Total RNA-seq overview

Rapid sequencing libraries from full-length and degraded total RNA samples

Good science leaves no possibility uninvestigated, no matter how small the sample size or how varied the sample composition. In support of those wide-ranging opportunities, we offer several SMARTer kits for total RNA-seq: the best-in-class tools for rapid and accurate RNA-seq from the entire transcriptome. With the variety of total RNA-seq solutions we offer, you can count on streamlined protocols and excellent technical expertise for all of your experiments.

This overview describes SMARTer total RNA-seq tools available for:

Pico-input mammalian total RNA: 250 pg–10 ng »

Low-input mammalian total RNA: 10 ng–100 ng »

High-input mammalian total RNA: 100 ng–1 µg »

Highly degraded, low-input RNA: 10–100 ng »

Non-mammalian, low-input samples: 200 pg–10 ng »

What is total RNA-seq?

Total RNA-seq uses random priming (rather than poly(dT) priming that is used in mRNA-seq) to generate transcriptomic data from an RNA sample. SMARTer products for total RNA-seq use random hexamers for priming. This method allows researchers to identify both non-coding and coding RNAs in their samples.

What are the challenges of total RNA-seq?

Since total RNA-seq uses random priming for first-strand cDNA synthesis, abundant transcripts, for example ribosomal RNA (rRNA), will dominate sequencing reads if they are not removed. Identifying antisense RNA (which is often non-coding RNA) is also a challenge if strand-of-origin information is not obtained. Finally, highly degraded RNA samples, like those from FFPE tissues, require particularly sensitive and robust strategies for accurate NGS library preparation. The SMARTer family of products is varied to accommodate RNA of different quality and quantity, while maintaining the same high level of sensitivity we demand from these superior products.

Tools for pico-input mammalian total RNA

The SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian has been optimized for stranded RNA-seq library construction from picogram amounts of total mammalian RNA, regardless of quality. cDNA libraries generated using this kit are ready for sequencing (no additional library prep needed).

- Recommended input range: 250 pg–10 ng of total mammalian RNA
- Sequencing libraries are generated in under 5 hours and are compatible with any Illumina platform
- Template-switching oligonucleotide incorporates locked nucleic acid (LNA) technology for improved sensitivity and reproducibility

Sequencing alignment metrics from 100 pg–10 ng total RNA								
RNA source	Mouse brain total RNA							
Input amount (ng)	10		1		0.25		0.1	
Library yield (ng/µl)	10.5	14.8	9.93	8.3	6.91	7.48	5.76	7.26
Number of reads (millions)	2.6 (paired-end reads)							
Number of transcripts FPKM >1	12,714	12,709	12,744	12,725	12,540	12,615	12,286	12,528
Pearson/Spearman correlations	0.99/0.93		0.99/0.93		0.98/0.92		0.97/0.90	
Correct strand per biological annotation (%)	97.7	97.7	97.7	97.7	97.7	97.7	97.7	97.6
Proportion of total reads (%):								
Exonic	22.6	22.8	23.4	23.5	23.3	23.1	23.1	22.8
Intronic	35.6	35.7	35.3	36.2	35.9	35.5	36.1	35.1
Intergenic	8.3	8.2	8.2	8.2	8.0	8.0	7.8	7.8
rRNA	11.2	10.5	10.8	9.9	9.7	9.7	8.8	9.5
Mitochondrial	8.8	8.7	8.3	8.5	8.3	8.4	7.5	7.9
Duplicate rate (%)	12.8	12.5	17.3	17.8	31.3	28.8	44.2	40.2

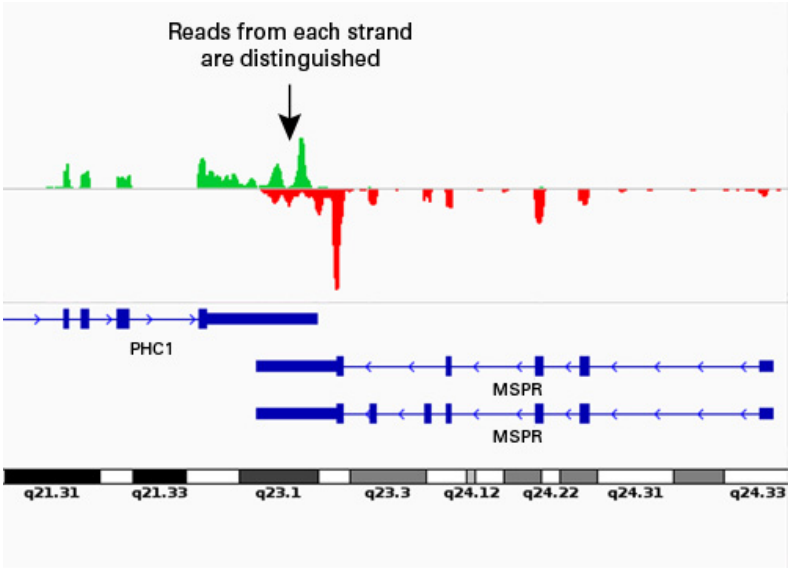
SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian shows high sensitivity and reproducibility over a 100-fold input range. Libraries were generated from mouse brain total RNA (100 pg–10 ng), with two technical replicates per input amount. Sequencing metrics show >12,000 transcripts detected with FPKM >1, good mapping to the genome, and preservation of strand-of-origin information even from 100 pg total RNA.

