Correct answers for Lab Quiz #3

1. The image shows a PCR tube and DNA undergoing replication; the question asked was "What is the name of the **cellular process** involved?". The name of the **cellular process is DNA replication**. The PCR allows for the "amplification" of DNA, but is not a cellular process because it occurs in vitro.

2. The "Taq" portion of the name Taq-polymerase is an abbreviation for ***Thermus aquaticus***, the hyperthermophilic bacteria found living in the hotspring.

3. What happens to DNA when heated to 94 degrees centigrade in the PCR machine? The **DNA is denatured** and this means the **hydrogen bonds between the complementary bases are broken** and **the two strands separate**.

4. Primers used in the PCR have multiple functions as follows:
   a) They bind to the DNA template (single strands) making small sections of duplex DNA. This will allow the DNA-polymerase enzymes to bind (it can only bind to duplex DNA).
   b) They provide the free 3' ends needed for phosphodiester bond formation so that new (incoming) nucleotides can be added.
   c) They determine which sections of template DNA will be amplified because they bind at either end of those sections (in our case the 16S rRNA genes).
   d) Their size and composition determine the anneal temperature required during the PCR.

5. During the "extension" process the **energy required is provided by dNTPs**. Again, the process involved is **DNA replication** and the energy required for that is provided by the incoming nucleotides (ATP, GTP, CTP and TTP).

6. The gene targeted during our PCR was the **16S rRNA gene**, but there are typically several of these within a bacterial genome.

7. During the Sanger chain termination method of nucleotide sequencing, replication is terminated by the addition of **NTPs containing dideoxyribose**.

8. The image shown was a section of an **electropherogram** or **chromatogram**.

9. The image shows four sections of electropherograms generated using four different primers (two forward and two reverse). **Only two of these sequences can be "flipped" to achieve the desired result**, i.e., all four sequences covering the same DNA strand. Either "flip" both reverse sequences (as instructed) or "flip" the two forward sequences. If you "flip" all four, you have accomplished nothing.

10. We use the BLAST algorithm to **compare our nucleotide sequences** (from PUNK2 and project cultures) **with those available in the NCBI GenBank** (the public database). We are looking for entry sequences with a high % similarity as these indicate what types of bacteria our PUNK2 and project cultures are likely to be.

11. Each student found a **different accession number** for their PUNK2 culture ID.