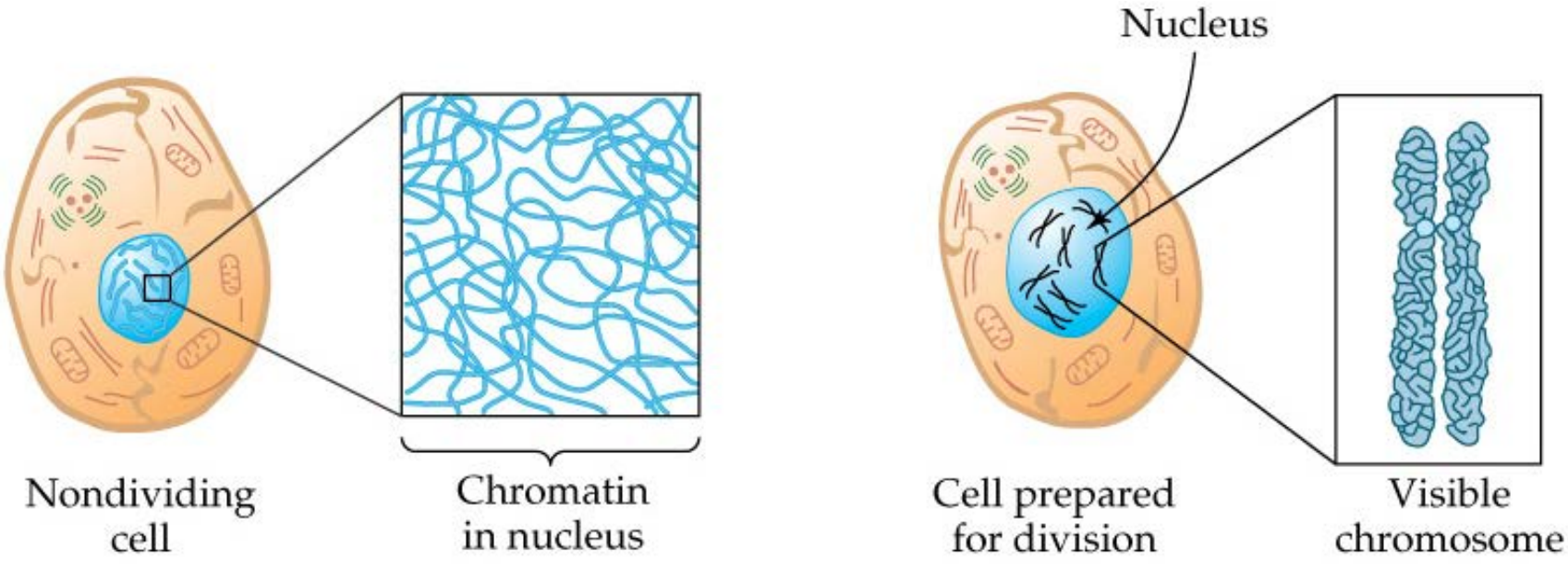
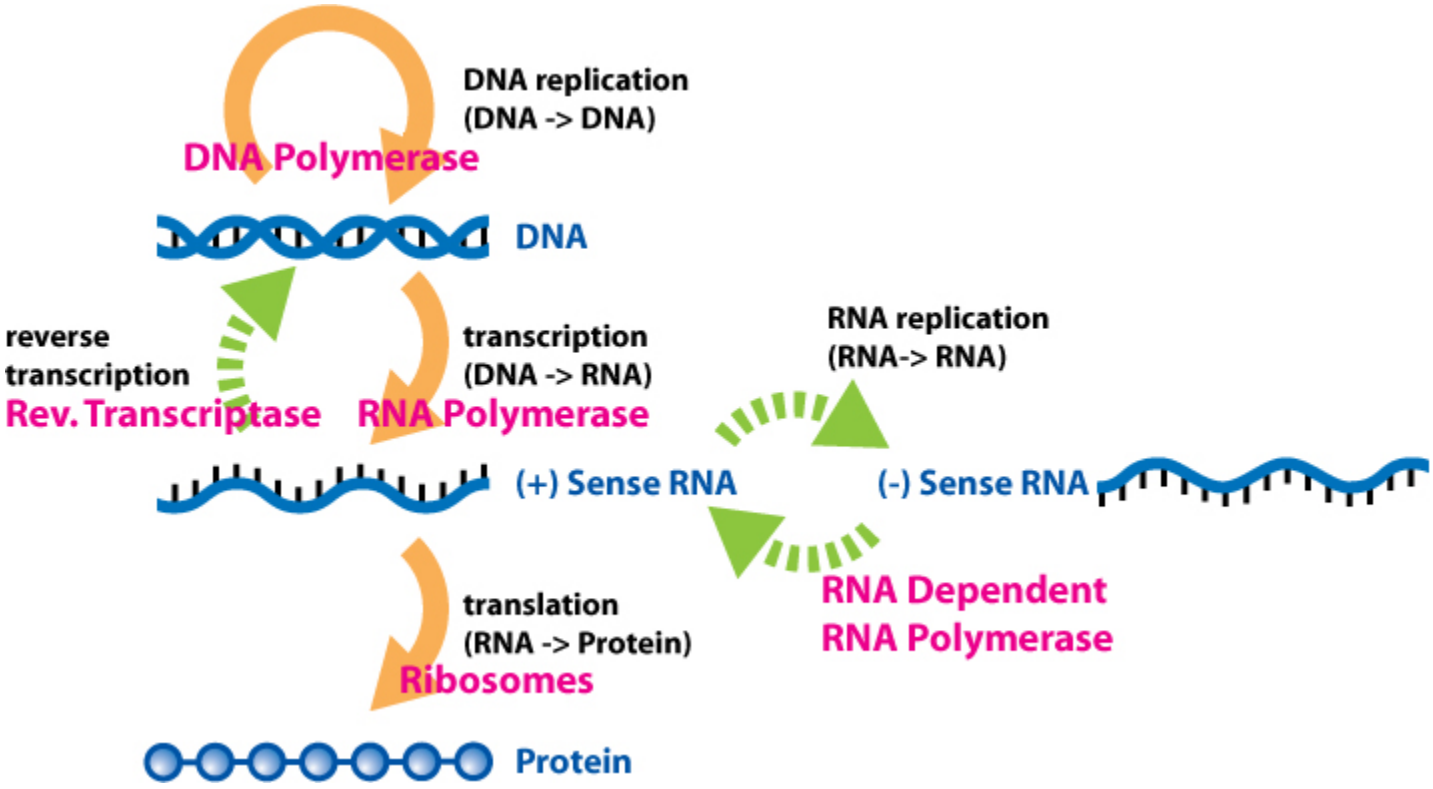
DNA


Nucleus
Nondividing cell
Chromatin in nucleus
Cell prepared for division
Visible chromosome

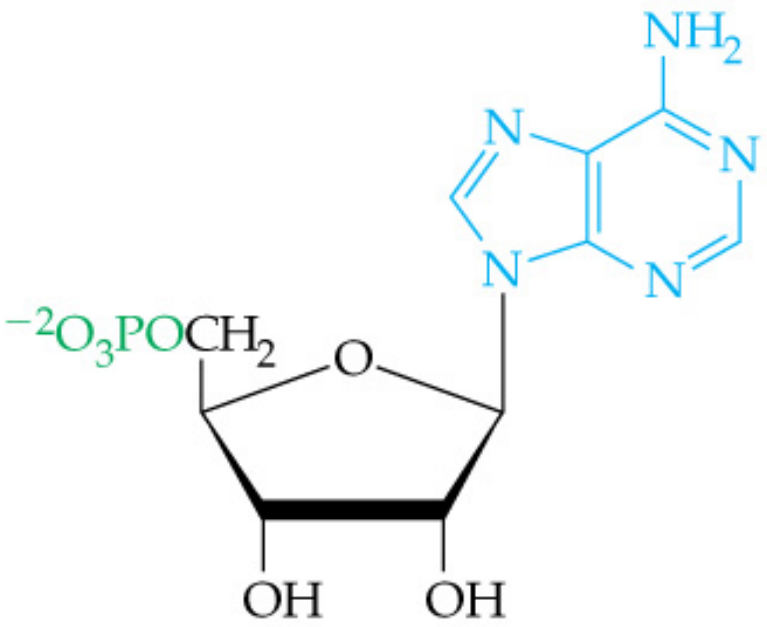
Central Dogma



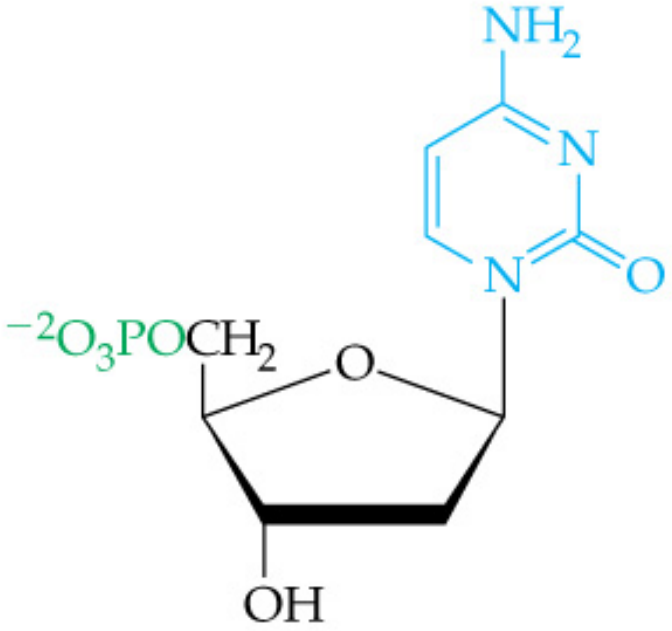
From DNA to Protein

https://www.youtube.com/watch?v=gG7uCskUOrA

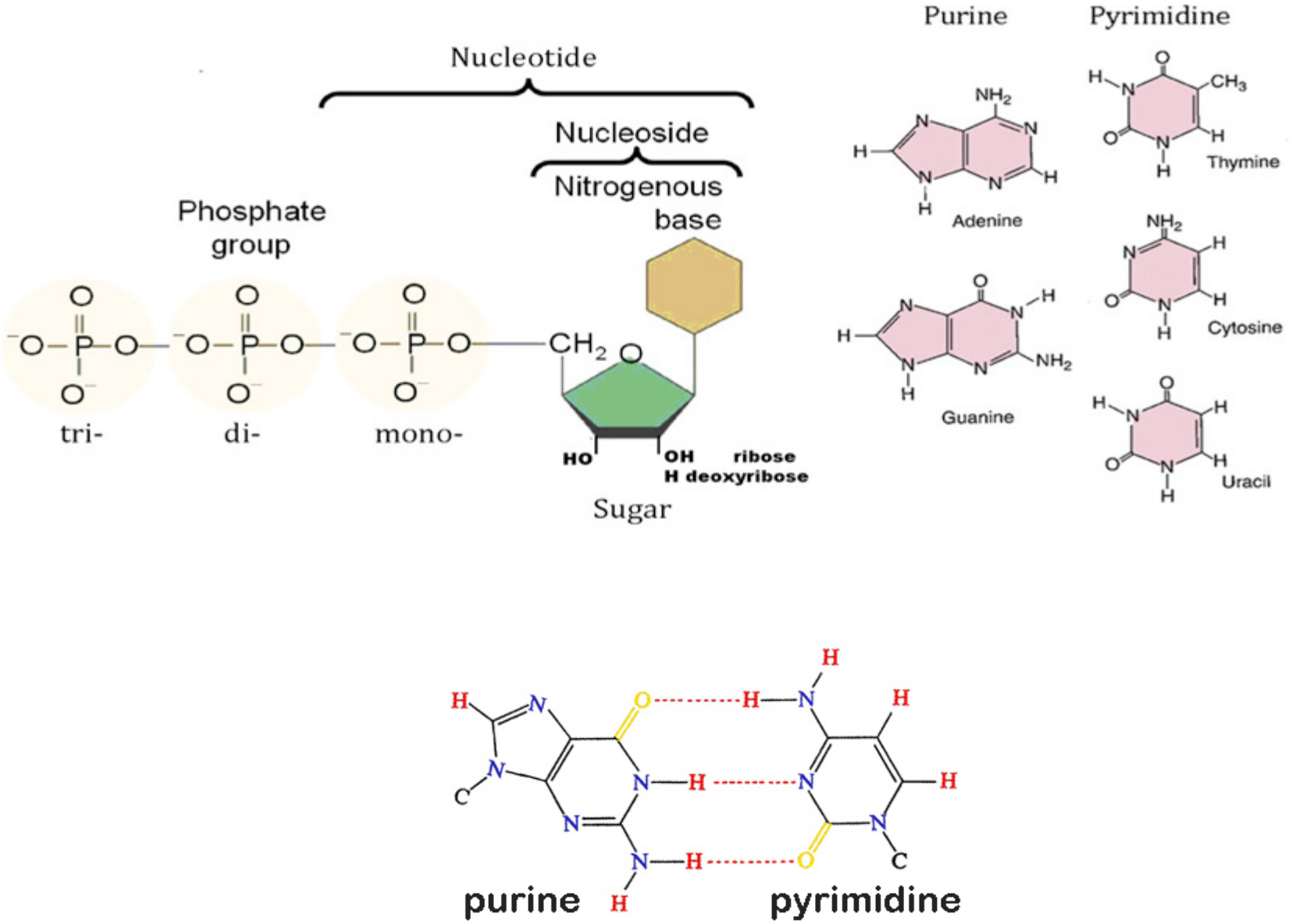
- In RNA, the sugar is ribose.
- In DNA, the sugar is deoxyribose.



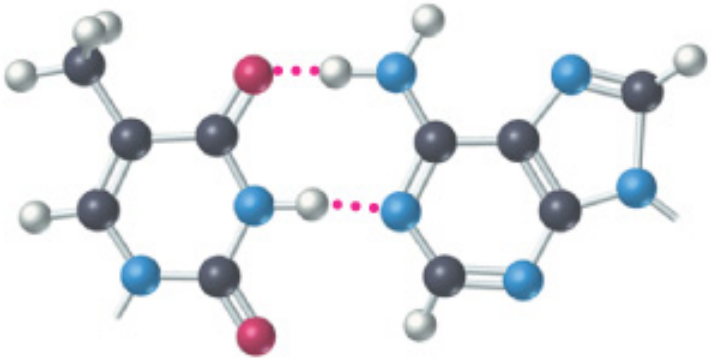
Adenosine 5′-monophosphate (AMP)
(a ribonucleotide)

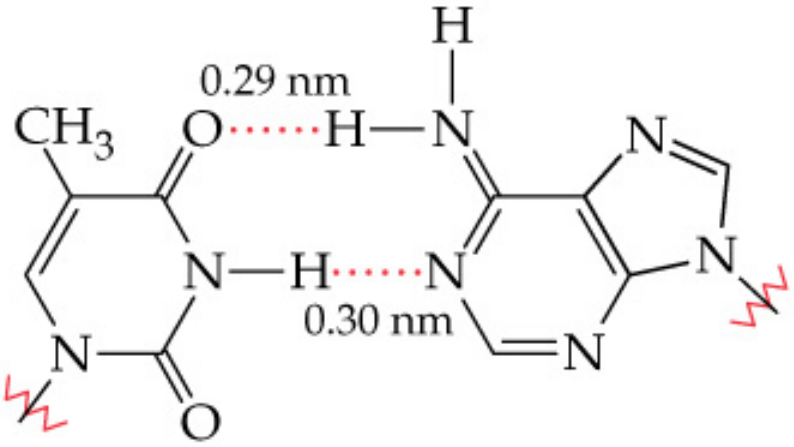


Deoxycytidine 5′-monophosphate (dCMP)
(a deoxyribonucleotide)

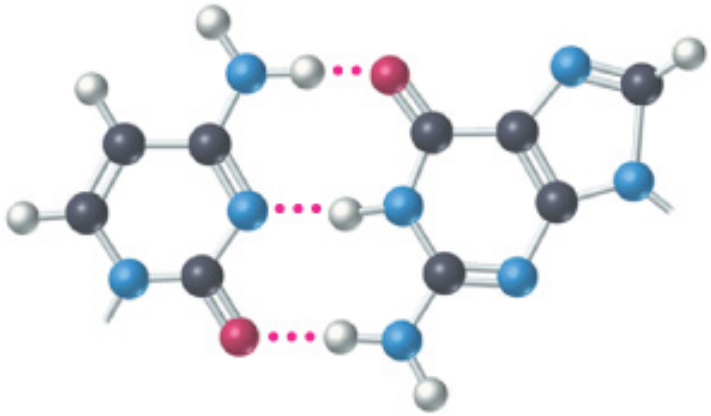

Purine
Pyrimidine
Nucleotide
Nucleoside
Nitrogenous base
Phosphate group
tri-
di-
mono-
HO
OH
H
ribose
deoxyribose
Sugar
Adenine
Thymine
Cytosine
Guanine
Uracil
purine
pyrimidine

