PCR BASED ANALYSIS
QA/QC MANUAL

Major Revision 6/05

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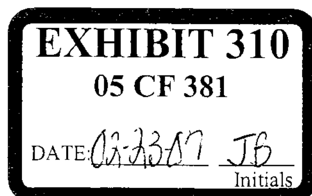

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(1)

Table of Reagent Forms

Alphabetical

Citrate Buffer (pH 5.0)
Dithiothreitol (DTT), 039M
Dithiothreitol (DTT), 1M
0.5M EDTA (pH 8.0)
Hair Digest Buffer/Pellet Wash
5M NaCl
10N NaOH
Phenol\Chloroform\Isoamyl Alcohol
Proteinase K
Sarkosyl
20% (w/v) Sodium Dodecyl Sulfate
Stain Extraction TBE
TE Buffer
TNE
1M TRIS-HCL (pH 7.5)
1 MTRIS-HCL (pH 8.0)

Ex 310
(2)

RECEIVING AND STORING CHEMICALS, REAGENTS AND MATERIALS

Receipt of commercially manufactured reagents and materials should be documented. This applies only to materials that have manufacturer's lot numbers and that are used as an integral part of the analytical process.

1. Check the items received against the items ordered to be sure that the shipment is correct.

2. Remove, date and file any package that have lot specific information on them (e.g., certificates of analysis, quality assurance information, etc.)

3. Store the items in the proper manner as defined by laboratory practice.

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(3)

CHEMICAL AND REAGENT LOG

DATE	ITEM	SOURCE	LOT#	RECEIVED BY

Ex 310
(4)

CITRATE BUFFER (pH 5.0)

FORMULA	AMOUNT
Trisodium citrate, dihydrate	18.4g
Citric acid, monohydrate to pH 5.0	

Dissolve trisodium citrate in 800mls distilled water. Adjust the pH to 5.0 by adding approximately 6g citric acid. Bring to a final volume of 1 liter with distilled water and autoclave.

DATE PREPARED	PREPARED BY	LOT NO. TRISODIUM CITRATE	LOT NO. CITRIC ACID	LAB LOT NO.

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(5)

DITHIOTHREITOL (DTT), 0.39 M

FORMULA	AMOUNT
Dithiothreitol	601.2 mg

Dissolve DTT in a small amount of distilled water. Bring to a final volume of 10 ml. Store in aliquots at -20^{0}C.

DATE PREPARED	PREPARED BY	LOT NO. DTT	LAB LOT NO.

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(6)

DITHIOTHREITOL (DTT), 1M

FORMULA	AMOUNT
Dithiothreitol	1.54 g

Dissolve DTT in 10 ml sterile deionized water in a sterile 15 ml disposable plastic tube. DO NOT AUTOCLAVE SOLUTION. Aliquot and store at -20^{0}C. Discard unused portion of thawed tube.

DATE PREPARED	PREPARED BY	LOT NO. DTT	LAB LOT NO.

Ex 310

(7)

0.5M EDTA (pH 8.0)

FORMULA	AMOUNT
$Na_2EDTA \cdot 2H_2O$	186.1 g
NaOH pellets	20 g

Slowly add EDTA to 800 mls distilled water. Stir vigorously on a magnetic stirrer. Add 20 g of NaOH pellets to aid in solution of the EDTA. Adjust the pH to 8.0 with more NaOH pellets. Bring to a final volume of 1 liter and autoclave. Store at RT.

DATE PREPARED	PREPARED BY	LOT NO. EDTA	LOT NO. NaOH	LAB LOT NO.

Ex 310
(8)

HAIR DIGEST BUFFER/PELLET WASH BUFFER

FORMULA	AMOUNT
1M Tris-HCl (pH 7.5)	1 ml
0.5M EDTA	2 ml
5M NaCl	1 ml
20% (w/v) SDS	10 ml

Mix Tris-HCl, EDTA, NaCl, SDS and bring to a final volume of 100 ml.

DATE PREPARED	PREPARED BY	LOT NO. TRIS	LOT NO. EDTA	LOT NO. NACL	LOT NO. SDS	LAB LOT NO.

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(9)

5M NaCl

FORMULA
NaCl

AMOUNT
292.2g

Dissolve NaCl in approximately 800 ml distilled water. Bring to a final volume of 1 liter. Autoclave and store at room temperature.

DATE PREPARED	PREPARED BY	LOT NO. SODIUM CHLORIDE	LAB LOT NO.

Ex 310
(10)

10N NAOH

FORMULA AMOUNT

NAOH 400 g

Dissolve NaOH in distilled water and bring to a volume of 1l with distilled water.

DATE PREPARED	PREPARED BY	LOT NO. NAOH	LAB LOT NO.

Ex 310

(11)

PHENOL\CHLOROFORM\ISOAMYL ALCOHOL

LOT NUMBER	DATE RECEIVED	LOT NUMBER	DATE RECEIVED

Ex 310
(12)

PROTEINASE K

FORMULA	AMOUNT
Proteinase K	100 mg

Dissolve proteinase K in 10 ml distilled water. Aliquot and store frozen at - 20^0C.

DATE PREPARED	PREPARED BY	LOT NO. PROTEINASE K	LAB LOT NO.

Ex 310
(13)

SARKOSYL

FORMULA	AMOUNT
Sarkosyl (N-laurylsarcosine, sodium salt)	20 g

Dissolve sarkosyl in 50 ml of distilled water. When in solution, bring to a final volume of 100 ml with distilled water. Filter sterilize.

DATE PREPARED	PREPARED BY	LOT NO. OF SARKOSYL	LAB LOT NO.

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(14)

20% (w/v) SODIUM DODECYL SULFATE

FORMULA	AMOUNT
SDS	200 g

Dissolve SDS in 800mls. DI water and bring to volume 1 liter.

DATE PREPARED	PREPARED BY	LOT NO. OF SDS	LAB LOT NO.

Ex 310
(15)

STAIN EXTRACTION BUFFER

FORMULA	AMOUNT
Tris Base	1.21 g
0.5 M $Na_2EDTA \cdot 2H_2O$	20 ml
NaCl	5.84 g
20% SDS	100 ml

Dissolve Tris and NaCl in approximately 500 ml of water. Adjust the PH to 8.0 with HCl. Add SDS and EDTA. Bring to a final volume of 1 l with distilled water.

DATE PREPARED	PREPARED BY	LOT NO. EDTA	LOT NO. NACL	LOT NO. TRIS	LOT NO. SDS	LAB LOT NO.

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TE BUFFER

FORMULA	AMOUNT
Tris-HCl (pH 8.0)	10 ml
0.5M EDTA	0.2 ml

Dilute the Tris HCl and EDTA to a final volume of 1 l with distilled water. Autoclave and store at room temperature.

DATE PREPARED	PREPARED BY	LOT NO. TRIS-HCl	LOT# EDTA	LAB LOT NO.

Ex 310
(17)

TNE

FORMULA	AMOUNT
Tris Base	0.121 g
NaCl	0.548 g
$Na_2EDTA \cdot 2H_2O$	0.037 g

Dissolve the above in approximately 80 ml of distilled water. Adjust PH to 8.0. Bring to a final volume of 100 ml with distilled water. Autoclave.

DATE PREPARED	PREPARED BY	LOT NO. TRIS	LOT NO. NACL	LOT NO. EDTA	LAB LOT NO.

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(18)

1M TRIS-HCL (pH 7.5)

FORMULA	AMOUNT
Tris base	121.1 g

Add Tris base to 800 ml of distilled water and adjust pH to 7.5 with concentrated HCl. Bring to a final volume of 1 liter. Autoclave and store at room temperature.

DATE PREPARED	PREPARED BY	LOT NO. TRIS HCL	LOT NO. EDTA	LAB LOT NO.

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(19)

1M TRIS-HCL (pH 8.0)

FORMULA	AMOUNT
Tris base	121.1 g

Add Tris base to 800 ml of distilled water. Adjust pH to 8.0 with concentrated HCl. Bring to a final volume of 1 liter. Autoclave and store at room temperature.

DATE PREPARED	PREPARED BY	LOT NO. TRIS HCL	LOT NO. EDTA	LAB LOT NO.

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PROTOCOLS FOR THERMAL CYCLER CALIBRATION

I. Temperature Verification Systems

There are a variety of Temperature Verification Systems available. Each consists of a digital thermometer and a specially designed probe that fits the sample wells of a particular type of thermal cycler. These systems are used to perform two tests on the thermal cyclers: the Calibration Verification Test (performed monthly) and the Temperature Non-Uniformity Test (performed semi-annually).

The Temperature Verification Systems themselves are subject to factory-approved recalibration on an annual basis. Follow the manufacturer's instructions for the proper decontamination, packaging, payment and shipping protocols. Use the Temperature Verification System Calibration Record form to document the recalibration.

II. Calibration Verification Tests

This test checks the temperature of the sample block against the equipment specifications for temperature accuracy. This test should be performed on each thermal cycler on a monthly basis.

DNA Thermal Cycler 480

1. Turn on the Thermal Cycler 480 and let it warm up for 15 minutes.

2. Create a two-temperature Step-Cycle file with the following parameters:

   Segment #1 Temperature = 95 °C
   Segment #1 Time = 3:30 minutes
      Optional step: Hold 1 minute at 20 °C (recommended for instruments with serial numbers above 30000)

   Segment #2 Temperature = 40 °C
   Segment #2 Time = 3:30 minutes

   Segment #3 Temperature = 0 °C
   Segment #3 Time 0:00 minutes

   Auto segment extension: Off
   Cycle count: 99
   Link to shut-off: (0)

   Note: Three minutes is the recommended time for the probe to reach its maximum accuracy. The block, however, reaches the setpoint within the first minute.

3. Coat wells C1 and C2 with mineral oil using a cotton swab.

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4. Place the probe in the sample block so that the black cone fits into sample well C1 and the silver cone fits into sample well C2. Make sure the probe is connected to the digital thermometer.

5. Close the sample block cover and place the one pound weight on the cover. This ensures that the probes are seated correctly in the sample wells.

6. Turn on the digital thermometer.

7. Run the two-temperature Step-Cycle file that was set up in step 2.

8. On the third cycle, measure the temperature of well C1 when the time remaining in Segment #1 is 30 seconds. Record this temperature on the Calibration Verification Test Data Sheet.

9. Also on the third cycle, measure the temperature of well C1 when the time remaining in Segment #2 is 30 seconds. Also record this temperature on the test data sheet.

10. Use the following formulae to calculate the average block temperature at the 95 °C and 40 °C holds:

    Block average at 95 °C = Recorded Value – High Offset*

    Block average at 40 °C = Recorded Value – Low Offset*

    *Obtained from the Thermal Cycler Users Manual (the offset is the number of degrees Celsius that the temperature of well C1 differed from the average temperature of the block when the instrument was calibrated at the factory. Record these values on the test data sheet also.

11. If a block average is within 1 °C of the target temperature, the test is passed. If a block average is not within this range the test is failed and the thermal cycler needs to be serviced. Record the pass/fail test result on the test data sheet and on the calibration record sheet.

12. Remove the probe from the sample block, turn off the digital thermometer and swab any residual oil from the sample wells. Turn off the thermal cycler.

GeneAmp PCR System 2400

1. Place the probe frame on to the sample block. Coat well B4 of the sample block with mineral oil and place temperature probe this well. (Note: If the probe assembly has a "dummy" probe, position this in well B5.) Thread the probe lead wire through the slot in the frame and close the heated cover.

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2. Connect temperature probe to the digital thermometer and turn the thermometer on. Turn on the thermal cycler.

3. Press the following keys in order to select the appropriate function on the thermal cycler:

   F4 – Util.
   F3 – Diag.
   F3 – TmpVer.
   F1 – Temp.
   F1 – Run.

4. The thermal cycler will ramp to 92 °C and stabilize at this temperature for three minutes. When prompted, record the actual temperature displayed on the digital thermometer on the Calibration Verification Test Data Sheet and via the thermal cycler keypad.

5. The thermal cycler will repeat the same process for the 56 °C and 20 °C setpoints. Record and enter the actual temperatures when prompted.

6. The thermal cycler will then compare the temperatures entered to verify that they are within range for the calibration. If the calibration is good, the screen will display a "Calibration is Good" message. If the results are deemed to be outside of parameters, the thermal cycler will display an, "Instrument may require service. Contact your Perkin Elmer Service Engineer", message. The thermal cycler needs to be serviced. Record the pass/fail result on the test data sheet and on the calibration record sheet.

7. When finished, press "F5 – Exit", remove the probe and turn off the digital thermometer. Remove the probe frame and any residual oil from the wells. Turn off the thermal cycler.

GeneAmp PCR System 9600

1. Place the probe frame on to the sample block. Coat well E1 of the sample block with mineral oil and place the temperature probe in this well. (Note: If the probe assembly has a "dummy" probe, position this in well D1.) Thread the probe lead wire through the slot in the frame and close the heated cover.

2. Connect the temperature probe to the digital thermometer and turn the thermometer on. Turn on the thermal cycler.

3. Press the following keys in order to select the appropriate function:

   Hit OPTION three times to select "UTIL", press enter
   Hit OPTION two times to select "DIAG", press enter

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Press “5” to select the “VERIFY CALIBRATION” test, press enter.

4. The sample block and the heated cover will go to 40 °C. When the temperature is reached, the screen will display a message instructing you to wait 3 minutes. After 3 minutes, a “Record Temperature” message will appear. At this time, record the temperature displayed on the digital thermometer on the Calibration Verification Test Data Sheet. Press Enter.

5. The sample block and heated cover will then go to 95 °C. Repeat the steps taken in step 4 for this temperature and record this value on the test data sheet. Press Enter.

6. Use the following formulas to calculate the average block temperature at the 40°C and 95°C holds:

   Block average at 40 °C =Recorded Value – Low Offset*
   Block average at 95 °C = Recorded Value – High Offset*

   *From the Thermal Cycler User’s Manual, the offset is the number of degrees Celsius that the temperature of well E1 differed from the average temperature of the block when the instrument was calibrated by the factory. Record these values on the test data sheet also.

7. If a block average is within 0.75 °C of its target temperature, the test is passed. If a block average is not within this range, the test is failed and thermal cycler needs to be serviced. Record the pass/fail result on the test data sheet and on the calibration record sheet.

8. Remove the probe from the sample block and turn off the digital thermometer. Remove the probe frame and swab any residual oil from the sample wells. Turn off the thermal cycler.

GeneAmp PCR System 9700

1. Place the probe frame on the sample block. Coat well A6 of the sample block with mineral oil and place the temperature probe in this well. Thread the probe lead wire through the slot in the frame and close the heated cover.

2. Connect the temperature probe to the digital thermometer and turn on the digital thermometer.

3. Turn on the thermal cycler. Press the following keys in order to select the appropriate function:

   Press F4 (Util).
   Press F1 (Diag).
   Press F3 (TmpVer).

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Press F1 (Temp).
Press F1 (Run).

4. The Calibration Verification screen will appear and the setpoint value will be displayed. The lid will warm to within 1 °C of 105 °C before the block heats to 85 °C. When the temperature is reached and stabilized, read the thermometer and enter the value (rounded to three digits) via the keypad. Also enter this value on the Calibration Verification Test Data Sheet to document the results.

5. The sample block will then cool to 45 °C. Once the temperature has stabilized, read the thermometer and enter the value (rounded to three digits) via the keypad. Also enter this value on the test data sheet.

6. Confirm that the displayed values match those recorded on the test data sheet and press F1 (Accept).

7. The 9700 will evaluate the calibration of the sample block based on the setpoint reading you entered and will display the results of the test. Record the pass/fail results on the test data sheet and on the calibration record sheet. If the test fails, repeat the test and if it fails again, the thermal cycler need to be serviced.

8. Turn off the digital thermometer, Remove the probe from the sample block and turn off the digital thermometer. Remove the frame and swab any residual oil from the sample well. Turn off the thermal cycler.

III. Temperature Non-Uniformity Tests

This test checks the uniformity of the sample block temperature from well to well. This test should be performed on each thermal cycler on a semi-annual basis.

DNA Thermal Cycler 480

1. Turn on the Thermal cycler and let it warm up for at least 15 minutes.

2. Create a two-temperature Step-Cycle file with the following parameters:

   Segment #1 Temperature = 95 °C
   Segment #1 Time = 1:00 minutes

   (Optional: Hold for 1 minute at 20 °C)

   Segment #2 Temperature = 40 °C
   Segment #2 Time = 1:00 minutes

   Segment #3 Temperature = 0 °C
   Segment #3 Time 0:00 minutes

   Auto Segment Extension: Off

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Cycle Count = 99
Link to the Shut-off (0)

3. Coat all the wells in sample block rows A, C, and F with mineral oil using a cotton swab.

4. With the probe connected to the digital thermometer, place the probe in the sample block so that the black cone fits into sample well A1 and the silver cone fits into sample well A2.

5. Close the sample block cover and place the one-pound weight on the cover. This ensures that the probe is seated correctly in the sample wells. Do not use excessive force when closing the covers.

