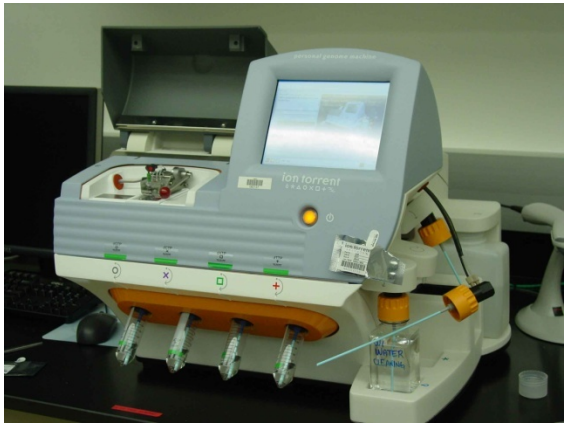
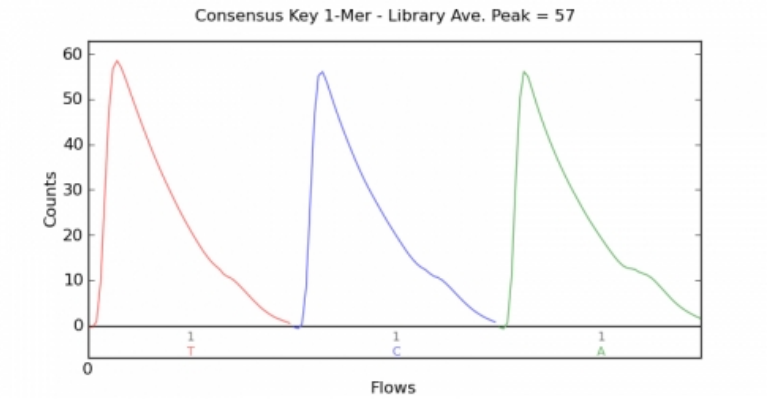
BINF 6350 ITSC 8350
Fall 2011
Biotechnology & Genomics Lab
Creating DNA libraries

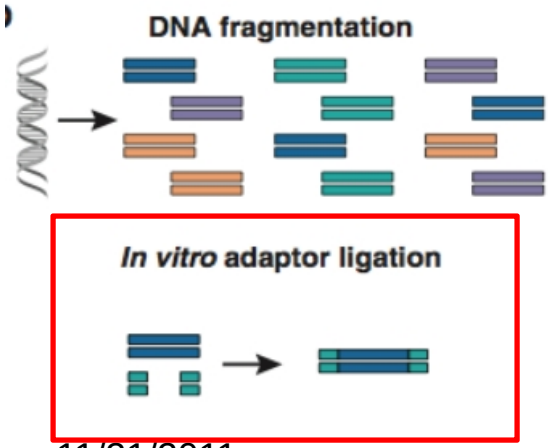
http://webpages.uncc.edu/~jweller2

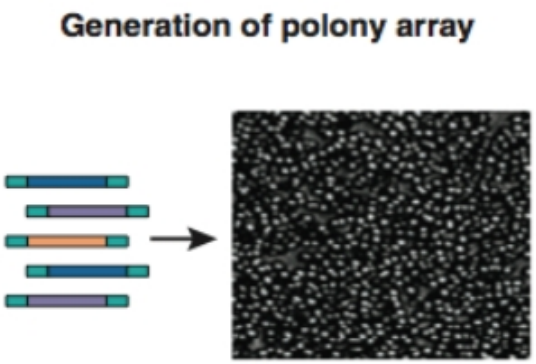


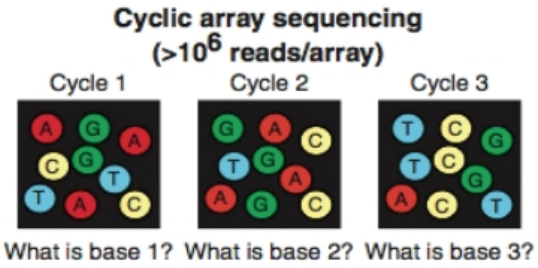


NGS data production steps

- Produce the fragments to be sequenced, clean up
- Modify fragments for processing, clean up
- Separate the modified fragments *from each other*
  - so they don't compete in the PCR process: emulsion reactors, dispersed on a surface
- Amplify each fragment to a base mass
- Sequence-by-synthesis (flowgrams, PPi with reversible terminators, etc.)
- Analyze

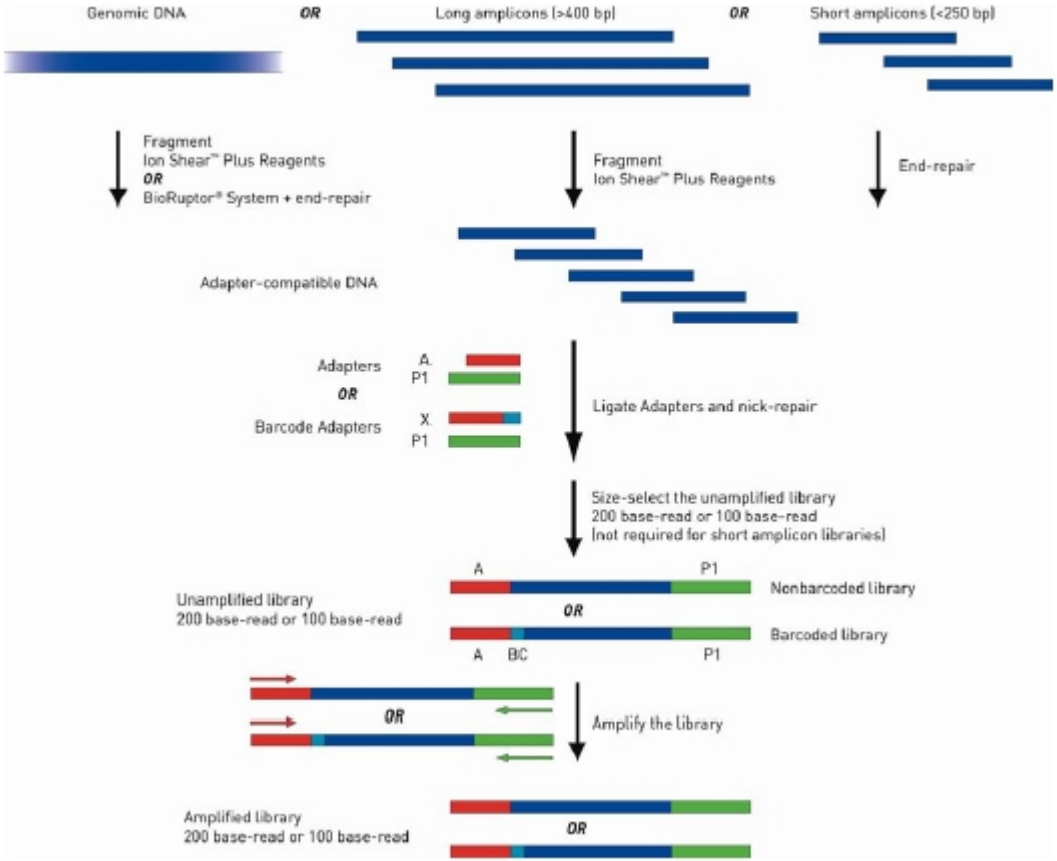






Shendure and Ji, Nat Biotech, 2008

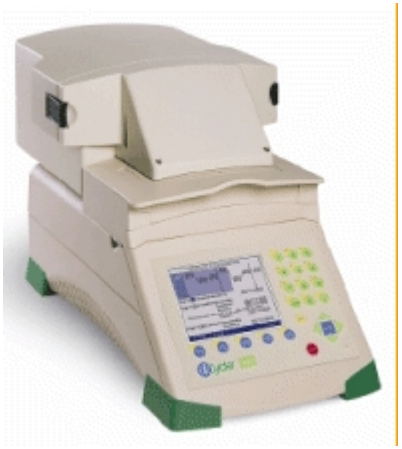
NGS libraries



Shendure and Ji, Nat Biotech, 2008

QC Of input library

- How do you know if the adaptors have ligated to your product?
- You can perform qPCR - but you need a control fragment.
  - Titrate the fragment with the other reagents
  - Use SYBR Green as the detection standard.
  - Include a DNA polymerase that is SYBR Green tolerant.
- qPCR will only amplify products with adaptors on both ends
- If you want to check the level of pure adaptor dimers you can do a melt phase as well.



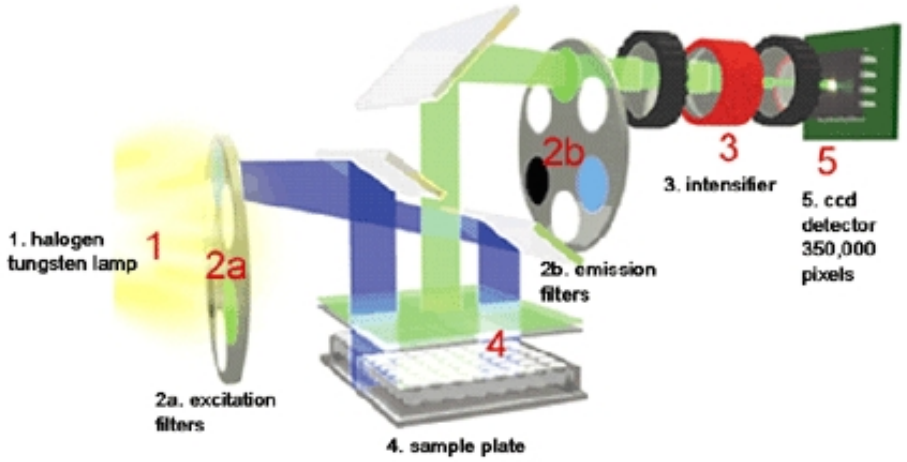
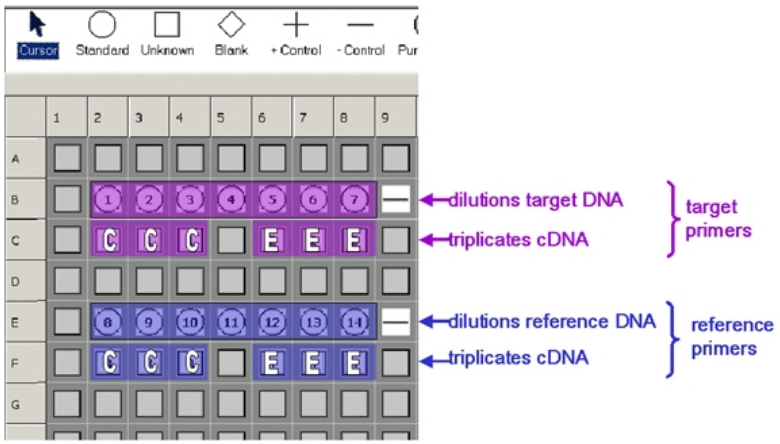
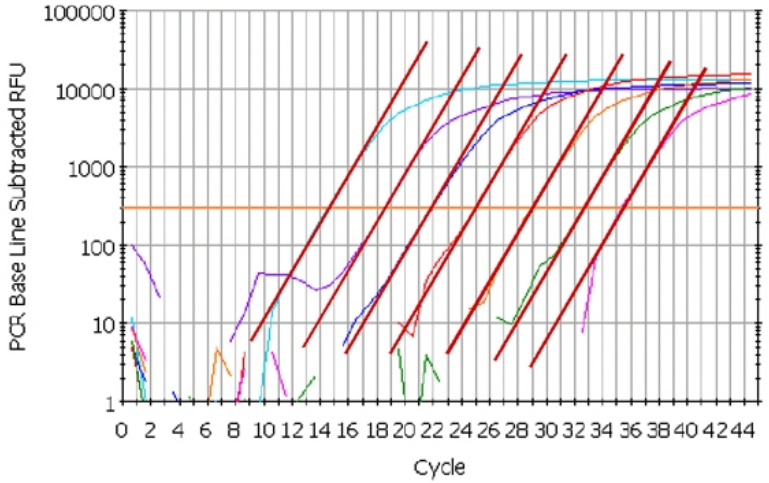


Fig. 1.2. Representation of Optical Detection System layout.

