Forensic Molecular Biology

Solutions Manual

Version 4.0

Initials: RCJ Date: 2/9/98

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S113 Acrylamide Solution, 40% lot number:_____

standard batch sizes: 250 mL, 375 mL

Ingredients	amount	amount
RM2085 polyacrylamide bisacrylamide (19:1) (premixed)	100 ± 1 g	150 ± 1.5 g
RM0409 mixed bed ion exchange resin	20 ± 1 g	30 ± 1.5 g
Initial amount deionized water	150 ± 5 mL	225 ± 5 mL
Final Volume	250 ± 5 mL	375 ± 5 mL

Procedure

Add initial amount deionized water to the original polyacrylamide bisacrylamide bottle.

Stir at low heat (position 2 on hot plate) until dissolved.

Adjust to the final volume with deionized water.

Pour back into bottle.

Add mixed bed ion exchange resin and stir for 30 minutes.

Remove the stir bar.

Store at 2-8°C for one month. Label with expiration date.

Data Log	source	lot	amount
RM2085 Polyacrylamide Bisacrylamide	_____	_____	_____
RM0409 mixed bed ion exchange resin	_____	_____	_____

made by:_____ date:_____

Initials: RG Date: 10/7/96

S115 Ammonium Persulfate (0.5g Aliquot) lot number: _____

standard batch size: ~ 25 tubes x 0.5g

Ingredients	aliquot	total amount
RM 0016 ammonium persulfate electrophoresis grade	0.5 ± 0.05 g	12.5 ± 1 g

Procedure

NOTE: WHEN WORKING WITH POWDERED AMMONIUM PERSULFATE WEAR GLOVES, EYE PROTECTION, LAB COAT, AND RESPIRATOR FOR SAFETY.

Fill out chemical logbook.

Using weigh paper, weigh 0.5± 0.05 g aliquots of ammonium persulfate.

Transfer the aliquots to 15mL conical tubes.

Cap all tubes tightly and label rack containing tubes with contents, lot number, date, initials, and safety data.

Store at room temperature.

Data Log	source	lot	amount
RM0016 ammonium persulfate	_____	_____	_____

made by:_____ date:_____

Initials: RCI Date: 10/2/96

S018 Analytical Gel Loading Buffer lot number: _____

standard batch size: 100 mL

Ingredients	final concentration	amount
RM020 bromophenol blue	0.25%	0.25 ± 0.01 g
RM217 xylene cyanol	0.25%	0.25 ± 0.01 g
RM040 Ficoll 400	12.5%	12.5 ± 0.1 g
S009 EDTA, 0.5M	50. mM	10.0 ± 0.1 mL
RM083 TAE, 10X	5.0 X	50.0 ± 0.5 mL

Procedure

Combine the TAE, EDTA, and Ficoll.
Mix well. The solution may need to be heated gently to dissolve the Ficoll.
Add the bromophenol blue and xylene cyanol.
Mix well.
When all the solids are dissolved, bring up to volume using deionized water.
Filter sterilize.
Dispense 1.5 mL aliquots into 1.5 mL eppendorf tubes.
Store at -20°C.

Data Log	source	lot	amount
RM020 bromophenol blue	_____	_____	_____
RM217 xylene cyanol	_____	_____	_____
RM040 Ficoll 400	_____	_____	_____
S009 EDTA, 0.5M	_____	_____	_____
RM083 TAE, 10X	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 2/9/98

S131 Blue Formamide + Loading Buffer (5:1) lot number: _____

standard batch size: 20 x 1200 µL

Ingredients	amount	final ratio
RM2099 Formamide	1000 ± 20 µL	5
S132 Sequencing Loading Buffer	200 ± 10 µL	1

Procedure

NOTE: PREPARE AWAY FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

Label 20 1.5mL reaction tubes.

Add Formamide to each tube. Add blue sequencing buffer to each tube.

Close all tubes and mix.

Store at 2-8°C.

Data Log

	source	lot	amount
RM2099 Formamide	_____	_____	_____
S132 Sequencing Loading Buffer	_____	_____	_____

Quality Control

QC032 STR gel electrophoresis

made by:_____date:_____

Initials: RS Date: 10/7/21

S117 BSA Solution, 5 mg/mL lot number:_____

standard batch size: 25 mL

Ingredients	amount
RM222 Bovine serum albumin	125 mg
S059 Sterile water	25 mL (guideline)

Procedure

Autoclave a 50 mL glass beaker with a stir bar in it.

Add the BSA to 20 mL of sterile water in the glass beaker.

Stir gently over **very low** heat until the BSA is completely dissolved.

Add the solution to a 50 mL disposable conical tube.

Add sterile water to a final volume of 25 mL.

Aliquot approximately 0.5 mL of BSA solution into 1.5 mL microcentrifuge tubes.

Label each tube with "BSA" and the lot number. Label the rack with expiration date.

Store at -20°C.

Data Log	source	lot	amount
RM222 BSA	_____	_____	_____
S059 Sterile water	_____	_____	_____

Quality Control

QC023 QuantiBlot Quality Control of Solutions- test 20 µL of solution

made by:_____date:_____

Initials: RCJ Date: 10/7/81

S060 Calibration Control lot number: _____

Ingredients	initial concentration (ng/µL)	initial volume (µL)	final concentration	final volume (µL)
RM221 K562 DNA			7.5 ng/µL	
S021 yield gel loading buffer	5 X		1 X	----
S059 sterile water	-----		----	----

Calculations

Record the initial concentration in ng/µL and the initial volume in µL of the K562 DNA received from the manufacturer.

Calculate the final volume according to equation 1.

$$\text{(final volume)} = \frac{\text{(initial DNA concentration)(initial DNA volume)}}{\text{(7.5 ng/µL)}} \qquad \textbf{equation 1}$$

Record the final volume above. The final volume is the total batch size.

Calculate the amount of buffer to be added according to equation 2.

$$\text{(buffer volume)} = 0.2(\text{final volume}) \qquad \textbf{equation 2}$$

Calculate the amount of sterile water to be added according to equation 3.

$$\text{(water volume)} = [0.8 * (\text{final volume})] - (\text{initial DNA volume}) \qquad \textbf{equation 3}$$

Record the buffer and water volumes above.

To check the calculations, add together the initial volumes of DNA, loading buffer, and sterile water.

The sum of the initial volumes must be equal to the calculated final volume.

Initials: RCJ Date: 10/7/96

S060 Calibration Control lot number: _____

Procedure

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 200 µL aliquots into sterile 1.5 mL eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM221 K562 DNA	_____	_____	_____
S021 yield gel loading buffer	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC026 Gel Electrophoresis

made by: _____ date: _____

Initials: CRC Date: 10/7/96

S010 Cell Lysis Buffer (CLB) lot number: _____

standard batch size: 2 L

Ingredients	final concentration	amount
RM068 sucrose	320 mM	219 ± 3 g
RM073 TRIS	10 mM	2.4 ± 0.1 g
RM046 magnesium chloride, hexahydrate	5 mM	2.0 ± 0.1 g
RM075 Triton X-100	1.0 %	20 ± 1 mL
RM096 hydrochloric acid	---	

Procedure

Dissolve the sucrose, TRIS, and magnesium chloride in approximately 1.5 L deionized water.
Add the Triton to the solution.
Adjust the pH to 7.6 with hydrochloric acid
Mix well.
Adjust the volume to 2 L with deionized water.
Filter sterilize.
Dispense into sterile 50 mL centrifuge tubes.
Store at 2-8°C.

Data Log	source	lot	amount
RM068 sucrose	_____	_____	_____
RM073 TRIS	_____	_____	_____
RM046 magnesium chloride, hexahydrate	_____	_____	_____
RM075 Triton X-100	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

Quality Control

QC023 QuantiBlot Quality Control of Solutions- test 150 µL of solution

final pH: _____ spec: 7.6 ± 0.1

made by: _____ date: _____

Initials: RY Date: 10/7/96

S064 Cell Pellet Control lot number: _____

Ingredients	concentration of cells	total volume (mL)	cells per aliquot	aliquot volume (mL)
RM243 K562 cells			1×10^6	
S034 phosphate buffered saline (PBS)	----	----	----	----

Calculations

Record the concentration of K562 cells in the suspension received from the manufacturer.

Record the total volume. This is the batch size.

Calculate the volume (in mL) which yields 1×10^6 cells according to equation 1.

$$\text{(aliquot volume)} = \frac{(1 \times 10^6 \text{cells})}{\text{(concentration of cells)}} \qquad \textbf{equation 1}$$

The aliquot volume must fit into a 1.5 mL eppendorf tube. The concentration of the cell suspension may have to be adjusted.

If the cell concentration is too low, the cells may be spun at 180 x g for 5 minutes at 4°C. Remove the excess media to give the desired concentration.

If the cell concentration is too high, PBS may be added to reach the desired concentration. After adding PBS, make sure the cells are well suspended before aliquoting.

Record the calculated aliquot volume.

Procedure

The following steps must be done on ice or at 4°C.

Bring the cell suspension up to the desired final volume.

Suspend the cells evenly by pipetting up and down or by gently inverting the container.

Initials: RG Date: 10/7/96

S064 Cell Pellet Control lot number: _____

Add aliquots of cell suspension to 1.5 mL eppendorf tubes.

Spin the tubes at 180 x g for 1 minute at 4°C, and remove the excess supernatant.

The tubes can be aliquoted and spun in sets of 52. Each set should be packaged separately in a seal-a-meal bag, labeled with the lot number and numbered sequentially.

Store the bags at -70°C.

Data Log	source	lot	amount
RM243 K562 cells	_____	_____	_____
S034 phosphate buffered saline	_____	_____	_____

Quality Control

QC024 Non-Organic Extraction

QC027 Southern Blotting and Hybridization

made by: _____ date: _____

Initials: RG Date: 10/7/96

S022 Chelex, 5% lot number: _____

standard batch size: 500 mL

Ingredients	final concentration	amount
RM027 Chelex 100	5. %	25 ± 2 g
S059 sterile water	---	450 ± 50 mL (guideline)

Procedure

Filter sterilize approximately 600 mL deionized water.

Pour the water into a 500 mL bottle.

Save the bottom container from the disposable filter unit.

Autoclave the water at 250°F for 30 minutes.

Add the Chelex to the bottom container of the filter unit.

Allow the water to cool after autoclaving.

Add sterile water to the Chelex to a volume of 500 mL using the graduation markings on the disposable filter container.

Mix on a magnetic stir plate.

While the stock solution is mixing, aliquot 10 mL each into 15 mL centrifuge tubes.

Store at 2-8°C.

Data Log	source	lot	amount
RM027 Chelex 100	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC014 Chelex Extraction

made by: _____ date: _____

Initials: RG Date: 10/7/81

S022 Chelex, 5% lot number: _____

standard batch size: 400 mL

Ingredients	final concentration	amount
RM536 DNA Extraction Reagent	5 %	100 mL
S059 sterile water	---	300 mL

Procedure

Filter sterilize approximately 300 mL deionized water.

Pour the water into a 500 mL bottle.

Save the bottom container from the disposable filter unit.

Autoclave the water at 250°F for 30 minutes.

Add 100 mL of DNA Extraction Reagent to the bottom container of the filter unit.

Allow the water to cool after autoclaving.

Add sterile water to the the DNA Extraction Reagent to a volume of 400 mL using the graduation markings on the disposable filter container.

Mix on a magnetic stir plate.

While the stock solution is mixing, aliquot 10 mL each into 15 mL centrifuge tubes.

Store at 2-8°C.

Data Log	source	lot	amount
RM536 DNA Extraction Reagent	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC014 Chelex Extraction

made by: _____ date: _____

Initials: RY Date: 10/7/96

S082 Chelex, 20% lot number: _____

standard batch size: 500 mL

Ingredients	final concentration	amount
RM027 Chelex 100	20. %	100 ± 2 g
S059 sterile water	---	450 ± 50 mL (guideline)

Procedure

Filter sterilize approximately 600 mL deionized water.

Pour the water into a 500 mL bottle.

Save the bottom container from the disposable filter unit.

Autoclave the water at 250°F for 30 minutes.

Add the Chelex to the bottom container of the filter unit.

Allow the water to cool after autoclaving.

Add sterile water to the Chelex to a volume of 500 mL using the graduation markings on the disposable filter container.

Mix on a magnetic stir plate.

While the stock solution is mixing, aliquot 10 mL each into 15 mL centrifuge tubes.

Store at 2-8°C.

Data Log	source	lot	amount
RM027 Chelex 100	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC017 Differential Extraction

made by: _____ date: _____

Initials: RU Date: 10/7/96

S027 Chloroform-Isoamyl Alcohol lot number: _____

standard batch size: 500 mL

Ingredients	final concentration	amount
RM088 chloroform	96. %	480 ± 3 mL
RM089 isoamyl alcohol	4. %	20 ± 3 mL

Procedure

NOTE: Use only glass graduated cylinders and containers.

Measure the isoamyl alcohol into a 500 mL brown bottle.

Add the chloroform.

Store at 2-8°C in a flammable materials refrigerator.

Data Log	source	lot	amount
RM088 chloroform	_____	_____	_____
RM089 isoamyl alcohol	_____	_____	_____

made by: _____ date: _____

Initials: RG Date: 10/7/16

S104 Chromogen Solution lot number: _____

standard batch size: 30 mL

Ingredients	final concentration	amount
RM435 chromogen:TMB	_____	60 mg
RM009 ethanol, 100% reagent grade	_____	30 mL

Procedure

Bring bottle of chromogen:TMB to room temperature.

Before opening, lightly tap the bottle on the counter to bring its contents to the bottom.

Carefully remove the stopper and reconstitute the chromogen:TMB with the room temperature ethanol.

CAUTION: DO NOT USE ETHANOL STORED IN A METAL CONTAINER; ONLY USE 100% REAGENT GRADE ETHANOL.

Recap the bottle and seal with Parafilm.

Tilt the bottle several times to ensure that all the powder is removed from within the rubber cap.

Shake on an orbital shaker for about 30 minutes.

Store at 2-8°C and away from rust.

The solution is stable for six months.

Data Log	source	lot	amount
RM435 chromogen	_____	_____	_____
RM009 ethanol, 100%	_____	_____	_____

made by: _____ date: _____

Initials: RCY Date: 10/17/96

S013 Denaturation Solution lot number: _____

standard batch size: 20 L

Ingredients	final concentration	amount
RM005 sodium chloride	1.5 M	1750 ± 50 g
RM004 sodium hydroxide, 10N	0.5 M	1000 ± 100 mL

Procedure

Measure the sodium chloride into a 20 L carboy with approximately 5-10 L deionized water.

Add the sodium hydroxide solution.

Mix well on magnetic stir plate using a stir bar.

Raise to the final volume with deionized water.

Mix well.

Measure and record the pH using pH paper.

Store at room temperature.

Data Log	source	lot	amount
RM005 sodium chloride	_____	_____	_____
RM004 sodium hydroxide, 10N	_____	_____	_____

Quality Control

final pH: _____ spec: ≥ 12

made by: _____ date: _____

Initials: RCJ Date: 10/2/96

S094 Digest Buffer lot number: _____

standard batch size: 2 L

Ingredients	final concentration	amount
S009 EDTA, 0.5M	10. mM	40 ± 2 mL
RM073 TRIS	10. mM	2.4 ± 0.2 g
RM0005 sodium chloride	50. mM	5.8 ± 0.4 g
S001 SDS, 20%	2.0 %	200 ± 2 mL
RM096 hydrochloric acid	---	---

Procedure

Add the EDTA, TRIS, sodium chloride, and SDS to approximately 1.5 L deionized water.
Adjust the pH to 7.5 with hydrochloric acid.
Bring up to the final volume with deionized water.
Mix well.
Measure and record the final pH.
Aliquot into 50 mL centrifuge tubes.
Store at room temperature.

Data Log	source	lot	amount
S009 EDTA, 0.5M	_____	_____	_____
RM073 TRIS	_____	_____	_____
RM0005 sodium chloride	_____	_____	_____
S001 SDS, 20%	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

Quality Control

final pH: _____ specification: 7.5 ± 0.1

QC023 QuantiBlot Quality Control of Solutions- Test 150 µL of solution

made by: _____ date: _____

Initials: RE Date: 10/4/96

S061 Digestion Control lot number: _____

INGREDIENTS	initial concentration (ng/µL)	initial volume (µL)	final concentration	final volume (µL)
RM221 K562 DNA			2 ng/µL	
S128 TE^{-4}, 1X	1 X		----	----

Calculations

Record the initial concentration in ng/µL and the initial volume in µL of the K562 DNA received from the manufacturer.

Calculate the final volume according to equation 1.

$$\text{(final volume)} = \frac{\text{(initial DNA concentration)(initial DNA volume)}}{\text{(2 ng/µL)}} \qquad \textbf{equation 1}$$

Record the final volume above. The final volume is the total batch size.

Calculate the amount of 1X TE^{-4} to be added according to equation 2.

$$(TE^{-4}\text{ volume}) = \text{(final volume)} - \text{(initial DNA volume)} \qquad \textbf{equation 2}$$

Record the TE^{-4} volume above.

To check the calculations, add together the initial volumes of DNA and 1X TE^{-4}.

The sum of the initial volumes must be equal to the calculated final volume.

Initials: Date:

S061 Digestion Control lot number: _____

Procedure

Combine the DNA and 1X TE^{-4}. **Make sure the DNA is in solution. If the DNA is frozen, resuspend for at least 2 hr RT or 4°C overnight**

Mix well.

Using sterile pipet tips, dispense 250 µL aliquots into 1.8 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
RM221 K562 DNA	_____	_____	_____
S128 TE^{-4}, 1X	_____	_____	_____

Quality Control

QC026 Gel Electrophoresis

made by: _____ date: _____

Initials: RH Date: 10/7/96

S061 Digestion Control lot number: _____

INGREDIENTS	initial concentration (ng/µL)	initial volume (µL)	final concentration	final volume (µL)
RM221 K562 DNA			2 ng/µL	
S039 TE, 1X	1 X		----	----

Calculations

Record the initial concentration in ng/µL and the initial volume in µL of the K562 DNA received from the manufacturer.

Calculate the final volume according to equation 1.

$$\text{(final volume)} = \frac{\text{(initial DNA concentration)(initial DNA volume)}}{\text{(2 ng/µL)}} \qquad \textbf{equation 1}$$

Record the final volume above. The final volume is the total batch size.

Calculate the amount of 1X TE to be added according to equation 2.

(TE volume) = (final volume) - (initial DNA volume) **equation 2**

Record the TE volume above.

To check the calculations, add together the initial volumes of DNA and 1X TE.

The sum of the initial volumes must be equal to the calculated final volume.

Initials: RY Date: 10/7/96

S061 Digestion Control lot number: _____

Procedure

Combine the DNA and 1X TE. **Make sure the DNA is in solution. If the DNA is frozen, resuspend for at least 2 hr RT or 4°C overnight**

Mix well.

Using sterile pipet tips, dispense 250 µL aliquots into 1.8 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
RM221 K562 DNA	_____	_____	_____
S039 TE, 1X	_____	_____	_____

Quality Control

QC026 Gel Electrophoresis

made by: _____ date: _____

Initials: RH Date: 10/7/96

S003 DQα Citrate Buffer lot number: _____

standard batch size: 4 L

Ingredients	final concentration	amount
RM001 trisodium citrate	-----	73.6 ± 0.1 g
RM002 citric acid	-----	24 ± 1 g (guideline)

Procedure

Dissolve the sodium citrate in approximately 3 liters deionized water.

Adjust the pH to 5.0 by addition of citric acid (approximately 24 g).

Adjust the final volume to 4 liters with deionized water.

Mix well.

Measure and record the final pH.

Dispense into a 4 L bottle.

Store at room temperature.

Data Log	source	lot	amount
RM001 trisodium citrate	_____	_____	_____
RM002 citric acid	_____	_____	_____

Quality Control

final pH: _____ specification 5.0 ± 0.2

made by: _____ date: _____

Initials: RV Date: 10/7/96

S004 DQα Hybridization Solution lot number: _____

standard batch size: 4 L

Ingredients	final concentration	amount
S002 SSPE, 20X	5.0 X	1000 ± 10 mL
S001 SDS, 20%	0.50 %	100 ± 1 mL

Procedure

Combine the SSPE and 2.9 L deionized water in a 4 L flask.

Add the SDS.

Warm the solution until all solids are dissolved.

Mix well.

Dispense into 1 L bottles.

Store at room temperature.

Data Log	source	lot	amount
S002 SSPE, 20X	_____	_____	_____
S001 SDS, 20%	_____	_____	_____

Quality Control

QC016 DQα Hybridization

made by: _____ date: _____

Initials: RD Date: 10/2/96

S005 DQα Wash Solution lot number: _____

standard batch size: 4 L

Ingredients	final concentration	amount
S002 SSPE, 20X	2.5 X	500 ± 10 mL
S001 SDS, 20%	0.10 %	20 ± 1 mL

Procedure

Measure 20 mL 20% SDS in a 50 mL graduated cylinder.

Raise the volume of the SDS solution to 50 mL by adding 30 mL deionized water.

Pour the SDS into a 4 L bottle.

Add 500 mL SSPE and 3450 mL deionized water.

Cap and mix well by inverting.

Store at room temperature.

Data Log	source	lot	amount
S002 SSPE, 20X	_____	_____	_____
S001 SDS, 20%	_____	_____	_____

Quality Control

QC016 DQα hybridization

made by: _____ date: _____

Initials: RJ Date: 10/7/96

S093 DTT, 1M lot number: _____

standard batch size: 5 mL

Ingredients	final concentration	amount
RM101 dithiothreitol	1.0 M	0.77 ± 0.05 g
S059 sterile water	-----	-----

Procedure

Add the DTT to approximately 4 mL sterile, deionized water in a 15 mL centrifuge tube.

Mix well.

When the DTT is dissolved, bring up to volume with sterile, deionized water.

Filter sterilize.

Dispense 250 µL aliquots into sterile 0.5 mL eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM101 dithiothreitol	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC023 QuantiBlot Quality Control of Solutions- Test 20 µL of solution

made by: _____ date: _____

Initials: AY Date: 10/7/96

S030 DTT, 0.39M lot number: _____

standard batch size: 1.5 mL

Ingredients	final concentration	amount
RM101 dithiothreitol	0.39 M	0.090 ± 0.001 g
S059 sterile water	__	1.5 mL (Guideline)

Procedure

Add the DTT to approximately 1 mL sterile water in a sterile 1.5 mL eppendorf tube.

Mix well.

When the DTT is dissolved, bring up to volume with sterile water.

Dispense 500 µL aliquots into sterile 0.5 mL eppendorf tubes.

Store at -20°C.

Discard after 6 months

Data Log	source	lot	amount
RM101 dithiothreitol	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC023 QuantiBlot Quality Control of Solutions- Test 40 µL of solution

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S009 EDTA, 0.5M lot number: _____

standard batch size: 500 mL

Ingredients	final concentration	amount
RM003 EDTA	0.50 M	93 ± 1 g
RM004 sodium hydroxide, 10N	------	------

Procedure

Add the EDTA to approximately 250 mL deionized water.

Adjust the pH to 8.0 with sodium hydroxide solution.

Mix well.

When the EDTA is dissolved, adjust the pH to 8.0.

Bring up to volume with deionized water.

Check and record the final pH.

Dispense into 125 mL bottles.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
RM003 EDTA	_____	_____	_____
RM004 sodium hydroxide, 10N	_____	_____	_____

Quality Control

final pH: _____ specification: 8.0 ± 0.1

made by: _____ date: _____

Initials: RU Date: 10 Aug

S110 EDTA, 200 mM lot number: _____

standard batch size: 25 mL

Ingredients	final concentration	amount
S009 EDTA, 0.5 M	200 mM	10.0 ± 0.5 mL

Procedure

Add the EDTA to 15 mL deionized water.

Mix thoroughly.

Filter sterilize or autoclave at 250°F for 20 minutes.

Dispense into 300 µL aliquots.

Store at room temperature.

Data Log	source	lot	amount
S009 EDTA, 0.5 M	_____	_____	_____

Quality Control

QC016 PCR Kit Hybridization- Test 20 µL of solution

made by: _____ date: _____

Initials: RU Date: 10/7/96

S056 Ethanol, 70% lot number: _____

standard batch size: 500 mL

Ingredients	final concentration	amount
RM009 ethanol, 100%	70%	350 ± 10 mL

Procedure

Measure the ethanol in a graduated cylinder.

Bring up to volume with deionized water.

Dispense into a storage container.

Store at room temperature or at 2-8°C.

Data Log	source	lot	amount
RM009 ethanol, 100%	_____	_____	_____

made by: _____ date: _____

Initials: RCS Date: 10/7/96

S031 Gel Neutralization Buffer lot number: _____

standard batch size: 8 L

Ingredients	final concentration	amount
RM073 TRIS	0.20 M	194 ± 4 g
RM005 sodium chloride	0.30 M	140 ± 4 g
RM096 hydrochloric acid	-----	100 ± 10 mL (guideline)

Procedure

Add the TRIS and NaCl to approximately 6 L deionized water in an 8 L carboy.

Adjust the pH to 7.6 using concentrated HCl.

Bring up to the final volume with deionized water.

Mix well.

Check and record the final pH.

Store at room temperature.

Data Log	source	lot	amount
RM073 TRIS	_____	_____	_____
RM005 sodium chloride	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

final pH: _____ specification: 7.6 ± 0.1

made by: _____ date: _____

Initials: RC Date: 2/9/98

S116 GS500 + Loading Buffer (1:3) lot number: _____

standard batch size: 10 x 800 µL

Ingredients	amount	ratio
RM1062 GS 500 Rox labelled	200 ± 2 µL	1
RM0451 Loading Buffer	600 ± 6 µL	3

Procedure

NOTE: PREPARE AWAY FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

Label a screw cap tube with GS500, add 200μL GS 500 and 600μL loading buffer to the tube.

Close and mix the tube.

Store at 2-8°C.

Data Log

	source	lot	amount
RM1062 ABI GS500 Rox labeled	_____	_____	_____
RM0451 ABI Loading Buffer	_____	_____	_____

made by:_____date:_____

Initials: RCJ Date: 10 Dec

S116 GS500 + Loading Buffer (1:3) lot number: _____

standard batch size: 800 µL

Ingredients	amount
RM1062 GS 500 Rox labelled	200 ± 2 µL
RM0451 Loading Buffer	600 ± 6 µL

Procedure

NOTE: PREPARE AWAY FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

Add the loading buffer to the original GS500 tube.

Mark the tube.

Store at 2-8°C.

Data Log

	source	lot	amount
RM1062 ABI GS500 Rox labeled	_____	_____	_____
RM0451 ABI Loading Buffer	_____	_____	_____

made by:_____date:_____

Initials: RCJ Date: 10/7/96

S016 Hae III Buffer, 10X lot number: _____

standard batch size: 100 mL

Ingredients	final concentration	amount
RM073 TRIS	500 mM	6.0 ± 0.2 g
RM046 magnesium chloride, hexahydrate	100 mM	2.0 ± 0.1 g
RM005 sodium chloride	500 mM	2.9 ± 0.1 g
RM096 hydrochloric acid	---	

Procedure

Add the TRIS, magnesium chloride, and sodium chloride to approximately 75 mL deionized water.
Mix well.
Adjust the pH to 8.0 with hydrochloric acid.
Bring up to the final volume with deionized water.
Dispense into a sterile 125 mL bottle.
Autoclave at 250°F for 20 minutes.
Using sterile pipet tips, dispense 1 mL aliquots into sterile 1.5 mL eppendorf tubes.
Store at -20°C.

Data Log	source	lot	amount
RM073 TRIS	_____	_____	_____
RM046 magnesium chloride, hexahydrate	_____	_____	_____
RM005 sodium chloride	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

Quality Control

final pH: _____ spec: 8.0 ± 0.1

made by: _____ date: _____

Initials: RD Date: 10/7/96

S102 Heparin Solution lot number:_____

standard batch size: 50 mL

Ingredients	final concentration	amount
S035 SP, 25X	5 X	10 ± 0.5 mL
RM028 Heparin	50 mg/mL	2.5 ± 0.05 g
RM061 Na Azide	0.2%	0.1 ±0.005 g
SO59 Sterile Water	___	

Procedure

Weigh heparin in a sterile 50 mL tube.

Add the Na Azide, SP and mix.

Add the sterile water to volume on tube.

Mix.

The solution may be heated to help dissolve the heparin.

Filter sterilize.

Dispense into a new sterile 50 mL tube.

Store at 4°C.

Data Log	source	lot	amount
S035 SP, 25X	_____	_____	_____
RM028 Heparin	_____	_____	_____
RM061 Na Azide	_____	_____	_____
SO59 Sterile Water	_____	_____	_____

made by:_____ date:_____

Initials: RH Date: 10/7/96

S026 Hybridization Solution, RFLP lot number: _____

standard batch size: 1 L

Ingredients	final concentration	amount
S035 SP, 25X	1.5 X	60 ± 2 mL
S001 SDS, 20%	7 %	350 ± 10 mL

Procedure

Add the SP to 590 mL deionized water.

Add the SDS to the solution.

Heat the solution to dissolve the SDS.

Mix well.

Rinse the filter of a disposable filter unit with approximately 500 mL sterile, deionized water.

Filter sterilize the warm hybridization solution.

Dispense into 250 mL aliquots.

Store at room temperature.

Data Log	source	lot	amount
S035 SP, 25X	_____	_____	_____
S001 SDS, 20%	_____	_____	_____

Quality Control

QC027 Southern Blotting and Hybridization

made by: _____ date: _____

Initials: RS Date: 10/7/96

S079 Hydrogen Peroxide, 3% lot number: _____

standard batch size: 30 X 0.5 mL

Ingredients	final concentration	amount
RM176 hydrogen peroxide, 30%	3 %	1.5 mL ± 0.1 mL
deionized water	-----	13.5 mL (guideline)

Procedure

Add hydrogen peroxide to a 15 mL disposable tube.

Add deionized water to a final volume of 15 mL.

Aliquot approximately 0.5 mL of hydrogen peroxide into 1.5 mL microcentrifuge tubes.

Label each tube with "H_2O_2" and the lot number. Label the rack with expiration date.

Store at 4°C in the dark.

Discard after 2 months.

Data Log	source	lot	amount
RM176 hydrogen peroxide, 30%	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S032 Lambda Marker lot number: _____

Ingredients	initial concentration (ng/µL)	initial volume (µL)	final concentration	final volume (µL)
RM155 lambda Hind IIIfragments			20 ng/µL	
S021 yield gel loading buffer	5 X		1 X	----
S059 sterile water	-----		----	----

Calculations

Record the initial concentration in ng/µL and the initial volume in µL of the lambda Hind III DNA received from the manufacturer.

Calculate the final volume according to equation 1.

$$\text{(final volume)} = \frac{\text{(initial DNA concentration)(initial DNA volume)}}{\text{(20 ng/µL)}} \qquad \textbf{equation 1}$$

Record the final volume above. The final volume is the total batch size.

Calculate the amount of buffer to be added according to equation 2.

(buffer volume) = 0.2(final volume) **equation 2**

Calculate the amount of sterile water to be added according to equation 3.

(water volume) = [0.8 * (final volume)] - (initial DNA volume) **equation 3**

Record the buffer and water volumes above.

To check the calculations, add together the initial volumes of DNA, loading buffer, and sterile water.

The sum of the initial volumes must be equal to the calculated final volume.

Initials: RCJ Date: 10/7/96

S032 Lambda Marker lot number: _____

Procedure

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 500 µL aliquots into sterile 1.5 mL eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM155 lambda Hind III fragments	_____	_____	_____
S021 yield gel loading buffer	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC026 Gel Electrophoresis

made by: _____ date: _____

Initials: RCJ Date: 10/17-18

S015 Lithium Chloride, 7.5 M lot number: _____

standard batch size: 100 mL

Ingredients	final concentration	amount
RM032 lithium chloride	7.5 M	31.8 ± 0.2 g

Procedure

Dissolve the lithium chloride in approximately 75 mL deionized water.

Mix well.

When the lithium chloride has dissolved, bring up to the final volume with deionized water.

Dispense 10 mL aliquots into 15 mL centrifuge tubes.

Autoclave at 250°F for 20 minutes.

Store at -20°C.

Data Log	source	lot	amount
RM032 lithium chloride	_____	_____	_____

made by: _____ date: _____

Initials: RCS Date: 10/7/96

S055 Magnesium Chloride, 0.1M lot number: _____

standard batch size: 250 mL

Ingredients	final concentration	amount
RM046 magnesium chloride, hexahydrate	0.10 M	5.1 ± 0.3 g

Procedure

Dissolve the magnesium chloride in approximately 200 mL deionized water.

Mix well.

When the magnesium chloride has dissolved, bring up to the final volume with deionized water.

Dispense into 125 mL bottles.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
RM046 magnesium chloride, hexahydrate	_____	_____	_____

made by: _____ date: _____

Initials: RCS Date: 10/7/96

S112 dNTP's (10mM stock) **lot number:** _____

standard batch size: ~ 25 tubes x 250 µL

Ingredients	final concentration	amount
dATP, 10 mM, 320 µL/tube	2.5 mM	1600 µL (5 tubes)
dCTP, 10 mM, 320 µL/tube	2.5 mM	1600 µL (5 tubes)
dGTP, 10 mM, 320 µL/tube	2.5 mM	1600 µL (5 tubes)
dTTP, 10 mM, 320 µL/tube	2.5 mM	1600 µL (5 tubes)
Autoclaved, microcentrifuge tubes	_____	~25 tubes

Procedure

NOTE: ALIQUOT ALL TUBES AT ONE TIME AND IN A ROOM FREE FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED. USE ONLY FILTER PIPET TIPS OR A REPEAT PIPETTOR FOR ALL PIPETTING.

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

Add the dNTP's together in a 15 mL sterile centrifuge tube and mix.

While wearing clean gloves, remove all tubes from the bag and place them in a clean rack designated for the PCR preparation room only.

Aliquot 250 µL of dNTP mix into each tube.

Once aliquotting is complete, cap all tubes and store in a labeled rack away from all sources of DNA. Store at 2-8°C.

Data Log	source	lot	amount
RM0211 Geneamp dNTP's	_____	_____	_____
dATP	_____	_____	_____
dCTP	_____	_____	_____
dGTP	_____	_____	_____
dTTP	_____	_____	_____

made by:_____ date:_____

Initials: RJ Date: 10/7/96

S006 Phenol lot number: _____

standard batch size: 500 mL

Ingredients	final concentration	amount
RM112 phenol	---	500 ± 10 g
S052 TRIS, 0.1 M - pH 7.8	---	400 ± 10 mL
RM036 m-cresol	---	25 ± 1 mL
RM049 2-mercaptoethanol	---	1.0 ± 0.1 mL
RM029 4-hydroxyquinoline	---	0.50 ± 0.01 g

Procedure

Place a 500 g bottle of phenol crystals in a 65°C waterbath to dissolve (about 10-15 minutes).

When dissolved, add 100 mL TRIS solution to the bottle. Invert several times to mix thoroughly.

Return the bottle to the 65°C water bath and allow the temperature to equilibrate another 10-15 minutes.

Pour the solution into a separatory funnel.

When the phases have separated, return the organic phase (the bottom phase) to the bottle. Discard the aqueous phase into an organic waste container.

To the organic phase, add another 100 mL TRIS.

Separate the phases as before, isolating the organic phase (the bottom phase) each time and discarding the aqueous phase into organic waste.

Repeat the 100 mL TRIS wash one more time.

To the final organic phase, add the m-cresol, mercaptoethanol, hydroxyquinoline, and 100 mL TRIS.

Store at 2-8°C.

Initials: RCJ Date: 10/2/95

S006 Phenol lot number: _____

Data Log	source	lot	amount
RM112 phenol	_____	_____	_____
S052 TRIS, 0.1M - pH 7.8	_____	_____	_____
RM036 m-cresol	_____	_____	_____
RM049 2-mercaptoethanol	_____	_____	_____
RM029 4-hydroxyquinoline	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S053 Phenol-Chloroform-Isoamyl Alcohol lot number: _____

standard batch size: 1 L

Ingredients	final concentration	amount
S006 phenol	50 %	500 ± 5 mL
S027 chloroform-isoamyl alcohol	50 %	500 ± 5 mL

Procedure

NOTE: Use only glass containers and graduated cylinders.

Take 500 mL of the phenol mixture from the bottom phase and place it in a brown 1 L bottle.

Add 500 mL chloroform-isoamyl alcohol.

Store at 2-8°C in the flammable materials refrigerator.

Data Log	source	lot	amount
S006 phenol	_____	_____	_____
S027 chloroform-isoamyl alcohol	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S042 Phi-X Marker lot number: _____

Ingredients	initial concentration (ng/µL)	initial volume (µL)	final concentration	final volume (µL)
RM156 phi-X-174, Hae III fragments			50 ng/µL	
S018 analytical gel loading buffer	5 X		1 X	----
S059 sterile water	-----		----	----

Calculations

Record the initial concentration in ng/µL and the initial volume in µL of the phi-X-174 Hae III received from the manufacturer.

Calculate the final volume according to equation 1.

$$\text{(final volume)} = \frac{\text{(initial DNA concentration)(initial DNA volume)}}{\text{(50 ng/µL)}} \qquad \textbf{equation 1}$$

Record the final volume above. The final volume is the total batch size.

Calculate the amount of buffer to be added according to equation 2.

(buffer volume) = 0.2(final volume) **equation 2**

Calculate the amount of sterile water to be added according to equation 3.

(water volume) = [0.8 * (final volume)] - (initial DNA volume) **equation 3**

Record the buffer and water volumes above.

To check the calculations, add together the initial volumes of DNA, loading buffer, and sterile water.

The sum of the initial volumes must be equal to the calculated final volume.

Initials: RCS Date: 10/7/96

S042 Phi-X Marker lot number: _____

Procedure

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 500 µL aliquots into sterile 1.5 mL eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM156 phi-X-174 Hae III fragments	_____	_____	_____
S018 analytical gel loading buffer	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC026 Gel Electrophoresis

made by: _____ date: _____

Initials: RCJ Date: 10/7-16

S034 Phosphate Buffered Saline (PBS) lot number: _____

standard batch size: 4 L

Ingredients	final concentration	amount
RM005 sodium chloride	137 mM	32.0 ± 0.1 g
RM053 potassium chloride	3.0 mM	0.90 ± 0.01 g
RM065 sodium phosphate, dibasic	6.0 mM	3.41 ± 0.03 g
RM056 potassium phosphate, monobasic	1.5 mM	0.82 ± 0.02 g

Procedure

Add all the components to approximately 3 L deionized water.
Mix well.
Adjust the pH to 7.5.
Bring up to the final volume with deionized water.
Measure and record the final pH.
Dispense into 50 mL centrifuge tubes.
Autoclave at 250°F for 20 minutes.
Store at room temperature.

Data Log	source	lot	amount
RM005 sodium chloride	_____	_____	_____
RM053 potassium chloride	_____	_____	_____
RM065 sodium phosphate, dibasic	_____	_____	_____
RM056 potassium phosphate, monobasic	_____	_____	_____

Quality Control

final pH: _____ spec: 7.5 ± 0.1

QC023 QuantiBlot Quality Control of Solutions- Test 150 µL of solution

made by: _____ date: _____

Initials: RU Date: 10/7/96

S109 PM PCR Reaction Mixture **lot number:** _____

standard batch size: ~ 65 tubes x 40 µL

Ingredients	final concentration	amount
PM PCR reaction mix	_____	2.4 mL
Autoclaved, PCR reaction tubes	_____	55 tubes

Procedure

NOTE: ALIQUOT ALL TUBES AT ONE TIME AND IN A ROOM FREE FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

While wearing clean gloves, remove all tubes from the bag and place them in a clean rack designated for the PCR preparation room only.

Using a dedicated positive displacement repeat pipettor or tips with hydrophobic filters, carefully aliquot 40 µL of PCR reaction mixture into each tube.

Once aliquotting is complete, cap all tubes and store in a labeled rack away from all sources of DNA.

Store at 2-8°C.

Data Log	source	lot	amount
PM reaction mix	_____	_____	_____
PCR reaction tubes	_____	_____	_____

Quality Control

QC015 PCR Kit Amplification- Only for the first kit of each shipment/lot

made by:_____ date:_____

Initials: RCI Date: 10/7/96

S126 Positive Control-External lot number_____

standard batch size ~50 tubes

Procedure

Collect EDTA blood from a volunteer. Prepare bloodstains from this blood sample.

Extract two 3x3 cm portions of the dried bloodstain following the organic extraction procedure in the RFLP manual.

Run an aliquot of each fraction on a yield gel. Pool both fractions. The DNA has to be of good quality that means of high molecular weight

Re-quantitate using the Quantiblot procedure.

Dilute with sterile water an aliquot of the DNA based on the yield gel result to a concentration of 1.25-2.5ng/20-25ul.

Quantitate dilution using Quantiblot.

Data Log	Source	Yield Gel Fract. 1	Yield Gel Fract. 2	Quantiblot Pool	Quantiblot Dilution
EDTA Blood	_____	_____	_____	_____	_____

	Source	Lot	Amount
S059 Sterile Water	_____	_____	_____

Quality Control

QC031- QUAD STR/PCR Amplification

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S127 Positive Control-Internal batch number_____

standard batch size 24 tubes

Procedure

All previously typed samples can be used as an internal positive control (reanalysis sample) if they meet the following criteria:

- the sample has been successfully typed for all STR loci so that all alleles for each locus are known.
- the DNA concentration according to Quantiblot was greater or equal to1.25ng/20ul.
- a sufficient amount of the DNA extract is left.
- the peak height for VWA was greater or equal 200 f.u. during the first analysis.

The internal positive control samples are prepared by **two** analysts together as follows:

Select 24 appropriate DNA samples

Fill out the Positive Control-Internal solution sheet. Note: One person should read the allele designations from the electropherograms, not the table, while the second person fills out the sheet

Calculate the amounts of DNA and deionized water according to the STR Quad amplification sheet.

Label 24 tubes adhering to this format: 1.1 to 1.24 for the first batch, 2.1 to 2.24 for the second batch and so on. Label tubes for 1:10 dilutions if required.

Pipet the calculated amount of water in all tubes and close the tubes.

Spin down Chelex extracts. With one analyst watching, pipet the required amount of DNA solution into the correct tube. Make 1:10 dilutions and use these where required.

Mix tubes, spin down shortly, and place them in the Positive Control Internal rack in the PCR set up room.

Initials: RH Date: 10/14/96

S127 Positive Control-Internal batch number_____

Batch. PI #	MB / ex. #	Conc. ng/20μl	DNA vol μl	dH_2O vol μl	VWA	F13A1	THO1	FES
.1								
.2								
.3								
.4								
.5								
.6								
.7								
.8								
.9								
.10								
.11								
.12								
.13								
.14								
.15								
.16								
.17								
.18								
.19								
.20								
.21								
.22								
.23								
.24								

S059 Sterile Water_____

Prepared by _____ Witness _____ Date _____

Initials: RCJ Date: 10/7/96

S097 Pre-Wetting Solution lot number: _____

standard batch size: 4 L

Ingredients	**final concentration**	**amount**
RM004 NaOH, 10 N	0.4 N	160 ± 10 mL
S009 EDTA, 0.5 M	25 mM	200 ± 10 mL

Procedure

Measure 3640 mL deionized water into a 4 L bottle.

Add 160 mL NaOH and 200 mL EDTA.

Cap and mix well by inverting.

Dispense into 1 L bottles or store in bulk.

Store at room temperature.

Data Log	source	lot	amount
RM004 NaOH, 10 N	_____	_____	_____
S009 EDTA, 0.5 M	_____	_____	_____

made by: _____ date: _____

Initials: RH Date: 10/7/96

S118 Primer, VWA 1 (50 µM) lot number: _____

Physical data

Sequence JOE - 5' CC CTA GTG GAT GAT AAG AAT AAT CAG TAT 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
VWA 1	9272.0	30.1	3246.3

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H_2O (µL)
RM 1087 VWA 1 primer			50 pM/µL	-----
S059 Sterile Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 3246 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RH Date: 10/7/96

S118 Primer, VWA 1 (50 µM) lot number: _____

Procedure

Add the sterile water to the original primer tube.

Mix well.

Dispense 50 µL aliquots into 1.8 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
RM1087 Primer VWA 1	_____	_____	_____
S059 sterile water	_____	_____	_____

Calculation checked by _____

Quality Control

QC031 QUAD STR/PCR Amplification

QC023 Quantiblot- test 1µL of solution

made by: _____ date: _____

Initials: RD Date: 10/7/96

S119 Primer, VWA 2 (50 µM) lot number: _____

Physical data

Sequence 5' GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
VWA 2	9383.0	29.4	3133.3

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H_2O (µL)
RM 1080 VWA 2 primer			50 pM/µL	-----
S059 Sterile Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 3133 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RCJ Date: 10/7/96

S119 Primer, VWA 2 (50 µM) lot number: _____

Procedure

Add the sterile water to the original primer tube.

Mix well.

Dispense 50 µL aliquots into 1.8 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
RM1080 Primer VWA 2	_____	_____	_____
S059 sterile water	_____	_____	_____

Calculation checked by _____

Quality Control

QC031 QUAD STR/PCR Amplification

QC023 Quantiblot- test 1µL of solution

made by: _____ date: _____

Initials: AC Date: 10/7/96

S120 Primer THO1 1 (50 µM) lot number: _____

Physical data

Sequence FAM - 5' GT GGG CTG AAA AGC TCC CGA TTA T 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
THO1 1	7386.1	32.3	4373.1

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H_2O (µL)
RM 1086 Tho1 1 primer			50 pM/µL	-----
S059 Sterile Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 4373 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: CLS Date: 10/7/96

S120 Primer, Tho1 1 (50 µM) lot number: _____

Procedure

Add the sterile water to the original primer tube.

Mix well.

Dispense 50 µL aliquots into 1.8 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
RM1086 Primer Tho1 1	_____	_____	_____
S059 sterile water	_____	_____	_____

Calculation checked by _____

Quality Control

QC031 QUAD STR/PCR Amplification

QC023 Quantiblot- test 1µL of solution

made by: _____ date: _____

Initials: RU Date: 10/7/96

S121 Primer THO1 2 (50 µM) lot number: _____

Physical data

Sequence 5' GTG ATT CCC ATT GGC CTG TTC CTC 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
THO1 2	7257.8	35.1	4836.2

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H_2O (µL)
RM 1079Tho1 2 primer			50 pM/µL	-----
S059 Sterile Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 4836 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RG Date: 10/7/96

S121 Primer, Tho1 2 (50 µM) lot number: _____

Procedure

Add the sterile water to the original primer tube.

Mix well.

Dispense 50 µL aliquots into 1.8 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
RM1079 Primer Tho1 2	_____	_____	_____
S059 sterile water	_____	_____	_____

Calculation checked by _____

Quality Control

QC031 QUAD STR/PCR Amplification

QC023 Quantiblot- test 1µL of solution

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S122 Primer F13A1 1 (50 µM) lot number: _____

Physical data

Sequence JOE - 5' AT GCC ATG CAG ATT AGA AA 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
F13A1/1	5841.8	29.8	5101.2

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H_2O (µL)
RM 1085 F13A1 1 primer			50 pM/µL	-----
S059 Sterile Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 5101 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RCJ Date: 10/7/96

S122 Primer, F13A1 1 (50 µM) lot number: _____

Procedure

Add the sterile water to the original primer tube.

Mix well.

Dispense 50 µL aliquots into 1.8 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
RM1085 Primer, F13A1 1	_____	_____	_____
S059 sterile water	_____	_____	_____

Calculation checked by _____

Quality Control

QC031 QUAD STR/PCR Amplification

QC023 Quantiblot- test 1µL of solution

made by: _____ date: _____

Initials: RS Date: 10/7/96

S123 Primer, F13A1 2 (50 µM) lot number: _____

Physical data

Sequence 5' GAG GTT GCA CTC CAG CCT TT 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
F13A1/2	6080.0	34.1	5608.6

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H_2O (µL)
RM 1076 F13A1 2 primer			50 pM/µL	-----
S059 Sterile Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 5609 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RCJ Date: 10/7/96

S123 Primer, F13A1 2 (50 µM) lot number: _____

Procedure

Add the sterile water to the original primer tube.

Mix well.

Dispense 50 µL aliquots into 1.8 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
RM 1076 Primer F13A1 2	_____	_____	_____
S059 sterile water	_____	_____	_____

Calculation checked by _____

Quality Control

QC031 QUAD STR/PCR Amplification

QC023 Quantiblot- test 1µL of solution

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S124 Primer, FES/FPS 1 (50 µM) lot number: _____

Physical data

Sequence 5' GG GAT TTC CCT ATG GAT TGG 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
FES 1	6173	32.8	5313.5

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H_2O (µL)
RM 1084 FES 1 primer			50 pM/µL	-----
S059 Sterile Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 5314 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RCS Date: 10/7/96

S124 Primer, FES/FPS (50 µM) lot number: _____

Procedure

Add the sterile water to the original primer tube.

Mix well.

Dispense 50 µL aliquots into 1.8 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
RM1084 Primer FES/FPS 1	_____	_____	_____
S059 sterile water	_____	_____	_____

Calculation checked by _____

Quality Control

QC031 QUAD STR/PCR Amplification

QC023 Quantiblot- test 1µL of solution

made by: _____ date: _____

Initials: RCS Date: 10/7/96

S125 Primer FES/FPS 2 (50 µM) lot number: _____

Physical data

Sequence FAM - 5' GCG AAA GAA TGA GAC TAC AT 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
FES 2	6179	29.7	4806.6

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H_2O (µL)
RM 1075 FES 2 primer			50 pM/µL	-----
S059 Sterile Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 4807 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RCJ Date: 10/7/96

S125 Primer, FES/FPS (50 µM) lot number: _____

Procedure

Add the sterile water to the original primer tube.

Mix well.

Dispense 50 µL aliquots into 1.8 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
RM1075 Primer FES/FPS 2	_____	_____	_____
S059 sterile water	_____	_____	_____

Calculation checked by _____

Quality Control

QC031 QUAD STR/PCR Amplification

QC023 Quantiblot- test 1µL of solution

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S011 Protein Lysis Buffer (PLB) lot number: _____
standard batch size: 2 L

Ingredients	final concentration	amount
S009 EDTA, 0.5M	10 mM	40 ± 2 mL
RM073 TRIS	10 mM	2.4 ± 0.1 g
RM005 sodium chloride	10 mM	1.2 ± 0.05 g
RM096 hydrochloric acid	---	

Procedure
Add the TRIS, EDTA, and sodium chloride to approximately 1.5 L deionized water.
Mix well.
Adjust the pH to 7.9 with hydrochloric acid
Raise to the final volume with deionized water.
Mix well.
Dispense into 15 mL centrifuge tubes.
Autoclave at 250°F for 30 minutes.
Store at 2-8°C.

Data Log	source	lot	amount
S009 EDTA, 0.5M	_____	_____	_____
RM073 TRIS	_____	_____	_____
RM005 sodium chloride	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

Quality Control
final pH: _____ spec: 7.9 ± 0.1

QC023 QuantiBlot Quality Control of Solutions- test 150 µL of solution

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S014 Proteinase-K Enzyme, 10mg/mL lot number: _____
standard batch size: 10 mL

Ingredients	final concentration	amount
RM119 proteinase-K, lyophilized	10 mg/mL	100 ± 1 mg
S059 sterile water	_____	10 ± 0.5 mL

Procedure
Add 10 mL sterile, deionized water to one bottle (100 mg) lyophilized proteinase-K enzyme.

Mix by slowly inverting until completely reconstituted.

Dispense 500 µL aliquots into 1.5 mL eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM119 proteinase-K, lyophilized	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC023 QuantiBlot Quality Control of Solutions- Test 10 µL of solution

QC024 Non-Organic Extraction

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S037 Proteinase-K Enzyme, 20mg/mL lot number: _____
standard batch size: 5 mL

Ingredients	final concentration	amount
RM119 proteinase-k, lyophilized	20 mg/mL	100.0 ± 0.5 mg
S059 sterile water	—	5 mL

Procedure
Add 5 mL sterile water to one bottle (100 mg) lyophilized proteinase-k enzyme.

Mix by slowly inverting until completely reconstituted.

Dispense 500 ul aliquots into 1.5 mL eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM119 proteinase-k, lyophilized	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC023 QuantiBlot Quality Control of Solutions- Test 10 µL of solution

QC024 Non-Organic Extraction

made by: _____ date: _____

Initials: QCJ Date: 10/7/96

S111 QUAD STR/PCR Reaction Mixture lot number:_____
standard batch size:50-200 tubes **page 1of 2**

Ingredients:	Final Concentrtion	1 Tube Amount	50 Tubes	100 Tubes	200 Tubes
RM0275 10X PCR Buffer II	1X	5 µL	250 µL	500 µL	1000 µL
S112 dNTP's (2.5 mM)	200 mM	4 µL	200 µL	400 µL	800 µL
S059 sterile dH20	--------	6.8 µL	340 µL	680 µL	1360 µL
S117 BSA (5mg/mL)	160ug/ml	1.6 µL	80 µL	160 µL	320 µL
S118 VWA/1 (50pM/µL)	0.22 mM	0.22 µL	11 µL	22 µL	44 µL
S119 VWA/2 (50pM/µL)	0.22 mM	0.22 µL	11 µL	22 µL	44 µL
S120 THO1/1 (50pM/µL)	0.22 mM	0.22 µL	11 µL	22 µL	44 µL
S121 THO1/2 (50pM/µL)	0.22 mM	0.22 µL	11 µL	22 µL	44 µL
S122 F13A1/1 (50pM/µL)	0.16 mM	0.16 µL	8 µL	16 µL	32 µL
S123 F13A1/2 (50pM/µL)	0.16 mM	0.16 µL	8 µL	16 µL	32 µL
S124 FES/1/(50pM/µL)	0.20 mM	0.20 µL	10 µL	20 µL	40 µL
S125 FES/2 (50pM/µL)	0.20 mM	0.20 µL	10 µL	20 µL	40 µL
RM0275 AmpliTaq (5u/µL)	5 U	1 µL	50 µL	100 µL	200 µL
TOTAL		20 µL	1 mL	2 mL	4 mL

Procedure

NOTE: ALIQUOT ALL TUBES AT ONE TIME AND IN A ROOM FREE FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper
Add the ingredients to either a microcentrifuge tube or a 15 mL centrifuge tube using pipetmen dedicated to PCR preparation area only.
While wearing clean gloves, remove sufficient amount of tubes from the bag and place them in a clean rack designated for the PCR prep room only.
Vortex and spin briefly. Add 20 µL per tube using a dedicated repeat pipettor or tips with hydrophobic filters.
Cap all tubes and store in a labeled rack away from all sources of DNA.
Store at 2-8°C.

Initials: RG Date: 10/7/96

S111 QUAD STR/PCR Reaction Mixture lot number:_____

Data Log	source	lot	amount
RM0275 10X PCR Buffer II	_____	_____	_____
S112 dNTP's (2.5 mM)	_____	_____	_____
S059 sterile dH20	_____	_____	_____
S117 BSA (5mg/mL)	_____	_____	_____
S118 VWA/1 (50pM/µL)	_____	_____	_____
S119 VWA/2 (50pM/µL)	_____	_____	_____
S120 THO1/1 (50pM/µL)	_____	_____	_____
S121 THO1/2 (50pM/µL)	_____	_____	_____
S122 F13A1/1 (50pM/µL)	_____	_____	_____
S123 F13A1/2 (50pM/µL)	_____	_____	_____
S124 FES/1/(50pM/µL)	_____	_____	_____
S125 FES/2 (50pM/µL)	_____	_____	_____
RM0275 AmpliTaq (5u/µL)	_____	_____	_____

made by:_____date:_____

Initials: RCJ Date: 10/24/96

S100 QuantiBlot DNA Standards lot number: _____
standard batch size: variable

Ingredients	final concentration	amount
RM442 DNA Standard A	varies	varies
S128 TE^{-4}, 1X	1X	varies

Procedure

Each lot of QuantiBlot DNA Standards is prepared by pooling up to 10 DNA Standard A's (from the QuantiBlot kit) and serially diluting according to the following procedure:

1. Pool the contents of five or ten DNA Standard A tubes (use all one lot number).
2. Vortex to mix thoroughly.
3. Label seven sterile microfuge tubes, 1A - 1G.
4. If five DNA Standard A tubes were pooled:
   Transfer 600 µL of DNA Standard A into the tube labeled 1A. This is now DNA Standard 1A.
   Aliquot 300 µL of 1X TE^{-4} into each of the six remaining tubes labeled 1B-1G.
   Add 300 µL of DNA Standard 1A to the 300 µL of 1X TE^{-4} in tube 1B. Vortex to mix thoroughly.
   Add 300 µL of diluted DNA Standard (tube 1B) to the 300 µL of 1X TE^{-4} in tube 1C. Vortex to mix thoroughly.
   Add 300 µL of diluted DNA Standard (tube 1C) to the 300 µL of 1X TE^{-4} in tube 1D. Vortex to mix thoroughly.
   Continue the serial dilution through tube 1G.
5. If ten DNA Standard A tubes were pooled:
   Transfer 1200 µL of DNA Standard A into the tube labeled 1A. This is now DNA Standard 1A.
   Aliquot 600 µL of 1X TE^{-4} into each of the six remaining tubes labeled 1B-1G.
   Add 600 µL of DNA Standard 1A to the 600 µL of 1X TE^{-4} in tube 1B. Vortex to mix thoroughly.

Initials: RCJ Date: 10/21/96

S100 QuantiBlot DNA Standards lot number: _____
standard batch size: variable

Add 600 µL of diluted DNA Standard (tube 1B) to the 600 µL of 1X TE^{-4} in tube 1C.
Vortex to mix thoroughly.
Add 600 µL of diluted DNA Standard (tube 1C) to the 600 µL of 1X TE^{-4} in tube 1D.
Vortex to mix thoroughly.
Continue the serial dilution through tube 1G.

6. Store at 2° to 8°C.
7. DNA Standards are stable for at least 3 months as 2° to 8°C.

If the dilution steps are performed as described above, the seven DNA Standard tubes will have the following concentrations of human DNA:

DNA Standards		
Standard Tube	Conc (ng/µL)	Quantity (ng/5µL)
1A	2	10
1B	1	5
1C	0.5	2.5
1D	0.25	1.25
1E	0.125	0.625
1F	0.0625	0.3125
1G	0.03125	0.15625

Data Log source lot amount

RM442 DNA Standard A _____ _____ _____

S128 TE^{-4}, 1X _____ _____ _____

Quality Control
QC018 QuantiBlot Hybridization.

made by: _____ date: _____

Initials: RCJ Date: 10/4/96

S100 QuantiBlot DNA Standards lot number: _____
standard batch size: variable

Ingredients	final concentration	amount
RM442 DNA Standard A	varies	varies
S039 TE, 1X	1X	varies

Procedure

Each lot of QuantiBlot DNA Standards is prepared by pooling up to 10 DNA Standard A's (from the QuantiBlot kit) and serially diluting according to the following procedure:

1. Pool the contents of five or ten DNA Standard A tubes (use all one lot number).
2. Vortex to mix thoroughly.
3. Label seven sterile microfuge tubes, 1A - 1G.
4. If five DNA Standard A tubes were pooled:
   Transfer 600 µL of DNA Standard A into the tube labeled 1A. This is now DNA Standard 1A.
   Aliquot 300 µL of 1X TE into each of the six remaining tubes labeled 1B-1G.
   Add 300 µL of DNA Standard 1A to the 300 µL of 1X TE in tube 1B. Vortex to mix thoroughly.
   Add 300 µL of diluted DNA Standard (tube 1B) to the 300 µL of 1X TE in tube 1C. Vortex to mix thoroughly.
   Add 300 µL of diluted DNA Standard (tube 1C) to the 300 µL of 1X TE in tube 1D. Vortex to mix thoroughly.
   Continue the serial dilution through tube 1G.
5. If ten DNA Standard A tubes were pooled:
   Transfer 1200 µL of DNA Standard A into the tube labeled 1A. This is now DNA Standard 1A.
   Aliquot 600 µL of 1X TE into each of the six remaining tubes labeled 1B-1G.
   Add 600 µL of DNA Standard 1A to the 600 µL of 1X TE in tube 1B. Vortex to mix thoroughly.

Initials: RCS Date: 10/7/26

S100 QuantiBlot DNA Standards lot number: _____
standard batch size: variable

Add 600 µL of diluted DNA Standard (tube 1B) to the 600 µL of 1X TE in tube 1C. Vortex to mix thoroughly.
Add 600 µL of diluted DNA Standard (tube 1C) to the 600 µL of 1X TE in tube 1D. Vortex to mix thoroughly.
Continue the serial dilution through tube 1G.

6. Store at 2° to 8°C.
7. DNA Standards are stable for at least 3 months as 2° to 8°C.

If the dilution steps are performed as described above, the seven DNA Standard tubes will have the following concentrations of human DNA:

DNA Standards		
Standard Tube	Conc (ng/µL)	Quantity (ng/5µL)
1A	2	10
1B	1	5
1C	0.5	2.5
1D	0.25	1.25
1E	0.125	0.625
1F	0.0625	0.3125
1G	0.03125	0.15625

Data Log source lot amount

RM442 DNA Standard A _____ _____ _____

S039 TE, 1X _____ _____ _____

Quality Control
QC018 QuantiBlot Hybridization.

made by: _____ date: _____

Initials: RCS Date: 10/7/96

S099 QuantiBlot Wash Solution lot number: _____

standard batch size: 4 x 4 L

Ingredients	**final concentration**	**amount/ 4 Liter**
S002 SSPE, 20X	1.5 X	300 ± 10 mL
S001 SDS, 20%	0.5 %	100 ± 5 mL

Procedure

Measure 3600 mL deionized water into four 4 L bottles.

Add 300 mL SSPE and 100 mL SDS to each bottle.

Cap and mix well by inverting.

Store at room temperature.

Data Log	source	lot	amount
S002 SSPE, 20X	_____	_____	_____
S001 SDS, 20%	_____	_____	_____

made by: _____ date: _____

Initials: RU Date: 10/7/96

S054 Sarkosyl, 10% lot number: _____

standard batch size: 100 mL

Ingredients	final concentration	amount
S040 sarkosyl, 20%	10. %	50 ± 2 mL

Procedure

Dilute 50 mL of 20% sarkosyl with 50 mL deionized water.

Mix well.

Filter sterilize.

Dispense into sterile 15 mL tubes.

Store at 2-8°C.

Data Log	source	lot	amount
S040 sarkosyl, 20%	_____	_____	_____

made by: _____ date: _____

Initials: RY Date: 10/7/96

S040 Sarkosyl, 20% lot number: _____

standard batch size: 100 mL

Ingredients	final concentration	amount
RM057 sarkosyl	20 %	20 ± 0.5 g

Procedure

Add the sarkosyl to approximately 75 mL deionized water.

Mix until the solution is completely clear.

Bring up to volume with deionized water.

Filter sterilize.

Dispense into sterile 15 mL tubes.

Store at 2-8°C.

Data Log	source	lot	amount
RM057 sarkosyl	_____	_____	_____

made by: _____ date: _____

Initials: AC Date: 2/9/98

S132 Sequencing loading buffer lot number: _____

standard batch size: 25mL

Ingredients	final	amount
S009 500mM EDTA, pH8.0	25 mM	1.25 ± 0.05mL
RM Blue Dextran	50 mg/mL	1250 mg ± 10 mg

Procedure

NOTE: PREPARE AWAY FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

Pipette EDTA into a 25mL cylinder. Fill up to 25mL using deionized water.

Decant into an 100mL Erlenmeyer flask. Add Blue Dextran. Stir at room temperature until dissolved.

Label 25 1.5mL reaction tubes.

Add 1000μL of the sequencing loading buffer to each tube. Close all tubes.

Store at 2-8°C.

Data Log

	source	lot	amount
S009 500mM EDTA, pH8.0	_____	_____	_____
RM Blue Dextran	_____	_____	_____

Quality Control

QC033 STR gel electrophoresis

made by:_____date:_____

Initials: RSJ Date: 10/7/96

S101 SDS, 0.1% lot number: _____

standard batch size: 20 L

Ingredients	**final concentration**	**amount**
S001 SDS, 20%	0.1 %	100 ± 10 mL

Procedure

Add approximately 15 L of deionized water into a 20 L carboy.

Add 100 mL 20% SDS.

Mix .

Bring up to a final volume of 20 L with deionized water.

Mix.

Store at room temperature.

Data Log	source	lot	amount
S001 SDS, 20%	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S045 SDS, 10% lot number: _____
standard batch size: 100 mL

Ingredients	final concentration	amount
RM007 sodium dodecyl sulfate	10 %	10.0 ± 0.3 g

Procedure
CAUTION: AN AEROSOL MASK OR FUME HOOD MUST BE USED WHEN MAKING THIS SOLUTION.. WEAR GOGGLES FOR EYE PROTECTION.

Dissolve the SDS in approximately 75 mL deionized water.

Warm the solution until all the solids have dissolved and the solution is clear.

Bring up to volume with deionized water.

Filter sterilize the warm solution.

Dispense into sterile 100 mL bottles.

Store at room temperature.

Data Log	source	lot	amount
RM007 sodium dodecyl sulfate	_____	_____	_____

Quality Control

QC023 QuantiBlot Quality Control of Solutions- Test 25 µL of solution

made by: _____ date: _____

Initials: RCJ Date: 10/17/96

S001 SDS, 20% lot number: _____
standard batch size: 1 L

Ingredients	final concentration	amount
RM007 sodium dodecyl sulfate	20 %	200 ± 5 g

Procedure
CAUTION: AN AEROSOL MASK OR FUME HOOD MUST BE USED WHEN MAKING THIS SOLUTION. WEAR GOGGLES FOR EYE PROTECTION.

Warm approximately 750 mL deionized water on a stirring hot plate.

Add a fraction of the SDS, allowing the solids to dissolve before adding more.

Add the SDS until it is all in solution.

When the solution is clear, bring up to volume with deionized water.

Filter sterilize the warm solution.

Dispense into sterile 500 mL bottles.

Store at room temperature.

Data Log	source	lot	amount
RM007 SDS	_____	_____	_____

made by: _____ date: _____

Initials: RCS Date: 10/7/85

S046 SLS, 20% lot number: _____
standard batch size: 4 L

Ingredients	final concentration	amount
RM218 sodium lauryl sulfate	20 %	800 ± 5 g

Procedure
CAUTION: AN AEROSOL MASK OR FUME HOOD MUST BE USED WHEN MAKING THIS SOLUTION. WEAR GOGGLES FOR EYE PROTECTION.

Warm approximately 2.5 L deionized water on a stirring hot plate.

Add a fraction of the SLS, allowing the solids to dissolve before adding more.

Add SLS until it is all in solution.

Mix well.

When the solution is clear, bring up to volume with deionized water.

Sterile filter each 2 L with a sterile unexpired cellulose nitrate filter.

Dispense into 1 L bottles.

Store at room temperature.

Data Log	source	lot	amount
RM218 sodium lauryl sulfate	_____	_____	_____

made by: _____ date: _____

Initials: RJ Date: 10/7/86

S043 Sodium Acetate, 2 M lot number: _____
standard batch size: 100 mL

Ingredients	final concentration	amount
RM059 sodium acetate, anhydrous	2.0 M	16.4 ± 0.4 g

Procedure

Slowly add the sodium acetate to approximately 50 mL deionized water.

Mix well.

Bring up to volume with deionized water.

Mix well.

Dispense into 100 mL bottles.

Autoclave at 250°F for 30 minutes.

Dispense into 15 mL tubes.

Store at room temperature.

Data Log	source	lot	amount
RM059 sodium acetate, anhydrous	_____	_____	_____

Quality Control

QC023 QuantiBlot Quality Control of Solutions- Test 20 µL of solution

made by: _____ date: _____

Initials: RCJ Date: 10/7/15

S044 Sodium Acetate, 0.2M lot number: _____
standard batch size: 250 mL

Ingredients	final concentration	amount
RM059 sodium acetate, anhydrous	0.2 M	4.1 ± 0.1 g

Procedure

Slowly add the sodium acetate to approximately 200 mL deionized water.

Mix well.

Bring up to volume with deionized water.

Mix well.

Dispense into 100 mL bottles.

Autoclave at 250°F for 30 minutes.

Store at room temperature.

Data Log	source	lot	amount
RM059 sodium acetate, anhydrous	_____	_____	_____

Quality Control

QC023 QuantiBlot Quality Control of Solutions- Test 150 µL of solution

made by: _____ date: _____

Initials: RU Date: 10/7/96

S035 SP, 25X lot number: _____
standard batch size: 4 L

Ingredients	final concentration	amount
RM003 EDTA	25 mM	37.2 ± 0.8 g
RM004 sodium hydroxide, 10N	-----	100 mL (guideline)
RM005 sodium chloride	3.75 M	877 ± 1 g
RM006 sodium phosphate, monobasic	0.25 M	138 ± 3 g

Procedure
Dissolve the EDTA in approximately 2 liters deionized water.
Adjust the pH to approximately 8.0 with 10N NaOH to help dissolve the EDTA.
Add the sodium phosphate. Mix until dissolved.
Add the sodium chloride.
Adjust the pH to 7.4 ± 0.2 with 10N NaOH (about 80 mL).
Adjust the final volume to 4 liters with deionized water.
Measure and record the final pH.
Sterile filter each 2 liters with a sterile unexpired cellulose nitrate filter.
Store at room temperature.

Data Log	source	lot	amount
RM003 EDTA	_____	_____	_____
RM004 sodium hydroxide, 10N	_____	_____	_____
RM005 sodium chloride	_____	_____	_____
RM006 sodium phosphate, monobasic	_____	_____	_____

Quality Control

final pH: _____ specification 7.4 ± 0.2

made by: _____ date: _____

Initials: RG Date: 10/17/96

S108 SP, 2X lot number: _____

standard batch size: 1 L

Ingredients	final concentration	amount
S035 SP, 25X	2.0 X	80.0 ± 0.8 mL

Procedure

Add the SP to approximately 800 mL deionized water.

Bring up to the final volume with deionized water.