6. Turn on the digital thermometer.

7. Run the two-temperature Step-Cycle file that was created in step 2.

8. In the third cycle, measure the temperature of well A1 when the time remaining in Segment #1 is 0:00 seconds. Record the temperature on the Temperature Non-Uniformity Test Data Sheet.

9. Still in the third cycle, measure the temperature of well A1 when the time remaining in Segment #2 is 0:00 seconds. Record this temperature on the test data sheet.

10. Remove the weight, lift the block cover, and move the black cone of the probe to well A3 and place the silver cone in well A4.

11. Close the cover, replace the weight, and repeat the measurements in Segment #1 and Segment #2 in the fourth cycle.

12. Repeat these measurements on wells A6, A8, C1, C3, C6, C8, F1, F3, F6, and F8. Be sure to place the black cone of the probe assembly into these wells and the silver cone into an adjacent well in the same horizontal row.

13. Subtract the lowest measured temperature from the highest measured temperature at both the 95 °C hold and the 40 °C hold. Record these values on the test data sheet. If any result is greater than 1 °C the thermal cycler needs to be serviced. Record the pass/fail result on the test data sheet and on the calibration record sheet.

14. Compute the average of the 12 measurements at both the 95 °C hold and the 40 °C hold. Record these values on the test data sheet. If either average is more that 1 °C above or below the target temperature, perform a temperature calibration verification test.

15. Remove the probe from the sample block and turn off the thermometer. Swab any residual oil from the sample wells and turn off the thermal cycler.

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GeneAmp PCR System 2400

1. Place the probe frame on to the sample block. Coat all wells of the sample block with mineral oil and place temperature probe in well A2. (Note: If the probe assembly has a "dummy" probe, position this in well A3.) Thread the probe lead wire through the slot in the frame and close the heated cover.

2. Connect temperature probe to the digital thermometer and turn the thermometer on. Turn on the thermal cycler.

3. Press the following keys in order to select the appropriate function:

   F4 – Util.
   F3 – Diag.
   F3 – TmpVer.
   F2 – TNU.
   F1 – Run.

4. The thermal cycler will ramp to 94°C and stabilize at this temperature for thirty seconds. When prompted, record the actual temperature reading displayed on the digital thermometer on the Temperature Non-Uniformity Test Data Sheet and via the keypad.

5. The thermal cycler will repeat the same process for the 37°C setpoint. Record and enter the actual temperature when prompted.

6. The thermal cycler will then ask you to move the temperature probe to well A7 (dummy probe in A6). Repeat steps 4 and 5 for this position.

7. Repeat steps 4 and 5 for the remaining positions as prompted on the screen.

8. After all positions have been checked, a summary of the temperatures entered will be displayed. If this is correct, press "Accept." If incorrect, press "Cancel" and repeat the test.

9. The thermal cycler will then compare the temperatures entered to verify that they are sufficiently uniform. The screen should display a message telling you whether the thermal cycler has passed the test. Record this result on the test data sheet and on the calibration record sheet.

10. If the results are deemed to be outside of parameters, the thermal cycler will display a "Fail" message. The thermal cycler needs to be serviced.

11. When finished, press "F5 – Exit", remove the probe and probe frame, swab any residual oil from the wells. Turn off the thermal cycler.

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GeneAmp PCR System 9600

1. Place the probe frame on to the sample block. Coat all wells in rows A, C, E, and H of the sample block with mineral oil and place the temperature probe in well A1. (Note: If the probe assembly has a "dummy" probe, position this in well A2.) Thread the probe lead wire through the slot in the frame and close the heated cover.

2. Connect the temperature probe to the digital thermometer and turn the thermometer on. Turn on the thermal cycler.

3. Set up a two-temperature cycle program with the following parameters (this program may be saved and reused in the future)

   Setpoint #1 Temperature = 95 °C
   Hold time = 2:00 minutes
   Ramp time = 0:00 minutes

   Setpoint #2 Temperature = 40 °C
   Hold time = 2:00 minutes
   Ramp time = 0:00 minutes

   Cycles = 99

4. Run the program created in step 3. On the third cycle, measure the temperature of well A1, using the digital thermometer, when the time remaining in setpoint #1 (95 degrees) is 30 seconds. Record the temperature on the Temperature Non-Uniformity Test Data Sheet.

5. Still in the third cycle, measure the temperature of well A1 when the time remaining in setpoint #2 (40 degrees) is 30 seconds. Record the temperature on the test data sheet.

6. After completing the measurement, open the heated cover and move the probe into well A4 (dummy probe in well A5). Close the heated cover and repeat the temperature measurements for this well during the next cycle.

7. Repeat step #4 for the following wells: A8, A12, C1, C4, C8, C12, E1, E4, E8, E12, H1, H4, H8, and H12 (dummy probe in a well adjacent to each well you are measuring). Record all temperatures on the test data sheet.

8. Subtract the lowest measured temperature from the highest measured temperature at both the 95 °C and 40 °C hold. Record these values on the test data sheet. If any result is greater than 1 °C, the thermal cycler needs to be serviced. Record the pass/fail result on the test data sheet and on the calibration record sheet.

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9. Remove the probe from the sample block and turn off the thermometer. Remove the frame and swab any residual oil from the sample wells. Turn off the thermal cycler.

GeneAmp PCR System 9700

1. Place the probe frame on the sample block. Coat wells A1, A12, C4, C9, F4, F9, H1 and H12 of the sample block with mineral oil. Place the temperature probe in well A1. Thread the probe lead wire through the slot in the frame and close the heated cover.

2. Connect the temperature probe to the digital thermometer and turn the thermometer on. Turn on the thermal cycler.

3. Press the following keys in order to select the appropriate function:

   Press F4 (Util).
   Press F1 (Diag).
   Press F3 (TmpVer).
   Press F2 (TNU).
   Press F1 (Run).

4. The TNU (Temperature Non-Uniformity) screen will appear and the setpoint value will be displayed. The lid will warm to within 1 °C of 35 °C before the block heats to 37 °C. Once the temperature has stabilized, read the thermometer and enter the value (rounded to three digits) via the keypad. Also enter this value on the Temperature Non-Uniformity Test Data Sheet to document the results.

5. The sample block will then cycle to 94 °C and back to 37 °C for sequential readings. Follow the instructions on the screen for moving the probe to each of the remaining test wells, entering the values via the keypad and on the test data sheet.

6. Once each of the 37 °C readings have been entered, the heated lid will warm to within 1 °C of 105 °C and the sample block will cycle between 37 °C and 94 °C for additional readings. Follow the instructions on the screen for moving the probe to each of the eight test wells, entering values via the keypad and on the test data sheett.

7. Once all the readings have been entered, confirm that the displayed values match those recorded on the test data sheet and press F1 (Accept).

8. The 9700 will display the results of the test. Record the pass/fail result on the test data sheet and on the calibration record sheet. If the test fails, repeat the test and if it fails again, the thermal cycler needs to be serviced.

9. Press F5 (Cancel) to exit the test.

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10. Remove the probe from the sample block and turn off the digital thermometer. Remove the probe frame and swab any residual oil from the sample wells. Turn off the thermal cycler.

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IV. Thermal Cycler Test Forms

Test Data Sheets

The following Test Data Sheets may be found on the following pages. Use these sheets to record data obtained during the testing procedures.

Calibration Verification Test – 480
Calibration Verification Test – 2400
Calibration Verification Test – 9600
Calibration Verification Test – 9700

Temperature Non-Uniformity Test – 480
Temperature Non-Uniformity Test – 2400
Temperature Non-Uniformity Test – 9600
Temperature Non-Uniformity Test – 9700

Record Sheets

The following Record Sheets are found on the following pages. Use these sheets to document that the tests have been performed on the equipment.

Temperature Verification System Calibration – for all thermometers and probes
Thermal Cycler Temperature Tests – for all thermal cyclers

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Data Sheet: Calibration Verification Test – 480

DOJ Number: ____________ **High Offset Value:** ______

Low Offset Value: ______

Instructions: Run the Calibration Verification Test monthly. Record the setpoint values for well C1 on this data sheet. At the end of the Calibration Verification Test, calculate the block average at each setpoint by subtracting the appropriate offset value. The block averages must be within 1 °C of the setpoints in order to pass the test. Also record the pass/fail result.

Date	Tested By	Temperature Verification System DOJ #	Setpoint Value: Well C1		Result
			95 °C	40 °C	
February					
April					
June					
August					
October					
December					

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Data Sheet: Calibration Verification Test – 2400

DOJ Number: ____________

Instructions: Run the Calibration Verification Test monthly. Record the setpoint values for well B4 on this data sheet. At the end of the Calibration Verification Test, check the values displayed on the system 2400 against the values recorded here. Also record the pass/fail test result.

Date	Tested By	Temperature Verification System DOJ #	Setpoint Value: Well B4			Result
			92 °C	56 °C	20 °C	
February						
April						
June						
August						
October						
December						

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Data Sheet: Calibration Verification Test – 9600

DOJ Number: ____________ **High Offset Value:** ______

Low Offset Value: ______

Instructions: Run the Calibration Verification Test monthly. Record the setpoint values for well C1 on this data sheet. At the end of the Calibration Verification Test, calculate the block average at each setpoint by subtracting the appropriate offset value. The block averages must be within 0.75 °C of the setpoint values in order to pass the test. Also record the pass/fail result.

Date	Tested By	Temperature Verification System DOJ #	Setpoint Value: Well E1		Result
			40 °C	95 °C	
February					
April					
June					
August					
October					
December					

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Data Sheet: Calibration Verification Test – 9700

DOJ Number: ____________

Instructions: Run the Calibration Verification Test monthly. Record the setpoint values for well A6 on this data sheet. At the end of the Calibration Verification Test, check the values displayed on the system 9700 against the values recorded here. Also record the pass/fail test result.

Date	Tested By	Temperature Verification System DOJ #	Setpoint Value: Well A6		Result
			85 °C	45 °C	
February					
April					
June					
August					
October					
December					

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Data Sheet: Temperature Non-Uniformity Test – 480

DOJ Number: ____________

Instructions: Run the Temperature Non-Uniformity Test semi-annually. Record the setpoint values for the wells listed on this data sheet. Calculate and record the greatest difference between wells at each setpoint and the average of all wells at each setpoint. Determine and record the pass/fail test results (±1 °C).

Date		
Tested by		
Temp.Ver.Sys DOJ #		
	Setpoint Values	
Sample Well	95 °C	40 °C
A1		
A3		
A6		
A8		
C1		
C3		
C6		
C8		
F1		
F3		
F6		
F8		
Max – Min		
Average		
Results		

Date		
Tested by		
Temp.Ver.Sys DOJ #		
	Setpoint Values	
Sample Well	95 °C	40 °C
A1		
A3		
A6		
A8		
C1		
C3		
C6		
C8		
F1		
F3		
F6		
F8		
Max – Min		
Average		
Results		

Ex 310
(36)

Data Sheet: Temperature Non-Uniformity Test – 2400

DOJ Number: ____________

Instructions: Run the Temperature Non-Uniformity Test semi-annually. Record the setpoint values for the wells listed on this data sheet. At the end of the Temperature Non-Uniformity Test, check the values displayed on the system 2400 against the values recorded here. Also record the pass/fail test result.

Date		
Tested by		
Temp.Ver.Sys DOJ #		
	Setpoint Values	
Sample Well	94 °C	37 °C
A2		
A7		
B1		
B4		
B8		
C2		
C7		
Results		

Date		
Tested by		
Temp.Ver.Sys DOJ #		
	Setpoint Values	
Sample Well	94 °C	37 °C
A2		
A7		
B1		
B4		
B8		
C2		
C7		
Results		

Ex 310
(37)

Data Sheet: Temperature Non-Uniformity Test – 9600

DOJ Number: ____________

Instructions: Run the Temperature Non-Uniformity Test semi-annually. Record the setpoint values for the wells listed on this data sheet. Calculate and record the greatest difference between wells at each setpoint. Determine and record the pass/fail test results (±1 °C).

Date		
Tested by		
Temp.Ver.Sys DOJ #		
	Setpoint Values	
Sample Well	95 °C	40 °C
A1		
A4		
A8		
A12		
C1		
C4		
C8		
C12		
E1		
E4		
E8		
E12		
H1		
H4		
H8		
H12		
Max - Min		
Results		

Date		
Tested by		
Temp.Ver.Sys DOJ #		
	Setpoint Values	
Sample Well	95 °C	40 °C
A1		
A4		
A8		
A12		
C1		
C4		
C8		
C12		
E1		
E4		
E8		
E12		
H1		
H4		
H8		
H12		
Max - Min		
Results		

Ex 310
(38)

Data Sheet: Temperature Non-Uniformity Test – 9700

DOJ Number: ____________

Instructions: Run the Temperature Non-Uniformity Test semi-annually. Record the setpoint values for the wells listed on this data sheet. At the end of the Temperature Non-Uniformity Test, check the values displayed on the system 9700 against the values recorded here. Also record the pass/fail test result.

Date		
Tested by		
Temp.Ver.Sys DOJ #		
	Setpoint Values	
Sample Well	37 °C	94 °C
A1		
A12		
C4		
C9		
F4		
F9		
H1		
H12		
Results		

Date		
Tested by		
Temp.Ver.Sys DOJ #		
	Setpoint Values	
Sample Well	37 °C	94 °C
A1		
A12		
C4		
C9		
F4		
F9		
H1		
H12		
Results		

Ex 310
(39)

Calibration Record: Temperature Verification System - __________

DOJ Number: ____________

Instructions: The Temperature Verification Systems for all thermal cyclers must be returned for factory approved calibration every year.

Probe SN	Thermometer SN	Initials	Calibration Date	Comments

Ex 310
(40)

Calibration Record: Thermal Cycler Temperature Tests - __________

DOJ Number: ____________

Instructions: The Calibration Verification Test must be performed monthly. The Temperature Non-Uniformity Test must be performed semi-annually.

Calibration Verification Test			Temperature Non-Uniformity Test			Comments
Initials	Date	Results	Initials	Date	Results	

Ex 310
(41)

STR TYPING KITS

1. Each lot of kits must be tested in-house using the routine positive and negative controls from the kits and additionally one positive control whose types have been repeatedly characterized in-house.

2. If any of the controls do not produce the expected types, the Supervisor must be notified and the kits cannot be used for casework.

Ex 310
(42)

PROFILER PLUS TYPING KIT
(PERKIN ELMER)

LOT NO. OF KIT	QC PERFORMED BY:/PASS OR FAIL	DATE OF QC	EXP. DATE OF KIT

Ex 310
(43)

COFILER TYPING KIT
(PERKIN ELMER)

LOT NO. OF KIT	QC PERFORMED BY:/PASS OR FAIL	DATE OF QC	EXP. DATE OF KIT

Ex 310
(44)

POWERPLEX 16
PROMEGA

LOT NO. OF KIT	QC PERFORMED BY:/PASS OR FAIL	DATE OF QC	EXP. DATE OF KIT

Ex 310
(45)

TAQ GOLD

New lots of Taq must be tested to ensure correct amplification of target DNA. Each new lot of Taq must be used to amplify the positive DNA control provided with each commercial kit. If the positive control samples fail to give the correct types, the Technical Unit Leader and/or Supervisor should be notified and the lot may not be used for casework.

Kit Lot number	TAQ lot number	QC completed By/Date	Beginning Use Date

Ex 310
(46)

Promega DNA™ IQ System QC

New lots of IQ kits must be tested to ensure they are functioning properly prior to use on casework samples. A non-casework standard sample (e.g. and IPS sample) should be extracted and quantitated to show human DNA was obtained using the IQ extraction kit. Identification of DNA by the human quantitation system will qualify a new IQ kit lot for use in casework. If human DNA is not obtained, the new lot may not be used for casework.

LOT NUMBER	QC COMPLETED BY/DATE	BEGINNING USE DATE
	Ex 310	

POWERPLEX 16 AND POWERPLEX Y
PROMEGA

LOT NO. OF KIT	QC PERFORMED BY:PASS OR FAIL	DATE OF QC	EXP. DATE OF KIT

Ex 310
(48)

EQUIPMENT MAINTENANCE, CALIBRATION, AND TEMPERATURE MONITORING SCHEDULE FOR PCR BASED EQUIPMENT

ANNUALLY

Temperature Verification Units (External Calibration)
Rainin pipettes (External Calibration)
Balances (External Calibration)
Water systems - Madison (External)

SEMIANNUALLY

Thermal Cycler (Non) Uniformity Test
Water Systems - (change final filter)

MONTHLY

Clean amplification labs
Temperature Verification Calibration of Thermal Cyclers
Balances (Internal calibration)

WEEKLY

Incubators (record temperature)
Refrigerators (record temperature)

DAY OF USE

Shaking Water Bath
Dry Block Heaters
pH meters - Milwaukee (calibration)
Autoclave - Madison
pH meters - Madison (calibration)

AS INDICATED

Water Systems - Milwaukee

Rainin pipettes

Pipettes will be calibrated annually by an outside vendor.

Ex 310
(49)

Temperatures

Temperatures of the equipment listed below will be monitored weekly using NIST traceable thermometers. If the temperature read by thermometer is not within an acceptable range, appropriate adjustments should be made and the temperature re-checked at a later time. If after re-checking the temperature it remains unacceptable, the Supervisor or Unit Leader should be notified.

1. INCUBATORS AND OVENS

   Incubators set to 37 ^{0}C (37 ^{0}C ± 2 ^{0}C)
   Incubators set to 56 ^{0}C (56 ^{0}C ± 2 ^{0}C)

2. REFRIGERATORS AND FREEZERS

   Refrigerators (4^0 C ± 3 ^{0}C)
   Freezers (-20 ^{0}C ± 5 ^{0}C)

   Evidence Freezers (-5^0 C or lower)
   Evidence Refrigerators (5^0 C or lower)

Thermometers

NIST traceable thermometers must be used to calibrate all equipment. A list of all thermometers and their certification numbers will be maintained. Thermometers will be replaced on or before the certification expiration date.

Ex 310
(50)

Water Baths/Dry Block Heaters

Temperatures of the water baths and dry block heaters must be monitored on the day of use. When in use, water bath temperatures should be maintained at 50 ^{0} C ± 1^0 C. If this temperature is not maintained while in use, appropriate adjustments should be made to ensure that the water bath maintains the proper temperature.

Balances

Balances are calibrated monthly in-house. Balances are serviced by an external source on an annual basis.

Water Systems

Maintenance is based on electronic monitoring devices located in each instrument. The final filters will be changed once every six months or sooner.

pH meters

pH meters should be calibrated in accordance with the manufactures recommendations.

Ex 310
(51)

EQUIPMENT RECORD

INSTRUMENT	DOJ NO.	TEMP DATE/BY	TEMP DATE/BY	TEMP DATE/BY	TEMP DATE/BY

Ex 310
(52)

THERMOMETERS

NIST traceable thermometers must be used to calibrate all equipment whose temperature is critical to the analysis. A list of those thermometers and their certification numbers will be maintained.

TYPE	NIST #	TEMP. RANGE	LOCATION	RECORDED BY

Ex 310
(53)

AUTOCLAVE

Each time that the autoclave is used, it must be checked with an appropriate temperature indicator.

DATE	Temperature OK	DATE	Temperature OK	DATE	Temperature OK

Ex 310
(54)

BALANCES

Balance performance must be verified on a monthly basis. External calibration and cleaning is done on an annual basis.

DOJ Number	Date of Performance Verification/Calibration	Performed by:

Ex 310
(55)

pH METER

The pH meter should be calibrated according to the manufactures recommendations.

Date	Performed by	Date	Performed by

Ex 310
(56)

RAININ PIPETTE CALIBRATION LOG

MODEL	SERIAL NO.	USER	DATE CALIBRATED			

Ex 310
(57)

TEMPERATURE RECORD

Equipment:________________ Temperature Range:_________to_________ Year:____________

Month												
Day	°C	Initials	°C	Initials	°C	Initials	°C	Initials	°C	Initials	°C	Initials
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
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30												
31							Ex 310					

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CHAPTER V

CRITICAL REAGENTS

(See Chapter III for QC of STR typing kits)

Ex 310
(59)

QUANTIFILER HUMAN AND Y QUANTIFICATION KIT QC

New lots of Human and Y Quantifiler kits must be tested to ensure all kit components work properly. A printout of each kit lot should include one set of standards in duplicate, the slope and R^2 values. All values should be within the expected range. If any values fall outside the expected ranges, the technical leader and/or supervisor should be notified and the kit lot should be re-evaluated before use on casework.

LOT NO. OF KIT	QC PERFORMED BY:/PASS OR FAIL	DATE OF QC	EXP. DATE OF KIT

Ex 310
(60)

PCR BASED ANALYSES

A. EXTRACTION METHODS

1. ORGANIC EXTRACTION OF BODY FLUID STAINS
2. ORGANIC EXTRACTION OF HAIR (CENTRICON)

2a ORGANIC EXTRACTION OF HAIR (MICROCON)

3. DIFFERENTIAL EXTRACTION OF SPERM CELL AND NON SPERM CELL DNA (ORGANIC EXTRACTION)
4. EXTRACTION USING THE DNA IQ KIT
5. EXTRACTION OF BONES

B. QUANTITATION

1. QUANTITATION USING ABI QUANTIFILER KITS

C. PROFILER PLUS/COFILER

1. AMPLIFICATION
2. SET UP OF THE 310 INSTRUMENT
3. SET UP OF THE 3100-*AVANT* INSTRUMENT

D. POWERPLEX 16 & Y

1. AMPLIFICATION
2. SET UP OF THE 310 INSTRUMENT
3. SET UP OF THE 3100 INSTRUMENT

E. INTERPRETATION AND REPORT WRITING

1. INTERPRETATION
2. DOCUMENTATION AND REPORT WRITING

A. EXTRACTION METHODS

Ex 310
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A1. ISOLATION OF DNA FROM BODY FLUID STAINS USING ORGANIC EXTRACTIONS

NOTE A: LIQUID BLOOD STANDARDS ARE CONVERTED INTO STAINS SO THAT ALL SAMPLES ARE PROCESSED SIMILARLY. SATURATE A STAIN CARD WITH THE LIQUID BLOOD. ALLOW TO AIR DRY WITHOUT APPLICATION OF HEAT.

NOTE B: FOR EACH SET OF EXTRACTIONS, A MANIPULATION CONTROL MUST BE PROCESSED IN THE SAME MANNER AS ALL OTHER SAMPLES.

1. Sample the stain using a sterile scalpel blade or a pair of scissors that have been cleaned with an appropriate solvent and distilled water. Place the pieces into a microcentrifuge tube with a minimum volume of 1.5 ml. If the stain is on a swab remove a portion of the swab from the applicator stick. Begin processing the manipulation control at this time.

2. Add the following reagent amounts to the tubes if Microcon 100 microconcentrators are to be used:

   300 μl stain extraction buffer (SEB)
   7.5 μl Proteinase K (Pro K) (10 mg/ml)
   40 μl 0.39M DTT (optional)

   Add the following reagent amounts to the tubes if Centricon 100 concentrators are to be used:

   500 μl stain extraction buffer (SEB)
   12 μl Proteinase K (Pro K) (10 mg/ml)

   Cap tube, mix and centrifuge for 2 seconds to force cut pieces into the liquid.

3. Incubate tubes at 56°C for two hours or overnight.

4. Place the solid material into a spin basket and insert the basket into the microcentrifuge tube. Cap and centrifuge for 5 minutes.

5. In a fume hood, add 300 μl (Microcon 100) or 500 μl (Centricon 100) phenol/chloroform/isoamyl alcohol to the microcentrifuge tube. Shake vigorously by hand or vortex for 2-3 seconds until a milky emulsion is achieved. Centrifuge for 2 to 5 minutes to separate the aqueous and organic phases. If the aqueous phase is cloudy, the sample can be extracted again with phenol/chloroform/isoamyl alcohol.

   Alternatively a 2 ml Phase Lock Gel Tube (PLG) may be used for the organic extraction stage. Pellet PLG at 13,500 x g (max. speed) for 30 sec. Transfer the sample from the extraction tube to the PLG tube. In a fume hood add 300 μl

(Microcon 100) or 500 μl (Centricon 100) phenol/chloroform/isoamyl alcohol to the microcentrifuge tube. Mix by inverting tube until a milky emulsion is achieved. Do not vortex. Centrifuge at maximum speed for 5 minutes to separate the aqueous and organic phases.

6. To a Microcon 100 microconcentrator add 100 μl of TE buffer. Next, transfer the aqueous phase from the microcentrifuge tube to the microconcentrator. (Avoid pipetting organic solvent and contaminates from the interface to the microconcentrator and do not touch the membrane with the pipette tip). Place the microconcentrator on a filtrate tube, cap and centrifuge at 500 x g^1 for 10 minutes.

   To a Centricon 100 concentrator add 1.5 ml of TE buffer. Next, transfer the aqueous phase from the microcentrifuge tube to the concentrator. Place the concentrator on a filtrate tube, cap and centrifuge at 1000 x g^1 for 20 minutes.

   Discard the microcentrifuge tube containing the organic solvent into a biohazard waste container in the hood.

   Carefully remove the concentrator and discard the fluid from the filtrate tube. If fluid remains in the concentrator, the centrifugation time can be extended.

7. Return the concentrator to the filtrate tube and add 200 μl (Microcon) or 2 ml (Centricon) of TE buffer. Replace the cap and centrifuge at 500 x g^1 for 10 minutes (Microcon) or 1000 x g^1 for 20 minutes (Centricon). This wash step can be repeated several times.

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(64)

8. Remove the cap and add 10μl -100 μl (or an appropriate amount) of TE buffer to the concentrator. Remove the concentrator and carefully invert onto a labeled retentate tube. Discard the filtrate tube. Centrifuge the assembly at 500 x g[1] for 5 minutes (Microcon) and 1000 x g[1] for 20 minutes (Centricon). Remove and discard the concentrator and cap the retentate tube.

9. Estimate the quantity of DNA in the sample.

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[1] It may be necessary to convert from centrifugal force (measured in g) to RPMs if your microcentrifuge does not provide this function. This can be accomplished using the following formula:

$RCP = (1.118 \times 10^{-6}) \times radius \times (rpms)^2$ where the radius is the distance measured in mm from the center of rotation to the base of the filtrate tube

A2. ORGANIC EXTRACTION OF HAIR (CENTRICON)

1. Wash hair to reduce surface dirt and contaminants as follows:

   For unmounted hairs, remove hair from evidence bag with clean forceps and wash by immersing it in a 50 ml beaker of sterile deionized water. Wash each hair separately in fresh water.

   For mounted hairs, freeze the slide in a -20^{0}C. freezer for 20 minutes. Remove the cover slip with a scalpel. Alternatively, the hair may be removed by soaking the slide in xylene for several hours after cracking the cover slip with a diamond scribe. Wash away the mounting medium using a pasteur pipette filled with xylene. Remove the hair with a clean forceps and wash in a microfuge tube with absolute ethanol, then rinse, using a microfuge tube filled with sterile water.

2. Examine the hair under a stereo microscope for the presence of any contaminating body fluids. If present, repeat the above washing procedure. Cut off about 5-10 mm of the proximal (root) end for digestion, and 5-10 mm of the adjacent shaft as a control. The remainder may be retained for remounting.

3. Add the following reagent amounts to the microcentrifuge tube along with the hair sample:

   500 μl hair digestion buffer
   20 μl 1M dithiothreitol
   15 μl Proteinase K

   Incubate at 56^{0}C. for 6 to 8 hours and vortex for 30 seconds.

4. Add to the sample an additional 20 μl of 1M Dithiothreitol and 15 μl of Proteinase K. Incubate at 56^{0}C. for 6 to 8 hours or overnight until hair is completely dissolved. Vortex for 30 seconds.

5. Centrifuge the sample for 1 minute at 10,000 to 15,000 rpms (maximum speed) at room temperature to remove pigments and particles. Transfer supernatant to a new microcentrifuge tube with sterile 1 ml disposable pipette tip.

6. To the 600 μl lysed and digested hair sample, add 600 μl of the phenol/chloroform/isoamyl alcohol reagent. Cap and vortex for 15 seconds or until an emulsion forms.

   Alternatively a 2 ml Phase Lock Gel Tube (PLG) may be used for the organic extraction stage. Pellet PLG at 13,500 x g (max. speed) for 30 sec. Transfer the sample from the extraction tube to the PLG tube. In a fume hood add 600 μl (Centricon 100) phenol/chloroform/isoamyl alcohol to the microcentrifuge tube. Mix by inverting tube until a milky emulsion is achieved. Do not vortex. Centrifuge at

maximum speed for 5 minutes to separate the aqueous and organic phases.

7. Spin the sample in a microcentrifuge for 3 to 5 minutes at 10,000 to 15,000 rpms (maximum speed) at room temperature, to separate the two phases.

8. Wash and concentrate the DNA solution as follows:

   Assemble the Centricon 100 unit according to the manufacture's directions and label the units. Add 1.5 ml TE buffer to the upper reservoir of the Centricon 100 concentrator. Add the entire aqueous layer to the top of the TE buffer. Cover the Centricon units loosely with Centricon caps. Centrifuge in a fixed angle centrifuge at 1000g for 20 minutes. Discard the tube containing the organic solvent into a biohazard waste container in the hood.

9. Discard the effluent from the lower reservoir of the Centricon 100 units. Add 2 ml of TE buffer to the upper reservoir and spin 1000g for 20 minutes. Repeat this step one time.

10. Discard the effluent from lower reservoirs of the Centricon 100 units, invert the unit and centrifuge at 500g for 2 minutes to concentrate the approximately 40 μl samples into labeled retentate cups. If the samples have not reduced to about 40 μl the centrifuge times can be increased. Estimate the quantity of DNA in the sample.

11. Estimate the quantity of DNA in the sample.

A2a. ORGANIC EXTRACTION OF HAIR (MICROCON)

1. Wash hair to reduce surface dirt and contaminants as follows:

   For unmounted hairs, remove hair from evidence bag with clean forceps and wash by immersing it in a 50 ml beaker of sterile deionized water. Wash each hair separately in fresh water.

   For mounted hairs, freeze the slide in a -20^{0}C. freezer for 20 minutes. Remove the cover slip with a scalpel. Alternatively, the hair may be removed by soaking the slide in xylene for several hours after cracking the cover slip with a diamond scribe. Wash away the mounting medium using a pasteur pipette filled with xylene. Remove the hair with a clean forceps and wash in a microcentrifuge tube with absolute ethanol, then rinse, using a microcentrifuge tube filled with sterile water.

2. Examine the hair under a stereo microscope for the presence of any contaminating body fluids. If present, repeat the above washing procedure. Cut off about 5-10 mm of the proximal (root) end for digestion, and 5-10 mm of the adjacent shaft as a control. The remainder may be retained for remounting.

3. Add the following reagent amounts to the microcentrifuge tube along with the hair sample:

   300 μl hair digestion buffer
   12μl 1M dithiothreitol
   9μl Proteinase

   Incubate at 56^{0}C. for 6 to 8 hours and vortex for 30 seconds.

4. Add to the sample an additional 12 μl of 1M Dithiothreitol and 9 μl of Proteinase K. Incubate at 56^{0}C. for 6 to 8 hours or overnight until hair is completely dissolved. Vortex for 30 seconds.

5. Centrifuge the sample for 1 minute at 10,000 to 15,000 rpms (maximum speed) at room temperature to remove pigments and particles. Transfer supernatant to a new microcentrifuge tube.

6. To the 342 μl lysed and digested hair sample, add 350 μl of the phenol/chloroform/isoamyl alcohol reagent to the microcentrifuge tube The microcentrifuge tube can be shaken vigorously by hand or vortex for 2-3 seconds until a milky emulsion is achieved.

   Alternatively a 2 ml Phase Lock Gel Tube (PLG) may be used for the organic extraction stage. Pellet PLG at 13,500 x g (max. speed) for 30 sec. Transfer the sample from the extraction tube to the PLG tube. In a fume hood add 350 μl (Microcon 100) phenol/chloroform/isoamyl alcohol to the microcentrifuge tube. Mix

by inverting tube until a milky emulsion is achieved. Do not vortex. Centrifuge at maximum speed for 5 minutes to separate the aqueous and organic phases.

7. Centrifuge at maximum speed for 5 minutes to separate the aqueous and organic phases.

8. Wash and concentrate the DNA solution as follows:

   Assemble the Microcon 100 unit according to the manufacture's directions and label the units. Add 100 μl TE buffer to the upper reservoir of the microcon 100 concentrator. Add the entire aqueous layer to the top of the TE buffer. Cover the microcon units loosely with microcon caps. Centrifuge in a fixed angle centrifuge at 500g for 10 minutes. Discard the tube containing the organic solvent into a biohazard waste container in the hood.

9. Discard the effluent from the lower reservoir of the microcon 100 units. Add 200 μl TE buffer to the upper reservoir and spin at 500xg for 10 minutes. Repeat this step at least one time.

10. Remove the cap from the microcon 100 unit and add 10-100 μl (or an appropriate amount) of TE buffer to the unit. Remove the concentrator from the filtrate tube and carefully invert the concentrator into a retentate tube. Discard the filtrate tube. Centrifuge at 500xg for 5 minutes. Discard the concentrator. Cap the retentate tube.

11. Estimate the quantity of DNA in the sample.

A3. DIFFERENTIAL EXTRACTION OF SPERM CELL AND NON SPERM CELL DNA (ORGANIC EXTRACTION)

1. Place the outer layer of the swab from the applicator stick or the fabric cutting into a microcentrifuge tube and add the following amount of reagents.

   400 μl Tris-NaCl-EDTA (TNE)
   25 μl 20% Sarkosyl
   75 μl H_2O
   5 μl ProK (10 mg/ml)

   Begin processing the manipulation control at this time.

   Mix and incubate at 37^0C for 2 hours.

