



Techniques for Genetic Analysis Using Agarose Gel Electrophoresis

PCR (Polymerase Chain Reaction):

Definition:

PCR is a technique for identifying differences in the DNA of individuals by selectively amplifying specific target sequences.

Advantages and Disadvantages:

The advantages of using PCR are that 1) it can be done rapidly (results within hours), and 2) it can be done on old, degraded DNA or tiny quantities of DNA. The disadvantage of PCR is that it generally yields less variation among individuals within a population than more time-consuming techniques such as RFLP and DNA sequencing analysis.

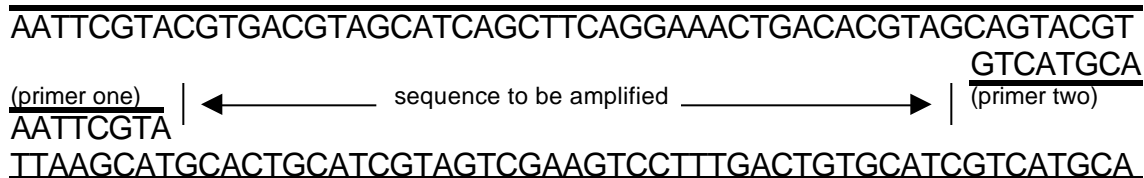
Uses:

It is often used in criminal forensics cases where only tiny amounts of biological evidence are available. It is also used widely in human genetics and evolutionary and ecology genetics research because of its speed and efficiency. It has been a particularly useful tool for researchers working with old or fossilized biological materials.

How it Works:

The DNA from an individual is extracted and purified. It is then combined with a mixture of the following reagents:

Primers = oligonucleotides, which are complementary to the flanking regions on opposite sides of the DNA sequence to be amplified



dNTP's (dATP, dCTP, dGTP, dTTP) = deoxy-nucleotides for building the copies of the sequence to be amplified

Taq-polymerase = enzyme which catalyzes the replication of the sequence to be amplified and which does not degrade at high temperature

This mixture of reagents is then subjected to many cycles of temperature change. During one cycle, the following steps would occur:

1.) Denaturation -- 94°C -- strands of DNA pull apart

AATTCGTACGTGACGTAGCATCAGCTTCAGGAAACTGACACGTAGCAGTACGT
TTAAGCATGCACTGCATCGTAGTCGAAGTCCTTTGACTGTGCATCGTCATGCA



AATTCGTACGTGACGTAGCATCAGCTTCAGGAAACTGACACGTAGCAGTACGT

TTAAGCATGCACTGCATCGTAGTCGAAGTCCTTTGACTGTGCATCGTCATGCA

2.) Annealing -- 40 - 60°C -- primers anneal to DNA template strands

AATTCGTACGTGACGTAGCATCAGCTTCAGGAAACTGACACGTAGCAGTACGT
TTAAGCATGCACTGCATCGTAGTCGAAGTCCTTTGACTGTGCATCGTCATGCA
(primer one) AATTCGTA GTCATGCA
(primer two)

3.) Amplification -- 72°C -- polymerase catalyzes replication of new strands

AATTCGTACGTGACGTAGCATCAGCTTCAGGAAACTGACACGTAGCAGTACGT
TTAAGCATGCACTGCATCGTAGTCGAAGTCCTTTGACTGTGCATC GTCATGCA
(primer one) AATTCGTA GTCATGCA
(primer two)
AATTCGTACGTGACGTAGCATCAGCTTCAGGAAACTGACACGTAGCAGTACGT
TTAAGCATGCACTGCATCGTAGTCGAAGTCCTTTGACTGTGCATCGTCATGCA

The cycle repeats with a mixture of new and old double-stranded molecules.

Figure from *Laboratory DNA Science*, Bloom, Freyer, and Micklos, 1995

The resulting mixture of DNA fragments after 30-50 cycles contains millions of copies of the target sequence, which can then be run directly on an agarose gel. The length of the target sequence amplified often varies among individuals. There may be any number of varying lengths of the target sequence within a population. Nevertheless, a single individual may inherit only two copies of the sequence. An individual may inherit two copies that are the same length or two different copies, each with a different length.

Therefore, when run on a gel, the amplification of one locus for one individual will always have either one band or two bands.

Individual with two copies
of the same length



Individual with two copies
of different lengths



Isozyme analysis

Definition:

Analysis by separation of similar but different forms of enzymes coded for by different alleles at the same gene locus. Isozymes are visualized using either agarose, acrylamide, cellulose, or starch gel electrophoresis and staining.

Uses:

The techniques of protein isozyme analysis have been in wide use somewhat longer than DNA techniques. Isozyme analysis is used by research scientists in determining measures of genetic diversity in populations, assessing paternity, establishing evolutionary relationships, and detecting inbreeding in populations.

How it Works:

A sample of tissue from each individual is homogenized.

Each sample is loaded into the gel and subjected to electrophoresis.

The surface of the gel is stained with reagents that cause the enzyme in question to become visible. (If desired, the gel can be sectioned into thin horizontal slices, and each slice can be stained for a different enzyme).

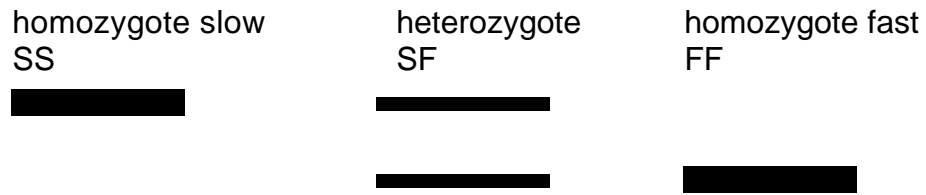
Interpreting Isozyme Results:

Different forms of an enzyme will migrate at different speeds through a gel based on both size and charge. There may be more than one allele for any given enzyme within a population, and these alleles are generally described by the speed with which they migrate through the gel (i.e. slow, medium, or fast). Any one individual, however, may have only two copies of the same allele or one copy each of two different alleles.

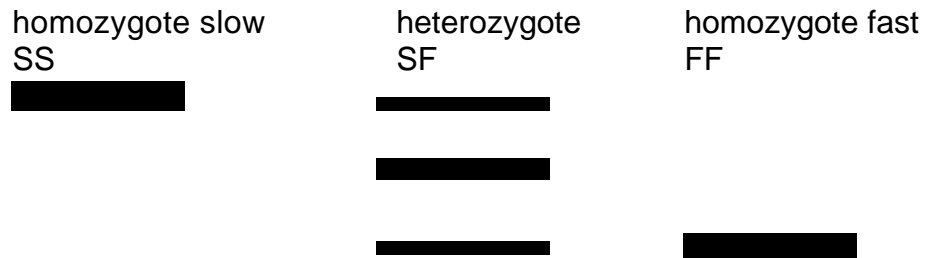
The interpretation of isozyme data is complicated by the fact that some enzymes are composed not of single units, but of two or more subunits, which migrate separately on a gel.

An enzyme with one subunit is called a monomer, an enzyme with two subunits is called a dimer, an enzyme with three subunits is called a trimer, etc.

A monomer would migrate according to the following pattern:



A dimer would migrate according to the following pattern:



A trimer would migrate according to the following pattern:

