TruSeq® Custom Amplicon v1.5

A new and improved amplicon sequencing solution for interrogating custom regions of interest.

Highlights

- **Simple assay customization**
  Easily create projects that include up to 1536 amplicons in a single reaction, across a range of amplicon sizes and reference genomes
- **Optimized reagents**
  Achieve high performance from as little as 50 ng of DNA, even in challenging GC-rich regions
- **Rapid sample processing**
  Prepare up to 96 reactions simultaneously using simple plate-based format, and standard lab equipment
- **Easy, integrated analysis**
  Access automated, design-specific variant calling, and data analysis with preconfigured MiniSeq® and MiSeq® software

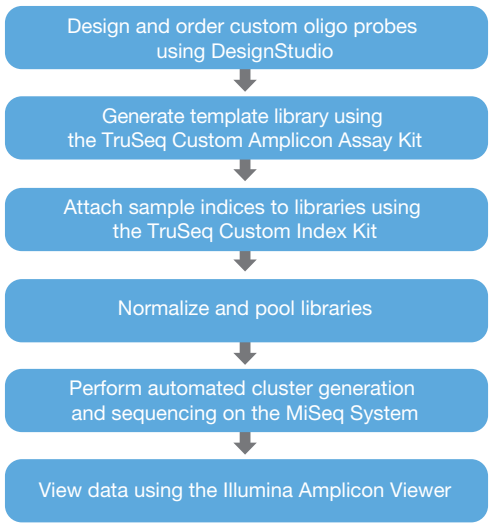

Figure 1: TruSeq Custom Amplicon Workflow—The TruSeq Custom Amplicon assay is a fully integrated end-to-end amplicon sequencing solution, including online probe design and ordering, assay, sequencing, automated data analysis, and offline software for reviewing results.

Introduction

The TruSeq Custom Amplicon Library Preparation Kit allows researchers to sequence hundreds of genomic regions covering as little as 2 kb up to 650 kb of cumulative sequence. This highly targeted approach enables a wide range of applications for discovering, validating, and screening genetic variants in a rapid and efficient manner.

Requiring as little as 50 ng of starting gDNA, the TruSeq Custom Amplicon assay is amenable to a wide range of samples, even those available in limited quantity. With the ability to generate up to 1,536 amplicons per reaction and integrated indexes to support up to 96 samples per MiSeq run, the TruSeq Custom Amplicon assay provides an unprecedented level of sample multiplexing.* DesignStudio™ supports TruSeq Custom Amplicon designs with a range of amplicon sizes optimized for different sample types (eg formalin-fixed paraffin-embedded) and reference genomes (eg human, mouse, rat, and bovine). Entire projects can now be accomplished in only days, leveraging the long paired-end–read capability, speed, and high data quality of the MiSeq System.

The TruSeq Custom Amplicon Library Preparation Kit offers a fully integrated DNA-to-data solution, including convenient online probe design and ordering, a streamlined workflow, and automated data analysis. The Illumina Amplicon Viewer software allows researchers to easily review project data, including variants detected, across multiple MiSeq runs.

TruSeq Custom Amplicon Workflow Overview

The entire TruSeq Custom Amplicon process takes only 2 days to go from DNA to data. Researchers can initiate a project by entering target regions of the genome into DesignStudio software (Figure 1). After a custom design has been ordered, oligonucleotide probes are synthesized and pooled into a Custom Amplicon Tube (CAT) containing all the probes necessary to generate up to 1536 attempted amplicons per reaction. Sample-specific indexes are then added to each library by PCR using common primers from the TruSeq Amplicon Index Kit. An integrated bead-based normalization procedure enables simple volumetric pooling of libraries before sequencing on the MiSeq System and avoids laborious quantification methods. Pooled libraries are then loaded into the MiSeq System for automated cluster generation, sequencing, and data analysis, including variant calling. Finally, the Illumina Amplicon Viewer can be used to view project data across many sequenced samples.

* Also compatible with the MiniSeq, NextSeq® Series, and HiSeq® Series Systems.

Simplified Probe Design

Design TruSeq Custom Amplicon probes targeting user-defined regions using DesignStudio, a free, easy-to-use online software tool that provides optimized coverage and estimates total project pricing (Figure 2). After logging on to a personalized account and naming the project, researchers can select coordinates for targeting genomic regions of interest. Probe design is automatically performed by using an algorithm that considers a range of factors, including GC content, specificity, probe interaction, and coverage. Candidate amplicons are visualized and assessed using estimated success scores. Probes can be filtered with user-defined tags, and then added to, or removed from, the design. After visualization and QC, the CAT design can then be ordered along with suggested accessory kits.

Typical design success, depending upon custom-selected content, is 90% or better for desired bases (Table 1). In some cases, higher or lower success rates can be achieved, depending upon the sequence context (eg, regions of homology or %GC content). To use DesignStudio, customers will need an account on the Illumina ecommerce system. Request an account at icom.illumina.com/Account/Register.

Optimized Assay Chemistry

The TruSeq Custom Amplicon v1.5 assay chemistry begins with hybridizing 2 custom-designed probes upstream and downstream of the region of interest (Figure 3). Each probe contains unique, target-specific sequence as well as a universal adapter sequence that is used in a subsequent amplification reaction. The addition of an improved hybridization buffer, OHS2, ensures that the probes are optimally bound across regions of varying GC-richness and improves assay performance in potentially difficult- to-address regions (Figure 4). A proprietary extension-ligation reaction extends across the region of interest, followed by ligation to unite the 2 probes and yield a library of new template molecules with common ends. During this step, an enhanced extension-ligation mix, ELM4, increases library yield and uniformity (Table 2).

While the increase in uniformity may appear modest, many amplicons that are poorly represented when using ELM3 achieve significantly higher read counts with ELM4, enabling higher confidence for variant calling in these regions. Extension-ligation templates are PCR-amplified and 2 unique, sample-specific indexes are incorporated. Final reaction products are converted to a single-stranded, adapter-ligated, normalized library using a bead-based protocol. The sequence-ready library can be stored in the optimized LNS2 storage buffer for up to 3 weeks, or loaded directly onto the MiSeq System without additional processing.†

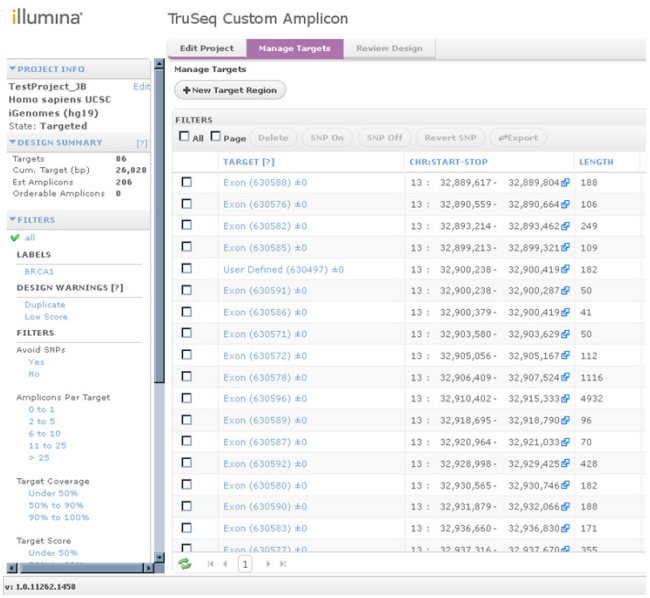

Figure 2: DesignStudio for Custom Probe Design—The Manage Targets Screen shows a Target Region view in DesignStudio software. Easily visualize targeted genomic regions and attempted amplicons to assess design coverage and score. Summary metrics for the entire project are on the left pane, along with project information and user-defined labels for convenient data sorting during the design phase.

Table 1: DesignStudio Parameters for Custom Amplicon

Parameters	Project A	Project B	Project C	Project D
Total Sequence Input (bp)	34,767	69,423	205,441	4,572
Total Number of Amplicons	293	264	1,434	114
Designability Score (% Base Coverage)	98.3	97.0	93.2	91.3

† Also compatible with the MiniSeq, NextSeq Series, and HiSeq Series Systems.

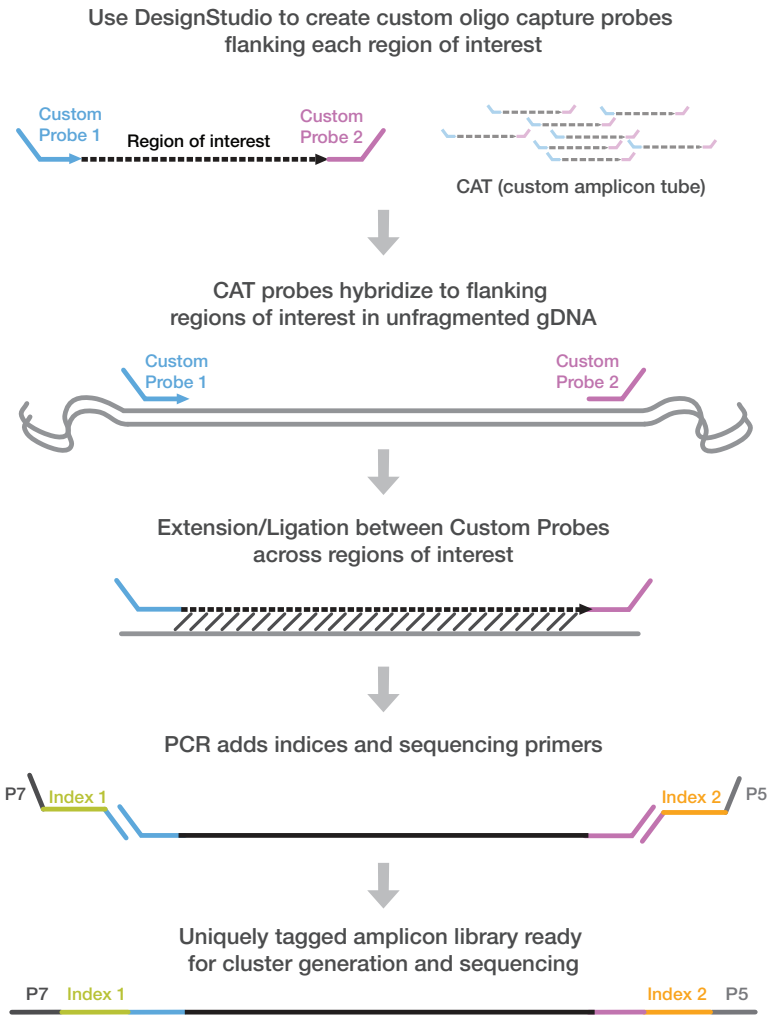

Figure 3: TruSeq Custom Amplicon Workflow—The TruSeq Custom Amplicon assay is a simple and streamlined method for capturing and amplifying targeted regions of interest.

