Jonah Ventures Lesson Plan Activity

Lesson Topic: The Environmental DNA Process: Sampling, Extraction, PCR, Next Generation Sequencing, and Analysis

Teacher Instructions

Objectives:

- The students will understand what eDNA is, how it is measured, why it is useful, and what some of the limits are.
- The students will learn the process necessary to determine the species DNA present in eDNA samples.
- The students will understand how primers work, and that they are specific to groups of species.
- The students will be able to replicate the PCR process on their own, beginning with a single strand of DNA.
- The students will understand how Next Generation Sequencing works and how sequences are matched to species.
- The students will be able to think critically and extrapolate conclusions from the information.

Main Activity:

Supplies:

- Scissors and glue stick for each pair of students
- Printed and cut out sequence slips
- Printed and stapled PCR Cycle Packets, one packet for each pair of students
- Printed and cut out Plant Sequence Library Cards
- Printed student worksheet packets, one for each pair of students
- A large bucket
- A large, clear plastic bag
- Printed and cut out primer slips, one for each pair of students

Guide:

- A: Plant Sequence Library Cards (one set of four for each group of students, this page will need to be printed once for each group)
- B: Primer Slips (cut out on the grey line and give one to each group of students, this page will only need to be printed once for a group of 32 split into pairs)
- C: Sequence Slips (cut out on the grey line and keep together in a plastic bag, these pages will only need to be printed once for a group of 32 split into pairs)
    - Note: For a group of 32, there are 8 Junegrass sequences, 3 willow sequences, 4 clover sequences, and 1 aster sequence. Do not reveal this to the students.

- PCR Cycle 1-4: Print out one of each set for each group of students. Staple the pages together. Do not cut these worksheets, the students will do that.

Introduction:

- It is highly recommended that teachers go through the series of educational slides that accompany this activity to familiarize the students with the topic before the activity.
- Split the students into pairs.
- Prior to European colonization, an estimated 30-60 million American Bison roamed North America. By 1884, only 325 wild bison remained, due to habitat loss and mass slaughter for sport, skins, and to make way for railroads and grazing land for cattle. Now, thanks to public bison preservation efforts, there are an estimated 500,000 domestic and wild bison in North America, though most of these are genetically impure, having been bred extensively with domestic cattle. Today, only three herds of genetically pure, wild America Bison occur in North America; a herd of 250-400 individuals in Wind Cave National Park of South Dakota, a herd of 250-400 individuals in the Henry Mountains of Utah, and a herd of around 5,000 individuals at Yellowstone National Park in Idaho, Montana, and Wyoming.
- Explain to the students that they are environmental DNA scientists. Wildlife researchers are monitoring one of these few remaining herds of genetically pure wild bison in the United States. They are interested in knowing what plants these bison eat at different times of year so they can help sustain the bison herd by promoting commonly eaten species. Bison are traditionally considered grass grazers, though this is based on relatively little information. Recent research suggests that they may be feeding on other plants as well. The researchers also want to disturb the animals as little as possible, which is why they are turning to eDNA to examine the bison's diets. The researchers have taken fecal samples and have asked the class to find out which species of plants the bison are eating and in what amounts.
- Hand out the PCR packet, one set of plant library cards, and one primer slip to each group of two students.

Sampling:

- At this time, place the bag of sequence slips into the bucket. Explain that the plastic bag is the feces, holding DNA within it, and the bucket is the sampling cup.

Extraction:

- Now they are headed back to the lab to complete extraction. Pour the bag into the bucket to extract the DNA from the filter.

PCR:

- At this time, walk around the room, and have each student match their primer slip to a sequence in the bucket. Their primer must be complementary to the DNA that they select. A pairs with T, G pairs with C. There will be sequences left over in the bucket. Show them to the students and explain that these are sequences that belong to species other than plants, because their primer is specific to plants. Perhaps the left-over sequences belong to parasites, fungi, bacteria, insects, or the bison itself.

- At this point, they must perform PCR with their primer and sequences. On the blank spaces on their sequence, they must write their primer and then figure out the missing letters on the opposite side. Remind them, A pairs with T, G pairs with C.
- Once this is complete, have the students cut on the thick dotted line between the paired strands. In their PCR packet, have the students glue each separate strand into a different pair of boxes in the PCR cycle 1 section. Now they must once again write down the base pairs that match with their sequence on the opposite side. This process is repeated through PCR cycle 4. At the beginning, they begin with one set of sequences, but by the end, they should have sixteen sets of sequences, explaining the exponential growth that the DNA experiences during PCR. They can attach these final two sheets to their worksheet.

Next Generation Sequencing:

- Direct the students' attention to the plant sequence library cards. Have them go through each possible plant species. There is a line of colored circles on each card with letters that correspond to some of the students' sequences. A is blue, T is orange, G is yellow, and C is purple. Have the students match up the sequences from their worksheet to the decoded sequence on the slide. Explain that during the Next Generation Sequencing stage, the machine registers colors and then translates them to letters. Have them write down their species on their worksheet.

Analysis:

- Go around the room and have each group say which species they had and have the students take a tally. Have them enter in the number of each species from the class on their worksheet and create a pie chart of their classroom species assemblage.

Discussion:

- Which plant species was most abundant? Which was least abundant?
- Determine the species richness with the class (how many species in the diet)
- Did they find anything surprising?
- American bison are now found throughout the US and Canada. Based on this knowledge and the plant range maps, what are the possible states where the sample was collected?
- There are more than just four plant species in this area. What are some reasons for why you were unable to detect these other species in your fecal sample?
- Have them record the answers on their worksheet.

For Further Reading on Bison Diet:

- Craine, Joseph M., et al. "Climatic Warming and the Future of Bison as Grazers." *Scientific Reports*, vol. 5, no. 16738, 2015, doi:10.1038/srep16738.
- Jorns, Tami, et al. "Climate Structures Bison Dietary Quality and Composition at the Continental Scale." *Environmental DNA*, vol. 00, 2019, pp. 1–14, doi:10.1002/edn3.47.

Jonah Ventures eDNA Group Worksheet

Names:_____ Date:_____

Supplies: This worksheet, PCR packet, primer slip, glue stick, scissors, Plant Library cards

Introduction:
Prior to European colonization, an estimated 30-60 million American Bison roamed North America. By 1884, only 325 wild bison remained, due to habitat loss and mass slaughter for sport, skins, and to make way for railroads and grazing land for cattle. Now, thanks to public bison preservation efforts, there are an estimated 500,000 domestic and wild bison in North America, though most of these are genetically impure, having been bred extensively with domestic cattle. Today, only three herds of genetically pure, wild America Bison occur in North America; a herd of 250-400 individuals in Wind Cave National Park of South Dakota, a herd of 250-400 individuals in the Henry Mountains of Utah, and a herd of around 5,000 individuals at Yellowstone National Park in Idaho, Montana, and Wyoming.

You and your team are environmental DNA scientists. Wildlife researchers are monitoring one of these few remaining herds of genetically pure wild bison in the United States. They are interested in knowing what plants these bison eat at different times of year so they can help sustain the bison herd by promoting commonly eaten species. Though bison are traditionally considered grass grazers, this is based on relatively little information and recent research suggests that they may be feeding on other plants as well. The researchers also want to disturb the animals as little as possible, which is why they are turning to eDNA to examine the bison's diets. The researchers have taken fecal samples and have asked your class to find out which species of plants the bison are eating and in what amounts.

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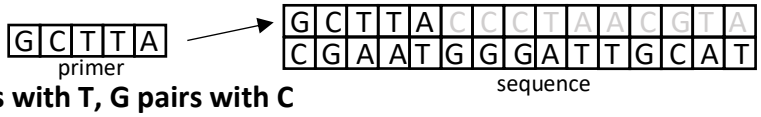
Instructions:
Take your primer slip, and as your teacher moves around the classroom, match up the primer slip to one of the sequences, or segments of DNA, in the bucket.

Example:



Remember: A pairs with T, G pairs with C

Write in the missing letters of the sequence (as in gray above).

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Now you will perform PCR with your sequence. Cut along the black dotted line and split the sequence into two strands. When you put DNA into a reaction and the temperature is increased, known as denaturation, the two strands of DNA split apart. Take each of these two strips, and glue one each to one side of the paired boxes on your PCR worksheet in the PCR cycle 1 section. This is the annealing portion of PCR. Fill in the missing letters of the sequence once again on the opposite side, which is called elongation, completing the sequence. Now you should have two identical paired segments of DNA. Cut each of the sequences apart, and repeat the steps above. By the end of PCR cycle 2 you should have four identical segments, in PCR cycle 3 you will have eight, and in PCR cycle 4, you will have sixteen identical sequences. The amount of DNA doubles in each cycle, and the number of sequences increases exponentially, making it easier for scientists and machines to detect and decode.

Please attach the completed PCR cycle 4 with all of your sequences to the back of this worksheet.

Use the Plant Library cards and compare them to your group of sequences. One of the Plant Library cards has the exact match of one side of your sequence on it in different colors. Next Generation sequencing reads the base pairs in colors instead of letters. Find what species of plant your group has, and record information below.

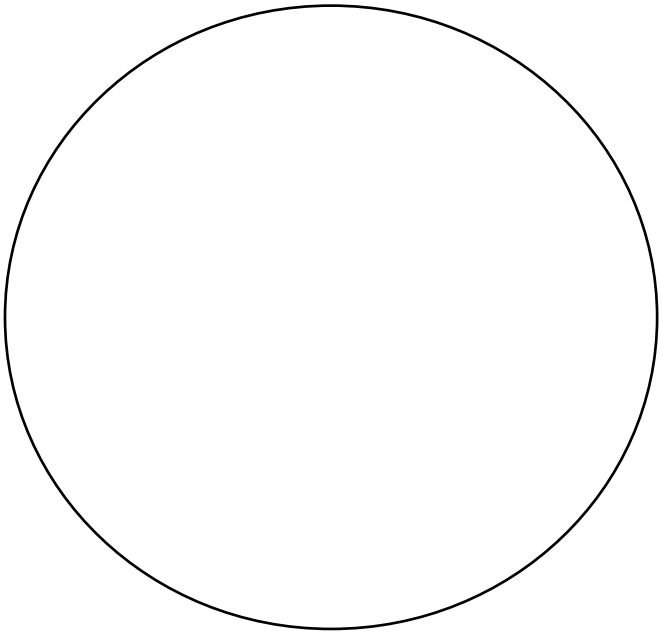
What species of plant did your group have? _____

List some facts about this plant (range, habitat, native/invasive, etc.): _____
_____
_____

Tally of plant species the bison ate:

Species	**Tallies**	**Percentages**
June grass		
Willow		
Aster		
Clover		
Total		100%

Make a pie chart, complete with percentages:



Which species were most, and least abundant in this bison diet? _____

_____

What is the species richness (number of species) in this diet? _____

If a true “grazer” relies on grass for more than 75% of its diet, would you say that your bison is a grazer? Other categories are “mixed feeders” (25-50% grass) and “browsers” (<25% grass).

_____

Based on what you now know about the bison that are being sampled and the plant range maps, what are some possible states where the sample was collected?

_____

_____

There are more than just four plant species in the area. What may be some reasons for why you were unable to detect these other plant species in your sample?

_____

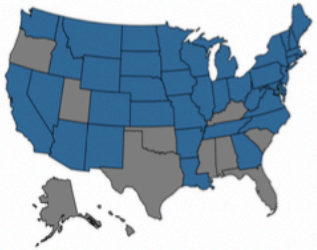
_____

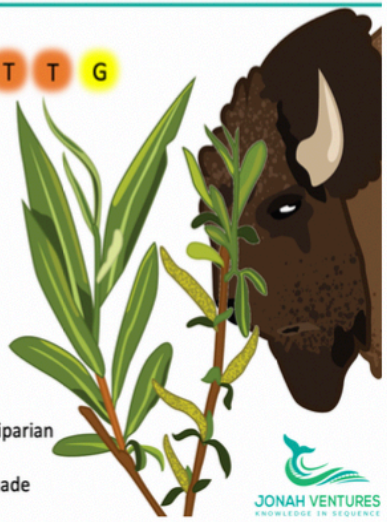
White Willow (*Salix alba*)

Plant Sequence Library

- **Barcode Sequence:**

T A C C C G A A G T A C T T G





- Introduced to the United States
- Native to Europe, northern Africa, and central Asia
- This plant has naturalized in many areas and provides food and riparian habitat to wildlife species
- Grow in average to wet, well-drained soils in full sun to partial shade

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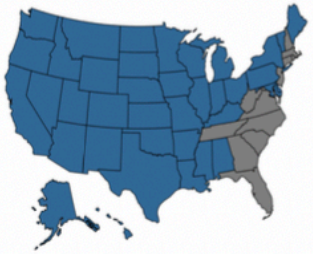
Prairie Junegrass (*Koeleria macrantha*)

Plant Sequence Library

- **Barcode Sequence:**

T A C C C T C T T G G G A A T



- Endangered in Ohio and Kentucky, critically impaired in Louisiana
- Native to the United States but introduced to Hawaii
- Good forage
- Grows in rocky or sandy, well drained soils within forests or plains in direct sunlight

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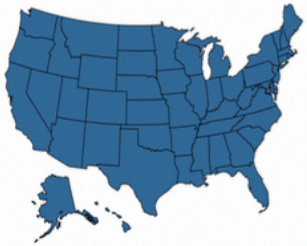
KNOWLEDGE IN SEQUENCE

Red Clover (*Trifolium pratense*)

Plant Sequence Library

- **Barcode Sequence:**

T A C C C G A T A G T C C A A



- Introduced and permitted in the United States
- Native to Europe, Western Asia, and northwest Africa
- Widely grown as a fodder crop, and valued for its ability to increase soil fertility
- Prefers full sun and moderately moist soil, but is also drought tolerant

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KNOWLEDGE IN SEQUENCE

White Heath Aster(*Symphyotrichum ericoides*)

Plant Sequence Library

- **Barcode Sequence:**

T A C C C G T A T C G G A C T

- Threatened in Tennessee
- Native to the United States
- This plant grows wild but has little value as food or cover for wildlife
- Prefers open locations with full sunlight and sandy, gravelly, or disturbed soil

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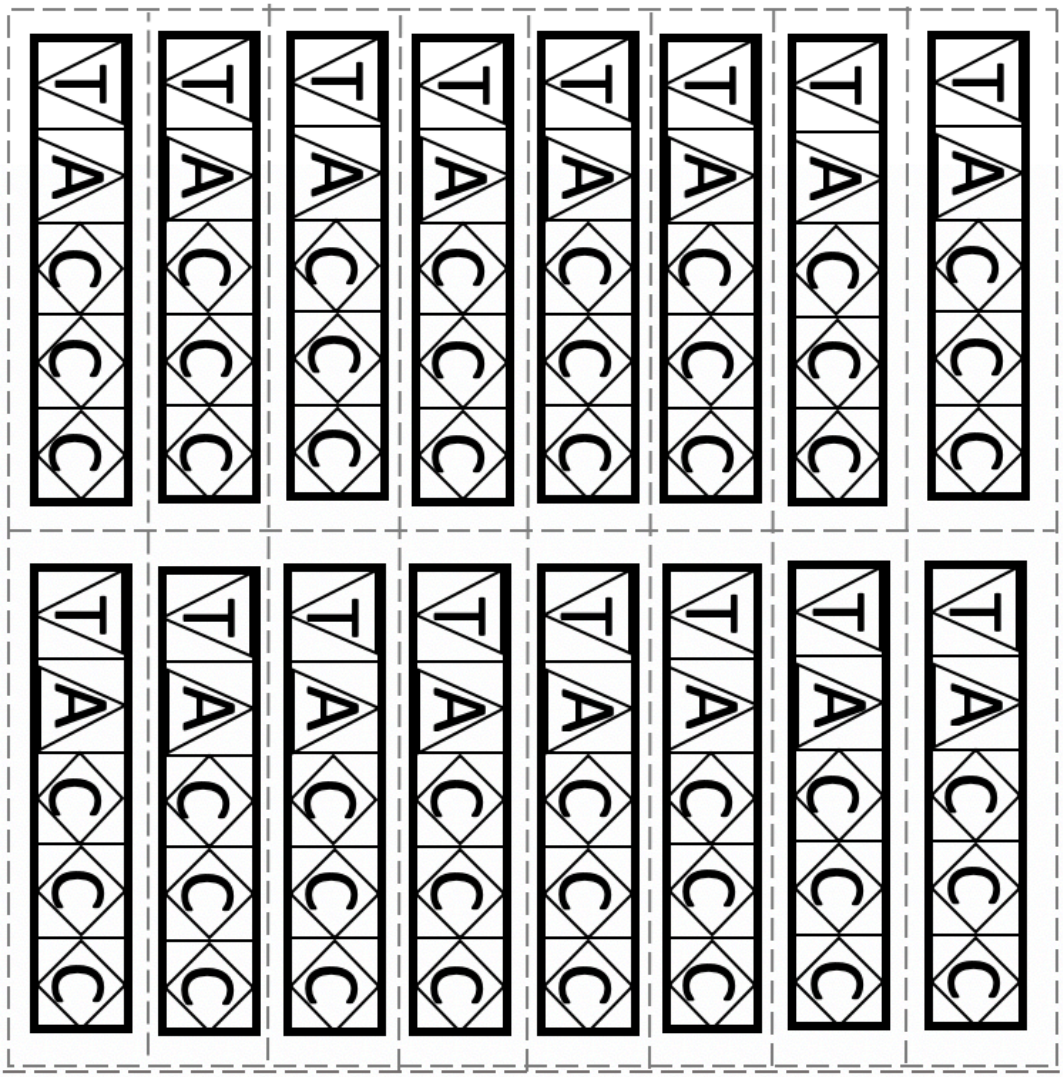
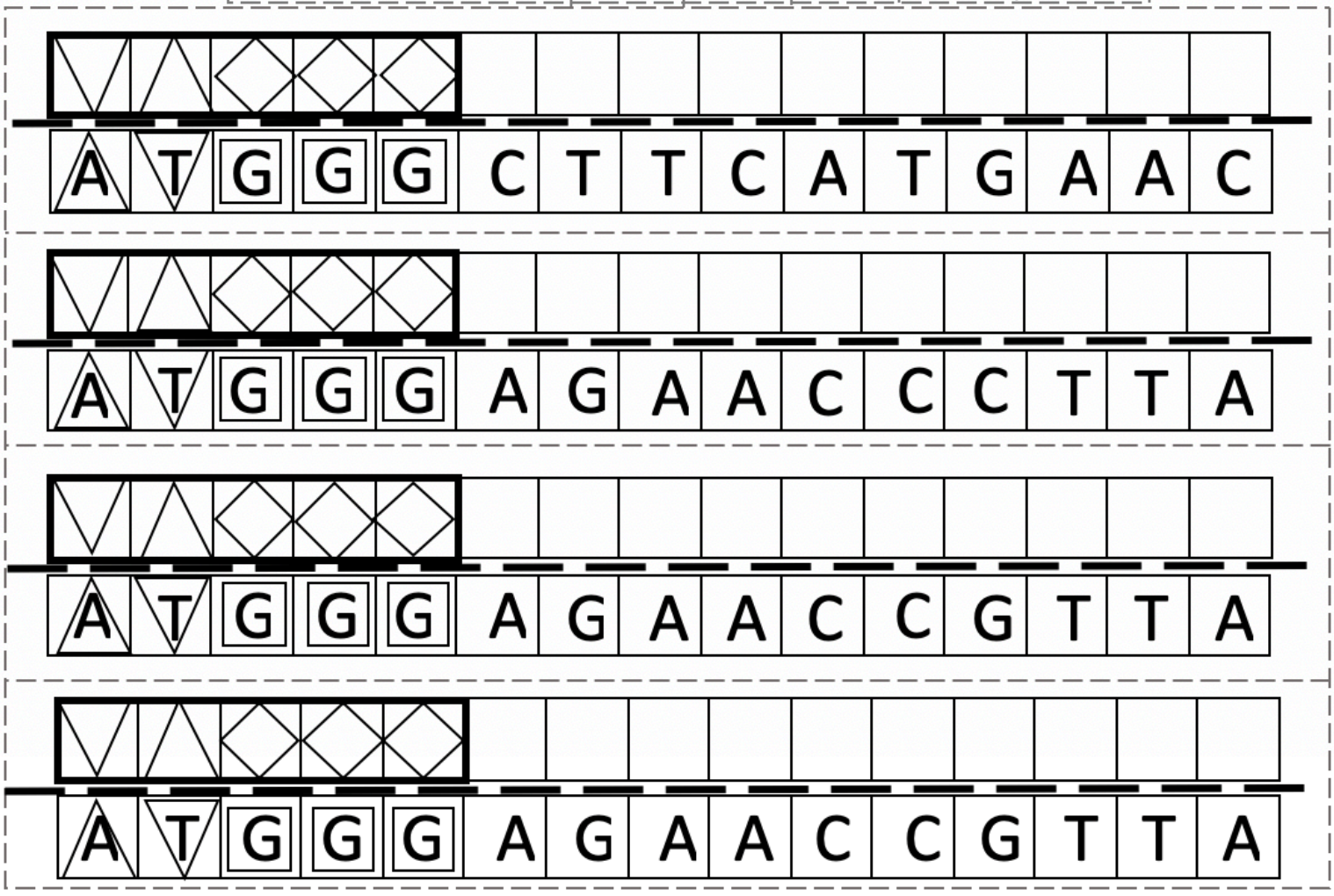
KNOWLEDGE IN SEQUENCE

B


T A C C C C

C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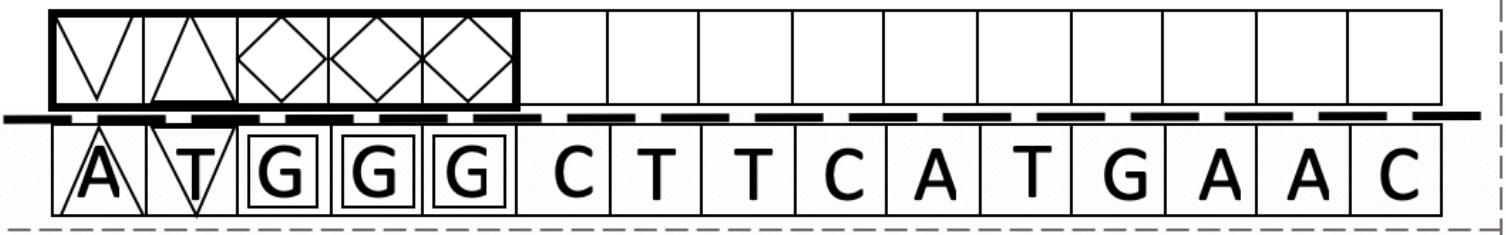
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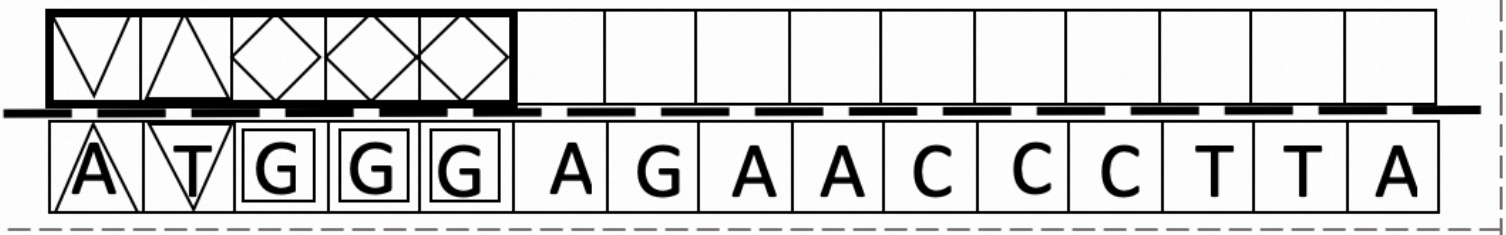
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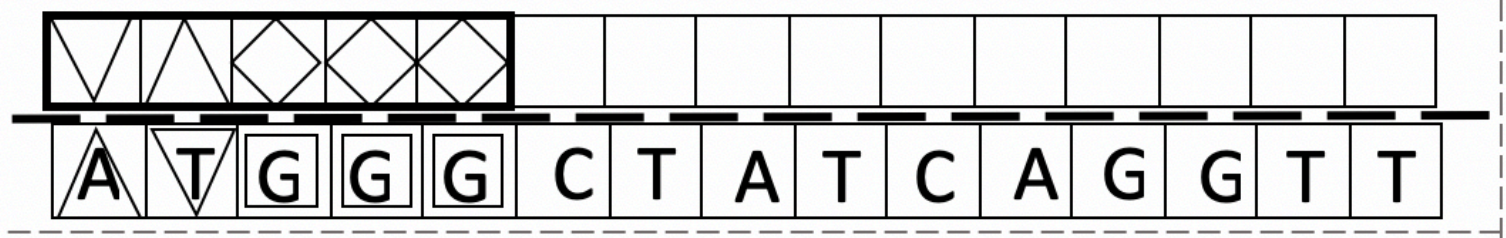
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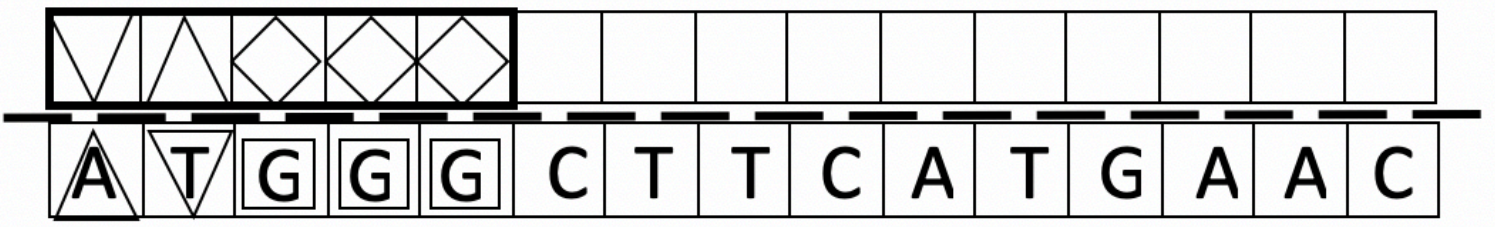
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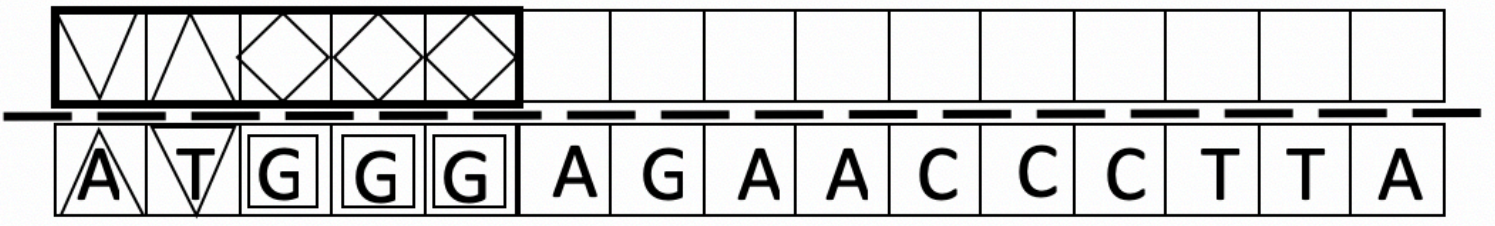
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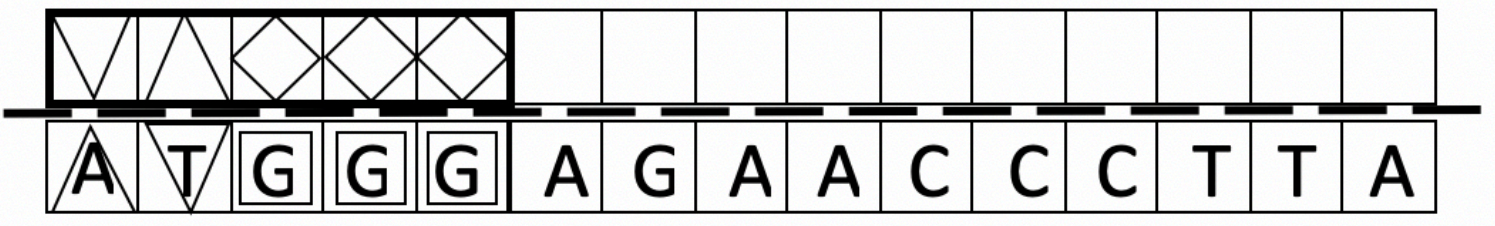
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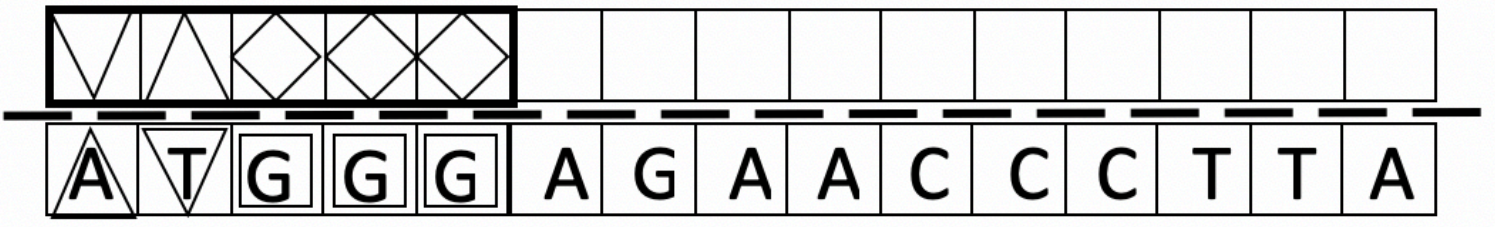
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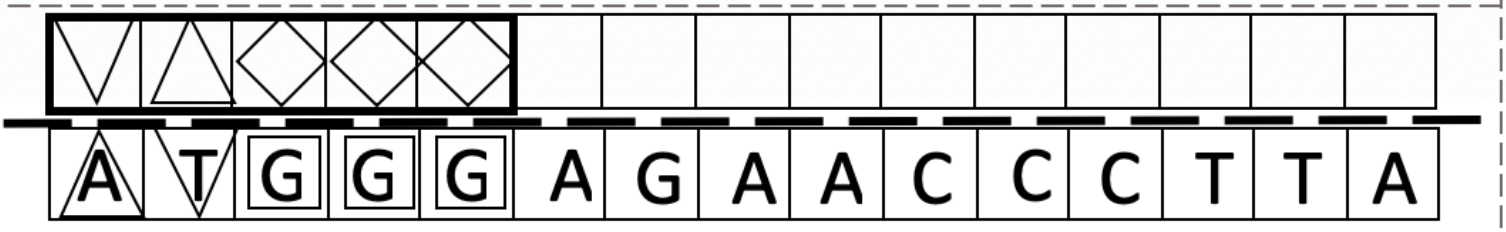
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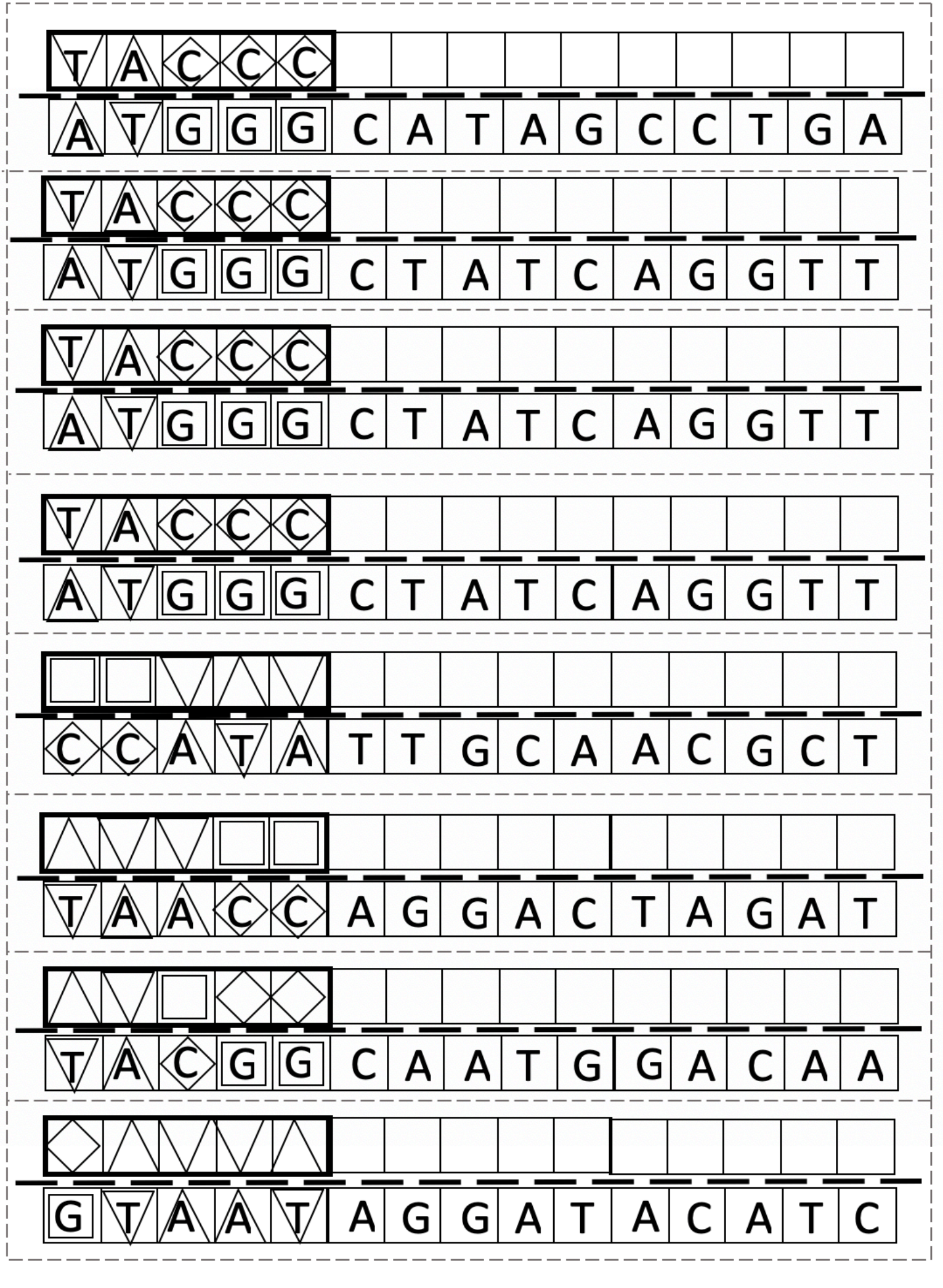
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ATGGGCATAGCCTGA
TACCC
ATGGGCTATCAGGTT
TACCC
ATGGGCTATCAGGTT
TACCC
ATGGGCTATCAGGTT
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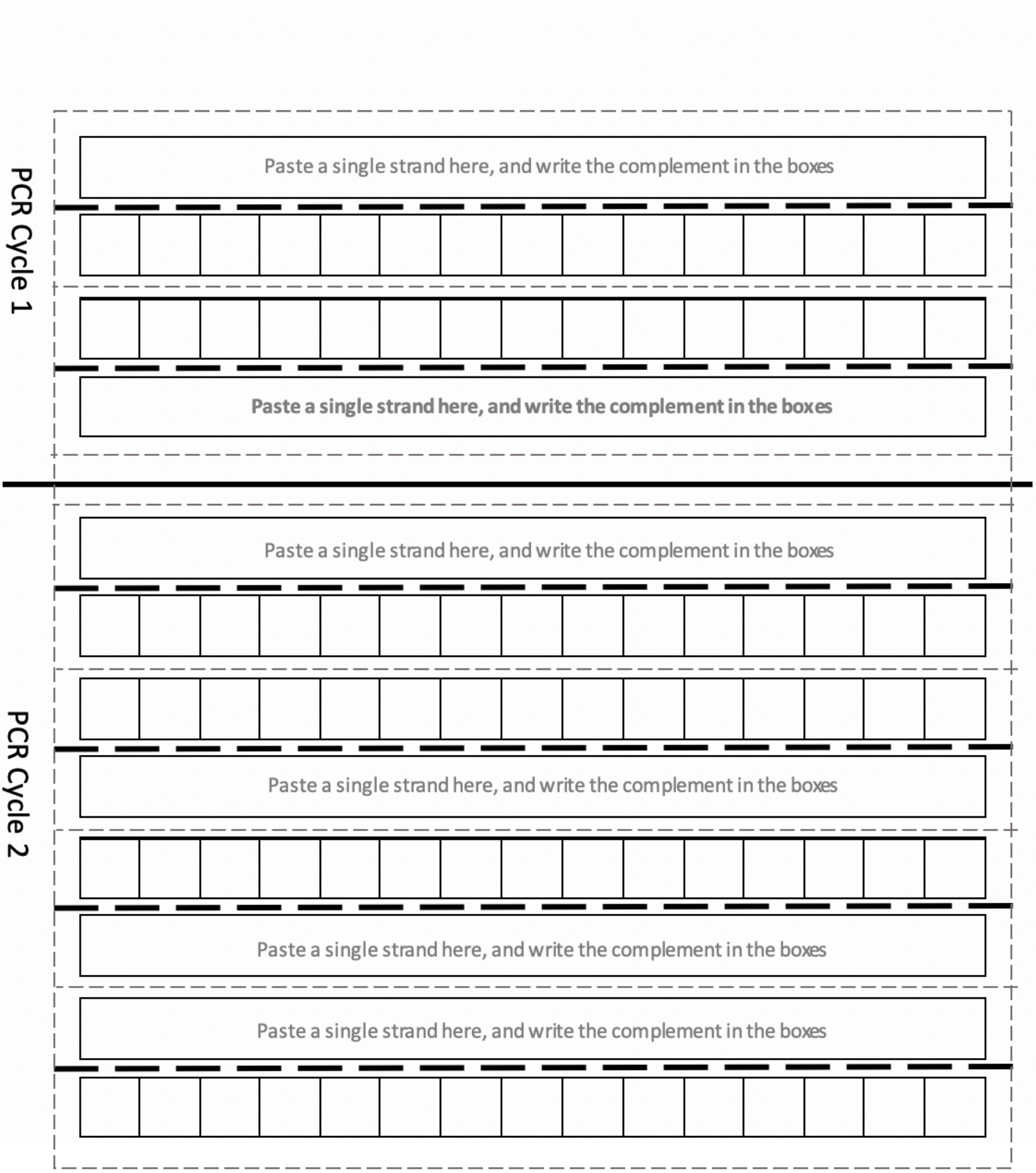
TAACCAGGACTAGAT
TACGGCAATGGACAA
GTAATAGGATACATC

PCR Packet


PCR Cycle 1
Paste a single strand here, and write the complement in the boxes
Paste a single strand here, and write the complement in the boxes
PCR Cycle 2
Paste a single strand here, and write the complement in the boxes
Paste a single strand here, and write the complement in the boxes
Paste a single strand here, and write the complement in the boxes
Paste a single strand here, and write the complement in the boxes

PCR Cycle 3

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PCR Cycle 4

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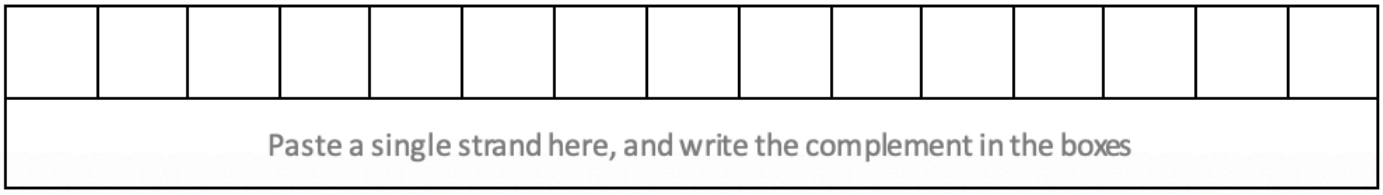
PCR Cycle 4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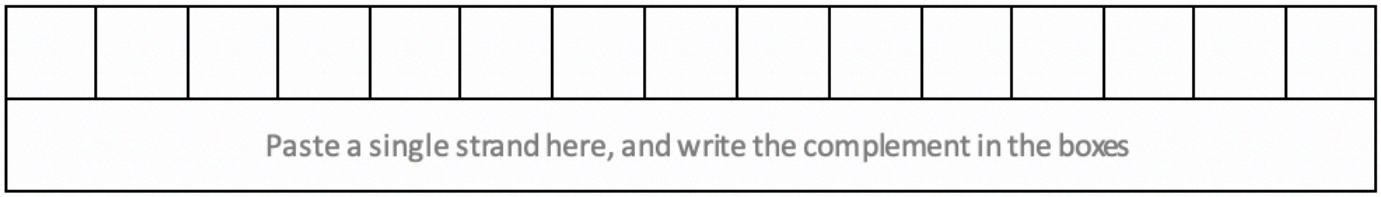
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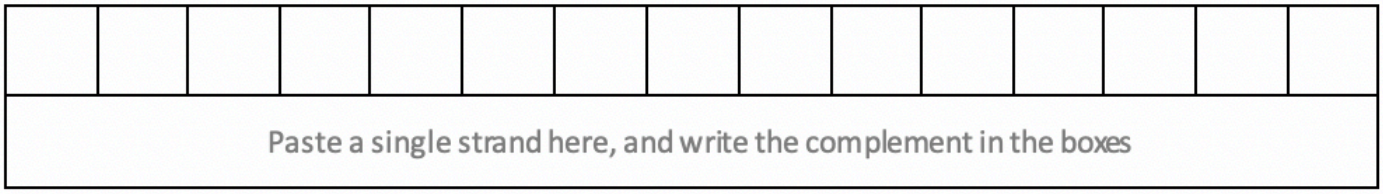
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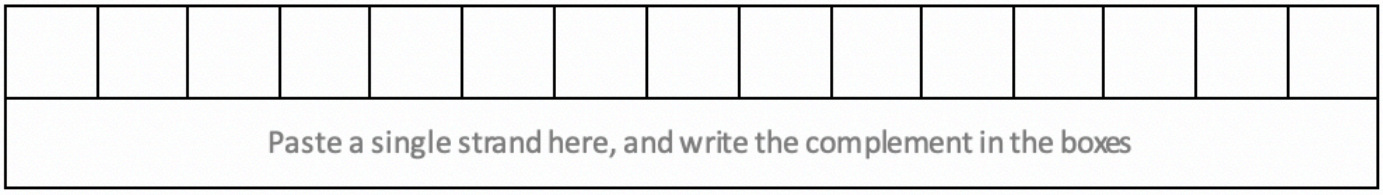
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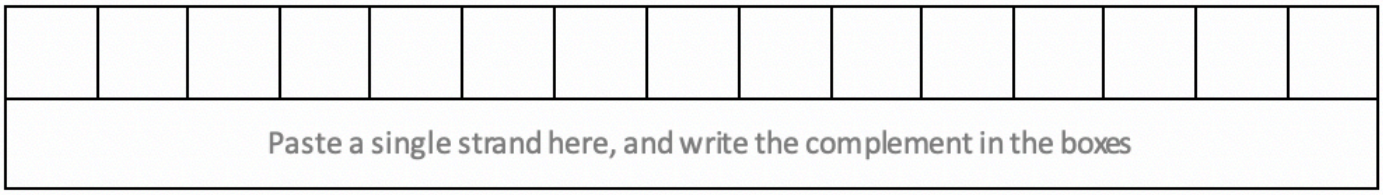
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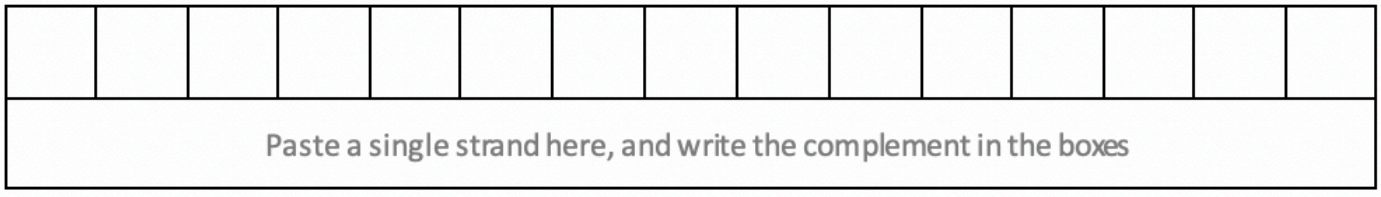
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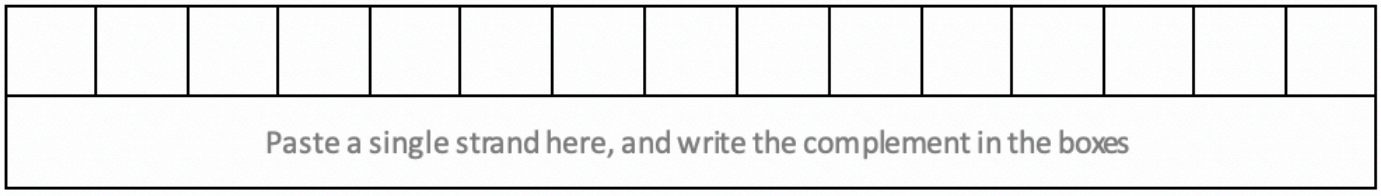
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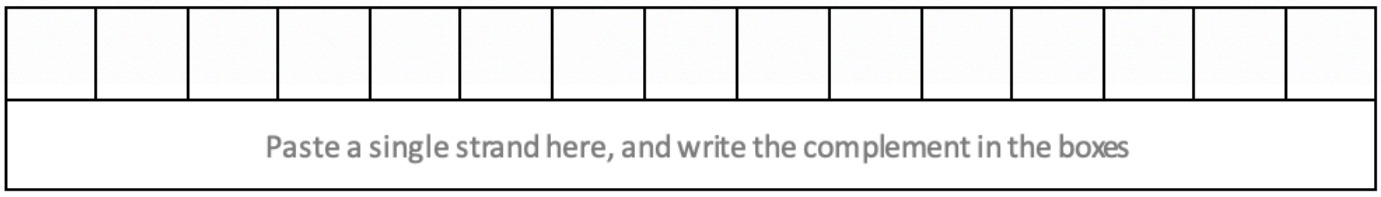
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