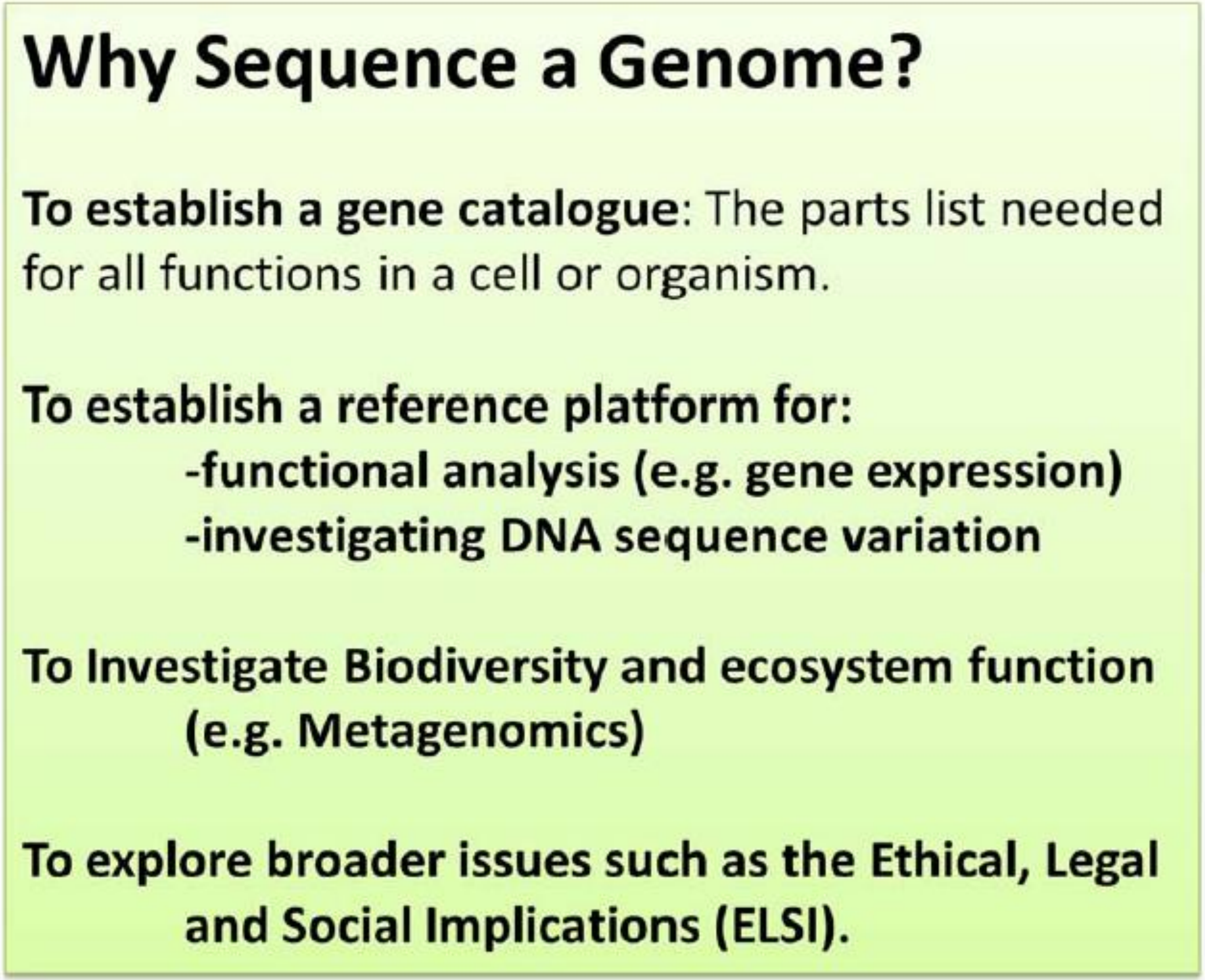
Principles of Genomics

The ability to sequence the genomes of organisms rapidly and cheaply has made genome sequencing one of the first ways we investigate the biology of a organism.



Why Sequence a Genome?

To establish a gene catalogue: The parts list needed for all functions in a cell or organism.

To establish a reference platform for:

-functional analysis (e.g. gene expression)
-investigating DNA sequence variation

To Investigate Biodiversity and ecosystem function (e.g. Metagenomics)

To explore broader issues such as the Ethical, Legal and Social Implications (ELSI).

There are many diverse reasons for sequencing genomes.

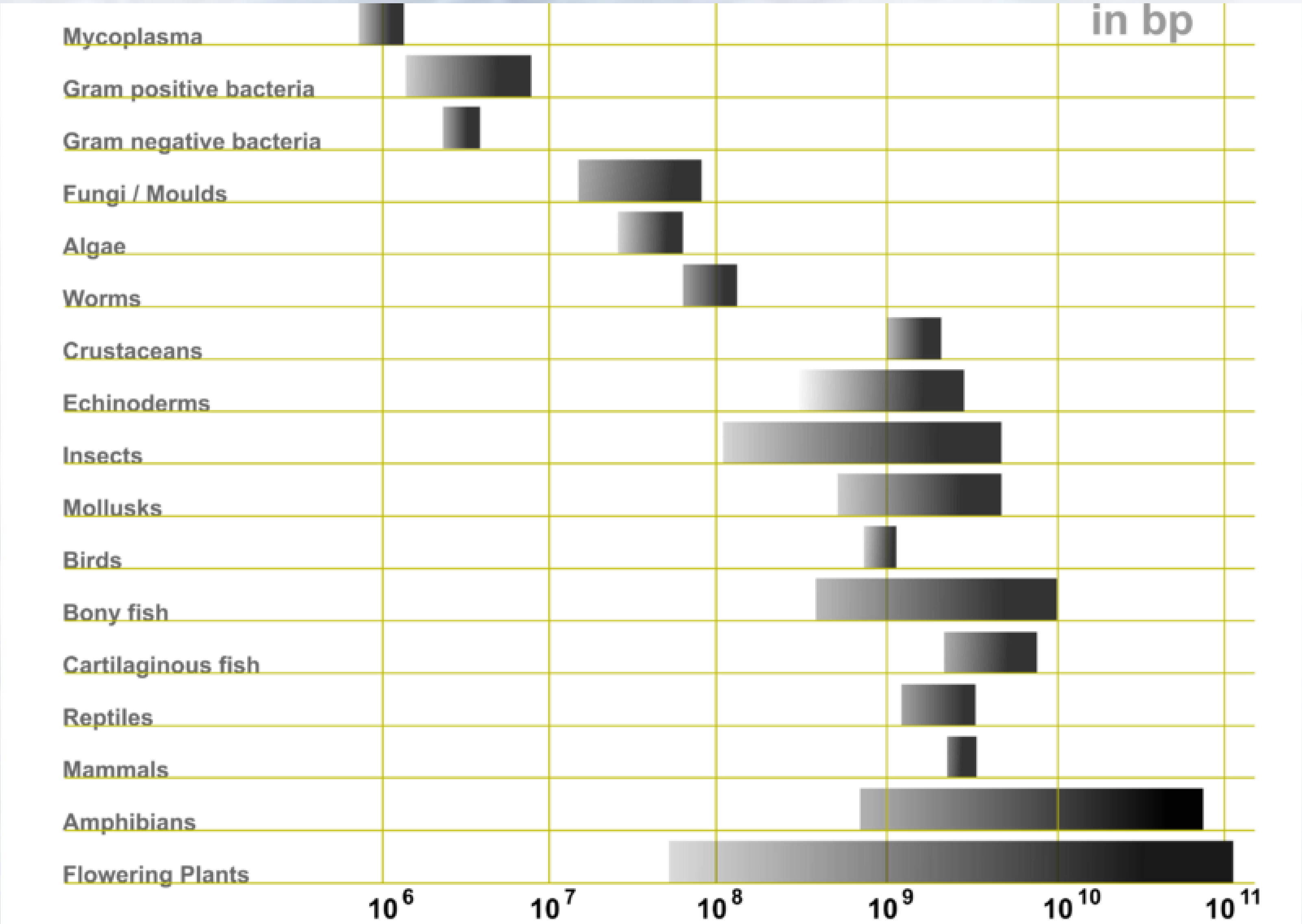
Variation in genome structure (bacteria/eukaryotes):

- Genomic DNA of all organisms is a DNA double helix.
  - Some viruses use ssDNA, dsRNA and ssRNA.
- The genomes of organisms vary extensively in size.
- Within a group of organisms there is often little correlation between the size of a genome and the number of genes (C-Value enigma).
- Larger genomes tend to have more repetitive DNA (e.g. transposable elements, simple sequence repeats and duplicated genes).
- The genomes of eukaryotes are often diploid and contain allelic variation (aka. heterozygosity).

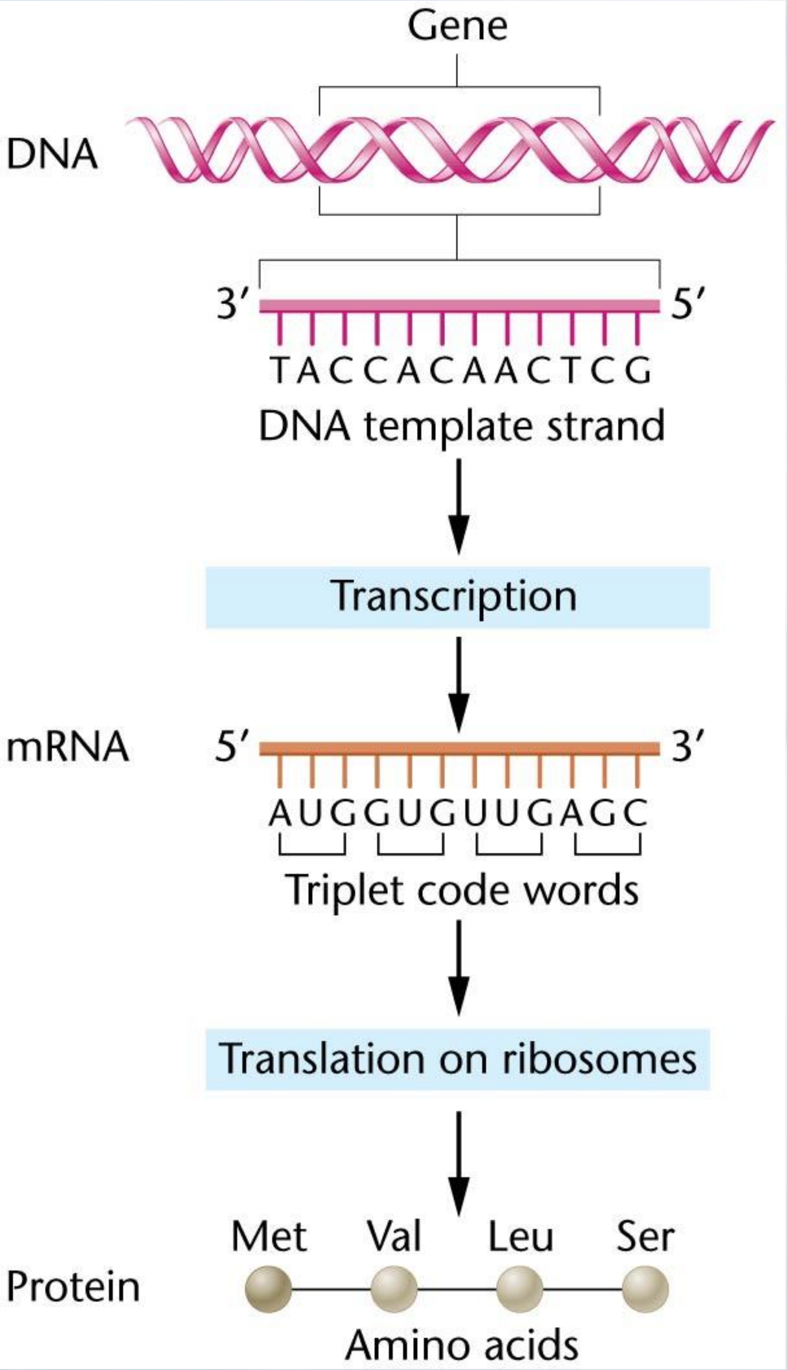
Genomes come in different sizes

Smallest Genome?
Nasuia deltocephalinicola
112,091 bp, 137 genes

Largest Genome?
Paris japonica
150 Gigabases



Bioinformatics of genomics uses our knowledge of cellular processes to “read” and understand DNA.



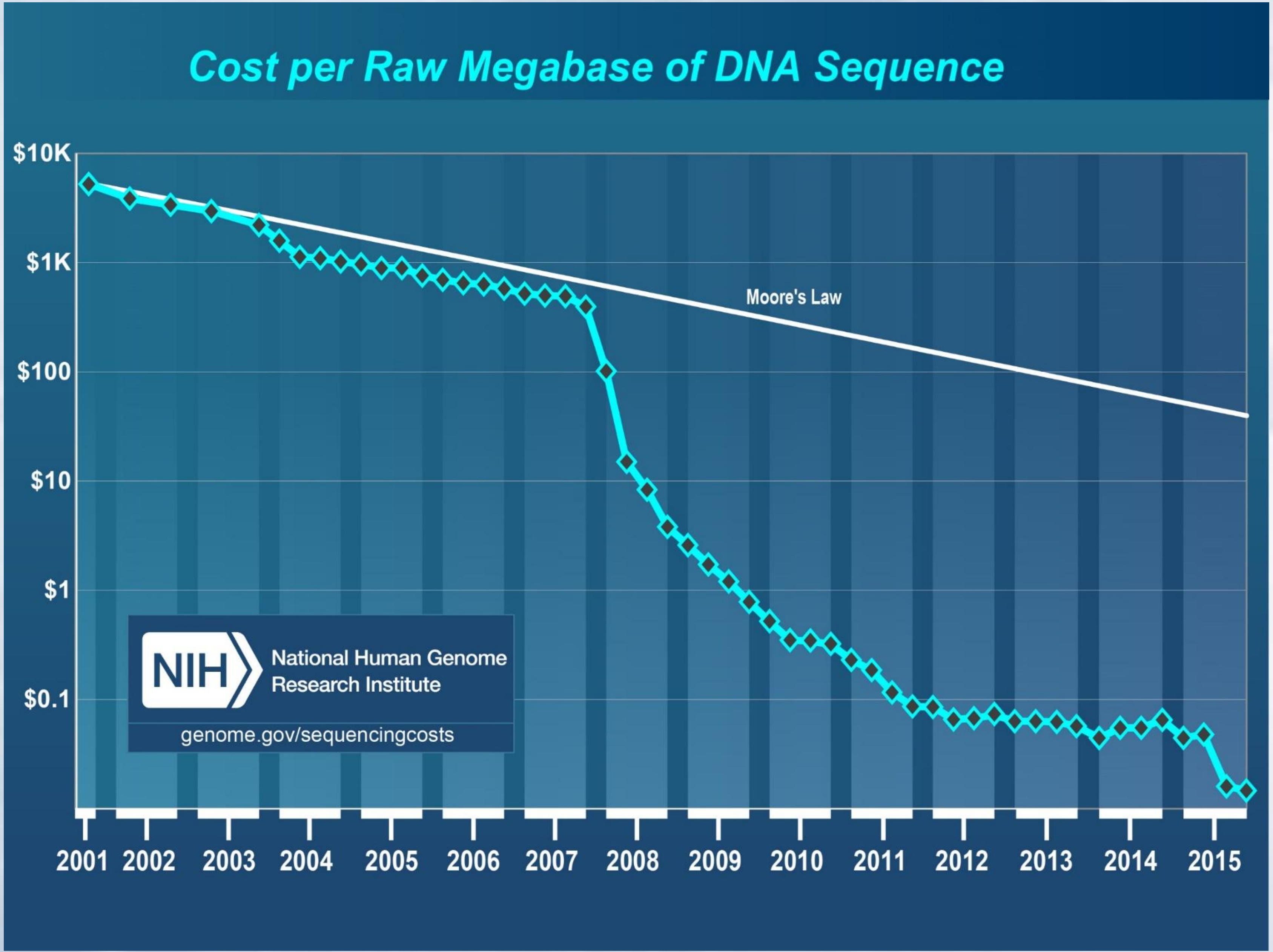
The generation of Genomic Data

Next Generation DNA sequencing technologies

A sea-change in genome-enabled biology

- The last several years have seen the development of fundamentally new sequencing technologies.
  - A process that continues…
- These technologies produce more data, and better data in many fewer steps.
- These changes make genomic analysis a core approach in diverse areas of biology