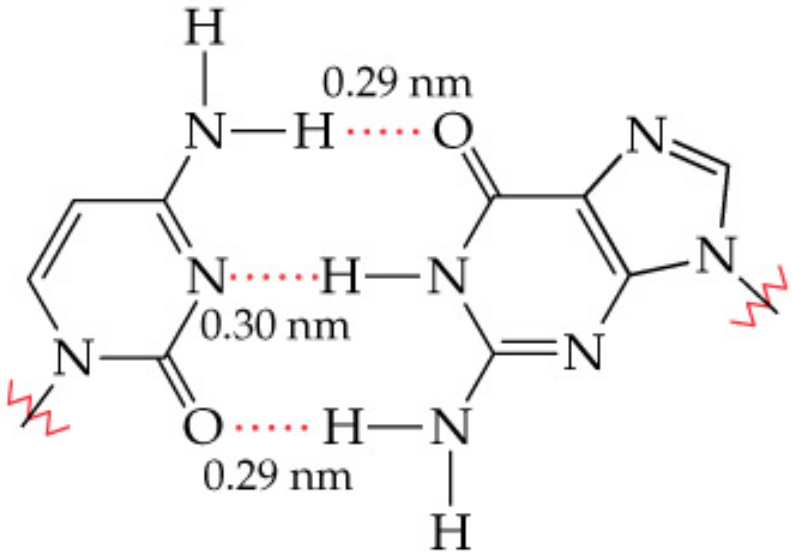
Thymine–Adenine

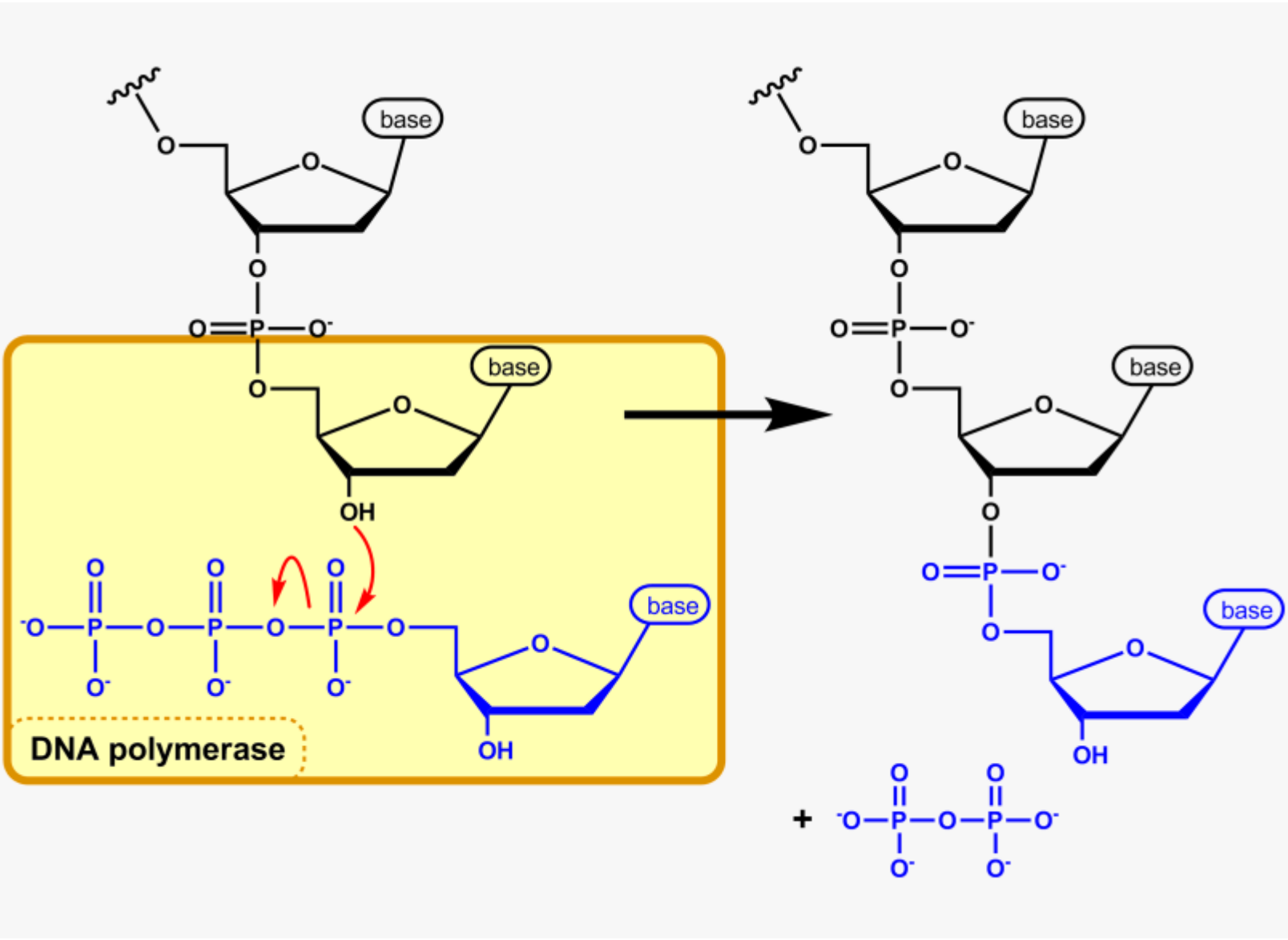


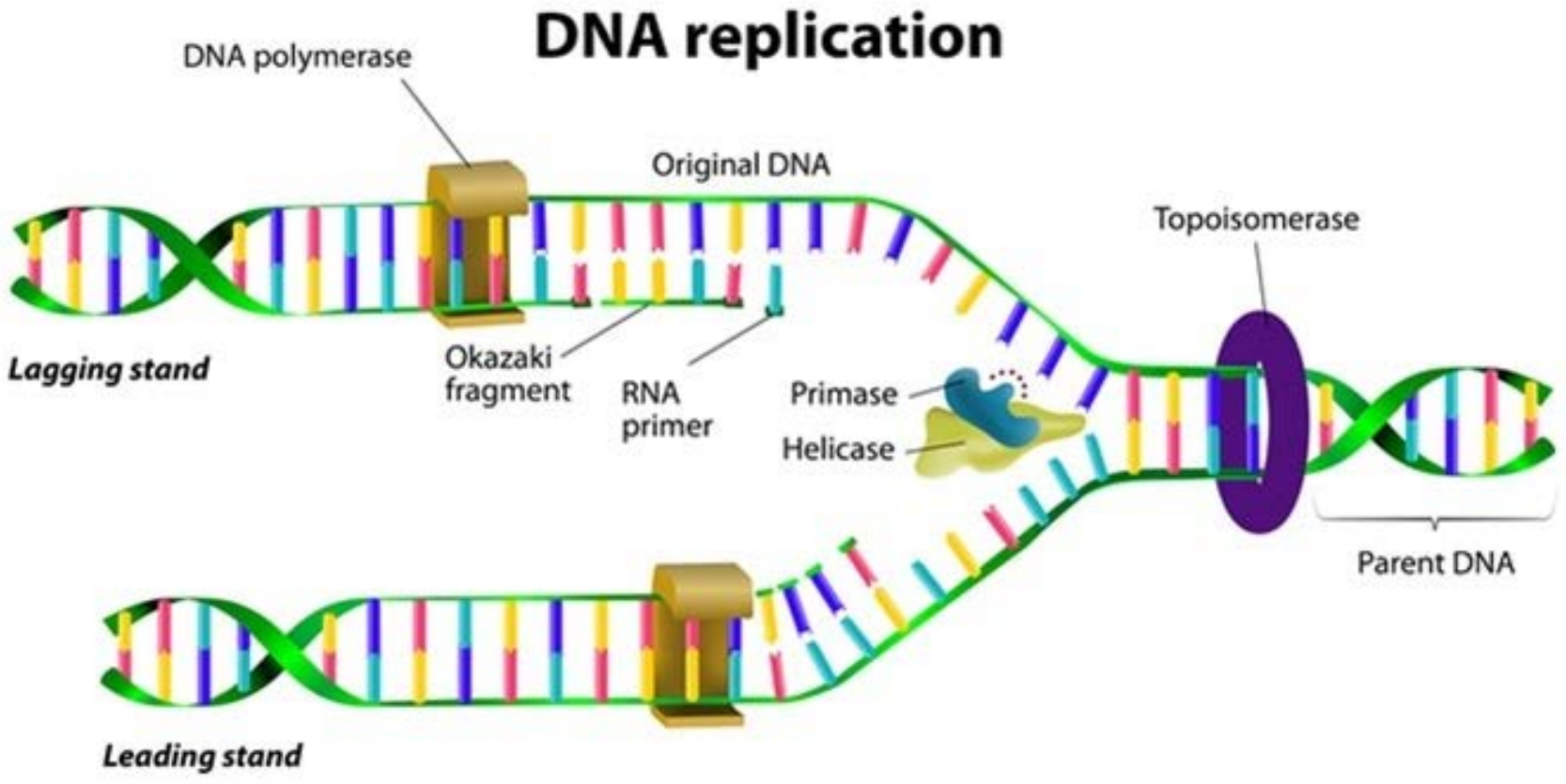


Cytosine–Guanine

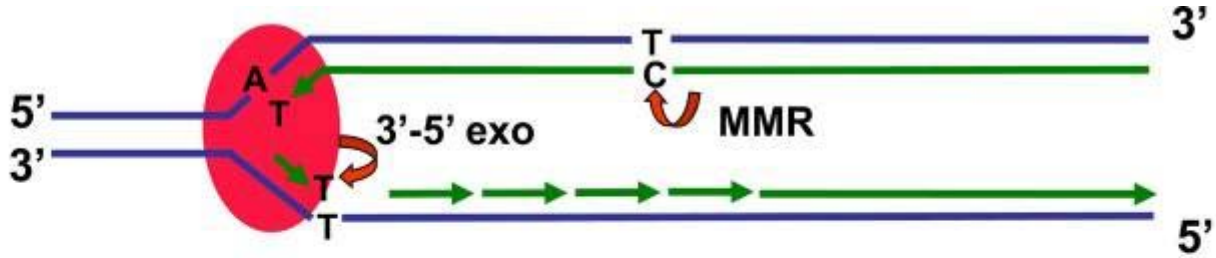





base
base
OH
base
DNA polymerase
OH
base
base
OH
+



https://youtu.be/TNKWgcFPHqw

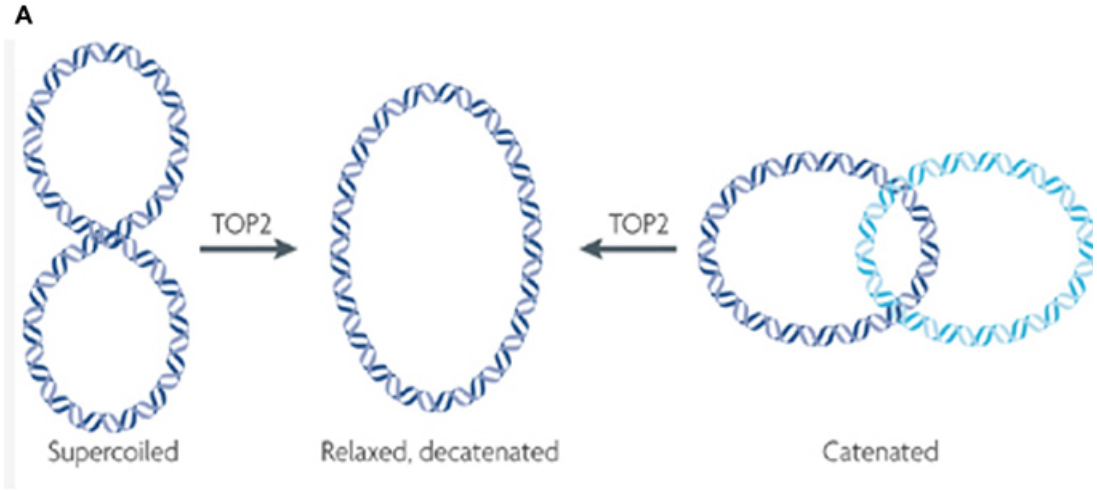


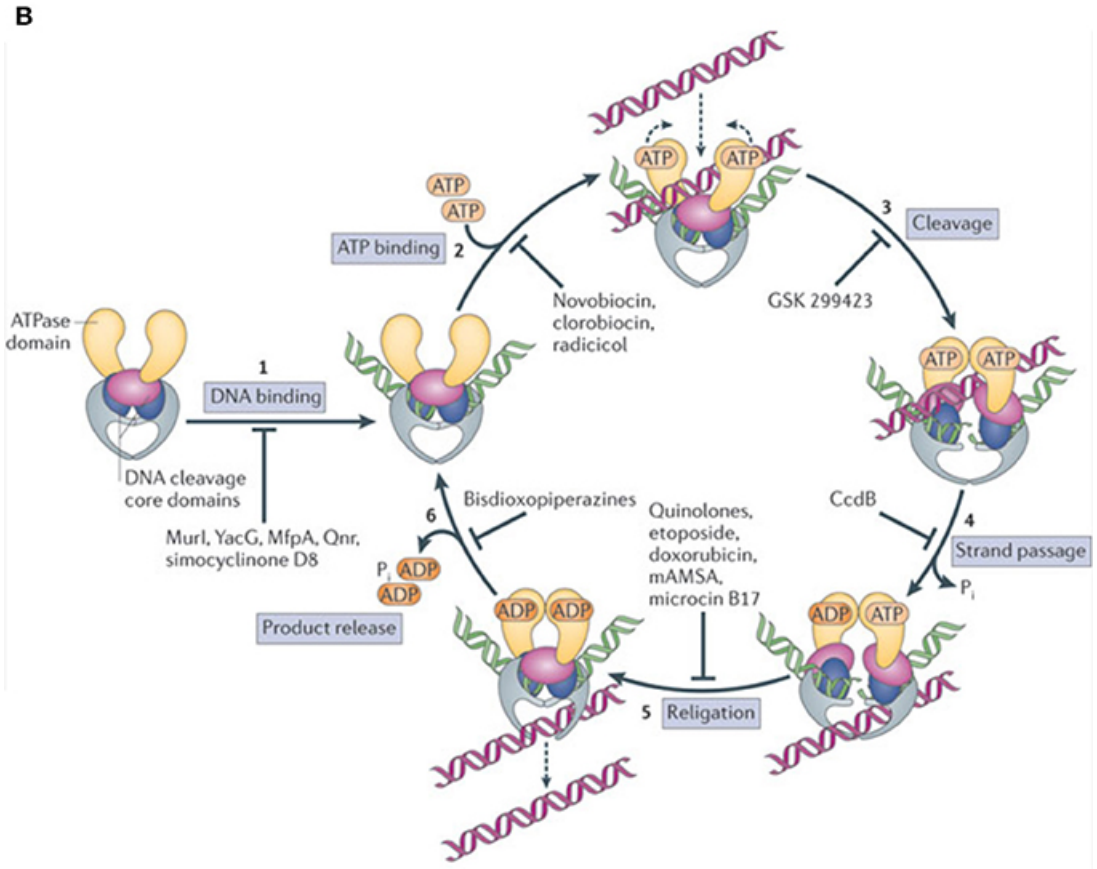
BASE SELECTION $10^{-4} – 10^{-5}$

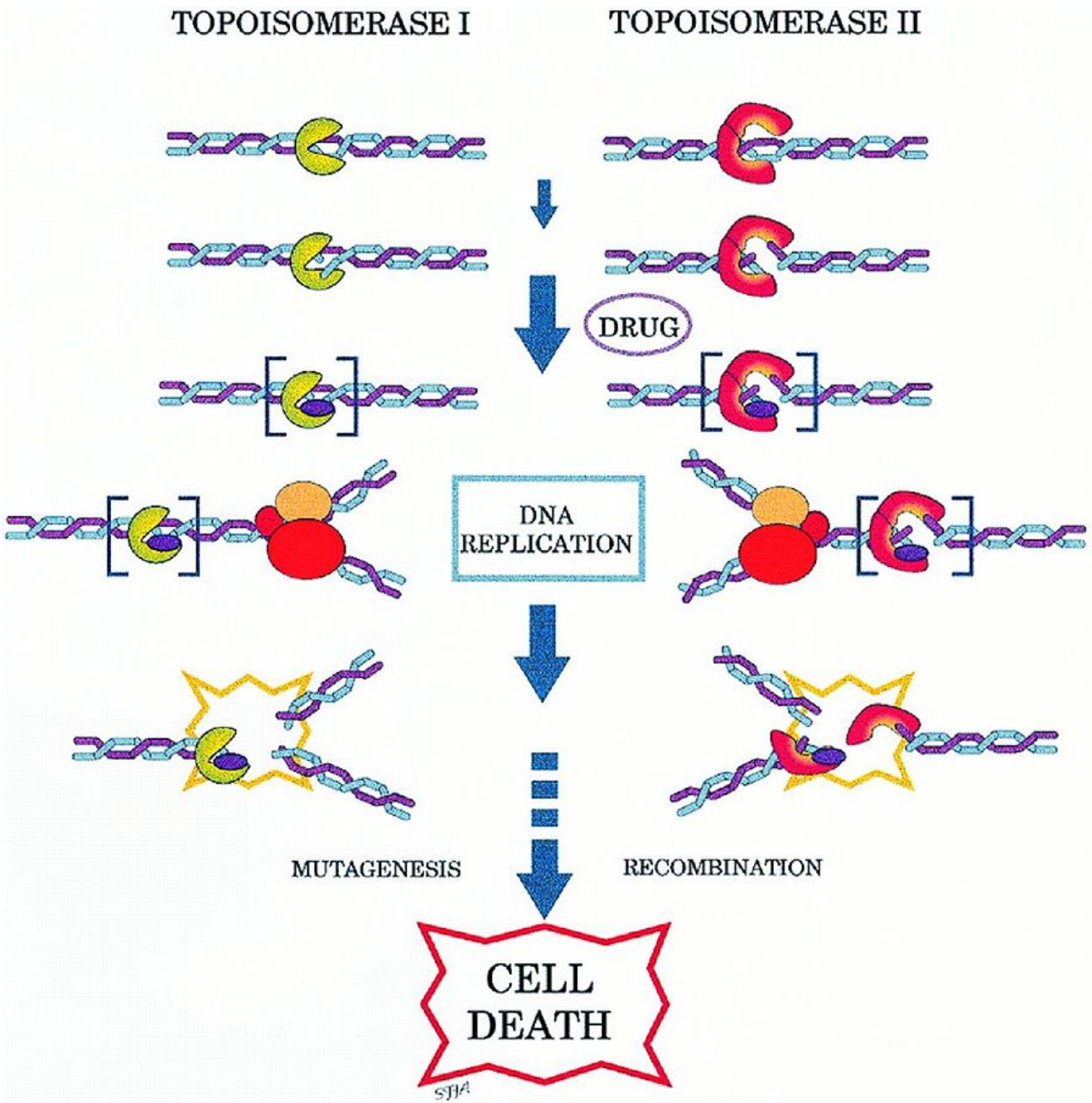
3'-5' EXONUCLEASE 10^{-2}

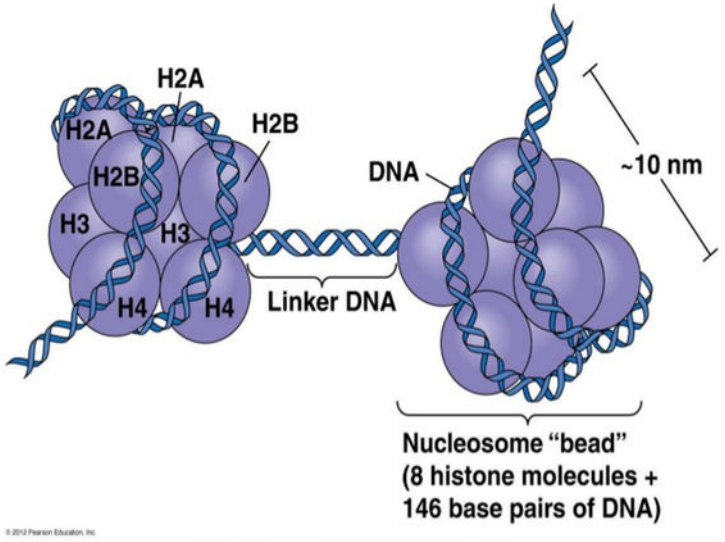
MISMATCH REPAIR 10^{-3}

DNA REPLICATION FIDELITY 10^{-10}

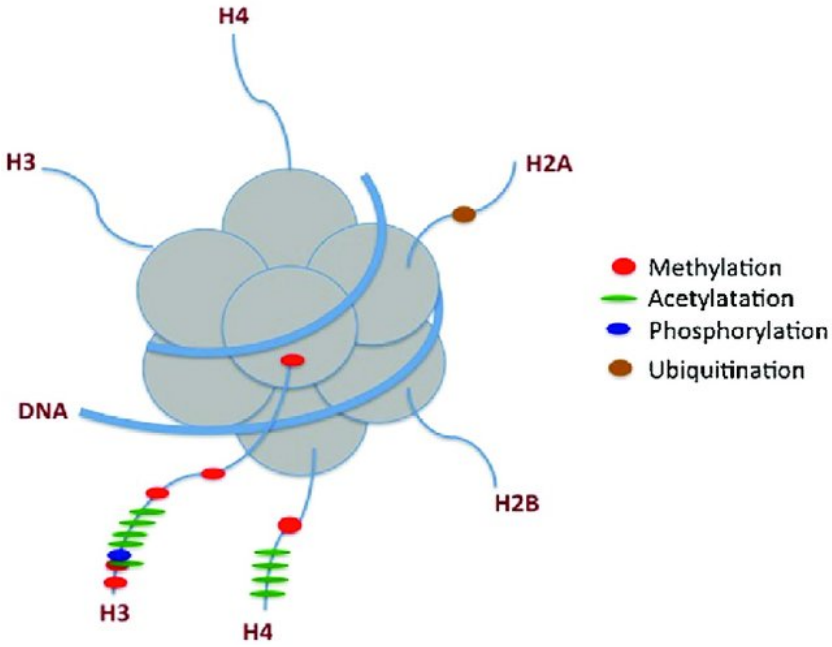

A
TOP2
TOP2
Supercoiled
Relaxed, decatenated
Catenated

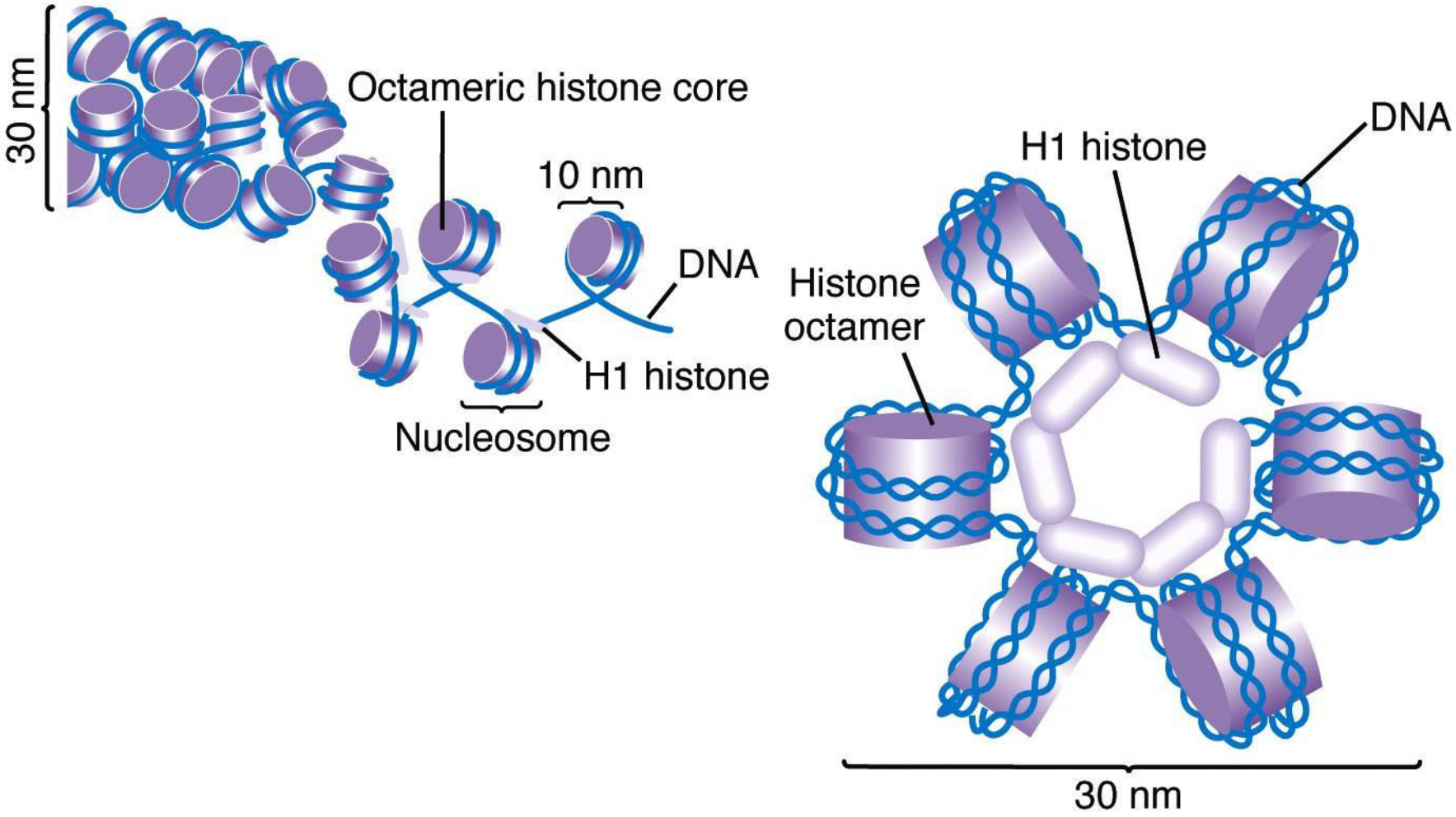

B
ATPase domain
DNA cleavage core domains
1
DNA binding
MurI, YacG, MfpA, Qnr, simocyclinone D8
ATP
ATP
ATP binding
2
Novobiocin, clorobiocin, radicicol
3
Cleavage
GSK 299423
4
Strand passage
CcdB
Pi
Quinolones, etoposide, doxorubicin, mAMSA, microcin B17
5
Religation
6
Bisdioxopiperazines
Pi
ADP
ADP
Product release

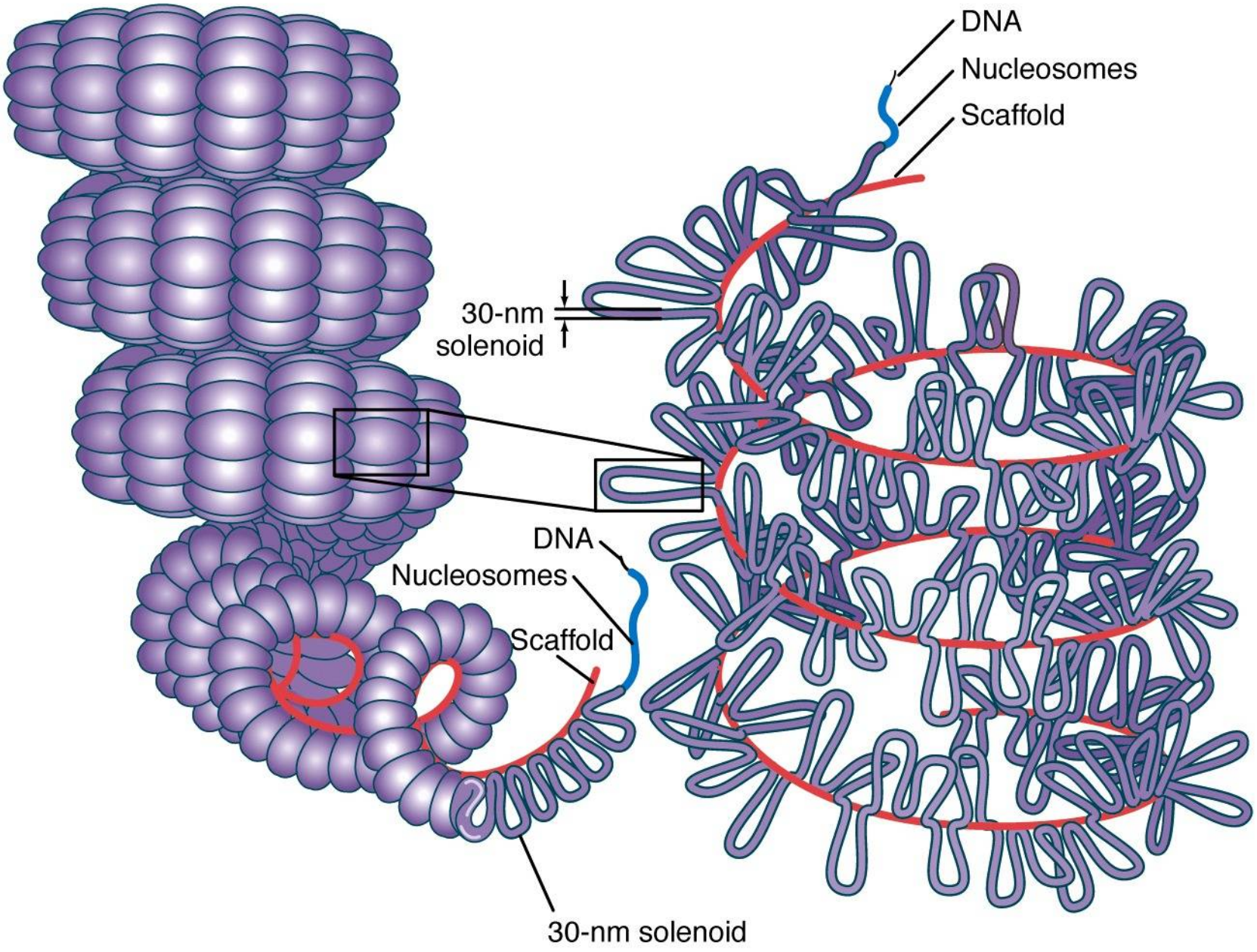

TOPOISOMERASE I
TOPOISOMERASE II
DRUG
DNA
REPLICATION
MUTAGENESIS
RECOMBINATION
CELL
DEATH
SJA

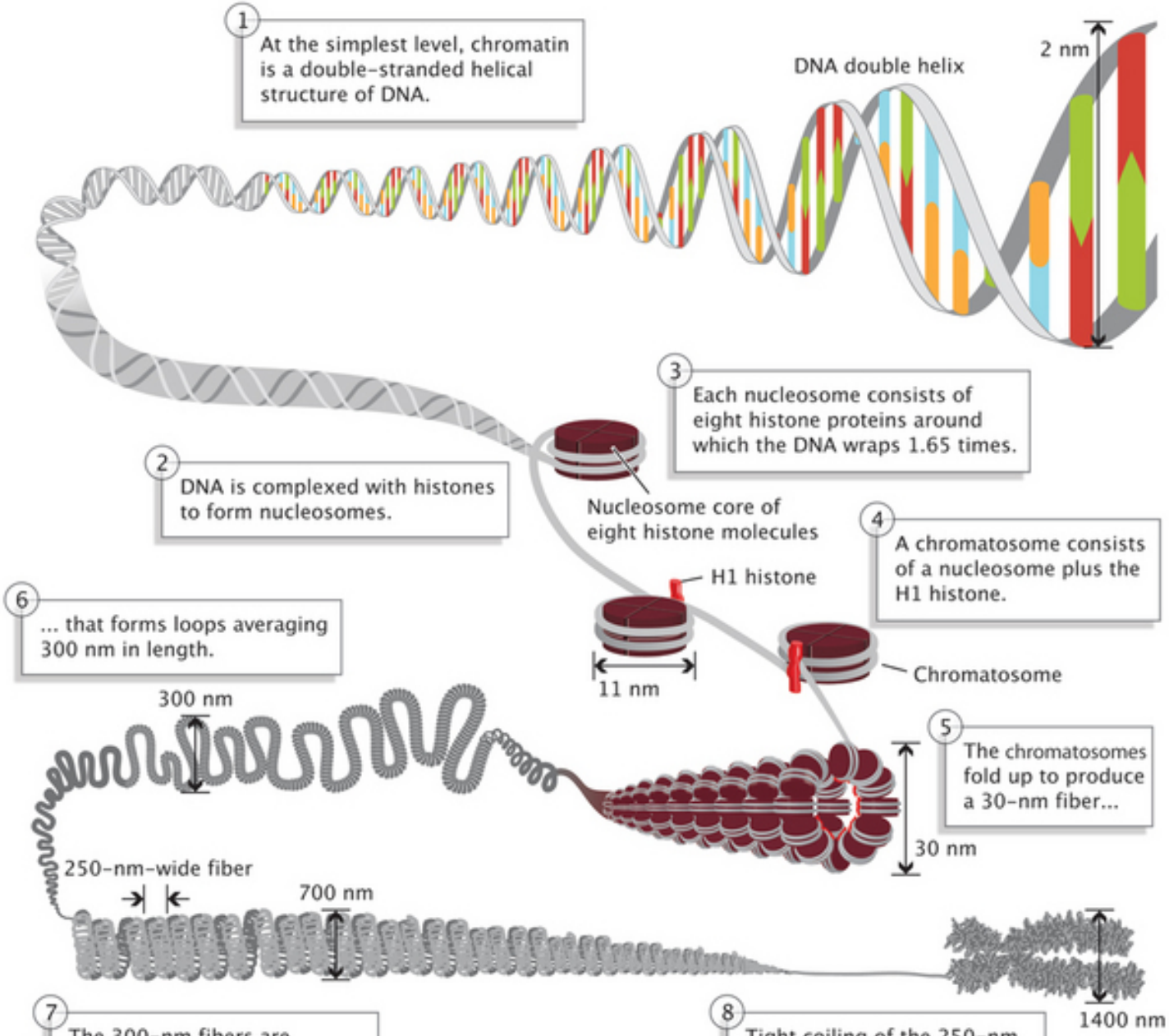


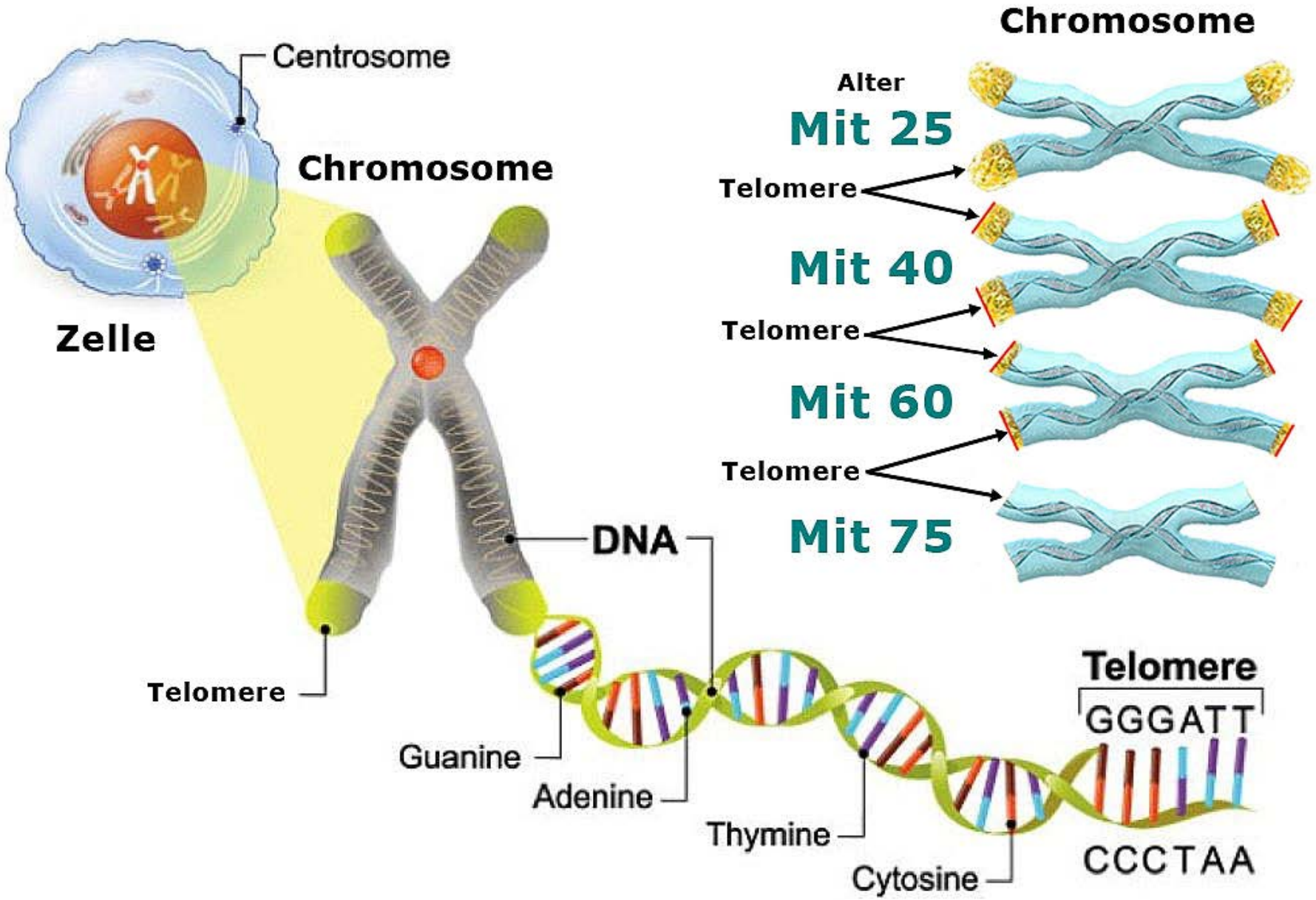
https://www.slideshare.net/jannatiftikhar/role-of-histone-in-dna-packaging

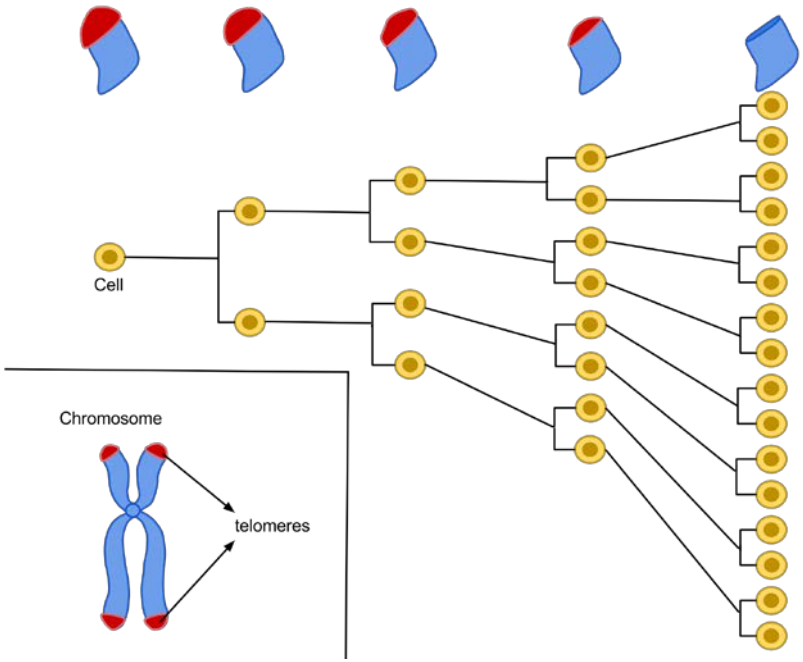


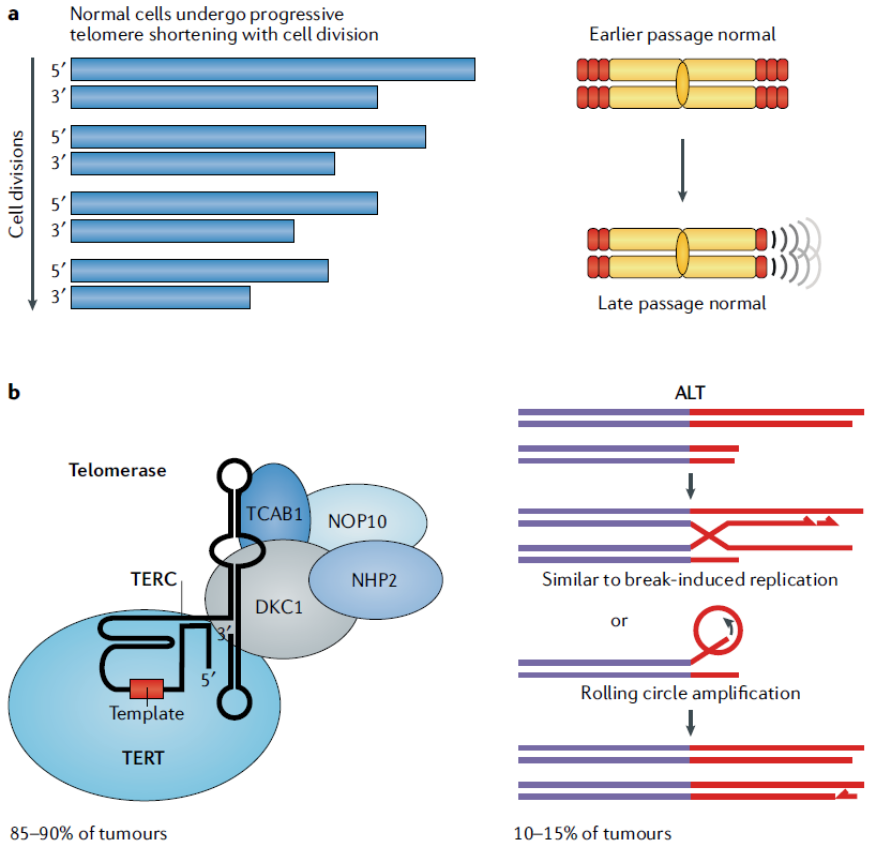

30 nm
Octameric histone core
10 nm
DNA
H1 histone
Nucleosome
DNA
H1 histone
Histone
octamer
30 nm

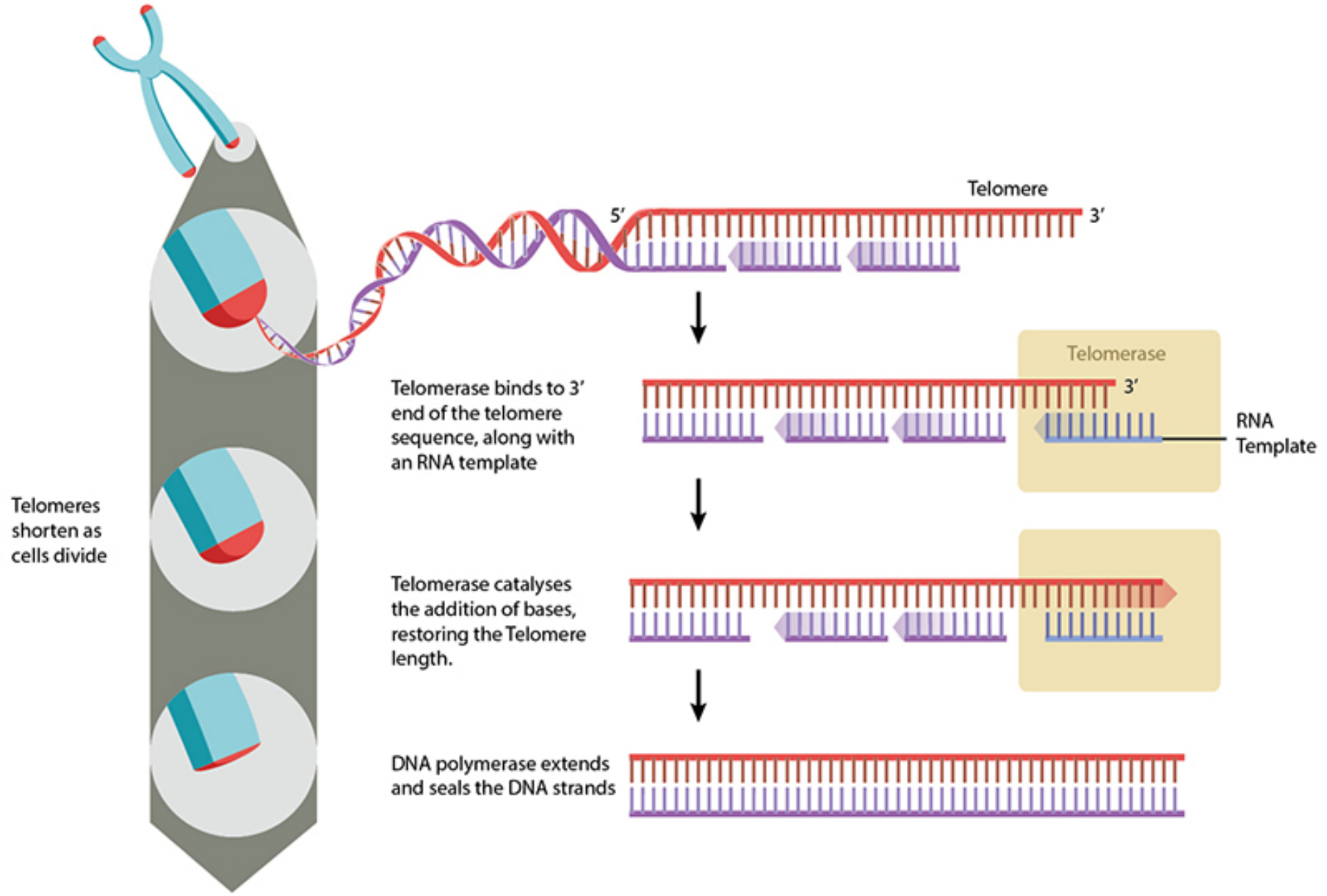

DNA
Nucleosomes
Scaffold
30-nm
solenoid
DNA
Nucleosomes
Scaffold
30-nm solenoid


1
At the simplest level, chromatin is a double-stranded helical structure of DNA.
DNA double helix
2 nm
2
DNA is complexed with histones to form nucleosomes.
3
Each nucleosome consists of eight histone proteins around which the DNA wraps 1.65 times.
Nucleosome core of eight histone molecules
4
A chromatosome consists of a nucleosome plus the H1 histone.
H1 histone
11 nm
Chromatosome
6
... that forms loops averaging 300 nm in length.
300 nm
5
The chromatosomes fold up to produce a 30-nm fiber...
30 nm
250-nm-wide fiber
700 nm
1400 nm
7
The 300-nm fibers are compressed and folded to produce a 250-nm-wide fiber.
8
Tight coiling of the 250-nm fiber produces the chromatid of a chromosome.


Chromosome
Alter
Mit 25
Telomere
Mit 40
Telomere
Mit 60
Telomere
Mit 75
Centrosome
Chromosome
Zelle
DNA
Telomere
Guanine
Adenine
Thymine
Cytosine
Telomere
GGGATT
CCCTAA

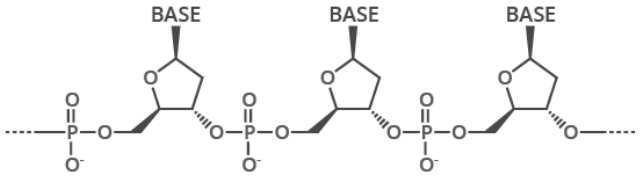

Cell
Chromosome
telomeres


a
Normal cells undergo progressive telomere shortening with cell division
Cell divisions
5′
3′
Earlier passage normal
Late passage normal
b
Telomerase
TCAB1
NOP10
NHP2
DKC1
TERC
3′
5′
Template
TERT
85–90% of tumours
ALT
Similar to break-induced replication
or
Rolling circle amplification
10–15% of tumours
c
T-loop
POT1
ACD
TIN2
TRF1
TRF2
RAP1
5′
(TTAGGG)n
3′
(AATCCC)n


Telomeres shorten as cells divide
Telomere
5′
3′
Telomerase binds to 3′ end of the telomere sequence, along with an RNA template
Telomerase
3′
RNA Template
Telomerase catalyses the addition of bases, restoring the Telomere length.
DNA polymerase extends and seals the DNA strands

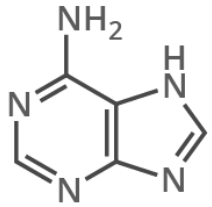
THE CHEMICAL STRUCTURE OF DNA

THE SUGAR PHOSPHATE 'BACKBONE'

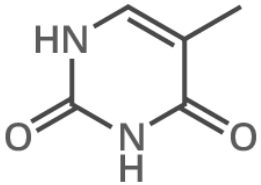


DNA is a polymer made up of units called nucleotides. The nucleotides are made of three different components: a sugar group, a phosphate group, and a base. There are four different bases: adenine, thymine, guanine and cytosine.

