Synthetic Biology for iGEM

Polymerase Chain Reaction (PCR)

History

- 1976: DNA polymerase isolated from T. aquaticus (Taq) was found to have activity at temperatures greater than 75 C
- 1977: Frederick Sanger described a process to determine DNA sequence using primers, DNA polymerase
- 1985: Researchers begin using Taq polymerase for DNA amplifications
- 1986: Patent for PCR with Taq polymerase filed, and paper describing PCR method is published
- 1986: Forensic scientists use PCR to amplify DNA evidence in criminal cases
- 1991: PCR patent sold for $300 million

What is PCR?

- Polymerase Chain Reaction, or PCR, is a technique used to amplify small amounts of DNA
- Reaction is performed in a thermocycler, which can automatically change temperatures for the different steps of PCR

Reagents

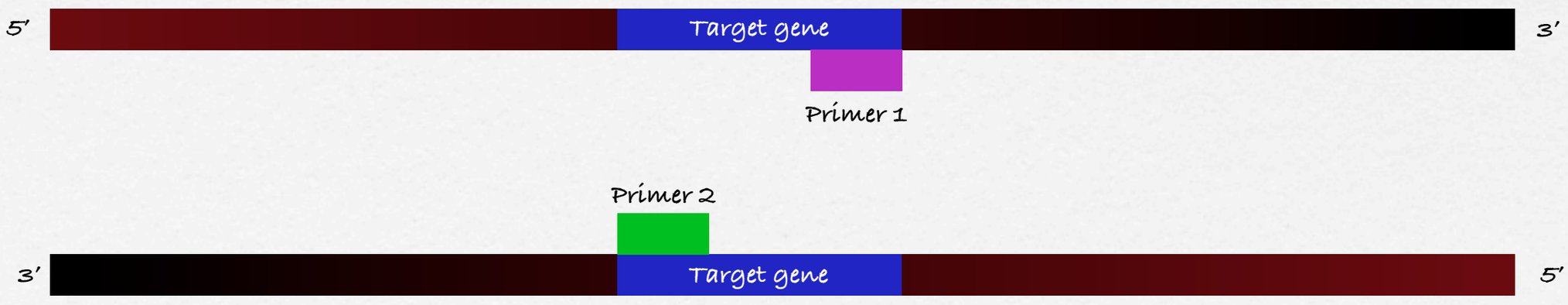
- PCR needs several things
  - target DNA to be amplified
  - Taq polymerase, a DNA polymerase that is stable at high temperatures
  - dNTPs, free nucleotides that DNA polymerase uses to make new strands of DNA
  - DNA primers, one for the 5' end of the target gene and a complement of the 3' end

PCR Process: Denaturation I

- Thermocycler heats reaction to 94 C
  - This denatures DNA, separating the double helix structure into two single strands

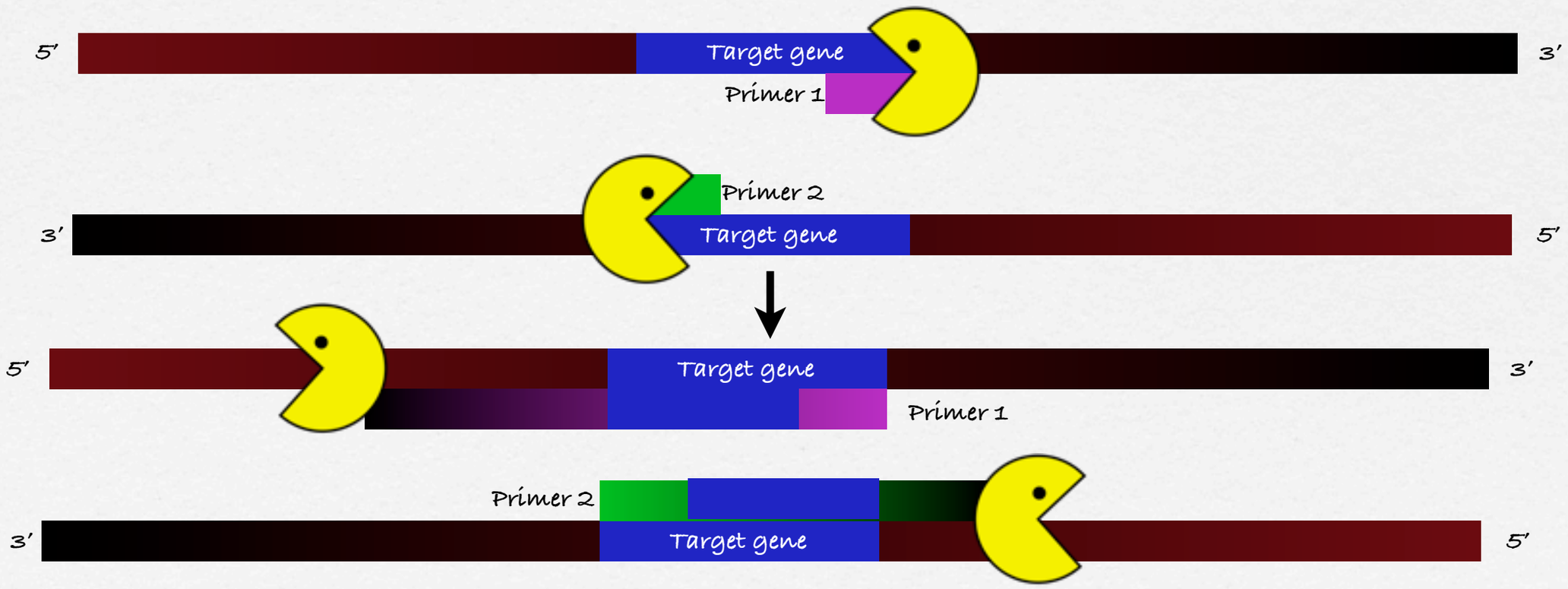
PCR: Annealing I

- Reaction is lowered to 50-65 C
- This causes DNA primers to anneal (bind) to DNA



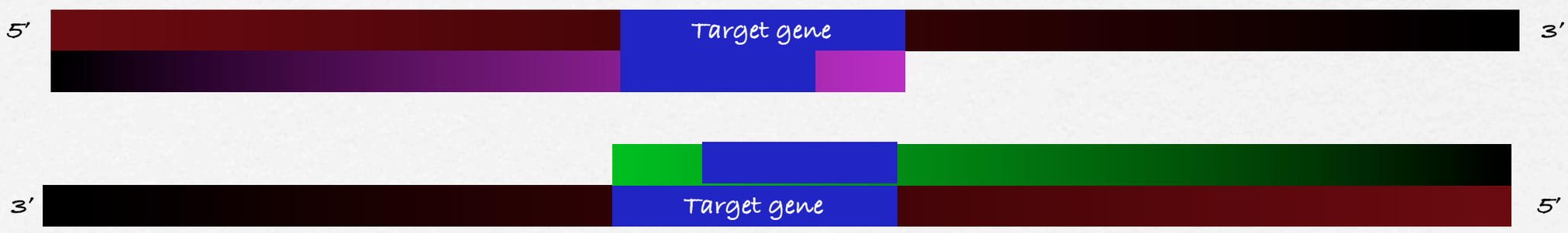
PCR: Elongation I

- Reaction is raised to 72 C
- Taq Polymerase proceeds with DNA replication



After Elongation I

- Two new strands of DNA
- Target genes have been amplified 2x
- Some DNA (not target) has also been amplified

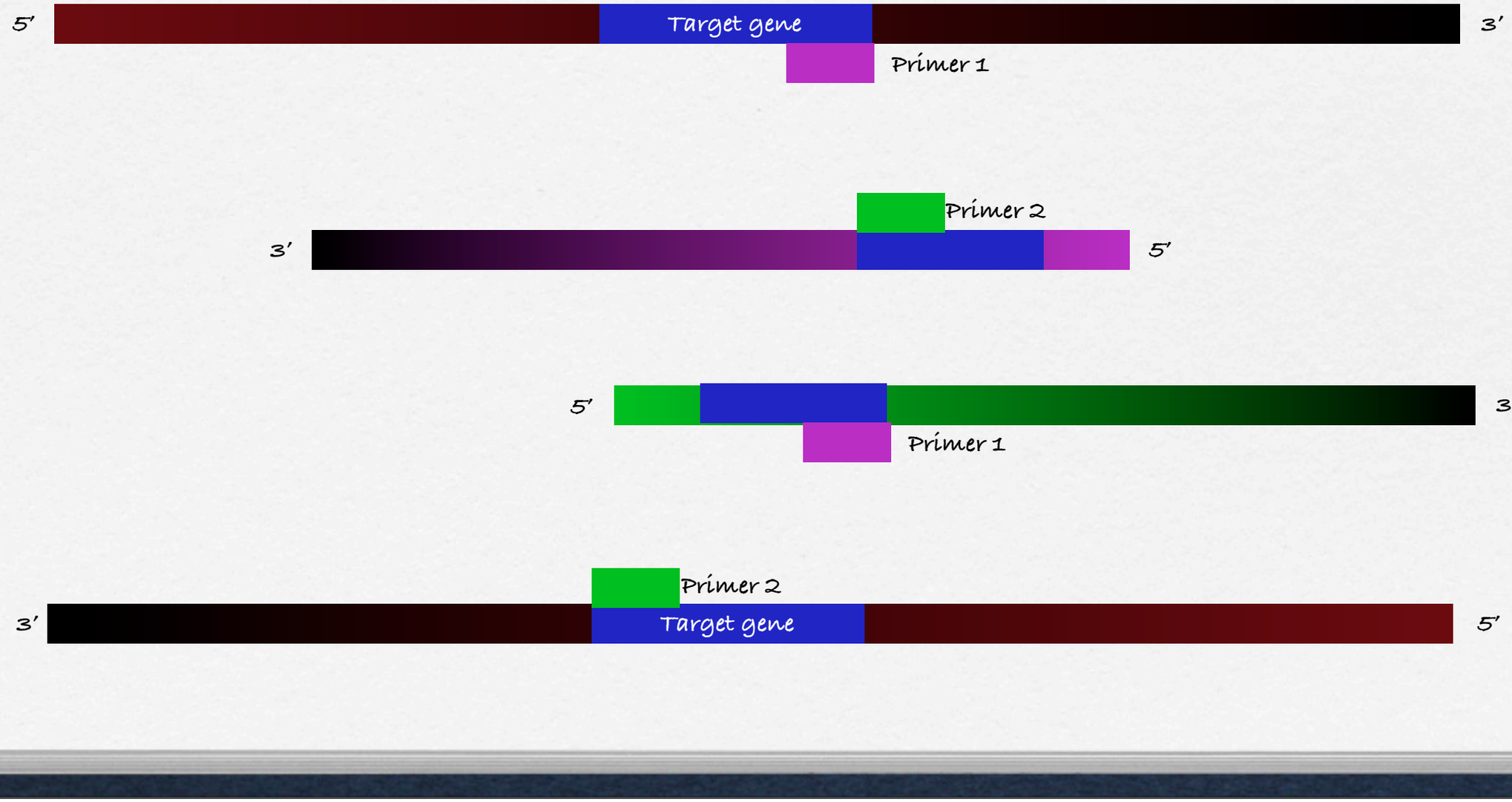


Denaturation II

- 96 C

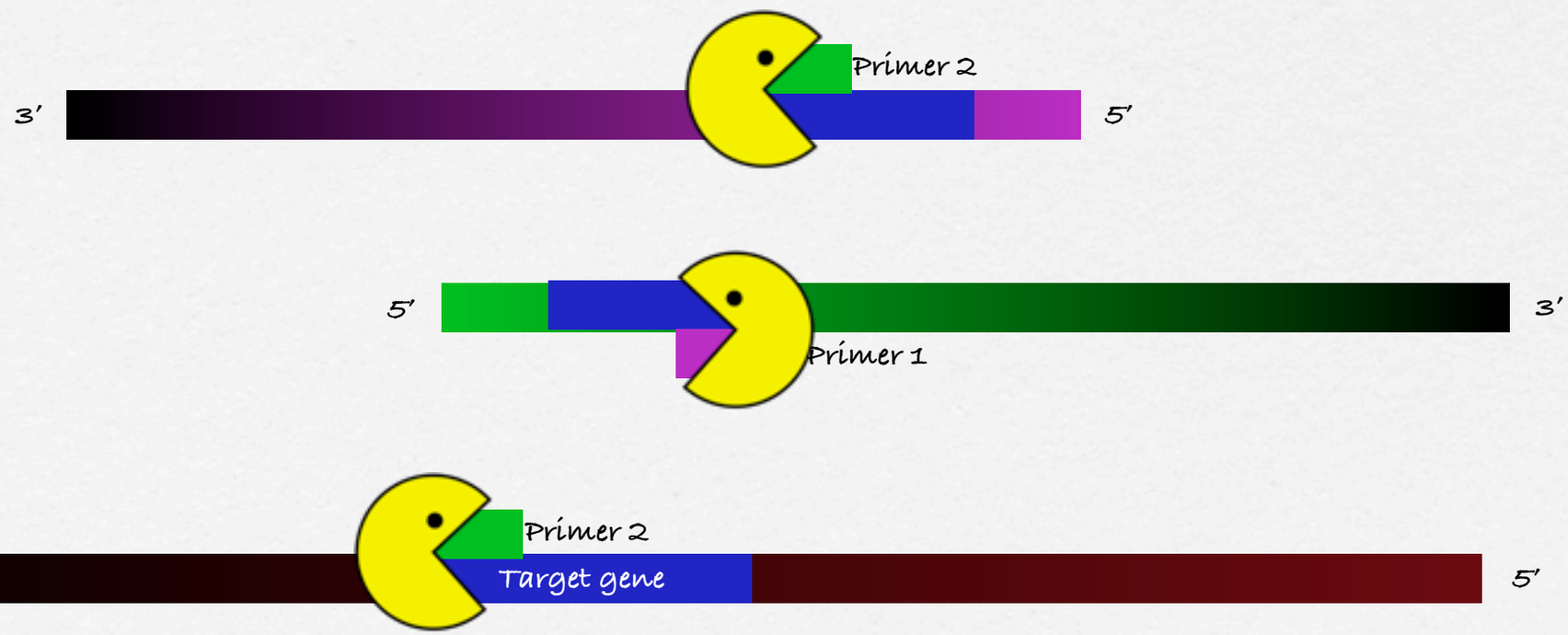
Annealing II

- 50-65 C



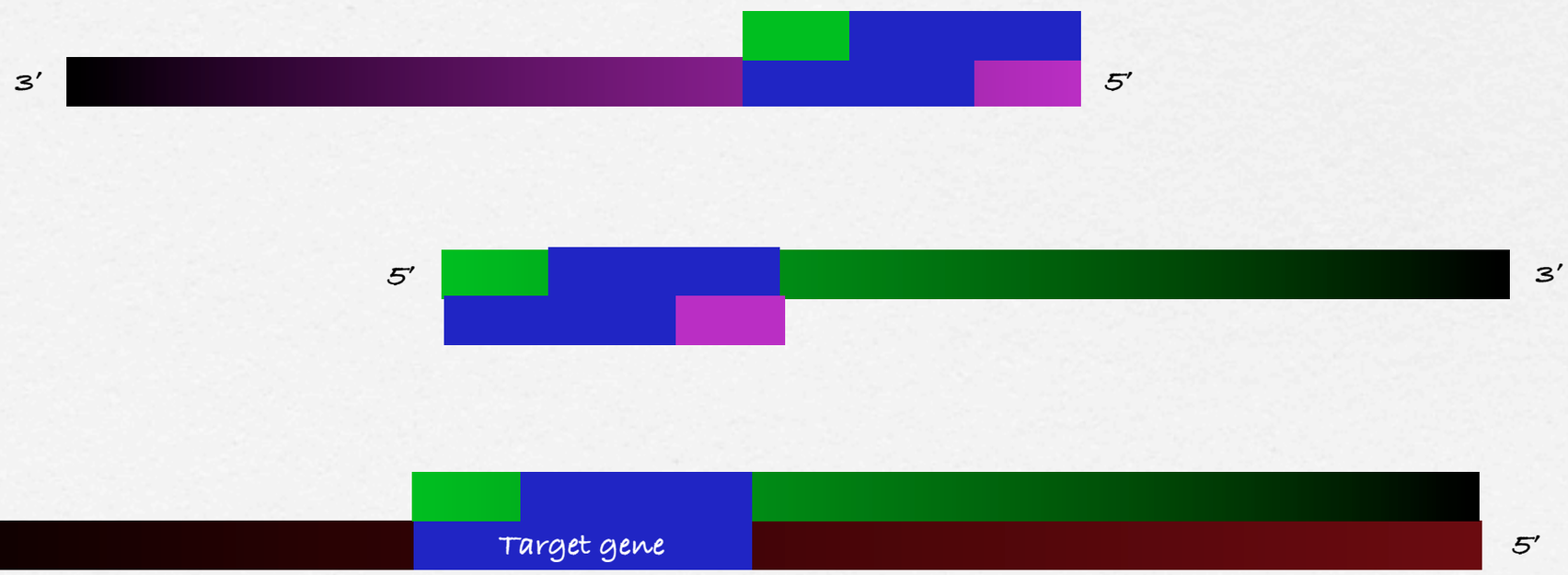
Elongation II

- 72 C



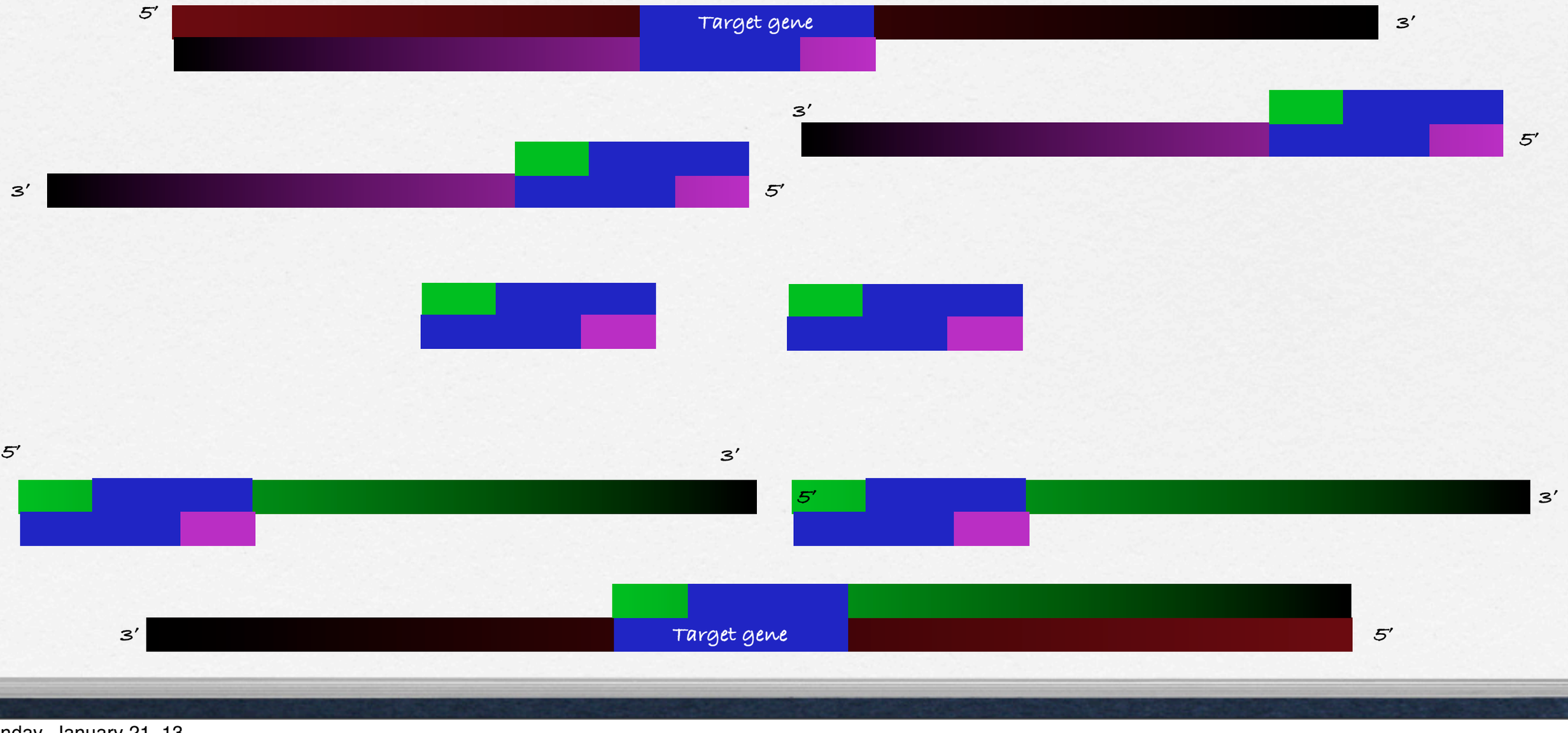
After Elongation II

- Notice that we now have two copies of only the target gene



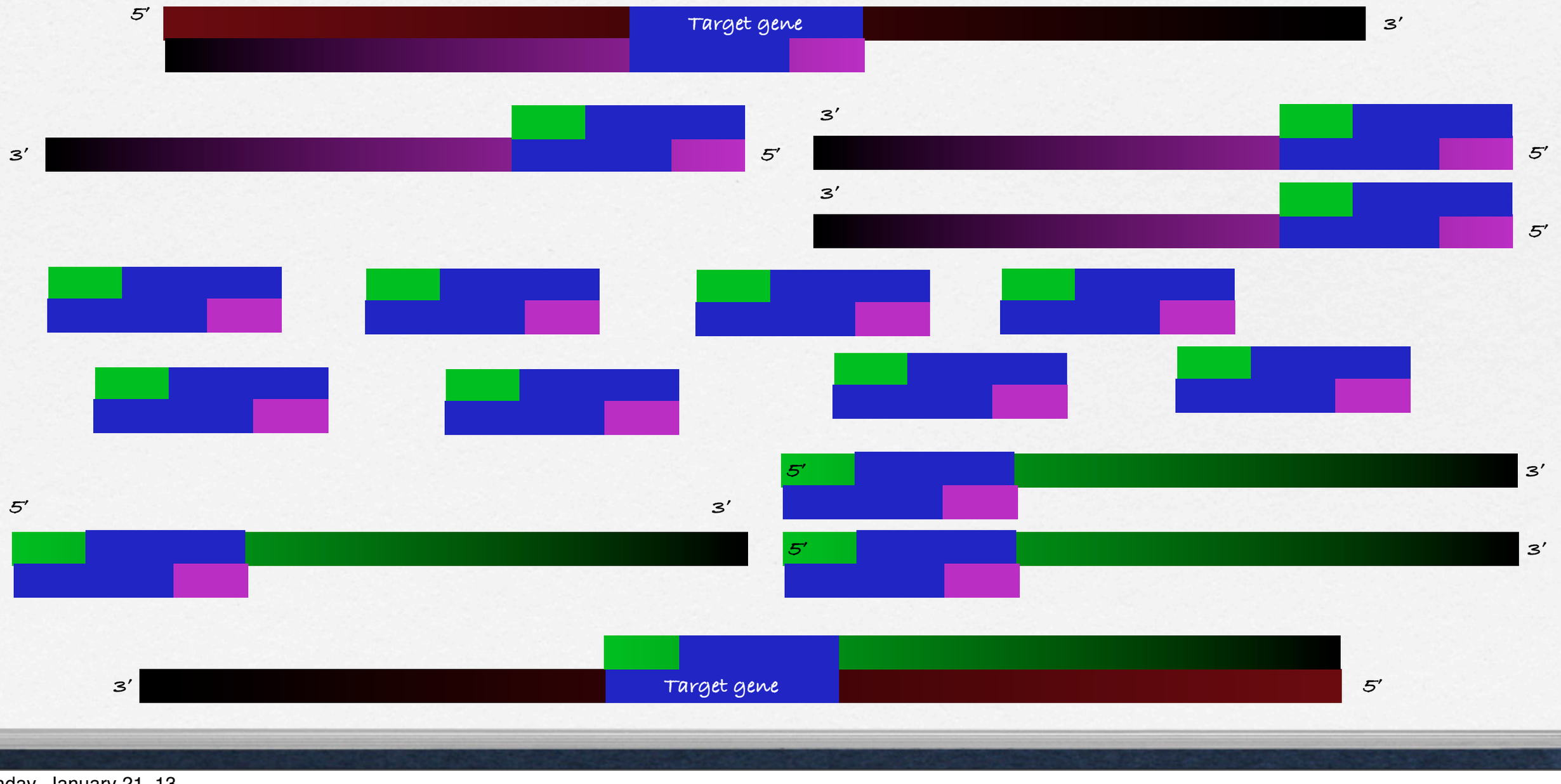
After Elongation III

- Notice that we now have eight copies of only the target gene



After Elongation IV

- Notice that we now have 22 copies of only the target gene



PCR Numbers

- Notice that the number of longer strands increases linearly, but the number of target only strands increases exponentially

Cycle	Template Strands	Long Strands	Target Only Strands
0	2	0	0
1	2	2	0
2	2	4	2
3	2	6	8
4	2	8	22
5	2	10	52
6	2	12	114

Example PCR Thermocycler Run

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	98°C	30 s	98°C	30 s	1
Denaturation	98°C	5-10 s	98°C	5-10 s	25-35
Annealing (see 6.3)	-	-	X°C	10-30 s	
Extension (see 6.4)	72°C	15-30 s /1 kb	72°C	15-30 s /1 kb	
Final extension	72°C	5-10 min	72°C	5-10 min	1
	4°C	hold	4°C	hold	

Protocol from New England Biolabs Phusion High-Fidelity DNA Polymerase kit