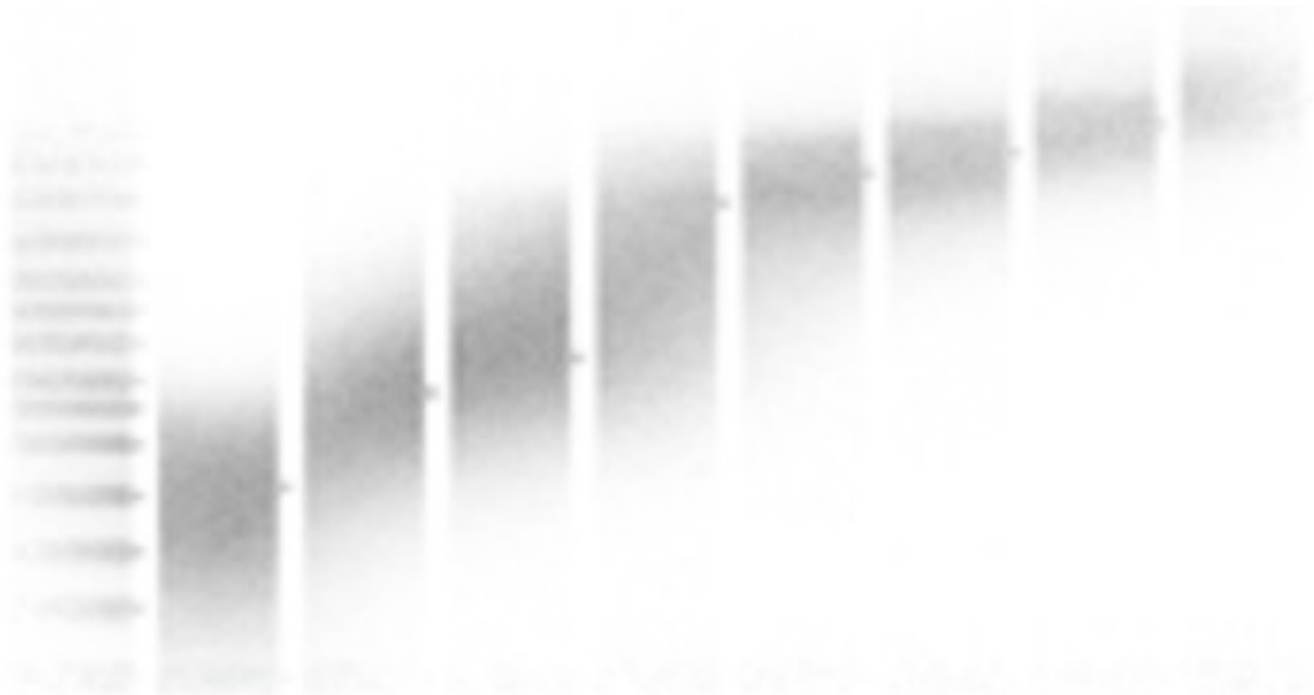
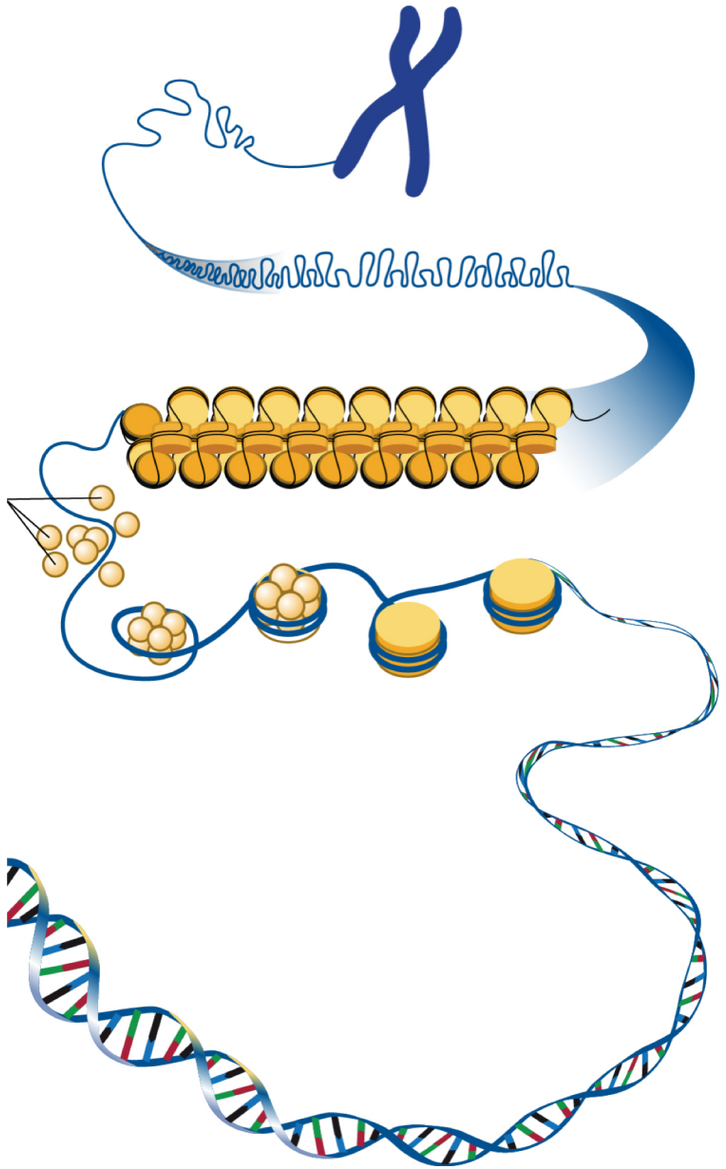
Nucleic Acid Fragmentation



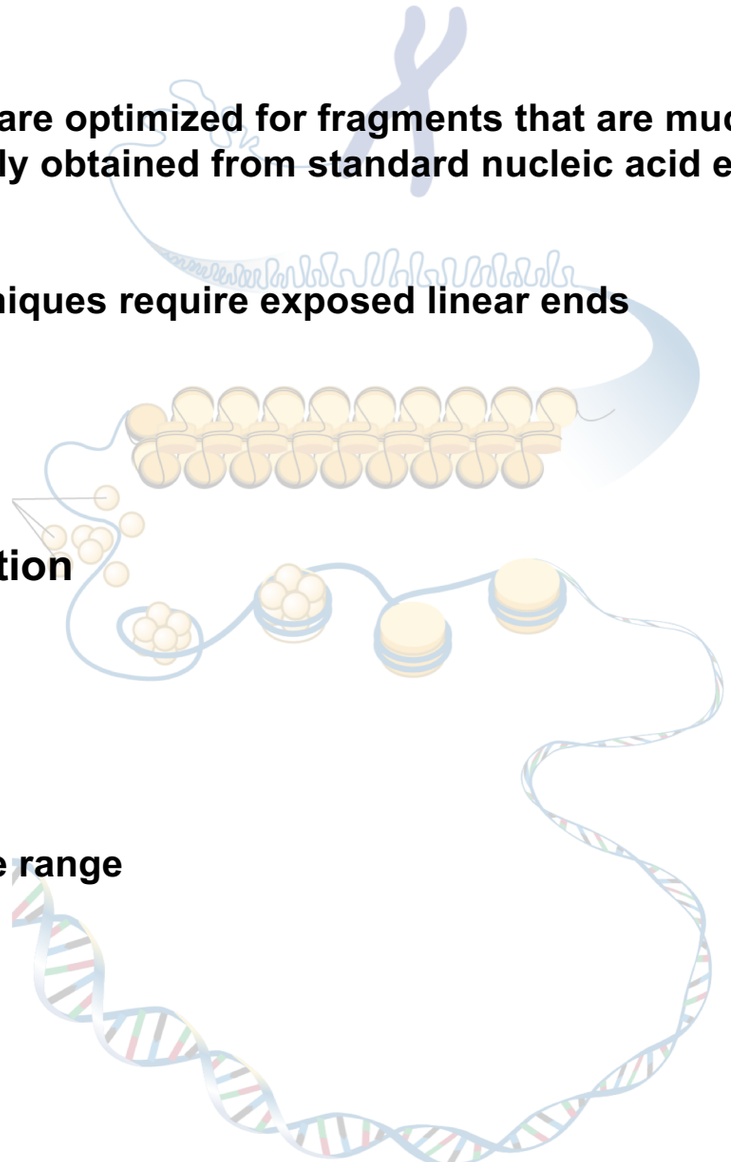
Why does library construction require DNA/RNA fragmentation?