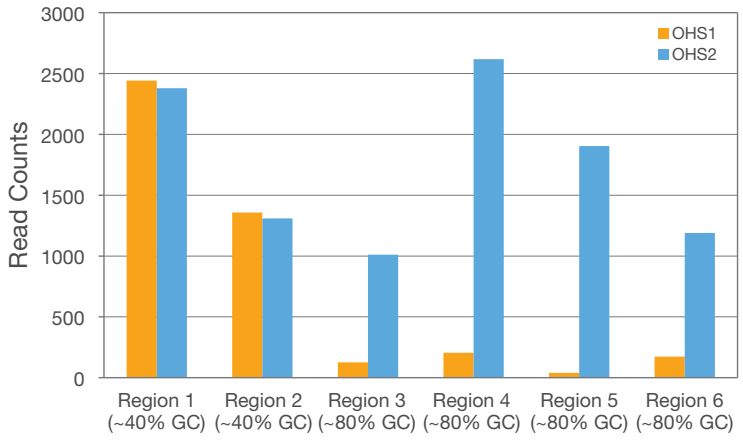

Figure 4: DeOHS2 Increases Read counts for GC-Rich Targets—The optimized OHS2 hybridization buffer with TruSeq Custom Amplicon v1.5 significantly improves assay performance in challenging GC-rich regions.

Table 2: DesignStudio Parameters for Custom Amplicon

		Coverage Uniformity[a]			Read Count[b]		
Design	# Amplicons	ELM3	ELM4	Increase	Amplicon	ELM3	ELM4
1	1483	93%	94%	1%	1	10	315
2	217	89%	91%	2%	2	6	63
3	1483	86%	87%	1%	3	8	28
4	1420	86%	87%	1%	4	8	22
5	115	83%	85%	2%			
Average		87%	89%	2%			

a. Coverage uniformity (defined as total bases present at > 0.2× mean coverage), across designs and in poorly represented regions, increases with ELM4 extension-ligation mix.

b. Read count for representative under-performing amplicons increases significantly with the addition of ELM4 extension-ligation mix.

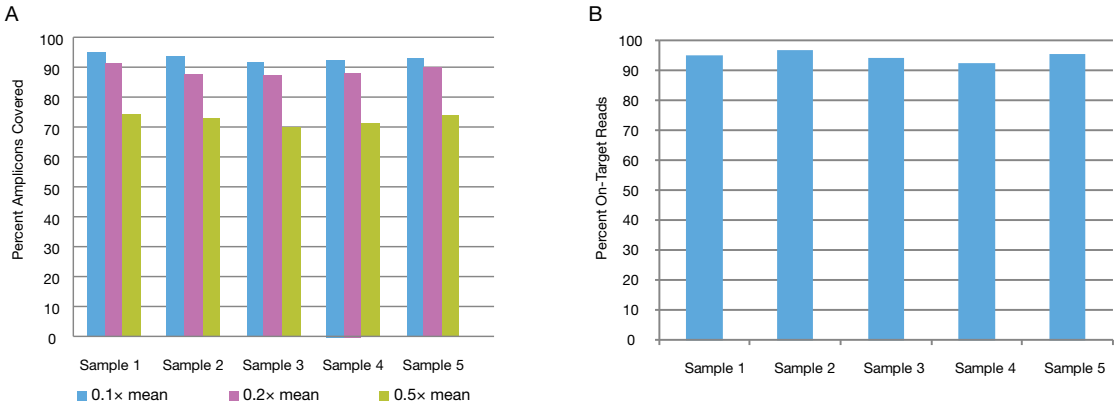

Figure 5: High Coverage Uniformity and Specificity—A. Uniformity measured as reads mapping to target regions out of total reads per run, is shown for 5 samples of a representative target panel. For all samples, > 85% of bases are covered at 0.1× and 0.2× of the mean coverage (blue and purple bars, respectively), and > 65% of bases are covered at 0.5× of the mean coverage (green bars). B. Specificity defined as percentage of filtered reads corresponding to user-defined targets. > 90% of reads were on target.

