Forensic Molecular Biology

**Solutions Manual** 

Version 4.0

# Alphabetical Table of Contents

	0
S113 Acrylamide Solution, 40%	
S115 Ammonium Persulfate (0.5g Aliquot)	7
S018 Analytical Gel Loading Buffer	8
S131 Blue Formamide + Loading Buffer (5:1)	. 0A
S117 BSA Solution, 5 mg/mL	9
S060 Calibration Control	. 10
S010 Cell Lysis Buffer (CLB)	
S064 Cell Pellet Control	13
	. 10
S022 Chelex, 5%	. 15
S082 Chelex, 20%	. 17
S027 Chloroform-Isoamyl Alcohol	. 18
S104 Chromogen Solution	. 19
S013 Denaturation Solution	20
	. 20
S094 Digest Buffer	. 21
S061 Digestion Control	. 22
S003 DQα Citrate Buffer	. 24
S004 DQα Hybridization Solution	. 25
S005 DQ $\alpha$ Wash Solution	. 26
S093 DTT, 1M	
	20
S030 DTT, 0.39M	. 20
S009 EDTA, 0.5M	. 29
S110 EDTA, 200 mM	. 30
S056 Ethanol, 70%	. 31
S031 Gel Neutralization Buffer	. 32
S116 GS500 + Loading Buffer (1:3)	. 33
S016 Hae III Buffer, 10X	. 34
S102 Heparin Solution	. 35
S026 Hybridization Solution, RFLP	36
S079 Hydrogen Peroxide, 3%	37
	28
S032 Lambda Marker	. 30
S015 Lithium Chloride, 7.5 M	
S055 Magnesium Chloride, 0.1M	
S112 dNTP's (10mM stock)	. 42
S006 Phenol	. 43
S053 Phenol-Chloroform-Isoamyl Alcohol	. 45
S042 Phi-X Marker	. 46
S034 Phosphate Buffered Saline (PBS)	48
S109 PM PCR Reaction Mixture	19
	50
S126 Positive Control-External	
S127 Positive Control-Internal	. 51
S097 Pre-Wetting Solution	. 53
S118 Primer, VWA 1 (50 μM)	. 54
S119 Primer, VWA 2 (50 μM)	. 56
S120 Primer THO1 1 (50 μM)	. 58
S120 Primer THO1 2 (50 μM)	
$0400 \text{ Dimer E40A4.4} (50 \text{ µW}) \dots \dots$	 63
S122 Primer F13A1 1 (50 μM)	. 02

	þ
	8

S123 Primer, F13A1 2 (50 μM)	64
S124 Primer, FES/FPS 1 (50 µM)	66
S125 Primer FES/FPS 2 (50 µM)	68
S011 Protein Lysis Buffer (PLB)	70
S014 Proteinase-K Enzyme, 10mg/mL	/1
S037 Proteinase-K Enzyme, 20mg/mL	72
S111 QUAD STR/PCR Reaction Mixture	73
S100 QuantiBlot DNA Standards	75
S099 QuantiBlot Wash Solution	77
S054 Sarkosyl, 10%	78
S040 Sarkosyl, 20%	
S132 Sequencing Loading Buffer	
S101 SDS, 0.1%	80
S045 SDS, 10%	81
S001 SDS, 20%	
S046 SLS, 20%	83
S043 Sodium Acetate, 2 M	
S044 Sodium Acetate, 0.2M	85
S035 SP, 25X	
S108 SP, 2X	8/
S098 Spotting Solution	
S002 SSPE, 20X	
S047 Stain Extraction Buffer	
S059 Sterile Water	
S023 Stripping Solution	
S017 TRIS-HCI, 1M - PH 8.0	
S128 TE <sup>-4</sup> , 1X	
S050 Test Gel Loading Buffer	
S062 Test Gel Standard	
S051 TNE, 10X	
S107 TNE, 1X	
S052 TRIS, 0.1M - pH 7.8	100
S114 Urea (25 g Aliquot)	
S114a (18 g Aliquot-377 Sequencer)	101A
S114b Urea Urea (10.8 g Aliquot-377 Sequencer)	1018
S024 Wash Solution #1	102
S025 Wash Solution #2	
S020 Yield Calibrators	
S021 Yield Gel Loading Buffer	106

#### Numerical Table of Contents

S001 SDS, 20%	82
S002 SSPE, 20X	89
S003 DQα Citrate Buffer	
S004 DQα Hybridization Solution	25
S005 DQ $\alpha$ Wash Solution	
S006 Phenol	
S009 EDTA, 0.5M	29
S010 Cell Lysis Buffer (CLB)	12
S011 Protein Lysis Buffer (PLB)	70
S013 Denaturation Solution	20
S014 Proteinase-K Enzyme, 10mg/mL	71
S015 Lithium Chloride, 7.5 M	
S016 Hae III Buffer, 10X	
S017 TRIS-HCI, 1M - PH 8.0	
S018 Analytical Gel Loading Buffer	8
S020 Yield Calibrators	
S021 Yield Gel Loading Buffer	
S022 Chelex, 5%	
S023 Stripping Solution	
S024 Wash Solution #1	
S025 Wash Solution #2	. 103
S026 Hybridization Solution, RFLP	
S027 Chloroform-Isoamyl Alcohol	
S030 DTT, 0.39M	
S031 Gel Neutralization Buffer	32
S032 Lambda Marker	38
S034 Phosphate Buffered Saline (PBS)	48
S035 SP, 25X	
S037 Proteinase-K Enzyme, 20mg/mL	72
S040 Sarkosyl, 20%	79
S042 Phi-X Marker	46
S043 Sodium Acetate, 2 M	84
S044 Sodium Acetate, 0.2M	
S045 SDS, 10%	
S046 SLS, 20%	. 83
S047 Stain Extraction Buffer	. 90
S050 Test Gel Loading Buffer	
S051 TNE, 10X	. 98
S052 TRIS, 0.1M - pH 7.8	
S053 Phenol-Chloroform-Isoamyl Alcohol	. 45
S054 Sarkosyl, 10%	. 78
S055 Magnesium Chloride, 0.1M	
S056 Ethanol, 70%	
S059 Sterile Water	
S060 Calibration Control	
S061 Digestion Control	. 22

Initials: RS

Ŵ

S062 Test Gel Standard
S064 Cell Pellet Control
S079 Hydrogen Peroxide, 3%
S082 Chelex, 20%
S093 DTT, 1M
S094 Digest Buffer
S097 Pre-Wetting Solution
S098 Spotting Solution
S099 QuantiBlot Wash Solution
S100 QuantiBlot DNA Standards
S101 SDS, 0.1%
S102 Heparin Solution
S104 Chromogen Solution
S107 TNE, 1X
S108 SP, 2X
S109 PM PCR Reaction Mixture
S110 EDTA, 200 mM
S111 QUAD STR/PCR Reaction Mixture
S112 dNTP's (10mM stock) 42
S113 Acrylamide Solution, 40%
S114 Urea (25 g Aliquot)
S114a (18 g Aliquot-377 Sequencer) 101A
S114b Urea Urea (10.8 g Aliquot-377 Sequencer) 101B
S115 Ammonium Persulfate (0.5g Aliquot)
S116 GS500 + Loading Buffer (1:3)
S117 BSA Solution, 5 mg/mL
S118 Primer, VWA 1 (50 μM)
S119 Primer, VWA 2 (50 µM)
S120 Primer THO1 1 (50 µM)
S121 Primer THO1 2 (50 µM) 60
S122 Primer F13A1 1 (50 μM)
S123 Primer, F13A1 2 (50 μM)
S124 Primer, FES/FPS 1 (50 μM)
S125 Primer FES/FPS 2 (50 μM)
S126 Positive Control-External
S127 Positive Control-Internal
S128 TE <sup>-4</sup> , 1X
S131 Blue Formamide + Loading Buffer (5:1)
S132 Sequencing Loading Buffer

# Alphabetical Table of Contents

S113 Acrylamide Solution, 40%	6
S115 Ammonium Persulfate (0.5g Aliquot)	7
S018 Analytical Gel Loading Buffer	8 0
S117 BSA Solution, 5 mg/mL	9
S060 Calibration Control	10
S010 Cell Lysis Buffer (CLB)	12
S064 Cell Pellet Control	13 15
S022 Chelex, 5%	15 17
S082 Chelex, 20%	17 10
S027 Chloroform-Isoamyl Alcohol	10 10
S104 Chromogen Solution	פו סר
S013 Denaturation Solution	20 21
S094 Digest Buffer	ZI
S061 Digestion Control	22
S003 DQα Citrate Buffer	24 25
S004 DQα Hybridization Solution	20 26
S005 DQ $\alpha$ Wash Solution	20 27
S093 DTT, 1M	ZI
S030 DTT, 0.39M	20 20
S009 EDTA, 0.5M	29
S110 EDTA, 200 mM	00 21
S056 Ethanol, 70%	ວາ ວາ
S031 Gel Neutralization Buffer	პ∠ იე
S116 GS500 + Loading Buffer (1:3)	33 24
S016 Hae III Buffer, 10X	34
S102 Heparin Solution	30 26
S026 Hybridization Solution, RFLP	30
S079 Hydrogen Peroxide, 3%	37 28
S032 Lambda Marker	30 40
S015 Lithium Chloride, 7.5 M	40
S055 Magnesium Chloride, 0.1M	41 12
S112 dNTP's (10mM stock)	42
S006 Phenol	40 15
S053 Phenol-Chloroform-Isoamyl Alcohol	40
S042 Phi-X Marker	40
S034 Phosphate Buffered Saline (PBS)	40 10
S109 PM PCR Reaction Mixture	49 50
S126 Positive Control-External	50
S127 Positive Control-Internal	53
S097 Pre-Wetting Solution	55 54
S118 Primer, VWA 1 (50 μM)	56
S119 Primer, VWA 2 (50 μM)	
S120 Primer THO1 1 (50 μM)	00 NA
S121 Primer THO1 2 (50 μM)	00 CA
S122 Primer F13A1 1 (50 μM)	υZ ΑΛ
S123 Primer, F13A1 2 (50 μM)	04

