Next Generation Sequencing

Illumina Sequencing

NOTE: These slides are taken from
http://www.slideshare.net/USDBioinformatics/illumina-sequencing

Illumina Diagram

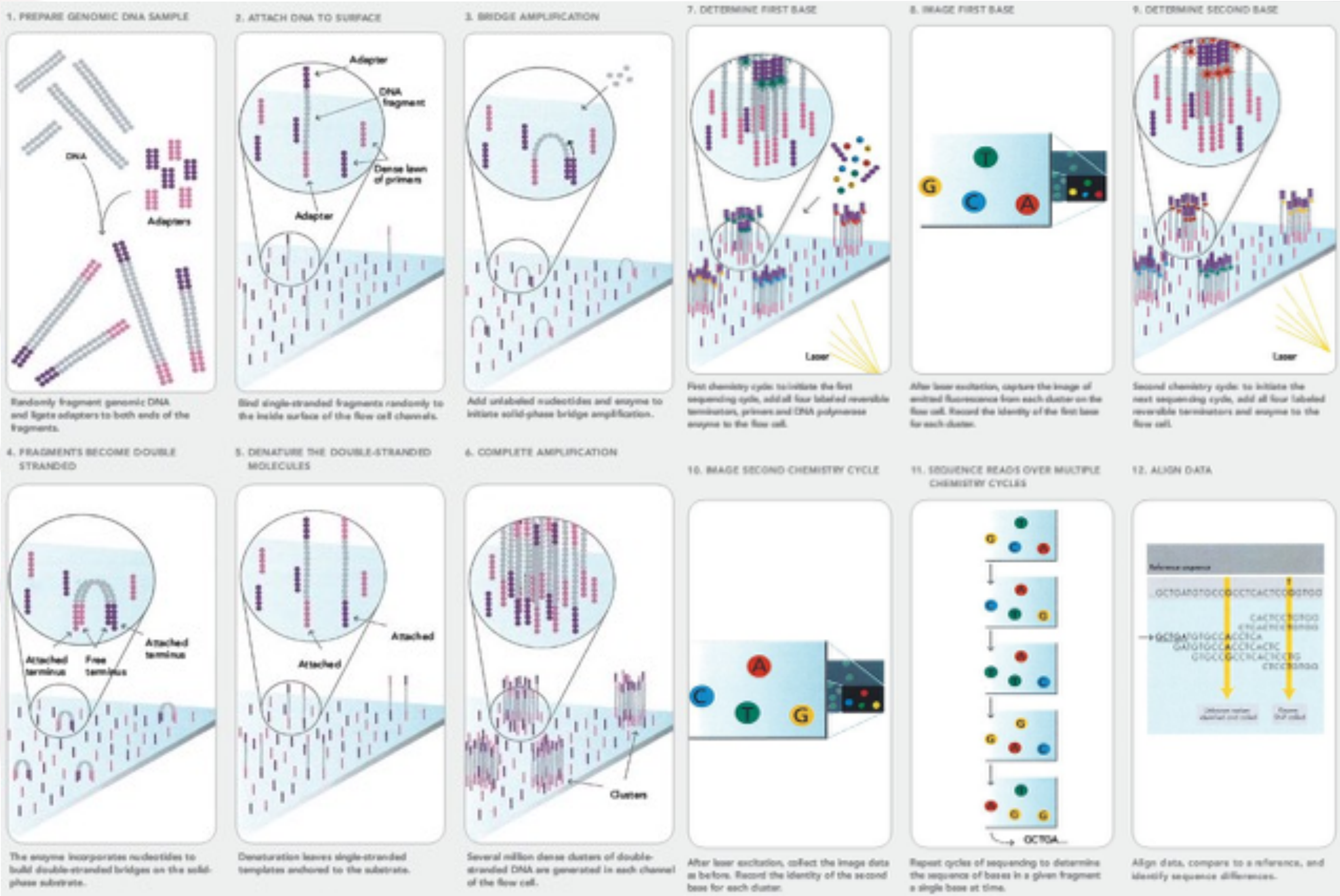


Image retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

Prepare Genomic DNA Sample

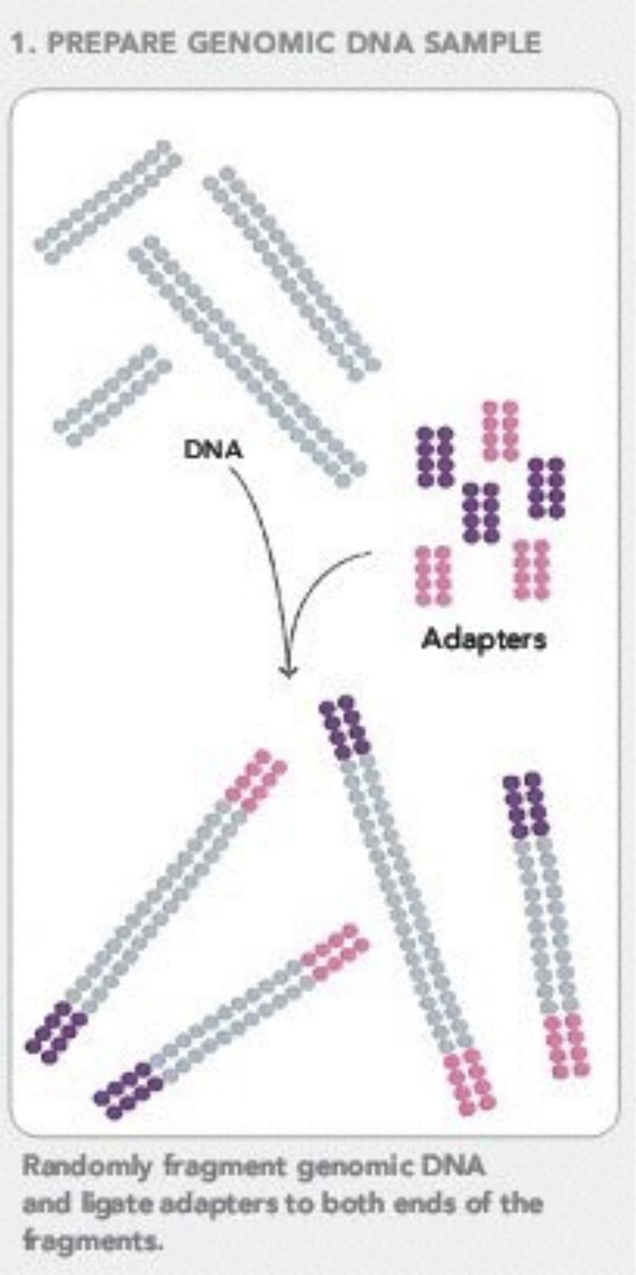
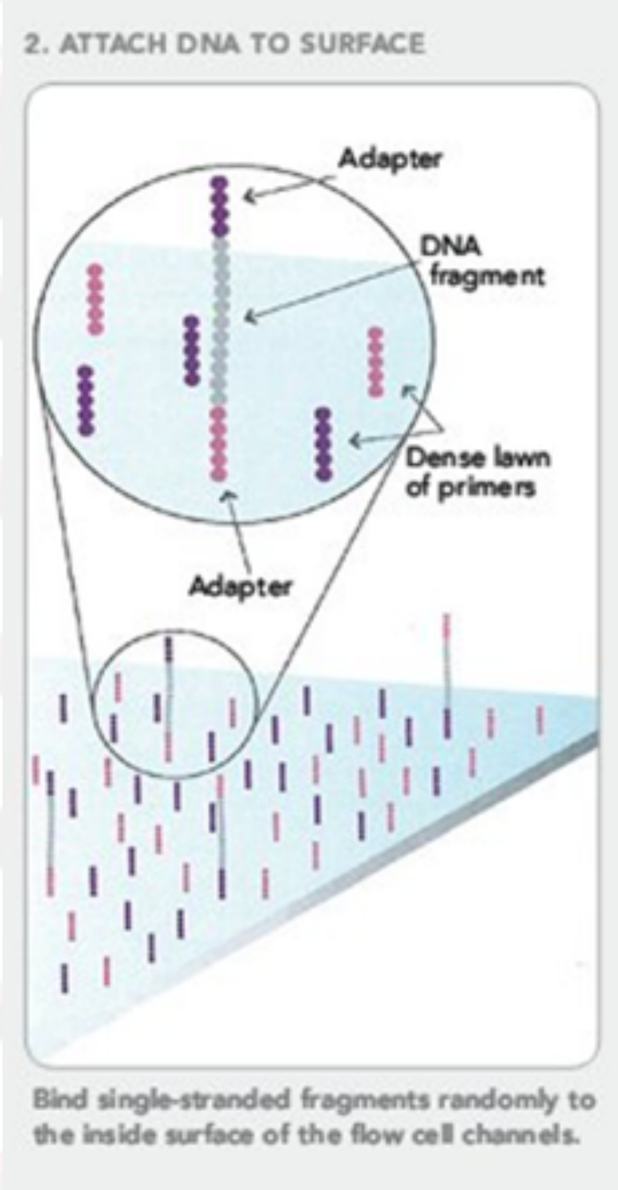


Image retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

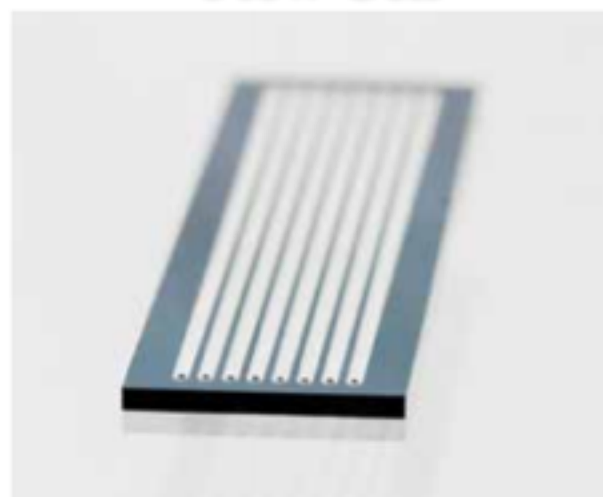
- Fragment DNA of interest into smaller strands that are able to be sequenced
    - Sonication
    - Nebulization
    - Enzyme digestion
- Ligate Adapters
- Denature dsDNA into ssDNA by heating to 95° C

Attach DNA to Surface



- ssDNA is then bound to inside surface of flow cell channels
- Dense lawn of primer on the surface of the flow cell

Flow Cell



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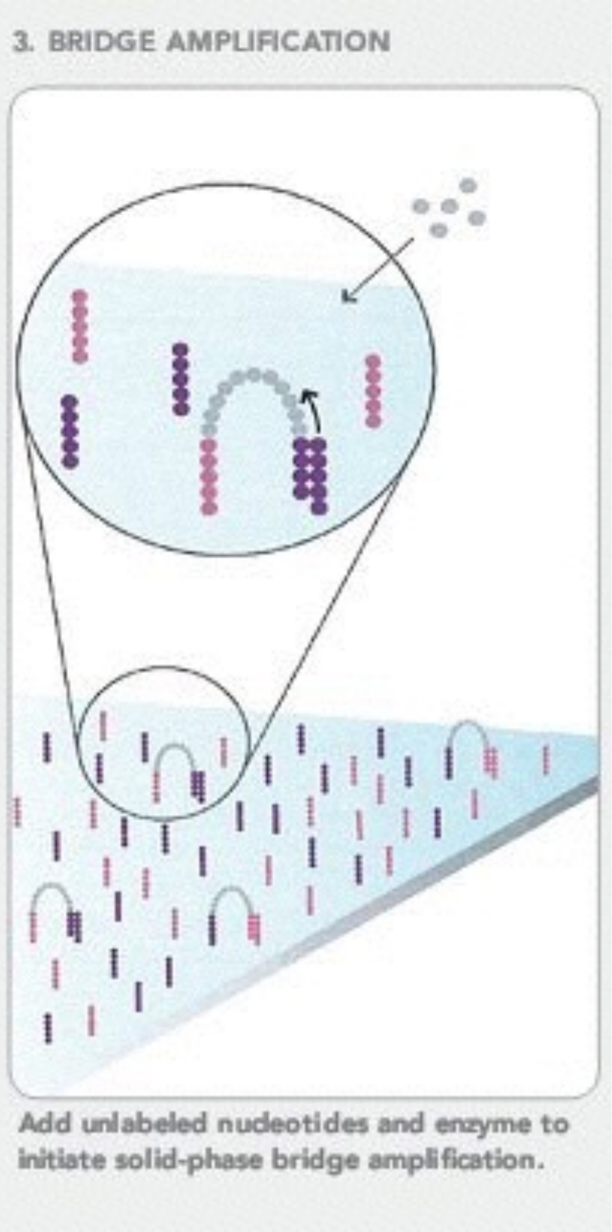

