Genomics and the use of Next Generation Sequencing (NGS) Techniques

IMBB 2016

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Genomics

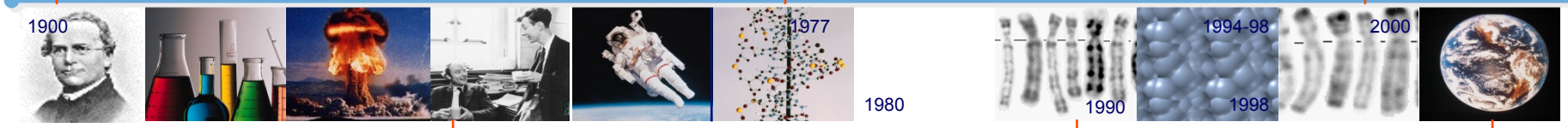
The study of the structure, organization, function, evolution of genomes (including interactions with the environment)

Genomics before NGS

Rediscovery of Mendel's laws helps establish the science of genetics

Sanger and Gilbert derive methods of sequencing DNA

Working Draft of the human genome sequencing complete



Watson and Crick identify DNA (the double helix) as the Chemical basis of heredity

Human Genome Projects (HPG) begins-an international effort to map and sequence all the genes in the human genome

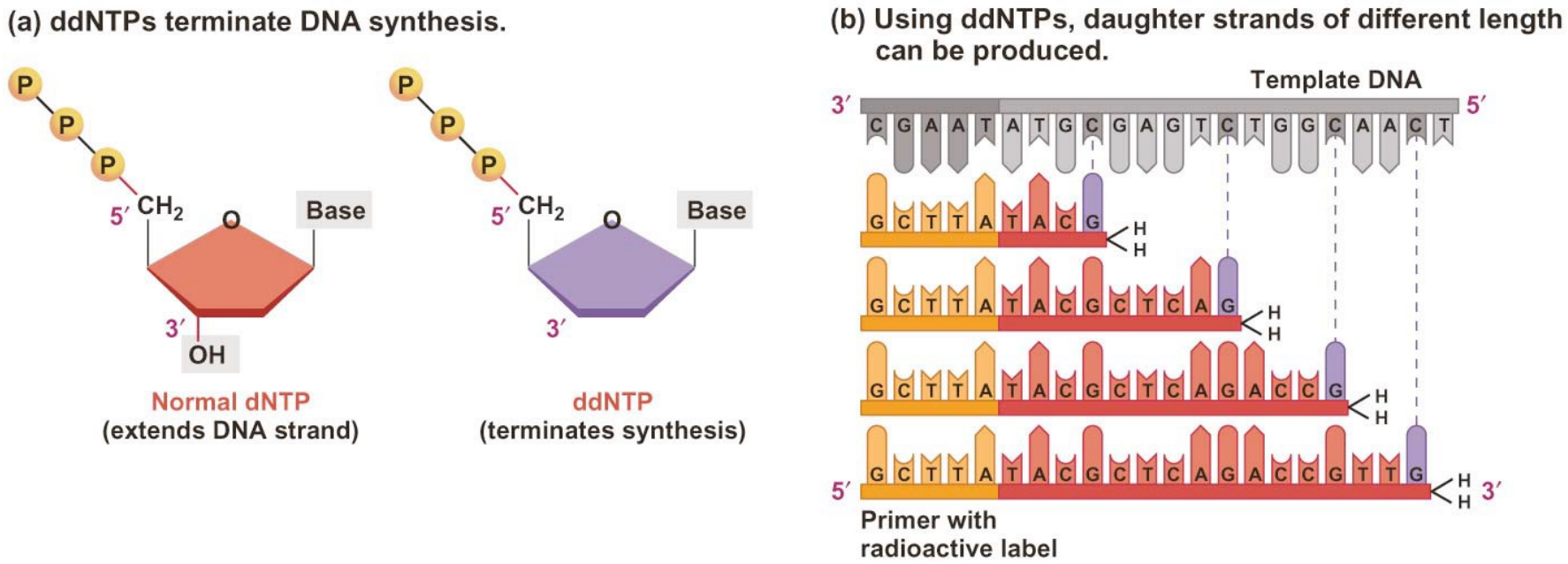
Human genome project completed 2003

Methods of sequencing

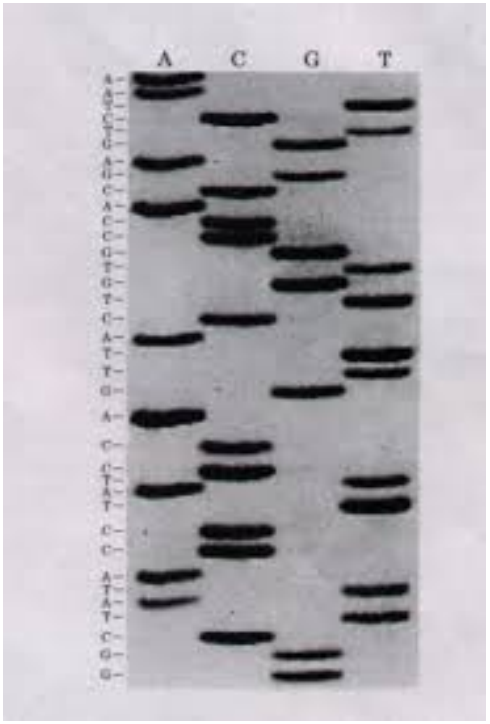
- Sanger dideoxy method
  (primer extension – termination)

