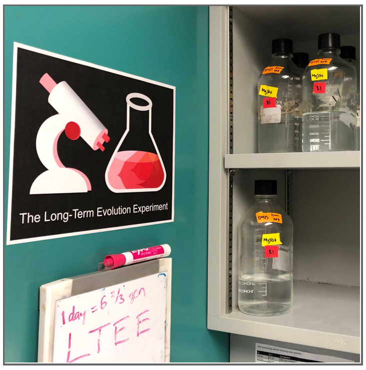

LTEE Media Recipes V.3

Forked from a private protocol

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the-ltee

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Version created by Jeffrey E Barrick

ABSTRACT

This protocol describes recipes to prepare growth media and reagents used in the *E. coli* long-term evolution experiment (LTEE).

Section 1: **DM-glucose**, Davis-Mingioli liquid medium supplemented with glucose
Section 2: **Sterile Saline**
Section 3: **TA agar**, Tetrazolium Arabinose agar
Section 4: **MG agar**, Minimal Glucose agar (equivalent to DM agar)
Section 5: **MA agar**, Minimal Arabinose agar
Section 6: **MC agar**, Minimal Citrate agar
Section 7: **CC agar**, Christensen Citrate agar
Section 8: **Stock Solutions**, detailed instructions for stock solutions needed for media preparation

DM-glucose: Davis-Mingioli medium (or sometimes called Davis Minimal medium) supplemented with glucose is used for propagating the LTEE populations and for performing related experiments. For propagating the LTEE, glucose is added to a concentration of 25 mg/L, which we refer to as "DM25". DM25 supports a stationary-phase density of about 5×10^7 cells/mL for *E. coli* REL606 and REL607, the founding strains of the LTEE. (The stationary-phase density of evolved LTEE clones varies, but tends to be approximately half that of the ancestral strains.) DM with higher concentrations of glucose is used for reviving cells from freezer stocks or for growing many cells to harvest for certain experiments. These other DM formulations are named in an analogous fashion of DMX, where X is the concentration of glucose in mg/L (e.g., 1000 mg/L glucose in DM1000).

Sterile Saline: Used to dilute *E. coli* cultures, for instance when plating on agar to isolate colonies or to count CFUs to determine cell titers.

TA agar: Tetrazolium Arabinose agar plates are used for distinguishing *E. coli* cells

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

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that can grow on the sugar arabinose (Ara^+) from those that cannot (Ara^-). Plating dilutions that give 150-250 colonies are used for monitoring the LTEE for contamination and also for co-culture competition assays that measure the relative fitness of two strains. Colonies grown from Ara^- cells appear red on TA agar, while those of Ara^+ strains appear pinkish-white. These phenotypes are very clear after 24 hours of incubation at 37°C for the REL606 (Ara^-) and REL607 (Ara^+) ancestors of the LTEE. Colonies of evolved clones can exhibit a wide variation of these color phenotypes. Some evolved clones may take longer than 24 hours to form visible colonies on TA.

MG agar: Minimal Glucose agar has the same base composition as DM-glucose liquid medium, except agar is added as a solidifying agent, and the glucose concentration is increased to 4 g/L to support the growth of colonies. Plating dilutions that give 150-250 colonies or streaking out on MG-agar is used to isolate colonies from LTEE populations. Dilutions of the LTEE populations can also be plated on MG agar to monitor for unexpected growth, colony appearance, or CFU numbers that could indicate contamination. Ancestral clones form colonies within 24 hours on MG agar. Evolved clones typically also form colonies within 24 hours on MG agar, but some may take longer. Evolved clones also generally produce larger colonies than the ancestors on MG.

