Next-Generation Sequencing

Breaking perfomance barriers in NGS library preparation

that's GOOD science!®

TaKaRa

Clontech TaKaRa cellartis

SMARTer NGS

Speed your journey to the most elusive answers.

We understand that cutting-edge research requires high-performance products that provide the sensitivity necessary to detect meaningful biological phenomena. For translational research and clinical studies, we recognize the need for user-friendly solutions that are amenable to high-throughput applications and automation, and that enable analysis of clinically relevant samples with high reproducibility. That is why we focus on developing best-in-class tools—backed with expert support—to help you achieve the results you need regardless of your research application.

Our broad NGS portfolio provides unmatched sensitivity for all of your demanding sequencing applications—regardless of sample type or input amount. While our NGS roots began with the adaptation of our SMART® cDNA synthesis technology for RNA-seq applications, our SMARTer NGS solutions now also leverage the innovations of ThruPLEX® and PicoPLEX® technologies for DNA-seq.

Sensitivity. Reproducibility. Reliability. Ease of use. Scalability.

Breaking performance barriers

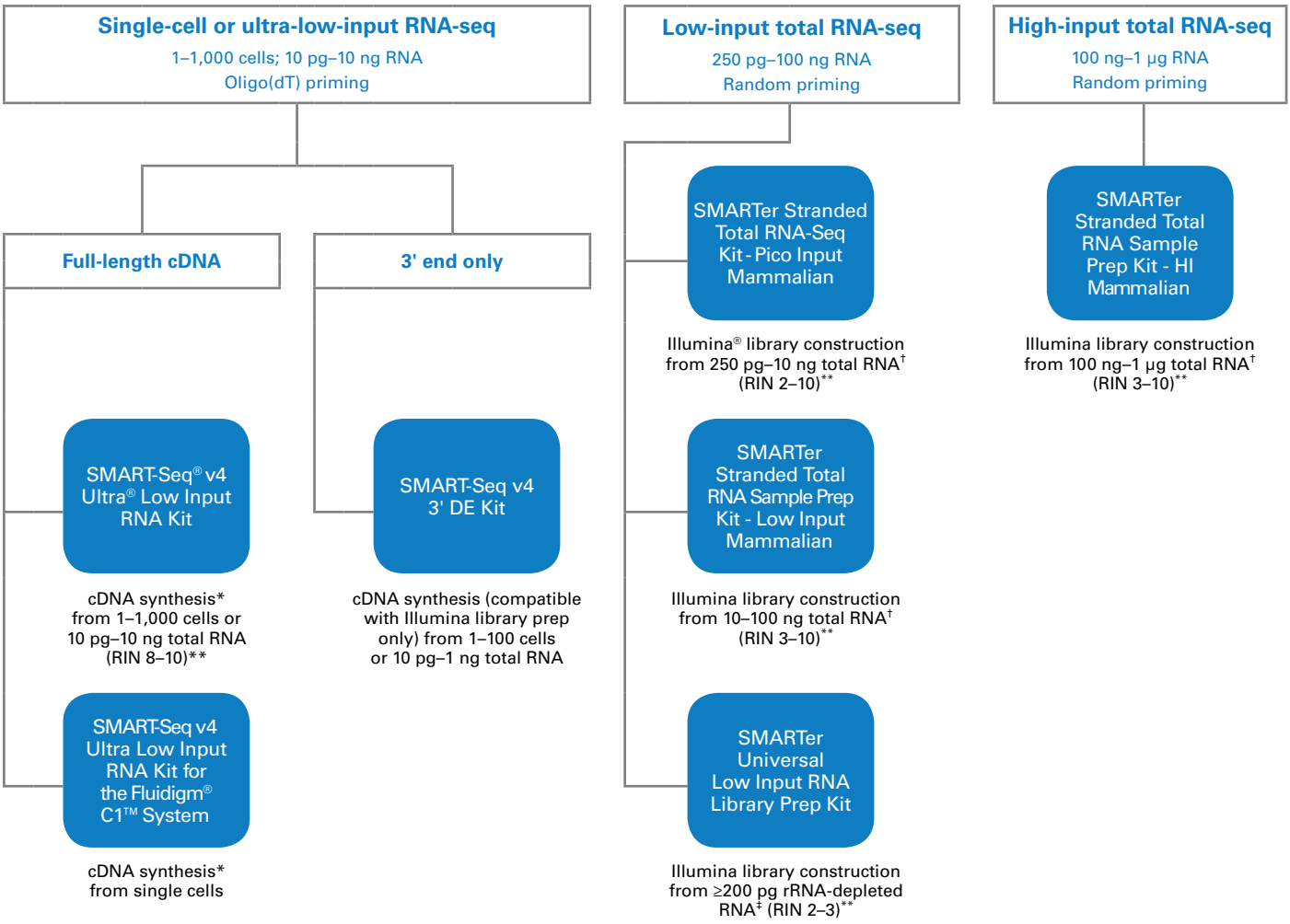
Whole transcriptome analysis

Targeted sequencing

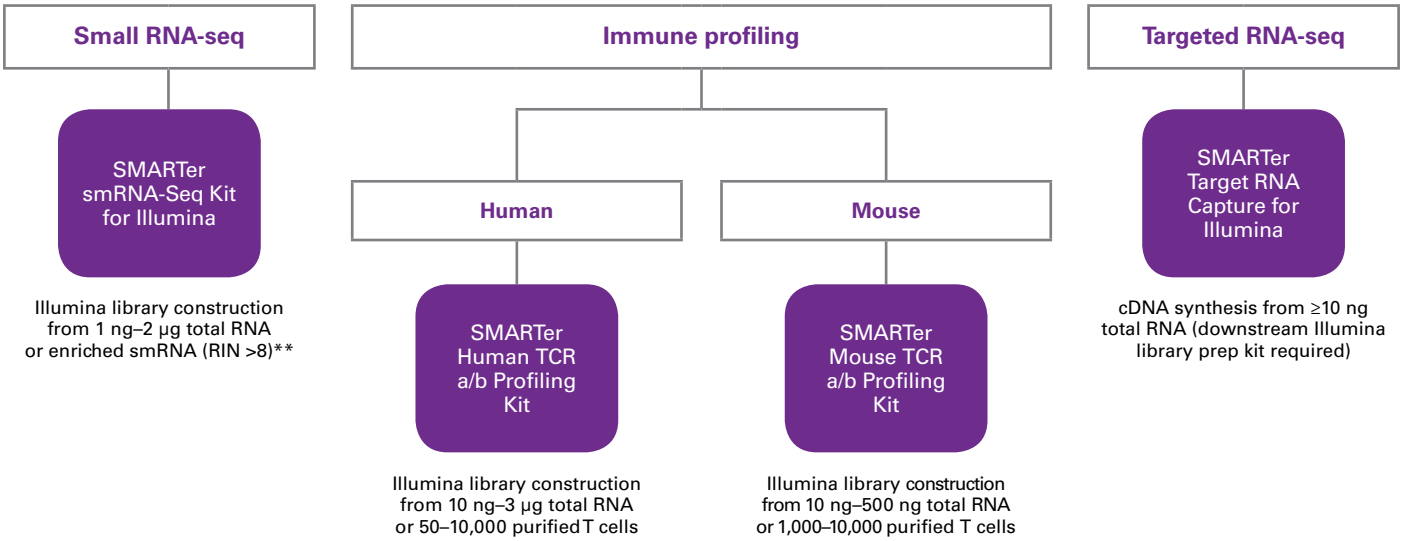
DNA sequencing

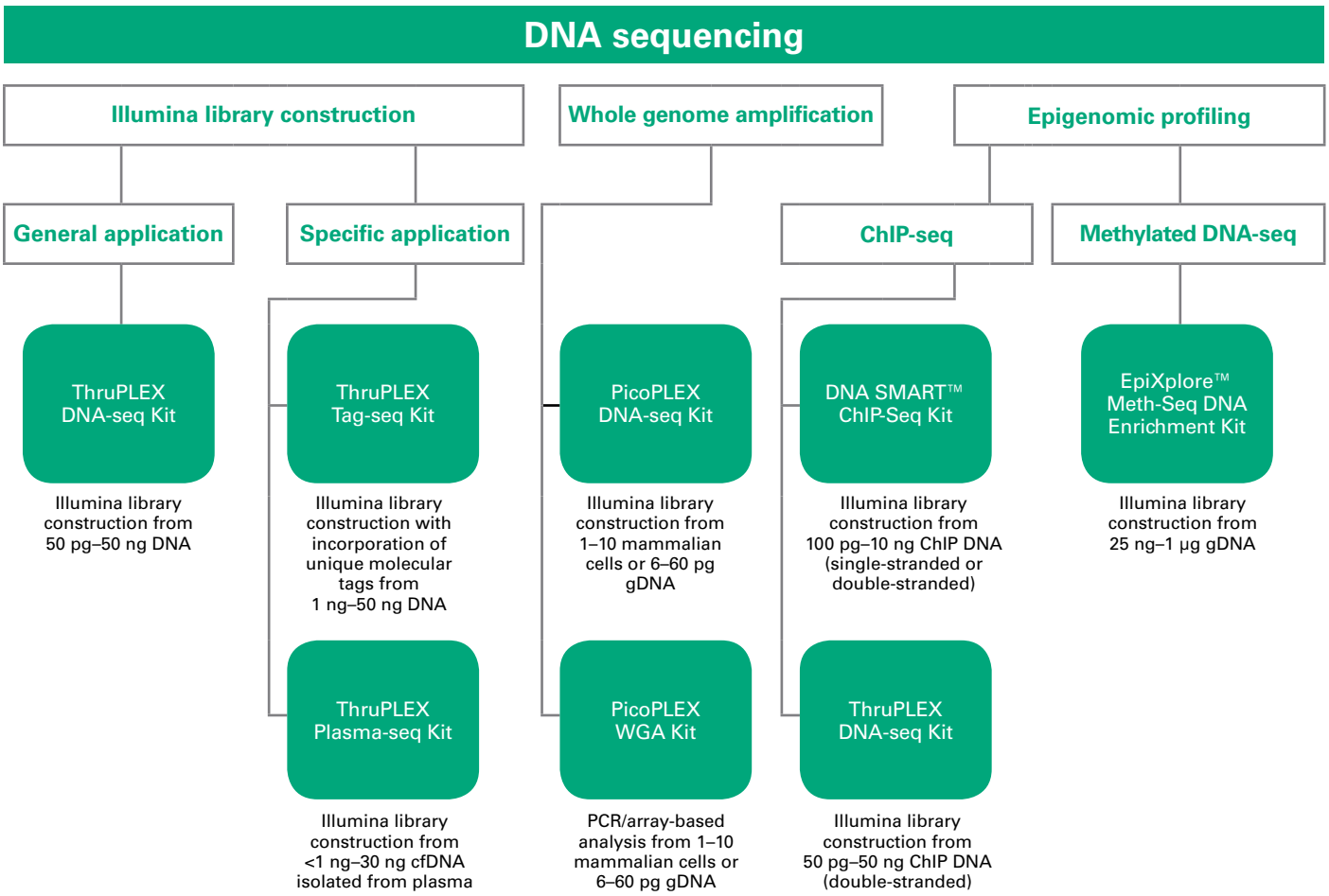
that's GOOD science!®

Whole transcriptome analysis (from mammalian samples)



