Polymerase Chain Reaction

Making multiple copies
of small segments of DNA

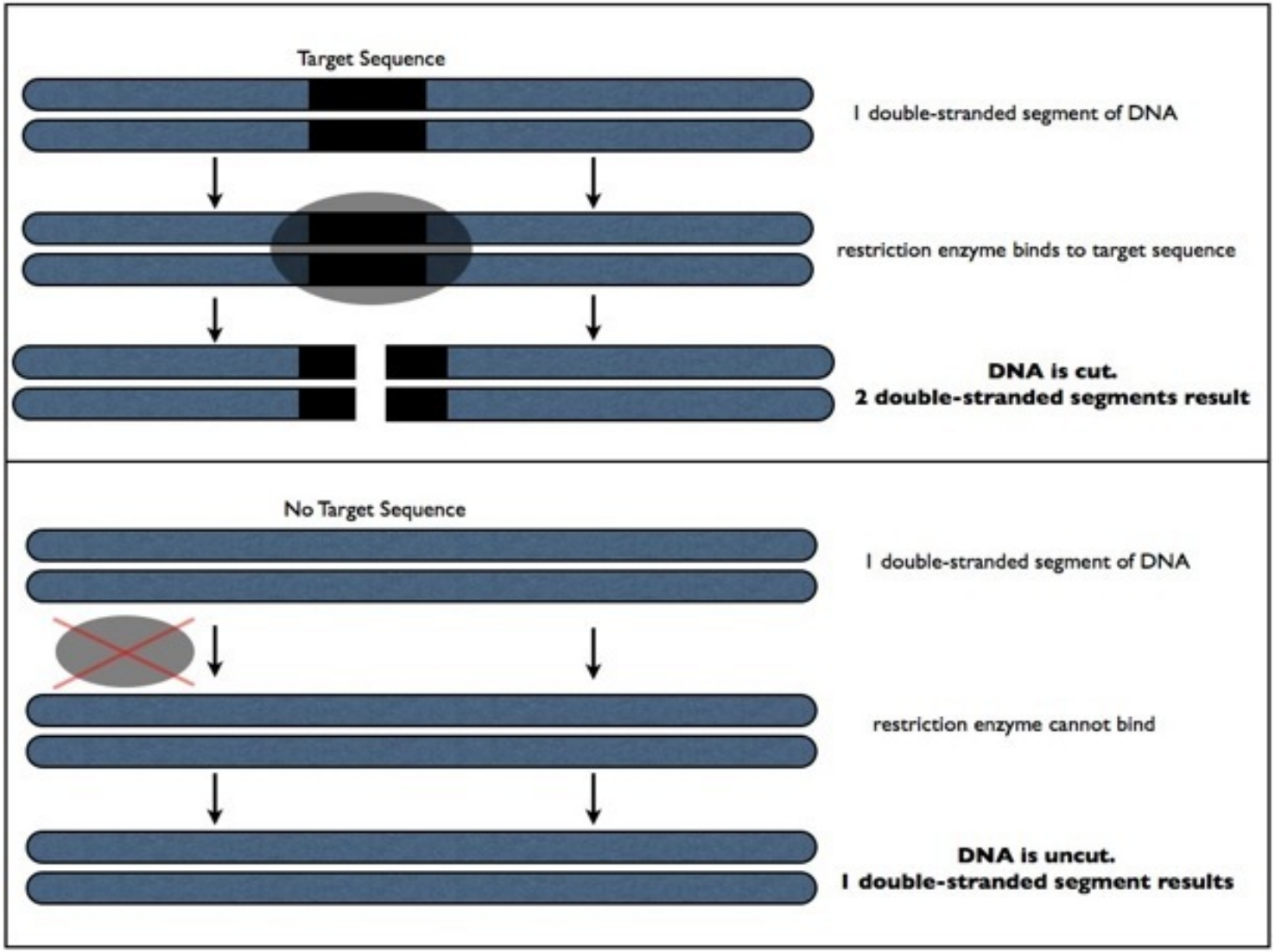
PCR

- Polymerase Chain Reaction
  - Copy machine for DNA
  - Makes multiple copies of a gene of interest
    - Amplify = copying = duplicate

PCR uses (applications)

1. **Research (enough DNA to study)**
2. **DNA fingerprinting (forensics)**
3. **Detection of a target DNA**
4. **Diagnosing a disease vs. cultures (e.g. tuberculosis)**
5. **Determine base sequence of DNA molecule**

Detecting a target DNA



PCR

- **Requires many components**
  - Enzyme – Taq polymerase
  - Nucleotides –all based in solution
  - Buffer (Mg)- makes the enzyme active
  - DNA- from your source
  - Primers- selected short pieces of DNA

The steps

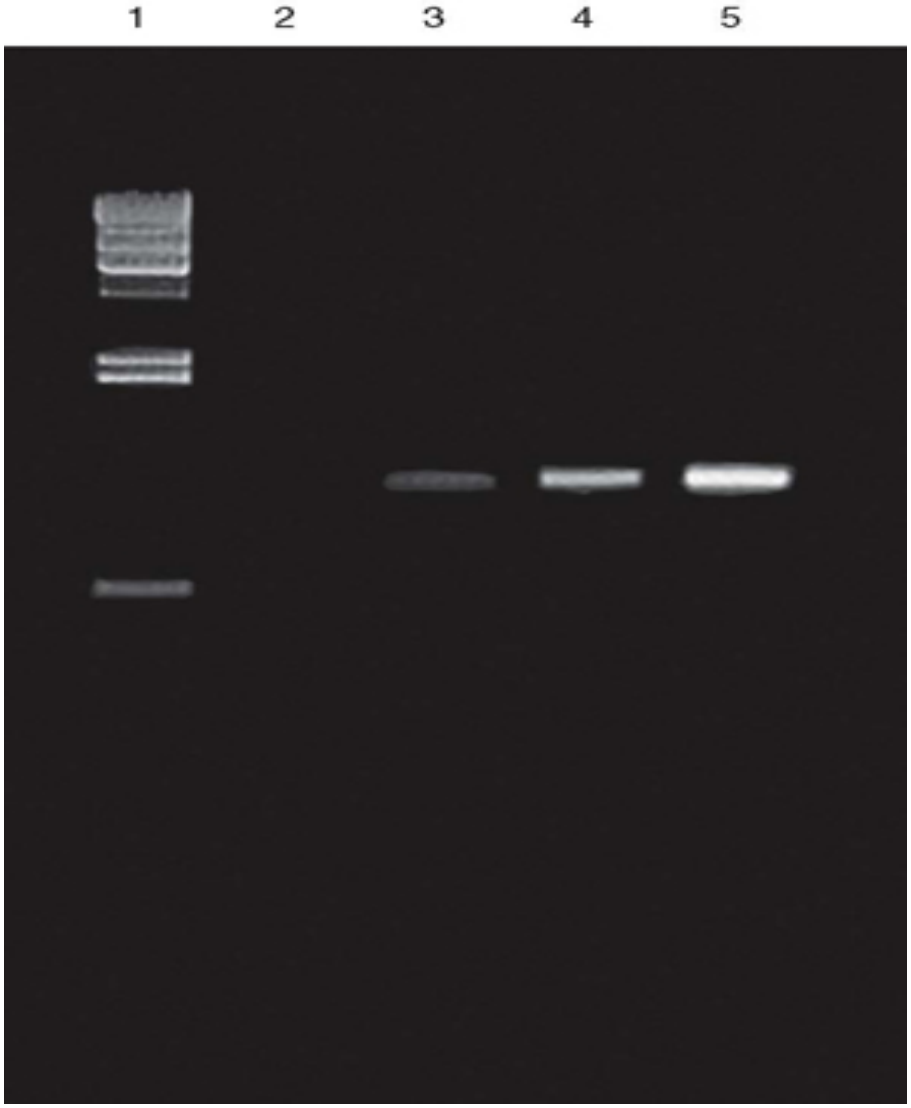
- Denaturation- 95 C breaks apart the DNA double helix strands
- Annealing (cooling) - 50-65 C allows the primers to bind a section of separated DNA
- Synthesis (extension) - allows DNA polymerase to add DNA nucleotide bases to the growing strand
- Repeat 25-40 times
- Get millions of copies

PCR & Gel electrophoresis

Shows the ability to copy DNA segments

Example: lambda DNA

- Lane 1- Hind III
- Lane 2 – before PCR
- Lane 3-5: 5x, 10x, 15x PCR cycles



Comparing components in PCR to photocopying a page in a book.

Photocopier items	**PCR components**
The book	The entire genome (called the DNA template)
The page	A portion of the genome (fragment) we are interested in
A bookmark	Primers that "mark" the specific fragment
The copy machine	The enzyme that copies DNA (called a polymerase)
Paper and toner	The four bases that make up DNA (called nucleotides)

http://www.extension.org/pages/32364/introduction-to-the-polymerase-chain-reaction-pcr

PCR process – Resources
Animations & Songs

- Introduction to the Polymerase Chain Reaction (PCR) - eXtension

http://www.extension.org/pages/32364/introduction-to-the-polymerase-chain-reaction-pcr

PCR animation

- Polymerase Chain Reaction

PCR Animation – U of Nebraska

- ::eLearn & Grow Library:: PCR

Paper PCR Activity

- Polymerase chain reaction
  - Standard laboratory procedure in biotechnology
  - Makes copies useful for detection & cloning
- Using paper model, show how DNA segments are copied in the PCR process

Reading: Paper PCR

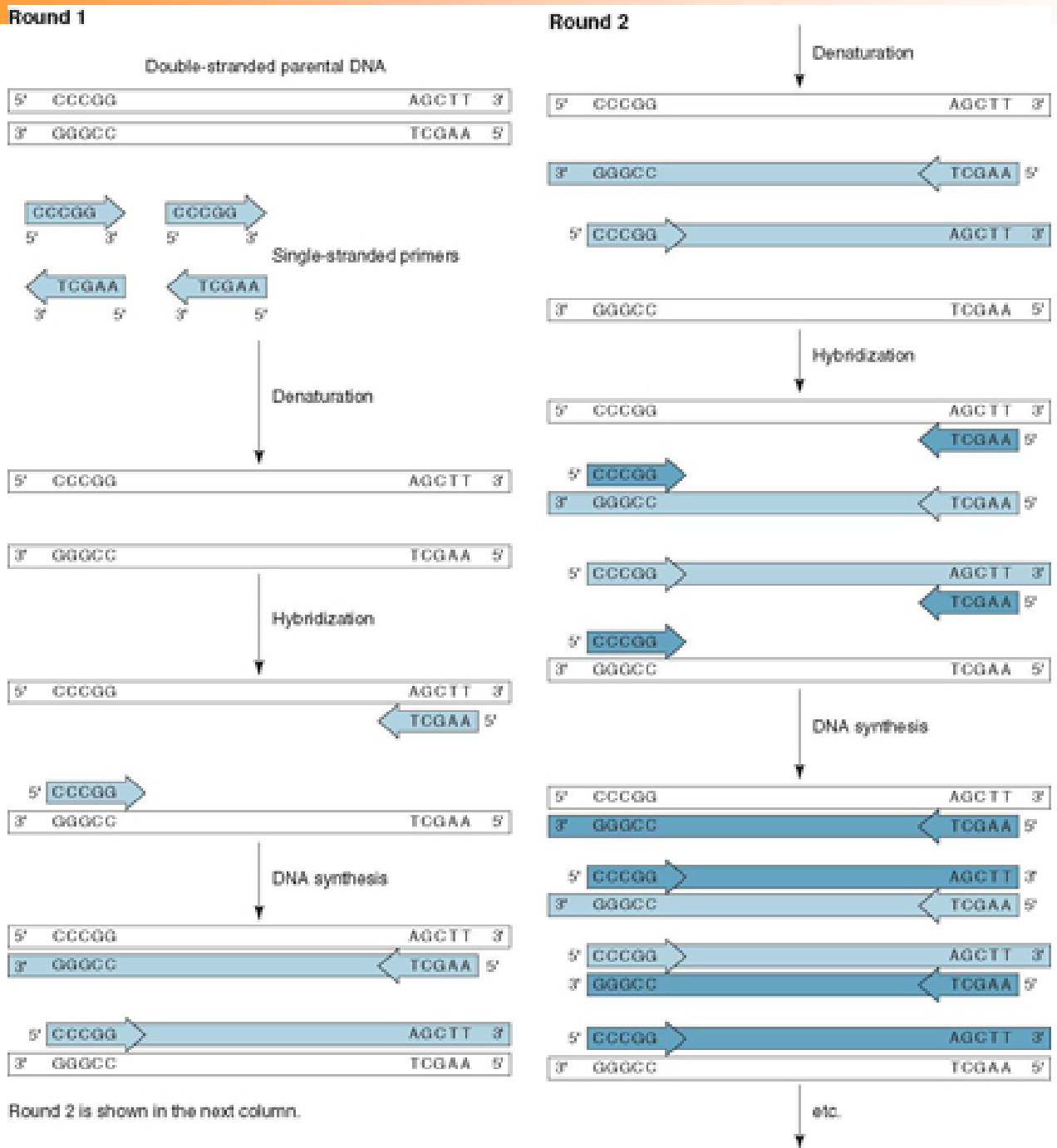
In your notebook, answer the following:

- What is the essence of the PCR process?
- Define the terms (see list on next slide)

- Determine the steps in PCR

PCR terms

- Chain reaction:
- Hybridization: formation of base pairs between 2 strands of DNA that are untwisted
- Denaturation (not in “natural state”):
- Synthesis:
- Amplify:
- Primer:
- 3’ and 5’: orientation of the DNA strands based on ?
- Annealing:


Round 1
Double-stranded parental DNA
5' CCCGG AGCTT 3'
3' GGGCC TCGAA 5'
CCCGG
CCCGG
5' 3'
5' 3'
Single-stranded primers
TCGAA
TCGAA
3' 5'
3' 5'
Denaturation
5' CCCGG AGCTT 3'
3' GGGCC TCGAA 5'
Hybridization
5' CCCGG AGCTT 3'
TCGAA 5'
5' CCCGG
3' GGGCC TCGAA 5'
DNA synthesis
5' CCCGG AGCTT 3'
3' GGGCC TCGAA 5'
5' CCCGG AGCTT 3'
3' GGGCC TCGAA 5'
Round 2 is shown in the next column.
Round 2
Denaturation
5' CCCGG AGCTT 3'
3' GGGCC TCGAA 5'
5' CCCGG AGCTT 3'
3' GGGCC TCGAA 5'
Hybridization
5' CCCGG AGCTT 3'
TCGAA 5'
5' CCCGG
3' GGGCC TCGAA 5'
5' CCCGG AGCTT 3'
TCGAA 5'
5' CCCGG
3' GGGCC TCGAA 5'
DNA synthesis
5' CCCGG AGCTT 3'
3' GGGCC TCGAA 5'
5' CCCGG AGCTT 3'
3' GGGCC TCGAA 5'
5' CCCGG AGCTT 3'
3' GGGCC TCGAA 5'
5' CCCGG AGCTT 3'
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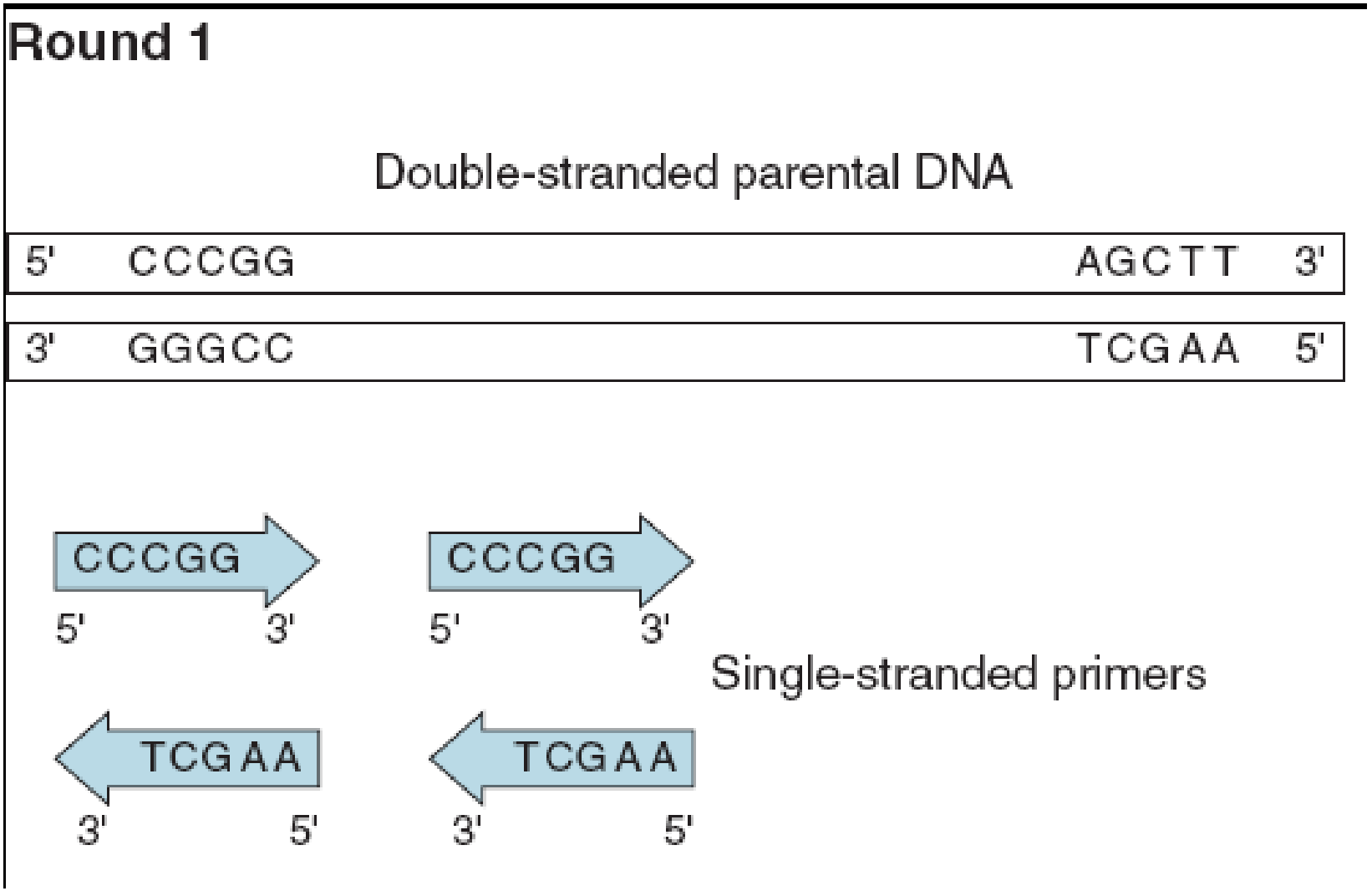
etc.

Round 1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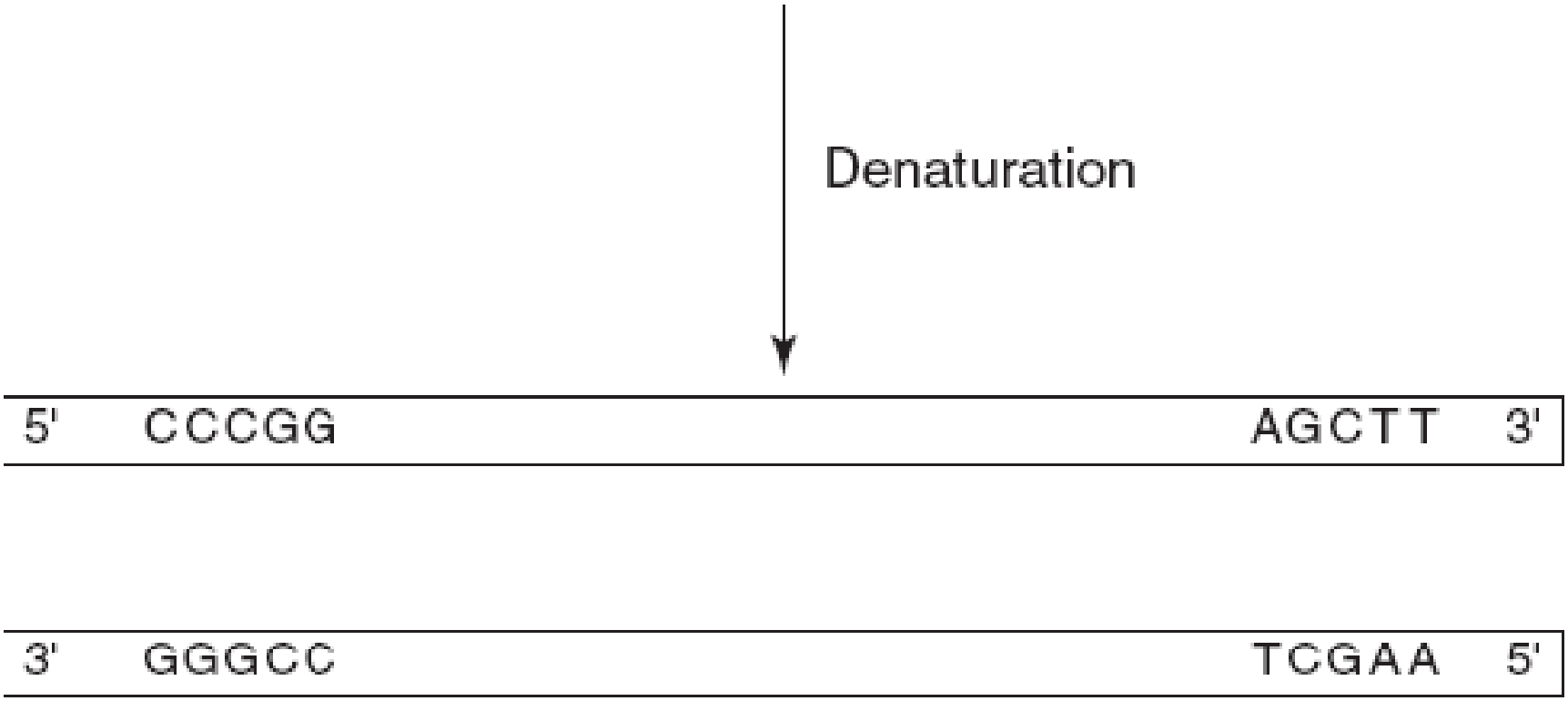
5' 3'
Single-stranded primers
TCGAA
3' 5'
TCGAA
3' 5'

Round 1: What is happening in this step?



