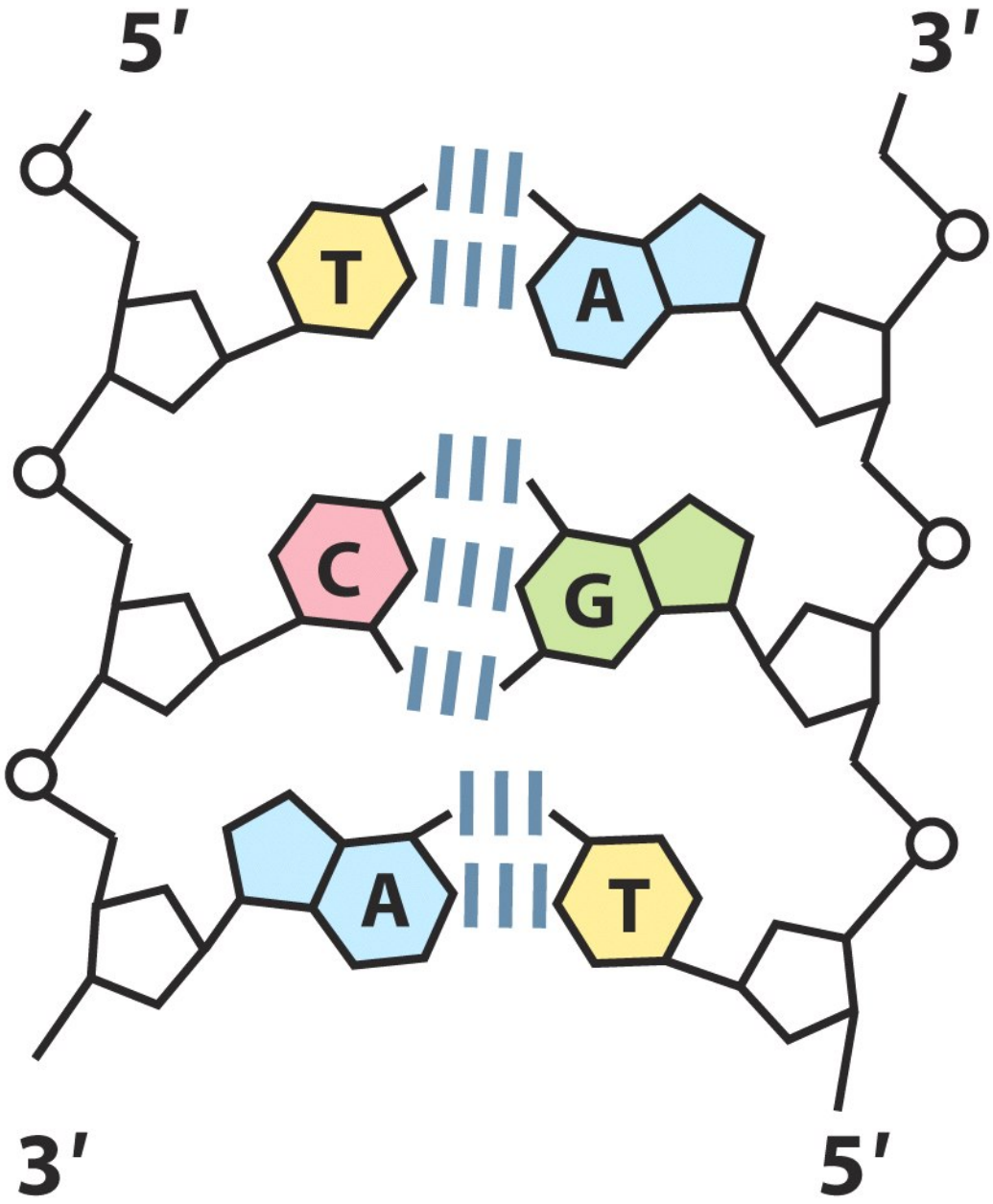
David L. Nelson and Michael M. Cox

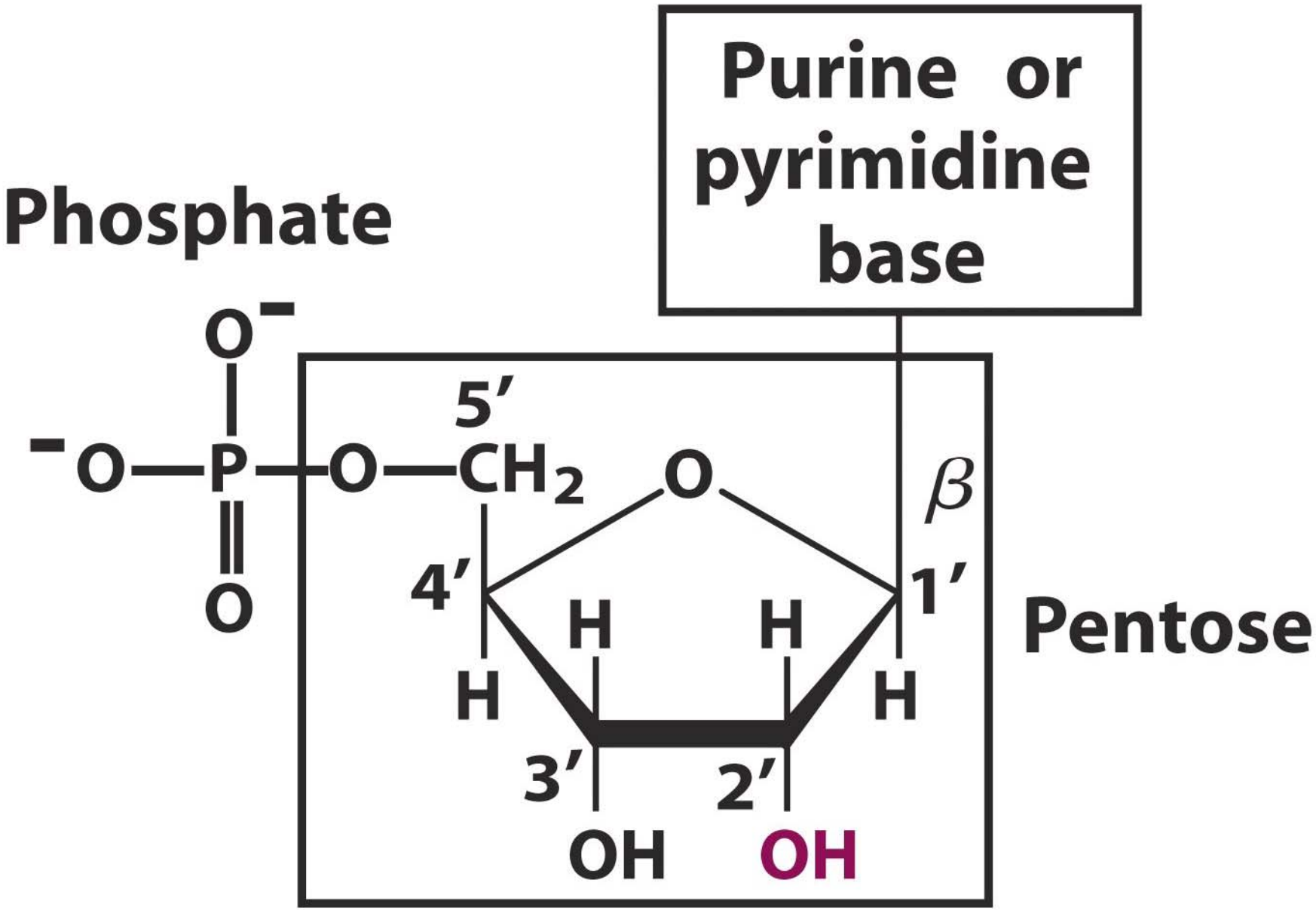
Lehninger Principles of Biochemistry

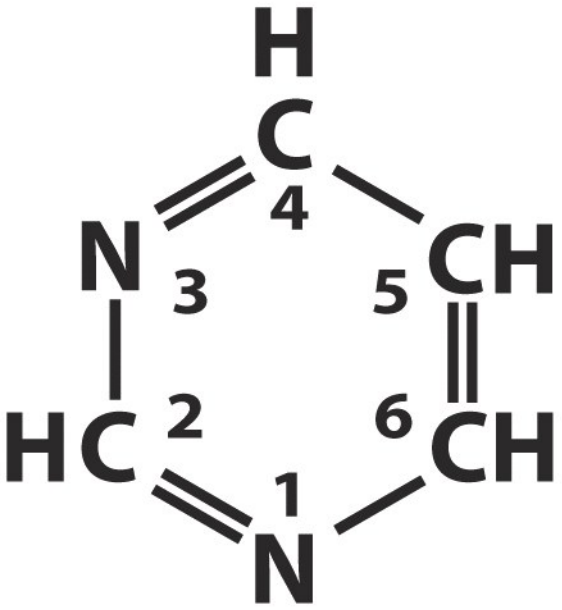
Fourth Edition

Chapter 8:

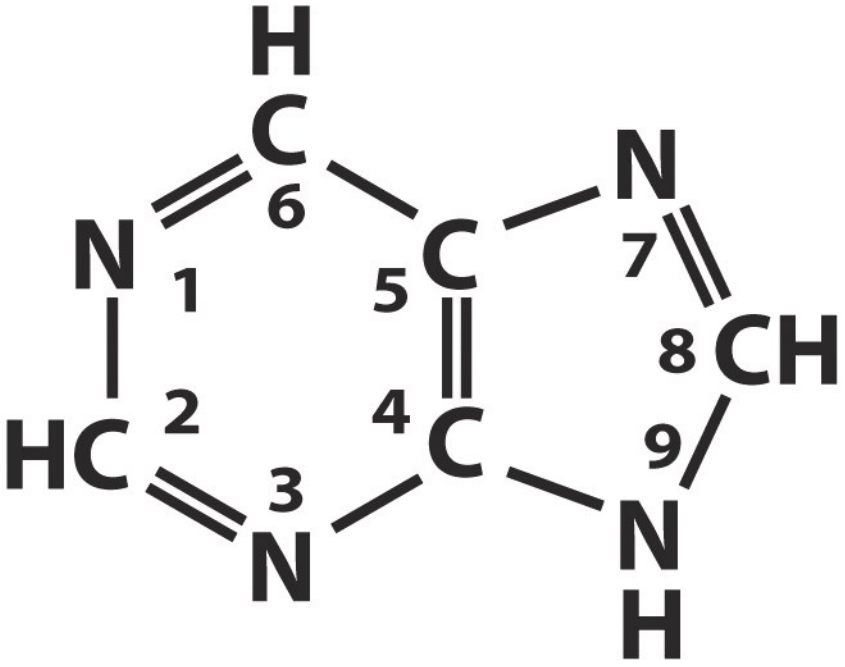
Nucleotides and Nucleic Acids


5′
3′
T
A
C
G
A
T
3′
5′

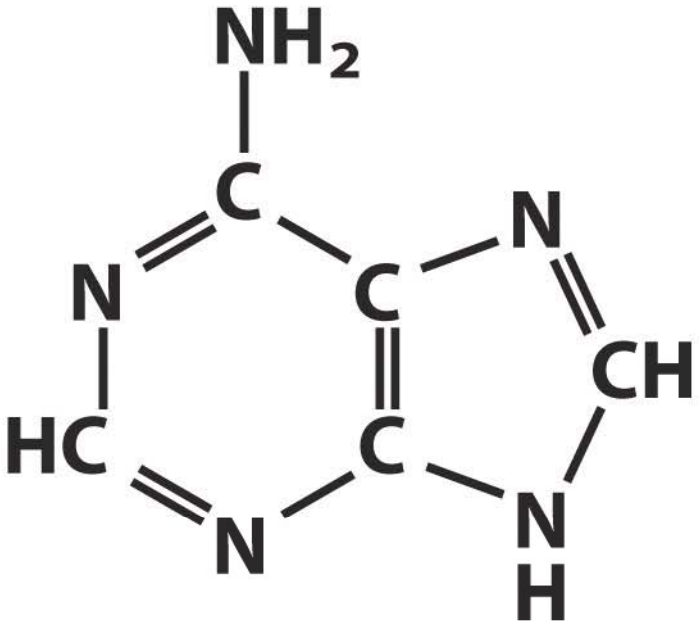

Purine or pyrimidine base
Phosphate
O⁻
⁻O—P—O—CH₂
O
O
5′
4′
1′
β
H
H
H
H
3′
2′
OH
OH
Pentose



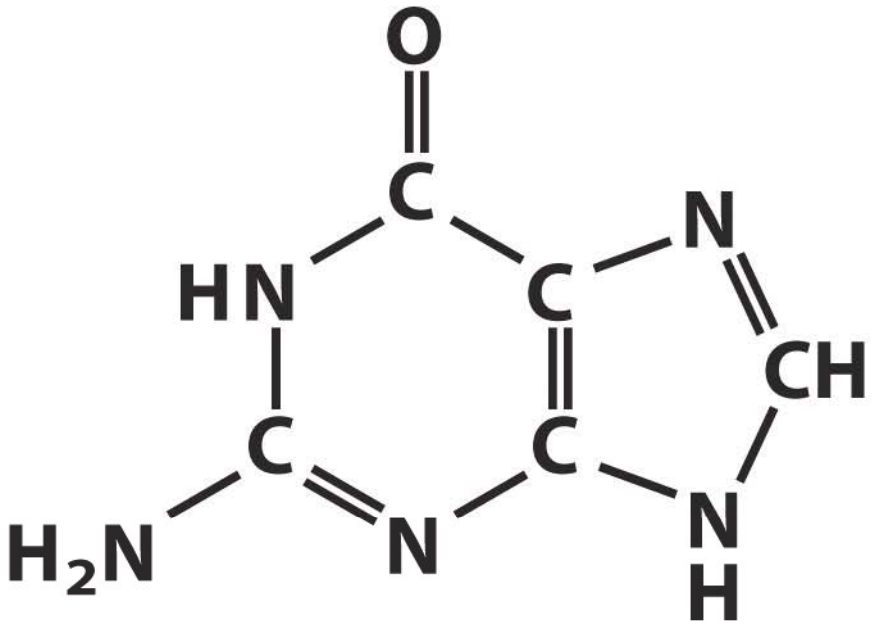
Pyrimidine



Purine

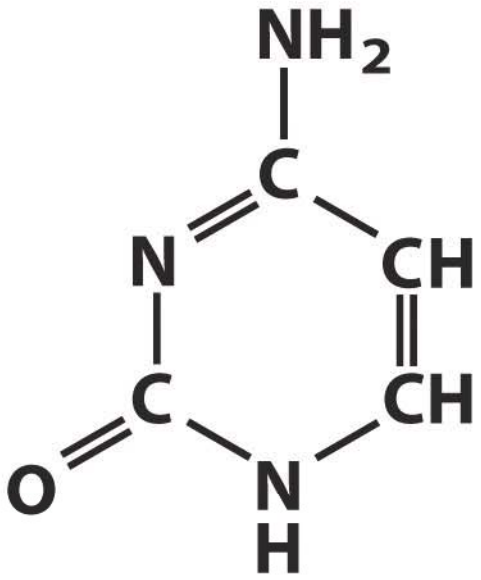


Adenine

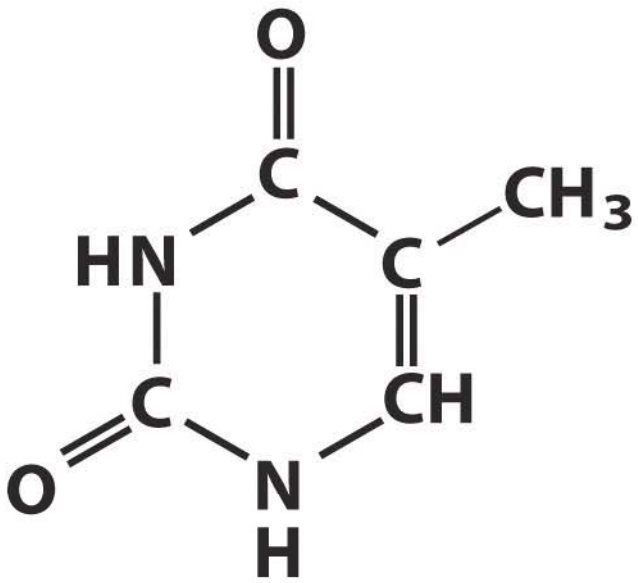


Guanine

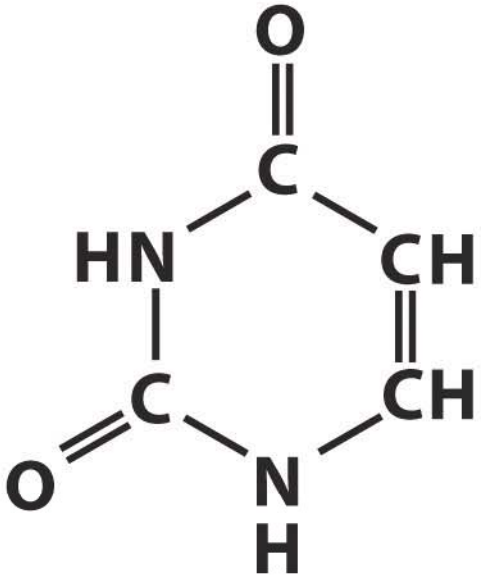
Purines



Cytosine

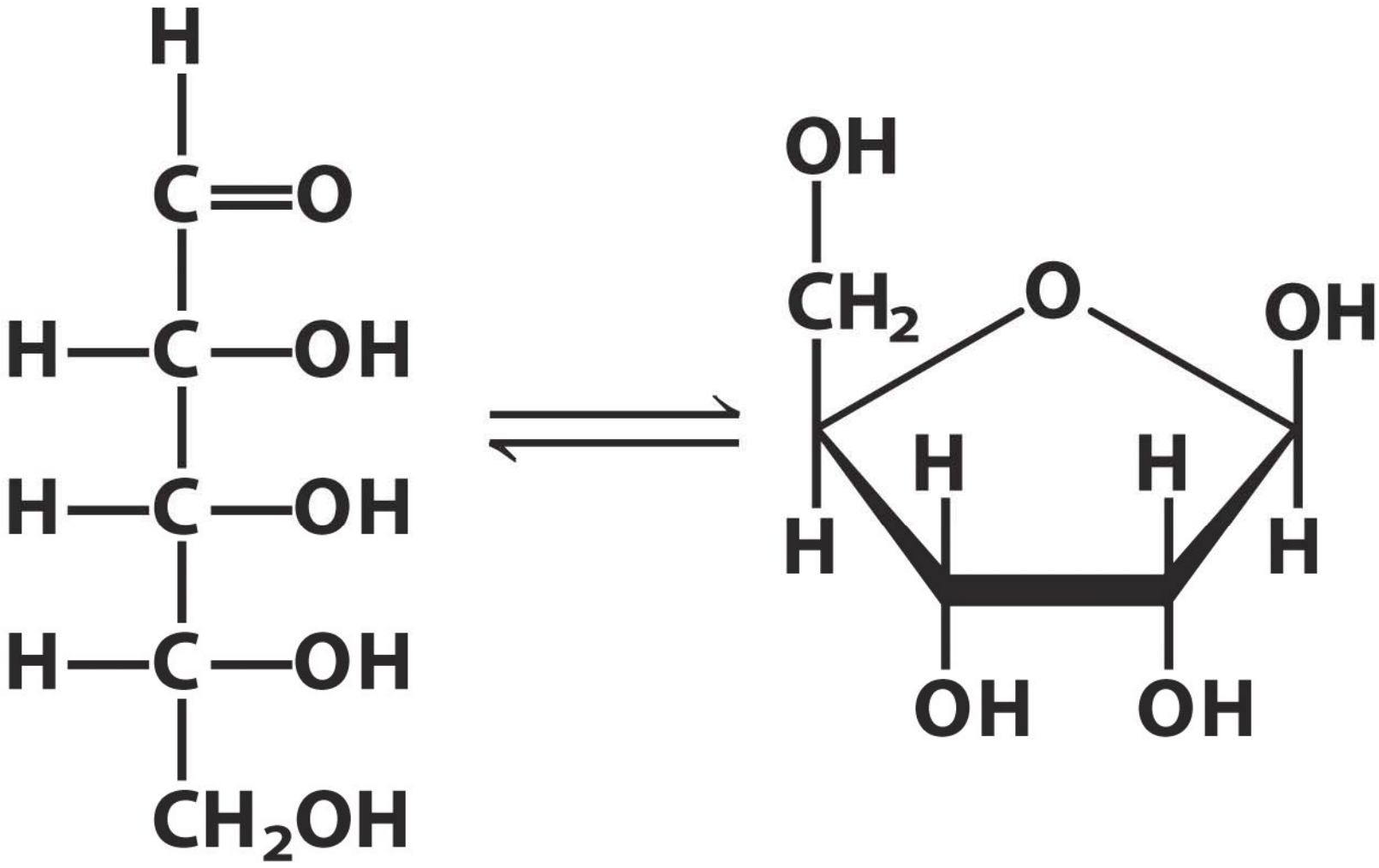


Thymine
(DNA)

