AmpliSeq™ for Illumina Custom RNA Sequencing Panel

Consistent and accurate RNA quantitation for multiplexing up to 1200 human gene targets combining AmpliSeq amplicon chemistry and Illumina sequencing technology.

Highlights

- **Consistent, reproducible RNA quantitation**
  High replicate-to-replicate reproducibility with a range of sample types
- **Highly accurate RNA quantitation across a broad dynamic range**
  Enable higher transcript coverage for low-expressing genes
- **High-quality data from low-quality, or low-input samples**
  Compatible with a range of sample types included FFPE samples
- **Fast, scalable workflow**
  High multiplexing capacity supports 384 samples per sequencing run

Introduction

Advances in next-generation sequencing (NGS) have accelerated the pace of RNA sequencing studies and have increased our understanding of normal and disease physiology.[1] While NGS-based whole-transcriptome sequencing is a powerful method for discovery applications, more and more researchers are turning to targeted applications such as targeted resequencing or custom RNA sequencing.[2] With custom RNA sequencing, a select set of genes or regions of interest are sequenced, focusing the power of NGS on a smaller subset of the transcriptome for deeper interrogation or as a replacement for quantitative polymerase chain reaction (qPCR) assays. A focused approach enables greater sensitivity for detection of low-expressing genes,[3,4] faster turnaround times, lower data analysis requirements, and lower sequencing costs.[5]

To harness these advantages Illumina offers the AmpliSeq for Illumina Custom RNA Panel. The AmpliSeq for Illumina Custom RNA Panel combines amplicon chemistry and Illumina sequencing technology to deliver accurate and consistent RNA expression profiling (Figure 1). The PCR-based method amplifies regions of interest to investigate detection of transcript isoforms, gene fusions, single nucleotide variants, and more. AmpliSeq chemistry can successfully amplify up to 1200 amplicons in a single reaction and can multiplex up to 384 libraries in a single sequencing run. Supporting just 1 ng of starting RNA, the AmpliSeq for Illumina Custom RNA Panel is also compatible with formalin-fixed paraffin-embedded (FFPE) samples (Table 1). The AmpliSeq for Illumina Custom RNA Panel is part of a fully integrated solution, including convenient online assay design and ordering, rapid library preparation, Illumina sequencing, and user-friendly data analysis.

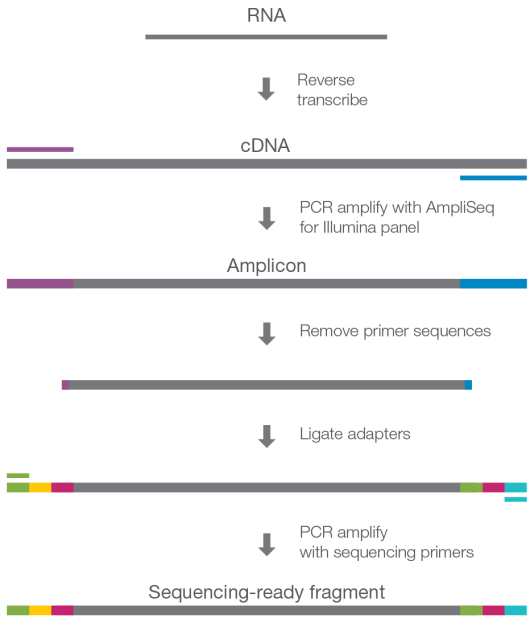

Figure 1: AmpliSeq for Illumina Custom RNA library prep chemistry—The highly multiplexed, PCR-based assay amplifies up to 1200 amplicons in a single reaction.

