Next Generation Sequencing

Basic Steps of NGS Method

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Terminology

- Next Generation Sequencing (NGS)
  - DNA sequencing methods that involve chemical assays other than the traditional Sanger deoxy-chain-termination method (1st Gen Seq)
- NGS AKAs
  - Deep Sequencing
  - Massively Parallel Sequencing
  - Second and Third Generation Sequencing
    - 2nd: Undergoes amplification of the template molecules
    - 3rd : Single molecule sequencing

Generic Overview of NGS

Library Preparation

Input DNA

Fragmentation

End repair and adapter ligation

Fragment library

Clonal Amplification

Clonal amplification of each fragment (2 Types)

A

A B

Emulsion PCR

Bridge Amplification

Sequencing of clonal amplicons in a flow cell

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Sequencing

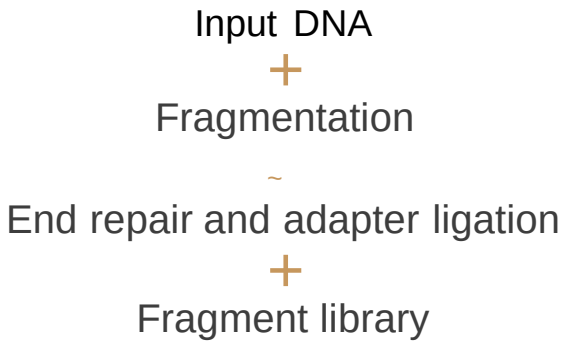
or

Generation of luminescent or fluorescent images

Conversion to Sequence

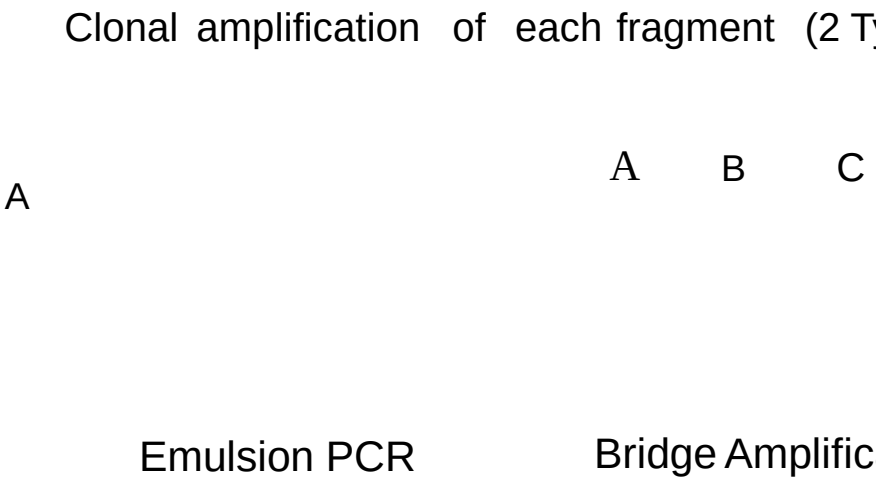
1. Library Preparation

- **Input DNA Fragmented**
  - Shearing by
    - Sonication
    - Nebulization
    - Enzyme digestion



- **Fragments have terminal overhangs**
  - Blunt-end repair and phosphorylation
- **Adapter ligation**
  - Platform-specific adapter are ligated to the fragments
- **Final Library**
  - Short DNA fragments with platform-specific adaptors

2. Clonal Amplification



- **Amplify the fragments**
  - Emulsion PCR – oil-in-water based
    - One Bead = One Fragment = One Sequence Bead
  - Bridge Amplification – solid surface, flow-cell based
    - One Cluster = One Fragment = One Sequence Bead

Voelkerding KV (2010) Next Generation Sequencing for Clinical Diagnostics-Principles and Application to Targeted Resequencing for Hypertrophic Cardiomyopathy. Journal of Molecular Diagnostics 5(12): 539-551

