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 Fish 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: Fish Sequence Library Cards (one set of three for each group of students)
- B: Primer Slips (cut out on the grey line and give one to each group of students)
- C: Sequence Slips (cut out on the grey line and keep together in a plastic bag)
- 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.

- Explain to the students that they are environmental DNA researchers. An environmental consulting agency has called and would like them to sample a small river in the United States. A contractor has bought the property alongside the river and wants to build an apartment complex. This might threaten any sensitive local fish species in the river if there are any. The consulting company is interested in the species of fish in the river, and would like the students to investigate it using environmental DNA.
- Hand out the PCR packet, one set of fish library cards, and one primer slip to each group of students.

Sampling:

- They now must go to the river and collect a sample of water. At this time, place the bag of sequence slips into the bucket. Explain that with water samples, the DNA must be filtered out of the water before extraction and tell them that the plastic bag is the filter holding DNA. This completes the sampling and water filtration step.

Extraction:

- Now they are headed back to the lab to complete extraction. Explain to the students that the bag is the filter, holding all of the DNA. 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 fish, because their primer is specific to fish. Perhaps the left-over sequences belong to algae, mammals, or amphibians.
- 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 fish sequence library cards. Have them go through each possible fish species. There is a line of colored circles on each card with letters that correspond to some of the students' sequences. Go through each species slide and decode the sequences on each slide together. 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.

Discussion:

- Which species was most abundant? Which was least abundant?
- Determine the species richness with the class (how many species in the ecosystem)
- Do they think this system is healthy? Why/why not?
- What were the invasive, rare, or common species? Are there primarily invasive, rare, or common species, or a mixture of all three?
- Did they find anything surprising?
- Based on the range maps and the fish found in the river, where do you think this river occurs? List possible states.
- There are more than just four fish species in this body of water. What are some reasons for why you were unable to detect these other fish species in your water sample?
- Have them record the answers on their worksheet.

Jonah Ventures eDNA Group Worksheet

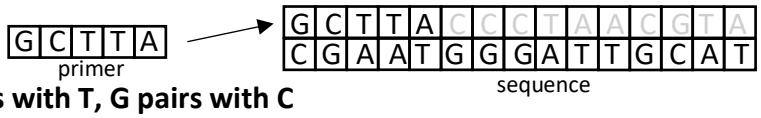
Names:_____ Date:_____

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

Introduction: You are environmental DNA researchers. An environmental consulting agency has called and would like you to sample a small river in the United States. A contractor has bought the property alongside the river and wants to build an apartment complex. This might threaten any sensitive local fish species in the river. The consulting company is interested in the species of fish in the river, and would like you to investigate it using environmental DNA.

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).

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 16 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 Fish Library cards and compare them to your group of sequences. One of the Fish 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 fish you have, and record information below.

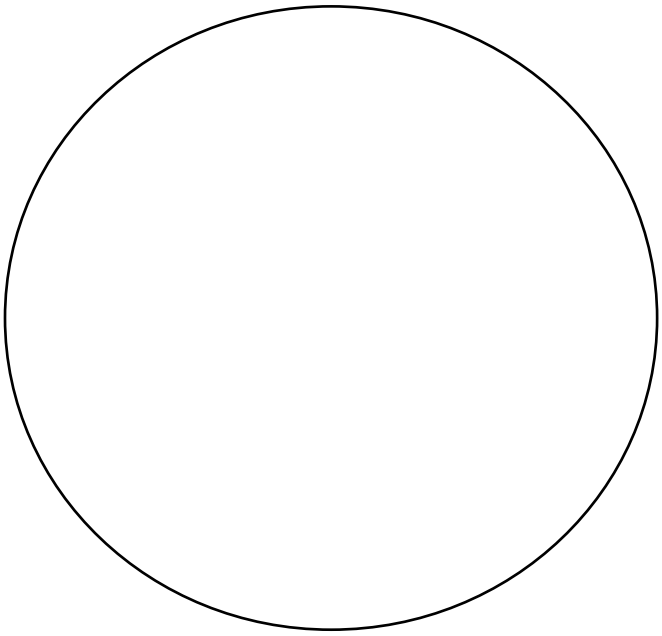
What species of fish did your group have? _____

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

Tally of fish species in the classroom:

Species	**Tallies**	**Percentages**
Northern Pike		
Bighead Carp		
Bluegill		
Gilt Darter		
Total		100%

Make a pie chart, complete with percentages:



Which species was most abundant in this ecosystem? _____

Which species was least abundant?_____

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

What are the invasive/native, rare/common species? _____
_____

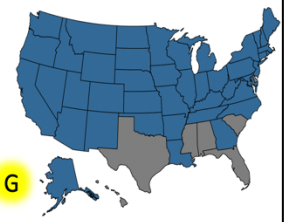
Based on the range maps and the fish found in the river, where do you think this river occurs? List possible states. _____
_____

There are more than just four fish species in this body of water. What are some reasons for why you were unable to detect these other fish species in your water sample?
_____
_____
_____
_____

Northern Pike (*Exos lucius*)

