LABORATORY PROCEDURES FOR GENOMIC LIBRARIES

REFERENCE NO: GLI/1998/3/9

TITLE: BUFFERS, SOLUTIONS AND REAGENTS

Ampicillin

Make 50mg/ml stock = 5g Ampicillin di-sodium salt (Sigma A 9518)
[Sanger stores]
[store powder at 4°C]
in 100ml MilliQ water

Stir to dissolve
Filter through a 0.2 micron filter unit, 100ml size, using the vacuum pump attached to the two large Buchner funnels.
Keep sterile
Divide into

one 50ml aliquot into a sterile 50ml Falcon tube (label the tube with; Ampicillin, 50mg/ml and the date)
and 100 x 500 µl aliquots, in sterile 1.5ml Sarstedt tubes, using a P1000 Gilson pipette

Label all the tubes on the lids, with **A**
Store frozen at -20°C
Use at 1/1000 dilution i.e. 500µl aliquot in 500ml media = 50µg/ml final concentration

Ampicillin is added to all broth/agar used for growing the YAC libraries.

Tetracycline

Make 5mg/ml stock = 500mg Chlortetracycline (Sigma C4881)
[store powder at -20°C]
in 50ml MilliQ water
+ 50ml 99% Alcohol (i.e. 50% alcohol)

Stir to dissolve
Do not filter (the 50% alcohol will sterilise the solution)
Using a 12.5ml combitip, at setting 5, aliquot 1.25ml into 50 sterile 1.5ml Sarstedt tubes
Label all the tubes on the lids, with **T**

Store the remainder in a sterile 50ml Falcon tube
(label the tube with; Tetracycline, 5mg/ml and the date)
Use at 1/400 dilution i.e. 1.25ml aliquot in 500ml media = 12.5µg/ml final concentration

Tetracycline is added to all broth/agar used for growing the YAC libraries.

Kanamycin

Make 25mg/ml stock = 2.5g Kanamycin monosulfate (Sigma K1377)
[store powder at -20°C]
in 100ml MilliQ water

Stir to dissolve
Filter through a 0.2 micron filter unit, 100ml size, using the vacuum pump attached to the two large Buchner funnels.
Keep sterile
Divide into

15 x 5ml aliquots into sterile 7ml Bijou tubes
(label the tubes with; Kanamycin, 25mg/ml and the date)
and 50 x 500µl aliquots, in sterile 1.5ml Sarstedt tubes, using a P1000 Gilson pipette

Label all the tubes on the lids, with **K**
Store frozen at -20°C

For the PAC library:	use at 1/1000 dilution i.e. 500µl aliquot in 500ml media	= 25µlg/ml final concentration
For the cFugu library:	use at 1/833 dilution i.e. 600µl aliquot in 500ml media	= 30µg/ml final concentration

Kanamycin is added to all broth/agar used for growing the PAC and cFugu libraries in *E.coli* cells.

0.5M EDTA, pH8

Dissolve 186.1g EDTA (Ethylenediaminetetra-acetic acid disodium salt)
in 900ml MilliQ water.

Stir, on a magnetic stirrer with a 'flea' (magnetic stirring bar), and with the pH electrode in the buffer gradually add solid sodium hydroxide pellets until it is stable at pH8. (As you add the NaOH the pH will come down. This allows more of the EDTA to dissolve and the pH will rise again - so you have to continue until the solution is clear and the pH is stable)
Use a 1 L measuring cylinder to bring the volume up to 1 L, with MilliQ water.

Pour into labelled Duran bottles, and autoclave before use.

1m Tris/HCl, pH 7.4 or 8.

Dissolve 121.1g Tris (Tris(Hydroxymethyl)Aminomethane) or (TRIZMA Base)
in 900ml MilliQ water

Stir, on magnetic stirrer with a 'flea' (magnetic stirring bar), and with the pH electrode in the buffer gradually add conc. HCl (concentrated hydrochloric acid) drop by drop using a pasteur pipette, continually recording the pH, until the pH is stable at pH7.4 or pH8. (NB. Tris requires a special pH electrode - the only one that we have in the Resource Centre is the Mettler Toledo Inlab 407 electrode attached to the Corning pH meter 240)

Wear safety spectacles when adding the conc. HCl.

When the pH is correct use a 1 L measuring cylinder to bring the volume up to 1 L, with MilliQ water.

Pour into labelled Duran bottles, and autoclave before use.

1 x TE pH8

[TE = Tris/EDTA] 1 x TE = 10mM Tris
1mM EDTA
pH8

Measure approx. 800ml milliQ water into a 1 L measuring cylinder

Add 10ml of 1M Tris/HCl pH8
2ml of 0.5M EDTA pH8 using pipettes

Make the volume up to 1L with milliQ water
Mix

Pour into labelled Duran bottles, and autoclave before use

5mM EDTA pH8

1 L autoclaved MilliQ water
Add 10ml 0.5M EDTA pH8

Spheroplasting Solution

1M Sorbitol
20mM EDTA
10mM Tris/HCl pH 7.4

For 5 L, dissolve 911g Sorbitol in 4 L MilliQ water

(it is easiest to do this by gradually adding the sorbitol to the water as it is strirring)

Add 200ml 0.5 EDTA pH8
Add 50 ml 1M Tris/HCl pH7.4
Make up to the 5L mark in the beaker, and stir to mix thoroughly.
Carefully pour into 10 x 500 ml Duran bottles
Label and data the bottles, and autoclave before use

YLS

1% Lithium dodecyl sulphate (Sigma L4632)
100mM EDTA
10mM Tris/Hcl

For 2 L

Wear gloves and face mask
Very carefully weigh out 20g of LDS (Lithium dodecyl sulphate = Docecyl lithium sulfate = Lauryl sulfate, Lithium salt) in a weighing boat. Carefully pour this into a 2L beaker, without allowing the powder to puff up. Cover the beaker with cling film and carry back to the laboratory. Add 1L of MilliQ water, cover with the cling film and stir to dissolve. It is safe to remove the face mask when LDS has dissolved.

Add 400ml 0.5M EDTA pH8
Add 20ml 1M Tris/Hcl pH8

Pour into a 2L measuring cylinder, and adjust the volume to 2L by adding MilliQ water. Put fresh cling film over the top of the measuring cylinder to mix.

Filter through a 0.2 micron filter unit, 500 ml size, using the vacuum pump attached to the two large Buchner funnels. You will need to use 4 filter units for each 2L of solution made.

Store at RT

70% Alcohol

For 1L

Using 99% Alcohol: 700ml alcohol + 300ml MilliQ water
Using 36% Alcohol: 730ml alcohol + 270 ml MilliQ water