Group Final Project Topics

Project topics have been posted as DISCUSSION TOPICS in Canvas; please form your project teams in the discussion area of Canvas.

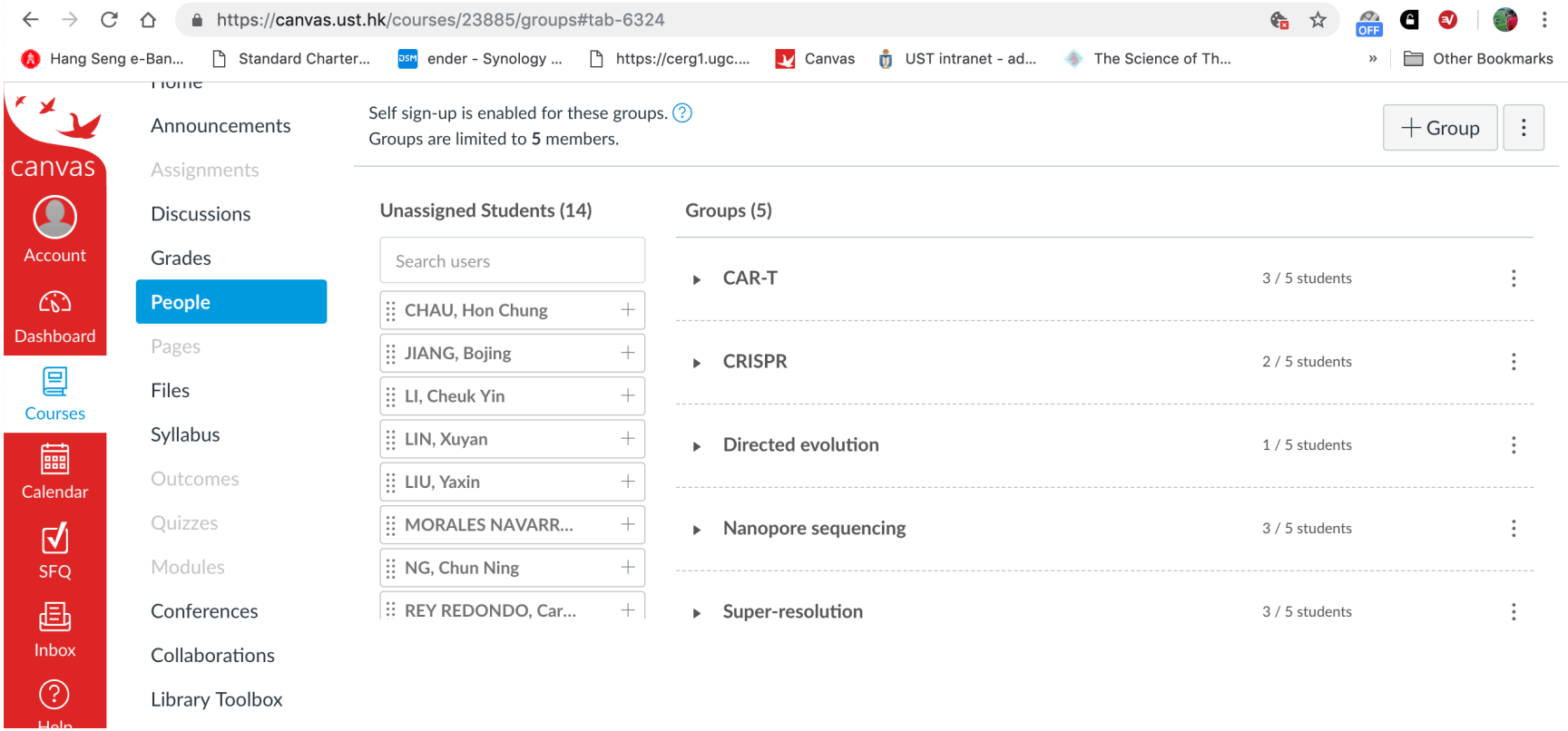
Guidelines for team formation:

Form teams of 4; you can form your own teams, but teams MUST contain members from at least 2 different research labs, and 2 different departments.

You must let me know your topic of choice before **23 March**

Topic choices:

- CRISPR
- Nanopore sequencing
- Mass spectrometry
- Directed evolution
- Super-resolution fluorescence microscopy
- CAR-T
- Cryo-EM
- AI-assisted diagnostics (not limited to digital pathology)
- Optogenetics
- *Propose your own topic – subject to my approval*



Once your group is decided, I will set up your group on canvas, so you can submit assignments and receive communications as a team

Other logistics

Tips on writing paper reviews will be given out the week before midterm

- **About the midterm:**
  - Slight change in format from previous years
  - **The midterm will be based on a paper:**
    - I will upload the paper to be reviewed to Canvas 48 hours before the class when Midterm takes place (midterm is 31 March; paper will be uploaded on 29 March at 9 am)
    - You have 48 hours to read the paper and make notes; you may use any resources to help your reading, including lecture notes, internet, etc.
  - **Part I will consist of short and long answer questions** relevant to the paper to directly assess knowledge learned in class
  - **Part II will be a short review of the paper**
    - You will write this short review during the exam time (i.e. March 31 9am – 11:50am)
  - Exam invigilation will be carried out through zoom video

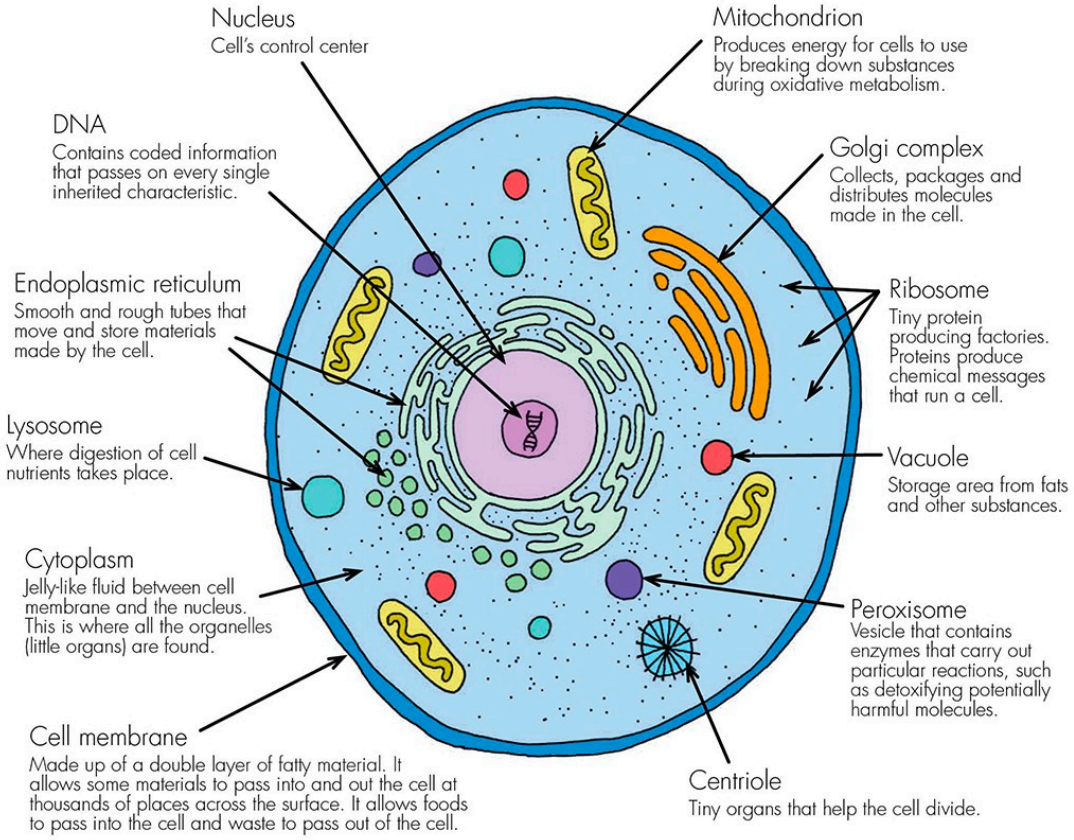
TRANSCRIPTION AND TRANSLATION; GENETICS AND EPIGENETICS

How does one set of genetic instructions generate so many different outcomes?

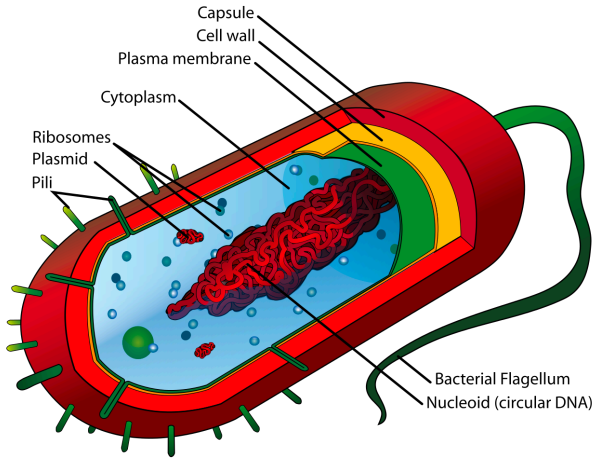
Quick Recap of last lecture

- Cellular structure
- Cellular organelles
- Cellular specialization (morphology, cellular and molecular characteristics)

Cells!



Eukaryotes:
~10-40 um in diameter



Prokaryotes:
~1-2 um in diameter

Biological Macromolecules

- The "chemical building blocks of life"

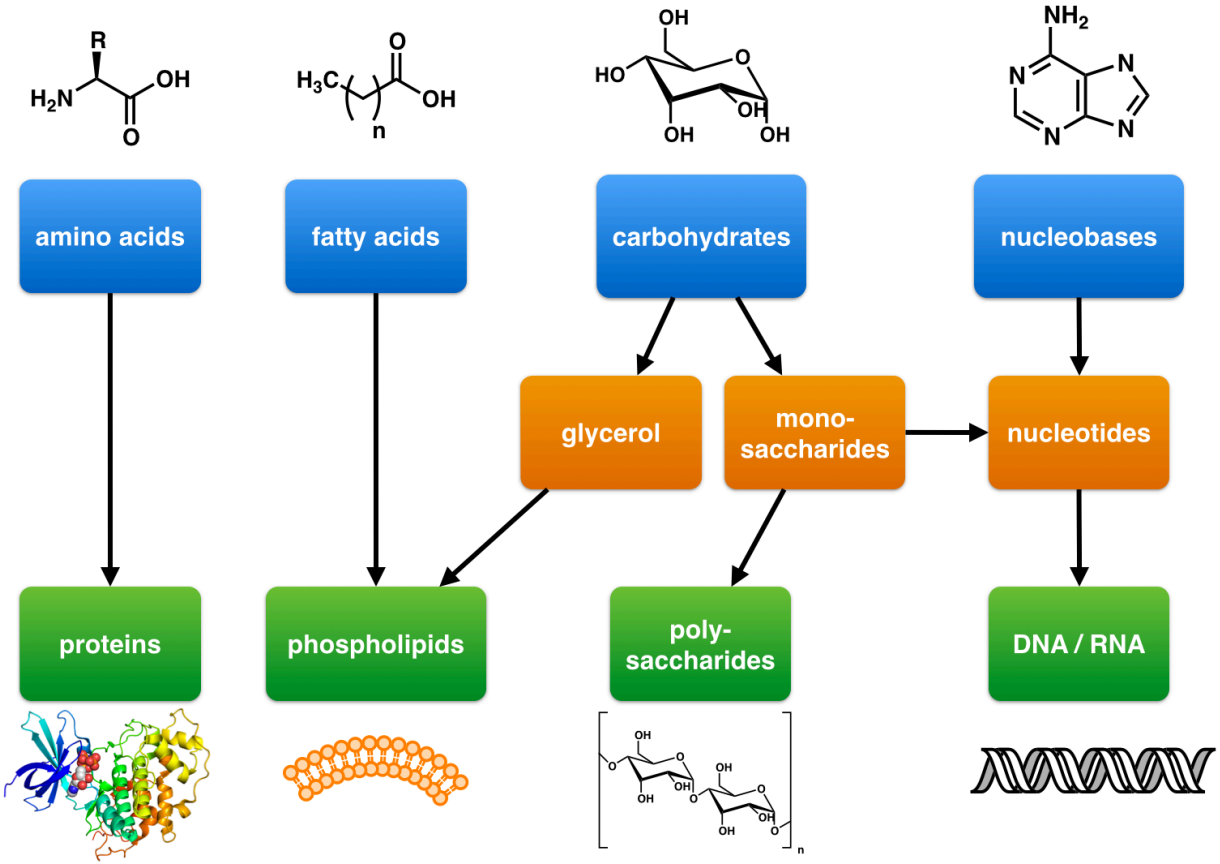
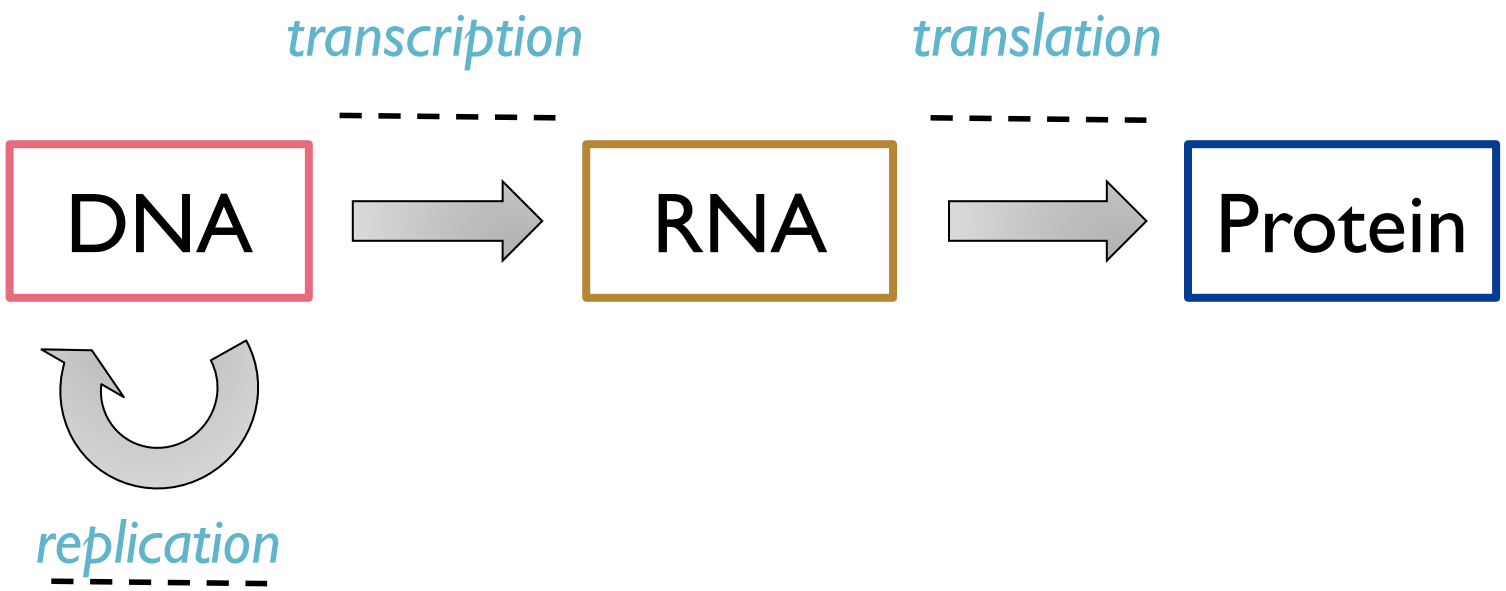
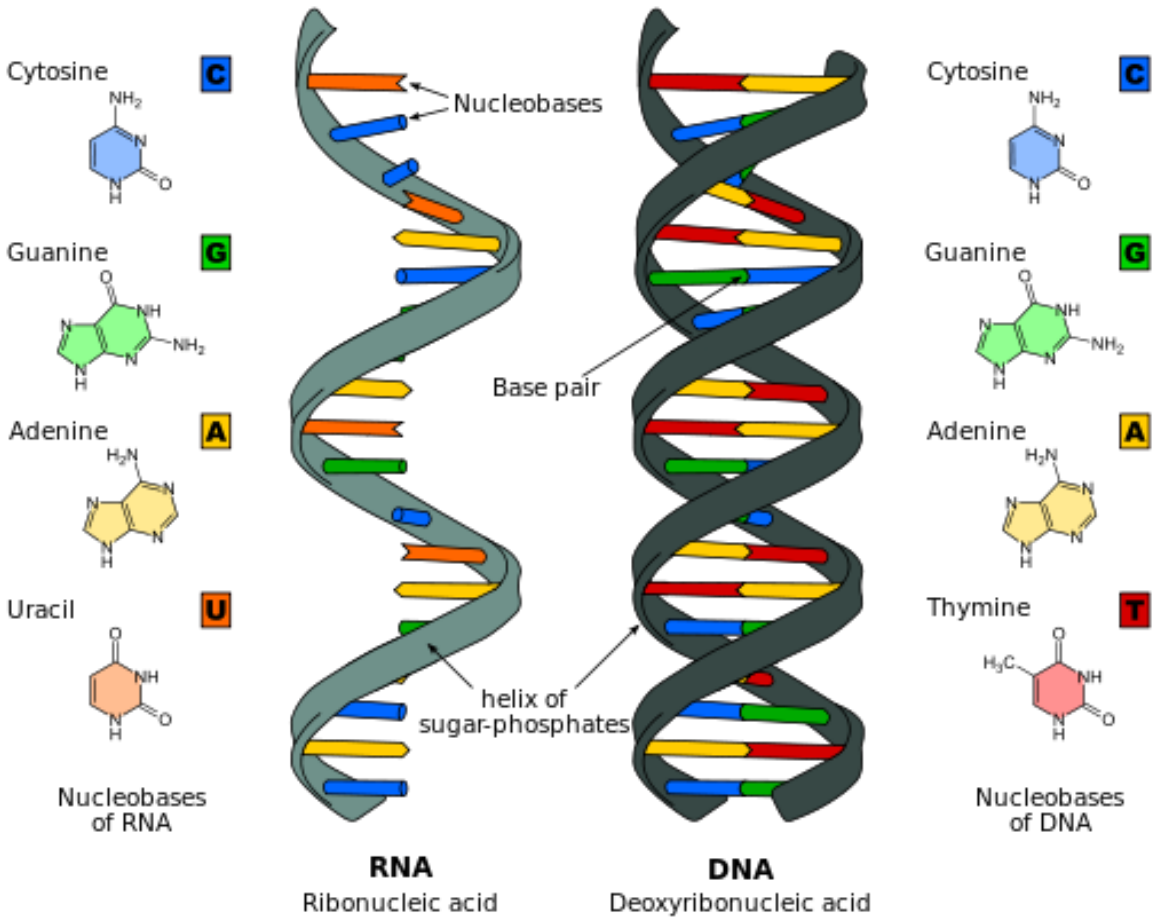


