**Miniprep, QVEU**

*Walker Orr, 03/21/2023*

**Required Equipment**

Eppendorf Tabletop Centrifuge (5430)

Benchtop Allegra X14 Bucket-Rotor Centrifuge

**Required Reagents**

Kit reagents from QIAprep® Spin Miniprep Kit

* **Buffer P1** 250 µL/ sample, at 4°C
* **Buffer P2** 250 µL/sample
* **Buffer N3** 350 µL/sample
* **Buffer PB** 500 µL/sample
* **Buffer PE** 750 µL/sample
* **Buffer EB** 50 µL/sample

**Sample Workflow**

Total time: 50 minutes

Active time: 35 minutes

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (h)** | **(m)** | **Main steps** | **Prep steps** |
| 0 |  | Make Glycerol Stock **(1)** |  |
|  | 5 |  |  |
|  | 10 | Pellet Culture **(2)** |  |
|  | 15 | Suspend, lyse, neutralize **(3)(4)(5)** |  |
|  | 20 |  |  |
|  | 25 | Centrifuge **(6)** |  |
|  | 30 |  |  |
|  | 35 | Bind **(7)** |  |
|  | 40 | Wash **(8)(9)** | Label tubes for elution |
|  | 45 | Dry **(10)** |  |
|  | 50 | Elute **(11)** |  |

**General Comments**

* Your overall yield—and the quality of your DNA—depends on culturing sufficient bacteria. Using too small a colony from an agar plate, for example, will penalize yields.

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| **Steps** |  | **Helpful Hints** |
| 1. If making a glycerol stock, do this now, before centrifuging the culture! |  |  |
| 1. Pellet 1-5 mL overnight culture by centrifugation for **3 minutes** at **room temperature** and **4,000 g**. |  | 4,150 rpm on the tabletop Allegra X14-R bucket centrifuge. |
| 1. Add **250 µL Buffer P1 (4°C)** and transfer to a microcentrifuge tube. |  |  |
| 1. Add **250 µL** **Buffer P2** and mix thoroughly by inverting 4-6 times until the solution becomes clear (if using LyseBlue, the solution will turn blue). Do not allow the reaction to proceed longer than **≤ 5 minutes**. |  |  |
| 1. Add **350 µL** **Buffer N3** and mix immediately and thoroughly by inverting the tube 4-6 times. If using LyseBlue reagent, the solution will turn colorless. |  |  |
| 1. Centrifuge for **10 minutes** at **room temperature** and **17,900 x g.** |  |  |
| 1. Apply **800 µL** supernatant from Step 6 to the QIAprep column. Centrifuge for **1 minute** at **17,900 x g** and discard flow-through. |  |  |
| 1. Wash the QIAprep 2.0 spin column by adding 500 µL **Buffer PB**. Centrifuge for **1 minute** at **17,900 x g** and discard flow-through. |  |  |
| 1. Was the QIAprep 2.0 spin column by adding 750 µL **Buffer PE**. Centrifuge for **1 minute** at **17,900 x g** and discard flow-through. |  |  |
| 1. Centrifuge dry for **1 minute** at **17,900 x g**. |  | It’s helpful to use a sacrifice tube with the lid snipped off for this. |
| 1. Transfer QIAprep 2.0 spin column to a clean, labeled 1.5 mL microcentrifuge tube. To elute DNA, add **50 µL** **Buffer EB** to the center of the spin column, let stand for **1 minute,** and centrifuge for **1 minute** at **17,900 x g.** |  |  |
| 1. Assess DNA concentration using Nanodrop or Qubit. |  |  |