High throughput sequencing revolution



Diverse Applications of Next-Generation Sequencing

Illumina 2014

PARS-SEQ
SHAPE-SEQ
MDA
MALBAC
CHIRP-SEQ
RNA-SEQ
MAINE-SEQ
RRBS-SEQ
RIBO-SEQ
ICE
OS-SEQ
CLASH-SEQ
TIF-SEQ/PEAT
IN-SEQ
TAB-SEQ
CHIP-SEQ
5-C
SMMIP
OXBS-SEQ
RIP-SEQ
4-C
TC-SEQ
NET-SEQ
UMI
CAP-SEQ
DNASE-SEQ
FAIRE-SEQ
DUPLEX-SEQ
PAR-CLIP-SEQ
BS-SEQ
MEDIP-SEQ
GRO-SEQ
MERIP-SEQ
DIGITAL
CHIA-PET
ATAC-SEQ
CIP-TAP
ICLIP
MBDCAP-SEQ
HITS-CLIP
PARE-SEQ/GMUT
HI-C/3-C
TRAP-SEQ
RC-SEQ
FRAG-SEQ

Next Generation Sequencing Technologies

- The dominant sequencing approach today is Sequencing by Synthesis (SBS) from Illumina. This technology produces the vast majorities of datasets and will be the method used for your sequencing.
- Other interesting and useful approaches include the Single Molecule Real Time (SMRT) Sequencing approach by Pacific Biosciences and Nanopore Sequencing by Oxford Nanopore Technologies. Both of these technologies can produce much longer “reads” or continuous runs of sequence information.

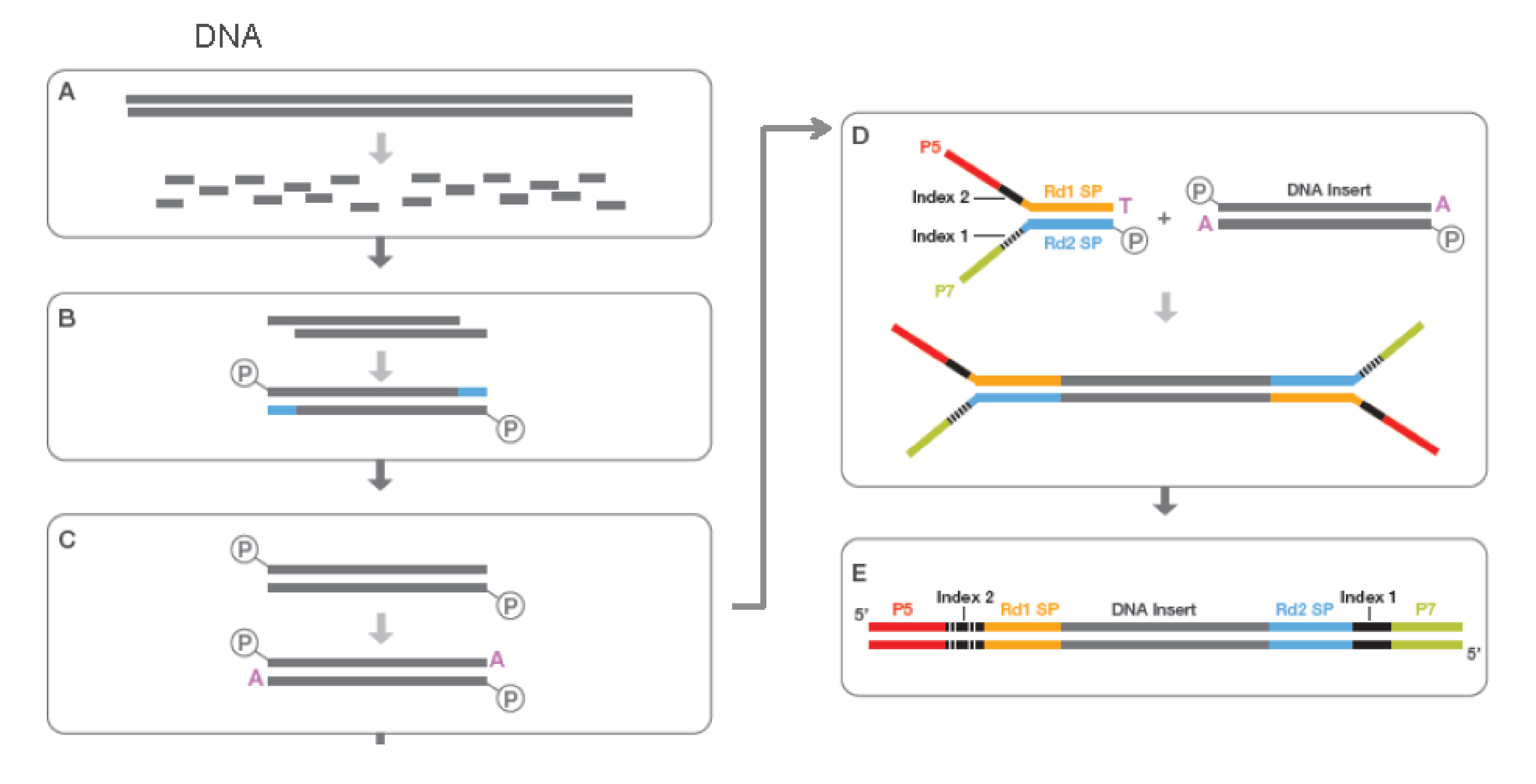
Illumina Sequencing by synthesis

Watch a Video showing the basics of the technology

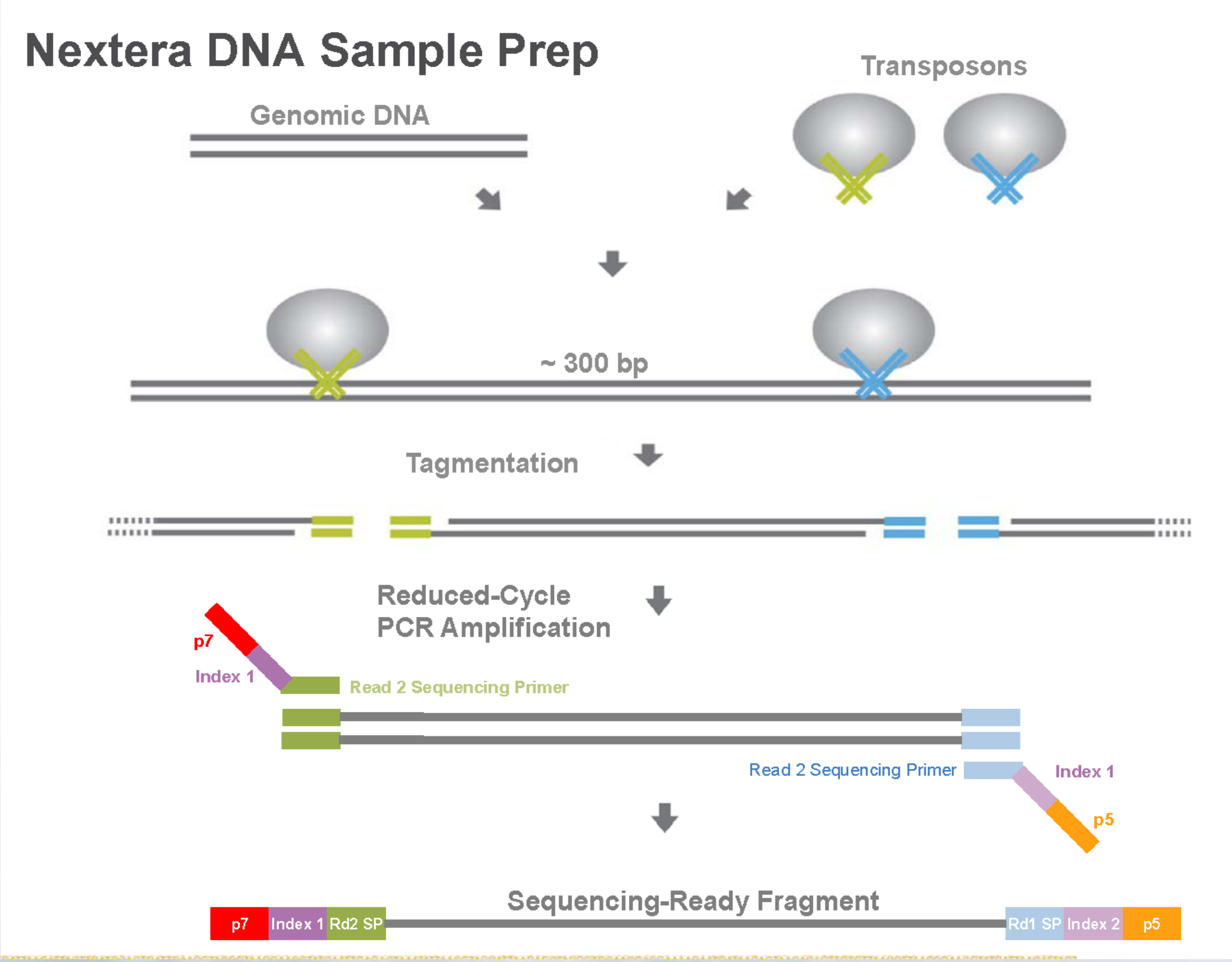
Serious
https://www.youtube.com/watch?v=womKfikWlxM

