Protocol Name: Miniprep of transformed colonies
Category: Naringenin Operon Biosynthesis
Date: 12/10/18
Author: Heather Bottomley
Source(s): Adapted from QIAprep Spin Miniprep Kit Quick- Start Protocol
Time Required: 2 hours

Additional Notes:
Materials:

- Overnight culture of transformed colonies.
- QIAprep Spin Miniprep Kit.
- 1.5ml Eppendorf tubes.

Procedure:

- Pellet 1-5ml of bacterial overnight culture from each colony by centrifugation >8,000rpm for 3 minutes between 15-25°C.
- Resuspend pelleted bacterial cells in 250µl Buffer P1 and transfer to a microcentrifuge tube.
- Add 250µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 minutes. If using LyseBlue reagent, the solution will turn blue.
- Add 350µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times. If using LyseBlue reagent, the solution will turn colorless.
- Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.
- Apply 800µl supernatant from step 5 to the QIAprep 2.0 spin column by pipetting. Centrifuge for 1 minute and discard the flow-through.
- Wash the QIAprep 2.0 spin column by adding 0.75 ml Buffer PE. Centrifuge for 1 minute and discard the flow-through. Transfer the QIAprep 2.0 spin column to the collection tube.
- Centrifuge for 1 minute to remove residual wash buffer.
- Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM TrisCl, pH 8.5) or water to the center of the QIAprep 2.0 spin column, let stand for 1 minute, and centrifuge for 1 minute.