2. Place the solid material in a spin basket. Centrifuge for 5 minutes (maximum speed).

3. Spin the tube in a microcentrifuge on high for 1 minute to further solidify the pellet.

4. Remove and save the supernatant fluid in a separate tube (non-sperm fraction). Be careful not to disturb the pellet on the bottom of the tube.

5. Wash the cell pellet by resuspending it in 500 μl cell pellet wash buffer, vortex the tube briefly, and centrifuge at maximum speed for 5 minutes. Remove and discard the supernatant, being careful not to disturb the cell pellet. Repeat this step for a total of three to five washes of the cell pellet. (Note: A sperm slide can be made from a small portion of the pellet and viewed microscopically.) To the tube containing the cell pellet add the following reagents:

   150 μl TNE
   50 μl 20% Sarkosyl
   40 μl 0.39M dithiothreitol (DTT)
   150 μl H_2O
   10 μl ProK

6. Cap the tube, vortex briefly, and spin briefly. Incubate at 37^0C for at least two hours.

7. Centrifuge for 5 minutes to pellet any debris. The supernatant now contains the sperm DNA fraction.

8. Extract the non-sperm DNA fraction and the sperm DNA fraction tubes with 400 μl of phenol/chloroform/isoamyl alcohol. This step must be done in the fume hood. Vortex (low speed) the tube to achieve a milky suspension in the tube. Microcentrifuge for 3 minutes (maximum speed). If the aqueous phase is cloudy

the samples can be extracted again with phenol/chloroform/isoamyl alcohol.

Alternatively a 2 ml Phase Lock Gel Tube (PLG) may be used for the organic extraction stage. Pellet PLG at 13,500 x g (max. speed) for 30 sec. Transfer the sample from the extraction tube to the PLG tube. In a fume hood add 400 μl phenol/chloroform/isoamyl alcohol to the microcentrifuge tube. Mix by inverting tube until a milky emulsion is achieved. Do not vortex. Centrifuge at maximum speed for 5 minutes to separate the aqueous and organic phases.

9. To a Microcon 100 concentrator add 100 μl of TE buffer. Next, transfer the aqueous phase from the tubes to the concentrators. Avoid pippetting organic solvent and contaminates from the interface to the concentrator. Place the spin cap on the concentrator and spin at 500g for ten minutes.

   If Centricon 100 concentrators are used, then use 1.5 ml TE buffer and spin 1000g for 20 minutes. Discard the tube containing the organic solvent into a biohazard waste container in the hood

10. If Microcon 100 concentrator are used, carefully remove the microconcentrator units from the assembly and discard the fluid from the filtrate cups. Return the microconcentrators to the top of the filtrate cups. Remove the spin caps and add 200 μl of TE buffer to the microconcentrator units. Replace the spin caps and spin the assembly in a microfuge at 500g for 10 minutes.

    If Centricon 100 concentrators are used, then discard the effluent from the lower reservoirs. Add 2 ml TE buffer to the upper reservoirs and spin at 1000g for 20 minutes.

    If the samples have not reduced to about 40 μl the centrifuge times can be increased. If PCR inhibitors are believed to be present then multiple washes with TE buffer can be done.

11. Remove the spin cap from the microcon 100 and add 10-100 μl of TE buffer (or an appropriate amount) to the microconcentrator unit. Remove the concentrator from the filtrate cup and carefully invert the concentrator into a labeled retentate cup. Discard the filtrate cup. Spin the assembly in a microcentrifuge at 500g for 5 minutes. Discard the concentrator. Cap the retentate cup.

    If Centricon 100 concentrators are used, then discard the effluent from lower reservoirs, invert the centricon 100 concentrators and centrifuge at 500g for 2 minutes to concentrate the 40 μl samples into labeled retentate cups.

12. Estimate the quantity of DNA in the sample.

A4. ISOLATION OF DNA FROM BODY FLUID STAINS USING THE DNA IQ™ EXTRACTION

NOTE A: LIQUID BLOOD STANDARDS ARE CONVERTED INTO STAINS SO THAT ALL SAMPLES ARE PROCESSED SIMILARLY. SATURATE STAIN CARD WITH THE LIQUID BLOOD. ALLOW TO AIR DRY WITHOUT APPLICATION OF HEAT

NOTE B: FOR EACH SET OF EXTRACTIONS, A MANIPULATION CONTROL MUST BE PROCESSED IN THE SAME MANNER AS ALL OTHER SAMPLES.

NOTE C: FOR SEMEN STAINS THAT REQUIRE A DIFFERENTIAL EXTRACTION OF SPERM CELL AND NONSPERM CELL DNA THE ORIGINAL PROCEDURE (A3) MUST BE FOLLOWED UNTIL THE SEPARATION OF THE FRACTIONS. AT THIS POINT BOTH FRACTIONS WILL BE TREATED AS SEPARATE SAMPLES. BEGIN AT STEP 5.

1. Sample the stain using a sterile scalpel blade or a pair of scissors that have been cleaned with an appropriate solvent and distilled water. Place the pieces into a microcentrifuge tube with a minimum volume of 1.5 ml. If the stain is on a swab remove a portion of the swab from the applicator stick. For databank samples place the sample directly into a spin basket in the microcentrifuge tube. *Note: Small casework samples may also be placed directly in spin baskets.*

   Begin processing the manipulation control sample at this time.

2. Add the appropriate amount of prepared Lysis Buffer to the sample.

Sample	Amount of Lysis Buffer
Cotton swab	125 - 250 µl
Stain (cloth up to 25mm^2)	125 - 250 µl
Databank Samples	125 µl

   If a spin basket is used at this step, most of the prepared Lysis Buffer should remain in the spin basket soaked into the sample material.

   Close the lid and place the tube, with the spin basket if used, in a heat block at 95°C for 30 minutes.

- **PREPARED LYSIS BUFFER: The Lysis Buffer is prepared by adding 1µl of 1M DTT for every 100µl of Lysis Solution (supplied in kit). Make up a suitable amount before beginning the extraction. This solution can be stored at room temperature for up to a month if sealed.**

3. Remove the tube from the heat block and transfer the Lysis Buffer and sample to a spin basket, if not already in a spin basket.

4. Centrifuge at room temperature for 2 minutes at maximum speed. Remove the spin basket.

5. Vortex the stock Resin bottle to resuspend Resin, and then add 7µl of Resin to the DNA solution for casework stains or 3.5 µl of resin for databank samples. Keep the Resin resuspended while dispensing to obtain uniform results.

6. Briefly vortex sample/Lysis Buffer/Resin mix and incubate at room temperature for 5 minutes.

7. Place tube in the Magnetic Stand. Separation will occur instantly.

8. Carefully remove all of the solution without disturbing the resin on the side of the tube.

9. Add 100 μl of prepared Lysis Buffer for casework stains or 50 μl for databank samples. Remove the tube from the Magnetic Stand and vortex briefly.

10. Return tube to the Magnetic Stand and discard all Lysis Buffer.

11. Add 100 μl of prepared Wash Buffer for casework samples or 50 μl for databank samples. Remove tube from the Magnetic Stand and vortex briefly.

- **PREPARED WASH BUFFER: WASH BUFFER IS PREPARED BY ADDING 35ML OF 95-100% ETHANOL AND 35ML OF ISOPROPYL ALCOHOL TO THE 2X WASH BUFFER THAT IS SUPPLIED IN THE KIT. ONCE PREPARED THIS WASH BUFFER IS GOOD FOR THE LIFE OF THE KIT. REMEMBER TO KEEP THE LID OF THE WASH BUFFER BOTTLE CLOSED TIGHTLY.**

12. Return tube to the Magnetic Stand and discard all Wash Buffer.

13. For casework samples repeat steps 11 and 12 two more times for a total of 3 washes. For databank samples the last wash may be omitted. Make sure that all of the solution has been removed after the last wash.

14. With lid open, air-dry the Resin in the Magnetic Stand for 5 minutes.

15. Add 25-100µl of Elution Buffer, depending on how much biological material was used. For databank samples add 50 μl. Lower elution volume ensures a higher final concentration of DNA.

16. Close the lid, vortex the tube and place it at 65°C for 5 minutes.

17. Remove the tube from the heat block (or incubator) and vortex briefly. Immediately place on the Magnetic Stand.

18. Transfer the solution to a container of choice.

19. Estimate the amount of DNA in the sample.

For databank samples and standard samples a quantitation need not be performed. The concentration can be approximated at 1 ng/μl and a dilution of the DNA will need to be made before amplification.

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A5. ISOLATION OF DNA FROM BONES

NOTE A: FOR EACH SET OF EXTRACTIONS, A MANIPULATION CONTROL MUST BE PROCESSED IN THE SAME MANNER AS ALL OTHER SAMPLES.

Before extraction, a bone specimen should be cleaned from soft tissue and dirt using a combination of picking, scraping and soaking with low heat and gentle agitation. Bone will be air dried and may be ground by hand using a mortar and pestle that have been cleaned with an appropriate solvent and distilled water.

1. Use up to 1 g of bone fragments/powder. Place the pieces into a microcentrifuge tube with a minimum volume of 1.5 ml. Begin processing the manipulation control at this time.

2. The bone samples must be covered with liquid.

   Add the following reagent amounts to the tubes if Microcon 100 microconcentrators are to be used:

   500 μl stain extraction buffer (SEB)
   12.5 μl Proteinase K (Pro K) (10 mg/ml)
   67 μl 0.39M DTT

   Add the appropriate larger volumes of each of the reagents, maintaining the same ratios, if Centricon 100 microconcentrators are to be used.

   Cap tube, mix and centrifuge for 2 seconds to force bone fragments/powder into the liquid.

3. Incubate tubes at 56°C for two hours or overnight.

4. After incubation, centrifuge samples for 5 minutes. Transfer clear supernatant into fresh tubes of the appropriate size.

5. In a fume hood, add 500 μl (Microcon 100) or the appropriate larger volume (Centricon 100) phenol/chloroform/isoamyl alcohol to the microcentrifuge tube. Shake vigorously by hand or vortex for 2-3 seconds until a milky emulsion is achieved. Centrifuge for 2 to 5 minutes to separate the aqueous and organic phases. If the aqueous phase is cloudy, the sample can be extracted again with phenol/chloroform/isoamyl alcohol.

   Alternatively a 2 ml Phase Lock Gel Tube (PLG) may be used for the organic extraction stage. Pellet PLG at 13,500 x g (max. speed) for 30 sec. Transfer the sample from the extraction tube to the PLG tube. In a fume hood, add 500 μl (Microcon 100) or the appropriate larger volume (Centricon 100) phenol/chloroform/isoamyl alcohol to the microcentrifuge tube. Mix by inverting

tube until a milky emulsion is achieved. Do not vortex. Centrifuge at maximum speed for 5 minutes to separate the aqueous and organic phases.

6. To a Microcon 100 microconcentrator add 100 μl of TE buffer. Next, transfer the aqueous phase from the microcentrifuge tube to the microconcentrator. (Avoid pipetting organic solvent and contaminates from the interface to the microconcentrator and do not touch the membrane with the pipette tip). Place the microconcentrator on a filtrate tube, cap and centrifuge at 500 x g[1] for 10 minutes.

   To a Centricon 100 concentrator add 1.5 ml of TE buffer. Next, transfer the aqueous phase from the microcentrifuge tube to the concentrator. Place the concentrator on a filtrate tube, cap and centrifuge at 1000 x g[1] for 20 minutes.

   Discard the microcentrifuge tube containing the organic solvent into a biohazard waste container in the hood.

   Carefully remove the concentrator and discard the fluid from the filtrate tube. If fluid remains in the concentrator, the centrifugation time can be extended.

7. Return the concentrator to the filtrate tube and add 200 μl (Microcon) or 2 ml (Centricon) of TE buffer. Replace the cap and centrifuge at 500 x g[1] for 10 minutes (Microcon) or 1000 x g[1] for 20 minutes (Centricon). This wash step can be repeated several times.

9. Remove the cap and add 200 μl of TE buffer to the concentrator. Remove the concentrator and carefully invert onto a labeled retentate tube. Discard the filtrate tube. Centrifuge the assembly at 500 x g[1] for 5 minutes (Microcon) and 1000 x g[2] for 20 minutes (Centricon). Remove and discard the concentrator and cap the retentate tube.

10. Add 2 volumes of DNA IQ™ Lysis Buffer to the solution.

11. Vortex the stock Resin bottle to resuspend Resin, and then add 15µl of Resin to the sample. Keep the Resin resuspended while dispensing to obtain uniform results.

12. Briefly vortex sample/Lysis Buffer/Resin mix and place tube in the Magnetic Stand. Separation will occur instantly.

13. Carefully remove all of the solution without disturbing the resin on the side of the tube.

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[2] It may be necessary to convert from centrifugal force (measured in g) to RPMs if your microcentrifuge does not provide this function. This can be accomplished using the following formula:

RCP = $(1.118 \times 10^{-6}) \times \text{radius} \times (\text{rpms})^2$ where the radius is the distance measured in mm from the center of rotation to the base of the filtrate tube

14. Add 100 μl of prepared Lysis Buffer to the sample. Remove the tube from the Magnetic Stand and vortex briefly.

15. Return tube to the Magnetic Stand and discard all Lysis Buffer.

16. Add 100 μl of prepared Wash Buffer to the sample. Remove tube from the Magnetic Stand and vortex briefly.

- **PREPARED WASH BUFFER: WASH BUFFER IS PREPARED BY ADDING 35ML OF 95-100% ETHANOL AND 35ML OF ISOPROPYL ALCOHOL TO THE 2X WASH BUFFER THAT IS SUPPLIED IN THE KIT. ONCE PREPARED THIS WASH BUFFER IS GOOD FOR THE LIFE OF THE KIT. REMEMBER TO KEEP THE LID OF THE WASH BUFFER BOTTLE CLOSED TIGHTLY.**

17. Return tube to the Magnetic Stand and discard all Wash Buffer.

18. Repeat steps 15 and 16 two more times for a total of 3 washes. Make sure that all of the solution has been removed after the last wash.

19. With lid open, air-dry the Resin in the Magnetic Stand for 5 minutes.

20. Add 25-100µl of Elution Buffer, depending on how much biological material was used. A lower elution volume ensures a higher final concentration of DNA.

21. Close the lid, vortex the tube and place it at 65°C for 5 minutes.

22. Remove the tube from the heat block (or incubator) and vortex briefly. Immediately place on the Magnetic Stand.

23. Transfer the solution to a container of choice.

24. Estimate the amount of DNA in the sample.

B. QUANTITATION

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B. Quantitation using the Applied Biosystems Quantifiler Kits

NOTE A: At this point, samples may be stored frozen or refrigerated. Prior to use, vortex and centrifuge all tubes. Also, prior to Quantifiler Human DNA Standard preparation, be sure to move the Quantifiler kits from the freezer to room temperature to ensure complete thawing of the primer.

NOTE B: Standard samples and manipulation controls need not be quantitated.

Note C: the same standard set can be used for both Quantifiler kits.

Preparation of Human DNA Standards:

This must be done prior to a sample run, and may last for five to ten days. Prepare a serial dilution of the Quantifiler Human Standard.

1. Label and date eight tubes for the standard series.

2. Vortex the tube of Quantifiler Human Standard.

Prepare serial dilutions with TE (or TE with glycogen at a ratio of 1000:1) as described below. Be sure to use a new pipette tip with each new dilution in the series.

Standard	Concentration (ng/μL)	Example Amounts	Minimum Amounts	Dilution Factor
Std. A	50.000	50 μL (200ng/uL stock) + 150 μL TE	10 μL (200 ng/μL stock) + 30 μL TE	4X
Std. B	16.700	50 μL (Std. A) + 100 μL TE	10 μL (Std. A) + 20 μL TE	3X
Std. C	5.560	50 μL (Std. B) + 100 μL TE	10 μL (Std. B) + 20 μL TE	3X
Std. D	1.850	50 μL (Std. C) + 100 μL TE	10 μL (Std. C) + 20 μL TE	3X
Std. E	0.620	50 μL (Std. D) + 100 μL TE	10 μL (Std. D) + 20 μL TE	3X
Std. F	0.210	50 μL (Std. E) + 100 μL TE	10 μL (Std. E) + 20 μL TE	3X
Std. G	0.068	50 μL (Std. F) + 100 μL TE	10 μL (Std. F) + 20 μL TE	3X
Std. H	0.023	50 μL (Std. G) + 100 μL TE	10 μL (Std. G) + 20 μL TE	3X

Preparation of Reactions:

In addition to the forensic samples, each Quantifiler sample run must contain a complete set of the Human DNA Standard Dilutions in duplicate for each type of kit used. If using only the Quantifiler Human kit, only one set of Human Standard must be run in duplicate. If using both Quantifiler Human and Y kits, one set of standards must be run

in duplicate for each kit.

1. Prepare the master mix. Be sure to prepare enough master mix for all samples and standards, as well as compensating for any master mix volume that might be lost during pipetting transfers. Before use, vortex the primer mix well. Swirl the reaction mix by hand. Do not vortex the reaction mix bottle!

Column	Volume per reaction (μL)
Quantifiler Human Primer Mix or Y Primer Mix	10.5
Quantifiler PCR Reaction Mix	12.5

2. Once the master mix is prepared, vortex briefly. Dispense 23 μL of the master mix into each reaction well needed.

3. Add 2 μL of standard or sample to its respective well. (Make sure the standards are set up in duplicate.) Seal the plate with the optical adhesive cover, sealing all edges and between-well spaces with the sealing tool. Remove any air bubbles that are resting on the bottom of the sample wells. (Air bubbles on the top of the wells will not affect sample amplification.)

Instrument Preparation:

1. Turn on the computer power. Once the computer has finished starting up, turn on the 7000 instrument power.

2. Open the ABI Prism 7000 SDS Software.

3. Set up the plate document for the sample run. If a template plate document has been previously set up, this can be opened and used at this time.

4. Make sure the thermal cycler conditions match the following:

Initial Incubation	95°C for 10 minutes
40 cycles at	95°C for 15 sec
	60°C for 1 minute
	Sample volume 25 μl
	Check box 9600 Emulation

. Be sure to save the document when this is complete.

5. Place the compression pad on top of the sealed amplification plate, gray side down, directly over the reaction wells. Place the plate in the instrument, close the door, and start the run.

Data Analysis and Results:

1. View the amplification plot and verify that the default analysis settings are correct.

Threshold should be set at 0.2, Baseline Start is set at 6, and Baseline End is set at 15. Analyze the data.

2. View the Standard Curve Tab and examine the results for each detector used. For each curve, the R^2 Value should be $\geq$0.98. The slope range for the Quantifiler Human detector should be from –2.9 to –3.3 ±0.3, and the slope range for the Quantifiler Y detector should be from –3.0 to –3.6±0.3. If any of the duplicate standard samples are outliers for the standard curve, that outlying standard well may be removed. Its duplicate must remain in place. If the R^2 value for the Quantifiler Y standard curve only is below 0.98, both sample wells for Standard H may be omitted to correct the R^2 value.

3. Examine the Amplification Plot. Be sure to check that the Internal PCR Control (IPC) has amplified properly for all samples and standards. If the IPC has failed for any sample, that sample must be evaluated.

Note: if any changes are made to the plate document after the initial analysis, the data must be reanalyzed.

4. Print out the sample quantity results.

C. PROFILER PLUS/COFILER

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C1. AMPLIFICATION – AMPFISTR PROFILER PLUS AND/OR CO-FILER

1. Turn on the thermal cycler and select the appropriate program. The set parameters will be displayed. Verify that the set parameters match the following:

Initial Incubation Step	95° C for 11 minutes
28 cycles Temperature cycle:	94° C for 1 minute
	59° C for 1 minute
	72° C for 1 minute
Final Extension	60° C for 45 minutes
Final Step	Hold at 25° C

2. Label sufficient amplification tubes to accommodate each sample and, in addition, one each for the positive and negative controls.

3. VORTEX the AmpFISTR PCR Reaction Mix, AmpFISTR Profiler Plus (or Co-Filer) Primer Set, and AmpliTaq Gold DNA Polymerase for 5 seconds. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.

   IMPORTANT: The fluorescent dyes attached to the primers are light sensitive. Store the AmpFISTR Profiler Plus (or Co-Filer) Primer Set protected from light while not in use. Amplified product should also be protected from light.

4. Prepare a Profiler Plus (or Co-Filer) Master Mix by adding the following volumes of reagents to a microcentrifuge tube:

   Number of samples X 21.0 µL of PCR Reaction Mix
   Number of samples X 1.0 µL of AmpliTaq Gold DNA Polymerase
   Number of samples X 11.0 µL of Profiler Plus (or Co-Filer) Primer Set

   Optional: The number of samples amplified may be multiplied by 0.5 for known databank and for standard casework samples to prepare half reactions.

5. Mix thoroughly by vortexing for 5 seconds. Spin tube briefly to remove any liquid from the cap.

6. Dispense 30 µL of Master Mix into each PCR tube.

   Optional: Dispense 15 µl of the Master Mix into each PCR tube for the optional procedure

The **optimum** sample concentration of template DNA should be within a range of 1.0 to 2.5 ng of DNA in a volume of 20 μL. A sample concentration of template DNA greater to the **optimum** concentration may be added if determined to be necessary by the analyst. Prepare appropriate dilutions of all samples in TE Buffer. However, results may be obtained for samples that contain less than 1 ng of DNA. In that case, do not dilute the sample. Add 20 μL of the appropriate concentration of sample to the PCR tube, already containing 30 μL of Master Mix.

Optional: For databank samples and standard casework samples the sample concentration of template DNA should be within a range of 0.5 to 1 ng of DNA in a volume of 10 μl. Add 10 μl of the appropriate concentration of sample to the PCR tube, already containing 15μl of the Master Mix.

7. Prepare the positive control. Vortex the Control DNA tube contained in the kit. Spin the tube briefly to remove any liquid from the cap. Add approximately1-2ng of Control DNA to the labeled Positive Control Tube.

   Optional: Add approximately 0.5-1 ng of Control DNA to the labeled positive control for half reactions.

8. Prepare the negative control by adding 20 μL of TE buffer to the labeled Negative Control Tube.

   NOTE: The final reaction volume in each PCR tube is 50 μL.

   Optional: Prepare the negative control by adding 10 μl of TE buffer to the Negative Control Tube for half reations.

9. Transfer the tubes to the thermal cycler. Verify that the thermal cycler is set to the correct program and begin the Amplification.

10. When the cycling is completed, remove the samples from the instrument and store the amplified products protected from light. The amplified products can be stored at 2-6° C for short periods of time (less than 2 weeks). For longer periods, store the tubes at –15 to –25° C.

C2. SET- UP OF THE ABI 310 INSTRUMENT

PREPARATION OF AMPLIFIED DNA SAMPLES FOR 310 ANALYSIS

1. Prepare a fresh master mix solution by mixing appropriate volumes of the GS 500 size standard with De-ionized formamide for a total volume of 25μl. The amount of De-ionized formamide/ GS 500 size standard may very depending on the relative concentration of the GS 500 size standard.

2. Label appropriate number of sample tubes (including a system blank, a positive and negative control and allelic ladders).

3. Aliquot 25 μl of the master mix into each sample tube.

4. To each tube add the appropriate amount of either:

   a. amplified DNA.

   b. allelic ladder.

   c. positive control.

   d. negative control.

6. Seal each tube with rubber septum. The tubes may need to be vortexed lightly and spun down briefly.

7. Pre-heat the heat block on the 310 to 60^0C.

8. Denature samples for 3-5 minutes at 95^0C.

9. Snap cool denatured samples for at least 3 minutes.

10. Place tubes in sample tray. Sample tubes must include blank, case samples, positive control, negative control and appropriate allelic ladder(s). There can be no more than 10 sample injections between injections of allelic ladder.

11. Load sample tray into the 310.

12. Create sample sheet and injection list (see below).

13. Start electrophoresis run.

CREATING A SAMPLE SHEET AND INJECTION LIST

1. Open a new Sample Sheet and create.
2. Save the Sample Sheet File.
3. Open New File to make Injection List.
4. Import Sample Sheet onto Injection List.
5. Verify Parameters:
   a. Module: GS STR POP4 (1 ml) F
   b. Injection time: 5 seconds
   c. Run temperature: 60^0C.

   NOTE: The injection times may be varied depending on the concentration of the sample. The injection time should never exceed 10 seconds.
6. Select appropriate Matrix.
7. If using Auto-analysis for Genescan, select now.
8. Save Injection List file.

GENESCAN ANALYSIS (when Auto-analysis/Auto-print is not performed)

1. Retrieve Project File.
2. Open Project File.
3. Create a new size standard (optional)
   A. Under the File menu, choose New.
   B. Choose the "Size Standard" icon
   C. Select Internal Size Standard and apply appropriate values to peaks.
   D. Save new size standard, close and apply to samples.
4. Check that appropriate Analysis Parameters are selected.
5. Select all 4 dyes for each sample.
6. Analyze.
7. Print Genescan data for analysis by analyst and second reader.

8. Save Project File.

9. Close Project File.

GENOTYPER ANALYSIS

1. Open appropriate Genotyper Program.

2. Import GeneScan File

3. Analyze project with the appropriate Kazam Macro.

4. Generate and print Genotyper electropherograms and/or table.

C3. SET-UP OF THE ABI 3100-*Avant* INSTRUMENT
PREPARATION OF AMPLIFIED DNA SAMPLES FOR 3100-*Avant* ANALYSIS

1. Prepare a fresh master mix solution by mixing appropriate volumes of the GS 500 size standard with De-ionized formamide for a total volume of 9 μL or 25µl. The amount of De-ionized formamide/ GS 500 size standard may very depending on the relative concentration of the GS 500 size standard.

2. Label a plate for the appropriate number of samples (including a system blank, an allelic ladder, positive and negative controls).

3. Aliquot 9μLor 25μL of the master mix into each sample tube/well.

4. To each tube/well add the appropriate amount of either:

    a. nothing (at least one per plate as a reagent blank)
    b. allelic ladder (at least two per plate, recommended one per capillary)
    c. amplified DNA
    d. positive control
    e. negative control.

5. Seal the tubes/wells with rubber septa.

6. Denature samples for 3 minutes at 95°C.

7. Snap cool denatured samples for 3 minutes.

8. Place the plate into the plate base provided with the instrument, cover with plate retainer then place onto the 3100-*Avant* auto sampler.

9. Create a plate record (see additional procedure).

10. Link plate. Go to Run Scheduler. Highlight the pending plate record click the plate position (B) to link the plate record to the sample plate.

11. Start electrophoresis run.

CREATING A PLATE RECORD AND LINKING TO A SAMPLE PLATE

1. Open Plate Manager to create a new plate record and enter:

    a) Name for plate
    b) Description of plate (optional)
    c) Select appropriate Application from drop down menu (3100-Avant, Spectral)
    d) Select 96-well in Plate Type drop down menu
    e) Type owner name

f) Type operator name
g) Click OK

2. The GeneMapper Plate Editor opens. Type in the following into the Plate Record

   a. Sample Name
   b. Comment (optional)
   c. Priority (default value of 100)
   d. Sample Type (select appropriate type from drop down menu)
   e. Size Standard (select appropriate standard from drop down menu)
   f. Panel (select appropriate panel from drop down menu, profiler plus, COfiler, identifiler, ect.)
   g. Analysis Method (select appropriate method from drop down menu)
   h. Results Group1 (select appropriate group from drop down menu)
   i. Instrument Protocol 1 (select appropriate protocol from drop down menu)

3. Click [OK] to save the plate record. (The plate name will appear in the pending plate record window.)

GeneMapper ANALYSIS

1. Open the GeneMapper software.
2. Open project.
3. Add the sample files to the project.
4. Specify a size standard (or create a new size standard).
5. Check that the appropriate Analysis Method, parameters, panels, are selected.
6. Analyze.
7. Check analyzed data. Highlight samples in the Project window; select the HID Genotyping plot from the drop down list.
8. Print the analyzed GeneMapper data for analysis by analyst and second reader.
9. Save Project File.
10. Close Project File.

D. POWERPLEX ™ 16 & Y SYSTEMS

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D1. AMPLIFICATION - POWERPLEX™ 16 and Y SYSTEMS (PROMEGA)

1. Turn on the thermal cycler and select the appropriate program. The set parameters will be displayed. Verify that the set parameters match the following:

PowerPlex™ 16 System

Perkin-Elmer GeneAmp® PCR System **9600** Thermal Cycler:

Initial Incubation Step: 95°C for 11 minutes
96°C for 1 minute

10 Cycles of: 94°C for 30 seconds
ramp 68 seconds to 60°C, hold for 30 seconds
ramp 50 seconds to 70°C, hold for 45 seconds

22 Cycles of: 90°C for 30 seconds
ramp 60 seconds to 60°C, hold for 30 seconds
ramp 50 seconds to 70°C, hold for 45 seconds

60°C for 30 minutes
4°C soak

Perkin-Elmer GeneAmp® PCR System **2400** Thermal Cycler:

Initial Incubation Step: 95°C for 11 minutes
96°C for 1 minute

10 Cycles of: ramp 100% to 94°C for 30 seconds
ramp 100% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds

22 Cycles of: ramp 100% to 90°C for 30 seconds
ramp 100% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds

60°C for 30 minutes
4°C soak

Perkin-Elmer GeneAmp® PCR System **9700** Thermal Cycler:

Initial Incubation Step: 95°C for 11 minutes
96°C for 1 minute

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10 Cycles of: ramp 100% to 94°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds

22 Cycles of: ramp 100% to 90°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds

60°C for 30 minutes
4°C soak

PowerPlex™ Y System

Perkin-Elmer GeneAmp® PCR System **9600** Thermal Cycler:

Initial Incubation Step: 95°C for 11 minutes
96°C for 1 minute

10 Cycles of: 94°C for 30 seconds
ramp 68 seconds to 60°C, hold for 30 seconds
ramp 50 seconds to 70°C, hold for 45 seconds

18-22 Cycles of: 90°C for 30 seconds
ramp 60 seconds to 58°C, hold for 30 seconds
ramp 50 seconds to 70°C, hold for 45 seconds

60°C for 30 minutes
4°C soak

Perkin-Elmer GeneAmp® PCR System **2400** Thermal Cycler:

Initial Incubation Step: 95°C for 11 minutes
96°C for 1 minute

10 Cycles of: ramp 100% to 94°C for 30 seconds
ramp 100% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds

18-22 Cycles of: ramp 100% to 90°C for 30 seconds
ramp 100% to 58°C for 30 seconds
ramp 23% to 70°C for 45 seconds

60°C for 30 minutes
4°C soak

Perkin-Elmer GeneAmp® PCR System **9700** Thermal Cycler:

Initial Incubation Step:	95°C for 11 minutes 96°C for 1 minute
10 Cycles of:	ramp 100% to 94°C for 30 seconds ramp 29% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds
18-22 Cycles of:	ramp 100% to 90°C for 30 seconds ramp 29% to 58°C for 30 seconds ramp 23% to 70°C for 45 seconds
	60°C for 30 minutes 4°C soak

2. Label sufficient amplification tubes to accommodate each sample and, in addition, one each for the positive and negative controls.

3. VORTEX the Gold STAR 10X Buffer and the PowerPlex™ 10X Primer Pair Mix for 5-10 seconds. Do not centrifuge the 10X Primer Pair Mix as this may cause the primers to be concentrated in the bottom of the tube.

   A precipitate may form in the Gold STAR 10X Buffer. If this occurs, warm the solution at 37°C, then vortex until it is in solution.

IMPORTANT: The fluorescent dyes attached to the primers are light sensitive. Store the PowerPlex™ 10X Primer Pair Mix protected from light while not in use. Amplified product should also be protected from light.

4. Prepare a PowerPlex™ Master Mix by adding the following volumes of reagents to a microcentrifuge tube:

PowerPlex™ 16 System

Number of samples X Nuclease-Free Water or TE-4 buffer (See step 7 for concentration)
Number of samples X 2.5μL of Gold STAR 10X Buffer
Number of samples X 2.5μL of PowerPlex™ 16 10X Primer Pair Mix
Number of samples X .8μL of AmpliTaq Gold® DNA Polymerase

PowerPlex™ Y System

Number of samples X Nuclease-Free Water or TE-4 buffer (See step 7 for concentration)
Number of samples X 2.5μL of Gold STAR 10X Buffer

Number of samples X 2.5μL of PowerPlex™ Y 10X Primer Pair Mix
Number of samples X .55μL of AmpliTaq Gold® DNA Polymerase

- Assumes the AmpliTaq Gold® DNA Polymerase is at 5u/μL concentration. If the enzyme concentration is different, the volume of enzyme used must be adjusted accordingly.
- If a larger volume of template is required, the water or TE-4 volume should be adjusted accordingly.

5. Add the final volume of each reagent into a tube. Mix well.

6. Dispense the appropriate volume of PCR Master Mix into each PCR tube.

7. The optimum sample concentration of template DNA should be within a range of .5ng to1ng of DNA in a sample volume not less than 2.5μL or greater than 19.2 μL (Powerplex™ 16 System) or 19.45 μL (Powerplex™ Y System). If sample dilution is necessary, prepare appropriate dilutions of all samples in deionized water or TE-4 buffer. Results **may** be obtained for samples that contain less than .5ng of DNA.

Note: The final reaction volume in each PCR tube is 25μL.

8. Add the appropriate volume and concentration of samples to the PCR tubes, already containing the PCR Master Mix.

9. Amplification controls:

Positive controls

PowerPlex™ 16 System:

Prepare the positive amplification control. Dilute the 9947A female DNA sample to 0.5ng to 1ng in the template volume. Pipette the diluted 9947A female DNA into the reaction tube containing the PCR master mix.

PowerPlex™ Y System:

Prepare the positive amplification control. Dilute the 9948 male DNA sample to 0.5ng to 1ng in the template volume. Pipette the diluted 9948 male DNA into the reaction tube containing the PCR master mix.

