Qiagen - MaxiPrep - Maggie Trout. March 2022.

mostly followed the protocol from the handbook (starting on page 14)

1. Harvest cells by centrifugation at 6,000 x g for 15 mins at 4°C
    a. **Did 3,500 rpm for 15 min**s

2. Resuspend the pellet in 10 mL buffer P1
    a. **If you split the culture to spin, DO NOT FORGET to combine them again (add 5 mL to each tube then combine).**

3. Add 10 mL P2, invert the tube 4-6 times, then incubate at RT for 5 mins

4. Prepare QIAfilter Cartridge by screwing the cap onto the outlet.

5. Add 10 mL chilled P3, invert the tube 4-6 times.

6. Pour lysate into the cartridge barrel, incubate at RT for 10 min.

7. Remove cap, then insert plunger and filter lysate into 50mL tube

8. Add 2.5 mL Buffer ER, mix by inverting approx. 10 times, incubate on ice for 30 minutes.

9. Equilibrate tip by applying 10 mL buffer QBT and allowing the column to empty

10. Apply filtered lysate to tip, allow to flow through via gravity

11. Wash 2x with 30 mL Buffer QC

12. Elute DNA with 15 mL Buffer QN into a 30 mL tube

13. Precipitate by adding 10.5 mL RT isopropanol, centrifuge at >15,000 g for 30 min at 4°C, decant supernatant
    a. pellet appears small and fairly transparent

14. Wash with 5 mL 70% ethanol and centrifuge at 15,000 g for 10 min, decant the supernatant

15. Air dry pellet for 5-10 minutes and redissolve DNA in Buffer TE (used 50 uL).

Let me know if you need anything else!
-Maggie