Not so serious
https://www.youtube.com/watch?v=-7GK1HXwCtE

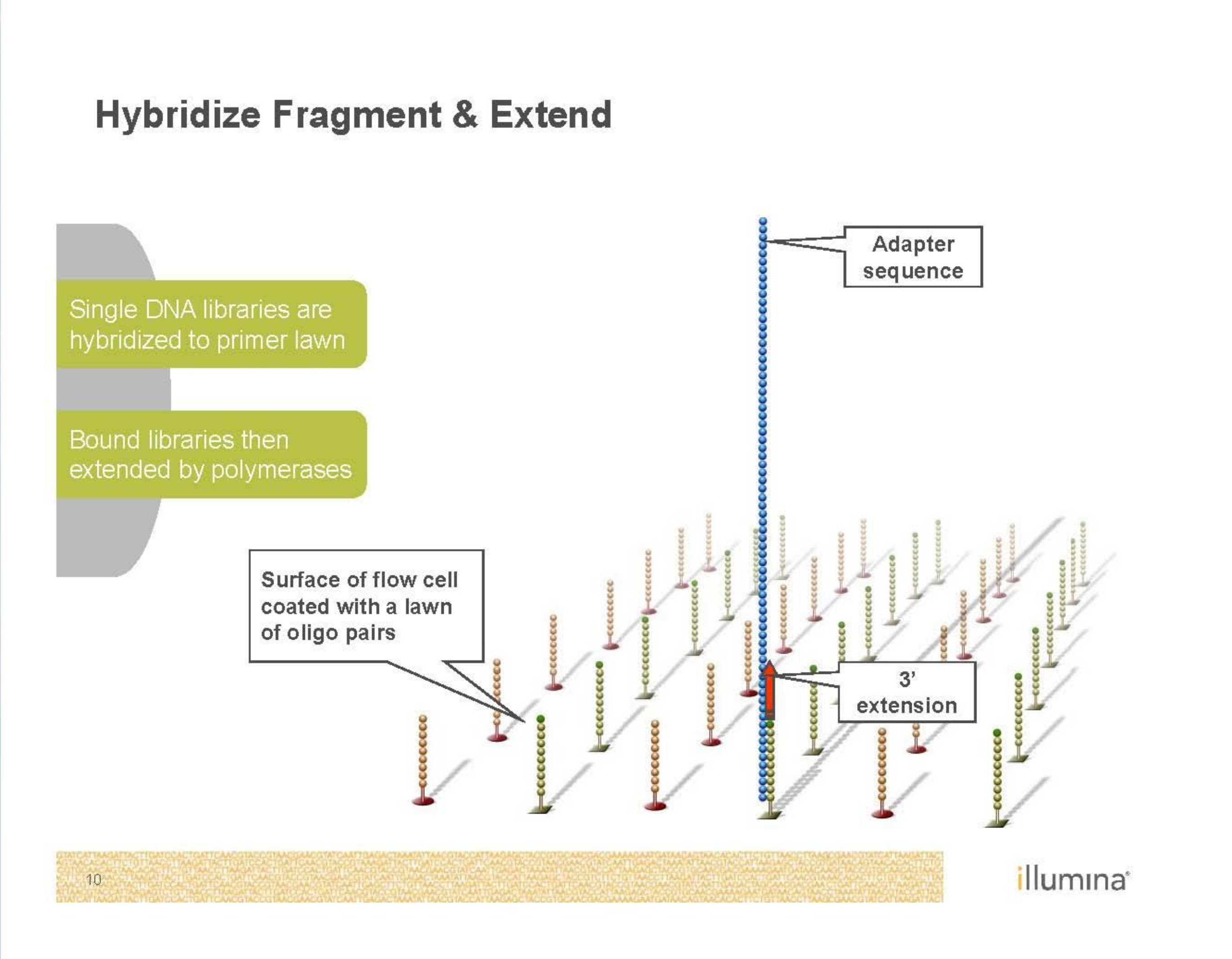
Basic Process



Alternative approach



Sequencing on the inside surface of a flowcell



Hybridize Fragment & Extend

Single DNA libraries are hybridized to primer lawn

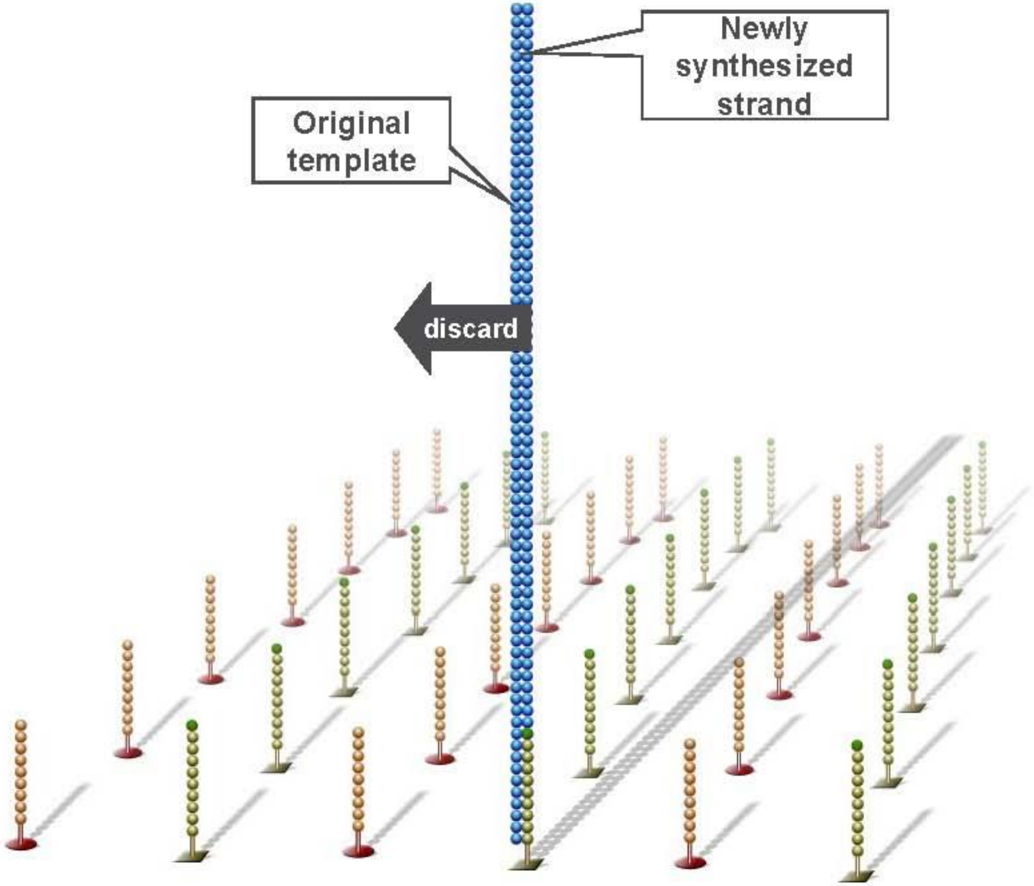
Bound libraries then extended by polymerases

Denature Double-stranded DNA

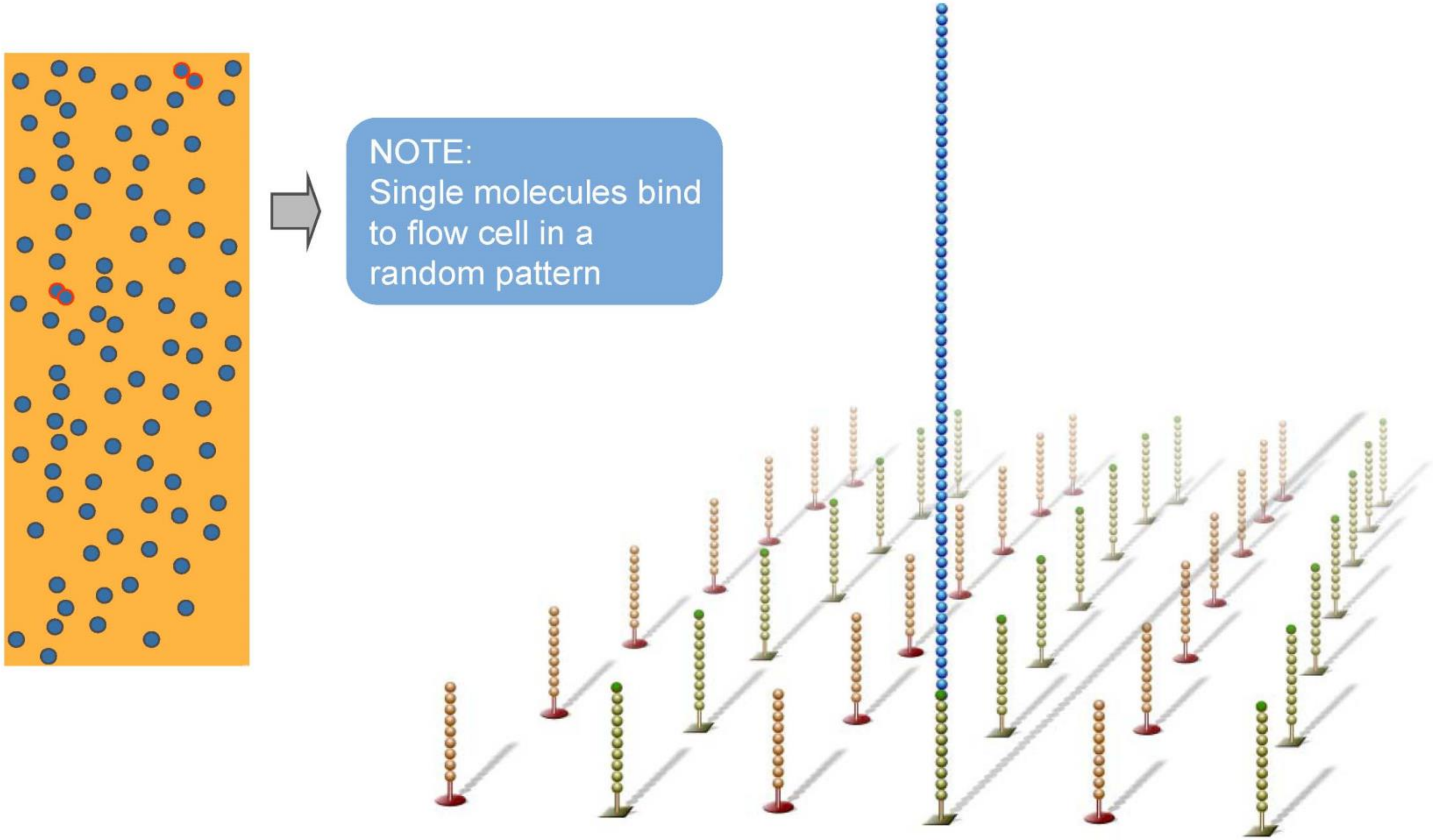
Double-stranded molecule is denatured

Original template washed away

Newly synthesized strand is covalently attached to flow cell surface



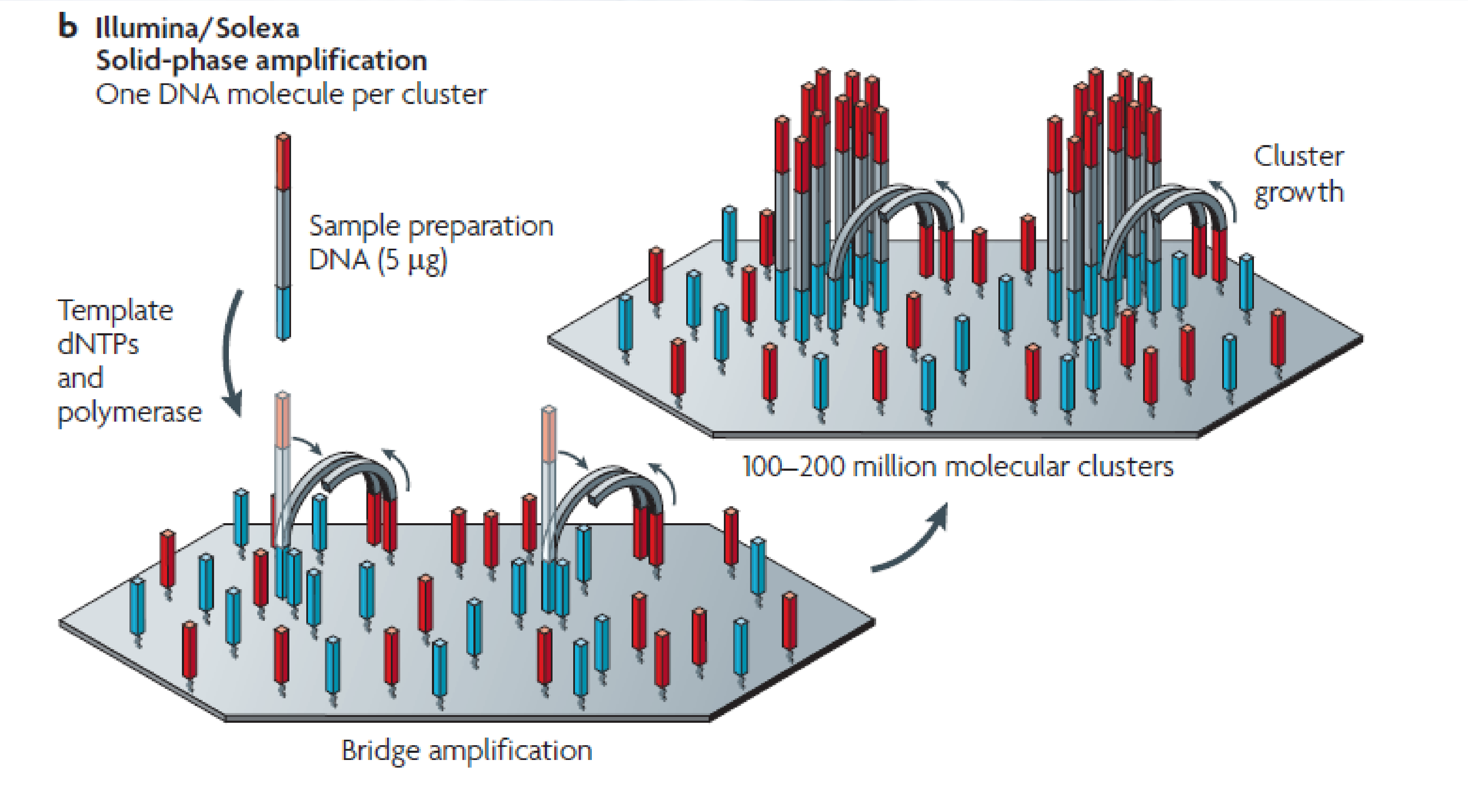
Hybridize Fragment & Extend



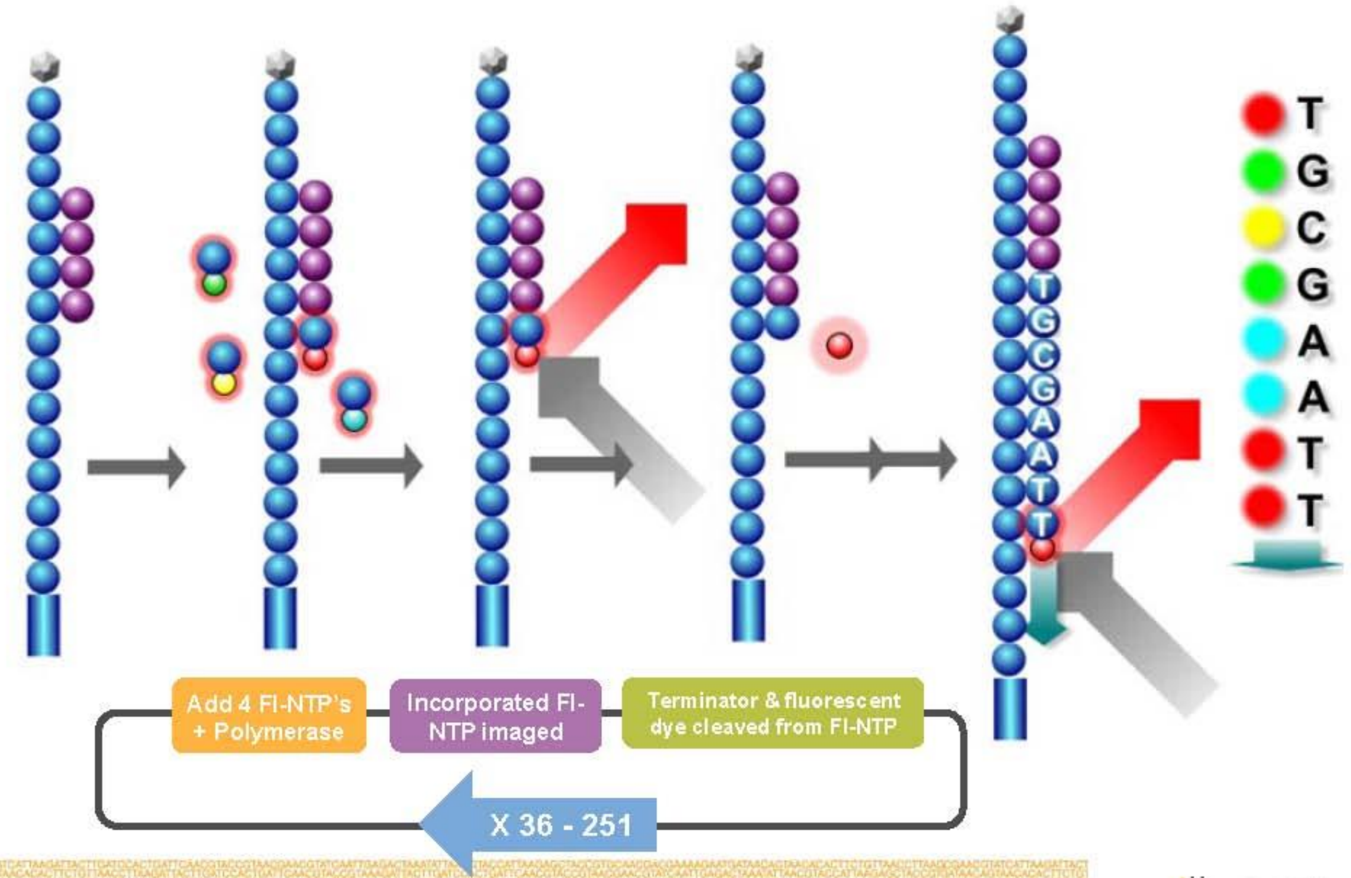
NOTE:
Single molecules bind to flow cell in a random pattern

Making sequencing massively parallel

- Solid Phase


b Illumina/Solexa
Solid-phase amplification
One DNA molecule per cluster
Sample preparation DNA (5 μg)
Template dNTPs and polymerase
Cluster growth
100–200 million molecular clusters
Bridge amplification

Sequencing By Synthesis



Add 4 Fl-NTP's + Polymerase

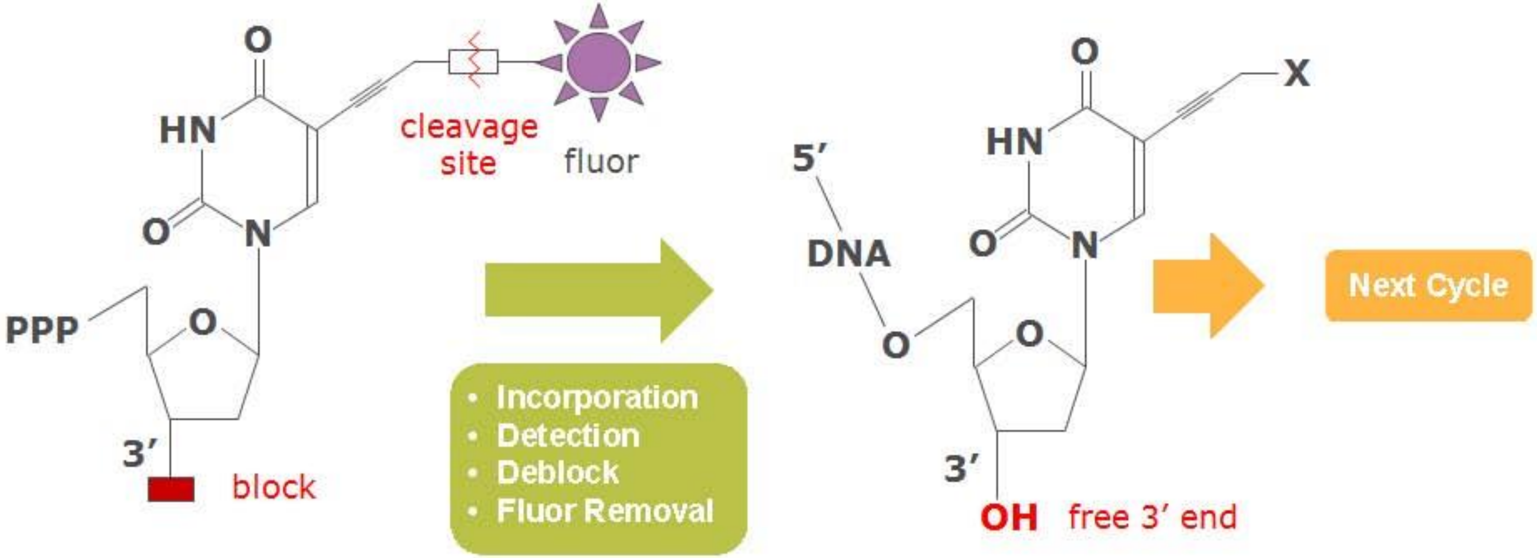
Incorporated Fl-NTP imaged

Terminator & fluorescent dye cleaved from Fl-NTP

X 36 - 251

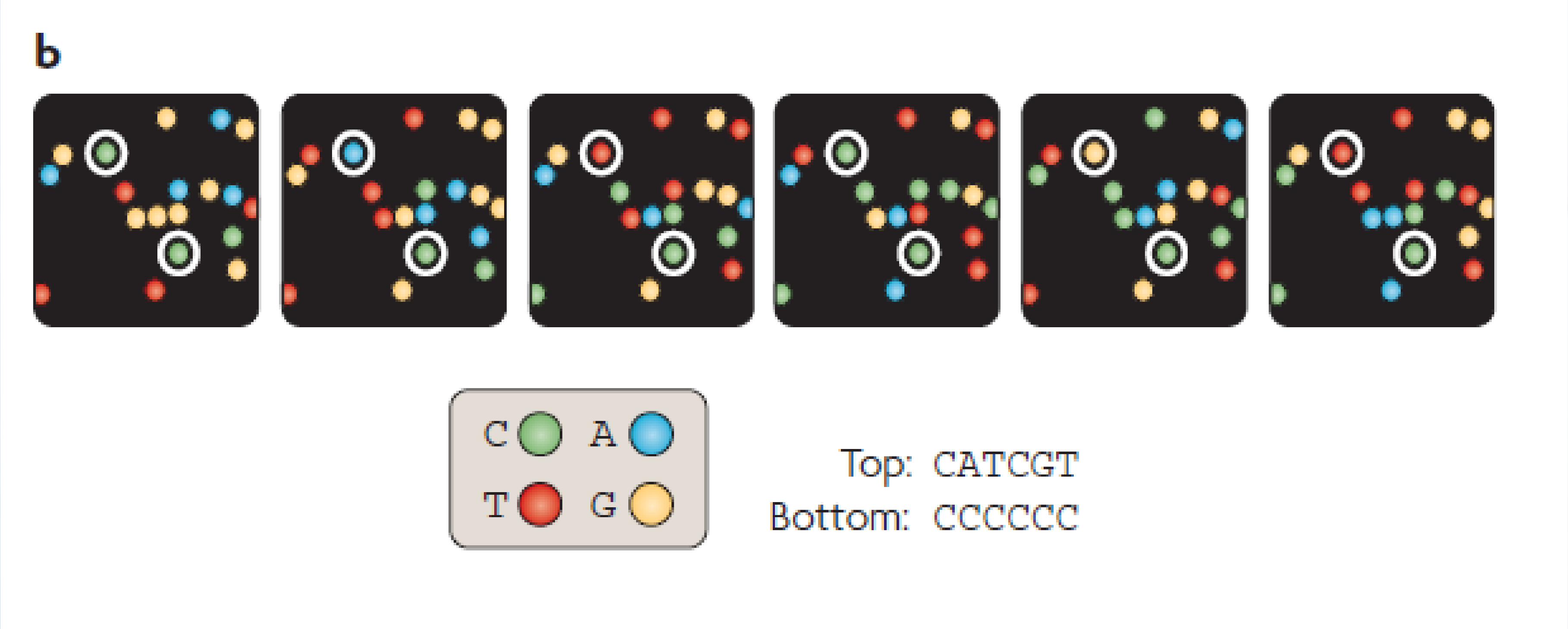
Reversible Terminator Chemistry

- All 4 labeled nucleotides in 1 reaction
- Higher accuracy
- No problems with homopolymer repeats

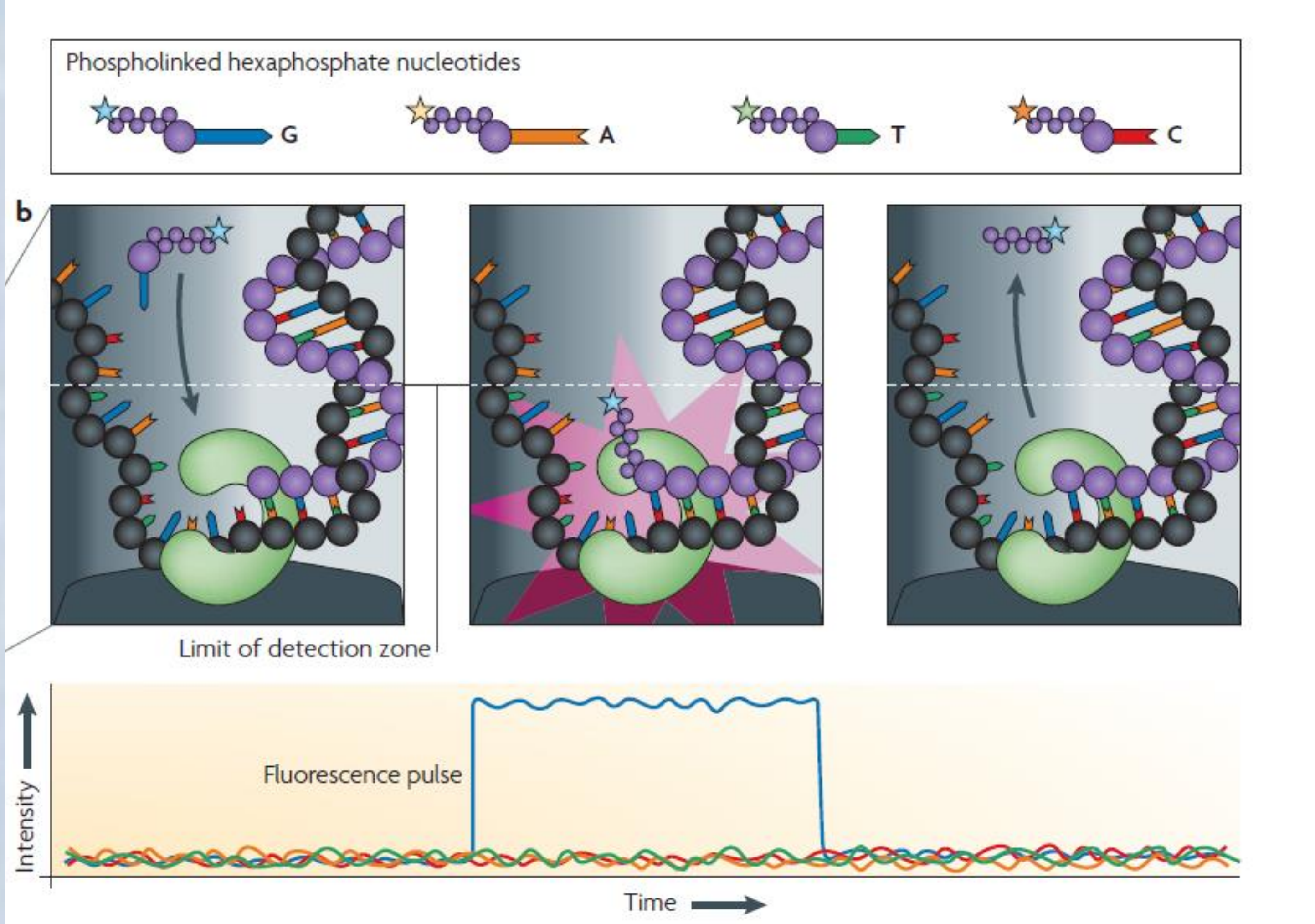


- Incorporation
- Detection
- Deblock
- Fluor Removal

Illumina Stargazing



Pac Bio



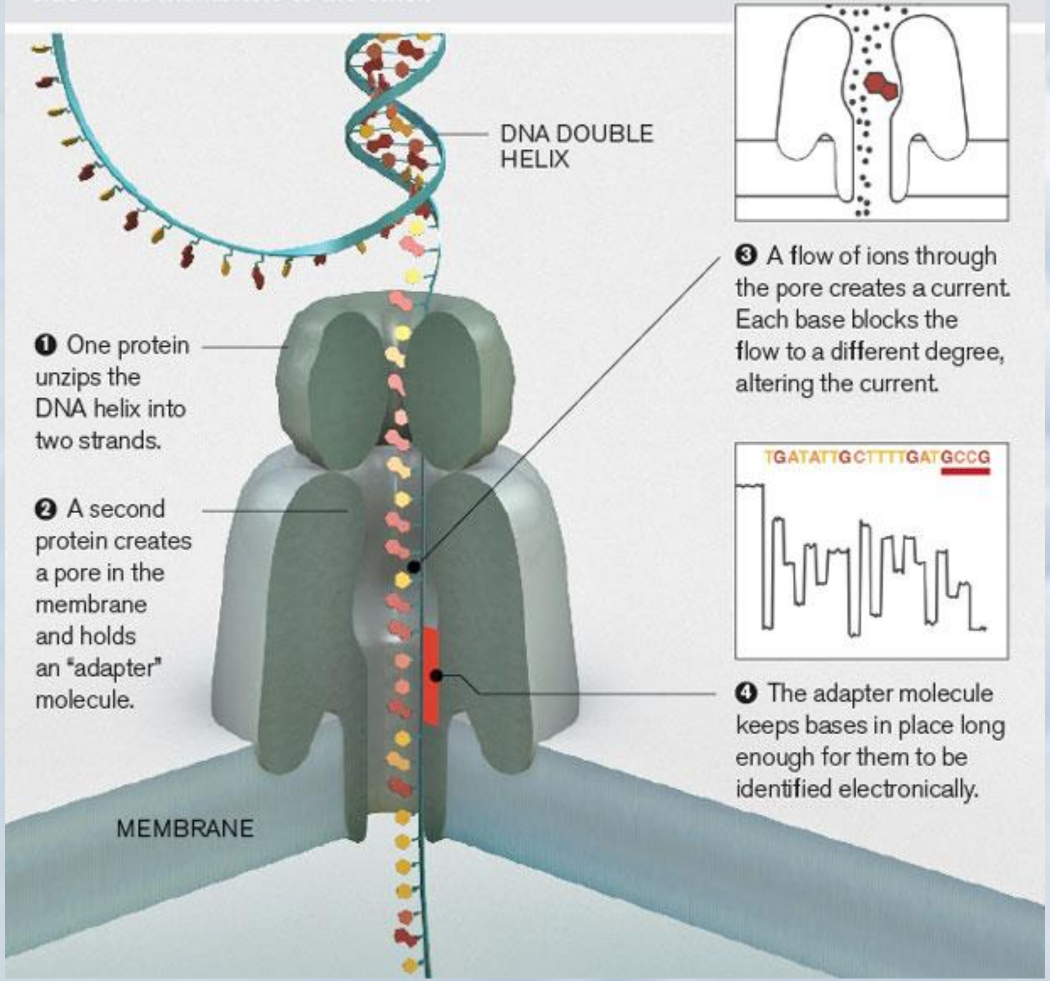
Watch a Video showing the basics of the technology

https://www.youtube.com/watch?v=hBr0TJg-N6U

Nanopore Sequencing

https://www.youtube.com/watch?v=3UHw22hBpAk

DNA can be sequenced by threading it through a microscopic pore in a membrane. Bases are identified by the way they affect ions flowing through the pore from one side of the membrane to the other.

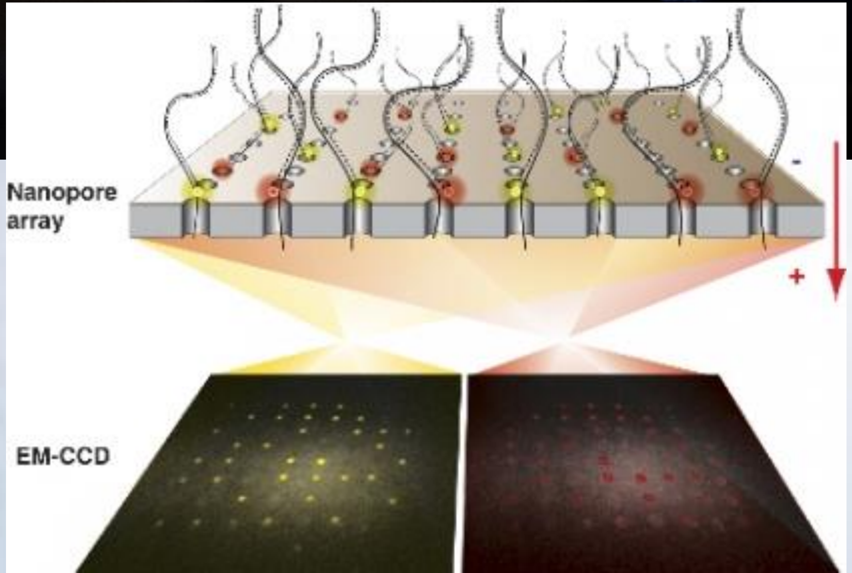


❶ One protein unzips the DNA helix into two strands.

❷ A second protein creates a pore in the membrane and holds an “adapter” molecule.

❸ A flow of ions through the pore creates a current. Each base blocks the flow to a different degree, altering the current.

❹ The adapter molecule keeps bases in place long enough for them to be identified electronically.



Data structures and conventions

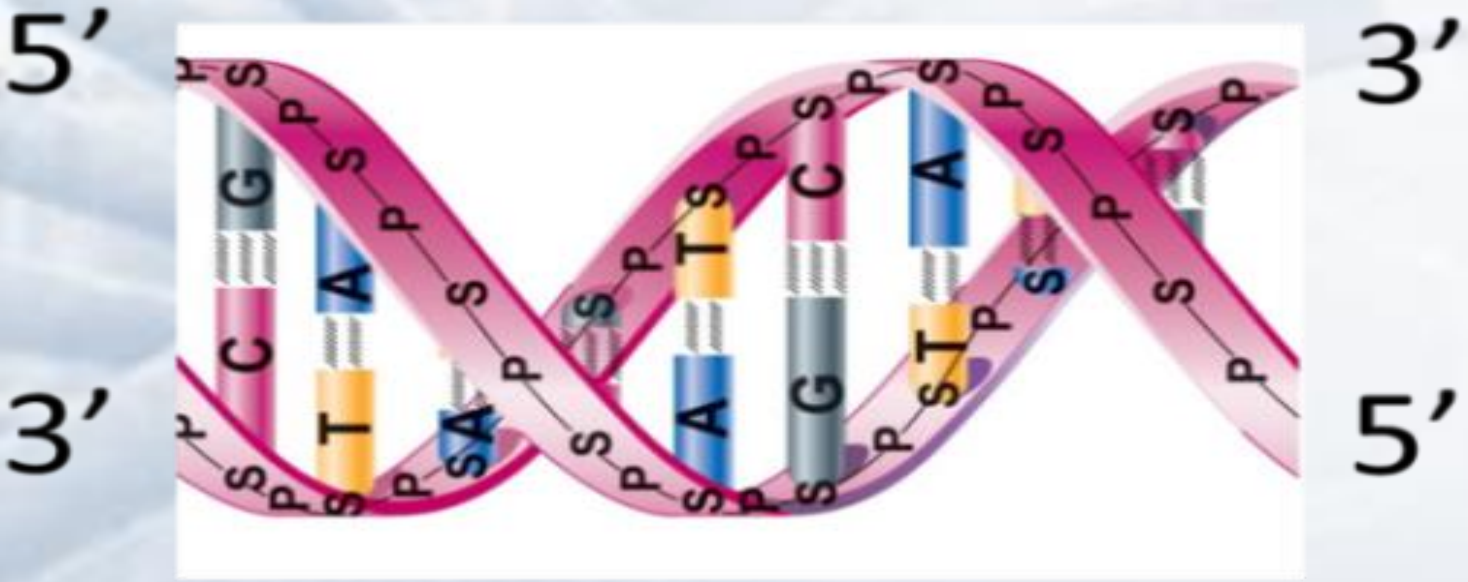
- The interaction of computers and data requires that we follow strict conventions for how we communicate genomic data.
- Bioinformatics relies on the use of common file formats.

Some simple concepts about DNA sequence data

In bioinformatics, DNA or RNA is depicted in a single string from 5’ to 3’ and only one strand shown (saves space). Similarly, protein sequences are always written from the N-terminus to the C- terminus.

“It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.”

In J.D. Watson and F.H.C. Crick, 'A Structure for Deoxyribose Nucleic Acid,' Letter in *Nature* (25 Apr 1953)



The FASTA File Format

n FASTA

9295|sp|P01013|OVAX_CHICK GENE X PROTEIN (OVALBUMIN-RELATED)
LVSSSTDLDTTLVLVNAIYFKGMWKTAFNAEDTREMPFHVTKQESKPVQMMCMNNSFNVATLPAE
ELPFASGDLSMLVLLPDEVSDLERIEKTINFEKLTEWTNPNTMEKRRVKVYLPQMKIEEKYNLTS
LGMTDLFIPSANLTGISSAESLKISQAVHGAFMELSEDGIEMAGSTGVIEDIKHSPESEQFRADHP
KHNPTNTIVYFGRYWSP

otide FASTA

014849.1 EIXKN4201CFU84 length=93
GGGGGGGGGGGCTTTTTTTGTTTGGAACCGAAAGG
TGAATTTCAAACCCTTTTCGGTTTCCAACCTTCCAA
ATGCCAATA

y Files with PHRED quality scores are often created in parallel

014849.1 EIXKN4201CFU84 length=93
5 3 2 1 1 1 1 1 1 1 1 1 1 1 22 37 31 22 16 11 6 1 26 34 30 11 33 26 30 21
25 36 32 16 36 32 16 36 32 20 6 24 33 25 30 25 2 24 36 32 15 35 31 17
20 6 25 29 20 30 25 4 32 26 32 2332 26 30 24 33 26 35 31 14 28 27 30 22
27 17 32 23 28 28

Extension	Meaning
.fna	fasta nucleic acid
.ffn	FASTA nucleotide of gene regions
.fasta (.fas)	generic fasta
.faa	fasta amino acid
.fq (.fastq)	FASTQ file

Quality scores and meaning. Quality scores = -log$_{10}$(probability of error)

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90 %
20	1 in 100	99 %
30	1 in 1000	99.9 %
40	1 in 10000	99.99 %
50	1 in 100000	99.999 %

FASTQ file format

The general format is similar in style to FASTA but has quality score attached in same file and given as ASCII characters to save space. This is the common format for raw sequence data.

@title and optional description
sequence line(s)
+optional repeat of title line
quality line(s)

```
@SRR014849.1 EIXKN4201CFU84 length=93
GGGGGGGGGGGGGGGGCTTTTTTTGTTTGGAACCGAAAGG
GTTTTGAATTTCAAACCCTTTTCGGTTTCCAACCTTCCAA
AGCAATGCCAATA
+SRR014849.1 EIXKN4201CFU84 length=93
3+&$#"""""""""""7F@71,’";C?,B;?6B;:EA1EA
1EA5’9B:?:#9EA0D@2EA5’:>5?:%A;A8A;?9B;D@
/=<?7=9<2A8==
```

Sequences are expected to be represented in the standard IUB/IUPAC amino acid and nucleic acid codes.

The nucleic acid codes are:

A	adenosine	C	cytidine	G	guanine
T	thymidine	N	A/G/C/T (any)	U	uridine
K	*G/T (keto)*	*S*	*G/C (strong)*	*Y*	*T/C (pyrimidine)*
M	*A/C (amino)*	*W*	*A/T (weak)*	*R*	*G/A (purine)*
B	*G/T/C*	*D*	*G/A/T*	*H*	*A/C/T*
V	*G/C/A*	*-*	*gap of indeterminate length*		

The amino acid codes are:

A	alanine
B	aspartate/asparagine
C	cystine
D	aspartate
E	glutamate
F	phenylalanine
G	glycine
H	histidine
I	isoleucine
K	lysine
L	leucine
M	methionine
N	asparagine
P	proline
Q	glutamine
R	arginine
S	serine
T	threonine
U	*selenocysteine*
V	valine
W	tryptophan
Y	tyrosine
Z	glutamate/glutamine
X	any
*	translation stop
-	*gap of indeterminate length*

GFF files describe the position of genes in a FASTA file.

The **general feature format** (**gene-finding format**, or **generic feature format**, **GFF**) is a file format used for describing genes and other features of DNA, RNA and protein sequences. The filename extension is .GFF.

In a GFF file there are 9 columns of information for each feature in a DNA sequence (FASTA file).

Position	Name	
1	sequence	
2	source	
3	feature	
4	start	
5	end	
6	score	
7	strand	
8	frame	
9	attributes.	

Sequence Alignment Map (SAM) Files or BAM for binary version

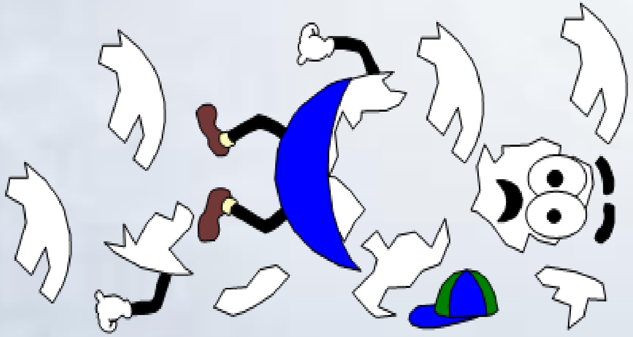
11 mandatory fields in each row: separated by spaces in a text file

1=Read Name, 2=bitwise flag, 3=Reference Sequence, 4=start position of read in reference, 5= Map Quality, 6= CIGAR string, 7= paired end read reference, 8=position of mate, 9=distance between reads, 10=Read sequence, 11=read quality.

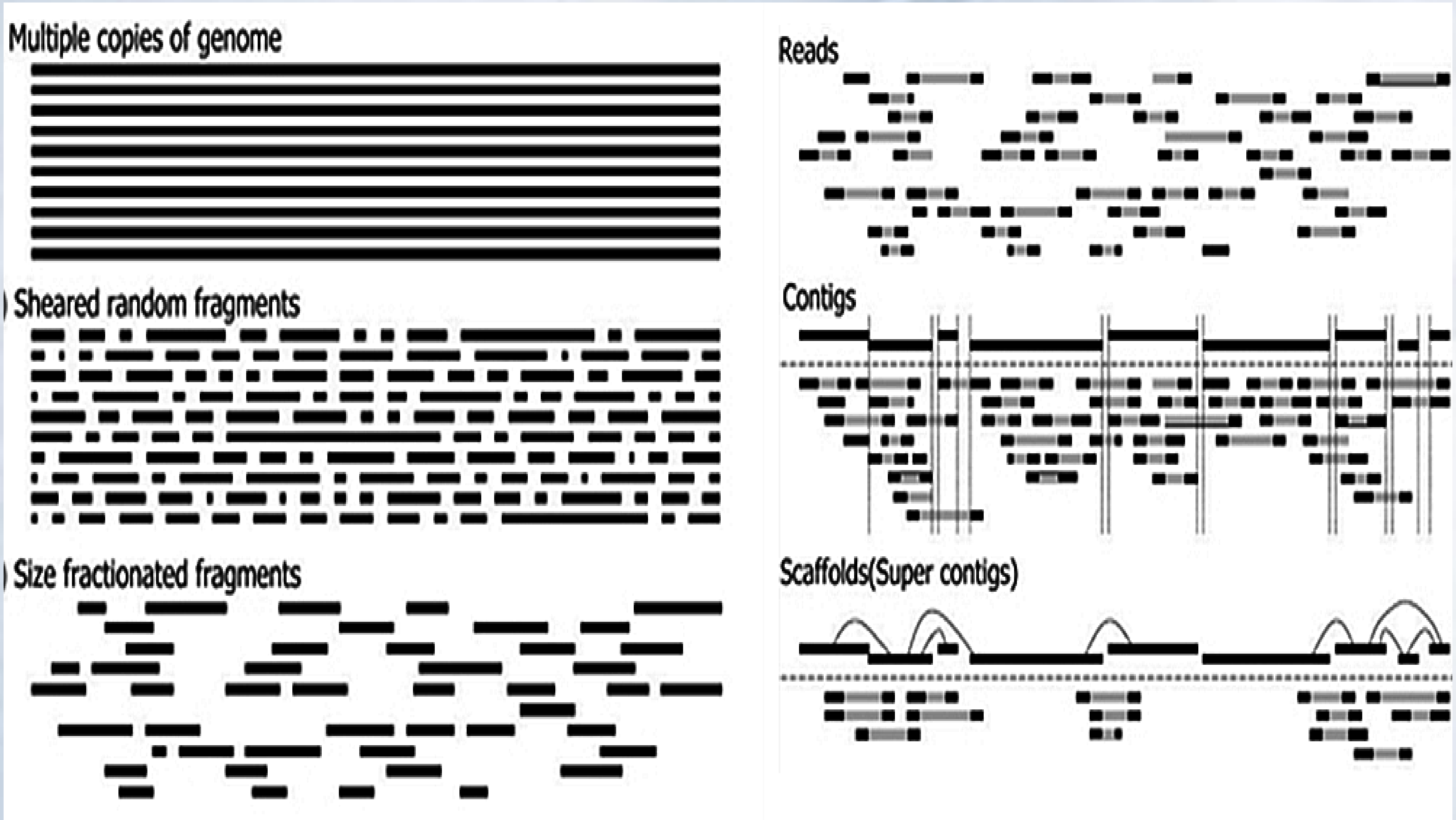
```
1:497:R:-272+13M17D24M 113 chr1 497 37 37M chr15 100338662 0

CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG

0;==-==9;>>>>>>=>>>>>>>>>>>>>=>>>>>>>>>>
```

Sequencing and assembling a genome

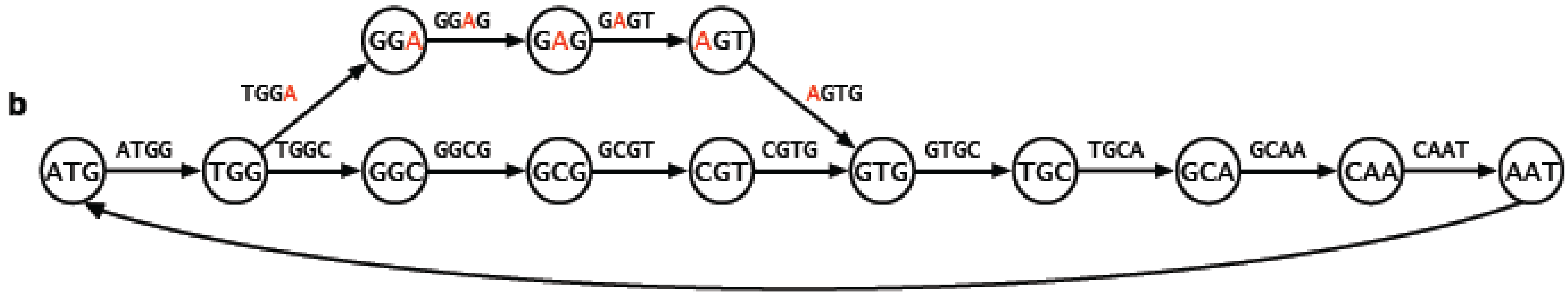
The process of sequencing genomes typically involves breaking the genome up and then attempting to put Humpty-Dumpty back together again.



