Lecture #5

“DNA repair & recombination”

Jan 22, 2018

RECAP

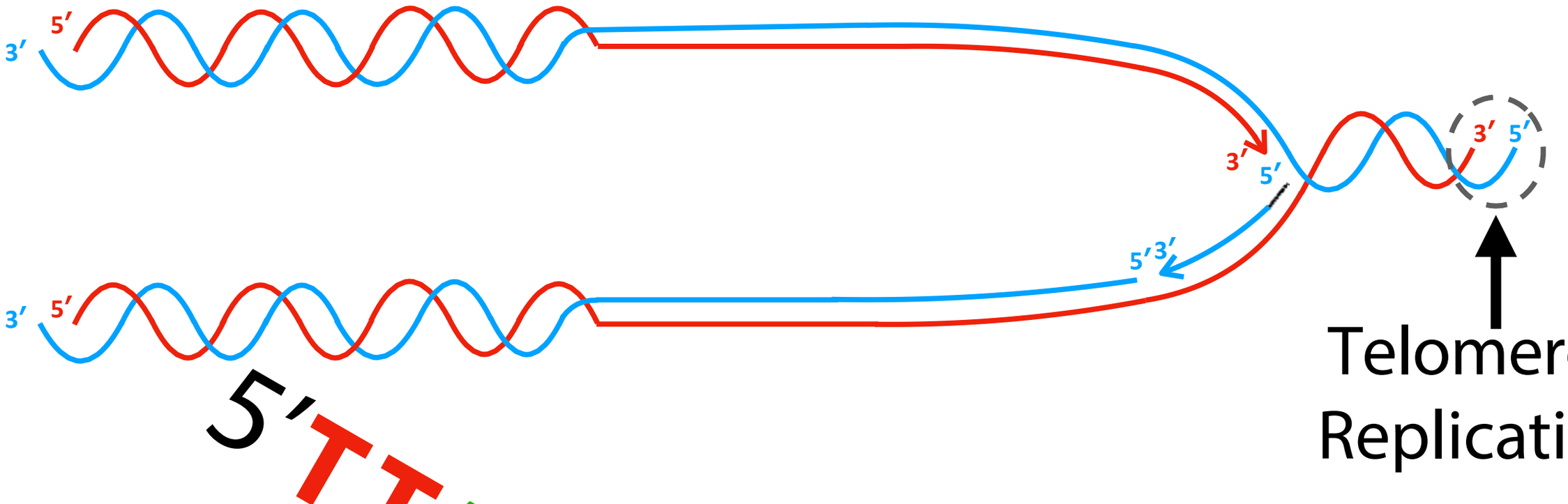
How cells protect DNA ends (telomeres)

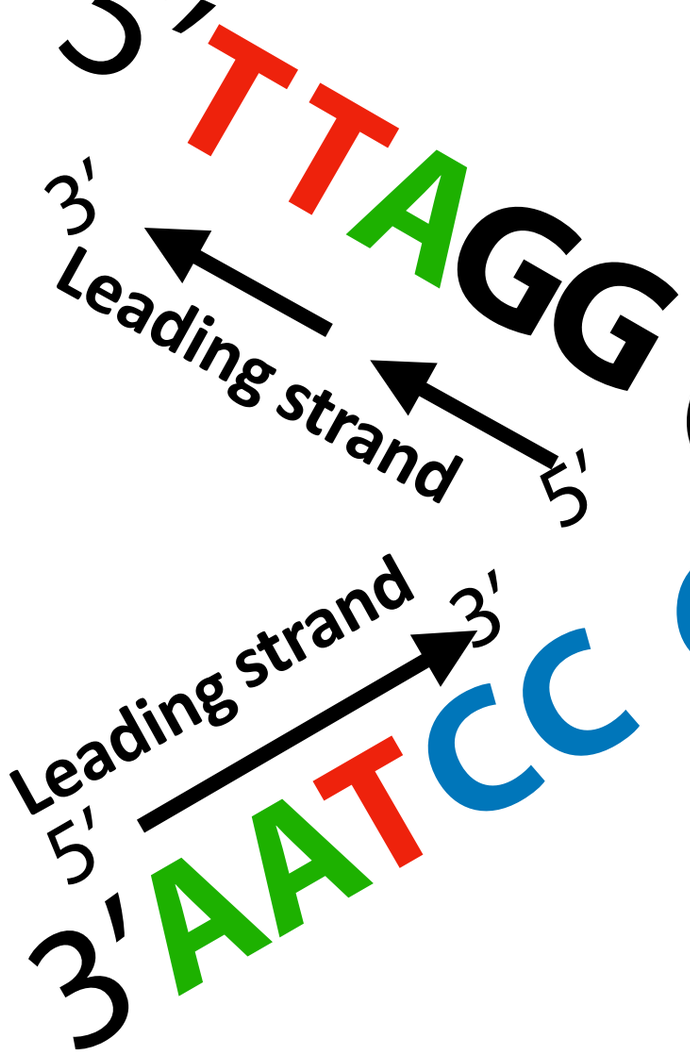
2009

Elizabeth H. Blackburn, Carol W. Greider & Jack Szostak (2009)

How chromosomes are protected by telomeres and the enzyme telomerase

Replication at the ends of chromosome





GTTAGGG 3′

CAATCCC 5′

Telomeres -
repetitive nucleotide sequence
~2500 times in humans

Replication at the ends of chromosome

Telomere shortening

GTTAGGG3′
CAAUCCC5′

GTTAGGG3′
CAATCCC 5′

How cells protect telomeres

5′**TTAGGGTTAGGG**3′

3′**AATCCCAAUCCC**5′

Human TERT

TElomerase Reverse Transcriptase

5′**TTAGGGTTAGGGTTAG** 3′

3′**AATCCCAATCCCAATCCC**-

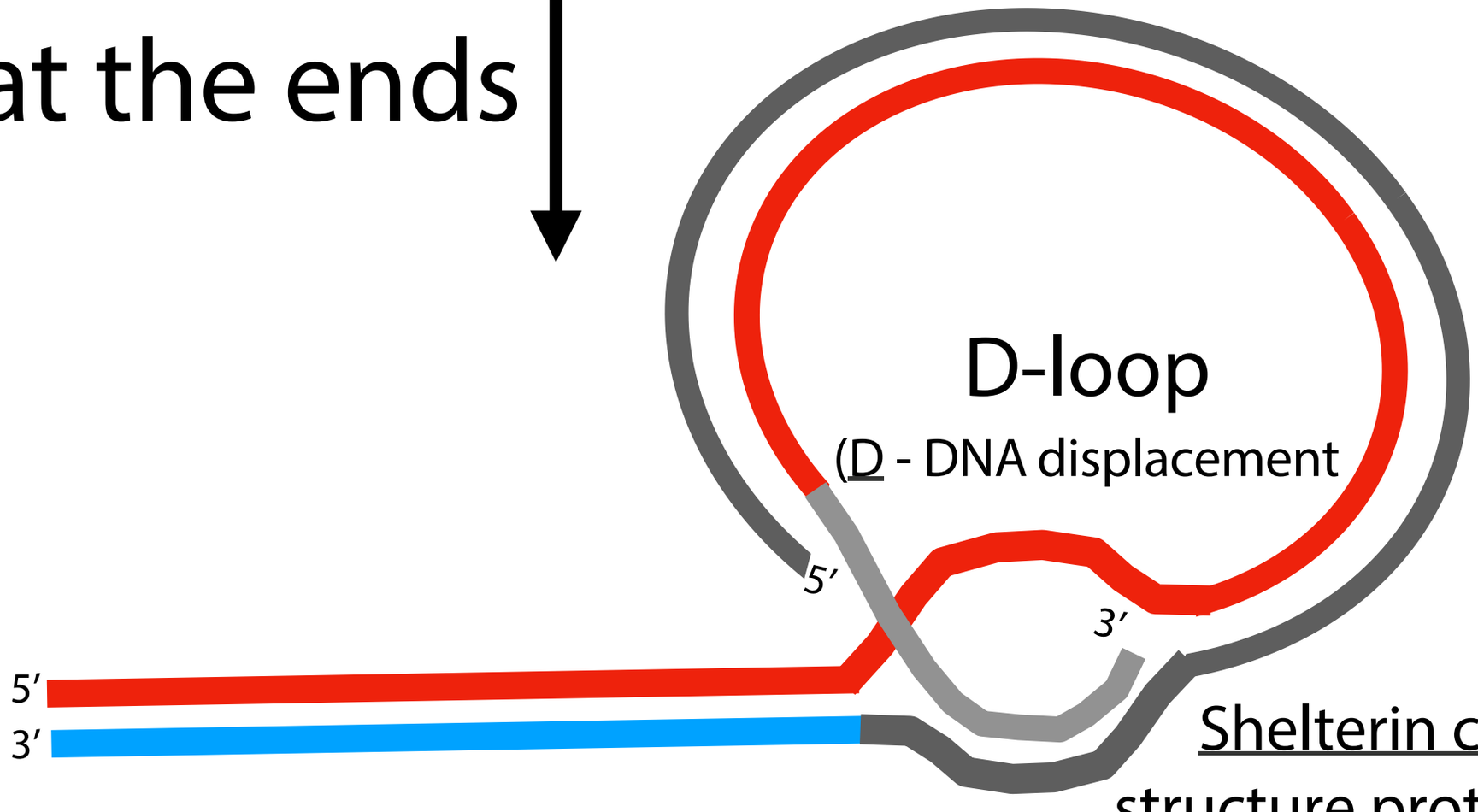
hTERT

RNA component used as a template

Protection at the ends of DNA

Protection at the ends

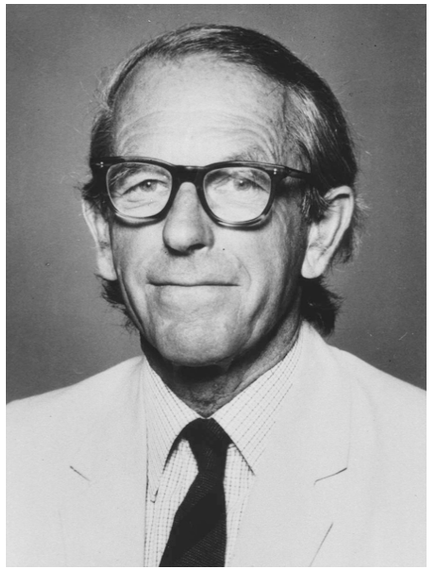
T-loop (T - Telomere)



Shelterin complex binds this structure protecting the ends from damage-signaling pathways

DNA sequencing

Paul Berg



Frederick Sanger

Walter Gilbert

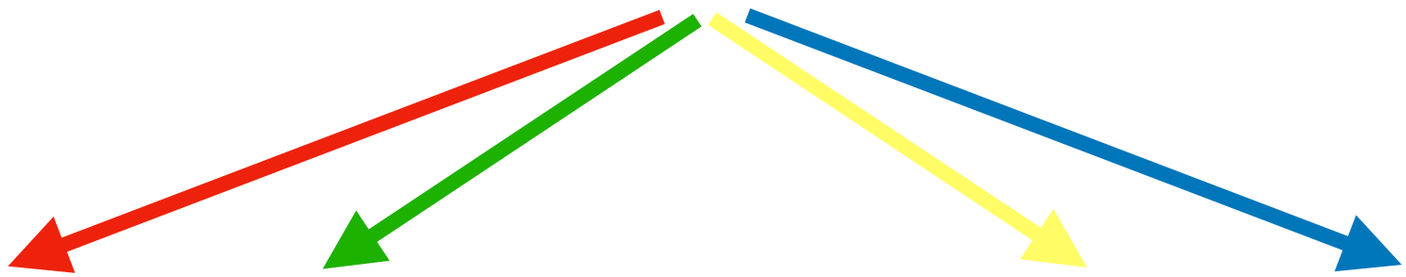
1980

2 Nobel prizes

One-half to Berg for his studies of nucleic acids relevant to recombinant DNA. The second half shared between Sanger and Gilbert for their work of determining base sequence of nucleic acids.

DNA sequencing (Sanger)

5’t t t t**TATCGCTCTGAC**3’
3’aaaa**ATAGCGAGACTG**5’



Separate the two DNA strands
Add DNA polymerase
Add dNTPs

ddTTP* **ddATP*** **ddGTP*** **ddCTP***

Add primer

5’t t t t
3’aaaa
ATAGCGAGACTG5’

DNA sequencing

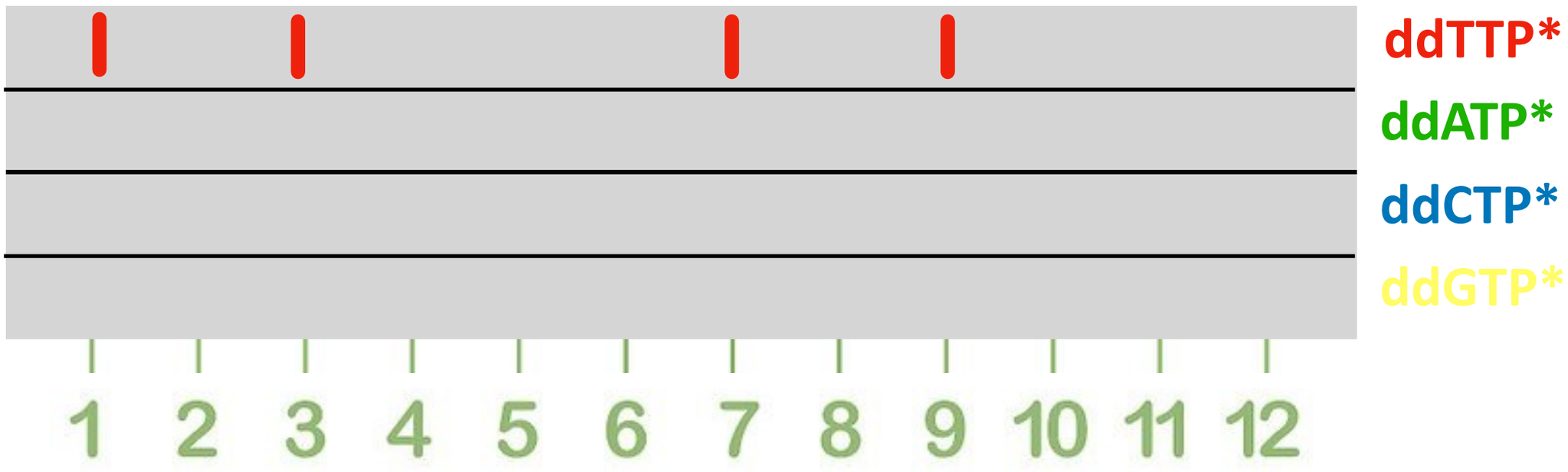
ddTTP*

5’ T
3’ A T A G C G A G A C T G 5’

5’ T A T
3’ A T A G C G A G A C T G 5’

5’ T A T C G C T
3’ A T A G C G A G A C T G 5’

5’ T A T C G C T C T
3’ A T A G C G A G A C T G 5’

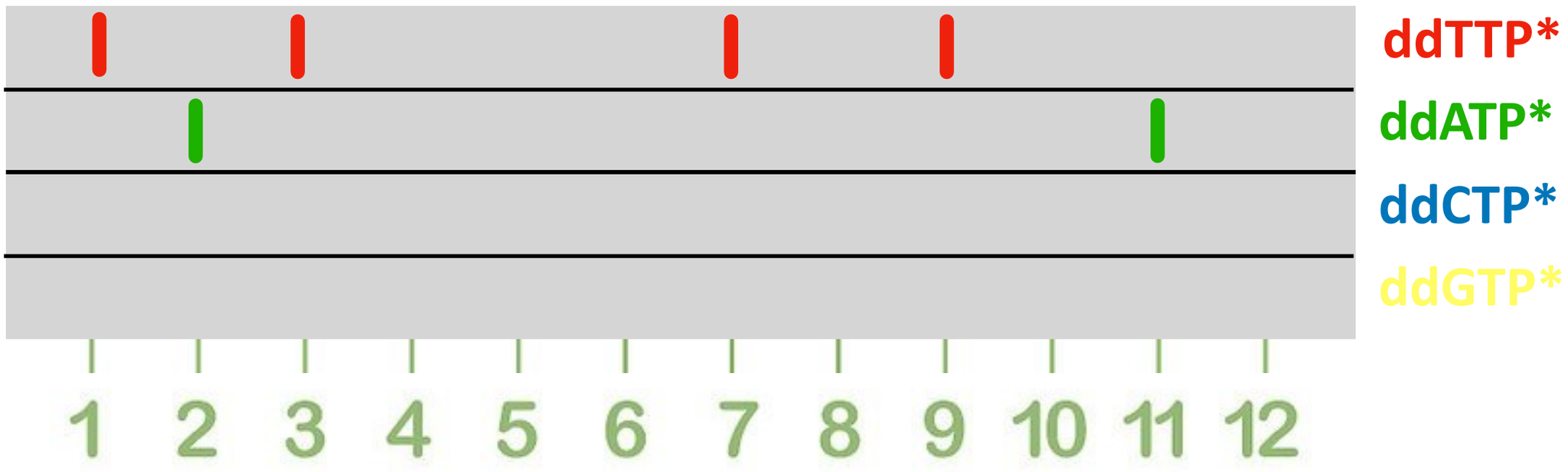


DNA sequencing

ddATP*

5’ T A
3’ A T A G C G A G A C T G 5’

5’ T A T C G C T C T G A
3’ A T A G C G A G A C T G 5’



DNA sequencing

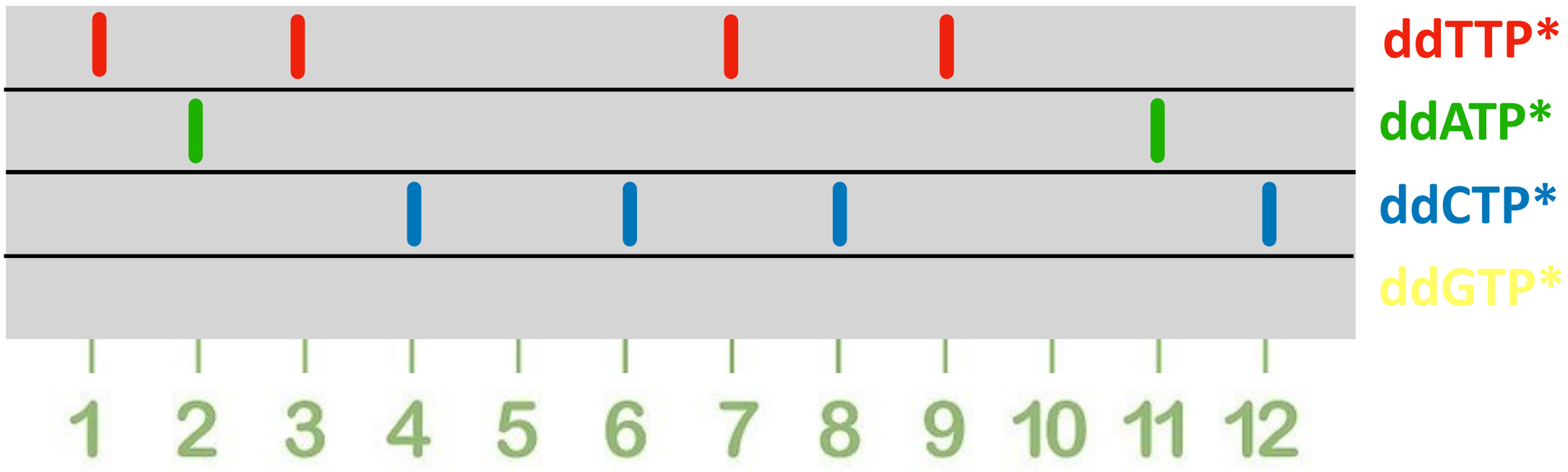
ddCTP*

5’ T A T **C**
3’ A T A G C G A G A C T G 5’

5’ T A T C G **C**
3’ A T A G C G A G A C T G 5’

5’ T A T C G C T **C**
3’ A T A G C G A G A C T G 5’

5’ T A T C G C T C T G A **C**
3’ A T A G C G A G A C T G 5’

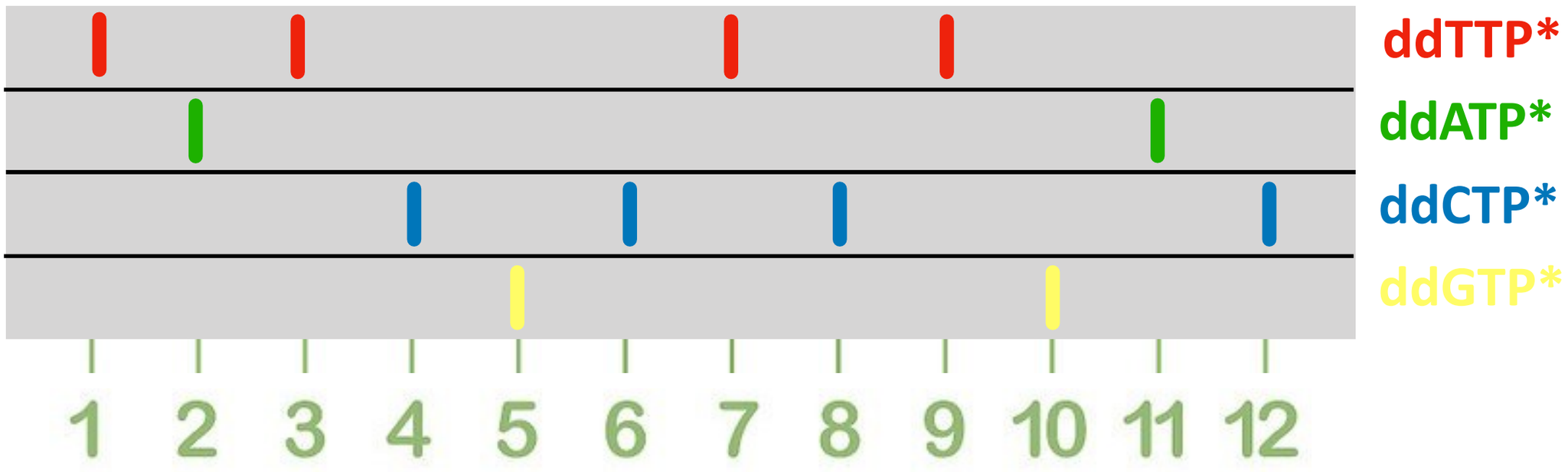


DNA sequencing

ddGTP*

5’ T A T C G
3’ A T A G C G A G A C T G 5’

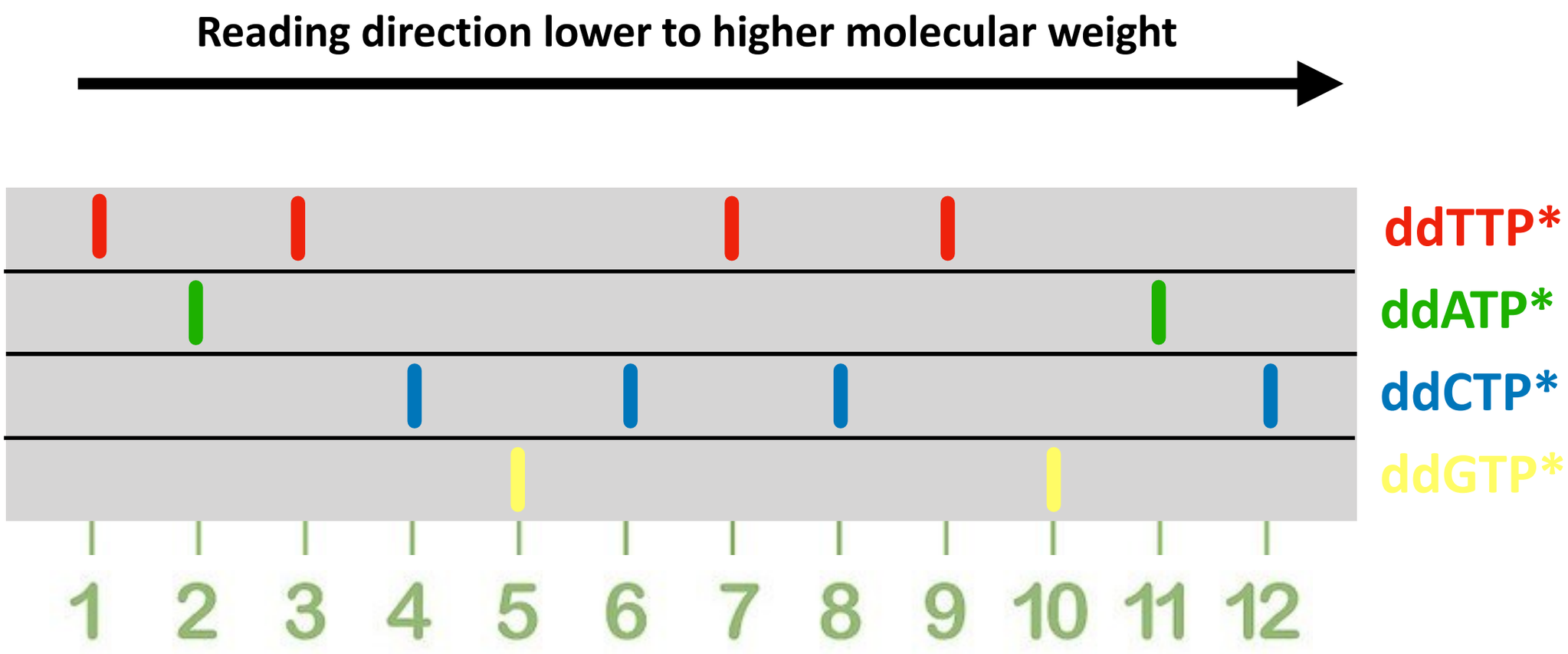
5’ T A T C G C T C T G
3’ A T A G C G A G A C T G 5’



DNA sequencing

5′**TATCGCTCTGAC**3′

3′**ATAGCGAGACTG**5′



DNA sequencing

Sequencing method	Read length	Accuracy	Reads per run	Time per run
Sanger	~1000bp	99.9%	500-1000bp	~1-2hrs
Single molecule pacific biosciences	~14,000bp - 40,000bp	87%	50,000bp-~1000 Mbp	~2-4hrs
Sequencing by synthesis (Illumina)	~75-1000bp	99.9%	1-3billion bp	1-10 days
Nanopore sequencing	~500 kbp	~90%	variable	In real time as sequencing takes place

DNA sequencing

Pop quiz

Sequencing the first human genome cost $1 billion and took >10 years. Now the cost has dropped by a factor of:

(A) ten

(B) a hundred

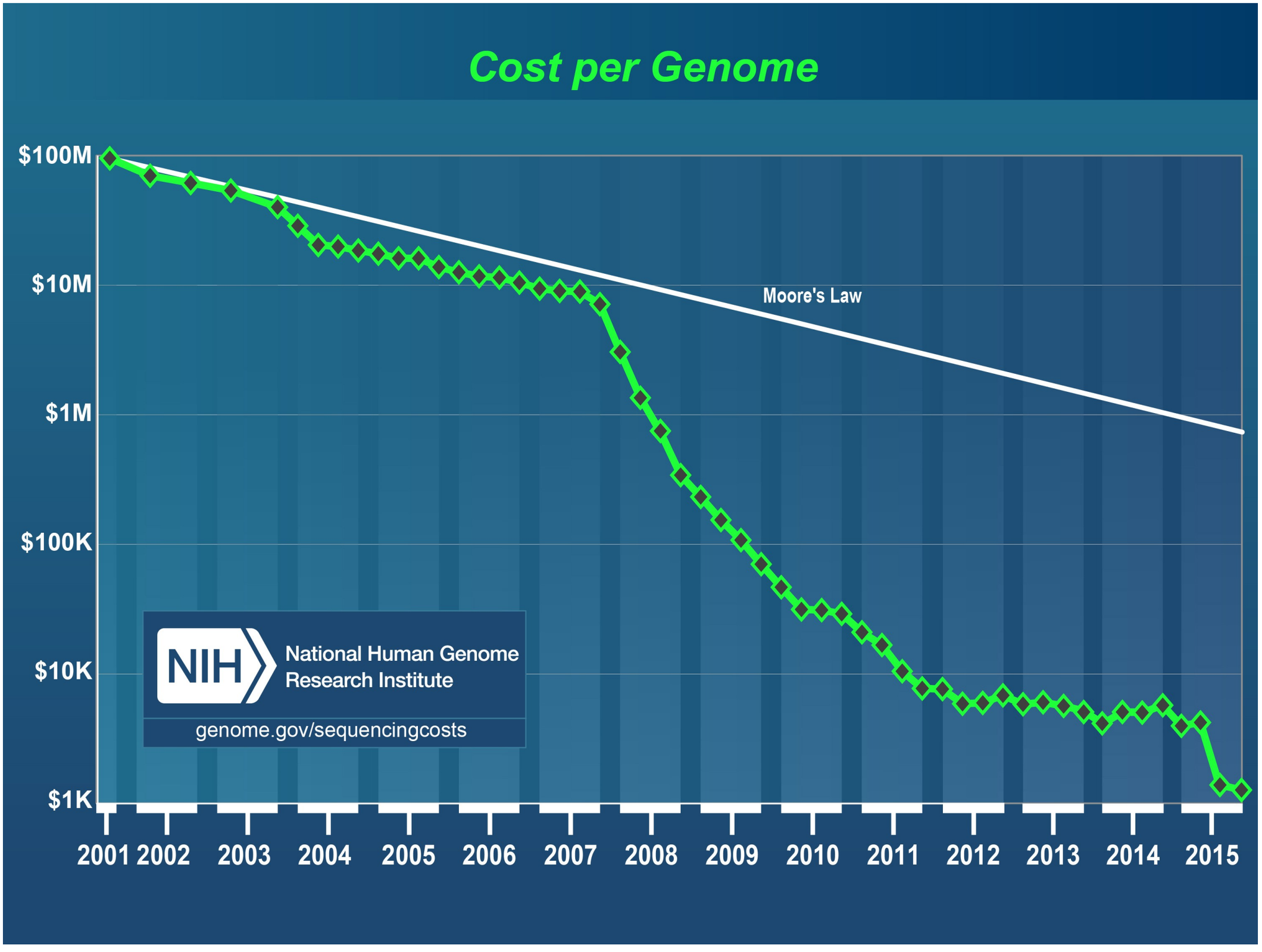
(C) a thousand

(D) a hundred-thousand

(E) a million

& only takes few days. Now, millions of genomes are being sequenced

DNA sequencing cost



Human genome project

www.yourgenome.org/facts/timeline-the-human-genome-

Nature 409, 860-921(2001)

Now


High Output
Please select the appropriate option to start
SEQUENCE
WASH
CHECK
MODE SELECT
High Output
Please select the appropriate option to start
SEQUENCE
WASH
CHECK
MODE SELECT
HiSeq™ 2500
Amelia Earhart
illumina

Future of DNA sequencing

Personalized DNA sequencing

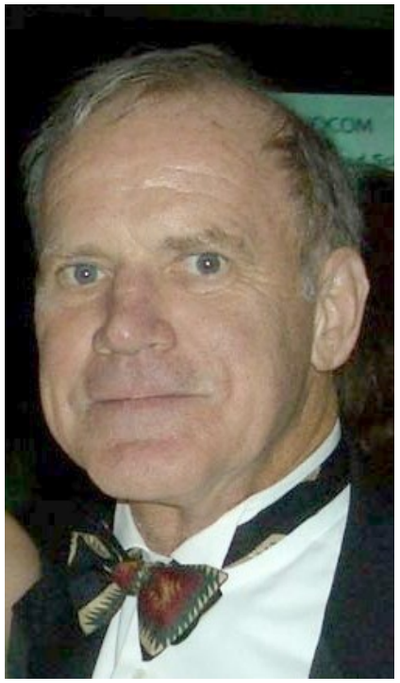
Helicos BioSciences Corporation

Sequencing fetus using DNA from the mother

Sequence your genome for $99

Polymerase Chain Reaction

How to make many copies of DNA in a test tube?



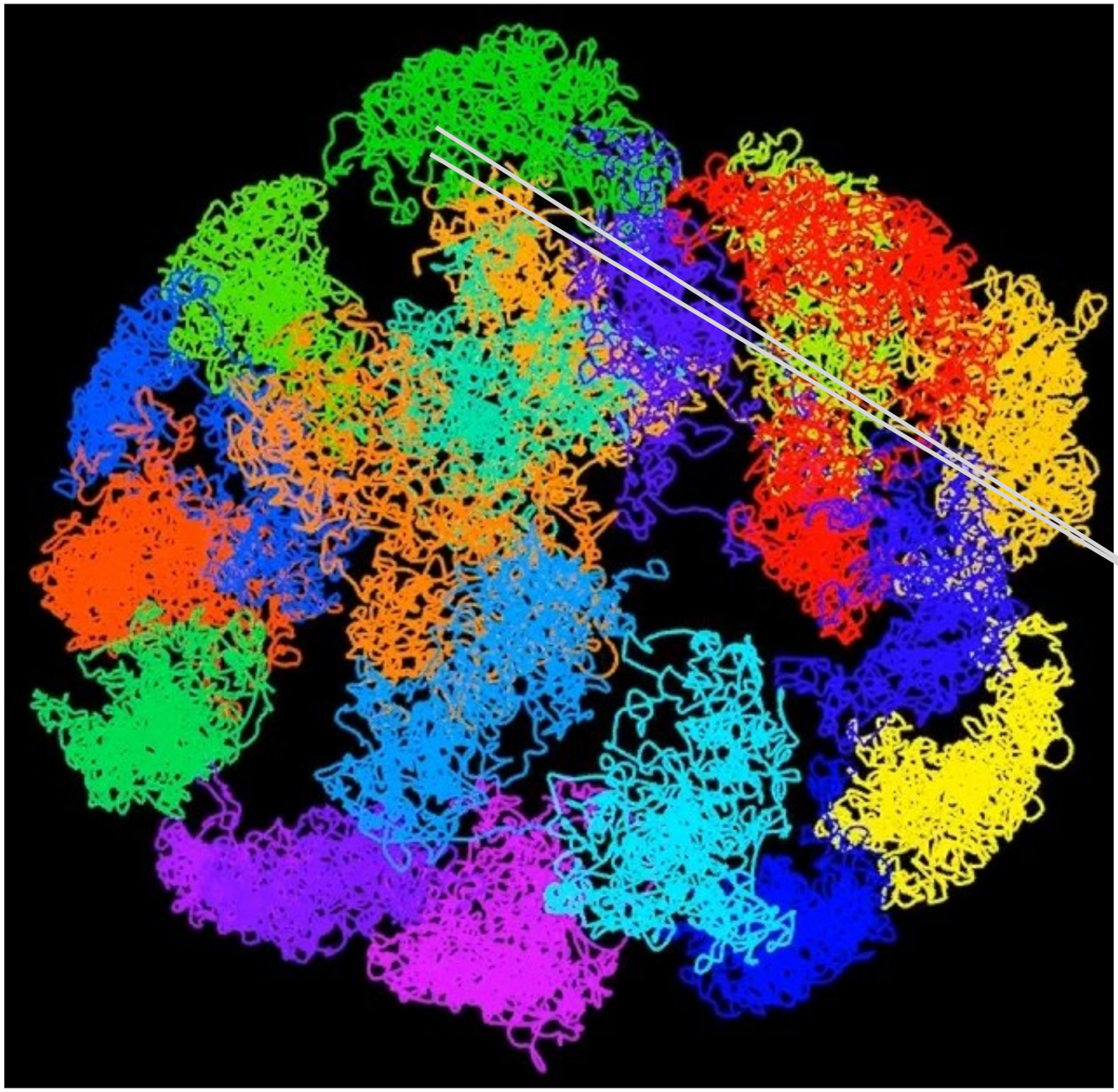
199

3

Micheal Smith & Kary Mullis

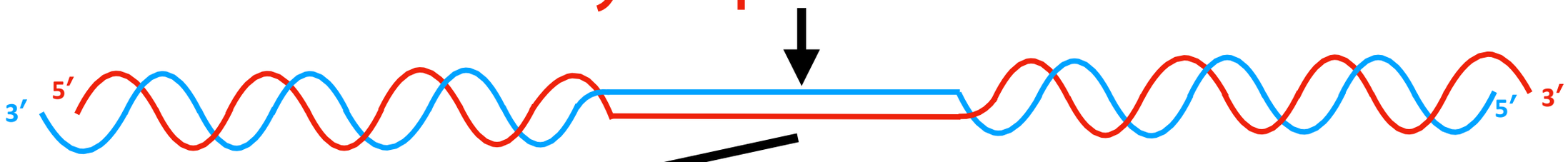
Site-directed mutagenesis & PCR

The human genome in 3D



How to make a copy of this region in our genome?

How to make many copies of DNA in a test tube?

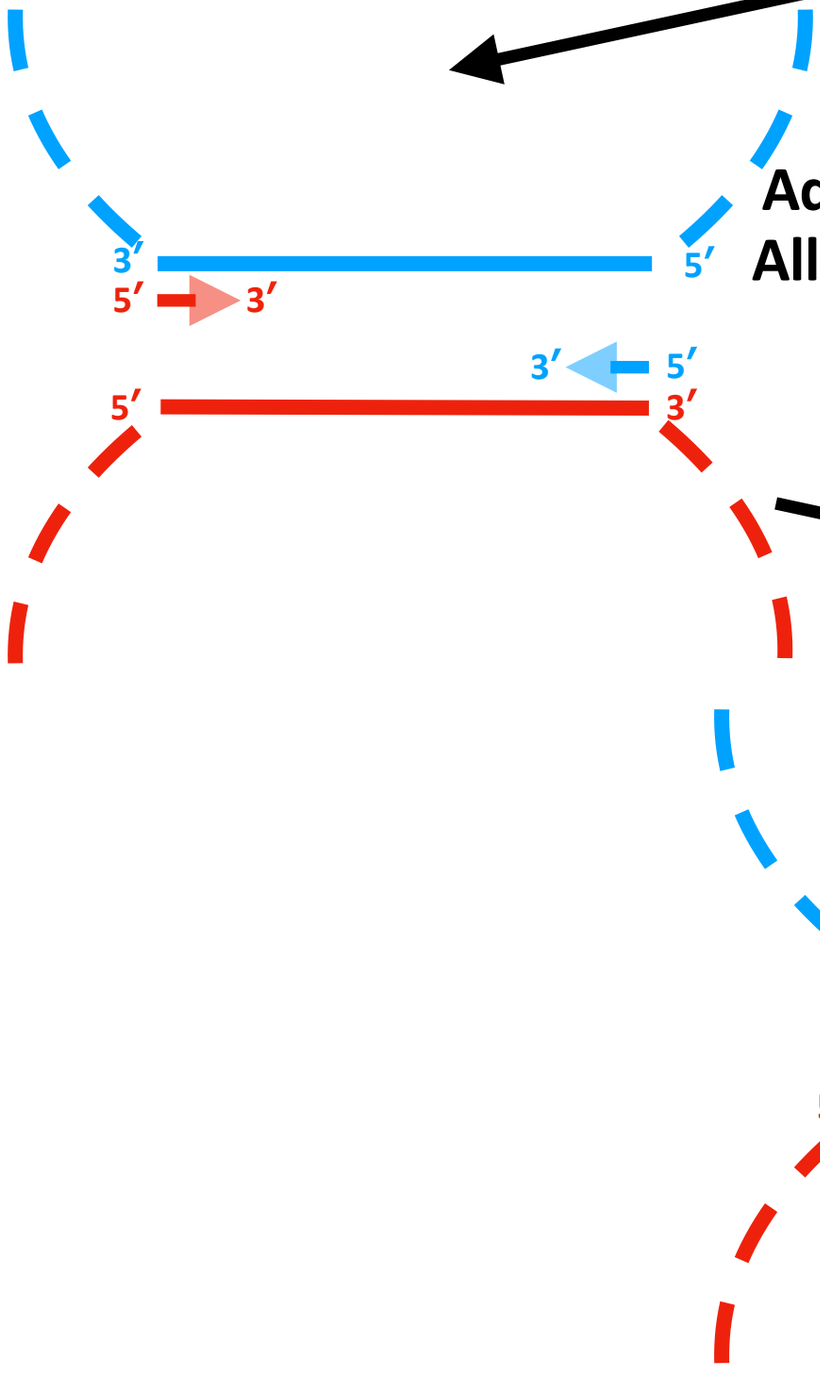


Add DNA polymerase + dNTPs

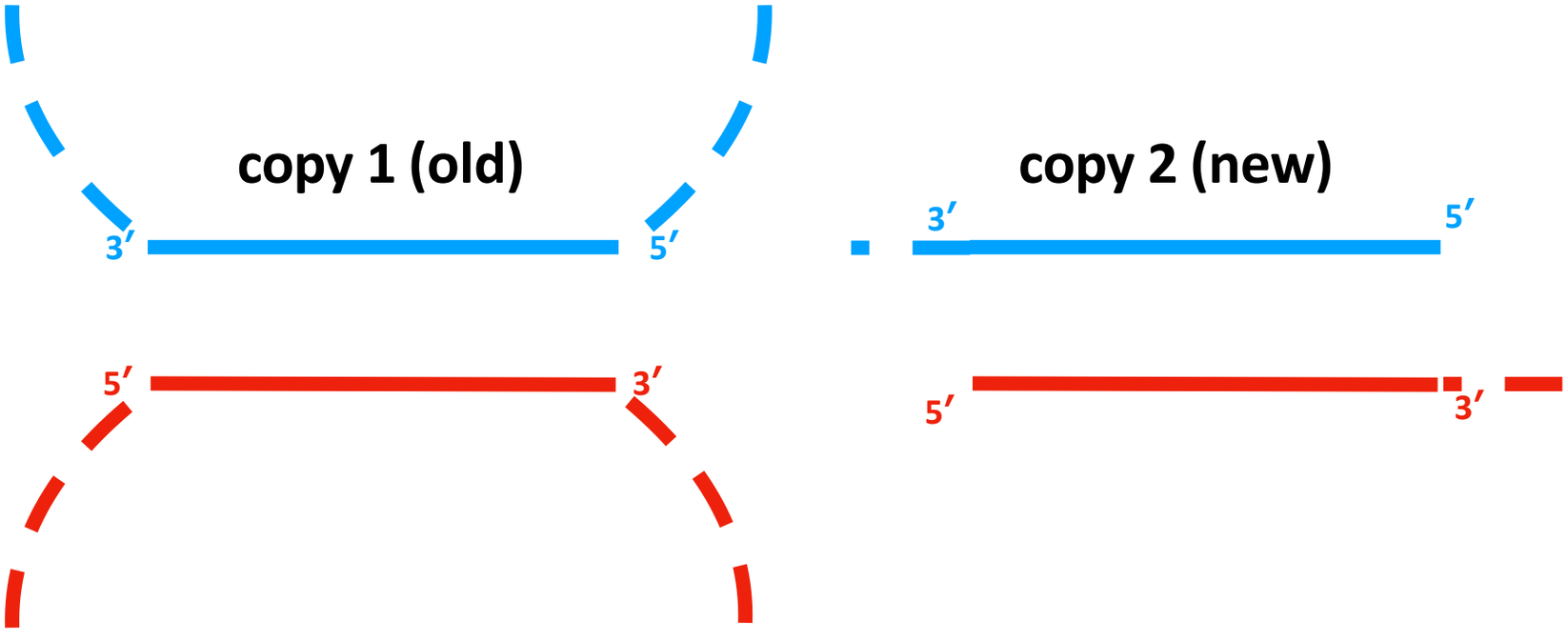
Denature (heat at >95°C)

Add primers (allow them to anneal DNA by cal. Tm) (say 44°C)

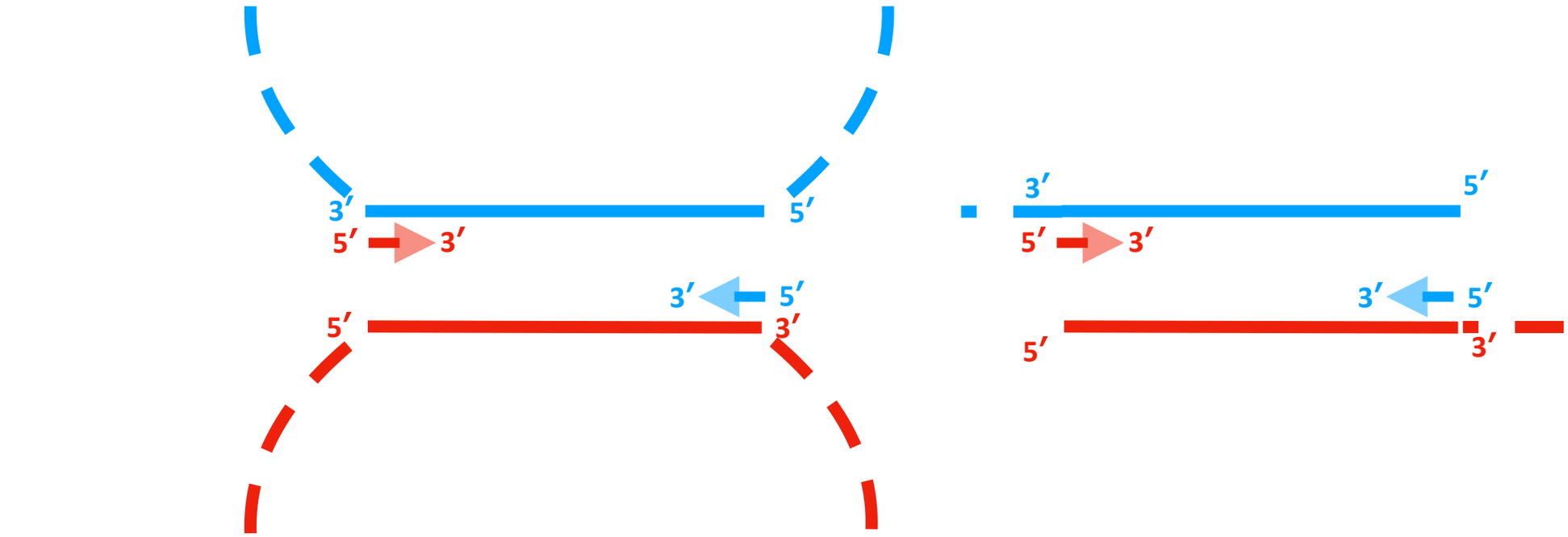
Allow DNA polymerase to do replication (say ~60°C)

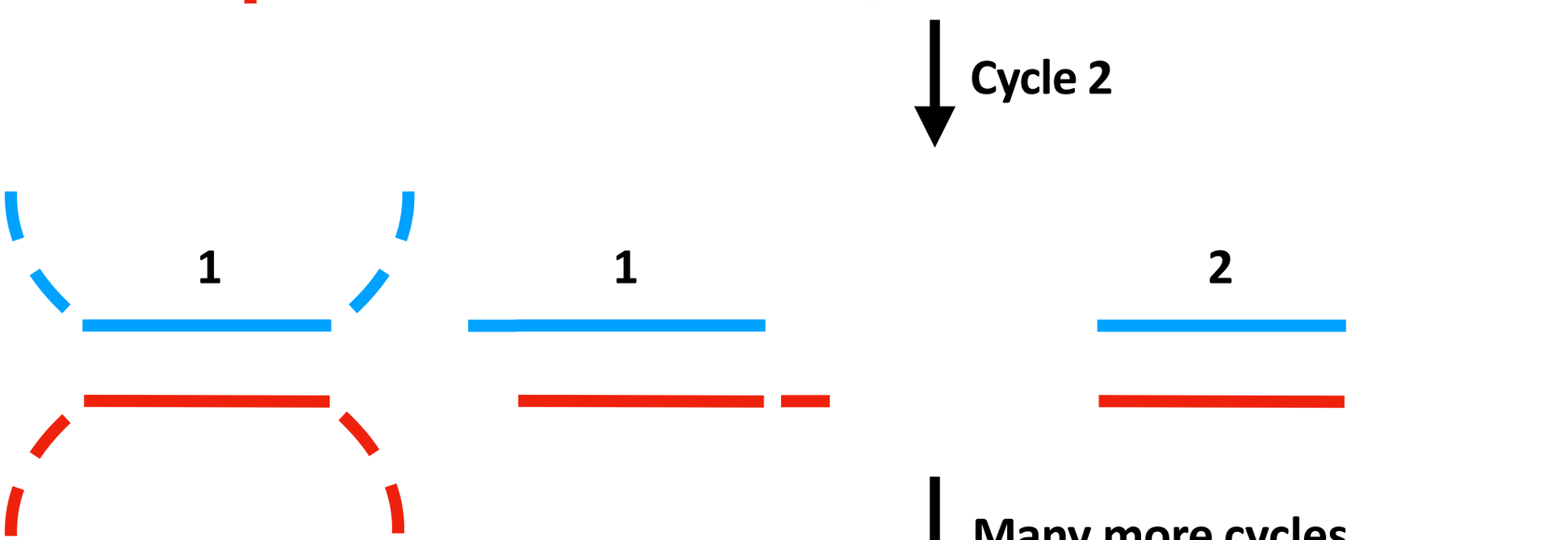


Cycle 1



Polymerase Chain Reaction

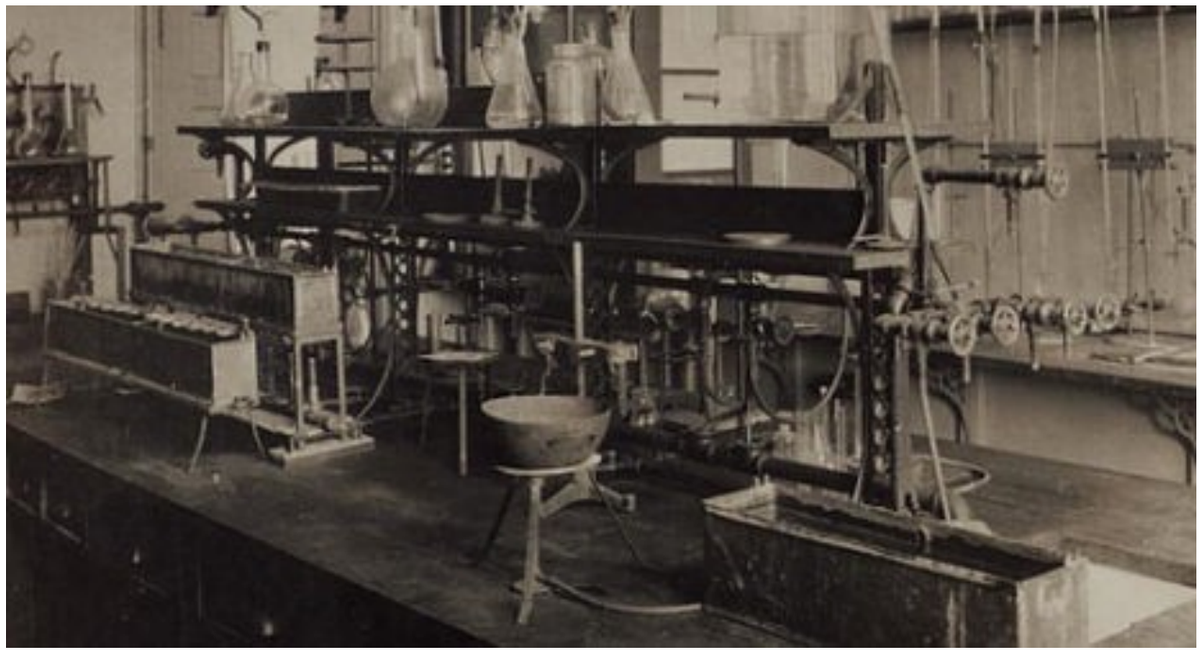




Polymerase Chain Reaction

>10^9 copies

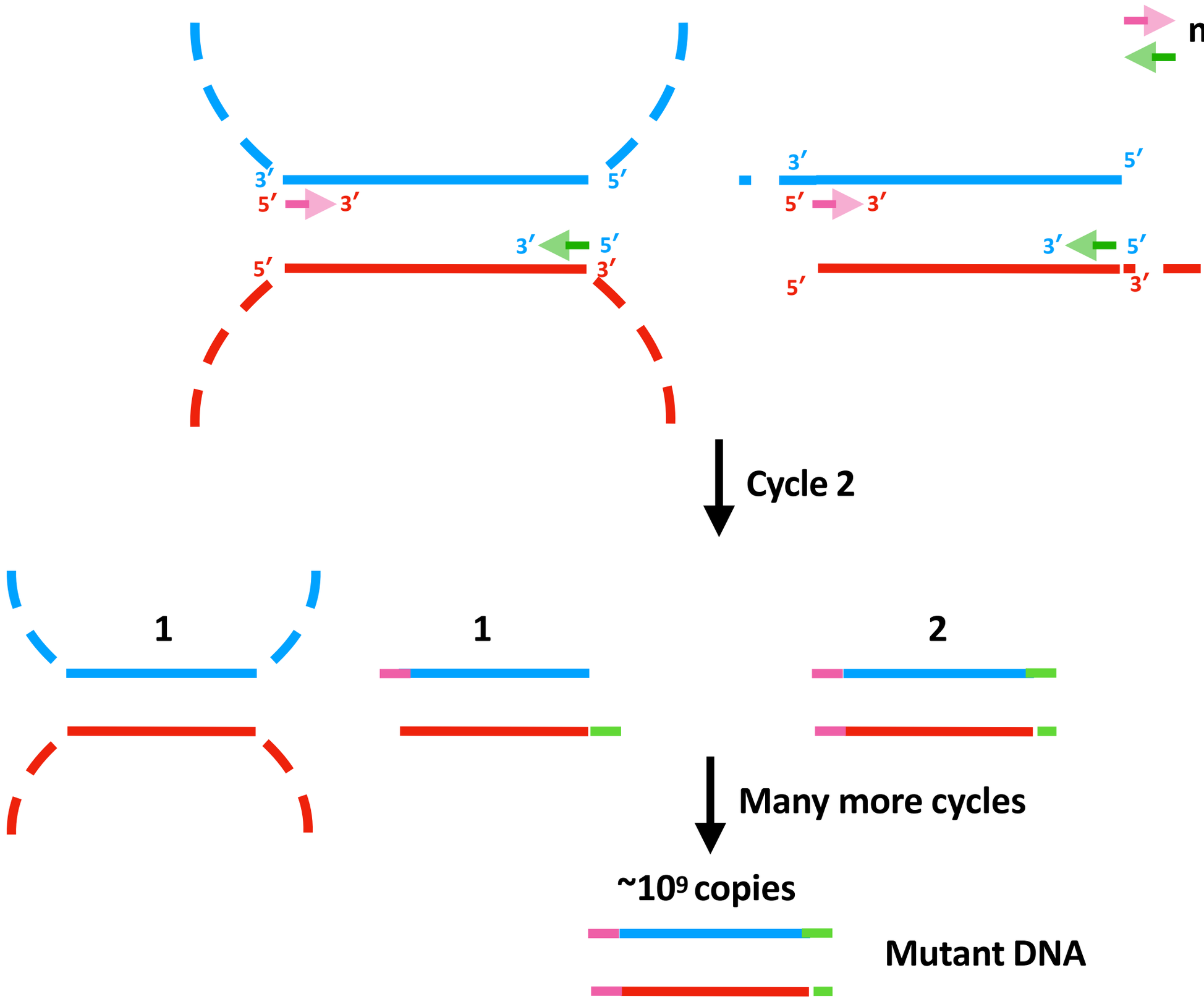
PCR machines



Running around the water baths (1980s)

And now!!!

Site-directed mutagenesis



Lecture #5

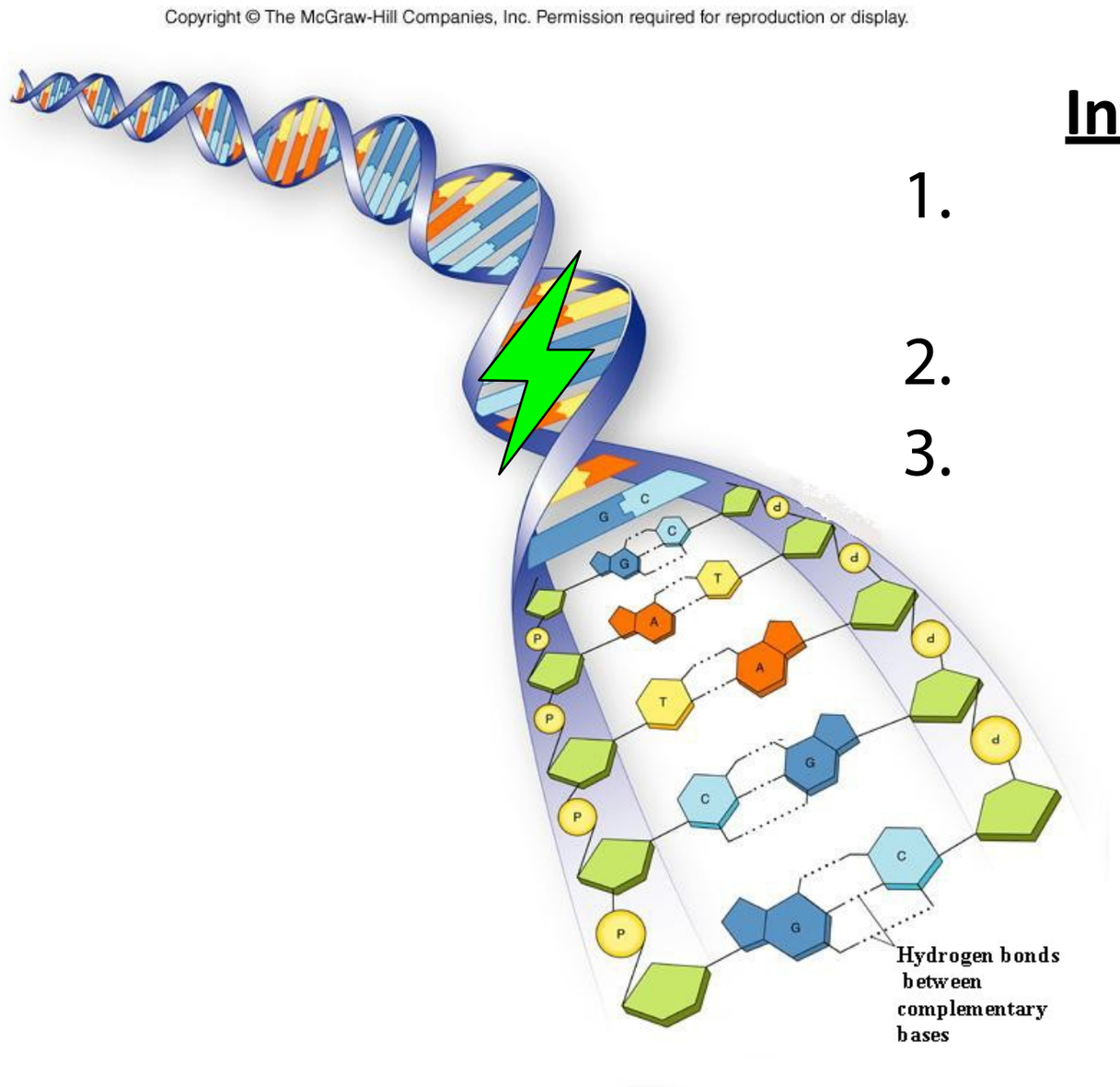
“DNA repair & recombination”

Jan 22, 2018

DNA Damage

External agents:

1. Sunlight
2. Tobacco smoke
3. Pollution
4. Toxins in food

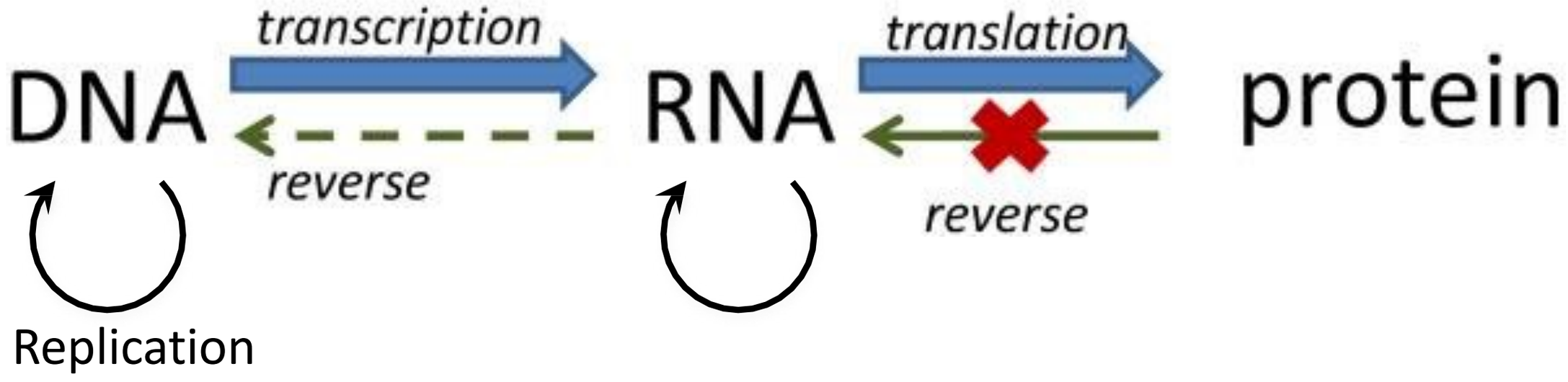


Internal agents:

1. Oxidation (ROS)
2. Reactive
3. Nitrogen species - NO
   Aging

DNA is constantly damaged by endogenous or exogenous agents

The central dogma



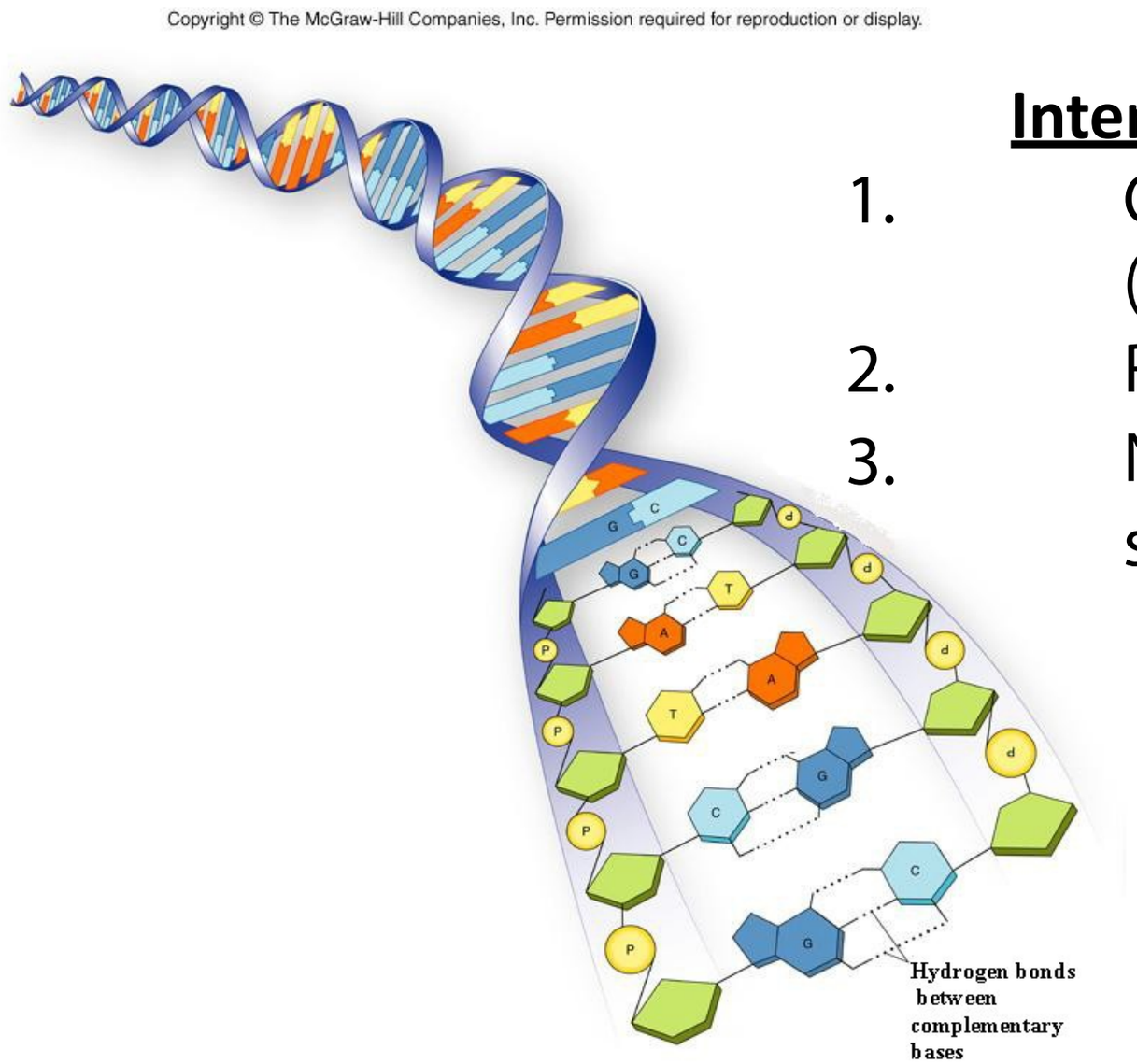
Damaging DNA results in a permanent change in the genetic material

Nature **389**, 537 (09 October 1997)

DNA Damage is Bad

External agents:

1. Sunlight
2. Tobacco smoke
3. Pollution
4. Toxins in food



Internal agents:

1. Oxidation (ROS)
2. Reactive
3. Nitrogen species - NO

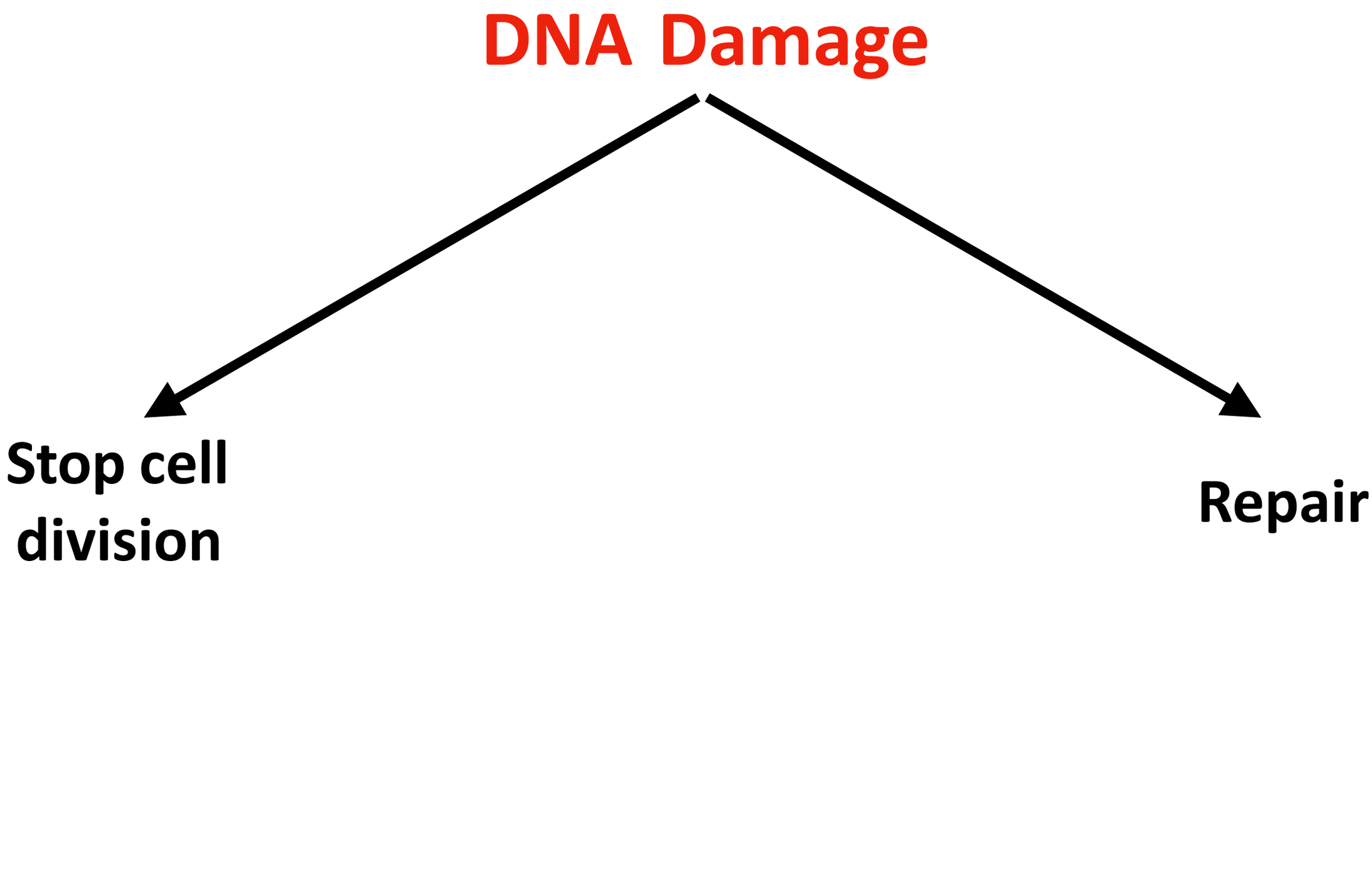
Aging

DNA damage causes mutations that may lead to Disease!!!

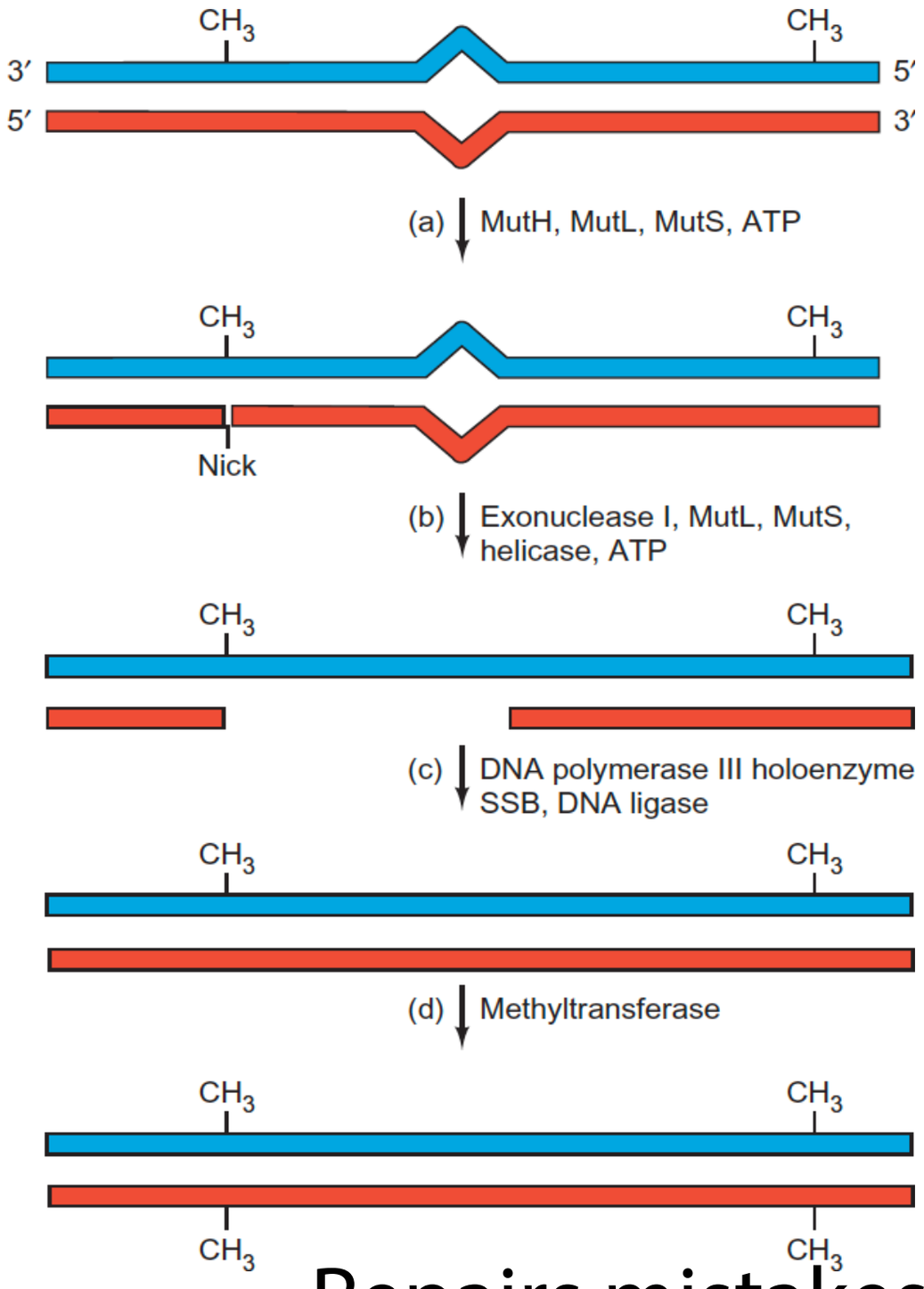
Mutation


TATCGTTCTGAC3′
ATAGCGAGACTG5′
Mismatch
Replication
with out repair
TATCGTTCTGAC3′
ATAGCAAGACTG5′
Mutation
TATCGCTCTGAC3′
ATAGCGAGACTG5′

What do cells do to repair DNA damage



Mismatch Repair



Parental strand

5′TATCGATCTGAC3′

3′ATAGATAGACTG5′

Nick

mismatch

Newly synthesized strand

Mismatch repair pathway

5′TATCGATCTGAC3′

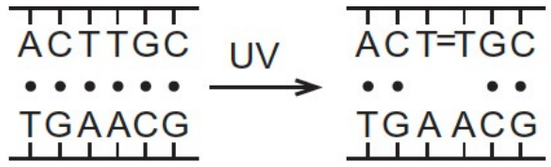
3′ATAGCTAGACTG5′

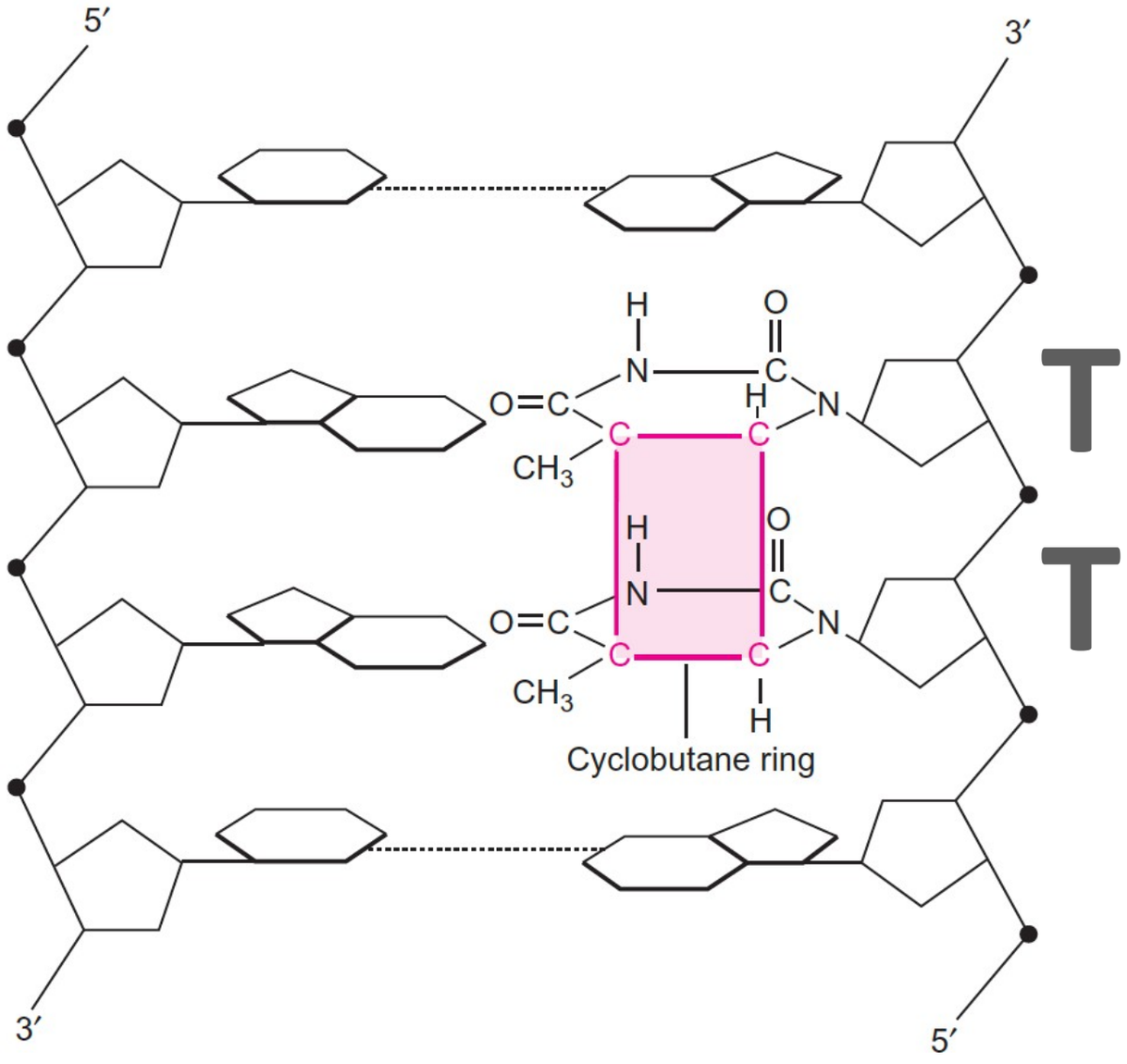
Repairs mistakes made by DNA polymerase

Mutation in mismatch repair causes Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome

Types of DNA damage

Ultra violet light

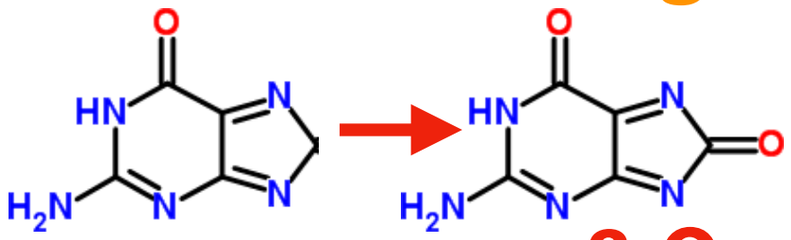




Cyclobutane Pyrimidine Dimer (CPD) formation

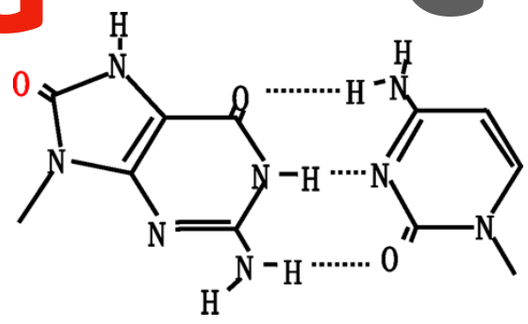
Types of DNA damage

Oxidative damage



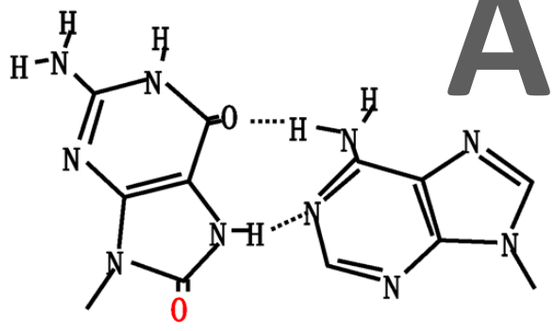
8-O

G **C**



8-O

G **A**



TC8-OGATCT 3′

AGATAGA *5′*

TCGATCT 3′

AGCTAGA *5′*

GC to AT

Transition

TC T ATCT 3′

AGATAGA *5′*

Types of DNA damage

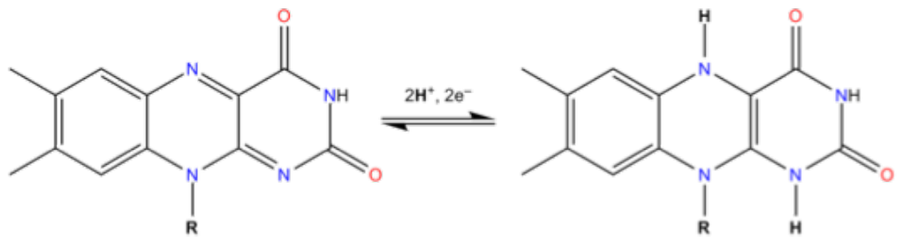
Table 1. DNA Lesions Generated by Endogenous and Exogenous DNA Damage

Endogenous DNA Damage	DNA Lesions Generated	Number Lesions/Cell/Day	
Depurination	AP site	10000[a]	
Cytosine deamination	Base transition	100–500[a]	
SAM-induced methylation	3meA	600[a]	
	7meG	4000[a]	
	O^6meG	10–30[b]	
Oxidation	8oxoG	400–1500[c]	
Exogenous DNA Damage	**Dose Exposure (mSv)**	**DNA Lesions Generated**	**Estimated Number Lesions/Cell**
Peak hr sunlight	—	Pyrimidine dimers, (6–4) photoproducts	100,000/day[d]
Cigarette smoke	—	aromatic DNA adducts	45–1029[e]
Chest X-rays	0.02[f,g,h]	DSBs	0.0008[i]
Dental X-rays	0.005[f,g,h]	DSBs	0.0002[i]
Mammography	0.4[f,g,h]	DSBs	0.016[i]
Body CT	7[f]	DSBs	0.28[i]
Head CT	2[f,g]	DSBs	0.08[i]
Coronary angioplasty	22[h]	DSBs	0.88[i]
Tumor PET scan (^{18}F)	10[h]	DSBs	0.4[i]
^{131}I treatment	70–150[h]	DSBs	2.8–6[i]
External beam therapy	1800–2000[j]	DSBs	72–80
Airline travel	0.005/hr[f]	DSBs	0.0002/hr[i]
Space mission (60 days)	50[k]	DSBs	2[i]
Chernobyl accident	300[l]	DSBs	12[i]
Hiroshima and Nagasaki atomic bombs	5–4000[k]	DSBs	0.2–160[i]

DOI: http://dx.doi.org/10.1016/j.molcel.2010.09.019

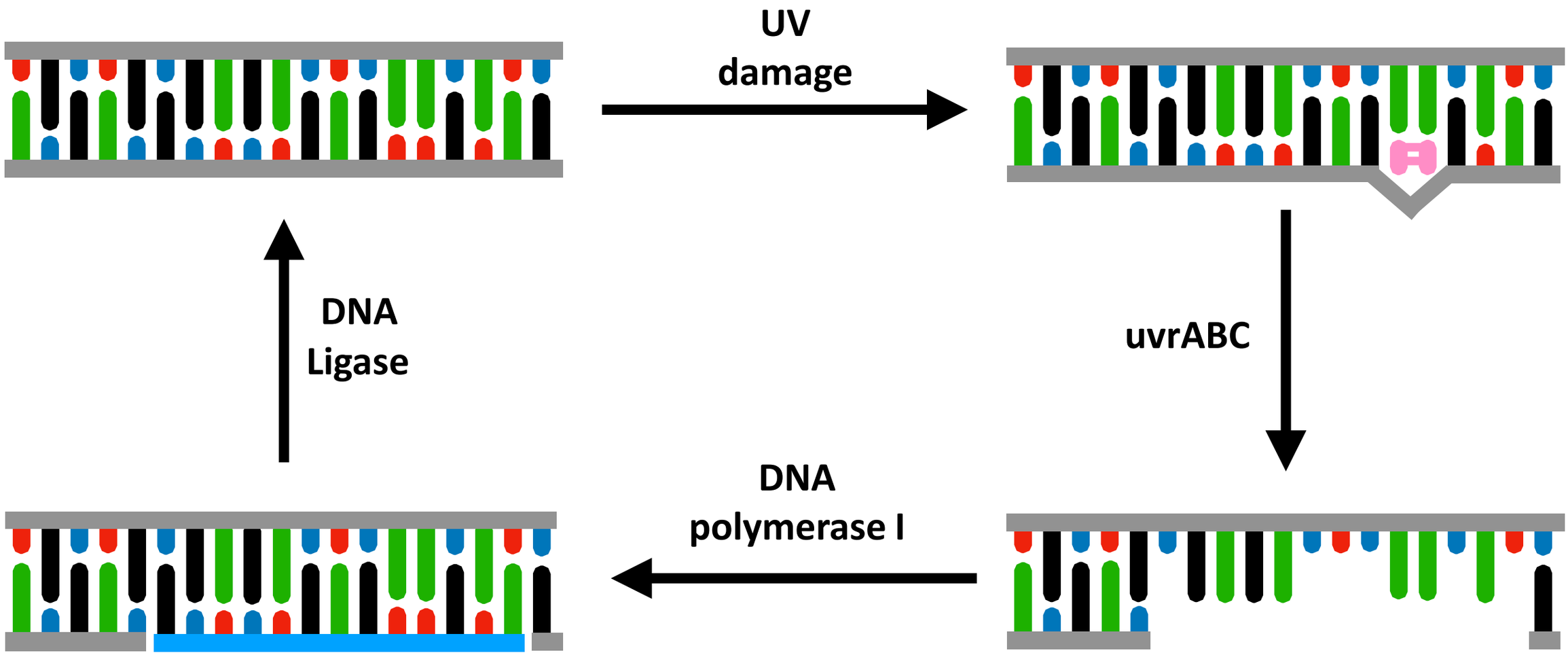
DNA repair by light

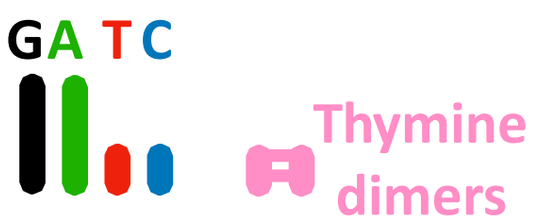
FAD to FADH2



10.1073/pnas.1101026108 PNAS **June 7, 2011**

Nucleotide excision repair



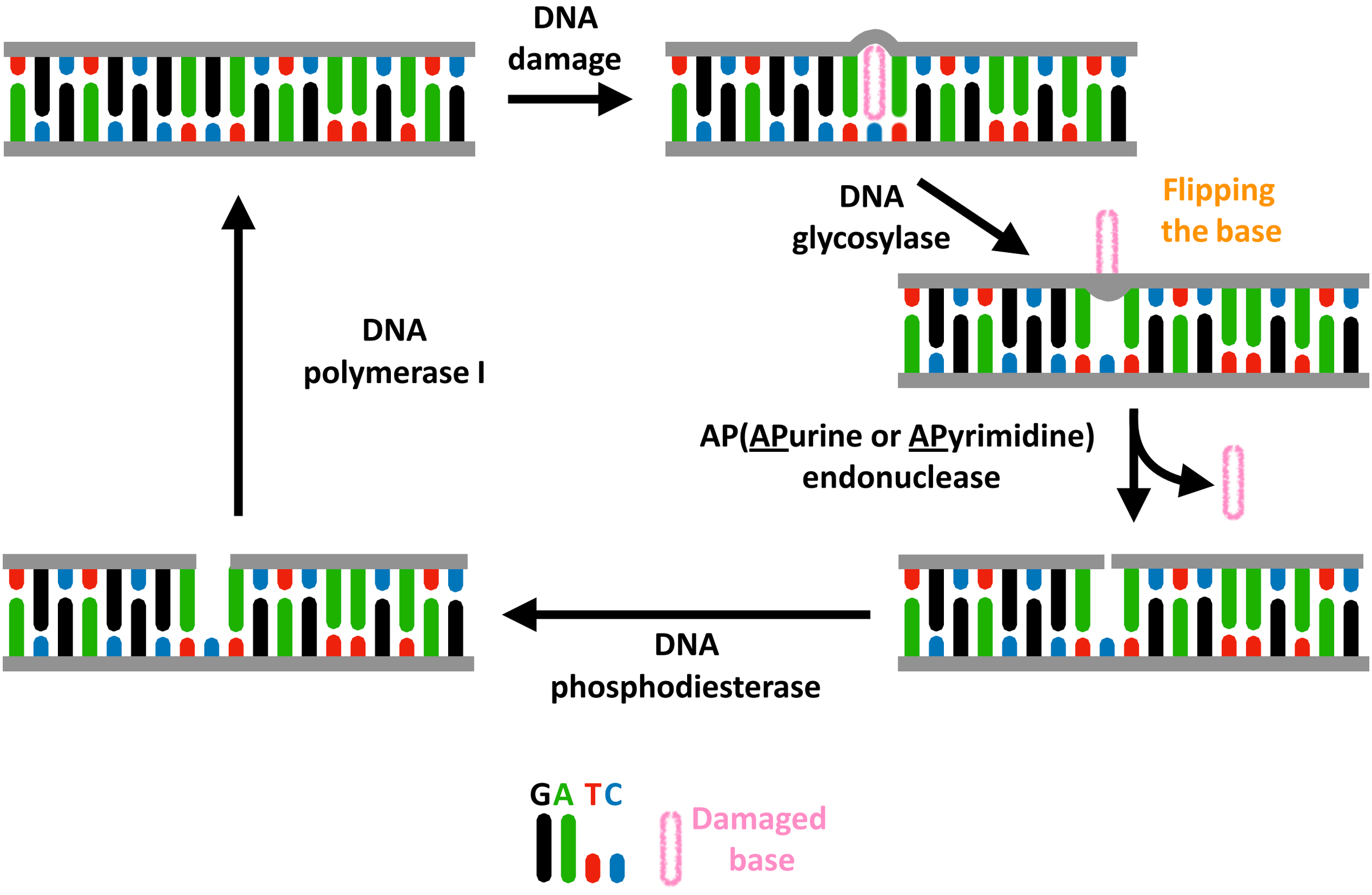


Inability to repair DNA damage causes disease

Xeroderma pigmentosum (XP)
mutation in DNA repair prevents skin cells to repair damage by ultra violet light.

Inability to repair the DNA damage is bad news

Base excision repair



Cytosine deamination

Cytosine deamination

Replication without repair

Mutation

TCCATCT TCUATCT TCAATCT

AGGTAGA → AGTTAGA → AGTTAGA

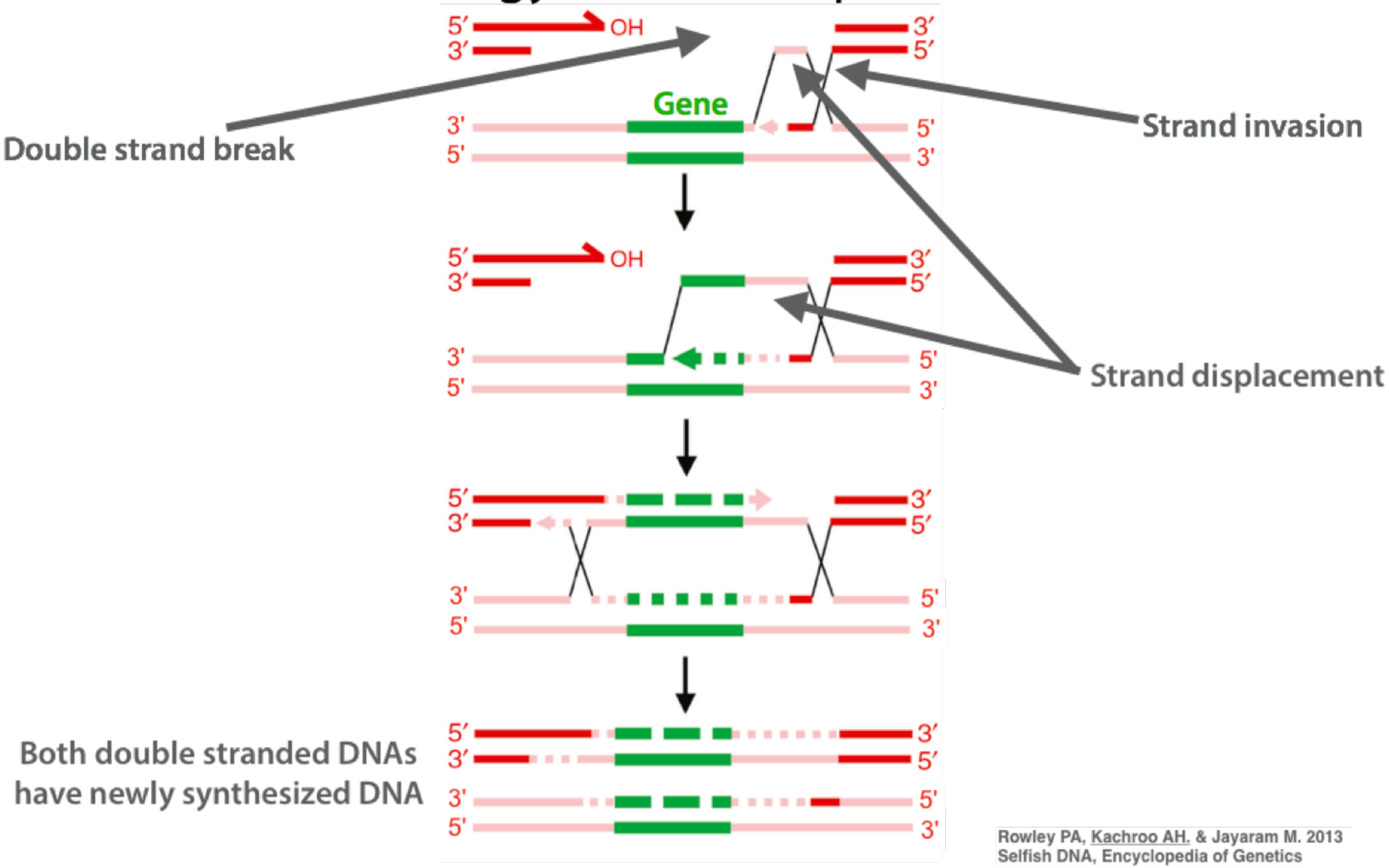
TCUATCT —BER→ **TC ATCT TCCATCT**

AGGTAGA → AGGTAGA → AGGTAGA

Uracil-DNA-glycosylase (BER)

Double Strand Break Repair (DSBR)

Homology Directed Repair (HDR)

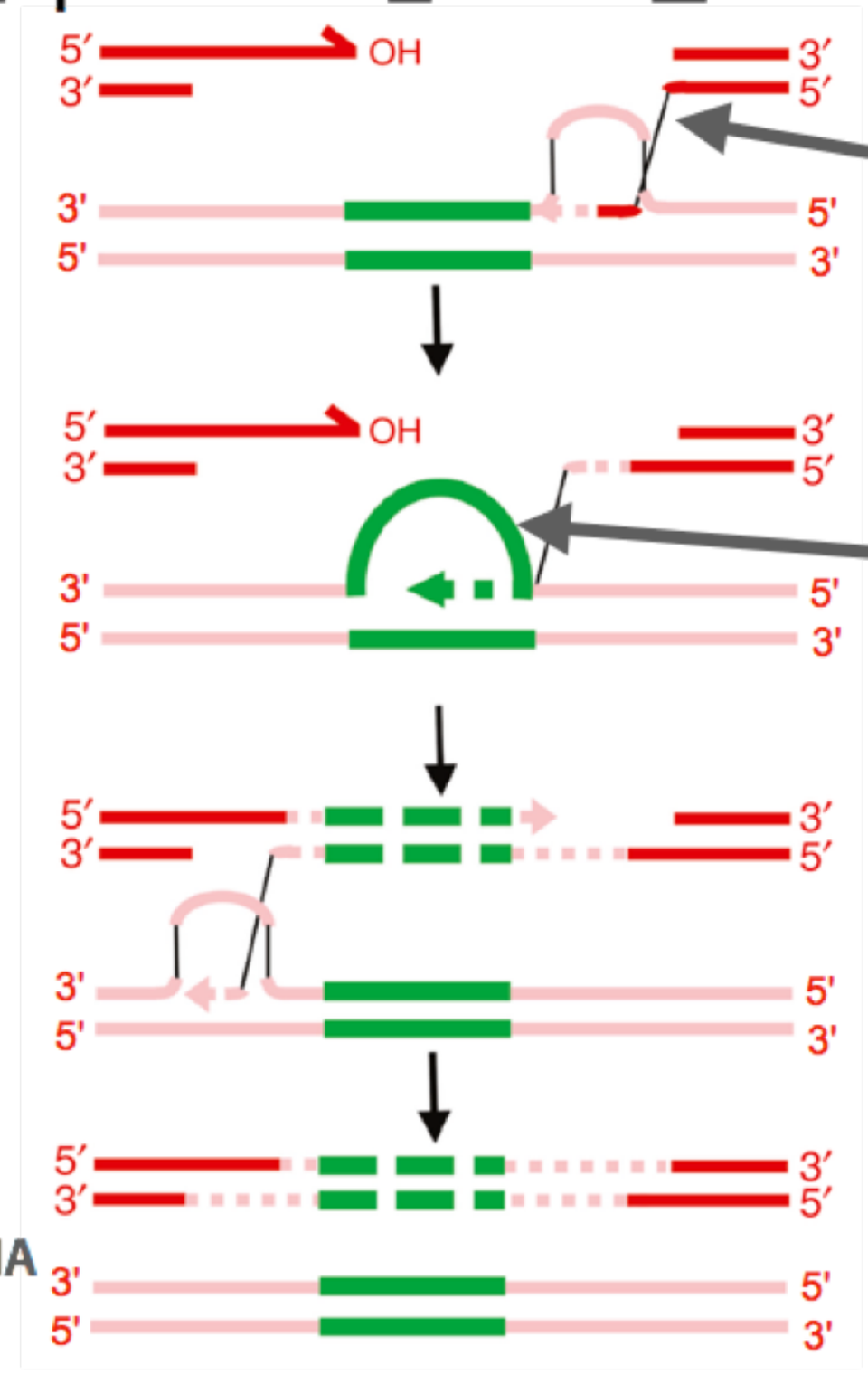


Both double stranded DNAs have newly synthesized DNA

Rowley PA, Kachroo AH. & Jayaram M. 2013
Selfish DNA, Encyclopedia of Genetics

Double Strand Break Repair (DSBR)

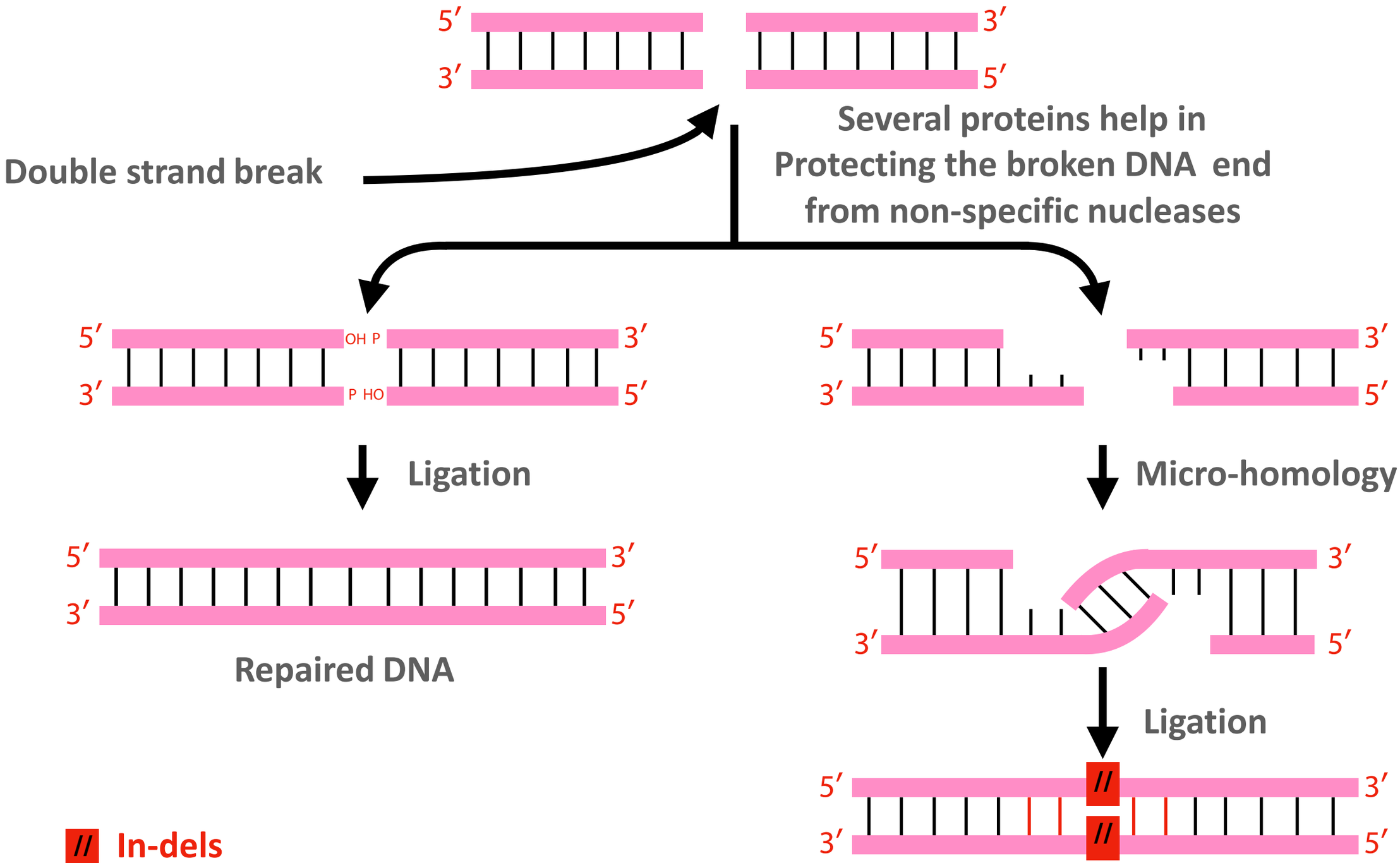
Synthesis Dependent Strand Annealing (SDSA)



Only the DSB DNA
has the newly synthesized DNA

Double Strand Break Repair (DSBR)

Non Homologous End Joining (NHEJ)



“DNA repair movie”