
Appendix 1

1. Details of the genotyping technique:

PCR was prepared in 50 μ l reaction volume named Master Mix.

1.1 Set reaction master mix for genotyping XRCC1 codon399.

Needed volume for Master mix	
1.5mM MgCl ₂	3 μ l
200 μ M dNTPs	1 μ l
0.8 μ M XRCC1 primer forward	1.6 μ l
0.8 μ M XRCC1 primer reverses	1.6 μ l
GoTaq DNA Polymerase5U	0.5 μ l
5xGreen GoTaq Flexi Buffer	1 μ l
Genomic DNA	0.1 μ l < DNA
Volume of dH ₂ O	33.4 > dH ₂ O
Total	50 μ l

50-100ng of genomic DNA was added. Adding the DNA is according to concentration of DNA. dH₂O was added to master mix by gently tapping the tubes and quick spin to collect the reaction at the bottom of the tube, and PCR reaction mix were placed in a GeneAmp PCR system 2727 (Perkin-Elmer, Applied Biosystems) programmed for the temperatures as in below table.

1.2. Conditions for the reaction mixtures following incubation in PCR.

Program	Temperature	Duration	Cycle
Hot start(pre heated)	95°C	5 min	1
Denaturation	95 °C	30 sec	40
Annealing	55 °C	1min	40
Elongation	72 °C	1min	40
Final Elongation	72 °C	5 min	1
Hold/Soak	4 °C		

At the end, PCR products including the XRCC1 gene was separated by Agarose gel (2%) and visualized with ethidium bromide to determine the presence or absence of genotype. If the gene is present, there is a band in 615 bp.

5x Buffer, MgCl₂, dNTP and Taq Polymerase were purchased from Promega, USA.

The XRCC1, codon 399 primers were purchased from Helix Biotech (M) SDN BHD. Before going to next stage the DNA was purified. DNA purification was done by using QIAamp DNA purification Kit (QIAGEN, USA). Digestion was done after purification.

Appendix 2

The detailed method of digesting the PCR Product with restriction enzyme and the preparation of:

1. RE Mix:

Needed volume	
10x Buffer	5 μ l
Msp1	0.5 μ l

The RE Mix was added to the PCR product and dH₂O.

2. Restriction enzyme reaction master mix.

Needed volume	
RE Mix	5.5 μ l
dH ₂ O	19.5 μ l
PCR product	24 μ l
Total	50 μ l

If the initial volume of Msp1 was 0.1 μ l which is very low, the volume will be increased to 0.5 μ l. After purification, digestion was done with 20 μ l PCR product and RE Mix (5.5 μ l) at 37°C for 16 hours for incubation.

3. Conditions for restriction enzyme reaction in PCR (PCR-RFLP).

A PCR product was used as positive control in electrophoresis. Msp1 Enzyme was purchased from New England Biolabs, Inc.

37°C	37°C	37°C	37°C	4°C
30 min	30 min	30 min	30 min	()

Appendix 3

1. Solutions and reagents used for Agarose gel electrophoresis

Agarose gel (2%)

1X TBE buffer	100 ml
Agarose powder	2 g
Ethidium bromide (10 mg/ml)	10 μ l

TBE buffer (10x)

All of the ingredients of TBE buffer (10x) were weighed and suspended in 500 ml of dH₂O by stirring with magnetic stirrer on the hot plate. pH of the buffer was measured and adjusted to pH 8.0 by adding additional NaOH or HCL. Then ddH₂O was added to top up 1000 ml and sterilized by autoclave at 121°C for 15 min.

Tris base	121.2 g
Orthoboric acid	61.8 g
EDTA	0.745 g
dH ₂ O	1000 ml

TBE buffer (1x)

All the ingredients were measured and mixed together thoroughly.

10x TBE buffer	100 ml
ddH ₂ O	900 ml

Ethidium bromide (10 mg/ml)

Ethidium bromide	100 mg
Deionised water	10 ml

The solution was then stored at 26°C in a dark container and diluted to 1.0 μ g/ml with distilled water before use.

