II

Forensic Molecular Biology

Solutions Manual

Version 2.0

Initials: RCJ Date: 4/7/94

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Initials: RCJ Date: 4/7/94

Numerical Table of Contents

Initials: RCJ Date: 4/7/94

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: RCS Date: 4/7/94

S060 Calibration Control lot number: _____

Ingredients	initial concentration (ng/μl)	initial volume (μl)	final concentration	final volume (μl)
RM221 K562 DNA			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.

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

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.

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

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

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

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: RS Date: 4/7/94

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	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/7/94

S010 Cell Lysis Buffer (CLB) lot number: _____

standard batch size: 2 L

Ingredients	final concentration	amount
RM068 sucrose	320 mM	219 ± 3 g
S007 TRIS-HCl, 1M - pH 7.6	10. mM	20 ± 1 ml
S008 magnesium chloride, 1M	5. mM	10 ± 1 ml
RM075 triton X-100	1.0 %	20 ± 1 ml

Procedure

Dissolve the sucrose in approximately 1.5 L deionized water.

Add the TRIS, magnesium chloride, and triton to the solution.

Mix well.

Adjust the volume to 2 L with deionized water.

Filter sterilize.

Dispense into sterile 500 ml bottles.

Store at 2-8°C.

Data Log	source	lot	amount
RM068 sucrose	_____	_____	_____
S007 TRIS-HCl, 1M - pH 7.6	_____	_____	_____
S008 magnesium chloride, 1M	_____	_____	_____
RM075 triton X-100	_____	_____	_____

made by: _____ date: _____

Initials: RH Date: 4/7/94

S064 Cell Pellet Control lot number: _____

Ingredients	concentration of cells	total volume (ml)	cells per aliquot	aliquot volume (ml)
RM243 K562 cells			$1 \cdot 10^6$	
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 $1 \cdot 10^6$cells according to equation 1.

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

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 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.

Add aliquots of cell suspension to 1.5 ml eppendorf tubes.

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S064 Cell Pellet Control lot number: _____

Spin the tubes at 180 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	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/7/94

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: QCJ Date: 4/7/94

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: RCJ Date: 4/2/94

S104 Chromogen Solution lot number: _____

standard batch size: 30 ml

Ingredients	final concentration	amount
RM435 chromogen: TMB	_____	60 mg
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	_____	_____	_____
ethanol, 100%	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 5/23/94

S094 Digest Buffer lot number: _____

standard batch size: 6 L

Ingredients	final concentration	amount
S009 EDTA, 0.5M	10. mM	120 ± 6 ml
S036 TRIS-HCl, 1M-pH 7.4	10. mM	60 ± 3 ml
S012 sodium chloride, 5M	50. mM	60 ± 1 ml
S001 SDS, 20%	2.0 %	600 ± 15 ml
RM096 hydrochloric acid	---	---

Procedure

Add the EDTA, TRIS, sodium chloride, and SDS to approximately 4 L deionized water.
Adjust the pH to 7.5.
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	_____	_____	_____
S036 TRIS-HCl, 1M-pH 7.4	_____	_____	_____
S012 sodium chloride 5M	_____	_____	_____
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: RCJ Date: 4/7/94

S094 Digest Buffer lot number: _____

standard batch size: 6 L

Ingredients	final concentration	amount
S009 EDTA, 0.5M	10. mM	120 ± 6 ml
S036 TRIS-HCl, 1M-pH 7.4	10. mM	60 ± 3 ml
S012 sodium chloride, 5M	50. mM	60 ± 1 ml
S001 SDS, 20%	2.0 %	600 ± 15 ml
RM096 hydrochloric acid	---	---

Procedure

Add the EDTA, TRIS, sodium chloride, and SDS to approximately 4 L deionized water.
Adjust the pH to 7.5.
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	_____	_____	_____
S036 TRIS-HCl, 1M-pH 7.4	_____	_____	_____
S012 sodium chloride 5M	_____	_____	_____
S001 SDS, 20%	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

Quality Control

final pH: _____ specification: 7.5 ± 0.1

QC017 Differential Extraction

made by: _____ date: _____

Initials: BCJ Date: 5/13/94

S093 DTT, 1M lot number: _____

standard batch size: 20 ml

Ingredients	final concentration	amount
RM101 dithiothreitol	1.0 M	3.1 ± 0.2 g
S059 sterile water	-----	-----

Procedure

Add the DTT to approximately 15 ml sterile, deionized water in a 50 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: RG Date: 4/7/94

S093 DTT, 1M lot number: _____

standard batch size: 20 ml

Ingredients	final concentration	amount
RM101 dithiothreitol	1.0 M	3.1 ± 0.2 g
S059 sterile water	-----	-----

Procedure

Add the DTT to approximately 15 ml sterile, deionized water in a 50 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

QC017 Differential Extraction

made by: _____ date: _____

Initials: RCJ Date: 4/7/94

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: RCS Date: 4/2/94

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: RG Date: 4/8/94

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

QC003 DQα hybridization

made by: _____ date: _____

Initials: RG Date: 4/7/94

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: RC Date: 4/7/94

S105 HLA-DQα PCR Reaction Mixture **lot number:** _____

standard batch size: ~ 55 tubes x 50 μl

Ingredients	final concentration	amount
HLA-DQα PCR reaction mix	_____	3 ml
HLA-DQα 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 50 μl of PCR reaction mixture into each tube.

