

Name: \_\_\_\_\_

# PCR Basics Worksheet

## Write your PCR recipe

Write a recipe for PCR. First list the ingredients (reagents) needed. Next write the role of each ingredient in PCR.

Ingredients	Role of ingredient in reaction
1) Template DNA	
2)	
3)	
4)	
5)	

## Base pairing - be the polymerase!

Primers are usually about 20 nucleotides long. For this activity we will simplify things and use a 5 base-pair primer.

Use the forward primer: 5' - G A T A C - 3'

Locate where the primer binds to your template DNA. Next, act as the polymerase and fill in the rest of the new strand of DNA.

New DNA strand: 5' - \_\_\_\_\_ - 3'

Template DNA: 3' - T A G C T A T G C G G A C C T C A T G C A T T A G A G T A G - 5'

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## PCR primers

Below is a 300 base-pair fragment of DNA. The top strand is written in the 5' to 3' direction. The bottom strand is written 3' to 5'.

5' ACCGTAGCTATATGCTATCGTGACGTATCGGCGCATTAATCGGGATCGAT 3'	50
3' TGGCATCGATATACGATAGCACTGCATAGCCGCGTAATTAGCCCTAGCTA 5'	
5' AGCTCGCTAGCAGGAGAGATATCGCTCATAGCTCCGATCGATGCCGCTAA 3'	100
3' TCGAGCGATCGTCCTCTCTATAGCGAGTATCGAGGCTAGCTACGGCGATT 5'	
5' TATAGCTCTCTGCGGATATCGCATATACCAAGGCCCTACGTATGTAGCTA 3'	150
3' ATATCGAGAGACGCCCTATAGCGTATATGGTTCCGGGATGCATACATCGAT 5'	
5' TGCGTATATCGGAGAGTCCTGGATATGGAGCTTGACTGCAGAGAGCTCGA 3'	200
3' ACGCATATAGCCTCTCAGGACCTATACCTCGAACTGACGTCTCTCGAGCT 5'	
5' TATGCGCTTAGGCCGTATATGCTTGGGGAAAGCTCTATGTATGCTATGTG 3'	250
3' ATACGCGAATCCGGCATATACGAACCCCTTTCGAGATACATACGATACAC 5'	
5' TGCATGTGCTATGCAACGTTCCGGATTGCGTAGCAGTAATAGCGCCGATTG 3'	300
3' ACGTACACGATACGTTGCAAGCCTAACGCATCGTCAATTATCGCGGGCTAAC 5'	

Forward Primer      5' CTATCGTGACGTATCGGC 3'

Reverse Primer      5' TGCTACGCAATCCGAACG 3'

The forward primer is complementary to the bottom strand of the DNA and the reverse primer is complementary to the top strand of DNA. Note that it is standard to write all DNA sequences in the 5' to 3' direction. Pay attention to the 5' to 3' direction when looking for a match between each primer and the DNA.

Find in the sequence where each primer binds. Draw a box around where each primer will bind.

In this case, exactly how many base pairs do you expect your PCR product to be? \_\_\_\_\_

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### PCR and exponential growth

In PCR, the number of copies of your template doubles every cycle. Use the table below to explore what that means. The first five boxes have been started for you.

