BENG 230A - Biochemistry

Midterm Review Fall 2022

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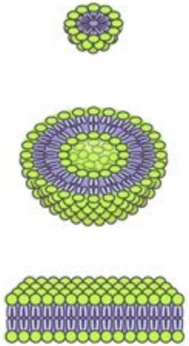
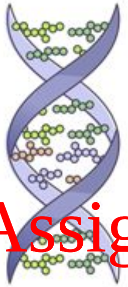
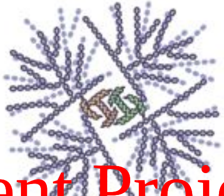
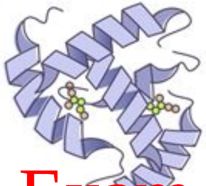
Biomolecules & Cell Chemistry

What is in a cell?

	 LIPIDS	 NUCLEIC ACID	 CARBOHYDRATES	 PROTEINS
Structure	Glycerol + Fatty Acids + Phosphate group	Sugar (Ribose/Deoxyribose) + Phosphate group + Nitrogenous base (A/T/G/C/U)	Polymers of monosaccharides linked by glycosidic bonds	Polymers of amino acids linked by peptide bonds
Functions	Plasma Membrane, Signaling Molecules, Hormones	Genetic Material (DNA and RNA)	Energy production and storage (glycolysis), Post translational modifications (glycosylation)	Enzymes, Antibodies, Hormones, Cytoskeleton

Water

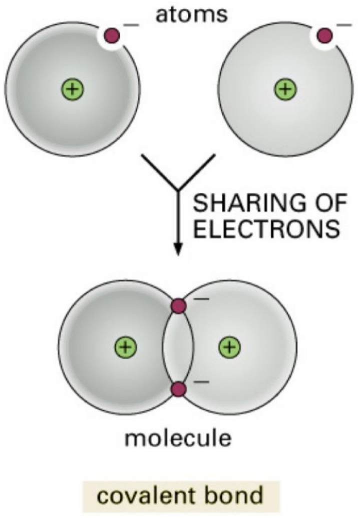
H_2O

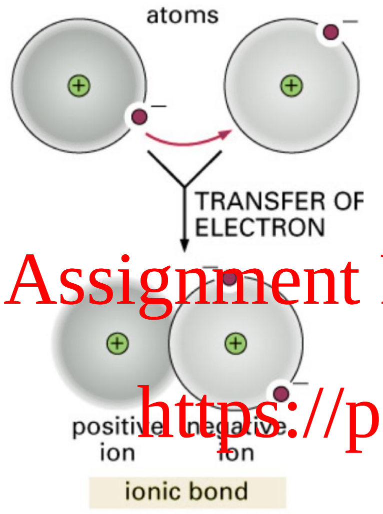
Most abundant molecule (70% of cell mass)

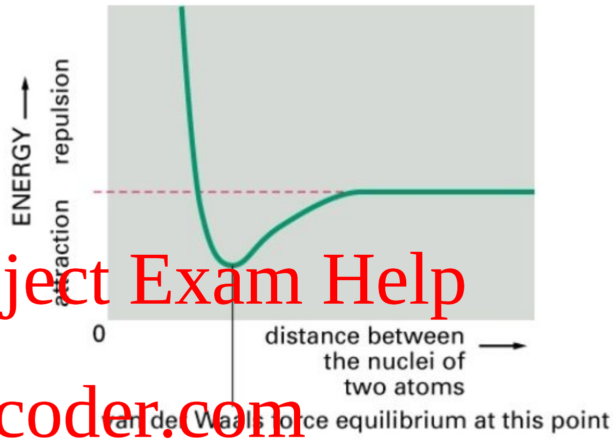
Universal solvent, medium of transportation between intracellular and extracellular compartments

Fun drinking game:
Take a shot of water every couple hours to make sure that you are healthy and hydrated

Covalent and non-covalent interactions in biological systems





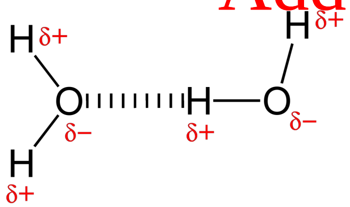


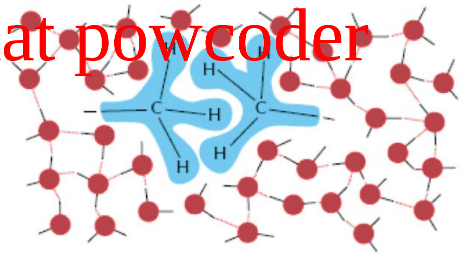
Van der Waals Interactions

Distance dependent interaction of oppositely polarized electron clouds

Hydrogen bonding

Difference in electronegativity





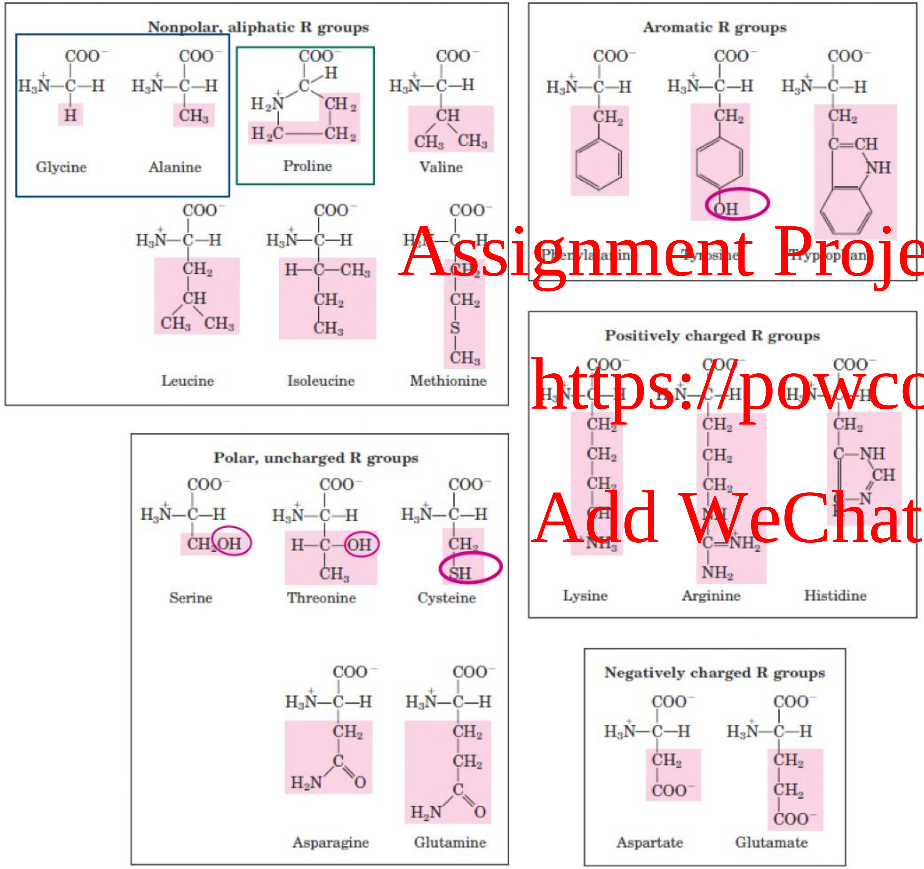
Hydrophobic Interactions

Polar groups - Hydrophilic
Non-polar groups - Hydrophobic

Strength: Covalent > Ionic > Hydrogen Bond > Hydrophobic > Van der Waals

Protein Structure & Function

Amino Acids Classification



AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

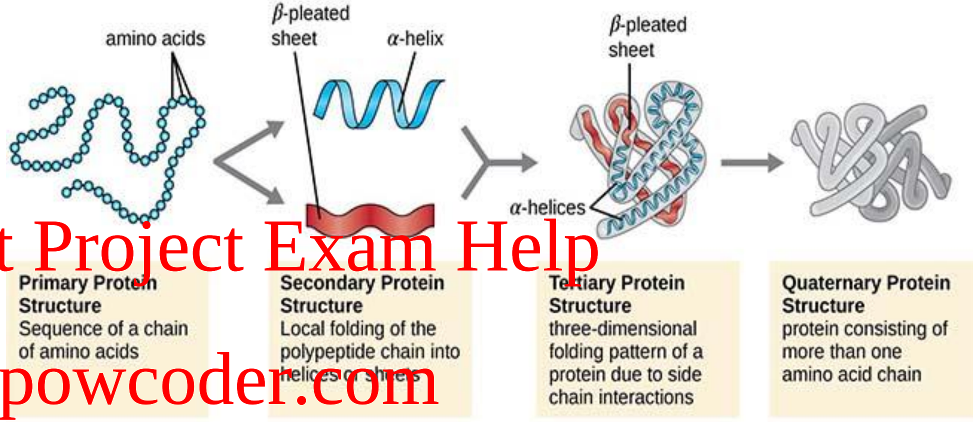
POLAR AMINO ACIDS

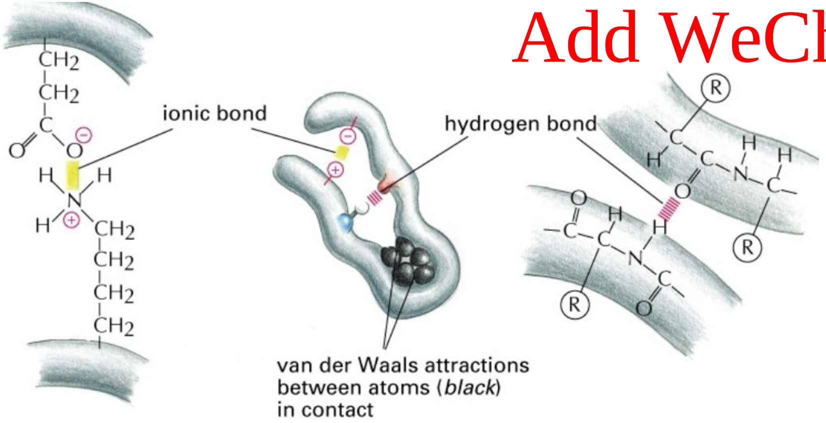
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

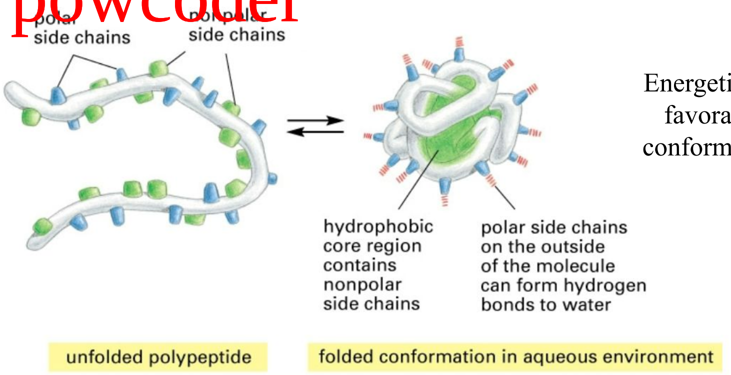
NONPOLAR AMINO ACIDS

3D Structure of Proteins

- Amino acids + Interactions: Determine protein structure
- Changes in amino acid sequence -> Misfolding of protein -> Loss of function

