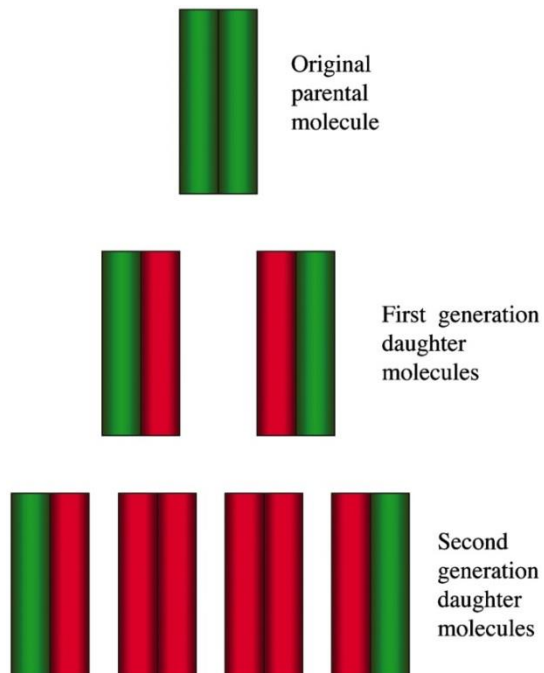


## Understandings:

### 1. Explain how DNA replicates in a semi-conservative manner and how it is achieved.

- First of all, we replicate DNA because our cells replicate through mitosis. Since daughter cells should have same genome as mother cell, DNA replication is crucial.

An overview is like this:



The original DNA splits and serves as a template for the daughter DNA.

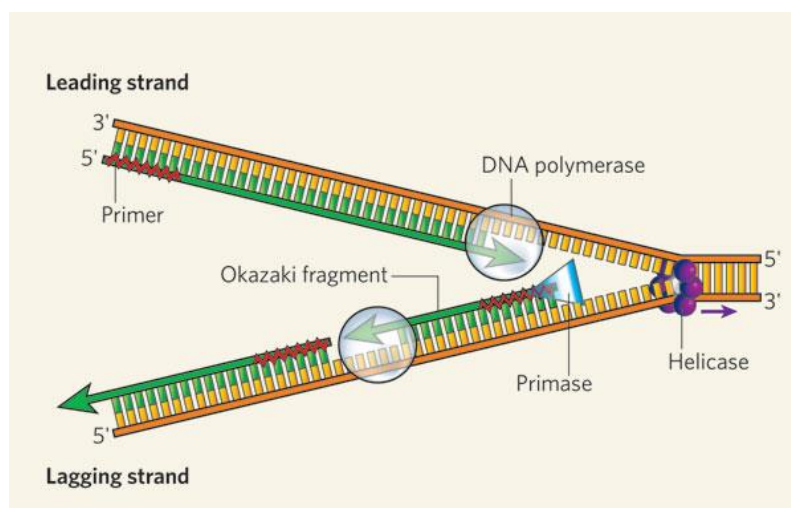
This works due to the complementary base pairing. Since it follows the rule of A-T and G-C, we preserve the sequence and hence the information.

### 2. Explain the role of helicase.

- This is an enzyme (-ase) that essentially breaks the hydrogen bonds and splits the strands. Helicase is the first step of replication since the DNA must split in order to be replicated. The key here is that DNA cannot split while it is still helical, but helicase unwinds and splits at the same time (but you will see in HL that gyrase helps out with the unwinding part too)!

Fantastic!

Studies have shown that helicase is composed of 6 globular polypeptides like many of enzymes, and they travel through the centre of DNA in the formation of a donut. Look at the picture below.



### 3. Explain the role of DNA polymerase.

- The picture above even includes polymerase, and it is quite easy to guess what it does. Yes, it links the free nucleotides, one by one, into these old strands, using it as a template. Thanks to its slow, meticulous process, few mistakes are made (no this is false lol. 2015 Nobel Prize showed that there were thousands of errors per cell each day).

The polymerase basically does two things. It ensures hydrogen bonding between complementary pairs, and then also the covalent bonds between the phosphate and 3' in the sugar.

