

**II**

**Forensic Molecular Biology**

**Solutions Manual**

**Version 3.0**

Initials: *RD*

Date: *1/16/96*

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PCJ

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2/2/95

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S105 HLA-DQ $\alpha$ PCR Reaction Mixture	31
S107 TNE, 1X	72
S108 SP, 2X	60

Initials: RCJDate: 2/2/95**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: RLJ

Date: 2/2/95

**S060 Calibration Control**

lot number: \_\_\_\_\_

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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.

$$(\text{final volume}) = \frac{(\text{initial DNA concentration})(\text{initial DNA volume})}{(7.5 \text{ ng}/\mu\text{L})} \quad \text{equation 1}$$

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.

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

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

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

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: RY

Date: 2/2/95

**S060 Calibration Control**

lot number: \_\_\_\_\_

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**Procedure**

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 200  $\mu$ L 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: hd

Date: 5/9/95

**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: RCDate: 2/2/95**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 500 mL bottles.

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: *RCJ*

Date: *2/2/95*

**S064 Cell Pellet Control**

lot number: \_\_\_\_\_

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

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

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  $180 \times g$  for 5 minutes at  $4^\circ \text{C}$ . 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  $4^\circ \text{C}$ .

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: RCS

Date: 2/2/95

**S064 Cell Pellet Control**

lot number: \_\_\_\_\_

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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: RC

Date: 2/2/95

**S022 Chelex, 5%**

lot number: \_\_\_\_\_

standard batch size: 500 mL

**Ingredients**

final  
concentration

amount

RM027 Chelex 100

5. %

25 ± 2 g

S059 sterile water  
(guideline)

---

450 ± 50 mL

**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: RCJ

Date: 2/2/95

**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: RCJ

Date: 2/2/95

**S082 Chelex, 20%**

lot number: \_\_\_\_\_

standard batch size: 500 mL

**Ingredients**

final  
concentration

amount

RM027 Chelex 100

20. %

100  $\pm$  2 g

S059 sterile water

---

450  $\pm$  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: *RCS*

Date: *2/2/95*

**S027 Chloroform-Isoamyl Alcohol**

lot number: \_\_\_\_\_

standard batch size: 500 mL

**Ingredients**

final  
concentration

amount

RM088 chloroform

96. %

480  $\pm$  3 mL

RM089 isoamyl alcohol

4. %

20  $\pm$  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: RCJ

Date: 2/2/95

**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: RCJ

Date: 2/2/95

**S013 Denaturation Solution**

lot number: \_\_\_\_\_

standard batch size: 20 L

**Ingredients**

final  
concentration

amount

RM005 sodium chloride

1.5 M

1750  $\pm$  50 g

RM004 sodium hydroxide, 10N

0.5 M

1000  $\pm$  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:  $\geq$  12

made by: \_\_\_\_\_ date: \_\_\_\_\_

Initials: RCJ

Date: 2/2/95

**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: *RC*Date: *10/31/95***S061 Digestion Control**

lot number: \_\_\_\_\_

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INGREDIENTS	initial concentration (ng/ $\mu$ L)	initial volume ( $\mu$ L)	final concentration	final volume ( $\mu$ L)
RM221 K562 DNA			2 ng/ $\mu$ L	
S039 TE, 1X	1 X		----	----

**Calculations**

Record the initial concentration in ng/ $\mu$ L and the initial volume in  $\mu$ L of the K562 DNA received from the manufacturer.

Calculate the final volume according to equation 1.

$$(\text{final volume}) = \frac{(\text{initial DNA concentration})(\text{initial DNA volume})}{(2 \text{ ng}/\mu\text{L})} \quad \text{equation 1}$$

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.

$$(\text{TE volume}) = (\text{final volume}) - (\text{initial DNA volume}) \quad \text{equation 2}$$

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: RCJ

Date: 10/31/95

**S061 Digestion Control**

lot number: \_\_\_\_\_

page 2 of 2

**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: *RCJ*Date: *2/2/95***S061 Digestion Control**

lot number: \_\_\_\_\_

page 1 of 2

INGREDIENTS	initial concentration (ng/ $\mu$ L)	initial volume ( $\mu$ L)	final concentration	final volume ( $\mu$ L)
RM221 K562 DNA			1 ng/ $\mu$ L	
S039 TE, 1X	1 X		----	----

**Calculations**

Record the initial concentration in ng/ $\mu$ L and the initial volume in  $\mu$ L of the K562 DNA received from the manufacturer.