Go to the SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian product page »

Tools for low-input mammalian total RNA

SMARTer Stranded RNA-Seq Kits pair seamlessly with RiboGone - Mammalian rRNA removal kits to provide a low-input solution for total RNA-seq from 10–100 ng mammalian total RNA. Additionally, Illumina sequencing library adaptors are added directly during cDNA synthesis, meaning that no additional library preparation is necessary.

- High-quality RNA-seq libraries for Illumina sequencing in less than 5 hours
- dUTP-independent identification of strand-of-origin information; over 99% accurate
- Extremely low rRNA reads when paired with RiboGone kits



With the SMARTer Stranded RNA-Seq Kit, short, overlapping reads originating from different strands of the genomic DNA, can be distinguished from each other. Thus, enabling quantitative expression analysis and accurate genome annotation. Libraries were prepared from Human Brain Poly A$^+$ RNA spiked with ERCC control RNA and sequencing was performed on an Illumina HiSeq® platform with 2 x 100 bp paired end reads.

Go to the SMARTer Stranded RNA-Seq Kit product page »

Tools for high-input mammalian total RNA

SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian is an all-in-one kit for rRNA removal, cDNA synthesis, and sequencing library preparation for higher-input (100 ng–1 µg) total RNA samples. This kit incorporates RiboGone and SMARTer stranded technology into a single kit, maintaining the low percentage of rRNA reads and high accuracy in identifying strand of origin found in these two technologies.

- Illumina sequencing libraries in 5 hours
- Input range of 100 ng–1 µg full-length or degraded mammalian RNA
- Up to 96 different Illumina indexes

Sequence alignment metrics from RNA of varying quality				
RNA source	Mouse liver			
RNA quality (RIN)	RIN 3		RIN 7	
Input amount	100 ng	1 µg	100 ng	1 µg
Number of reads (millions)	1.7 (paired end reads)			
Percentage of reads (%):				
rRNA	2%	2%	1%	1%
Mapped to genome	82%	86%	81%	88%
Mapped uniquely to genome	73%	75%	72%	77%
Exonic	55%	53%	54%	54%
Intronic	32%	31%	33%	32%
Intergenic	12%	14%	12%	13%
Number of genes identified	12,079	12,172	12,099	12,212
Percent biological strandedness	95.5%	97.2%	95.6%	98.1%

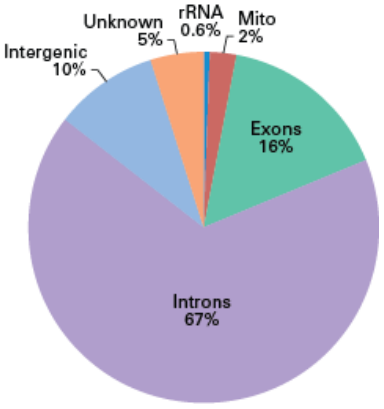
SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian for high-input samples generates high-quality libraries from total RNA of varying quality. Libraries were generated from chemically sheared Mouse Liver Total RNA (sheared to a RIN of 3 or 7). Sequencing metrics (as defined by Picard analysis) showed high mapping to the genome, low numbers of rRNA reads, and excellent identification of the correct strand of origin.

Go to the SMARTer Stranded Total RNA Sample Prep Kit - HI Mammalian product page »

Tools for highly degraded, low-input samples

Highly degraded RNA samples (e.g., from FFPE tissues) tend to have many RNA fragments less than 200 bp. The SMARTer Universal Low Input RNA Kit for Sequencing preserves these small fragments, making it ideal for low-input degraded RNA. For low-input (10–100 ng) mammalian samples we recommend depleting rRNA using the RiboGone - Mammalian kit prior to cDNA synthesis. The cDNA generated with this kit is compatible with downstream sequencing library preparation for either Illumina or Ion Torrent sequencing.

- Excellent sequencing metrics from low-input and degraded FFPE RNA samples
- Compatible with both the ThruPLEX DNA-Seq Kit (for preparation of Illumina sequencing libraries) and library prep kits for Ion Torrent platforms
- Generate sequencing data from both coding and non-coding RNAs



The SMARTer Universal Low Input RNA Kit for Sequencing is an excellent solution for RNA-seq from highly degraded RNA and FFPE samples. Total RNA from breast carcinoma FFPE tissue was rRNA-depleted using **RiboGone - Mammalian** and used to generate RNA-

seq libraries with the **SMARTer Unversal Low Input RNA Kit for Sequencing** and the **Low Input Library Prep Kit**. rRNA reads were reduced to 0.6% of total reads. The number of reads that mapped to introns, exons, intergenic regions, rRNA, mitochondrial RNA, and unknown sources are shown as percentages of the total reads.

Go to the SMARTer Universal Low Input RNA Kit for Sequencing product page »

Tools for low-input, non-mammalian samples

SMARTer Universal Low Input RNA kits and SMARTer Stranded RNA-Seq Kits are compatible with low-input non-mammalian samples. The SMARTer Universal kits are compatible with 200 pg–10 ng of RNA, while the SMARTer Stranded kits may be used with 100 pg–100 ng of RNA. We strongly recommend that total RNA samples are rRNA-depleted or poly(A)-purified prior to use. These kits utilize random priming and, therefore, up to 90% of sequencing reads are expected to map to rRNA without removal of rRNA prior to library preparation.

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