CLARETBIO

SRSLY™ A versatile, simple, and efficient NGS library prearation for the analysis of highly fragmented double-stranded and single-stranded DNA

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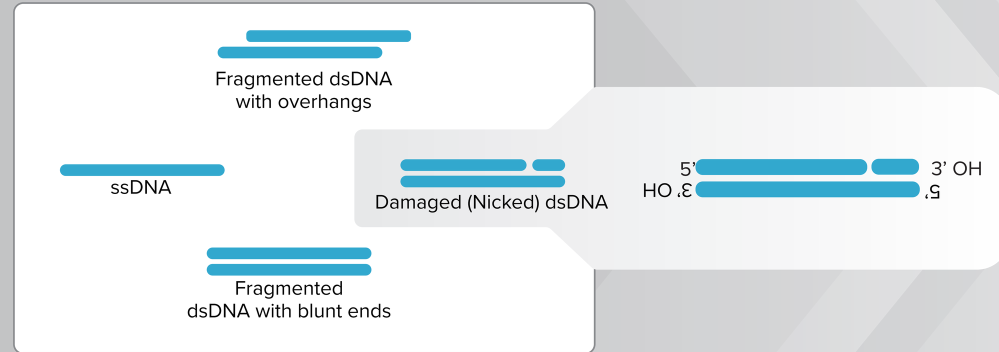
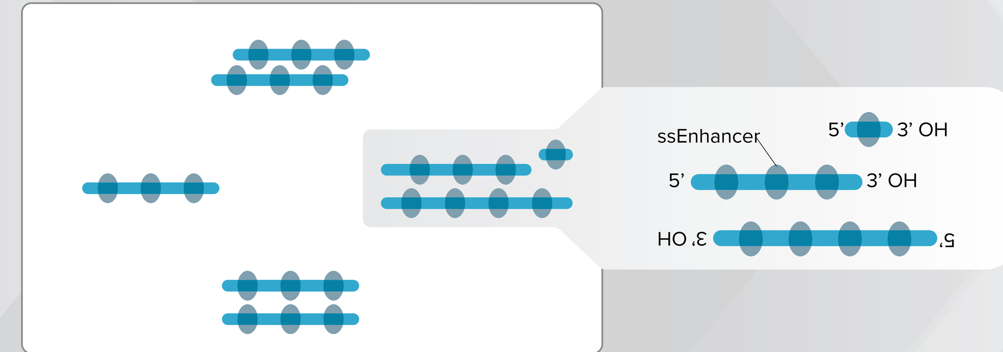
LIBRARY PREPARATION WORKFLOW

