Plasmid Minipreps

Start with 2ml of overnight cultures (if ampicillin is used, amp: 100µg/ml).

1. Transfer culture to 1.5 ml tubes. Spin down the cells at 6,000 rpm for 6 min. Discard supernatant well using vacuum trap.

2. Add 200µl 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_2O			45.7 ml

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

3. Add 400µl 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_2O			46.5 ml

   ! Add Water first, otherwise SDS will precipitate irreversibly.

4. Add 300 µl of solution III, inverting 4-6 times. Incubate on ice for 5 min.

   Solution III: 3M Potassium Acetate pH 5.2

   Weigh 147.21 g of Potassium Acetate, dissolve in 300 ml di H_2O, adjust pH to 5.2 using Glacial Acetic Acid, adjust volume to 500 ml by adding di H_2O.

5. Centrifuge for 5 min at 14,000rpm at 4°C.

6. Transfer the supernatant to a new tube; add 1 volume (900 µl) of 100% Isopropanol; mix by inversion.

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

8. Discard supernatant. Wash the pellets with 500 µl of 70% ethanol.

9. Dry the pellets and dissolve the DNA in 50-100µl of TE, as desired.

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

From Step 9:

Resuspend pellets in 200 µl TE, **PAUL's advice**: "**VORTEX 30 second REALLY HARD, SPIN AT MAX SPEED 3 MINS, VORTEX 30s REALLY HARD, SPIN AT MAX SPEED, VORTEX AGAIN FOR 5s"**

Then add RNase A to a final concentration of 20 µg/ml.
Incubate at 37°C for 10 min

10. Add 200µl of 10M LiCl, mix by inverting 4-6 times.

11. Incubate for >= 20min. at -20°C (For extra clean DNA, use the overnight sermon).

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

13. Transfer the supernatant to a clean 1.5 ml tube.

14. Add 2.2 volume of cold 100% ethanol (1ml), mix by inverting. **PAUL: "FREEZE 1 HOUR in -20C so the DNA will precipitate, then thaw out, then freeze 1 hour then thaw again. It's because there was a paper that showed a curve about how DNA precipitate many times more after 1 hour of freezing and thawing."**

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

16. Wash pellet twice with 70% cold ethanol.

17. Dry the pellet and dissolve in 10 mM Tris HCl pH 7.5. **Usually the volume is 50µl, but it depends on what concentration of DNA you want. If you're not sure you have a lot of DNA then dissolve in smaller amount so you get good concentration (e.g. for sequencing it's 400ng/µl, so if you didn't see a lot of pellet then dissolve in 5µl, for example).**