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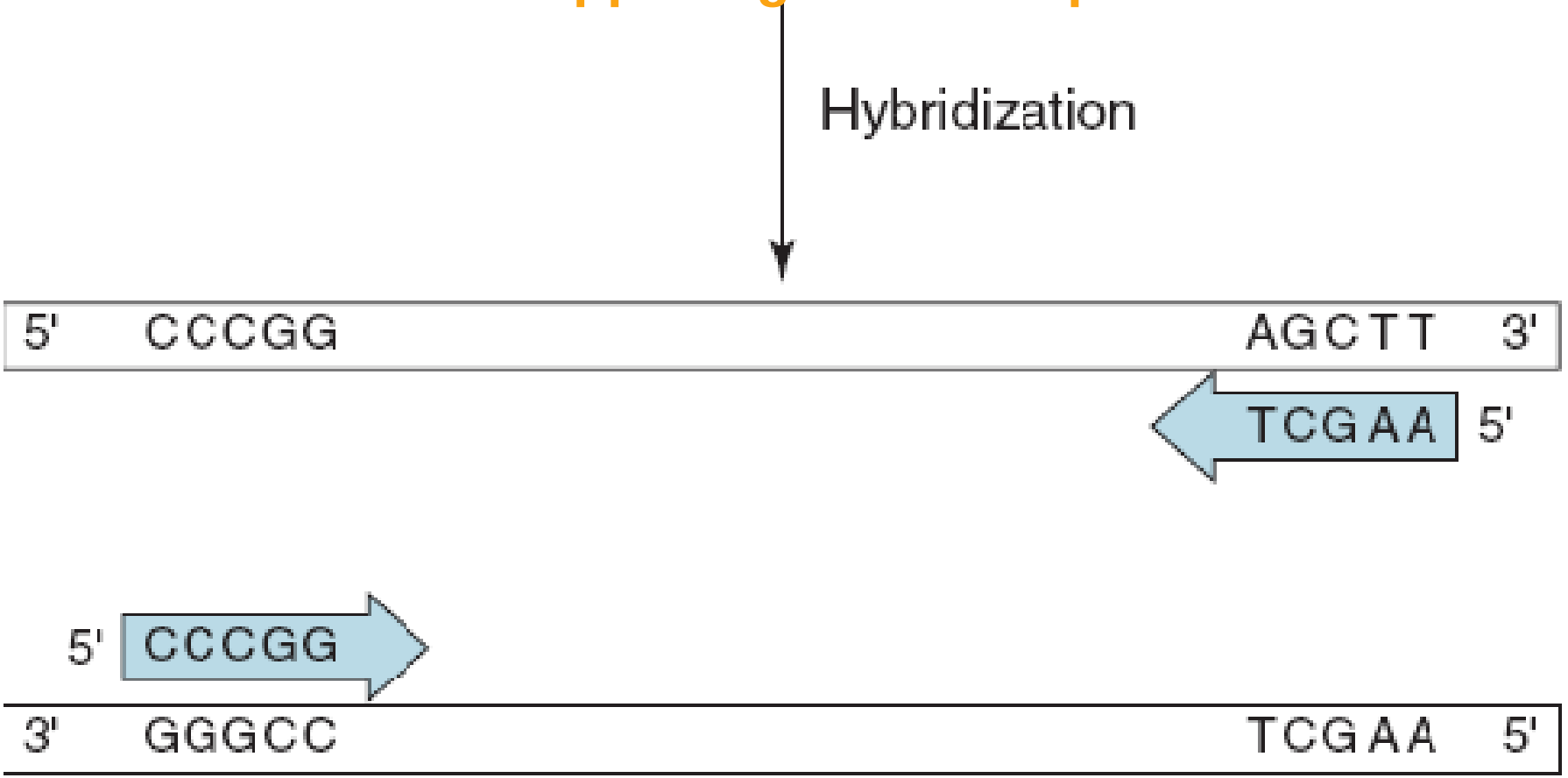

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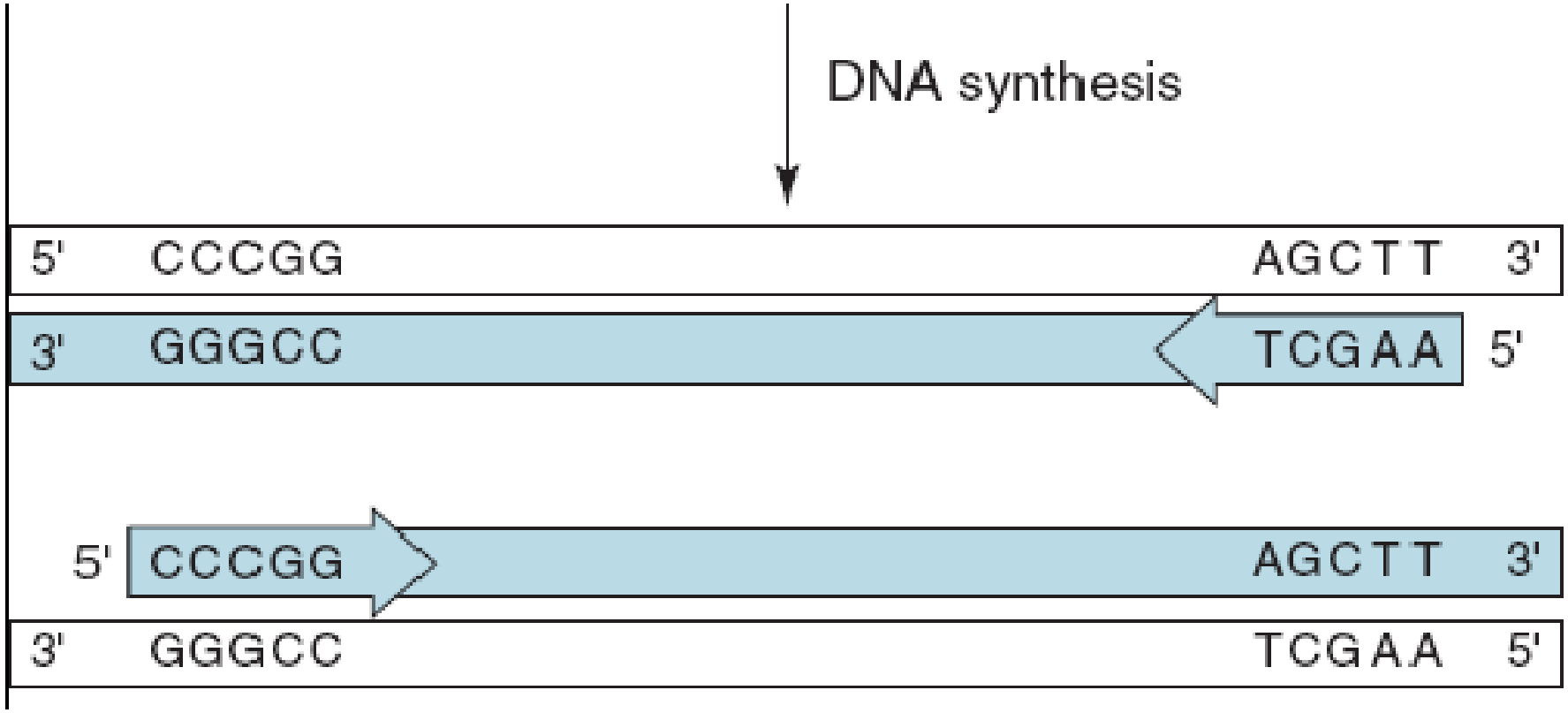

