

# NEXT GENERATION SEQUENCING

Summary

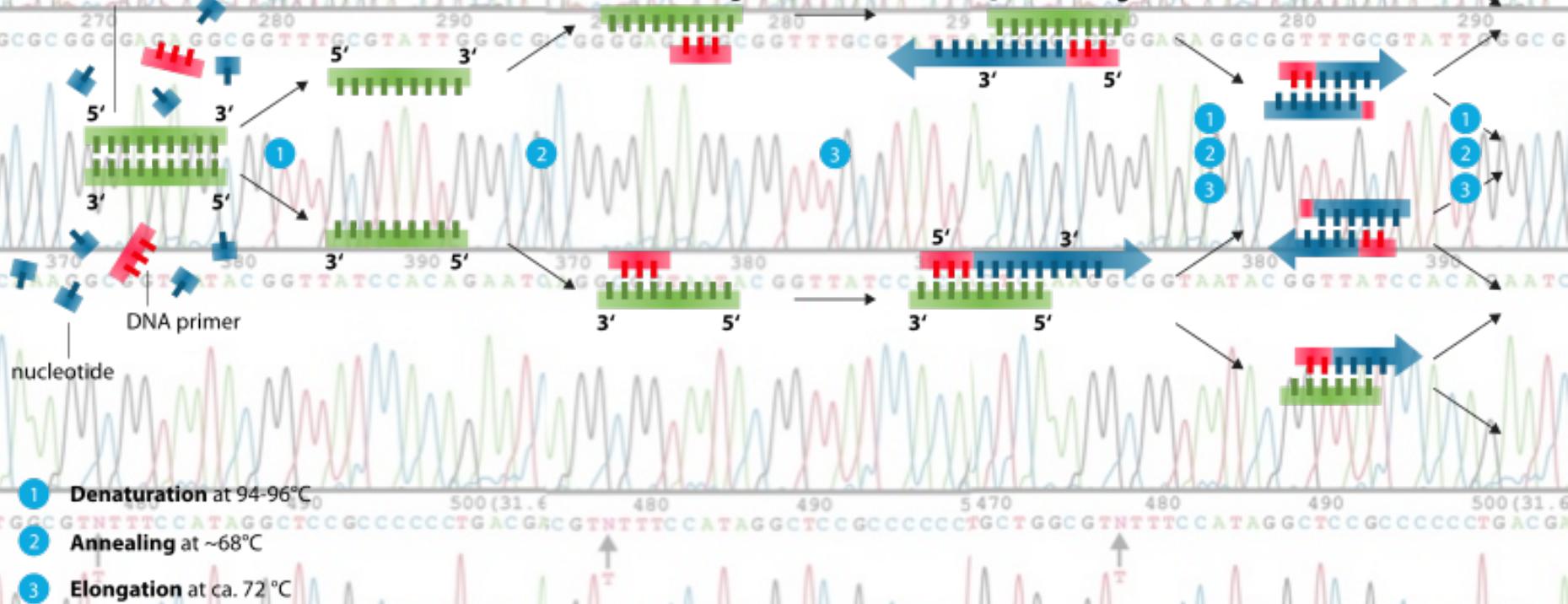
# Extracting DNA



# Remember what happens in PCR?

## Polymerase chain reaction - PCR

original DNA  
to be replicated



1 Denaturation at 94-96°C

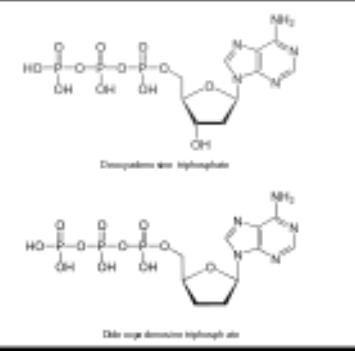
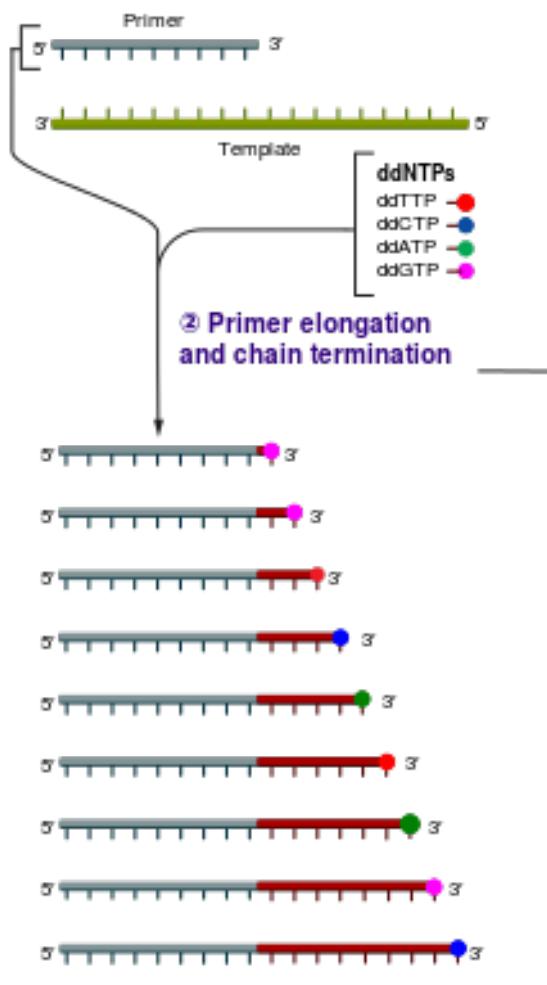
2 Annealing at ~68°C

3 Elongation at ca. 72 °C

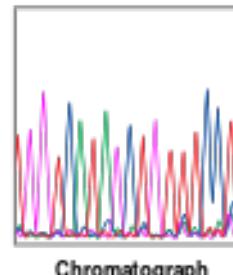
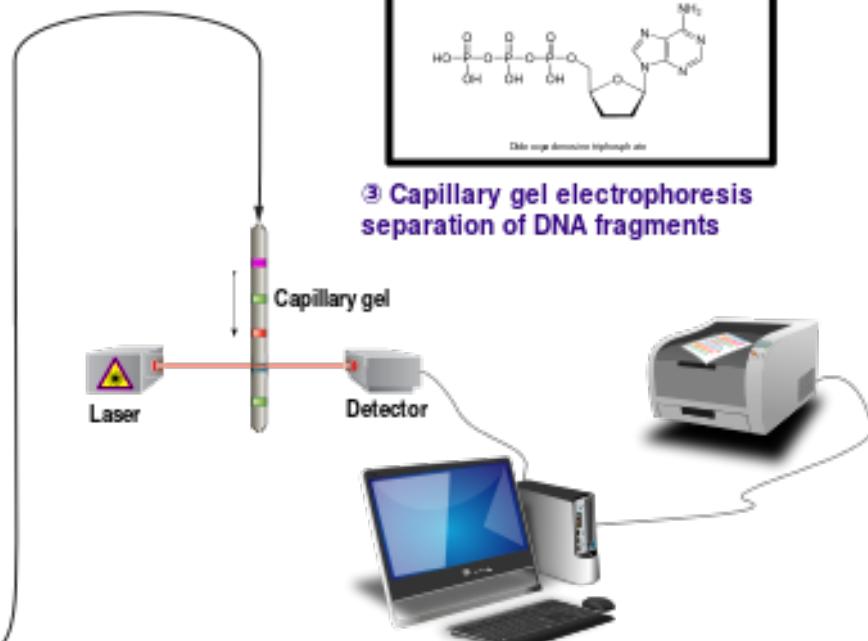
# Sanger Sequencing

