II

Forensic Molecular Biology

Solutions Manual

Version 2.0

Initials: RC

Date: 4/7/94

Alphabetical Table of Contents

S018 Analytical Gel Loading Buffer	
S060 Calibration Control	
S010 Cell Lysis Buffer (CLB)	
S064 Cell Pellet Control	. (
S022 Chelex, 5%	
S104 Chromogen Solution	12
S094 Digest Buffer	13
S093 DTT, 1M	14
	15
	16
S005 DQα Wash Solution	17
S009 EDTA, 0.5M	18
	19
	20
	21
	23
	24
	26
S097 Pre-Wetting Solution	27
	28
	29
	30
	32
	33
	3 5
	36
	37
	38
	39
	40
	41
	42
	43
	13 44
S007 TRIS-HCl, 1M - pH 7.6	
S020 Yield Calibrators	
S021 Yield Gel Loading Buffer	

Initials: RC)

Date: 4/7/29

Numerical Table of Contents

Initials: Rel

Date: 4/7/84

S018 Analytical Gel Loading Buffer

lot number:

standard batch size: 100 ml

Ingredients	final concentration	amount
RM020 bromophenol blue	0.25%	$0.25 \pm 0.01 \text{ g}$
RM217 xylene cyanol	0.25%	$0.25 \pm 0.01 \text{ g}$
RM040 ficol1 400	12.5%	$12.5 \pm 0.1 \text{ g}$
S009 EDTA, 0.5M	50. mM	$10.0 \pm 0.1 \text{ ml}$
RM083 TAE, 10X	5.0 X	$50.0 \pm 0.5 \text{ ml}$

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	44		
RM217 xylene cyanol	-		
RM040 ficoll 400		nonceleocological accusada a socieda especial es	
S009 EDTA, 0.5M	***************************************	Walter Control of the	
RM083 TAE, 10X			
mada huu			
made by:	d	ate:	

Initials: RG

Date: 4/7/84

S060 Calibration Control

lot number:

page 1 of 2

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	ner ske me ske me			

Calculations

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

Calculate the final volume according to equation 1.

(final volume) = $\frac{\text{(initial DNA concentration)(initial DNA volume)}}{\text{(5 ng/<math>\mu$ l)}} 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: (4)	Date:	417-194	/	
S060 Calibration Control		,	lot number:	
				page 2 of 2
Procedure				
Combine the DNA, loading buffer, ar	nd sterile v	vater.		
Mix well.				
Using sterile pipet tips, dispense 200	μ l aliquots	into steril	e 1.5 ml eppe	endorf tubes.
Store at -20°C.				
Data Log	sou	ırce	lot	amount
RM221 K562 DNA	***************************************			AMARIA
S021 yield gel loading buffer	**************************************			***************************************
S059 sterile water				

made by: _____ date: _____

Initials: Red

Date: 4/7/84

lot number:

S010 Cell Lysis Buffer (CLB)

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-HC1, 1M - pH 7.6			
S008 magnesium chloride, 1M		Water to the second second	Marine
RM075 triton X-100		And the commission of the comm	
made by:	date: _		

Initials: Ru

Date: 4/7/84

S064 Cell Pellet Control

lot number:

page 1 of 2

Ingredients	concentration of cells	total volume (ml)	cells per aliquot	aliquot volume (ml)
RM243 K562 cells			1·10 ⁶	
S034 phosphate buffered saline (PBS)			an as an	

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 1.106cells according to equation 1.

(aliquot volume) = $\frac{(1\cdot10^6\text{cells})}{\text{(concentration of cells)}}$ 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.

Initials: RC	Date: 4/3/2	94		
S064 Cell Pellet Control	•	lot numbe	r:	
			page 2 of 2	
Spin the tubes at 180 g for 1 minu	ute at 4°C, and remov	e the excess	supernatant.	
The tubes can be aliquoted and sp seal-a-meal bag, labeled with the				ely in a
Store the bags at -70°C.				
Data Log	source	lot	amount	
RM243 K562 cells	***************************************		Man Total Control Cont	
S034 phosphate buffered saline				

