Introduction to high-throughput experiments and data analysis

Outline

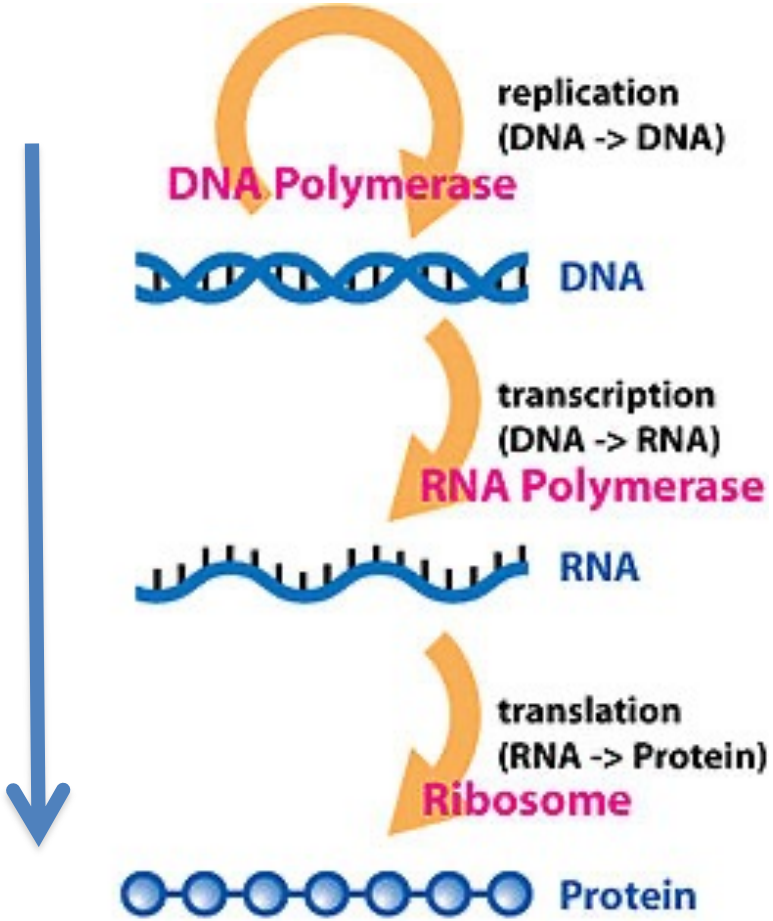
- Biology in a nutshell.
- High-throughput experiments:
  - microarrays.
  - Second generation sequencing.
- R and Bioconductor.
- Online resources: genome browser and public data repositories.

Biology in a nutshell

Central dogma of molecular biology

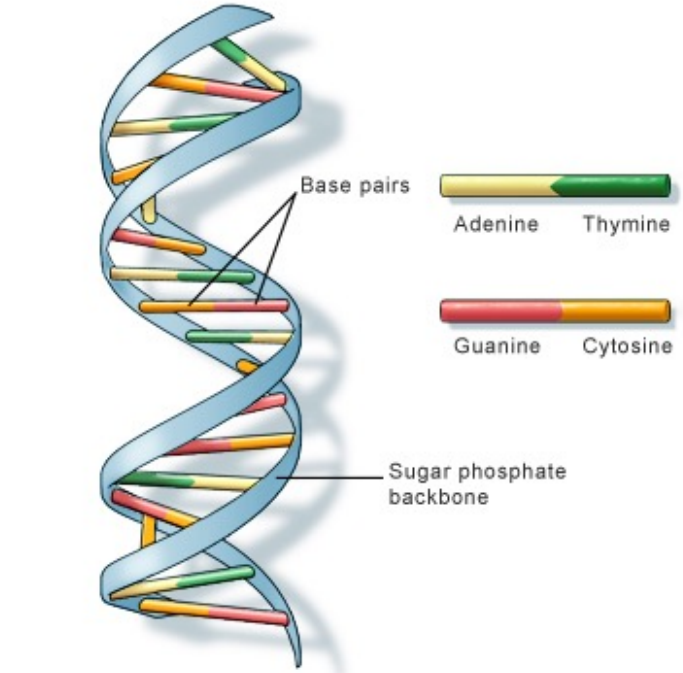
- By Francis Crick (1970) *Nature*:

  "The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that information cannot be transferred back from protein to either protein or nucleic acid."



DNA (DeoxyriboNucleic Acid)

- A molecule contains the genetic instruction of all known living organisms and some viruses.
- Resides in the cell nucleus, where DNA is organized into long structures called **chromosomes**.
- Most DNA molecule consists of two long polymers (**strands**), where two strands entwine in the shape of a double helix.
- Each strand is a chain of simple units (**bases**) called **nucleotides**: A, C, G, T.
- The bases from two strands are complementary by **base pairing**: **A-T, C-G.**



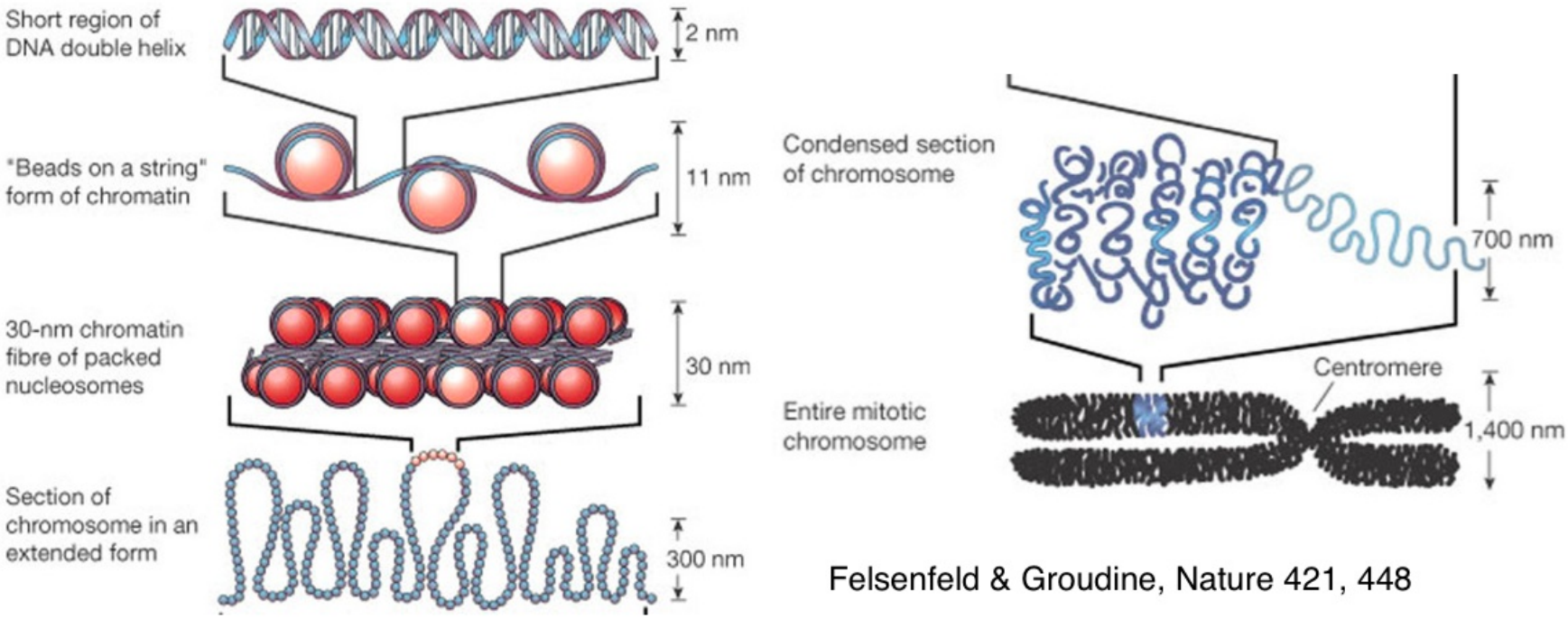
DNA sequence

- The order of occurrence of the bases in a DNA molecule is called the **sequence** of the DNA. The DNA sequence is usually store in a big text file:

```
ACAGGTTTGCTGGTGACCAGTTCTTCATGAGGGACCATCTATCACAACAG
AGAAAGCACTTGGATCCACCAGGGGCTGCCAGGGGAAGCAGCATGGGAGC
CTGAACCATGAAGCAGGAAGCACCTGTCTGTAGGGGGAAGTGATGGAAGG
ACATGGGCACAGAAGGGTGTAGGTTTTGTGTCTGGAGGACACTGGGAGTG
GCTCCTGGCATTGAAACAGGTGTGTAGAAGGATGTGGTGGGACCTACAGA
CAGACTGGAATCTAAGGGACACTTGAATCCCAGTGTGACCATGGTCTTTA
AGGACAGGTTGGggccaggcacagtggctcatgcctgtaatcccagcact
```

- Some interesting facts:
  - Total length of the human DNA is **3 billion bases**.
  - Difference in DNA sequence between two individuals is less than 1%.
  - Human and chimpanzee have 96% of the sequences identical. Human and mouse: 70%.