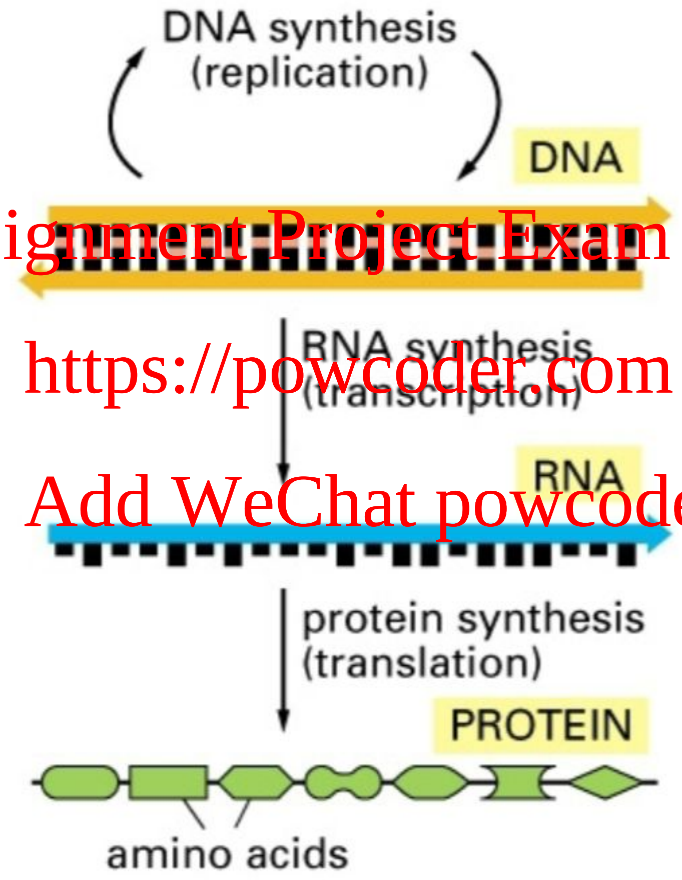






Energetically favorable conformation

Central Dogma of Molecular Biology



From DNA to DNA: Replication

DNA replication
Separation, Base pair

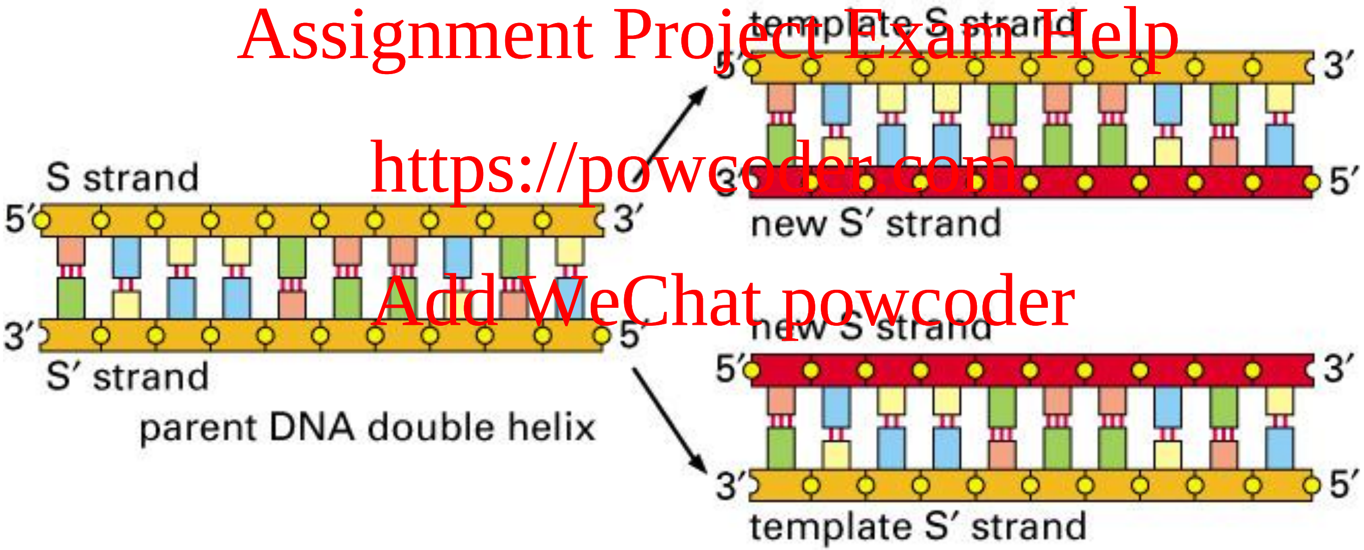
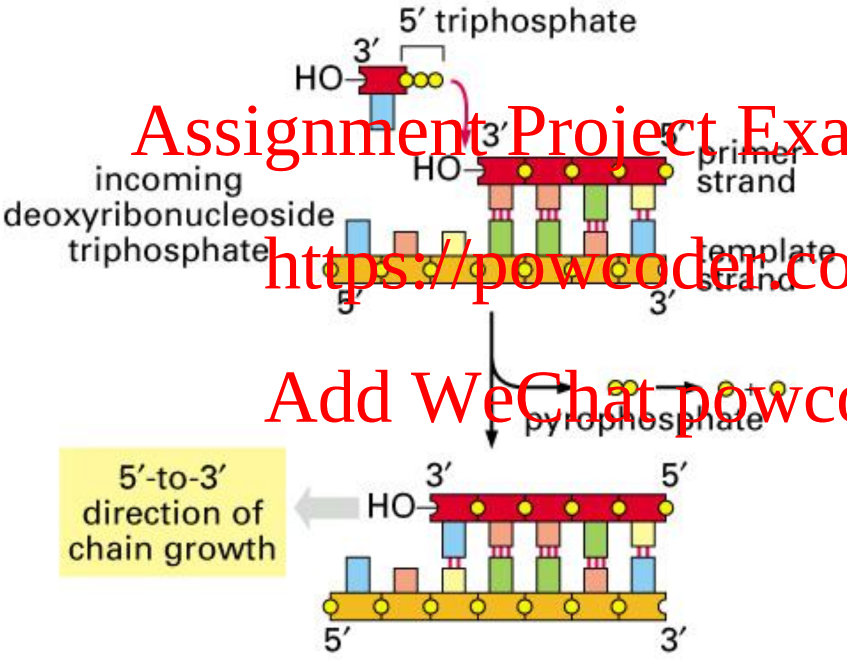


Figure 5–2. Molecular Biology of the Cell, 4th Edition.

DNA Synthesis by DNA polymerase



(A)

Figure 4–5 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

DNA replication Fork

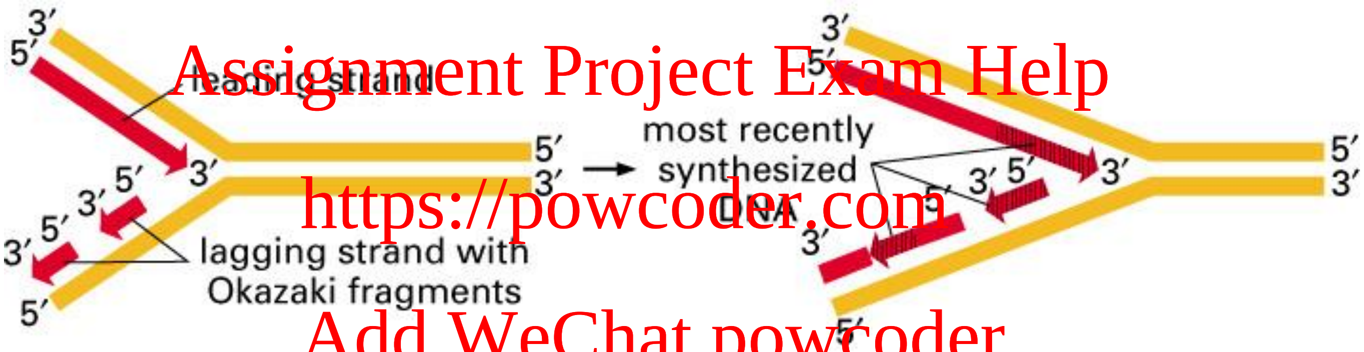


Figure 5–8. Molecular Biology of the Cell, 4th Edition.

DNA Proofreading

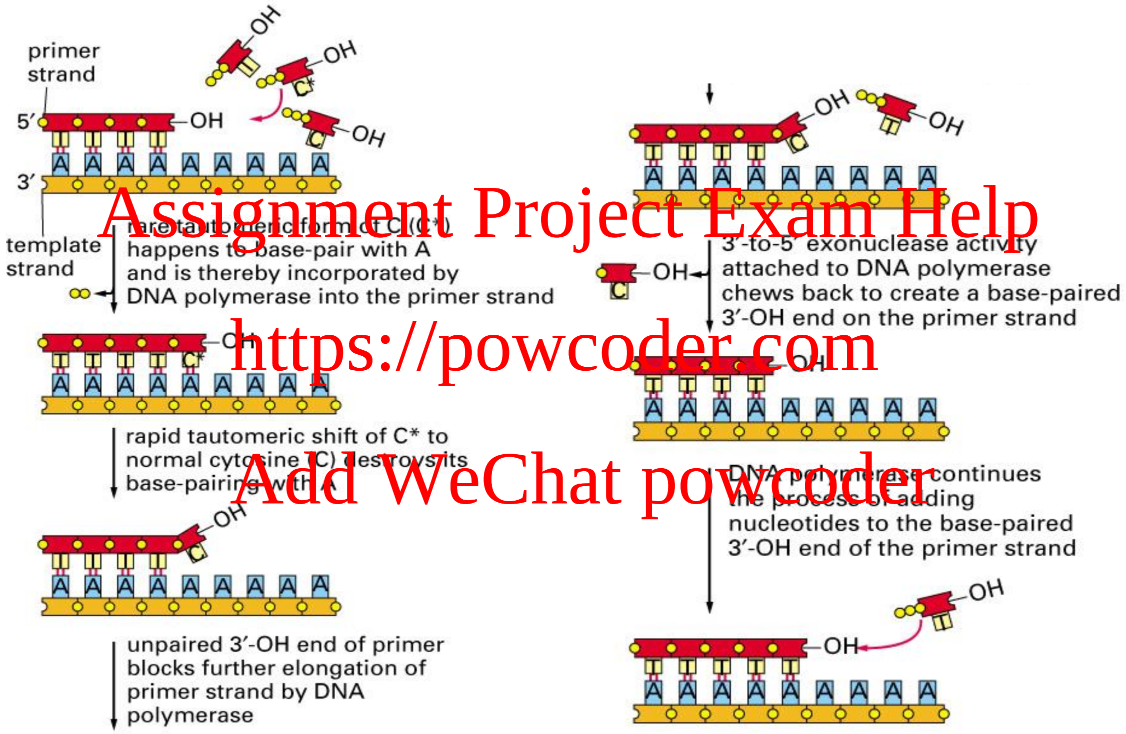


Figure 5–9 part 1 of 2. Molecular Biology of the Cell, 4th Ec Figure 5–9 part 2 of 2. Molecular Biology of the Cell, 4t

Why 5’->3’?

The need for accuracy

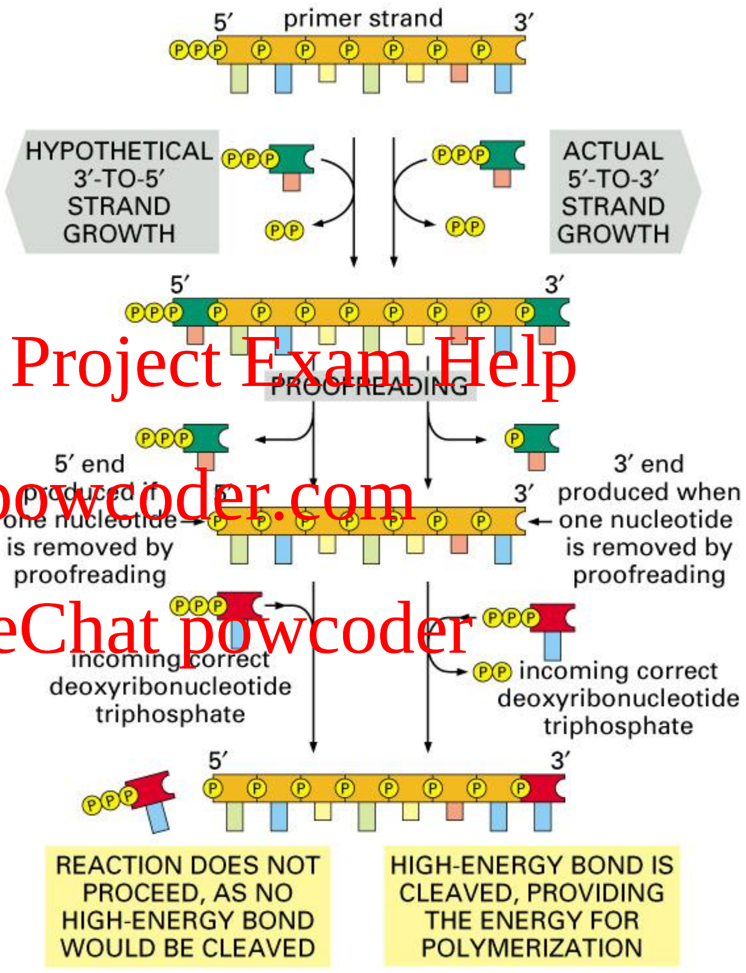


Figure 5–11. Molecular Biology of the Cell, 4th Edition.

