EDVOTEK® INQUIRY GUIDE:

PCR

TEACHERS:
For the answer key, please contact us at **info@edvotek.com**

EDVOTEK®

www.edvotek.com

Key Terms

DEFINE the following terms before you begin the guide then refer to them as needed. Use any resources you want!

PCR:

Primer:

dNTP:

DNA Template:

Base Pairs:

Taq Polymerase:

Denaturation:

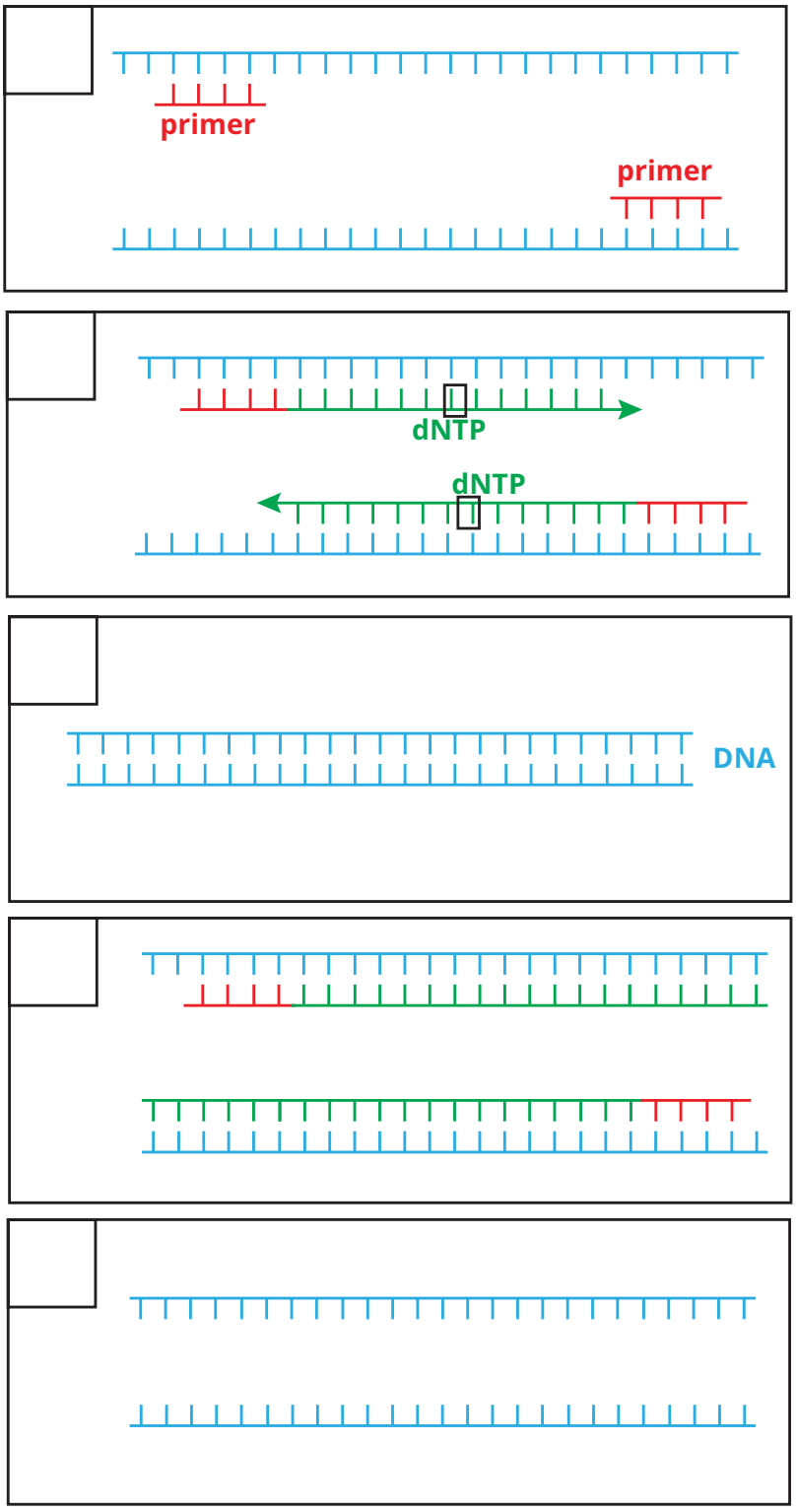
Annealing:

Extension:

Target Sequence:

DNA Replication:

1. Below are 5 tiles depicting the first cycle in a PCR experiment. **NUMBER** each box to reflect the correct sequence of events.