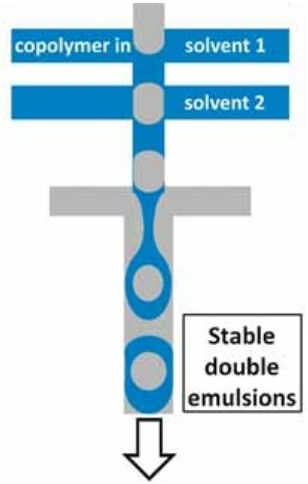


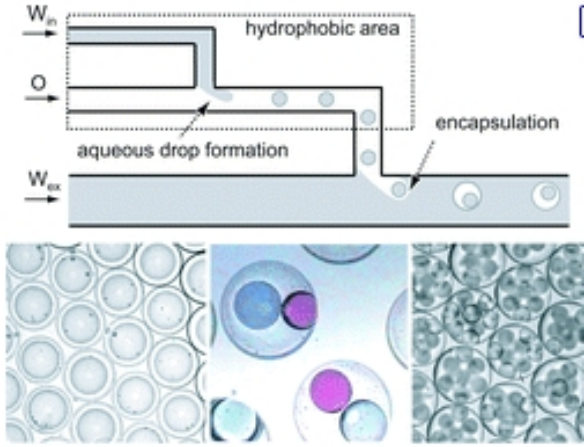
Standard curve method

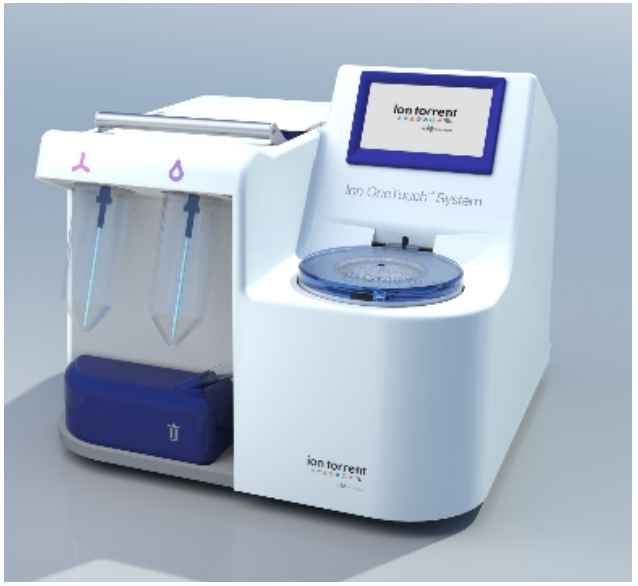


SERIES OF 10-FOLD DILUTIONS

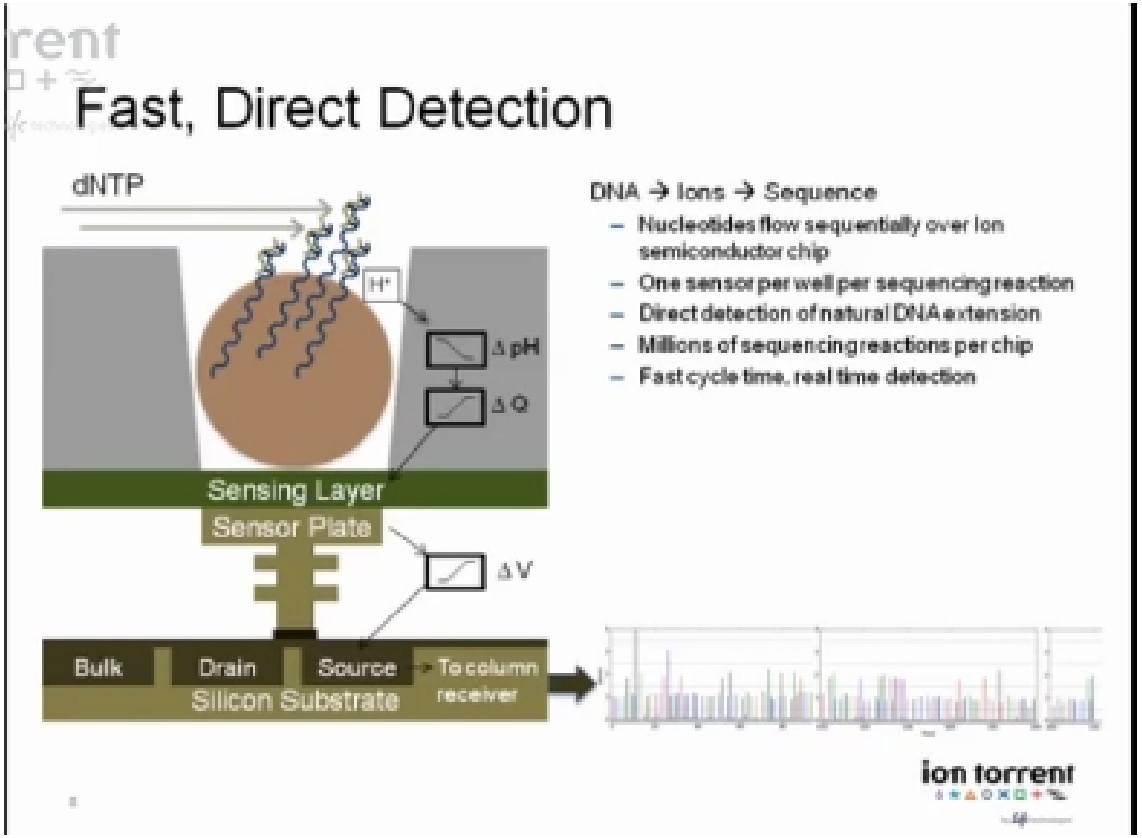
Amplifying the library – Emulsion Reactors





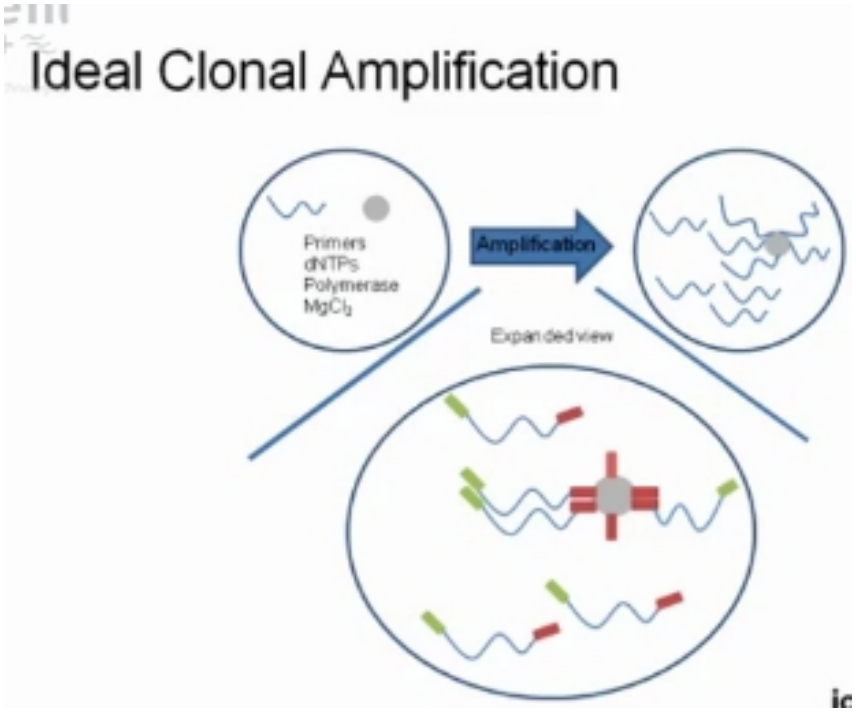


Ion Torrent sequencing chemistry

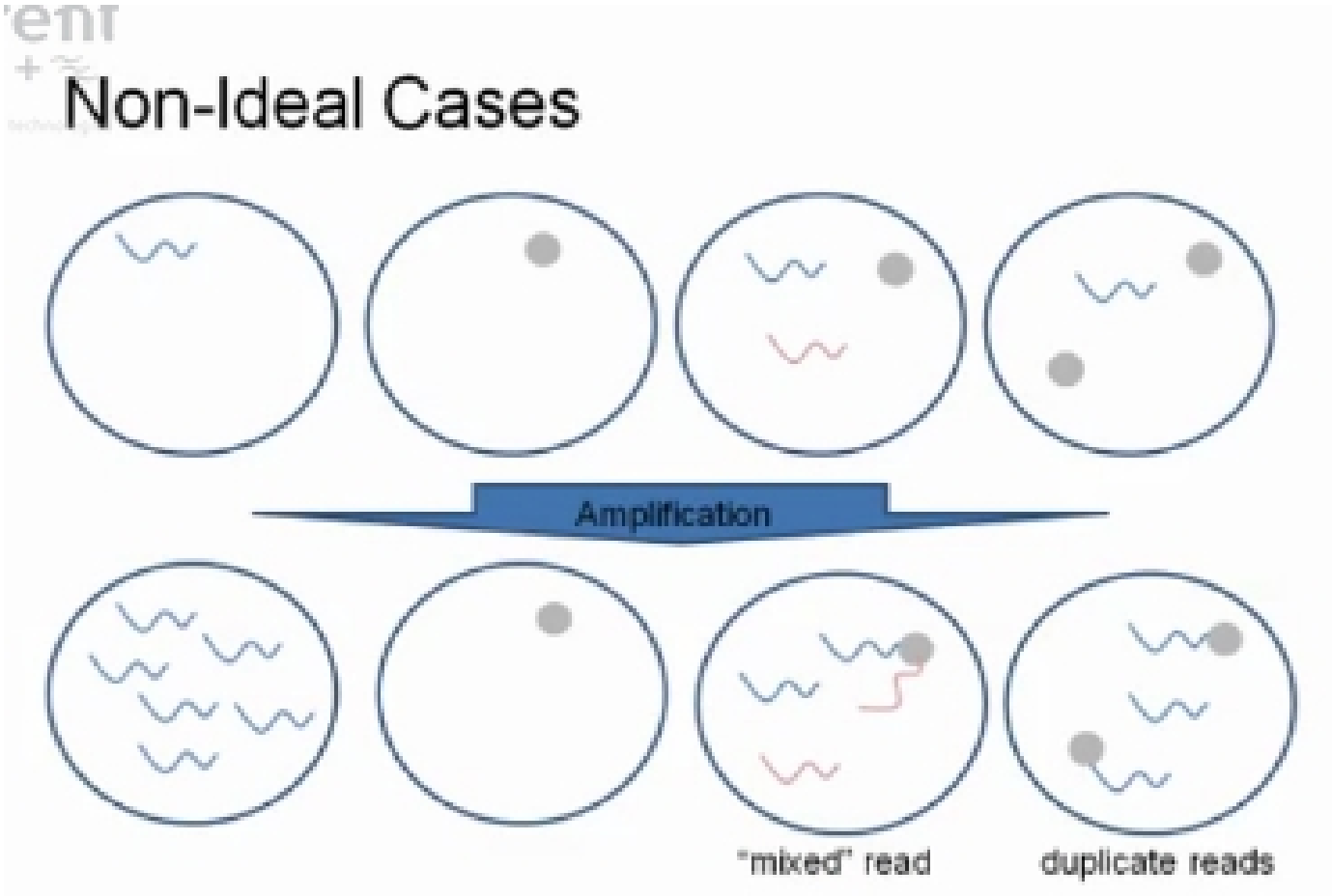


Emulsion and PCR

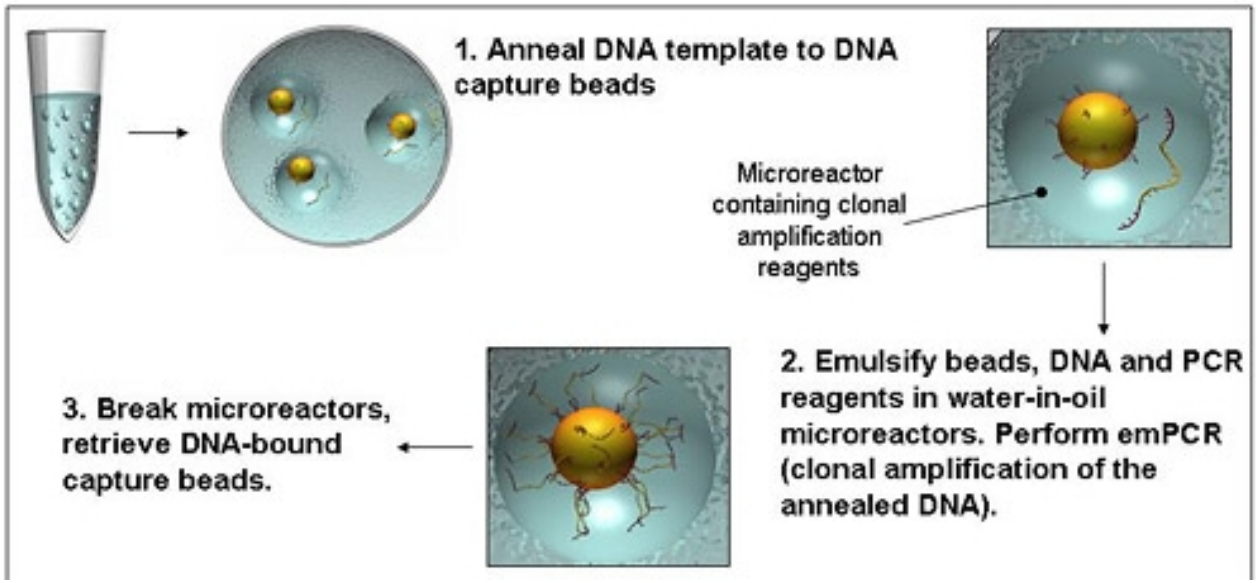
- emPCR



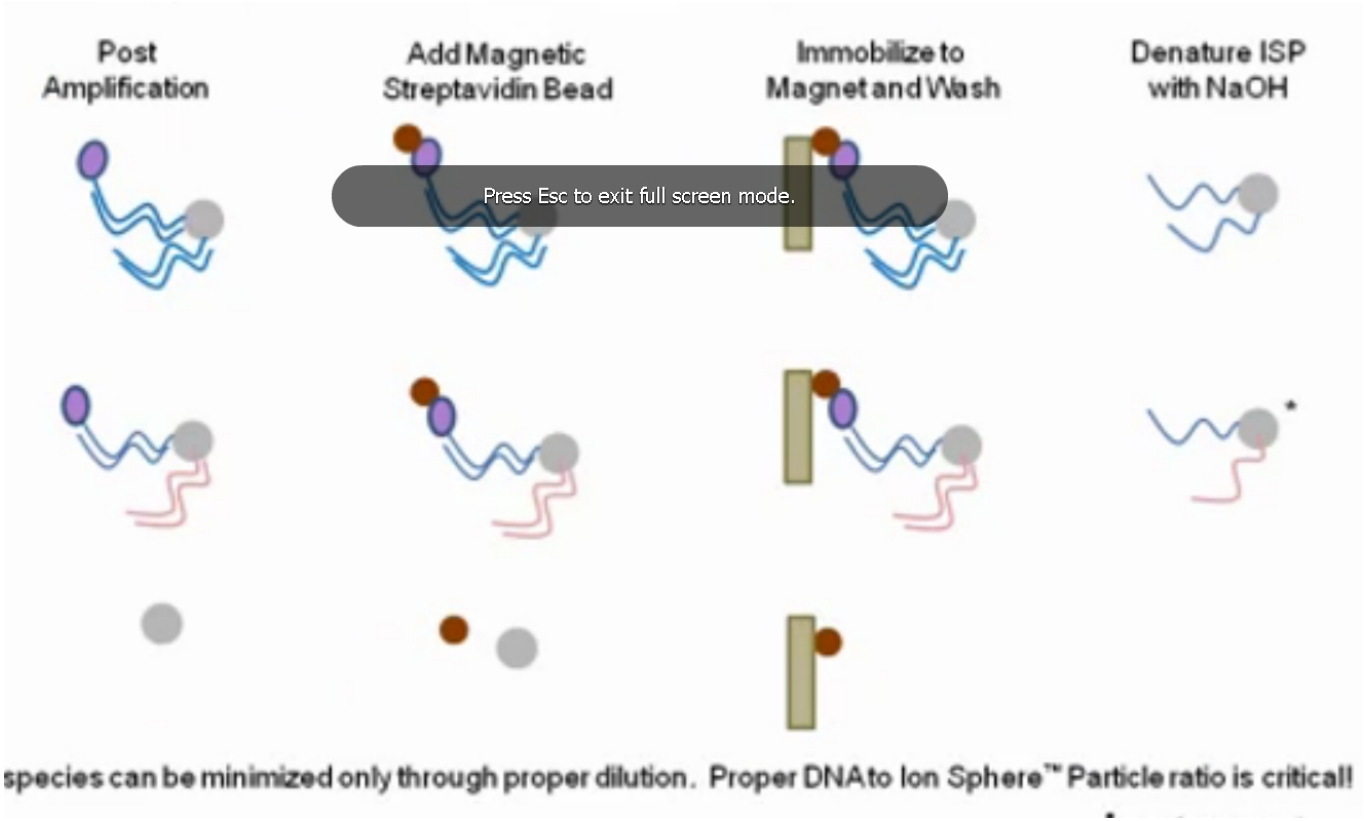
Non-Ideal Cases



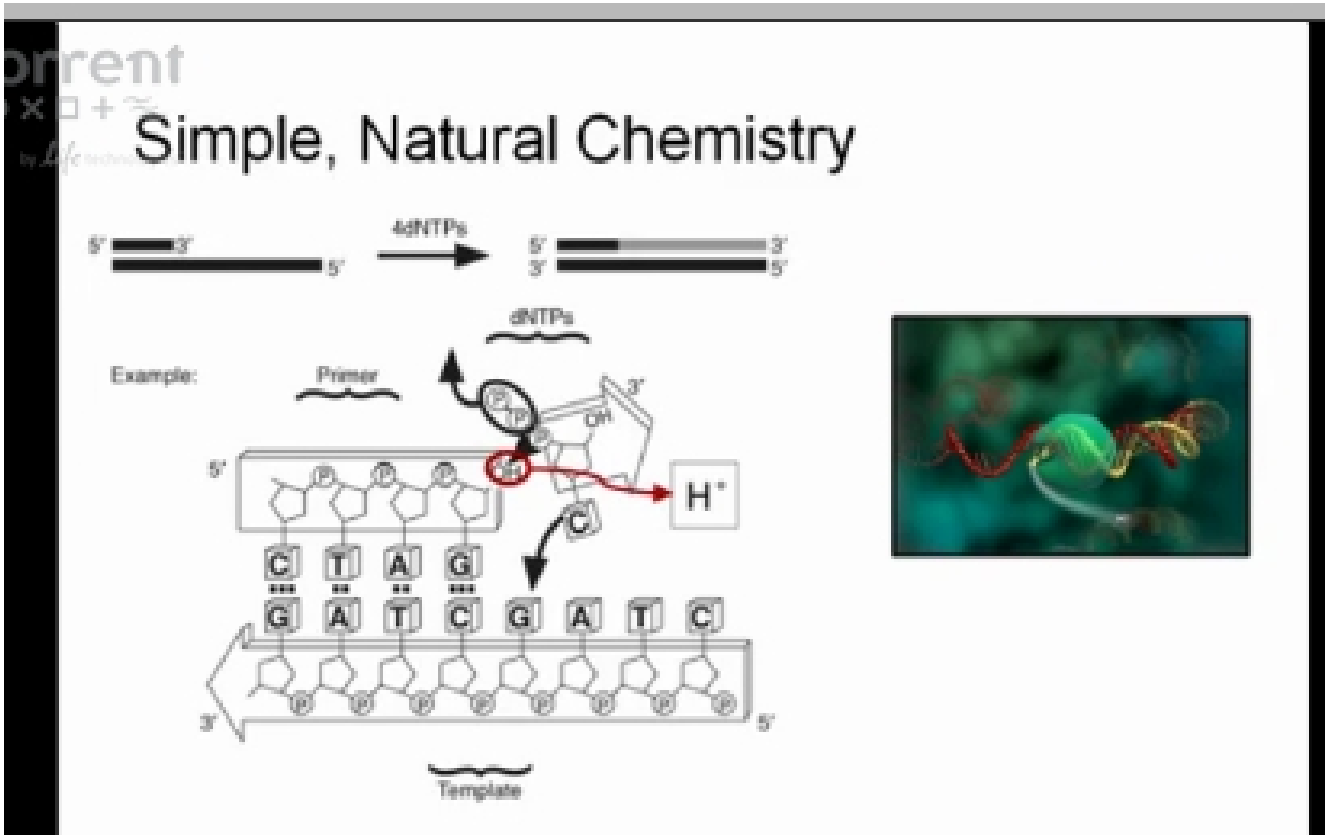
emPCR


1. Anneal DNA template to DNA capture beads
Microreactor containing clonal amplification reagents
2. Emulsify beads, DNA and PCR reagents in water-in-oil microreactors. Perform emPCR (clonal amplification of the annealed DNA).
3. Break microreactors, retrieve DNA-bound capture beads.

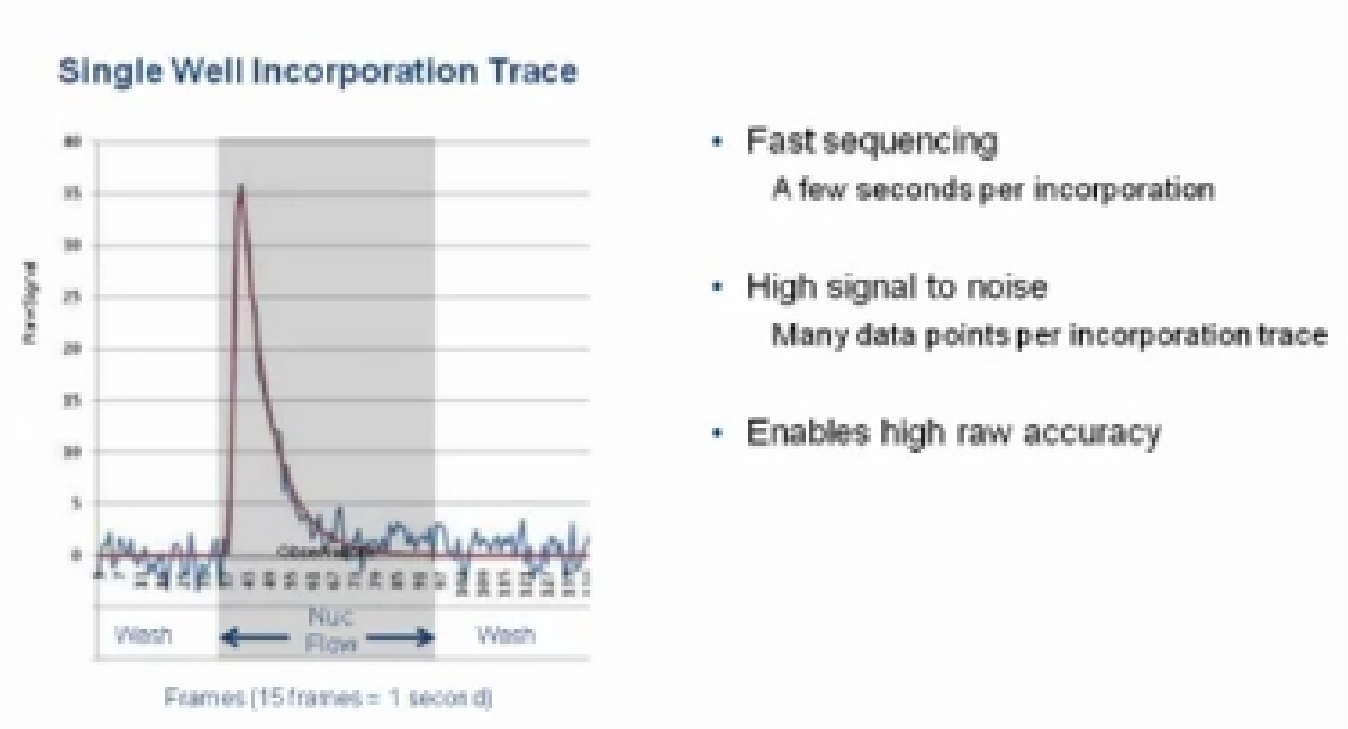
Enrichment


Post
Amplification
Add Magnetic
Streptavidin Bead
Immobilize to
Magnet and Wash
Denature ISP
with NaOH
Press Esc to exit full screen mode.
species can be minimized only through proper dilution. Proper DNA to Ion Sphere™ Particle ratio is critical!

