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Appendix

Appendix A

Glossary

Allele one of two or more alternative forms of a gene

Allele frequency the proportion of a particular allele among the chromosomes carried by individuals in a population

Amplicons PCR products

Autoradiograph a photographic recording of the positions on a film where radioactive decay of isotopes has occurred

Band the visual image representing a particular DNA fragment on autoradiograph

Basepair two complementary nucleotides held together by hydrogen bonds; basepairing occurs between A and T and between G and C

Controls tests performed in parallel with experimental samples and designed to demonstrate that a procedure worked correctly

Degradation the breaking down of DNA by chemical or physical means

Denaturing the process of unfolding of the complementary double strands of DNA to form single strands

Diallelic DNA variation showing only two forms with a frequency of more than 1 %

DNA database a collection of DNA typing profiles of selected or randomly chosen individuals

DNA polymerase an enzyme that catalyzes the synthesis of double-stranded DNA

DNA probe a short segment of single-stranded DNA labeled with a radioactive or chemical tag that is used to detect the presence of a

particular DNA sequence through hybridization to its complementary sequence

Electrophoresis a technique in which different molecules are separated by their rate of movement in an electric field

Ethidium bromide an organic molecule that binds to DNA and fluoresces under ultraviolet light and is used to identify DNA

Gel semisolid matrix (usually agarose or acrylamide) used in electrophoresis to separate molecules

Genome the total genetic makeup of an organism

Genotype the genetic makeup of an organism, as distinguished from its physical appearance or phenotype

Hardy-Weinberg equilibrium the condition, for a particular genetic locus and a particular population, with the following properties: allele frequencies at the locus are constant in the population over time and there is no statistical correlation between the two alleles possessed by individuals in the population; such a condition is approached in large randomly mating populations in the absence of selection, migration, and mutation

Heterozygote a diploid organism that carries different alleles at one or more genetic loci on its homologous chromosomes

Heterozygous having different alleles at a particular locus; for most forensic DNA probes, the autoradiogram displays two bands if the person is heterozygous at the locus

Homozygote a diploid organism that carries identical alleles at one or more genetic loci on its homologous chromosomes

Homozygous having the same allele at a particular locus; for most forensic DNA probes, the autoradiogram displays single bands if the person is homozygous at the locus

Human leukocyte antigen (HLA) protein-sugar structures on the surface of most cells, except blood cells, that differ among individuals and are important for acceptance or rejection of tissue grafts or organ transplantation; the locus of one particular class, HLA DQ∝, is used for forensic analysis with PCR

Hybridization the reassociation of complementary strands of nucleic acids, nucleotides, or probes

Locus (pl. loci) the specific physical location of a gene on a chromosome

Marker a gene with a known location on a chromosome and a clear-cut phenotype that is used as a point of reference in the mapping of other loci

Molecular weight size marker DNA fragments of known size, from which the size of an unknown DNA sample can be determined

Multilocus probe a DNA probe that detects genetic variation at multiple sites; an autoradiogram of a multilocus probe yields a complex, stripe-like pattern of 30 or more bands per individual

Phenotype the physical appearance of functional expression of a trait

Polymerase chain reaction (PCR) an in vitro process that yields millions of copies of desired DNA through repeated cycling of a reaction that involves the enzyme DNA polymerase

Polymorphism the presence of more than one allele of a gene in a population at a frequency greater than of a newly arising mutation; operationally, a population in which the most common allele at a locus has a frequency of less than 99%

Probe a short segment of single-stranded DNA tagged with a reporter molecule, such as radioactive phosphorus atom, that is used to detect a particular complementary DNA sequence

Restriction fragment length polymorphism (RFLP) variation in the length of DNA fragments produced by a restriction endonuclease that cuts at a polymorphic locus

Southern blot the nylon membrane to which DNA adheres after the process of Southern blotting

Southern blotting the technique for transferring DNA fragments that have been separated by electrophoresis from the gel to a nylon membrane

Tandem repeats multiple copies of an identical DNA sequence arranges in direct succession in a particular region of a chromosome

Taq polymerase a DNA polymerase used to form double-stranded DNA from nucleotides and a single-stranded DNA template in the PCR technique

Variable number of tandem repeats (VNTR) repeating units of a DNA sequence for which the number varies between individuals

Appendix B

Buffers and Reagents

1. 1 M Tris-Cl (pH 7.0)

Tris base [tris(hydroxymethyl)aminomethane]	121.14	g
dd.H_2O	800	ml

Adjust to desired pH with concentrated HCl

Add H_2O to 1 liter and sterilize by autoclaving.

2. 0.5 M Na_2EDTA (pH 8.0)

Disodium ethylenediamine tetraacetate.2 H_2O	186.12	g
dd. H_2O	700	ml

Adjust pH to 8.0 with concentrated NaOH

Add H_2O to 1 liter and sterilize by autoclaving.

3. $T_{10}E_{10}$ (pH 7.0)

1 M Tris-Cl	10	ml	(10 mM final)
0.5 M Na_2EDTA	20	ml	(10 mM final)
dd. H_2O	700	ml	

Adjust pH to 7.0 with concentrated HCl

Add H_2O to 1 liter and sterilize by autoclaving.

4. $T_{20}E_5$ (pH 8.2)

1 M Tris-Cl	10	ml	(20 mM final)
0.5 M Na_2EDTA	5	ml	(5 mM final)
dd. H_2O	400	ml	

Adjust pH to 8.2

Add H_2O to 500 ml and sterilize by autoclaving.

5. 10% SDS (w/v) (pH7.2)

Sodium dodecyl sulfate	10	g
dd. H_2O	80	ml

Adjust pH to 7.2 with concentrated HCl

Add H_2O to 100 ml.

6. 20 mg/ml Proteinase K (stock solution)

Dissolve Proteinase K	20	mg
Sterile dd. H_2O	1	ml

Store at -20 °C

7. Saturated NaCl (6 M)

NaCl	350.7	g

Add H_2O to 1000 ml and sterilize by autoclaving.

8. STR 10X buffer

KCl	500	mM
Tris-HCl, pH 9.0 at 25 °C	100	mM
$MgCl_2$	15	mM
Triton® X-100	1%	
Each dNTP	2	mM

9. 10X TBE buffer (pH 8.3)

Tris base	121.14	g	(1 M final)
$Na_2EDTA.\ 2H_2O$	3.7	g	(10 mM final)

d.H_2O	800	ml	
Slowly add the Boric acid, anhydrous	55.6	g	(0.9 M final)

Adjust pH to 8.3

Add d.H_2O to 1 liter.

10. 2% (w/v) Agarose gel

Agarose	1	g
1X TBE	50	ml

Dissolve by heating and occasionai mixing until no granules of agarose are visible.

Add Ethidium bromide 25 μg (0.5 μg/ml).

11. 10 mg/ml Ethidium bromide

Ethidium bromide	0.2	g
H_2O	20	ml

Store at 4 ^{o}C in dark or foil-wrapped bottle.

12. STR 6X Loading buffer

Bromophenol blue	0.25%
Xylene cyanol FF	0.25%
Ficoll (type 400) in distilled water	15%

Storage at room temperature.

13. 4% Denaturing polyacrylamide gel (total volume 60 ml)

Urea (Uitrapure)	25.2	g	(7 M final)
deionized H_2O	32	ml	
10X TBE	3	ml	(0.5X final)

40% Acrylamide:Bis (19:1)	6	ml	(4% final)
TEMED	40	µl	
10% $(NH_4)S_2O_8$	400	µl	

14. 10% $(NH_4)S_2O_8$

Ammonium persulfate	0.5	g
Add deionized H_2O to	5	ml

Store at 4 °C.

15. STR 2X Loading solution

NaOH	10	mM
Formamide	95%	
Bromophenol blue	0.05%	
Xylene cyanol FF	0.05%	

16. 0.5% Acetic acid in 95% ethanol

Add 0.5 ml of glacial acetic acid to 99.5 ml of 95% ethanol.

17. Fix/Stop solution (10% acetic acid)

Glacial acetic acid	100	ml
deionized H_2O	900	ml

18. Staining solution

Silver nitrate ($AgNO_3$)	0.5	g
37% Formaldehyde (H_2CO)	750	µl
deionized H_2O	500	ml

19. Developer solution

Sodium carbonate (Na_2CO_3)	30	g
37% Formaldehyde (H_2CO)	1.5	ml
10 mg/ml Sodium thiosulfate ($Na_2S_2O_3.5H_2O$)	200	µl
deionized H_2O	1000	ml

** Prepare fresh and chill to 10 ^{o}C before use.

Use only high quality deionized H_2O and sodium carbonate. Prepare fresh before each use.

Biography

Miss Unchalee Kongsrisook was born on September 4, 1971 in Nokornrachasrima, Thailand. She received the Degree of Bachelor of Nursing Science in 1993 from the Faculty of Nursing, Mahidol University, Bangkok, Thailand. She has enrolled at Chulalongkorn University in the graduate programme for the Degree of Master of science in Medical Science in 1995.