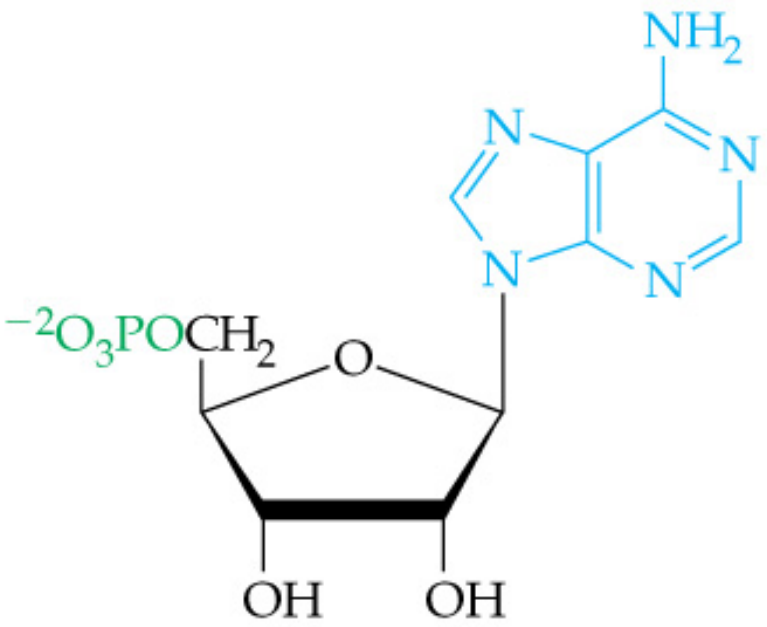
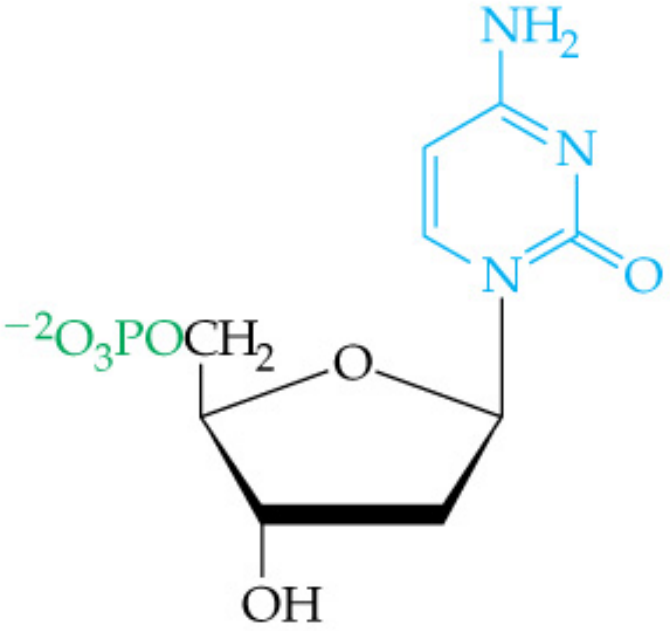
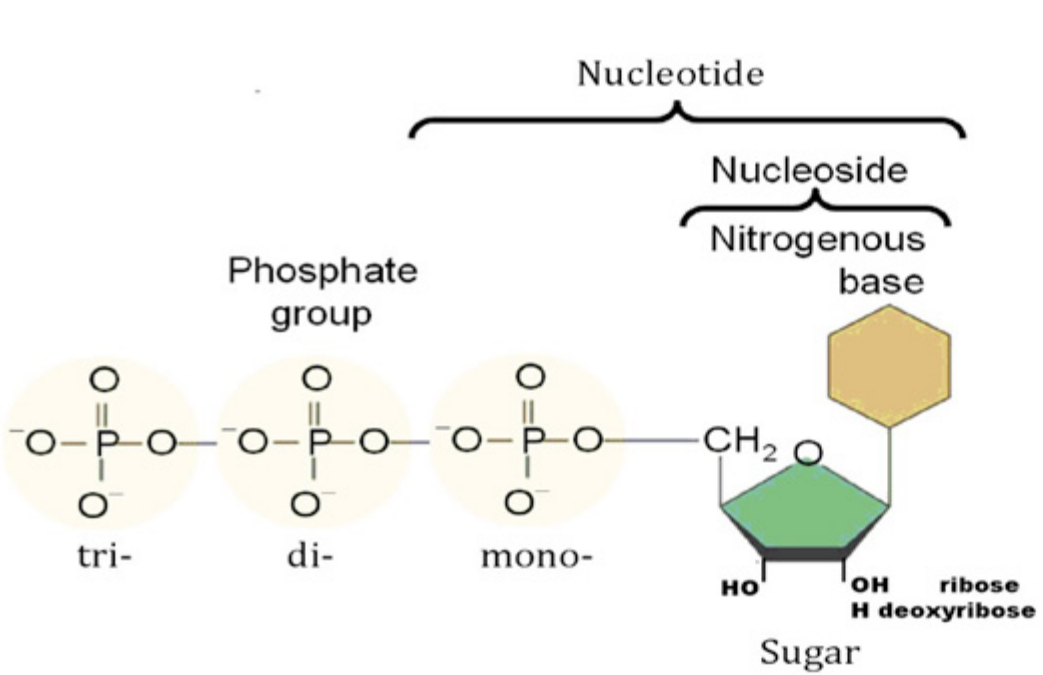
- In RNA, the sugar is ribose.
- In DNA, the sugar is deoxyribose.

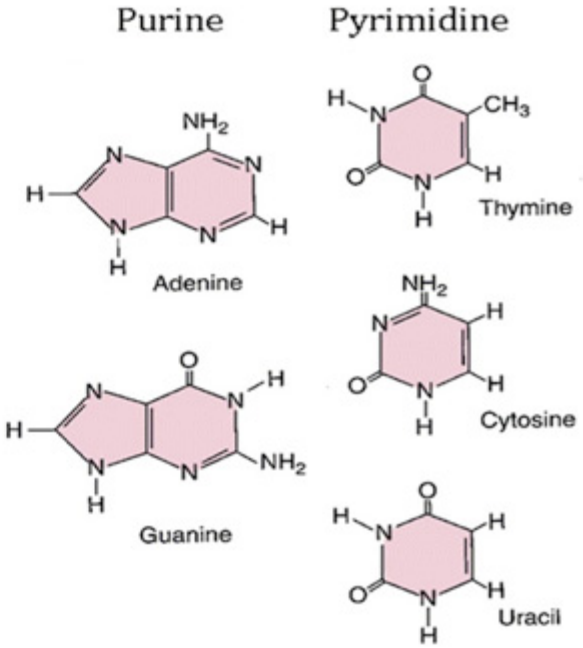


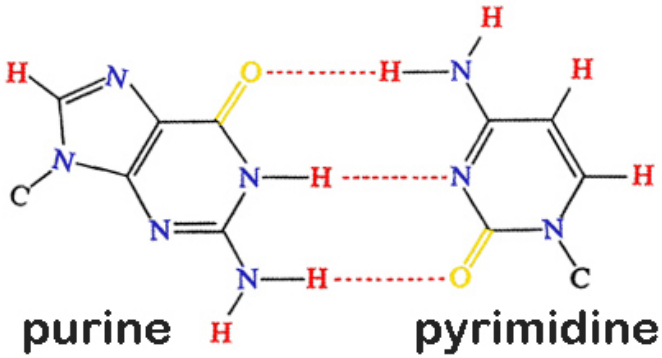
Adenosine 5′-monophosphate (AMP)
(a ribonucleotide)



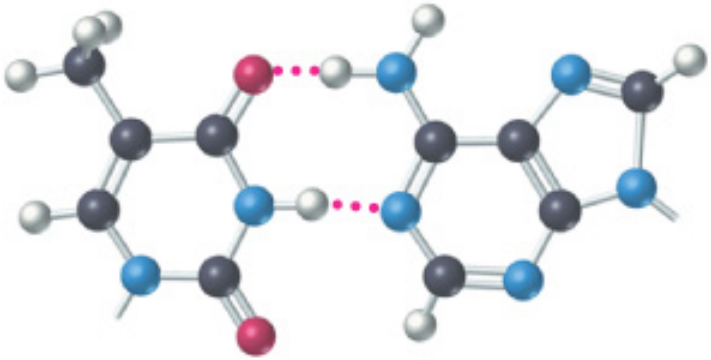
Deoxycytidine 5′-monophosphate (dCMP)
(a deoxyribonucleotide)

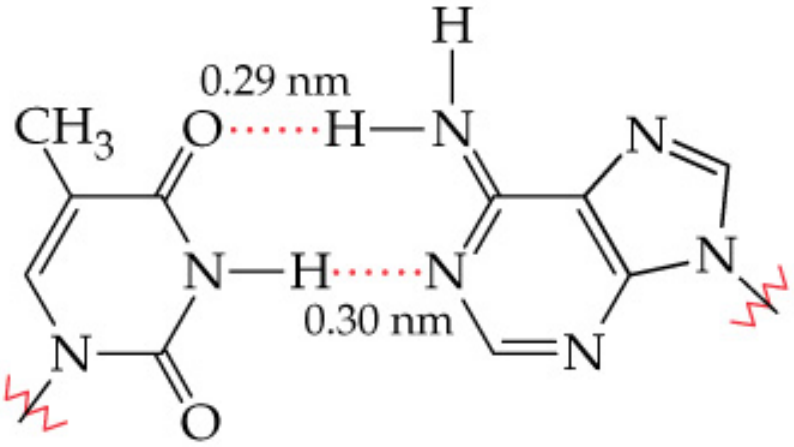

Nucleotide
Nucleoside
Nitrogenous base
Phosphate group
tri-
di-
mono-
HO
OH
H
ribose
deoxyribose
Sugar