made by: _____ date: _____

Initials: RC	Date:	1/2/00		
S022 Chelex, 5%	Date: 4/7/9/			
standard batch size: 500 ml				and the state of t
Ingredients	final concentra	ation	amount	
RM027 chelex 100	5. %	6	25 ± 2 g	
S059 sterile water			450 ± 50 ml	(guideline)
Procedure				
Filter sterilize approximately 600 m	l deionized	water.		
Pour the water into a 500 ml bottle.				
Save the bottom container from the	disposable f	filter unit.		
Autoclave the water at 250°F for 30	0 minutes.			
Add the chelex to the bottom contain	ner of the fi	ilter unit.		
Allow the water to cool after autocl	aving.			
Add sterile water to the chelex to a disposable filter container.	volume of 5	500 ml using th	e graduation ma	arkings on the
Mix on a magnetic stir plate.				
While the stock solution is mixing,	aliquot 10 n	nl each into 15	ml centrifuge tu	ıbes.
Store at 2-8°C.				
		•		
Data Log	source	lot	amount	
RM027 chelex 100		tion to the state of the state		
S059 sterile water				
Quality Control				
QC014 Chelex Extraction				
made by:		date:		manio processori al assessora bassa bossa.

		,		
Initials: \mathcal{A}	Date:	4/7-184		
S082 Chelex, 20%		lot number:	***************************************	AMORE CONTROL
standard batch size: 500 ml				
Ingredients	final concentration		amount	
RM027 chelex 100	20. %		100 ± 2 g	
S059 sterile water			450 ± 50 ml (gu	uideline)
Procedure				
Filter sterilize approximately	600 ml deionized	water.		
Pour the water into a 500 ml	bottle.			
Save the bottom container fro	om the disposable t	ilter unit.		
Autoclave the water at 250°F	for 30 minutes.			
Add the chelex to the bottom	container of the fi	lter unit.		
Allow the water to cool after	autoclaving.			
Add sterile water to the chele disposable filter container.	x to a volume of 5	00 ml using the	graduation markin	gs on the
Mix on a magnetic stir plate.				
While the stock solution is mi	xing, aliquot 10 m	al each into 15 r	nl centrifuge tubes.	
Store at 2-8°C.				
Data Las			•	
Data Log		source	lot	amount
RM027 chelex 100			Well-time introduction and a street of the s	
S059 sterile water	vieni/annocedatai/mod			Marc
Quality Control				
QC017 Differential Extraction				
made by:		date:		

Initials: RG	Date	: 4/2/84	/			
S104 Chromogen Solution		lot number:				
standard batch size: 30 ml						
Ingredients		nal ntration		amount		
RM435 chromogen: TMB			(60 mg		
ethanol, 100% reagent grade				30 ml		
Procedure						
Bring bottle of chromogen:TM	MB to room tem	perature.				
Before opening, lightly tap the	e bottle on the c	ounter to bri	ng its contents	to the bottom.		
Carefully remove the stopper ethanol.	and reconstitute	the chromog	en:TMB with	the room temperature		
CAUTION: DO NOT USE I 100% REAGENT GR	ETHANOL STO RADE ETHANO	ORED IN A	METAL CON	TAINER; ONLY USE		
Recap the bottle and seal with	parafilm.					
Tilt the bottle several times to	ensure that all t	the powder is	removed from	within the rubber cap.		
Shake on an orbital shaker for	about 30 minut	es.				
Store at 2-8°C and away from	rust.					
The solution is stable for six n	months.					
Data Log	source	lot	amount			
RM435 chromogen	NAME CHEST CONTRACT CO	998844666666666666666666666666666666666	######################################			
ethanol, 100%						
made by:		date:				

Т	n	÷	+	÷	3	7	s	•
1	11	1	L	1	а	1	S	•

RCI

Date: 5/23/54

S094	Digest	Buffer
------	--------	--------

lot number: _____

standard batch size: 6 L

Ingredients	final concentration	amount
S009 EDTA, 0.5M	10. mM	$120 \pm 6 \text{ ml}$
S036 TRIS-HCl, 1M-pH 7.4	10. mM	$60 \pm 3 \text{ ml}$
S012 sodium chloride, 5M	50. mM	$60 \pm 1 \text{ ml}$
S001 SDS, 20%	2.0 %	$600 \pm 15 \text{ ml}$
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.

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:	spec	eification: 7.5	± 0.1
QC023 QuantiBlot Quality Control of Solu	tions- Test 15	$0 \mu l$ of solution	n
made by:	d	ate:	

Initials: RC/ S094 Digest Buffer	Date: 4	17/94 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		$60 \pm 3 \text{ ml}$
S012 sodium chloride, 5M	50. mM		$60 \pm 1 \text{ ml}$
S001 SDS, 20%	2.0 %		600 ± 15 ml
RM096 hydrochloric acid			
Procedure Add the EDTA, TRIS, sodium chloradjust the pH to 7.5. Bring up to the final volume with a Mix well. Measure and record the final pH. Aliquot into 50 ml centrifuge tubes Store at room temperature.	deionized water.	approximately 4	L deionized water.
Data Log	source	lot	amount
S009 EDTA, 0.5M	****		
S036 TRIS-HCl, 1M-pH 7.4			
S012 sodium chloride 5M			
S001 SDS, 20%			

