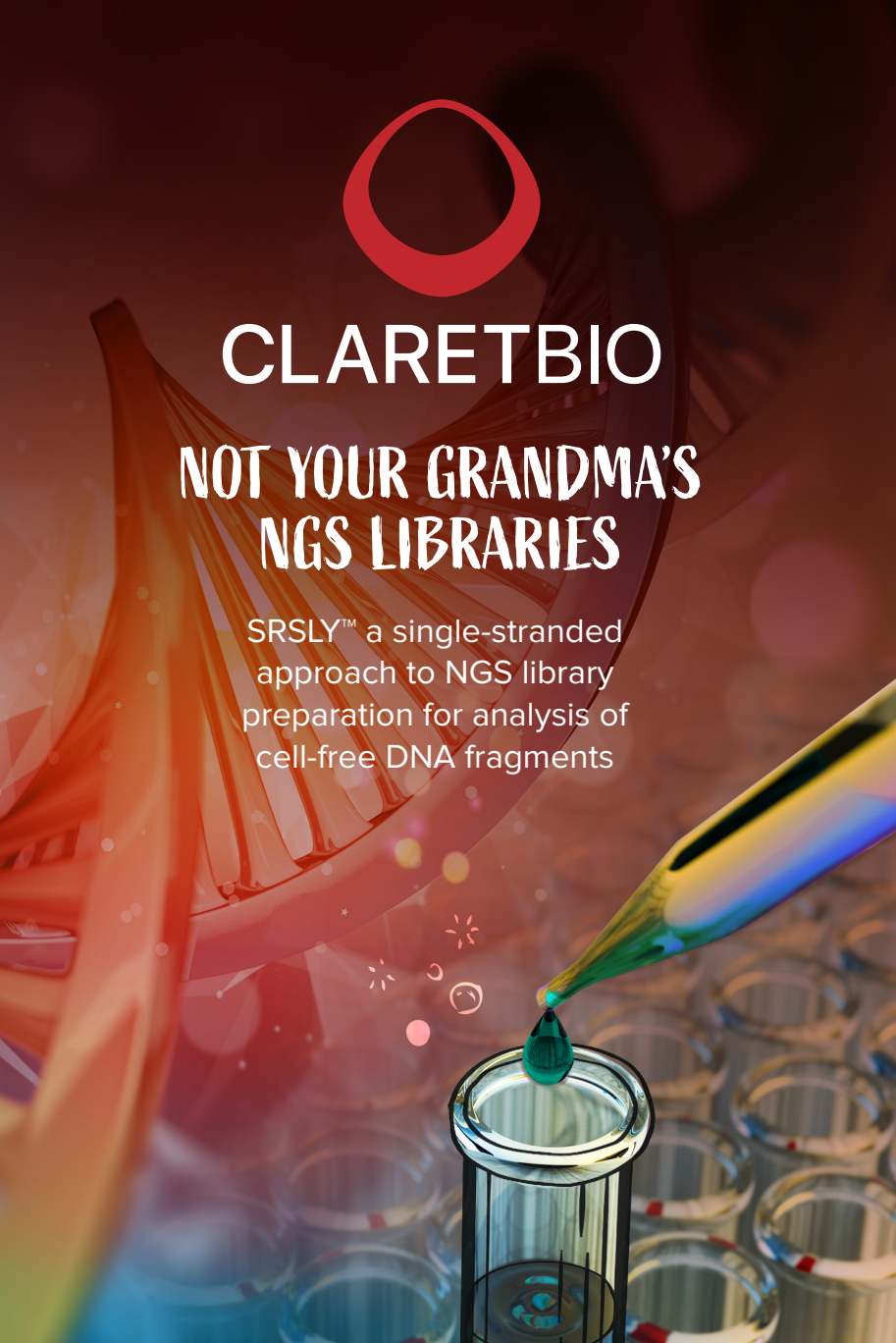

CLARETBIO
NOT YOUR GRANDMA'S NGS LIBRARIES
SRSLY™ a single-stranded approach to NGS library preparation for analysis of cell-free DNA fragments

WHY SHOULD YOU INVESTIGATE SHORT cfDNA FRAGMENTS AND THEIR ENDS?

