Genome Informatics 2020

Lesson 1 - An introduction

Lesson overview

- Course info
- Bioinformatics and genomics definitions
- Molecular biology basics
- Genome sequencing technologies
- Introduction to Python

**Why slides in English?*

Course info

- 13 classes (lecture + exercise) - ~2.5h
- Exam will have both theoretical and practical part
  - 40% on the exam
  - 60% during the semester
    - 40% project assignment (with presentation)
    - 20% - 2 exercises
- Last class - presentation of student assignments
- Exercise will follow lectures - examples in python Jupyter notebook

Communication

github.com/vladimirkovacevic/gi-2020-etf

(All info about the course. Create an issue!)

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General info (not relevant to all)

Questions about lessons 1-6

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Questions about lessons 7-10

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Questions about lessons 11-12

Course info - syllabus

1	Course info. Bioinformatics and genomics definitions. Molecular biology basics. Genome sequencing technologies. Exercise: Introduction to python and Jupiter environment.
2	Portable and reproducible bioinformatic analysis. Describing bioinformatic tools in Common Workflow Language. Exercise: Python structures. Pandas library. Writing tests.
3	Executing bioinformatic analysis locally and on the cloud. Variant calling. Cancer analysis.
4	Exact string matching: Boyer-Moore, indexing structures, hash tables
5	Exact string matching: Suffix trie, suffix tree. Pigeonhole principle
6	Burrows-Wheeler Transform and FM Index
7	Approximate string matching, Edit distance, Dynamic programming, Global alignment
8	Variation on global alignment (end-space-free variant, longest common substring) , local alignment, gaps. Practical: BLAST, Bowtie
9	Shortest common superstring, Overlap graph
10	De-Bruijn graph, scaffolding, error correction
11	The central dogma of molecular biology, RNA-Seq motivation and technologies for gene expression measurement; RNA-Seq alignment.
12	Normalization procedures; Differential expression: statistical inference, multiple testing corrections; Biological pathways.
13	Presentation of the student projects.

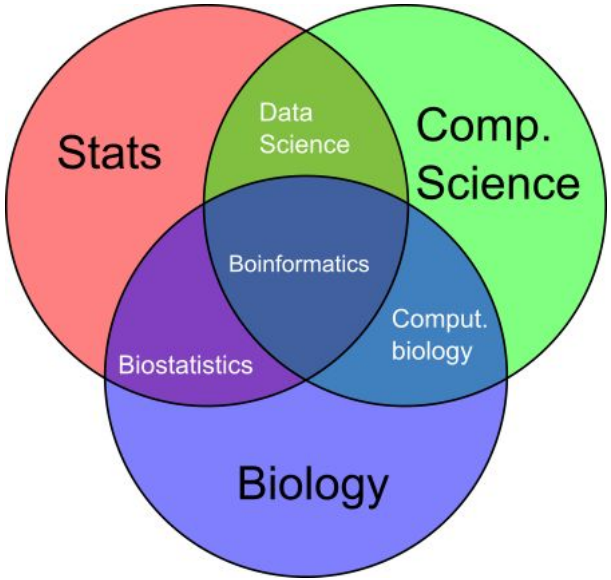
Literature

- Vince Buffalo: **Bioinformatics Data Skills**
- Dan Gusfield: **Algorithms on Strings, Trees, and Sequences: Computer Science and Computational Biology,** Cambridge University Press
- Pavel Pevzner, Neils Jones: **An Introduction to Bioinformatics Algorithms (Computational Molecular Biology)**, MIT Press
- R. Durbin, S. Eddy, A. Krogh, G. Mitchinson: **Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids** , Cambridge University Press
- Veli Mäkinen, Djamal Belazzougui, Fabio Cunial, Alexandru I. Tomescu: **Genome-Scale Algorithm Design: Biological Sequence Analysis in the Era of High-Throughput Sequencing**, Cambridge University press

What is bioinformatics

Bioinformatics, n. The science of information and information flow in biological systems, esp. of the use of computational methods in genetics and genomics. *(Oxford English Dictionary)*

Bioinformatics - using statistical and computing methods that aim to solve biological problems.



What is bioinformatics

"I do not think all biological computing is bioinformatics, e.g. mathematical modelling is not bioinformatics, even when connected with biology-related problems. In my opinion, bioinformatics has to do with management and the subsequent use of biological information, particular genetic information."

-- Richard Durbin

Bioinformatics in practice: Develops methods and software tools for storing, retrieving, organizing and analyzing biological data.

Bioinformatics principles

- Golden rule of bioinformatics: **Never ever trust your tools (or data)**
- Adapt robust and reproducible practices
  - Document each step
  - Write down key facts
  - Conclude the work done to easy understand it again in few months (make figures, reports)
  - Automate manual tasks with script
  - Always ask yourself: How much time would it take me to do it one more time?
  - Release or publish your code and tools
- Write code for humans, Write (meta)data for computers
  - Make assertions (be loud)
  - Let the code test the code
  - Use existing libraries whenever possible
  - Frequently used scripts -> tools
  - Use code versioning (git)

**Vince Buffalo: Bioinformatics Data Skills*

Genomics 101

Genome: “The complete set of genes or genetic material present in a cell or organism.” *(Oxford English Dictionary)*

- “Blueprint” or “recipe” of life
- Human genome - 6 billions of base-pairs (A, C, T, G) letters
  - Can be imagined as a sting 6 billion letters long

ACTGTGTCACATCGAGAGAGATCACAACACATAGATTACGATCGTAACGT
AACGTAACACCCAAATATACGAGTGAGGGGTGGGGACCCCCCCCCCCCCAC
ACACATTTTTACAAACCTAGATCACCATACAGATATAAGAGAGAGANACG
TACGTACACAATTACAAATTAACAACACAAAGTACTTATACATACACATG
GGACCCATAGCACACACAGATATTTATAATATATAGAGAGACAATGTCGT
GCTGCAGTAA...

Genomics: contrast with biology & genetics*

* Everything on this slide is a gross generalization

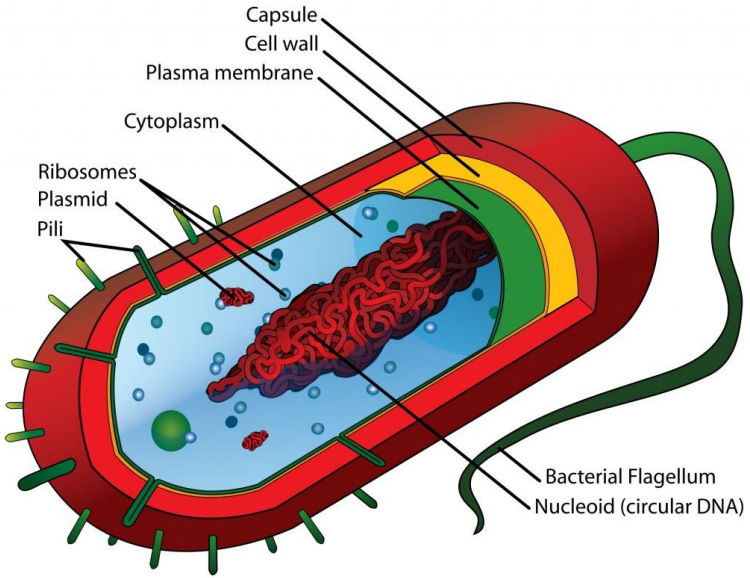
Biology & Genetics		Genomics
Targeted studies of one or a few genes	scope	Studies considering all genes in a genome
Targeted, low-throughput experiments	technology	Global, high-throughput experiments
Clever experimental design, painstaking experimentation	hard part	Tons of data, uncertainty, computation

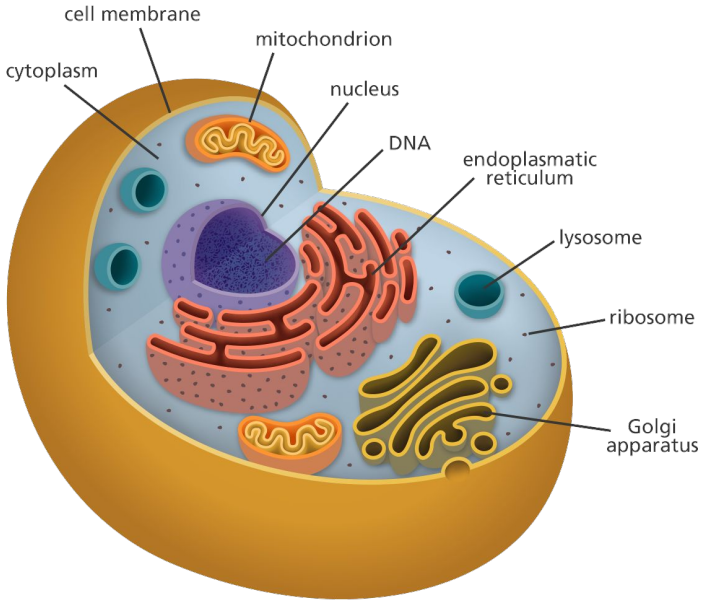
Used with permission of Ben Langmead

The cell

Fundamental working units of every living system.

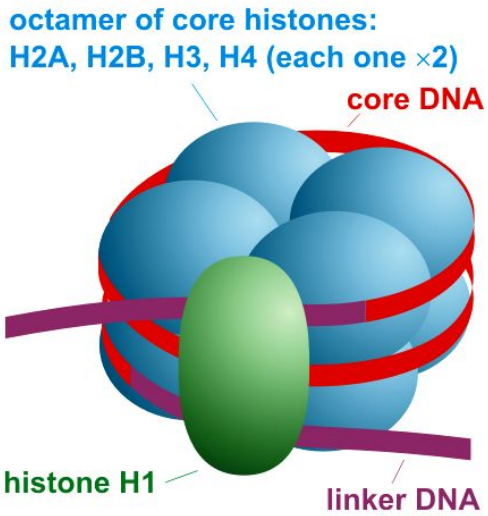
- Prokaryotic (bacteria)
- Eukaryotic (higher organisms - animals, plants)

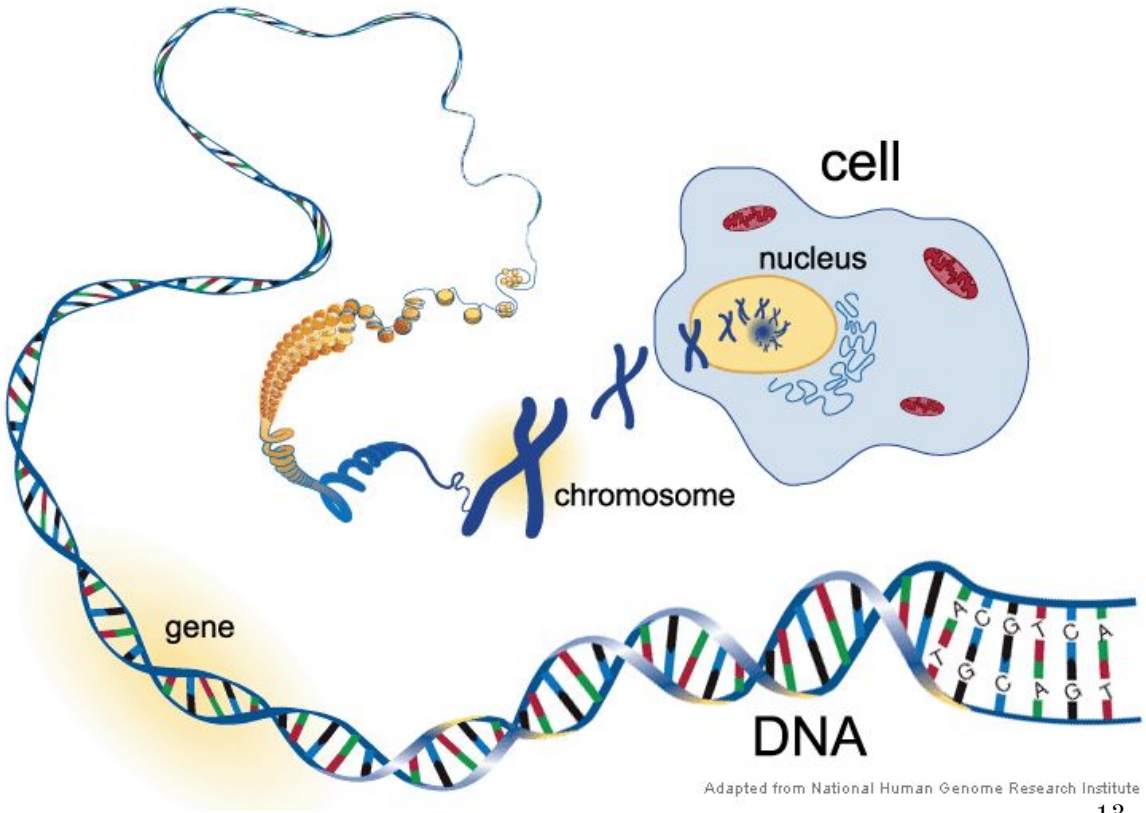




“And inside the nucleus thou lays the mighty DNA”

