RUN SRSLY® NGS LIBRARIES ON THE AGILENT® BRAVO LIQUID HANDLER

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 tissue (FFPE) derived DNA, ancient DNA, single-stranded oligonucleotides. The method can generate libraries from inputs that contain as low as 100 pg of fragmented DNA. Sequence-ready libraries are generated in 4 simple steps with an optional UMI Addition.

While the protocol is very easy to perform manually, with increased sample numbers, automated workflows are required for high-throughput library prepration. Particularly in clincial and biotech settings, automation helps standardize workflows by reducing human error. SRSLY is compatible with several liquid handlers; learn more at www.claretbio.com/automation.

AGILENT BRAVO AUTOMATED LIQUID HANDLING PLATFORM

The Agilent Bravo Liquid Handling Platform is a state-of-the-art instrument that combines advanced technology, intelligent software, and modular flexibility to meet the diverse needs of genomics, proteomics, and other scientific disciplines. The system comprises a range of independently operating robotic arms, each equipped with high-precision pipetting capabilities. This modular configuration enables multiple liquid handling tasks simultaneously, reducing experimental timelines and increasing throughput. The instrument is equipped with various features to prevent cross-contamination, such as disposable pipette tips and liquid-level sensing. Moreover, the instrument's compact footprint optimizes bench space utilization, making it an excellent choice for laboratories with limited space. The platform features a user-friendly interface empowers researchers to easily design and execute protocols, develop methods, and track experiments.

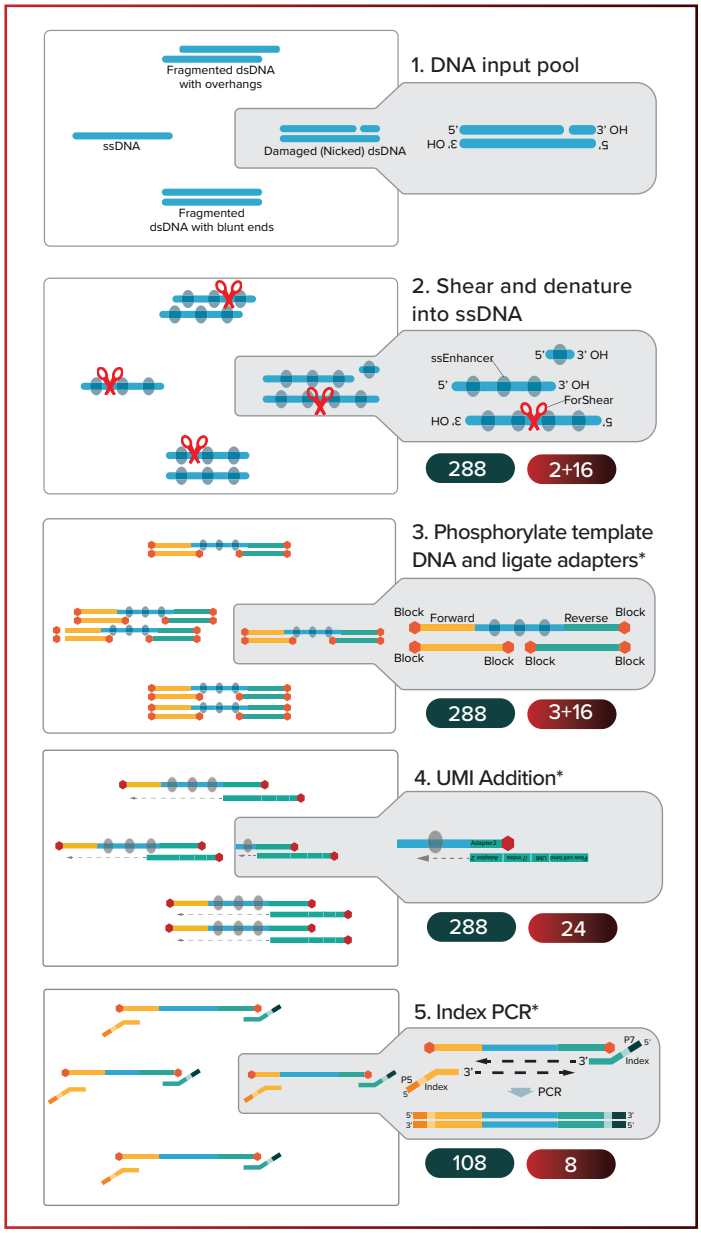

Figure 1. ***SRSLY library preparation protocol.*** *Input DNA is denatured in a single step and single-stranded DNA is stabilized. For high-molecular weight DNA, enzymatic shearing is performed as a part of the denaturation step. Next, the template is simultaneously phosphorylated and ligated with specialized adapters. Optional UMI addition may be performed. Finally, the libraries are amplified via Index PCR to generate sequencing-ready libraries. The number of pipetting steps for reaction set-up are indicated for each step manual (teal), Bravo Option A (red). For the automation workflow, all steps require reagent plate set-up, along with preparation of master mixes in steps 2 and 3. Bead purifcation is performed after steps 3,4 & 5 - the manual workflow requires addition 72 pipetting steps, wherease the Bravo requires only 8.*

Combining the simplicity of the SRSLY workflow with the efficiency of the Agilent Bravo system ensures high-throughput and consistent next-generation sequencing library preparation. Here we show the performance of SRSLY library preparation on the Agilent Bravo Instrument. The scripts to perform the various SRSLY workflows and the required liquid class files are available at upon request.

METHODS

We generated libraries with 1 ng cell-free DNA (cfDNA) extracted from blood plasma from healthy individuals or 20ng of the Horizon Discovery® Quantitative Multiplex Reference Standard formalin compromised DNA (fcDNA) and NA12878 as input. The fcDNA was either severely or mildly damaged (DIN <2.5, 7 respectively).

Both manual and automation workflows were performed for all inputs. For both workflows, all incubations were performed on a BioRad® Thermocycler with time and temperatures set up according to the standardized SRSLY protocol (Figure 1). For the automation method, reagent transfers and DNA bead purification (including incubations) were performed on the Agilent Bravo Instrument. The reagents were placed in a BioRad Hard-sheel 96 well plates, on the dedicated position on the Bravo deck with appropriate cooling. As the pipetting head transfers reagents in multiples of each, each reagent or master mix is thoroughly mixed and transferred to eight wells in a column of a reaction plate. The reactions were set up in the the BioRad hardshell 96 well plates, prior to transfering the plates to the thermocycler, plates were briefly spun at 300g for 3 minutes at 4°C using a bench-top centrifuge. The manual workflow was performed as per the SRSLY SOP. The manual protocol requires 10 times more number of pipetting than an automation workflow, we did not observe significant differences in overall protocol time.

For cfDNA, we performed the main SRSLY library preparation protocol i.e. Denaturation, Ligation and Index PCR steps using the SRSLY PicoPLus kit, Clarefy Beads and UDI Index Primers with 1ng cfDNA inputs . For the high-molecular weight inputs we performed shearing using the ForShear™ Enzymatic fragmentation module followed by library preparation with the SRSLY NanoPlus kit - our dedicated kit for 10-50ng input ranges. We also included the Unique Molecular Index addition step and the downstream bead purification steps for for these inputs.

Libraries were pooled and sequenced on an Illumina MiSeq instrument (2X76 using v3 kit). The raw fastq data were analyzed using ClaretBio's opensource srsly-run and srsly-umi software that perform the standard steps in sequencing data analyses i.e. Read trimming, mapping, UMI deconvolution and read-group assignment and deduplication. For more details visit *www.claretbio.com/software/*

RESULTS - MOLECULAR METRICS

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). While we saw slighty higher amounts of adapter dimers in the automation protocol, these amounts are well within our QC metrics of < 1% dimers. Comparison of the blanks run with each workflow show minimal carryover of material between adjacent wells, as indicated by higher amount of adapter dimers. Comparison of blank wells showed that there was no carry-over between wells.

Workflow	Input DNA	Protocol	Input	PCR Cycles	Yield (ng)	% Dimers	Avg Lib Size
Bravo	cfDNA	SRSLY PicoPlus	1 ng	12	1114	0.025	355
Manual					834	0.21	313
Bravo	NA12878	ForShear>> SRSLY NanoPlus With UMI Addition	20 ng	9	307	0.39	288
Manual					300	0.04	326
Bravo	fcDNA-Mild				250	0.86	251
Manual					345	0.22	269
Bravo	fcDNA-Severe				321	0.34	286
Manual					318	0.05	339

Table 1. Experimental set up and molecular metrics of libraries generated with SRSLY workflow for cfDNA and high molecular weight DNA. Average values are provided for Bravo generated libraries.

RESULTS - SEQUENCING METRICS

The sequencing data showed that for each input type tested, SRSLY libraries generated with automation or manually had comparable mapping rates, base quality and duplication percent (Table 2). For all inputs tested, we observed higher chimeric rates for samples using the manual protocol.. Chimeric reads are generated when the template molecules ligate to each other. The SRSLY ligation master mix is viscous and improper mixing can promote template-template ligation and results in higher chimeric reads. Our data suggests that the mixing cycles incorporated within the automation workflow mitigates concatamer formation unlike manual workflow were mixing is prone to user-error.

Workflow	Input	Read Pairs Sequenced	Read Pairs Kept	Read Pairs Kept That Map	Map q20	Duplication Percent	Estimated Library Size	Chimeric Rates
Bravo	cfDNA	932719	922112	99.88	92.16	0.0869	530938011	0.95
Bravo		441794	435203	99.87	92.10	0.0929	235034642	0.89
Manual		648255	644345	99.90	92.70	0.0358	1612163375	4.047
Bravo	NA12878	505573	486825	99.72	89.79	0.1253	198672938	2.34
Bravo		633679	619153	99.91	90.83	0.1148	280096799	2.96
Manual		473346	467588	99.94	91.66	0.1123	216805207	4.74
Bravo	fcDNA-Mild	612208	591928	99.80	90.99	0.1225	247365088	6.61
Bravo		581297	565069	99.9	91.37	0.1074	270443053	1.83
Manual		534584	528446	99.93	92.25	0.1058	267674644	4.74
Bravo	fcDNA - Severe	536811	518759	99.81	88.26	0.1182	222997751	2.57
Bravo		500385	485071	99.77	88.56	0.1245	196793336	2.71
Manual		617273	605686	99.94	89.59	0.1016	307473872	4.45

Table 2. Sequencing metrics of SRSLY libraries. Libraries were sequenced shallow on an Illumina MiSeq instrument and analyzed using a standardized data analysis pipeline. Reads were mapped to the human hg38 geneome using Burrows-Wheeler Aligner. Duplicates were called using GATK Picard and chimeric reads were determined using Samtools by counting the number reads that mapped to different chromosomes.

The insert size distribution demonstrated that both the automation and manual workflow generated libraries with expected size range for all input types and for their corresponding protocol. Minimal differences were observed in fragment size distribution for cfDNA, with only minor variations detected in low-abundance fragments that were under 100 nt. For the high molecular weight DNA, we observed that the automation workflow generated slightly shorter fragments than the manual workflow for the NA12878 and fcDNA-Mild inputs. This may be attributed to the fact that enzymatic DNA shearing initiates immediately after reaction is set-up. The automation protocol requires that the reaction is mixed thoroughly on the liquid-handler and centrifuged (to collect all the liquid at the bottom of the plate); this presumably increases the overal incubation period. This variation was not observed with the severly damaged inputs, which have inherently degraded DNA that do not undergo further fragmentation.

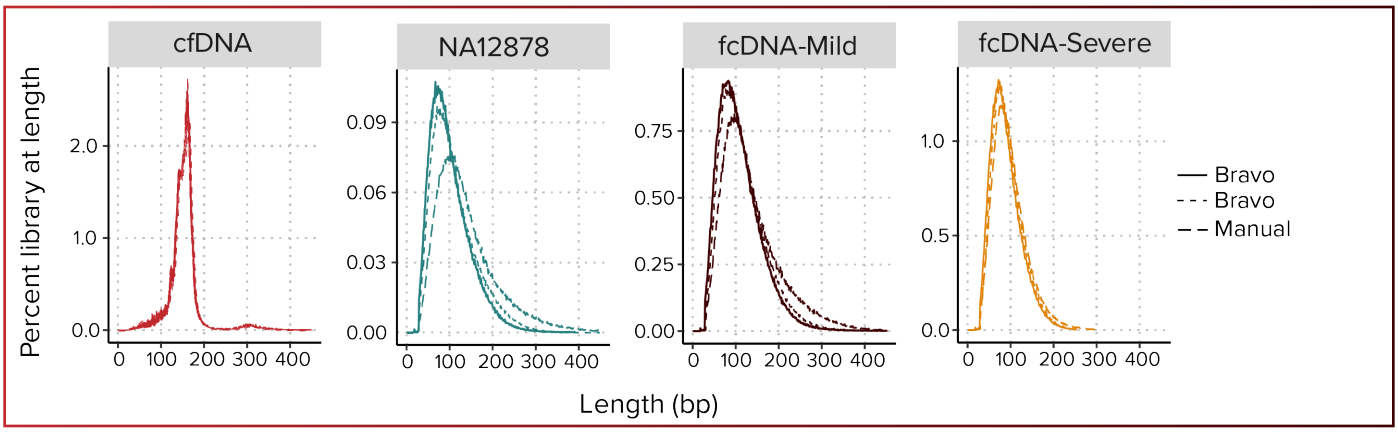

Figure 2. Insert size distribution of libraries generated by SRSLY with both automation and manual protocol. Percent reads of each length per library was calculated for mapped reads and plotted based on input type. Minimal variation is seen for cfDNA libraries generated by Bravo automation workflow vs manual. For NA12878 and fcDNA-mild, automation increases the overall shearing time, leading to shorter fragments.

SUMMARY

The data shown here demonstrate that the Bravo liquid handling platform rapidly generates high-quality sequencing libraries that are at par with manual workflows. SRSLY is ideal for highly fragmented DNA from clinical samples such as liquid biopsies and FFPE tissues and work with low inputs of samples. These samples are often collected serially *and* require rapid turnaround time in a clinical setting. The ease of set up on the Agilent Bravo enable high-throughput SRSLY library preparation, allowing laboratories to scale-up operations, whereas the simple protocol and low sample requirment make SRSLY an ideal method for limiting clinical samples. Together the two technologies can synergistically reduce turnaround time and human errors in NGS library preparation in both research and clinical setttings.

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

Agilent Techologies

For more information, please visit www.agilent.com

Product	Catalog
Bravo Automated Liquid Handling Platform	Enquire
Bravo specific Consumables	Enquire

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