## ① Reaction mixture

- Primer and DNA template ► DNA polymerase
  - ddNTPs with flourochromes ► dNTPs (dATP, dCTP, dGTP, and dTTP)



### ③ Capillary gel electrophoresis separation of DNA fragments



# Why do we use NGS?

Community Amplicon sequencing

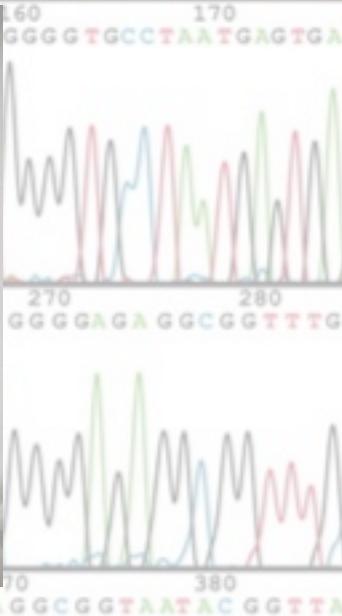
Metagenomes

Whole genome sequencing

Transcriptome sequencing

# NGS Platforms

Ion Torrent PGM



454 Life Sciences

Illuminaria MiSeq



# Which one?

Platform	Capacity	Speed	Read Length	Homopolymers	Cost/ run
454 Roche	450 - 700 Mb	10-23 hours	400-700bp	-	5000 euros
Illumina	15 Gb - 850 MB	5.5 - 65 hours	100-250bp	+	15000 euros
Ion Torrent	1.2-2 Gb	4-5 hours	200-400bp	-	1500 euros

