Polymerase Chain Reaction (PCR)

Dr Murtakab Y Al-Hejjaj

PCR

- Polymerase chain reaction (PCR) is a common molecular biology technique that enables researchers to amplify (make multiple copies of) a specific region of DNA in vitro.
- PCR has been invented by Kary Mullis and colleagues in 1983, which set the stage for a scientific revolution.
- PCR is efficient, rapid and can amplify DNA or RNA sequences (templates) from various sources.
- Once the DNA has been sufficiently amplified, the resulting product can be sequenced, analysed by gel electrophoresis, or cloned into a plasmid for experimental purposes.

PCR Recipe List, Hints, and Tips

- **Template DNA**: Decide how you will obtain this; will you use a commercially available kit or an “in-house” extraction? Check the concentration of the template material using either by UV spectroscopy or by electrophoresis with a quantitative molecular weight marker
- **Primers** (forward and reverse):
- **dNTPs:** These are usually purchased individually or as a premixed preparation containing equal quantities of all four bases.

- **MgCl2/MgSO4**: It is vital for PCR to work; it acts as an enzyme cofactor and also impacts the specificity and stringency of primer annealing.
- **Reaction buffer:** This is usually supplied with the enzyme as a 5 or 10 concentration that is diluted as you prepare the reaction; the reaction buffer may or may not contain **Mg2+**
- **Nuclease-free water:**
- **Polymerase:** There is a number of different polymerase available, the most common is Taq. Polymerases with a number of different characteristics are available and so that can be selected according to application.

PCR stapes

Initial Denaturation

Cycling
X 20-35

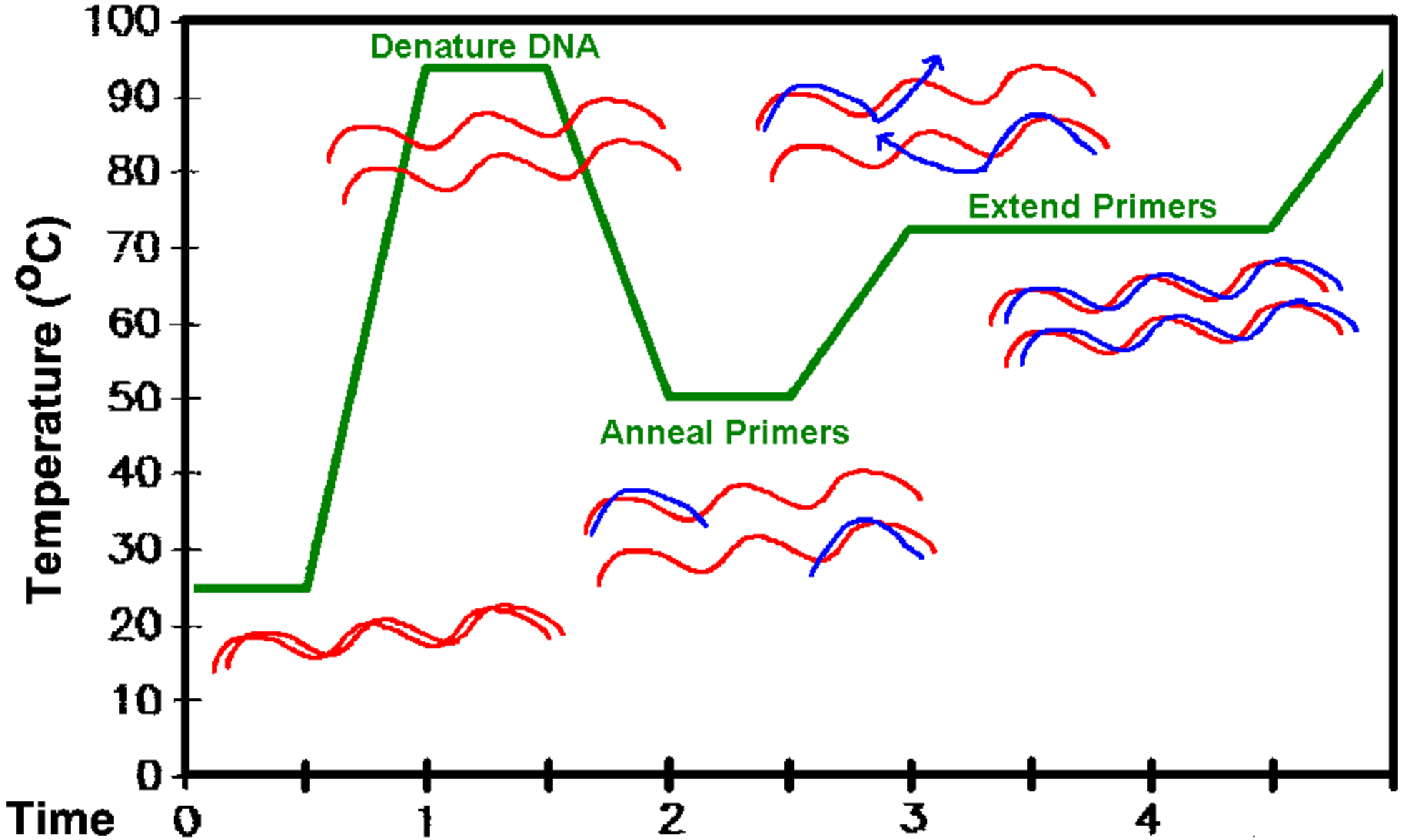
Denaturation

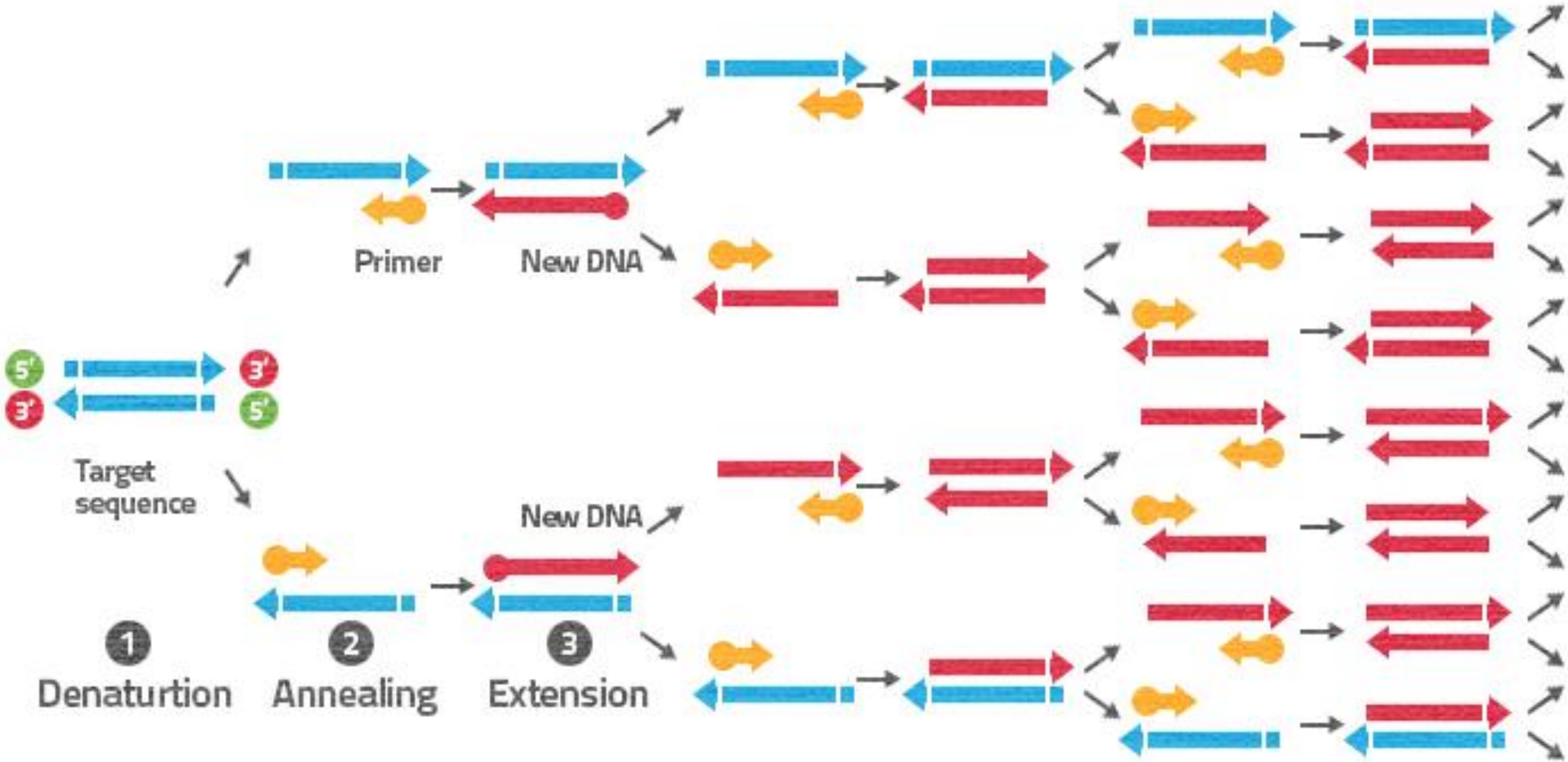
Annealing

Extension

Final Extension

Holding

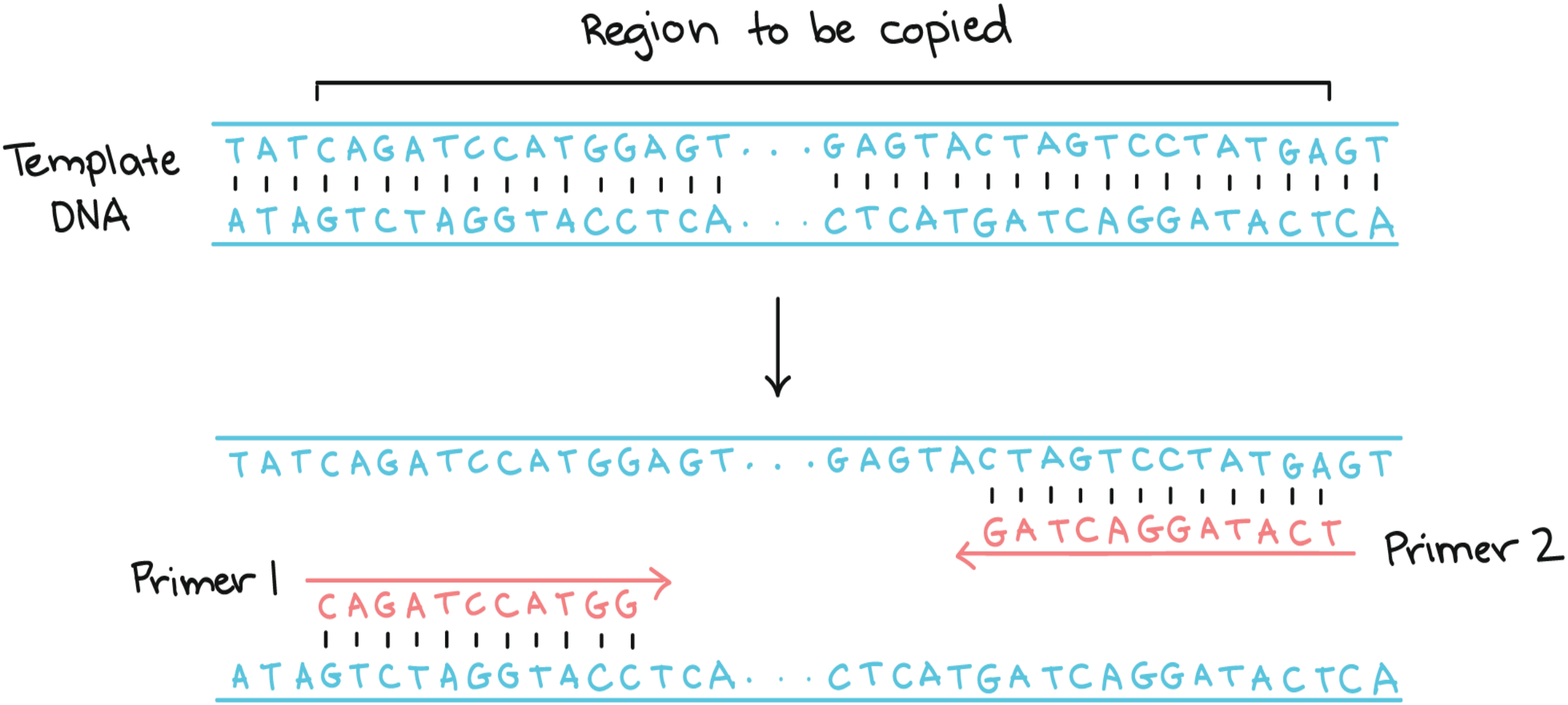

Denature DNA
Anneal Primers
Extend Primers
Temperature (°C)
100
90
80
70
60
50
40
30
20
10
0
Time
0
1
2
3
4


5'
3'
3'
5'
Target sequence
Primer
New DNA
New DNA
1
Denaturtion
2
Annealing
3
Extension

Factors effect PCR

- **Magnesium Concentration**
- **Buffer Considerations**
- **Enzyme Concentration**
- **PCR Primer Design**
- **Template Quality and Quantity**
- **Cycling Parameters**
  - annealing temperature
  - extension time.
- **PCR Enhancers and Additives**
- **Nucleic Acid Cross-Contamination**

PCR Primer Design



Enzyme Concentration

- It is recommended using 1 unit of *Taq* DNA polymerase in a 50µl amplification reaction. In most cases, this is an excess of enzyme, and adding more enzyme will not significantly increase product yield. In fact, increased amounts of enzyme increase the likelihood of generating artifacts associated with the intrinsic 5′→3′ exonuclease activity of *Taq* DNA polymerase, resulting in smeared bands in an agarose gel.

