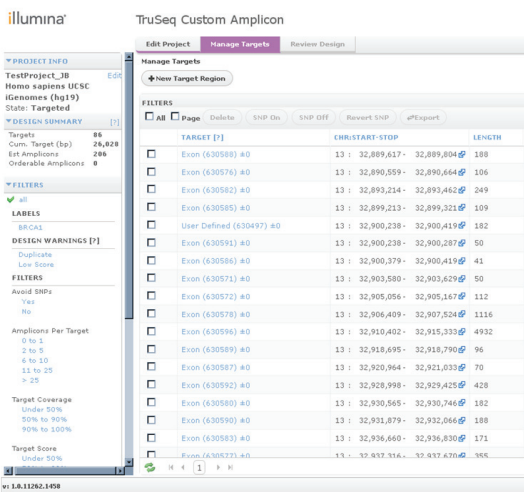


Table 1: DesignStudio Parameters for Custom Amplicon

Parameters	Project A	Project B	Project C	Project D
Total Sequence Input (bp)	34,767	69,423	20,984	4,572
Total Number of Amplicons	293	264	315	114
Designability Score (% Base Coverage)	98.3	97.0	91.1	91.3

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

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

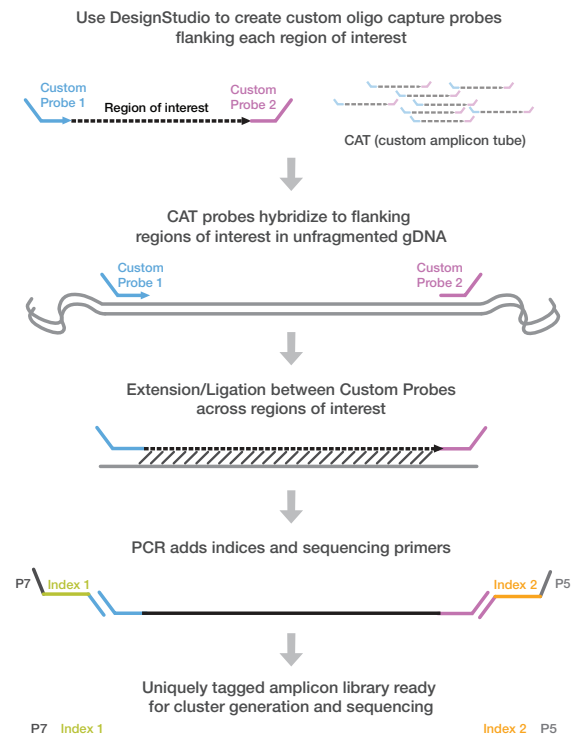
Revolutionary Assay Chemistry

The TSCA assay chemistry begins with two custom-designed probes hybridizing upstream and downstream of the region of interest (Figure 3). Each probe includes sequence designed to capture regions of interest and an adapter sequence used in a subsequent amplification reaction. A proprietary extension-ligation reaction extends across the region of interest, followed by ligation to unite the two probes. This creates a new template strand and gives the assay excellent specificity. Extension-ligation templates are then amplified by PCR, which also incorporates two unique sample-specific indexes. The final reaction product contains desired amplicons with the necessary sequencing adapters and indices for sequencing on the MiSeq system. TruSeq Custom Amplicon libraries can be loaded directly onto the MiSeq system without any additional processing.

Excellent Data Quality

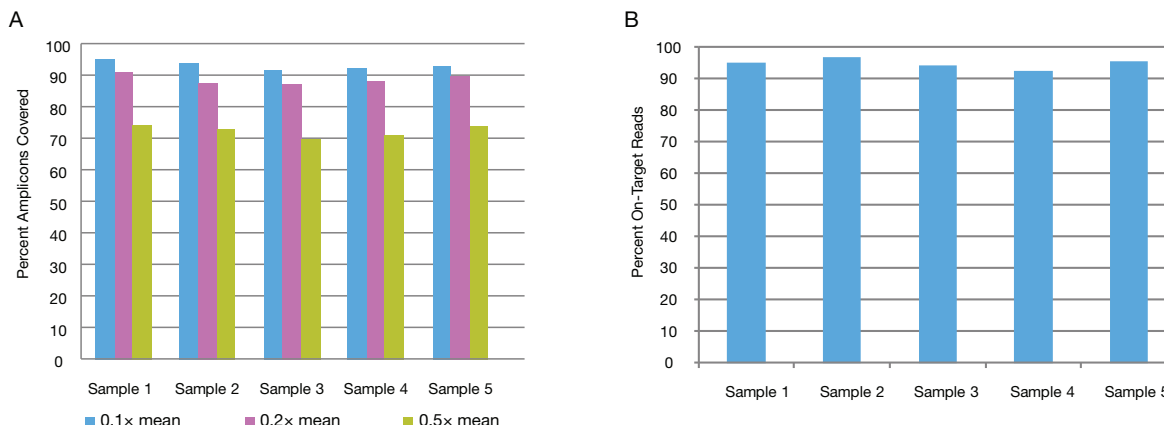
The TSCA assay supports unprecedented multiplexing, while providing excellent specificity and uniformity. An example TSCA experiment was performed following the workflow described in Figure 1. Representative uniformity data, with percent of bases at least 0.2x the mean sequencing depth, are shown for five samples prepared with TSCA and sequenced using the MiSeq system (Figure 4A). Specificity data, percent of sequenced bases passing filter aligning to desired target regions, for samples are shown in Figure 4B. Coverage uniformity and specificity were excellent, with > 85% of bases covered at > 0.2x 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.

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: 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 0.1x and 0.2x of the mean coverage (blue and purple bars, respectively), and >65% of bases are covered at 0.5x 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.

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 5, read counts per amplicon are very highly reproducible across different users, with $R^2 = 0.92$.

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 6, 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.

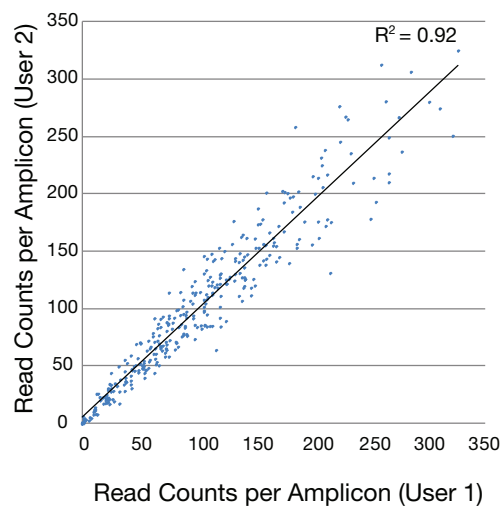
Summary

TruSeq Custom Amplicon provides unprecedented multiplexing to amplify hundreds of genomic targets in a single reaction and sequencing of up to 96 samples in a single MiSeq run. With MiSeq, TSCA allows researchers to easily sequence targeted regions of the genome in a fraction of the time and cost than previously possible—a new era in amplicon sequencing has begun.

Learn More

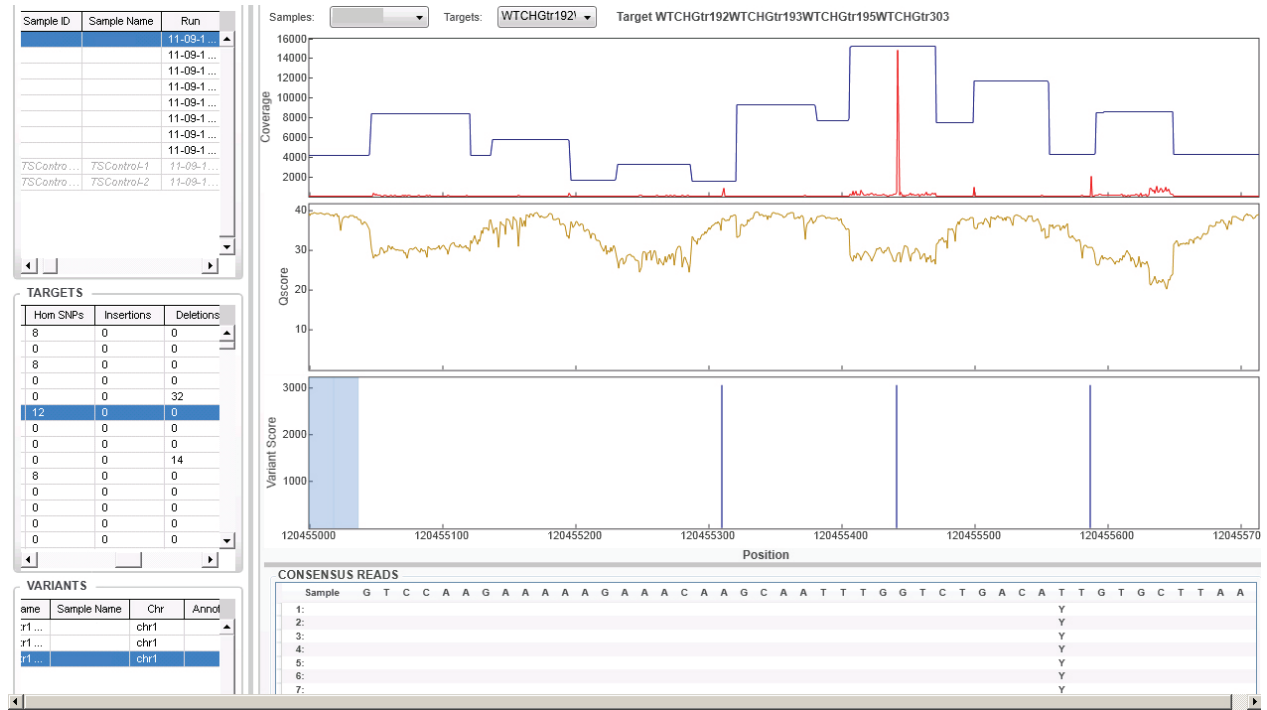
Go to www.illumina.com/miseq to learn more about the next revolution in personal sequencing.

Figure 5: High Assay Reproducibility



High reproducibility of Project A read counts across two different users ($R^2 = 0.92$).

Figure 6: 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.

TruSeq Custom Amplicon Specifications

Specification	Value
Input DNA	250 ng
Attempted amplicons per sample*	16–384
Content range*	4–96 kb
Specificity*	> 70%
Coverage uniformity (> 0.2x mean)*	> 80%

*Target values will vary due to custom designs.

Ordering Information

Kit*	Catalog No.
TruSeq Custom Amplicon 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
MiSeq Reagent Kit (300-cycles - PE)	MS-102-1001

*Use the DesignStudio Project Calculator to determine the number of recommended MiSeq reagent kits.

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