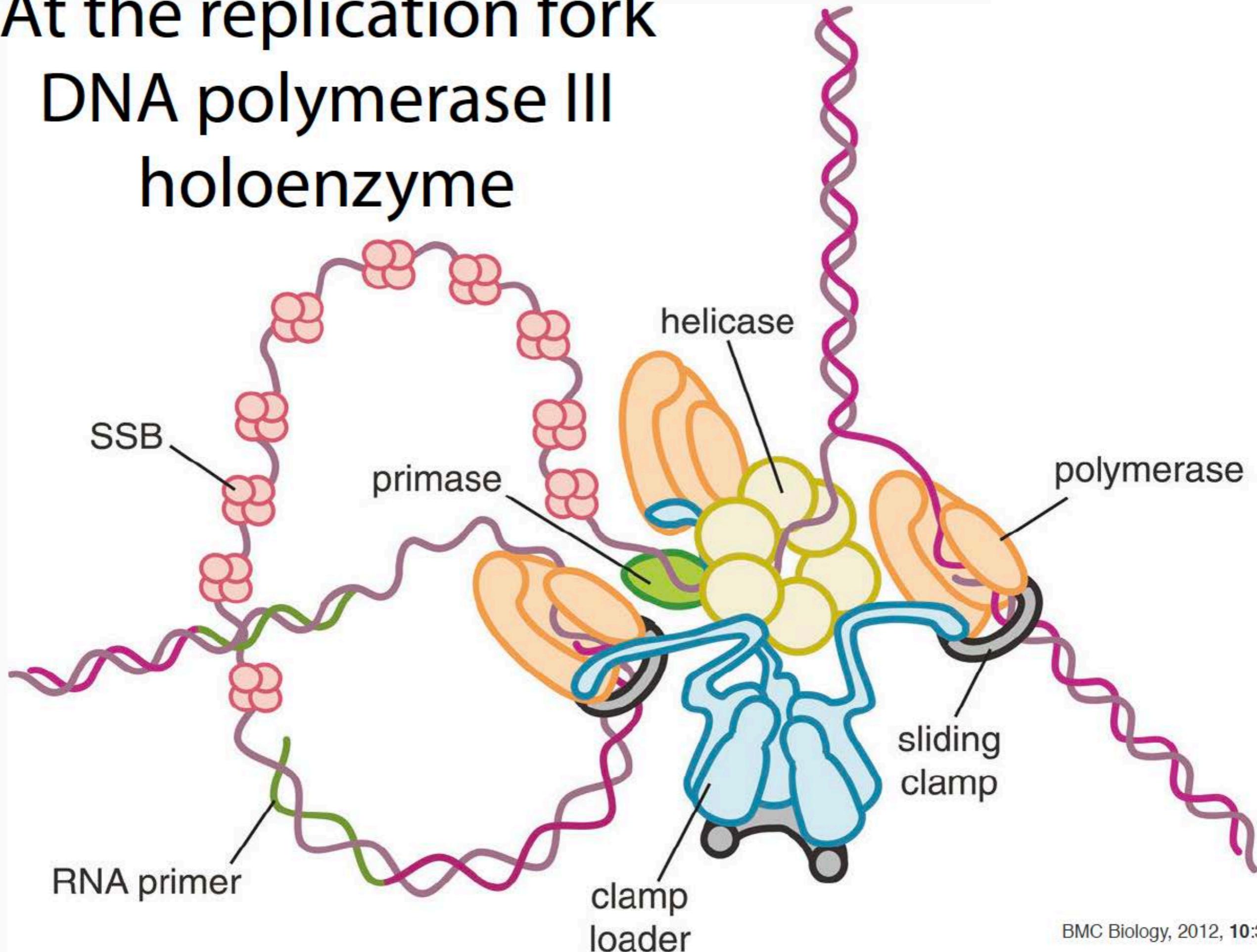


Lecture #5

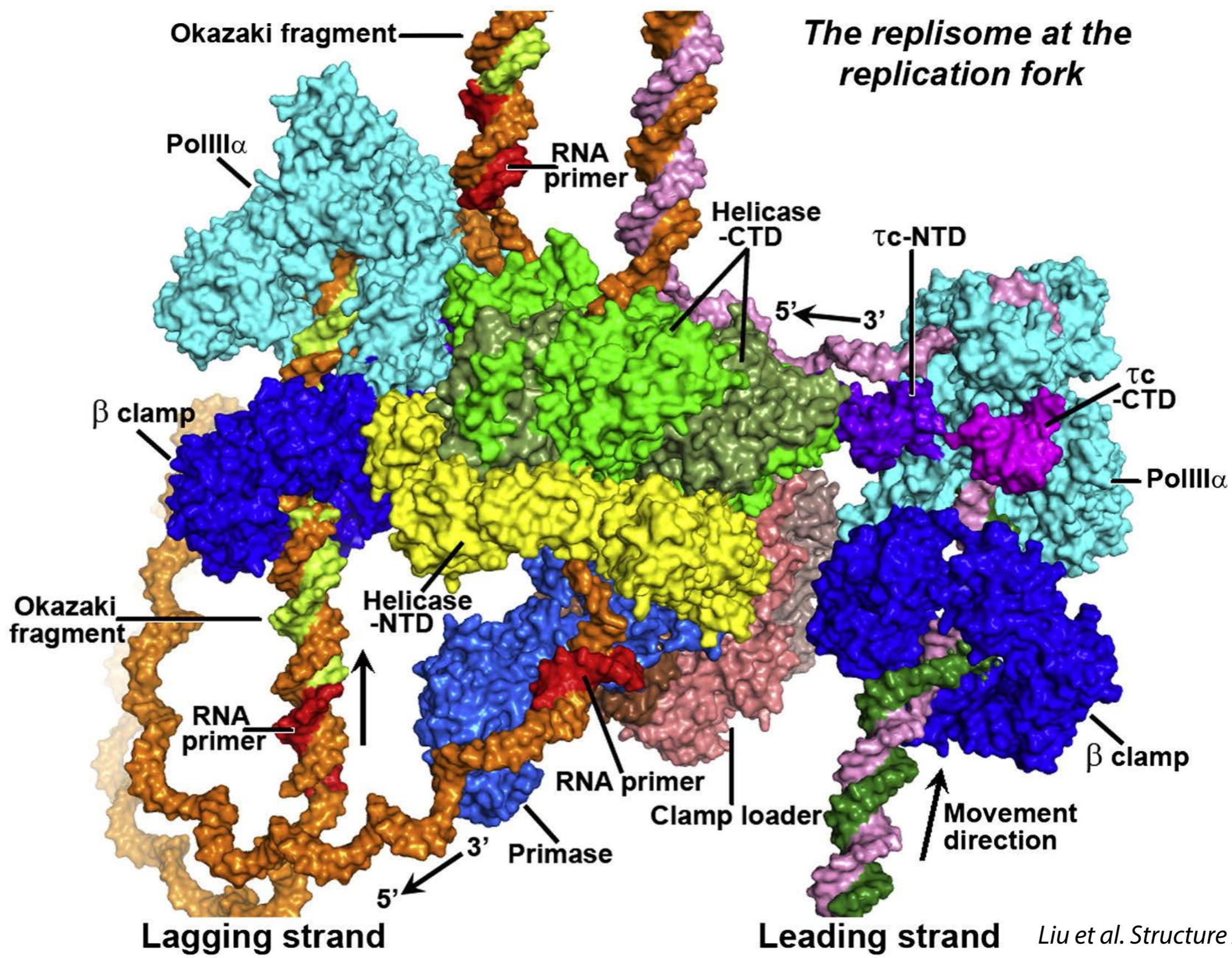
“RECAP”

At the replication fork

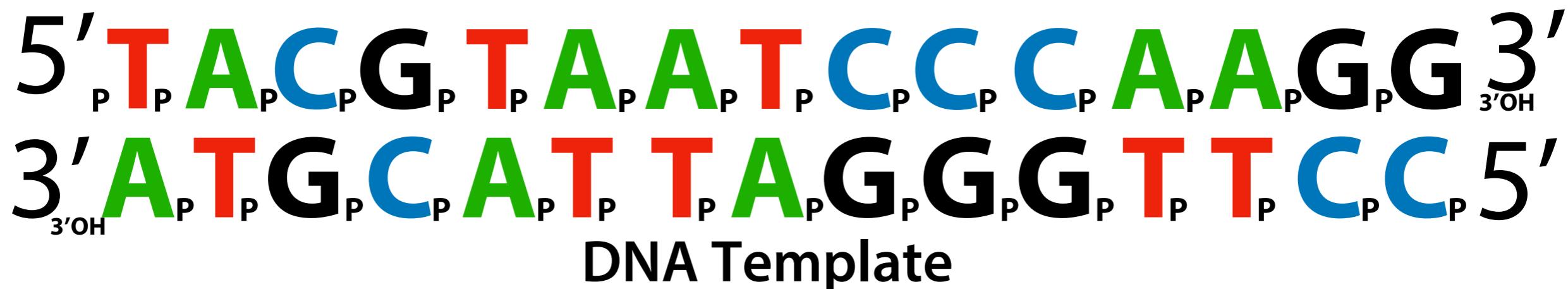
DNA polymerase III holoenzyme



DNA Pol III holoenzyme structure



DNA Replication “fidelity”



K_{eq} of incorporating the correct base = **Error rate = 10^{-3}**
 $10^3 - 10^4$ /bp/cell division

Proofreading

← DNA Polymerase also has



New error rate = 10^{-5} - 10^{-6}

DNA Replication “fidelity”



“Mismatch” repair system



New error rate = $\sim 10^{-9} - 10^{-10}$

How do cells protect telomeres

5' **TTAGGGTTAGGG** 3'

3' **AATCCCCAAUCCC** 5'



Human TERT

Telomerase Reverse Interpretase

5' **TTAGGGTTAGGGTTAG** 3'

