OBJECTIVE LEARNING:

(1- Gel Electrophoresis - 2- Preparation of primers solution- 3- PCR Reaction)

1- Agarose Gel Electrophoresis:

After genomic DNA extraction, agarose gel electrophoresis was adopted to confirm the presence and integrity of the extracted DNA. The methods were done according (Lee et al. 2012).

A- Reagents of Gel Electrophoresis:

- Agarose.
- 1X TBE Buffer.
- Bromophenol Blue (loading dye).
- Ethidium Bromide (2 µl of 10mg/ml).

B- Protocol of Gel Electrophoresis:

- **Preparation of agarose gel:**

1- The amount of 1 X TBE (100 ml) was taken in a beaker.

2- Agarose powder (1.2gm) was added to the buffer.

3- The solution was heated to a boiling point until all gel particles were dissolved by microwave oven.

4- Ethidium Bromide (2µl of 10mg/ml) was added to the agarose solution.

5- The agarose was stirred in order to be mixed and to avoid bubbles.

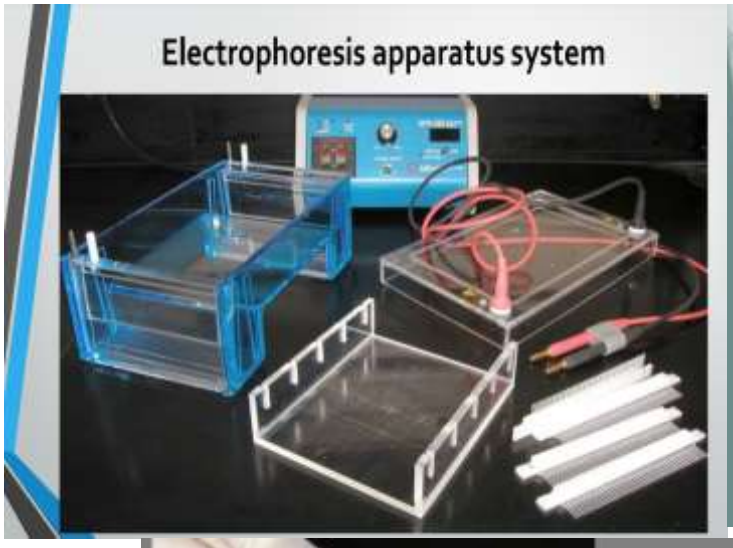
6- The solution was left to cool down at 50 – 60^{o}C.

- **Casting of the horizontal agarose gel:**

After sealing both edges of the gel tray with a cellophane tape and after fixing the comb in 1 cm away from one edge, the agarose solution was poured into the gel tray. The agarose was allowed to solidify at room temperature for 30 minutes. The fixed comb was carefully removed and the gel tray was placed in the gel tank. The tank was filled with 1 X TBE buffer until it reached 1-2 mm over the surface of the gel.

- **DNA Loading and Electrophoresis:**

DNA (5 µl) was mixed with (2 µl) loading dye. Samples have been uploaded carefully into individual wells of the gel, electrical energy was then turned on at 7 V/cm for 40 min. The DNA was then transferred from the negative electrode (-) to the positive electrode (+). Ethidium bromide colored bands in the gel were visualized using UV light (365 nm) and then photographed.



2- Preparation of primers solution:

The lyophilized primers were dissolved in deionized distilled water (DDH2O) in the master tube to achieve 100 pmol/µl, and then 10 pmol/ µl was created as a working solution by transferring 10µl from the master tube to another tube and completing the volume to 100µl by adding DDH2O. Then saved at -20 ^{o}C.

3- PCR Reaction:

The targeted SNPs will be involved in this region. Twenty-five microliters of PCR amplification reaction contains 12.5 µl of OneTaq (NEB®) mastermix, 3 µl of DNA sample, 1.5 µl 10 pmol/µl of each primer and 6.5 µl of free nuclease water. The reaction was carried out under optimal PCR conditions for this gene as described in table.

PCR conditions for amplification of *Gene*s.

Step	Temperature (˚C)	Time	No.of cycle
Initial denaturation	95	5 min	**1**
Denaturation	95	30 sec	**35**
Annealing	48	48sec	
extension	72	50sec	
Final extension	72	7min	**1**
Hold temperature	4	**-**	**-**