# Workflow

454 Life Sciences  
Library preparation



emPCR



Pyrosequencing

Illumina  
Library preparation



Bridge Amplification



Reversible  
Termination  
Sequencing

Ion Torrent PGM  
Library preparation

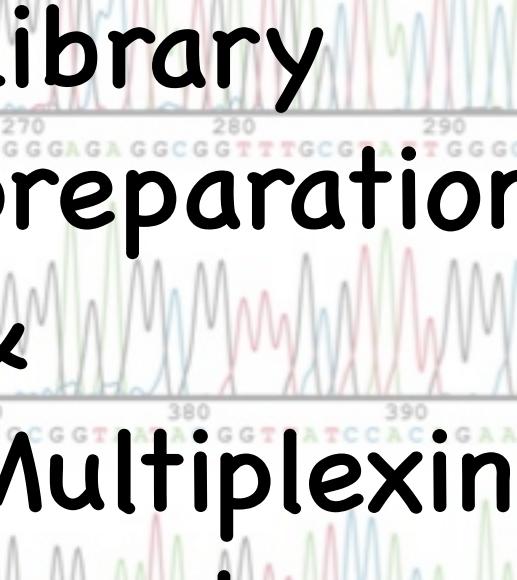


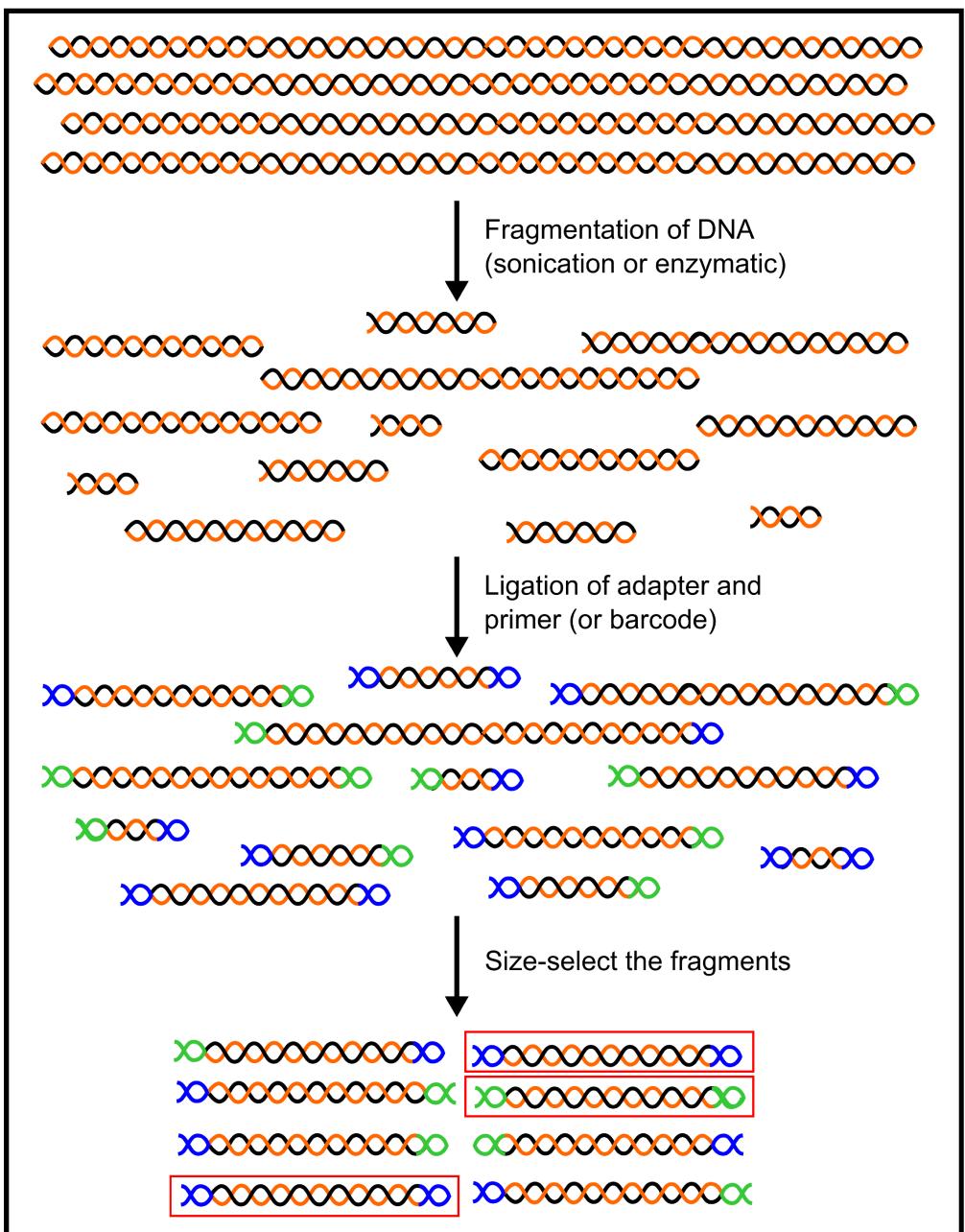
emPCR



Semiconductor  
Sequencing

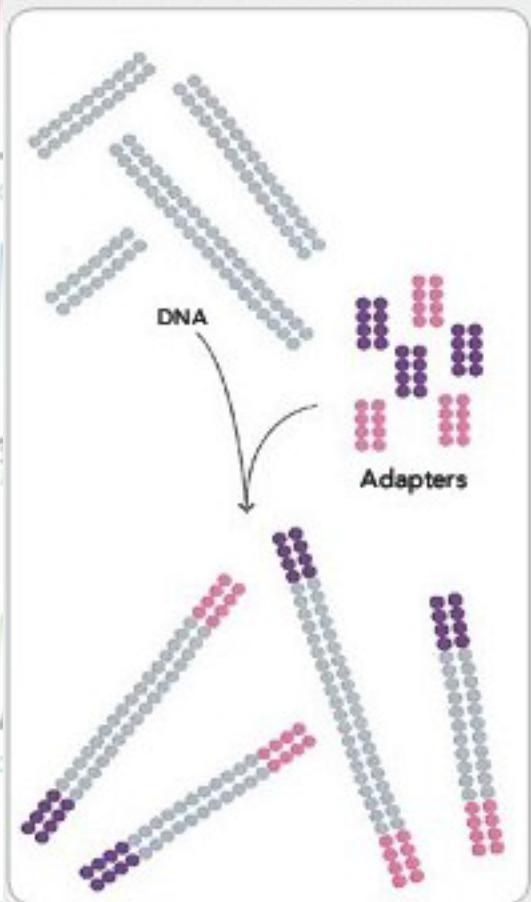
# Workflow: Library preparation & Multiplexing samples





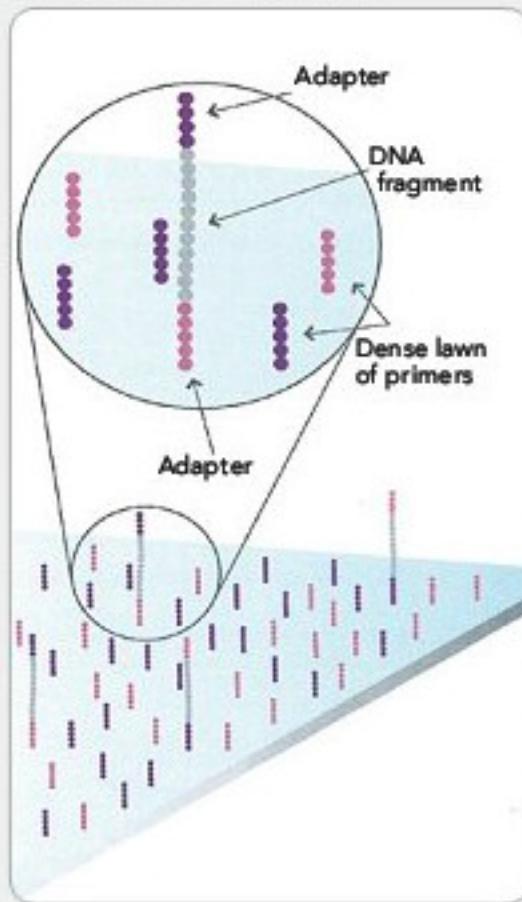
# Workflow: Illumina Bridge Amplification

## 1. PREPARE GENOMIC DNA SAMPLE



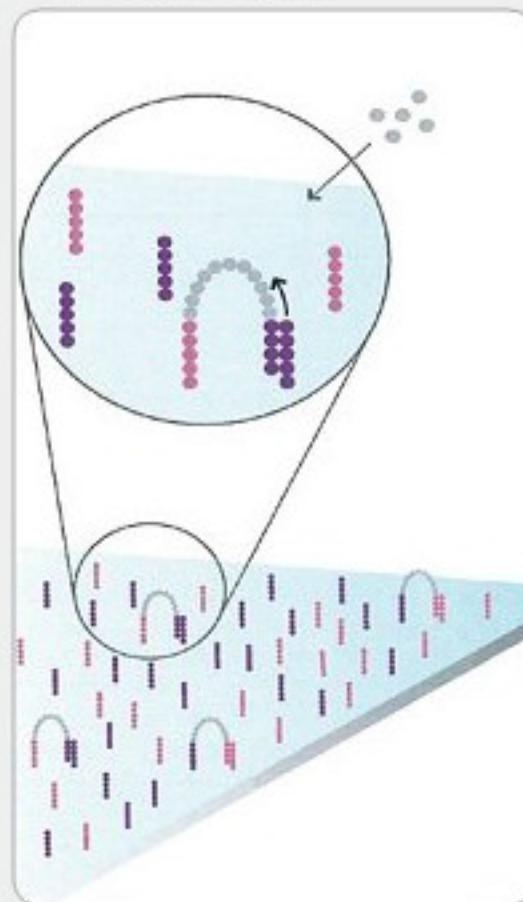
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

