

Chloramphenicol - amplification of plasmids

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When low-copy-number plasmids containing the pMB1 or ColE1 origin of replication are prepared, plasmid DNA yields can be improved by adding chloramphenicol to the culture medium.

Chloramphenicol inhibits host protein synthesis and thus prevents replication of the host chromosome. Plasmid replication, however, is independent of newly synthesized proteins and continues for several hours until up to 2000–3000 copies per cell are accumulated.

Alternatively, the cell culture can be grown with only partial inhibition of protein synthesis under low chloramphenicol concentrations (10–20 g/ml) resulting in a 5–10- fold greater yield of plasmid DNA. Both methods show the positive side effect of much less genomic DNA per plasmid, but they obviously work only with plasmids that do not carry the chloramphenicol resistance gene.

Furthermore, the method is only effective with low copy number plasmids under stringent control (pBR322). All modern high copy number plasmids (pUC) are already under relaxed control due to mutations in the plasmid copy number control genes and show no significant additional increase in their copy number.

Chloramphenicol is a powder stored at 4°C.

Make up in ethanol at 125mg/ml and store this at 4°C.

Chloramphenicol should be handled with care; wear gloves and avoid inhalation of powder.

Method 1:

1. Grow a 200 ml prep in terrific broth to saturation by vigorous shaking at 37°C (6-8 hrs or overnight).
2. Add 200 ml more of prewarmed terrific broth (to avoid shocking cells), then add chloramphenicol to a final concentration of 125 ug/ml.
3. Grow to saturation.

Method 2:

1. Inoculate terrific broth containing appropriate antibiotic with 4 ml of bacterial culture.
2. Incubate with vigorous shaking at 37° C until the OD600 is approximately 0.4.
3. Add chloramphenicol to a final concentration of 170ug/ml.
4. Incubate for a further 12 to 16 hours (ie overnight) at 37° C with vigorous shaking.

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

5. Grow 100ml prep in terrific broth for 6 to 8 hours at 37° C in the appropriate antibiotic with vigorous shaking. (For best results inoculate with 200ul of a 5 ml overnight culture.)
6. Add chloramphenicol to 170 ug/ml final concentration and incubate for a further 12 to 16 hours with vigorous shaking.