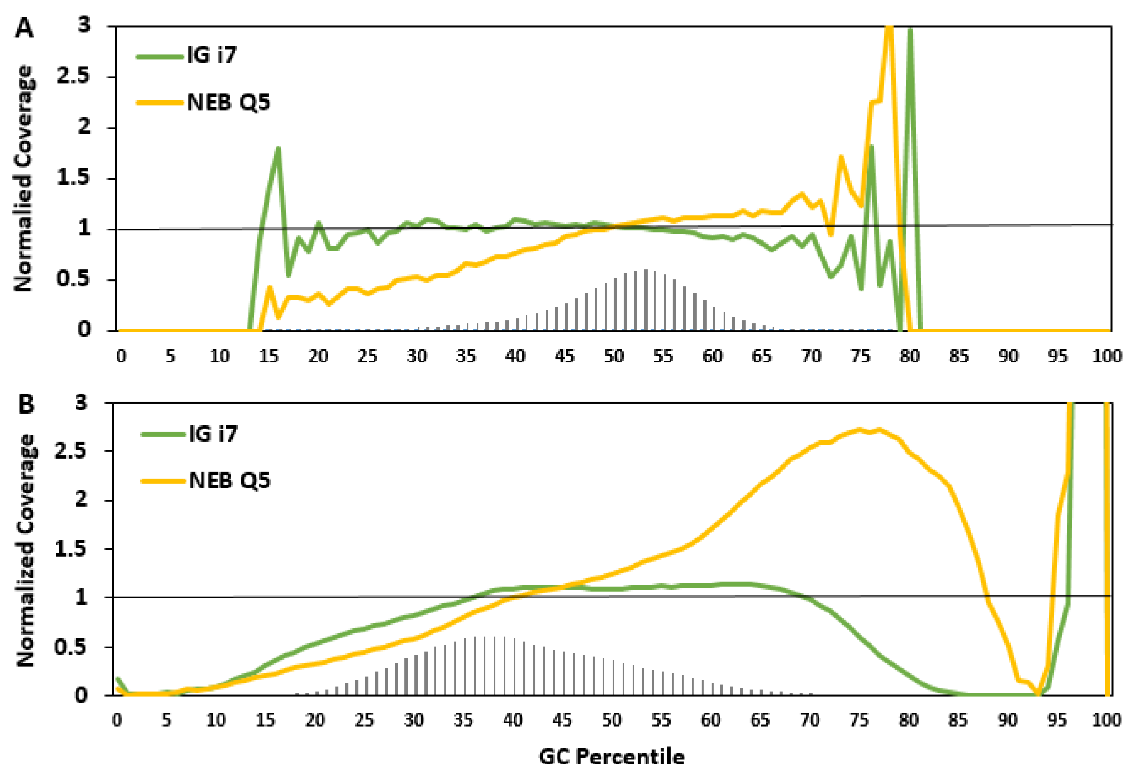
Total Mapped: The percentage of reads mapped to Human GRCh37 reference.

Duplication: The percentage of reads concordantly mapped to reference more than once

Chimeras: The percentage of reads which can mapped to reference but over the maximum insert size.

- **igNext™ NGS Library Prep Kit provides uniform coverage across the GC content of *E.coli* and Human genomes**

Figure 4. Normalized coverage across the GC content of human and E. coli genomic DNA

