

BAC prep Using Quiagen plasmid mini prep kit (do not need spin columns)

1. Cell culture overnight in 5 ml LB + 3ul CMP stock
2. Harvest 3ml in 2ml tubes
3. resuspend by adding 250 ul Solution P1 (stored at 4°C)
4. Lysis by adding 250 ul solution P2, sit at room temp 5 min
5. Precipitate by adding 350 ul of N3 Buffer 10-20 minutes on ice
6. Centrifuge at max speed for 10 min

Remove lysate to a new tube

7. Repeat step 6 (use a 1.5 ml tube for the final lysate)
8. DNA precipitate by adding 600 ul isopropanol mix and let stand at room temp for 5-10 min.
9. Centrifuge at max speed for 20 min
10. Remove isopropanol add 200 ul PE buffer
11. Centrifuge at max speed for 10 min.
12. Decant the PE Buffer and Dry for 20-30 minutes
13. Add 20 ul water and incubate at 37° for one hour

use immediately for sequencing

may freeze for later use for PCR or digestion

5 ul for sequencing
2-3ul for PCR
20 ul for digestion

Notes:

Higher yields are achieved by performing all centrifugation at 4°C