3.1 Verification of Reagents

3.1.1 Verification of specific critical reagents is outlined in the following procedures:

- 3.2 Verification of Qiagen DNA Investigator kits
- 3.3 Verification of Plexor ® HY Quantitation kits
- 3.12 Verification of PowerPlex® Fusion
- 3.15 Verification of WEN Internal Lane Standard 500 (for PowerPlex® Fusion and PowerPlex® Y23)
- 3.18 Verification of PowerPlex® Y23

In addition to the critical reagent verification forms identified in each verification procedure, a log indicating the reagent, lot number, and date/initials of supervisor (or designee) approval shall be maintained.

If for any reason acceptable data are not obtained upon completion of the verification steps associated with each critical component, another attempt shall be completed. If acceptable data are still not obtained, the DNA Technical Leader shall be contacted for approval or further direction.

3.1.2 Verification of In-House Prepared Batch and Purchased Reagents

A batch of in-house prepared reagents is assigned a new LOT number each time they are assembled. A new lot number is not required if a purchased reagent is simply aliquotted for frozen storage over a period of time. Each reagent lot number shall be reliability checked prior to their use in casework by utilizing an applicable positive control, Internal Laboratory Control (ILC) or known semen stain, and negative reagent blank. The verification shall begin at the point in which the reagent is first used and carry through the the remainder of the full DNA analytical process. A log, and supporting worksheet and data documentation, shall be retained that includes (as applicable) the date prepared, date received, preparer's initials, individual components (amount and lot number(s)), and date/initials of supervisor (or designee) approval. The performance of the reagent blank and Internal Laboratory Control (ILC) shall be determined based as defined below. Beyond the initial performance verification, continued performance of the reagent shall be documented in each individual case file record utilizing appropriate controls.

3.1.3 Negative Controls

Purpose: To provide a documented control to monitor reagents, test environment and processing procedures associated with the complete PCR analytical process. All negative controls shall correlate with the most restrictive samples in the DNA batch such as extract recovery volume, amplification volume and electrophoresis injection times. All negative controls shall be amplified on the same thermal cycler model and analyzed on the same genetic analyzer model as the samples they are controlling. All extraction sets where potential additional testing using chemistries or testing conditions necessitate

additional amplifications shall include two reagent controls for each extraction type. The replicate with the highest DNA concentration for the amplification chemistry being utilized (autosomal-STR or Y-STR) shall by continued through STR typing. The replicate with the lowest DNA concentration shall be archived for possible future additional testing. If both replicates of the reagent control result in an undetected DNA concentration, one reagent control is archived and the other is carried forward to STR amplification.

STAIN REAGENT CONTROLS - A blank control that is processed with the stain extraction, Chelex extraction or Promega's Tissue and Hair Extraction Kit procedure.

The Stain Reagent Control is set up with each of the stain extraction sets.

DIFFERENTIAL REAGENT CONTROL - A blank control that is processed with the differential extraction procedure.

The Differential Reagent Control is set up with each differential extraction set.

Negative Controls - Performance Criteria

Alleles should not be observed in these samples above the 250 RFUs reporting threshold (analytical/detection).

Peaks greater than 75 RFUs and corresponding to allele positions should be evaluated carefully. If it is determined that peaks correspond to alleles and a potential genotype proceed to the Failure of Negative Controls section, below.

Failure of Negative Controls

Failure of negative controls can result from a variety of situations. Each failure will be evaluated on a case by case basis.

The point and source of the failure should be determined, when possible.

If the alleles and/or genotypes observed in the negative control(s) for a set of reactions are the same as the genotype that supports an inclusion, the data for those related samples is voided and may require re-extraction of the original item of evidence.

If the alleles or genotype observed are not associated with a genotype that supports an inclusion, the data may be interpreted cautiously. If the results are reported, the failed control must be reported in the written report.

3.1.4 Internal Laboratory Control (ILC): NIST-Traceable Standard

Purpose: The Internal Laboratory Control originated as an unknown male whole blood sample preserved in K_3-EDTA. Bloodstains were prepared from the whole blood on filter paper. The Internal Laboratory Control was analyzed against the NIST SRM 2391C, making it a NIST-traceable standard.

The Internal Laboratory Control is intended to provide a documented positive human DNA control to monitor the complete DNA analytical process. The Internal Laboratory Control is run for quality assurance purposes.