MA agar: Minimal Arabinose agar is the same as MG agar except that the sugar arabinose is used instead of glucose. Ara^- cells like those of strain REL606 will not form colonies on MA agar. Only Ara^+ cells like those of strain REL607 will. Plating dilutions from the LTEE populations that give 150-250 CFUs on MA agar can be used to monitor the Ara^- populations for contamination from Ara^+ populations. The Ara^+ ancestor and evolved strains generally form colonies within 24 hours on MA agar. However, some Ara^+ populations have lost the ability to form colonies at later generations. Plating a large number of Ara^- cells ($>10^9$) on an MA plate can also be used to select spontaneous mutants that have reverted from the Ara^- marker state to the Ara^+ marker state. Reversion to Ara^+ among LTEE clones is usually via a single nucleotide substitution mutation in the *araC* gene. This mutation occurs at a rate of $\sim 10^{10}$ cells/generation among non-mutator clones, and much higher among clones with mutator phenotypes.

Note

(1) Ara$^-$ clones will typically form microcolonies on MA that are at the limits of visual inspection owing to trace substrates present in agar. (2) Because MA contains citrate, Cit$^+$ clones from the Ara−3 population will form visible colonies on MA even if they have an Ara$^-$ phenotype. When isolating Ara$^+$ revertant mutants or inspecting the Ara− phenotype of Cit+ clones, use of MA formulated without citrate is recommended.

MC agar: Minimal Citrate agar is the same as MG/MA agar except that citrate is used as the carbon source. Strains that have evolved citrate utilization (Cit+) can form colonies on MC agar.

CC agar: Christensen Citrate agar is an indicator medium that can be used to detect weak citrate utilization in colonies on the basis of a color change even, for strains that may not be able to form colonies on MC agar.

PROTOCOL MATERIALS

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

In 3 steps

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

In 2 steps

L-Cysteine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C1276**

In 2 steps

Ammonium sulfate **Catalog #97061-184** In 3 steps

Potassium phosphate (dibasic) **P212121** In 2 steps

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

In 2 steps

Magnesium Sulfate **P212121** In 3 steps

Bacto™ Yeast Extract **Thermo Fisher Catalog #212750** In 2 steps

Sodium Chloride **Fisher Scientific Catalog # MK-7581-212** In 4 steps

Phenol Red **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3532-25G**

Step 7.1

Sodium Thiosulfate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #72049-250G**

Step 7.1

Sodium Thiosulfate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #72049-250G**

Step 7.4

Potassium phosphate dibasic trihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9666**

Step 1.1

Thiamine HCl **P212121** In 3 steps

Glucose **P212121 Catalog #Glucose** In 4 steps

Bacto™ Tryptone **Thermo Fisher Catalog #211705** Step 3.1

Agar **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A1296** In 3 steps

ammonium iron(III) citrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #F5879-100G**

In 2 steps

Potassium phosphate (monobasic) **P212121** In 3 steps

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

In 3 steps

DM-glucose: Davis-Mingioli liquid medium supplemented with...

1 To prepare 1 L of DM, follow these steps.

Note

DM media are described here:

CITATION

B. C. Carlton and B. J. Brown (1981). Gene mutation. Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C..

The DM-glucose medium formulation used in the LTEE is further described here:

CITATION

Richard E. Lenski, Michael R. Rose, Suzanne C. Simpson, Scott C. Tadler (1991). Long-term experimental evolution in Escherichia coli. I. Adaptation and divergence during 2,000 generations. American Naturalist.

LINK

https://www.jstor.org/stable/2462549

NOTE: There is a typo in the Lenski et al. paper. The actual amount of thiamine hydrochloride in this recipe and what has been used throughout the LTEE is 0.0002% (w/v), not the 0.000002% (w/v) stated in the paper.

Note

DM History and Notes on Citrate

DM medium was developed by Bernard D. Davis (1916-1994) and Elizabeth S. Mingioli (1926-1997) at the U.S. Public Health Service Tuberculosis Research Laboratory at Cornell University for use in the isolation of auxotrophic mutants of *E. coli* using penicillin (Davis and Mingioli 1950). Developed independently by Davis and by Joshua Lederberg (1925-2008) and Norton Zinder (1928-2012), the "penicillin method" takes advantage of the fact that penicillin only kills actively growing cells. Addition of penicillin to a culture growing in a minimal medium selects for auxotrophic mutants that are not growing owing to the inability to synthesize one or more needed nutrients. DM was a refinement of the medium that Davis had originally used for the penicillin method. It included the addition of 0.5 mg/L of citrate as a standard part of the recipe because Davis had previously found that adding citrate increased the efficiency with which penicillin killed growing cells. The medium came to be commonly used in microbiology and molecular biology as the penicillin method spread in the community.