# Alphabetical Table of Contents

		6
S113 Acrylamide Solution, 40%		
S115 Ammonium Persulfate (0.5g Aliguot)		
S018 Apolytical Gel Loading Buffer		0
S117 BSA Solution 5 mg/mL		
S060 Calibration Control		
S010 Cell Lysis Buffer (CLB)		
S064 Cell Pellet Control		
S022 Chelex 5%		
S022 Chelex, 5%		16
S082 Chelex, 20%		17
S027 Chloroform-Isoamyl Alcohol		
S104 Chromogen Solution		19
S104 Chromogen Solution		20
S013 Denaturation Solution		21
S094 Digest Buffer		
S061 Digestion Control		
S003 DQ $\alpha$ Citrate Buffer		
S004 DQ $\alpha$ Hybridization Solution		
S005 DOg Wash Solution		
S093 DTT 1M		Z/
S110 EDTA 200 mM		
S056 Ethanol 70%		
S031 Get Neutralization Buffer		
S116 GS500 + Loading Buffer (1:3)		33
S016 Hae III Buffer, 10X		34
S102 Heparin Solution		35
S026 Hybridization Solution, RFLP		
S079 Hydrogen Peroxide, 3%	•••••	
S032 Lambda Marker		38
S032 Lambda Marker		40
S015 Lithium Chloride, 7.5 M		41
S055 Magnesium Chloride, 0.1M		42
S112 dNTP's (10mM stock)		43
S006 Phenol		
S053 Phenol-Chloroform-Isoamyl Alcohol		
S042 Phi-X Marker		
S034 Phosphate Buffered Saline (PBS)		40
S109 PM PCR Reaction Mixture		
S126 Positive Control-External		
S127 Positive Control-Internal		51
S097 Pre-Wetting Solution		53
S118 Primer VWA 1 (50 uM)		
S119 Primer VWA 2 (50 $\mu$ M)		
S120 Primer THO1 1 (50 $\mu$ M)		58
S121 Primer THO1 2 (50 µM)	, , <i>, , , , , , , , , , , , , , , , , </i>	60
S122 Primer F13A1 1 (50 μM)		62

S124 Primer, FES/FPS 1 (50 μM)
S125 Primer FES/FPS 2 (50 μM)
S011 Protein Lysis Buffer (PLB)
S014 Proteinase-K Enzyme, 10mg/mL
S037 Proteinase-K Enzyme, 20mg/mL
S111 QUAD STR/PCR Reaction Mixture
S100 QuantiBlot DNA Standards
S099 QuantiBlot Wash Solution
S054 Sarkosyl, 10%
S040 Sarkosyl, 20%
S101 SDS, 0.1%
S045 SDS, 10%
S001 SDS, 20%
83 83 846 SLS, 20%
S043 Sodium Acetate, 2 M
S044 Sodium Acetate, 0.2M
S035 SP, 25X
S108 SP, 2X
S098 Spotting Solution         88           S002 SSPE, 20X         89
S002 SSPE, 20X
S047 Stain Extraction Buller
S039 Sterne Water
S023 Stripping Solution
S017 TRIS-RG, TM - FH 8.0
S050 Test Gel Loading Buffer
S050 Test Gel Standard
S052 Test Ger Standard
S107 TNE, 1X
S052 TRIS, 0.1M - pH 7.8
S114 Urea (25 g Aliquot)
S024 Wash Solution #1
S024 Wash Solution #1
S020 Yield Calibrators
S020 Tield Calibrators
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ate:	10	7-196	
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S123 Primer, F13A1 2 (50 μM)	4
S124 Primer, FES/FPS 1 (50 μM)	b
S125 Primer FES/FPS 2 (50 µM) 6	8
S011 Protein Lysis Buffer (PLB)	0
S014 Proteinase-K Enzyme, 10mg/mL	1
S037 Proteinase-K Enzyme, 20mg/mL	2
S111 QUAD STR/PCR Reaction Mixture	3
S100 QuantiBlot DNA Standards	5
S099 QuantiBlot Wash Solution	1
S054 Sarkosyl, 10%	8
S040 Sarkosyl, 20%	9
S101 SDS, 0.1%	0
S045 SDS, 10%	-
S001 SDS, 20%	_
S046 SLS, 20%	3
S043 Sodium Acetate, 2 M	4
S044 Sodium Acetate, 0.2M	5
S035 SP, 25X	0
S108 SP, 2X	1
S098 Spotting Solution	ð
S002 SSPE, 20X	9
S047 Stain Extraction Buffer	0
S059 Sterile Water	1
S023 Stripping Solution	2
S039 TE, 1X	С И
S049 TE, 100X	4
S050 Test Gel Loading Buffer	C C
S062 Test Gel Standard	0
S051 TNE, 10X	0
S107 TNE, 1X	3
S052 TRIS, 0.1M - pH 7.8	1
S114 Urea (25 g Aliquot)	ן כו
S024 Wash Solution #1	2
S025 Wash Solution #2	0
S020 Yield Calibrators	4
S021 Yield Gel Loading Buffer 10	0

RCS Date: 10/20195

# Numerical Table of Contents

S001 SDS, 20%	. 82
S002 SSPE, 20X	. 89
S003 D0g Citrate Buffer	. 24
S004 DQα Hybridization Solution	. 25
S005 DQ $\alpha$ Wash Solution	. 26
S006 Phenol	. 43
S009 EDTA, 0.5M	. 29
S010 Cell Lysis Buffer (CLB)	. 12
S011 Protein Lysis Buffer (PLB)	. 70
S013 Denaturation Solution	. 20
S014 Proteinase-K Enzyme, 10mg/mL	. 71
S015 Lithium Chloride, 7.5 M	. 40
S016 Hae III Buffer, 10X	. 34
S017 TRIS-HCl, 1M - PH 8.0	. 95
S018 Analytical Gel Loading Buffer	8
S020 Yield Calibrators	104
S021 Yield Gel Loading Buffer	106
S022 Chelex, 5%	. 15
S022 Chelex, 5%	92
S023 Stripping Solution #1	102
S024 Wash Solution #1	103
S026 Hybridization Solution, RFLP	36
S028 Hybridization Solution, REEF	18
S030 DTT, 0.39M	28
S030 DTT, 0.39M	. 20
S031 Gen Neutralization Buller	38
S032 Phosphate Buffered Saline (PBS)	48
S034 Phosphate Buneled Saline (1 B3)	. 86
S039 SP, 25A	. 22
S037 Proteinase-K Enzyme, 20mg/mL	79
S040 Sarkosyl, 20%	46
	. 84
S043 Sodium Acetate, 2 M	
S044 Sodium Acetate, 0.210	. 81
S045 SDS, 10%	83
S046 SLS, 20% Solution Suffer Solution	90
S050 Test Gel Loading Buffer	95
	98
S051 TNE, 10X	100
S052 TRIS, 0.1M - pH 7.6 S052 TRIS, 0.1M - pH 7.6 S052 TRIS, 0.1M - pH 7.6 S053 Phenol-Chloroform-Isoamyl Alcohol	45
	78
S054 Sarkosyl, 10%	. 70 
S055 Magnesium Chloride, 0.1M	
S056 Ethanol, 70%	. 01
S059 Sterile Water	
S060 Calibration Control	
S061 Digestion Control	. baa baa

### Numerical Table of Contents

	80
S001 SDS, 20%	00
S002 SSPE, 20X	07
S003 DQα Citrate Buffer	
S004 DQα Hybridization Solution	25
S005 DQα Wash Solution	26
S006 Phenol	43
S009 EDTA, 0.5M	29
S010 Cell Lysis Buffer (CLB)	12
S011 Protein Lysis Buffer (PLB)	68
S013 Denaturation Solution	20
S014 Proteinase-K Enzyme, 10mg/mL	69
S015 Lithium Chloride, 7.5 M	40
S016 Hae III Buffer, 10X	34
	2. 01 8
S018 Analytical Gel Loading Buffer	102
S020 Yield Calibrators	. 102
S021 Yield Gel Loading Buffer	. 104
S022 Chelex, 5%	16
S022 Chelex, 5%	15
S023 Stripping Solution	90
S024 Wash Solution #1	. 100
S025 Wash Solution #2	. 101
S026 Hybridization Solution, RFLP	36
S027 Chloroform-Isoamyl Alcohol	18
S030 DTT, 0.39M	28
S031 Gel Neutralization Buffer	32
S032 Lambda Marker	38
S034 Phosphate Buffered Saline (PBS)	48
S035 SP, 25X	84
S037 Proteinase-K Enzyme, 20mg/mL	70
S037 Froteinase-K Enzyme, 20mg/mL	91
S039 TE, TA	77
	46
S042 Phi-X Marker	
S043 Sodium Acetate, 2 M	02
S044 Sodium Acetate, 0.2M	03
S045 SDS, 10%	79
S046 SLS, 20%	81
S047 Stain Extraction Buffer	88
S049 TE, 100X	92
S050 Test Gel Loading Buffer	93
S051 TNE, 10X	96
S052 TRIS, 0.1M - pH 7.8	98
S053 Phenol-Chloroform-Isoamyl Alcohol	45
S054 Sarkosyl, 10%	76
S055 Magnesium Chloride, 0.1M	. 41
S056 Ethanol, 70%	31
S059 Sterile Water	89
JUJJ JUJI VVALOI	

S062 Test Gel Standard	)6
S064 Cell Pellet Control	3
S079 Hydrogen Peroxide, 3%	37
S082 Chelex, 20%	7
S093 DTT, 1M	27
S094 Digest Buffer	!1
S097 Pre-Wetting Solution	3
S098 Spotting Solution	8
S099 QuantiBlot Wash Solution	7
S100 QuantiBlot DNA Standards	5
S101 SDS, 0.1%	
S102 Heparin Solution	5
S104 Chromogen Solution	9
S107 TNE, 1X	19
S108 SP, 2X	97 10
S109 PM PCR Reaction Mixture	9
S110 EDTA, 200 mM	U N
S111 QUAD STR/PCR Reaction Mixture	с n
S112 dNTP's (10mM stock)	:Z 6
S113 Acrylamide Solution, 40%	0
S114 Urea (25 g Aliquot) 10	'  7
S115 Ammonium Persulfate (0.5g Aliquot)	1
S116 GS500 + Loading Buffer (1:3) 3	o a
S117 BSA Solution, 5 mg/mL	Э Л
S118 Primer, VWA 1 (50 μM)	4
S119 Primer, VWA 2 (50 μM)	2
S120 Primer THO1 1 (50 μM)	0
S121 Primer THO1 2 (50 μM)	2
S122 Primer F13A1 1 (50 μM)	Ζ.
S123 Primer, F13A1 2 (50 μM)	6
S124 Primer, FES/FPS 1 (50 μM)	38
S125 Primer FES/FPS 2 (50 µm)	50
S126 Positive Control-External	1
S127 Positive Control-Internal $$	14
5120 IE , IA	, r