The Internal Laboratory Control (ILC) profile is:

Genetic Marker	ILC Genotype		Genetic Marker	ILC Haplotype
Amelogenin	X,Y		DYS576	16
D3S1358	14,16		DYS389I	14
D1S1656	14,16		DYS448	20
D2S441	13,15		DYS389II	31
D10S1248	15,17		DYS19	14
D13S317	11,12		DYS391	11
Penta E	8,16		DYS481	23
D16S539	11		DYS549	13
D18S51	12,21		DYS533	12
D2S1338	19,20		DYS438	12
CSF1PO	8,10		DYS437	16
Penta D	2.2		DYS570	17
TH01	7		DYS635	24
vWA	13,16		DYS390	23
D21S11	30,33.2		DYS439	13
D7S820	10,12		DYS392	14
D5S818	11		DYS643	10
TPOX	11		DYS393	13
DYS391	11		DYS458	16
D8S1179	13,14		DYS385	12,14
D12S391	18,23		DYS456	15
D19S433	14.2,15		YGATAH4	13
FGA	24			

Genetic Marker	ILC Genotype		Genetic Marker	ILC Haplotype
D22S1045	14,15			

Positive Control Performance Criteria - (Internal Laboratory Control)

Genotypes must match the expected genotype at each locus. The relative fluorescent intensity must meet the minimum analytical threshold of 250 RFUs. Exceptions to meeting the minimum analytical threshold of 250 RFUs are outlined below. Because the Internal Laboratory Control samples are single source in nature, it is acceptable to have homozygous alleles below the 900 RFUs Stochastic Threshold. It is acceptable for the Internal Laboratory Control samples to have Peak Height Ratios (PHRs) less than the expected 50% for heterozygous markers. Alleles with very high RFUs that exhibit potential oversaturation of the genetic analyzer detector as represented by flat peak tops and/or associated elevated stutter peaks may be interpreted if the peaks are symmetrical, sharp and potential artifacts do not interfere with the interpretation of the electropherogram. Alleles that do exhibit oversaturation as described previously must be reanalyzed by reamplification and/or reinjection at reduced injection conditions.

Performance failures of Positive Controls

Incorrect genotypes - If any positive control (Internal Laboratory Control) has genotypes that are not the expected genotypes at all genetic markers, all samples within the batch must be re-amplified with the same kit initially used. A different lot of the amplification kit may be used if the initial lot is no longer available. Results from samples that do not have sufficient material to re-amplify are invalid and shall not be interpreted or reported.

If any positive control (Internal Laboratory Control) has alleles at some or all loci that do not meet the 250 RFUs analytical threshold, the entire amplification set shall be evaluated to see if the trend is observed across all samples within the batch. If there is a trend that the entire run is showing characteristics exhibited in the positive control, the amplification is voided and the set shall be re-amplified.

Instances where the positive control performance did not meet acceptable standards (greater than or equal to 250 RFUs and/or expected genotype), a statement in the report shall be included indicating the positive control performance failure. Instances where additional action is taken to achieve acceptable positive control performance (increased injection time, re-amplification of the ILC etc...), may be reported without a statement in the report.

3.2 Verification of Qiagen DNA Investigator Kits

3.2.1

The purpose of this procedure is to evaluate the performance of the Qiagen DNA Investigator Kit prior to use in the extraction and purification of casework samples on the QIAsymphony® SP instrument.

3.2.2 Procedure

Extract, purify, and collect four Internal Laboratory Controls (ILCs) and four reagent blanks, alternating between ILC and blank, as outlined in Biology procedure manual section 2.3 QIAsymphony Extraction, using the Qiagen DNA Investigator Kit, and CW500_ADV_HE CR21380 ID1710 protocol.

Proceed to Biology procedure manual section 2.19 Plexor® HY with the Applied Biosystems 7500 Real-Time PCR System to determine DNA concentrations in the Internal Laboratory Controls and reagent blanks.

3.2.3 Assessment of Results

The DNA quantitation results should be as expected, with quantitation values representative of the size of the sample. The reagent blanks should have a quantitation value that is either undetected or negligible.

The calculated DNA concentrations, kit components used, and expiration dates shall be recorded in STACS-CW. The verification shall be approved in STACS-CW by the individual reviewing the kit performance prior to use in casework. The reviewing supervisor or scientist shall not approve the kit in STACS-CW without acceptable results and shall consult with the DNA Technical Leader if the acceptability of the results may be in question.

3.3 Verification of Plexor® HY Quantitation Kits

3.3.1

The purpose of this procedure is to evaluate the performance of the Plexor® HY kit. This procedure shall be followed for each lot number of Plexor® HY kits or subsequent shipments of a previously verified lot number. An acceptable range for the standard curve slope and y-intercept values based on the kit verification will be generated and communicated. NIST SRM 2372 containing components A, B and C shall be utilized in this procedure.

