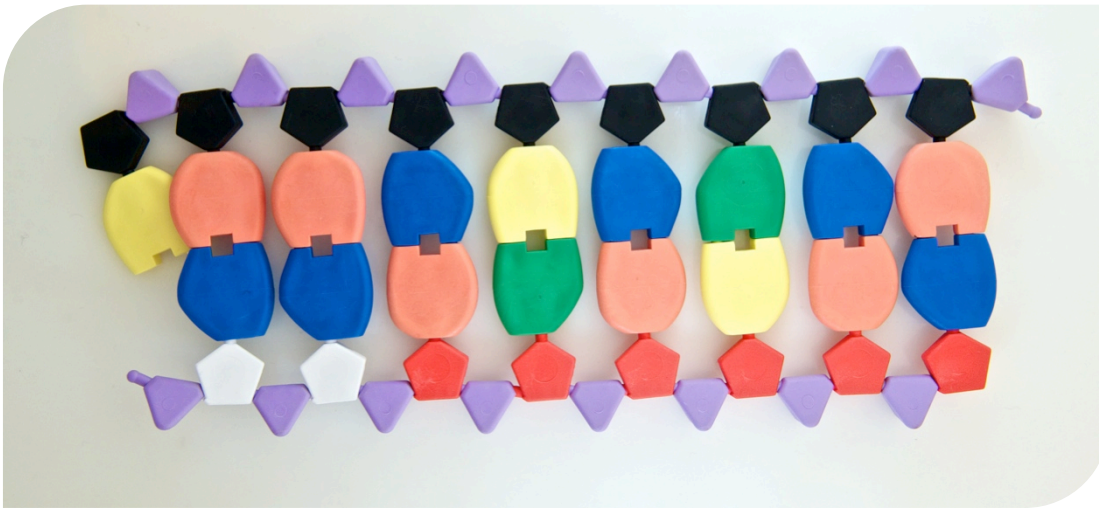
Discovering DNA

Molymod® miniDNA®

PCR Puzzle^{tm}

Instructions and teachers guide

Cat no MDNA-PCR-366



We'd love to hear any feedback, comments or questions you have!

Please email Discovering DNA on:

info@discoveringdna.com

Molymod® miniDNA® PCR Puzzle instructions

Contents:

Black sugar	18	Cytosine (Yellow)	20
White sugar	28	Guanine (Green)	17
Red sugar	76	Adenine (Blue)	44
Phosphate (Purple)	122	Thymine (Orange)	41

Learning objective

The technique of Polymerase Chain Reaction (PCR) is used in many contexts – DNA fingerprinting, genetic testing, paternity testing, and many more. PCR makes lots of copies of a specific region of DNA using the enzyme DNA polymerase. Kary Mullis won the Nobel Prize for his invention in 1993.

You will use PCR to amplify a short target sequence (9 bases) to copy just the region you want and remove the unwanted overhangs at each end of the template. You will use two primers (2 bases each) to specify the region to copy. This hands-on activity will help you remember many key points about PCR and DNA replication.

The set provides enough materials to carry out the lesson with 30 students working in groups.

By the end of the session, your students will have learnt:

- PCR has three steps to each cycle
- You double the number of copies with each PCR cycle
- The primers must match only one region in the template DNA
- Primers must match each end of the target
- Primers are incorporated into every copy of the target
- Some copies still retain the overhang
- The template remains unchanged at the end of the PCR process
- Complementary base pairing is critical for PCR
- You need some knowledge of the sequence to be able to design PCR primers
- The difference between 5′ and 3′ prime ends of DNA bases
- DNA polymerase only works in the 5′-3′ prime direction
- DNA polymerase needs a primer to start DNA replication
- DNA polymerase will only work when it has a DNA template to copy

Time requirements

Preparation
The first time will take about 25 minutes but after this preparation is no more than 5 minutes as you can keep the components assembled in the storage box for future use.

Lesson
20 minutes is sufficient to carry out the activity with more time to explain the stages and analyse the outcomes as required.

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

PCR Puzzle - Preparation

1 Assemble template bases

You only need to do this once as the bases can be kept for future lessons!

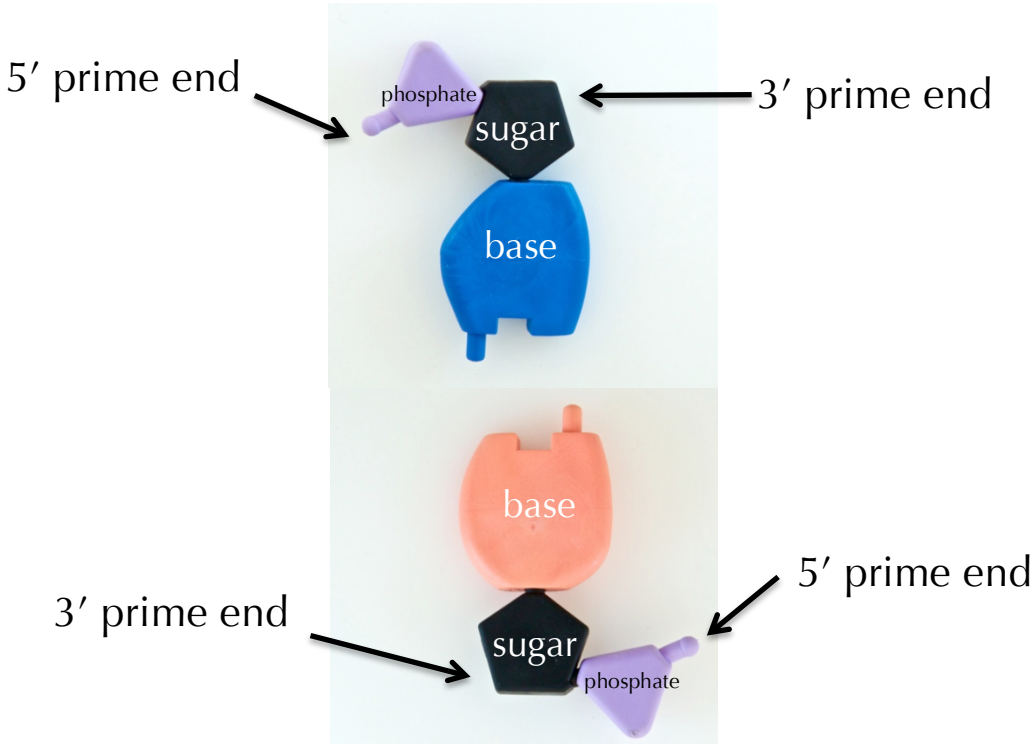
The bases comprise of a sugar (red, black or white), a phosphate (purple) and a base (yellow, green, blue or orange). Please note the different sugar colours are included to make it easier to see the primer and template they do not represent different chemicals.

Use black sugars for the template bases.

Attach the purple phosphate to the black sugar by pushing the bent knob from the sugar into the hole in the purple phosphate.

Make sure you add the sugar to the 5′ prime end as shown so the knob still sticks out of the purple phosphate not out of the sugar.

Push the coloured base (green, orange, blue or yellow) onto the straight knob on the sugar.



Assemble the following bases:

Cytosine (Yellow)	3
Guanine (Green)	3
Adenine (Blue)	6
Thymine (Orange)	6

Helpfully, C, G, A, and T are embossed on the edge of the base.

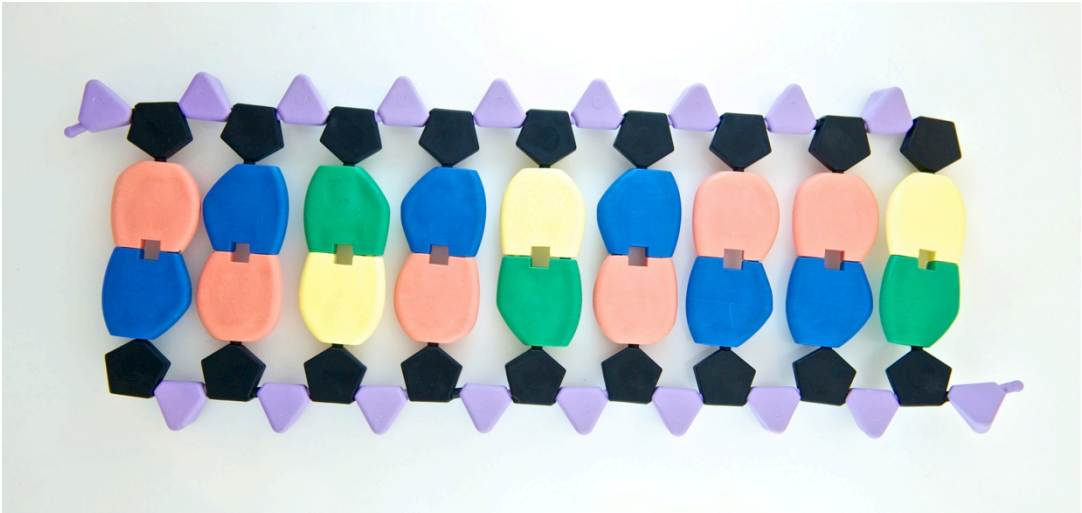
Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

Put them into this 9 base sequence. The 5′ end is the phosphate side of the sugar, the 3′ end is the hole in the sugar.

5′ - T A G A C A T T C - 3′
3′ - A T C T G T A A G - 5′

Do both strands so the template is double stranded.

T pairs with A (orange with blue)
C pairs with G (yellow with green)



2 Assemble primer bases

Use white sugars for the primer bases. Assemble the following bases:

Guanine (Green)	7
Adenine (Blue)	21

Put them into this sequence:

5′ - AA - 3′	x 7
5′ - AG - 3′	x 7

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

Typically, PCR primers are between 10 and 20 bases long. Remember PCR primers are single stranded!

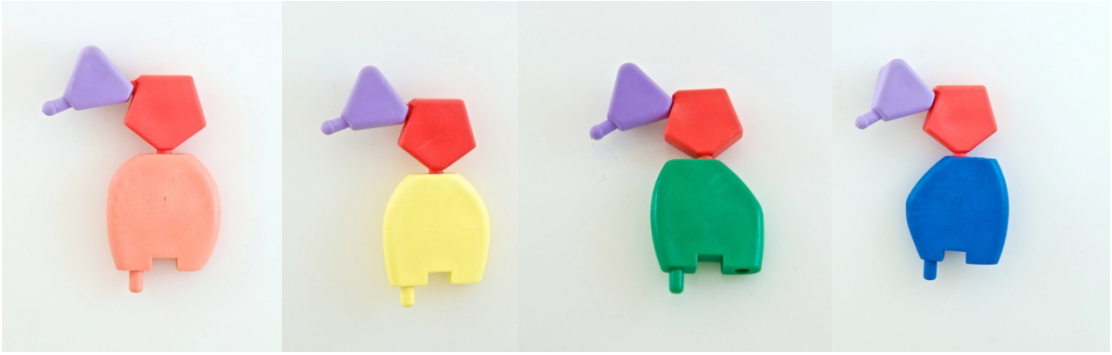
Optional: The primers can be glued together

3 Assemble bases for extension

Use red sugars for these bases.

Assemble the following bases:

Cytosine (Yellow)	17
Guanine (Green)	7
Adenine (Blue)	17
Thymine (Orange)	35



After completion of the lesson

Store the template strands (with black sugar) intact in the storage box.

Remove the primers (with white sugar) and keep them intact in the storage box.

Remove the rest of the bases (with red sugar) and keep them in the storage boxes for use next time.

Preparation for subsequent uses (about 5 minutes)

Sort out the bases (with red sugars) by colour (yellow, green, blue or orange)

Separate the 2 types of primers (with white sugars)

Rejoin the 2 template strands (with black sugars) so the green-yellow pair and the blue-orange pair

Then you are ready to go!

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

PCR puzzle – teacher's notes

Lesson tips

Important - ensure students have bases correctly orientated so 5′ and 3′ are opposite each other when bases are paired (shown on page 2) & don't let your students take the bases apart!

Cycle one - split the class in half and give one strand to each half. Get each student to add a base to the growing strand. Put the completed strands next to each other so the students can make their observations.

Cycle two – after this, split the groups in half again and each group gets a strand.

Cycle three – when complete put the completed strands at the front of the room so students can make their observations again.

We have found it is useful for students to have images of completed rounds so they can check the primer position and orientation is correct.

1 PCR cycle one

Denaturing

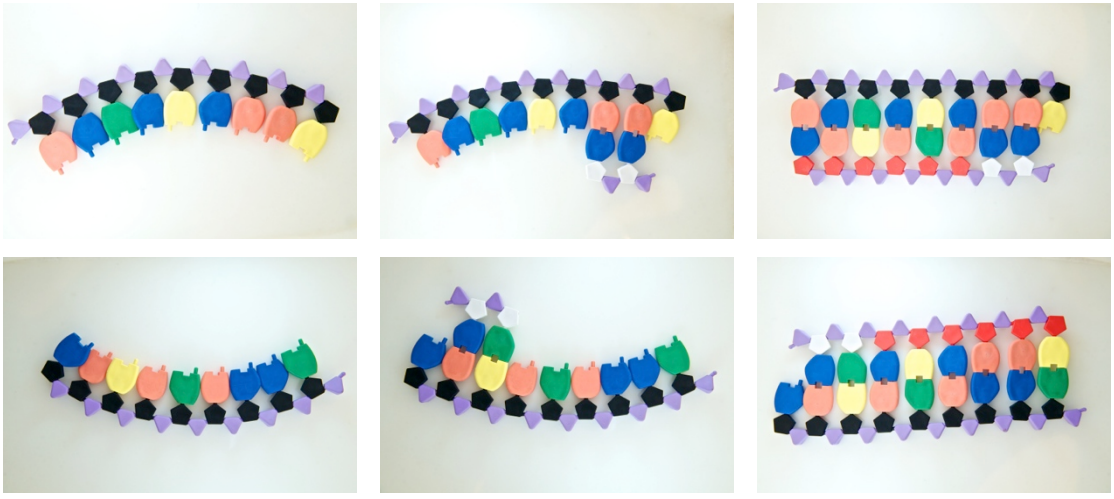
Start by separating the template double stranded DNA molecule into two separate strands. This is caused by 95°C heat in the first step of PCR. A special heat resistant form of DNA polymerase is used (called Taq Polymerase) that does not denature at 95°C.

Annealing

Anneal the primers onto each strand, one at each end where it matches by complementary base pairing (A-T; C-G). This happens at around 45°C in the second step of PCR. The ratio of A-T and C-G determines the annealing temperature.

Extension

Extend the primer by copying the template on both strands. Use the bases with the red sugars for this. Stop when you reach the end of the template. This happens at 72°C in the third step of PCR. 72°C is the normal temperature for Taq Polymerase.



Congratulations! You have completed the first cycle (made up of three steps of PCR – denature, anneal, extend). Next you repeat the 3 steps in the second cycle.

Before moving on, observe the following:

- How many copies do you get?
- How many are just the target sequence (ie begin and end with primers)?

Comments on PCR cycle 1

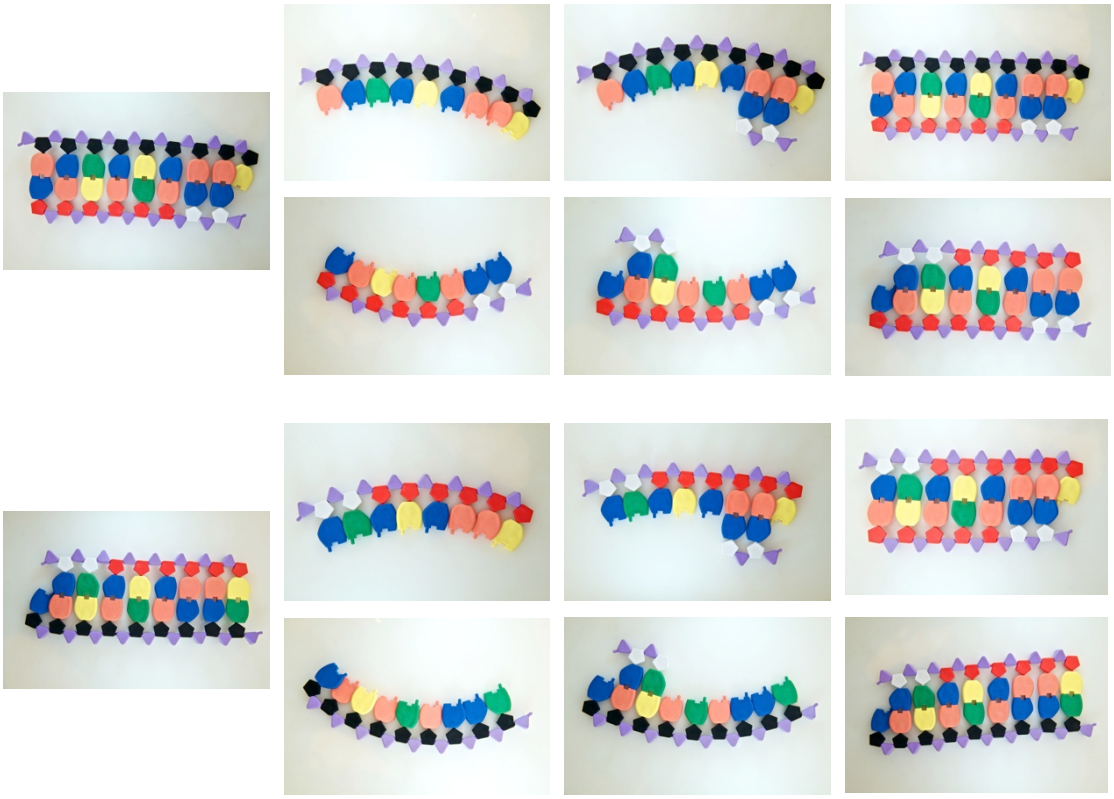
- You get two double stranded copies
- There is an overhang on both copies

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

- The primer is part of both copied strands
- Red sugars are the minority (thus most strands are comprised of template or primer)

2 PCR cycle two

Using both template strands from round one, repeat the denature, anneal and extension steps.



Observe the following:

- How many copies do you get?
- How many are just the target sequence (ie begin and end with primers)?

Comments on PCR cycle 2

- Four double stranded copies are produced
- There is an overhang on all four copies
- Two strands are correct target sequence
- The primer is in all copies
- Red sugars majority (thus most strands are comprised of copies)
- Template unchanged

3 PCR cycle three

Using all four template strands from round two, repeat the denature, anneal and extension steps.

Observe the following:

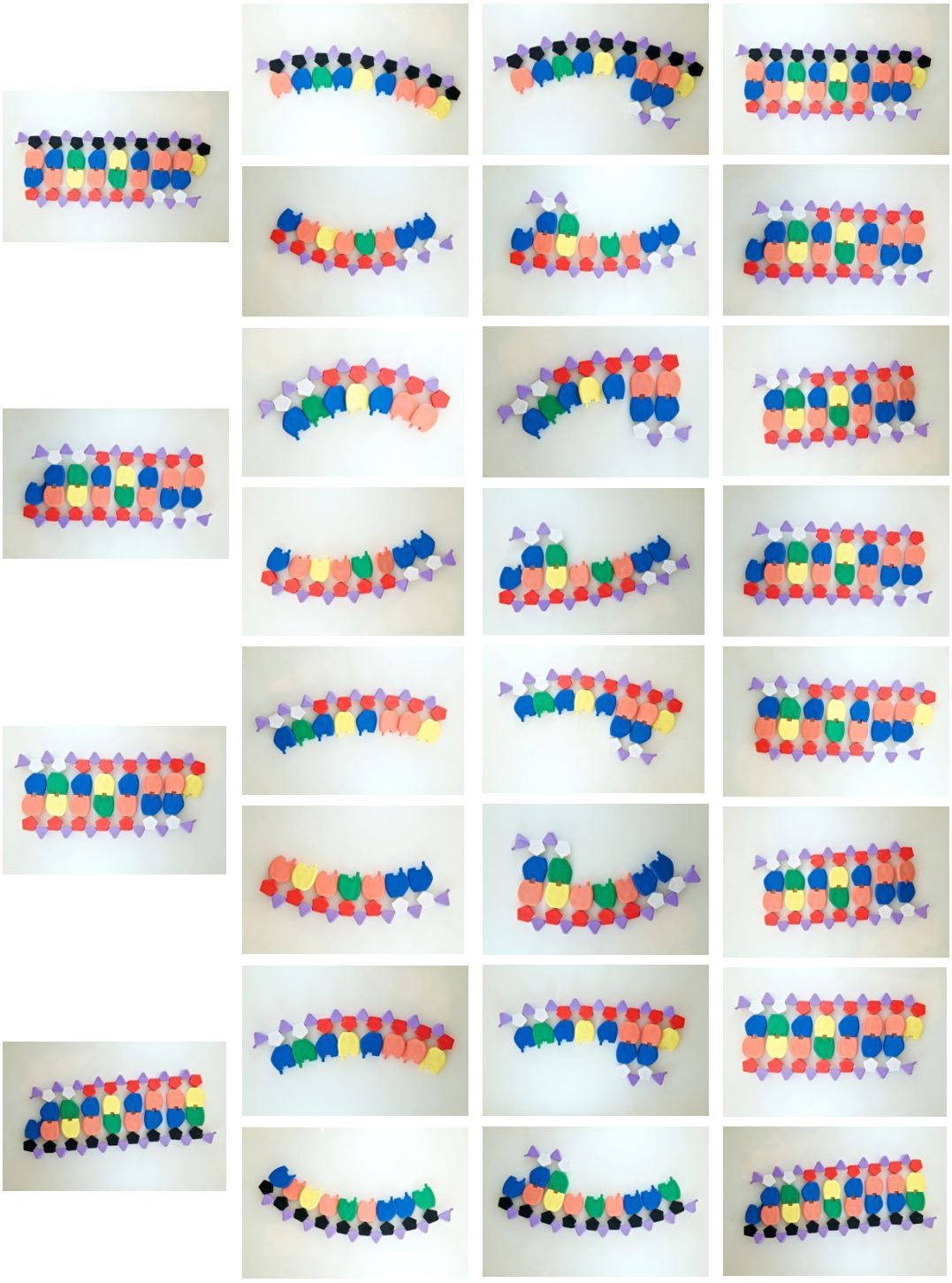
- How many copies do you get?
- How many are just the target sequence (ie begin and end with primers)?
- What if anything, has happened to the template?
- How many bases with red, black and white do you now have?

Comments on PCR cycle 3

- Eight double stranded copies are produced
- There is an overhang on six of the copies
- For the first time, two copies are the correct double stranded product

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

- Red sugars majority (thus most strands are comprised of copies)
- The primer is in all copies
- Template unchanged



Extension ideas

Your student's could design their own primers for the sequence given or they could rearrange the sequence and design primers for this new sequence.

Your student's could investigate what decides the annealing temperature of the primer.

PCR Puzzle – student instructions

Important - Ensure you have the bases correctly orientated so 5′ and 3′ are opposite to each other when the bases are paired and please don't take the bases apart!

1 PCR cycle one

Denaturing
Start by separating the template double stranded DNA molecule into two separate strands. This is caused by 95°C heat in the first step of PCR.

Annealing
Anneal the primers onto each strand, one at each end where it matches by complementary base pairing (A-T; C-G). This happens at around 45°C in the second step of PCR.

Extension
Extend the primer by copying the template on both strands. Use the bases with the red sugars for this. Stop when you reach the end of the template. This happens at 72°C in the third step of PCR.

You have completed the first cycle (made up of three steps of PCR – denature, anneal, extend). Next you repeat the 3 steps in the second cycle.

Before moving on, observe the following:

- How many copies do you get?
- How many are just the target sequence (ie begin and end with primers)?

2 PCR cycle two

Using both template strands from round one, repeat the denature, anneal and extension steps.

Observe the following:

- How many copies do you get?
- How many are just the target sequence (ie begin and end with primers)?

3 PCR cycle three

Using all four template strands from round two, repeat the denature, anneal and extension steps.

Observe the following:

- How many copies do you get?
- How many are just the target sequence (ie begin and end with primers)?
- What if anything, has happened to the template?
- How many bases with red, black and white do you now have?