

Hendry Lab Protocols & Recipes

Contents

Autoclave Procedures	3
Abbreviations	4
Antibiotics & Antifungals	5
Rifampicin stocks (100 mg / mL)	6
3M Sodium Hydroxide (NaOH) stocks	7
Nystatin (anti-fungal) stocks	8
1M Magnesium Chloride (MgCl ₂) stock	9
M9 Minimal Salts 10X stock (for M9 media)	10
10X DNA gel-loading dye, 10mL	11
0.5M EDTA, 1L	12
1x Phosphate buffered saline (PBS), 1L	13
Tris-acetate-EDTA (TAE) Buffer (50X), 1L	14
Tris-borate-EDTA (TBE) Buffer (10X), 1L	15
King's B (KB) agar or media (500 ml)	16
Liquid Broth (LB) media (500mL)	17
Yeast extract, Peptone, & Dextrose (YPD) Media (500mL)	18
Nitrogen Limited Leeds Agar	19
Leeds Acinetobacter Agar Base (500mL)	20
M9 Minimal Media (500mL)	21

Pseudomonas Minimal Media (PMM) (500mL)	22
863 Media (500mL), liquid	23
868 Media (500mL), agar	24
Nitrofurantoin (NFT) stocks (50 mg/mL)	25
Naladoxic Acid (NA) stocks (30 mg/mL)	26
Aphid diet ingredients	27
Planting & Aphids	28
<i>Pseudomonas</i> Epiphytic Growth Assays	31
Glycerol Stocks	33
King's Broth (KB) Media (500 mL)	34
-80C Freezer Strain Retrieval	35
MgCl ₂ (1M) Stock	36
Pseudomonas Overnight Liquid Cultures	36

Autoclave Procedures

To autoclave liquids and waste:

1. Make sure all bottles containing liquid are slightly unscrewed such that steam can escape.
2. Set autoclave to 'Liquid cycle' and change sterilization time to 30 min, exhaust time to 0 min.
 - Liquid cycle
 - 30 min sterilization
 - 0 min exhaust (none)
3. Make sure to reset the jacket steam (red button) after the run is completed (about 1 hour later)

To autoclave solids and glassware:

- Set autoclave to 'solids' or 'gravity' cycle. Sterilization time should be set to 20 min, and exhaust time to 20 min.
 - Gravity/solid cycle
 - 20 min sterilization
 - 20 min exhaust

Abbreviations

- ‘Liquid media’ vs ‘agar plates’ – Media or liquid media is the broth form of a growth medium. To make liquid media, follow the normal recipe but make sure to NOT include agar. Agar media (ex. ‘KB agar’) refers to when agar is added to a liquid media to make petri dishes of media. Typically, we add about 7.5g of agar to a 500mL media. But this is not always the case, so make sure to check the recipe.
- KB media – King’s B Media. Used to cultivate pseudomonads. Iron limiting media which results in fluorescent siderophore expression.
- YPD media – Yeast, Peptone, and Dextrose media. A broad media used to cultivate yeasts and fungi. In our lab, antibiotics are added to select for *Yarrowia*-like yeast isolates.

KB	King’s B media	Used to grow pseudomonads. Iron limited.
LB	Liquid broth media	Broad media for environmental bacteria
TSB	Tryptic soy broth	Broad media
TSA	Tryptic soy agar	Broad media
YPD	Yeast, Peptone, and Dextrose media	Used to grow yeasts/fungi
BHI	Brain Heart Infusion media	Broad media, used to grow host associated bacteria
863		Special medium for <i>Serratia symbiotica</i>
Leeds	Leeds <i>Acinetobacter</i> media	Agar media with a pH indicator. Used to identify <i>Acinetobacter</i> .
M9 Minimal media	M9 Minimal media	Media with minimal nutrients used to test growth on various carbon sources. A carbon source must be added for growth.
PMM	<i>Pseudomonas</i> Minimal media	A nutrient limited minimal media specific for pseudomonads

Antibiotics & Antifungals

Abbreviation	Name	Stock concentration	Typical media concentration
Rif	Rifampicin	100 mg/mL	50 µg/mL
Tet	Tetracycline	50 mg/mL	10 µg/mL
Gent	Gentamycin	50 mg/mL	25 µg/mL
Kan	Kanamycin	50 mg/mL	50 µg/mL
Strep	Streptomycin	100 mg/mL	
Chlor	Chloramphenicol	50 mg/mL	30 µg/mL
Amp	Ampicillin	50 mg/mL	
Nal	Nalidixic Acid	30 mg/mL	15 µg/mL
NFT	Nitrofuratoin	50 mg/mL	50 µg/mL
Nyst	Nystatin (antifungal)	35 mg/mL	35 µg/mL

Rifampicin stocks (100 mg / mL)

- Dissolve 1 g in 10 mL of DMSO in hood.
- Vortex (and heat gently if needed) to dissolve
- Filter through 0.2 μm and aliquot.
- Store in -20 freezer.
- Add 500 μl / L for final concentration of 50 μg / mL

3M Sodium Hydroxide (NaOH) stocks

- Make 200 mL
- For 3M:
 - $1\text{M} = 40\text{ g in } 1\text{ L}$ $3 = 120\text{ g} / 5 = 24\text{g}$
- Dissolve slowly in DI water in fume hood.
- Store at room temp.

Nystatin (anti-fungal) stocks

- Dissolve 350 mg in 10 mL of 70% EtOH.
- Add 1 mL / L to cool medium
- => 35 µg / mL
- Store in -20 freezer

1M Magnesium Chloride (MgCl₂) stock

- Molecular weight = 95.21
- Make 200 mL
- $0.2 \times 95.21 = 19.04$ g
- Dissolve in DI water in the fume hood.
- NB: it does warm up a bit.
- Autoclave on fluid run for 30 mins.
- Aliquot into 1 mL tubes for daily use.

M9 Minimal Salts 10X stock (for M9 media)

- DI H₂O 500 mL
- Na₂HPO₄ · 7H₂O 35 g
- KH₂PO₄ 15 g
- NaCl 2.5 g
- NH₄Cl 5 g

10X DNA gel-loading dye, 10mL

- Glycerol 3.9 mL
- 10% (w/v) SDS 500 μ L
- 0.5 M EDTA 200 μ L
- Bromophenol blue 0.025 g
 - (Borrowed from Peter's lab)
- Xylene cyanol 0.025 g
 - (Can be skipped)
- Bring to 10 mL total volume with DI H₂O
- Filter sterilize with syringe and 15mL falcon tube

0.5M EDTA, 1L

- MilliQ H₂O 800 mL
- EDTA disodium salt 186.1 g
- NaOH tablets up to 2 g, added SLOWLY.

Add the EDTA to approximately 800mL of Milli-Q water and stir vigorously on magnetic stirrer. Adjust volume to 1L with Milli-Q water. Slowly add NaOH tablets (a few at a time) to adjust the pH to 8.0. The EDTA will not dissolve until the pH reaches 8.0.

1x Phosphate buffered saline (PBS), 1L

- DI H₂O
- NaCl 8 g
- KCl 0.2 g
- Na₂HPO₄ 1.44 g
- K₂HPO₄ 0.24 g

pH to 7.4 using HCL

Add the ingredients to approximately 900 mL of distilled water and stir vigorously on a magnetic stirrer to dissolve. Adjust volume to 1L using distilled water. Adjust pH to 7.4 using HCL. Autoclave for 20min.

Tris-acetate-EDTA (TAE) Buffer (50X), 1L

- Tris base 242.0 g
- Glacial acetic acid (Open in hood!) 57.1 g
- EDTA disodium salt 18.6 g
- DI H₂O

Add the tris, EDTA, and glacial acetic acid to approximately 700mL of distilled water and stir until the contents are dissolved. Adjust the volume to 1L using distilled water.

To make 1L of 1X TAE, add 20 mL 50X TAE to 980 mL distilled H₂O

Tris-borate-EDTA (TBE) Buffer (10X), 1L

- Tris base 108 g
- Boric acid 55 g
- EDTA disodium salt 7.5 g
- DI H₂O

Add the tris, EDTA, and boric acid to approximately 800mL of distilled water and stir until contents are dissolved. Adjust volume to 1L using distilled water.

To make 1L of 1X TBE, add 100mL of 10X TBE to 900mL DI H₂O

King's B (KB) agar or media (500 ml)

- Distilled H₂O 500mL
- Peptone 10 g
- K₂HPO₄ 0.75 g
- MgSO₄ • 7H₂O 0.75 g
- Glycerol 5.0 ml
- Agar (if needed) 7.5 g

Measure 500 ml DI water into a 1 L glass media bottle. Add ingredients in order above (use syringe for glycerol) and swirl to mix. Autoclave on liquid run for 30 mins. Place in waterbath at 56°C until cool enough to pour.

If adding rifampicin or other antibiotics/antifungals, wait until bottle is temperature of a warm cup of tea before adding. For a final concentration of 25µg/mL rifampicin in 500mL of media, add 250µL of the 100mg/mL rif stock and swirl to stir.

Optional additions:

- Rifampicin (25ug/ml) add 250µL stock to 500mL media.
- Nystatin (35 µg/mL) add 500µL stock to 500mL media.

Liquid Broth (LB) media (500mL)

- DI H₂O 500 mL
- Tryptone 5.0 g
- Yeast Extract 2.5 g
- NaCl 5.0 g
- Agar (if needed) 6.5 g

Adjust pH to 7.4

autoclave on liquid cycle for 20min

Yeast extract, Peptone, & Dextrose (YPD) Media (500mL)

For the cultivation of *Yarrowia* and other fungi.

- DI H₂O 500 mL
- Peptone 10 g
- Yeast extract 5 g
- Dextrose 10 g
- Agar 7.5 g

Antibiotics (for selection of *Yarrowia* isolates)

To get: Add:

15ug/mL tetracycline 250uL of stock

15ug/mL nalidixic acid 150uL of stock

25ug/mL chloramphenicol 250uL of stock

Nitrogen Limited Leeds Agar

(Katie's frankenmedia for Yarrowia)

(500mL)

DI H ₂ O	500 mL
Casein acid hydrolysate	7.5 g
Peptone	2.5 g
Sodium Chloride	2.5 g
L-phenylalanine	0.50 g
Ferric ammonium citrate	0.20 g
Phenol red	0.01 g
Glycerol	6.65 mL
Agar	6.0 g
pH to 7.0 +/- 0.2	

Leeds Acinetobacter Agar Base (500mL)

DI H2O	500 mL
Casein acid hydrolysate	7.5 g
Soya peptone	2.5 g
Sodium Chloride	2.5 g
Fructose	2.5 g
Sucrose	2.5 g
Mannitol	2.5 g
L-phenylalanine	0.50 g
Ferric ammonium citrate	0.20 g
Phenol red	0.01 g
Agar	6.0 g
pH to 7.0 +/- 0.2	

M9 Minimal Media (500mL)

- DI H₂O 450 mL
- M9 Salts (1x) 50 mL
- Agar 15 g
Autoclave, then add:
- 1M MgSO₄ 1.0 mL
- 1M CaCl₂ 0.1 mL (100uL)
- Carbon source
Possible carbon sources:
10% glucose 5mL
20% sucrose 10mL
20% fructose 10mL

Pseudomonas Minimal Media (PMM) (500mL)

- DI H₂O 492.5 mL
- Glycerol 7.5 mL (leave out if testing other carbon source)
- L-Glutamine 2.5 g
- K₂HPO₄ 0.75 g
- MgSO₄ 0.1 g
- Agar 7.5 g

863 Media (500mL), liquid

For the cultivation of *serratia symbiotica*

- DI H₂O 500mL
- Glucose 5g
- Yeast Extract 5g
- Casein peptone (or tryptone) 5g

868 Media (500mL), agar

(same as 863 media, but with 1.7% agar)

- DI H₂O 500mL
- Glucose 5g
- Yeast Extract 5g
- Casein peptone (or tryptone) 5g
- Agar 8.5 g

Nitrofurantoin (NFT) stocks (50 mg/mL)

1. In the hood, make solution of:
 - 1500 mg Nitrofurantoin
 - 30 mL ddH₂O (sterile)
2. Aliquot into 2 mL tubes.
3. Store at 4C.

Naladoxic Acid (NA) stocks (30 mg/mL)

1. In the hood, using a 50 mL conical, make a solution of:
 - 900 mg Naladoxic Acid
 - 30 mL ddH₂O (sterile)
2. Aliquot into 2 mL tubes.
3. Store at 4C.

Aphid diet ingredients

NAME	Douglas (g/L)
Alanine	0.4455
Arginine	2.178
Asparagine, H ₂ O	1.652
Aspartic Acid	1.664
Cysteine	0.3029
Glutamic Acid	1.103
Glutamine	2.192
Glycine	0.0751
Histidine, HCl, H ₂ O	1.5722
Isoleucine	0.9839
Leucine	0.9839
Lysine mono HCl (182.65g/mol)	1.3699
Methionine	0.373
Phenylalanine	0.413
Proline	0.5757
Serine	0.5255
Threonine	0.8934
Tryptophan	0.5106
Tyrosine	0.0906
Valine	0.8786
Sucrose	171.15
p-aminobenzoic acid	0.1
L-ascorbic acid	1
Biotin	0.001
D-calcium pantothenate	0.05
Choline chloride	0.5
Folic acid	0.01
i-Inositol	0.42
Nicotinamide (amide of niacin)	0.1
Pyridoxin HCl	0.025
Thiamine di-HCl	0.025
CuSO ₄ 5H ₂ O (0.1M)	0.00254
FeCl ₃ 6 H ₂ O (0.1M)	0.01336
MnCl ₂ 4H ₂ O (0.1M)	0.00504
NaCl	0.01271
ZnCl ₂ (0.1M)	0.00417
Calcium citrate	0.1
Cholesteryl benzoate	0.025
MgSO ₄ , 7H ₂ O	2.42
KH ₂ PO ₄	2.5

THEN ADJUST pH TO 7.5 WITH ~14 ML NaOH 3M

Planting & Aphids

Updated: 2023 November 13

Procedure

1. Evaluate plants

- Dryness
- Deadness
- Aphid contamination
 - If you find an aphid: text Tory or Katie.
 - Isolate the plant, ideally in an aphid cage.
 - Check if there is a backup of that plant.
 - If so, plant may have to be moved to the aphid room.
- *Note: never go into the plant room after being in the aphid room on the same day.*

2. Pots

- Clear the sink.
- Empty soil from the old pots (after freezing) into the trash bag.
 - *Note: do not leave defrosting trash bags out for >3 days.*
- Place empty old pots in sink and scrub/rinse to remove dirt.
- Add 1:10 bleach to water (by eye) into a tub in the sink and soak scrubbed/rinsed pots for ~12 hours.
- Rinse off the bleach from the pots and set on bench to dry.
 - *Note: be careful not to get bleach on clothes or hands.*

3. Planting

- Use dry pots with two trays underneath and up to 6 pots/tray.
 - Use smaller pots for plants like fava bean/pea and bigger pots for plants like barley/cucumber.
- Loosen a bag of soil and fill pots up to inner rim.
- Seeds are located in the fridge on the second shelf.
 - Use Johnny's seeds.
- Plant seeds according to the post-it note on the countertop shelf.
 - *Note: calculate any fava plants 2 weeks before experiments*
 - *Note: always make sure there are at least 12 fava bean plants for the aphids.*

Plant	Amount
Cucumbers	3 seeds/pot
Beans	4 beans/pot
Turnips	1 pinch/pot
Barley	1 pinch/pot
Dock	1 pinch/pot

4. Watering

- The hose is in the adjacent room.
- Turn on the tap in the adjacent room and the switch for the water in located on the hose nozzle.
 - 3 secs/plant on high setting.

5. Sacrificing Plants to the Aphids

- Take out $\frac{3}{4}$ of the plants in a cage and put them in a trash bag.
 - Use a different trash bag for each aphid species.
- Replace those 3 old plants with fresh plants.
- When watering plants, wash can between aphid cages.

Tory's tips for minimally maintaining all non pea aphids at once

Monday

- Plants
 - 6 pea
 - 1 dock
 - 2 turnip/cuc
- Aphids
 - pea aphids (replace 3 plants in each tent, 6 total)
 - goldenrod (just water)
 - dock (water, replace 1 plant)
 - barley (just water)
 - turnips (water plants, OR replace most plants, depending on which are sadder, turnip or cucumbers)
 - cucumber (water plants, OR replace most plants, depending on which are sadder, turnip or cucumbers)

Thursday

- Plants
 - 6 pea
 - 2 barley
 - 2 cuc/turnip
- Aphids
 - pea aphids (replace 3 plants in each tent, 6 total)
 - goldenrod (water, replace 1 plant on weeks with)
 - dock (just water)
 - barley (replace most plants)
 - turnips (water plants, OR replace most plants, depending on which are sadder, turnip or cucumbers)
 - cucumber (water plants, OR replace most plants, depending on which are sadder, turnip or cucumbers)

Notes

- Water goldenrod twice per week and change some plants once a month
- Aphids on barley, dock, cucumber and turnip will slowly build up density even if most of the plants are changed each week, so that every third week or so we get an explosion of winged adults on the tops of the cages.
 - When this happens, remove ALL plants and replace, let density build up again
- Follow this order for going into cages:
 1. pea

2. goldenrod
3. dock
4. barley
5. cucumber or turnip (cage that is being watered only)
6. cucumber or turnip (cage with plant change last)
 - Do *not* change plants in both turnip and cucumber on the same day.
 - * Water the cage with the better looking plant and then change plants in the worse looking cage
 - After going into a cage, wash and wipe down your hands and arms, rinse aphids of watering can, before going into a new cage

Pseudomonas Epiphytic Growth Assays

Date Updated: 2023 November 24

Day -3: Plating

1. From -80°C/current plate, plate *Pseudomonas* strains/individual colonies on KB + NA (15 ug/mL) + NFT (50 ug/mL) plates.
2. Incubate for 24-72 hours at 28°C.

Day 0: Overnights of *Pseudomonas*

1. Pipette 10 mL of KB + NA (15 ug/mL) + NFT (50 ug/mL) media in 15 mL culture tube.
 - *Note: Make 1 tube/pot and don't forget a blank!*
2. Transfer a colony of *Pseudomonas* from plate to media.
3. Incubate in benchtop shaker at 28°C overnight (~18 hrs).

Day 1: Spraying

1. Transfer overnight *Pseudomonas* culture from culture tube to falcon tube.
2. Pellet cells by centrifuging for 10 min on max speed
3. Discard supernatant.
4. Add 1 mL 10mM MgCl₂ buffer and resuspend/vortex.
5. Pellet and discard supernatant again.
6. Add ~25 mL 10mM MgCl₂ buffer and resuspend/vortex.
7. Transfer 100 ul of sample to a blank cuvette.
8. Measure OD of bacterial resuspension (include blank!).
 - Goal: OD = 0.2
9. Transfer 20 mL of bacterial resuspension into autoclaved spray bottle.
 - Clean tops using ethanol and sterile water.
10. Retrieve 14 day old plants from plant room and place in biosafety cabinet.
11. Spray ~20 mL bacterial solution onto all the leaves and all plant surfaces (stem, top, bottom) of pot until runoff (starting to drip).
12. Allow plant to dry in the biosafety cabinet.
13. Move plant to tent downstairs and note time.
14. Incubate for 72 hours in a tent at 70°F and 85% humidity.

Day 4: Sampling

1. Retrieve plants from plant room and place in biosafety cabinet.
2. Sterilize cork borer and forceps using ethanol flame and place in a sterile petri dish in biosafety cabinet.
3. Take 10 samples from all over each plant in the pot.
 - Place all samples from a plant in a single falcon tube, 10 discs/tube.
 - Will end with 3 falcon tubes and 30 samples if sampling 3 plants (1 pot).
 - Add 10 mL of 10mM MgCl₂ buffer to falcon tubes.
4. Sonicate falcon tubes for 10 min.
5. Vortex to dislodge epiphytic bacteria.
6. Make serial dilutions (2 technical replicate/sample) in 96 well plate using multichannel pipette.

- 450 uL buffer and 50 uL sample/dilution.
7. Plate serial dilutions on KB + NA (15 ug/mL) + NFT (50 ug/mL) plates.
 - Each sample should be plates undiluted, 10^{-1} , and 10^{-2}
 - 100 ul per plate
 8. Allow plates to dry then flip upside and incubate at 28C for 48 hrs.

Day 5: Counting

1. Photograph plates.
2. Count colonies on each plate and note in spreadsheet.
3. Calculate CFU/10 mL bacterial resuspension (ie. per sample).

Glycerol Stocks

Updated: 2022 November 4 by Vivi Sanchez

1. Add 1mL of LB to sterile glass culture tube.
2. Select one colony of interest from your nutrient plate and inoculate culture media.
3. Grow culture overnight at 28C in shaker.
4. The next day, label 2 cryotubes with isolate information on the side of the tube (i.e Isolate ID, isolate name, date collected, Collector) and label the top of the tube with the Isolate ID.
5. Check the -80C master sheet on Lab archives to determine Isolate ID.
6. Dispense 900 uL of sterile 20% glycerol in each cryotube.
7. Dispense 100 uL of overnight culture to its designated cryotube.
8. Invert to mix and let sit for at least five minutes.
9. Store at -80C.

King's Broth (KB) Media (500 mL)

Ingredients

ddH ₂ O	500mL
Peptone	10 g
K ₂ HPO ₄	0.75 g
MgSO ₄ • 7H ₂ O	0.75 g
Glycerol	5.0 ml
Agar (if needed)	7.5 g

Procedure

1. Measure 500 ml DI water into a 1 L glass media bottle.
2. Add ingredients in order above (use syringe for glycerol) and swirl to mix.
3. Autoclave on liquid run for 30 mins.
4. Place in waterbath at 56°C until cool enough to pour.
5. If adding antibiotics/antifungals, wait until bottle is temperature of a warm cup of tea before adding.

-80C Freezer Strain Retrieval

Updated: 2022 November 4 by Vivi Sanchez

1. Grab -80C freezer key from its designated drawer in room 257.
 - *Please remember to return the key to this spot when you are done using it.*
2. Fill an ice bucket with ice and retrieve stocks of interest.
 - *Glycerol stocks will thaw quickly, so please remove a few stocks at a time to prevent unnecessary thawing.*
3. Plate stocks on LB and incubate overnight at 28C.
4. Return stocks to -80C freezer and return key to its drawer.
5. Check pockets to make sure you did not accidentally take the key. :)

MgCl₂ (1M) Stock

Ingredients

MgCl ₂ anhydrous (Molecular weight = 95.21)	19.04 g
dH ₂ O	200 mL

Procedure

1. In the fume hood, dissolve MgCl₂ in dH₂O
 - *Note: this is an exothermic reaction and it will heat up.*
2. Autoclave on fluid for 30 mins.

Pseudomonas Overnight Liquid Cultures

Note: the inoculation should be performed in the hood.

1. Pipette 10 mL of KB + NA (15 ug/mL) + NFT (50 ug/mL) media in 15 mL culture tube.
2. Transfer a colony of *Pseudomonas* from plate to media using either a sterile flamed loop or sterile pipette tip.
3. Incubate in benchtop shaker at 28C overnight (~18 hrs).