Calculate the final volume according to equation 1.

$$(\text{final volume}) = \frac{(\text{initial DNA concentration})(\text{initial DNA volume})}{(1 \text{ ng}/\mu\text{L})} \quad \text{equation 1}$$

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.

$$(\text{TE volume}) = (\text{final volume}) - (\text{initial DNA volume}) \quad \text{equation 2}$$

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.

**Procedure**

Combine the DNA and 1X TE.

Mix well.

Using sterile pipet tips, dispense 1.0 mL aliquots into sterile 1.5 mL eppendorf tubes.

Store at  $-20^{\circ}\text{C}$ .

Initials: RCJ

Date: 2/2/95

**S061 Digestion Control**

lot number: \_\_\_\_\_

page 2 of 2

**Data Log**

source

lot

amount

RM221 K562 DNA

\_\_\_\_\_

S039 TE, 1X

\_\_\_\_\_

**Quality Control**

QC026 Gel Electrophoresis

made by: \_\_\_\_\_ date: \_\_\_\_\_

Initials: RCJ

Date: 2/2/85

**S003 DQ $\alpha$  Citrate Buffer**

lot number: \_\_\_\_\_

standard batch size: 4 L

Ingredients	final concentration	amount
RM001 trisodium citrate	-----	73.6 $\pm$ 0.1 g
RM002 citric acid	-----	24 $\pm$ 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  $\pm$  0.2

made by: \_\_\_\_\_ date: \_\_\_\_\_

Initials: RG

Date: 2/2/95

**S004 DQ $\alpha$  Hybridization Solution**

lot number: \_\_\_\_\_

standard batch size: 4 L

**Ingredients**

final  
concentration

amount

S002 SSPE, 20X

5.0 X

1000  $\pm$  10 mL

S001 SDS, 20%

0.50 %

100  $\pm$  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 DQ $\alpha$  Hybridization

made by: \_\_\_\_\_ date: \_\_\_\_\_

Initials: RCJ

Date: 2/2/95

**S005 DQ $\alpha$  Wash Solution**

lot number: \_\_\_\_\_

standard batch size: 4 L

**Ingredients**

final  
concentration

amount

S002 SSPE, 20X

2.5 X

500  $\pm$  10 mL

S001 SDS, 20%

0.10 %

20  $\pm$  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 DQ $\alpha$  hybridization

made by: \_\_\_\_\_ date: \_\_\_\_\_

Initials: RCJ

Date: 5/9/95

**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: RCJ

Date: 2/2/85

**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: RCW

Date: 2/2/95

**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: 1/16/96

**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: RD

Date: 2/2/85

**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: 2/2/95

**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: RCJ

Date: 2/2/85

**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: RCJ

Date: 2/2/95

**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: PCJ

Date: 2/2/95

**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: RS

Date: 1/16/96

**S105 HLA-DQ $\alpha$  PCR Reaction Mixture**

**lot number:** \_\_\_\_\_

standard batch size: ~ 55 tubes x 50  $\mu$ L

Ingredients	final concentration	amount
HLA-DQ $\alpha$ PCR reaction mix	_____	3 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 50  $\mu$ L 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
PCR reaction mix	_____	_____	_____
PCR reaction tubes	_____	_____	_____

**Quality Control**

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

made by: \_\_\_\_\_ date: \_\_\_\_\_



Initials: RG

Date: 2/2/95

**S105 HLA-DQ $\alpha$  PCR Reaction Mixture**

lot number: \_\_\_\_\_

standard batch size: ~ 55 tubes x 50  $\mu$ L

**Ingredients**

final  
concentration

amount

HLA-DQ $\alpha$  PCR reaction mix

\_\_\_\_\_

3 mL

HLA-DQ $\alpha$  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  $\mu$ L 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

PCR reaction mix

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

PCR reaction tubes

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**Quality Control**

QC015 DQ $\alpha$  Amplification

made by: \_\_\_\_\_ date: \_\_\_\_\_

Initials: RD

Date: 2/2/95

**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: RCJ

Date: 2/2/85

**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  $\pm$  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<sub>2</sub>O<sub>2</sub>" 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: *RD*

Date: *2/2/80*

**S032 Lambda Marker**

lot number: \_\_\_\_\_

page 1 of 2

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.

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

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.

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

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

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

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: RD

Date: 2/2/81

**S032 Lambda Marker**

lot number: \_\_\_\_\_

page 2 of 2

### Procedure

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 500  $\mu$ L aliquots into sterile 1.5 mL eppendorf tubes.