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:

standard batch size: 20 ml			
Ingredients	final concentration	amo	ount
RM101 dithiothreitol	1.0 M	3.1	± 0.2 g
S059 sterile water		-	
Procedure			
Add the DTT to approximately	15 ml sterile, deionized v	vater in a 50) ml centrifuge tube
Mix well.			
When the DTT is dissolved, br	ing up to volume with ster	rile, deioniz	ed water.
Filter sterilize.			
Dispense 250 μ l aliquots into st	erile 0.5 ml eppendorf tub	oes.	
Store at -20°C.			
Data Log	source	lot	amount
RM101 dithiothreitol			· · · · · · · · · · · · · · · · · · ·
S059 sterile water			
Quality Control			
QC023 QuantiBlot Quality Cor	ntrol of Solutions- Test 20	$0 \mu l$ of solut	ion
made by:	da	ate:	

Date: 5/13/85

lot number:

Initials:

S093 DTT, 1M

S093 DTT, 1M	lot number:				
standard batch size: 20 ml					
Ingredients	final concentration	am	ount		
RM101 dithiothreitol	1.0 M	3.3	1 ± 0.2 g		
S059 sterile water					
Procedure					
Add the DTT to approximately 15	5 ml sterile, deionized	l water in a 5	0 ml centrifuge to	ıbe.	
Mix well.					
When the DTT is dissolved, bring	g up to volume with s	terile, deioniz	zed water.		
Filter sterilize.					
Dispense 250 μ l aliquots into steri	le 0.5 ml eppendorf t	ubes.			
Store at -20°C.					
Data Log	source	lot	amount		
RM101 dithiothreitol	-	***************************************			
S059 sterile water		***************************************			
Quality Control					
QC017 Differential Extraction					
made by:		date:			

Date: 4/7/84

Initials: RG

S093 DTT, 1M

Initials:	Date:	4/2/94		
S003 DQ α Citrate Buffer		lot	number:	
standard batch size: 4 L				
Ingredients	final concentration	on	amount	
RM001 trisodium citrate	and the sale and		$73.6 \pm 0.1 \text{ g}$	
RM002 citric acid			24 ± 1 g (guideline)
Procedure				
Dissolve the sodium citrate in app	proximately 3	liters deionize	d water.	
Adjust the pH to 5.0 by addition	of citric acid (approximately	7 24 g).	
Adjust the final volume to 4 liters	with deionize	ed 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		#764197Ababaanaanaanaanaanaanaanaanaa		
Quality Control				
final pH:		specification	5.0 ± 0.2	
made by:		date:		

Initials: ρc	Date:	1/2/84			
S004 DQ\alpha Hybridization Solutio	lot number:				
standard batch size: 4 L					
Ingredients	final concentration	ı	amount		
S002 SSPE, 20X	5.0 X		$1000 \pm 10 \text{ ml}$		
S001 SDS, 20%	0.50 %		$100 \pm 1 \text{ ml}$		
Procedure					
Combine the SSPE and 2.9 L deio	nized water in	a 4 L flask.			
Add the SDS.					
Warm the solution until all solids a	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 α Hybridization					

made by: _____ date: _____

Initials: RCJ	Date: 4	16/94	
S005 DQ α Wash Solution		lot numb	oer:
standard batch size: 4 L			
Ingredients	final concentration		amount
S002 SSPE, 20X	2.5 X		$500 \pm 10 \text{ ml}$
S001 SDS, 20%	0.10 %		20 ± 1 ml
Procedure			
Measure 20 ml 20% SDS in a 50 m	l graduated cy	linder.	
Raise the volume of the SDS solution	on to 50 ml by	adding 30 ml de	eionized water.
Pour the SDS into a 4 L bottle.			
Add 500 ml SSPE and 3450 ml deic	onized water.		
Cap and mix well by inverting.			
Store at room temperature.			
Data Log S002 SSPE, 20X	source	lot	amount
S001 SDS, 20%			in the second se

Quality Control

QC003 DQ α hybridization

made by: _____ date: ____

Initials: fy	Date:	*		
S009 EDTA, 0.5M		lot n	umber:	
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 2	250 ml deionize	d water.		
Adjust the pH to 8.0 with sodium	hydroxide solut	ion.		
Mix well.				
When the EDTA is dissolved, adju	st 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	ı .			
Store at room temperature.				
Data Log		source	lot	amount
RM003 EDTA				
RM004 sodium hydroxide, 10N		Microbrycolla escribe a bombind literatur den adurabilisti wind del distri	rephrolerent/selectricity describit accordinate accord	works statement with the last and despectation in the despectation in the despectation in the despectation is a despectation of the despectation in the despectation in the despectation is a despectation of the despectation in the despectation is a despectation of the despectation in the despectation is a despectation of the despectation in the despectation is a despectation of the despectation in the despectation is a despectation of the despectation in the despectation is a despectation of the despectation in the despectation is a despectation of the despectation in the despectation is a despectation of the despectation is a despectation of the despectation of the despectation is a despectation of the despectation o
Quality Control				
final pH:	specification	n: 8.0 ± 0.1		
made by:		date:		

Initials: fc	Date	e: 4/7/94	r		
S105 HLA-DQα PCR React		ě	ber:		
standard batch size: ~ 55 tu	ibes x 50 μ l				
Ingredients		final concentration		amount	
HLA-DQα PCR reaction mi	x			3 ml	
HLA-DQ $lpha$ autoclaved, PCR	reaction tubes	estation and the second second		55 tubes	
Procedure					
NOTE: ALIQUOT ALL TU AMPLIFIED DNA TO MI GLOVES IS ESSENTIA	NIMIZE CON	TAMINATIO	N. USING	CLEAN	
Clean the bench top thorough	ly using a 10%	bleach solutio	n, and cover	it with new bench p	aper.
While wearing clean gloves, a designated for the PCR prepare			and place th	nem in a clean rack	
Using a dedicated positive disaliquot 50 μ l of PCR reaction			ips with hyd	rophobic filters, care	fully
Once aliquotting is complete, DNA.	cap all tubes ar	nd store in a la	belled rack a	away from all source	s of
Store at 2-8°C.					
Data Log	source	lot	amount		
PCR reaction mix		delinate anti-continuous anti-			
PCR reaction tubes		oodaalaanahtiinkiiloottaanahtiinkiinkiinkiinkiinkiinkiinkiinkiinkiin	nitration assessment of the Committee in the Committee of		
Quality Control					
QC015 DQ α Amplification					
made by:		date:			

Initials: Ry	Date: 4/2/84		
S079 Hydrogen Peroxide, 3%	lo	t number:	
standard batch size: 30 X 0.5 ml			
Ingredients	final concentration		amount
RM176 hydrogen peroxide, 30%	3 %		$1.5 \text{ ml} \pm 0.1 \text{ ml}$
deionized water	****		13.5 ml (guideline)
Procedure			
Add hydrogen peroxide to a 15 ml dis	sposable tube.		
Add deionized water to a final volume	e of 15 ml.		
Aliquot approximately 0.5 ml of hydro	ogen peroxide into 1.5	ml micro	centrifuge tubes.
Label each tube with "H2O2" and the l	ot number. Label the	rack with	expiration date.
Store at 4°C in the dark.			
Discard after 2 months.			
Data Log	source 1	ot	amount
RM284 hydrogen peroxide, 3%		······································	
made by:	date:		

Initials: Ry

Initials: fc

S032 Lambda Marker

Date: 4/2/94

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			400 NOV NOV	

Calculations

Record the initial concentration in $ng/\mu l$ and the initial volume in μl of the lambda Hind III DNA received from the manufacturer.

Calculate the final volume according to equation 1.

(final volume) = $\underline{\text{(initial DNA concentration)(initial DNA volume)}}$ equation 1 (20 ng/ μ l)

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.

Date:

Sold Lambda Marker

Date:

Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:
Date:

Data Log	source	lot	amount
RM155 lambda Hind III fragments			
S021 yield gel loading buffer	-		-
S059 sterile water			
made by:		date:	

T-161-1 //(/-		
Initials: AU	Date: 4/7/			
S008 Magnesium Chloride, 1M		lot numb	er:	-
standard batch size: 250 ml				
Ingredients	final concentration		amount	
RM046 magnesium chloride, hexahydrate	1.00 M		$50.8 \pm 0.3 \text{ g}$	
Procedure				
Dissolve the magnesium chloride	in approximately 200 i	ml deionize	d water.	
Mix well.				
When the magnesium chloride has	dissolved, bring up to	the final v	olume with deionize	d water
Dispense into 125 ml bottles.				
Autoclave at 250°F for 20 minutes	S.			
Store at room temperature.				
Data Log	source	lot	amount	
RM046 magnesium chloride, hexahydrate		***************************************		

made by: _____ date: ____

Initials: RU

S042 Phi-X Marker

Date: 4/3/04

lot number:

page 1 of 2

Ingredients	initial concentration $(ng/\mu l)$	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	40 MA AND AND AND AND AND AND AND AND AND AN			

Calculations

Record the initial concentration in $ng/\mu l$ 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.

