

## CHAPTER III

### MATERIALS AND EQUIPMENTS

#### 1. Specimens

The specimens used in this study were consisted of both fresh tumour tissues and peripheral blood leukocytes obtained from twenty three previously histologically confirmed nasopharyngeal carcinoma patients who had been diagnosed at Chulalongkorn hospital during 1994-1995.

#### 2. Material

- 2.1 Pipette tip : 10  $\mu$ l, 100  $\mu$ l, 1,000  $\mu$ l (Elkay, USA)
- 2.2 Microcentrifuge tube : 0.2 ml, 0.5 ml, 1.5 ml (Bio-rad, Elkay, USA)
- 2.3 Polypropylene conical centrifuge tube : 15 ml, 50 ml  
(Elkay, USA)
- 2.4 Beaker : 50 ml, 100 ml, 200 ml, 500 ml, 1,000 ml (Pyrex)
- 2.5 Flask 250 ml, 500 ml, 1,000 ml (Pyrex)
- 2.6 Reagent bottle : 100 ml, 250 ml, 500 ml, 1,000 ml (Duran, USA)
- 2.7 Cylinder : 25 ml, 50 ml, 100 ml, 250 ml, 500 ml, 1,000 ml  
(Witeg, Germany)
- 2.8 Glass pipette : 5 ml, 10 ml (Witeg, Germany)
- 2.9 Pipett tip rack (Autopack, USA)
- 2.10 Microcentrifuge Tube rack (USA/Scientific plastics)
- 2.11 Thermometer (Precision, Germany)
- 2.12 Parafilm

- 2.13 Polaroid film (Polaroid,UK)
- 2.14 X-ray film (Kodak,USA)
- 2.15 3MM Chromatography paper (Whatman,UK)
- 2.16 Plastic wrap
- 2.17 Syringe 20 ml and needle (Terumo,Japan)
- 2.18 Sterring-magnetic bar

### 3. Equipment

- 3.1 Automatic adjustable micropipette : P2 (0.1-2  $\mu$ l), P10 (0.5-10  $\mu$ l), P20 (5-20  $\mu$ l), P100 (20-100  $\mu$ l), P1,000(0.1-1 ml) (Gilson,France)
- 3.2 Pipette boy (Tecnomara,Switzerland)
- 3.3 X-ray film cassette (Kodak,USA)
- 3.4 Vortex (Scientific Industry,USA)
- 3.5 pH meter (Eutech Cybernetics)
- 3.6 Stirring hot plate (Barnstead/Thermolyne,USA)
- 3.7 Balance (Precisa,Switzerland)
- 3.8 Microcentrifuge (Fotodyne,USA)
- 3.9 DNA Thermal cycle 480 (Perkin Elmer,Cetus USA0)
- 3.10 Thermal cyclcr (Omnigene,Hybaid USA)
- 3.11 Horizontal electrophoresis apparatus (Gibco BRL,USA)
- 3.12 Sequencing gel electrophoresis apparatus (Bio-rad,USA)
- 3.14 Power supply model 250 (Gibco BRL,USA)
- 3.15 Power supply model pac 3000 for Sequencing gel electrophoresis (Bio-rad,USA)

- 3.16 Heat block (Boekel, Germany )
- 3.17 Incubator (Mettler)
- 3.18 Thermostat shaking-water bath (Heto,Denmark)
- 3.19 Liquid nitrogen tank
- 3.20 Spectronic spectrophotometers(Genesys 5,Milton Roy USA)
- 3.21 UV-Transilluminator (Foto/prep,Fotodyne USA)
- 3.22 Polaroid camera
- 3.23 UV-absorbing face shield
- 3.24 Radiation safety shielding-screen
- 3.25 Beta microcentrifuge tube racks
- 3.26 Beta waste safes
- 3.27 Refrigerator 4°C (Misubishi,Japan)
- 3.28 Deep freeze -20°C , -80°C (Revco)
- 3.29 Water purification equipment (Water Pro Ps,labconco USA)

#### 4. Reagent

##### 4.1 General reagent

- 4.1.1 Absolute ethanol (Merck)
- 4.1.2 Absolute methanol (Merck)
- 4.1.3 Acetic acid (Merck)
- 4.1.4 Acrylamide-bis solution (Bio-rad)
- 4.1.6 Agarose,molecular grade (Promaga)
- 4.1.7 Ammonium persulfate (Merck)
- 4.1.8 Ammonium acetate (Merck)
- 4.1.9 Boric acid (Merck)

- 4.1.10 Bromophenol blue (Pharmacia)
- 4.1.11 Developer solution for X-ray film (Kodak)
- 4.1.12 Disodium ethylenediamine tetracetic acid:EDTA(Merck)
- 4.1.13 Ethidium bromide (Gibco BRL)
- 4.1.14 Fixer solution for X-ray film (Kodak)
- 4.1.15 Formamide (Pharmacia)
- 4.1.16 Hydrochloric acid (Merck)
- 4.1.17 Mineral oil (Sigma)
- 4.1.18 N,N,N',N'-Tetra-methylethylenediamine : TEMED  
(Promega)
- 4.1.19 Phenol-Chloroform-isoamyl alcohol (Sigma)
- 4.1.20 Potassium chloride (Merck)
- 4.1.21 Sodium acetate (Merck)
- 4.1.22 Sodium chloride (Merck)
- 4.1.23 Sodium dodecyl sulfate (Sigma)
- 4.1.24 Sodium hydroxide (Merck)
- 4.1.25 Sigmacote (Sigma)
- 4.1.26 Tris hydroxymethyl aminomethane (Amresco)
- 4.1.27 Urea (Promega)
- 4.1.28 Xylene cyanol FF (Amresco)

## 4.2 Reagent kits

- 4.2.1 Deoxynucleotide triphosphate kit (Pharmacia)
- 4.2.2 PCR kit (Perkin Elmer Cetus)
- 4.2.3 Polynucleotide kinase kit (Biolabs, New England)

#### 4.2.4 STR primers (Map pairs, Research genetics USA)

(List of primers are shown in Appendix C)

### 4.3 Enzymes

4.3.1 Proteinase K (Amresco)

4.3.2 *Taq* polymerase (Perkin Elmer)

4.3.3 T4 polynucleotide kinase (Biolabs, New England)

## 5. Radioactive redivue

$\gamma$  - <sup>32</sup> P dATP >3,000 Ci/mmol, 10 mCi/ml (Amersham)