Plasmid Amplification

Plasmid Amplification Protocol

abm supplies purified plasmid DNA in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), optimized for efficient amplification through direct transformation into standard *E. coli* cloning strains such as DH5a or DH10B derivatives.

Transformation Protocol

The following protocol serves as a general guideline and may require optimization.

1. Thaw a vial of competent cells on ice for 10 min. Gently flick tube to thoroughly mix any settled cells. Aliquot 60 µl of cells per transformation into sterile 1.5 ml tubes. *Note: once thawed, cells should not be re-frozen as transformation efficiency will be compromised.*

2. Add 1 µl of plasmid DNA to the cells and mix gently by flicking the tube 4-5 times. Do not vortex. Incubate reaction at 4°C for 30 min.

3. Heat shock the cells by placing the tube into a 42°C water bath for 45 s. Remove tubes immediately and place on ice for 1-2 min.

4. Add 150 µl of room temperature SOC media to the mixture. Incubate tubes at 37°C with vigorous shaking (250 rpm) for 1 h.

5. Prepare several dilutions of the mixture and spread 50-100 µl of each dilution onto appropriate antibiotic selection plates. Incubate at 37°C overnight.

6. Inoculate a single colony into 5-10 ml of LB broth with antibiotic selection. Incubate overnight at 200 rpm in a 37°C shaking incubator for 16-18 h.

7. Isolate plasmid DNA by standard miniprep protocol.

E. coli Antibiotic Selection Table

	Kanamycin	Ampicillin	Spectinomycin	Tetracycline	Chloramphenicol
Working Concentration	50 µg/ml	100 µg/ml	50 µg/ml	12.5 µg/ml	25 µg/ml