Common Solutions

All solutions must be consistent. Reagents must be measured accurately and added to the solution at the appropriate time. These protocols should be followed closely to prevent damaging the pH meter and other laboratory equipment.

Solutions are generally made using the following protocol:

1) Add distilled H_2O to account for about ¾ of the final volume to a flask containing a magnetic stir bar. Never add H_2O to the final volume before dissolving reagents.
2) Add reagents to the flask and allow them to completely dissolve.
3) Measure the pH using the Corning pH Meter 140; adjust the pH of the solution by adding HCl or NaOH solution.
4) Transfer the solution from the Erlenmeyer flask to a graduated cylinder, and add distilled H_2O to the desired final volume.
5) (VARIES) Add any reagents that either damage the pH probe (e.g. CTAB) or do not dissolve before autoclaving (e.g. agar).
6) Transfer the solution to a vessel (usually a screw cap Pyrex bottle), and autoclave on the liquids cycle (see autoclave protocol).

Important guidelines:

1. **Make solutions safely**. Always wear gloves when preparing solutions. If a solution produces hazardous vapors, then measure it inside of the fume hood. Read safety warnings on all containers.
2. **Reagents are arranged in alphabetical order on the chemical shelves**. Put all reagents away when finished. The bench holding the balance and pH meter also serves as the undergraduate workspace. Don't clutter the already limited space with containers.
3. **Before adding, check and recheck reagent amounts**. Make sure to have adequate amounts of each reagent before starting a solution.
4. **Make sure enough empty bottles are available before starting to make a solution**. Solutions must be stored in the proper containers to prevent evaporation and contamination. Check containers and lids before transferring a solution.
5. **When mixing reagents, use a flask has at least twice the volume of the solution**. Using a flask that is too small may cause solution to spray out of the flask during mixing.
6. **Avoid making large batches of seldom-used solutions**. Ask other members of the lab if they need any of the solution made as well as the volume. Solutions like 10x TBE and 0.5 M Na-EDTA are used often and should be kept in good supply. Recipes can be scaled up or down proportionally with the volume of H_2O.

7. **Clean up any spills**. Spilled chemicals present a hazard to everyone in lab. Even if you spill a substance as harmless as NaCl, clean the area thoroughly. The lab bench should be clean of powders and residues.
8. **Put concentrated acids back into the acid storage when finished**. Strong bases can be stored in standard chemical storage.
9. **Mark bottles clearly**. Write the following neatly on a piece of pressure sensitive tape: name of solution and concentration, pH, date prepared, preparer's initials. Place the tape on the side of the bottle.
10. **Peel off old autoclave tape before placing a fresh piece**. All items that need to be autoclaved are marked with autoclave tape. The tape stripes darken when exposed to an autoclave cycle. Darkened tape should be removed from items going into the autoclave to prevent confusion. Also, tape hardens and becomes very sticky after repeated cycles.
11. **When finished, clean all glassware and measuring equipment**. Spatulas should be removed from near the balance. Do not leave graduated cylinders in the sink; they can tip easily and break.

10X TBE, pH 8.0 – 1 L

Component	Amount	Concentration in solution
Tris base	108 g	0.89 M
Boric Acid	55 g	0.9 M
0.5 M Na-EDTA	40 mL	20 mM

Add 700 mL of distilled H_2O to a 2000 mL Erlenmeyer flask containing a magnetic stir bar. Place the flask on a stir plate, and set the bar stirring at a high setting. Add the above quantities of reagents to the flask. When the reagents are fully dissolved, place a calibrated pH meter into solution, and adjust the pH to 8.0. Remove the magnetic stir bar, pour the solution into a 1 L graduated cylinder, and add water to 1 L. Return the solution to the flask, wrap a square piece of aluminum foil over the mouth of the flask, and place a small piece of autoclave tape on the foil.
10X TBE is diluted to 1X for running gels. Add 500 mL 10X TBE to the carboy labeled "1X TBE". Then add 4500 mL of distilled H_2O to the carboy. Seal the top and spigot, pick up the carboy and shake it vigorously to mix.

