Library Preparation

NGS Library Preparation: Molecular Details

1. DNA Fragmentation

Input DNA (High MW)

Physical

Sonication

Enzymatic

DNase

Tagmentation

Fragmented DNA (200-600 bp)

Target: 300-500 bp

2. End Repair & A-tailing

End Repair (Blunt ends)

5'   3'   T4 Pol   5'   3'

3'   5'   3'   5'

A-tailing (3' overhang)

5'   3'   Taq   5'   A

3'   A   5'

Reaction conditions:
- T4 DNA Polymerase + T4 PNK
- Taq Polymerase + dATP @ 72°C

3. Adapter Ligation

Y-shaped Adapters

P5   Index1   Index2

Ligation Reaction

T4 Ligase

P5   P7

Adapter Components:
Sequencing primers • Indices • Flow cell bind...

4. Size Selection

Gel/Bead Selection

300-500 bp

SPRI Beads

Size-selective binding
Buffer/bead ratio

Quality Control Checkpoints

- Fragment size: 300-500 bp
- Concentration: >10 ng/μL
- A260/280: 1.8-2.0
- Bioanalyzer profile

5. PCR Amplification

Limited Cycle PCR (8-12 cycles)

95°C

60°C

72°C

0   Time   End

Final Library

Ready for sequencing

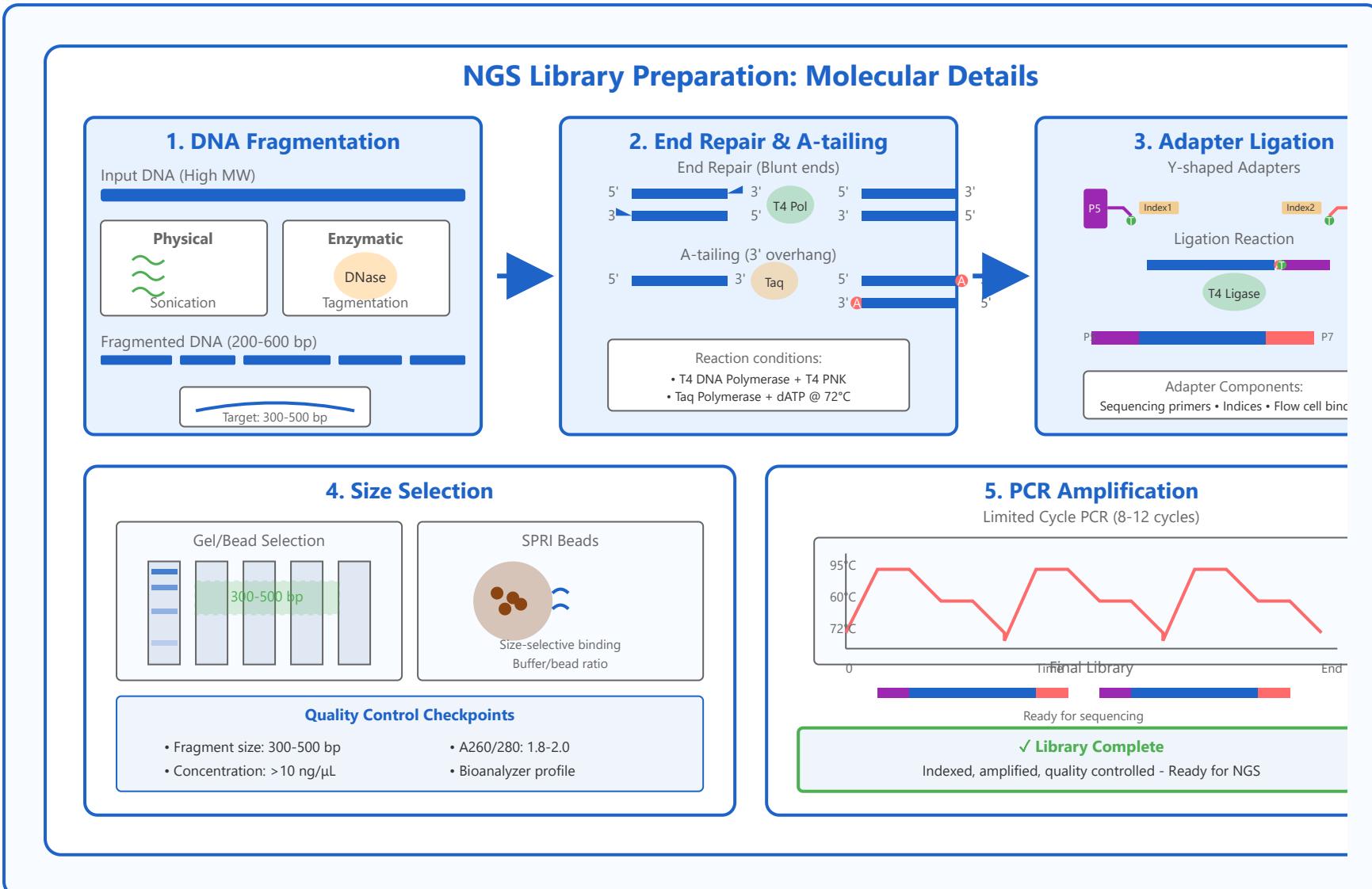✓ **Library Complete**

Indexed, amplified, quality controlled - Ready for NGS

Critical Factors

Library Types

- Input DNA quality and quantity
- Fragment size distribution
- Adapter ligation efficiency
- Minimal PCR cycles to avoid bias

- Whole genome libraries
- PCR-free libraries (reduce bias)
- Mate-pair libraries (long-range)
- Targeted capture libraries