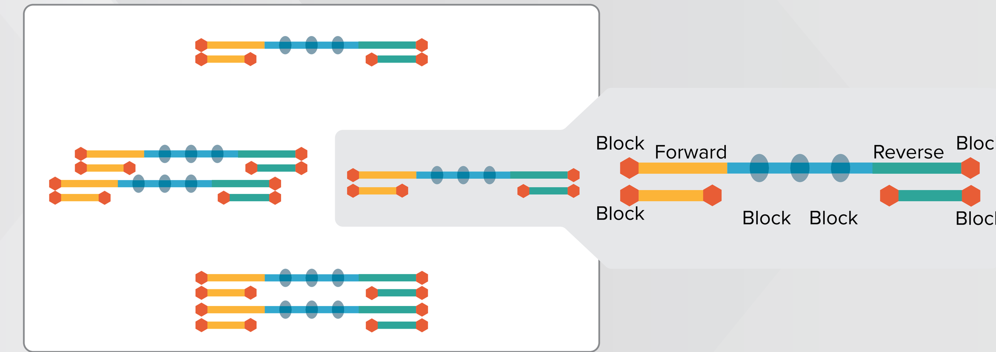
1. DNA input pool



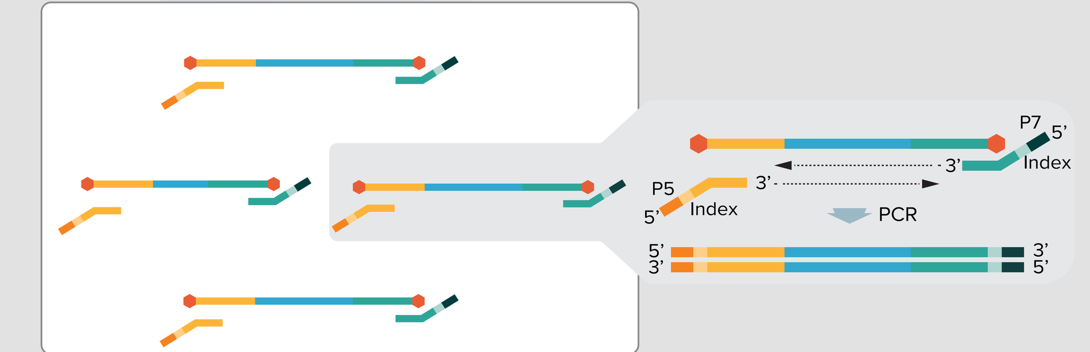
2. Denature and capture as single-stranded DNA



3. Phosphorylate template DNA and ligate adapters



4. Index PCR



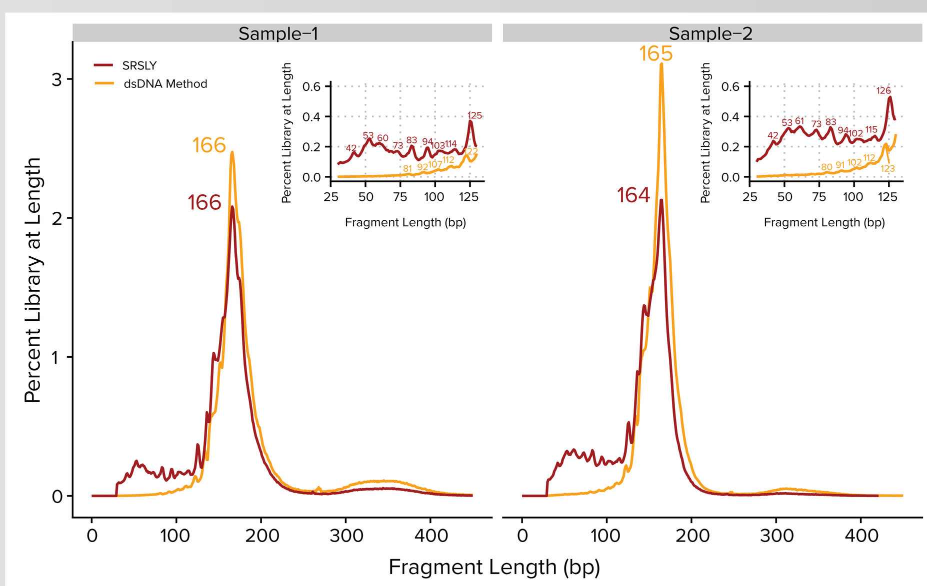
SRSLY FAMILY OF APPLICATIONS

cfDNA

cfDNA library preparation with SRSLY captures short fragments and retains native ends unlike double-stranded approaches. Here, libraries were generated with equal amount of cfDNA inputs using the SRSLY-PicoPlus Kit and an end-repaired/ A-tailed double-stranded method (ds-method) and sequenced on Illumina NextSeq500. Comparable mapping metrics and library complexities were obtained from both protocols. However, SRSLY outperformed the dsDNA method in capturing short fragments < 130 bp. Downstream analyses such as Window Protection Score calculations (Snyder et al 2016) to evaluate nucleosome positioning and transcription factor binding can be performed using data from libraries generated with SRSLY.