Negative controls

PowerPlex™ 16 System:

Prepare the negative amplification control. Pipette deionized water or TE-4 buffer (instead of the template DNA) into a reaction tube containing the PCR master mix.

PowerPlex™ Y System:

Prepare the negative amplification control. Pipette deionized water or TE-4 buffer (instead of the template DNA) into a reaction tube containing the PCR master mix. Optional male specificity control: dilute the 9947A female DNA sample to 0.5ng to

1ng in the template volume. Pipette the diluted 9947A female DNA into the reaction tube containing the PCR master mix.

10. Transfer the tubes to the thermal cycler. Verify that the thermal cycler is set to the correct program and begin the Amplification.

11. When the cycling is completed, remove the samples from the instrument and store the amplified products protected from light. The amplified products can be stored at 2-6°C for short periods of time (less than 2 weeks). For longer periods, store the tubes at –15 to -25°C.

D2. SET-UP OF THE ABI 310 INSTUMENT
PREPARATION OF AMPLIFIED DNA SAMPLES FOR 310 ANALYSIS

1. Prepare a fresh master mix solution by mixing appropriate volumes of the ILS600 size standard with de-ionized formamide (24μL of formamide plus 1μL of ILS600 for each sample and standard plus an additional two for waste).
2. Label appropriate number of sample tubes (including a system blank, a positive and negative control and allelic ladders).
3. Aliquot 25μL of the master mix into each sample tube.
4. To each tube add the appropriate amount of either:
    a. amplified DNA
    b. allelic ladder
    c. positive control
    d. negative control.
5. Seal each tube with a rubber septum. The tubes may need to be vortexed lightly and spun down briefly
6. Pre-heat the heat block on the 310 to 60°C.
7. Denature samples for 3 minutes at 95°C.
8. Snap cool denatured samples for 3 minutes.
9. Place tubes in the sample tray.

NOTE: There can be no more than ten samples between ladder injections.

10. Load sample tray into the 310.
11. Create a sample sheet and injection list (see below).
12. Start electrophoresis run.

CREATING A SAMPLE SHEET AND INJECTION LIST

1. Open a new Sample Sheet and create.
2. Save the Sample Sheet File.
3. Open New File to make Injection List.

4. Import Sample Sheet onto Injection List.

5. Verify Parameters:

   a. Module: GS STR POP4 (1ml)A
   b. Injection time: 3 seconds
   c. Run Temperature: 60°C
   d. Run length 30 minutes (Powerplex™ 16 System)
   e. Run length 24 minutes (Powerplex™ Y System)

   NOTE: The injection time may be varied depending on the concentration of the sample. Injection time may not exceed 10 seconds.

6. Select appropriate Matrix.

7. If doing Auto-analysis for Genescan, select now.

8. Save Injection List file.

GENESCAN ANALYSIS (when Auto-analysis/Auto-print is not performed)

1. Retrieve Project File.

2. Open Project File.

3. Create a new size standard (optional)

   a. Under the File menu, choose New.
   b. Choose the "Size Standard" icon
   c. Select Internal Size Standard and apply appropriate values to peaks.
   d. Save new Size Standard, close and apply to samples.

4. Check that appropriate Analysis Parameters are selected.

5. Select all 4 dyes for each sample.

6. Analyze

7. Print Genescan data for analysis by analyst and second reader.

8. Save Project File

9. Close Project File.

GENOTYPER ANALYSIS

1. Open appropriate Genotyper Program.

2. Import GeneScan File.

3. Analyze project with PowerTyper Macro.

4. Generate and print PowerTyper results.

D3. SET-UP OF THE ABI 3100 INSTRUMENT

PREPARATION OF AMPLIFIED DNA SAMPLES FOR 3100 ANALYSIS

1. Prepare a fresh master mix solution by mixing appropriate volumes of the ILS600 size standard with de-ionized formamide (24μL of formamide plus 1μL of ILS600 for each sample for possible re-injection on a 310, or 10μL of formamide plus 1μL of ILS600 for use on the 3100 only).

2. Label a plate for the appropriate number of samples (including a system blank, an allelic ladder, positive and negative controls).

3. Aliquot 25μL (or 10μL of the 3100-only mixture) of the master mix into each sample tube/well.

4. To each tube/well add the appropriate amount of either:

   a. nothing (at least one per plate as a reagent blank)
   b. allelic ladder (at least two per plate)
   c. amplified DNA
   d. positive control
   e. negative control.

5. Seal the tubes/wells with rubber septa.

6. Denature samples for 3 minutes at 95°C.

7. Snap cool denatured samples for 3 minutes.

8. Lock the plate into a retainer and place into the 3100 .

9. Create a plate record and link to the sample plate (see additional procedure).

10. Start electrophoresis run.

CREATING A PLATE RECORD AND LINKING TO A SAMPLE PLATE

1. Open a new plate record and enter:

   a) Sample_name (no spaces allowed)
   b) BioLIMS project name (arbitrary)
   c) Color Info (same as sample name)
   d) Dye set "Z" (according to the Promega User Bulletin – PowerPlex® Matrix Standards, 3100, 10/01)
   e) Run module "PPX_15sec" (or a modification thereof)
   f) Run length can be shortened from 1500 seconds to 1100 seconds for the Powerplex™ Y System

1. Run Voltage after an initial series of step-ups: 15,000 Volts
2. Injection Voltage: 1,000 Volts
3. Injection Duration: 15 Seconds (This is equivalent to a 3 second injection on the 310 with the 310 formamide master mix. The time may be varied depending on the anticipated concentration of the sample.)
4. Temperature: 60°C
5. Laser Power: 10 mWatts.

g. Analysis module "PPX Analysis" (if auto-analysis is used).

2. Click [OK] to save the plate record. (The plate name will appear in the pending plate record window.)

3. Highlight the pending plate record name and click on the appropriate plate position (A or B) to link them.

GENESCAN ANALYSIS (when Auto-analysis is not performed)

1. Open the GeneScan software.

2. Create a new project.

3. Add the sample files to the project.

4. Specify a size standard (or create a new size standard).

   a. Under the File menu, choose New.
   b. Choose the "Size Standard" icon
   c. Select Internal Size Standard and apply appropriate values to peaks.
   d. Save new Size Standard, close and apply to samples.

5. Check that appropriate Analysis Parameters are selected.

6. Select all 4 dyes for each sample.

7. Analyze.

8. Print the analyzed Genescan data for analysis by analyst and second reader.

9. Save Project File.

10. Close Project File.

GENOTYPER ANALYSIS

1. Open the Genotyper software.

2. Import GeneScan project file.

3. Run the PowerTyper Macro.

4. Print the PowerTyper results.

E. INTERPRETATION AND REPORT WRITING

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E1. INTERPRETATION OF STR RESULTS

The interpretation of results from casework is a matter of professional judgement and expertise. Not every situation can nor should be covered by a preset rule. This Laboratory's interpretation guidelines are based upon validation studies, data from the literature, instrumentation used and/or casework experience. This section is to serve as a general guideline for the interpretation of STR profiles generated from casework samples. Situations may occur that require an analyst to deviate from the stated guidelines. Any deviations must be documented in written form prior to peer review (See Unified Quality Assurance Manual - Appendix E). In those situations, any deviations must be approved by the Technical Leader and the Supervisor.

In order to assess the success of the amplification and electrophoresis process, both analyst and the second reviewer will review a printout of the GeneScan® and Genotyper® results for the electrophoresis run. The analyst and the case examiner must agree on the status given or types assigned to each peak. In cases where an agreement cannot be reached, the Technical Leader will resolve the issue.

1) PRELIMINARY EVALUATION OF DATA:

Description of Peak Height Thresholds:

For any given locus, peaks above 150 RFU's may be used for inclusionary as well as exclusionary purposes. Peaks between 100 and 150 RFU's must be interpreted with caution. Peaks in this range may be used for inclusionary purposes with the approval of the peer reviewer and/or the technical leader. Peaks between 50 and 100 RFU's may be used for exclusionary purposes only.

Analyzed (Genescan) peaks to be called should normally fall within the range of 150 to 4500 RFU. Analyzed peaks above 4500 RFU will be interpreted with caution. When viewing the raw data, any peaks greater than 8100 RFU are off-scale.

Evaluation of Internal lane size standards and Ladders:

The internal lane marker GS 500 (Profiler Plus™ and/or COfiler™) must have correct sizes assigned to the peaks used for sizing. It is noted that the 250 bp peak is not used for sizing purposes. The 250bp peak must not vary greater than 1bp throughout the sample project. The 75 and 350 bp peaks must be captured for all samples. The 400 bp peak must be captured for alleles greater than 340 bp in size. The internal lane marker ILS600 (PowerPlex™ 16 and Y) must have correct sizes assigned to the peaks used for sizing.

A ladder, corresponding to the kit used to amplify the samples, must be present with each run. No more than ten case samples should be between each ladder injection.

Account for **all** peaks (in the allele typing areas) of the ladder that are detected by the GeneScan® program. Using the Genotyper program a ladder is used to assign allele designations to the peaks in the sample. This is done by comparing the base pair size of the unknown peak to the base pair size of the known alleles found in the ladder. The base pair size of the unknown peak should be within 0.5bp of a base pair of the known allele peak seen in the ladder.

Evaluation of Controls:

Reagent Blank and/or Manipulation Control: A reagent blank and/or manipulation control test for the possible presence of contamination of the extraction reagents and/or supplies by an adventitious source of DNA. The adventitious DNA can be non-amplified DNA or PCR product. If, in the allele typing areas, a reagent blank and/or manipulation control exhibits identifiable allele peaks (greater than the analysis parameters) not attributable to an artifact, the DNA specimens extracted or amplified with the reagents contained in the reagent blank and/or manipulation control will be considered inconclusive for match purposes. However, these specimens may be used for exclusionary purposes. Random anomalous peaks such as off ladder or 1 or 2 random peaks without a recognizable pattern will not be considered contamination. In these cases results maybe interpreted with the agreement between analyst and peer reviewer.

Negative Control: A negative control is a test for the possible presence of contamination occurring during amplification set-up. If, in the allele typing areas, a negative control exhibits identifiable alleles peaks (greater than the analysis parameters) not attributable to an artifact, the DNA specimens amplified at the same time as the negative control will be considered inconclusive for match purposes. However, these specimens may be used for exclusionary purposes. Random anomalous peaks such as off ladder or 1 or 2 random peaks without a recognizable pattern will not be considered contamination. In these cases results maybe interpreted with the agreement between analyst and peer reviewer..

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The negative amplification control and manipulation control will be examined for any extraneous peaks in two or more dyes (spikes). If spikes occur outside an allele typing area it is not necessary to reinject. However, if spikes do occur within an allele typing area it **may** be necessary to reinject the sample.

NOTE: If analysis parameters are changed for any given sample, the appropriate manipulation control and negative amplification control must also be subjected to the same parameters.

Positive Control: A positive control is a test for the proper amplification of specimens. A positive DNA control is included in the Profiler Plus™, COfiler™ and PowerPlex™ amplification kits. This control must exhibit the following typing results:

Profiler Plus™:

D3S1358	vWA	FGA	AMEL	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820

14,15	17,18	23,24	X,X	13,13	30,30	15,19	11,11	11,11	10,11

COfiler™:

D3S1358	D16S539	AMEL	TH01	TPOX	CSF1PO	D7S820
14,15	11,12	X,X	8,9.3	8,8	10,12	10,11

PowerPlex™ 16.2 (9947A):

D3S1358	TH01	D21S11	D18S51	PENTA E	D5S818	D13S317	D7S820
14,15	8.,9.3	30,30	15,19	12,13	11,11	11,11	10,11

D16S539	CSF1PO	PENTA D	AMEL	vWA	D8S1179	TPOX	FGA
11,12	10,12	12,12	X,X	17,18	13,13	8,8	23,24

PowerPlex™ Y (9948)

DYS391	DYS389I	DYS439	DYS389II	DYS438	DYS437	DYS19	DYS392
10	13	12	31	11	15	14	13

DYS393	DYS390	DYS385
13	24	11,14

If the positive control does not exhibit the expected STR results, the following steps must be taken.

1) If there appears to be an injection or electrophoretic problem, reinject the positive control with a ladder.
2) If reinjection of the positive control does not resolve the problem and may be due to amplification issues, all samples set-up and amplified with this control will be considered inconclusive for matching purposes, but can be used for purposes of exclusion. If sufficient DNA remains of samples co-amplified with a failed control, then it is appropriate to re-amplify them. If the positive control yields accurate typing results when re-amplified, then the samples re-amplified with this control will be considered conclusive for matching purposes.

Redundant loci from Profiler Plus™ and COfiler™ amplification kits:

When using STR multiplexes certain loci are typed in both Profiler Plus™ and COfiler™ systems. Within a sample, genotypes generated for D3S1358 and D7S820 must be concordant for interpretable data in both amplification systems. Situations may occur where one or more alleles at D3S1358 and D7S820 are absent and therefore the profiles for Profiler Plus™ and COfiler™ will differ. Provided all criteria for interpretation are fulfilled, the most informative profile may be used for matching purposes.

2) DESIGNATION OF LOCI AND ALLELES:

The AmpFISTR Profiler Plus™ and AmpFISTR COfiler™ or Geneprint®PowerPlex™ 16.2 amplification kits are used in order to generate STR profiles for the 13 loci required by the FBI for inclusion in the national DNA database. Power Plex™ 16.2 also has two additional penta-repeat loci. These loci are D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, THO1, TPOX, CSF1PO, Penta E and Penta D. In addition the amelogenin gene, located within the X and Y chromosomes is also analyzed to allow for gender determination. The PowerPlex™ Y amplification kit generates a haplotype profile for 12 loci from the Y chromosome. These loci are DYS391, DYS389I, DYS439, DYS389II, DYS438, DYS437, DYS19, DYS392, DYS393, DYS390, and DYS385 (a and b). The amplified DNA fragments are separated according to size by capillary electrophoresis.

The size of each peak is determined by GeneScan® Analysis software by comparing the internal size standard (ROX500 or ILS600) using Local Southern analysis. Sample peaks are labeled by comparison of their size to the size of the ladder alleles. The ladders are composed of the most common alleles in the general population. The ladder does not represent all alleles present in the population.

Alleles are designated in accordance with the recommendations of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH). The alleles are named by the number of full repeats of the repetitive motif. In the case of a variant presumed to contain a partial repeat, that partial repeat unit is designated by the number of complete repeats separated by a decimal followed by the number of bases contained in the partial repeat unit. For example, a THO1 9.3 allele contains 9 complete repeat units and one partial repeat unit containing only 3 bases.

Off ladder (OL) alleles are first converted to size in base pairs (bp), compared to the size of the appropriate ladder alleles and finally the allelic designation is determined. If the OL allele is not a 4 (or 5 for penta loci) base pair repeat, but rather varies by 1, 2, 3 or 4 bp from a ladder allele, it will then be designated as an integer of that variation. For example, if a green OL peak size is 236.39 bp, and the 36 allele of the D21S11 ladder is 234.32, then the peak will be designated as D21S11 36.2. If an allele is identified as off-ladder (OL) it should be reinjected to confirm the size of the allele. If an allele falls above the largest or below the smallest peak of the sizing ladder, the allele will be designated as either greater than (>) or less than (<) the respective ladder allele for up loading into CODIS and for use in statistical interpretations. Virtual alleles (alleles not present in the actual ladder, but assigned an allele designation by Genotyper®/Powertyper®) above the largest or below the smallest peak of a sizing ladder may be assigned a numerical designation (if appropriate) for reporting purposes.

Artifacts:

Artifacts or anomalies can occur during STR analysis and should be noted. If these occur outside an allele typing area it is not necessary to repeat. However, if these

artifacts or anomalies occur within allele typing areas it may be necessary to reinject the sample. It is within the scientist's own discretion (with agreement of the peer reviewer) whether or not a sample should be re-injected.

Spikes are potential artifacts of the capillary electrophoresis process. Generally spikes are present in two or more colors and have the same data points.

Off-scale data refers to the raw data on the 310 Genetic Analyzer, which has peaks greater than 8100 RFU's. If a peak is off-scale, the injection time may be shortened, the sample may be diluted (to bring the data on-scale) and rerun or the sample may be re-amplified with a smaller amount of template DNA.

Pull-up peaks refer to a situation in which a signal from one dye "pulls up" a small signal in another dye because the fluorescent intensity exceeds the linear dynamic range of the instrument. This situation may occur due to a number of reasons. (Some examples are an incorrect matrix file being applied or from too much template DNA being added to the PCR reaction mixture resulting in off-scale peaks).

Stutter is a minor product peak four bp (or five for the penta loci) shorter than the corresponding allele peak. These product peaks will be considered stutter if their peak height percentages to the larger peaks are equal to or less than the following "Stutter Cutoffs": 7% at the THO1 and TPOX loci, 10% at the CSF1PO locus, 12% at the D8S1179, D5S818, D13S317 and D7S820 loci, 15% for the D3S1358, D21S11, vWA, FGA and D16S539 loci and 18% at the D18S51 locus. For any given locus, minor peaks that are above the RFU cutoff and are located at a position one repeat unit smaller than a major peak will be considered as a real allele if their peak heights exceed the stutter percentage established for that locus as indicated above and evidence of a mixed sample is present at additional loci. [Note: the stutter comparison will be done automatically through the Genotyper® /Powertyper® computer program; it must be done manually with the GeneScan® data.]