Purine
Pyrimidine
Adenine
Thymine
Guanine
Cytosine
Uracil

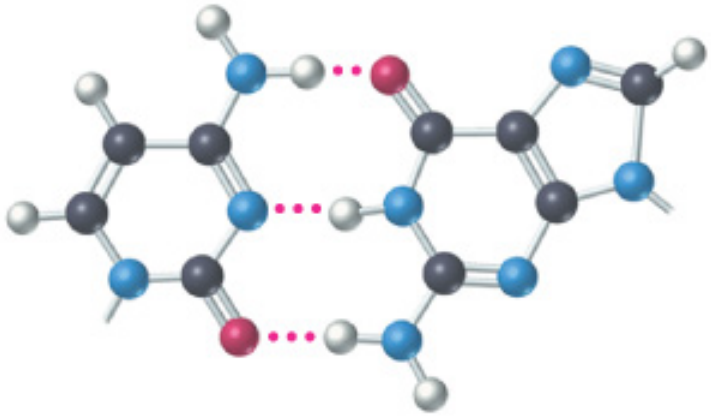

purine
pyrimidine

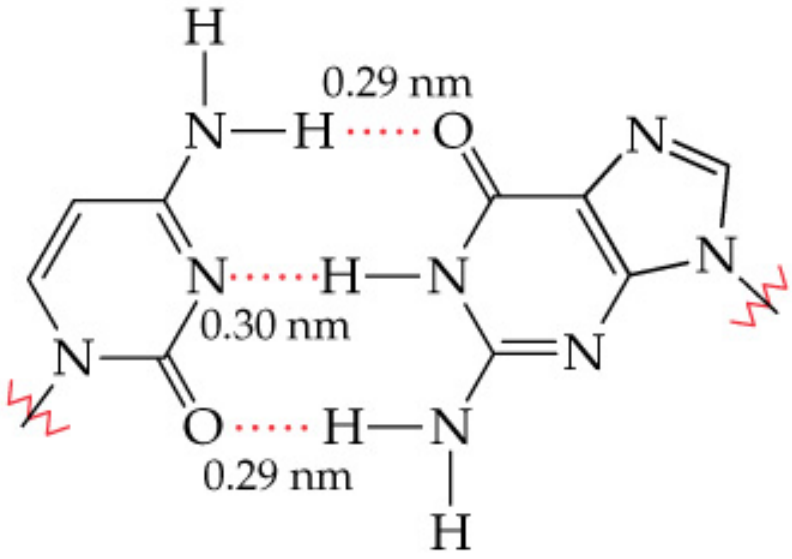
Thymine–Adenine

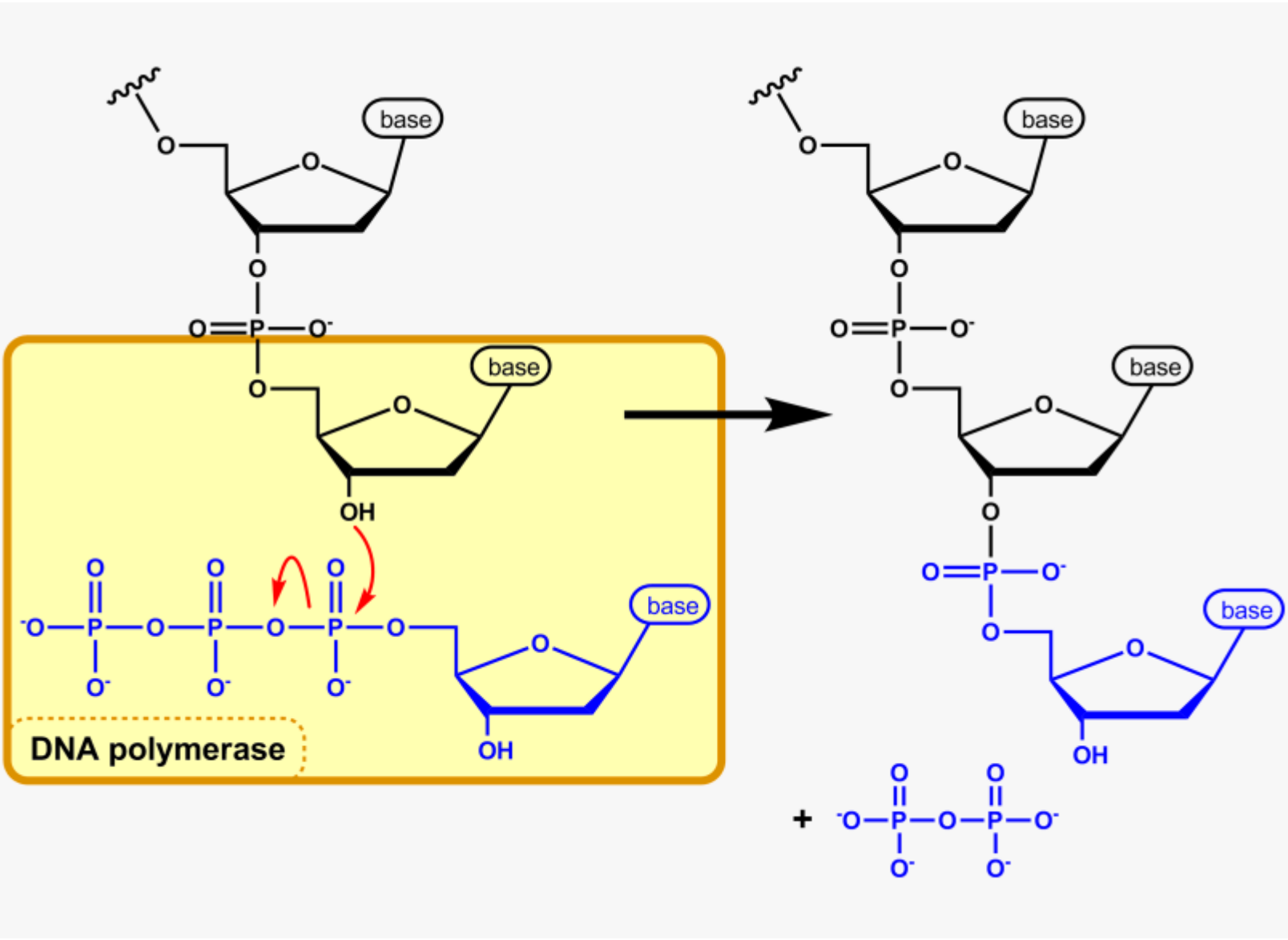


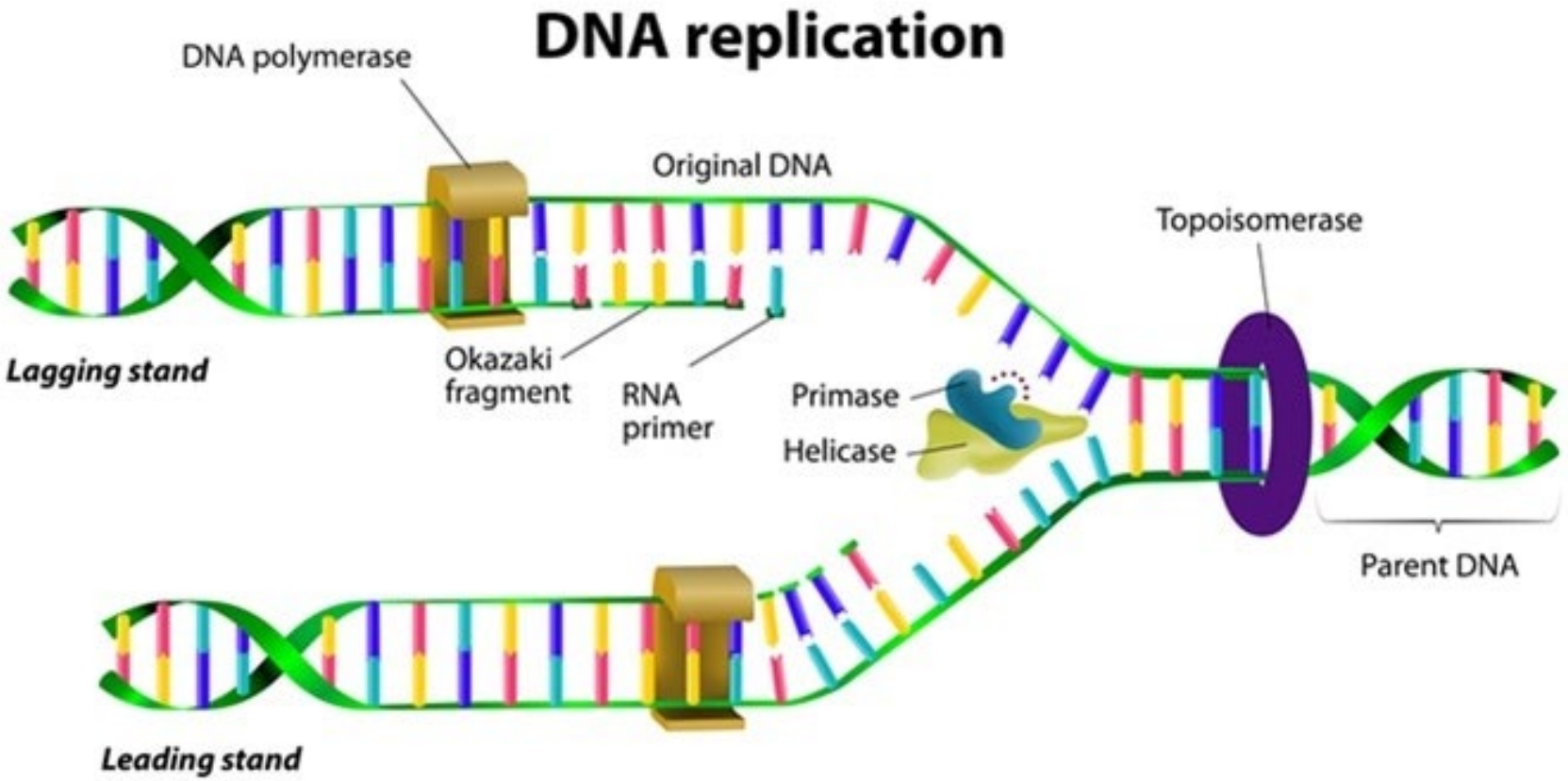


Cytosine–Guanine

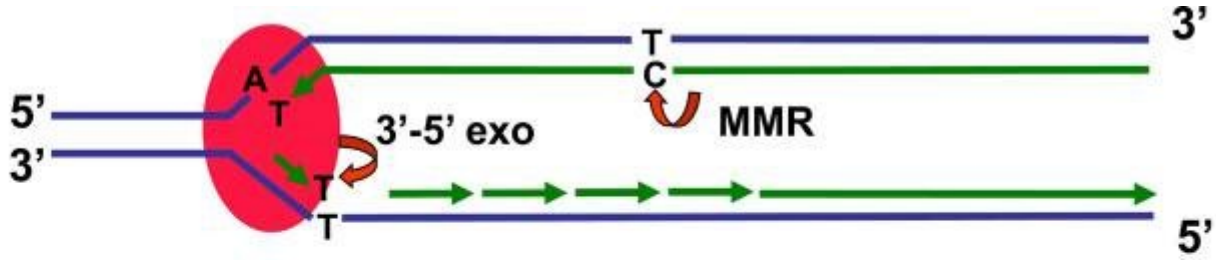





base
O
O
O
O=P—O⁻
OH
DNA polymerase
+



https://youtu.be/TNKWgcFPHqw

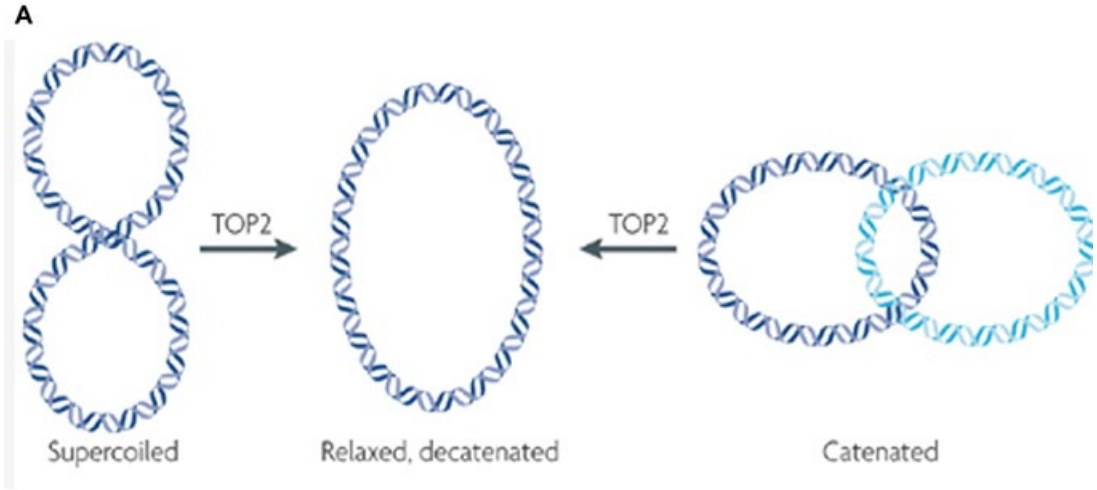


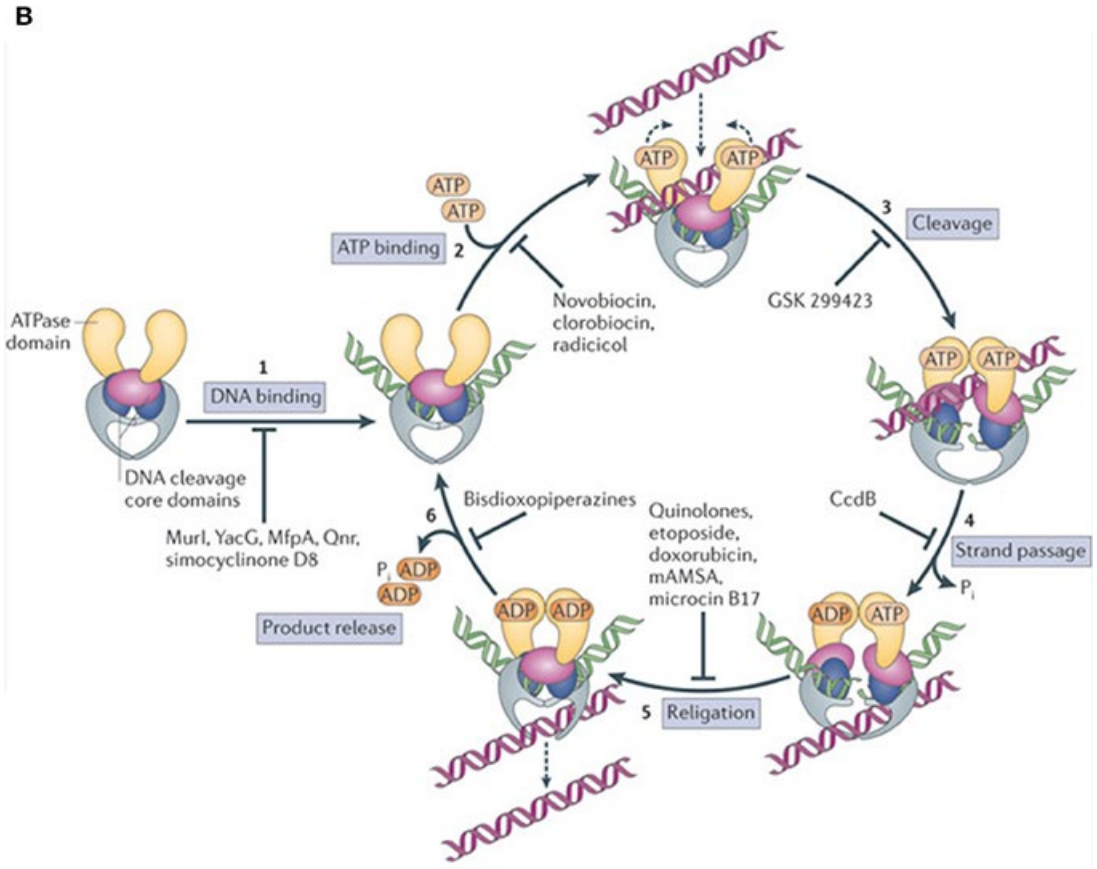
BASE SELECTION $10^{-4} – 10^{-5}$

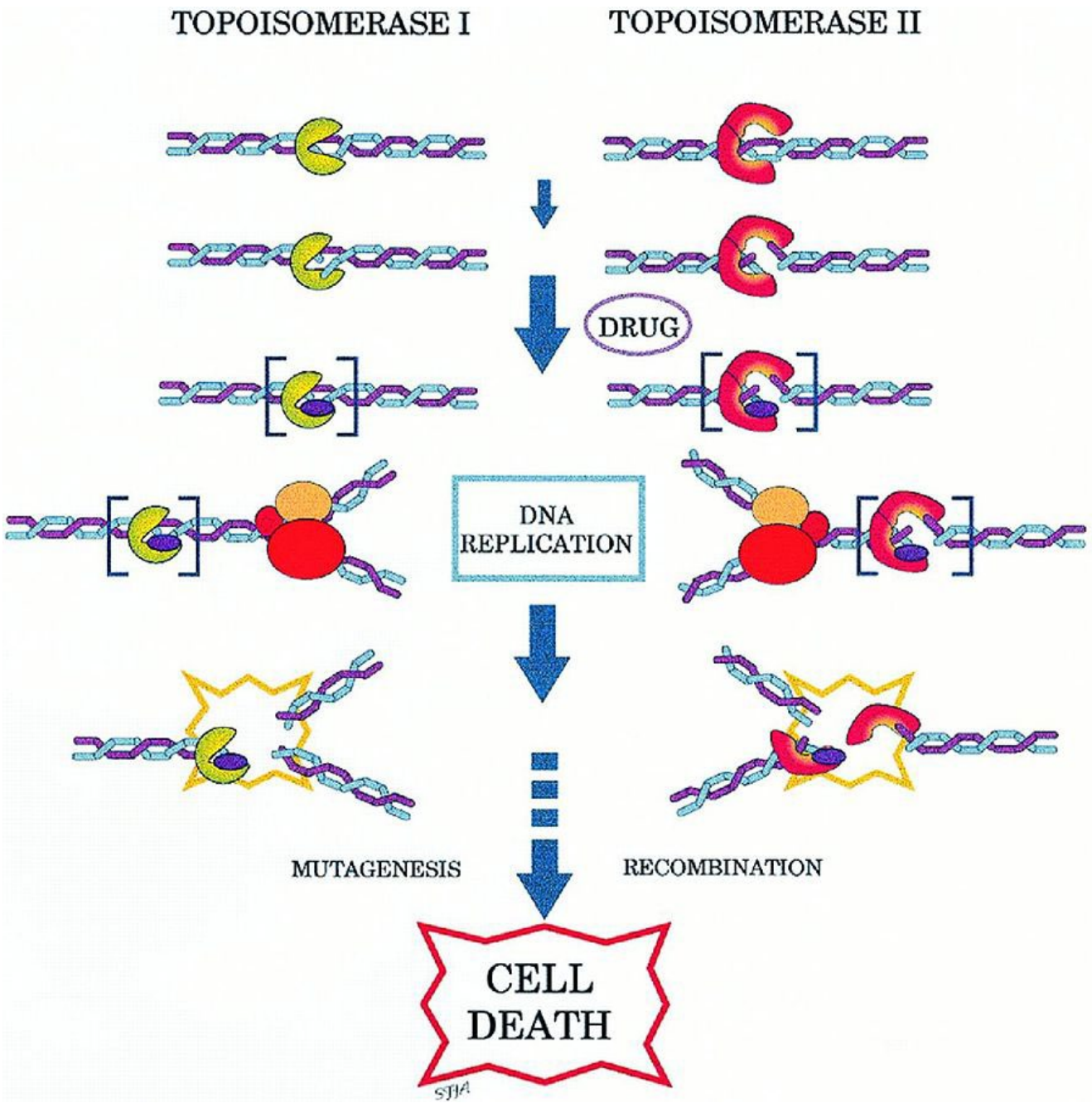
3'-5' EXONUCLEASE 10^{-2}

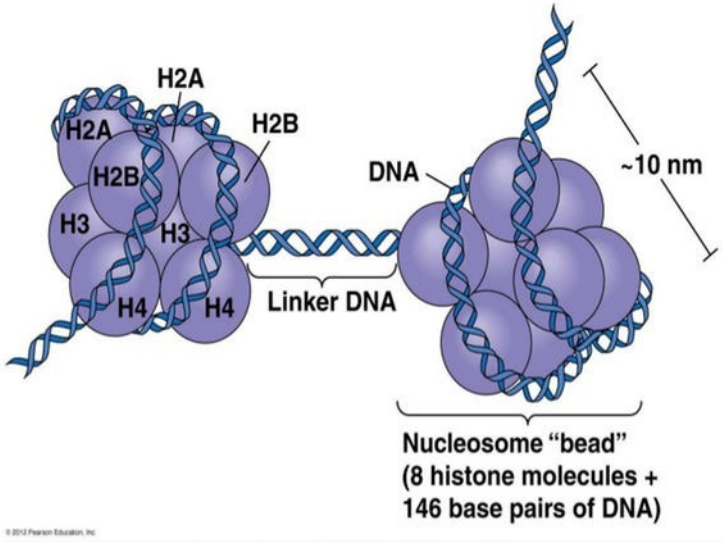
MISMATCH REPAIR 10^{-3}

DNA REPLICATION FIDELITY 10^{-10}

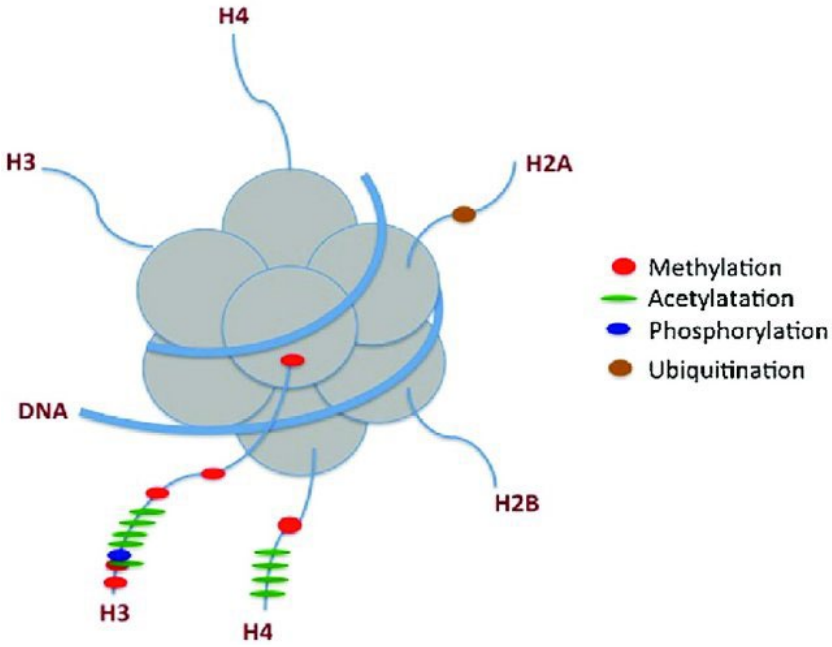

A
TOP2
TOP2
Supercoiled
Relaxed, decatenated
Catenated

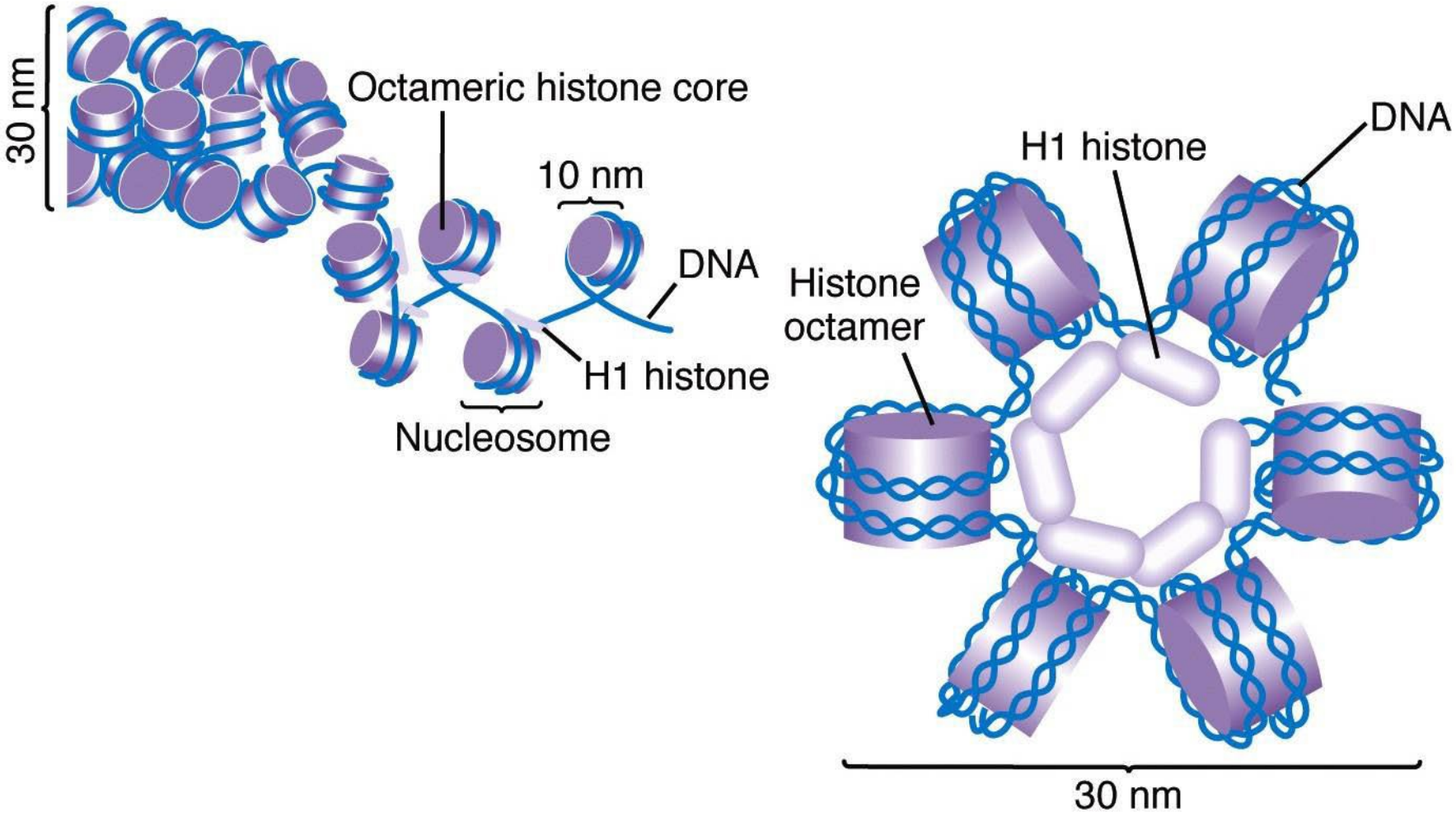

B
ATPase domain
DNA cleavage core domains
1
DNA binding
MurI, YacG, MfpA, Qnr, simocyclinone D8
ATP
ATP
ATP binding
2
Novobiocin, clorobiocin, radicicol
3
Cleavage
GSK 299423
CcdB
4
Strand passage
Quinolones, etoposide, doxorubicin, mAMSA, microcin B17
5
Religation
Bisdioxopiperazines
6
Product release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

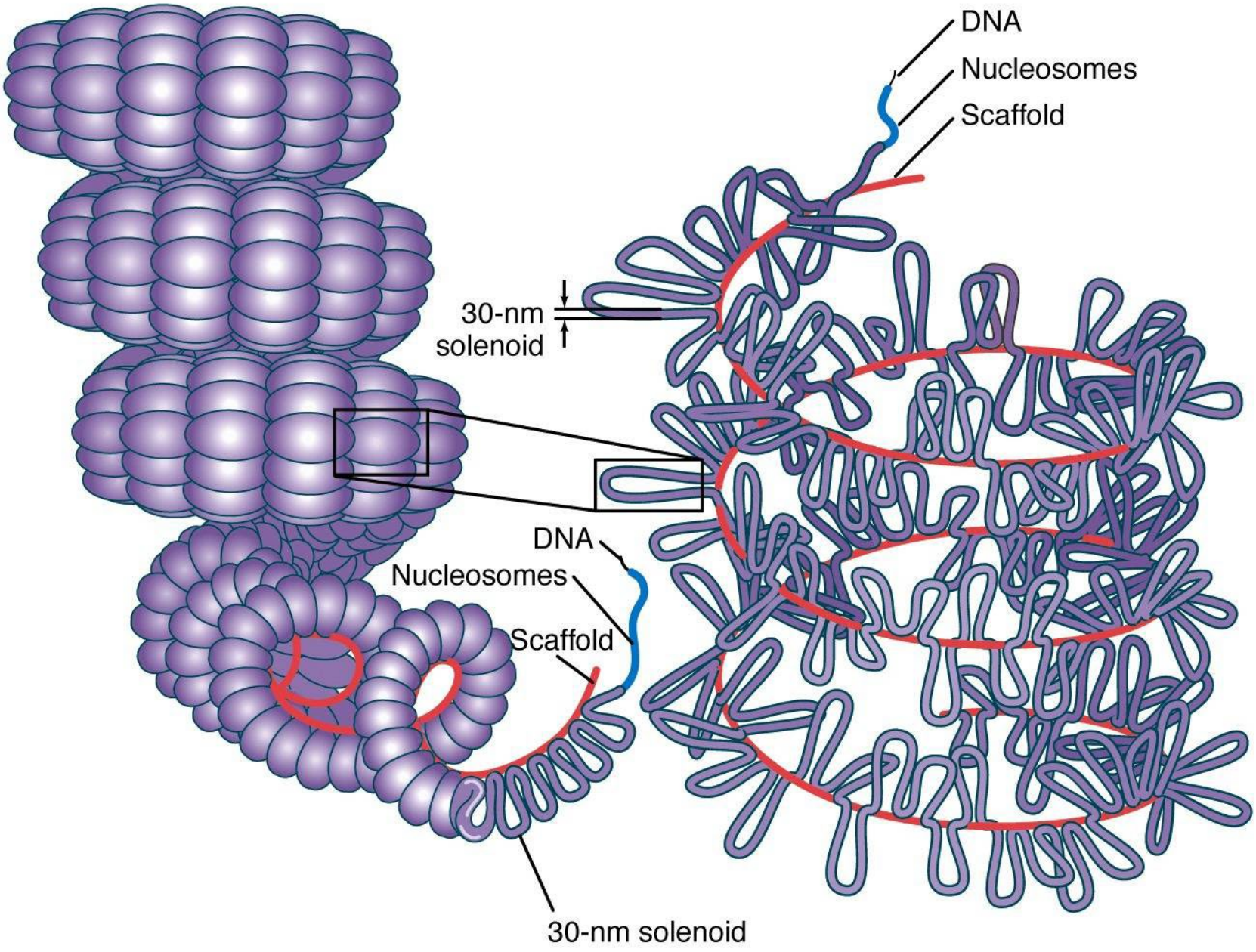

TOPOISOMERASE I
TOPOISOMERASE II
DRUG
DNA REPLICATION
MUTAGENESIS
RECOMBINATION
CELL DEATH

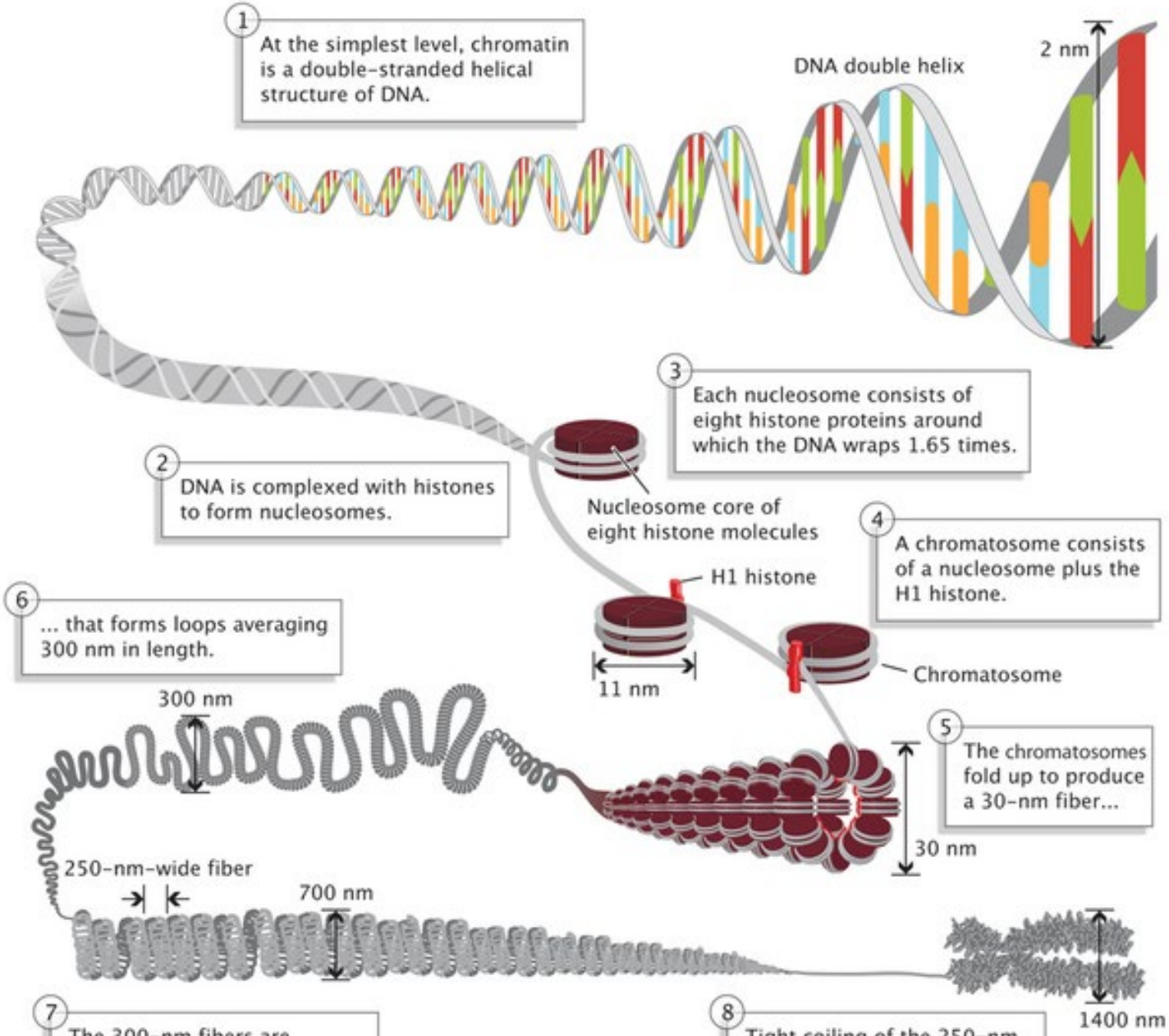


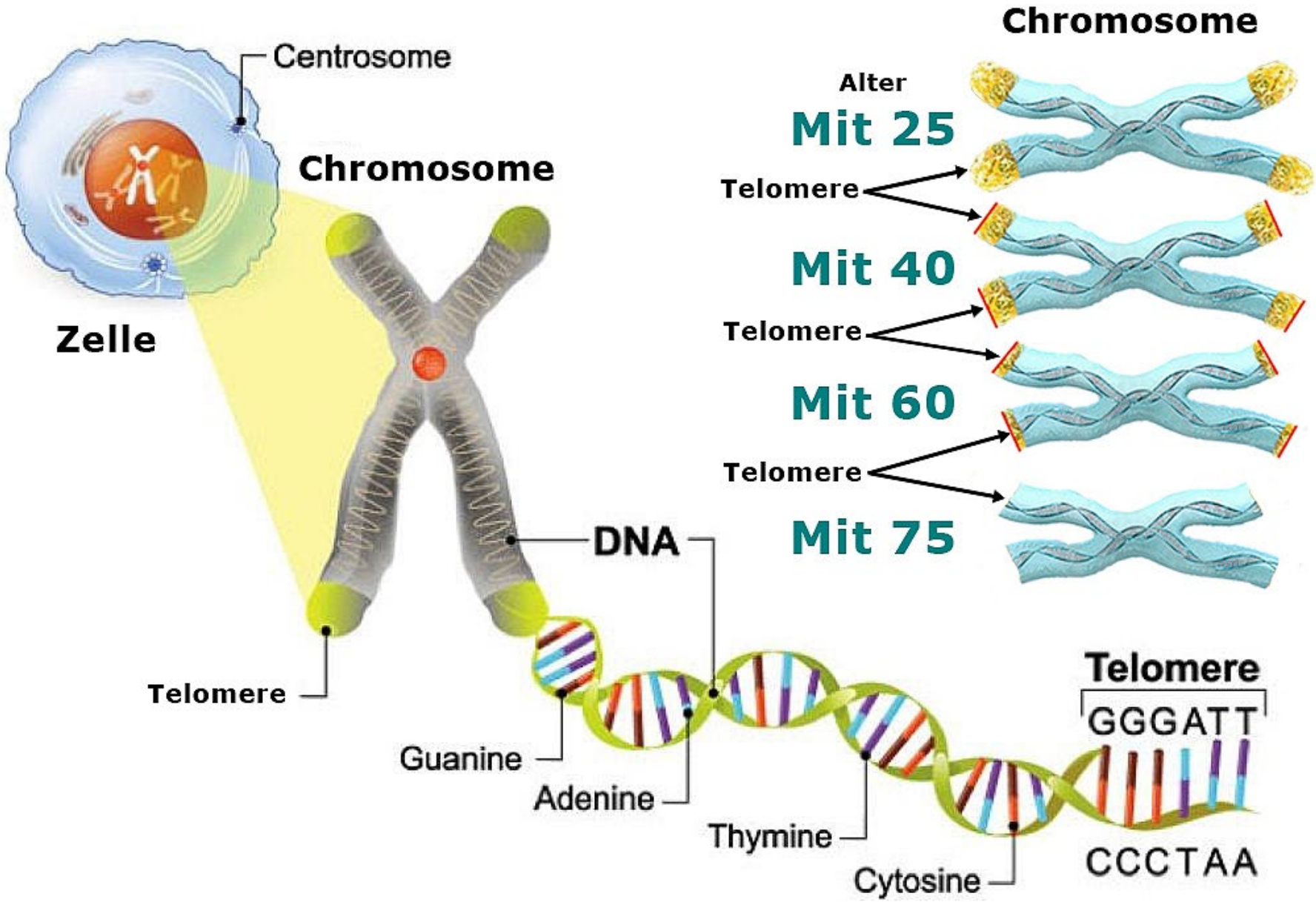
https://www.slideshare.net/jannatiftikhar/role-of-histone-in-dna-packaging

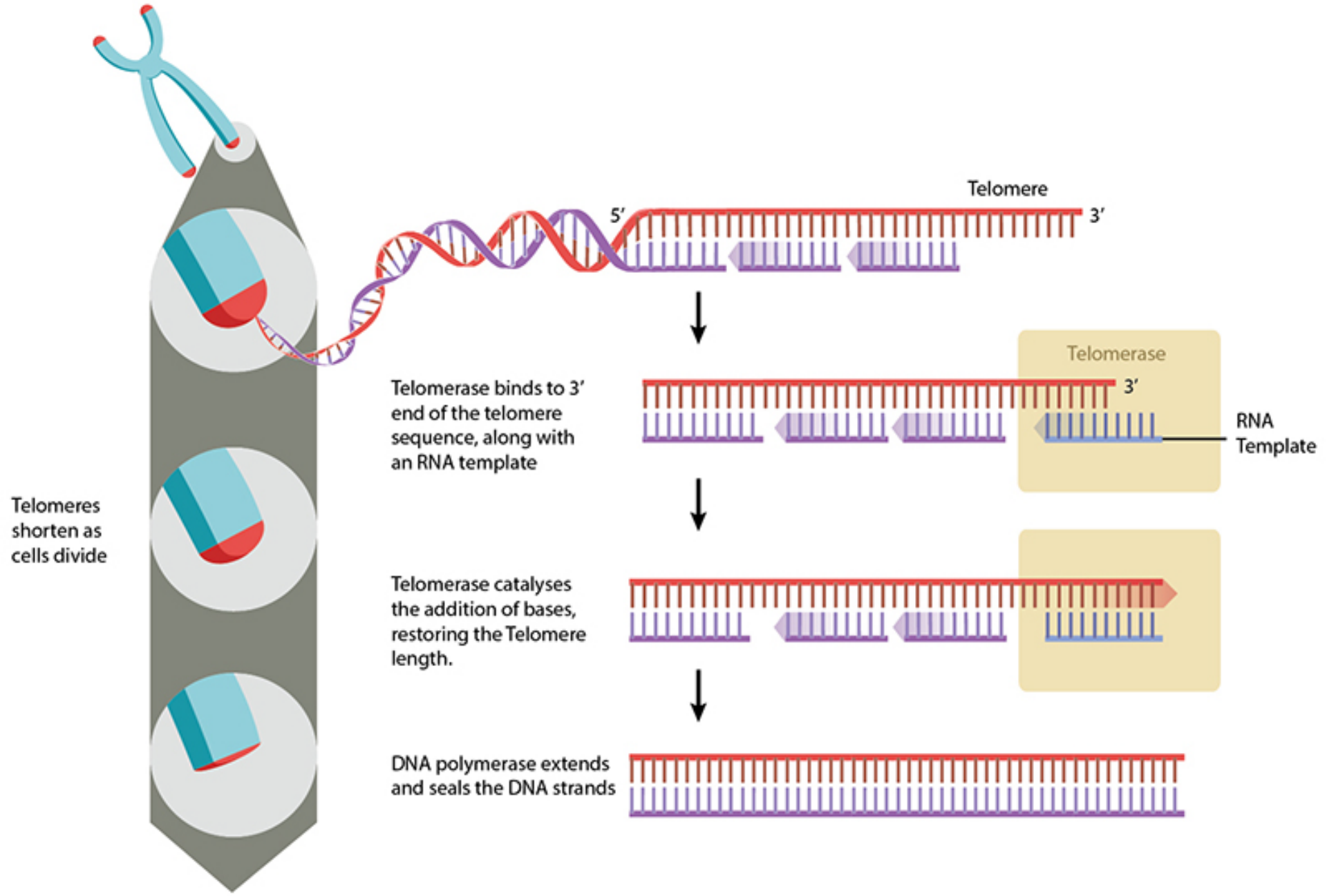



30 nm
Octameric histone core
10 nm
DNA
H1 histone
Nucleosome
DNA
H1 histone
Histone
octamer
30 nm


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

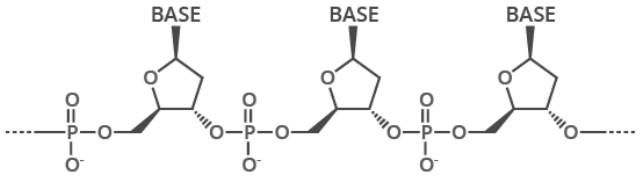

1
At the simplest level, chromatin is a double-stranded helical structure of DNA.
DNA double helix
2 nm
3
Each nucleosome consists of eight histone proteins around which the DNA wraps 1.65 times.
2
DNA is complexed with histones to form nucleosomes.
Nucleosome core of eight histone molecules
4
A chromatosome 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.


