College of the Canyons: Introduction to Biotechnology

PCR Post Lab Write Up

1. What is an alu insert? How many alu inserts do humans have? What were the two alu inserts looked at in lab?

ALU insert is a remnant of a retrovirus, tPA-25-ALU is unique to humans. Humans have over 300,000 copies of alu, they are thought to have been inserted into vertebrate line early in primate evolutionary history.

2. What (if any) link do the two alu inserrts have to human biology? (i.e. harmless, affect disease, gene expression, etc).

a. Usually harmless as most alu inserts lad in portions of the genome that have no impact on gene expression.

b. Occasionally land in areas near genes and may impact gene expression / physiology. One alu insert lands near the region called TPA, which stands for tissue plasminogen activator. The TPA gene relates to proteins expressed after recovered from a cardiac incident. Another affect the gene which can cause "neurofibromatosis". This gene's product is related to bone growth and in extreme cases, causes the condition know as "elephant man's disease".

3. What purpose does the Chelex solution serve? Why is it needed when the cells are first ruptured? Why must it NOT be present in the final PCR reaction tube.

Chelex is a cationic resin that removes divalent cations: Mg^{++} or Ca^{++}. These ions are cofactors in proteases and DNA-ases which could cut up DNA (so chelex effectively deactivates them). So this is a good thing when the cells are first ruptured as the DNA will not be degraded from the cells own enymes. The Chelex bead will bind with Mg++ and effectively deactivate DNA polymerase (the TAQ in PCR). So..if it is present in the PCR tube, no PCR reaction.

4. Describe the function of DNA primers in PCR reactions.

To provide 3'-OH for polymerase and some double stranded (DS) nucleotide, and the DNA polymerase will recognize the DS nucleic acid and 3'OH as the active site and start to build a DNA strand using the template to guide it. The primer also functions to allow the researcher to decide what part of the DNA the PCR reaction will amplify (make copies). See answers to questons 5 and 9 for more information…

5. How does a primer help the research identify a / the specific gene?

Next to the gene is a specfic sequence of bases. This seqencee will randomly appear if it isshort (say 5-10 bases). Once a primer reaches 20 plus bases, the liklihood that it will appear is very remote, unless of course it is supposed to be in this location base on DNA sequencing information.

6. What is the function of TAQ polymerase (as opposed to human polymerase)in PCR reactions? TAQ comes from the bacteria Thermus aquaticus. This bacteria lives in hot springs, and has evolved a form of DNA polymerase that can function at very high temperatures, like those seen in the PCR thermal cycle.

7. What occurs at the three different steps of the PCR cycle? What instrument is used to perform these repeated temperature changes?

94^{o} : DNA separates from dsDNA to ssDNA
72^{o} : TAQ elongates growing DNA strand
54^{o} : primers bind
The instrument used is PCR thermocycler.

8. How many base pairs will be present in your sample if the ALU insert is present? How many base pairs will be present in your sample if the ALU insert is absent?

a) ~400 if present
b) 100 if absent

9. Assume a primer is 14 bases long. How often will this primer attach to a sequence of DNA? If the creature has a genome of 6.4 billion base pairs, how many fragments would you expect to see amplified? Show the math… a 14 base long primer will appear about every 268 million bases. Divide this into the 3.1 billion in the genome and about 11 times, this sequence will appear, or about 24 times in the creature with the genome of 6.4 billion.