## 2. ATTACH DNA TO SURFACE



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

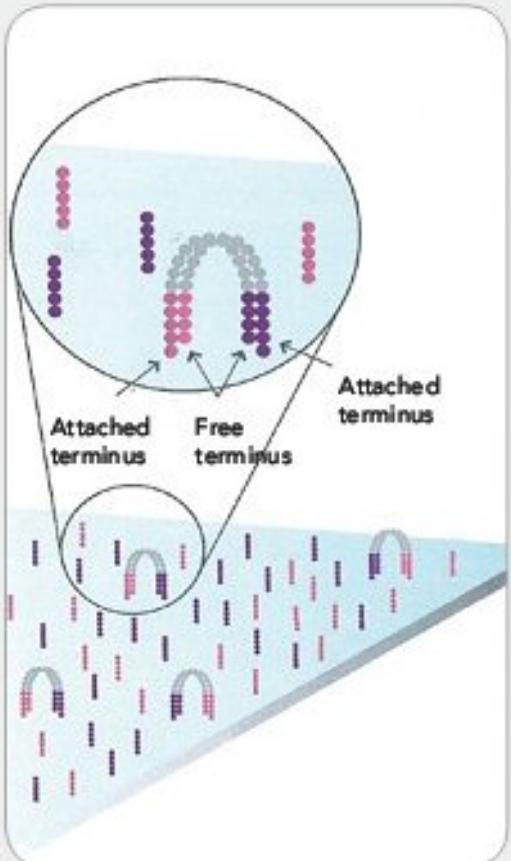
## 3. BRIDGE AMPLIFICATION



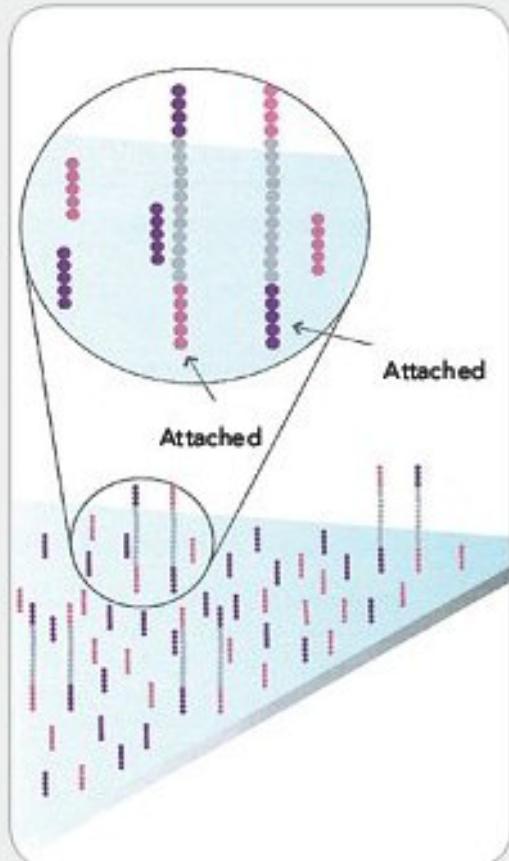
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

