

## **Competent bugs protocol**

**We use HB101 or Stbl2 (Invitrogen) as they grow retroviral plasmids better. HB101 are RecA- so you must use the additional chaotropic salt step (PB) in DNA preps.**

### **Day 1**

Take a tip and streak four ways from an aliquot (NO antibiotic plate)

### **Day 2**

Pick a colony and grow up o/n cultures in 10mls no antibiotic LB

### **Day 3**

Inoculate 200mls No antibiotic LB with 5mls o/n culture

Grow at 37°C on shaker until OD550 =0.45-0.55

Or OD 595 =0.5-0.6

Use LB to blank

check after 60min, 90min and then check regularly

When density right pour into 50ml falcons

*We usually grow at 37 but if you grow for longer at lower temp eg room temp or even 16°C if you can, they take longer to reach the right density eg 6hrs at 28° but then they become a lot more competent.*

Chill on ice 10 min

Pellet at 3,500rpm 10mins

Pour off LB –PUT BACK ON ICE

Resuspend gently in 20mls cold TFB1 (first 2mls, then 18mls)

Leave on ice 5 min

Spin at 3,500rpm 10mins

Pour off supernatant –PUT BACK ON ICE

Resuspend gently in 2mls cold TFB2

Leave on ice for 10 mins

Using a multidispenser – aliquot 100ul aliquots into 500µl tubes whilst the tubes are on dry ice. Store at -80°C

### Stock solutions

All solutions made up in water

\*\*\*Use plastic containers where possible – some glassware have had trigene, which prevents the bugs growing

	g/L	g/50ml	g/100ml
1M Kac	98.14	4.907	9.814
1M RbCl	120.90	6.045	12.090
0.1M Cacl <sub>2</sub>	14.70	0.735	1.470
1M MnCL <sub>2</sub>	197.90	9.895	19.790
*0.1M PIPES (pH6.5)	30.24	1.512	3.024

\*Need to add KOH to adjust pH to 6.5 to dissolve

### **Working solutions TFB1**

Make up in T75 flask

\*\*\*Filter sterilize

Keep on ICE

	50ml	100ml
30mM Kac	1.5ml	3ml
100mM RbCl	5ml	10ml
10mM Cacl <sub>2</sub>	5ml	10ml
50mM MnCL <sub>2</sub>	2.5ml	5ml
15% Glycerol (from 50%)	15ml	30ml

Water

21ml

42ml

### **Working solutions TFB2**

Make up in 50ml falcon

\*\*\*Filter sterilize

Keep on ICE

10mM PIPES	1ml
75mM Cacl	7.5ml
10mM RbCL	0.1ml
15% glycerol (100%)	1.5ml

## **Test HB101 competent bugs**

Dry 2 Amp plates in incubator

Thaw out bugs on ice -30min

Add either no DNA or 10pg PUC19 (1ul) (Invitrogen transformation control)  
Leave on ice 10 min

Heat shock  
42° C for 45sec  
2min ice

If you are selecting in Kanamycin. you need to recover the bugs in 2ml LB at 37°C for 45mins after heat shock and 2min ice. After recovery spin 30s low speed to pellet bugs, resuspend in 50-100ul and plate. If you're using Ampicillin you don't need the recovery period and can plate directly after heat shock and 2min on ice.

Plate out – o/n 37°C incubator

## **Day 3**

Good efficiency – should have around 100 colonies