PCR

(Polymerase Chain Reaction)
Long Target DNA Sequence

Intro:
PCR allows a researcher to locate a specific fragment of DNA within a sample. The DNA fragment could be a section of DNA specific to one individual in a blood sample, a section of DNA from a disease-causing bacteria in a water sample or any fragment of DNA the researcher is interested in. This fragment of "**target DNA**" is isolated from all of the rest of the DNA in the sample and copied many times over. At the end of the process, the researcher is left with a billion copies of the **target DNA** and almost none of the other DNA.
This process allows a researcher to identify the presence of a target organism within a sample - if the organism is present, its **target DNA** will be copied. If the organism is not present, there is no **target DNA** to be copied.
PCR is also used to provide the researcher enough of the **target DNA** for experiments. While a few strands of the **target DNA** extracted from a few organisms might not be enough for the researchers' experiment, a billion copies of the **target DNA** made through PCR will probably suffice.

PCR is a three step process:

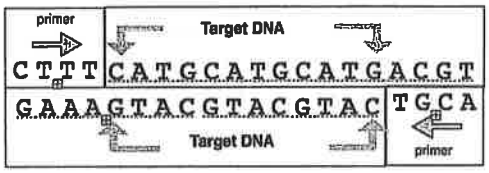
1. heating the DNA to denature - or break apart the double strand to create two single strands of DNA
2. cooling the DNA to attach primers - stretches of DNA that will match up with and attach to sections of the single-stranded DNA on either side of the **target DNA**.
3. reheating to extend the DNA -add bases to the single-stranded DNA to create double-sided DNA. This process begins at the primer that was attached to the DNA and continues in one direction only.

This process is repeated 30 times - or cycles. After the 3rd cycle, 2 stretches of DNA have been created that contains only the **target DNA**. Each cycle after this doubles the number of **target DNA**, but not the rest of the DNA in the sample. The 4th cycle gives 4 stretches of **target DNA**, then 8, then 16 and so on until, after 30 cycles there are a billion copies of the **target DNA** and almost none of the rest of the DNA in the sample.

This activity takes the students through three cycles only - enough to get their **target DNA**. If desired, more cycles can be completed to get more copies of the target DNA

*Note: The **target DNA** isolated in this activity will also include the primers at each end of the Target DNA section.*

This is what you are trying to produce



The steps that are modeled in this activity:

1. Denature the double-stranded DNA

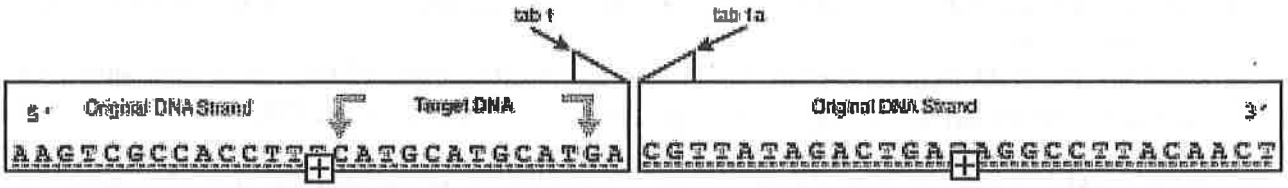
2. Anneal primers
3. Extend the DNA

Teacher Prep for Activity

1. Print one set of this activity for each group.
2. Provide the following materials to each group:
   - Scissors
   - tape

Student Prep for Activity

1. Cut out the four **Original DNA Strands** on page 7.
2. Fold back the tabs on each strand. Tape the top two strands together at tab 1 and tab 1a to make one long DNA strand.