Citrate was later established to improve iron availability for *E. coli*, which in turn improves growth in the medium and thus the killing efficiency of penicillin. Under neutral pH and oxic conditions, iron largely occurs as the insoluble ferric ion (Fe^{3+}). Citrate chelates ferric ions to form a soluble complex of ferric dicitrate. *E. coli* is able to bind ferric di-citrate and import the iron from it. The concentration of citrate needed for this role is ~10 µM, far lower than the 1700 µM found in DM. The excess amount of citrate in DM is likely owing to 0.5 mg/L being a convenient concentration that Davis did not see a need for optimizing owing to *E. coli*'s inability to grow aerobically on it.

While *E. coli* can grow in DM formulated without citrate, that growth is inconsistent and highly variable, likely reflecting sensitivity to minor fluctuations in the dissolved iron content of the water used. It is therefore not recommended.

The original citation for DM medium may be found here:

CITATION

Bernard D. Davis, Elizabeth S. Mingioli (1950). Mutants of Escherichia coli requiring methionine or vitamin B-12. Journal of Bacteriology.

LINK

https://doi.org/10.1128/jb.60.1.17-28.1950

This note is drawn from the following:

CITATION

Blount ZD (2016). A case study in evolutionary contingency. Studies in history and philosophy of biological and biomedical sciences.

LINK

https://doi.org/10.1016/j.shpsc.2015.12.007

1.1 Weigh dry components:

a. 5.34 g of Potassium phosphate (dibasic) **P212121** or 7 g of Potassium phosphate dibasic trihydrate **Merck Millipore Sigma 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 **Sigma-aldrich Catalog #S1804**

1.2 Add distilled water to a final volume of 1 L

1.3 Autoclave for 1 hour at 121°C and a pressure of 15 psi or higher.

1.4 After autoclaving add the following stock solutions:

a. 1 mL of previously autoclaved 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 desired final concentration:

per 1L of DM	DMX	[Glucose] (w/v)	[Glucose] (mg/L)	[Glucose] (M)
250 µL	DM25	0.0025%	25 mg/L	139 µM
1 mL	DM100	0.010%	100 mg/L	694 µM
2.5 mL	DM250	0.025%	250 mg/L	1.39 µM
5 mL	DM500	0.05%	500 mg/L	2.78 mM
10 mL	DM1000	0.1%	1000 mg/L	5.55 mM
20 mL	DM2000	0.2%	2000 mg/L	11.1 mM
0 mL	DM0	0.0%	0 mg/L	0 mM

Note

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

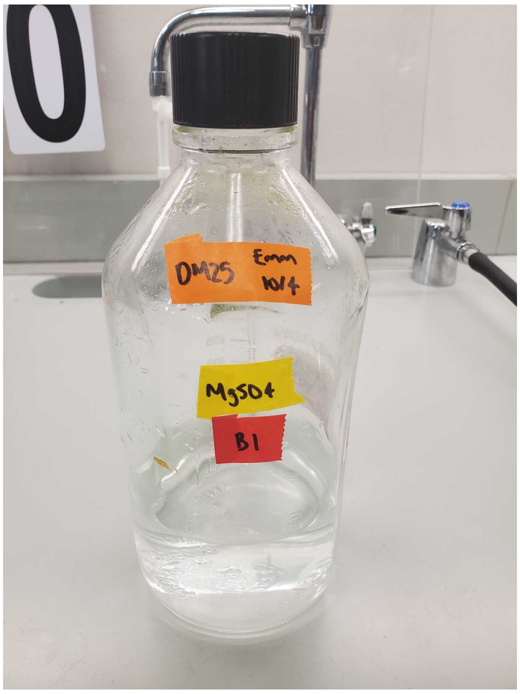
Note

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)