Kit	Type of Prep Method	Sample	Input cfDNA Amount	Yield Post Index PCR (9c)	% Reads Discarded	% Reads Mapped	% Mapped Reads 30-130 bp	Est. Unique molecules in 30,000,000
SRSLY™ PicoPlus	Single-stranded	SRSLY-1	5 ng	816 ng	3.9	96.9	14.6	32.1 M
		SRSLY-2	2.5 ng	364 ng	6.4	96.4	22.5	23.6 M
dsDNA method	Double-stranded	dsDNA-1	5 ng	1200 ng	0.0	95.8	2.5	22.7 M
		dsDNA-2	2.5 ng	692 ng	0.0	96.9	4.1	24.0 M

RETAIN NATIVE ENDS OF DNA AND CAPTURE SHORTER CELL-FREE DNA FRAGMENTS



Insert size distribution plots for mapped reads from sequencing libraries generated with SRSLY (red) and a double-stranded method - dsDNA (yellow) from two human cfDNA extracts. Reads were filtered for PCR duplicates and a quality score equal to or greater than q20. Inset - size distributions for insert < 130 bp demonstrate better capture of short fragments with SRSLY over double-stranded methods.

ANALYZE NUCLEOSOME AND TRANSCRIPTION FACTOR BINDING CHANGES

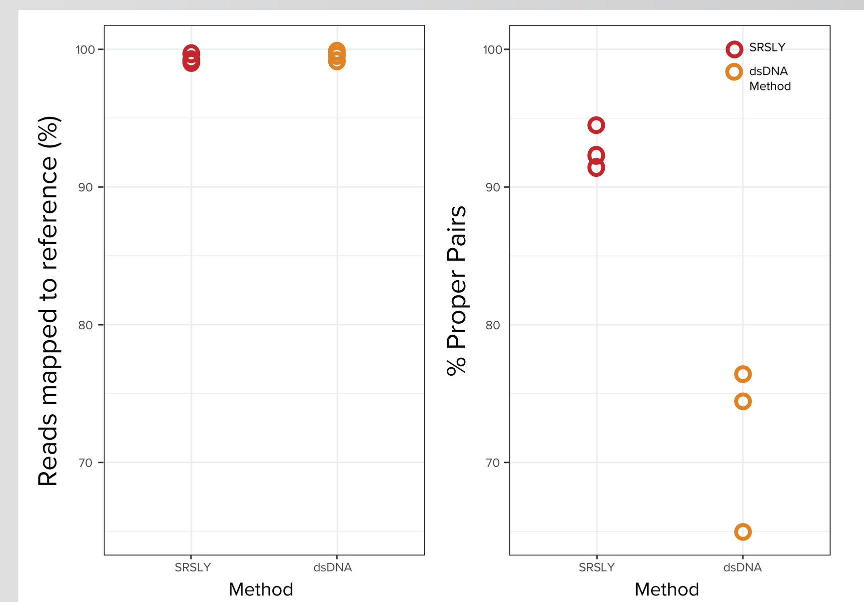
Window Protection Scores were calculated as described in Snyder et al., 2015 (PMID: 26771485). A bed file containing a list of putative TF binding sites was downloaded from UCSC Genome Browser Table Browser (JASPAR 2018 table, hub_186875_Jaspar TFBS) and filtered to include only CTCF sites. Long - WPS for nucleosome binding; Short - WPS for CTCF binding. SRSLY - WPS data generated with SRSLY libraries, Expected WPS data obtained from the Snyder et al. paper.

FFPE

SRSLY generates sequence-ready libraries from damaged or degraded DNA derived from FFPE tissue samples. Libraries were generated from three tissue samples using the SRSLY NanoPlus NGS kit and a ds-method (that performs end-repair and A-tailing). Even with small amounts of input DNA (5ng – 7.5ng) SRSLY generates libraries of greater complexity, despite lower yields. Though similar percentages of reads map to the reference genome with both methods, SRSLY shows more reads in proper pairs. Additionally, the GC percentage of mapped reads is more uniform for SRSLY than for dsDNA method.

