

Outline

- High-throughput sequencing (NextGen) technologies
- Sequencing coverage
- Multiplexing

---

---

---

---

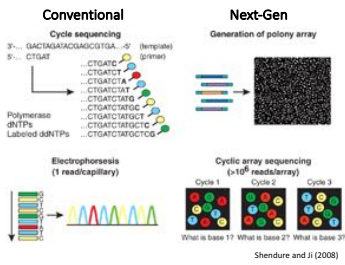
---

---

---

---

NextGen Sequencing




---

---

---

---

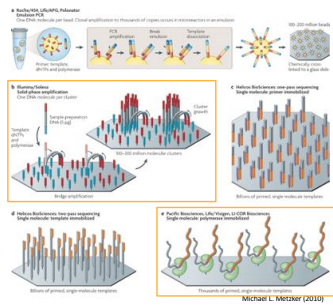
---

---

---

---

NextGen Sequencing Technologies




---

---

---

---

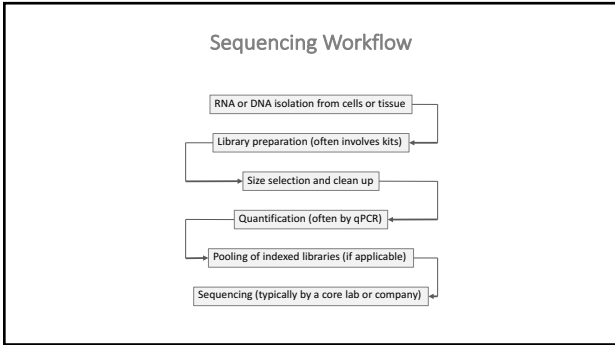
---

---

---

---





---

---

---

---

---

---

---

---

### Illumina Sequencing

Some slides used with permission from Illumina

---

---

---

---

---

---

---

---



---

---

---

---

---

---

---

---

### Library Prep is Critical for Successful Sequencing



The aim of the sample prep step is to obtain nucleic acid fragments with adapters attached on both ends

---

---

---

---

---

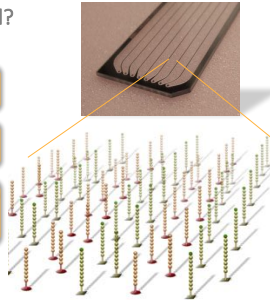
---

---

---

### What is a Flow Cell?

- Cluster generation occurs on a flow cell
- A flow cell is a thick glass slide with channels or lanes
- Each lane is randomly coated with a lawn of oligos that are complementary to library adapters



---

---

---

---

---

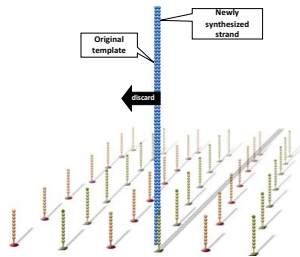
---

---

---

### Denature Double-stranded DNA

- Double-stranded molecule is denatured
- Original template washed away
- Newly synthesized strand is covalently attached to flow cell surface



---

---

---

---

---

---

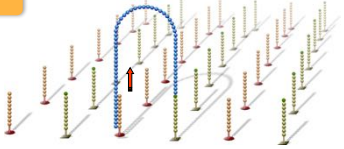
---

---

### Bridge Amplification

Single-stranded molecule flips over and forms a bridge by hybridizing to adjacent complementary primer.

Hybridized primer is extended by polymerases.



---

---

---

---

---

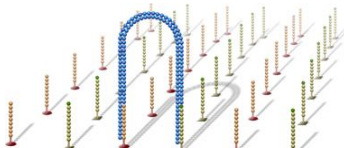
---

---

---

### Bridge Amplification

Double-stranded bridge is formed.



---

---

---

---

---

---

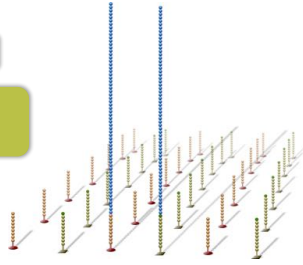
---

---

### Denature Double-stranded Bridge

Double-stranded bridge is denatured.

Result: Two copies of covalently bound single-stranded templates.



