

Answer Booklet to SYNBIO 1.0

an initiative
by the iGEM team of
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Answers to "Think further" Questions

Question: Imagine I have a DNA fragment with 100 base pairs. 22 of the nucleotides which I have are guanine. How many thymine nucleotides are found in my DNA?

Solution:

The number of thymine nucleotides found in the given DNA fragment are **78**.

Pairing rule:

Guanine pairs with cytosine
Adenine pairs with thymine

100 base pairs => 200 bases

22 guanine bases => 22 cytosine bases

Now, the remaining number of bases:

$$200 - (22 + 22) = 156$$

No. of adenine bases (x) = No. of thymidine bases (y)

$$156 = x + y$$

$$156 = 2x$$

$$x = 78$$

Question: We know that our DNA is double-stranded. Since gene is basically a small piece of DNA will both strands of the small piece undergo transcription to form RNA or is it just one strand?

Solution: Transcription of both strands would produce **two complementary RNA strands**. Being complementary, they can join together to form a double stranded RNA. Molecules involved in the subsequent steps would not be able to recognise this dsRNA fragment.

Also, the two strands of DNA presumably would produce **two different kinds of protein** (the transcribed RNA fragment will be different which means upon translation they will produce different amino acid sequences).

Question: What happens if the cloning vector do not posses the Ori ?

Solution: The replication of DNA begins at ori (origin of replication). If the cloning vector does not possess ori then **it cannot replicate**. Thus, the subsequent generations of bacterial cell will not possess the cloning vector as it did not undergo replication.

Question: Why do we have to make the host competent? Why doesn't the host take up DNA spontaneously?

Solution: The cells have to be made competent in order to take up the foreign DNA. **DNA, being a negatively charged hydrophilic molecule cannot pass through the cell membrane easily.** The process of making competent cells introduces pores into the cell membrane which allow them to uptake extracellular DNA more readily.

Bacterial cells can be made competent by treating it with a specific concentration of a divalent cation such as calcium as it increases the efficiency with which DNA can enter the bacteria through the pores of the cell wall.

Question:

Why did bacteria evolve to have restriction enzymes?

Solution:

Restriction enzymes are present in bacteria as a **natural defence** to protect bacteria from invading foreign DNAs. These enzymes cut the foreign DNA at restriction sites causing its destruction and thus losing its ability to attack the host cell.

Question: Find the restriction site where a hypothetical restriction enzyme would probably cleave the given DNA strand 5'- A T G C A G C G C T C C T -3' (look for a 6 nucleotide sequence)

Solution:

The restriction sites are **palindromic sequences**. Write the sequence of the other strand and check for the palindromic sequence.



Question:

What will be the consequence if I add cellulase instead of chitinase to isolate the genetic material from fungi?

Solution:

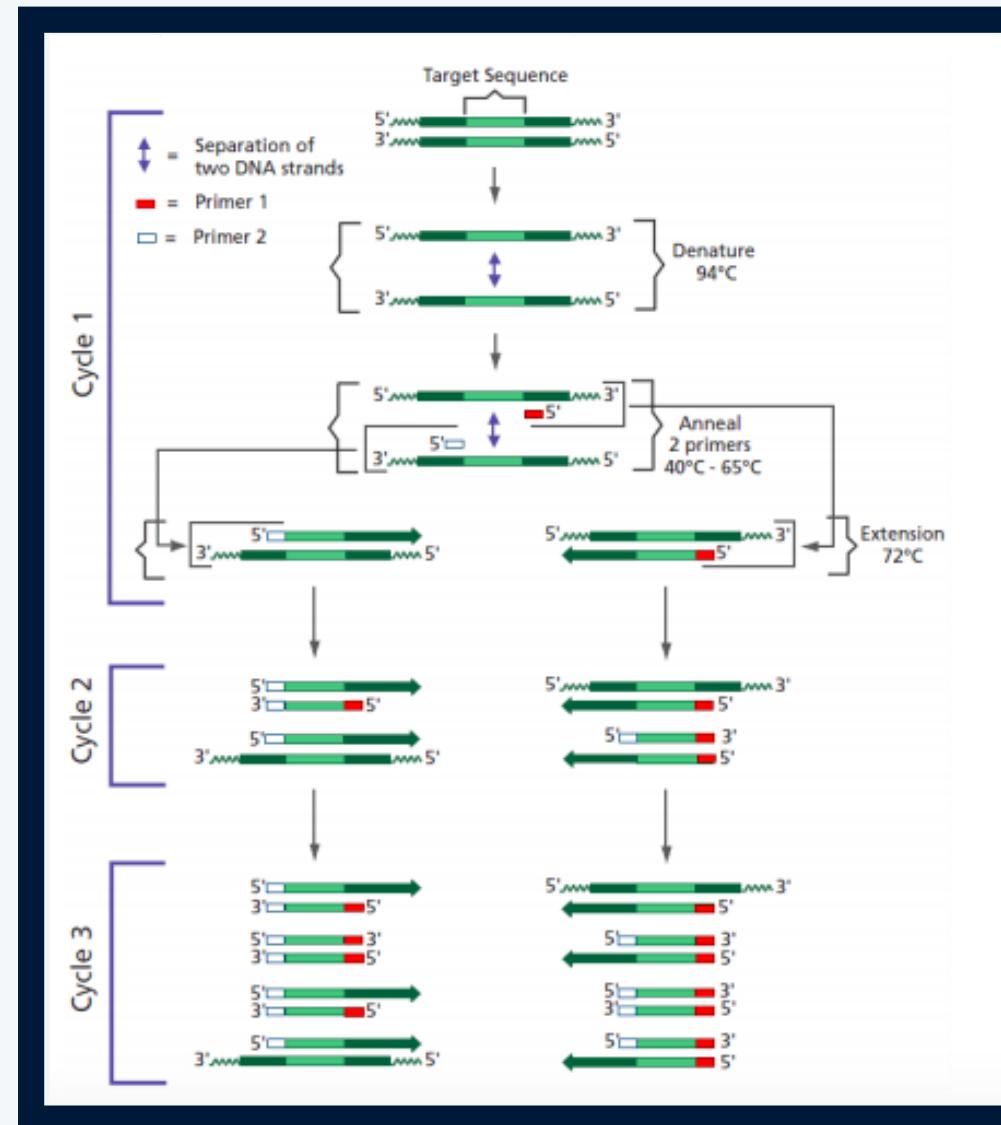
The fungal cell wall is composed of chitin. Cellulase cannot break down chitin. So cell wall cannot be broken. Thus, isolation of genetic material becomes difficult.

Question: What is the minimum number of PCR cycles required to obtain the desired length of DNA-the target gene sequence without any adjacent DNA contamination?

Solution: *The minimum number of PCR cycles required to obtain the target gene sequence without any adjacent DNA contamination is 3.*

Consider a single copy of the DNA sequence. This double-stranded molecule undergoes denaturation, annealing and extension. At the end of cycle 1, we thus have two partially double-stranded molecules that contain the target sequence but also some adjacent DNA. In cycle 2, the steps are repeated and we will have four partially double-stranded molecules that contain the target sequence along with adjacent DNA. At the end of cycle 3, we get two double-stranded molecules with the target sequence and

no contaminating adjacent DNA alongside six partially double-stranded molecules with the target sequence and adjacent DNA molecules. So at the end of cycle 3, we have in total 8 molecules.



Source: <https://blog.edvotek.com/2014/05/01/pcr/>

Question:

How do we know if a bacterium or cell has taken up our desired gene?

Solution:

The **selectable marker** in the cloning vector can help to know if the host has taken up the desired gene. For example, consider the selectable marker is a particular antibiotic resistance and the host naturally does not have that particular antibiotic resistance. Culture the cells on that particular antibiotic medium. Only the transformed host cells (cells that have taken up the cloning vector) will grow because the cloning vector has conferred the property of antibiotic resistance to the transformant.

Question: The plasmid "Z" has the property of Kanamycin and Chloramphenicol resistance. The desired gene of interest is inserted into the Chloramphenicol resistant gene of Z. The bacteria is transformed with this plasmid. Match the following figures under the following conditions.

- a. If the bacteria are grown in a medium that contains Chloramphenicol.
- b. If the bacteria are grown in a medium that contains Kanamycin.
- c. If bacteria are grown in a medium that contains both Kanamycin and Chloramphenicol.

Figure 1

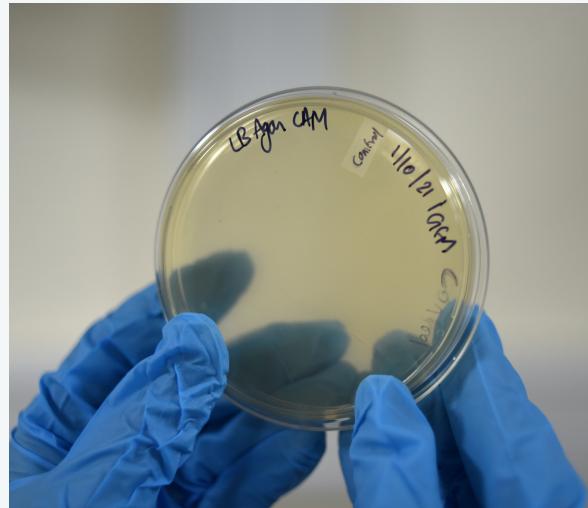
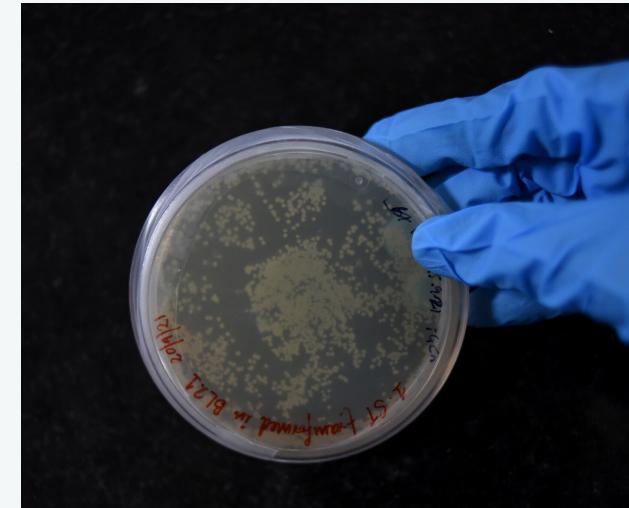


Figure 2



Solution: The desired gene of interest is inserted to the site that confers the property of Chloramphenicol resistance. This gene insertion will make the site lose its property of chloramphenicol resistance. Hence now the plasmid only has kanamycin resistance. If a bacteria has to grow in a particular antibiotic medium it must have resistance against it.

- a. The bacteria won't grow because the plasmid has lost its chloramphenicol resistance property. So **Figure 1** will be the most appropriate answer here.
- b. The bacteria will grow because the plasmid still has its kanamycin resistance. **Figure 2** will be the matching one here.
- c. The bacteria won't grow because of the presence of chloramphenicol in the medium. The bacteria can grow in this medium only when it has resistance to both antibiotics.
Figure 1 is the answer here.



Answers to Questions in Feedback Form

Question: A linear DNA and a plasmid DNA have 2 and 3 restriction sites for the enzyme EcoRI. How many DNA fragments will I get, when I perform restriction digestion using EcoRI of the linear DNA and the plasmid DNA respectively?

Solution: The linear DNA has **2 restriction sites** for EcoRI.
=> During restriction digestion, EcoRI will cut the linear DNA at 2 sites, generating **3 linear DNA fragments**.

Now, the plasmid DNA has **3 restriction sites** for EcoRI. We know that **plasmids are circular**.
=> During restriction digestion, EcoRI will cut the circular plasmid DNA at 3 sites, generating **3 linear DNA fragments**.

Question: A double stranded DNA has 100 base pairs. It has 34 adenine nucleotides. How many hydrogen bonds will be present in total for this DNA? Hint: 'Base pairs' are not the same as 'bases'.

Solution:

34 adenine nucleotides mean 34 adenine-thymidine nucleotide base pairs. Out of 100 base pairs remaining 66 [100-34] base pairs will be guanine-cytosine base pairs.

Two hydrogen bonds exist between adenine and cytosine.
Three hydrogen bonds exist between guanine and cytosine.

$$\begin{aligned}\text{Therefore, total number of hydrogen bonds} &= (2*34) + (3*66) \\ &= \mathbf{266}\end{aligned}$$

Question: What is the minimum number of PCR cycle(s) required to get the desired DNA fragment (target gene sequence) without adjacent DNA contamination?

Solution:

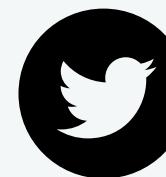
The minimum number of PCR cycles required to get the desired DNA fragment without adjacent DNA contamination is **three**.

Question: Why restriction endonucleases present in a bacterium does not cleave their own genetic material?

Solution: *The recognition sequences are modified (methylated) and hence they cannot be cleaved by the enzymes.*

The host bacterial genome contains restriction sites/recognition sequences for these restriction endonucleases. These sites are highly methylated hence they cannot be recognised by the enzymes. The enzyme cannot cleave the genetic material if it cannot recognise the sites. Hence bacterium protects its own genome from degrading.

Thank You!



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