Polymerase Chain Reaction

Introduction

Most DNA analysis situations require fairly large amounts of DNA. Usually the amount in a few cells is not enough to fully analyze. A method called the polymerase chain reaction (PCR) has been developed to amplify the amount of DNA in a sample.

Background

Since DNA polymerase is an enzyme it will denature under high heat situations. A special form of DNA polymerase was isolated from *Thermus aquiphilus,* a thermophilic (heat loving) bacterium found in hot springs in Yellowstone National Park. This DNA polymerase, *Taq* polymerase, does not denature under high heat situations and is used in PCR.

When a DNA molecule is heated the two strands will separate.

DNA polymerase cannot initiate replication it can only add nucleotides to an existing nucleotide chain that is paired to the template. Therefore previously synthesized primers are needed for PCR. The primers in PCR are DNA sequences (different than the RNA primers synthesized by primase in DNA replication).

It is imperative that primers are selected to bracket the region of DNA to be amplified.

The third requirement for PCR is a supply of DNA nucleotides in the form of nucleoside triphosphates, called DNTPs.

$MgCl_2$ is added to serve as a cofactor in the bonding of *Taq* polymerase to the primer.

PCR is performed in a machine called a thermal cycler which alternately heats and cools the DNA.

The Process

To begin:	A sequence of dsDNA to be amplified
	Primers
	DNA polymerase
	DNA nucleotides (DNTPs)
	$MgCl_2$
Cycle one:	DNA is heated to 95^{o}C separate (denature) the strands
	It is allowed to cool to 55^{o}C which allows the primers to base pair (anneal)
	DNA polymerase then copies rest of strand
	In five minutes there are two molecules of dsDNA
Cycle Two	Repeat steps of cycle one
	Now there are four molecules of dsDNA
Cycle Three	Eight molecules

The Simulation

Label a sheet of blank paper Cycle One

Cut out your double stranded DNA molecule;

Cut it into two strands. (Heat the DNA to denature)
Paste each strand to the page labeled Cycle One

Attach your primers in the correct location. (Cool to anneal the primers)
Remember the two strands of DNA are antiparallel.

It will make your life easier if you keep orientation like this:

5' – 3'
3' – 5'

Cut out the correct replicated sequence and attach it to the end of the primer.
You will need to cut the replicated sequence on the third cycles.

Repeat for a total of three cycles.
The results from cycle one and two are provided
as stating points for cycles 2 & 3, respectively.
Use a fresh sheet of paper for Cycle Two and Cycle Three.

Starting DNA to be amplified:

5’ ATTCGGTTAACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAAGTTTAGCCA 5’

Primers

GTTTAG 5’   GTTTAG 5’   GTTTAG 5’   GTTTAG 5’

GTTTAG 5’   GTTTAG 5’   GTTTAG 5’   GTTTAG 5’

5’ CGGTTA   5’ CGGTTA   5’ CGGTTA   5’ CGGTTA

5’ CGGTTA   5’ CGGTTA   5’ CGGTTA   5’ CGGTTA

Replicated Sequences

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAA

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAA

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAA

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAA

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAA

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAA

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAA

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAA

ACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

ACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

ACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

ACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

ACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

ACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

ACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

ACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

Use for Cycle Two
Results from First Cycle

5’ ATTCGGTTAACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAAGTTTAG 5’

5’ CGGTTAACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAAGTTTAGCCA 5’

Use for Cycle Three
Results from Cycle Two

5’ ATTCGGTTAACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAAGTTTAG 5’

5’ CGGTTAACCGTCGTATCGATTAGGCAACCTTGATTCAAATC 3’

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAAGTTTAG 5’

5’ CGGTTAACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

3’ GCCAATTGGCAGCATAGCTAATCCGTTGGAACTAAGTTTAG 5’

5’ CGGTTAACCGTCGTATCGATTAGGCAACCTTGATTCAAATCGGT 3’

3’ TAAGCCAATTGGCAGCATAGCTAATCCGTTGGAACTAAGTTTAGCCA 5’