illumina®

Nextera® Custom Enrichment Kit

Customizable probe design, rapid library prep, highly specific enrichment. All wrapped into one simple solution.

Highlights

- **Rapid Nextera Library Preparation**
  Prepare sample libraries in less than 3 hours with no need for mechanical DNA shearing
- **End-to-End Enrichment Protocol**
  Enrich 500 kb to 25 Mb of custom content using the most streamlined library preparation and enrichment solution available
- **Low DNA Input**
  Process samples with only 50 ng of DNA; Get superior data quality

Figure 1: Nextera Custom Enrichment Kit

A fully customizable, end-to-end targeted resequencing solution for generating custom-enriched pooled sample libraries.

Introduction

The Nextera Custom Enrichment Kit is a complete library preparation and a fully customizable enrichment solution that enables researchers to prepare samples in less than 3 hours with no need for mechanical DNA shearing, and deliver fully enriched libraries in just 2.5 days (Figure 1). Based on ground-breaking Nextera technology, sample libraries can be rapidly generated from 50 ng of DNA. Now researchers can easily sequence and enrich samples that yield very low amounts of DNA, while obtaining excellent data quality. Developed and optimized for Illumina sequencing technology, this end-to-end solution provides the fastest and simplest approach for sequencing specific subsets of the genome to discover and validate novel variants, or examine specific genes in pathways.

Simplified Probe Design

Researchers can initiate a custom enrichment experiment by designing a panel of interest using DesignStudio™, a freely available and simple online tool that provides dynamic feedback to optimize target coverage and estimate total project pricing (Figure 2). After logging on to a personalized account, naming the project, and choosing the reference genome build of choice, you can select coordinates or gene names (individually or batch uploaded), and the probe design option of exons or full genomic regions. Capture probe design is automatically performed using an algorithm that considers a range of factors, including GC content, specificity, spacing, and coverage. Candidate probes are then visualized and assessed using estimated success scores. The probe design success rate is typically 95% or better, depending on the sequence. Factors like sequence homology and the percentage of GC content can cause higher or lower success rates.

Probes can be filtered with user-defined tags, added to, or removed from the design. After visualization and QC, the custom probe library is added to the final design, and can then be ordered along with the recommended sequencing reagents (cluster generation and sequencing by synthesis kits). All the information can be saved, reordered, or copied to a new project. To use DesignStudio, a MyIllumina account is required. Accounts can be requested at https://icom.illumina.com/Account/Register.

Streamlined Workflow

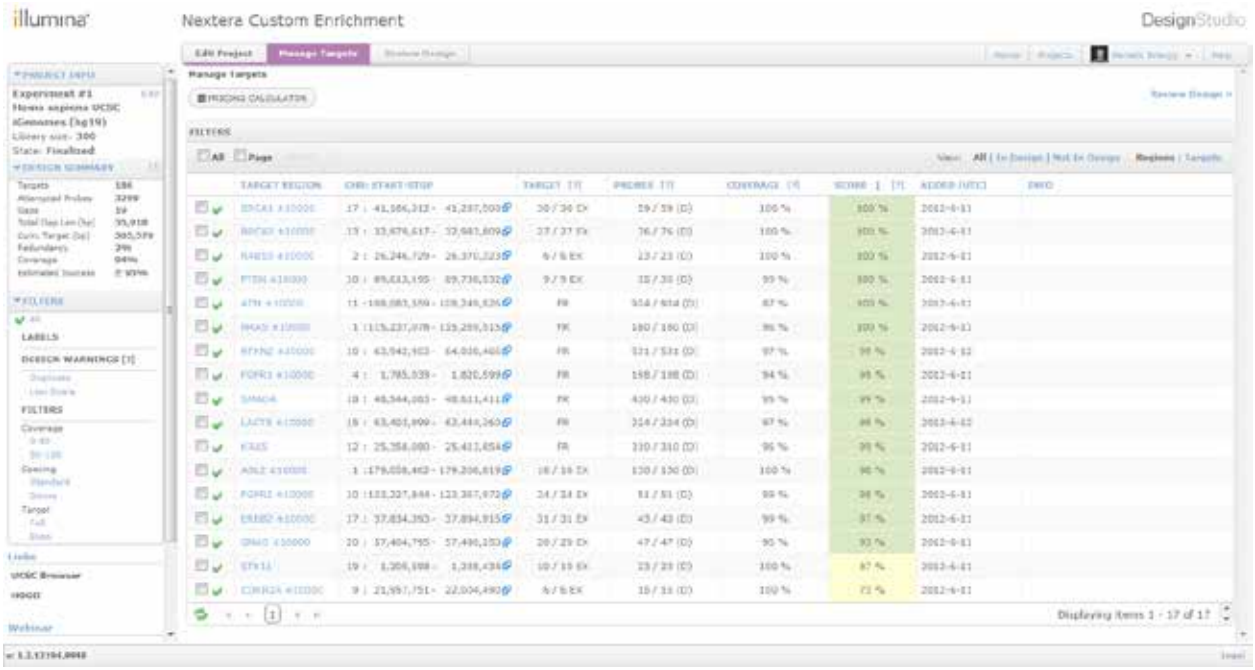
Leveraging the speed of Nextera sample library preparation technology and unique 12-plex pre-enrichment sample pooling, the Nextera Custom Enrichment method reduces hands-on time for a cost-effective, high-throughput workflow that saves at least one full day over all other currently available enrichment workflows (Figure 3). Master-mixed reagents are coupled with plate-based processing for up to 8 enrichment reactions (96 samples), and volumes are optimized for liquid handlers to make the process automation-friendly for even higher throughput.

Library Preparation

The workflow begins with breakthrough Nextera library preparation technology. Using a single “tagmentation” enzymatic reaction, sample DNA is simultaneously fragmented and tagged with adapters. An optimized, limited-cycle PCR protocol amplifies tagged DNA and adds sequencing indexes. From start to finish, the complete Nextera protocol is over 80% faster than other available library preparation methods, and requires the least amount of hands-on time.

Nextera Custom Enrichment kit provides an innovative solution for pre-enrichment pooling of up to 12 sample simultaneously. During library preparation, each gDNA fragment is tagged with a unique combination of two indices, which will be used to demultiplex the data post-sequencing. The indexed sample libraries are then pooled prior to enrichment.

Figure 2: DesignStudio for Custom Probe Design



Manage Targets screen showing Target Region view in DesignStudio. Easily visualize genomic target regions and probes, and assess coverage and score. Design Summary metrics for the entire project are located on the left sidebar, along with project information and user defined labels for convenient data sorting during the design phase.

Library Enrichment

During enrichment, the pooled libraries are denatured into single-stranded DNA, then hybridized to biotin-labeled custom oligonucleotide capture probes specific to the targeted region(s). Streptavidin beads are added to bind to the biotinylated probes. Biotinylated DNA fragments bound to the streptavidin beads are magnetically pulled down from the solution. The enriched DNA fragments are then eluted from the beads and re-hybridized for a second enrichment reaction. After amplification of the enriched regions, the targeted library is ready for cluster generation and sequencing.

Data Analysis

Sequence data from custom enrichment samples are used to generate two sets of statistics: post-alignment and post-CASAVA (Consensus Assessment of Sequence and Variation) analysis. Post-alignment analysis counts the number of reads that overlap any targeted region and defines whether a read falls within a target. Post-CASAVA analysis calculates the coverage at each base within a region. Data can be visualized using GenomeStudio® Data Analysis Software to examine the on-target and off-target coverage in a sample.

Unsurpassed Data Quality

With Nextera Custom Enrichment, researchers can be confident in the quality of sequencing data generated from pooled multi-sample libraries. Shown in Figure 4 is a custom panel targeting approximately 1 Mb of total content with ~6,000 oligos. Each sample is sequenced with high coverage uniformity across the target region, with > 80 % of bases covered at 0.2x the mean read depth. The Nextera enrichment protocol is also highly specific for the target region of interest. In a 12-sample experiment, each demulitplexed sample library demonstrated > 80% target enrichment across all samples (Figure 5).

