

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

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

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 ₂	5ml	10ml
50mM MnCL ₂		

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