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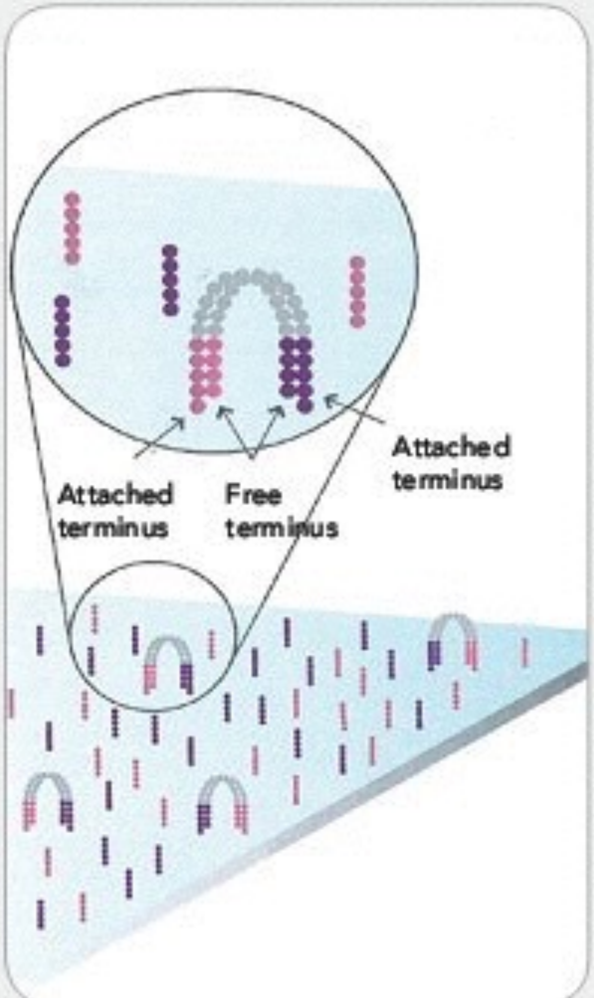
Bridge Amplification

- Unlabeled nucleotides and polymerase enzyme are added to initiate the solid phase bridge amplification

4. FRAGMENTS BECOME DOUBLE STRANDED



The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

