PCR CAG HUNTING'ton Activity

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.

5'…CTC AAG TCC TTC $(CAG)_n$ CAA CAG CCG CCA… 3'

Which of the following set of primers would you use to amplify the CAG repeat in the brackets $(CAG)_n$?

5'-CTC AAG TCC TTC $(CAG)_n$ CAA CAG CCG CCA-3'

A. 5'-GAG TTC AGG AAG-3' & 5'-TGG CGG CTG TTG-3'
B. 3'-GAA CTC CCT GAG-5' & 5'-CCG CCA CCG CCG-3'
C. 5'-GAA CTC CCT GAG-3' & 3'-TGG CGG CTG TTG-5'
D. 5'-CTC AAG TCC TTC-3' & 3'-TGG CGG CTG TTC-5'
E. 5'-CTC AAG TCC TTC-3' & 5'-TGG CGG CTG TTG-3'
F. 5'-CTG CTG CTG CTG-3' & 5'-CAG CAG CAG CAG-3'
G. None of the above

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

If you started with 0.5 ng/ml of the DNA sequence shown above, what would be the expected amount of each of the original DNA templates after 10 PCR cycles?

A. 5.02 ng/ml
B. 502 ng/ml
C. 512 ng/ml
D. 1004 ng/ml
E. 1024 ng/ml
F. None of the above

If you started with 0.5 ng/ml of the DNA sequence shown above, what would be the expected amount of single stranded DNA fragments of indeterminate lengths after 10 PCR cycles?

A. 1 ng/ml
B. 10 ng/ml
C. 20 ng/ml
D. 100 ng/ml
E. None of the above

If you started with 0.5 ng/ml of the DNA sequence shown above, what would be the expected amount of DNA duplex fragments of defined length (amplified CAG repeats and their complementary repeats) only after 10 PCR cycles?

A. 1004 ng/ml
B. 1024 ng/ml
C. 2008 ng/ml
D. 2048 ng/ml
E. None of the above