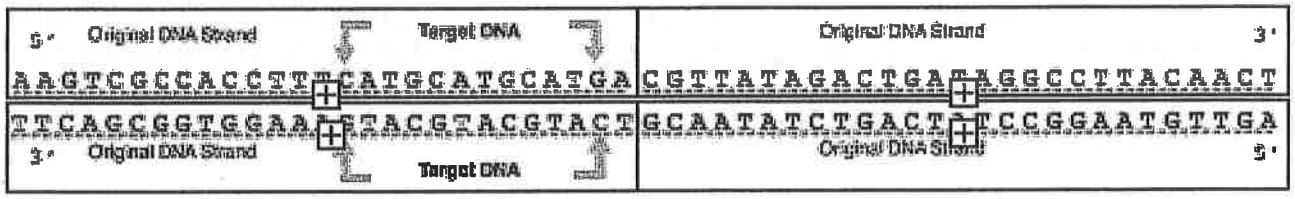
3. Repeat this process with the bottom two strands, taping the strands together at tabs 2 and 2a.
   You should end up with two equal lengths of single stranded DNA.

4. Cut out the six pieces of **Nucleotides** on page 9.
5. Fold back the tabs on the four strands that have tabs. Tape the strands together at tab 3 and 3a.

6. Repeat the process for the **Nucleotides** on pages 11- 15.

*In a real PCR reaction, individual nucleotides would be added to the PCR mix and, during the reaction, they would be added to the **original DNA strands** one at a time. However, for ease of handling, the nucleotides have been combined in a strand and the whole strand will be added all at once.*

7. Cut out the **Primers** on page 17.
8. Place all of your pieces on a large flat surface. These represent the important parts of the PCR reaction in the experiment.
9. Find the two **Original DNA Strands** and place them one above the other, as shown below. Make sure the base pairs match up - **A** to **T** and **G** to **C**. This represents the double-stranded DNA from which you wish to find the Target DNA to amplify.



Perform the PCR Reaction

Denature the double-stranded DNA
Heat to 94□ (201□)

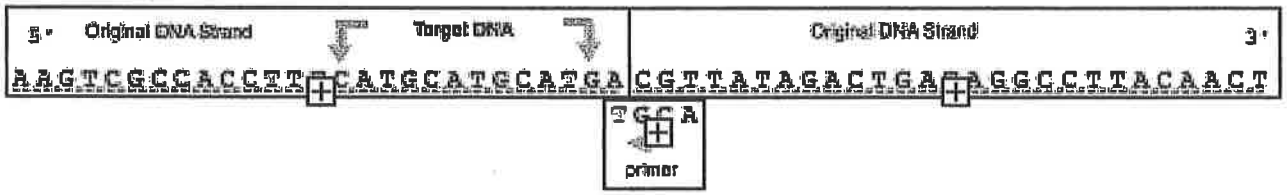
1. Separate all double stranded DNA on the table.

□□*In this first cycle, the only double-stranded DNA is the one piece of* ***Original DNA Strand****. In successive cycles, there will be more pieces of double-stranded DNA. These will also be denatured in this step in future cycles.*

Anneal the Primers
Cool to 20□ (20□)

2. There are two different primers: **TGCA** and **CTTT**. Following the base pair ruling of **A** to **T** and **G** to **C**, find where each primer pairs on a strand of DNA that was denatured in the last step – one primer on each strand.□
Hint: Look right next to the Target DNA.□

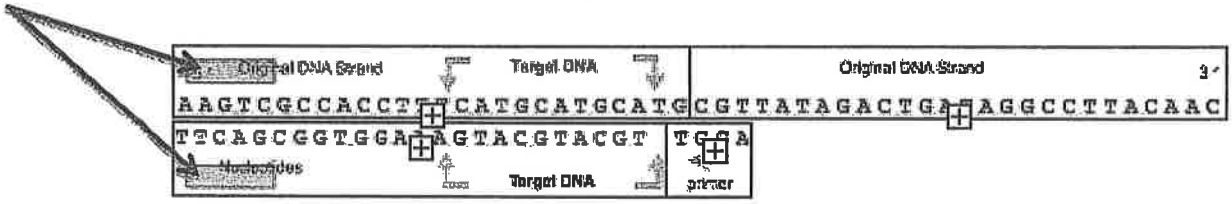
3. Place the primer in place. Do not tape it to the strand of DNA it binds to because you will soon be heating it to denature (see step one above).



Extend the DNA
Heat to 72°C (162□)

4. Find the **nucleotide** strand that pairs with one of the single strands of DNA directly adjacent to the primer. The **nucleotides** will always attach to the side of the primer that the arrow is pointing to.□

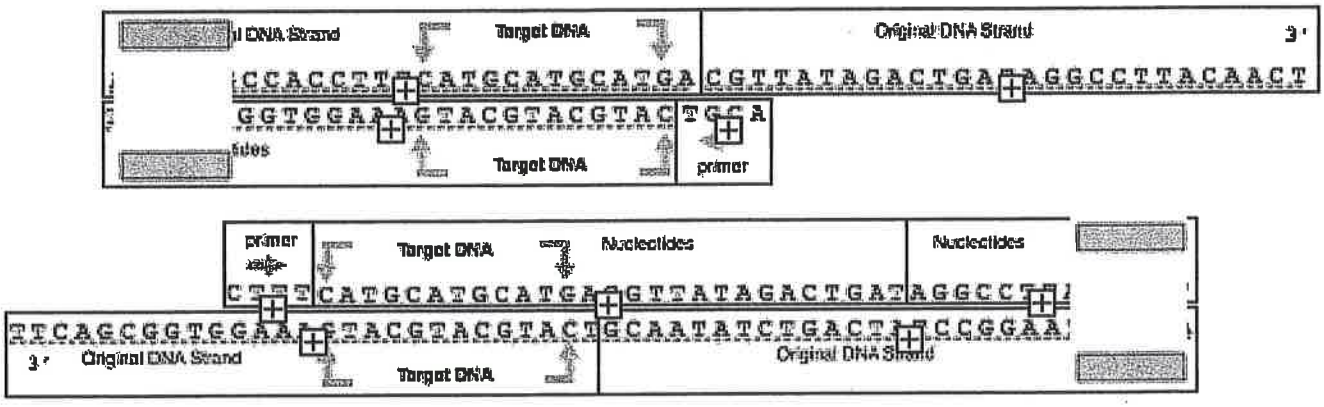
5. Tape the **Nucleotides** strip to the **Primer**. To prevent confusion in future cycles, mark each half of this double strand with a marker.□



6. Repeat this process with the other denatured strands.

7. If any of the **Nucleotides** strand extends past the DNA strand it is attaching to, cut it off with the scissors (This will not happen during the first cycle, but will happen in successive cycles).□

(Note: In real life, the nucleotides will be attached to the DNA strand one at a time. But for this activity, the nucleotides are added all together as the **nucleotide** strip.)



You should end up with two double strands of DNA that look like this:

8. Repeat the 3 steps of the PCR process two more times until you end up with some double-stranded fragments that contain only the Target DNA with the **Primers** attached at either end. If you were to repeat this process 30 times, you would end up with over a million copies of your target DNA -- enough to use in your experiment.

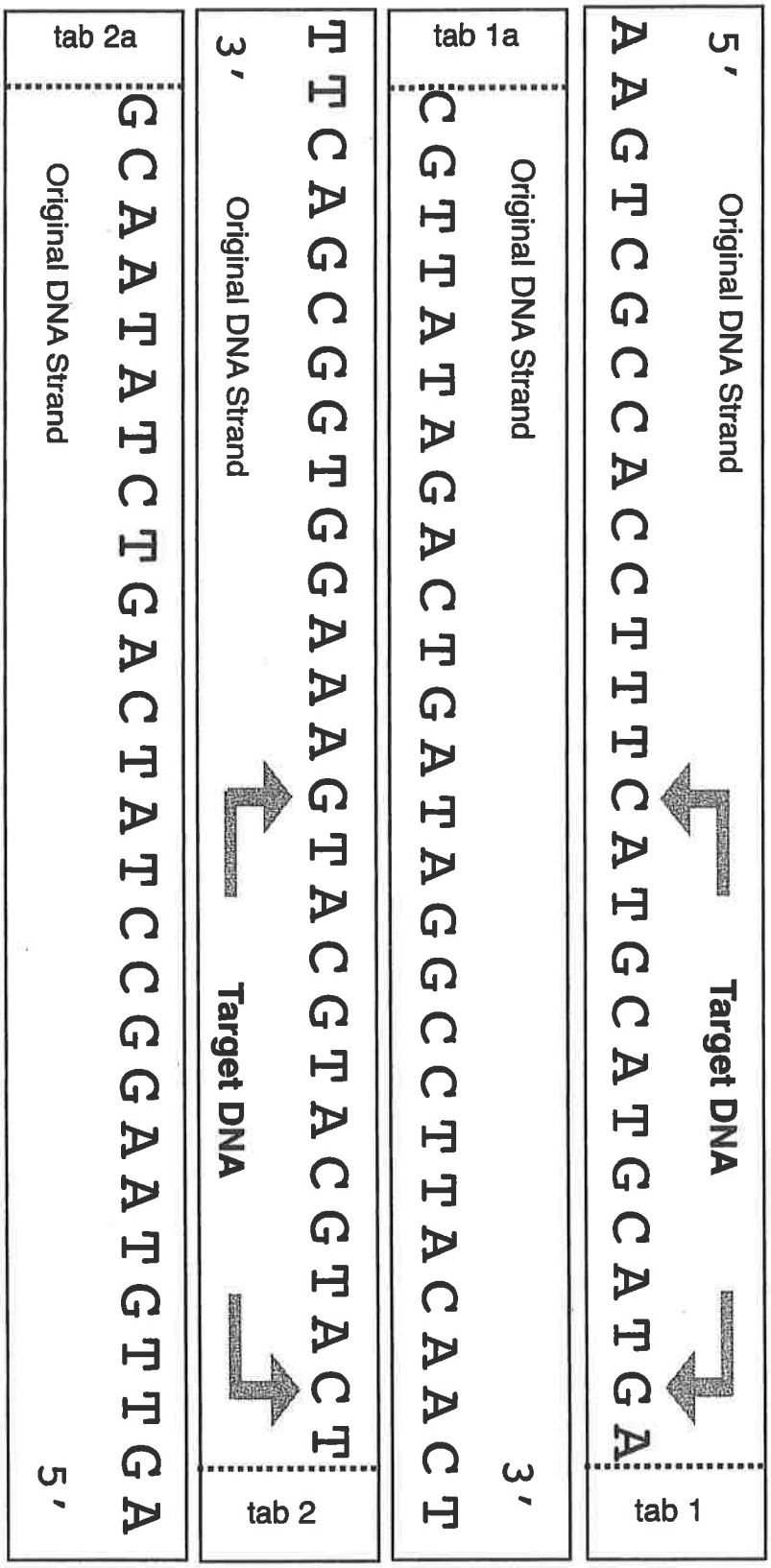
This is your final product

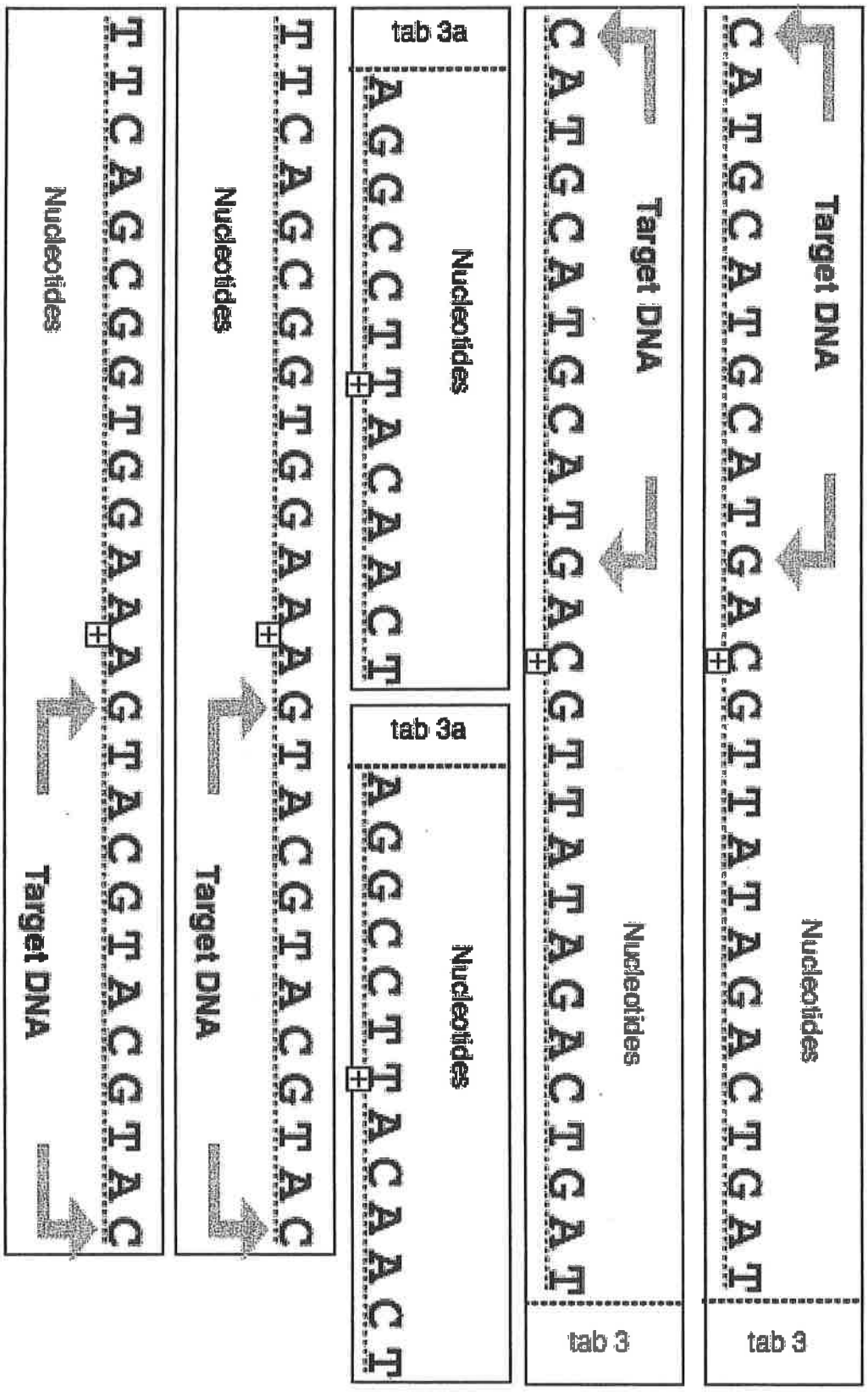
20 base pairs

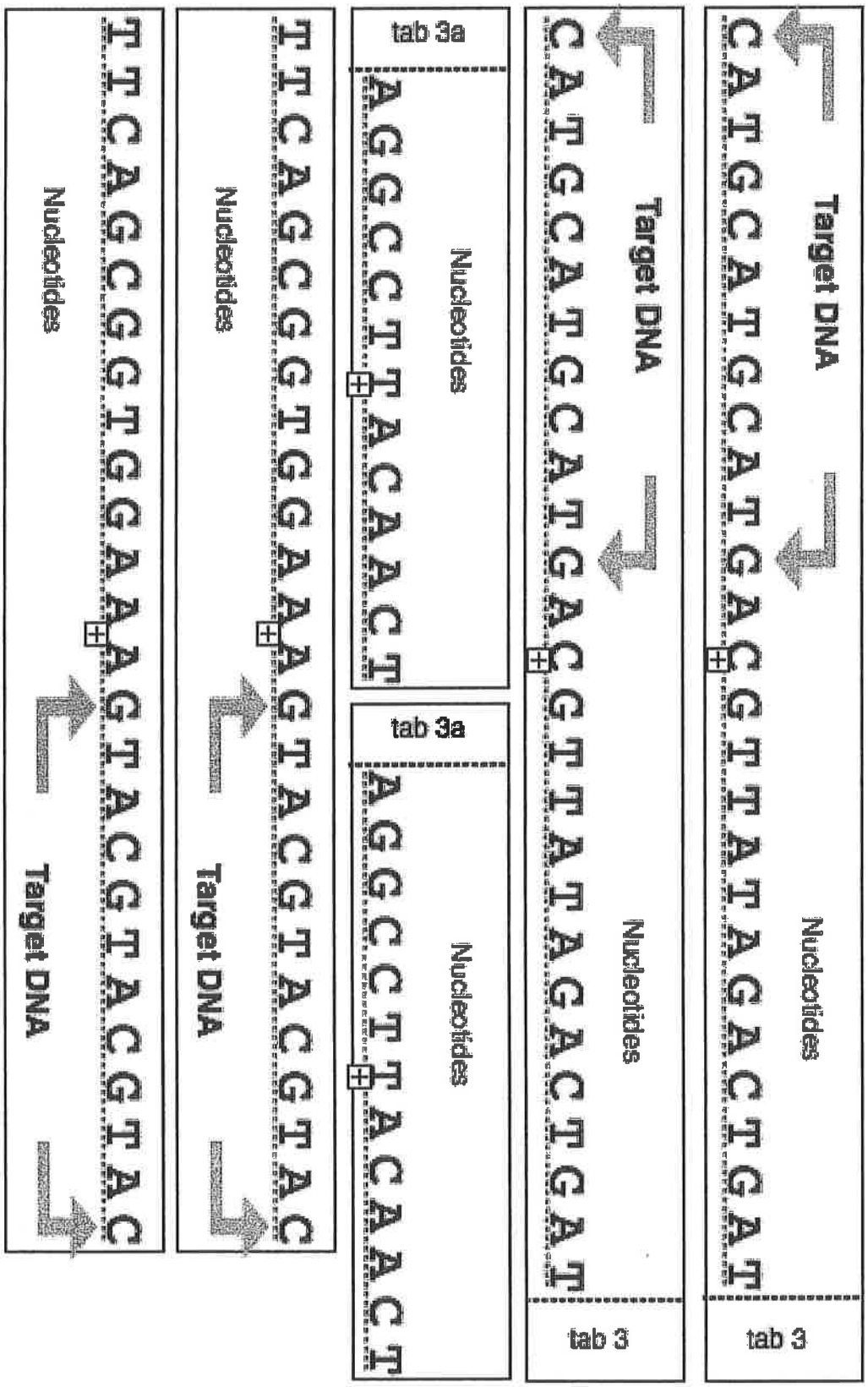
Extension

Have half the class perform this activity with the Long Target DNA sequence and half with the Short Target DNA Sequence. These two Target DNA sequences can then be used in the DNA Fingerprinting activity to demonstrate how the PCR can be used to differentiate slightly different fragments of DNA.