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

Store at 2-8°C.

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

Quality Control

QC015 DQα Amplification

made by:_____ date:_____

Initials: RCJ Date: 4/7/94

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
RM284 hydrogen peroxide, 3%	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/7/94

S032 Lambda Marker lot number: _____

Ingredients	initial concentration (ng/μl)	initial volume (μl)	final concentration	final volume (μl)
RM155 lambda Hind III fragments			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.

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

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: 4/12/94

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	_____	_____	_____

made by: _____ date: _____

Initials: RG Date: 4/7/94

S008 Magnesium Chloride, 1M lot number: _____

standard batch size: 250 ml

Ingredients	final concentration	amount
RM046 magnesium chloride, hexahydrate	1.00 M	50.8 ± 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: RS Date: 4/7/94

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.

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

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.

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

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

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

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: 4/7/94

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	_____	_____	_____

made by: _____ date: _____

Initials: KC/ Date: 5/23/18

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: RCJ Date: 4/7/94

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

QC017 Differential Extraction

made by: _____ date: _____

Initials: RG Date: 4/12/94

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: RCJ Date: 4/7/94

S011 Protein Lysis Buffer (PLB) lot number: _____

standard batch size: 2 L

Ingredients	final concentration	amount
S036 TRIS-HCl, 1M - pH 7.4	10 mM	20 ± 1 ml
S009 EDTA, 0.5M	10 mM	40 ± 2 ml
S012 sodium chloride, 5M	10 mM	4.0 ± 0.2 ml

Procedure

Add the TRIS, EDTA, and sodium chloride to approximately 1.5 L deionized water.

Raise to the final volume with deionized water.

Mix well.

Dispense into 500 ml bottles.

Autoclave at 250°F for 30 minutes.

Store at 2-8°C.

Data Log	source	lot	amount
S036 TRIS-HCl, 1M - pH 7.4	_____	_____	_____
S009 EDTA, 0.5M	_____	_____	_____
S012 sodium chloride, 5M	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 5/23/94

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

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	_____	_____	_____

Quality Control

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

made by: _____ date: _____

Initials: RS Date: 4/8/94

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

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	_____	_____	_____

Quality Control

QC017 Differential Extraction

made by: _____ date: _____

Initials: RD Date: 4/7/94

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.

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
RM221 K652 DNA	_____	_____	_____
RM442 DNA Standard A	_____	_____	_____
S039 TE, 1X	_____	_____	_____

Quality Control
QC018 QuantiBlot Hybridization.

made by: _____ date: _____

Initials: RCJ Date: 5/19/99

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: RCJ Date: 4/7/94

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 2600 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: RCJ Date: 5/23/94

S106 Quantitation Check Standards lot number: _____

Ingredients	initial concentration (ng/μl)	initial volume (μl)	final concentration	final volume (μl)
RM221 K562 DNA			0.4 ng/μl	750 μl
S039 TE, 1X	-----		----	----

Calculations

Record the initial concentration in ng/μl of the K562 DNA as described in the procedure below.

Calculate the initial DNA volume according to equation 1.

$$\text{(initial DNA volume)} = \frac{(0.4\ \text{ng}/\mu\text{l})\ (750\ \mu\text{l})}{\text{(initial DNA concentration)}} \qquad \text{equation 1}$$

Record the initial DNA volume above.

Calculate the amount of S039 TE, 1X to be added according to equation 2.

$$\text{(TE volume)} = (750\ \mu\text{l}) - \text{(initial DNA volume)} \qquad \text{equation 2}$$

Record the TE volummes above.

Procedure

Let the DNA thaw in the freezer overnight. Mix gently.

Check the DNA concentration by yield gel and fluorimetry. Compare to the manufacturers concentration. If there is a discrepancy use the fluorimetry data.

Label three tubes Q1, Q2, Q3.

Combine the DNA and TE in Q1.

Mix well.

Initials: RC Date: 4/7/94

S106 Quantitiation Check Standards lot number: _____

Ingredients	initial concentration (ng/μl)	initial volume (μl)	final concentration	final volume (μl)
RM221 K562 DNA			0.4 ng/μl	750 μl
S039 TE, 1X	-----		----	----

Calculations

Record the initial concentration in ng/μl of the K562 DNA as described in the procedure below.

