Jul 25, 2024 Version 2

Library Bottlenecking Protocol (Basic Microbial Culture Equipment) V.2

DOI
dx.doi.org/10.17504/protocols.io.x54v9227pl3e/v2

David Ross[1]

[1]NIST

Align to Innovate | Pooled, Growth-Based As…

Open Datasets Initiative
Align to Innovate

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.x54v9227pl3e/v2

Protocol Citation: David Ross 2024. Library Bottlenecking Protocol (Basic Microbial Culture Equipment). **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9227pl3e/v2Version created by **Open Datasets Initiative**

Protocol status: Working
We use this protocol and it's working

Created: May 31, 2024

Last Modified: July 25, 2024

Protocol Integer ID: 101038

Keywords: Deep mutational scanning, protein sequence-function relationships, fitness landscape, laboratory automation

Disclaimer

The protocol outlined in this document was created as a part of the *Pooled, Growth-Based Assays for Protein Function Measurements* pipeline for Align to Innovate's Open Dataset Initiative. Align to Innovate is a non-profit research organization operating under open science principles with the goal of improving science research with programmable experiments. The Open Datasets Initiative is working to accelerate community-driven science with the use of automated labs to pioneer robust data collection methods and curated, high-fidelity, public biological datasets amenable to machine learning. This work was supported by Align to Innovate's Open Datasets Initiative which receives philanthropic funding in part from Griffin Catalyst.

Abstract

Protocol for bottlenecking a library using a basic microbial culture equipment.

Materials

Starting cultures:

- glycerol stock of variant library

Reagents:

- M9 Media (ThermoFisher A1374401)
- Glycerol (MilliporeSigma G5516)

Consumables:

- Six 15 mL snap cap tubes (Corning 352059)
- Eight 250mL baffled flasks (ThermoFisher 4116-0250)
- Eighteen 1.5 mL Microcentrifuge tubes (ThermoFisher 69715)
- Eighteen Agar Plates with LB and Kanamycin-50 (MilliporeSigma L0543)
- Multiple 1.2 mL Cryogenic vials (Corning 430487)

Create the first overnight culture

1 Dilute a full 1 mL vial of the library glycerol stock into 50 mL of media in a 250 mL baffled flask.

2 Incubate the resulting culture at 37 C with shaking (300 rpm) for 12 - 24 hours (to reach stationary phase).
- This generates the **overnight flask**

Prepare all flasks and tubes

3 Prepare one baffled flask (125 mL or 250 mL) with 49.9 mL media
- This is the **dilution flask**

4 Prepare six 15 mL snap-cap culture tubes, each with 2 mL media
- Number the tubes 1 - 6

5 Prepare six new 250-mL baffled flasks, each with 50 mL media.
- Number the flasks 1 - 6

6 Prepare 18 1.5 mL microcentrifuge tubes
- Number the tubes 1.1, 1.2, 1.3, 2.1, 2.2, 2.3, ..., 6.1, 6.2, 6.3
- Leave tubes 1.1, 2.1, 3.1, ..., 6.1 empty
- Add 900 uL media to each of the other tubes

7 Prepare 18 agar plates (with LB + kan)
- Number the plates to match the microcentrifuge tubes (1.1, 1.2, 1.3, 2.1, 2.2, 2.3, ..., 6.1, 6.2, 6.3)

Make culture dilutions

8 Mix the 50 mL **overnight flask** well, then pipette 0.1 mL of the extra culture into the **dilution flask** (with 49.9 mL media).

9 Swirl the **dilution flask** to mix well.

10 Make a serial dilution series from the **dilution flask** into culture tubes:
- Pipette 1 mL from **dilution flask** to culture tube no. 1
- Mix culture tube no. 1 well
- Pipette 1 mL from culture tube no. 1 to culture tube no. 2

- Mix culture tube no. 2 well
- Pipette 1 mL from culture tube no. 2 to culture tube no. 3
- Mix culture tube no. 3 well
- Continue this for the remaining tubes 4-6.

11
- Transfer 1 mL of each culture from tubes 1-6 to flasks 1-6 and microcentrifuge tubes 1-6 :
- Mix well
- Pipette 1 mL from each culture tube to corresponding baffled flask
- Mix baffled flask well
- Pipette 1 mL from flask to corresponding empty microcentrifuge tube (X.1)

12 For each set of microcentrifuge tubes:
- Mix microcentrifuge tube X.1
- Pipette 100 uL from tube X.1 to tube X.2
- Mix microcentrifuge tube X.2
- Pipette 100 uL from tube X.2 to tube X.2
- Mix microcentrifuge tube X.2
- Pipette 100 uL from tube X.2 to tube X.3
- Mix microcentrifuge tube X.3

Plate and incubate all cultures

13 Plate 150 uL from each microcentrifuge tube onto the corresponding agar plate.

14 Incubate all agar plates at 37 C for 16-24 hours or until colonies are visible and incubate all flasks at 37 C with shaking (300 rpm) for 16-24 hours (to stationary phase)

Count colonies and estimate colony forming units

15 The next day, count the colonies on every agar plate to get colony forming unit (CFU) estimates for the starting point for each culture.

16 Choose the culture with an estimated starting CFU count closest to the target diversity (100,000).
- The true CFU count should be an overestimate of the library diversity because some variants will have multiple copies in the culture. But in our experience, the diversity resulting from this method is just as likely to be higher or lower than the estimated CFU count.

Make glycerol stocks

17 Make several 1 mL glycerol stocks (0.5 mL cells + 0.5 mL 40% glycerol) from the chosen bottlenecked culture, label appropriately, and store at -80 C until use.

- Also, consider making glycerol stocks from some of the other bottlenecked cultures in case you decide to run a similar library with higher or lower diversity after the initial pilot-scale pooled assay.