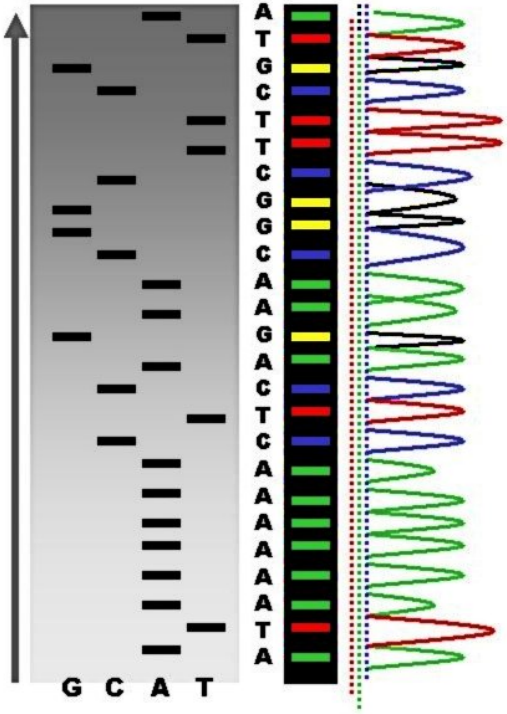
- Maxam-Gilbert chemical cleaved method
  (DNA labelled, chemically cleaved in sequence-dependent manner.

- Next Generation Sequencing (pyrosequencing, reversible dye-terminators, etc.)



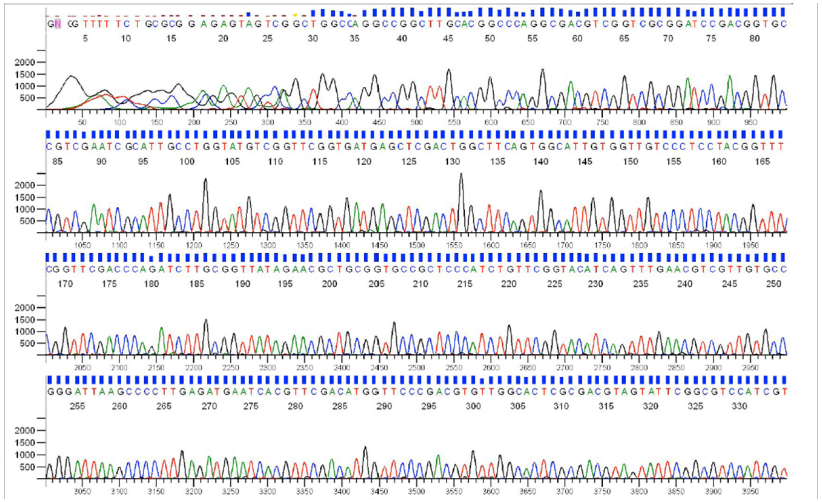
- Single stranded DNA template
- A primer for DNA synthesis
- DNA polymerase
- Deoxynucleoside triphosphates (normal dNTP) – extends DNA strand
- Dideoxynucleotide triphosphates (ddNTP) – terminates synthesis



