

Name \_\_\_\_\_ Section (day) \_\_\_\_\_

1) Name the organism that is the source of the cDNA clones that you are sequencing. (one point)

*Caenorhabditis brenneri*

2) How are the DNA chains separated for DNA sequence analysis? (one point)

Chains are electrophoresed on a gel or column, fluorescent products coming off the gel are read with a laser.

3) Can this nucleotide be used for **chain termination** in sequence analysis or not? **STATE WHY**, or you will not get credit (six points).

A. No—it has a 3' OH on the ribose sugar and will not terminate the chain.

B. Yes--this is a ddNTP, with no 3' OH the chain cannot be extended.

C. No—this ddNTP lacks the tri-phosphate residue needed to provide the energy to join the chain in the first place.

4) Name the two critical features of di-deoxy NTPs that enable DNA sequence to be determined. (3 points)

-The lack of the 3' OH on the ddNTP sugar causes chain termination.

-Fluorescent tags are included on each ddNTP (4 different colors for 4 different bases) to enable the identification of the terminated chain as ending in A, C, G, T.

5) Consider these potential outcomes of mistakes in sequencing:

-No sequence at all - Very small fragments- Missing information for one nucleotide- -mostly illegible sequence data- -average fragment length becomes shorter- -Average fragment length becomes longer- -No apparent sequence but long fragments are synthesized- sequence reaction is fine

(4 points) Describe what happens and **WHY** if:

A) You add the PCR forward and reverse primers to your DNA sequencing reaction.

Mostly illegible data since you would get two sequence reactions, one primed on the forward primer and one primed on the reverse primer, but products would run on top of each other.

B) Your miniprep DNA was not eluted from the column.

No sequence as a template is needed for DNA sequence analysis.

C) Your plasmid did not include a cDNA insert.

In this case, you will still read the sequence, although it will be of the cloning vector. The sequence reaction is fine, however.

D. You forgot to add the ddNTPs until your lab partner reminded you—so you added them after the first half hour of the reaction.

This would mean that chains would not terminate for a half hour. Exactly what would happen would depend on the kinetics of the reaction, but you could assume that the average chain becomes longer since termination was not possible in the first 30 minutes.