

Optimization of annealing temperature

The title is positioned above a series of horizontal lines that span the width of the slide. These lines include a thick teal bar, followed by a thin white bar, and then two thin teal bars, creating a layered, modern design element.

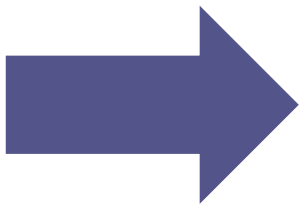
PCR



Example : you want to study a mutation in a DLG3 gene and how it relate to memory,
Find you're the region from any website, eg.Ensebmle

The segment that you want to amplified is in the blue square

5'
CATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT
GTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA
3'



Design the primers using Primer3, then send them to ant companey who will synthesize them

Make sure that the area that you want to study is between the primers

The region to be studied should be between the forward and reverse

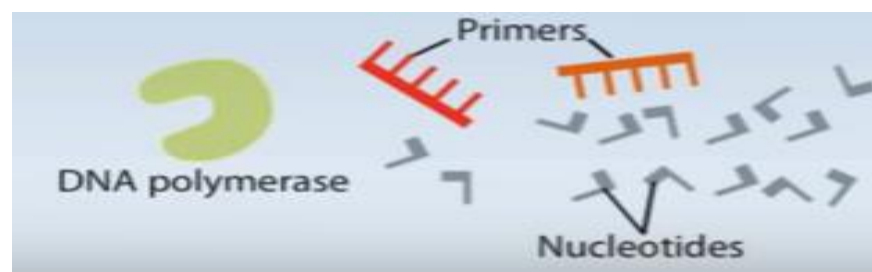
PCR cycle steps

1-Denaturation:

The purpose is.....

CATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT

GTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA



2-Annealing:

The purpose is.....

GATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT GATTGAGGT
GGTGGTACAATAGTACGCTA

CTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA CTA
ATAAGAGTGATTGAGGT



3-Extension:

GATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT GATTGAGGT
GGTGGTACAATAGTACGCTA TTCTCACTAACTCCA

CACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA CTA
CACCATGTTATCATGCGATAAGAGTGATTGAGGT

3-Extension:

GATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT GATTGAGGT
GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA CTA ACTCCA

GATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT CCACCATGTTATCATGCGATAAGAGTGATTGAGGT
CACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA GGTGGTACAATAGTACGCTATTCTCACTAACTCCA CTA ACTCCA

PCR Optimization

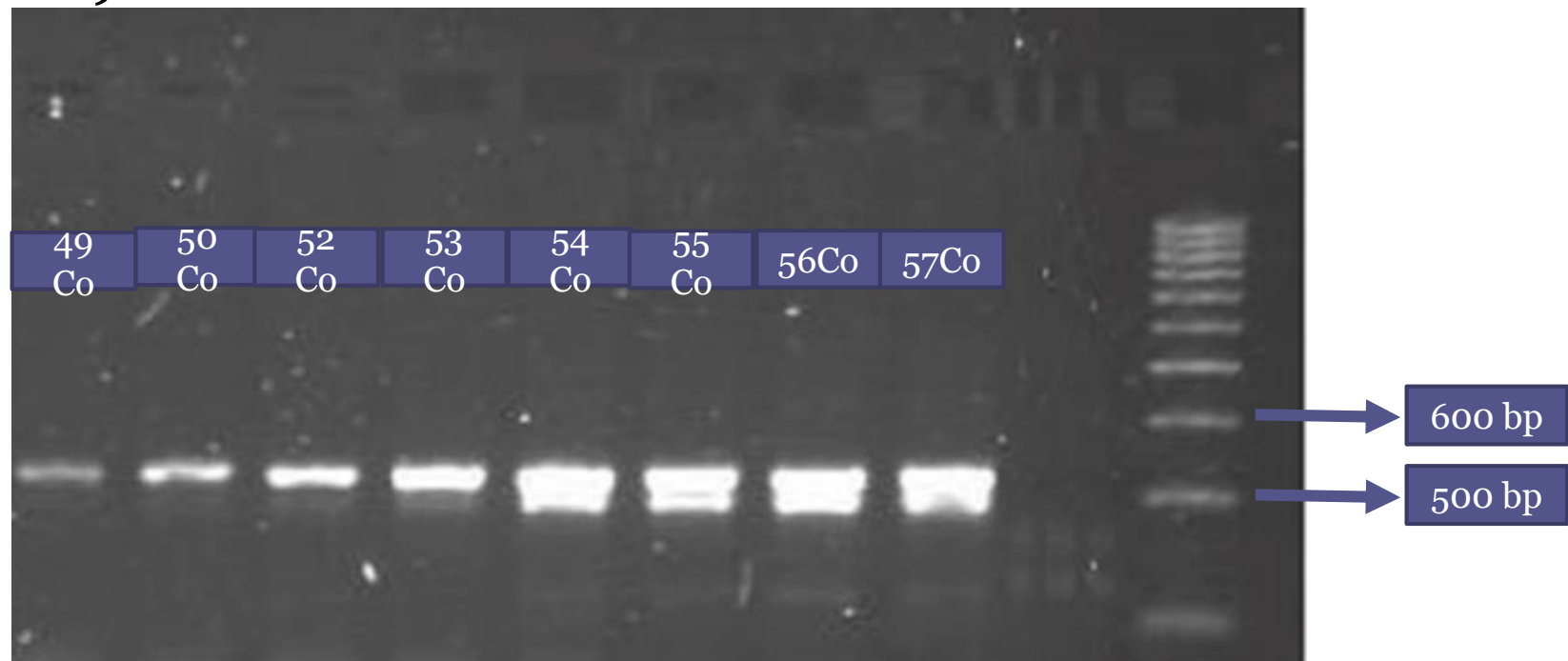
- When developing a protocol for PCR amplification of a new target, it may be important to optimize all parameters including reagent concentrations, cycling temperatures, and cycle number.

