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Growing overnight culture of OP50 as worm food

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Protocol status: Working

We use this protocol and it's working

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Abstract

Luria broth (LB) is a nutrient-rich media commonly used to culture bacteria in the lab. LB agar plates are frequently used to isolate individual (clonal) colonies of bacteria carrying a specific plasmid. However, a liquid culture is capable of supporting a higher density of bacteria and is used to grow up sufficient numbers of bacteria necessary to isolate enough plasmid DNA for experimental use. The following protocol is for inoculating an overnight culture of liquid LB with bacteria.

This protocol is specifically for making a liquid bacterial culture of OP50 and the following parameters are specific for this bacterial strain:

1. Name of the bacterial strain: E.coli (OP50)
2. Growth temperature: 37C
3. Incubation time: 16-18hrs (Overnight)
4. Rpm of the shaking incubator: 200-220 rpm

Troubleshooting

- 1 Obtain LB Broth from the Media kitchen- Make sure to make at least 500ml in volume for standardisation purposes

LB Broth contents:
4gNaCl
4 g Tryptone
2 g Yeast Extract dH₂O to 400 mL
- 2 Obtain sterile Erlenmeyer flask from the media kitchen
- 3 Wipe the work area with 70% ethanol and create a relatively sterile environment on the laboratory bench by using a simple gas-powered burner
- 4 Add the desired volume of liquid LB to the flask
- 5 Using a sterile inoculation loop, select a single colony from your bacteria streaked LB agar plate (One streaked plate can be used for about a month and stored at 4C during that time period)
- 6 Dip the inoculation loop into the liquid LB and swirl. Discard the inoculation loop
- 7 Loosely cover the culture with sterile aluminium foil or a cap that is not air tight as bacteria needs air
- 8 Incubate the bacterial culture at the required growth temperature overnight in a shaking incubator
- 9 After incubation, check for growth, which is characterized by a cloudy haze in the media
- 10 Measure the optical density of the bacterial culture at 600nm wavelength using a spectrophotometer. Record the OD₆₀₀ three times and calculate average, use LB Broth as Blank