3.3.2 Procedure

3.3.2.1

Prepare Plexor® HY standards and reactions as outlined in Biology Procedures Manual sections 2.19.3 through 2.19.5

3.3.2.2

Verification of Plexor® HY must include three sets of standards prepared independently (Set A, Set B, Set C) and ran in duplicate with a negative control (TE-4 or water) and NIST SRM 2372 components. The NIST SRM 2372 components must be diluted 2µl into 18µl TE-4 prior to quantitation.

3.3.2.3

Operate the Applied Biosystems 7500 Real-Time PCR System and review data as outlined in Biology Procedures Manual sections 2.19.6 through 2.19.8

3.3.2.3.1

Following Biology Procedures Manual section 2.19.8.6, assign standards Set A as standards and all other samples as either No Template Control or unknowns.

3.3.2.3.2

Evaluate and document the standard curve values for slope and y-intercept (Autosomal and Y) as outlined in Biology Procedures Manual section 2.19.8.10.

3.3.2.3.3

Repeat steps above for standards Set B and Set C.

3.3.2.4

Assessment of Results

3.3.2.4.1

The standard curves R^2 value should be >0.990

3.3.2.4.2

The calculated concentrations of the negative controls (NTC) and NIST SRM 2372 components should be consistent with the respective sample types (diluted Component A 5.24 ng/µl, Component B 5.36 ng/µl and Component C 5.43 ng/µl).

3.3.2.4.3

The negative control and NIST SRM 2372 concentrations, kit components used and expiration dates shall be recorded on the Verification of Plexor® HY Kit form (FS-19). The form shall be signed and dated by the person performing the verification and the person reviewing the kit performance prior to use in casework. The reviewing scientist shall not sign the FS-19 or put the kit into use without acceptable results or consulting the DNA Technical Leader. Upon approval of Plexor® HY kit verification, the lot/shipment-specific standard curve slope and y-intercept values shall be communicated to all Forensic Scientists that may utilize the specific Plexor® HY kit.

3.4 5% Chelex (W/V) Stock Solution

3.4.1

Weigh out 0.05 grams of dry Chelex 100 beads and place into 1.5 ml tubes.

3.4.2

Add 1ml of sterile distilled water to create a 5% Chelex solution.

3.4.3

Alternatively, a larger preparation of a stock solution of 5% Chelex may be prepared as long as the pH is checked prior to each use. The pH must be >9. 5% Chelex shall be discarded if it is outside of this range. Following preparation of a large stock, remaining aliquots in 2ml tubes can be stored frozed for up to six months.

3.4.4

Use a large bore pipette tip (P-1000) when pipetting Chelex beads.

3.5 0.39M DTT

3.5.1

601.2 mg DTT

3.5.2

Q.S. to 10 mL with H_2O

3.5.3

Aliquot into convenient size volumes and freeze. Remix after thawing.

3.6 0.5M EDTA

3.6.1

Dissolve 18.6 g Na 2 EDTA-2H 2 O in 60 mL H2O.

3.6.2

Adjust pH to 8.0 (Note: EDTA will not go into solution at acid or neutral pH) when fully dissolved. Q.S. to 100 mL with H_2O. Filter.

3.6.3

Alternatively, 0.5M EDTA may be purchased from VWR (catalog number CA82023-102, supplier number R013).

3.7 Hi-Di™ Formamide

3.7.1

Hi-Di™ Formamide shall be purchased commercially from Applied Biosystems using part number 4311320 (25 mL).

3.8 2M Na Acetate

3.8.1

16.4 g NaOAc

3.8.2

Q.S. to 100 mL with H_2O. Filter

3.9 5M NaCL

3.9.1

29.22 g NaCl

3.9.2

Q.S. to 100 mL with H_2O. Filter

3.9.3

As an alternative, 5 M NaCl may be purchased commercially. BRL catalog # 24740-011

3.10 10N NaOH

3.10.1

Purchase commercially.

3.12 Phenol Chloroform Isoamyl Alcohol

3.11 Phenol/Chloroform/Isoamyl Alcohol

3.11.1

Purchase commercially and store according to manufacturer's recommendations.

3.11.2

Caution! Phenol is very Caustic. Use in a chemical fume hood.

3.12 Verification of PowerPlex® Fusion

3.12.1

PowerPlex® Fusion is classified as a critical reagent, requiring verification of each lot and shipment prior to use in casework applications.

