Problem Set #7
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Tech and Methods 2

Question 1: List the materials required for running a PCR.

Answer: Sequence to be amplified, DNA polymerase, primers, dNTPs, thermal cycler, buffer, PCR tubes

Question 2: Name the steps that constitute one PCR cycle and indicate what the nominal temperature of these steps are for a standard PCR. What is a standard cycle number for a standard PCR?

Answer: Denaturation (~94-98°C, depends on polymerase being used), Annealing (~50-55°C, depends on primer), and Extension (72°C, depends on polymerase being used). A standard PCR reaction normally runs for 30 cycles.

Question 3: In agarose gel electrophoresis one can use different percentages of agarose. What influence does the agarose concentration have on DNA migration through the gel?

Answer: Higher percentage agarose gels reduce the mobility of DNA fragments through the gel, whereas lower percentages give rise to higher migration. This is due to the different gel pore sizes that are a function of agarose percentage (higher percentage agarose = smaller pores, lower percentage agarose = larger pores). Lower percentage agarose gels are generally used to separate lower molecular weight DNA fragments, whereas higher percentage agarose gels are used for higher molecular weight DNA.

Question 4: In agarose gel electrophoresis, does DNA migrate towards the anode or cathode, and why?

Answer: DNA migrates toward the anode, which is positively charged, because DNA molecules carry a net negative charge due to their phosphate backbone. When an electric field is applied, the negatively charged DNA is attracted to the positive electrode (anode), enabling its movement through the gel matrix.

Question 5: What is a DNA ladder, what is it made of, and what is it used for?

Answer: A DNA ladder is a mixture of DNA fragments of known sizes (lengths), often produced by restriction enzyme digestion or by synthesis. It is used as a molecular size

marker in agarose gel electrophoresis to estimate the size of DNA fragments in experimental samples by comparing their migration distance to that of the ladder bands.

Question 6: In real-time PCR, what gives an indication of the original DNA concentration that was amplified?

Answer: The cycle number provides an indication of the template DNA concentration present at the beginning of the reaction. (lower cycle number = higher starting DNA concentration, higher cycle number = lower starting DNA concentration).

Question 7: What is the main difference between a “standard” PCR and an isothermal PCR?

Answer: The main difference lies in the fact that isothermal PCRs can be run at a constant temperature and do not require thermal cycling.

Question 8: How does Gibson assembly work?

Answer:
Gibson Assembly is a method for joining multiple DNA fragments in a single, isothermal reaction. It works by using three enzymatic activities:

- Exonuclease chews back the 5' ends of DNA fragments, creating single-stranded 3' overhangs with complementary sequences.

- DNA fragments with overlapping ends anneal to each other at these complementary regions.

- DNA polymerase fills in any gaps.

- DNA ligase seals the nicks in the sugar-phosphate backbone, resulting in a complete, covalently bonded DNA molecule.

This technique is widely used for cloning and synthetic biology because it allows seamless joining of multiple fragments without restriction sites.

Question 9: What are the major advantages and disadvantages of microarray based oligo synthesis compared to standard column synthesis?

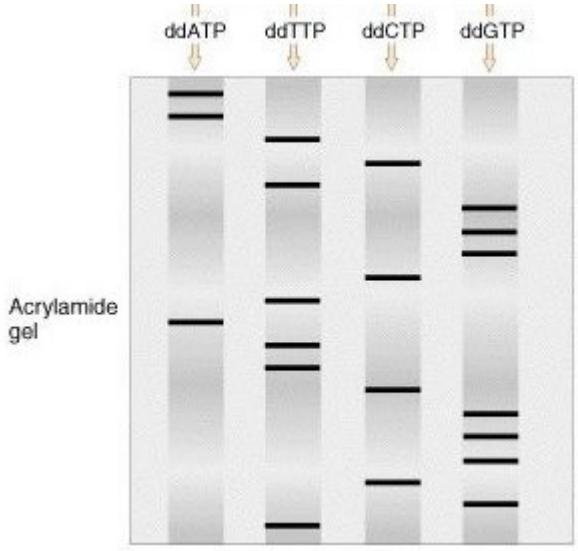
Answer:
Advantages:
- Much higher throughput as thousands to tens of thousands of different oligos can be synthesized on a single microarray

- The above advantage also leads to much better economies of scale, drastically reducing synthesis cost

Disadvantages:

- Oligos need to be eluted as pools from microarrays whereas in column synthesis each oligo can be eluted into its own test tube
- Microarray synthesis produces only small quantities of oligos, which may need to be amplified post synthesis

Question 10: What is the DNA sequence based on the Sanger DNA sequencing gel shown below?



Answer: AATCTGGGCTATTCGGGCGT