5

S060 Calibration Control
S061 Digestion Control
S062 Test Gel Standard 94
S064 Cell Pellet Control
S079 Hydrogen Peroxide, 3%
S082 Chelex 20%
S093 DTT 1M
S094 Digest Buffer
S097 Pre-Wetting Solution
S098 Spotting Solution
S099 QuantiBlot Wash Solution
S100 QuantiBlot DNA Standards
S101 SDS 0.1%
S102 Heparin Solution
S104 Chromogen Solution
S107 TNF 1X
S108 SP 2X
S109 PM PCR Reaction Mixture
S110 EDTA 200 mM
S111 QUAD STR/PCR Reaction Mixture
S112 dNTP's (10mM stock)
S113 Acrylamide Solution, 40%
S114 Urea (25 g Aliquot)
S115 Ammonium Persulfate (0.5g Aliguot)
S116 GS500 + Loading Buffer (1:3)
S117 BSA Solution, 5 mg/mL
S118 Primer, VWA 1 (50 μM)
S119 Primer, VWA 2 (50 μM)
S120 Primer THO1 1 (50 μM)
S121 Primer THO1 2 (50 μM)
S122 Primer F13A1 1 (50 μM)
S123 Primer, F13A1 2 (50 μM)
S124 Primer, FES/FPS 1 (50 µM)
S125 Primer FES/FPS 2 (50 μM)
S126 Positive Control-External
S127 Positive Control-Internal

Initials: RC	Date: $(\circ) \neq \ell \in \ell$		
S113 Acrylamide Solution, 40%		lot number:	
standard batch sizes: 250 mL	, 375 mL		
Ingredients		amount	amount
RM2085 polyacrylamide bisa (premixed)	crylamide (19:1)	100 ± 1 g	150 ± 1.5 g
RM0409 mixed bed ion excha	ange resin	20 ±1g	30 ± 1.5 g
Initial amount deionized wate	ŗ	<u>150 ± 5 mL</u>	<u>225 ± 5 mL</u>
Final Volume		250 ± 5 mL	375 ± 5 mL

#### Procedure

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

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

Adjust to the final volume with deionized water.

Pour back into bottle.

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

#### Remove the stir bar.

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

Data Log	source	lot	amount
RM2085 Polyacrylamide Bisacrylamide			
RM0409 mixed bed ion exchange resin			
made by:		date:	

Initials: RG

Date:  $l^{0}/7/26$ 

S115 Ammonium Persulfate (0.5g Al	lot number:	
standard batch size: ~ 25 tubes x 0.5g		
Ingredients	aliquot	total amount
RM 0016 ammonium persulfate electrophoresis grade	0.5 ± 0.05 g	12.5 ± 1 g

#### Procedure

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

Fill out chemical logbook.

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

Transfer the aliquots to 15mL conical tubes.

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

Store at room temperature.

Data Log	source	lot	amount	
RM0016 ammonium persulfate				

Initials: RCI

Date: 10/2/96

# S018 Analytical Gel Loading Buffer lot number: \_\_\_\_\_

standard batch size: 100 mL

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

#### Procedure

Combine the TAE, EDTA, and Ficoll.

Mix well. The solution may need to be heated gently to dissolve the Ficoll. Add the bromophenol blue and xylene cyanol. Mix well. When all the solids are dissolved, bring up to volume using deionized water.

Filter sterilize.

Dispense 1.5 mL aliquots into 1.5 mL eppendorf tubes. Store at -20°C.

Data Log	source	lot	amount
RM020 bromophenol blue			
RM217 xylene cyanol			
RM040 Ficoll 400			
S009 EDTA, 0.5M			
RM083 TAE, 10X			
made by:		_ date:	

Initials: RCJ

Date: 2/9/28

### S131 Blue Formamide + Loading Buffer (5:1)

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

standard batch size: 20 x 1200  $\mu L$ 

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

#### Procedure

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

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

Label 20 1.5mL reaction tubes.

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

Close all tubes and mix.

Store at 2-8°C.

#### Data Log

	source	lot	amount
RM2099 Formamide			
S132 Sequencing Loading Buffer			
Quality Control			
QC032 STR gel electrophoresis			
made by:	date	9:	

Initials: RS

Date: (0/7/2/

S117 BSA Solution, 5 mg/mL lot number: standard batch size: 25 mL Ingredients amount 125 mg RM222 Bovine serum albumin 25 mL (guideline) S059 Sterile water **Procedure** Autoclave a 50 mL glass beaker with a stir bar in it. Add the BSA to 20 mL of sterile water in the glass beaker. Stir gently over very low heat until the BSA is completely dissolved. Add the solution to a 50 mL disposable conical tube. Add sterile water to a final volume of 25 mL. Aliquot approximately 0.5 mL of BSA solution into 1.5 mL microcentrifuge tubes. Label each tube with "BSA" and the lot number. Label the rack with expiration date. Store at -20°C.

Data Log	source	lot	amount
RM222 BSA			<b></b>
S059 Sterile water			Magnayar tanan tahun ya ana dalah ya birta
Quality Control			

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

made by:\_\_\_\_\_date:\_\_\_\_\_

Initials: RCI

Date: 10/7/81

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			7.5 ng/µL	
S021 yield gel loading buffer	5 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 received from the manufacturer.

Calculate the final volume according to equation 1.

(final volume) = (initial DNA concentration)(initial DNA volume) equation 1 (7.5 ng/µL)

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.

October 3, 1996

Date: 6 7/26 Initials: RCJ lot number: S060 Calibration Control page 2 of 2 Procedure Combine the DNA, loading buffer, and sterile water. Mix well. Using sterile pipet tips, dispense 200 µL aliquots into sterile 1.5 mL eppendorf tubes. Store at -20°C. source lot Data Log amount RM221 K562 DNA ------S021 yield gel loading buffer S059 sterile water \_\_\_\_\_ **Quality Control** QC026 Gel Electrophoresis made by: \_\_\_\_\_ date: \_\_\_\_\_ Initials: RC

Date: 10	l	7	1	Ç
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S010 Cell Lysis Buffer (CLB)

lot number:

6

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

October 3, 1996

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 α	of solution	
final pH:	spec: 7	7.6 ± 0.1	
made by:	date		

Initials: 24

Date: (\$ 17/26

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 X 10 <sup>6</sup>	
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 X 10<sup>6</sup> cells according to equation 1.

(aliquot volume) =

(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  $180 \times g$  for 5 minutes at  $4^{\circ}C$ . Remove the excess media to give the desired concentration.

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

Record the calculated aliquot volume.

#### Procedure

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

Bring the cell suspension up to the desired final volume.

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

Initials: 25

Date: UD A-184

S064 Cell Pellet Control

lot number:

page 2 of 2

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 Hybridizat	tion		
mada hvr		data	
made by:		_ date:	

Initials: RU Date:

: LO/7/86

S022 Chelex, 5%

lot number:

standard batch size: 500 mL

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

#### Procedure

Filter sterilize approximately 600 mL deionized water.

Pour the water into a 500 mL bottle.

Save the bottom container from the disposable filter unit.

Autoclave the water at 250°F for 30 minutes.

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

Allow the water to cool after autoclaving.

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

Mix on a magnetic stir plate.

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

Store at 2-8°C.

Data Log	source	lot	amount
RM027 Chelex 100	-		
S059 sterile water			
Quality Control			
QC014 Chelex Extraction			
made by:		da	ate:
October 3, 1996	15		

Initials: 29

Date: Lo Ffe;

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:	
October 3, 1996	16			

Initials: /Lt

Date:  $(\circ) \neq / \epsilon \zeta$ 

S082 Chelex, 20%

lot number:

standard batch size: 500 mL

Ingredients	final concentration	amount
RM027 Chelex 100	20. %	100 ± 2 g
S059 sterile water		$450 \pm 50 \text{ mL}$ (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:	
made by:				
October 3, 1996	17			

Initials: 📈 Date: (0/7/96 S027 Chloroform-Isoamyl Alcohol lot number: standard batch size: 500 mL Ingredients final amount concentration RM088 chloroform 96. % 480 ± 3 mL RM089 isoamyl alcohol 4. % 20 ± 3 mL Procedure NOTE: Use only glass graduated cylinders and containers. Measure the isoamyl alcohol into a 500 mL brown bottle. Add the chloroform. Store at 2-8°C in a flammable materials refrigerator. Data Log source lot amount RM088 chloroform ------RM089 isoamyl alcohol -----\_\_\_\_ made by: \_\_\_\_\_ date: \_\_\_\_\_ Initials: 19

Date: CO /HEF

#### S104 Chromogen Solution

lot number:

standard batch size: 30 mL

Ingredients	final Incentration	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	enverseten synamical and an		- California a succession and a succession of the succession of th
RM009 ethanol, 100%			
made by:		date:	

Initials: RG

Date: 10/2/20

S013 Denaturation Solution       lot number:	Comment J			
Ingredients       final concentration       amount         RM005 sodium chloride       1.5 M       1750 ± 50 g         RM004 sodium hydroxide, 10N       0.5 M       1000 ± 100 mL         Procedure         Measure the sodium chloride into a 20 L carboy with approximately 5-10 L deionize 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	S013 Denaturation Solution	lot	number:	
RM005 sodium chloride       1.5 M       1750 ± 50 g         RM004 sodium hydroxide, 10N       0.5 M       1000 ± 100 mL         Procedure         Measure the sodium chloride into a 20 L carboy with approximately 5-10 L deionize 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         RM004 sodium hydroxide, 10N         Quality Control         final pH:	standard batch size: 20 L			
RM004 sodium hydroxide, 10N       0.5 M       1000 ± 100 mL         Procedure       Measure the sodium chloride into a 20 L carboy with approximately 5-10 L deionize 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         RM004 sodium hydroxide, 10N	Ingredients		e	imount
Procedure         Measure the sodium chloride into a 20 L carboy with approximately 5-10 L deionize 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         RM005 sodium chloride         RM004 sodium hydroxide, 10N         Quality Control         final pH:	RM005 sodium chloride	1.5 M	17	50 ± 50 g
Measure the sodium chloride into a 20 L carboy with approximately 5-10 L deionize 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         RM005 sodium chloride         RM004 sodium hydroxide, 10N         Quality Control         final pH:       spec: ≥ 12	RM004 sodium hydroxide, 10N	0.5 M	10	00 ± 100 mL
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   Iot   amount   RM005 sodium chloride   RM004 sodium hydroxide, 10N   Guality Control   final pH:   spec: ≥ 12	Procedure			
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:		o a 20 L carboy wi	th approximate	ly 5-10 L deionized
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   Iot amount   RM005 sodium chloride   RM004 sodium hydroxide, 10N   Guality Control   final pH:	Add the sodium hydroxide solution	on.		
Mix well. Measure and record the pH using pH paper. Store at room temperature. Data Log source lot amount RM005 sodium chloride RM004 sodium hydroxide, 10N Quality Control final pH: spec: ≥ 12	Mix well on magnetic stir plate us	sing a stir bar.		
Measure and record the pH using pH paper.         Store at room temperature.         Data Log       source       lot       amount         RM005 sodium chloride	Raise to the final volume with de	ionized water.		
Store at room temperature.         Data Log       source       lot       amount         RM005 sodium chloride	Mix well.			
Data Log       source       lot       amount         RM005 sodium chloride	Measure and record the pH using	g pH paper.		
RM005 sodium chloride	Store at room temperature.			
RM004 sodium hydroxide, 10N	Data Log	source	lot	amount
Quality Control final pH: spec: ≥ 12	RM005 sodium chloride			-
final pH: spec: ≥ 12	RM004 sodium hydroxide, 10N			
	Quality Control			
made by: date:	final pH:		_ spec: ≥ 12	
	made by:		date:	

Date: LO / 7/56 Initials: 20 S094 Digest Buffer lot number: standard batch size: 2 L final amount Ingredients concentration 10. mM  $40 \pm 2 \, mL$ S009 EDTA, 0.5M 10. mM  $2.4 \pm 0.2$  g RM073 TRIS  $5.8 \pm 0.4$  g 50. mM RM0005 sodium chloride  $200 \pm 2 \,\text{mL}$ 2.0 % S001 SDS, 20% 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 Sc	olutions- Test	150 µL of solu	ution
made by:		date:	

Initials:  $\mathcal{R}$ 

Date: 78/4/96

**S061 Digestion Control** 

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

page 1 of 2

INGREDIENTS	initial concentration (ng/µL)	initial volume (µL)	final concentration	final volume (μL)
RM221 K562 DNA			2 ng/µL	
S128 TE <sup>-4</sup> , 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.

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

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

(TE<sup>-4</sup> volume) = (final volume) - (initial DNA volume) equation 2

Record the  $TE^{-4}$  volume above.

To check the calculations, add together the initial volumes of DNA and 1X TE<sup>-4</sup>.

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

Initials:

Date:

**S061 Digestion Control** 

lot number:

page 2 of 2

#### Procedure

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

Mix well.

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

Store at -20°C.

Data Log	source	lot	amount
RM221 K562 DNA			
S128 TE <sup>-4</sup> , 1X	-		-
Quality Control			
QC026 Gel Electrophoresis			
made by:		date:	

Initials: RU

Date: (0/7/80

**S061 Digestion Control** 

lot number:

page 1 of 2

INGREDIENTS	initial concentration (ng/µL)	initial volume (µL)	final concentration	final volume (μL)
RM221 K562 DNA			2 ng/µL	
S039 TE, 1X	1 X		440 KW 100 KW	

#### 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.

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

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

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

Record the TE volume above.

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

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

Initials: Ry

Date: 10792

**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: المعلم S003 DQα Citrate Buffer	Date: t० lot		
standard batch size: 4 L			
Ingredients	final concentra	tion	amount
RM001 trisodium citrate			73.6 ± 0.1 g
RM002 citric acid			24 ± 1 g (guideline
Procedure			
Dissolve the sodium citrate ir	n approximately	3 liters deio	nized water.
Adjust the pH to 5.0 by additi	on of citric acid	(approximat	ely 24 g).
Adjust the final volume to 4 lit	ters with deioni	zed 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:		_ specificat	ion 5.0 ± 0.2
made by:		date: _	

Initials: RS Date:  $LO \neq EG$ **S004 DQ**α Hybridization Solution lot number: \_\_\_\_\_ standard batch size: 4 L Ingredients final amount concentration S002 SSPE, 20X 5.0 X 1000 ± 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 lot source amount S002 SSPE, 20X S001 SDS, 20% \_\_\_\_\_ **Quality Control** QC016 DQ<sub>α</sub> Hybridization made by: \_\_\_\_\_ date: \_\_\_\_\_
Initials: Ad

Date: 10/2/96

# S005 DQ $\alpha$ Wash Solution

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

standard batch size: 4 L

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

## Procedure

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

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

Pour the SDS into a 4 L bottle.

Add 500 mL SSPE and 3450 mL deionized water.

Cap and mix well by inverting.

Store at room temperature.

Data Log	source	lot	amount
S002 SSPE, 20X			
S001 SDS, 20%		****	
Quality Control			
QC016 DQ $\alpha$ hybridization			
made by:		date:	

Initials: RS	Date: いろそしてん		
S093 DTT, 1M	lot number:	Man dan managaran da	
standard batch size: 5 mL			
Ingredients	final concentration	amo	punt
RM101 dithiothreitol	1.0 <b>M</b>	0.77	± 0.05 g
S059 sterile water		nini kan ant ann ann	
Procedure			
Add the DTT to approximat	ely 4 mL sterile, deionized	water in a <sup>2</sup>	15 mL centrifuge tu
Mix well.			
When the DTT is dissolved	, bring up to volume with s	terile, deion	ized water.
Filter sterilize.			
Dispense 250 µL aliquots in	to sterile 0.5 mL eppendo	rf tubes.	
Store at -20°C.			
Data Log	source	lot	amount
RM101 dithiothreitol			
S059 sterile water			Mantasansana sa
Quality Control			

QC023 QuantiBlot Quality Control of Solutions- Test 20  $\mu L$  of solution

made by:	date:	······································
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Date: (0/7/96 Initials: AU S030 DTT, 0.39M lot number: \_\_\_\_\_ standard batch size: 1.5 mL Ingredients final amount concentration RM101 dithiothreitol 0.39 M  $0.090 \pm 0.001 \text{ g}$ 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 lot source amount RM101 dithiothreitol . S059 sterile water ------**Quality Control** QC023 QuantiBlot Quality Control of Solutions- Test 40 µL of solution

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

Initials: RC

Date: 10/2/66

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			Manahan Manahang mangkang mangkan sa kabang kabupatén kabang kabang kabang kabang kabang kabang kabang kabang k
Quality Control			
final pH:	specification:	8.0 ± 0.1	
made by:		date:	

October 3, 1996

Initials:  $\mathcal{RU}$ 

Date: ( ) Ales

S110 EDTA, 200 mM	lot num	nber:	
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 2	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 $\mu$	L of solution		
made by:		date:	

Initials: RU	Date: (	0/2/26		
S056 Ethanol, 70%		lot numb	er:	
standard batch size: 500 n	nL			
Ingredients	final concentra	ition	amount	
RM009 ethanol, 100%	709	%	350 ± 10 mL	
Procedure				
Measure the ethanol in a g	aduated cylinde	r.		
Bring up to volume with dei	onized water.			
Dispense into a storage cor	ntainer.			
Store at room temperature	or at 2-8°C.			
Data Log	source	lot	amount	
RM009 ethanol, 100%				
made by:			date:	

Initials: 205

Date: (0 (7/26

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 ±4g
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 HCI.

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:	specificat	tion: 7.6 ± 0.	1
made by:		data:	
made by:			

Initials: RCJ

# S116 GS500 + Loading Buffer (1:3)

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

standard batch size: 10 x 800 µL

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

#### Procedure

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

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

Label a screw cap tube with GS500, add 200 $\mu L$  GS 500 and 600 $\mu L$  loading buffer to the tube.

Close and mix the tube.

Store at 2-8°C.

## Data Log

	source	lot	amount
RM1062 ABI GS500 Rox labeled			-
RM0451 ABI Loading Buffer			••••••••••••••••••••••••••••••••••••••
made by:	date	):	

Initials: 20

Date: COALEC

S116 GS500 + Loading Buffer (1:3)

lot number:

standard batch size: 800 µL

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

## Procedure

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

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

Add the loading buffer to the original GS500 tube.

Mark the tube.

Store at 2-8°C.

# Data Log

5	source	lot	amount
RM1062 ABI GS500 Rox labeled			
RM0451 ABI Loading Buffer			*****

made by: date:

Initials: RU

Date: 10/7/26

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:	Mitter and the second	spec: 8.0 ± 0.	1
made by:		date:	
October 3, 1996	34		

Initials: RU

Date:  $(0) ( + ( \varepsilon ) )$ 

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			8-94-9-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-
made by:			date:
October 3, 1996		35	

Initials: RSJ

Date: 10/7/96

 S026 Hybridization Solution, RFLP
 lot number: \_\_\_\_\_\_

 standard batch size: 1 L
 1

ngredients 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 Hyl	bridization		
made by:		date:	

Initials: $\mathcal{M}$ Date: $( \circ \big) \neq \ell \ell ($ S079 Hydrogen Peroxide, 3%Iot number:standard batch size: $30 \times 0.5 \text{ mL}$ Ingredientsfinal concentrationRM176 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 disposable tube.

Add deionized water to a final volume of 15 mL.

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

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

Store at 4°C in the dark.

Discard after 2 months.

Data Log	source	lot	amount
RM176 hydrogen peroxide, 30%			

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

RS Initials:

Date: 10 796

# 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 IIlfragments			20 ng/µL	
S021 yield gel loading buffer	5 X		1 X	
S059 sterile water				aut has air sin

# Calculations

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

Calculate the final volume according to equation 1.

(final volume) = <u>(initial DNA concentration)(initial DNA volume)</u> equation 1 (20 ng/µL)

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: 10/7/25 Initials: RCJ 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 µL aliquots into sterile 1.5 mL eppendorf tubes. Store at -20°C. **Data Log** source lot amount RM155 lambda Hind III fragments S021 yield gel loading buffer \_\_\_\_\_ S059 sterile water \_\_\_\_\_ **Quality Control** QC026 Gel Electrophoresis made by: \_\_\_\_\_ date: \_\_\_\_\_

Initials: RCI Dat	e: col>-lss		
S015 Lithium Chloride, 7.5 M	lot number	•	
standard batch size: 100 mL			
Ingredients	final concentrati	ion	amount
RM032 lithium chloride	7.5 M		31.8 ± 0.2 g
Procedure			
Dissolve the lithium chloride in appr	oximately 75 mL	. deionized	water.
Mix well.			
When the lithium chloride has disso	lved, bring up to	the final vo	olume with deionized water.
Dispense 10 mL aliquots into 15 mL	centrifuge tube	S.	
Autoclave at 250°F for 20 minutes.			
Store at -20°C.			
Data Log	source	lot	amount
RM032 lithium chloride			
made by:		date:	

Initials: 205 Date:  $(0/) \rightarrow (\varepsilon_6)$ S055 Magnesium Chloride, 0.1M lot number: standard batch size: 250 mL Ingredients final concentration

## 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.

0.10 M

amount

 $5.1 \pm 0.3$  g

Dispense into 125 mL bottles.

Autoclave at 250°F for 20 minutes.

RM046 magnesium chloride, hexahydrate

Store at room temperature.

Data Log	source	lot	amount
RM046 magnesium chloride, hexahydrate			
made by:	da	ate:	

Initials: RCS

Date: (0/2-(86

## S112 dNTP's (10mM stock)

lot number:

standard batch size: ~ 25 tubes x 250 µL

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

#### Procedure

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

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

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

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

Aliquot 250 µL of dNTP mix into each tube.

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

Data Log	source	lot	amount
RM0211 Geneamp dNTP's			
dATP			
dCTP			
dGTP			
dTTP			
made by:		date:	

Initials: Rd	Date: 10/7/26	
S006 Phenol	lot number:	
standard batch size: 500 mL		page 1 of 2
Ingredients	final concentration	amount
RM112 phenol		500 ± 10 g
S052 TRIS, 0.1 M - pH 7.8		400 ± 10 mL
RM036 m-cresol		25 ± 1 mL
RM049 2-mercaptoethanol		1.0 ± 0.1 mL
RM029 4-hydroxyquinoline		0.50 ± 0.01 g

## Procedure

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

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

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

Pour the solution into a separatory funnel.

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

To the organic phase, add another 100 mL TRIS.

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

Repeat the 100 mL TRIS wash one more time.

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

Store at 2-8°C.

Initials: ACS	Date:	(1)/2/8	s	
S006 Phenol		lot n	umber:	
				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:		da	ate:	

Initials: 24

Date: 10/7/20

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 alcoho	1 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: RG

Date: (0)7/26

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/ $\mu$ L and the initial volume in  $\mu$ L of the phi-X-174 Hae III received from the manufacturer.

Calculate the final volume according to equation 1.

(final volume) = <u>(initial DNA concentration)(initial DNA volume)</u> equation 1 (50 ng/μL)

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: ACS

Date: ( ) ( ) ( ) ( )

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 µL aliquots into sterile 1.5 mL eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM156 phi-X-174 Hae III fragments			
S018 analytical gel loading buffer			
S059 sterile water		· ······	
Quality Control			
QC026 Gel Electrophoresis			
made by:	da	ite:	

Initials: RC

Date: 2017-(96

# 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			#9990000000000000000000000000000000000
Quality Control			
final pH:	spec:	7.5 ± 0.1	
QC023 QuantiBlot Quality Control of Solutions	- Test 150 μL	of solution	
made by:	date:		
October 3, 1996 48			

Initials: RU

Date: (01+(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:
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Initials: RC

Date: (0/7/EL

S126 Positive Control-External

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

standard batch size ~50 tubes

## Procedure

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

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

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

Re-quantitate using the Quantiblot procedure.

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

Quantitate dilution using Quantiblot.

Data Log	Source	Yield Gel Fract. 1	Yield Gel Fract. 2	Quantiblot Pool	Quantiblot Dilution
EDTA Blood					
	Source	Lot	Amount		
S059 Sterile Water					
Quality Control					
QC031- QUAD STR	R/PCR Amplifi	cation			
made by:			dat	e:	

Initials: RG

S127 Positive Control-Internal

batch number\_\_\_\_\_ page 1 of 2

standard batch size 24 tubes

## Procedure

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

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

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

Select 24 appropriate DNA samples

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

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

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

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

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

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

Initials: RS

Date: ColHEE

S127	7 Positive Co	Positive Control-Internal			batch number page 2 of 2			
Batch. PI #	MB / ex. #	Conc. ng/20µl	DNA vol µl	dH <sub>2</sub> O vol µl	VWA	F13A1	THO1	FES
.1								
.2								
.3								
.4								
.5								
.6								
.7								
.8								
.9								
.10								
.11								
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.24								

# S059 Sterile Water\_\_\_\_

Prepared by \_\_\_\_\_ Witness \_\_\_\_\_ Date \_\_\_\_\_

October 3, 1996

Initials: RC	Date: ر ک	017-186	
S097 Pre-Wetting Solution		lot number:	
standard batch size: 4 L			
Ingredients	final ncentration		amount
RM004 NaOH, 10 N	0.4 N		160 ± 10 mL
S009 EDTA, 0.5 M	25 mM		200 ± 10 mL
Procedure			
Measure 3640 mL deionized wa	ater 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 stor	re in bulk.		
Store at room temperature.			
Data Log	source	lot	amount
RM004 NaOH, 10 N			
S009 EDTA, 0.5 M		-	
made by:		date:	

Initials: 24

Date: (0)7/9(

S118 Primer, VWA 1 (50 μM)

lot number: \_\_\_\_

page 1 of 2

## Physical data

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

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

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H <sub>2</sub> O (µL)
RM 1087 VWA 1 primer			50 p <b>M</b> /µL	
S059 Sterile Water				

#### Calculations

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

Calculate the total amount in pmoles according to equation 1.

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

Record the amount in pmoles above.

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

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

Record the water volume above.

Have somebody check the calculation.

October 3, 1996

Initials: 21 Date	: 10/7/8	6	
S118 Primer, VWA 1 (50 μM)		lot number:	page 2 of 2
			page 2 of 2
Procedure			
Add the sterile water to the original prim	er tube.		
Mix well.			
Dispense 50 µL aliquots into 1.8 mL mic	rocentrifuge	tubes.	
Store at -20°C.			
Data Log	source	lot	amount
RM1087 Primer VWA 1		-	
S059 sterile water		-	
Calculation checked by			
Quality Control			
QC031 QUAD STR/PCR Amplification			
QC023 Quantiblot- test 1µL of solution			

made by: o	date:
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Initials: Ad

Date: 10/7/26

S119 Primer, VWA 2 (50 µM)

lot number:

page 1 of 2

# Physical data

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

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

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

## Calculations

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

Calculate the total amount in pmoles according to equation 1.

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

Record the amount in pmoles above.

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

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

Record the water volume above.

Have somebody check the calculation.

Initials: $\mathcal{R}^{CJ}$ Date	: co/7-(91		
S119 Primer, VWA 2 (50 μM)		lot number:	
			page 2 of 2
Procedure			
Add the sterile water to the original prin	ner tube.		
Mix well.			
Dispense 50 µL aliquots into 1.8 mL mic	crocentrifuge	tubes.	
Store at -20°C.			
Data Log	source	lot	amount
RM1080 Primer VWA 2		-	
S059 sterile water			
Calculation checked by			
Quality Control			
QC031 QUAD STR/PCR Amplification			
QC023 Quantiblot- test 1µL of solution			

made by:	date:	
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Initials: acl

Date: (0/+/85

S120 Primer THO1 1 (50 µM)

lot number:

page 1 of 2

## Physical data

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

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

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H <sub>2</sub> O (µL)
RM 1086 Tho1 1 primer			50 pM/µL	
S059 Sterile Water				

## Calculations

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

Calculate the total amount in pmoles according to equation 1.

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

Record the amount in pmoles above.

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

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

Record the water volume above.

Have somebody check the calculation.

Initials: CLJ Date	e: (6/+/	86	
S120 Primer, Tho1 1 (50 μM)		lot number:	
			page 2 of 2
Procedure			
Add the sterile water to the original print	mer tube.		
Mix well.			
Dispense 50 µL aliquots into 1.8 mL m	icrocentrifuge	tubes.	
Store at -20°C.			
Data Log	source	lot	amount
-	Source	101	aniount
RM1086 Primer Tho1 1			
S059 sterile water			
Calculation checked by	-		
Quality Control			
QC031 QUAD STR/PCR Amplification			
QC023 Quantiblot- test 1µL of solution			

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

Initials: RU

Date: 10/7/25

S121 Primer THO1 2 (50 µM)

lot number:

page 1 of 2

# Physical data

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

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

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

#### Calculations

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

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) =  $(O.D.) \times 4836$ 

equation 1

Record the amount in pmoles above.

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

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

Record the water volume above.

Have somebody check the calculation.
Initials: Ref	Date: ८०/-	7185	
S121 Primer, Tho1 2 (50 <b>բ</b>	(ML	lot number:	
			page 2 of 2
Procedure			
Add the sterile water to the	original primer tube.		
Mix well.			
Dispense 50 µL aliquots in	to 1.8 mL microcentri	fuge tubes.	
Store at -20°C.			
Data Log	SOURCE	e lot	amount
RM1079 Primer Tho1 2			
S059 sterile water			
Calculation checked by			
Quality Control			
QC031 QUAD STR/PCR Ar	nplification		
QC023 Quantiblot- test 1µL	of solution		

made by:	date:	Milletinistanisaisisisis
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Initials: RU

Date: (>/7/85

S122 Primer F13A1 1 (50 µM)

lot number: \_\_\_\_\_ page 1 of 2

Physical data

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

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

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H <sub>2</sub> O (µL)
RM 1085 F13A1 1 primer			50 p <b>M/</b> μL	
S059 Sterile Water				

### Calculations

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

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) =  $(O.D.) \times 5101$ equation 1

Record the amount in pmoles above.

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

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

Record the water volume above.

Have somebody check the calculation.

Initials: AS Dat	te: $(o/+l)$	6	
S122 Primer, F13A1 1 (50 µM)	lot nu	mber:	
			page 2 of 2
Procedure			
Add the sterile water to the original pri	mer tube.		
Mix well.			
Dispense 50 µL aliquots into 1.8 mL m	nicrocentrifuge t	ubes.	
Store at -20°C.			
Data Log	source	lot	amount
RM1085 Primer, F13A1 1			
S059 sterile water		·····	
Calculation checked by	_		
Quality Control			
QC031 QUAD STR/PCR Amplification			
QC023 Quantiblot- test 1µL of solution			

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

Initials: 24 Date: c6/HE6

S123 Primer, F13A1 2 (50 µM)

lot number:

page 1 of 2

### **Physical data**

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

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

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

### Calculations

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

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) =  $(O.D.) \times 5609$ equation 1

Record the amount in pmoles above.

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

 $(dH_2O volume) = (amount in pmoles)$ equation 2 50

Record the water volume above.

Have somebody check the calculation.

Initials: RCS	Date: 10/7/2	6		
S123 Primer, F13A1 2 (50 μM)	lot nu	mber:	page 2 of 2	
			page 2 of 2	
Procedure				
Add the sterile water to the original	primer tube.			
Mix well.				
Dispense 50 µL aliquots into 1.8 ml	_ microcentrifuge	tubes.		
Store at -20°C.				
Data Log	source	lot	amount	
RM 1076 Primer F13A1 2				
S059 sterile water		-		
Calculation checked by				
Quality Control				
QC031 QUAD STR/PCR Amplificati	on			
QC023 Quantiblot- test 1µL of solut	ion			
made by:		date:		

Initials: RS

Date: 10/7/80

S124 Primer, FES/FPS 1 (50 µM)

lot number: \_\_\_\_

page 1 of 2

# Physical data

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

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

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H <sub>2</sub> O (µL)
RM 1084 FES 1 primer			50 pM/µL	
S059 Sterile Water				

# Calculations

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

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 5314

equation 1

Record the amount in pmoles above.

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

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

Record the water volume above.

Have somebody check the calculation.

Initials: Rcs	Date: CO	17/25		
S124 Primer, FES/FPS (50	) μM)	lot number	•	
				page 2 of 2
Procedure				
Add the sterile water to the	original primer tub	e.		
Mix well.				
Dispense 50 µL aliquots int	to 1.8 mL microcen	trifuge tubes	S.	
Store at -20°C.				
Data Log	soul	rce	lot	amount
RM1084 Primer FES/FPS	1			
S059 sterile water	and in the second second			*****
Calculation checked by				
Quality Control				
QC031 QUAD STR/PCR An	nplification			
QC023 Quantiblot- test 1µL	of solution			

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

Initials: RCS

Date:  $(\circ/2/\epsilon)$ 

# S125 Primer FES/FPS 2 (50 µM)

lot number: \_\_\_\_

page 1 of 2

# Physical data

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

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

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H <sub>2</sub> O (µL)
RM 1075 FES 2 primer			50 p <b>M</b> /µL	
S059 Sterile Water				

### Calculations

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

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) =  $(O.D.) \times 4807$ 

# equation 1

Record the amount in pmoles above.

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

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

Record the water volume above.

Have somebody check the calculation.

lot nu	ımber:	a f 0
		page 2 of 2
imer tube.		
nicrocentrifuge	tubes.	
source	lot	amount
	-	
I		
	lot nu imer tube. nicrocentrifuge	nicrocentrifuge tubes.

made	by:	date:	

Initials: RS	Date: (6	17/20				
<b>S011 Protein Lysis Buffer (PL</b> standard batch size: 2 L	В)	lot number	-			
Ingredients	final concentratio	on	amount			
S009 EDTA, 0.5M RM073 TRIS RM005 sodium chloride RM096 hydrochloric acid	10 mM 10 mM 10 mM 		40 ± 2 mL 2.4 ± 0.1 g 1.2 ± 0.05 g			
<ul> <li>Procedure</li> <li>Add the TRIS, EDTA, and sodium chloride to approximately 1.5 L deionized water.</li> <li>Mix well.</li> <li>Adjust the pH to 7.9 with hydrochloric acid</li> <li>Raise to the final volume with deionized water.</li> <li>Mix well.</li> <li>Dispense into 15 mL centrifuge tubes.</li> <li>Autoclave at 250°F for 30 minutes.</li> <li>Store at 2-8°C.</li> </ul>						
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:		da	ate:			

Initials: RS	Date: (0/7/96			
<b>S014 Proteinase-K Enzyme, 10</b> standard batch size: 10 mL	ng/mL	lot number		
Ingredients	final concentration		amount	
RM119 proteinase-K, lyophilized	10 mg/mL	1	00 ± 1 mg	
S059 sterile water		1	0 ± 0.5 mL	
<b>Procedure</b> Add 10 mL sterile, deionized wate	er to one bottle (100 r	ng) lyophil	ized proteinase-	K enzym
Mix by slowly inverting until comp	letely 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 Contro	l of Solutions- Test 1	0 μL of sol	ution	
QC024 Non-Organic Extraction				
made by:	(	tate <sup>.</sup>		

Initials: RC	Date: (0/7/2	
<b>S037 Proteinase-K Enzyme, 20m</b> standard batch size: 5 mL	ig/mL lot num	ber:
Ingredients	final concentration	amount
RM119 proteinase-k, lyophilized	20 mg/mL	100.0 ± 0.5 mg
S059 sterile water		5 mL
<b>Procedure</b> Add 5 mL sterile water to one bottle	e (100 mg) lyophilized prote	einase-k enzyme.
Mix by slowly inverting until comple	etely reconstituted.	
Dispense 500 ul aliquots into 1.5 m	nL 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 $\mu$ L of	solution
QC024 Non-Organic Extraction		
made by:	date:	

Initials: 201

Date: Co A/26

lot number:

#### S111 QUAD STR/PCR Reaction Mixture

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

#### Procedure

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

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper Add the ingredients to either a microcentrifuge tube or a 15 mL centrifuge tube using pipetmen dedicated to PCR preparation area only.

While wearing clean gloves, remove sufficient amount of tubes from the bag and place them in a clean rack designated for the PCR prep room only.

Vortex and spin briefly. Add 20  $\mu$ L per tube using a dedicated repeat pipettor or tips with hydrophobic filters.

Cap all tubes and store in a labeled rack away from all sources of DNA. Store at 2-8°C.

Initials: Rd

Date: 00 / 7/ 8/

S111 QUAD STR/PCR Reaction Mixture

lot number:\_\_\_\_\_ page 2 of 2

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

made by:\_\_\_\_\_date:\_\_\_\_\_

Initials: RCJ

Date: 10/21/26

S100 QuantiBlot DNA Standards

standard batch size: variable

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

page 1 of 2

Ingredients	final concentration	amount	
RM442 DNA Standard A	varies	varies	
S128 TE <sup>-4</sup> , 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<sup>-4</sup> 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<sup>-4</sup> 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<sup>-4</sup> 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<sup>-4</sup> 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<sup>-4</sup> 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<sup>-4</sup> in tube 1B. Vortex to mix thoroughly.

Initials: RCJ

Date: 10/21/96

S100 QuantiBlot DNA Standards

standard batch size: variable

lot number: \_\_\_\_\_page 2 of 2

Add 600  $\mu$ L of diluted DNA Standard (tube 1B) to the 600  $\mu$ L of 1X TE<sup>-4</sup> 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<sup>-4</sup> in tube 1D.

Vortex to mix thoroughly.

Continue the serial dilution through tube 1G.

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

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

Data Log	source	lot	amount
RM442 DNA Standard A			
S128 TE <sup>-4</sup> , 1X			
<b>Quality Control</b> QC018 QuantiBlot Hybridization.			
made by:		date:	

Initials: RC

Date: co A/20

S100 QuantiBlot DNA Standards

standard batch size: variable

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

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 µL 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 µL 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: RCS

Date: 00 /7/26

S100 QuantiBlot DNA Standards

standard batch size: variable

lot number: \_\_\_\_\_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 µL of diluted DNA Standard (tube 1C) to the 600 µL of 1X TE in tube 1D. Vortex to mix thoroughly.

Continue the serial dilution through tube 1G.

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

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

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

Data Log	source	lot	amount
RM442 DNA Standard A			
S039 TE, 1X			
<b>Quality Control</b> QC018 QuantiBlot Hybridization.			
made by:		date:	

Initials: RC

Date: (0/7/26

# 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: Rts	Date:	10/7/98		
S054 Sarkosyl, 10%		lot number:		
standard batch size: 100	mL			
Ingredients	final concent	tration	amount	
S040 sarkosyl, 20%	10. %	, D	50 ± 2 mL	
Procedure				
Dilute 50 mL of 20% sarko	osyl with 50 mL o	deionized water.		
Mix well.				
Filter sterilize.				
Dispense into sterile 15 m	L tubes.			
Store at 2-8°C.				
<b>Data Log</b> S040 sarkosyl, 20%		lot amoun		
made by:				

Initials: Ry	Date:	CO/7/ES	
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 appro	oximately 75 mL o	deionized water.	
Mix until the solution is co	mpletely clear.		
Bring up to volume with de	eionized water.		
Filter sterilize.			
Dispense into sterile 15 m	L tubes.		
Store at 2-8°C.			
Data Log	source	lot amount	
RM057 sarkosyl			
made by:		date:	

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Initials: ACI

Date: 219198

S132 Sequencing loading buffer	lot nu	umber:
standard batch size: 25mL		
Ingredients	final	amount
S009 500mM EDTA, pH8.0 RM Blue Dextran	25 m <b>M</b> 50 mg/mL	1.25 ± 0.05mL 1250 mg ± 10 mg

### Procedure

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

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

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

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

Label 25 1.5mL reaction tubes.

Add 1000 $\mu$ L of the sequencing loading buffer to each tube. Close all tubes.

Store at 2-8°C.

Data Log

	source	lot	amount
S009 500mM EDTA, pH8.0			
RM Blue Dextran			***
Quality Control			
Quality Control			
QC033 STR gel electrophoresis			
made by:	date:		
February 6, 1998	79A		

Initials: RS	Date: 10/7/20			
S101 SDS, 0.1%	lot number:			
standard batch size: 20 l	L			
Ingredients	final concentra		amount	
S001 SDS, 20%	0.1 %	6	100 ± 10 mL	
Procedure				
Add approximately 15 L o	f deionized w	ater into a 20	L carboy.	
Add 100 mL 20% SDS.				
Mix .				
Bring up to a final volume	of 20 L with o	deionized wat	er.	
Mix.				
Store at room temperature	9.			
Data Log	SOURCO	lot	amount	
-	source	101	amount	
S001 SDS, 20%				
made by:			date:	

Initials: RCJ

Date: (0/7/26

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

# Ingredients

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

## **Procedure**

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

Dissolve the SDS in approximately 75 mL deionized water.

