



DYS SEE

**Miniprep Plasmid
Isolation.**

Protocols

Plasmid DNA isolation – Alkaline lysis – Miniprep

1. Pellet 1.5ml of bacterial culture (1 min, full speed) [take the 1.5 ml from the liquid colony while near fire and sterile conditions]]
2. Discard supernatant (SN) completely
3. Resuspend in 100µl of Solution 1 (S1/P1/B1) (with RNaseA)
Note: It's better to prepare a master mix (S1 with RNase on ice) to avoid pipetting error
4. Add 200µl S2 lysis solution, invert 5X gently, and then incubate MAXIMUM 3 min at room temperature (RT) (3 mins are strict)
5. Add 150µl of S3, invert gently
6. Incubate on ice for 10 min
7. Spin, full speed for 10 min (during that time prepare clean eppies and label them for the next step)
8. Transfer SN to a clean eppie(In order to increase the precision of the supernatant removal, while retaining the advantage of a big pipette volume, you can use double tips (blue first, then yellow)
9. Spin at full speed for 5 min (again, prepare clean and labeled eppies for the next step)
10. Transfer SN to a clean eppie and precipitate plasmid with 2 volumes of ice cold 100% EtOH (or 600µl 2-propanol), invert
11. Spin 15 min, full speed → After that you see a DNA pellet
12. Remove SN completely
13. Wash pellet with 70% EtOH (You just add the 70% EtOH, shake gently. It does not dissolve, you need to secure it)
14. Spin 10 min, full speed (At this step you secure your plasmid DNA pellet at the bottom of the eppie)
15. After centrifuging, remove EtOH immediately and dry the pellet. Resuspend in ddH₂O (15µl or 20, depending on how concentrated you want your DNA to be)

Solutions

S1 cell suspension solution	150ml
50mM glucose	
25mM Tris-Cl (pH 8.0)	0.909gr
19mM EDTA	0.558gr
pH=8.00 (adjust with HCL)	
+3mg/ml RNaseA (10mg/ml stock)	2µl per 100µl Add directly before use!

- Autoclave and store at 4 degrees Celsius

S2 lysis solution (always fresh)	5ml working solution
20mM NaOH	2ml from 0.5N stock solution
1% SDS	250µl from 20% stock solution

S3 neutralization solution	150ml
3 M potassium acetate	44,175gr
pH=5.5 (adjust with glacial acetic acid)	(about 16.5ml)

- Store at 4 degrees Celsius and keep on ice before use!



Protocols