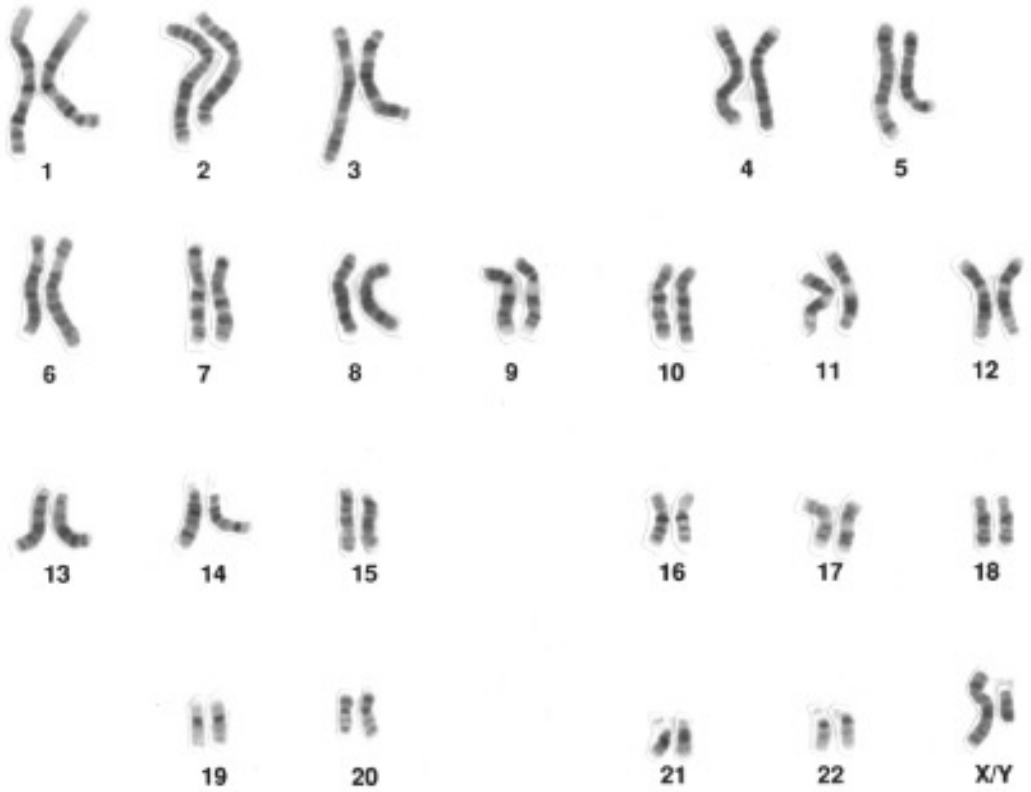
Chromosome: organized structure of DNA and proteins



- **Ploidy**: number of set of chromosomes in a cell.
  - monoploid, diploid or polyploid.
  - Human are diploid: cells have two copies of each chromosome, one from mother and one from father.

Genome

- All of the heritable biological information needed to build and maintain a living example of that organism.
- Or simply, the full set of chromosomes.

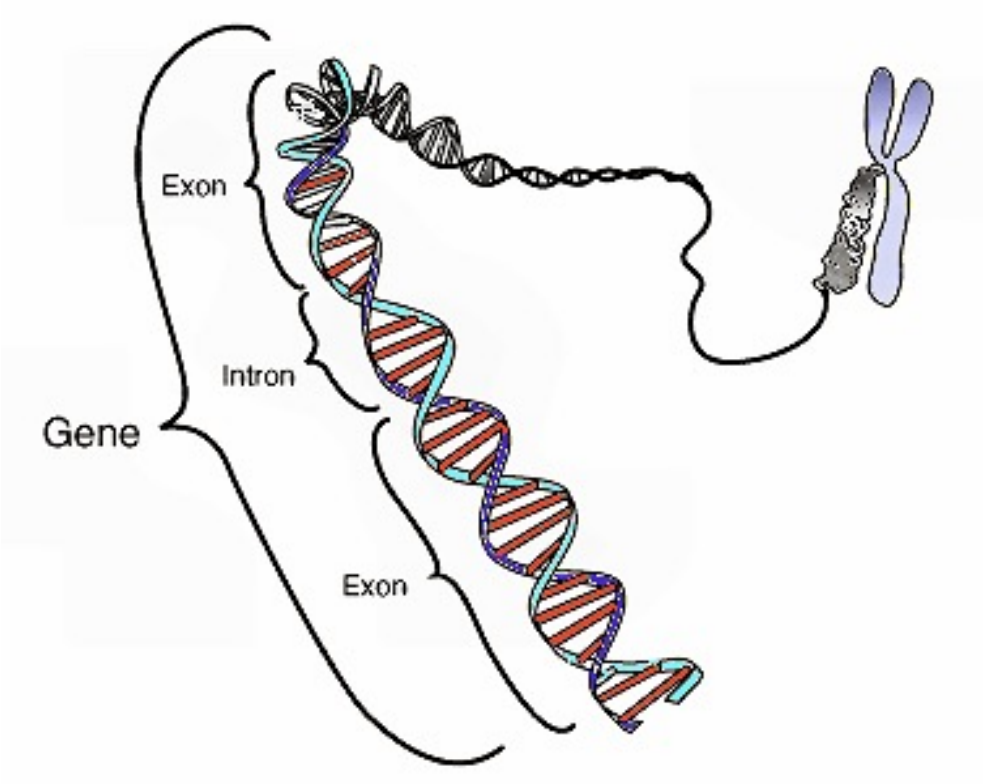


Genomes of different model organisms

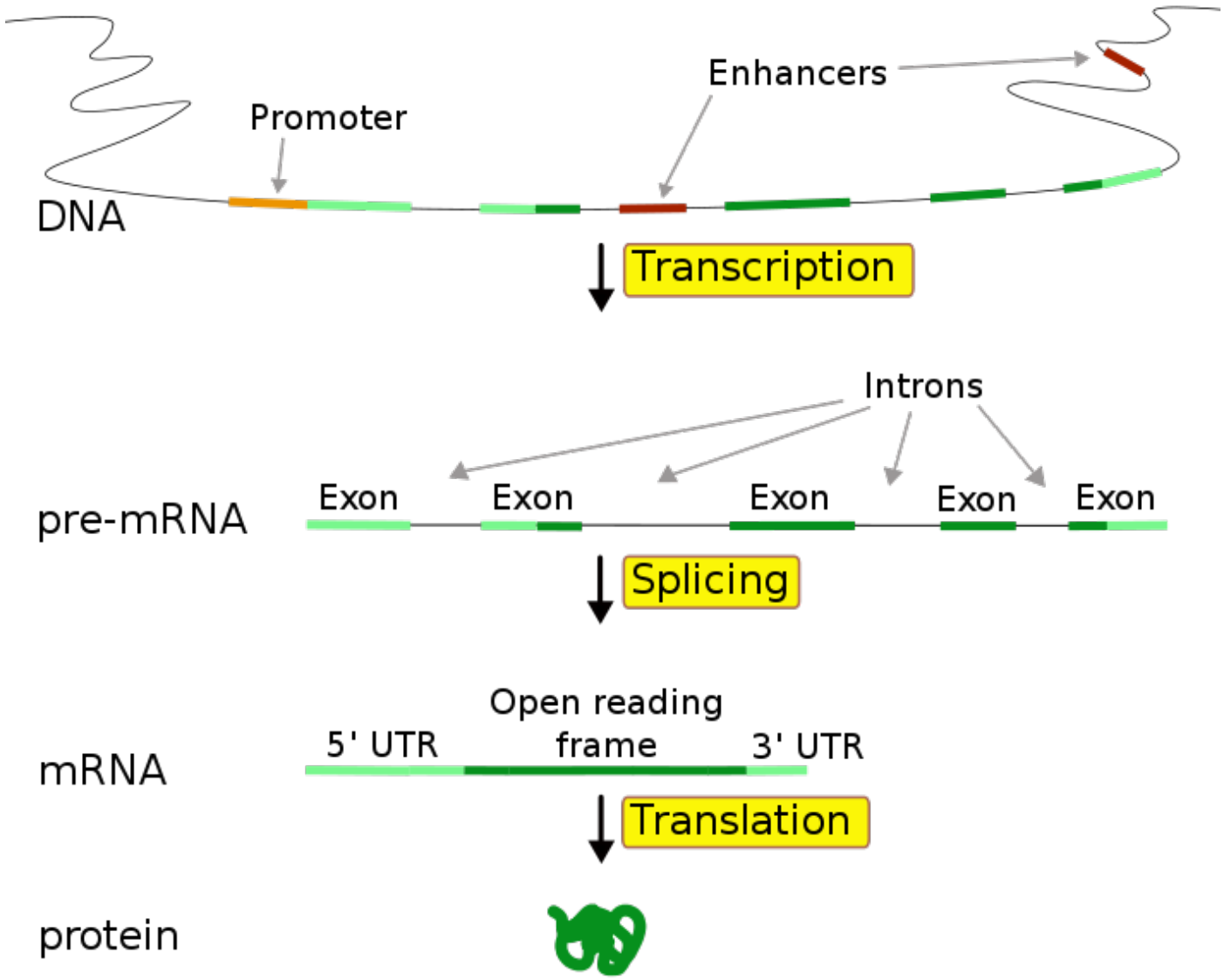
Organism	Genome size (bp)	# genes
E. coli	4.6M	4,300
S. cerevisiae (yeast)	12.5M	5,800
C. elegans (worm)	100M	20,000
A. thaliana (plant)	115M	28,000
D. melanogaster (fly)	123M	13,000
M. musculus (mouse)	3G	23,800
H. sapiens (human)	3.3G	25,000

Gene

- A locatable region of genomic sequence, corresponding to a unit of inheritance, which is associated with regulatory regions, transcribed regions, and or other functional sequence regions.
- Or simply, a piece of “useful” DNA sequence.



Gene structure and splicing



- In a nutshell (for biostatisticians):
  - **enhancer**: a region for enhancing gene expression. Not necessarily closes to the gene.
  - **promoter**: at the beginning of the gene, helps transcription.
  - **exons**: the “useful” part of the gene, will appear in the mRNA product.
  - **introns**: the “spacer” between exons, will NOT be in the mRNA product.
  - **splicing**: the process to remove introns and join exons.
  - **alternative splicing**: different splicing pattern for the same pre-mRNA. For example, mRNA could be from exons 1 and 2 or exons 1 and 3. Those are different “transcript” of the same gene.

RNA (Ribonucleic acid)

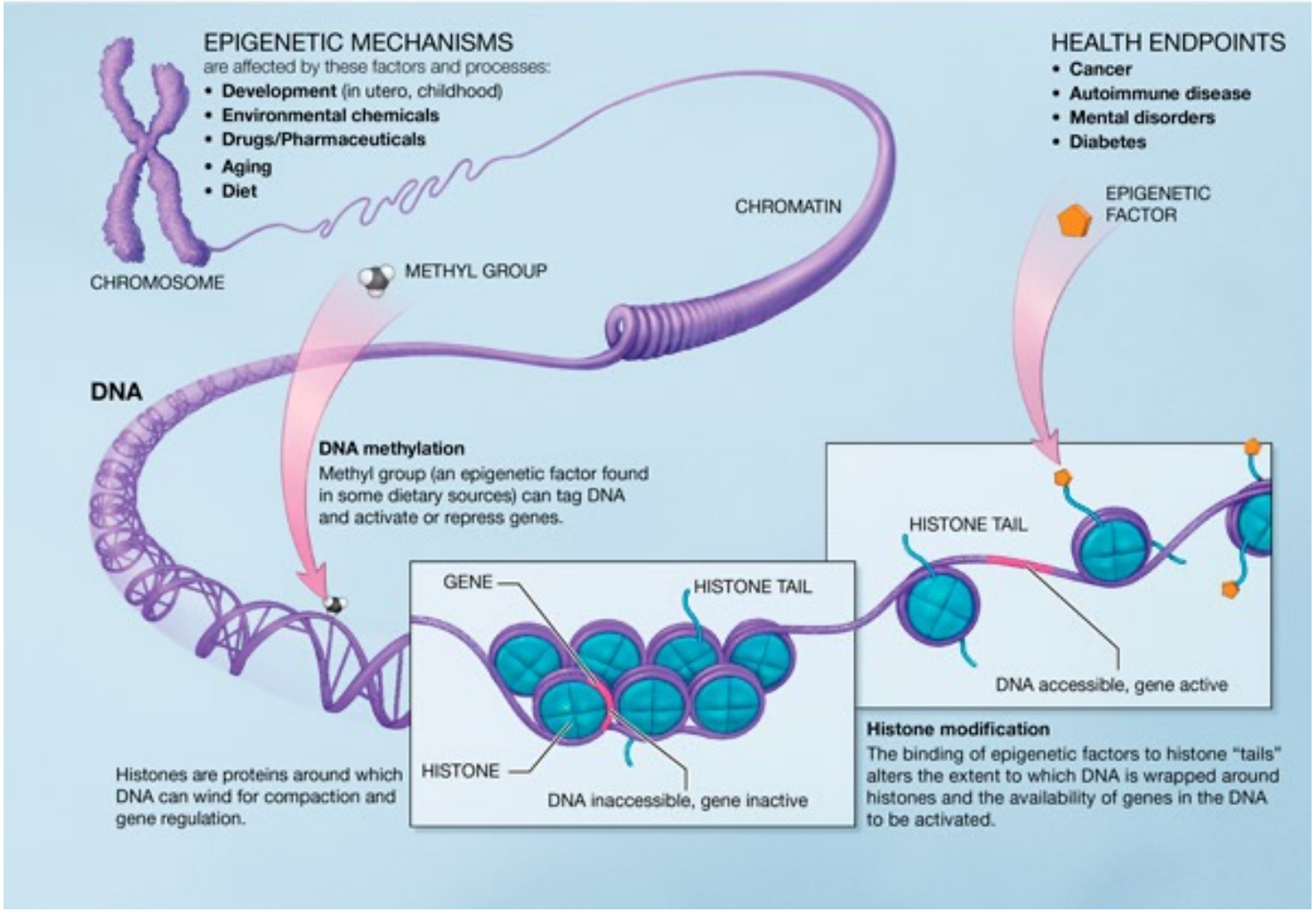
- Similar to DNA, but
  - RNA is usually single-stranded.
  - The base U is used in place of T.
  - The backbone is different.
- Many different types: mRNA, tRNA, rRNA, miRNA, snoRNA, etc.