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


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

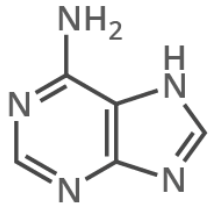
THE CHEMICAL STRUCTURE OF DNA

THE SUGAR PHOSPHATE 'BACKBONE'

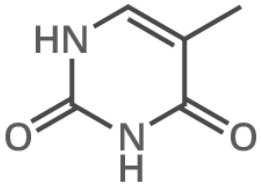


DNA is a polymer made up of units called nucleotides. The nucleotides are made of three different components: a sugar group, a phosphate group, and a base. There are four different bases: adenine, thymine, guanine and cytosine.

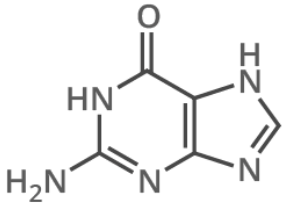
A ADENINE



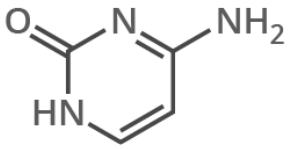
T THYMINE



G GUANINE

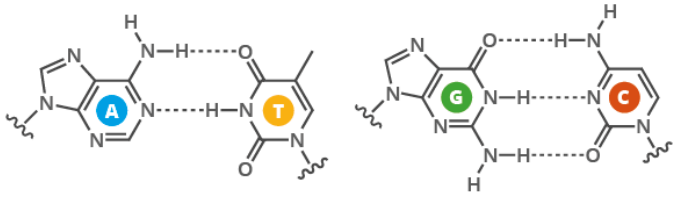


C CYTOSINE



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.



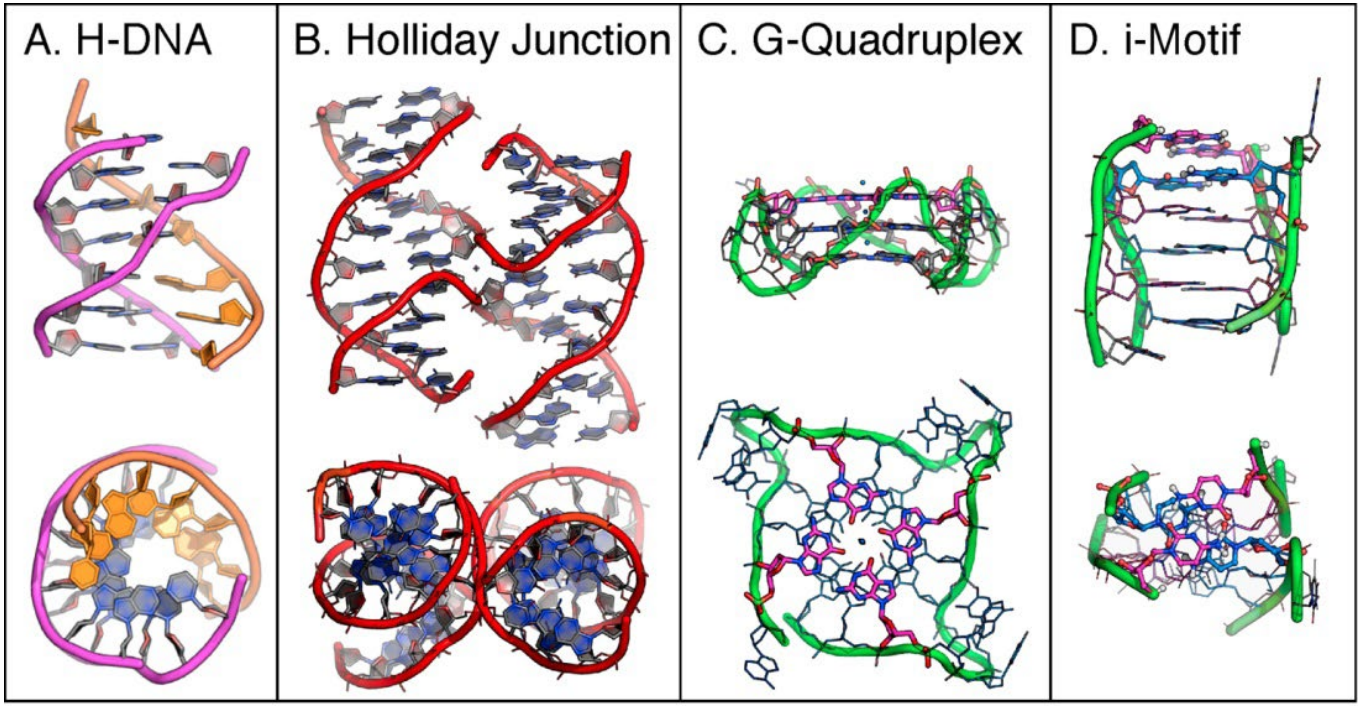
FROM DNA TO PROTEINS

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.

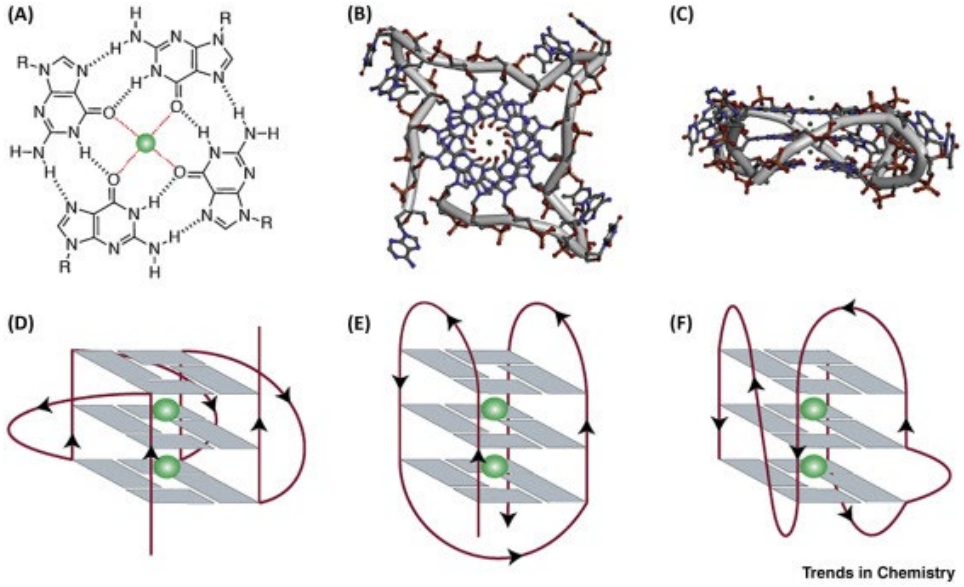
An enzyme, RNA polymerase, transcribes DNA into mRNA (messenger ribonucleic acid). It splits apart the two strands that form the double helix, then reads a strand and copies the sequence of nucleotides. The only difference between the RNA and the original DNA is that in the place of thymine (T), another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

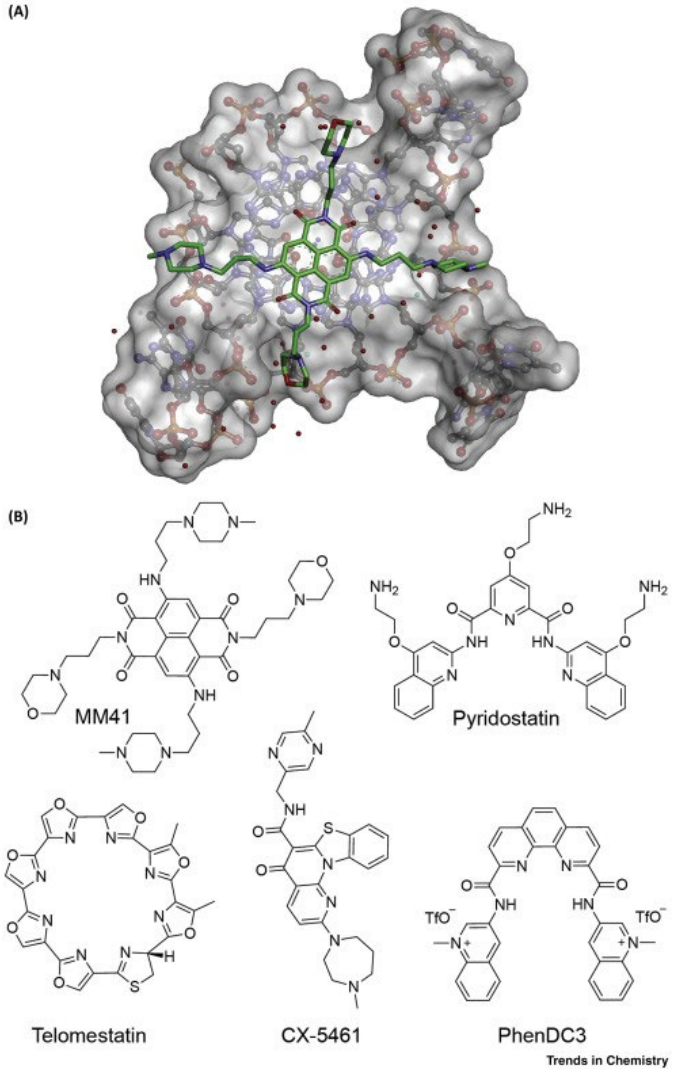
In multicellular organisms, the mRNA carries genetic code out of the cell nucleus, to the cytoplasm. Here, protein synthesis takes place. 'Translation' is the process of turning the mRNA's 'code' into proteins. Molecules called ribosomes carry out this process, building up proteins from the amino acids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

Triple and Quadruple Strained DNA

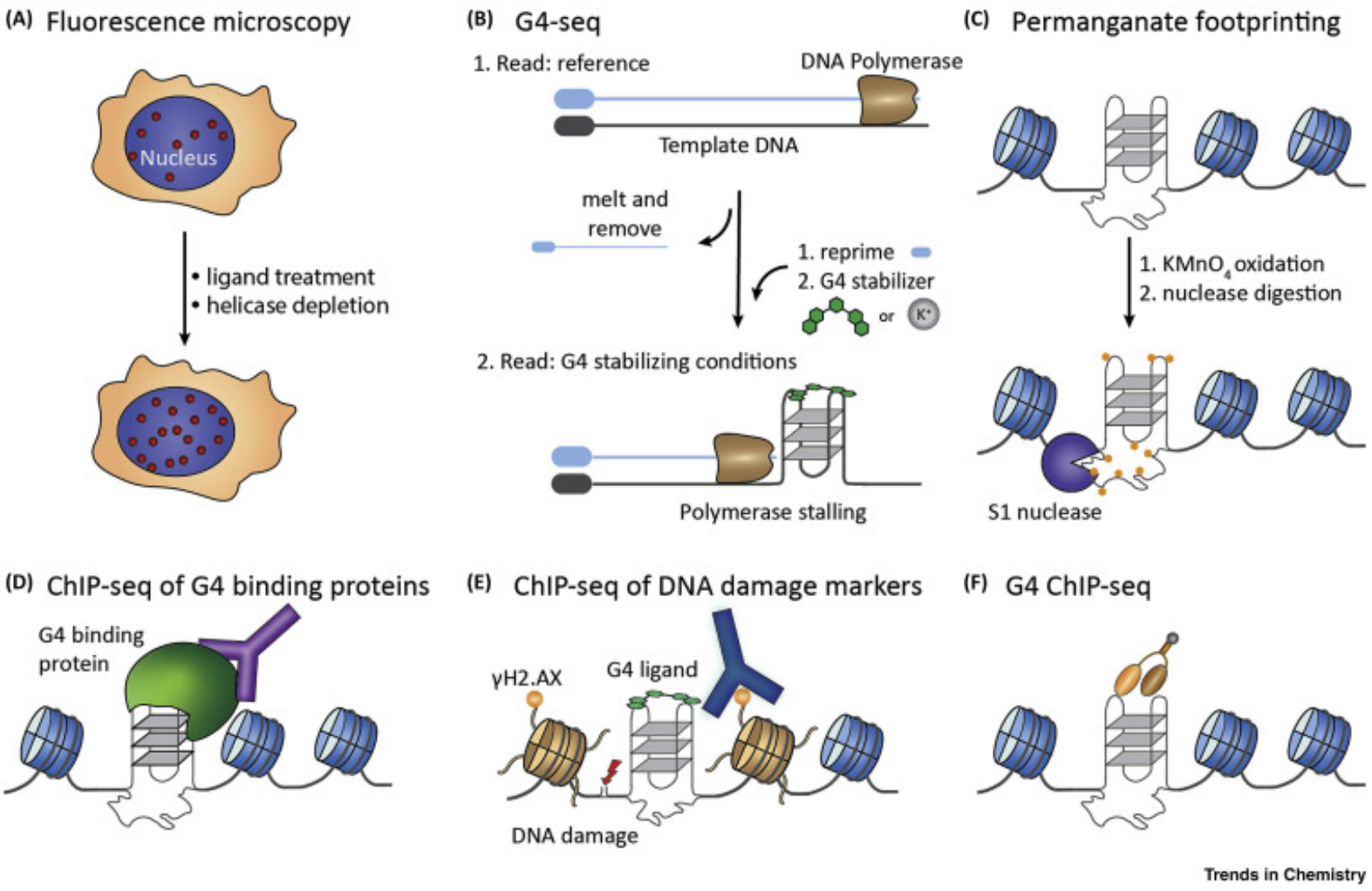


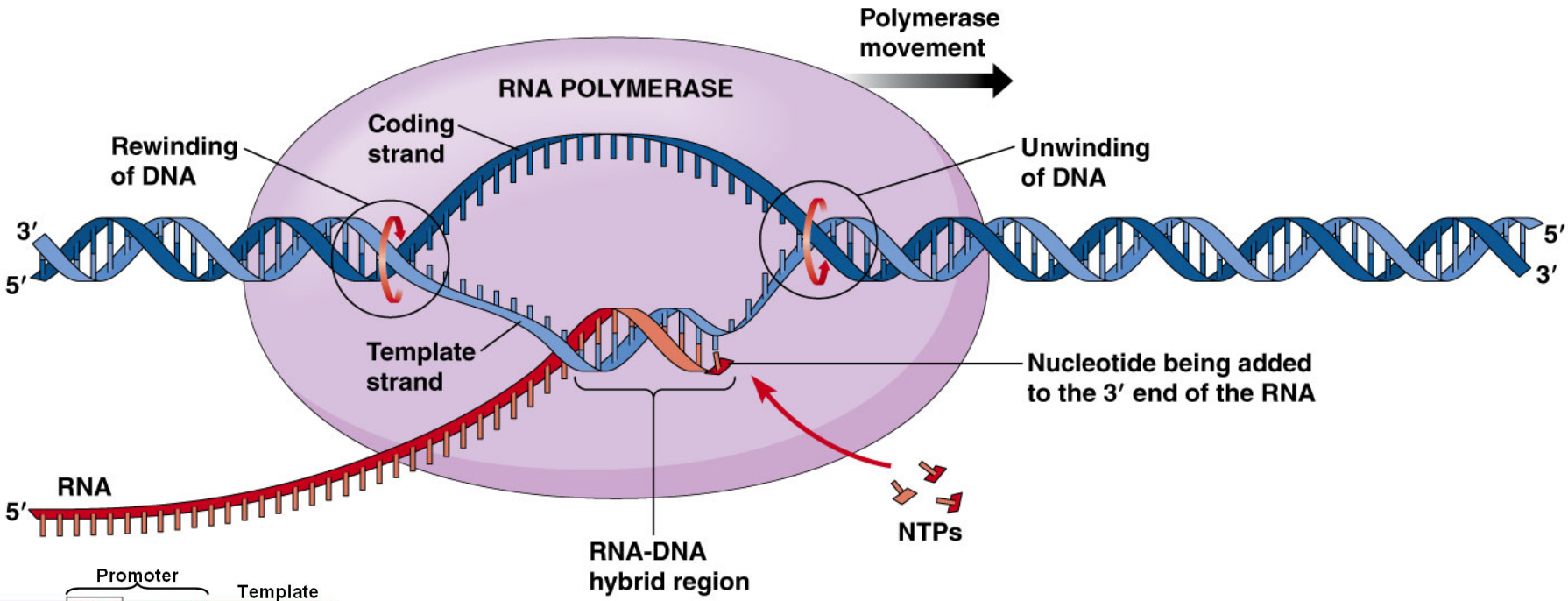
DNA G-quadruplex (G4)

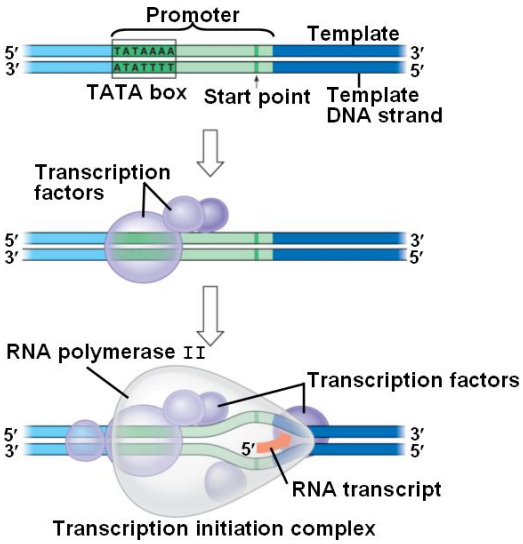


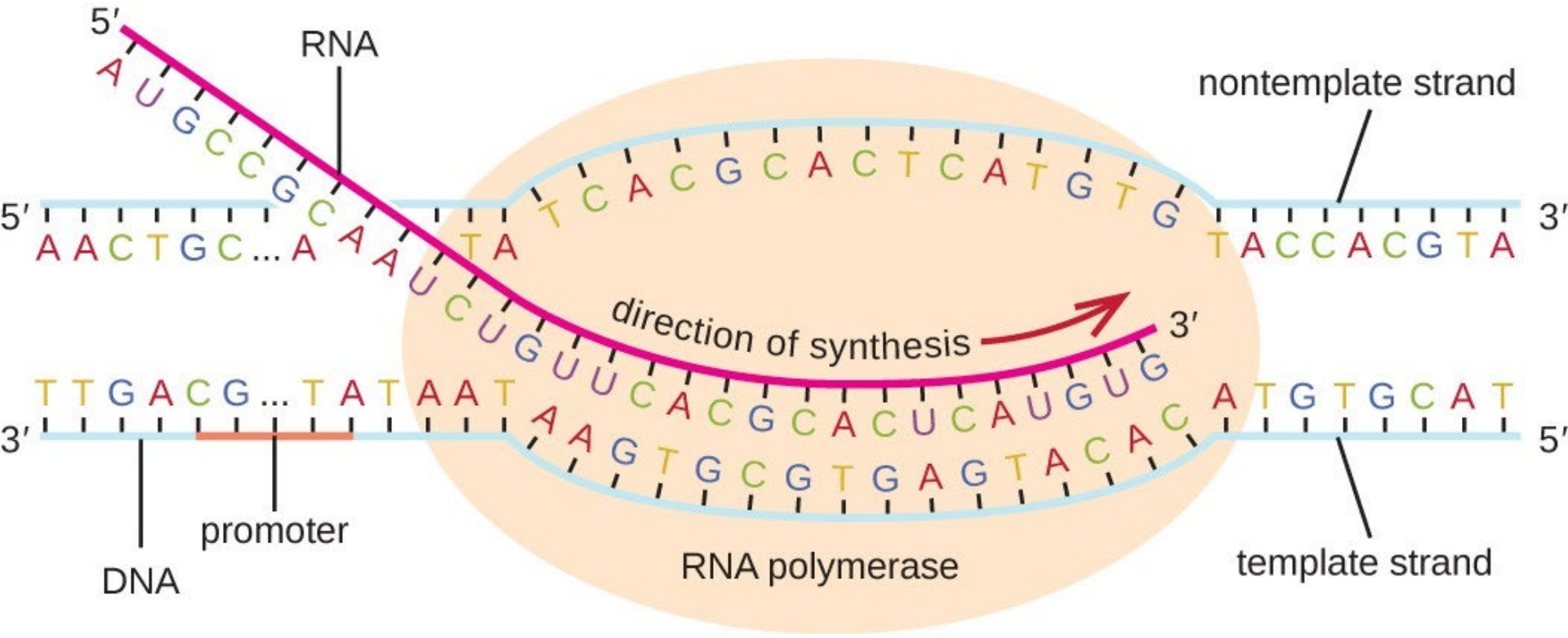


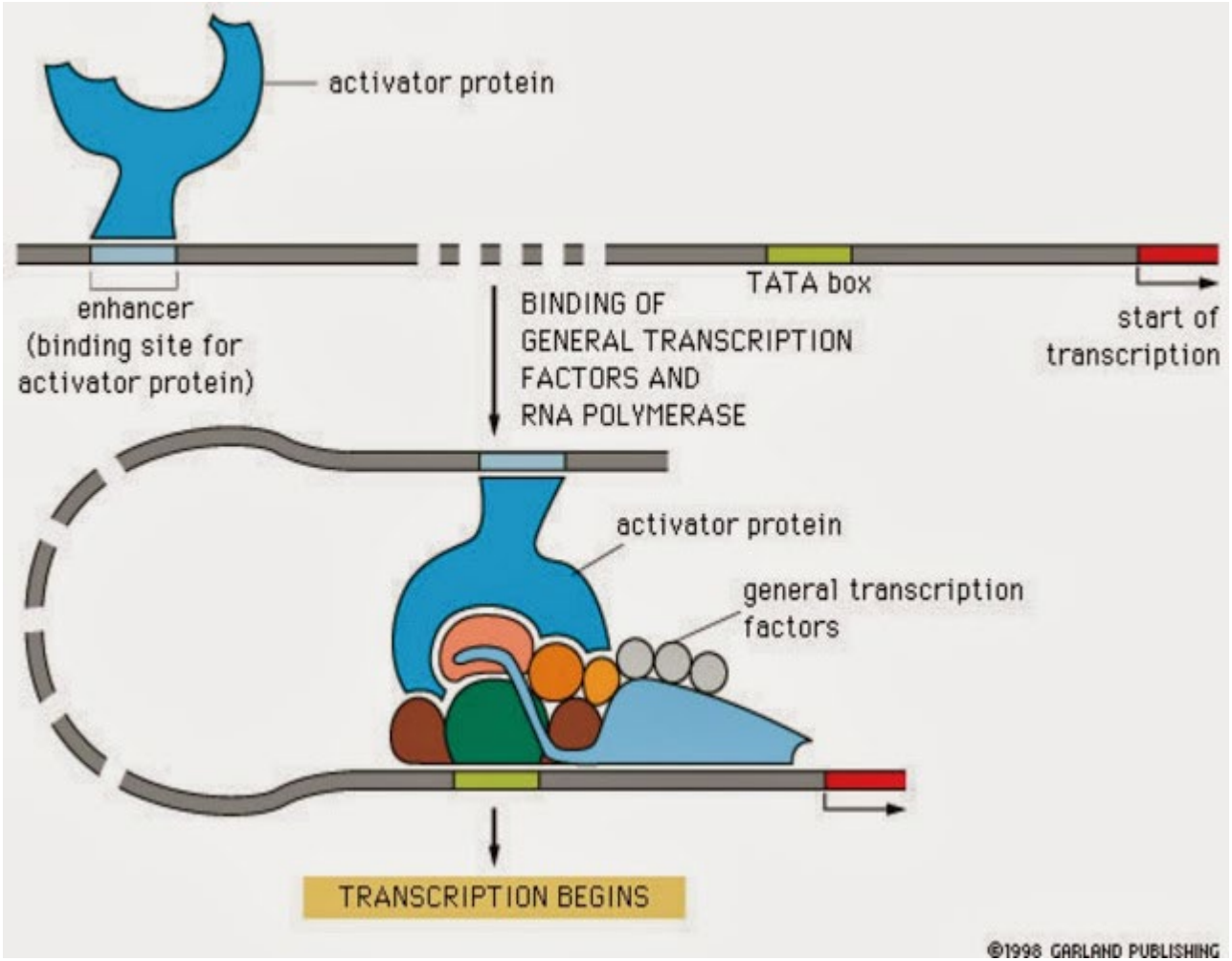
Trends in Chemistry, February 2020, Vol. 2, No. 2 P123

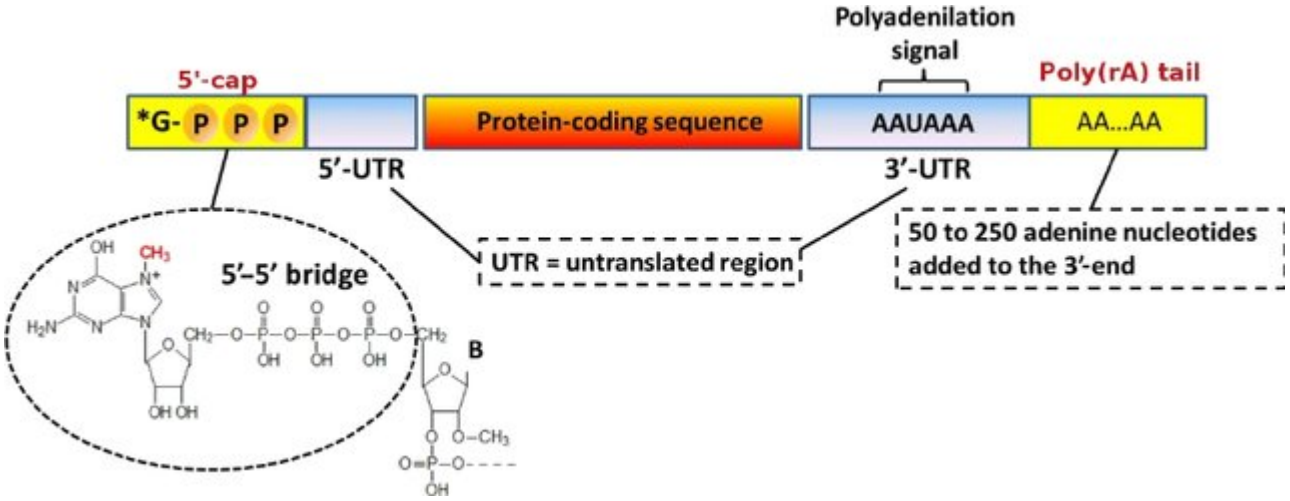

(A) Fluorescence microscopy
Nucleus
• ligand treatment
• helicase depletion
(B) G4-seq
1. Read: reference
DNA Polymerase
Template DNA
melt and remove
1. reprime
2. G4 stabilizer
or
K+
2. Read: G4 stabilizing conditions
Polymerase stalling
(C) Permanganate footprinting
1. KMnO4 oxidation
2. nuclease digestion
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

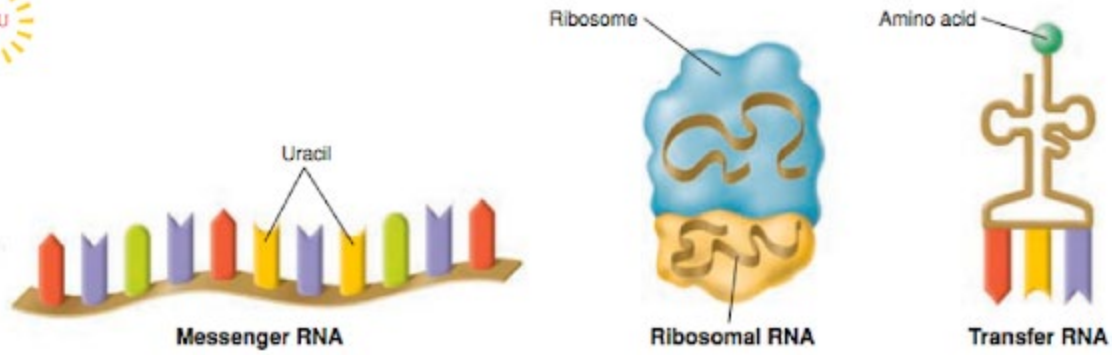

Promoter
Template
5′
3′
TATAAAA
ATATTTT
3′
5′
TATA box
Start point
Template DNA strand
Transcription factors
RNA polymerase II
Transcription factors
RNA transcript
Transcription initiation complex


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
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


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

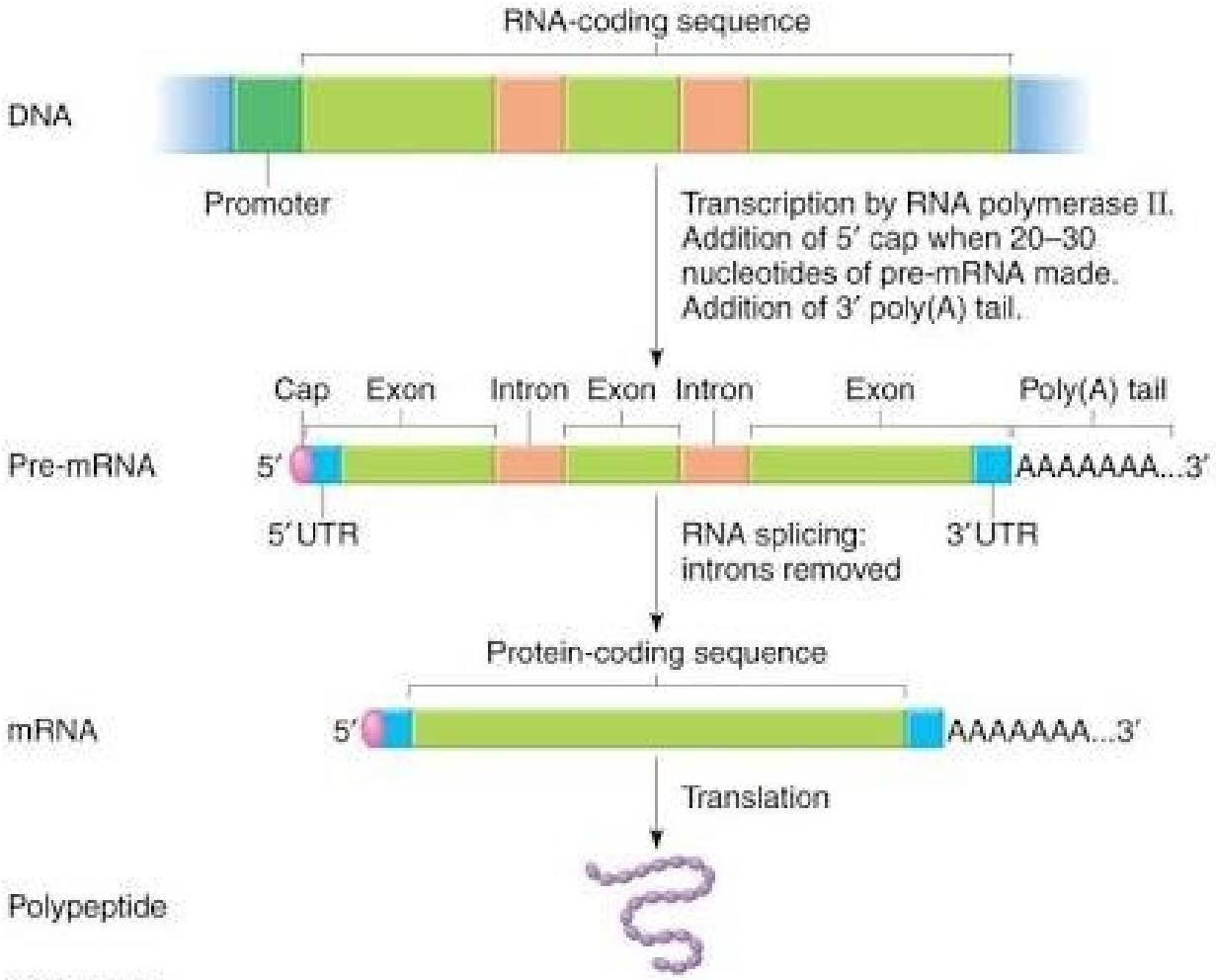
11 DIFFERENT TYPES OF RNA IN A CELL

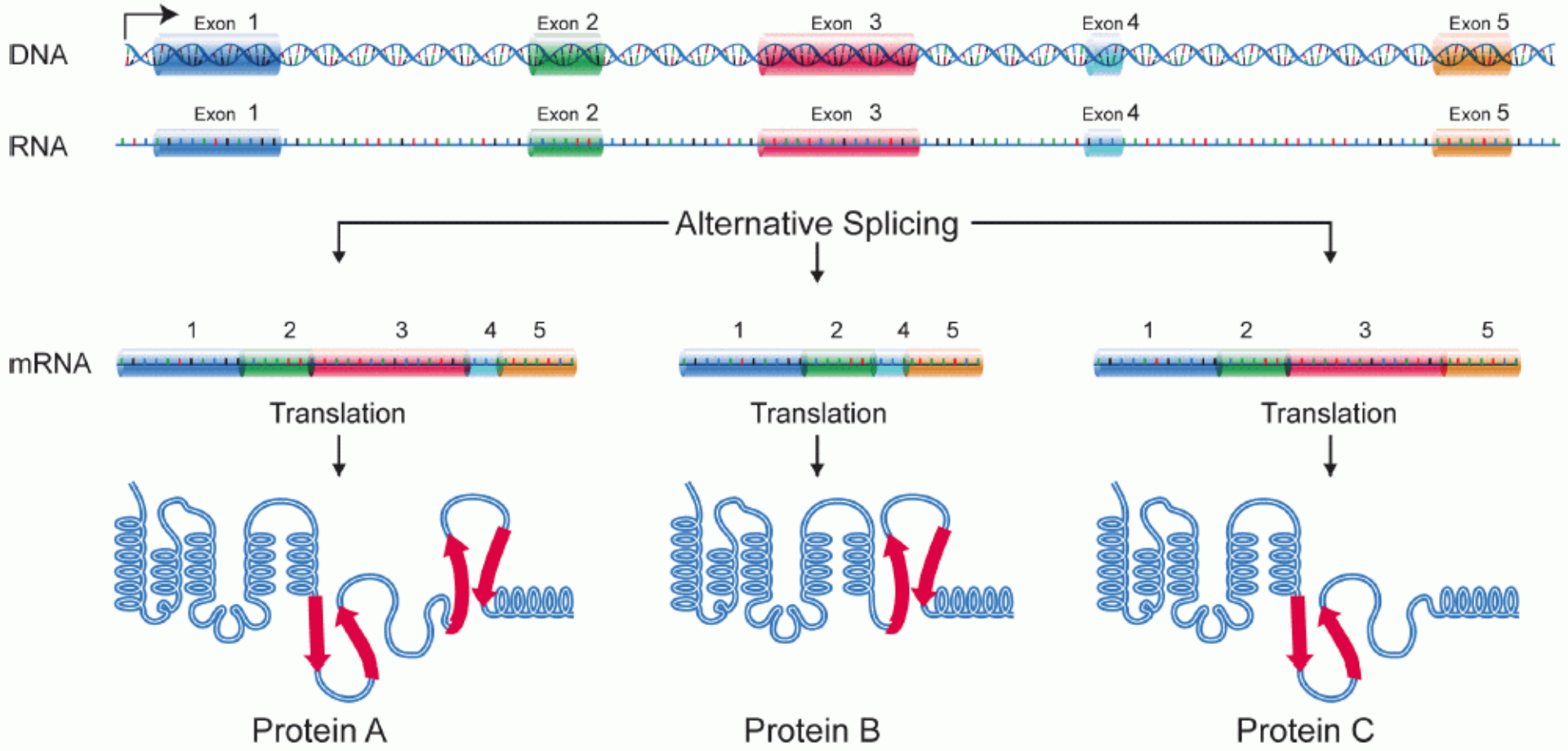


WWW.BIOLOGYEXAMS4U.COM

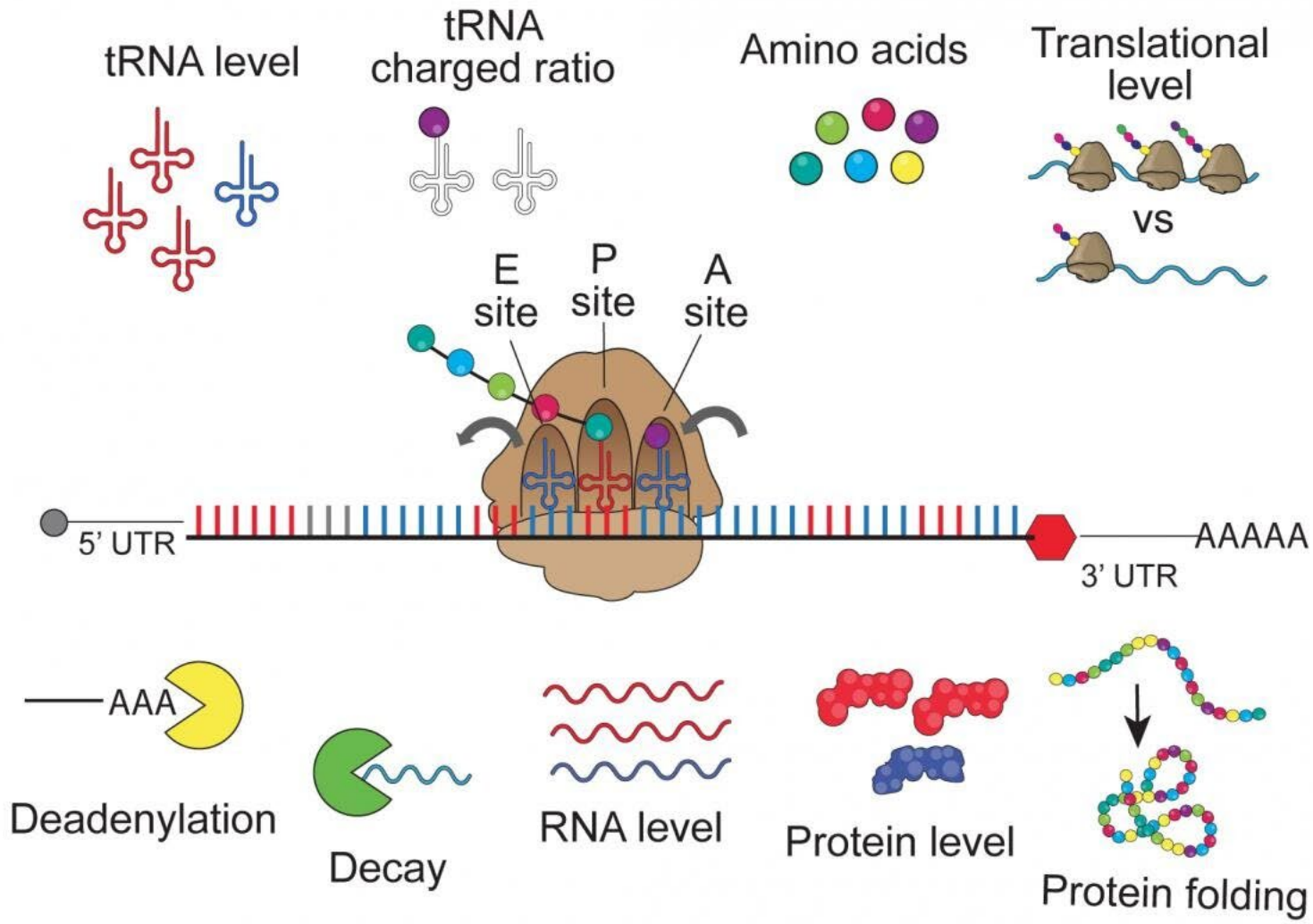
Post Transcription Modification of RNA

1. RNA capping
2. PolyA tail
3. Splicing



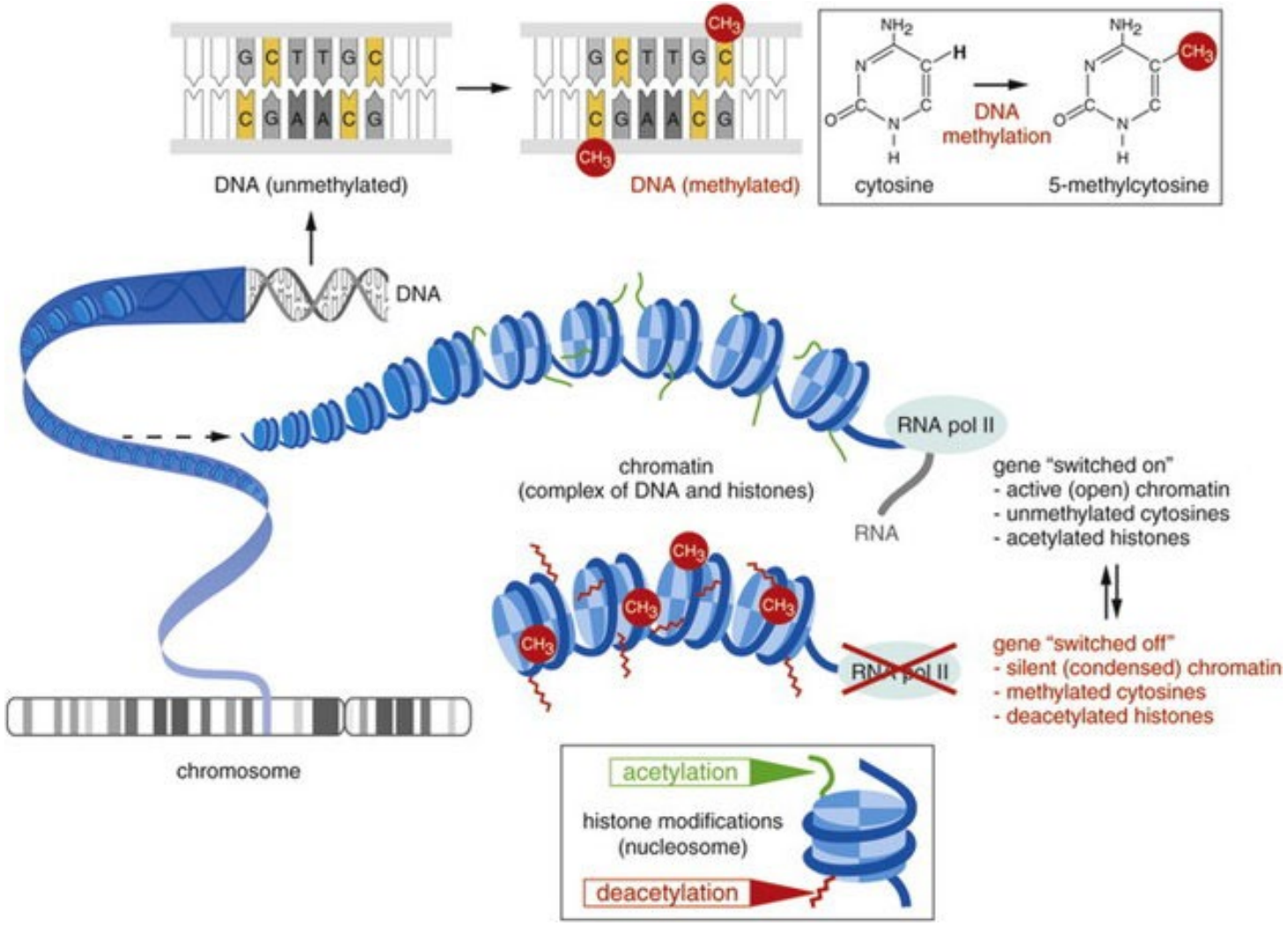

DNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
RNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Alternative Splicing
mRNA
1 2 3 4 5
1 2 4 5
1 2 3 5
Translation
Translation
Translation
Protein A
Protein B
Protein C

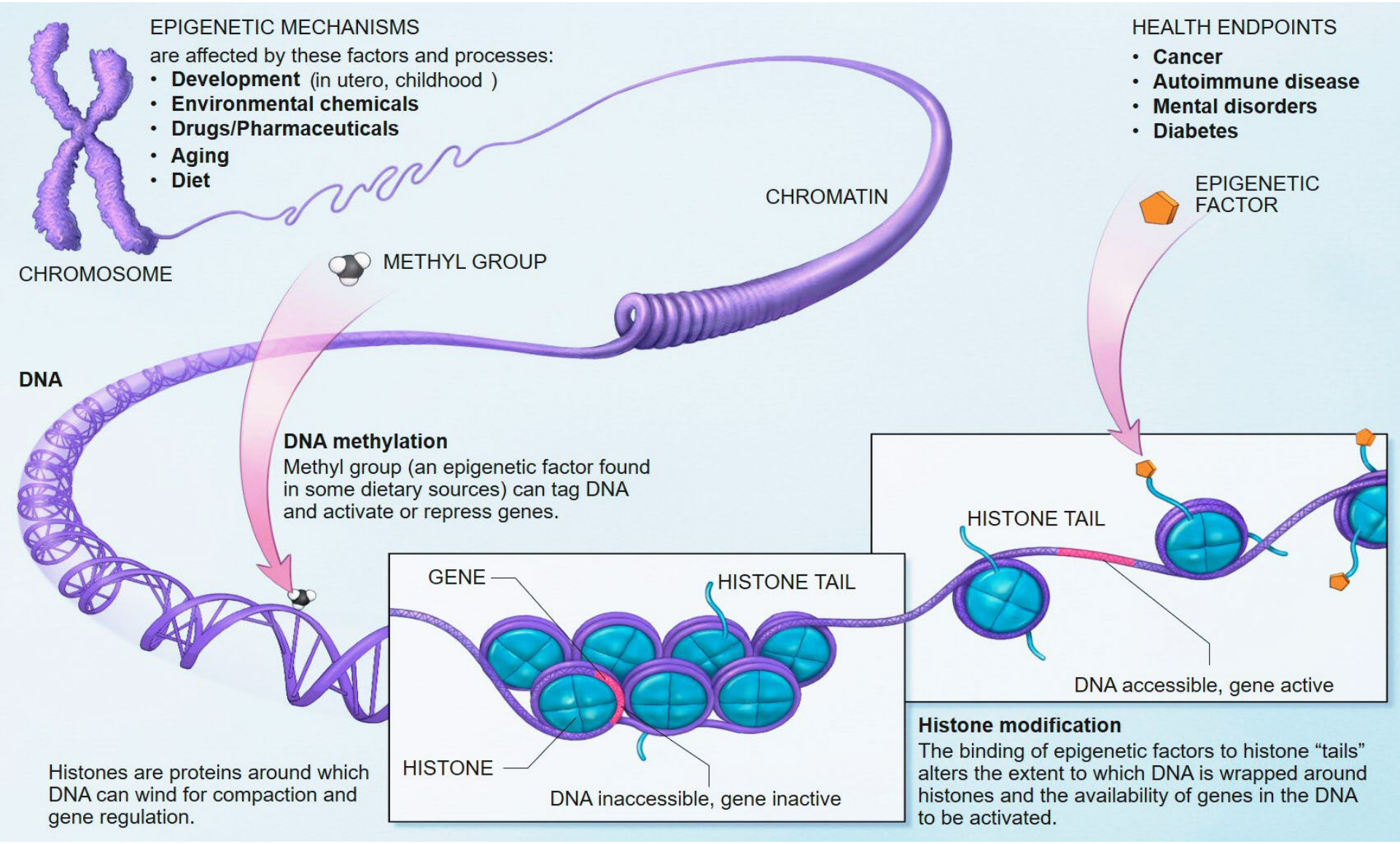
Upstream regulator



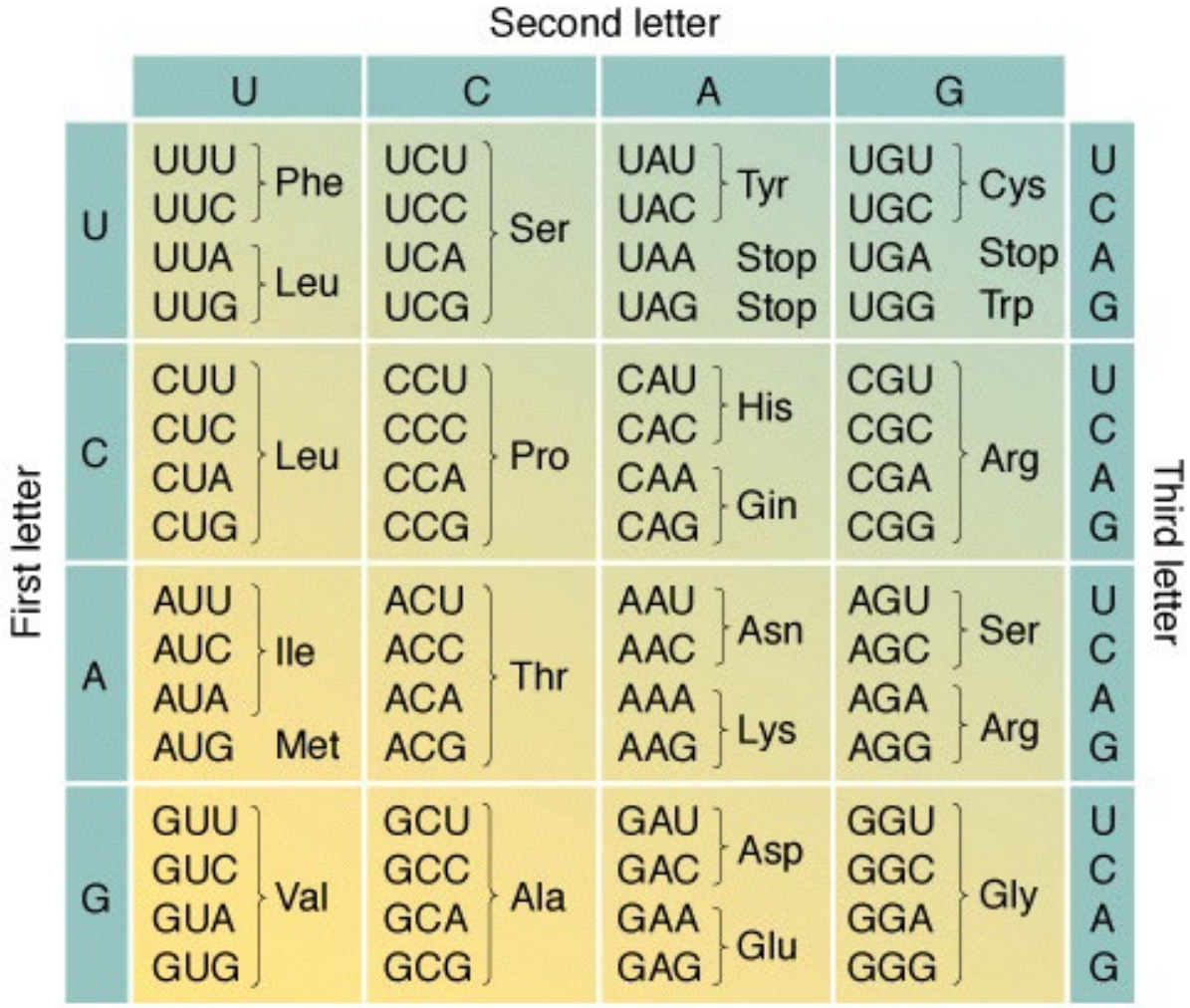
Downstream effects

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.

