

Amplification of plasmids with chloramphenicol

Method 1:

Grow a 200 ml prep in superbroth to saturation by vigorous shaking at 37 C (6-8 hrs or overnight).

Add 200 ml more of prewarmed superbroth (to avoid shocking cells), then add chloramphenicol to a final concentration of 125 ug/ml.

Make chloramphenicol stock solution at 125 mg/ml in ethanol, add 1 ul per ml for final concentration of 125 ug/ml. Chloramphenicol should be handled with care; wear gloves and avoid inhalation of powder. Consult the MSDS sheet for further information.

Method 2: (modified from Maniatis, 2nd ed., p. 1.33)

Innoculate SB containing appropriate antibiotic with 4 ml of bacterial culture.

Incubate with vigorous shaking at 37 degrees until the OD600 is approximately 0.4.

Add chloramphenicol to a final concentration of 170 ug/ml.

Incubate for a further 12 to 16 hours at 37 degrees with vigorous shaking.

Note: This method only works well for 500 ml preps; for 100 ml preps use the following modification:

Grow a 100ml prep in SB for 6 to 8 hours at 37 degrees in the appropriate antibiotic with vigorous shaking. (For best results inoculate with 200ul of a 5 ml overnight culture.)

Add chloramphenicol to 170 ug/ml final concentration and incubate for a further 12 to 16 hours with vigorous shaking.