TruSeq® Custom Amplicon Low Input Library Prep Kit

A scalable amplicon sequencing solution that delivers sensitive and specific analytical results from both low-input and FFPE DNA samples.

Highlights

- **Low DNA Input and FFPE Compatibility**
  Accurate variant detection from as low as 10 ng of input gDNA and challenging FFPE samples
- **Confidence in High Data Quality**
  Optimized protocol yields high uniformity and analytical specificity
- **Fast Workflow**
  Automation-friendly workflow can be completed in 6.5 hours, with only 3 hours of hands-on time
- **Comprehensive Workflow Solution**
  Fully supported, integrated workflow solution includes simple data analysis options

Introduction

The TruSeq Custom Amplicon Low Input Library Prep Kit is a fully customizable, amplicon-based assay for targeted resequencing. With only 10 ng of genomic DNA (gDNA), this assay allows researchers to capture multiple targets of interest simultaneously, sequencing up to 1536 amplicons in a single reaction. The TruSeq Custom Amplicon Low Input Library Prep Kit offers the flexibility to accommodate formalin-fixed, paraffin-embedded (FFPE) samples, such as preserved tumor tissue. Optimized for the MiniSeq™, MiSeq®, and NextSeq® Systems, this assay delivers the high analytical sensitivity and uniform coverage needed to detect low-frequency somatic variants. With an integrated workflow and consistent results, the TruSeq Custom Amplicon Low Input Library Prep Kit offers a reliable, fast, and easy-to-use solution for targeted resequencing.

Comprehensive Solution

The Illumina workflow for amplicon sequencing enables biologists to access a fully-supported solution, from design through analysis (Figure 1). Illumina provides technical specialists for library preparation, sequencing, and data analysis, ensuring rapid resolution and minimizing potential laboratory downtime. Illumina Concierge Services offers additional support for probe design and functional evaluation of custom panels.*

Low DNA Input

The TruSeq Custom Amplicon Low Input Library Prep Kit accommodates various DNA input amounts from as low as 10 ng of gDNA, allowing researchers to successfully interrogate both standard and challanging sample types. It is compatible with FFPE samples, starting with 10–50 ng of FFPE DNA (depending on DNA quality). Whether starting with high or low DNA input amounts or challenging sample types, the assay delivers consistant, high-quality performance, allowing researchers to be confident in their results (Figure 2).

Confident Assay Design

TruSeq Custom Amplicon Low Input oligonucleotide probes are designed in Illumina DesignStudio™ software, a free, easy-to-use, online tool (Figure 3). DesignStudio Software provides optimized coverage of user-defined regions and estimates total project pricing. After logging in to a personalized account and naming the project, researchers can select targets or genomic regions of interest. Probe design is performed automatically using an algorithm that considers a range of factors, including GC content, analytical specificity, probe interaction, and coverage. Candidate regions 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 quality control (QC), the probe designs can be ordered and saved for future orders. Typical design success, depending upon custom-selected content, is 90% or better for desired bases. In some cases, higher or lower success rates can be achieved, depending upon the sequence context (eg, regions of homology or %GC content). Illumina Concierge Services offers additional support for increasing target coverage.

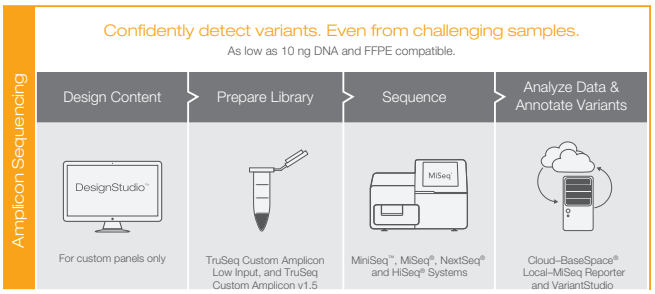

Figure 1: Comprehensive Amplicon Sequencing Workflow—The TruSeq Custom Amplicon Low Input workflow is an integrated, fully supported workflow for amplicon sequencing that guides researchers from design through data analysis.

* Illumina Concierge Services offers expert assistance, from *in silico* design optimization, functional performance evaluation, and improvement to final shipment. For more information, contact an Illumina representative.

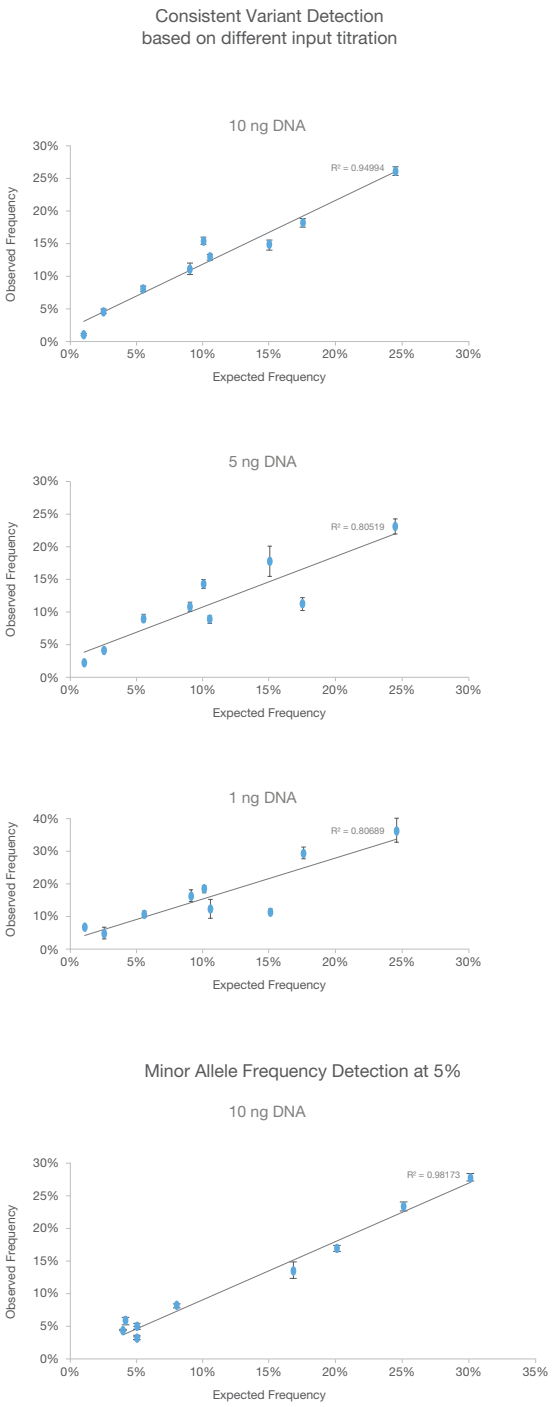

Figure 2: (A) Consistent Variant Detection Based on Different Input Titration—The TruSeq Custom Amplicon Low Input Library Prep Kit demonstrates high concordance between expected and observed variant frequencies for 10 ng, 5 ng, and 1 ng of high-quality FFPE DNA. Reference DNA samples were obtained from Horizon Diagnostics HD200, prepared following the TruSeq Custom Amplicon Low Input workflow with the TruSeq Amplicon - Cancer Panel primer pool, and sequenced on the MiSeq System in replicates of 4. Variants were called using MiSeq Reporter software. R^2 values are shown. **(B) Minor Allele Frequency Detection at 5%**—TruSeq Custom Amplicon Low Input Library Prep Kit shows robust and reproducible mutant allele detection. Using Horizon Diagnostics FFPE samples HD729, 6 of 6 SNPs were detected as low as 5% at 10 ng input amount.

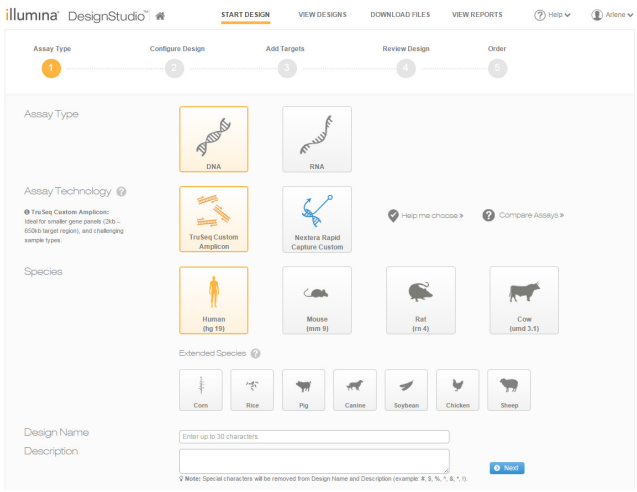

Figure 3: Custom Probe Design—Researchers can use DesignStudio Software to visualize targeted genomic regions and attempted amplicons to assess design coverage and design scores.