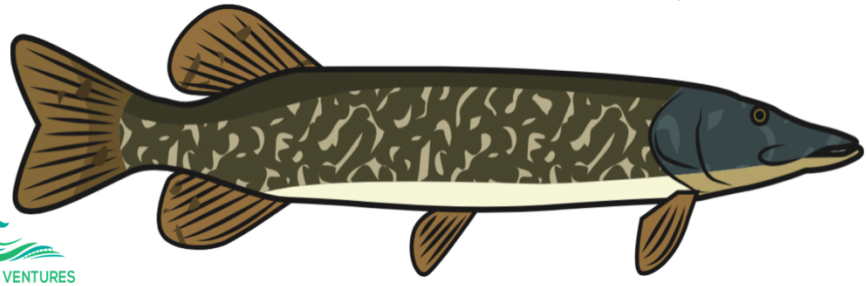
Fish Sequence Library

- Least Concern, Native
- Feeds on fish, invertebrates, amphibians, birds, and small mammals
- This fish usually occurs in clear small lakes, shallow vegetated areas of larger lakes, marshes, creeks, and small to large rivers



- Barcode Sequence

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



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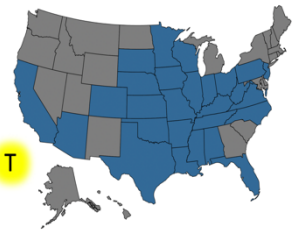
Bighead Carp (*Hypophthalmichthys nobilis*)

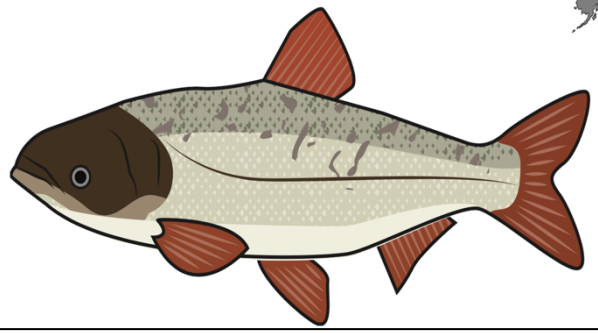
Fish Sequence Library

- Least Concern, Invasive
- Feeds primarily on plankton and detritus
- This fish is native to large rivers and the floodplain lakes of Asia



- Barcode Sequence

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



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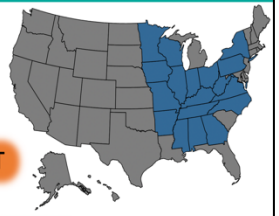
Gilt Darter (*Percina evides*)

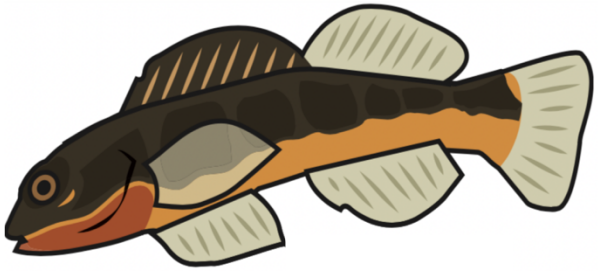
Fish Sequence Library

- Endangered, Native
- Feeds on aquatic insect larvae
- This fish usually occurs in cool-water streams



- Barcode Sequence

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



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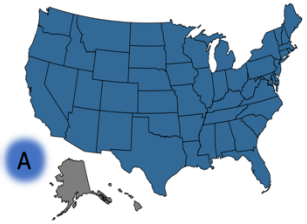
Bluegill (*Lepomis macrochirus*)

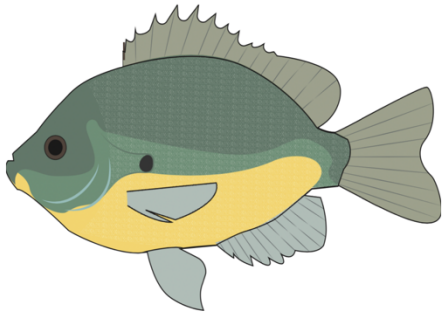
Fish Sequence Library

- Least Concern, Native
- Omnivore; feeds on insects, smaller fish, and aquatic vegetation
- This fish lives in ponds, rivers, streams, and lakes



- Barcode Sequence

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



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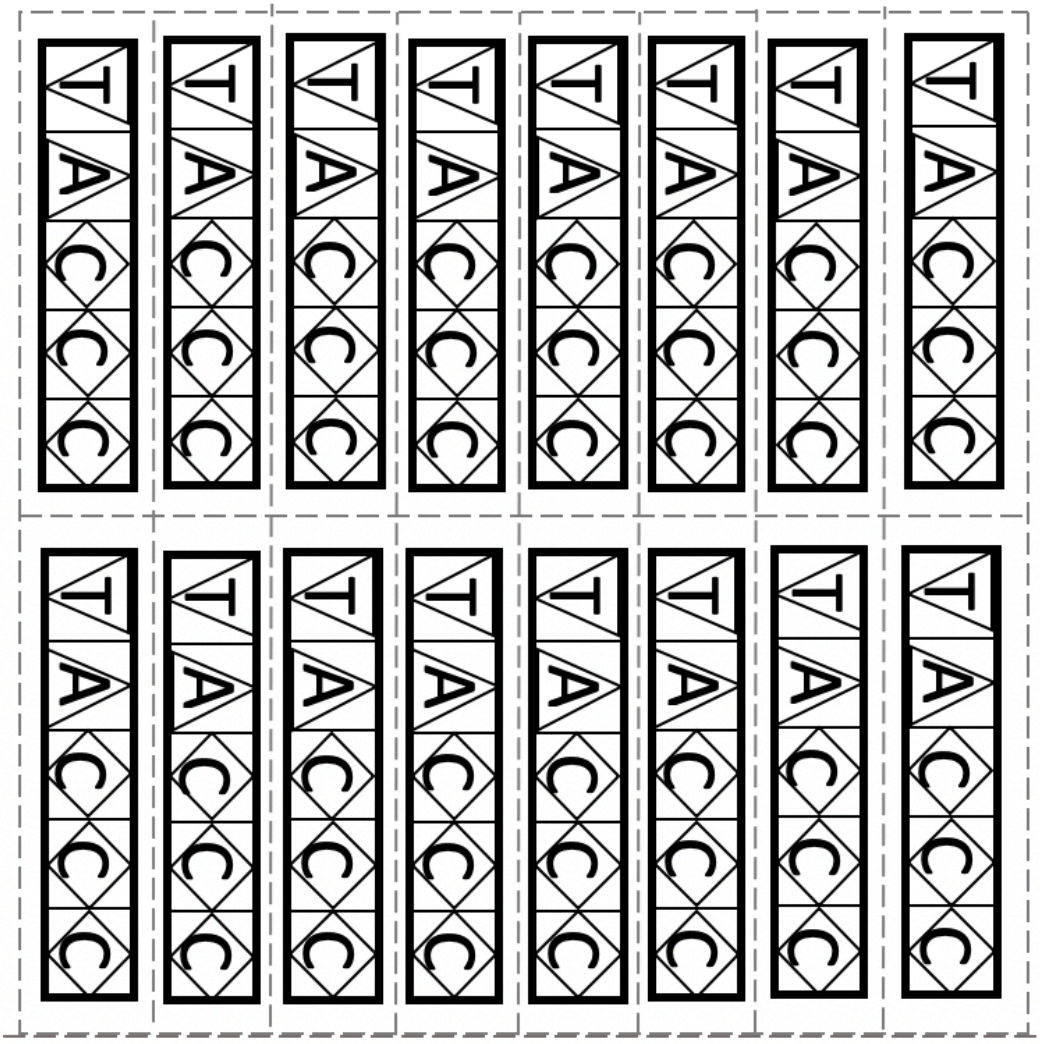
B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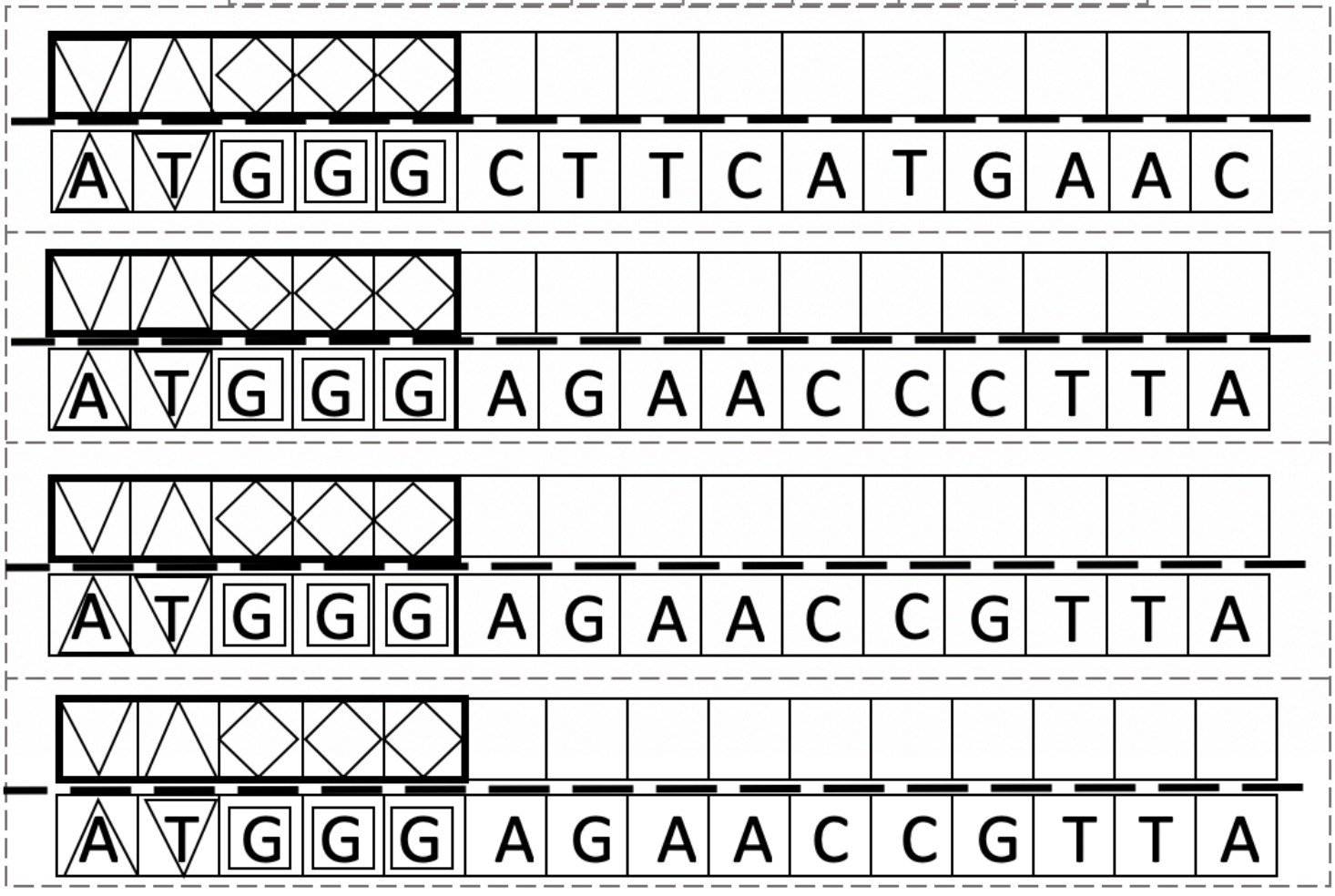
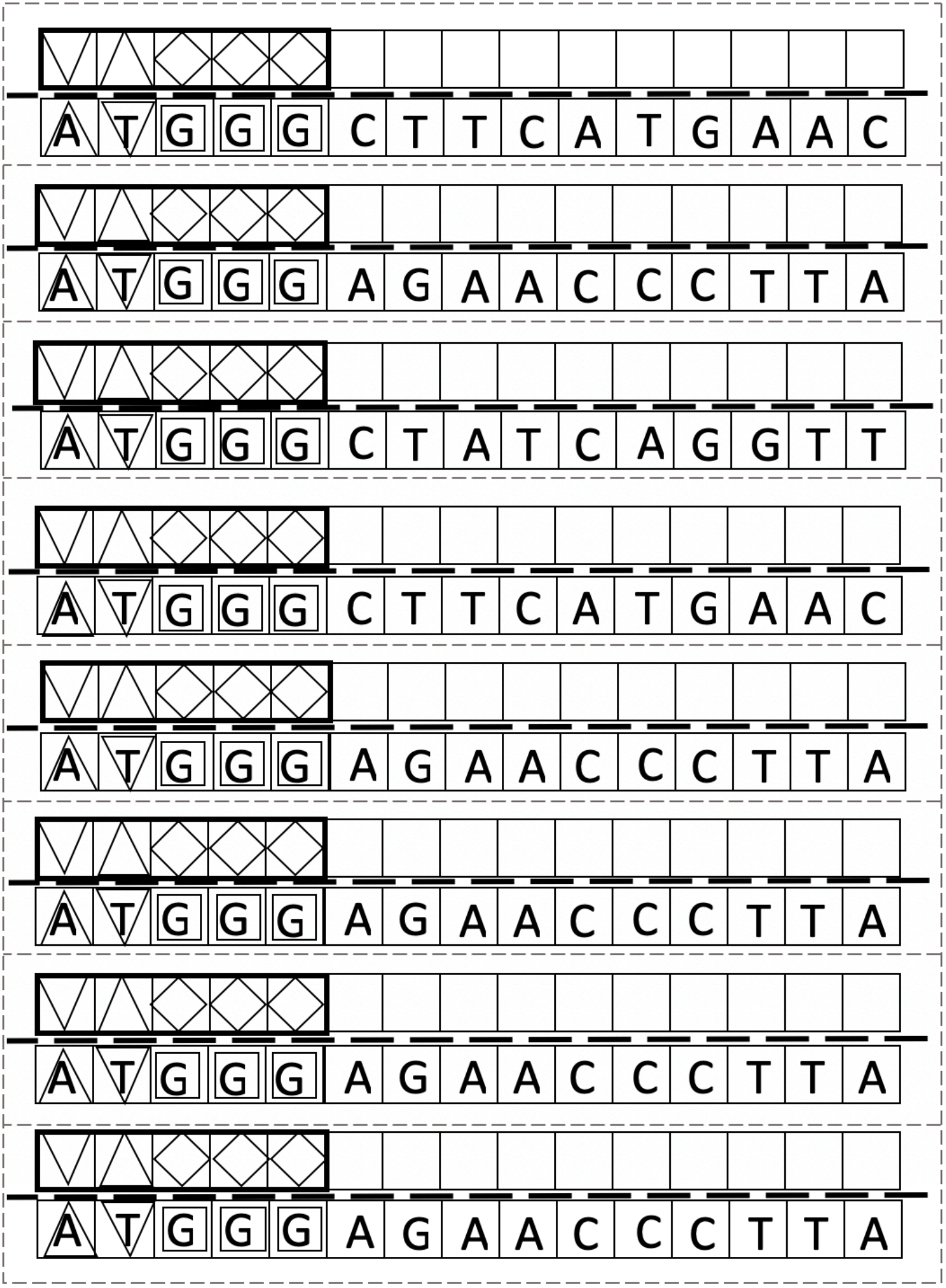
C