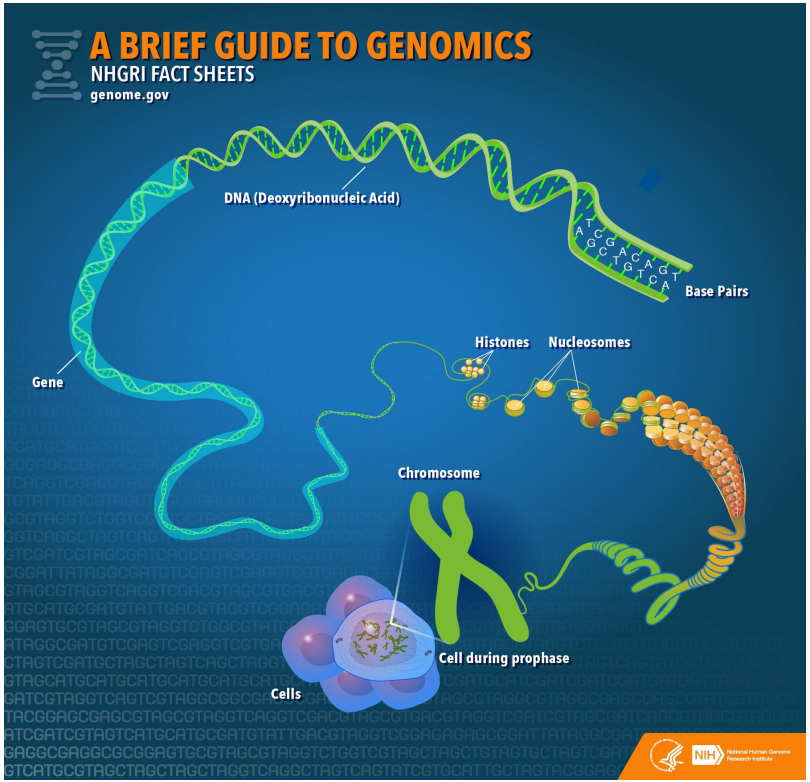
- Chromatin - tightly packed DNA
- A **nucleosome** is a basic unit of DNA packaging in eukaryotes, consisting of a segment of DNA wound in sequence around eight histone protein cores.
- Current model





DNA - the code of life

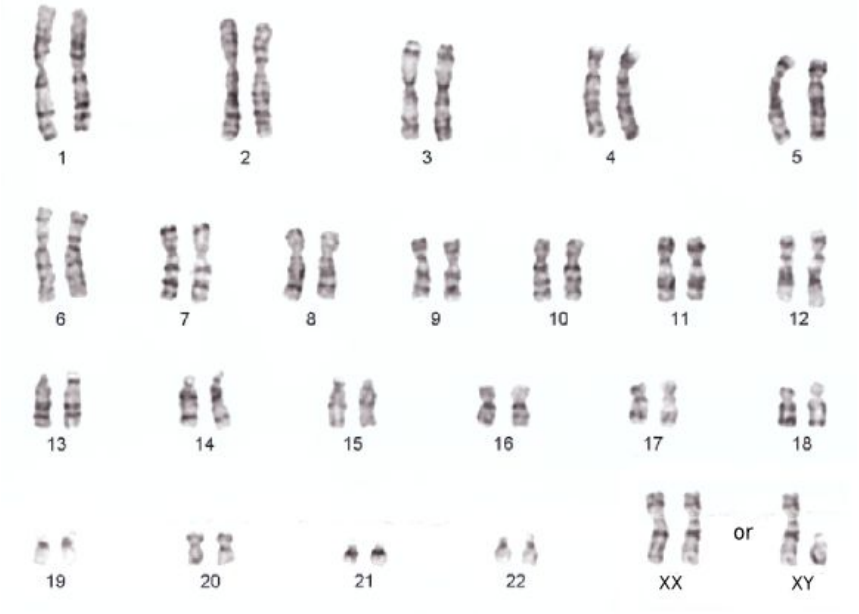
- DNA (deoxyribonucleic acid) - double stranded molecule
- Same in every cell - DNA replication during cell division
- More stable, redundant information - complementary double helix chain
- ~99.6% same between 2 individuals
- Base (nucleotide) pairs (complementary bases)
  - A - T (adenine and thymine)
  - C - G (cytosine and guanine)



Genome

- Set of all pairs of chromosomes

- Human genome:
  - 23 pair of chromosomes (diploid)
  - 22 autosomes
  - 1 sex chromosome (X and/or Y)
  - 3 billion base-pairs x 2
  - CTGGATTATATATCGAAGGGACTAT... etc
  - Intron and exom (2%)

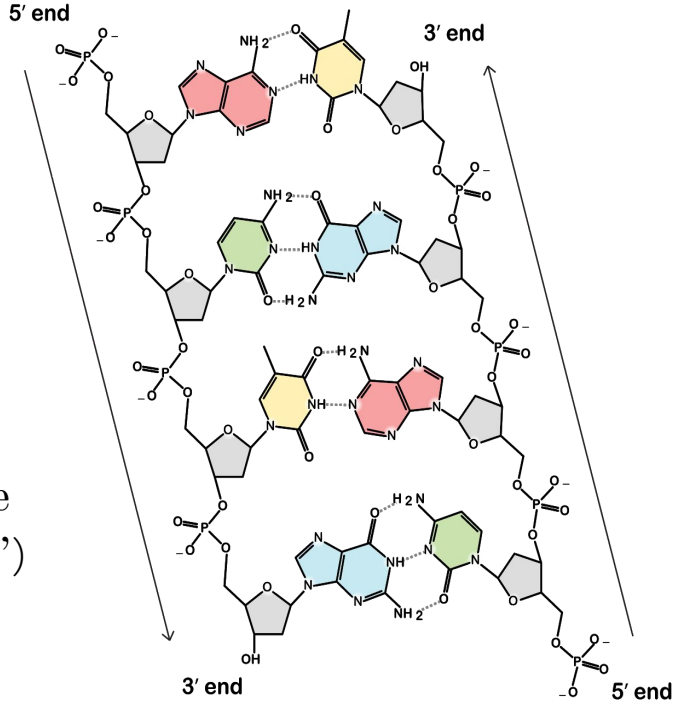


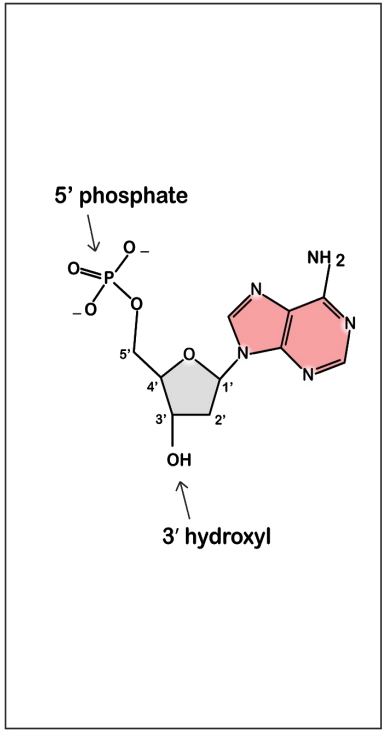
Karyogram

DNA - structure

- Consists of:
  - Phosphate group
  - Sugar (deoxyribose)
  - Nitrogen base
- Hydrogen bonds
- Forward and reverse strand
- DNA direction:
  - 5’ head and 3’ tail
  - Transcribed from 5’ to 3’ end
- In bioinformatics we write just one strand (by convention from 5’ to 3’)

5’ ACTG *3’*
↕
3’ TGAC *5’*
(reverse complement)





DNA - discovery

1952-1953 James D. Watson and Francis H. C. Crick deduced the double helical structure of DNA from X-ray diffraction images by Rosalind Franklin (provided by M. Wilkins) and data on amounts of nucleotides in DNA.

“Molecular structure of nucleic acids. A structure for deoxyribose nucleic acid”

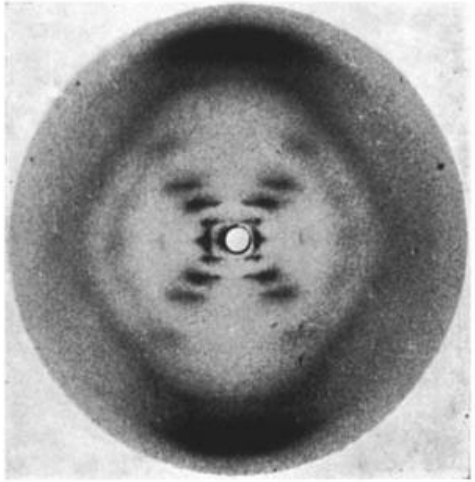


Photo 51

Central dogma of molecular biology

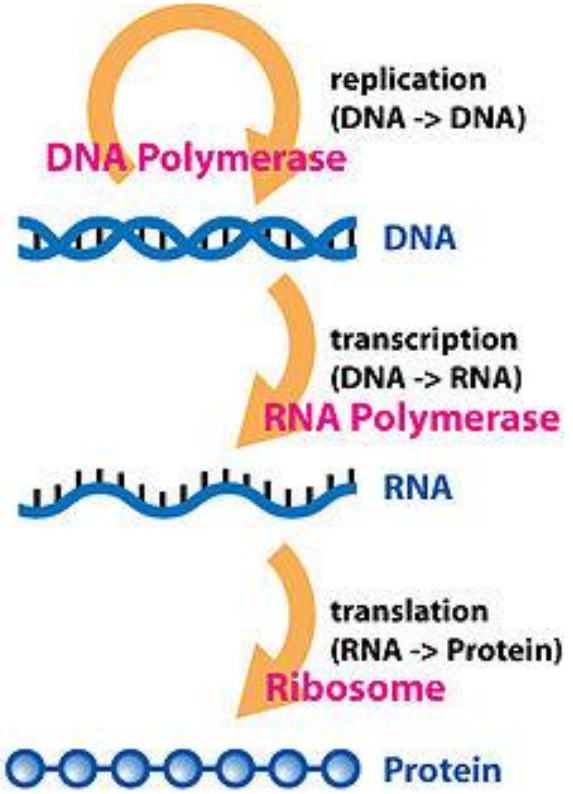
DNA ----> RNA -----> Protein

Transcription: DNA ->RNA

- particular segment of DNA is copied into RNA (especially mRNA) by the enzyme RNA polymerase.

Translation: RNA -> Protein

- process in which ribosomes synthesize proteins after the process transcription of DNA to RNA in the cell's nucleus.



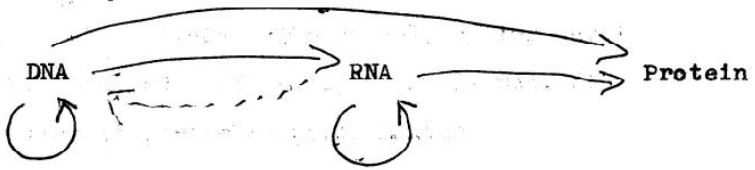
Central dogma of molecular biology

Ideas on Protein Synthesis (Oct. 1956)

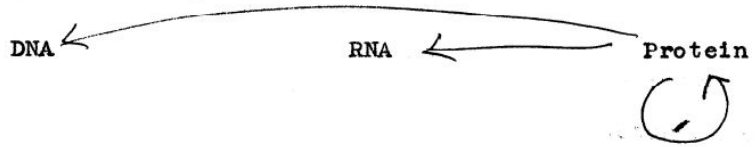
The Doctrine of the Triad.

The Central Dogma: "Once information has got into a protein it can't get out again". Information here means the sequence of the amino acid residues, or other sequences related to it.

That is, we may be able to have



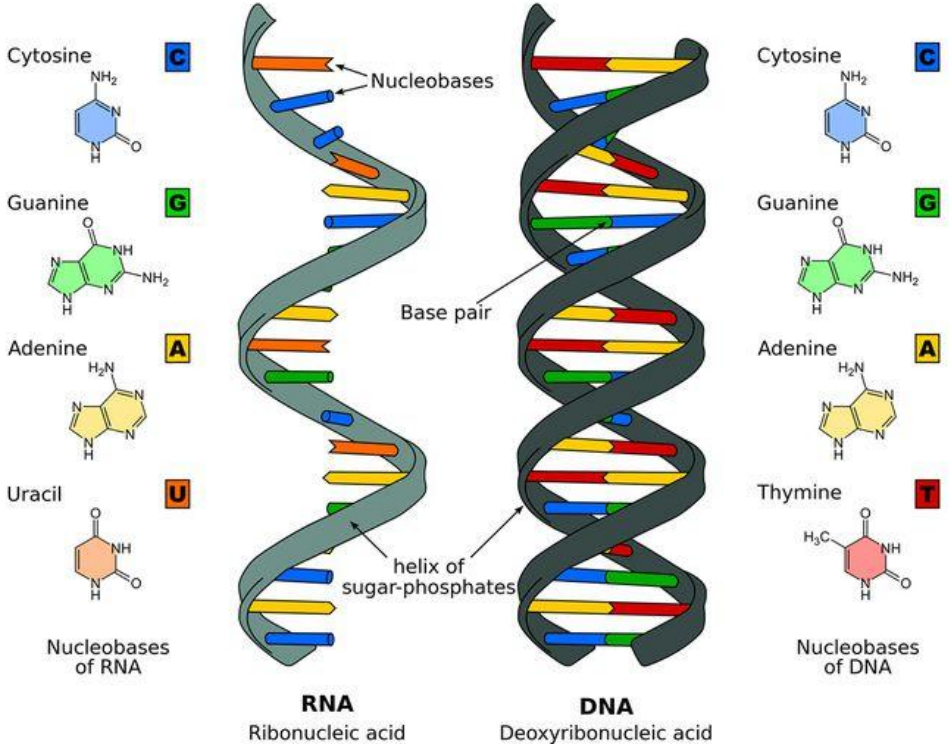
but never



where the arrows show the transfer of information.

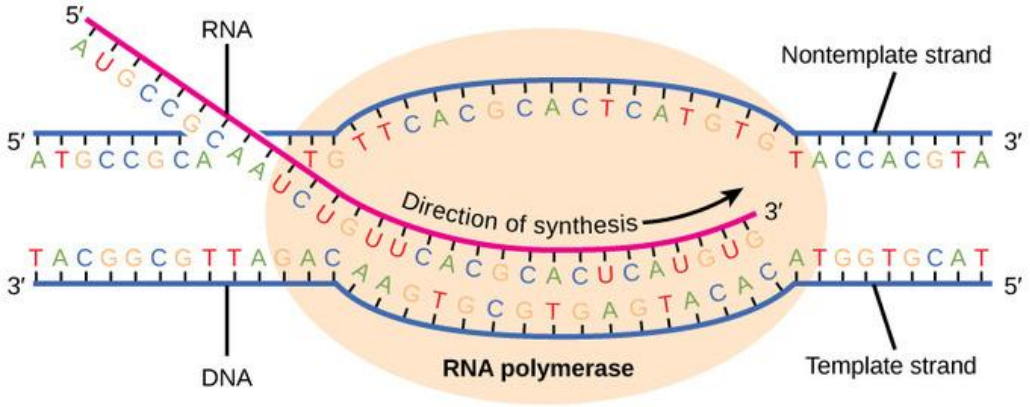
RNA

- Single stranded
- Sugar:
  - ribose (instead of deoxyribose)
- Uracil instead of Thymine

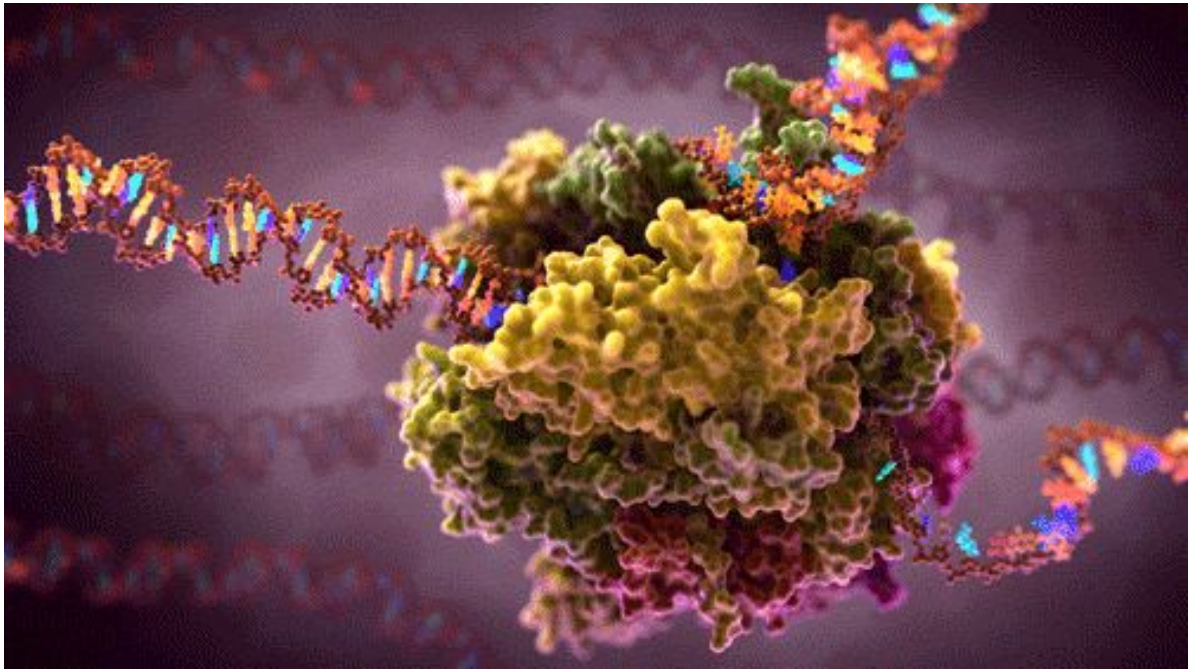


Transcription

- Template (noncoding) strand
  - One which is transcribed by RNAP (RNA polymerase)

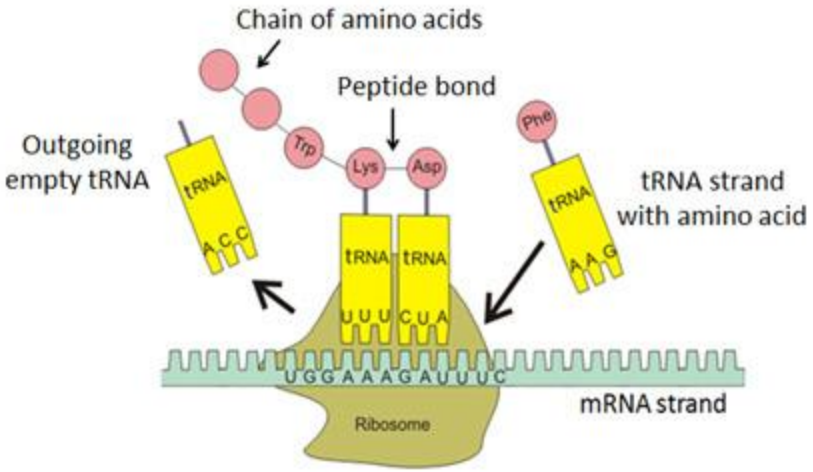


Transcription



Translation

- Occurs in ribosome
- Each triplet of nucleotides (codon) codes for specific amino-acid
  - “Letters of protein code”
  - 20 amino-acid (some redundancy)

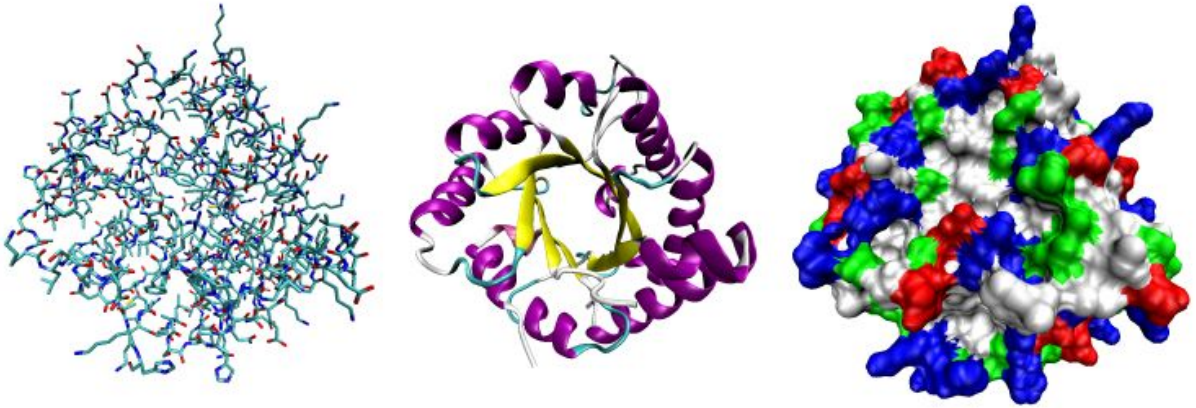


Second letter

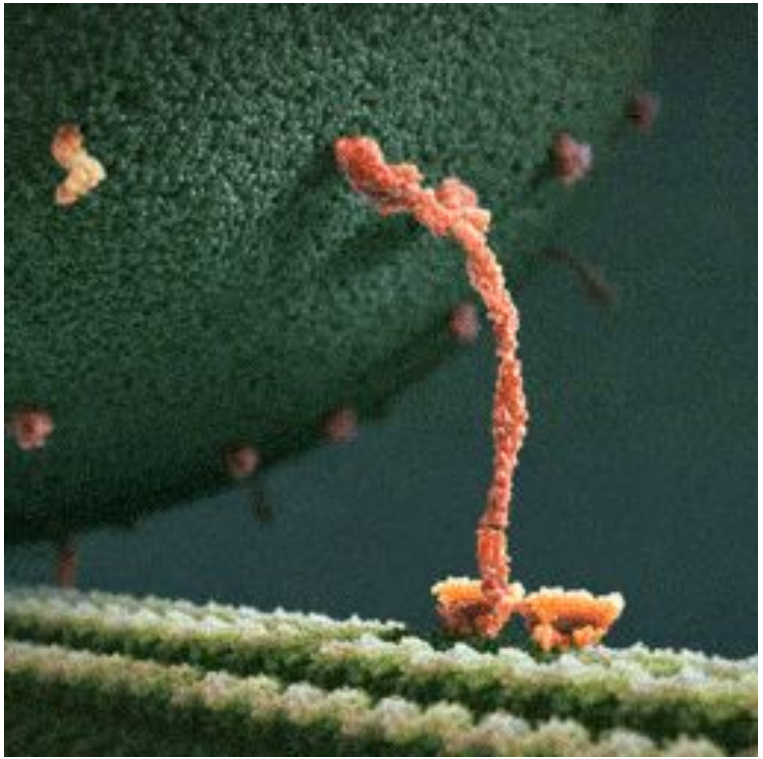
First letter	U	C	A	G	Third letter
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr **UAA Stop** **UAG Stop**	UGU Cys UGC Cys **UGA Stop** UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

Proteins

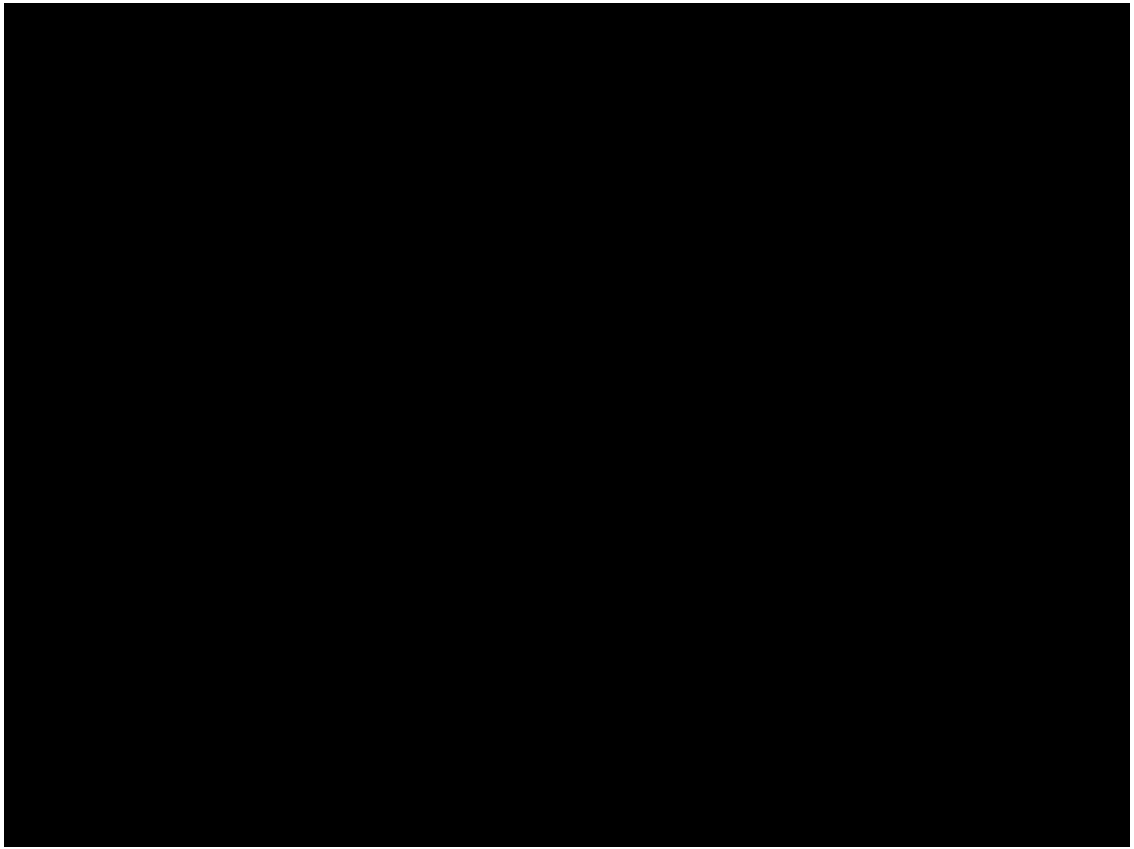
- Building blocks of life
  - Various functions in the organism (transportation, regulation, metabolism, DNA replication)
- Long chains of amino-acids, that also fold into complicated 3D structures
  - We often distinguish protein primary, secondary, tertiary and quaternary structure



Proteins



Proteins



Transcription & Translation: The Central Dogma of Biology - DNA Learning Center

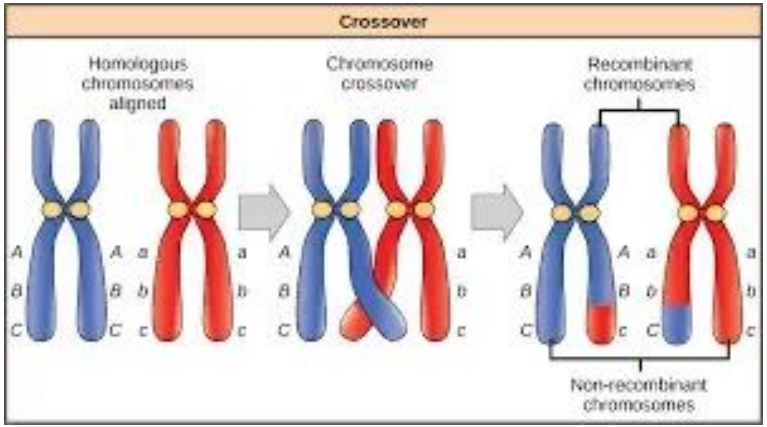
Biology 101 - genotype vs phenotype

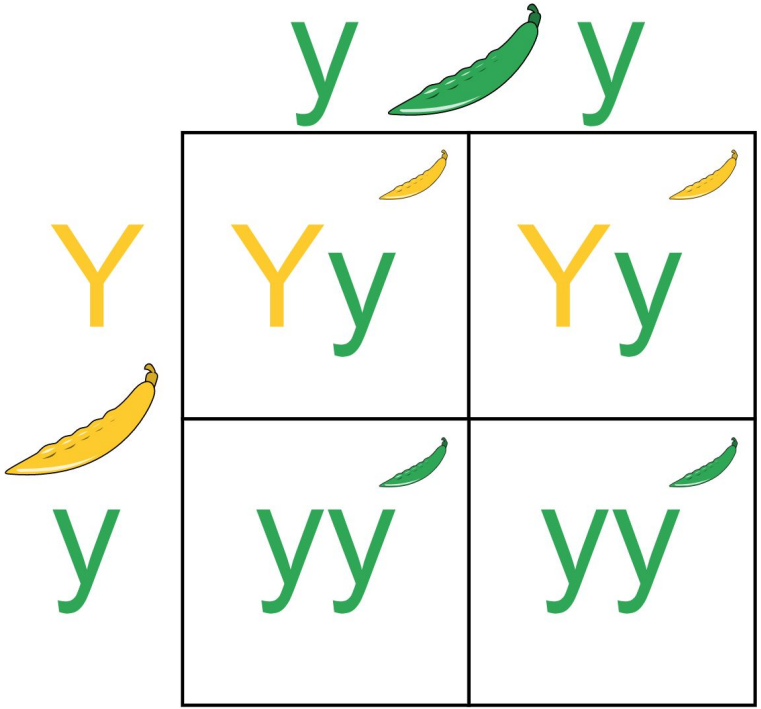
The **genotype** is the part of the genetic makeup of a cell, and therefore of an organism or individual, which determines one of its characteristics (phenotype).

A **phenotype** (from Greek *phainein* , meaning 'to show ', and *typos* , meaning 'type') is the composite of an organism's **observable characteristics** or traits, such as its morphology, development, biochemical or physiological properties, behavior, and products of behavior (such as a bird's nest).

Rules of inheritance

- Mendelian inheritance
- Multiple alleles of the same gene
  - One allele per chromosome
  - Dominant/recessive allele

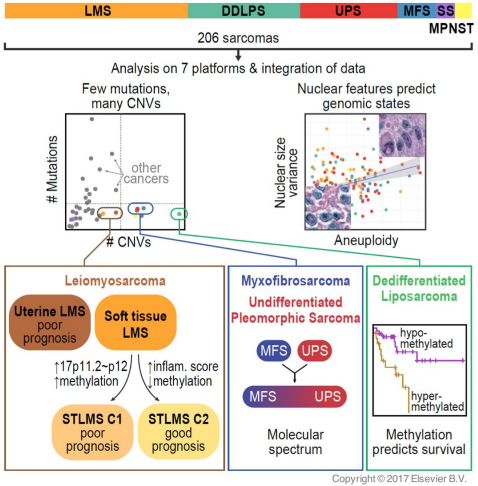


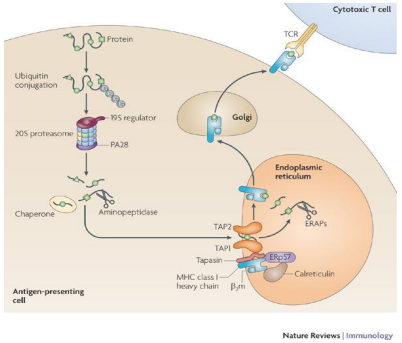


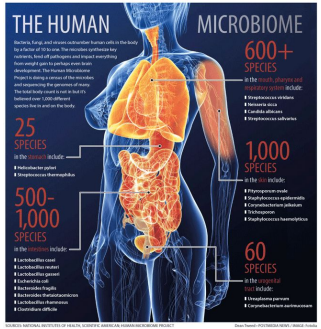
Example of inheritance combinations

Why perform DNA sequencing?

- Rare genetic diseases
- Origins of humans
- Precision medicine- Cancer treatment (immunotherapy)
- Microbes that live inside us (microbiome)
- Study ways that genomes work
- Gene editing
- Forensics





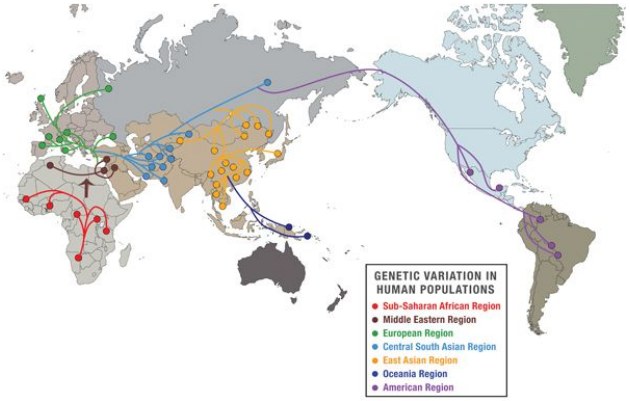


MEDICAL DISPATCH JULY 21, 2014 ISSUE

ONE OF A KIND

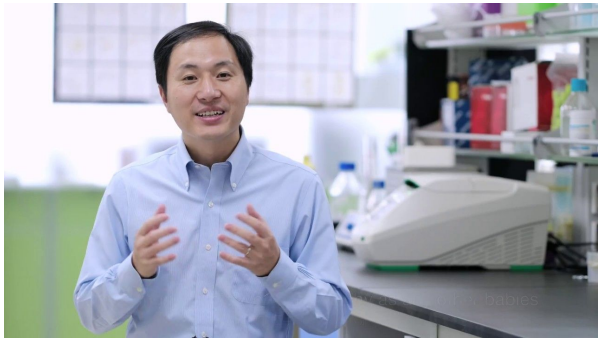
What do you do if your child has a condition that is new to science?

By Seth Mnookin



The world's first germline genetically edited babies

- Clinical project: standard in vitro fertilization
- + CRISPR-Cas9 (technology that can modify DNA)
- Mother was HIV positive
- Modify the CCR5 gene on single egg cell before fertilization to confer genetic resistance to the HIV virus
- CCR5 codes for a protein that HIV uses to enter cells
- Clinical project was conducted secretly until November 2018
- Lulu and Nana are born healthy crying babies



He Jiankui

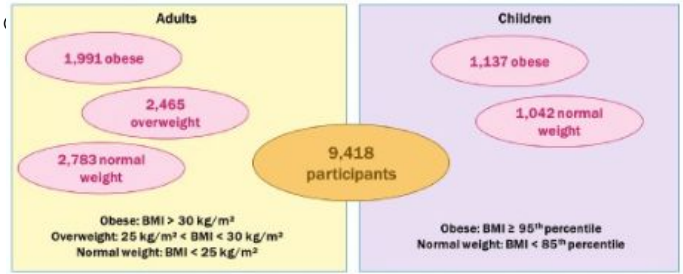
Exom sequencing of 25000 schizophrenia cases and 100 000 controls

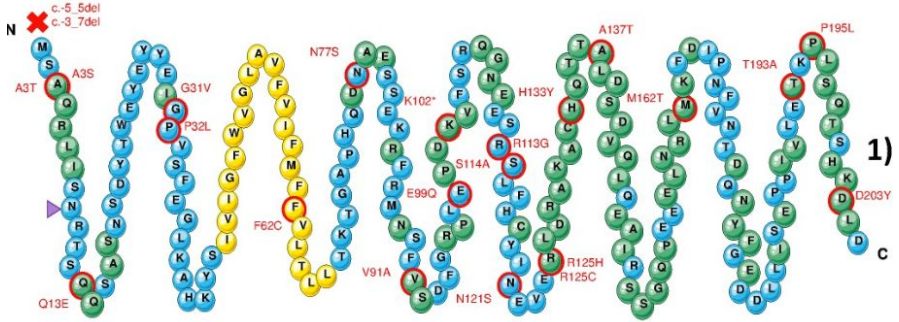
Tarjinder Singh

- The Schizophrenia Exome Sequencing Meta-analysis (SCHEMA) consortium (2017) - aggregating and analyzing high-throughput sequencing data
- Understand the genetic causes of schizophrenia
- Motivate the development of new therapeutics
- Life expectancy - 12-15 years shorter life expectancy
- **10 genes** that when disrupted, dramatically increase risk for schizophrenia
- Odds ratios 4 - 50, $P < 2e\text{-}6$
- 2 genes code glutamate receptors - crucial in brain cells communication
- 10 genes have no protein-truncating variant signal
- Significant overlap between autism ($n = 102$, FDR $< 10\%$) and neurodevelopmental delay risk genes ($n = 34$, FDR $< 5\%$)

Pathogenics loss-of-function in MRAP2 cause metabolic syndrome - A. Bonnefond

- Melanocortin receptor accessory protein 2 (MRAP2) is a transmembrane accessory protein predominantly expressed in the brain
- Deletion of Mrap2 results obesity in both mice and human
- 23 rare mutations in MRAP2
  - 2 frameshift
  - 1 non-sense





Insight into genetic architecture of autism

Adam Rocke

- Autism sequencing consortium (SPARK)
- ASD affects 1–1.5% of individuals and is hi
- 18,381 autism spectrum disorder (ASD) cas
- identifies 5 risk loci

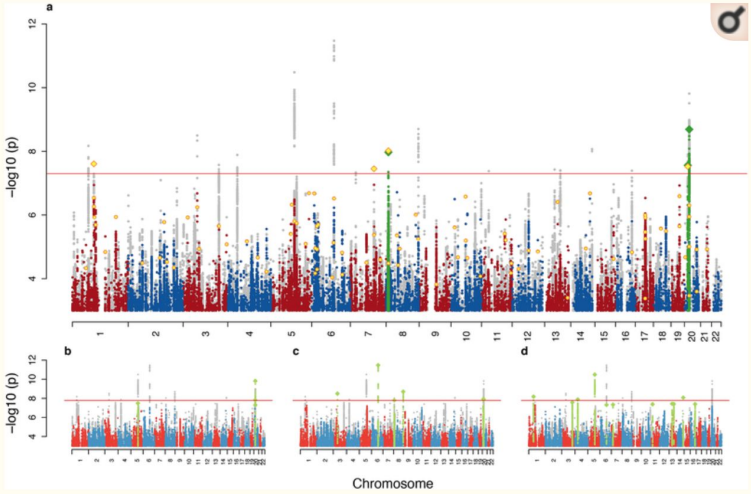


Figure 1.

Manhattans plots:

with the x axis showing genomic position (chromosomes 1–22) and the y axis showing statistical significance as −log10 (*P*) of z statistics. **a:** The main ASD scan (18,381 cases and 27,969 controls) with the results of the combined analysis with the follow-up sample (2,119 cases and 142,379 controls) in yellow in the foreground. Genome-wide significant clumps are painted green with index SNPs as diamonds. **b-d**: Manhattan plots for three MTAG scans of ASD together with, respectively, schizophrenia[12] (34,129 cases and 45,512 controls), educational attainment[29] (*N* = 328,917) and major depression[29] (111,902 case and 312,113 controls). See Supplementary Figures 45–48 for full size plots. In all panels the results of the composite of the five analyses (consisting for each marker of the minimal p-value of the five) is shown in grey in the background.

Insight into genetic architecture of autism

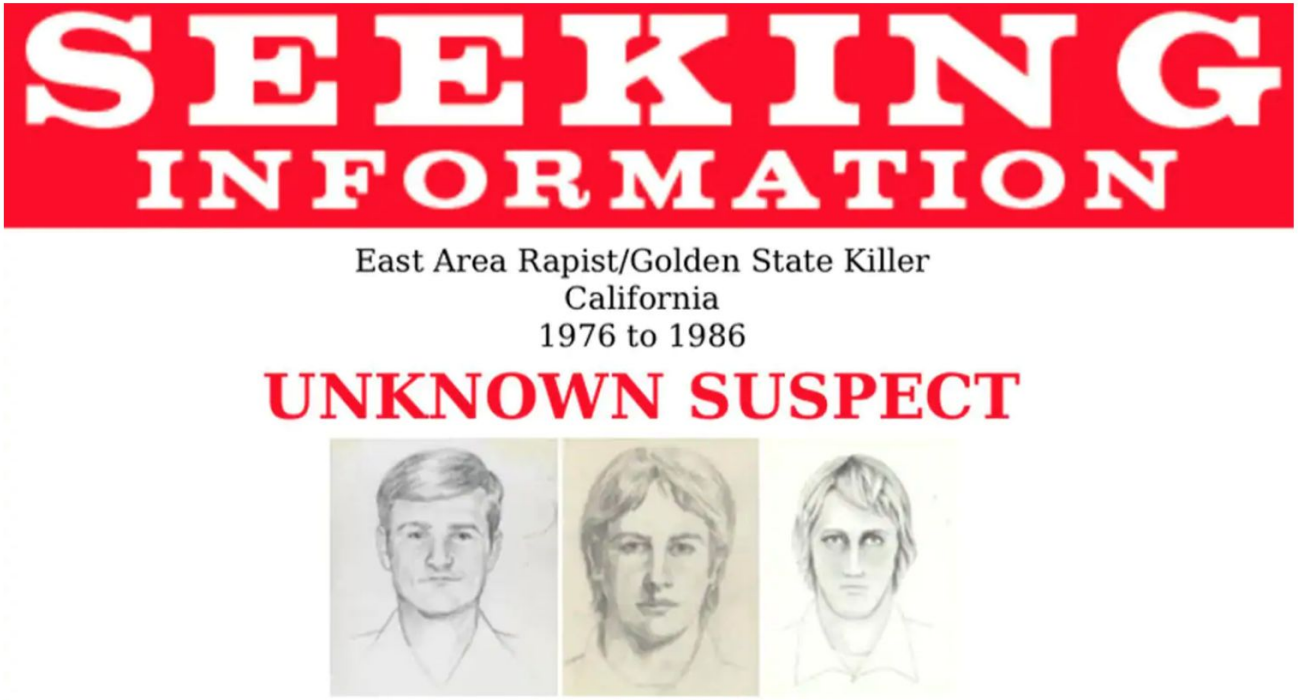
Adam

Supplementary Table 4: Review of the top loci identified in the single SNP analysis with and without follow-up sample, the three MTAG analyses, and the gene-wise MAGMA analysis (see also main Table 1, Table 3, 10 and Box 1 for further details and explanations of significance tests). Loci are $r^2 = 0.1$ clumps and the genes listed are the protein coding genes in the locus except marked with * (see below) or on chromosome 8 where the locus around rs10099100 was restricted to a $r^2 = 0.6$ clump plus 50kb flanks. See section 2.1.4 for details.
* Nearest protein coding gene within ±1 Mb from locus.
** Results based on samples overlapping the present ASD sample, hence not independent.
† Previous GWS hits for brain disorders in locus.

Chr	Gene	Index SNP (BP)	Analysis P-value	Function	Tissue specificity	Disease	Previous GWS hits in locus†
1	*NEGR1*	rs1620977 (72729142)	MTAG MD: $6.7 \cdot 10^{-9}$	*NEGR1* encodes the Neuronal growth regulator 1, a GPI-anchored IgLON protein belonging to the immunoglobulin superfamily of cell adhesion molecules. *NEGR1* has been identified as a raft-associated component of the brain that is involved in neurite outgrowth[72–74] and neurodevelopmental determination of synapse number in the hippocampus[75]. *NEGR1* expression levels are affected by nutritional state in brain areas relevant to feeding[76] and *NEGR1* may also serve a role in intracellular cholesterol trafficking[77].	NEGR1 is localized at postsynaptic sites of dendritic and somatic synapses[78] and is expressed at high levels during postnatal development in cerebral cortex, hippocampus, cerebellum, and hypothalamus[78–80].	A 1p31.1 deletion including a part of *NEGR1* (and no other genes) has been identified in two siblings with a history of neuropsychiatric and behavioral problems, learning difficulties, hypotonia, mild aortic root dilatation, hypermobility and scoliosis[81].	Meta-analysis of autism spectrum disorder and schizophrenia[24]**, Schizophrenia[82], Educational attainment[50], Depressive symptoms[62], Major depression[48], Intelligence[83]
1	*PTBP2**	rs201910565 (96561801)	Comb: $2.5 \cdot 10^{-8}$	*PTBP2* encodes Polypyrimidine tract-binding protein 2. PTBP2 is a PTBP1 (Polypyrimidine tract-binding protein 1) paralog and is also known as nPTB (neuronal PTB) or brPTB (brain PTB). PTBP1 and PTBP2 binds to intronic polypyrimidine clusters in pre-mRNA molecules and each target large sets of exons to coordinate programs of splicing events during development[84]. During neuronal development and differentiation, several switches in the expression of *PTBP1* and *PTBP2* activates networks of new spliced isoforms[85–88].	*PTBP2* is expressed at high levels in adult brain, testis, myoblasts and lymphocytes[89, 90]. Different isoforms generated by alternative splicing of *PTBP2* are expressed in a tissue specific manner[90, 91]. In neuronal cells, *PTBP2* acts by autoregulating its own exon 10 inclusion, leading to an increased expression level of the PTBP2 protein[92]. PTBP1 promotes the exon 10 exclusion from *PTBP2* transcripts, leading to NMD of *PTBP2* transcripts[93].	Hemizygous deletion of varying genomic regions containing *PTBP2*, *DPYD* and *MIR137* have been reported in cases with ASD, severe speech delay and intellectual disability[94, 95]. Two brothers with ASD and intellectual disability born to consanguineous parents were found to be homozygous for a novel 5 bp indel variant located in a human accelerated region (HAR) between *DPYD* and *PTBP2*[96]. This intergenic indel variant was suggested to affect the PTBP2-directed enhancer activity of the region and that this effect was relatively specific to neurons[96]. Further evidence pointing to *PTBP2* as a ASD risk gene include identification of a *de novo PTBP2* potentially damaging missense variant in an ASD proband[97] as well as identification of a *de novo PTBP2* intronic insertion in a ASD proband from a simplex family[98].	Educational attainment[50]

Table

Golden state killer



Golden state killer

- https://www.gedmatch.com, tutorial
- applications for comparing your DNA test results with other people
- Genealogical Data Communication
- Software developed by the Church of the Latter Day Saints

- Site found 10-20 distant relatives of the killer, roughly, equivalent of third cousins
- “When you go that far back in time, you have trees that grow huge,” Holes said.
- Census data, old newspaper and a gravesite locator to find the deceased relatives, websites such as LexisNexis.

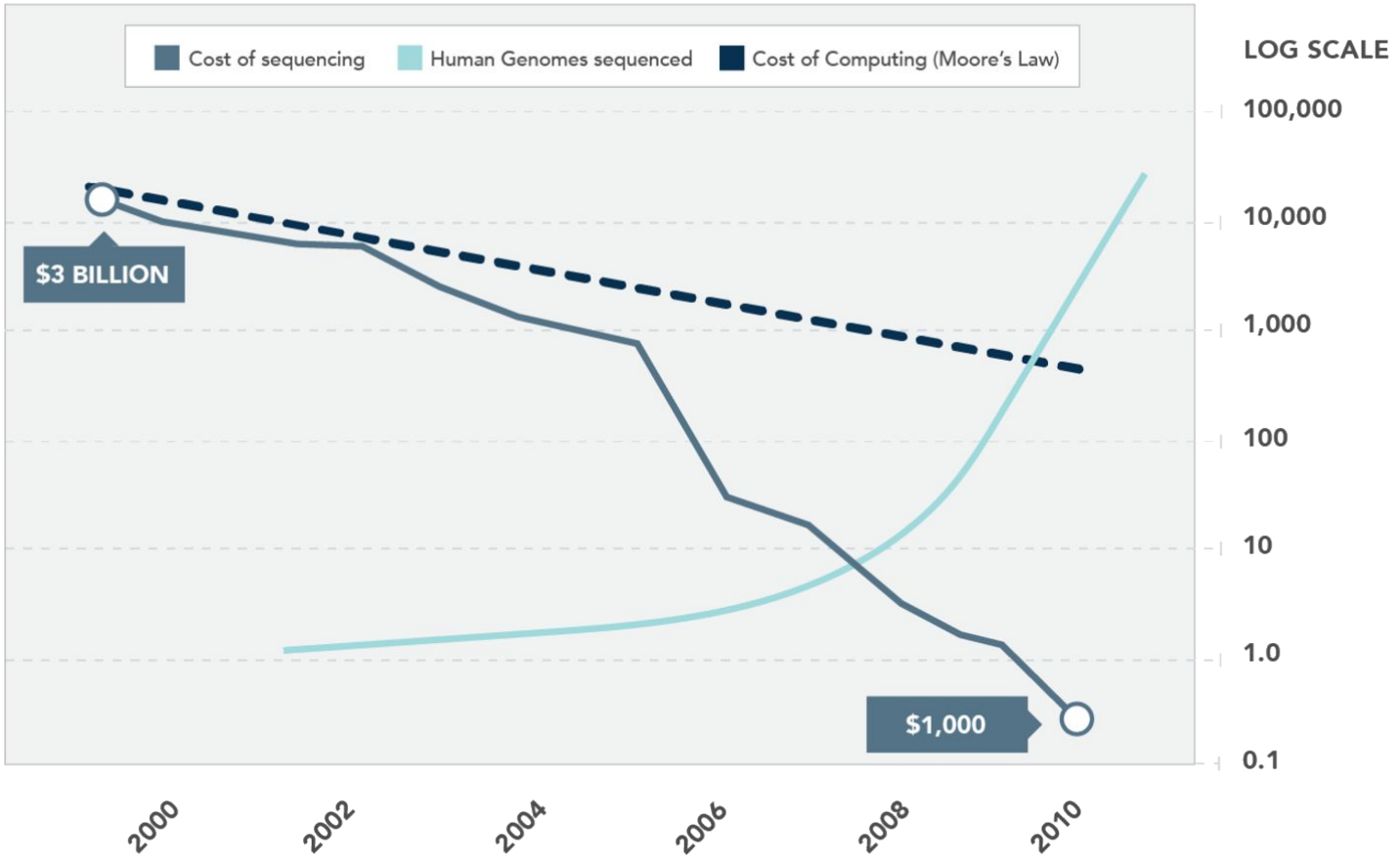
Golden state killer

Joseph James DeAngelo

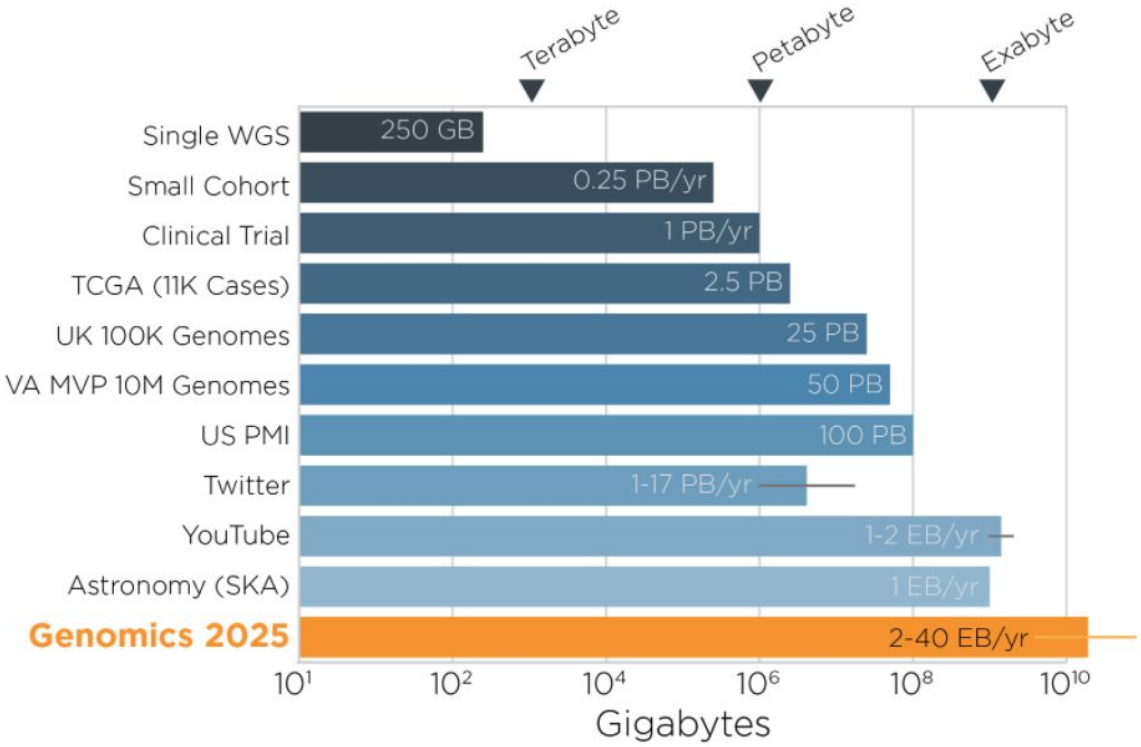
Genome sequencing

- Digitalization of genome
- **Human Genome Project** (1990-2003), 3B $
- Birth of bioinformatics
- Sanger sequencing (First generation sequencing)
  - Long (took 13 years)
  - Costly (3B$ for one human genome)
- Currently NGS (next generation sequencing)
  - Illumina
  - Around 200$ and 1 day needed to sequence the genome
- Also third generation sequencing in use
  - Longer read-length (up to 50k base)
  - Oxford nanopore, PacBio
  - Higher error rate
  - Smaller in size
  - Sequencing in space

GROWTH OF DNA SEQUENCING



Genomics is Big Data



Source: "Big Data: Astronomical or Genomical?" *PLoS Biology* (2015).

Sequences:
1 zetta-bases/yr

Storage needs:
2-40 exabytes

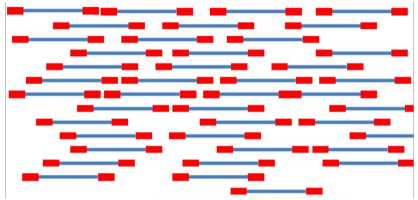
Compute for Alignment:
10,000 trillion CPU hrs
= 83x time since Big Bang

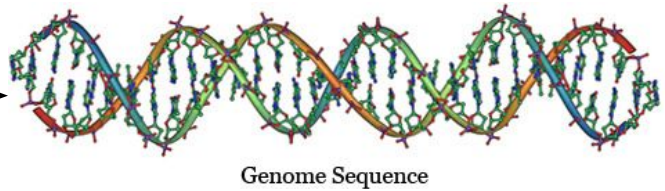
Variant Calling:
~**2 trillion** CPU hrs

Tertiary Analysis:
~**4 trillion** CPU hrs
= time since land-breathing mammals evolved

Bioinformatics to the rescue!

- Genomes of the all species are arrays of nucleotides (A, T, C, G) - strings
- The process of DNA sequencing returns only fragments of it
- Our mission: RECONSTRUCT IT!

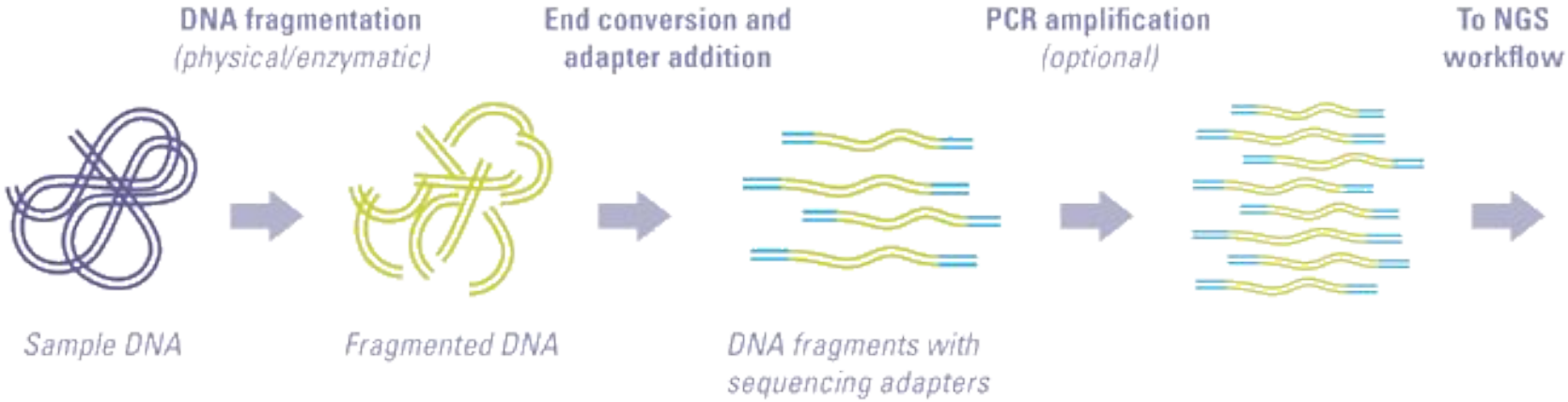




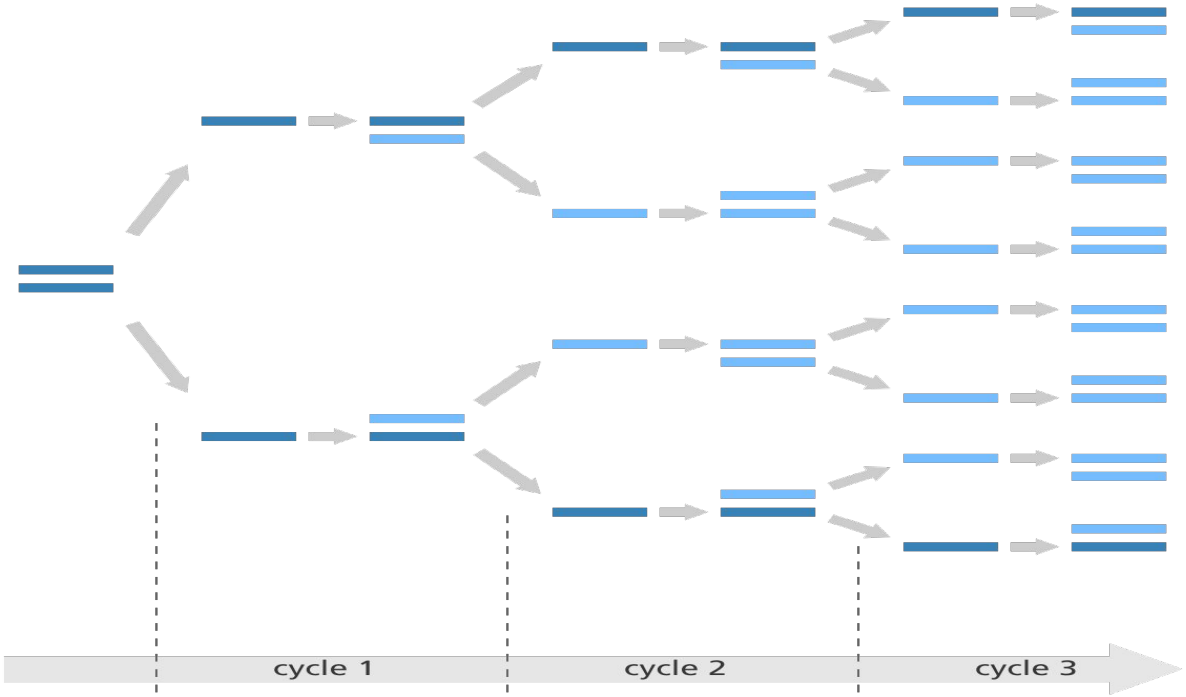
Genome Sequence

Illumina sequencing

- Read - DNA fragment after reading it in sequencer
- Typical whole genome sequencing experiment:
  - 200-500 million reads
  - 50-150 bases (letters long)

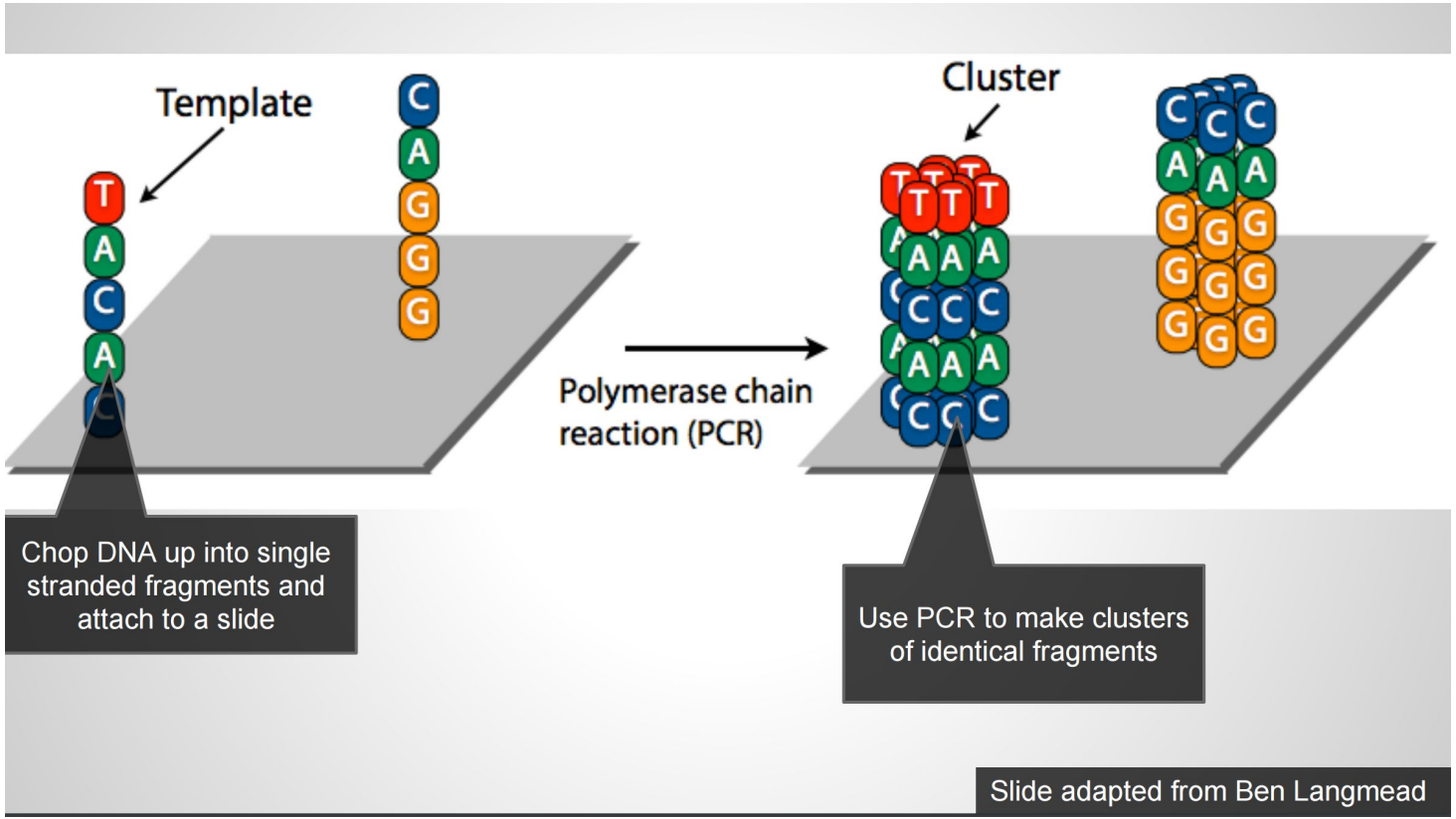


Sequencing - PCR (polymerase chain reaction)

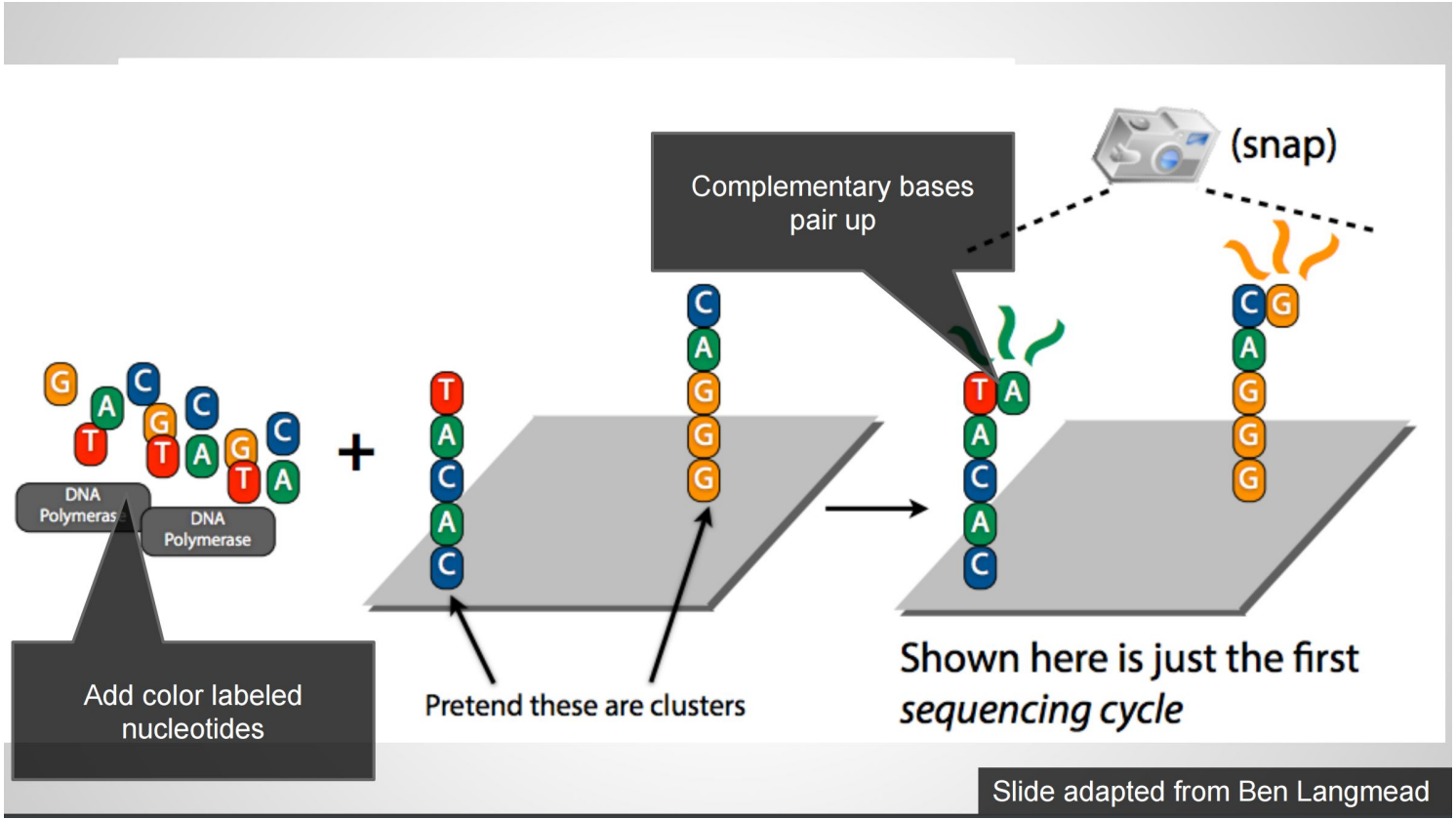


Bridge amplification

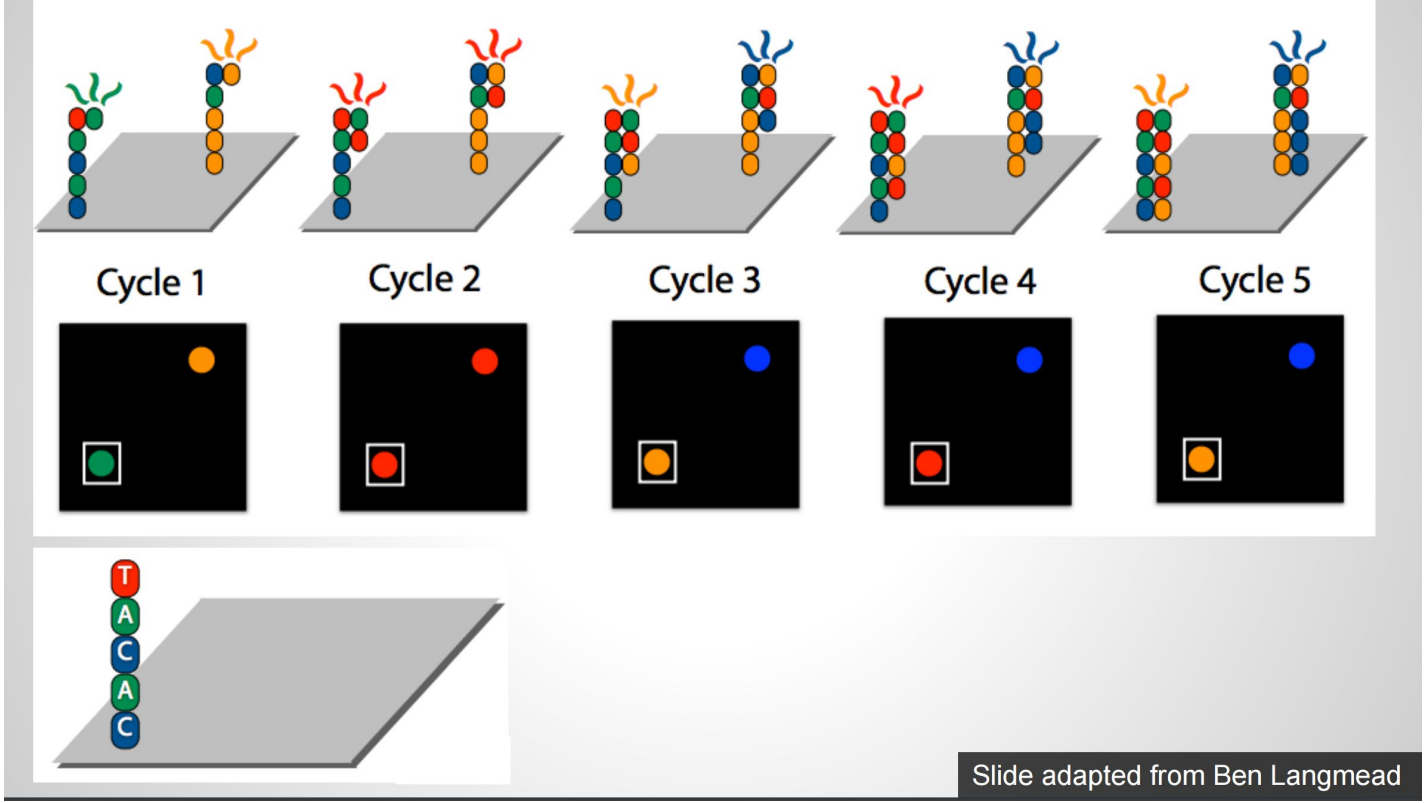
Sequencing (Illumina)



Sequencing (Illumina)

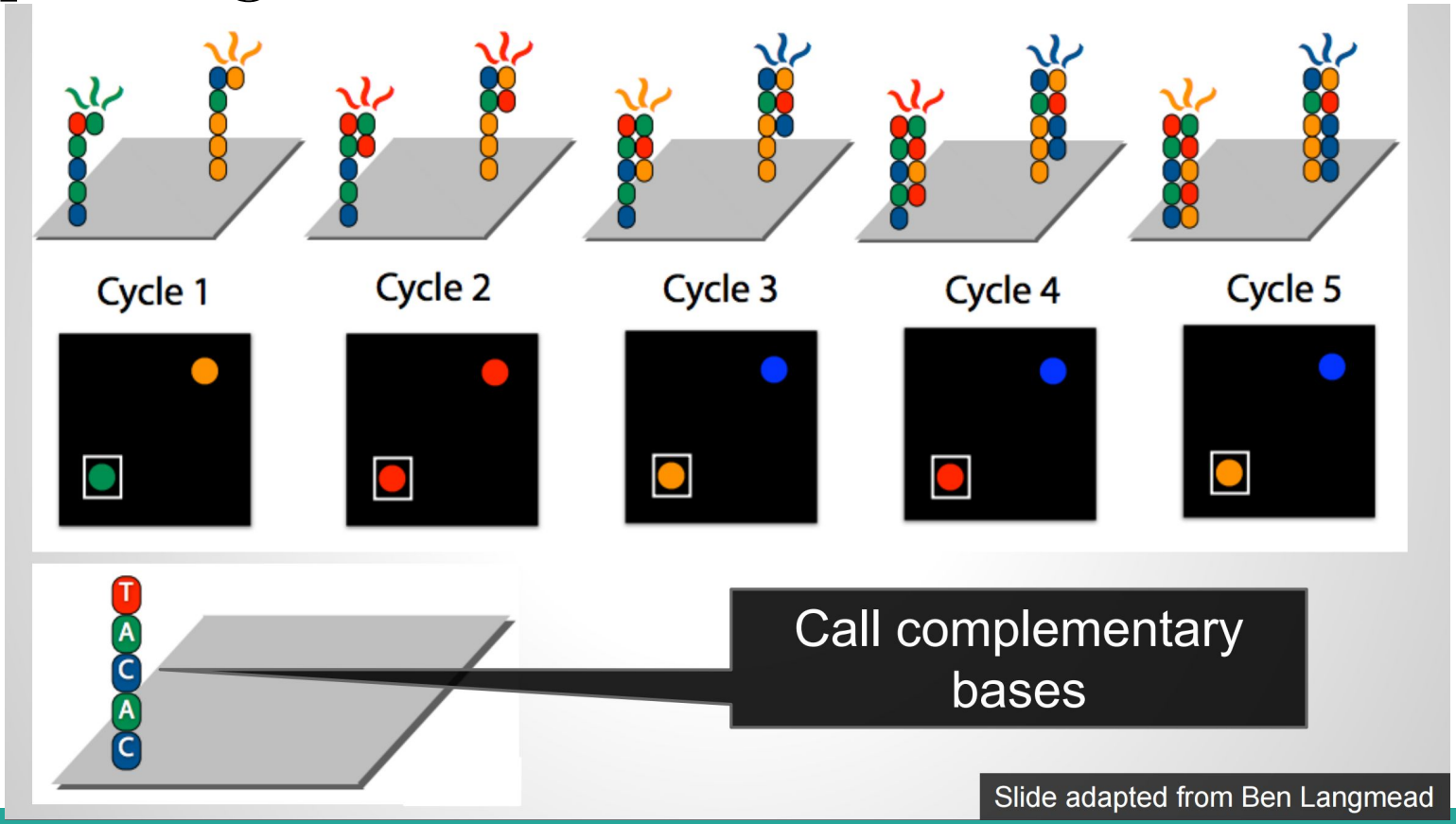


Sequencing (Illumina)



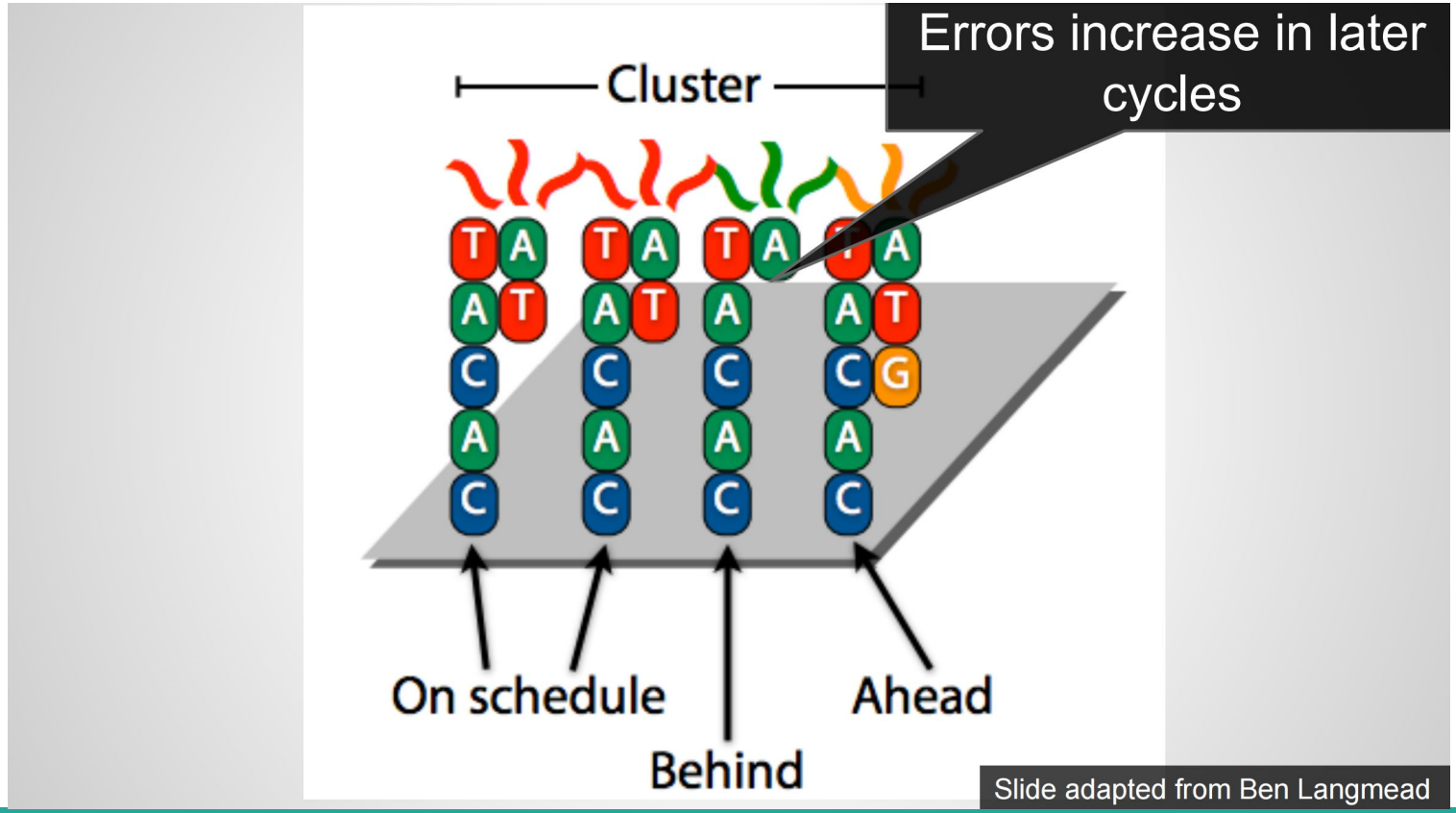
Slide adapted from Ben Langmead

Sequencing (Illumina)



Slide adapted from Ben Langmead

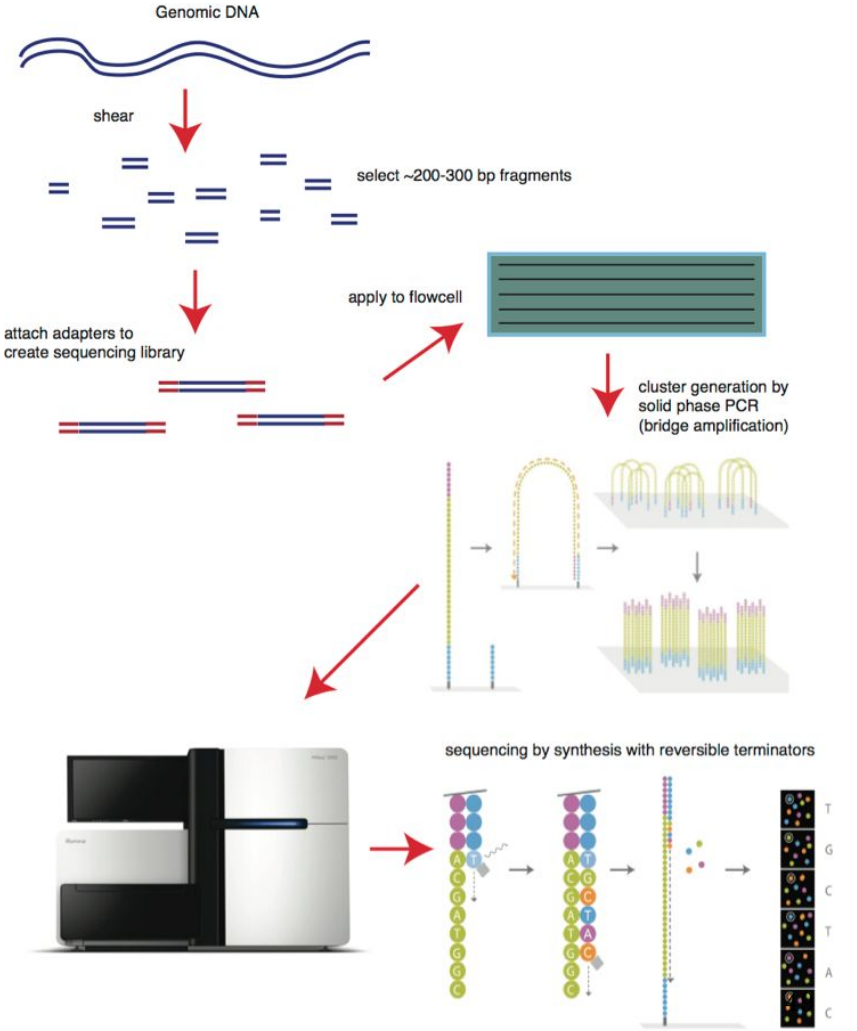
Sequencing error



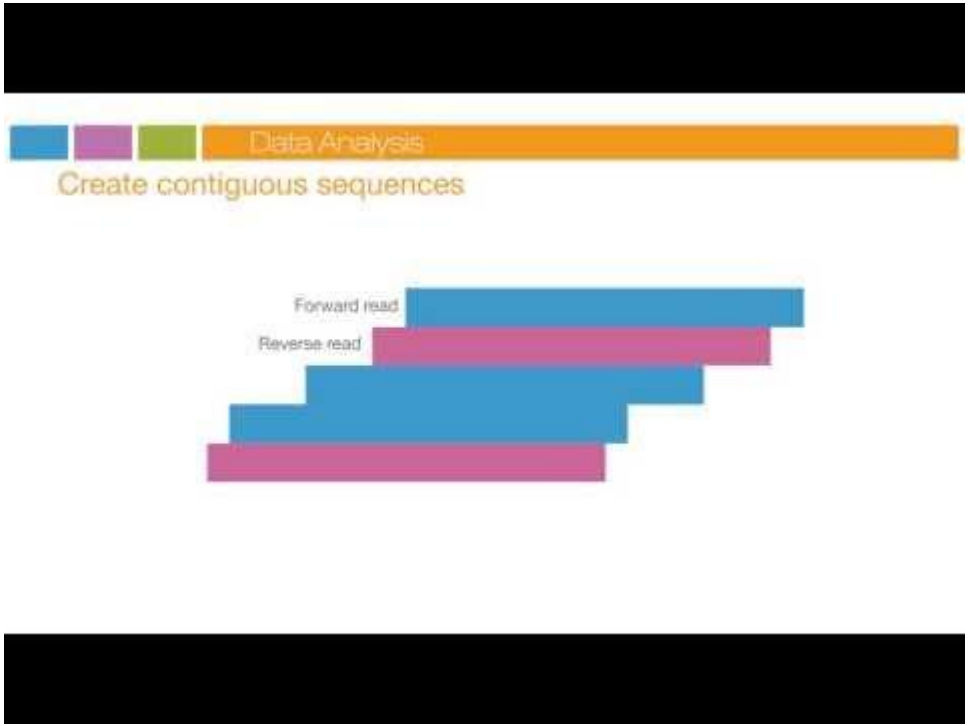
Slide adapted from Ben Langmead

Sequencing (sum up)

1. Shearing (fragmentation of the genome)
2. Attaching adapters
3. PCR amplification (optional)
4. Attaching template to surface/flowcel
5. PCR/bridge amplification (cluster creation)
6. Adding fluorescent bases and taking a picture after each cycle (repeat this many times)
7. Stack up images and read the sequence



Illumina sequencing



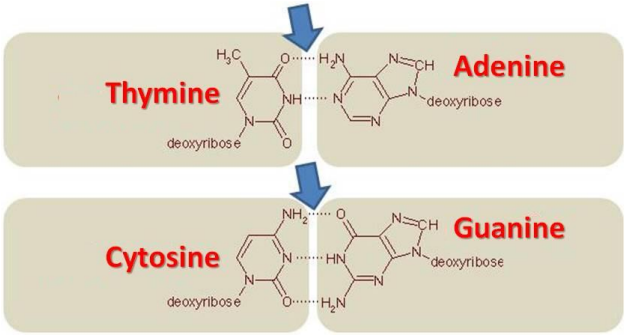
https://www.youtube.com/watch?v=womKfikWlxM

