Methods to Fragment DNA for Next-Generation Sequencing

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Next Generation Sequencing Applications in Functional Genomics

Fragmentation in Next- Generation Sequencing

Next Generation Sequencing (NGS) is comprised of a three step workflow:

1. Library Preparation
2. (Template amplification)
3. Sequencing

Library Preparation is characterized by three steps (Figure 1):

1. Fragmentation of DNA
2. Ligation of adapters
3. Fragments size selection

There are six techniques used in the fragmentation of DNA, these can be classified into three approaches:

1. Physical
2. Enzymatic
3. Chemical

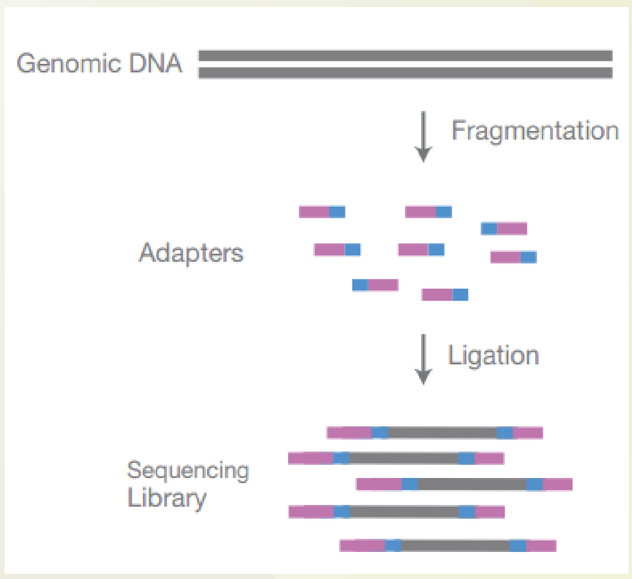

Figure 1. NGS sequencing llibraries are prepared by fragmenting genomic DNA and ligating specialized adapters. illumina ®

It is critical to identify the bias, advantages, and requirements of each technique so that the most appropriate fragmentation procedure for each sequencing project can be selected.

Physical Fragmentation

✓ Acoustic shearing (Covaris)
✓ Sonication
✓ Nebulization

How it works

These techniques shear DNA using force. Fragments contain 5′- or 3′-overhangs, so T4 DNA polymerase is required to fill in the 5’ overhang or cut off the 3’ overhang to produce blunt ends for primer ligation (Poptsova et al. 2014).

Bias

Common in all three techniques is the C enriched break point bias (Figure 3) (Poptsova et al. 2014).

Acoustic and Sonication

Three main advantages:

- Low DNA input requirement
- High sample recoveries
- Non-contact-based technique (reduced risk for contamination)

Nebulization

Atomizes liquid using compressed air causing DNA to shear.

Advantages:

- Low cost/ No instrument required
- Quick

Disadvantages:

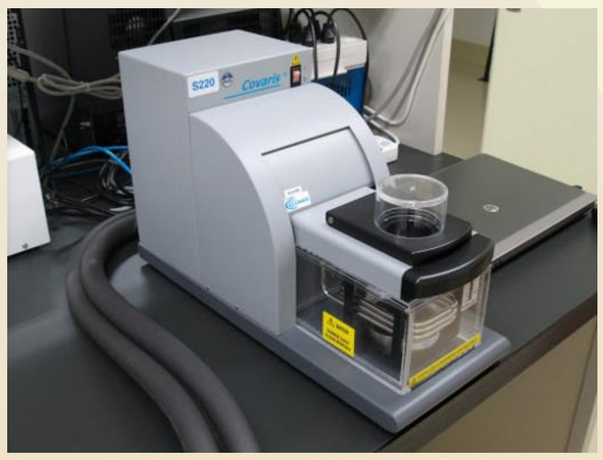

Figure 2. Covaris ® S220 involved in the fragmentation of DNA for NGS by acoustic shearing.

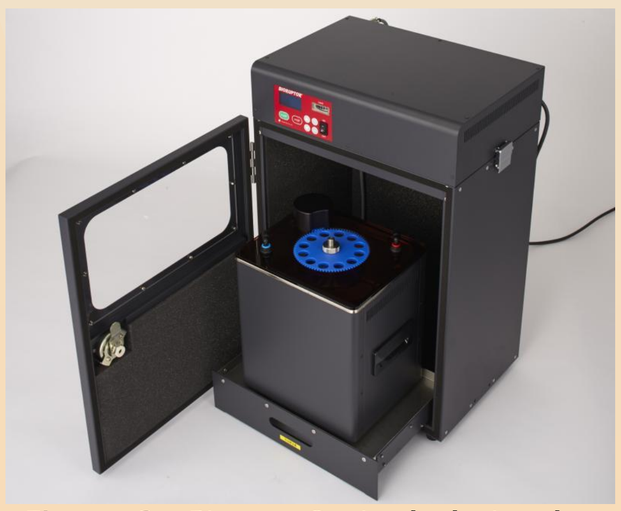

Figure 3. Bioruptor® involved in the fragmentation of DNA for NGS by sonication.

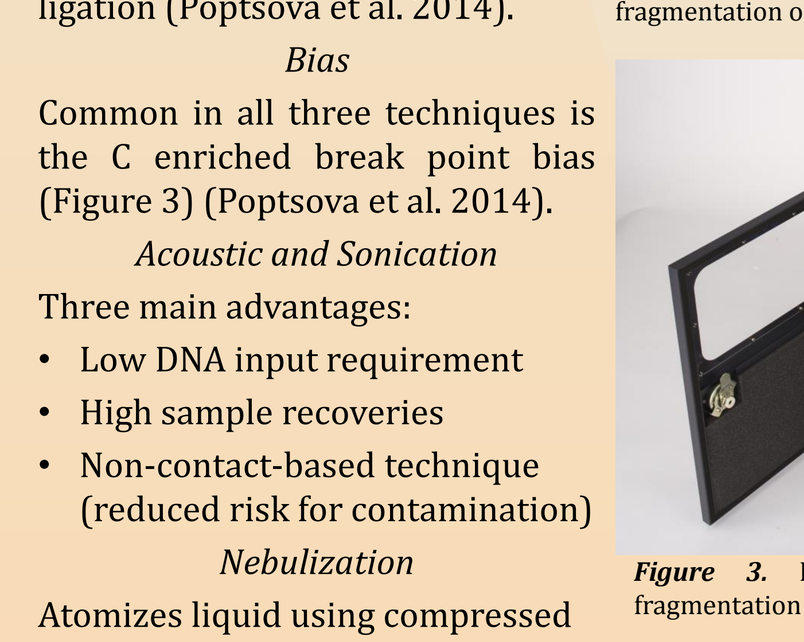

Figure 5. Relative mononucleotide frequencies around break point (+/−100 bp) for sonication method (Poptsova et al. 2014).

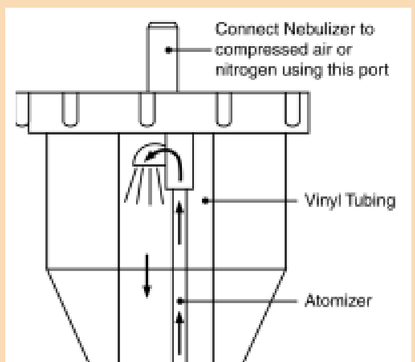

Figure 4. Diagram of Invitrogen™'s nebulizer involved in the fragmentation of DNA for NGS by nebulization.

Enzymatic Fragmentation

✓ DNase I, restriction endonuclease, non-specific nuclease
✓ Transposase/ Tagmentation

How it works

NEBNext™ dsDNA Fragmentase enzyme mix contains two enzymes. One randomly nicks and the other cuts the strand opposite to the nicks. The short overhangs and nicked DNA are repaired by *E.coli* DNA ligase (Knierim et al. 2011).

Nextera XT transposome tagments the DNA. In a single step, DNA is fragmented and adapter tags are added (Figure 6) (illumina®).

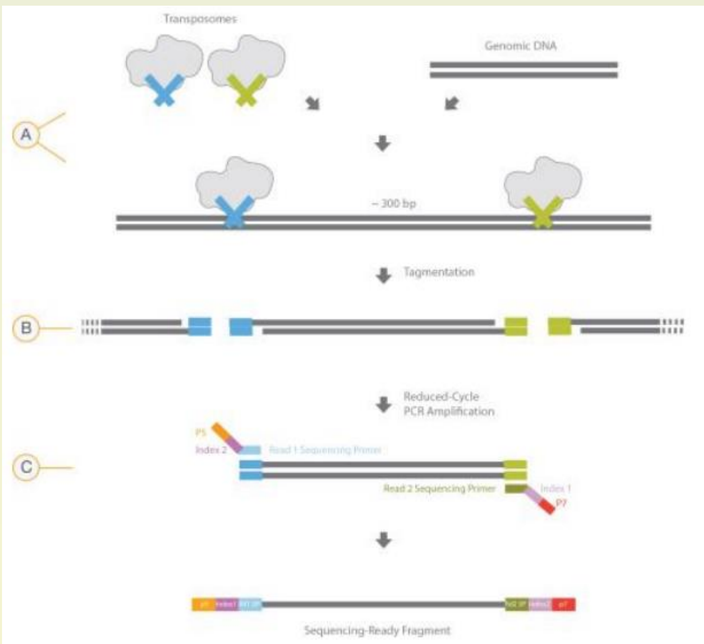

Figure 6. Library preparation using Nextera XT transposome. (A) Partial adapters are combined with template DNA. (B) Tagmentation - DNA fragmented and partial adapters are added. (C) Sequencing primer added for indexing. illumina ®

Bias

NEBNext™ dsDNA Fragmentase does not introduce any detectable bias (NEB unpublished).

A more prominent GC bias is found in Nextera XT protocols because of transposase insertion bias and subsequent high number of PCR cycles (Lan et al. 2015)

Side Notes

Knierim et al (2011) found that use of NEBNext™ dsDNA Fragmentase may result in short deletions if insufficient ligase is added.

Nextera XT has an ultra-low DNA input of only 1 ng, and the reaction can take place in a single enzymatic reaction.

Chemical Fragmentation

✓ Heat and divalent metal cation

How it works

Desired length of RNA fragmentation can be adjusted by increasing incubation time of heated digestion of RNA with a divalent metal cation (Mg or Zn) (Head et al. 2014). Chemical digestion is known to show less bias and good reproducibility (Head et al 2014).

Summary

Technique	DNA input	Frag. Size	Requires an Instrument?	End point?
Acoustic Shearing	LOW	100-5kb	YES	No Req. T4 polym
Sonication	LOW	150-1kb	YES	No Req. T4 polym
Nebulization	HIGH (several micrograms)	100-3kb	NO	No T4 polym
NEBNext™ dsDNA Fragmentase	LOW	50–1,000 bp	NO	No *E.coli* DNA ligase
Nextera XT transposome	LOW (1 ng)	175-700 bp	NO	Yes!
Divalent metal cation	LOW	115 - 350	NO	NO Req. conversion to cDNA

References

Knierim E, Lucke B, Schwarz JM, Schuelke M, Seelow D (2011) Systematic Comparison of Three Methods for Fragmentation of Long-Range PCR Products for Next Generation Sequencing. PLoS ONE 6(11): e28240. doi:10.1371/journal.pone.0028240

Head, Steven R, Komori Kiyomi, LaMere, Sarah, Whisenant, Thomas, Nieuwerburgh, Filip Vam, Salomon, Daniel R., Ordoukhanian, Philip (2014) Library construction for next-generation sequencing: Overviews and challenges. BioTechniques,. 56,. 2:61–77.

Lan, James H., Yin, Yuxin, Reed, Elaine F., Moua, Kevin, Thomas, Kimberly, Zhang, Qiuheng. (2015) Impact of three Illumina library construction methods on GC bias and HLA genotype calling. *Human Immunology 76:2-3*

Poptsova, Maria S. et al. "Non-Random DNA Fragmentation in next-Generation Sequencing." *Scientific Reports* 4 (2014): 4532. *PMC*. Web. 29 Sept. 2016.

Nextera® XT DNA Library Prep Reference Guide. Illumina®

Genoscribe. http://blog.genohub.com/fragmentation-of-dna-rna-for-next-gen-sequencing-library-prep/