**Electroporation, QVEU**

*Walker Orr, 03/02/2023*

**Purpose:** transform >10 kb libraries into bacteria.

**Required Equipment:**

* Eppendorf 5430 centrifuge

**Required Reagents:**

* Kit reagents from NEB Monarch PCR & DNA Cleanup Kit (5 µg)
* Selective antibiotics; LB
* Kit reagents from NEB® 10-beta electrocompetent cells

**Sample Workflow**

Total time: 3 hours

Active time: 1.5 hours

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (h)** | **(m)** | **Main steps** | **Prep steps** |
| 0 |  |  | Prewarm elution buffer (nanopure water) to **50°C**. Prewarm SOM **(B1; 37°C)**. |
|  | 15 | Dilute **(A1)** |  |
|  | 30 | Spin **(A2)**, wash **(A3, A4)** |  |
|  | 45 | Dry **(A5)**, Elute **(A6, A7)** | Get ice and thaw 10-beta cels **(B4;** Jesse James shelf 1 box 3**)** |
| 1 |  | Dilute pUC19 **(B3)** | Chill cuvettes and tubes  Prepare culture tubes |
|  | 15 | Aliquot; add plasmid **(B4)** |  |
|  | 30 | Transfer to cuvettes **(B5)** |  |
|  | 45 | Electroporate **(B6)**, suspend **(B7)** |  |
| 2 |  | Culture | Prepare and prewarm plates **(B1; 37°C)** |
|  | 15 |  | Prepare dilution/plating setup **(C1)**  Prepare flasks with media for overnight culture **(C2)** |
|  | 30 |  |  |
|  | 45 |  |  |
| 3 |  | Dilute cells and plate **(C1)** |  |
|  | 15 | Make overnight culture **(C2)** |  |
|  | 30 |  |  |

**General Comments**

**DNA Cleanup (A)**

This workflow starts by cleaning & concentrating the ligation reaction. Use “Monarch PCR & DNA Cleanup Kit (5 ug).

|  |  |  |
| --- | --- | --- |
| **Steps** |  | **Helpful Hints** |
| 1. Dilute 1 part sample in **2 parts Binding Buffer**. Mix well by pipetting up and down; don’t vortex. |  | For a 20 µL ligation reaction, this means 40 µL binding buffer. Warm (N + 1) \* 8 µL water for elution **(50°C)**. |
| 1. Insert column into collection tube and load sample. Spin at **16,000 x g** for **1 minute.** Discard flow-through. |  | Use **Eppendorf 5430.** |
| 1. Reinsert column into collection tube. Add **200 µL** **DNA Wash Buffer** and spin at **16,000 x g** for **1 minute.** |  |  |
| 1. Repeat step 3. |  |  |
| 1. \*Discard flow-through and centrifuge dry at **16,000 x g** for **1 minute**. |  |  |
| 1. Transfer column to a clean, labeled 1.5 mL microcentrifuge tube. |  | While transferring, don’t let the tip of the column touch the flow-through; if it does, re-centrifuge. |
| 1. Add **8 µL** warmed nanopore water to the center of the filter. Let stand at **50C\*** for **3 minutes\***. Spin at **16,000 x g** for **1 minute** to elute DNA. |  | Touch the pipette tip to the filter when loading elution buffer. |

**Electroporation (B)**

Transform cleaned-up ligation reaction product into *E. coli*.

|  |  |  |
| --- | --- | --- |
| **Steps** |  | **Helpful Hints** |
| 1. Prewarm NEB® 10-beta/Stable Outgrowth Medium (B9035S) in a water bath (**37°C**). Also prewarm selective plates at **37°C** for **1 hour**. Prepare round-bottom culture tubes. |  | Aliquot S.O.M… do not use directly from the stock container. |
| 1. Prechill electroporation cuvettes **(1 mm)** and microcentrifuge tubes **on ice**. |  |  |
| 1. As a positive control for transformation, dilute control pUC19 by 1:5 for a final concentration of 10 pg/µL using sterile water. |  |  |
| 1. Thaw NEB 10-beta electrocompetent cells **on ice** (**about 10 minutes**) and mix cells by flicking gently. Transfer 25 µL of the cells to a chilled microcentrifuge tube. Add 1 µL of purified plasmid. |  |  |
| 1. Carefully transfer the cell/DNA mix to a chilled cuvette without introducing air bubbles. Make sure cells deposit across the bottom of the cuvette. |  | Use a 20 uL tip, because you need to be able reach through the narrow aperture at the bottom of the cuvette. |
| 1. Electroporate using the following conditions: 2.0 kV, 200 , 25 µF. Time constant after shocking should be 4.8 – 5.1 ms. |  | Program WB on our electroporator. |
| 1. Immediately add 975 µL **37°C** 10-beta/Stable Outgrowth Medium to the cuvette, gently mix up and down twice, then transfer to round-bottom culture tube. |  |  |
| 1. Shake vigorously (**225 rpm**) at **37°C** for **1 hour**. |  |  |

**Plating and Culturing (C)**

Plate cells to assess transformation efficiency and library coverage; grow cultures for Midiprep DNA harvesting.

|  |  |  |
| --- | --- | --- |
| **Steps** |  | **Helpful Hints** |
| 1. Dilute cells as appropriate and spread **100 µL** on warmed selective plates. |  | 1:100; 1:10,000 by serial dilution are good places to start. For plating (-) control as well, you’ll want to do 2µL first culture:198 µL fresh LB so you have enough for both plates. |
| 1. Transfer remaining first culture volume to **50 mL** **LB** (with selective antibiotics). Grow at **37°C** for **16 hours**. |  | Carb is 1:500, so **100 µL** per flask. |
| 1. If you need to start a Midiprep later than 16 hours, transfer cultures to falcon tubes and **refrigerate**. |  |  |