Image retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

Fragments Become Double Stranded

- In this step it demonstrates the work done by the sequencing reagents
  - Primers
  - Nucleotides
  - Polymerase enzymes
  - Buffer

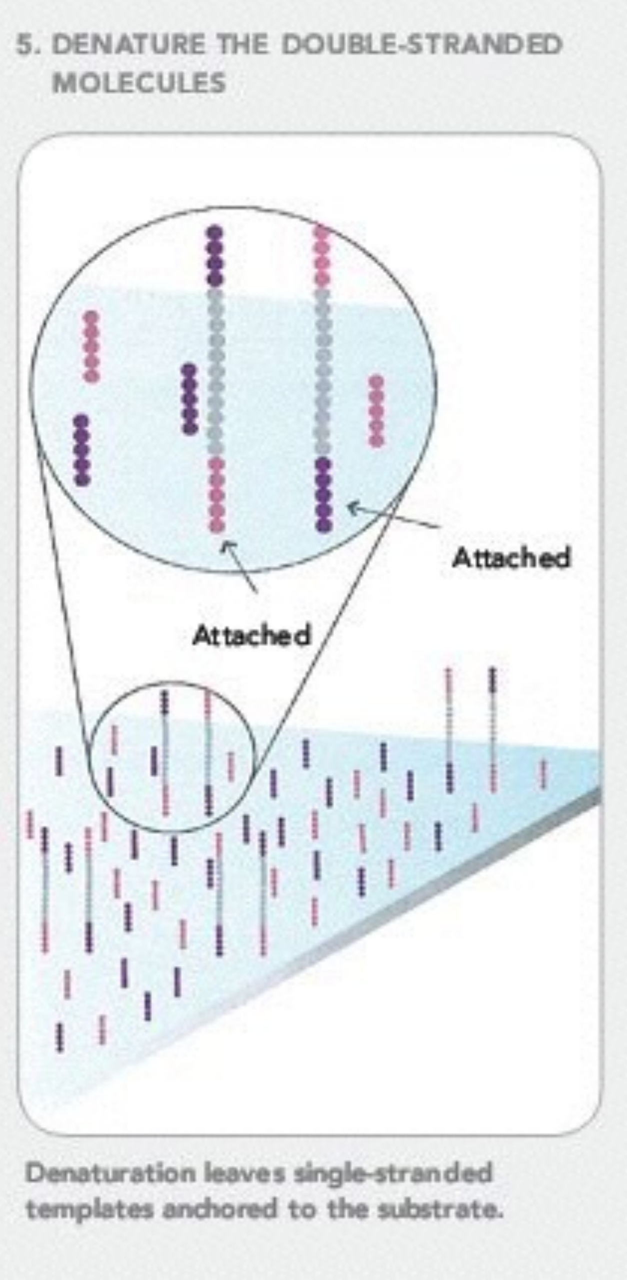
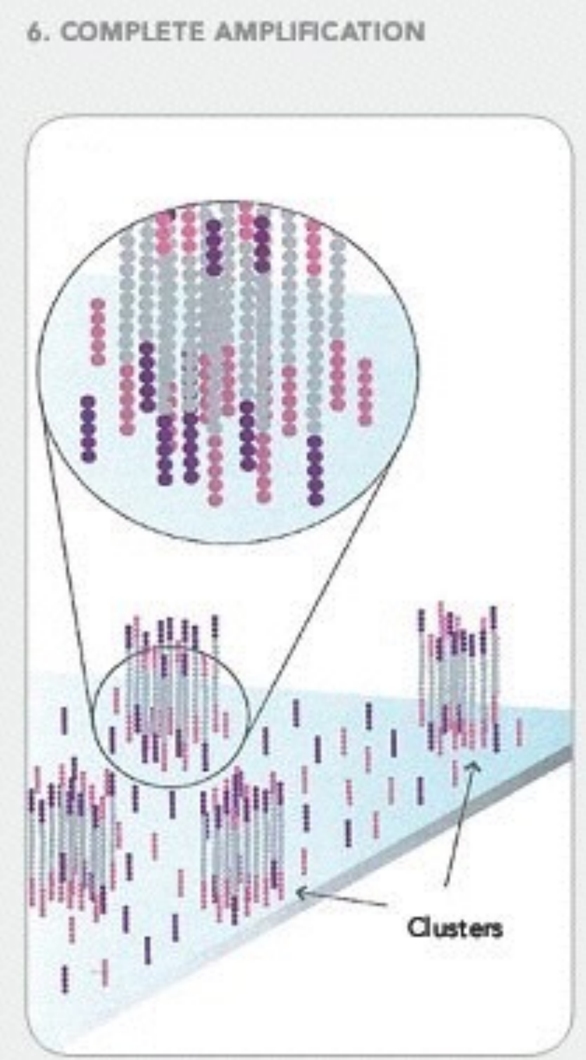