Calculate the initial DNA volume according to equation 1.

$$\text{(initial DNA volume)} = \frac{(0.4\ \text{ng}/\mu\text{l})\ (750\ \mu\text{l})}{\text{(initial DNA concentration)}} \qquad \text{equation 1}$$

Record the initial DNA volume above.

Calculate the amount of S039 TE, 1X to be added according to equation 2.

$$\text{(TE volume)} = (750\ \mu\text{l}) - \text{(initial DNA volume)} \qquad \text{equation 2}$$

Record the TE volums above.

Procedure

Let the DNA thaw in the freezer overnight. Mix gently.

Check the DNA concentration by yield gel and fluorimetry. Compare to the manufacturers concentration. If there is a discrepancy use the fluorimetry data.

Label three tubes Q1, Q2, Q3.

Combine the DNA and TE in Q1.

Mix well.

Initials: RCJ Date: 4/7/94

S106 Quantitiation Check Standards lot number: _____

Combine 250 μL of Q1 to 250 μl of TE in tube Q2.

Add 50 μl of Q1 to 450 μl of TE in tube Q3

Mix well

Aliquot each tube into 100 μl aliquots

Store one aliquot of each tube at -80°C for reference. Store the remainder at 4°C.

Q1 has 2ng/5μl, Q2 has 1ng/5μl, and Q3 has 0.2 ng/5μl

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

	yield gel	fluorimetry	manufacturer
RM221 K562 DNA	_____	_____	_____

Quality Control

QC018 QuantiBlot Hybridization- Compare to the previous lot of Quantitation Check Standard which was stored at -80°C.

made by: _____ date: _____

Initials: RJ Date: 4/7/94

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: RCI Date: 4/7/94

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: RCJ Date: 4/7/94

S080 Sodium Acetate, 1M lot number: _____

standard batch size: 100 mL

Ingredients	final concentration	amount
RM059 sodium acetate, anhydrous	1.0 M	8.2 ± 0.4 g
RM093 acetic acid, glacial	-----	-----

Procedure

Add the sodium acetate to approximately 75 ml deionized water.

Mix well.

Adjust the pH to 5.2 with glacial acetic acid.

Bring up to volume with deionized water.

Measure and record the final pH.

Dispense into a 100 ml bottle.

Autoclave at 250°F for 30 minutes.

Store at room temperature.

Data Log	source	lot	amount
RM059 sodium acetate, anhydrous	_____	_____	_____
RM093 acetic acid, glacial	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/7/94

S012 Sodium Chloride, 5M lot number: _____

standard batch size: 4 L

Ingredients	final concentration	amount
RM005 sodium chloride	5.0 M	1170 ± 10 g

Procedure

Slowly add the sodium chloride to approximately 2 L deionized water.

Raise the volume to just under 4 L so that the sodium chloride will go into solution.

Mix well.

Bring up to volume with deionized water.

Dispense into 1 L bottles.

Store at room temperature.

Data Log	source	lot	amount
RM005 sodium chloride	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/7/94

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: RCJ Date: 4/7/94

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: RCJ Date: 5/23/94

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: RCJ Date: 4/7/94

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.

made by: _____ date: _____

Initials: RCJ Date: 4/7/94

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

made by: _____ date: _____

Initials: RCI Date: 4/7/94

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: RCJ Date: 4/7/94

S036 TRIS-HCl, 1M - pH 7.4 lot number: _____

standard batch size: 250 ml

Ingredients	final concentration	amount
RM073 TRIS	1.00 M	30.3 ± 0.1 g
RM096 hydrochloric acid	------	------

Procedure

Add the TRIS to approximately 200 ml deionized water.

Mix well.

Adjust the pH to 7.4 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.

Dispense the 1M TRIS-HCl into 125 ml bottles.

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: _____ specification: 7.4 ± 0.1

1:100 pH: _____ specification: 7.4 ± 0.1

made by: _____ date: _____

Initials: RCJ Date: 4/7/94

S007 TRIS-HCl, 1M - pH 7.6 lot number: _____

standard batch size: 250 ml

Ingredients	final concentration	amount
RM073 TRIS	1.00 M	30.3 ± 0.1 g
RM096 hydrochloric acid	------	------

Procedure

Add the TRIS to approximately 200 ml deionized water.

Mix well.

Adjust the pH to 7.6 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.

Dispense the 1M TRIS-HCl into 125 ml bottles.

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: _____ specification: 7.6 ± 0.1

1:100 pH: _____ specification: 7.6 ± 0.1

made by: _____ date: _____

Initials: RCJ Date: 4/7/94

S020 Yield Calibrators lot number: _____

standard batch size: 5 X 400μl each

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 (guideline)

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.

S020 Yield Calibrators lot number: _____

Procedure

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

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

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.

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	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/7/94

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: RCI Date: 4/7/94

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: _____