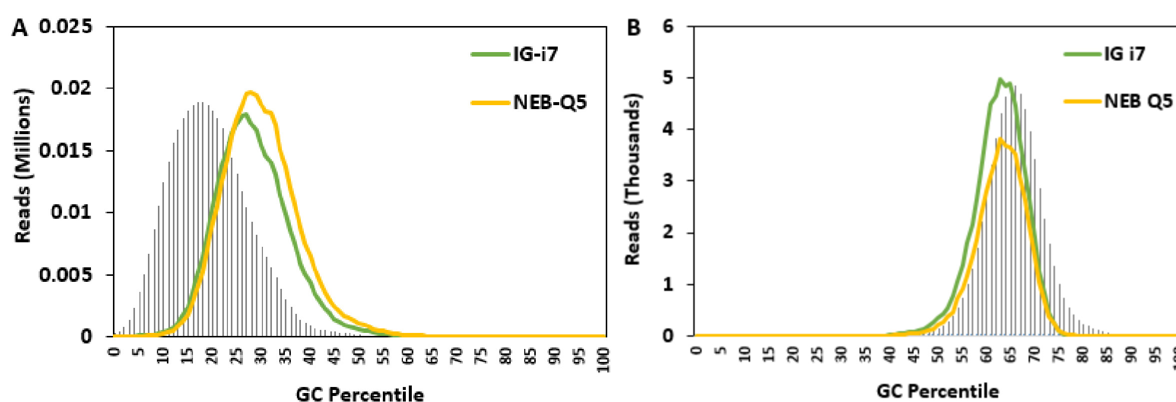


Libraries were prepared from 100 ng *E.coli* (A) and Human (B) genomic DNA using the igNext™ NGS Library Prep Kit, with substitutions between i7 High Fidelity DNA Polymerase and Q5 High Fidelity DNA Polymerase. Sequencing was performed on an Illumina MiSeq. Bowtie 2.4.2 was applied to map reads to the reference genome and GC coverage information was analyzed using Picard's Collect GC Bias Metrics(v2.25). The black horizontal solid line indicates the expected normalized coverage of 1.0. The grey cluster bars represent the number of 100 bp regions at each GC percentile. The normalized coverage for different libraries is indicated by color lines (Fig 4).

- **igNext™ NGS Library Prep Kit performs equivalently or better for low and high GC content genomic DNA**

In this test, due to the limited amount of genomic DNA from *P. falciparum* and *M. tuberculosis*, whole genome application (WGA) was applied to these samples. Libraries were prepared from 100 ng purified WGA amplicons.

Figure 5. The reads distribution of libraries from low and high GC content DNA



Libraries were prepared from 100 ng purified whole genome amplification amplicons of two microbes: *Plasmodium falciparum* (A) and *Mycobacterium tuberculosis* (B), then sequenced on an Illumina MiSeq. The reads were mapped utilizing Bowtie 2.4.2 and read distribution on GC content was analyzed by Picard's Collect GC Bias Metrics(v2.25). The grey cluster bars represent the number of 100 bp regions at each GC percentile. The number of reads for different libraries was indicated by color lines (Fig. 5).

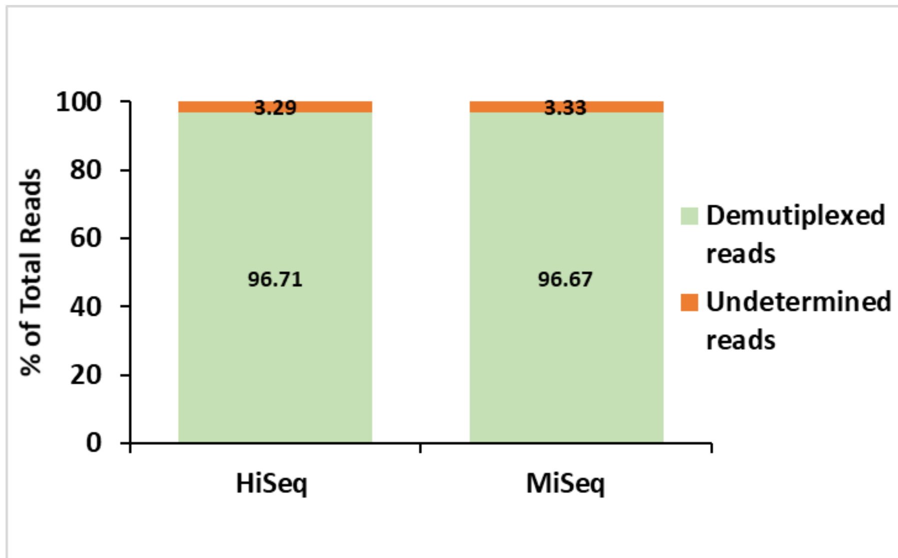
- **Incorporating igNext™ Dual Index Oligos and igNext™ NGS Library Prep Kit enables large-scale multiplexing.**

Many NGS DNA library prep kit providers only offer index primer sets to allow up to 384 sample multiplexing of Illumina libraries. This is a limitation for certain applications that require pooling of extra-large numbers of samples. However, this challenge can be overcome by using the igNext™ NGS Library Prep Kit and igNext™ Dual Index Oligos.