3.12.2

Standard Amplification Method

Prepare and amplify approximately 0.5 ng of DNA from the positive control (2800M) and Internal Laboratory Control (ILC). Include a negative amplification control, water or TE^{-4}, with the kit verification. Amplify the samples according to DNA Procedure Manual 2.24. Record all necessary information on the PowerPlex® Fusion amplification worksheet.

Direct Amplification Method

Prepare a buccal swab from an individual with a known DNA profile. Extract and amplify the known buccal sample and reagent blank control. Include a negative amplification control, water or TE^{-4}, with the kit verification. Amplify the samples according to DNA Procedure Manual 2.24. Record all necessary information on the PowerPlex® Fusion amplification worksheet.

3.12.3

Run the samples using the ABI 3500/3500xL Genetic Analyzer according to the DNA Procedure Manual.

3.12.4

Evaluate the prepared samples using the GeneMapper® ID-X v. 1.4 software system. Print the electropherograms with the allele call, peak height (RFUs) and base pair size labeled. Compare the base pair designations of all alleles from each locus in the positive controls. Record the necessary information on the Verification of PowerPlex® Fusion worksheet.

3.12.5 Assessment of Results

No observable allelic peaks exceeding the 275 RFUs analytical threshold or artifacts that are unable to be resolved should be noted in the negative controls (water or TE^{-4}). The base pair sizes of all alleles in the 2800M and ILC must be compared to corresponding alleles from the current lot of PowerPlex® Fusion and be within +/- 0.5 base pairs. It may be necessary to compare the base pair sizes of alleles generated with a single allelic ladder in the GeneMapper® IDx project to achieve this range. If acceptable results are not achieved, a second attempt shall be conducted. The DNA Technical Leader shall be contacted if the second attempt does not yield acceptable results.

The positive control genotypes, as applicable, shall be:

Genetic Locus	2800M Genotype	ILC Genotype
Amelogenin	X,Y	X,Y
D3S1358	17,18	14,16
D1S1656	12,13	14,16
D2S441	10,14	13,15
D10S1248	13,15	15,17
D13S317	9,11	11,12
Penta E	7,14	8,16
D16S539	9,13	11,11
D18S51	16,18	12,21
D2S1338	22,25	19,20
CSF1PO	12,12	8,10
Penta D	12,13	2.2,2.2
TH01	6,9.3	7,7
vWA	16,19	13,16

D21S11	29,31.2	30,33.2
D7S820	8,11	10,12
D5S818	12,12	11,11
TPOX	11,11	11,11
DYS391	10	11
D8S1179	14,15	13,14
D12S391	18,23	18,23
D19S433	13,14	14.2,15
FGA	20,23	24,24
D22S1045	16,16	14,15

3.13 Proteinase K

3.13.1

500 mg Proteinase K

3.13.2

Dissolve in 25 mL H_2O

3.13.3

Aliquot and freeze.

3.13.4

As an alternaive, Proteinase K may be purchased as commercially prepared. Aliquot in 200 µL units and store at -20ºC.

3.14 20% Sarkosyl

3.14.1

Purchase molecular grade from Sigma-Aldrich.

Part Number L7414 (50ml or 10ml sizes).

3.14.2

Stock may be aliquotted to smaller volumes

3.14.3

Refrigerate.

3.15 Verification of WEN Internal Lane Standard 500

3.15.1 Procedure

Prepare PowerPlex ® Fusion (WEN), PowerPlex ® Y23 (WEN) allelic ladder, positive controls and negative controls according to the applicable procedure.

Run the prepared samples using an ABI Genetic Analyzer following the applicable procedures. Evaluate the prepared samples using GeneMapper ® ID-X software.

Compare the base pair designation of all alleles from each locus in the positive control and in the ILC from the new lot of the WEN Internal Lane Standard 500 to the previously processed corresponding samples from teh current kit.

3.15.2 Assessment of Results

The peaks corresponding to the analysis range 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 base pair fragments must be sharp and well defined with RFUs of 75 or higher.

Positive controls must exhibit known allele types.

No observable allelic peaks or irresolvable artifacts should be observed in the negative controls. Careful evaluation will be performed when the base pair values between the new and current lots differ by more than 0.5 base pairs. (Samples migrate differently with previously used CC5 dye compared to the WEN dye).

Document the values, component lot numbers and expiration dates on the In-Lane Sizing Standard Verification form. The form shall be signed and dated by the person performing the verification and the person reviewing the kit performance prior to casework.

3.16 10% SDS

3.16.1

Purchase commercially, BRL catalog #24730-020 (1L)

3.16.2
Alternatively, dissolve 10 g SDS in 70 mL of H_2O.

3.16.3
Heat to ~65°C to aid.

3.16.4
Q.S. to 100 mL with H_2O.

3.16.5

DO NOT ALLOW TEMP. OF SDS >65°C!!