Table 1: AmpliSeq for Illumina Custom RNA Panel specifications at a glance

Parameter	Specification
Input RNA requirement	1-100 ng (10 ng recommended)
Methods	Amplicon sequencing, targeted RNA sequencing
Mechanism of action	Multiplex PCR
Multiplexing	Up to 384-plex
Amplicons per reaction	12-1200 amplicons
Target insert size	150 bp
Number of reactions per order	1125 reactions
Recommended sequencing systems	iSeq 100, MiniSeq, MiSeq, NextSeq 550 Systems
Supported sample types	FFPE tissue, blood, cell lines,
Species	Human
Assay time	5.5-7.5 hours
Hands-on time	< 1.5 hours

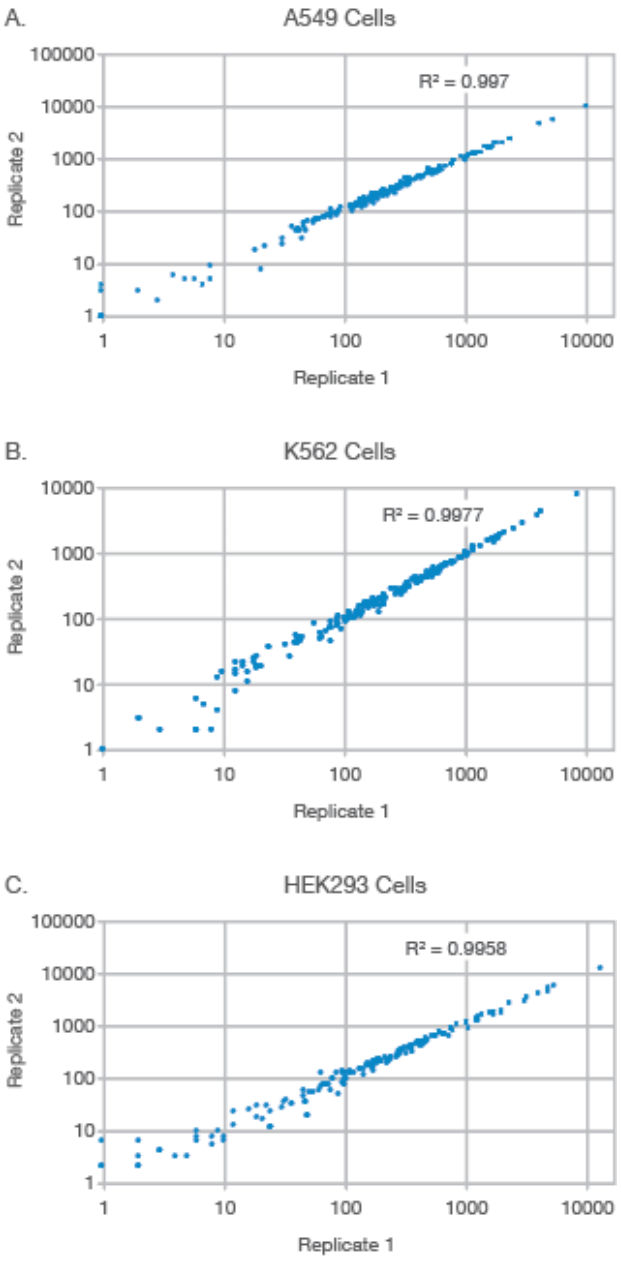

Figure 2: High replicate-to-replicate reproducibility—Comparison between Replicate 1 read counts (x-axis) and Replicate 2 read counts(y-axis) in (A) A549 human lung carcinoma cells (B) K562 human chronic myeloid leukemia cells and (C) HEK293 human embryonic kidney cells. The dynamic range covers five orders of magnitude between amplicons showing the lowest and highest numbers of read counts.

Consistent, reproducible RNA expression profiling

To demonstrate the consistent reproducibility of the AmpliSeq for Illumina Custom RNA Panel, a 168-custom RNA gene expression panel targeting the Mitogen-Activated Protein Kinase (MAPK) pathway was used to generated libraries. The MAPK custom panel was designed using DesignStudio™ Software and can also be designed as an AmpliSeq Community Panel. Libraries were prepared from 10 ng of total RNA from the well-characterized cell lines A549 human lung carcinoma cells (ATCC, CCL-185), K562 human chronic myeloid leukemia cells (ATCC, CCL-243), and HEK293 human embryonic kidney cells (ATCC, CRL-1573). Libraries were sequenced on an iSeq™ 100 System with a run configuration of 2 × 151 bp. The RNA expression profiling results comparing two replicates per library type show consistent, highly reproducible results with Pearson correlation coefficients between 0.997-0.998 (Figure 2). Furthermore, the amplicon read counts span five orders of magnitude, indicating a broad dynamic range and high sensitivity.

Highly accurate RNA expression profiling

To illustrate the highly accurate expression profiling of theAmpliSeq for Illumina Custom RNA Panel, experiments were conducted on samples containing known quantities of RNA from the AmpliSeq External RNA Controls Consortium (ERCC) RNA Spike-In Mix (Illumina, Cat. no. 20030697). The ERCC control is a preformulated blend of transcripts with known molar concentrations for each transcript. ERCC controls are designed to mimic natural eukaryotic mRNAs across a range of expression levels and are commonly used to establish standard baseline measurements in gene expression experiments. Detection and measurement of ERCC spike-in was performed with the AmpliSeq ERCC Companion Panel (Illumina, Cat. no. 20030696), which is a predesigned panel composed of primers that target the ERCC spike-in molecules.

ERCC control libraries were pooled and sequenced on a MiSeq™ System with a run configuration of 2 × 151 bp. Read counts from the AmpliSeq ERCC library were compared to known molar concentrations in the ERCC RNA Spike-In Mix with the RNA Amplicon App BaseSpace™ Sequence Hub, the cloud-based Illumina genomics computing and storage platform (Figure 3). With a correlation coefficient of 0.99, the data show extremely high accuracy and sensitivity spanning over five orders of magnitude.

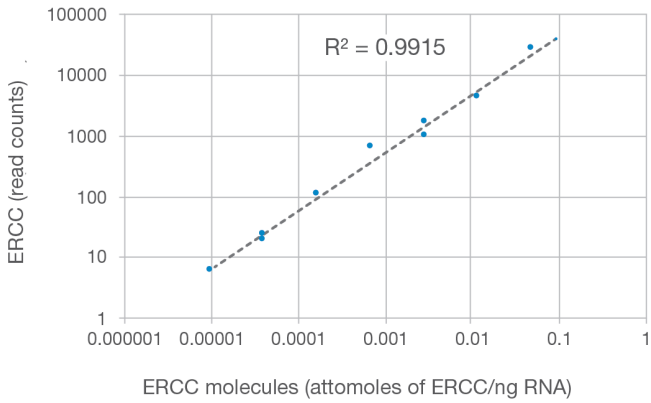

Figure 3: Accurate RNA expression profiling—Comparison of known ERCC transcript molarity (x-axis) and sequencing read counts from the AmpliSeq for Illumina ERCC RNA Companion Panel (y-axis).

Excellent performance from a broad range of RNA input and RNA sample quality

The AmpliSeq for Illumina Custom RNA Panel generates high-quality data across a range of RNA input amounts. To demonstrate consistent performance across a range of inputs, an 827-target AmpliSeq for Illumina Custom RNA Panel was designed. Libraries were prepared in triplicate using Human Brain Reference RNA and Universal Human Reference RNA samples. By comparing the brain vs. reference log2 fold change expression levels from 1 ng, 10 ng, and 100 ng input libraries, the data illustrate high consistency and high correlation (Figure 4).

Gene expression profiling studies often rely on low-input, or low-quality samples. In cancer research, samples may come from precious tumor-normal specimens or FFPE archival samples. While FFPE tissue samples provide a rich source of biological information, they can be difficult to study due to nucleic acid degradation from the fixation and storage process.[6,7] The AmpliSeq for Illumina Custom RNA Panel addresses this challenge by direct amplification of transcripts, rather than using poly(A) capture.*

Comprehensive design-to-data workflow

The AmpliSeq for Illumina Custom RNA Panel is part of a comprehensive design-to-data workflow including solutions for every step of the process (Figure 5). The AmpliSeq for Illumina Custom RNA Panel generates indexed, sequence-ready libraries targeting specific genes or regions of interest. Starting with as little as 1 ng of total RNA, all targets are amplified in a single reaction, minimizing potential bias and eliminating workflow steps compared to methods such as qPCR. Following panel design, library preparation through data analysis takes less than two days.

Easy, online assay design with DesignStudio Software

Easily design and order custom content with DesignStudio Software, a free web-based assay design tool. DesignStudio Software provides dynamic feedback to optimize target region sequencing coverage, reducing the time required to design custom projects. Choose from over 20,000 RefSeq genes to create fully custom panels of 12–1200 amplicons. To access DesignStudio Software, log in to your MyIllumina account.

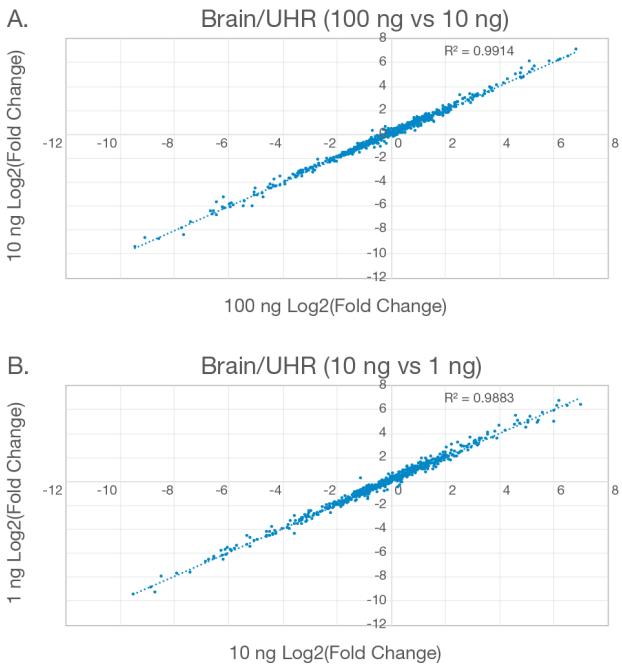

Figure 4: Excellent performance across a range of input amounts—Comparison of Human Brain Reference RNA vs. Universal Human Reference log2 fold changes expression between libraries generated from (A) 100 ng and 10 ng RNA samples and between libraries generated from (B) 10 ng and 1 ng RNA samples.

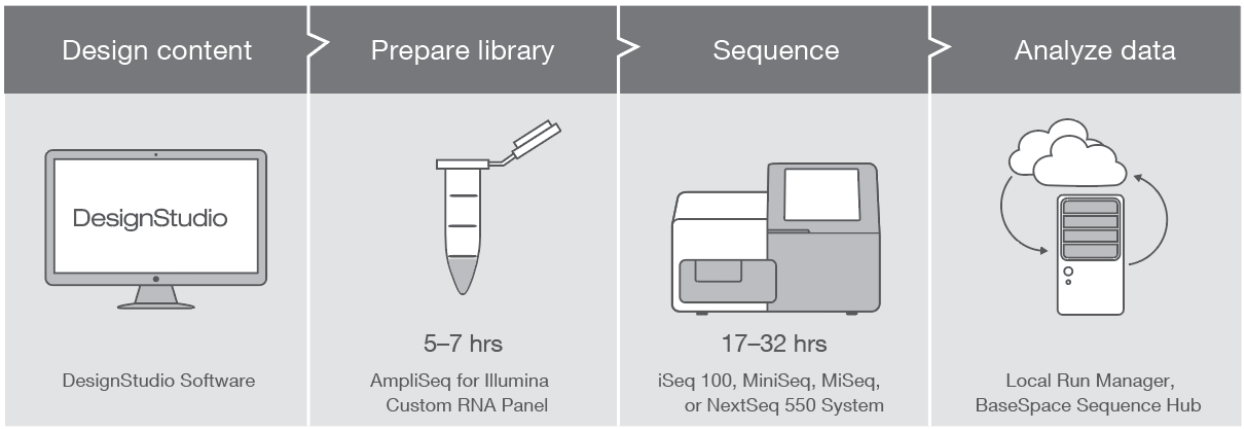

Figure 5: AmpliSeq for Illumina Custom RNA workflow—The AmpliSeq for Illumina Custom RNA Panel is part of a comprehensive, integrated sequencing solution from amplicon design to data analysis.

*Performance on FFPE samples will be dependent on sample quality, input amounts, and panel design. Studies with FFPE samples may require higher RNA inputs for library preparation and higher mean sequence coverage.

Table 2: Multiplexing with the AmpliSeq for Illumina Custom RNA Panel

Custom content	Reads / experiment[a]	Instrument (reads/run)	Samples /run
12-gene panel	50 K	iSeq 100 Sytem(4 M)	80
		MiniSeq System, HO (25 M)	384
		MiSeq System v3 chemistry (25 M)	384
		NextSeq 550 System MO (260 M)	384
NF-kB / cell cycle (168 targets)	200 K	iSeq 100 System	20
		MiniSeq System HO	125
		MiSeq System v3 chemistry	125
		NextSeq 550 System MO (260 M)	384
MAPK (197 targets)	1 M	iSeq 100 System	4
		MiniSeq System HO	25
		MiSeq System v3 chemistry	25
		NextSeq 550 System MO (260 M)	260
Immune response (398 targets)	1 M	iSeq 100 System	4
		MiniSeq System HO	25
		MiSeq System v3 chemistry	25
		NextSeq 550 System MO (260 M)	260

a. Number of reads needed per experiment can vary based on panel content, sample type, transcriptome profile, and experimental design goals.

Abbreviations: mid output, MO; high output, HO.

Higher target and sample capacity with library multiplexing

Library multiplexing is a process that allows many libraries to be pooled together and sequenced simultaneously. Multiplexing is a powerful way to exponentially increase the number of samples analyzed in a single run, without drastically increasing run time or cost. The AmpliSeq for Illumina Custom RNA Panel supports multiplexing up to 384 libraries within a single run, depending on sequencing system capacity and custom panel size (Table 2). With 25 million reads, the MiSeq system can generate 25,000 data points per run (at an average of 1000 reads per target), which is equivalent to 65 × 384-well plates. Multiplexing up to 384 samples requires the AmpliSeq CD Indexes Sets A-D (Illumina, Cat. No. 20031676).

User-friendly data analysis

AmpliSeq for Illumina Custom RNA Panel data analysis does not require highly trained bioinformatics support or dedicated high-performance computing infrastructure. Raw sequence data may be streamed directly from the sequencing system to BaseSpace Sequence Hub. Secondary analysis, including read alignment and expression profiling, can be performed in BaseSpace Sequence Hub with the RNA Amplicon App. This app includes Differential Expression Analysis (DESeq2) for read counting and differential gene expression analysis.

On-instrument analysis

The same secondary analysis workflows can be performed with the RNA Amplicon Module in Local Run Manager. Local Run Manager is an on-site software platform used to create a run, monitor status, and analyze sequencing data. Local Run Manager is available both on-instrument for select sequencing systems (iSeq 100, MiniSeq™, MiSeq, and NextSeq™ 550 Systems) and off-instrument for installation on separate computers.

Ordering information

Order AmpliSeq for Illumina products online at www.illumina.com

Product	Catalog Number
AmpliSeq for Illumina Custom RNA Panel	20020496
AmpliSeq for Illumina ERCC RNA Spike-In Mix	20030697
AmpliSeq for Illumina ERCC Companion Panel	20030696
AmpliSeq for Illumina CD Indexes Set A-D	20031676

Learn more

To learn more about the AmpliSeq for Illumina Custom RNA Panel, visit www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/ampliseq-custom-rna-panel.html

References

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