Image from Wikipedia, by BogHog
https://commons.wikimedia.org/wiki/File:Building_blocks_of_life.png

The Central Dogma

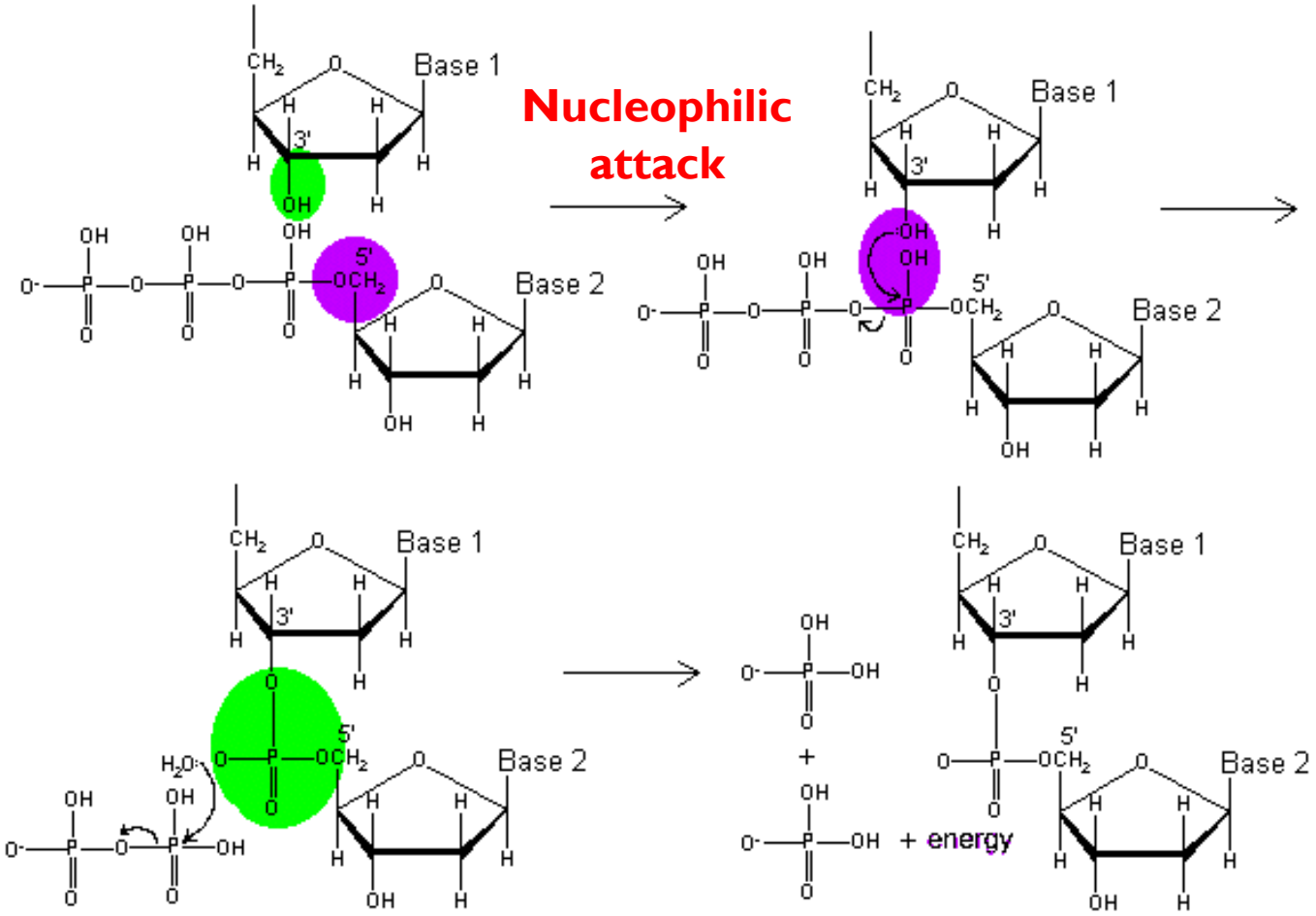


DNA double helix



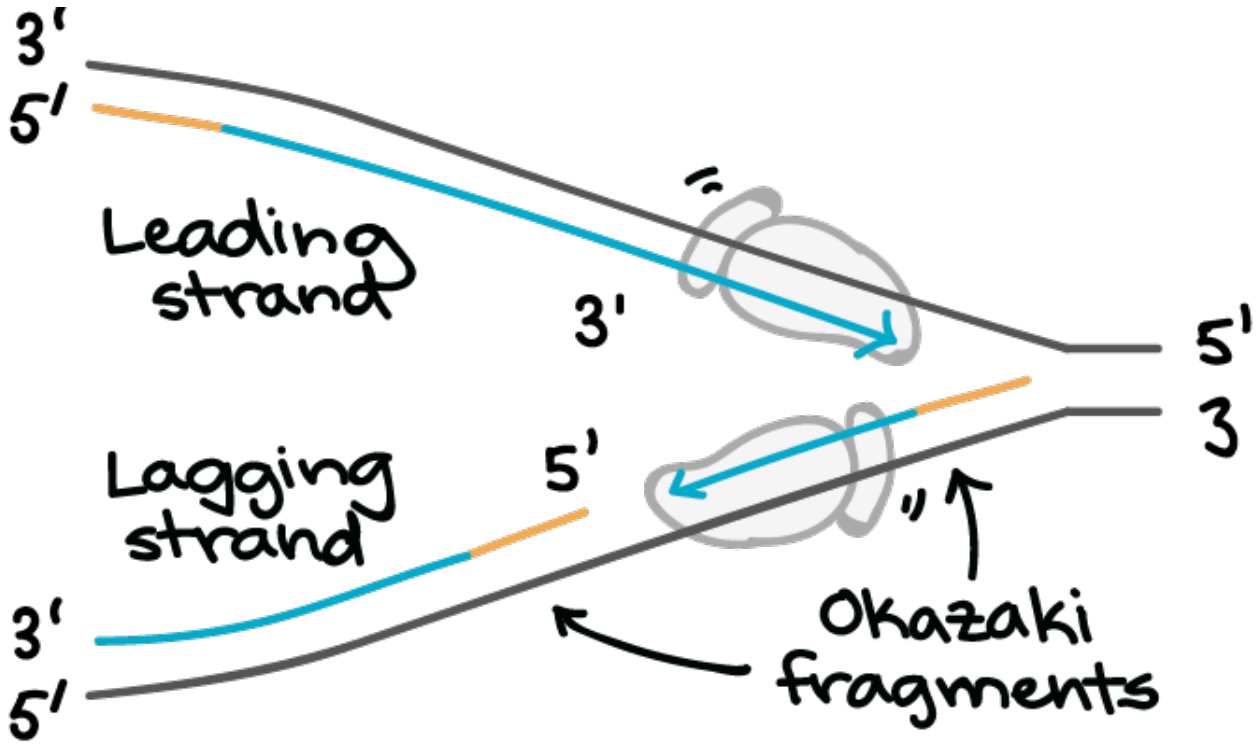
By Difference_DNA_RNA-DE.svg: Sponk (talk) translation: Sponk [CC BY-SA 3.0 (http://creativecommons.org/licenses/by-sa/3.0, via Wikimedia Commons

DNA replication chemistry



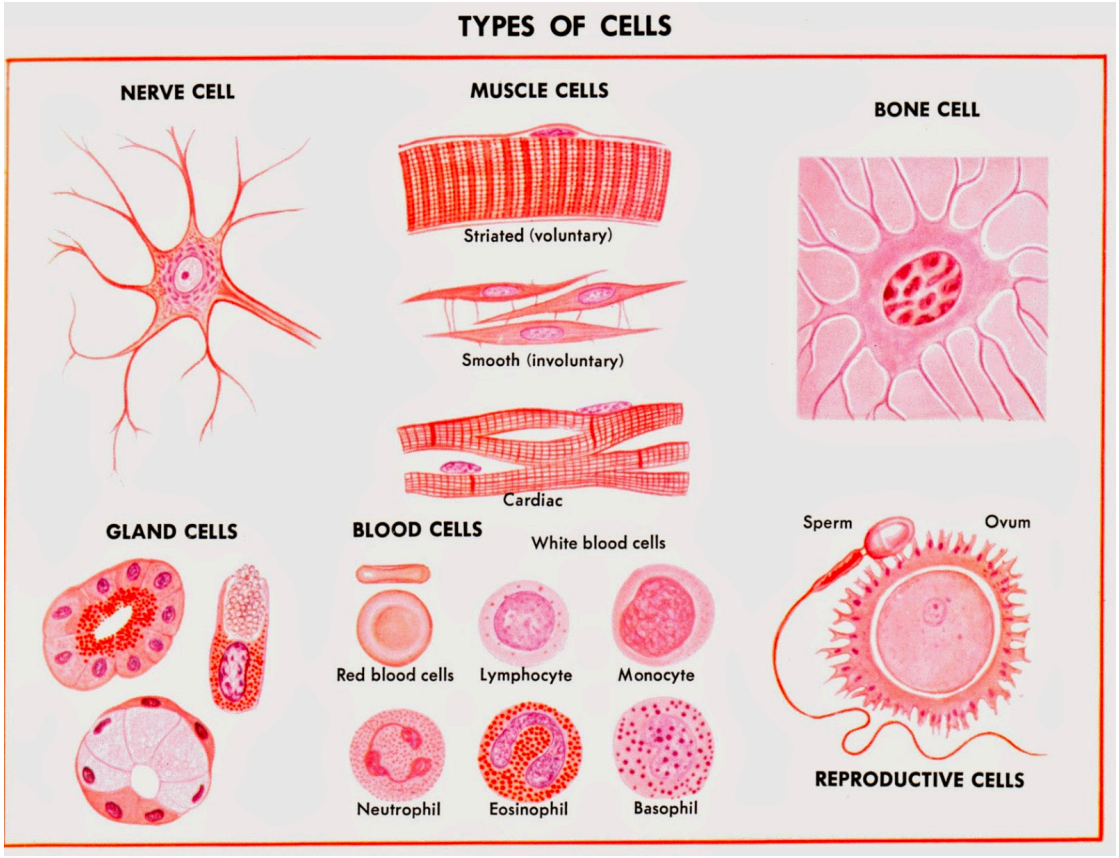
https://chem.libretexts.org/Bookshelves/General_Chemistry/Book%3A_ChemPRIME_(Moore_et_al.)/20Molecules_in_Living_Systems/20.20%3A_DNA_Replication