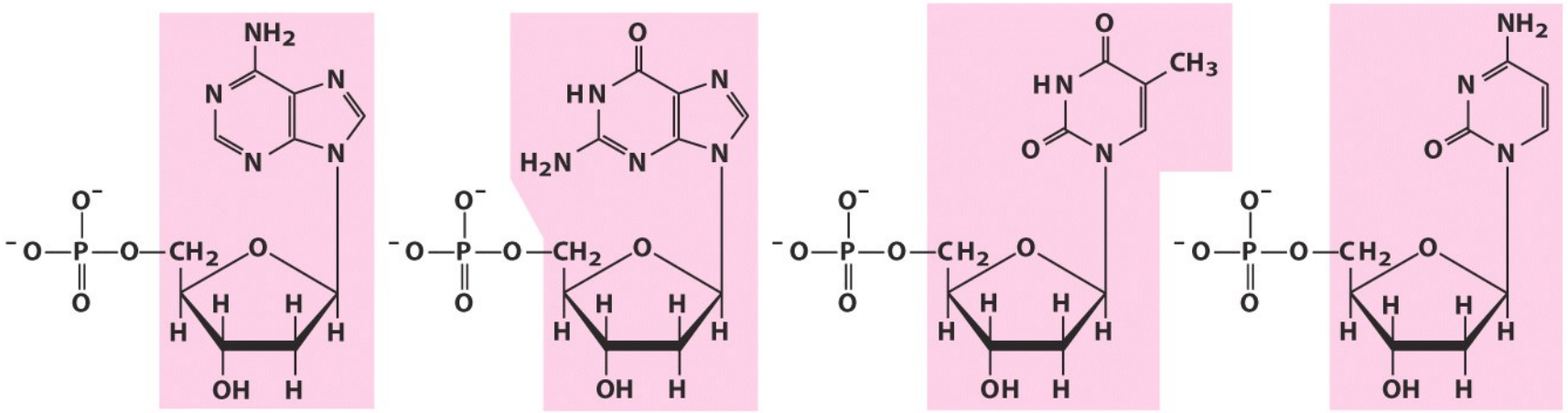


Uracil
(RNA)

Pyrimidines

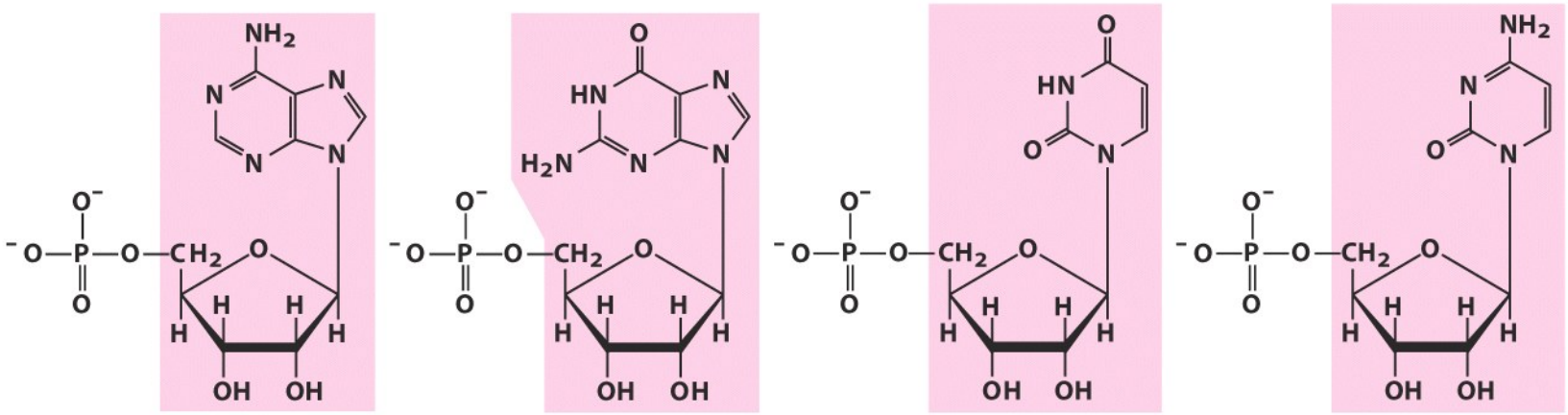


Aldehyde $\rightleftharpoons$ **β-Furanose**



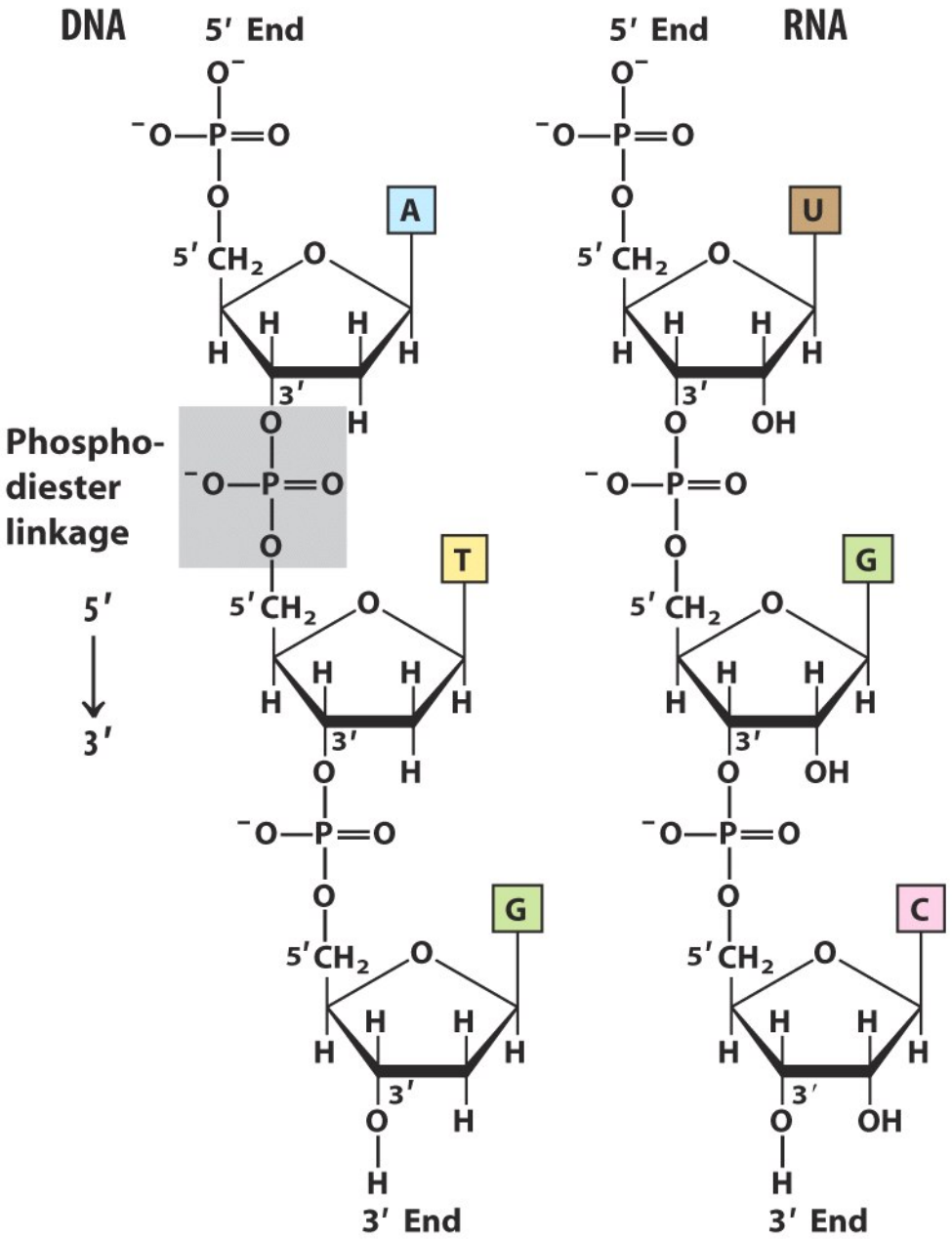
	Deoxyadenylate (deoxyadenosine 5′-monophosphate)	Deoxyguanylate (deoxyguanosine 5′-monophosphate)	Deoxythymidylate (deoxythymidine 5′-monophosphate)	Deoxycytidylate (deoxycytidine 5′-monophosphate)
Nucleotide:				
Symbols:	A, dA, dAMP	G, dG, dGMP	T, dT, dTMP	C, dC, dCMP
Nucleoside:	Deoxyadenosine	Deoxyguanosine	Deoxythymidine	Deoxycytidine

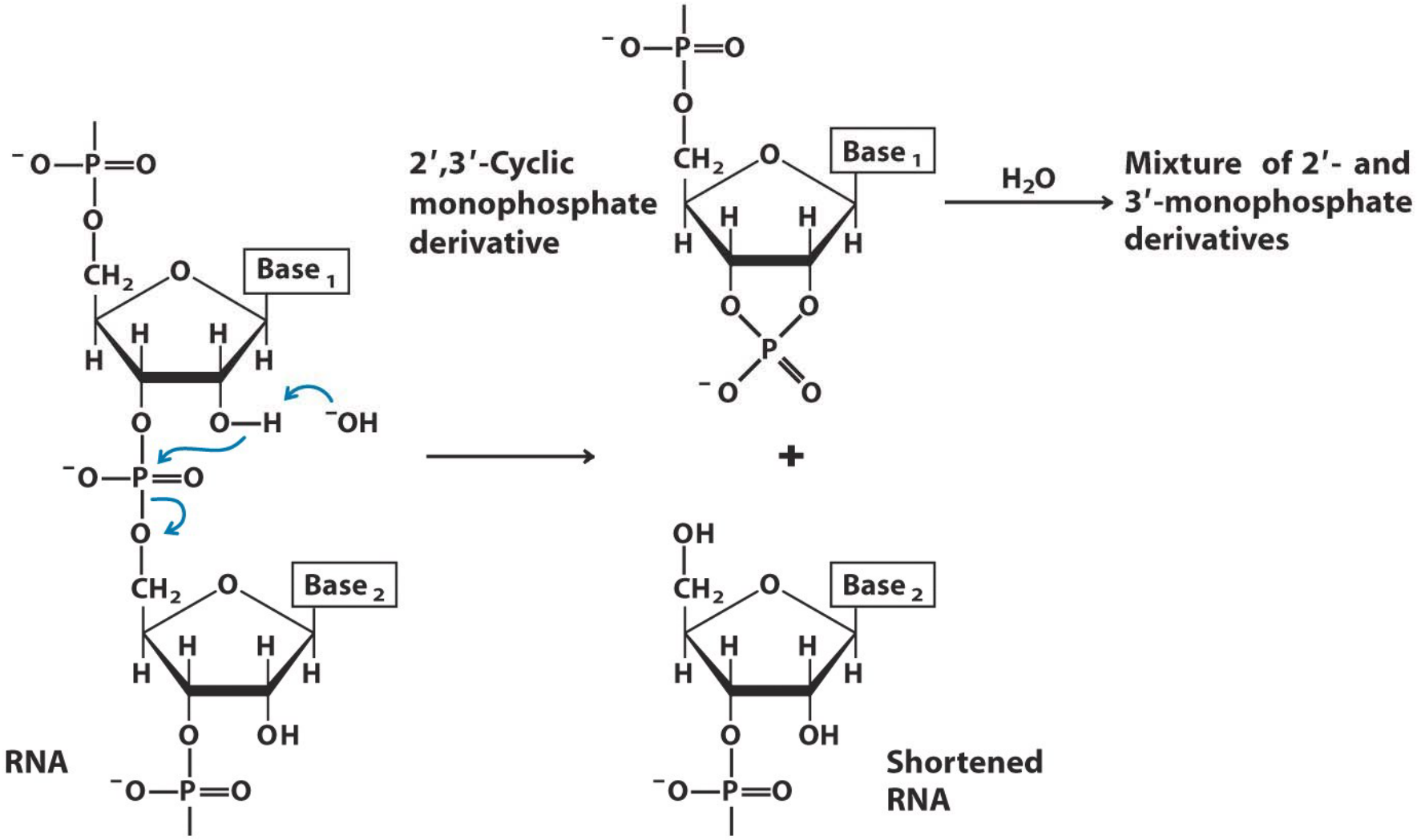
(a) Deoxyribonucleotides

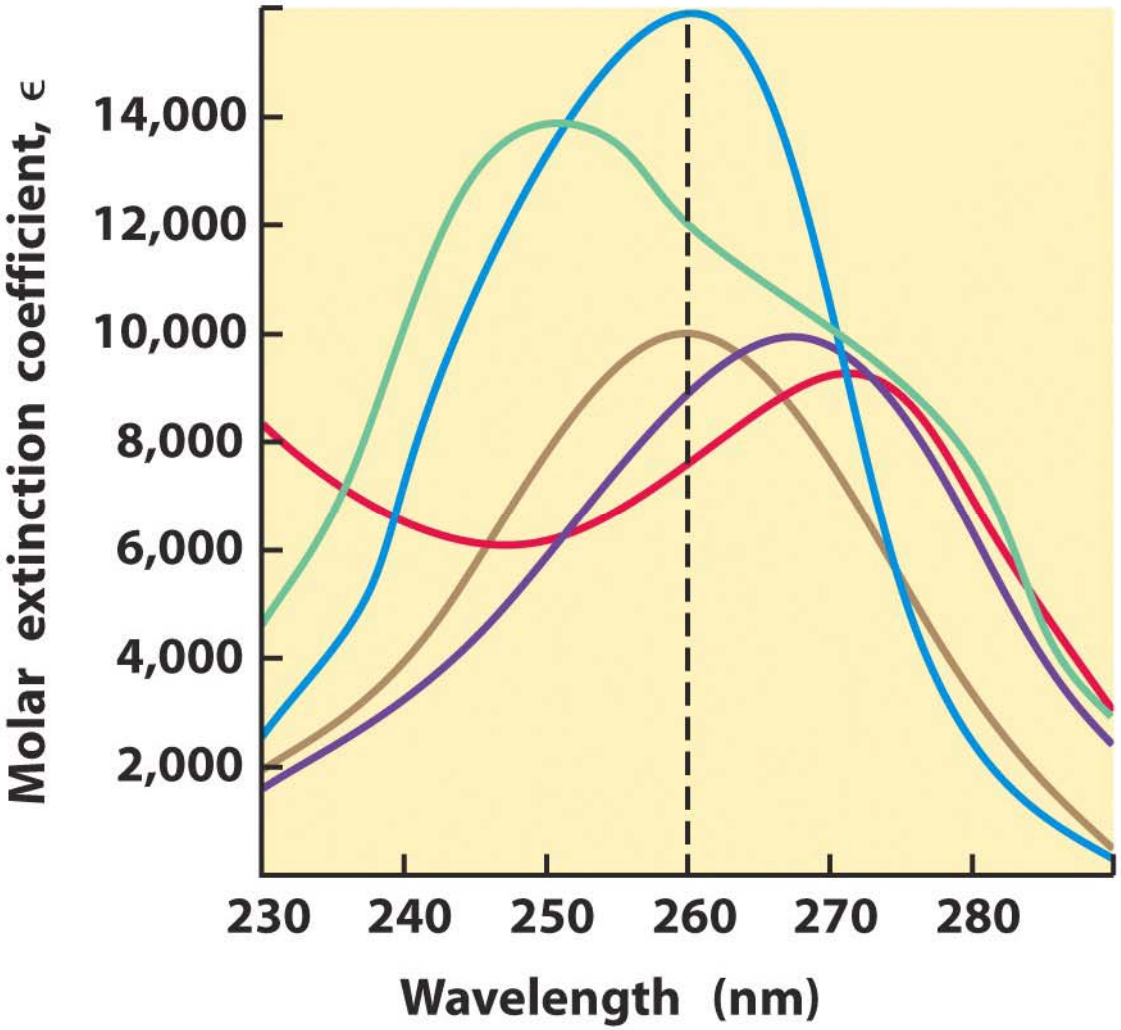


	Adenylate (adenosine 5′-monophosphate)	Guanylate (guanosine 5′-monophosphate)	Uridylate (uridine 5′-monophosphate)	Cytidylate (cytidine 5′-monophosphate)
Nucleotide:				
Symbols:	A, AMP	G, GMP	U, UMP	C, CMP
Nucleoside:	Adenosine	Guanosine	Uridine	Cytidine

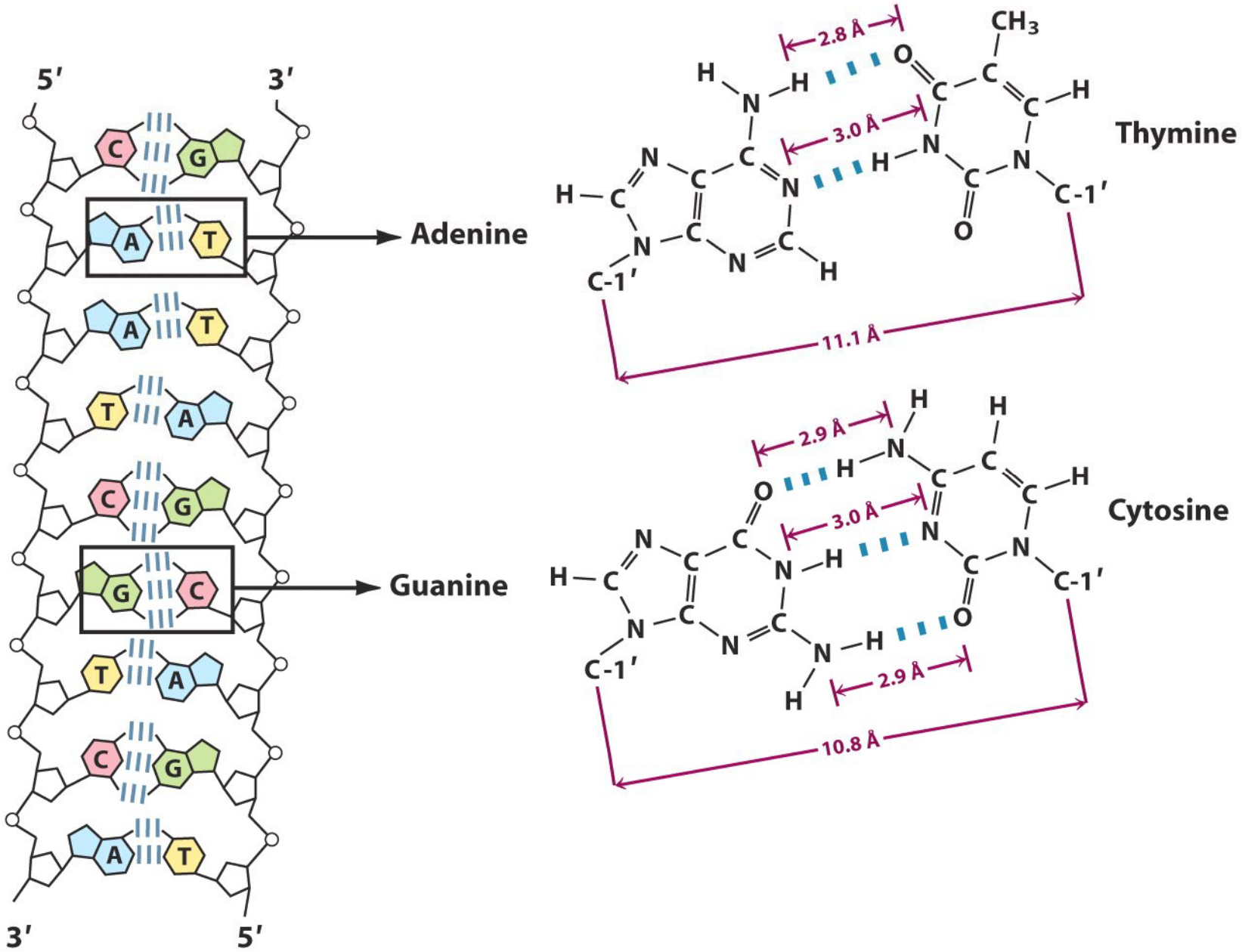
(b) Ribonucleotides

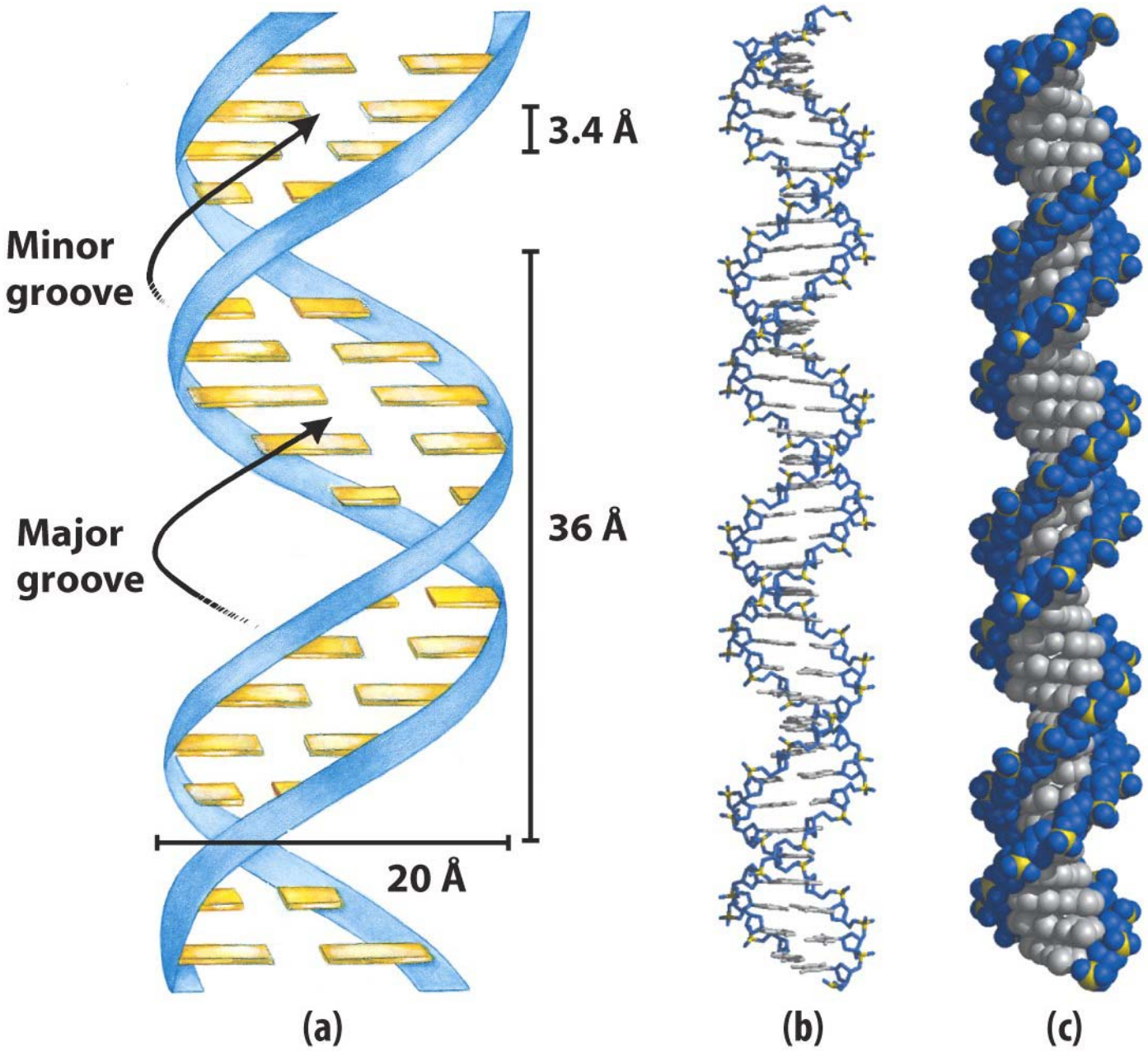

DNA
5′ End
RNA
5′ End
A
U
T
G
G
C
Phospho-
diester
linkage
5′
3′
3′ End
3′ End

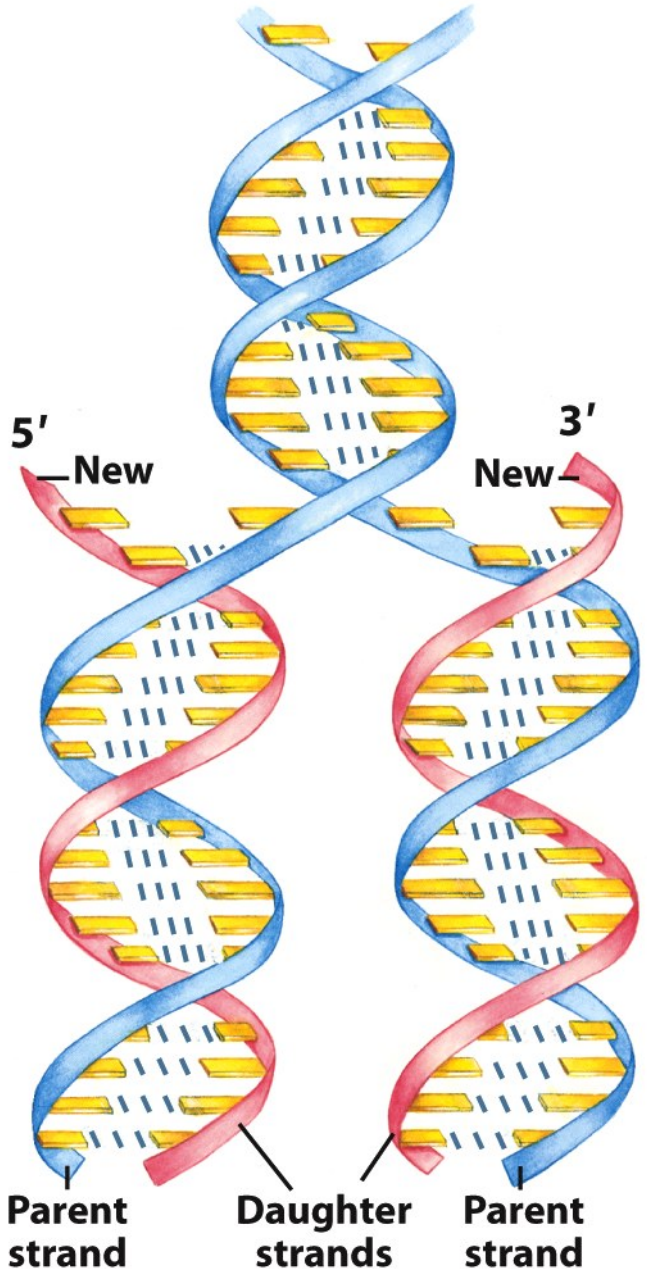

RNA
Base 1
Base 2
2′,3′-Cyclic monophosphate derivative
H2O
Mixture of 2′- and 3′-monophosphate derivatives
Shortened RNA

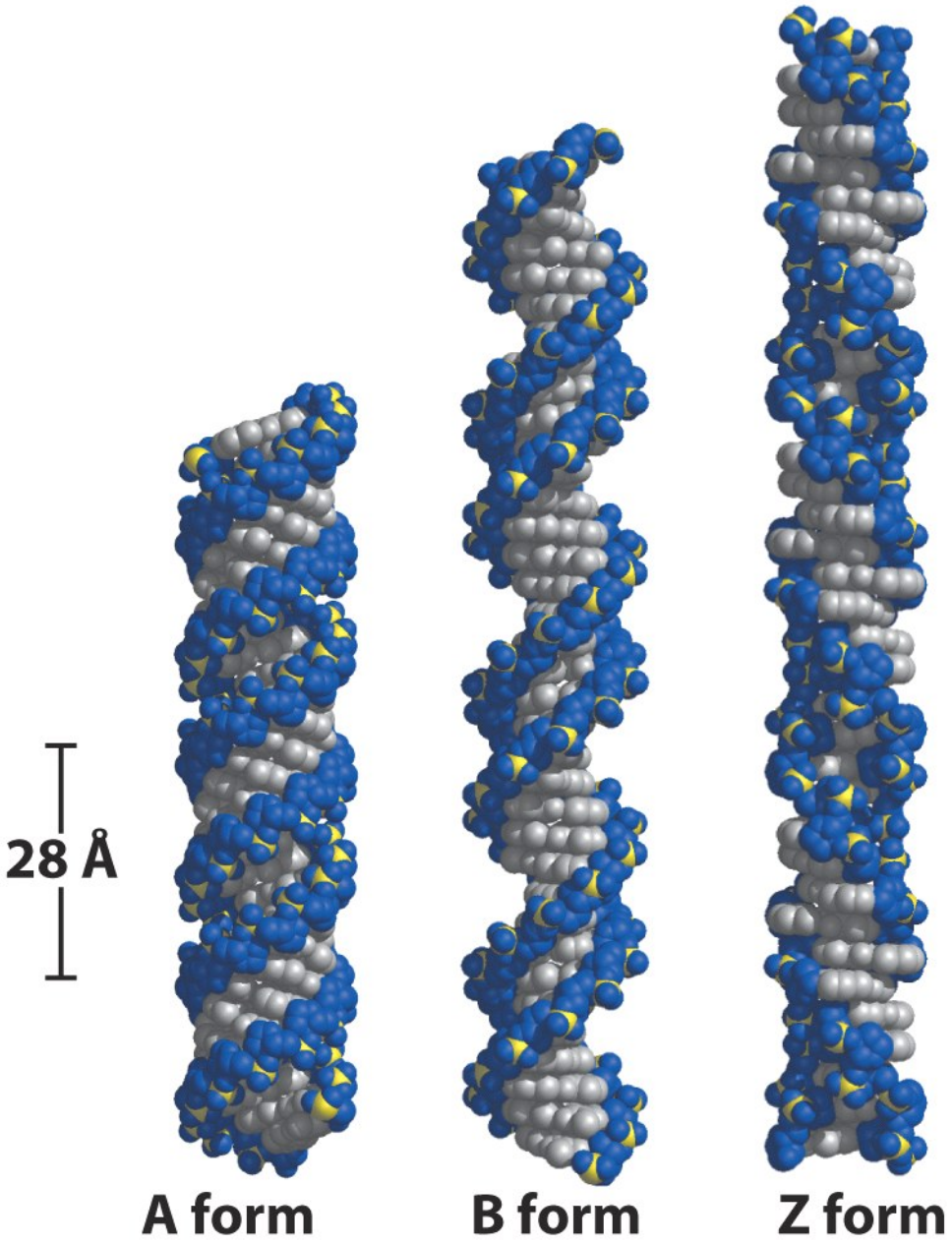


Molar extinction coefficient at 260 nm, ϵ_{260} ($M^{-1}cm^{-1}$)	
AMP	15,400
GMP	11,700
UMP	9,900
dTMP	9,200
CMP	7,500


5′
3′
C
G
A
T
Adenine
T
A
T
A
C
G
G
C
Guanine
T
A
C
G
A
T
3′
5′
2.8 Å
3.0 Å
CH3
Thymine
C-1′
11.1 Å
2.9 Å
3.0 Å
Cytosine
2.9 Å
C-1′
10.8 Å

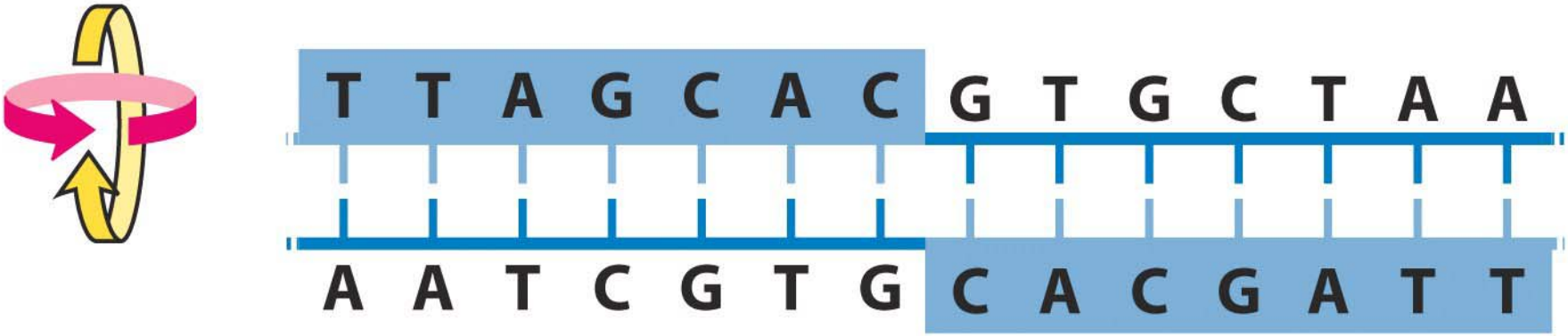

3.4 Å
Minor groove
Major groove
36 Å
20 Å
(a)
(b)
(c)


5′
New
3′
New
Parent strand
Daughter strands
Parent strand

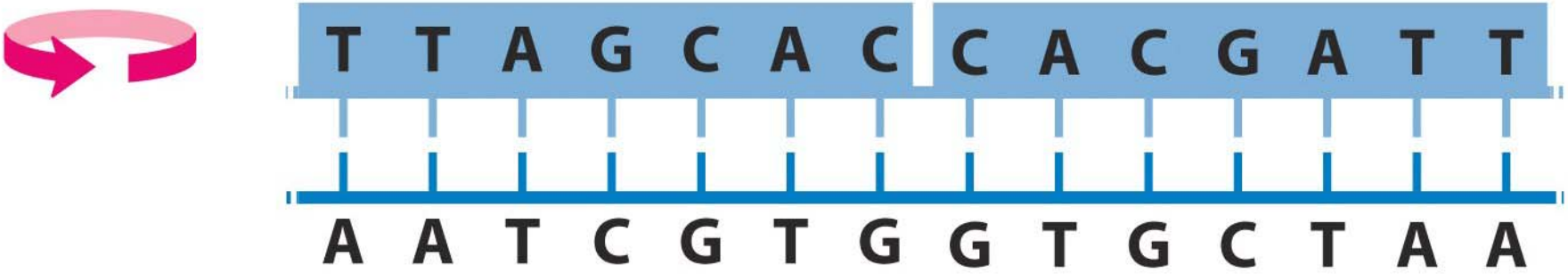

28 Å
A form
B form
Z form

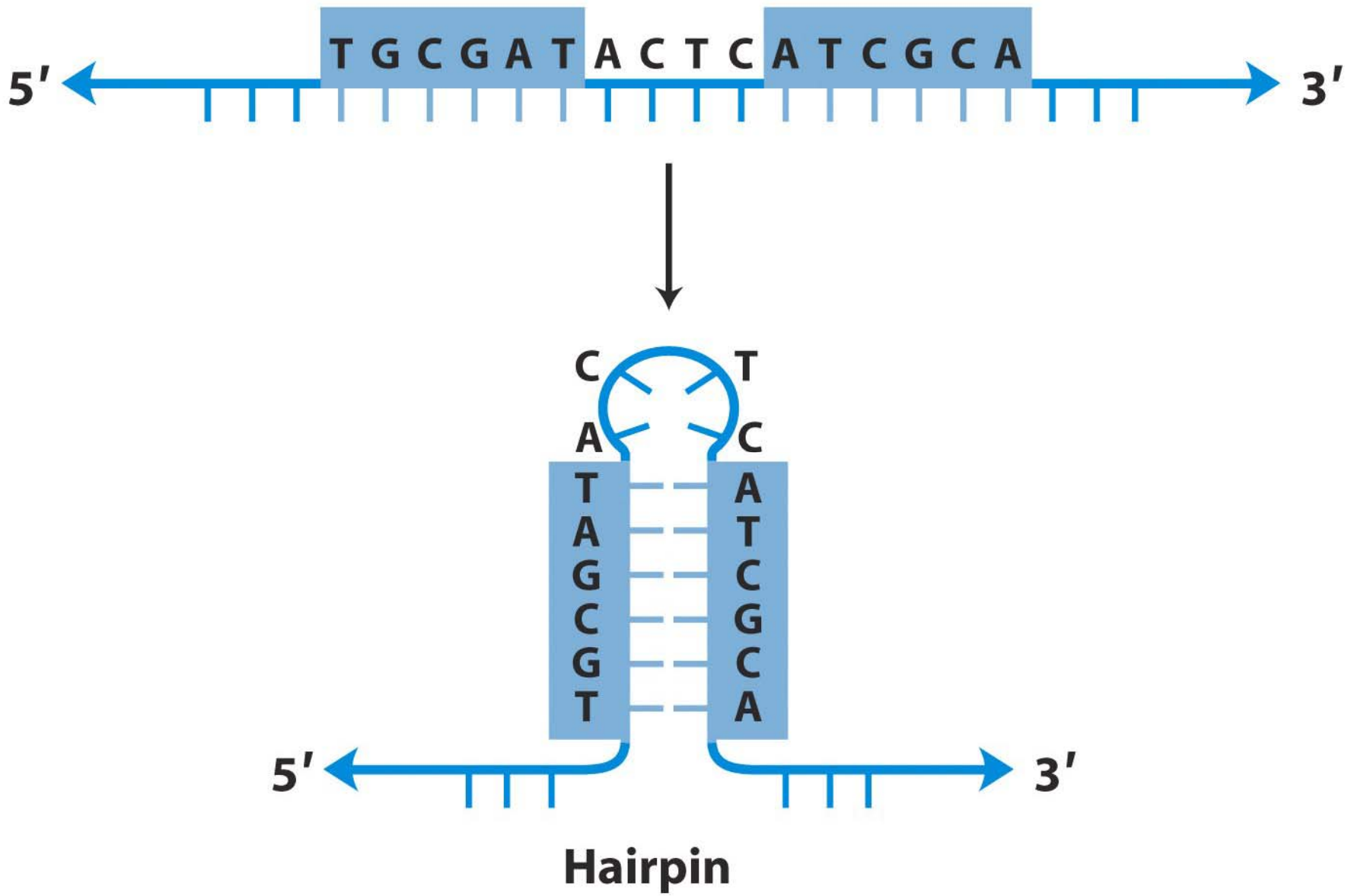
	A form	*B form*	*Z form*
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines

Palindrome

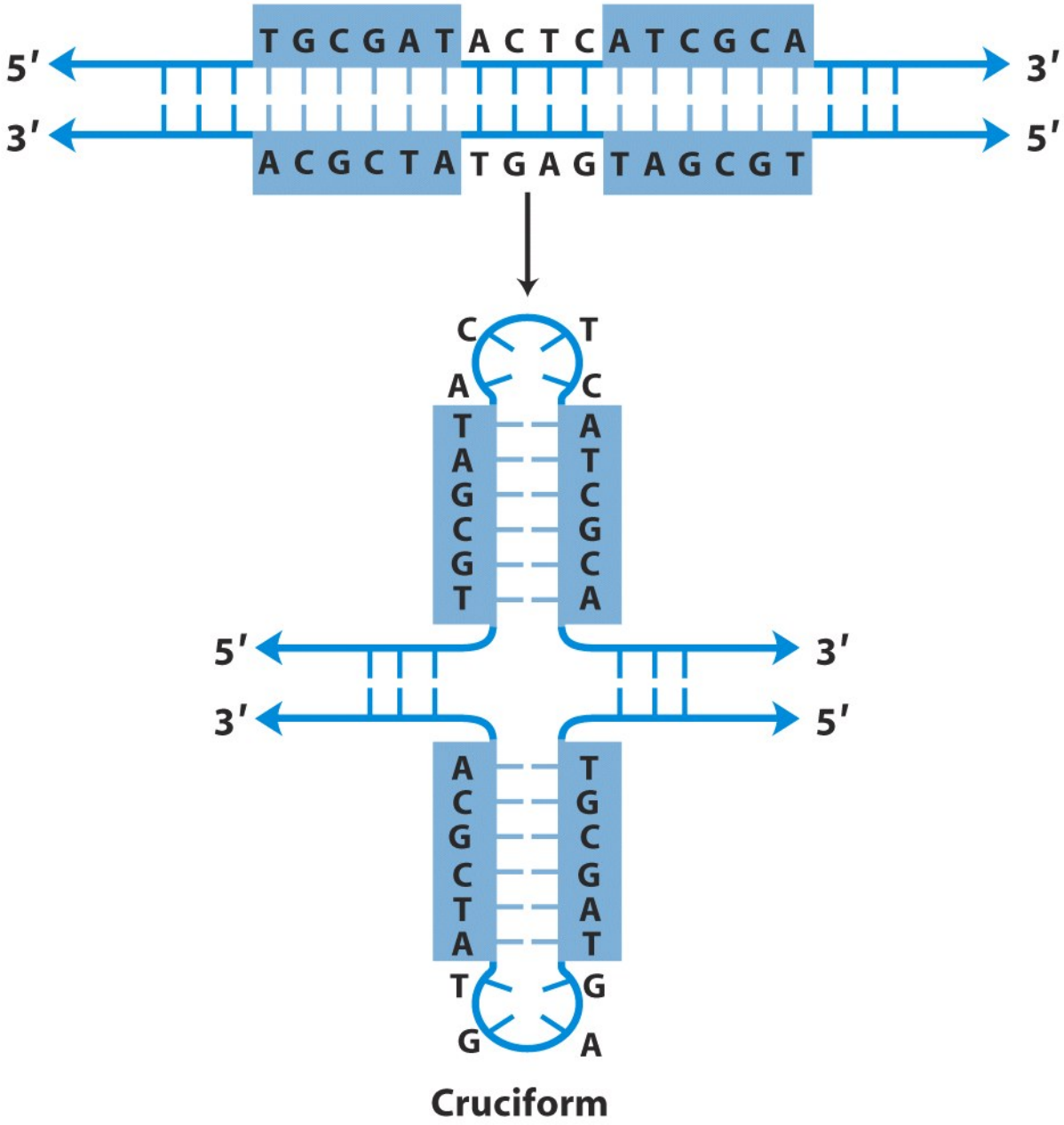


Mirror repeat

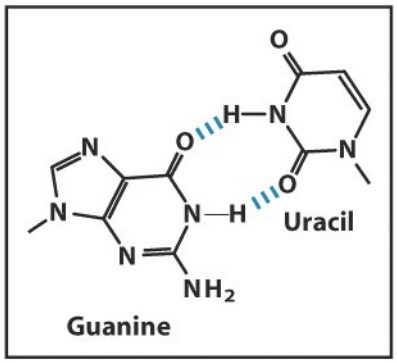


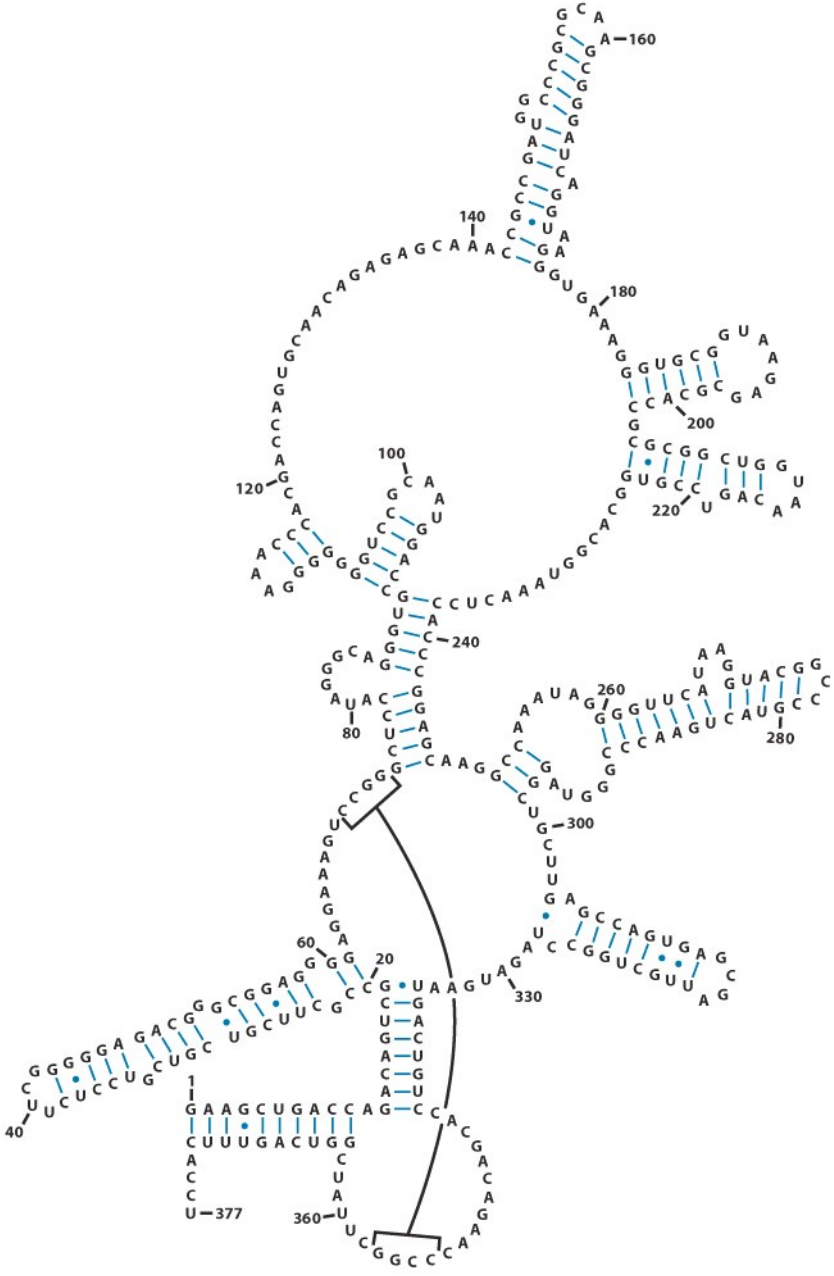


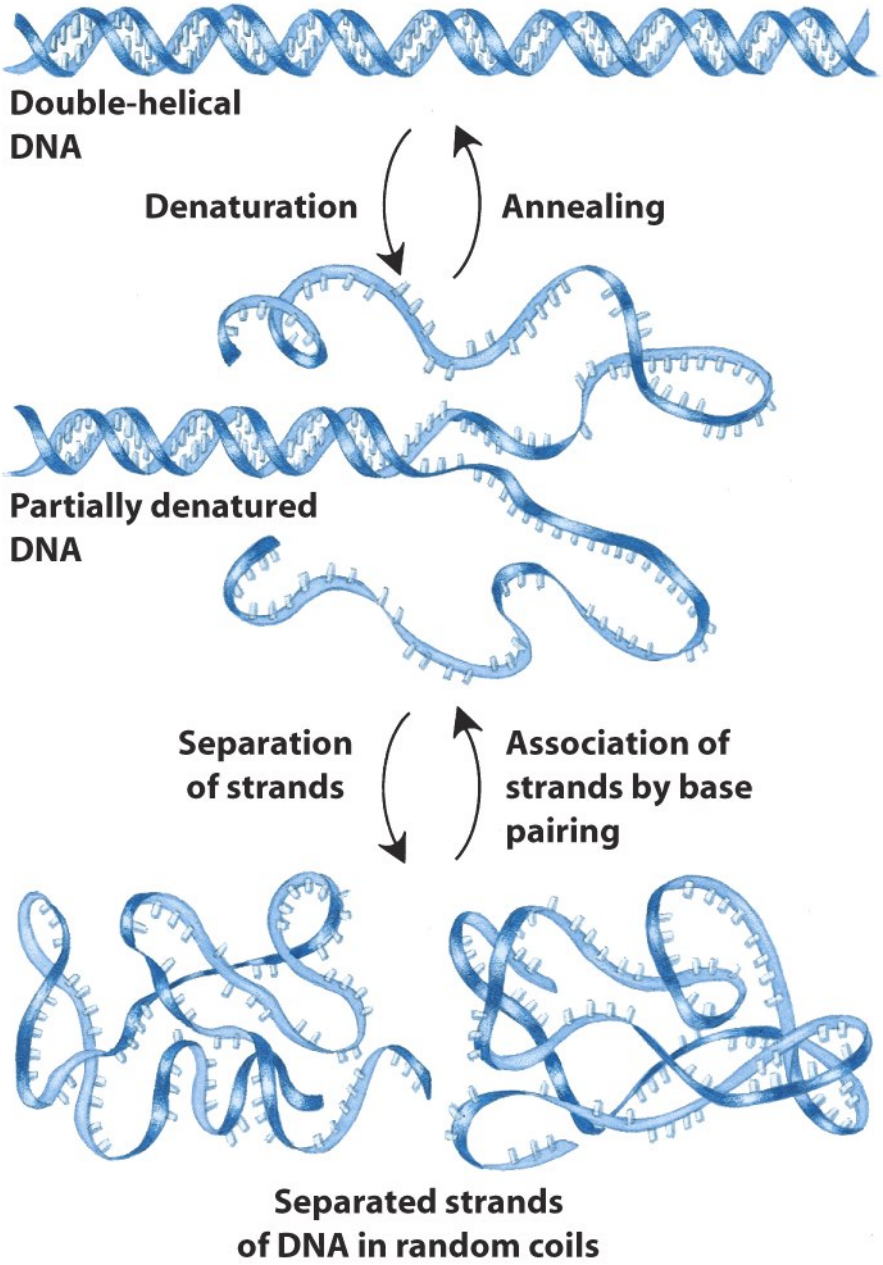
Hairpin

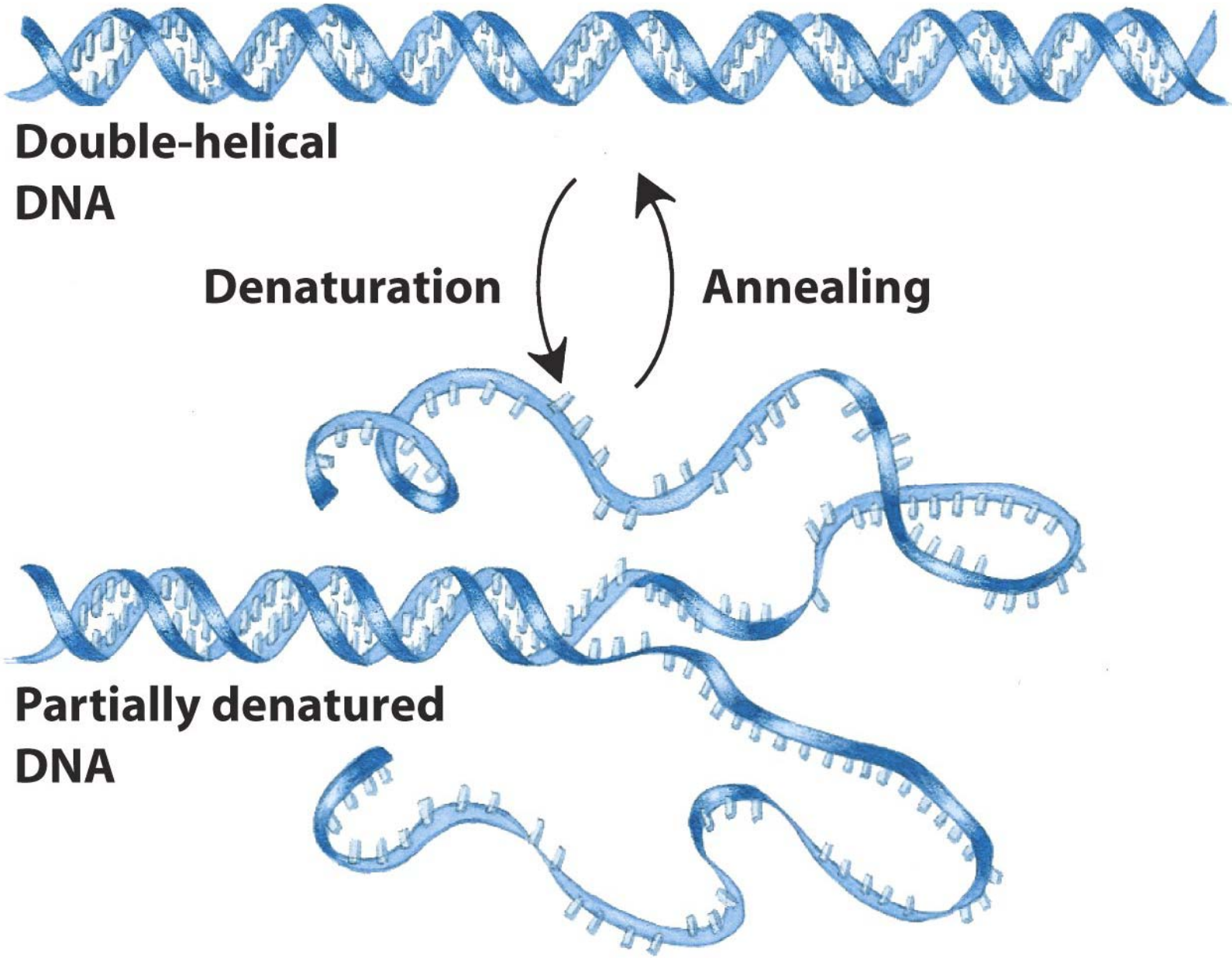

5′
3′
T G C G A T A C T C A T C G C A
A C G C T A T G A G T A G C G T
3′
5′
C
T
A
C
T A G C G T
A T C G C A
5′
3′
3′
5′
A C G C T A
T G C G A T
T
G
G
A