1.6 DM-glucose medium is stable for more than a year at room temperature.

Expected result



Bottle of DM25

Sterile Saline

2 To prepare 1 L of Sterile Saline (0.85% w/v), follow these steps.

2.1 Add 8.5 g of Sodium Chloride **Fisher Scientific Catalog # MK-7581-212** to a 2 L flask.

2.2 Add distilled water to 1000 mL

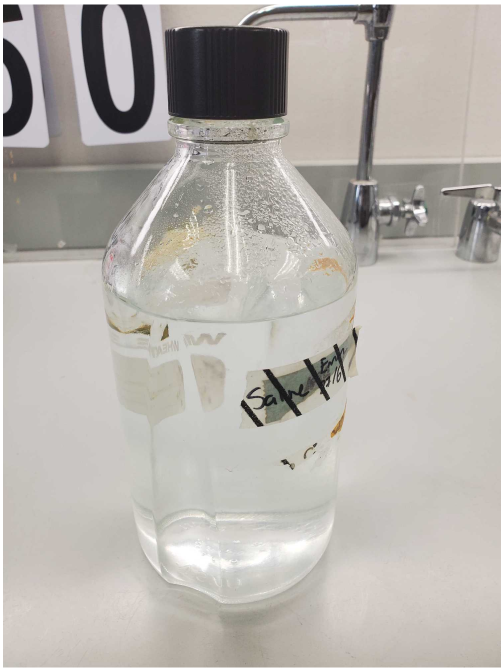
2.3 Autoclave for 1 hour at 121°C and a pressure of 15 psi or higher.

Note

It's also possible to make 5 L at a time by adding 42.5 g of Sodium Chloride **Fisher Scientific Catalog # MK-7581-212** to a 6 L flask.

2.4 Sterile saline is stable for years at room temperature.

Expected result



Bottle of Sterile Saline

TA agar: Tetrazolium Arabinose agar

3 To prepare 1 L of TA agar, follow these steps.

Note

Tetrazolium sugar agar recipe used in the LTEE is derived from (Levin, Stewart and Chao, 2015)

CITATION

Bruce R. Levin, Frank M. Stewart and Lin Chao (1977). Resource-Limited Growth, Competition, and Predation: A Model and Experimental Studies with Bacteria and Bacteriophage. The American Naturalist.
LINK
http://www.jstor.org/stable/2459975

Note

How TA Works
On TA, bacteria may grow on amino acids from the tryptone in the medium or on arabinose or whatever other sugar is provided. Cells that are unable to grow on the provided sugar grow on the amino acids, which produces ammonia and thus increases the pH. At alkaline pH, TTC is converted to the intensely red, insoluble dye, formazan, which is sequestered in a granule in the cell. Owing to this accumulation of formazan, bacteria that are unable to grow on the provided sugar thus form red colonies. By contrast, cells that can grow on the sugar do so preferentially. They do not form ammonia and thus do not cause conversion of TTC to formazan, causing them to form colonies that are white to pinkish in color. If incubated over long periods of time, however, colonies formed by sugar-using bacteria will turn more red. This occurs for two reasons. First, there is some small amount of growth on the amino acids, and so formazan still builds up, albeit slowly. Second, over time, the cells in the colony can exhaust the sugar and switch to growing more on the amino acids, resulting in greater accumulation of formazan.

TA Origins
TTC is used in a wide variety of indicator media. Its use to distinguish between sugar-using and non-sugar using bacteria traces to Joshua Lederberg. In 1948, Lederberg described using it to distinguish wild type *E. coli* that were able to ferment glucose, maltose, or lactose from mutants that were not. TA is a logical modification of this original use.

