SEQUENCE SRSLY® LIBRARIES ON THE SINGULAR GENOMICS G4™ PLATFORM

CLARETBIO'S SINGLE-STRANDED LIBRARY PREPARATION WORKFLOW

SRSLY or Single Reaction Single-Stranded LibrarY is a next-generation sequencing library preparation protocol developed by Claret Bioscience (Figure 1). This simple workflow excels at generating high quality libraries from a variety of challenging inputs such as cell-free DNA, Formalin-fixed Paraffin Embedded (FFPE) tissue derived DNA, ancient DNA, single-stranded oligonucleotides. The method is highly sensitive and can generate libraries from inputs that contain as low as 100 pg of fragmented DNA. The method was first developed for sequencing on Illumina sequencing technologies, however the SRSLY workflow is sequencing platform agnostic and can be easily made compatible with emerging sequencing technologies. Here, we report seamless integration and performance metrics of SRSLY libraries sequenced on the Singular Genomics G4™ Sequencing Platform.

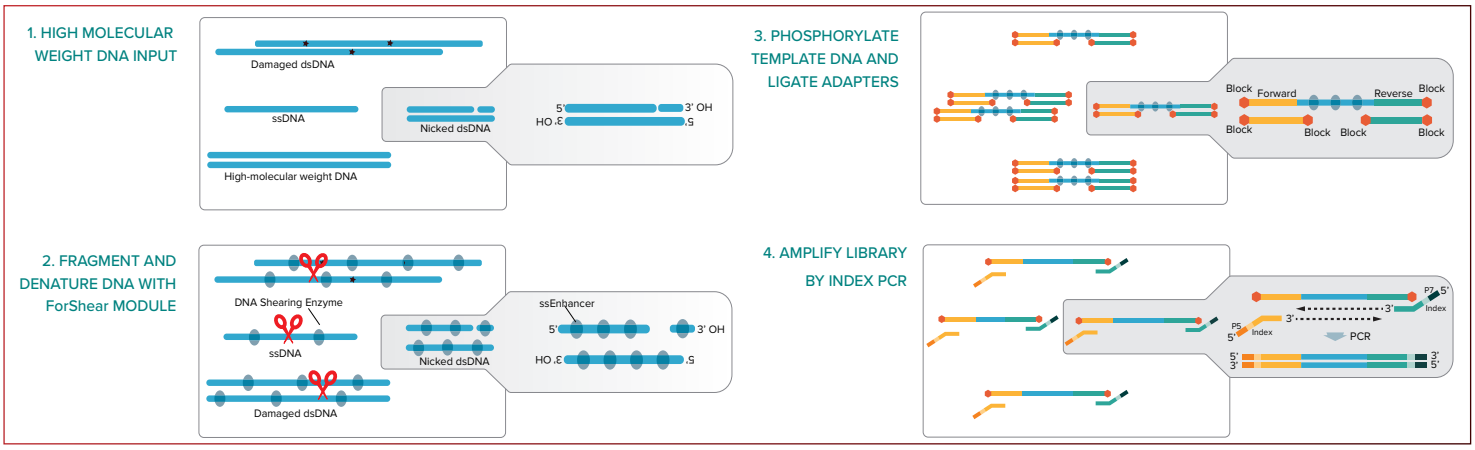

Figure 1. ***SRSLY library preparation protocol with ForShear ezymatic fragmentation upstream.*** *For cfDNA application, the protocol is performed without shearing. Libraries are generated in 4 simple steps - input DNA is denatured (and fragmented) in a single step and single-stranded DNA is stabilized. Specialized adapters are ligated without any end-repair of input DNA. Following magnetic bead-based purification, libraries are amplified via Index PCR. the index PCR step also incorporates nucleotides that confer sequencer-compatiblility. A final bead purification results in sequencing ready libraries. Here, G4 Platform compatible primer pairs were used during index PCR.*

SINGULAR GENOMICS G4 SEQUENCING PLATFORM

The G4 Sequencing Platform is a highly versatile benchtop sequencer that is well suited for demanding research applications. The G4 Platform leverages a novel 4-color rapid sequencing by synthesis (SBS) chemistry to deliver highly accurate reads (single or paired-read format with optional index reads) with a single-day turnaround. To maximize flexibility, the G4 Platform enables users to load up to four flow cells at a time, with each flow cell comprising four fluidically independent lanes, thereby enabling sample multiplexing without the need for index reads. The G4 Platform outputs FASTQ format files that integrate seamlessly with existing bioinformatics tools. Users may elect to automatically demultiplex samples on-instrument via sample indices provided by the sample sheet or off-instrument using the Singular Genomics rapid demultiplexing tool. More information about G4 specifications, such as run time, accuracy, and quality metrics, can be found on the Singular Genomics website.

METHODS

We prepared SRSLY libraries using the Horizon® Discovery's OncoSpan cfDNA standard or the Quantitative Multiplex Reference Standard formalin compromised DNA (fcDNA) as described in Table 1. Formalin compromised DNA and NA12878 contain fragments >500bp and we included a shearing step upstream using the ForShear™ Enzymatic fragmentation module. To make the libraries compatible with the Singular Genomics G4 sequencing platform, we used primer pairs (5µM each) during the index PCR step, provided by Singular Genomics. All other library preparation parameters remained unchanged.

Libraries were pooled and sequenced on the G4 Platform using an F2 flow cell with sequencing format of 2X150 bp (plus 12 bp dual indices). 238M read pairs were generated (82.1% and 80.2% of base calls >=Q30 for Read 1 and Read 2, respectively). The raw fastq data were analyzed using the srsly-run software that performs the standard steps in sequencing data analyses i.e. adapter trimming, read mapping. For more details visit *www.claretbio.com/software/srsly-run*

RESULTS

The resultant libraries passed the quality metrics expected of SRSLY libraries with respect to yield, adapter dimer percent and average molecular weight for both fcDNA and cfDNA standards (Table 1). The cfDNA libraries had slightly higher adapter dimers as the protocol is designed to capture short fragments often found in cfDNA.

Libraries	Input DNA	Protocol	Input	Singular Primer	PCR Cycles	Yield	% Dimers	Avg Lib Size
SR4500-221013	Oncospan cfDNA	SRSLY PicoPlus	5ng	1	8	204	0.44	336
SR4501-221013			10ng	2	7	256	0.24	341
SR4502-221013			20ng	3	7	586	2.09	340
SR4503-221013	NA12878	ForShear>SRSLY NanoPlus	HMW, 20ng	4	9	810	0.08	381
SR4504-221013	Formalin Compromised DNA		Mild, 20ng	5	9	550	0.43	386
SR4505-221013			Moderate, 20ng	6	9	636	0.36	348
SR4506-221013			Severe, 20ng	7	9	772	0.26	296

Table 1. Experimental set up and molecular metrics of libraries generated with SRSLY

The sequencing metrics of the libraries showed high quality sequencing data for all libraires from both input types with comparable mapping rates, base quality, and chimeric rates (Table 2), metrics estimated from a single lane are shown.

Libraries	Read Pairs Sequenced	Read Pairs Kept	Read Pairs Kept That Map	Map q20	Duplication Percent	Estimated Library Size	Chimeric Rates
SR4500-221013	7254846	5397547	96.3	90.3	3.1	109820654	0.40
SR4501-221013	7896832	5791995	97.9	90.7	3.1	123487927	0.90
SR4502-221013	8409557	6079422	93.3	90.6	3.0	126173502	1.07
SR4503-221013	10712942	6447964	97.4	91.0	2.7	187787002	0.47
SR4504-221013	7524002	4249132	96.3	91.6	2.5	140361650	0.29
SR4505-221013	8414103	5742949	97.0	90.3	2.8	142374038	0.33
SR4506-221013	8527664	7510405	97.2	87.9	2.7	150096405	0.46

Table 2. Mapping metrics of the SRSLY libraries sequenced on the G4 Platform

SUMMARY

The data shown here demonstrate that the G4 Platform rapidly generates accurate sequencing from SRSLY libraries. SRSLY is ideal for highly fragmented DNA from clinical samples such as liquid biopsies and FFPE tissues. These samples are often collected serially and require rapid turnaround time in a clinical setting. The unique flow cell flexibility and unmatched run times of the G4 Sequencing Platform offer labs the ability to scale operations to match demand. Together the two technologies can synergistically reduce turnaround time and cost burden in research applications and future clinical applications.

ORDERING INFORMATION

Claret Bioscience Products

To place an order visit www.claretbio.com/srsly-quote or write to *info@claretbio.com*

Modules	Catalog	Components
ForShear™	**CBS-ESM**	Dilution Buffer, Activity Buffer, Enzyme
SRSLY® Base Kit	CBS-K155B, CBS-K250B	ssEnhancer, Adapters, Ligation Master Mix, Index-PCR Master Mix
Clarefy Beads	CBS-BD	DNA Purification beads

Singular Genomics Products

For more information, please visit www.singulargenomics.com or email us at care@singulargenomics.com

Product	Catalog
Singular Genomics Indexed Primer Pairs: SG UDI - Set of 24 [Set A]	**700,135**
Singular Genomics G4 Sequencing Platform	Enquire

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