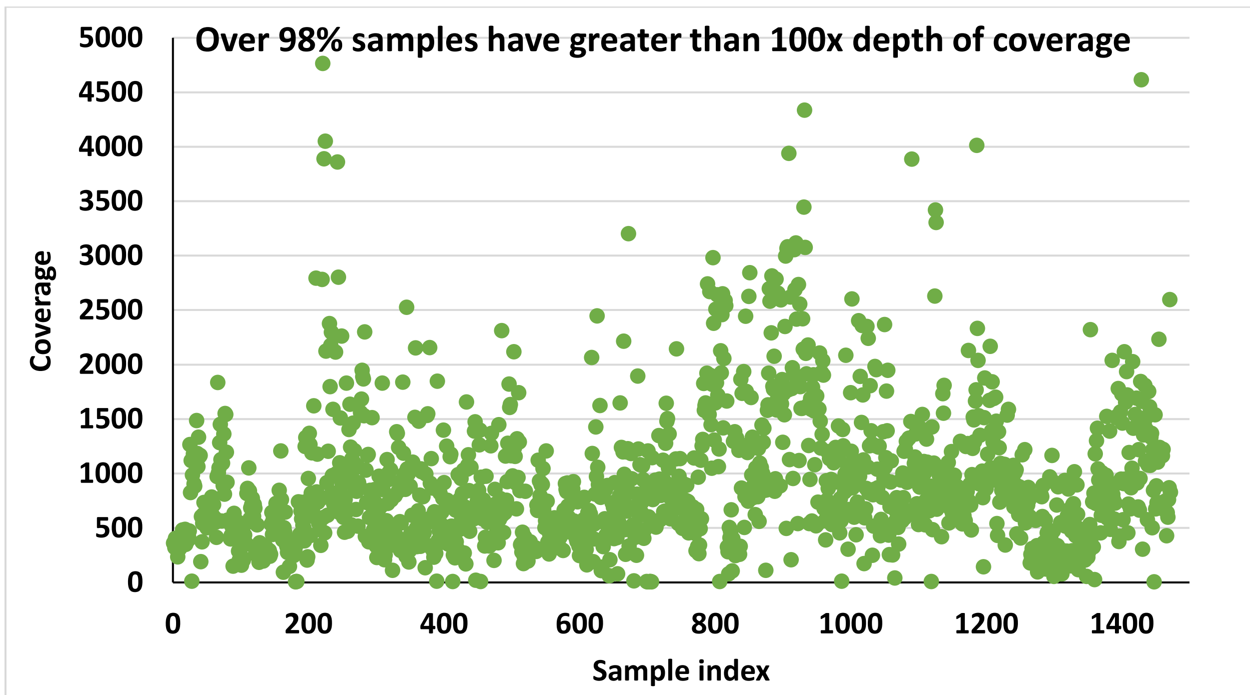
igNext™ Dual Index Oligos contain 48 x 48 unique indexes, these primer sets can be used with IG products as well as with other standard Illumina-compatible library preparation protocols, enabling high yield multiplex Illumina library production.

