**Instructions for making stocks**

**Updated February 2012**

**Media**

*General notes:*

* The three types of media we keep stocks of are LB, TB, and 2X YT.
* Always use the black-topped bottles for making media.
* Make 1 L and then split into 4 bottles of 100 mL and 3 bottles of 200 mL.
* Autoclave media (with tops loosened) as soon as possible, or things will grow in them.

*Instructions:*

1. Fill a 1 L beaker with ~800 mL of dI water (from the white hose).
2. Place weigh boat on scale, tare, and use a spoon to measure out the appropriate amount of powder.
	1. LB: 25 g
	2. TB: 50.8 g
	3. 2X YT: 31 g
	4. Wear gloves. *Wipe down scale and surrounding bench with a damp paper towel when done.*
3. Carefully pour the powder into the beaker, while stirring.
4. Squirt a small amount of water from a squeeze bottle to wash powder off the sides.
5. Add water until the level reaches 1 L.
6. Let stir until all dissolved.
7. Label **black-topped** bottles with type of media and date, and place autoclave tape on bottle top.
8. Pour into bottles (using a funnel) in appropriate amounts (can judge by marks on bottles).
9. Screw tops on loosely and autoclave on **liquid** setting (P4) (place paper towels in the bottom of the autoclave tray).
10. Remove from autoclave and let cool. Tighten lids until barely tight (not super tight!) and place on shelf.

**LB Agar**

*General notes:*

* Make 250 mL mixtures individually.
* Use 500 mL black-topped bottles.
* Autoclave as soon as possible or things will grow in them.

*Instructions:*

1. Label desired number of bottles with “LB Agar” and date, and place autoclave tape on bottle top.
2. Into each bottle add the following:
	1. 6.25 g LB powder
	2. 3.75 g bacto agar
	3. 250 mL dI water (from white hose)
3. Swirl gently to dissolve LB and distribute agar.
4. Screw tops on loosely and autoclave on **liquid** setting (P4) (place paper towels in the bottom of the autoclave tray).
5. Remove from autoclave and let cool. Swirl gently to distribute agar before mixtures solidify. Tighten lids until barely tight (not super tight!) and place on shelf. If a lid has cracks, wrap the neck in parafilm.

**Sterile Water**

*Instructions:*

1. Label orange-topped bottles with “sterile water” and date. Place autoclave tape on bottle tops.
2. Rinse bottles a few times with distilled water from Hydro hose before filling them up (don’t fill all the way).
3. Screw tops on loosely and autoclave on liquid setting (P4).
4. Remove from autoclave and cool. Tighten lids until barely tight and place on shelf.

**80% Glycerol**

*General notes:*

* Make 50 mL in little black-topped bottles.
* Glycerol is very viscous so pipet slowly and wait for residue to drip out.

*Instructions:*

1. Label bottles with “80% glycerol” and date. Place autoclave tape on top.
2. Pipet 10 mL dI water into each bottle.
3. Pipet 40 mL of glycerol into each bottle. Pipet glycerol slowly up out of the stock bottle, waiting for liquid level to stop moving. After emptying into little bottle, remove the pipet and let it sit in the bottle while residue drips out.
4. MIX WELL.
5. Screw tops on loosely and autoclave on liquid setting (P4).
6. Remove from autoclave and let cool. Tighten lids until barely tight and place on shelf.

**5X SDS Running Buffer**

*General notes:*

* We reuse the same bottle for this buffer which has the amounts written on it.
* When running a lot of gels it’s good to have a second bottle of stock ready to go.
* Sometimes the empty bottle is crusty so you can rinse off the outside, inside, and bottle cap before pouring in the new solution.
* Be careful when weighing and pouring SDS powder, as it is an irritant.

*Instructions:*

1. Fill a 1 L beaker with ~800 mL of dI water (from the white hose).
2. Separately weigh out each component and add to the water while stirring:
	1. 15.1 g Tris base
	2. 94 g Glycine (electrophoresis grade)
	3. 5 g SDS (try not to inhale the SDS powder as it will make you cough)
	4. Wear gloves. Wipe down scale and surrounding bench with a damp paper towel when done.
3. Squirt a small amount of water from a squeeze bottle to wash powder off the sides.
4. Let stir until all dissolved (SDS will take a little while).
5. Pour solution into a 1 L graduated cylinder and bring volume to 1 L.
6. Carefully pour into designated bottle. Mix well and place on shelf.

**2X Coomassie Stain**

*General notes:*

* Make 400 mL of 2X stain
* Filtering will take the whole day, or it can be left to go overnight.