Store at  $-20^{\circ}\text{C}$ .

### 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: *RD*

Date: *2/2/85*

**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: RW

Date: 2/2/85

**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: *RV*

Date: *2/2/85*

**S006 Phenol**

lot number: \_\_\_\_\_

standard batch size: 500 mL

page 1 of 2

**Ingredients**

final  
concentration

amount

RM112 phenol	---	500 $\pm$ 10 g
S052 TRIS, 0.1 M - pH 7.8	---	400 $\pm$ 10 mL
RM036 m-cresol	---	25 $\pm$ 1 mL
RM049 2-mercaptoethanol	---	1.0 $\pm$ 0.1 mL
RM029 4-hydroxyquinoline	---	0.50 $\pm$ 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: 2/2/95

**S006 Phenol**

lot number: \_\_\_\_\_

page 2 of 2

**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: RD

Date: 2/2/95

**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: *PC*

Date: *2/2/95*

**S042 Phi-X Marker**

lot number: \_\_\_\_\_

page 1 of 2

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.

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

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.

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

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

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

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: 2/2/85

**S042 Phi-X Marker**

lot number: \_\_\_\_\_

page 2 of 2

### Procedure

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 500  $\mu$ L 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: RDDate: 2/2/95**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: REJ

Date: 1/16/96

**S109 PM PCR Reaction Mixture**

lot number: \_\_\_\_\_

standard batch size: ~ 65 tubes x 40  $\mu$ L

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  $\mu$ L 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: RD

Date: 2/2/85

**S097 Pre-Wetting Solution**

lot number: \_\_\_\_\_

standard batch size: 4 L

Ingredients	final concentration	amount
RM004 NaOH, 10 N	0.4 N	160 $\pm$ 10 mL
S009 EDTA, 0.5 M	25 mM	200 $\pm$ 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: RG

Date: 1/16/96

**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: ResDate: 5/8/95**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 7.4 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:   LO  Date:   2/2/85  **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 7.4 with hydrochloric acid

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
S009 EDTA, 0.5M	_____	_____	_____
RM073 TRIS	_____	_____	_____
RM005 sodium chloride	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

**Quality Control**

final pH: \_\_\_\_\_ spec: 7.4 ± 0.1

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

made by: \_\_\_\_\_ date: \_\_\_\_\_

Initials: ACJ

Date: 2/2/95

**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: 2/2/95

**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: *RCF*

Date: *2/2/95*

**S100 QuantiBlot DNA Standards**

lot number: \_\_\_\_\_

standard batch size: variable

page 1 of 2

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  $\mu$ L of DNA Standard A into the tube labeled 1A. This is now DNA Standard 1A.

Aliquot 300  $\mu$ L of 1X TE into each of the six remaining tubes labeled 1B-1G.

Add 300  $\mu$ L of DNA Standard 1A to the 300  $\mu$ L of 1X TE in tube 1B. Vortex to mix thoroughly.

Add 300  $\mu$ L of diluted DNA Standard (tube 1B) to the 300  $\mu$ L of 1X TE in tube 1C. Vortex to mix thoroughly.

Add 300  $\mu$ L of diluted DNA Standard (tube 1C) to the 300  $\mu$ L 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  $\mu$ L of DNA Standard A into the tube labeled 1A. This is now DNA Standard 1A.

Aliquot 600  $\mu$ L of 1X TE into each of the six remaining tubes labeled 1B-1G.

Add 600  $\mu$ L of DNA Standard 1A to the 600  $\mu$ L of 1X TE in tube 1B. Vortex to mix thoroughly.

Initials: RC

Date: 2/2/85

**S100 QuantiBlot DNA Standards**

lot number: \_\_\_\_\_

standard batch size: variable

page 2 of 2

Add 600  $\mu$ L of diluted DNA Standard (tube 1B) to the 600  $\mu$ L of 1X TE in tube 1C. Vortex to mix thoroughly.