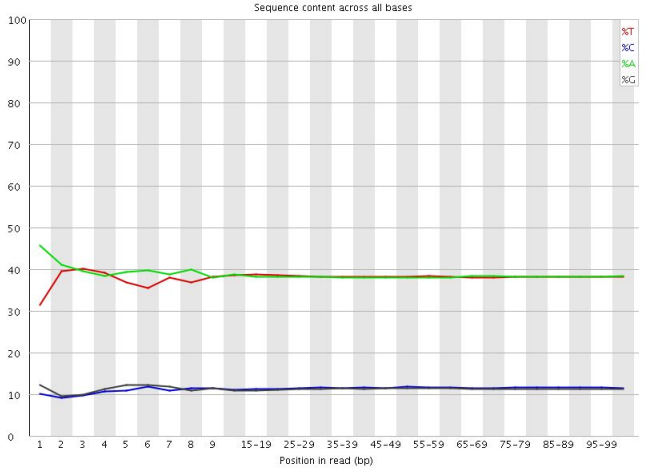
Sequencing errors

1. GC bias

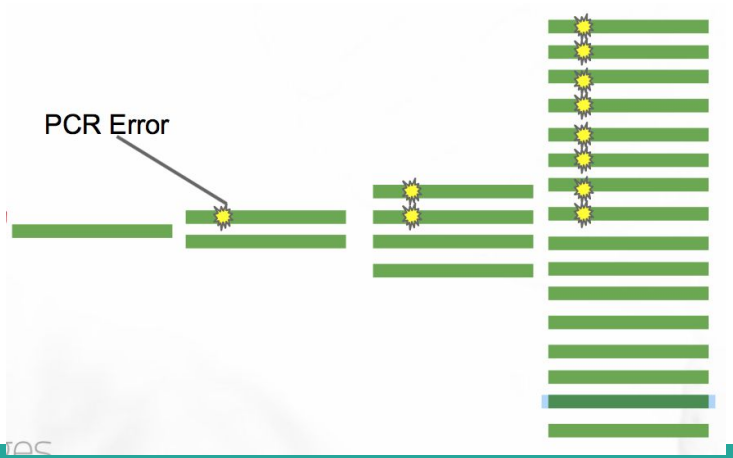
35% to 60% - human

~20% - Plasmodium falciparum



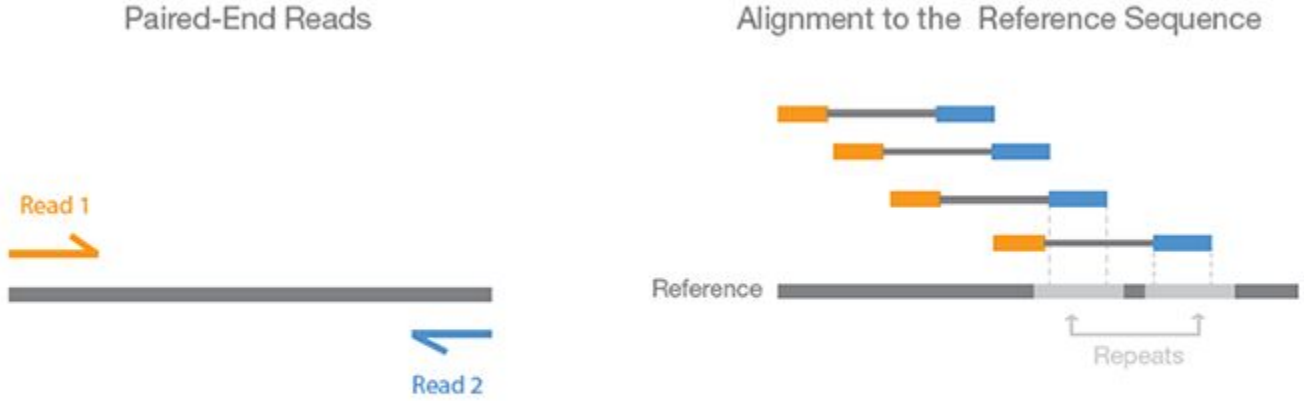


2. Error propagation (1 in 10.000 error rate)



Paired-end sequencing

Figure 4. Paired-End Sequencing and Alignment

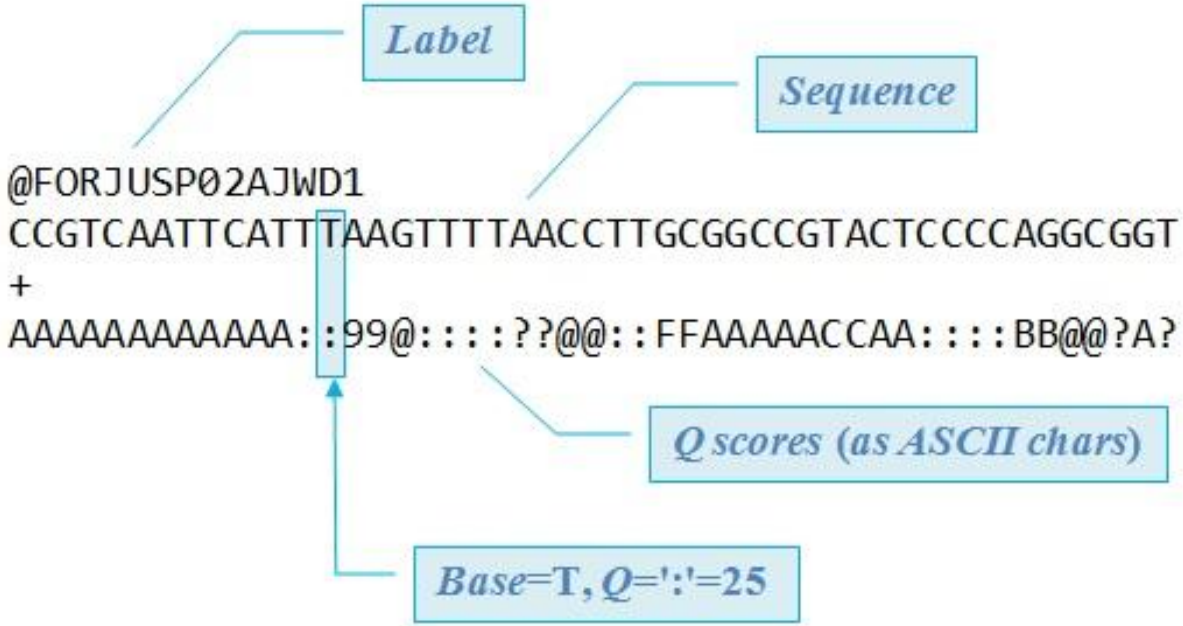


Paired-end sequencing enables both ends of the DNA fragment to be sequenced. Because the distance between each paired read is known, alignment algorithms can use this information to map the reads over repetitive regions more precisely. This results in much better alignment of the reads, especially across difficult-to-sequence, repetitive regions of the genome.

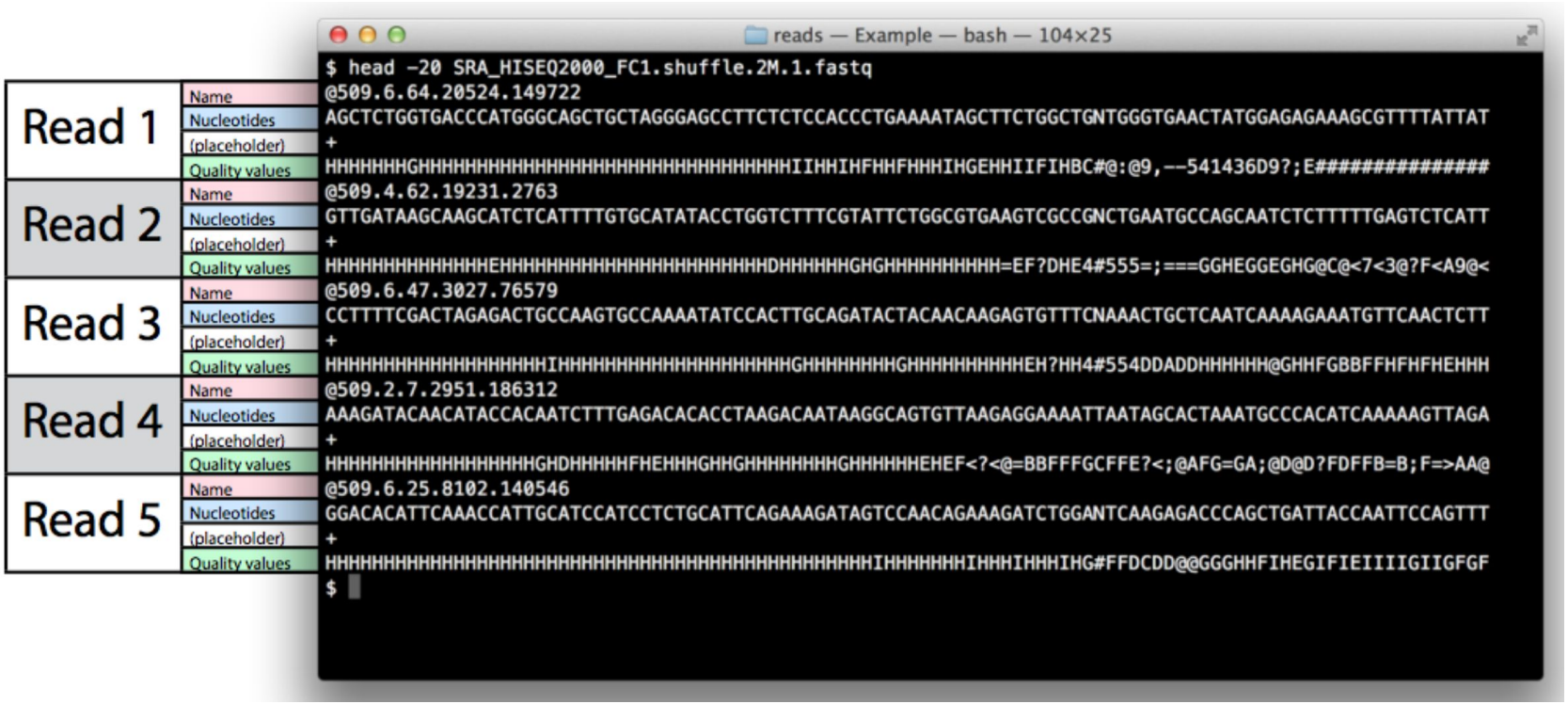
Sequencing data - FASTQ file

4 lines for each read

- Read id
- Read sequence
- + sign
- ASCII encoded quality



Sequencing data - FASTQ file



Genome reconstruction

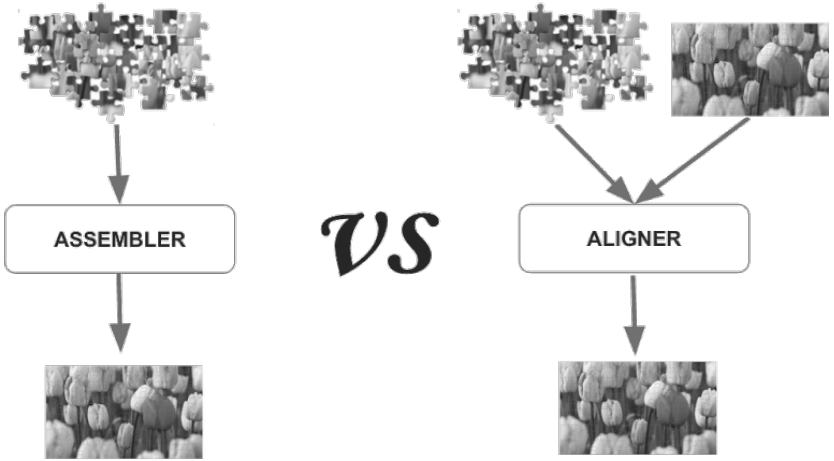
Result of sequencing experiment

- FASTQ file
- 100-500 GB
- Each read(line) containing a genome sequence 50-250 bp long

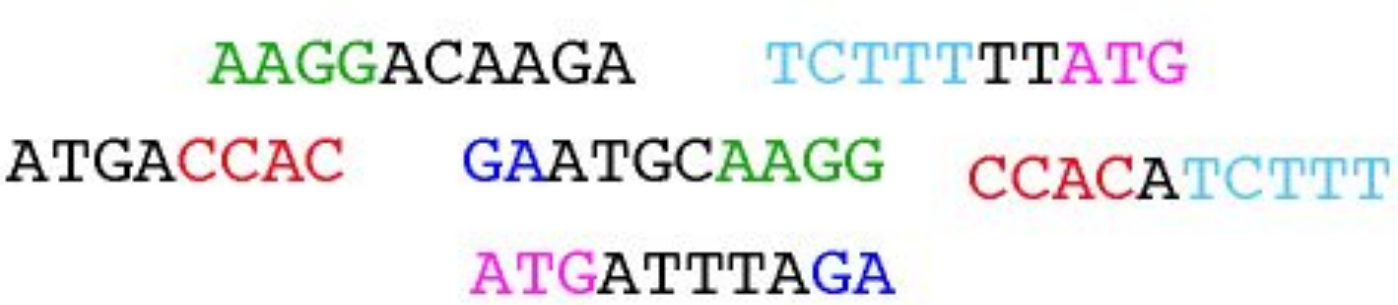
Genome reconstruction

How do we reconstruct genome from reads?

1. Alignment
    - Using reference genome to map the position of the reads
2. Assembly
    - Reconstructing the genome by finding the links between the reads



Alignment


AAGGACAAGA
TCTTTTTTATG
ATGACCAC
GAATGCAAGG
CCACATCTTT
ATGATTTAGA

Assembly


AAGGACAAGA
TCTTTTTATG
ATGACCAC
GAATGCAAGG
CCACATCTTT
ATGATTTAGA

Resources and additional reads

Presentation available at: github.com/vladimirkovacevic/gi-2020-etf

- A Computer Scientist's Guide to Cell Biology, A Travelogue from a Stranger in a Strange Land
- Genomics 101, Edition 2016
- Bioinformatics at COMAV - SNP Calling
- Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM
- High-Throughput Sequencing Technologies - Review paper
- Vince Buffalo: Bioinformatics Data Skills
- Dan Gusfield: Algorithms on Strings, Trees, and Sequences: Computer Science and Computational Biology, Cambridge
- Pavel Pevzner, Neils Jones: An Introduction to Bioinformatics Algorithms (Computational Molecular Biology), MIT
- R. Durbin, S. Eddy, A. Krogh, G. Mitchinson: Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids , Cambridge University Press
- Veli Mäkinen, Djamal Belazzougui, Fabio Cunial, Alexandru I. Tomescu: Genome-Scale Algorithm Design: Biological Sequence Analysis in the Era of High-Throughput Sequencing, Cambridge University press