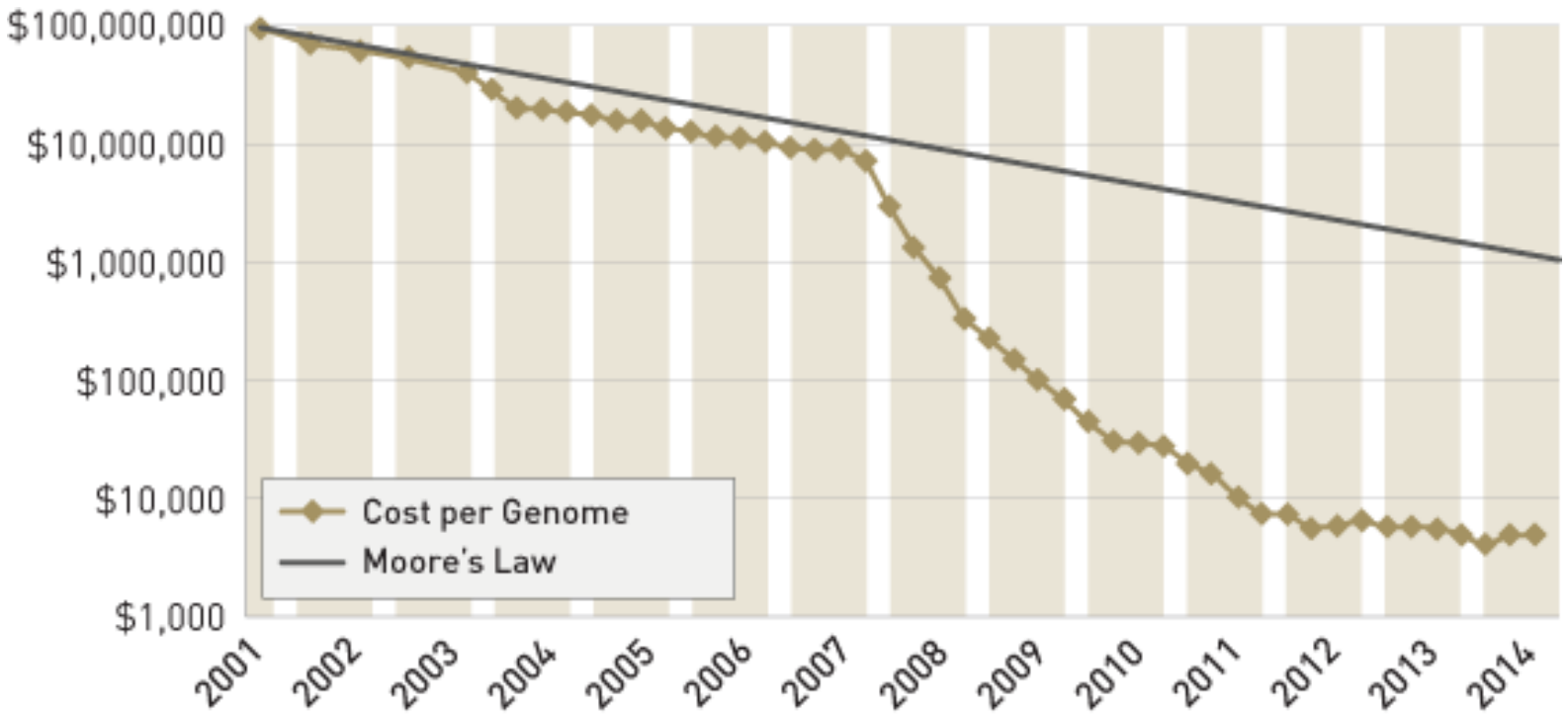


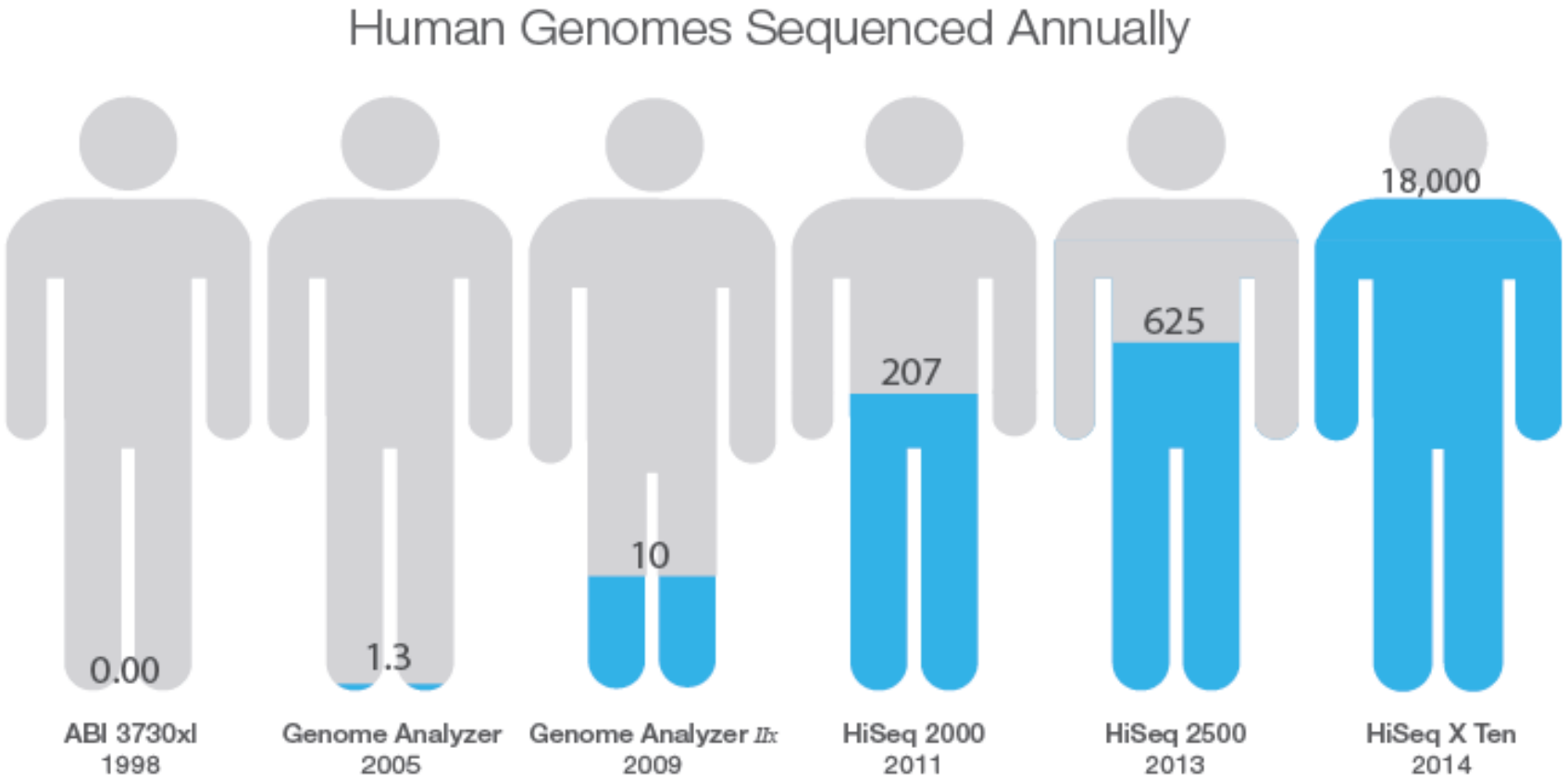
Sanger Sequencing Output

Each sequencing reaction gives us a **chromatogram**, usually ~600-1000 bp:



Cost for sequencing genomes rapidly reduced




Human Genomes Sequenced Annually
0.00
1.3
10
207
625
18,000
ABI 3730xl
1998
Genome Analyzer
2005
Genome Analyzer IIx
2009
HiSeq 2000
2011
HiSeq 2500
2013
HiSeq X Ten
2014

Current research: Comparative genomics

Some of the animals whose genomes are being or have been sequenced:

Platypus
Ornithorhynchus anatinus

Dog
Canis familiaris
Photo: Courtesy of The Broad Institute of MIT and Harvard

African elephant
Loxodonta africana

Fruitfly
Drosophila melanogaster

Silkworm
Bombyx mori Daza
Photo: Alden M. Johnson, California Academy of Sciences

Cow
Bos taurus
Photo: Courtesy of Terri Hobbs (www.crazyforcows.com)

European rabbit
Oryctolagus cuniculus

Honey Bee
Apis mellifera
Photo: Courtesy of Scott Bauer, USDA/ARSLaboratories

Armadillo
Dasypus novemcinctus
Photo: Courtesy of B. Bagatto, Department of Biology, University of Akron

Guinea pig
Cavia porcellus

Roundworm
Caenorhabditis elegans
Photo: Courtesy of Erik Jorgensen, University of Utah

Zebrafish
Danio rerio

Mouse
Mus musculus
Photo: Courtesy of Jackson Laboratories

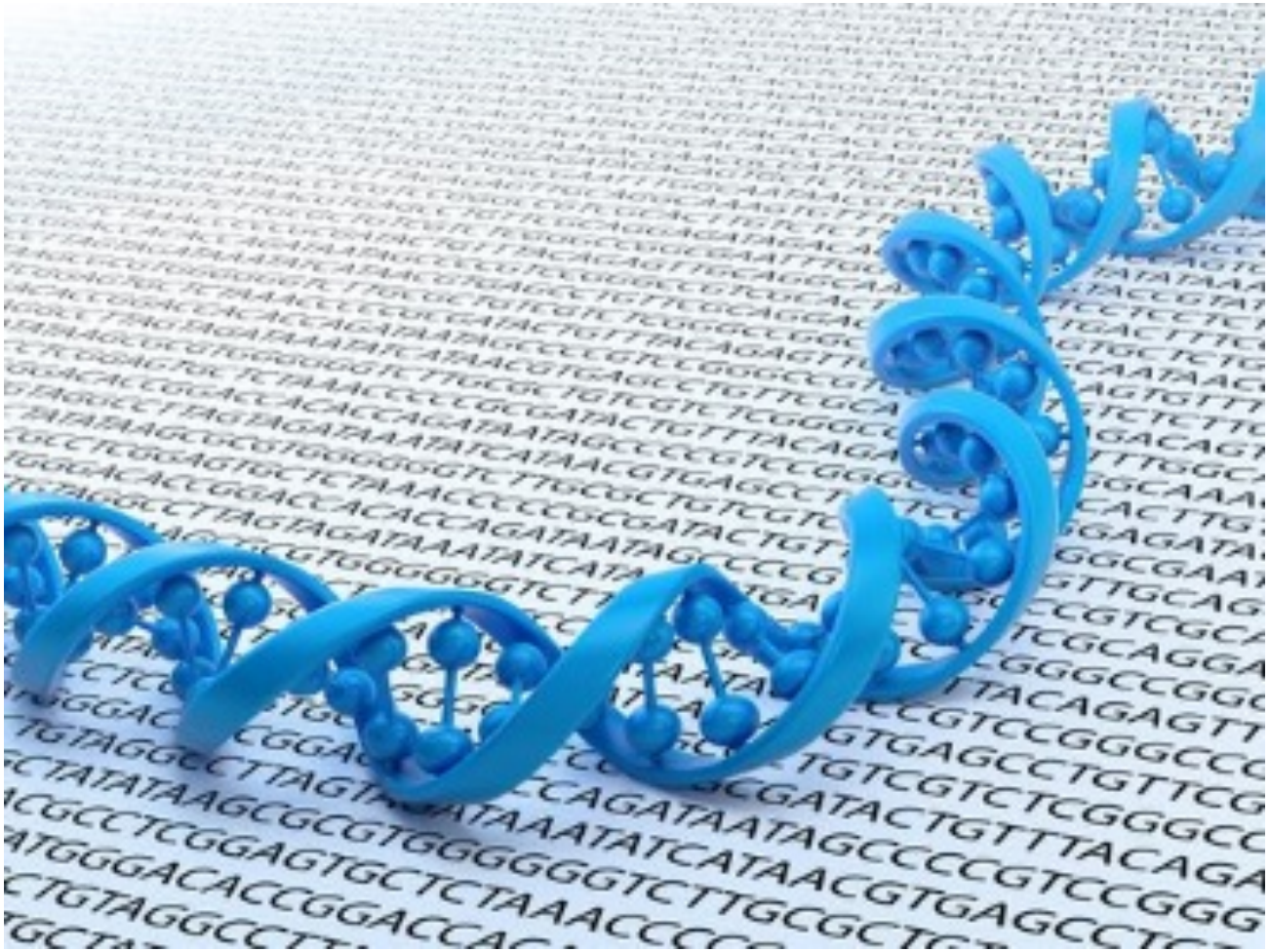
Chicken
Gallus gallus

Opossum
Monodelphis domestica
Photo: Courtesy of Don Sakaguchi

Cat
Felis domesticus
Photo: Courtesy of Dr. Kristina Narfstrom, University of Missouri-Columbia

National Human Genome Research Institute

What is NGS?



