Lab: 5

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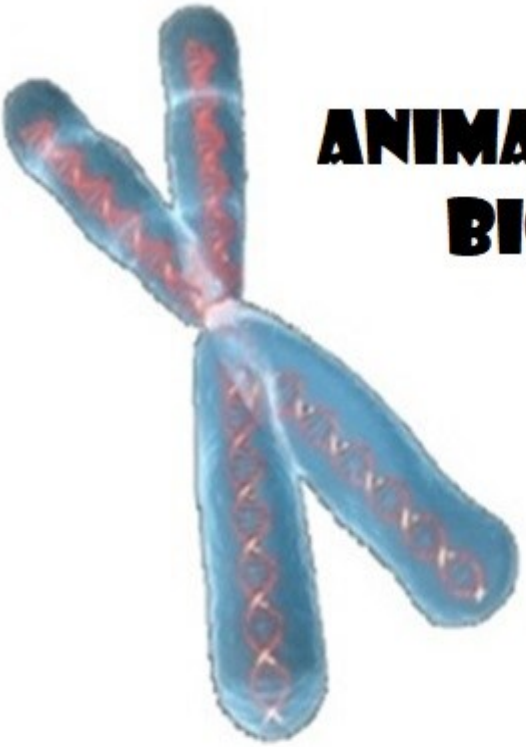
PRACTICAL



ANIMAL BIOTECHNOLOGY

Subject : MULTIPLEX PCR

Department: Animal Resources

Stage : 4

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Introduction of Multiplex PCR

Multiplex PCR is a widespread molecular biology technique for amplification of multiple targets in a single PCR experiment. In a multiplexing assay, more than one target sequence can be amplified by using multiple primer pairs in a reaction mixture. As an extension to the practical use of PCR, this technique has the potential to produce considerable savings in time and effort within the laboratory without compromising on the utility of the experiment.

Types of Multiplex PCR

Multiplexing reactions can be broadly divided in two categories:

1. Single Template PCR Reaction

This technique uses a single template which can be a genomic DNA along with several pairs of forward and reverse primers to amplify specific regions within a template.

2. Multiple Template PCR Reaction

It uses multiple templates and several primer sets in the same reaction tube. Presence of multiple primers may lead to cross hybridization with each other and the possibility of mis-priming with other templates.

Primer for Multiplex PCR

1. Primer Length

Multiplex PCR assays involve designing of large number of primers, hence it is required that the designed primer should be of appropriate length. Usually, primers of short length, in the range of 18-22 bases are used.

2. Melting Temperature

Primers with similar Tm, preferably between 55°C-60°C are used. For sequences with high GC content, primers with a higher Tm (preferably 75°C-80°C) are recommended. A Tm variation of between 3°-5° C is acceptable for primers used in a pool.

3. Specificity

It is important to consider the specificity of designed primers to the target sequences, while preparing a multiplex assay, especially since competition exists when multiple target sequences are in a single reaction vessel.

4. Avoid Primer Dimer Formation

The designed primers should be checked for formation of primer dimers, with all the primers present in the reaction mixture. Dimerization leads to unspecific amplification.

All other parameters are similar to standard PCR primer design guidelines.

Applications of Multiplex PCR

1. Pathogen Identification
2. High Throughput SNP Genotyping
3. Mutation Analysis

4. Gene Deletion Analysis
5. Template Quantitation
6. Linkage Analysis
7. RNA Detection
8. Forensic Studies