

Name: Asma

Date: 09/04/2019

Goal:

1. Miniprep of original mCherry overnights

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Protocol:

- a. Centrifuged 9\ bacterial overnight cultures in 15 ml falcon tubes in the centrifuge at 5000 rpm for 10 minutes at room temperature. **ALL FALCON TUBES BROKE DURING SPIN, HOWEVER PELLETS FORMED, SO PROTOCOL WAS CONTINUED.**
- b. Discard the supernatant and resuspend pelleted bacterial cells with 250  $\mu$ L Buffer P1.
- c. Add 250  $\mu$ L of Buffer P2 and invert 5 times.
- d. Add 350  $\mu$ L of Buffer N3 and immediately mix by inverting 5 times.
- e. Centrifuge for 10 minutes at 13,000 rpm.
- f. Micropipette 800  $\mu$ L of the clear supernatant into a spin column and centrifuge for 60 seconds and discard the excess liquid.
- g. Add 500  $\mu$ L of PB and centrifuge the spin columns for 60 seconds. Discard the flow through.
- h. Add 750  $\mu$ L of PE to the spin columns, centrifuge for 60 seconds, and discard the flow through.
- i. Centrifuge the spin columns again for 60 seconds to remove residual wash buffer and discard the flow through.
- j. Transfer the spin columns to a clean eppendorf tube and add 50  $\mu$ L of EB to the center of the spin column to elute the DNA.
- k. Allow the spin column to stand for one minute and then centrifuge for one minute.
- l. Record the concentrations for each sample.

Results:

sample	2-6a	1-4	2-9	1-1	2-3	2-6
Concentration (ng/ul)	25.0	12.5	37.5	25	55.0	52.5