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

Bring up to volume with deionized water.

Filter sterilize the warm solution.

Dispense into sterile 100 mL bottles.

Store at room temperature.

Data Log	source	lot	amount
Data Log	source	IUL	amount

RM007 sodium dodecyl sulfate

# **Quality Control**

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

made by:	C	date:	
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Initials: 2cs	Date: Col	$\neq \ell \epsilon_{\zeta}$		
<b>S001 SDS, 20%</b> standard batch size: 1 L		lot number:		
Ingredients	final concentratio	amount n		
RM007 sodium dodecyl sulfate	20 %	200 ± 5 g		
<b>Procedure</b> CAUTION: AN AEROSOL MASK OR FUME HOOD MUST BE USED WHEN MAKING THIS SOLUTION. WEAR GOGGLES FOR EYE PROTECTION.				
Warm approximately 750 mL de	eionized water	on a stirring hot plate.		
Add a fraction of the SDS, allow	ving the solids t	o dissolve before adding more.		
Add the SDS until it is all in solu	ution.			
When the solution is clear, brin	g up to volume	with deionized water.		
Filter sterilize the warm solution	1.			
Dispense into sterile 500 mL bo	ttles.			
Store at room temperature.				
Data Log source	lot	amount		
RM007 SDS				
made by:	date:			

	Initia	als:	RUS
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Date: (17/25

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

# In

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

## Procedure

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

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

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

Add SLS until it is all in solution.

Mix well.

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

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

Dispense into 1 L bottles.

Store at room temperature.

Data Log	source	lot	amount
RM218 sodium lauryl sulfate		,	
made by:		_ date:	

Initials: 오네		0/7/86	
<b>S043 Sodium Acetate, 2 M</b> standard batch size: 100 m		lot number:	
Ingredients		final concentration	amount
RM059 sodium acetate, anh	ydrous	2.0 M	16.4 ± 0.4 g
Procedure			
Slowly add the sodium aceta	ate to approxima	tely 50 mL deionized	water.
Mix well.			
Bring up to volume with deio	nized water.		
Mix well.			
Dispense into 100 mL bottle:	3.		
Autoclave at 250°F for 30 mi	inutes.		
Dispense into 15 mL tubes.			
Store at room temperature.			
Data Log	source	lot amo	punt
RM059 sodium acetate, anhydrous			
Quality Control			
QC023 QuantiBlot Quality C	ontrol of Solution	ns- Test 20 µL of solu	ution
		date:	

October 3, 1996

84

Initials: RCI	Date:	$coh(\epsilon)$	
<b>S044 Sodium Acetate, 0.2M</b> standard batch size: 250 mL		lot number	
Ingredients		final concentrat	ion amount
RM059 sodium acetate, anhyo	drous	0.2 M	
Procedure			
Slowly add the sodium acetate	e to approxi	mately 200 mL d	eionized water.
Mix well.			
Bring up to volume with deioni	ized water.		
Mix well.			
Dispense into 100 mL bottles.			
Autoclave at 250°F for 30 min	utes.		
Store at room temperature.			
Data Log	source	lot	amount
RM059 sodium acetate, anhydrous	*****		
Quality Control			
QC023 QuantiBlot Quality Co	ntrol of Solu	itions- Test 150	uL of solution

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

Initials: 20

Date: 00/7/26

S035 SP, 25X

standard batch size: 4 L

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

Ingredients	final ncentration	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 ± 0.2
made by:		date:	

Initials: RY	Date:	10/7/2	6	
S108 SP, 2X		lot num	1ber:	
standard batch size: 1 L				
Ingredients		nal icentration		amount
S035 SP, 25X	2	2.0 X		80.0 ± 0.8 mL
Procedure				
Add the SP to approximately 800 i	mL deioniz	zed water.		
Bring up to the final volume with d	eionized v	vater.		
Dispense into a 500 mL bottles.				
Autoclave at 250°F for 20 minutes	i.			
Store at room temperature.				
Data Log	SOL	ırce	lot	amount
S035 SP, 25X				
made by:			date:	

Initials: RCS	Date: 🔿	13-680	
S098 Spotting Solution lot number		lot number:	
standard batch size: 75 mL			
Ingredients	final concentration	amount	
S097 Pre-Wetting Solution		74.85 mL ± 1 mL	
RM443 Bromothymol	0.00008%	150 μL ± 1 μL	

# Procedure

Blue, 0.04%

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

Add 150 µL bromothymol blue.

Cap and mix well by inverting.

Store at room temperature.

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

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

date: \_\_\_\_\_

Initials: 29

Date: 10/7/86

S002 SSPE, 20X standard batch size: 4 L

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

# Ingrediente

Ingredients	final	amount
	concentratior	1
RM003 EDTA	20. mM	29.8 ± 0.7 g
RM004 sodium hydroxide, 10N		$40 \pm 5 \text{mL}$ (guideline)
RM005 sodium chloride	3.6 M	840 ± 10 g
RM006 sodium phosphate, monobasic	200 mM	110±3g

# 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	9	lot	amount
RM003 EDTA				
RM004 sodium hydroxide, 10N				
RM005 sodium chloride				
RM006 sodium phosphate, monobasic		<u></u>		
Quality Control				
final pH:		specifi	cation 7.4 :	± 0.2
made by:		date:		

Initials: 20

Date: (0/7/E1

### S047 Stain Extraction Buffer

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

standard batch size: 1 L

Ingredients	final	amount
	concentration	
S009 EDTA, 0.5M	10. mM	20 ± 1 mL
S052 TRIS-HCI, 0.1M - pH 7.8	10. m <b>M</b>	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 <u>except</u> 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 S009 EDTA, 0.5M S052 TRIS-HCI, 0.1M - pH 8.0 RM005 sodium chloride RM101 dithiothreitol S046 SDS, 20% RM004 sodium hydroxide, 10N	source	lot 	amount
Quality Control			
final pH:		specification	8.0±0.2
QC023 QuantiBlot Quality Control of Solutions- Test 150 µL of solution			

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

Initials: 20

Date: 10/2/20

**S059 Sterile Water** 

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

standard batch size: 500 mL

### Procedure

Filter sterilize 500 mL of deionized water.

Aliquot 10 mL each into 15 mL centrifuge tubes.

Autoclave at 250°F for 30 minutes.

Store at room temperature.

**Quality Control** 

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

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

Initials: AG

Date: co/HEC

S023	Stripping	Solu	ition
stand	ard batch	size:	2 L

lot number:

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%		-	
			dete
made by:			date:

Initials:	Date: 10/21/94	
S017 TRIS-HCI, 1M - PH 8.0	lot nu	umber:
standard batch size: 500 mL		
Ingredients	final concentration	amount
RM073 TRIS	1.00 M	60.5 ± 0.1 g
RM096 hydrochloric acid		
<b>Procedure</b> Add the TRIS to approximately 40	0 mL deionized water.	
Mix well.		
Adjust the pH to 8.0 with concentr	ated hydrochloric acid.	
Bring up to final volume with deior	nized water.	
Measure and record the final pH.		
Prepare a 1:100 dilution (10 mM TF water.	RIS-HCI) by mixing 1 mL TF	RIS-HCI solution and 99 mL deionized
Measure and record the pH of the	dilution.	
Autoclave at 250°F for 20 minutes	i.	
Store at room temperature.		
Data Log	source lo	t amount
RM073 TRIS		
RM096 hydrochloric acid		
Quality Control		
final pH: spec: 8.0 ± 0	0.1 1:100 pH:	spec: 8.0 ± 0.1
made by:	date:	
October 18, 1996	93	
Initials:	ŖIJ	
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Date: coladec

**S039 TE, 1X** standard batch size: 500 mL

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

Ingredients	final	~ ~	amount		
S049 TE, 100X	concentrati 1.0 X		5.0 ± 0.3 mL		
<b>Procedure</b> Add the TE to approximately 400 mL de	eionized wate	er.			
Bring up to the final volume with deioni	zed 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 S	olutions- Tes	t 150 µL of solu	ution		

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

Initials: RCJ	Date: 10/21/91		
S128 TE <sup>-4</sup> , 1X	lot number:		
standard batch size: 500 mL			
Ingredients	final concentration	amount	
S017 TRIS-HCI, pH 8.0, 1 M	10 mM	5.0 ± 0.3 mL	
S009 EDTA, 0.5 M	0.1 mM	100± 2 µL	
Procedure			
Add the TRIS and EDTA to 495 m	nL deionized water. Mix well		
Filter Sterilize			
Dispense into 15 mL sterile centri	ifuge tubes.		
Autoclave at 250°F for 20 minute	PS.		
Store at room temperature.			
Data Log	source lot	amount	
S017 TRIS-HCI, pH 8.0, 1 M			
S009 EDTA, 0.5 M			
Quality Control			
final pH:	specification:	8.0 ± 0.2	
QC023 QuantiBlot Quality Contro	ol of Solutions- Test 150 µL of solu	ution	
made by:	date:		

October 18, 1996

Initials: Ad Date	e: (8/7	186		
<b>S049 TE, 100X</b> standard batch size: 250 mL	lot n	umber:		
Ingredients	final	<b>a</b> n	amou	unt
	concentrati	on	02105	~
RM003 EDTA	0.10 M		$9.3 \pm 0.5$	-
RM073 TRIS	1.00 M		30.3 ± 0.1	i g
RM004 sodium hydroxide, 10N	and the same and the same			-
RM096 hydrochloric acid			Not have seen and have been	-
Add the EDTA to approximately 200 ml Adjust the pH to approximately 8.0 with until totally dissolved. Add the TRIS and mix well. Use hydrochloric acid or sodium hydrox Bring up to final volume with deionized Measure and record the final pH. Dispense into 125 mL bottles. Autoclave at 250°F for 30 minutes. Store at room temperature.	n sodium hyd kide to adjust	roxide to get th		,
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: RSF

Date: 10/7/es

## S050 Test Gel Loading Buffer

lot number:

standard batch size: 100 mL

Ingredients	final concentration	amount
RM020 bromophenol blue	0.10%	$0.10 \pm 0.01$ g
RM217 xylene cyanol	0.10%	$0.10 \pm 0.01$ g
RM040 Ficoll 400	5.0%	$5.0 \pm 0.1$ g
S009 EDTA, 0.5M	20. mM	$2.00 \pm 0.05  \text{mL}$
RM083 TAE, 10X	2.0 X	$20.0 \pm 0.5$ 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 $^{\circ}$ C.