NGS platforms

454

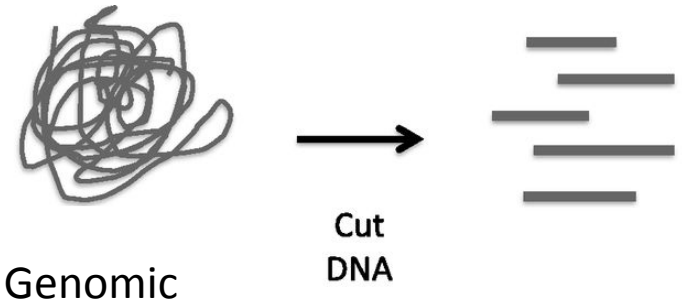
Illumina

ABI SOLiD

General steps of illumina NGS technologies

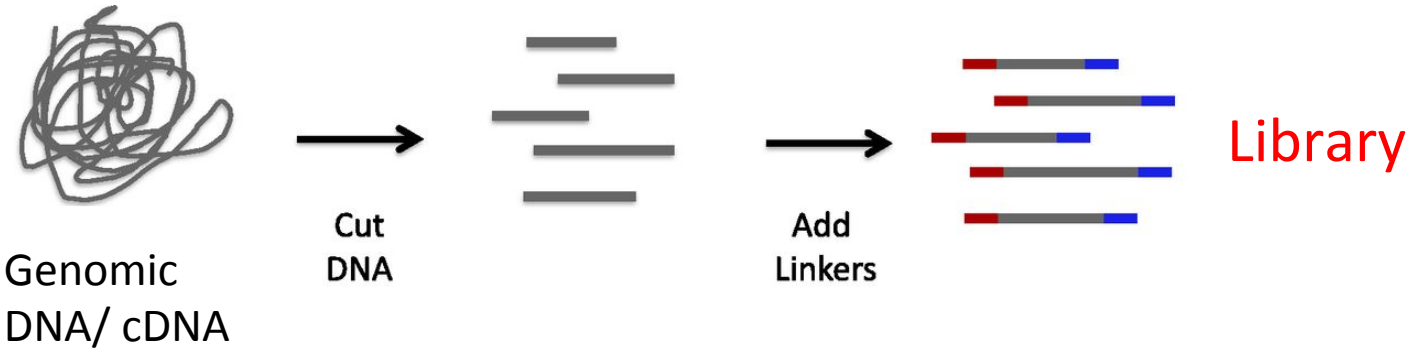
Genomic
DNA/ cDNA

General steps of illumina NGS technologies

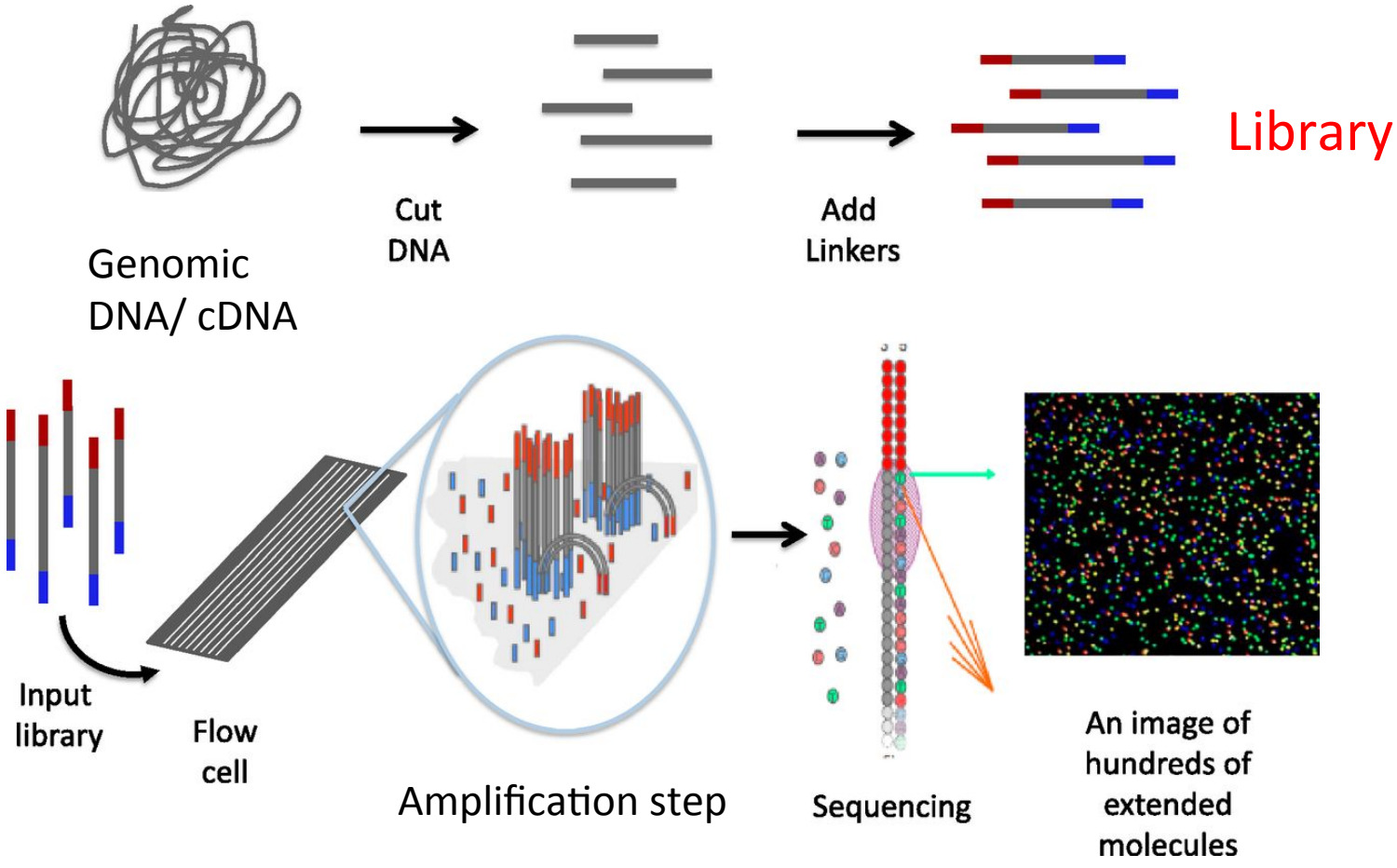


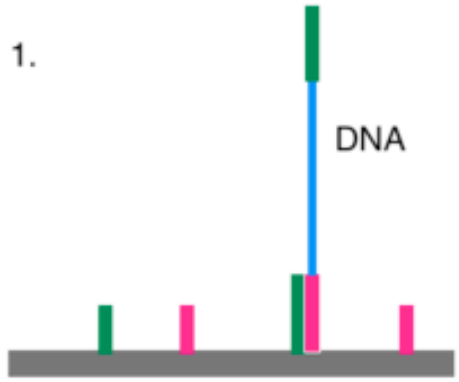
Genomic
DNA/ cDNA

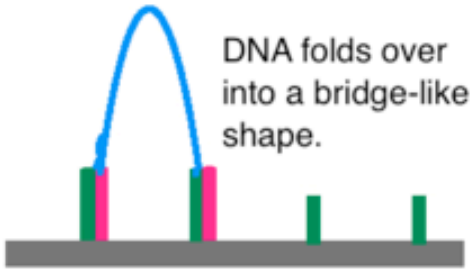
General steps of illumina NGS technologies

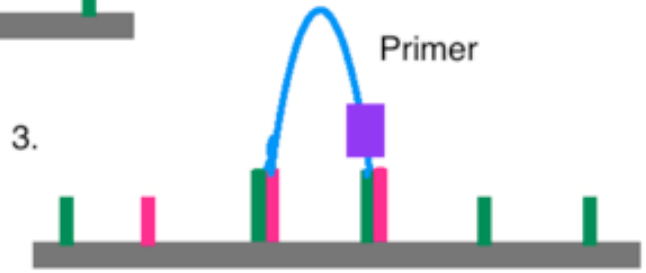


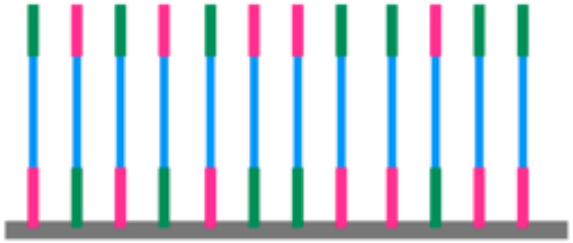
General steps of Illumina NGS technologies

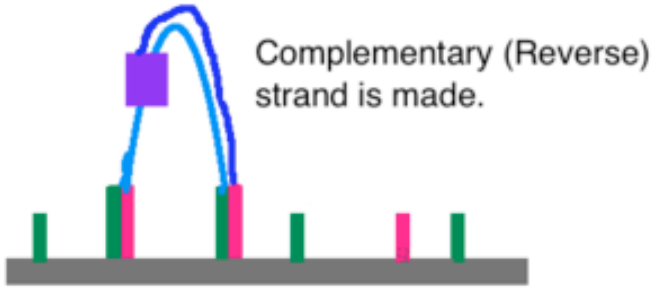


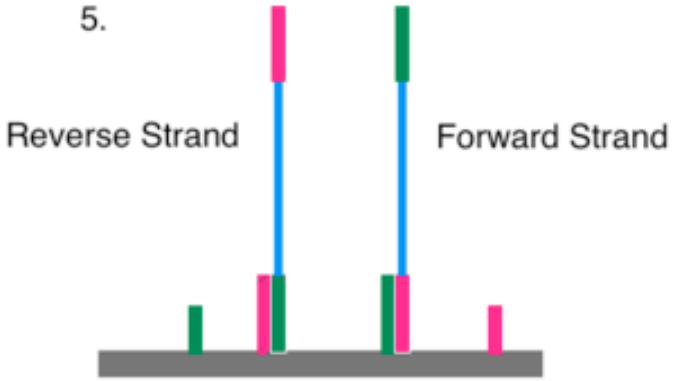

1.
DNA
Flow cell


2.
DNA folds over into a bridge-like shape.


Primer
3.