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


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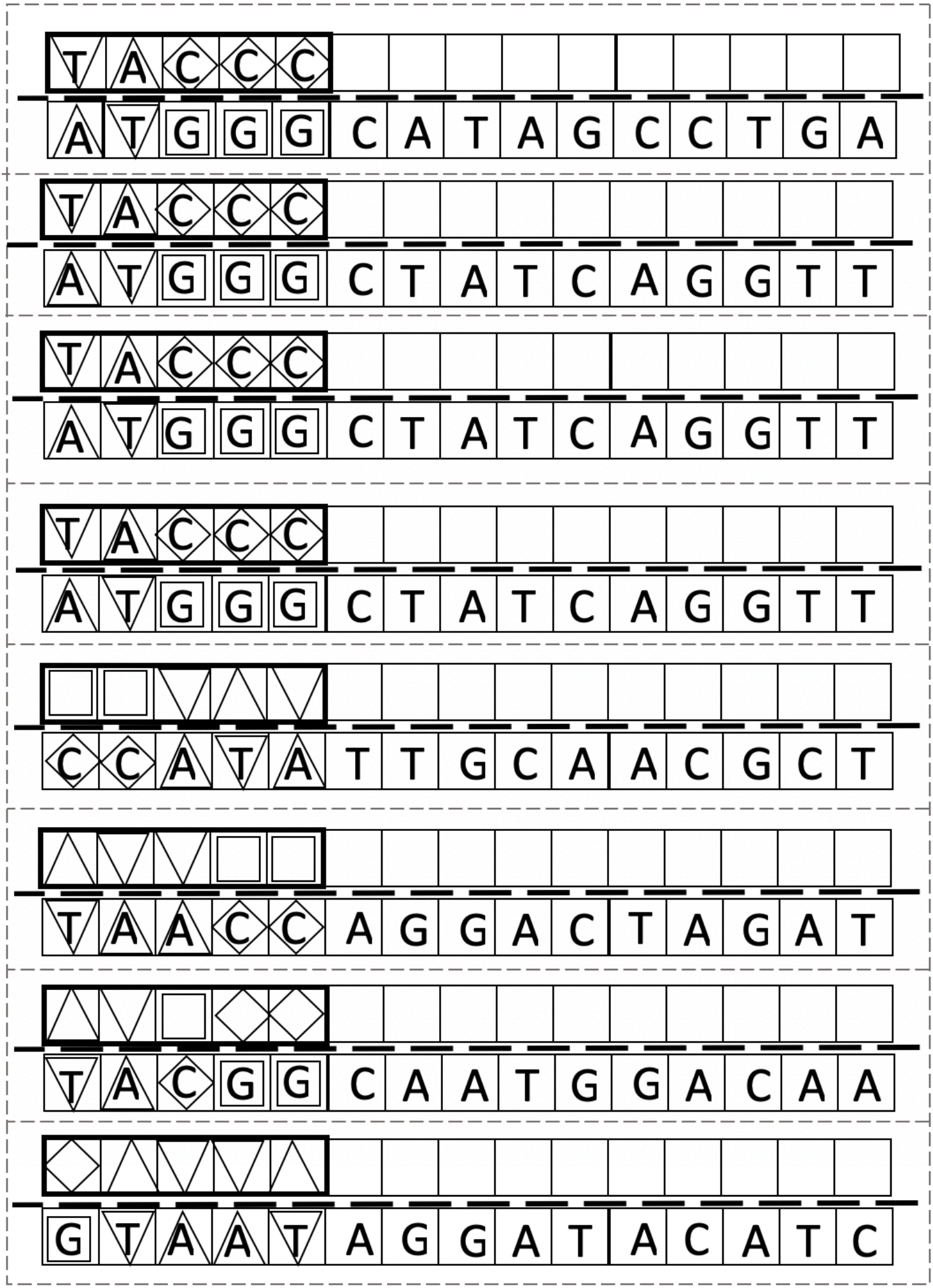
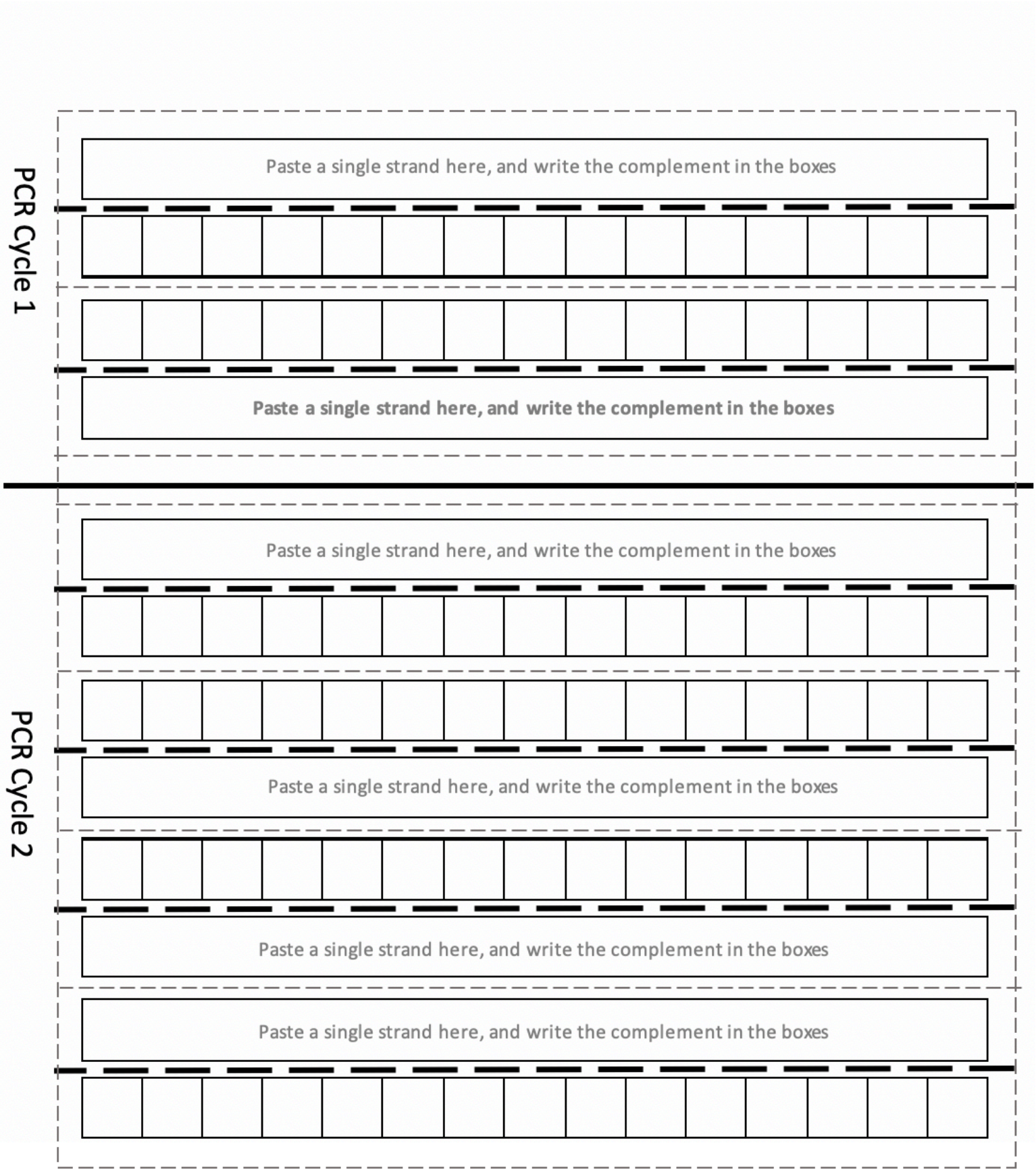

T A C C C
A T G G G C A T A G C C T G A
T A C C C
A T G G G C T A T C A G G T T
T A C C C
A T G G G C T A T C A G G T T
T A C C C
A T G G G C T A T C A G G T T
C C A T A T T G C A A C G C T
T A A C C A G G A C T A G A T
T A C G G C A A T G G A C A A
G T A A T A G G A T A C A T C

PCR Packet


PCR Cycle 1
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Paste a single strand here, and write the complement in the boxes
PCR Cycle 2
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PCR Cycle 3


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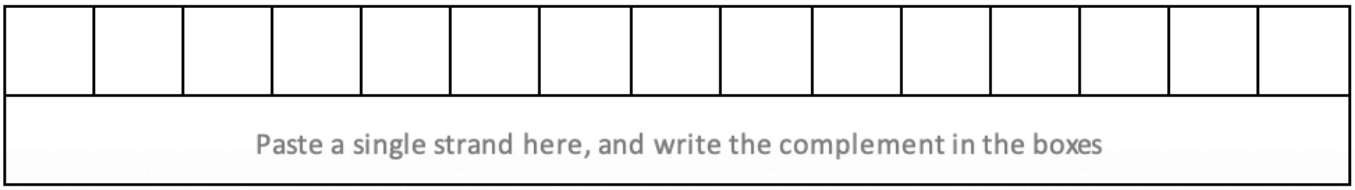
PCR Cycle 4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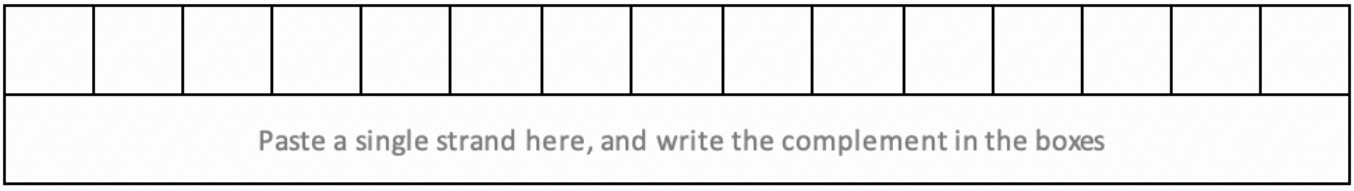