Rapid Workflow

The streamlined, simplified TruSeq Custom Amplicon Low Input workflow offers several advantages. It uses simple bead-based purification, making it automation friendly (Figure 4). The TruSeq Custom Amplicon Low Input assay can be completed in 6.5 hours, with only 3 hours of hands-on time.

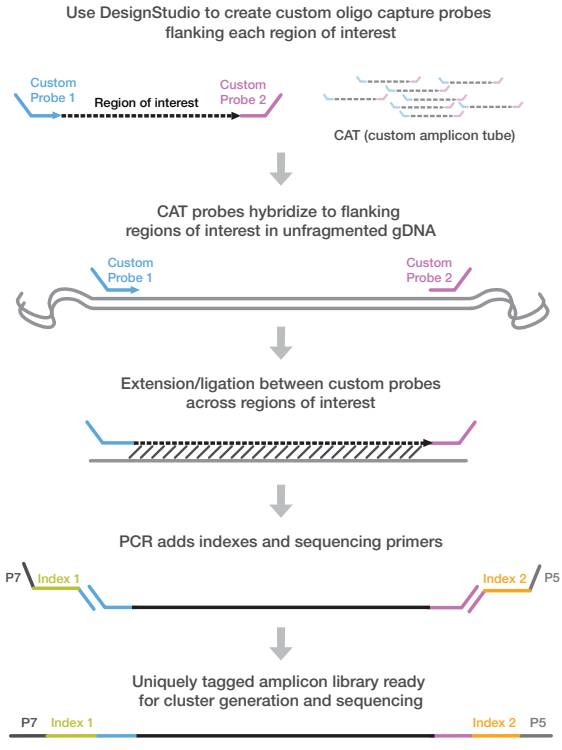

Figure 4: TruSeq Custom Amplicon Low Input Workflow—The TruSeq Custom Amplicon Low Input workflow can be completed in 6.5 hours.

Exceptional Data Quality

The TruSeq Custom Amplicon Low Input Library Prep Kit is compatible with MiniSeq, MiSeq, and NextSeq Systems which use sequencing by synthesis (SBS) chemistry, the most widely adopted sequencing technology. Illumina systems generate the highest percentage of sequenced bases over Q30† in the industry.[1–5] When paired with Illumina sequencing systems, the TruSeq Custom Amplicon Low Input Library Prep Kit delivers high coverage uniformity and analytical specificity, regardless of amplicon length and pool plexity (Figure 5). With consistent high-quality results, researchers can be confident in the accuracy of their data, even with challenging targets of interest (eg, repetitive regions, GC-rich content).[6] For increased confidence in variant calls, 3 replicates can be run in parallel.

FFPE Quality Assessment

For FFPE samples, Illumina offers the TruSeq FFPE DNA Library Prep QC Kit. It uses a simple qPCR‡ reaction to determine DNA quality and provides guidance on sequencing parameters. This step ensures that only samples that will achieve the necessary sequencing metrics are prepared. The results of the QC step determine the recommended amount of input DNA. The TruSeq FFPE DNA Library Prep QC Kit can be bundled with the TruSeq Custom Amplicon Low Input Library Prep Kit to maximize lab budgets.

Expanded Features With Illumina Concierge

Illumina Concierge Services offer additional design support and expanded features for the TruSeq Custom Amplicon Low Input Library Prep Kit. This kit can incorporate unique molecular identifiers for enhanced allelic detection and increased analytical sensitivity.[7] Unique molecular identifiers allow removal of PCR duplicates, which enables detection of individual molecules. The TruSeq Custom Amplicon Low Input assay is compatible with dual-strand sequencing, which virtually eliminates false positives that can arise from deamination events during formalin fixation. Illumina Concierge also offers the capability to design smaller amplicons (~100 bp), increasing compatibility with fragmented DNA. Contact an Illumina representative for access to Illumina Concierge Services.