The direction that the polymerase moves is in the direction of 5' 3' since that is the right direction for the new strand.

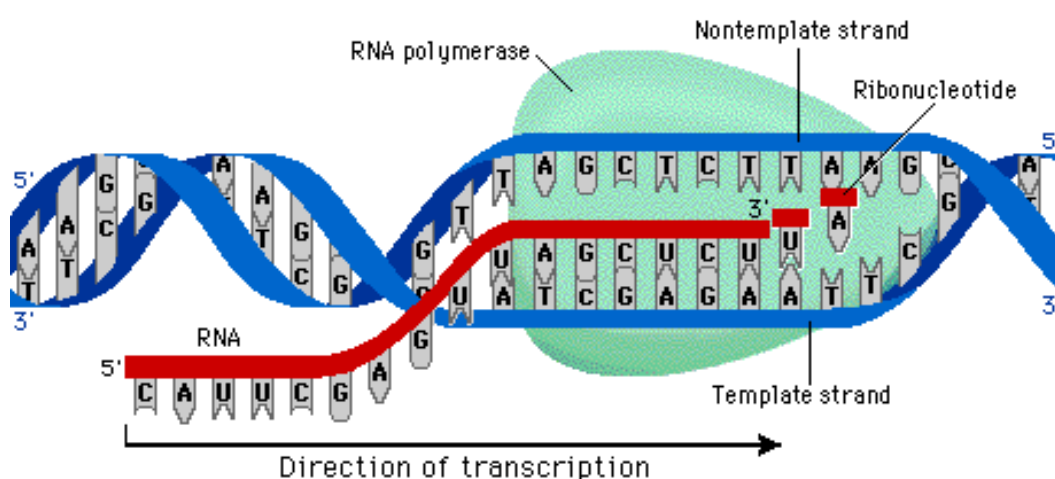
### 4. Explain the role and process of transcription.

- What is transcription? Transcription is just rewriting the language into similar sentences. In this case, it is from DNA to RNA. Why do we do this? Well, in order to make proteins, we need to somehow convert the information in our genes (section of DNA that stores information for amino acids) into real proteins! Transcription is the first step out of two.

Firstly, an RNA polymerase binds to DNA and moves along while splitting and pairing RNA nucleotides. Yes, it does both splitting and pairing so helicase is not needed apparently. So helicase is only for replication? Remember that RNA has uracil instead of thymine.

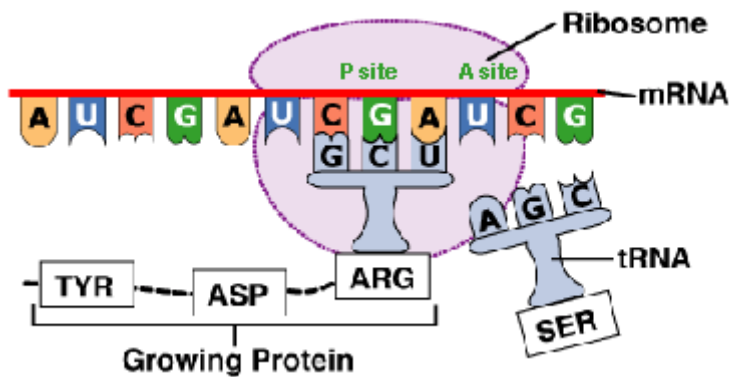
Secondly, the polymerase forms the covalent bond between the RNA nucleotides as well. So essentially, RNA polymerase does three things: split, form hydrogen bond, and covalent bond.

Thirdly, RNA molecule separates and DNA binds back into its double helical shape. It is only a section of the DNA that gets transcribed, not the whole DNA, thus RNA is released.



### 5. Explain the role and process of translation.

- This time, we need to take the RNA strand and make it into polypeptide. Essentially, the needed parts of RNA are transferred as mRNA (messenger RNA) to the ribosomes in our cells. Then, depending on the complementary codon, we form amino acid and eventually polypeptides.



### 6. Explain the role of mRNA and how it determines the sequence of amino acids.

- Briefly mentioned above, mRNA is RNA moving to ribosome for translation. And proteins are produced according to the sequence of mRNA. Why do we have it? As mentioned, we have bunch of different genes that code for different polypeptides. But we do not use all of them at the same time right? So it would be such a waste of energy and time to completely transcribe DNA into RNA if we are only going to use a fraction of it. Cells might have different jobs and we might need different proteins at a particular instant. Then the cell can just make more copies of that mRNA rather than taking directly from the DNA.

#### Extra notes

- We might be asked to explain the structure of the ribosome and there are few things to mention here.

1. It is a protein structure.
2. It contains rRNA.
3. It is made of small and large subunit, and mRNA binds to the small subunit.
4. It has three binding sites: A, P and E.
5. Sizes are different in eukaryotes and prokaryotes.
6. Depending on where protein is going to be used, ribosome may be free or in rough ER.

## 7. Explain what codons are.

- Codons are sequences of three bases in mRNA that correspond to 1 amino acid. Why three? Well, we have 20 (most common) amino acids and only 4 bases. 1 base coding for 1 amino acid only makes 4 amino acids. 2 bases coding for 1 amino acid only makes  $4 \times 4 = 16$  amino acids. 3 bases coding will give us  $4 \times 4 \times 4 = 64$  amino acids, yes!

But notice that 64 are now too much, which means that there must be overlaps.

Notice that there are stop codons. These determine where to start the triplet/where to end.

		Second base of codon									
		U		C		A		G			
First base of codon	U	UUU	Phenylalanine phe	UCU	Serine ser	UAU	Tyrosine tyr	UGU	Cysteine cys	U	Third base of codon
		UUC		UCC		UAC		UGC		C	
		UUA	Leucine leu	UCA		STOP codon	UGA STOP codon	UGG Tryptophan trp	A		
		UUG		UCG					UAG	G	
	C	CUU	Leucine leu	CCU	Proline pro	CAU	Histidine his	CGU	Arginine arg	U	
		CUC		CCC		CAC		CGC		C	
		CUA		CCA		CAA	Glutamine gin	CGA		A	
		CUG		CCG		CAG		CGG		G	
	A	AUU	Isoleucine ile	ACU	Threonine thr	AAU	Asparagine asn	AGU	Serine ser	U	
		AUC		ACC		AAC		AGC		C	
		AUA		ACA		AAA	Lysine lys	AGA	Arginine arg	A	
		AUG met (start codon)	ACG	AAG		AGG		G			
	G	GUU	Valine val	GCU	Alanine ala	GAU	Aspartic acid asp	GGU	Glycine gly	U	
		GUC		GCC		GAC		GGC		C	
		GUA		GCA		GAA	Glutamic acid glu	GGA		A	
		GUG		GCG		GAG		GGG		G	

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## 8. Explain how codons and anticodons work together.

- To finish off the translation part, when mRNA gives the template for amino acid, tRNA comes with anticodons (complementary codons) and binds. The location of this is as mentioned in the ribosome.

## Applications and skills:

### 1. Explain the use of Taq DNA in the PCR.

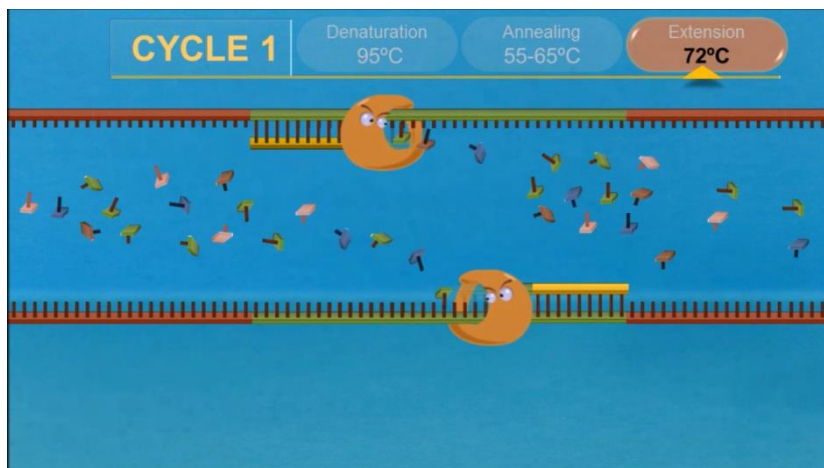
- What is PCR? It stands for polymerase chain reaction, which is basically a DNA copy machine. You put in a desired sequence and get out very many! But how exactly does it do?

Well, instead of using helicase to separate, the DNA is heated (in 95°C for 15 seconds) until the hydrogen bonding is broken, just like boiling water! Then it is cooled quickly (down to 54°C) to enable a small sequence called primers to bind with the desired DNA sequence.

There are two reasons for primers:

1. Polymerase can only know where to start when primers are present (the picture above shows that as well).
2. It prevents re-annealing of the parent DNA strands.

Now that we have two DNA with a primer, a special polymerase, called Taq (heat resistant polymerase, found in bacteria living in hot springs) rapidly replicates the DNA. 1000 nucleotides/minute!



This whole process takes around 2 minutes and this can be repeated over and over again to grow the target DNA sequence into millions and billions.

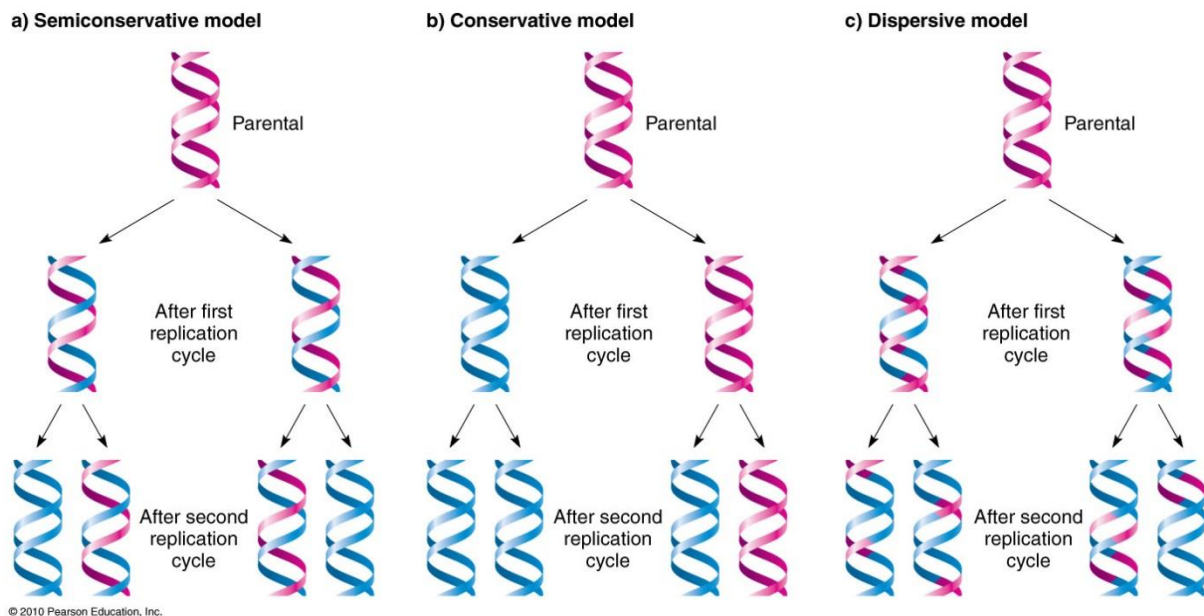
### 2. Explain how we use the universality of genetic code, give example using human insulin.

- The codon map showed above for mRNA is fundamentally very similar to all living organisms, and due to this phenomenon, genetic engineers have been able to treat problems regarding proteins.

An example is diabetes. Diabetes type 1 is when the immune system does not recognize beta-cells and kill them. Thus injections of insulin are needed for those suffering. However, where do we get the insulin? Due to the universal codon map for most of the organisms, we have been able to grow and harvest same polypeptide as human insulin in E.Coli and probably other as well. Thus without making harm to other humans, we have been able to copy the insulin.

### 3. Understand the Meselson and Stahl experiment, and the underlying principles they used.

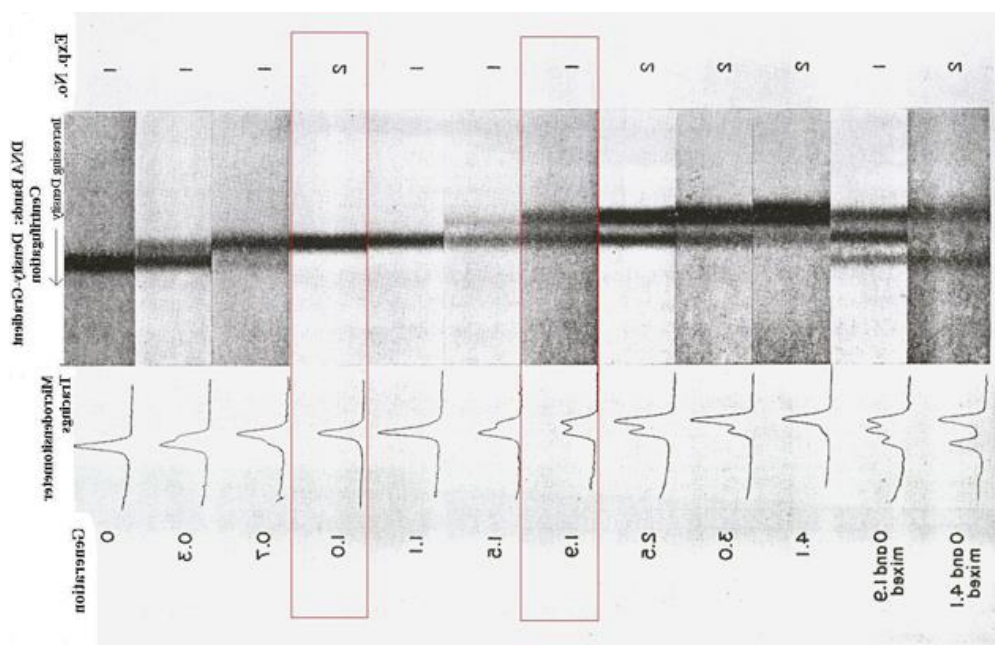
- Up until the middle of 20<sup>th</sup> century, people were unsure how the DNA was replicating. Some options were semi-conservative, conservative and dispersive.



However, Meselson and Stahl came up with an elegant experiment that could determine which method DNA used.

What they did was to use the principle of mass and density of isotopes. Here is a step by step what they did.

1. They exposed DNA strand to N-15 medium for several generations to ensure that most of the DNA was now containing N-15. (Nitrogen is essential for the base in nucleotide.).
2. The DNA if N-15 was dumped in a N-14 medium. They knew that replication time was 50 minutes; hence each generation took 50min. They collected sample for each generation.
3. Now what did they do with the samples for each generation? They just centrifuged it in CsCl! The picture is original results for the experiment.



At generation 0, all DNA is heavy.

At generation 1 (highlighted in red) is a hybrid of N-14 and N-15.

At generation 2, the ones with N-15 is still hybrid but N-14 will form newer, lighter ones.

Hence two different densities.

At later generations, the thickness of N-14 will grow, while N-15 hybrid will remain the same.

**4. Be able to analyse the data from Meselson and Stahl's experiment.**

- Yes, it is done above.

**5. Be able to use a table of mRNA codons to deduce which codon represents which amino acid.**

- Yes, the table is shown above.

**6. Deduce DNA sequence for mRNA strand.**

- DNA sequence for mRNA strand is just its antisense strand. This just means the mRNA's complementary sequence, with T instead of U!

But be aware that they may ask you for the sequence of the DNA sense strand from the given RNA. In that case, it is not its complementary sequence. It is exactly the same sequence with T instead of U. Read the questions carefully!