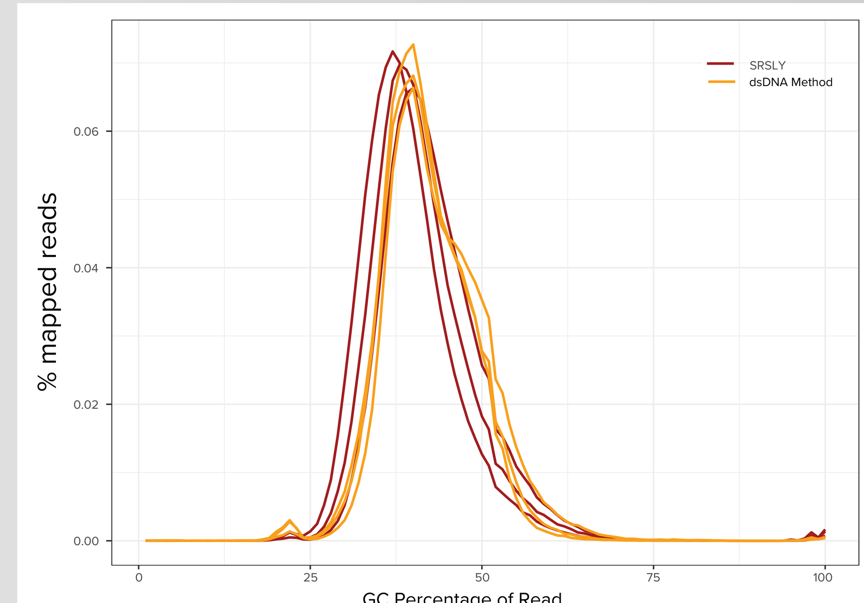
Kit	Type of Prep Method	Sample	Tissue Source	Input cfDNA amount	Yield Post Index PCR (9c)	Unique Reads @ 30M Sequenced
SRSLY™ PicoPlus	Single-stranded	SRSLY-1	Liver	5 ng	35.4 ng	22.2 M
		SRSLY-2	Colon	7.5 ng	77.6 ng	23.0 M
		SRSLY-3	Spleen	5 ng	332 ng	23.7 M
dsDNA method	Double-stranded	dsDNA-1	Liver	5 ng	125 ng	16.2 M
		dsDNA-2	Colon	7.5 ng	238 ng	16.0 M
		dsDNA-3	Spleen	5 ng	376 ng	18.4 M

CAPTURE MORE PROPERLY PAIRED READS



Read mapping statistics for three FFPE samples prepared with SRSLY and a dsDNA technique. Paired end reads were mapped with bwa mem and mapping statistics were calculated with samtools stats. Though mapping rates are similar, the dsDNA preparation results in more discordant pairs.

CREATE LIBRARIES WITH UNIFORM GC CONTENT



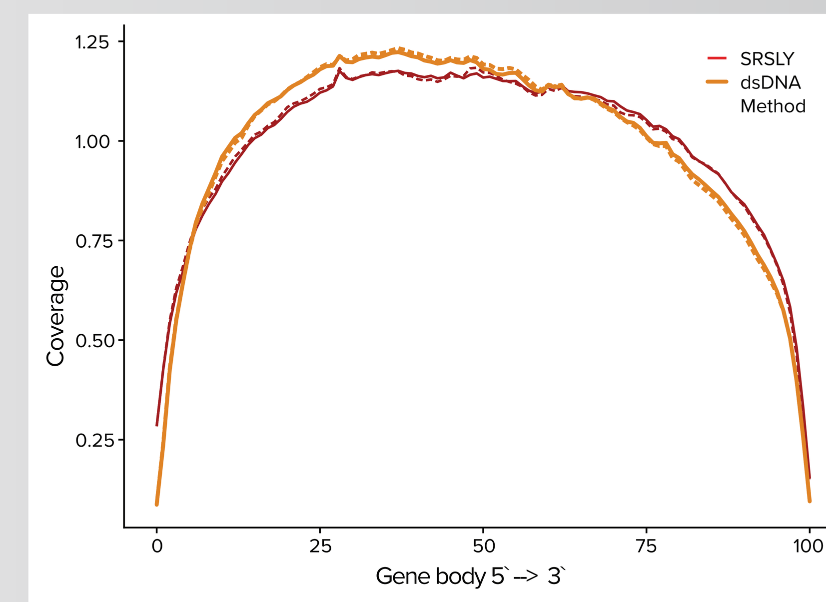
GC content of read sequences. The GC content of each read in a library was counted in bins of 1% width. The dsDNA library preparation (yellow) shows a shoulder of higher GC content, unlike SRSLY (red)

cDNA (RNA-Seq)