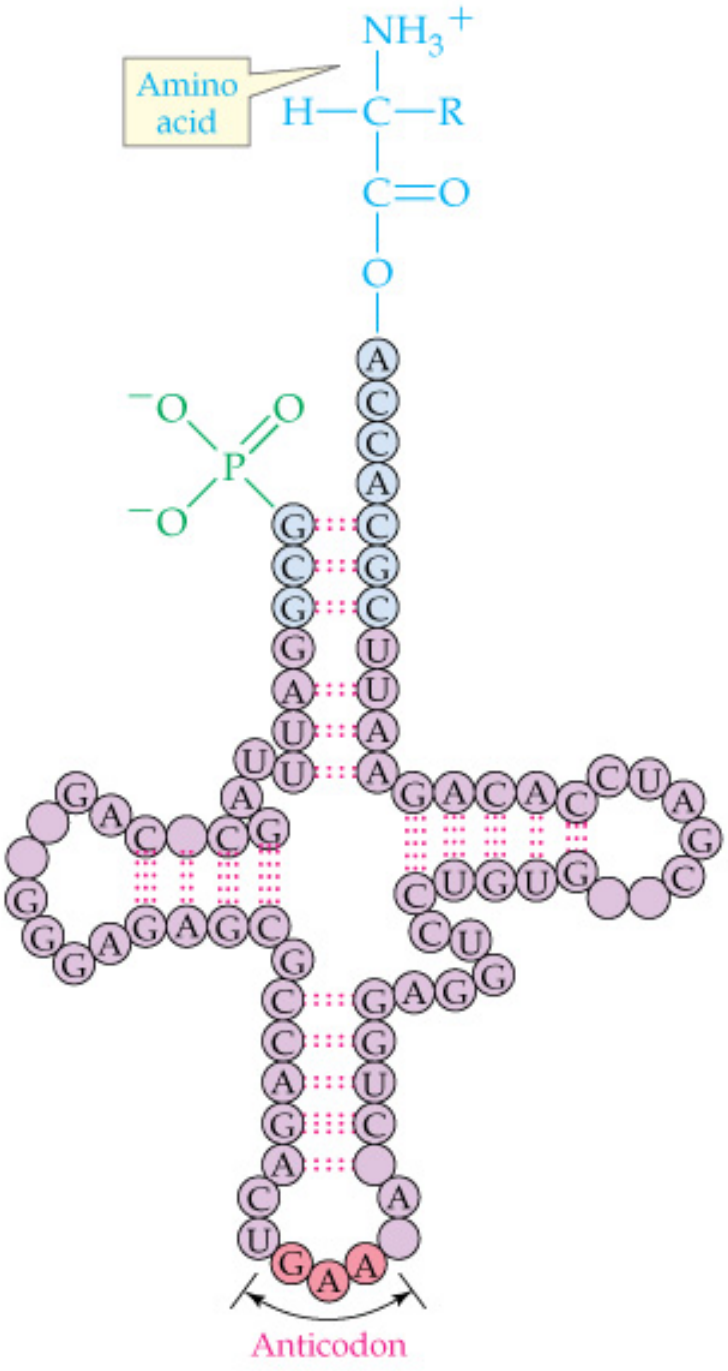
RNA Sequence



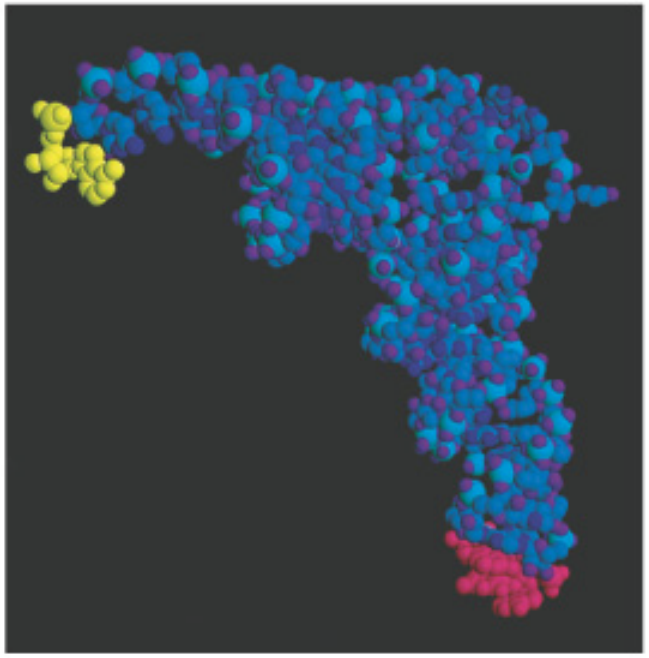
Base	G	C	U	A	C	G	G	A	G	C	U	U	C	G	G	A	G	C	U	A	G
Codon	Codon 1			Codon 2			Codon 3			Codon 4			Codon 5			Codon 6			Codon 7		
Aminoacid	Alanine			Threonine			Glutamate			Leucine			Arginine			Serine			Stop		

Second letter

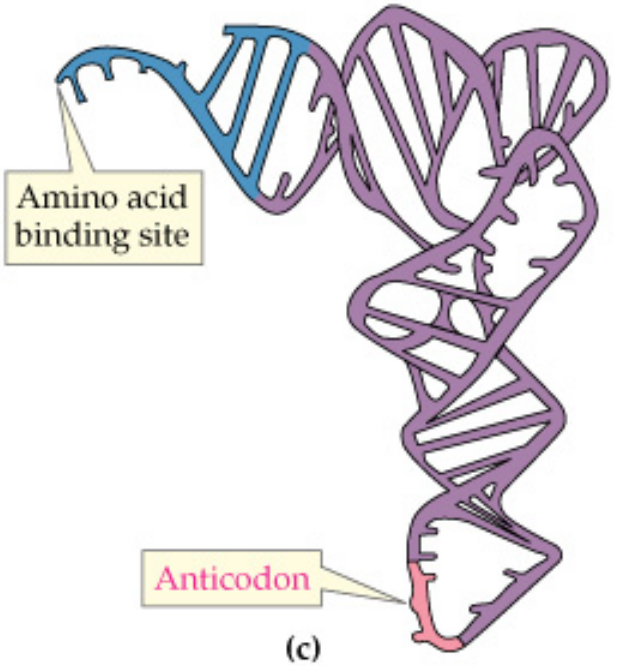
First letter	U	C	A	G	Third letter
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr **UAA Stop** **UAG Stop**	UGU Cys UGC Cys **UGA Stop** UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg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


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

(a)

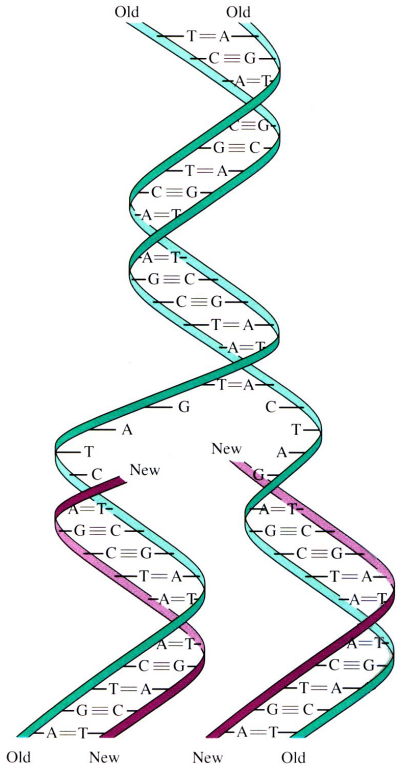


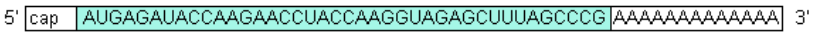
(b)


Amino acid binding site
Anticodon

(c)

Self-Assembly Process in Nature

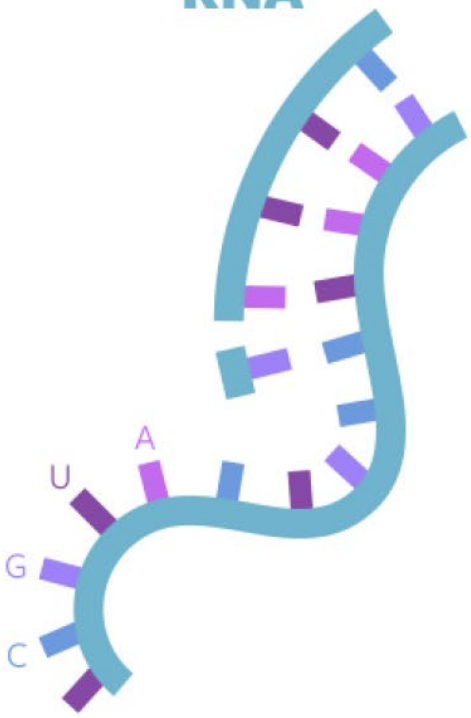




Definition of Life

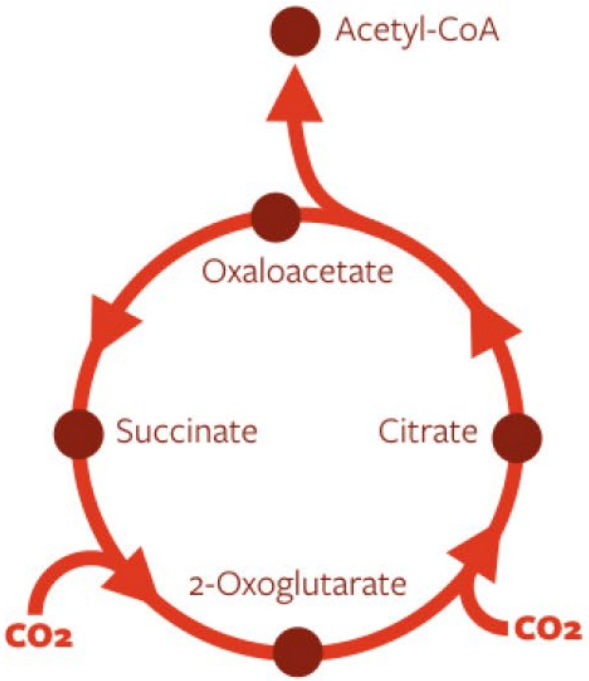
REPLICATION

RNA



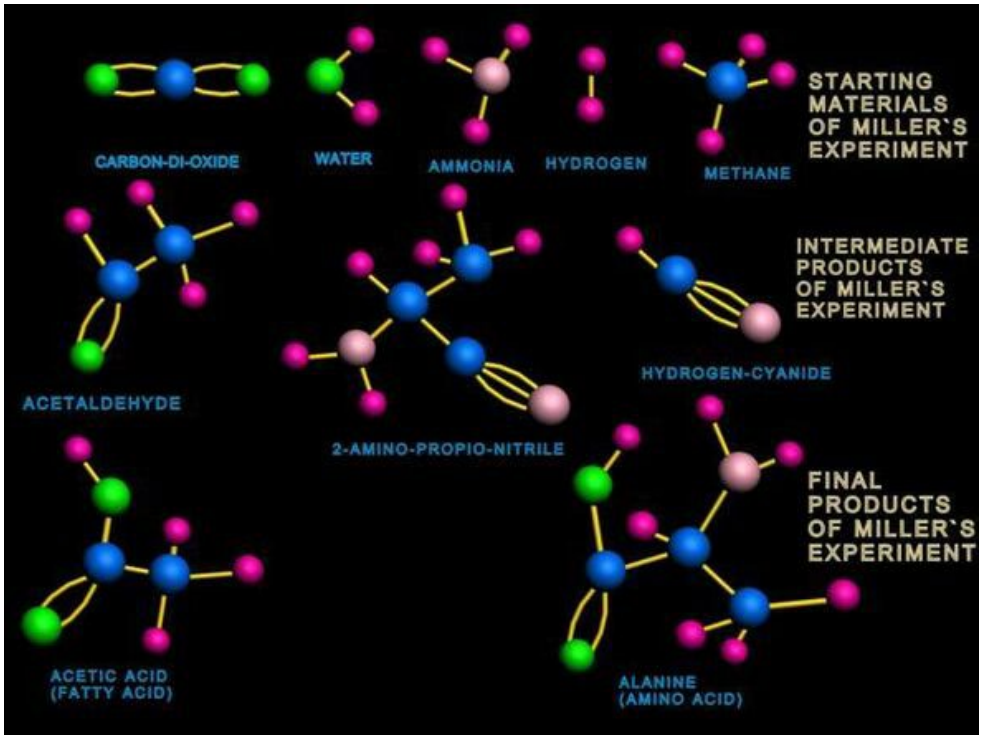
METABOLISM

rTCA CYCLE



COMPARTMENTS

LIPOSOME



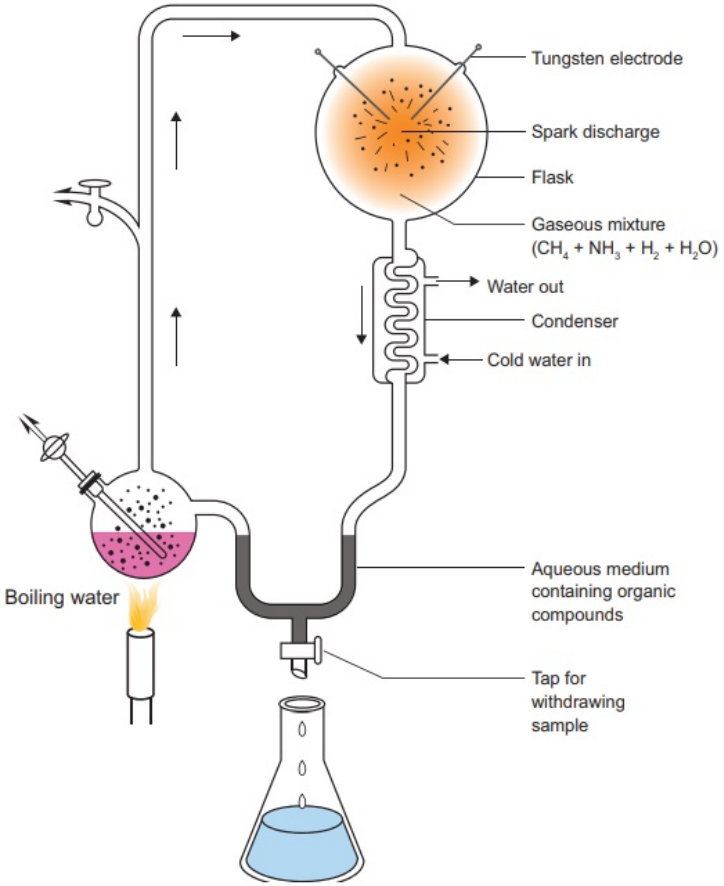
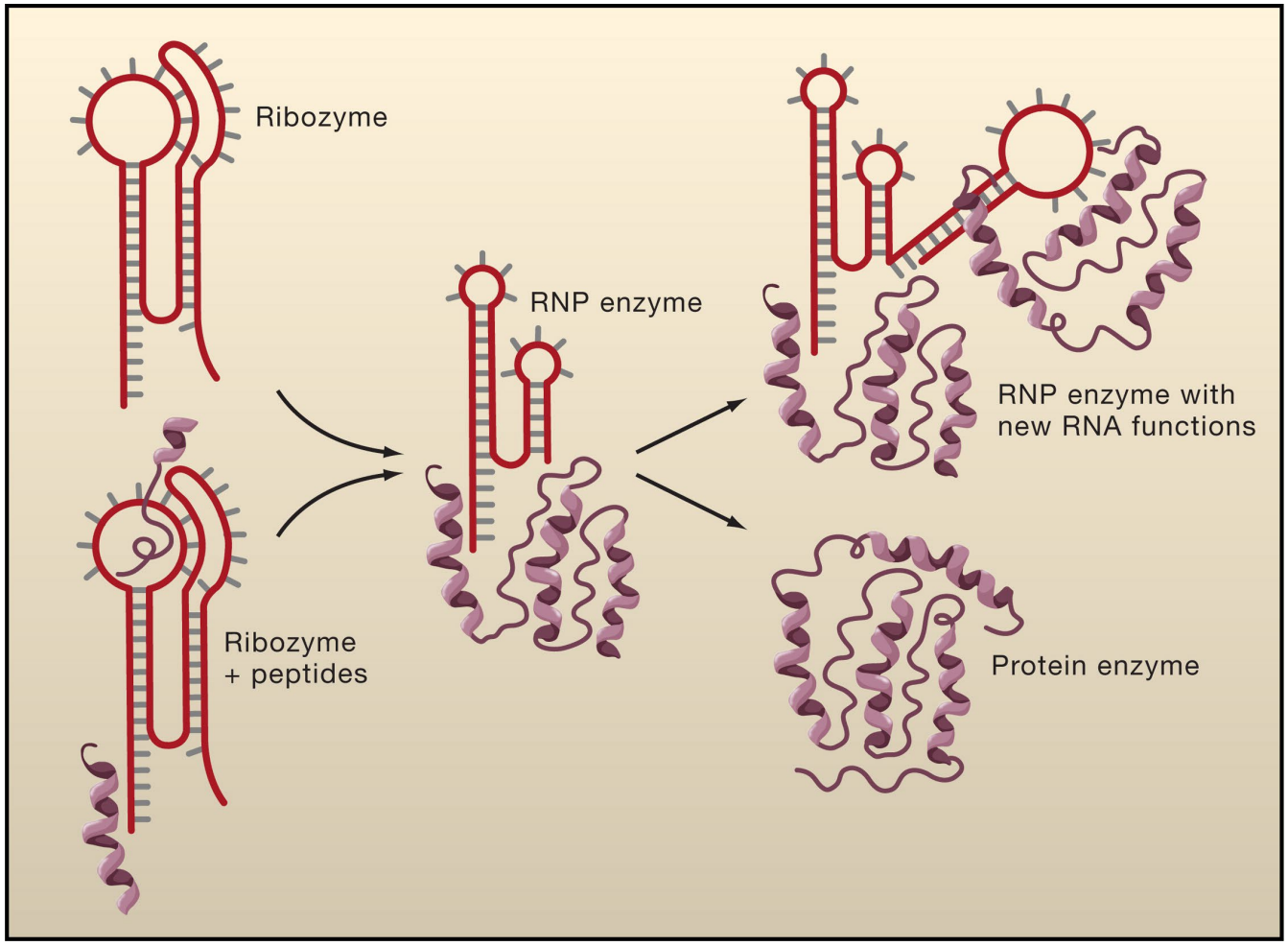
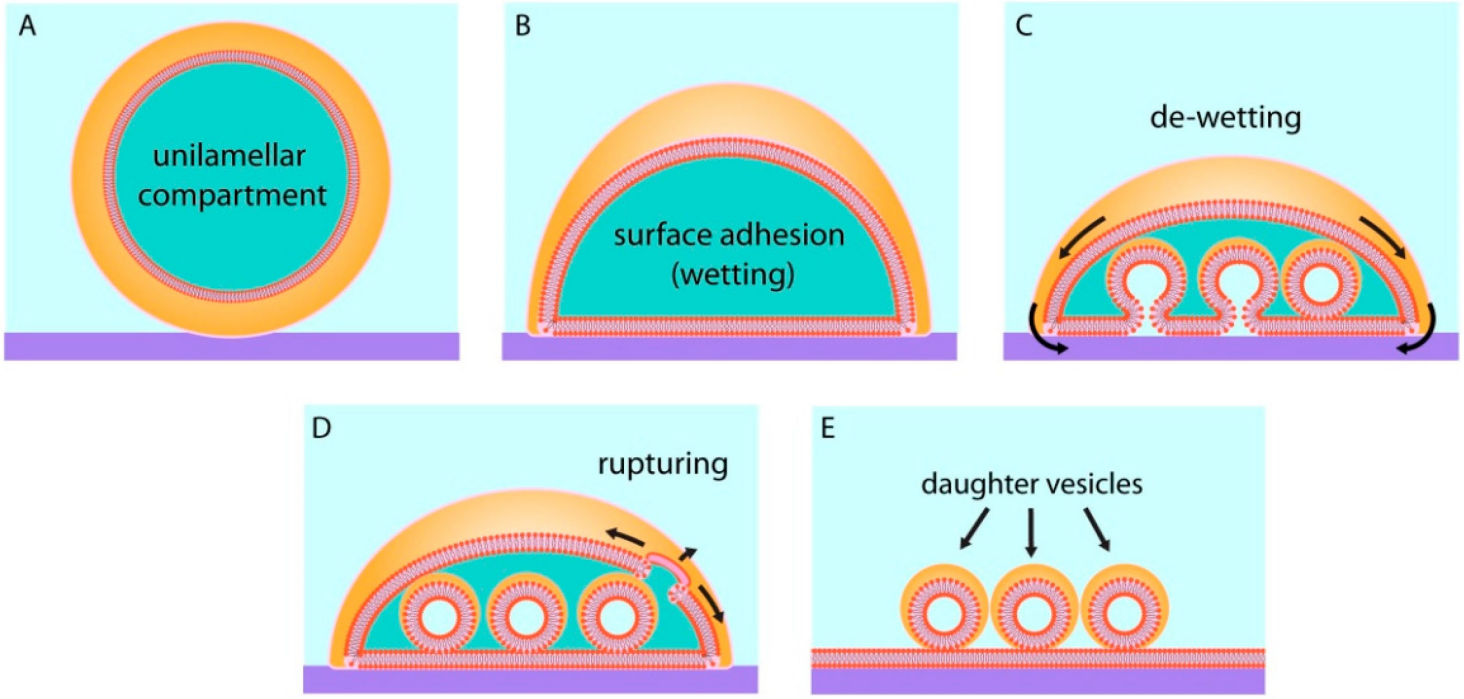