3.17 20% SDS

3.17.1
200 g SDS

3.17.2
Dissolve in 700 mL of H_2O.

3.17.3
Heat to ≈65°C to aid.

3.17.4
Q.S. to 1 Liter with H_2O.

3.17.5

DO NOT ALLOW TEMP. OF SDS >65°C!!

3.18 Verification of PowerPlex® Y23

3.18.1 Critical Reagent
PowerPlex® Y23 is classified as a critical reagent, requiring verification of each lot and shipment prior to use in casework applications.

3.18.2 Sample Preparation and Amplification

Standard Amplification Method

Prepare and amplify 0.5ng of DNA from the positive control (2800M). Include a negative amplification control, water or TE^{-4}, with the kit verification. Amplify the samples according to DNA Procedure Manual 2.23. Record all necessary information on the Y-STR Amplification worksheet.

Direct Amplification Method

Prepare a buccal swab from an individual with a known haplotype. Extract the known buccal sample and reagent blank control following the direct amplification procedure. Include a negative amplification control, water or TE-4, with the kit verification. Amplify the samples according to DNA Procedure Manual 2.23. Record all necessary information on the Y-STR Amplification worksheet.

3.18.3 Genetic Analyzer

Run the samples using the ABI 3500 Genetic Analyzer according to DNA Procedure Manual 2.23.

3.18.4 Documentation

Evaluate the prepared samples using the GeneMapper™ ID-X software system. Print the electropherograms with the allele call, peak height (RFUs) and base pair size labeled. Compare the base pair designations of all alleles from each locus in the positive control. Record the necessary information on the Verification of PowerPlex® Y23 worksheet.

3.18.5 Evaluation

Assessment of Results-the 2800M positive control haplotype must be:

Genetic Locus	Haplotype 2800M
DYS576	18
DYS389I	14
DYS448	19
DYS389II	31
DYS19	14
DYS391	10
DYS481	22
DYS549	13
DYS533	12
DYS438	9
DYS437	14

DYS570	17
DYS635	21
DYS390	24
DYS439	12
DYS392	13
DYS643	10
DYS393	13
DYS458	17
DYS385a/b	13,16
DYS456	17
YGATAH4	11

The Y-STR haplotype of the positive direct amplification extraction control must match the expected haplotype.

No observable allelic peaks exceeding the analytical threshold or artifacts that are unable to be resolved should be noted in the negative controls. The base pair sizes of all alleles in the 2800M must be compared to corresponding alleles from the current kit lot of PowerPlex® Y23 and be within +/- 0.5 base pairs. If acceptable results are not achieved, a second attempt shall be conducted. The DNA Technical Leader shall be contacted if the second attempt does not yield acceptable results.

3.19 Vacant

3.20 Vacant

3.21 Stain Extraction Buffer

3.21.1

1.21 g Trizma base

3.21.2

20 mL 0.5 EDTA

3.21.3
20 mL 5M NaCl

3.21.4
Dissolve in 400 mL H_2O.

3.21.5
pH to 8.0 with HCl.

3.21.6
200 mL 10% SDS

3.21.7
Q.S. to 1000 mL with H_2O.

3.21.8
Aliquot into convenient size volumes and freeze. Remix after thawing.

3.22 1X TAE

3.22.1
20 mL 50X TAE

3.22.2
980 mL MQ H_2O

3.23 50X TAE

3.23.1
Purchase commercially.

3.24 TE-4

3.24.1
1.21 g 10 mM Trizma base

3.24.2
Dissolve in 500 mL molecular grade H_2O. pH to 7.5 with HCl.

3.24.3
0.2 mL 0.5 M EDTA

3.24.4
pH to 7.5. Q.S. to 1000 mL with molecular grade H_2O.

3.24.5
Aliquot into convenient size volumes and freeze. Remix after thawing.

3.25 2 M TRIS, pH 7.5

3.25.1
121.1 g Trizma base

3.25.2
Dissolve in 250 mL H_2O. pH to 7.5 with HCl.

3.25.3
Q.S. to 500 mL with H_2O. Filter.

3.26 TNE

3.26.1
1.21 g Trizma base

3.26.2
20 mL 5 M NaCl

3.26.3
2 mL 0.5 M EDTA

3.26.4
Dissolve in 800 mL H_2O. pH to 8.0.

3.26.5

Q.S. to 1000 mL with H_2O. Filter.

3.26.6

Aliquot into convenient size volumes and freeze. Remix after thawing.