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Keywords: PCR; Sex determination; DNA polymerase

Introduction

Polymerase chain reaction is one type of the method used for the determination of the sex. Polymerase chain reaction method (PCR) was invented by Kary B. Mullis, they was also received the Nobel prize in chemistry for PCR method invention on 10 December, 1993 [1]. PCR Method also used in other detection like mutations detecting, monitoring cancer therapy, detecting bacterial, viral infection and also sex determination [1].

Sex determination is one of the important methods, which help to determine the sex of the developing embryo. Mostly x-y system is use for the sex determination in human and mammals. Males have 'x' chromosome (xy) and female had both (xx) chromosomes. So with the help of the chromosome sex is determined. It is not true for all the organisms, for example in bird and reptiles male have homogametic (ZZ) and female had heterogametic (ZW). Generally in male x and y

First Place the 16 sample comb in the MIDI Gel electrophoresis. A er that we poured gel solution (1.5% agarose) to a depth of 5 mm and allow setting for 15 min. en we add 5 µl of sample loading bu er to each 0.2 ml tube from the PCR reaction. When the gel has set, take the comb and add electrophoresis bu er (TAE) to cover the gel by approximately 0.5 cm. Add 20 µl of all sample to individual well and then run the gel at 100 V until the blue dye runs half way down the gel.

DNA quantification

DNA quanti cation helps to determine the quantity of ampli able DNA [3]. Quantities analysis is a procedure where the concentration of a particular bimolecule in a sample is determined [4]. With the help of a spectrophotometer the DNA and proteins are determine by absorbance of UV light. At 260 nm absorbance DNA determined and 280 nm absorbance protein contamination was estimated. e blank sample was used before measuring each sample absorbance.

Results

e rst lane in the gure above is marker sample.

Second lane is male

ird lane is female

Forth lane is sample 1

Fi h lane is sample 2

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Copyright: ©f DNA. In this method, we used PCR method for determination of the sex of

Six lane is unknown sample 3

Seven lane is negative control

In the second lane, it is for male control. Ideally two bands should be detected. One bands at the level of 218 bp and second band at 741 bp, First band for GAPDH gene and second band for SRY gene. In our experiment result two week bands are visible (Figure 1).

ird lane is for female. ere is only one band are visible at 218 bp, which is for GAPDH gene. In female there is no SRY gene. In our experiment we detected week band at 218 bp in second lane.

Forth lane is for unknown sample 1. is sample was female, there visible only one band at 218 bp and there is no SRY gene visible. In our experiment one band is visible at 218 and no other bands are visible.

Fivth lane is for unknown sample 2. is sample was male, there should two bands are visible, one band at 218 bp for housekeeping GAPDH gene and second band at 741 bp level for SRY gene. In our experiment one week band at 218 bp and no other band is visible.

Sixth lane is for unknown sample 3. is sample was male. ere should two bands are visible, one band at 218 bp for housekeeping GAPDH gene and second band at 741 bp level for SRY gene. In our experiment two bands are visible; rst band at 218 bp for GAPDH and second week bands at 741 bp for SRY gene.

Seventh lane is for negative control. ere should be no band are visible. In our experiment bright band is shown, which is some similar to the primer dimer.

1. e terms regarding denaturing, annealing and extension refer to in PCR.

   Denaturing means heating of the PCR products upto 94°C for 30 sec, because of these hydrogen bonds between complementary bases is broken and get single stranded DNA molecules. Annealing means lowering the temperature at 66°C for 1 min which are help in the binding of primers to single-stranded DNA templates. Extension means heating of mixture to 72°C for 1 min to which helps to DNA polymerase to synthesize a new DNA strand complementary to DNA template.

2. Explanation regarding the role of GAPDH primers in this experiment.

   GAPDH genes are housekeeping genes. GAPDH primers were provided to amplify these genes.

3. Explanation regarding the role of SRY primers in this experiment.

   SRY gene which is sex determining region on Y chromosome. For di erentiation of testis in human embryo, the presence of SRY gene and the protein encoded by it are necessary. If SRY gene is absent, it results in development of ovaries SRY primers were provided to amplify these genes (Table 1).

4. Calculation of GC content of each primer. Show your workings.

   GC content= G+C/ A+T+G+C × 100

   GAPDH F: 12/12+12 × 100 = 50

   GAPDH R: 12/12+12 × 100 = 50

   SRY F: 11/13+11 × 100 = 45.83

   SRY R: 11/13+11 × 100 = 45.83

5. Calculation of the volume of each reagent in the master mix that

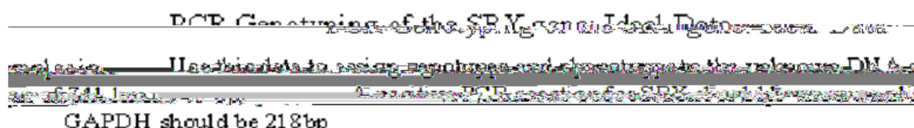

Figure 1: A positive PCR reaction for SRY should have an amplicon of 741bp, GAPDH should be 218bp.
The frst lane in the fgure above is marker sample.
Second lane is male

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