1. Scope

   This protocol outlines the frequency and type of quality control checks that are to be conducted on critical reagents to ensure the high quality of work suitable for a forensic casework laboratory.

2. References
   - 2.1. Applied Biosystems™ GlobalFiler™ PCR Amplification Kit, User's Manual, Rev. E, 2016.
   - 2.2. Applied Biosystems™ Quantifiler® Kits: Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit, User's Manual, Rev. F, 2012.
   - 2.3. Qiagen® QIAamp® DNA Investigator Handbook, 2012.

3. Equipment

   See individual reagent sections in Appendix A.

4. Safety / Quality Assurance

   See individual reagent sections in Appendix A.

5. Procedure
   - 5.1. Documentation
     - 5.1.1. All paperwork related to quality checks shall be kept in the *Reagent Quality Control Log* and/or electronically.
     - 5.1.2. User manuals shall be stored with the reagent, in the *Reagent Quality Control Log,* and/or electronically.
   - 5.2. Quality Checks
     - 5.2.1. Unless otherwise stated, the quality control check will consist of the evaluation or a test for function listed in Appendix A.
     - 5.2.2. A quality control check will be conducted on any critical reagent prior to its use in casework for:
       - 5.2.2.1. New reagents
       - 5.2.2.2. Reagents that have been stored greater than 8 hours outside of their recommended temperature range
     - 5.2.3. No reagent will be used for casework unless it passes its designated quality control check.
   - 5.3. Review
     - 5.3.1. All worksheets and data associated with a quality control check will be reviewed by a qualified casework analyst and either approved or rejected for use in casework. A second qualified individual will perform and document a review of the data.

5.3.1.1. Approval

5.3.1.1.1. If the quality control check for a given lot of a reagent meets all requirements listed in Appendix A and the work is both technically and administratively sound, that reagent lot shall be approved for use in casework.

5.3.1.1.2. If approved, the cover sheet shall be initialed by the approver and reviewer. The packet, including all associated worksheets and data, will be stored in the appropriate log and/or electronically.

5.3.1.1.2.1. Electropherograms may be generated and stored electronically.

5.3.1.2. Rejection

5.3.1.2.1. If the quality control check for a given lot of a reagent does not meet the specific requirements listed in Appendix A, that reagent lot will not be used in casework until it passes.

5.3.1.2.2. The DNA Technical Leader will be notified.

5.3.1.2.3. Further tests will be performed at the discretion of the analyst/technical leader to include:

5.3.1.2.3.1. Additional laboratory evaluations and/or;

5.3.1.2.3.2. Contact the manufacturer for replacement.

5.4. Reagents

5.4.1. See Appendix A for reagent specific quality control procedures for the following reagents:

5.4.1.1. Phenolphthalein (Kastle-Meyer) Kit

5.4.1.2. 200 Proof Ethanol

5.4.1.3. Tris-EDTA^{-4} (TE^{-4})

5.4.1.4. Qiagen® QIAamp® DNA Investigator Kit

5.4.1.5. Proteinase K (ProK)

5.4.1.6. Carrier RNA (cRNA)

5.4.1.7. Dithiothreitol (DTT)

5.4.1.8. Quantifiler® Quantification Kit

5.4.1.9. GlobalFiler™ Amplification Kit

5.4.1.10. GeneScan™ 600 LIZ™ Size Standard v2.0

5.4.1.11. 3130 POP-4™

5.4.1.12. Hi-Di™ Formamide

5.4.1.13. 1x Sequencing Buffer

5.4.1.14. Molecular Grade Water

APPENDIX A – Reagent Specific Quality Control Procedures

Reagent: Phenolphthalein (Kastle-Meyer) Test Kit

Manufacturer: Various

1. Equipment
- 1.1. Disposavble gloves
- 1.2. Eye protection
- 1.3. Lab coat
- 1.4. Swabs or filter paper

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The expiration date will be set 12 months from the date of receipt.

4. Procedure
- 4.1. Make a serial dilution using liquid whole blood and water at the following dilutions: neat, 1:100, 1:500, 1:1,000, 1:5,000, 1:10,000, 1:50,000, and 1:100,000.
- 4.2. Spot 10 µL of each dilution on a swab or filter paper.
- 4.3. Test a known non-human positive control (supplied in kit), a negative control, and the full dilution series from Section 4.1 using the reagents from the new kit.
- 4.4. Record the results.
  - 4.4.1.A positive result is indicated by a pink color change within 5 seconds.
  - 4.4.2.A negative result is indicated by no color change within 5 seconds.

5. Acceptable Results
- 5.1. The positive control must produce a positive result.
- 5.2. The negative control must produce a negative result.
- 5.3. At a minimum, the neat, 1:100, 1:500, and 1:1,000 dilutions must produce a positive result.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: Ethanol (200 Proof)

Manufacturer: Various

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat
- 1.4. Safety cabinet

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The expiration date will be set 9 years from the date of receipt or the manufacturer's expiration date, whichever is sooner.

4. Procedure
- 4.1. Extract at least one sample using 1-5 µL of known liquid blood or an oral swab from an individual with a known DNA profile and a reagent blank using the ATF Laboratory QIAmp® DNA Investigator protocol and the new lot of ethanol at all appropriate steps throughout the extraction process.

  NOTE: The 100% ethanol used to initially create the AW1 and AW2 buffers can be a different previously QC'd lot than the 100% ethanol used during the extraction protocol.

- 4.2. Quantify, amplify, and genetically type these samples using the appropriate controls and the standard ATF Laboratory protocols for each step of the process.

5. Acceptable Results
- 5.1. Correct DNA profiles must be obtained for the QC sample(s) and positive control.
- 5.2. No additional peaks above the analytical threshold (AT) can be observed in the QC sample or positive control.
- 5.3. The negative control and reagent blank should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: Tris-EDTA-4 (TE-4)

Manufacturer: Various

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The expiration date will be set 12 months from the QC date or the manufacturer's expiration date, whichever is sooner.

4. Procedure
- 4.1. Add 300 µL TE^{-4} to a Microcon filtration device and concentrate the sample to approximately 15µL.
- 4.2. Amplify all 15µL of concentrated TE^{-4} buffer and genetically type the sample using the appropriate controls using the standard ATF Laboratory protocols for each step of the process.

NOTE: The negative amplification control should be made with the new TE^{-4} buffer.

5. Acceptable Results
- 5.1. The correct DNA profile must be obtained for the positive control. No additional peaks above the analytical threshold (AT) can be observed in the positive control.
- 5.2. The QC sample, negative control, and reagent blank should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: QIAamp® DNA Investigator Kit

Manufacturer: Qiagen®

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat
- 1.4. 200 proof ethanol

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The expiration date will be set 12 months from the date of receipt or the manufacturer's expiration date, whichever is sooner.

4. Procedure
- 4.1. Optional: Prepare new cRNA:
  - 4.1.1. Add 310 µL of ATE to each cRNA tube.
    - 4.1.1.1. If multiple tubes from the same lot are reconstituted, combine and mix all tubes before proceeding to next step.
  - 4.1.2. Aliquot 30 µL of vortexed solution into capped 1.5 ml tubes.
  - 4.1.3. Spin down and place in freezer.
- 4.2. Extract at least two samples using 1-5 µL of known liquid blood or an oral swab from an individual with a known DNA profile and three reagent blanks using the new lot of QIAmp® DNA Investigator Kit.
- 4.3. Quantify, combine, and concentrate the three reagent blanks. Amplify and genetically type these samples using the appropriate controls and the standard ATF Laboratory protocols for each step of the process.

5. Acceptable Results
- 5.1. Correct DNA profiles must be obtained for the QC samples and positive control.
- 5.2. No additional peaks above the analytical threshold (AT) can be observed in the QC samples or positive control.
- 5.3. The negative control and reagent blank should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: Proteinase K

Manufacturer: Various

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The manufacturer derived expiration date will be used.

4. Procedure
- 4.1. Extract at least one sample using 1-5 µL of known liquid blood or an oral swab from an individual with a known DNA profile and a reagent blank using the new lot of proteinase K.
- 4.2. Quantify, amplify, and genetically type these samples using the appropriate controls and standard ATF Laboratory protocols for each step of the process.

5. Acceptable Results
- 5.1. Correct DNA profiles must be obtained for the QC sample(s) and positive control.
- 5.2. No additional peaks above the analytical threshold (AT) can be observed in the QC sample or positive control.
- 5.3. The negative control and reagent blank should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: Carrier RNA (cRNA)

Manufacturer: Qiagen®

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The expiration date will be set 12 months from the date of receipt of the associated QIAamp® DNA Investigator Kit or the expiration date of the TE^{-4}, whichever is shortest.

4. Procedure
- 4.1. Prepare new cRNA:
  - 4.1.1. Add 310 µl of ATE in each cRNA tube.
    - 4.1.1.1. If multiple tubes from the same lot are re-constituted, combine and mix all tubes before proceeding to next step.
  - 4.1.2. Aliquot 30 µl of vortexed solution into capped 1.5 ml tubes.
  - 4.1.3. Spin down and place in freezer.
- 4.2. Extract at least one sample using 1-5 µL of known liquid blood or an oral swab from an individual with a known DNA profile and a reagent blank using the procedure for the QIAmp® DNA Investigator Kit.
- 4.3. Quantify, amplify, and genetically type these samples using the appropriate controls and the standard ATF Laboratory protocols for each step of the process.

5. Acceptable results
- 5.1. Correct DNA profiles must be obtained for the QC sample(s) and positive control.
- 5.2. No additional peaks above the analytical threshold (AT) can be observed in the QC sample or positive control.
- 5.3. The negative control and reagent blank should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: Dithiothreitol (DTT)

Manufacturer: Various

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat
- 1.4. Scale

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The expiration date will be set 12 months from the date of receipt or the manufacturer's expiration date, whichever is sooner.

4. Procedure
- 4.1. Prepare new DTT:
  - 4.1.1. Measure 0.154 g of DTT powder using scale paper.
  - 4.1.2. Put DTT in 2 mL tube along with 1 mL molecular grade water and vortex until DTT is fully dissolved.
  - 4.1.3. Aliquot 100 µL of DTT solution into 1.5 mL tubes and label caps with "DTT."
  - 4.1.4. Place tubes in freezer.
- 4.2. Extract two freshly plucked hairs from an individual with a known DNA profile and a reagent blank using the ATF Laboratory QIAmp® DNA Investigator Kit protocol and the new lot of DTT.
- 4.3. Quantify, amplify, and genetically type these samples using the appropriate controls and the standard ATF Laboratory protocols for each of the processes.

5. Acceptable Results
- 5.1. Correct DNA profiles must be obtained for the QC samples and positive control.
- 5.2. No additional peaks above the analytical threshold (AT) can be observed in the QC sample or positive control.
- 5.3. The negative control and reagent blank should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: Quantifiler® Quantification Kit

Manufacturer: Applied Biosystems™

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat
- 1.4. 7500 thermal cycler

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The manufacturer derived expiration date will be used.

4. Procedure
- 4.1. Using one of the components of the NIST SRM 2372, create the following dilutions for a standard curve:

1 - 50 ng/µl	5 - 0.62 ng/µl
2 - 16.7 ng/µl	6 - 0.21 ng/µl
3 - 5.56 ng/µl	7 - 0.068 ng/µl
4 - 1.85 ng/µl	8 - 0.023 ng/µl

NOTE: If the NIST SRM 2372 component DNA concentration is between 45 ng/µL and 55 ng/µL,

proceed with the dilutions as if the concentration is 50 ng/µL.

4.2. Combine all of the standard tubes from the new lot of Quantifiler® Human DNA Standard to create one pooled tube of Quantifiler® Human DNA Standard. Using the pooled standard, create the following dilutions: 1:20, 1:10, and 1:2.
4.3. Run the following in duplicate using the new lot of reagents included in the Quantifiler® kit: the standard curve components, the dilutions of the pooled Quantifiler® Human DNA Standard, and a neat and/or 1:10 dilution of the NIST SRM 2372. A plate blank must be included in the run.

5. Acceptable Results
5.1. The plate blank value should be "undetected."
5.2. The standard curve should have a slope between -2.9 and -3.5 with an R^2 value greater than 0.98.
5.3. The NIST SRM 2372 sample (neat and/or 1:10 dilution) should be within 20% of its published value.

6. Documentation
6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.
6.2. The calculated concentration of the Quantifiler® standard and its associated recommended dilution factor will be recorded in the *Reagent Quality Control Log* and/or electronically.

7. Dilution Calculation Examples
7.1. Pooled human DNA standard concentration / Target concentration of initial dilution (50 ng/µl) = Dilution factor.
7.2. Example: Actual concentration is 200 ng/µL, then 200 / 50 = 4.
7.2.1. Dilution would then be 10 µL of DNA Std + 30 µL of TE.

Reagent: GlobalFiler™ PCR Amplification Kit

Manufacturer: Applied Biosystems™

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat
- 1.4. 9700 or Veriti™ thermal cycler
- 1.5. AB 3130 Genetic Analyzer

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The expiration date will be set 12 months from the date of receipt for the kit, or the manufacturer's expiration date, whichever is sooner.
- 3.2. Individual tubes will be given an expiration date of 6 months from the date they are thawed not to exceed the kit expiration date.

4. Procedure
- 4.1. Using the new kit, amplify the following samples using the GlobalFiler™ PCR Amplification Kit protocol:
  - 4.1.1. 5 replicates of the same QC sample (previously characterized and quantified standard sample)
  - 4.1.2.2 replicates of the new GlobalFiler™ Control DNA 007.
  - 4.1.3.5 replicates of the negative amplification control (15 µL TE.)
- 4.2. Type the above listed samples on the AB 3130 Genetic Analyzer using the allelic ladder from the new kit.
- 4.3. Calculate the average total RFU / locus for the five replicate amplifications using the new kit and the QC sample.
  - 4.3.1. Sum the RFU across all loci and divide by 24 for each sample (average total RFU / locus), then calculate the global average across all five replicate samples.
  - 4.3.2. Compare the global average total RFU / locus for the five replicates of the new kit to the global average total RFU / locus for the five replicates of the standard reference set (baseline sensitivity set).

5. Acceptable Results

5.1. Difference in average total RFU / locus must be within 20% between the new lot and the standard reference set.

5.2. Correct DNA profiles must be obtained for the QC and positive control samples.

5.3. No additional peaks above the analytical threshold (AT) can be observed in the QC or positive control samples.

5.4. The negative controls should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation

6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: GeneScan™ 600 LIZ™ Size Standard v2.0

Manufacturer: Applied Biosystems™

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat
- 1.4. Genetic analyzer

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The expiration date will be set 12 months from the date of receipt or the manufacturer's expiration date, whichever is sooner.

4. Procedure
- 4.1. A separate 3130 injection will be set-up to include a positive control, negative control, and an allelic ladder using the new lot of GeneScan™ 600 LIZ™ Size Standard v2.0.

5. Acceptable Results
- 5.1. The 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, and 460bp fragments are present and sized correctly.
- 5.2. The positive control must type correctly with no additional peaks above the analytical threshold (AT).
- 5.3. The negative control should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: 3130 POP-4™

Manufacturer: Applied Biosystems™

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat
- 1.4. Genetic analyzer

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The manufacturer derived expiration date will be used.

4. Procedure
- 4.1. A separate 3130 injection will be set-up to include a positive control, negative control, and an allelic ladder using the new lot of 3130 POP-4™.

5. Acceptable Results
- 5.1. The positive control must type correctly with no additional peaks above the analytical threshold (AT).
- 5.2. The negative control should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: Hi-Di™ Formamide

Manufacturer: Applied Biosystems™

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat
- 1.4. Genetic analyzer

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The manufacturer derived expiration date will be used.
  - 3.1.1. Call Applied Biosystems™ (1-800-955-6288) and follow the prompts to speak to a customer service representative to get the expiration date for the lot number. You will need to provide the service representative the catalog and/or lot number.

4. Procedure
- 4.1. A separate 3130 injection will be set-up to include a positive control, negative control, and an allelic ladder using the new lot of Hi-Di™ Formamide.

5. Acceptable Results
- 5.1. The positive control must type correctly with no additional peaks above the analytical threshold (AT).
- 5.2. The negative control should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: 1x Sequencing Buffer

Manufacturer: Various

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat
- 1.4. Genetic analyzer

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The manufacturer derived expiration date will be used.

4. Procedure
- 4.1. A separate 3130 injection will be set-up to include a positive control, negative control, and an allelic ladder using the new lot of 1x sequencing buffer.

5. Acceptable Results
- 5.1. The positive control must type correctly with no additional peaks above the analytical threshold (AT).
- 5.2. The negative control should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.

Reagent: Molecular Grade Water

Manufacturer: Various

1. Equipment
- 1.1. Disposable gloves
- 1.2. Eye protection
- 1.3. Lab coat

2. Safety
- 2.1. Lab coat, eye protection, and disposable gloves must be worn at all times while performing this procedure.

3. Expiration
- 3.1. The expiration date will be set 12 months from the QC date or the manufacturer's expiration date, whichever is sooner.

4. Procedure
- 4.1. Add 300 µL of water to a Microcon® filtration device and concentrate the sample to approximately 15µL (washed and eluted with TE-4).
- 4.2. Amplify the concentrated sample and genetically type the sample using the appropriate controls using the standard ATF Laboratory protocols for each step of the process.

5. Acceptable Results
- 5.1. The correct DNA profile must be obtained for the positive control. No additional peaks above the analytical threshold (AT) can be observed in the positive control.
- 5.2. The QC sample, negative control, and reagent blank should not contain any true peaks over the AT or any pattern of peaks below the AT. Pull-up peaks from the LIZ™ size standard can be disregarded.

6. Documentation
- 6.1. Records will be maintained in the *Reagent Quality Control Log* and/or electronically.