User-Friendly Data Analysis

Data can be transferred directly from Illumina systems to BaseSpace® Sequence Hub, the Illumina genomics computing environment. Within this platform, push-button BaseSpace Apps simplify data analysis by offering optimized analysis pipelines packaged in a user-friendly interface. The TruSeq Amplicon App streamlines analysis of custom panels and delivers results in an easy-to-read format (Figure 6). BaseSpace Variant Interpreter (Beta) enables rapid filtering, identification, and classification of disease-associated variants from sequencing data. Example data sets are available in BaseSpace Sequence Hub, so researchers can explore and evaluate data generated by Illumina kits and systems before committing to a solution.

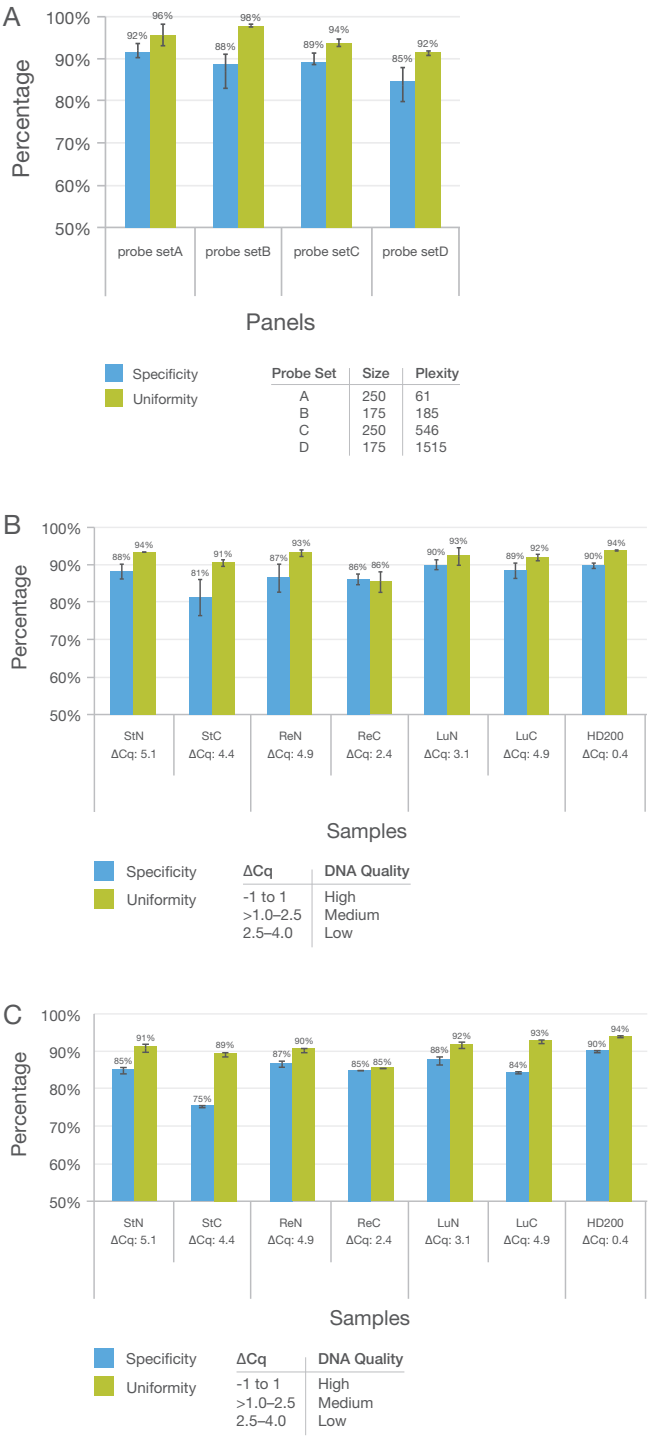

Figure 5: High Coverage Uniformity and Analytical Specificity— (A) Consistent Performance of Different Size Panels, at 10 ng Input. Analytical specificity and uniformity are high, regardless of the amplicon length and plexity. Reference DNA was obtained from Coriell samples. (B) Robust Performance of Highly Degraded FFPE Samples at 50 ng Input for 3 Tissues (Stomach, Rectal, and Lung Tumor/Normal Samples) at Low Plexity (125). High analytical specificity and uniformity of customer A panel across poor-quality FFPE tumor/normal sample pairs, in replicates of 3. (C) Robust Performance of Highly Degraded FFPE Samples at 50 ng Input at Medium Plexity (577). High analytical specificity and uniformity of customer B panel across poor-quality FFPE tumor/normal sample pairs.

† Q30 = Refers to a base call quality score (Q-Score) of 30, which represents an estimate or prediction of 1 error in base calling per 1000 base calls

‡ Quantitative polymerase chain reaction (qPCR).

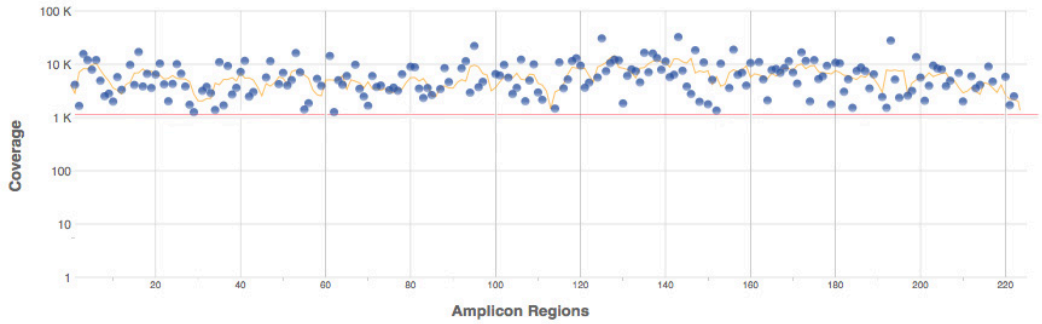

Figure 6: TruSeq Amplicon App—The BaseSpace TruSeq Amplicon App simplifies data analysis, delivering results in an intuitive format. Amplicon coverage for a representative run using the TruSeq Amplicon - Cancer Panel run and the MiSeq System is shown.

Table 1: TruSeq Custom Amplicon Low Input Assay Specifications

Parameter	Specification
Available preloaded reference genomes	*Homo sapiens* *Mus musculus* *Rattus norvegicus* *Bos taurus*
Extended species	Corn, rice, pig, canine, soybean, chicken, sheep
Custom reference genomes	Leverage the expertise of Illumina Concierge services to design custom panels for any species of interest
Preloaded amplicon lengths	150 bp, 175 bp, and 250 bp
Recommended amplicon length for FFPE DNA	150 bp or 175 bp
Oligo pools per panel	• 1 for standard designs • 2 for mirrored-strand designs
Amplicon plexity (single pool)	• 16 to 1536
Input required for standard gDNA	10 ng
Input required for FFPE DNA	10–50 ng, depending on QC results
Sequencing data quality	Over 90% of sequenced bases ≥ Q30
Dual-strand sequencing	Available in DesignStudio Software

Summary

The TruSeq Custom Amplicon Low Input Library Prep Kit provides a comprehensive workflow solution for amplicon sequencing. With low DNA input requirements and high-quality data, the TruSeq Custom Amplicon Low Input Library Prep Kit enables researchers to process both standard and challenging samples with confidence.

Ordering Information

Product	Catalog No.
TruSeq Custom Amplicon Low Input Library Prep Kit (96 samples)	FC-134-2001
TruSeq Custom Amplicon Low Input Library Prep Kit (16 samples)	FC-134-2002
TruSeq FFPE DNA Library Prep QC Kit	FC-121-9999
TruSeq Custom Amplicon Index Kit (96 indices, 384 samples)	FC-130-1003

References

1. Perkins TT, Tay CY, Thirriot F, Marshall B. Choosing a benchtop sequencing machine to characterize *Helicobacter pylori* genomes. *PLoS One.* 2013;8:e67539.
2. Quail MA, Smith M, Coupland P, et al. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics.* 2012;13:341.
3. Ross MG, Russ C, Costello M, et al. Characterizing and measuring bias in sequence data. *Genome Biol.* 2013;14:R51.
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6. Wong SQ, Fellowes A, Doig K, et al. Assessing the clinical value of targeted massively parallel sequencing in a longitudinal, prospective population-based study of cancer patients. *Br J Cancer.* 2015;112:1411-1420.
7. Kivioja T, Vähärautio A, Karlsson K, et al. Counting absolute numbers of molecules using unique molecular identifiers. *Nat Methods.* 2011;9:72-74.

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