Additional PCR Resources (e.g. interesting links, video links, worksheets, etc.)

PCR Basics Worksheet:

https://www.genesinspace.org/documents/11/PCR_basics_worksheet_V2.pdf

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Activity 3: PCR Story Board

Use the three boxes to illustrate the steps of PCR as if they were a comic strip. On the lines 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:

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'. There are also two primer sequences; both primers are written 5' to 3'. Note that we are displaying a double-stranded DNA fragment, but primers will only bind to one of the two displayed strands.

```
5'  ACCGTAGCTATATGCTATCGTGACGTATCGGCGCATTAATCGGGATCGAT  3'
    ....|....|....|....|....|....|....|....|....|....|      50
3'  TGGCATCGATATACGATAGCACTGCATAGCCGCGTAATTAGCCCTAGCTA  5'
                   forward primer

5'  AGCTCGCTAGCAGGAGAGATATCGCTCATAGCTCCGATCGATGCCGCTAA  3'
    ....|....|....|....|....|....|....|....|....|....|     100
3'  TCGAGCGATCGTCCTCTCTATAGCGAGTATCGAGGCTAGCTACGGCGATT  5'

5'  TATAGCTCTCTGCGGATATCGCATATACCAAGGCCCTACGTATGTAGCTA  3'
    ....|....|....|....|....|....|....|....|....|....|     150
3'  ATATCGAGAGACGCCTATAGCGTATATGGTTCCGGGATGCATACATCGAT  5'

5'  TGCGTATATCGGAGAGTCCTGGATATGGAGCTTGACTGCAGAGAGCTCGA  3'
    ....|....|....|....|....|....|....|....|....|....|     200
3'  ACGCATATAGCCTCTCAGGACCTATACCTCGAACTGACGTCTCTCGAGCT  5'

5'  TATGCGCTTAGGCCGTATATGCTTGGGGAAAGCTCTATGTATGCTATGTG  3'
    ....|....|....|....|....|....|....|....|....|....|     250
3'  ATACGCGAATCCGGCATATACGAACCCCTTTCGAGATACATACGATACAC  5'
                   reverse primer

5'  TGCATGTGCTATGCAACGTTCGGATCGCGTAGCAGTAATAGCGCCGATTG  3'
    ....|....|....|....|....|....|....|....|....|....|     300
3'  ACGTACACGATACGTTGCAAGCCTAACGCATCGTCATTATCGCGGCTAAC  5'

          Forward Primer    5'CTATCGTGACGTATCGGC 3'
          Reverse Primer    5'TGCTACGCAATCCGAACG 3'
```

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? 270

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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.**

*Note: Illustrations will vary but should depict double-stranded DNA separating into two single strands.

Name of Step:	Denaturation
Temperature:	95 degrees Celsius
Description:	The temperature in the thermocycler is raised to near boiling. High temperature separates double-stranded DNA into two single strands

*Note: Illustrations will vary but should depict primers binding to single-stranded DNA.

Name of Step:	Annealing
Temperature:	50-65 degrees Celsius
Description:	The thermocycler temperature is lowered to allow primers to bind to the complementary sequence in the template DNA

*Note: Illustrations will vary but should depict Taq polymerase synthesizing new DNA by adding nucleotides to the end of the primer.

Name of Step:	Extension
Temperature:	72 degrees Celsius
Description:	The temperature is raised to activate the enzyme Taq polymerase. Taq reads the sequence of the template DNA after the primer, adding dNTPs to the new strand according to the rules of complementary base pairing. This step completes one cycle in the amplification process.

