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Nextera® Rapid Capture Custom Enrichment

Leverage a superior library preparation and enrichment workflow for unparalleled access to your regions of interest.

Highlights

- **Integrated Library Preparation and Enrichment Workflow**
  Nextera tagmentation and optimized hybridization reduce workflow duration, generating data faster
- **Target Regions of Interest**
  Choose 0.5–15 Mb of custom content, and pool up to 12 samples per enrichment reaction
- **Evolve Design With Add-On Content**
  Supplement existing panels and keep adding on as research needs expand

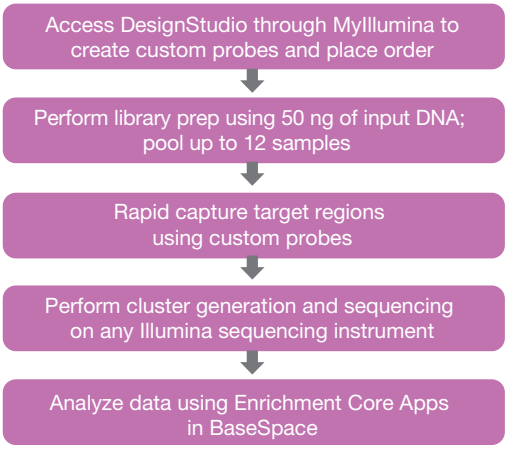

Figure 1: Overview of Nextera Rapid Capture Custom Enrichment. The Nextera Rapid Capture Custom Enrichment Kit is an integral part of a complete and fully supported solution for targeted resequencing.

Introduction

Nextera Rapid Capture Custom Enrichment is an all-in-one assay for library preparation and custom target enrichment. Nextera tagmentation coupled with optimized target capture ensures the fastest enrichment workflow time for custom content. The flexible, fully customizable design accommodates up to 15 Mb of custom content so you can focus on the regions of the genome that you care about. The new add-on feature in the DesignStudio™ tool allows you to expand content iteratively as new discoveries are made.

Custom Probe Design

The first step in developing any Nextera Rapid Capture Custom Enrichment assay is to design your custom probe set. DesignStudio is a free, online, user-friendly tool accessed through your MyIllumina account. Designate your regions of interest, refine your custom probe set, and place an order for your custom design. DesignStudio uses a complex algorithm to optimize probe set design and alert you to any potential coverage gaps or challenging regions. Desired targets can be added individually or in batches by chromosomal coordinate or gene name.

Unmatched Ease of Workflow

Nextera Rapid Capture Custom Enrichment allows researchers to maximize the productivity of their lab personnel and Illumina sequencing technology (Figure 1). The simplicity and speed of the Nextera Rapid Capture assay enables a single technician to prepare and enrich 12 samples in only 1.5 days.

Nextera-based library preparation generates adapter-tagged libraries from 50 ng input genomic DNA (Figure 2A). Nextera tagmentation of DNA simultaneously fragments and tags DNA without the need for mechanical shearing. Integrated sample barcodes allow the pooling of up to 12 of these adapter ligated sample libraries into a single, hybridization-based, pulldown reaction. The pooled libraries are then denatured into single-stranded DNA (Figure 2B) and biotin-labeled probes complementary to the targeted region are used for the Rapid Capture hybridization (Figure 2C). Streptavidin beads are added, which bind to the biotinylated probes that are hybridized to the targeted regions of interest (Figure 2D). Magnetic

pulldown of the streptavidin beads enriches the targeted regions that are hybridized to biotinylated probes. (Figure 2E). The enriched DNA fragments are then eluted from the beads and a second round of Rapid Capture is completed to increase enrichment specificity. The entire process is completed in only 1.5 days, enabling a single researcher to process up to 12 samples efficiently and at one time—all without automation.