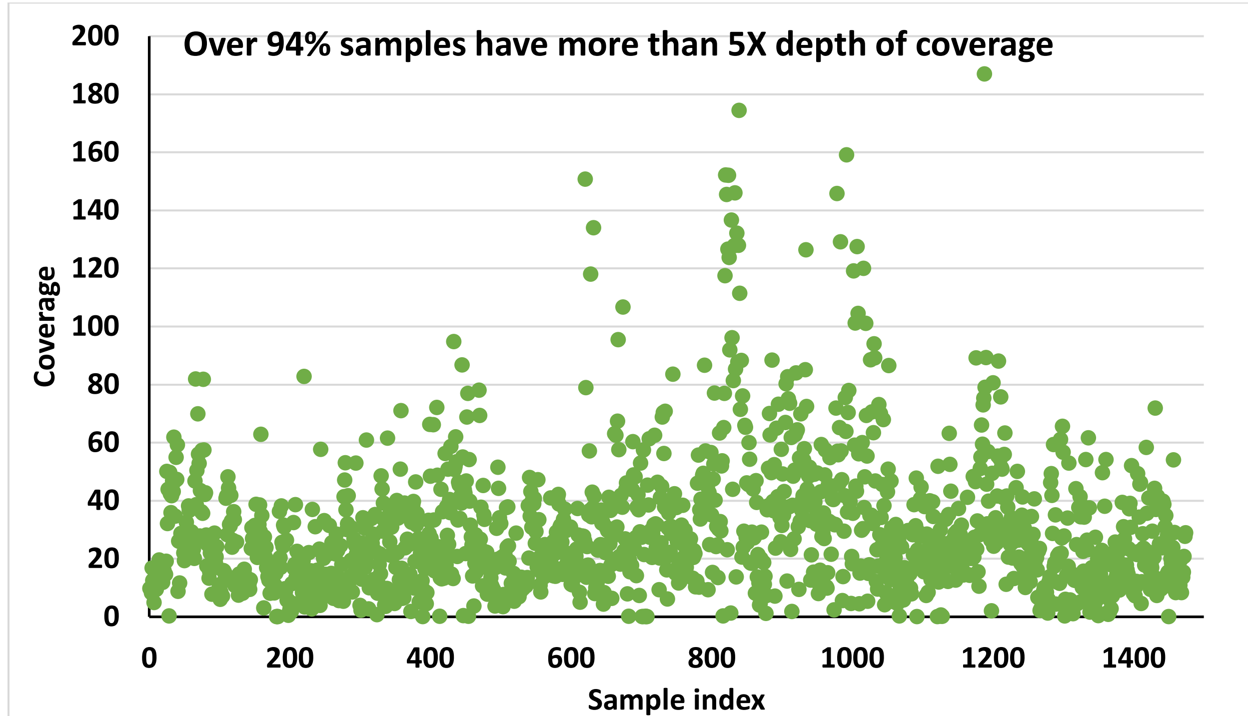
Figure 6. High demultiplexing rate and excellent coverage for pooling of 1472 Illumina libraries

A



B HiSeq





This example shows one set of IG NGS DNA libraries prepared from 1,472 Fungal artificial chromosome (FAC) clones (each clone averaging 100kb) was used to assess the performance of igNext™ Index Primer sets. The individual 1,472 FAC clones were grown independently in 1 mL of culture media in 96-well blocks with antibiotic selection. DNAs of the 1,472 FACs were prepared in 96-well plate format, dissolved in equal volume and sheared to ~600bp by sonication. The DNA concentrations are relatively even, however all differ randomly without justification. The next-gen sequencing (NGS) libraries were prepared with the igNext™ NGS Library Prep Kit according to the manual instructions. A simplified method of NGS library QC was performed by randomly selecting 3 NGS libraries of every 96-well plate and performing QC analysis by Bioanalyzer. The 1,472 NGS libraries various concentrations were pooled at equal volume and the pool was sequenced on both one Hiseq Xten lane (2x150 bp) and one Miseq run (2x75 bp). Reads were demultiplexed using the Illumina bcl2fastq tool (version 1.8.4). On both the Hiseq and Miseq instruments, more than 96% reads have been demultiplexed based on expected dual indexing barcode combinations. On the HiSeq X instrument deep coverage was achieved; 98% FACs have greater than 100X depth of coverage. While on the MiSeq, more shallow coverage was achieved; 94% FACs having more than 5X depth of coverage. To date, we have used igNext™ Index Primer sets (upto 2,304 dual indexes) and igNext™ NGS prep kit for a multitude of runs with the similar results above.