PCR and exponential growth

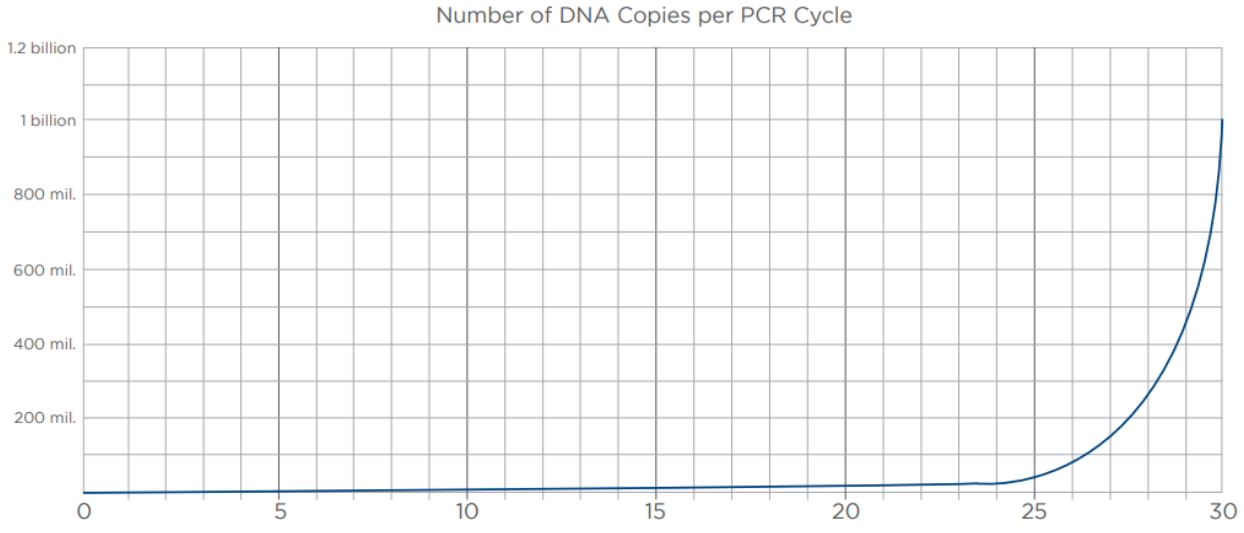
In PCR, the number of copies of your template doubles every cycle. Use the table below to explore what that means. The first five boxes have been started for you.

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	64	128	256	512	1024	2048	4096	8192	16348	32768

PCR Cycle	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Copies of DNA	65536	131,072	262,144	524,288	1,048,576	2,097,152	4,194,304	48,388,608	16,777,216	33,554,432	67,108,864	134,217,728	268,435,456	536,870,912	1,073,741,824

Theoretically, after 30 PCR cycles, how many copies of your template DNA would you expect to have for every one you started with? 1,073,741,824

Graph your results below



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.**

*Note: Illustrations will vary but should depict double-stranded DNA separating into two single strands.

Name of Step: Denaturation

Temperature: 95 degrees Celsius

Description: The temperature in the thermocycler is raised to near boiling. High temperature separates double-stranded DNA into two single strands

*Note: Illustrations will vary but should depict primers binding to single-stranded DNA.

Name of Step: Annealing

Temperature: 50-65 degrees Celsius

Description: The thermocycler temperature is lowered to allow primers to bind to the complementary sequence in the template DNA

*Note: Illustrations will vary but should depict Taq polymerase synthesizing new DNA by adding nucleotides to the end of the primer.

Name of Step: Extension

Temperature: 72 degrees Celsius

Description: The temperature is raised to activate the enzyme Taq polymerase. Taq reads the sequence of the template DNA after the primer, adding dNTPs to the new strand according to the rules of complementary base pairing.

This step completes one cycle in the amplification process.

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.	High temperature is used to denature double-stranded DNA, separating it into two single strands.
Replication begins at short RNA primers that bind to the DNA. The RNA primers are later removed and replaced with DNA.	Replication begins at DNA primers complementary to the beginning and end of the sequence of interest. These primers are not removed and remain incorporated in the PCR product.
Typically occurs at 37°C. (Body temperature)	The thermocycler raises and lowers temperature to coordinate the amplification process. Temperature varies between roughly 50 and 95 degrees Celsius.
Makes a single copy of all of the DNA in the nucleus.	Makes millions of copies of only the DNA sequence that falls between the forward and reverse primers.

PCR Glossary

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

Annealing: The PCR step during which primers bind to complementary regions of the template DNA strand	PCR product: What you have at the end of a PCR experiment. It contains the original DNA sample you started with, plus many copies of your sequence of interest
Base pair: A matched pair of complementary nitrogenous bases (e.g., adenine and thymine)	Primer: A short sequence of DNA designed to match the beginning or end of the sequence of interest
Denaturation: The PCR step during which double-stranded DNA is separated into two single strands	Taq DNA polymerase: The enzyme that replicates DNA in a PCR reaction. Taq is a special polymerase that functions best at high temperatures
Extension: The PCR step during which the sequence of DNA between the forward and reverse primers is copied	Template DNA: The original DNA sequence that you'd like to amplify
Nucleotide: A building block of a strand of DNA. It consists of part of the phosphate backbone, plus one of four nitrogenous bases: adenine, cytosine, guanine, or thymine	Thermocycler: A machine that is used to carry out PCR. It coordinates the reaction by changing temperatures to initiate the steps of the amplification process

Understanding how viruses work link for kids:
https://www.science-sparks.com/what-is-a-virus/

Interesting link about the PCR inventor:
https://www.nobelprize.org/prizes/chemistry/1993/mullis/facts/

Understanding genes link for kids:
https://kids.britannica.com/kids/article/genetics/353170