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

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 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
Atoms liquid using compressed air causing DNA to shear.

Advantages:
- Low cost/ No instrument required
- Quick

Disadvantages:
- Partially sheared DNA
- Potential for contamination

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 fragments the DNA. In a single step, DNA is fragmented and adapter tags are added (Figure 6) (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NEBNext™ dsDNA Fragmentase does not introduce any detectable bias (NEB unpublished).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 (Jan et al. 2015)

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

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

References

Nextera® XT DNA Library Prep Reference Guide. Illumina®

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

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 4. Diagram of Invitrogen™’s nebulator involved in the fragmentation of DNA for NGS by nebulization.

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

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®

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.