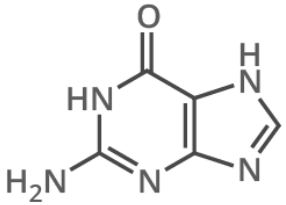
A ADENINE



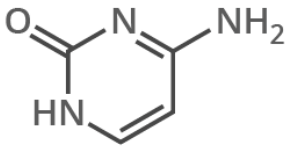
T THYMINE



G GUANINE

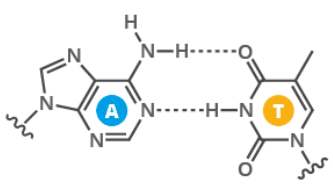


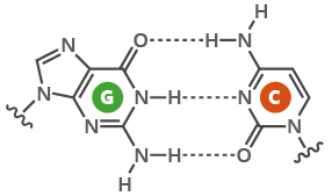
C CYTOSINE



WHAT HOLDS DNA STRANDS TOGETHER?

DNA strands are held together by hydrogen bonds between bases on adjacent strands. Adenine (A) always pairs with thymine (T), while guanine (G) always pairs with cytosine (C). Adenine pairs with uracil (U) in RNA.





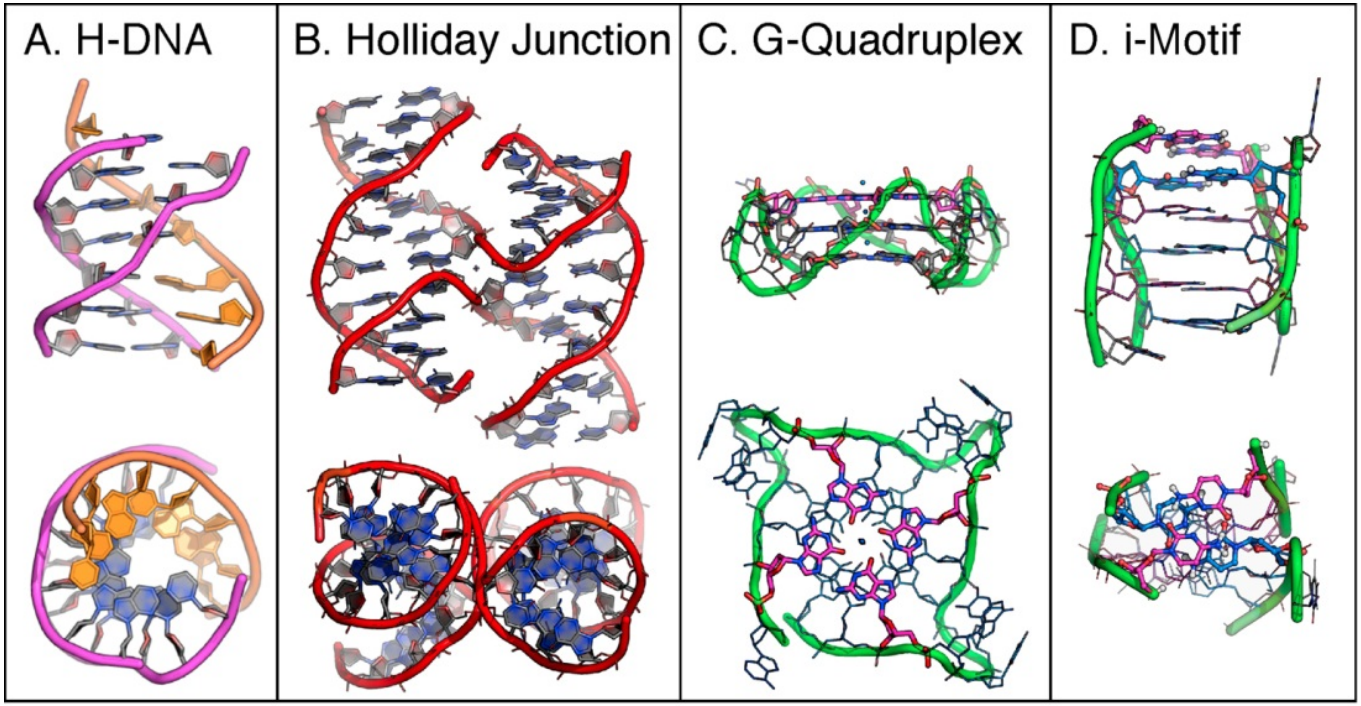
FROM DNA TO PROTEINS

The bases on a single strand of DNA act as a code. The letters form three letter codons, which code for amino acids - the building blocks of proteins.

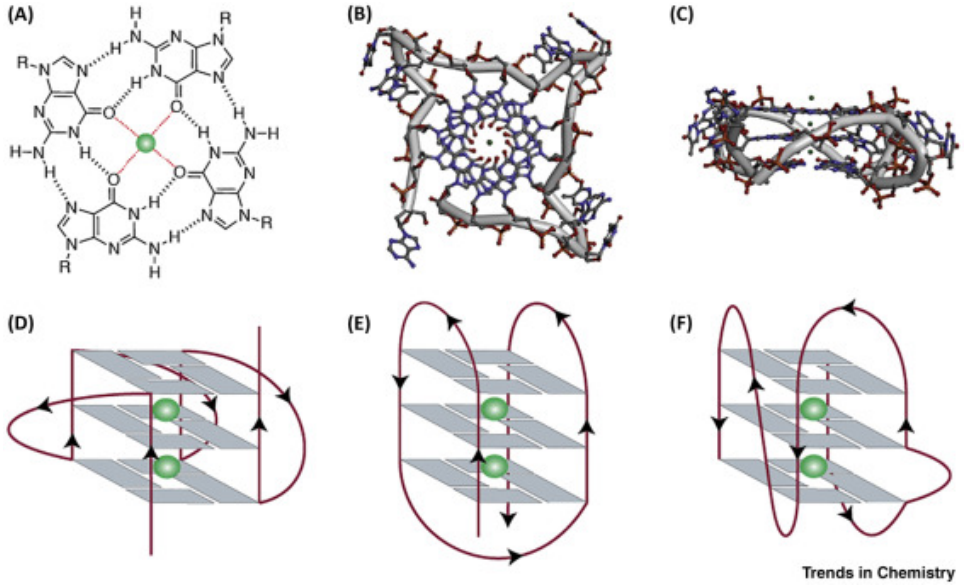
An enzyme, RNA polymerase, transcribes DNA into mRNA (messenger ribonucleic acid). It splits apart the two strands that form the double helix, then reads a strand and copies the sequence of nucleotides. The only difference between the RNA and the original DNA is that in the place of thymine (T), another base with a similar structure is used: uracil (U).

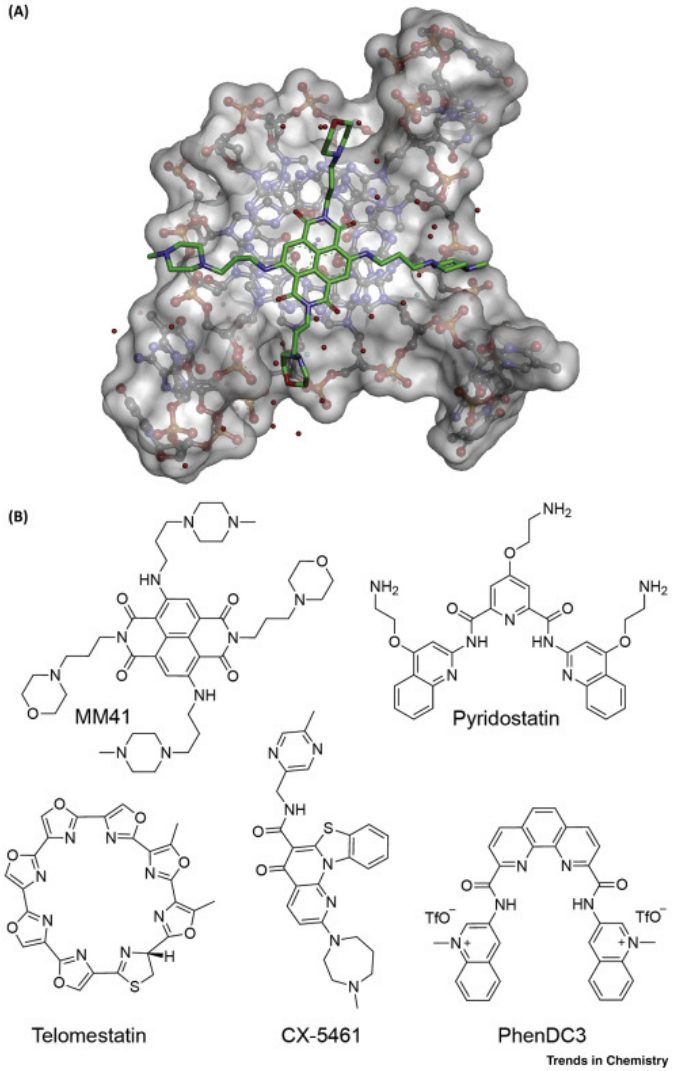
In multicellular organisms, the mRNA carries genetic code out of the cell nucleus, to the cytoplasm. Here, protein synthesis takes place. 'Translation' is the process of turning the mRNA's 'code' into proteins. Molecules called ribosomes carry out this process, building up proteins from the amino acids coded for.

Triple and Quadruple Strained DNA

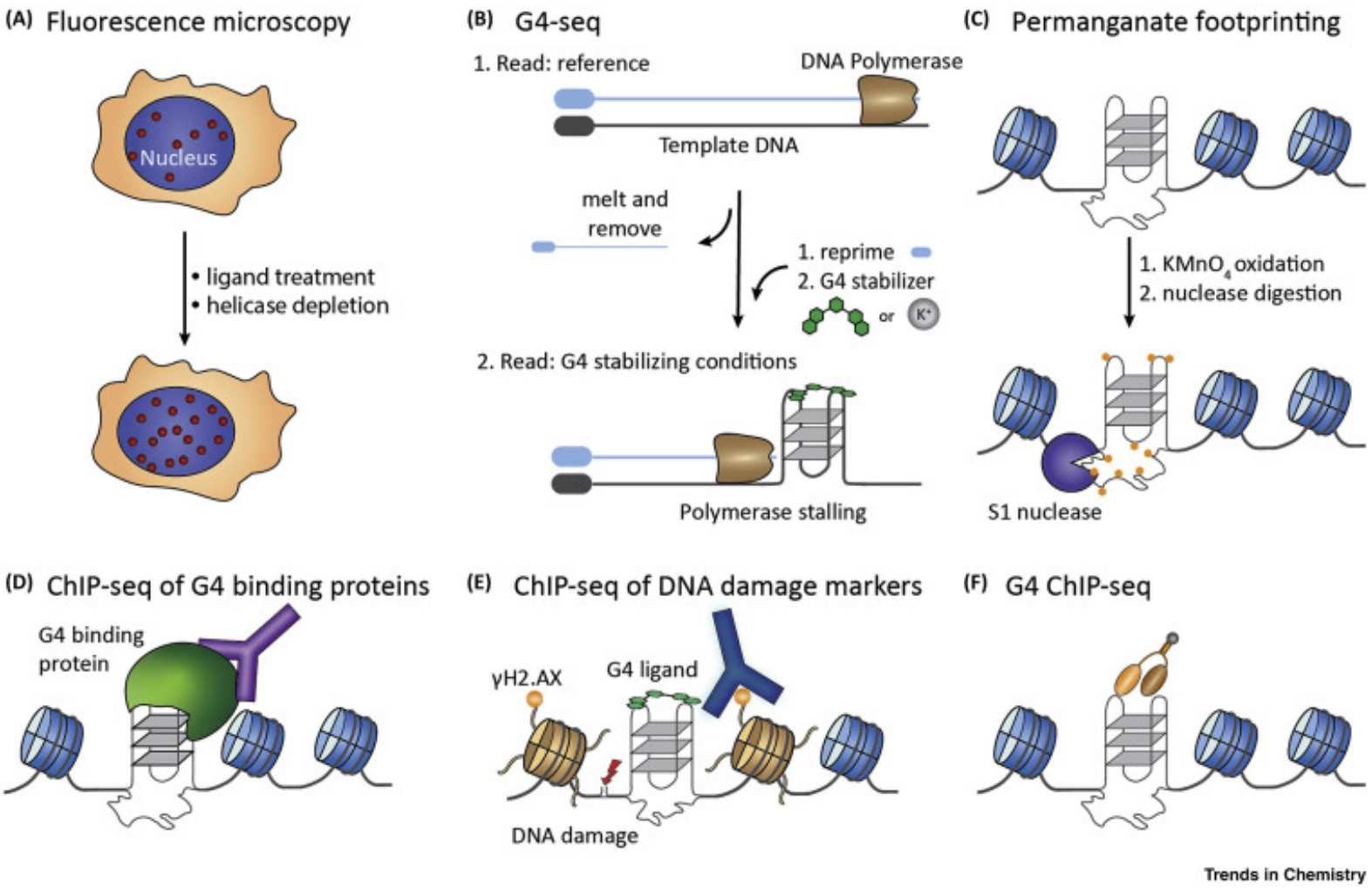


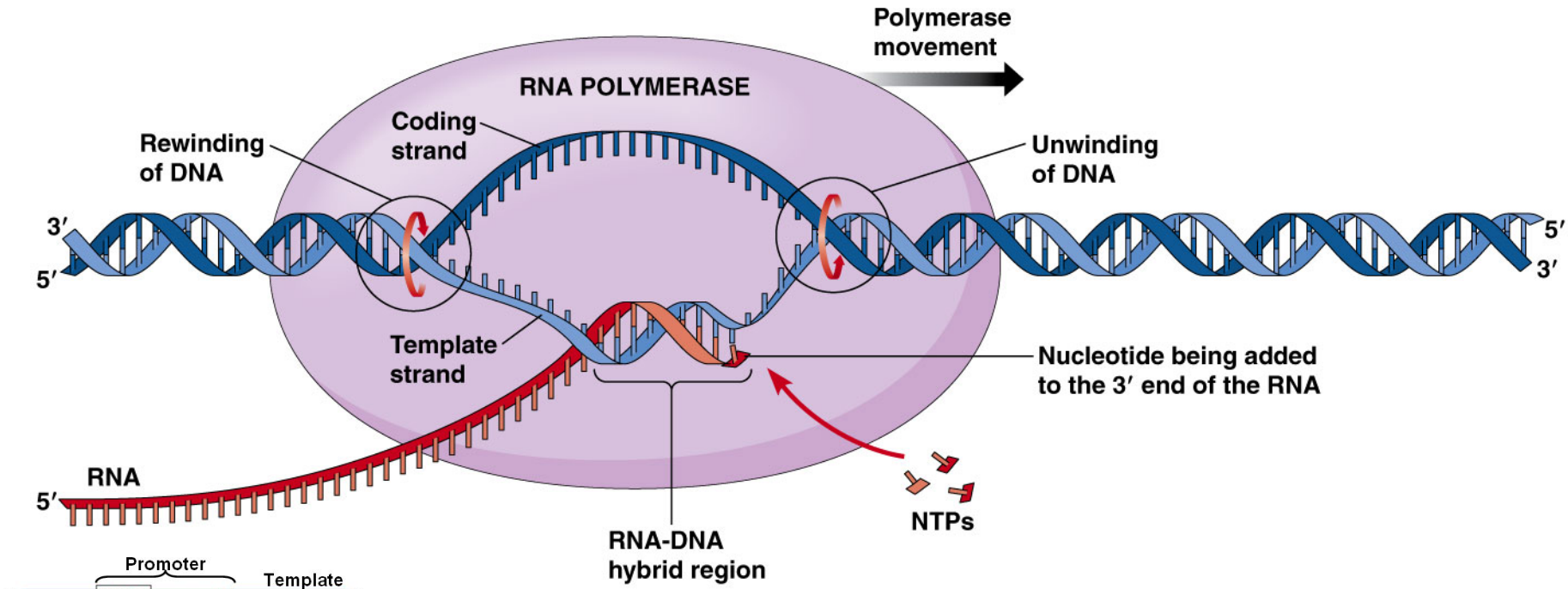
DNA G-quadruplex (G4)

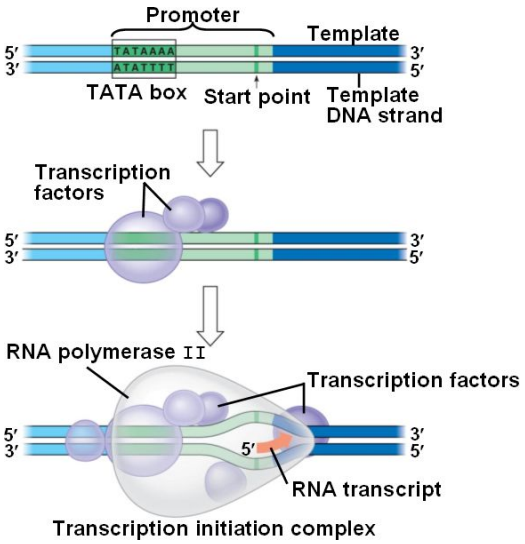


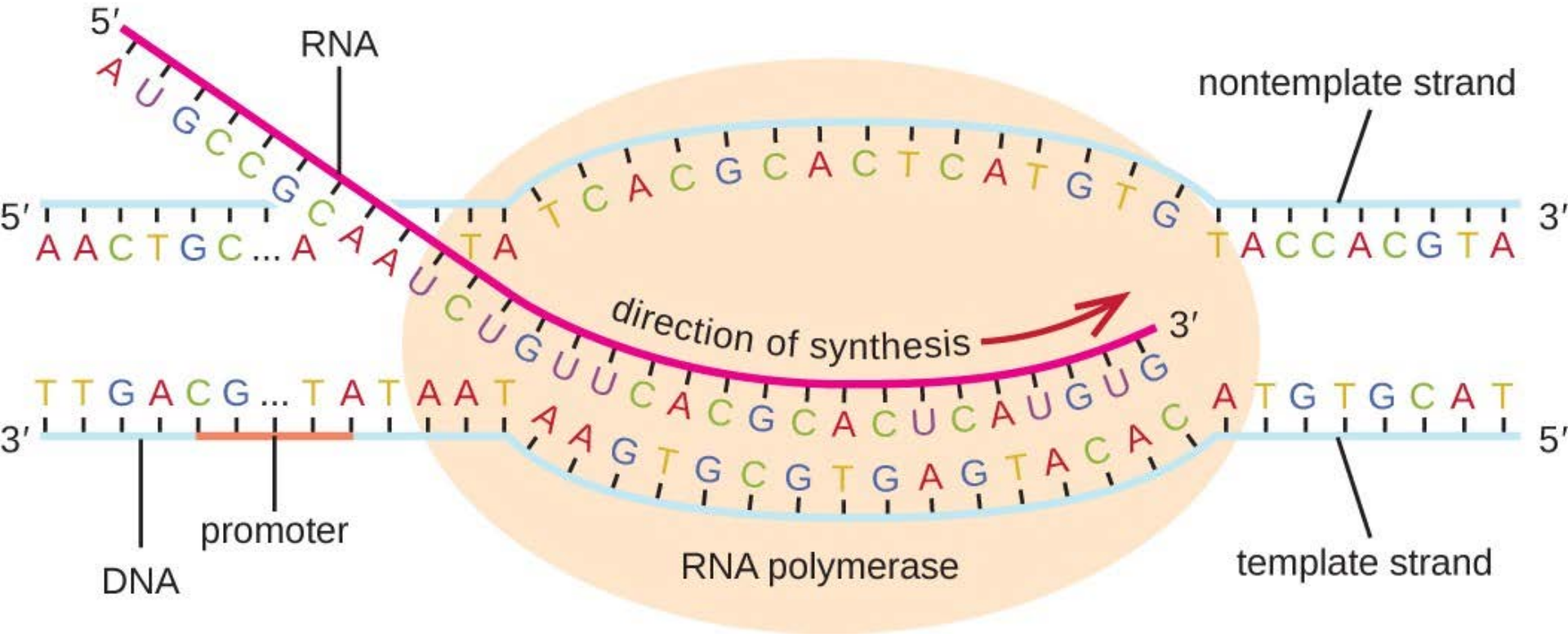


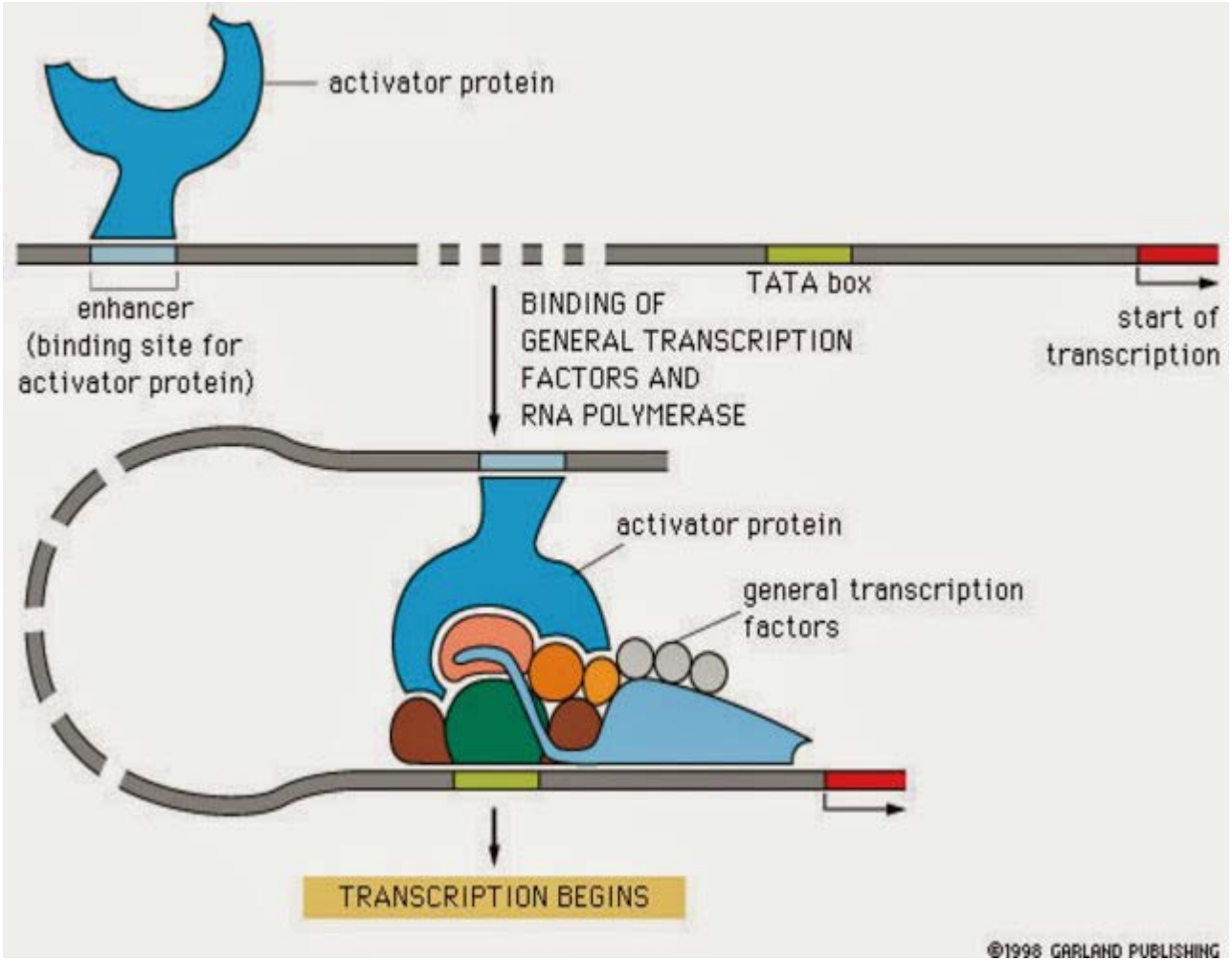
Trends in Chemistry, February 2020, Vol. 2, No. 2 P123

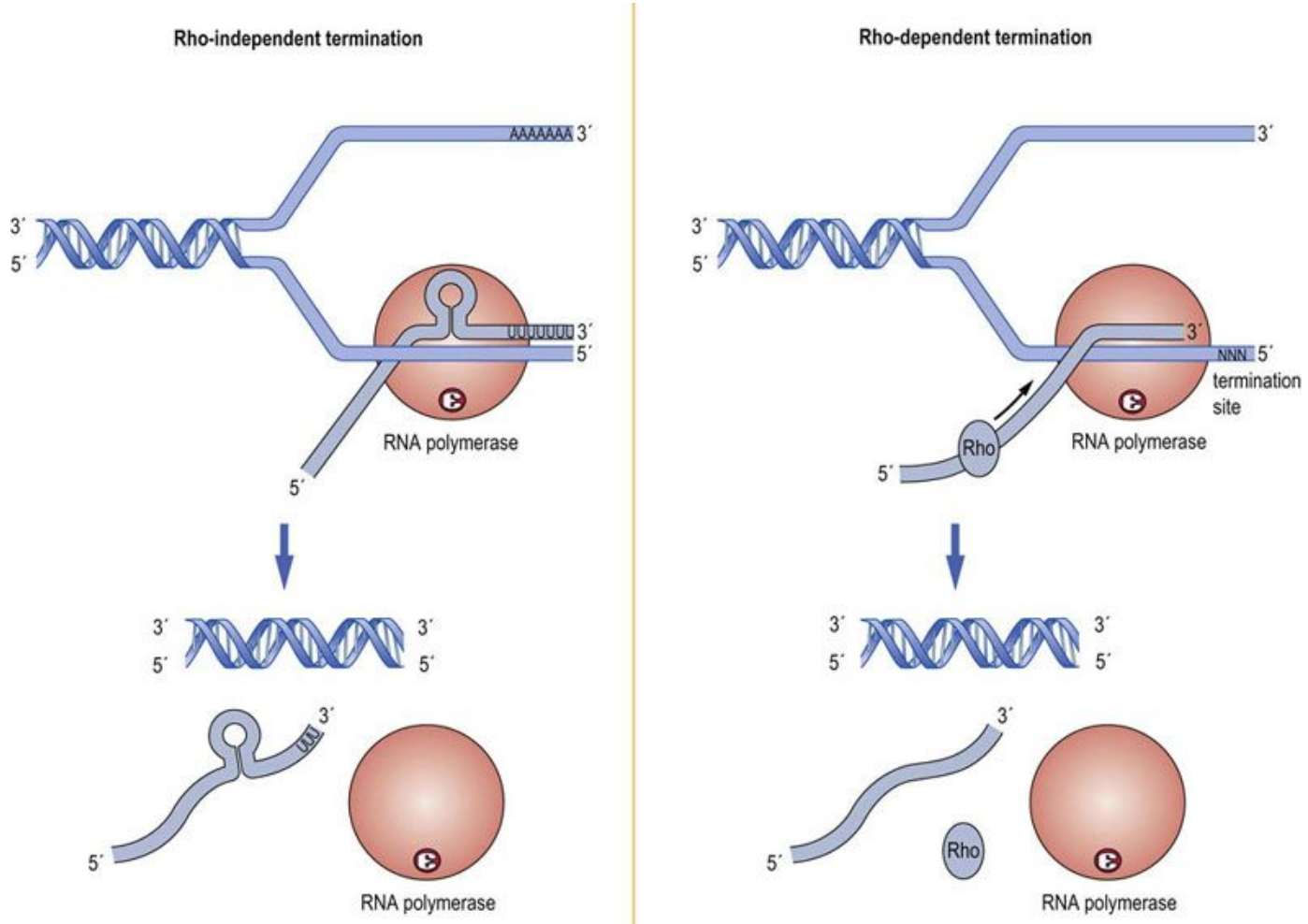

(A) Fluorescence microscopy
Nucleus
• ligand treatment
• helicase depletion
(B) G4-seq
1. Read: reference
DNA Polymerase
Template DNA
melt and remove
1. reprime
2. G4 stabilizer
or
K+
2. Read: G4 stabilizing conditions
Polymerase stalling
(C) Permanganate footprinting
1. KMnO4 oxidation
2. nuclease digestion
S1 nuclease
(D) ChIP-seq of G4 binding proteins
G4 binding protein
(E) ChIP-seq of DNA damage markers
γH2.AX
G4 ligand
DNA damage
(F) G4 ChIP-seq
Trends in Chemistry

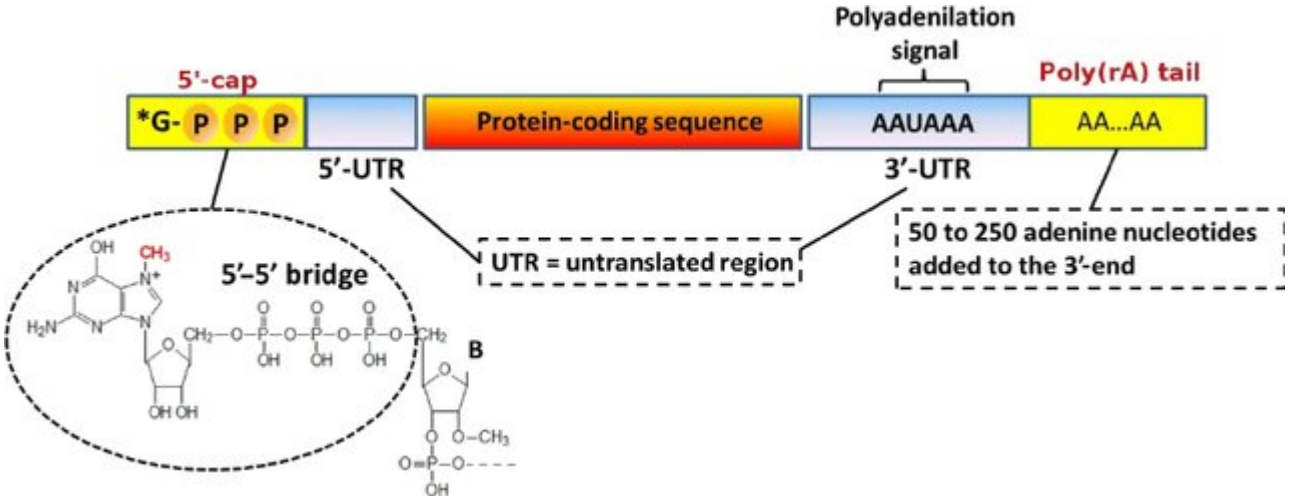

Polymerase movement
RNA POLYMERASE
Coding strand
Rewinding of DNA
Unwinding of DNA
3′
5′
5′
3′
Template strand
Nucleotide being added to the 3′ end of the RNA
RNA
5′
NTPs
RNA-DNA hybrid region

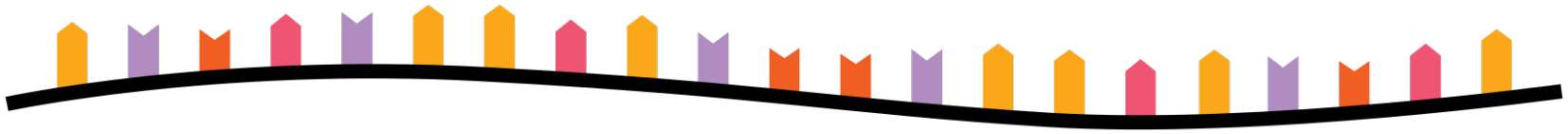

Promoter
Template
5′
3′
TATAAAA
ATATTTT
3′
5′
TATA box
Start point
Template DNA strand
Transcription factors
RNA polymerase II
Transcription factors
RNA transcript
Transcription initiation complex


5′
RNA
A U G C C G C A A U C U G U U U C A C G C A C U C A U G U G C
nontemplate strand
5′
A A C T G C ... A T A
T C A C G C A C T C A T G T G
T A C C A C G T A
3′
direction of synthesis
3′
T T G A C G ... T A T A A T
A A G T G C G T G A G T A C A C
A T G T G C A T
3′
5′
promoter
DNA
RNA polymerase
template strand


activator protein
enhancer
(binding site for
activator protein)
TATA box
start of
transcription
BINDING OF
GENERAL TRANSCRIPTION
FACTORS AND
RNA POLYMERASE
activator protein
general transcription
factors
TRANSCRIPTION BEGINS
©1998 GARLAND PUBLISHING


Rho-independent termination
AAAAAAA 3′
3′
5′
UUUUUUU 3′
5′
RNA polymerase
5′
3′
5′
3′
5′
3′
UUU
5′
RNA polymerase
Rho-dependent termination
3′
3′
5′
3′
NNN 5′
termination site
Rho
5′
RNA polymerase
3′
5′
3′
5′
3′
5′
Rho
RNA polymerase


Polyadenilation signal
5'-cap
Poly(rA) tail
*G- P P P
Protein-coding sequence
AAUAAA
AA...AA
5'-UTR
3'-UTR
5'–5' bridge
UTR = untranslated region
50 to 250 adenine nucleotides added to the 3'-end
B

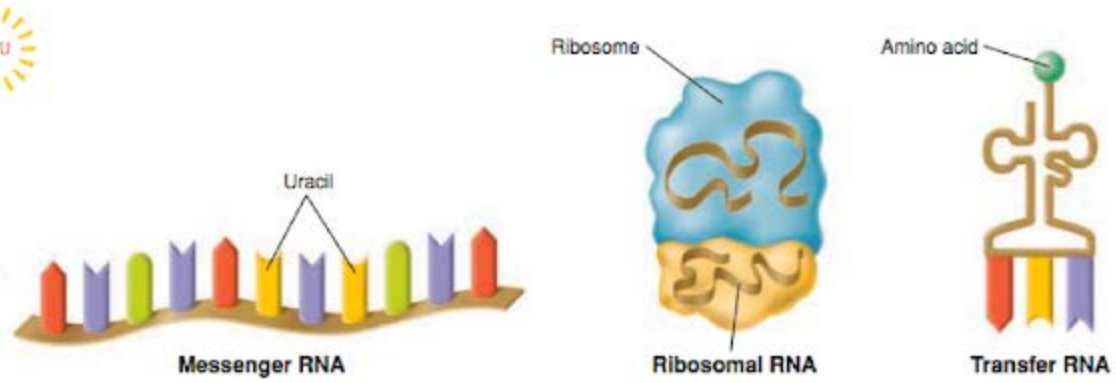


Base	G C U	A C G	G A G	C U U	C G G	A G C	U A G
Codon	Codon 1	Codon 2	Codon 3	Codon 4	Codon 5	Codon 6	Codon 7
Aminoacid	**Alanine**	**Threonine**	**Glutamate**	**Leucine**	**Arginine**	**Serine**	**Stop**

Second letter

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

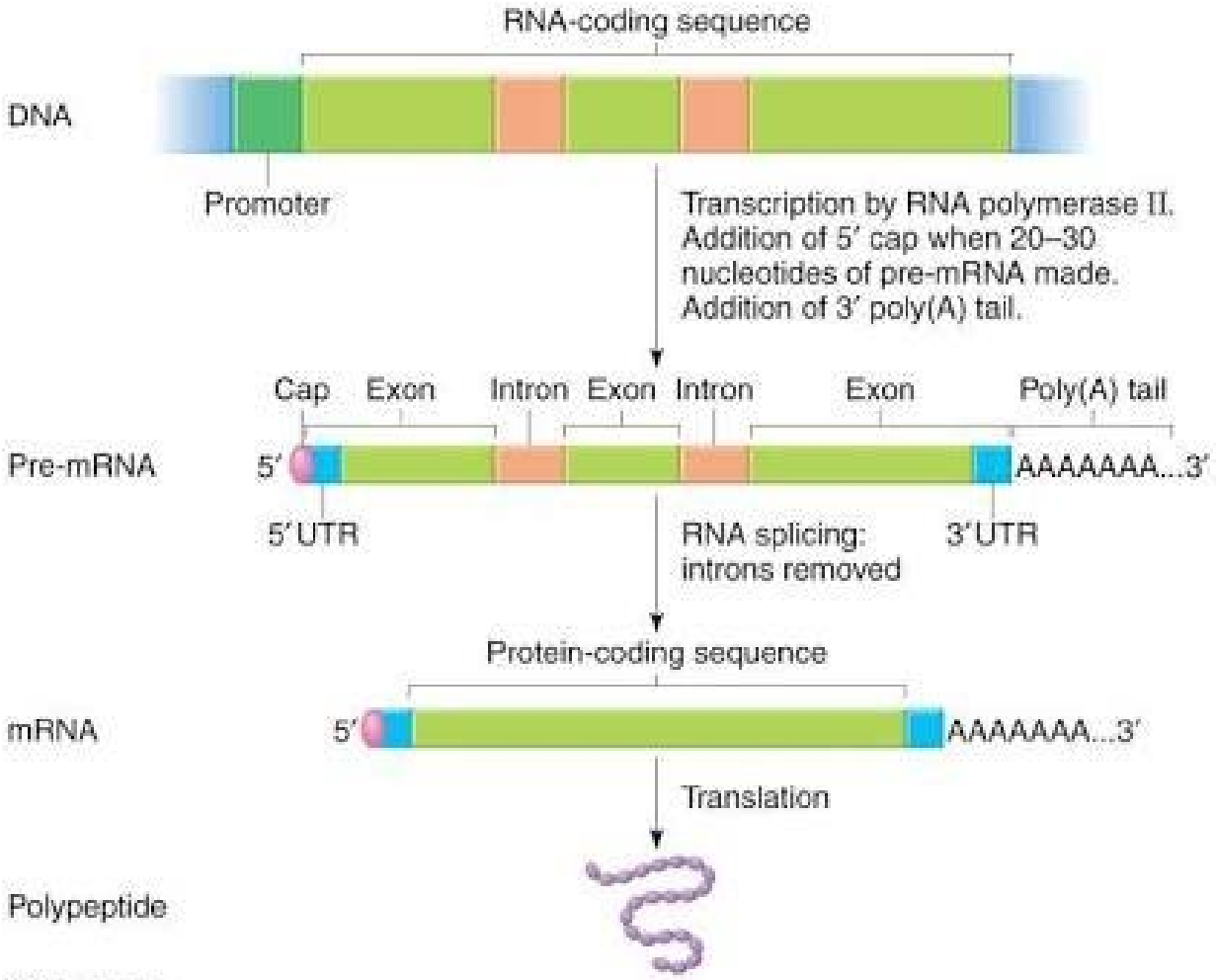
11 DIFFERENT TYPES OF RNA IN A CELL

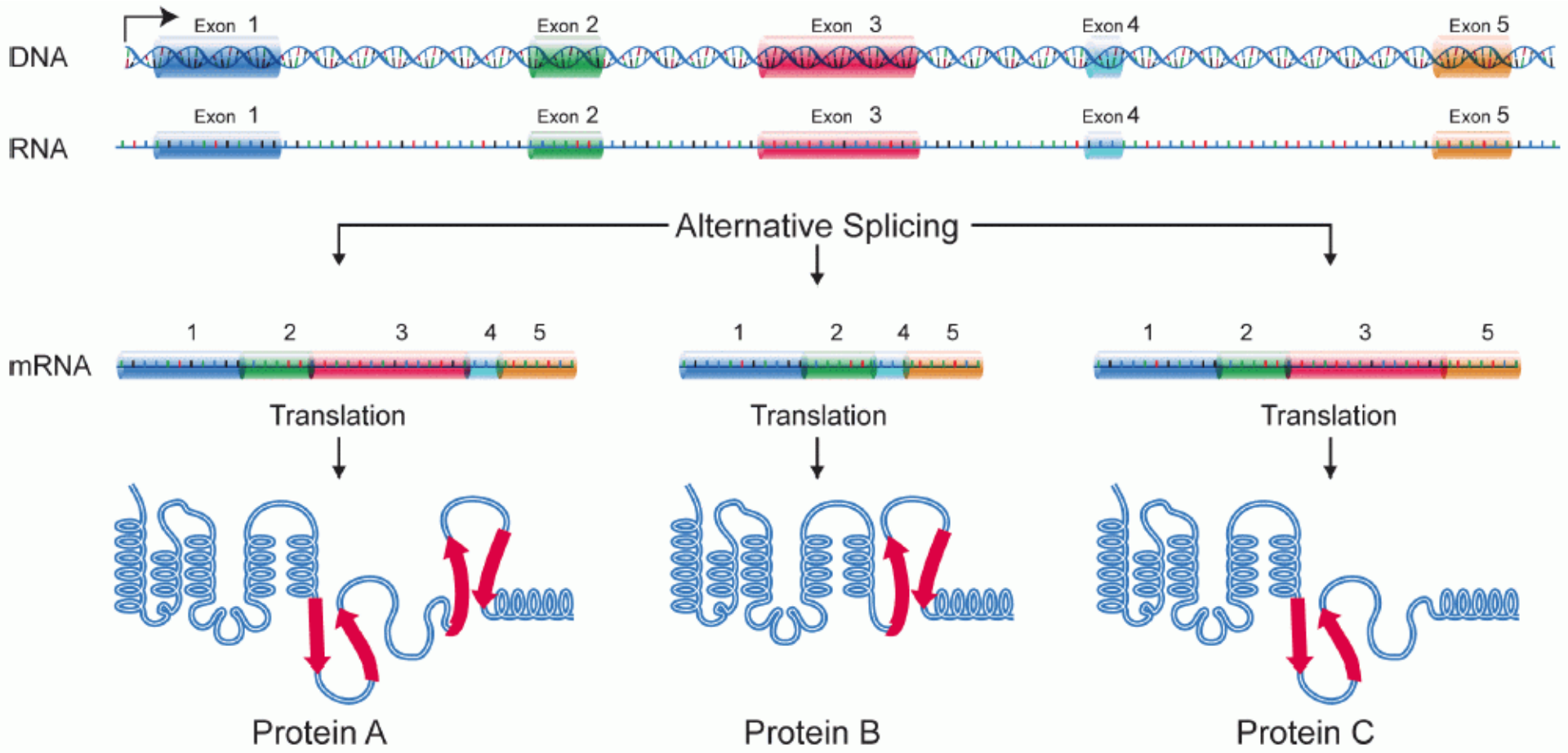


WWW.BIOLOGYEXAMS4U.COM

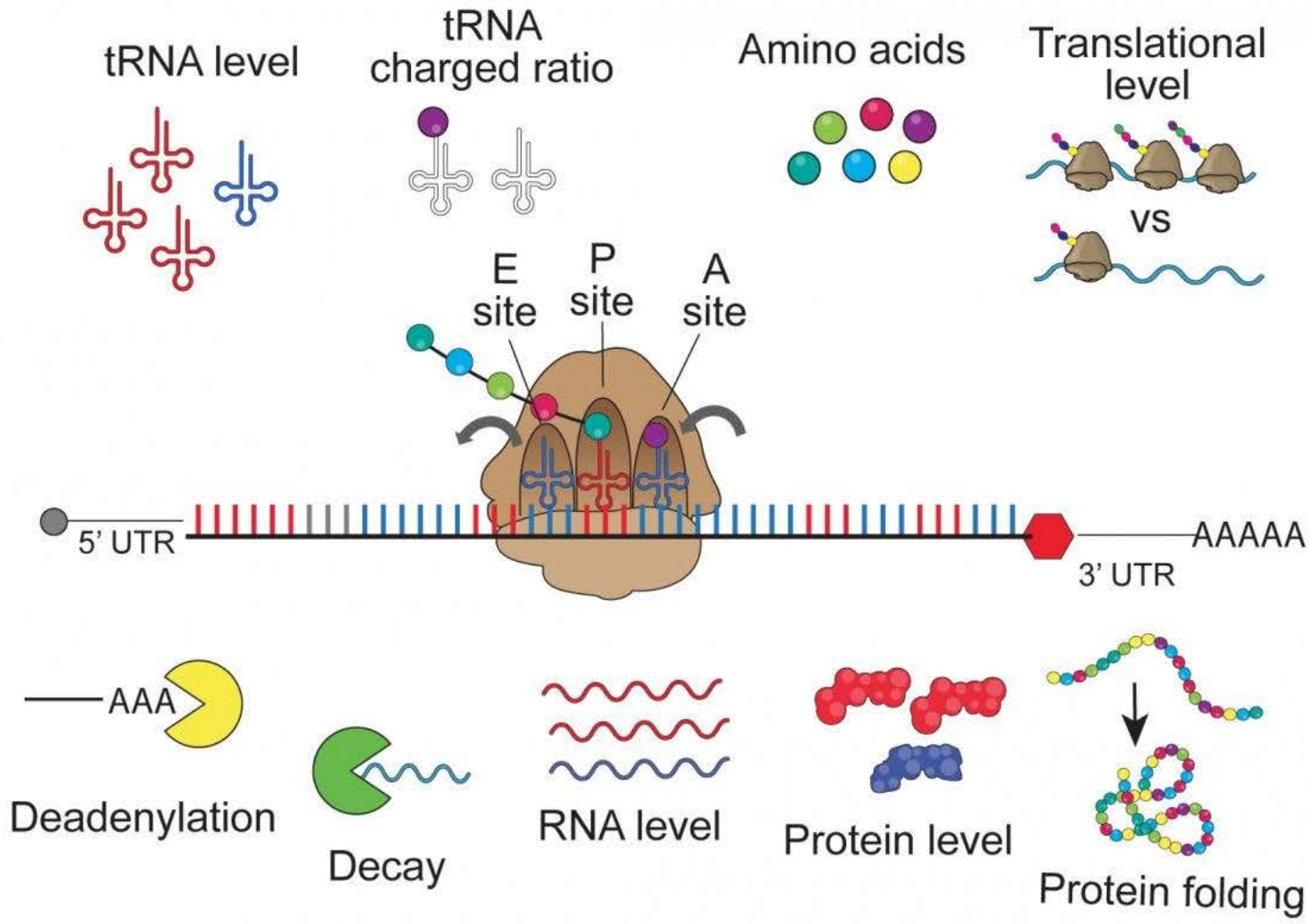
Post Transcription Modification of RNA

1. RNA capping
2. PolyA tail
3. Splicing

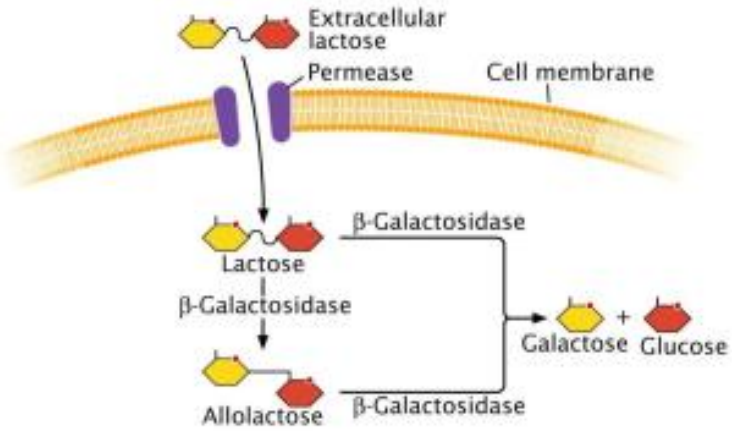



DNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
RNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Alternative Splicing
mRNA
1 2 3 4 5
1 2 4 5
1 2 3 5
Translation
Translation
Translation
Protein A
Protein B
Protein C

Upstream regulator



Downstream effects



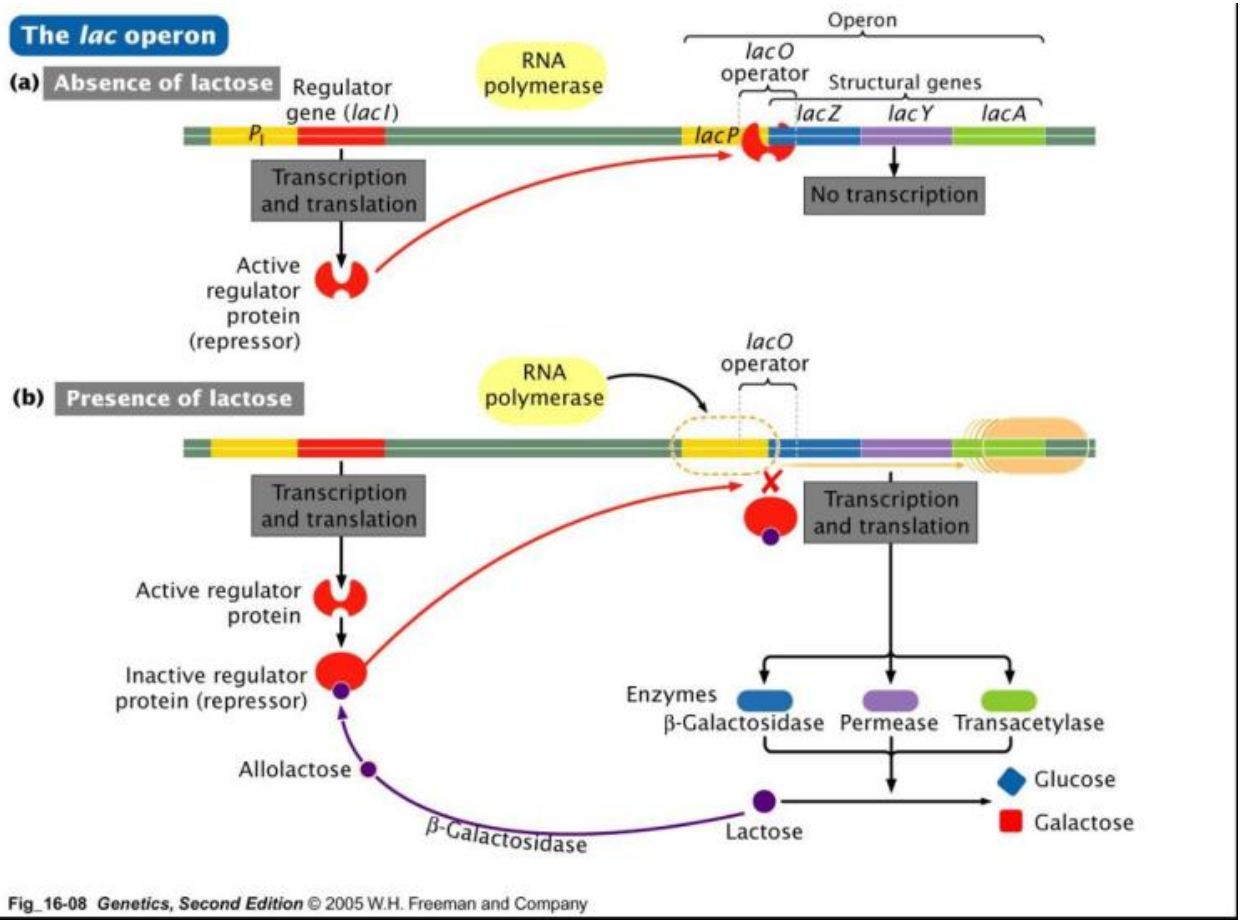
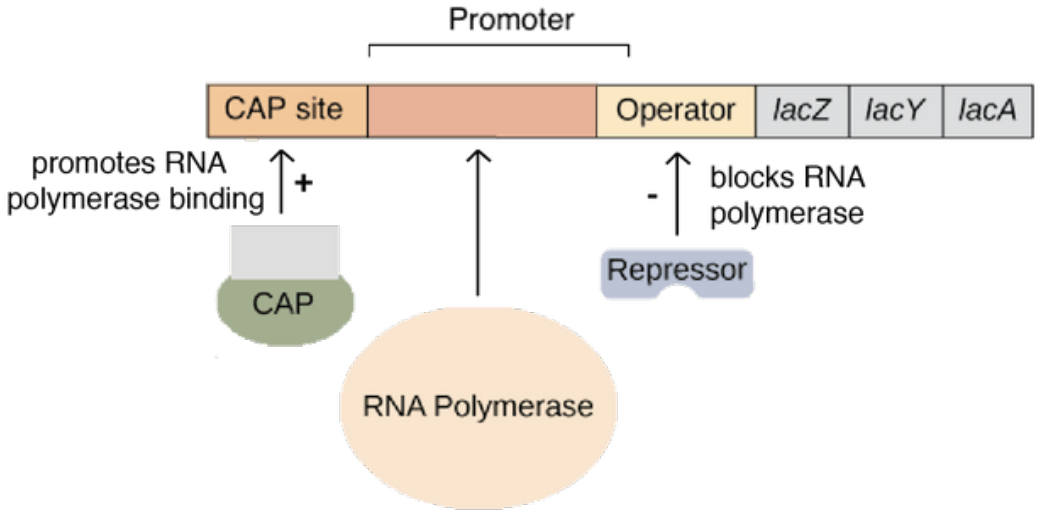
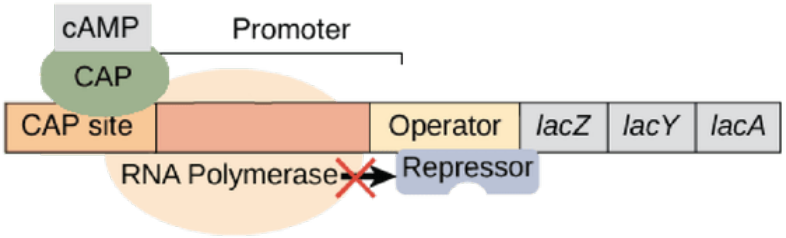


Fig_16-08 *Genetics, Second Edition* © 2005 W.H. Freeman and Company

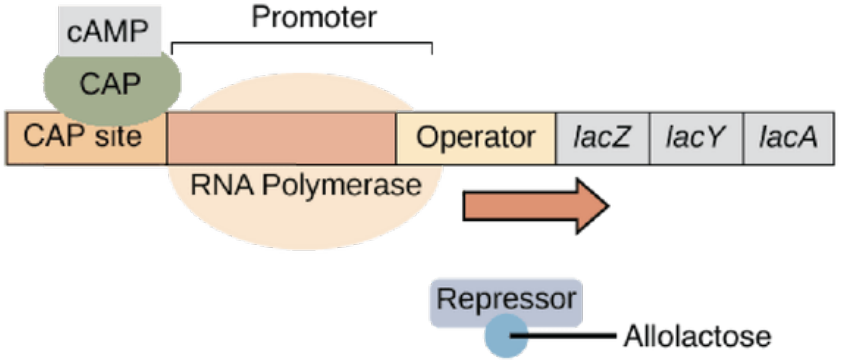
The *lac* operon:



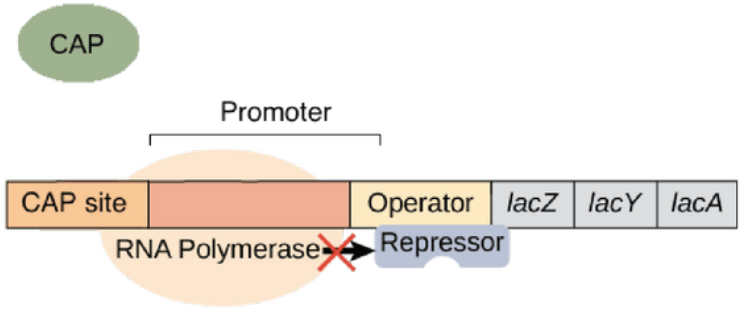
Glucose absent, lactose absent:



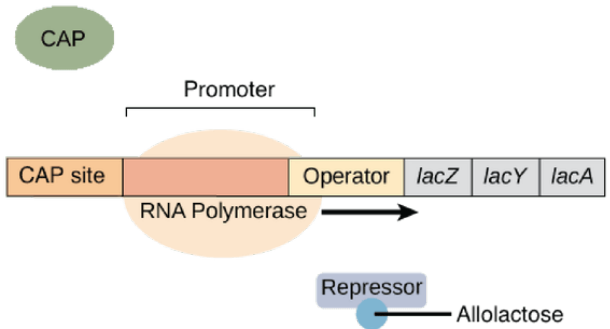
Glucose absent, lactose present:



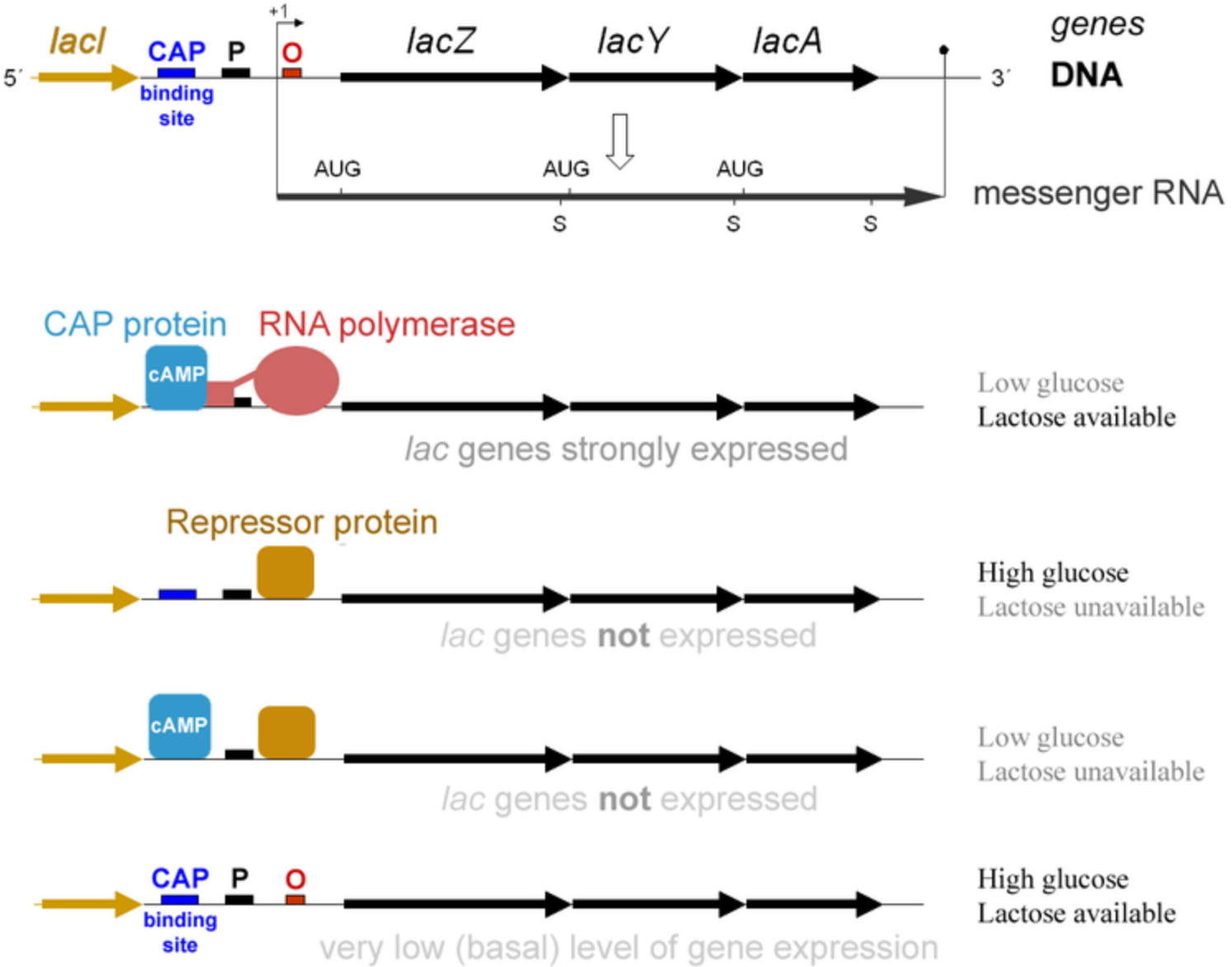
Glucose present, lactose absent:



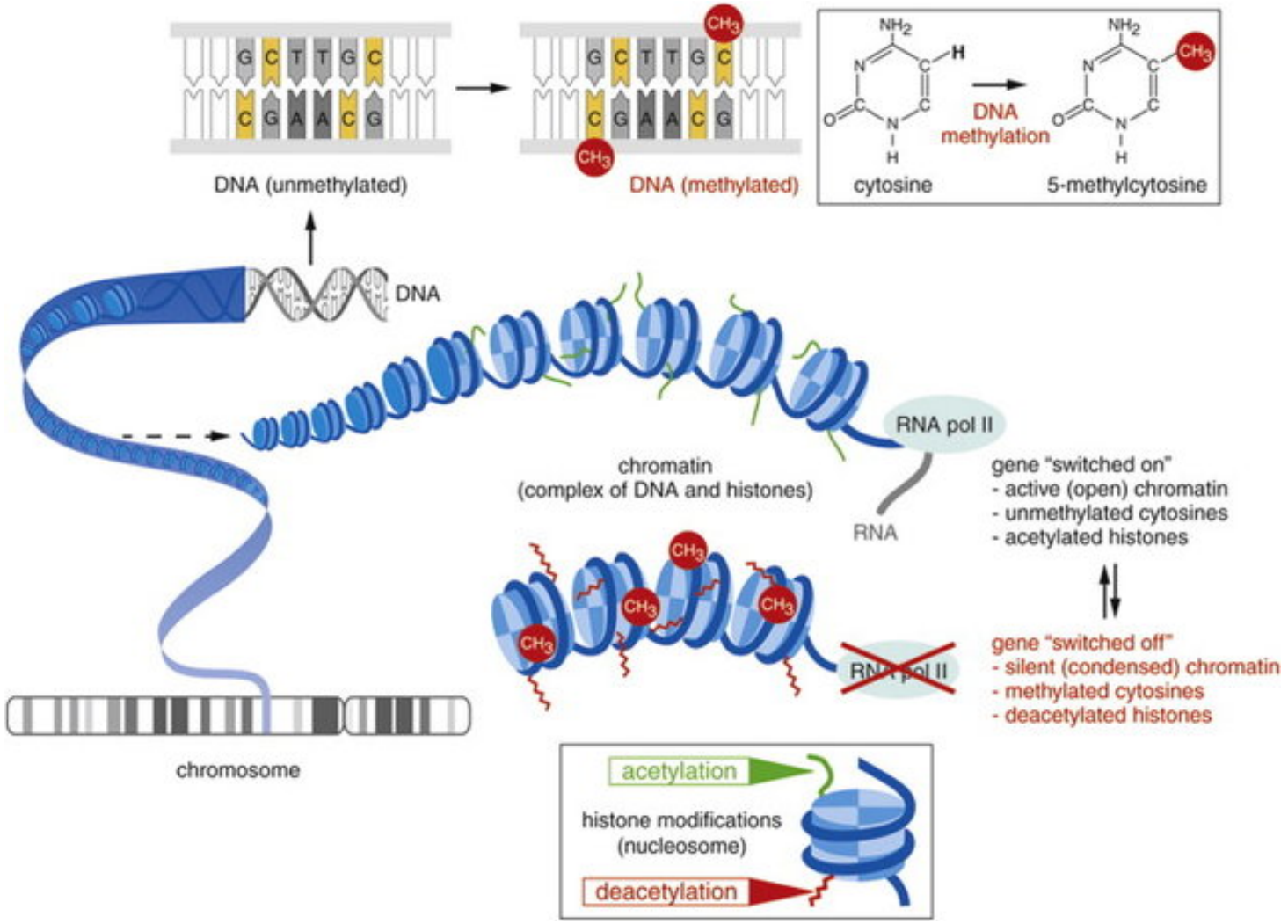
Glucose present, lactose present:

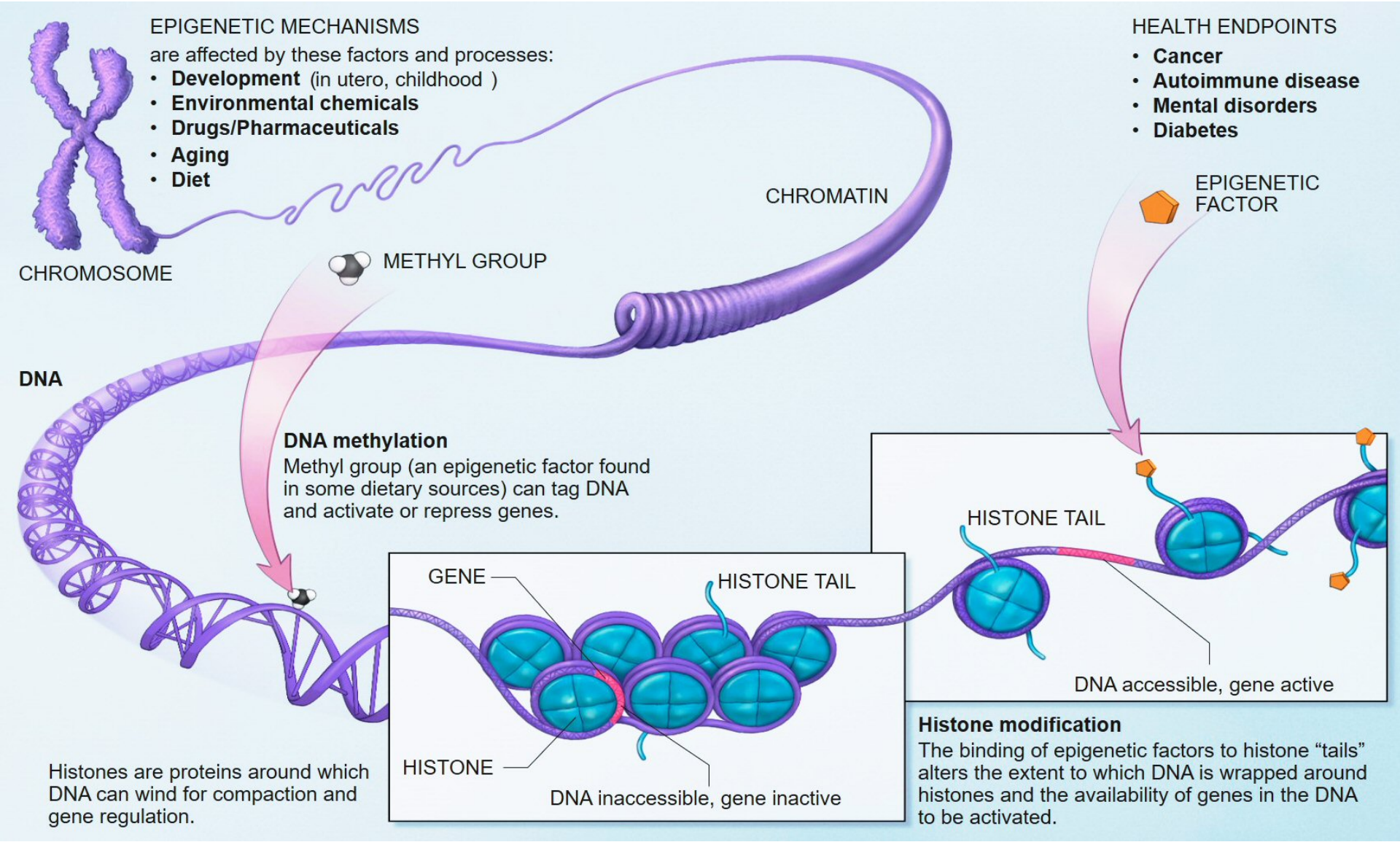


The *lac* Operon and its Control Elements

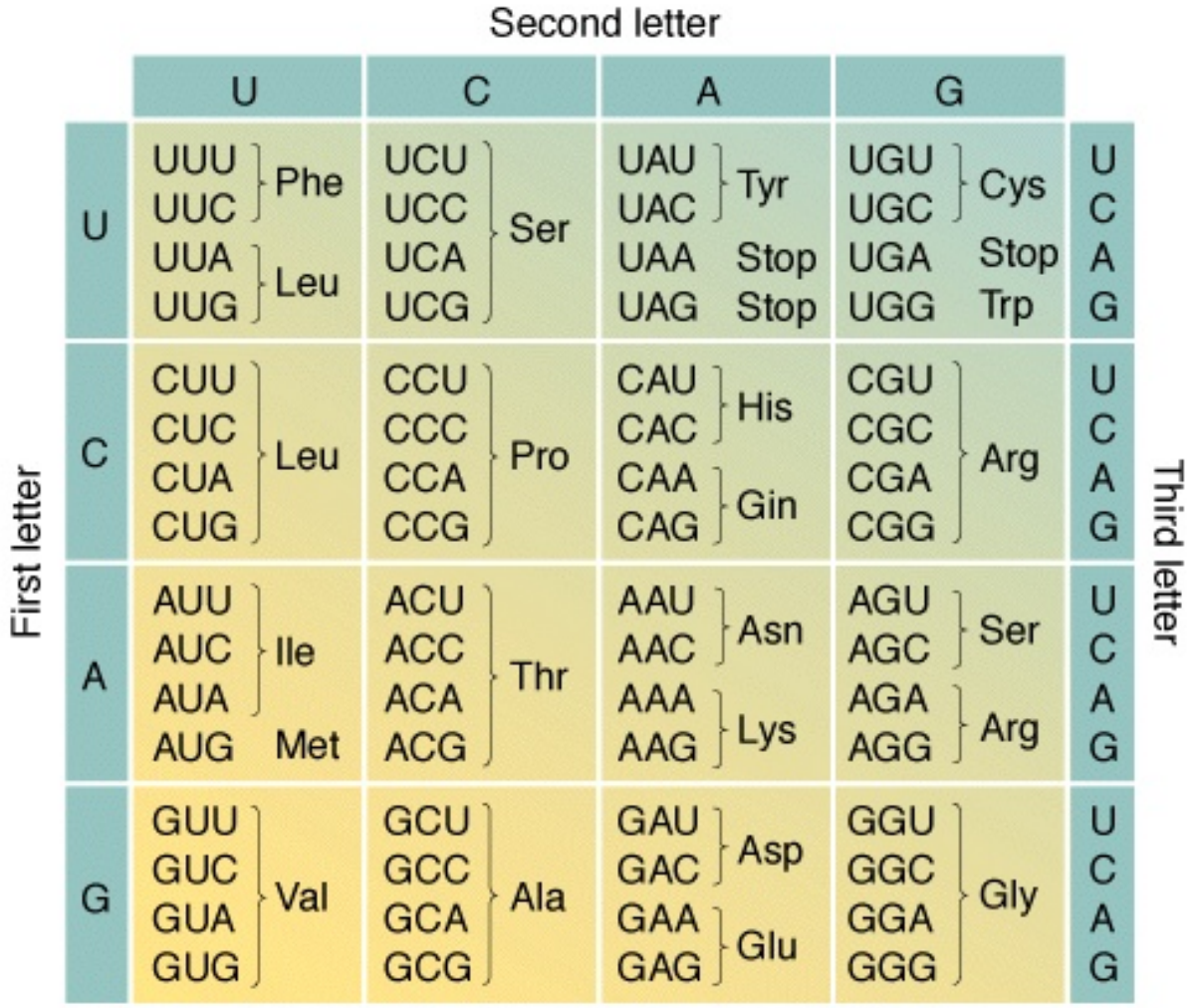


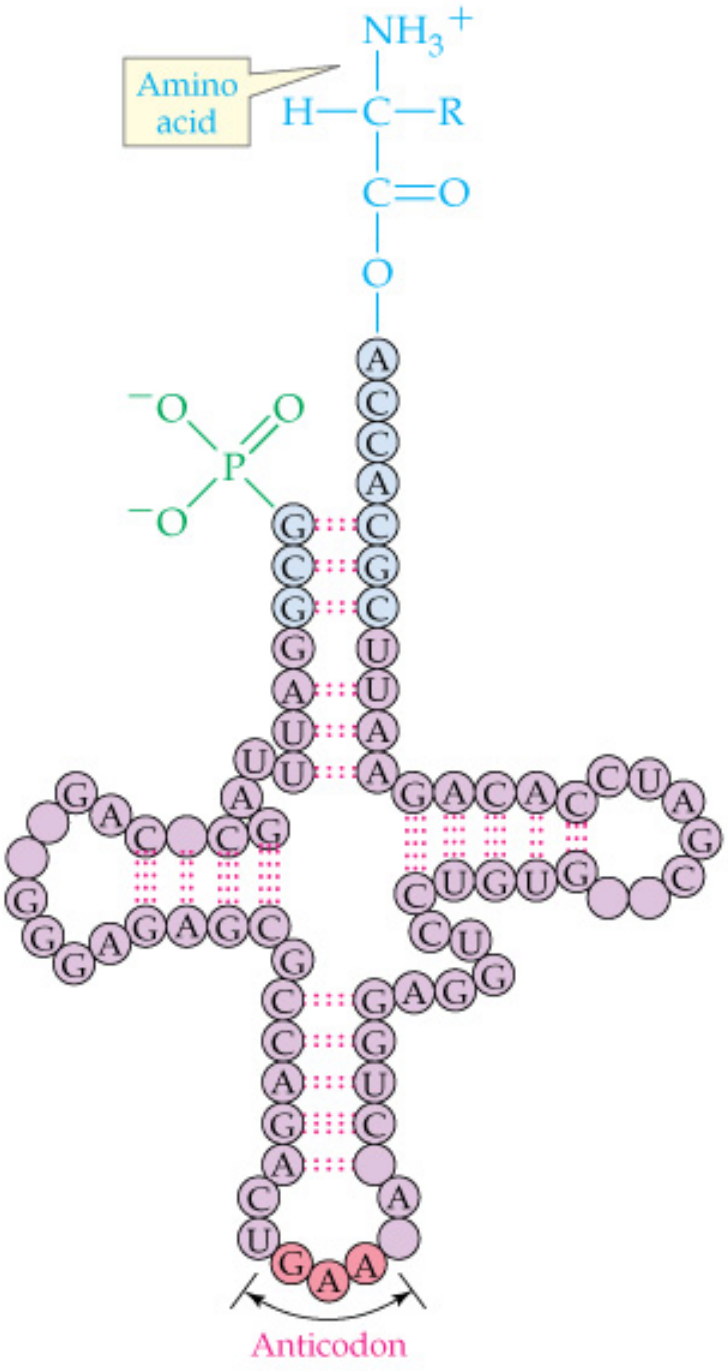
DNA Methylation and Histone Acetylation



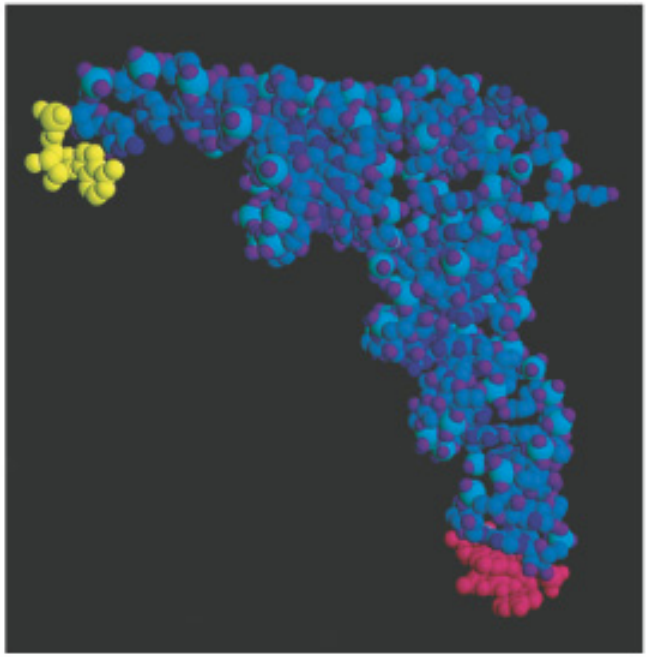

EPIGENETIC MECHANISMS
are affected by these factors and processes:
• Development (in utero, childhood)
• Environmental chemicals
• Drugs/Pharmaceuticals
• Aging
• Diet
HEALTH ENDPOINTS
• Cancer
• Autoimmune disease
• Mental disorders
• Diabetes
CHROMOSOME
CHROMATIN
METHYL GROUP
EPIGENETIC FACTOR
DNA
DNA methylation
Methyl group (an epigenetic factor found in some dietary sources) can tag DNA and activate or repress genes.
GENE
HISTONE TAIL
HISTONE
DNA inaccessible, gene inactive
HISTONE TAIL
DNA accessible, gene active
Histones are proteins around which DNA can wind for compaction and gene regulation.
Histone modification
The binding of epigenetic factors to histone "tails" alters the extent to which DNA is wrapped around histones and the availability of genes in the DNA to be activated.

DNA Sequence

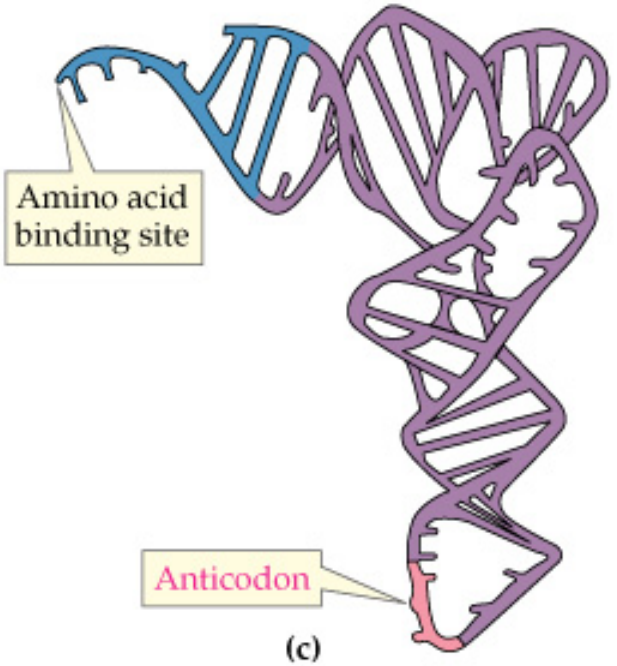



Amino acid
NH_3^+
H—C—R
C=O
O
^-O
O
P
^-O
Anticodon

(a)

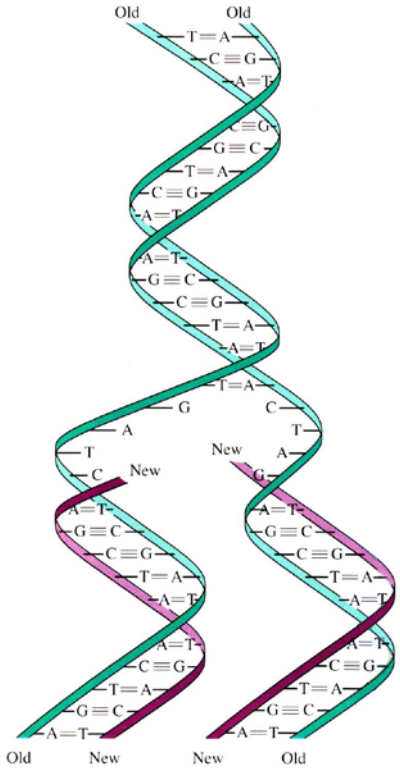


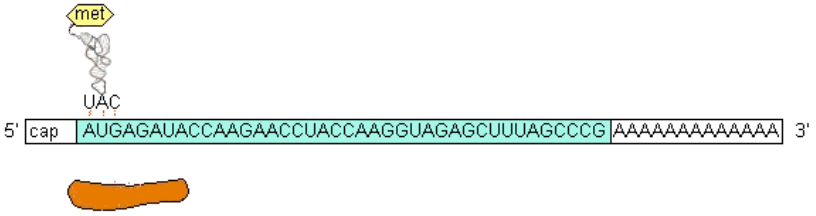
(b)


Amino acid binding site
Anticodon

(c)

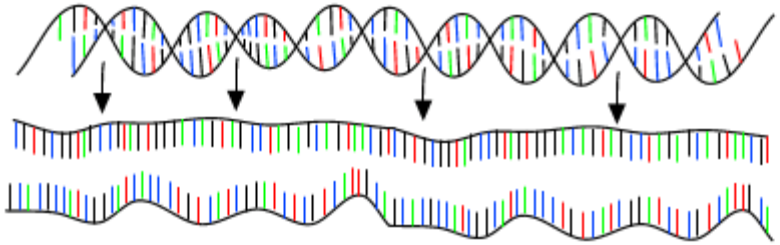
Self-Assembly Process in Nature





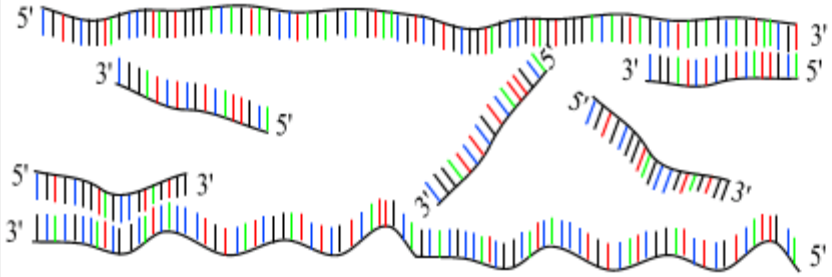
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

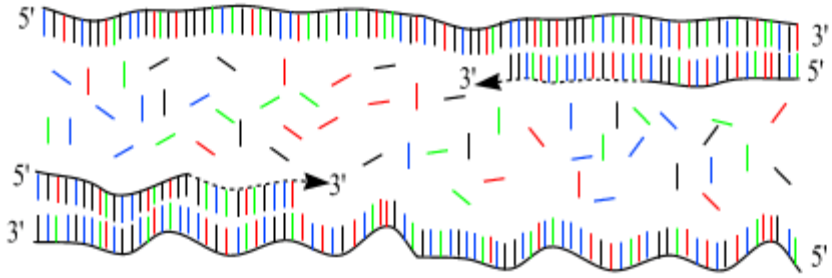
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

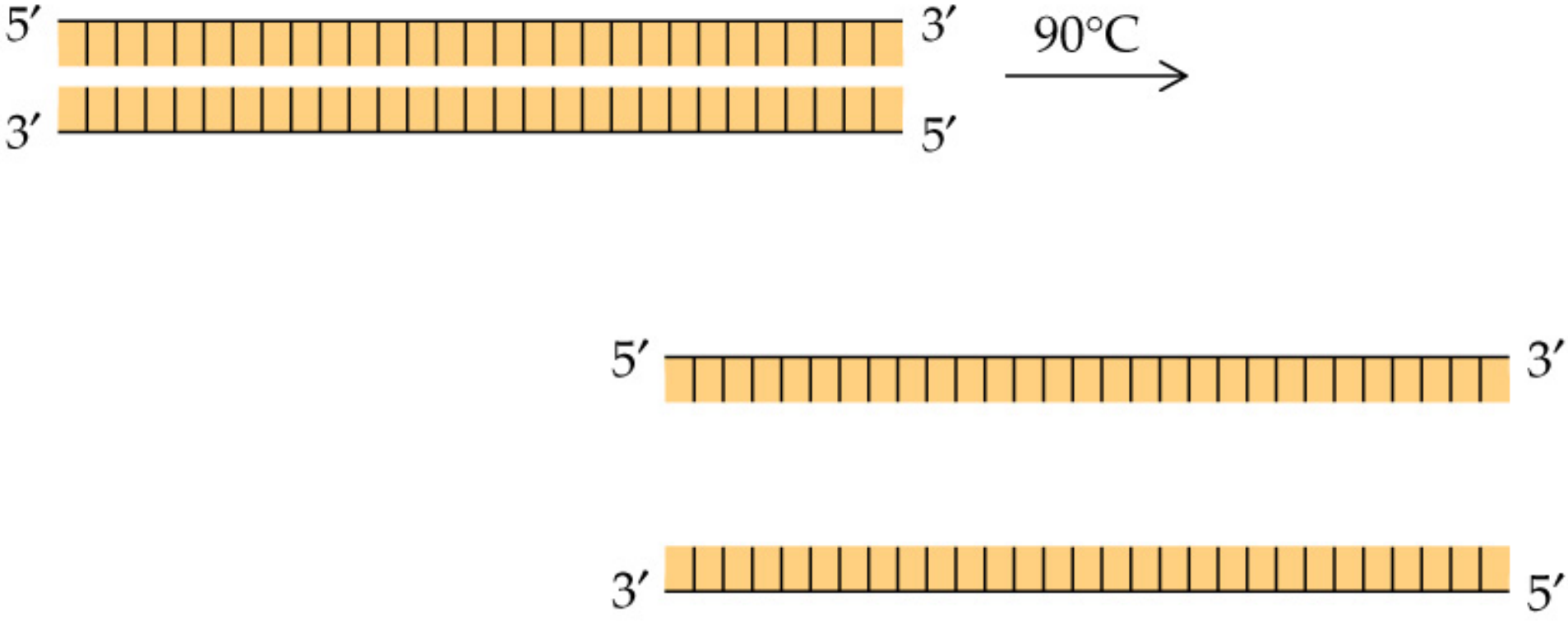
2 minutes 72 °C

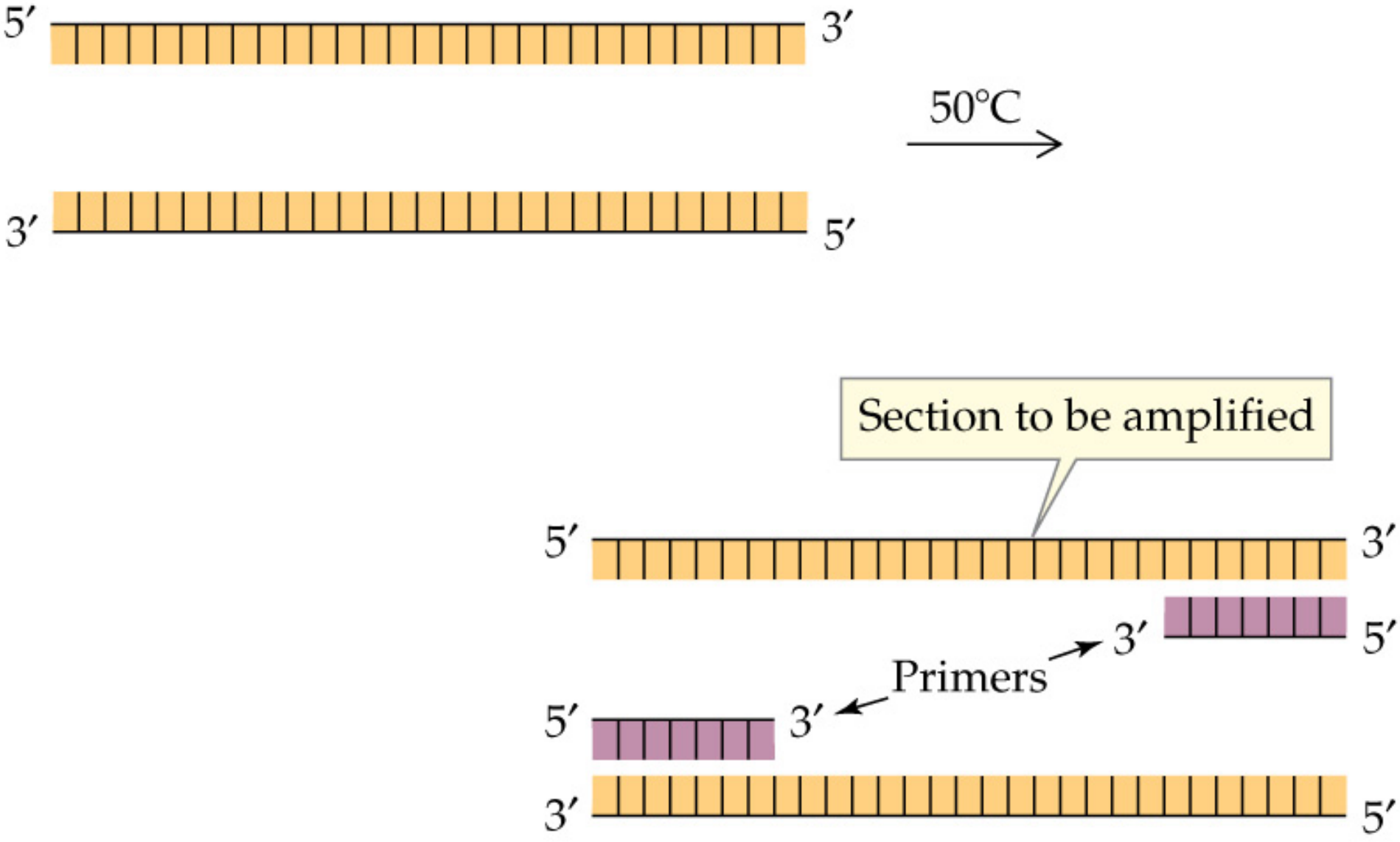
only dNTP's

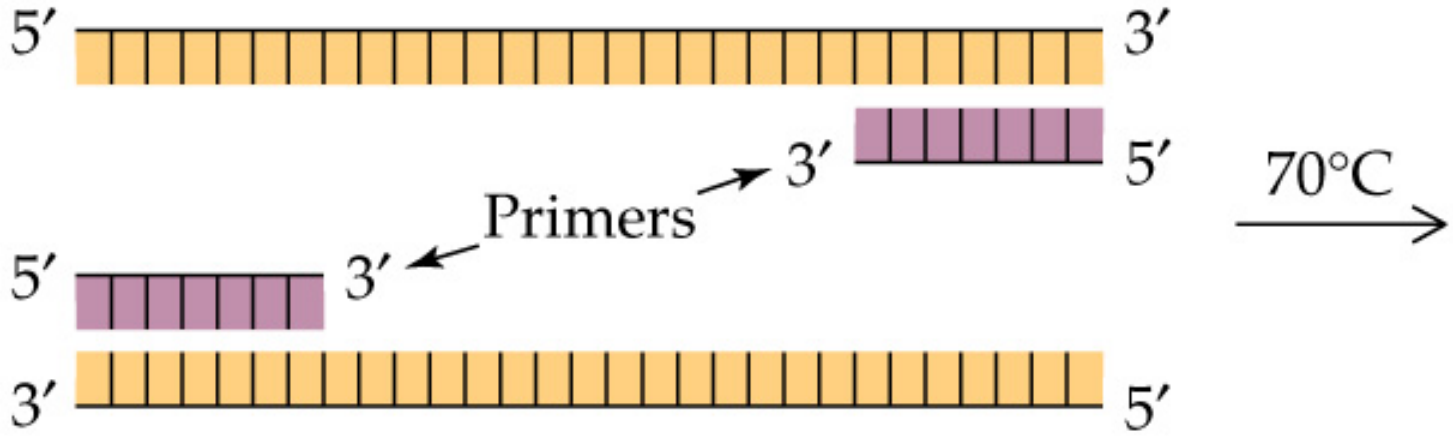
(Andy Vierstraete 1999)

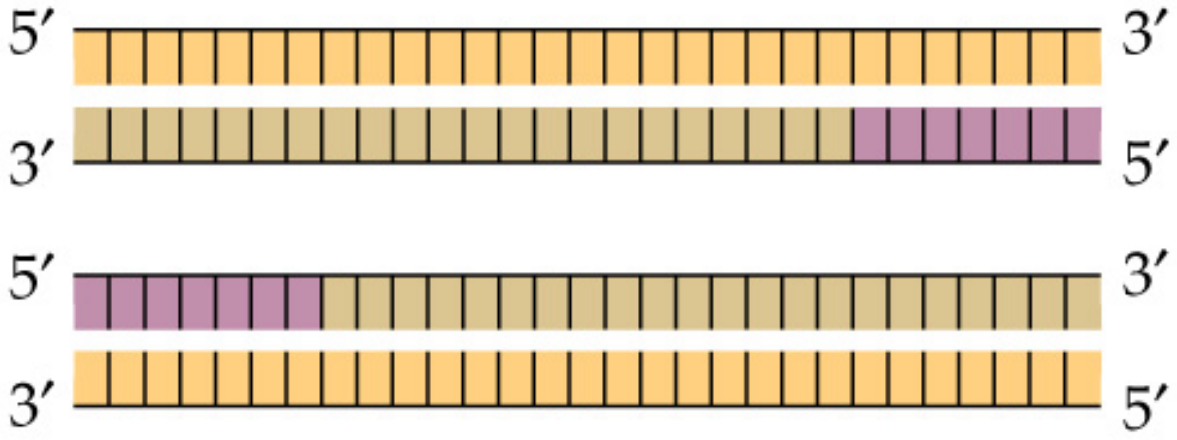
PCR

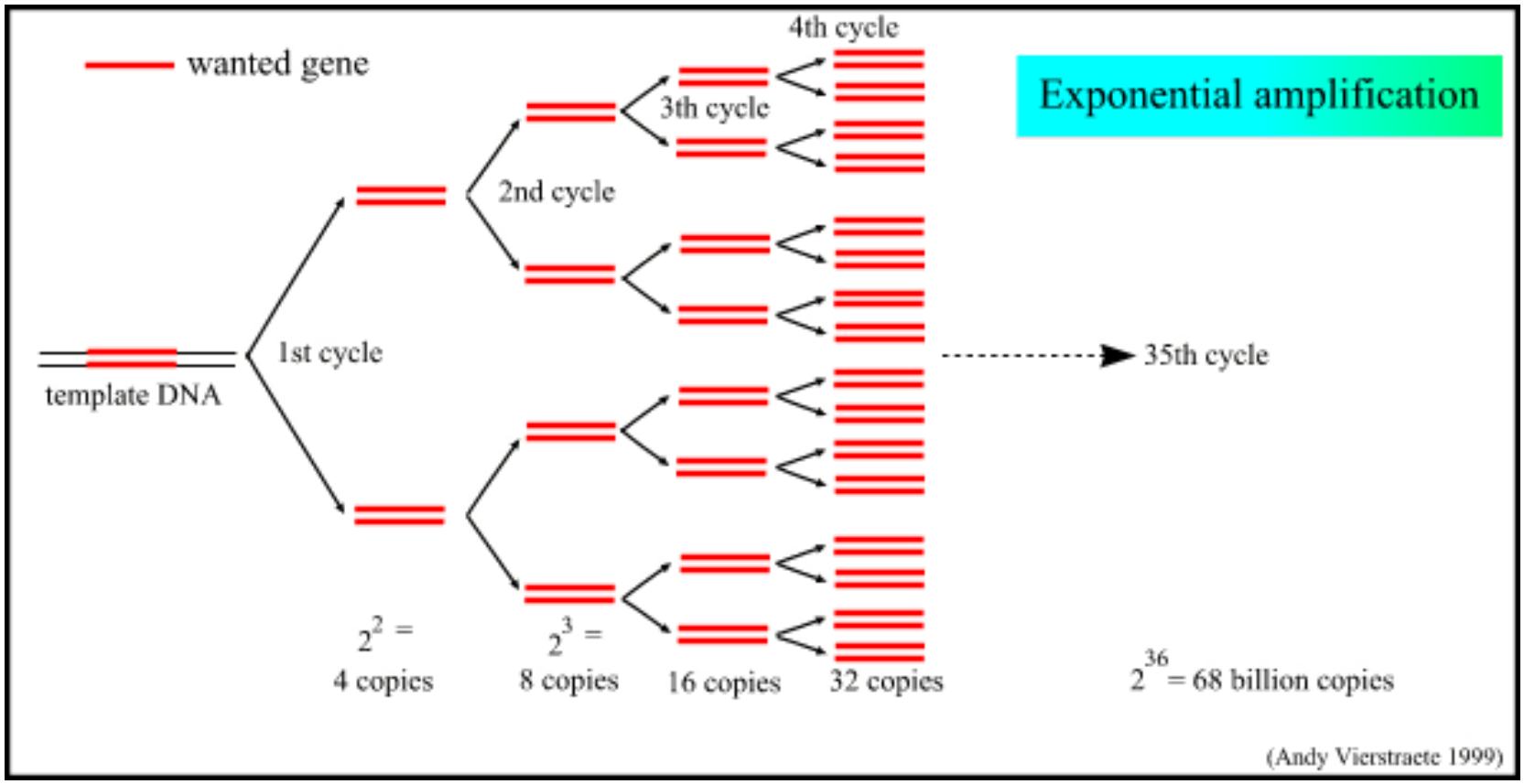
https://www.youtube.com/watch?v=MyLrs_h1OlE


5′
3′
90°C
3′
5′
5′
3′
3′
5′


5′
3′
50°C
3′
5′
Section to be amplified
5′
3′
3′
5′
Primers
5′
3′
3′
5′

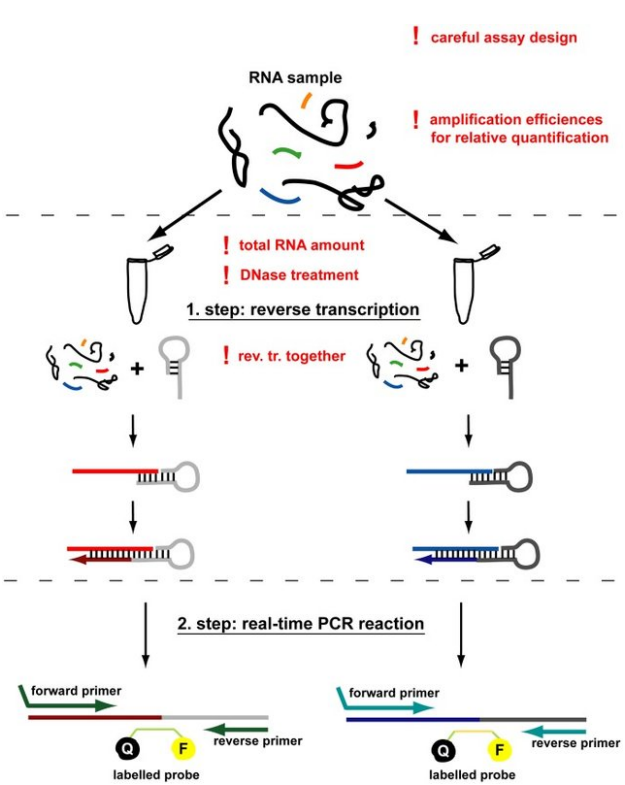

5′
3′
3′
5′
Primers
5′
3′
3′
5′
70°C

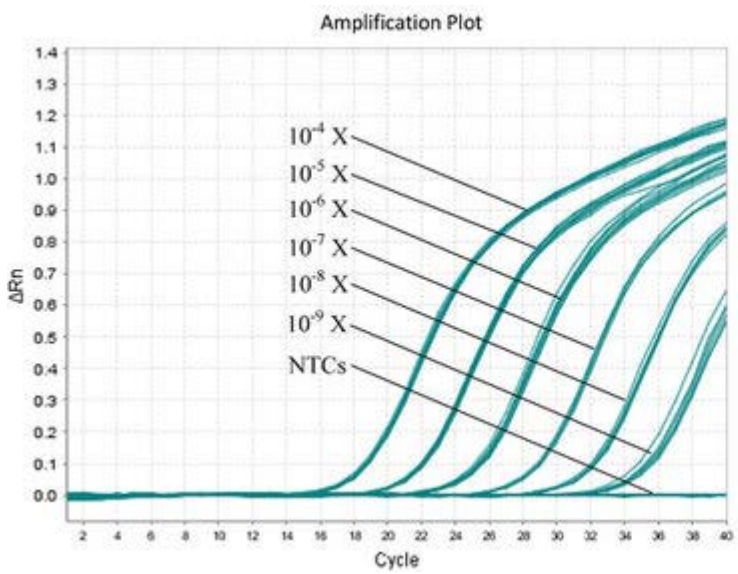

5′
3′
3′
5′
5′
3′
3′
5′

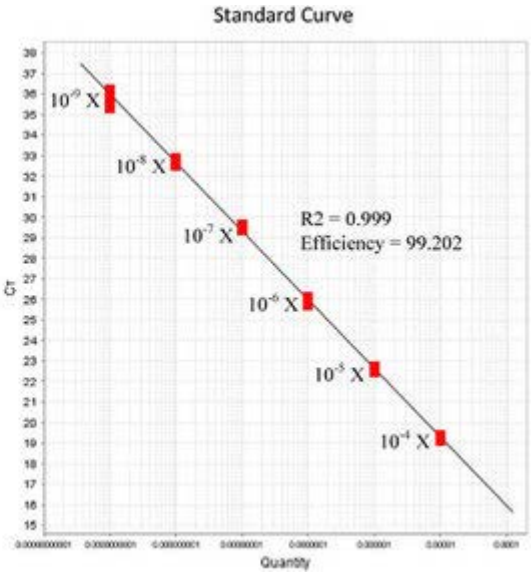

wanted gene
Exponential amplification
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies
(Andy Vierstraete 1999)

Real-time PCR

https://www.youtube.com/watch?v=1kvy17ugI4w


! careful assay design
RNA sample
! amplification efficiences
for relative quantification
! total RNA amount
! DNase treatment
1. step: reverse transcription
! rev. tr. together
2. step: real-time PCR reaction
forward primer
reverse primer
Q
F
labelled probe
forward primer
reverse primer
Q
F
labelled probe


Amplification Plot
ΔRn
Cycle
10-4 X
10-5 X
10-6 X
10-7 X
10-8 X
10-9 X
NTCs


Standard Curve
Ct
Quantity
10-9 X
10-8 X
10-7 X
10-6 X
10-5 X
10-4 X
R2 = 0.999
Efficiency = 99.202

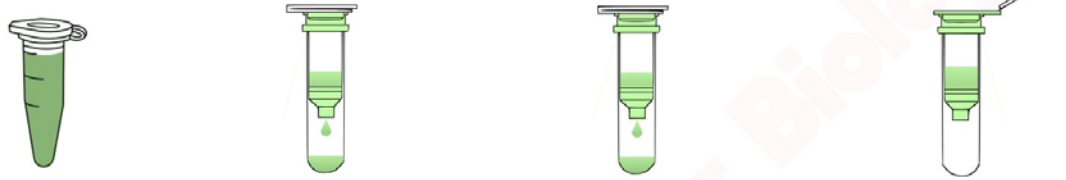
PROTOCOL OF SARS-COV-2 DETECTION USING REAL-TIME RT-PCR

Target gene → RdRp gene (Corman *et al.* 2020)

PCR amplification regions → nCoV_IP2/12621-12727 and nCoV_IP4/14010-14116 (Institut Pasteur, Paris)

Primer sets and probes → designed based on the first sequences of SARS-CoV-2 available on the GISAID database

RNA extraction → NucleoSpin® RNA Virus or viral RNA mini kit (QIAGEN)



Sample lysis

5 min incubation of sample in Lysis Buffer containing Proteinase K

Binding of viral RNA

Ethanol addition and transfer of lysate to Column

Washing

1st Wash Buffer (high salt concentration)
2nd Wash Buffer (low salt concentration)

Elution of viral RNA

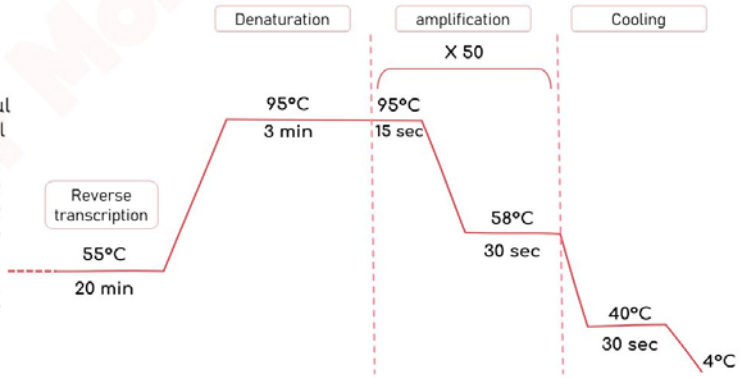
Elution in 20-50 µl RNase-free water or Elution Buffer

Real-time Multiplex RT-PCR (Institut Pasteur, Paris)

Multiplex Mix (nCoV_IP2&IP4)

Sample RNA	5 µl
H20	1.3 µl
Reaction mix 2X	12.50 µl
MgSO4 (50mM)	0.40 µl
Forward Primer1 (10µM)	1.00 µl
Reverse Primer1 (10µM)	1.00 µl
Forward Primer2 (10µM)	1.00 µl
Reverse Primer2 (10µM)	1.00 µl
Probe 1 (10µM)	0.4 µl
Probe 2 (10µM)	0.4 µl
SuperscriptIII RT/Platinum Taq Mix	1.00 µl

Amplification Cycles (Lightcycler System)



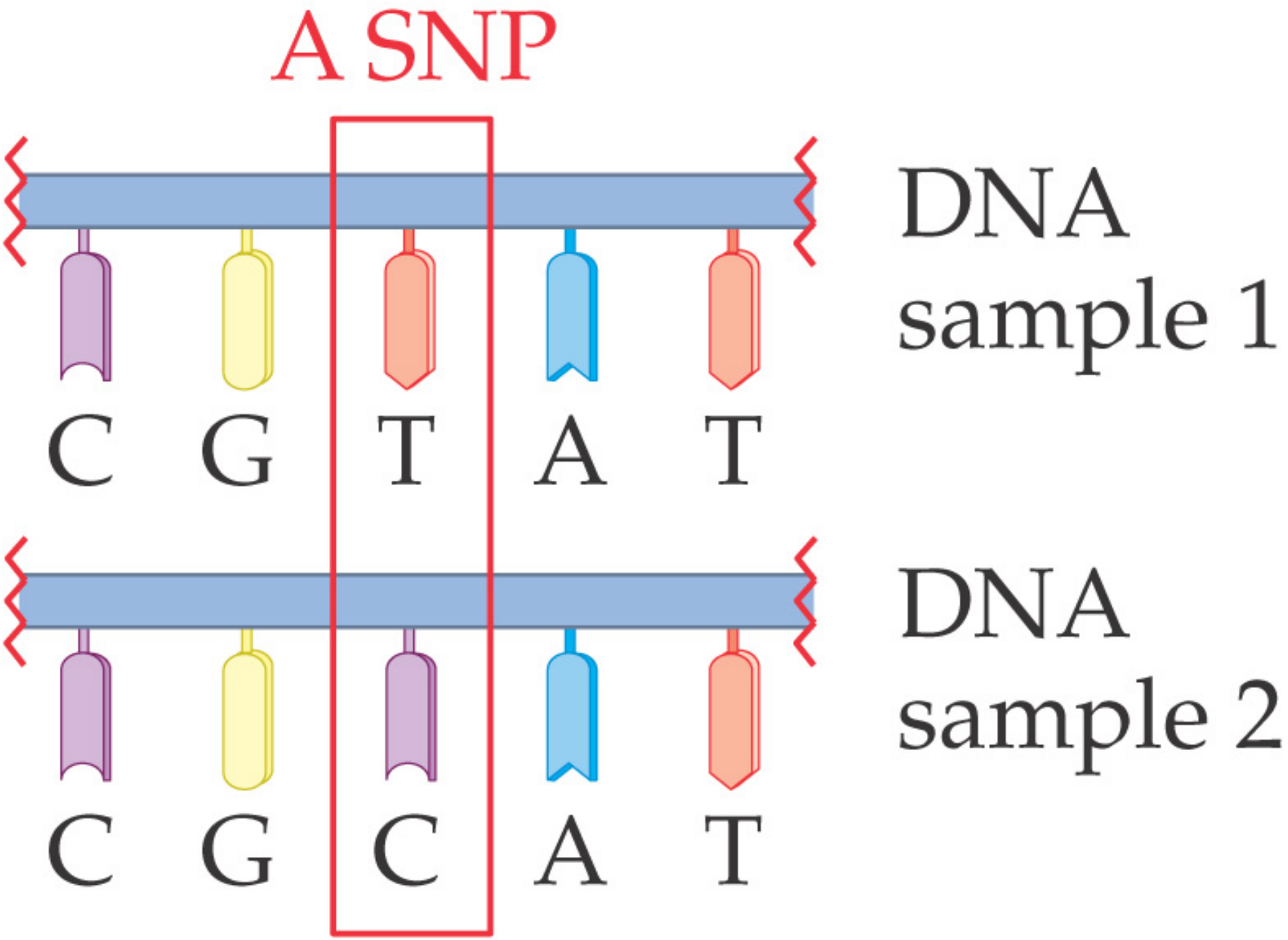
POSITIVE CONTROL

Positive control for real-time RT-PCR is the in vitro transcribed RNA derived from strain BetaCoV_Wuhan_WIV04_2019. The transcript contains the **amplification regions** of the RdRp and E gene as positive strand.

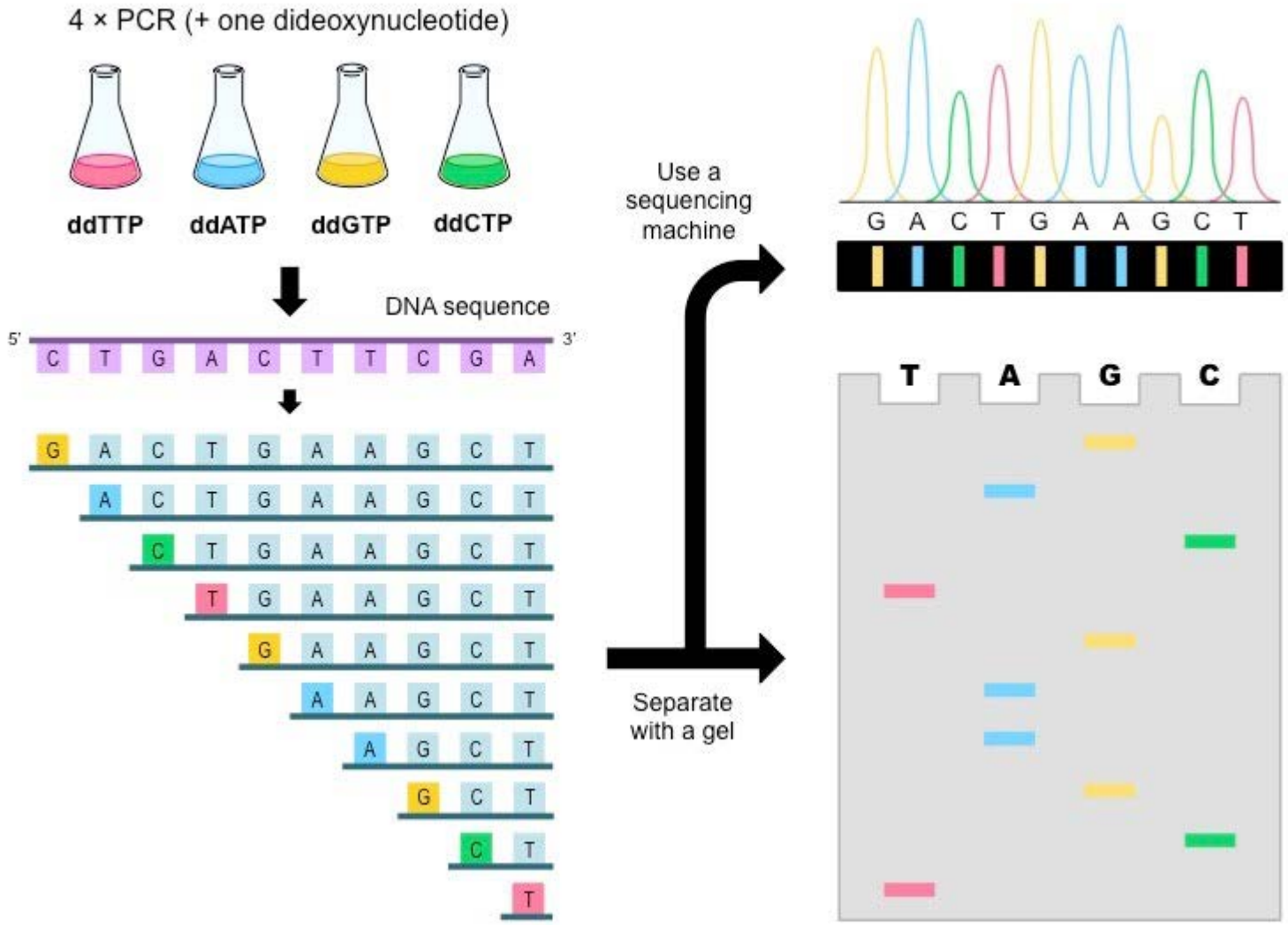
References

1. Institut Pasteur, Paris. « Protocol: Real-time RT-PCR assays for the detection of SARS-CoV-2 ». OMS. 2 mars 2020.

2. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25.

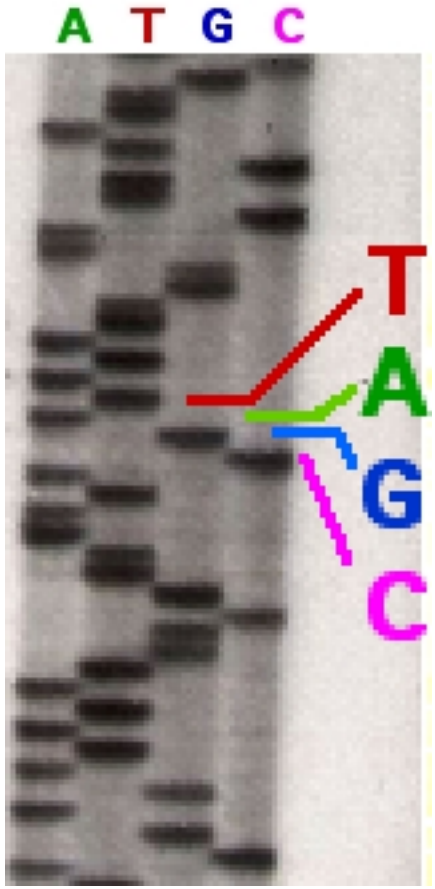

A SNP
C G T A T
C G C A T
DNA sample 1
DNA sample 2

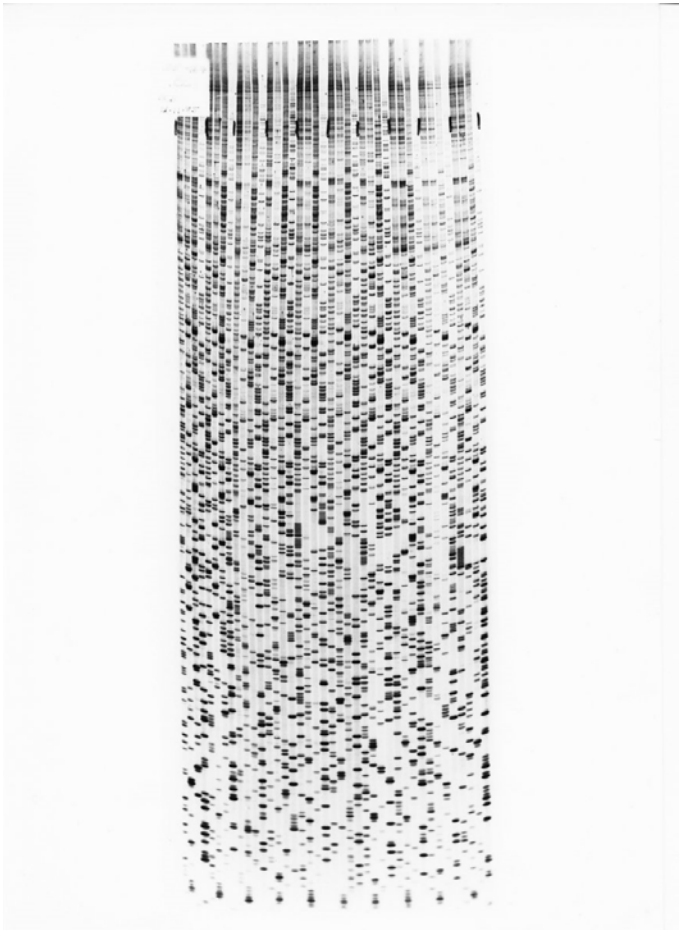
DNA Sequencing



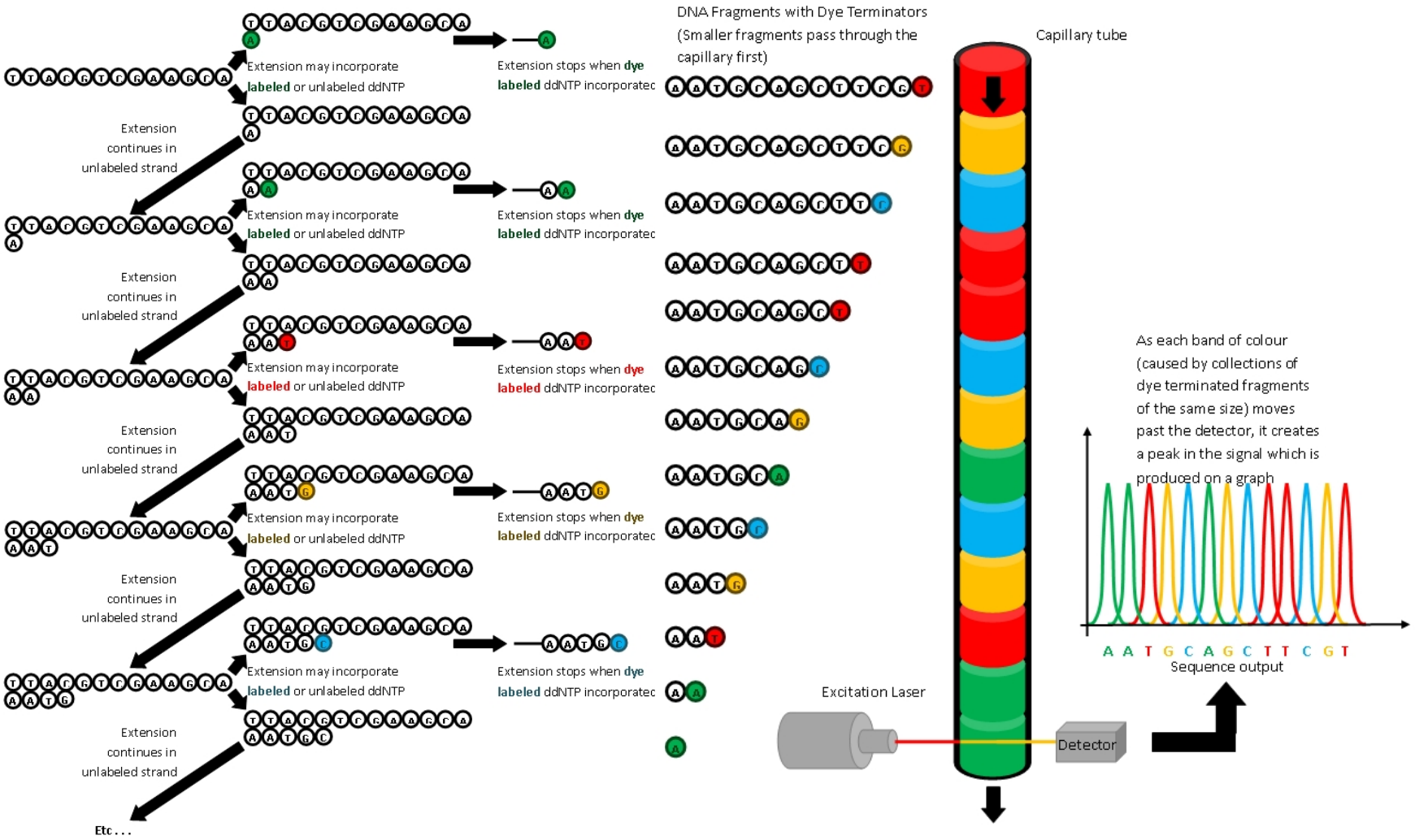
DNA Sequencing

https://www.youtube.com/watch?v=vK-HlMaitnE

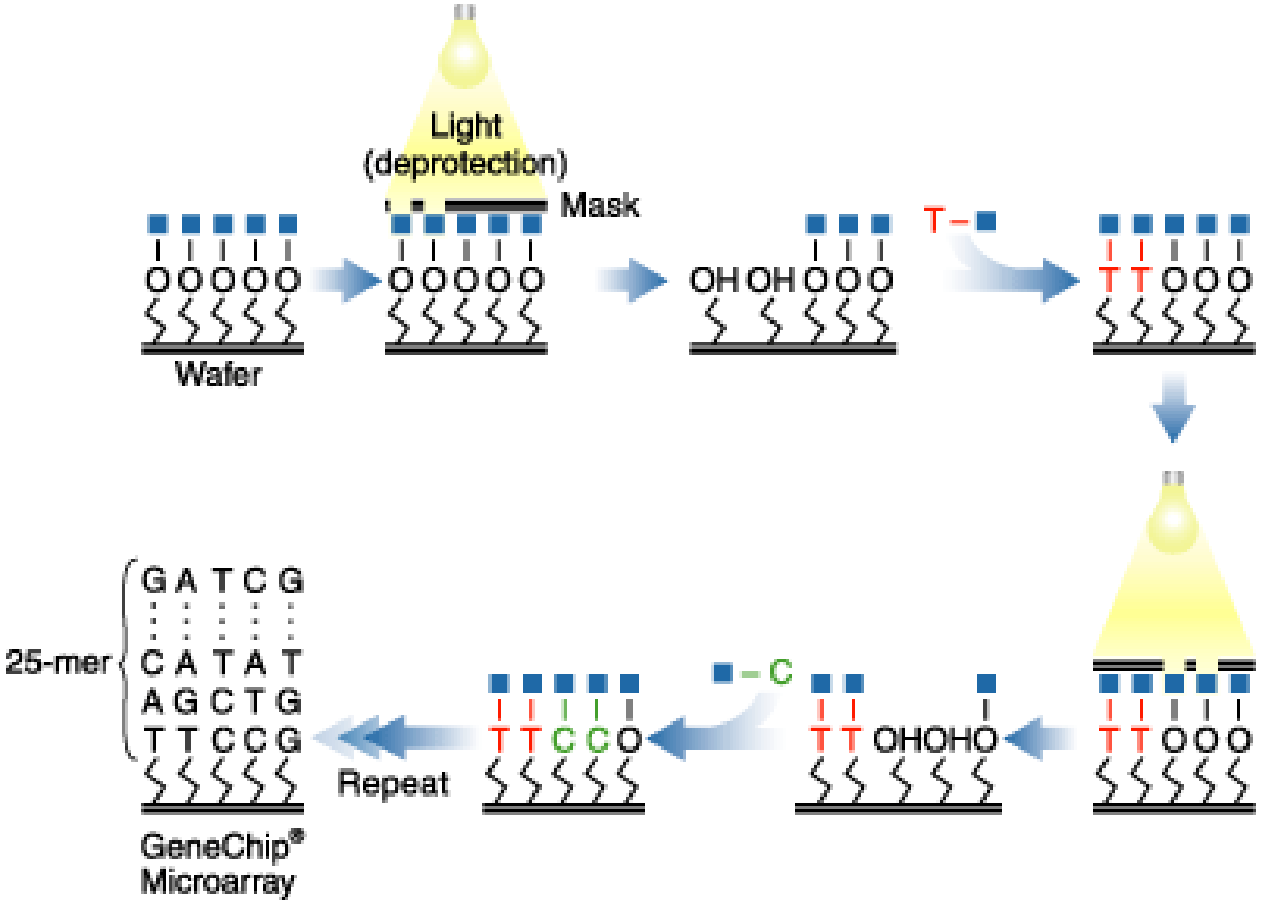




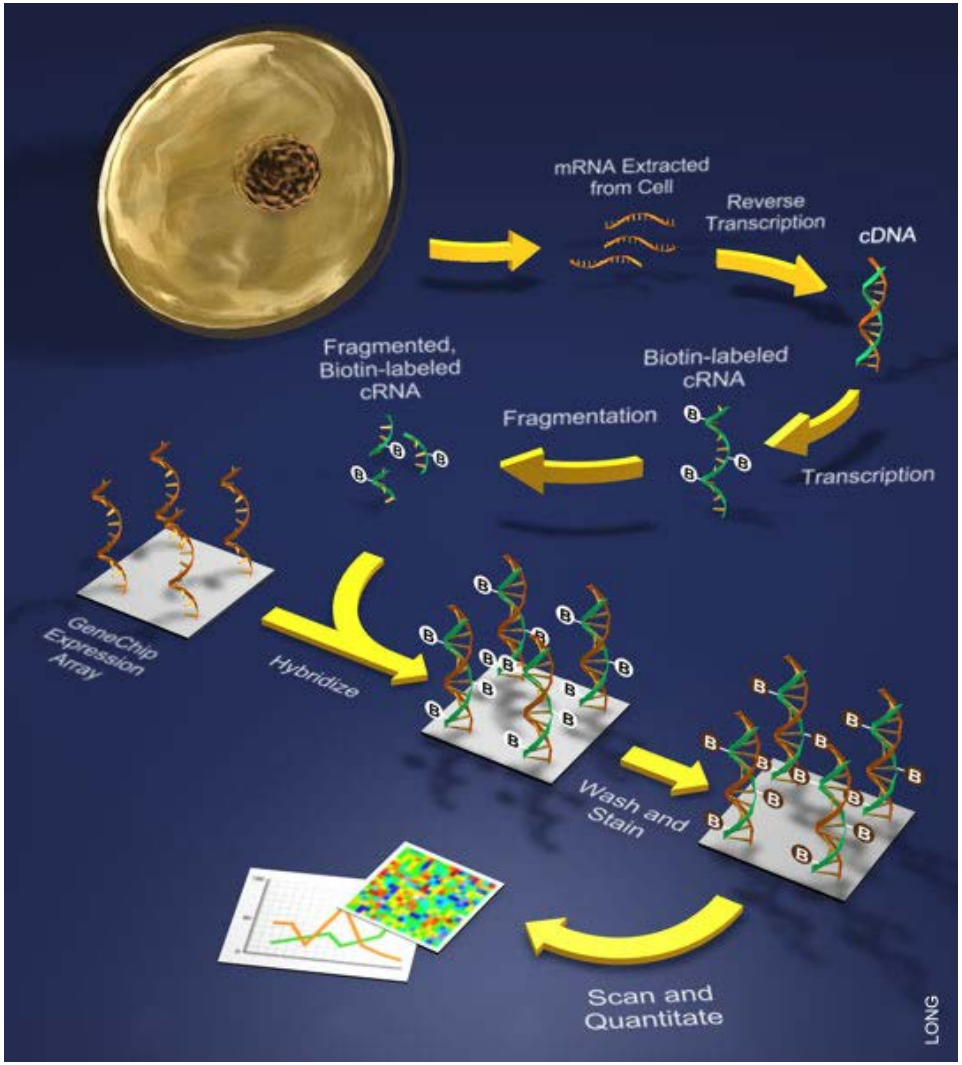
Dye Terminations



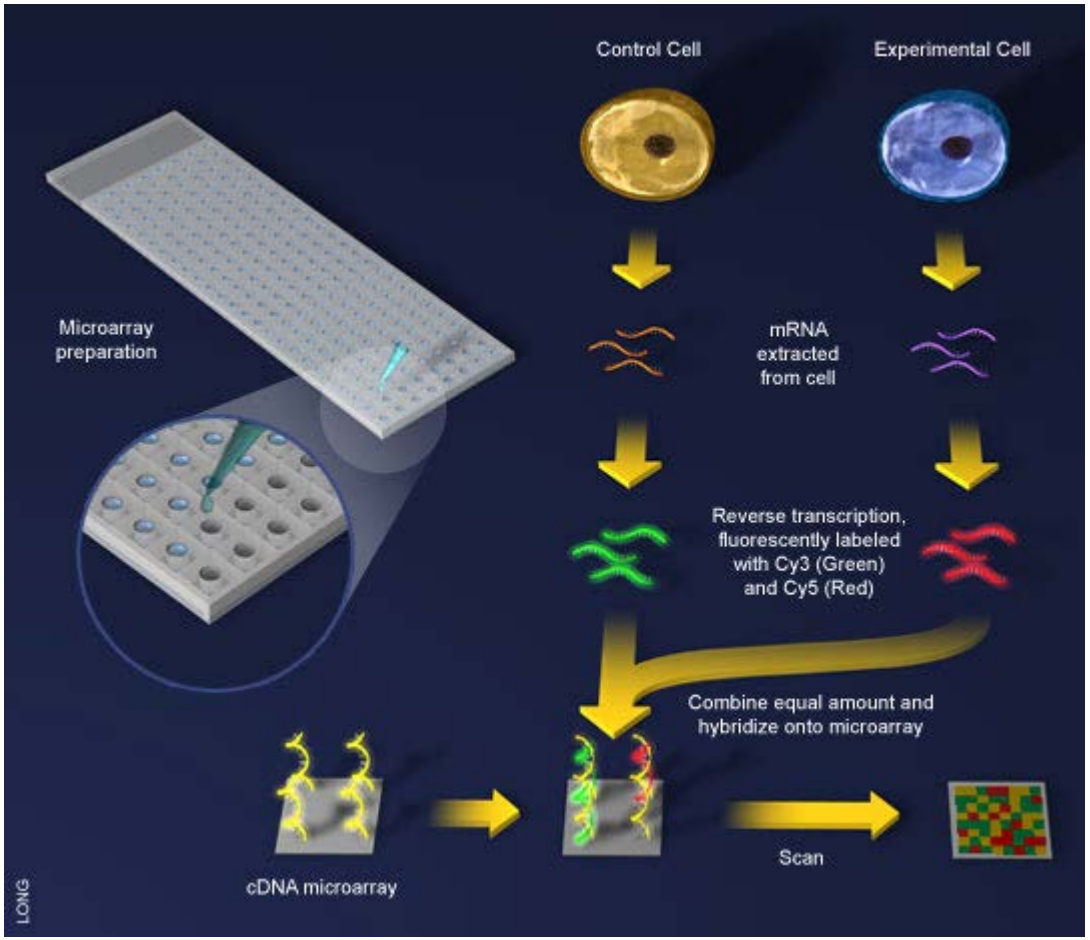
GeneChip


Light
(deprotection)
Mask
Wafer
T –
– C
Repeat
25-mer
G A T C G
C A T A T
A G C T G
T T C C G
GeneChip®
Microarray

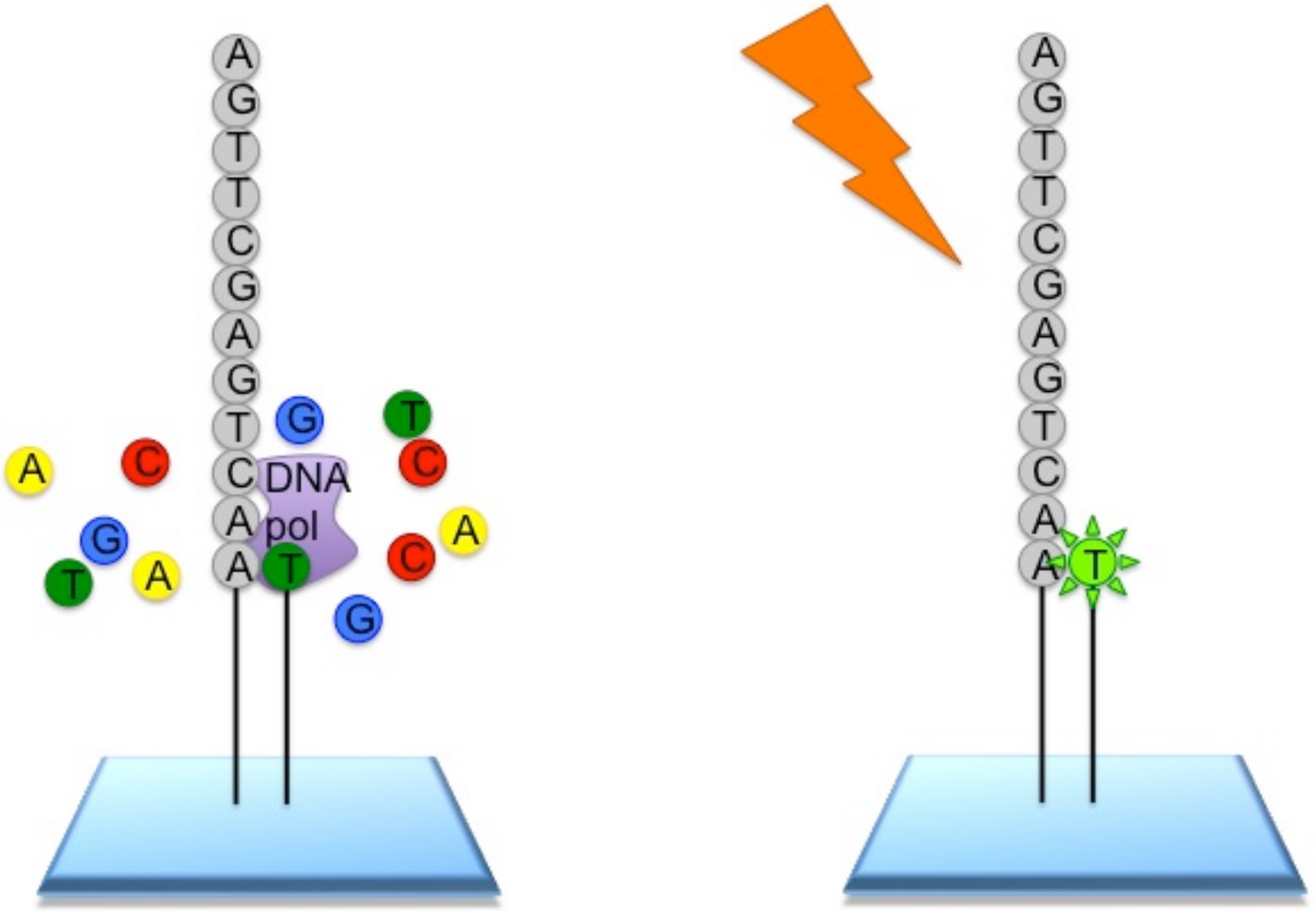
Scheme


mRNA Extracted from Cell
Reverse Transcription
cDNA
Fragmented, Biotin-labeled cRNA
Biotin-labeled cRNA
Fragmentation
Transcription
GeneChip Expression Array
Hybridize
Wash and Stain
Scan and Quantitate
LONG

cDNA Microarray

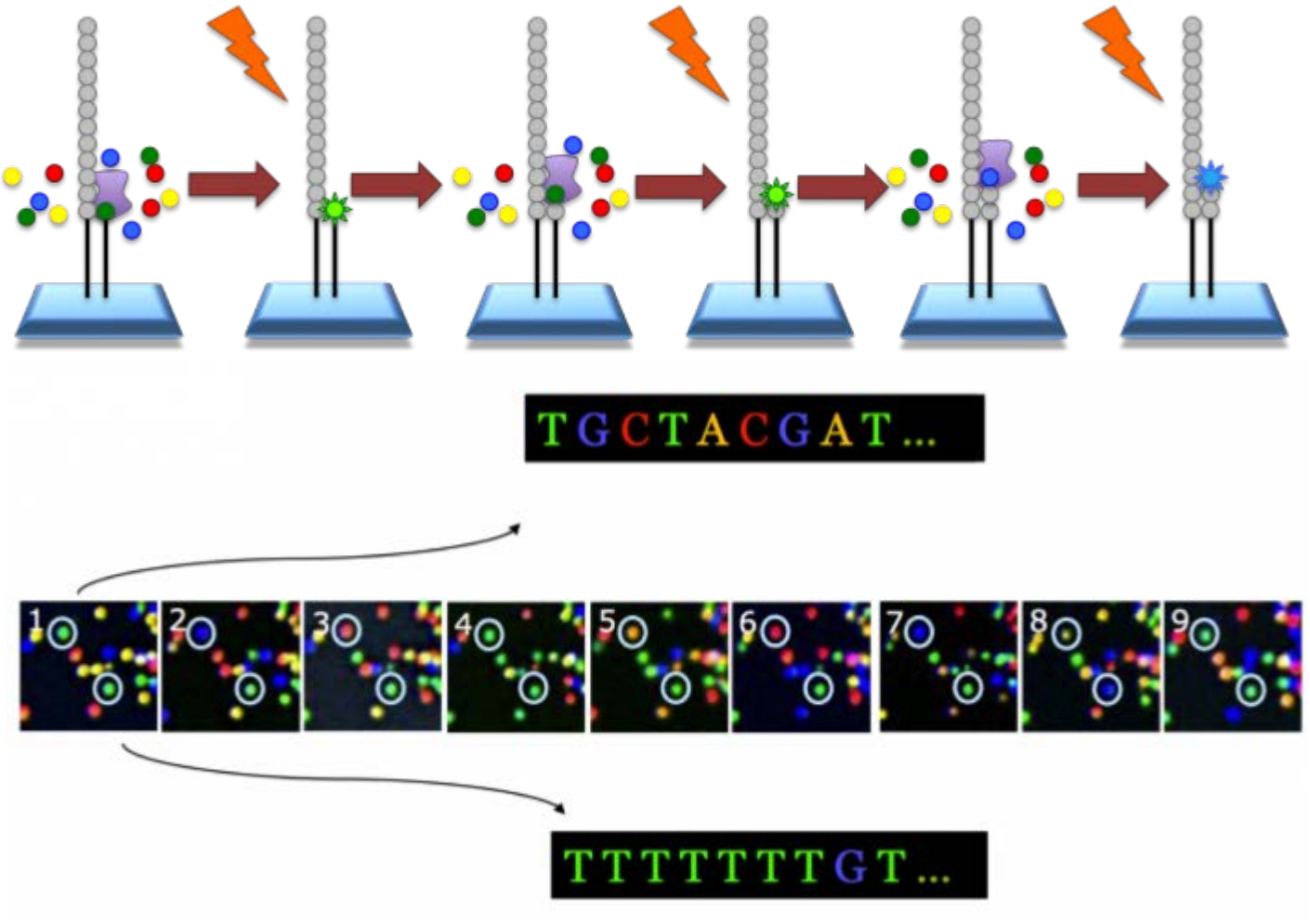


NGS Illumina

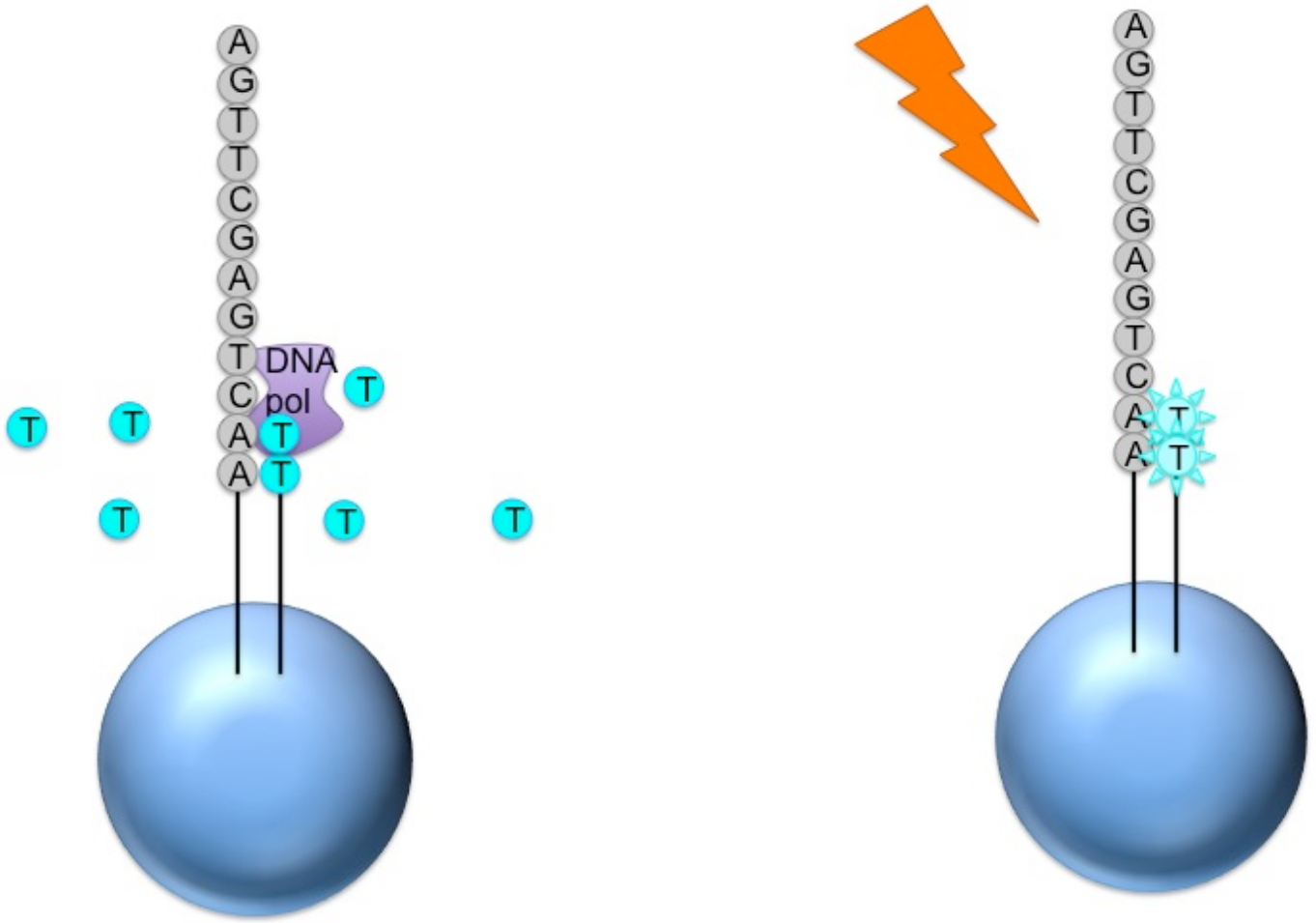


100-150 bp

NGS Illumina

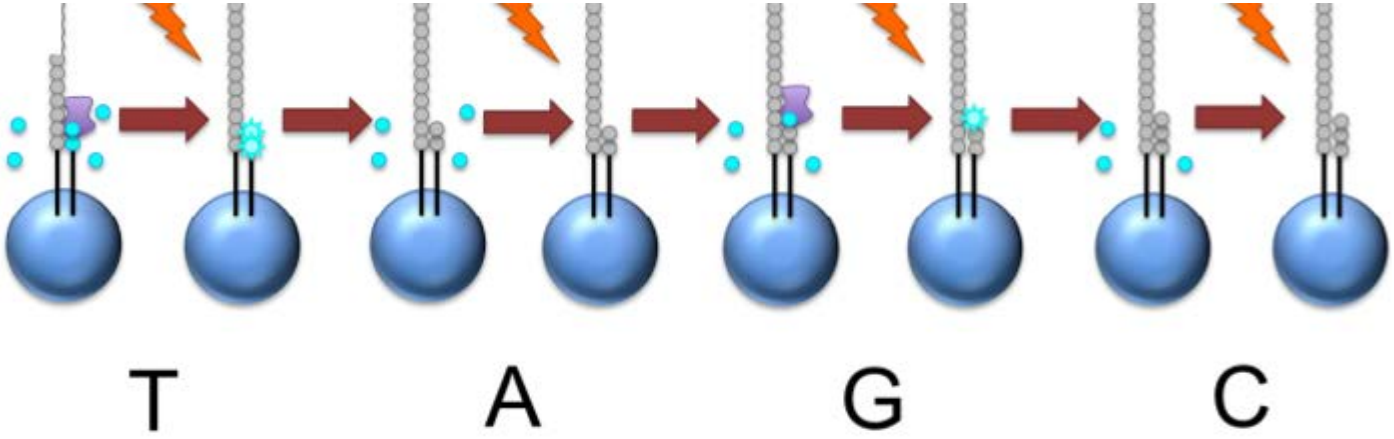


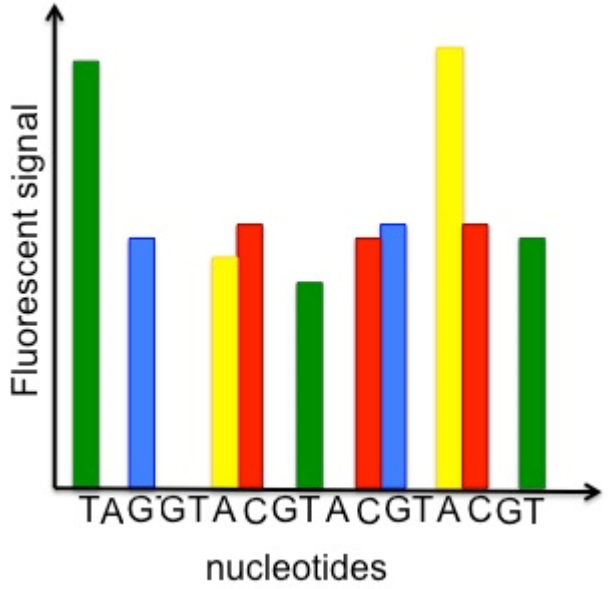
Roche 454 sequencing



1000 bp

Roche 454 sequencing





The four main advantages of NGS over classical Sanger sequencing are:

speed
cost
sample size
accuracy

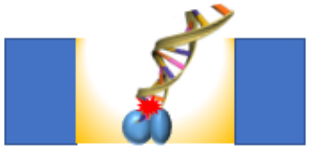
NGS is significantly cheaper, quicker, needs significantly less DNA and is more accurate and reliable than Sanger sequencing.

NGS is quicker than Sanger sequencing in two ways. Firstly, the chemical reaction may be combined with the signal detection in some versions of NGS, whereas in Sanger sequencing these are two separate processes. Secondly and more significantly, only one read (maximum ~1kb) can be taken at a time in Sanger sequencing, whereas NGS is massively parallel, allowing 300Gb of DNA to be read on a single run on a single chip.
The first human genome sequence cost in the region of £300M. Using modern Sanger sequencing methods, aided by data from the known sequence, a full human genome would still cost £6M. Sequencing a human genome with Illumina today would cost only £6,000.

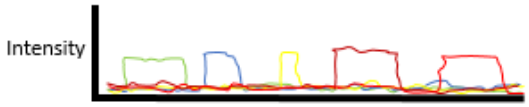
Third Generation Sequencing

PacBio SMRT seq

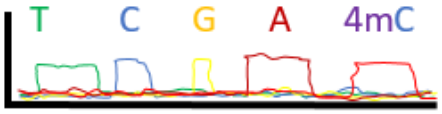
DNA passes thru polymerase in an illuminated volume



Raw output is fluorescent signal of the nucleotide incorporation, specific to each nucleotide

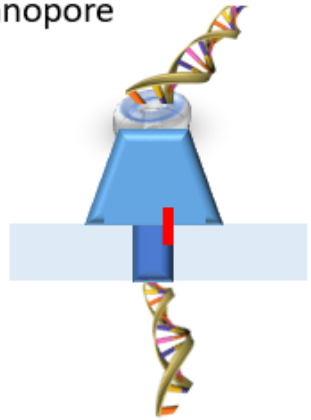


A,C,T,G have known pulse durations, which are used to infer methylated nucleotides

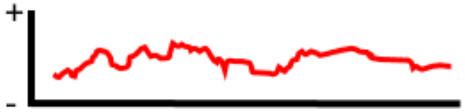


Oxford Nanopore

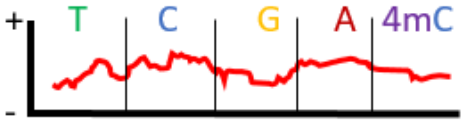
DNA passes thru nanopore



Raw output is electrical signal caused by nucleotide blocking ion flow in nanopore

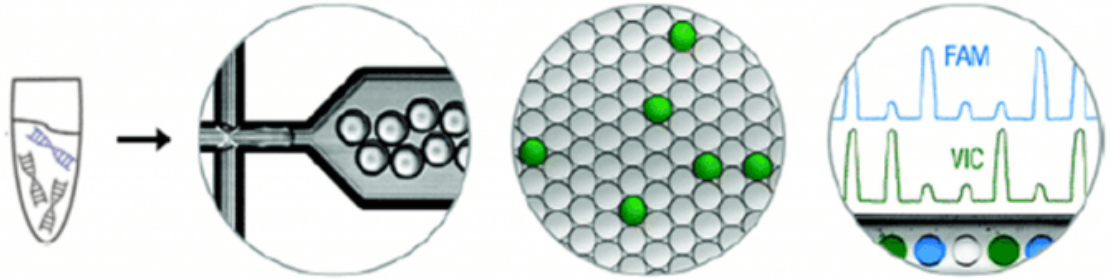


Each nucleotide has a specific electric “signature”



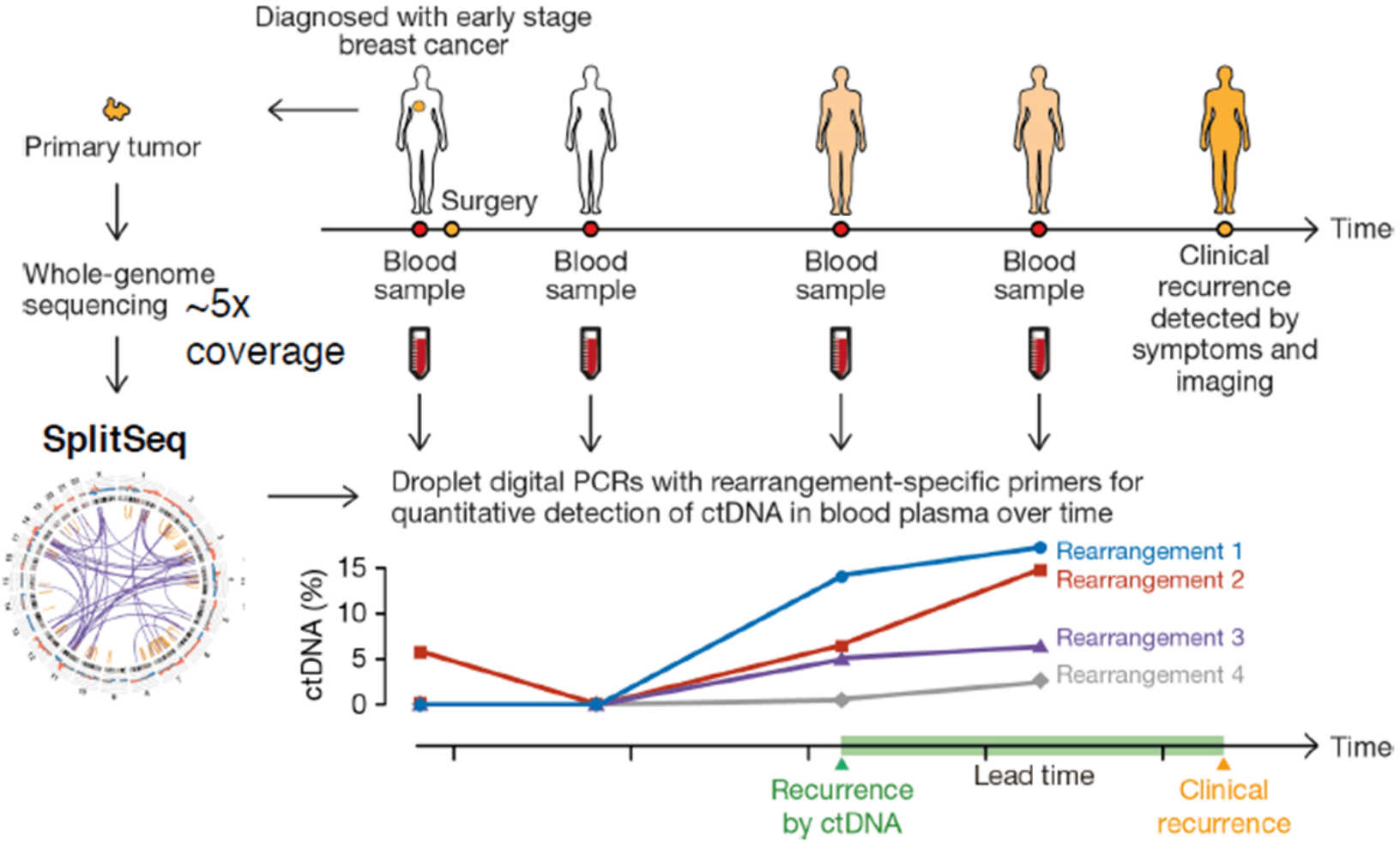
Digital PCR

Droplet digital PCR

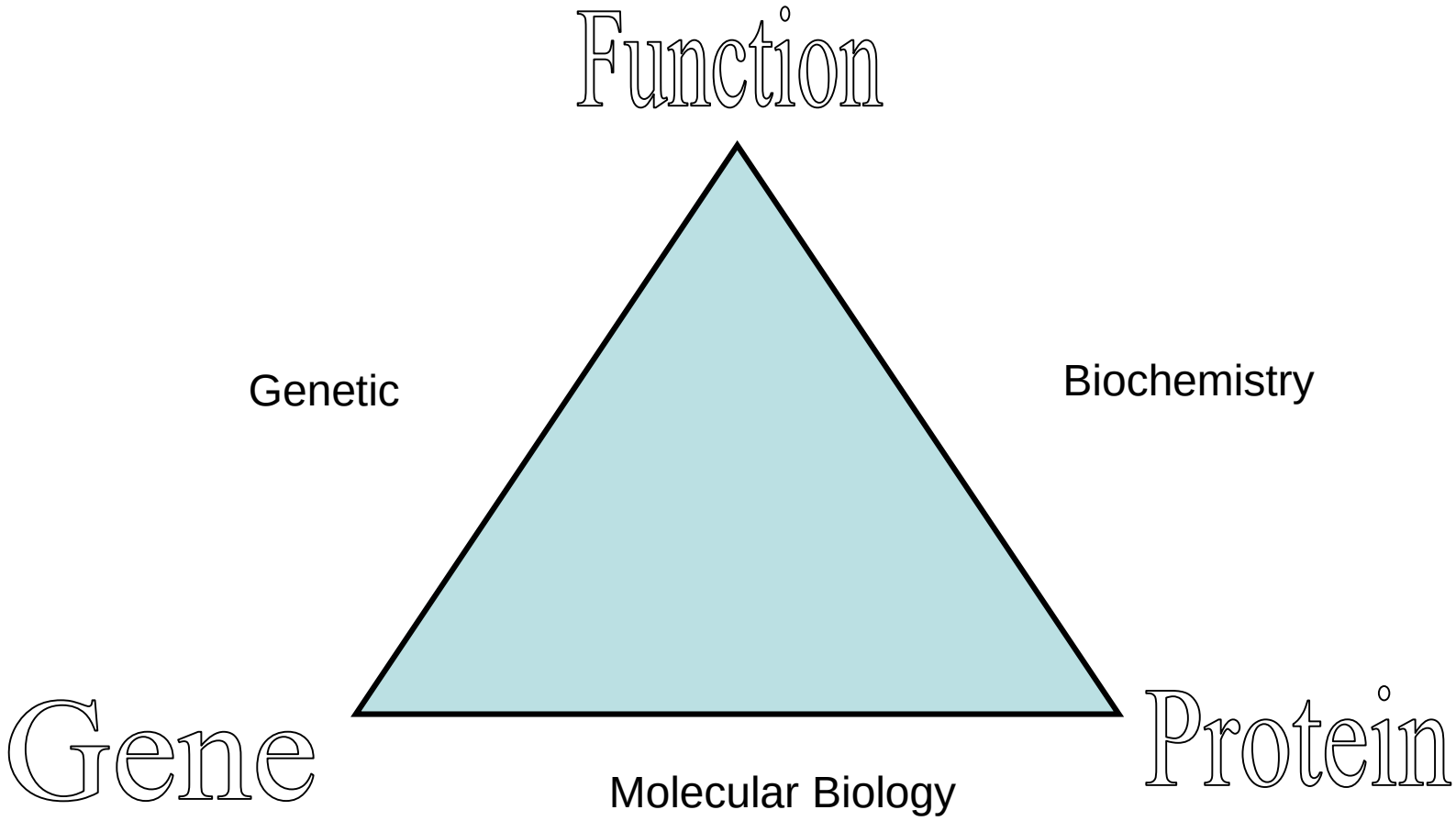


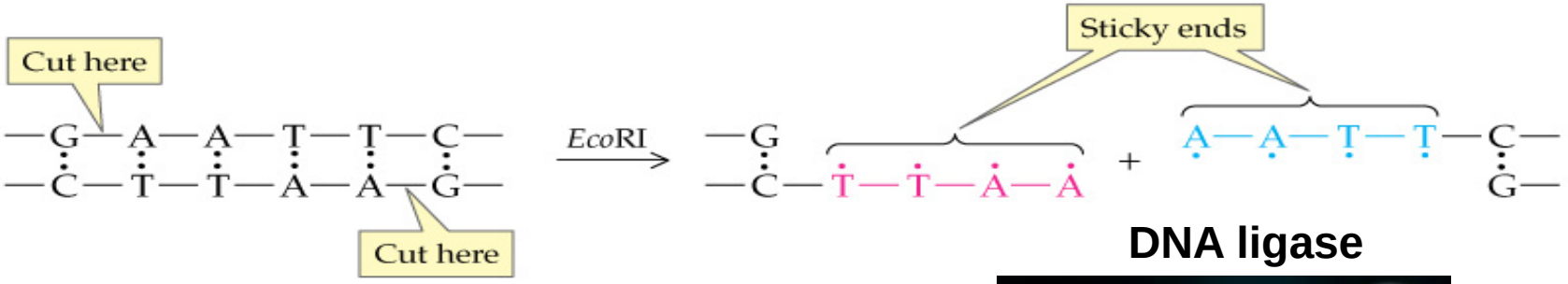
Bio-Rad QX100

1. MAKE	2. CYCLE	3. READ	
Sample is partitioned into 20,000 droplets	Run PCR cycles in all droplets simultaneously	Measure fluorescence intensity in each droplet	Calculate concentration from number of positive droplets

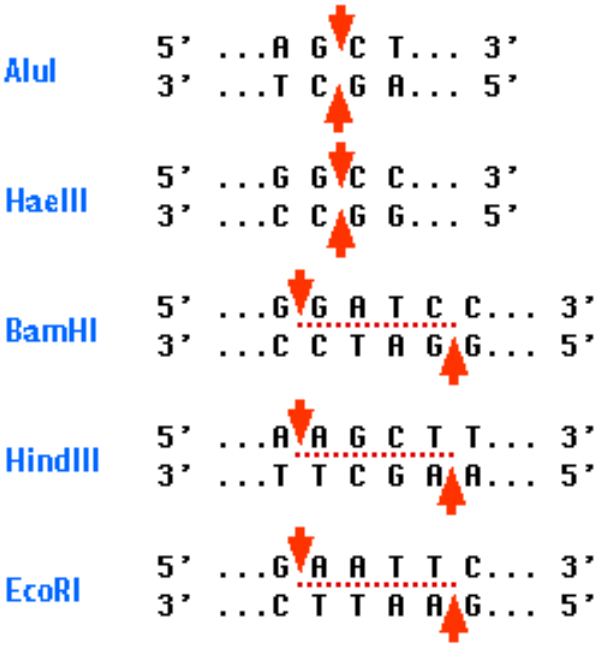

Diagnosed with early stage breast cancer
Primary tumor
Surgery
Time
Whole-genome sequencing
~5x coverage
Blood sample
Blood sample
Blood sample
Blood sample
Clinical recurrence detected by symptoms and imaging
SplitSeq
Droplet digital PCRs with rearrangement-specific primers for quantitative detection of ctDNA in blood plasma over time
ctDNA (%)
15
10
5
0
Rearrangement 1
Rearrangement 2
Rearrangement 3
Rearrangement 4
Time
Lead time
Recurrence by ctDNA
Clinical recurrence

Recombinant DNA





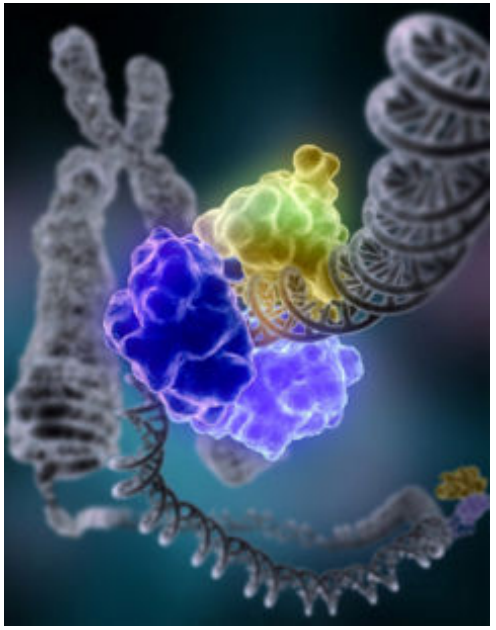
Restriction Enzyme

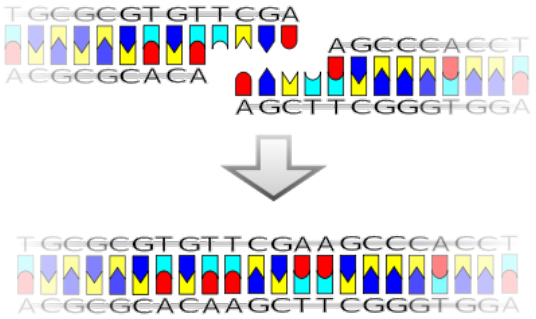


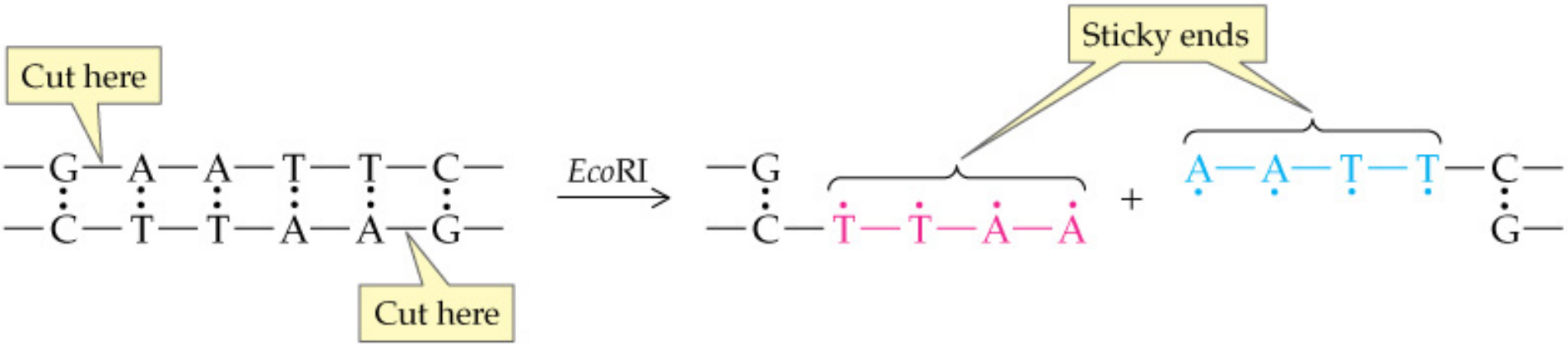
AluI and **HaeIII** produce blunt ends

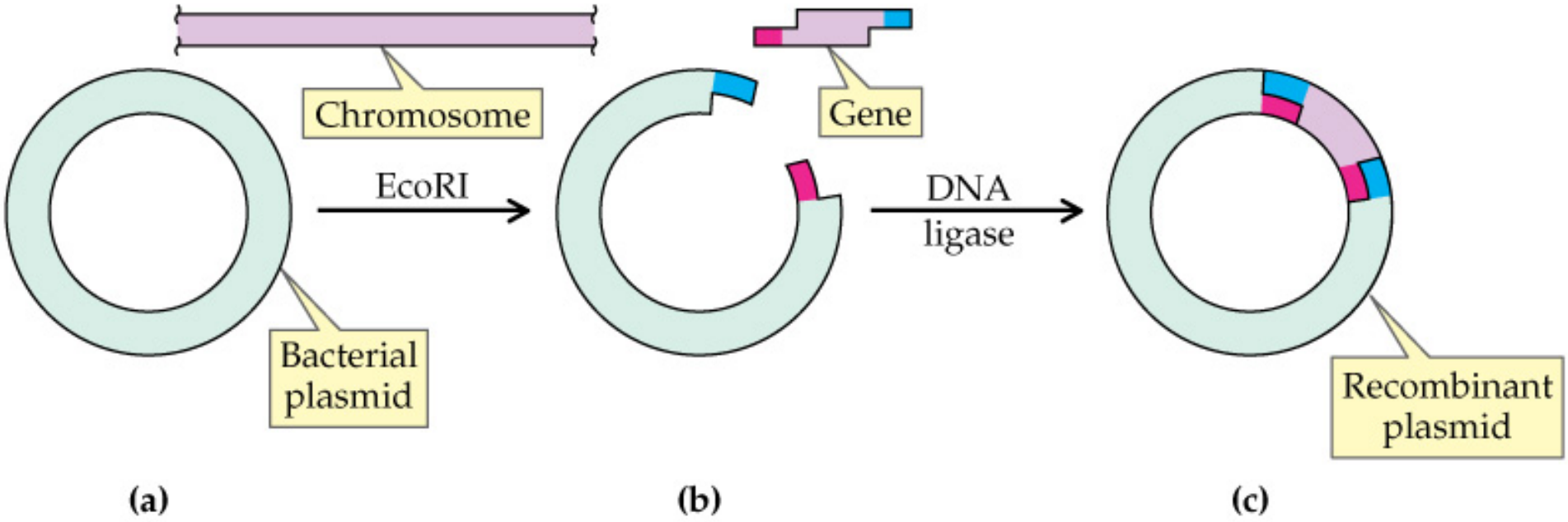
BamHI **HindIII** and **EcoRI** produce "sticky" ends

DNA ligase



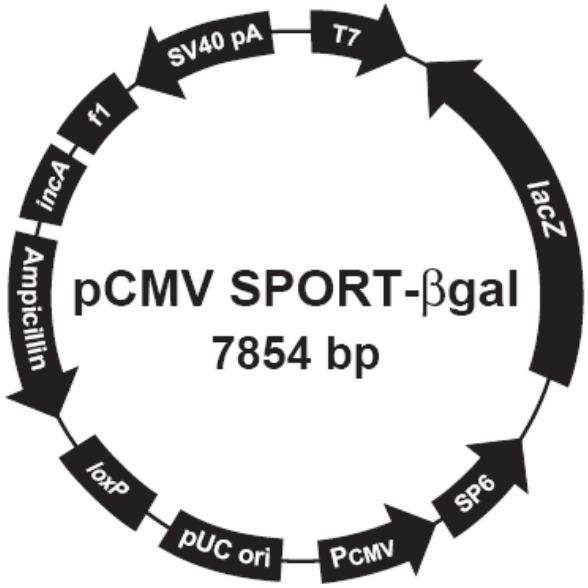


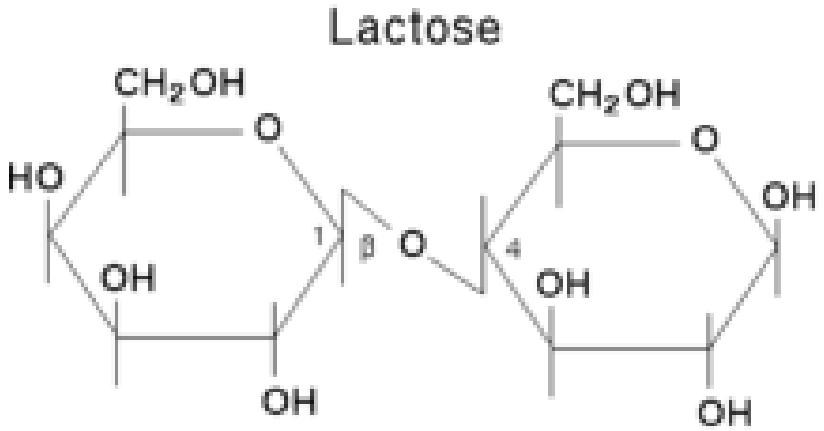

Cut here
G A A T T C
C T T A A G
Cut here
EcoRI
Sticky ends
G
C T T A A
+
A A T T C
G


Chromosome
Gene
EcoRI
DNA
ligase
Bacterial
plasmid
Recombinant
plasmid
(a)
(b)
(c)

β-Galactosidase

The enzyme that splits lactose into glucose and galactose. Coded by a gene (lacZ) in the lac operon of Escherichia coli.

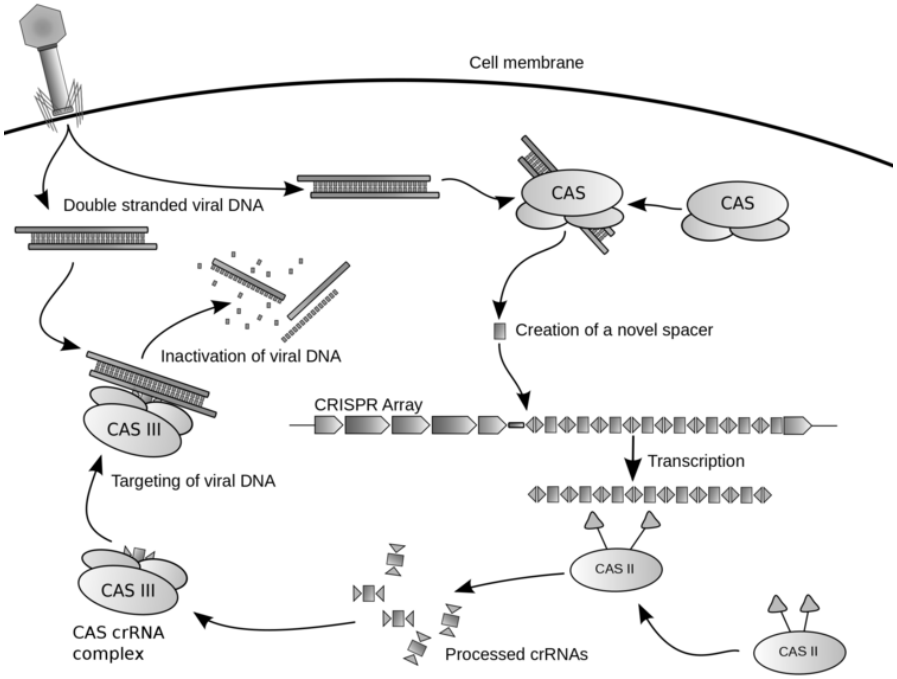




PUC is a family of plasmids that have an ampicillin resistance gene and more importantly a *lacZ* gene. A functional lacZ gene will produce the protein β - galactosidase. Bacterial colonies in which β - galactosidase is produced, will form blue colonies in the presence of the substrate 5 - bromo - 4 - chloro - 3 - indolyl - b - D - galactoside or as it is more commonly referred to, X-gal.

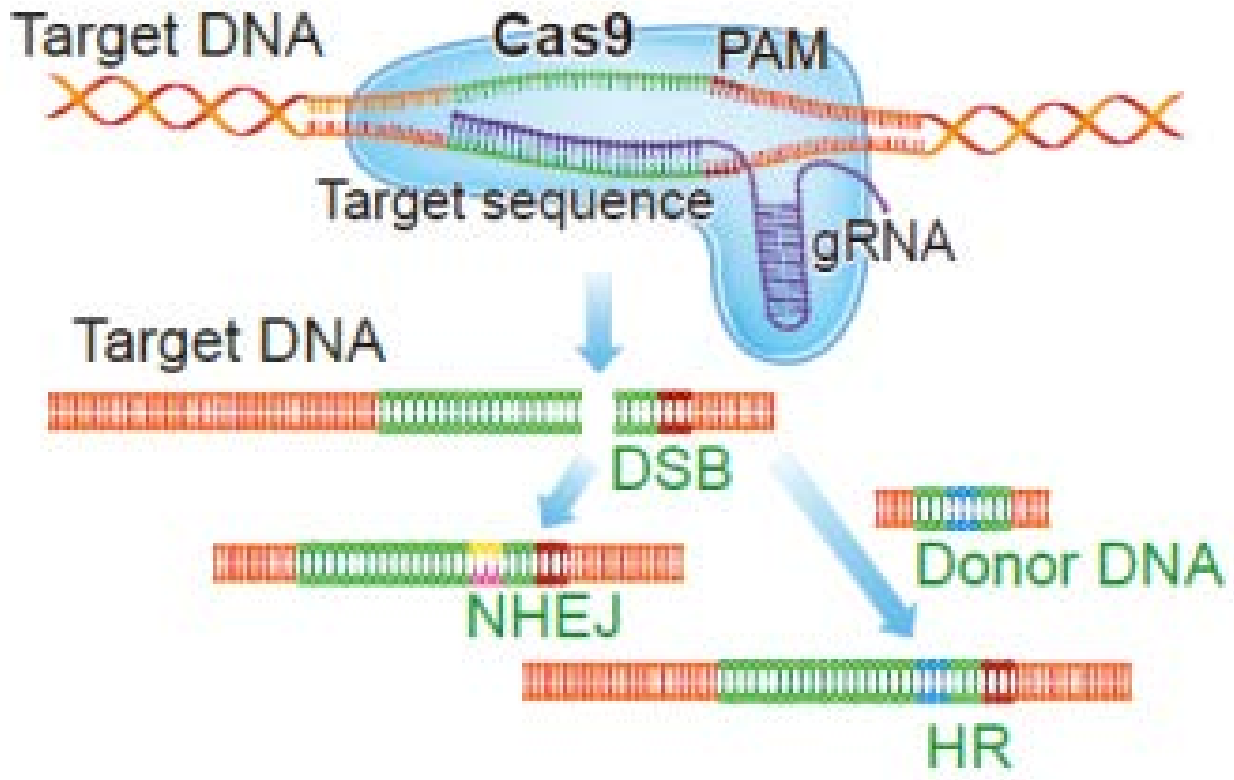
CRISPR CAS9

CRISPRs (**clustered regularly interspaced short palindromic repeats**) are segments of prokaryotic DNA containin g short repetitions of base sequences. Each repetition is followed by short segments of "spacer DNA" from previous exposures to a bacterial virus or

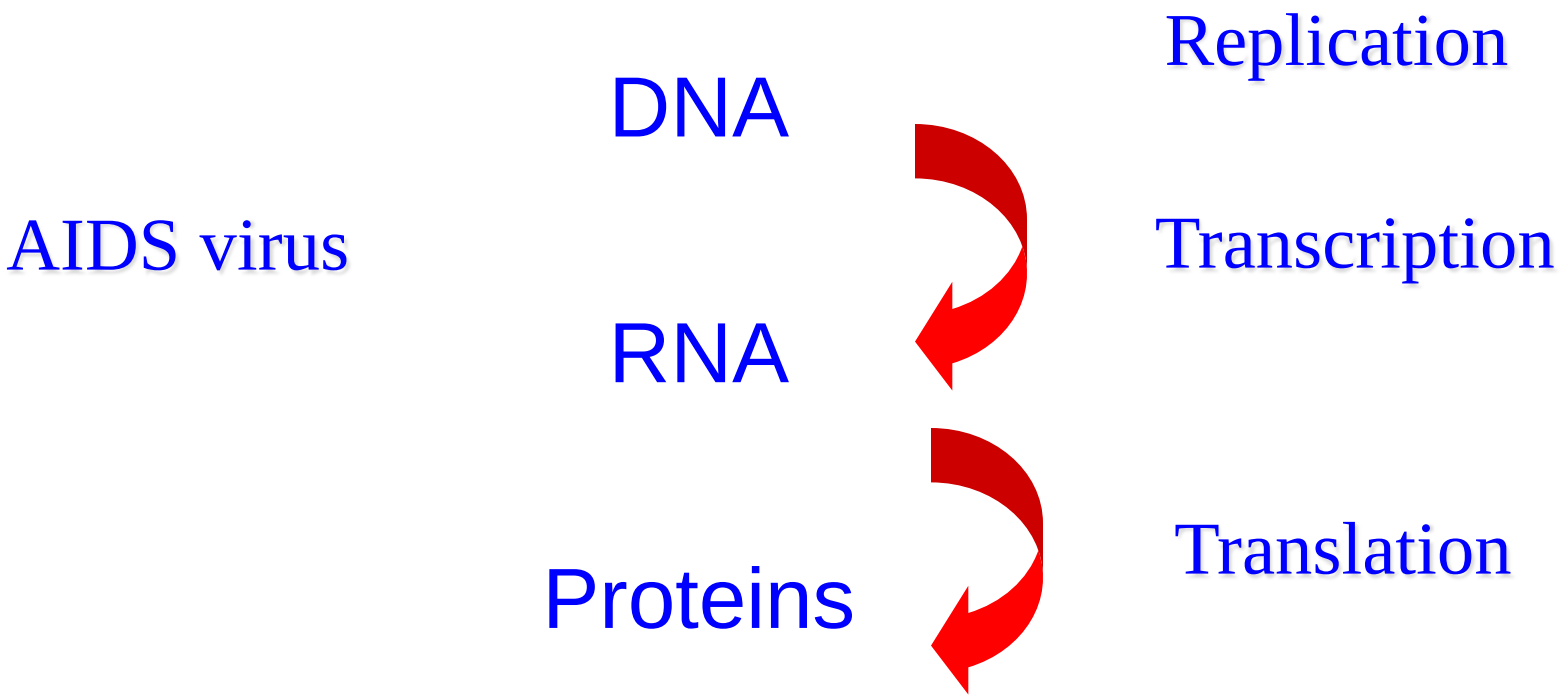


Movie

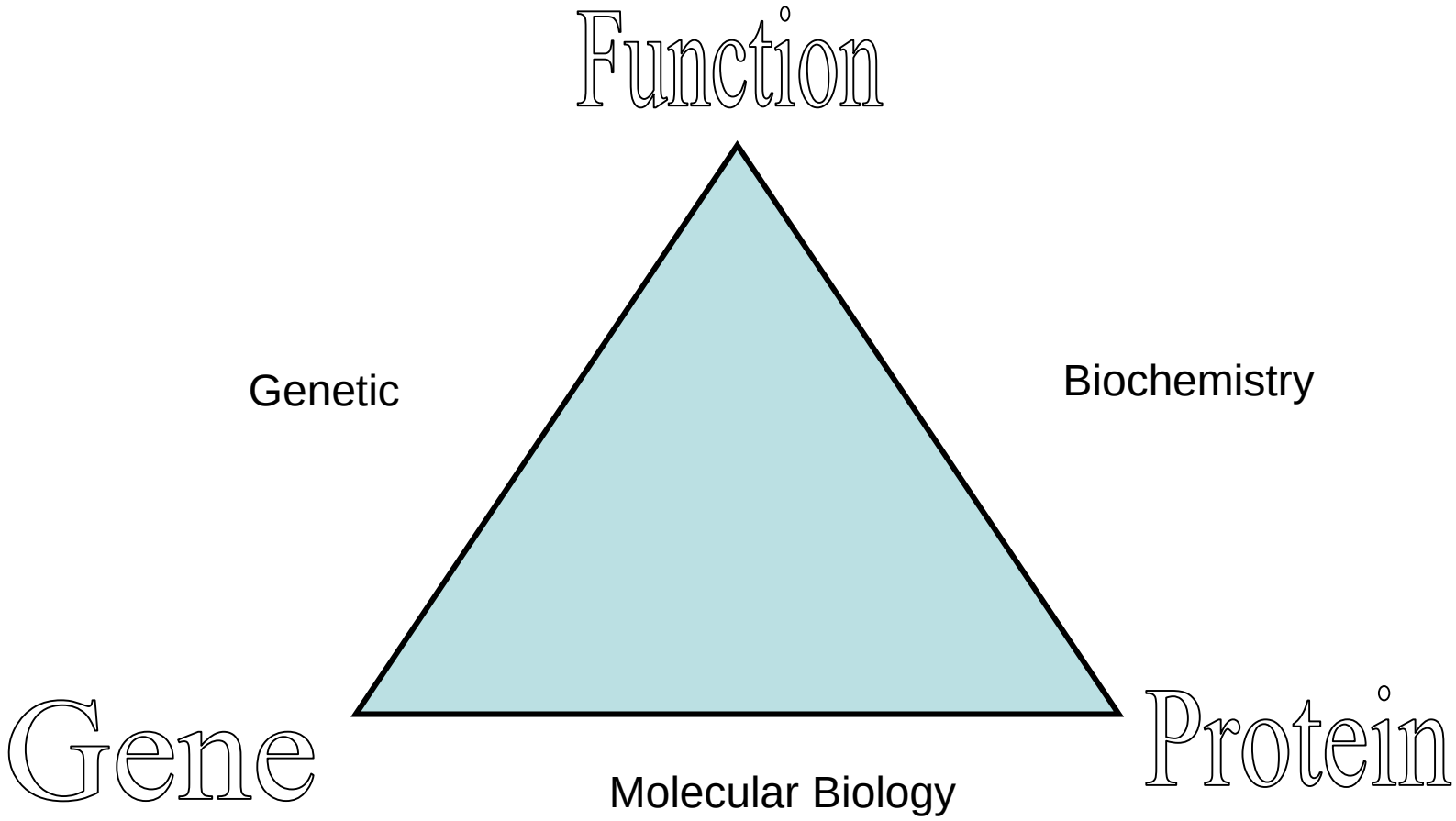
- https://youtu.be/2pp17E4E-O8

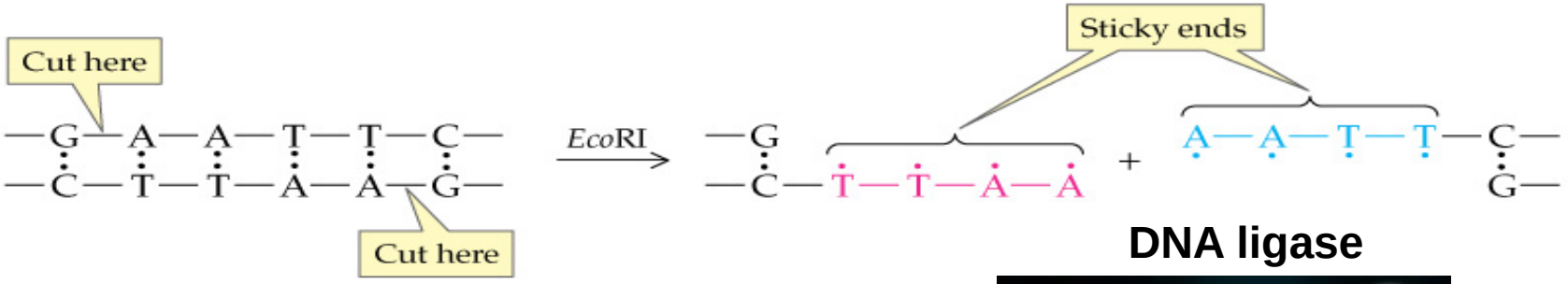

Target DNA
Cas9
PAM
Target sequence
gRNA
Target DNA
DSB
NHEJ
Donor DNA
HR

Central Dogma



Recombinant DNA





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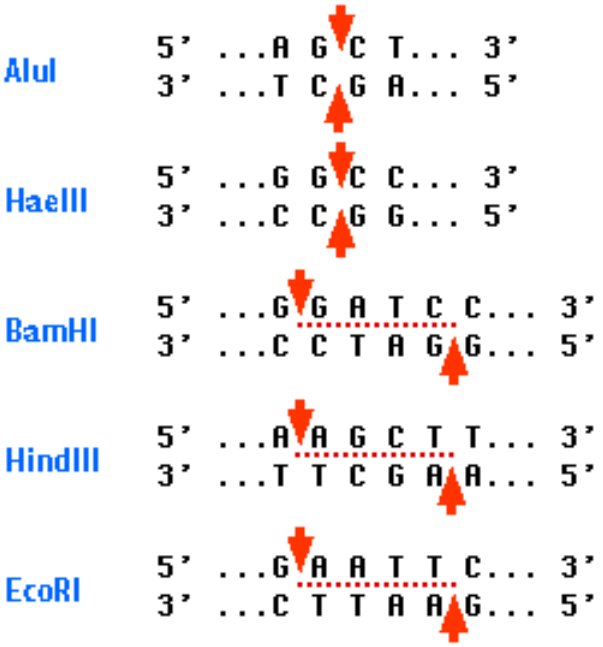

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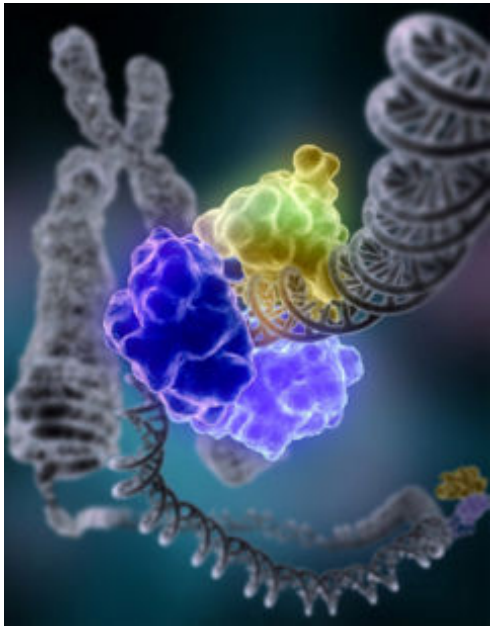

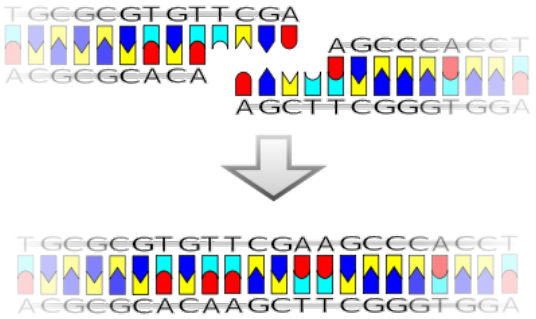

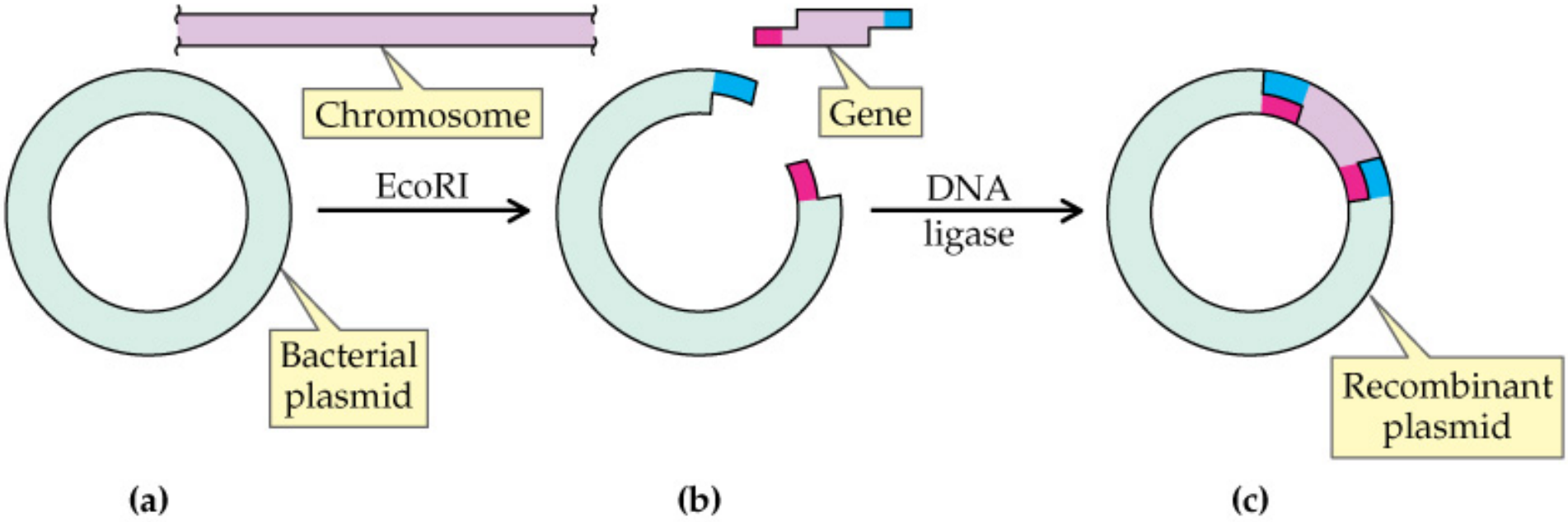
Chromosome
Gene
EcoRI
DNA
ligase
Bacterial
plasmid
Recombinant
plasmid
(a)
(b)
(c)

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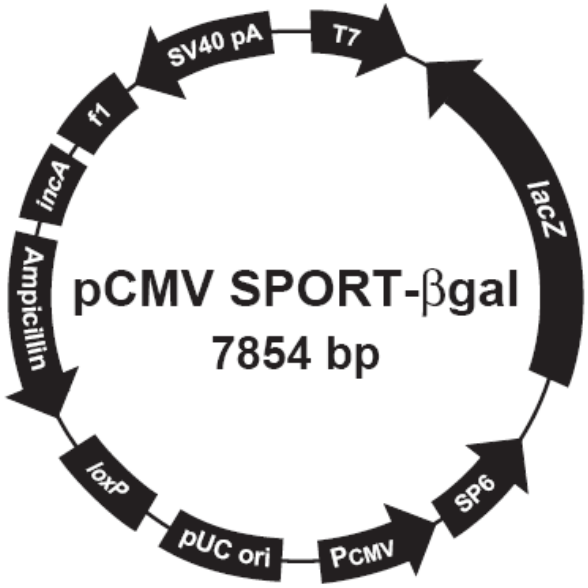

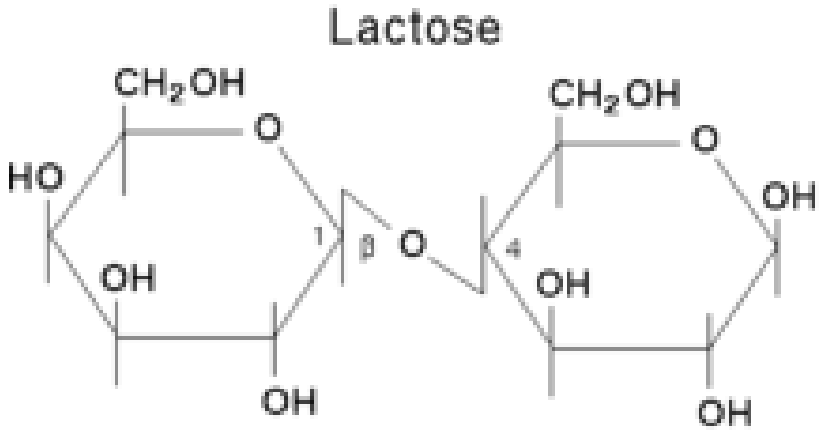

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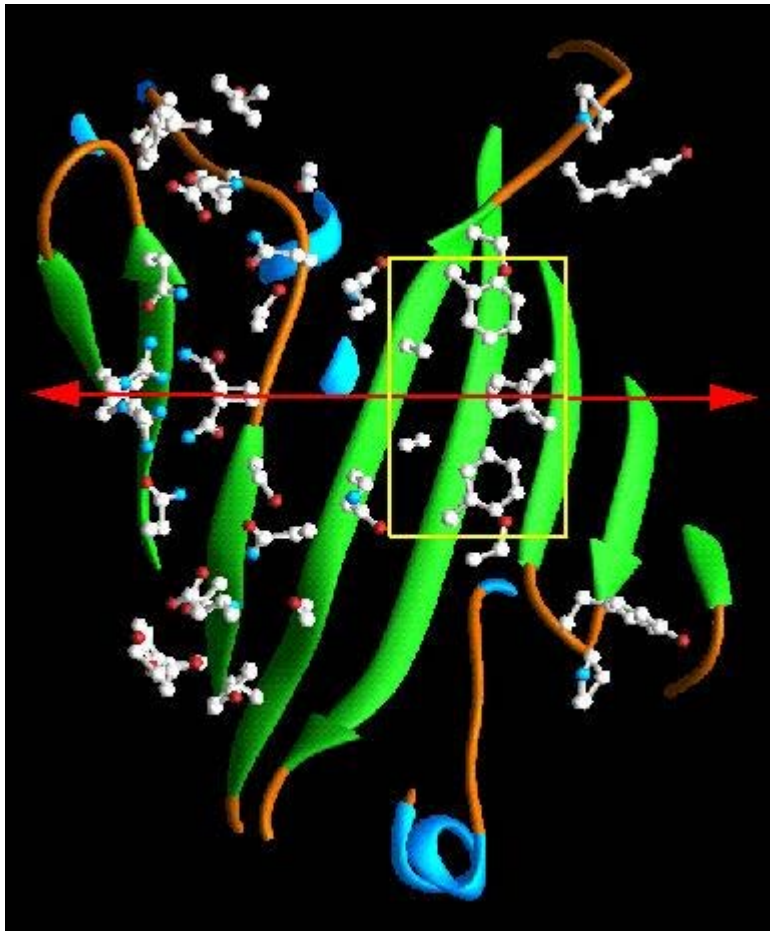
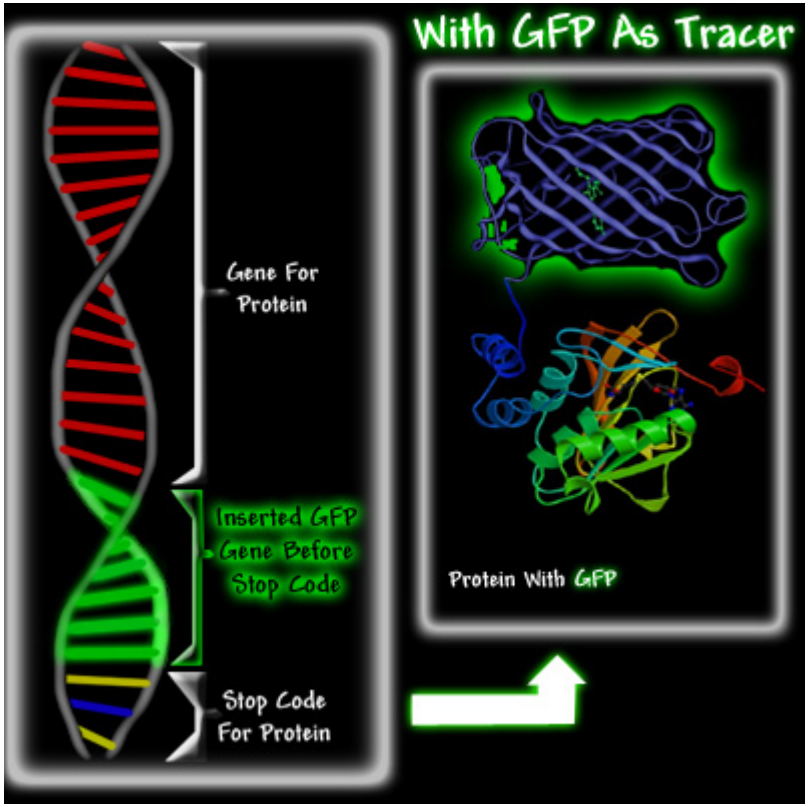
Green Fluorescent Protein (GFP)



The **green fluorescent protein** (**GFP**) is a protein from the jellyfish *Aequorea victoria* that fluoresces green when exposed to blue light.

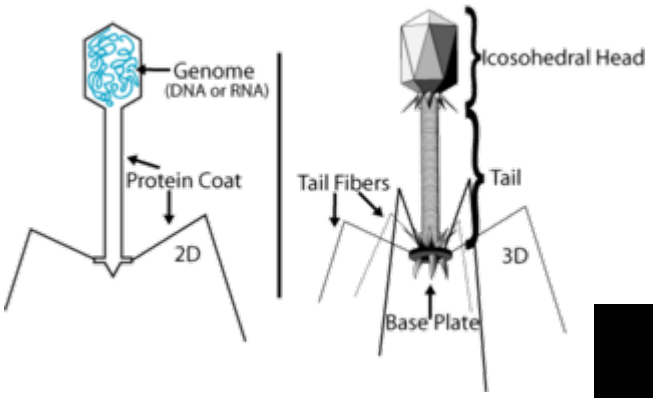


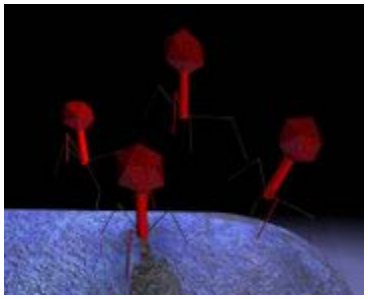
GFP Rats

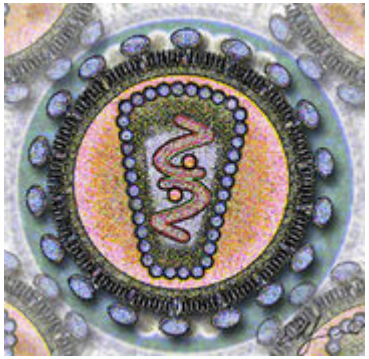
Life

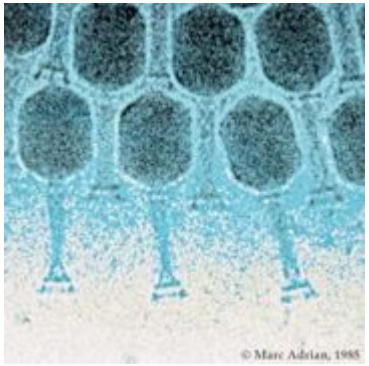
- Replication: reproduction
- Function: catalytic functions
- RNA world:
- Virus is not alive

Virus


Genome
(DNA or RNA)
Protein Coat
2D
Icosohedral Head
Tail Fibers
Tail
3D
Base Plate



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Virus Reproduction