Sanger Sequencing Activity

The purpose of this activity is to help develop understanding of the basic concept underlying dideoxy, chain-termination sequencing. This idea is the basis for many of the sequencing techniques commonly used today. This reaction is almost identical to a PCR, except that dideoxy forms of the nucleotides (the letters A, C, T, G) are included. When these ddNTPs are added to a growing strand, the Taq polymerase cannot add any more dNTPs and the chain is terminated. The resulting PCR products are fragments of all different length. If only one ddNTP is added to a reaction, then you know what letter ends each strand. Then by sorting the fragments by length, you can decipher the sequence.

Materials:

- 1 page of the sequence per student, working in teams of 4
- Sheet of graph paper on which to arrange sequences
- Glue, for sticking fragments to paper
- Ruler, for lining up fragments

Instructions:

- On the following sheet are several copies of the same sequence.
- First, cut out each line. Next, cut each of the lines/sequences at one random place. Make fragments of all different lengths, being sure that each one ends in a letter (i.e. don't cut through the middle of a letter).
- Throw out the 5' end and save the 3' end. These are your reaction products.
- Combine your fragments with those of your team-members and sort ALL the fragments into four groups:
    - End in A
    - End in T
    - End in C
    - End in G
- If these were dideoxy sequencing reactions, these are the kind of fragments you would find in your reactions.
- Each team member picks a "letter" and then arranges all the fragments in order of length
    - Glue the fragments to the graph paper
    - Make sure the 3' end is lined up evenly so lengths are easy to compare
- Sequence your gene by determining the last letter of the shortest strand, then find the last letter of the next longest strand
    - If there are any "holes", mark them with an X
    - The earliest sequences were done by hand, much in the same way you have sequenced this gene

Alaska BioPREP is funded by Science Education Partnership Award (SEPA) 5R25RR024282-02
from the National Institutes of Health (NIH)

Questions:

Did you get a full sequence?

- If you didn't, what do you think you could do to make sure you got a full sequence?

Why do you need ddNTPs?

- Why can't you just read off the letters?

Why do you need to set up four different reactions?

- Why not just put all four ddNTPs in the same reaction?

What is one easy way to sort all these fragments by size?

- Do you see any potential problems with this method if you were sequencing all 3 billion base pairs of the human genome?

Alaska BioPREP is funded by Science Education Partnership Award (SEPA) 5R25RR024282-02 from the National Institutes of Health (NIH)

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

3’ – gttctttcatgggggagcagatttgggtacacccaagtattgactcacccatcagcaaccgct – 5’

Alaska BioPREP is funded by Science Education Partnership Award (SEPA) 5R25RR024282-02 from the National Institutes of Health (NIH)