(final volume) = (initial DNA concentration)(initial DNA volume) equation 1 (50 ng/ μ l)

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: RG	Date:	462-684		
S042 Phi-X Marker		lot n	umber:	
				page 2 of 2
Procedure				
Combine the DNA, loading buffer, a	and sterile	water.		
Mix well.				
Using sterile pipet tips, dispense 500	μ l aliquots	s into sterile 1.5	ml eppendo	orf tubes.
Store at -20°C.				
Data Log				
Data Log		source	lot	amount
RM156 phi-X-174 Hae III fragments		***************************************		
S018 analytical gel loading buffer			***************************************	
S059 sterile water		***************************************		
made by:		date:		

Initials: /c/

Date: 5/23/8 4

S034 Phosphate Buffered Saline (PBS)

lot number:

standard batch size: 4 L

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

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.

Data Log	source	lot	amount
RM005 sodium chloride		Section 1997 and the section of the	
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 Sol	lutions- Test 1	150 μ l of solution	on
made by:		date:	

Initials: RC

Date:

1/2/94

S034 Phosphate Buffered Saline (PBS)

lot number:

standard batch size: 4 L

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

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.

Data Log	source	lot	amount
RM005 sodium chloride	****		
RM053 potassium chloride	***************************************		
RM065 sodium phosphate, dibasic			
RM056 potassium phosphate, monobasic			MMS-CO-CO-CO-CO-CO-CO-CO-CO-CO-CO-CO-CO-CO-
Quality Control			
final pH:	And the second s	spec: 7.5 ±	0.1
QC017 Differential Extraction			
made by:	and incommunity of well-the derivative before the contract of	date:	

Initials: RY	Date: 4/5/89
S097 Pre-Wetting Solution	lot number:
standard batch size: 4 L	

Ingredients	final concentration	amount 160 ± 10 mL	
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.

Data Log	source	lot	amount
RM004 NaOH, 10 N			WWW.compromented and construction and co
S009 EDTA, 0.5 M	-	***************************************	
made by:	da	nte:	

Initials: (C) S011 Protein Lysis Buffer (PLB)	Date: 4/3/99 lot number:
standard batch size: 2 L	

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

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			NAMES OF THE PROPERTY OF THE P
S009 EDTA, 0.5M			Mile Construction
S012 sodium chloride, 5M	Marie Control of Marie Control of	Manager and the second	
made by:		date:	

Initials: RC	Date: ರ	1/23/29	
S014 Proteinase-K Enzyme, 10mg/	ml	lot n	umber:
standard batch size: 10 ml			
Ingredients	fin conce	al ntration	amount
RM119 proteinase-K, lyophilized	10 n	ng/ml	$100 \pm 1 \text{ mg}$
Procedure			
Add 10 ml sterile, deionized water to	o one bottle ((100 mg) lyop	hilized proteinase-K enzyme
Mix by slowly inverting until comple	etely reconsti	tuted.	
Dispense 500 μ l aliquots into 1.5 ml	eppendorf tu	ibes.	
Store at -20°C.			
Data Log	source	lot	amount
RM119 proteinase-K, lyophilized			
Quality Control			

QC023 QuantiBlot Quality Control of Solutions- Test 10 μ l of solution

made by: _____ date: ____

•	g/ml lot	number:
standard batch size: 10 ml		
Ingredients	final concentration	amount
RM119 proteinase-K, lyophilized	10 mg/ml	$100 \pm 1 \text{ mg}$
Procedure		
Add 10 ml sterile, deionized water	to one bottle (100 mg) lyc	philized proteinase-K enzy
	1.4.1	
Mix by slowly inverting until comp	detery reconstituted.	
Dispense 500 μ l aliquots into 1.5 m		
Mix by slowly inverting until comp Dispense 500 μ l aliquots into 1.5 m Store at -20°C.		amount

made by: _____ date: ____

QC017 Differential Extraction

Initials: Rd

Date: 4/7/84

S100 QuantiBlot DNA Standards

lot number:

standard batch size: variable

page 1 of 2

Ingredients final amount

concentration

RM442 DNA Standard A varies varies

S039 TE, 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 μ L of DNA Standard A into the tube labeled 1A. This is now DNA Standard 1A.