Why does library construction require DNA/RNA fragmentation?

- **Sequencing technologies are optimized for fragments that are much smaller than those that are typically obtained from standard nucleic acid extractions.**

- **Library construction techniques require exposed linear ends**



Goals of DNA/RNA fragmentation

- **Random breakpoints**

- **Narrow and definable size range**

- **Minimal loss of sample**

Fragmentation Methods

- **Enzymatic**
  - NEB dsDNA Fragmentase
  - DNase I
  - Restriction endonucleases (for RAD-tags)

Low start-up costs (no big equipment purchases)

- **Chemical**
  - Heat and salts (RNA fragmentation)

Standard practice in Illumina RNA-seq library construction.

- **Mechanical**
  - Hydroshear
  - Nebulizers

- **Sonication/Ultrasonication**
  - Bioruptor
  - Covaris

Good repeatable, random shearing. Large start-up cost (instrument) and marginal costs (tubes).

- **Transposase**
  - Nextera Tagmentation

Simultaneous “fragmentation” and Illumina adapter ligation. Limited flexibility/customization.

NEB dsDNA Fragmentase

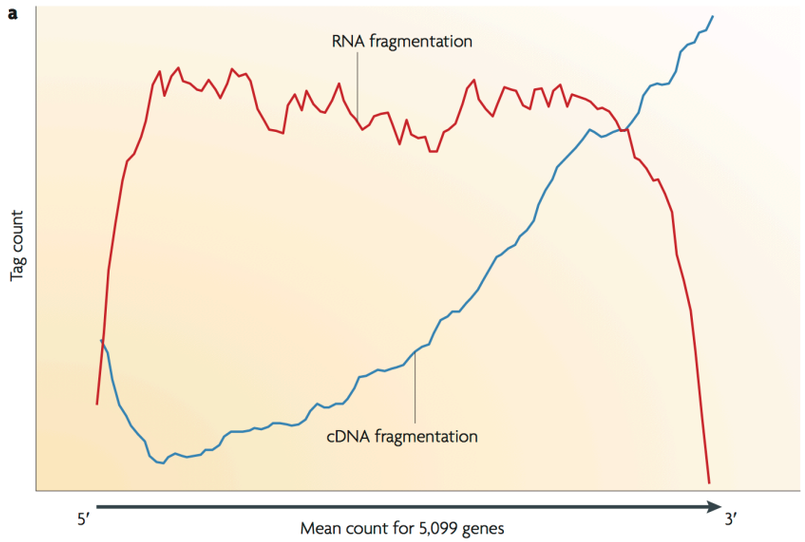
2-Enzyme Cocktail

- Nickase
- Enzyme that recognizes nick and induces double-stranded break

mRNA-seq Libraries

Fragment RNA rather than cDNA

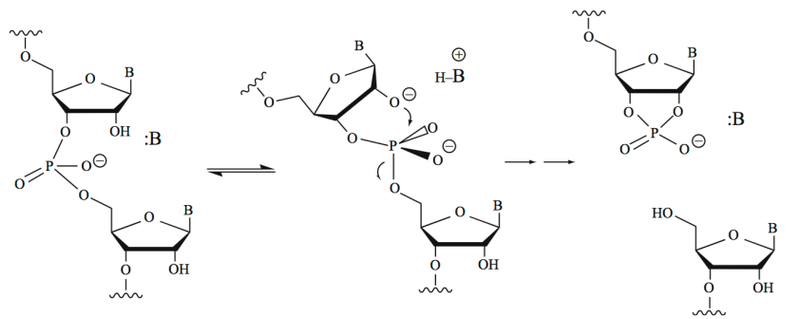
- Less bias in coverage



Wang et al. 2009 NRG

Chemical fragmentation of RNA with heat and salts (divalent cations)

- Quick, cheap, and easy
- Fine-tunable: adjust incubation time



Forconi and Herschlag 2009

Covaris Ultrasonicators – Adaptive Focused Acoustics

Shearing Principle

- Ultrasound waves induce strand breaks in nucleic acids
- Waves are focused on a specific target position at the base of the tube containing your sample

