

E. coli Filter Paper Blots for Shipping Strains

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Materials:

Shipping

Sterile Whatman #42 filter paper cut into 2 x 2 cm squares (in foil packets)

Media to grow E. coli with appropriate antibiotic (usually MM1 10 g/L glucose, 5 g/L yeast)

Sterile 80% glycerol

Sterile 2 ml cryovials with silicone gasket

Sterile forceps (can sterilize over Bunsen burner)

Sterile 1.5 ml microfuge tubes; you only need one for each strain you are using

Parafilm (optional)

Ziplock bag

Receiving

LB plates with appropriate antibiotic

Media to grow E. coli with appropriate antibiotic (usually MM1 10 g/L glucose, 5 g/L yeast)

Sterile 80% glycerol

Procedure:

Shipping

1. Grow E. coli sample overnight with shaking to saturation
2. In a sterile 2 ml microfuge tube add 230 μ l sterile 80% glycerol
3. Add one 1 ml of E. coli culture to 2 ml microfuge tube with glycerol and mix well with up and down pipetting, Glycerol concentration is now 15%
4. Using sterile forceps, carefully open a filter paper foil packet and place one drop of culture in 15% glycerol onto the filter paper. Do not over do this we don't want the filter paper to be sopping wet just barely moist
5. Place filter paper square into 2 ml cryovial, may want to seal cryovial with Para film
6. Label cryovial and place in Ziploc bag for shipping

Receiving

1. Prepare LB plates with appropriate antibiotic
2. Open cryovial with sterile forceps and press both sides of filter paper to media
3. Leave filter paper on plate and incubate over night at 30 – 37°C
4. The next morning if you have colonies use colonies to inoculate a liquid medium to make a glycerol stock

Glycerol Stock

1. To make glycerol stock prepare a sterile cryovile by adding 800 μ l of 80% sterile glycerol
2. To the cryovile add 1 ml of E.Coli liquid culture (try to catch culture in exponential phase of growth, usually OD \approx 2)
3. Snap freeze in LN₂

The shipping protocol is based on the method of Sanderson and Zeigler in their Methods Enzymol. 1991;204:248-64. "Storing, shipping, and maintaining records on bacterial strains"

E. coli Filter Paper Blots for Shipping Strains Reagents

LB Plates, 200 mlIn 200 ml dH₂O

+ 5 g LB Broth (Difco, Luria-Bertani)

+ 3 g Agar (Fishger, BP 1423-500)

Autoclave for 20 min, swirl immediately, allow to cool to touch

Pour plates (≈ 35 ml) label, store at 4°C

80% Glycerol, 80 mlIn 16 ml dH₂O

+ 64 ml Glycerol

Autoclave for 20 min

Antibiotic	Stock (mg/ml)	Working (µg/ml)	µl of stock in 5 ml	µl of stock in 20 ml	µl of stock in 40 ml	ml of stock in 200 ml	ml of stock in 500 ml	ml of stock in 1 L
Ampicillin	10	50	25	100	200	1	2.5	5
Chloramphenicol (methanol)	10	20	10	40	80	0.4	1	2
Kanamycin	10	25	12.5	50	100	0.5	1.25	2.5
Rifampicin* (methanol)	30	150	25	100	200	1	2.5	5
Spectinomycin	10	100	50	200	400	2	5	10
Streptomycin	10	30	15	60	120	0.6	1.5	3

Note: If using multiple antibiotics you can cut the concentration of each by half

* Rifampicin is light sensitive cover with aluminum foil

10 mg/ml Antibiotic, 10 mlIn 10 ml of sterile dH₂O

+ 0.1g Antibiotic

Filter sterilize using 0.2 - 0.45 µm syringe filter

Aliquot into sterile 1.5 ml microfuge tubes and store at -20°C