Targeted sequencing





For non-mammalian or rRNA-depleted samples, use

SMARTer Stranded RNA-Seq Kit for Illumina library construction from 100 pg–100 ng rRNA-depleted or polyA⁺-enriched RNA

SMARTer Universal Low Input RNA Kit for Sequencing* for cDNA synthesis from ≥200 pg rRNA-depleted RNA derived from 2–100 ng of total RNA

SMARTer Universal Low Input RNA Library Prep Kit for Illumina library construction from ≥200 pg rRNA-depleted RNA (RIN 2–3)**

* Compatible with Illumina or Ion Torrent library preparation

† rRNA removal included

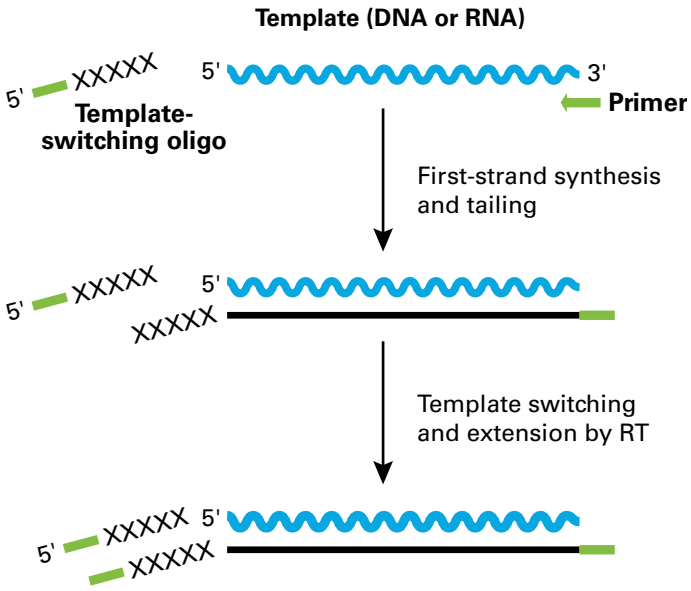
‡ For mammalian rRNA removal, we recommend **RiboGone™ - Mammalian**, specifically designed to remove rRNA from low-input samples (10–100 ng of total RNA)

** Recommended RNA quality

SMART technology overview

What is SMART technology?

SMART (**S**witching **M**echanism **a**t the 5′ end of **R**NA **T**emplate) technology leverages the ability of certain reverse transcriptases (RTs) to add non-templated nucleotides (depicted by Xs in the diagram) upon reaching the 5′ end of a template. A carefully designed template-switching oligo can pair with these additional nucleotides, providing a new template for the RT to continue cDNA synthesis. This process allows for the incorporation of adapter sequences for subsequent PCR amplification (depicted in green) at both ends of a template, ensuring full-length template coverage.



Workflow for SMART technology

Why adopt SMART technology?

- Exquisite sensitivity and reproducibility
- Full-length template coverage
- Ligation-free adapter incorporation
- High-quality sequencing libraries
- Simplified workflows, single-tube protocols

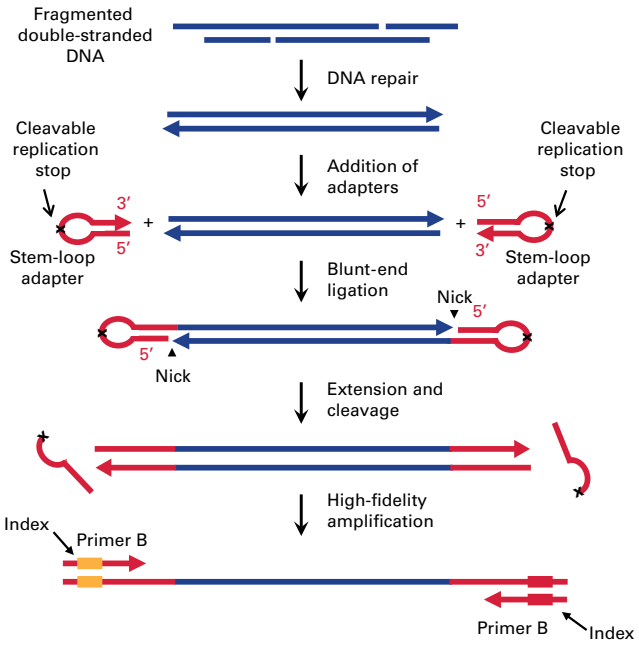
SMARTer NGS applications

	DNA SEQUENCING	TARGETED SEQUENCING	WHOLE TRANSCRIPTOME ANALYSIS
SMART Technology	• ChIP-seq • Meth-seq	• Targeted RNA-seq • Small RNA-seq • Immune profiling	• Single-cell/ultra-low-input RNA-seq • Total RNA-seq
ThruPLEX Technology	• Illumina library construction • Targeted sequencing • ChIP-seq	• Targeted DNA-seq with major enrichment platforms	
PicoPLEX Technology	• Whole genome amplification • Aneuploidy/CNV detection		

ThruPLEX technology overview

What is ThruPLEX technology?

ThruPLEX technology starts with fragmented, double-stranded DNA (blue), which is repaired in a highly efficient process. Stem-loop adapters (red) with blocked 5′ ends are ligated to the 5′ ends of the DNA, leaving nicks at the 3′ ends. The adapters cannot ligate to each other and do not have single-strand tails, reducing non-specific background in sequencing data. The 3′ ends of the DNA are then extended to complete library synthesis, and Illumina indexes are added through a high-fidelity amplification.



Workflow for ThruPLEX technology

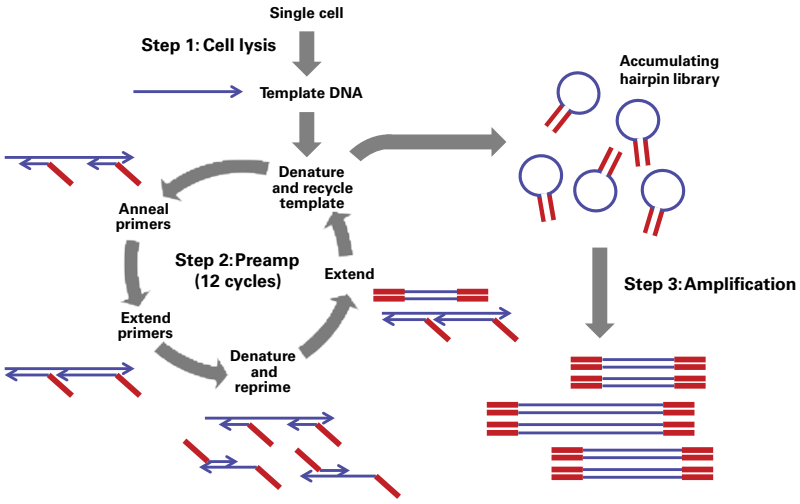
Why adopt ThruPLEX technology?

- High sensitivity with low background
- Accurate representation of GC-rich sequences and high library diversity
- Analysis of diverse inputs, including FFPE DNA, gDNA, ChIP DNA, cfDNA, cDNA, amplicons, etc.
- Compatible with major target-enrichment platforms
- Simplified workflows, single-tube protocols

PicoPLEX technology overview

What is PicoPLEX technology?

PicoPLEX technology uses single-cell or picogram inputs of genomic DNA as a template for multiple cycles of quasi-random priming and linear whole genome amplification, followed by exponential library amplification.



Workflow for PicoPLEX technology

Why adopt PicoPLEX technology?

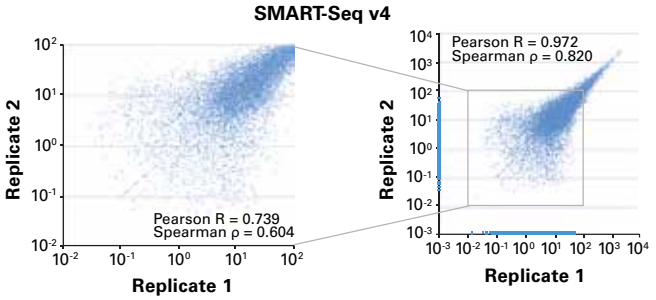
- Robust, highly reproducible whole-genome amplification from single-cell or ultra-low inputs
- Ligation-free adapter incorporation
- Simplified workflows, single-tube protocols

Single-cell RNA-seq

Single-cell RNA-seq using oligo(dT) priming is a powerful approach for resolving biologically relevant differences between individual cells and allows for analysis of rare or precious samples, including stem cells, circulating tumor cells, and tissue biopsies. In order to yield meaningful data from single cells, NGS library preparation methods must be robust and sensitive enough to reproducibly capture RNA molecules present in minute quantities.

About SMART-Seq v4 kits

- Solutions for single-cell and ultra-low inputs
- Ligation-free protocol
- Improved chemistry relative to previous versions and locked nucleic acid (LNA)-enhanced oligos
- Accurate representation of GC-rich transcripts
- Compatible with Illumina and Ion Torrent sequencing platforms



High reproducibility between replicates for data generated with the SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing. For sequencing data generated from 10 pg of mouse brain total RNA, comparison of FPKM values between replicates indicates strong correlations, both overall (right panel) and among low-abundance transcripts (FPKM <100, left panel).

SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing

- Inputs of 1–1,000 cells or 10 pg–10 ng of total RNA
- Single-tube protocol
- Even gene-body coverage

SMART-Seq v4 3′ DE Kit

- Inputs of 1–100 cells or 10 pg–1 ng of total RNA
- Designed specifically for differential expression analysis
- 3′ focusing of sequencing reads reduces costs and saves time

SMART-Seq v4 Ultra Low Input RNA Kit for the Fluidigm C1 System

- For analysis of single cells isolated on the Fluidigm C1 platform
- Library preparation can be completed in two working days

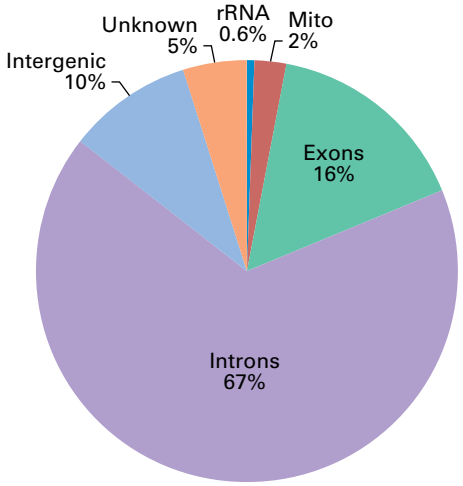
Reduced variability among cDNA libraries using the SMART-Seq v4 (SS-v4) protocol. Sequencing data were generated with the SS-v4 method (11 libraries x 2 replicate experiments) and the Ultra Low v1 (UL-v1) method (11 libraries). Pearson correlation coefficients were higher and tighter for data generated with SS-v4.

Total RNA-seq

Total RNA-seq using random priming is the preferred method for analyzing both coding and non-coding RNA and for generating libraries from FFPE or degraded samples. However, the typical abundance of ribosomal RNA (rRNA) in total RNA inputs and the identification of non-coding and antisense RNA without strand-of-origin information pose significant challenges for this approach.

About SMARTer total RNA-seq kits

- Solutions for inputs of varying quantity and quality, including degraded RNA (e.g., FFPE samples)
- Ligation-free protocols
- Illumina-ready sequencing libraries
- Multiplexing of up to 96 samples



High-quality sequencing data generated from FFPE samples. rRNA depletion and random-primed cDNA synthesis technologies included in the SMARTer Universal Low Input RNA Library Prep Kit yield a high proportion of usable sequencing reads and low proportions of reads mapping to rRNA, mtRNA, etc., from degraded samples.

SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian

- Inputs of 250 pg–10 ng of mammalian total RNA
- Strand-of-origin information
- Compatible with degraded inputs
- Generates libraries in ~5 hours

SMARTer Stranded Total RNA Sample Prep Kit - Low Input Mammalian

- Inputs of 10–100 ng of mammalian total RNA
- Strand-of-origin information
- Compatible with degraded inputs
- Includes RiboGone - Mammalian rRNA-depletion technology
- Generates libraries in ~5 hours

SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian

- Inputs of 100 ng–1 µg of mammalian total RNA
- Strand-of-origin information
- Includes RiboGone - Mammalian rRNA-depletion technology
- Generates libraries in ~5 hours

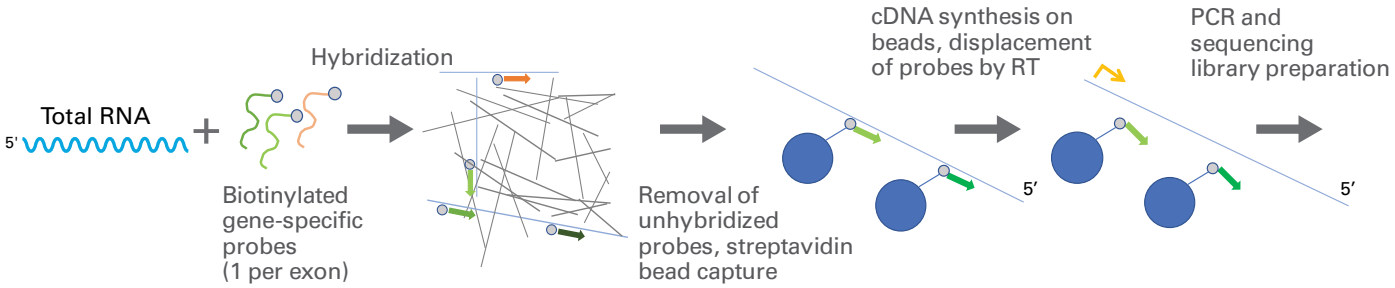
SMARTer Universal Low Input RNA Library Prep Kit

- Inputs of ≥200 pg of rRNA-depleted RNA of any species
- Compatible with degraded inputs

Targeted RNA-seq

The ability to analyze the entire transcriptome in a single RNA-seq experiment has revolutionized biomedical research, but for some applications a targeted approach is more practical. For studies involving a pre-determined set of genes, targeted RNA-seq allows for the analysis of rare transcripts—including those resulting from alternative splicing or gene fusion events—using fewer sequencing reads than would be required in the context of a global analysis.

SMARTer Target RNA Capture for Illumina employs biotinylated DNA probes that are designed by the user to hybridize with transcripts of interest and are bound by streptavidin-coated capture beads (indicated below by blue circles). cDNA synthesis is performed on hybridized transcripts using SMART-Seq v4 chemistry with oligo(dT) priming. The Nextera® XT DNA Library Preparation Kit can then be used to generate Illumina-ready sequencing libraries



Workflow for SMARTer Target RNA Capture for Illumina

About SMARTer Target RNA Capture for Illumina

- Inputs of 10 ng–1 µg of total RNA
- User-designed DNA probes enrich for transcripts of interest
- Ligation-free protocol
- Requires ~3 hours of active time
- Recommended for analysis of up to 100 genes per reaction

Targeted transcript enrichment from various cell types/tissues					
	Fold-Enrichment				
	K562	KBM-7	HURR	HBR	
GAPDH	0.52	0.33	0.23	0.47	Not targeted
ERCC-002	0.45	0.07	0.02	0.13	
ABL1	324	325	656	329	Targeted
ALK	20	1	563	302	
CDKN2A	0.20	205	135	16	
FGFR1	27	603	644	437	
HPRT1	270	314	294	282	
KRAS	227	158	203	144	
RB1	323	585	604	108	
RET	6	29	459	369	
TP53	24	1455	943	119	
	Percentage of reads on target				
With capture	16%	37%	28%	14%	
No capture	0.07%	0.10%	0.07%	0.05%	

Demonstrated enrichment of targeted transcripts with SMARTer Target RNA Capture for Illumina. Sequencing reads for transcripts targeted with the kit (ABL1, ALK, etc.) are highly enriched relative to data for untargeted transcipts (GAPDH and ERCC-002).

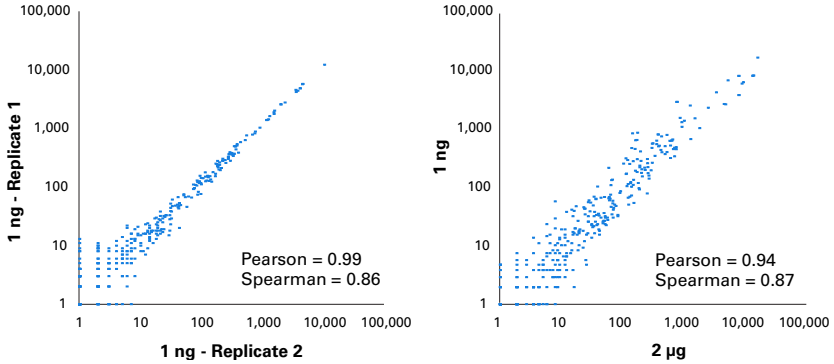
Small RNA-seq

The ability to identify and analyze diverse small RNA species—including miRNAs, siRNAs, piRNAs, and snoRNAs—has benefited greatly from the development of NGS technology. However, small RNA-seq libray preparation is not without its challenges, which can include time-consuming enrichment steps prior to cDNA synthesis, and sample misrepresentation due to biases in small RNA end modification, reverse transcription, and PCR amplification.

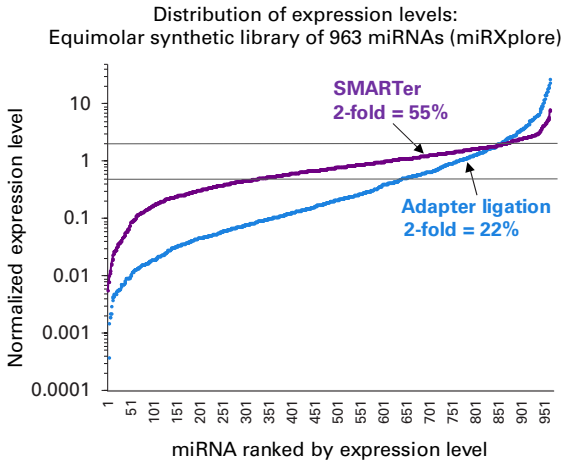
The SMARTer smRNA-Seq Kit for Illumina leverages SMART technology coupled with 3′ polyadenylation to generate Illumina-ready small RNA-sequencing libraries. In contrast with library construction methods that isolate small RNAs via adapter ligation, this approach uses 3′ polyadenylation and SMART technology to minimize the likelihood of sample representation bias.

About the SMARTer smRNA-Seq Kit for Illumina

- Inputs of 1 ng–2 µg of total RNA or enriched small RNA
- Suitable for analysis of diverse smRNAs 15–150 nt in size, including miRNAs, siRNAs, piRNAs, and snoRNAs
- Ligation-free, single-tube protocol
- Multiplexing of up to 96 samples



High reproducibility across technical replicates and varying input amounts for data generated with the SMARTer smRNA-Seq Kit for Illumina. Strong correlations are observed for sequencing data generated from human brain total RNA, both in replicate (1 ng inputs, left panel) and across varying input amounts (1 ng vs. 2 µg inputs, right panel).



Improved accuracy of SMARTer small RNA-seq vs. an adapter ligation-based approach. Sequencing data generated for an equimolar pool of 963 synthetic miRNAs using the SMARTer smRNA-Seq Kit for Illumina (purple line) more closely approaches known normalized expression values (expression = 1) relative to data produced using an adapter-ligation approach (blue line).

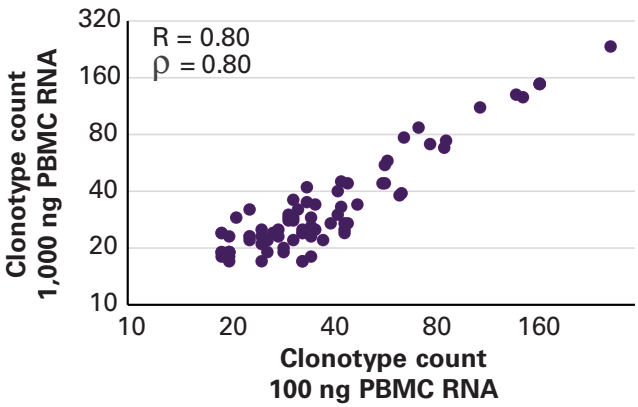
Immune profiling

The seemingly endless diversity of T-cell receptor and antibody sequences found in higher organisms poses a unique challenge to researchers studying the adaptive immune system. NGS has dramatically expanded the capacity to profile immune repertoires in a comprehensive manner, but concerns regarding the accuracy of sample representation and the identification of functionally relevant sequences remain.

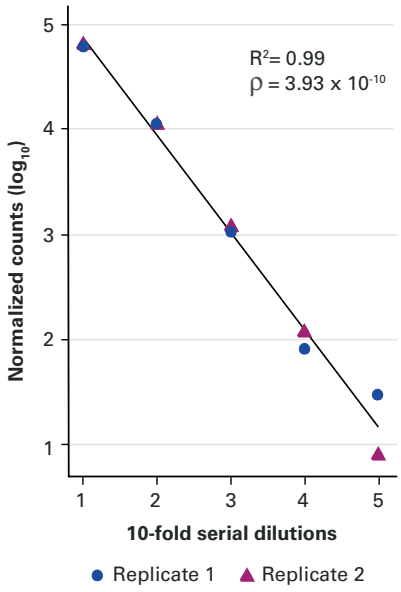
The SMARTer Human TCR a/b Profiling Kit and the SMARTer Mouse TCR a/b Profiling Kit leverage SMART technology and a 5′ RACE-based approach to capture transcribed TCR sequences with high sensitivity and reproducibility.

About SMARTer TCR a/b profiling kits

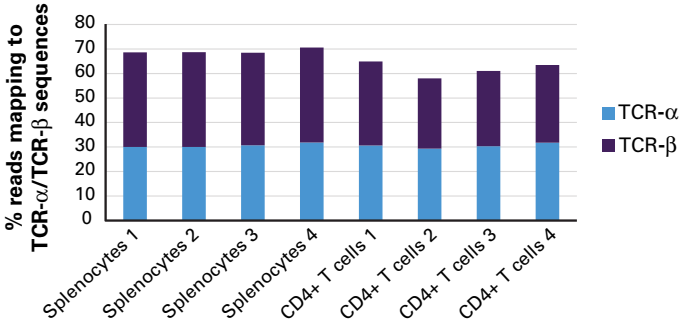
- Inputs of 10 ng–3 µg of human blood total RNA or 50–10,000 T cells (human kit), and 10 ng–500 ng mouse blood total RNA or 1,000–10,000 T cells (mouse kit)
- Full-length sequence information for TCR-α and/or TCR-β
- Library amplification without PCR multiplexing
- Ligation-free protocol
- Illumina-ready sequencing libraries
- Multiplexing of up to 96 samples



Consistent transcript representation across varying input amounts for data generated with the SMARTer Human TCR a/b Profiling Kit. Measured frequencies of various TCR clonotypes correlate strongly for libraries generated from 1,000 ng or 100 ng inputs of PBMC total RNA.



Reproducible representation of low-abundance TCR clonotypes with the SMARTer Human TCR a/b Profiling Kit. A TCR clonotype corresponding to spiked-in Jurkat RNA is reproducibly quantified down to a concentration of 0.1% (X-axis = 3).



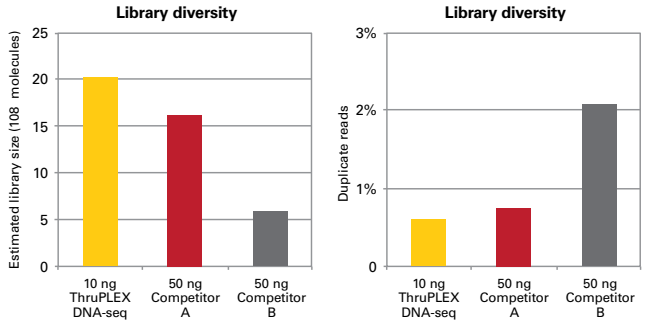
High proportions of on-target reads mapping to mouse TCR-α and TCR-β CDR3 sequences for data generated with the SMARTer Mouse TCR a/b Profiling Kit. Sequencing libraries were generated from total RNA extracted from total splenocytes or enriched CD4+ T cells. High proportions of on-target reads mapping to TCR-α or TCR-β CDR3 were observed for each input.

Illumina library construction

In a typical DNA-seq workflow, input DNA is first purified and mechanically sheared to yield short, double-stranded DNA molecules. For analysis on Illumina platforms, sequencing libraries containing proper adapters and indexes must then be generated from this input material. To ensure that sequencing libraries are of the highest quality, especially when working with limiting DNA inputs, the library preparation method must be highly efficient and accurate, and it must avoid purification or transfer steps where DNA can be lost or contaminated.

About ThruPLEX kits

- Solutions for diverse inputs, including FFPE DNA, gDNA, ChIP DNA, cfDNA, cDNA, amplicons, etc.
- Illumina-ready sequencing libraries
- Multiplexing of up to 96 samples
- Compatible with major target-enrichment platforms such as Agilent SureSelect, Roche Nimblegen SeqCap EZ, and IDT xGen Lockdown probes
- Three-step, single-tube protocol can be completed in ~2 hours



The ThruPLEX DNA-seq Kit outperforms other NGS library prep kits. The ThruPLEX DNA-seq Kit yielded more diverse libraries (left panel) with less input gDNA and generated fewer duplicates (right panel) at 25M read pairs than other kits.

ThruPLEX DNA-seq Kit

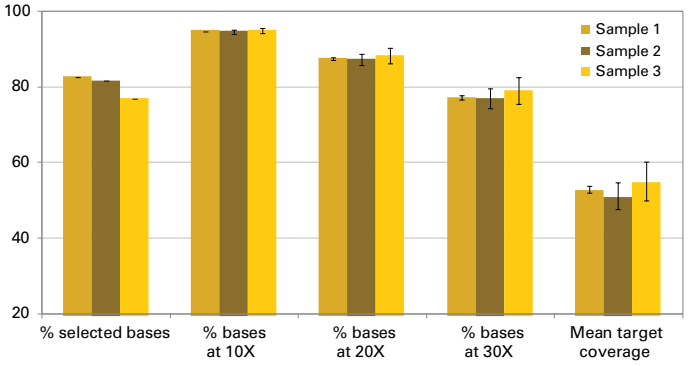
- Inputs of 50 pg–50 ng of FFPE DNA, gDNA, ChIP DNA, cfDNA, cDNA, amplicons, etc.
- Suitable for a broad array of DNA-seq applications

ThruPLEX Tag-seq Kit

- Inputs of 1 ng–50 ng of FFPE DNA, gDNA, ChIP DNA, cfDNA, etc.
- Incorporation of up to 16 million unique molecular tags (UMTs) during library construction

ThruPLEX Plasma-seq Kit

- Inputs of <1 ng–30 ng of cfDNA isolated from plasma
- Automation-friendly: Beckman FX^{P} Workstation



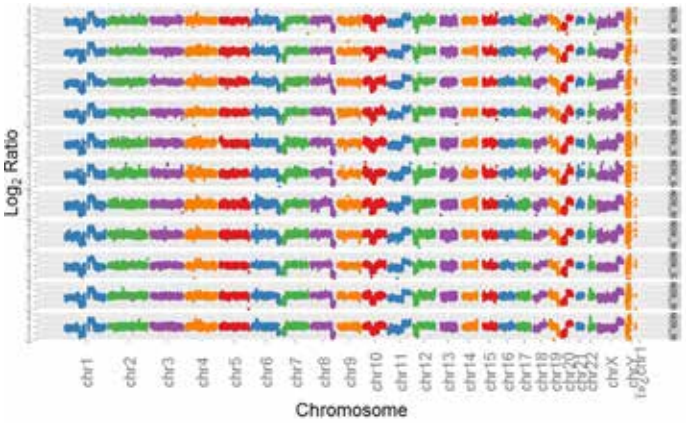
Outstanding target-enrichment performance with the ThruPLEX Plasma-seq Kit. ThruPLEX Plasma-seq Kit libraries generated in triplicate from three different plasma samples (5–10 ng inputs) were captured at high efficiency using the ClearSeq Human DNA Kinome Panel for SureSelectXT2 and generated data with deep coverage of the kinome for mutation detection. Selected bases were successfully captured bases that were in or within 250 bp of the baits.