DNA replication machinery



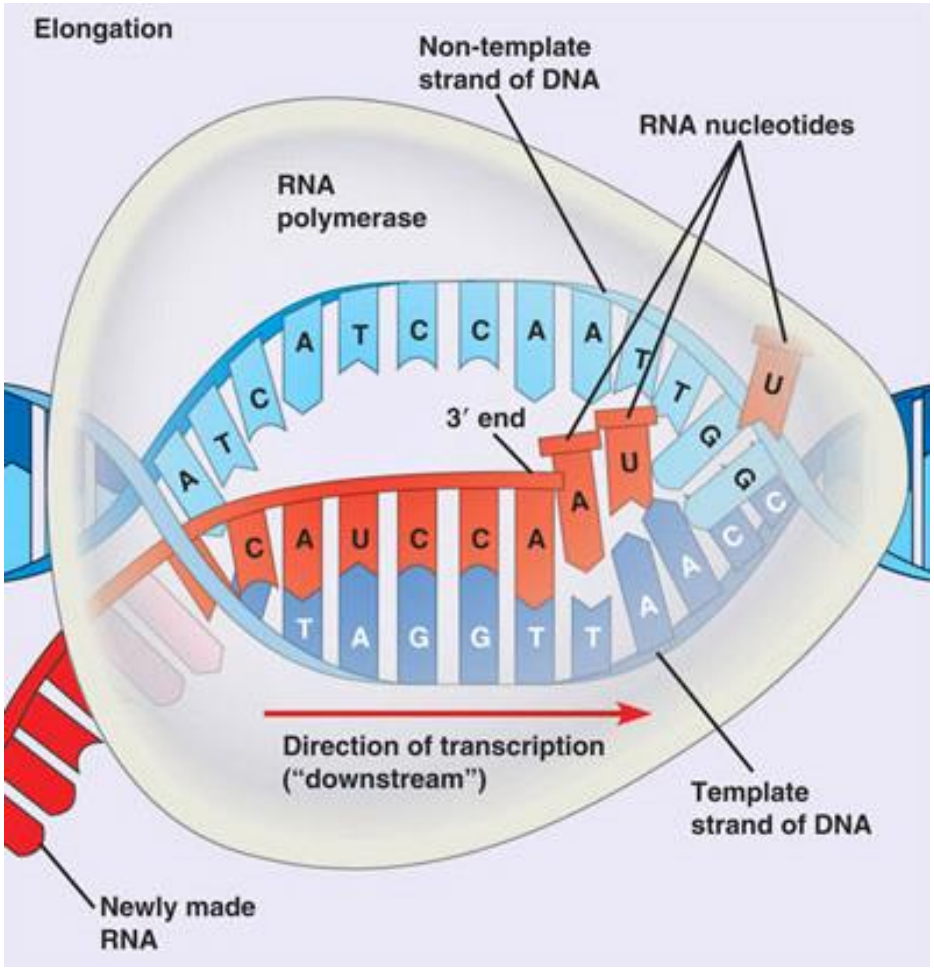
Question

- So many cell types, so few genomes…

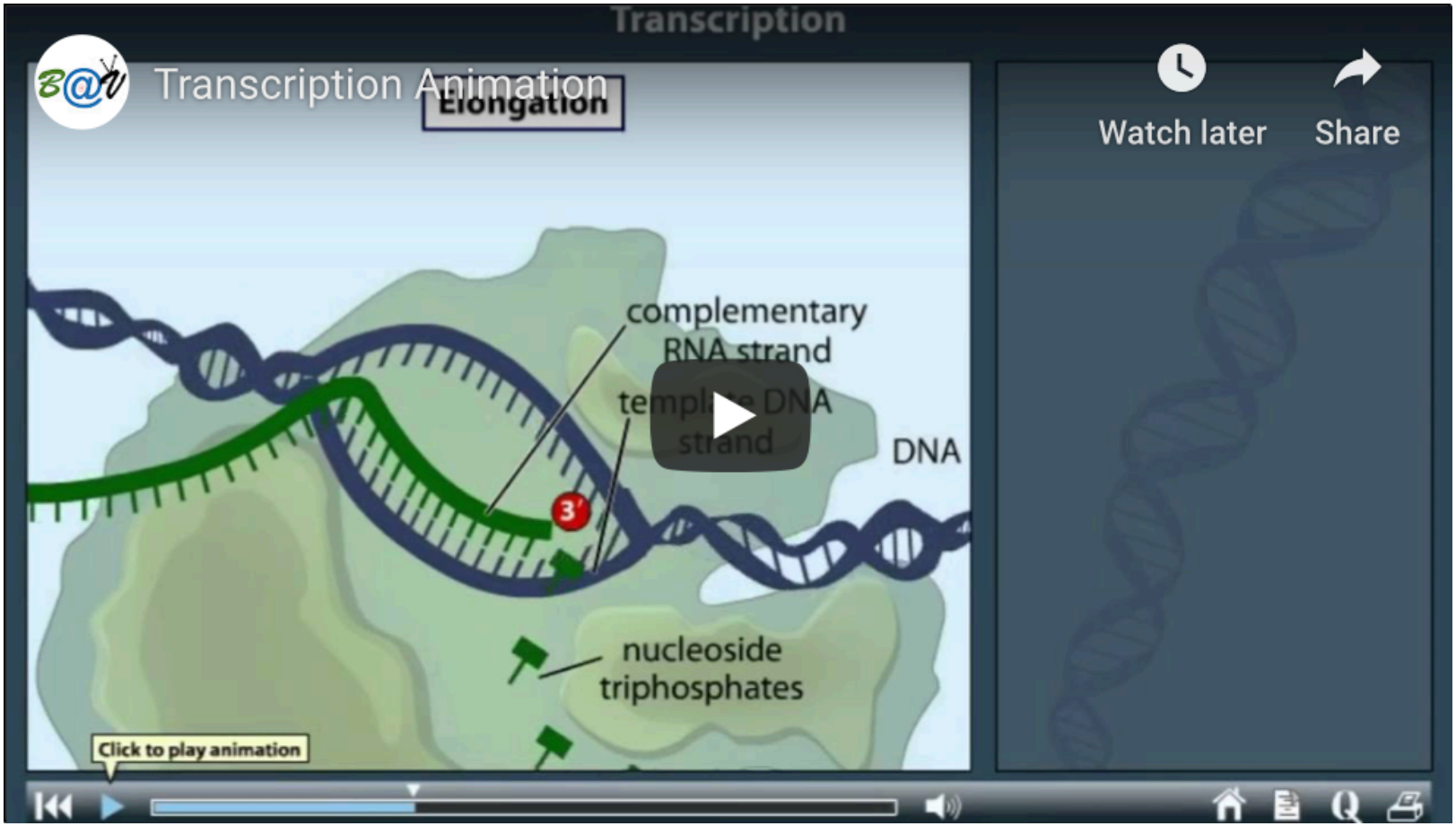


https://wikis.engrade.com/a6thgradescience2/body

Transcription: Blueprint to Messages

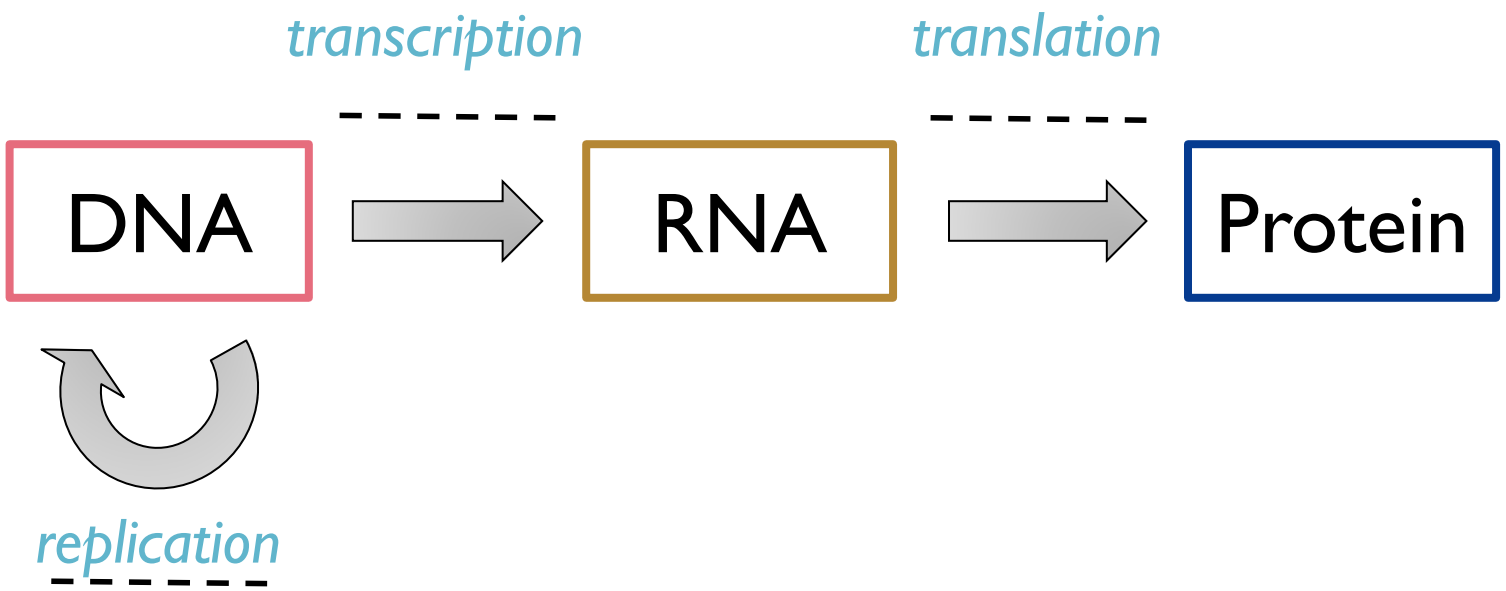


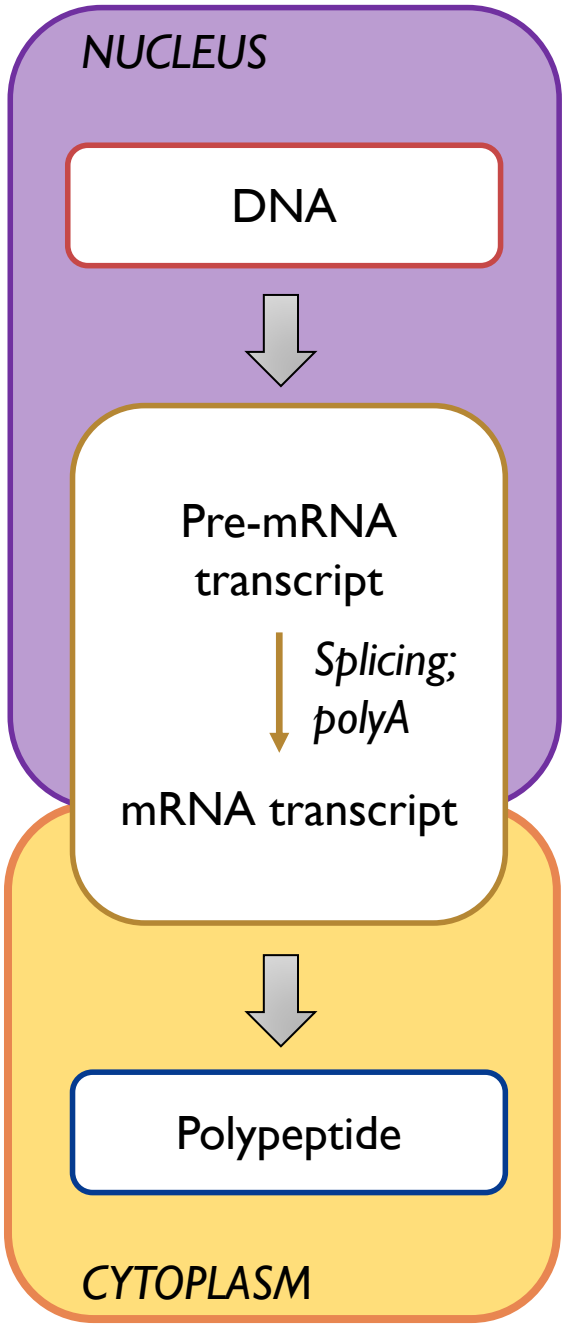
Molecular Biology of the Cell, 5th Edition Garland Science, 2008.

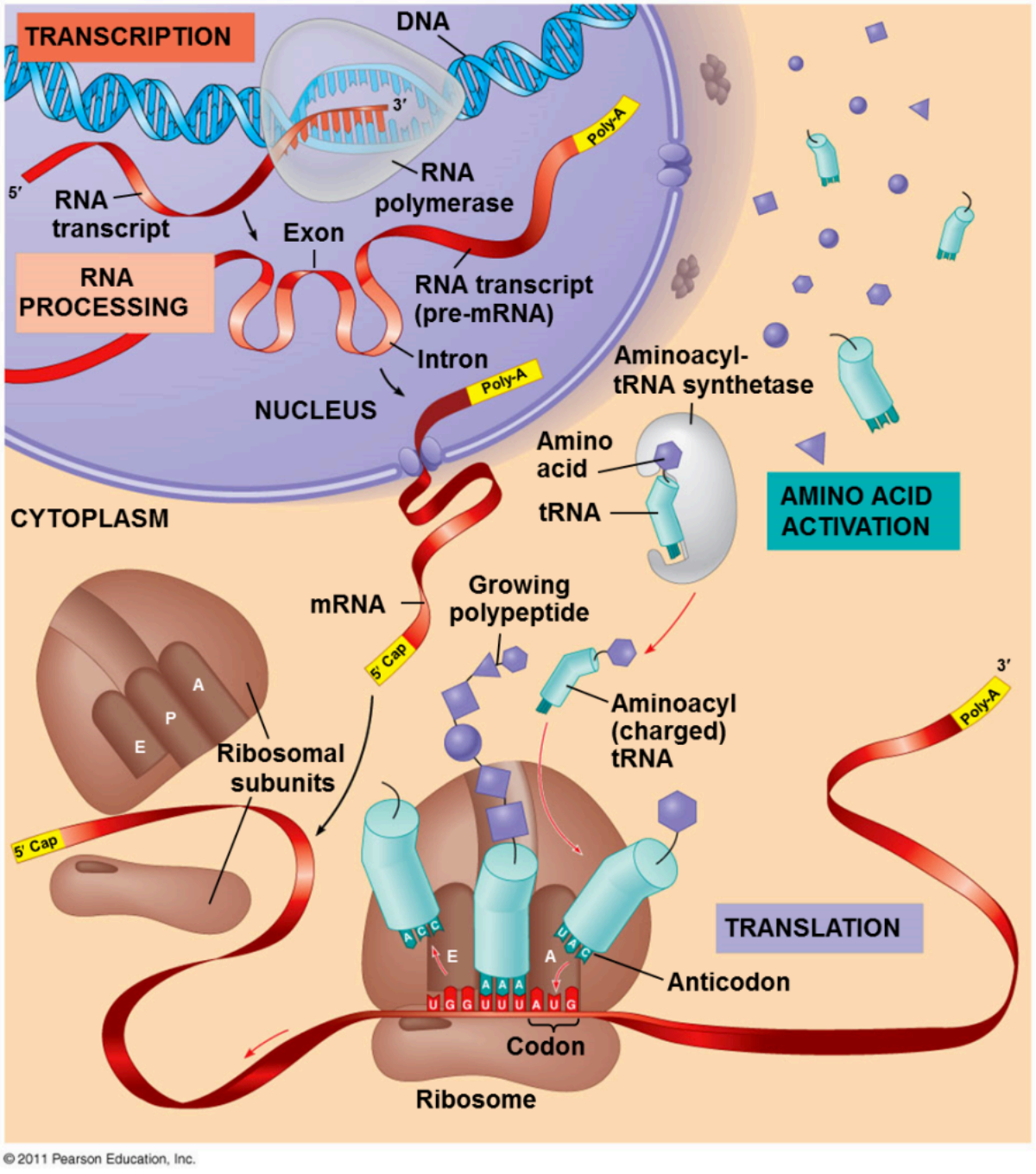


https://youtu.be/vLz2A1cjPH8

The Central Dogma

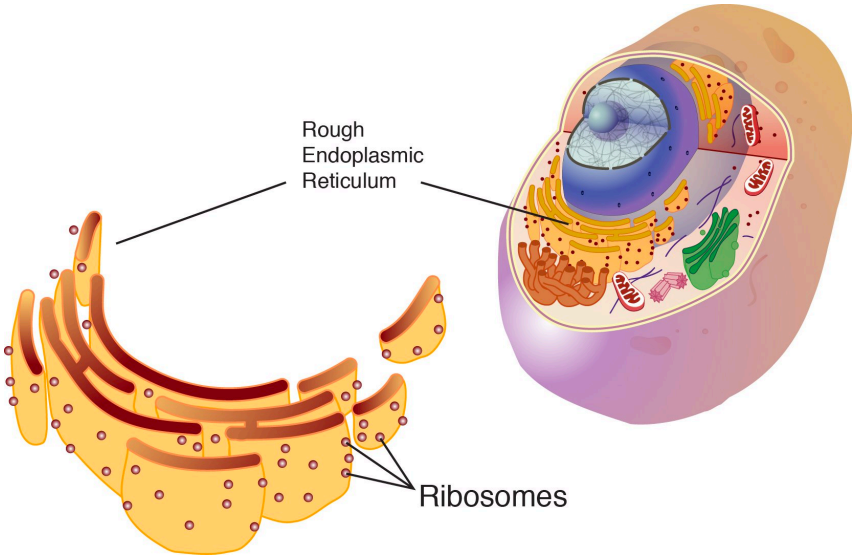


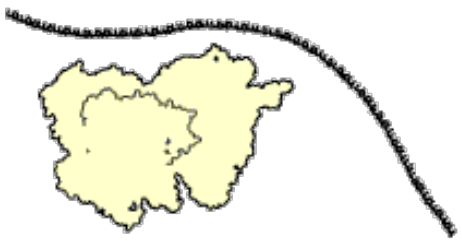

NUCLEUS
DNA
Pre-mRNA transcript
Splicing; polyA
mRNA transcript
Polypeptide
CYTOPLASM

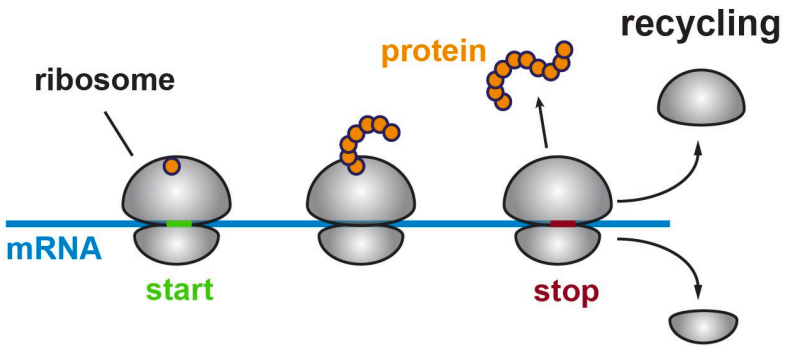

TRANSCRIPTION
DNA
3′
5′
RNA transcript
RNA polymerase
Poly-A
Exon
RNA PROCESSING
RNA transcript (pre-mRNA)
Intron
NUCLEUS
Poly-A
CYTOPLASM
Aminoacyl-tRNA synthetase
Amino acid
tRNA
AMINO ACID ACTIVATION
mRNA
5′ Cap
Growing polypeptide
A
P
E
Ribosomal subunits
5′ Cap
Aminoacyl (charged) tRNA
3′
Poly-A
TRANSLATION
Anticodon
Codon
Ribosome
© 2011 Pearson Education, Inc.

Ribosome

- It makes proteins!
- Needs help from other things in the cell like tRNA

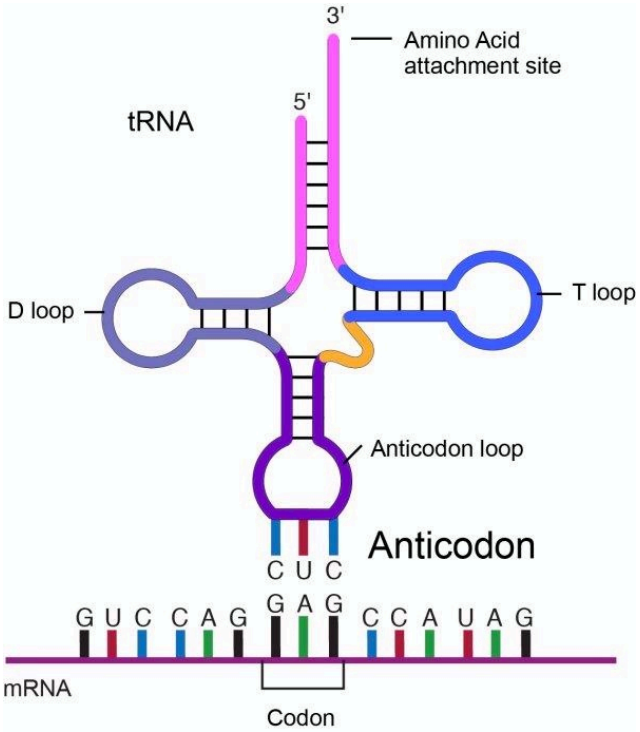


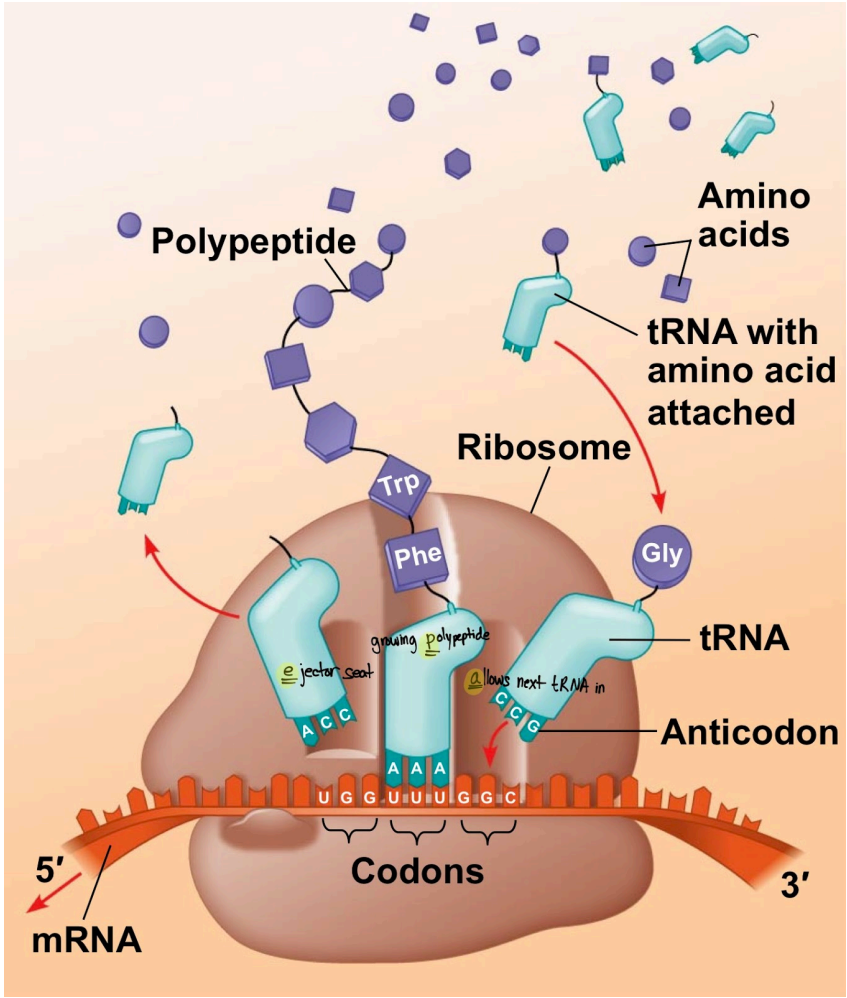




Animated Gif By Bensaccount at en.wikipedia, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=8287100

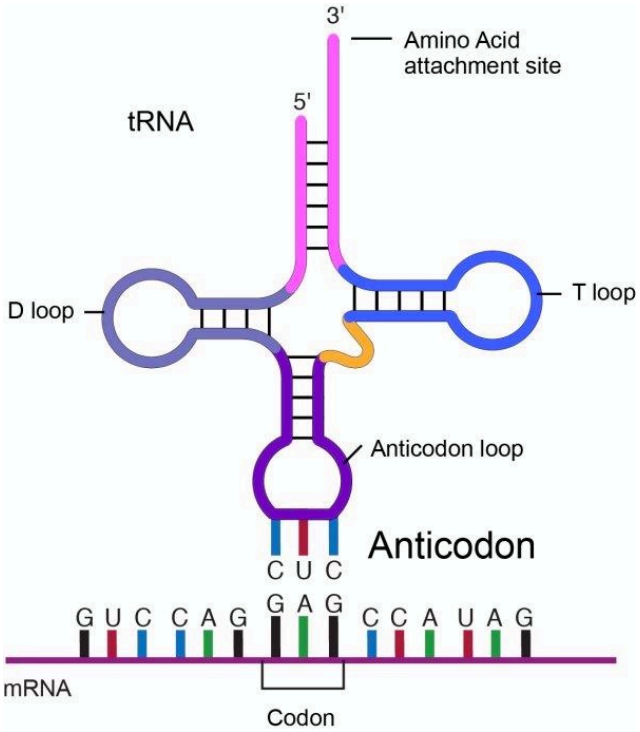
Translation: Messages to end products





Molecular Biology of the Cell, 5th Edition Garland Science, 2008.
Molecular Biology of the Cell, 7th Edition Garland Science, 2013.

Translation codon: The genetic code



Second letter

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

Putting everything together

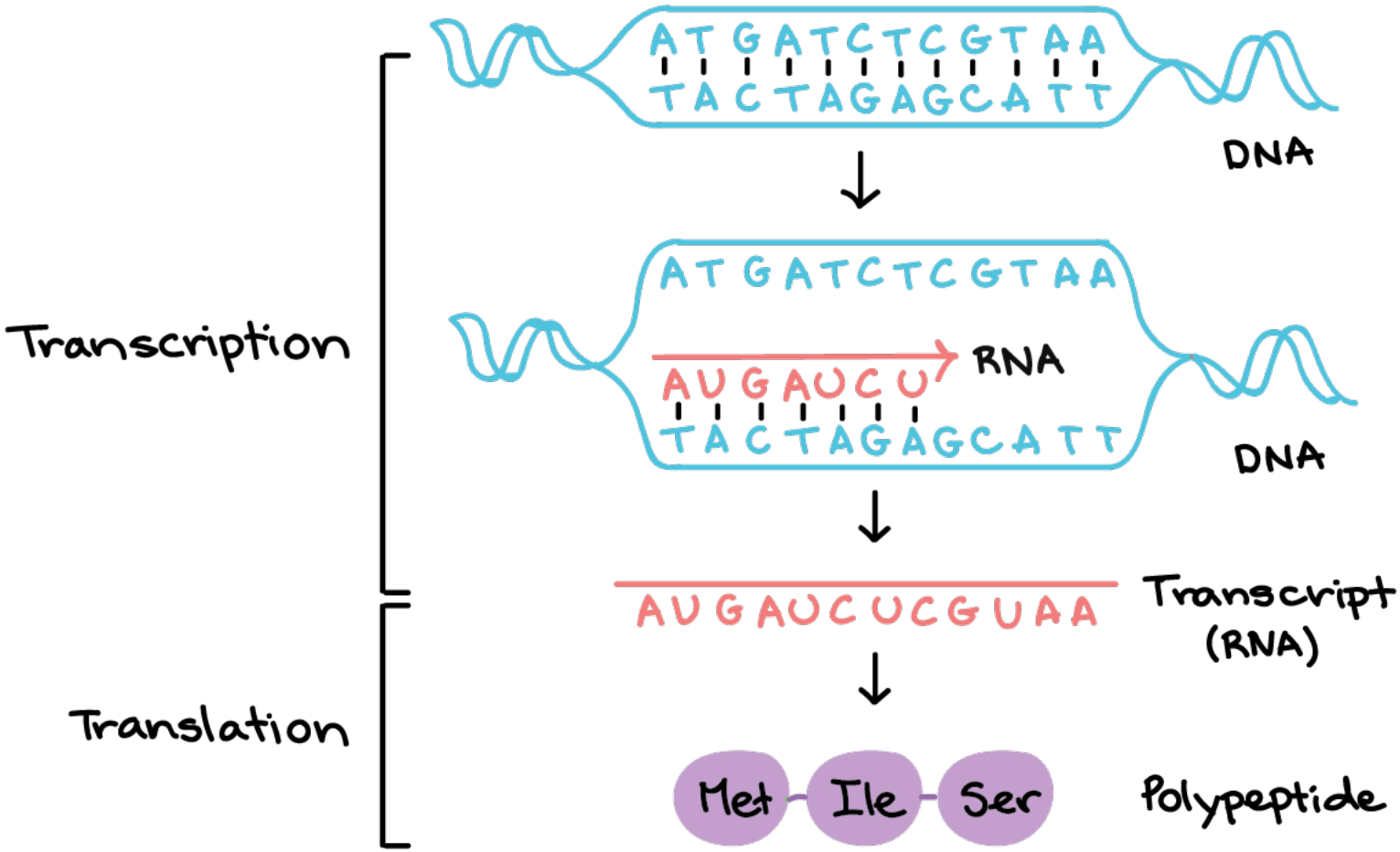


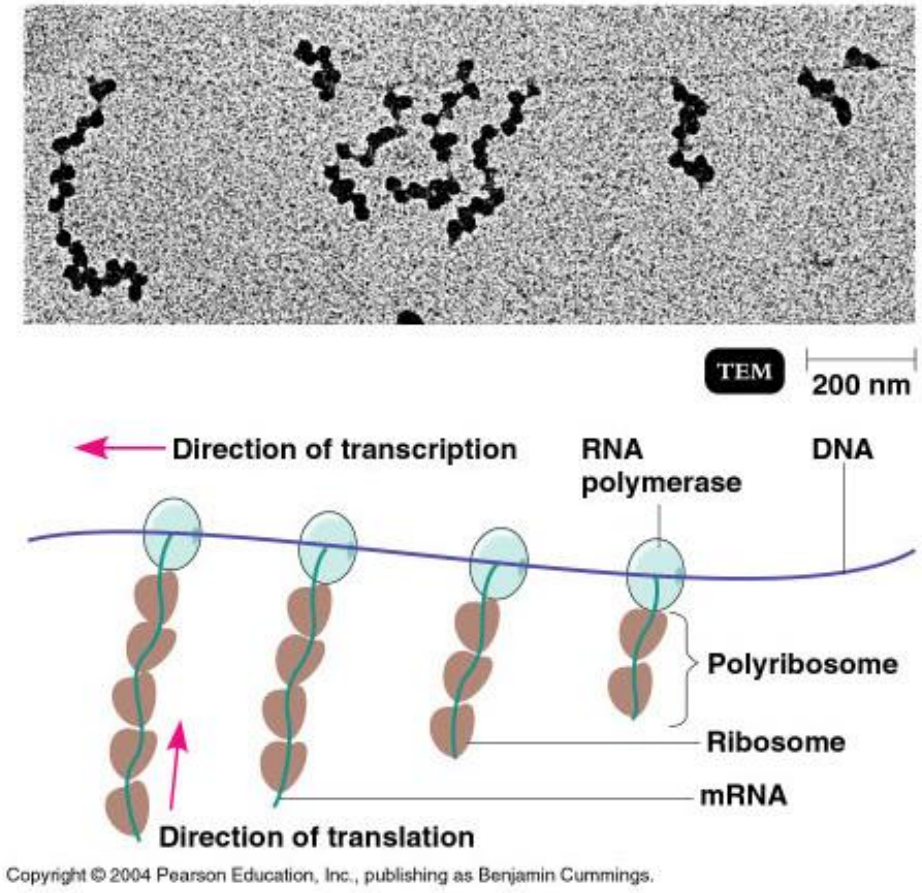
Image from Khan Academy

A review!

- Which main molecular components are needed for:
  - DNA replication
  - RNA transcription
  - Protein translation?

- How might these processes be different in prokaryotes compared to eukaryotes?

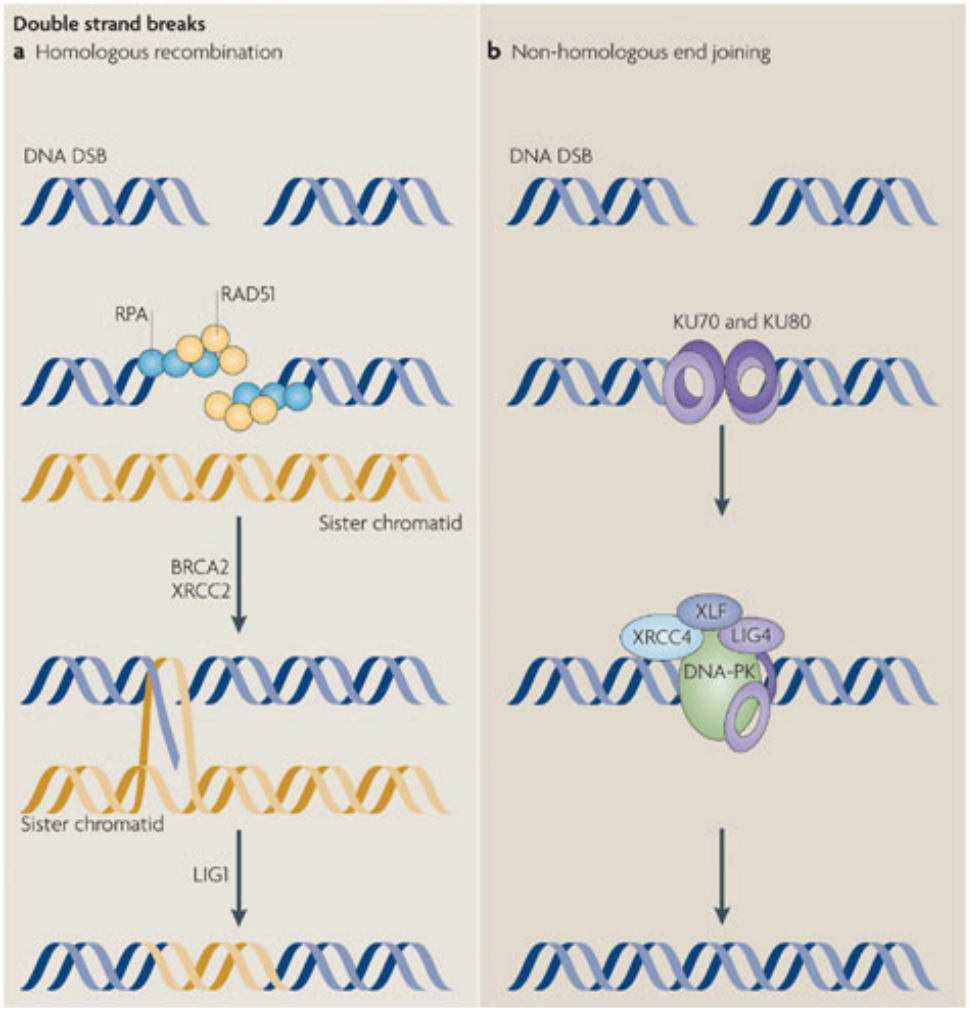
In prokaryotes, translation can begin before transcription is complete due to lack of compartmentalization




It's Time For A Break
EXMEN

Genetics - DNA repair

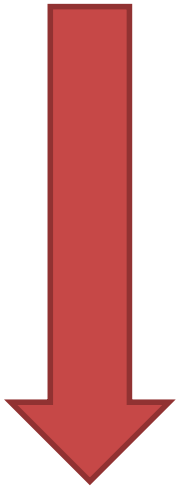
- Homology directed repair (HDR)
  - Requires homologous DNA to be present
  - https://www.youtube.com/watch?v=86JCMM5kb2A
- Non-homologous end joining (NHEJ)
  - https://www.youtube.com/watch?v=31stiofJjYw



Genetics – DNA repair is a crap-shoot

- Mutations
  - What kinds of mutations would affect gene function?

Large scale changes

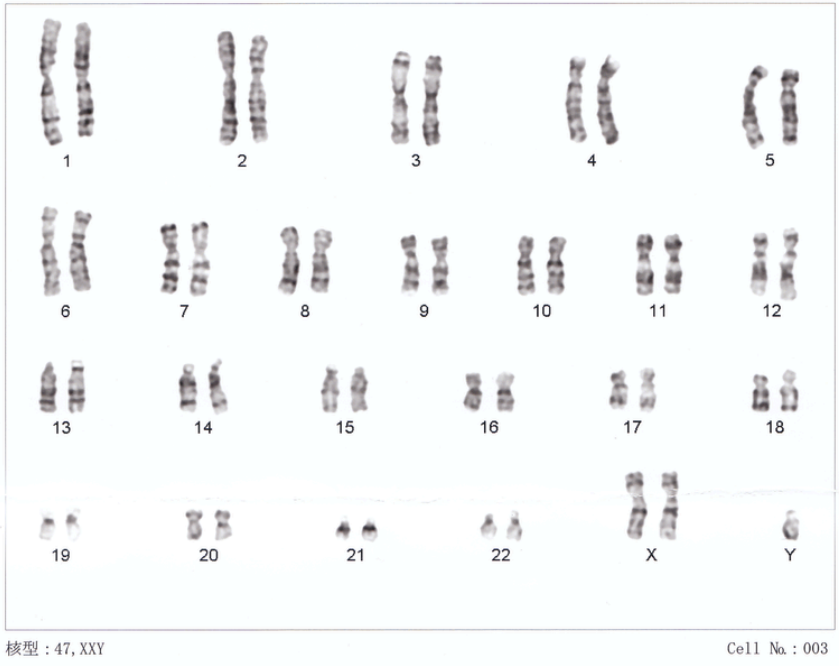


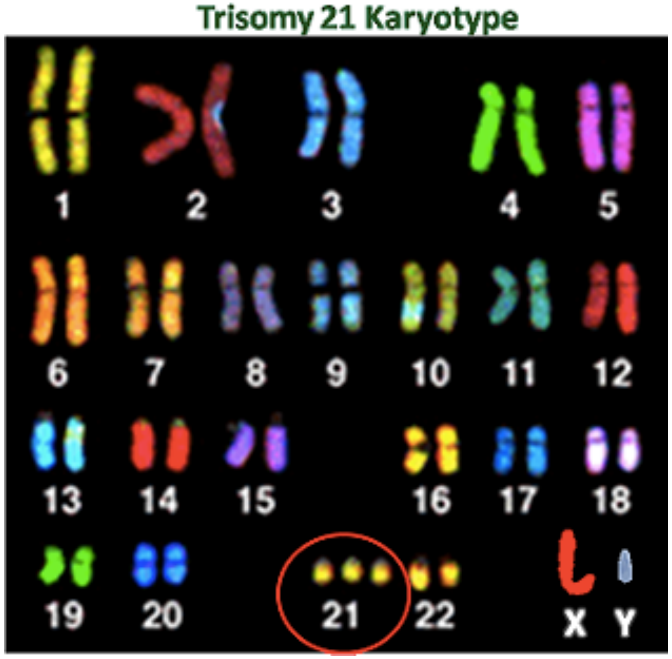
Small scale changes

- Aneuploidy
- Large chromosomal translocations/truncations
- Other inversions, translocations
- Copy number variations (CNVs)
- Point mutations – non-sense, missense, frame-shift, silent…
- Single nucleotide variations/polymorphisms (SNVs/SNPs)

Genetics - Aneuploidy

- Down Syndrome
- XXY – Klinefelter Syndrome



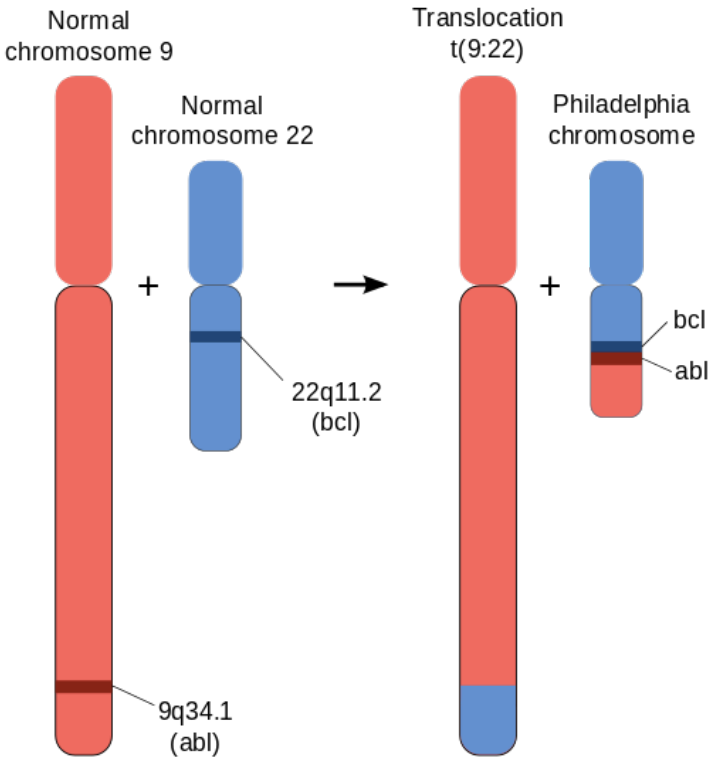


http://study.com/academy/lesson/aneuploidy-definition-disorders-quiz.html

By User:Nami-ja, via Wikimedia Commons

Genetics – Large chromosomal aberrations

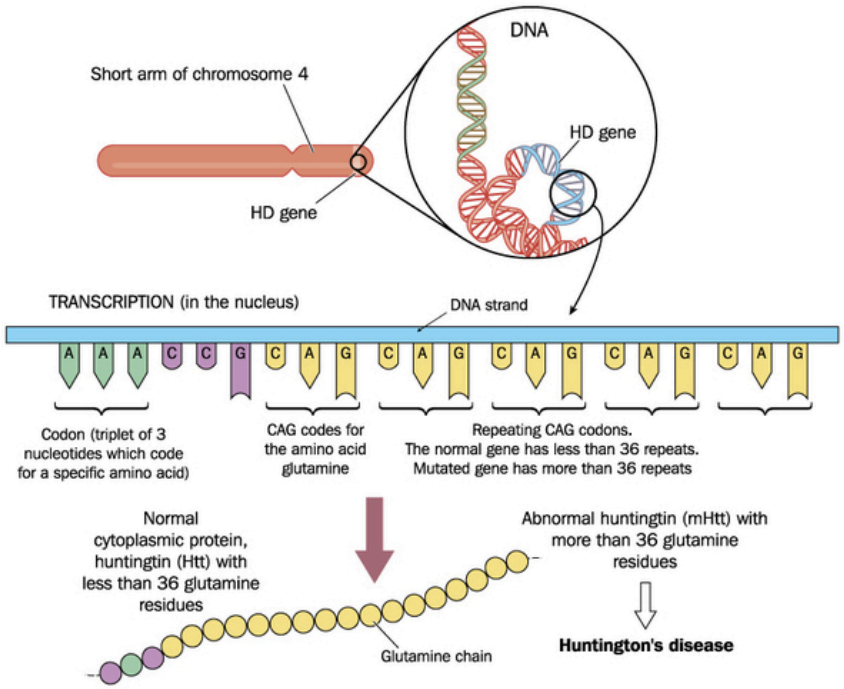
- Philadelphia chromosome and CML; BCR-ABL fusion



By Aryn89 (Own work) [CC BY-SA 4.0 (http://creativecommons.org/licenses/by-sa/4.0)], via Wikimedia Commons