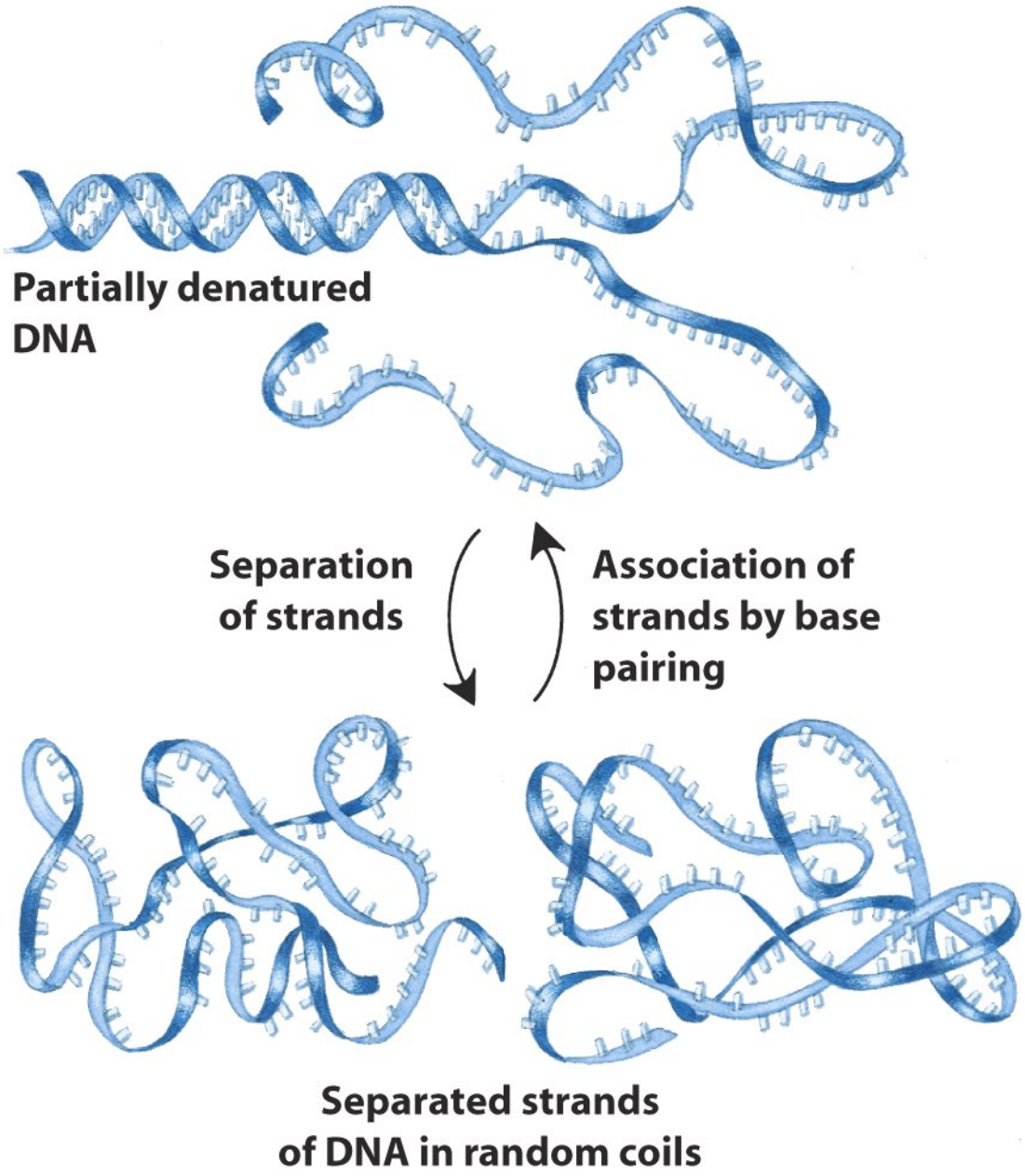
Cruciform

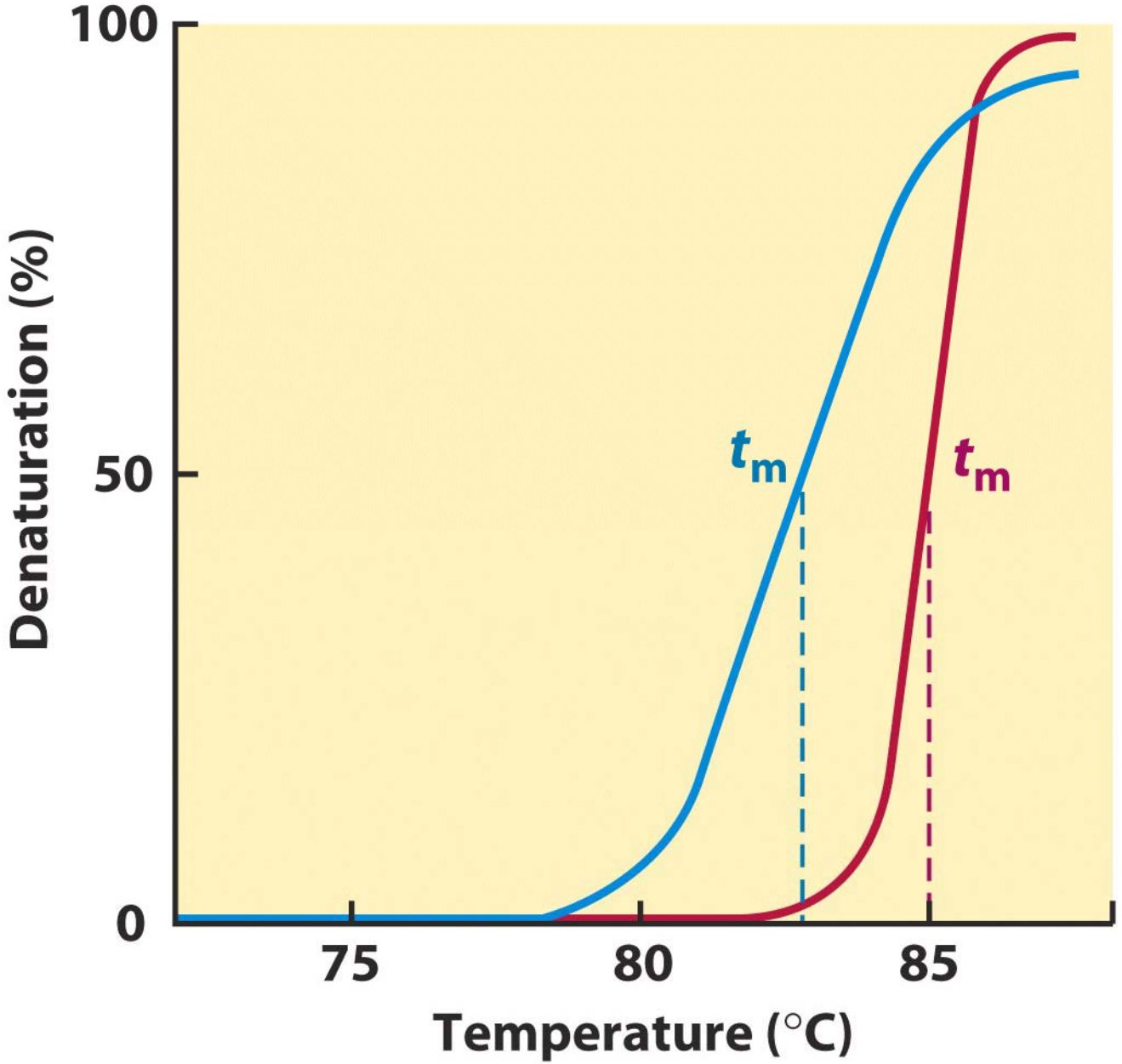

Guanine
Uracil

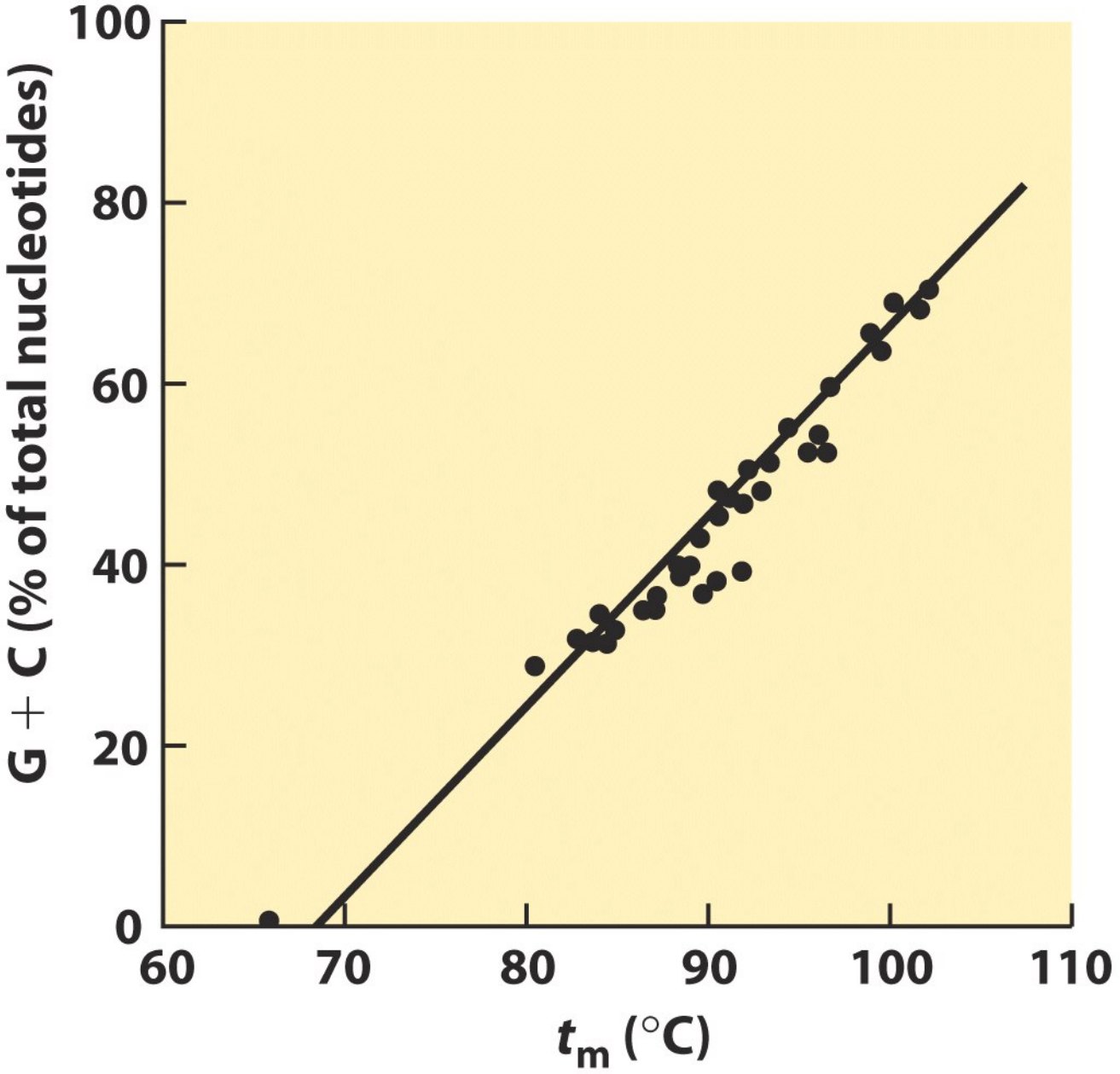


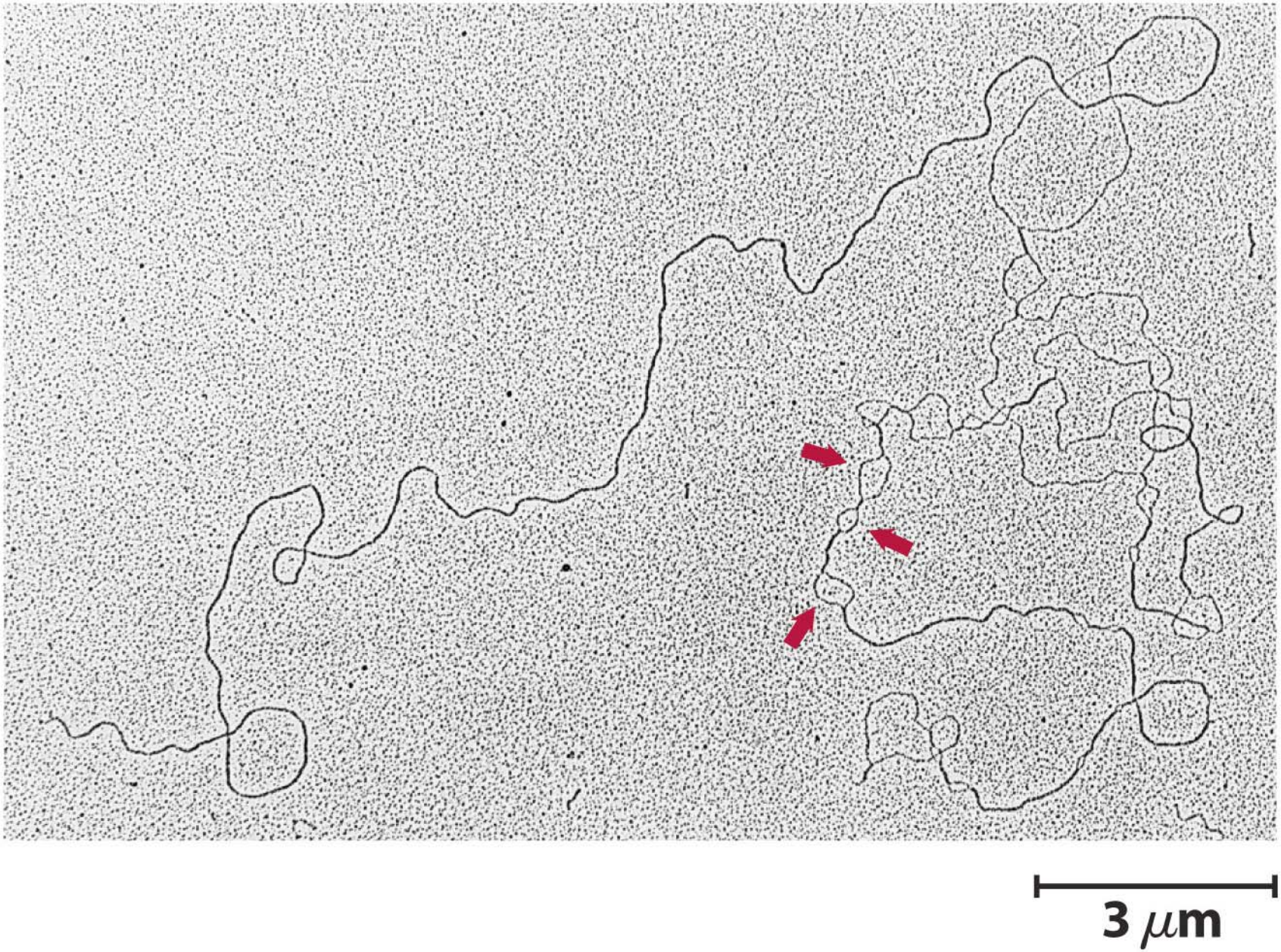

Double-helical
DNA
Denaturation
Annealing
Partially denatured
DNA
Separation
of strands
Association of
strands by base
pairing
Separated strands
of DNA in random coils

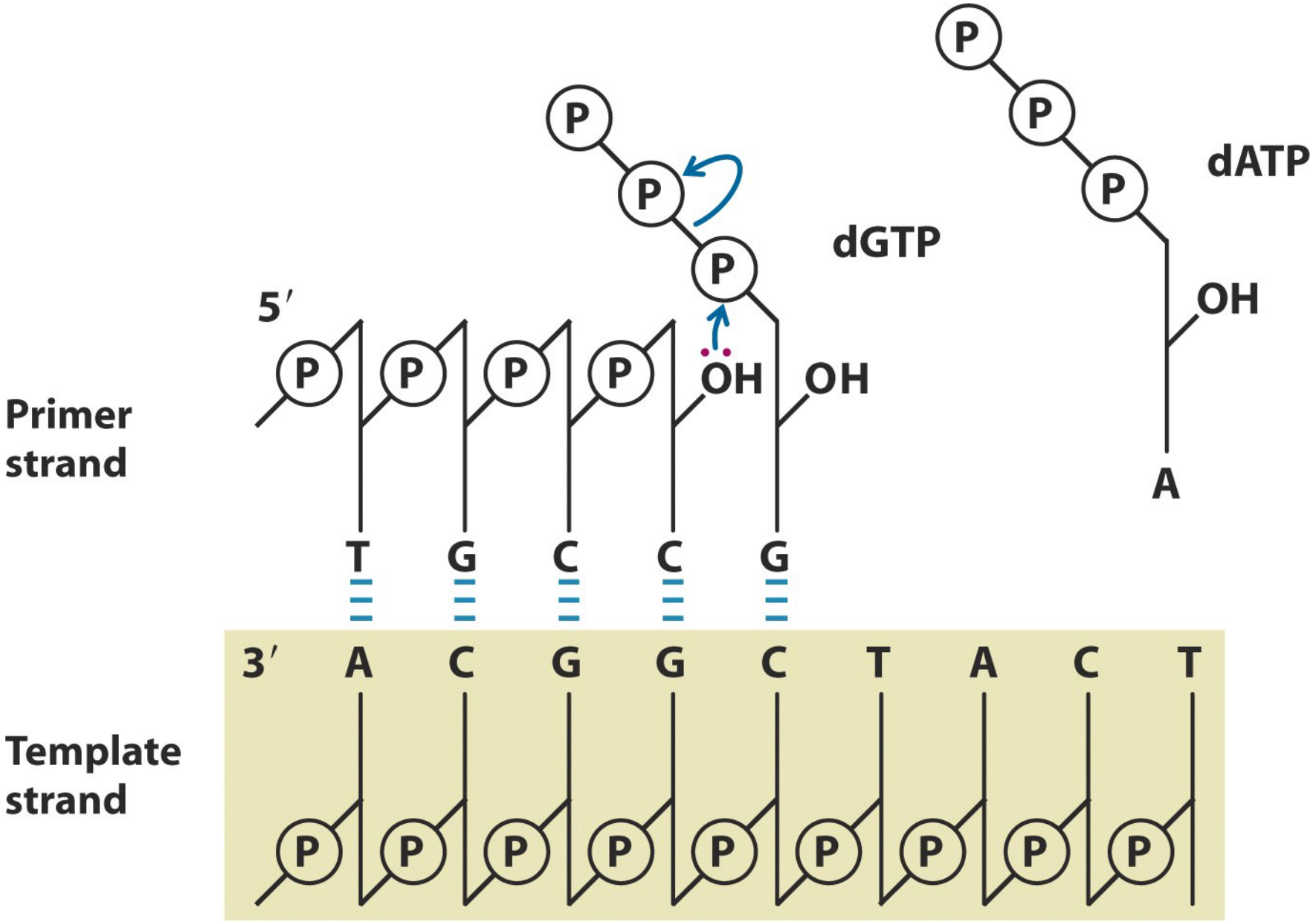

Double-helical
DNA
Denaturation
Annealing
Partially denatured
DNA

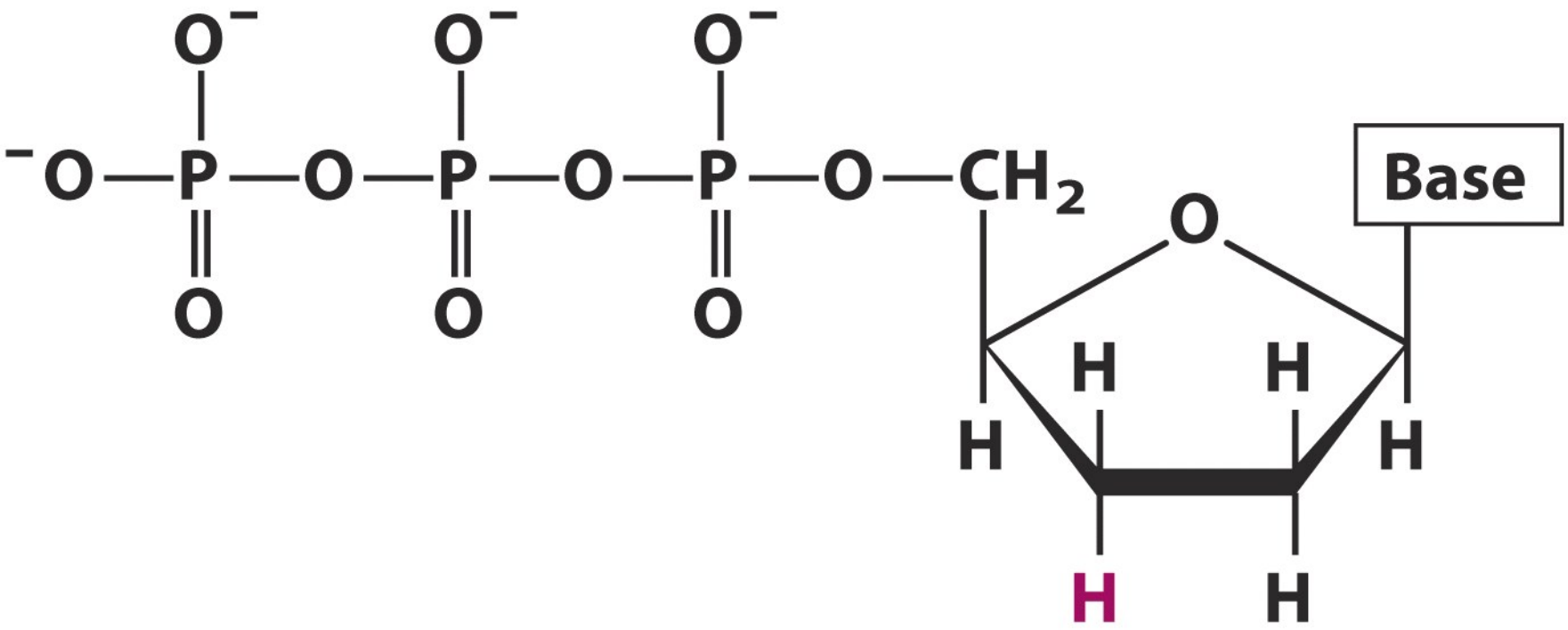

Partially denatured
DNA
Separation
of strands
Association of
strands by base
pairing
Separated strands
of DNA in random coils


100
50
0
Denaturation (%)
t_m
t_m
75
80
85
Temperature (°C)

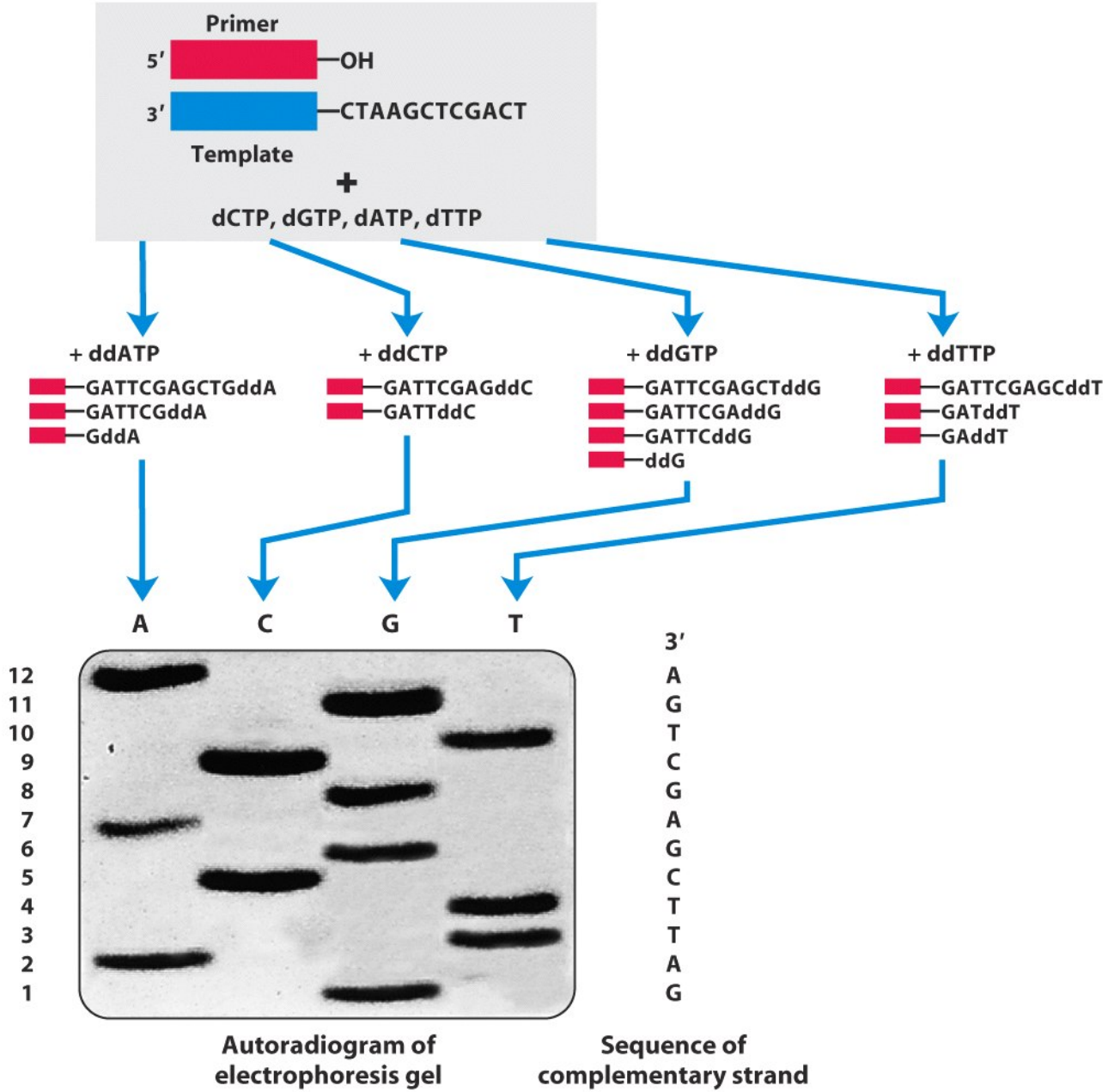

100
80
60
40
20
0
G + C (% of total nucleotides)
60
70
80
90
100
110
t_m (°C)

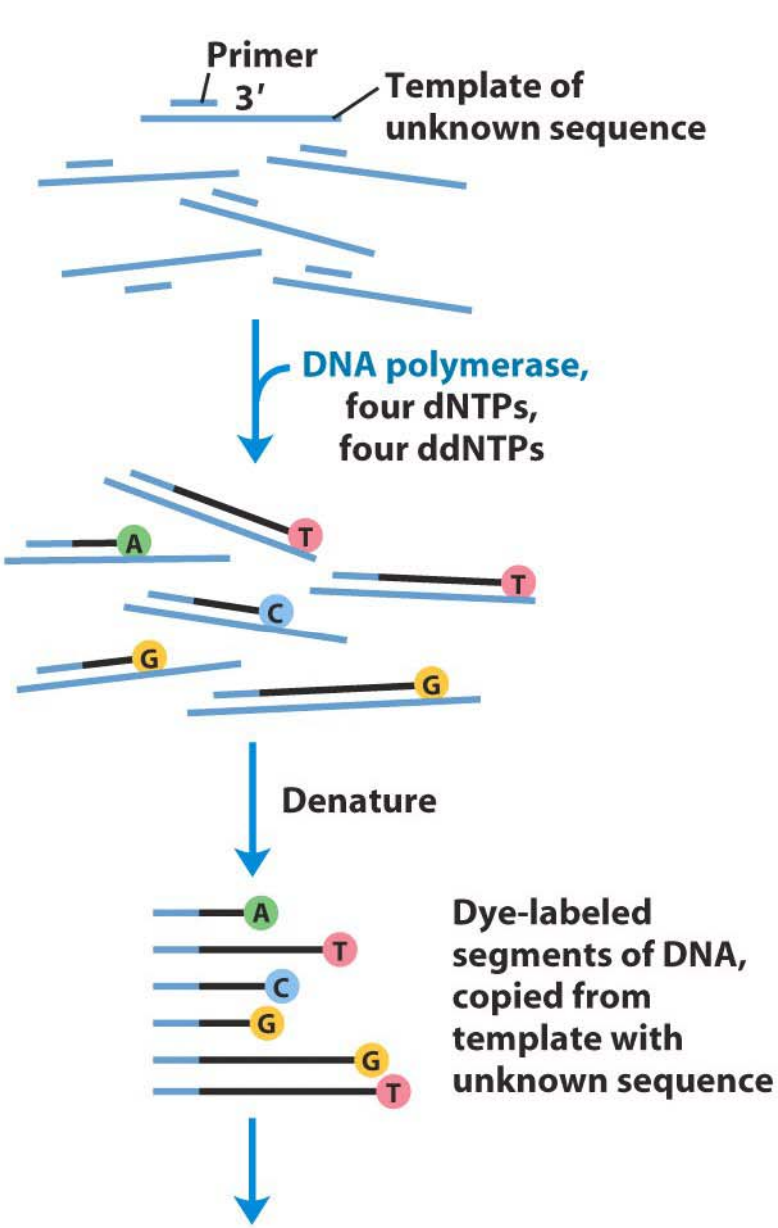

3 μm

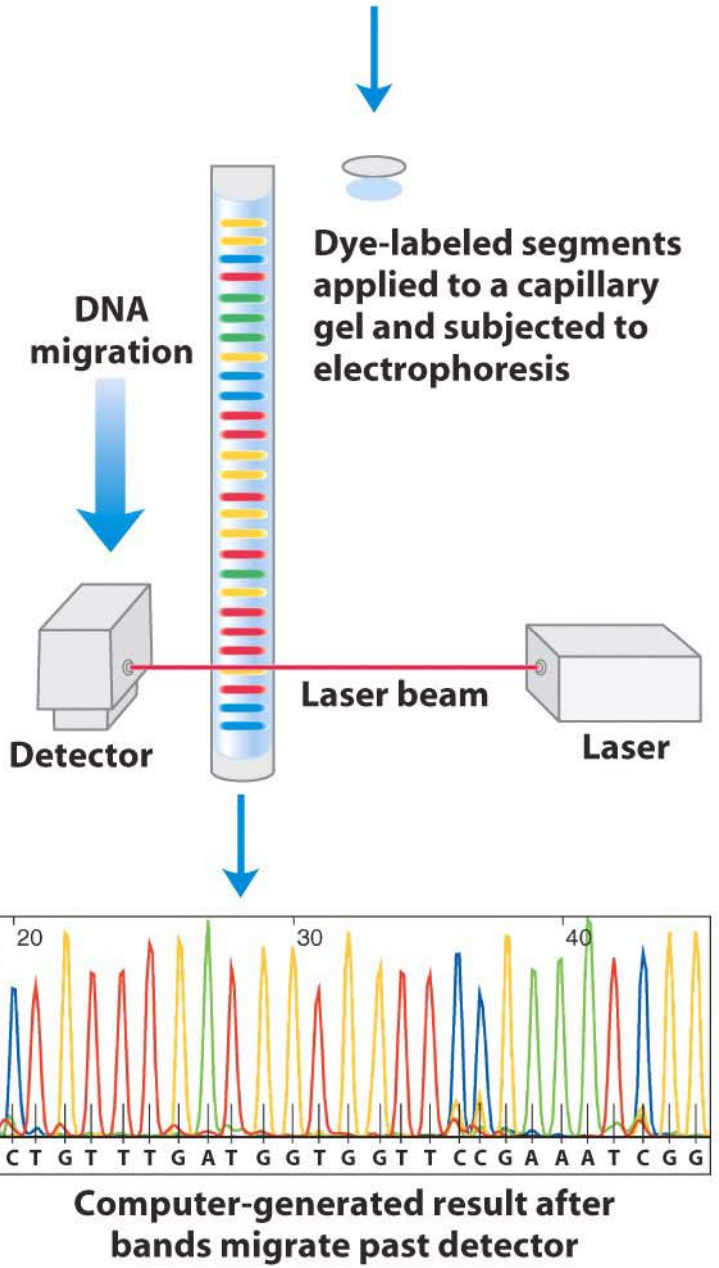

P
P
P
dATP
OH
A
P
P
P
dGTP
5′
P
P
P
P
OH
OH
Primer strand
T
G
C
C
G
3′
A
C
G
G
C
T
A
C
T
Template strand
P
P
P
P
P
P
P
P
P

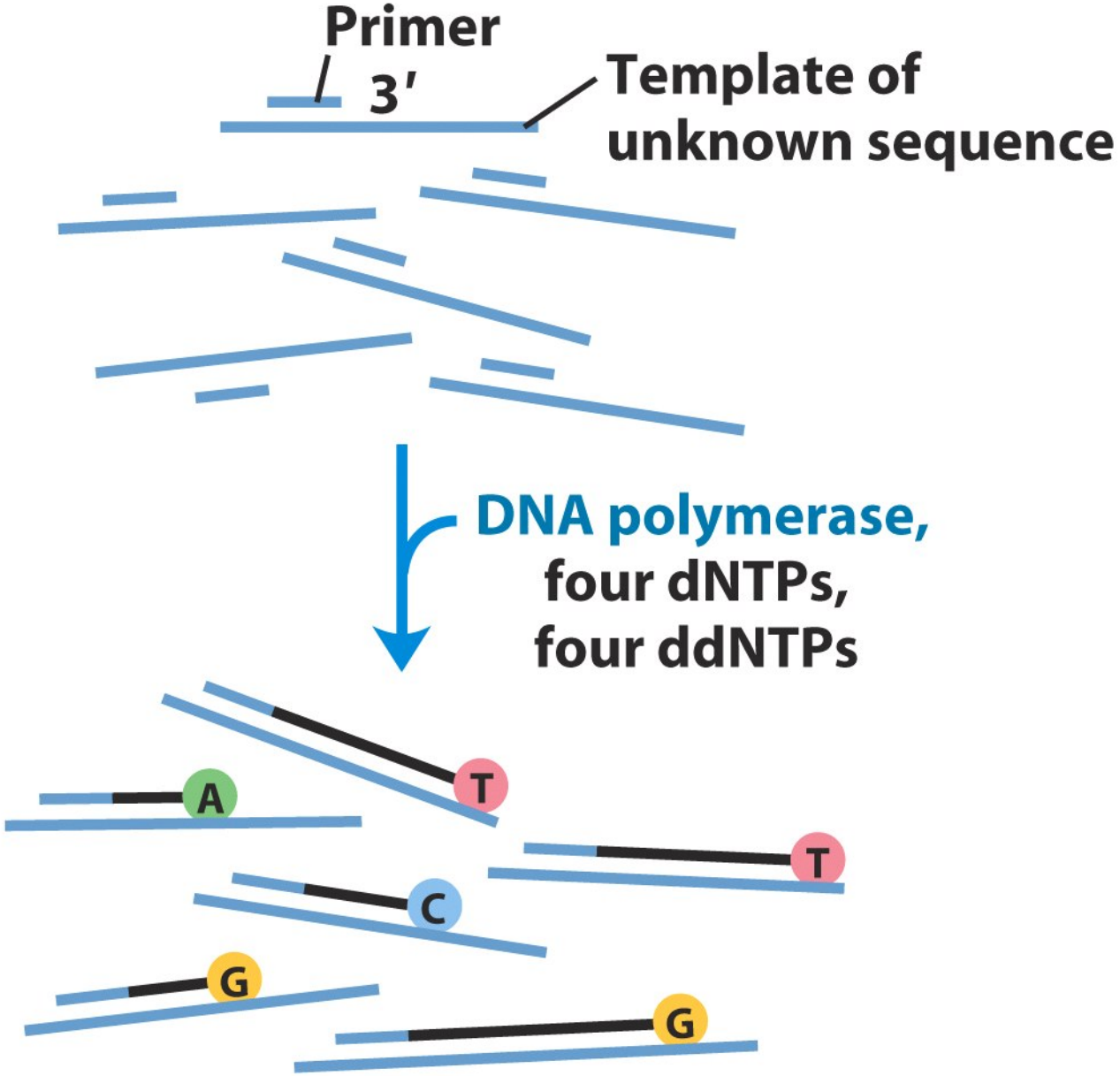


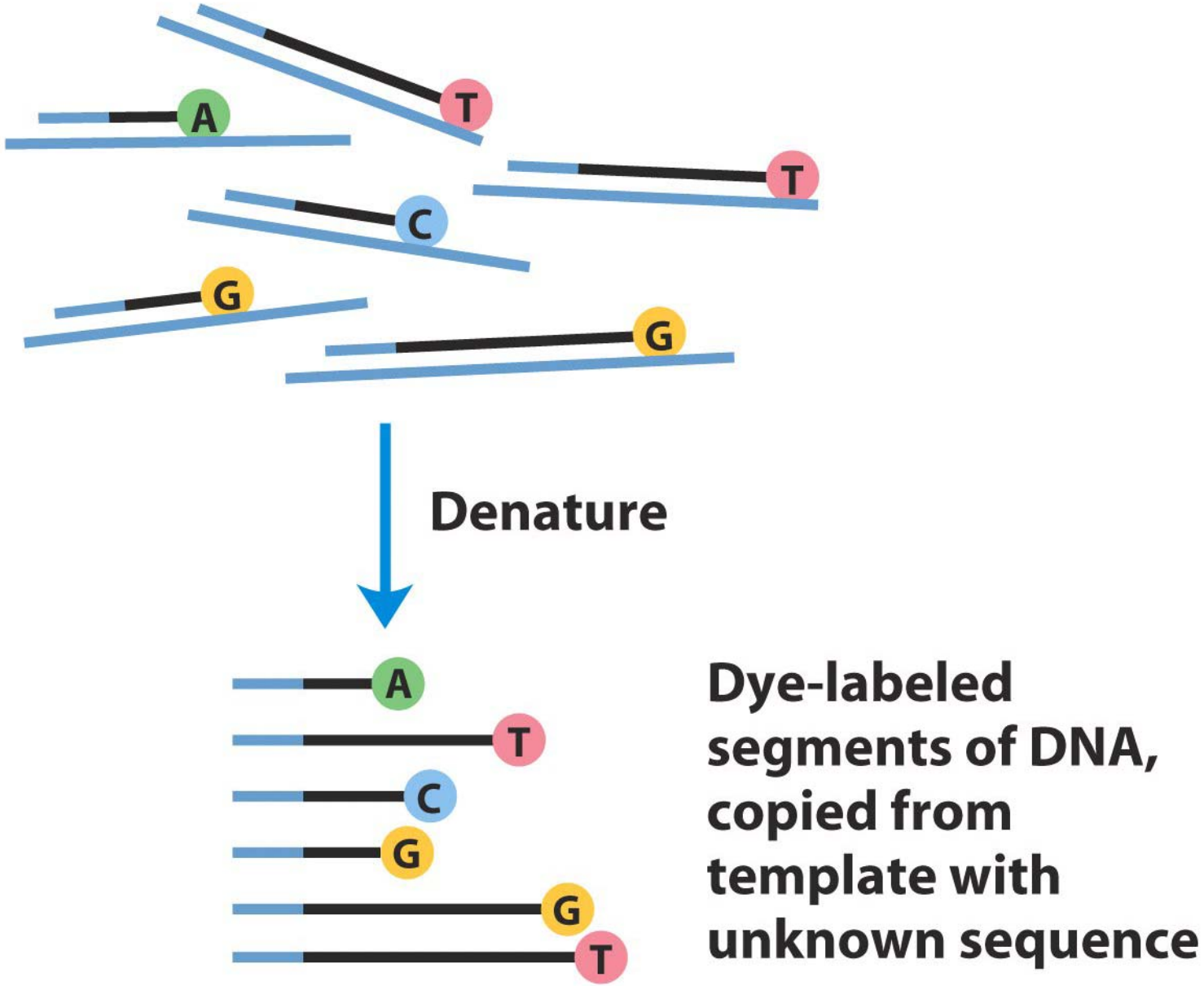
ddNTP analog

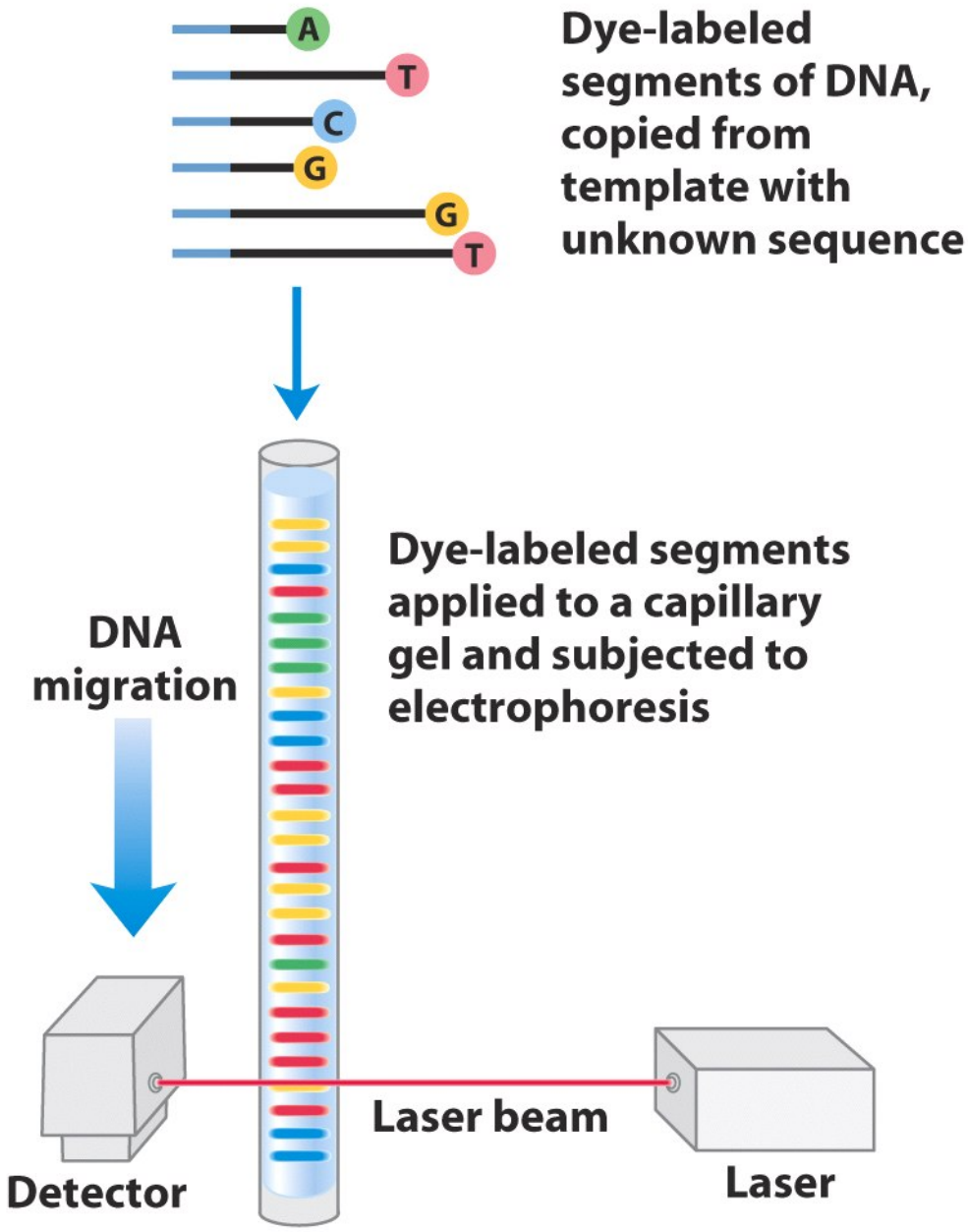

Primer
5′
OH
3′
CTAAGCTCGACT
Template
+
dCTP, dGTP, dATP, dTTP
+ ddATP
GATTCGAGCTGddA
GATTCGddA
GddA
+ ddCTP
GATTCGAGddC
GATTddC
+ ddGTP
GATTCGAGCTddG
GATTCGAddG
GATTCddG
ddG
+ ddTTP
GATTCGAGCddT
GATddT
GAddT
A
C
G
T
12
11
10
9
8
7
6
5
4
3
2
1
3′
A
G
T
C
G
A
G
C
T
T
A
G
Autoradiogram of electrophoresis gel
Sequence of complementary strand