Lederberg's original note is here:

CITATION

Joshua Lederberg (1948). Detection of fermentative variants with tetrazolium. Journal of Bacteriology.
LINK
https://doi.org/10.1128%2Fjb.56.5.695-695.1948

More information on the biochemistry of tetrazolium indicator dye may be found here:

CITATION

Nikki Turner, W. E. Sandine, P.R. Elliker, E. A. Day (1963). Use of tetrazolium dyes in an agar medium for differentiation of streptococcus lactis and streptococcus cremoris. Journal of Dairy Science.
LINK
https://doi.org/10.3168/jds.S0022-0302(63)89059-1

3.1 Prepare **basal medium** by combining in a 2 L flask into which a stir bar has been placed:

a. 1 g of Bacto™ Tryptone **Thermo Fisher Catalog #211705**

b. 1 g of Bacto™ Yeast Extract **Thermo Fisher Catalog #212750**

c. 5 g of Sodium Chloride **Fisher Scientific Catalog # MK-7581-212**

d. 16 g of Agar **Sigma-aldrich Catalog #A1296**

e. 1 mL of Antifoam B Emulsion **Sigma-aldrich Catalog #A5757-250ML**

3.2 Add distilled water to 800 L

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

a. 10 g of L-( )-Arabinose **Sigma-aldrich Catalog #A3256-500G**

b. 200 mL of distilled water

Note

It is also possible to make indicator plates for other sugars such as rhamnose, maltose, lactose, etc..

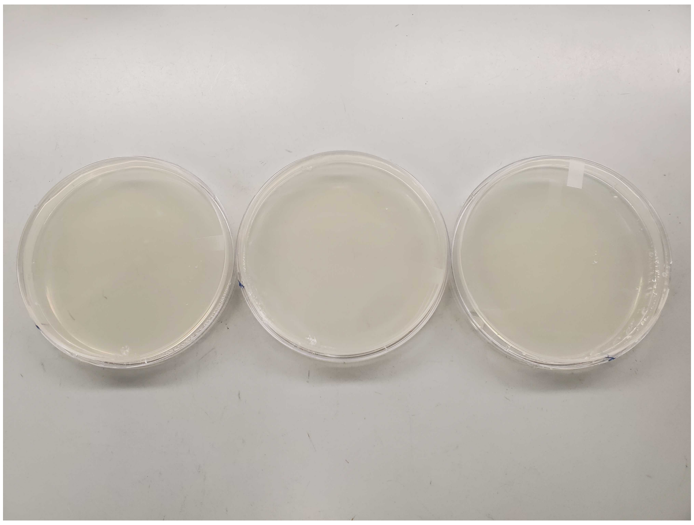
3.4 Autoclave both the **basal medium** and the **sugar solution** separately for 01:00:00 at 121°C an 1h 15 psi or higher.

3.5 When the **basal medium** has cooled to the point at which its flask can be touched with the back of the hand for 5 second without pain, add 1 mL of 235-Triphenyltetrazolium chloride **Sigma-aldrich Catalog #T8877** (TTC) at [M] 5 Mass / % volume and **sugar solution** for a total of 1 L. Place flask with combined medium on a stir plate and stir at medium speed for 5 minutes to fully mix.

Note: TTC stock should be filtered sterilized and stored at 4 °C. TTC is sensitive to light, so bottles of TTC stock solution should be stored in the dark and wrapped in foil to reduce light exposure.

3.6 Pour plates. 1 L will make approximately 35-45 plates. TA agar plates can be stored for at least one to two months at 4°C or one to two weeks at room temperature. While bacterial colonies will form on older medium, the TTC breaks down over time, reducing color differentiation between sugar$^-$ and sugar$^+$ colonies. Exposure to light increases the rate at which TTC in the medium breaks down.

Expected result



TA Agar Plates

MG agar: Minimal glucose agar

4 To prepare 1 L of MG agar, follow these steps.

4.1 MG agar is composed of **3 solutions (basal salt solution, agar solution, and sugar solution), which must be prepared and autoclaved separately**. If combined and autoclaved together, the components will react to produce compounds that will inhibit bacterial growth.

