COMMONWEALTH OF VIRGINIA

DEPARTMENT OF CRIMINAL JUSTICE SERVICES

DIVISION OF FORENSIC SCIENCE

FLUORESCENT DETECTION PCR-BASED STR

DNA PROTOCOL:

POWERPLEX® 1.1, 2.1 AND 16 BIO SYSTEMS

FORENSIC BIOLOGY SECTION PROCEDURE MANUAL SECTION III

ISSUE 2

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1.1.7 The QIAamp® extraction procedure uses spin columns to extract/purify DNA from both liquid blood samples and dried blood stains. The QIAGEN® AL lysis buffer, included in the QIAamp® extraction kit, is a guanidine-based buffer. The guanidine helps to set up the binding conditions needed for the DNA to adhere to the spin column membrane. The QIAGEN® AL lysis buffer also contains a detergent to rupture leukocyte nuclear membranes which exposes the nucleic acids.

1.1.8 The QIAGEN® protease is similar to, but less stringent than, the above mentioned ProK, and serves the same purpose for the breakdown of proteins into their constituent amino acids. The DNA yield reaches a maximum after lysis for 10 min at 56^0 C. Longer incubation times have no effect on the overall yield of the purified DNA.

1.1.9 The QIAGEN® AW1 wash buffer is an ethanol-based stringent wash solution containing a low concentration of guanidine. This wash step removes any non-specific binding to the spin column membrane.

1.1.10 The QIAGEN® AW2 wash buffer is a Tris-based solution containing ethanol which will wash away any salts that are present.

1.1.11 The QIAGEN® AE elution buffer is a Tris-EDTA solution which elutes the DNA attached to the membrane and serves as a stable storage medium.

1.1.12 A random sample will be run with each extraction to serve as an extraction, amplification, and a typing gel migration control. The random sample is a previously analyzed convicted offender sample for which the identity of the sample is known to the Forensic Biology Program Manger and Laboratory Directors. This sample serves as an internal laboratory control since the DNA profile is not known to the examiner/analyst and must be verified by the Forensic Biology Program Manger or the Laboratory Director prior to the sizing data being considered acceptable. If a sample must be re-extracted a new random sample must be extracted along with the sample.

1.1.13 Routinely the organic or DNA IQ™ extraction methods are used to isolate DNA from blood/buccal samples obtained in criminal cases and the QIAGEN® BioRobot™ 9604 is used to isolate DNA from convicted offenders blood/buccal or arrestee buccal samples in accordance with the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section QIAGEN® BioRobot™ 9604 Procedure Manual (March 3, 2003). However, when necessary convicted offender blood/buccal and arrestee buccal samples may also be extracted using the organic extraction procedure outlined in section 1.5, the Organic Extraction Method For Blood Stains and Tissue Samples, the DNA IQ™ extraction method for buccal cells and bloodstains outlined in section 1.6, the spin column extraction procedure outlined in section 1.12, QIAamp® Extraction Procedure, or section 1.13, the QIAamp® 96 Well Extraction Procedure.

1.2 EQUIPMENT

1.2.1. Heat block or incubator, 37^0 C

1.2.2 Heat block or incubator, 56^0 C

9.2.14.1 Only the allele(s) for the locus in question will be sized.

9.2.14.2 The allele designations generated from this typing gel will be the official allele designation.

NOTE: If the typing gel will not be run for a sufficiently longer period of time to resolve the possible coalesced band(s), the locus must be reported as inconclusive.

9.2.15 To indicate the gender of the contributor of a particular biological sample the Amelogenin locus may be used. A biological sample exhibiting a single band at approximately 106 bp (X allele) will generally be considered to have originated from a female individual. A biological sample exhibiting a band at approximately 106 bp (X allele) and a band at approximately 112 bp (Y allele) will generally be considered to have originated from a male individual. Refer to Technical Notes 9.1.7 and 9.1.9 for additional information about the Amelogenin locus.

9.2.16 For a typing result to be reported the controls must work appropriately.

9.2.16.1 Reagent Blanks

9.2.16.1.1 If a weak signal is detected in a reagent blank at a single locus, the test results associated with the reagent blank will be considered inconclusive at that locus. If it is imperative that the locus be used, the samples will be re-extracted and/or re-amplified.

9.2.16.1.2 If a weak signal is detected in a reagent blank at multiple loci, the test results for all loci will be considered inconclusive and all samples associated with the reagent blank, if possible, will be re-extracted and/or re-amplified.

9.2.16.1.3 If a strong signal is detected in a reagent blank at a single locus, the test results for all loci will be considered inconclusive and all samples associated with the reagent blank, if possible, will be re-extracted and/or re-amplified.

9.2.16.2 The Random Sample profile when searched in CODIS must elicit the correct result. If the DNA sample number identified as a result of the search is different from the DNA sample number on the master list (maintained by the Forensic Biology Section Program Manager and/or the Laboratory Director), all samples associated with the Random Sample will be re-extracted **and/or** re-amplified if possible.

9.2.16.3 The Control DNA (K562 or GM9947A Cell Line) must elicit the "Known" genotypes for all loci as specified in Technical Note 9.1.9. If a DNA profile is detected in the Control DNA that is not consistent with the known genotype(s), the test will be considered inconclusive at that locus. If it is imperative that the locus be used, the samples will be re-amplified.

9.2.16.4 Negative Amplification Control

9.2.16.4.1 If a weak signal is detected in the negative amplification control at a single locus, the test results associated with the negative amplification control will be considered inconclusive at that locus. If it is imperative that the locus be used, the samples will be re-amplified.

9.2.16.4.2 If a weak signal is detected in the negative amplification control at multiple loci, the test results for all loci will be considered inconclusive and all samples, if possible, will be re-amplified.

9.2.16.4.3 If a strong signal is detected in the negative amplification control at a single locus, the test results for all loci will be considered inconclusive and all samples, if possible, will be re-amplified.

9.2.16.5 If no typing result is observed for the Random Sample or the Control DNA (K562 or GM9947A Cell Line) at a particular locus all samples at that locus will be considered inconclusive. If it is deemed necessary the sample(s) associated with the Random Sample or Control DNA will be re-amplified and typed.

9.2.17 When a band stronger in intensity is accompanied by a band weaker in intensity that has migrated one allele position (n-4) farther than the more intense band, this may be a stutter band.

NOTE: Stutter may also be seen at a n+4 position to that of the intense band. In addition, if the sample is overloaded or a high concentration of DNA was amplified, artifactual bands may also be seen at n-1, n-2, n-3 and n-8 positions to the intense band.

9.2.17.1 Both the strong and weak band will be sized using the STaRCall allele calling software. (Refer to Appendix F for the FMBIO Fluorescent Imaging Analysis System procedure).