Any good story has a beginning, middle, and end. Yet, current commercial library preparation kits jump straight into the middle of the plot without providing the whole picture. Cell-free DNA (cfDNA) circulating in biofluids and assayed by next-generation sequencing (NGS) provides a wealth of information about disease status through minimally-invasive sampling. As shown in the model below (Figure 1), cfDNA is non-randomly fragmented primarily exhibiting nucleosome footprints (~167 bp). In addition, regions protected by smaller biological structures, such as transcription factors and DNA binding protein complexes (~30-100 bp) are also observed. Genomic positions of cfDNA fragment ends are increasingly appreciated for their potential utility as a tumor tissue-of-origin biomarker. Double-stranded DNA library preparation kits are neither designed to capture these native ends nor smaller cFDNA fragments.

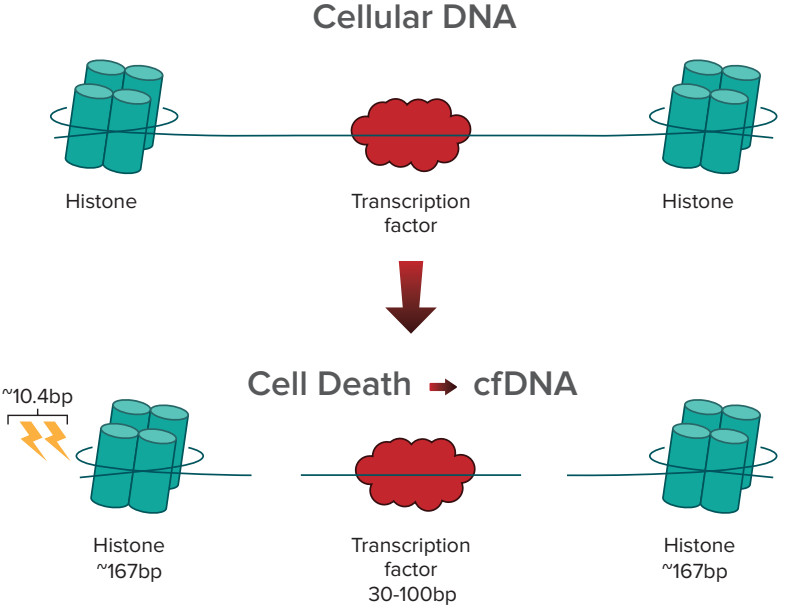
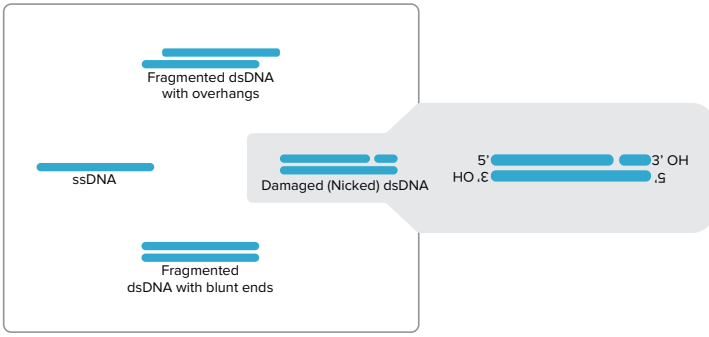


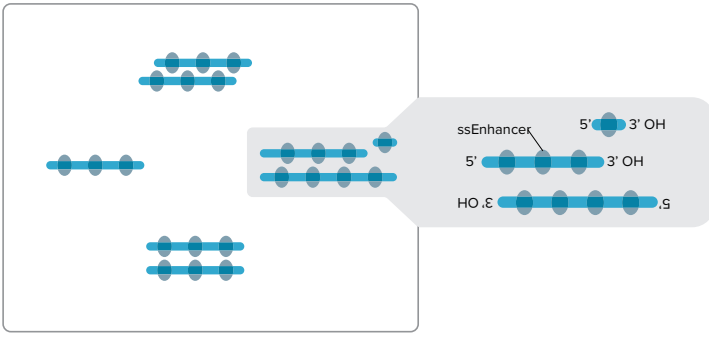
Figure 1: The cfDNA protection model (Figure adapted from Snyder et al, 2016. Cell 164, 57–68).

SRSLY HOW SIMPLE IS YOUR WORKFLOW?

DNA input pool



Denature and capture single-stranded DNA



Phosphorylate template DNA and ligate adapters

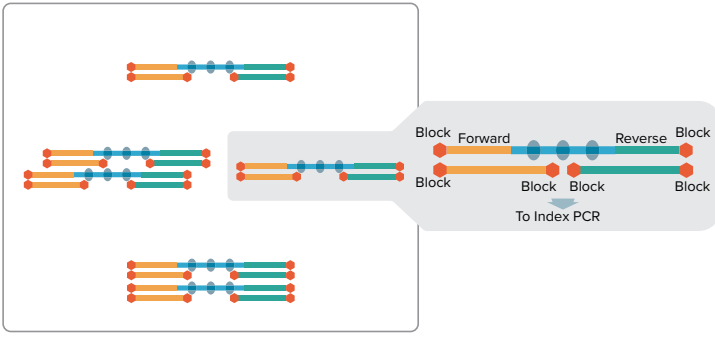


Figure 2: The SRSLY workflow is a one tube process taking less than 2.5 hours.

HOW DOES YOUR LIBRARY PERFORM?

SRSLY performance was compared against three commercial NGS kits using two healthy cfDNA extracts (Sample 1 and Sample 2). The results show SRSLY metrics not only rival commonly used kits but outperform them. SRSLY captures more short fragments than dsDNA preparations and retains the 10.4 bp DNA helix periodicity lost to data trimming required by the Swift Biosciences™ single-stranded approach (Swift Biosciences Technical note: Accel-NGS 1S Plus and Methyl-Seq Tail Trimming).

SRSLY Captures Small Fragments

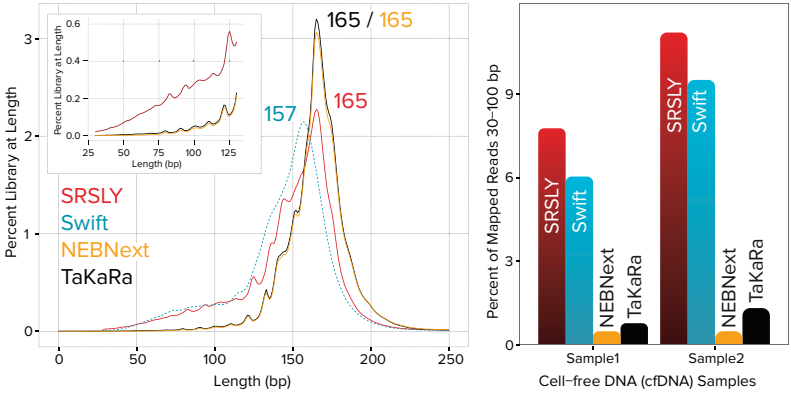


Figure 3: Size distribution of cfDNA molecules captured by SRSLY and three commercial NGS kits. SRSLY demonstrates maximum capture of short fragments.

Kit	Type of Prep Method	Input cfDNA Amount	Total Time	Input cfDNA Sample ID	Yield Post 10c Index PCR (Total)	Merged Read Pairs Sequenced	Mapping Rate
ClaretBio SRSLY	Single-stranded	1ng	2.5hrs	Sample 1	569ng	73,077,812	94.35%
				Sample 2	525ng	63,449,167	93.52%
NEBNext® Ultra II	Double-stranded	1ng	3hrs	Sample 1	278ng	78,705,033	94.66%
				Sample 2	310ng	54,734,560	88.88%
TaKaRa ThruPLEX®	Double-stranded	1ng	2.5hrs	Sample 1	476ng	74,429,274	94.01%
				Sample 2	470ng	71,294,525	87.97%
Swift Accel NGS® 1S Plus	Double-stranded	1ng	3.5hrs	Sample 1	432ng	78,018,897	93.66%
				Sample 2	534ng	69,959,246	93.73%

Table 2: Comparison of library yields and sequencing data.

HOW DO YOU GET MORE FROM YOUR cfDNA?

Start with a library preparation engineered to produce complex libraries from low DNA inputs (100 pg – 2 ng) while retaining the shortest fragments, whether duplexed, nicked, or single-stranded. Combine this with SRSLY (Single Reaction Single-stranded LibrarY), a rapid library preparation that eliminates DNA end repair resulting in:

- Higher efficiency ligation
- Broader recovery of molecules
- Retention of native ends

WHAT DO dsDNA LIBRARY PREPARATIONS LOSE?

Input DNA Molecule Type	SRSLY™ Kits	dsDNA Kits
 dsDNA	✓	✓
 ssDNA	✓	✗
 Nicked/Abasic dsDNA	✓	✗
 Native ends at 3’ overhangs	✓	✗

Table 1: Molecule diversity recovered by SRSLY and other ssDNA library preparations vs dsDNA preparation methods.

HOW DOES A SINGLE-STRANDED APPROACH IMPROVE LIBRARY PREPARATION?

Single-stranded approaches to NGS library preparation, initially developed for ancient DNA, handle lower DNA inputs. Compared to traditional dsDNA library methods, they improve short and degraded DNA fragment recovery, resulting in greater library complexity and improved yields. This makes them ideally suited for cfDNA and other degraded clinical samples such as FFPE. Capturing shorter fragments and, in the case of SRSLY, native ends offers more precise determination of genomic coordinates for improved fragmentomic analyses and clinical utility.

WHAT STORY DO THE DATA TELL?

SRSLY facilitates analysis of nucleosome positioning and transcription factor binding.

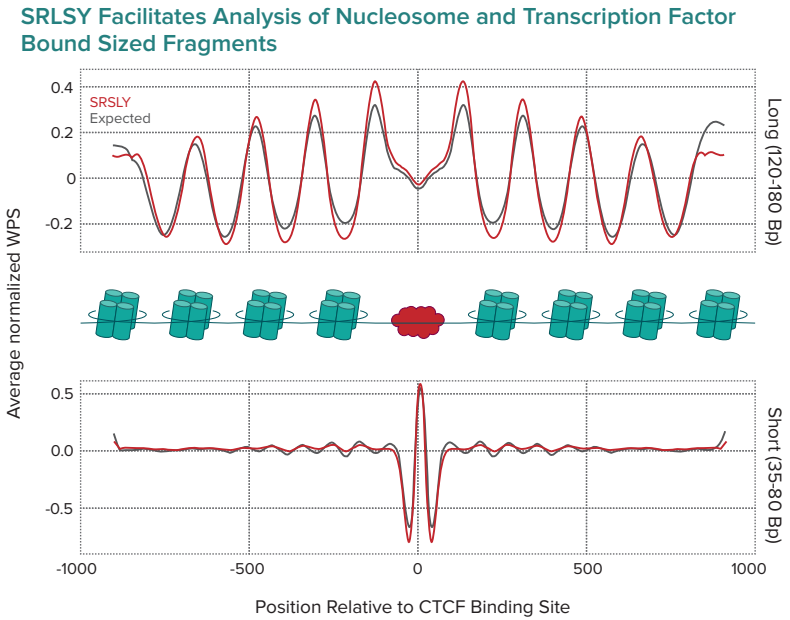


Figure 4: Analysis of nucleosome (long) and transcription factor (short) sized fragments (WPS score methods and expected distribution data from Snyder et al, 2016. Cell 164, 57-68).