3-D Molecular Designs
...where molecules become real ™

Biotechnology Kit©

PCR Activity Guide: Part 1

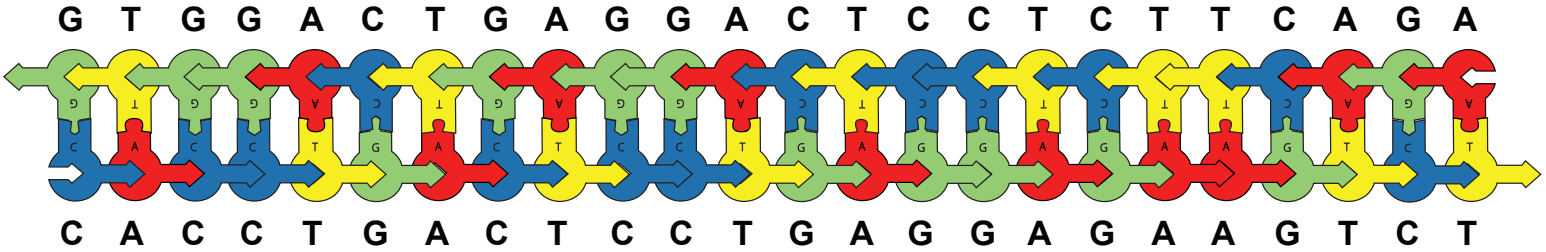
Exploring Polymerase Chain Reaction (PCR)

Step-By-Step Instructions

Create double-stranded template DNA sequence:

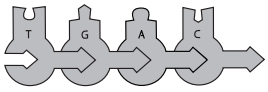
3’ GTGGACTGAGGACTCCTCTTCAGA 5’
5’ CACCTGACTCCTGAGGAGAAGTCT 3’

Assembly Guide



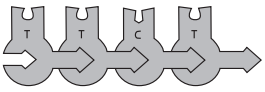
Assemble 7 sets of each primer and apply tape to the back of the nucleotides.

Primer Sequence I



T G A C

Primer Sequence II



T T C T

Premodeling Set-up

Before beginning the modeling, it is highly suggested that teachers either illustrate how to set up a modeling station, or encourage their students to set up one themselves. This enables all material to be easily accessible and only the necessary materials to be present on a student lab desk/station.

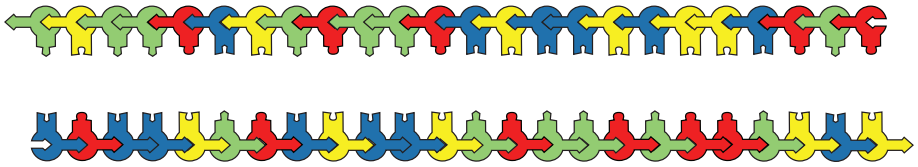
[Optional: Add 2 flags labeled “Target Sequence” to one end of each target DNA strand to mark original template DNA strands.]

PCR Activity Guide: Part 1

Cycle 1: Denaturation

Denaturation: Double-stranded template DNA is heated to separate it into two single strands.

Separate the two template DNA strands.

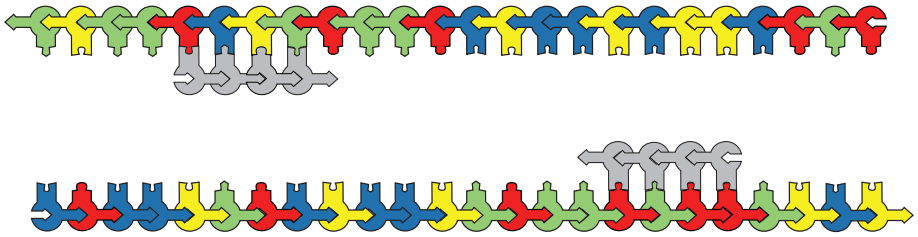


Cycle 1: Annealing

Annealing: The temperature is lowered to enable the DNA primers to attach to the template DNA strands.

Add **Primer 1** to matching complementary strand of template DNA. Attach “Cycle 1” flag to 5’ end of Primer 1.

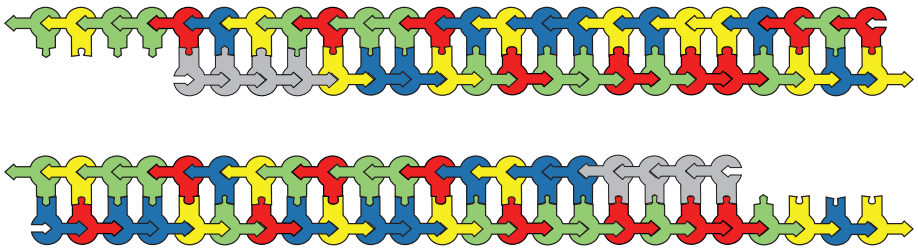
Add **Primer 2** to the complementary region of the opposite strand of template DNA. Attach “Cycle 1” flag to 5’ end of Primer 2.