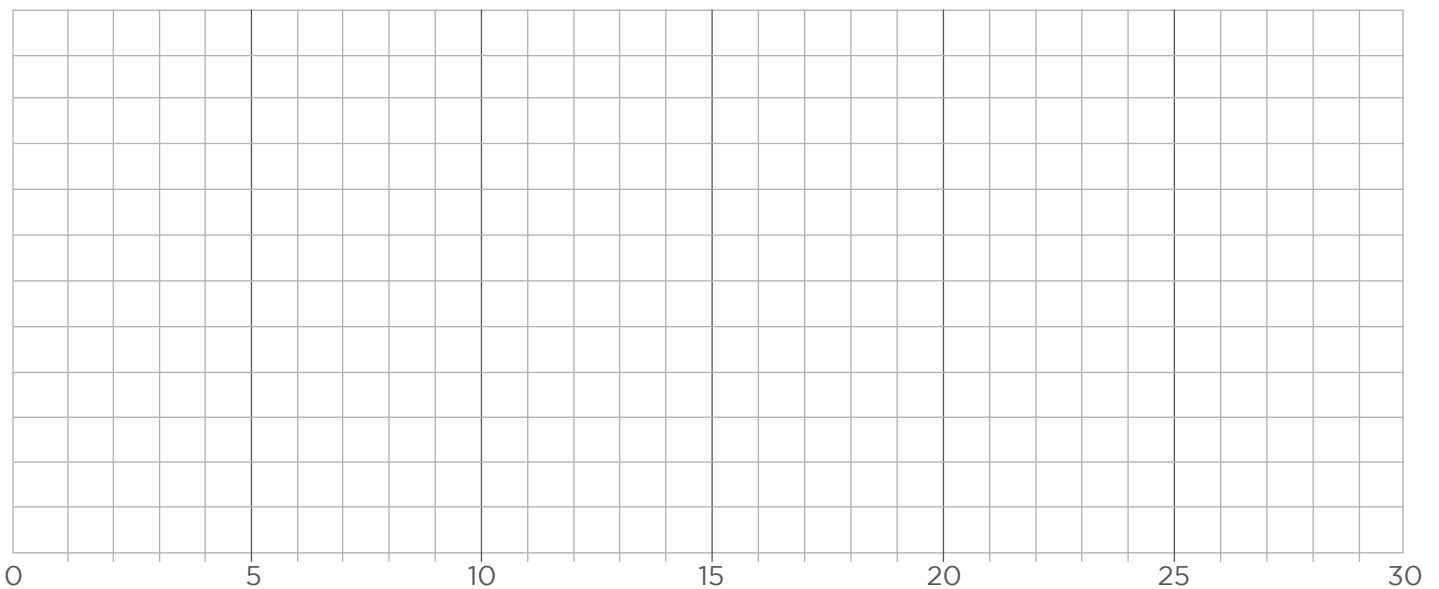
PCR Cycle	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Copies of DNA	2	4	8	16	32										

PCR Cycle	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Copies of DNA															

Theoretically, after 30 PCR cycles, how many copies of your template DNA would you expect to have for every one you started with? \_\_\_\_\_

### Graph your results below

Number of DNA Copies per PCR Cycle



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### PCR story board

Use the three boxes to illustrate the 3 basic steps of PCR as if they were a comic strip. Beside each box, describe what is happening in each drawing. Use and underline the following words: **template**, **primers**, **dNTPs**, **Taq DNA polymerase**, **thermocycler**, **denaturation**, **annealing**, **extension**, **amplification**.

Name of Step: \_\_\_\_\_  
Temperature: \_\_\_\_\_  
Description: \_\_\_\_\_

Name of Step: \_\_\_\_\_  
Temperature: \_\_\_\_\_  
Description: \_\_\_\_\_

Name of Step: \_\_\_\_\_  
Temperature: \_\_\_\_\_  
Description: \_\_\_\_\_

Name: \_\_\_\_\_

## DNA replication vs PCR

PCR relies on many of the same principles as DNA replication, the process by which your genome is copied during cell division. However, PCR often uses slightly different mechanisms to achieve the same results. To further understand the connection between PCR and DNA replication, complete the comparison table below.

DNA replication in cell	PCR
The hydrogen bonds between the two strands of DNA are broken by the enzyme DNA helicase.	
Replication begins at short RNA primers that bind to the DNA. The RNA primers are later removed and replaced with DNA.	
Typically occurs at 37°C. (Body temperature)	
Makes a single copy of all of the DNA in the nucleus.	

## PCR Glossary

Test your PCR vocabulary by defining the following terms in your own words.

Annealing:	PCR product:
Base pair:	Primer:
Denaturation:	Taq DNA polymerase:
Extension:	Template DNA:
Nucleotide:	Thermocycler: