Student Exercise on Polymerase Chain Reaction (PCR)

# **Prepared by the Office of Biotechnology, Iowa State University**

## Part I.

**Segment of interest**

(01) Original-1 3' T C G G C T A C A G C A G C A G A T G G T A C G T A 5'

(02) Original-2 5' \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ 3'

**1.** The purpose of PCR is to make copies of a DNA segment of interest. In our exercise, one strand of the double helix of DNA will be designated Original-1. Write the nucleotide sequence of the complementary strand designated Original-2.

## Part II.

(01) Original-1 3' T C G G C T A C A G C A G C A G A T G G T A C G T A 5'

5' \_ \_ \_ \_ \_

(Primer-1)

(02) Original-2 5' A G C C G A T G T C G T C G T C T A C C A T G C A T 3'

3' \_ \_ \_ \_ \_ 5'

(Primer-2)

**2.** A piece of DNA known as the primer is artificially made that has a nucleotide sequence complementary to the bases adjacent to the segment of interest on the 3' end of Original-1. Write the nucleotide sequence of the five bases of Primer-1.

**3.** A primer is artificially made that has a nucleotide sequence complementary to the bases adjacent to the segment of interest on the 3' end of Original-2. Write the nucleotide sequence of the five bases of Primer-2.

## Part III.

(01) Original-1 3' T C G G C T A C A G C A G C A G A T G G T A C G T A 5'

(C1) Copy-1 5' C C G A T \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ 3'

(O2) Original-2 5' A G C C G A T G T C G T C G T C T A C C A T G C A T 3'

(C2) Copy-2 3' \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ A T G G T 5'

**4.** In cycle 1 and all subsequent cycles of the PCR reaction, a copy of each of the two original strands will be made beginning at the 3' end of the primer and continuing to the 5' end of the original strand. Write the sequence of the copies that are made from the strands of O1 and O2.

## Part IV.

(01) Original-1 3' T C G G C T A C A G C A G C A G A T G G T A C G T A 5'

(C1) Copy-1 5' C C G A T \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ 3'

(C1) Copy-1 5' C C G A T G T C G T C G T C T A C C A T G C A T 3'

(CC1) Copy-C1 3' \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ 5'

(O2) Original-2 5' A G C C G A T G T C G T C G T C T A C C A T G C A T 3'

(C2) Copy-2 3' \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ A T G G T 5'

(C2) Copy-2 3' T C G G C T A C A G C A G C A G A T G G T 5'

(CC2) Copy-C2 5' \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ 3'

**5.** During the second cycle of PCR, a copy is made of each of the strands of Original-1, Copy-1, Original-2, and Copy-2 obtained in cycle 1. Write the sequence of Copy-1 formed during the replication of Original-1 in cycle 2. Does the sequence differ from that of Copy-1 made in the first cycle? Write the sequence of Copy-2 formed during the replication of Original-2 in the second cycle. Does the sequence differ from that of Copy-2 made in the first cycle?

**6.** To make a copy of the Copy-1 (C1) strand, a primer attaches to appropriate sequences on the strand. Note that only one of the two primers will be appropriate. Write the sequence of the primer. Complete the sequence of CC1.

**7.** To make a copy of the Copy-2 strand, write the primer sequence at the appropriate place on CC2. Complete the sequence of CC2.

**8.** How many strands of each of the following are present after the second cycle?

O1 \_\_\_ O2 \_\_\_

C1 \_\_\_ C2 \_\_\_

CC1 \_\_\_ CC2 \_\_\_

## Part V.

O1

\_\_\_ ?

C1

\_\_\_ ?

C1

\_\_\_ ?

CC1 3' G G C T A C A G C A G C A G A T G G T 5'

CC2 5' \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ 3'

O2

\_\_\_ ?

C2

\_\_\_ ?

C2

\_\_\_ ?

CC2 5' C C G A T G T C G T C G T C T A C C A 3'

CC1 3' \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ \_ 5'

**9.** For the third cycle of PCR, each of the eight strands produced by Cycle 2 are replicated. Write the symbol of the strand produced by replication of O1, C1, O2, and C2. You may refer to part IV of the exercise for assistance.

**10.** To replicate CC1 and CC2, a primer attaches to an appropriate sequence on each of the strands. Write the primer sequence at the appropriate place on the new strands. Complete the sequence of the two strands.

**11.** How many strands of each of the following types are present after the third cycle?

Total number \_\_\_\_\_

O1 \_\_\_ O2 \_\_\_

C1 \_\_\_ C2 \_\_\_

CC1 \_\_\_ CC2 \_\_\_

## Part VI.

**12.** During the fourth cycle of PCR, the 16 strands produced by cycle 3 will each be copied in the same manner as the previous cycles. Use Part IV as a reference and determine the number of strands of each of the following that will be present after cycle 4 is completed. When making your determination, remember to count the number of new strands made as well as the number of strands in the solution at the beginning of the cycle.

Total number \_\_\_\_

O1 \_\_\_ O2 \_\_\_

C1 \_\_\_ C2 \_\_\_

CC1 \_\_\_ CC2 \_\_\_

## Part VII.

**13.** The total number of strands present at the end of each cycle is equal to (2)cycle no.+1 For example, the total number at the end of cycle 5 is (2)6. Use this principle to calculate and record the total number of strands in the table below.

**14.** The PCR reaction began with a double helix of DNA that was separated into two strands designated O1 and O2. The number of strands of O1 and O2 does not change during PCR. Record the number of strands of O1 and O2 in the table below.

**15.** The number of copies of each of C1 and C2 increases by one each cycle. As a result, the number of copies of each C1 and C2 is equal to the cycle number. Record the number of strands of C1 and C2 in the table below.

**16.** The number of copies of each of CC1 and CC2 in each cycle can be determined by subtracting the copies of O1, O2, C1 and C2 from the total number and dividing the difference by 2. For example, the number of copies of each CC1 and CC2 after cycle 4 is equal to 32 minus 10 divided by 2, which is 11 copies of CC1 and 11 of CC2. Use this principle to complete the table below.

Number of Strands

Cycle Total O1 O2 C1 C2 CC1 CC2

O \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_

1 \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_

2 \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_

3 \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_

4 \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_

5 \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_

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10 \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_

:

:

:

20 \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_