Jan 26, 2026 Version 2

Culturing of *E.coli* stocks for Whole Genome Sequencing V.2

DOI
https://dx.doi.org/10.17504/protocols.io.5jyl88p6rl2w/v2

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Protocol Citation: Lindsay Newbold, Susheel Bhanu Busi, Manasa Suresh, Denise Pallett, Wiebke Schmidt, Martin Spurr 2026. Culturing of E.coli stocks for Whole Genome Sequencing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl88p6rl2w/v2Version created by **Lindsay Kate Newbold**

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

Created: January 22, 2026

Last Modified: January 26, 2026

Protocol Integer ID: 239134

Keywords: culturing methodology, Whole Genome Sequencing, whole genome sequencing, culturing methodology

Funders Acknowledgements:
The Environment Agency
Grant ID: SC250010

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Abstract

This protocol outlines a simple culturing methodology for the growth of *E.coli* prior to Whole Genome Sequencing.

Safety warnings

Ensure good laboratory practice is followed throughout waste is suitably disposed of either through treatment with Rely+ Virkon (liquid waste) or via autoclave (solid waste).

Before start

All culture vessels and plates should be labeled prior to use. Luria Bertani (LB) broth (Lennox) should be pre-prepared following manufacturers instructions. All reagents and plasticware should be sterile prior to use.

Reviving culture from stock

1 Take stock sample out of the -80°C Freezer and transfer to upright -80°C Freezer in microbiology lab.
Alternatively keep stock on ice whilst in use to minimize thawing.

2 Take sub-set of samples from box, place sample and plate in laminar flow cabinet and use a sterile loop to take small amount of glycerol stock. Return to -80°C

3 Lift plate to 45 degree angle and swipe loop over pre-prepared Tryptone Bile X-Glucuronide (TBX) agar plate using streak plating method.

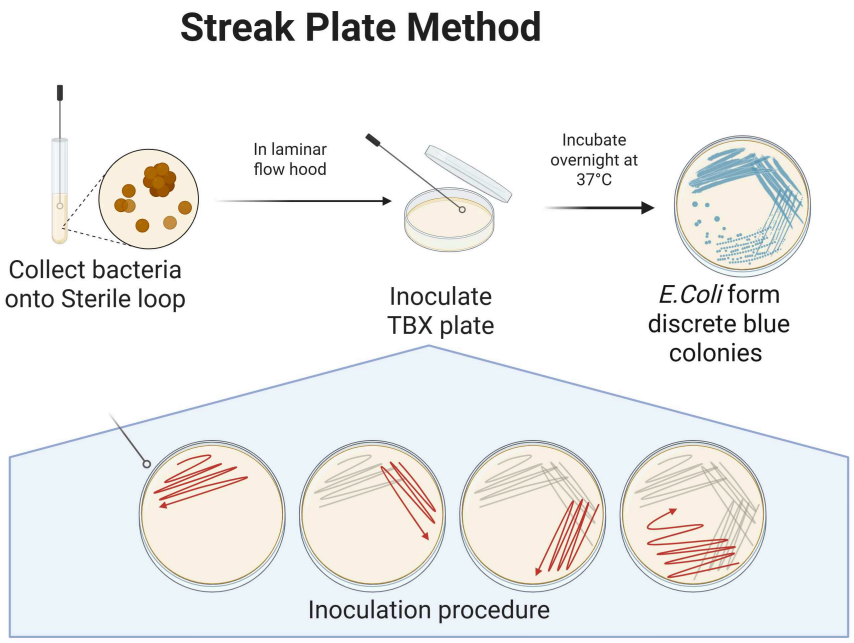

Figure 1: Streak plate method for revival of *E.coli* isolates.

4 Incubate plates at 37°C overnight.

Colony culture

5 Remove individual colony from overnight culture using sterile loop.
Note: E.coli on TBX will be blue, only select blue colonies.

6 Add loop to 5ml LB (Lennox) Broth in 15ml centrifuge tube, and swirl to remove colony. Dispose of loop.

7 Seal centrifuge tube with lid, then slighty release to allow some airflow. Place in tube rack, and incubate overnight in a shaking incubator pre-set to 37^{O}C and 200 rpm.

8

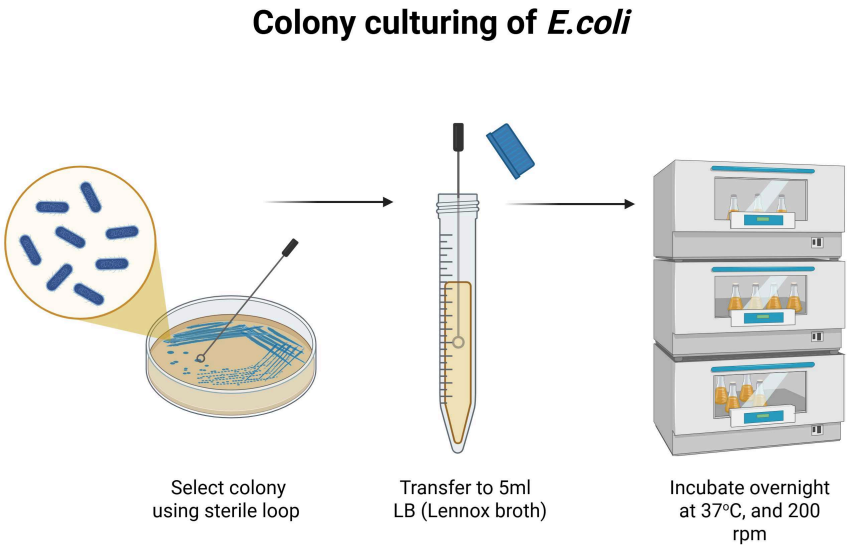

Figure 2: Colony culture of *E.coli* in LB (Lennox) Broth.

Culture pellet and preparation for sequencing

9 Remove overnight culture from shaking incubator.

10 Tighten tube lid and place in centrifuge capable of holding rotor with 15ml tube adapter. Such as Beckman Coulter Avanti J-E, and JS 5.3 Rotor with 15ml adapters. Centrifuge at 4500 rpm for 20 minutes.

11 Remove tubes from centrifuge, and pour supernatant into waste bottle. For later treatment with Rely+ Virkon (one tablet per 500mls).

12 Suspend resultant pellet in Zymo DNA/RNA shield preservation buffer, in 2ml screw top tubes provided by microbes ng.

13 Add pellet suspension, back into 2ml tube (provided by microbes ng).

14 Secure preparation with 2ml tube lid and store in the fridge until dispatch.