4.2

Prepare **basal salt solution** by combining:

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 **Sigma-aldrich Catalog #S1804**

e. 400 mL of distilled water

4.3

Prepare **agar solution** by combining:

a. 16 g of Agar **Sigma-aldrich Catalog #A1296**

b. 1 mL of [M] 5 % (v/v) Antifoam B Emulsion **Sigma-aldrich Catalog #A5757-250ML**

c. 400 mL of distilled water

4.4

Prepare **sugar solution** by combining:

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

b. 200 mL of distilled water

Note

Any sugar of interest can be substituted for glucose.

4.5

Autoclave basal salt, agar, and sugar solutions for 01:00:00 at 121°C and at least 15 psi. 1h

4.6

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:

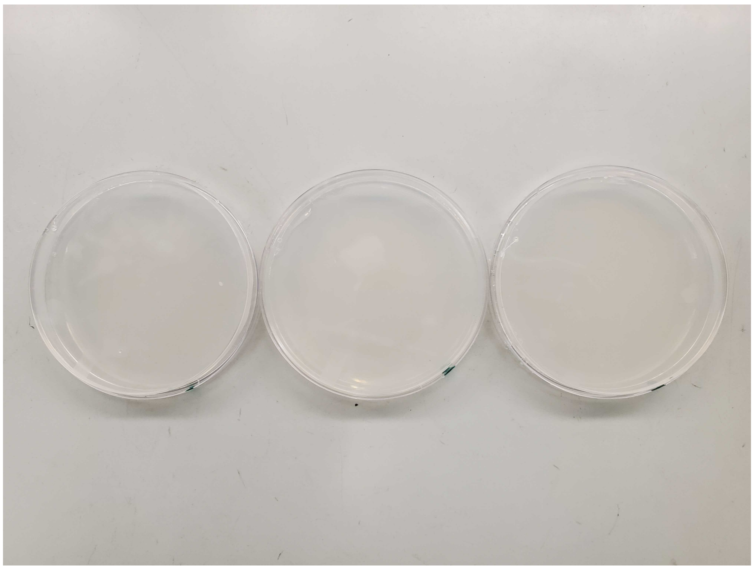
a. 1 mL of Magnesium Sulfate **P212121** at [M] 10 Mass / % volume (separately autoclaved stock)

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

4.7

Pour plates. 1 L will make approximately 35 - 45 plates. MG agar plates can be stored for at least one to two months at 4°C or one to two weeks at room temperature.

Expected result



MG Agar Plates

MA agar: Minimal Arabinose agar

5 To prepare 1 L of MA agar, follow these steps.

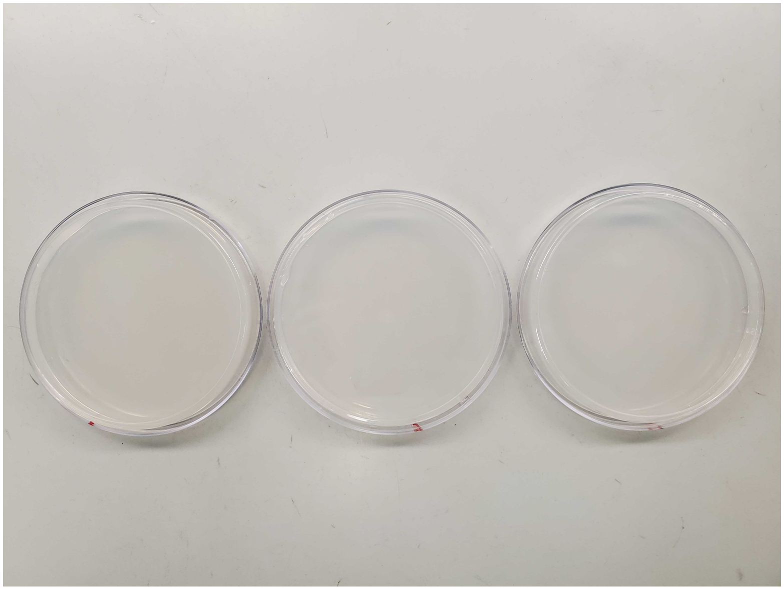
5.1 Follow the instructions for MG agar, with the exception of the substitution of L-( )-Arabinose **Sigma-aldrich Catalog #A3256-500G** in the place of glucose.

