

Next Generation Sequencing

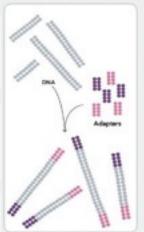
Illumina Sequencing

NOTE: These slides are taken from

http://www.slideshare.net/USDBioinformatics/illumina-sequencing

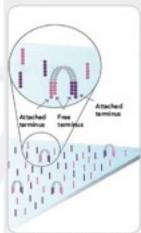
Illumina Diagram





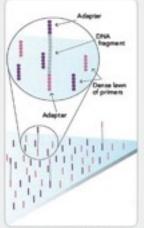
Randomly fragment genomic DNA. and lights adapters to both ends of the

4. FRASMENTS BECOME DOUBLE



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

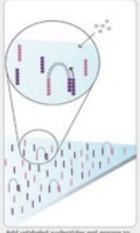
2. ATTACH DNA TO SURFACE



Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

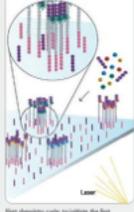
5. DENATURE THE DOUBLE-STRANDED

3. BRIDGE AMPLIFICATION



Add unlabeled nudeotides and enzyme to initiate solid-phase bridge amplification.

6. COMPLETE AMPLIFICATION



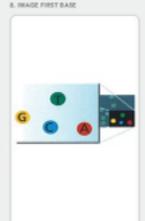
7. DETERMINE FIRST BASE

First chamistry cycle: to initiate the first sequencing cycle, add all four labeled revenible terminators, primers and CNA polymerase anapene to the flow cell.

10. MAGE SECOND CHEMISTRY CYCLE

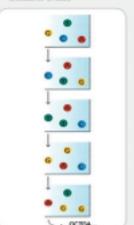


After leser excitation, collect the image data as before. Record the identity of the second



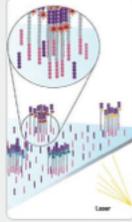
After laser excitation, capture the image of enitted fluorescence from each sluster on the flow cell. Record the identity of the first bese-

11. SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES



Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at time.

9. DETERMINE SECOND BASE

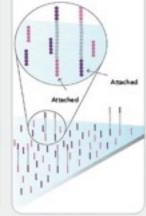


Second chemistry cycle: to initiate the next sequencing cycle, add all four falseled reverable terminators and evapme to the

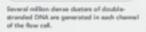
12. ALIGN DATA



Align data, compare to a reference, and identify sequence differences.

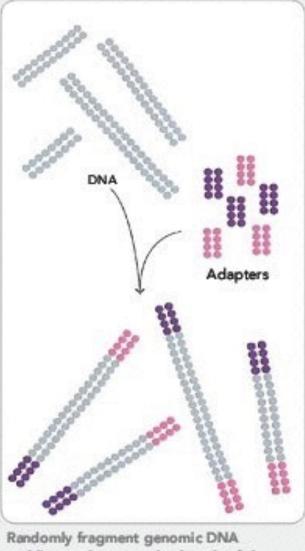


Denaturation leaves single-stranded templates androved to the substrate.



Gusters

1. PREPARE GENOMIC DNA SAMPLE



Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

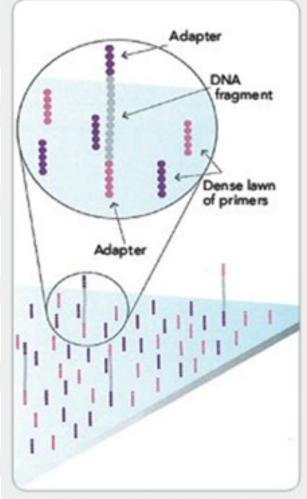
Image retrieved from http://res.illumina.com/
documents/products/techspotlights/
techspotlight_sequencing.pdf

Prepare Genomic DNA Sample



- 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

2. ATTACH DNA TO SURFACE



Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

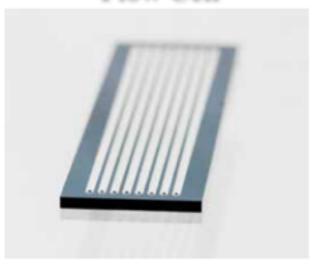
Images retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

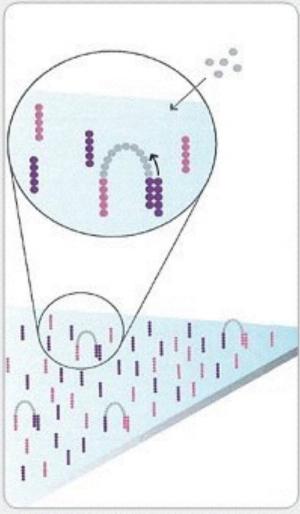
Attach DNA to Surface



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

Flow Cell





Add unlabeled nudeotides and enzyme to initiate solid-phase bridge amplification.

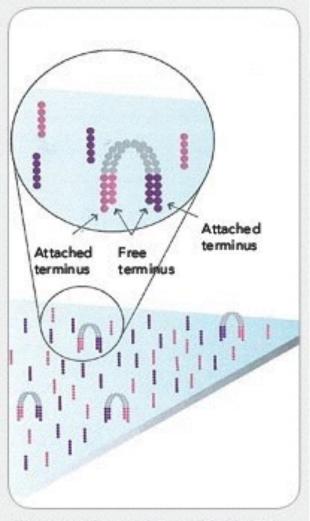
Image retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

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 solidphase substrate.

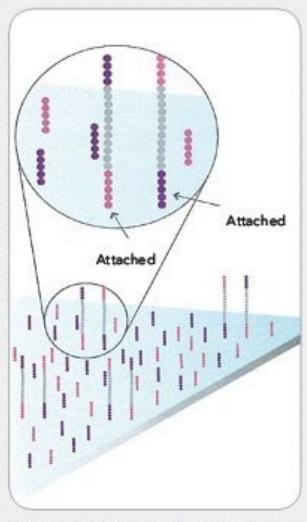
Image retrieved from http://res.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf

Fragments Become Double Stranded



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

5. DENATURE THE DOUBLE-STRANDED MOLECULES



Denaturation leaves single-stranded templates anchored to the substrate.

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

Denature the Double Stranded Molecules

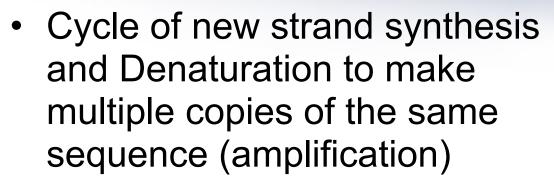


 The original strand is then washed away, leaving only the strands that had been synthesized to the oligos attached to the flow cell

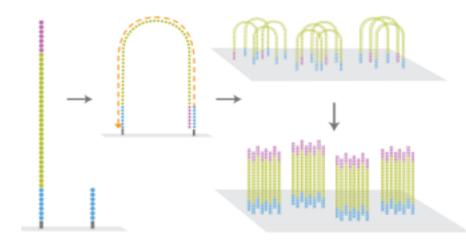
Several million dense dusters of doublestranded DNA are generated in each channel of the flow cell.

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

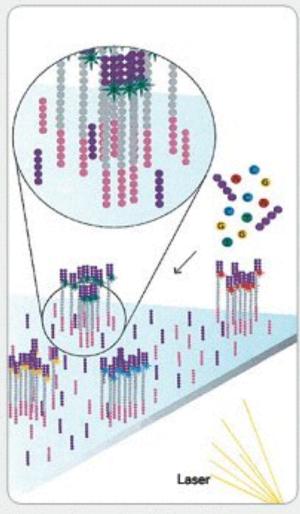
Steps 5-7 Repeats



- Fragments Become Double Stranded
- Denature the Double Strand Molecules



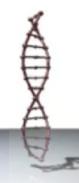
7. DETERMINE FIRST BASE



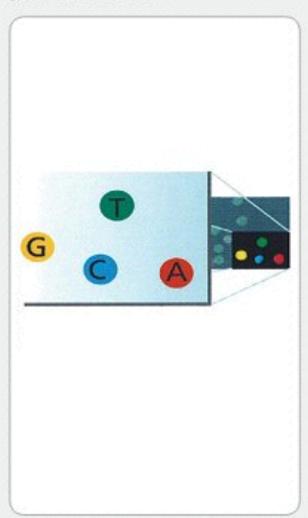
First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

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

Determine First Base



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



After laser excitation, capture the image of emitted fluorescence from each duster on the flow cell. Record the identity of the first base for each duster.

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

Image First Base



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

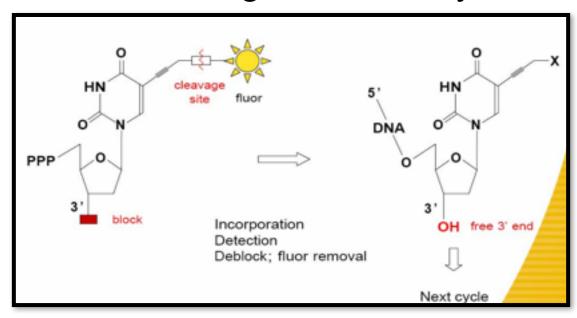
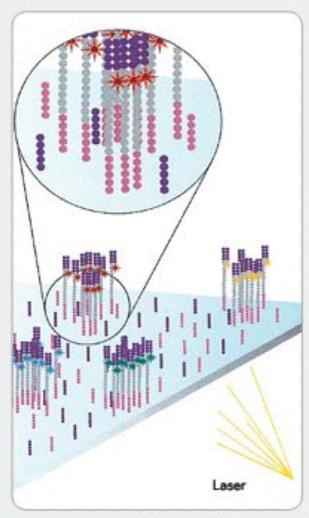


Image retrieved from http://research.stowers-institute.org/microscopy/external/
PowerpointPresentations/ppt/Methods_Technology/
KSH_Tech&Methods_012808Final.pdf

9. DETERMINE SECOND BASE



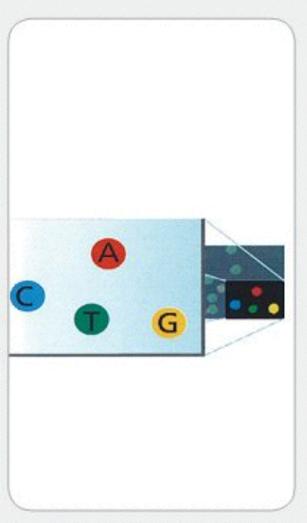
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

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

Determine Second Base



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



After laser excitation, collect the image data as before. Record the identity of the second base for each duster.

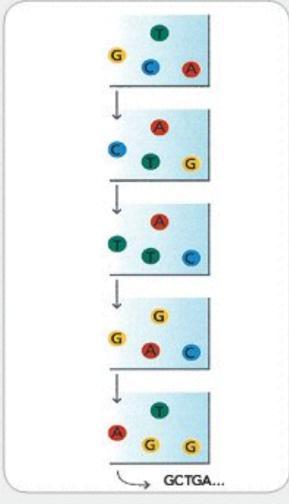
Image retrieved from http://res.illumina.com/
documents/products/techspotlights/
techspotlight_sequencing.pdf

Image Second Chemistry Cycle



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

11. SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES



Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at time. Sequence Reads Over Multiple Chemistry Cycles

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

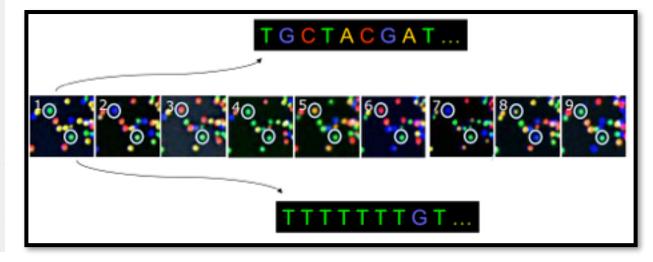


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PowerpointPresentations/ppt/Methods Technology/KSH Tech&Methods 012808Final.pdf

Work Cited



- Illumina Sequencing Technology. (2010). In Techonlogy Spotlight: Illumina Sequencing. Retreived July 30, 2014, from http://res.illumina.com/documents/products/techspotlights/techspotlightsequencing.pdf
- Illumina Solexa Sequencing. (Jan 22, 2010). On YouTube uploaded by Draven1983101.
 Retrieved July 30, 2014, from https://www.youtube.com/watch?v=77r5p8IBwJk
- Overview of Illumina Chemistry. In Massachusetts General Hospital. Retreived July 30, 2014, from http://nextgen.mgh.harvard.edu/IlluminaChemistry.html
- Introduction to Next Generation Sequencing Using the Illumina 1G Genome Analyzer (Solexa). (Jan 31, 2008). Retrieved July 30, 2014, from http://research.stowers-institute.org/microscopy/external/PowerpointPresentations/ppt/Methods_Technology/KSH_Tech&Methods_012808Final.pdf
- Sequencing technology Past, Present and Future. (2013). Wei Chen. Berlin Institute for Medical Systems Biology. Max-Delbrueck-Center for Molecular Medicine Retrieved July 30, 2014, from http://www.molgen.mpg.de/899148/OWS2013_NGS.pdf
- DNA barcoding. (2014, July 30). In *Wikipedia, The Free Encyclopedia*. Retrieved July 30, 2014, from http://en.wikipedia.org/w/index.php?title=DNA_barcoding&oldid=619163634
- Reference genome. (2014, June 22). In Wikipedia, The Free Encyclopedia. Retrieved 19:54, August 4, 2014, from http://en.wikipedia.org/w/index.php?
 title=Reference genome&oldid=613984719