Data Analysis

Sequence data generated from custom enrichment samples on HiSeq® and NextSeq® Systems are analyzed using the Enrichment Core Apps in the BaseSpace® environment. There are 2 Core Apps available for analysis: BWA Enrichment App v2.0, which provides industry-standard BWA alignment and GATK variant calling, and Isaac™ Enrichment App v2.0, the Illumina pipeline optimized for rapid analysis.

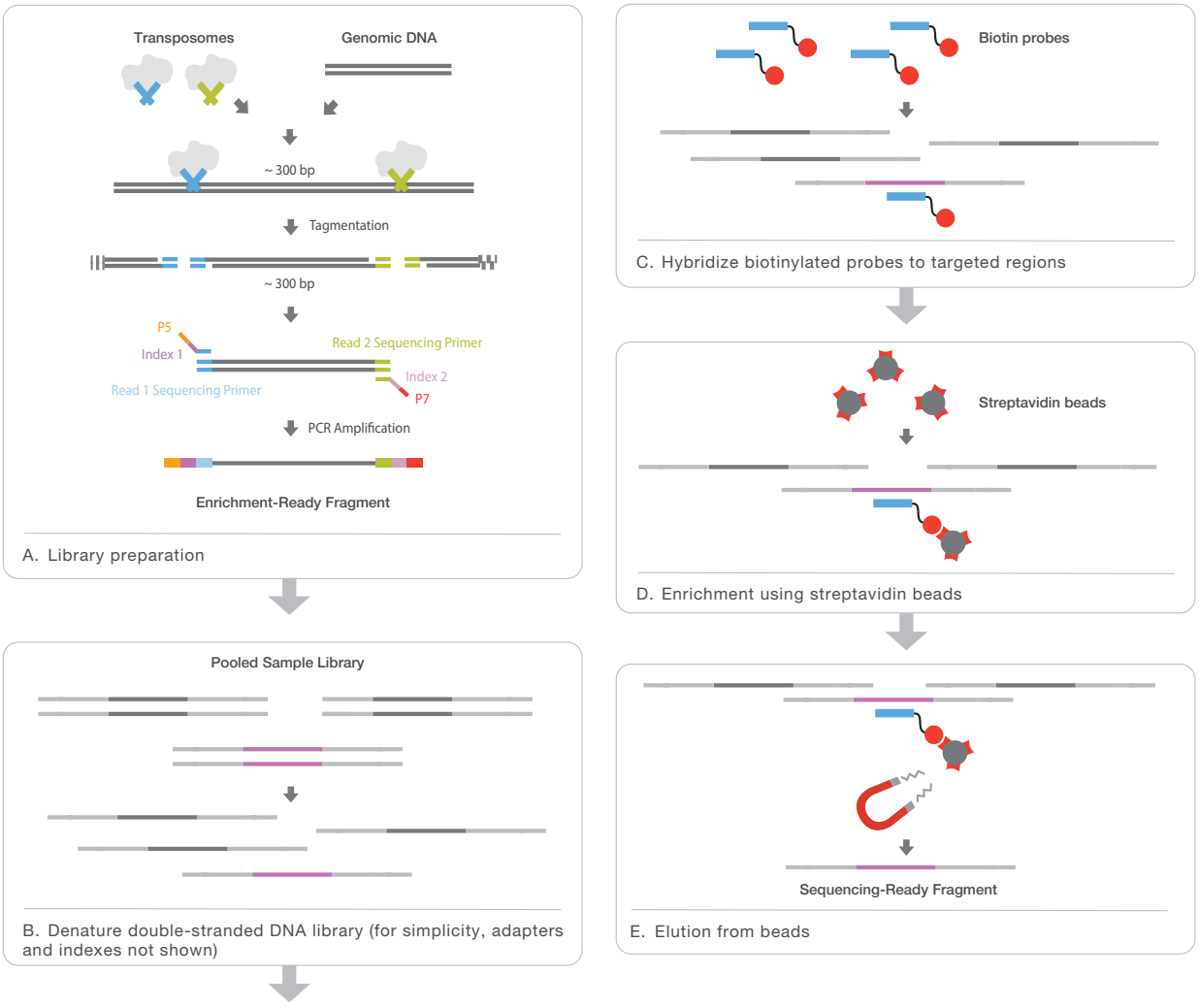

Figure 2: Nextera Rapid Capture Workflow. Nextera Rapid Capture Custom Enrichment provides a simple and streamlined in-solution method for isolating and enriching targeted regions of interest. The workflow combines library preparation and exome enrichment steps, and can be completed in 1.5 days with minimum hands-on time.

Custom pools sequenced on the MiSeq® System can be analyzed using the BaseSpace Core Apps or MiSeq Reporter. All analysis workflows generate aligned sequence reads in BAM format. Variant calling occurs in the target regions specified in the manifest file. The variant callers generate VCF files that contain genotype, annotation, and other information across all sites in the specified target region. Coverage files containing coverage depth in the genome and within gaps is also generated (.CoverageHistogram.txt, .gaps.csv). Additionally, enrichment summary statistics are provided via the .enrichment_summary.csv file or through the CalculateHSMetrics.jar tool within the Picard Suite (.HSmetrics.txt). The enrichment files contain a summary of the on-target and off-target reads/base, average coverage in the target region, % reads that are present at 1×, 10×, 20×, and 50× coverage, read/base enrichment, and variant call information. Variant call information includes the number of variants (SNPs and indels), Het/Hom, and Ts/Tv ratios as well as the overlap with a standard curated database.

Data Examples

Four different Nextera Rapid Capture Enrichment experiments were performed following the workflow described in Figure 2. Each project included different target regions and coverage depths (Table 1). Representative enrichment and coverage data are shown in Figure 3. In all multiplexed projects, high percent enrichment was achieved, and mean normalized coverage plots show that > 85% of bases are covered at 0.2× of the mean coverage. Figure 4 shows that supplementing an existing design (Nextera Rapid Capture Exome) with custom add-on content does not notably decrease coverage uniformity.

Table 1: Sequencing Details for Example Projects.

Project	Content	Mean Coverage	% On-Target Bases[a]
1[b]	0.5 Mb	1500×	88.6
2[b]	0.5 Mb	146×	79.5
3[c]	3.5 Mb	300×	80.1
4[b]	7 Mb	152×	72.5

a. Calculated using Picard Hybrid Selection tool with 250 bp padding[1]
b. Sequenced on HiSeq
c. Sequenced on MiSeq

