Appendix 1

1.Details of the genotyping technique:

PCR was prepared in 50µl reaction volume named Master Mix.

1.1 Set reaction master mix for genotyping XRCC1 codon399.

	Needed volume for Master mix
1.5mM $MgCl_2$	3µl
200µM dNTPs	1µl
0.8µMXRCC1 primer forward	1.6µl
0.8µMXRCC1 primer reverses	1.6µl
GoTaq DNA Polymerase5U	0.5µl
5xGreen GoTaq Flexi Buffer	1µl
Genomic DNA	0.1µl < DNA
Volume of dH_2O	33.4> dH_2O
Total	50µl

50-100ng of genomic DNA was added. Adding the DNA is according to concentration of DNA. dH_2O 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)	95ºC	5 min	1
Denaturation	95 ºC	30 sec	40
Annealing	55 ºC	1min	40
Elongation	72 ºC	1min	40
Final Elongation	72 ºC	5 min	1
Hold/Soak	4 ºC		

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, $MgCl_2$, 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 µl
Msp1	0.5µl

The RE Mix was added to the PCR product and dH_2O.

2. Restriction enzyme reaction master mix.

	Needed volume
RE Mix	5.5µl
dH_2O	19.5µl
PCR product	24µl
Total	50µl

If the initial volume of Msp1 was 0.1µl which is very low, the volume will be increased to 0.5µl.After purification, digestion was done with 20µl PCR product and RE Mix (5.5µl) at 37ºC 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.

37ºC	37ºC	37ºC	37ºC	4ºC
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)	10µl

TBE buffer (10x)

All of the ingredients of TBE buffer (10x) were weighed and suspended in 500 ml of dH_2O 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 ddH_2O was added to top up 1000 ml and sterilized by autoclave at 121ºC for 15 min.

Tris base	121.2 g
Orthoboric acid	61.8 g
EDTA	0.745 g
dH2O	1000 ml

TBE buffer (1x)

All the ingredients were measured and mixed together thoroughly.

10x TBE buffer	100 ml
ddH_2O	900 ml

Ethidium bromide (10 mg/ml)

Ethidium bromide	100 mg
Deionised water	10 ml

The solution was then stored at 26ºC in a dark container and diluted to 1.0 µg/ml with distilled water before use.