Dispense into a 500 mL bottles.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
S035 SP, 25X	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 10/17/96

S098 Spotting Solution lot number: _____

standard batch size: 75 mL

Ingredients	**final concentration**	**amount**
S097 Pre-Wetting Solution	---	74.85 mL ± 1 mL
RM443 Bromothymol Blue, 0.04%	0.00008%	150 µL ± 1 µL

Procedure

Measure 74.85 mL Pre-Wetting Solution into a graduated cylinder and pour into a 100 mL bottle.

Add 150 µL bromothymol blue.

Cap and mix well by inverting.

Store at room temperature.

Data Log	source	lot	amount
S097 Pre-Wetting Solution	_____	_____	_____
RM443 Bromothymol Blue, 0.04%	_____	_____	_____

made by: _____ date: _____

Initials: RY Date: 10/7/96

S002 SSPE, 20X lot number: _____
standard batch size: 4 L

Ingredients	final concentration	amount
RM003 EDTA	20. mM	29.8 ± 0.7 g
RM004 sodium hydroxide, 10N	-----	40 ± 5 mL (guideline)
RM005 sodium chloride	3.6 M	840 ± 10 g
RM006 sodium phosphate, monobasic	200 mM	110 ± 3 g

Procedure
Dissolve the EDTA in approximately 3 liters deionized water.
Adjust the pH to approximately 6.0 with 10N sodium hydroxide to help dissolve the EDTA.
Add the sodium phosphate first and then the sodium chloride.
Adjust the pH to 7.4 with 10N sodium hydroxide (about 40 mL).
Adjust the final volume to 4 liters with deionized water.
Measure and record the final pH.
Dispense into 1 L bottles.
Store at room temperature.

Data Log	source	lot	amount
RM003 EDTA	_____	_____	_____
RM004 sodium hydroxide, 10N	_____	_____	_____
RM005 sodium chloride	_____	_____	_____
RM006 sodium phosphate, monobasic	_____	_____	_____

Quality Control

final pH: _____ specification 7.4 ± 0.2

made by: _____ date: _____

Initials: RU Date: 10/7/96

S047 Stain Extraction Buffer lot number: _____
standard batch size: 1 L

Ingredients	final concentration	amount
S009 EDTA, 0.5M	10. mM	20 ± 1 mL
S052 TRIS-HCl, 0.1M - pH 7.8	10. mM	100 ± 0.5 mL
RM005 sodium chloride	100 mM	5.8 ± 0.2 g
RM101 dithiothreitol	33.9 mM	5.227 ± 0.008 g
S046 SDS, 20%	2.0 %	100 ± 3 mL
RM004 sodium hydroxide, 10N	-----	-----

Procedure
Add all the ingredients except for the SDS to approximately 400 mL deionized water. Mix well.
Adjust the pH to 8.0 with 10N NaOH.
Record the pH.
Add the SDS. Mix well.
Bring up to the final volume with deionized water.
Dispense into sterile 125 mL bottles.
Store at 2-8°C.

Data Log	source	lot	amount
S009 EDTA, 0.5M	_____	_____	_____
S052 TRIS-HCl, 0.1M - pH 8.0	_____	_____	_____
RM005 sodium chloride	_____	_____	_____
RM101 dithiothreitol	_____	_____	_____
S046 SDS, 20%	_____	_____	_____
RM004 sodium hydroxide, 10N	_____	_____	_____

Quality Control

final pH: _____ specification 8.0 ± 0.2

QC023 QuantiBlot Quality Control of Solutions- Test 150 µL of solution

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S059 Sterile Water lot number: _____

standard batch size: 500 mL

Procedure

Filter sterilize 500 mL of deionized water.

Aliquot 10 mL each into 15 mL centrifuge tubes.

Autoclave at 250°F for 30 minutes.

Store at room temperature.

Quality Control

QC023 QuantiBlot Quality Control of Solutions- Test 150 µL of solution

made by: _____ date: _____

Initials: RCS Date: 10/7/96

S023 Stripping Solution lot number: _____
standard batch size: 2 L

Ingredients	final concentration	amount
RM102 formamide	55 %	1100 ± 10 mL
S035 SP, 25X	2.0 X	160 ± 4 mL
S046 SLS, 20%	1.0 %	100 + 6 mL

NOTE: S001 SDS, 20% can be substituted for 20% SLS in this solution.

Procedure
Add the SP and formamide to 1280 mL deionized water.

Mix well.

Add the SDS and mix gently.

Dispense into a 4 L brown bottle.

Store at 2-8°C.

Data Log	source	lot	amount
RM102 formamide	_____	_____	_____
S035 SP, 25X	_____	_____	_____
S046 SLS, 20%	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 10/21/96

S017 TRIS-HCl, 1M - PH 8.0 lot number: _____

standard batch size: 500 mL

Ingredients	final concentration	amount
RM073 TRIS	1.00 M	60.5 ± 0.1 g
RM096 hydrochloric acid	------	------

Procedure

Add the TRIS to approximately 400 mL deionized water.

Mix well.

Adjust the pH to 8.0 with concentrated hydrochloric acid.

Bring up to final volume with deionized water.

Measure and record the final pH.

Prepare a 1:100 dilution (10 mM TRIS-HCl) by mixing 1 mL TRIS-HCl solution and 99 mL deionized water.

Measure and record the pH of the dilution.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
RM073 TRIS	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

Quality Control

final pH: _____ spec: 8.0 ± 0.1 1:100 pH: _____ spec: 8.0 ± 0.1

made by: _____ date: _____

Initials: RS Date: 10/7/96

S039 TE, 1X lot number: _____
standard batch size: 500 mL

Ingredients	final concentration	amount
S049 TE, 100X	1.0 X	5.0 ± 0.3 mL

Procedure
Add the TE to approximately 400 mL deionized water.

Bring up to the final volume with deionized water.

Dispense into 125 mL bottles.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
S049 TE, 100X	_____	_____	_____

Quality Control

final pH: _____ specification: 8.0 ± 0.2

QC023 QuantiBlot Quality Control of Solutions- Test 150 µL of solution

made by: _____ date: _____

Initials: RCJ Date: 10/21/96

S128 TE^{-4}, 1X lot number: _____

standard batch size: 500 mL

Ingredients	final concentration	amount
S017 TRIS-HCl, pH 8.0, 1 M	10 mM	5.0 ± 0.3 mL
S009 EDTA, 0.5 M	0.1 mM	100± 2 µL

Procedure

Add the TRIS and EDTA to 495 mL deionized water. Mix well

Filter Sterilize

Dispense into 15 mL sterile centrifuge tubes.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
S017 TRIS-HCl, pH 8.0, 1 M	_____	_____	_____
S009 EDTA, 0.5 M	_____	_____	_____

Quality Control

final pH: _____ specification: 8.0 ± 0.2

QC023 QuantiBlot Quality Control of Solutions- Test 150 µL of solution

made by: _____ date: _____

Initials: RC Date: 10/7/96

S049 TE, 100X lot number: _____
standard batch size: 250 mL

Ingredients	final concentration	amount
RM003 EDTA	0.10 M	9.3 ± 0.5 g
RM073 TRIS	1.00 M	30.3 ± 0.1 g
RM004 sodium hydroxide, 10N	------	-------
RM096 hydrochloric acid	------	-------

Procedure
Add the EDTA to approximately 200 mL deionized water.
Adjust the pH to approximately 8.0 with sodium hydroxide to get the EDTA into solution. Mix until totally dissolved.
Add the TRIS and mix well.
Use hydrochloric acid or sodium hydroxide to adjust the pH of the solution to 8.0.
Bring up to final volume with deionized water.
Measure and record the final pH.
Dispense into 125 mL bottles.
Autoclave at 250°F for 30 minutes.
Store at room temperature.

Data Log	source	lot	amount
RM003 EDTA	_____	_____	_____
RM073 TRIS	_____	_____	_____
RM004 sodium hydroxide, 10N	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

Quality Control

final pH: _____ specification: 8.0 ± 0.2

made by: _____ date: _____

Initials: RS Date: 10/7/96

S050 Test Gel Loading Buffer lot number: _____
standard batch size: 100 mL

Ingredients	final concentration	amount
RM020 bromophenol blue	0.10%	0.10 ± 0.01 g
RM217 xylene cyanol	0.10%	0.10 ± 0.01 g
RM040 Ficoll 400	5.0%	5.0 ± 0.1 g
S009 EDTA, 0.5M	20. mM	2.00 ± 0.05 mL
RM083 TAE, 10X	2.0 X	20.0 ± 0.5 mL

Procedure
Combine the TAE, EDTA, and Ficoll.
Mix well. The solution may need to be heated gently to dissolve the Ficoll.
Add the bromophenol blue and xylene cyanol.
Mix well.
When all the solids are dissolved, bring up to volume using deionized water.
Filter sterilize.
Dispense 1.5 mL aliquots into 1.5 mL eppendorf tubes.
Store at -20°C.

Data Log	source	lot	amount
RM020 bromophenol blue	_____	_____	_____
RM217 xylene cyanol	_____	_____	_____
RM040 Ficoll 400	_____	_____	_____
S009 EDTA, 0.5M	_____	_____	_____
RM083 TAE, 10X	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S062 Test Gel Standard lot number: _____

INGREDIENTS	initial concentration (ng/µL)	initial volume (µL)	final concentration	final volume (µL)
RM242 K562 DNA, Hae III fragments			5 ng/µL	
S050 test gel loading buffer	2 X		1 X	----
S059 sterile water	-----		----	----

Calculations

Record the initial concentration in ng/µL and the initial volume in µL of the K562 DNA, Hae III fragments received from the manufacturer.

Calculate the final volume according to equation 1.

$$\text{(final volume)} = \frac{\text{(initial DNA concentration)(initial DNA volume)}}{\text{(5 ng/µL)}} \qquad \textbf{equation 1}$$

Record the final volume above. The final volume is the total batch size.

Calculate the amount of buffer to be added according to equation 2.

$$\text{(buffer volume)} = 0.5(\text{final volume}) \qquad \textbf{equation 2}$$

Calculate the amount of sterile water to be added according to equation 3.

$$\text{(water volume)} = [0.5 * (\text{final volume})] - (\text{initial DNA volume}) \qquad \textbf{equation 3}$$

Record the buffer and water volumes above.

To check the calculations, add together the initial volumes of DNA, loading buffer, and sterile water.

The sum of the initial volumes must be equal to the calculated final volume.

Initials: RU Date: 10/7/96

S062 Test Gel Standard lot number: _____

Procedure

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 500 µL aliquots into sterile 1.5 mL eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM242 K562 DNA, Hae III fragments	_____	_____	_____
S050 test gel loading buffer	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC026 Gel Electrophoresis

made by: _____ date: _____

Initials: RCS Date: 10/2/96

S051 TNE, 10X lot number: _____

standard batch size: 100 mL

Ingredients	final concentration	amount
RM073 TRIS	100 mM	1.2 ± 0.02 g
S009 EDTA, 0.5M	10 mM	2.0 ± 0.1 mL
RM005 sodium chloride	1.0 M	5.8 ± 0.2 g
RM096 hydrochloric acid	---	

Procedure

Add the TRIS, EDTA, and sodium chloride to approximately 75 mL deionized water.
Mix well.
Adjust the pH to 7.4 with hydrochloric acid
Bring up to the final volume with deionized water.
Measure and record the final pH. Adjust with concentrated HCl if necessary.
Filter to remove any particulates.
Dispense into a sterile 125 mL bottle.
Store at room temperature.

Data Log	source	lot	amount
RM073 TRIS	_____	_____	_____
S009 EDTA, 0.5M	_____	_____	_____
RM005 sodium chloride	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

Quality Control

final pH: _____ specification: 7.4 ± 0.1

made by: _____ date: _____

Initials: RY Date: 10/7/96

S107 TNE, 1X lot number: _____

standard batch size: 100 mL

Ingredients	final concentration	amount
S051 TNE, 10X	1.0 X	10.0 ± 0.3 mL

Procedure

Add the TNE to approximately 80 mL deionized water.

Bring up to the final volume with deionized water.

Dispense into a 125 mL bottles.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
S051 TNE, 10X	_____	_____	_____

Quality Control

QC023 QuantiBlot Quality Control of Solutions- Test 150 µL of solution

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S052 TRIS, 0.1M - pH 7.8 lot number: _____
standard batch size: 1 L

Ingredients	final concentration	amount
RM073 TRIS	0.1 M	12.1 ± 0.2 g
RM096 hydrochloric acid	---	

Procedure
Add the TRIS to approximately 750 mL deionized water.

Mix well.

Adjust the pH to 7.8 with hydrochloric acid.

Bring up to the final volume with deionized water.

Mix well.

Dispense into a 1 L bottles.

Store at room temperature.

Data Log	source	lot	amount
RM073 TRIS	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

Quality Control

final pH: _____ spec: 7.8 ± 0.1

made by: _____ date: _____

Initials: RY Date: 10/7/96

S114 Urea (25 g Aliquot) lot number: _____

standard batch size: ~ 25 tubes x 25 g

Ingredients	aliquot	total amount
RM0079 Urea electrophoresis grade	25 ± 0.2 g	625 ± 6 g

Procedure

NOTE: WHEN WORKING WITH POWDERED UREA WEAR GLOVES, EYE PROTECTION, LAB COAT, AND RESPIRATOR FOR SAFETY.

Fill out chemical logbook.

Using small weigh boat, weigh 25 ± 0.2 g aliquots of urea.

Transfer the aliquots to 50 mL conical tubes

Cap all tubes tightly and label rack containing tubes with contents, lot number, date, initials, and safety data.

Store at room temperature.

Data Log	source	lot	amount
RM0079 urea	_____	_____	_____

made by:_____date:_____

Initials: RCJ Date: 2/9/98

S114a Urea (18 g Aliquot-377 Sequencer) lot number: _____

standard batch size: ~ 25 tubes x 18 g

Ingredients	aliquot	total amount
RM0079 Urea electrophoresis grade	18 ± 0.1 g	450 ± 4 g

Procedure

NOTE: WHEN WORKING WITH POWDERED UREA WEAR GLOVES, EYE PROTECTION, LAB COAT, AND RESPIRATOR FOR SAFETY.

Fill out chemical logbook.

Using small weigh boat, weigh 18 ± 0.1 g aliquots of urea.

Transfer the aliquots to 50 mL conical tubes

Cap all tubes tightly and label rack containing tubes with contents, lot number, date, initials, and safety data.

Store at room temperature.

Data Log	source	lot	amount
RM0079 urea	_____	_____	_____

made by:_____date:_____

Initials: RCJ Date: 2/9/98

S114b Urea (10.8 g Aliquot-377 Sequencer) lot number: _____

standard batch size: ~ 25 tubes x 10.8 g

Ingredients	aliquot	total amount
RM0079 Urea electrophoresis grade	10.8 ± 0.1 g	450 ± 4 g

Procedure

NOTE: WHEN WORKING WITH POWDERED UREA WEAR GLOVES, EYE PROTECTION, LAB COAT, AND RESPIRATOR FOR SAFETY.

Fill out chemical logbook.

Using small weigh boat, weigh 10.8 ± 0.1 g aliquots of urea.

Transfer the aliquots to labeled 50 mL conical tubes.

Cap all tubes tightly and label rack containing tubes with contents, lot number, date, initials, and safety data.

Store at room temperature.

Data Log	source	lot	amount
RM0079 urea	_____	_____	_____

made by:_____date:_____

Initials: RCJ Date: 10/7/96

S024 Wash Solution #1 lot number: _____

standard batch size: 4 L

Ingredients	final concentration	amount
S035 SP, 25X	2.0 X	320 ± 8 mL
S046 SLS, 20%	0.50 %	100 ± 1 mL

NOTE: S001 SDS, 20% can be substituted for 20% SLS in this solution.

Procedure

Add the SP to approximately 3 L deionized water.

Add the SLS to the solution.

Mix gently.

Bring up to the final volume with deionized water.

Dispense into a 4 L bottle.

Store at room temperature.

Data Log	source	lot	amount
S035 SP, 25X	_____	_____	_____
S046 SLS, 20%	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 10/7/96

S025 Wash Solution #2 lot number: _____

standard batch size: 4 L

Ingredients	final concentration	amount
S035 SP, 25X	0.10 X	16.0 ± 0.8 mL
S046 SLS, 20%	0.50 %	100 ± 1 mL

NOTE: S001 SDS, 20% can be substituted for 20% SLS in this solution.

Procedure

Add the SP to approximately 3 L deionized water.

Add the SLS to the solution.

Mix gently.

Bring up to the final volume with deionized water.

Dispense into a 4 L bottle.

Store at room temperature.

Data Log	source	lot	amount
S035 SP, 25X	_____	_____	_____
S046 SLS, 20%	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 10/21/96

S020 Yield Calibrators lot number: _____
standard batch size: 5 X 400µL each

Ingredients	final concentration	amount
S128 TE^{-4}, 1X	1 X	------
RM148 lambda DNA	-----	140 ± 10 µg (guideline)
S021 yield gel loading buffer	1.25 X	3.0 ± 0.5 mL
S059 Sterile Water	----	

Calculations

Stock Solution

Final DNA Concentration	Final Volume	Initial DNA Concentration	Volume Lambda DNA	Volume 1X TE^{-4}
50 ng/µL	2800 µL			

Calibrators

Calibrator	Final DNA Concentration	Stock DNA Concentration	Volume Stock DNA	Volume Water	Volume Buffer
A	300 ng/10 µL	50 ng/µL	1200 µL	300 µL	500 µL
B	200 ng/10 µL	50 ng/µL	800 µL	700 µL	500 µL
C	100 ng/10 µL	50 ng/µL	400 µL	1100 µL	500 µL
D	50 ng/10 µL	50 ng/µL	200 µL	1300 µL	500 µL
E	25 ng/10 µL	50 ng/µL	100 µL	1400 µL	500 µL
F	10 ng/10 µL	50 ng/µL	40 µL	1460 µL	500 µL

Procedure

Each lot of yield calibrators is prepared as a batch of five sets. Each batch requires 2800 µL of 50 ng/µL stock lambda DNA solution.

Record the concentration in ng/µL of the lambda DNA received from the manufacturer under initial DNA concentration.

Initials: RO Date: 10/21/96

S020 Yield Calibrators lot number: _____

Procedure

Calculate the volume of lambda DNA required for the stock solution according to equation 1.

$$\text{(volume lambda DNA)} = \frac{\text{(final DNA concentration)(final volume)}}{\text{(initial DNA concentration)}} \qquad \textbf{equation 1}$$

Calculate the volume of 1X TE^{-4} to add to the stock solution according to equation 2.

(volume 1X TE^{-4}) = (final volume) - (volume lambda DNA) **equation 2**

Prepare the stock solution by diluting the lambda DNA in a sterile centrifuge tube with 1X TE^{-4} and mix well.
Label six sterile eppendorf tubes, one for each of the six yield calibrator levels.
Pipet the appropriate amounts of DNA stock solution and sterile water into the labeled tubes.
The combined volume of DNA and water is 1500 µL for each level.
Mix well.
Divide each level into five 300 µL aliquots, and dispense into labeled, sterile eppendorf tubes.
Add 100 µL of yield gel loading buffer to each tube. The final volume of each aliquot is 400 µL.
Store at -20°C.

Data Log	source	lot	amount
S128 TE^{-4}, 1X	_____	_____	_____
RM148 lambda DNA	_____	_____	_____
S021 yield gel loading buffer	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC026 Gel Electrophoresis

made by: _____ date: _____

Initials: RG Date: 10/7/96

S020 Yield Calibrators
standard batch size: 5 X 400µL each lot number: _____

Ingredients

	final concentration	amount
S039 TE, 1X	1 X	------
RM148 lambda DNA	-----	140 ± 10 µg (guideline)
S021 yield gel loading buffer	1.25 X	3.0 ± 0.5 mL
S059 Sterile Water	----	

Calculations

Stock Solution

Final DNA Concentration	Final Volume	Initial DNA Concentration	Volume Lambda DNA	Volume 1X TE
50 ng/µL	2800 µL			

Calibrators

Calibrator	Final DNA Concentration	Stock DNA Concentration	Volume Stock DNA	Volume Water	Volume Buffer
A	300 ng/10 µL	50 ng/µL	1200 µL	300 µL	500 µL
B	200 ng/10 µL	50 ng/µL	800 µL	700 µL	500 µL
C	100 ng/10 µL	50 ng/µL	400 µL	1100 µL	500 µL
D	50 ng/10 µL	50 ng/µL	200 µL	1300 µL	500 µL
E	25 ng/10 µL	50 ng/µL	100 µL	1400 µL	500 µL
F	10 ng/10 µL	50 ng/µL	40 µL	1460 µL	500 µL

Procedure
Each lot of yield calibrators is prepared as a batch of five sets. Each batch requires 2800 µL of 50 ng/µL stock lambda DNA solution.

Record the concentration in ng/µL of the lambda DNA received from the manufacturer under initial DNA concentration.

Initials: KCJ Date: 10/7/96

S021 Yield Gel Loading Buffer lot number: _____

standard batch size: 100 mL

Ingredients	final concentration	amount
RM020 bromophenol blue	0.25%	0.25 ± 0.01 g
RM217 xylene cyanol	0.25%	0.25 ± 0.01 g
RM040 Ficoll 400	12.5%	12.5 ± 0.1 g
S009 EDTA, 0.5M	50. mM	10.0 ± 0.1 mL
RM083 TAE, 10X	5.0 X	50.0 ± 0.5 mL
S001 SDS, 20%	0.20 %	1.00 ± 0.02 mL

Procedure

Combine the TAE, EDTA, SDS, and Ficoll.

Mix well. The solution may need to be heated gently to dissolve the Ficoll.

Add the bromophenol blue and xylene cyanol.

Mix well.

When all the solids are dissolved, bring up to volume using deionized water.

Filter sterilize.

Dispense 1.5 mL aliquots into sterile 1.5 mL eppendorf tubes.

Store at -20°C.

Initials: RY Date: 10/7/95

S021 Yield Gel Loading Buffer lot number: _____

standard batch size: 100 mL

Data Log	source	lot	amount
RM020 bromophenol blue	_____	_____	_____
RM217 xylene cyanol	_____	_____	_____
RM040 Ficoll 400	_____	_____	_____
S009 EDTA, 0.5M	_____	_____	_____
RM083 TAE, 10X	_____	_____	_____
S001 20% SDS	_____	_____	_____

made by: _____ date: _____

Initials: RS Date: 10/7/96

S020 Yield Calibrators lot number: _____

Procedure

Calculate the volume of lambda DNA required for the stock solution according to equation 1.

$$\text{(volume lambda DNA)} = \frac{\text{(final DNA concentration)(final volume)}}{\text{(initial DNA concentration)}} \qquad \textbf{equation 1}$$

Calculate the volume of 1X TE to add to the stock solution according to equation 2.

(volume 1X TE) = (final volume) - (volume lambda DNA) **equation 2**

Prepare the stock solution by diluting the lambda DNA in a sterile centrifuge tube with 1X TE and mix well.
Label six sterile eppendorf tubes, one for each of the six yield calibrator levels.
Pipet the appropriate amounts of DNA stock solution and sterile water into the labeled tubes. The combined volume of DNA and water is 1500 µL for each level.
Mix well.
Divide each level into five 300 µL aliquots, and dispense into labeled, sterile eppendorf tubes.
Add 100 µL of yield gel loading buffer to each tube. The final volume of each aliquot is 400 µL.
Store at -20°C.

Data Log

	source	lot	amount
S039 TE, 1X			
RM148 lambda DNA			
S021 yield gel loading buffer			
S059 sterile water			

Quality Control

QC026 Gel Electrophoresis

made by: _____ date: _____