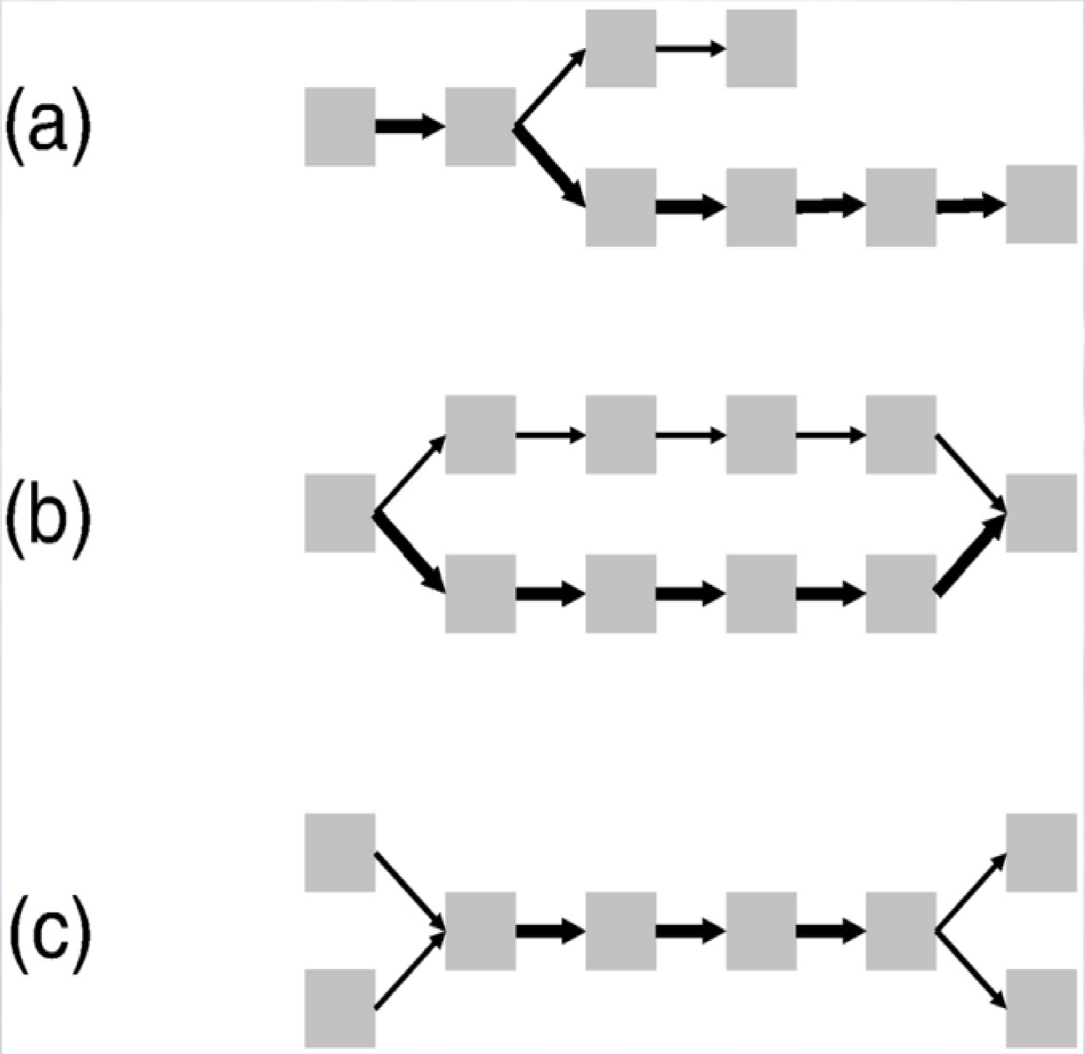
Sequencing a genome


Multiple copies of genome
) Sheared random fragments
) Size fractionated fragments
Reads
Contigs
Scaffolds(Super contigs)

In real data, there will be sequencing errors and polymorphisms. In the figure below, a single base difference results in two paths that diverge and then converge. This could be caused by a sequencing error in the middle of a read or polymorphisms. If this represents heterozygosity, the paths may have equal representation.



In the diagram below, the path complexities include spurs that will result from a sequencing error at the end of a read, bubbles as shown above and “rope ends”. Rope ends depict two different paths that share a common set of k-mers. These are the result of repeats that are greater than the length of a k-mer.



Spur

Bubbles

Rope ends

Assembly output and assessing the quality of an assembly:

De novo and assembly produces two main outputs.

- **Contig file** (FASTA or multi FASTA)
- **SAM file:** SAM (Sequence Alignment/Map) format is a generic format for storing large nucleotide sequence alignments.

How do we assess the quality of an assembly? There are three basic measures of assembly quality:

1. **N50:** A measure of average contig size. Specifically, ½ of the genome is assembled in contigs of this size or greater.
2. **Depth of coverage:** A measure of how much information is available for each base call.
3. **Completeness of the gene catalogue:** What percentage of the genes are assembled into contigs?

Key challenges for genome assembly:

Intrinsic Challenges:

1. ***Heterozygosity:*** The alleles of a gene are not the same, yet we typically force them into a single consensus sequence.
2. ***Paralogy vs. Alleleism:*** Genes come from other genes by a process of duplication. This results in two or more similar genes in an organism. There are two alleles in a diploid organism that are very similar. How do you tell a duplicated gene from alleles of a gene?
3. ***Sequence complexity:*** Simple sequence repeats (SSR), large-scale repeats like transposable elements (TEs).

Extrinsic Challenges:

1. ***Quality of DNA sequences (sequencing errors):*** Each sequencing technology has specific patterns of error. For example, pyrosequencing typically has high error rates associated with runs of a single nucleotide.
2. ***Length of DNA sequence reads:*** Shorter reads are less likely to be unique or to include many unique K-mers (see below).
3. ***Coverage:*** Depth of Coverage is a random process at best. Consequently some regions of the genome will have low levels of coverage.
4. ***Memory intensive:*** Inherently requires large amounts of RAM for assembly and storage for input and output.
5. ***Software:*** Need for approaches that are flexible, user friendly and powerful.

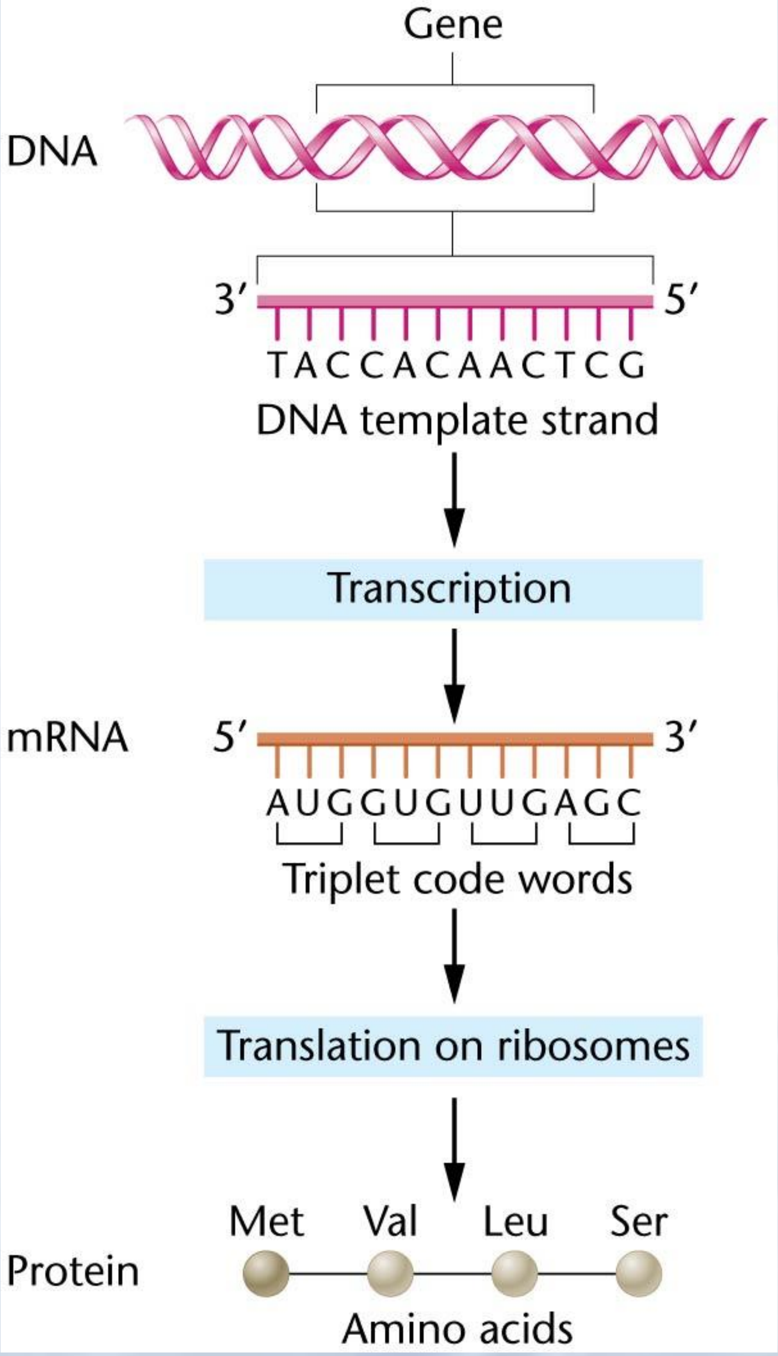
Genome annotation and inferring function

Once we have assembled a genome into one or more large “contigs” how do we “read” the DNA sequences and predict the genes and their functions

Inferring Function from a DNA Sequence

- We use our understanding of cellular processes and evolution to predict the existence and function of genes in DNA sequences.
  - The near universal nature of the genetic code makes it possible to predict what protein sequences can be encoded by any DNA molecule.
  - Evolution allows us to compare genes and their proteins from one species to another.
  - When we have demonstrated the function of a gene in a model organism we often assume it will serve the same or similar role in other species

Most concepts in Bioinformatics rely on core knowledge of Genetics



Gene

DNA

3′ 5′

TACCACAACTCG

DNA template strand

Transcription

mRNA

5′ 3′

AUGGUGUUGAGC

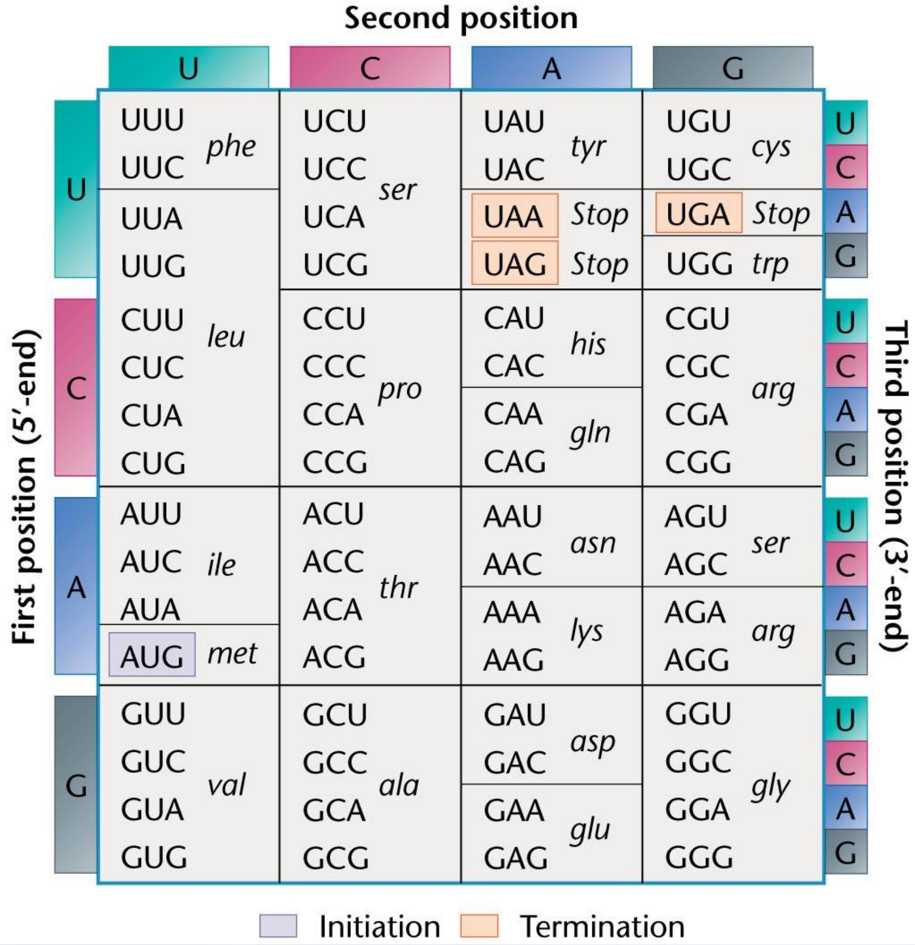
Triplet code words

Translation on ribosomes

Protein

Met Val Leu Ser

Amino acids

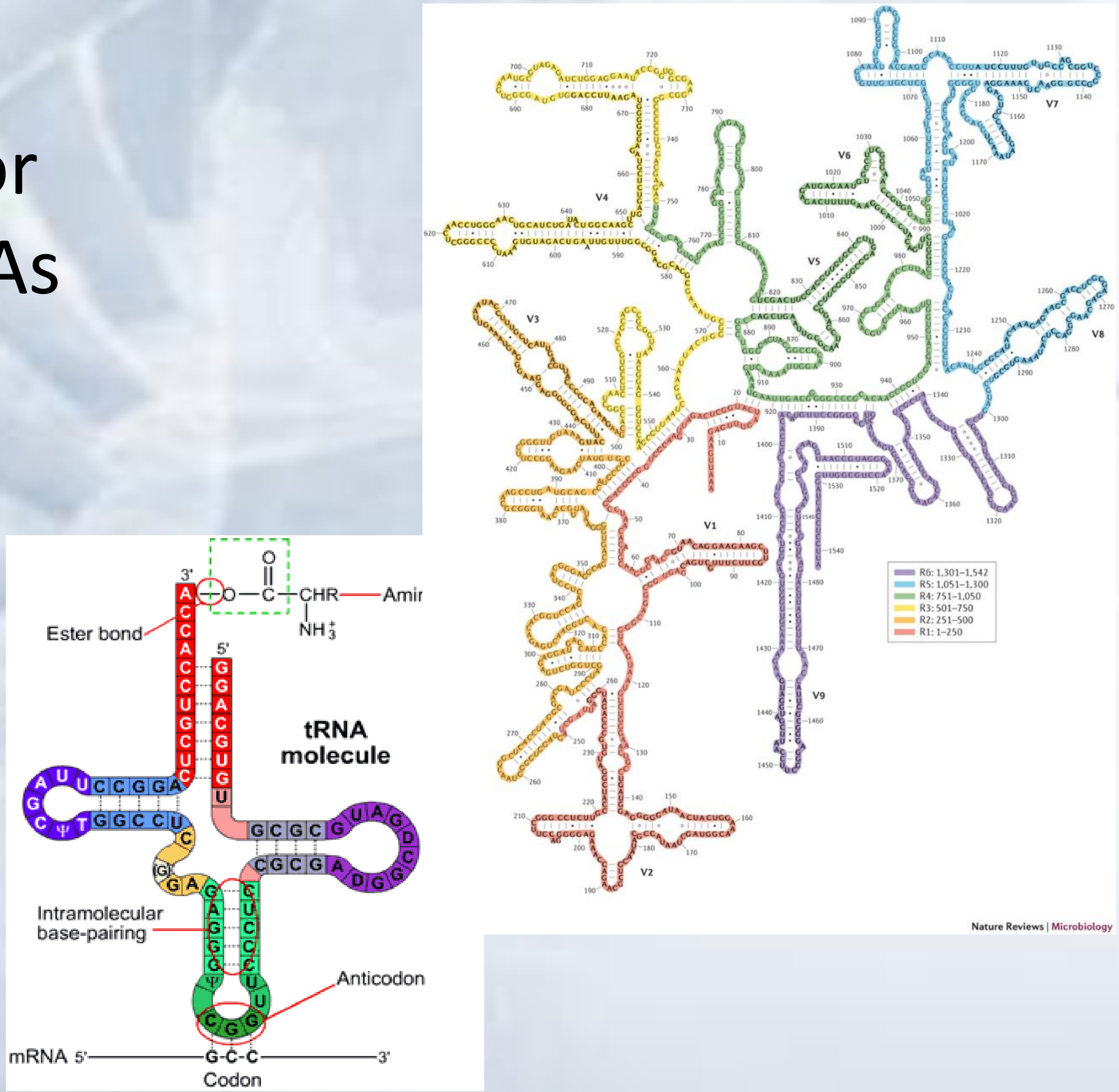


	Second position								
First position (5′-end)	U		C		A		G		Third position (3′-end)
U	UUU	*phe*	UCU	*ser*	UAU	*tyr*	UGU	*cys*	U
	UUC		UCC		UAC		UGC		C
	UUA	*leu*	UCA		UAA	*Stop*	UGA	*Stop*	A
	UUG		UCG		UAG	*Stop*	UGG	*trp*	G
C	CUU	*leu*	CCU	*pro*	CAU	*his*	CGU	*arg*	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	*gln*	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	*ile*	ACU	*thr*	AAU	*asn*	AGU	*ser*	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	*lys*	AGA	*arg*	A
	AUG	*met*	ACG		AAG		AGG		G
G	GUU	*val*	GCU	*ala*	GAU	*asp*	GGU	*gly*	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	*glu*	GGA		A
	GUG		GCG		GAG		GGG		G

▨ Initiation ▨ Termination

Beyond protein coding genes

- Not all genes encode proteins
- How would you find the genes for transfer RNAs and ribosomal RNAs in a DNA molecule?
  - You can look for similar sequences identified in related organisms
  - You can consider their special features like secondary structures



Nature Reviews | Microbiology

Learning to do “Bioinformatics”

- Most tools for bioinformatics are open source.
- The web is a great source of information
- Most tools are run from the command line
- Operating in the command line environment is not hard but it is foreign and a hurdle.

Metagenomics

- The study of genetic information gathered directly from the environment
- Focus on taxonomic identification of all the tiny creatures of the unknown Biome
  - A product of molecular evolution and phylogenetics
  - Transformed by the advent of PCR
  - Transformed again by the advent of NGS

Major Workflows

"Environmental" sample

Marker Gene Surveys

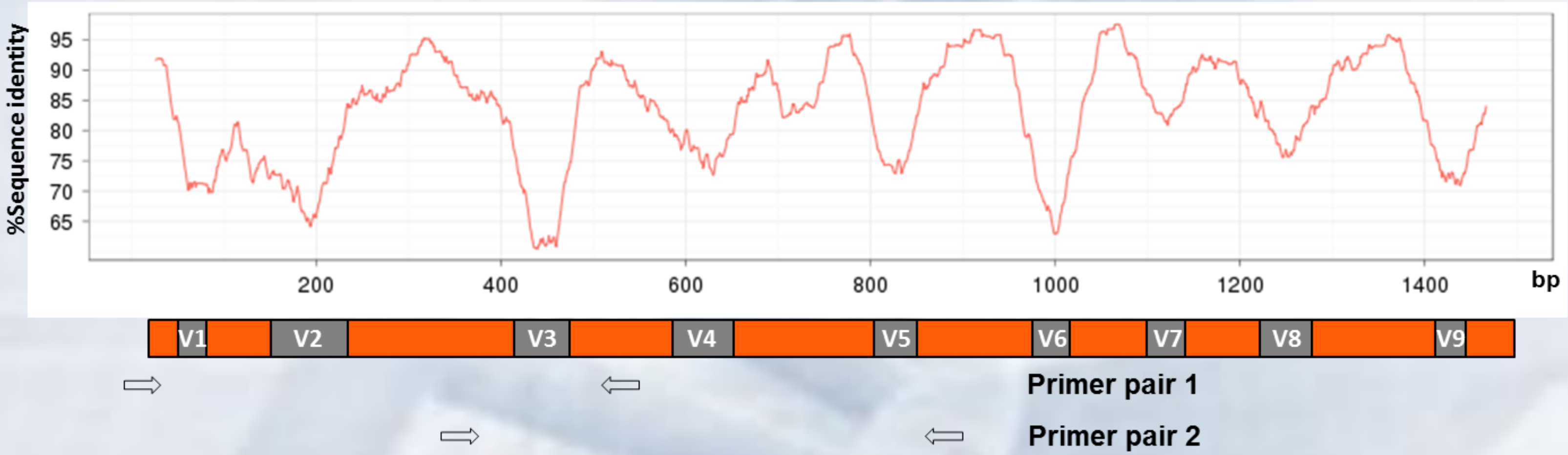
- Positive Aspects
  - Efficient
- Negative Aspects
  - PCR Limitations
  - Limited by databases
  - Not directly related to ecosystem function

Whole Metagenome Shotgun

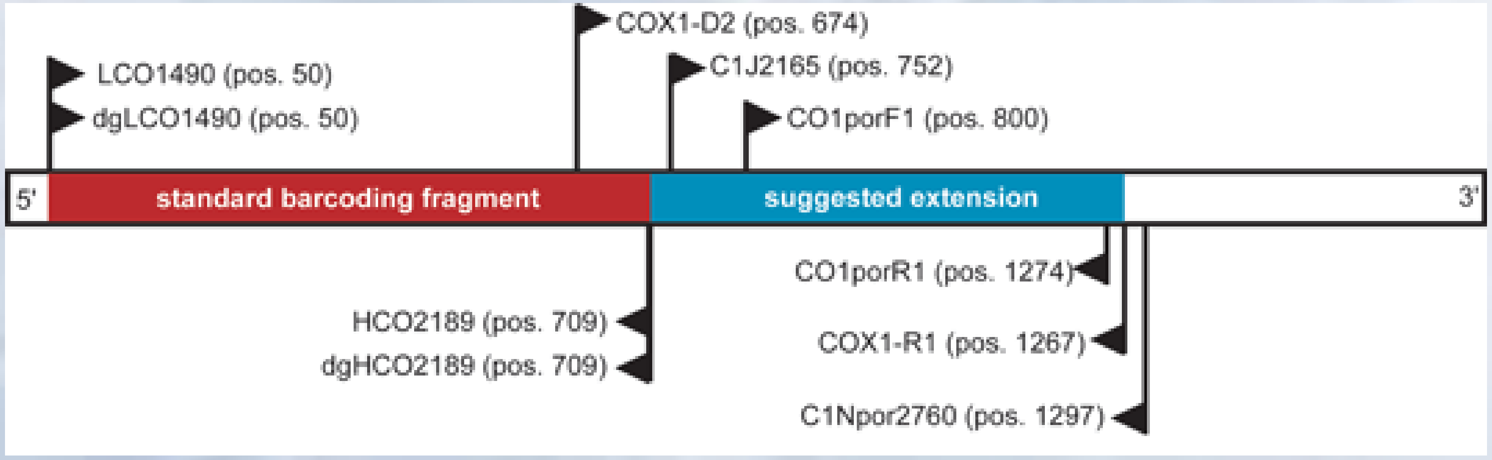
- Positive Aspects
  - Produces functional and taxonomic data
  - Not restricted by PCR "All" "organisms"
- Negative Aspects
  - Not restricted by PCR "All" "organisms"
  - Limited by databases
  - Inefficient

Marker Genes/Barcodes

Ribosomal RNA

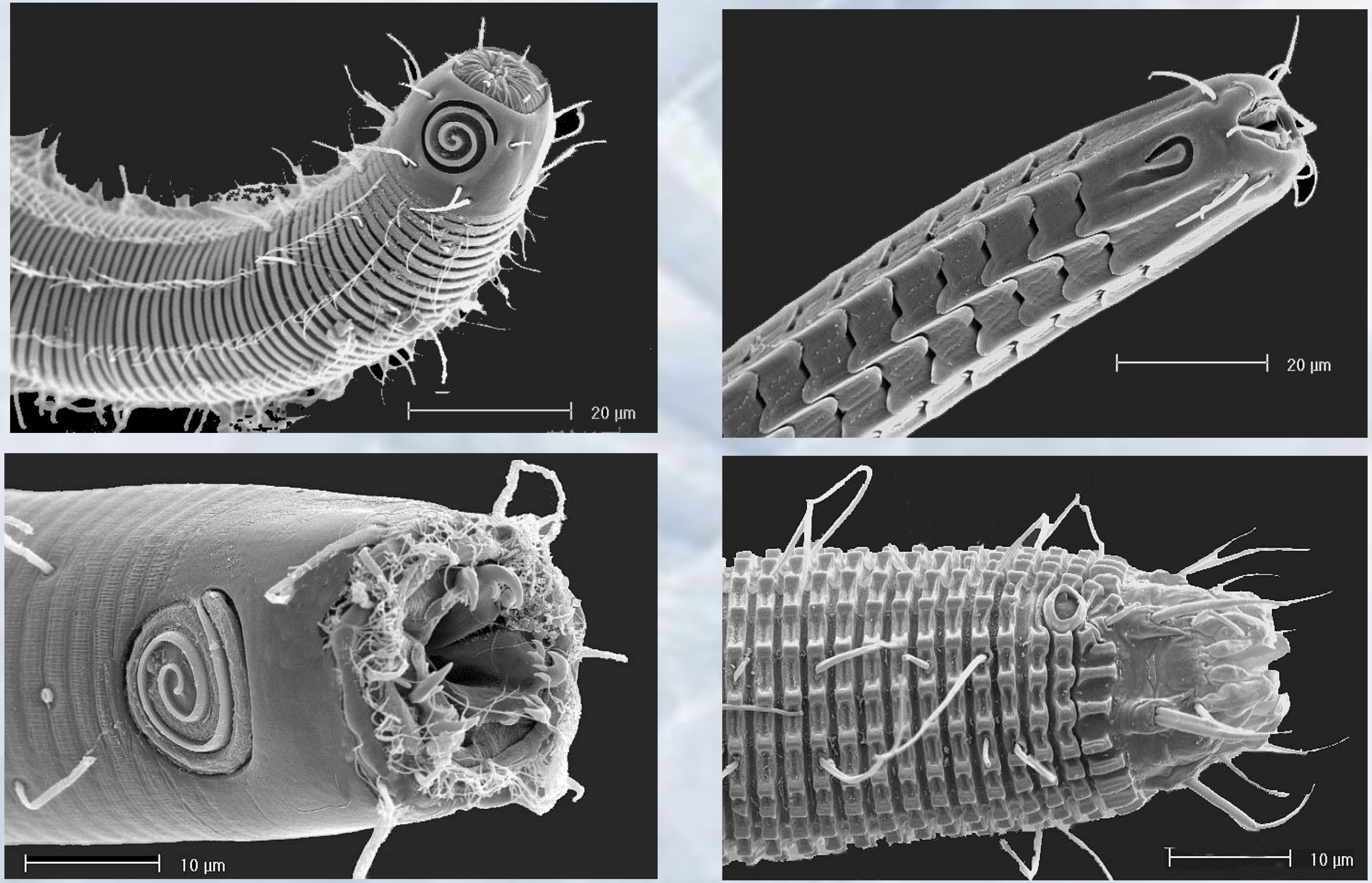


Mitochondrial DNA



Marker gene case study: Consequences of the DWH oil spill

Metagenomic analysis of the meiofauna

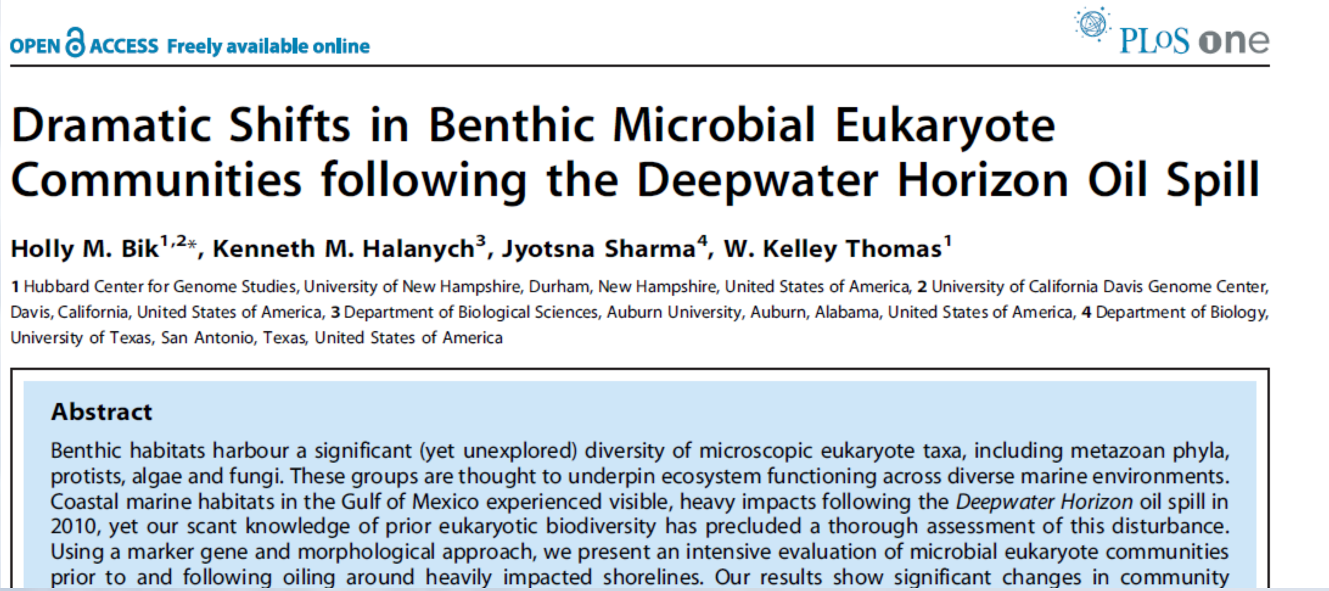


James Baldwin and Manuel Mundo, UCR

Approach

- Extract DNA from environmental samples
- PCR amplify 18s w/conserved primers).
- Next-Generation Sequencing
- Infer biologically meaningful diversity units
- Describe community changes
- Publish