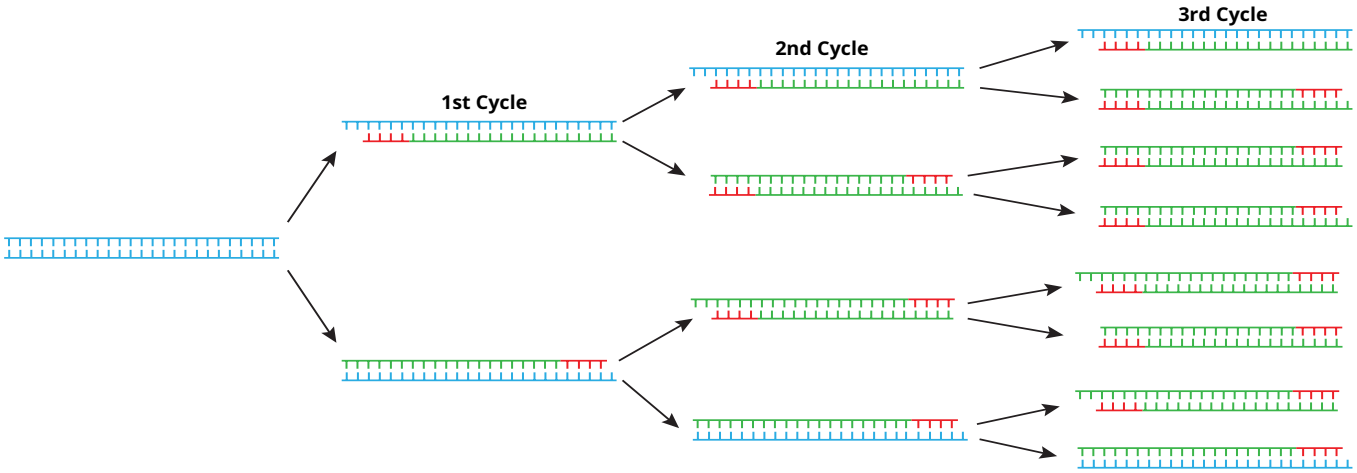


If you are struggling it can help to physically rearrange the tiles. For a printable version, ask your teacher or visit: https://edvotek.com/storytiles/pcr

2. Use the following definitions to **LABEL** the key steps of denaturation, annealing, and extension in question #1's illustration. Next, **WRITE** in a good temperature for each labeled box (94-96°C, 45-65°C, or 72°C). *Hint: Two boxes will remain unlabeled (they represent the pre-denaturation and post-extension state of the DNA).*

- Denaturation: The first step in a PCR cycle which involves heating the reaction mixture to a high temperature in order to separate the double-stranded DNA template into two single strands.
- Annealing: The second step in a PCR cycle where a reaction mixture is cooled. During this step, short DNA primers that are complementary to the template DNA sequence bind to their complementary sites on the single-stranded template DNA.
- Extension: The final step of the PCR cycle where the temperature of the reaction mixture is increased and the heat-stable Taq polymerase enzyme uses the primers as a starting point to synthesize a new complementary DNA strand.

3. PCR's power lies in repetition. Typically, a PCR experiment will involve 30-35 cycles of denaturation, annealing, and extension. Each cycle approximately doubles the amount of target DNA. **CHOOSE** one side of the table below and **COMPLETE** the target DNA column for that side to illustrate the amplifying power of cycling.



Cycle Number:	Target DNA:
1	2
2	4
3	8
4	16
5	32
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	

Cycle Number:	Target DNA:
16	65536
17	131072
18	262144
19	524288
20	1048576
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	

4. Search and Find DNA edition!

   Below are the sequences for a forward primer and reverse primer. **SEARCH** the DNA sequence and **MARK** where each primer would attach.

   Forward Primer:
   5'– GATGTAAATACAGCTTC

   Reverse Primer:
   5'– CAAACTACTTGACGACTACG

   DNA Sequence:

IMPORTANT HINTS:

To find the forward primer's target sequence, it can be useful to write the complementary base pairs below the primer and then search for that sequence. To find the reverse primer write the complementary base pairs below and then reverse their order.

By convention, forward primers bind near the start of a written sequence and to the bottom strand (3' to 5') while reverse primers bind at the end of the sequence and to the top strand (5' to 3').

Base Pair Rules:
A → T, T → A, C → G, G → C

```
5'–AGCTGGGATGTAAATACAGCTTCGTCTCCGTTGCACTCTACATAGCTTCTAGGCACTCTGCACCCGGAGGTTGCTCTTGACGTCGTAGTCGTCAAGTAGTTTGGG–3'
   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3'–TCGACCCTACATTTATGTCGAAGCAGAGGCAACGTGAGATGTATCGAAGATCCGTGAGACGTGGGCCTCCAACGAGAACTGCAGCATCAGCAGTTCATCAAACCC–5'
```

- In PCR what step would this happen in?

- What step would have to happen first?

5. Here's another close-up of a PCR experiment, this time at the beginning of the extension step. **WRITE** in the correct base pairs to complete the extension process.

```
5'  ATCGTTAGCTAGCTGACTGCTAGCTGACGTTACGTAGCTAGCTAGCACGTGAATTCTTGACGCACTT  3'
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3'  TAGCAATCG_____________________________________________________________  5'
```

- In a PCR experiment where do the "A", "C" ,"T", and "G" come from?

- What enzyme enables this extension?

6. You've been asked to amplify a DNA region containing a 50 bp target sequence using PCR. The first step is to design primers. Use the sequence and tips below to **CREATE** a forward primer that will amplify the highlighted DNA region. A reverse primer is provided below as an example.

TIPS:
- The amplification region can be larger than the target.
- Aim for the GC content of between 40 and 60%.
- Have the primer's 3' end be G or C to promote binding.
- Optimize annealing by having have primers between 18-30 bps long and similarly sized.
- Avoid runs of 4 or more identical base pairs (for example, ACCCC).
- Avoid dinucleotide repeats (for example ATATATAT).
- Avoid inter-primer homology (forward and reverse primers having complementary sequences) to reduce the risk of nonspecific products like primer-dimers.

NOTE:
Following all seven tips/ suggestions may be challenging depending on the DNA region, but the more you implement, the more reliable and robust your PCR results become.

DNA Sequence:

```
5'-ATCGTACGTAACGTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAG
   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3'-TAGCATGCATTGCATCGATCGATCGATCGATCGATCGATCGATCGATCGATCGATCGATCGATCGATCGATCGATCGATCGATCGATC

                                                    CTAGCTAGCTAGGTCTGTGGAAGAGGATTCACCTAGCT-3'
                                                    ||||||||||||||||||||||||||||||||||||
                                                    GATCGATCGATCCAGACACCTTCTCCTAAGTGGATCGA-5'
```

Forward Primer:_____

Example:
Reverse primer `CAGACACCTTCTCCTAAGTG` (binds to the underlined region above)

- 20 bp
- 10 bp G or C = 50% GC content
- 3' ends in C
- No runs of more than 4 identical base pairs & no dinuclear repeats

7. In labs, many PCR experiments are often performed together. To do this, researchers create a 'master mix' containing all the key ingredients (except for the DNA sample) for all the experiments. **CREATE** your own master mix for a 96 reaction PCR experiment by finishing the table below. On the side, brainstorm and **WRITE** 2-3 reasons why creating a master mix might be such a popular practice.

Ingredient	Amount for 1 Experiment	Amount for 100 Experiments
10X Buffer	5 µL	
dNTP Mix	1 µL	
Forward Primer	2.5 µL	
Reverse Primer	2.5 µL	
Taq DNA Polymerase	0.2 µL	
Sterile Water	3.8 µL	

8. DNA replication and PCR are very similar but also have their differences.
For statements below, **WRITE** "P" for PCR, "D" for DNA Replication, or "B "for both.

- Relies on DNA polymerase. _____
- Three steps: Initiation, elongation, termination. _____
- Three steps: denaturation, annealing, extension. _____
- Polymerase is generally thermophilic (heat loving). _____
- Polymerase has proofreading and repair abilities. _____
- General Error rate of 1 in 9000. _____
- General Error rate of 1 in 100,000. _____
- Uses exiting DNA as the template for the synthesis of new DNA. _____
- Copies the whole genome. _____
- Occurs at three different temperatures. _____
- Occurs at body temperature within the body of a living organism. _____
- Proceeds in the 5' to 3' direction of DNA strands, so polymerization of the two strands occur in opposite direction. _____
- A widely used method in molecular biology to make many copies of a specific DNA segment. _____
- The biological process of producing two identical replications of DNA. _____
- DNA is separated by heat. _____
- DNA is separated by enzymes. _____
- Requires artificial primers. _____
- In vitro process (occurring in a test tube). _____
- In vivo process (occurring in a cell). _____
- Produces many copies of a single and short DNA fragment. _____