Power Plex™ 16.2: The above listed "stutter cutoffs" are appropriate to use with the Power Plex™ 16.2 system with the exception of CSF1PO. The cutoff for this locus will be 12%. For the additional penta loci the following "stutter cutoffs" will be applied: 7% at Penta D and 12% at the Penta E locus.

Power Plex™ Y: Some of the stutter or stutter-like peaks encountered are described in *Promega's Powerplex® Y System Technical Manual (pg. 25).* Cutoff values for the following commonly encountered peaks for each locus were established as follows: DYS391 (-4: 10.2%), DYS389I (-8: 11.7%, -4: 10.2%), DYS439 (-4: 10.2%), DYS389II (-8: 11.7%, -4: 19.0%), DYS438 (-5: 6.5%), DYS437 (-8:14.4%' –4: 12.4%), DYS19 (-4: 12.4%, -2: 12.7%), DYS392 (-3: 19.6%, +3: 14.2%), DYS393 (-4: 12.8%), DYS390 (-8: 12.5%, -4: 12.8%), and DYS385 (a and b) (-9: 18.1%, -4: 15.1%, +3: 18.6%, +8: 18.8%).

VWA Artifact: A small peak, which is typically 12-20 bp smaller than its corresponding vWA allele peak, may be detected when amplifying samples with Promega Powerplex ® 16.2 amplification kit. If a vWA artifact peak is causing an issue with the mixture

interpretation of a minor DNA component at this locus, the sample can be reamplified using the Profiler Plus amplification kit.

Template independent nucleotide addition (-A) occurs when Taq DNA Polymerase adds an additional nucleotide to the 3' ends of double stranded PCR products. This results in PCR product that is 1 base longer than the actual sequence size of the allele. The PCR reaction parameters are set-up to drive the addition of a nucleotide to the 3' end of the PCR product. Incomplete "A" nucleotide addition may occur when too much amplification product is generated during amplification for the polymerase to complete non-template nucleotide addition. A possible resolution of –A peaks is to incubated at 60°C for 45 minutes.

3) INTERPRETATION OF RESULTS:

Autosomal STR Analysis

Single Source

A sample can be considered to have originated from a single source if:

- only one or two alleles are present at all loci examined, although three peak patterns may occur
- the peak height ratios of heterozygous individuals at a locus are within expected values. (ref. Heterozygote Peak Height Ratios)

Mixtures

Evidence samples may contain DNA from more than one individual. The possibility of multiple contributors should be considered when interpreting the results. Presence of one or more of the following conditions could indicate the presence of a mixture:

- the presence of more than two alleles at a locus
- the presence of a peak at a stutter position that is significantly greater in percentage than what is typically observed in a single source sample
- significantly imbalanced alleles for a heterozygous genotype

A sample containing DNA from two sources can be comprised (at a single locus) of any of the seven genotypic combinations listed below.

- ♦ heterozygote + heterozygote, no overlapping alleles (four peaks)
- ♦ heterozygote + heterozygote, one overlapping allele (three peaks)
- ♦ heterozygote + heterozygote, two overlapping alleles (two peaks)
- ♦ heterozygote + homozygote, no overlapping alleles (three peaks)
- ♦ heterozygote + homozygote, overlapping allele (two peaks)
- ♦ homozygote + homozygote, no overlapping alleles (two peaks)
- ♦ homozygote + homozygote, overlapping allele, (one peak)

Mixtures with Major/Minor Contributors

A sample may be considered to consist of a "mixture with major/minor contributor(s)" if there is a distinct contrast in peak intensities between the alleles. Additionally the alleles with large peak height values must satisfy the conditions of a single source specimen. All loci must be considered in making this determination. These conditions are subject to agreement between the analyst and peer reviewer. Providing these conditions exist, a genotype frequency can be reported for the major contributor. At some loci, interpretation of the major contributor may not be possible. These loci will not be used in a single source statistical interpretation.

The genotype of the minor donor can be determined if the peaks, after the major contributor's alleles have been accounted for, meet expected peak height ratios. Providing these conditions exist, a genotype frequency can be reported for the minor contributor. Interpretation of the minor contributor may be limited to inclusion or exclusion at some loci.

Specific genotype combinations and input DNA ratios of the samples contained in a mixture determine whether it is possible to resolve the genotypes of the major and minor component(s) at a single locus. The ability to obtain and compare quantitative values for the different allele peak heights on the instrument provides additional valuable data to aid in resolving mixed genotypes.

The interpretation and deduction of alleles for single-source contributors in a mixture must be made without knowledge or consideration of the profiles from the standard samples, with the exception of samples of an intimate nature. If the profiles cannot be deduced without consideration of the standard samples, then a mixture calculation will be used. Loci that do not include the contributor will not be used in the statistical calculation.

Mixtures with a Known Contributor(s)

In samples of an intimate nature, such as vaginal swabs or underwear, when one of the contributors is known, the genetic profile of the unknown contributor may be inferred. Depending on the profiles in the specific instance, this can be accomplished by subtracting the contribution of the known donor from the mixed profile.

Mixtures in a Differential Extraction

If the two profiles obtained in the sperm and non-sperm fractions from a single differential extraction indicate one or more common contributors, the types of each contributor may, if feasible, be inferred. This may be performed by subtracting the major contributor of one fraction from the mixed profile of the other, or by deducing the contributing single-source profiles using both mixed profiles.

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Mixtures with Indistinguishable Contributors or Multiple Contributors

If a major or minor contributor cannot be distinguished because of similarity in peak heights or overlapping alleles are present that cannot be assigned, individuals may be included or excluded and a combined genotype frequency may be determined using the mixture calculation defined in section 5 (Statistical Interpretation).

In samples with partial profiles (degraded, low levels, and inhibitors) the data will be interpreted following the stated laboratory guidelines. Individual loci with peaks that fall below 100 RFU's may be called inconclusive for inclusionary purposes. A statistical interpretation will be made on the types obtained for the loci used for an inclusion.

If the profile obtained for a sample is highly degraded, consists of multiple contributors (3 or more), and/or is obtained from low levels of DNA, it may be impossible to make an inclusionary statement or a statistical interpretation. When it is not possible to make an inclusionary statement individuals may be excluded as possible contributors to the results obtained or will be considered inconclusive and will be reported as such.

Heterozygote Peak Height Ratios

This ratio is determined by dividing the peak height of the allele with the lower relative fluorescent unit (RFU) value, by the peak height of the allele with the larger RFU value and multiplying this value by 100 to obtain a percentage. It is expected that from a single-source sample the peak height ratio between two sister alleles at a heterozygote locus would not be less than 60% for Profiler Plus and COfiler. For the Power Plex ™ 16.2 system, a peak height ratio of 50% is expected for all loci with the exception of the Penta E locus. When the amount of template added to the amplification set-up is below a level to produce sufficient product to produce peaks above 150 RFU, then a greater imbalance between sister alleles at a heterozygote locus may be observed. Extremely low levels of template may lead to stochastic processes which may under-represent one of the alleles or may even lead to allele drop out. When peak heights for alleles are low the data must be interpreted with caution and the possibility of stochastic effects and allele drop out considered.

Y-STR Analysis:

Single Source

A sample can be considered to have originated from a single source if:

- only one allele is present at all loci examined (although two or three peak patterns may occur) except for the DYS385 locus where two allele patterns are commonly encountered due to gene duplication

Mixtures

Evidence samples may contain DNA from more than one individual. The possibility of multiple contributors should be considered when interpreting the results. Presence of one or more of the following conditions could indicate the presence of a mixture:

- the presence of more than one allele at a locus
- the presence of a peak at a stutter position that is significantly greater in percentage than what is typically observed in a single source sample
- a peak height ratio greater than 60% at the DYS385 locus when two allele peaks are present

Mixtures with Major/Minor Contributors

A sample may be considered to consist of a "mixture with major/minor contributor(s)" if there is a distinct contrast in peak intensities between the alleles. Additionally the alleles with large peak height values must satisfy the conditions of a single source specimen. All loci must be considered in making this determination which is subject to analyst and peer reviewer's discretion. Providing these conditions exist, a genotype frequency can be reported for the major contributor. At some loci interpretation of the major contributor may not be possible. These loci will not be used in a single source statistical interpretation.

Specific genotype combinations and input DNA ratios of the samples contained in a mixture determine whether it is possible to resolve the genotypes of the major and minor component(s) at a single locus. The ability to obtain and compare quantitative values for the different allele peak heights on the instrument provides additional valuable data to aid in resolving mixed genotypes.

The interpretation and deduction of alleles for single-source contributors in a mixture must be made without knowledge or consideration of the profiles from the standard samples, with the exception of samples of an intimate nature. If the profiles cannot be deduced without consideration of the standard samples, then a mixture calculation will be used. Loci that do not include the contributor will not be used in the statistical calculation.

Mixtures with a Known Contributor(s)

In samples of an intimate nature, such as penile swabs or underwear, when one of the male contributors is known, the genetic profile of the unknown contributor can be inferred. Depending on the profiles in the specific instance, this can be accomplished by subtracting the contribution of the known donor from the mixed profile.

Mixtures in a Differential Extraction

If the two profiles obtained from the sperm and non-sperm fraction of a single differential

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extraction indicate one or more common contributors, the types of each contributor may, if feasible, be inferred. This may be performed by subtracting the major contributor of one fraction from the mixed profile of the other, or by deducing the contributing single-source profiles using both mixed profiles.

Mixtures with Indistinguishable Contributors or Multiple Contributors

If a major or minor contributor cannot be distinguished because of similarity in peak heights or overlapping alleles are present that cannot be assigned, individuals may still be included or excluded as possible contributors to the mixture. A "failed to exclude" conclusion may be reported without a statistical interpretation.

In samples with partial profiles (degraded, low levels, and inhibitors) the data will be interpreted following the stated laboratory guidelines. Individual loci with peaks that fall below 100 RFU's may be called inconclusive for inclusionary purposes.

If the profile obtained for a sample is highly degraded, consists of multiple contributors (3 or more), and/or is obtained from low levels of DNA, it may be impossible to make an inclusionary statement or a statistical interpretation. When it is not possible to make an inclusionary statement individuals may be excluded as possible contributors to the results obtained or will be considered inconclusive and will be reported as such.

4) **CONCLUSIONS**:

For any given locus, peaks above 150 RFU's may be used for inclusionary as well as exclusionary purpose. Peaks between 100 and 150 RFU's must be interpreted with caution. Peaks in this range may be used for inclusionary purposes with the approval of the peer reviewer and/or the technical leader. Peaks between 50 and 100 RFU's may be used for exclusionary purposes only.

INCLUSIONS:

An inclusion indicates that the DNA profile of the individual in question is represented in the types obtained from a questioned and/or reference sample(s). Inclusions will be based on peaks with heights of at least 100 RFU. A statistical interpretation as to the strength of the inclusion must accompany the report of an inclusion for a questioned sample.

When making a direct comparison between a reference sample and a single source questioned sample an inclusion may be reported if the DNA profile of the reference sample is consistent with the DNA profile of the questioned sample.

If a direct comparison is not possible as in issues of parentage and missing persons, an indirect comparison may be made by considering the DNA profile of the questioned sample, along with the profiles of related reference samples in the context of the passage of alleles from parent(s) to offspring. An inclusion may be reported when all alleles present in the offspring's profile are represented in the parental profile(s).

EXCLUSIONS:

An exclusion indicates that the DNA profile of the individual in question is not represented in the types obtained from a questioned and/or reference sample(s). Peaks with heights of 50 RFU or above may be used for reporting an exclusion.

When making a direct comparison to a single source profile, an exclusion may be reported if there is a single difference between the questioned sample profile and the reference sample profile. Certain conditions may occur within the questioned sample that does not necessarily result in an exclusion if there is a single difference between the questioned sample profile and the reference sample profile. Examples of some of these conditions would be degradation, low levels of DNA, and amplification inhibition.

When making an indirect comparison using related reference samples, an exclusion may be reported when there are three or more alleles present in the offspring's profile that are not represented in a parental profile.

INCONCLUSIVE RESULTS:

Peaks with heights below 100 RFU are inconclusive for inclusionary purposes. Peaks with heights below 50 RFU will not be interpreted. They are inconclusive for any purpose.

When making an indirect comparison, the results are inconclusive when there i s one or two alleles present in the offspring's profile that are not represented in a parental profile. The submitting agency will be referred to an expert in parentage interpretation if a mutational analysis is desired.

The results may also be inconclusive if any of the profiles involved in the indirect comparison consist of a non-resolvable mixture. The submitting agency will be referred to an expert in parentage interpretation if an inclusionary interpretation is desired.

5) STATISTICAL INTERPRETATION:

Autosomal STR analysis:

Once a comparison between a reference sample(s) and a questioned sample is completed and an inclusion reported, the significance of that inclusion is estimated to allow the triers of fact to place the appropriate emphasis on that conclusion.

Databases:

Allele frequencies for the 13 core STR loci are available from databases provided by the FBI (Caucasian, African-American, Southeast Hispanic, Southwest Hispanic, American Indian, Asian and Caribbean), Additionally, other databases are also available in the literature, if needed.

Minimum Allele Frequency:

A minimum allele frequency estimation is calculated for STR loci which demonstrate alleles with a low frequency of occurrence. The intent of this application is to set a lower limit for the frequency for such rare alleles and, consequently, produce a conservative allele frequency estimation that does not underestimate the allele's frequency of occurrence.

The approach utilized is a basic procedure described previously by Budowle et al, 1991 and NRC II (National Research Council), 1996. The minimum allele frequency is calculated using the following expression:

$$p_{min} = 5/2n$$

where n represents the sample size (individuals) (ref: National Research Council II, 1996, pg. 148)

Formulas available for calculating population frequencies:

For direct comparisons of questioned profiles that meet the criteria for a single source, a major component or a deduced component of a mixture, the following formulas will be utilized for calculations:

(1) Heterozygote formula: $2pq = f$

(2) Homozygote formula: $p^2 + p(1-p)\theta = f$

Alternatively, formulas with a correction for population substructure (National Research Council II, 1996, pg. 114-115; formulas 4.10a, 4.10b) may be used.

NOTE: θ is an empirically determined measure of population subdivision.
$\theta = .01$ (recommended value, NRC II, pg.29)
$\theta = .03$ (recommended value for very small, isolated populations of interrelated people, NRC II, pg.29).

Genetic frequencies obtained for DNA samples at one STR locus can be multiplied by the frequencies found for the same samples at the other STR loci to obtain a combined frequency estimate. Using the "multiplication rule", the frequencies at each locus are multiplied together to generate the frequency of the entire profile.

$F = (f_1 x\ f_2\ x\ f_3\ x\ \ldots f_n)$, where n is the number of loci.

For indirect comparisons (issues of parentage and missing persons) involving profiles that meet the criteria for a single source, a major component or a deduced component of a mixture, the formulas in the following tables are used by the FBI's POPSTATS program for calculations:

(a) Parentage Trio - An offspring and two parental profiles versus an offspring and one known and one unknown parental profile – according to the table below (as described by I. W. Evett & B. S. Weir in Interpreting DNA Evidence, pg. 168 by Sinauer Associates Inc. 1998):

Offspring	Parent 1	Parent 2 (purported)	Numerator	Denominator	LR formula
P	P	P	1	p	1/p
P	PR	P	½	½ p	1/p
P	P	PR	½	p	1/(2p)
P	PR	PR or PS	¼	½ p	1/(2p)

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Offspring	Parent 1	Parent 2 (purported)	Numerator	Denominator	LR formula
PQ	P	Q	1	q	1/q
PQ	PR	Q	½	½ q	1/q
PQ	P	PQ or QR	½	q	1/(2q)
PQ	PR	PQ, QR or QS	¼	½ q	1/(2q)
PQ	PQ	P, Q or PQ	½	½ p + ½q	1/(p+q)
PQ	PQ	PR or QR	¼	½ p + ½q	1/(2p+2q)

Alternatively, formulas with a correction for population substructure (I. W. Evett & B. S. Weir in Interpreting DNA Evidence, pg. 179 by Sinauer Associates Inc. 1998) may be used.

(b) Single Parent Kinship - An offspring, one known parental profile and one unknown parental profile versus an offspring and two unknown parental profiles – according to the table below (as described by C. H. Brenner in Transfusion 1993; 33: 51-54):

Offspring	Parent 1 (purported)	Numerator	Denominator	LR formula
P	P	p	p^2	1/p
P	PR	½ p	p^2	1/(2p)
PQ	P	q	2pq	1/(2p)
PQ	PR	½ q	2pq	1/(4p)
PQ	PQ	½ p + ½ q	2pq	1/(4p) + 1/(4q)

(c) Reverse Parentage - An offspring and two parental profiles versus an offspring and two unknown parental profiles – according to the table below (essentially as described by M. N. Hochmeister, et al., Journal of Forensic Science **41**(1): pg. 158 (1996)).