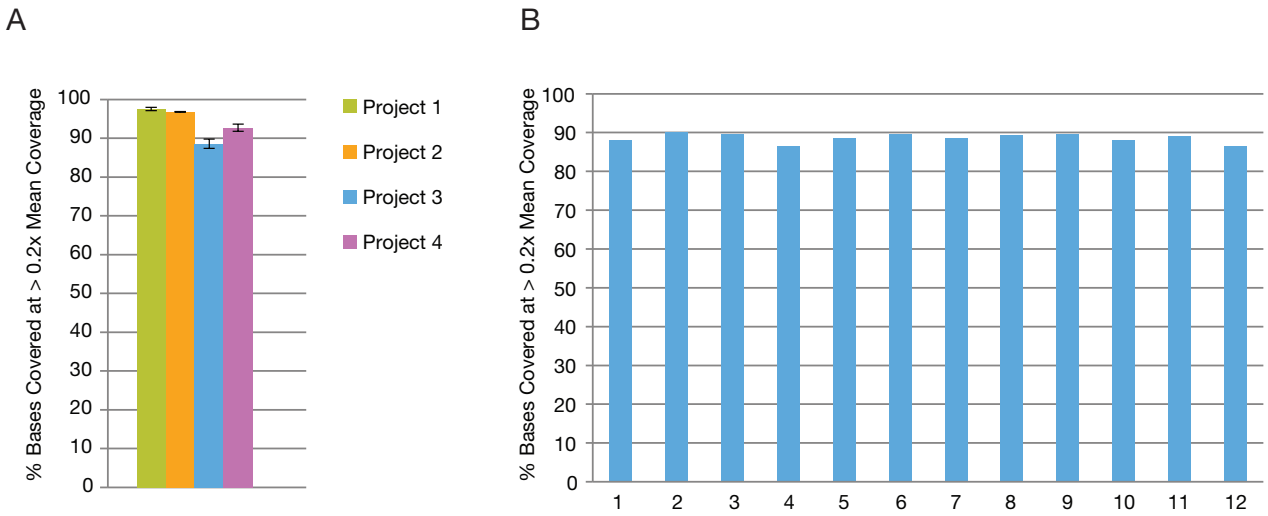

Figure 3: High Coverage Uniformity Across Custom 12-Plex Pools. Nextera Rapid Capture Custom Enrichment provides uniform target enrichment across different custom probe sets and individual samples within a 12-plex pool. **A.** Coverage uniformity is shown as % of targeted bases represented by > 0.2× mean coverage. Mean coverage for these custom probe sets can be found in Table 1. Error bars show SD of uniformity across the 12 pooled samples for each project. **B.** Coverage uniformity for each of 12 pooled samples within Project 3 is shown. Mean coverage for this run was 300×, and % of targeted bases that were covered at > 60× are shown.

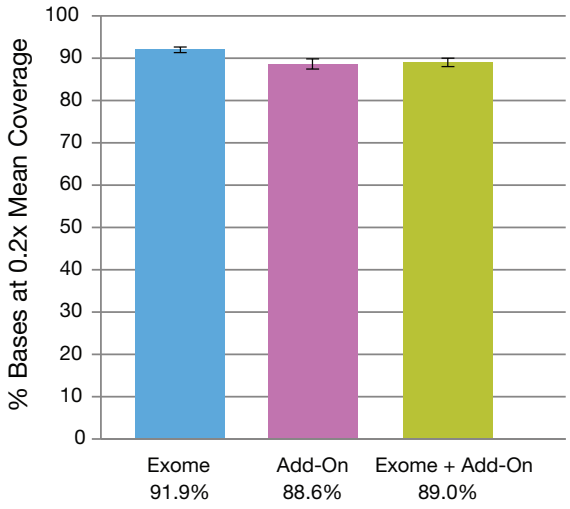

Figure 4: Add-On Content Retains High Coverage. High coverage uniformity is maintained when 3.5 Mb of add-on content is added to the Nextera Rapid Capture Exome. All samples were run as 12-plex pools.

Summary

Nextera Rapid Capture Custom Enrichment leverages a superior, integrated library preparation and enrichment workflow to provide unparalleled access to genomic regions of interest. Not only are researchers able to perform targeted sequencing using only 50 ng of input DNA—they can now do so faster and more efficiently than ever before. Take advantage of robust add-on functionality to refine content over time, or add regions of unique interest to established panels such as Nextera Rapid Capture Exome or TruSight® content sets.

Learn More

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

Nextera Rapid Capture Custom Enrichment Details.

Parameter	Specification
Enrichment efficiency[d]	> 70%
Coverage uniformity (0.2× mean)	> 85%
Content range	0.5–15 Mb
Samples in pre-enrichment pooling	Up to 12
Sample input	50 ng
Library insert size	230 bp

d. Target values will vary due to custom designs.

Ordering Information.

Product	Catalog No.
Nextera Rapid Capture Custom (48 samples) Compatible with designs of 3000–10,000 custom enrichment probes	FC-140-1007
Nextera Rapid Capture Custom (96 samples) Compatible with designs of 3000–10,000 custom enrichment probes	FC-140-1008
Nextera Rapid Capture Custom (288 samples) Compatible with designs of 3000–67,000 custom enrichment probes	FC-140-1009

Reference

1. Picard Tools - By Broad Institute (broadinstitute.github.io/picard) Accessed 12 January 2015.

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