Question 1:

a) What reagents are needed to carry out a polymerase chain reaction?

Template DNA, synthetic oligonucleotide primers complementary to the template DNA. dNTPs, buffer/magnesium ions, thermostable DNA polymerase.

b) PCR can be used to fuse different DNA fragments together. Explain why it cannot be used to directly construct megabase-scale DNA constructs.

PCR reactions cannot produce fragments more than a few kilobases in length because the DNA polymerases used are not processive enough.

c) A PCR reaction is set up to amplify a human exon from a blood sample. When the products of the PCR reaction are separated by agarose gel electrophoresis two bands are seen: one is the expected size and the other is slightly smaller. Provide a possible explanation for this result.

Many possible answers but likely ones are: there has been a mis-priming event somewhere else in the genome, possibly with a related gene or pseudogene; one primer may recognise a repetitive sequence in the gene allowing two priming events; there are two variants of the gene of different length.

d) A PCR reaction is set up but inadvertently the researcher adds 100 fold too little of one of the primers. Describe the course of the subsequent reaction and the most likely dominant product that will be generated.

The PCR reaction will start as usual with exponential amplification of the target region. However when the lower concentration primer is exhausted, the remaining primer will result in linear amplification of one strand, which is therefore likely to be the dominant product of the reaction.

Question 2:

a) What are the three key components of evolution by natural selection?

mutation/ variation in population; consequent phenotype change/ variation; natural selection for the fittest (most reproductively successful)

b) Suppose two independent mutations arise in a large (~10^12 cells) population of bacterial cells grown in continuous liquid culture, seeded from one colony.

Both mutations allow the cells containing them to grow faster but the effect is significantly stronger in mutation 'A' than in mutation 'B'. How do you expect the frequency of each mutation within the population to change over time?

The frequency of both variants should rise in the population. This will be quicker for variant 'A' which is likely to take over the entire population.

c) In the same population as in part (b) a mutation arises that does not confer any growth advantage or disadvantage. How do you expect the frequency of this variation to vary with time?

The frequency of the variation will take a random walk. When it arises it is present at a very low frequency and is likely to be lost

on dilution of the culture during growth. However in principle it could eventually take over the population.

d) A cell-surface protein similar to an antibody binds gold with low affinity. A researcher would like to evolve variants that bind gold more strongly with a view to purifying gold from sea water. Outline a directed evolution strategy to achieve this.

Various answers possible but a good one would involve repeated rounds of: mutation followed selection for ability to bind immobilised gold. Cells with higher binding efficiency are enriched in the population in successive cycles of mutation and selection.

Question 3 = JC's question

Question 4:

a) A genetic engineer wants to express the peptide encoded by the DNA sequence below. Referring to the codon table supplied at the end of the paper, what amino-acid sequence is encoded in the DNA sequence below? Assume that the first codon starts at the first base.

GCCCUUAUCGGCAAUGACAACGCG

Answer: A L I G N D N A

b) How would the above sequence need to be adapted so that the peptide is properly translated?

Ribosome binding site then start codon (ATG) at the start, and stop codon at the end.

c) The researcher wishes to maximise expression of the peptide in malaria, the codon usage table of which is given below. Write down the DNA sequence most likely to result in high expression given the information available.

Students should pick the most-used codons i.e.
A L I G N D N A
Optimised:
GCA UUG AUU GGA AAU GAU AAU GCA

d)

i) Outline what factors, other than codon usage, could affect protein expression yield.

Many possible - most likely are: promotor strength, ribosome binding site strength, growth conditions (log phase is best), RNA secondary structure in the transcript can inhibit translation.

ii) A different codon optimised recombinant DNA construct was expressed in Bacillus, but although the protein was produced, in fact it was at lower levels than an identical non-optimised construct. What is the most likely explanation for this?

RNA secondary structure as the constructs are otherwise identical.

Plasmodium vivax codon table showing codon usage

Columns:
Codon, single letter amino-acid code, frequency of codon usage

UUU F 22.6	UCU S 9.1	UAU Y 15.1	UGU C 9.9
UUC F 17.0	UCC S 13.7	UAC Y 26.8	UGC C 10.5
UUA L 13.7	UCA S 9.2	UAA * 0.9	UGA * 0.2

UUG	L	18.9	UCG	S	8.2	UAG	*	0.3	UGG	W	4.9
CUU	L	9.0	CCU	P	5.7	CAU	H	6.9	CGU	R	1.5
CUC	L	14.0	CCC	P	9.4	CAC	H	10.2	CGC	R	2.7
CUA	L	10.0	CCA	P	13.4	CAA	Q	20.6	CGA	R	2.3
CUG	L	17.4	CCG	P	4.1	CAG	Q	14.8	CGG	R	1.4
AUU	I	22.7	ACU	T	11.8	AAU	N	34.8	AGU	S	12.7
AUC	I	16.9	ACC	T	15.4	AAC	N	32.7	AGC	S	17.8
AUA	I	16.5	ACA	T	14.3	AAA	K	53.7	AGA	R	10.9
AUG	M	19.6	ACG	T	11.1	AAG	K	51.3	AGG	R	8.6
GUU	V	12.8	GCU	A	11.7	GAU	D	28.6	GGU	G	10.9
GUC	V	10.0	GCC	A	16.3	GAC	D	26.2	GGC	G	12.4
GUA	V	15.0	GCA	A	26.6	GAA	E	54.1	GGA	G	22.4
GUG	V	19.7	GCG	A	13.3	GAG	E	34.4	GGG	G	10.6