CH1010 Tutorial 9 Answers

1. Provide the **codons** in the **DNA template** and **mRNA** transcribed from it for the following decapeptide: VHCIGAKAPN
   - Make sure that you include a start codon and a stop codon.

Peptide:	Start	Val	His	Cys	Ile	Gly	Ala	Lys	Ala	Pro	Asn	STOP
mRNA:	AUG	GUU	CAU	UGU	AUU	GGU	GCU	AAA	GCU	CCU	AAU	UAA
DNA template:	TAC	CAA	GTA	ACA	TAA	CCA	CGA	TTT	CGA	GGA	TTA	ATT

There are many other possibilities as none of these AAs are coded by 1 codon only, there are 4 triplets for Val for instance.. There are 3 STOP codons (UAA, UAG, UGA).

2. Illustrate what effect the following mutations would have on the **transcribed peptide**:

ATG ACT AAA AGT GTT DNA coding strand
ACU AAA AGU GUU mRNA
Thr Lys Ser Val

ATG AC**C** AA**C** AGT GTT DNA coding strand, point mutations to C
Thr Asn
Silent

ATG **G**ACT AAA AGT GTT DNA coding strand, insertion mutation, inserting G
ATG **G**AC TAA AAG TGT TUA A reading frame offset, Stops after 1 triplet
Asp STOP

- What **physico-chemical factors** produce mutations?

Mutation: a heritable change in the base sequence of DNA. Caused by either: 1) mistakes made by DNA polymerase during replication, 2) UV light or 3) ionizing radiation, 4) mutagenic chemicals, such as halogenated organic compounds – CCl_4, aromatic compounds – C_6H_6, methylating agents – $(CH_3O)_2S(=O)_2$ DMS and intercalating agents – ethidium bromide.

3. Explain why **PCR methods** are an example of cloning and how the PCR method works.

A cloned population is a a genetically identical population. In molecular biology, cloning is used in two contexts: cloning a gene (DNA fragment), or cloning of an organism. Cloning in the case of PCR is then read in the context of cloning a gene or fragment of DNA.

Into a PCR Thermocycler are placed the DNA fragments, *Taq* polymerase and the 4 DNA nucleotides (dATP, dTTP …).
1) Denature: the fragment is unwound to form 2 template strands at 95^{o}C for a couple of minutes.
2) Anneal: Two primers (around 14 nucleotides long) are added at 50^{o}C and hydridized with the templates.
3) Synthesize: The temperature is raised to 72^{o}C and *Taq polymerase* extends the chains from where the primers stop using available nucleotides.
There are now 2 complete copies of the DNA fragments. Now loop back to 1.
After 25 cycles there will be enough DNA (2^{25} copies) from 1 hair follicle to carry out a separation of the fragments for DNA fingerprinting. Around 30 cycles can be carried out in an hour and so from 1pb $\rightarrow$ 1ug can be obtained using the usual 10^6 amplification.

4. **DNA replication** is a semi-conservative, semi-discontinuous process – what does this mean? In your answer go over the major steps in replication and indicate where the process is semi-discontinuous.

DNA replication is semi-conservative as a template strand is used to create the new daughter strand ensuring a very low error count in the replication process.

DNA replication occurs in 6 major steps: opening superstructures (chromosomes), relaxing higher DNA structures (supercoil DNA), unwinding (DNA double strands), complexation of primers (lagging strand synthesis), DNA polymerase syntheses, ligation (Okazaki fragments joined). The protein machine that carries this out is called a replisome which contains all the enzymes required for the process.

In the unwinding of DNA two strands are formed a leading strand (5' $\rightarrow$ 3') and a lagging strand (3' $\rightarrow$ 5'). Each strand is then read as a template strand and so the process is semi-conservative on both strands of DNA>
The DNA polymerase has two sliding clamps which move along the unwound DNA strands (powered by ATP). In the leading strand then synthesis proceeds in a continuous manner towards the replication fork as the template unwinds in the direction 5' $\rightarrow$ 3' (only 1 RNA primer is needed at the start for the entire strand). DNA polymerase III is a homodimer, there are two cores that carry out the DNA polymerisation. Leading strand synthesis occurs at one polymerase core. In the lagging strand synthesis the complexation of a short stretch of nucleotides primers (RNA) occurs once every second at the replication fork (catalysed by primase). Another 200 nucleotides are added – in the 5' $\rightarrow$ 3' direction (the Okazaki fragment) using the other core of the DNA polymerase III. This is running in the opposite direction to the leading strand synthesis, away from the replication fork. Once this core reaches the previous primer section the core releases from the template and returns to the new primer to begin the synthesis of another Okazaki fragment. This process then is semi-discontinuous in the lagging strand synthesis as the DNA polymerase III runs along the lagging strand for a stretch, stops and then jumps to the next position and runs again.