Original DNA Strand




Nucleotides
Target DNA
C A T G C A T G A C G T T A T A G A C T G A T
Nucleotides
tab 3
Target DNA
C A T G C A T G A C G T T A T A G A C T G A T
Nucleotides
tab 3
tab 3a
A G G C C T T A C A A C T
Nucleotides
tab 3a
A G G C C T T A C A A C T
Nucleotides
T T C A G C G G T G G A A A G T A C G T A C
Target DNA
Nucleotides
T T C A G C G G T G G A A A G T A C G T A C
Target DNA
Nucleotides
T T C A G C G G T G G A A A G T A C G T A C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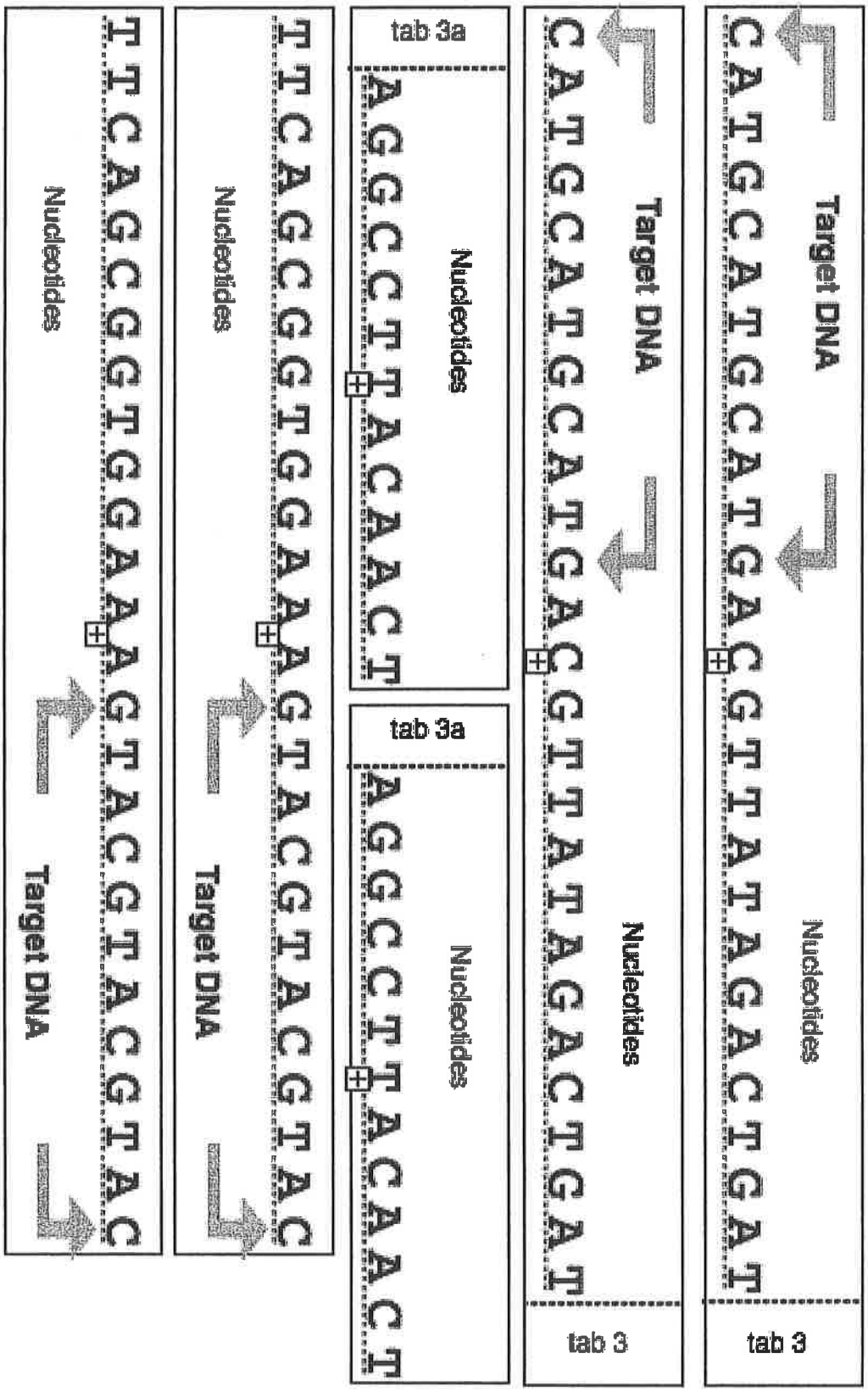
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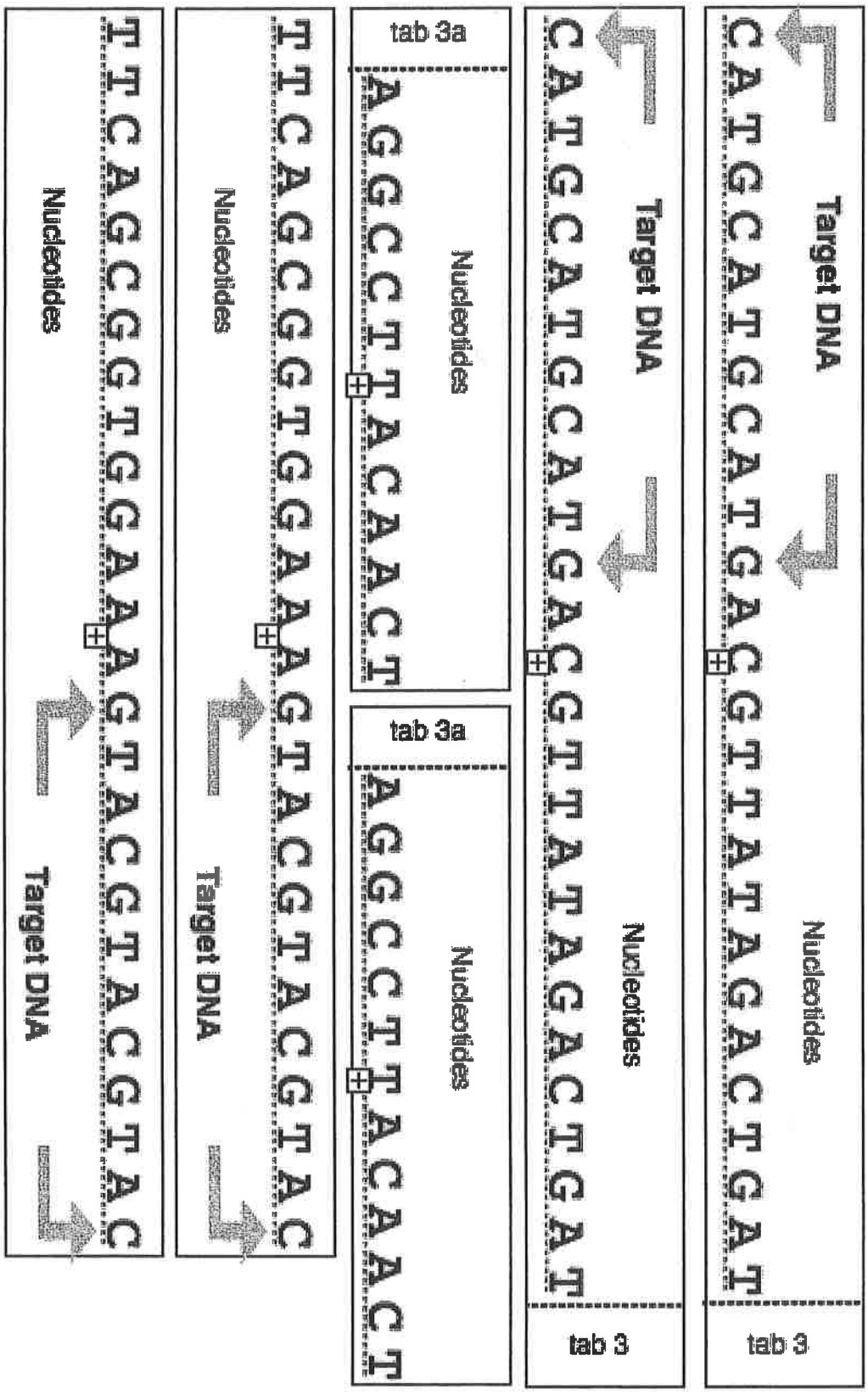
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Primers