TruSeq[®] Custom Amplicon v1.5

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

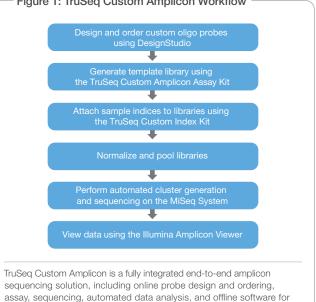
Highlights · Simple assay customization Easily create projects that include up to 1,536 amplicons in a single reaction, across a range of amplicon sizes and reference genomes · Optimized reagents Achieve high performance from as little as 50 ng of DNA, even in challenging GC-rich regions Rapid sample 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 pre-configured MiSeq® software

Introduction

TruSeg Custom Amplicon (TSCA) 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, TSCA 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 indices to support up to 96 samples per MiSeq® run, TSCA provides an unprecedented level of sample multiplexing. DesignStudio supports TSCA designs with a range of amplicon sizes optimized for different sample types (e.g. formalin-fixed paraffin-embedded) and reference genomes (e.g. human, mouse, rat, and bovine). Entire projects can now be accomplished in only days, leveraging the long pairedend-read capability, speed, and high data quality of the MiSeq system.

TSCA 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 MiSeg runs.



TruSeq Custom Amplicon Workflow Overview

reviewing results.

The entire TSCA process takes only two 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 1,536 attempted amplicons per reaction. Sample-specific indices are then added to each library by PCR using common primers from the TruSeg Amplicon Index Kit. An integrated bead-based normalization procedure enables simple volumetric pooling of libraries prior to sequencing on the MiSeq system and avoids laborious quantification methods. Pooled libraries are then loaded into the MiSeg 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.

Figure 1: TruSeq Custom Amplicon Workflow

Simplified Probe Design

Design TSCA oligonucleotide 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 (e.g., regions of homology or %GC content). To use DesignStudio, customers will need an account on the Illumina e-commerce system.

Request an account at https://icom.illumina.com/Account/Register.

Figure 2: DesignStudio for Custom Probe Design

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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 sidebar, along with project information and user-defined labels for convenient data sorting during the design phase.

Table 1: DesignStudio Parameters for Custom Amplicon

Project A	Project B	Project C	Project D
34,767	69,423	205,441	4,572
293	264	1,434	114
98.3	97.0	93.2	91.3
	34,767 293	34,767 69,423 293 264	34,767 69,423 205,441 293 264 1,434

*Designability score for each amplicon based on Tm, %GC content, length, secondary structure, and other factors. Refer to DesignStudio online help for more information.

Optimized Assay Chemistry

The TSCA v1.5 assay chemistry begins with hybridizing two customdesigned 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 two 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 two unique, sample-specific indices 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 three weeks, or loaded directly onto the MiSeq system without additional processing.

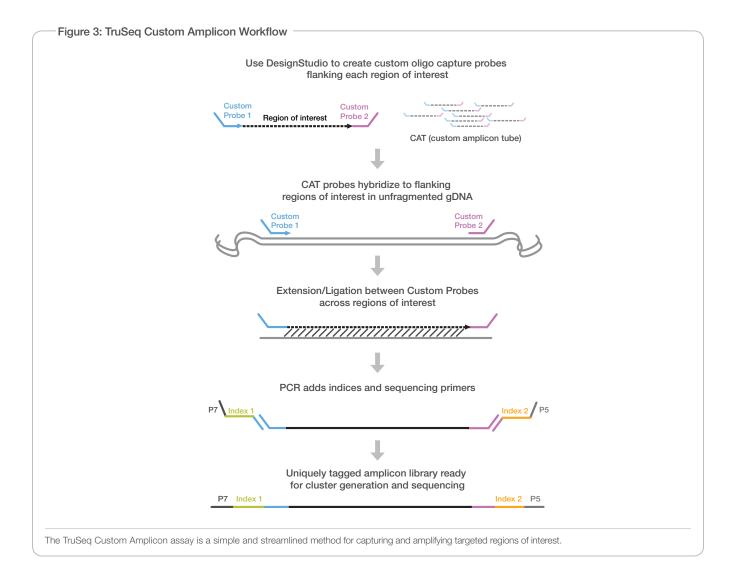
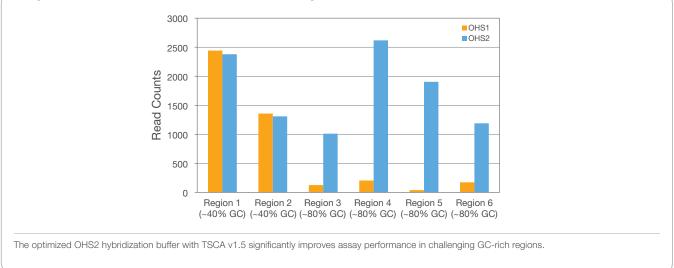


Figure 4: OHS2 Increases Read Counts for GC-Rich Targets

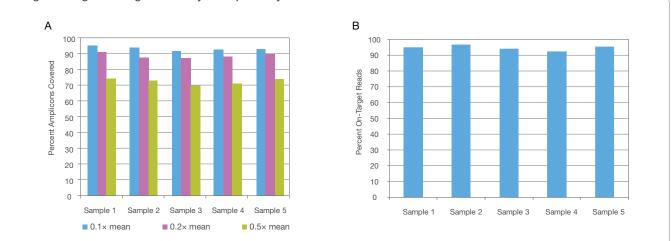


					В		
		с	overage Uniform	ity		Read	Count
Design	# Amplicons	ELM3	ELM4	Increase	Amplicon	ELM3	ELM4
1	1483	93%	94%	1%	1	10	315
2	217	89%	91%	2%	2	6	63
3	1483	86%	87%	1%	3	8	28
4	1420	86%	87%	1%	4	8	22
5	115	83%	85%	2%			

A. Coverage uniformity (defined as total bases present at >0.2× mean coverage), across designs and in poorly represented regions, increases with ELM4 extensionligation 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 five samples of a representative target panel. For all samples, >85% of bases are covered at $0.1 \times$ and $0.2 \times$ of the mean coverage (blue and purple bars, respectively), and >65% of bases are covered at $0.5 \times$ of the mean coverage (green bars).

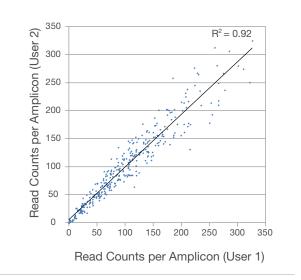
B. Specificity defined as percentage of filtered reads corresponding to user-defined targets. > 90% of reads were on target.

Excellent Data Quality

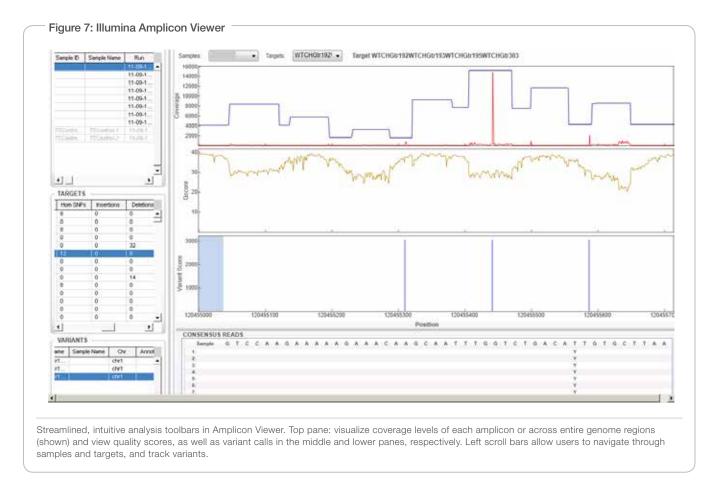
The TSCA assay supports high levels of multiplexing, while providing excellent specificity and uniformity. An example TSCA experiment was performed by following the workflow described in Figure 1. Representative uniformity data, with percent of bases at least 0.2× the mean sequencing depth, are shown for five samples prepared with TSCA 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 TSCA can be used to rapidly generate desired amplicons in a highly multiplexed reaction for sequencing with the MiSeq system.

The TSCA 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 two different users (R 2 = 0.92).



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 TSCA 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, TSCA v1.5 produces remarkably high-quality data for up to 96 samples in a single run. The robust combination of TruSeq Custom Amplicon 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.

Learn More

Go to www.illumina.com/products/truseq_custom_amplicon.ilmn to learn more about the new and improved TSCA v1.5.

TruSeq Custom Amplicon Specifications

Specification	Value
Minimum input DNA	50 ng
Attempted amplicons per reaction*	16-1,536
Content range*	4–650 Kb
Specificity*	> 70%
Coverage uniformity (> 0.2x mean)*	> 80%
Species available	Human, mouse, rat, and bovine
Samples types	Fresh, frozen, and FFPE
Amplicon sizes	150, 175, 250, and 425 bp

- Ordering Information

Kit	Catalog No.
TruSeq Custom Amplicon v1.5 Kit (96 Samples)	FC-130-1001
TruSeq Custom Amplicon Index Kit (96 Indices, 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

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