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

Add 300 μ l of DNA Standard 1A to the 300 μ l of 1X TE in tube 1B. Vortex to mix thoroughly.

Add 300 μ l of diluted DNA Standard (tube 1B) to the 300 μ l of 1X TE in tube 1C. Vortex to mix thoroughly.

Add 300 μ l of diluted DNA Standard (tube 1C) to the 300 μ 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 μ l of DNA Standard A into the tube labeled 1A. This is now DNA Standard 1A.

Aliquot 600 μ l of 1X TE into each of the six remaining tubes labeled 1B-1G.

Add 600 μ l of DNA Standard 1A to the 600 μ l of 1X TE in tube 1B. Vortex to mix thoroughly.

Initials: FC

Date: 4/7/84

S100 QuantiBlot DNA Standards

lot	number:	

standard batch size: variable

page 2 of 2

Add 600 μ l of diluted DNA Standard (tube 1B) to the 600 μ l of 1X TE in tube 1C. Vortex to mix thoroughly.

Add 600 μ l of diluted DNA Standard (tube 1C) to the 600 μ 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	tandard Tube Conc (ng/µl)			
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			Otherwise Contraction and Cont
RM442 DNA Standard A		***************************************	
S039 TE, 1X	**************************************	deministrative and a second and a second	
Quality Control QC018 QuantiBlot Hybridization.			
made by:	imaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa	date:	

						_		
т	n	÷	+	÷	~	1	S	٠
1	11	1	L	_	а	ㅗ	2	



Date: 5/18/89

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	$300 \pm 10 \text{ mL}$
S001 SDS, 20%	0.5 %	$100 \pm 5 \text{ mL}$

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.

Data Log	source	lot	amount
S002 SSPE, 20X	was the state of t	·	
S001 SDS, 20%			
made by:		date: _	

Initials:

Date: 4/7/84

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	$300 \pm 10 \text{ mL}$
S001 SDS, 20%	0.5 %	$100 \pm 5 \text{ mL}$

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.

Data Log	source	lot	amount
S002 SSPE, 20X	***************************************		
S001 SDS, 20%	**************************************		
made by:		date: _	

Initials:

RY

Date: 5/23/94

S106 Quantitation Check Standards

lot number:

Page 1 of 2

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 $ng/\mu l$ of the K562 DNA as described in the procedure below.

Calculate the initial DNA volume according to equation 1.

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

Record the initial DNA volume above.

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

(TE volume) = $(750 \mu l)$ - (initial DNA volume)

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:

Page 1 of 2

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			W0 40 00 00	***

Calculations

Record the initial concentration in $ng/\mu l$ of the K562 DNA as described in the procedure below.

Calculate the initial DNA volume according to equation 1.

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

Record the initial DNA volume above.

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

(TE volume) = $(750 \mu l)$ - (initial DNA volume) 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: RO

Date: 4/7/99

S106 Quantitiation Check Standards

lot number:

Page 2 of 2

Combine 250 μ L of Q1 to 250 μ l of TE in tube Q2. Add 50 μ l of Q1 to 450 μ l 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 $2ng/5\mu l$, Q2 has $1ng/5\mu l$, and Q3 has $0.2 ng/5\mu l$ 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.

Initials: Rd

Date: 4/7-194

S101 SDS, 0.1%

lot number: _____

standard batch size: 20 L

Ingredients final amount concentration

S001 SDS, 20%

0.1 %

 $100 \pm 10 \text{ mL}$

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.

Data Log	source	lot	amount
S001 SDS, 20%	**************************************	***************************************	
made hv:		data:	

Initials: RCI		Date:	4/2/99		
S001 SDS, 20%			lot number:		
standard batch size:	1 L				
Ingredients	C	final concentratio	on	amount	
RM007 sodium dode	cyl sulfate	20 %		200 ± 5 g	
Procedure					
THIS SOLUTION.	ROSOL MASK			E USED WHEN MAKING	
Warm approximately	750 mL deioniz	ed water or	n a stirring hot plat	e.	
Add a fraction of the	SDS, allowing	the solids to	o dissolve before ac	dding more.	
Add the SDS until it	is all in solution	i .			
When the solution is	clear, bring up t	to volume v	vith deionized wate	r.	
Filter sterilize the war	rm solution.				
Dispense into sterile 5	500 mL bottles.				
Store at room tempera	nture.				
Data Log	source	lot	amount		
RM007 SDS					