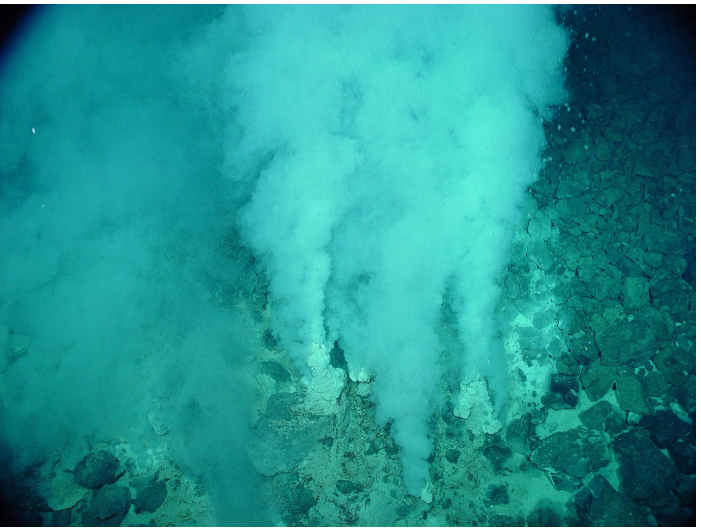
Fig. 6.1 Diagrammatic representation of Urey-Miller's experiment

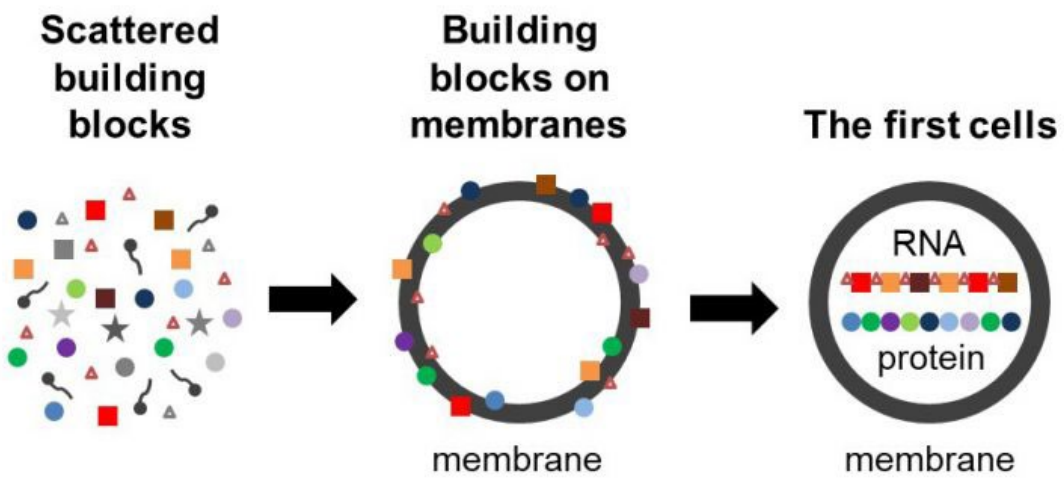
RNA World

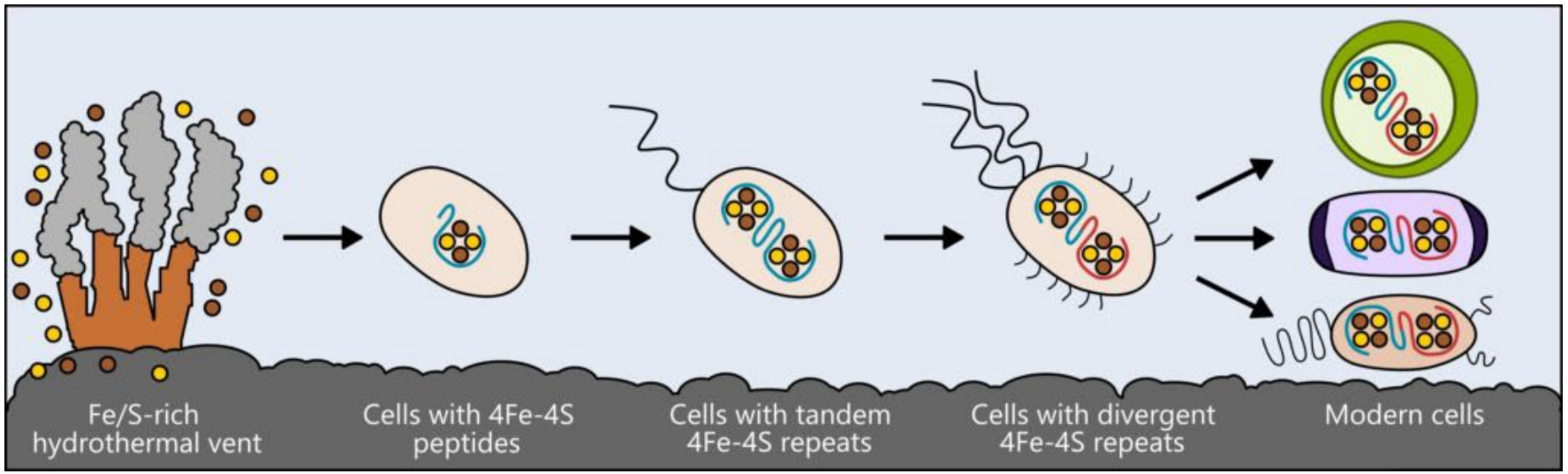


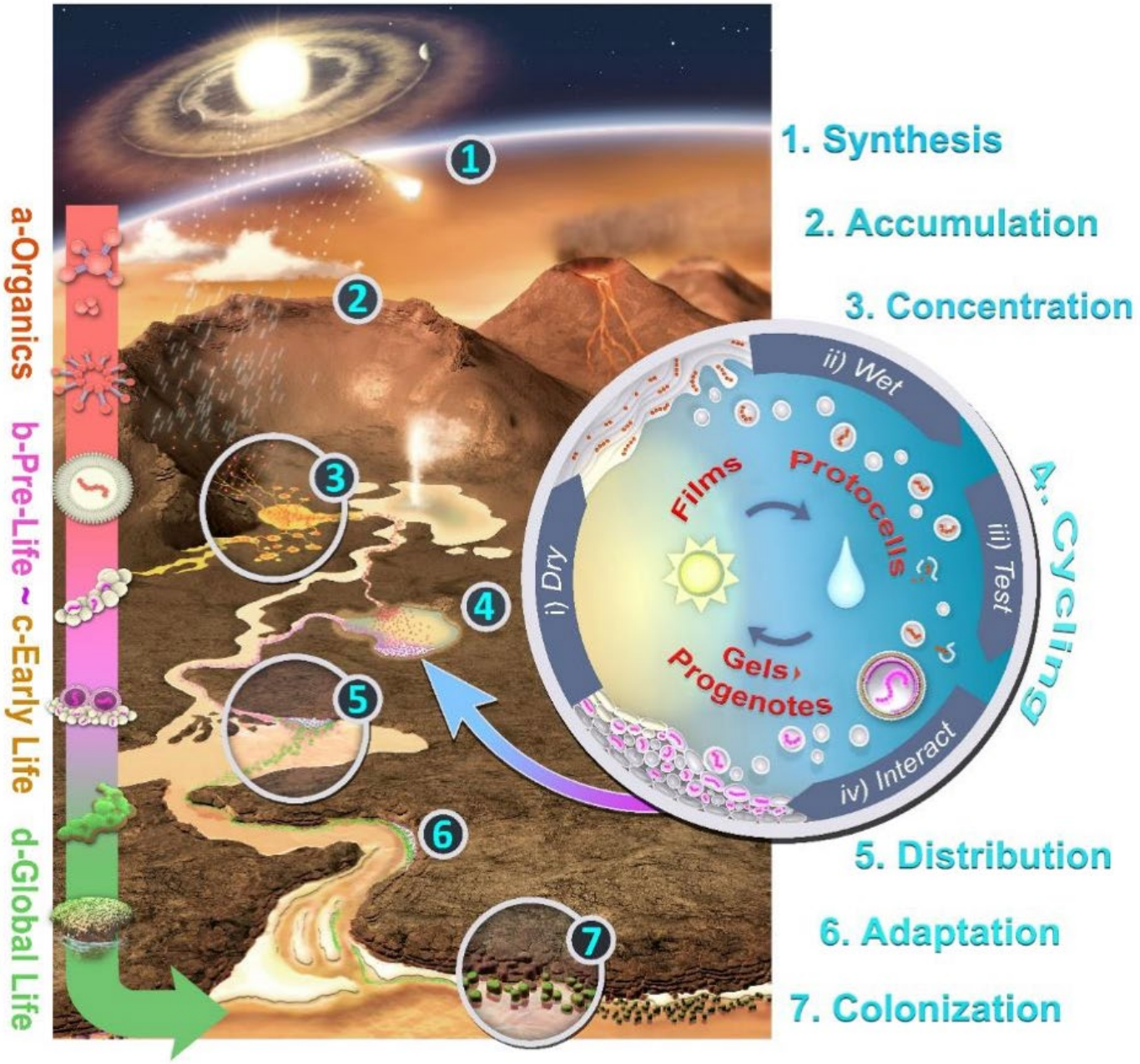
Cell 2009 136599-602DOI: (10.1016/j.cell.2009.02.002)


A
unilamellar compartment
B
surface adhesion (wetting)
C
de-wetting
D
rupturing
E
daughter vesicles
F
z y x
10 µm
G
z x
10 µm



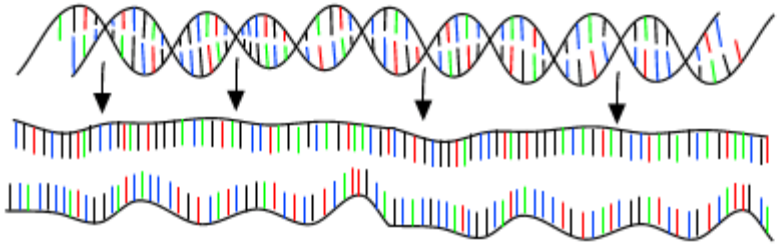

Scattered building blocks
Building blocks on membranes
The first cells
RNA
protein
membrane
membrane


Fe/S-rich hydrothermal vent
Cells with 4Fe-4S peptides
Cells with tandem 4Fe-4S repeats
Cells with divergent 4Fe-4S repeats
Modern cells


a-Organics
b-Pre-Life ~ c-Early Life
d-Global Life
1
2
3
4
5
6
7
ii) Wet
iii) Test
iv) Interact
i) Dry
Films
Protocells
Gels
Progenotes
4. Cycling
1. Synthesis
2. Accumulation
3. Concentration
5. Distribution
6. Adaptation
7. Colonization

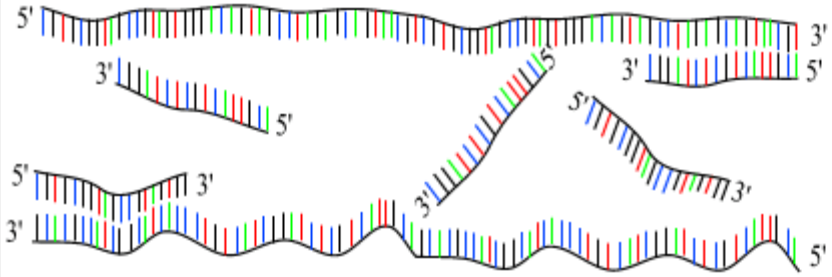
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

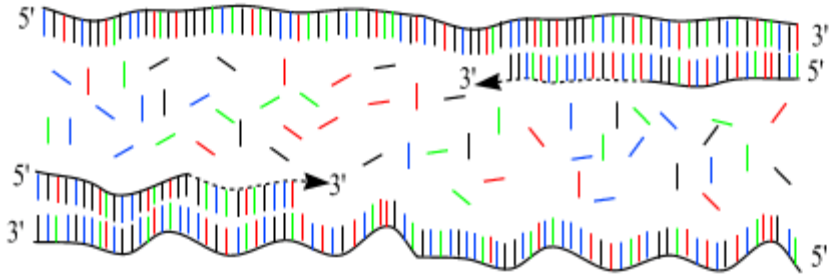
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

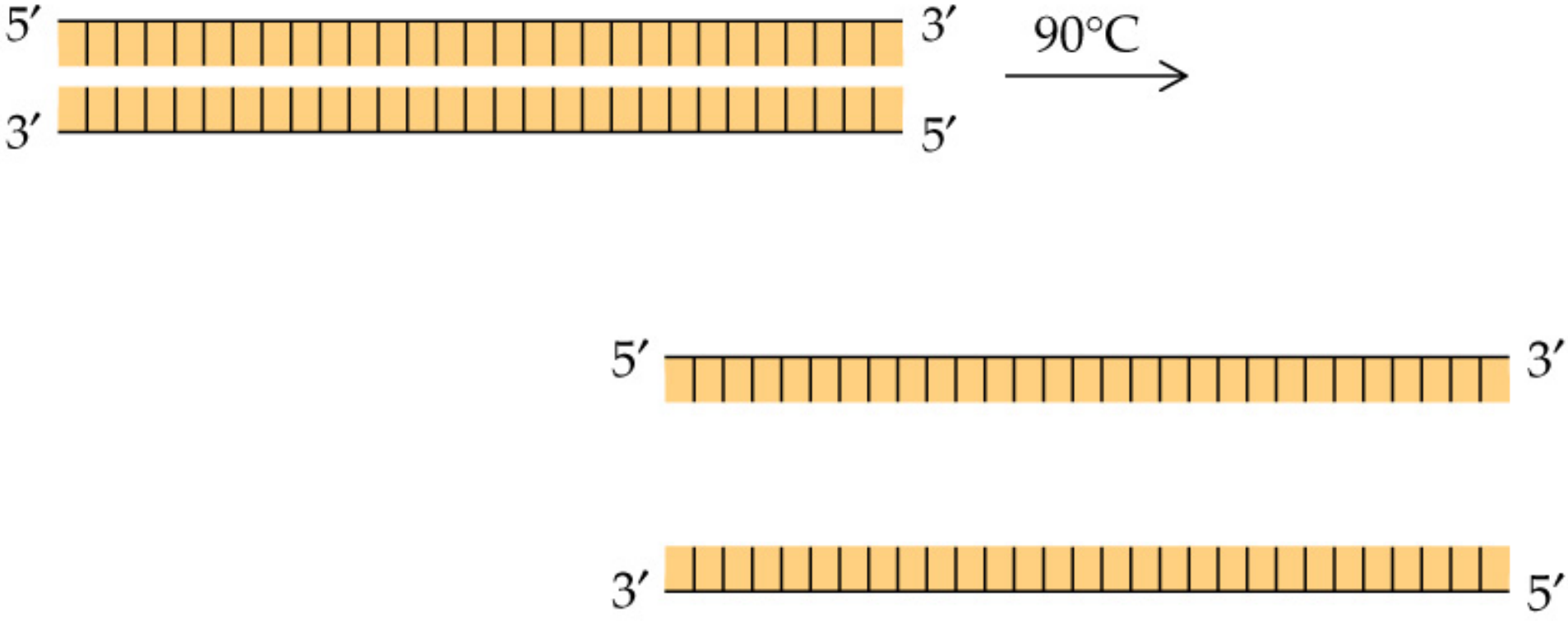
2 minutes 72 °C

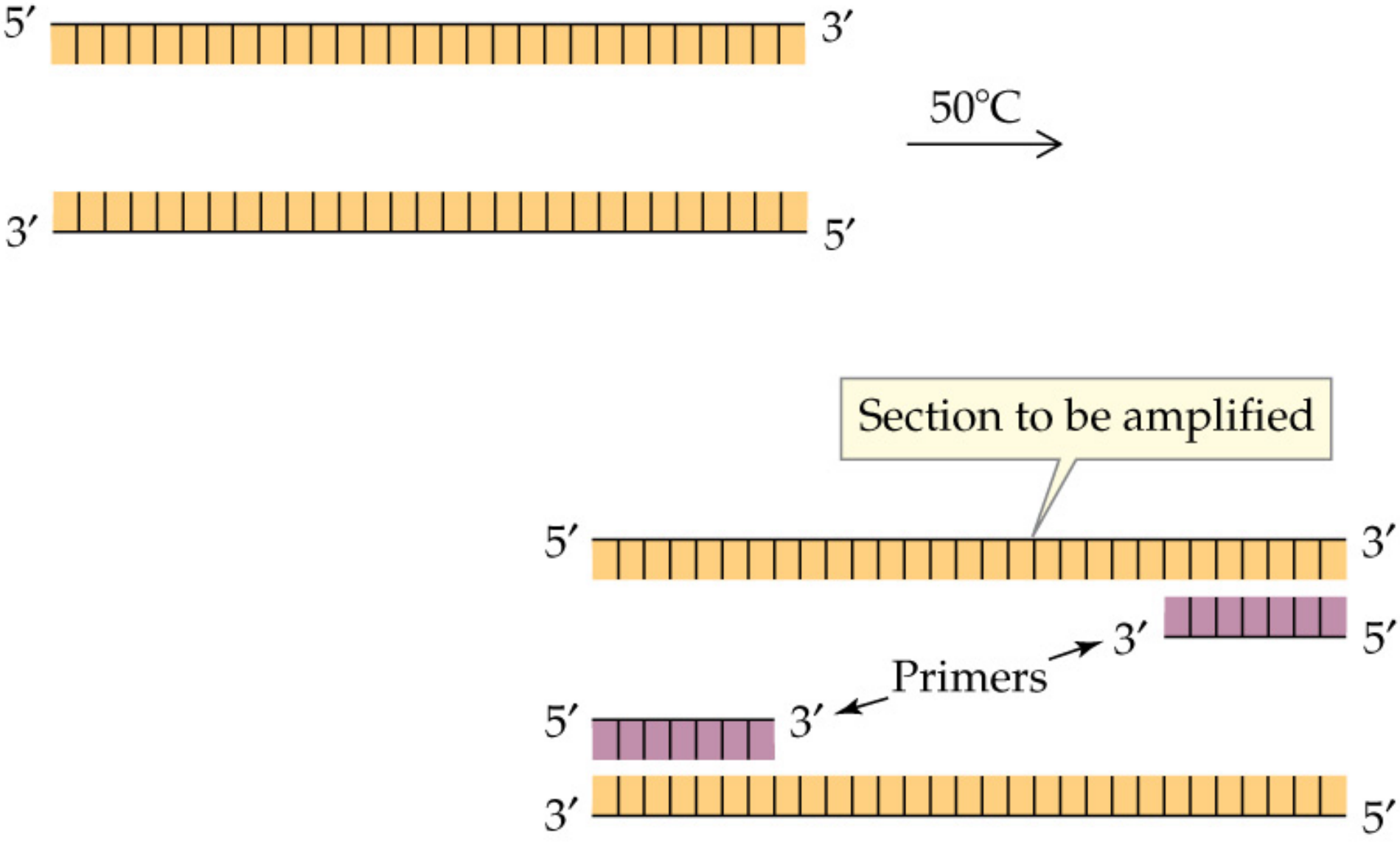
only dNTP's

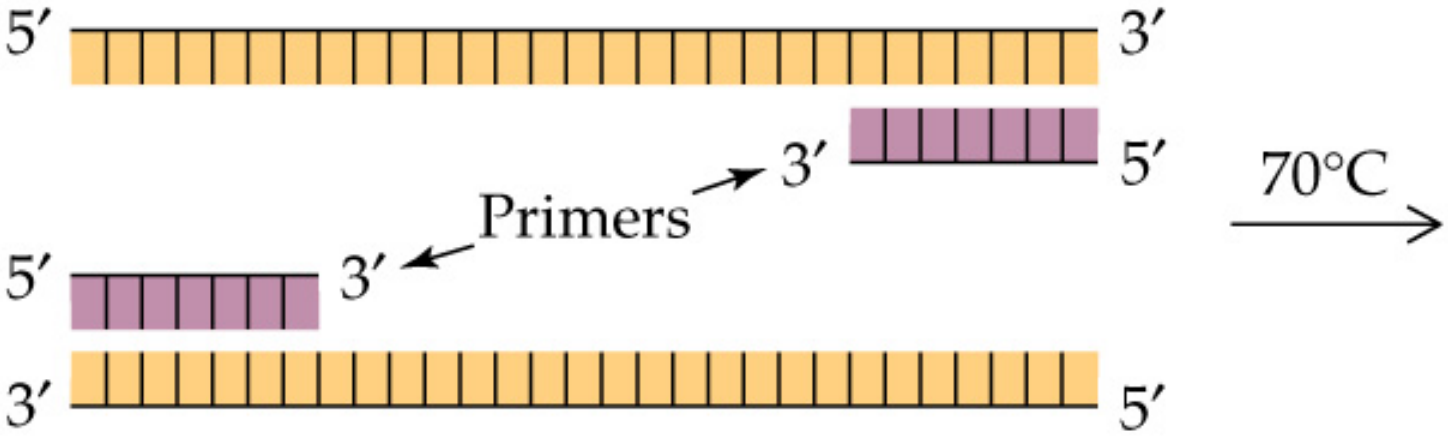
(Andy Vierstraete 1999)

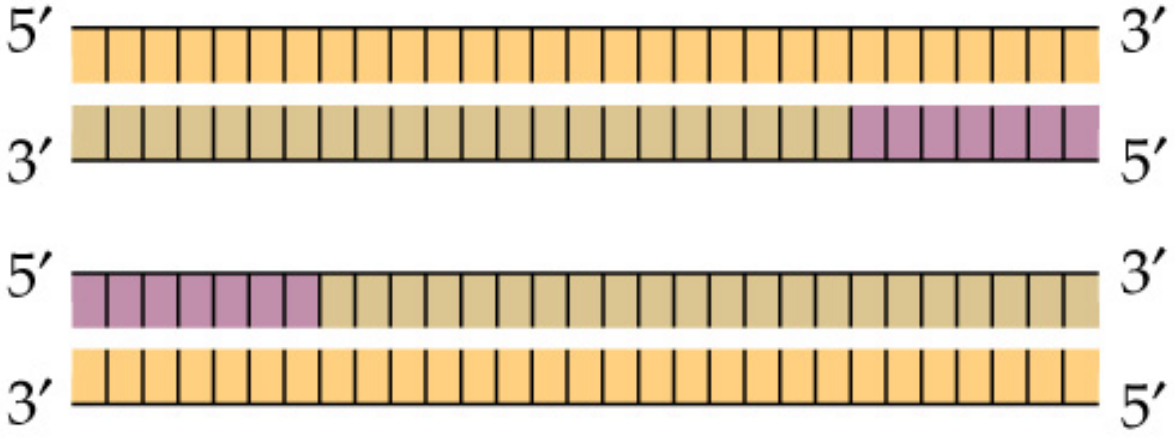
PCR

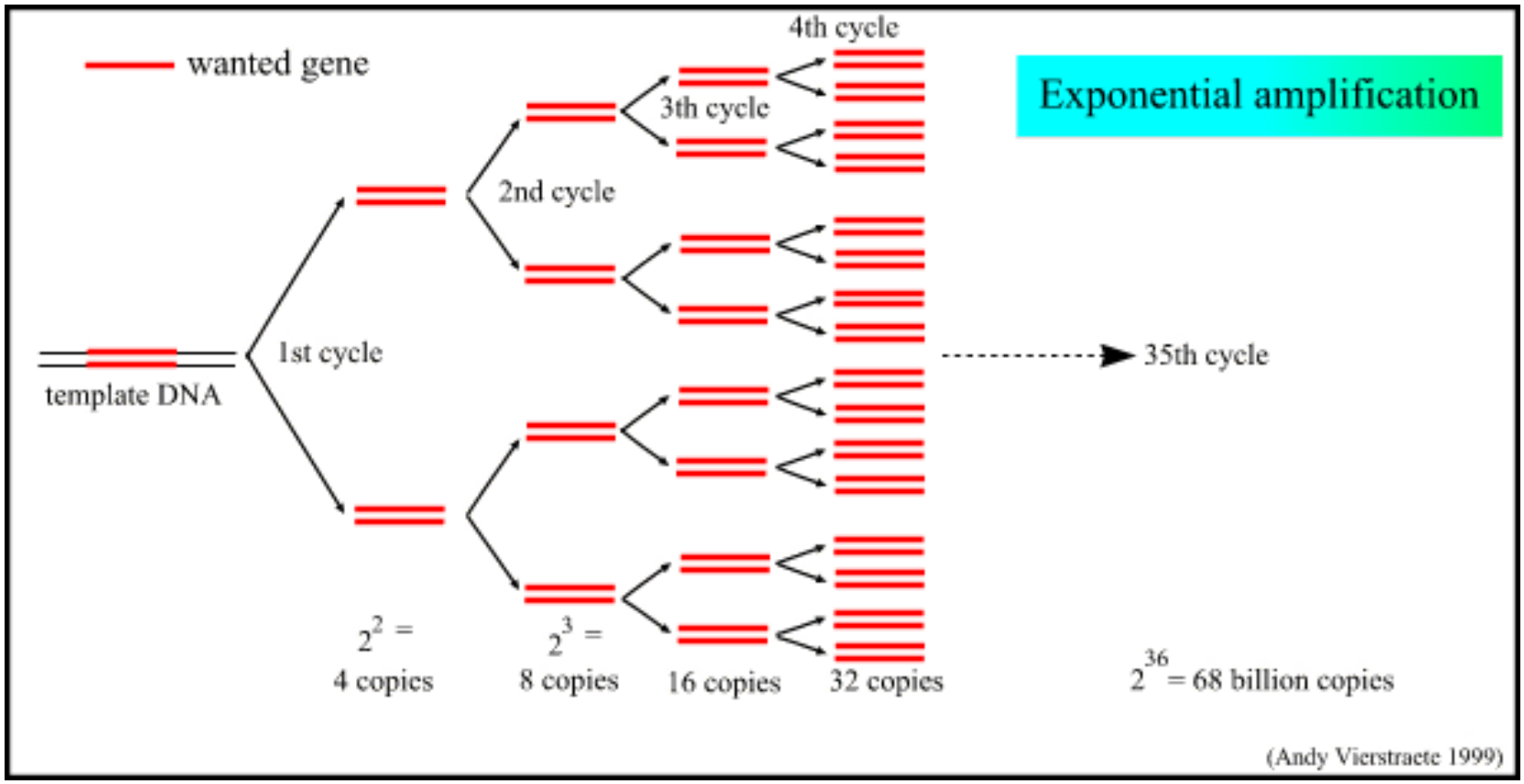
https://www.youtube.com/watch?v=MyLrs_h1OIE