3' **AATCC** CAAUCCC AAUCCC

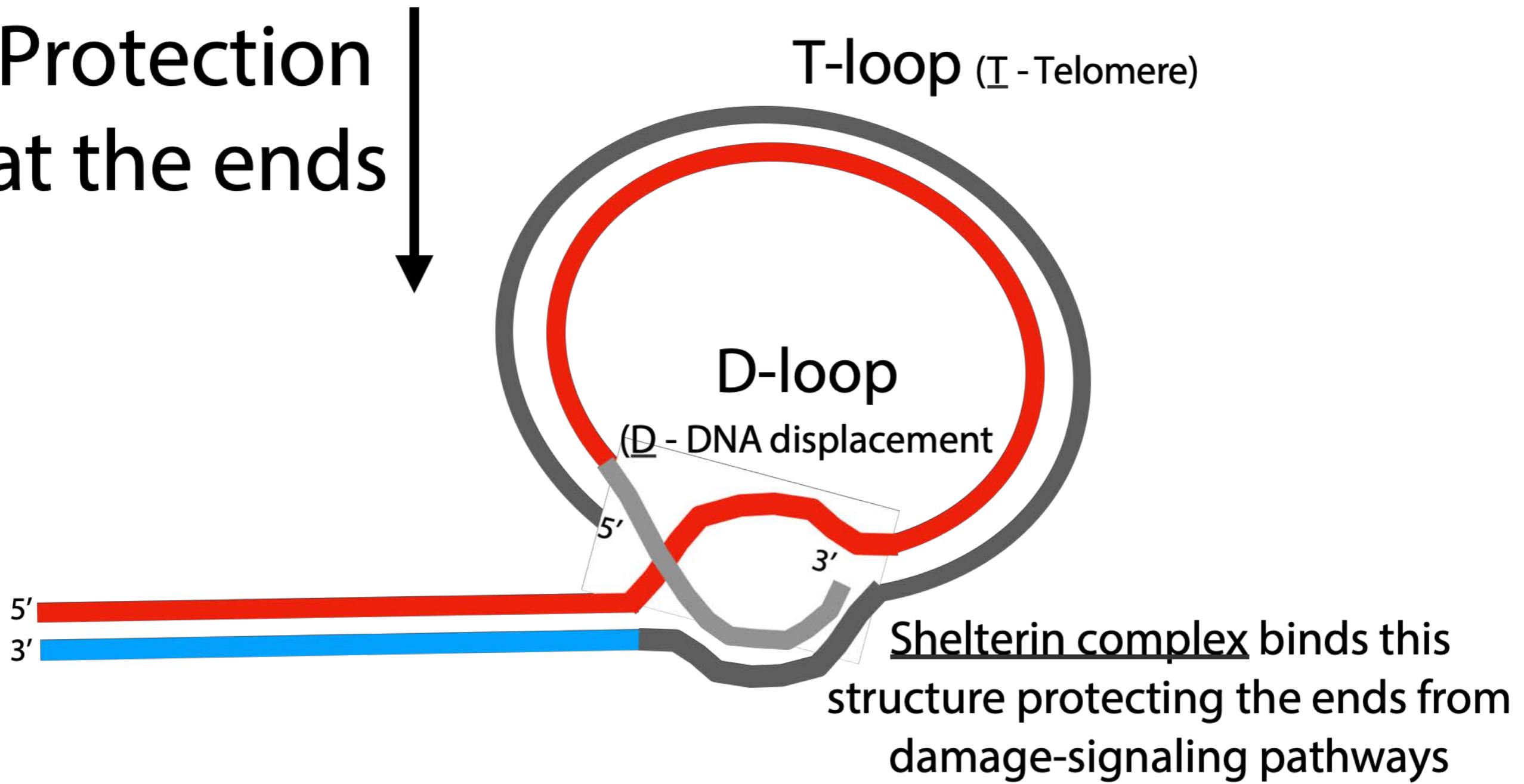
hTERT

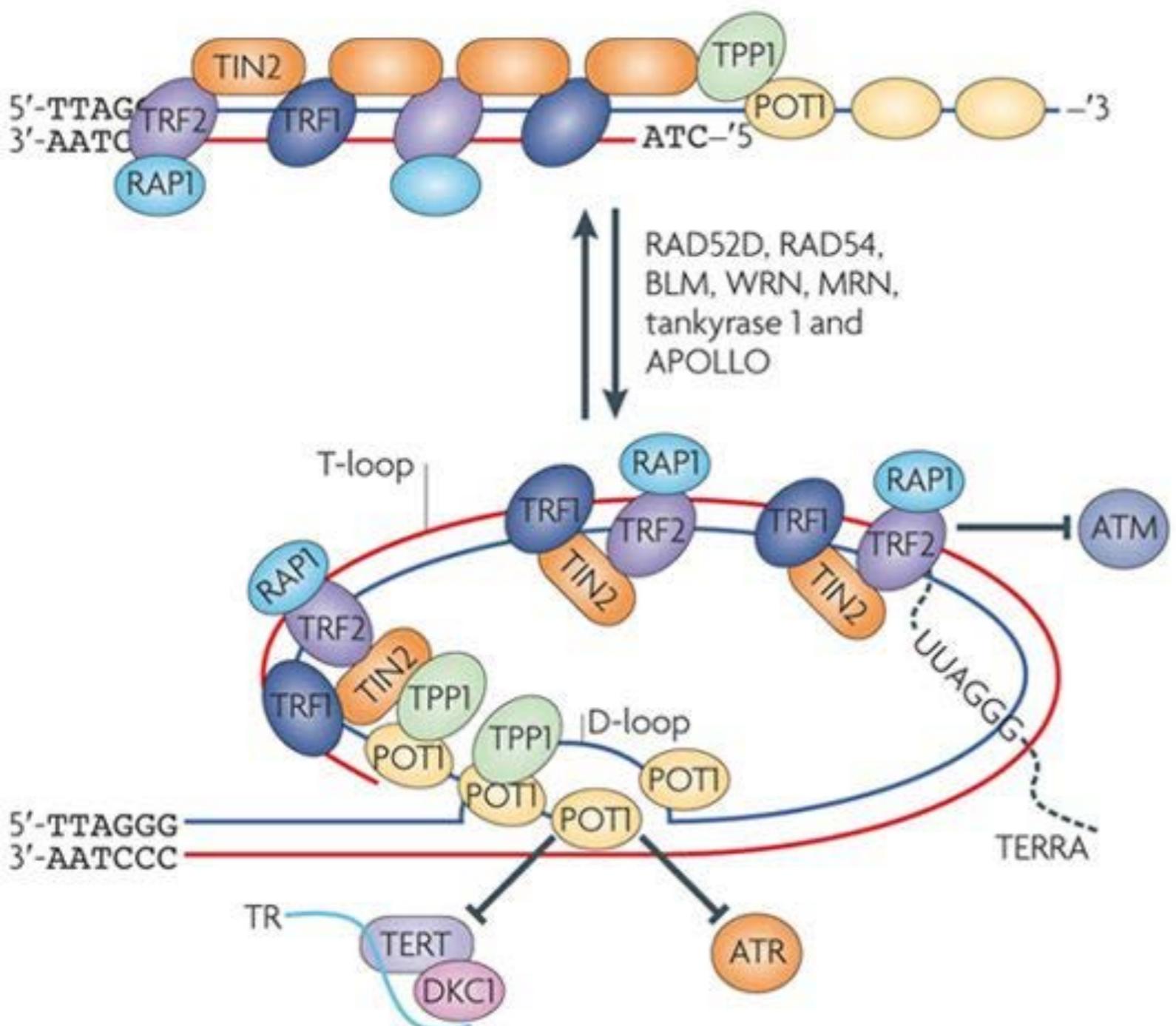
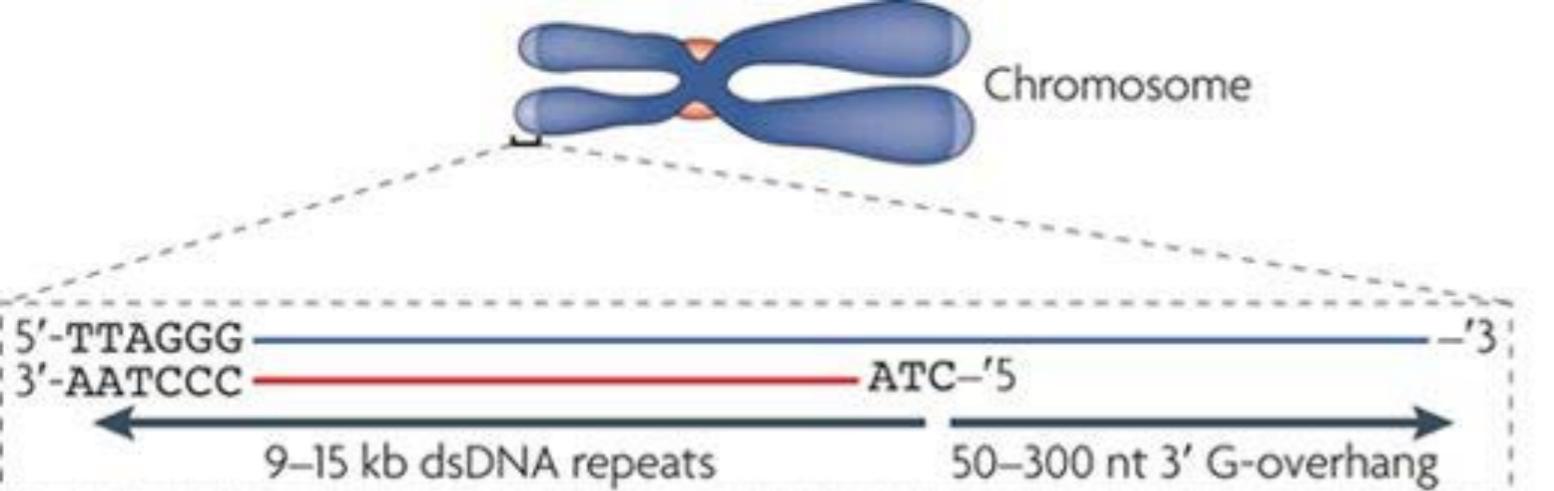
RNA used
as a template

Protection at the ends of DNA



Protection
at the ends





Shelterin complex

Lecture #6

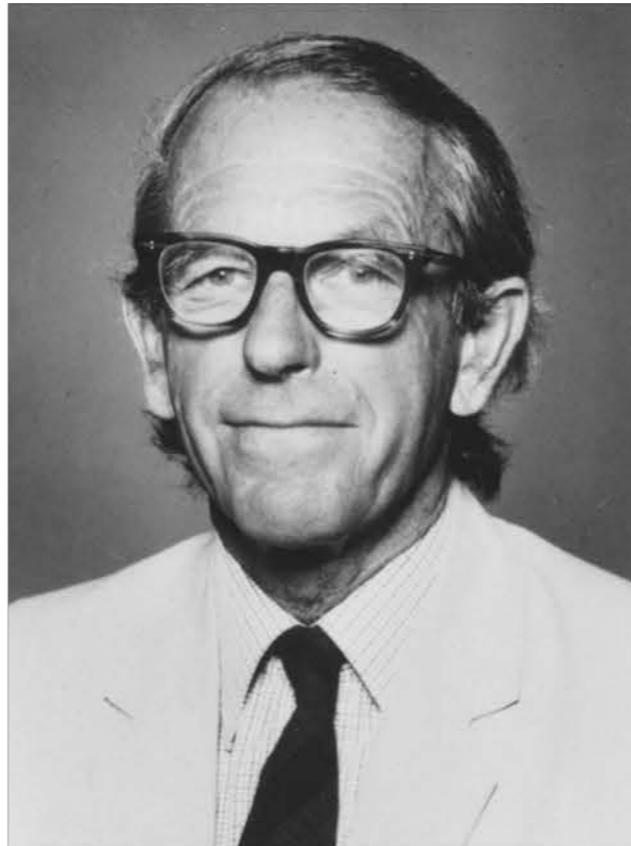
September 24, 2018

DNA sequencing

DNA sequencing



Paul Berg



Frederick Sanger
2 Nobel prizes



Walter Gilbert



1980

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 TATCGCTCTGAC3'
3'aaaaaATAGCGAGACTG5'

↓ Denature

Add DNA polymerase
Add dATP, dCTP, dTTP and dGTP

Add a radiolabeled primer

*5't t t t

3'aaaaaATAGCGAGACTG5'

DNA sequencing (Sanger)

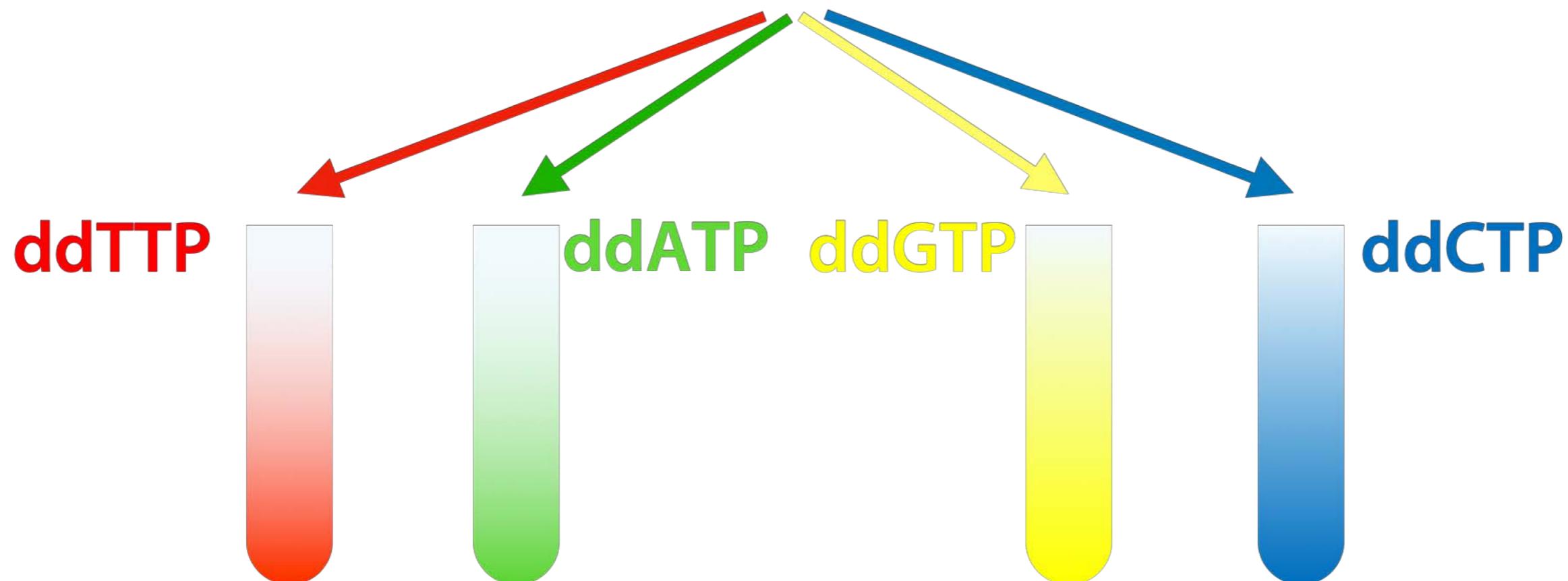
*5't t t t

3'aaaaa**ATAGCGAGACTG5'**

Add DNA polymerase

Add dTTP, dATP, dGTP and dCTP

Add a radiolabeled primer



DNA sequencing (Sanger)