Holly Bik



OPEN ACCESS Freely available online

PLoS one

Dramatic Shifts in Benthic Microbial Eukaryote Communities following the Deepwater Horizon Oil Spill

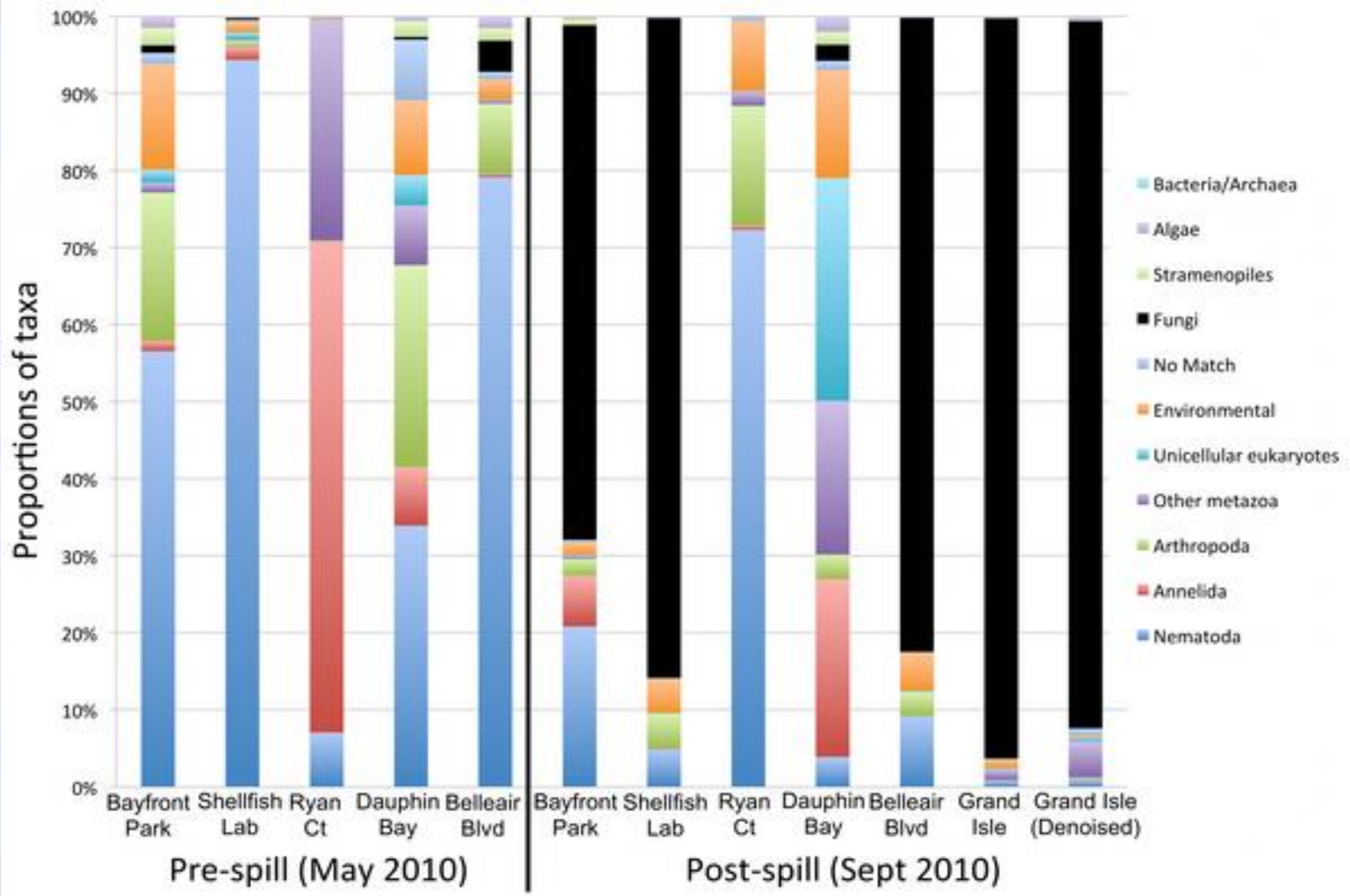
Holly M. Bik[1,2]*, Kenneth M. Halanych[3], Jyotsna Sharma[4], W. Kelley Thomas[1]

1 Hubbard Center for Genome Studies, University of New Hampshire, Durham, New Hampshire, United States of America, **2** University of California Davis Genome Center, Davis, California, United States of America, **3** Department of Biological Sciences, Auburn University, Auburn, Alabama, United States of America, **4** Department of Biology, University of Texas, San Antonio, Texas, United States of America

Abstract

Benthic habitats harbour a significant (yet unexplored) diversity of microscopic eukaryote taxa, including metazoan phyla, protists, algae and fungi. These groups are thought to underpin ecosystem functioning across diverse marine environments. Coastal marine habitats in the Gulf of Mexico experienced visible, heavy impacts following the *Deepwater Horizon* oil spill in 2010, yet our scant knowledge of prior eukaryotic biodiversity has precluded a thorough assessment of this disturbance. Using a marker gene and morphological approach, we present an intensive evaluation of microbial eukaryote communities prior to and following oiling around heavily impacted shorelines. Our results show significant changes in community

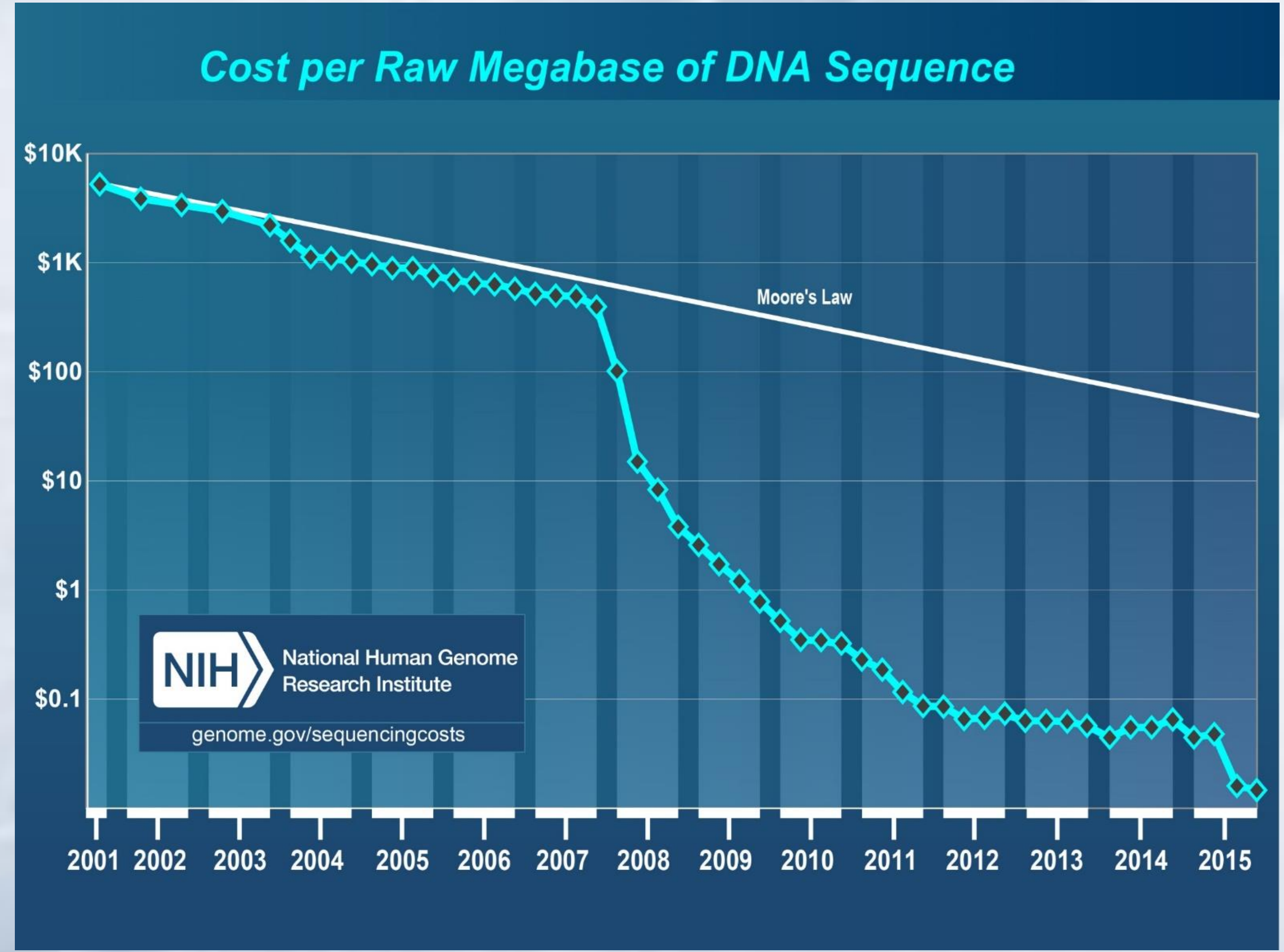
Figure 1. Pre-spill and Post-spill taxonomic comparisons of microbial eukaryote communities.



Bik HM, Halanych KM, Sharma J, Thomas WK (2012) Dramatic Shifts in Benthic Microbial Eukaryote Communities following the Deepwater Horizon Oil Spill. PLoS ONE 7(6): e38550. doi:10.1371/journal.pone.0038550
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0038550

PLOS | ONE

A sea-change in genome-enabled biology is shifting the paradigm



Whole Metagenome “shotgun” approach

- Avoids PCR issues (but not differential library generation issues)
- Can avoid function = taxonomy assumption
- Massive amounts of data that is challenging to analyze
- Still lacking reference databases

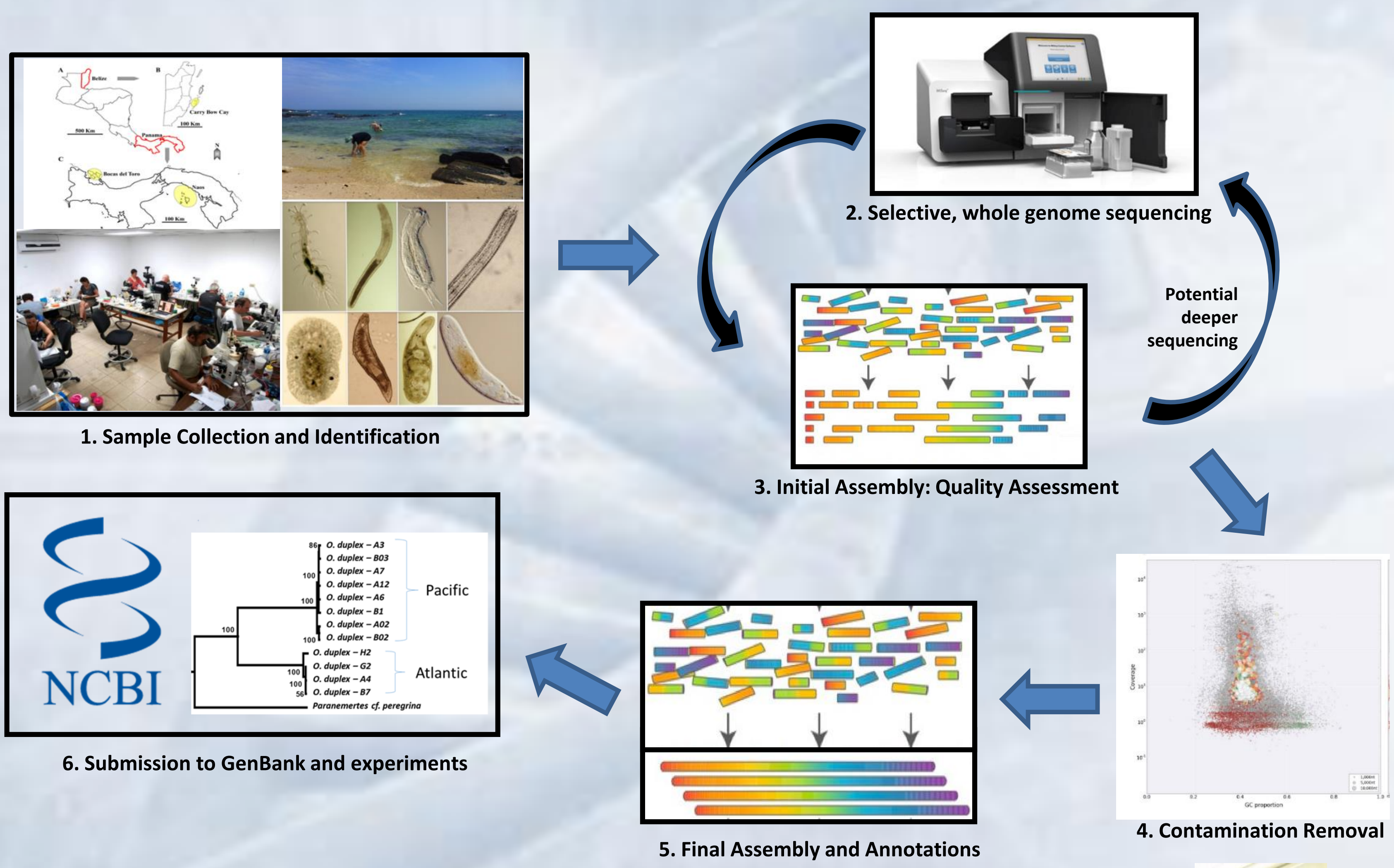
Preparing for the future

GULF OF MEXICO RESEARCH INITIATIVE

Develop procedures to effectively use metagenomics to understand the impacts of future spills in the GoM.

1. **Establish effective methods of analysis**
2. **Draft reference genomes for 200 new families of meiofaunal phyla**

Generating reference genomes for 200 meiofaunal families



1. Sample Collection and Identification

2. Selective, whole genome sequencing

Potential deeper sequencing

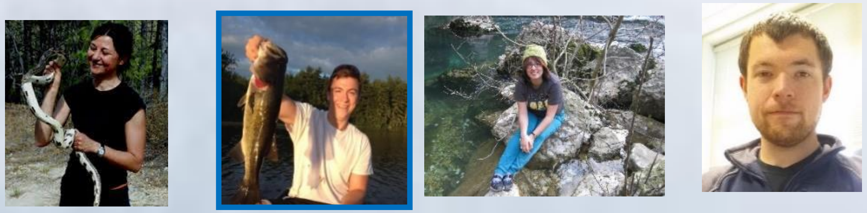
3. Initial Assembly: Quality Assessment

4. Contamination Removal

5. Final Assembly and Annotations

6. Submission to GenBank and experiments

Franci, Joe, Krystalle, and Jordan



Thanks

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- RAPID (NSF): DBI 1262470
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- GoMRI: 2015-V-449