Note

Cit+ evolved clones from the Ara−3 population will form visible colonies on MA owing to the presence of citrate. When selecting for Ara^+ revertant mutants of Cit^+ clones, use of MA agar with citrate excluded from the formulation is recommended.

5.2 Pour plates. 1 L will make approximately 35 - 45 plates. MA agar plates can be stored for up to two months at 4°C or one to two weeks at room temperature.

Expected result



MA Agar Plates

MC agar: Minimal Citrate agar

6 To prepare 1 L of MC agar, follow these steps.

6.1 Follow the same steps as for making MG agar, but with the following modifications:

1. **Do not prepare a sugar flask**. You will thus have only two flasks: one for the **basal salts solution** and one for the **agar solution**. You will thus divide the 1 L of distilled water between just these two flasks.

2. To the basal salts solution, you will add 4.5 g of Trisodium citrate dihydrate **Sigma-aldrich Catalog #S1804** instead of 0.5 g of a sugar.

When done,

CC agar: Christensen Citrate agar

1h

7 To prepare 1 L of CC agar, follow these steps.

7.1 In a 2 L flask into which has been placed a stir bar, combine the following:

a. 3 g of Sodium Chloride **Fisher Scientific Catalog # MK-7581-212**

b. 0.2 g of Glucose **P212121 Catalog #Glucose**

c. 0.5 g of Bacto™ Yeast Extract **Thermo Fisher Catalog #212750**

d. 0.1 g of L-Cysteine hydrochloride **Sigma Aldrich Catalog #C1276**

e. 0.4 g of ammonium iron(III) citrate **Sigma Aldrich Catalog #F5879-100G**

f. 1.0 g of Potassium phosphate (monobasic) **P212121**

g. 5.0 g of Sodium Chloride **Fisher Scientific Catalog # MK-7581-212**

h. 0.08 g g of Sodium Thiosulfate **Sigma Aldrich Catalog #72049-250G**

i. 0.012 g g of Phenol Red **Sigma Aldrich Catalog #P3532-25G**

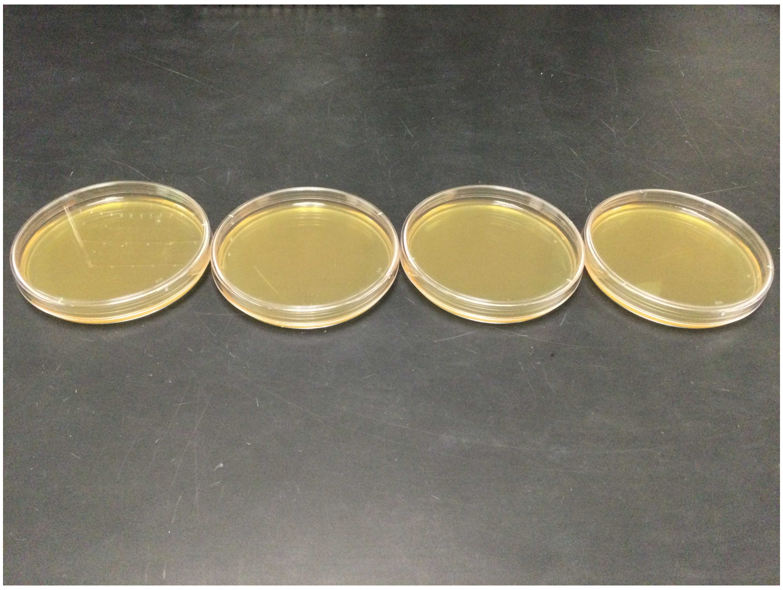
j. 15 g of Agar **Sigma-aldrich Catalog #A1296**

k. 1 L of distilled water

7.2 Adjust pH to 6.7 with NaOH (1 N or 10 N).

7.3 Autoclave solution for 01:00:00 at 121°C and at least 15 psi. 1h

7.4 Pour plates. 1 L will make approximately 35 - 45 plates. CC agar plates can be stored at room temperature for up to 4 months or at 4ºC for up to a year.



