**DNA Replication and PCR**

James is a Huntington disease patient who has recently been institutionalized and requires constant nursing care. His wife is unaffected, and they have a 38-year-old daughter, Delilah, who has 4 children ranging in age from 8 to 15 years. Delilah wishes to know whether she has inherited the disease-producing allele from her father.

Mutations in the HTT gene cause Huntington disease. The HTT mutation that causes Huntington disease involves a DNA segment known as a CAG trinucleotide repeat. This segment is made up of a series of three DNA building blocks (cytosine, adenine, and guanine) that appear multiple times in a row. Normally, the CAG segment is repeated 10 to 35 times within the gene. In people with Huntington disease, the CAG segment is repeated 36 to more than 120 times.

A blood sample from Delilah was tested for the CAG mutation using PCR. PCR amplification is carried out on the region containing the CAG repeat shown below.

1. Which of the following set of primers would you use to amplify the CAG repeat in the brackets (CAG)n? Explain.

**5’-CTC AAG TCC TTC (CAG)n CAA CAG CCG CCA-3’**

PRIMER OPTIONS:

1. 5’-GAG TTC AGG AAG-3’ & 5’-TGG CGG CTG TTG-3’
2. 3’-GAA CTC CCT GAG-5’ & 5’-CCG CCA CCG CCG-3’
3. 5’-GAA CTC CCT GAG-3’ & 3’-TGG CGG CTG TTG-5’
4. 5’-CTC AAG TCC TTC-3’ & 3’-TGG CGG CTG TTC-5’
5. 5’-CTC AAG TCC TTC-3’ & 5’-TGG CGG CTG TTG-3’
6. 5’-CTG CTG CTG CTG-3’ & 5’-CAG CAG CAG CAG-3’

2. Once PCR is completed, how can you tell whether the amplicons contain >36 CAG repeats?

3. If you started with 5 molecule of the DNA sequence shown above (*only one strand is shown*), how many molecules of DNA in total would you obtain at the end of 15 PCR cycles? Show your calculations.

4. If you started with 0.5 ng/ml of the DNA sequence shown above, what would be the expected amount of the following after 10 PCR cycles (show your calculations):

a- Each of the original DNA templates:

b- Single stranded DNA fragments of indeterminate lengths:

c- DNA duplex fragments of defined length (amplified CAG repeats and their complementary repeats) only: