
CLARETBIO
GET THE BEST DATA FROM THE WORST SAMPLES
ForShear™ and SRSLY® a robust combination for NGS library preparation **from severely damaged**
FFPE DNA

WHY IS NGS FROM FFPE DNA CHALLENGING?

Sample preservation can significantly damaged the quality of DNA.

Quality of genomic DNA is highly dependent on the source, storage and extraction methods. DNA from formalin-fixed praffin embedded (FFPE) samples and herbarium specimens can be highly degraded and damaged. These inputs types perform poorly with tradiitonal NGS library preparation methods. SRSLY® is a single-stranded method that efficiently generates high-quality libraries from damaged DNA inputs with significant reductions in protocol steps and times.

By combining SRSLY with a robust DNA shearing module, we now provide an optimized workflow that generates high-quality sequencing libaries from gDNA that are compatible with Illumina® sequencing platforms.

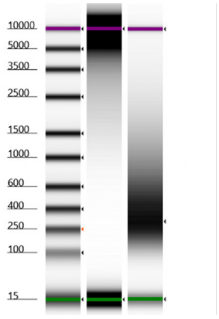
Here we introduce **ForShear**™ - our robust enzymatic DNA shearing module that integrates seamlessly with SRSLY®.

Module	Shearing method	Library	Input DNA	Input Amount	Index PCR conditions
ForShear	Enzymatic	**SRSLY**	Horizon fcDNA Mild, Severe	20 ng	9 cycles
dsPrep	Enzymatic	**ds-Prep**			

We compared the performance of ClaretBio's ForShear module and SRSLY library preparation or a double-stranded enzymatic shearing and library preparation method. We used the Horizon Discovery® formalin compromised DNA (fcDNA) standards which show mild or severe of damage by formalin as input.

The Quantitative Multiplex Reference Standard (QMRS) portfolio used to develop these standards includes 11 mutations at 0.8-24.5% allelic frequency in genomic DNA. With the Formalin-Compromised format, the robustness of your NGS workflow may be tested to ensure that your library protocol and informatics pipeline can accommodate these highly degraded samples.

Horizon Discovery's Formalin Compromised DNA



HOW DO **ForShear>SRSLY** LIBRARIES COMPARE?

Capture the most degraded and fragmented DNA, lost to standard preparation

Protocol	Input DNA	PCR Cycles	SPRI Clean	Total Lib Yield Post index PCR	% Dimers	Avg Lib Size
ForShear>SRSLY	Mild fcDNA	9	Dual sided	564ng	1.9	364
	Severe fcDNA	9	Dual sided	676ng	1.4	279
dsPrep	Mild fcDNA	9	single sided	1012ng	0.04	396
	Severe fcDNA	14*	single sided	524ng	0.79	324

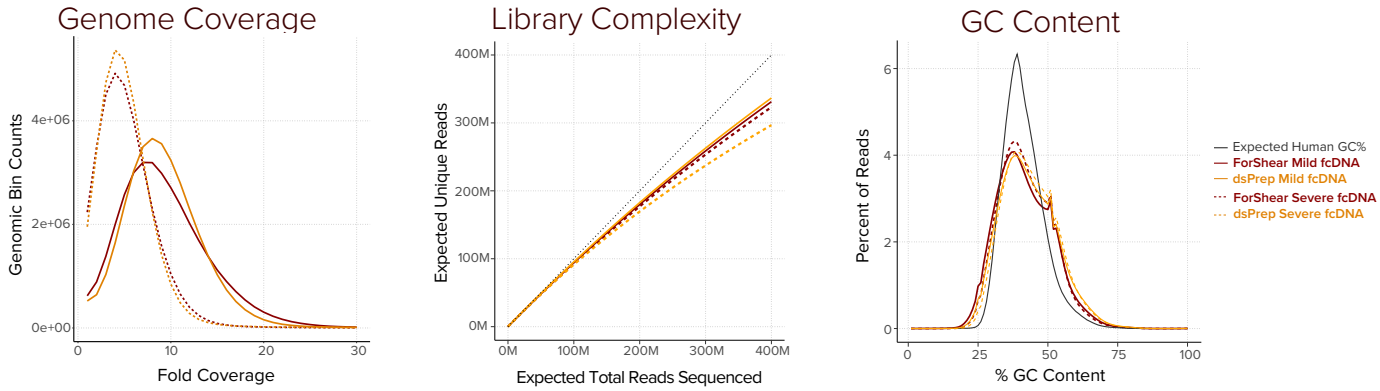
For Mild fcDNA, both SRSLY-ForShear protocol and the dsPrep protocol showed equivalent molecular method. However for the severe fcDNA, SRSLY-ForShear generated high-quality libraries at lower PCR cycles with minimal adapter dimers. Overall the SRSLY approach generated smaller libraries presumably due to the capture of nicked and short ssDNA.

Libraries were sequenced on Illumina NovaSeq and analyzed using a comprehensive bioformatic pipeline called srsly-run that includes UMI deconvolution. **Available for download at www.claretbio.com/software/srsly-run**

HOW DO **ForShear>SRSLY** LIBRARIES MAP?

Make libraries of high mapping quality, even from severely degraded DNA

Protocol	Input DNA	Read Pairs Sequenced	Read Pairs Kept	Read Pairs Kept that Map	Map q20	Duplication Percent	Estimated Library Size	Percent Map Diff Chr
ForShear>SRSLY	Mild fcDNA	191607319	94.0	99.8	85.7	7.9	1405053063	1.67
	Severe fcDNA	187897380	93.2	99.8	83.4	7.1	1514579488	1.77
dsPrep	Mild fcDNA	160859867	99.1	99.9	87.2	6.7	1528615578	1.01
	Severe fcDNA	121869185	98.1	99.5	80.1	10.5	607140658	1.60

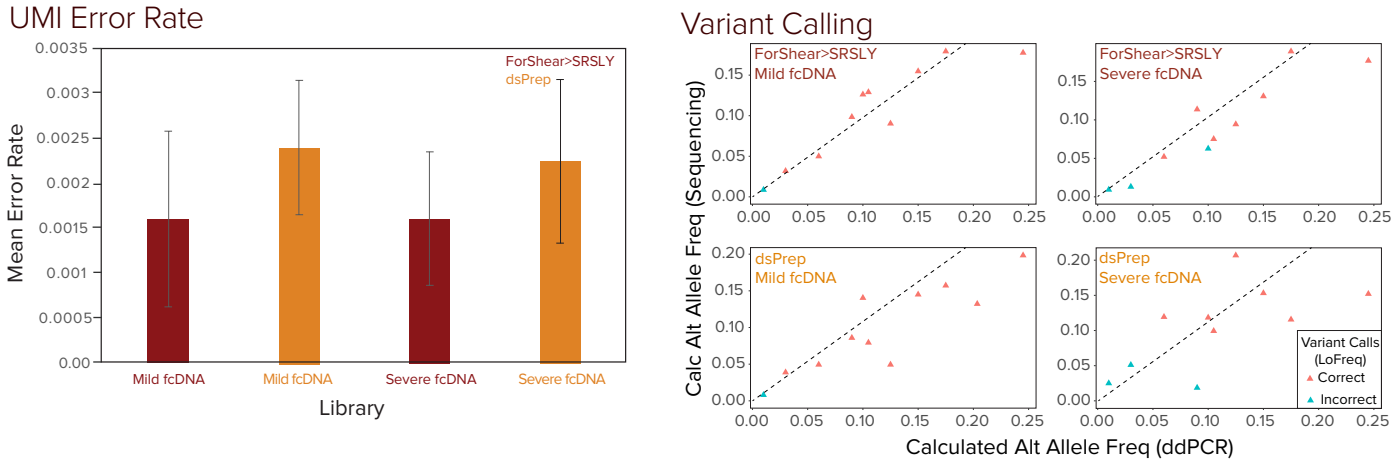


The mapping metrics of the libraries showed that both approaches generated good quality libraires for both input types with comparable chimeric rates, genome coverage and GC content. However, ForShear>SRSLY generated libraries with lower duplication rates and higher complexity with the severly damaged input types.

WHAT ABOUT TARGET ENRICHMENT?

