

Preparation of competent bacteria and transformations

Ref: Hanahan, D. J. Mol. Biol. 1983. 166:558

Solutions

<u>SOB/SOC</u>		For 1 liter	
		<u>SOB</u>	<u>SOC</u>
Bactotryptone	2%	20g	20g
Yeast Extract	0.5%	5g	5g
NaCl (5M stock)	10 mM	2 ml	2 ml
KCl (1M stock)	2.5 mM	2.5 ml	2.5 ml
Mg Salts	20 mM	10 ml	10 ml
Glucose	20 mM	--	10 ml

Prepare without Mg salts and glucose and autoclave. Then add sterile-filtered Mg salts and glucose.

Mg salts

1 M $MgCl_2$
1 M $MgSO_4$

<u>FSB</u>		<u>500 ml</u>	<u>250 ml</u>
KAc (1 M stock)	10 mM	5 ml	2.5 ml
KCl	100 mM	3.728 g	1.864 g
$MnCl_2 \cdot 4H_2O$	45 mM	4.453 g	2.227 g
$CaCl_2 \cdot 2H_2O$	10 mM	735 mg	368 mg
$HACo(III)Cl_3$	3 mM	401 mg	200.5 mg
Glycerol	10%	50 ml	25 ml

1 M KAc is adjusted to pH 7.0, sterile filtered and stored at -20. Salts are added as solids and pH of the solution adjusted to 6.4 with 0.1 M HCl. Sterile filter and store at 4°. Use Millipore water directly from filter for preparing solutions. Make fresh each time you prepare cells.

DMSO Use tissue culture grade from Sigma. Comes in 5 ml glass vials. Use fresh.

Procedure for preparing frozen stocks

For 500 ml culture. Enough to prepare approximately 80 0.5 ml aliquots of competent cells.

Inoculate 25 ml SOB with 25 μ l of previously competent DH5 α . Grow O/N.

Inoculate 6.25 ml into 500 ml SOB in 1 liter sterile flask. Grow with shaking @ 300 RPM at 37⁰.

Grow until OD550 is 0.35 to 0.5

Transfer to pre-chilled 250 ml centrifuge tubes and keep on ice for 15 min.

Centrifuge at 2500 RPM, 4^oC, for 12 min

Gently resuspend in 1/3 volume FSB (167 ml); ice for 15 min

Centrifuge at 2500 RPM, 4^oC, for 12 min

Gently resuspend in 1/12.5 original volume FSB (40 ml)

Add DMSO to 3.5% (1.4 ml)

Ice 5 minutes

Add same volume DMSO (1.4 ml)

Ice 5 minutes

Aliquot cells into Eppendorf tubes; 0.5ml/tube

Freeze in dry ice/EtOH bath

Store at -70^oC

Transformation

Thaw an aliquot of cells at room temperature until just liquid.

Aliquot 200 μ l/transformation into prechilled 12x75 polypro tubes

Add 25 μ l DNA and swirl to mix

Incubate on ice for 1 hour

Heat shock @ 42° C for 90 seconds

Ice 1-2 minutes

Add 800 μ l **SOC** and incubate at 37° C for 1 hour

Plate 100 μ l onto LB plate containing the appropriate antibiotic

Spin down remaining 900 μ l

Remove 800 μ l of supernatant and plate remaining 100 μ l onto LB plate.

Incubate O/N at 37° C