3. Sequencing

Sequencing of clonal amplicons in a flow cell

+

or

Generation of luminescent or fluorescent images

Conversion to Sequence

- **Pyrosequencing**
  - Sequence incorporation of nucleotides → luminescence
- **Sequencing by Ligation**
  - Introduction of oligonucleotide probes → fluorescence
- **Reversible dye terminators**
  - Incorporation of reversible dye terminators → fluorescence

Voelkerding KV (2010) Next Generation Sequencing for Clinical Diagnostics-Principles and Application to Targeted Resequencing for Hypertrophic Cardiomyopathy. Journal of Molecular Diagnostics 5(12): 539-551

4. Analysis

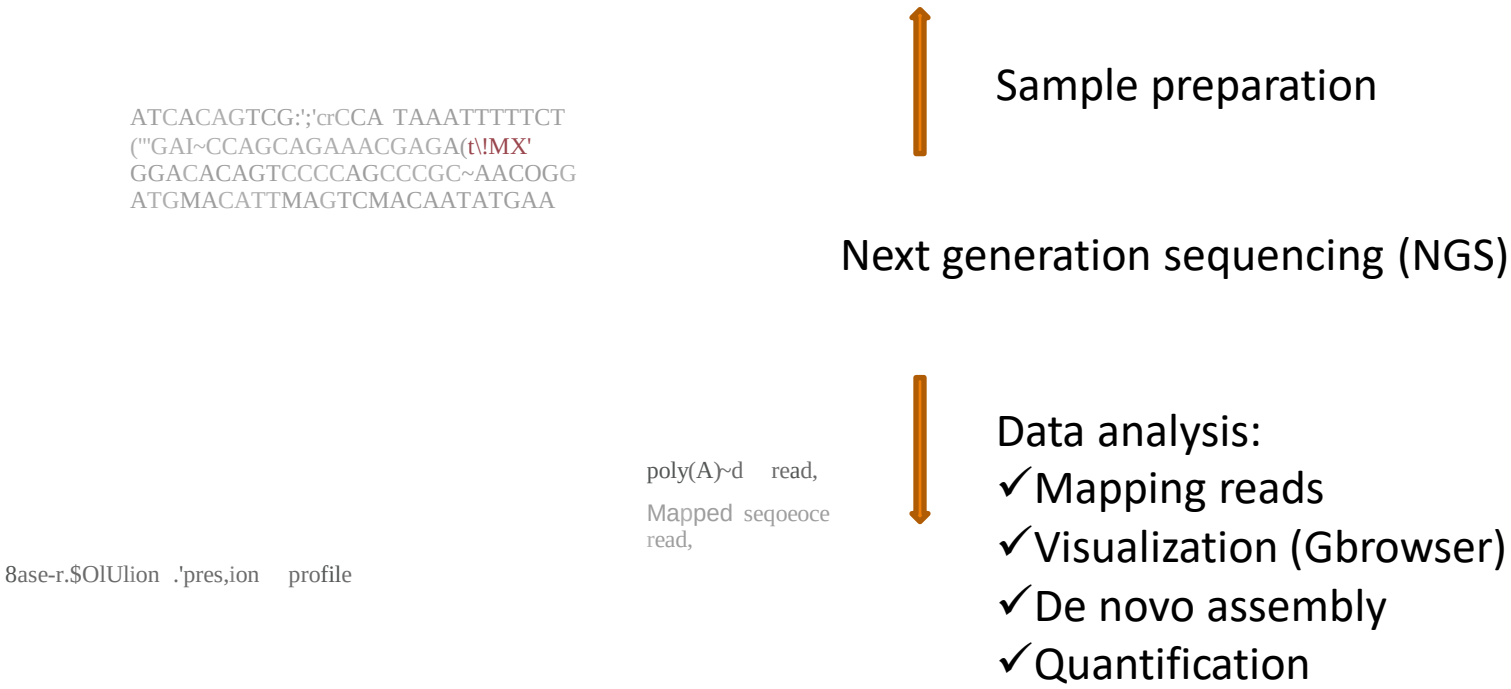


Figure from Wang et. al, **RNA-Seq: a revolutionary tool for transcriptomics,** Nat. Rev. Genetics 10, 57-63, 2009).

Next Generation Sequencing

Sanger Dideoxy