*Instructions:*

1. Place two tablets of PhastGel Blue R (stored on door of fridge) in a 500 mL beaker along with a stir bar.
2. Measure 160 mL of dI water in graduated cylinder and pour in.
3. Measure 240 mL of Methanol (stored in flammables cabinet) in graduated cylinder and pour in.
4. Stir well for a while.
5. Fold and place large Whatman filter in glass funnel and place in “2X coomassie” storage bottle. Pour in stain solution and allow it to drip through the filter for several hours, adding more of the solution as room becomes available.
6. Continue stirring remaining solution on stir plate while filtering.
7. Filter all of stain solution (may take overnight). Cover funnel with saran wrap while waiting.
8. When finished, place bottle of stain on shelf above sink.
9. Throw out filter paper and rinse funnel, beaker, and stir bar well. Use methanol to remove coomassie stain.

**20% Acetic Acid**

*General notes:*

* Make 400 mL for mixing with 2X coomassie stain.
* Use caution when handling glacial acetic acid! Wear gloves, labcoat, and goggles, and pour in hood.

*Instructions:*

1. Measure 320 mL of dI water with a graduated cylinder and pour into “20% acetic acid” bottle on shelf.
2. Glacial acetic acid is stored in the acids cabinet below the fume hood. Pour a small amount into a little beaker, in the hood. Measure 80 mL in a graduated cylinder from this little beaker. Pour into “20% acetic acid” bottle.
3. Mix well inside of bottle. Place bottle on shelf.
4. Pour any extra acetic acid down the drain with copious water. Rinse beaker and cylinder well with water. Avoid breathing fumes.

**Destain**

*General notes:*

* Destain is stored in a large carboy on the edge of the sink with the amounts needed for 10 L written on it.
* Use caution when handling glacial acetic acid! Wear gloves, labcoat, and goggles.
* Large volumes of methanol and acetic acid are required so make sure we have enough of these on hand before starting.

*Instructions:*

1. Measure 6 L of dI water (fill 2 L graduated cylinder 3 times) and pour into carboy.
2. Measure 3 L of Methanol (can use same 2 L graduated cylinder) and pour into carboy.
3. Measure 1 L of glacial acetic acid. Pour into 2 L graduated cylinder while it is down in the sink. Wear gloves, labcoat and goggles and pour slowly. Try not to breathe fumes. Pour into carboy CAREFULLY.
4. Screw top on and give carboy a few shakes to mix contents. Make sure to leave top on but unscrewed or liquid won’t come out bottom.

**50X TAE Buffer (for agarose gels)**

*General notes:*

* This stock is made at 50X so it does not need to be made very often.
* 500 mM EDTA should be made beforehand and the pH adjusted to 8.0 with NaOH (this is a slow and painful process).

*Instructions:*

1. Fill a 1 L beaker with ~600 mL of dI water (from the white hose).
2. Add the following to the water while stirring:
	1. 242 g Tris base
	2. 57.1 mL glacial acetic acid
	3. 100 mL 500 mM EDTA (pH 8.0)
3. Pour the solution into a 1 L graduated cylinder and bring volume to 1 L.
4. Carefully pour into designated bottle. Mix well and place on shelf.

**Antibiotics**

*General notes:*

* Ampicillin is stored on the door of the fridge. Kanamycin and Chloramphenicol are stored with other chemicals at room temp.
* Make 10 mL and aliquot out 510 µL per tube (~20 tubes).

*Instructions:*

1. We make stocks of these antibiotics at the following concentrations:
	1. Ampicillin: 100 mg/mL in water
	2. Kanamycin: 50 mg/mL in water
	3. Chloramphenicol: 34 mg/mL in ethanol
2. Weigh out appropriate amount of antibiotic powder and carefully pour into 15 mL conical tube.
3. Add dI water (or ethanol for chloramphenicol) to a volume of 10 mL.
4. Vortex until dissolved.
5. For Amp and Kan, filter with a 0.22 µM yellow filter and disposable 10 mL syringe into a fresh conical (not necessary for Chloramphenicol).
6. Aliquot 510 µL into labeled eppendorf tubes and place in “Antibiotics” box in -20 C freezer.

**IPTG**

*General notes:*

* IPTG is stored on door of freezer (full name is Isopropyl--D-thiogalactopyranoside)
* Make 10 mL of 1M solution (MW=238.31).

*Instructions:*

1. Weigh out 2.38 g of IPTG and carefully pour into 15 mL conical tube.
2. Add dI water to a volume of 10 mL.
3. Vortex until dissolved.
4. Filter with 0.22 µM yellow filter and disposable 10 mL syringe into a fresh conical.
5. Aliquot 510 µL into labeled eppendorf tubes and place in “IPTG” box in -20 C freezer.

**X-gal**

*General notes:*

* X-gal is stored on door of freezer (full name is 5-Bromo-4-chloro-3-indolyl--D-galactoside)
* Make 5 mL of 20 mg/mL solution in dimethylformamide (DMF).

*Instructions:*

1. Weigh out 0.1 g of X-gal and carefully pour into 15 mL conical tube.
2. Add DMF to a volume of 5 mL.
3. Vortex until dissolved (not necessary to filter).
4. Aliquot 500 µL into labeled eppendorf tubes and place in box in -20 C freezer.