*5'tttT

3'aaaa**ATAGCGAGACTG5'**

*5'tttTATCGCT

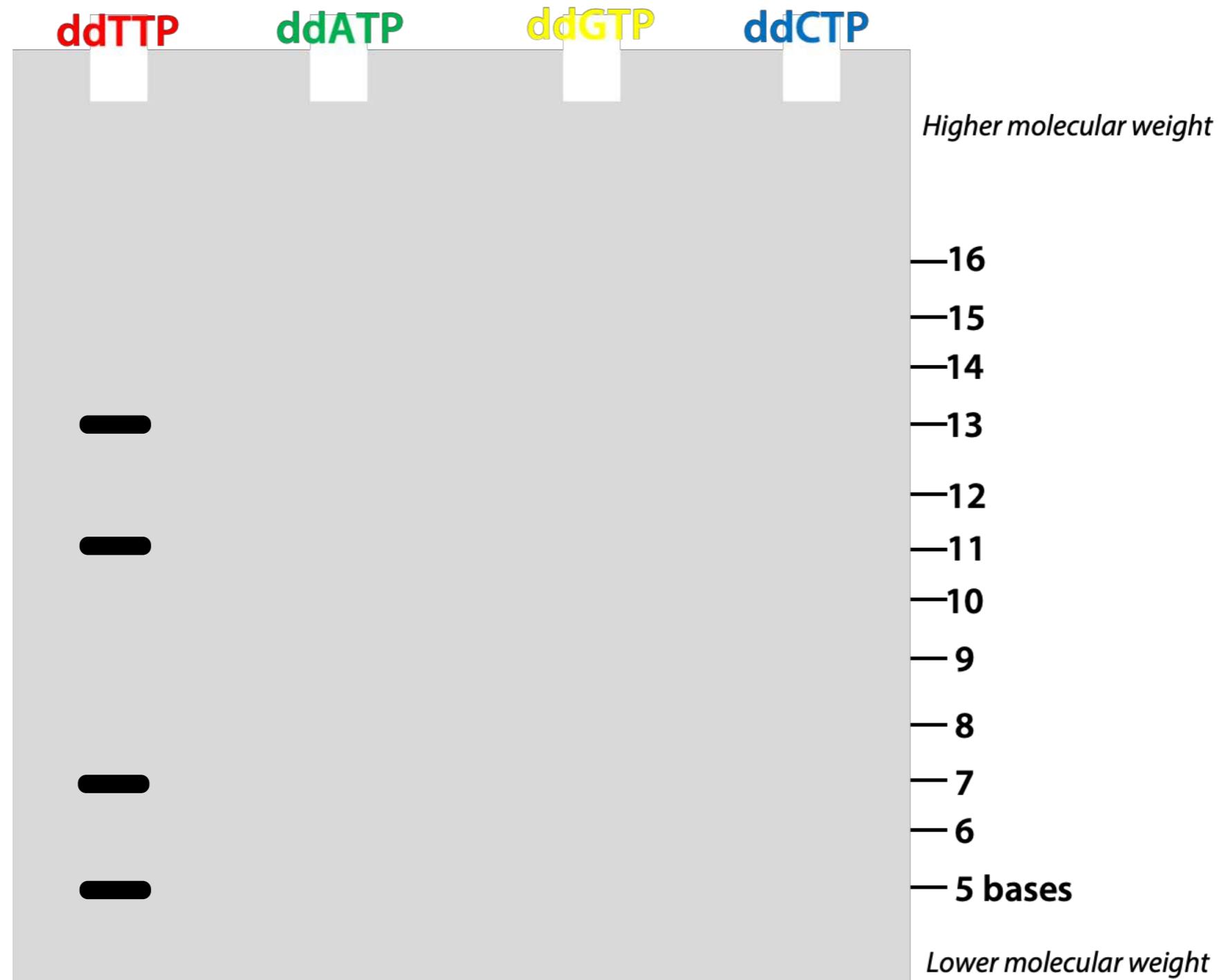
3'aaaa**ATAGCGAGACTG5'**

*5'tttTAT

3'aaaa**ATAGCGAGACTG5'**

*5'tttTATCGCT C

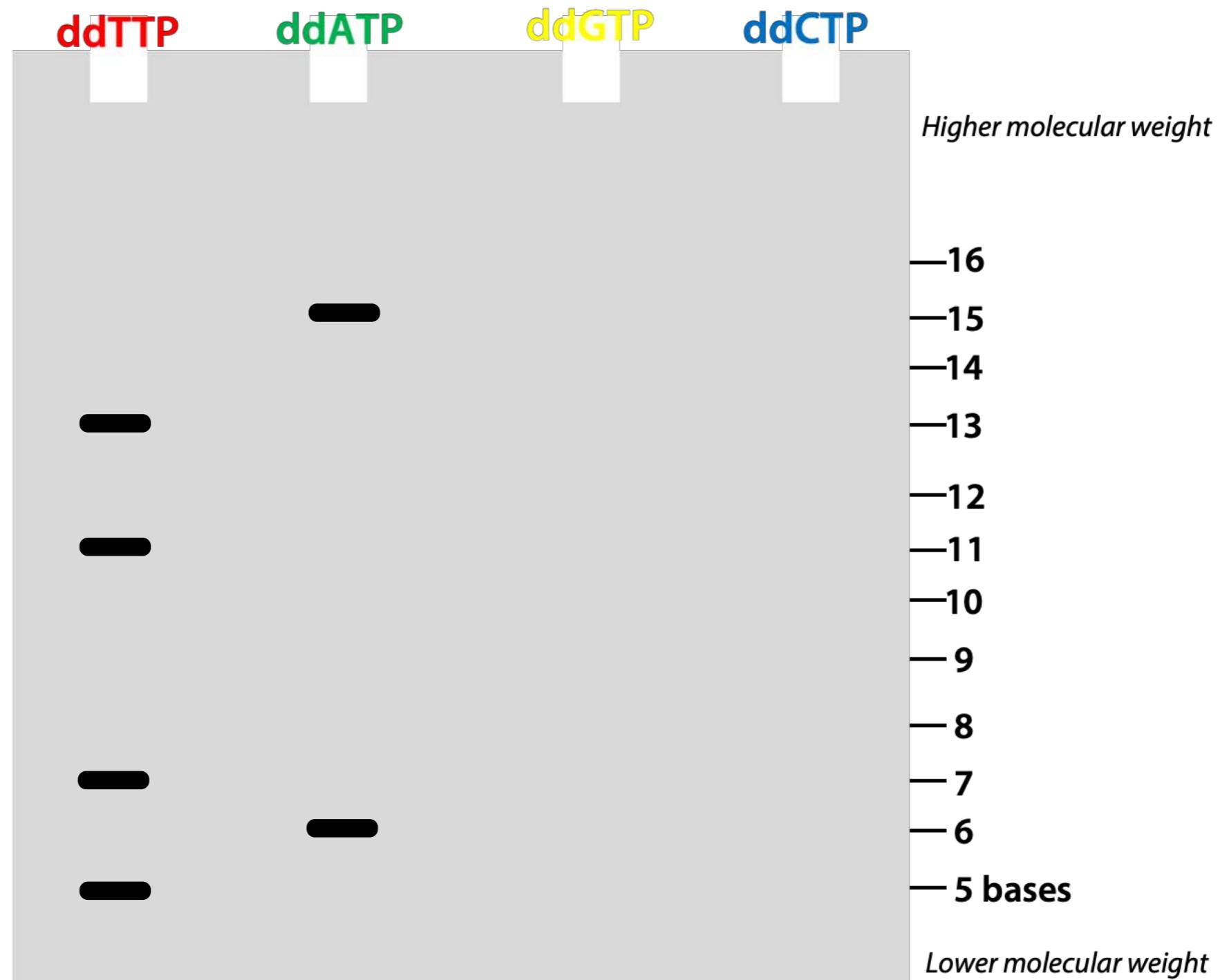
3'aaaa**ATAGCGAGACTG5'**



DNA sequencing (Sanger)

*5'tttt**TA**
3'aaaa**ATAGCGAGACTG5'**

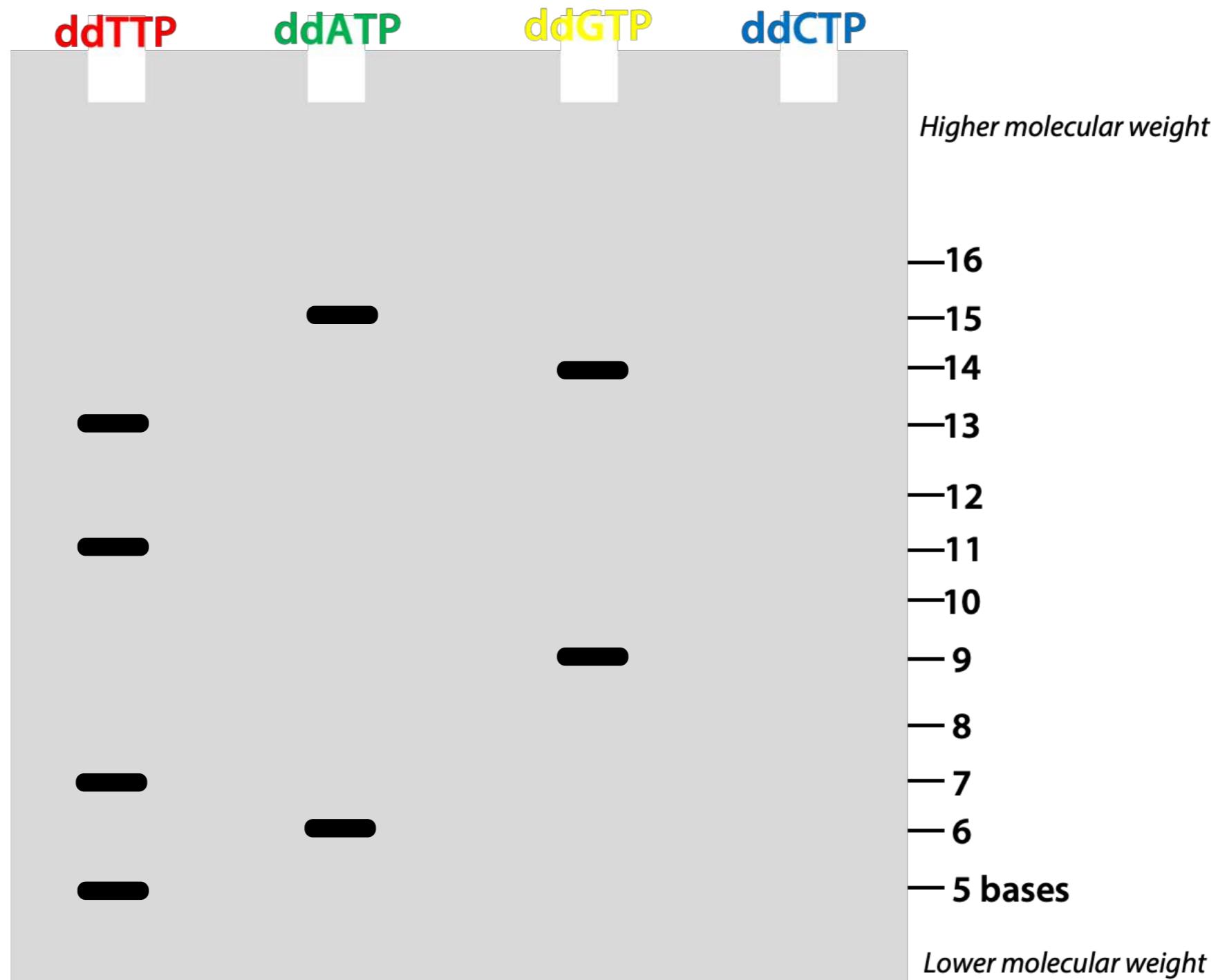
*5'tttt**TAT CGCT CTGA**
3'aaaa**ATAGCGAGACTG5'**



DNA sequencing (Sanger)

*5'tttt**TATCG**
3'aaaa**ATAGCGAGACTG5'**

*5'tttt**TAT CGCT CTG**
3'aaaa**ATAGCGAGACTG5'**



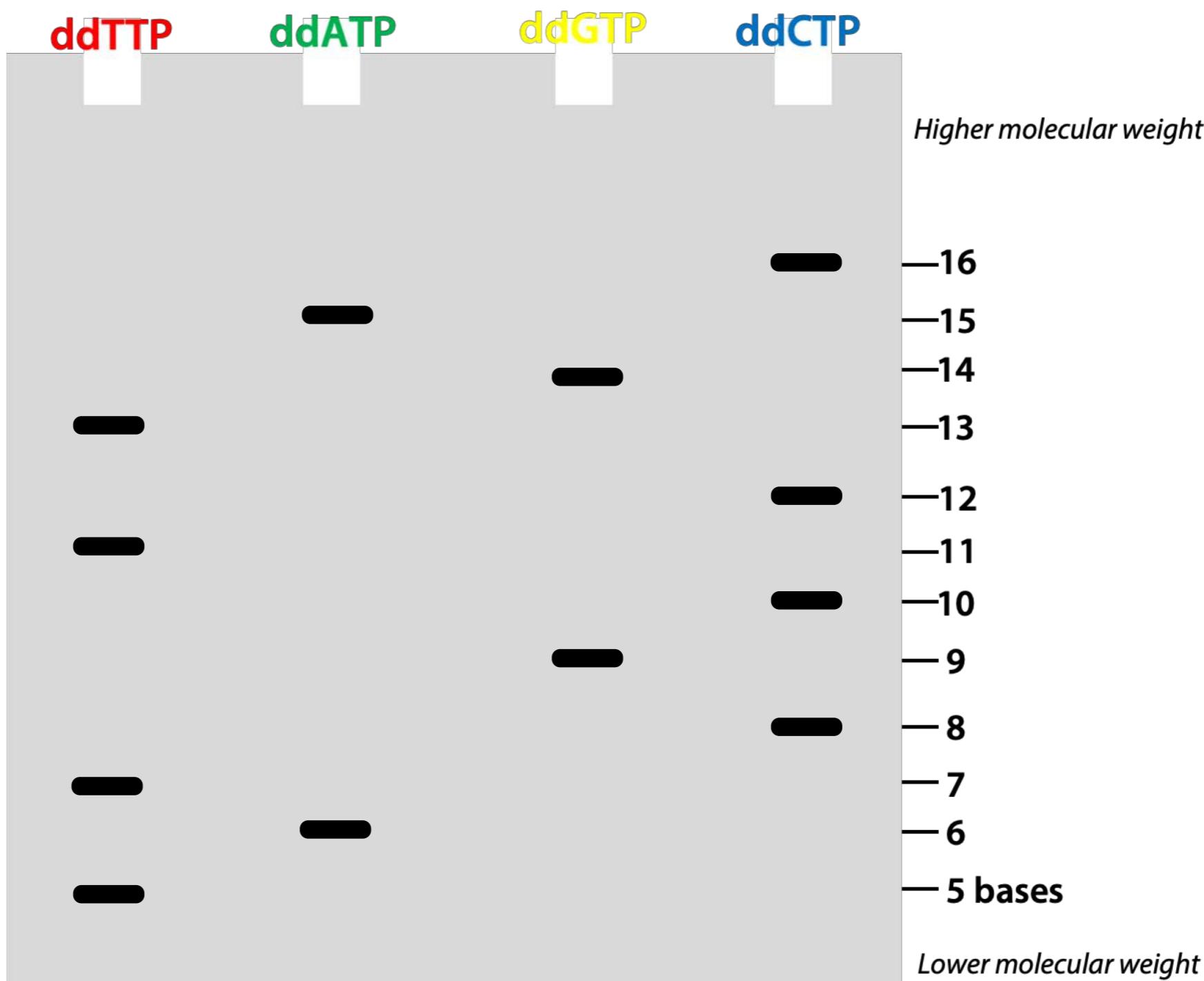
DNA sequencing (Sanger)