Detection and flowgrams



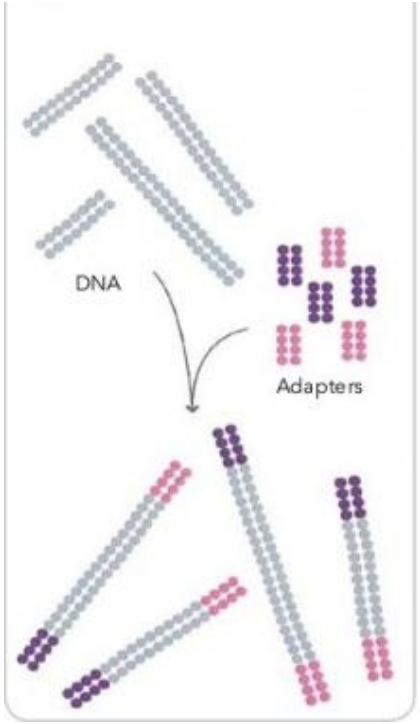
Signal characteristics

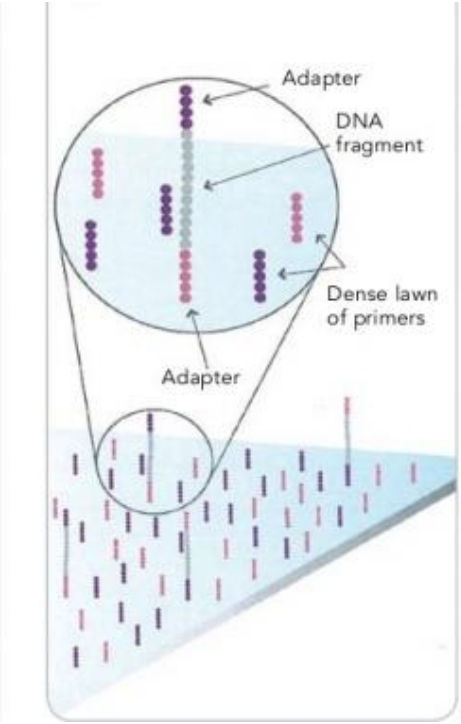


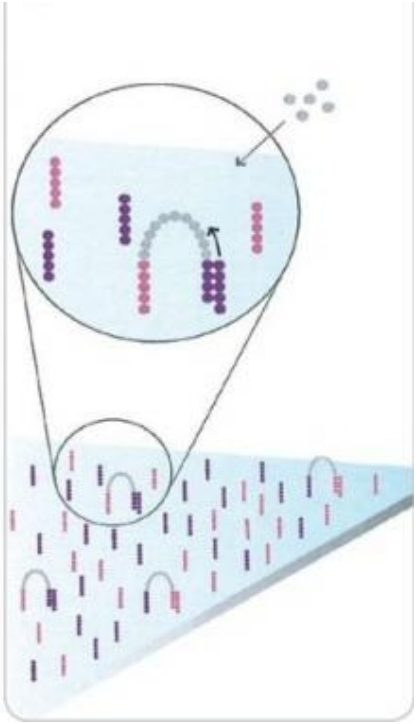
Other surface attachment methods

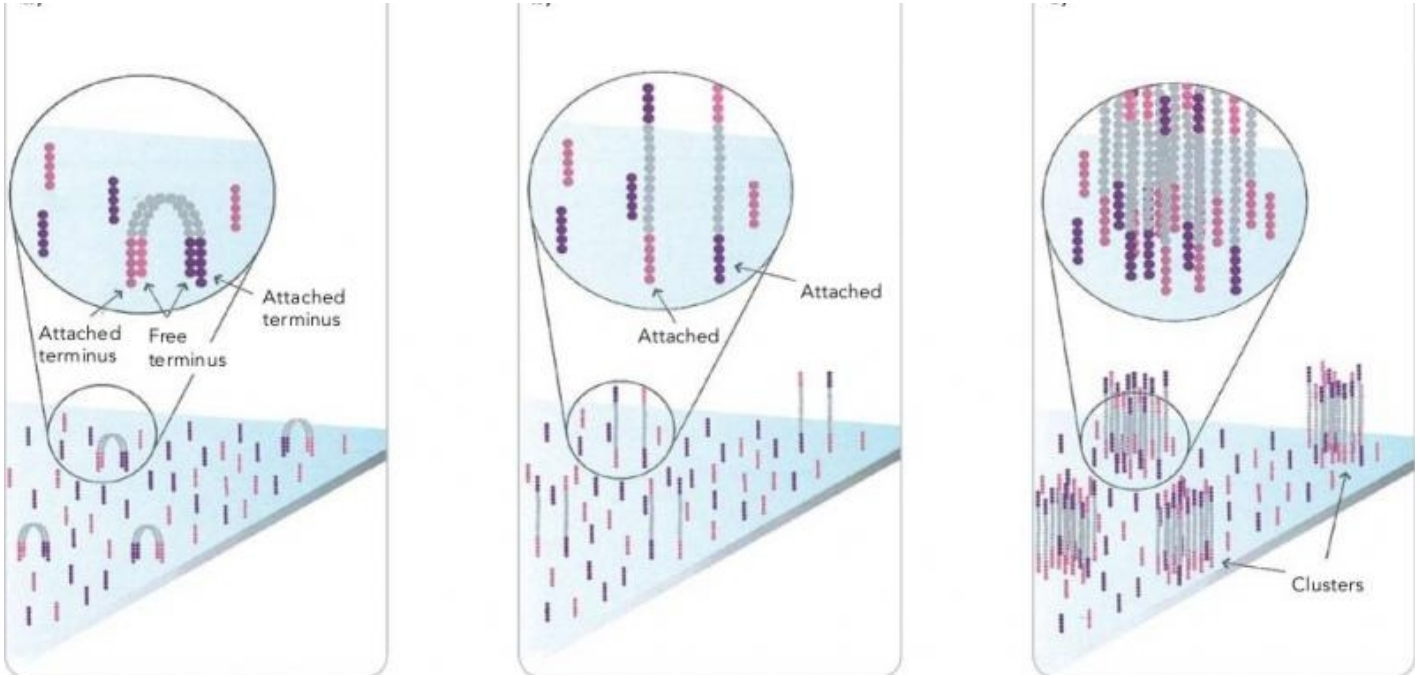
- Fragments are distributed randomly on a surface that is covered with DNA complementary to the adaptor sequences.
- The fragments are attached to the surface and then multiplied by bridge amplification
  - The form small clusters or ssDNA (because they don't diffuse very far).
  - Once the clusters are formed sequencing can begin
  - In Illumina, the nucleotides have reversible terminators and carry fluorescent dyes. After incorporation the spot is imaged, then the terminator is rnatpr is removed so along with the dye, extension can happen.

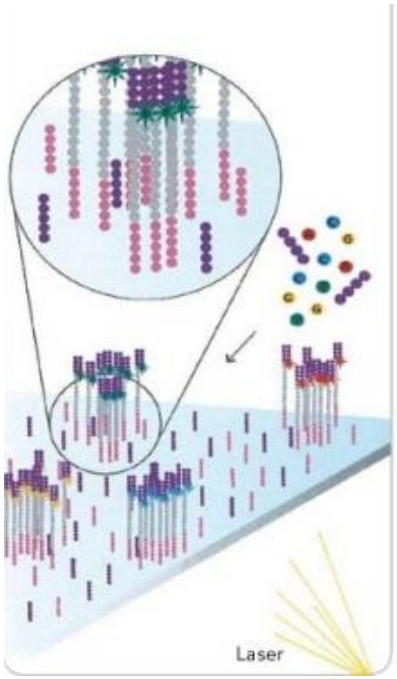
Illumina

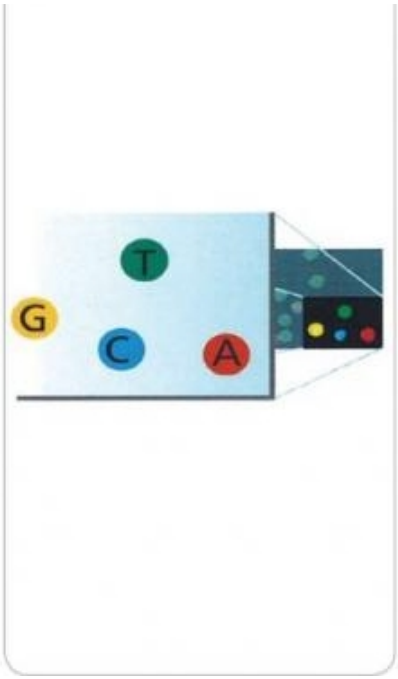

DNA
Adapters

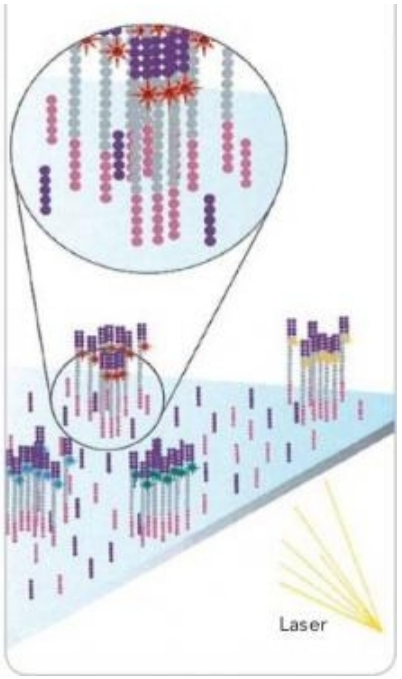

Adapter
DNA fragment
Dense lawn of primers
Adapter




Attached terminus
Attached terminus
Free terminus
Attached terminus
Attached
Attached
Clusters

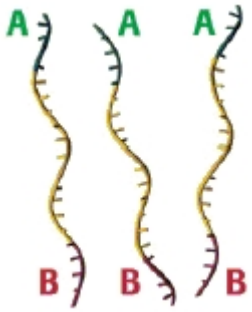

Laser

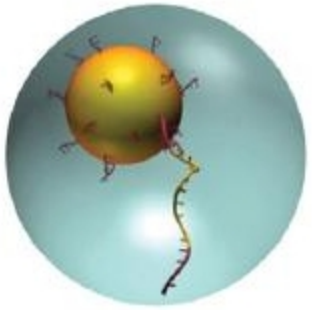
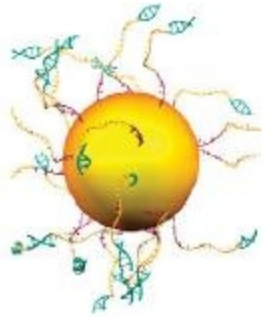
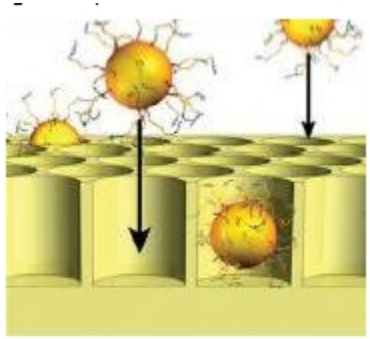

T
G
C
A


Laser

Roche 454 sequencing

- The GS FLX sequencer uses 454 chemistry and technology.
- Adaptors are added. One strand has a bitoin group, so it can be removed, leaving ssDNA behind
- The adaptor allows bead capture.
- A water-oil emulsion makes a microreactor so one target has a bead all to itself, which it colonizes.
- After several million-fold amplification the emulsion is broken.
- The beads are deposited in pico-lieter fiber-optic wells fore sequencing.
- The complementary strand is synthesized by adding the primer anmer and nuclecotides.
- An inorganic phosphate cleavage leads to an enzymatic cascade that releases light.
- Figures from Roche Diagnostics.



454 Chemistry (GS FLX)