Polymerase Chain Reaction
Biology Laboratory

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 heat loving bacterium found in hot springs in Yellowstone National Park. This DNA 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.

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

The third requirement for PCR is a supply of DNA nucleotides.

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

The Process

To begin: A sequence of DNA to be amplified
Primers
Taq DNA polymerase
DNA nucleotides

Cycle one: 1) DNA is heated to 95°C to separate (denature) the strands.
2) It is allowed to cool to 55°C
which allows the primers to base pair
3) DNA polymerase copies rest of strand

In five minutes there are two molecules of DNA

Cycle Two Repeat steps 1-3

Now there are four molecules of DNA

Cycle Three Repeat steps 1-3

Now there are eight molecules of DNA

After 2 hours there are more than a million molecules of DNA

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 separate)
Paste each strand to the page labeled Cycle One

Attach your primers in the correct location. (Cool to attach primer)
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.

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.

You may need to cut the replicated sequence after two cycles.

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’