Practical works

**Modern methods in biotechnology**

Specialty «7M05201 - Environmental Biotechnology», 1 course.

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**Practical class 11.**

Content for consideration:

 Different types of PCR.

 Determination optimal concentration of sample DNA,

Optimization of concentration of primers,

Regime of PCR for amplification of PCR product.

Principe of correct choice of primers

1. polymerase chain reaction (PCR): ¬ It is a molecular technology aim to amplify a single or few copies of the DNA to thousands or millions of copies. ¬ Developed in 1983 by Kary Mullis, PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications. These include diagnosis of infectious diseases, DNA sequencing and DNA-based phylogeny. ¬ In 1993, Mullis was awarded the Nobel prize in Chemistry along with Michael Smith for his work on PCR.
2. Types of PCR ?? • Conventional (Qualitative)PCR. • Multiplex PCR. • Nested PCR. • RT-PCR and qRT-PCR. • Quantitative PCR. • Hot-start PCR. • Touchdown PCR. • Assembly PCR. • Colony PCR. • Methylation-specific PCR. • LAMP assay.
3. Multiplex-PCR: It is a special type of the PCR used for detection of multiple pathogens by using Multiple primers sets each one targets a particular pathogen. Uses: This permits the simultaneous analysis of multiple targets in a single sample.
4. Nested-PCR: ¬ Used to increase the specificity of DNA amplification. ¬ Two sets of primers are used in two successive reactions. ¬ In the first PCR, one pair of primers is used to generate DNA products, which will be the target for the second reaction.
5. ¬ Using one ('hemi-nesting') or two different primers whose binding sites are located (nested) within the first set, thus increasing specificity. Uses: Detection of pathogens that occur with very few amount.
6. RT-PCR (Reverse Transcription PCR, Real Time - PCR) ¬ Used to reverse-transcribe and amplify RNA to cDNA. ¬ PCR is preceded by a reaction using reverse transcriptase, an enzyme that converts RNA into cDNA. ¬ The two reactions may be combined in a tube. Uses: 1-Detection of RNA virus like (HCV). 2-Detection of other M.O. through targeting of their Ribosomal RNA.
7. Reverse Transcription PCR, Real Time - PCR
8. Reverse Transcription PCR, Real Time - PCR
9. Quantitative Real-Time PCR (qRT-PCR) •Method use fluorescent dyes, such as Sybr Green, or fluorescence-containing DNA probes, such as TaqMan, to measure the amount of amplified product as the amplification progresses.
10. Progress of DNA amplification during real time (RT-PCR) by measuring the release of fluorescent "flashes" during amplification. A computer measures the rate of "flashing" in 96 simultaneous experimental PCR reactions relative to a control reaction
11. [12.](https://image.slidesharecdn.com/typesofpcr-161203045658/95/types-of-pcr-12-638.jpg?cb=1480741044)Quantitative – PCR: ¬Used to measure the specific amount of target DNA (or RNA) in a sample. ¬By measuring amplification only within the phase of true exponential increase, the amount of measured product more accurately reflects the initial amount of target. ¬Special thermal cyclers are used that monitor the amount of product during the amplification.
12. [13.](https://image.slidesharecdn.com/typesofpcr-161203045658/95/types-of-pcr-13-638.jpg?cb=1480741044)Hot-start PCR: ¬ It is a technique performed manually by heating the reaction components to the DNA melting temperature (e.g. 95°C) before adding the polymerase.
13. [14.](https://image.slidesharecdn.com/typesofpcr-161203045658/95/types-of-pcr-14-638.jpg?cb=1480741044)Touchdown PCR: ¬In this type the annealing temperature is gradually decreased in later cycles. ¬The annealing temperature in the early cycles is usually 3-5°C above the standard Tm of the primers used, while in the later cycles it is a similar amount below the Tm. ¬ The initial higher annealing temperature leads to greater specificity for primer binding, while the lower temperatures permit more efficient amplification at the end of the reaction.
14. [15.](https://image.slidesharecdn.com/typesofpcr-161203045658/95/types-of-pcr-15-638.jpg?cb=1480741044)Assembly-PCR (also known as Polymerase Cycling Assembly or PCA) ¬ In this type synthesis of long DNA structures by performing PCR on a pool of long oligonucleotides with short overlapping segments, to assemble two or more pieces of DNA into one piece. ¬ It involves an initial PCR with primers that have an overlap and a second PCR using the products as the template that generates the final full-length product. ¬ This technique may substitute for Ligation-based assembly
15. [16.](https://image.slidesharecdn.com/typesofpcr-161203045658/95/types-of-pcr-16-638.jpg?cb=1480741044)Assembly PCR:
16. [17.](https://image.slidesharecdn.com/typesofpcr-161203045658/95/types-of-pcr-17-638.jpg?cb=1480741044)Assembly PCR:
17. [18.](https://image.slidesharecdn.com/typesofpcr-161203045658/95/types-of-pcr-18-638.jpg?cb=1480741044)Colony PCR ¬ Bacterial colonies are screened directly by PCR, for example, the screen for correct DNA- vector constructs. ¬ Colonies are sampled with a sterile pipette tip and a small quantity of cells transferred into a PCR mix.
18. [19.](https://image.slidesharecdn.com/typesofpcr-161203045658/95/types-of-pcr-19-638.jpg?cb=1480741044)Methylation-specific PCR (MSP) ¬ Used to identify patterns of DNA methylation at cytosine guanine islands (C&G islands) in genomic DNA. CpG islands, are concerned in regulation of gene expression in mammalian cells. ¬ Target DNA is first treated with sodium bisulfite, which converts unmethylated cytosine bases to uracil, which is complementary to adenosine in PCR primers. ¬ Two amplifications are then carried out on the bisulfite-treated DNA:
19. [20.](https://image.slidesharecdn.com/typesofpcr-161203045658/95/types-of-pcr-20-638.jpg?cb=1480741044)¬One primer set anneals to DNA with cytosine (corresponding to methylated cytosine), ¬The other set anneals to DNA with uracil (corresponding to unmethylated cytosine). ¬ MSP used in quantitative PCR provides quantitative information about the methylation state of a given CpG island. Methylation-specific PCR (MSP)
20. [21.](https://image.slidesharecdn.com/typesofpcr-161203045658/95/types-of-pcr-21-638.jpg?cb=1480741044)Methylation-specific PCR (MSP)
21. [22.](https://image.slidesharecdn.com/typesofpcr-161203045658/95/types-of-pcr-22-638.jpg?cb=1480741044)LAMP assay: (Loop-mediated isothermal amplification) ¬ It is a Modified type of the PCR using 3:6 primers sets one of them is loop like primer. ¬ This test use Bst- polymerase enzyme (Bacillus stearothermophilus DNA Polymerase). ¬ Using only two temperatures (63°C for 45 min. then 85°C for 5 min.), may be carry out in water path.