COMPETENT CELLS (GM119, dam-, dcm-)

Most strains of E. coli contain two site specific methylases. The methylase encoded by the dam gene transfers a methyl group from S-adenosylmethionine to the N^6 position of adenine residues in the sequence GATC. The dcm methylase methylates the internal cytosine residues in the sequence CCAGG and CCTGG. Methylation by these methylases inhibits cleavage of DNA by certain restriction endonucleases whose recognition sequence is identical to the recognition of dam and dcm methylases.

dam sensitive enzymes:	sequence:*
Bcl 1	TGATCA
Cla 1	gATCGATc
Hph 1	GGTGAtc
Mbo 1	GATC
Mbo II	GAAGAtc
Nru 1	gaTCGCGA
Taq 1	gaTCGAtc
Xba 1	TCTAGAtc

dcm sensitive enzymes:	sequence:*
Ava II	GG(A/T)CC(a/t)gg
EcoR II	CC(A/T)GG
Sau 961	GGNCC(a/t)gg
Scr F1	CC(A/T)GG
Stu 1	AGGCCTgg

* Capital letters indicate restriction enzyme recognition site.

All DNA isolated from E. coli is not methylated to the same extent. However, if you are dependent on complete digestion of your DNA by a methylase sensitive enzyme, you would want to grow up your plasmid in a dam-, dcm- strain such as GM119.

Solutions: keep ice cold; also chill Corex tubes, pipets, and HB-4 rotor

Buffer A 10 mM Tris pH 8
50 mM $CaCl_2$

Buffer B 10 mM Tris pH 8
50 mM $CaCl_2$
15% glycerol

Buffer C 10mM Tris pH 8
10mM CaCl2
10mM Mg SO4

1. Grow GM119 cells in LB to a density of 5 X 10^7 cells/ml (approx. 0.5 A_{595}) 100 mls

2. Pour into four chilled Corex tubes

3. Spin at 4,000 g for 5 min at 4°C (5k rpm in HB-4 rotor)

4. Resuspend in 4 X 12.5 mls ice cold fresh Buffer A

5. Ice for 4 hrs

6. Centrifuge as above (4,000 g for 5 min at 4°C)

7. Resuspend in (4X) 1.66 mls of ice cold fresh Buffer B

8. Aliquot 200 µl into prechilled tubes

9. Freeze at -70°C.

For transformation ~ do as usual except add DNA to cells in Buffer C (instead of TE)