*5'tttt**TAT C**
3'aaaa**ATAGCGAGACTG5'**

*5'tttt**TAT CGC TC**
3'aaaa**ATAGCGAGACTG5'**

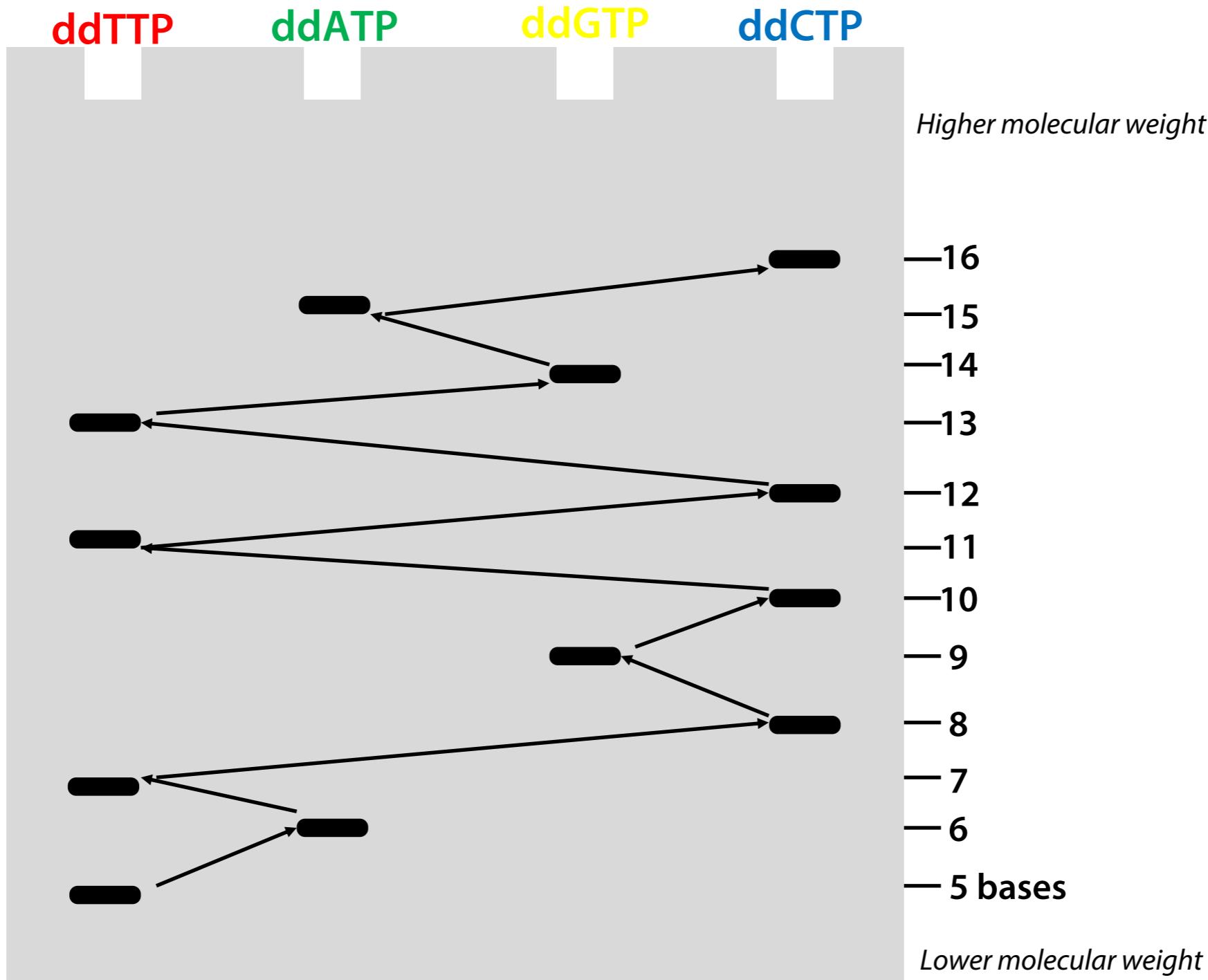
*5'tttt**TAT CGC**
3'aaaa**ATAGCGAGACTG5'**

*5'tttt**TAT CGC TC TGAC**
3'aaaa**ATAGCGAGACTG5'**



DNA sequencing (Sanger)

5' T A T C G C T C T G A C 3'



A typical sanger sequencing gel

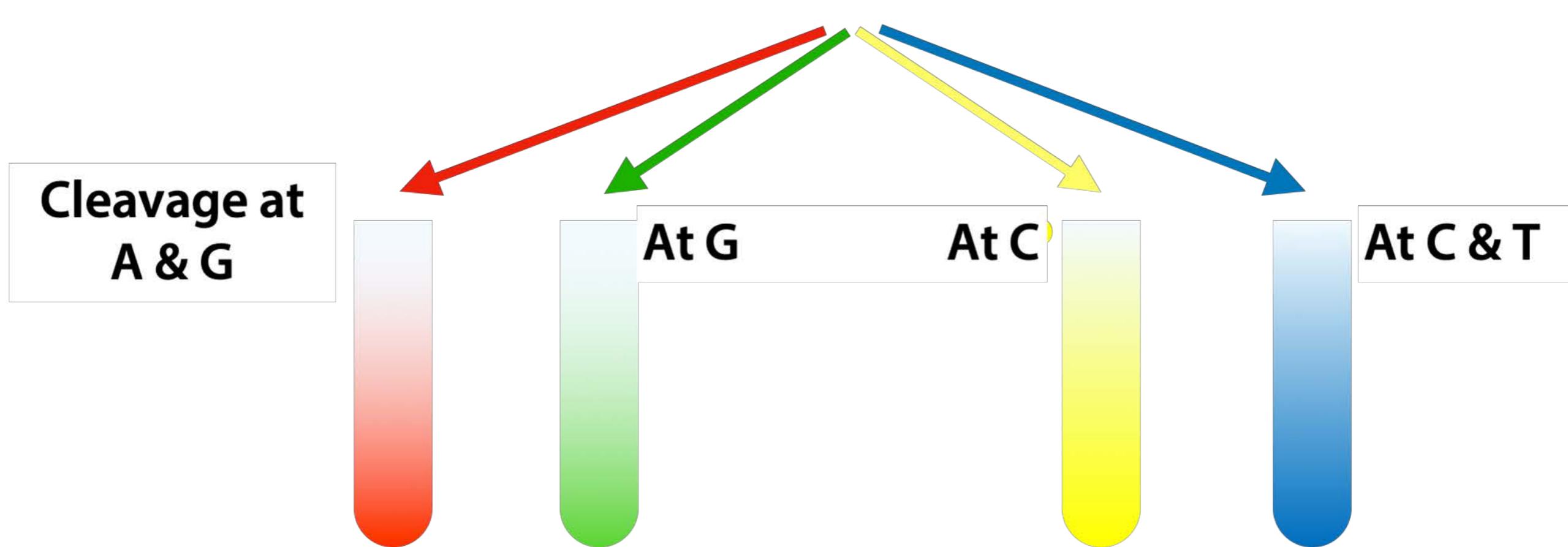


Maxam-Gilbert sequencing

3'ATAGCGGAGACTG5'

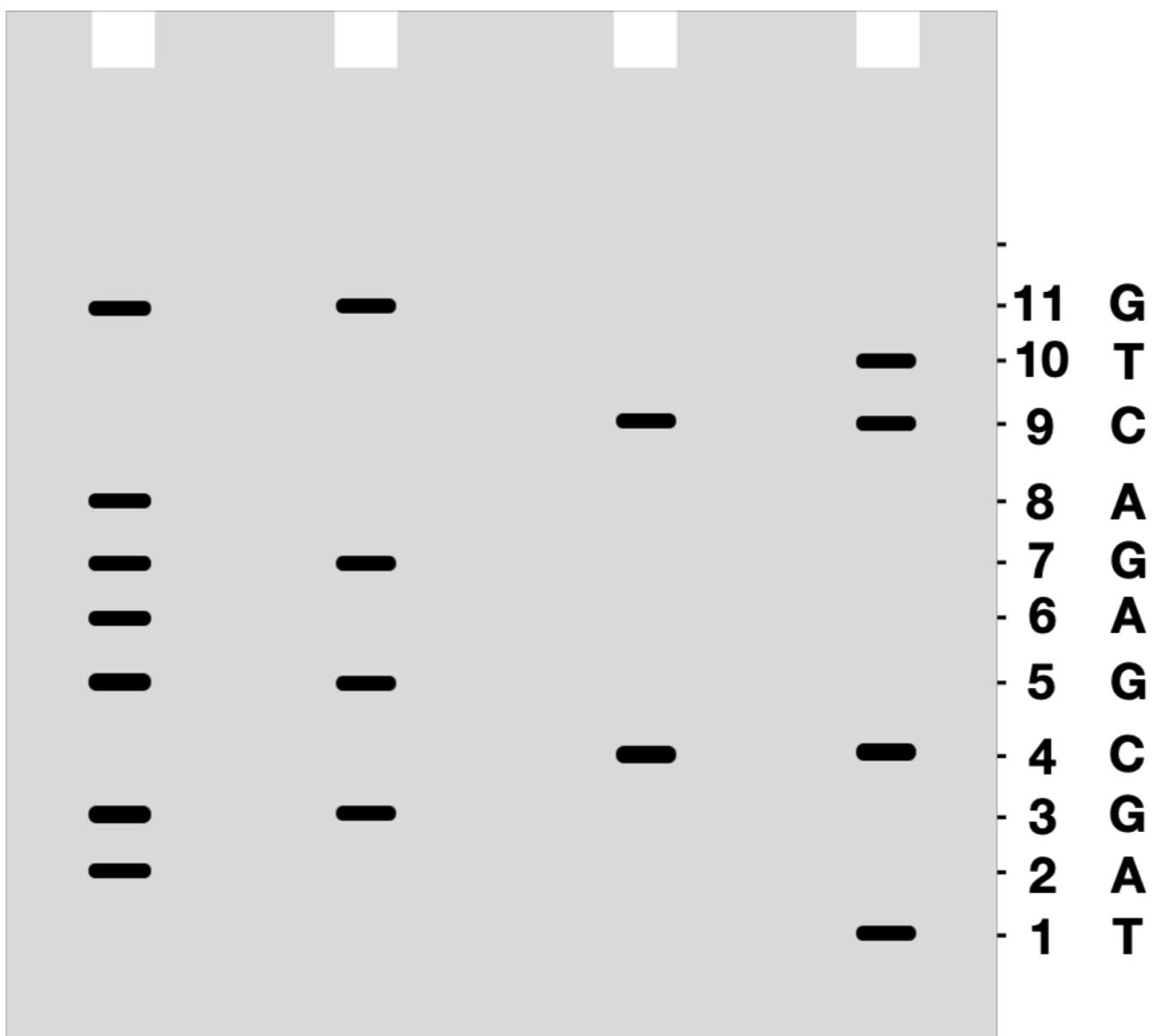
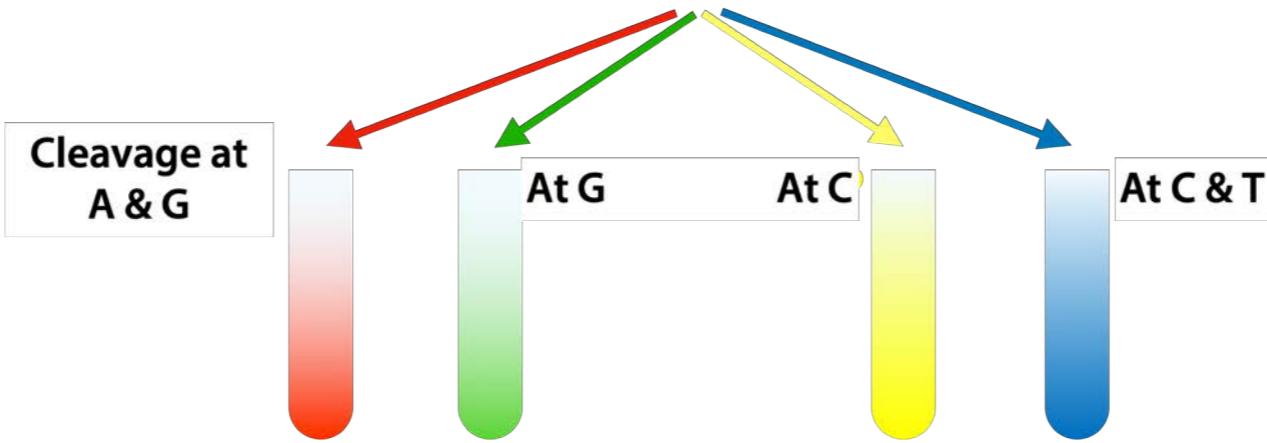
3'ATAGCGGAGACTG5'P³²

Label the end of DNA



Maxam-Gilbert sequencing

^{32}P 5'ATAGCGAGACTG3'



Maxam-Gilbert sequencing - modifications

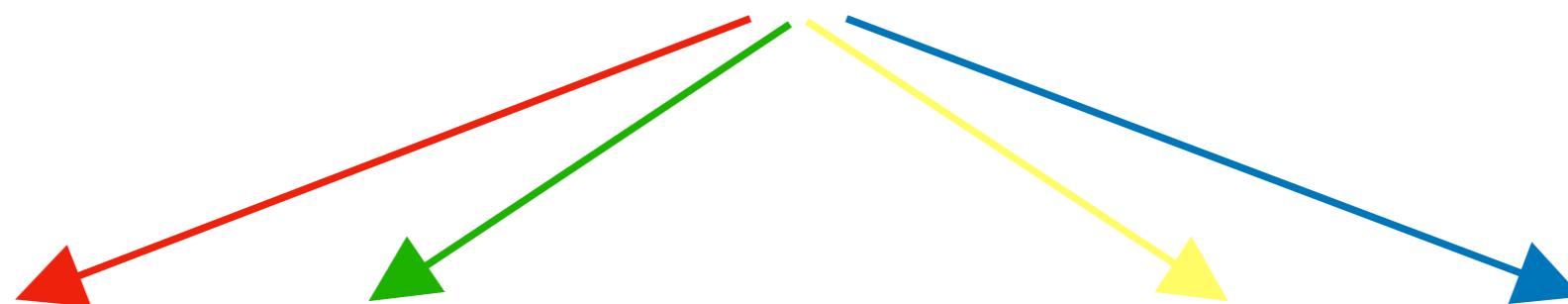
TABLE 13.2 Chemical Modifications Used in the Maxam-Gilbert Method

Base	Specific modification ^a
G	Methylation of N ₇ with dimethyl sulfate at pH 8.0 makes the C ₈ —C ₉ bond specifically susceptible to cleavage by base
A + G	Piperidine formate at pH 2.0 weakens the glycosidic bond of adenine and guanine by protonating nitrogen atoms in the purine rings resulting in depurination
C + T	Hydrazine opens pyrimidine rings, which recyclize in a five-membered form that is susceptible to removal
C	In the presence of 1.5 M NaCl, only cytosine reacts appreciably with hydrazine
A > C	1.2 N NaOH at 90°C results in strong cleavage at A and weaker cleavage at C

^a Hot (90°C) piperidine (1 M in H₂O) is used to cleave the sugar-phosphate chain of DNA at the sites of chemical modifications.