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

Initials: RCS

10/7/85 Date:

S062 Test Gel Standard

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

## page 1 of 2

INGREDIENTS	initial concentration (ng/µL)	initial volume (µL)	final concentration	final volume (µL)
RM242 K562 DNA, Hae III fragments			5 ng/µL	
S050 test gel loading buffer	2 X		1 X	
S059 sterile water	ar 10 ar 10 ar		<b></b>	

#### 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.

(final volume) = <u>(initial DNA concentration)(initial DNA volume)</u> equation 1 (5 ng/µL)

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) = 0.5(final volume) equation 2

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

(water volume) = [0.5 \* (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.

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	11124	18	
	iua	-	

RU

Date: 10/7/26

S062 Test Gel Standard

lot number:

page 2 of 2

## Procedure

Combine the DNA, loading buffer, and sterile water.

Mix well.

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

Store at -20°C.

Data Log	source	lot	amount
RM242 K562 DNA, Hae III fragments			
S050 test gel loading buffer			
S059 sterile water			
Quality Control			
QC026 Gel Electrophoresis			
made by:		date:	

Initials: RS

Date: 10/2/15

S051 TNE, 10X standard batch size: 100 mL

lot number:

Ingredients	final concentration	amount
RM073 TRIS	100 mM	1.2 ± 0.02 g
S009 EDTA, 0.5M	10 mM	$2.0 \pm 0.1 \text{ mL}$
RM005 sodium chloride	1.0 M	5.8 ± 0.2 g
RM096 hydrochloric acid		C C

### 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.

Data Log	source	lot	amount
RM073 TRIS	******		
S009 EDTA, 0.5M	*****		
RM005 sodium chloride			
RM096 hydrochloric acid		-	
Quality Control			
final pH:		specification:	7.4 ± 0.1
made by:		date:	

	Initials: 24	Date: co /	7186	
	S107 TNE, 1X	lo	t 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 w	ater.	
	Bring up to the final volume with c	leionized water.		
	Dispense into a 125 mL bottles.			
	Autoclave at 250°F for 20 minutes	S.		
	Store at room temperature.			
	Data Log	source	lot	amount
	S051 TNE, 10X			
	Quality Control			
	QC023 QuantiBlot Quality Contro	l of Solutions- T	est 150 µL of	solution
	made by		date <sup>.</sup>	

Initials: RJ	Date: Col7/E	۷			
<b>S052 TRIS, 0.1M - pH</b> standard batch size: 1					
Ingredients	final	amount			
RM073 TRIS RM096 hydrochloric ac	concentration 0.1 M d	12.1 ± 0.2 g			
<b>Procedure</b> Add the TRIS to approx	imately 750 mL deionized wa	ater.			
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 aci	1				
Quality Control					
final pH:		spec: 7.8 ± 0.1			
made by:	da	ate:			

Ò

Initials: Ry

Date:  $co/7/\epsilon c$ 

S114 Urea (25 g Aliquot)

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

standard batch size: ~ 25 tubes x 25 g

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

#### Procedure

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

Fill out chemical logbook.

Using small weigh boat, weigh  $25 \pm 0.2$  g aliquots of urea.

Transfer the aliquots to 50 mL conical tubes

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

lot

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200	

Store at room temperature.

Data	Loa	
Dara		

source

amount

RM0079 urea

made by:\_\_\_\_\_date:\_\_\_\_\_

Initials: RCJ Date	219128				
S114a Urea (18 g Aliquot-377 Sequencer) lot number:					
standard batch size: ~ 25 tubes x 18 g	standard batch size: ~ 25 tubes x 18 g				
Ingredients	aliquot	total amount			
RM0079 Urea electrophoresis grade	18 ± 0.1 g	450 ± 4 g			

#### Procedure

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

Fill out chemical logbook.

Using small weigh boat, weigh  $18 \pm 0.1$  g aliquots of urea.

Transfer the aliquots to 50 mL conical tubes

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

Data Log	source	lot	amount	
RM0079 urea				
made by:		date	2	

Initials: $\mathcal{L}\mathcal{L}$ Date: $\mathcal{L}(\mathcal{L}\mathcal{L})$ S114b Urea (10.8 g Aliquot-377 Sequencer)Iot number:standard batch size: $\sim 25$  tubes x 10.8 gIngredientsaliquottotal amountRM0079 Urea<br/>electrophoresis grade $10.8 \pm 0.1$  g $450 \pm 4$  g

#### Procedure

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

Fill out chemical logbook.

Using small weigh boat, weigh  $10.8 \pm 0.1$  g aliquots of urea.

Transfer the aliquots to labeled 50 mL conical tubes.

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

Data Log	source	lot	amount	
RM0079 urea				
made by:		dat	e:	

Initials: RCJ

Date: 10/7/20

S024 Wash Solution	n	#1
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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.

Data Log	source	lot	amount
S035 SP, 25X			
S046 SLS, 20%			
made by:			date:

Initials: 20

Date:

10/7/21

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.

Data Log	source	lot	amount
S035 SP, 25X			
S046 SLS, 20%			
made by:			date:

### Initials: RU

Date: 10/21/26

## S020 Yield Calibrators

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

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

## page 1 of 2

Ingredients	final	amount
	concentration	
S128 TE <sup>-4</sup> , 1X	1 X	
RM148 lambda DNA	ann sur air air	140 ± 10 µg (guideline)
S021 yield gel loading buffer	1.25 X	$3.0 \pm 0.5 \text{ mL}$
S059 Sterile Water		

#### Calculations

### **Stock Solution**

Final D Concent	 Final Volume	Initial DNA Concentration	Volume Lambda DNA	Volume 1X TE <sup>-4</sup>
50 ng/µL	2800 µL			

	Cal	ib	rate	ors
--	-----	----	------	-----

Calibrato r	Final DNA Concentration	Stock DNA Concentration	Volume Stock DNA	Volume Water	Volume Buffer
А	300 ng/10 μL	50 ng/µL	1200 µL	300 µL	500 µL
В	200 ng/10 μL	50 ng/µL	800 µL	700 µL	500 µL
С	100 ng/10 μL	50 ng/µL	400 µL	1100 µL	500 µL
D	50 ng/10 µL	50 ng/µL	200 µL	1300 µL	500 µL
E	25 ng/10 μL	50 ng/µL	100 µL	1400 µL	500 µL
F	10 ng/10 μL	50 ng/µL	40 µL	1460 µL	500 µL

### Procedure

Each lot of yield calibrators is prepared as a batch of five sets. Each batch requires 2800  $\mu L$  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: RO

Date: 1 \* /21/8\*

**S020 Yield Calibrators** 

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) = <u>(final DNA concentration)(final volume)</u> equation 1 (initial DNA concentration)

Calculate the volume of 1X TE<sup>-4</sup> to add to the stock solution according to equation 2.

(volume 1X TE<sup>-4</sup>) = (final volume) - (volume lambda DNA) equation 2

Prepare the stock solution by diluting the lambda DNA in a sterile centrifuge tube with 1X TE<sup>-4</sup> 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  $\mu$ L for each level.

Mix well.

Divide each level into five 300  $\mu$ L aliquots, and dispense into labeled, sterile eppendorf tubes. Add 100  $\mu$ L of yield gel loading buffer to each tube. The final volume of each aliquot is 400  $\mu$ L. Store at -20°C.

Data Log S128 TE <sup>-4</sup> , 1X RM148 lambda DNA S021 yield gel loading buffer S059 sterile water	source	lot	amount  
Quality Control QC026 Gel Electrophoresis			
made by:			date:

#### Initials: Ry

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

Date: (0/7/86

6	In an unit batch size: 5 X 400µL each	lot number	r.	
	Ingredients			
	S039 TE, 1X RM148 lambda DNA S021 vield gold	final <sup>concentration</sup> 1 X	page 1 of 2 amount	
	S021 yield gel loading buffer S059 Sterile Water	 1.25 X	 140 ± 10 µg (guideline 3.0 ± 0.5 ml	
(	Calculations		$3.0 \pm 0.5 \text{ mL}$ (guideline	<del>)</del> )

ŀ	50 pg/ul		Volume	Volume 1X TE	77
$\Gamma$					

Calibrat	• Final DNA	Ca	librators			
	Concentration		Volume	No.		
A	300 ng/10 μL	50 ng/µL	n Stock DN	A Volume A Water	∍   Volume   Buffer	•
В	200 ng/10 µL	μ	1200 µL	300 µL		4
С	100 ng/10 µL	50 ng/µL	800 µL	700 µL		-
D	50 ng/10 μL	50 ng/μL	400 µL	1100 µL		4
E	25 ng/10 μL	50 ng/µL	200 µL			
F	10 ng/10 μL	50 ng/µL	100 µL	1300 µL	500 µL	
		50 ng/µL	40 µL	1400 µL	500 µL	
<b>rocedure</b> ach lot of vie	ld collin		μL	1460 µL	500 µL	

Pr Ea

of 50 ng/µL stock lambda DNA solution.

of yield calibrators is prepared as a batch of five sets. Each batch requires 2800  $\mu$ L Record the concentration in ng/µL of the lambda DNA received from the manufacturer under

4 -

	Initials: KS	Date: () /2-/86	
	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 ± 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.

October 3, 1996

Initials:	Date:	10/71	25	
S021 Yield Gel Loading Buffer		lot nu	ımber:	
standard batch size: 100 mL				Page 2 of 2
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 <sup>.</sup>	

Date: 18/7/86

## S020 Yield Calibrators

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

page 2 of 2

#### Procedure Calculate th

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

(volume lambda DNA) = <u>(final DNA concentration)(final volume)</u>	equation 1
(initial DNA concentration)	•

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

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

Prepare the stock solution by diluting the lambda DNA in a sterile centrifuge tube with 1X TE and mix well.

Label six sterile eppendorf tubes, one for each of the six yield calibrator levels.

Pipet the appropriate amounts of DNA stock solution and sterile water into the labeled tubes. The combined volume of DNA and water is 1500  $\mu$ L for each level.

Mix well.

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

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

Store at -20°C.

<b>Data Log</b> S039 TE, 1X RM148 lambda DNA S021 yield gel loading buffer S059 sterile water	source	lot 	amount 	
Quality Control QC026 Gel Electrophoresis				
made by:		d	ate:	