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′

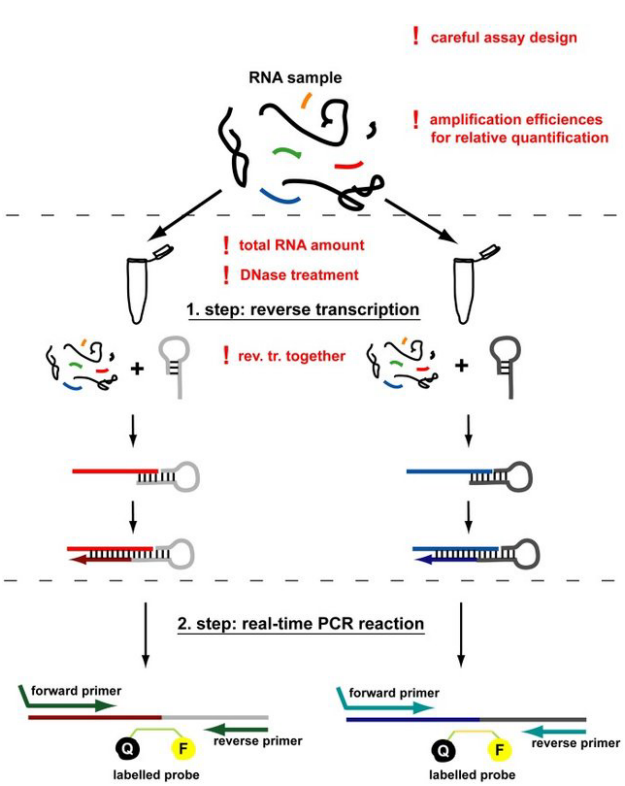

5′
3′
3′
5′
Primers
3′
5′
5′
3′
70°C

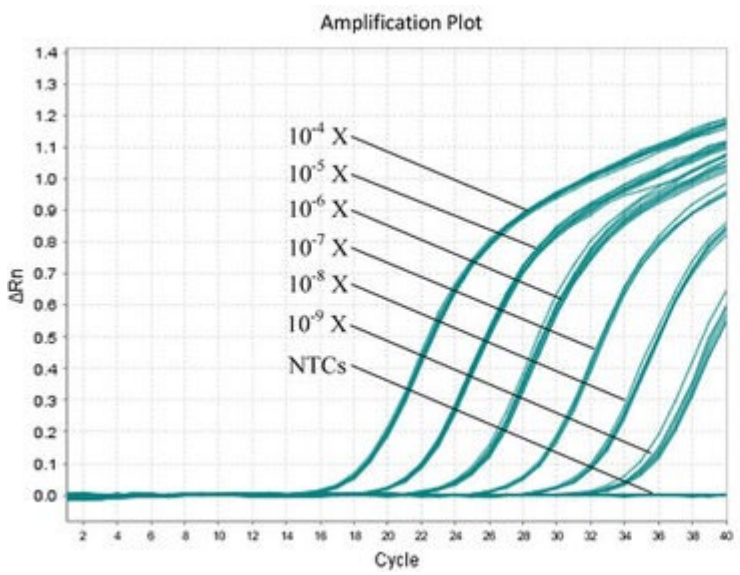

5′
3′
3′
5′
5′
3′
3′
5′

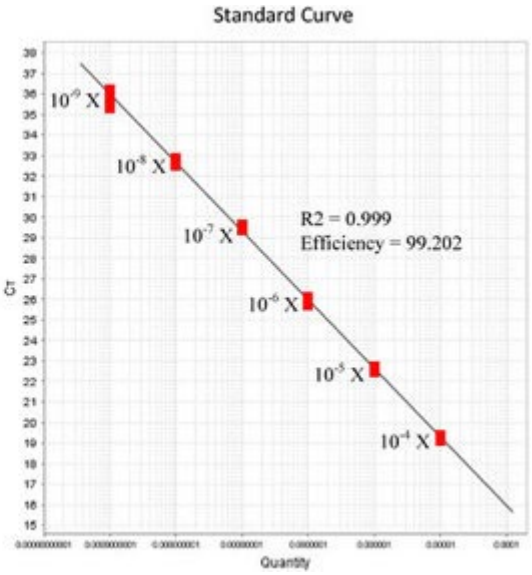

wanted gene
Exponential amplification
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies
(Andy Vierstraete 1999)

Real-time PCR

https://www.youtube.com/watch?v=1kvy17ugl4w


! 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

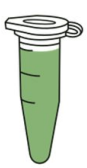
PROTOCOL OF SARS-COV-2 DETECTION USING REAL-TIME RT-PCR

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

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

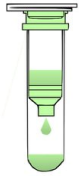
Primer sets and probes → designed based on the first sequences of SARS-CoV-2 available on the GISAID database

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



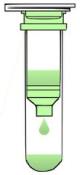
Sample lysis

5 min incubation of sample in Lysis Buffer containing Proteinase K



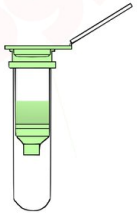
Binding of viral RNA

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

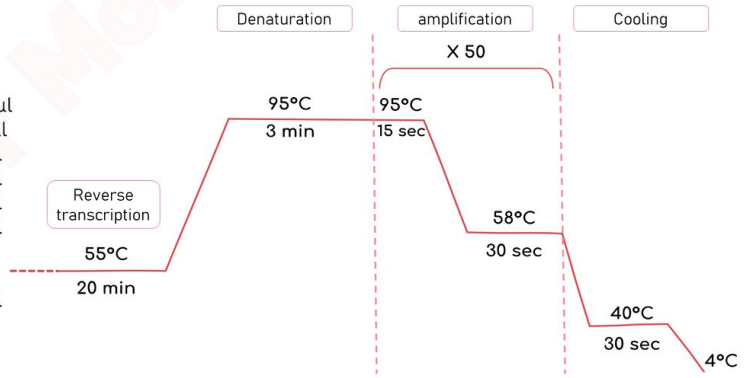
Elution in 20-50 µl RNase-free water or Elution Buffer

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)



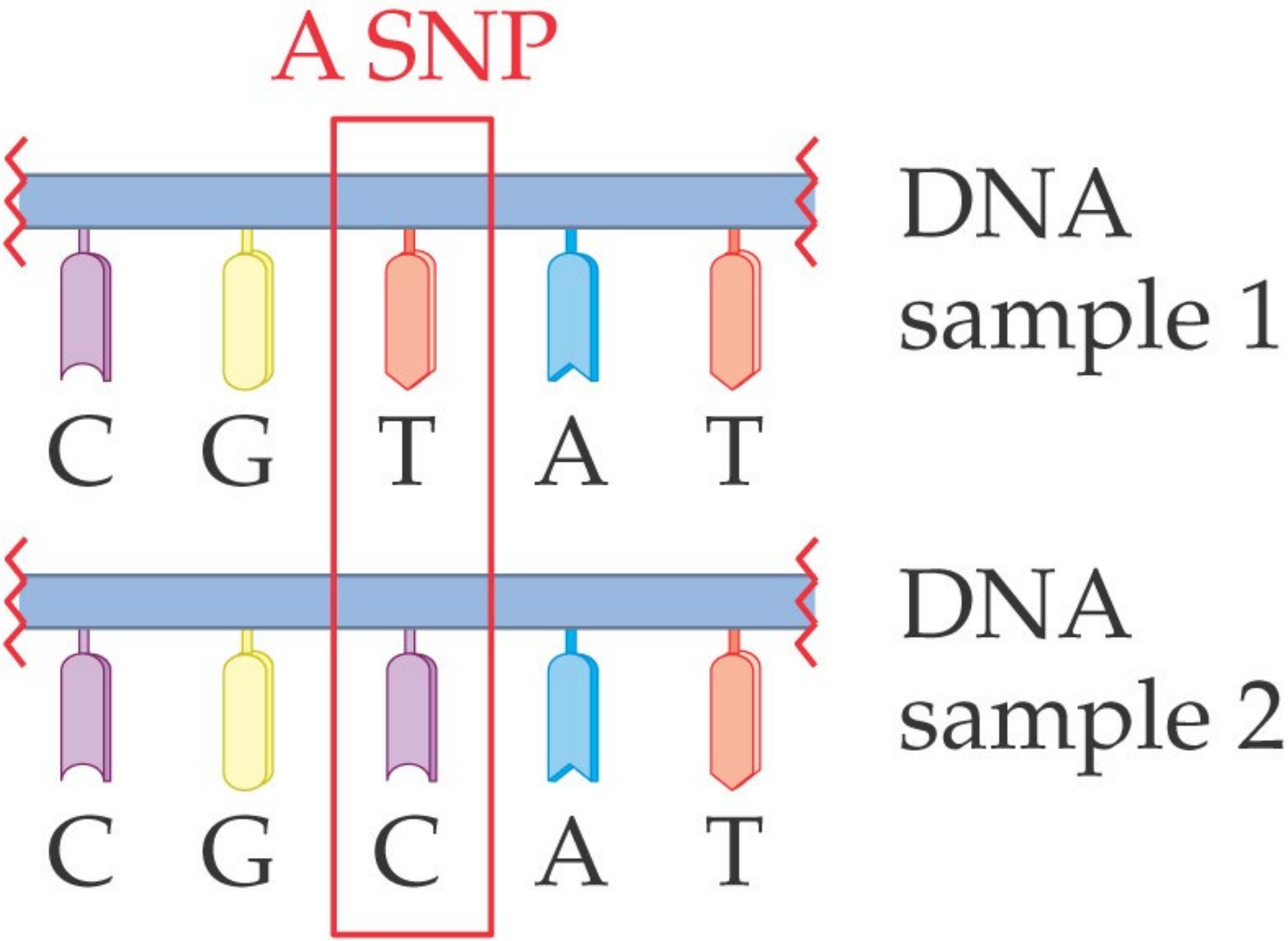
POSITIVE CONTROL

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.

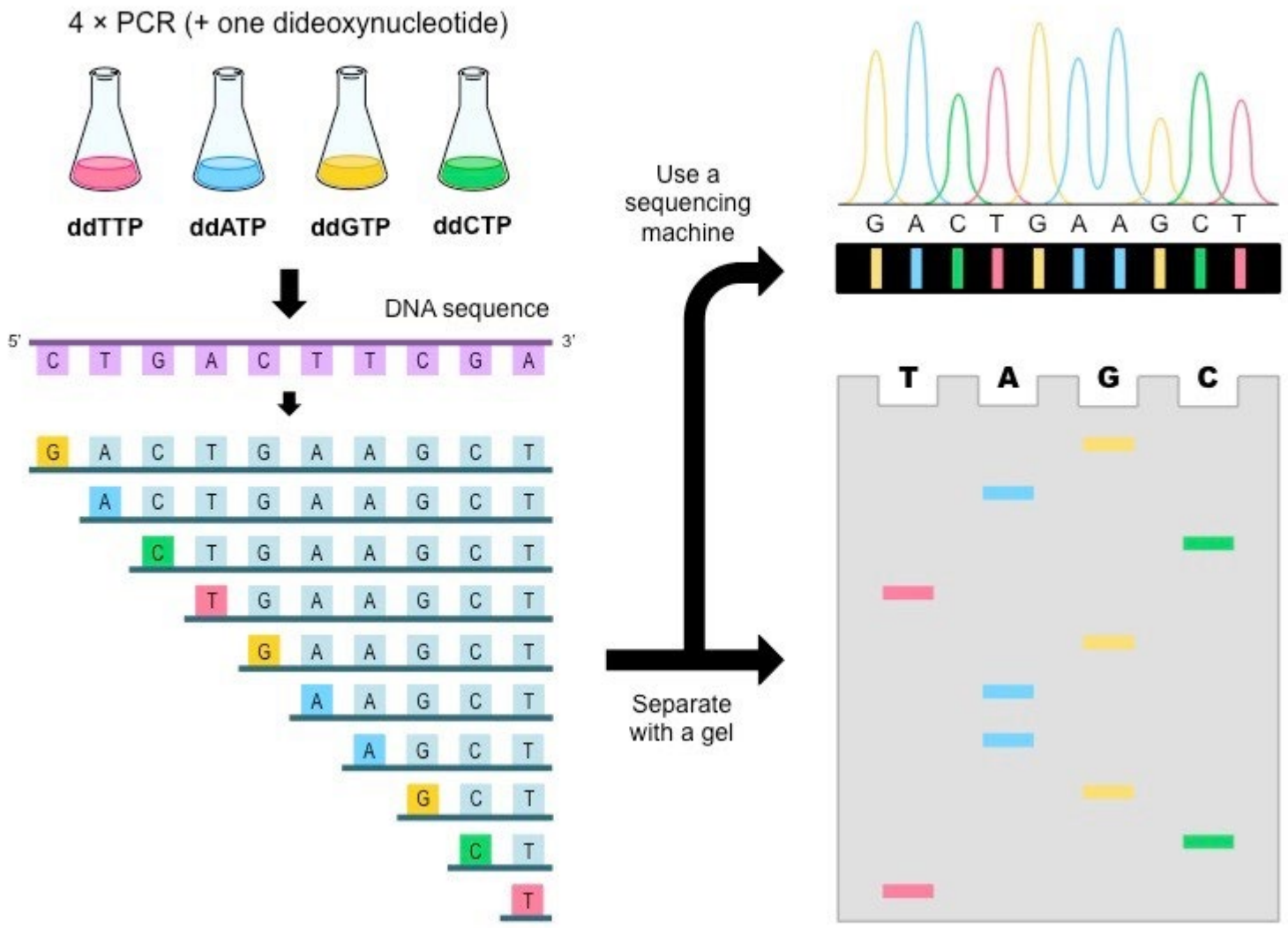
M. MERZOUG

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.

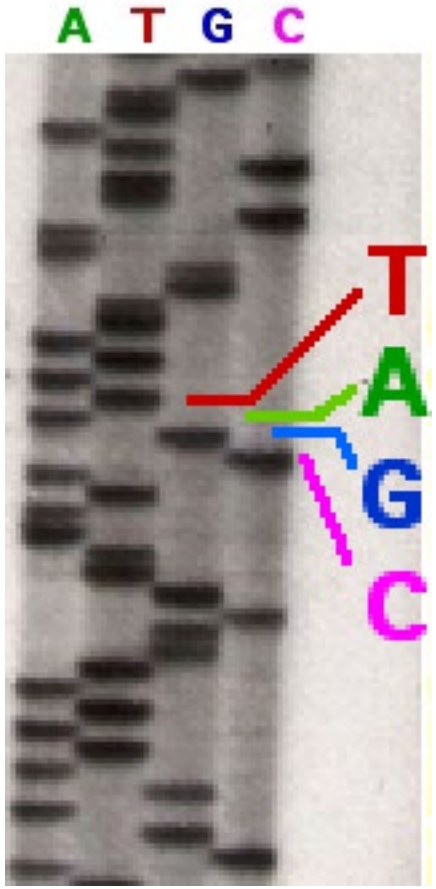

A SNP
C G T A T
DNA sample 1
C G C A T
DNA sample 2

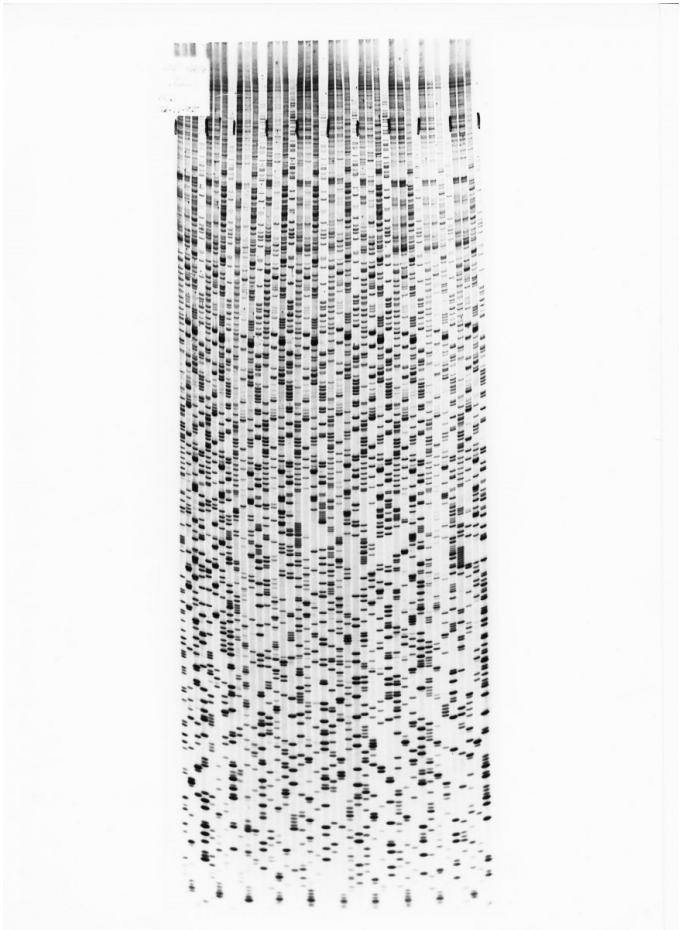
DNA Sequencing



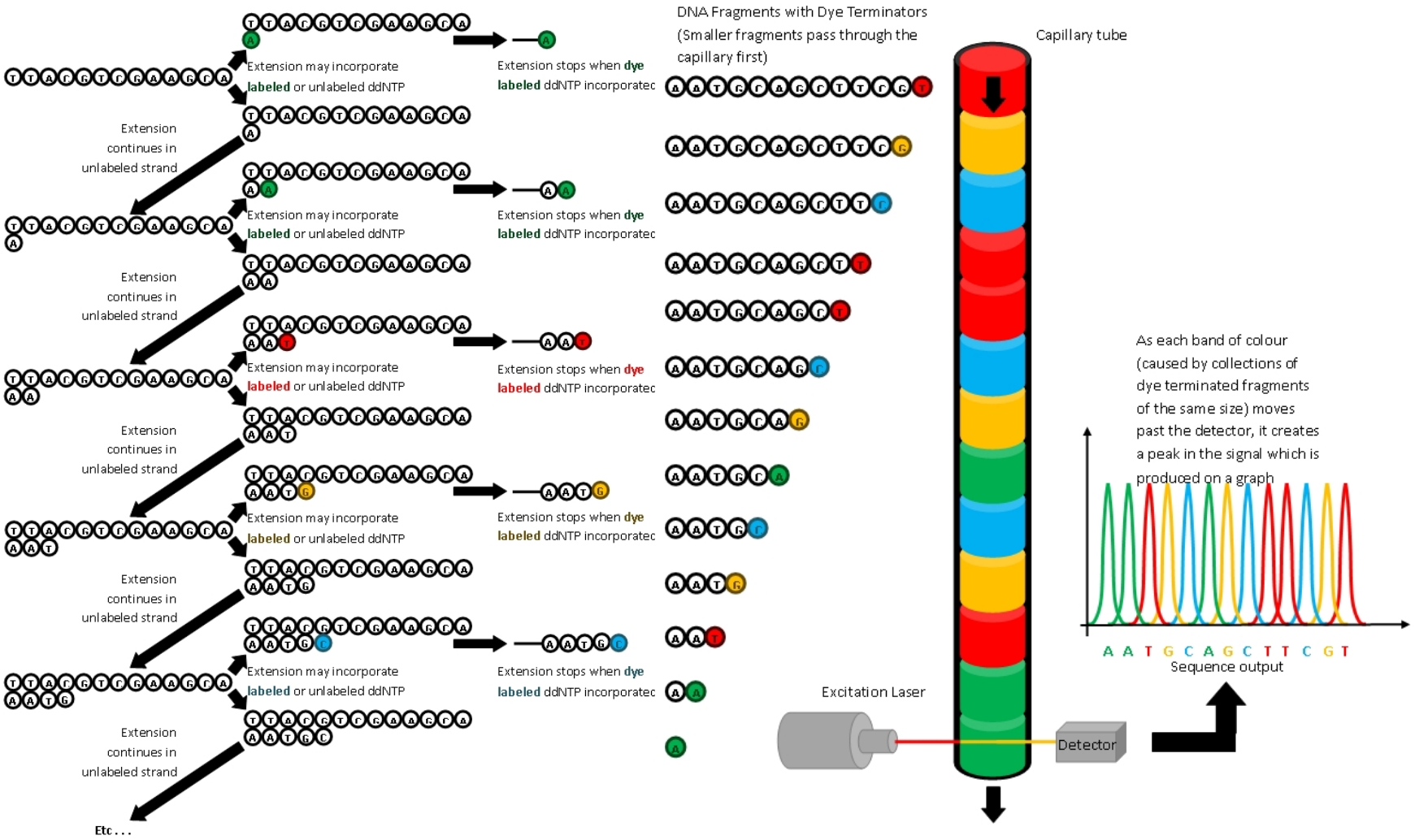
DNA Sequencing

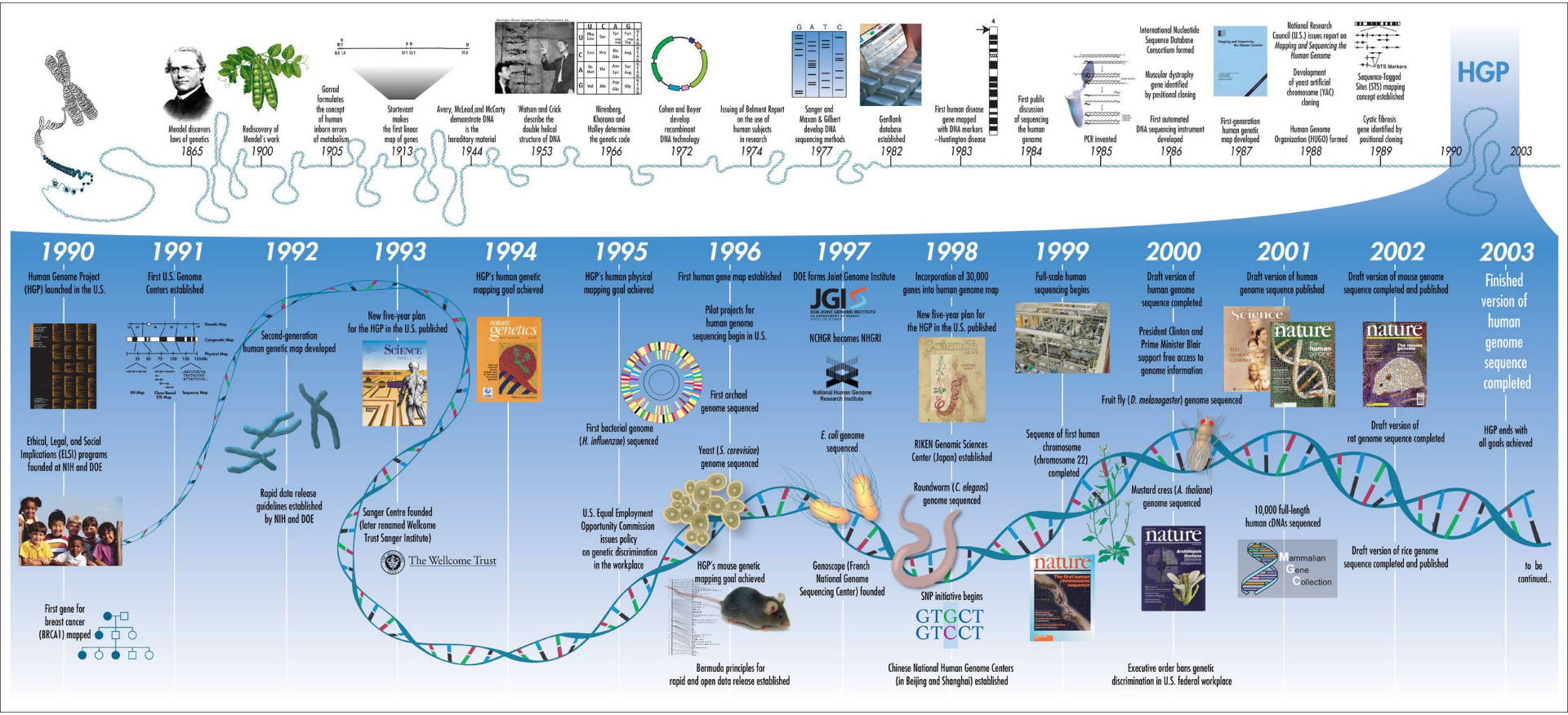
https://www.youtube.com/watch?v=vK-HlMaitnE



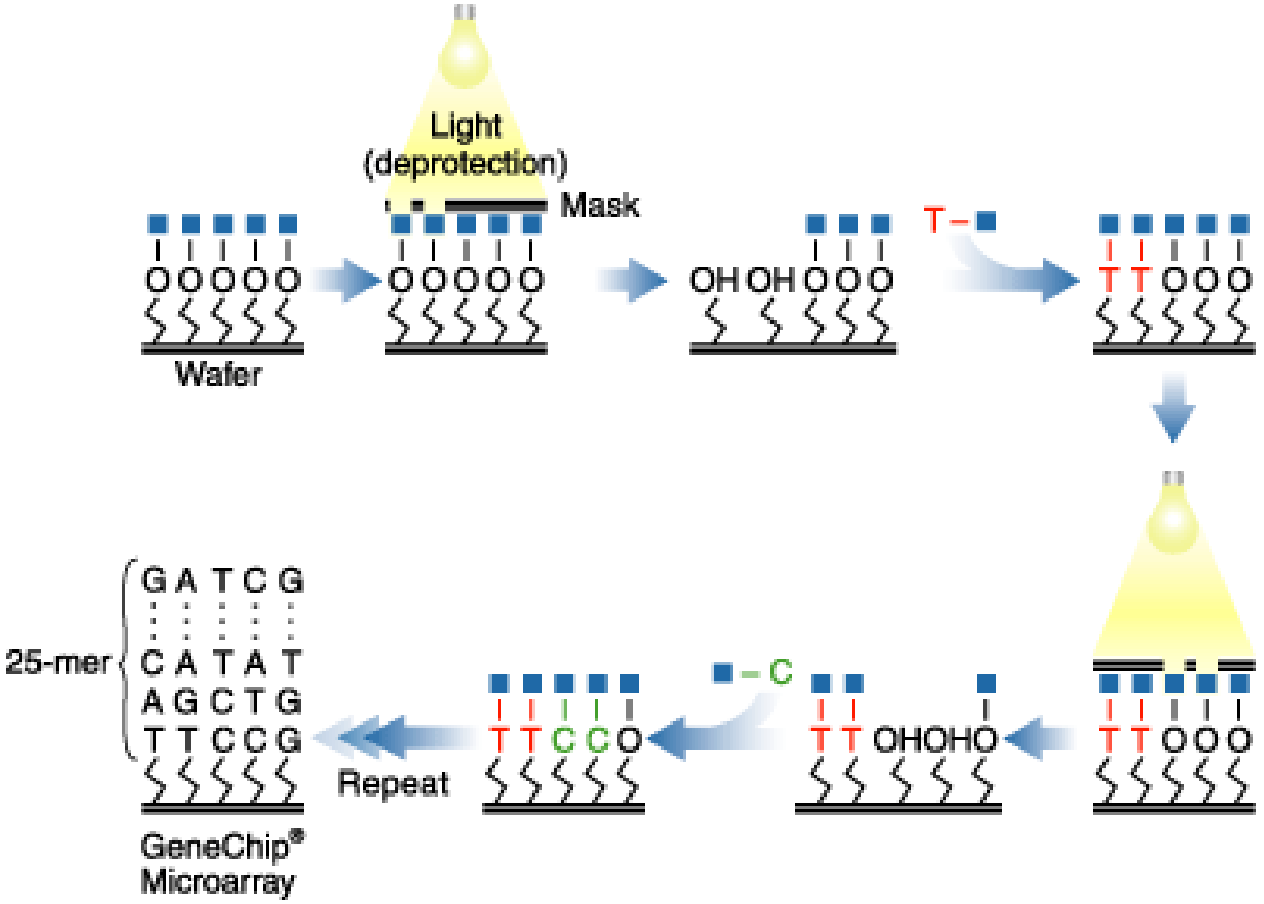


Dye Terminations

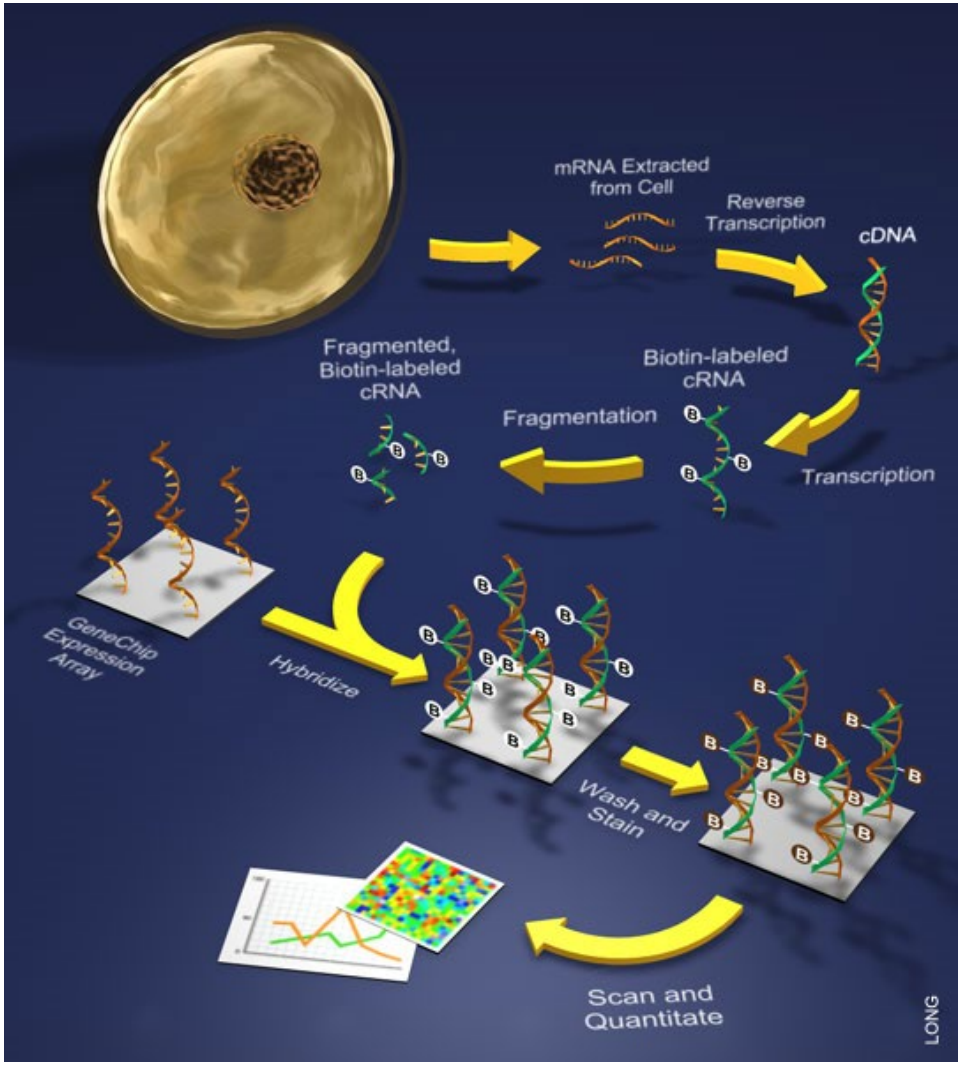



HGP
1865 Mendel discovers laws of genetics
1900 Rediscovery of Mendel's work
1905 Garrod formulates the concept of human inborn errors of metabolism
1913 Sturtevant makes the first linear map of genes
1944 Avery, McLeod, and McCarty demonstrate DNA is the hereditary material
1953 Watson and Crick describe the double helical structure of DNA
1966 Nirenberg, Khorana and Holley determine the genetic code
1972 Cohen and Boyer develop recombinant DNA technology
1974 Issuing of Belmont Report on the use of human subjects in research
1977 Sanger and Maxam & Gilbert develop DNA sequencing methods
1982 GenBank database established
1983 First human disease gene mapped with DNA markers –Huntington disease
1984 First public discussion of sequencing the human genome
1985 PCR invented
1986 International Nucleotide Sequence Database Consortium formed
Muscular dystrophy gene identified by positional cloning
First automated DNA sequencing instrument developed
1987 First-generation human genetic map developed
1988 National Research Council (U.S.) issues report on Mapping and Sequencing the Human Genome
Development of yeast artificial chromosome (YAC) cloning
Human Genome Organization (HUGO) formed
1989 STS Markers
Sequence-Tagged Sites (STS) mapping concept established
Cystic fibrosis gene identified by positional cloning
1990
2003
1990
Human Genome Project (HGP) launched in the U.S.
Ethical, Legal, and Social Implications (ELSI) programs founded at NIH and DOE
First gene for breast cancer (BRCA1) mapped
1991
First U.S. Genome Centers established
1992
Second-generation human genetic map developed
Rapid data release guidelines established by NIH and DOE
1993
New five-year plan for the HGP in the U.S. published
Sanger Centre founded (later renamed Wellcome Trust Sanger Institute)
The Wellcome Trust
1994
HGP's human genetic mapping goal achieved
1995
HGP's human physical mapping goal achieved
First bacterial genome (H. influenzae) sequenced
U.S. Equal Employment Opportunity Commission issues policy on genetic discrimination in the workplace
1996
First human gene map established
Pilot projects for human genome sequencing begin in U.S.
First archael genome sequenced
Yeast (S. cerevisiae) genome sequenced
HGP's mouse genetic mapping goal achieved
Bermuda principles for rapid and open data release established
1997
DOE forms Joint Genome Institute
JGI
NCHGR becomes NHGRI
National Human Genome Research Institute
E. coli genome sequenced
Genoscope (French National Genome Sequencing Center) founded
1998
Incorporation of 30,000 genes into human genome map
New five-year plan for the HGP in the U.S. published
RIKEN Genomic Sciences Center (Japan) established
Roundworm (C. elegans) genome sequenced
SNP initiative begins
GTGCT
GTCCT
Chinese National Human Genome Centers (in Beijing and Shanghai) established
1999
Full-scale human sequencing begins
Sequence of first human chromosome (chromosome 22) completed
2000
Draft version of human genome sequence completed
President Clinton and Prime Minister Blair support free access to genome information
Fruit fly (D. melanogaster) genome sequenced
Mustard cress (A. thaliana) genome sequenced
Executive order bans genetic discrimination in U.S. federal workplace
2001
Draft version of human genome sequence published
10,000 full-length human cDNAs sequenced
Mammalian Gene Collection
2002
Draft version of mouse genome sequence completed and published
Draft version of rat genome sequence completed
Draft version of rice genome sequence completed and published
2003
Finished version of human genome sequence completed
HGP ends with all goals achieved
to be continued..

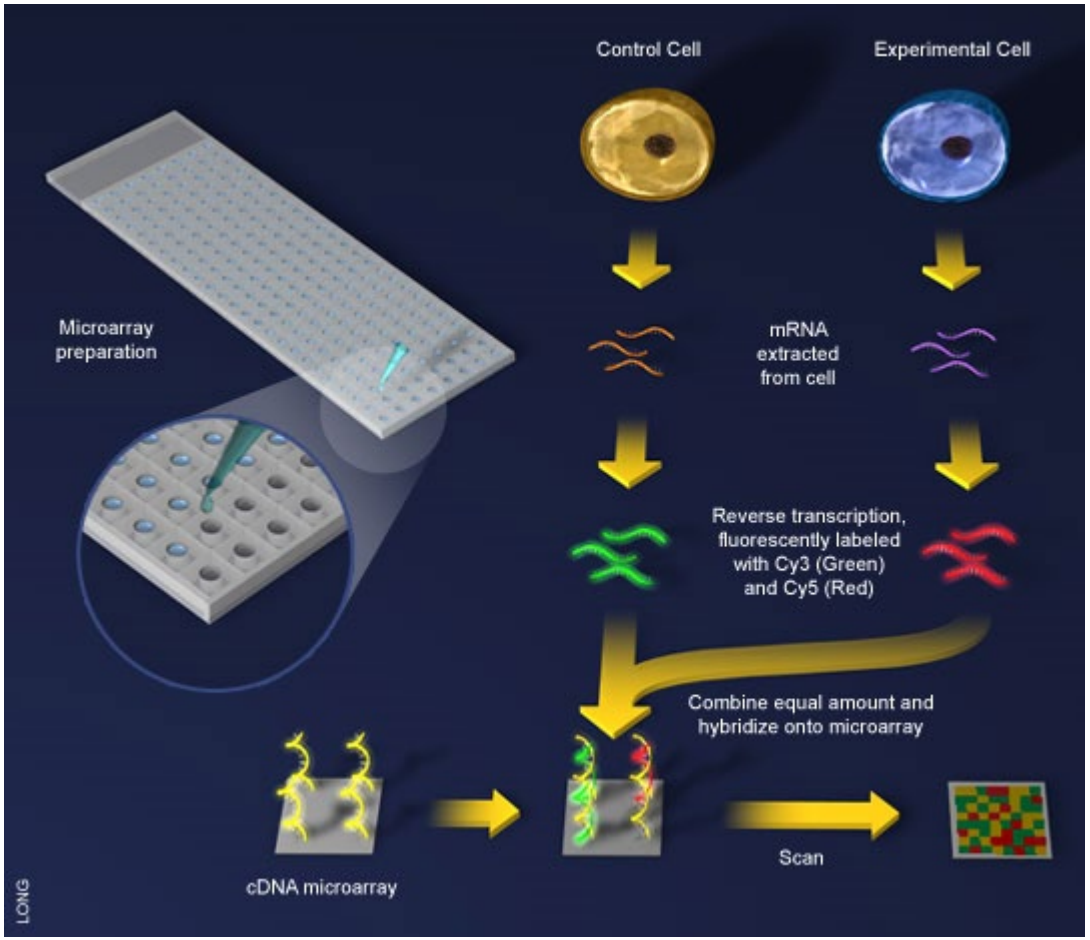
GeneChip


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

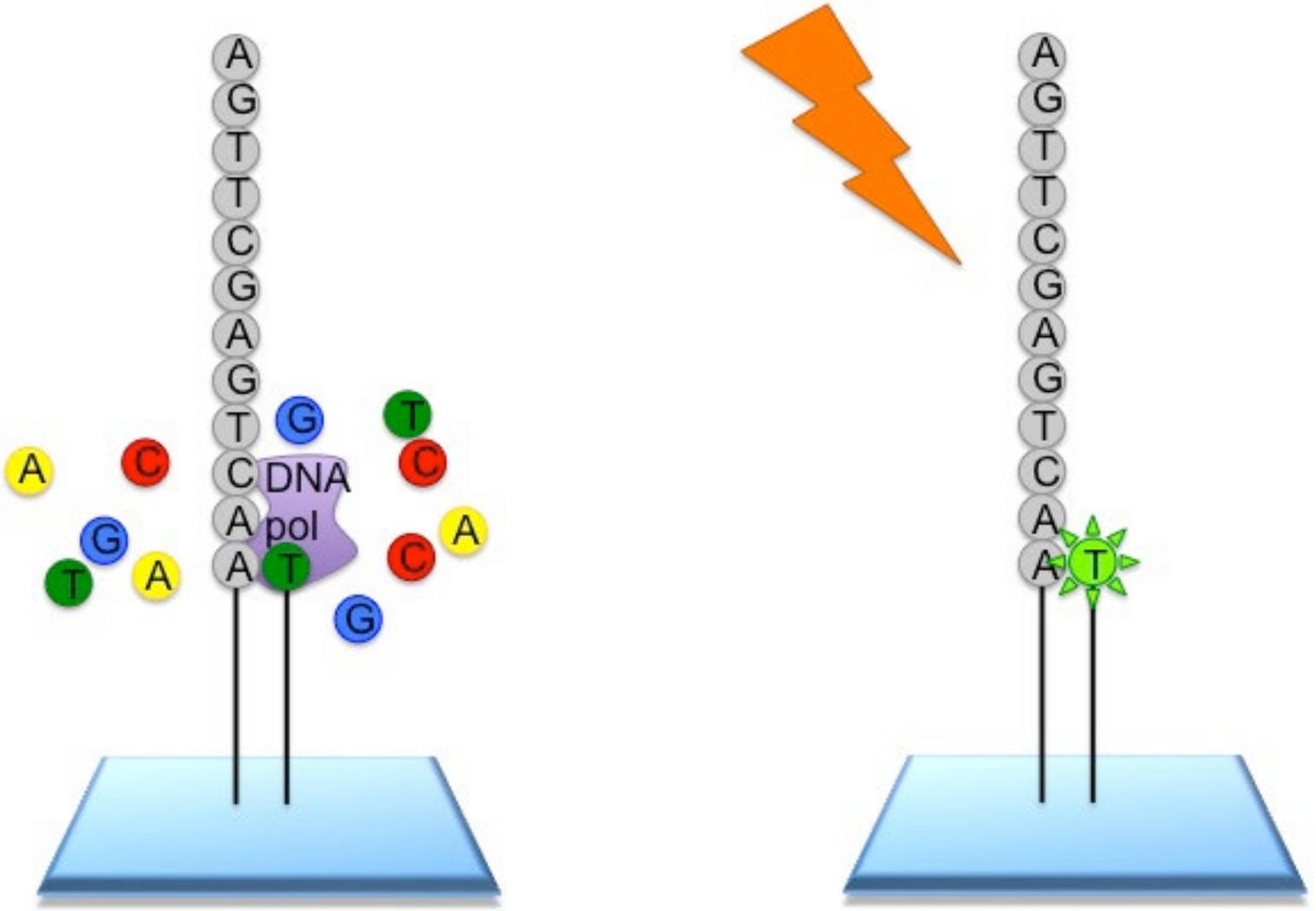
Scheme


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



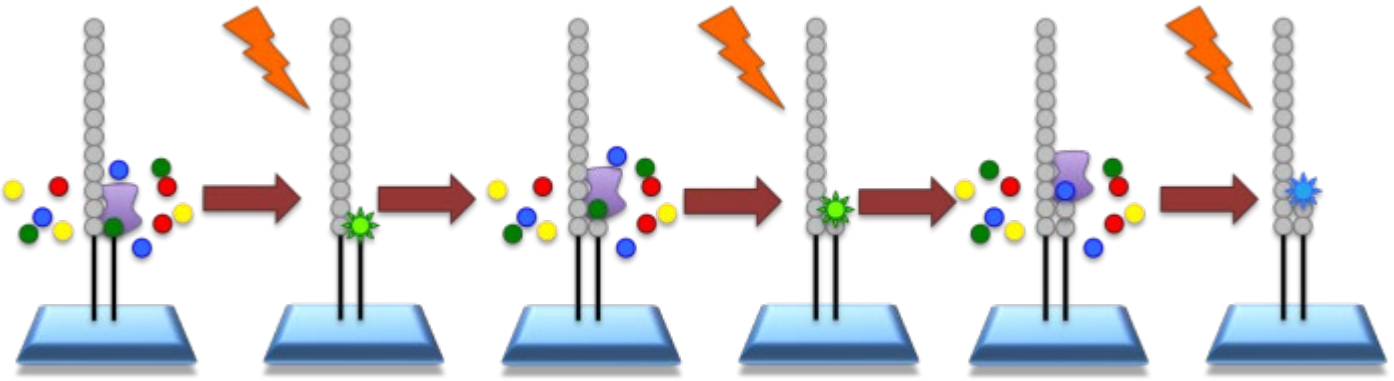
NGS Illumina

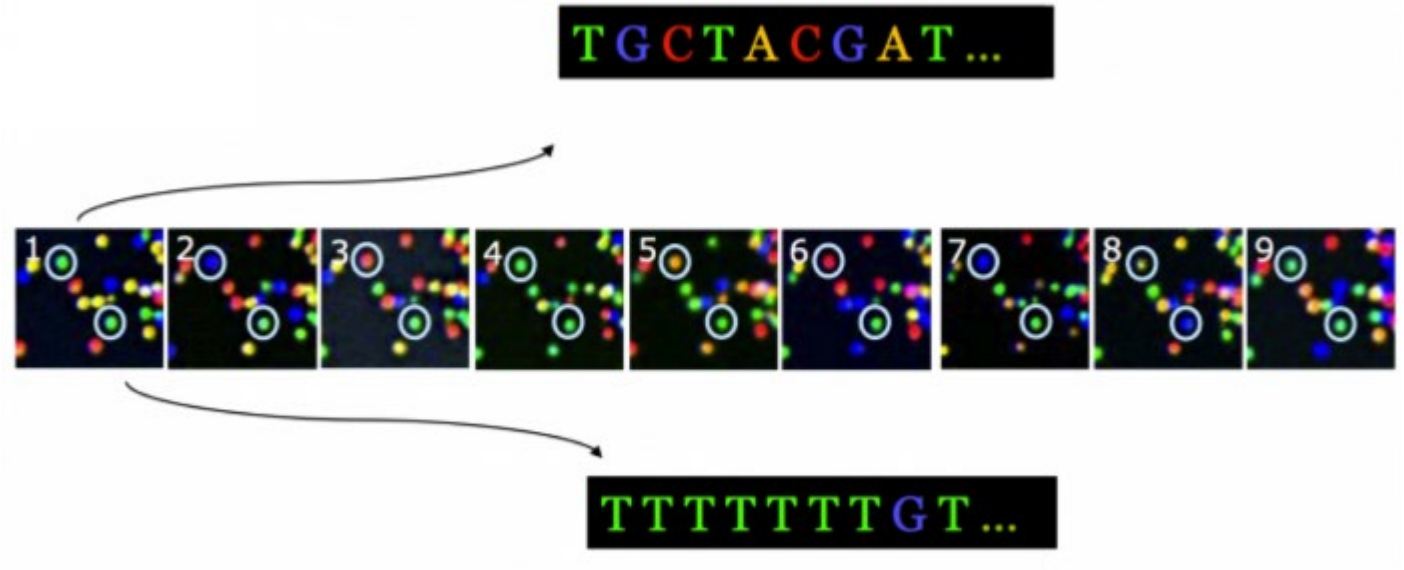


100-150 bp

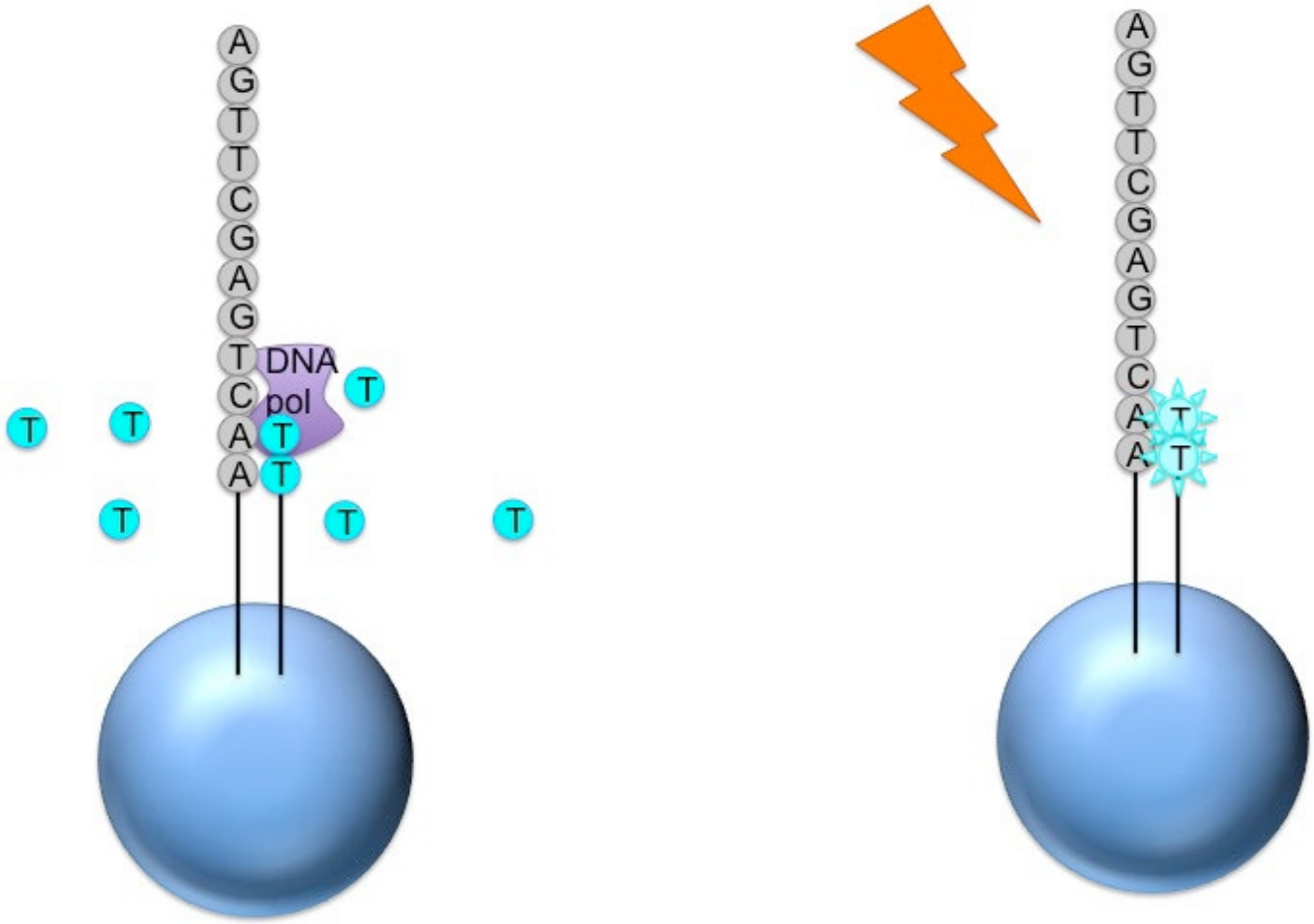
NGS Illumina

https://www.youtube.com/watch?v=fCd6B5HRaZ8





Roche 454 sequencing



1000 bp

Roche 454 sequencing