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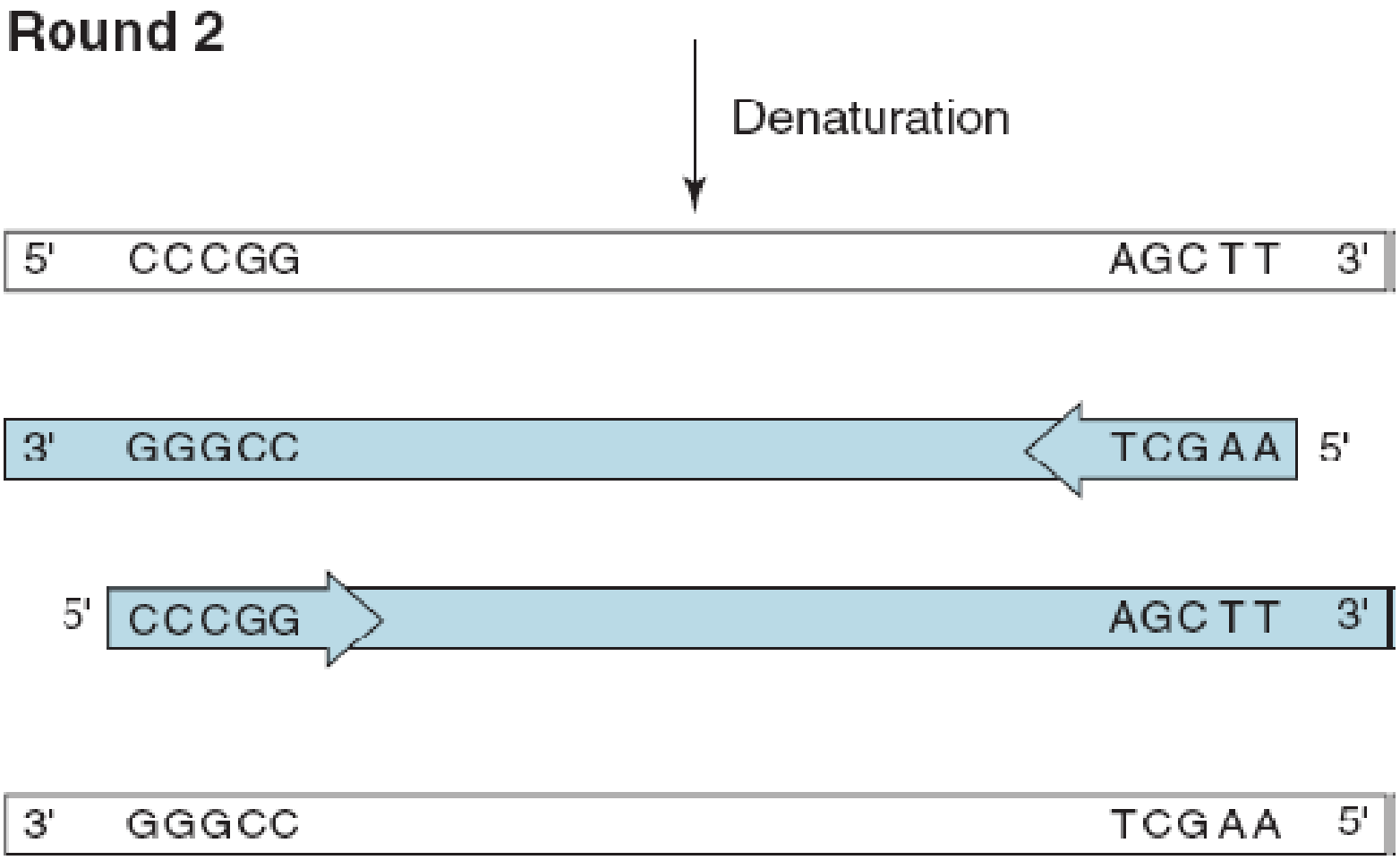
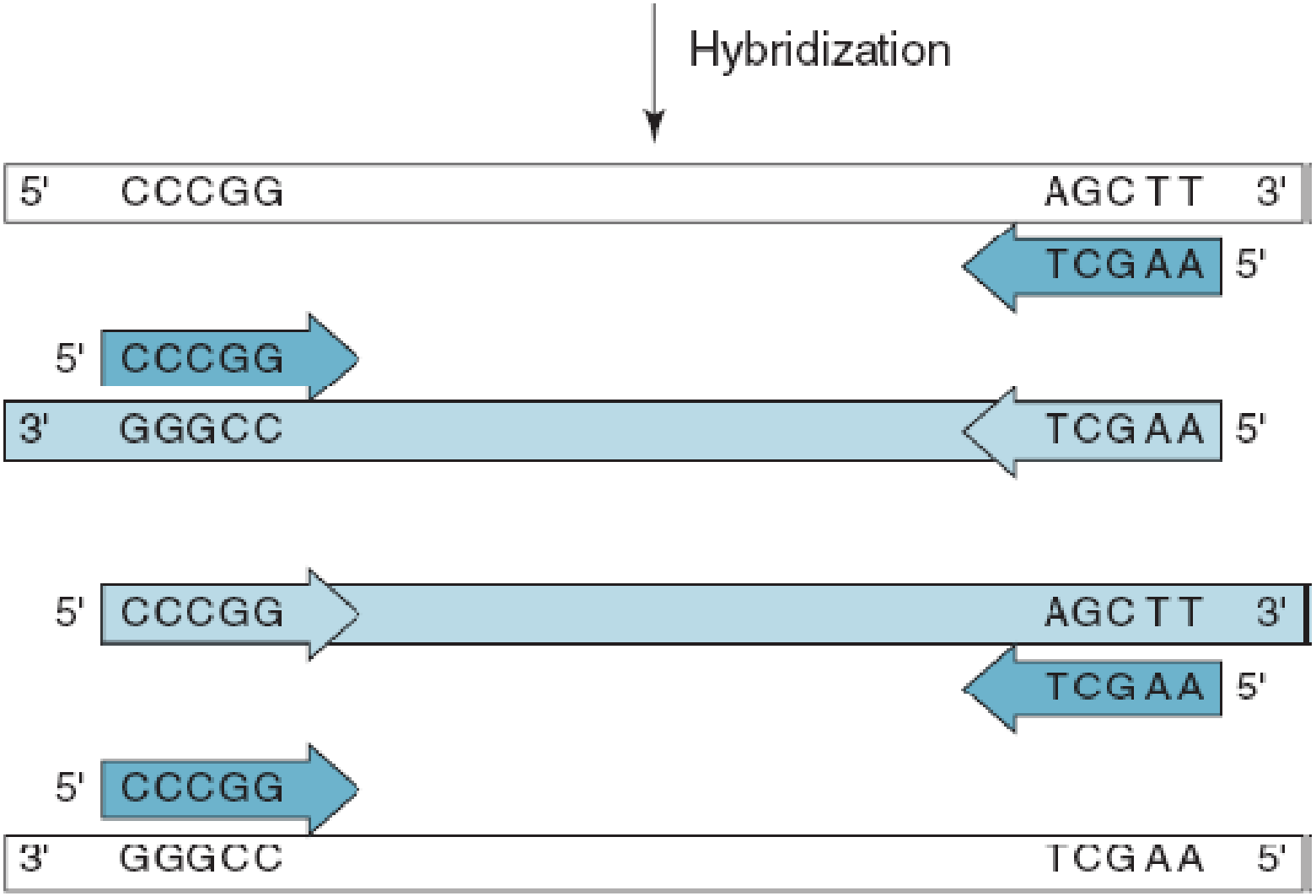
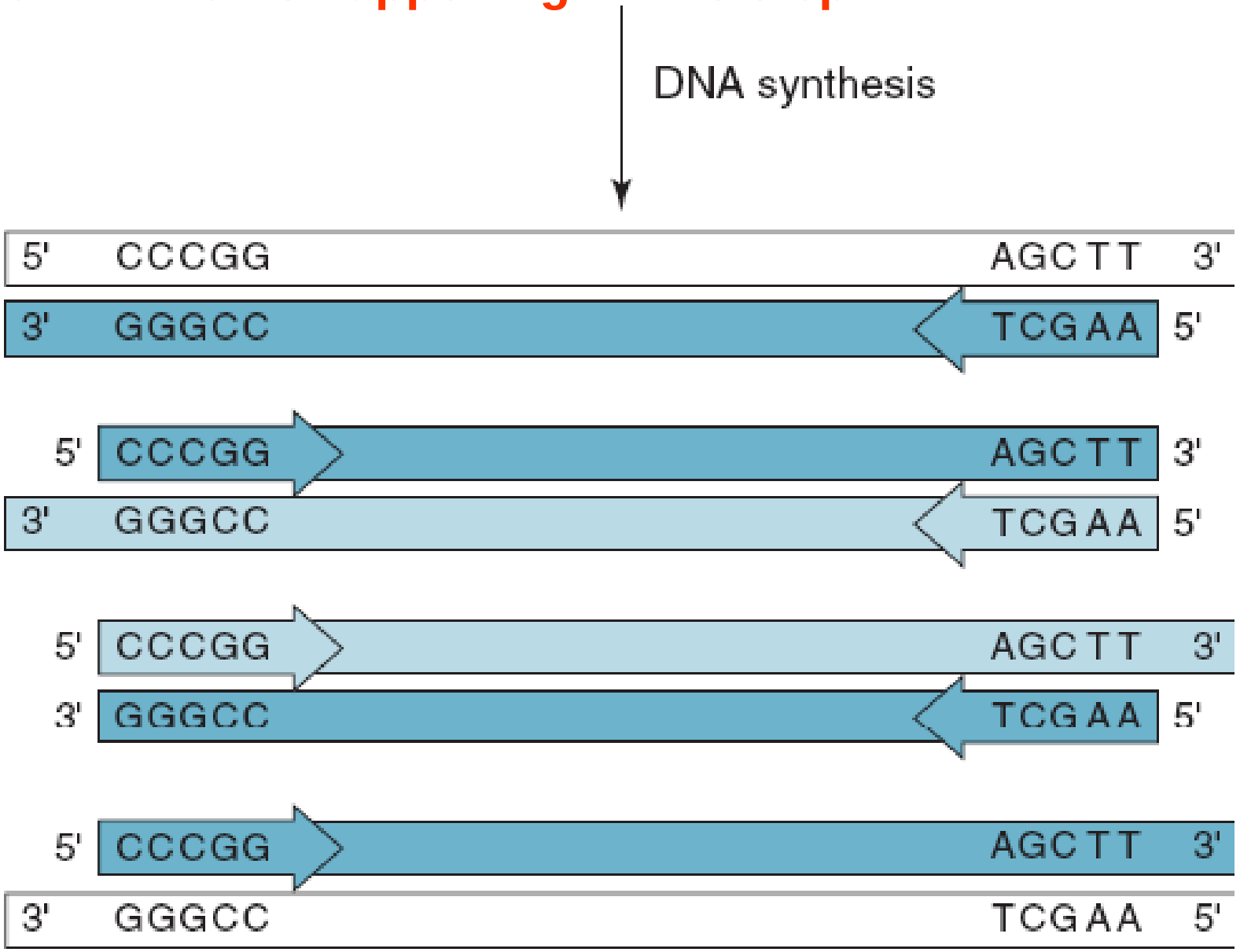


Round 2: What is happening in this step?



Round 2: What is happening in this step?



- Dolan - 3D animation

Paper PCR Activity

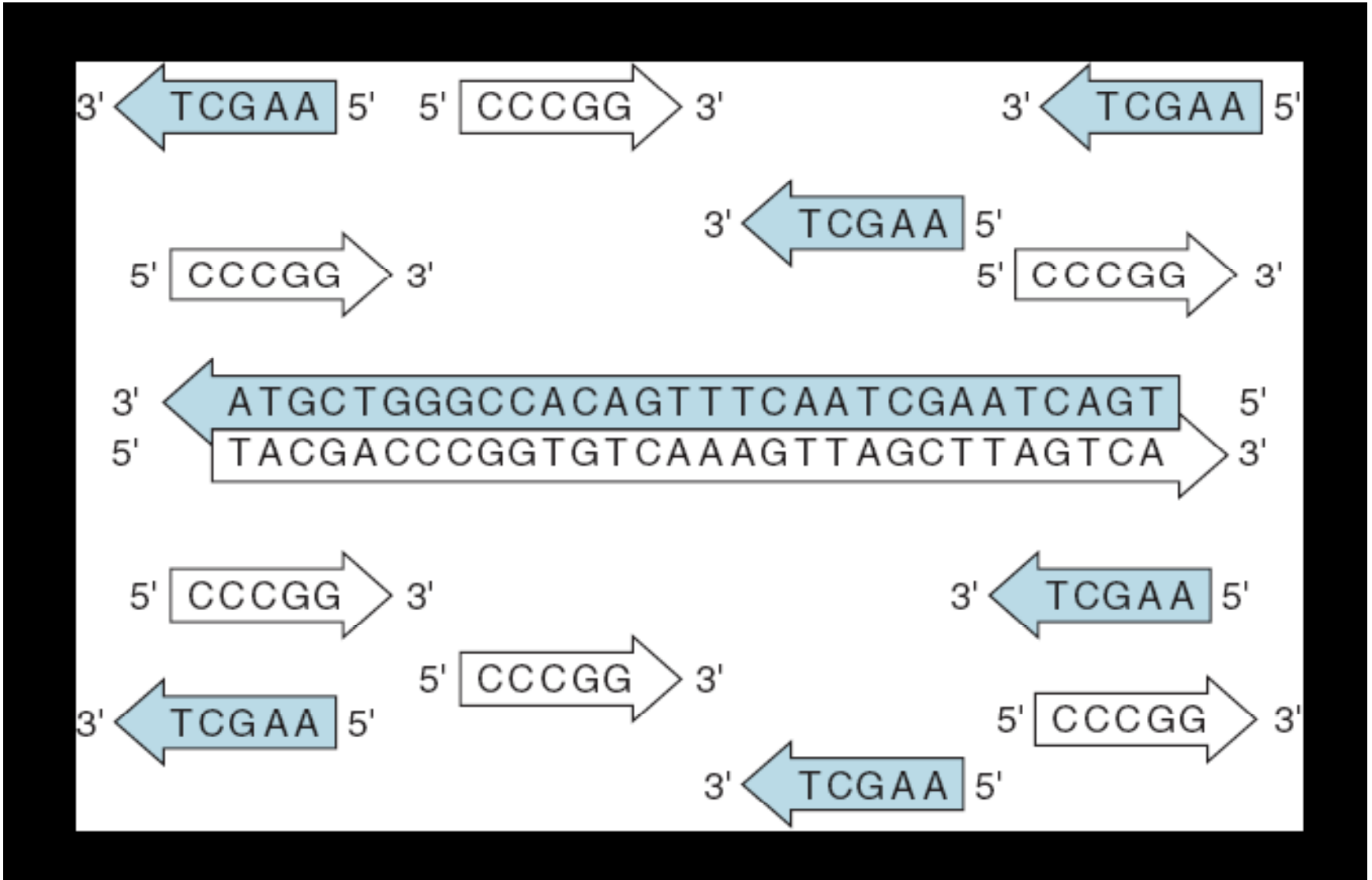
1) Cut out the DNA strands & primers
2) Align the 2 strands of DNA
    1) Note the orientation of 3’ and 5’ ends

3) As a class,
    1) Denaturation
    2) Hybridization
    3) DNA synthesis

Starting the PCR process- paper model

- Place the DNA strands as double strands
  - Align the complementary bases
  - Remember that the DNA strands are in a solution in the nucleus with the primers

Starting the PCR process – the paper model should look like this:



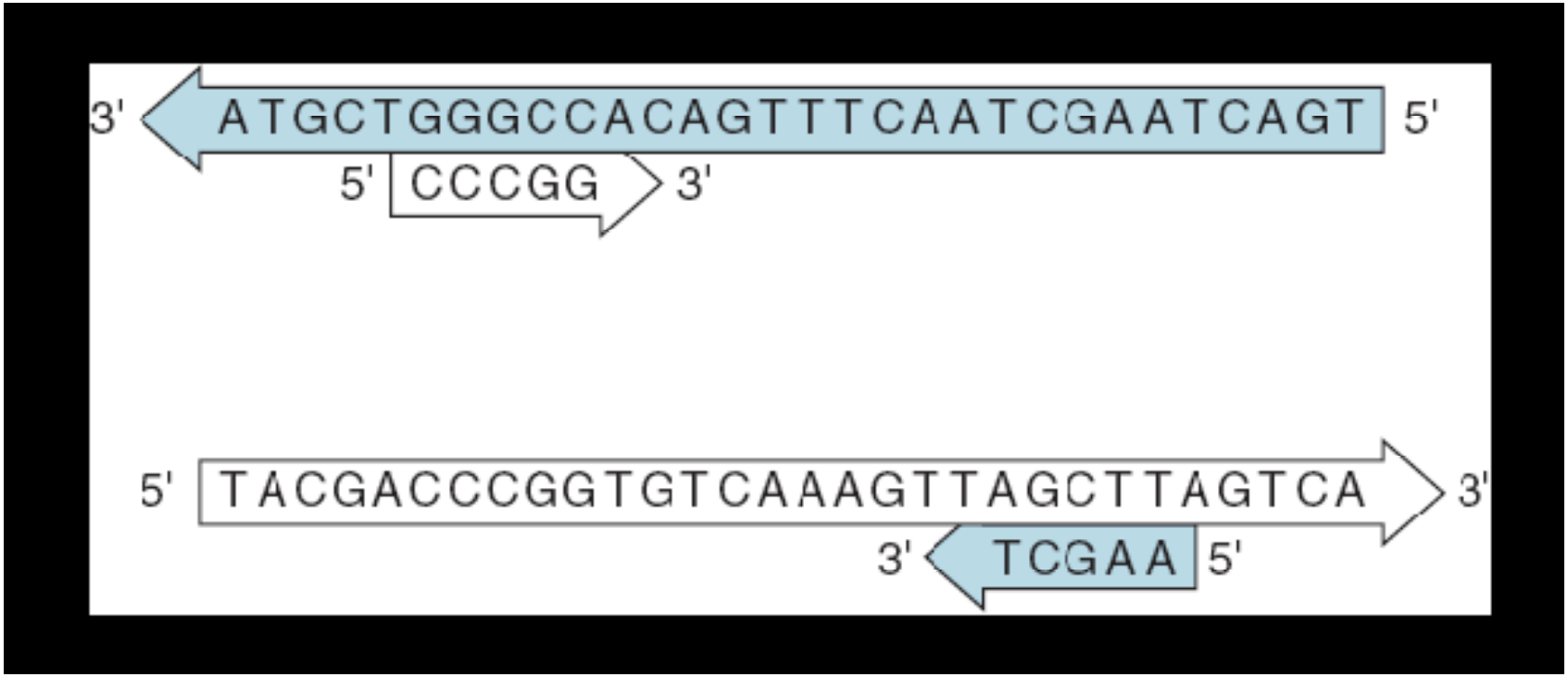
PCR Step 1: Denaturation

- Denature the DNA by increasing the temperature to 95°C
- Place all your primers into solution

PCR Step 2: Hybridization

- Cool your sample (anneal) to 50-60°C
- Hybridize your sample by adding the primers to the DNA separated strands
  - Check the 5’ and 3’ ends
  - Note: Letters can be upside down
- Why did the DNA strand hybridize to the primer rather than the other strand?

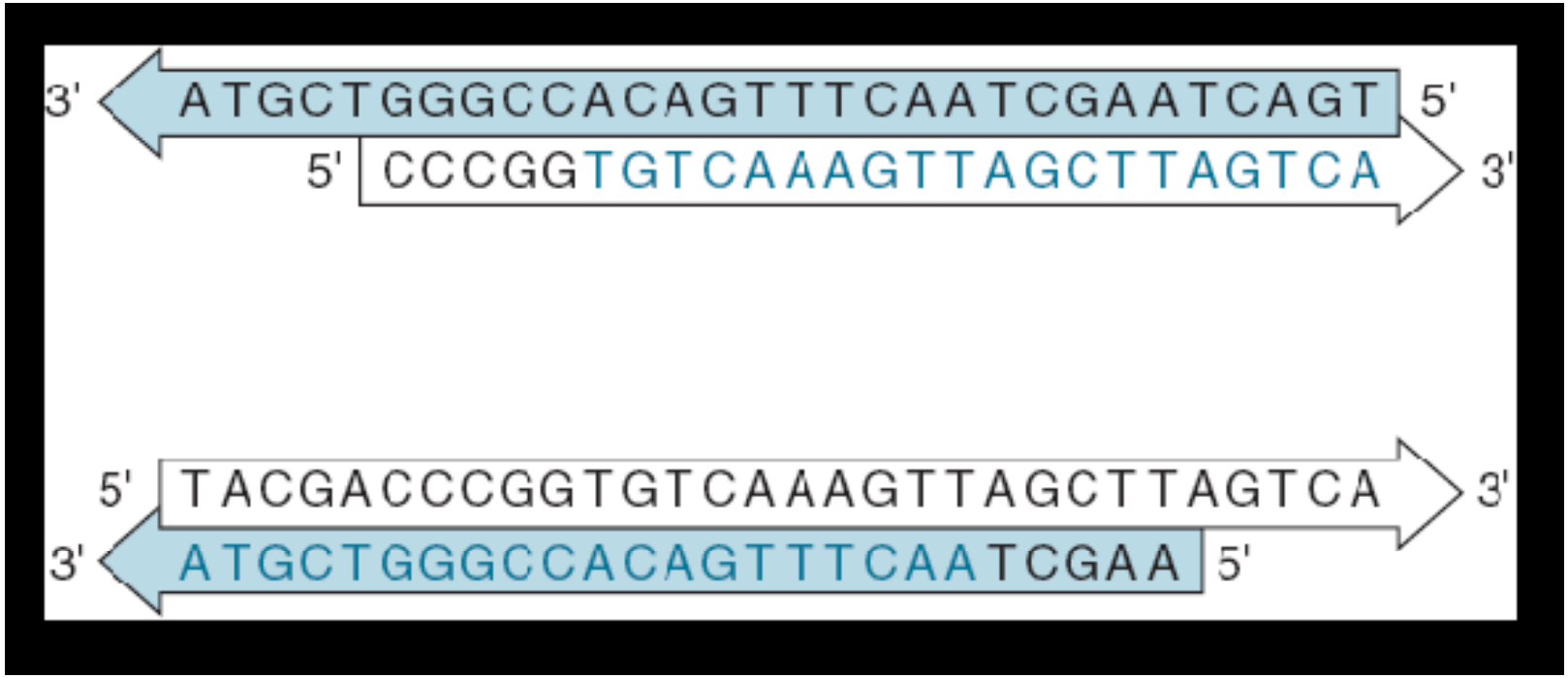
After hybridization, your model should look like this



PCR Step 3: DNA Synthesis

- Synthesize your DNA strands by adding the correct nucleotide bases after the primer. (These will be free bases in solution)
  - Note: You are acting as the DNA polymerase
  - Use white or blue colored strips that are taped to the end of the primer to make the complementary strand.
  - Write the correct DNA sequence of nucleotide bases on the new strand
- **End of Round 1**

After synthesis, your model should look like this



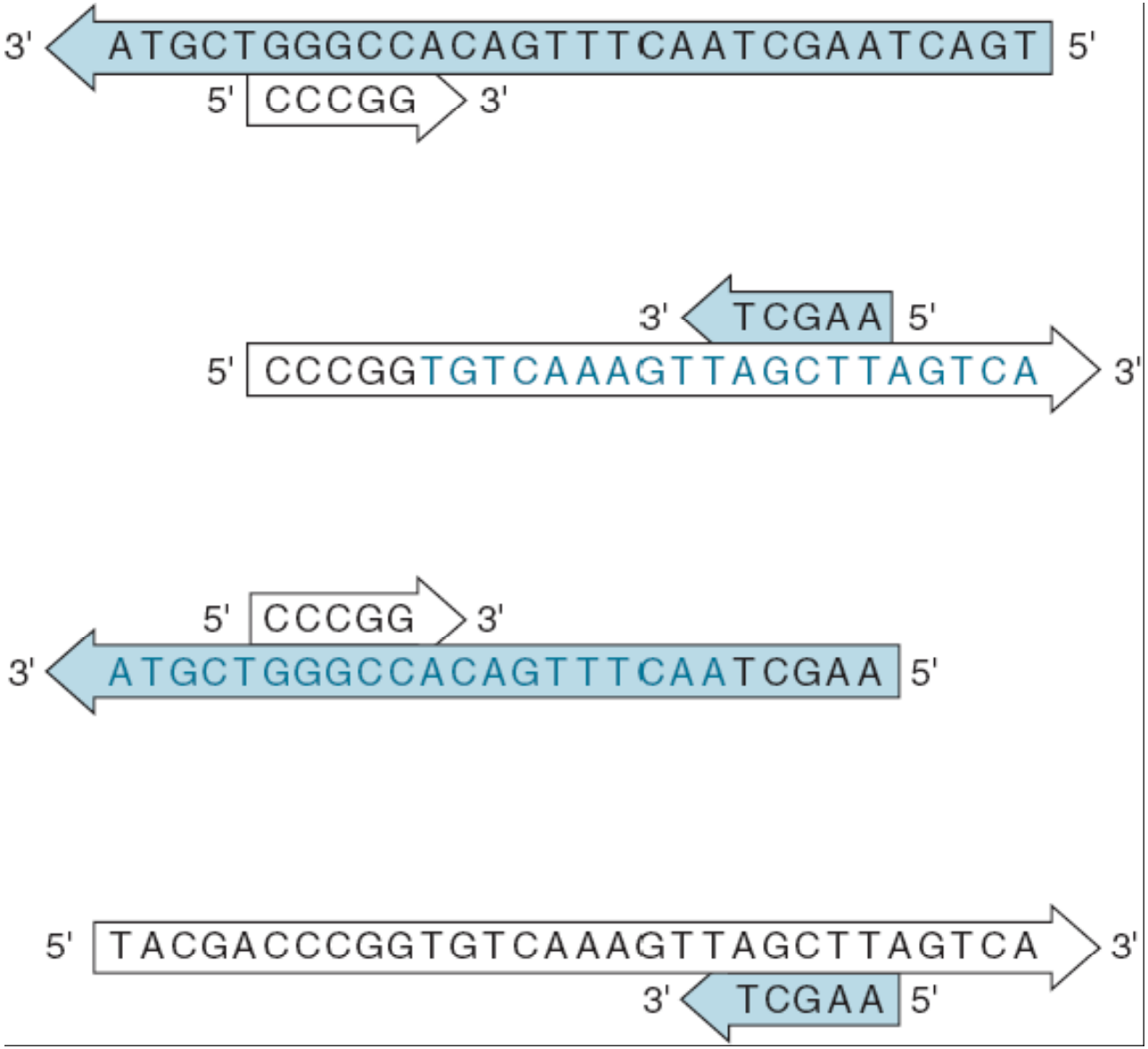
Round 2

- Go through the PCR steps
- Predict products for Round 3
  - Select students – go to Round 3
- At the end, glue your models into your notebook
  - Label your samples
  - Explain what the samples represent

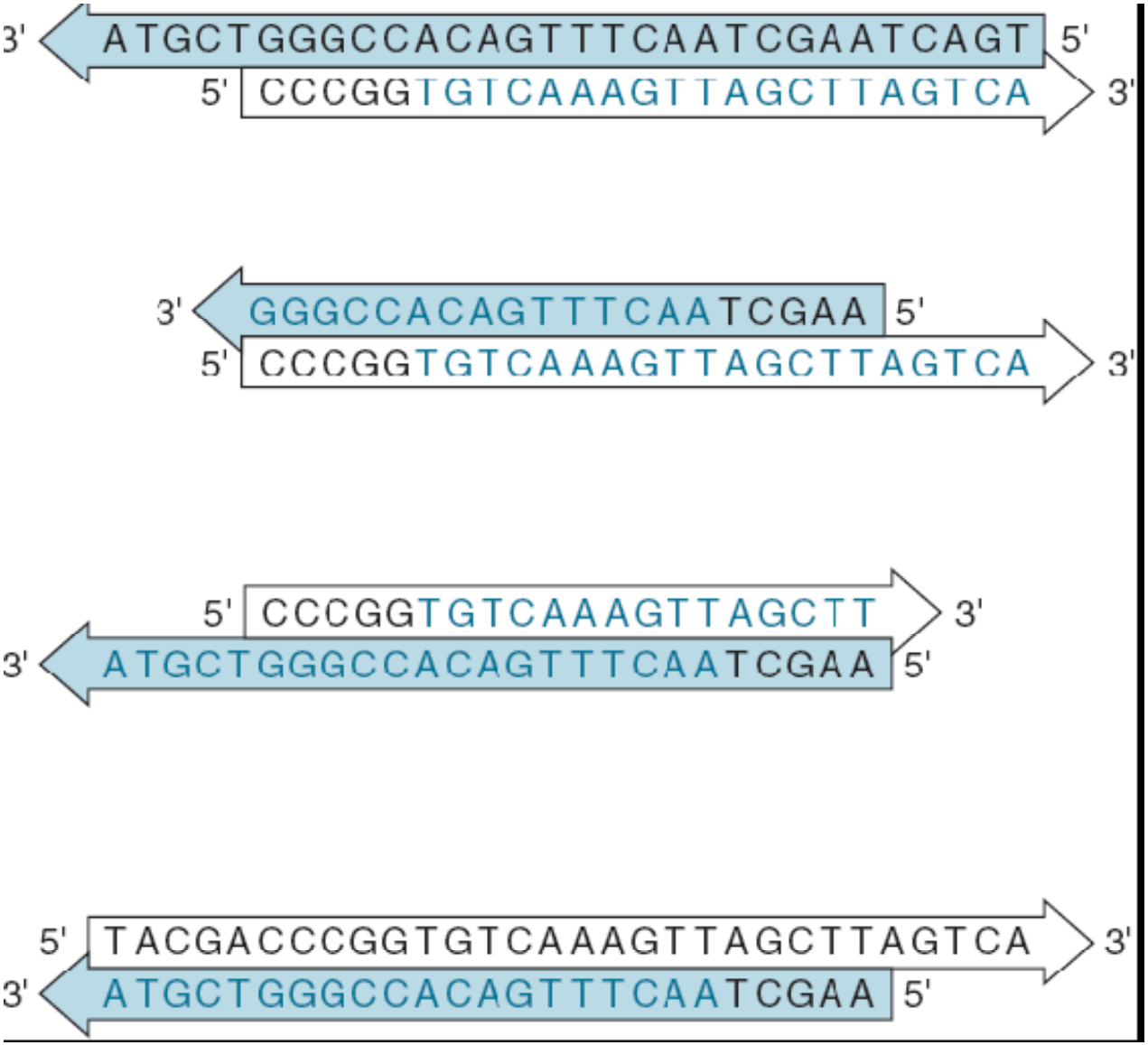
Round 3 - PCR

- Predict the products of another round of PCR

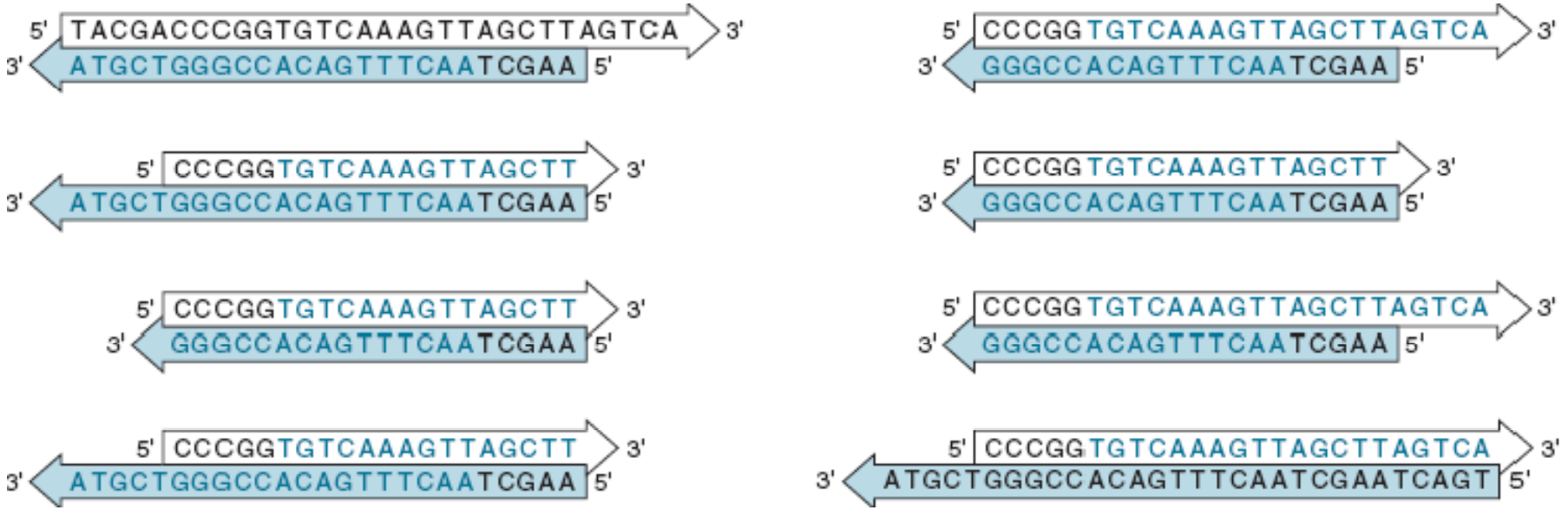
Round 2: Paper model hybridization



Round 2: DNA Synthesis



Round 3 DNA Synthesis should look like this



PCR Song (from BioRad)

- Biocompare Funny Science Videos