DNA Replication at the Lagging strand

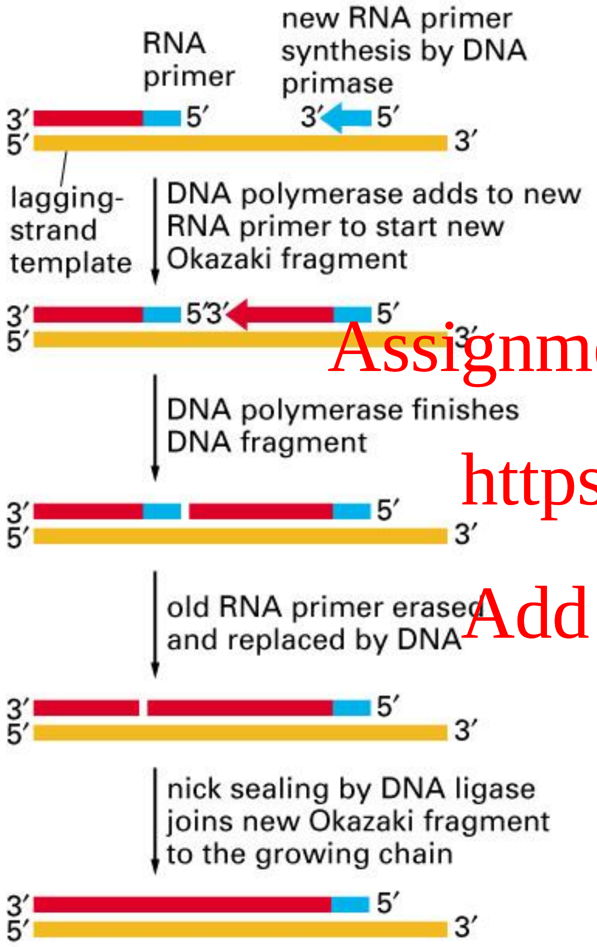


Figure 5–13. Molecular Biology of the Cell, 4th Edition.

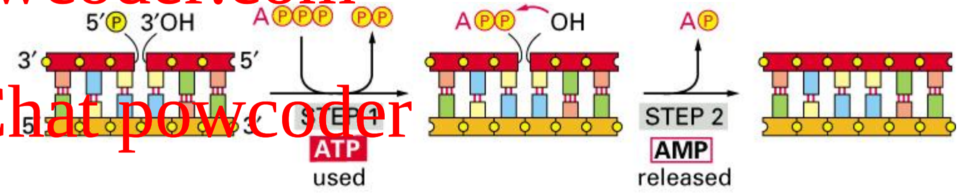


Figure 5–14. Molecular Biology of the Cell, 4th Edition.

Done by DNA Ligase

DNA Helicase

DNA double helix are tightly coupled. High temperature is needed to break them (95ºC)

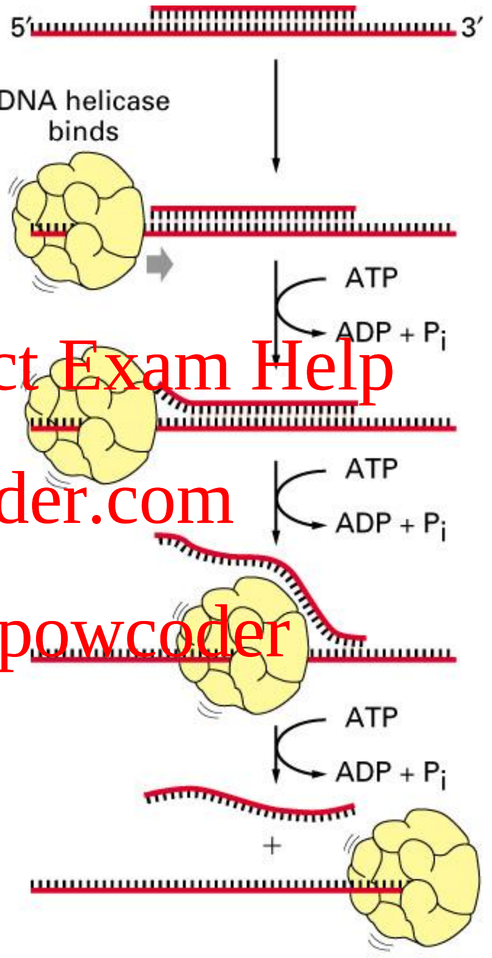


Figure 5–15. Molecular Biology of the Cell, 4th Edition.

DNA Binding Protein

SSB: Single Strand DNA-binding Proteins, also called helix destabilizing proteins

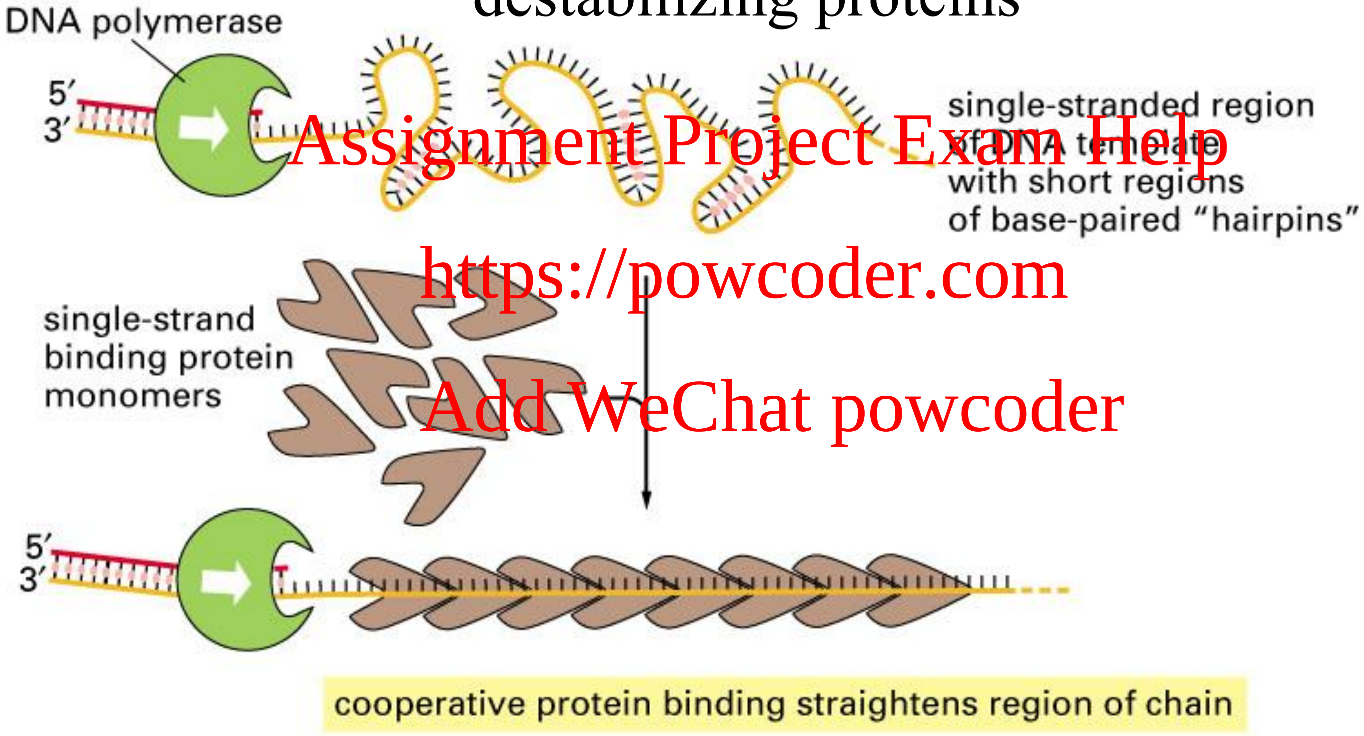


Figure 5–17. Molecular Biology of the Cell, 4th Edition.

DNA Clamping Protein

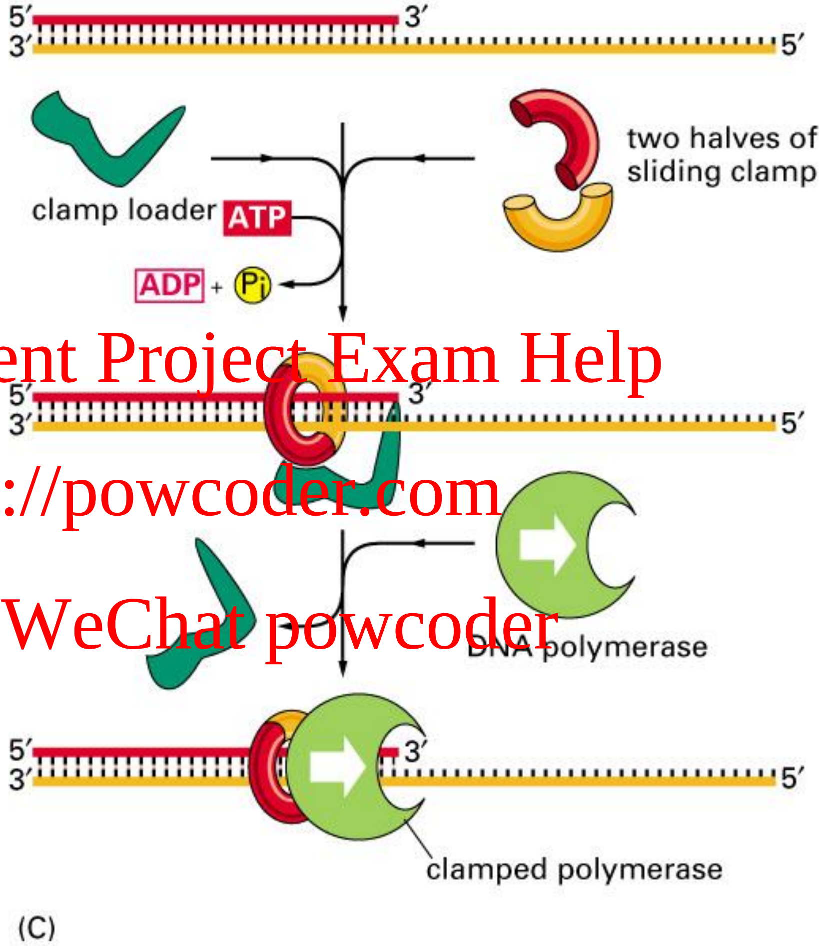


Figure 5–19 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Protein machinery for DNA replication

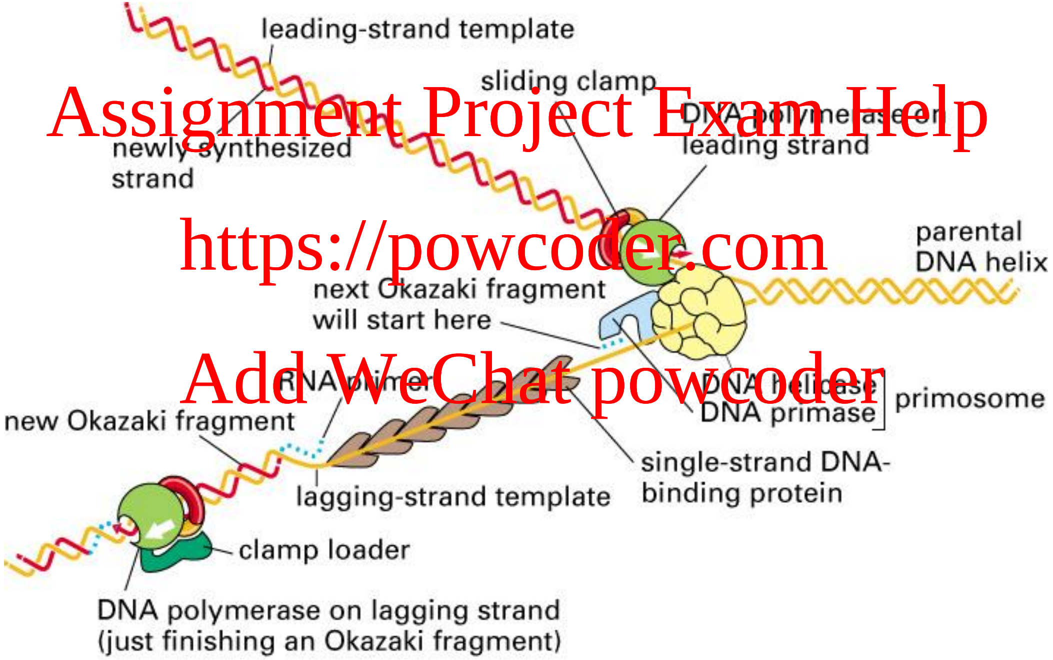
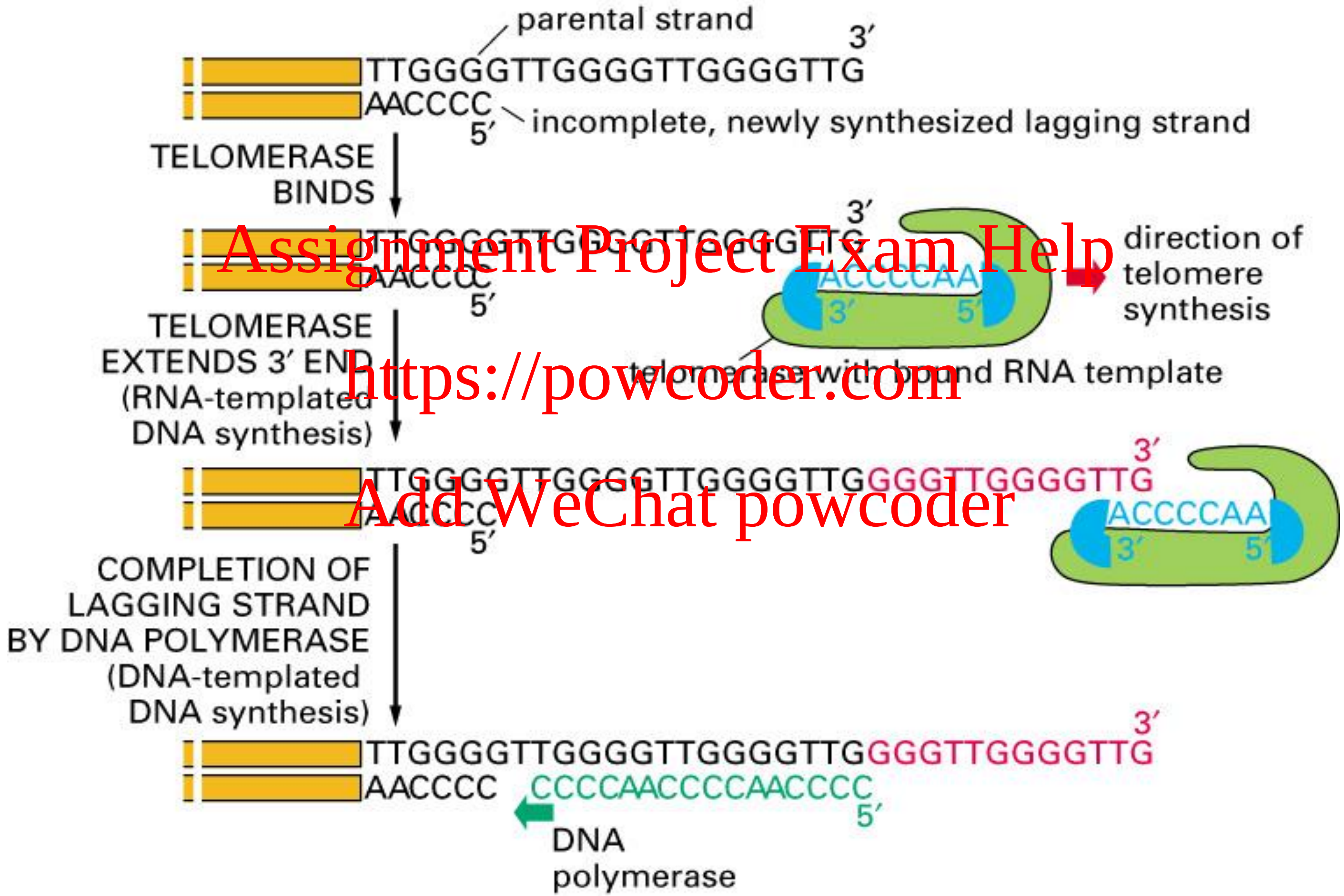