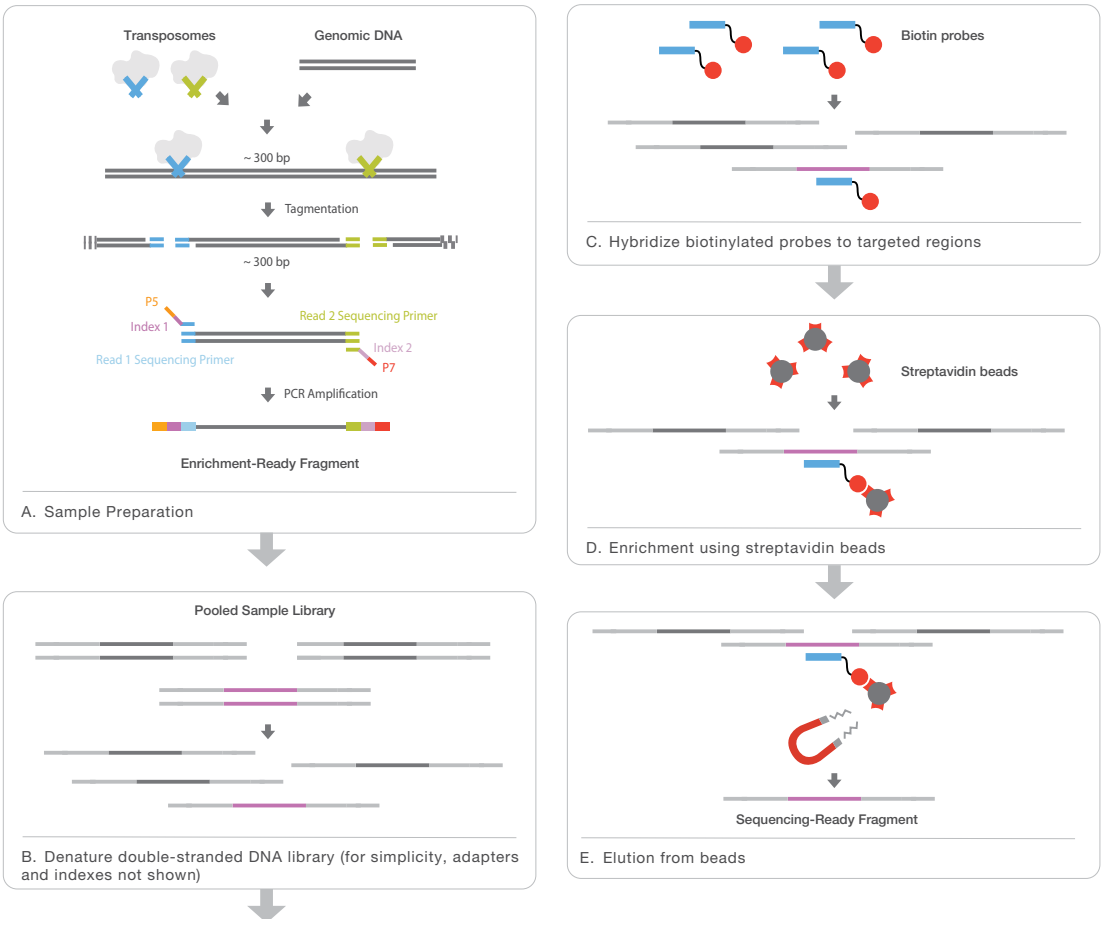
Optimizing Targeted Resequencing

To maximize the efficiency of targeted resequencing studies and to ensure that sufficient coverage is obtained for highly sensitive variant calling, three key factors should be taken into account:

1. Sum length of targeted regions, equaling the total amount of targeted sequence
2. Enrichment efficiency (percentage of reads passing filter and mapping to targeted regions)
3. Distribution of coverage depth for targeted regions