Christensen's Citrate Agar Plates

Note

Alternate Formulations:
Modified Christensen's Citrate Agar (MCCA):
This version is a simplified formulation that excludes some of the more trace components. It works just as well for detecting citrate-users.

For MCCA, exclude the L-Cysteine hydrochloride **Sigma Aldrich Catalog #C1276**, ammonium iron(III) citrate **Sigma Aldrich Catalog #F5879-100G**, and Sodium Thiosulfate **Sigma Aldrich Catalog #72049-250G**, and substitute 0.5 g of Ammonium sulfate **Catalog #97061-184**. Otherwise prepare exactly as for the original formulation.

Christensen's Citrate Broth (CCB):
Prepare exactly as with CCA, but exclude the agar. Using the formulation of MCCA, sans agar, also works.

Appendix: Stock solutions

8 This section describes in more detail how to make each the stock solutions required for the above recipes.

8.1 10% (w/v) $MgSO_4$

Add 25 g of Magnesium Sulfate **P212121** to a 500 mL or 1 L graduated cylinder. Add distilled water to a final volume of 250 mL . Fully mix by swirling as you do so. Pour the solution into a 250 mL bottle. Autoclave to sterilize. Store at Room temperature .

8.2 0.2% (w/v) Thiamine

Add 0.5 g of Thiamine HCl **P212121** to a 500 mL or 1 L graduated cylinder. Add distilled water to a final volume of 250 mL . Fully mix by swirling as you do so. Filter sterilize the solution into a pre-autoclaved 250 mL bottle. Store at 4 °C .

Note: This solution is 0.2% (w/v) in thiamine•HCl — NOT in thiamine. The concentration of thiamine is actually 89.2% of this or 0.178% (w/v) if you account for the fact that the reagent being dissolved is not pure thiamine. (The molecular weight of thiamine•HCl is 337.27, and the molecular weight of HCl is 36.46).

8.3 10% (w/v) D-Glucose

Add 25 g of Glucose **P212121 Catalog #Glucose** to a 500 mL or 1 L graduated cylinder. Add distilled water to a final volume of 250 mL . Fully mix by swirling as you do so. Pour solution into a 250 mL bottle. Autoclave to sterilize. Store at Room temperature .

Note: D-Dextrose is a synonym for D-glucose that you may find on many reagent containers. Typically, if D- is not specified in the labeling, you can assume that it is the correct D-sugar.

8.4 5% (w/v) Triphenyltetrazolium chloride (TTC)

Add 5 g of 235-Triphenyltetrazolium chloride **Sigma-aldrich Catalog #T8877** (TTC) to a 250 mL graduated cylinder. Add distilled water to a final volume of 100 mL . Fully mix by swirling as you do so. Filter sterilize the solution into a pre-autoclaved 100 mL bottle. Store at 4 °C . Wrap the bottle in foil. TTC is sensitive to light and can degrade over time.

Note: The final solution should be a pale yellow color.

8.5 **5% (v/v) Antifoam solution**

Add 12.5 mL of Antifoam B Emulsion **Sigma-aldrich Catalog #A5757-250ML** to 237.5 mL of distilled water in a 250 mL glass bottle. Autoclave to sterilize.

Note: The reagent from Sigma-Aldrich comes as a 10% emulsion in water. This stock solution is 5% (v/v) of the reagent, so it is actually 0.5% v/v of Antifoam B.