Add 600  $\mu$ L of diluted DNA Standard (tube 1C) to the 600  $\mu$ L 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/ $\mu$ L)	Quantity (ng/5 $\mu$ L)
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: 2/2/95

**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: 2/2/85

**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: RC

Date: 2/2/85

**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: RCJ

Date: 2/2/95

**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: 2/2/95

**S045 SDS, 10%**

lot number: \_\_\_\_\_

standard batch size: 100 mL

**Ingredients**

final  
concentration

amount

RM007 sodium dodecyl sulfate

10 %

10.0  $\pm$  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  $\mu$ L of solution

made by: \_\_\_\_\_ date: \_\_\_\_\_

Initials: BC

Date: 2/2/95

**S001 SDS, 20%**

lot number: \_\_\_\_\_

standard batch size: 1 L

**Ingredients**

final  
concentration

amount

RM007 sodium dodecyl sulfate

20 %

200  $\pm$  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: RS

Date: 2/2/85

**S046 SLS, 20%**

lot number: \_\_\_\_\_

standard batch size: 4 L

**Ingredients**

final  
concentration

amount

RM218 sodium lauryl sulfate

20 %

800  $\pm$  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: RU

Date: 2/2/85

**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: 2/2/81

**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: RCDate: 2/2/85**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  $7.4 \pm 0.2$  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  $7.4 \pm 0.2$ 

made by: \_\_\_\_\_ date: \_\_\_\_\_



Initials: RCJ

Date: 2/2/95

**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: PC

Date: 2/2/95

**S098 Spotting Solution**

lot number: \_\_\_\_\_

standard batch size: 75 mL

Ingredients	final concentration	amount
S097 Pre-Wetting Solution	---	74.85 mL $\pm$ 1 mL
RM443 Bromothymol Blue, 0.04%	0.00008%	150 $\mu$ L $\pm$ 1 $\mu$ L

**Procedure**

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

Add 150  $\mu$ L 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: RCJDate: 2/2/95**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: RCJDate: 2/2/95**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: 2/2/95

**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  $\mu$ L of solution

made by: \_\_\_\_\_ date: \_\_\_\_\_

Initials: 201

Date: 2/2/95

**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: 2/2/85

**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: 2/2/85

**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: RCJDate: 2/26/95**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: *2/2/95***S062 Test Gel Standard**

lot number: \_\_\_\_\_

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

**Calculations**

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

Calculate the final volume according to equation 1.

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

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.

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

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

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

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: 2/2/95

**S062 Test Gel Standard**

lot number: \_\_\_\_\_

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**Procedure**

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 500  $\mu$ L 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: RCJDate: 2/2/80**S051 TNE, 10X**

lot number: \_\_\_\_\_

standard batch size: 100 mL

**Ingredients**final  
concentration

amount

RM073 TRIS

100 mM

1.2  $\pm$  0.02 g

S009 EDTA, 0.5M

10 mM

2.0  $\pm$  0.1 mL

RM005 sodium chloride

1.0 M

5.8  $\pm$  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  $\pm$  0.1

made by: \_\_\_\_\_ date: \_\_\_\_\_

Initials: RCJ

Date: 2/2/80

**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: 2/2/95

**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: RD

Date: 2/2/95

**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: PCI

Date: 2/2/95

**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: *2/2/95***S020 Yield Calibrators**

lot number: \_\_\_\_\_

standard batch size: 5 X 400  $\mu$ L each

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**Ingredients**final  
concentration

amount

S039 TE, 1X

1 X

-----

RM148 lambda DNA

-----

140  $\pm$  10  $\mu$ g (guideline)

S021 yield gel loading buffer

1.25 X

3.0  $\pm$  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/ $\mu$ L	2800 $\mu$ L			

**Calibrators**

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

**Procedure**

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

Record the concentration in ng/ $\mu$ L of the lambda DNA received from the manufacturer under initial DNA concentration.

Initials: RCJ

Date: 2/2/95

**S020 Yield Calibrators**

lot number: \_\_\_\_\_

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**Procedure**

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

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

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

$$(\text{volume 1X TE}) = (\text{final volume}) - (\text{volume lambda DNA}) \quad \text{equation 2}$$

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  $\mu\text{L}$  for each level.

Mix well.

Divide each level into five 300  $\mu\text{L}$  aliquots, and dispense into labeled, sterile eppendorf tubes.

Add 100  $\mu\text{L}$  of yield gel loading buffer to each tube. The final volume of each aliquot is 400  $\mu\text{L}$ .

Store at  $-20^{\circ}\text{C}$ .

**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: \_\_\_\_\_

Initials: *RCD*

Date: *2/2/95*

**S021 Yield Gel Loading Buffer**

lot number: \_\_\_\_\_

standard batch size: 100 mL

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**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: ACJ

Date: 2/2/95

**S021 Yield Gel Loading Buffer**

lot number: \_\_\_\_\_

standard batch size: 100 mL

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**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: \_\_\_\_\_