Settings and Controls

- Duration
- Power intensity
- Duty factor
- Sample volume: 15-200 ul (different tubes)
- Individual tubes vs. 96 well plates (different instruments)

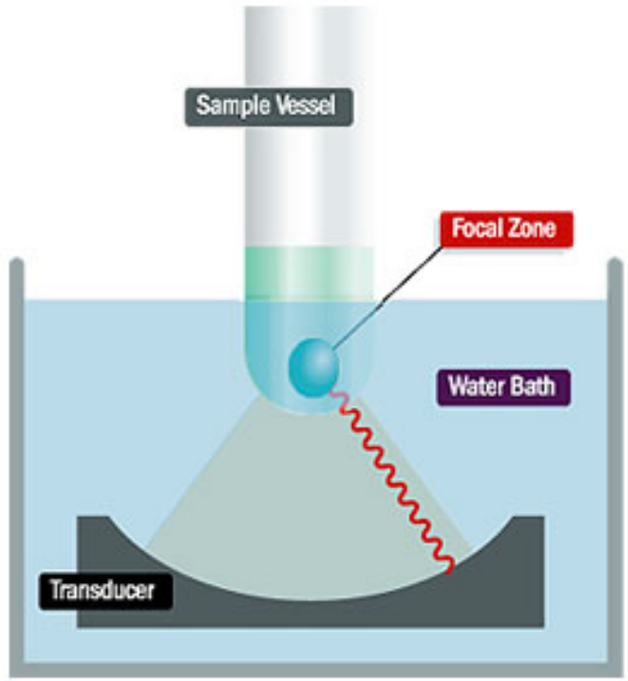
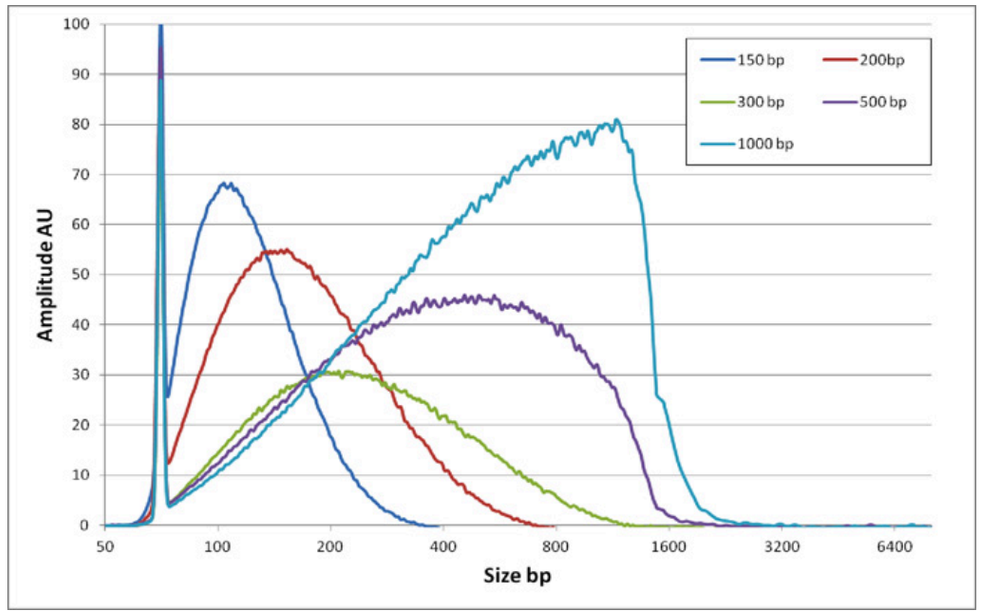


Image Source: Covaris

Covaris Ultrasonicators – Adaptive Focused Acoustics

Advantages

- Repeatable, customizable target size ranges for DNA, RNA, or chromatin
- Robust to differences in concentration, buffers, contamination
- “Random” shearing
- Isothermal
- Quiet (ultrasound!)
- Fast (~2 min and no switching buffers)



Disadvantages

- Cost (instrument and tubes)
- One samples at a time for smaller instruments
- Oxidative damage and increase sequencing error rate (especially at fragment ends)