PCR Optimization

- There is no single set of conditions that is optimal for all PCR.
Why?
- PCR Optimization: is to find the most effective condition (eg. primer conc., T_a , $MgCl_2$..) in which the amplification of your target sequence will occur.
- How will you know which condition is the best of amplification?
And how the result should be?

PCR Optimization

- Example:
- What is the best annealing temp.? (you know previously that PRC product is 520 bp)



Annealing Temperature optimization

- Optimizing the annealing temperature of your PCR assay is one of the most critical parameters for reaction specificity
- It is done by using different T_a until you find the best one that will cause a good band.
- When optimizing T_a what you should do with other PCR component?

1. Start by applying the standard concentration of PCR component that work with majority of PCR reaction.
2. Try at first 8 different annealing temperature.
3. (Choose it depending on T_m) (Example: If the T_m of a primer is 60 Co, try 55-63)

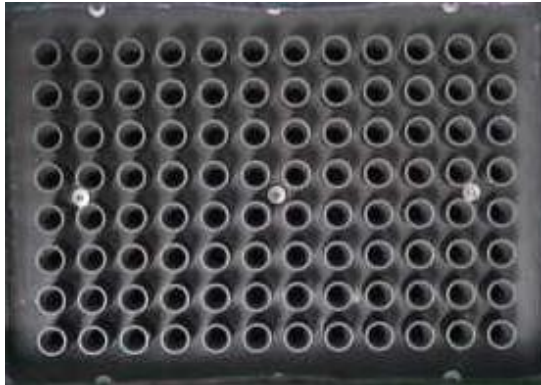
PCR components

	Stock concentration	Final Concentration	Volume of each
PCR buffer	10 X	1 X	
dNTPs	10 mM	200 μ M	
Mg ²⁺	25 mM	1.5 mM	
Forward Primer	10 μ M	0.4 μ M	
Reverse Primer	10 μ M	0.4 μ M	
Template DNA	45 ng/ μ l	90 ng	
Taq DNA Polymerase	5 Units/ μ l	0.05 Units/ μ l	
Total volume for each tube			50 μ l

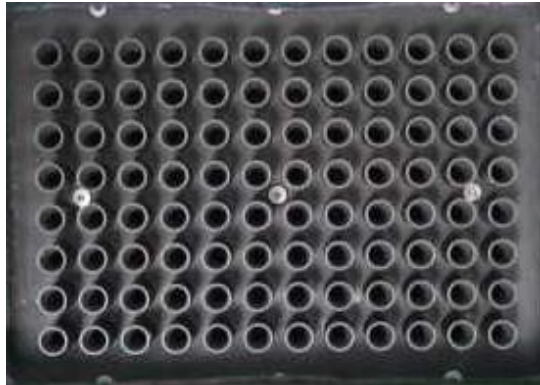
Cycling condition

Initial denaturation	95° C	3 min	
Denature	90° – 95° C	0.5 min	} 25 – 40 cycles
Primer annealing° –° C	0.5 min	
extension	72 C	0.5 min	
Final extension	72° C	5 – 10 min	

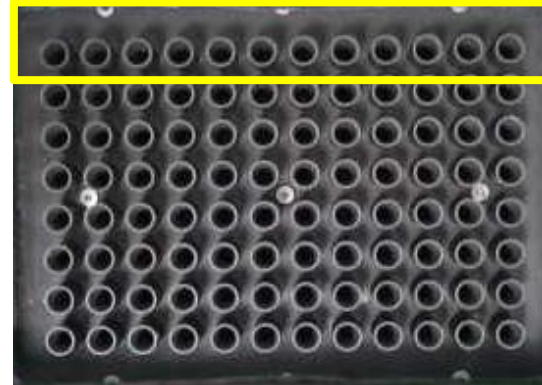
How Thermal Cycler will control the temperature?



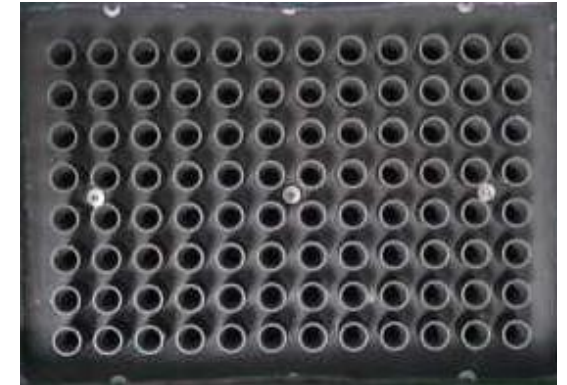
Initial
denaturation
All the row the
same temp



Denature
All the row the
same temp



Annealing
Each row will have
different annealing



Extention
All the row the
same temp

- You will calculate the volume needed
- Each group will do the reaction of one annealing temp.