Genetics - CNVs

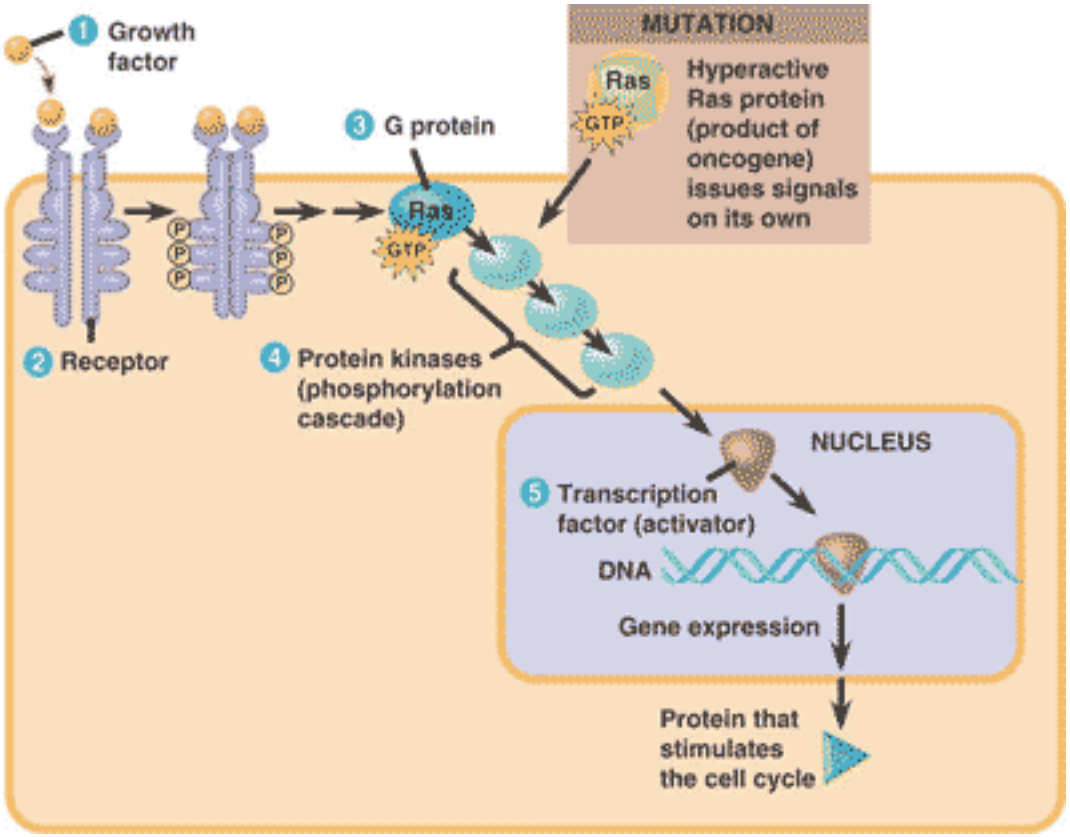
- Huntington's disease
  - CAG repeats, more than 44-46 times → likely to develop disease
- Breast cancer HER2/NEU/ERBB2 amplification



https://ghr.nlm.nih.gov/condition/huntington-disease

Genetics – Point mutations

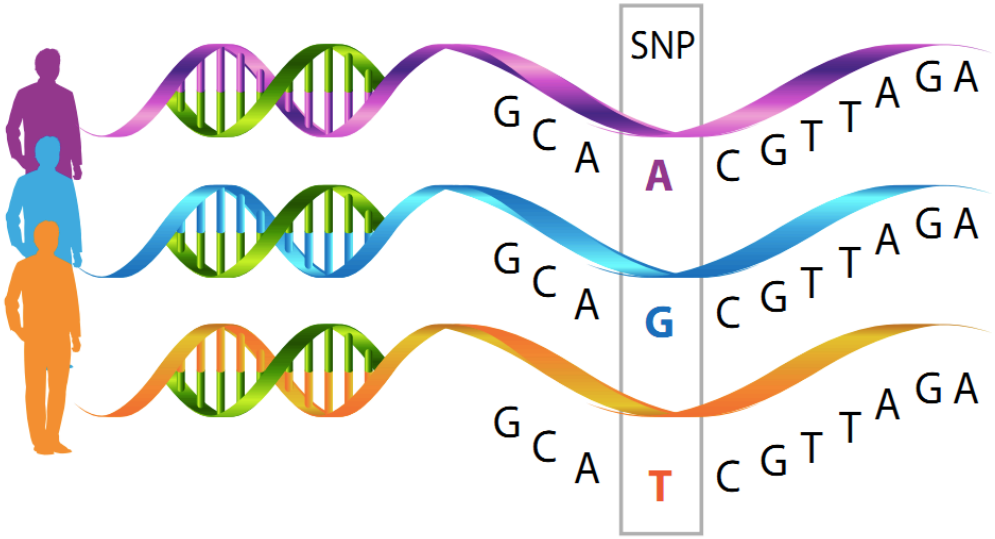
- RAS mutations leading to cancer



https://www.quia.com/jg/1276704list.html

Genetics - SNPs

- CCR5 receptor and immunity to HIV infection
- Difference between SNPs and 'just a mutation'



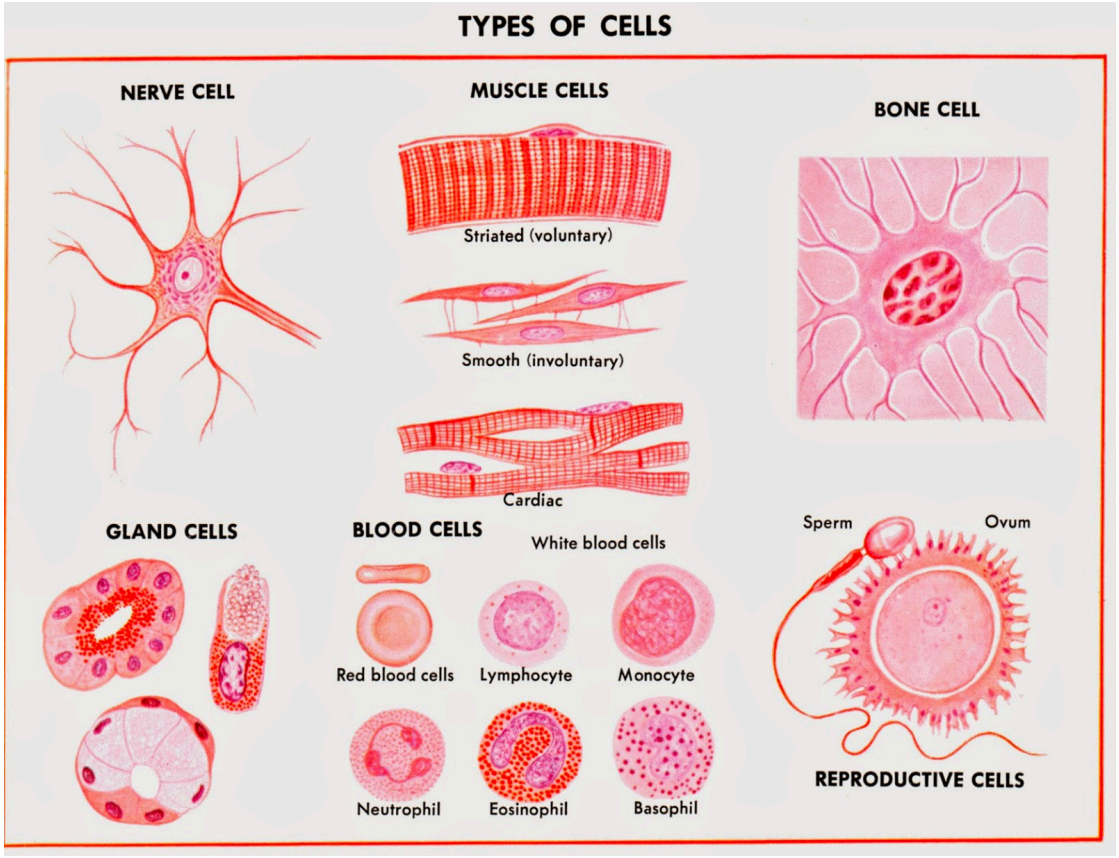
>1% abundance in population

Humans have over 3 million recorded SNPs

Link to personalized medicine

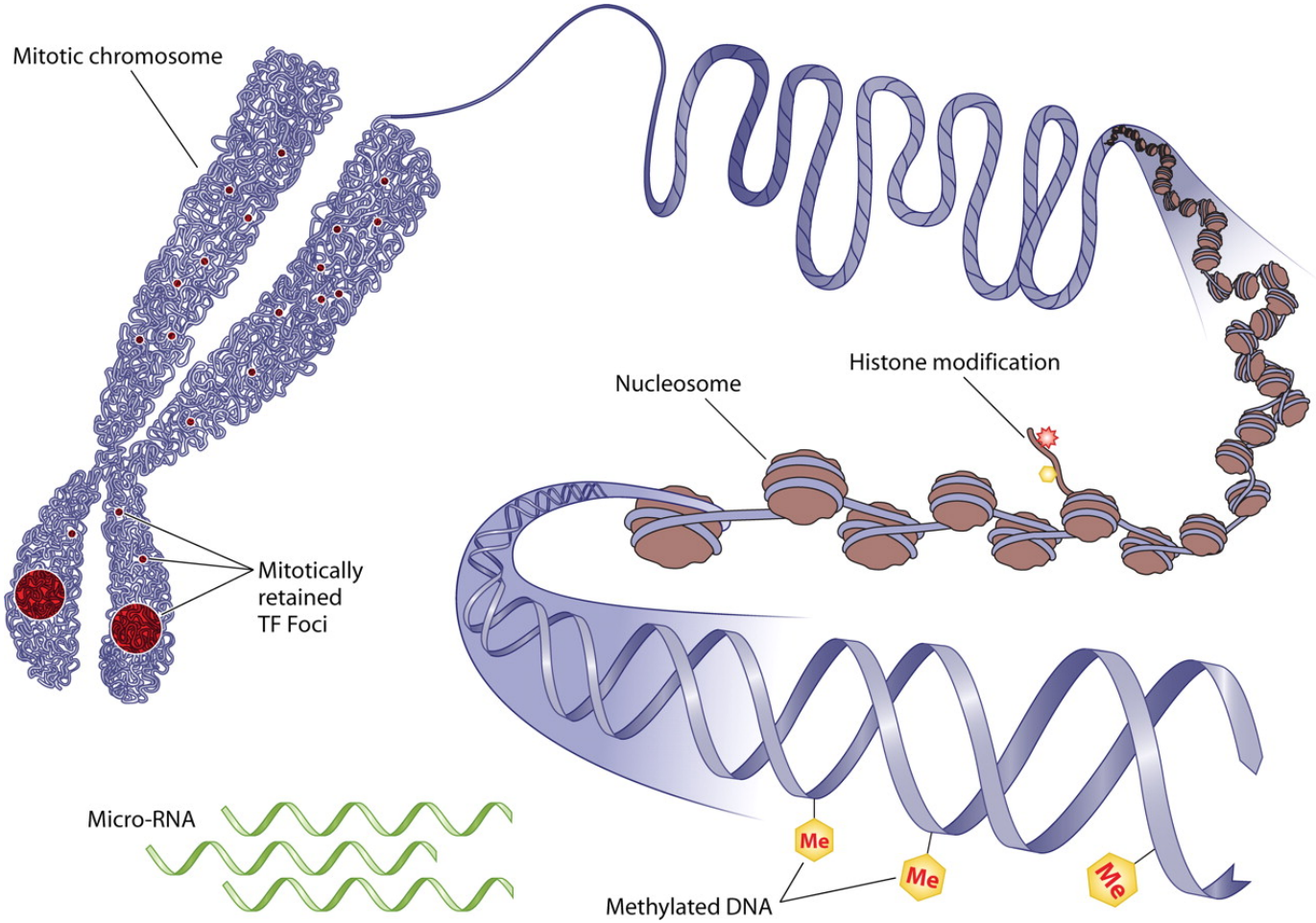
Question

- So many cell types, so few genomes…



https://wikis.engrade.com/a6thgradescience2/body

Epigenetics – Layers upon layers of information



Epigenetics – controlling transcription

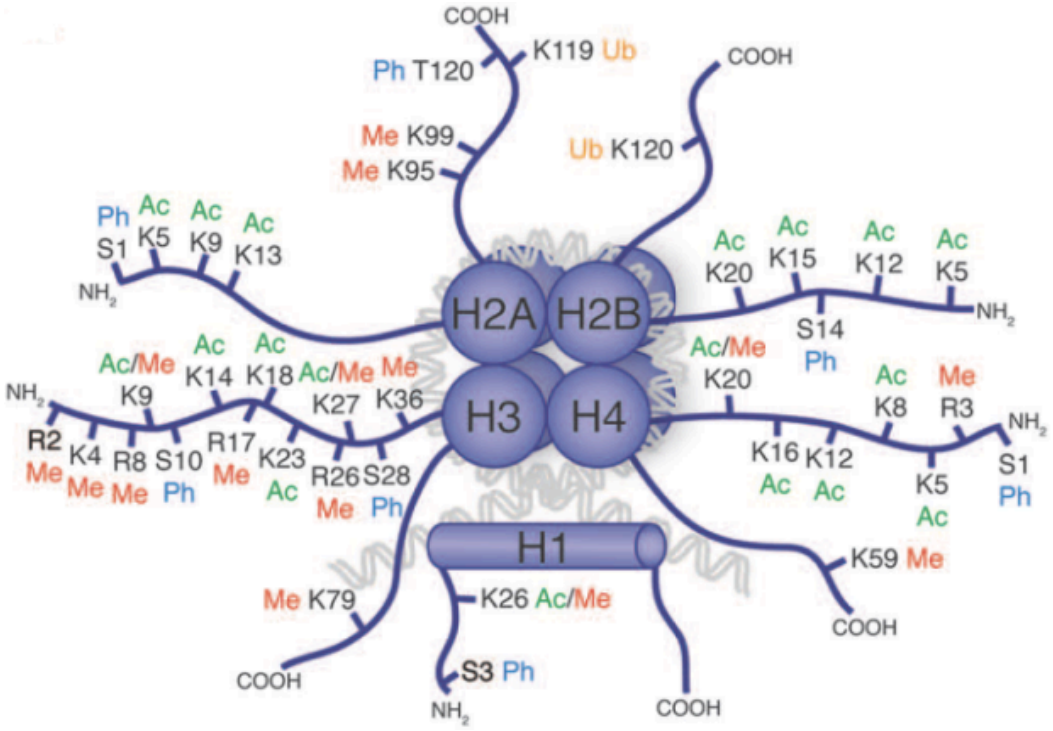
- Control of protein binding to DNA
- Control of DNA accessibility
  - Allele specific? X-inactivation?
- Control of coordinated expression of genes through 3D chromatin structure (Hi-C)
- Control of mRNA degradation

- Mis-regulation results in bad things:
  - Down syndrome
  - Many many many cancers

Epigenetics

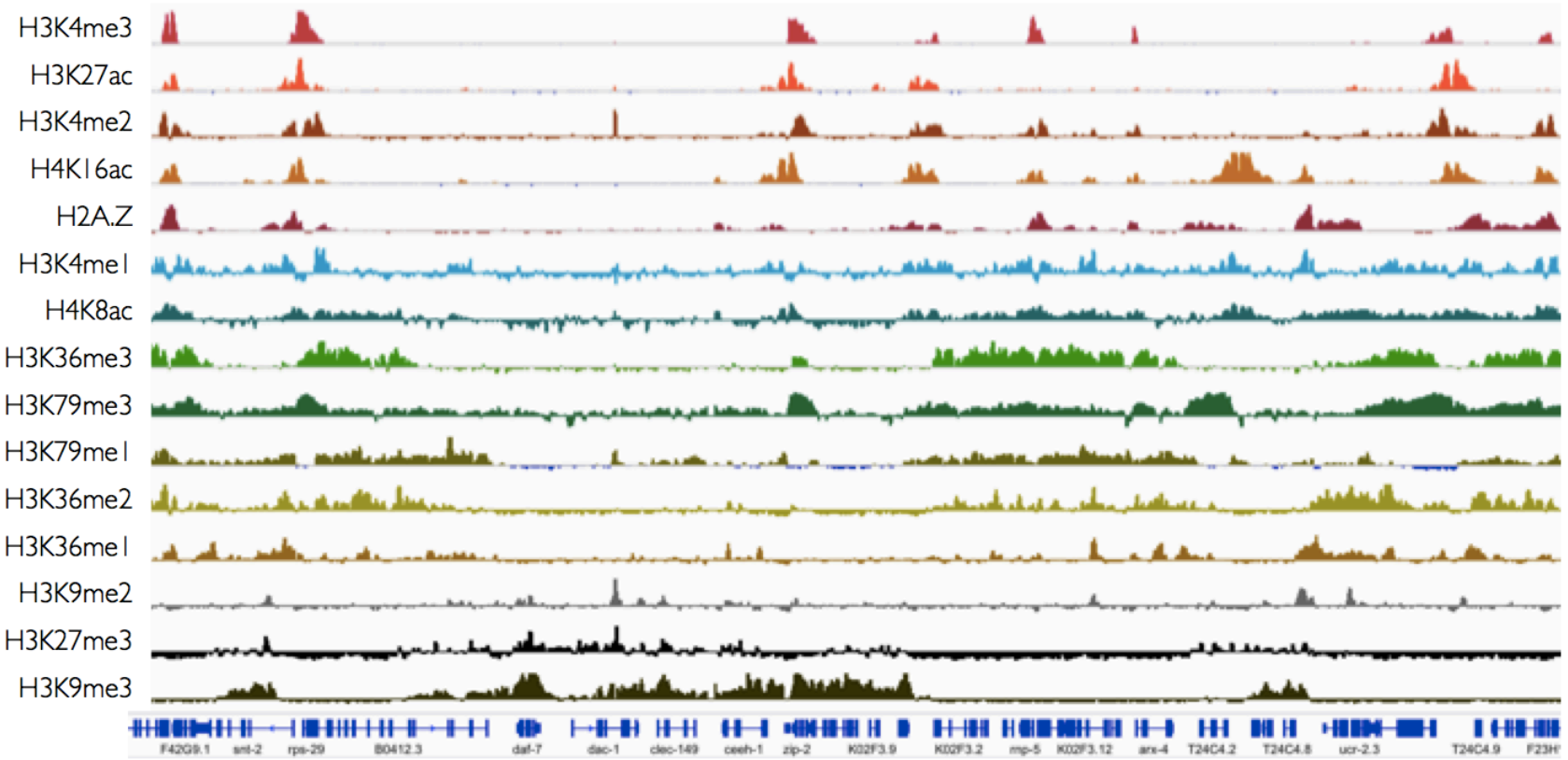
- Mechanisms of regulation:
  - DNA methylation
  - DNA hydroxymethylation
  - DNA XXX-ylation…
  - Histone modifications
  - miRNA silencing
  - lncRNA
  - Enhancers, repressors, ERVs,

Epigenetics – The histone code



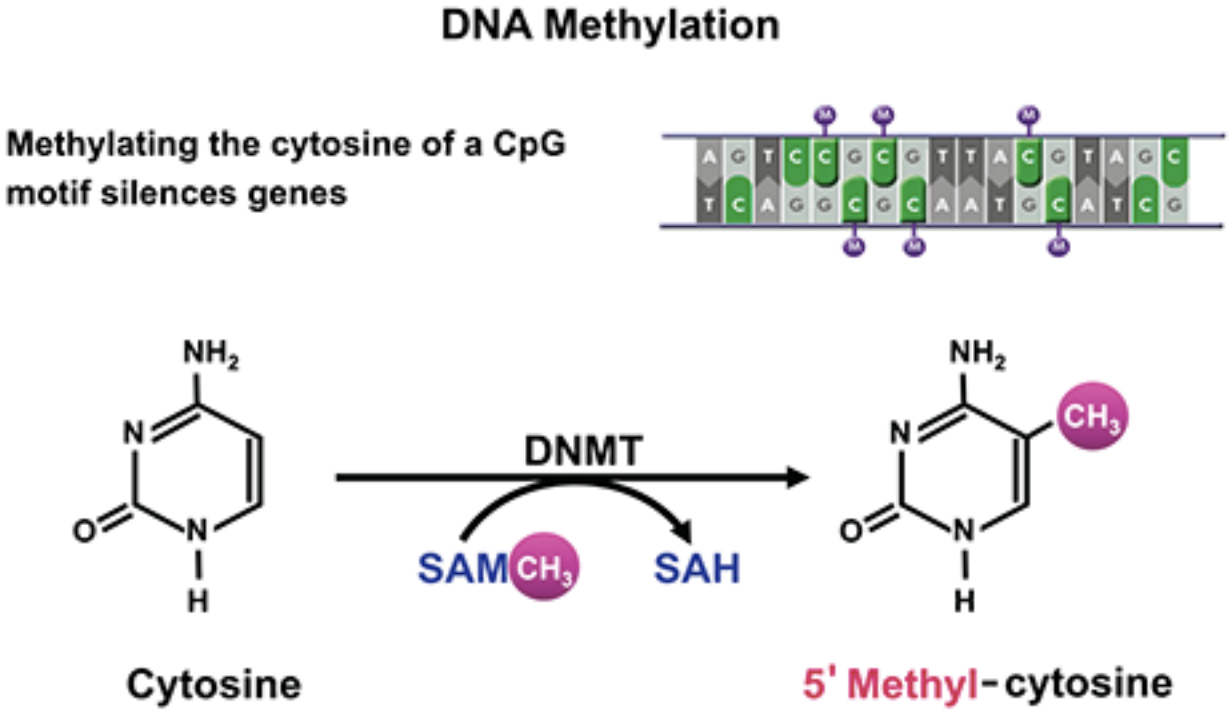
Perla Cota, Mehdi Shafa and Derrick E. Rancourt (2013). Stem Cells and Epigenetic Reprogramming, Pluripotent Stem Cells, Dr. Deepa Bhartiya (Ed.), InTech, DOI: 10.5772/55983. Available from: http://www.intechopen.com/books/pluripotent-stem-cells/stem-cells-and-epigenetic-reprogramming