Excellent Data Quality

The TruSeq Custom Amplicon Library Preparation Kit supports high levels of multiplexing, while providing excellent specificity and uniformity. An example TruSeq Custom Amplicon experiment was performed by following the workflow described in Figure 1. Representative uniformity data, with percent of bases at least 0.2× the mean sequencing depth, are shown for 5 samples prepared with the TruSeq Custom Amplicon assay and sequenced using the MiSeq System (Figure 5A). Specificity data, percent of sequenced bases passing filter aligning to desired target regions, for samples are shown in Figure 5B. Coverage uniformity and specificity were excellent, with > 85% of bases covered at > 0.2× of the mean coverage and > 90% of reads on target. These data illustrate how the TruSeq Custom Amplicon assay can be used to rapidly generate desired amplicons in a highly multiplexed reaction for sequencing with the MiSeq System.‡ The TruSeq Custom Amplicon assay is highly concordant with genotype information using the Infinium® assay (99.4%, 27,500/27,654 calls, data not shown). As shown in Figure 6, read counts per amplicon are highly reproducible across different users, with $R^2 = 0.92$.

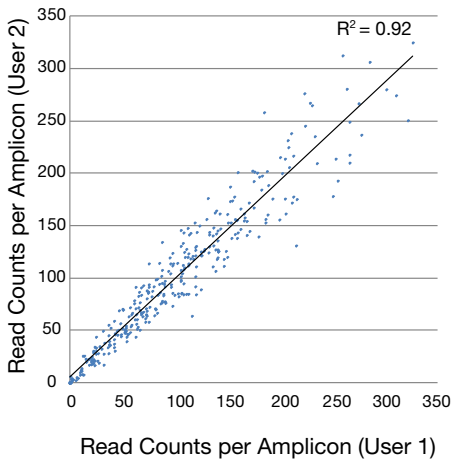

Figure 6: High Assay Reproducibility—High reproducibility of Project A read counts across 2 different users ($R^2 = 0.92$).

‡ Also compatible with the MiniSeq, NextSeq Series, and HiSeq Series Systems.