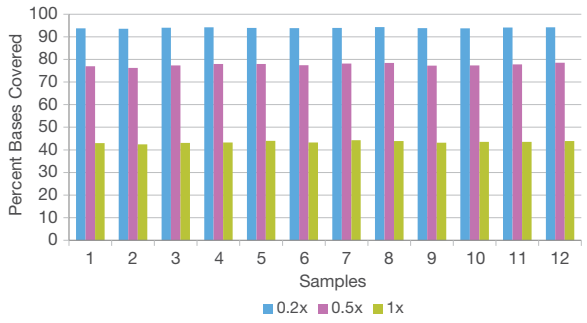
These key parameters and a method for pre-calculating the amount of sequencing and mean coverage required to fully optimize any targeted sequencing study is described in greater detail in the Optimizing Coverage for Targeted Resequencing Technical Note.[1]

Figure 3: Nextera Custom Enrichment Workflow



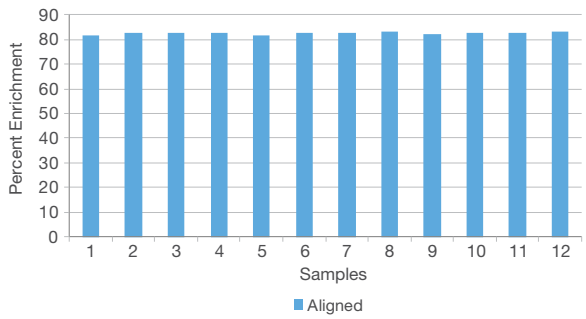
The Nextera Custom Enrichment Kit provides a simple and streamlined in-solution method for isolating and enriching targeted regions of interest.

Figure 4: Excellent Coverage Uniformity



Coverage uniformity is given for 12 samples with respect to the percentage of bases covered across the 1 Mb target region at varying mean normalized read depths. The 12 samples were prepared and then simultaneously enriched using the Nextera Custom Enrichment Kit. The pooled samples were sequenced across one lane of a HiSeq® flow cell, generating mean read depths of 128–158× (varying for each sample). Over 80% of bases were covered at 0.2× mean coverage.

Figure 5: High-Target Specificity



Percent Enrichment is defined as the number of reads mapping to the targeted regions out of the total reads produced in a sequencing run (on a per-sample basis). The 12 samples shown here were prepared and enriched using the Nextera Custom Enrichment Kit, then sequenced on the Genome Analyzer IIx. More than 80% enrichment was achieved across all 12 samples in the pool.

Summary

Nextera Custom Enrichment Kits offer unmatched speed and simplicity for targeted resequencing. Starting from just 50 ng of DNA, researchers can prepare samples in < 3 hours and complete enrichment of up to 96 samples in 2.5 days. Designing and ordering custom probes to target any region of interest is conveniently done using DesignStudio, an intuitive online tool that provides step-by-step guidance. Researchers will enjoy remarkable performance with superior data quality that they can rely on.

Learn More

To learn more about complete solutions for targeted resequencing, visit www.illumina.com/applications/sequencing/targeted_resequencing.ilmn.

References

1. Optimizing Coverage for Targeted Resequencing Technical Note.

Nextera Custom Enrichment Details

Enrichment Efficiency*	> 55-60% (on target) > 60-65% (+/-150 bp)
Coverage Uniformity (0.2× mean)*	> 80%
Content Range	500 kb to 25 Mb
Number of Oligos	2,500–67,200
Samples in Pre-Enrichment Pooling	Up to 12
Library Prep Input	50 ng
Library Size	250–450 bp

*Target values will vary due to custom designs.

Ordering Information

Product	Description	Catalog No.
Custom Oligo Set	Custom oligos designed against target regions of interest	FC-121-0200
Nextera Custom Enrichment Kit (48 Samples)	Contains reagents for preparing and enriching up to 48 samples at 12-plex. Includes 14 dual-indexes supporting 24 index cominations.	FC-121-1204
Nextera Custom Enrichment Kit (96 Samples)	Contains reagents for preparing and enriching up to 96 samples at 12-plex. Includes 14 dual-indexes supporting 24 index cominations.	FC-123-1208
Nextera Custom Enrichment Kit (288 Samples)	Contains reagents for preparing and enriching up to 288 samples at 12-plex. Includes 20 dual-indexes supporting 96 index cominations.	FC-123-1224

Illumina • 1.800.809.4566 toll-free (U.S.) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

FOR RESEARCH USE ONLY

Pub. No. 770-2012-015 Current as of 12 November 2014

illumina®