---

---

---

---

---

---

---

---

### Bridge Amplification

Single-stranded molecules flip over to hybridize to adjacent primers

Hybridized primer is extended by polymerase

The diagram shows a grid of vertical DNA strands. Some strands are red, some are yellow, and some are blue. Red and yellow strands are hybridized to each other. Blue strands are shown flipping over to hybridize to adjacent red and yellow strands. Red arrows indicate the direction of strand flipping.

---

---

---

---

---

---

---

---

### Bridge Amplification

Bridge amplification cycle repeated until multiple bridges are formed

The diagram shows the same grid of DNA strands as in the first step, but now multiple blue double-stranded bridges have formed between the red and yellow strands.

---

---

---

---

---

---

---

---

### Linearization

dsDNA bridges are denatured

The diagram shows the same grid of DNA strands, but the blue double-stranded bridges have been denatured into single-stranded molecules. Red arrows indicate the direction of denaturation.

---

---

---

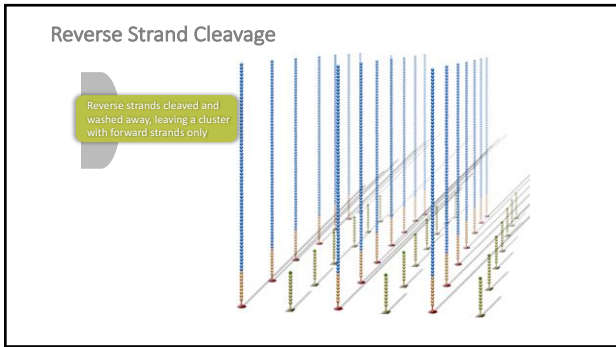
---

---

---

---

---



---

---

---

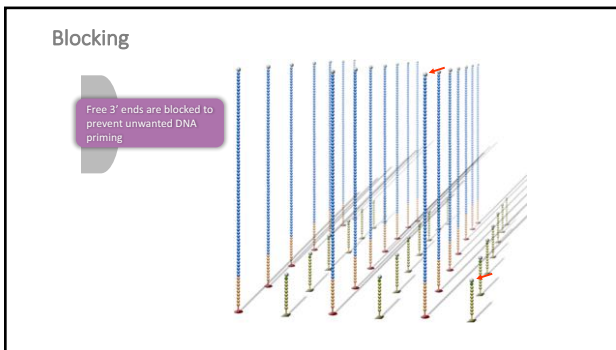
---

---

---

---

---



---

---

---

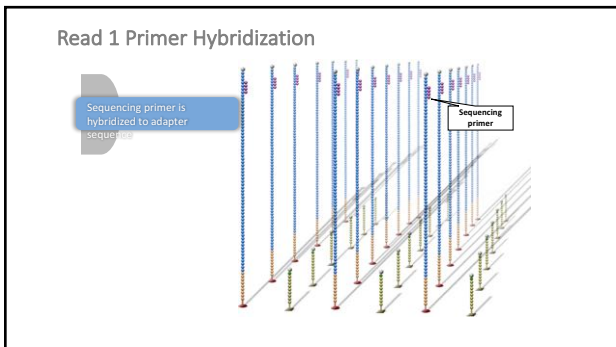
---

---

---

---

---



---

---

---

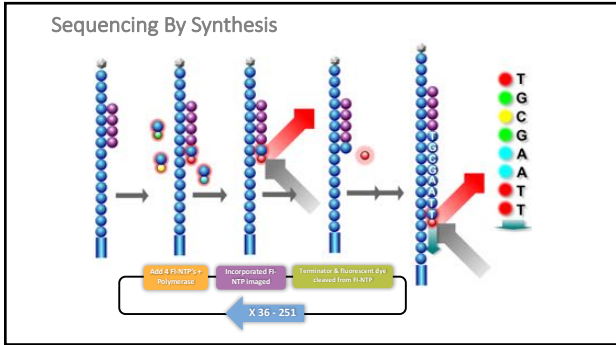
---

---

---

---

---



---

---

---

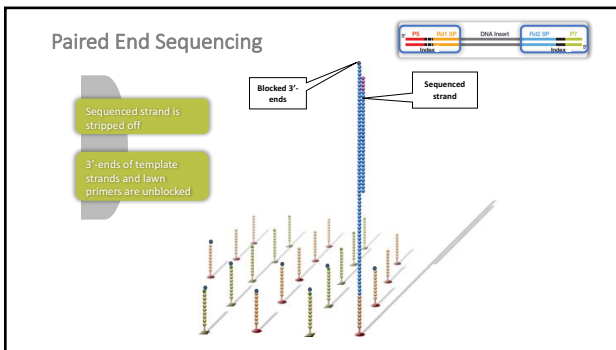
---

---

---

---

---



---

---

---

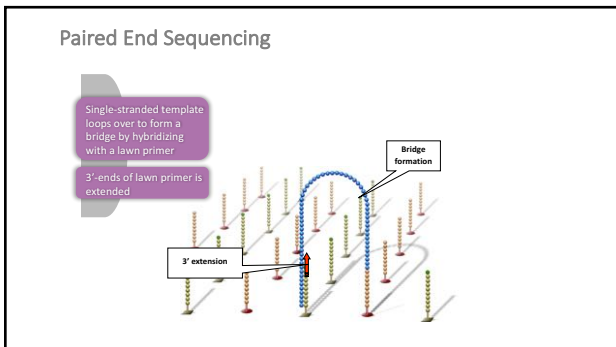
---

---

---

---

---



---

---

---

---

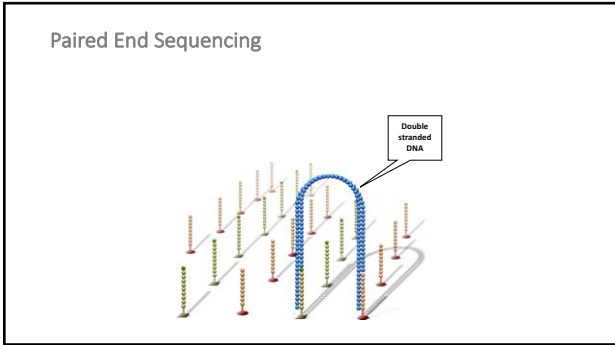
