Next-Generation Sequencing (NGS)

Rob Colautti

Biological Sciences

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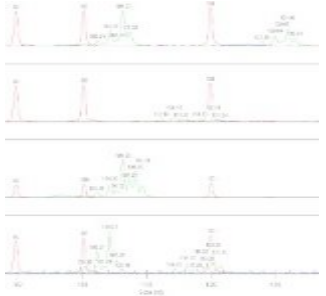
Research Background

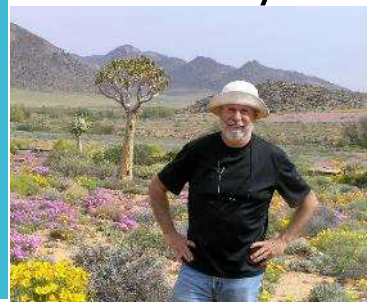
University of Windsor & GLIER – Hs Bsc, MSc

Hugh MacIsaac

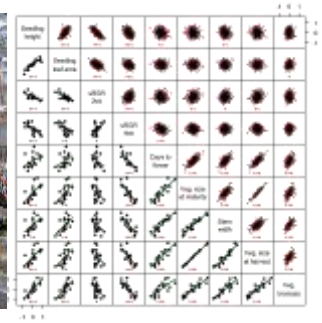
Dan Heath

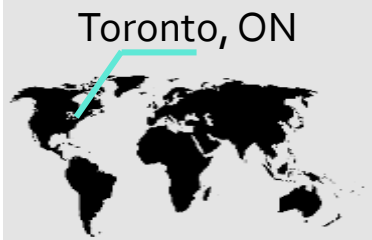


University Toronto – PhD



Spencer Barrett





Research Background

Duke University – Postdoc




Tom Mitchell-Olds

Jill Anderson

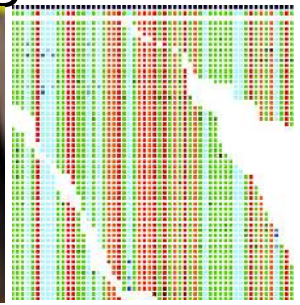
University of British Columbia – Postdoc




Loren Rieseberg

University of Tuebingen – Postdoc





Oliver Bossdorf

Durham, NC

Vancouver, BC

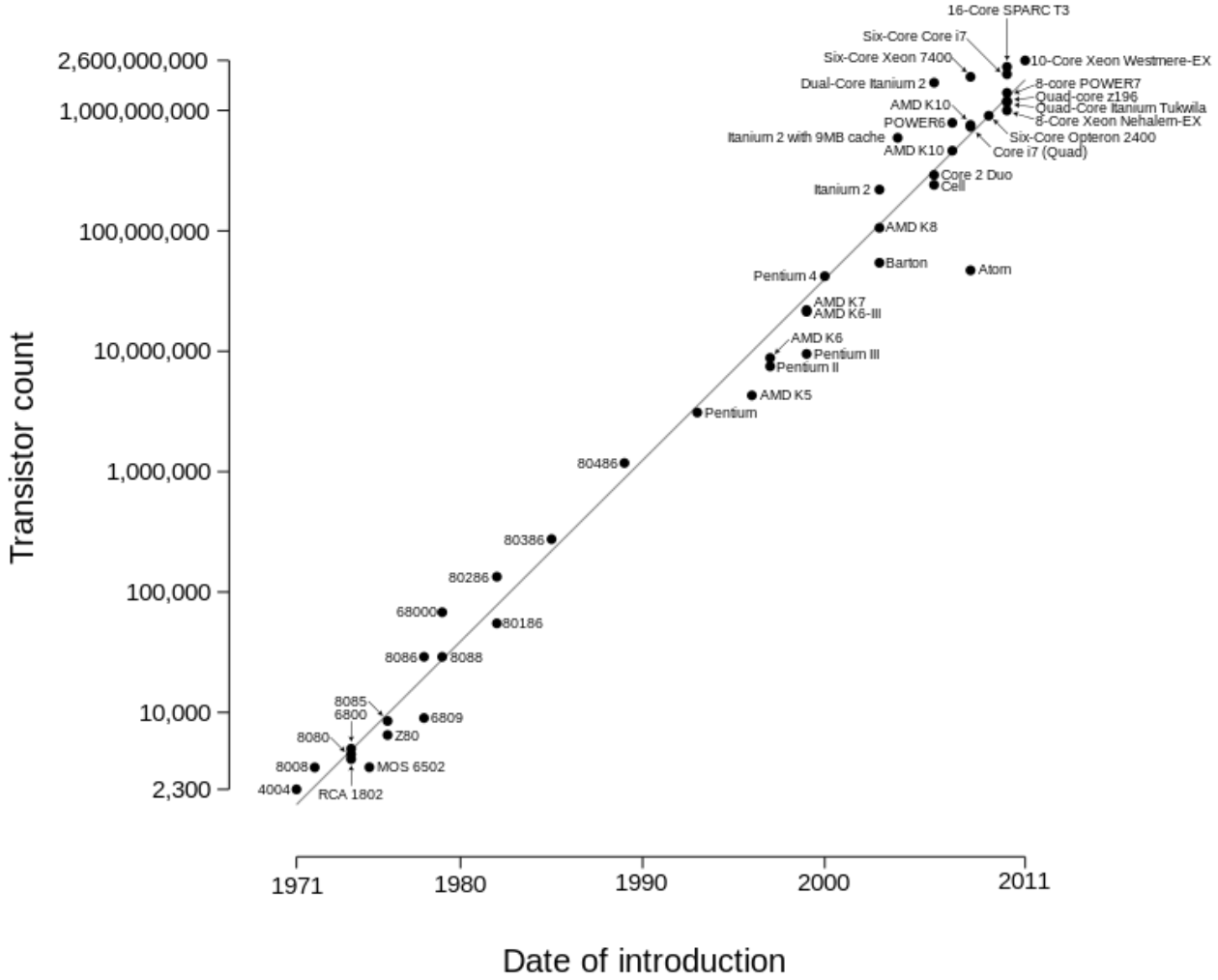
Tuebingen, DE

Overview

- What is Next-Generation Sequencing (NGS)?
- How does NGS work?
- Why is NGS revolutionizing the biological sciences?

Introduction to NGS

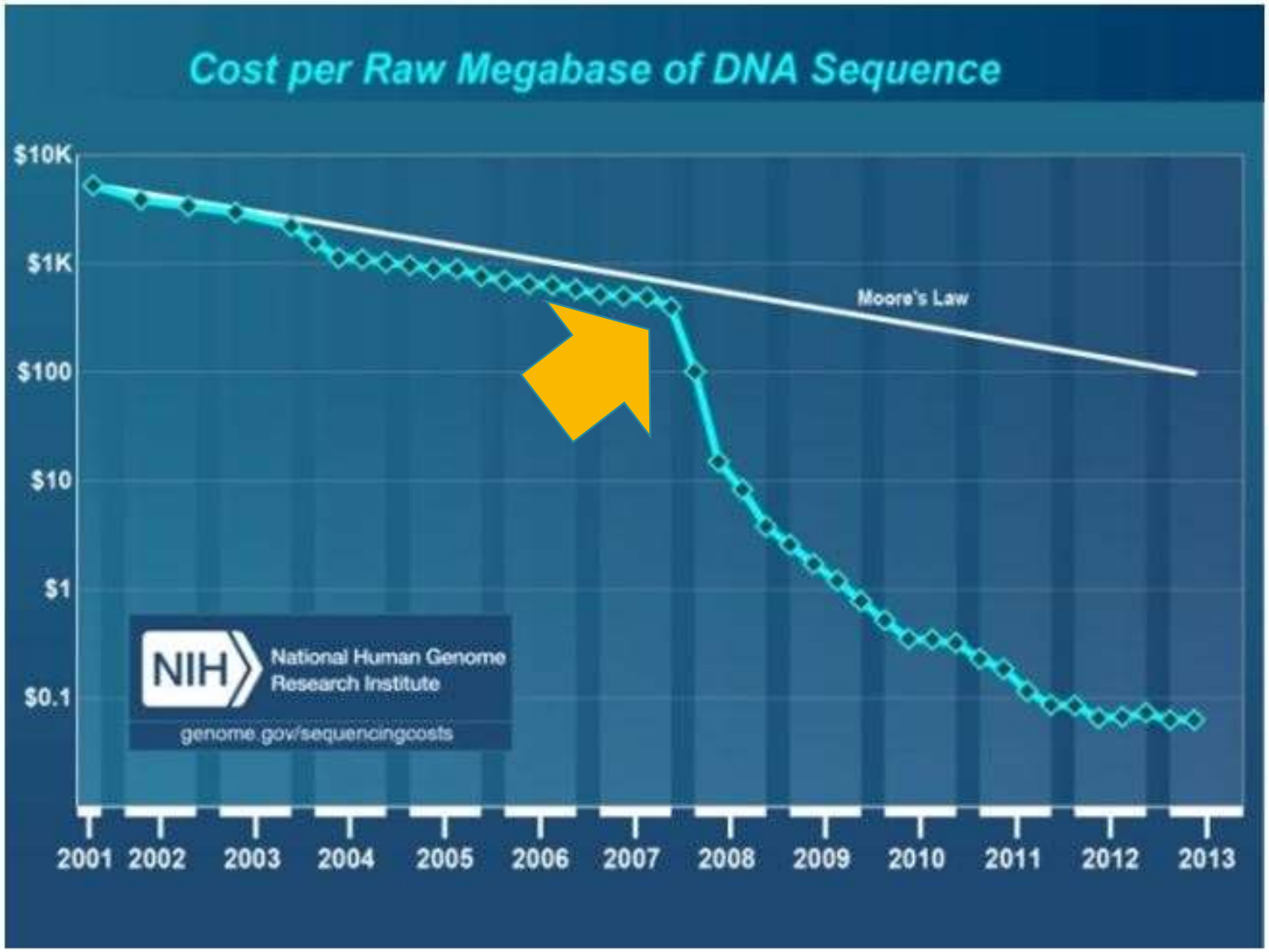
Moore's Law



- "the number of transistors in a dense integrated circuit doubles approximately every two years"

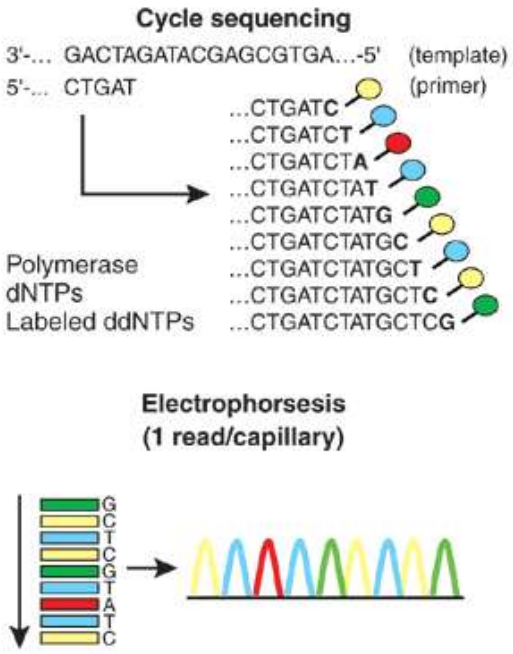
-- Wikipedia (Feb 2, 2016)

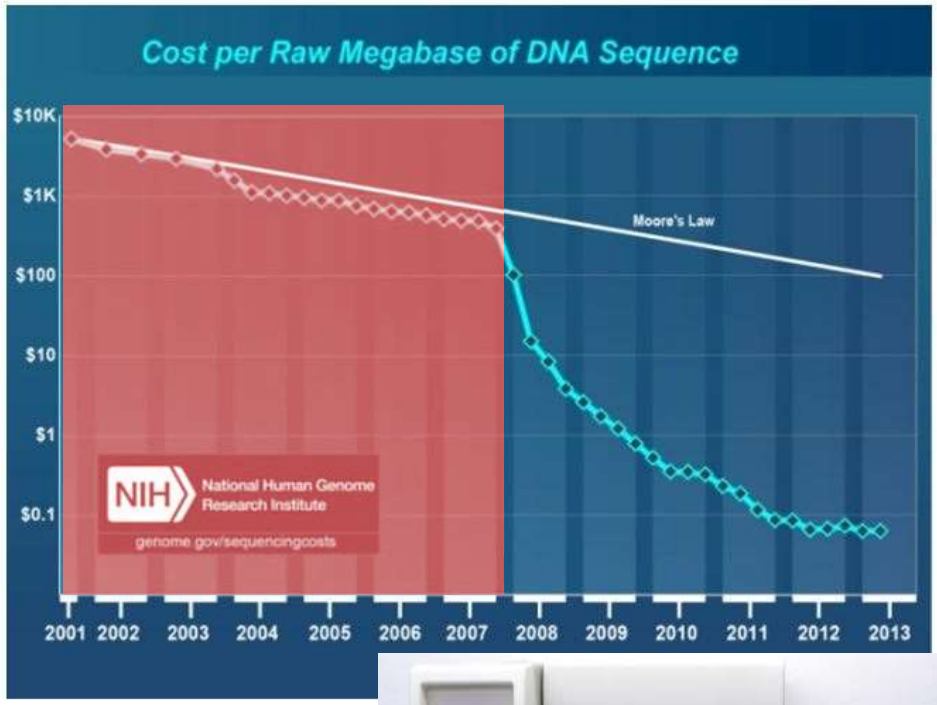
Moore's Law vs DNA Sequencing technology



Sanger Sequencing

Dideoxy chain termination sequencing

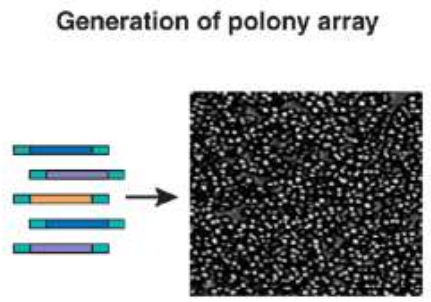


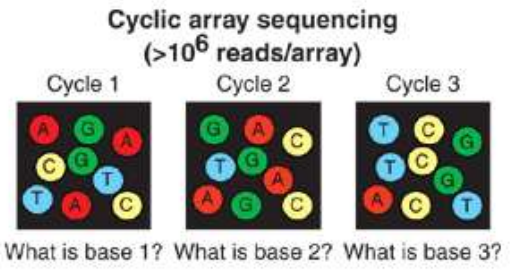


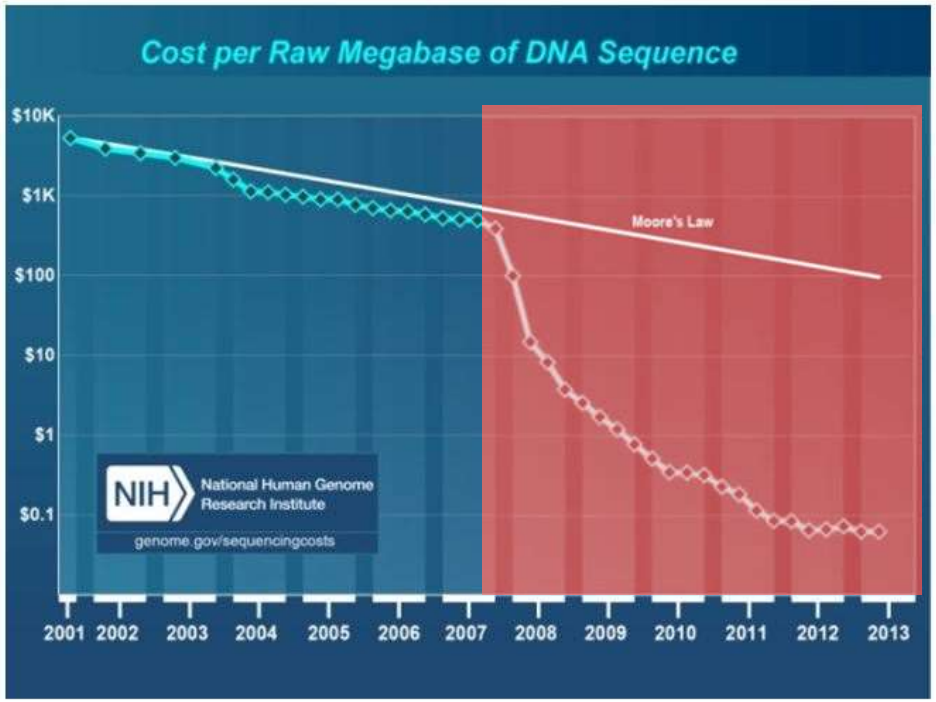
Beckman Coulter CEQ 8000

Next-generation sequencing (NGS)

Sequencing by synthesis

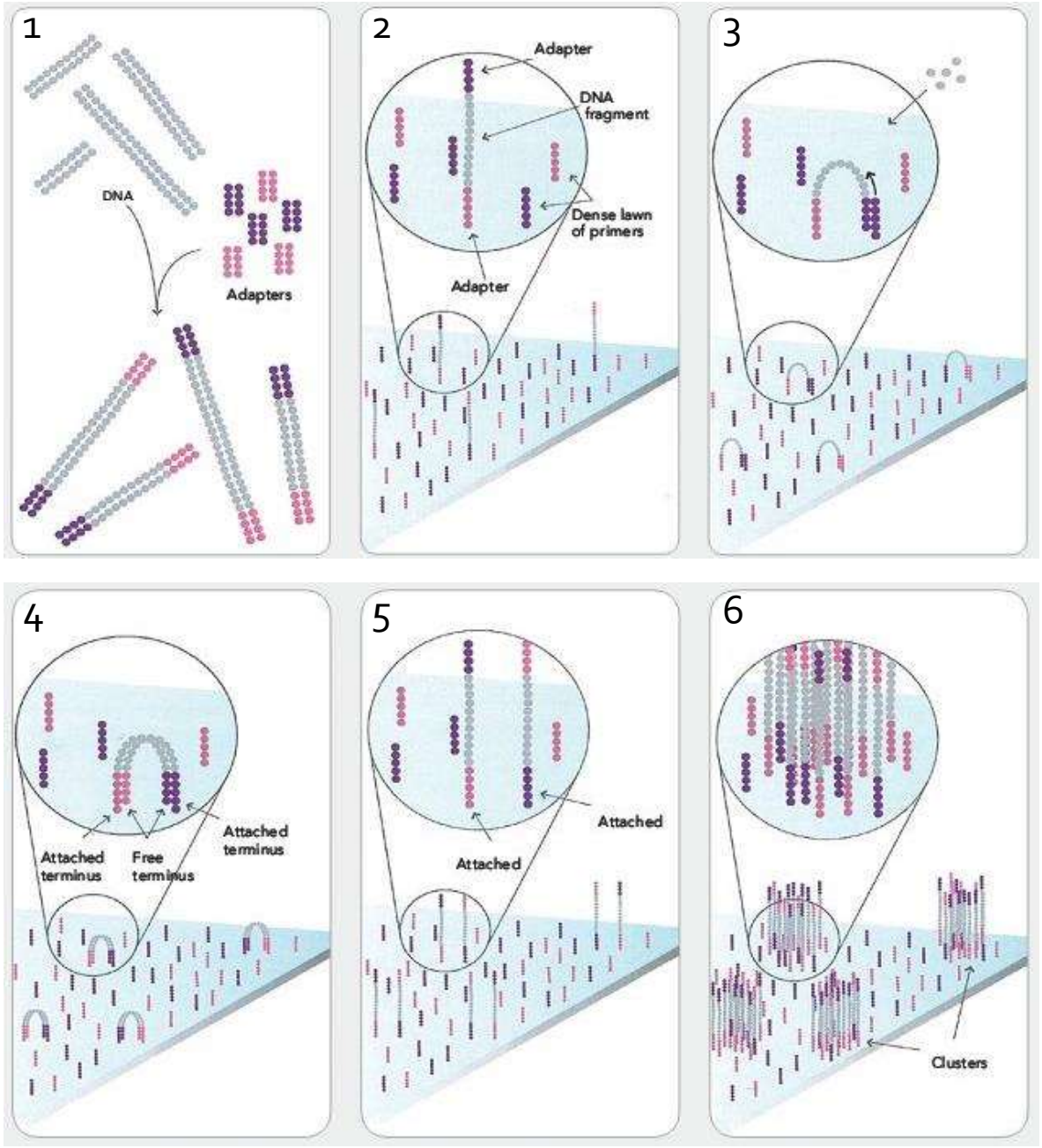






Illumina HiSeq & MiSeq

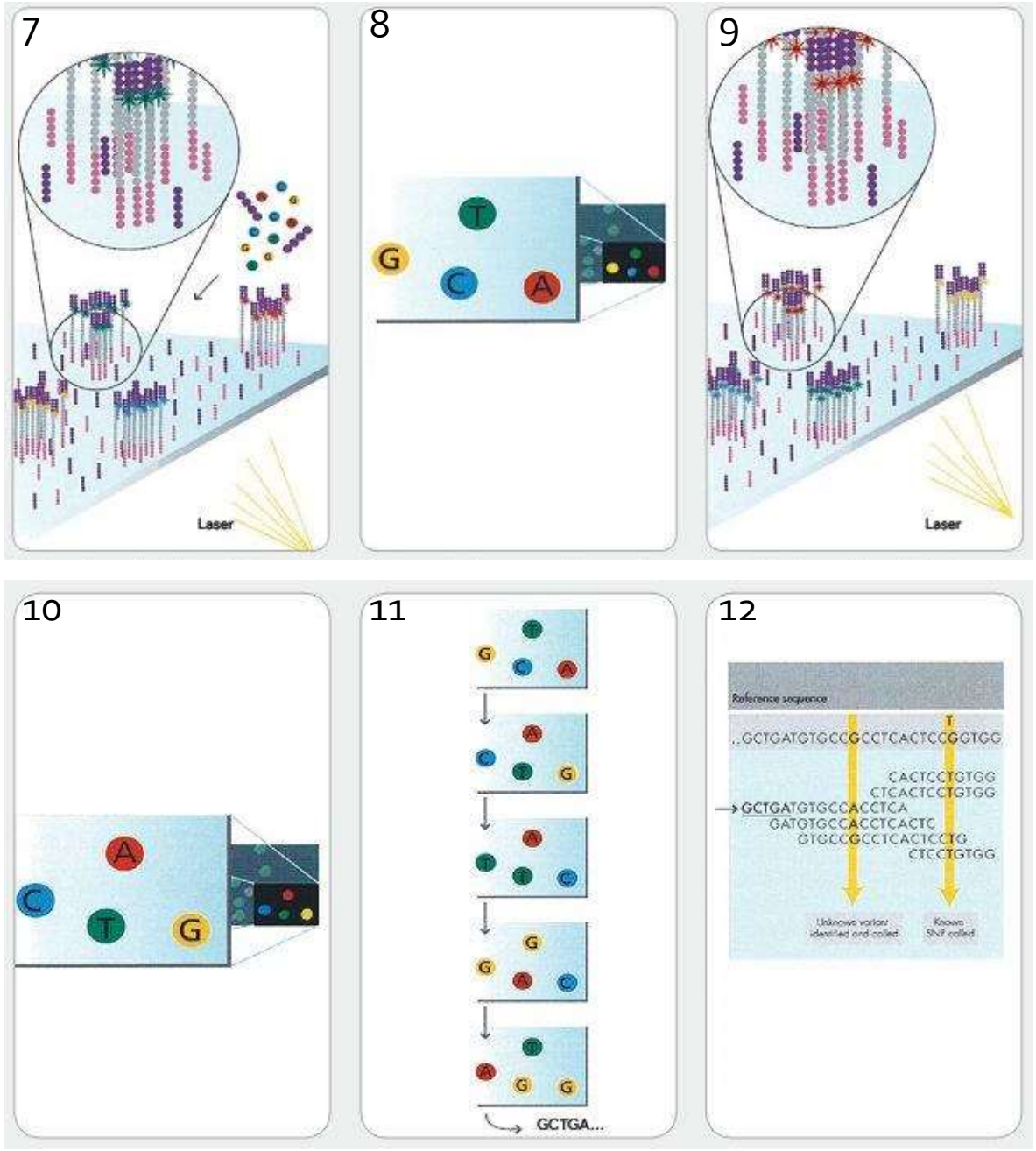
Illumina (Sequencing by synthesis)



1. Shear DNA into short fragments (~100-500bp) & ligate sequencing primers (adapters)
2. Attach DNA to surface
3. Bridge amplification
4. Fragments become double stranded
5. Denature the double stranded molecules
6. Cluster amplification

Illumina.com

Illumina



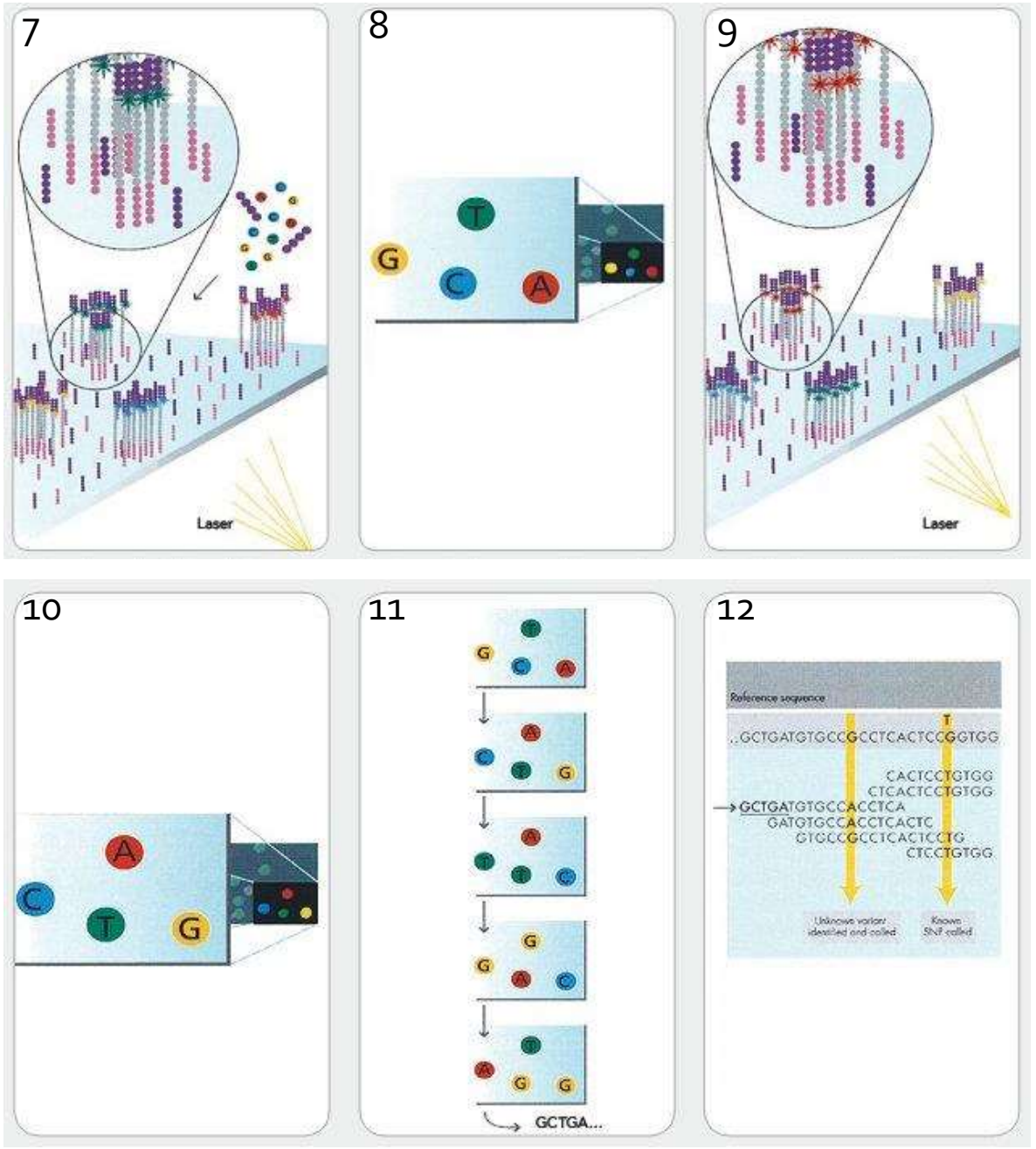
7. Determine first base
8. Image first base
9. Determine second base
10. Image second base
11. Sequence reads over multiple cycles
12. Align data

