Department of Forensic Biology

Forensic Molecular Biology Laboratory

DNA
Quality Control Manual
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QUALITY CONTROL SYSTEMS AND PROCEDURES

The DNA quality program maintains performance standards with six systems:

1. Material Flow and Inventory
2. General Laboratory Quality Control
3. Equipment and Supplies Quality Control
4. Raw Material Quality Control
5. Solution Quality Control
6.. PCR Contamination Control

Each system describes a flow of information. The system defines an area of interest, outlines what information is routinely collected, how it is collected, and how it is evaluated.

Unless otherwise specified, raw materials are quality controlled one time. They are quality controlled before the first time the raw material is used. However if the raw material is unstable or degrades additional quality controls may be required and they will be specified in the appropriate Quality Control Procedure. Each time a solution which requires quality control is prepared, it is quality controlled. Any quality control procedure listed on a solution sheet is required. Critical reagents are defined as those raw materials and solutions requiring a quality control procedure.

A system may incorporate one or more procedures. A procedure is a list of instructions for a specific task within the laboratory. Examples of procedures are QC008 Thermocycler Block Cleaning and QC015 Chelex Extraction. Worksheets used for documentation are located in the appendix. This manual describes how the systems and procedures operate and how they are integrated to make up the QA/QC program for DNA testing.

The quality program has both a bookkeeping function and a revision function. The information collected during routine testing is recorded and compiled to support typing results from the laboratory. The information is also used to evaluate the success of the quality program. Changes may be necessary to improve the efficiency of data collection or to ensure that the system is comprehensive. Consequently, the quality program modifies itself based upon the information it collects during routine testing.

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INVENTORY AND MATERIAL FLOW WITHIN THE LABORATORY

To standardize definitions and to make inventory simpler, every material (equipment, supply and chemical) which passes through the laboratory is assigned a raw material number (RM#). The same raw material procured from different manufacturers is assigned the same RM#. For example, white, 1.8 mL microcentrifuge tubes from Fisher and VWR are assigned the same RM#. Red tubes are assigned a different RM#. If raw material from one manufacturer can freely substitute for raw material from another manufacturer in the laboratory they are assigned the same RM# even if they have slight differences. For example, 1.8 and 1.9 mL white microcentrifuge tubes are assigned the same RM# because they can be used interchangeably (or 100 mL beakers from Pyrex, Kimble or Corning). Different raw material with the same RM# are distinguished by date of receipt, lot #, and/or manufacturer.

The inventory system tracks the receipt and usage of raw material. It is not concerned with the location of the raw material, only the total amount of raw material available. The management of the laboratory (and accreditation) is concerned with the location of the raw material and not the quantity of the raw material available. These two systems appear to contradict each other but actually they are complementary.

Each line in the inventory database reflects one day's receipt of a single raw material. The amount of raw material initially received is entered and never changes. The amount of raw material on hand steadily decreases. At each inventory, a new field is added to the table to reflect the date of inventory. When an item is used up, the line is moved from the current inventory database to an archival database. I will move each of the lines of used up items. The location of each item is tracked in a separate database and the two databases are joined by forms.

All items when they enter the lab are marked with the RM# and date or receipt. If a case of raw materials is opened for use then the individual items are marked with RM# and date of receipt. Inventory is performed by recording the RM#, # of units, and definition of units. The name of the item is secondary during inventory. Only the RM#'s are important. That way there is only one name for each item and the person taking inventory does not have to know how to name an item.

The following are the steps to be followed when an item enters the laboratory:

1. Receipt of raw material

2. Compare raw material to packing slip and check off items received and quantity. Initial and date. Notify Procurement of any inconsistencies or damage. Xerox packing list.

3. Compare raw material to requisition AND purchase order. Check off items received and quantity. Initial and date. Notify Procurement of any inconsistencies. Attach packing list to requisition and purchase order. If order is complete move to completed order folder.

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4. Look up in the database the RM# for the raw material. If it is a new raw material, assign a new RM number.

5. Write on each item, the RM# and date received. This can be cases or individual items.

6. Enter in the database, the RM#, date received, quantity received, unit definition, location, expiration date, manufacturer, cat # etc..

7. Disperse the raw material to the appropriate storage location.

8. If an item is used up enter zero in the quantity on hand column.

9. During inventory, record RM#, date of receipt, quantity, unit, and location. The RM# and date of receipt is written on the outside of each item. Inventory will go a lot faster this way because there is less to write and think about.

10. In the database, add a new field for the inventory date. Match up the RM# and date of receipt of the inventory with the database. Enter the quantity in the new field using the same unit definition as previously. Quantities may need to be converted to match a unit definition.

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GENERAL LABORATORY QUALITY CONTROL

QC001 Glassware Cleaning

General Procedure

Most pieces of laboratory glassware can be cleaned by washing and brushing with a solution of detergent. Detergent is available from the OCME stockroom.

Rinse each piece at least three times with tap water to remove all detergent residue.

Rinse each piece three times with deionized water. If the surface is clean, the water will wet the surface uniformly. On soiled glass the water stands in droplets. If spotting is observed during the deionized water rinse, the detergent wash should be repeated. If spotting is observed after a second detergent wash, a nitric acid rinse may be necessary (see below).

Allow the glassware to dry at room temperature on a drying rack.

Dishwasher

Load the dishwasher with glassware and put a scoop (approx. 42 g) of non-foaming, laboratory dishwasher detergent in the detergent cup. **Do not use regular laboratory detergent!**

Turn on the dishwasher using the steam scrubbing cycle. When the cycle is finished, remove the clean glassware.

Alternative Cleaning Procedures

When glassware cannot be completely cleaned by scrubbing with a detergent solution, other cleaning methods must be used.

Agarose

Solidified agarose in flasks can be redissolved by adding water to the flask and heating in the microwave. Solidified agarose in graduated cylinders can be removed with a brush. It is best not to use boiling water to redissolve solidified agarose in graduated cylinders, since this may affect the calibration of the cylinder over time.

Nitric Acid

Stubborn films and residues which adhere to the inside of flasks and bottles may often be removed by rinsing with dilute nitric acid. Some glassware may need to soak in dilute nitric acid overnight. Any nitric acid rinse must be followed by multiple rinses with distilled water to remove acid residues.

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QC002 Autoclaving

GLASSWARE/EQUIPMENT

All glassware must be clean and dry prior to autoclaving (refer to QC001 for standard glassware cleaning procedure).

Cover glassware openings loosely with foil.

Attach a strip of autoclave time tape to the foil on each piece.

Flasks should be loosely capped.

Small items may be autoclaved inside a beaker covered with foil.

SOLUTIONS

Flasks should be loosely capped.

Do not fill flasks more than 75% of capacity.

OPERATION

The drain should be closed. The chamber should be filled with deionized water to the fill line (approx. 4-6 qt). Load the chamber and close the door. Select temperature, exhaust and set the timer. The autoclave starts automatically. The autoclave should not be opened until all of the pressure is released.

MAINTENANCE

After each day's use, the chamber should be drained of water by opening the drain knob, wahsed with soap and water, rinsed, dried and the door should be left open.

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QC003 Temperature Control

REFRIGERATORS/ FREEZERS/ VWR OVENS

Either a printing thermometer or the Cole-Parmer 1559-72 Multimeter temperature probe (type K) is used to measure refrigerator/freezer/VWR oven temperatures. Refrigerator/freezer temperatures are recorded daily during the work week and the VWR oven temperature is recorded before blot washing and other uses.

Place the probe into the refrigerator/freezer/VWR oven and close the door. Make sure the door seal closes tightly around the probe wire. Allow the probe to equilibrate 5 - 10 minutes. The probe should not be removed from the unit.

Measure the temperature and log or tape the reading on the monthly TEMPERATURE CONTROL LOG (F015) sheet for that unit.

WATER BATHS/HYBAID OVENS/ HEAT BLOCKS

A recording thermometer or an Omega thermistor probe (type T) is used to measure the temperature of the Bellco water baths, HYBAID ovens, and heat blocks. The probe is mounted in the water bath , HYBAID oven or heat block. Temperature measurements are recorded each day the waterbath, oven or heatblock is used and before each hybridization/wash.

To measure the temperature, turn the water bath, HYBAID oven or heat block on (if necessary) and allow it to equilibrate for at least 15 minutes.

When the temperature has stabilized, record or tape the temperature reading on the appropriate TEMPERATURE CONTROL LOG sheet. To measure the thermistor temperature, plug the probe into the correct position on the meter. Record the reading. The thermistor reading can be corrected using the slope and y-intercept values calculated from the probe calibration (QC005). The corrected thermistor reading must be 55 ± 1 °C for PCR hybridizations.. The corrected thermistor reading must be 65 ± 1 °C for RFLP hybridizations. The corrected thermistor reading must be 50 ± 1 °C for QuantiBlot.

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QC030 Thermocouple Verification (Type T-brown and Type K)

Temperature probe operation is verified twice a year.

Place the temperature probe in an ice water slurry. Allow the temperature to equilibrate. The probe must read between -1 and 1°C.

If the probe is going to be used in the 0 to 100°C range, place the temperature probe in a boiling water bath. Allow the temperature to equilibrate. The probe must read between 99 and 101°C.

If the probe is going to be used in the -80 to 0°C range, place the temperature probe in a dry ice acetone slurry. Allow the temperature to equilibrate. The probe must read between -78 and -74°C.

Record the results of the temperature check on the appropritate Thermocouple Verification Sheet (F023 or F023A) . The probe must meet the above specifications to be ceritified for use.

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QC004 Omega Type T Thermocouple Preparation

Introduction

Temperature probes have two components. The thermocouple, which responds to temperature changes, is mounted inside either an 0.5 mL amplification tube or a 1.5 mL centrifuge tube. Thermocouples are used because of their fast response time and the availability of thin thermocouple wire. Sensors made with thin wire will not significantly affect the temperature reading. The laboratory probes are made from Teflon coated, 36 gauge, type T thermocouples purchased from Omega Engineering.

The thermocouple is plugged into an electronic thermometer which displays the temperature read-out. An Omega electronic thermometer is used for its speed, sensitivity, and accuracy. The combination of the thermocouple and electronic thermometer must be calibrated together as the response with different thermocouples will vary.

Procedure

Poke a small hole through the center of the cap of a sterile reaction tube using a sterile needle.

Without bending the wire, pass the thermocouple through the hole from the top of the cap, so the soldered tip of the wire will be inside the tube when the cap is closed.

Tie an overhand knot in the insulated part of the wire. Carefully tighten the knot so that it fits inside the cap of the tube. The knot should not be so tight as to kink or break the wire. The knot prevents the wire from being pulled out of the tube during temperature measurements.

Check the length by closing the tube and pulling the knot against the inside of the cap. Enough of the thermocouple wire should remain below the knot so that the thermocouple is within 1 mm or so of the bottom of the tube; it may touch the tube wall slightly. Adjust if the length is too long or too short.

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The temperature probe is calibrated twice a year against an ASTM mercury thermometer, graduated to 1°C over the range 25-100°C. Before beginning the calibration procedure, the mercury thermometer is checked by measuring two standard temperatures.

Mercury Thermometer Standardization

Place the thermometer in an ice water slurry. The etched line around the bottom of the thermometer must be at or below the level of the liquid. Allow the temperature to equilibrate. The thermometer must read between -0.2 and 0.2°C.

Place the thermometer in a boiling water bath. The etched line around the bottom of the thermometer must be at or below the level of the liquid. The thermometer must read between 99.8 and 100.2°C.

Record the results of the temperature check on the Thermocouple Calibration Sheet (F010) .

Thermocouple Temperature Response

Add 3 liters of distilled water to a 4 liter glass beaker.

Place the beaker on a stir plate.

Set up a clamp and ring stand behind the beaker.

Clamp the mercury thermometer onto the ring stand and position it so that the thermometer can be submerged in the water.

Open the cap of the tube with the thermocouple and slide the tube up the wire far enough to be out of the way. The performance of the thermocouple should be checked directly, without interference from the tube.

With a twist tie, attach thermocouple near the bulb of the thermometer so that the thermocouple bead is close to but not touching the bulb.

Lower the mercury thermometer, with attached thermocouple and wire, into the water. Tighten the clamp to hold the thermometer at the correct depth. The thermometer has an etched line 17 cm from the bulb which is the minimum level the thermometer must be

immersed for accurate readings. Failure to immerse at the correct depth will result in incorrect results.

Plug the thermocouple into the socket of the electronic thermometer to be used during routine measurements.

Turn on the stir plate. Stir the water to the point where a shallow vortex forms. If necessary, adjust the stirrer during the procedure to keep the water well stirred. Thorough mixing will reduce temperature gradients near the thermometer.

Seven or eight comparisons of the mercury thermometer and the electronic thermometer should be made, over a range of 25°C to 94°C. Temperatures must not be taken above 95°C because the formation of small vapor bubbles can cause fluctuations leading to variable temperatures.

The first measurement is made at room temperature. Record the reading from the thermocouple and the mercury thermometer on the Thermocouple Calibration Sheet (F010). The probe measurements are recorded under the x-axis column, and the readings from the mercury thermometer are recorded under the y-axis column.

Raise the temperature of the water approximately 10°C above room temperature by heating the stir plate.

When the temperature has risen several degrees, turn down the heat.

Check the immersion level of the thermometer. The position of the thermometer may have to be adjusted to compensate for evaporative loss of water.

If gas bubbles have formed on the thermometer or the thermocouple, gently tap the lower part of the thermocouple wire with a pencil to release them.

Check the temperature of the thermometer until successive readings show changes of less than 0.2°C in a 15 second period.

Once the temperature has stabilized, but at least one minute after any adjustment of the probe, record the readings of both thermometers.

Heat the water about 10°C more. Lower the heat until the temperature stabilizes, check the immersion level, remove any gas bubbles, and record the second set of readings.

Repeat this process until seven or eight temperature measurements have been recorded from 25°C to 95°C. For best results, the number of comparisons within a set should be a bit greater at the top of the range to compensate for a higher uncertainty of measurement. The multiple readings will partially overcome the uncertainty in reading the mercury thermometer and provide some confidence in the performance of the system over a range of temperatures.

Calibration Line

If the pairs of readings taken during the calibration procedure were plotted on a graph, thermocouple values along the x-axis and thermometer values along the y-axis, the points would fall along a straight line. This line is the calibration curve which relates observed temperature values measured by the thermocouple probe to standard temperatures. The calibration line is defined mathematically by the equation

$$y = mx + b$$

where m is the slope and b is the y-intercept.

The best fit line for the data can be calculated directly using a least squares method. The least squares calculation yields the slope and intercept necessary to convert thermocouple readings into standard temperatures as well as the correlation coefficient, r. The correlation coefficient gives a quantitative estimate of the goodness of fit. The closer the data points are to the best fit line, the higher the correlation coefficient. A perfect fit has a correlation coefficient of 1.

Calculations

The following are calculated and recorded on the Thermocouple Calibration Sheet (F010). The variable n is the number of data points collected during the calibration experiment, typically seven or eight.

The following are calculated the same way for the sets of x and y values. The discussion describes the calculations with respect to the x values only, assuming parallel calculations for the y values will be performed. Summation (x) is calculated by adding

together the x-axis values. This is written in standard notation as

$$\text{sum}(x) = \Sigma\, x_i$$

Mean x equals summation (x) divided by n. This is written

$$\bar{x} = \frac{\text{sum}(x)}{n}$$

Summation (x^2) is the sum of the squares of the x values. All of the x values are squared first and then the squares are added together. This is written

$$\text{sum}(x^2) = \Sigma\, (x_i^2)$$

S_{xx} is defined as the sum of the squares of the x values minus the sum of the x values squared divided by n.

$$S_{xx} = \text{sum}(x^2) - \frac{[\text{sum}(x)]^2}{n}$$

Summation (XY) is calculated by multiplying the pairs of x and y values together and adding the products together.

$$\text{sum}(xy) = \Sigma\, x_i y_i$$

S_{xy} is defined as the sum of the x and y products minus the sum of the x values times the sum of the y values divided by n.

$$S_{xy} = \text{sum}(xy) - \frac{\text{sum}(x)\ \text{sum}(y)}{n}$$

The slope of the best fit line, m, is defined as

$$m = \frac{S_{xy}}{S_{xx}}$$

The intercept is calculated using the mean x and y values.

$$b = \bar{y} - m\bar{x}$$

Finally, the correlation coefficient is calculated using

$$r = \frac{S_{xy}}{(S_{xx} S_{yy})^{1/2}}$$

The slope is written with three significant figures. The intercept is rounded to the tenth's place. The correlation coefficient has a specification of >0.9999. If the calibration passes specification, the probe is ready for use.

Final Adjustments

Release the thermocouple wire from the mercury thermometer.

Slide the cap of the tube down the thermocouple wire until it is near the knot.

For the thermocycler probe, place 120 µL of deionized water into the tube and overlay with two drops of mineral oil. The mineral oil prevents evaporative cooling of the liquid inside the tube.

For the water bath probe, place approximately 1 mL of mineral oil into the tube.

Close the cap of the tube. The thermocouple tip should be just above or lightly touching the end of the tube. Do not seal the hole in the cap. If the cap is sealed around the thermocouple wires, the pressure in the tube at high temperatures will force liquid up between the sheath and the wire.

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EQUIPMENT QUALITY CONTROL AND OPERATING INSTRUCTIONS

QC006 Balances **Page 1 of 2**

ROUTINE WEIGHT MEASUREMENTS

Press the control bar once to turn on the power. Allow the readout to stabilize.

Place the weigh paper or weigh boat on the pan of the balance. Allow the readout to stabilize.

Press the control bar once to tare the balance.

Make the desired measurement.

When finished, pull the control bar up to turn off the power. Clean out the weighing chamber with the small brush or a damp paper towel, being careful not to disturb the pan.

METTLER AE260 ANALYTICAL BALANCE TWO-POINT CALIBRATION

A two-point standardization can be performed before routine measurements to ensure consistent performance day to day.

Press the control bar once to turn on the power. Allow the readout to stabilize.

Close all the doors surrounding the weighing chamber.

Press and hold the control bar until the readout says CALIB. The balance is calibrating at zero grams.

When the readout flashes 100, slide the lever on the right side back to release the internal 100 gram standard weight. Allow the balance to calibrate at 100 grams.

When the readout flashes 0, slide the lever forward. Allow the readout to stabilize.

The balance is calibrated for weekly use.

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BALANCE FOUR-POINT STANDARDIZATION

Each week, the balance is standardized using four standard weights.

Weigh the first standard. Record the standard weight and the measured weight on the BALANCE LOG sheet.

Repeat the measurements for the other three standard weights. Record all measurements.

MAINTENANCE

This balance is serviced as needed by an outside contractor.

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QC022 pH Meter **Page 1 of 2**

TWO-POINT CALIBRATION

Choose standard buffer solutions for a two-point calibration which bracket the expected final pH of the solution to be measured. i.e. use pH 7 and 10 standard buffers for a solution with final pH of 8.

Press STNDBY/MEAS button. Allow the reading to stabilize.

Press TWO POINT CAL button.

Fill the electrode with saturated KCl solution if necessary.

Rinse the electrode with deionized water. Blot dry.

Place the electrode in fresh standard buffer solution.

The display asks for the pH of the first standard solution. Enter the pH value of the standard solution and press ENTER.

The meter will stabilize the mV reading at that pH.

When the readout is stable, press ENTER.

The display asks for the temperature of the reading. Enter the room temperature (a value of 25 °C is adequate for our measurements).

Rinse the electrode with deionized water. Blot dry.

Place the electrode in the second standard buffer solution.

The display asks for the pH of the second standard solution. Enter the pH value and press ENTER.

Allow the meter to stabilize the mV reading at that pH.

When the readout is stable, press ENTER.

Enter the temperature.

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Rinse the electrode with deionized water. Blot dry.

The meter is calibrated before routine measurements.

ROUTINE PH MEASUREMENTS

Fill the electrode with saturated KCl solution if necessary. When fresh KCl is added, it is a good idea to mix the solution in the electrode by slowly inverting the electrode several times before continuing.

Calibrate the pH meter.

Rinse the electrode with deionized water. Blot dry.

Place the electrode in solution. Allow the reading to stabilize and record the measurement.

MAINTENANCE

The pH electrode must be kept filled with saturated KCl solution. This solution is approximately 30% KCl. The electrode is stored in a 2% KCl solution made from the saturated KCl filling solution (NOT deionized water or pH 7.00 standard solution).

When measuring the pH of large volumes, the pH electrode must be held in place. The electrode can be damaged if it is hung over the edge of the container and allowed to stir with the solution.

If the pH reading drifts or requires a long time to stabilize, the electrode bulb may need to be regenerated or the electrode may need to be replaced. Refer to the Beckman insert for further details of electrode maintenance.

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Definitions

Errors in analysis may be classified as systematic (determinant) or random (indeterminate). Systematic errors are caused by a defect in the analytical method or by an improperly functioning instrument or analyst. For example, a dirty balance pan will cause a systematic error. Systematic error is consistent. It represents the effect of instrument calibration, operating characteristics, and conditions that are constant or that change only in a consistent, predictable way during a series of liquid measurements. Systematic error normally biases all measurements at a given setting toward volumes that are either higher or lower than the set value. The only way to deal with this type of error is to rectify the cause.

Random errors are unavoidable because there is some uncertainty in every physical measurement. The careful analyst can only read a 50 mL graduated cylinder accurately to the nearest 0.5 or 1 mL, for example. However a truly random error is just as likely to be positive as negative. This fact makes the average of several replicate measurements more reliable than any individual measurement. Unfortunately, random errors do set a definite limit on accuracy even when the measurement is repeated many times.

Statistics

The gravimetric method is used most often to assess the performance of a micropipette. Distilled water is used as the standard. The water measured at a given volume setting is weighed on an analytical balance. The actual volume measured can be determined from the weight by correcting for the density. If the frequency of occurrence is plotted versus measured volume for a large number of samples, a distribution similar to that in Figure 1 is obtained.

Two calculated statistical values are useful in summarizing and interpreting this type of data. The mean (V_{mg}) is calculated in milligrams according to Equation 1.

$$\text{mean (mg)} = \bar{V}_{mg} = \frac{\Sigma V_i}{n} \qquad \text{Equation 1}$$

The V_i are the individual measurements in milligrams, and n is the total number of measurements recorded.

This mean weight is converted to volume by dividing by the density of water (δ) at the recorded temperature.

$$\text{mean } (\mu L) = \bar{V}_{\mu L} = \frac{\bar{V}_{mg}}{\delta} \qquad \text{Equation 2}$$

The mean volume is the peak of the normal distribution of measured volumes. The standard deviation quantifies the magnitude of scatter due to random error. Volumes close to the mean volume will occur with much higher frequency than volumes farther from the mean volume.

The standard deviation (SD) is calculated according to Equation 3.

$$SD = \left[\frac{\Sigma (V_i - \bar{V}_{mg})^2}{n-1} \right]^{1/2} \qquad \text{Equation 3}$$

Specifications

Each micropipette must pass specifications for both accuracy and precision before it can be released to the laboratory for general use. Accuracy in liquid measurement refers to how close a measurement is to the volume specified by the setting of the pipette. Precision refers to the reproducibility of a volume measurement.

Mean error is the difference between the mean volume of actual measurements and the volume set on the instrument. Mean error is a measure of the systematic error component of individual liquid measurements. The accuracy specification defines an acceptable range of systematic error.

The precision specification places an upper limit on the standard deviation of measurements performed at a given volume setting. It defines an acceptable range of random error.

Both accuracy and precision may be expressed as percent figures. Percent error is the mean error expressed as a percent of set volume. The percent error is calculated according to Equation 4.

$$\text{percent error} = \frac{(\bar{V}_{\mu L} - V_{set})}{V_{set}} \cdot 100 \qquad \text{Equation 4}$$

where V_{set} is the volume setting on the instrument.

The coefficient of variation (CV) gives the standard deviation as a proportion of the mean.

$$CV = \frac{SD}{\bar{V}_{mg}} \cdot 100 \qquad \text{Equation 5}$$

Method

Two times a year, either critical pipets are sent to an outside vendor for calibration or the following procedure is followed. Ten measurements of distilled water are made at three volume settings for each micropipette. For Eppendorf repeater pipettes, five settings are measured.

Record the model and serial number for the instrument to be calibrated on a Micropipette Calibration Sheet (F009).

Measure and record the temperature of the distilled water.

Record the density of water at the measured temperature. Refer to Table 2 for the density of water at one atmosphere.

Calibrate the analytical balance at zero and 100 grams using the internal standard weight.

Place the weighing vessel on the pan. The weighing vessel should not exceed 25 times the volume of the sample to be measured. Vessels which are as small as possible relative to the set volume reduce the error due to evaporation. Ideally, the vessel should be cylindrical so that the liquid surface area will stay fairly constant as it fills. The caps from screw-top microcentrifuge tubes are useful for the smallest volumes (1-10µL), and caps from larger bottles are used for larger volumes.

Close the glass panels on the balance and allow the reading to stabilize.

Adjust the volume of the pipette to the desired setting. Refer to Table 1 for the volume settings used for each of the different models.

Tare the balance.

Open the top panel of the balance.

Carefully measure a volume of deionized water into the weighing vessel.

Close the top panel and allow the balance to equilibrate.

Record the weight. The reading must be taken as soon as possible after the measurement has been made so that significant evaporation does not occur. Typically, the reading is taken when the "off-balance" light goes out.

Using a new pipet tip each time, repeat the procedure until ten measurements have been recorded at that volume setting.

Repeat the procedure for the two remaining volume settings.

Calculate the percent accuracy and coefficient of variation for each set of measurements.

Compare the results to the specifications for that pipette model. Refer to Table 1 for the specifications.

If the measurements are within specification, relabel the handle with the new date of calibration and return the pipette to the laboratory. File the completed Pipette Calibration Sheet in the Micropipette Performance Log (F008).

If any of the measurements is outside specification, the pipette must be withheld while the results are reviewed. Often when an instrument fails a performance test, the problem is not due to a loss of proper calibration. Consistent measurements require practice and technical ability. Frequently, a pipette may not pass specifications because of inexperience on the part of the technician performing the test. Instruments may be retested and a decision may be reached upon additional results.

To convert the mean sample weights to mean volumes, the sample weights are divided by the density of water (see table 2) under the test conditions. The table of densities assumes that the air temperature is the same as that of the water in the sampling reservoir and that the barometric pressure is one atmosphere.

Because of evaporative cooling, the steady-state temperature of water in the sampling reservoir will be lower than the air temperature by as much as two or three degrees. Also, the density of air will vary slightly with barometric pressure and humidity. These smaller effects are not taken into account since they are significant at a level well below the level of discrimination needed to monitor pipette performance.

Correction for evaporation will also increase the accuracy of the data and can be important for small volume measurements. An evaporation blank is used to estimate the amount of water lost during a single measurement. The amount of evaporative loss is estimated by performing the identical sequence of manipulations for a single sample measurement without sample aspiration. This amount is then added to the mean sample weight. Evaporation blanks are currently not performed as a routine part of pipette standardization since at the smallest volumes, the accuracy of the Mettler AE-260 balance is limiting. Evaporative loss is minimized by using the smallest practical weighing vessels.

Table 1: Pipette Performance Specifications

model	volume setting (µL)	percent error	CV
P-1000	1000	≤ ±2.0	≤ 1.0
	500	≤ ±2.0	≤ 1.0
	200	≤ ±2.0	≤ 1.0
P-200	200	≤ ±2.0	≤ 1.0
	100	≤ ±2.0	≤ 1.0
	50	≤ ±2.0	≤ 1.0
P-100	100	≤ ±2.0	≤ 1.0
	50	≤ ±2.0	≤ 1.0
	20	≤ ±2.0	≤ 1.0
P-20	20	≤ ±2.0	≤ 1.0
	10	≤ ±2.0	≤ 1.0
	2	≤ ±10	≤ 5.0
E-10	10	≤ ±2.0	≤ 1.0
	5	≤ ±5.0	≤ 2.0
	2	≤ ±10	≤ 5.0
Repeater	10 (500µL tip)	≤ ±2.0	≤ 1.0
	30 (500µL tip)	≤ ±2.0	≤ 1.0
	50 (500µL tip)	≤ ±2.0	≤ 1.0
	50 (2.5mL tip)	≤ ±2.0	≤ 1.0
	250 (12.5mL tip)	≤ ±2.0	≤ 1.0

P - Rainin Pipetman
E - Eppendorf Ultra-micropipette
Repeater - Eppendorf Repeater Pipette

Table 2: Density of Water

temp (°C)	density δ (mg/µL)	temp (°C)	density δ (mg/µL)
4	1.00000	17	0.99880
5	0.99999	18	0.99862
6	0.99997	19	0.99844
7	0.99993	20	0.99823
8	0.99988	21	0.99802
9	0.99981	22	0.99780
10	0.99973	23	0.99757
11	0.99963	24	0.99733
12	0.99953	25	0.99708
13	0.99941	26	0.99681
14	0.99927	27	0.99654
15	0.99913	28	0.99626
16	0.99897	29	0.99598

References

Adapted from CRC Handbook of Chemistry and Physics, 53rd ed., R.C. Weast, Ed., CRC Press, Inc., 1972-1973, page F-5.

Initials: RCJ Date: 9/11/96

QC020 SAVANT UVS400 Freeze Drier/Vacuum Pump

1. Turn on main power to allow unit to cool. Wait 30 minutes before use.
2. Place samples in centrifuge
3. Set drying rate at medium.
4. Turn rotor on.
5. Turn on vacuum switch.
6. Place arrow perpendicular to hose 90° clockwise. Check to make sure cover on rotor cannot open.
7. Allow samples to dry for appropriate time.
8. Turn off vacuum. Place arrow parallel with hose. (270° turn clockwise)
9. Shut off rotor and remove samples.
10. Turn off power.
11. Detach condensation bottle from unit and check for condensation. If condensation is present, dry bottle and reattach to unit.**

**** THIS STEP MAY BE DONE PERIODICALLY**

Initials: RJ Date: 9/4/96

QC021 Operation of the Biosafety Hood

Routine Use

NOTE: Do not work with any organic solvents (except ethanol) in the biosafety hood.

Turn the blower on and **WAIT** 15 minutes before using the hood. Leave the blower on while you are working in the hood.

Turn on the fluorescent light, NOT the UV light.

Wipe all exposed hood surfaces with 70% ethanol. This must be done by every individual, each time they start to work in the hood.

Line the work surface with absorbent pads. Put the plastic side down and the paper side up. Do not block the vents.

Work on the absorbent pads following all of the safety precautions listed above.

In case of a spill onto the hood surface, decontaminate with 10% bleach for 10 minutes. Absorb the bleach onto a paper towel and rinse the surface with 70% ethanol.

NOTE: All the bleach must be rinsed from the hood surface with the ethanol. Otherwise the hood will corrode.

If the blower stops running, DISCONTINUE all work and safely seal up all samples. **The hood no longer offers any protection.**

When you are done working, discard the absorbent pads and change your top layer of gloves.

Wipe all exposed surfaces with 70% ethanol and then discard your gloves layer by layer in the red biohazard bags.

Turn the UV on for 1 hour. Do NOT expose yourself to the UV.

Shut off the blower and UV Do NOT leave on overnight.

Maintenance

The hood is inspected by an outside vendor once a year. The airflow should be recorded monthly.

Initials: RCS Date: 9/11/96

QC028 Verification of Centrifuge Rotation **Page 1 of 3**

Centrifuges and each of their rotors should have the speed of their rotation verified at least once a year. Speed verification is done using a tachometer. It is easiest and safest to verify the speed using the photo tachometer setting.

No samples should be in the centrifuge rotor during rotation verification.

The rotor (inside and out) and the centrifuge should be cleaned before rotor speed verification. Otherwise the laboratory will be exposed to a biological aerosol during verification.

If the rotor has a lid, the lid must fit securely and tightly on the centrifuge. If the lid is not tight and secure, do not use during speed verification.

The rotor must be firmly on the centrifuge shaft. If not, **do not** verify the rotors speed.

Before verification, operate the centrifuge and rotor for 2 minutes at the verification speed with the centrifuge lid locked and closed to ensure that the rotor lid and rotor are firmly attached to the centrifuge

Photo Tachometer Operation

1. Cut a strip of the reflective tape into a 0.5 inch square.

2. Stop the Rotating object you wish to measure and place the reflective tape on the object to be measured. The tape may be placed on either the end or the circumference (surface) of the rotating object.

   The non-reflective area must always be greater than the reflective area. If the rotating object is normally reflective, it must be covered with black tape or black paint before attaching the reflective tape. The surface should be smooth and clean to make certain the reflective tape will adhere to it.

3. Set the Mode switch to PHOTO RPM.

4. If a centrifuge has a clear top, measure the RPM through the top. If the centrifuge has an opaque or solid top, measure the RPM through the hole in the centrifuge lid. If the above measurements are not possible, measure the RPM with the centrifuge lid open. If the centrifuge lid has a safety interlock, push down on the interlock with a screw driver to allow the centrifuge to spin with the lid open. **Be careful and make sure you are wearing eye protection.**

5. Allow your object to rotate. Press and hold the ON (Measure) button and point the light beam at the reflective tape.

6. Verify that you are properly aligned by making certain the Monitoring Indicator is flashing each time the tape passes through the beam of light. The indicator flashes once for every observed rotation.

7. Record the result displayed when the reading stabilizes in approximately 2 to 10 seconds. Release the **ON** (Measure) button.

8. If the rotation is less than 50 RPM, increase your accuracy by applying additional reflecting squares located approximately 180 degrees from each other. Divide the displayed result by the number of squares to arrive at the proper RPM reading.

8. Use particular care when measuring the rotation of the flat end of a shaft with a relatively small diameter. Completely cover the flat end with non-reflective black tape. Place the 0.5 inch square reflective strip on top of the black tape, as close to the edge as possible.

Contact RPM Tachometer Operation

1. Set the Mode switch to Contact RPM

2. Place the cone or funnel on the contact end of the tachometer.

3. Press and hold the **ON** (Measure) button.

4. Place the cone of funnel in contact with the rotating object. The cone is particularly useful when measuring the rotation of the flat end of a shaft with a hole or dimple in the center. Place and hold the cone in the center of the shaft.

5. Record the result displayed when the reading stabilizes in approximately 2 to 10 seconds. Release the **ON** (Measure) button.

Memory Recall Operation

To recall the last reading, highest reading, and lowest reading use the MEMORY button. Readings are normally retained in memory for several minutes. The first press of the **MEMORY** button will display a reading and then LA (indicating the last reading). The second press of the **MEMORY** button will display a reading and then **UP** (indicating the maximum or highest reading obtained). The third press of the **MEMORY** button will display a reading and then **DN** (indicating the minimum or lowest reading obtained after the highest reading was reached).

Low Battery

If LO BAT is indicated on the display, remove the two screws from the battery cover located on the back on the unit. Remove the battery cover. Remove the old batteries and replace them with four new **AA** 1.5V alkaline batteries. Use alkaline batteries, do not use regular or heavy duty batteries. Place the batteries in the proper direction (as indicated by the diagram in the battery compartment). Incorrectly installed batteries may damage the electronics. Replace the battery cover and the two battery cover screws.

Initials: RCJ Date: 9/11/96

QC029 Autorad Processing

Frequency

Weekly test of the x-ray processor

Samples

Two small pieces of x-ray film cut from a sheet
Two x-ray film standards

Procedure

Expose one piece of x-ray film to the light.

Develop both pieces of film

Specifications

The pieces of x-ray film should match the standards.
The exposed piece of film should be black and have a uniform intensity.
The unexposed piece of film should be translucent, light grey in color, and uniform with no streaks.
If the sample films do not meet the specifications, the processor should be serviced.

Documentation
The sample x-ray films dated, initialed and retained in a file.

Initials: RCJ Date: 9/11/96

QC030 ABI 373 Sequencer Tests **Page 1 of 2**

There are two diagnostic tests run once a month. The test results are recorded on a 373 Sequencer Diagnostics Specification Sheet (F027). These tests can only be run if there is no gel in the machine. Make sure that the machine is empty and clean, and that the beam stop bar and the lid are closed.

Switch the machine on.
To access the diagnostic test files, use the following commands.

LASERTEST

Press **Main Menu**.
The following will appear on the display.

Set-up Run
Start Prerun
Choose Run
Self Test
Calibration

Press **Self Test**.
The following will appear on the display.

Select Test
Memory
Battery
REPEAT
ALL
More

Press **More**.
The following will appear on the display.

Select Test
Keys
Display
Clock
Tones
More

Press **More**
The following will appear on the display.

Select Test
Datacom
Laser
Pmt OFF
Fw-mtr
More

Initials: RLS Date: 2/9/98

QC036 ABI 377 Sequencer QC Check And Maintenance

There are no diagnostic tests to be performed. Check, and if necessary clean all instruments, and sign the maintenance log.

A **Refilling the Water Reservoir**- once a month and if the water level drops below one third. The ideal level for the water reservoir is between one third and two thirds full.

1. The water reservoir is located in a compartment on the right side of the instrument.

2. Make sure the pump is not running.

3. Open the compartment door. Unscrew the plastic bottle and remove it by pulling downward. Place a papertowel under the tubes connetcting the reservoir to the pump.

4. Discard the old fluid, and rinse out the bottle. Fill the reservoir up to the mark (corresponds to 600 mL) with dH_2O, and add 50 mL of antifreeze.

5. Replace the reservoir, being sure to insert the two tubes before you screw it into place.

B **Review The QC Check Log**- once a month

1. Review the actual Prerun and Run values for all instruments, starting with the last QC check off. The values should be in the following range:

	Prerun	Run
E.Voltage (kV) -	1.00 ±0.05	3.00 ±0.05
Current (mA) -	10 - 15	30 - 50
Power (W) -	9 - 15	95 - 160
Laser Power (W) -	40.00 ±0.05	40.00 ±0.05

2. If any values are out of range, review the laboratory sheets, and the analysis results for the run in question. Determine possible sources for the out of range values, test and discard suspicious reagents lots.

3. Date and initial last entry that was checked.

Initials: RH Date: 4/3/98

QC034 Capillary Electrophoresis

Test Materials:

RM2400	50μm capillary	S130 formamide, deionized
RM 2391	performance optimized polymer 4	RM 2376 CXR size standard
RM2393	310 genetic analyzer buffer with EDTA	
RM2374	AmpFlSTR Green kit Allelic ladder	
RM2375	AmpFlSTR Blue kit Allelic ladder	

Samples

The QC test can be performed using either the AmpflSTR Blue or Green allelic ladder, and amplified products. Amplified products from two DNA samples known to be heterozygous at all blue or green loci; an allelic ladder, amplified positive control DNA, and a reagent blank, where no amplified product is added.

Procedure

Electrophorese samples according to the capillary electrophoresis protocol.

Analyse samples according to the Genescan Analysis and Genotyper Instructions protocols.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

Documentation

Write up the test on appropriate Capillary Electrophoresis run Worksheets.

Attach the completed worksheets to the Solution Log Sheet (S130).

Attach the completed worksheets to a copy of the product/lot information (RM2400, RM 2391, RM 2376, RM2393, RM2374, RM2375).

Initials: RCJ Date: 4/3/98

There are two diagnostic tests run every two months. The test results are recorded on a 310 Capillary Electrophoresis Diagnostics Specification Sheet (F037A). These tests can be run while there is a capillary in the instrument. Make sure that the capillary is not damaged during the testing. Especially since the second test requires the removal of the capillary from the laser window. The first test cannot be run with the 310 Collection Software open!

LASERTEST

1.) Quit 310 Collection Software if necessary.

2.) To access the diagnostic test files, open the **310 diagnostics** folder located on the hard drive. And click on the 310 diagnostics icon. At this point you will receive a warning, that the 310 diagnostics software cannot run if the Prism collection software is already running. You can check this by going to the upper left hand corner, and clicking on the finder icon. If it is not running, click **Continue,** otherwise click **Quit** and start with step1).

At this point you may receive the message "Establishing serial communication link with 310 instrument. This may take several seconds. Do not click Abort!!! Afterwards you might get the message "Instrument is not responding. Wait 10 seconds and then click o.k." Do wait and click **o.k.**.

From the first menu of options choose **Test Components.** From the second menu of test components choose **Laser Power**.

3.) Click on **start**. The values for the laserpower mW and the laserpower Amps will appear on the screen, ignore the first two readings and record the 3rd, the 4th, and the 5th reading on sheet F037A.
Also record the pass or fail status.

4.) After the 5th set of values appeared, wait till the indicator on the left side shows 100% done, then click on **Done**. The message that will appear says results not logged. To the question "log now" click **no**.

5.) On the 310 components menu press **Return.**
On the main diagnostics menu press **Quit.**

If the laser fails readings 3-5 take the instrument out of service and call the PE/ABD technical service representative.

CCD CAMERA SENSITIVITY TEST

For this test the regular capillary is replaced with a sensitivity standard capillary and a mock run is performed. The capillary does not have to be taken out, it is sufficient to temporarily remove it from the CCD camera lens window.

1.) Open the 310 Collection Software.

2.) Under **file** select **new** then select **sequence sample sheet**. In the first row (A1) put one sample name e.g. CCD test. If there is no module and no matrix selected, import any of the existing possibilities. The sections have to be filled, but the files will not be applied and are just fake. Close the sample sheet and save it as e.g. CCD test.

3.) Under **file** select **new** then select **sequence run.** Import the sample sheet that was created under 2.). Select **Test CCD sensitivity** as run module. Deselect Autoanalyze if necessary.

4.) Open the 310 instrument door, open the heat plate cover door, and the laser window door. Be careful not to damage the regularly installed capillary during the next steps. Move the capillary out of the laser window notch and bend it out of the way so that the laser window door and the heat plate cover can be closed without damaging the capillary.

5.) Take the sensitivity standard capillary provided by ABD/PE (part # 401928) and place its window in front of the camera lens. The yellow tag should be on top. Carefully close the laser window door, the heat plate cover and the instrument door.

6.) Click on **Run**. Under **Window** open **Status** to observe the progress. The program will collect data for 5 min. Then a second data collection set for 2.5 min will start. An alert message "EP current is zero" will pop up, click **o.k.**. Data collection will continue.

Initials: RC Date: 4/3/98

7.) When the alert prompt " Remove capillary" appears, open the instrument door, open the heat plate cover and the laser window door and remove the sensitivity standard. Do not put the old capillary back yet!! Close all doors, click **o.k.**, the run will resume automatically. Data will be collected for 2.5 minutes. Click **o.k.** to the alert prompt that the EP current is zero.

8.) After the data collection is completed, close the run, save the injection list, and quit the data collection program.

9.) On the hard drive open the **310 diagnostics** folder and click on the **310 diagnostics icon**. From the main menu select **Analysis**. From the Analysis menu select **Signal to Noise Auto**.

10.)Click on **Start**. Import the mock run from before, which should be in the current run folder. Highlight the sample file and click ok. The data will be analyzed automatically.
Record the relevant values on form F037B, the relevant values are 586 S/N ratio, 625 S/N ratio, 586 noise w/cap, 625 noise w/cap. These are the only ones listed on this form.

11.) Click on **done**. On the 310 components menu press **Return.**
On the main diagnostics menu press **Quit.**

12.) Open the instrument door, the heat plate door, and the laser window door and place the regular capillary in front of the camera lens. Close all doors.

If any of the values fail call technical service.

Initials: RCS Date: 9/11/96

There are six diagnostic tests run once a month. The test results are recorded on a Thermocycler Diagnostics Specification Sheet (F016/F016A).

To access the diagnostic test files, use the following commands.

Press **File**, **Yes**.

The following will appear on the display.

Select Function
CONFIG-DIAGNOSTIC

Press **No**.

This moves the cursor to the "Diagnostic" option.

Press **Enter**.

The following will appear on the display.

Diagnostic Tests
Enter test # (1-8)

Type the number of the test you want and press **Enter**.
To leave a test, press **Stop**.

Test 1: Display/Keypad Test

The machine first illuminates each block on the display board. The operator must watch to see that all the dots light up across the screen. Next, the operator checks each of the keys on the control board. As each key is pressed, the machine should display the corresponding command or number.

Test 3: Heater Test

This test measures the maximum heating rate. At the end of the test, the machine displays the time in seconds required for the first 15 degrees of temperature change, the temperature difference between the upper and lower temperature sensors just before the heaters go off (if applicable), and the heating rate. The heating time is a measure of the thermal time constant of the sensor/block assembly. If its value is not correct, a mechanical problem is indicated. The temperature difference is an indication of proper sensor operation and installation. Before conducting the test, measure the line voltage with a voltmeter. Compare the results to the specifications.

Test 4: Chiller Test

This test measures the maximum cooling rate. The machine displays the sensor difference and cooling time similar to the heating test. Allow the machine to idle for at least 30 minutes before this test is run so that the coolant has time to reach operating temperature. Compare the results to the specifications.

Test 5: Sensor Test

To check the sensor difference, allow the sample block to soak at a set temperature for at least 10 minutes. For example, run file 1 with a setpoint of 35°C. Record the soak temperature on the Thermocycler Diagnostics Specification Sheet. At the end of the incubation period, quickly abort the file and enter this diagnostic test. The machine will display the current temperature readings of the two sensors and their difference. Compare the results to the specifications.

Test 7 (F016); 6 (F016A): Overshoot Test

This test measures the temperature overshoot on a setpoint step from 37 to 94°C. The block is set to 37°C for 1 minute then ramps up to 94°C. The overshoot past 94°C is shown on the display after 15 seconds. Compare the results to the specifications.

Test 8 (F016); 7(F016A): Undershoot Test

This test measures the temperature undershoot on a setpoint step from 94 to 55°C. The block is set to 94°C for 1 minute and then ramps down to 55°C. The undershoot past 55°C is shown on the display after 15 seconds. Compare the results to the specifications.

Evaluation of Results

If all the results meet specifications, the thermocycler passes diagnostic testing. The Thermocycler Diagnostics Specification Sheet (F016/F016A) is filed in the Thermocycler Calibration Log.

If the results for any of the diagnostic tests fail to meet specifications, the thermocycler must be taken off-line for casework. Recent casework must be reviewed and selected samples may be retyped to confirm the results. Further testing may be necessary to rule out the possibility of human error. The test may not have been run properly or the results may not have been interpreted correctly. If after review the results fall consistently outside specification, the thermocycler must be tested before it can be put back on-line (see QC012 Thermocycler Well Test). If all the wells pass the test, casework may resume. If any of the wells fail the test, those wells must be taken out of service. The wells which pass the test can still be used event if there are wells on the same machine out of service.

Initials: RCJ Date: 9/11/96

QC010 Thermocycler Cycle Time

The amplification cycle on a thermocycler is timed once a month to confirm the reproducibility of the thermocycler.

Procedure

To measure the cycling time, load amplification program file 14- 94°C for 1 min; 60°C for 30 sec; 72°C for 30 sec; 32 cycles.

Start the program.

Allow the machine to complete three amplification cycles before making any measurements.

Begin timing the fourth cycle when the machine first ramps to 94°C from the third incubation at 72°C.

Stop the timer at the end of the 72°C incubation.

Record the time for a single cycle (F016/F016A).

Repeat this procedure for an additional cycle.

Calculate the difference in seconds between the first and second cycle measurements.

Compare the results to the specifications.

Evaluation of Results

If the results meet specifications, the thermocycler passes the cycle time test. The Thermocycler Diagnostics Specification Sheet (F016/F016A) is filed in the Thermocycler Calibration Log.

If the results fail to meet specifications, the thermocycler must be taken off-line for casework. Recent casework must be reviewed and selected samples may be retyped to confirm the results. Further testing may be necessary to rule out the possibility of human error. The test may not have been run properly or the results may not have been interpreted correctly. If after review the results fall consistently outside specification, the thermocycler must be tested before it can be put back on-line. QC012 Thermocycler Well Test must be performed for every third well of the block. If all the wells pass the test, casework may resume. If any of the theermocycler fails the test, the thermocycler must be serviced and retested before going back on-line.

Initials: RG Date: 9/4/96

QC011 Thermocycler Well Temperature Profile

The thermocouple probe is used to approximate the temperature response of a sample during an actual amplification cycle. Each well is measured during two cycles, listed as run 1 and run 2 on the Thermocycler Well Calibration Sheet (F017).

Procedure

Allow the thermocycler to warm up at least 15 minutes.

Make sure that the tip of the thermocouple is immersed in water and that mineral oil has not collected in the bottom of the tube. There must be sufficient oil above the water to prevent evaporative cooling.

Place one drop of mineral oil in the well to be measured.

Place the tube in the well. Press down on the top of the tube without disturbing the thermocouple wire so that the tube is seated firmly in the well.

Load amplification file 14- 94°C for 1 min; 60°C for 30 sec; 72°C for 30 sec; 32 cycles

Begin the program. Once the block has reached 94°C, push the tube as far into the well as possible. Allow the machine to complete the first cycle.

Wait until the sample block reaches 94°C for the second time.

When the instrument begins the 60 second countdown, record the probe temperature on the Thermocycler Well Calibration Sheet in the column headed 94, the set temperature for this step of the amplification cycle. This is the measurement at time zero.

Record the probe temperature every 15 seconds until the 94°C incubation is complete. The readings must be timed carefully in order for the temperature measurements to be valid.

Wait until the sample block reaches 60°C.

When the instrument begins the 30 second countdown, record the probe temperature on the Thermocycler Well Calibration Sheet in the column headed 60. This is the measurement at time zero.

Record the probe temperature every 15 seconds until the 60°C incubation is complete.

Initials: RCS Date: 9/11/96

Wait until the sample block reaches 72°C.

When the instrument begins the 30 second countdown, record the probe temperature on the Thermocycler Well Calibration Sheet in the column headed 72. This is the measurement at time zero.

Record the probe temperature every 15 seconds until the 72°C incubation is complete.

Record temperatures for a second cycle.

Calculate the standard mercury temperatures for each of the observed probe temperatures using

$$y = \bar{m}x + \bar{b}$$

where x is the observed probe temperature, m is the average slope, and b is the average intercept from the current Thermocouple Calibration Summary for the thermocycler probe. The y values are the standard temperatures. The corresponding standard temperatures are recorded in the 'std' columns of the Thermocycler Well Calibration Sheet (F017).

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SOLUTION QUALITY CONTROL

The laboratory prepares several solutions. Every solution has a corresponding Solution Sheet. The sheet indicates the standard batch size, the ingredients of the solution, the procedure to follow when preparing the solution, a section where data is recorded, and a section which lists the quality control procedures to be performed before the solution is released for use in the laboratory. Blank solution sheets are listed in the solution manual.

3.1 Solution Numbers

Each solution has been assigned a unique solution number along with the name (format S003 DQα Citrate Buffer). The solution numbers identify a solution with a specific recipe. They can be used as a double check for analysts performing procedures with which they are relatively unfamiliar. They are also a useful labeling shorthand for intermediate vessels during solution preparation.

3.2 Standard Batch Size

Each sheet indicates the standard batch size which is routinely prepared for each lot. The quantities listed in the ingredients section have been calculated for this standard batch. Occasionally, it may be convenient to prepare a batch larger or smaller than the standard batch size. In such cases, the analyst must note the total volume clearly on the solution sheet and carefully record the adjusted amount of each ingredient added to the solution. If changes in demand persist over time, the solution sheet may be adjusted to a new batch size.

3.3 Lot Numbers

Each batch of a solution is assigned a lot number beginning with 1. Information about each lot of the solutions is recorded in the Solution Inventory (F003/F003A). The inventory indicates the date each solution lot was prepared, who prepared it, and where it is stored. The solution sheets for each lot are filed in the Solution Inventory along with any supporting quality control documentation.

3.4 Ingredients

The ingredients required for the solution are listed at the top of the page. They indicate the final concentration of the ingredient and the amount of that ingredient required for the standard batch size. An ingredient may be either a raw material, something purchased from an outside vendor, or another solution prepared in the laboratory.

Each amount is listed with an uncertainty of measurement. The uncertainties are calculated to define an acceptable range of variation which will not significantly change the final

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concentration. In a few cases, narrower ranges have been adopted based upon recommendations for optimum performance.

3.5 Procedure

The procedure describes how to prepare the solution step by step and includes important notes regarding the safe handling of hazardous chemicals. The completed sheets must document exactly how the solution was prepared. Any deviation from the printed procedure must be clearly noted.

3.6 Data Log

The data log is where information is recorded about the ingredients of the solution. Every raw material ingredient is labeled with a QA sticker when it is received in the laboratory. The label lists an assigned RM number for the material (format RM000), the vendor, the vendor's lot number, and the date. On the solution sheet, the vendor is recorded as the source of the material, the vendor's lot number is recorded, and the amount of the ingredient measured and added to the solution is recorded. The amount measured must fall within the specified range listed in the ingredients section unless the range is marked guideline. A guideline is a suggested range used to make preparation easier and faster. Other ranges are specifications of tolerance.

Solutions prepared in the laboratory may also be listed as ingredients. In those cases, the source is listed as DNA and the laboratory lot number is recorded. Volume measurements which are made in the appropriate size graduated cylinders and which appear to the eye to be exact fall well within the ranges of tolerance listed in the ingredients section. The solution volumes must be recorded in the data log to keep track of the ingredients as they are added.

3.7 Quality Control

The quality control section lists the tests to be performed, if any, before the solution is released for use in the laboratory. These test procedures have been assigned QC numbers and names (format QC014 Chelex Extraction).

Quality tests are started at different stages of protocols, depending upon the reagent under study. For example, for HLA-DQα, there are three QC procedures beginning with the extraction, amplification, and hybridization steps. The QC procedure assigned to a solution represents the appropriate level of testing required to pass the reagent. (i.e. QC016 DQα Hybridization is listed in the quality control section for DQα Wash Solution. To evaluate the performance of this component, it is not necessary to run through the entire test. Only the hybridization procedure is critical. QC016 begins with samples which have previously been amplified and repeats the hybridization using the new wash solution. A hybridization negative is the appropriate negative control. No amplified extract is added to a hybridization negative, but in every other respect, the strip is processed the same as the positive controls.)

Initials: RCS Date: 9/11/96

More than one solution may be tested at a time. In this case, the quality test must be sufficient for all of the components. For example, if a single run is to be performed for 5% Chelex and DQα Wash Solution, the quality test must begin with the extraction. QC014 Chelex Extraction is the appropriate test for the Chelex, and the procedure encompasses the hybridization necessary for the wash solution.

3.8 Documentation

After a quality test has been performed, the supporting documentation is attached to the original solution sheet and submitted for review. If the solution performance is satisfactory, it will be released for general use in the laboratory. If the solution fails to meet the standards set forth in the QC procedure, it may be submitted for further testing or discarded.

After a solution has passed quality control and been released, the solution sheet and quality control documentation are filed in the Solution Inventory. If more than one solution has been quality controlled in a single test run, the original quality control documents will be filed with one solution sheet and a copy of the original will be filed with each additional solution sheet.

Initials: RCJ Date: 2/9/98

3.9 Raw Materials Testing

In addition to solution quality control, each lot of the raw materials listed in the table below require quality control to ensure that its performance meets specifications. These quality control records are filed in the Inventory Control Log with raw materials information.

RM#	Raw Material	QC Procedure
RM0014	Agarose	QC027
RM0016	Ammonium Persulfate	QC032
RM0022	BSA	QC031
RM0070	TEMED	QC032
RM0079	Urea	QC032
RM0083	TAE, 10X	QC027
RM0102	Formamide	QC032
RM0134	Hae III	QC025
RM0138	BRL Sizing Standard	QC027
RM0165	Biodyne B Membranes	QC027
RM0166	Hae III Buffer, 10X	QC025
RM0211	dNTP's Set	QC023, QC031
RM0275	Taq/ PCR Buff II/$MgCl_2$	QC023, QC031
RM0378	QuantiBlot Kits	QC018
RM0451	ABI Loading Buffer	QC032
RM2070, 2071, 2626, 2527	Sequencer Glass Plate	QC033
RM1062	GS500 ROX Lablelled	QC032
RM1088	Proteinase K Solution	QC023, QC024
RM2031	HLA DQA1+PM Kits	QC015
RM2085	Polyacrylamide:Bisacrylamide mix 19:1	QC032
RM2473	Long Ranger	QC032

Initials: RG Date: 9/16/96

3.9 Raw Materials Testing

In addition to solution quality control, each lot of the raw materials listed in the table below require quality control to ensure that its performance meets specifications. These quality control records are filed in the Inventory Control Log with raw materials information.

RM#	Raw Material	QC Procedure
RM0014	Agarose	QC027
RM0016	Ammonium Persulfate	QC032
RM0022	BSA	QC031
RM0070	TEMED	QC032
RM0079	Urea	QC032
RM0083	TAE, 10X	QC027
RM0102	Formamide	QC032
RM0134	Hae III	QC025
RM0138	BRL Sizing Standard	QC027
RM0165	Biodyne B Membranes	QC027
RM0166	Hae III Buffer, 10X	QC025
RM0211	dNTP's Set	QC023, QC031
RM0275	Taq/ PCR Buff II/$MgCl_2$	QC023, QC031
RM0378	QuantiBlot Kits	QC018
RM0451	ABI Loading Buffer	QC032
RM0538, 0539,2070, 2071	Sequencer Glass Plate	QC033
RM1062	GS500 ROX Lablelled	QC032
RM1088	Proteinase K Solution	QC023, QC024
RM2031	HLA DQA1+PM Kits	QC015
RM2085	Polyacrylamide:Bisacrylamide mix 19:1	QC032

Initials: RCJ Date: 9/11/96

QC014 Chelex Extraction

Test Materials

S022 Chelex, 5%

Samples

Two whole blood or stain samples of known type.
One negative control sample.
One positive control sample from the typing kit (if applicable).

Procedure

Extract the two known samples and the negative control sample according to the Chelex extraction procedure for whole blood and bloodstains in the forensic DNA manual.

Amplify the samples and a positive control from the kit according to the appropriate amplification protocol.

Hybridize or electrophorese the samples according to the appropriate protocol.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The negative control sample must show no evidence of contamination.

Documentation

Write the test up on a set of the appropriate worksheets.

Attach the completed worksheets to the Solution Log Sheet.

Initials: RCS Date: 9/11/96

QC015 PCR Kit Amplification

Test Materials

RM2031 HLA DQA1 + PM Amplitype Kit

Samples

Two whole blood or stain samples of known type.
One amplification negative.
One positive control sample from the PCR typing kit.

Procedure

Amplify the samples and a positive control from the kit according to the amplification protocol. No extract is added to the amplification negative.

Hybridize the samples according to the hybridization protocol or run a sizing gel according to the sizing gel protocol.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Write the test up on appropriate Amplification and Hybridization Worksheets.

Attach the completed worksheets to the Solution Log Sheet.

Initials: RCS Date: 9/11/96

QC016 PCR Kit Hybridization

Test Materials

S004 DQα Hybridization Solution
S110 EDTA, 200 mM

Samples

Three amplified samples of known type .
One hybridization negative.
S110 EDTA, 200 mM

Procedure

Hybridize the samples according to the kit hybridization protocol. No amplified extract is added to the hybridization negative. In all other respects, this strip is processed the same way as the positive control samples.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The hybridization negative and EDTA must show no evidence of contamination.

Documentation

Write the test up on the appropriate Hybridization Worksheet.

Attach the completed worksheet to the Solution Log Sheet.

Initials: Date:

QC035 AmpflSTR Kit Amplification

Test Materials

RM2374 AmpFlSTR Green kit
RM2375 AmpFlSTR Blue kit

Samples

Two whole blood or stain samples of known type.
One amplification negative.
One positive control sample from the PCR typing kit.

Procedure

Amplify the samples and a positive control from the kit according to the amplification protocol. No extract is added to the amplification negative.

Separate the amplification products on the capillary electrophoresis instrument following the manual.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Write the test up on appropriate Amplification and Electrophoresis Worksheets.

Attach the completed worksheets to the Solution Log Sheet.

Initials: RG Date: 9/4/96

QC017 PCR Differential Extraction

Test Materials

S082 Chelex, 20%

Samples

One swab with epithelial and sperm cells of known type.
One negative control sample.
One positive control sample from the DNA typing kit (if applicable).

Procedure

Extract the known swab and the negative control sample according to the differential extraction procedure in the forensic DNA manual.

Amplify the samples and a positive control from the kit according to the appropriate amplification protocol.

Hybridize or electrophorese the samples according to the appropriate protocol.

Specifications

Each sample fraction must match the assigned type within the current interpretation guidelines.

The negative control sample must show no evidence of contamination.

Documentation

Write the test up on a set of appropriate worksheets.

Attach the completed worksheets to the Solution Log Sheet.

Initials: RCJ Date: 10/7/96

QC031 QUAD STR/PCR Amplification

Test Materials

S111 Quad STR/PCR Reaction Mixture
RM022 BSA
RM0211 dNTP's set
RM0275 Taq/ PCR Buff II/ $MgCl_2$
S118, S119 VWA Forward and Reverse Primers
S120, S121 Tho1 Forward and Reverse Primers
S122, S123 F13A1 Forward and Reverse Primers
S124, S125 FES Forward and Reverse Primers
S126 Positive Control-External

Samples

Two whole blood or stain samples of known type.
One amplification negative.
One positive control sample from STR/Quad amplification materials.

Procedure

Amplify the samples and a positive control using the Quad STR/PCR Reaction Mixture (S111) according to the amplification protocol. No extract is added to the amplification negative.

Electrophorese samples according to the gel electrophoresis protocol.

Analyse samples according to the STR Gel Analysis and Genotyper Instructions protocols.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Write the test up on appropriate Amplification and STR Gel Worksheets.

Attach the compled worksheets to the Solution Log Sheet.

Initials: RCS Date: 9/11/96

QC031 QUAD STR/PCR Amplification

Test Materials

S111 Quad STR/PCR Reaction Mixture
RM022 BSA
RM0211 dNTP's set
RM0275 Taq/ PCR Buff II/ $MgCl_2$
S118, S119 VWA Forward and Reverse Primers
S120, S121 Tho1 Forward and Reverse Primers
S122, S123 F13A1 Forward and Reverse Primers
S124, S125 FES Forward and Reverse Primers

Samples

Two whole blood or stain samples of known type.
One amplification negative.
One positive control sample from STR/Quad amplification materials.

Procedure

Amplify the samples and a positive control using the Quad STR/PCR Reaction Mixture (S111) according to the amplification protocol. No extract is added to the amplification negative.

Electrophorese samples according to the gel electrophoresis protocol.

Analyse samples according to the STR Gel Analysis and Genotyper Instructions protocols.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Write the test up on appropriate Amplification and STR Gel Worksheets.

Attach the compled worksheets to the Solution Log Sheet.

Initials: RC Date: 2/9/98

QC032 Acrylamide Gel Electrophoresis

Test Materials:

S113 40% Acrylamide Stock
S116 GS500 and Loading Buffer
RM0102 Formamide
RM2085 Polyacrylamide:Bisacrylamide Mix 19:1
S131 Blue Formamide + Loading Buffer (5:1)
S115 Ammonium Persulfate
RM0070 Temed
RM0079 Urea
RM2473 Long Ranger
S132 Sequencing Loading Buffer

Samples

Amplified products from four DNA samples known to be heterozygous at all STR/Quad loci; the four samples should be combined in a way so that four different alleles are present at each locus.

Procedure

Electrophorese samples according to the gel electrophoresis protocol.

Analyse samples according to the STR Gel Analysis and Genotyper Instructions protocols.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

Documentation

Write up the test on appropriate STR Gel Worksheets.

Attach the completed worksheets to the Solution Log Sheet (S113, S114, S115, S116, S131, S132).

Attach the completed worksheets to a copy of the product/lot information (RM 070, RM 079, RM0102, RM2085, RM2473).

Initials: RCL Date: 4/3/97

QC032 Acrylamide Gel Electrophoresis

Test Materials:

S113 40% Acrylamide Stock
S116 GS500 and Loading Buffer
RM0102 Formamide
RM2085 Polyacrylamide:Bisacrylamide Mix 19:1
S115 Ammonium Persulfate
RM0070 Temed
RM0079 Urea

Samples

Amplified products from four DNA samples known to be heterozygous at all STR/Quad loci; the four samples should be combined in a way so that four different alleles are present at each locus.

Procedure

Electrophorese samples according to the gel electrophoresis protocol.

Analyse samples according to the STR Gel Analysis and Genotyper Instructions protocols.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

Documentation

Write up the test on appropriate STR Gel Worksheets.

Attach the completed worksheets to the Solution Log Sheet (S113, S114, S115, S116).

Attach the completed worksheets to a copy of the product/lot information (RM 070, RM 102).

Initials: RCJ Date: 9/11/96

QC018 QuantiBlot Hybridization

Test Materials

S100 QuantiBlot DNA Standards
RM378 QuantiBlot DNA Quantitation Kits

Samples

QuantiBlot Calibrators 1 and 2.

Procedure

Hybridize the samples according to the Quantiblot protocol.

Specifications

Each Calibrator must have an intensity bounded by the appropriate QuantiBlot DNA standard.
All of the QuantiBlot standards must be visible.
The QuantiBlot Standard intensities must show a correlation with DNA concentrations.
The negative control must show no evidence of contamination.

Documentation

Write the test up on a QuantiBlot Hybridization Worksheet.
Attach the completed worksheet to the Solution Log Sheet.

Initials: RD Date: 10/21/96

QC023 QuantiBlot Quality Control of Solutions

Test Materials

S010 Cell Lysis Buffer
S011 Protein Lysis Buffer
S014 Proteinase-K Enzyme 10 mg/mL
S030 DTT, 0.39 M
S034 Phosphate Buffered Saline (PBS)
S037 Proteinase-K Enzyme, 20 mg/mL
S128 TE^{-4}, 1X
S043 Sodium Acetate, 2M
RM0211 dNTP's Set
RM1088 Proteinase K Solution
S118, S119 VWA Primers
S122, S123 F13A1 Primers
S044 Sodium Acetate, 0.2M
S045 SDS, 10%
S04, Stain Extraction Buffer
S059 Sterile Water
S093 DTT, 1 M
S094 Digest Buffer
S107 TNE, 1X
S117 BSA, 5 mg/mL
RM0275 Taq/PCR Buff II/$MgCl_2$
S120, S121 Tho1 Primers
S124, S125 FES Primers

Samples

Solution to be tested for the presence of DNA at the volume indicated in the QC section of the solution sheet. Test 20 µL of S112 for dNTP's set, 5 µL Taq, 25 µL PCR Buff II, 25 µL $MgCl_2$ QuantiBlot Calibrators 1 and 2

Procedure

Hybridize the samples according to the Quantiblot protocol.

Specifications

Each QuantiBlot Calibrator must have an intensity bounded by the appropriate QuantiBlot DNA standard.
All of the QuantiBlot standards must be visible.
The tested solution must show no evidence of contamination. There must be no hybridization to the slot containing the tested solution.
The negative control must show no evidence of contamination.

Documentation

Write the test up on a QuantiBlot Hybridization Worksheet.
Attach the completed worksheet to the Solution Log Sheet.

Initials: RG Date: 9/11/96

QC023 QuantiBlot Quality Control of Solutions

Test Materials

S010 Cell Lysis Buffer
S011 Protein Lysis Buffer
S014 Proteinase-K Enzyme 10 mg/mL
S030 DTT, 0.39 M
S034 Phosphate Buffered Saline (PBS)
S037 Proteinase-K Enzyme, 20 mg/mL
S039 TE, 1X
S043 Sodium Acetate, 2M
RM0211 dNTP's Set
RM1088 Proteinase K Solution
S118, S119 VWA Primers
S122, S123 F13A1 Primers
S044 Sodium Acetate, 0.2M
S045 SDS, 10%
S04, Stain Extraction Buffer
S059 Sterile Water
S093 DTT, 1 M
S094 Digest Buffer
S107 TNE, 1X
S117 BSA, 5 mg/mL
RM0275 Taq/PCR Buff II/$MgCl_2$
S120, S121 Tho1 Primers
S124, S125 FES Primers

Samples

Solution to be tested for the presence of DNA at the volume indicated in the QC section of the solution sheet. Test 20 µL of S112 for dNTP's set, 5 µL Taq, 25 µL PCR Buff II, 25 µL $MgCl_2$ QuantiBlot Calibrators 1 and 2

Procedure

Hybridize the samples according to the Quantiblot protocol.

Specifications

Each QuantiBlot Calibrator must have an intensity bounded by the appropriate QuantiBlot DNA standard.
All of the QuantiBlot standards must be visible.
The tested solution must show no evidence of contamination. There must be no hybridization to the slot containing the tested solution.
The negative control must show no evidence of contamination.

Documentation

Write the test up on a QuantiBlot Hybridization Worksheet.
Attach the completed worksheet to the Solution Log Sheet.

Initials: RCS Date: 8/14/96

QC024 RFLP DNA Extraction

Test Materials

S064 Cell Pellet Control
SO14 Proteinase K (10 mg/mL)
S037 Proteinase K (20 mg/mL)
RM1088 Proteinase K Solution

Samples

One neat semen stain
One blood stain
One negative control sample.
One S064 Cell Pellet Control.

Procedure

Extract the known samples, cell pellet control and the negative control sample according to the appropriate RFLP extraction procedure in the forensic DNA manual.

Electrophorese the samples with controls on a yield gel according to the forensic DNA protocol.

Specifications

High molecular weight DNA must be isolated from each sample except very little sperm DNA should be in the epithelial fraction. There must be very little degradation.

The negative control sample must show no evidence of contamination.

Documentation

Write the test up on a set of the appropriate worksheets.

Attach the completed worksheets to the Solution Log Sheet.

Initials: RCJ Date: 10/21/96

QC025 Restriction Digestion **Page 1 of 3**

Reagents

PhiX 174 DNA, Intact- Prepare at 0.50 µg/µL in TE^{-4}, 1X
Hae III-Digested PhiX 174- Prepare at 0.25 µg/mL in TE^{-4}, 1X
RM0134 Hae III- Prepare dilutions with TE^{-4}, 1X to give 1 and 10 unit/µL

Sample preparation:

	Sample Number				
Reagent (µL per Sample)	**1**	**2**	**3**	**4**	**5**
PhiX 174 DNA Intact 0.5 µg/µL	4	4	4	4	4
Water	30	30	28	28	32
10X Hae III Buffer	4	4	4	4	4
HAE III @ 1U/µL	2	2	-	-	-
HAE III @ 10U/µL	-	-	4	4	-
Incubate (hours) at 37°C	1	1	5	5	5

Post-restriction handling of samples 1 through 5:

1. To the 40 µL digest, add 20 µL of 7.5M lithium chloride and mix.
2. Add 120 µL cold absolute EtOH and mix by hand.
3. Place the tube -20^0 for 15-30 minutes.
4. Spin tube for 15 minutes. Decant the alcohol.
5. Rinse the pellet with 1000 µL room temperature 70% EtOH. Spin the tube for 5 minutes and decant the alcohol. Remove remaining alcohol with a pipette.
6. Put tube in Speed-Vac for 5 minutes.
7. Dissolve DNA in 4 µL TE^{-4}, 1X.
8. After DNA is dissolved, add 2 µL test gel loading buffer and mix.

Initials: RCJ Date: 9/4/96

QC025 Restriction Digestion **Page 1 of 3**

Reagents

PhiX 174 DNA, Intact- Prepare at 0.50 µg/µL in TE, 1X
Hae III-Digested PhiX 174- Prepare at 0.25 µg/mL in TE, 1X
RM0134 Hae III- Prepare dilutions with TE, 1X to give 1 and 10 unit/µL

Sample preparation:

	Sample Number				
Reagent (µL per Sample)	**1**	**2**	**3**	**4**	**5**
PhiX 174 DNA Intact 0.5 µg/µL	4	4	4	4	4
Water	30	30	28	28	32
10X Hae III Buffer	4	4	4	4	4
HAE III @ 1U/µL	2	2	-	-	-
HAE III @ 10U/µL	-	-	4	4	-
Incubate (hours) at 37°C	1	1	5	5	5

Post-restriction handling of samples 1 through 5:

1. To the 40 µL digest, add 20 µL of 7.5M lithium chloride and mix.
2. Add 120 µL cold absolute EtOH and mix by hand.
3. Place the tube -20^0 for 15-30 minutes.
4. Spin tube for 15 minutes. Decant the alcohol.
5. Rinse the pellet with 1000 µL room temperature 70% EtOH. Spin the tube for 5 minutes and decant the alcohol. Remove remaining alcohol with a pipette.
6. Put tube in Speed-Vac for 5 minutes.
7. Dissolve DNA in 4 µL TE, 1X.
8. After DNA is dissolved, add 2 µL test gel loading buffer and mix.

Preparation of predigested PhiX 174 DNA:

	Sample Number		
Reagent (µL per Sample)	**6**	**7**	**8**
Hae III digested PhiX 174	2.5	2.5	2.5
TE, 1X	1.5	1.5	1.5
Test gel loading buffer	2.0	2.0	2.0

Electrophoresis of samples:

1. Prepare a 100 mL 14 cm gel that is 1.5% agarose in TAE buffer supplemented with ethidium bromide. You will need at least 8 wells in this gel.

2. Add samples to the gel in the following arrangement:

Well	Sample # (composition)
1	1 (HAE III @ 1U/µg-hr)
2	6 (predigested PhiX174)
3	2 (HAE III @ 1U/µg-1hr)
4	7 (predigested PhiX174)
5	3 (HAE III @ 10U/µg-5hr)
6	8 (predigested PhiX174)
7	4 (HAE III @ 10U/µg-5hr)
8	5 (intact PhiX174)

3. Electrophorese for 2-3 hours at 100 volts.

4. Place the gel on the UV transilluminator and photograph.

Specifications

Using the photograph as the experimental data, compare the location of the bands.

A. Look first at lanes 2,4, and 6. These samples are PhiX174 DNA that was restricted by the manufacturer. Ten bands should be visible in each lane.

B. Look at lanes 1 and 3. Ten bands should be visible and in the same positions as the bands in lanes 2,4, and 6. The DNA in these samples was cut with an amount of HAE III that should completely digest the DNA. If the digestion is incomplete, then the unitage of the HAE III is lower than desired.

C. Look at lanes 5 and 7. The bands in these lanes should compare exactly with the bands in lanes 2,4, and 6. The DNA in these samples was digested with a 20-fold excess of HAE III and for a five-fold excess in incubation time. These digestions will reveal any low level contamination of the HAE III by another endonuclease(s). Such contamination would present itself as extra bands that will not line up with the control bands in lanes 2,4, and 6.

D. The DNA in lane 8 is intact PhiX 174 that has not been cut. You should see one, or possibly two bands that have not migrated very far from the origin.

Initials: RCS Date: 9/11/96

QC026 Gel Electrophoresis

Test Materials

	Gel Lane
S032 Lambda Marker	2
S042 PhiX Marker	3
S020 Yield Calibrators	4-9, old lot(15-20)
S060 Calibration Control	10
S062 Test Gel Standard	11
S061 Digestion Control-undigested	12
(Digestion Control- Hae III digested	13)
S042 PhiX Marker	13 (14)
S032 Lambda Marker	14 (15)

Procedure

For Quality Control of the Digestion Control, Hae III digest the Digestion Control according to the RFLP Manual. For all other QC's, the Digestion Control does NOT have to be Hae III digested.

Prepare a yield gel (substituting 2.0 g agarose/ 200 mL) according to the protocol in the DNA manual.

Add 4µL of yield gel loading buffer to 20 µL of the undigested Digestion Control (and 5 µL of Test Gel Loading Buffer to 5 µL of the Hae III digested Digestion Control, if applicable).

The test material and standards should be heated to 65°C and centrifuged as specified in the manual.

Load the gel as specified above, loading 24 µL of the digestion control and test gel standard and loading 10 µL of rest of the test materials. For Quality Control of the Digestion Control, load the gel as specified in parentheses.

For quality control of S020 Yield Calibrators, the previous lot of yield calibrators should also be electrophoresed as specified above.

Electrophorese and photograph as specified for a yield gel.

Specifications

S032 Lambda Marker-	The photograph should display the banding pattern specified by the manufacturer
S042 PhiX Marker-	The photograph should display the banding pattern specified by the manufacturer.
S020 Yield Calibrators-	From the photograph, the new lot should have comparable intensities to the old lot. Each calibrator should have the correct relative intensity compared to the other calibrators. Each calibrator should appear as a single band with no trailing, at or above the highest band of the lambda standard. The calibration control should quanitate correctly.
S060 Calibration Control-	From the photograph, the calibration control should appear as a single band with no trailing, at or above the highest band of the lambda standard. The calibration control should quanitate correctly.
S061 Digestion Control-	From the photograph, the undigested digestion control should appear as a single band with no trailing, at or above the highest band of the lambda standard. It should have an intensity between yield calibrators E and F. When applicable, the Hae III digested digestion control should appear as a smear with no apparent bands.
S062 Test Gel Standard-	From the photograph, the test gel standard should appear as a smear with no apparent bands.

Documentation

Write the test up on the appropriate worksheets.

Attach the completed worksheet to the Solution Log Sheet.

Initials: RCJ Date: 9/11/96

QC027 Southern Blotting and Hybridization **Page 1 of 2**

Test Materials

S064 Cell Pellet Control
RM083 TAE, 10X
RM014 Agarose
RM138 BRL Sizing Ladder
RM165 Biodyne B Membranes
S026 Hybridization Solution

Samples

A. Cell Pellet QC-	Restriction digested cell pellet control (see procedures manual, digest 2 aliquots of CPC)
B. Other QC-	Hae III digested K562 DNA
C. All QC-	Sizing Ladder and PhiX Marker

Procedure

Load an analytical gel with 200, 100, 50, 25, 10 ng of DNA and appropriate sizing ladders and PhiX markers according to the DNA procedures manual.

Electrophorese, southern blot, hybridize, and size according to the manual.

Five probes are required for cell pellet QC and one probe is required for other QC.

Specifications

In the lanes containing the Cell Pellet Control or K562 DNA, there must be either 1 or 2 dark bands, depending on which RFLP locus has been probed. There should be no extra bands.

The Cell Pellet Control band(s) should be located relative to the size standards within 2.5% of their expected position on the autorad.

In the BRL Sizing Standard lanes, thirty bands should be present and resolved in each lane; however, it is possible for the smallest bands to run off the gel during an extended run. The bands in these lanes must be of sufficient intensity to enable them to be used as size references for the Cell Pellet Control or K562 DNA.

Documentation

Write the test up on the appropriate worksheets.

Attach the completed worksheet to the Solution Log Sheet.

Initials: RJ Date: 9/11/96

PCR CONTAMINATION CONTROL

4.1 Prevention

Several measures have been taken to prevent contamination problems. The laboratory is divided into at least three physically isolated areas for extraction, pre-amplification and post-amplification. Each of these areas has its own dedicated equipment used only for PCR. Samples, once they are accepted into the laboratory, move through these three areas in one direction only. Samples are first processed in the extraction area. They are then moved into the pre-amplification area which is a low DNA concentration area. Here fresh kit reagents are stored and samples are prepared for amplification. Finally, the samples are amplified and hybridized in the third area, which is a high DNA concentration area. This laboratory set-up helps eliminate cross contamination from high concentration DNA areas back into low concentration DNA areas.

To avoid cross contamination between specimens, exemplar samples with high concentrations of higher quality DNA are processed separately from evidence samples which are expected to have lower concentrations of partially degraded and/or degraded DNA. Also only one sample is processed at a time when the sample is in an unsealed container, instruments are thoroughly cleaned between each sample and single use disposable supplies are used whenever possible. (See the PCR Protocol Manual for additional procedures to avoid cross contamination).

By far the best defense against contamination is the training program for the analysts. The analysts must understand what is happening to the DNA at every step of the procedure. They must understand the rationale behind the laboratory set-up and the methods of sample handling so they are able to prevent problems before they arise. In this way, they are equipped to assess and to modify their individual habits as they practice the test.

4.2 Contamination Protocol

Contamination problems reflect a system failure or contamination of the samples by an outside source. The source may be equipment, the samples themselves, materials, or the working environment. Contamination can either be a single isolated event such as cross contamination between two samples or it can be persistent such as contamination of a reagent or equipment. Persistent contamination may be sporadic and not appear in each run. Contamination is identified as the presence of extraneous dots, bands, or alleles in the amplification negative, extraction negative or positive controls. To remedy contamination caused by a single isolated event, the appropriate extraction, amplification and/or hybridization is repeated. (See the Results Interpretation Section of the PCR Protocol Manual).

If the contamination persists or if several laboratory members are experiencing the same contamination, the QC coordinator or designee must be notified. The source of contamination should be identified, if possible, and eliminated. To demonstrate the elimination of the persistent

Initials: RCJ Date: 9/4/96

contamination, a clean run (QC019) should be performed. Recent casework may be reviewed, and selected samples may be repeated later to verify the results. The analysts will be informed of any corrective action adopted to prevent the recurrence of the problem.

4.3 Troubleshooting

Often the source of a contamination problem can be identified on the basis of experience. For example, in the HLA-DQA1 test, persistent appearance of a light signal without a visible 'c' dot in the extraction negative control may indicate slight contamination of the Chelex or the sterile water used during the extraction procedure or contamination by the analyst during extraction. This contamination may represent a build up of DNA in the reagents over the course of many extractions. The weak signal appears when the concentration of DNA in the amplified extraction negative control is greater than the threshold of delectability for the hybridization. Generally, fresh reagents will eliminate this problem.

HLA-DQA1 strips which appear to have the same mixture of DNA types across all the samples indicate a more serious contamination problem at the amplification step. If tubes or reagents are contaminated during the pre-amplification set-up, the contaminant DNA will be amplified along with the sample. The sample signals may even be overwhelmed by the contaminant. To solve this problem, the pre-amplification room must be cleaned out and the bench washed with a 10% bleach solution. All of the kit reagents must be changed and new reaction tubes must be aliquoted.

In some cases, the source of contamination may be more elusive. Problems which persist may be addressed by performing a clean run (QC019). During a clean run, control samples are processed along with a series of negative controls. Negative controls are run at the extraction, amplification, and hybridization steps. The results from these samples will indicate the area in which contamination appears. By focusing attention on one area at a time, the source or sources of contamination can be systematically eliminated.

Initials: RS Date: 9/11/96

QC019 Clean Run

This procedure is used to pinpoint sources of contamination when a typing problem arises.

Samples

two whole blood or bloodstain samples of known type.
one extraction negative.
one amplification negative.
one hybridization or electrophoresis negative.
one positive control sample from the DNA typing kit (if applicable).

Procedure

Extract the control samples and the extraction negative according to the Chelex extraction procedure for whole blood and bloodstains from the forensic DNA manual. The extraction negative control is a reagent control, containing distilled water in place of sample. This sample should be handled the same way as the other samples, but no substrate should be added.

Amplify the samples with the positive control from the kit (if applicable) and an amplification negative according to the appropriate amplification protocol. No Chelex extract is added to the amplification negative. This negative is used to evaluate contamination from the reagents and equipment in the amplification area.

Hybridize or electrophorese the samples with a hybridization or electrophoresis negative according to the appropriate protocol. No amplified extract is added for the hybridization or electrophoresis negative.

Evaluation

If only the extraction negative shows contamination, the problem has occurred during the extraction step.

If the amplification negative shows contamination, the problem has occurred during the amplification set-up. The extraction negative may or may not appear contaminated as well.

If only the positive controls appear contaminated, the problem might be the stringency of the hybridization or a contaminated positive control.

Individual clean runs have to be evaluated on a case by case basis. It may be useful to determine what components have been changed since the last successful typing and to work from there.

Documentation

Write the clean run up on a set of appropriate worksheets.

Initials: RD Date: 9/11/96

REFERENCES

Amplitype User Guide, Version 2, Cetus Corporation, Emeryville, California, 1990.

HLA-DQα Forensic DNA Amplification and Typing Kit Package Insert, Cetus Corporation, Emeryville, California, 1990.

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Nowaczyk, Ronald H., *Introductory Statistics*, Holt, Rinehart and Winston, Inc., 1988, pp. 120-125.

Rice, John A., *Mathematical Statistics and Data Analysis*, Wadsworth and Brooks, 1988, pp. 472-473.

Fritz, James S. and Schenk, George H., *Quantitative Analytical Chemistry*, Allyn and Bacon, Inc., 1979, p. 25.

APPENDIX A- FORENSIC DNA FORMS, SHEETS AND LOGS

This appendix contains blank copies of the worksheets used to document the quality control program for DNA. These documents may be amended individually as the needs of the program change.

Press **Laser.**

Using the keypad enter **40** as "mw-target". Press **Enter**.

Wait until the "mw-actual" value stops changing. Record the "mw-actual" and the "amps" values on the sheet.

SCANNER MOTOR TEST

Press **Main Menu**.

If this test is performed immediately after the laser test the following display should appear. Otherwise repeat the above steps until the right display is shown.

On this display:	Select Test Datacom Laser Pmt OFF Fw-mtr More

Press **More.**

The following will appear on the display.	Go right Scan Current Limits More

Press **Current.**

Record the reading for "milliamps right".

Press **Current** again.

Record the reading for "milliamps left".

Press main menu and shut machine .

Initials: RCS Date: 9/11/96

QC033- Preparation of Sequencing Gel Electrophoresis Plates

Each new set of plates has to be treated with NaOH. This process does not have to be repeated.

A set of plates consists of one backplate and a notched front plate. The insides that will be in contact with the gel have to be treated. To mark which sides have to be the insides, the outside of the plates get etched the following way:

Notched plate - an "L" for left on the left upper side, an "R" for right on the right upper side.
Plain plate - a mirror image "L" on the right side, and a mirror image "R" on the left side.
This way the "L"s and "R"s should be readable when the plates are placed correctly.

Place the plates on a sheet of bench paper with the side of the plates that is not etched facing upwards. Wear protective goggles, gloves and a lab coat before handling sodium hydroxide!!! Pour 10mL of 10N NaOH on the plate and distribute it evenly using a bundle of large Kimwipes. Rub the plate for approx. one minute in every direction. Rinse the plate off with plenty of tap water followed by a final rinse with deionized water. Repeat for the second plate.

The set can be used immediately.

Initials: RCJ Date: 9/11/96

2.1 Thermocycler

The performance of the Perkin Elmer thermocyclers is routinely monitored. Once a month, six internal diagnostic tests are run (QC009), the time for the HLA-DQA1 amplification cycle is measured (QC010), and the thermocycler block is cleaned (QC008). Each run ,the positive control is placed into a different well of the thermocycler to monitor well performance.

Temperature profiles for every well of the Perkin Elmer thermocyclers are measured before a thermocycler is put into service (TC011). The profile is compared to profiles and specification of the other thermocyclers. The hotest and coldest well at each step is then tested with known exemplars as part of the thermocycler validation. Potential problem exemplars such as 1.1, 4.1 in HLA-DQA1 or large allele size diefferences with STR's are preferred. These profiles are used as specifications of performance. Well temperature profiles can then be re-measured in the future to determine if there is a shift in performance.

Initials: RCJ Date: 9/4/96

QC008 Thermocycler Block Cleaning

The wells of the sample block must be cleaned each month. Dirt, oil, and other contaminating agents collect in the sample wells, preventing the reaction tubes from seating properly. Maximum contact ensures optimum heat transfer from the block to the sample.

Procedure

NOTE: PROTECTIVE EYEWEAR MUST BE WORN WHEN CLEANING THE SAMPLE BLOCK. LIQUID MAY SPRAY OUT OF THE SAMPLE WELLS AS THEY ARE CLEANED WITH COTTON SWABS.

Prepare a 50% v/v isopropanol/water solution.

Clean excess oil out of the wells using kimwipes or cotton swabs.

Add one or two drops of the isopropanol solution to each well and carefully clean using cotton swabs. Rotating the swab helps to loosen material dried in the bottom. Wash the sides of each well with the isopropanol solution.

Remove excess liquid using a kimwipe or a dry cotton swab.

Check that there are no deposits left in the sample wells.

Clean the channels between the rows of the block using the same procedure.

If the deposits of dirt are heavy, it may be difficult to clean the wells. In this case, set the thermocycler to soak at 37°C. At a slightly warmer temperature, hardened deposits are easier to remove.

If the sample block has been contaminated with biological material, clean the wells using a 10% bleach solution, followed by a distilled water rinse. Dry the sample wells with dry cotton swabs or kimwipes.

Initials: RCJ Date: 9/11/96 A-2

MAINTENANCE LOG (F001- 4/4/94) Unit: _____

Room: _____

date		initials

Initials: RCJ Date: 9/4/96 A-3

SOLUTION CONTROL SHEET (F003- 4/4/94) solution: _____

date of manufacture (DOM)					
lot number					
quantity					
location					

review date						stock on hand

Initials: RCJ Date: 9/11/96 A-4

SOLUTION CONTROL SHEET (F003A- 4/4/94) solution: ______________________

date of manufacture (DOM)					
lot number					
quantity					
location					

date of manufacture (DOM)					
lot number					
quantity					
location					

date of manufacture (DOM)					
lot number					
quantity					
location					

date of manufacture (DOM)					
lot number					
quantity					
location					

date of manufacture (DOM)					
lot number					
quantity					
location					

BALANCE LOG (F005- 4/4/94)

Unit: _____

date	std 1 (g)	meas 1 (g)	std 2 (g)	meas 2 (g)	std 3 (g)	meas 3 (g)	std 4 (g)	meas 4 (g)	init	comments

Initials: RCJ Date: 9/11/96

Initials: RCJ Date: 9/11/96 A-6

PH METER LOG (F006- 4/4/94) Unit: ______________

date	standard 1	pH	standard 2	pH	init	comments

Initials: RW Date: 9/11/96

POWER SUPPLY LOG **(F007- 4/4/94)** Power Supply: ______________

date	purpose	gel box	init	comments

MICROPIPETTE PERFORMANCE LOG (F008- 4/4/94)

Serial Number _____ Model _____

Location _____

date	initial	comments

MICROPIPETTE CALIBRATION SHEET (F009- 4/4/94) Model: _____ Serial #: _____

Temperature: _____ Density: _____ Date: _____ Analyst: _____

test volume		sample		sample	mean (mg)	mean (µl)	% error	spec	p/f	SD	CV	spec	p/f
	1		6										
	2		7										
	3		8										
	4		9										
	5		10										
	1		6										
	2		7										
	3		8										
	4		9										
	5		10										
	1		6										
	2		7										
	3		8										
	4		9										

Initials: RS Date: 9/11/96

THERMOCOUPLE (TYPE T, blue) CALIBRATION SHEET (F010- 2/1/95)

probe _____ date _____ position _____

meter _____ thermometer _____ performed by _____

Mercury Thermometer Standardization

Ice Water Bath Measured Temp. _____ spec. -0.2-0.2°C

Boiling Water Bath Measured Temp. _____ spec. 99.8-100.2°C

Thermistor Calibration

x probe reading (°C)	y thermometer reading (°C)

n =_____

sum (x) =_____ sum (y) = _____

$\bar{x}$ =_____ $\bar{y}$ = _____

sum (x^2) =_____ sum (y^2) = _____

S_{xx} =_____ S_{yy} = _____

sum (xy) =_____ S_{xy} = _____

r = _____ spec > 0.9999

m = _____ b = _____

THERMOCOUPLE (TYPE T, blue) CALIBRATION SUMMARY (F011- 2/1/95)

probe _____ date _____

meter _____ position _____

1. calibration date _____ m = _____ b = _____

2. calibration date _____ m = _____ b = _____

3. calibration date _____ m = _____ b = _____

4. calibration date _____ m = _____ b = _____

5. calibration date _____ m = _____ b = _____

6. calibration date _____ m = _____ b = _____

7. calibration date _____ m = _____ b = _____

8. calibration date _____ m = _____ b = _____

9. calibration date _____ m = _____ b = _____

10. calibration date _____ m = _____ b = _____

$\bar{m}$ = _____ $\bar{b}$ = _____

Reference Table

probe reading (°C)	standard temperature (°C)

Initials: ALS Date: 9/11/96

TEMPERATURE CONTROL LOG (F012- 4/4/94)

Unit: __________ Room: __________ Month: __________

Initials: RCG Date: 9/4/96 A-13

HEAT BLOCK CONTROL LOG **(F013- 4/4/94)** Unit: __________

Probe: __________ Room: __________ Month: __________

date	thermocouple reading	standard temperature	comments	init

Initials: RB Date: 9/11/94

THERMOCYCLER FILE LOG (F014- 4/4/94) INSTRUMENT ____________

FILE NO.	FILE TYPE	USER ID	FILE DESCRIPTION

Initials: RCS Date: 9/11/96 A-15

THERMOCYCLER LOG (F015- 4/4/94) Thermocycler: ______________

date	purpose	init	comments

THERMOCYCLER 480 DIAGNOSTICS SPECIFICATION (F016A- 3/1/95)

Thermocycler _____ Date _____ Performed By _____

QA002 Diagnostic Tests

Test 1: Display/Keypad Test

All panels of display illuminate properly yes_____ no_____
Comments

All keys correspond to the correct command yes_____ no_____
Comments

Test 3: Heater Test

Line Voltage	_____	Rate (°C/s) _____	Diff (°C) _____	Time (s) _____
specifications	108-117	≥0.67°C/s	0.0-12.0°C	≤30s
	118-124	≥0.80°C/s	0.0-12.0°C	≤25s
	125-132	≥0.90°C/s	0.0-12.0°C	≤23s

Test 4: Chiller Test Rate (°C/s) _____ specification 0.85-1.90°C/s

Test 5: Sensor Test Temp (°C) _____

Diff (°C) _____ specification <±0.5°C

Test 6: Overshoot Test Over (°C) _____ specification <0.5°C

Test 7: Undershoot Test Under (°C) _____ specification <2°C

QA003 Cycle Time

Run 1 (s) _____ Run 2 (s)_____ Diff (s)_____
specifications 3:30-4:00 3:30-4:00 <5s

WELL CALIBRATION SHEET (F017- 4/4/94)

Thermocycler _____ Well # _____ Date _____ Performed By _____

time (s)	RUN 1 temperature (°C)								
	94	std	spec	60	std	spec	72	std	spec
0			81.3-90.5			53.9-72.8			63.5-67.8
15			86.1-93.2			55.4-66.1			66.9-70.8
30			89.2-94.4			56.4-62.9			68.6-72.0
45			90.9-94.5	---	---	---	---	---	---
60			91.9-94.4	---	---	---	---	---	---

time (s)	RUN 2 temperature (°C)								
	94	std	spec	60	std	spec	72	std	spec
0			81.3-90.5			53.9-72.8			63.5-67.8
15			86.1-93.2			55.4-66.1			66.9-70.8
30			89.2-94.4			56.4-62.9			68.6-72.0
45			90.9-94.5	---	---	---	---	---	---
60			91.9-94.4	---	---	---	---	---	---

Initials: RG Date: 9/11/96

WELL CALIBRATION SHEET THERMOCYCLER 480A TC#3 (F017a- 12/18/95)

Thermocycler _____ Well # _____ Date _____ Performed By _____

time (s)	RUN 1 temperature (°C)								
	94	std	spec	60	std	spec	72	std	spec
0			81.3-90.9			53.9-74.4			63.5-68.8
15			86.1-93.2			55.4-66.5			66.9-70.8
30			89.2-94.4			56.4-62.9			68.6-72.0
45			90.9-94.5	---	---	---	---	---	---
60			91.8-94.4	---	---	---	---	---	---

time (s)	RUN 2 temperature (°C)								
	94	std	spec	60	std	spec	72	std	spec
0			81.3-90.9			53.9-74.4			63.5-68.8
15			86.1-93.2			55.4-66.5			66.9-70.8
30			89.2-94.4			56.4-62.9			68.6-72.0
45			90.9-94.5	---	---	---	---	---	---

Initials: RCJ Date: 9/11/97

WELL CALIBRATION SHEET THERMOCYCLER 480B TC#5 (F017b- 12/18/95)

Thermocycler _____ Well # _____ Date _____ Performed By _____

time (s)	RUN 1 temperature (°C)								
	94	std	spec	60	std	spec	72	std	spec
0			84.9-87.4			73.2-80.1			65.7-67.7
15			88.9-90.8			65.4-70.5			68.3-69.9
30			91.0-91.9			62.6-66.0			70.0-70.6
45			92.3-93.0	---	---	---	---	---	---
60			92.9-93.5	---	---	---	---	---	---

time (s)	RUN 2 temperature (°C)								
	94	std	spec	60	std	spec	72	std	spec
0			84.9-87.4			73.2-80.1			65.7-67.7
15			88.9-90.8			65.4-70.5			68.3-69.9
30			91.0-91.9			62.6-66.0			70.0-70.6
45			92.3-93.0	---	---		---	---	---

Initials: RCJ Date: 9/11/96

POSITIVE CONTROL LOG SHEET (F018- 4/4/94)

TUBE	DATE	CASE #	ANALYST	"C" DOT VISIBLE?

DNA PCR PROFICIENCY TEST REVIEW (F019- 4/3/95) **Test:** _____ **Analyst:** _____

Data Sheets

Were Extraction, Amplification, & Hybridization/ Electrophoresis sheets filled out correctly? **yes** **no**

Were all reagent lot numbers recorded? **yes** **no**

Procedure

Were reagent negatives processed? **yes** **no**

Were positive controls processed? **yes** **no**

Were substrate controls processed? **yes** **no**

Were exemplars extracted separately from evidence? **yes** **no**

Were duplicate analyses performed? **yes** **no**

Results

Were the appropriate photos attached to each Hybridization/Electrophoresis sheet? **yes** **no**

Did the positive controls type correctly? **yes** **no**

Were the alleles written down by the analyst consistent with the photos? **yes** **no**

Report

Is the report written in the standard format? **yes** **no**

Does the table of results accurately describe each specimen? **yes** **no**

Was the evidence matched correctly to the exemplars? **yes** **no**

Is the table of results complete? **yes** **no**

Is the summary consistent with the table of results? **yes** **no**

Initials	Error Class*	Description

Initials	Error Class*	Description

*Error Class= Class I- The nature and cause of the discrepancy raises immediate concern regarding the quality of the individual's work product. A class I error is cause for failure of the proficiency test. A class I error requires suspension from performing the test in casework and re-training. Casework can be resumed after passing a new proficiency test.

Class II- The discrepancy is due to a problem which may affect the quality of the work, but is not persistent or serious enough to cause immediate concern for the over-all quality of the individual's work product. A class II error requires re-training. A second consecutive class II error requires a new proficiency test and an unratable evaluation. A third consecutive class II error results in proficiency test failure.

Class III- The discrepancy is determined to have only minimal effect or significance, be unlikely to recur, is not systematic and does not significantly affect the fundamental reliability of the individual's work. No further follow-up will normally be required.

Analysts Response:

Evaluation pass fail unratable

I have read the proficiency summary, and I understand the comments and corrective actions outlined above.

Analyst :_____ Date: _____ Assist. Director: _____ Date: _____

Supervisor: _____ Date: _____ Director: _____ Date: _____

Initials: RG Date: 9/4/96

WATER BATH CONTROL LOG (F020- 4/4/94) Unit: __________

Room: __________ Probe: __________ Month: __________

date	water bath setting	thermistor reading	corrected temperature	checked by

DNA RFLP PROFICIENCY TEST REVIEW (F021- 4/3/95)

Test: _____ **Analyst:** _____

Data Sheets

Were worksheets/logs filled out correctly? **yes** **no**

Were all reagent lot numbers recorded? **yes** **no**

Procedure

Were reagent negatives processed? **yes** **no**

Were K562 & digestion controls processed? **yes** **no**

Were substrate controls processed? **yes** **no**

Results

Were the yield & test gels interpreted correctly? **yes** **no**

Were the appropriate photos attached to each worksheet? **yes** **no**

Were the reagent negatives clean? **yes** **no**

Did the K562 type correctly for each probe? **yes** **no**

Results (cont.)

Were the sizes written down by the analyst and the matches consistent with the autorads? **yes** **no**

Did the autorads have any artifacts, band shifting, and/or extra bands? **yes** **no**

Were all of the results double read? **yes** **no**

Were the D7Z2 results within range? **yes** **no**

Report

Is the report written in the standard format? **yes** **no**

Does the table of results accurately describe each specimen? **yes** **no**

Was the evidence matched correctly to the exemplars with the correct statistics? **yes** **no**

Is the table of results complete? **yes** **no**

Is the summary consistent with the table of results? **yes** **no**

Initials	Error Class*	Description

Initials	Error Class*	Description

*Error Class= Class I- The nature and cause of the discrepancy raises immediate concern regarding the quality of the individual's work product. A class I error is cause for failure of the proficiency test. A class I error requires suspension from performing the test in casework and re-training. Casework can be resumed after passing a new proficiency test.

Class II- The discrepancy is due to a problem which may affect the quality of the work, but is not persistent or serious enough to cause immediate concern for the over-all quality of the individual's work product. A class II error requires re-training. A second consecutive class II error requires a new proficiency test and an unratable evaluation. A third consecutive class II error results in proficiency test failure.

Class III- The discrepancy is determined to have only minimal effect or significance, be unlikely to recur, is not systematic and does not significantly affect the fundamental reliability of the individual's work. No further follow-up will normally be required.

Analysts Response:

Evaluation pass fail unratable

I have read the proficiency summary, and I understand the comments and corrective actions outlined above.

Analyst :_____ Date: _____ Assist. Director: _____ Date: _____

Supervisor: _____ Date: _____ Director: _____ Date: _____

RADIOACTIVE STATION LOG #1 (F022- 2/1/95)

USER	DATE	TIME IN	TIME OUT

RADIOACTIVE STATION LOG #2 (F022- 2/1/95)

USER	DATE	TIME IN	TIME OUT

THERMOCOUPLE (TYPE T-brown & TYPE K) VERIFICATION FOR OVENS AND REFRIGERATORS (F023-5/2/95)

probe _____ position _____

meter _____ Specification ±1°C

calibration date _____ 0°C = _____ 100°C = _____

calibration date _____ 0°C = _____ 100°C = _____

calibration date _____ 0°C = _____ 100°C = _____

calibration date _____ 0°C = _____ 100°C = _____

calibration date _____ 0°C = _____ 100°C = _____

calibration date _____ 0°C = _____ 100°C = _____

calibration date _____ 0°C = _____ 100°C = _____

calibration date _____ 0°C = _____ 100°C = _____

calibration date _____ 0°C = _____ 100°C = _____

calibration date _____ 0°C = _____ 100°C = _____

THERMOCOUPLE (TYPE T-brown & TYPE K) VERIFICATION FOR FREEZERS
(F023A-5/2/95)

probe _____ position _____

meter _____ Specification ±1 °C at 0 °C
±2 °C at -76 °C

calibration date _____ 0 °C = _____ -76 °C = _____

calibration date _____ 0 °C = _____ -76 °C = _____

calibration date _____ 0 °C = _____ -76 °C = _____

calibration date _____ 0 °C = _____ -76 °C = _____

calibration date _____ 0 °C = _____ -76 °C = _____

calibration date _____ 0 °C = _____ -76 °C = _____

calibration date _____ 0 °C = _____ -76 °C = _____

calibration date _____ 0 °C = _____ -76 °C = _____

calibration date _____ 0 °C = _____ -76 °C = _____

calibration date _____ 0 °C = _____ -76 °C = _____

Initials: RG Date: 9/11/96 A-32

USAGE LOG (F024- 5/19/95) Unit: _____

Room: _____

date		initials

Initials: RG Date: 2/11/95 A-33

HOOD FLOW RATE MONTHLY MEASUREMENT (F026 - 12/12/95)

Hood _____

Location _____

Date	1 Inch	6 Inch	12 Inch	18 Inch	24 Inch	By

373 SEQUENCER DIAGNOSTICS SPECIFICATION (F027-9/9/96) Sequencer _____

Lasertest

mw target (spec=40)	mw actual (spec=38-42)	amps (spec < 9.5 amps)	date	initials

Scanner motor test

mAmps right (spec < 300)	mAmps left (spec < 300)	date	initials

CHECK OF AUTORAD PROCESSOR (F028- 9/9/96)

Test Date					
Analyst					
Exposed piece film black?					
Uniform intensity of exposed filem?					
Unexposed film translucent?					
Unexposed film light grey in color and uniform?					
Do both x-ray films match standards?					

Comments: _____

THERMOCYCLER WELL TEMPERATURE - PM/HLADQA1 PROGRAM (F029- 9/9/96)

Thermocycler_____ Themrocouple _____

	Well						
	Analyst						
	Date						
Denaturation (spec =94°C)	Time (Sec)						
	0						
	15						
	30						
	45						
	60						
Annealing (spec= 60°C)							
	0						
	15						
	30						
Extension (spec =72°C)							
	0						
	15						
	30						

THERMOCYCLER WELL TEMPERATURE - STR QUAD PROGRAM (F030- 9/9/96)

Thermocycler_____ Themrocouple _____

	Well						
	Analyst						
	Date						
Denaturation (spec =94°C)	Time (Sec)						
	0						
	15						
	30						
	45						
	60						
Annealing (spec= 54°C)							
	0						
	15						
	30						
	45						
	60						
Extension (spec =72°C)							
	0						
	15						
	30						
	45						
	60						

THERMOCYCLER WELL POSITIVE CONTROL PLACEMENT (F031-9/9/96)

Well	Analyst	Date	Case #	System	Pos. Contrl. Lot #	Acceptable Result
A1						
A2						
A3						
A4						
A5						
A6						
A7						
A8						
B1						
B2						
B3						
B4						
B5						
B6						
B7						
B8						

THERMOCYCLER WELL POSITIVE CONTROL PLACEMENT (F031-9/9/96)

Well	Analyst	Date	Case #	System	Pos. Contrl. Lot #	Acceptable Result
C1						
C2						
C3						
C4						
C5						
C6						
C7						
C8						
D1						
D2						
D3						
D4						
D5						
D6						
D7						
D8						

THERMOCYCLER WELL POSITIVE CONTROL PLACEMENT (F031-9/9/96)

Well	Analyst	Date	Case #	System	Pos. Contrl. Lot #	Acceptable Result
E1						
E2						
E3						
E4						
E5						
E6						
E7						
E8						
F1						
F2						
F3						
F4						
F5						
F6						
F7						
F8						

Initials: RG Date: 9/11/96 A-41

GENESCAN LOG (F032- 9/9/96) Genescan Model: ________________

date	purpose	init	comments

Initials: RCJ Date: 2/9/98 A-42

377 LOG (F033- 12/14/97) 377 Model: _____

Run folder date and time	purpose	STR multiplex	Gel name	init.	comments

Keys: CW = casework → B = bloodstains; S = semen; E = exemplars
QC= Quality control T= Training R= research

Initials: RCJ   Date: 2/9/98   A-43

377 QC CHECK LOG (F034- 12/14/97)   377 Model: _______________

Run folder date and time	Prerun set	Prerun actual	Run set	Run actual	init.
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		

377 QC CHECK LOG **(F034- 12/14/97)**

377 Model:_______________

Run folder date and time	Prerun set	Prerun actual	Run set	Run actual	init.
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		
	E.Voltage (kV) - 1.00 Current (mA) - 35.0 Power (W) - 50 Laser Power (W) - 40		E.Voltage (kV) - 3.00 Current (mA) - 60.0 Power (W) - 200 Laser Power (W) - 40		

377 LOG (F033- 12/14/97) 377 Model: _____

Run folder date and time	purpose	STR multiplex	Gel name	init.	comments

Keys: CW = casework → B = bloodstains; S = semen; E = exemplars
QC= Quality control T= Training R= research