

Preparing solutions for BAC DNA preparation

1X LB (to make 2L) **best for making BAC fingerprints ******

Tryptone	1%	20g
Yeast extract	0.5%	10g
NaCl	1 %	20g

Chloramphenicol 12.5 ug/ml 0.8 ml (Add only after autoclave rest)

For freezing media, also add 140 ml of glycerol to make 6%. This should be added prior to autoclaving.

Chloramphenicol stock (25mg/ml):

0.25g chloramphenicol

10ml EtOH

Use closed scale to measure chloramphenicol, mix & store in freezer

To make up 1X LB:

Put small amount distilled water in bottom of container mixing broth in, add NaCl first, then tryptone, then yeast. Use distilled water to wash down any powder on the sides of the container, cover with plastic wrap, let mix on magnetic stirrer for 5 minutes. Pour into graduated cylinder, wash down bucket with distilled water & add to cylinder until reach 2000 ml. Return to bucket, pour back & forth several time to make sure well mixed, use graduated cylinder to measure 400ml, pour into 500ml bottle. Place cover on, make aluminum foil cap to go on top. Put into plastic tray, lift cover/aluminum cap to make sure just sitting on bottle. Put in autoclave on the bottom shelf. Close door, set to P5, then start.

Let cool before adding chloramphenicol

For 2L batch:

For each 400 ml bottle of LB add 200 ul of stock chloramphenicol
($12.5 / 25000 * 400 = 0.2\text{ml} = 200 \text{ ul}$ use P200)

Solution 1

50 mM TrisHCL

10 mM EDTA

100ug RNase

Autoclaved water until reach desired volume

If making a large batch, put smaller amount into purple top tube & add the RNase to only the smaller amount. RNase stock (from freezer) is 30.5 mg protein/ml. Desired final concentration is 100 ug/ml

**Draw RNase up slowly (viscous) and wipe outside of the tip on the inside of the vial
**When add RNase, rinse pipette tip to get all RNase out Example: to make 40 ml

Final concentration	Stock conc	Amount
50mM TrisHCl	1M TrisHCl	2 ml (P1000)
10mM EDTA	0.5 M EDTA	0.80 ml
100 ug/ml RNase	30.5 mg/ml	0.131 ml

Sample calculation

For Tris: 50 mM Tris HCl / 1000 (1M) Tris HCl * 40 (final volume) = 2 ml (P1000)

For EDTA: 10 mM EDTA / 500 (0.5M) * 40 = 0.80 ml (P1000)

For RNase: 100 ug/ml / 30500 (30.5 mg/ml) * 40 = 0.131 ml = 131 ul (P200)

Solution 2

200 mM NaOH

1% SDS

Weigh 2 tablets NaOH, add small amount autoclaved water, mix until dissolved. Add rest of water and required volume of 10% SDS

200mM NaOH = 8 g/l

final solution volume = (wt tab*1000)/8

Need 10% of this volume to be made up with 10% SDS to get final conc of 1% SDS

ex. Tablets weigh 0.175 g so need to add 21.8 ml of solution.

Add 19.6 ml of water and 2.2 ml 10% SDS

Solution 3

3 M KOAc

To make 500ml:

Weigh potassium acetate (in anhydrous cabinet) $98.14 \text{ MW} * 3\text{M} = 294.42/2.0275 = 145.21\text{g}$

Wash down sides with distilled water, add 400 ml, put on magnetic mixer

Calibrate pH meter

Plug in, wash off pH meter with distilled water. Calibrate pH meter with pH 4 & 7 standards

Hold in solution standard until reads the standard, press calibrate key

Wash probe, repeat with second standard

Turn on mag mixer, then put pH meter in solution, hit pH button, add acetic acid until reach pH of 5.5. Then wash meter & return to original storage solution (yellow pH7).

Pour solution into graduated cylinder. Add distilled water to bring to 500ml. Pour back & forth between cylinder & beaker twice. Pour into bottle & make aluminum cover, leave cap loose, autoclave at P4 setting

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