# Workflow: Illumina Bridge Amplification

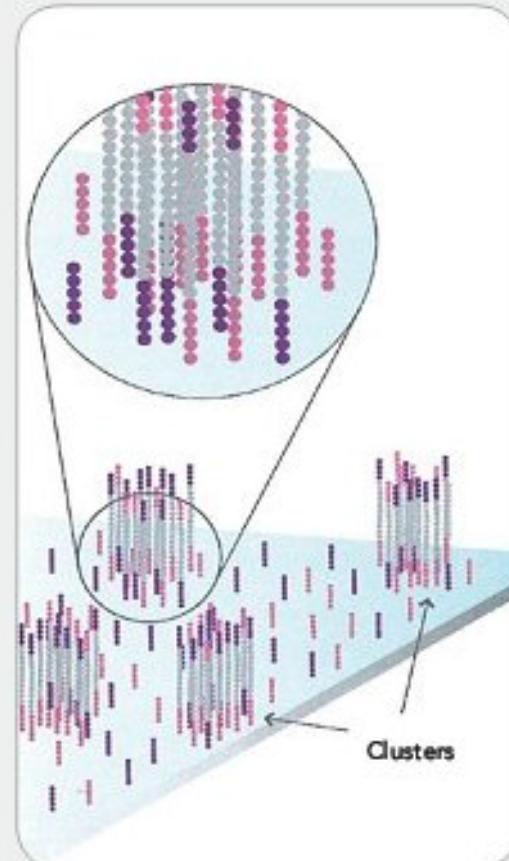
4. FRAGMENTS BECOME DOUBLE STRANDED



5. DENATURE THE DOUBLE-STRANDED MOLECULES



6. COMPLETE AMPLIFICATION

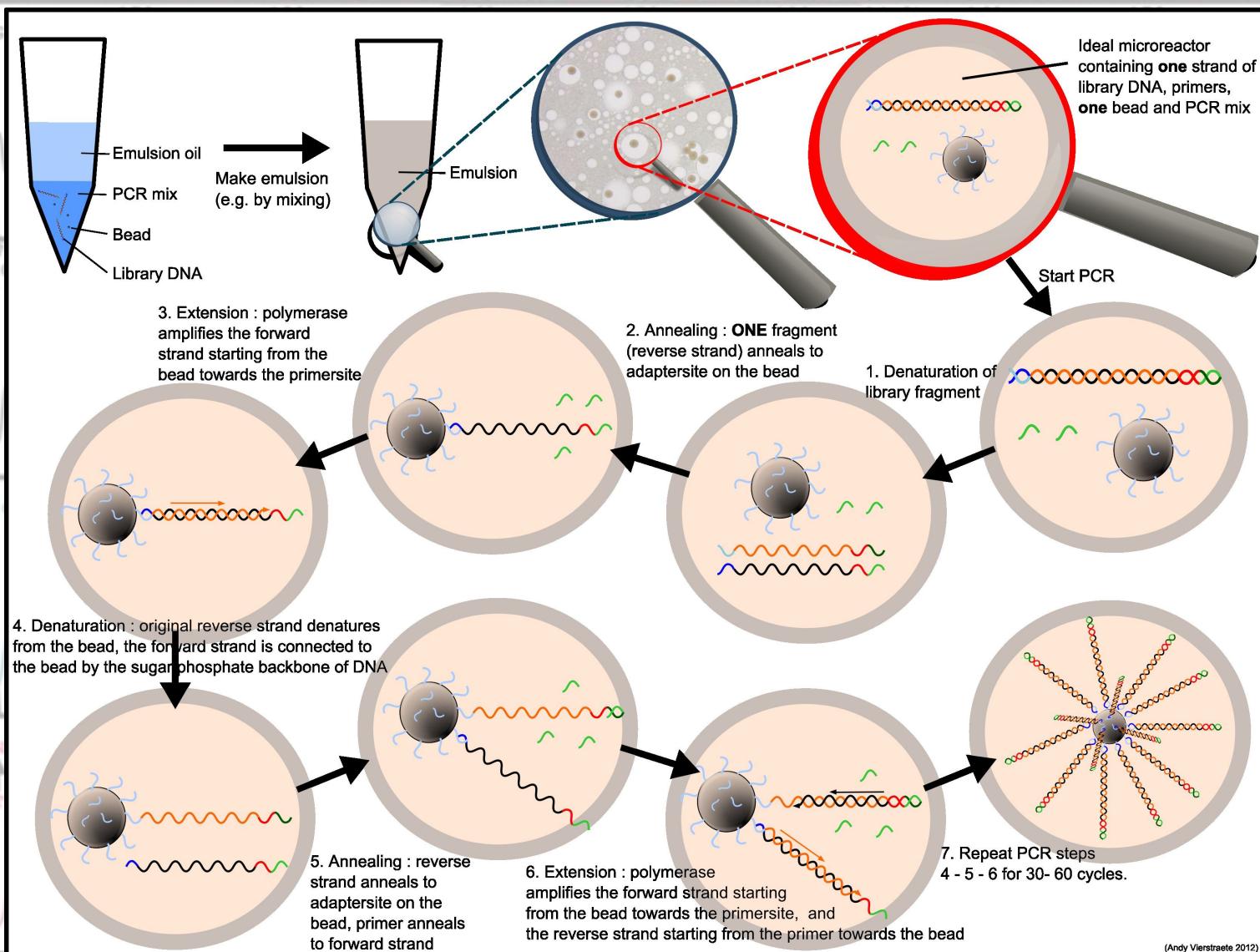


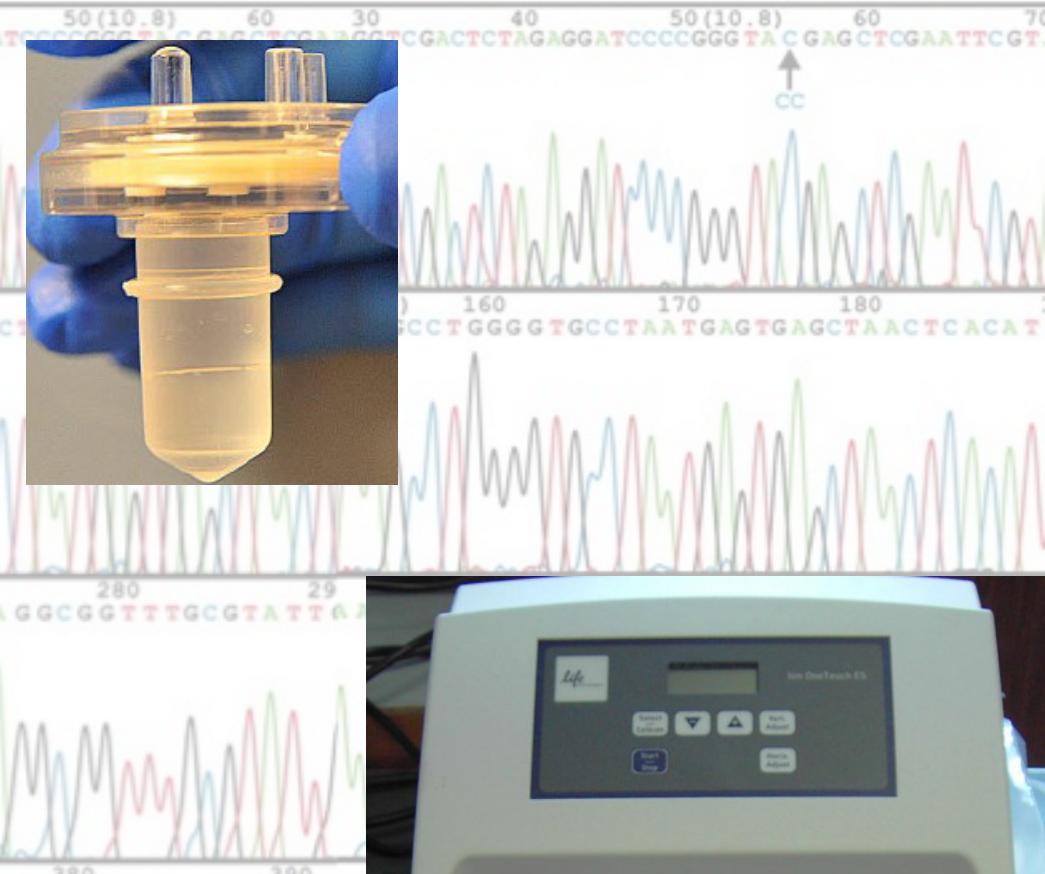
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

Denaturation leaves single-stranded templates anchored to the substrate.

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

# Workflow: Ion Torrent Emulsion PCR



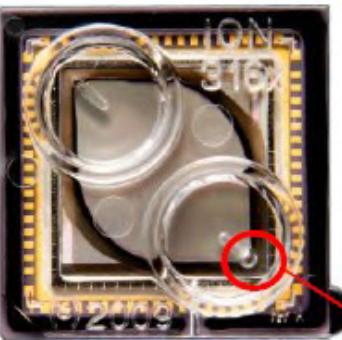


## Ion One Touch

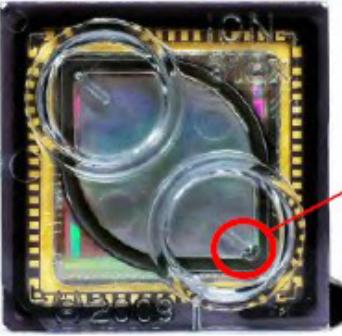


# Workflow: Loading the chip

Ion 316™ Chip:



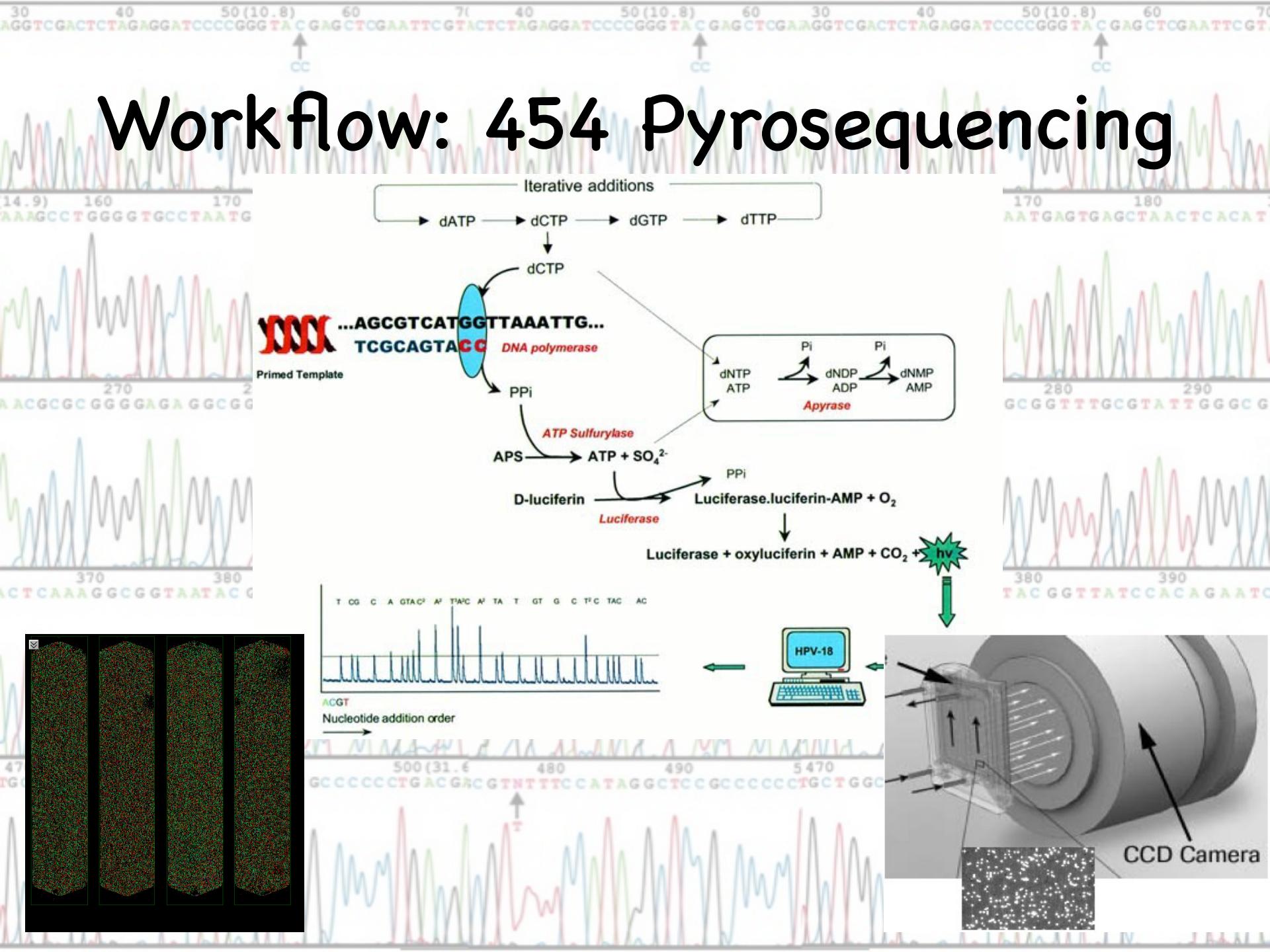
Ion 318™ Chip:



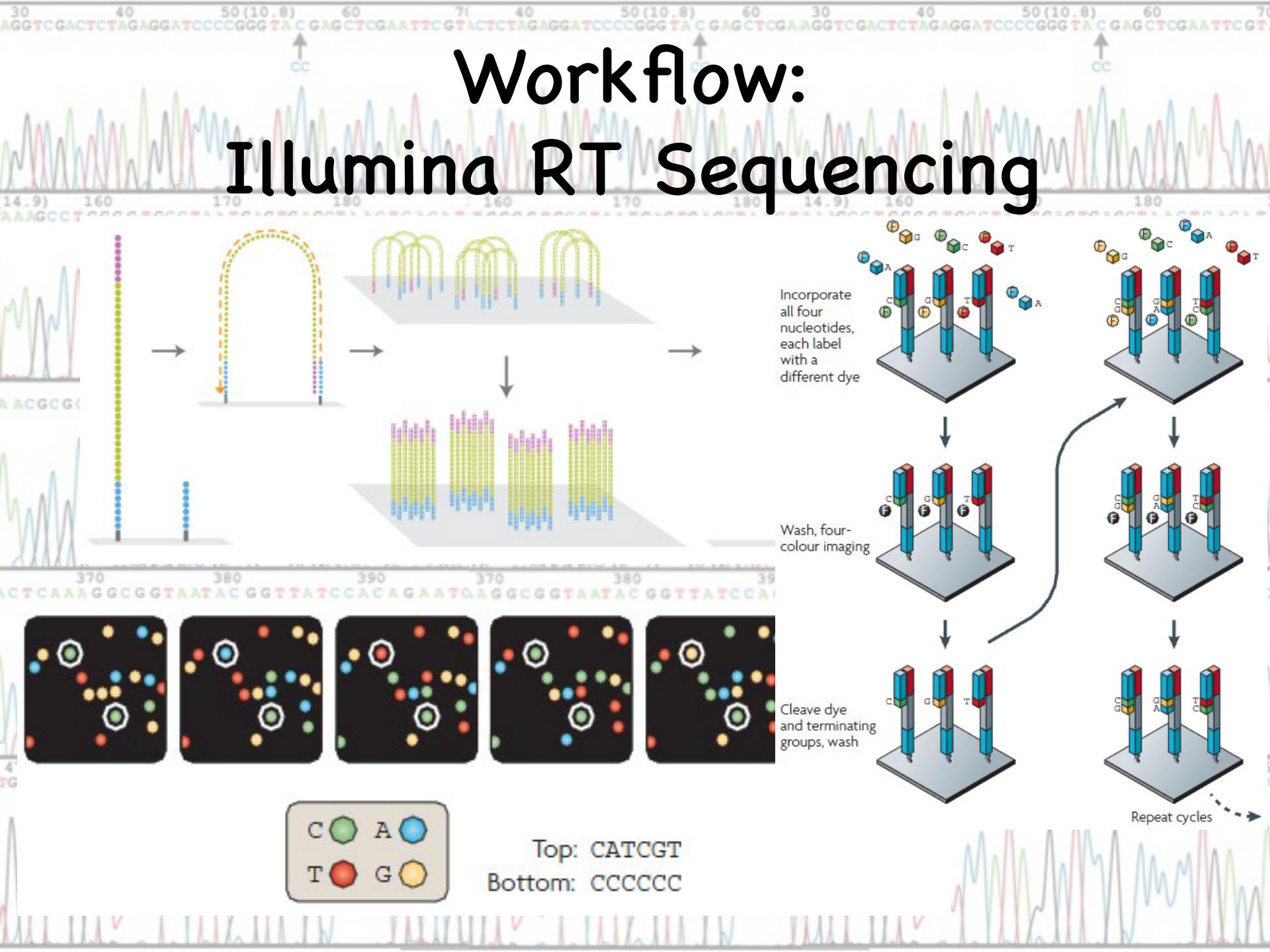
Centrifuge  
adapter  
bucket



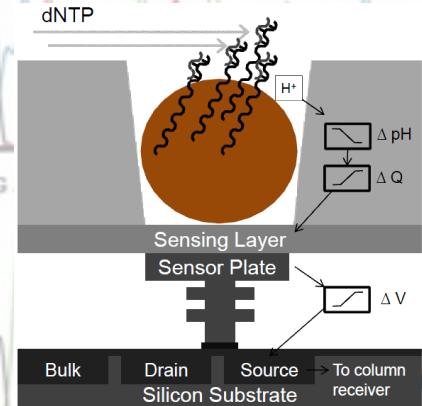
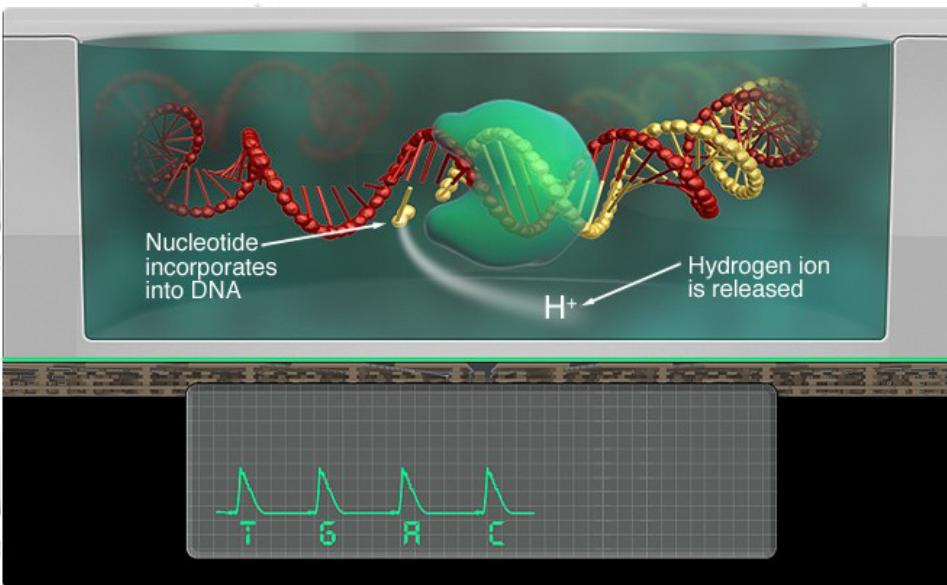
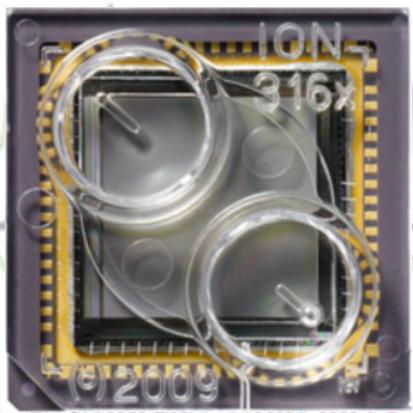
# Workflow: 454 Pyrosequencing



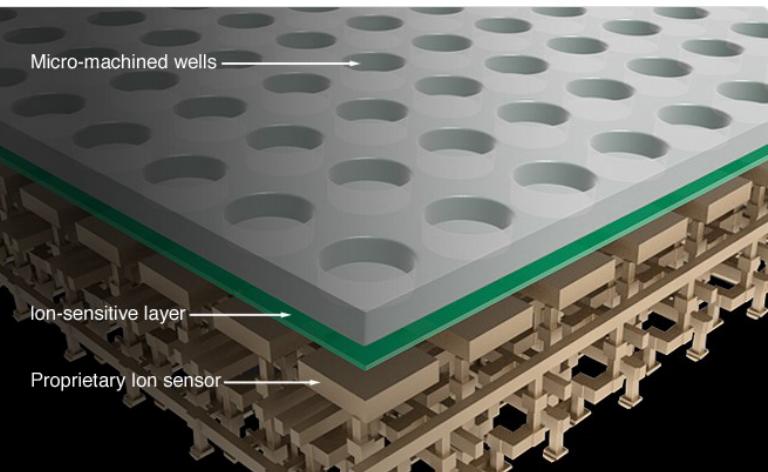
