**Midiprep, QVEU**

*Walker Orr, 03/01/2023*

**Required Equipment**

* Allegra X14R centrifuge, chilled to 4C
* Thermo-Fisher RC6+ High-speed centrifuge, chilled to 4C
* Eppendorf 5430R benchtop centrifuge, chilled to 4C

**Required Supplies**

**Required Reagents**

* QIAGEN Plasmid Midi kit reagents

*Buffer P1 and P3 stored at 4C*

* Isopropanol; freshly prepared 70% ethanol

**Sample Workflow**

Total time: ~5 hours

Active time: ~3 hours

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (h)** | **(m)** | **Main steps** | **Prep steps** |
| 0 |  |  | Chill Allegra X14-R |
|  | 15 |  | Prepare workstation |
|  | 30 | Harvest cells **(1)** |  |
|  | 45 | Resuspend cells **(2),** Incubate **(3)**, |  |
| 1 |  | Lyse **(4)** | Prepare ice; chill Thermo RC6+; bleach culture flasks |
|  | 15 | Centrifuge **(5)** |  |
|  | 30 |  | Prepare QIAGEN-100 tip **(6)** |
|  | 45 |  | Prepare Eppendorf 5430R; wash flasks |
| 2 |  | Load column **(7)** |  |
|  | 15 | Wash **(8)**, elute **(9)** |  |
|  | 30 |  |  |
|  | 45 | Precipitate **(10)** |  |
| 3 |  |  |  |
|  | 15 | Wash **(11)** |  |
|  | 30 | Dry **(12)** |  |
|  | 45 |  |  |
| 4 |  | Suspend and Nanodrop |  |
|  | 15 |  |  |

**General Comments**

* 4 is a good number for the first few times; later can do up to ~8 preps at a time.

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| **Steps** |  | **Helpful Hints** |
| 1. Transfer your culture to falcon tubes. Harvest at **4,150 x g\*** for **15** **minutes** at **4** **°C**. |  | Use the **Allegra X14-R;** 4150 x gis max speed. |
| 1. Drain supernatant. Resuspend pellet in **4 mL Buffer P1.** Transfer resuspended pellet to lidded reusable Nalgene autoclave tubes. |  | Invert tubes over Kimwipe for more complete supernatant removal. Use serological pipette to resuspend the pellet; tip can be used to physically break up the pellet. Air bubbles okay here. |
| 1. Add **4 mL** **Buffer P2** and mix by vigorously inverting 6-8 times. Incubate at **room temperature** for **5 minutes**. If using LyseBlue, the solution will turn blue. |  |  |
| 1. Add **4 mL** **Buffer P3** (4C) and mix by inverting 4-6 times. If using LyseBlue, the solution will turn white. Incubate **on ice** for **15 minutes**. |  |  |
| 1. Centrifuge at **20,000 x g** for **45 minutes\*** at **4 °C.** |  | Use the **Thermo-Fisher RC6+.** To tighten rotor nut, completely turn bottom knob, then top knob. Loosen in reverse. |
| 1. Prepare a QIAGEN-100 tip by washing it with **4 mL Buffer QBT**, and allow column to drain by gravity flow. |  |  |
| 1. Transfer supernatant yielded from step 5 to the column and let drain fully. |  | This takes around 15 minutes. |
| 1. Wash QIAGEN-tip with **10 mL** **Buffer QC.** Repeat, for a total of 2 washes. |  | This takes 15-20 minutes total. |
| 1. Elute DNA in 5 mL **Buffer QF**. |  | Capture the eluent in a clean 50-mL falcon tube. |
| 1. Precipitate DNA by adding **3.5mL room temperature isopropanol.** Centrifuge for **30 minutes\*** at **max rcf** and **4 °C**. |  | Use **Eppendorf 5430R** at **max rcf.** Chill with “fast temp.” Load tubes in same orientation. To drain, use the electric vacuum aspirator. Don’t aspirate pellet! |
| 1. Wash pellet with **2 mL fresh 70% ethanol** and centrifuge at **max rcf** and **4 °C** for **10 minutes.** Carefully decant supernatant. |  | Aspirate the ethanol. You need to be more thorough than in Step 10. If doing 8 preps, leave half in Step 10. |
| 1. Air-dry pellet for **30 minutes\***. Redissolve in a suitable volume of appropriate buffer. |  | Buffer is **nanopure water; 200 uL**. Redissolve first pellet and carry across to all four tubes to capture all of the DNA. |
| 1. Assess DNA concentration and purity by nanodrop. |  | Use **2 uL**. |