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LTEE Media Recipes V.1

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Jesus Chavarria-Palma[1], Jeffrey Barrick[2]

[1]University of Texas at Austin; [2]The University of Texas at Austin

the-ltee

Jeffrey E Barrick
Michigan State University

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We use this protocol and it's working

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Abstract

Growth media used by the long-term *E. coli* evolution experiment.

- TA: Tetrazolium Arabinose for distinguishing Ara- and Ara+ strains in most competition assays. Colonies of Ara- strains typically appear red on TA agar, while those of Ara+ strains appear white. TA plates are generally incubated at 37°C for 24 h.
- DM: The basic medium used for propagating the long-term lines is Davis Minimal broth supplemented with glucose at a concentration of 25 mg per L, which we refer to as DM25. This medium supports a stationary-phase density of about 5×10^7 cells per ml for the founding strain of E. coli B.
- MG: Same basic composition as for DM liquid medium, except that we: add agar (as solidifying agent), increase the sugar concentration (so that colonies are robust), and sometimes use arabinose (instead of glucose). Glucose is used to examine the colonies on the standard minimal medium.

Protocol materials

- Magnesium Sulfate **P212121**
- Thiamine HCl **P212121**
- Glucose **P212121** **Catalog #**Glucose
- L-( )-Arabinose **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A3256-500G
- 235-Triphenyltetrazolium chloride **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T8877
- Potassium phosphate (monobasic) **P212121**
- Trisodium citrate dihydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S1804
- Ammonium sulfate **Catalog #**97061-184
- Potassium phosphate (dibasic) **P212121**
- Agar **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A1296
- Antifoam B Emulsion **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A5757-250ML
- Potassium phosphate dibasic trihydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P9666
- Bacto™ Tryptone **Thermo Fisher** **Catalog #**211705
- Bacto™ Yeast Extract **Thermo Fisher** **Catalog #**212750
- Sodium Chloride **Fisher Scientific** **Catalog #** MK-7581-212

DM: Davis-Mingioli

1 To prepare 1 L of DM:

1.1 Weigh dry components:

a. 5.34 g of Potassium phosphate (dibasic) **P212121** or 7 g of Potassium phosphate dibasic trihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P9666

b. 2 g of Potassium phosphate (monobasic) **P212121**

c. 1 g of Ammonium sulfate **Catalog #**97061-184

d. 0.5 g of Trisodium citrate dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S1804

1.2 Add distilled water to a final volume of 1 L

1.3 Autoclave using liquid program. Sterilization times are based on total volume:

	Volume (ml)	Time (min)
	75-200	20
	200-500	25
	500-1000	30
	1000-1500	35
	1500-2000	40
	>2000	60

Sterilization time

1.4 After autoclaving add the following stock solutions:

a. 1 mL of previously sterilized Magnesium Sulfate **P212121** at [M] 10 Mass / % volume

b. 1 mL of filtered sterilized Thiamine HCl **P212121** at [M] 0.2 Mass / % volume

1.5 If preparing DM-glucose, add this volume of Glucose **P212121 Catalog #**Glucose (separately autoclaved stock) at [M] 10 Mass / % volume , to get the final concentration desired:

	per 1L of DM	DMX	[Glucose] (w/v)	[Glucose] (mg/L)	[Glucose] (M)
	5 ml	DM500	0.05%	500 mg/L	2.78 mM
	250 µl	DM25	0.0025%	25 mg/L	139 µM
	20 ml	DM2000	0.2%	2000 mg/L	11.1 mM
	2.5 ml	DM250	0.025%	250 mg/L	1.39 µM
	10 ml	DM1000	0.1%	1000 mg/L	5.55 mM
	1 ml	DM100	0.010%	100 mg/L	694 µM

Note

Remember: DMX = DM + X mg/L glucose. Glucose may no longer limit the final growth density above approximately DM1000. Remember: DMX = DM + X mg/L glucose.

Note

Final composition:

- Sodium (Na^+) = [M] 5.1 millimolar (mM)
- Potassium (K^+) = [M] 75.8 millimolar (mM)
- Ammonium (NH_4) = [M] 15.2 millimolar (mM)
- Magnesium (Mg^{2+}) = [M] 0.83 millimolar (mM)
- Sulfate (SO_4^{2-}) = [M] 8.41 millimolar (mM)
- Phosphate (PO_4^{3-}) = [M] 45.3 millimolar (mM)
- Citrate = [M] 1.7 millimolar (mM)
- (In DM25) Glucose = [M] 139 micromolar (µM)

TA: Tetrazolium Arabinose

2 To prepare 1.5 L of TA:

2.1 Prepare **media base** by combining in a 2L flask:

a. 15 g of Bacto™ Tryptone **Thermo Fisher Catalog #**211705

b. 1.5 g of Bacto™ Yeast Extract **Thermo Fisher Catalog #**212750

c. 7.5 g of Sodium Chloride **Fisher Scientific Catalog #** MK-7581-212

d. 24 g of Agar **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A1296

e. 1.5 mL of

Antifoam B Emulsion **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A5757-250ML

2.2 Add distilled water to 1.3 L

2.3 Separately, prepare **sugar solution** by combining:

a. 15 g of

L-( )-Arabinose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A3256-500G

b. 200 mL of distilled water

Note

Sugar could be substituted for any other sugar.

2.4 Autoclave both solutions, media base and sugar solution from go to step #2 and go to step #2.3 separatley and according to sterilization table in go to step #1.3 .

2.5 Combine sterile solutions, **media base** and **sugar solution** for a total of 1.5 L

2.6 Add 1.5 mL of (filter sterilized and stored at 4 °C)

235-Triphenyltetrazolium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8877

at [M] 5 Mass / % volume

MG: Minimal glucose

3 To prepare 1 L of MG:

3.1 When making these plates it is necessary to prepare and autoclave the **3 main parts (salt solution, agar base, and sugar solution) separately**. Compounds that inhibit growth are produced when agar and phosphate or phosphate and glucose are autoclaved together.

3.2 Prepare **salt solution**, combine:

a. 5.3 g of Potassium phosphate (dibasic) **P212121**

b. 2 g of Potassium phosphate (monobasic) **P212121**

c. 1 g of Ammonium sulfate **Catalog #**97061-184

d. 0.5 g of

Trisodium citrate dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S1804

e. 400 mL of distilled water

3.3 Autoclave salt solution according to go to step #1.3

3.4 Prepare **agar base** by combining:

a. 16 g of Agar **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A1296

b. 1 mL of

Antifoam B Emulsion **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A5757-250ML

c. 400 mL of distilled water

3.5 Autoclave agar base according to go to step #1.3

3.6 Prepare **sugar solution** by combining:

a. 4 g of Glucose **P212121 Catalog #**Glucose

b. 200 mL of distilled water

Note

Sugar could be substituted for any other sugar.

3.7 Autoclave sugar solution according to go to step #1.3

3.8 After the three parts have been autoclaved, combine the contents of the three flasks together while they are still warm add the following stock solutions:

a. 1 mL of Magnesium Sulfate **P212121** at [M] 10 Mass / % volume (separately autoclaved stock)

b. 1 mL of Thiamine HCl **P212121** at [M] 0.2 Mass / % volume (filter sterilized stock)