RNA-Seq library preparation with SRSLY does not require second strand synthesis. Following adapter ligation to cDNA and purification, library amplification is immediately performed and sequence ready libraries are generated in < 3 hours with SRSLY, unlike dsDNA methods which require 2nd strand synthesis for adapter ligation and subsequent removal of one strand to maintain directionality. Here, comparison metrics for replicate RNA-Seq libraries generated from 10ng of cDNA using either SRSLY or a double-stranded method are shown. Both methods demonstrated similar mapping rates and maintain directionality. However, SRSLY showed more uniform coverage across the gene body and better capture of untranslated regions of mRNA.

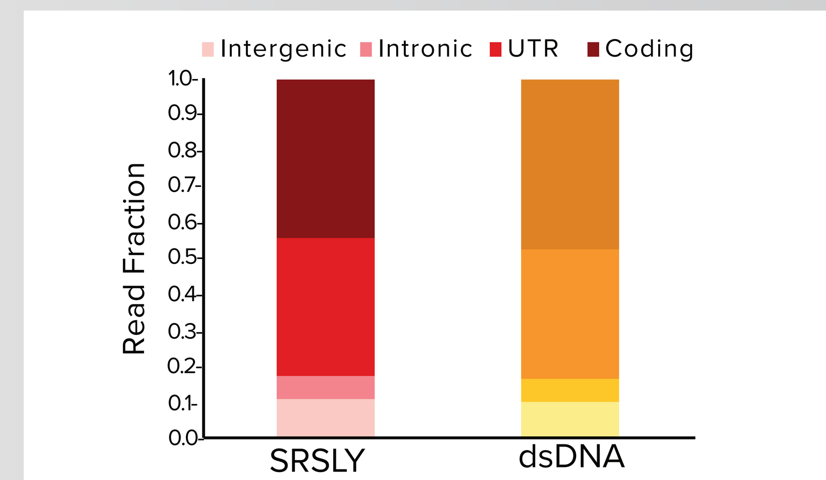
Kit	Type of Prep Method	Sample	Input cDNA Amount	Yield Post Index PCR	% Uniquely Mapped Reads	% Correct Strand
R-SRSLY™ (~3.5 hrs)	Single-stranded	SRSLY-1	10 ng	378 ng	90.2	93.72
		SRSLY-2	10 ng	370 ng	89.7	93.59
dsDNA method (~5.5 hrs)	Double-stranded	dsDNA-1	10 ng	386 ng	92.2	93.43
		dsDNA-2	10 ng	432 ng	92.2	93.54

OBTAIN MORE UNIFORM GENE BODY COVERAGE



Gene body coverage of RNA-Seq libraries generated with SRSLY and a double-stranded method. Each library was sequenced >10 million reads (Illumina MiSeq 2x76 bp). Reads were mapped to the human reference genome (GRCh.hg38) using STAR v 2.6.1d. Picardtools CollectRNASeqMetrics generated all other quality metrics using a bed file containing gene coordinates. dsDNA method (yellow) showed 5' bias unlike SRSLY (red) which had more uniform coverage across the gene body.

IMPROVED COVERAGE OF 3' UNTRANSLATED REGION



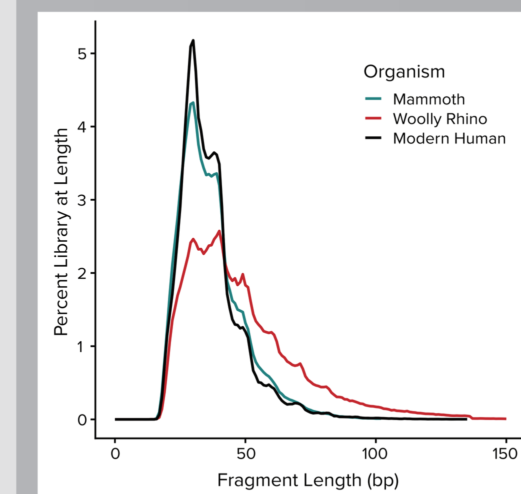
Genomic composiiton of RNA-Seq libraries generated with SRSLY and the dsDNA method showed minimal intergenic and ribosomal reads. SRSLY (red shades) showed higher capture of untranslated regions (UTR) unlike dsDNA methods (yellow shades)

OTHER APPLICATIONS

SRSLY offers significant advantages over traditional library preparation methods particularly for samples with degraded and low-input DNA. Apart from biomedical samples, we have successfully demonstrated the efficacy of this approach in evaluating challenging inputs such as highly fragmented DNA derived from rootless hair from both ancient animals and modern humans. The method can also be used to evaluate the purity of single-stranded synthetic oligonucleotides and measure the amount of molecules that arise from incomplete oligo synthesis.

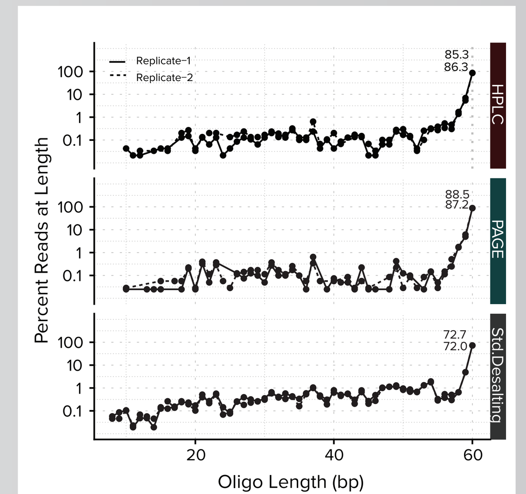
Kit	Sample Age	Organism	Input Amount	Yield Post Index PCR (14c)	% Reads Mapped	Average Mitochondrial Coverage
SRSLY™ PicoPlus	Modern	HUMAN	178.6 pg	224 ng	67.9	21.9X
SRSLY™ PicoPlus	Ancient	YUKA MAMMOTH	102.6 pg	72.4 ng	31.5	36.2X
		WOOLLY RHINO	104.4 pg	57.6 ng	47.4	39.0X

PERFORM GENOMIC ANALYSIS WITH DNA FROM ROOTLESS HAIR



Insert size distribution of SRSLY libraries prepared with ~100 pg DNA from rootless hair of a modern living human (2019) and two ancient organisms (10-30 kya). DNA extracted from hair shafts is shown to degrade rapidly but stay preserved, with peak sizes below 50 bp, while maintaining the "saw-tooth" pattern i.e. 10.4 bp periodicity typically observed in degraded DNA from rootless hair.

EVALUATION OF SYNTHETIC OLIGO PURITY



Effect of various purification methods on oligo purity as a function of oligo length. SRSLY libraries were generated with 60 nt synthetic single-stranded oligos purified by HPLC, PAGE or standard desalting protocols. The percent of full-length product for each purification type indicates that std. desalting has lower amount of full-length product. Solid and dashed lines represent replicate libraries.

SERIOUS ADVANTAGES

Features	SRSLY	DS-Method	Application
Single strand	✓✓	✗	cfDNA, oligo, methyl-seq, cDNA
Native ends	✓✓	✗	cfDNA,oligo
Nicked DNA	✓✓	✗	cfDNA, aDNA, FFPE
Short fragments	✓✓	✗	aDNA, hair DNA, cfDNA
Damage DNA capture	✓✓	✓	FFPE, aDNA, hair DNA
Short protocol time	✓✓	✓	cfDNA, RSRSLY
Directional	✓✓	✓	cDNA, Methy-seq

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