

Plasmid Midipreps

Start with 150ml of overnight cultures (if ampicillin is used, amp: 100µg/ml).

1. Transfer culture to 250 ml tubes. Spin down the cells at 6,000 rpm for 6 min. Discard supernatant.
2. Add 4 mls of solution I. Resuspend pellets by vortexing vigorously. Incubate at RT for 5-10 min.

Solution I:

	Final	Stock	for 50 ml
Glucose	50 mM	20% (=1.2 M)	2.1 ml
Tris-HCl pH 8	25 mM	1 M	1.25 ml
EDTA pH 8	10 mM	0.5 M	1 ml
di H ₂ O			45.7 ml

+ add RNase A to a final concentration of 20 µg/ml (stock is 10 mg/ml)

3. Add 8 mls of solution II (if too old (> 2 weeks), prepare fresh), mix by gently inverting 4-6 times (do not vortex). Place the tubes on ice and incubate for **5 min**. *Clear lysis.*

Solution II:

	Final	Stock	for 50 ml
NaOH	0.2 M	10 M	1 ml
SDS	1 %	20 %	2.5 ml
di H ₂ O			46.5 ml

! Add Water first, otherwise SDS will precipitate irreversibly.

4. Add 6 mls of solution III, inverting 4-6 times. Incubate on ice for 10 min.

Solution III: 3M Potassium Acetate pH 5.2

Weigh 147.21 g of Potassium Acetate, dissolve in 300 ml di H₂O, adjust pH to 5.2 using Glacial Acetic Acid, adjust volume to 500 ml by adding di H₂O.

5. Centrifuge for 10 min at 10,000 rpm at 4°C.
6. Transfer the supernatant to a new tube; add 1 volume (18 ml) of 100% Isopropanol; mix.
7. Centrifuge for 10 min. at 14,000 rpm at 4°C.
8. Discard supernatant. Wash the pellets with 5 ml of 70% ethanol.
9. Dry the pellets and dissolve the DNA in 1 ml of TE, as desired.

Additional steps for CLEAN midipreps (e.g. for sequencing purpose)

From Step 9:

Resuspend pellets in 1 ml TE, add RNase A to a final concentration of 20 µg/ml.

Incubate at 37°C for 30 min.

10. Add 1 ml of 10M LiCl, mix by inverting 4-6 times.

11. Incubate for $\geq$ 20min. at -20°C.

12. Centrifuge at 14,000rpm for 10 min at 4°C.

13. Transfer the supernatant to a clean tube.

14. Add 2.2 volume of ethanol 100%; mix by inverting.

15. Centrifuge at 14,000 rpm for 10 min at 4°C.

16. Wash twice with 70% ethanol.

17. Dry the pellet and dissolve in 500µl of 10 mM Tris HCl pH 7.5.