Protein

- The final product of gene expression process, workhorses in the cells.
- A chain of amino acid.
- Every 3 nucleotide is translated into one amino acid during translation.
- There are 20 types of amino acids, so a protein can be thought as a string from a 20-character alphabet.
- 3D protein structure is often important for its function.

Epigenetics

- Non-DNA sequence related, heritable mechanisms to control gene expressions. Examples: DNA methylation, histone modifications.



What is computational biology

- Use mathematical/statistical models to study biological mechanisms.
- Imagine biological system as a machine.
  - Bench biologists ("**web lab**") perform experiments to collect data to measure the outputs of the machine.
  - Computational biologists ("**dry lab**") make inferences about how the machine works based on data.

Examples of computational biology researches

- DNA sequence analysis:
  - sequence alignment and searching.
  - gene and motif finding.
  - evolution: phylogenetic trees.
- Transcriptional analysis:
  - compare gene expression by measuring mRNA quantity in different sample (expression microarrays, RNA-seq).
  - detect alternative splicing and gene fusion.
  - detect protein (transcription factor) binding or epigenetic modification.

- Epigenetics:
  - Detect, compare and characterize DNA methylation or histone modifications.
  - Epigenetic regulation of gene expression.
- Protein:
  - protein sequence alignment.
  - protein expressions (protein arrays).
- Joint analysis:
  - Jointly model multiple –omics data to decipher gene expression process or understand their relationships.
- Single cell omics

A brief introduction to High-throughput experiments

High-throughput experiments

- Methods to conduct a large number of experiments simultaneously.
- Examples:
  - Microarrays.
  - Second generation sequencing.
  - Flow cytometry
  - …
- Pros: quick, cheap.
- cons: lower accuracy, complicated data.

Microarray

- 2D array on a solid substrate that assays large amount of biological materials.
- Examples of microarrays:
  - DNA microarray:
    - Gene expression array.
    - SNP array.
    - Tiling arrays (ChIP-chip, array CGH).
    - Methylation array.
  - Protein microarray
  - Others …

DNA microarrays

- A collection of many spots, each has a certain type of probes (short segments of DNAs).
- Detect and quantify target sequence (e.g., mRNA) by **hybridization**: sequence-specific interaction between two complementary strands of nucleic acid.
  - An exmple:

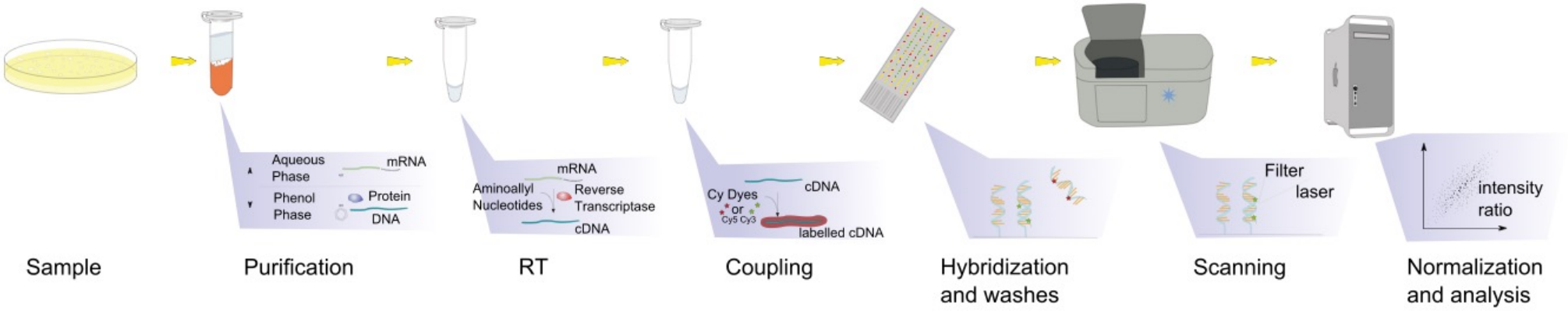
    `ATCGATTGAGCTCTAGCG`

    `TAGCTAACTCGAGATCGC`
- DNA segments in the sample will stick to probes with complementary sequences.
- Each probe has a reading (intensity), which measures the **RELATIVE** amount of target sequence in the sample.

Gene expression microarray

- Measure the gene expressions by the amount of mRNA.
- Each gene is targeted by many probes.
- Major manufactures:
  - Affymetrix
  - Illumina
  - Nimblegen (now acquired by Roche).

GE microarray procedures



- Data: a fluorescent intensity value (a non-negative floating-point number) for each probe.
- Goal: find genes that are differentially expressed (produce different amount of mRNAs) among samples.

