APPENDIX J – PREPARING NIST TRACEABLE SAMPLES

1 Take blood samples from at least 2 people, apply this material to blood stain cards (1 donor on each card) and dry the cards.

2 Demonstrate that the dried samples from each donor are homogenous by taking multiple cuttings from each stain card prepared and process the samples in accordance with the DNA extraction and typing procedures outlined in the Procedures Manual. All normal controls must be processed with samples (e.g., reagent blanks, negative blanks, positive controls, etc.). The same DNA profile should be obtained from the duplicate samples from each card.

3 Once the representative testing has been completed as outlined above take a single cutting from each stain card (e.g., if enough blood was drawn to make 2 stain cards from each donor for a total of 4 stain cards then a single cutting from each card is needed) and extract these samples in parallel with samples from a Standard Reference Material (SRM). If the correct DNA profiles are obtained from the in-house prepared samples and the SRM kit samples, then the in-house prepared samples are now considered National Institute of Standards and Technology (NIST) traceable.

4 To conduct the annual testing using the in-house prepared NIST traceable samples take a single cutting from each stain card (e.g., if enough blood was drawn from an individual to make 2 stain cards a single sample from 1 of the cards is needed). Process the samples in accordance with the DNA extraction and typing procedures outlined in the Procedures Manual. Maintain all documentation in a properly labeled binder or file.

5 The in-house prepared samples are good until the sample is consumed or a discrepancy is detected. New lots of NIST traceable samples have to be prepared as listed in step 1 above (meaning you cannot just bleed the same people and use their sample without going through the representative testing with all the applicable extraction and typing controls).

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APPENDIX K – GUIDE FOR REVIEW OF AUTOSOMAL DNA DATA

Extraction worksheet
- Samples extracted from low to high (batch or per case)
- Knowns extracted separately from evidence
- Appropriate reagent blanks used and correct volumes
- Extraction method, volume, buffer and analyst specified

BIOMEK® worksheet
- Samples & controls in proper location
- Knowns separated from evidence
- "Wells Loaded" box accurately recorded
- Operator/Analyst actions initialed and dated
- Crosscheck initialed and dated or otherwise marked
- Instrument ID recorded, if applicable

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Plexor® worksheet
- Operator initials present
- Proper documentation if STD curves adjusted
- R^2 values for Autosomal/Y curves recorded
- IPC value checked for possible inhibition
- Samples noted that are not going to be amplified here or on the sample dilution tracking form, if applicable
- Instrument ID recorded, if applicable

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Sample Dilution Tracking worksheet
- Operator initials present
- Deviations correctly noted, if applicable

Reagent / Amplification worksheet
- Lot #s & required expiration dates recorded
- Extraction / Quantitation / Amplification dates recorded
- TC # & QC (if applicable), unique positive and negative info recorded, date recorded

Manual Amplification form
- Manual dilutions checked
- Reagent blank volumes mimic the most sensitive samples they monitor

CE Plate map
- Reagent volumes, lot #s & expiration dates recorded, if applicable
- Polymer load date & buffer change date recorded, if applicable
- Sample load volumes & injection times recorded
- Plate name, instrument ID, plate loading date, and wells loaded recorded
- Controls evaluated and documented in box checked off and dated by examiner & tech reviewer
- Reagent blanks loaded and injected proper volume
- Minimum two ladders loaded per plate
- Reason for reinjection documented, if applicable

CE Data / Electronic Information
- Correct module file & peak threshold values used
- Verify ILS for each sample is suitable
- Verify ladder(s) allele values are correct
- Verify correct types obtained for at least one positive control associated with the amplification
- Negative control(s)/reagent blanks evaluated, proper action taken if expected values not obtained
    - Include a review of raw data to ensure primer peaks are observed indicating the sample was loaded

- Evaluate for off-scale data – use with caution if evidence or mixture sample
- Verify sample allele calls/edits/ST calculations are accurate, if applicable

Egram printouts

- Injection time recorded on egrams or in case file documentation
- ST, M/m calculation, if applicable, are correct

Landscape worksheet (if present)

- Verify allele calls against egrams
- INC loci addressed if not addressed elsewhere
- Profile suitability for comparison/searching documented, if not documented elsewhere

CODIS Specimen Detail report printouts

- All appropriate samples entered into CODIS
- Appropriate specimen nomenclature used
- Appropriate specimen category assigned
- Source ID appropriately chosen, if applicable
- Correct alleles entered
- CODIS review documented, if applicable & not documented elsewhere
- Reported NDIS suitability consistent with data entry

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CODIS search result printouts

- Staff index search conducted, if appropriate
- Local & state search sheets included, if appropriate
- Proper indices searched
- Matches properly evaluated & documented
- Appropriate alleles used

Statistics

- Appropriate calculation used
- For LR, appropriate assumptions made
- For CPI, loci used with all data above stochastic threshold
- For single source, profile evaluated for 2p
- Theta used as appropriate
- ST peaks considered, if appropriate
- Correct alleles entered
- Correct database used
- Sample description / identification listed

APPENDIX L: GUIDE FOR REVIEW OF Y-STR DNA DATA

Reagent/Amplification worksheet
- Lot #'s & expiration dates recorded
- Dilution/Amplification date recorded
- TC name & QC date recorded

CE Data worksheet
- Lot #'s recorded
- Proper controls

Electropherogram data
- Case number and item number documented
- Artifactual peaks addressed, if not done so in GeneMapper® ID/ GeneMapper® ID-X
- Controls evaluated, proper action taken if expected values not obtained
- Appropriate parameters used

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Statistics
- Correct alleles entered
- STH applied, if applicable
- Sample description/identification documented

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