Offspring	Parent 1 (purported)	Parent 2 (purported)	Numerator	Denominator	LR formula
P	P	P	1	p^2	$1/(p^2)$
P	P	PR	½	p^2	$1/(2p^2)$
P	PR	PR or PS	¼	p^2	$1/(4p^2)$
PQ	P	Q	1	2pq	1/(2pq)
PQ	P	PQ or QR	½	2pq	1/(4pq)
PQ	PQ	PQ	½	2pq	1/(4pq)
PQ	PR	PQ, QR or QS	¼	2pq	1/(8pq)

A likelihood ratio (numerator or denominator) calculated for one STR locus can be multiplied by the likelihood ratios (numerators or denominators) found for the same samples at the other STR loci to obtain a combined estimate. For those situations not

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directly calculable by the FBI's POPSTATS program, the analyst and the reviewer will each independently document the calculation in the case notes.

For any situation not covered by the scenarios described above, the submitting agency will be referred to an expert in parentage interpretation if further interpretation is desired. Likely situations might include but are not limited to the following:

- Analyses that consider more than one parent/offspring passage simultaneously (e.g. considering a questioned profile as both an offspring for a set of parental references and as the parent of an offspring reference; or considering a questioned profile as the possible parent of more than one offspring reference). The components of these relationships may be interpreted individually (as per the scenarios described above), but will not be considered simultaneously.
- Analyses involving individuals more distantly related than parent/offspring (e.g. siblings, grandparents, aunts, uncles, cousins, etc.).
- Analyses involving mutational events.
- Analyses involving one or more non-resolvable mixtures.

Mixture Calculations

If the evidence sample meets the mixture criteria as defined in **Interpretation of Results – Mixtures** and does not have a clearly defined major component or the types of the contributors cannot be deduced with (an) assumed known contributor(s), the following mixture calculations may be applied. Interpretation of DNA mixtures requires careful consideration of factors including, but not limited to, detectable alleles; variation of peak heights; and the number of alleles. The interpretation of each mixture will be evaluated on a case by case basis.

(1) Likelihood Ratios:

The LR (likelihood ratio) is the ratio of the probabilities of the evidence, given two competing hypotheses. It is the probability of a match if the DNA in the evidence sample and that from the suspect came from the same person to the probability of a match if they came from different persons. Specific calculations for a LR are referenced in the NRC II (National Research Council), 1996 (Appendix 5A) and as in the FBI's POPSTATS Mixture calculations.

(2) Combined Mixed Profile Probability:

A mixture calculation may be performed using the allele frequencies of each allele represented to determine the probability of selecting an unrelated individual in the population who could be a potential contributor to the mixture.

The allele frequency for each allele in the mixed DNA profile for an individual locus is determined.

The individual fragment frequencies are summed and this value is squared:

$$(p_1 + p_2 + \ldots p_n)^2 = P_{locus}$$

where n equals the number of alleles present, and P_{locus} is the probability of an unrelated individual being a contributor to that mixture at that locus.

The value of P_{locus} for each locus will be multiplied together to generate the probability of an unrelated individual in the population being a contributor to that mixture for all loci examined.

$$P_{locus1} \times P_{locus2} \times \ldots . P_{locus(n)} = P_{mix}$$

where n equals the number of loci analyzed and present in the mixture as in the FBI's POPSTATS Mixture calculations.

Biological relationships:

If the possible contributors of the evidence sample include relatives of the suspect, DNA profiles of those relatives should be obtained. If these profiles cannot be obtained, the probability of finding the evidence profile in those relatives should be calculated with formulas 4.8a, 4.8b or 4.9a, 4.9b (National Research Council II, 1996, pg. 113 and as in the FBI's POPSTATS Relatedness calculations).

Source Attribution:

A statement of source attribution may be made if the frequency of the profile is rarer than 1 person in 6 trillion. This figure is based on the approximate population of the world (6 billion) and a confidence interval of 0.999. The formula used for the calculation is:

$$p \leq 1 - (1 - \alpha)^{1/N}$$

(reference: "Source Attribution of a Forensic DNA Profile", Budowle, Carmody, Charkraborty, Monson; Forensic Science Communications, July 2000, Vol.2, Number 3).

Y-STR Analysis:

Once a comparison between a reference sample(s) and a questioned sample is completed and an inclusion reported, the significance of that inclusion is estimated to allow the triers of fact to place the appropriate emphasis on that conclusion. It is noted that all paternally related males relatives will share the same Y-STR profile.

Databases:

Statistically, the frequency of a haplotype profile is not addressed in the same manner as autosomal STRs. The questioned haplotype developed for the evidence sample is searched against the *Promega Powerplex® Y Haplotype Database* and found to occur "n" times in the population group(s) searched. The result of the questioned profile's occurrence in the database ("counting method") will be included in the Laboratory report.

It is also noted that the Y-STR haplotype database generated by the Wisconsin State Crime Laboratory was incorporated into *Promega's* database.

E2 DOCUMENTATION AND REPORT WRITING:

1. Documentation Requirements for DNA Cases

All steps of the DNA analysis process must be documented. This documentation allows each sample to be tracked through the process and each reagent used in the analysis of the samples to be identified. Examples of worksheets for accomplishing this are given in the Case Form Section of this manual. Documentation requirements for DNA cases must conform to the guidelines that are set in the Crime Laboratories' *Unified Policy and Procedure Manual.* The following specific documentation is required for DNA case files.

A. A case report describing the DNA work done on the case.

B. Copies of Sample Extraction Data Sheets for all samples from the case.

C. Copies of Quantifiler results sheet.

D. Copies of Amplification Sheets for all samples from the case.

E. Copies of the STR Data Sheet for each run in the case.

F. Copies of Genescan® electropherograms and Genotyper® or Powertyper® results for all samples in the case. A copy of the "Sample Information" sheet for a sample analyzed in the run showing the instrumental settings used for the run and the analytical conditions used to analyze the data. Any changes made to the standard conditions[3] must be documented. Initials of a second qualified DNA analyst must appear in the data set.

G. POPSTATs/ CODIS upload/ CODIS search results (if appropriate).

H. Any other documents that relate to the case but are not specified above.

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[2] Injection conditions: Run Voltage 15 kV, Inj. Voltage 15 kV, Inj. Duration 5 sec.(Profiler Plus and Cofiler) or 3 sec. (Power Plex ™ 16.2 & Y), Temp. 60°C, Laser Power 9 m Watts, Peak Detection Threshold: 150 RFU (for all dyes)

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2. Reporting Requirements for DNA Cases

DNA reports should communicate the results of PCR analyses effectively to the intended readers of the report. If the person reporting the DNA results is the person who examined the evidence from which the DNA samples were acquired, then the report must describe what was done to the evidence in the same way as a standard Serology report would. If the person reporting the DNA results did not examine the evidence from which the DNA samples were acquired, then the report must describe the nature of the DNA samples as received by the DNA scientist.

As of October 1, 1998, our laboratory must follow the *"Quality Assurance Standards for Forensic DNA Testing Laboratories"* promulgated by the DNA Advisory Board. Standard 11.1.2 states that "Reports ... shall include:

- (a) Case identifier
- (b) Description of evidence examined
- (c) A description of the methodology
- (d) Locus
- (e) Results and/or conclusions
- (f) An interpretative statement (either quantitative or qualitative)
- (g) Date issued
- (h) Disposition of evidence
- (i) A signature and title, or equivalent

The <u>case identifier</u>, <u>date issued</u> and <u>signature and title</u> are all routine parts of the report. They are included in the letterhead portion of the report and need no further clarification.

<u>Description of evidence examined</u> The description of the item should include

1. What the item was
2. Descriptive information if appropriate
3. The unique identifier of the item

<u>A description of the methodology</u> The typing systems used for the analysis should be mentioned in the body of the report.

<u>Locus</u> The tested loci must be included in the report. This can take the form of a typed listing of the loci or the loci can be identified as part of a table in which results are listed.

<u>Results and/or conclusions</u> A conclusion based on the results of the analysis must be included in the report. The typing results derived from each item relevant to the interpretation statement may be included. For partial profiles the number of loci used for the conclusion will be stated unless the quantitative interpretation statistic meets source attribution, in which case the source attribution statement is all that is required.

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An interpretative statement (either quantitative or qualitative) Qualitative interpretation statements for non-mixed samples can include language such as inclusion statements, exclusion statements, etc.

Quantitative interpretative statements would be statements of any probabilities that are calculated. All inclusionary statements must include a quantitative interpretative statement. Probability statements should include a description of the database that was used to calculate the statistics.

Disposition of Evidence Language should be included in the report stating what will be done with the evidence. Language stating whether any remaining sample is available for additional testing may be included in the report.

3. DNA Case Review

The Crime Laboratories' *Unified Policy and Procedure Manual* describes the purpose of case file review and the conditions under which it must be done. This section provides specific instructions for conduction of a review of DNA cases.

All DNA case files must be reviewed prior to issuance of the final report. This review process involves two steps, a technical review and a section administrative review. The technical review involves examination of the actual work done, the quality assurance measures, and the conclusions drawn. An examiner who is fully qualified to perform DNA STR analysis on casework must do this review. The section administrative review involves examination of the case file for completeness of documentation and the report for clarity, completeness, grammar and spelling.

A. Technical Review

1. Independent Reading

To start a technical review the reviewer must independently examine the printed electropherograms . The reviewer may find it helpful to use an STR Review Checklist during this process.

Any disagreement about the typing results must be resolved before continuing. The discrepancies must be discussed with the case examiner with the goal of determining why the discrepancy exists. If after discussion, the types can be agreed upon, allow the case analyst/case examiner to amend the report to reflect the acceptable types.

If no agreement in types can be reached or if there is disagreement about what needs to be done (i.e. extra work) to resolve the typing disagreement, the dispute must be settled by the unit leader.

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2. Quality Assurance Review

If all the types are acceptable, examine the scientific results and evaluate the quality of the case examiner's work using knowledge of the protocol and its quality assurance measures as a standard. The case examiner must be made aware of any deficiency and be allowed to correct it if possible.

If it is not possible to correct a deficiency, then any conclusions based on the results affected by the lack of quality assurance will be in question and a decision will have to be made as to whether a report can be issued with other than inconclusive results. This decision will be made by consensus of the case examiner, the reviewing examiner, the technical unit leader and the supervisor. All decisions to issue a report in the absence of quality assurance measures must be justified in compliance with the *Unified Policy & Procedure Manual*.

If all required quality assurance measures have been met satisfactorily, evaluate all conclusions made by the case examiner, whether positive (inclusions), negative (exclusions), or inconclusive. Any discrepancies should be brought to the attention of the case examiner and resolved in the manner indicated above.

If all conclusions appear to be valid and based on consideration of the required factors, initial and date the completed checklist or a copy of the laboratory report.

B. Administrative Review

Administrative review of the report, the case jacket and its contents should follow the procedure outlined in the Crime Laboratories' *Unified Policy and Procedure Manual*.

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CHAPTER VII

CASE FORMS

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DNA ANALYSIS STAIN EXTRACTION SHEET

CASE NO:
ANALYST:
DATE:

SAMPLE DESIGNATION		TYPE OF SAMPLE	RACE
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

EXTRACTION REAGENT	LOT NO.	EXTRACTION REAGENT	LOT NO.
EXTRACTION BUFFER		TE BUFFER	
PRO K		TNE BUFFER	
ϕOH/$CHCl_3$/IAA		SARKOSYL	
PELLET WASH BUFFER		DTT	
ETHANOL		ISOPROPYL ALCOHOL	
DNA IQ KIT			

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AMPLIFICATION DATA SHEET

CASE NO:
SCIENTIST:
DATE:

SAMPLE	AMOUNT AMPED (ng)	VOLUME SAMPLE (μl)	VOLUME DILUENT (μl)	AMPLIFICATION TYPE	THERMAL CYCLER POSITION

Type of Diluent:/Lot No.		Lot No. of Kit	

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AMPLIFICATION DATA SHEET (2)

CASE NO:
DATE:
SCIENTIST:
AMPLIFICATION TYPE:
THERMAL CYCLER:

SAMPLE	AMOUNT AMPED (ng)	VOLUME SAMPLE (µl)	VOLUME DILUENT (µl)	FINAL VOLUME AMPLIFIED (µl)	FINAL VOLUME DILUENT (µl)	VOLUME MASTER MIX (µl)

<table>
<tr><td rowspan="2">Type of Diluent
Lot No.</td><td rowspan="2"></td><td>Amp Kit Lot No.</td><td></td></tr>
<tr><td>Taq Pol. Lot No.</td><td></td></tr>
</table>

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AMPLIFICATION DATA SHEET (3)

CASE NO:
DATE:
SCIENTIST:
AMPLIFICATION TYPE:
THERMAL CYCLER:

SAMPLE	AMOUNT AMPED (ng)	VOLUME SAMPLE (μL)	VOLUME TE (μL)

TE Lot No.	

Profiler Plus Kit Lot No.		ProfilerTaq Lot No.	
Cofiler Kit Lot No.		Cofiler Taq Lot No.	

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STR DATA SHEET

CASE NO.:
DATE:
SCIENTIST:

REAGENT/INSTRUMENT	LOT/INVENTORY NUMBER
GENETIC ANALYZER (310/3100)	
CAPILLARY	
POLYMER	
10X GA BUFFER	
FORMAMIDE	
INTERNAL SIZE STANDARD	

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Equipment Maintenance Log

Serial Number or Other Equipment Identifier	Maintenance Action Taken	Action Taken By (Initials)	Action Date

QUANTIFILER SHEET

SCIENTIST:
DATE:
CASE NO:

HUMAN QUANTIFILER KIT:
Y QUANTIFILER KIT:
STANDARD PREP DATE:

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APPENDIX

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EVIDENCE HANDLING PROCEDURES

1. Stains prepared from standard blood samples to include those from the victim and suspect will be processed at a different time than the evidence samples. If evidence samples contain very small amounts of DNA such as hairs, these samples should be extracted at a different time than other samples from the same case. All samples can be united for the remainder of the procedure after the extraction step is completed.

2. Only one item of evidence will be open at any one time.

3. Examination paper and gloves will be changed between every item of evidence.

4. Benchtops used for evidence examination will be cleaned with an appropriate solvent. The examination of the evidence samples should be completed and the bench cleaned before the examination of the standard samples. Pipettes should be wiped down with an appropriate solvent after each case.

5. All tools (scissors, forceps, etc) should be rinsed with an appropriate solvent, then distilled water and then wiped off with a Kimwipe after each cutting or manipulation of a sample.

6. A manipulation control consisting of a clean piece of the appropriate type of substrate will be carried through the entire process. This should be the last sample in the set to be extracted. Each set of samples and standards extracted should have their own manipulation control.

7. Only one sample tube may be opened at a time. The only exception to this rule is during the wash steps when extraction with magnetic beads. At this time the DNA is bound to the beads and the possibility of carryover is minimal.

8. All reagents should be aliquoted into small amounts and all pipetting done from the aliquot to ensure that the stock quantity is never contaminated.

9. Dedicated pipettes will be used for the various stages of DNA analysis, extraction, amplification, and detection. Aerosol resistant tips will be used for all analyses. Tips must be changed after every pipeting.

10. Spin baskets will be used to extract DNA from the solid stain material. This will preclude centrifuging of open material containing DNA and possibly contaminating the centrifuge. The centrifuge should be wiped out with an appropriate solvent to minimize the possibility of carryover contamination.

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12. DNA extraction and amplification areas for PCR based systems will be separated in physical location. All reaction set-ups will be performed in an isolated area designated for that sole use.

13. Lab coats will be worn in the amplification rooms. These coats will be brought into the room and not leave until they are ready for disposal.

14. An amplified product free area can be designated in the amplification room. This area can be used for note taking or other activities that should remainfree of amplified product.

15. Contamination logs will be kept for each amplification room. They will record who contaminated/how severe the contamination was (i.e. an outbreak of contamination versus an incident)/what cases were involved/what was done to correct the contamination.

16. All counter tops, floors and benches in the amplification lab will be cleaned with an appropriate bleach solution monthly. Records of cleaning will be maintained.

Internal Validation Proposal

As per SWGDAM Guidelines, the purpose of the validation is to assess the ability of the procedure to obtain reliable results, to determine the conditions under which such results can be obtained, and define the limitations of the procedure. This is accomplished through developmental and internal validation studies as defined by the *TWGDAM Guidelines for a Quality Assurance Program for DNA Analysis* [Crime Laboratory Digest 1995:22(2): 18-43] revised by SWGDAM in July 2003.

System to be validated:

Developmental validation:

Internal validation studies:

(1) Known and Non-probative Evidence Samples:

(2) Reproducibility and Precision:

(3) Match Criteria:

(4) Sensitivity and Stochastic Studies:

(5) Mixture Studies:

(6) Contamination:

(7) Qualifying tests: