PROTOCOLS FOR FORENSIC PCR ANALYSIS

VERSION 3.0

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Initials: RC Date: 1/16/96

Initials: PU Date: 1/16/96

Initials: RG Date: 1/16/96

General PCR Guidelines

1. The general laboratory policy is to identify physiological fluids (see the Forensic Biochemistry and Hematology Laboratory Manual) before individualization is attempted. However, circumstances will exist when this is not be possible.

2. Duplicate analyses must be performed. Preferably, this should begin at the DNA extraction stage. However, if an additional extraction would consume >75% of the sample (or a complex mixture needs to be duplicated) then the duplicate analysis can begin at the amplification stage. An RFLP analysis with one probe which verifies the PCR results is considered a duplicate analysis.

3. Duplicate analyses are performed to verify the typing results. At least one run must have *no* visible dots in the extraction reagent control, amplification negative control and no extraneous dots in the positive control. The other run which is only used for verification, can have extraneous dots less than the "C"or "S" dots in the extraction reagent, amplification negative and positive controls.

4. The substrate control can have visible dots. These dots do *not* invalidate the results of the accompanying stains. The presence of dots in the substrate control should be noted in the report.

5. To minimize the potential for carry-over contamination, the laboratory is organized so that the areas for handling amplified DNA, for DNA extraction and for PCR set-up are physically isolated from each other. Each of the three areas is in a separate room. Dedicated equipment such as pipettors should not leave their designated areas. Only the samples should move between areas.

6. Samples that have not yet been amplified should never come in contact with equipment in the amplified DNA work area. Samples that have been amplified should never come in contact with equipment in the unamplified work area.

7. Handle all samples aseptically to prevent contamination by extraneous DNA.

8. The DNA extraction and PCR setup of evidence samples should be performed at a separate time from the DNA extraction and PCR setup of exemplars. This precaution will help to prevent potential cross-contamination between evidence samples and exemplars.

Initials: RCJ Date: 1/16/96

9. Change gloves frequently to avoid sample-to-sample contamination. Change them whenever they might have been contaminated with DNA and whenever exiting a work area.

10. Always change pipette tips between handling each sample.

11. Never "blow out" the last bit of sample from a pipettor. Blowing out increases the potential for aerosols, which may contaminate a sample with DNA from other samples. The accuracy of liquid volume delivered is not critical enough to justify blowing out.

12. Use filter pipet tips for pipetting all DNA and use whenever possible for other reagents. Use the appropriate filter tips for the different sized pipet men. The tip of the pipet man should never touch the filter.

13. Avoid splashes. Centrifuge all liquid to the bottom of the closed tube before opening it.

14. Avoid touching the inside surface of the tube caps.

15. Use disposable bench paper to prevent the accumulation of human DNA on permanent work surfaces. Bleach should be used periodically to decontaminate exposed work surfaces.

16. Limit the quantity of samples handled in a single run to a manageable number (approximately 16). This precaution will reduce the risk of sample mix-up and the potential for sample-to sample contamination.

17. Store the DNA Amplification Reagents together in the box provided which will serve as a barrier to possible contamination by exogenous DNA. The box should be stored in PCR set-up refrigerator.

18. Store evidence and unamplified DNA in a separate refrigerator or freezer from the amplified DNA.

19. Each sample handling area should have its own microfuge racks. The racks should only leave their designated area to transport samples to the next area. Immediately after transporting samples, the racks should be returned to their designated area.

20. Avoid exposing mineral oil to UV light. Exposure to UV light causes the mineral oil to inhibit PCR.

Initials: RCJ Date: 1/16/96

21. Use the Thermal Cycler only for amplification and denaturation of amplified DNA for typing.

22. Keep bleach away from the color development area. Small quantities of bleach can inhibit dot color development.

23. Make sure lab coat sleeves do not touch the caps of open tubes.

24. Discard pipette tips if they accidentally touch the bench paper or any other surface.

25. Wipe the outside of the pipet man with 10% bleach solution if the barrel goes inside a tube.

26. During analysis, all evidence, unamplified DNA, and amplified DNA should be stored refrigerated or frozen. Freezing is generally better for long term storage.

27. After the report is issued, amplified DNA's are discarded. **The remainder of the DNA extract's must be retained.**

28. Make sure worksheets and logbooks are completely filled out.

Initials: RCJ Date: 1/16/96

DNA Extraction

DNA Extraction Guidelines

Slightly different extraction procedures may be required for each type of specimen. Due to the varied nature of evidence samples, the user may need to modify procedures. See the trouble shooting guide (page 63) for suggestions on how to modify procedures.

1. Use a clean cutting surface for each sample such as a Kimwipe.

2. Clean scissors thoroughly with 70% ethanol or use fresh razor blades for cutting each evidence sample.

3. Swabs should be cut into two or three pieces of equal size. Sections which are not to be analyzed immediately should be stored frozen.

4. Use Kimwipes to open sample tubes and blood tubes.

5. Only one tube should be uncapped at a time. When a sample is added to a tube, the tube should be re-capped and the scissors and work area cleaned before the next tube is uncapped.

6. Try not to consume more than 75% of the sample, when possible.

7. When pouring or pipetting Chelex solutions, the resin beads must be distributed evenly in solution. This can be achieved by shaking or vortexing the tubes containing the Chelex stock solution before aliquoting.

8. Pour an aliquot of the Chelex solution from the stock tube into a sterile disposable working tube before adding to samples. The stock tube can be used multiple times. The working tube is discarded after each batch of extractions.

9. For pipetting Chelex, the pipette tip used must have a relatively large bore--1 mL pipet man tips are adequate.

10. Keep the Chelex extraction reagents and equipment separated from the rest of the laboratory equipment.

11. Be aware of small particles of fabric which may cling to the outside of tubes.

Initials: RCJ Date: 1/16/96

12. Include an extraction reagent control with each batch of extractions to demonstrate extraction integrity. The extraction reagent control contains water in place of biological fluids or stains. If DNA is found in the extraction reagent control by QuantiBlot analysis, the extraction of all the samples in the batch should be repeated and the samples should not be amplified. However, if no DNA is found then the extraction reagent controls are treated as normal samples and extracted, amplified and typed along with the test samples.

13. Obtain substrate controls from unstained fabric or substrate as close to each stain as feasible. It is not always possible to find an unstained substrate control for each evidence sample. If no DNA is found in a substrate control by QuantiBlot analysis, the substrate control is discarded without amplification because no DNA is present. However if DNA is found in the substrate control either a new substrate control should be extracted and quantitated or the substrate control should be amplified and typed along with the test samples.

14. If a sample is found to contain <0.15 ng of DNA by QuantiBlot analysis for HLA DQA1 or <0.31 ng of DNA for PM, the sample should not be amplified and it should be reported as containing no detectable DNA.

15. After extraction, the tubes containing the unamplified DNA should be transferred to a box and stored in the appropriate refrigerator or freezer. The microtube rack used to hold the DNA extraction tubes should be washed with 10% bleach. The tubes should *not* be stored in the extraction racks

Initials: RCJ Date: 1/16/96

Protein and DNA Extraction of Stains and Swabs

The following procedure is used for samples in which Biochemical, Hematological, and PCR analyses may be performed on the same sample.

1. Suspend the sample in approximately 75-200 μL of sterile dH_2O (or 0.05 M DTT if no semen is present) in a 1.5 mL microcentrifuge tube.

2. Centrifuge the tube in the microcentrifuge for 2 minute at 10,000 to 15,0000 x g.

3. Carefully pipet off all but 50 μL of the supernatant containing the enzyme fraction to a fresh microcentrifuge tube for serological analysis. Lyophilize or store the enzyme fraction frozen if it is not going to be analyzed immediately.

4. Proceed with the appropriate DNA extraction on the following pages adding the deionized water directly to the tube containing the cell debris pellet and the fabric or swab substrate.

Initials: RCJ Date: 1/16/96

Chelex DNA Extraction From Whole Blood, Bloodstains, or Blood Scraped or Swabbed Off a Surface

1. Fill out the extraction worksheet.

2. Pipette 1 mL of sterile deionized water into a 1.5 mL microcentrifuge tube for each sample. Cap all of the tubes.

3. A. Open one tube at a time with a Kimwipe and add one of the following:
   a) 3 μL whole blood
   b) portion of bloodstain or swab about 3 mm square
   c) enough scrapings to give a light straw colored extract

   B. Cap the tube before adding sample to the next tube.

4. Mix the tubes by inversion or vortexing.

5. Incubate at room temperature for 15 to 30 minutes. Mix occasionally by inversion or vortexing.

6. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

7. Carefully remove supernatant (all but 20 to 30 μL). If the sample is a bloodstain or swab, leave the substrate in the tube with pellet. (The supernatant can be frozen and retained for analysis according to the Forensic Biochemistry and Hematology Manual or it can be discarded if it is not needed).

8. Add 175 μL of 5% Chelex.

9. Incubate at 56°C for 15 to 30 minutes.

10. Vortex at high speed for 5 to 10 seconds.

11. Incubate at 100°C for 8 minutes using a screw down rack.

12. Vortex at high speed for 5 to 10 seconds.

13. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

Initials: RY Date: 1/16/96

14. Pipet 20 μL into a microcentrifuge tube for QuantiBlot Analysis to determine human DNA concentration.

15. Store the remainder of the supernatant at 2 to 8°C or frozen.

16. To re-use a sample, thaw and repeat steps 12-13.

Initials: RCJ Date: 1/16/96

Chelex DNA Extraction From Liquid Semen

1. Fill out the extraction worksheet.

2. Add 200 μL of 5% Chelex into a 1.5 mL microcentrifuge tube for each sample.

3. Add 1 μL of 20 mg/mL Proteinase K and 7 μL of 1 M DTT to each of the tubes. Cap all of the tubes and mix gently.

4. Open one tube at a time with a Kimwipe and add 3 μL of whole semen. Cap the tube before adding sample to the next tube.

5. Mix gently.

6. Incubate at 56°C for 30 to 60 minutes. Vortex at high speed 5 to 10 seconds.

7. Spin in a microcentrifuge for 10 to 20 seconds at 10,000 - 15,000 x g.

8. Follow the protocol for Whole Blood/Blood Stains (page 10) beginning with Step 10.

Initials: RC Date: 1/16/96

Chelex DNA Extraction from Semen Stains or Swabs

1. Fill out the extraction worksheet.

2. Pipette 1 mL of PBS into a 1.5 mL microcentrifuge tube for each sample. Cap all of the tubes.

3. A. Open one tube at a time with a Kimwipe and add one of the following:
   a) portion of semen stain about 3 mm square
   b) one third of a swab
   c) scrapings of a stain

   B. Cap the tube before adding sample to the next tube.

4. Mix by inversion or vortexing

5. Incubate at room temperature for 30 minutes.

6. Vortex or sonicate the substrate or swab for at least 2 minutes to agitate the cells off of the substrate or swab.

7. Remove the swab or other substrate from the sample tube, one tube at a time, using sterile tweezers and close tubes. Sterilize tweezers with ethanol before the removal of each sample.

   It is advisable not to discard the substrate until microscopic analysis (Step 11) shows that the sample contains sperm. Store swab or substrate in a sterile tube.

8. Spin in a microcentrifuge for 5 minutes at 10,000 to 15,000 x g.

9. Without disturbing the pellet, remove all but 50 μL of the supernatant. (The supernatant may be frozen or lyophilized and retained for analysis according to the Biochemistry and Hematology Laboratory Manual or discarded if not needed).

10. Resuspend the pellet in the remaining 50 μL by stirring with a sterile pipette tip.

Initials: RCJ Date: 1/16/96

11. Remove about 3 μL of the resuspended sample for a Christmas Tree Stain (see Biochemistry and Hematology Laboratory Manual). After staining, the slide should be labeled and saved as evidence. If sperm are not visible microscopically, the substrate may be put back into the PBS and vortexed more vigorously (step 6) to try to dislodge additional sperm.

    Note: If epithelial cells are detected, proceed with the differential extraction procedure beginning with step 12. If no epithelial cells are observed, the differential extraction procedure may be omitted and the sample may be processed beginning with step 21.

12. To the approximately 50 μL of resuspended cell debris pellet, add 150 μL sterile deionized water (final volume of 200 μL).

13. Add 1 μL of 20 mg/mL Proteinase K. Vortex briefly to resuspend the pellet.

14. Incubate at 56°C for about 60 minutes to lyse epithelial cells, but for no more than 75 minutes, to minimize sperm lysis.

15. During the incubation step do the following:

    a. Label a new tube for each sample, including the extraction reagent control. Mark each tube as an epithelial cell fraction
    b. Add 50 μL of 20% Chelex to each epithelial cell fraction tube
    c. Close tubes

16. Spin the extract in a microcentrifuge at 10,000 to 15,000 x g for 5 minutes.

17. Add 150 μL of the supernatant from each sample to its respective epithelial cell fraction sample tube. Store at 4°C or on ice until step 22.

18. Wash the sperm pellet with Digest Buffer as follows:
    a. Resuspend the pellet in 0.5 mL Digest Buffer.
    b. Vortex briefly to resuspend pellet.
    c. Spin in a microcentrifuge at 10,000 to 15,000 x g for 5 minutes.
    d. Remove all but 50 μL of the supernatant and discard the supernatant.
    e. Repeat steps a-d for a total of 5 times.

Initials: RSJ Date: 1/16/96

19. Wash the sperm pellet once with sterile dH_2O as follows:
    a. Resuspend the pellet in 1 mL sterile dH_2O.
    b. Vortex briefly to resuspend pellet.
    c. Spin in a microcentrifuge at 10,000 to 15,000 x g for 5 minutes.
    d. Remove all but 50 μL of the supernatant and discard the supernatant.

20. Resuspend the pellet by stirring with a sterile pipette tip. Remove about 3 μL of the resuspended sample and spot on a glass microscope slide for examination. Perform Christmas Tree stain (see Biochemistry and Hematology Laboratory Protocol). After staining the slide should be labeled and saved as evidence.

21. To the approximately 50 μL resuspended sperm fraction, add 150 μL of 5% Chelex, 1 μL of 20 mg/mL Proteinase K, and 7 μL of 1M DTT. Mix gently.

22. Vortex both the epithelial cell and sperm fractions. The following steps apply to both fractions.

23. Incubate at 56°C for approximately 60 minutes.

24. Vortex at high speed for 5 to 10 seconds.

25. Incubate in at 100°C for 8 minutes using a screw down rack.

26. Vortex at high speed for 5 to 10 seconds.

27. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

28. Pipet 20 μL into a microcentrifuge tube for QuantiBlot Analysis to determine human DNA concentration.

31. Store the remainder of the supernatant at 2 to 8°C or frozen.

32. To re-use a sample, thaw and repeat steps 26-27.

Initials: RG Date: 1/16/96

Chelex DNA Extraction From Hair

Microscopic examination of hair should be completed before attempts at DNA typing.

1. Fill out the extraction worksheet.

2. Handling hair with clean forceps, examine the hair under a dissecting microscope for the presence of sheath material. The hair may be placed on a clean piece of white paper. Note possible presence of body fluid on hair.

3. Wash the hair containing sheath material to reduce surface dirt and contaminants by immersing the hair in sterile, deionized water in a clean 50 mL beaker.

4. Return the hair to the dissecting microscope. Use a clean scalpel to cut a 1 cm portion from the root end of the hair. Because hair may contain cellular material on the surface which may or may not originate from the hair donor, it is advisable to cut off a 1 cm section of the shaft adjacent to the root portion for separate analysis as a control.

5. Add the root portion of the hair to 200 μL of 5% Chelex in a 1.5 mL microcentrifuge tube.

6. Incubate at 56°C (at least 6 to 8 hours) or overnight.

7. Vortex at high speed for 5 to 10 seconds.

8. Incubate at 100°C for 8 minutes using a screw down rack.

   Note: Check that the hair is completely immersed in the Chelex solution before boiling.

9. Vortex at high speed for 5 to 10 seconds.

10. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

11. Pipet 20 μL into a microcentrifuge tube for QuantiBlot Analysis to determine human DNA concentration.

12. Store the remainder of the supernatant at either 2 to 8°C or frozen. To re-use, repeat Steps 8 through 11.

Initials: RCS Date: 5/7/96

Chelex Extraction From Non-Organically and Organically Extracted Samples

The amount of DNA in non-organically and organically extracted samples is usually quantitated by yield gel. See the RFLP manual for organic extraction, non-organic extraction and yield gel analysis. The target amount of DNA to add to each tube for Chelex Extraction is 50 ng in 50 μL. Table I lists the volumes of (non-)organic extract to add to the Chelex tube for the various yield gel concentrations.

When a differential extraction has been performed on semen samples, both semen and epithelial cell fraction reagent blanks are generated. Both reagent blanks must be Chelex extracted and amplified if their corresponding fraction contains DNA.

Table I: Non-Organic or Organic Extract for Amplification

yield gel conc (ng/μL)	volume dH_2O (μL)	volume (non-)organic extract (μL)	volume 5% Chelex (μL)
100	49.5	0.5	150
50	49	1.0	150
40	48.7	1.3	150
25	48	2.0	150
20	47.5	2.5	150
12.5	46	4.0	150
10	45	5.0	150
5.0	40	10	150
2.5	30	20	150
2.0	25	25	150
≤1.0	0	50	150

Initials: RU Date: 1/16/96

Chelex Extraction From Non-Organically and Organically Extracted Samples

The amount of DNA in non-organically and organically extracted samples is usually quantitated by yield gel. See the RFLP manual for organic extraction, non-organic extraction and yield gel analysis. The target amount of DNA to add to each amplification tube is 5 ng in 20 μL. The amount of DNA is normalized by adding different volumes of non-organic extract to each Chelex tube, depending upon the yield of the sample. Table I lists the volumes of non-organic extract to add to the Chelex tube for the various yield gel concentrations.

Table I: Non-Organic Extract for Amplification

yield gel conc (ng/μL)	volume dH_2O (μL)	volume non-organic extract (μL)	volume Chelex (μL)
100	49.5	0.5	150
50	49	1.0	150
25	48	2.0	150
20	47.5	2.5	150
12.5	46	4.0	150
10	45	5.0	150
5.0	40	10	150
2.5	30	20	150
≤1.0	25	25	150

1. Fill out the extraction worksheet.

2. Vortex and briefly microfuge the tubes containing the non-organically extracted DNA samples.

3. Add in order, the appropriate amount of dH_2O, non-organic extract, and 5% Chelex solution to each sample tube (Table I) for a final volume of 200 μL.

4. Incubate at 56°C for 15-30 minutes.

Initials: RCJ Date: 5/7/96

1. Fill out the extraction worksheet.

2. Vortex and briefly microfuge the tubes containing the (non-)organically extracted DNA samples.

3. Add in order, the appropriate amount of dH_2O, (non-)organic extract, and 5% Chelex solution to each sample tube (Table I) for a final volume of 200 μL. The target amount of DNA to add to each Chelex Extraction Tube is 50 ng.

4. Add 50 μL dH_2O and 150 μL 5% Chelex to an empty tube marked as a Chelex Extraction Negative.

5. Incubate at 56°C for 15-30 minutes.

6. Vortex.

7. Incubate in at 100°C for 8 minutes using a screw down rack.

8. Vortex.

9. Microfuge for 2-3 minutes.

10. Pipet 20 μL into a microcentrifuge tube for QuantiBlot Analysis to determine human DNA concentration.

11. Store the remaining sample at 2-8°C or frozen.

Initials: RCJ Date: 1/16/96

5. Vortex.

6. Incubate in at 100°C for 8 minutes using a screw down rack.

7. Vortex.

8. Microfuge for 2-3 minutes.

9. Pipet 20 μL into a microcentrifuge tube for QuantiBlot Analysis to determine human DNA concentration. If QuantiBlot analysis is not performed, 20 μL of the supernatant may be added to the PCR reaction tube.

10. Store the remaining sample at 2-8°C or frozen.

Initials: RCJ Date: 1/16/96

Estimation of DNA Quantity from QuantiBlot Analysis

See the RFLP manual for the Estimation of DNA Quantity from a Yield Gel

Sample Blotting

1. Vortex samples including, if applicable, Cell Pellet Control(s), Extraction Reagent Control(s), Substrate Control(s), DNA Standards, and DNA Calibrators 1 and 2. Centrifuge briefly to bring the contents to the bottom of the tube. If Chelex extracts are being used, centrifuge for 2 minutes.

2. While wearing gloves, label enough microfuge tubes for all samples and standards.

   Pipet samples and standards into the microfuge tubes, using the following amounts of each:

   A. DNA Standards and Calibrators - 5 μL

   B. Chelex extracts intended for HLA-DQα - 20 μL

   C. Non-organic extracts - using the yield gel concentration, estimate the volume needed to apply 1-5 ng DNA; prepare a dilution with 1X TE if necessary.

   The samples can be aliquoted ahead of time and stored at 4°C.

3. Heat a shaking water bath to 50°C. The water level should be 1/4 to ½ inch above the shaking platform. The temperature should not go below 49°C or above 51°C. **It is essential to check the temperature with a calibrated thermistor probe before the hybridization is performed. Also remember to record the temperature.**

   Heat a stationary water bath to between 37°C and 50°C. Warm the HLA DQα Hybridization Solution and the QuantiBlot Wash Solution in the water bath. All solids must be in solution before use.

4. Once you begin the rest of the QuantiBlot assay, you must finish. Allow approximately 3 hours.

   Add 150 μL of Spotting Solution to each tube. Vortex and centrifuge briefly to bring the contents to the bottom of the tube.

Initials: RCJ Date: 1/16/96

5. While wearing gloves, cut a piece of Biodyne B membrane to 11.5 x 7.9 cm. Cut a small notch in the upper left corner to mark its orientation. Place the membrane in a container containing 50 mL of Pre-Wetting Solution and incubate at room temperature for 1-30 minutes.

6. Using forceps, remove the membrane from the Pre-Wetting solution. Place the membrane on the gasket of the slot blotter, then place the top plate of the slot blotter on top of the membrane. Turn on the vacuum source (house vacuum or vacuum pump) to a vacuum of approximately 8 to 10 inches Hg. Turn off the sample vacuum and turn on the clamp vacuum on the slot blot apparatus. Push down to ensure a tight seal.

7. Load the membrane as follows:

   Using a new pipet tip for each sample, apply all of each sample into a separate well of the slot blotter. For best results, slowly dispense each sample directly into the center of the wells, with the pipet tip approximately 5 mm above the membrane.

Slot	Sample
1A	10 ng standard
1B	5 ng standard
1C	2.5 ng standard
1D	1.25 ng standard
1E	0.625 ng standard
1F	0.3125 ng standard
1G	0.15625 ng standard
1H	extraction reagent control (negative)
2A	3.5 ng Calibration 1 Std.
2B	0.5 ng Calibration 2 Std.
2C	0.15625 ng standard
2D-6H	samples and controls

Initials: RS Date: 1/16/96

8. After all the samples have been applied, slowly turn on the sample vacuum. Leave the sample vacuum on until all samples have been drawn through the membrane. Inspect each slot that contains a sample for a uniform blue band. If a uniform blue band is not visible, make a note of it.

   Turn off the sample vacuum, the clamp vacuum, then the vacuum source.

10. Disassemble the slot blotter and remove the membrane. Proceed immediately to pre-hybridization. Do not allow the membrane to dry out.

    Clean the apparatus by soaking in enough 0.1% SDS to cover for 5-15 minutes. Using a Kimwipe, clean the gasket and the side of the top plate that contacts the membrane. Rinse the slot blotter in H_2O and allow to dry at room temperature. **Never use bleach.**

11. Transfer the membrane to 100 mL of pre-warmed HLA DQα Hybridization Solution in the hybridization tray. Add 5 mL of 30% H_2O_2. Place the lid on the tray. Put the tray into the 50°C shaking water bath. Place a weight (e.g. lead ring) on the covered tray to prevent the tray from sliding or floating.

    Shake at 50°C for 15 minutes at 50-60 rpm. Pour off the solution.

Initials: RS Date: 1/16/96

Hybridization

12. Add 30 mL of pre-warmed HLA DQα Hybridization Solution to the tray. Tilt the tray to one side and add 20 μL of QuantiBlot D17Z1 Probe to the HLA DQα Hybridization Solution. Cover tray with lid and weight.

    Shake at 50°C for 20 minutes at 50-60 rpm. Pour off the solution.

13. Add 100 mL of pre-warmed QuantiBlot Wash Solution to the tray. Rinse by rocking for several seconds, then pour off the solution.

14. Add 30 mL of pre-warmed QuantiBlot Wash Solution to the tray. Tilt the tray to one side and add 180 μL of Enzyme Conjugate. Cover tray with lid and weight.

    Shake at 50°C for 10 minutes at 50-60 rpm. Pour off the solution.

15. Add 100 mL of QuantiBlot Wash Solution to the tray. Rinse by rocking for 1 minute, then pour off the solution.

    Repeat for a total two washes.

16. Add 100 mL of QuantiBlot Wash Solution to the tray. Cover tray with lid and weight.

    Shake at room temperature for 15 minutes at 100-125 rpm. Pour off the solution. During this time, prepare the Color Development Solution (see below).

Initials: RY Date: 1/16/96

Color Development

17. In a glass flask, prepare the Color Development Solution. Add the reagents in order:

    60 mL of Citrate Buffer
    3 mL Chromogen
    60 μL 3% H_2O_2.

    Mix thoroughly by swirling (do not vortex).

    Note: Do not prepare the Color Development Solution more than 10 minutes before use. Use a new tube of hydrogen peroxide for each batch of Color Development Solution. Discard the remaining hydrogen peroxide after use. Wrap the Chromogen bottle in Parafilm after each use to prevent oxidation.

18. Add 100 mL of Citrate Buffer to the tray. Rinse by rocking for several seconds, then pour off the solution.

19. Add the Color Development Solution to the tray. Cover tray with lid.

    Develop the membrane by shaking at room temperature for 20-30 minutes at 50-60 rpm. Pour off the solution.

20. Stop the color development by washing in 100 mL deionized H_2O. Cover tray with lid and shake at room temperature for 5-10 minutes at 50-60 rpm.

    Repeat for a total of 3 washes.

Initials: RJ Date: 1/16/96

Photography

21. Photograph the membrane while wet. Place the membrane on a dark, flat, non-absorbent surface.

22. Use a Polaroid MP4 camera system with type 667 (preferred) or 665 film and a Wratten 23A or 22 (orange) filter.

23. Turn on the flood lights. Adjust the height of the camera and focus so that the membrane fills the entire viewing frame.

24. Photograph at 1/125 seconds and f22 for type 667 film. Photograph at ½ second and f16 for type 665 film.

25. Develop the film for 30-60 seconds. If the photograph is out of focus, not exposed properly, or does not accurately record the bands on the membrane, vary the exposure conditions and re-photograph.

    Discard the membrane once a good photograph is obtained.

26. Attach the photograph to the QuantiBlot worksheet. Once the photograph is reviewed, file in the appropriate binder or folder. For casework, the original and photocopies are retained in the case files.

Initials: KJ Date: 1/16/96

Interpretation

27. At least one of the 0.15 ng standards must be visible. If not, the samples on the membrane with an amount of <0.31 ng must be repeated.

28. Compare the intensity of Calibrator 1 (3.5 ng/5 μL) and Calibrator 2 (0.5 ng/5 μL) to the DNA Standards. The intensity of Calibrator 1 should be between 2.5 ng and 5 ng; the intensity of Calibrator 2 should be between 0.31 ng and 0.62 ng. If not, the membrane must be repeated.

29. Estimate the quantity (ng) and concentration (ng/μL) of DNA loaded for each sample by comparing the band intensity of the unknowns with those of the DNA Standards. If the band intensity is between two DNA Standards **match the sample to the DNA Standard with the lower amount of DNA.**

    The concentration (ng/μL) is the quantity (ng) divided by the amount of sample used (μL).

    For diluted samples (1/10), multiply DNA concentration by 10.

    If sample band intensity is >10 ng, it must be diluted 1/10 and quantitated again.

30. Show the photograph to another Analyst or Scientist to review the DNA quantity and concentration. Disagreements should be settled by another Scientist, the Assistant Director and/or the Director.

Initials: RCJ Date: 1/16/96

Troubleshooting of QuantiBlot

	Observation	Possible Cause	Recommended Action
1.	No signal or low sensitivity.	Use of a membrane other than Biodyne B.	Use Biodyne B nylon membrane. Do not use membranes that have neutral charge.
		Incorrect NaOH or EDTA concentrations in Spotting Solution.	Prepare Spotting Solution correctly.
		Water bath temperature too high.	Water bath temperature should be 50°C (±1°C).
		DNA Probe was not added at hybridization step.	Add QuantiBlot D17Z1 Probe.
		Enzyme conjugate was not added.	Add Enzyme conjugate: HRP-SA at indicated step in protocol. Use 180 μL of Enzyme Conjugate: HRP-SA.
		Hydrogen peroxide was inactive.	Prepare a new Color Development Solution using a fresh bottle of hydrogen peroxide.
		Presence of $MgCl_2$ in the DNA sample.	Concentrations of $MgCl_2$ >0.3 mM can result in reduced sensitivity. Prepare all DNA dilutions in TE Buffer. Any $MgCl_2$ can be removed from samples by microdialysis using Centricon 100 spin units (follow manufacture's directions).

Initials: RSJ Date: 1/16/96

	Observation	Possible Cause	Recommended Action
2.	Areas of low sensitivity across the membrane.	Membrane slipped up onto the side of the Hybridization Tray during Hybridization or Stringent Wash steps.	Reduce the rotation rate of the water bath to 50-60 rpm. Check that the membrane is fully submerged in the bottom of the Hybridization Tray before shaking.
		Membrane dried-out significantly at some point in the protocol.	Do not allow the membrane to dry at any point in the protocol.
3.	Non-uniform signal intensity within a slot.	Bubbles(s) in slot blot wells when sample was pipetted into well, or when vacuum was applied.	Slowly pipet the Spotting Solution directly over the center of the wells of the slot blot apparatus, with the pipet tip raised approximately 5 mm above the membrane.
			Turn on the sample vacuum slowly, not all at once. After being drawn through the membrane, the sample should appear as a uniform blue band on the membrane.
			If the entire sample is not drawn through the membrane, turn off the sample vacuum. Pipet the sample back into the pipet tip; then pipet the sample back into the well of the slot blot apparatus. Turn on the sample vacuum to draw the sample through the membrane.
4.	Filter background	No or low SDS in the Hybridization Solution or in the Wash Solution	Prepare solutions with proper concentrations of SDS.

Initials: RY Date: 1/16/96

	Observation	Possible Cause	Recommended Action
		Membrane was not pre-wetted prior to slot blotting.	Pre-wet the membrane in Pre-Wetting Solution prior to slot blotting.
		Too much Enzyme conjugate: HRP-SA was added.	Use 180 μL of Enzyme Conjugate:HRP-SA.
		Lack of thorough rinsing.	Thoroughly rinse twice, for 1 minute each, using 100 mL of pre-warmed Wash Solution. These two rinse times can be extended beyond 1 minute if necessary.
		Slot blot apparatus not cleaned thoroughly after last use.	Immediately after each use, soak the slot blot apparatus in a large volume of 0.1% SDS solution. Never use bleach.
5.	The DNA Calibrators do not quantitate correctly with respect to the DNA Standards.	DNA Standard serial dilutions prepared incorrectly.	Prepare two-fold serial dilutions of DNA Standard in TE Buffer as described. Add 5 μL of Spotting Solution for slot blotting.
6.	Signal obtained for non-human DNA samples.*	Water bath temperature too low.	Water bath temperature should be 50°C (±1°C).
		SSPE concentration too high in Wash Solution.	Check that the 20X SSPE solution and the Wash Solution were prepared correctly.

* DNA from primates species may give signals similar to those obtained from equivalent amounts of human DNA. In Roche Molecular Systems (RMS) laboratories, 30 ng to 300 ng quantities of non-primate DNA samples result in either no signals or signals that are less than or equal to the signal obtained for 0.15 ng of human DNA.

Initials: RCJ Date: 1/16/96

References for QuantiBlot

1. Walsh, P.S., Valaro, J., and Reynolds, R., 1992. A rapid chemiluminescent method for quantitation of human DNA. Nucleic Acids Research **20:** 5061-5065.

2. Wayne, J.S. and Willard, H.F., 1986. Structure, organization, and sequence of alpha satellite DNA from human chromosome 17: evidence for evolution by unequal Crossing-Over and an ancestral pentamer repeat shared with the human X chromosome. Molecular and Cellular Biology **6:** 3156-3165.

3. Whitehead, T.P., Thorpe, G.H.G., Carter, T.J.N., Groucutt, C., and Kricka, L.J., 1983. Enhanced luminescence procedure for sensitive determination of peroxidase-labeled conjugates in immunoassay. Nature **305**: 158-159.

4. Miller, S.A., Dykes, D.D., and Polesky, H.F., 1988. A simple salting out procedure for extraction DNA from human nucleated cells. Nucleic Acids Research **16:** 1215.

Initials: RSJ Date: 1/16/96

Reverse Dot Blot Test (HLA DQα, PM, and PM+DQA1)

Amplification of HLA DQα, PM, and PM+DQA1

A positive control, an amplification negative control, an extraction reagent control and substrate controls, if applicable, should be included with each batch of samples being amplified to demonstrate procedural integrity.

1. Turn on the Perkin Elmer Thermal Cycler. (See manufacturer's instructions). File #14 on all machines should include the following:

   Denature at 94°C for 1 minute
   Anneal at 60°C for 30 seconds
   Extend at 72°C for 30 seconds

   Repeat the above for 32 cycles

   Link to Time Delay File #13 on all machines for an additional 7 minutes incubation at 72°C.

   Link to Soak File #12 on both machines for a 4°C soak.

   If Files #12, 13, or 14 are not correct, bring this to the attention of the QC/QA coordinator and a supervisor. Re-program the incorrect file(s) and note that the file(s) was changed in the log book for that run.

   NOTE: Use only the Model 480 Thermal Cyclers for PM and/or PM+DQA1 kit amplifications.

2. Determine the number of samples to be amplified, including controls.

3. Fill out the amplification worksheet and record the appropriate lot numbers.

4. Ensure that the solution is at the bottom of each PCR reaction mix tube by tapping the tube down onto a clean work surface or by centrifuging briefly. Label the caps of the PCR Reaction Mix tubes. Open caps using the microcentrifuge tube de-capping tool or a new Kimwipe. **Avoid touching the inside surface of the tube caps.**

Initials: RS Date: 1/16/96

5. Note: **It is important to begin the cycling processing within 20 minutes after addition of the $MgC1_2$ Solution or PM Primer Set to the PCR Reaction Mix.**

HLA DQα	PM or PM+DQA1
50 μL $MgC1_2$ Solution	40 μL PM Primer Set

Pipet the appropriate solution (see above) into each tube including controls, with a sterile pipet tip. Pipet carefully at a slight angle to minimize mixing and to avoid splashing of solution. Use either a filter tip or a combi tip on the pipettor.

6. Carefully add 2 drops of the Mineral Oil from the dropper bottle provided in the kit to all tubes including the controls. **Do not actually touch tube.**

7. Close all of the tubes.

8. **Note: Use a new sterile filter pipet tip for each sample addition. Open only one tube at a time for sample addition.** For HLA DQα, the final aqueous volume in the PCR reaction mix tubes will vary between 101 μL and 140 μL, depending on the volume of DNA added. For PM and PM+HLA DQA1 The final volume is 100 μL.

Test Sample Tubes:
See Table II or III for the amount of DNA to add to each tube. For PM+HLA DQA1, it is preferable to amplify the undiluted extract rather than any dilution of the extract (see note to Table III). It is recommended that the target amount of DNA be amplified in the first attempt. Do not amplify samples in which no DNA was detected by QuantiBlot. Add the sample DNA to each labeled tube by inserting the pipet tip through the mineral oil layer. After the addition of the DNA, cap each sample before proceeding to the next tube. **Do not vortex or mix.**

Positive Control Tubes:
Add 20 μL of the 100 ng/mL Genomic Control DNA to the designated PCR Reaction Mix tube.

Extraction Reagent Controls:
Add 20 μL of the extraction reagent control to the designated PCR Reaction Mix tube.

Amplification Negative Control Tubes:
Do not add DNA. For PM and PM/HLA DQA1, add 20 μL sterile deionized H_2O. For HLA DQα the deionized H_2O is optional.

Initials: RCJ Date: 1/16/96

Table II- Amount of DNA to be Amplified for the HLA DQα Kit

QuantiBlot DNA Concentration (ng/20μL)	Target Volume (μL) to be amplified	Range of Volumes (μL) which can be amplified
≥10	Dilute 1:10 and re-quantitate	
5	5	1-40
2.5	10	2-40
1.25	20	3-40
0.62	40	10-40
0.31	40	20-40
0.15	40	20-40
<0.15	Do not amplify	-

Initials: RS Date: 1/16/96

Table III- Amount of DNA to be Amplified for the PM and PM+DQA1 Kits

DNA Concentration* (ng/20μL)	Target Volume (μL) to be amplified	Sterile H_2O (μL) for Target Volume	Range of Volumes (μL) which can be amplified
≥50	Dilute 1:10 and use the dilution for amplification		
25	1.6	18.4	0.5-8**
12.5	3.2	16.8	0.9-16**
6.2	6.5	13.5	1.6-20**
5	8	12	2-20**
2.5	16	4	4-20**
1.25	20	0	8-20**
0.62	20	0	-
0.31	20	0	-
<0.31	Do not amplify	-	-

* If the quantiblot result is ≥10 ng/20 μL, dilute the sample 1:10 and re-quantitate. Multiply the diluted concentration by 10 to obtain the original sample concentration. Repeat the procedure if the diluted sample is still ≥10 ng/20 μL.

Note: When a dilution is made to determine DNA concentration, it is preferable to calculate the DNA concentration in the undiluted DNA extract and to amplify the undiluted DNA extract and not amplify the dilution.

** Add sterile deionized H_2O to a final volume of 20 μL.

Initials: RJ Date: 1/16/96

9. **Note: PCR Reaction Mixes should be amplified in rows A-D in the Thermal Cycler (HLA DQα) or any row of the 480 Thermal Cycler. Only amplify the PM and PM+DQA1 kits in the 480 Thermal Cyclers**

   For each well of the Thermal Cycler heat block which will be used to amplify samples, add one drop of mineral oil to the well. Place the PCR Reaction Mix tubes into the Thermal Cycler. Push the tubes down completely into the heat block. Record the heat block position of each tube.

10. Start the Thermal Cycler amplification program. Verify the cycling parameters by monitoring the first cycle. The tubes should be checked after the first cycle and pressed further into the heat block so that they fit tightly.

11. Return the microtube rack used to set-up the samples for PCR to the PCR Set-Up Area.

12. **For PM+DQA1 Kits Only!**
    In the hybridization room, open the tubes one at a time and add 5 μL of 200 mM EDTA. Use a new pipet tip for each addition. Insert the pipet through the mineral oil layer. Discard the pipet tip and re-cap the tube before proceeding to the next tube. It may be convenient to remove a 5 μL aliquot for gel electrophoresis before recapping the tube.

13. After the amplification process, the samples are ready for DNA Hybridization and Color Development or they may be stored at 2-8°C for at least fourteen days, or at -20° for at least 6 months in the appropriate refrigerator or freezer.

Initials: RCJ Date: 1/16/96

Optional Step to Verify Amplification

The presence of amplified sequences can be verified by gel electrophoresis. The Amplitype HLA DQα gene amplification product will be either 239 or 242 base pairs long, depending on the particular allele.

Agarose Gel Preparation

1. Weigh out agarose for a 3% Nusieve™ plus 1% Seakem™ GTG or a 3% Metaphor gel

2. Add agarose to the appropriate amount of 1X TBE in a flask. Volume required will vary depending on size of the minigel apparatus. Prepare enough agarose to pour a 0.45cm gel.

3. To dissolve agarose, heat in a microwave oven for 1 to 2 minutes. Swirl flask to aid in dissolving agarose.

4. Cool the solution to 55°C in a water bath.

5. Add a volume of 10 mg/mL Ethidium Bromide to the molten agarose to achieve a final concentration of 0.5 μg/mL Ethidium Bromide.

6. To form the gel, pour liquified agarose/ethidium bromide solution in minigel apparatus for a 3 mm thick gel. Immediately insert slot forms and adjust to assure that the apparatus is level.

7. Allow the gel to set for 20 minutes at room temperature, or until completely solidified.

8. When gel is ready, add a sufficient volume of 1X TBE to fill the buffer tanks and cover the gel to a depth of about 2 mm.

9. Carefully remove slot forms. Avoid touching wells.

Initials: RJ Date: 1/16/96

Gel Loading and Electrophoresis

1. Add 2 μL Gel Loading Buffer to a 0.5 mL microcentrifuge tube for each sample or to a well of a 96 well microplate for each sample.. Add 5 μL of the DNA sample to the loading buffer and mix by tapping gently.

2. Dilute the Gibco BRL 123 bp ladder to 40 ng/μL with sterile water. Add 5 μL of the diluted ladder to 2 μL of Gel Loading Buffer.

3. Do not use the outside lanes of the gel. In the first and last useable lanes of the gel, pipet 123 bp ladder.

4. Carefully pipette samples into the remaining wells.

5. Connect leads so that the DNA migrates toward the positive (+) electrode. Run the minigel at 100 volts, (or 7.5 v/cm) at room temperature for about two hours or until the bromophenol blue (faster-migrating) dye is 7.5 cm from the wells of the gel.

6. Check for the extent of DNA migration by examining visually on a 300 nm UV transilluminator. **WEAR PROTECTIVE EYEWEAR AND HANDLE THE GEL WITH GLOVES.**

   To photograph gel, place on a UV transilluminator box under a stationary camera with a Kodak 23A Wratten orange filter. Photograph in the dark under UV illumination.

Interpretation of Gel Electrophoresis Patterns

The HLA DQα/ HLA DQA1 amplification products will appear as 242/239 bp bands. The PM products will appear as 214 bp (LDLR), 190 bp (GYPA), 172 pb (HBGG), 151 bp (D7S8), and 138 bp (GC). Primer dimer bands and unincorporated primers may appear as broad bands near the bottom of the gel in the region of lower molecular weight. Occasionally,, non-specific bands or smearing can be observed above and/or below the six specific bands, but they do not compromise the typing results.

For PM and PM+DQA1 six bands should be present. For HLA DQα one or two bands should be present. Otherwise do not proceed.

Initials: RSJ Date: 1/16/96

HLA DQα Hybridization

1. Heat a shaking water bath to 55°C. The water level should be 1/4 - 1/2 inch above the shaking platform. The water level should not be higher than 1/2 inch, as higher levels may result in water splashing into wells. **The temperature should not go below 54°C or above 56°C. It is essential to check the temperature with a calibrated thermistor probe before the hybridization is performed.**

2. Heat a stationary water bath to 37-55°C. Warm the HLA DQα Hybridization Solution and the HLA DQα Wash Solution in the water bath. All solids must be in solution before use.

3. Fill out the hybridization worksheet.

4. Using filter forceps, remove the required number of DNA Probe Strips from the glass tube. **Make sure the tube is at room temperature before removing the strips.** Place one Probe Strip in each clean well of the Amplitype DNA Typing Tray. With a waterproof marking pen, label each strip in the space provided.

5. Add one drop of oil to each well that will be sued. Place the tubes in a 95°C heat block. Press the tubes down tightly in the heat block. Denature the amplified DNA by incubation at 95°C for 3-10 minutes. Keep each tube at 95°C until use.

6. **Note: Do not prepare the DQα Hybridization/Enzyme conjugate Solution more than 15 minutes before use.**

   Prepare the hybridization solution in a glass flask either according to Table IV or the following formula:

   # Strips x 3.3mL pre-warmed DQα Hybridization Solution
   # strips x 27 μL Enzyme Conjugate

   Mix by swirling.

Initials: RCJ Date: 1/16/96

Table IV- DQα Hybridization Solution

number of strips	hyb solution	enzyme conjugate
3	9.9 ml	81 μl
4	13.2 ml	108 μl
5	16.5 ml	135 μl
6	19.8 ml	162 μl
7	23.1 ml	189 μl
8	26.4 ml	216 μl
9	29.7 ml	243 μl
10	33.0 ml	270 μl
11	36.3 ml	297 μl
12	39.6 ml	324 μl
13	42.9 ml	351 μl
14	46.2 ml	378 μl
15	49.5 ml	405 μl
16	52.8 ml	432 μl

7. Tilt the Typing Tray towards the labelled end of the strips. Add 3 mL of the freshly prepared DQα Hybridization/Enzyme Conjugate Solution at the labelled end of each strip.

Initials: RCJ Date: 1/16/96

8. **Note: Perform the following steps for each tube of amplified DNA. When pipeting amplified extract, wait for the bead of mineral oil to expel from the tip before drawing up. For each tube, perform steps a-c within 30 seconds.**

   a. Remove the tube from the 95°C heat block.
   b. Carefully open the tube. (Use the microcentrifuge tube decapping device or a new Kimwipe).
   c. Withdraw 35 μL amplified DNA from the aqueous (bottom) layer and immediately add to the contents of the well (pipet below the surface of the hybridization solution) at the labeled end of the corresponding Probe Strip.
   d. Cap the tube and set aside.
   e. Repeat until each amplified DNA sample has been added to the corresponding well. Use a new pipet tip for each addition.

   The remaining amplified DNA samples can be stored at 2-8°C for 2 months, at -20°C for 6 months or discarded when the analysis is complete.

9. Mix the tray by carefully rocking and place the clear plastic lid on the tray. Ensure that each strip is completely wet. Put the tray into the 55°C shaking water bath. Place a weight (e.g., lead ring) on the covered tray to prevent the tray from sliding or floating. **Once the hybridization has begun strips should remain wet through the conclusion of the Color Development and Photography steps.**

10. Hybridize the amplified DNA samples to the Probe Strips by incubating at 55°C for 20 minutes at 50-90 rpm. Adjust the water level and check the tray position so that water does not splash into the wells of the tray.

11. After hybridization, remove the tray from the water bath and pour out the contents of each well. Wipe the tray lid with a Kimwipe. Do not use a paper towel.

12. **Note: HLA DQα Wash Solution solids must be in solution before use.**

    Dispense 10 mL of HLA DQα Wash Solution into each well. Rinse by rocking for several seconds, then pour the solution from each well.

Initials: RJ Date: 1/16/96

13. **Note: The temperature and timing of the Stringent Wash are CRITICAL.**

    Stringent Wash:
    Dispense 10 mL of HLA DQα Wash Solution into each well. Cover tray with lid and weight. Place into the 55°C shaking water bath for 12 minutes, ±2 minutes, at about 50 rpm.

14. Remove the tray from the water bath, take off the lid and pour the solution from each well.

15. Dispense 10 mL of HLA DQα Wash Solution into each well. Cover and place on an orbital shaker at room temperature for 5 minutes at about 50 rpm.

16. Remove the tray from the orbital shaker, take off the lid and pour the solution from each well.

Initials: RSJ Date: 1/16/96

HLA DQα Color Development

1. Dispense 10 ml of citrate buffer into each well. Wipe the tray lid with a Kimwipe. Cover tray and place on an orbital shaker for 5 minutes at about 50 rpm.

2. **Note:** **Do not prepare the Color Development Solution more than 10 minutes before use. A tube of hydrogen peroxide can be used for one week. Discard the remaining hydrogen peroxide after one week. Cover the chromogen bottle with parafilm after each use to prevent oxidation.**

   During this wash step, prepare the Color Development Solution in an Erlenmeyer flask either according to Table V or the following formula. Add the reagents in order:

   # strips x 10 mL DQα Citrate Buffer
   # strips x 10 μL 3% Hydrogen Peroxide
   # strips x 0.5 mL Chromogen Solution

Mix by swirling 2-3 times. Do Not Vortex. Protect from light.

Initials: RCJ Date: 1/16/96

Table V- Development Solution

number of strips	citrate buffer	hydrogen peroxide	chromogen
3	30 ml	30 µl	1.5 ml
4	40 ml	40 µl	2.0 ml
5	50 ml	50 µl	2.5 ml
6	60 ml	60 µl	3.0 ml
7	70 ml	70 µl	3.5 ml
8	80 ml	80 µl	4.0 ml
9	90 ml	90 µl	4.5 ml
10	100 ml	100 µl	5.0 ml
11	110 ml	110 µl	5.5 ml
12	120 ml	120 µl	6.0 ml
13	130 ml	130 µl	6.5 ml
14	140 ml	140 µl	7.0 ml
15	150 ml	150 µl	7.5 ml
16	160 ml	160 µl	8.0 ml

3. **Note: Place lid on tray and cover lid with aluminum foil during steps, 3,5 and 6 to protect from strong light.**

   Remove the tray from the orbital shaker, remove the cover and slowly pour off the DQα Citrate Buffer. Add 10 mL of the newly prepared Color Development Solution to each well. Develop the strips at room temperature by shaking on the orbital shaker at about 50 rpm for 20-30 minutes.

4. Remove tray from shaker and slowly pour off the contents from each well.

Initials: RCJ Date: 1/16/96

5. Stop the color development by washing the strips in deionized water. Dispense approximately 10 mL of water into each well. Place tray on orbital shaker at about 50 rpm for 5-10 minutes. Slowly pour off the contents of each well.

6. Repeat Step 4 and Step 5 twice for a minimum of three water washes. Additional 5-10 minutes washes will reduce the potential for developing background color.

7. Photographs must be taken for a permanent record (see the next section).

8. Determine and record the HLA DQα type for each sample from the photograph. (See Results Interpretation section.)

Initials: RJ Date: 1/16/96

PM and PM+DQA1 Hybridization

1. Heat a shaking water bath to 55°C. The water level should be 1/4 - 1/2 inch above the shaking platform. The water level should not be higher than 1/2 inch, as higher levels may result in water splashing into wells. **The temperature should not go below 54°C or above 56°C. It is essential to check the temperature with a calibrated thermistor probe before the hybridization is performed.**

2. Heat a stationary water bath to 37-55°C. Warm the HLA DQα Hybridization Solution and the HLA DQα Wash Solution in the water bath. All solids must be in solution before use.

3. Fill out the hybridization worksheet.

4. Using filter forceps, remove the required number of DNA Probe Strips from the glass tube. **Make sure the tube is at room temperature before removing the strips.** Place one Probe Strip in each clean well of the Amplitype DNA Typing Tray. With a waterproof marking pen, label each strip in the space provided.

   Note: PM and DQA1 DNA Probe strips can be used to type PCR products products from the same PM amplfication reaction at the same time but the DNA probe strips must be placed in separate wells of the tray.

5. Add one drop of oil to each well that will be used. Place the tubes in a 95°C heat block. Press the tubes down tightly in the heat block. Denature the amplified DNA by incubation at 95°C for 3-10 minutes. Keep each tube at 95°C until use.

6. Tilt the Typing Tray towards the labelled end of the strips. Add 3 mL of DQα Hybridization Solution at the labelled end of each strip.

Initials: RC Date: 1/16/96

7. **Note: Perform the following steps for each tube of amplified DNA. When pipeting amplified extract, wait for the bead of mineral oil to expel from the tip before drawing up. For each tube, perform steps a-c within 20 seconds.**

   a. Remove the tube from the 95°C heat block.
   b. Carefully open the tube. (Use the microcentrifuge tube decapping device or a new Kimwipe).
   c. Withdraw 20 μL amplified DNA from the aqueous (bottom) layer and immediately add to the contents of the well (pipet below the surface of the hybridization solution) at the labeled end of the corresponding Probe Strip.
   d. Cap the tube.
      i. If using only PM strip or only DQA1 strips for each amplified sample, set the tube aside.
      ii. If using both PM **and** DQA1 strips for the same amplified sample, return the tube to the 95°C heat block for 3-10 minutes after probe additon to the first strip. Add the remaining DNA samples to the first strip of each PM and DQA1 set. After the required time has passed, add the probe to the second strip of each set. Set the tube aside after addition to the second strip.
   e. Repeat until each amplified DNA sample has been added to the corresponding well. Use a new pipet tip for each addition.

   The remaining amplified DNA samples can be stored at 2-8°C for 2 months, at -20°C for 6 months or discarded when the analysis is complete.

8. Mix the tray by carefully rocking and place the clear plastic lid on the tray. Ensure that each strip is completely wet. Put the tray into the 55°C shaking water bath. Place a weight (e.g., lead ring) on the covered tray to prevent the tray from sliding or floating. **Once the hybridzation has begun, strips should remain wet through the conclusion of the Color Development and Photography step.**

9. Hybridize the amplified DNA samples to the Probe Strips by incubating at 55°C for 15±2 minutes at 50-70 rpm. Adjust the water level and check the tray position so that water does not splash into the wells of the tray.

Initials: RC Date: 1/16/96

10. Approximately 5 minutes before the end of the hybridization step, prepare the Enzyme Conjugate Solution in a glass flask either according to Table VI or the following formula:

    # Strips x 3.3mL pre-warmed DQα Hybridization Solution

    # strips x 27 μL Enzyme Conjugate: HRP:SA

    Mix by swirling.

Table VI- DQα Hybridization Solution

number of strips	hyb solution	enzyme conjugate
3	9.9 ml	81 μl
4	13.2 ml	108 μl
5	16.5 ml	135 μl
6	19.8 ml	162 μl
7	23.1 ml	189 μl
8	26.4 ml	216 μl
9	29.7 ml	243 μl
10	33.0 ml	270 μl
11	36.3 ml	297 μl
12	39.6 ml	324 μl
13	42.9 ml	351 μl
14	46.2 ml	378 μl
15	49.5 ml	405 μl
16	52.8 ml	432 μl

11. After hybridization, remove the tray from the water bath and pour out the contents of each well. Wipe the tray lid with a Kimwipe. Do not use a paper towel. Replace the water bath cover and keep the water bath rotating between incubation steps to maintain the temperature at 55°C±1°C

Initials: RJ Date: 1/16/96

12. **Note: DQα Wash Solution solids must be in solution before use.**

    Dispense 5 mL of DQα Wash Solution into each well. Rinse by rocking for several seconds, then pour the solution from each well. Wipe the tray lid with a Kimwipe.

13. Dispense 3 mL of the enzyme Conjugate Solution prepared in Section 10 into each well. Cover tray with lid and weight. Place into the 55°C in the shaking water bath for 5±1 minutes at 50-70 RPM.

14. After incubation, remove the tray from the water bath and pour out the contents of each well. Wipe the tray lid with a Kimwipe. Do not use a paper towel. Replace the water bath cover and keep the water bath rotating between incubation steps to maintain the temperature at 55°C±1°C

15. Dispense 5 mL of DQα Wash Solution into each well. Rinse by rocking for several seconds, then pour the solution from each well. Wipe the tray lid with a Kimwipe.

16. **Note: The temperature and timing of the Stringent Wash are CRITICAL.**

    Stringent Wash:
    Dispense 5 mL of DQα Wash Solution into each well. Cover tray with lid and weight. Place into the 55°C shaking water bath for 12 ±1 minutes, at about 50-70 rpm.

17. Remove the tray from the water bath, take off the lid and pour the solution from each well.

18. Dispense 5 mL of DQα Wash Solution into each well. Rinse by rocking for several seconds, then pour the solution from each well. Wipe the tray lid with a Kimwipe.

Initials: RCJ Date: 1/16/96

PM and PM+DQA1 Color Development

1. Dispense 5 ml of citrate buffer into each well. Wipe the tray lid with a Kimwipe . Cover tray and place on an orbital shaker for 5 minutes at about 50 rpm.

2. **Note:** **Do not prepare the Color Development Solution more than 10 minutes before use. A tube of hydrogen peroxide can be used for one week. Discard the remaining hydrogen peroxide after one week. Cover the chromogen bottle with parafilm after each use to prevent oxidation.**

   During this wash step, prepare the Color Development Solution in an Erlenmeyer flask either according to Table VII or the following formula. Add the reagents in order:

   # strips x 5 mL HLA DQα Citrate Buffer
   # strips x 0.5 μL 30% Hydrogen Peroxide or 5μL 3% Hydrogen Peroxide
   # strips x 0.25 mL Chromogen Solution

Mix by swirling 2-3 times. Do Not Vortex. Protect from light.

Initials: RG Date: 1/16/96

Table VII- Development Solution

number of strips	citrate buffer	hydrogen peroxide 30% (3%)	chromogen
3	15 ml	1.5 (15) µl	0.75 ml
4	20 ml	2.0 (20) µl	1.0 0 ml
5	25 ml	2.5 (25) µl	1.25 ml
6	30 ml	3.0 (30) µl	1.50 ml
7	35 ml	3.5 (35) µl	1.75 ml
8	40 ml	4.0 (40) µl	2.00 ml
9	45 ml	4.5 (45) µl	2.25 ml
10	50 ml	5.0 (50) µl	2.50 ml
11	55 ml	5.5 (55) µl	2.75 ml
12	60 ml	6.0 (60) µl	3.00 ml
13	65 ml	6.5 (65) µl	3.25 ml
14	70 ml	7.0 (70) µl	3.50 ml
15	75 ml	7.5 (75) µl	3.75 ml
16	80 ml	8.0 (80) µl	4.00 ml

3. **Note: Place lid on tray and cover lid with aluminum foil during steps, 3, 5, and 6 to protect from strong light.**

   Remove the tray from the orbital shaker, remove the cover and slowly pour off the DQα Citrate Buffer. Add 5 mL of the newly prepared Color Development Solution to each well. Develop the strips at room temperature by shaking on the orbital shaker at about 50 rpm for 20-30 minutes.

4. Remove tray from shaker and slowly pour off the contents from each well.

Initials: RU Date: 1/16/96

5. Stop the color development by washing the strips in deionized water. Dispense approximately 5 mL of water into each well. Place tray on orbital shaker at about 50 rpm for 5-10 minutes. Slowly pour off the contents of each well.

6. Repeat Step 4 and Step 5 twice for a minimum of three water washes. Additional 5-10 minutes washes will reduce the potential for developing background color.

7. Photographs must be taken for a permanent record (see the next section).

8. Determine and record the PM and/or HLA DQA1 type for each sample from the photograph. (See Results Interpretation section.)

Initials: RH Date: 1/16/96

Photography And Storage

1. Place wet strips on a flat surface as close together as possible. Keep strips wet throughout the photographic procedure. Minimize exposure to strong light.

   For casework photograph up to approximately 8 strips at a time. For QC and validation photograph up to 16 strips at a time.

2. Use a Polaroid MP4 camera system with type 667 or 665 film and a Wratten 23A or 22 orange filter. Type 667 is the preferred film.

3. Turn on the flood lights. Adjust the height of the camera and focus so that the strips fill the **entire** viewing frame.

4. Photograph at 1/125 second and f22 for type 667 film. Photograph at 1/2 second and f16 for type 665 film.

5. Develop at room temperature for ≈30-60 seconds.

6. If the photograph is not exposed properly or does not accurately record the dots on the strips, vary the exposure conditions and re-photograph.

   Make sure there is at least one good photograph of each set of strips.

7. Attach the photographs to the hybridization worksheets and file in the appropriate binder or folder. For cases the worksheets are retained in the case file.

8 Place strips between sheets of blotting paper (**do not use paper towels**) and allow to dry; minimize exposure to light

9. When strips are dry, store the strips in a plastic folder in the appropriate binder or folder. Protect from light and oxidizing agents. Color may fade somewhat on drying.

Initials: RG Date: 1/16/96

Results Interpretation

Results are interpreted by observing the pattern and relative intensities of blue dots on the wet AmpliType PM, Amplitype HLA DQα, and AmpliType HLA DQA1 DNA Probe Strips to determine which alleles are present in the DNA sample.

1. Reading and Interpreting the AmpliType PM DNA Probe Strips

The AmpliType PM DNA Probe Strips have been spotted with a total of fourteen sequence-specific oligonucleotide probes to distinguish the alleles of five genetic loci (a mixture of two probes is spotted at the GYPA "A" allele position). Under the AmpliType hybridization conditions, the typing probes will bind specifically to PCR product containing the alleles designated on the AmpliType PM DNA probe strip.

To read the developed AmpliType PM DNA Probe Strips, the "S" dot is examined first and then each locus is examined separately. The standard probe "S" on the AmpliType PM DNA Probe Strip is identical in sequence to the control probe "C" on the AmpliType HLA DQA1 DNA Probe Strip and detects all of the HLA DQA1 alleles. The "S" dot is designed to be the lightest typing dot on the PM DNA Probe Strip and acts as a minimum dot intensity control for the remaining probes. A DNA probe strip with no visible "S" dot should not be typed for any locus.

When an "S" dot is visible on the AmpliType PM DNA Probe Strip, the intensities of the dots at the remaining twelve positions are compared to the intensity of the "S" dot. Those dots that appear either darker than or equivalent to the "S" dot are considered positive. Each positive dot indicates the presence of the corresponding allele. Dots with signals less than the "S" dot should be noted and interpreted with caution.[9,11,28 14,15]

Initials: RCJ Date: 1/16/96

The dots on the AmpliType PM DNA Probe Strip correspond to the following alleles:

The "A" dot for each locus is positive in the presence of the A allele.

Note: The "A" dot for the GYPA locus is positive in the presence of both the A allele and the A' allele. Both the GYPA AB and GYPA A'B heterozygous genotypes have balanced dot intensities, but additional GYPA A and B variant alleles (observed in <8% of the African American population) may produce a slightly imbalanced heterozygous signal.

The "B" dot for each locus is positive in the presence of the B allele.

The "C" dot for the HBGG and GC loci is positive in the presence of the C allele.

For LDLR, GYPA, and D7S8, three genotypes are possible (AA, BB, and AB). For HBGG and GC, six genotypes are possible (AA, BB, CC, AB, AC, and BC).

A sample from a single individual should produce balanced dot intensities within each locus for which the individual is heterozygous.

2. Reading and Interpreting the AmpliType HLA DQα and HLA DQA1 DNA Probe Strips

The AmpliType HLA DQα DNA Probe Strips have been spotted with a total of nine sequence-specific oligonucleotide probes to detect six alleles of the HLA DQA1 locus. The AmpliType HLA DQA1 DNA Probe Strips have been spotted with a total of eleven sequence-specific oligonucleotide probes to detect eight alleles of the HLA DQA1 locus. Under the AmpliType hybridization conditions, the typing probes will bind specifically to PCR product containing the alleles designated on the AmpliType HLA DQα or HLA DQA1 DNA Probe Strips.

To read the developed AmpliType HLA DQα or HLA DQA1 DNA Probe Strips, the "C" dot is examined first and then the remaining dots are examined. The control probe "C" on the AmpliType HLA DQα and HLA DQA1 DNA Probe Strips detects all of the HLA DQA1 alleles. The "C" dot is designed to be the lightest typing dot on the strip and it indicates that adequate amplification and typing of the HLA DQA1 alleles in the sample have occurred. If the "C" dot is absent, an accurate determination of the type cannot be made. HLA DQα and HLA DQA1 DNA Probe Strips with no visible "C" dot should not be typed.

Initials: RJ Date: 1/16/96

The "C" probe serves two functions.

A. To indicate adequate amplification and typing of the HLA DQA1 alleles in the sample.

The "C" dot is usually the weakest on the strip. If the "C" dot is absent, an accurate determination of the type cannot be made since there is a possibility that other probe signals are also below the threshold of detection. The presence of a "C" dot provides assurance that the appropriate typing and sub-typing dots should be clearly visible.

B. To indicate a possible procedural error, mixed sample, or DNA contamination.

The presence of visible dots with a signal intensity less than the "C" dot may indicate any of the above or amplification of HLA DQA2 pseudogene, HLA DQα type 1.3,4, or sub-types of the HLA DQα 4 allele (See page 60).

The accurate interpretation of the HLA DQα or HLA DQA1 results depends on the presence and intensity of the "C" dot. The intensities of the dots at the remaining eight or ten positions are compared to the intensity of the "C" dot. Those dots that appear either darker than or equivalent to the "C" dot are considered positive. Each positive dot indicates the presence of the corresponding HLA DQA1 allele. Dots with signals less than the "C" dot should be noted and interpreted with caution.[9,11,28]

The dots on the AmpliType HLA DQA1 DNA Probe Strip correspond to the following alleles:

The "1" dot is positive in the presence of the HLA DQA1, 1.1, 1.2, and 1.3 alleles.

The "2" dot is positive only in the presence of the HLA DQA1 2 allele.

The "3" dot is positive only in the presence of the HLA DQA1 3 allele.

The "4" dot is positive in the presence of the HLA DQA1 4.1, 4.2, and 4.3 alleles.

Four HLA DQA1 sub-typing probes differentiate the HLA DQA1 1.1, 1.2, 1.3 alleles.

The "1.1" dot is positive only in the presence of the HLA DQA1 1.1 allele.

Note: A faint "1.1" dot will appear with some HLA DQA2 pseudogene alleles. [30]

The "1.3" dot is positive only in the presence of the HLA DQA1 1.3 allele.

Note: There is no probe that detects only on HLA DQA1 1.2 allele.

The "1.2, 1.3, 4" dot is positive in the presence of HLA DQA1 1.2, 1.3, 4.1, 4.2, and 4.3 alleles.

Note: The "1.2, 1.3, 4" dot can be lighter that the "C" dot when the genotype has a HLA DQA1 4.2 or 4.3 allele paired with a HLA DQA1 1.1, 2, 3, 4.2, or 4.3 allele because the HLA DQA1 4.2 and 4.3 alleles each have a single partially destabilizing mismatch to the "1.2, 1.3, 4" probe[18]. The partially destabilizing mismatch allows these two alleles to bind to this probe weakly relative to the HLA DQA1, 1.2, 1.3, and 4.1 alleles.

The "All but 1.3" dot is positive in the presence of all HLA DQA1 alleles EXCEPT 1.3. This probe is necessary of differentiate the 1.2, 1.3 genotype from the 1.3, 1.3 genotype.

Note: The "All but 1.3" dot can be equal to or lighter than the "C" dot when the genotype has a HLA DQA1 1.3 allele paired with a HLA DQA1 4.1, 4.2, or 4.3 allele because the HLA DQA1 4.1, 4.2, and 4.3 alleles have a single partially destabilizing mismatch to the "All but 1.3" probe.[18] The partially destabilizing mismatch allows these three alleles to bind to this probe weakly relative to the HLA DQA1 1.1, 1.2, 2, and 3 alleles. The "All but 1.3" may also sometimes be weaker than the "C" dot for genotypes with two of the 4 alleles.

For the HLA DQA1 DNA Probe Strips, two additional HLA DQA1 sub-typing probes differentiate the HLA DQA1 4.1 allele from the HLA DQA1 4.2 and 4.3 alleles.

The "4.1" dot is positive only in the presence of the HLA DQA1 4.1, allele.

The "4.2, 4.3" dot is positive in the presence of HLA DQA1 4.2 and 4.3 alleles.

There is also variation among the typing dots on a single probe strip. For instance, for the genotype 1.1, 3 the "1" dot is often darker than the "3" dot for high, medium and low signal levels. The difference is most pronounced at the high level. These differences in intensity are quite consistent from typing to typing.

Initials: RCJ Date: 5/7/96

These HLA DQA1 allele designations correspond to the World Health Organization (WHO) nomenclature as shown in Table VIII.

Table VIII. World Health Organization (WHO) Nomenclature for HLA DQA1 alleles

Allele	WHO Designation	Allele	WHO Designation
1.1	0101	3	0301
1.2	0102	4.1	0501*
1.3	0103	4.2	0401*
2	0201	4.3	0601

* Note that the WHO number designations are out of sequence compared to the original allele designations.

2A. Reporting Procedures

All alleles which meet reporting criteria are listed in the laboratory report regardless of intensity differences. Genotypes are not reported and should not be inferred. Since an individual must have two alleles at each locus and there are no known null alleles in these systems, if a single allele is found for a locus, there must be two copies of the single allele and both copies should be reported. (i.e. if only a "3" allele is found, it should be reported as 3,3). The reporting criteria are as follows:

A. If an allele has an intensity greater than or equal to "C" or "S" in two runs (or one run if only one run is required and performed) then the allele is listed in the report. (i.e. 2, 3 or 2, 3, 4.1)

B. If an allele has an intensity greater than or equal to "C" or "S" in one run and an intensity of less than "C" or "S" in at least one additional run then the allele is listed in the report in brackets. (i.e. [2]). At the bottom of the table, the brackets are defined as "[ ]= The presence of the allele above a minimum threshold could not be duplicated."

C. If an allele has an intensity of less than "C" or "S" in at least one run and does not fit into categories A and B above than the allele is reported as **. The ** is defined at the bottom of the table as " ** = additional alleles were detected which did not meet laboratory criteria for allele identification; therefore, these additional alleles are not reported."

Initials: 2G Date: 5/7/96

D. If an allele has an intensity greater than or equal to "C" or "S" in one run and its presence can not be duplicated (greater or less than the "C" or "S") when duplication is required, then the allele is reported as **.

E. If due to the nature of the case, the presence of the 1.2 allele is suspected of being masked (see the Interpretation of Complex HLA DQA1 Results section), the possible presence of the 1.2 allele is reported as (1.2). The (1.2) is defined at the bottom of the table as "Indicates that this sample may contain a 1.2 allele; however, due to the number of alleles detected in the sample it is not possible to make a definitive determination.."

F. If no alleles are detected in a sample then the sample is reported as "Neg= no alleles detected."

G. If DNA below the minimum threshold is found on Quantiblot Analysis then the sample is reported as "no DNA =Insufficient human DNA was detected; therefore, this sample was neither amplified nor hybridized."

H. If there is a large intensity difference between alleles from a locus, the intensity difference should be noted on the report with ***. The *** is defined at the bottom of the table as "***= Large intensity difference between alleles suggests a mixture of DNA."

I. Other symbols or reporting procedures will be used if necessary depending on the details of the case.

Comparison of Samples and Interpretation of Results in Report

A. Determine whether it is likely that a sample contains a mixture of DNA (i.e. more than two alleles for a locus, intensity differences between alleles within a locus, reproducible pattern of alleles less than "C" or "S", or the facts of the case suggest a potential mixture.) State in the report whether a sample contains a mixture or possible mixture of DNA, determine the minimum number of individuals who could have contributed to a mixture and the likely source of each component of the mixture

B. Compare all possible evidence and exemplar pairs and all possible evidence pairs to determine inclusions and exclusions. For inclusions look at the examination table in the report and compare all alleles reported regardless of

whether they are in parentheses or brackets. For exclusions in addition to the preceding, consider all clear dots which in each of two runs have an intensity less than the "C" or "S" dots.

C. Assuming a single physiological fluid donor, two samples could derive from a common biological source (inclusion) if all the alleles in the evidence sample could be accounted for by the alleles in the exemplar sample. If however a mixture is possible in the evidence sample, there may be alleles in the evidence sample that are not accounted for by the exemplar sample. If an inclusion requires the presence of more than one physiological fluid donor, this should be stated in the report. (i.e.. (S) 4.1, 4.1; vaginal swab sperm fract. 1.1, 4.1 (4.1 > 1.1); Assuming a single semen donor, the suspect can be eliminated as the semen donor. However if there is more than one semen donor, the suspect can not be elminated as a possible semen donor.)

D. Statistics are calculated for probative inclusions only where: (1) The sample is apparently unmixed. (2) The sample appears to be a mixture of two components and the source of one component is known. (i.e. when vaginal epithelial cells are present in the sperm fraction from a vaginal swab.) See below for the calculation of statistics.

E. Statistics are not calculated for expected inclusions such as vaginal epithelial cells from a vaginal swab consistent with the victim.

3. Extraction Reagent, Amplification Negative and Substrate Controls

The extraction reagent control and amplification negative control are a check for the possible contamination of the reagents in the PM and/or HLA DQA1 test by other human DNA or by amplified PM and/or HLA DQA1 DNA. The extraction reagent control is performed by carrying out the extraction in a tube containing no sample. The amplification negative control contains no added DNA and checks for contamination at the amplification step.

The substrate control is a check for the possible contamination of substrate by an undetected stain containing biological material (e.g. human DNA). This contamination could be pre-existing, it could be deposited on the substrate during the commission of the crime, or it could be deposited during the handling and processing of the evidence. In addition, the substrate control extract can be used to verify that the substrate contains an extractable PCR inhibitor. The substrate control is performed by carrying out the DNA extraction on unstained substrates (e.g. piece of fabric) located as close to each stain as possible.

Initials: RCJ Date: 1/16/96

These HLA DQA1 allele designations correspond to the World Health Organization (WHO) nomenclature as shown in Table VIII.

Table VIII. World Health Organization (WHO) Nomenclature for HLA DQA1 alleles

Allele	WHO Designation	Allele	WHO Designation
1.1	0101	3	0301
1.2	0102	4.1	0501*
1.3	0103	4.2	0401*
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The substrate control is a check for the possible contamination of substrate by an undetected stain containing biological material (e.g. human DNA). This contamination could be pre-existing, it could be deposited on the substrate during the commission of the crime, or it could be deposited during the handling and processing of the evidence. In addition, the substrate control extract can be used to verify that the substrate contains an extractable PCR inhibitor. The substrate control is performed by carrying out the DNA extraction on unstained substrates (e.g. piece of fabric) located as close to each stain as possible.

The extraction reagent control, amplification negative control and substrate control are amplified and typed along with the test samples. The appearance of signals in the typing of these controls indicates any or all of the following:

a. The sample preparation reagents may have been contaminated.

Initials: RCJ Date: 5/7/96

The extraction reagent control, amplification negative control and substrate control are amplified and typed along with the test samples. The appearance of signals in the typing of these controls indicates any or all of the following:

a. The sample preparation reagents may have been contaminated.

b. Cross-contamination between samples may be occurring during preparation.

c. The substrate might have an undetected stain containing biological material.

d. Human DNA or amplified PM and/or HLA DQA1 DNA may be getting into the samples from some other source.

Clearly, if the test sample does not show any probe signal in common with the controls, the test sample is not affected by the same source of contamination.

If the signals on the typing strips for the extraction reagent, amplification negative or substrate controls are very faint with the "C" or "S" dots not visible, and the test samples are easily typeable with clearly visible "C" or "S" dots, the contamination problem is not serious. If the "C" or "S" dot is visible on the typing strip of the extraction reagent, amplification negative or substrate controls, the contamination problem is more serious. **See Table IX for interpretation guidelines.**

The appearance of signals in extraction reagent, amplication negative or substrate controls does not necessarily mean that the types obtained for the test samples are incorrect because of the following:

a. The contamination might be due to a single event limited to the control.

b. The level of contamination might be inconsequential compared to the amount of DNA being amplified and typed in the test samples.

c. The contamination might be easily distinguished from the test samples because the contamination and test samples do not have any alleles in common.

However, further testing may be necessary to support these possibilities.

See Table IX for interpretation guidelines.

Initials: RS Date: 1/16/96

b. Cross-contamination between samples may be occurring during preparation.

c. The substrate might have an undetected stain containing biological material.

d. Human DNA or amplified PM and/or HLA DQA1 DNA may be getting into the samples from some other source.

Clearly, if the test sample does not show any probe signal in common with the controls, the test sample is not affected by the same source of contamination.

If the signals on the typing strips for the extraction reagent, amplification negative or substrate controls are very faint with the "C" or "S" dots not visible, and the test samples are easily typeable with clearly visible "C" or "S" dots, the contamination problem is not serious. If the "C" or "S" dot is visible on the typing strip of the extraction reagent, amplification negative or substrate controls, the contamination problem is more serious. **See Table IX for interpretation guidelines.**

The appearance of signals in extraction reagent, amplication negative or substrate controls does not necessarily mean that the types obtained for the test samples are incorrect because of the following:

a. The contamination might be due to a single event limited to the control.

b. The level of contamination might be inconsequential compared to the amount of DNA being amplified and typed in the test samples.

c. The contamination might be easily distinguished from the test samples because the contamination and test samples do not have any alleles in common.

However, further testing may be necessary to support these possibilities.

See Table IX for interpretation guidelines.

Initials: ACS Date: 1/16/96

Table IX- Guideline to the Interpretation of Visible Dots in the Extraction Reagent and Substrate Controls

Control	"C" / "S" dot visible?	Interpretation of Test Sample
Extraction Reagent/ Amplification Neg.	yes	All test samples inconclusive
	no	Test samples are conclusive if there is a duplicate run with no visible dots.
Substrate	yes	Sample is conclusive and alleles matching the control are not attributed to the stain.
	no	Sample is conclusive. The presence of additional alleles is noted in the report.

4. Amplification Positive Control

Control DNA 1 which is provided in the HLA DQα, PM, and PM+DQA1 kits is a positive control which is used with each batch of samples typed to demonstrate that the kit is performing properly. Control DNA 1 has an HLA DQA1 type of **1.1, 4.1** and a PM type of LDLR **BB**, GYPA **AB**, HBGG **AA**, D7S8 **AB**, GC **BB**.

If the positive control does not produce a correct and readable type, the amplification must be repeated and the test samples are considered inconclusive.

5. Species Specificity

A positive and interpretable HLA DQα, PM, HLA DQA1 and/or QuantiBlot result can be considered primate positive. Identification of the specific physiological fluid may be accomplished using the procedures described in the Forensic Biochemistry and Hematology Laboratory Manual.

6. Population Frequencies

To interpret the significance of a match between genetically typed samples, it is necessary to know the population distribution of alleles at the locus in question. For example, if the HLA DQA1 or PM genotype of the relevant evidence sample is different from the type of the suspect's reference sample, then the suspect is "excluded", and cannot be the donor of the biological evidence being tested. An exclusion is independent of the frequency of the two genotypes in the population.

Initials: RS Date: 1/16/96

If the suspect and evidence samples have the same genotype, then the suspect is "included", and could be the source of the evidence sample. The probability that another, unrelated, individual would also match the evidence sample is equal to the frequency of that genotype in the relevant population. Population frequencies are calculated separately for the Black, Caucasian and Hispanic populations and each population's frequency is listed in the laboratory report regardless of the population group of subject(s) in the case. Additional population frequencies may be used for other population groups. If a source contains more than one frequency for a single population group, than the highest frequency is used for calculations. Observed frequencies are used whenever possible. For those loci for which there is no observed frequency and which are demonstrated to be in Hardy-Weinberg Equilibrium, the frequency for each locus is calculated according to the Hardy Weinberg Equation of $p^2+2*p*q+q^2=1$ (i.e. for homozygotes such as AA, the frequency is p^2 and for heterozygotes such as AB, the frequency is $2*p*q$ where p is the frequency of the A allele and q is the frequency of the B allele).

For those loci which have been demonstrated to be independent, the individual locus frequencies are multiplied to obtain an overall frequency. For those loci which are not independent, an overall, observed haplotype frequency is determined by counting. The overall frequency for each racial group is reported as occurring in 1 out of x individuals (i.e. 1/overall frequency). The overall frequency is reported to two significant digits (i.e. two non-zero digits). For example, 1 out of 12,345 would be reorted as 1 out of 12,000, 1 out of 1,234 would be reported as 1 out 1,200 and 1 out of 123 would be reported as 1 out of 120. A Quattro Pro Spreadsheet is used to automate the calculation of the racial specific loci and overall frequencies. The spreadsheet is located in the popstat subdirectory on the network and explanations for its use are included with the spreadsheet

The HLA-DQA1 and PM loci are all in Hardy-Weinberg equilibirium and each individual locus is independent. Therefore the overall frequency is determined by multiplying the individual locus frequencies. The population frequencies are derived from the following sources:

Locus	**Reference**
HLA-DQA1	Helmuth et. al (1990) Am J. Hum Genet. **47**: 515-523
HLA-DQA1 4 subtypes	PM+HLA DQA1 Kit Package Insert
PM loci	Budowle et. al (1995) J. For. Sciences **40**: 45-50.

Initials: RCJ Date: 1/16/96

Interpretation of Complex HLA DQA1 Results

Occasionally typing results may appear markedly different from the standard patterns. Such results could be due to a procedural error, mixtures of DNA's (multiple contributors to the sample) the presence of the uncommon subtypes 4.2 or 4.3, or the presence of an allele of the related pseudogene, HLA DQA2.

1. Mixtures of DNA: more than one genotype present in the DNA sample.

   A. General Mixtures

   Evidence samples may contain DNA from more than one individual either because of the nature of the sample or from contamination. The possibility of multiple contributors should be considered when interpreting the HLA DQA1 typing results. For any typing system in which heterozygous genotypes are analyzed, the detection of more than two alleles indicates a mixed sample. Furthermore, there is a possibility that a phenotype read for example as HLA DQA1 3,4 is a mixture of approximately equal contributions from a homozygous 3,3 individual and a homozygous 4,4 individual or from {3,3 + 3,4} or {4,4 + 3,4} mixtures. Such mixtures would not be detected from typing results alone, as they would reveal only two alleles.

   There are 210 possible mixtures of two different HLA DQA1 genotypes. Of these, 166 are mixtures that would contain three or more alleles and 44 are mixtures of the type that may not be easily determined to be mixtures as demonstrated by the example above.

   B. Mixtures containing the 1.2 allele.

   Since there is no probe uniquely specific for the 1.2 allele, it could be overlooked in some three-allele mixtures. There are five hybridization patterns corresponding to normal HLA DQA1 genotypes in which a three allele mixture could contain an undetected 1.2 allele. These five patterns are listed in Table X. These five patterns correspond to 25 of the 166 possible mixtures of two genotypes containing three alleles in total.

Initials: RCJ Date: 1/16/96

Table X: HLA DQA1 phenotypes that can contain hidden 1.2 alleles

three allele combinations in which 1.2 can be present but not specifically detected	apparent heterozygous type
1.1, 1.2, 4	1.1, 4
1.1, 1.2, 1.3	1.1, 1.3
1.2, 1.3, 2	1.3, 2
1.2, 1.3, 3	1.3, 3
1.2, 1.3, 4	1.3, 4

C. Mixtures with different level of starting DNA

Mixtures may be present in unequal amounts. For example, the typing result of a mixture of ten parts type HLA DQA1 3,3 and one part of type 4,4 is usually recognizable as being different than a true heterozygous type 3,4. When the dot intensities from the mixture are compared to the dot intensities in a heterozygous individual, the dot or dots corresponding to 3 are darker than they should be relative to the other dots, and the dots corresponding to 4 may be fainter than the "C" dot. This type of a result is a flag for the possible presence of a mixture or contamination.

D. Apparent mixtures containing a weak "1.1" allele

The presence of a weak "1.1" allele may not be the result of a mixture or contamination but instead could be due to the amplification of the related HLA DQA2 pseudogene.

2. Subtypes of the HLA DQA1 4 allele

Occasionally, the signal intensity of the "1.2, 1.3, 4" dot will be much weaker than the "C" dot, even though a strong "4" dot is present. This phenomena is due to the relatively rare subtypes of the HLA DQA1 4 allele. The 4.2 and 4.3 allele sequences contain a single mismatch to the "1.2, 1.3, 4" probe. This mismatch, close to one end of the probe sequence, is not completely destabilizing so that a reduced signal is obtained with these alleles.

Initials: RSJ Date: 1/16/96

3. Weak Amplification of the Related HLA DQA2 allele

Occasionally a very weak signal, much weaker than the "C" dot, is seen at the "1.1" dot of a probe strip on which other signals are strong, even when there is no dot present for the "1" probe. This may be due to the relatively low-level amplification of a related gene, HLA DQA2. **HLA DQA2 can not be used for comparison of samples.**

HLA DQA2 is a non-expressed pseudogene that has some sequence similarity to HLA DQA1. Sequence information shows that one HLA DQA1 primer has two base-pair mismatches with the second exon of HLA DQA2 and the other HLA DQA1 primer has a one base-pair mismatch. Under the primer annealing conditions of this kit, the efficiency of HLA DQA2 amplification is always very low compare to HLA DQA1; however signals at the "1.1" dot which can be attributed to weak HLA DQA2 amplification are observed sporadically. The amplified segment of HLA DQA2 will hybridized to the HLA DQA1 "1.1" dot but not to the "1" dot. This hybridization pattern - "1" dot negative, "1.1" dot weak - is an indication of HLA DQA2 amplification. This signal should not confuse the typing result since 1.1 signal due solely to HLA DQA2 will always be much weaker than the "C" probe and as such should be noted but not considered as part of the genotype.

Another possible indication of HLA DQA2 amplification is the presence of a weak "1.1" and strong hybridization to the other dots in types {1.2, 1.3}, {1.2, 1.2}, {1.3, 1.3}, {1.2, 3}, {1.2, 4}, {1.3, 3}, or {1.3, 4}. The "1" dot is positive (darker than the "C" dot) in all of these types because they contain the 1 allele. However the "1.1" dot is weaker than the "C" dot. Therefore the presence of a weak "1.1" may indicate amplification of the HLA DQA2 pseudogene and does <u>not</u> necessarily suggest that a sample is composed of a mixture or is contaminated.

4. Amplified Samples Which Have Been Stored in the Refrigerator Prior to Hybridization

Amplified samples which have been stored for even a short time at 4°C often show low intensity dots which were not visible if the samples had been hybridized immediately after amplification. These dots are less intense than the "C" dot and do not affect the typing results.

Initials: RCJ Date: 1/16/96

Troubleshooting

If the Chelex extracted DNA samples fail to amplify try one or several of the following: The choice of which procedure to use is up to the discretion of the Analyst in consultation with a Scientist or Assistant Laboratory Director.

(1) Amplify using an additional 10 units of Taq polymerase.

(2) Amplify a smaller aliquot of the DNA extract to dilute potential Taq polymerase inhibitors or amplify a larger aliquot of the DNA extract because the sample is highly degraded.

(3) Re-extract the sample using a smaller area of the stain or less biological fluid to prevent saturation of the Chelex and to dilute potential Taq polymerase inhibitors.

(4) Re-extract the sample using a larger area of the stain or more biological fluid to ensure sufficient high molecular DNA is present.

(5) Re-extract the samples following the appropriate extraction procedure in the RFLP manual followed by a Chelex extraction. Amplify 0.3-10 ng of material as calculated by QuantiBlot.

(6) Add BSA to a final concentration of 160 μg/mL in the amplification reaction.

(7) Purify the extracted DNA on a Centricon-100 ultrafiltration device as follows:

a) Assemble the Centricon-100 unit according to the manufacturer's directions and label each unit.

b) Add 1.5 mL TE Buffer to the upper Centricon-100 reservoir.

c) Add the entire extracted DNA sample to the top of the TE Buffer. Cover the tube with Parafilm™. Use a sterile needle to punch a pinhole in the Parafilm being careful not to touch the solution with the needle. Centrifuge in a fixed-angle rotor (see Centricon instructions) at 1000 x g at room temperature for 20 minutes. The DNA sample will remain concentrated in about 20 to 40 μL of TE Buffer in the bottom of the upper Centricon reservoir and molecules with molecular weights of less than about 100,00 daltons will pass through the filter. Discard the effluent in the lower reservoir.

Initials: RCJ Date: 1/16/95

Note: The Centricon units are sensitive to rotor force. Do not centrifuge above 1,000 x g. Centrifugation time can be increased if the volume does not reduce to 40 μL in the specified 20 minutes.

d) Add 2 mL of TE Buffer to the concentrated DNA solution in the upper Centricon reservoir.

e) Repeat the centrifugation and wash steps in c through d twice for a total of 3 washes. After the last wash cycle collect the approximately 40 μL concentrated DNA sample (as per Centricon instructions) by inverting the upper reservoir into the provided retentate cup, and centrifuging at 500 x g for 2 minutes to transfer concentrate into cup. Label retentate cup. QuantiBlot 5 μL. The sample is now ready for the PCR amplification process. Store the sample at 2 to 8°C or freeze at -20°C until ready to perform PCR.

Initials: RSJ Date: 1/16/96

Troubleshooting

Observation	Possible Cause	Recommended Action
1. No signal or faint signal from both the Control DNA 1 and the DNA test samples at all loci.	No PCR amplification or insufficient PCR amplification of all markers.	Check amplified DNA on agarose gel (see **Observation 1.1**).
	Improper hybridization or assay condition.	Repeat test from Hybridization step (see **Observation 1.2**).
1.1 No amplified product visible on gel.	No DNA added or insufficient DNA added to PCR Reaction Mix.	Quantitate DNA and add 2-10 ng DNA; repeat test.
	Mg Cl_2 or AmpliType PM Primer Set not added to AmpliType PCR Reaction Mix.	Add Mg Cl_2 or AmpliType PM Primer Set; repeat test.
	GeneAmp PCR Instrument System failure or wrong program.	See GeneAmp PCR Instrument System Manual and check instrument calibration.
	Tubes not seated tightly in the DNA Thermal Cycler 480 block during amplification.	Push tubes firmly into contact with block after first cycle; repeat test.
	MicroAmp Base used with tray and tubes in GeneAmp PCR System 9600.	Removed MicroAmp Base; repeat test.
1.2 Amplified product visible on gel but no signal or faint signal on AmpliType DNA Probe Strips.	Hybridization and/or Stringent Wash temperature too high.	Check that the rotating water bath temperature is at 55°C (±1°C) with an immersible thermometer; repeat test.

Initials: RY Date: 1/16/96

Observation	Possible Cause	Recommended Action
	DQα Hybridization and/or DQα Wash Solution salt concentration too low.	Prepare new solutions; repeat test.
	Stringent Wash time too long.	Repeat test, washing for 12 minutes (±1 minute) only.
	Inadequate agitation of the DNA probe strips during hybridization.	Check speed of rotating water bath (50 to 70 rpm); verify that hybridization solution is washing over the strips; repeat test.
	Amplified DNA was not added to DNA probe strips.	Repeat test, adding amplified DNA to DNA probe strips.
	Amplified DNA was not denatured.	Check GeneAmp+ PCR Instrument System block temperature is 95°C; leave sample in block >3 minutes. Repeat the test.
	Enzyme Conjugate: HRP-SA was not added to the appropriate solution.	Prepared new diluted Enzyme Conjugate: HRP-SA solution; repeat test.
	Hydrogen peroxide was not added or too much was added to the Color Development Solution.	Make new Color Development Solution with correct amount of hydrogen peroxide; repeat test.
	Hydrogen peroxide inactive.	Make new Color Development Solution using new bottle or dilution of hydrogen peroxide; repeat test.
	Chromogen: TMB was not added to the Color Development Solution.	Make new Color Development Solution adding Chromogen: TMB; repeat test.

Initials: RH Date: 1/16/96

Observation	Possible Cause	Recommended Action
	For HLA DQA1 kits, the original HLA DQα instead of PM or PM + DQA1 typing protocol was followed.	Repeat test following the PM + DQA1 typing protocol.
2. Positive signal from Control DNA 1, but no signal from DNA test sample.	Quantity of DNA test sample is below the assay sensitivity.	Quantitate DNA and add 2-10 ng DNA; repeat test.
	Test sample contains PCR inhibitor (e.g. heme compounds, certain dyes).	Any or all of the following actions may be taken: 1. Quantitate DNA and add minimum necessary volume; repeat test. 2. Wash the sample in Centricon+ 100 (see **Reference 11**); repeat test. 3. Add BSA to reaction and see **Reference 15 and 31**; repeat test.
	Test sample DNA is degraded	If possible evaluate the quality of the DNA sample by running an agarose gel (see **Reference 18**). If the DNA is degraded, reamplify with an increased amount of DNA.
3. High DNA probe strip background color.	Low or lack of SDS in DQα Hybridization and/or DQα Wash Solution.	Prepare new DQα Hybridization and/or DQα Wash Solution with correct amount of SDS; repeat test
	Inadequate agitation of the DNA probe strips during hybridization and steps.	Check speed of rotating water bath (50 to 70 rpm); verify that solutions are washing over strips; repeat test

Initials: RJ Date: 1/16/96

Observation	Possible Cause	Recommended Action
	Tray lid not wiped adequately.	Wipe lid; repeat test.
	Excess amounts of Enzyme Conjugate; HRP-SA added Solution.	Prepared new solution with correct amount of Enzyme Conjugate: HRP-SA; repeat test.
	Expose to light during Color Development.	Cover tray lid with foil during Color Development steps; repeat test.
	Use of water other than deionized or glass distilled water for water rinses.	Repeat test using deionized or glass distilled water for water rinses.
4. High DNA probe strip background color upon storage.	Exposure to strong light and oxidizing agents.	Store strips in the dark away from oxidizing agents.
	Insufficient water washes after Color Development.	Increase number of water washes in future assays.
5. Presence of unexpected or additional dots in the amplified Control DNA 1 sample.	Cross-hybridization caused by Hybridization and/or Stringent Wash temperature being too low.	Check that the rotating water bath temperature is at 55°C (±1°C) with a total immersion thermometer; repeat test.
	Cross-hybridization caused by DQα Hybridization and/or DQα Wash Solution salt concentration being too high.	Prepare new solutions; repeat test.
	Cross-hybridization caused by Stringent Wash time being too short.	Repeat test, washing for 12 minutes (±1 minute).

Initials: RCJ Date: 1/16/96

Observation	Possible Cause	Recommended Action
	Contamination by amplified product or samples.	See **Reference 18** and **28**.
6. Signals weaker than "S" or "C" dot on the same strip (also see Observations 12 and 13).	Hybridization and/or Stringent Wash temperature too high or too low.	Check that the rotating water bath temperature is at 55°C (±1°C) with an immersible thermometer; repeat test.
	DQα Hybridization and/or DQα Wash Solution salt concentration too high or too low.	Prepare new solutions; repeat test.
	Stringent wash time too long or too short.	Repeat test, washing for 12 minutes (±1 minute).
	Mixed sample or contamination.	See **Reference 14, 15, 17, 18,** and **28.**
	Amplification of HLA DQA2 pseudogene (faint "1.1" dot)	See **Reference 18** and **30**.
	EDTA not added to the reaction prior to the heat denaturation step of the DNA hybridization protocol.	Add EDTA to amplified sample (**Section 4.0**); repeat test.
7. More than two alleles present on the AmpliType HLA DQα or HLA DQA1 DNA Probe Strips or at the HBGG and/or GC marker on the AmpliType PM DNA Probe Strip.	Cross-hybridization caused by Hybridization and/or Stringent Wash temperature too low.	Check that the rotating water bath temperature is at 55°C (±1°C) with a total immersion thermometer; repeat test.
	Cross-hybridization caused by DQα Hybridization and/or DQα Wash Solution salt concentration too high.	Prepare new solutions; repeat test.

Initials: RO Date: 1/16/96

Observation	Possible Cause	Recommended Action
	Stringent Wash time too short.	Repeat test washing for 12 minutes (±1 minute).
	Mixed sample or contamination.	See **Reference 14, 15, 17, 18,** and **28**.
	Amplification of HLA DQA2 pseudogene (faint 1.1 dot).	See **Reference 18** and **30**.
8. Some, but not all, loci visible on gel of AmpliType PM PCR products.	Test sample DNA is degraded.	If possible, evaluate the quality of the DNA sample by running an agarose gel (see **Reference 18**). If the DNA is degraded, reamplify with an increased amount of DNA.
	Test sample contains PCR inhibitor (e.g. heme compounds, certain dyes).	Any or all of the following actions may be taken: 1. Quantitate DNA and add minimum necessary volume; repeat test. 2. Wash the sample in Centricon+ 100 (see **Reference 11**); repeat test. 3. Add BSA to reaction and see **Reference 15 and 31**; repeat test.
	Input DNA and/or PCR product was not denatured sufficiently during amplification.	Check calibration of the GeneAmp+ PCR Instrument System using the appropriate Temperature Verification System.
9. Some, but not all, loci visible on AmpliType PM DNA Probe Strip.	Not all loci amplified.	Verify the presence of amplified loci on an agarose gel (see **Observation 8**).

Initials: RSJ Date: 1/16/96

Observation	Possible Cause	Recommended Action
	Amplified DNA was not denatured.	Check GeneAmp PCR Instrument System block temperature is 95°C; leave sample in block >3 minutes. Repeat the test
10. Imbalanced dot intensities within a locus on the AmpliType PM DNA Probe Strip (this kit is designed to produce balance dot intensities when heterozygous samples are typed, except as described in interpretation section)	DQα Hybridization and/or DQα Stringent Wash temperature too high or too low.	Check that the rotating water bath temperature is at 55°C (±1°C) with a total immersion thermometer; repeat test.
	DQα Hybridization and/or DQα Wash Solution salt concentration too high or too low.	Prepare new solutions; repeat test.
	Stringent Wash time too long or too short.	Repeat test, washing for 12 minutes (±1 minute).
	Mixed sample or contamination.	See **Reference 14, 15, 17, 18,** and **28.**
	EDTA not added to the reaction prior to the heat denaturation step of the DNA hybridization protocol.	Add EDTA to amplified sample; repeat test.
11. Weak or absent "4.1" dot on the AmpliType HLA DQα or HLA DQA1 DNA Probe Strips in the amplified Control DNA 1 sample.	EDTA not added to the reaction prior to the heat denaturation step of the DNA hybridization protocol.	Add EDTA to amplified sample; repeat test.
12. "1.2, 1.3,4" dot weaker than "C" dot on AmpliType HLA DQα or HLA DQA1 DNA Probe Strips.	Genotype of sample has a HLA DQA1 4.2 or 4.3 allele paired with a HLA DQA1 1.1, 2, 3, 4.2, or 4.3 allele.	See **Reference 18**.

Initials: RG Date: 1/16/96

Observation	Possible Cause	Recommended Action
13. "1.1" dot weaker than "C" dot but on AmpliType HLA DQα or HLA DQA1 DNA Probe Strip.	Amplification of HLA DQA2 pseudogene (faint 1.1 dot).	See **Reference 18** and **30**.
14. "All but 1.3" signal weaker than "C" dot on AmpliType HLA DQA1 DNA Probe Strip.	Genotype of sample has a HLA DQA1 1.3 allele paired with a HLA DQA1 4.1, 4.2, or 4.3 allele.	See **Reference 18**.

Initials: RS Date: 1/16/96

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Initials: RCJ Date: 1/16/96

Appendix A

Storage and Stability of the Amplitype Kits

The AmpliType™ Forensic DNA Amplification and Typing Kit s should be stored at 2-8°C.

The Kit should be isolated from any sources of contaminating DNA, especially amplified PCR product.

The DNA Probe Strips should be stored with the desiccant in the glass tube with the screw-cap securely tightened, protected from light, at 2-30°C.

Under these conditions, components of the kit are stable until the printed expiration date.

Initials: RCJ Date: 1/16/95

Washing and Re-Use of the Hybridization Trays

1. To each well of the DNA typing tray, add approximately 10 mL of 95-100% ethanol.

2. Cover the DNA typing tray with the lid and carefully agitate for 15 to 30 seconds to dissolve any residual Chromogen.

3. Remove the lid and pour off the ethanol from each well. Visually inspect each well for the presence of Chromogen (faint blue color). If necessary repeat steps a and b to remove any residual Chromogen.

4. Rinse each tray well and the tray lid with dH_2O.

5. Allow trays and lids to air dry. The trays are now ready for re-use.

Initials: RS Date: 1/16/95

Appendix B- Equipment and Supplies for PCR Typing

General Equipment and Supplies

Autoclave
Balance
Deionizer column for water
Calibrated Thermocouples and Meter
Labware, glassware including beakers, graduated cylinders, Erlenmeyer flasks
Freezer, -80°C and -20°C
Refrigerator
Lab Coat
Microwave oven
pH meter with calomel (Tris) electrode
Reference buffers
Polaroid Camera with type 667 film
Stir plate, Stir bar
Thermometer (N.B.S or equivalent)
Timer, 60 minute (±1 minute)
Parafilm
U.V. Transilluminator
Absorbent bench paper
Absorbent tissues (e.g., Kimwipes)
Evidence bags or envelops (for storage)
Filter paper, Whatman 3M, Whatman #1
Lab marker, waterproof ink
Microscope slides and cover slips
Paper, white
Paper towels
Parafilm
Plastic disposable pasteur pipettes
Pipettes, plastic, sterile disposable to deliver 1 to 10 mL
Pipette bulbs or electric pipettors
Wash bottles

Initials: RCJ Date: 1/16/96

DNA Extraction Work Area Dedicated Equipment and Supplies

This work area should be used only for evidence handling and for extraction and isolation of DNA. Microscopy, photography, and any other activities that involve the handling of evidence samples before amplification should also be performed in this work area. These items should never leave the DNA extraction work area.

1. Pipettors: Adjustable 2 to 20 μL, 20 to 200 μL and 200 to 100 μL. If possible, reserve one set of pipettor for reagent use only, and another set for the handling of samples containing DNA.
2. Microcentrifuge tube racks
3. Microcentrifuge tubes (1.5 mL)
4. Microcentrifuge
5. Refrigerated Centrifuge and Rotors
6. Scissors
7. Disposable razor blades
8. Pipette tips and Filter pipette tips
9. Pipette bulb
10. Refrigerator
11. Hot/Stir plate
12. Laboratory glassware
13. Disposable gloves
14. Forceps
15. Vortex mixer
16. Freezer to -20°C
17. Heat blocks and Heater
18. Laminar flow/biological/biosafety cabinet with U.V. source
19. Centricon-100 microconcentrators
20. Toothpicks
21. Boiling Water bath racks
22. 1 L Pyrex glass beakers
23. Ice
24. Ice bucket

Initials: RC Date: 1/16/96

PCR Set Up Work Area Dedicated Equipment and Supplies

This work area is used only for adding $MgC1_2$ Solution, mineral oil, and sample DNA to the PCR Reaction Mix. These items should never leave the PCR Setup Work Area

1. Combitip™ repeat pipettor with combitips
2. Dedicated pipettor (adjustable 2 to 20 μL and 20 to 1000 μL for adding DNA samples to the PCR Reaction Mix.
3. Sterile filter pipette tips
4. Microtube de-capping devices, autoclavable
5. Microtube rack
6. Disposable gloves
7. Microcentrifuge
8. Heatblock and heater
9. Refrigerator
10. Vortex Mixer
11. Kimwipes

Initials: RCJ Date: 1/16/95

Amplified DNA Work Area Dedicated Equipment and Supplies

This work area(s) is used only for those activities that involve the handling of amplified DNA. This includes DNA typing (Hybridization and Color Development), DNA yield determination, gel electrophoresis of amplified DNA, waste disposal of amplified DNA solutions, and storage of amplified DNA. Amplified DNA or equipment and supplies used to handle amplified DNA should not be taken out of Amplified DNA Work Area.

Samples that have not yet been amplified should never come in contact with this equipment.

1. PE Cetus DNA Thermal Cycler
2. 1 to 20 μL and 20 to 200 μL adjustable pipettors
3. Disposable gloves
4. Microtube de-capping device
5. Disposable serological pipettes
6. Pipettor for serological pipettes
7. Towel wipes
8. Kimwipes
9. Microtube racks
10. Filter Pipette tips
11. Laboratory glassware
12. Gel electrophoresis apparatus
13. Power supply for electrophoresis
14. Disposable gloves
15. Sink
16. Refrigerator
17. Aluminum foil
18. Seal-a-meal bags
19. Waterproof marking pen
20. Water bath, stationary
21. Weight, (approx. 1kg)
22. Shaker, variable speed, orbital platform
23. Shaker, water bath
24. Filter forceps
25. Microcentrifuge
26. Heat block and heater
27. Vortex Mixer
28. Microcentrifuge tubes, 0.5 mL & 1.5 mL
29. 2 to 10 mL multi-dispensers
30. Hybridization Tray
31. Heat block and heater

Initials: RG Date: 1/16/96

QuantiBlot Equipment and Supplies

1. Bellco Hot Shaker Plus
2. Lead weights (approx. 1 kg)
3. Pipettors (P20, P200 and P1000)
4. Shaker, variable speed, orbital platform
5. Timer
6. Vortex
7. Water bath, stationary
8. Slot blot apparatus
9. Vacuum pump
10. Plastic Tubing
11. Vacuum trap
12. Biodyne B nylon membrane 0.45μM
13. Microcentrifuge tubes, 0.5 mL & 1.5 mL
14. Hybridization tray