Plug-in targeted-erichment to call low frequency allele at with lower error

Protocol	Input DNA	Read Pairs Mapped	Fold Enrichment	Target Bases 30X	% Dups	Mean Bait coverage	Mean Target coverage	On Bait vs Selected
ForShear>SRSLY	Mild fcDNA	186.9M	48.0	91%	15.6%	380.8	194.5	0.7
	Severe fcDNA	169.7M	54.0	89%	13.5%	318.4	139.3	0.8
dsPrep	Mild fcDNA	114.5M	45.7	90%	8.00%	230.8	131.5	0.6
	Severe fcDNA	83.3M	47.6	86%	37.20%	165.3	53.2	0.7



Both approaches perform equivalently in hybridization capture pipelines - here we used the Twist® Exome 2.0 capture panel. SRSLY had lower mean error rate based on consensus UMI groups. For severe fcDNA, the dsPrep showed lower mean target coverage and lower observed concordance with the expected allele frequency based on ddPCR.

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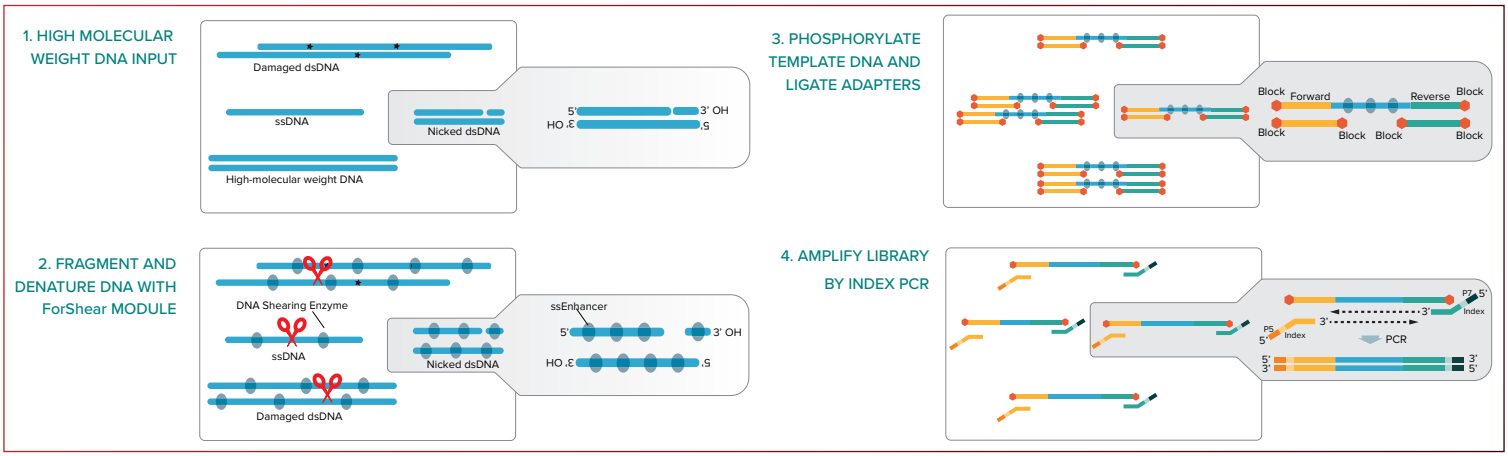
WORKFLOW



PRODUCT SPECIFICATIONS

- Generates final libraires from gDNA in ~4 hours
- Contains reagents for gDNA shearing, adapter ligation, indexing primers and magnetic beads for purification.
- Compatible with an optional step for Unique Molecule Indentifier (UMI) addition
- Enzymatic method; compatible with automation platforms
- **Reactions:** 24 and 96 reaction kits available

INPUT TYPES

Genomic DNA from

- Formalin-fixed paraffin embedded tissue
- Fresh-frozen tissue
- Cell-cuture
- Herbaria

KIT SPECIFICATIONS

All modules available in 24 and 96 reaction formats

Modules	Components
ForShear™	Dilution Buffer, Activity Buffer, Enzyme
SRSLY® Base Kit	ssEnhancer, Adapters, Ligation Master Mix, Index-PCR Master Mix
UDI Primers	12-UDI primer mix
UMI Add-on	UMI Extension Primer, Buffer, Index Primers
Clarefy Beads	DNA Purification beads

SRSLY. ORDER TODAY!

Early adapter and bulk discounts available

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