Figure 5–21. Molecular Biology of the Cell, 4th Edition.

Telomerase and its function



Retrovirus-based Transposition

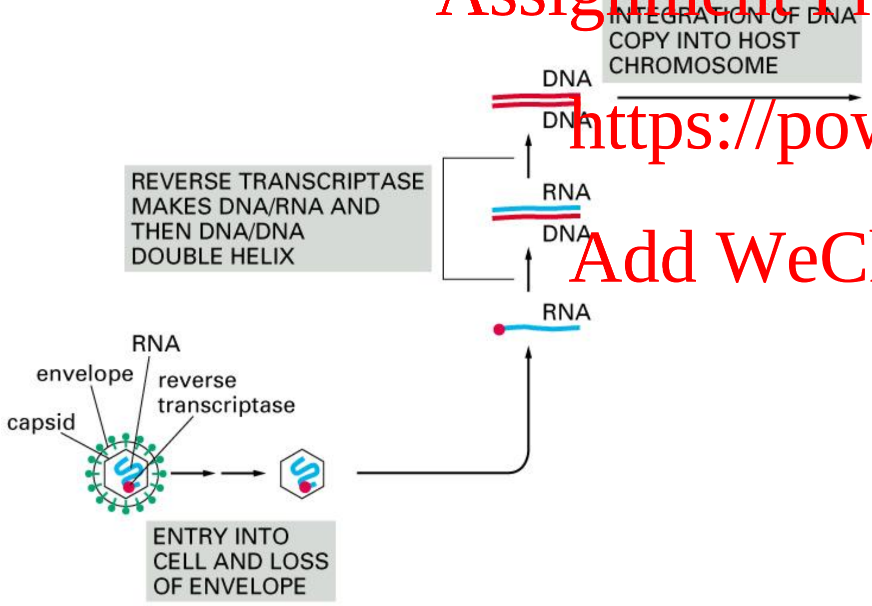


Figure 5–73 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

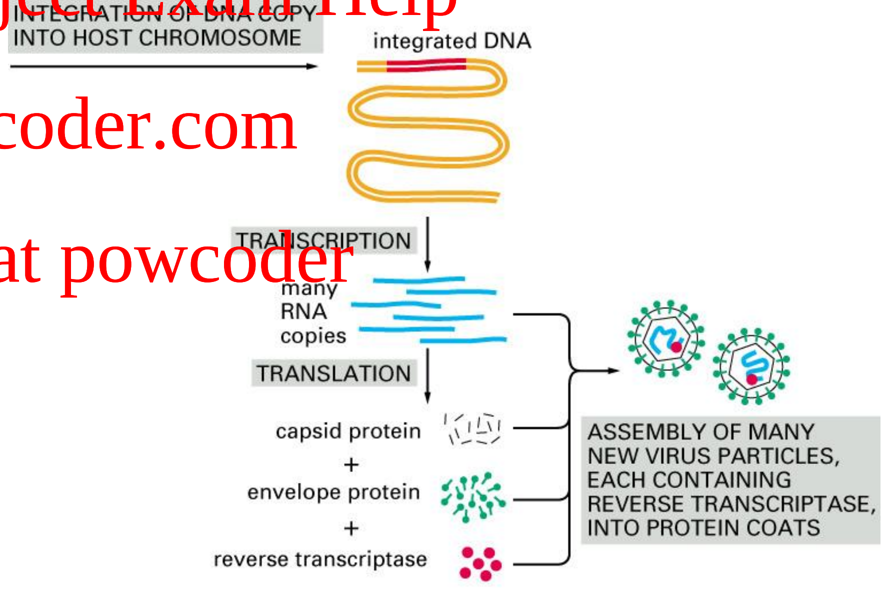


Figure 5–73 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Non-retroviral retrotransposition

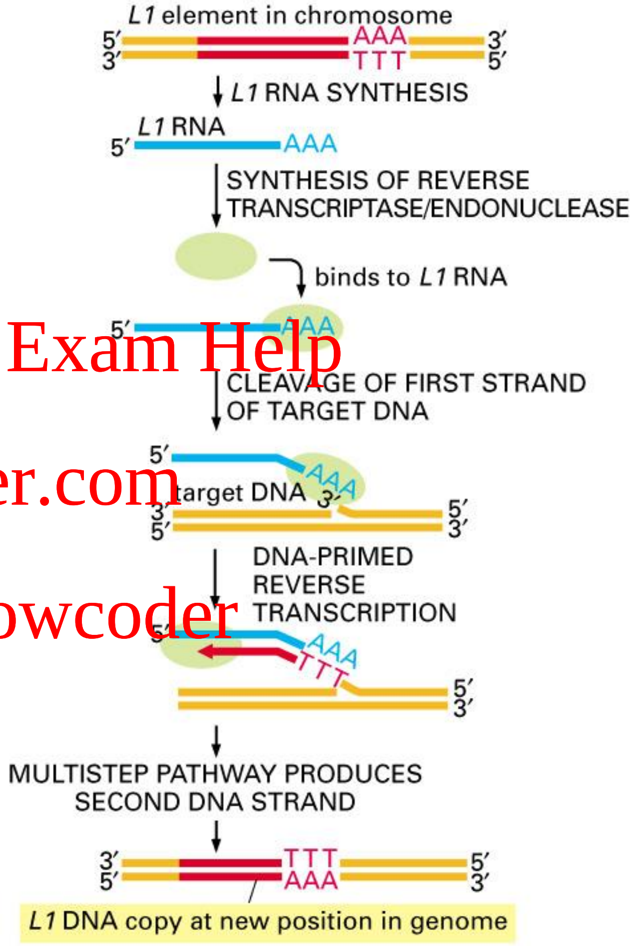


Figure 5–76. Molecular Biology of the Cell, 4th Edition.

From DNA to RNA: Transcription

DNA->RNA-> Proteins

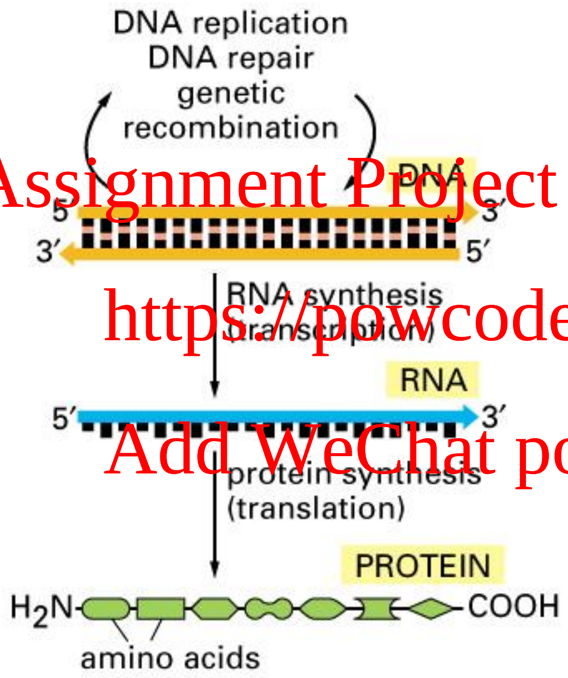


Figure 6–2. Molecular Biology of the Cell, 4th Edition.

Genes expressed with different efficiency

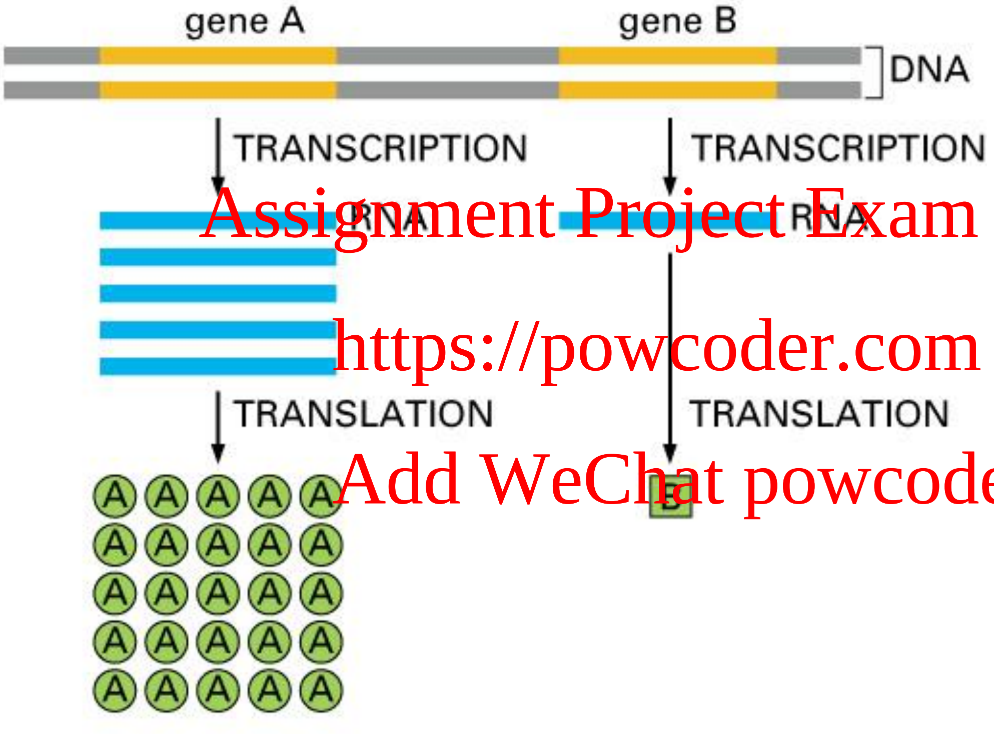


Figure 6–3. Molecular Biology of the Cell, 4th Edition.

The chemical structure differences between DNAs and RNAs

1. ribose, deoxyribose
2. Uracil and thymine

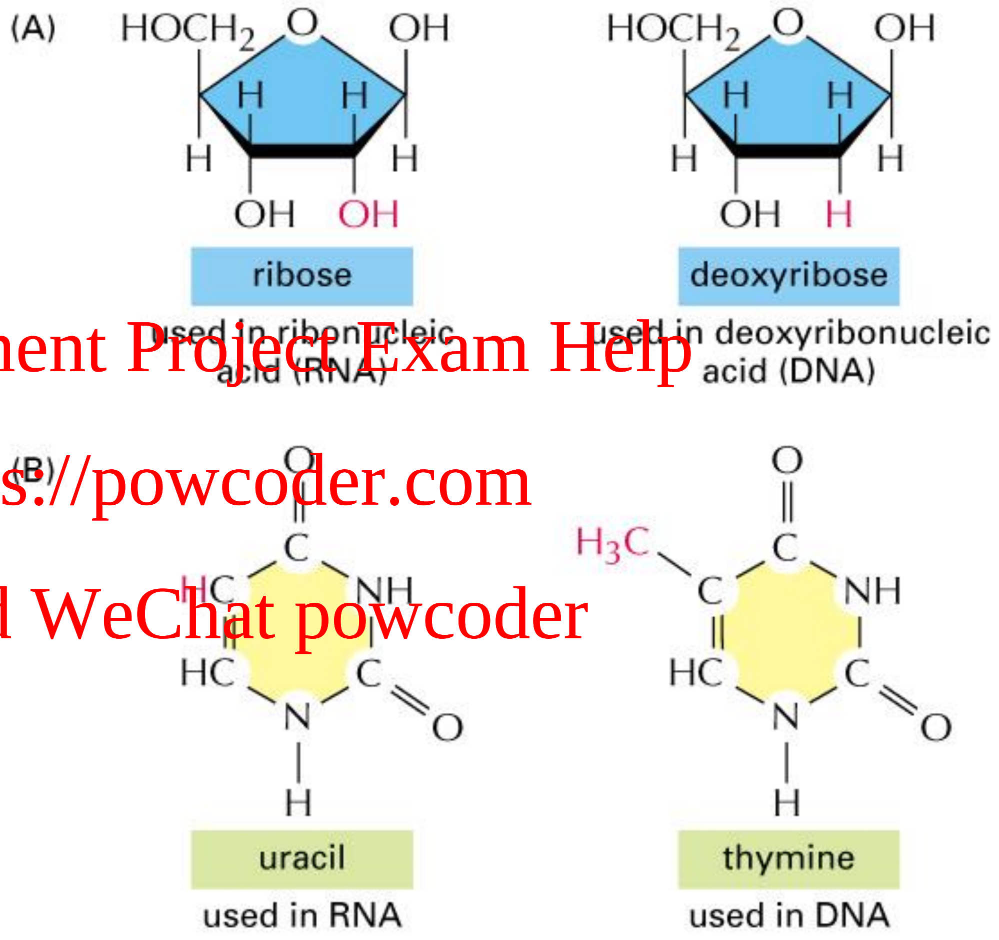
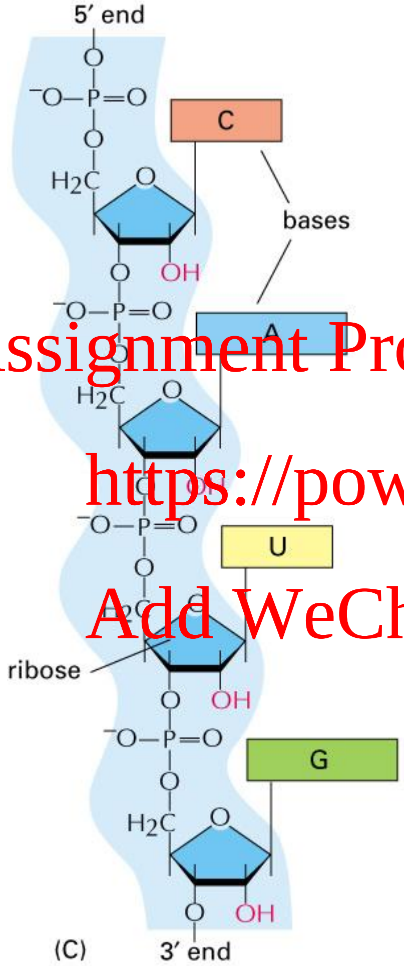


Figure 6–4 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

RNA base pairs
A-U; G-C



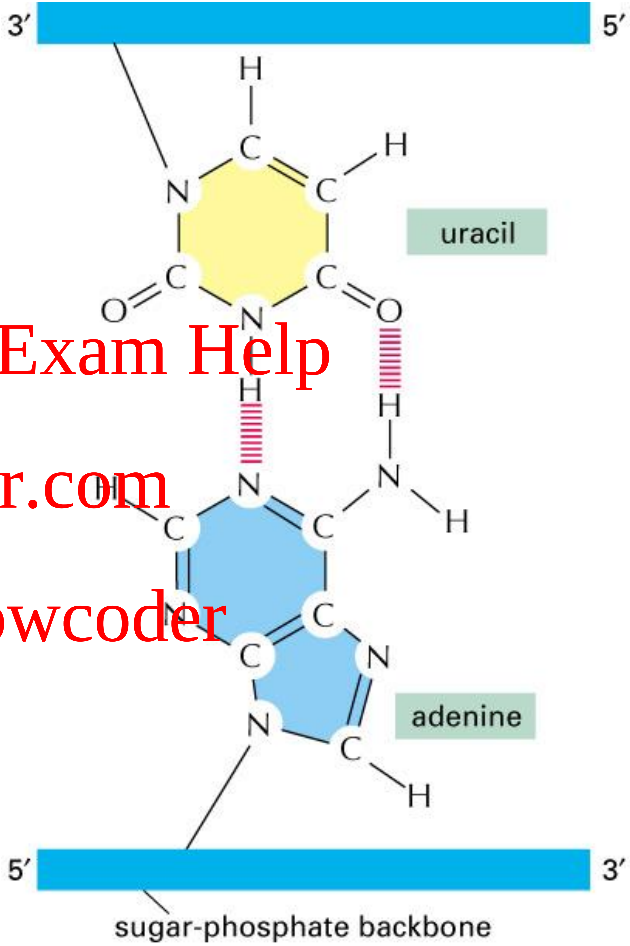


Figure 6–4 part 2 of 2. Molecular Biology of the Cell, Figure 6–5. Molecular Biology of the Cell, 4th Edition.

RNA Structures

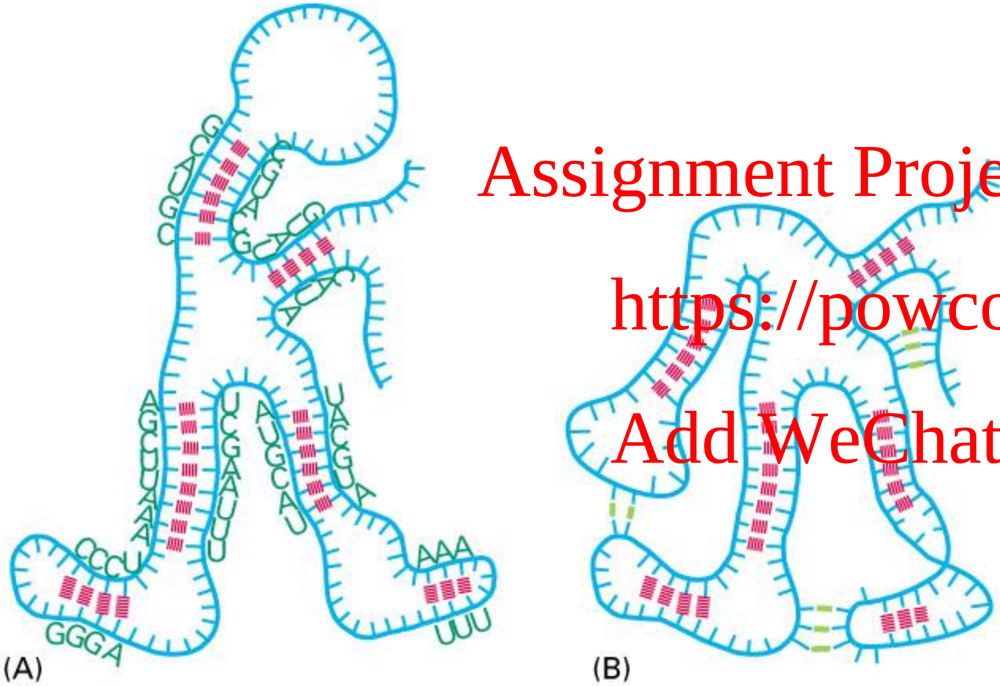


Figure 6–6 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

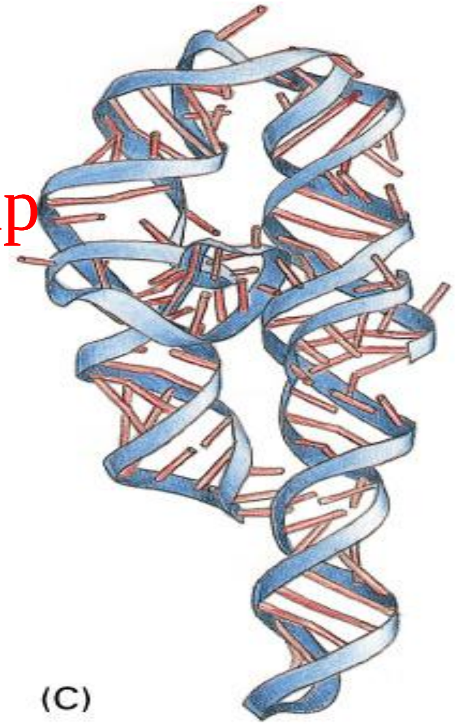
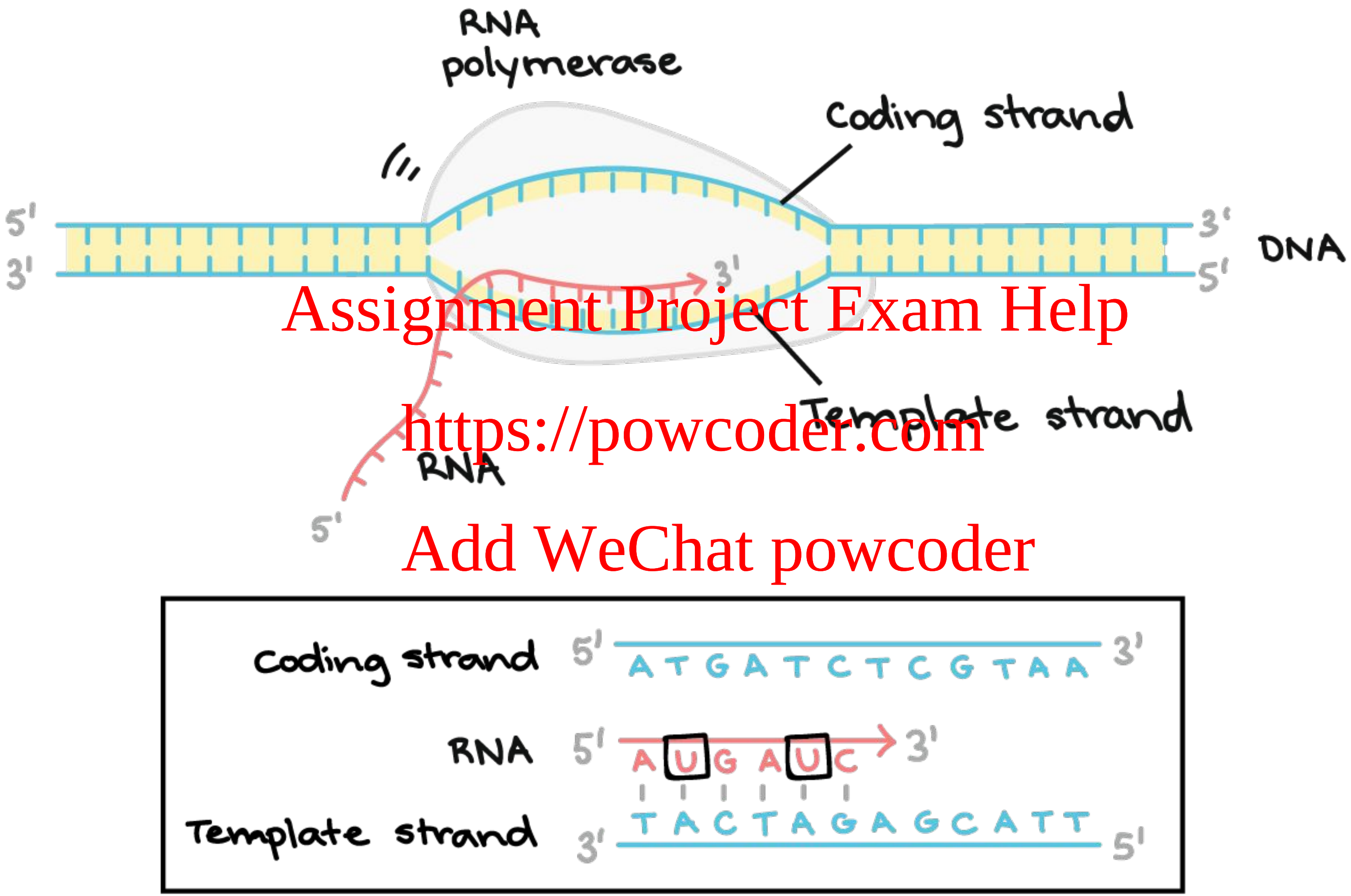


Figure 6–6 part 2 of 2. Mol


RNA polymerase
Coding strand
5'
3'
3'
5'
DNA
3'
Template strand
RNA
5'
Coding strand 5' A T G A T C T C G T A A 3'
RNA 5' A U G A U C 3'
Template strand 3' T A C T A G A G C A T T 5'

Initiation of transcription with RNA polymerase II in eucaryotes
TF: transcription factor
TBP: TATA box binding protein
Promoter upstream of real starting sequence of transcription

TFIIH open DNA double helix and phosphorylate C-tail of polymerase and allow the release and transcription

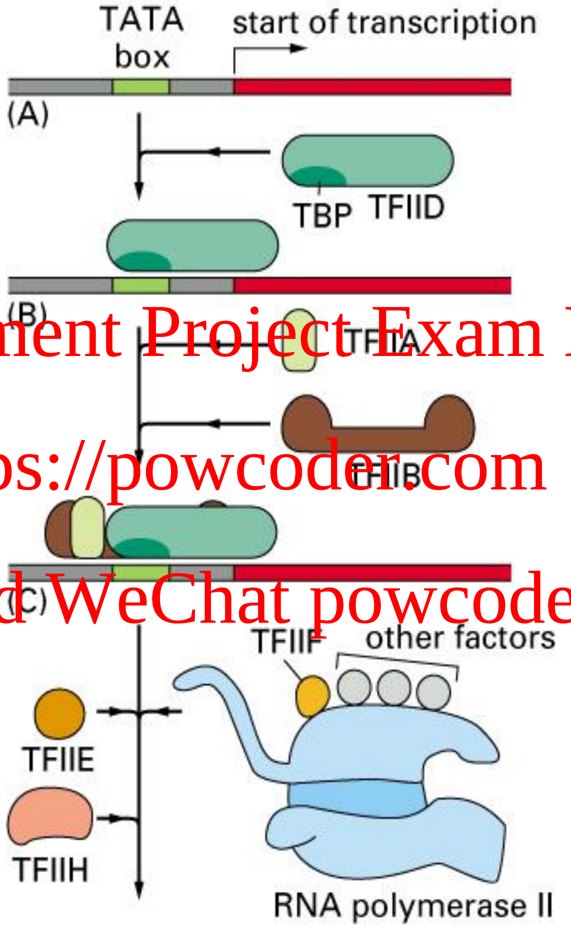


Figure 6–16 part 1 of 2. Molecular Biology

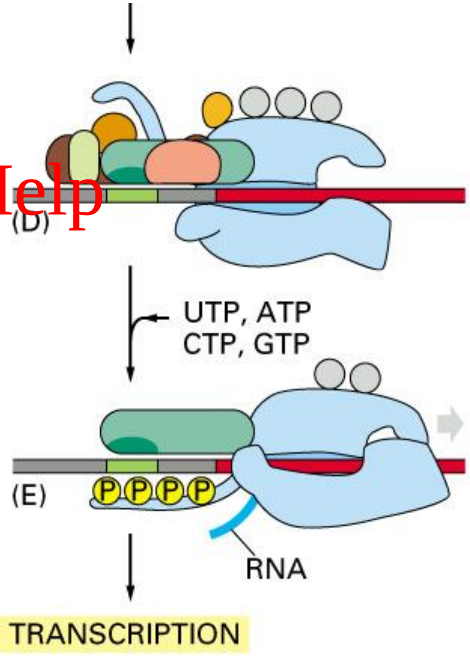


Figure 6–16 part 2 of 2. Molecular Biolog

The importance of RNA polymerase II tail

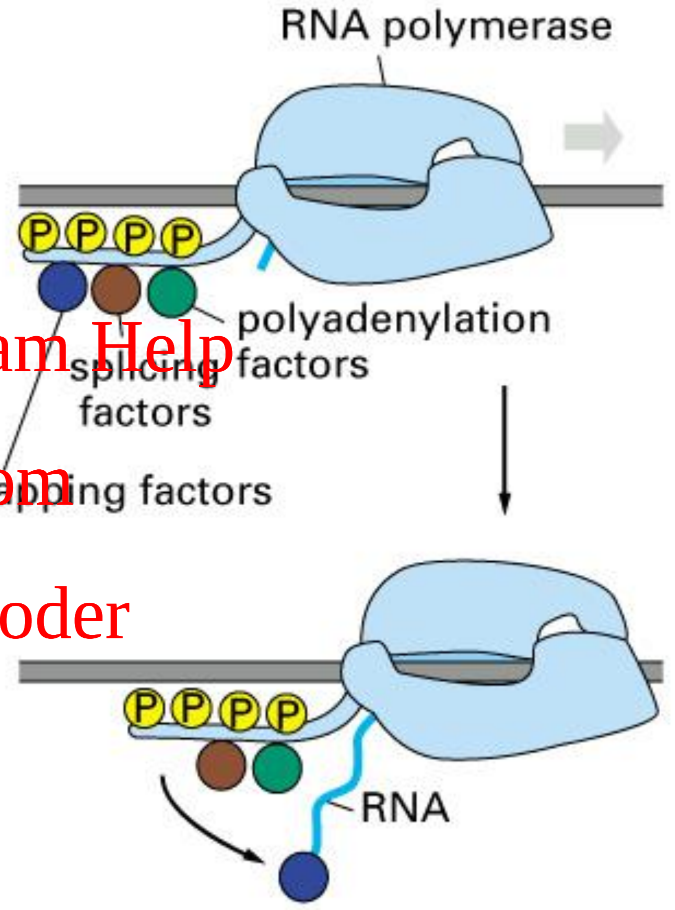
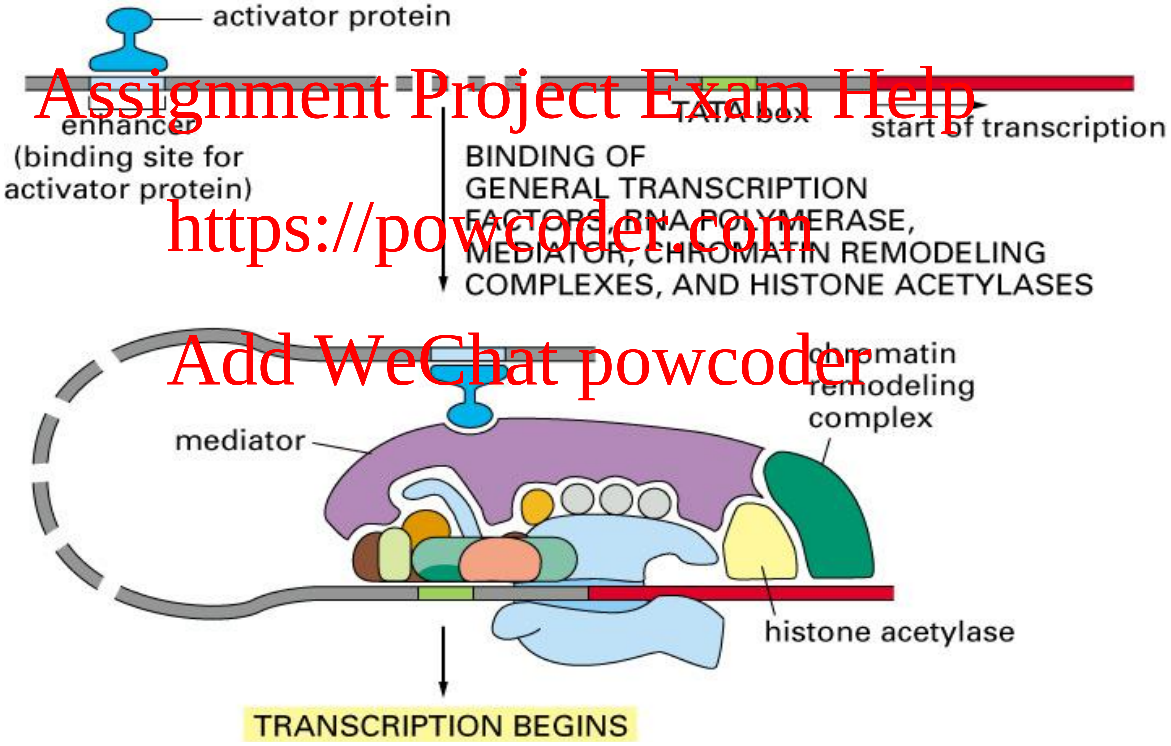
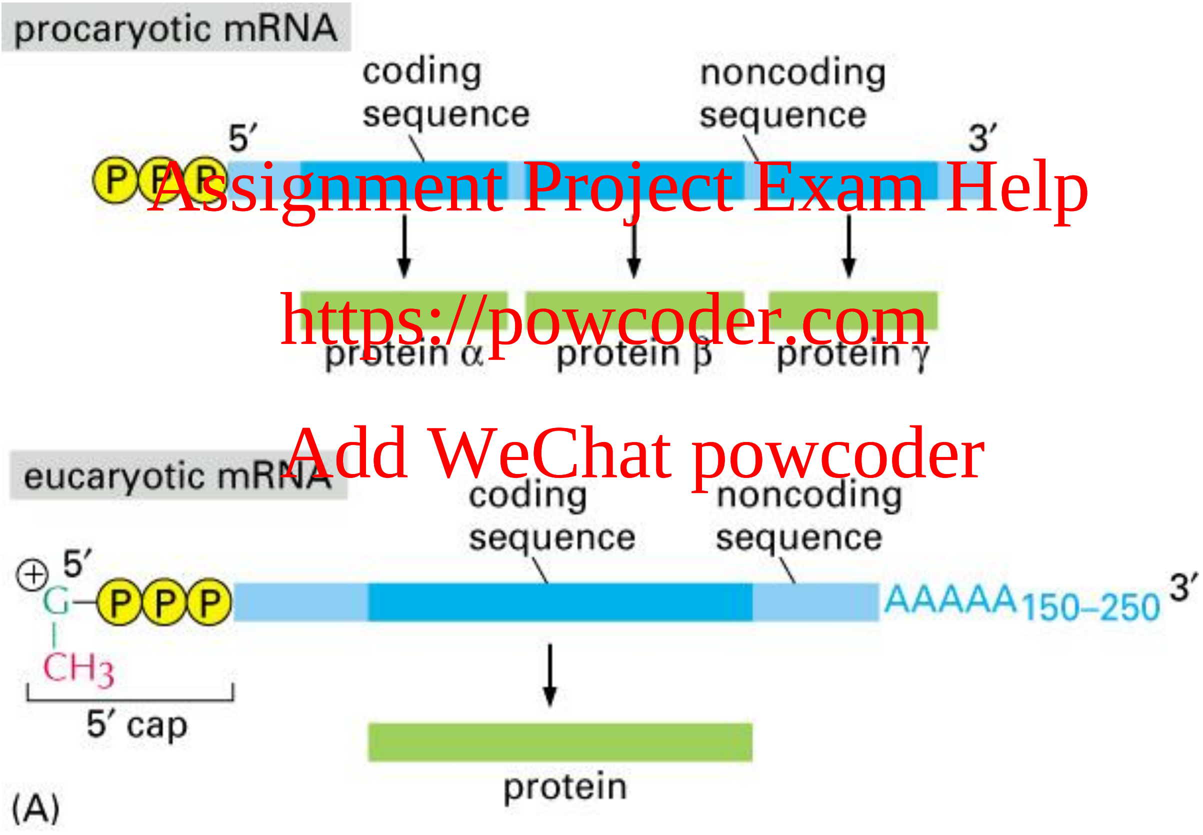


Figure 6–23. Molecular Biology of the Ce

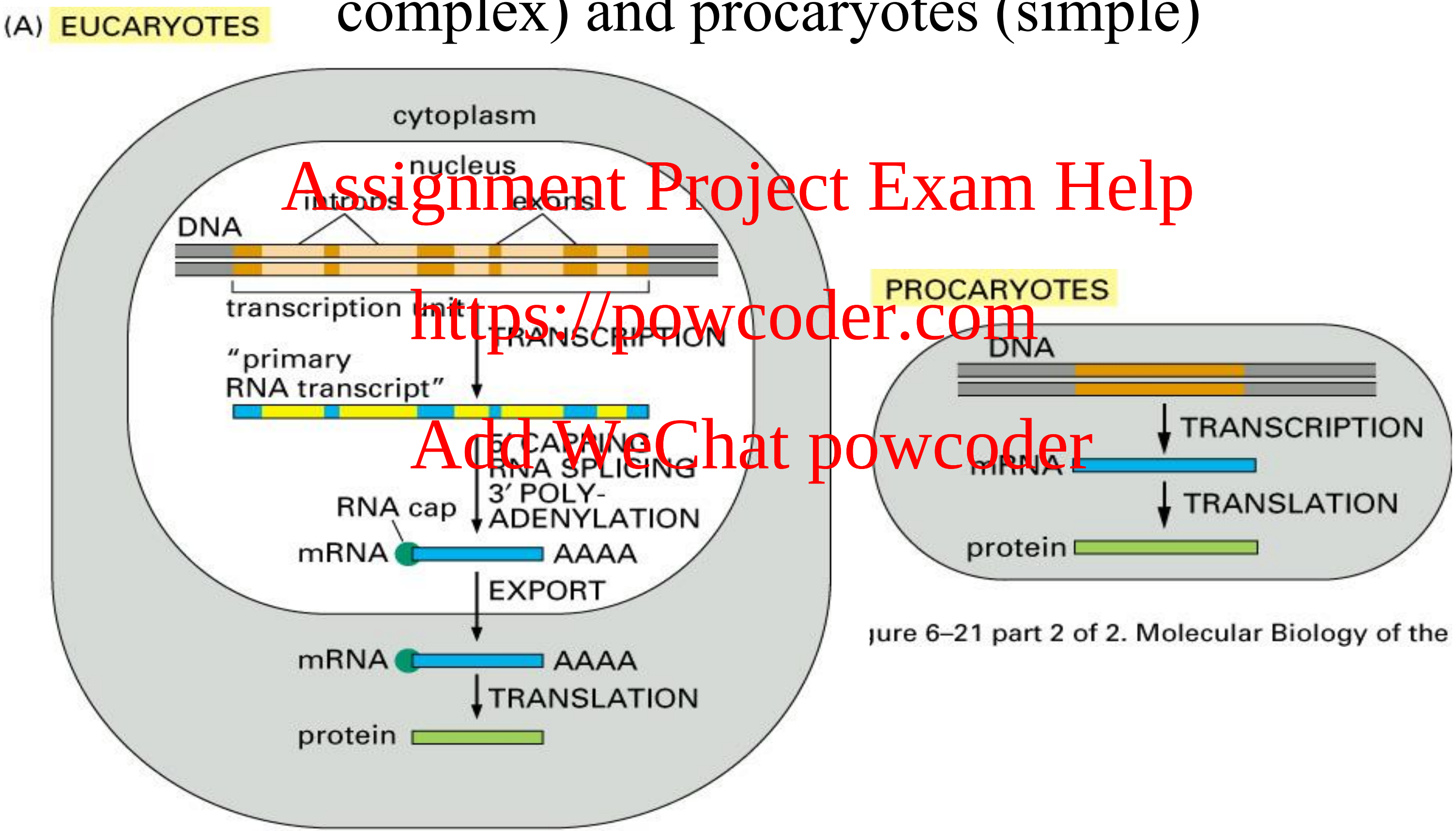
Initiation of transcription with RNA polymerase II in eucaryotic cells
Remember Nucleasomes
Enhancer, mediator, chromatin remodeling complex, histone acetylase



mRNA between procaryotic and eucaryotic cells
5’ capping and 3’ polyadenylation



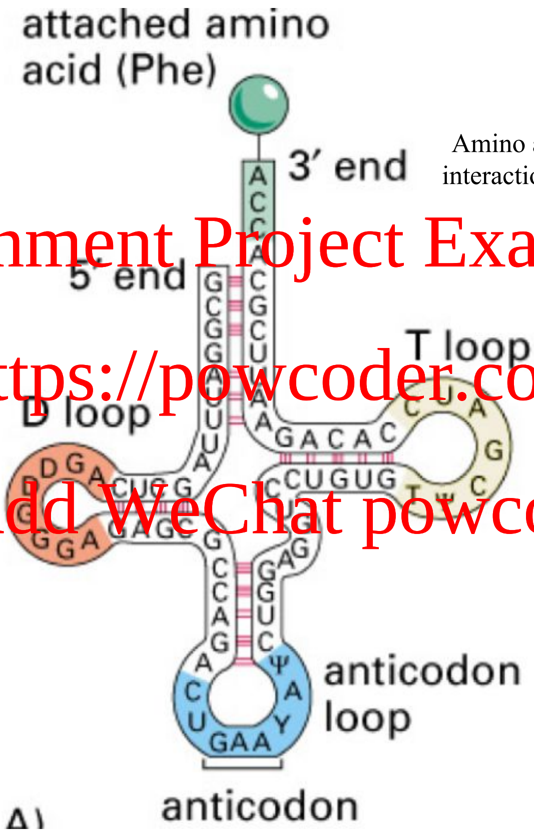
Genes to proteins
The comparison between eucaryotes (substantially complex) and procaryotes (simple)



ɟure 6–21 part 2 of 2. Molecular Biology of the

From RNA to Protein: Translation

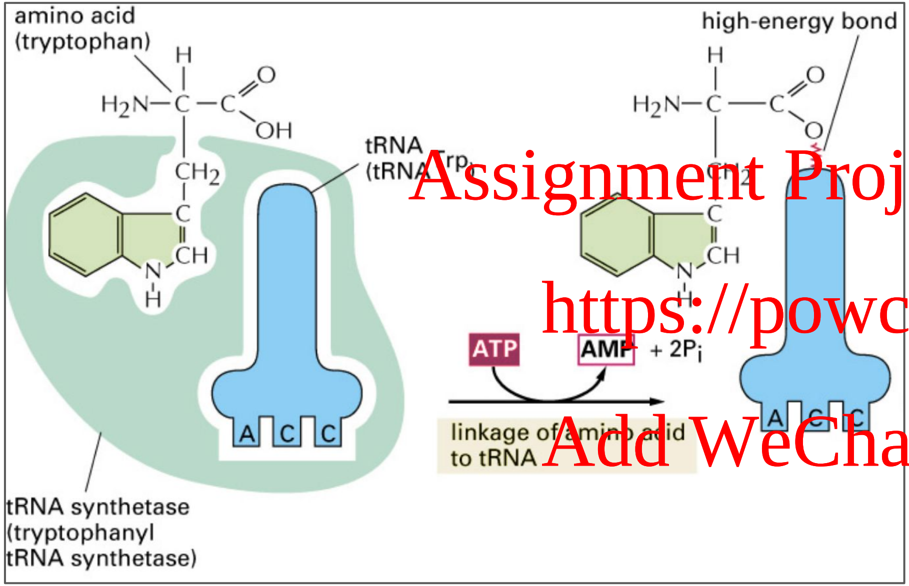
Key Player #1: Transfer RNA (tRNA)

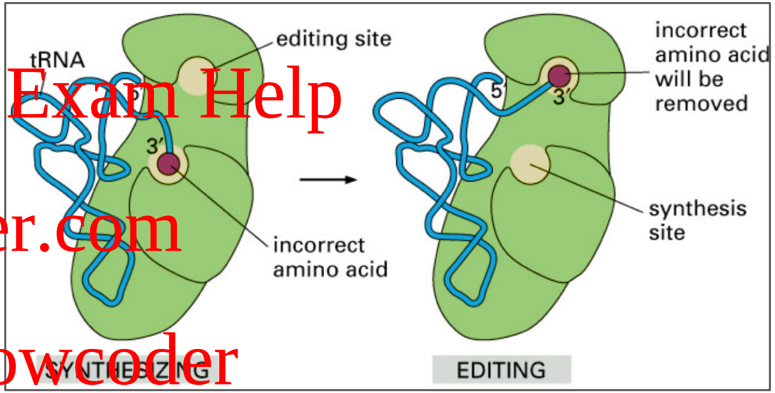


Amino acid attachment and interaction with the ribosome

Interacts with mRNA

Aminoacylation of tRNA by aminoacyl-tRNA synthetase





Proofreading ability for
translational fidelity

Key Player #2: Messenger RNA (mRNA)

The Genetic Code

Second letter

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

20 Amino Acids, 64 Codons
Redundancy but no ambiguity

Wobble position in codon

bacteria

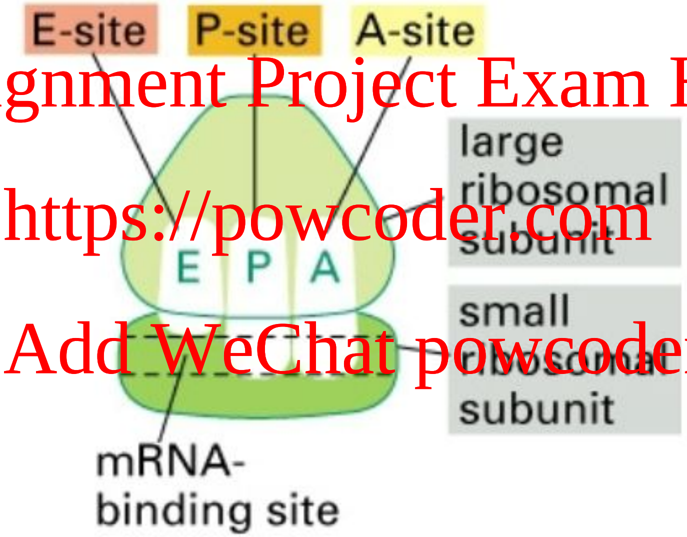
wobble codon base	possible anticodon bases
U	A, G, or I
C	G or I
A	U or I
G	C or U

eucaryotes

wobble codon base	possible anticodon bases
U	G or I
C	G or I
A	U
G	C

Key Player #3: Ribosome

Protein synthesizing organelle



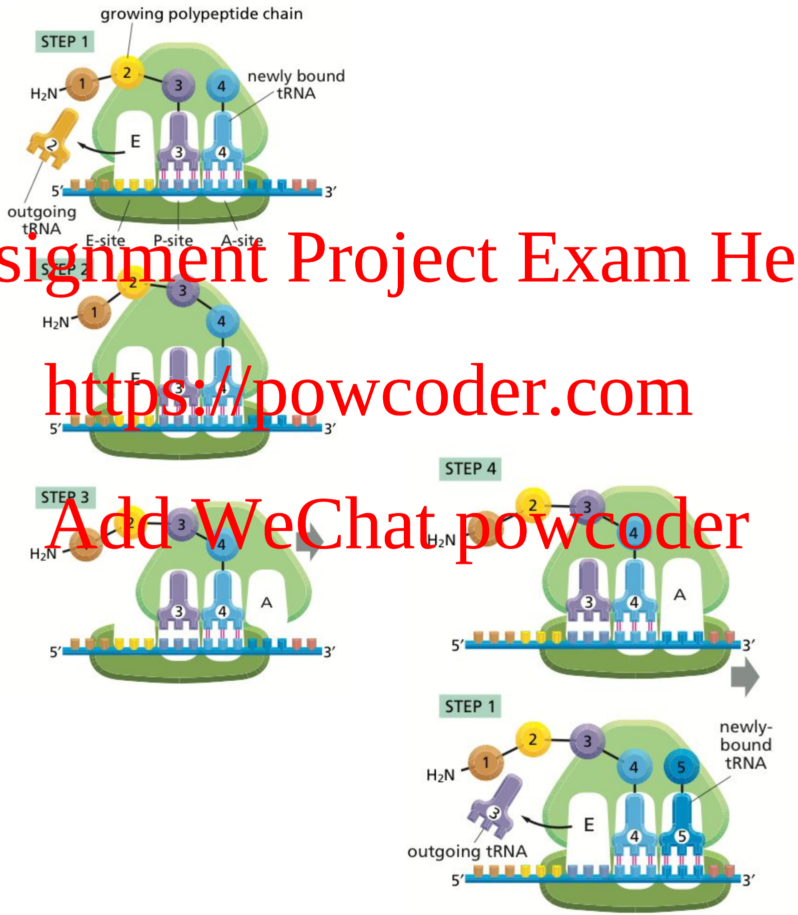
Aminoacyl site
Peptidyl site
Exit site

Ribosomal RNA (rRNA) - Binds to tRNA and mRNA to ensure accurate translation

Translation: Initiation, elongation, release

Start codon: AUG, codes for Methionine

Peptidyl transferase activity of large ribosomal subunit (ribozyme)



Ribosomal translocation

Stop Codons: UAA, UAG, UGA

From DNA to Protein: Techniques

Why do cells in your body behave differently despite having mostly identical genome?

Gene Expression Evaluation: An Overview

For each of the key technique, you need to master:

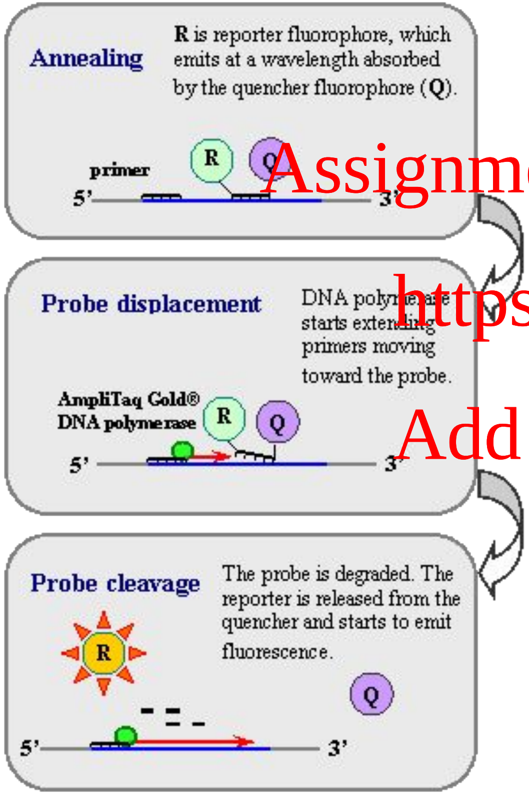
- Use cases
- Pitfalls
- Compensation methods

A good format to following when describing an experiment on the test:

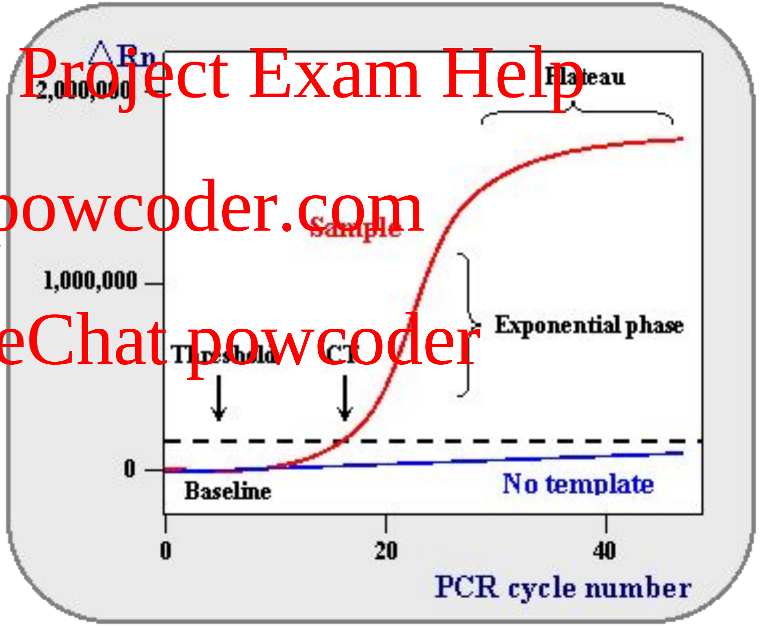
- Control/experimental groups
- Technique
- Expectation from analysis
- pitfalls

RT-qPCR: An Overview

TaqMan® Applied Biosystems



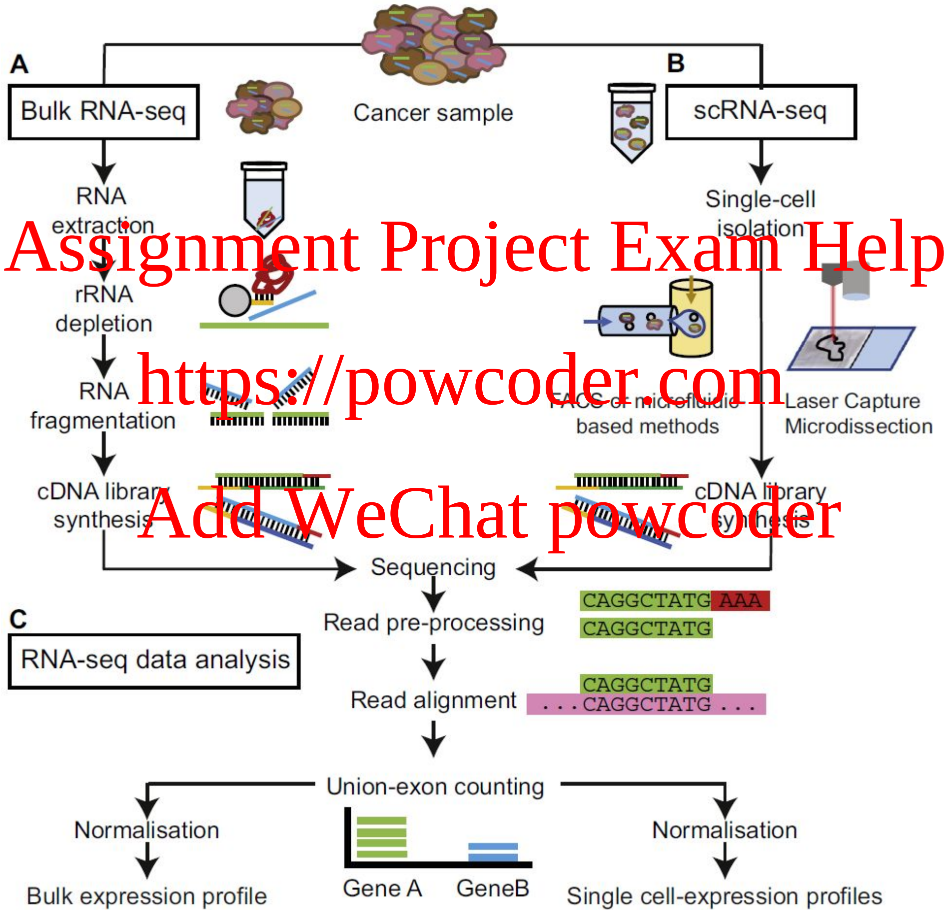
Model of real time quantitative PCR plot



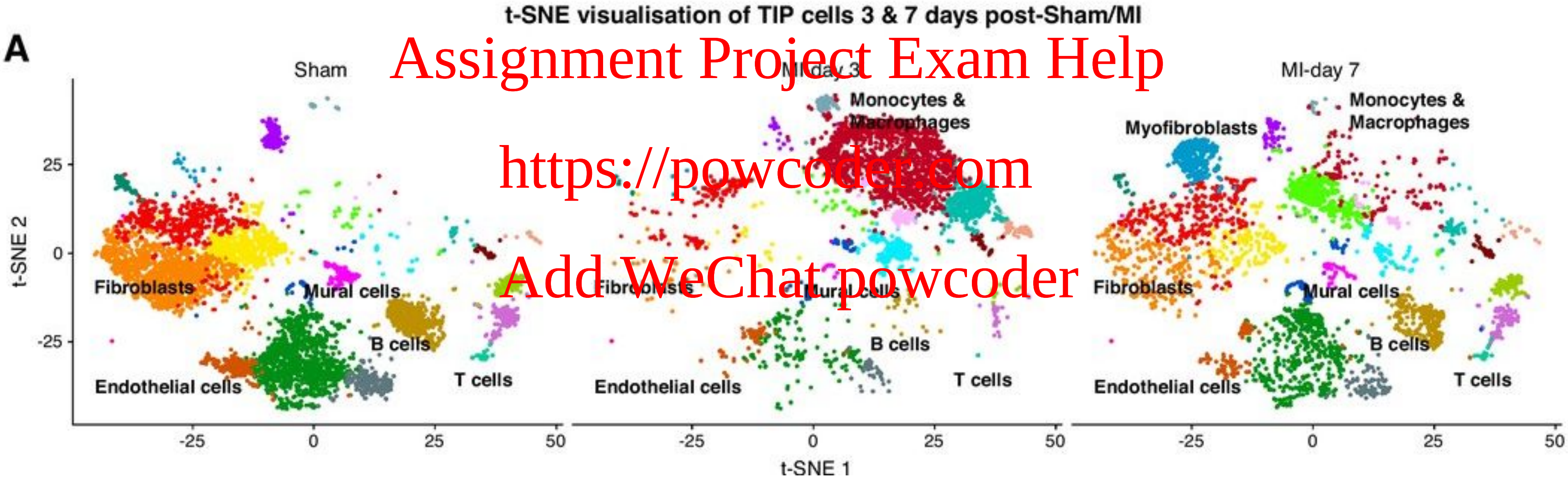
RT-qPCR: Things to Consider

- Limitations in target selection (primers)
- Scalability
- Normalization across samples/genes

Bulk v.s. Single-Cell RNA Sequencing

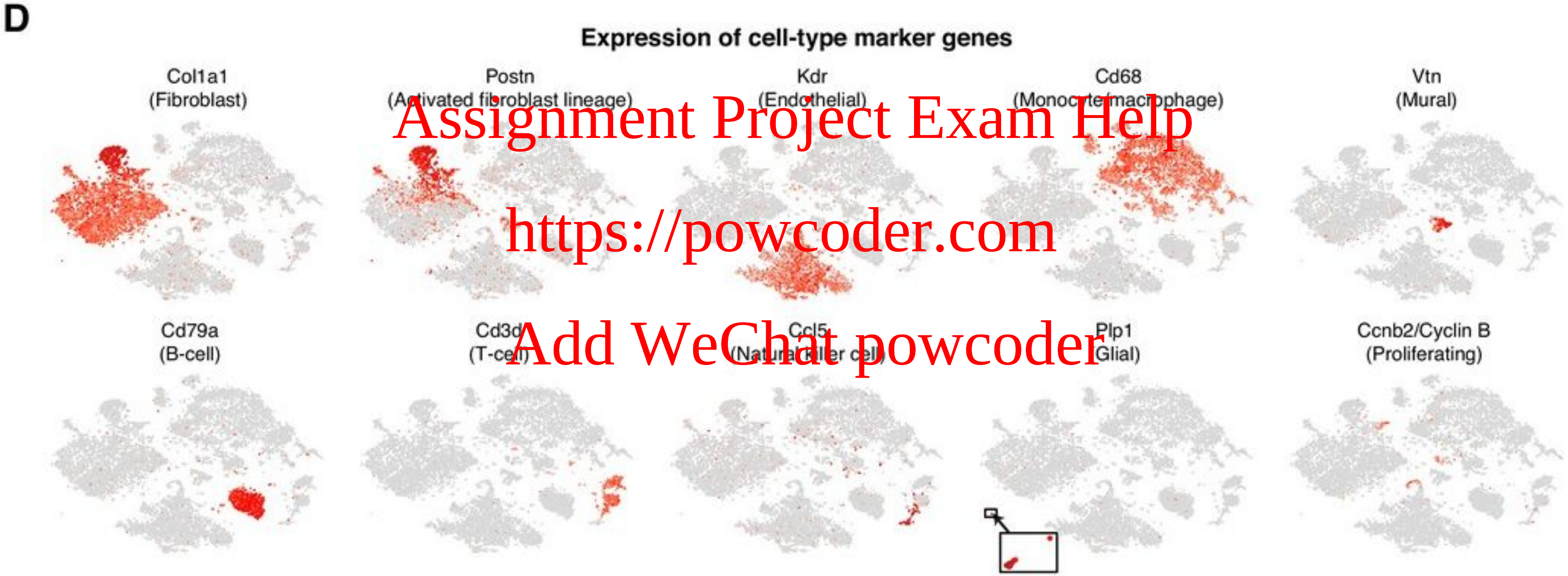


Sample scRNA-seq Output



Nona Farbehi, Ralph Patrick, Aude Dorison, Munira Xaymardan, Vaibhao Janbandhu, Katharina Wystub-Lis, Joshua WK Ho, Robert E Nordon, Richard P Harvey (2019) Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury eLife 8:e43882
https://doi.org/10.7554/eLife.43882

Sample scRNA-seq Output



Nona Farbehi, Ralph Patrick, Aude Dorison, Munira Xaymardan, Vaibhao Janbandhu, Katharina Wystub-Lis, Joshua WK Ho, Robert E Nordon, Richard P Harvey (2019) Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury eLife 8:e43882
https://doi.org/10.7554/eLife.43882

Bulk RNA-seq: Things to Consider

- Sequencing depth
- Read length
  - Gene1: ACAAA   Gene2: GGAAA
  - Read1: AAA   Read2: CAAA
- Normalization across samples
  - Gene1: 100/1000 reads Gene2: 200/2000
- Heterogeneity

scRNA-seq: Things to Consider

- Extreme cost
- Dropout effect
- Batch effect

Complete list of challenges can be found here:

[Eleven grand challenges in single-cell data science | Genome Biology | Full Text (biomedcentral.com)]

Cre and LoxP recombination