Whole genome amplification

By enabling highly accurate detection of aneuploidy and copy-number variation (CNV) from single cells, advances in whole genome amplification combined with NGS and array-based technologies have revolutionized clinical applications such as pre-implantation genetic screening and diagnosis (PGS/PGD). Widespread adoption of this approach has been driven by the development of user-friendly workflows that are cost-effective and yield reproducible results.

About PicoPLEX kits

- Compatible with single-cell or ultra-low inputs
- Reproducible, informative results with low sequence coverage
- Ligation-free protocol
- Solutions for both NGS and PCR/array-based approaches
- Three-step, single-tube protocol takes less than 3 hours



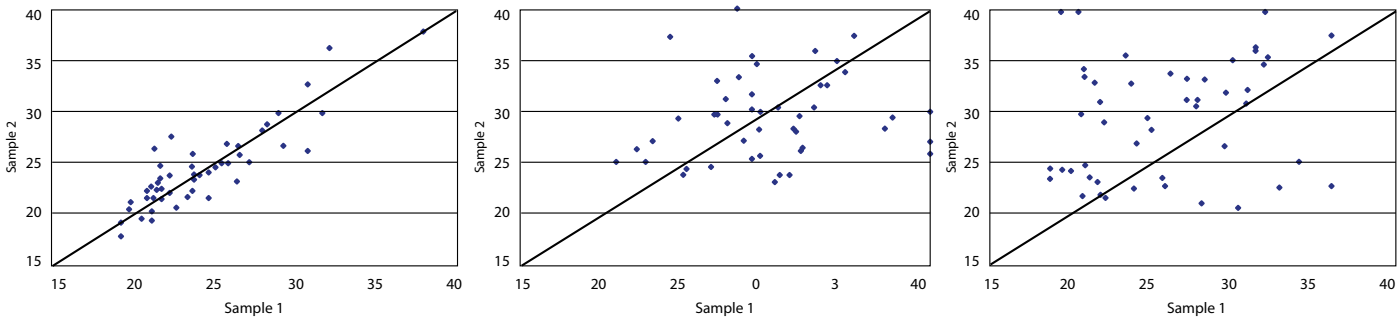
Highly reproducible CNV detection over the entire genome with the PicoPLEX DNA-seq Kit. Amplified libraries from 11 individual flow-sorted H929 cells were sequenced and downsampled to 250,000 total reads. 35-base single-end reads were mapped to the entire genome.

PicoPLEX DNA-seq Kit

- Inputs of 1–10 cells or 6 pg–60 pg of DNA
- Library quantification not required
- Illumina-ready sequencing libraries
- Multiplexing of up to 48 samples

PicoPLEX WGA Kit

- Inputs of 1–10 cells or 15 pg–50 pg of DNA
- Yields libraries for array or PCR-based analysis
- Unambiguous results at all resolutions



The PicoPLEX WGA Kit outperforms the competition in locus-specific qPCR assays. Locus-specific qPCR was used to quantify 48 loci in independent single-cell libraries. Data shown compares results from two individual samples amplified with (from left panel to right panel) the PicoPLEX WGA Kit, Competitor kit A, and Competitor kit B using 10 pg of input DNA. Stronger correlations were observed for libraries generated with the PicoPLEX WGA Kit relative to other methods.

Epigenomics

ChIP-seq

The capacity to analyze protein-DNA interactions on a genome-wide scale using chromatin immunoprecipitation sequencing (ChIP-seq) has facilitated tremendous progress for epigenetics research. Given that many proteins of interest (e.g., transcription factors and modified histones) are associated with a limited number of specific genomic loci, the amount of DNA recovered by chromatin immunoprecipitation can be quite low, posing challenges for the generation of representative ChIP-seq libraries.

About the ChIP Elute Kit

- A simple solution for elution, cross-linking reversal, and purification of ChIP DNA
- Yields single-stranded DNA that is ready for NGS library construction
- Protocol can be completed in less than 1 hour

About the DNA SMART ChIP-Seq Kit

- Inputs of 100 pg–10 ng of single or double-stranded DNA
- Ligation-free protocol
- Illumina-ready sequencing libraries
- Multiplexing of up to 48 samples
- Generates libraries in ~4 hours

Meth-seq

While it is well known that DNA methylation plays an important role in the regulation of gene expression, many outstanding questions remain regarding the establishment and biological significance of variation in DNA methylation across the genome. Bisulfite sequencing has proven useful for identifying DNA methylation patterns with single-base resolution at specific genomic locations, but is not an ideal approach for performing high-throughput genome-wide analyses.

The EpiXplore Meth-Seq DNA Enrichment Kit exploits the binding affinity of the MBD2 protein to separate methylated and unmethylated fractions of genomic DNA for NGS library preparation.

About the EpiXplore Meth-Seq DNA Enrichment Kit

- Inputs of 25 ng–1 µg of sheared genomic DNA
- Ligation-free protocol
- Illumina-ready sequencing libraries

Sequencing accessories

Ribosomal RNA removal

RiboGone - Mammalian enables removal of mammalian rRNA from 10–100 ng inputs, including degraded RNA samples. The kit eliminates ≥95% of ribosomal RNA sequences from NGS data, including sequences from *5S, 5.8S, 18S,* and *28S* nuclear rRNA, and *12S* mtRNA.

Library quantification

The **Library Quantification Kit** provides a highly sensitive, qPCR-based method specifically for quantification of Illumina libraries.

Magnetic bead separation

The **SMARTer-Seq® Magnetic Separator - PCR Strip** holds up to 24 0.2-ml PCR tubes in two rows, and is designed for capture of magnetic beads in 10–20 minutes. It is ideally suited for DNA purification and size-selection techniques used with most NGS library preparation kits.

NGS Learning Center

Visit the Takara Bio NGS Learning Center to find more information about our NGS kits.

- FAQs
- Selection Guide
- Technical Notes
- Citations
- Webinars
- ...and more

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