0.5 M Na-EDTA, pH 8.0 – 1 L

Component	Amount	Concentration in solution
$Na_2EDTA \cdot 2H_2O$	186.1 g	0.5 M

Add 700 mL of distilled H_2O to a 2000 mL Erlenmeyer flask containing a magnetic stir bar. Place the flask on a stir plate, and set the bar stirring at a high setting. Add $Na_2EDTA \cdot 2H_2O$ to the flask. The solid will not go into solution until the pH of the solution has been raised. Add pellets of dry NaOH to the flask slowly, allowing each to dissolve. When the Na-EDTA dissolves, place a calibrated pH probe into solution and adjust the pH of the solution to 8.0. Bring the volume to 1 L in a graduated cylinder, and make two 500 mL aliquots in 1 L screw-top bottles.

1.0 M Tris-HCl, pH 8.0 – 200 mL

Component	Amount	Concentration in solution
Tris-HCl	31.5 g	1.0 M

Add 170 mL of distilled H_2O to a 500 mL Erlenmeyer flask containing a magnetic stir bar. Place the flask on a stir plate, and set the bar stirring at a medium setting. Add Tris-HCl to the flask. When the reagents are fully dissolved, place a calibrated pH meter into solution, and adjust the pH to 8.0. Bring the volume to 200 mL in a graduated cylinder, and transfer to a 500 mL screw-top bottle.

2% CTAB buffer, pH 8.0 – 200 mL

Component	Amount	Concentration in solution
1 M Tris-HCl, pH 8	20 mL	0.1 M
0.5 M Na-EDTA	8 mL	20 mM
NaCl	16.4 g	1.4 M
CTAB*	4.0 g	2% (w/v)

*Hexadecyltrimethylammonium bromide (Sigma: H5882), add CTAB *after* bringing pH to 8.0

Dissolve the ingredients (except CTAB) in 150 mL of distilled H_2O in a 250 mL Erlenmeyer flask containing a magnetic stir bar. When the reagents are fully dissolved, place a calibrated pH meter into solution, and adjust the pH to 8.0. Bring the volume to 200 mL in a graduated cylinder, and transfer to a 500 mL screw-top bottle. Add the CTAB to the screw top bottle, screw on the cap tightly, and mix the solution by swirling.

Tris-EDTA (TE), pH 8.0 – 100 mL

Component	Amount	Concentration in solution
1 M Tris-HCl, pH 8.0	1 mL	10 mM
0.5 M Na-EDTA	200 µL	1 mM

Add 80 mL of distilled H_2O to a 500 mL Erlenmeyer flask containing a magnetic stir bar. Place the flask on a stir plate, and set the bar stirring at a medium setting. Add the above reagents to the flask. When

the reagents are fully mixed, place a calibrated pH meter into solution, and adjust the pH to 8.0. Bring the volume to 200 mL in a graduated cylinder, and transfer to a 500 mL screw-top bottle.

Do not autoclave the following solutions:

24:1 chloroform:octanol (or isoamyl alcohol) – 100 mL

Component	Amount	Concentration in solution
Chloroform	96 mL	96% (v/v)
Octanol	4 mL	4% (v/v)

Chloroform produced noxious vapors; use great care when pouring inside of the fume hood. Combine the chloroform and octanol in a screw-top light-resistant bottle; chloroform is sensitive to light. Mix the two by gently swirling the sealed container inside of the hood. Store the solution in a plastic tray within the fume hood.

Tracking (loading) dye – 50 mL

Component	Amount	Concentration in solution
Bromophenol blue	0.125 g	0.25% (w/v)
Xylene cyanol	0.125 g	0.25% (w/v)
Sterile 30% glycerol	50 mL	99.5%

Pour the 30% glycerol into a 250 mL Erlenmeyer flask. Add bromophenol blue and xylene cyanol to the flask and mix them thoroughly by swirling. Assemble a filtering apparatus by placing a Buchner funnel with rubber stopper into the lip of a 250 mL Erlenmeyer flask with vacuum adapter. Cut a circle of Whatman filter paper 4 (with pore size of 20-25 µm) to the size of the Buchner funnel. Run run a rubber hose from the flask to a yellow spigot labeled “vac” on the lab bench. Open the vacuum valve to create suction on the filter. Wet the filter slightly with distilled H_2O, causing it to seal to the funnel. Pour the dye mixture into the funnel slowly. When all dye solution has flowed through the filter, remove suction by sealing the vacuum valve. Remove the Buchner funnel, and pour the filtered dye into a 100 mL screw-top bottle. Store the dye at 4 °C.

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10 mg/mL Ethidium bromide (EtBr) stock – 50 mL

WARNING: Ethidium is a carcinogen. Wear gloves when handling staining solutions or working in an area where gels are stained.