HiSeq 3000/4000
up to 5 billion fragments/flow cell
2x 150bp fragment length

MiSeq
up to 25 million
2x300bp

Illumina.com

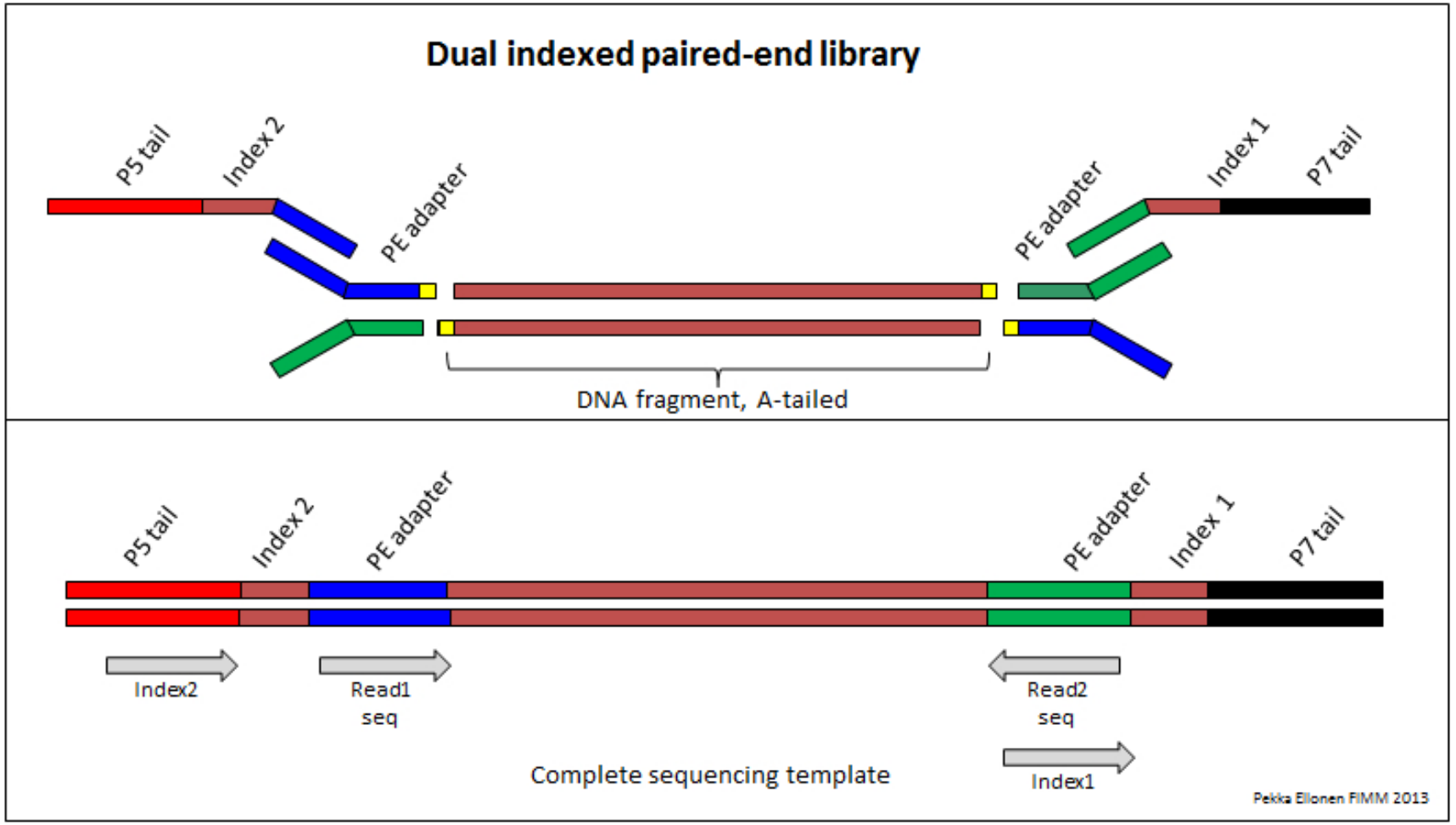
Illumina



7. Add fluorescent-labelled nucleotides
8. Image first base
9. Flush flow cell and repeat
10. Image second base
11. Sequence reads over multiple cycles
12. Align data

Illumina.com

DNA construct



Advantages of Illumina

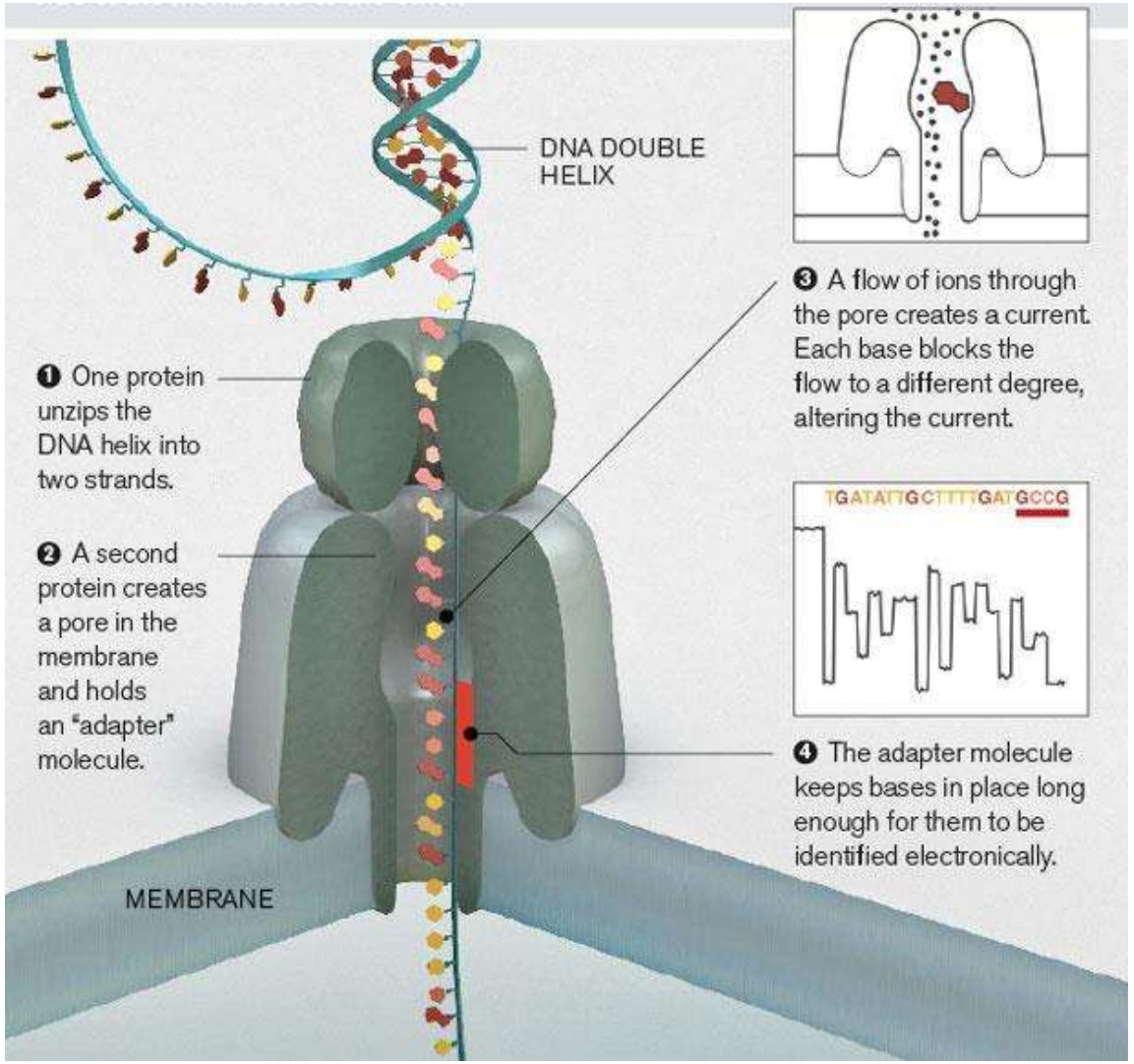
- The industry standard
- Versatile (see applications later)
- Economic (price per Mb of data)
  - **MiSeq** – up to 2 x 300bp x 15M reads = **9 Gb**
    - **~$2,500 ($0.27)**
  - **HiSeq** – up to 2 x 125bp x 200M reads = **50 Gb**
    - **~$3,000 ($0.06)**

Compare Platforms:
http://www.molecularecologist.com/next-gen-fieldguide-2016/

Other NGS Platforms

- **PacBio –** longest reads (>20kb)
  - genome assembly and full-length cDNA sequencing
  - DNA structural differences (e.g. inversions, transposon jumps)
  - BUT: see 10x genomics for Illumina
- **Ion Torrent** – Fewer reads (1 Gb)
  - Lower up-front investment ($80k vs $150k+ for Illumina)
  - pre-existing variant panels (model organisms)
  - Amplicon sequencing (e.g. microbiome)
  - User-friendly, fast turnaround
- **Roche 454** – Obsolete; Lowest throughput (1 Mb)
  - cheaper long reads (up to 1 kb)
  - Microbiome studies

Experimental: MinION (Oxford Nanopore)



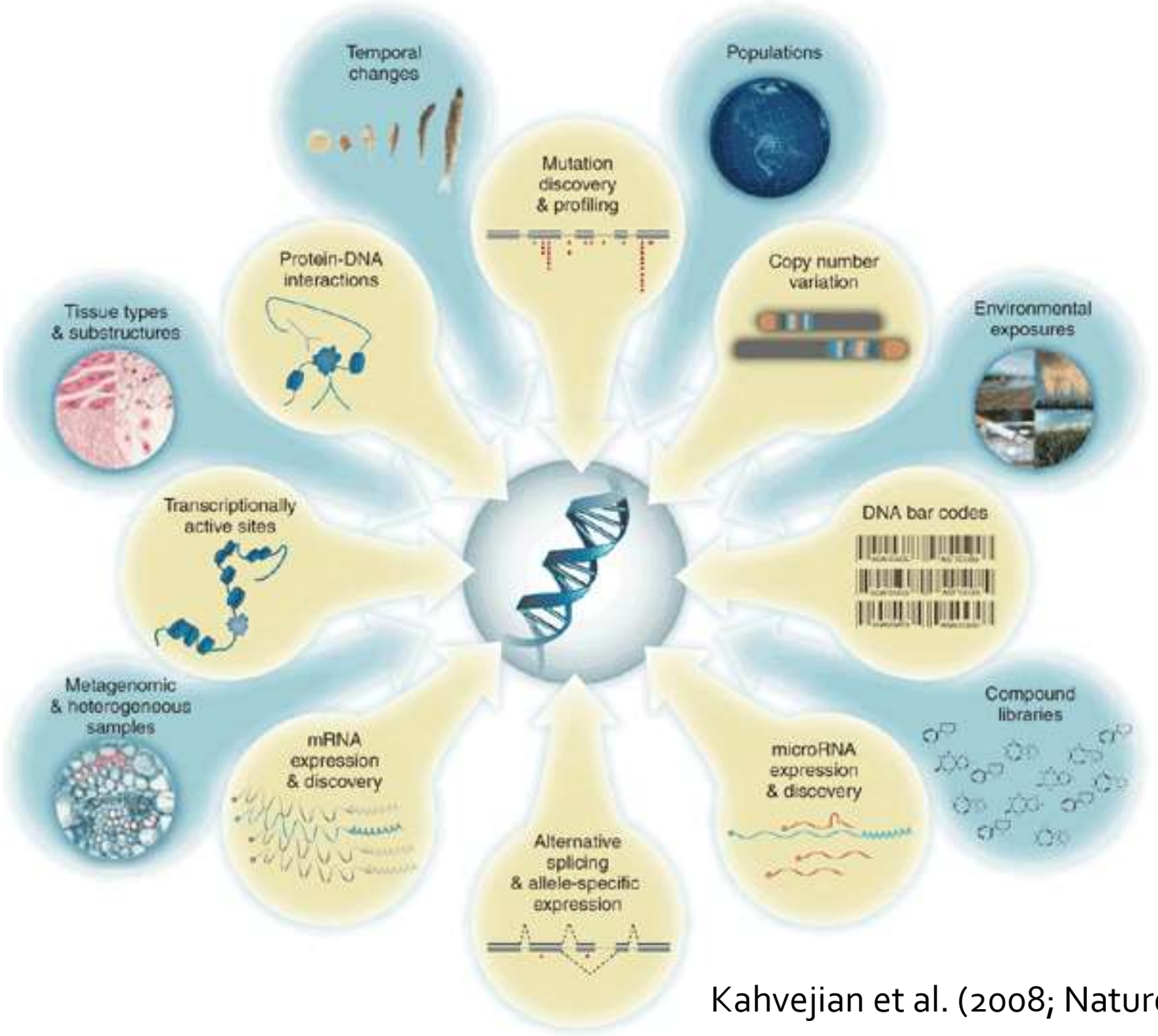
MIT Tech Review

Why is NGS revolutionizing Biology?

- NGS-tools translate across disciplines. For example:
- Metagenomics:
  - Soil, water, human gut microbiomes
- Variant detection & GWAS
  - QTL mapping for agriculture, adaptive traits, environmental stressors, human health

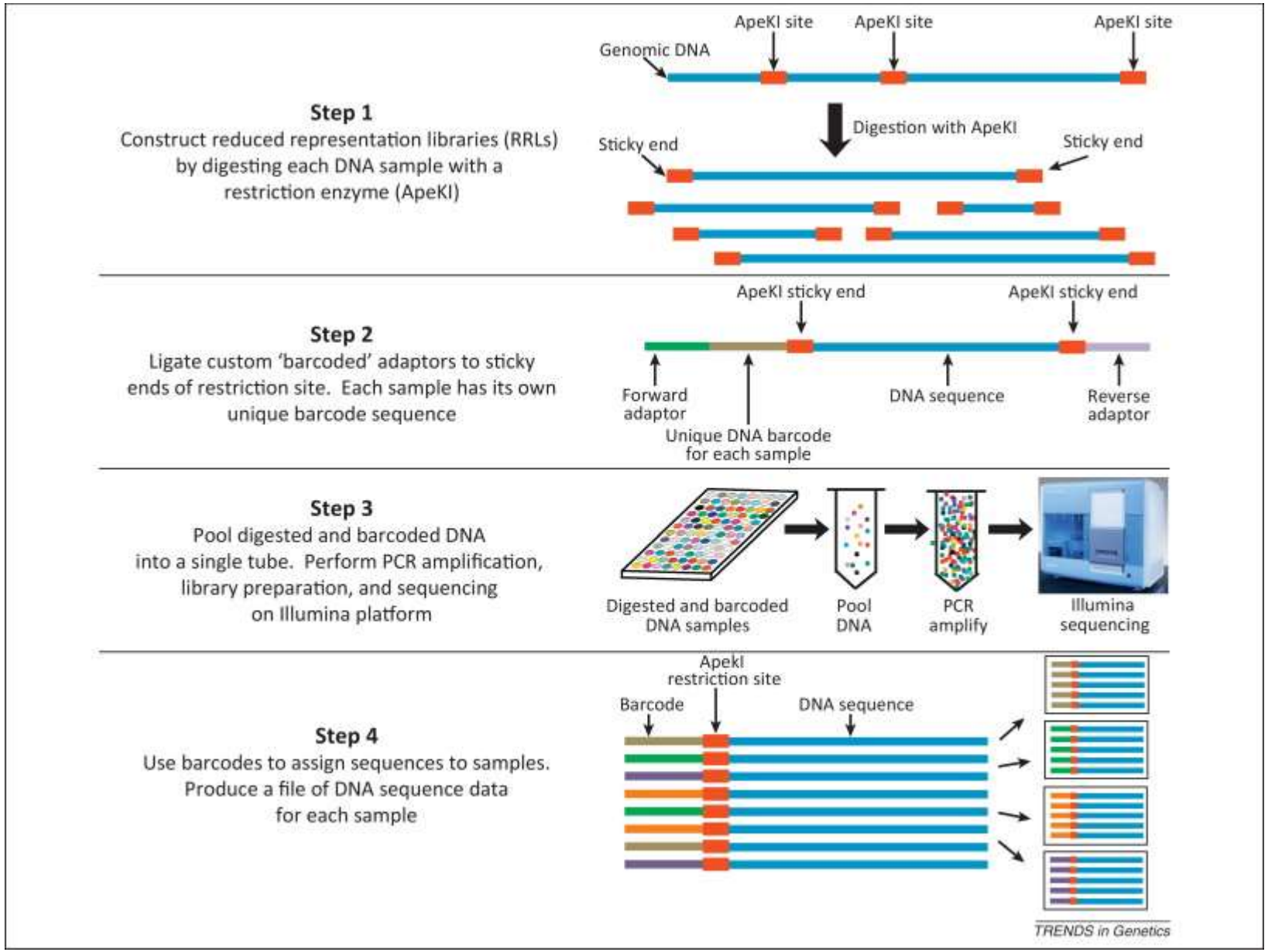
Applications

Applications



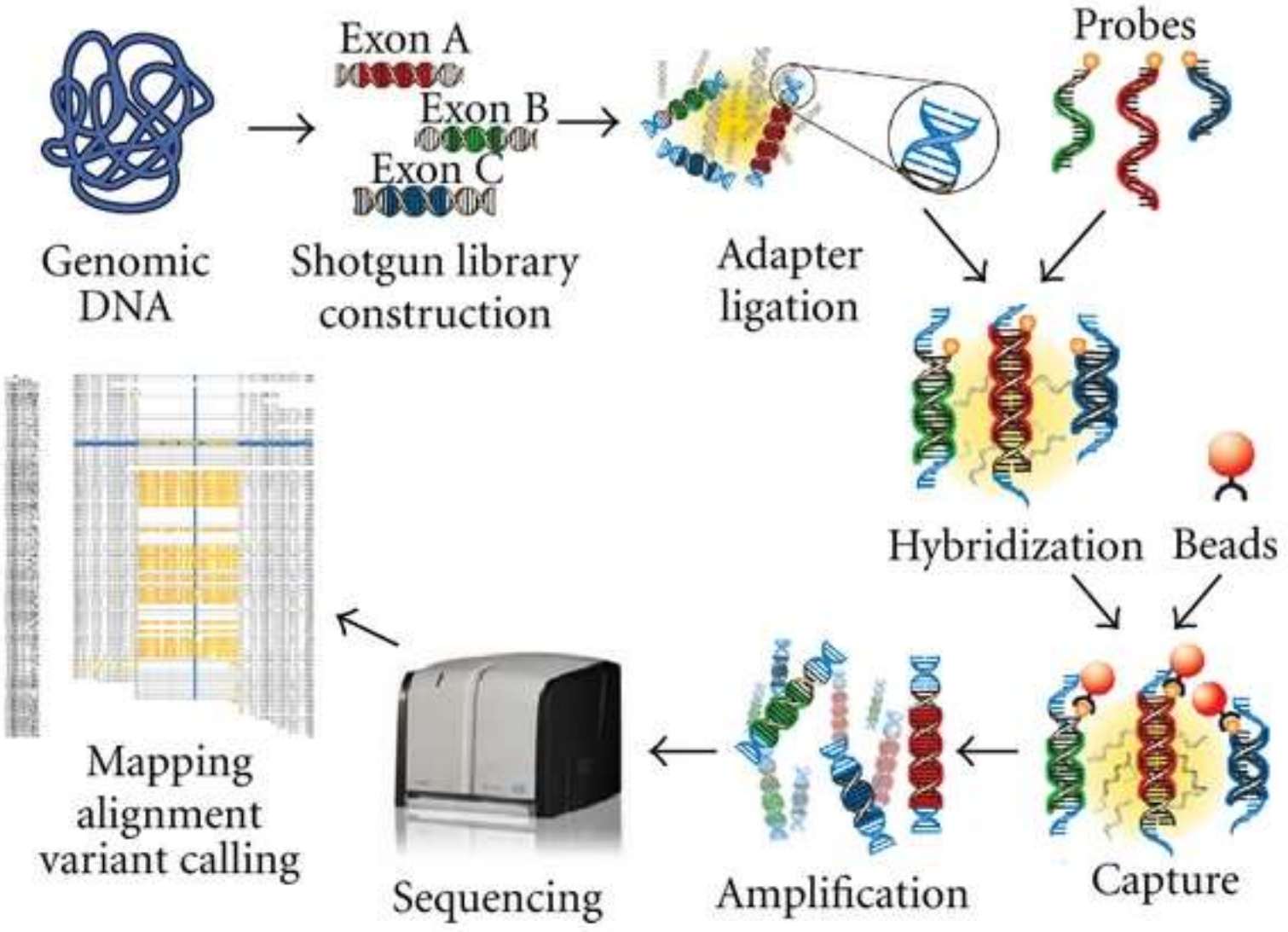
Kahvejian et al. (2008; Nature Biotech)

Genotype-by-sequencing (GBS)

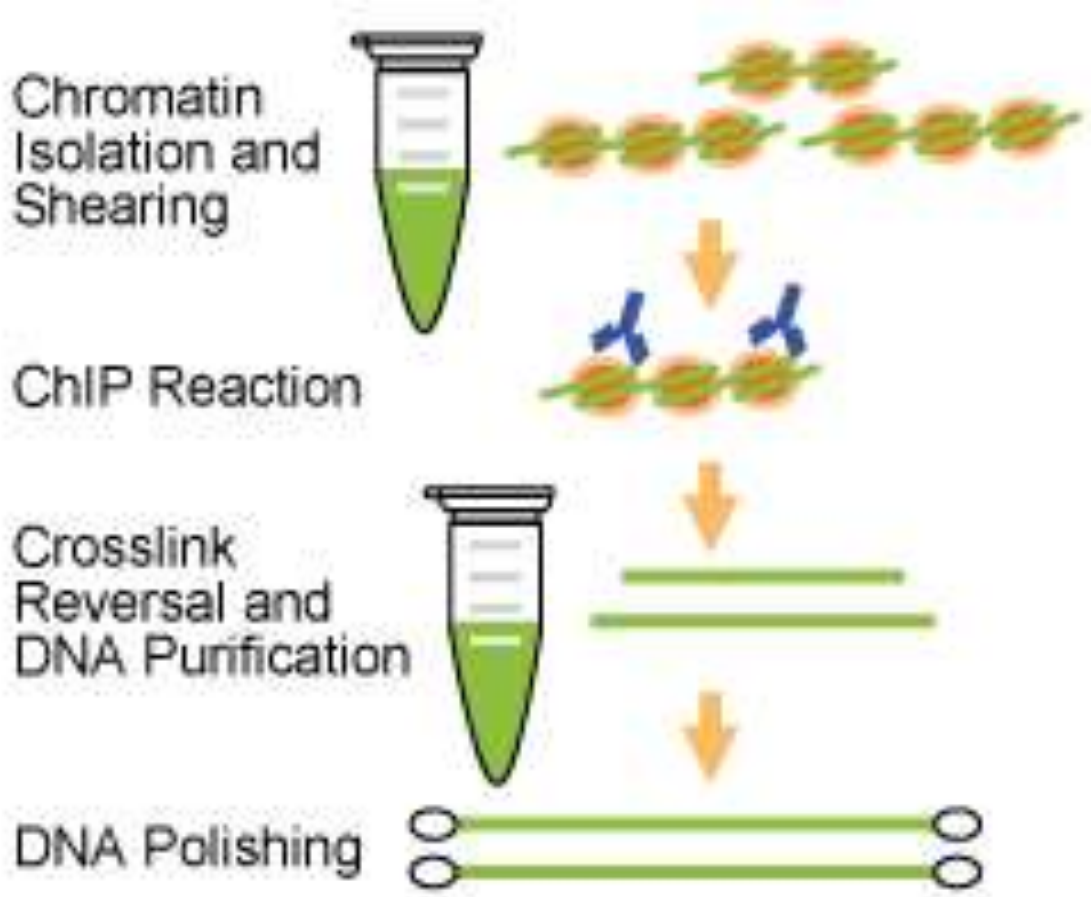


Myles (2013; Trends Genetics)

Target-capture sequencing

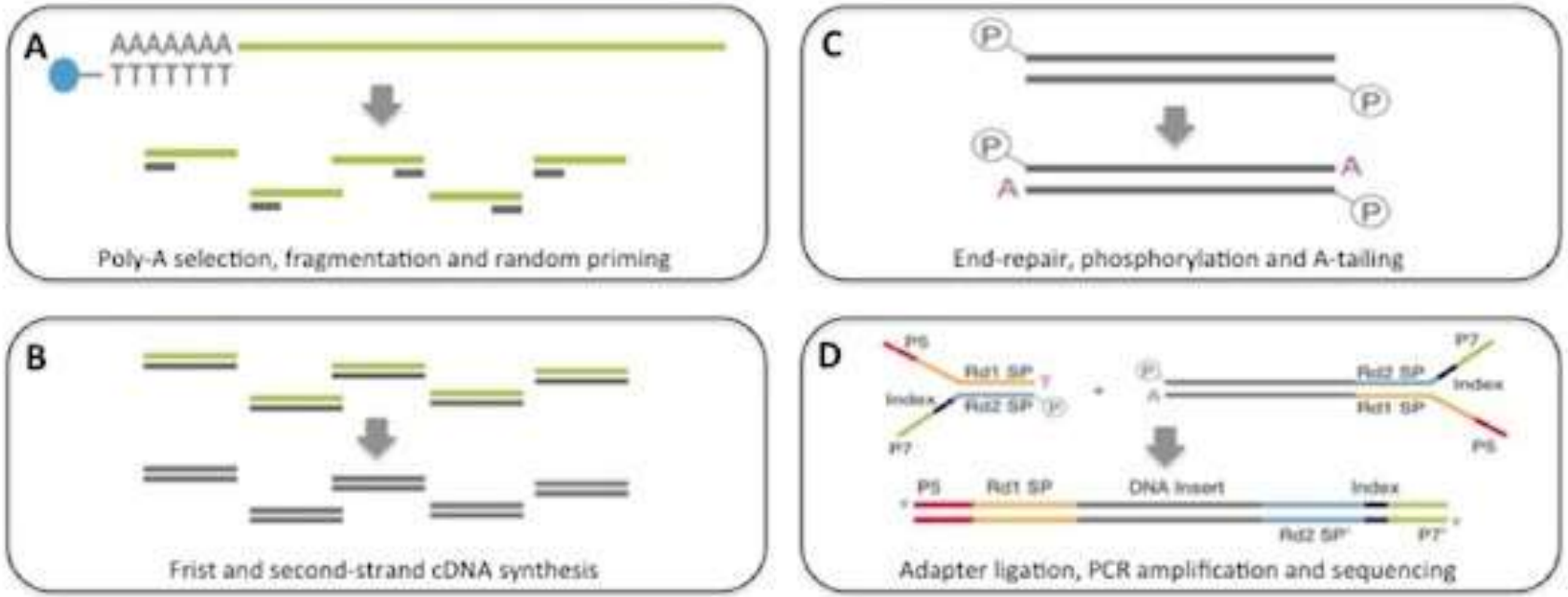


ChIP-seq (protein-targets)



RNA-Seq

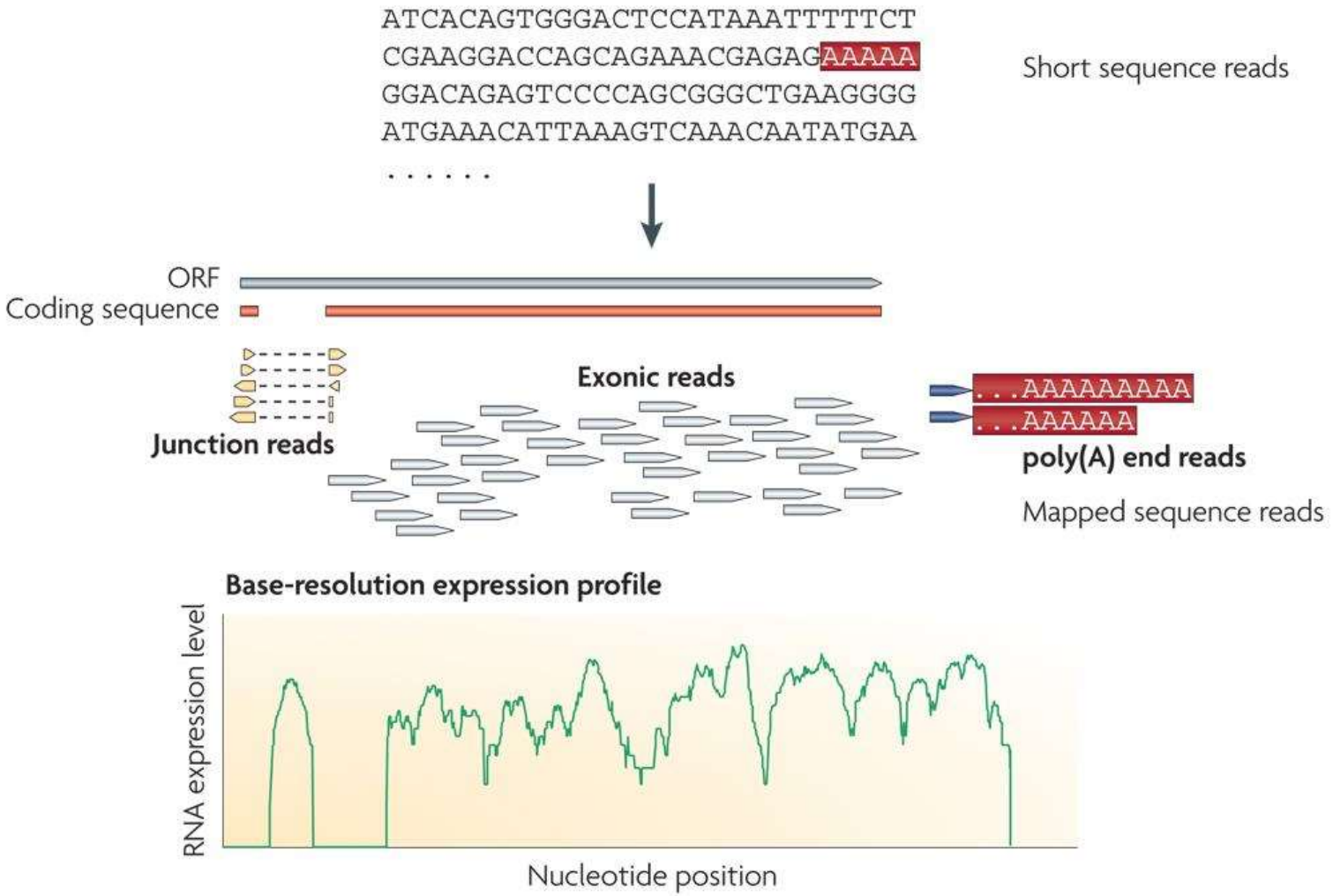
Illumina Tru-Seq RNA-seq protocol



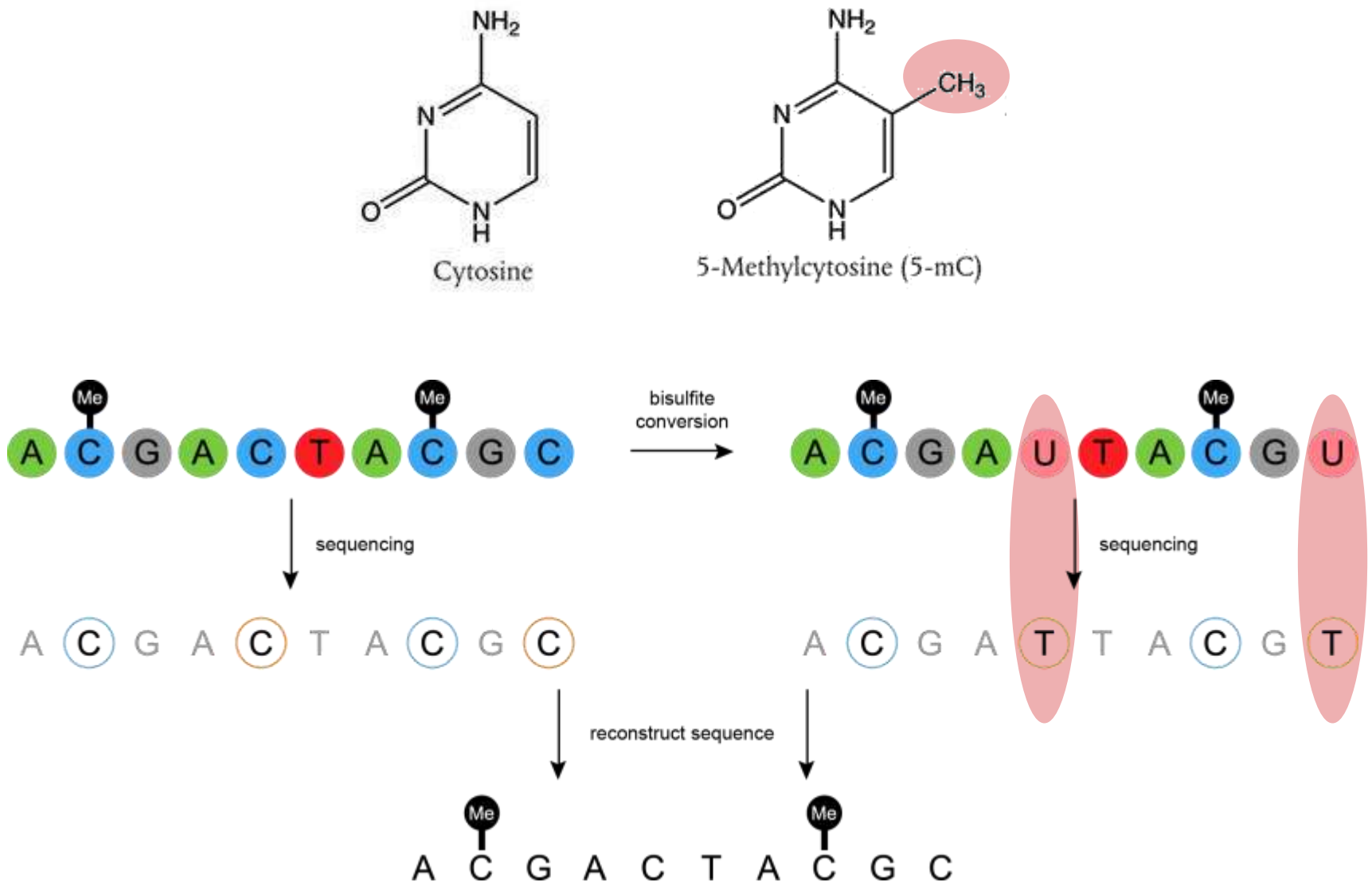
Library prep begins from 100ng-1ug of Total RNA which is poly-A selected (A) with magnetic beads. Double-stranded cDNA (B) is phosphorylated and A-tailed (C) ready for adapter ligation. The library is PCR amplified (D) ready for clustering and sequencing.

Illumina.com

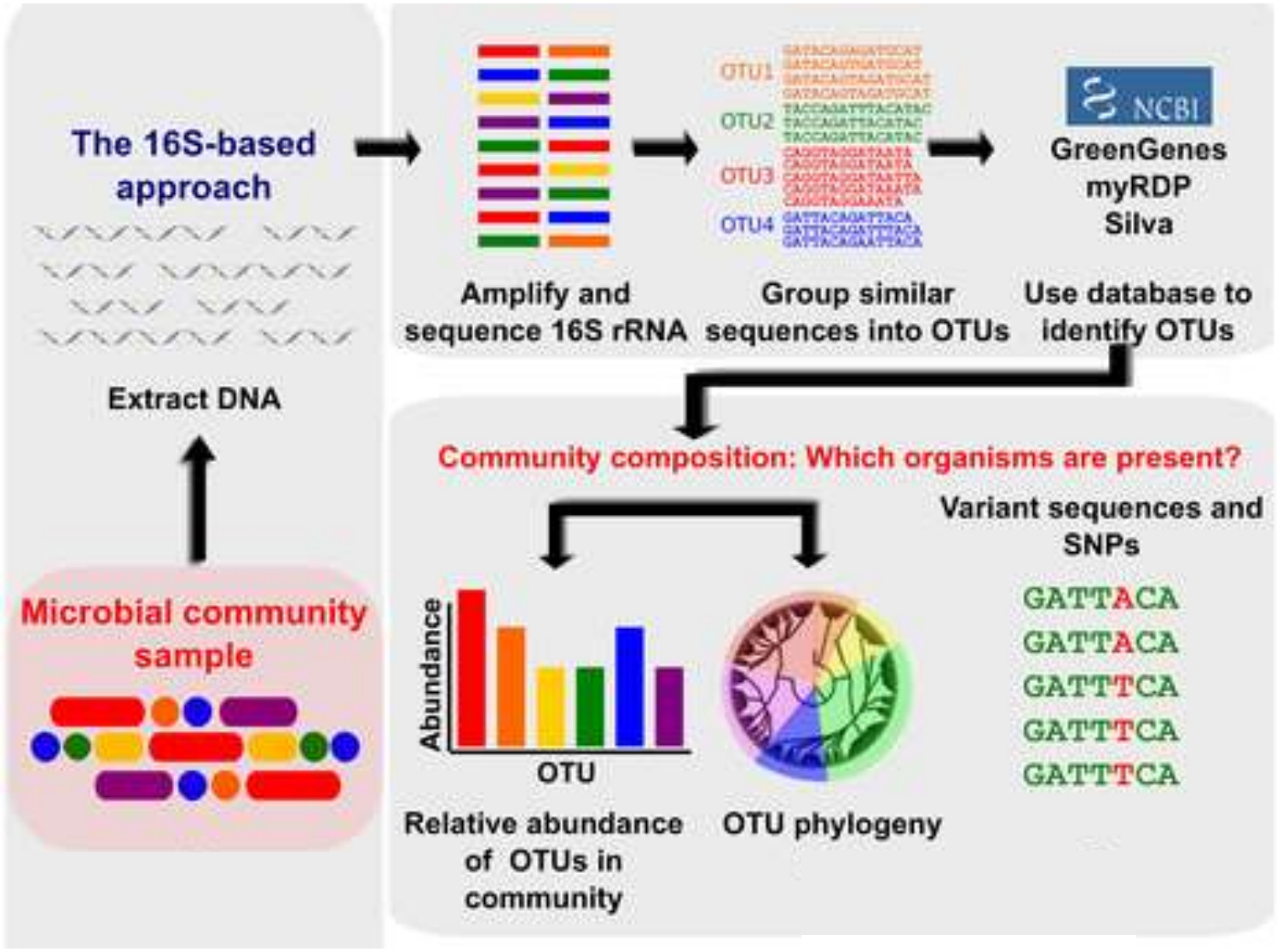
RNA-Seq expression profiles



Bisulfite sequencing

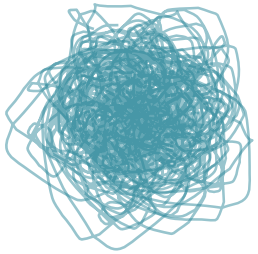


Metagenomics

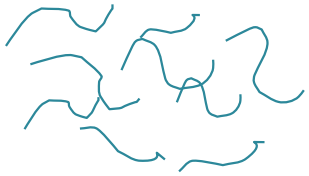


OUT = Operational Taxonomic Unit

Whole-Genome Shotgun (WGS) sequencing



Extract



Fragment

Sequence

Align

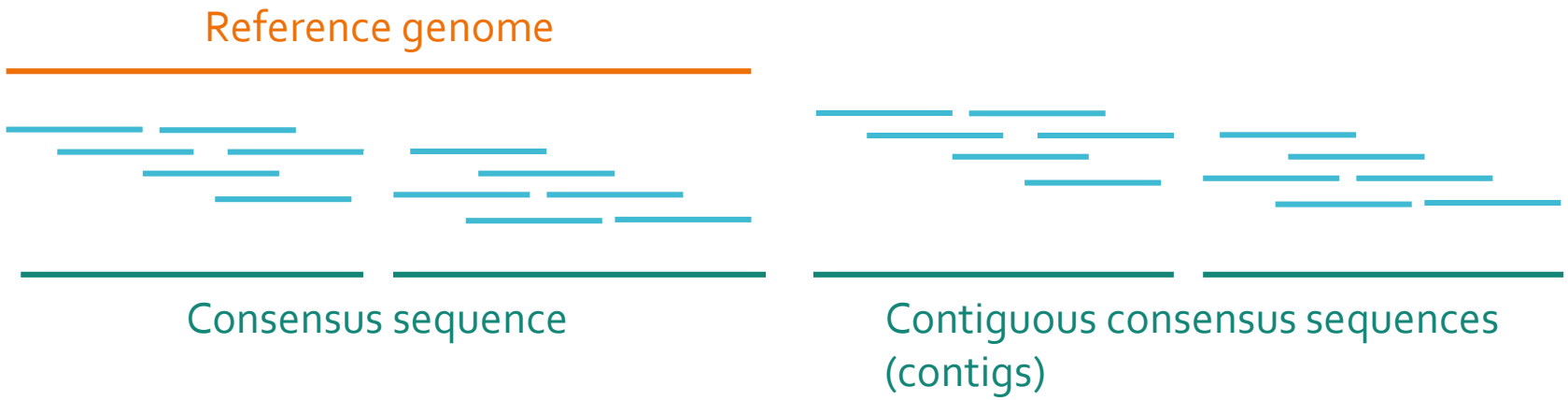
Assembly with or without a reference

Alignment to reference

- sometimes called 'resequencing'
- common in 'model systems'
- e.g. human, mouse, *Drosophila*, *C. elegans*, *Arabidopsis*

de novo assembly

- 'non-model' organisms



De novo assembly

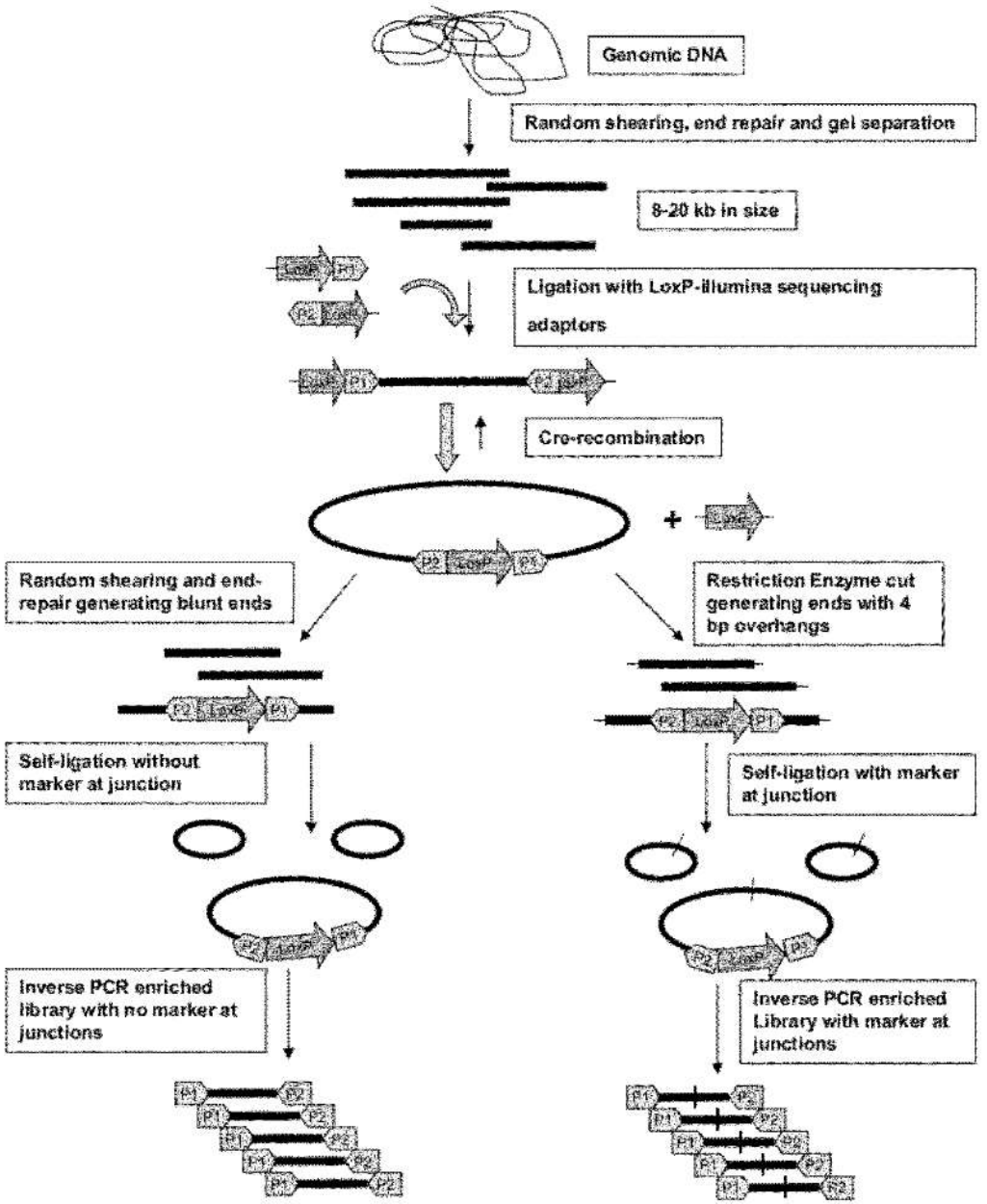
Figure 5. *De Novo* Assembly with Mate Pairs

Using a combination of short and long insert sizes with paired-end sequencing results in maximal coverage of the genome for de novo assembly. Because larger inserts can pair reads across greater distances, they provide a better ability to read through highly repetitive sequences and regions where large structural rearrangements have occurred. Shorter inserts sequenced at higher depths can fill in gaps missed by larger inserts sequenced at lower depths. Thus a diverse library of short and long inserts results in better de novo assembly, leading to fewer gaps, larger contigs, and greater accuracy of the final consensus sequence.

- IMPORTANT: Paired End vs Mate-Pair

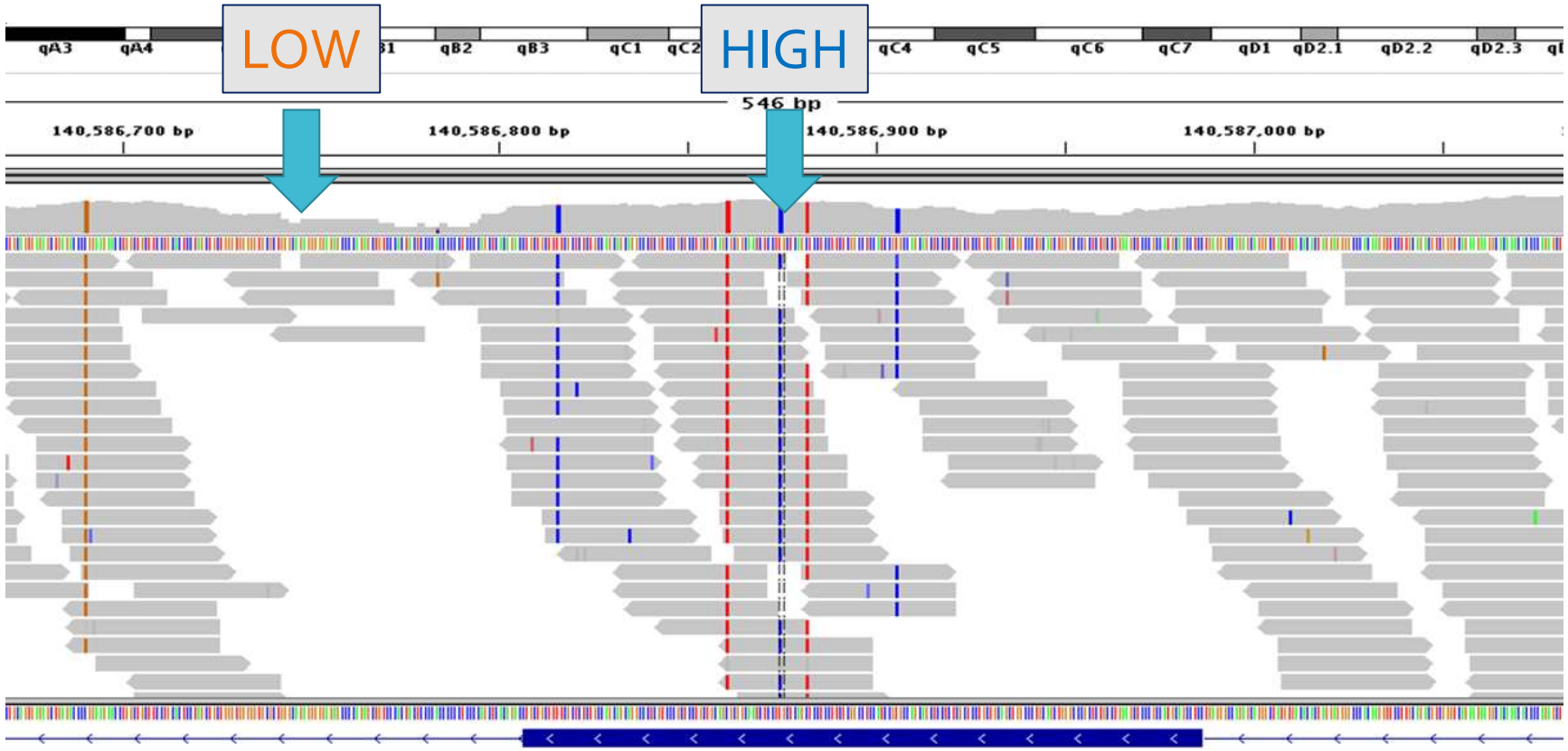
Illumina.com

Mate pair library



http://www.google.com/patents/US20120329678

Coverage



- = average # reads at any given location (base pair)
- A 'random' sampling process
- (Higher is better)

de novo assembly programs*

- ABySS
- ALLPATHS-LG
- CORTEX
- CLC Genomics Workbench
- DISCOVAR de novo
- Geneious
- IDBA
- MaSuRCA
- MIRA
- PLATANUS
- RAY
- SOAP de novo

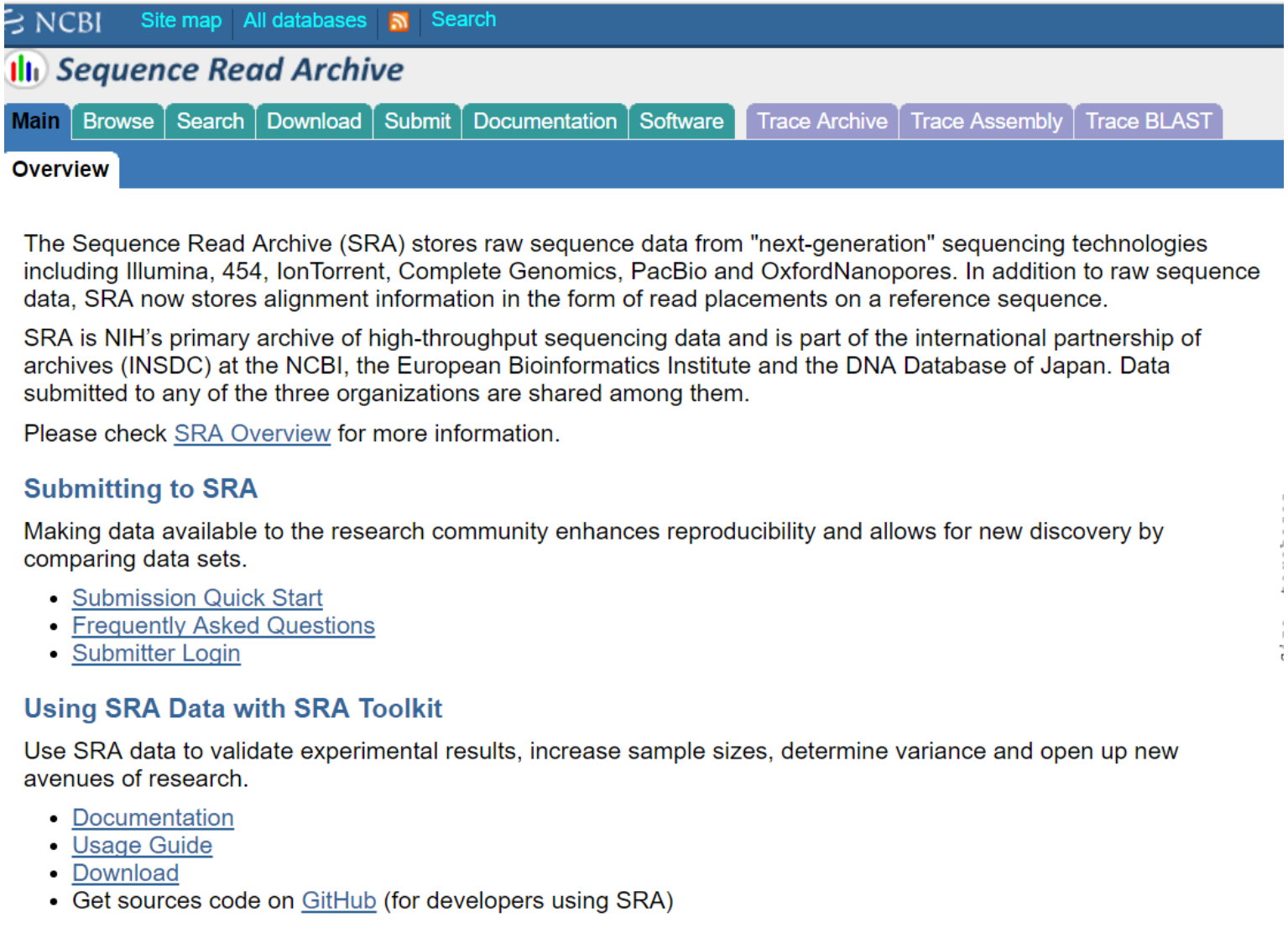
*Large genome assemblers (>0.5Gb)

Gold = commercial/restricted license

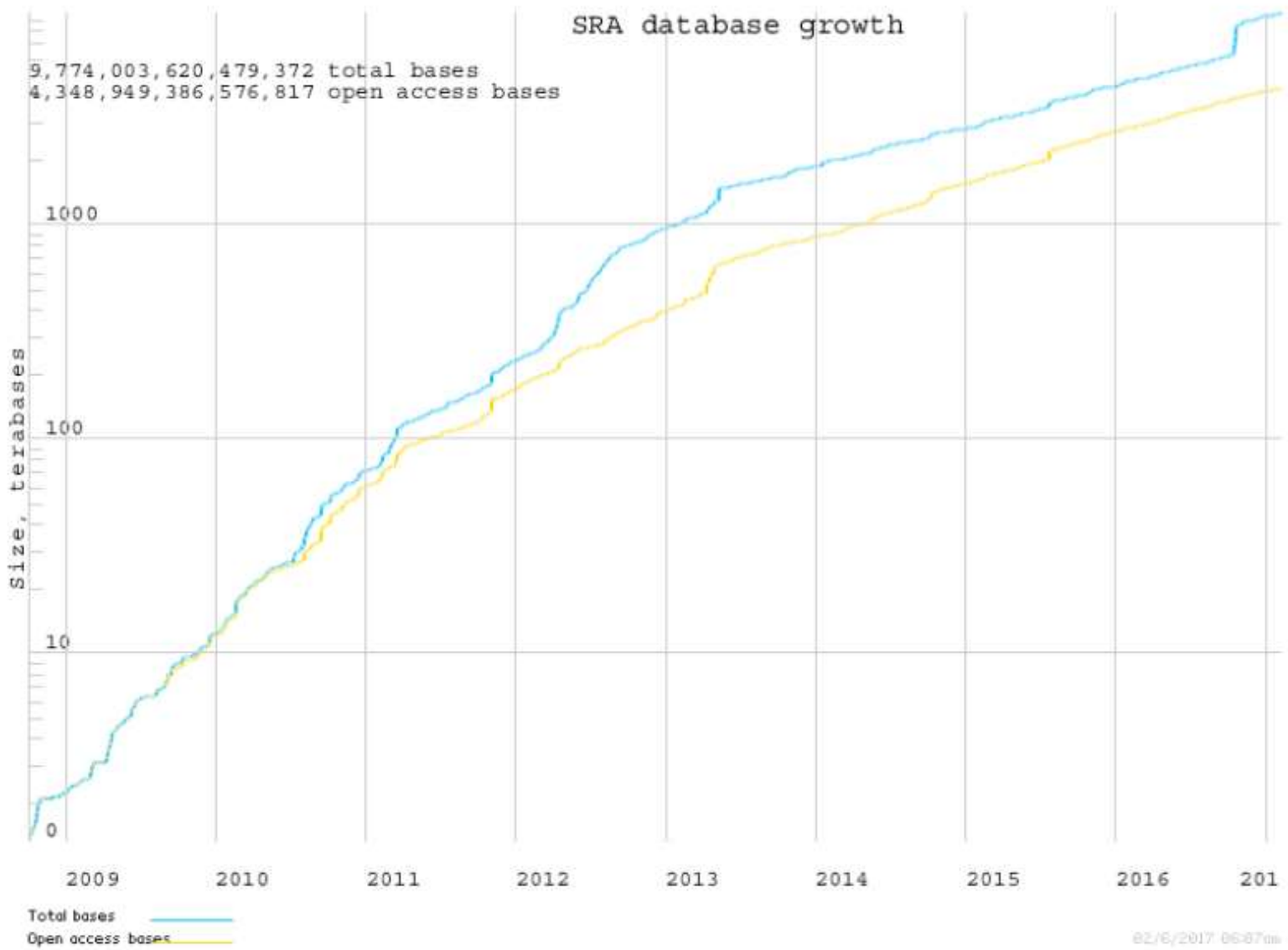
Challenges

‘Big Data’ Computation

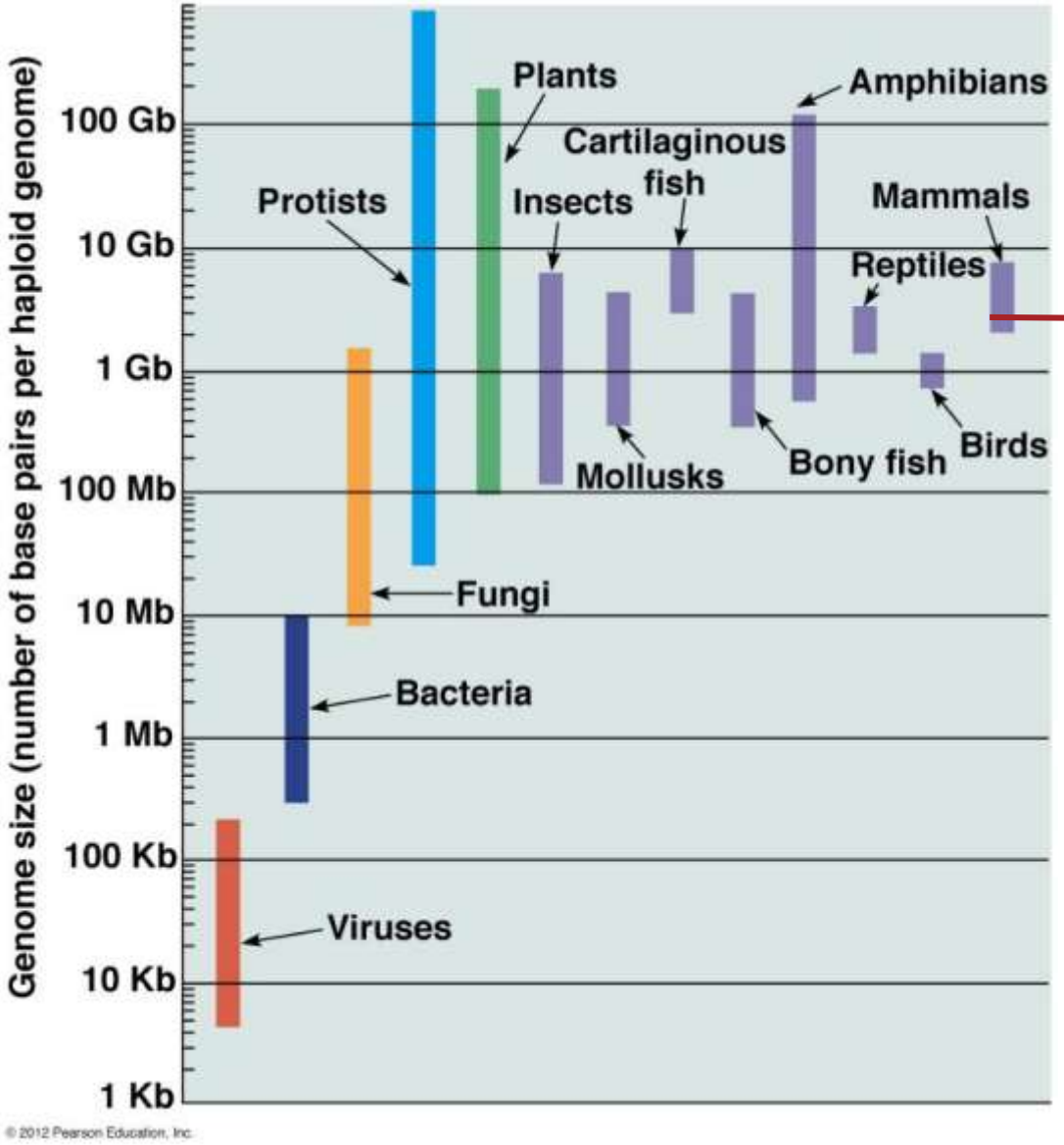
https://trace.ncbi.nlm.nih.gov/Traces/sra/



‘Big Data’ (~10 Petabytes)



Large genomes



Assembly challenges

- Large repeats – creates gaps
- Errors – cannot be assembled
- Low coverage – creates gaps
- Unequal coverage – confuses error correction

NGS and Ecological Genomics

in the Colautti Lab

Ecological Genomics

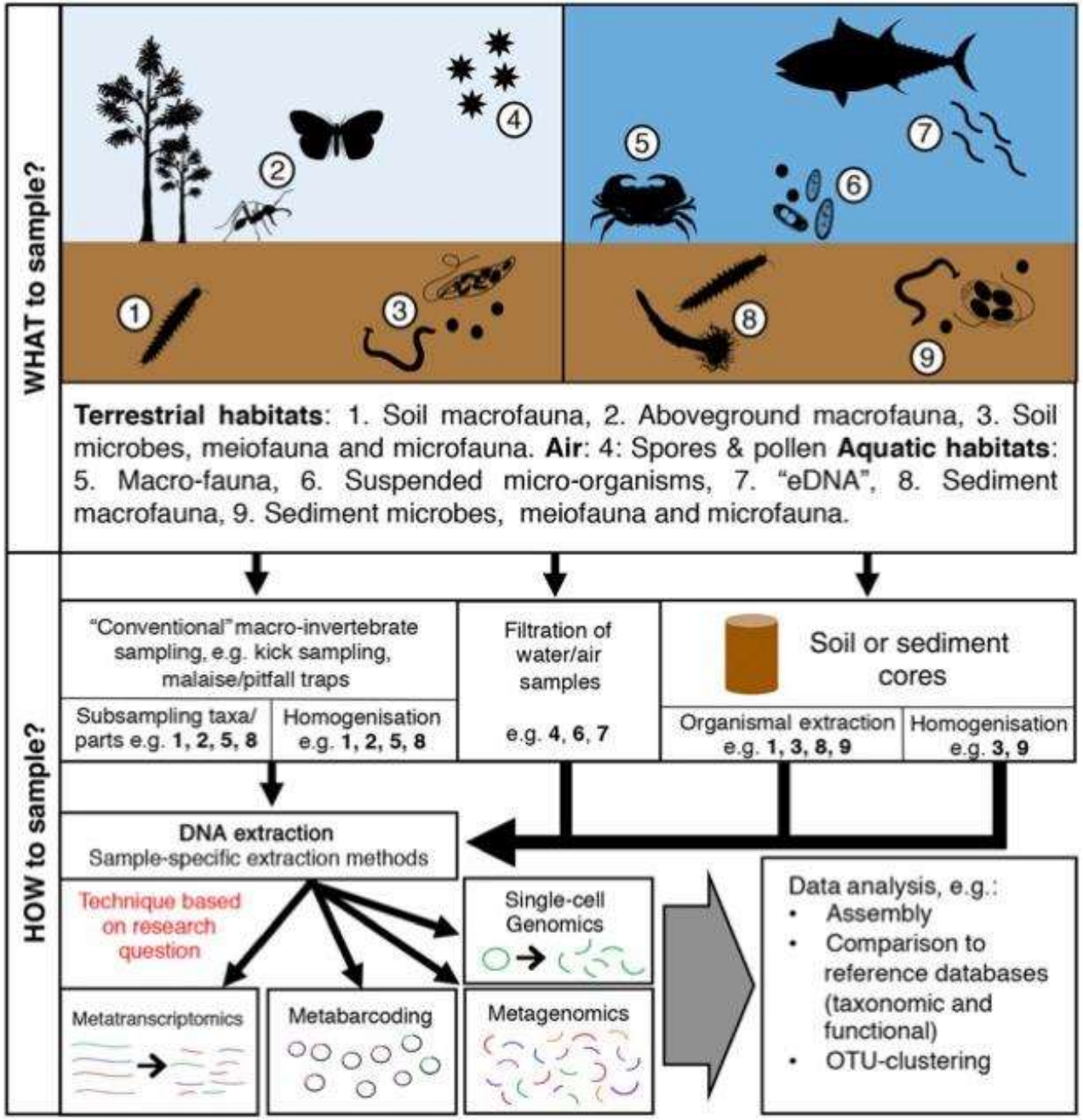


Figure 1. (Creer et al., 2016) Schematic of molecular ecology workflow.

Ecology and Evolution in the Anthropocene

Magellan I Sverige James Hoznik

Environment --> Natural Selection --> Genome Evolution

Photos by Ed Burtynsky (mostly)

Ecological genomics of invasive species

Dreissena polymorpha

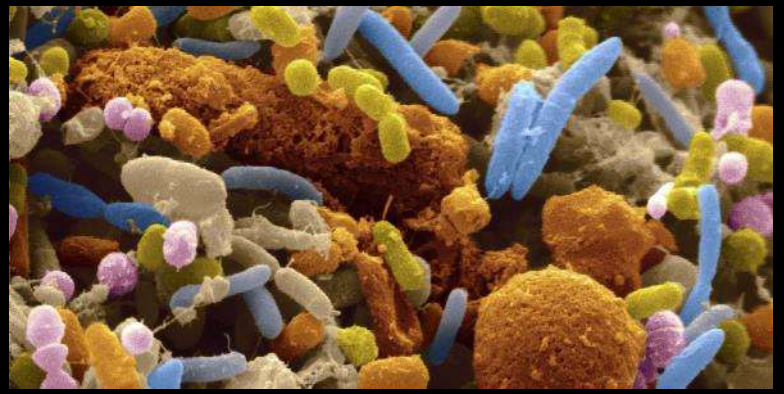
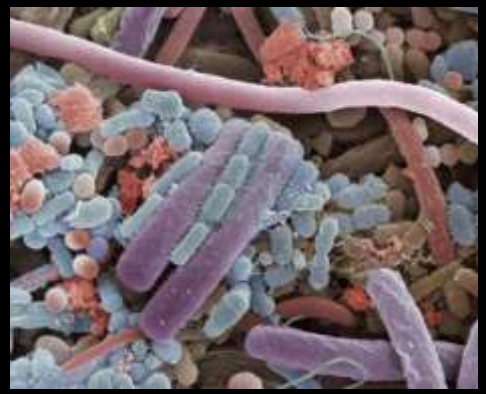
gbif.org

Invasions: grand, unplanned ecological experiments

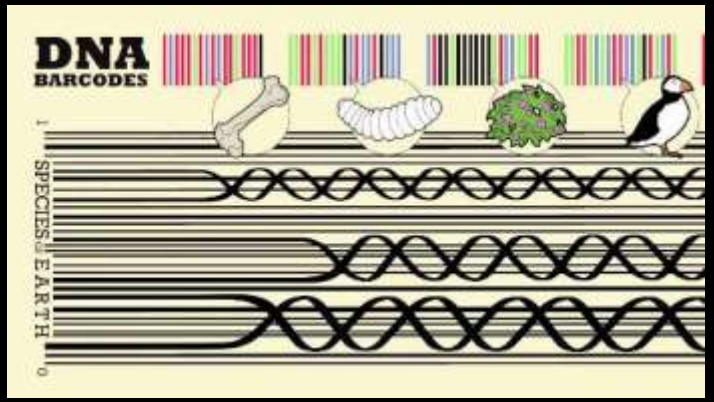
eDNA and DNA barcodes for environmental monitoring





Barcode of Life Project
www.boldsystems.org

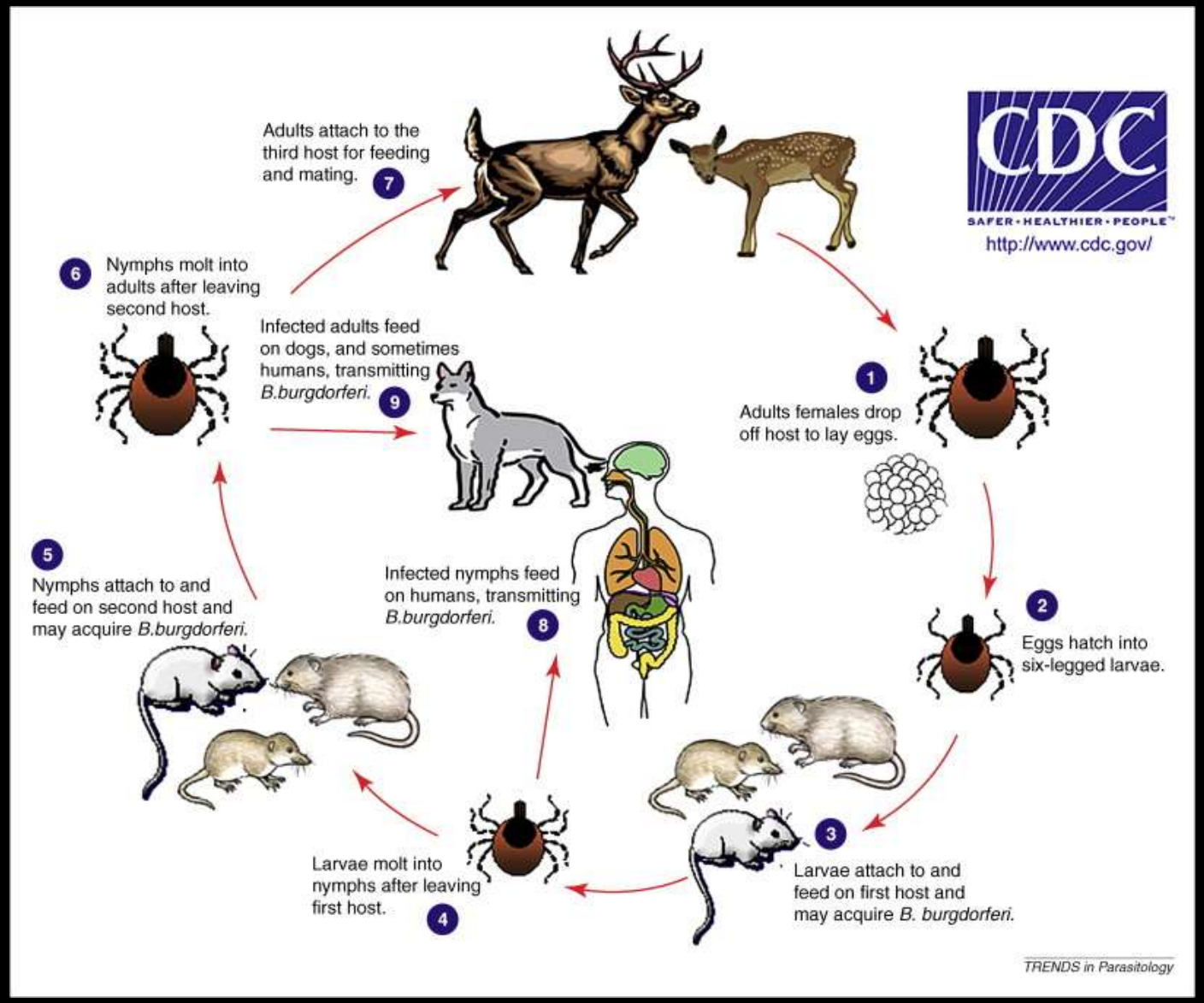


https://www.youtube.com/watch?v=ZImiXgU6bCk

Tick microbiome analysis

Lyme disease in Canada (cbc.ca)

Ixodes scapularis (black-legged tick)



NGS @Queen's

- Biology Department has the only Illumina sequencer on campus (MiSeq)
- Other machines (IonTorrent, microarrays) spread across campus
- Queen's has no university-wide genomics core facility – very unusual for a research-intensive university

NGS @ Queen's: Typical WGS sequencing workflow

Library Prep

- Extract & Purify DNA
- Fragment and size-select
- Ligate sequencing primers

Sequencing

- Generate FASTQ file – includes sequence data (short-reads) and quality score

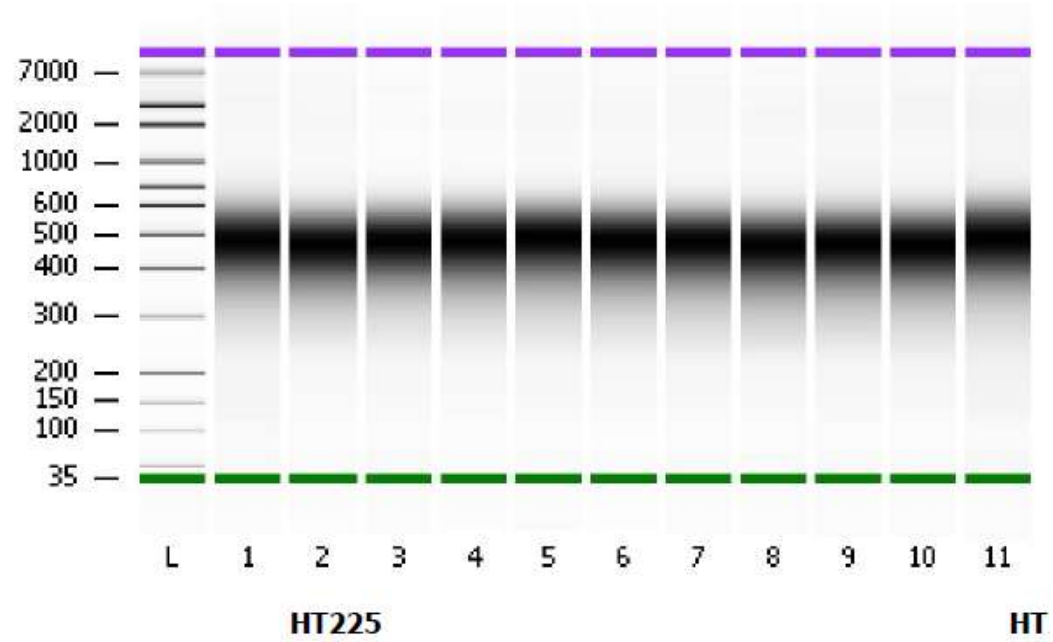
Assembly

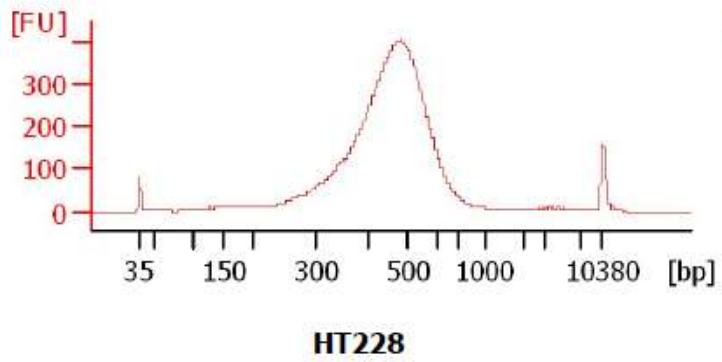
- Assemble short-reads into contigs and scaffolds

Analysis

- e.g. genome annotation; comparative genomics; structural variant detection; taxonomy ID (metagenomics); align short reads for variant detection (e.g. for GWAS)

QC @ Queen's





Agilent Bioanalyzer

MiSeq @ Queen's

Miseq Kits	Running time (est. in hours)	Max read length (bp)	Number of Reads (million)	Data generated (giga-bases)	Plan to Stock at the core?	Price $CDN (USD x 1.33)
MiSeq Reagent ver.3 2 x 300	65	600	25	15	Yes	2438
MiSeq Reagent ver.2 2 x 250	39	500	15	7.5	No	1850
MiSeq Reagent ver.2 2 x 150	24	300	15	4.5	Yes	1676
MiSeq Reagent ver.3 2 x 75	24	150	25	3.75	Yes	1470
MiSeq Micro 2 x 150	24	300	4	1.2	No	1432
MiSeq Reagent ver.2 2 x 25	6	50	15	0.75	No	1347
MiSeq Nano 2 x 250	39	500	1	0.5	No	1180
MiSeq Nano 2 x 150	24	300	1	0.3	No	1004

de novo sequencing Application

Ecological genomics of *Alliaria petiolata*

Loren Rieseberg

Oliver Bossdorf

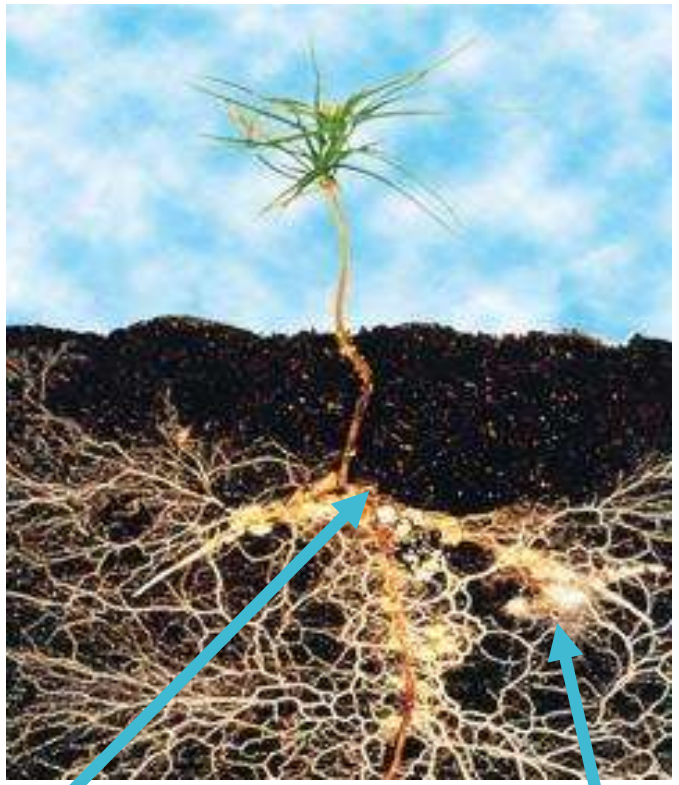
Ecological genomics of *Alliaria petiolata*

Why *Alliaria petiolata*?

- Problematic invasive species in North America
- Easy to identify
- Simple lifetime fitness estimate
- Brassicaceae, self-pollinated
- Ecologically important chemistry
  - (Enemy release; allelopathy/soil biota)

Alliaria petiolata ecology: plant-microbe interactions

Alliaria petiolata ecology: plant-microbe interactions



Alliaria petiolata ecology: plant-microbe interactions

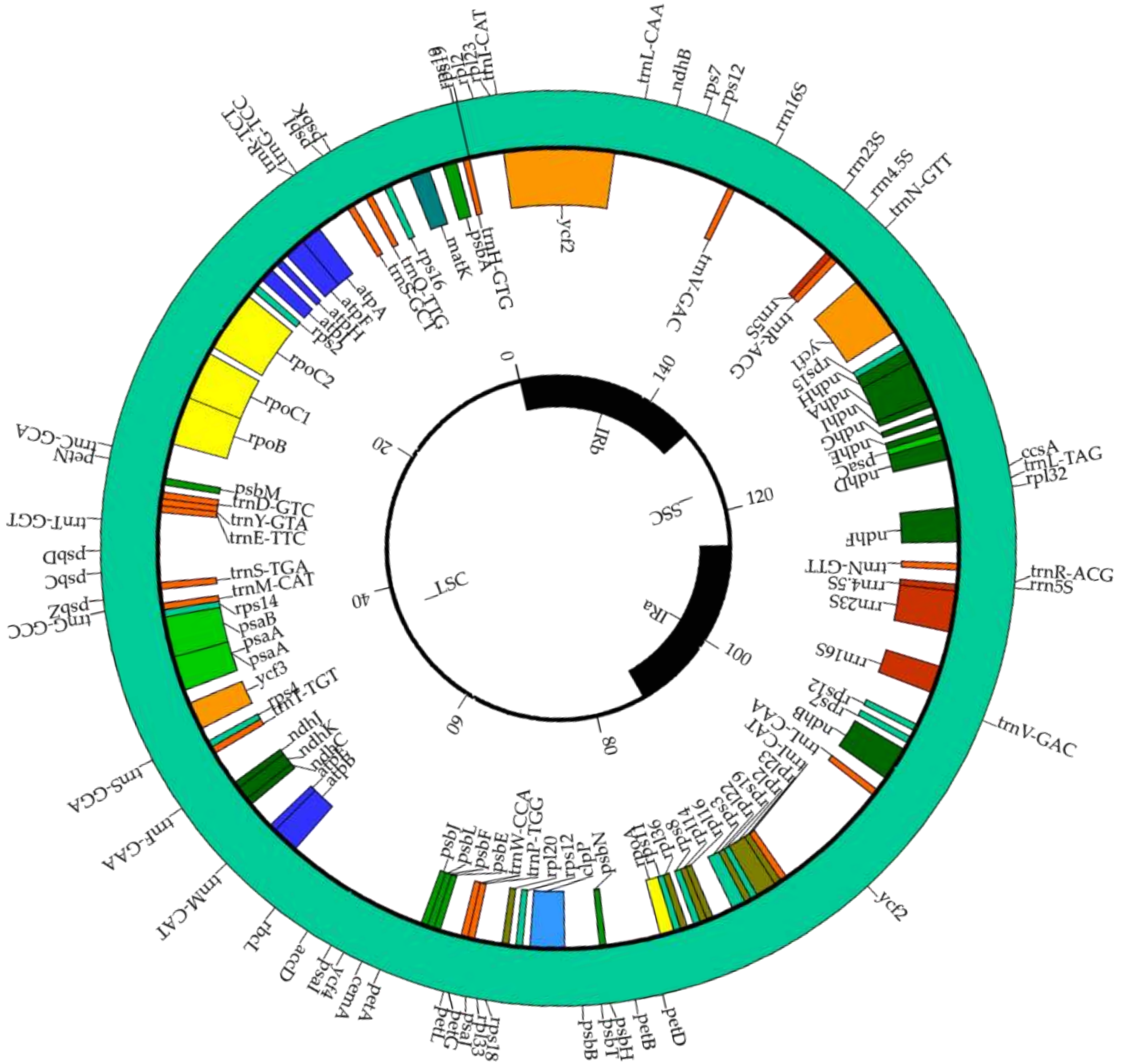
Global garlic mustard field survey

- International collaboration
  - 164 participants from 16 countries
- Field surveys
  - 45,000 field measurements
  - Plant performance traits
  - Herbivory & fungal damage
- Genetic resource
  - Seeds from >5,000 plants
  - 395 locations across Europe and North Am.

Population genomics: Some major questions

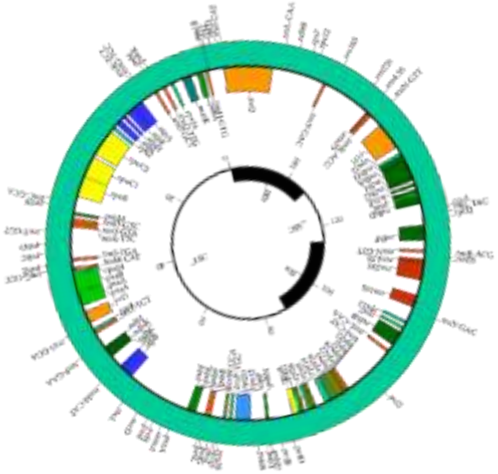
- How many introductions to North America, from which parts of Europe, and how has it been moved around?
- Which genes affect survival and reproduction in response to:
  - cold vs warm climates
  - wet vs dry conditions
  - native vs introduced range
  - herbivores and pathogens
  - competition with other plants
- Does selection act mainly on a few loci with large effects on phenotype, or many loci with smaller effects?
- How important is standing genetic variation vs new mutations?
- How important are SNPs vs genome rearrangements?

Alliaria petiolata draft chloroplast genome 153,190 bp



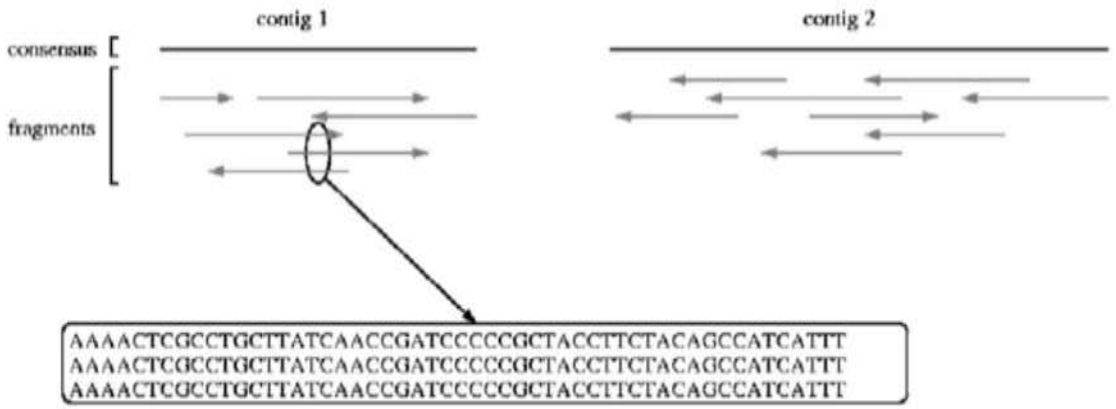
de novo assembly (Sanger)

Overlap/layout/consensus



Essentially,

1. Calculate all overlaps
2. Cluster based on overlap.
3. Do a multiple sequence alignment



UMD assembly primer (cbcb.umd.edu)

de novo assembly (NGS)

de Bruijn graph method

based on k-mers

K-mers

Break reads (of any length) down into multiple overlapping words of fixed length *k*.

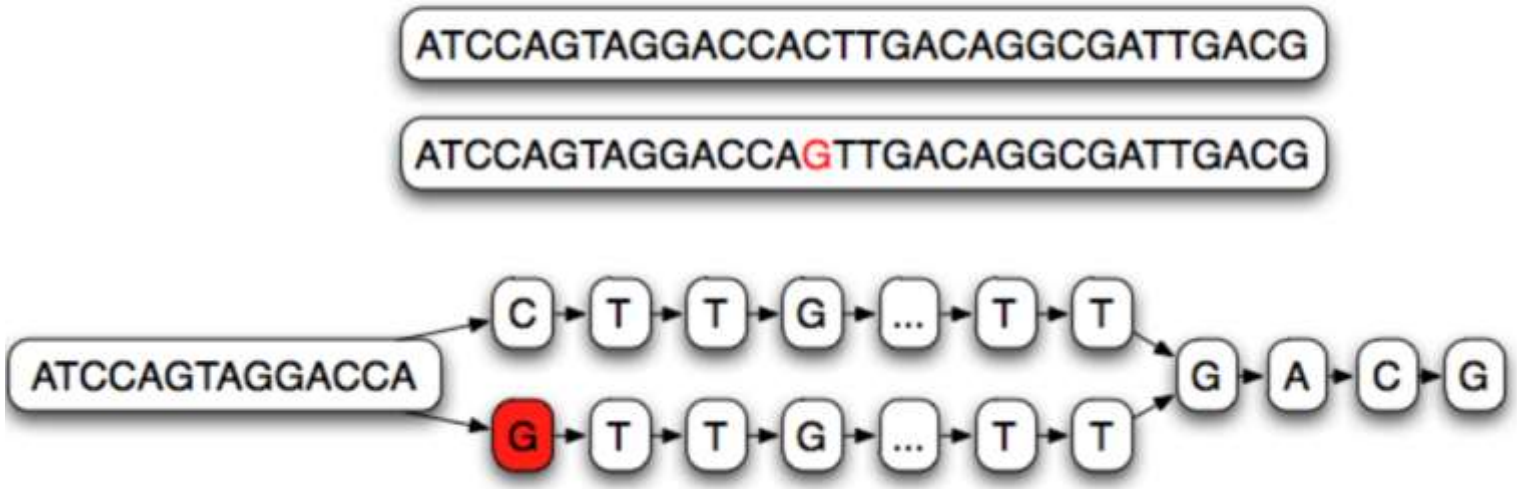
```
ATGGACCAGATGACAC (k=12) =>

ATGGACCAGATG
 TGGACCAGATGA
  GGACCAGATGAC
   GACCAGATGACA
    ACCAGATGACAC
```

de novo assembly (NGS)

de Bruijn graph

'bubbles' in the assembly



De novo assembly

In practice

```
$ tar -zxvf CAC_files.tar.gz /home/hpc####
```