Epigenetics – The Roadmap/ENCODE project



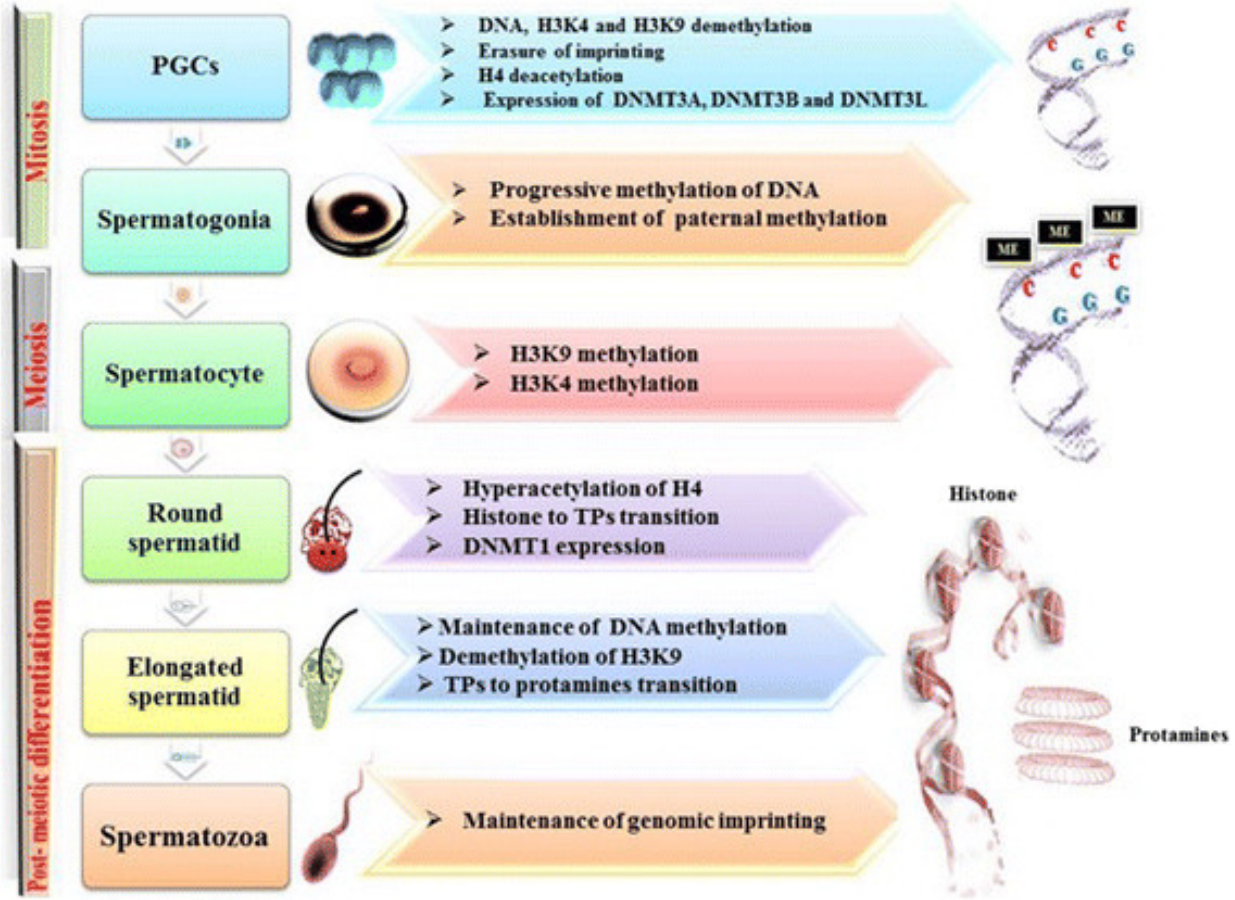
http://www2.gurdon.cam.ac.uk/~ahringerlab/research-3.html

Epigenetics – DNA methylation



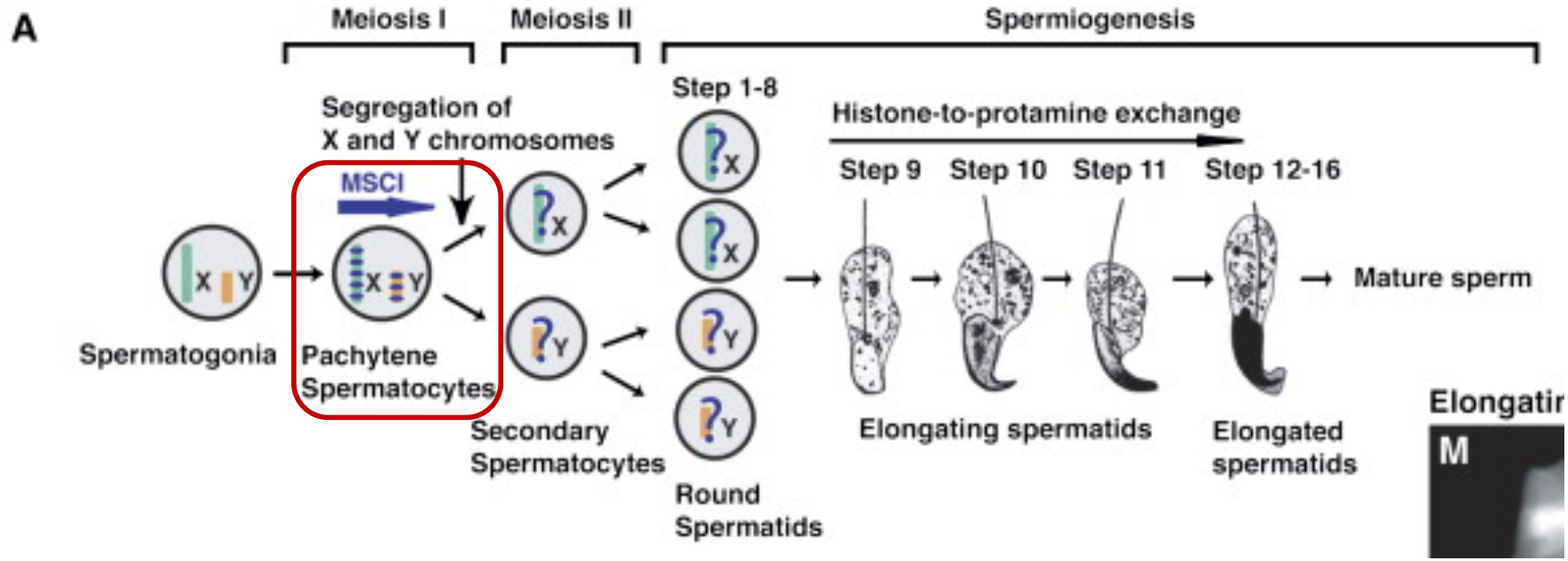
http://pubs.niaaa.nih.gov/publications/arcr351/6-16.htm

Illustration of epigenetics at work: Spermatogenesis



Stuppia et al. Clinical Epigenetics (2015) 7:120
DOI 10.1186/s13148-015-0155-4

Illustration of epigenetics at work: Spermatogenesis



MSCI – Meiotic Sex Chromosome Inactivation

Namekawa et al. Current Biology 16.7 (2006): 660-667. https://doi.org/10.1016/j.cub.2006.01.066

BASIC MOLECULAR BIOLOGY TOOLS AND TECHNIQUES

How to characterize DNA and protein

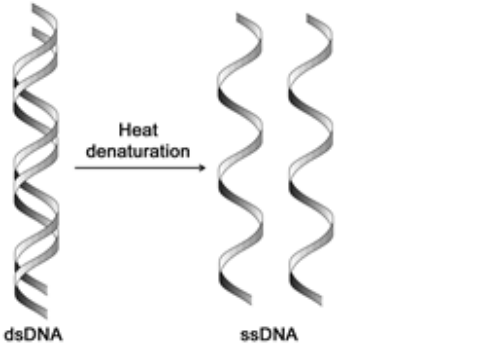
A tiny bit of biophysics to start: DNA denaturation

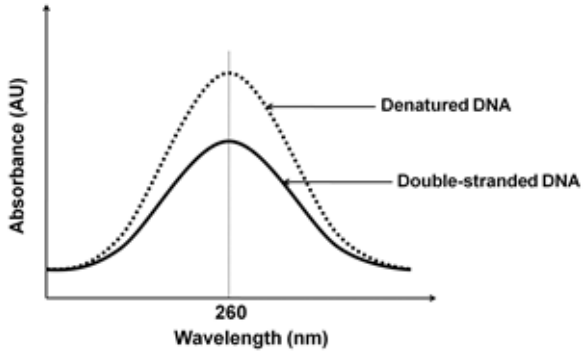
- Melting DNA from double stranded to single stranded

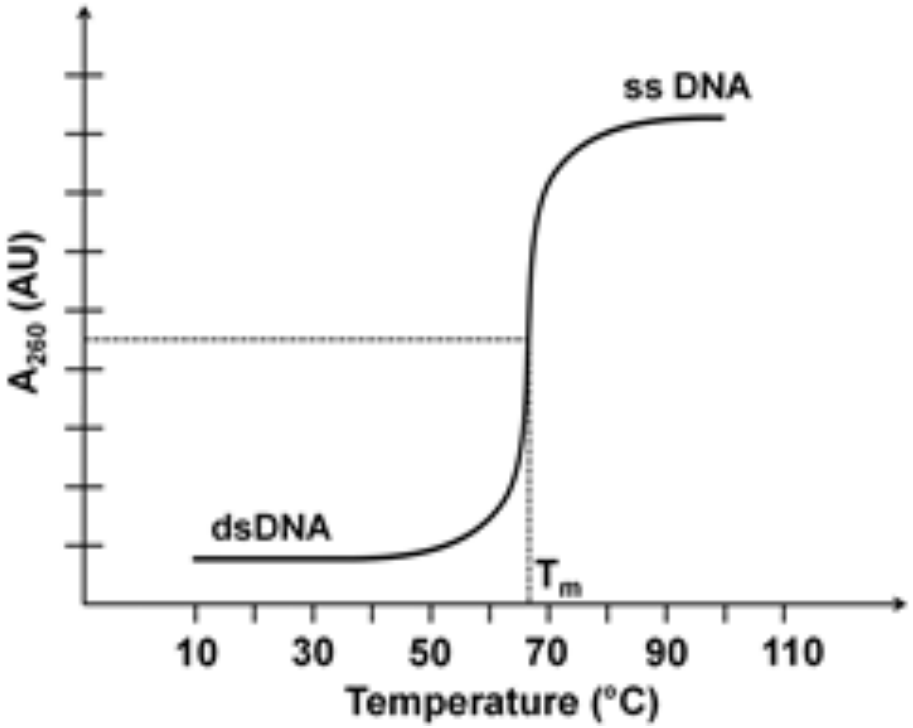
- [AB] ⇔ [A][B]

DNA denaturation

- The temperature where equilibrium state (half dsDNA, half ssDNA) is achieved is called Tm (DNA melting temp)







http://nptel.ac.in/courses/102103047/7

DNA denaturation

- Conditions favoring denaturation/melting:
  - **low salt** concentrations: DNA is a polyanionic molecule. High salt concentrations "shield" the negative charges on each phosphate. In low salt, the electrostatic repulsion of the negatively charged strands makes it energetically more favorable to separate the strands
  - **high pH** (basic conditions) also breaks hydrogen bonds
    - High pH = more free OH-
    - More free OH- → deprotonation → disrupt H-bonding

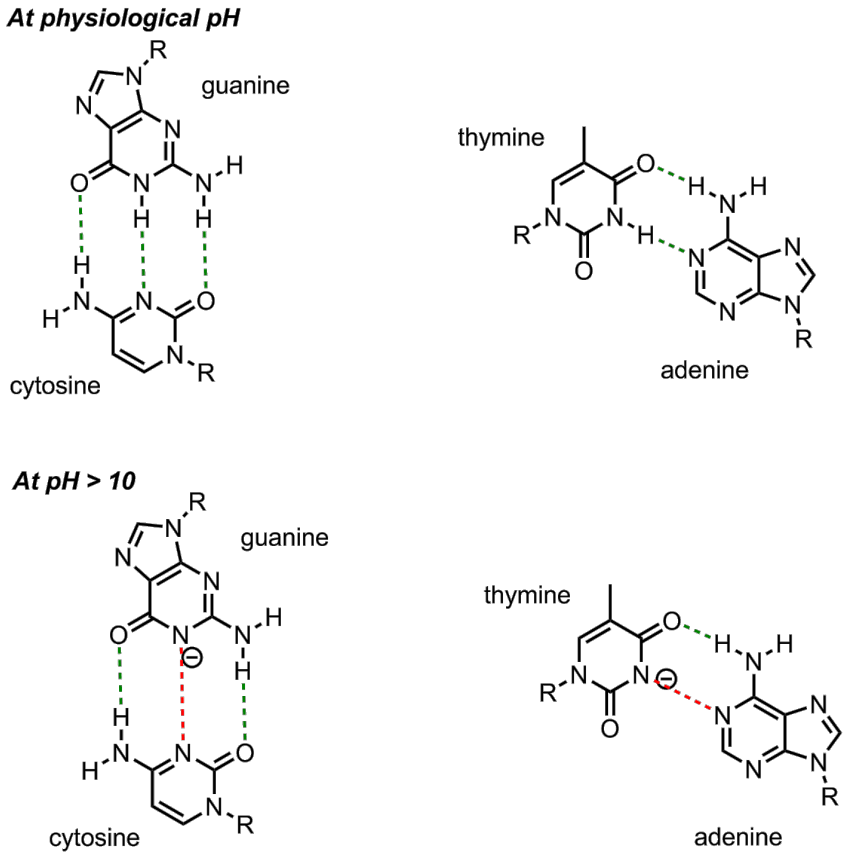
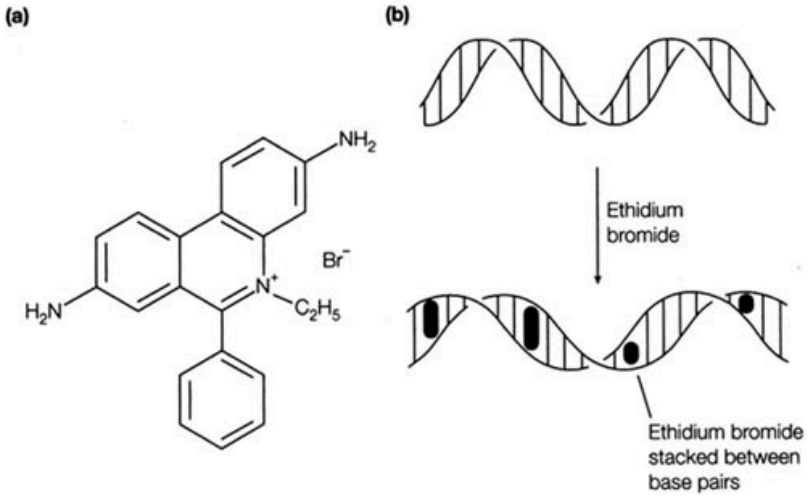


Figure from: https://biology.stackexchange.com/questions/29925/why-does-high-ph-result-in-the-denaturation-of-dna

DNA staining



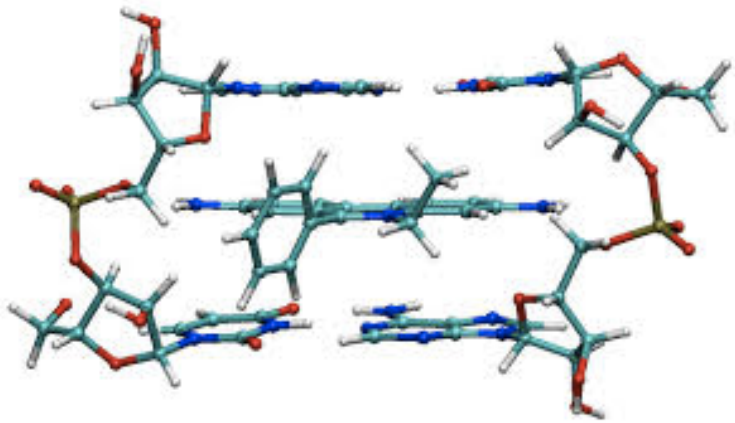
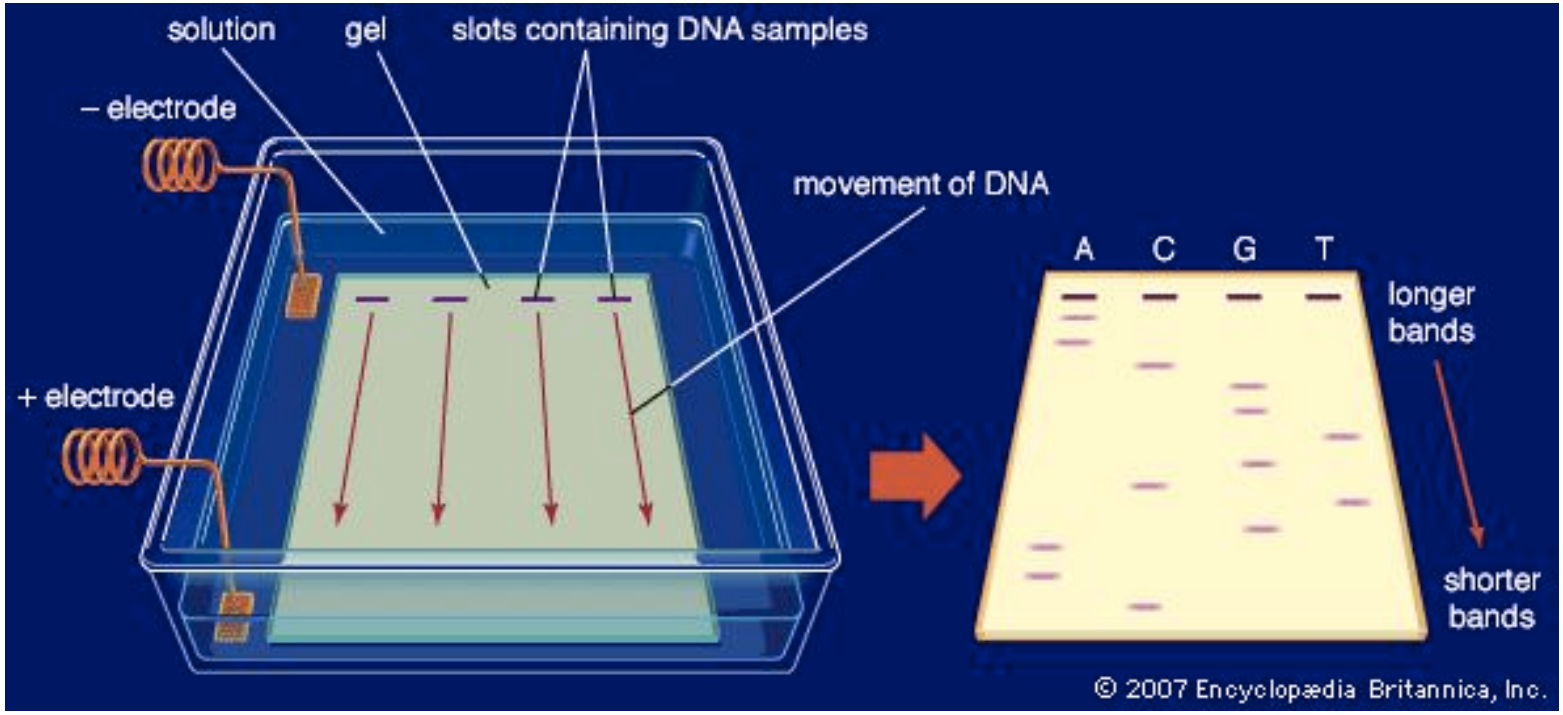


Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.

http://www.madsci.org/posts/archives/1999-02/919869466.Mb.r.html
https://commons.wikimedia.org/wiki/File:DNA_intercalation2.jpg

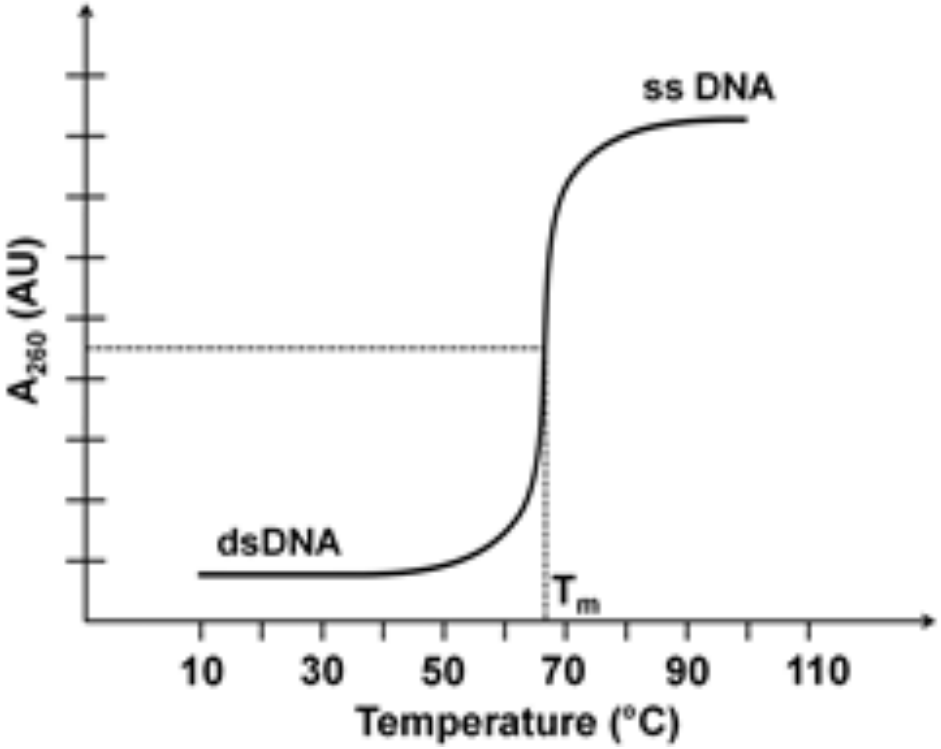
Gel Electrophoresis



https://global.britannica.com/science/gel-electrophoresis

Question 1 – DNA denaturation

- The average GC content of the human genome ranges from 35%-60% depending on the chromosome. We discover a new species of bacteria, and analyzed its genome. The bacteria's genome is 80% GC. What is the % of AT?



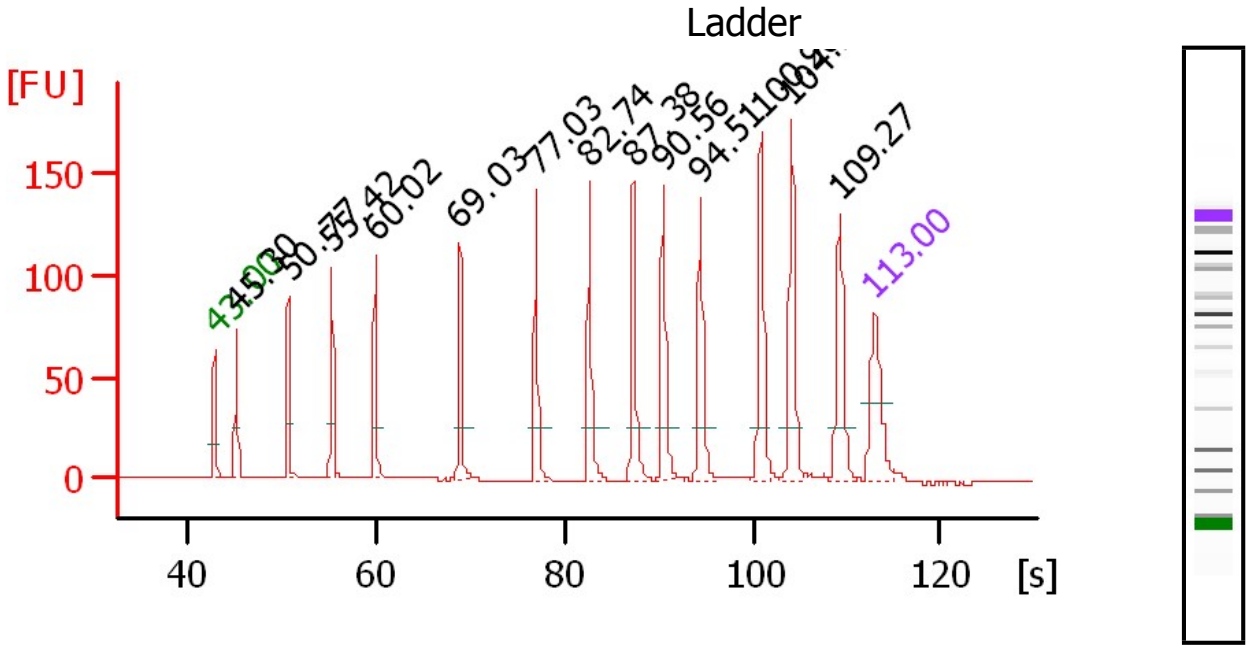
- If the figure on the right shows the dissociation curve for a long strand of human genomic DNA, what

Question 11 – DNA denaturation

- A trickier question:
  - During transcription, RNAP enzyme has a subunit that helps to unwind the DNA as it goes along, making template strand available for transcription.
  - Suppose we are trying to perform transcription in-vitro, but we only have access to the parts of RNAP without the DNA-unwinding component. What kinds of in-vitro/buffer/experimental conditions would you try to get this in-vitro transcription to work?

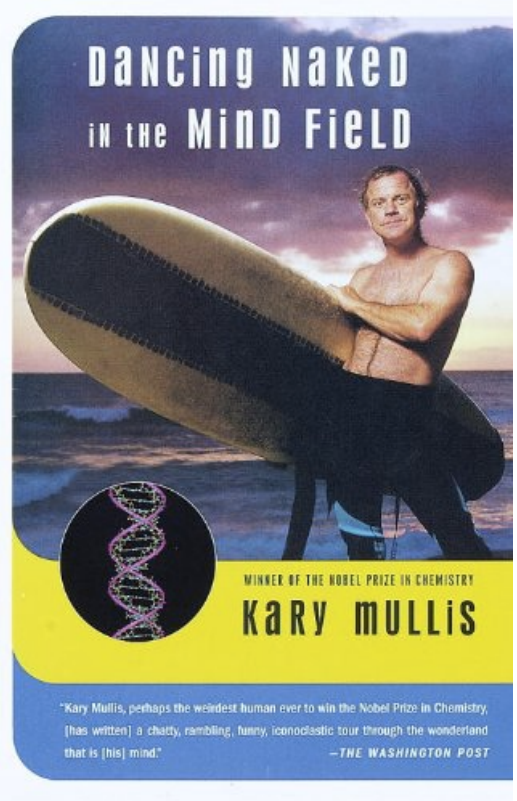
Question 2 – Capillary Electrophoresis

Based on this raw data from a DNA sizing experiment using capillary electrophoresis technology, can you suggest some possible mechanisms for how capillary electrophoresis works? Try to imagine that you are building a prototype capillary electrophoresis machine – what major components would you need, and what are they for?



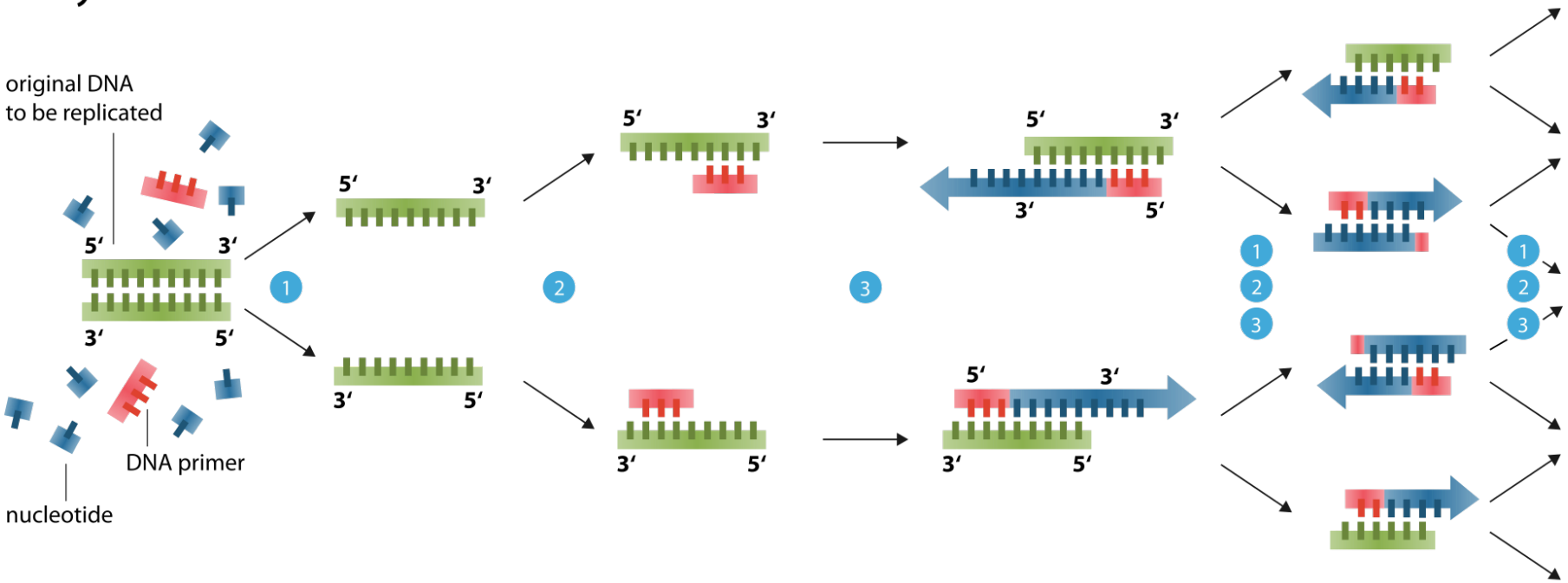
PCR Fun Fact

- Invented by Dr. Kary Mullis, who got the Nobel Prize for Chemistry in 1993
- "Nearly a year after he collected his Nobel, Mullis told California Monthly: "Back in the 1960s and early '70s I took plenty of **LSD**. A lot of people were doing that in Berkeley back then. And I found it to be a mind-opening experience. It was certainly much more important than any courses I ever took." And in 1997, he told the BBC, "What if I had not taken LSD ever; would I have still invented PCR? I don't know. I doubt it. I seriously doubt it.""



PCR

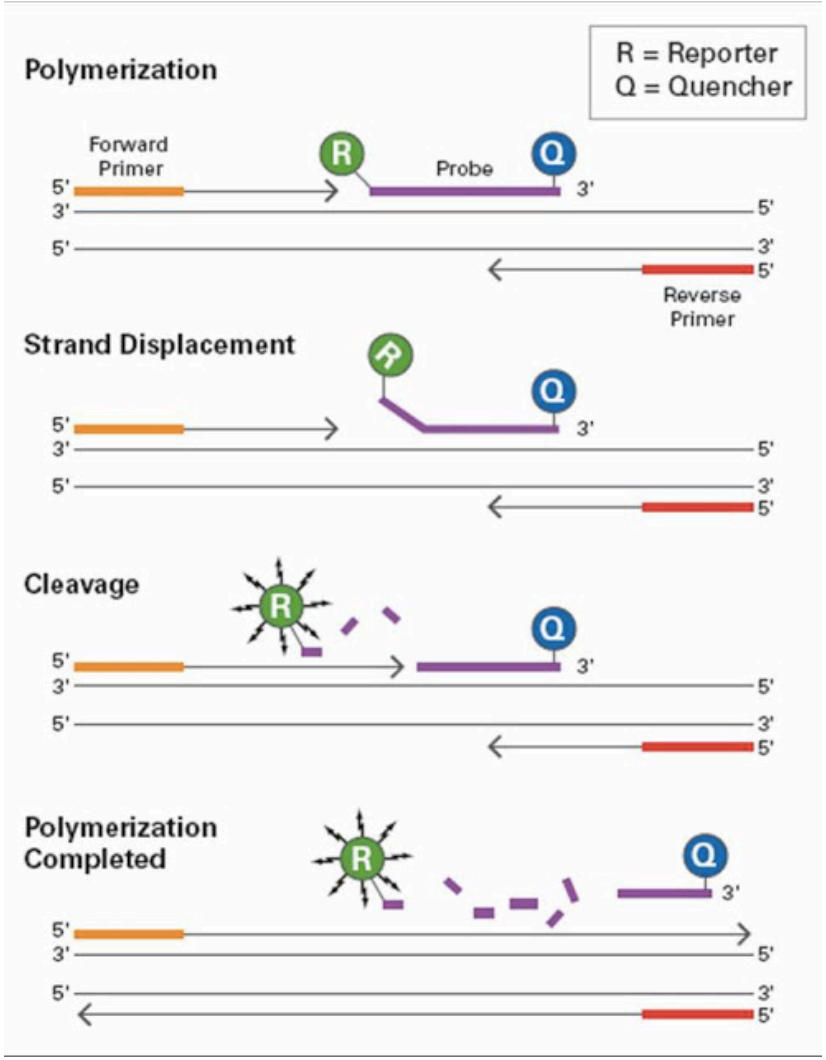
Polymerase chain reaction - PCR



1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

Quantitative PCR

- Using Taqman chemistry:
  - Fluorescent dye and quencher are on the same probe
  - In close proximity, fluorescence is quenched
  - With positive amplification, the polymerase will cleave the probe as it copies template
  - Cleavage releases dye from quencher, results in emission
  - More copies = more dye released = more fluorescent signal
  - Highly specific: probe + primers required for signal



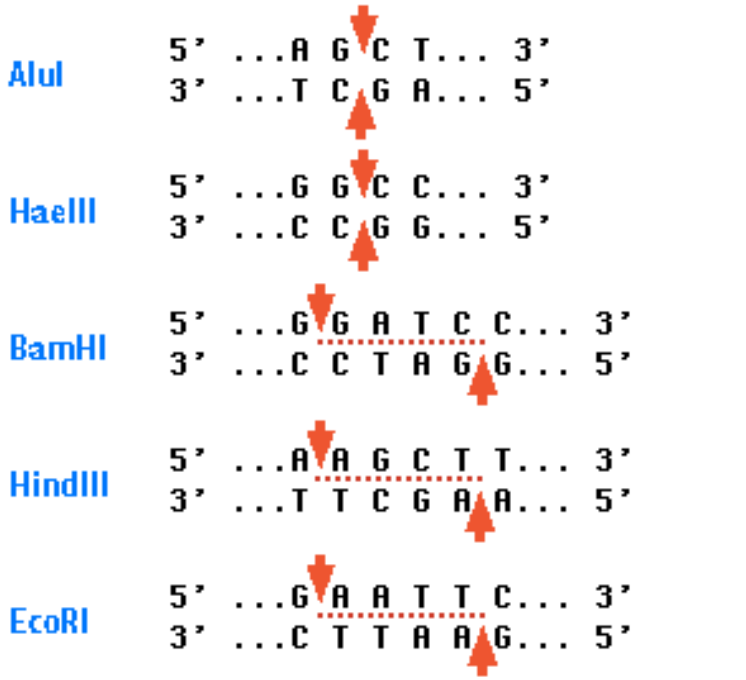
Recombinant DNA

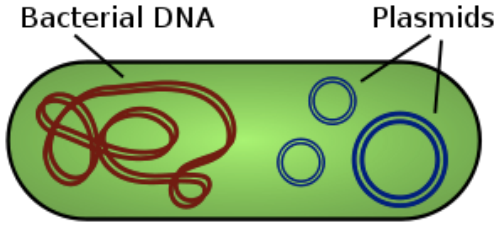
- "DNA molecules formed by laboratory methods of genetic recombination, such as molecular cloning, to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure. They differ only in the nucleotide sequence within that identical overall structure."
- **Cloning genes of interest**
- **Combining different DNA fragments into one**
- **Specific applications:** fusion proteins; expressing new protein in existing genome; making protein in large quantities (e.g. insulin)

https://en.wikipedia.org/wiki/Recombinant_DNA

Molecular Cloning

- **Restriction enzymes** – enzymes (mostly from bacteria) that make cuts in DNA at specific sequences (palindromic site); these are "Type II"
- **Plasmids** – small DNA separate from chromosomal DNA, and can replicate separately; commonly found in bacteria
- **Origin of Replication (ORI)** – DNA sequence which allows initiation of replication within a plasmid by recruiting transcriptional machinery proteins



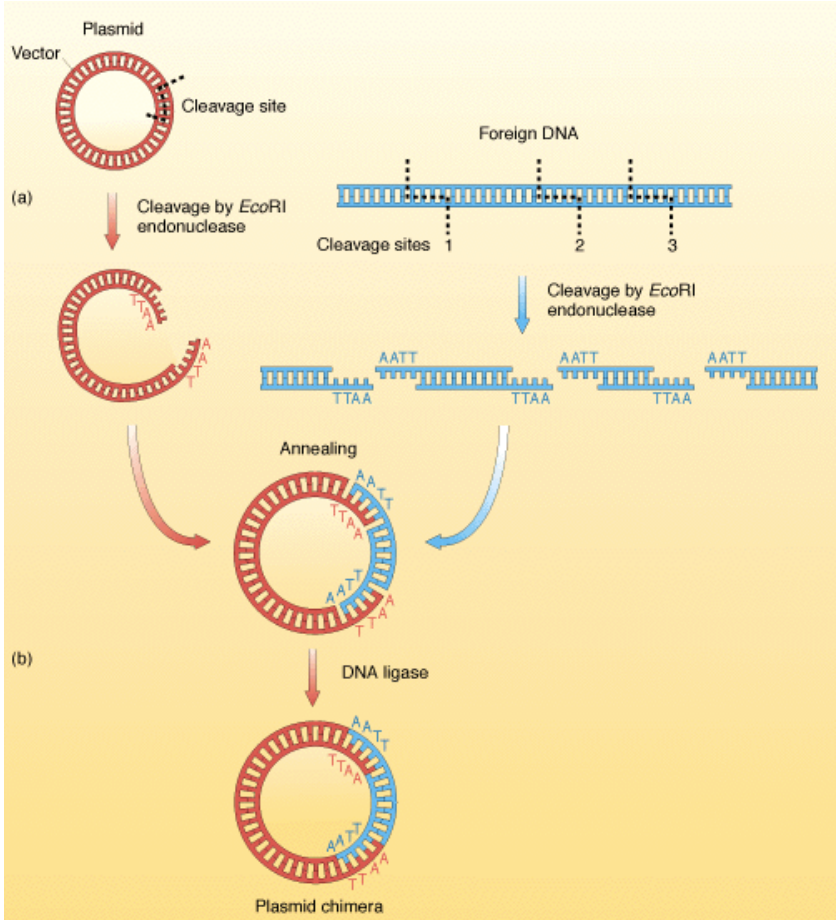


https://en.wikipedia.org/wiki/Recombinant_DNA

http://www.biology-pages.info/R/RestrictionEnzymes.gif
By User:Spaully on English wikipedia (Own work) [CC BY-SA 2.5 (http://creativecommons.org/licenses/by-sa/2.5)], via Wikimedia Commons

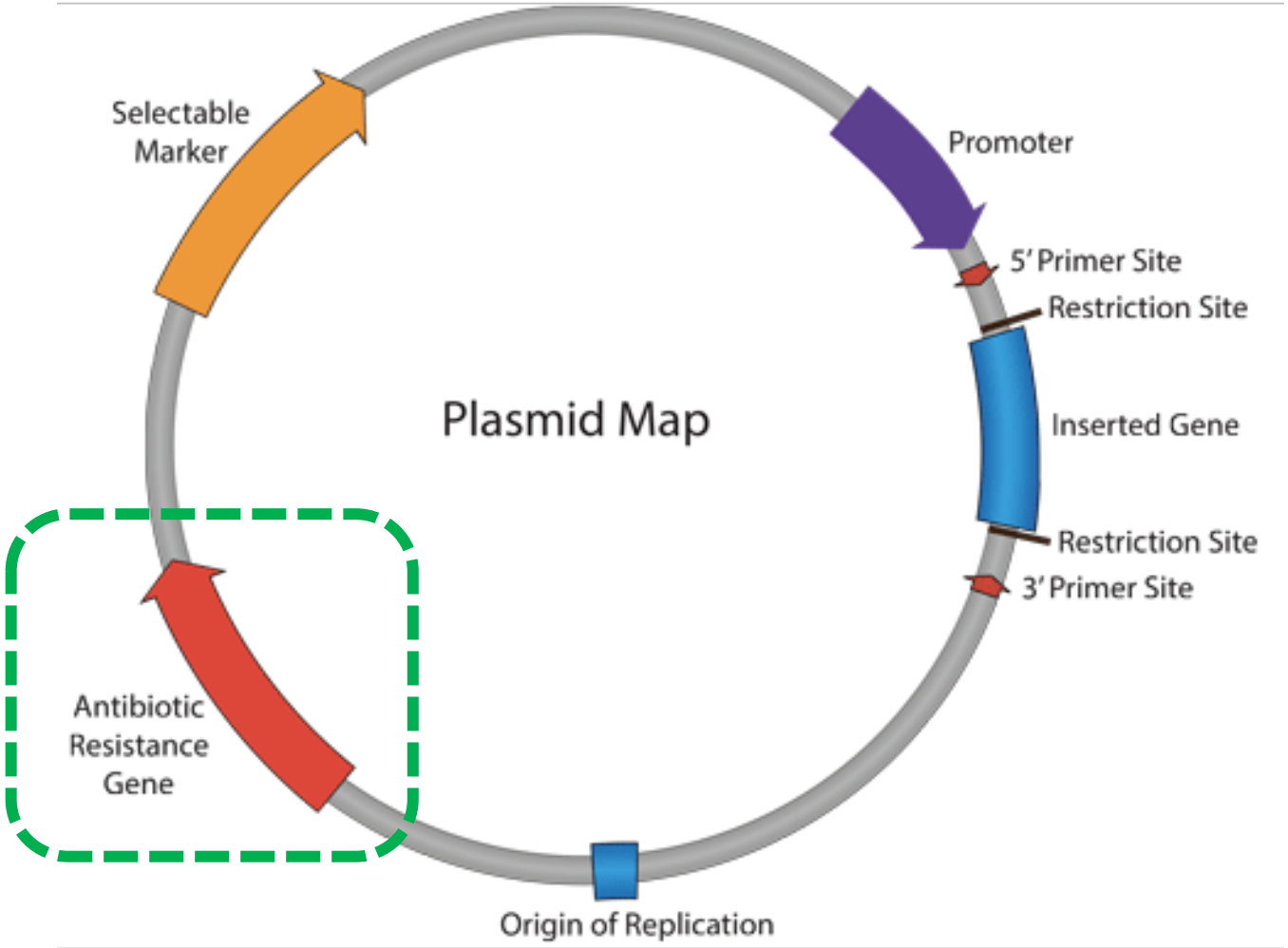
Molecular cloning

- **Vector -** DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated/expressed
- Cut the plasmid vector; Cut the insert sequence using the same restriction enzyme
- Join/"Ligate" the two together



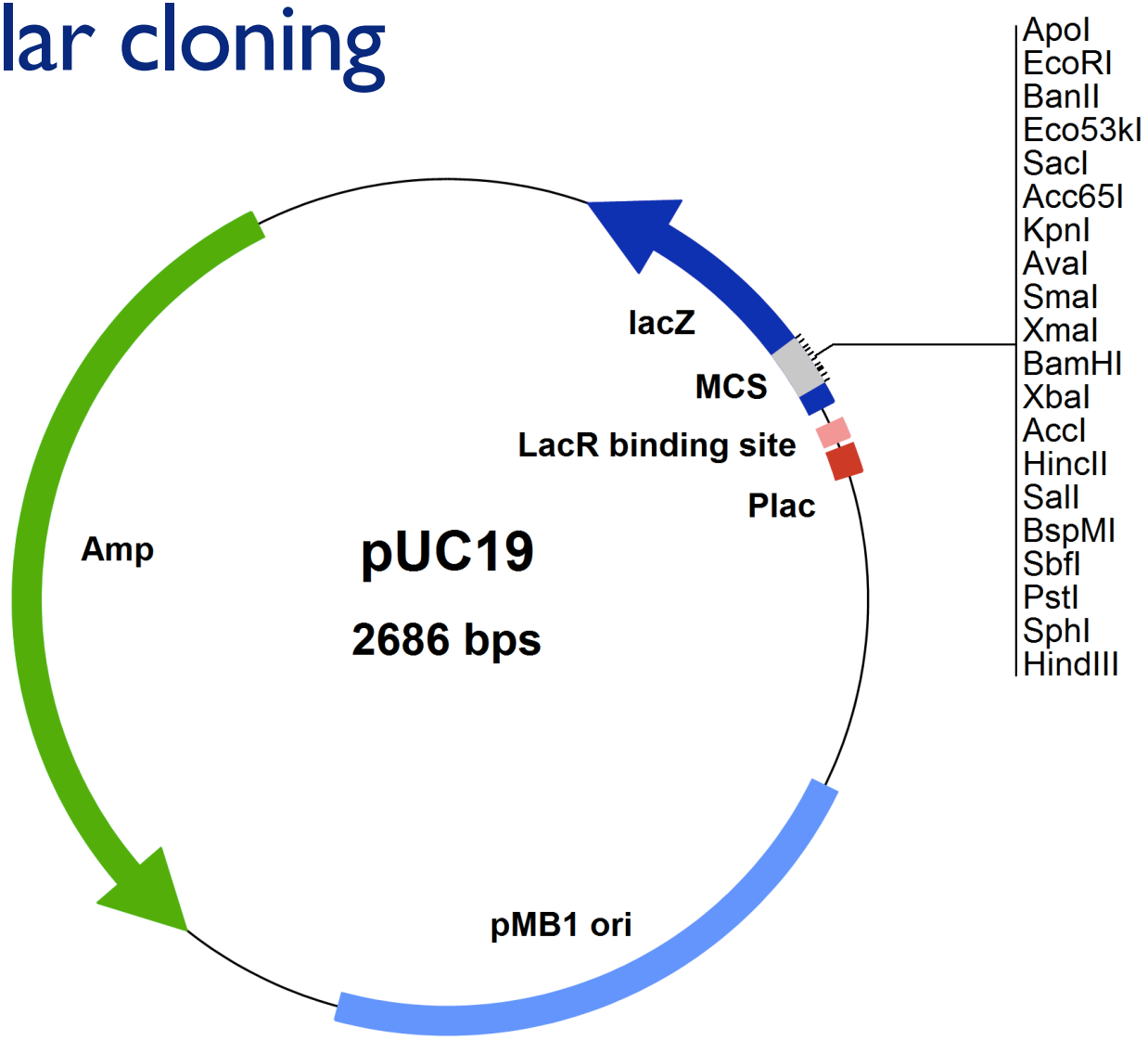
https://en.wikipedia.org/wiki/Recombinant_DNA
http://www.bio.miami.edu/dana/pix/chimericDNA.gif

Molecular cloning



http://blog.addgene.org/hs-fs/hub/306096/file-404153303-png/Plasmid_Map.png?t=1474663191759&width=350&name=Plasmid_Map.png

Molecular cloning

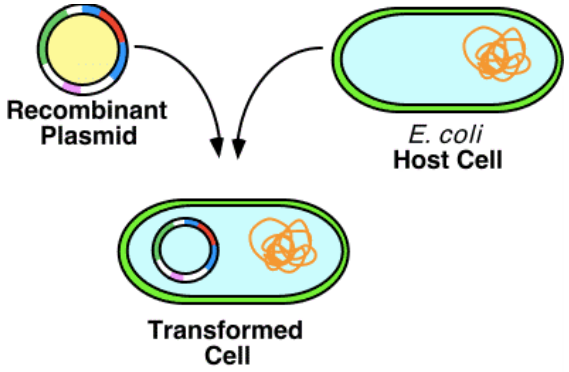


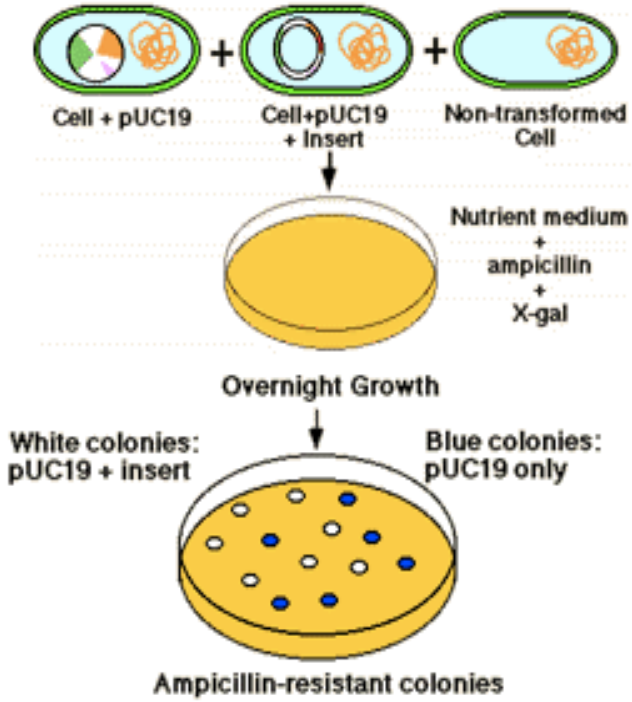
http://www.mobitec.com/cms/bilder/products/vector_sys/pUC19.png

Molecular cloning

- **Transformation** – genetic alteration of a cell resulting from direct uptake and incorporation of exogenous DNA through the cell membrane; typically achieved by heat shock, electroporation, or chemical treatment of cells (DNA precipitation)

- **Selection** – use of a selectable marker or antibiotic resistance gene to distinguish cells that did not take up plasmid, or did not insert the gene in the right place, or took up empty plasmid



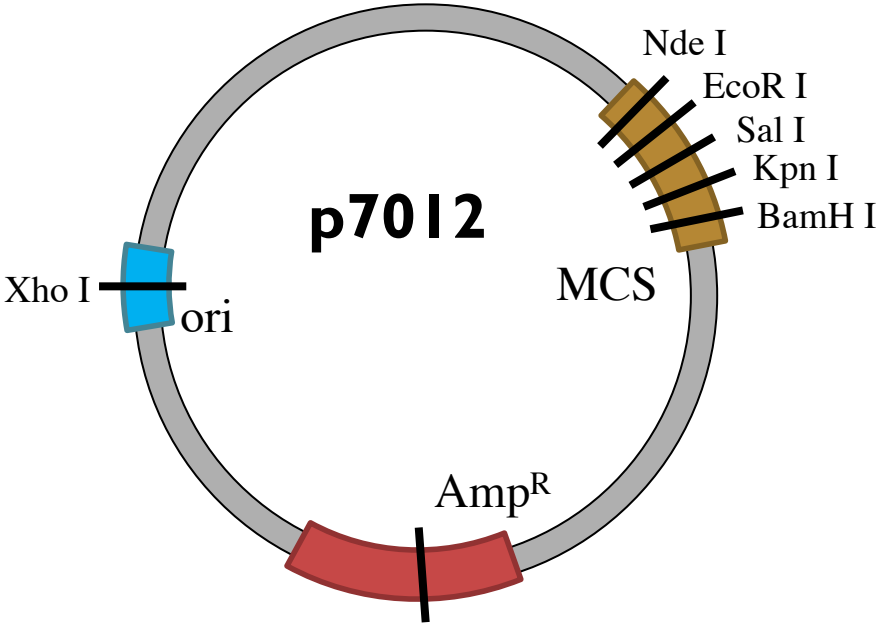


Question 3

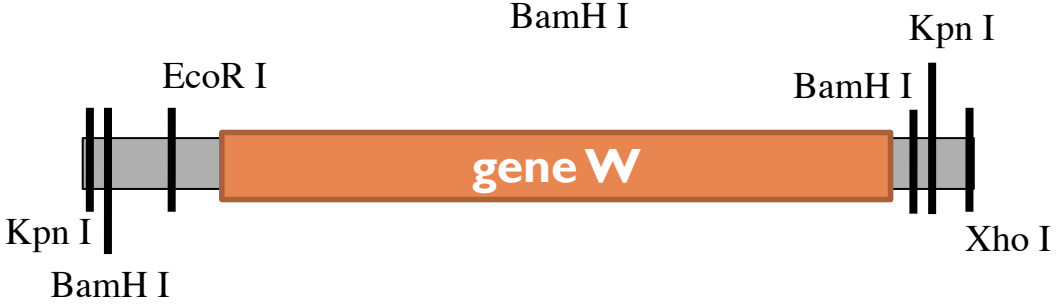
- Here is vector p7012:
- Here are the restriction enzymes:

Nde I:	Sal I:	Kpn I:
5' CATATG	5' GTCGAC	5' GGTACC
3' GTATAC	3' CAGCTG	3' CCATGG

EcoR I:	BamH I:	Xho I:
5' GAATTC	5' GGATCC	5' CTCGAG
3' CTTAAG	3' CCTAGG	3' GAGCTC



- Here is gene W:



a) *What are 3 strategies for cloning gene W into p7012?*

b) *In which strategies would gene W be inserted into the vector in only one direction?*

c) *After cloning, you transform and plate bacterial cells using your cloned plasmid. Onto what type of growth medium will you plate your cells in order to distinguish between bacterial cells that obtained the plasmid and those that did not?*

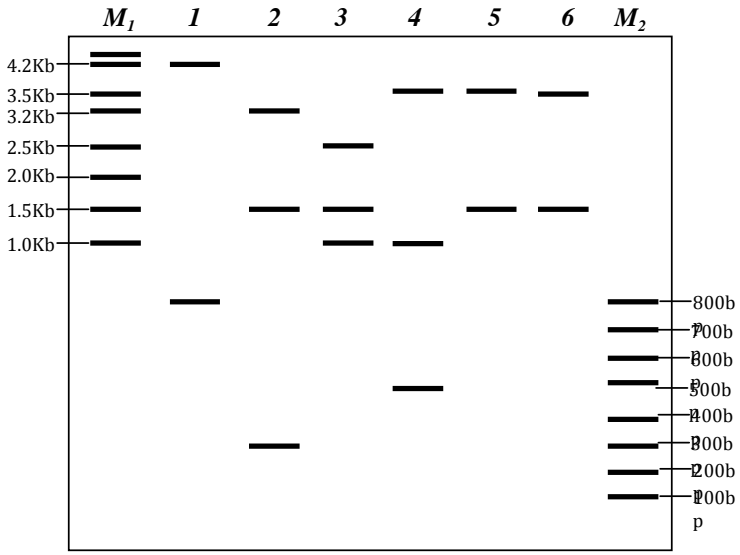
Sample question adapted from MIT OpenCourseware: 7-01sc-fundamentals-of-biology-fall-2011

Question 4

You are given a plasmid. In order to map this plasmid you set up a series of restriction digests and obtain the following results using agarose gel electrophoresis.

a) *What is the approximate size of the plasmid?*

b) *Add the SmaI, KpnI, BglII sites to plasmid map. On your map give the distances between each of the restriction sites.*



***M1 and M2 are DNA markers.**

Lane	*Digest*	*Size of fragments in bp*
1	Bam*HI* and Sma*I*	*4200, 800*
2	Sma*I* and Kpn*I*	*3200, 1500, 300*
3	Kpn*I* and Bgl*II*	*2500, 1500, 1000*
4	Bam*HI* and Kpn*I*	*3500, 1000, 500*
5	Kpn*I*	*3500, 1500*
6	Bgl*II* and Bam*HI*	*3500, 1500*

Sample question taken from MIT OpenCourseware: 7-01sc-fundamentals-of-biology-fall-2011