Sanger sequencing updated

5't t t t TATCGCTCTGAC 3'
3'aaaaa ATAGCGAGACTG 5'



Separate the two DNA strands
Add DNA polymerase
Add dNTPs

ddTTP* ddATP*

ddGTP* ddCTP*

Add primer

5't t t t

3'aaaaa ATAGCGAGACTG 5'

↓ Denature

Sanger sequencing updated

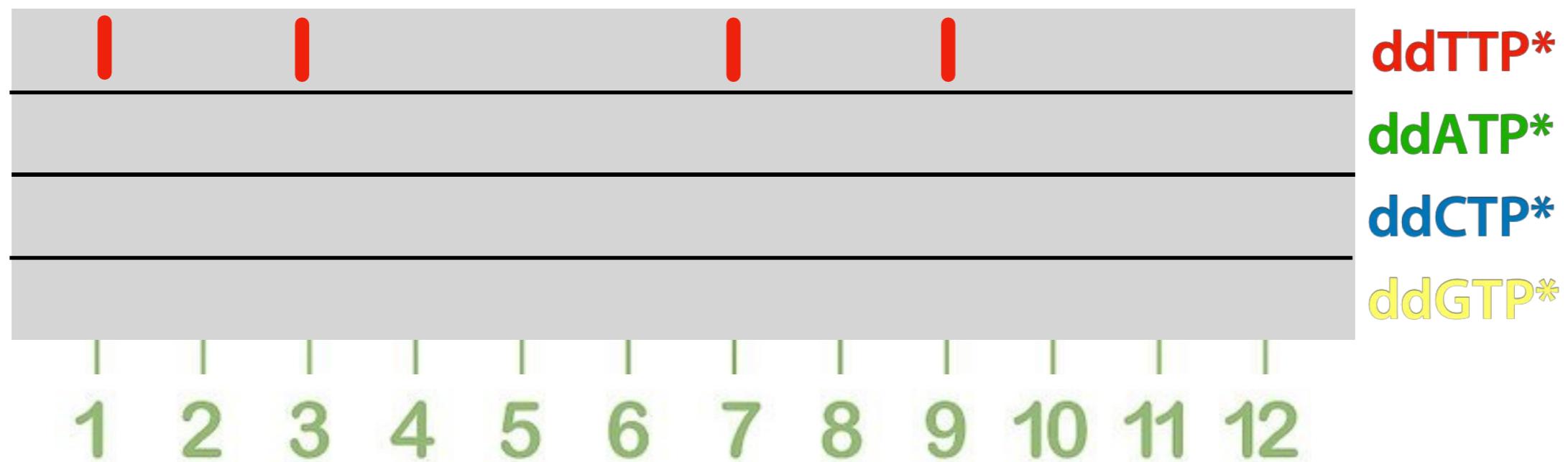
ddTTP*

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' TATCGCTCT
3' ATAGCGAGACTG 5'

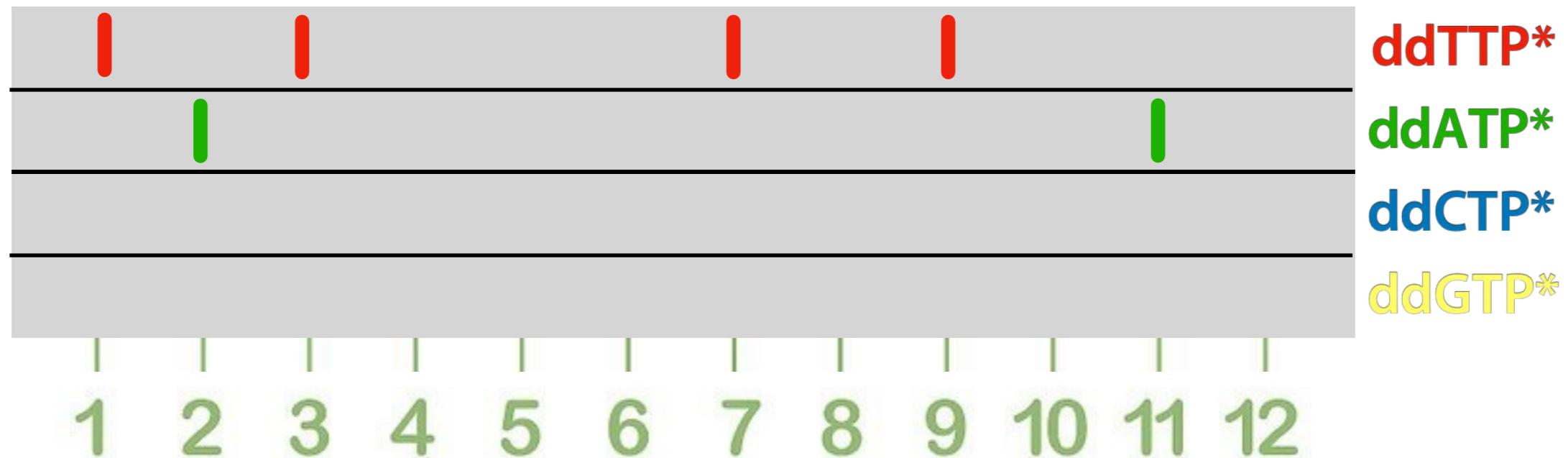


Sanger sequencing updated

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'



Sanger sequencing updated

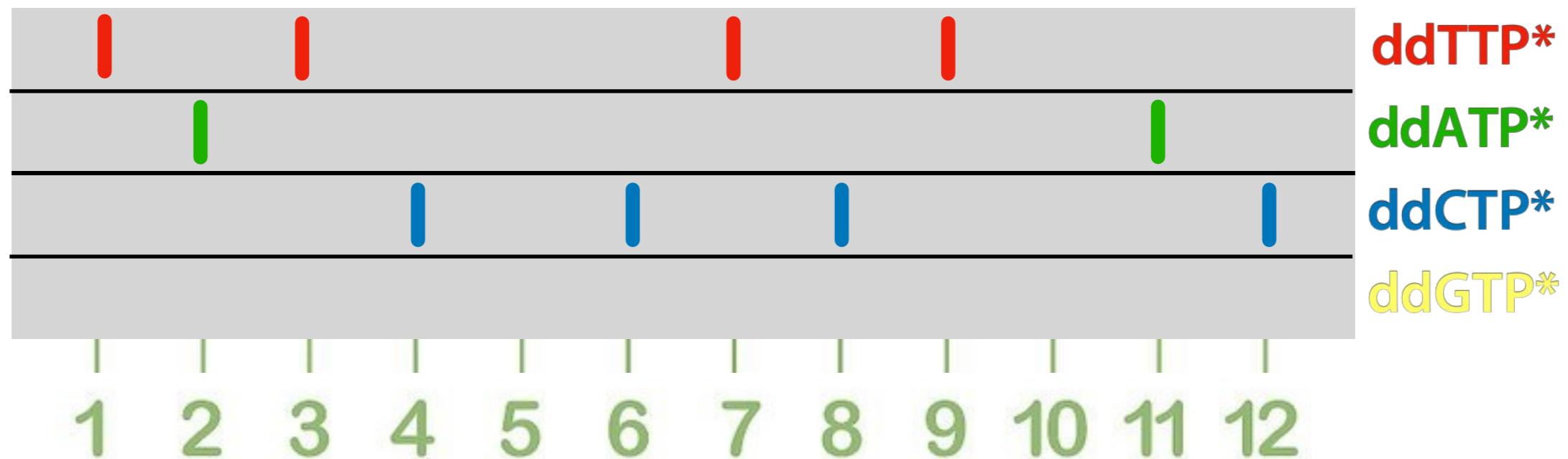
ddCTP*

5' T A T C
3' A T A G C G A G A C T G 5'

5' TATCG C
3' ATAGCGAGACTG 5'

5' TATCGCT C
3' ATAGCGAGACTG 5'

5' TATCGCTCTGA C
3' ATAGCGAGACTG 5'

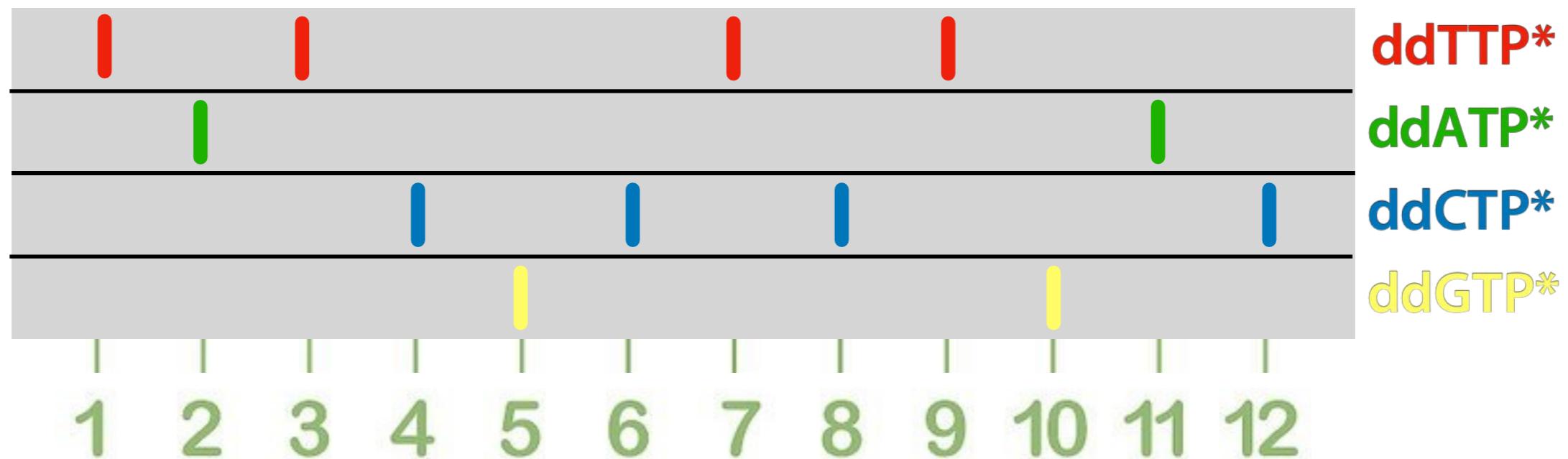


Sanger sequencing updated

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'

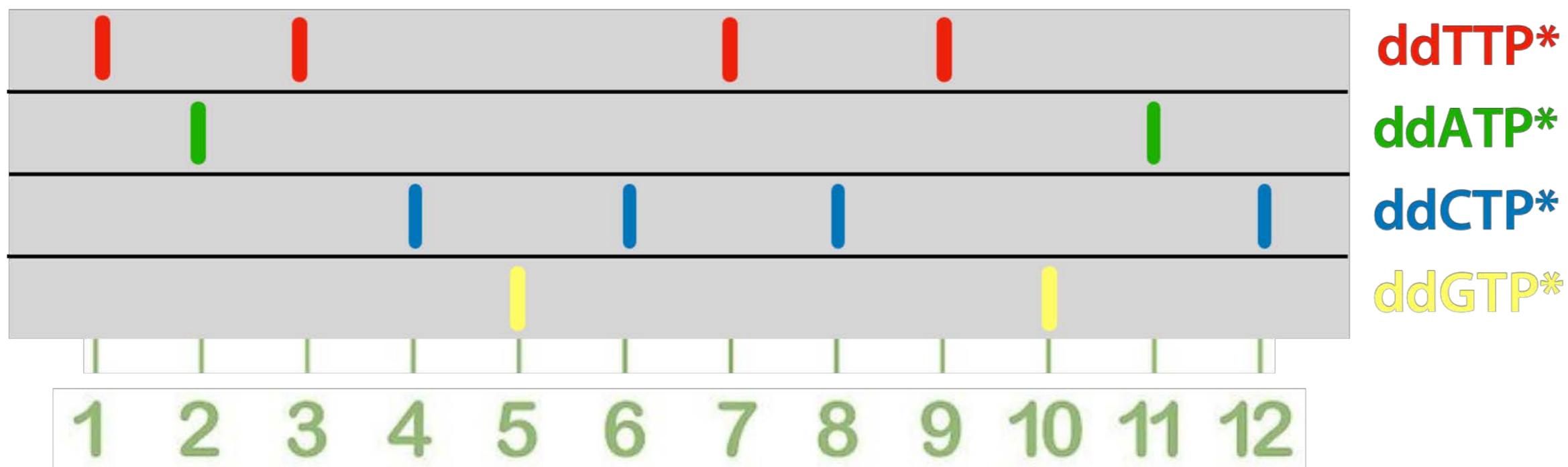


Sanger sequencing updated

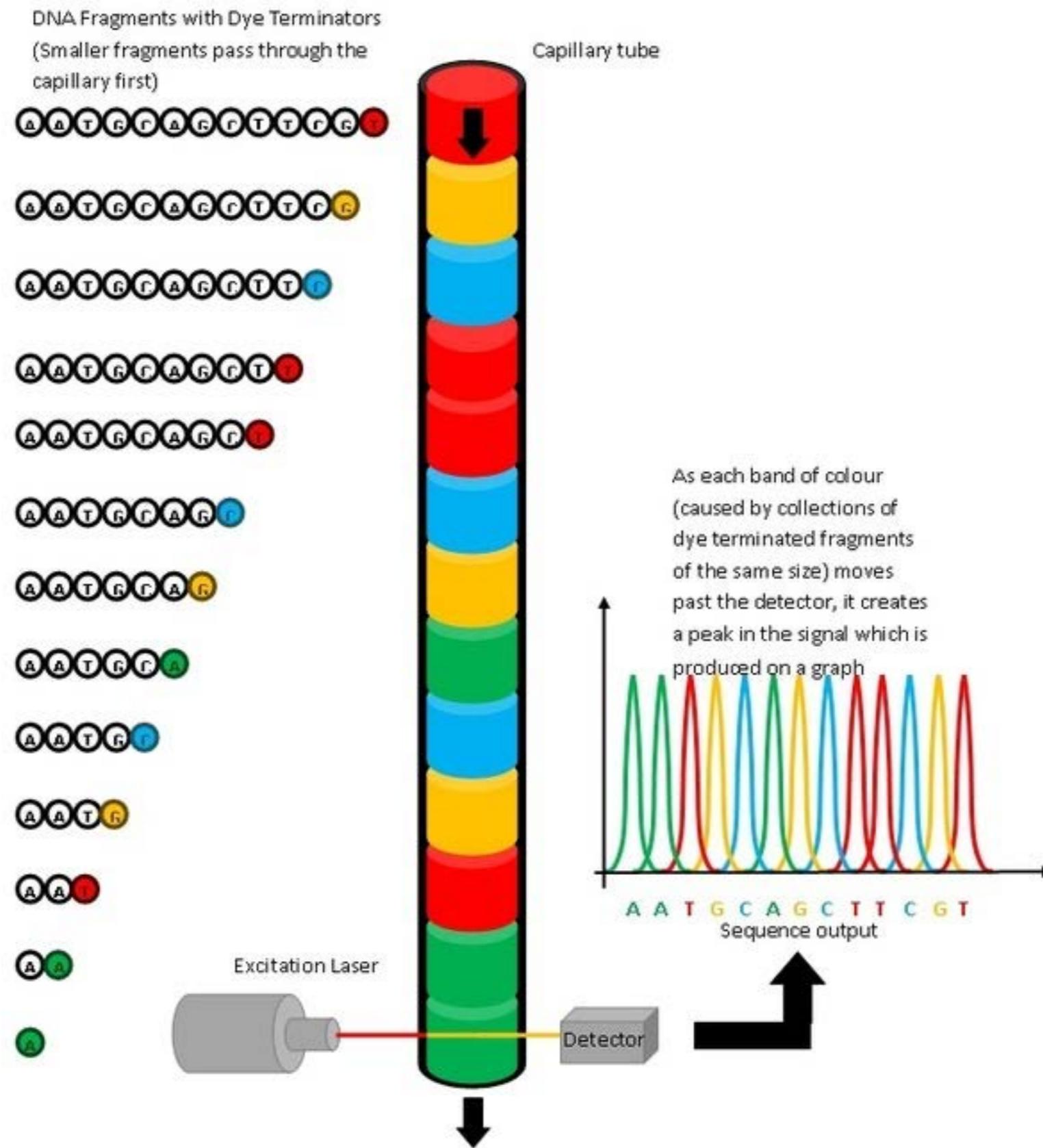
5' **TATCGCTCTGAC** 3'
3' ATAGCGAGACTG 5'

Reading direction lower to higher molecular weight

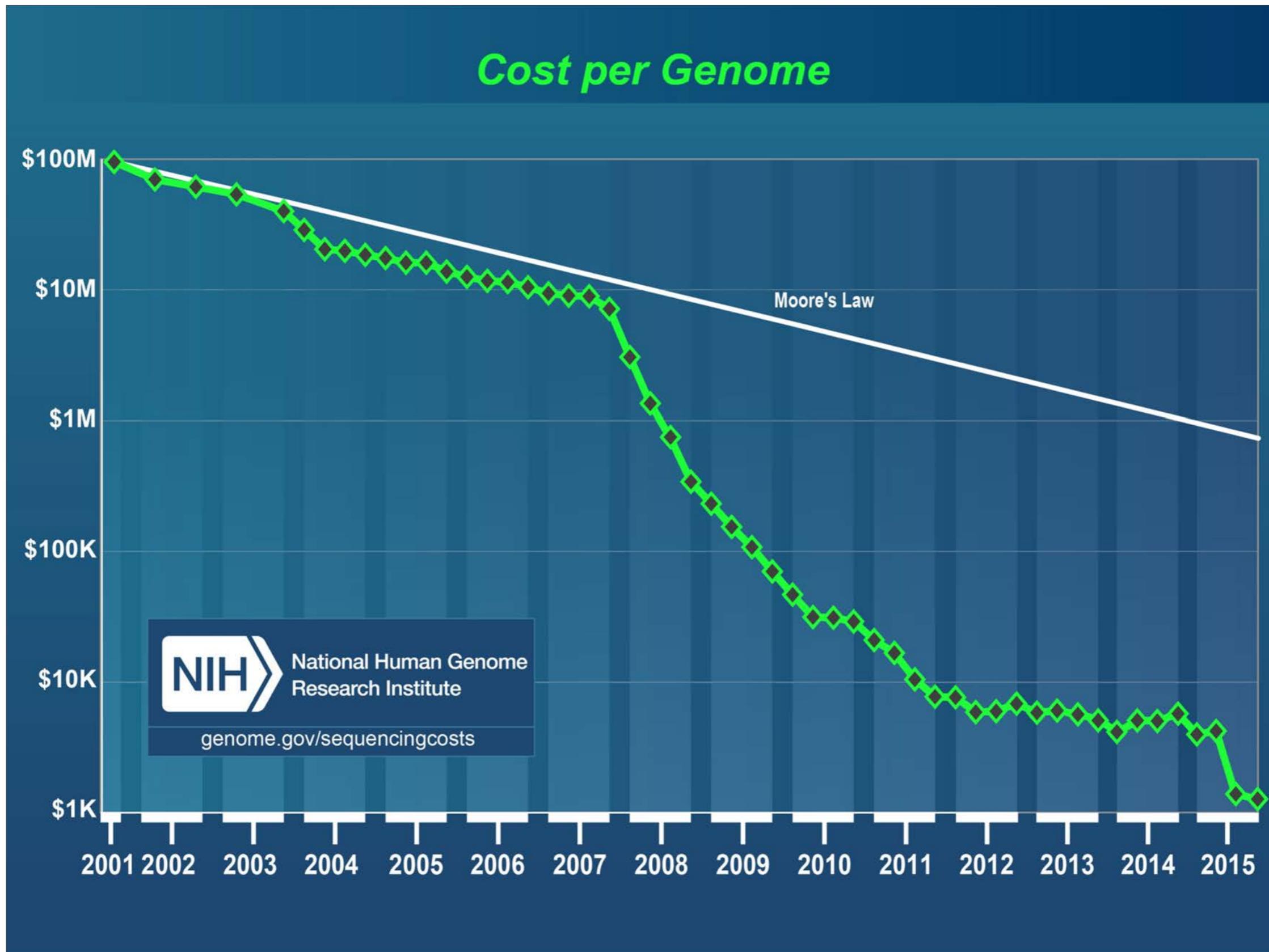
The diagram illustrates four sequencing lanes, each represented by a horizontal grey bar. Red vertical bars are placed at positions 1, 3, 7, and 9. Green vertical bars are placed at positions 2 and 11. Blue vertical bars are placed at positions 4, 6, and 10. Yellow vertical bars are placed at positions 5 and 12. A large black arrow points from left to right, indicating the reading direction from lower to higher molecular weight.



Sanger sequencing updated



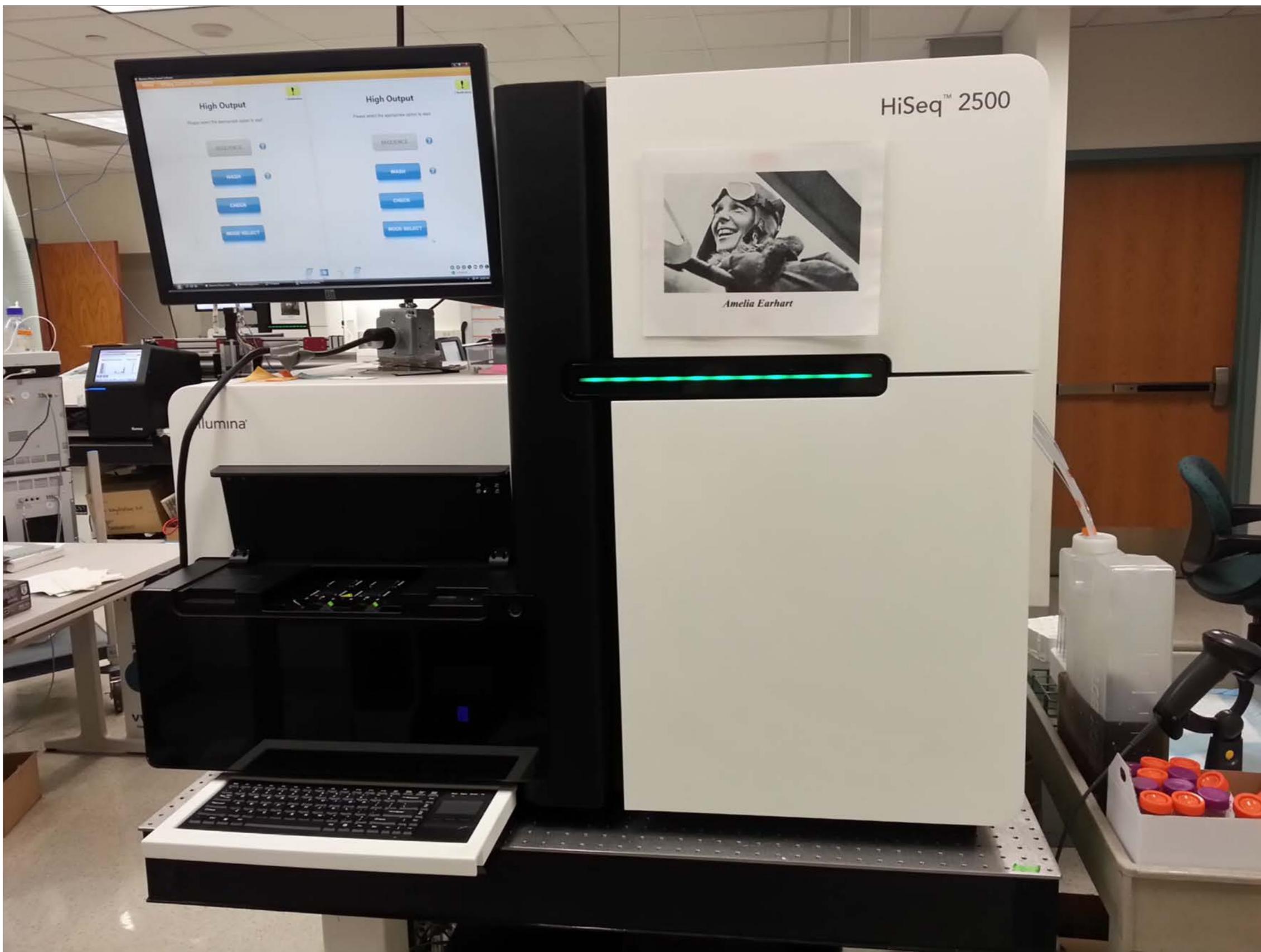
DNA sequencing cost



Human genome project



But now

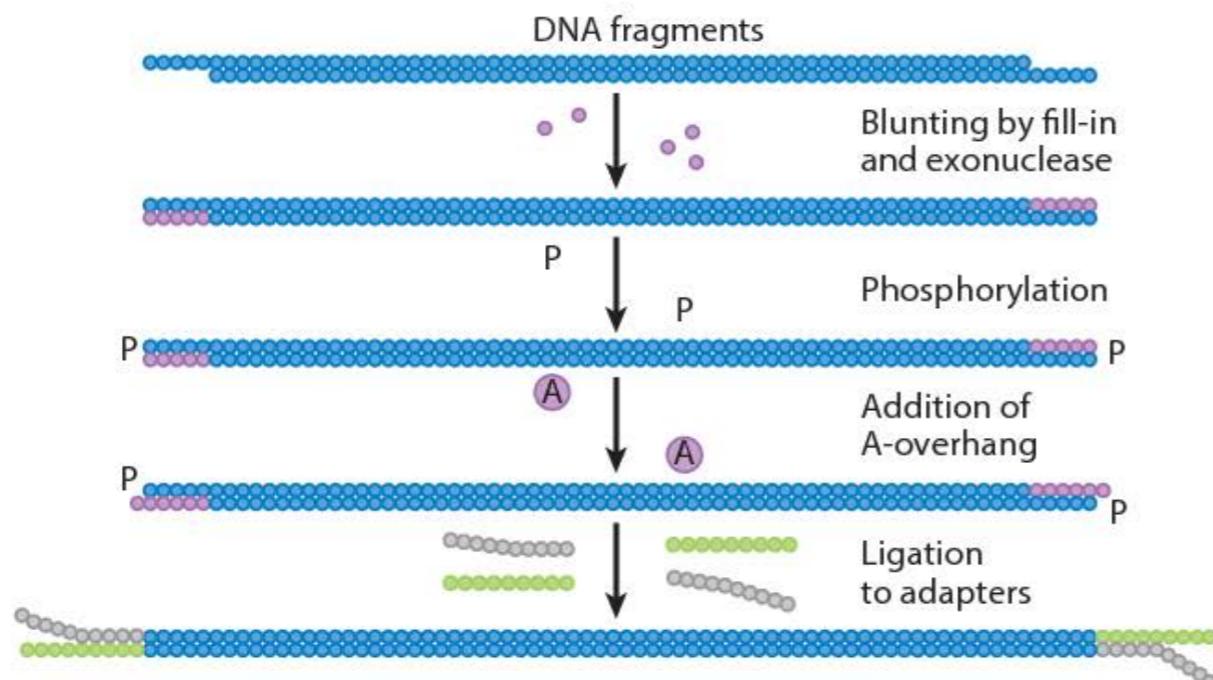
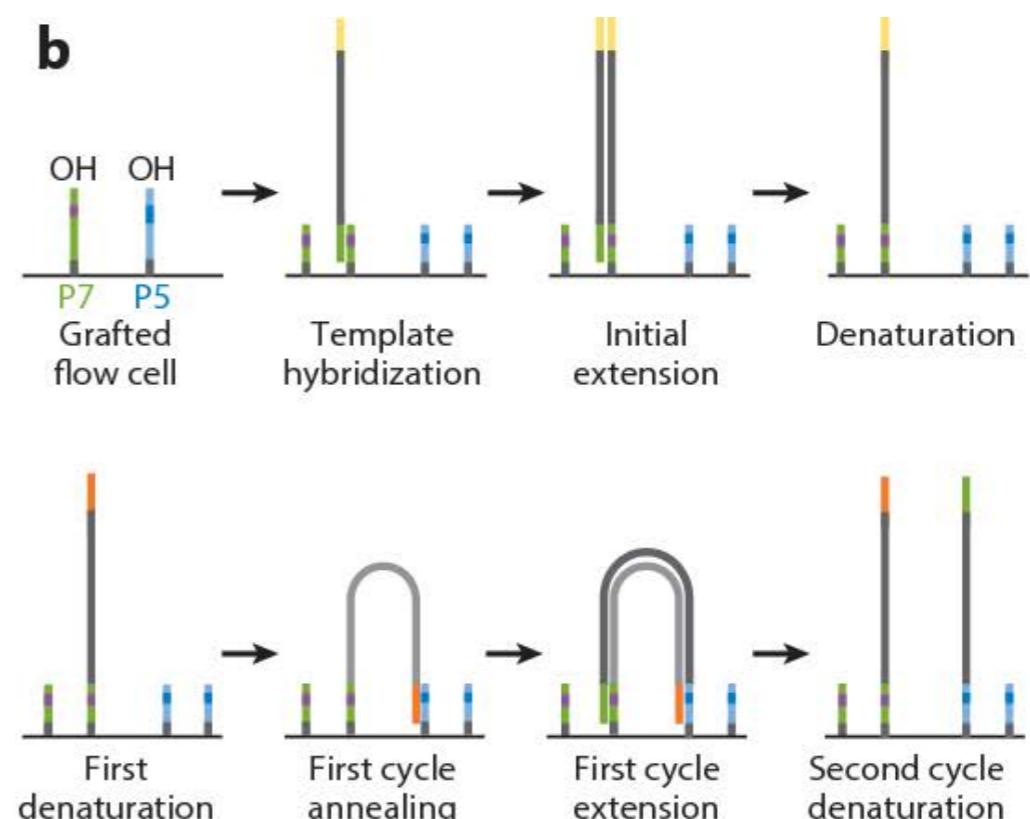
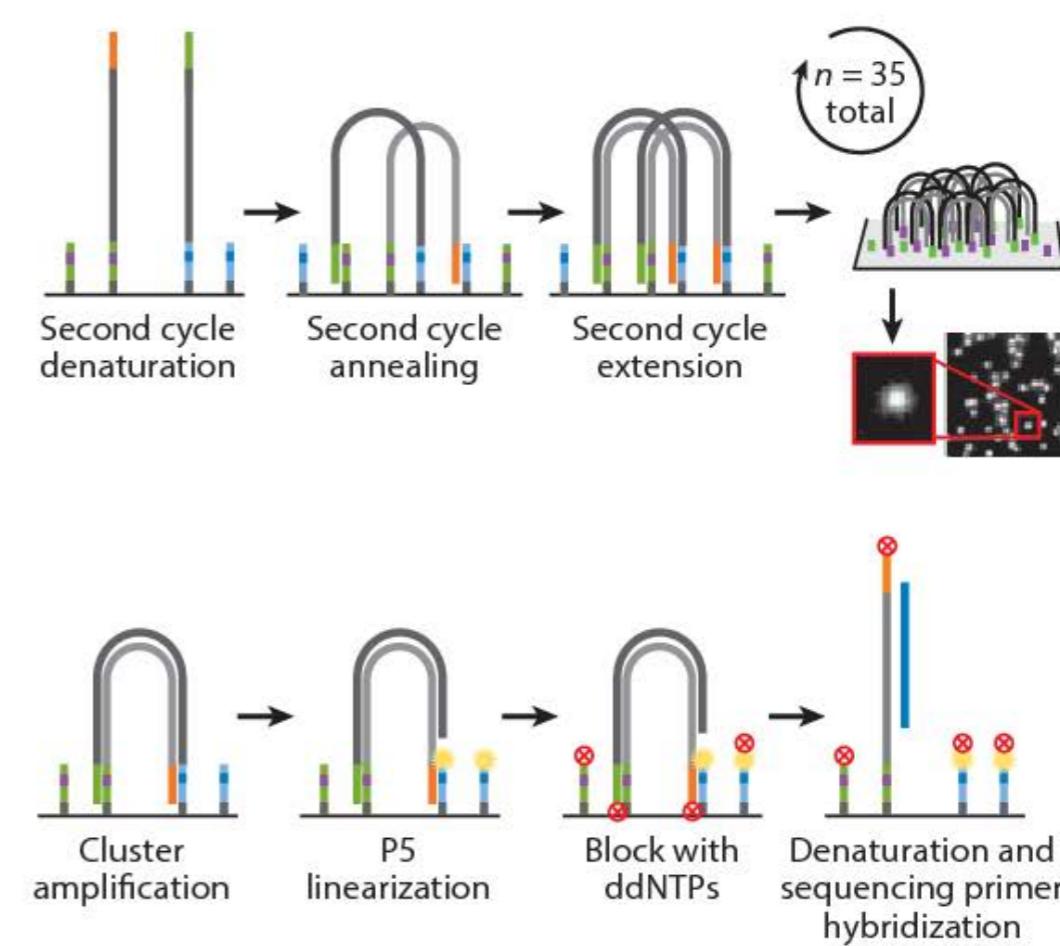
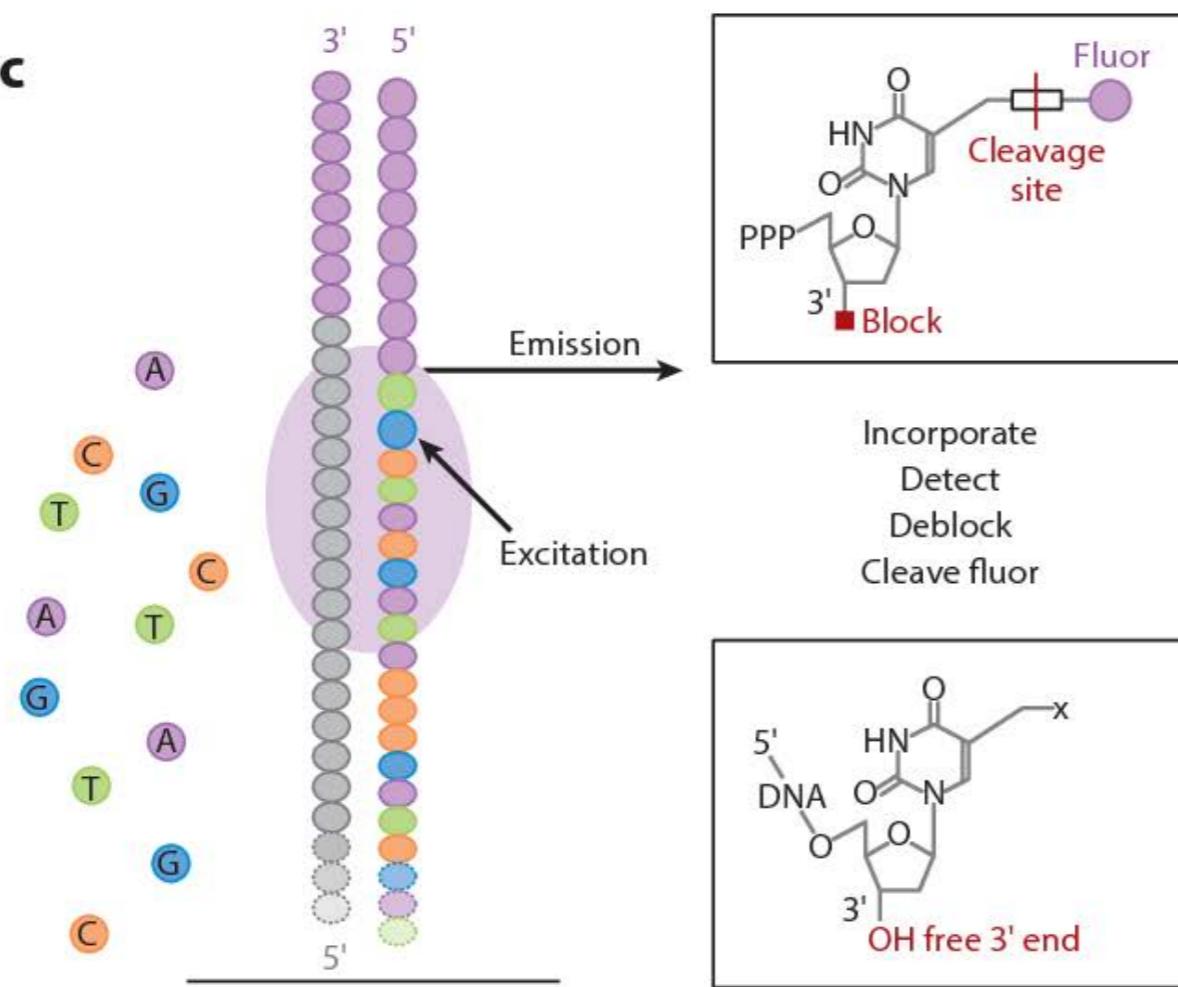


DNA sequencing

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

Sequencing by synthesis - illumina

a Illumina's library-preparation work flow

**b****c**

DNA illumina sequencing [video-1](#)

DNA illumina sequencing [video-2](#)