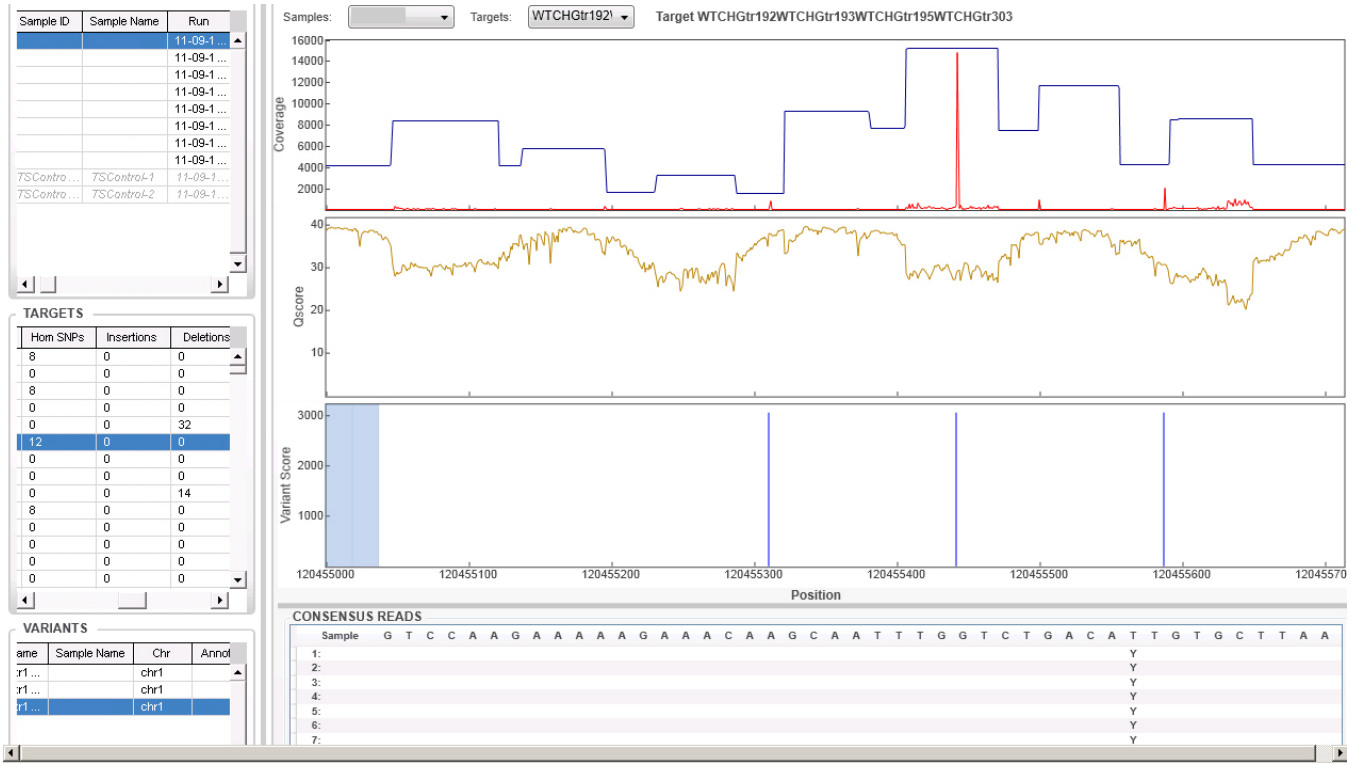

Figure 7: Illumina Amplicon Viewer—Streamlined, intuitive analysis toolbars in Amplicon Viewer. Top pane: visualize coverage levels of each amplicon or across entire genome regions (shown) and view quality scores, as well as variant calls in the middle and lower panes, respectively. Left scroll bars allow users to navigate through samples and targets, and track variants.

After the sequencing run on the MiSeq System, data are automatically aligned using the MiSeq Reporter and can be visualized using the Illumina Amplicon Viewer. As shown in Figure 7, large genomic regions containing multiple amplicons can be viewed, or zoomed in to view individual amplicons. Quality scores are tracked, and variant calls are easily and intuitively visualized. Target and sample statistics are managed in simple drop-down menus. From design to analysis, the TruSeq Custom Amplicon user experience is customized and streamlined, and keeps project data highly accessible.

Summary

TruSeq Custom Amplicon v1.5 is a powerful targeted resequencing solution that provides highly multiplexed amplicon sequencing performance with an optimized chemistry workflow and integrated analysis. Paired with the MiSeq System,§ The TruSeq Custom Amplicon v1.5 assay produces remarkably high-quality data for up to 96 samples in a single run. The robust combination of the TruSeq Custom Amplicon assay and the MiSeq System has driven studies comprising tens of thousands of samples, including hundreds of thousands of amplicons. Using TruSeq Custom Amplicon v1.5 with the MiSeq System enables the highest quality, most reliable, and simplest approach to deep interrogation of genomic regions of interest.

TruSeq Custom Amplicon Specifications

Specification	Value
Minimum input DNA	50 ng
Attempted amplicons per reaction[a]	16–1,536
Content range[a]	2–650 kb
Specificity[a]	> 70%
Coverage uniformity (> 0.2x mean)[a]	> 80%
Species available	Human, mouse, rat, and bovine
Samples types	Fresh, frozen, and FFPE
Amplicon sizes	150, 175, 250, and 425 bp

a. Target values will vary due to custom designs.

§ Also compatible with the MiniSeq, NextSeq Series, and HiSeq Series Systems.

Ordering Information

Kit	Catalog No.
TruSeq Custom Amplicon v1.5 Kit (96 Samples)	FC-130-1001
TruSeq Custom Amplicon Index Kit (96 indexes, 384 Samples)	FC-130-1003
TruSeq Index Plate Fixture Kit	FC-130-1005
TruSeq Index Plate Fixture and Collar Kit (2 Each)	FC-130-1007

Learn More

Go to www.illumina.com/products/truseq_custom_amplicon.ilmn to learn more about the new and improved TruSeq Custom Amplicon v1.5 Assay.

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