Image retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

Denature the Double Stranded Molecules

- The original strand is then washed away, leaving only the strands that had been synthesized to the oligos attached to the flow cell

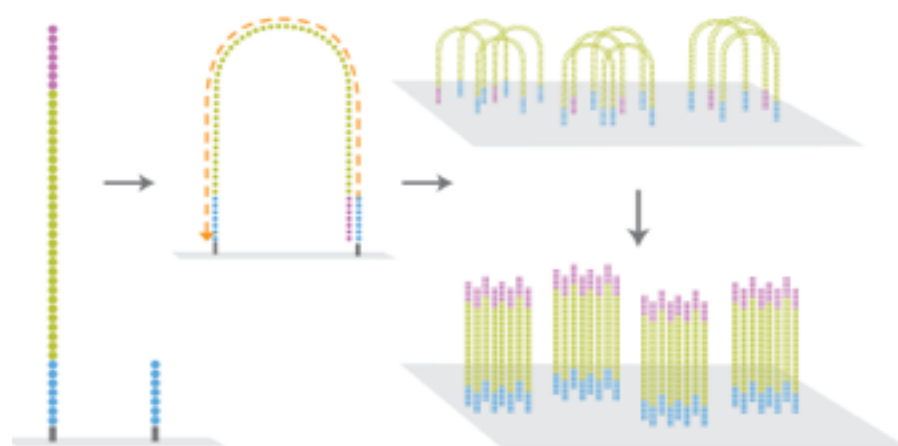
Steps 5-7 Repeats



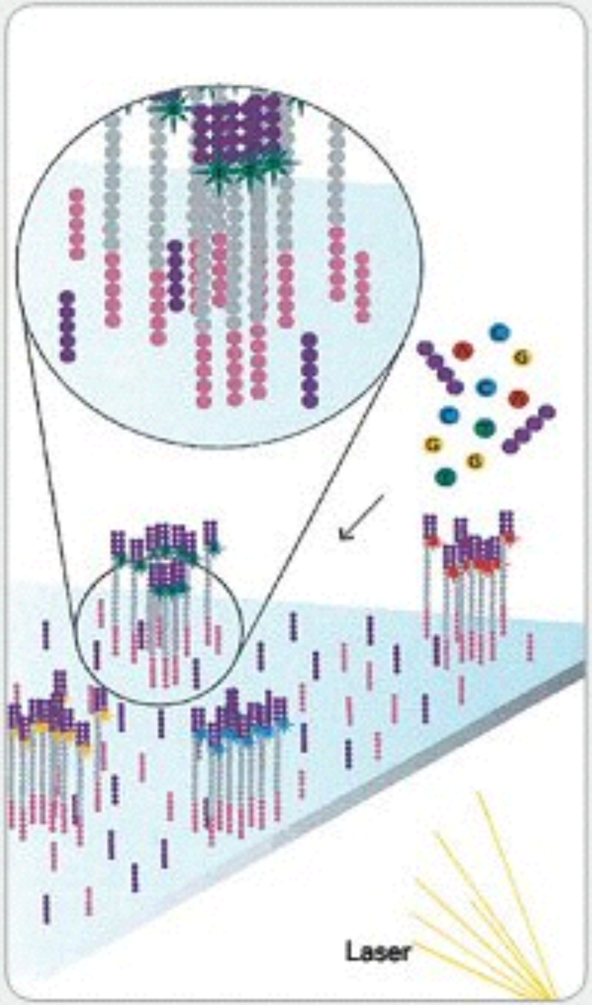
Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

Image retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

- Cycle of new strand synthesis and Denaturation to make multiple copies of the same sequence (amplification)
  - Fragments Become Double Stranded
  - Denature the Double Strand Molecules



7. DETERMINE FIRST BASE



First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

Determine First Base

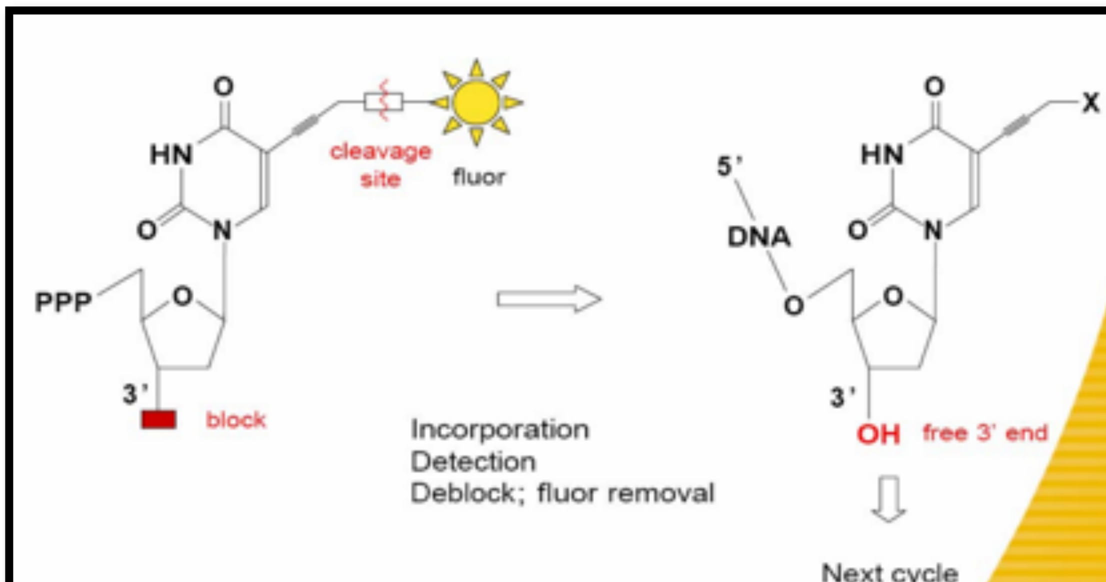
- The P5 region is cleaved
- Add sequencing reagents
  - Primers
  - Polymerase
  - Fluorescently labeled nucleotides
  - Buffer
- First base incorporated

Image retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

Image First Base

Image retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

- Remove unincorporated bases
- Detect Signal
- Deblock and remove the fluorescent signal →new cycle