Personalized DNA sequencing



**Sequencing fetus
using DNA from the
mother**

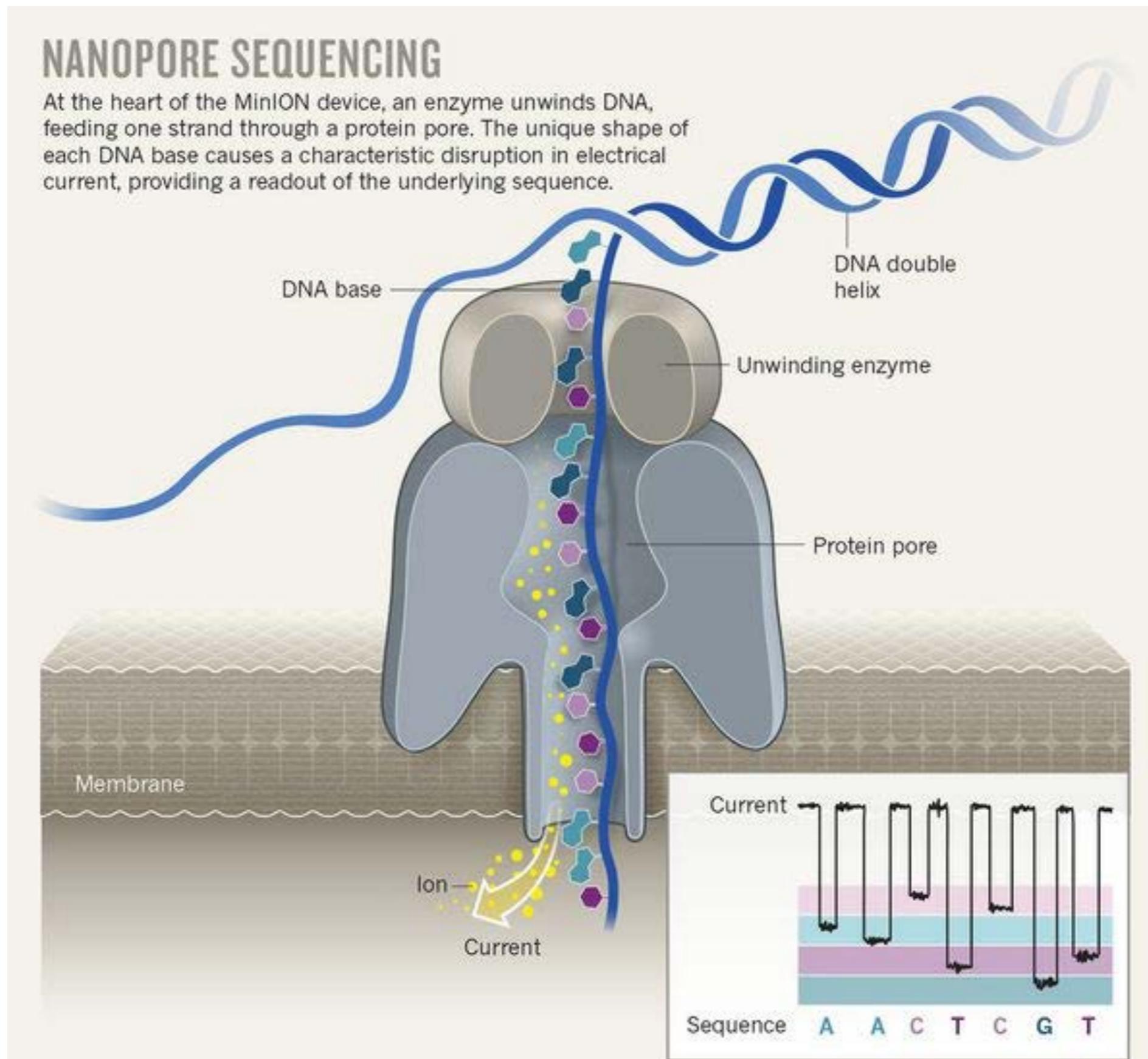


**Sequence your
genome for \$99**

Future of DNA sequencing



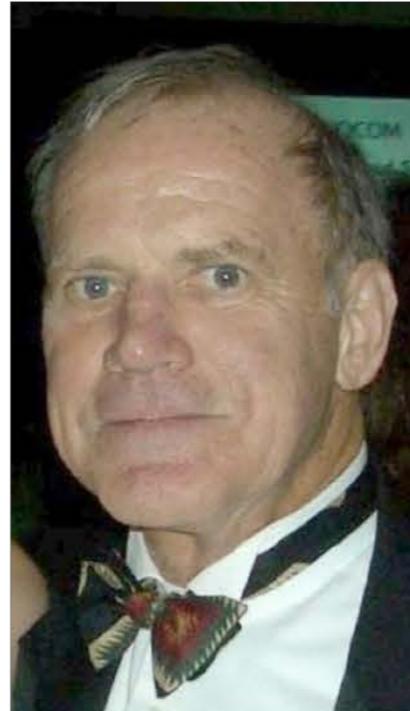
Single molecule sequencing



Nanopore sequencing [video-1](#)

Polymerase Chain Reaction

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

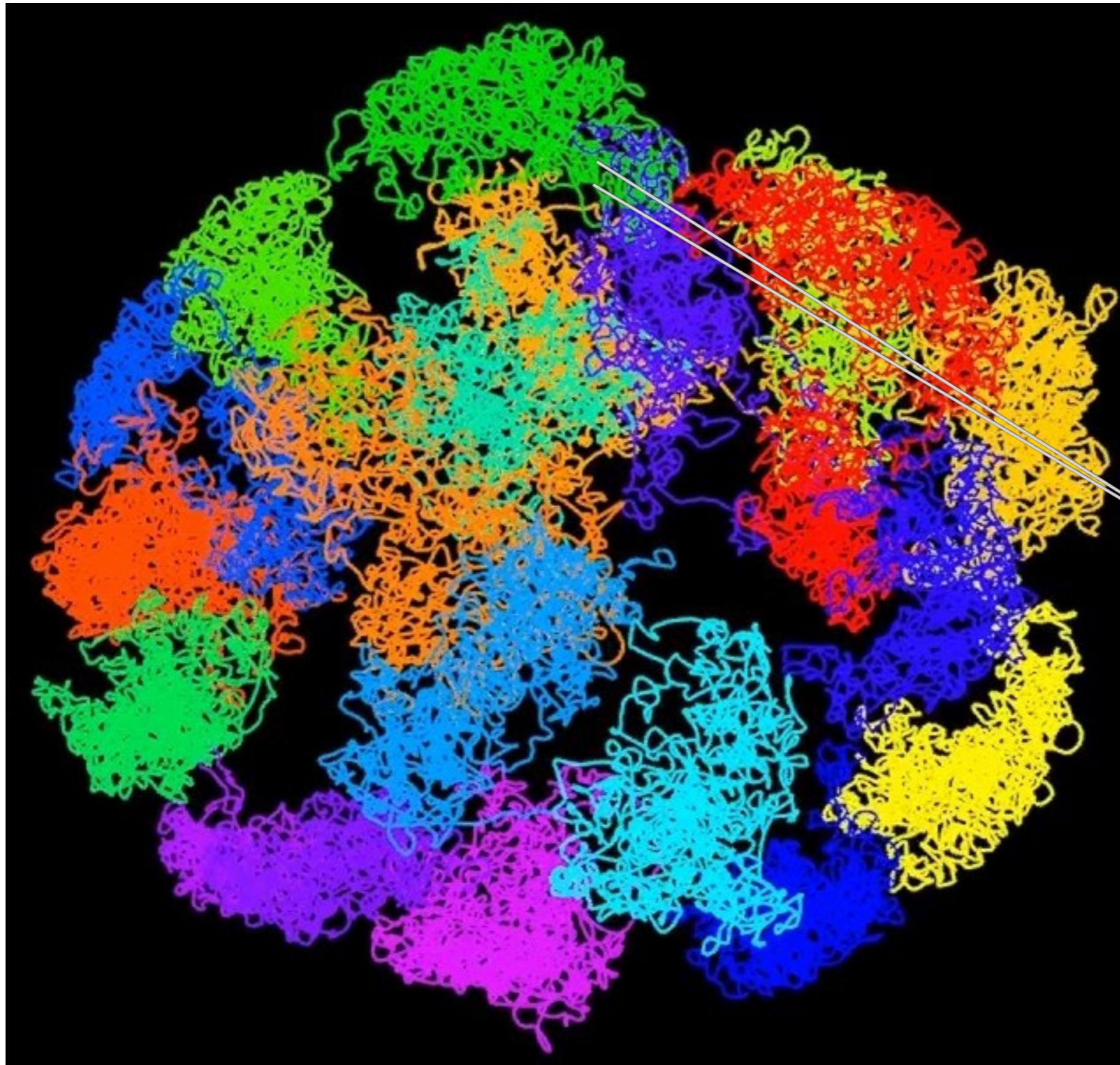


1993

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?



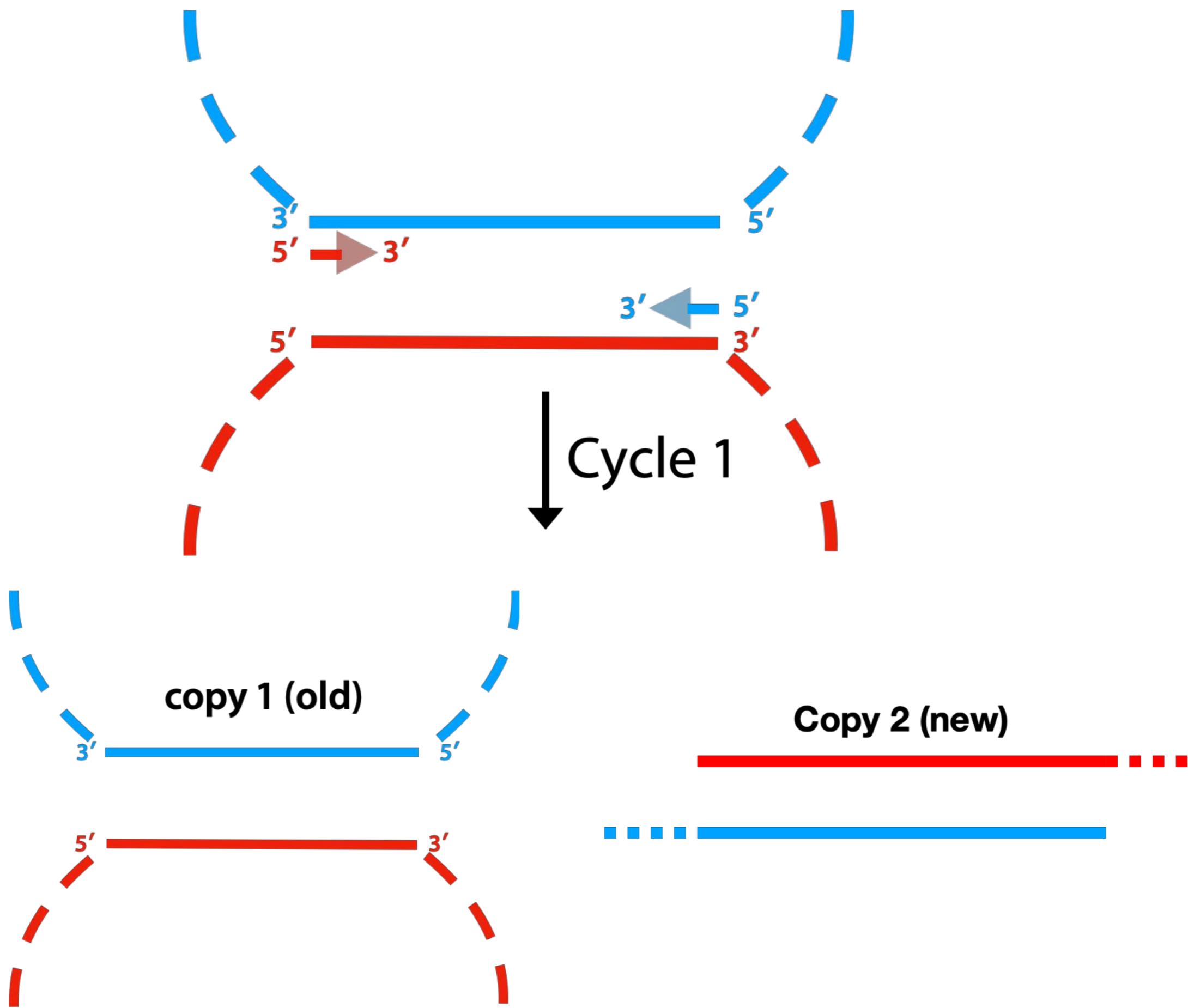
Denature DNA > 95°C

Add DNA polymerase + dNTPs

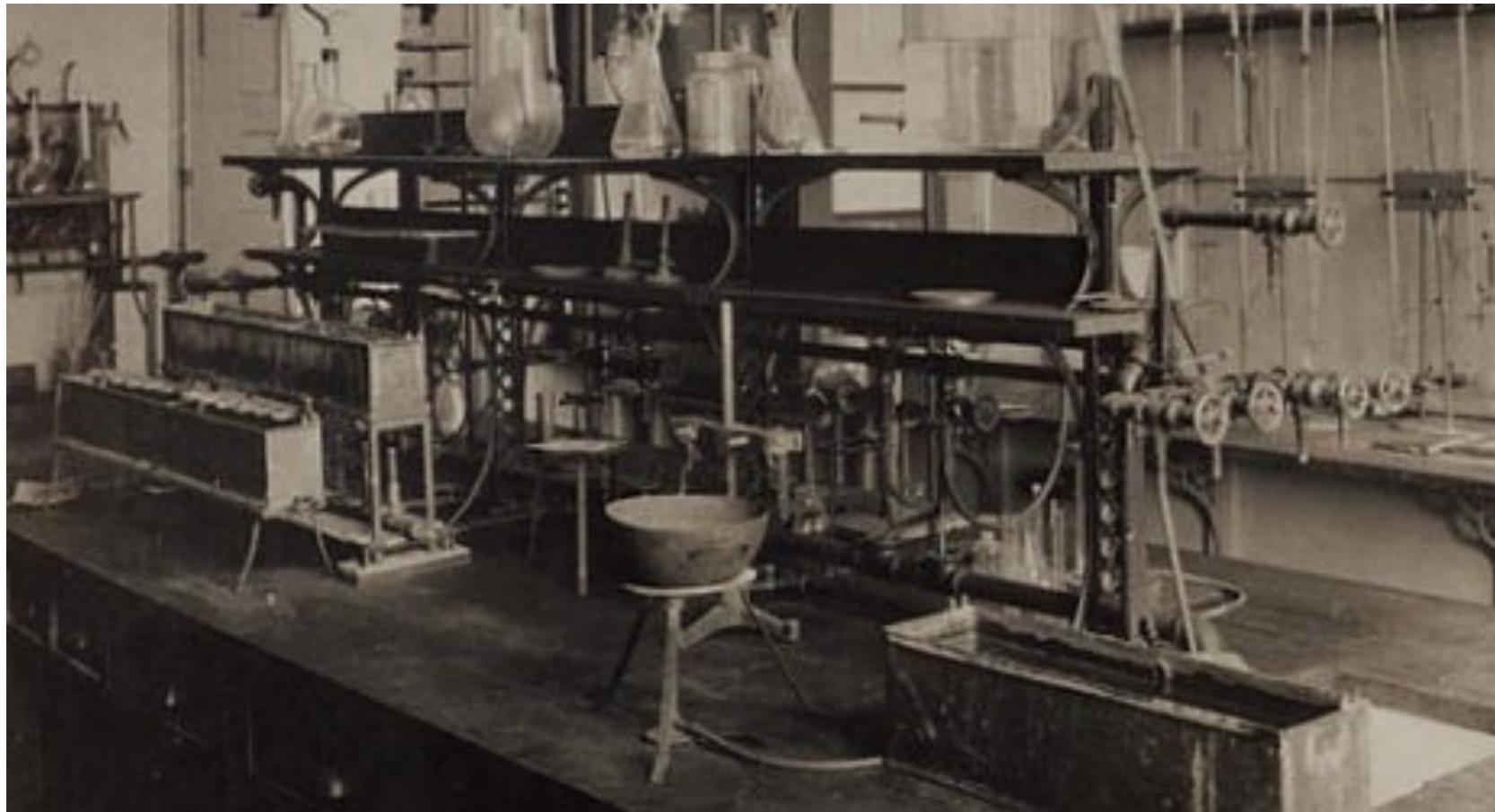
Add primers

Perform repeated cycles of denaturation, annealing & replication

PCR



PCR machines



Running around the water baths (1980s)



And now!!!