10	1/2/1	· ar
Initials: RO	Date: 4/7/9	
S080 Sodium Acetate, 1N	M lot number:	
standard batch size: 100 i	mL	
Ingredients	final concentration	amount
RM059 sodium acetate, anhydrous	1.0 M	$8.2 \pm 0.4 \text{ g}$
RM093 acetic acid, glacial	l	
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 de	eionized water.	
Measure and record the fin	al pH.	
Dispense into a 100 ml bot	tle.	
Autoclave at 250°F for 30	minutes.	
Store at room temperature.		
Data Log	source lot	amount
RM059 sodium acetate, anhydrous		
RM093 acetic acid,		

Initials: RG		Date:	417/99	
S012 Sodium Chloride, 5N	1		lot number:	
standard batch size: 4 L				
Ingredients	co	final ncentration	ı	amount
RM005 sodium chloride		5.0 M		$1170 \pm 10 \text{ g}$
Procedure				
Slowly add the sodium chlor	ride to app	roximately	2 L deionized w	rater.
Raise the volume to just und	ler 4 L so	that the soc	dium chloride wi	ll go into solution.
Mix well.				
Bring up to volume with dei	onized wat	er.		
Dispense into 1 L bottles.				
Store at room temperature.				
Data Log	source	lot	amount	t
RM005 sodium chloride				

S098 Spotting Solution		lot number:
standard batch size: 75 m	L	
Ingredients	final concentration	amount
S097 Pre-Wetting Solution		$74.85 \text{ ml} \pm 1 \text{ ml}$
RM443 Bromothymol Blue, 0.04%	0.00008%	$150 \ \mu l \ \pm \ 1 \ \mu l$
Procedure		
Measure 74.85 mL Pre-We	etting Solution into a į	graduated cylinder and pour into a 1
Measure 74.85 mL Pre-Webottle.		graduated cylinder and pour into a 1
Measure 74.85 mL Pre-Webottle. Add 150 μ L bromothymol	blue.	graduated cylinder and pour into a 1
Procedure Measure 74.85 mL Pre-Webottle. Add 150 μL bromothymol Cap and mix well by inverstore at room temperature.	blue. ting.	graduated cylinder and pour into a 1
Measure 74.85 mL Pre-We bottle. Add 150 μ L bromothymol Cap and mix well by inver	blue. ting.	
Measure 74.85 mL Pre-Webottle. Add 150 μ L bromothymol Cap and mix well by inversions at room temperature.	blue. ting.	

made by:

date:

Initials: RO

Date: 4/7/84

S002 SSPE, 20X

lot number: _____

standard batch size: 4 L

Ingredients	final concentration	amount
RM003 EDTA	20. mM	$29.8 \pm 0.7 \text{ g}$
RM004 sodium hydroxide, 10N		$40 \pm 5 \text{ ml (guideline)}$
RM005 sodium chloride	3.6 M	$840 \pm 10 \text{ g}$
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.

Data Log	source	lot	amount
RM003 EDTA	***************************************	***************************************	-
RM004 sodium hydroxide, 10N	***************************************		-
RM005 sodium chloride	-	***	THE THE PARTY OF T
RM006 sodium phosphate, monobasic	449940th distribution for facilities and an operation	minimized and a minima and a simple of the s	OT COMMUNICATION CONTRACTOR
Quality Control			
final pH:	specificat	ion 7.4 ± 0.3	2
made by:	date:		***************************************

Initials: LC/	Date: 5/23/2 4
S059 Sterile Water	lot number:
standard batch size: 500 ml	
Procedure	
Filter sterilize 500 ml of deio	nized water.
Aliquot 10 ml each into 15 m	l centrifuge tubes.
Autoclave at 250°F for 30 mi	nutes.
Store at room temperature.	
Quality Control	
QC023 QuantiBlot Quality Co	ontrol of Solutions- Test 150 μ l of solution

Initials: RC	Date: 4/7/94	
S059 Sterile Water	lot number:	
standard batch size: 500 ml		
Procedure		
Filter sterilize 500 ml of deion	nized water.	
Aliquot 10 ml each into 15 m	l centrifuge tubes.	
Autoclave at 250°F for 30 mi	nutes.	

Initials: RC Date: 4/7-184 S039 TE, 1X lot number: standard batch size: 500 ml **Ingredients** final amount concentration S049 TE, 100X 1.0 X $5.0 \pm 0.3 \, \text{ml}$ **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

final pH: _____ specification: 8.0 ± 0.2

made by:

lot

amount

date:

S049 TE, 100X

Quality Control

Initials: RC/

Date: 4/7/94

S049	TE.	100X
~		* * * * *

lot number:

standard batch size: 250 mL

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.

