EXPERIMENT 4:

DETERMINATION OF GSTP1 (Ala114Val) SINGLE NUCLEOTIDE POLYMORPHISM

A) Materials, devices and solutions used in the assay

For PCR

1) PCR main mixture: PCR buffer, $MgCl_2$, nucleotide mixture, *Taq* polymerase enzyme.

2) Primers: They are synthesized specifically for the amplified gene region.

3) DNA solution

4) Thermal cycler

5) Micropipette

6) Centrifuge

7) PCR tubes

For Gel

1) Electrophoresis tank

2) Agarose gel

3) Electrophoresis buffer (TBE)

4) DNA marker

5) Electrophoresis power supply

6) Gel imaging system

B) Procedure

Polymerase chain reaction: After adding primers and DNA solution into the PCR main mixture, PCR cycle programme have to be programmed in the thermal cycler as: 2min. at 95^{o}C for first denaturation, 40 cycles of 30 sec. at 94 ^{o}C (melting), 30 sec. at 59 ^{o}C (annealing) and 30 sec. at 72 ^{o}C (synthesis). During the running the gel is need to be prepared. After the run, the PCR products have to be applied to the gel.

1. Preparation of the gel: Prepare agarose at 1.5 % concentration in 40 ml of buffer. Add the weighed amount of the agarose into the 40 ml TBE buffer and then mix. Agarose must be in gel form so heat it. After heating, wait it to be cooled and lucid. Put the gel into the template and the put to comb in order to apply the PCR products easily. After the gel become frozen, fill the tank with the buffer solution.

2. Electrophoresis: After the application of PCR products into the gel, close the lid of the tank and connect to the power supply. Apply 100V electricity for 65 min. for electrophoresis. After the run, bring out the gel delicately.

3. To make the PCR products visible by nucleic acid gel dye: Incubate the gel with a help of low-speed shaker in a plate with diluted SYBR Green nucleic acid gel dye (Dilution ratio: 1/10000) for 30 minutes so that the lines of PCR products will be visible under UV light.

4. Gel imaging: Following the incubation, place the gel on the UV tray and take the photograph of the lines of the PCR products. Then compare these lines with the DNA marker in order to confirm whether the studied gene region is amplified or not.

5. After amplification of the gene region, it is processed with the DNA restriction enzyme specific to mutation region.