Statistical challenges for expression arrays

- Data normalization, transformation, and summarization.
- Statistical inferences: tests for DE (differentially expressed) genes.
- Pattern recognition, e.g., clustering.
- Biological/clinical implications.

DNA sequencing

- Technologies to determine the nucleotide bases from a DNA molecule.
- Traditional method: Sanger sequencing.
  - slow (low throughput) and expensive: took Human Genome Project (HGP) 13 years and $3 billion to sequence the entire human genome.
  - Relatively accurate.

Next-generation sequencing (NGS)

- Aka: high-throughput sequencing, second-generation sequencing.
- Able to sequence large amount of short sequence reads in a short period:
  - high throughput: hundreds of millions sequences in a run.
  - Cheap: sequence entire human genome costs a few thousand dollars.
  - short read length: up to several hundred bps.

NGS Applications

- **DNA-seq**: sequence the genomic DNA in order to find variants or assemble reference genome.
- **RNA-seq**: sequence the transcriptome (mRNA -> cDNA) in order to measure gene expressions or detect alternative splicing/gene fusion.
- **MeDIP/ChIP-seq**: detect protein-DNA binding or epigenitic modification sites.
- **BS-seq**: Single nucleotide resolution DNA methylation.

Single cell sequencing

- Traditional "**bulk**" sequencing: measures the average signal of many cells, which ignores the inter-cellular heterogeneities:
  - Difference among different cell types.
  - Biological variation among the same type of cell.
- Single cell sequencing:
  - Experiment is performed for each cell individually.
  - Provides more detailed, higher resolution information.

Available platforms

- Major player:
  - Illumina: Genome Analyzer, HiSeq, MiSeq
  - LifeTech: SOLiD, IonTorrent
- Third generation sequencing (long-read sequencing):
  - Oxford Nanopore
  - Pacific Bioscience (pacbio)

Statistical challenges for second generation sequencing data

- Sequence alignment.
- Data transformation and normalization.
- Goal specific:
  - RNA-seq: differential gene/isoform expression or splicing, new gene/exon discovery.
  - ChIP-seq: peak detection, differential peak,
  - BS-seq: differential methylation.

R and Bioconductor

R programing language

- THE programming language and environment for statisticians.
- Free and open source.
- Easy and intuitive.
- Contains a large collection of add-on “packages”.
- Provides extensive graphics capabilities and interfaces to lower level languages (C, Fortran, etc.)
- Evolve rapidly: several (7) years ahead of SAS.
- Relatively slow, but with easy interfaces with other languages.
- Visit www.r-project.org to download/install R and reference manuals.

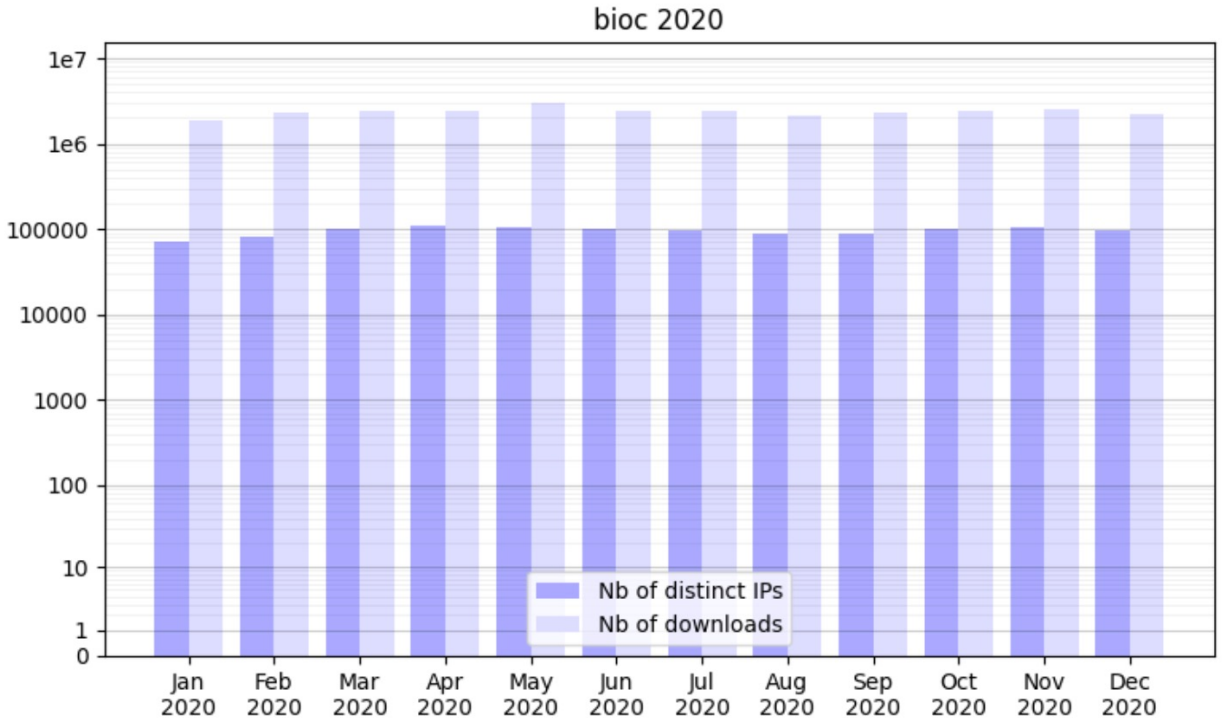
R environment

- You can write programs in a text editor, and copy/paste into R console.
- IDEs (Integrated Development Environment) available (such as **Rstudio**), which are much more convenient.
- For geeks, I recommend using emacs with ESS. See http://www.biostat.wisc.edu/~kbroman/Rintro/ for details.

Bioconductor: a collection of R packages

- Started by Rob Gentleman (Fred Hutch), with a few junior(at that time) faculty members.
- Becoming the *de facto* language for genomic data analysis.

2020



Month	Nb of distinct IPs	Nb of downloads
Jan/2020	71347	1863031
Feb/2020	82959	2327549
Mar/2020	100156	2437796
Apr/2020	109245	2445530
May/2020	107201	3059277
Jun/2020	99529	2406886
Jul/2020	96776	2409421
Aug/2020	86995	2119491
Sep/2020	90269	2280596
Oct/2020	100693	2427302
Nov/2020	103036	2572492
Dec/2020	95193	2225497
2020	**816065**	**28574868**

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Functionalities

- *"The mission of the Bioconductor project is to develop, support, and disseminate free open source software that facilitates rigorous and reproducible analysis of data from* ***current and emerging biological assays****…*

  Bioconductor uses the R statistical programming language, and is open source and open development. It has two releases each year, and an active user community. Bioconductor is also available as Docker images."
- Currently (Aug 2022, version 3.15) provides 2140 packages:
  - microarrays.
  - second generation sequencing.
  - other high-throughput assays.
  - annotation and data.
- Most of the packages are contributed.

Bioconductor installation

- Use `BiocManager::install()`.
- Basic installation: installing default (core) packages:

```
if (!requireNamespace("BiocManager"))
    install.packages("BiocManager")
BiocManager::install()
```

- Installing a specific package:

```
BiocManager::install("limma")
```

- Upgrading also use `BiocManager::install()`
  - It's a good habit to upgrade bioconductor periodically.

Online resources: genome browser and public data repositories

UCSC Genome Browser

- Initially developed by Jim Kent on 2000 when he was a Ph.D. student.
- Host genomic annotation data for many species.
- The genome browser is a graphical viewer for visualizing genome annotations.
- Provide other tools for genomic data analysis and interfaces for querying the database.

UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x

chr1:11,102,837-11,267,747 164,911 bp. enter position, gene symbol, HGVS or search terms go

Other genome browsers/databases

- General:
  - NCBI Map Viewer
  - Ensemble genome browser
- Other species specific genome browser
  - MGI: Mouse genome informatics
  - wormbase, Flybase, SGD (yeast), TAIR DB (arabidopsis), microbial genome database
- More or less the same, pick your favorite one.

Public high-throughput data repositories

- **GEO**: Gene expression omnibus.
  - Host array- and sequencing-based data.
- **ArrayExpress**: European version of GEO.
  - Better curated than GEO but has less data.
- **SRA**: sequence read archive.
  - Designed for hosting large scale high-throughput sequencing data, e.g., high speed file transfer.

Data are required to be deposited in one of the databases when paper is accepted!

Other public data resources

- The Cancer Genome Atlas (TCGA) data portal (https://portal.gdc.cancer.gov):
  - Host data generated by TCGA, a big consortium to study cancer genomics.
  - Huge collection of cancer related data: different types of genomic, genetic and clinical data for many different types of cancers.
- The **ENC**yclopedia **O**f **D**NA **E**lements (ENCODE) data coordination center: (https://www.encodeproject.org/about/data-access):
  - Host data generated by ENCODE, a big consortium to study functional elements of human genome.

- Human Cell Atlas (https://data.humancellatlas.org).
  - The most comprehensive single cell omics data repository, for human and mouse.
  - 28.1 million cells, 10 organs, thousands of donors.
- Many others: GTEx, Roadmap Epigenomics, UK biobank, Allen Brain Atlas, etc.

To do list after this class

1. Review slides.
2. Read wikipedia pages for DNA, gene, genome, DNA microarray and DNA sequencing.
3. Install R and Bioconductor on your computer.
4. Start to learn R by reading “R for beginners”: http://cran.r-project.org/doc/contrib/Paradis-rdebuts_en.pdf