Template Quality

- Successful amplification depends on DNA template quantity and quality. Reagents commonly used to purify nucleic acids (salts, guanidine, proteases, organic solvents and SDS) are potent inactivators of DNA polymerases. For example, 0.01% SDS will inhibit *Taq* DNA polymerase by 90%, while 0.1% SDS will inhibit *Taq* DNA polymerase by 99.9%.

Cycling Parameters

- The two most commonly altered cycling parameters are annealing temperature and extension time. The lengths and temperatures for the other steps of a PCR cycle do not usually vary significantly. However in some cases, the denaturation cycle can be shortened or a lower denaturation temperature used to reduce the number of depurination events, which can lead to mutations in the PCR products.

PCR Enhancers and Additives

- Addition of PCR-enhancing agents can increase yield of the desired PCR product or decrease production of undesired products. There are many PCR enhancers, which can act through a number of different mechanisms. These reagents will not enhance all PCRs; the beneficial effects are often template- and primer-specific and will need to be determined empirically. Addition of betaine, DMSO and formamide can be helpful when amplifying GC-rich templates and templates that form strong secondary structures, which can cause DNA polymerases to stall.

Nucleic Acid Cross-Contamination

- It is important to minimize cross-contamination between samples and prevent carryover of RNA and DNA from one experiment to the next. Use separate work areas and pipettors for pre- and post-amplification steps. Use positive displacement pipettes or aerosol resistant tips to reduce cross-contamination during pipetting. Wear gloves, and change them often.

- Once you have considered all of the above parameters, have designed your primers and prepared your template material, it is a good idea to write a reaction-specific “ingredient list” to pin up at your workstation so that you can easily refer to it when preparing your reaction.