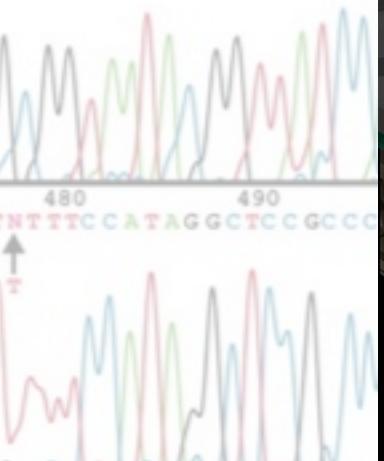
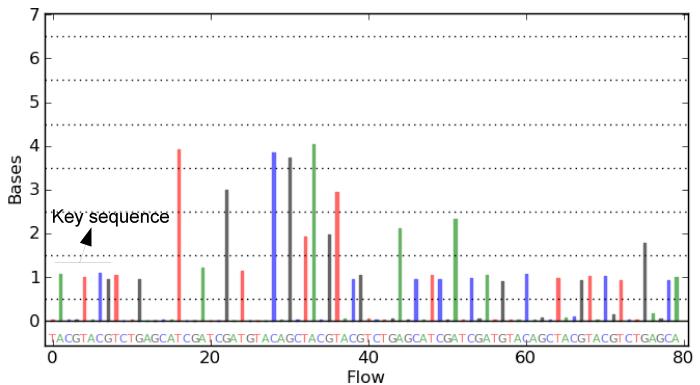
# Workflow: Illumina RT Sequencing



# Workflow: Ion Torrent Semiconductor Sequencing



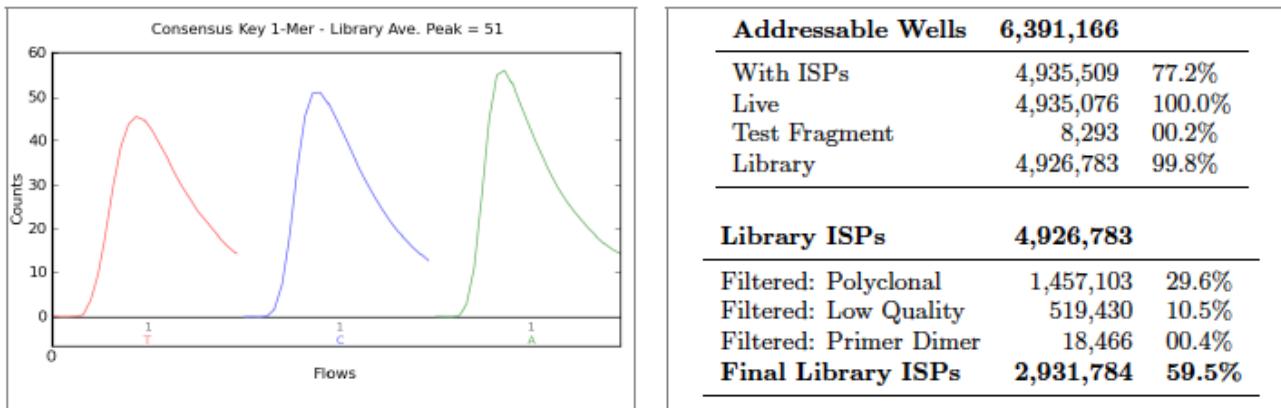
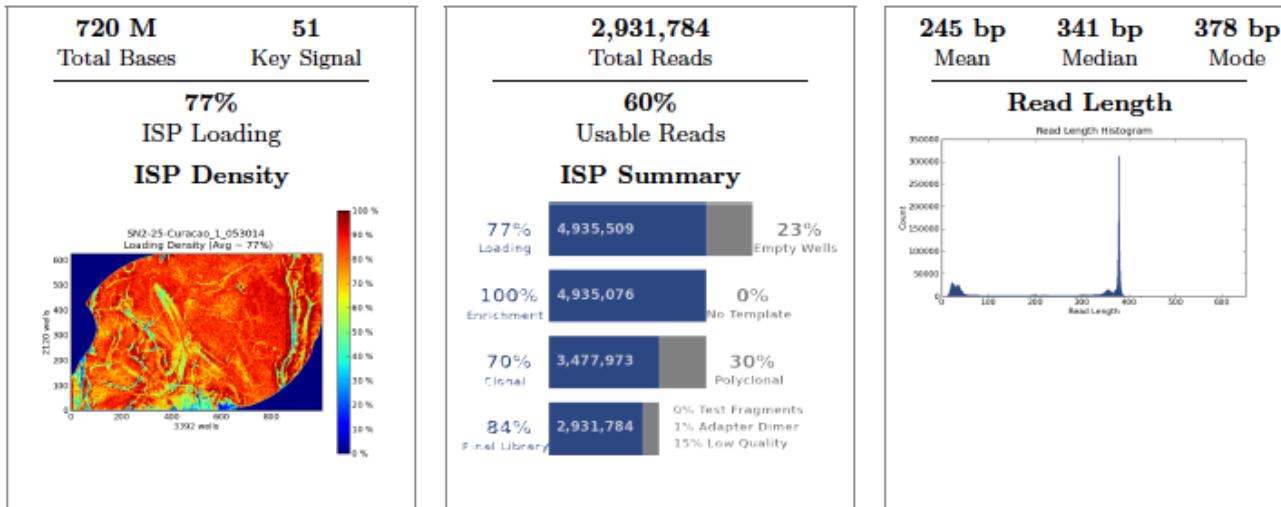
Average Corrected Ionogram



# What you get..

## Run Report for Auto\_user\_SN2-25-Curacao\_1\_053014\_52

### Run Summary



Notes: 23 percent enrichment. 1.22 ul of 3 and 7 at 50 pmol. 0.52 ul of 11 at 50 pmol.

# Good data... Bad data?

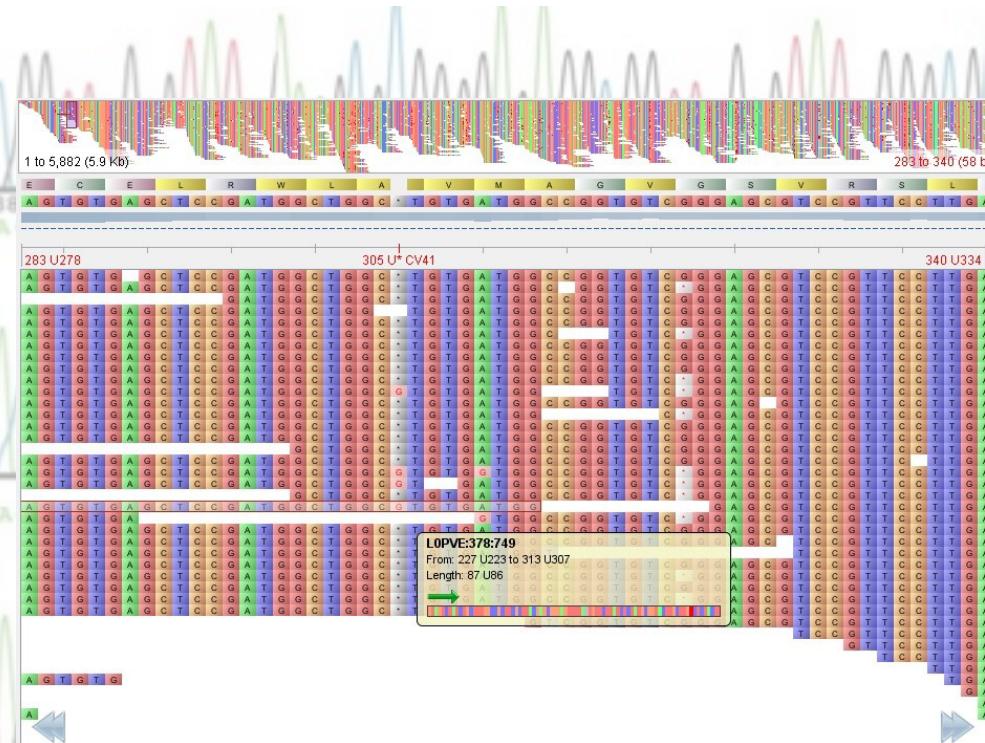
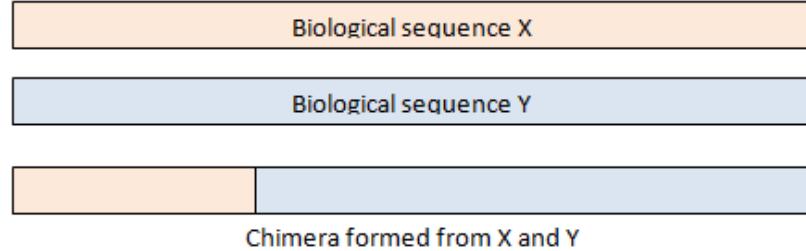
Polyclonality

Short reads

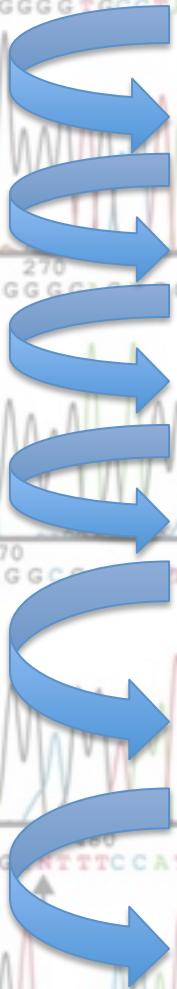
Homopolymer reads

Chimeras

Enough coverage?



# Basic Background to Bioinformatics



Raw sequences

Trim and align sequences

Detection of poor quality sequences

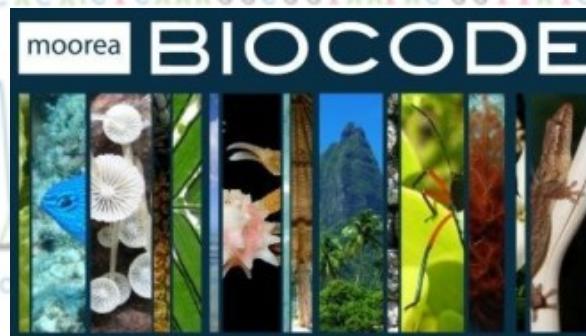
Removal of chimeric sequences

Cluster sequences into OTUs

Search for representative sequences in databases

Use probability based software to increase identities

A curated database is essential



<http://mooreabiocode.org>