Cycle 1: Extension

Extension: The temperature is raised and the new strand of DNA is made by the *Taq* polymerase enzyme.

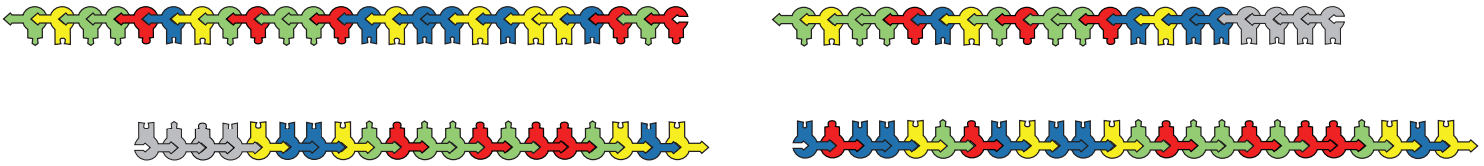
Starting from each 3’ end of the primer, add complementary DNA nucleotides extending from the primer until the end is reached. Repeat with other primer and exposed template strand, until 2 double-stranded molecules result.



PCR Activity Guide: Part 1

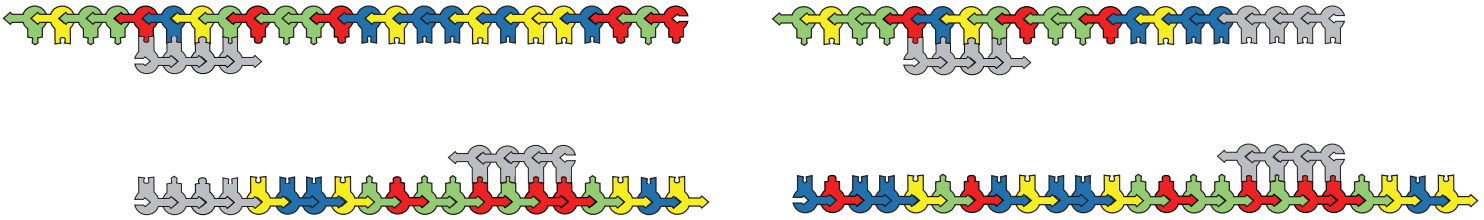
Cycle 2: Denaturation

Separate the 4 DNA strands.

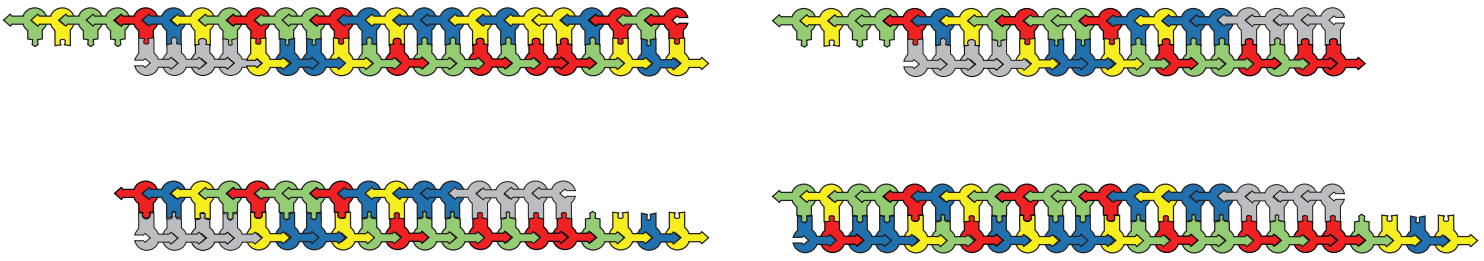


Cycle 2: Annealing

Repeat the annealing step as shown in the previous cycle. Attach “Cycle 2” flags to 5’ ends of Primer 1 and Primer 2.



Cycle 2: Extension



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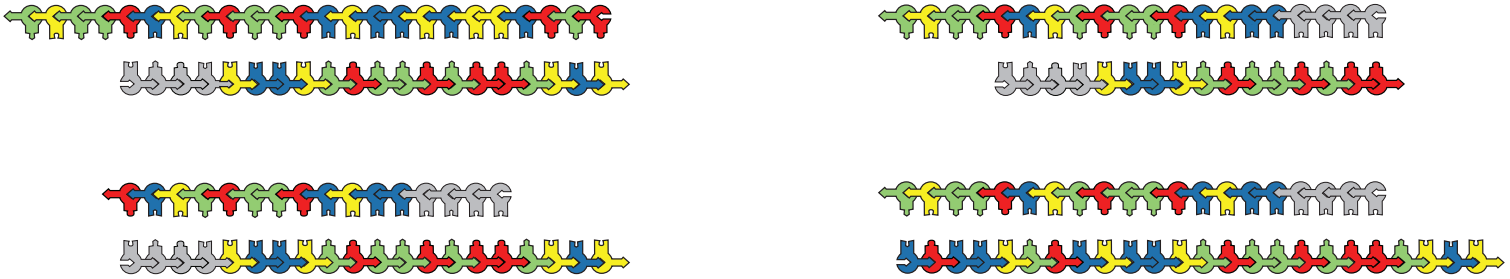
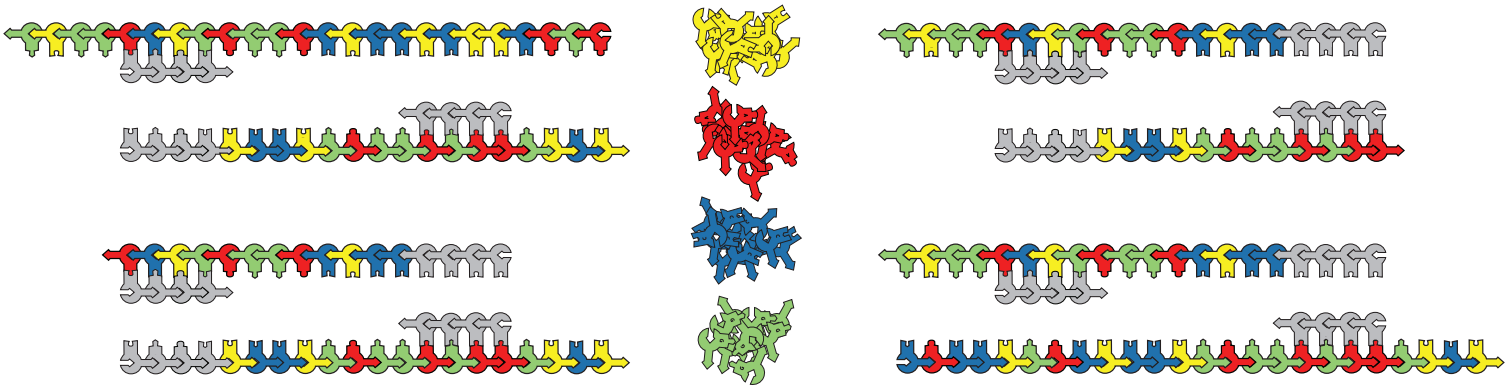
Cycle 3: Denaturation

Separate the 4 DNA strands.



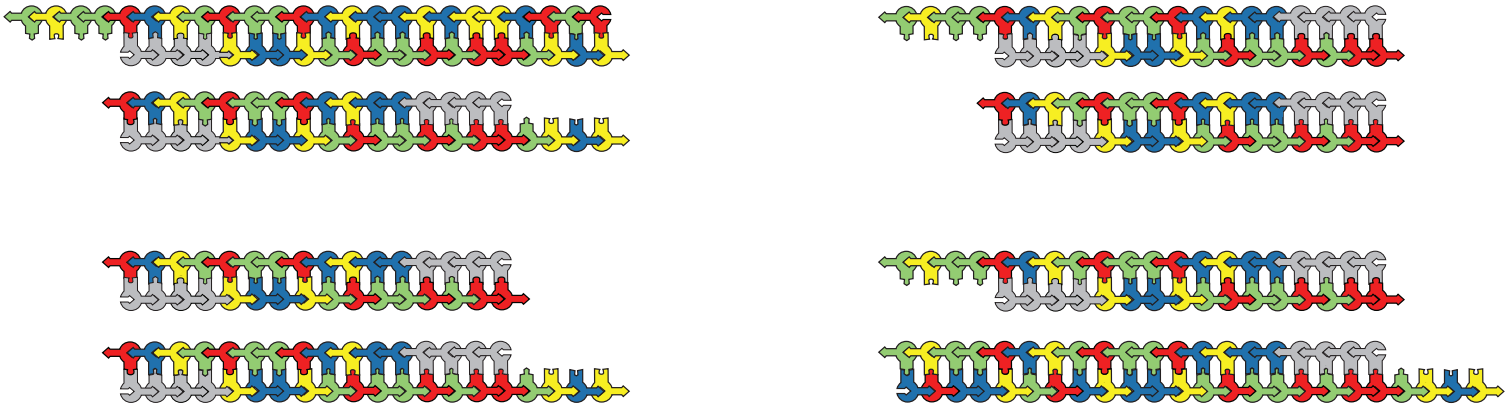
Cycle 3: Annealing

Attach “Cycle 3” flags to 5’ ends of Primer 1 and Primer 2 and repeat the annealing step.



Cycle 3: Extension

Final product: After 3 cycles, the result will be 8 double-stranded DNA molecules.



PCR Activity Guide: Part 1

Note:

- The 3 stages of (1) denaturation, (2) annealing, and (3) extension are repeated 20-40 times, doubling the number of DNA copies each time.
- A complete PCR reaction can be performed in a few hours, or even less than an hour with certain high-speed machines.
- After PCR has been completed, gel electrophoresis can be used to check the quanty and size of the DNA fragments produced.

Resources:

- *https://www.yourgenome.org/facts/what-is-pcr-polymerase-chain-reaction*