



Recipes for Media, Reagents, and Stock Solutions

THE SUCCESS OF THE LABORATORIES ON THIS SITE depends on the use of uncontaminated reagents. Follow the recipes with care and pay scrupulous attention to cleanliness. Measure each ingredient with a clean spatula or carefully pour each ingredient from its container.

The recipes are organized in sections. Stock solutions that are used in more than one laboratory are listed once, according to their first use in the laboratories.

I. Bacterial Culture

- 4 N Sodium Hydroxide (NaOH)
- 10 mg/mL Ampicillin
- 1:1 vol/vol Distilled or Deionized Water:Ethanol
- 12.5 mg/mL Tetracycline
- Luria-Bertani (LB) Broth
- LB Broth + Antibiotic
- LB Agar Plates
- LB Agar + Antibiotic

II. *C. elegans* Culture

- 5 mg/mL Cholesterol (dissolved in 100% ethanol)
- NGM-lite plates
- 10 mg/mL Ampicillin
- 1M Isopropyl- β ,D-thiogalactoside (IPTG)
- NGM-lite + Ampicillin + IPTG plates
- M9 Buffer
- Freezing Solution

III. Worm DNA Isolation

- 1 M Tris (pH 8.3)
- 5 M KCL
- 1 M Magnesium Chloride (MgCl₂)
- 10 \times PCR Buffer

IV. Polymerase Chain Reaction

- 1% Cresol Red Dye
- Cresol Red Loading Dye
- Primer/Loading Dye Mix
- Primer Sequences

V. Agarose Gel Electrophoresis

- 10 \times Tris/Borate/EDTA (TBE) Electrophoresis Buffer
- 1 \times Tris/Borate/EDTA (TBE) Electrophoresis Buffer
- 1.0% Agarose
- 1 μ g/ μ l Ethidium Bromide Staining Solution
- pBR322/BstNI Size Markers (0.1 μ g/ μ l)
- 100-bp or 1-kb DNA Ladder (0.1 μ g/ μ l)

VI. Ligation

1 M Tris (pH 7.6)
10× Ligation Buffer
0.1 M Adenosine Triphosphate (ATP)
2× Ligation Buffer + ATP
T-tailing L4440 Vector

VII. Bacterial Transformation

1 M Calcium Chloride (CaCl₂)
50 mM Calcium Chloride (CaCl₂)
Competent Cell Preparation

VIII. Plasmid Miniprep

0.5 M Ethylenediaminetetraacetic Acid (EDTA)
Tris/EDTA (TE) Buffer
Glucose/Tris/EDTA (GTE)
5 M Potassium Acetate (KOAc)
Potassium Acetate/Acetic Acid
10% Sodium Dodecyl Sulfate (SDS)
1% SDS/0.2 N NaOH

Notes on Buffers

1. Typically, solid reagents are dissolved in a volume of de-ionized or distilled water equivalent to 70-80% of the finished volume of buffer. This leaves room for the addition of acid or base to adjust the pH. Then, water is added to bring the solution up to the final volume.
2. Buffers typically are used as 1× or 10× solutions. Buffers are diluted when mixed with other reagents to produce a working concentration of 1×.
3. The buffer supplied with the commercial enzymes used in these laboratories should be used unless noted otherwise.
4. Storage temperatures of 4°C and -20°C refer to normal refrigerator and freezer (non-frost free) temperatures, respectively.
5. The final concentration of each liquid reagent is given in the right-hand column of the reagent list.

I. Bacterial Culture

4 N Sodium Hydroxide (NaOH)

Makes 100 mL.

Store at room temperature (indefinitely).

1. Slowly add 16 g of NaOH pellets (m.w. = 40.00) to 80 mL of deionized or distilled water, with stirring. The solution will get very hot.
2. When the NaOH pellets are completely dissolved, add water to a final volume of 100 mL.

10 mg/mL Ampicillin

Makes 100 mL.

Store at -20°C (1 year) or 4°C (3 months).

1. Add 1 g of ampicillin (sodium salt, m.w. = 371.40) to 100 mL of deionized or distilled water in a clean 250-mL flask. (The sodium salt dissolves readily; however, the free acid form is difficult to dissolve.)
2. Stir to dissolve.
3. Pre-wash a 0.45- or 0.22- μm sterile filter (Nalgene or Corning) by drawing through 50–100 mL of deionized or distilled water. Pass the ampicillin solution through the washed filter.
4. Dispense 10-mL aliquots in sterile 15-mL tubes (Falcon 2059 or equivalent), and freeze at -20°C .

1:1 vol/vol Distilled or Deionized Water:Ethanol

Makes 100 mL.

Store in a sealed container.

1. Add 50 mL of distilled or deionized water to 50 mL of 95-100% ethanol in a clean 200-mL bottle.

12.5 mg/mL Tetracycline

Makes 80 mL.

Store at -20°C (1 year) or 4°C (3 months).

1. Add 1.0 g of tetracycline (m.w. = 444.44) to 75 mL of 1:1 vol/vol distilled or deionized water:ethanol in a clean 250-mL flask.
2. Stir to dissolve.
3. Bring up the volume to 80 mL with 1:1 vol/vol distilled or deionized water:ethanol.
4. Pre-wash a 0.45- or 0.22- μm sterile filter (Nalgene or Corning) by drawing through 50–100 mL of deionized or distilled water. Pass the tetracycline solution through the washed filter.

5. Dispense 10-mL aliquots in sterile 15-mL tubes (Falcon 2059 or equivalent), wrap in aluminium foil (tetracycline is light sensitive), and freeze at -20°C .

Luria-Bertani (LB) Broth

Makes 1 liter.

Store at room temperature (indefinitely).

1. Weigh out:

- 10 g of tryptone
- 5 g of yeast extract
- 10 g of NaCl (m.w. = 58.44)

Alternatively, use 25 g of premix containing all of these ingredients.

2. Add all ingredients to a clean 2-liter flask that has been rinsed with deionized or distilled water.
3. Add 1 liter of deionized or distilled water to flask.
4. Add 0.5 mL of 4 N NaOH.
5. Stir to dissolve the dry ingredients, preferably using a magnetic stir bar.
6. *If preparing for mid-log cultures:* Split LB broth into two 500-mL aliquots in 2-liter flasks. Plug top with cotton or foam, and cover with aluminum foil. (Alternatively, cover with aluminum foil only.) Autoclave for 15–20 minutes at 121°C .

or

If preparing for general use in transformations: Dispense 100-mL aliquots into sterile 150–250-mL bottles using one of the following methods:

- a. Loosely put on the caps. Autoclave for 15–20 minutes at 121°C . (To help guard against breakage, autoclave the bottles in a shallow pan with a small amount of water.)

or

- b. Prewash a 0.45- or 0.22- μm sterile filter (Nalgene or Corning) by drawing through 50–100 mL of deionized or distilled water. Pass LB broth through the filter, and dispense aliquots into sterile bottles.

NOTE LB broth can be considered sterile as long as the solution remains clear. Cloudiness is a sign of contamination by microbes. Always swirl the solution to check for bacterial or fungal cells that may have settled at the bottom of the flask or bottle.

LB Broth + Antibiotic

Makes 100 mL.

Store at 4°C (3 months).

1. Sterilely add 1 mL of stock antibiotic to 100 mL of cool LB broth.
2. Swirl to mix.

LB Agar Plates

Makes 35–40 plates.

Store at 4°C (3 months) or room temperature (3 months).

1. Weigh out:
 - 10 g of tryptone
 - 5 g of yeast extract
 - 10 g of NaCl (m.w. = 58.44)
 - 15 g of agar

Alternatively, use 40 g of premix containing all of these ingredients.

2. Add all ingredients to a clean 2-liter flask that has been rinsed with deionized or distilled water.
3. Add 1 liter of deionized or distilled water.
4. Add 0.5 mL of 4 N NaOH.
5. Stir to dissolve dry ingredients, preferably using a magnetic stir bar. Any undissolved material will dissolve during autoclaving.
6. Cover flask mouth with aluminum foil, and autoclave solution for 15 minutes at 121°C.
7. Allow the solution to cool just until the flask can be held by bare hands (55–60°C). (If the solution cools too long and the agar begins to solidify, remelt by briefly autoclaving for 5 minutes or less or heating it in a microwave oven for a few minutes.)
8. While the agar is cooling, mark *culture plate bottoms* with the date and description of the media (e.g., LB). If using presterilized polystyrene plates, carefully cut the end of the plastic sleeves, and save the sleeves for storing the poured plates. Spread the plates out on the lab bench.
9. When the agar flask is cool enough to hold, lift the lid of a culture plate only enough to pour the solution. *Do not place the lid on the lab bench.* Quickly pour in enough agar to just cover the bottom of the plate (~25–30 mL). Tilt the plate to spread the agar evenly, and immediately replace the lid.
10. Continue pouring agar into plates. Occasionally flame the mouth of the flask to maintain sterility.

11. To remove bubbles in the surface of the poured agar, touch the plate surface with the flame from a Bunsen burner while the agar is still liquid.
12. Allow agar to solidify undisturbed.
13. If possible, incubate the plates *lidside down* for several hours at 37°C (overnight if convenient). This dries the agar, limiting condensation when plates are stored under refrigeration. It also allows the ready detection of any contaminated plates.
14. Stack plates in their original sleeves for storage.

LB Agar + Antibiotic

Makes 30–45 plates.

Store at 4°C (3 months).

1. Follow recipe above for LB agar plates through Step 8.
2. When the agar flask is cool enough to hold, sterilely add 10 mL of stock antibiotic. Ampicillin and tetracycline are destroyed by heat; therefore, *it is essential to cool the agar before adding the antibiotic.*
3. Swirl the flask to mix the antibiotic and agar solution.
4. Resume recipe above at Step 9.

NOTE In a pinch, antibiotic-containing plates can be made quickly by evenly spreading 200 µL of 10 mg/mL antibiotic on the surface of an LB agar plate. Allow the agar to absorb the antibiotic for 10–20 minutes before use. Outdated antibiotic plates can be refurbished in this manner.

II. *C. elegans* Culture

5 mg/mL Cholesterol (dissolved in 100% ethanol)

Makes 10 mL.

Store at -20°C (1 year) or 4°C (3 months).

1. Add 50 mg of cholesterol to 10 mL of 100% ethanol in a sterile 15-mL tube (Falcon 2059 or equivalent).
2. Stir to dissolve.
3. Freeze at -20°C .

NGM-lite Plates

Eric Lambie originally described NGM-lite plates in *Worm Breeder's Gazette* 13(5):11 (February 1, 1995). These plates contain a simple, complete medium for culturing *C. elegans*.

1. Weigh out:
 - 2 g of NaCl
 - 4 g of Bactotryptone
 - 3 g of KH_2PO_4
 - 0.5 g of K_2HPO_4
 - 20 g of agar
 - 1 mL of 5 mg/mL cholesterol (dissolved in 100% ethanol)
2. Add all ingredients to a clean 2-liter flask that has been rinsed with deionized or distilled water.
3. Add 1 liter of deionized or distilled water.
4. Stir to dissolve dry ingredients, preferably using a magnetic stir bar. Any undissolved material will dissolve during autoclaving.
5. Cover the flask mouth with aluminum foil, and autoclave the solution for 15 minutes at 121°C .
6. After autoclaving, cool this solution to 55°C in a water bath. (If the solution cools too long and the agar begins to solidify, melt by briefly autoclaving for 5 minutes or less or heating it in a microwave oven for a few minutes.)
7. Pour the solution into 6 cm plastic petri dishes. Add enough to fill the plates about half full. If the plate has large bubbles (that are large enough for worms to crawl into), pop the bubbles by briefly flaming the surface with a Bunsen burner. Occasionally flame the mouth of the flask to maintain sterility.
8. Allow the plates to cool for at least 1 day before seeding them.

1M Isopropyl- β ,D-thiogalactoside (IPTG)

Makes 5 mL.

Store at -20°C (indefinitely).

1. Add 1.19 g of Isopropyl- β ,D-thiogalactoside (IPTG, m.w. = 238.3) to 5 mL of deionized or distilled water in a clean 15-mL tube.
2. Mix or rock gently to dissolve.
3. Pass the IPTG solution through a 0.45 or 0.22 μm sterile filter (Nalgene or Corning).
4. Dispense the solution into a sterile 15-mL tube (Falcon 2059 or equivalent), and freeze at -20°C .

NGM-lite + Ampicillin + IPTG plates

1. Follow recipe above for NGM-lite plates through Step 6.
2. When the agar flask is cool enough to hold, sterilely add 10 mL of 10 mg/mL ampicillin and 0.4 mL of 1M IPTG. Ampicillin and IPTG are destroyed by heat; therefore, it is essential to cool the agar before adding them.
3. Swirl the flask to mix the ampicillin and IPTG with the agar solution.
4. Resume "NGM-lite Plates" recipe above at Step 8.

M9 Buffer

Makes 1 liter.

Store at room temperature (indefinitely).

1. Add the following ingredients to 500 mL of deionized or distilled water in a 1-liter bottle.
 - 3 g of KH_2PO_4
 - 6 g of Na_2HPO_4
 - 5 g of NaCl
 - 1 mL of 1M MgSO_4
2. Stir to dissolve, preferably using a magnetic stir bar.
3. Add deionized or distilled water to bring total solution to 1 liter.
4. Ensure the bottle cap is loose, and autoclave solution for 15 minutes at 121°C .
5. After autoclaving, cool the solution to room temperature, and tighten the cap for storage.

Hypochlorite Solution

Makes 300 μ L.

Make fresh daily.

Mix in a 1.5 mL tube:

200 μ L of 4 M NaOH

300 μ L of bleach with 10–20% hypochlorite (NaOCl)

Freezing solution

Makes 1 liter.

Store at room temperature (indefinitely).

1. Add the following ingredients to 500 mL of deionized or distilled water in a 2-liter flask:
 - 5.85 g of NaCl
 - 6.8 g of KH_2PO_4
 - 300 g of glycerol
 - 5.6 mL of 1N NaOH
2. Stir to dissolve, preferably using a magnetic stir bar.
3. Add deionized or distilled water to bring total solution to 1 liter.
4. Ensure the bottle cap is loose, and autoclave solution for 15 minutes at 121°C.
5. After autoclaving, cool the solution to room temperature, and tighten the lid for storage.

III. Worm DNA Isolation

1 M Tris (pH 8.3)

Makes 100 mL.

Store at room temperature (indefinitely).

1. Dissolve 12.1 g of Tris Base (m.w. = 121.10) in 70 mL of deionized or distilled water.
2. Adjust the pH by slowly adding concentrated hydrochloric acid (HCl); monitor with a pH meter.

5 M Potassium Chloride (KCl)

Makes 100 mL.

Store at room temperature (indefinitely).

1. Dissolve 37.275 g of KCl (m.w. = 74.55) in 70 mL of deionized or distilled water.
2. Add deionized or distilled water to make a total volume of 100 mL of solution.

1 M Magnesium Chloride (MgCl₂)

Makes 100 mL.

Store at room temperature (indefinitely).

1. Dissolve 20.3 g of MgCl₂ (6-hydrate, m.w. = 154.25) in 80 mL of deionized or distilled water.
2. Add deionized or distilled water to make a total volume of 100 mL of solution.

10× PCR Buffer

Makes 10 mL.

Store at -20°C (indefinitely).

Mix in a 15-mL tube:

1 mL of 1 M Tris (pH 8.3)

1 mL of 5 M KCl

0.15 mL of 1 M MgCl₂

7.85 mL of deionized water

IV. Polymerase Chain Reaction

1% Cresol Red Dye

Makes 50 mL.

Store at room temperature (indefinitely).

Mix in a 50-mL tube:

500 mg of cresol red dye

50 mL of distilled water

Cresol Red Loading Dye

Makes 50 mL.

Store at -20°C (indefinitely).

1. Dissolve 17 g of sucrose in 49 mL of distilled water in a 50-mL tube.
2. Add 1 mL of 1% cresol red dye and mix well.

Primer/Loading Dye Mix

Makes enough for 50 reactions.

Store at -20°C (1 year).

Mix in a 1.5-mL tube:

640 μL of distilled water

460 μL of Cresol Red Loading Dye

20 μL of 15 pmol/ μL 5' primer

20 μL of 15 pmol/ μL 3' primer

Primer Sequences

Lab 5: Examining the RNAi Mechanism

dpy-13 Primers:

5'-AGTCGTCTTCTCCGTTATCG-3' (Forward Primer)

5'-GAGCAACGCATAAGGCAAAG-3' (Reverse Primer)

V. Agarose Gel Electrophoresis

10× Tris/Borate/EDTA (TBE) Electrophoresis Buffer

Makes 1 liter.

Store at room temperature (indefinitely).

1. Add the following dry ingredients to 700 mL of deionized or distilled water in a 2-liter flask:

1 g of NaOH (m.w. 40.00)
108 g of Tris base (m.w. 121.10)
55 g of boric acid (m.w. 61.83)
7.4 g of EDTA (disodium salt, m.w. 372.24)

2. Stir to dissolve, preferably using a magnetic stir bar.
3. Add deionized or distilled water to bring total solution to 1 liter.

NOTE If stored 10× TBE comes out of solution, place the flask in a water bath (37°C to 42°C) and stir occasionally until all solid matter goes back into solution.

1× Tris/Borate/EDTA (TBE) Electrophoresis Buffer

Makes 10 liters.

Store at room temperature (indefinitely).

1. Into a spigoted carboy, add 9 liters deionized or distilled water to 1 liter of 10× TBE electrophoresis buffer.
2. Stir to mix.

1.0 % Agarose

Makes 200 mL.

Use fresh or store solidified agarose at room temperature (several weeks).

1. Add 2 g of agarose (low EEO electrophoresis grade) to 200 mL of 1× TBE in a 600-mL flask or beaker.
2. Stir to suspend agarose.
3. Cover beaker with aluminum foil, and heat in a boiling-water bath (double boiler) or on a hot plate until all agarose is dissolved (~10 minutes).

or

Heat *uncovered* in a microwave oven at high setting (3–5 minutes per beaker) until all agarose is dissolved.

NOTE Agarose will go into solution as the liquid begins to boil. Do not allow the solution to boil for more than a few seconds as this will alter the final concentration of the agarose.

- Swirl solution and check bottom of beaker to make sure that all agarose has dissolved. (Just prior to complete dissolution, particles of agarose appear as translucent grains.) Reheat for several minutes if necessary.

NOTES

- 1-g samples of agarose powder can be preweighed and stored in capped test tubes until ready for use.
- Solidified agarose can be stored at room temperature and then remelted over a boiling-water bath (15–20 minutes) or in a microwave oven (5–7 minutes per beaker) prior to use. Always loosen the cap when remelting the agarose in a bottle.
- Cover with aluminum foil, and hold in a hot-water bath (at ~60°C) until ready for use. Remove any “skin” of solidified agarose from surface prior to pouring.

1 µg/µL Ethidium Bromide Staining Solution

Makes 500 mL.

Store in dark at room temperature (indefinitely).

CAUTION! Ethidium bromide is a mutagen by the Ames microsome assay and a suspected carcinogen. Wear latex gloves when preparing and using ethidium bromide solutions. Review *Responsible Handling of Ethidium Bromide* in Laboratory 5.

- Add 100 µL of 5 mg/mL ethidium bromide to 500 mL of deionized or distilled water.
- Store in unbreakable bottles (preferably opaque). Label bottle CAUTION: Ethidium Bromide. Mutagen and cancer-suspect agent. Wear latex gloves when handling.

NOTE Ethidium bromide is light-sensitive; store in dark container or wrap container in aluminum foil.

pBR322/*Bst*NI Size Markers (0.1 µg/µl)

Makes 100 ml.

Store at –20°C for 1 year.

Either buy pBR322 pre-cut with restriction enzyme *Bst*NI from New England Biolabs (#N3031) or prepare it according to the following protocol:

- Add 1 µl of a solution of 10 µg/µl pBR322 to 75 µl deionized or distilled water.
- Add 10 µl 10× buffer (provided by supplier of the enzyme).
- Add 5 ml *Bst*NI restriction enzyme, and incubate at 60°C for 60 min.
- Electrophorese 5 µl (plus 1 µl loading dye) in a 1-2% agarose gel to check for complete digestion. Exactly 5 bands should be

visible, corresponding to 1,857 bp, 1,058 bp, 929 bp, 383 bp, and 121 bp. Any additional bands indicate incomplete digestion; add additional enzyme and incubate again at 60°C.

100-bp or 1-kb DNA Ladder (0.1 µg/µL)

Makes 100 µl.

Store at –20°C (1 year).

Obtain the stock DNA ladder solution through New England Biolabs (#N3231S). The sold concentration is 0.5 µg/µL and should be kept at –20°C. You can dilute a small amount of the stock at a time following this procedure:

1. Add 55 µL of deionized or distilled water to a 1.5-mL tube.
2. Add 25 µL of cresol red loading dye.
3. Add 20 µL of 100-bp or 1-kb DNA ladder stock.

VI. Ligation

1 M Tris (pH 7.6)

Makes 100 mL.

Store at room temperature (indefinitely).

1. Dissolve 12.1 g of Tris base (m.w. = 121.10) in 70 mL of deionized or distilled water.
2. Adjust the pH by slowly adding ~6.3 mL of concentrated hydrochloric acid (HCl); monitor with a pH meter. (If a pH meter is not available, adding 6.3 mL of concentrated HCl will yield a solution of ~pH 7.6.)
3. Add deionized or distilled water to make a total volume of 100 mL of solution.

10× Ligation Buffer

Makes 1000 μ L.

Store at -20°C (indefinitely).

Mix the following ingredients in a 1.5-mL tube:

600 μ L of 1 M Tris (pH 7.6) (600 mM)
100 μ L of 1 M MgCl_2 (100 mM)
70 μ L of 1 M DTT (70 mM)
230 μ L of deionized water

0.1 M Adenosine Triphosphate (ATP)

Makes 5 mL.

Store at -20°C (1 year).

1. Dissolve 0.3 g of ATP (disodium salt, m.w. = 605.19) in 5 mL of deionized or distilled water.
2. Dispense 500- μ L aliquots into 1.5-mL tubes.

2× Ligation Buffer + ATP

Makes 500 μ L.

Store at -20°C (1 month).

1. Mix 100 μ L of 10× ligation buffer and 10 μ L of 0.1 M ATP in a 1.5-mL tube.
3. Add 390 μ L of deionized or distilled water.

T-tailing L4440 Vector and Ligation

Makes 79 μ L.

Store at 4°C

1. Digest 5-10 μg (approximately 10-20 μL of a midi-prep) of DNA with EcoRV for 2-3hrs or overnight.

Sample digest:

Vector (L4440)	=	10 μL
10 x restriction buffer	=	20 μL
EcoRV enzyme	=	5 μL
Distilled water	=	165 μL

Incubate for 2-3hrs or overnight at 37°C

2a. Ethanol precipitation of DNA:

1. Add to the DNA solution 1/10 the volume of 3M sodium acetate and 3 volumes of ethanol.
2. Place the sample into the -70 °C freezer for 20 minutes
3. Spin in microcentrifuge for 10 minutes at max speed to pellet DNA.
4. Wash with 70% ethanol and spin for 10 minutes
5. Air-dry the DNA and resuspend in distilled water, or TE buffer.

or

- 2b. Utilize a gel purification kit to extract the desired fragment and then resuspend the DNA in 79 μL of distilled water.

3. T-tail the digested vector by preparing the following reaction:

DNA (vector)	=	79 μL
10 x PCR buffer	=	10 μL
25mM Mg Cl ₂	=	8 μL
100mM dTTP	=	2 μL
Taq Polymerase	=	1 μL

Incubate for 2hrs at 72°C

4. Ethanol precipitate the T-tailed vector as in step 2a, and resuspend the DNA in 30 μL of distilled water.

5. Ligate the T-tailed vector to the desired insert by preparing the following reaction:

Vector (L4440)	=	1.5 μ L
Insert	=	15.5 μ L
10 x ligase buffer	=	2 μ L
Ligase	=	1 μ L

Incubate overnight at room temperature.

6. Transform 1ul of ligation reaction into competent DH5 alpha bacterial cells.

Note: Be sure to use Taq Polymerase (non-proofreading) to ensure that an “A” will be placed at the 3’ end of the amplified product (insert). If a proofreading polymerase is used, then an “A” can be added to the ends of the amplified product using the above T-tailing reaction.

VII. Bacterial Transformation

1 M Calcium Chloride (CaCl₂)

Makes 100 mL.

Store at room temperature (indefinitely).

1. Dissolve 11.1 g of anhydrous CaCl₂ (m.w. = 110.99) or 14.7 g of the dihydrate (m.w. = 146.99) in 80 mL of deionized or distilled water.
2. Add deionized or distilled water to make a total volume of 100 mL of solution.

50 mM Calcium Chloride (CaCl₂)

Makes 1000 mL.

Store at 4°C or room temperature (indefinitely).

1. Mix 50 mL of 1 M CaCl₂ with 950 mL of deionized water.
2. Prerinse a 0.45- or 0.22- μ m sterile filter by drawing through 50–100 mL of deionized or distilled water.
3. Pass CaCl₂ solution through prerinsed filter.
4. Dispense aliquots into presterilized 50-mL conical tubes or autoclaved 150–250-mL bottles.

Classic preparation for preparing competent Cells

This entire experiment *must be performed under sterile conditions*.

Day 1 Overnight suspension

1. The day before this protocol, start an overnight culture of DH5 α or other *E.coli* strain.

Day 2 Competent cell preparation

2. Approximately 2-4 hours prior to preparation begin a Mid-log suspension. Add 1mL of overnight culture per 100mL of liquid LB at 37°C. Allow the culture to incubate at 37°C with continuous shaking.
3. *If a spectrophotometer is available:* approximately 1 hour after inoculation, sterily withdraw a 1 mL sample of the culture and measure absorbance (optical density at 550nm). Repeat procedure in 20 minute intervals. The culture should be grown to an OD₅₅₀ of 0.3-0.5. *If a spectrophotometer is not available:* It can safely be assumed that the culture has reached OD₅₅₀

- 0.3-0.5 after 2 hours, 15 minutes of incubation with continuous shaking.
4. Store mid-log culture on ice until ready to begin preparation of competent cells.
 5. Place sterile tube of CaCl_2 solution on ice.
 6. Obtain two 15-mL tubes each with 10 mL of mid-log cells, and label with your name.
 7. Securely close caps and place both tubes of cells in a balanced configuration in the rotor of the clinical centrifuge. Centrifuge at 3000 rpm for 10 minutes to pellet cells on the bottom-side of the culture tube.
 8. Sterilely pour off supernatant from each tube into a waste beaker for later disinfection. Do not disturb the cell pellet.
 - a. Remove cap from the culture tube, and briefly flame mouth. Do not place cap on lab bench.
 - b. Carefully pour off supernatant. Invert culture tube, and tap gently on the surface of a clean paper towel to drain thoroughly.
 - c. Reflame mouth of culture tube, and replace cap.
 9. Use a 5- or 10-mL pipette to sterilely add 5 mL of ice-cold CaCl_2 solution to each culture tube:
 - a. Remove cap from CaCl_2 tube. Do not place cap on lab bench.
 - b. Withdraw 5 mL of CaCl_2 and replace cap.
 - c. Remove cap of the culture tube. Do not place cap on lab bench.
 - d. Expel CaCl_2 into culture tube and replace cap.
 10. Immediately finger vortex to resuspend pelleted cells in each tube.
 - a. Close cap tightly.
 - b. Hold upper part of tube securely with thumb and index finger.
 - c. With the other hand, vigorously hit the bottom end of the tube with index finger or thumb to create a vortex that lifts the cell pellet off the bottom of the tube. Continue "finger vortexing" until all traces of the cell mass are completely

- resuspended. This may take a couple of minutes, depending on technique.
- d. Hold the tube up to the light to check that the suspension is homogeneous. No visible clumps of cells should remain.
11. Return both tubes to ice, and incubate for 20 minutes.
 12. Following incubation, re-spin the cells in a clinical centrifuge for 5 minutes at 2000–4000 rpm. This time the cell pellet will be more spread out on the bottom of the tube due to the CaCl₂ treatment.
 13. Sterilely pour off CaCl₂ from each tube into a waste beaker. Do not disturb the cell pellet.
 - a. Remove the cap from the culture tube. Do not place the cap on the lab bench.
 - b. Carefully pour off supernatant. Invert the culture tube, and tap gently on the surface of a clean paper towel to drain thoroughly.
 - c. Replace cap.
 14. Use a 100–1000- μ L micropipettor (or 1-mL pipette) to sterilely add 1000 μ L (1 mL) of fresh, ice-cold CaCl₂ to each tube.
 - a. Remove cap from CaCl₂ tube. Do not place cap on lab bench.
 - b. Withdraw 1000 μ L (1 mL) of CaCl₂ and replace cap.
 - c. Remove cap of culture tube. Do not place cap on lab bench.
 - d. Expel CaCl₂ into culture tube and replace cap.
 15. Close caps tightly, and immediately finger vortex to resuspend pelleted cells in each tube. Hold the tube up to the light to check that the suspension is homogeneous. No visible clumps of cells should remain.
 - a. Store cells in a beaker of ice in the refrigerator (~0°C) until ready for use in Part B. “Seasoning” at 0°C for up to 24 hours increases competency of cells five- to tenfold.

VIII. Plasmid Miniprep

0.5 M Ethylenediaminetetraacetic Acid (EDTA, pH 8.0)

Makes 100 mL.

Store at room temperature (indefinitely).

1. Add 18.6 g of EDTA (disodium salt, m.w. = 372.24) to 80 mL of deionized or distilled water.
2. Adjust pH by slowly adding ~2.2 g of sodium hydroxide pellets (m.w. = 40.00). (If a pH meter is not available, adding 2.2 g of NaOH pellets will make a solution of ~pH 8.0.)
3. Mix vigorously with a magnetic stirrer or by hand. EDTA will only dissolve when the pH has reached 8.0 or higher.
4. Add deionized or distilled water to make a total volume of 100 mL of solution.

Tris/EDTA (TE) Buffer

Makes 100 mL.

Store at room temperature (indefinitely).

Mix:

1 mL of 1 M Tris (pH 8.0) (10 mM)
200 μ L of 0.5 M EDTA (1 mM)
99 mL of deionized water

Glucose/Tris/EDTA (GTE)

Makes 100 mL.

Store at 4°C or room temperature (indefinitely).

Mix:

0.9 g of glucose (m.w. = 180.16) (50 mM)
2.5 mL of 1 M Tris (pH 8.0) (25 mM)
2 mL of 0.5 M EDTA (10 mM)
94.5 mL of deionized water

5 M Potassium Acetate (KOAc)

Makes 200 mL.

Store at room temperature (indefinitely).

1. Add 98.1 g of potassium acetate (m.w. = 98.14) to 160 mL of deionized water.

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2. Add deionized or distilled water to make a total volume of 200 mL of solution.

Potassium Acetate/Acetic Acid

Makes 100 mL.

Store at 4°C or room temperature (indefinitely).

Add 60 mL of 5 M potassium acetate and 11.5 mL of glacial acetic acid to 28.5 mL of deionized or distilled water.

10% Sodium Dodecyl Sulfate (SDS)

Makes 100 mL.

Store at room temperature (indefinitely).

1. Dissolve 10 g of electrophoresis-grade SDS (m.w. = 288.37) in 80 mL of deionized water.
2. Add deionized or distilled water to make a total volume of 100 mL of solution.

1% SDS/0.2 N NaOH

Makes 10 mL.

Store at room temperature (several days).

Mix 1 mL of 10% SDS and 0.5 mL of 4 N NaOH into 8.5 mL of distilled water.