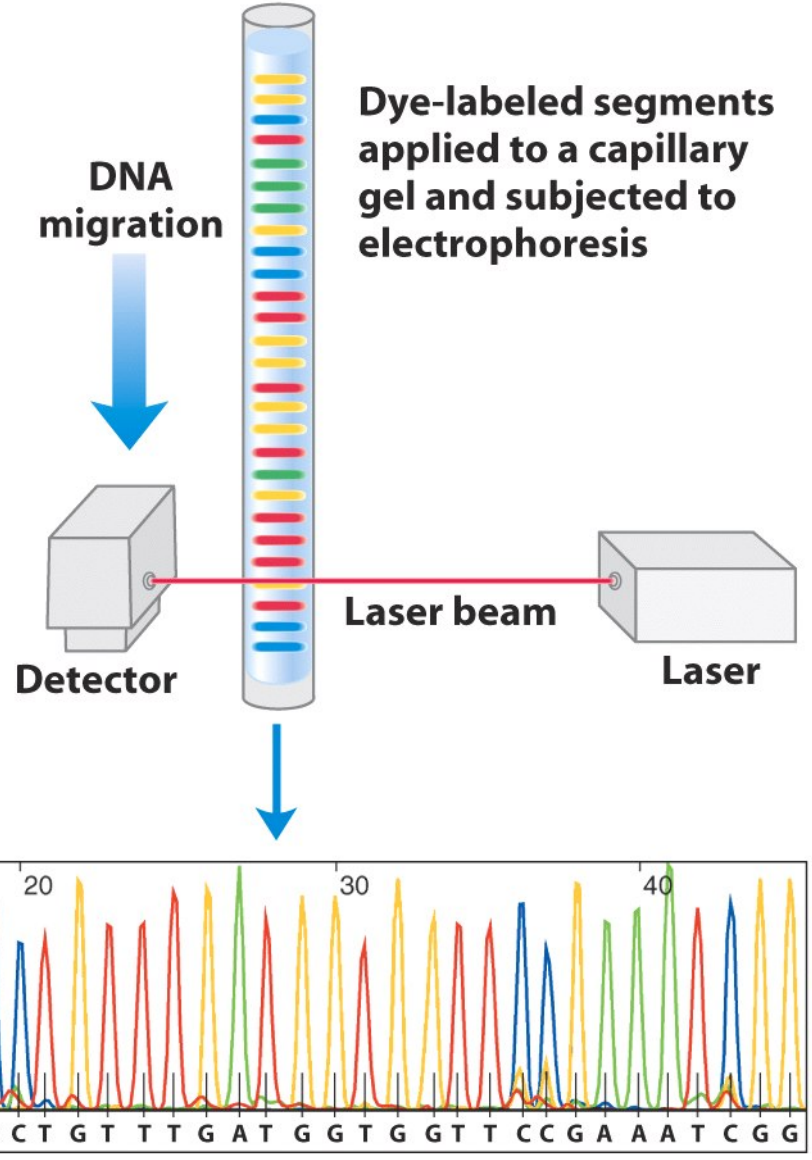

Primer
3′
Template of unknown sequence
DNA polymerase, four dNTPs, four ddNTPs
A
T
T
C
G
G
Denature
A
T
C
G
G
T
Dye-labeled segments of DNA, copied from template with unknown sequence


Dye-labeled segments applied to a capillary gel and subjected to electrophoresis
DNA migration
Detector
Laser beam
Laser
20
30
40
C C T G T T T G A T G G T G G T T C C G A A A T C G G
Computer-generated result after bands migrate past detector

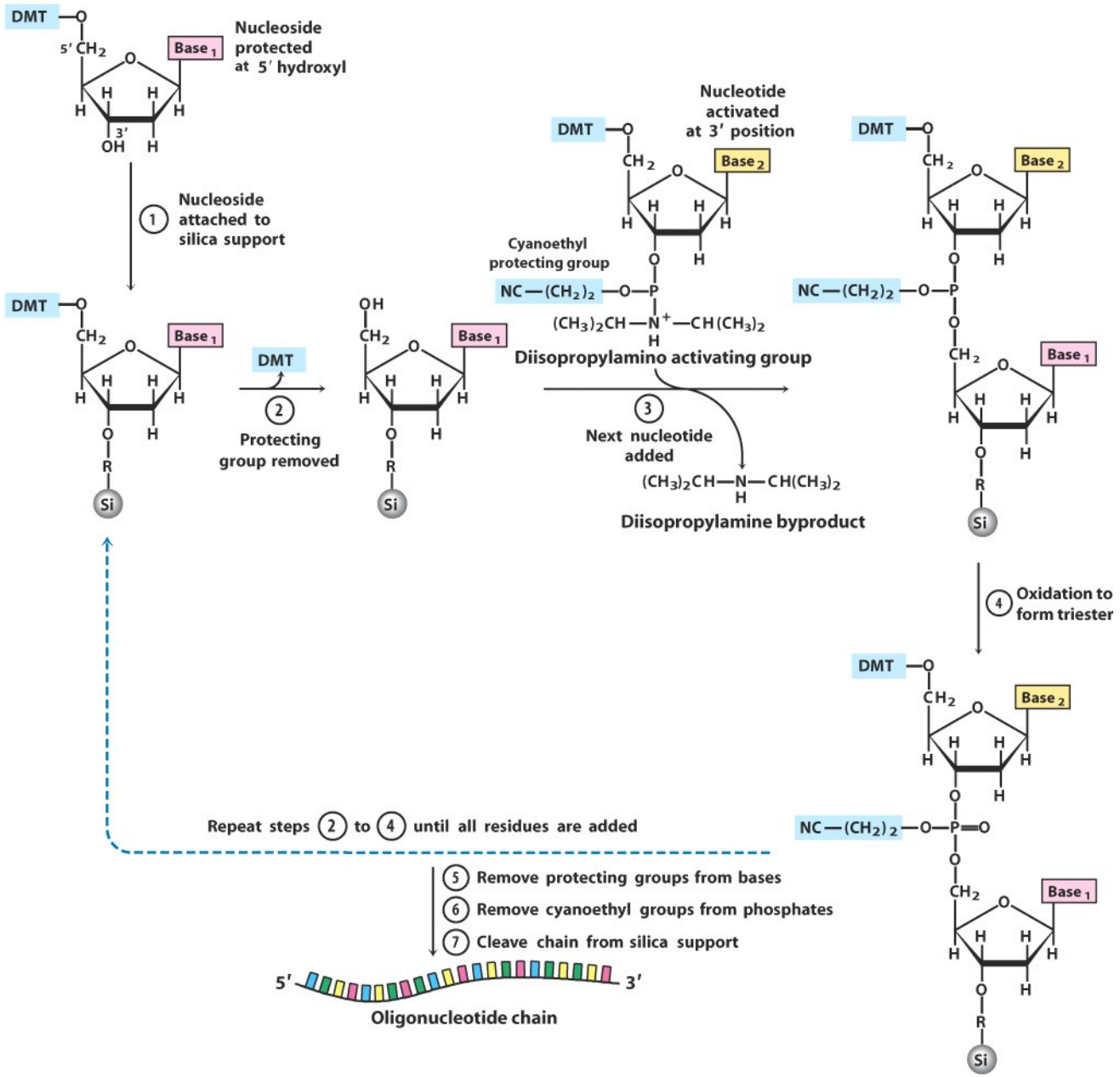

Primer
3′
Template of
unknown sequence
DNA polymerase,
four dNTPs,
four ddNTPs
A
T
T
C
G
G

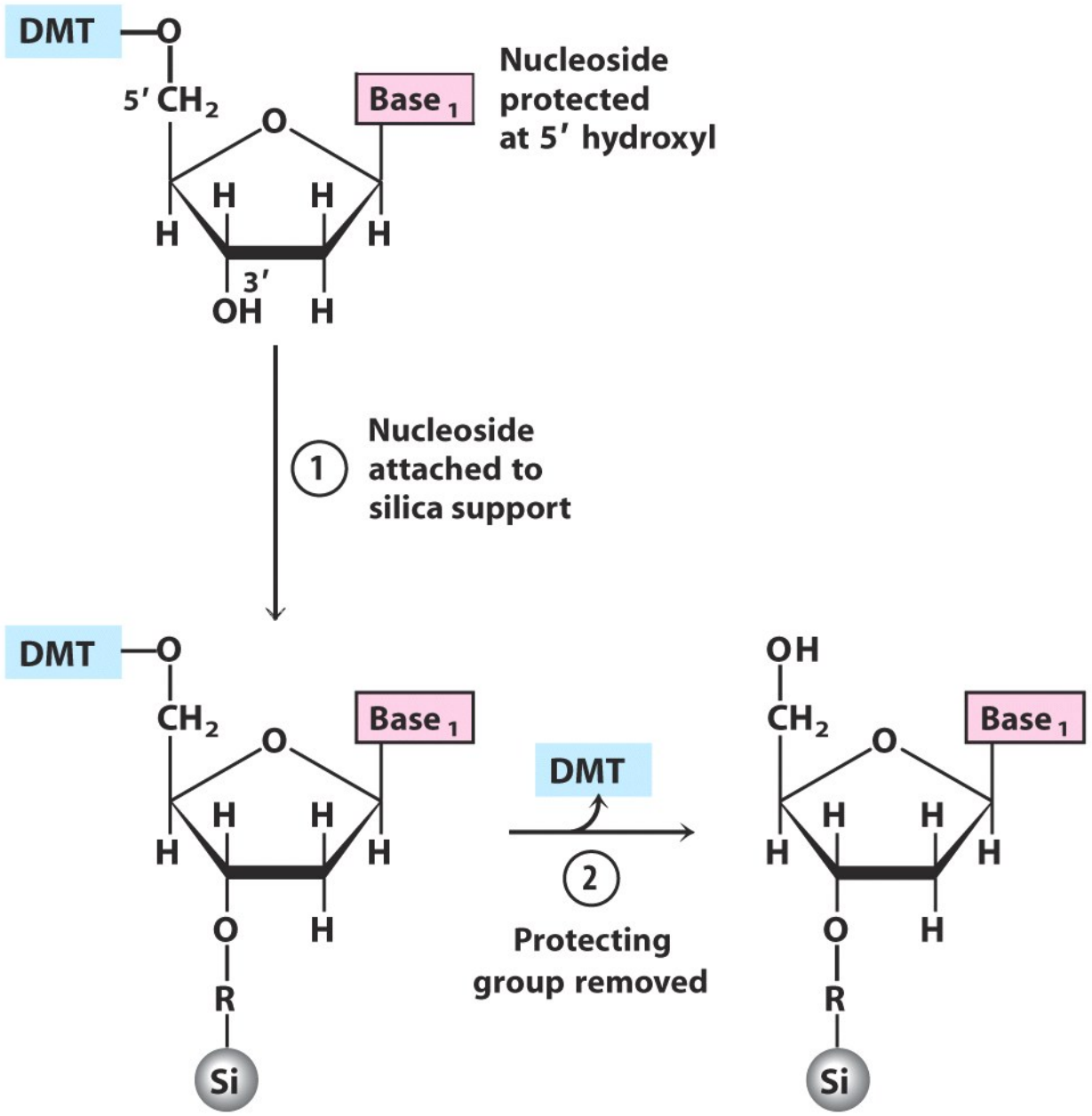

A
T
T
C
G
G
Denature
A
T
C
G
G
T
Dye-labeled segments of DNA, copied from template with unknown sequence

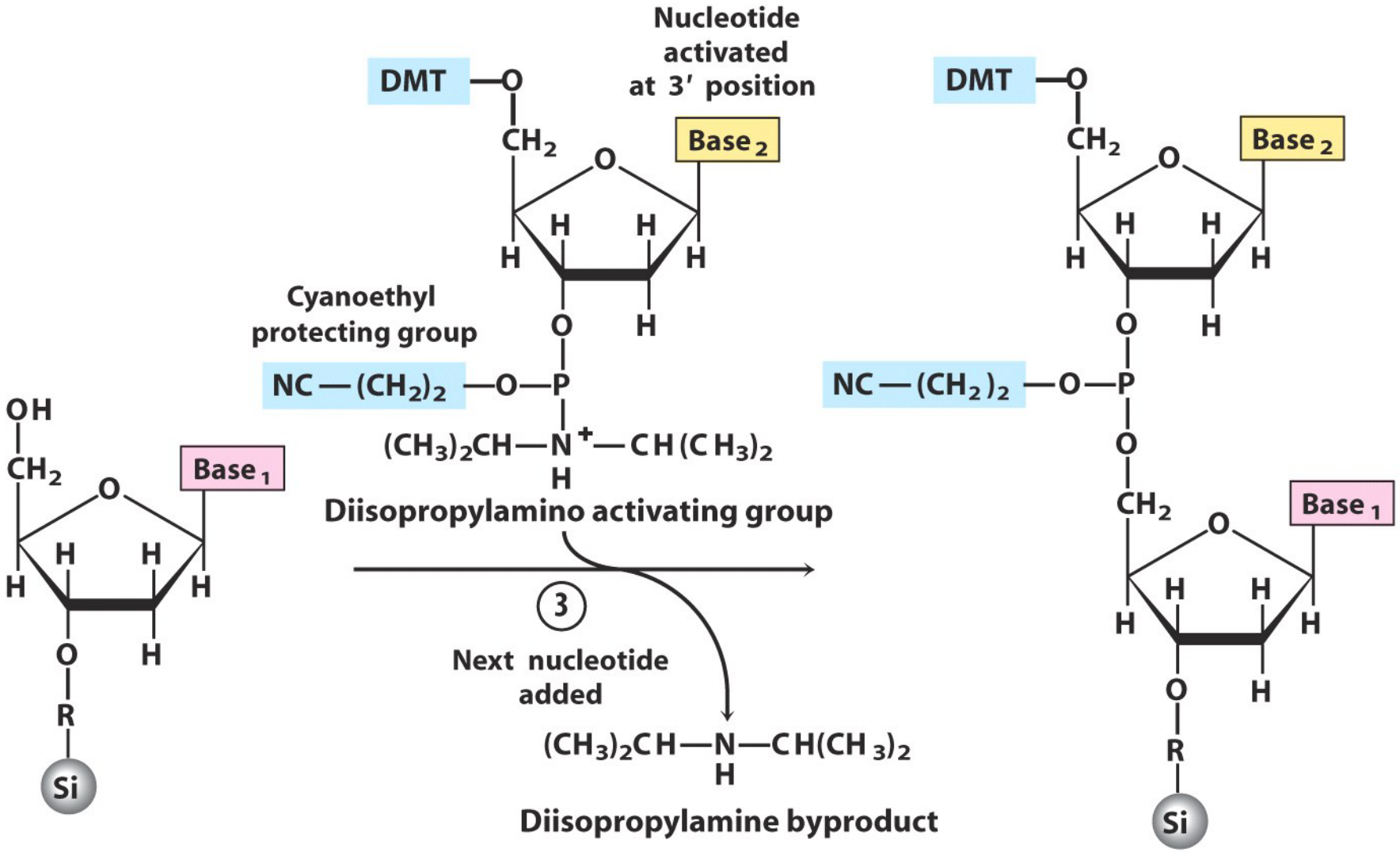

A
T
C
G
G
T
Dye-labeled segments of DNA, copied from template with unknown sequence
Dye-labeled segments applied to a capillary gel and subjected to electrophoresis
DNA migration
Laser beam
Detector
Laser