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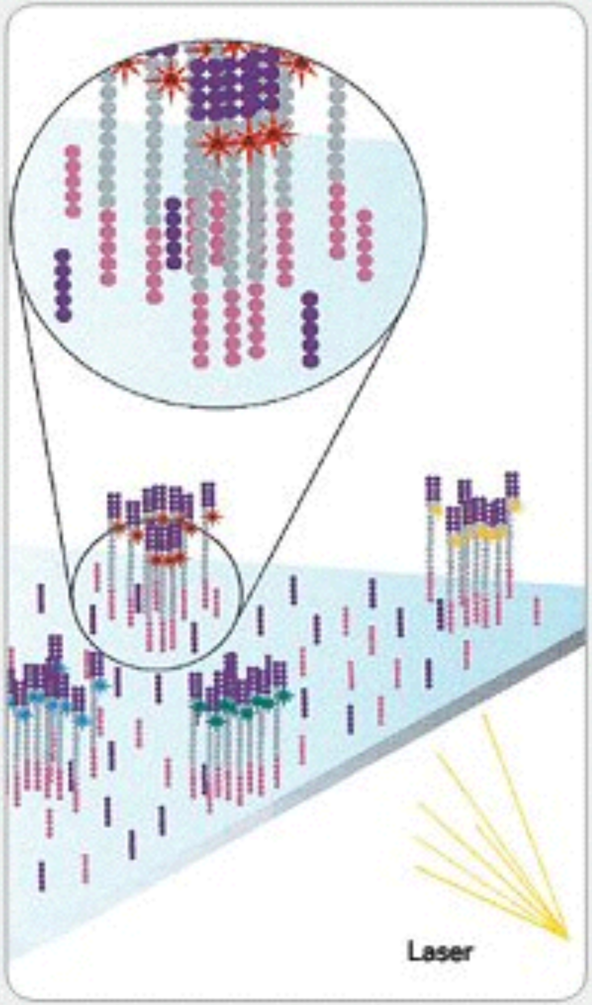


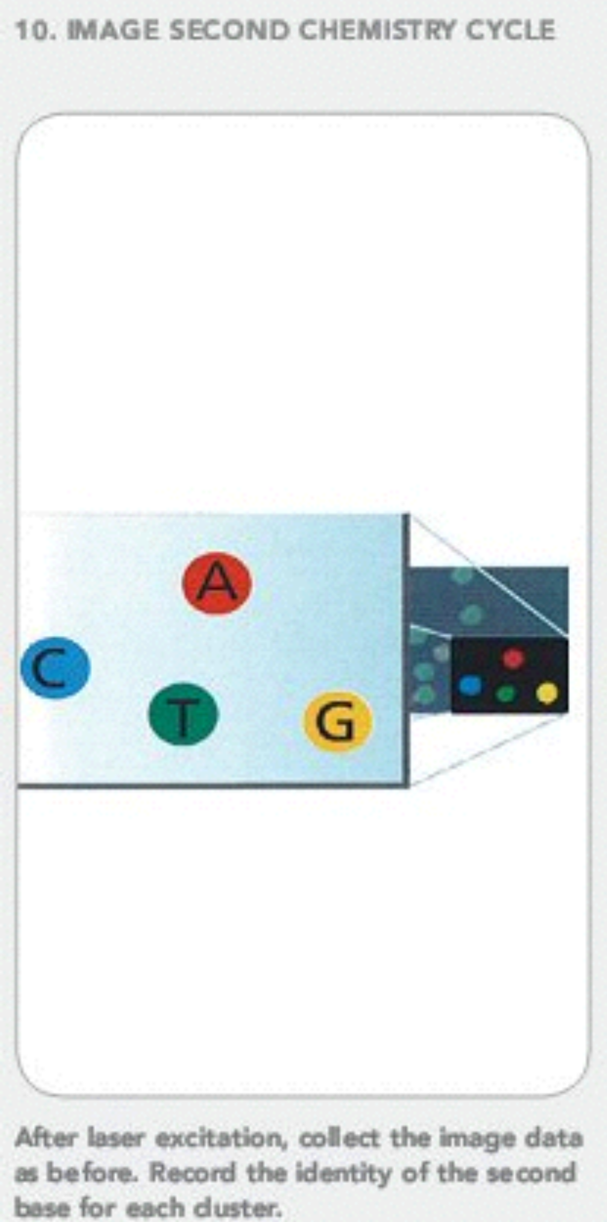
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

Determine Second Base

- Add sequencing reagents
    - Primers
    - Polymerase
    - Fluorescently labeled nucleotides
    - Buffer
- Second base incorporated

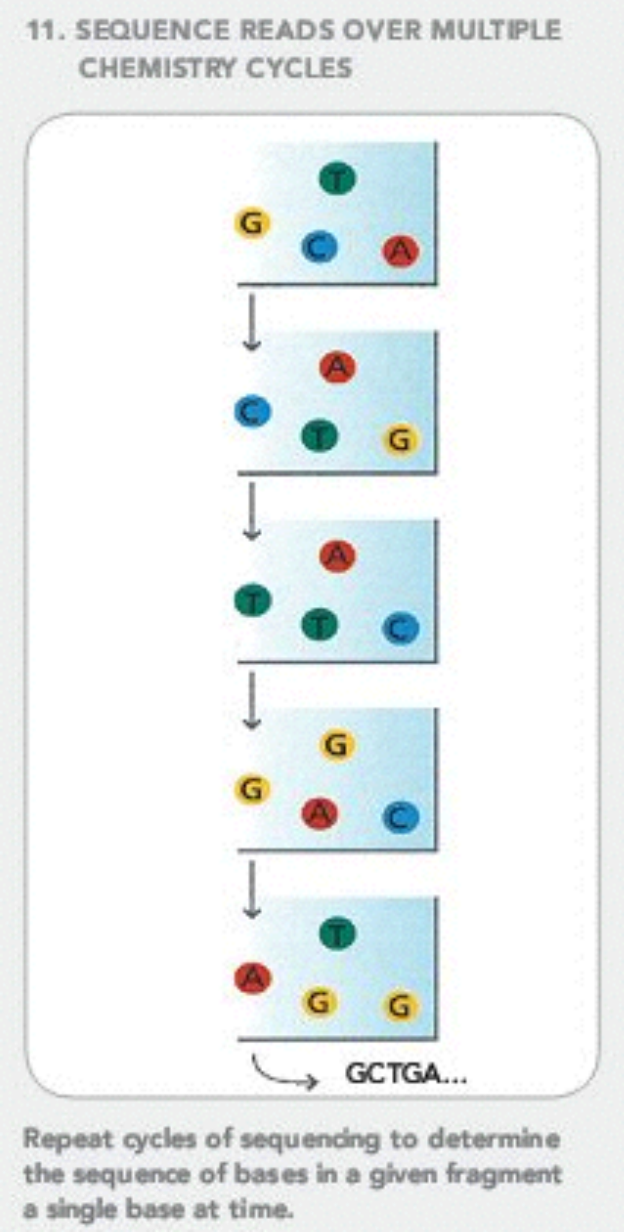
Image retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

Image Second Chemistry Cycle



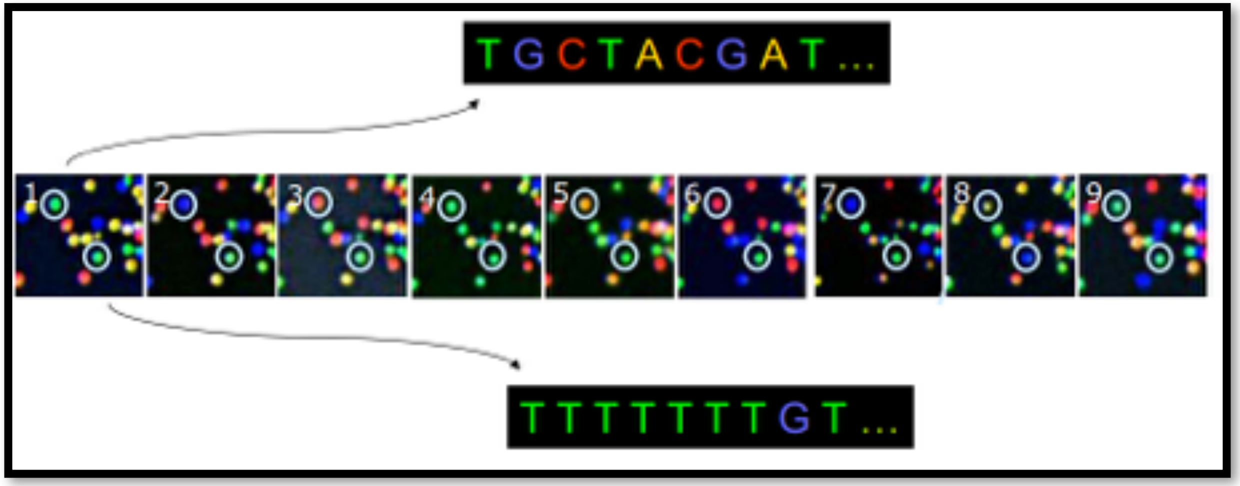
- Remove unincorporated bases
- Detect Signal
- Deblock and remove the fluorescent signal → new cycle

Image retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf



Sequence Reads Over Multiple Chemistry Cycles

- The identity of each base of a cluster is read off from sequential images



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