Data Log	source	lot	amount
RM003 EDTA		***************************************	
RM073 TRIS			
RM004 sodium hydroxide, 10N		and and a contract of the cont	DOMANUM 1002-00-00-00-00-00-00-00-00-00-00-00-00-
RM096 hydrochloric acid	MANAGEMENT AND ACCORDING TO A STATE OF THE S	***************************************	
Quality Control			
final pH:		specification:	8.0 ± 0.2
made by:		date:	

Initials: AC

Date: 4/7/84

S036 TRIS-HCl, 1M - pH 7.4

lot number: _____

amount

standard batch size: 250 ml

Ingredients

final concentration

Concontrativ

RM073 TRIS 1.00 M $30.3 \pm 0.1 \text{ g}$

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

Initials: RC)	Date:	4/7/99		
S007 TRIS-HCl, 1M - pH 7.6		lot nu	ımber:	
standard batch size: 250 ml				
Ingredients	final concentration	on	amount	
RM073 TRIS	1.00 M		$30.3 \pm 0.1 \text{ g}$	
RM096 hydrochloric acid	Tele seri des des des			
Procedure				
Add the TRIS to approximately 200) ml deionize	ed water.		
Mix well.				
Adjust the pH to 7.6 with concentra	ated hydroch	loric acid.		
Bring up to final volume with deion	nized water.			
Measure and record the final pH.				
Prepare a 1:100 dilution (10 mM T deionized water.	RIS-HCl) by	mixing 1 ml T	RIS-HCl solution and	l 99 ml
Measure and record the pH of the d	lilution.			
Dispense the 1M TRIS-HCl into 125	5 ml bottles.			
Autoclave at 250°F for 20 minutes.				
Store at room temperature.				
Data Log	source	lot	amount	
RM073 TRIS				
RM096 hydrochloric acid		- monor-control control contro	one and the state of the state	

Quality Control

final pH: _____ specification: 7.6 ± 0.1

1:100 pH: _____ specification: 7.6 ± 0.1

Initials: 20)

Date: 4/+/44

S020 Yield Calibrators

lot number:

standard batch size: 5 X 400 µl each

page 1 of 2

 3.0 ± 0.5 ml (guideline)

Ingredients final amount concentration S039 TE, 1X 1 X RM148 lambda DNA $140 \pm 10 \,\mu g$ (guideline)

Calculations

S021 yield gel loading buffer

Stock Solution

1.25 X

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 μ1	300 μ1	500 μ1
В	200 ng/10 μl	50 ng/μl	800 μl	700 μ1	500 μl
С	100 ng/10 μl	50 ng/μl	400 μ1	1100 μ1	500 μ1
D	$50~\mathrm{ng}/10~\mu\mathrm{l}$	50 ng/μl	200 μ1	1300 μ1	500 μ1
E	25 ng/10 μl	50 ng/μl	100 μ1	1400 μ1	500 μl
F	10 ng/10 <i>μ</i> l	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 μ l of 50 ng/ μ 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: $\rho()$

S020 Yield Calibrators

Date: 4/7/94

lot	number:	

page 2 of 2

Procedure

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

(volume lambda DNA) = (final DNA concentration)(final volume)
(initial DNA concentration)

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 μ l for each level.

Mix well.

Divide each level into five 300 μ l aliquots, and dispense into labeled, sterile eppendorf tubes.

Add 100 μ l of yield gel loading buffer to each tube. The final volume of each aliquot is 400 μ l. Store at -20°C.

date:

Data Log	source	lot	amount
S039 TE, 1X		***************************************	
RM148 lambda DNA			Non-military international control of the control o
S021 yield gel loading buffer	**Committee Anna An		

made by:

Initials: RS)

Dat

Date: 4/+/94

S021 Yield Gel Loading Buffer

lot number: _____

standard batch size: 100 ml

Page 1 of 2

Ingredients	final concentration	amount
RM020 bromophenol blue	0.25%	$0.25 \pm 0.01 \; \mathrm{g}$
RM217 xylene cyanol	0.25%	$0.25 \pm 0.01 \; \mathrm{g}$
RM040 ficoll 400	12.5%	$12.5~\pm~0.1~\mathrm{g}$
S009 EDTA, 0.5M	50. mM	$10.0 \pm 0.1 \text{ ml}$
RM083 TAE, 10X	5.0 X	$50.0 \pm 0.5 \text{ ml}$
S001 SDS, 20%	0.20 %	$1.00 \pm 0.02 \text{ ml}$

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.

made by: _____ date: ____

S009 EDTA, 0.5M

RM083 TAE, 10X

S001 20% SDS