PCR (Polymerase Chain Reaction)

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

PCR (Polymerase Chain Reaction) is a fundamental technique employed to amplify a specific segment of DNA. It was developed in 1983 and has since become one of the most utilized methodologies in the fields of molecular biology and genetics. By allowing the exponential amplification of specific DNA fragments *in vitro*, PCR enables researchers to generate abundant quantities of genetic material for further analysis. This enzymatic assay relies on the use of two flanking oligonucleotides called primers, which selectively bind to targeted regions of the DNA template, as well as a thermostable DNA polymerase.

The process of PCR involves cycling through different temperatures. Initially, the double-stranded template DNA is denatured and separated into its single-stranded strains. During the annealing step, the primers bind to the complementary single-stranded DNA molecules. In the extension step, the primers are elongated and DNA synthesis is performed by the DNA polymerase.

PCR finds extensive applications in research, particularly in the study of gene expression, mutations and genetic variations. It is an invaluable tool for detecting the presence of DNA from pathogens such as viruses and bacteria.

- 2 - Material

Material	
Template	DNA of interest with the region for amplification
Master Mix	Distilled nucleases free water (nuclease free) DNA Polymerase (thermostable) Forward primer Reverse primer dNTP Mix (dATP, dCTP, dGTP, dTTP) Polymerase Buffer
Vial	Thin walled 200 µl tubes

- 3 - Method

Sample Preparation

- Gather the necessary number of PCR tubes
- Prepare the PCR master mix with enough volume for the desired number of reactions
  Please refer to "Sample Approach" for required components
- Distribute the master mix evenly among sterile PCR tubes
- Add the DNA template to each tube
- Thoroughly mix the PCR reaction solution by gentle pipetting
- Briefly centrifuge the tubes
- Place the tubes into a thermal cycler and proceed with the amplification program

Sample Approach

Reagents	For 25 µl Approach	Final Concentration
10x Polymerase Buffer	2.5 µl	1x
10 mM dNTP Mix	0.5 µl	200 µM
Thermostable Polyermase	0.25 µl	2.5 Units
10 µM Forward Primer	0.5 µl	0.2 – 0.4 µM
10 µM Reverse Primer	0.5 µl	0.2 – 0.4 µM
Sample DNA	X µl	5 ng
Water	ad. 25 µl	

PCR Program

- Initiate the standard amplification program on the thermal cycler
- Set the "Primer Annealing" temperature to the melting temperature minus 5°C [TM -5] of utilized the primer
  Common primer annealing temperatures are between 48 – 60 °C
- Set the "Fragment Elongation" duration to expected fragment length
  Common duration for unknown fragments is 1 minute

Thermo Cycler Program

Steps	Temperature	Duration
Initial Denaturation	95°C	3 min
	Start Cycle of 35x	
Template Denaturation	95°C	30 sec
Primer Annealing	[TM – 5] °C	30 sec
Fragment Elongation	72°C	30 sec/kb
	End Cylce	

Final Elongation	72°C	5 min
Storage	4°C	-

Detection by Agarose gel

- Weigh the appropriate amount of agarose
  Concentration of 1.5% is suitable for PCR products ranging from 100 bp to 2000 bp
- Dissolve the agarose in TAE buffer, pH 8
- Heat the agarose solution to completely dissolve the agarose
  Please refer to the safety datasheets and handle Ethidium bromide with care
- Allow the agarose solution to cool down slightly
- Add 0.5 µg/ml Ethidium bromide
- *Approximately 1 µl per 100 ml of agarose solution*
- Carefully load your DNA samples into the gel
- Limit the current to a maximum of 5 mA for a single gel
- Run the gel at 100 V for 40 minutes
- To visualize the DNA bands, use a UV lamp at a wavelength of 302-365 nm
  Take necessary precautions, as exposure to UV light can be harmful to the skin and eyes

- 4 - Trouble Shooting

Reason	Solution
No Amplification	Ensure good DNA template quality and concentration. Verify primer design and sequences are appropriate. Confirm the thermal cycler settings (temperatures, ramp rates, and cycling program). Optimize annealing temperature by performing a temperature gradient or adjusting primer concentrations.
Non-specific Bands	Optimize primer design to ensure specificity Consider a Hot-Start PCR to minimize non-specific amplification, by increasing the initial denaturation to 15 minutes
High Background	Check for contamination in reagents or equipment Utilize high-quality DNA template Use nuclease-free water Reduce the cycle number
GC rich templates	Optimize annealing temperature Consider using additives such as DMSO Design new primers with adjusted melting temperatures Use a specialized DNA Polymerase with enhanced GC-rich amplification capabilities
Primer Dimer Contamination	Optimize primer design to reduce complementarity Adjust primer concentrations and optimize annealing temperature