EXPERIMENT 5:

ANALYSIS OF GSTP1 (Ala114Val) RESTRICTION PRODUCT

A) General Information:

The major portion of the genetic polymorphisms is identified by **Restriction Fragment Length Polymorphism (RFLP)** method. In this method, PCR products are restricted by a restriction enzyme. The restriction enzymes recognize about 4 or 6 base pairs of a specific DNA alignment and restrict this part. The region is called as **restriction region**. After restriction, according to the alignment of the interested DNA region, the restricted or not-restricted DNA products will come out.

B) Materials, devices and solutions used in the assay

For restriction

1) PCR product

2) Restriction enzyme

3) Reaction buffer

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

C) Procedure

Prepare the reaction medium containing reaction buffer, PCR product and *Alw26I* restriction enzyme in a PCR tube and put it in the thermal cycler. Arrange the heat to 37^{o}C. After 5 minutes, apply the restriction products into the agarose 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 determine which samples belong by to which genotype.