Appendix XV: Deoxyribonucleic Acid (DNA) Identification

DNA analysis is one of the most reliable molecular methods for CMM authentication. The genetic composition of each individual is unique; therefore molecular markers have the advantages in CMM authentication as they are less affected by age, physiological conditions, environmental factors, harvest, storage and processing. Using a Polymerase Chain Reaction (PCR) technique, nano-gram quantities of DNA can be amplified and yield sufficient amount of template DNA for molecular genetic analysis. After amplification of the region(s) of interest in the genome, subsequent gel electrophoresis is performed to size the PCR products. To date, a variety of PCR-based methods have been developed for the use in CMM authentication, including Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP), Random Amplification of Polymorphic DNA (RAPD) etc.

(1) Apparatus and materials –

a) **Thermal cycler** – Also known as PCR machine, is used to amplify segments of DNA via PCR.

b) **DNA gel tank** – A tank with electrodes (anode and cathode) that can hold an agarose gel used for gel electrophoresis.

c) **Power supply** – A high voltage power supply connects to the DNA gel tank setting up an electric field between the two electrodes.

d) **Ultraviolet (UV) transilluminator** – A transilluminator apparatus that use UV radiation to visualize DNA in a gel electrophoresis procedure. It may also contain image capture devices, such as a digital camera, that allow an image of the gel to be captured.

e) **Primers -** A strand of nucleic acid that serves as a starting point for DNA synthesis with the help of the DNA polymerase enzyme. The polymerase starts replication at the 3'- end of the primer, and copies the opposite strand.

f) **PCR reaction mix** – A mixture including $MgCl_2$ reaction buffer, deoxyribonucleotides (dATP, dTTP, dCTP and dGTP) and DNA polymerase.

g) **Restriction enzyme** – An enzyme that cuts DNA at specific recognition nucleotide sequences known as restriction sites.

(2) **PCR system** - Set up the PCR system according to the manufacturer's manual. The recommended PCR conditions are as follows (Table 1) –

Table 1 Recommended PCR conditions

PCR system	GeneAmp®PCR System 9700 (AB Applied Biosystems) or equivalent		
DNA Template	10 - 50 ng		
Initial denaturation	95°C, 4 min		
Number of cycles	30		
PCR profile of each cycle	**Temperature (°C)**	**Time (min)**	**Remarks**
	95	0.5	Denaturation
	58	0.5	Annealing
	72	0.5	Extension
	72	5	Final extension

(3) **Restriction analysis** - Perform the restriction analysis of PCR products with the recommended conditions as follows (Table 2) –

Table 2 Recommended restriction analysis conditions

Restriction enzyme	*Sma I* (10 U/µL)	
Incubation temperature	37°C	
Incubation time	1.5 h	
Reaction mixture (Total volume – 20 µL)	**Reaction components**	**Amount required**
	PCR product	6 µL
	Sma I	0.5 µL (10 U/µL)
	10 x digestion buffer	2 µL
	PCR grade water (ultra-pure)	11.5 µL

(4) **Electrophoresis and detection system** - Set up the electrophoresis and detection system according to the manufacturer's manual. The recommended electrophoresis and detection conditions are as follows (Table 3) –

Table 3 Recommended electrophoresis and detection conditions

Separation unit	1.2% agarose gel with 0.001% SYBR Safe DNA gel stain
Loading volume	10 μL
DNA size marker	1 kb DNA ladder (10 μL)
Detector	UV transilluminator
Running voltage	80V
Running time	30 min