---

---

---

---





---

---

---

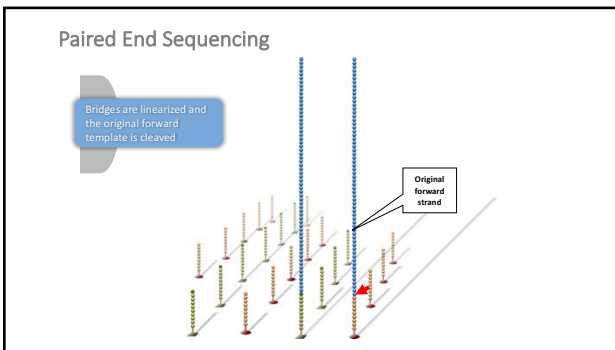
---

---

---

---

---



---

---

---

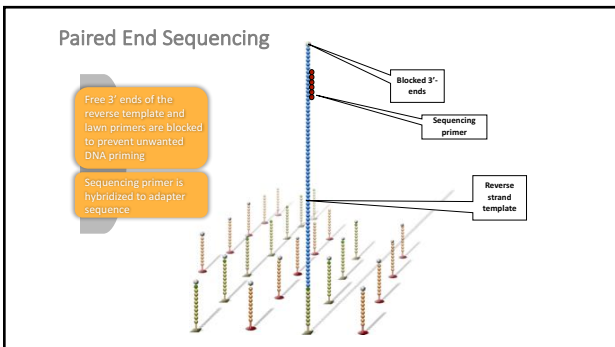
---

---

---

---

---



---

---

---

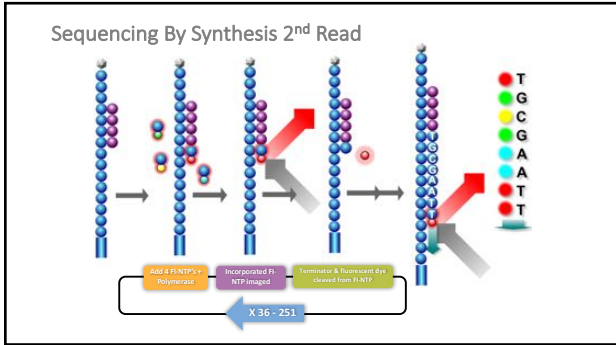
---

---

---

---

---




---

---

---

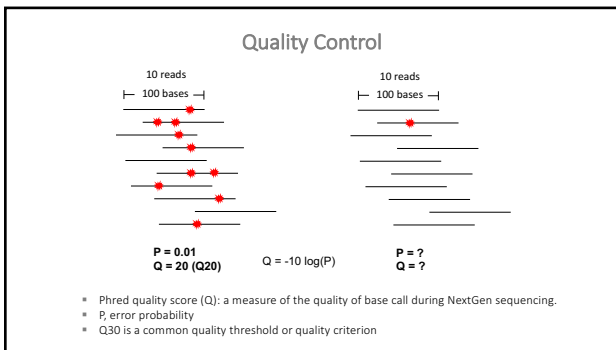
---

---

---

---

---




---

---

---

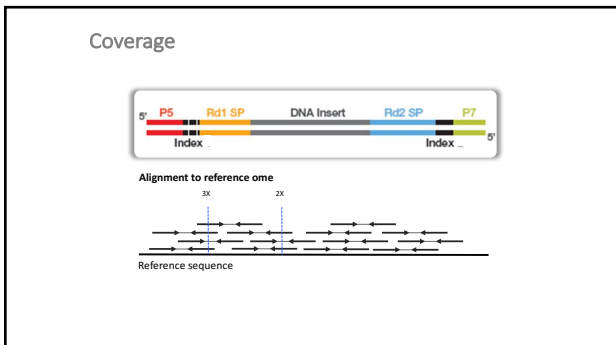
---

---

---

---

---




---

---

---

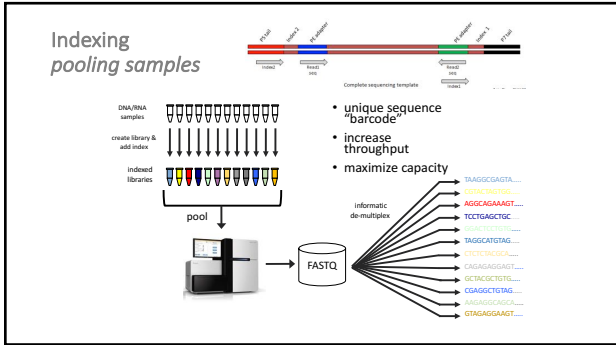
---

---

---

---

---



---

---

---

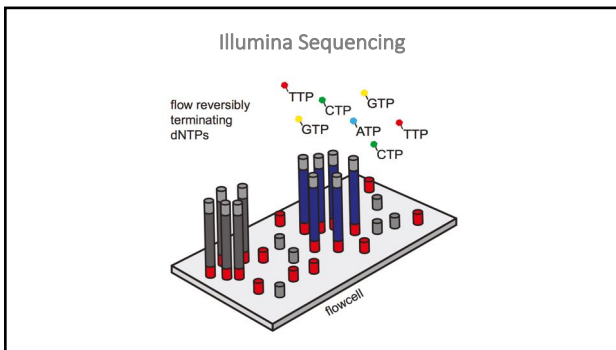
---

---

---

---

---



---

---

---

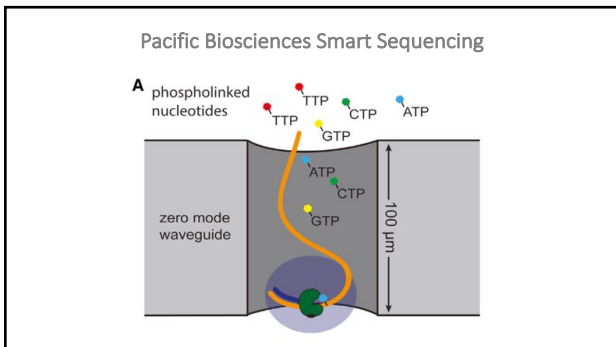
---

---

---

---

---



---

---

---

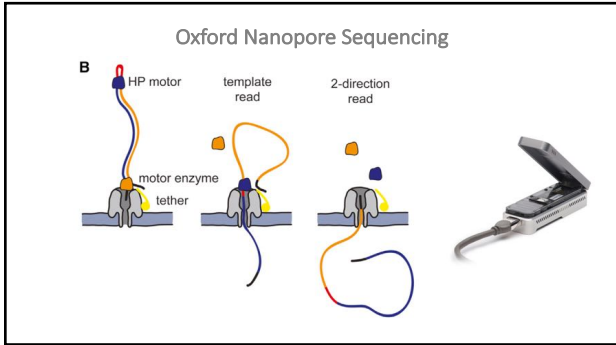
---

---

---

---

---



---

---

---

---

---

---

---