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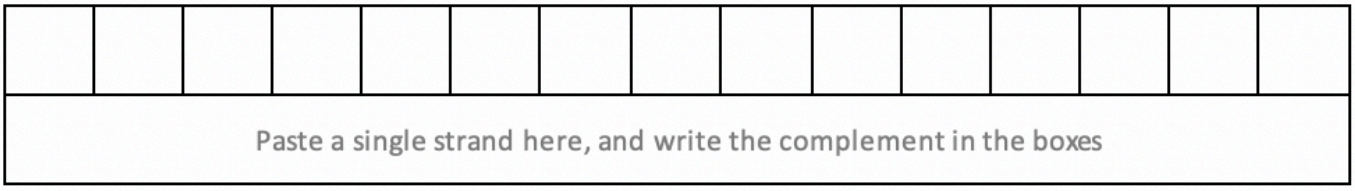
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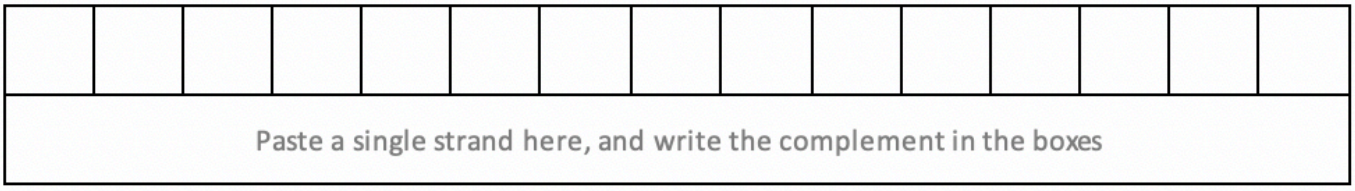
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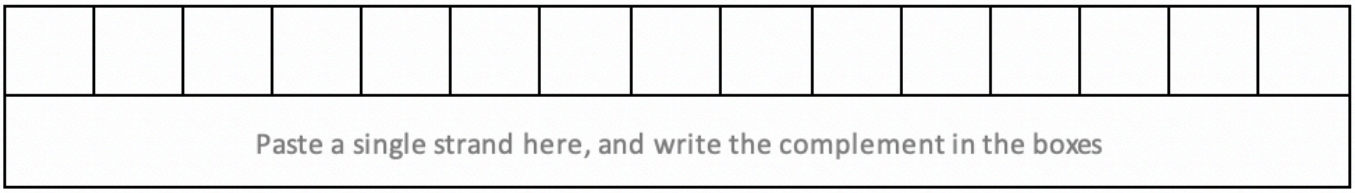
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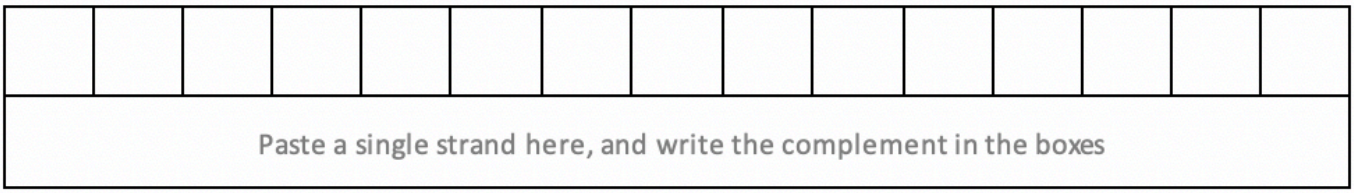
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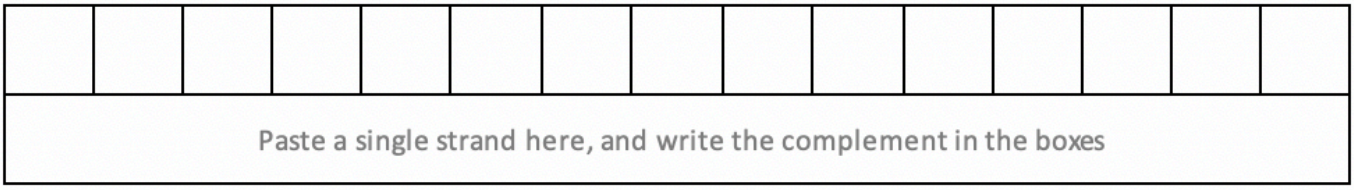
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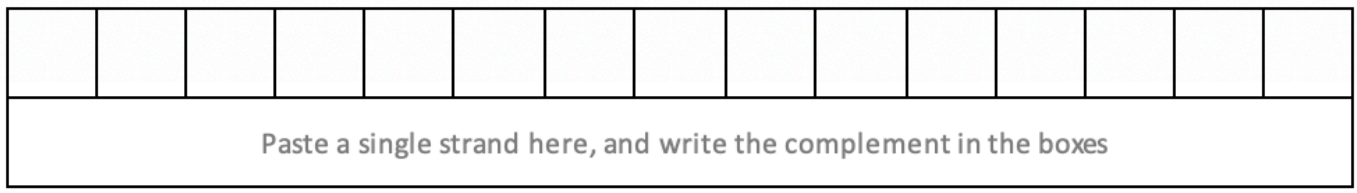
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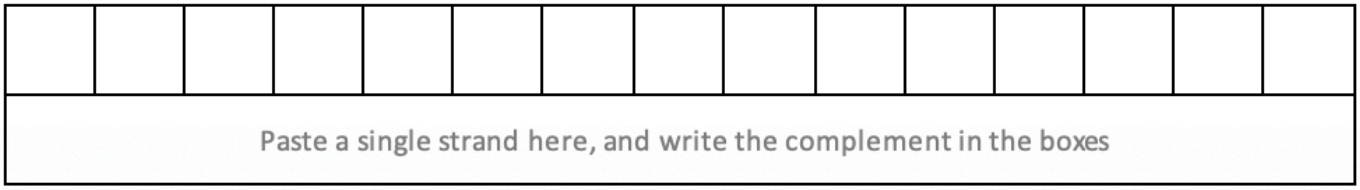
PCR Cycle 4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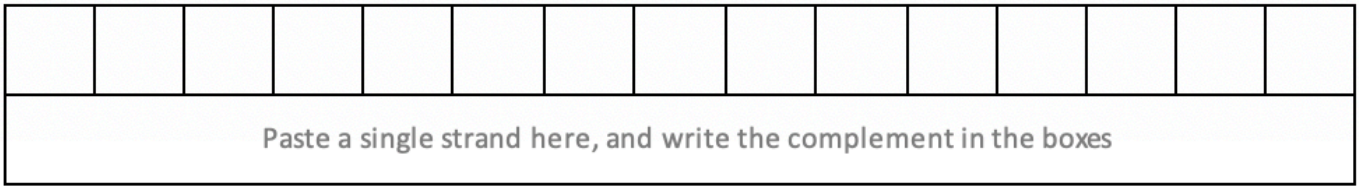
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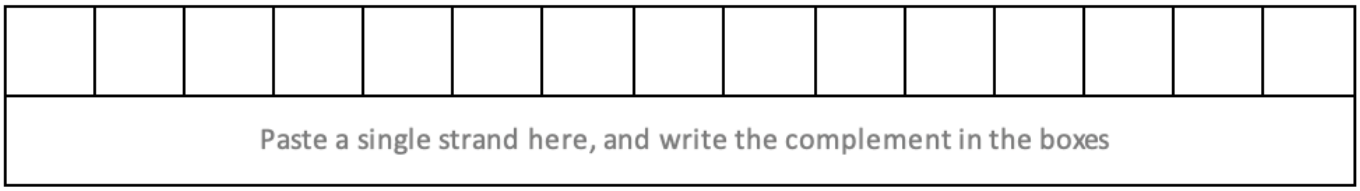
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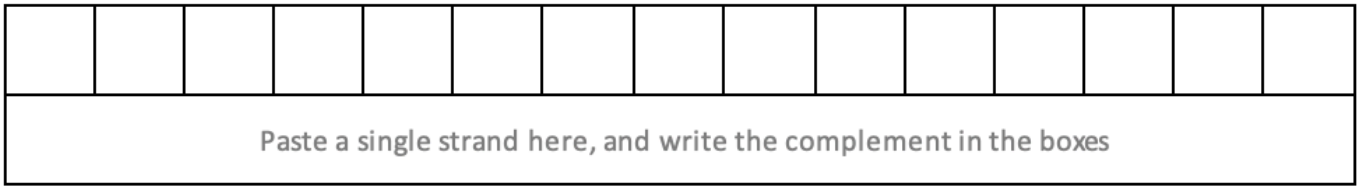
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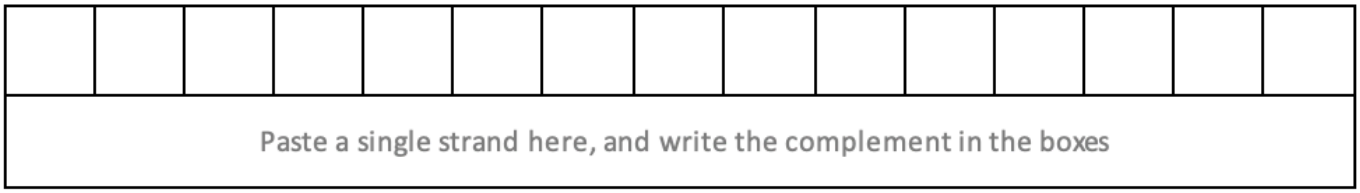
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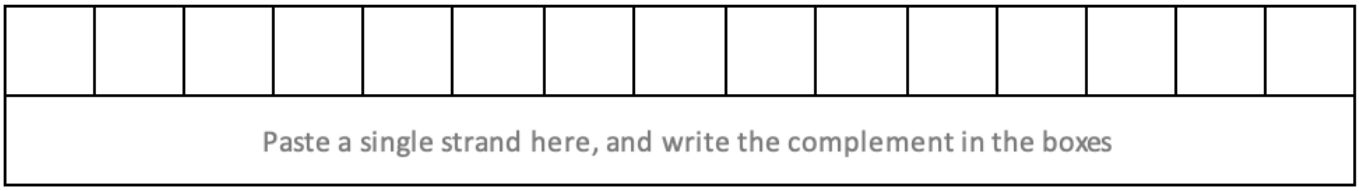
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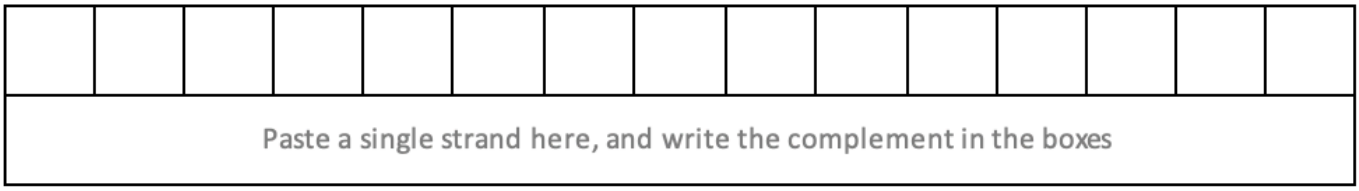
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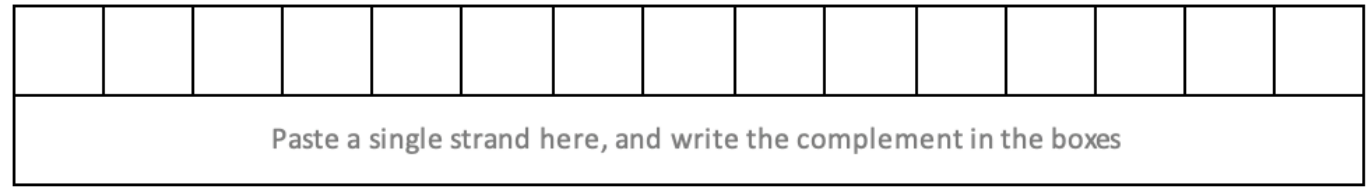
Paste a single strand here, and write the complement in the boxes