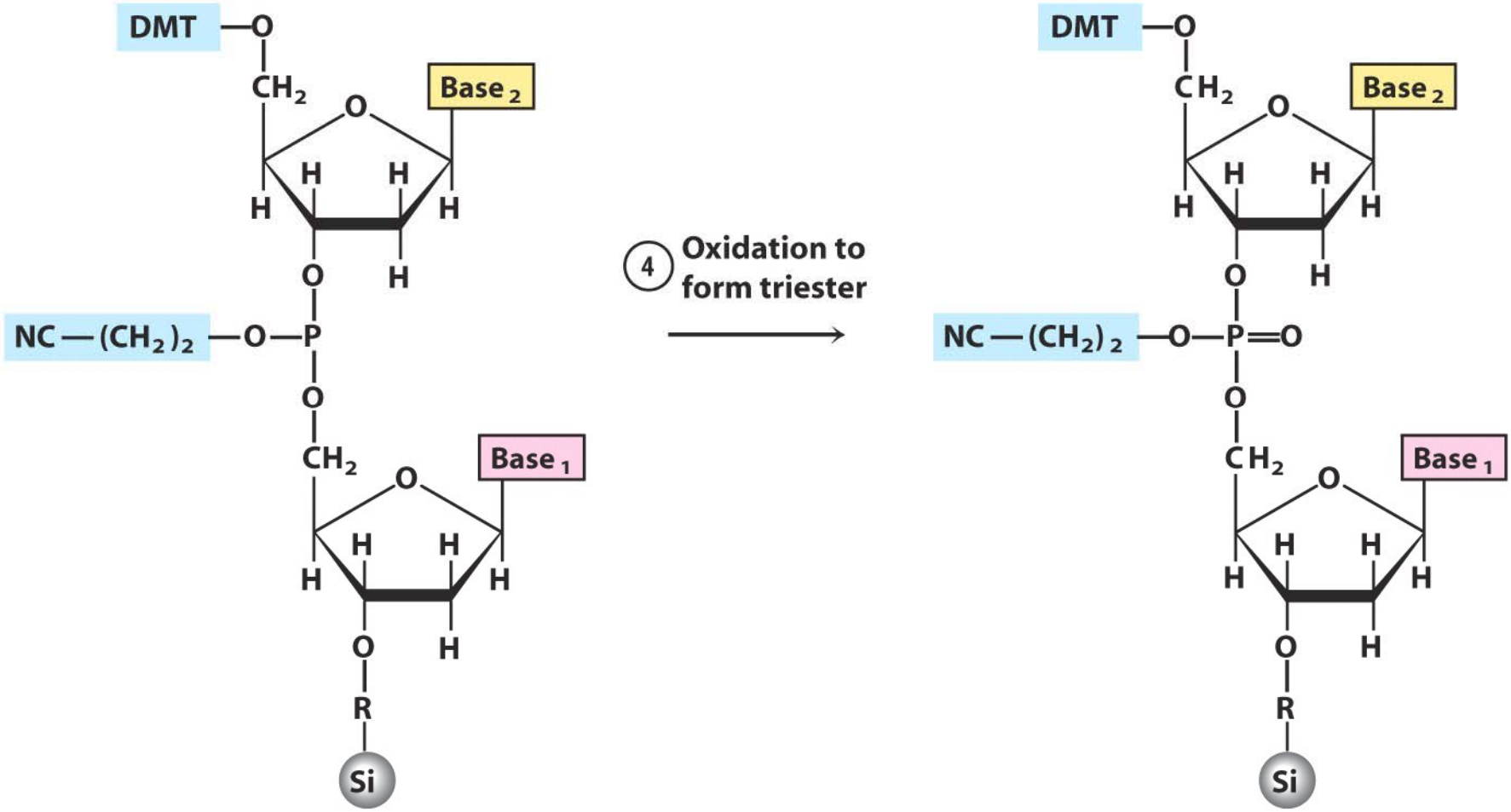


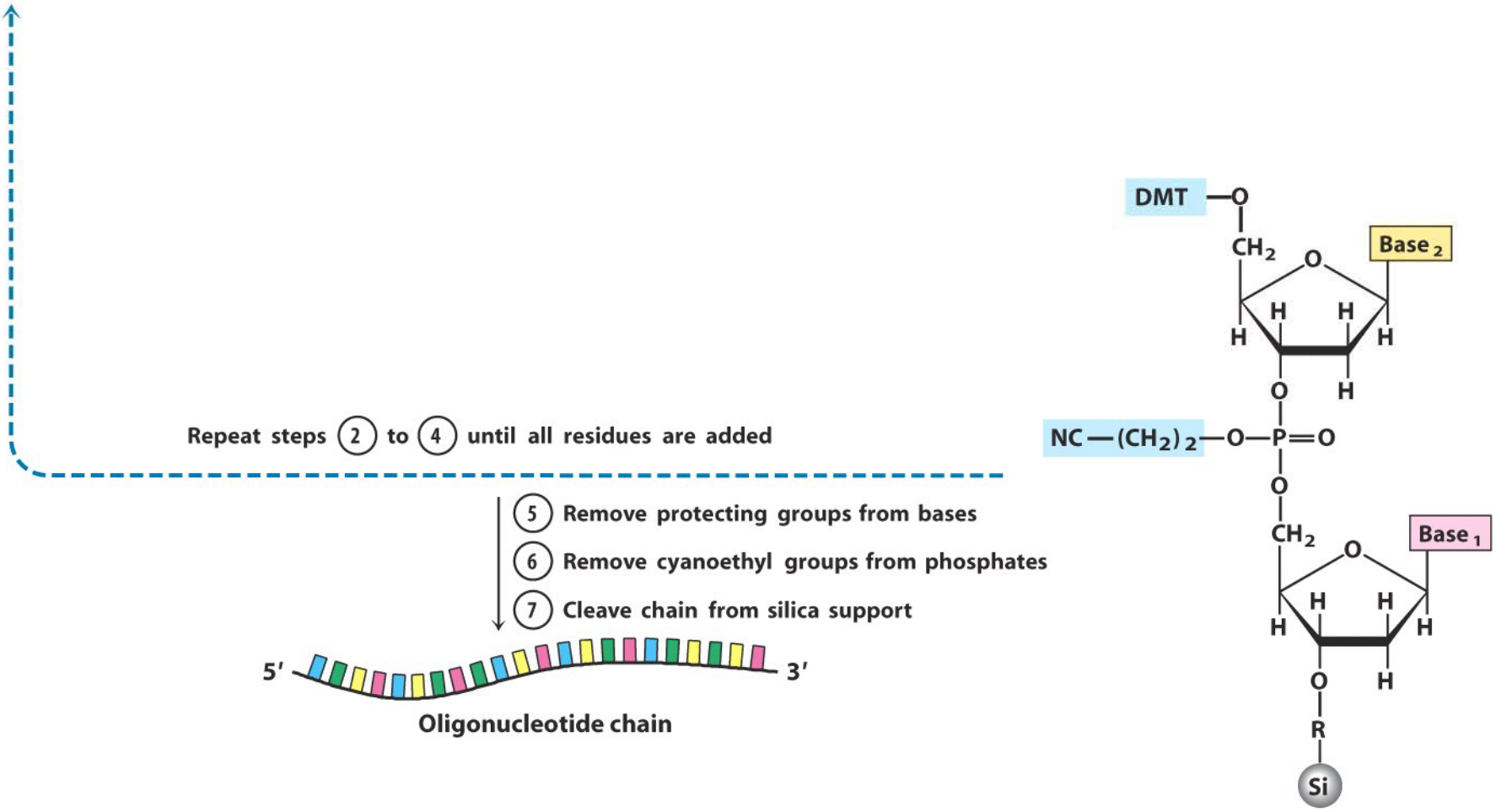
Computer-generated result after bands migrate past detector

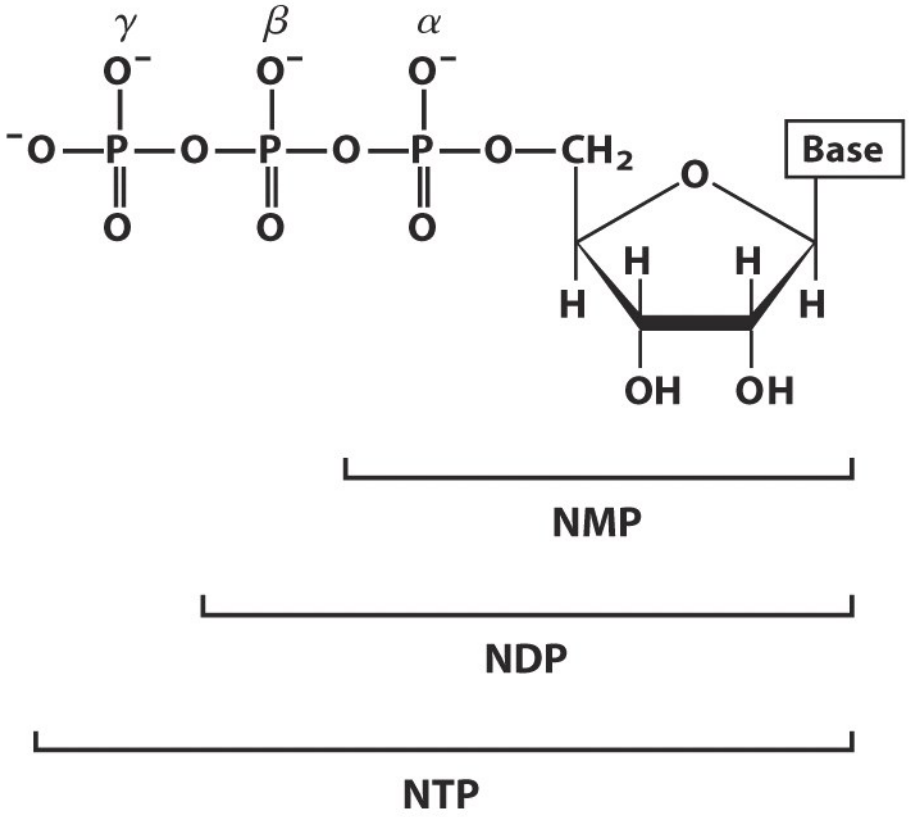

DMT
Base 1
Nucleoside protected at 5′ hydroxyl
1 Nucleoside attached to silica support
Si
2 Protecting group removed
Nucleotide activated at 3′ position
Base 2
Cyanoethyl protecting group
NC—(CH2)2—O—P
(CH3)2CH—N+—CH(CH3)2
Diisopropylamino activating group
3 Next nucleotide added
(CH3)2CH—N—CH(CH3)2
Diisopropylamine byproduct
4 Oxidation to form triester
NC—(CH2)2—O—P=O
Repeat steps 2 to 4 until all residues are added
5 Remove protecting groups from bases
6 Remove cyanoethyl groups from phosphates
7 Cleave chain from silica support
5′
3′
Oligonucleotide chain


DMT
O
5′ CH2
Base 1
Nucleoside
protected
at 5′ hydroxyl
H
H
H
H
3′
OH
H
1
Nucleoside
attached to
silica support
DMT
O
CH2
Base 1
H
H
H
H
O
H
R
Si
DMT
2
Protecting
group removed
OH
CH2
Base 1
H
H
H
H
O
H
R
Si


Nucleotide activated at 3′ position
DMT
Base 2
Cyanoethyl protecting group
NC — (CH2)2 — O — P
(CH3)2CH — N+ — CH(CH3)2
H
Diisopropylamino activating group
3
Next nucleotide added
(CH3)2CH — N — CH(CH3)2
H
Diisopropylamine byproduct
OH
Base 1
Si
R

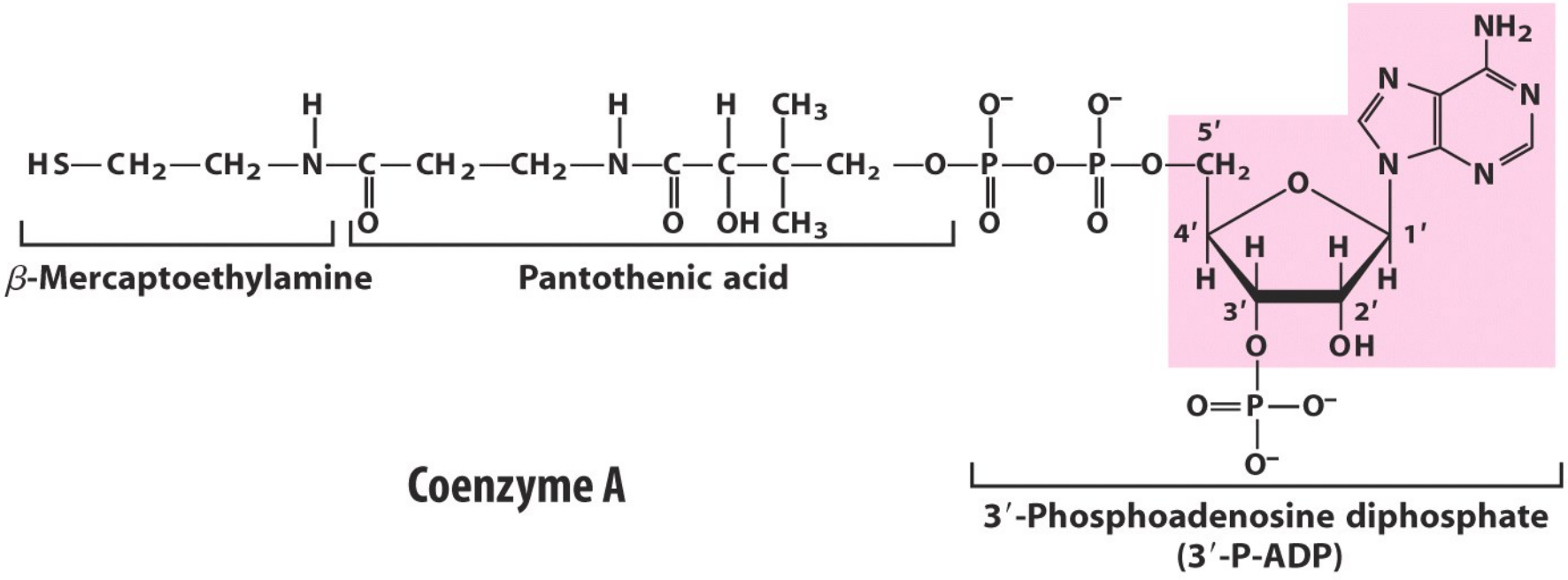

DMT
Base 2
NC — (CH2)2 — O — P
Base 1
Si
4
Oxidation to
form triester
DMT
Base 2
NC — (CH2)2 — O — P=O
Base 1
Si


Repeat steps 2 to 4 until all residues are added
5 Remove protecting groups from bases
6 Remove cyanoethyl groups from phosphates
7 Cleave chain from silica support
5′
3′
Oligonucleotide chain
DMT
Base 2
NC—(CH2)2—O—P=O
Base 1
Si

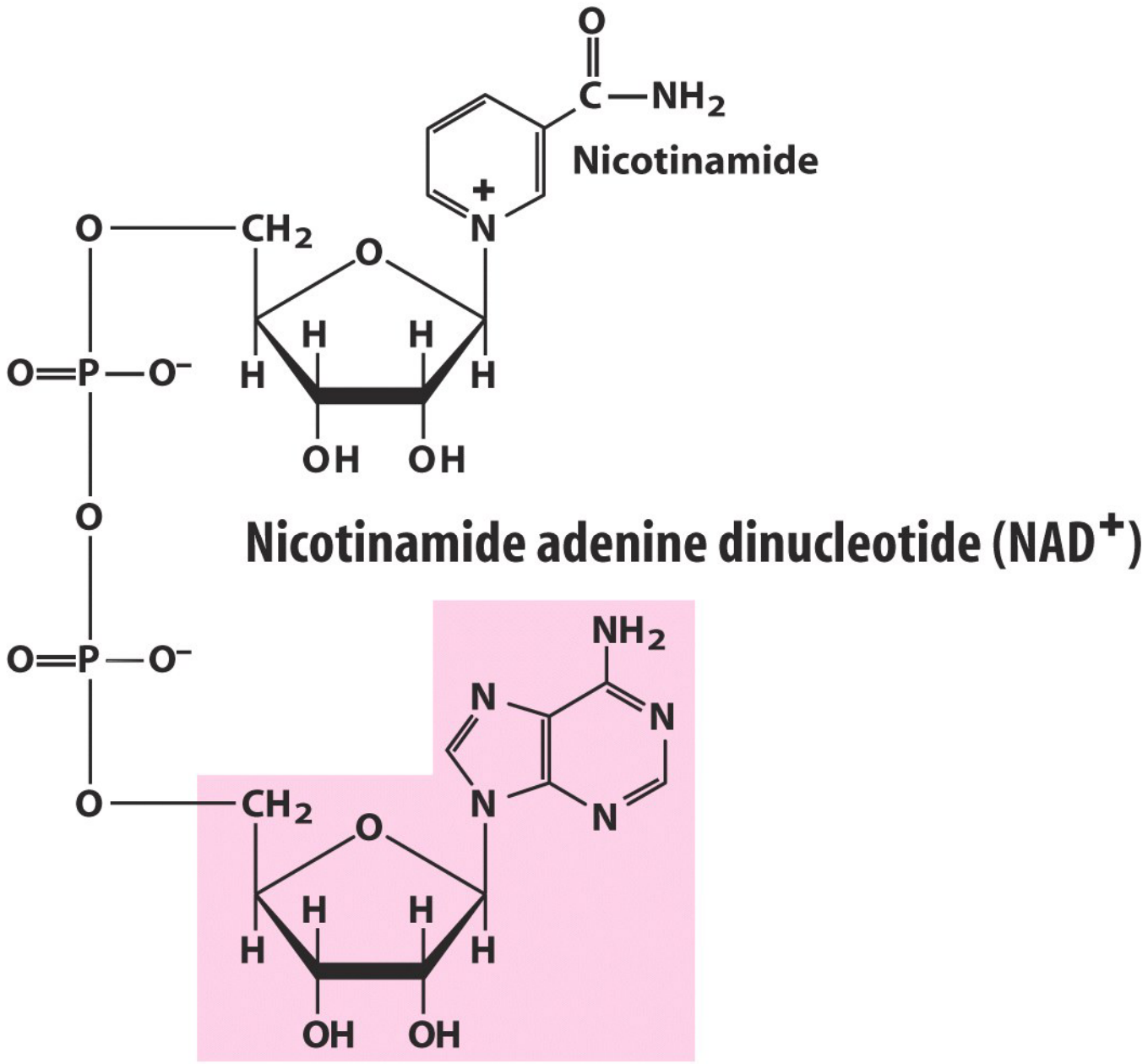


Abbreviations of ribonucleoside 5′-phosphates			
Base	**Mono-**	**Di-**	**Tri-**
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

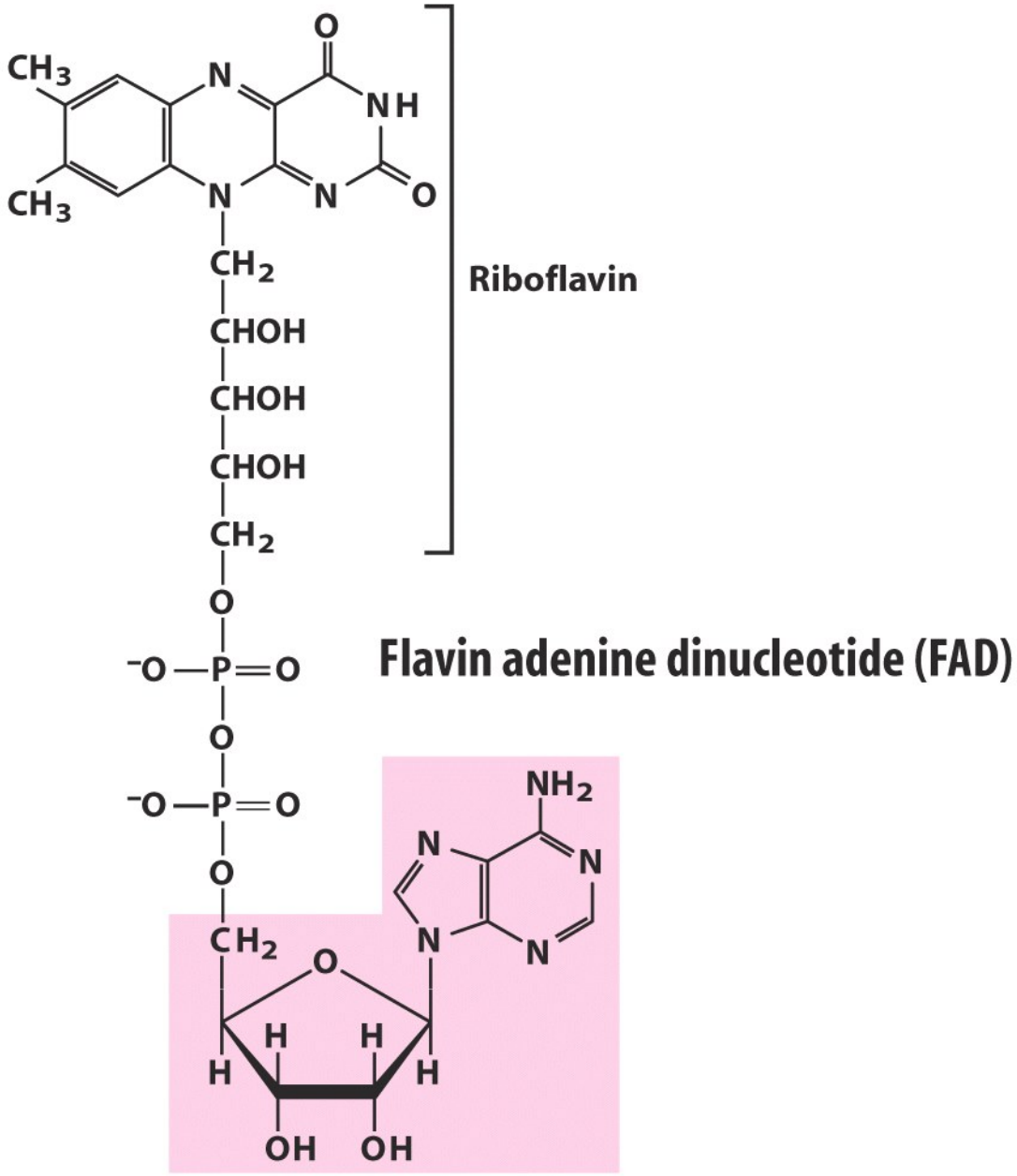
Abbreviations of deoxyribonucleoside 5′-phosphates			
Base	**Mono-**	**Di-**	**Tri-**
Adenine	dAMP	dADP	dATP
Guanine	dGMP	dGDP	dGTP
Cytosine	dCMP	dCDP	dCTP
Thymine	dTMP	dTDP	dTTP


β-Mercaptoethylamine
Pantothenic acid
3′-Phosphoadenosine diphosphate
(3′-P-ADP)

Coenzyme A



Nicotinamide adenine dinucleotide (NAD^+)


Riboflavin
Flavin adenine dinucleotide (FAD)