6.
Clonal copies of both forward and reverse strand in a cluster.


4.
Complementary (Reverse) strand is made.


5.
Reverse Strand
Forward Strand

	454 GS FLX	Illumina HiSeq 2000	SOLiD v4
Read length	700bp	2*100bp	50*2 bp
Accuracy	99.9%	98%	99.94%
Output data	0.7GB	600GB	120GB
Cost/million bases	$10	$0.07	$0.13

Illumina technologies

MiSeq Series

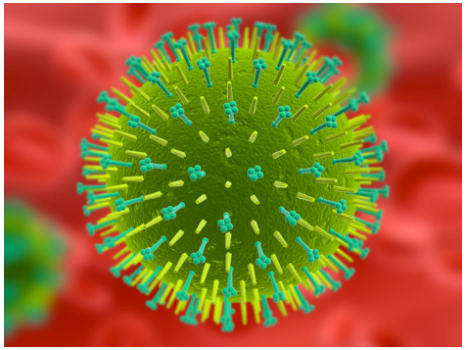
NextSeq Series

HiSeq Series

HiSeq X Series

Nextera	DNA
Nextera XT	small genomes
TruSeq	total RNA
TruSeq Stranded	RNA ribosomal RNA removed

Scope of Sequencing



Metagenomics (viral/bacterial)

The New Science of Metagenomics: Revealing the Secrets of Our Microbial Planet
http://www.nap.edu/catalog/11902.html

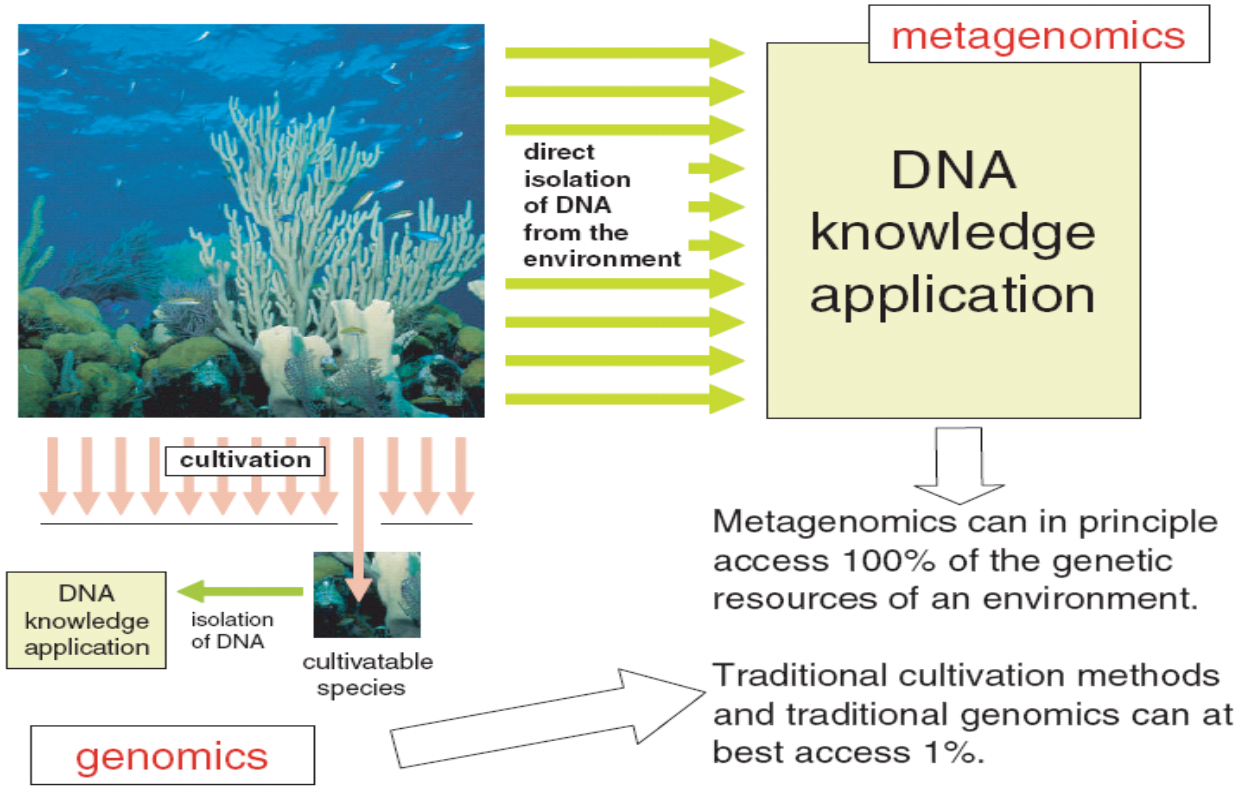
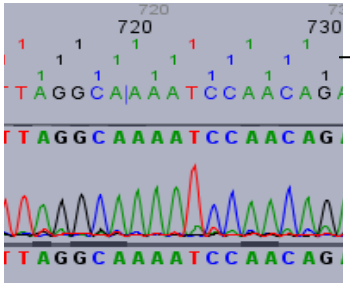


FIGURE 1-3 How metagenomics differs from microbial genomics. Image provided by W. Ford Doolittle.

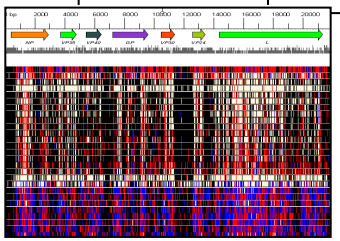
From Sequence to impact

(meta)genome sequencing

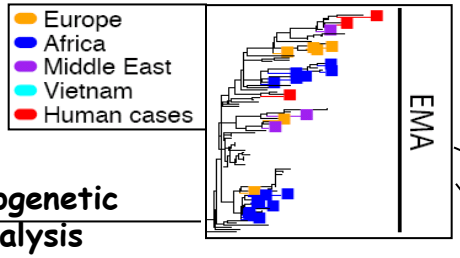


Compilation of complete genomes, metagenomes, annotation and curation of metadata

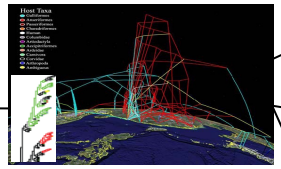
Databases



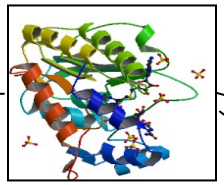
Extraction of important biological information



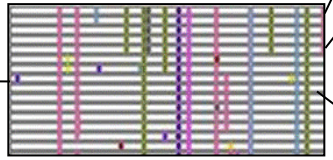
phylogenetic analysis



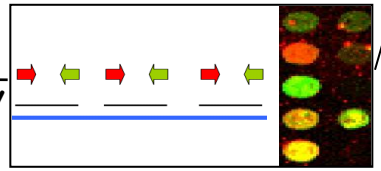
geographical mapping



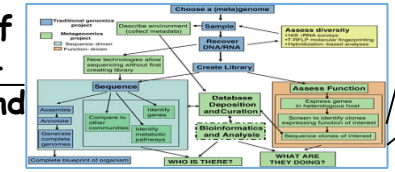
protein modeling



sequence variation analysis



Primer, microarray



discovery of new micro-organisms and pathways

Diagnostics

Global diseases surveillance

Vaccine dvlpmt

Drug dvlpmt

Improved drug selection

Environmental sustainability

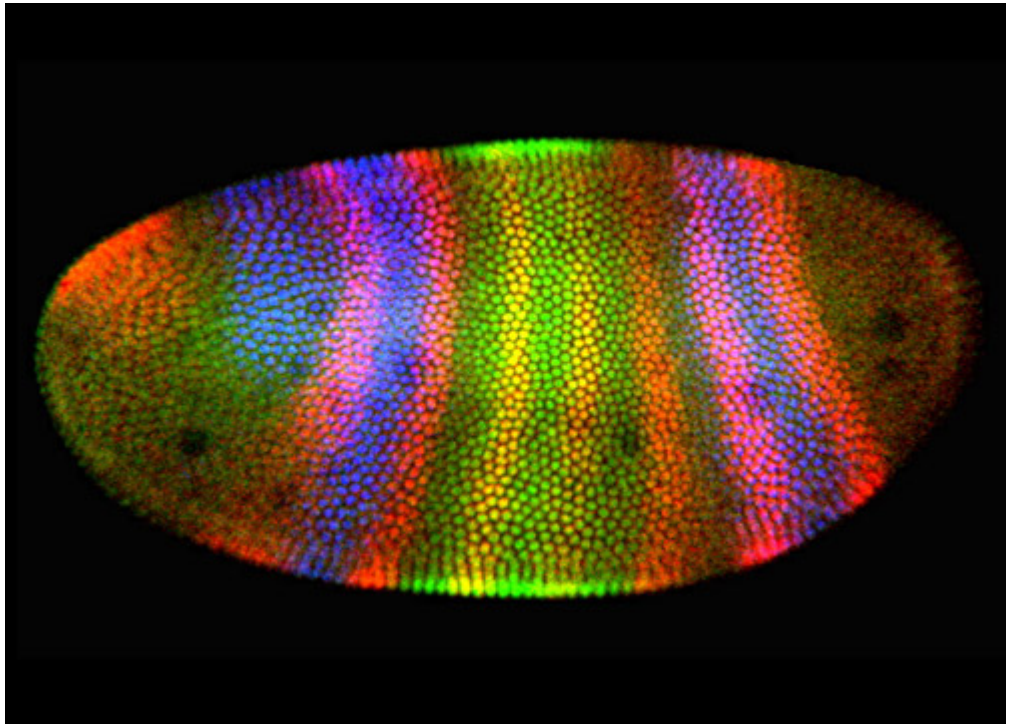
Improved Public health intervention

Diversity studies

A reference genome for common bean and genome-wide analysis of dual domestications

Schmutz et. al 2014 Nature Genetics 46

Expression studies



The developmental transcriptome of *Drosophila melanogaster*

Sequencing ancient genomes

Miller, W. *et al*. Sequencing the nuclear genome of the extinct woolly mammoth. Nature 456, 387–390 (2008).

Biological Question?

Thank you

IMBB 2016

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Francesca Stomeo

15th Anniversary biosciences eastern and central africa

NEPAD TRANSFORMING AFRICA

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