Stock Solutions

**IPTG**  
Isopropyl thiogalactoside, or isopropyl beta-D-thiogalactopyranoside. Sigma stock number I5502.  
0.1 M solution. The formula weight is 238.3, so this is 0.238 g in 10 ml of water. Sterilize by filtration, then store in the freezer.

**X-gal**  
5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside. Sigma stock number B4252.  
20 mg/ml solution. It must be dissolved in DMSO (dimethyl sulfoxide) or dimethyl formamide, not water! It must be wrapped in foil to protect it from the light, sterilize by filtration, and then stored in the freezer.

Using IPTG and X-gal for blue/white selection on Petri plates

There are three basic methods: *spread the chemicals on top of the plates before you use them*, *pour the plates with IPTG and X-gal in them*, or *incorporate the chemicals into top agar*.

- **Putting IPTG and X-gal on top of pre-made agar plates.** Spread 40 ul of IPTG and 40 ul of X-gal on top of the plate with a hockey stick spreader. Then, let the plates dry before you use them. This should take 30 minutes or so if the plate is dry (i.e. a day or two old), but up to several hours for freshly made plates. I definitely prefer this method for bacteria.

- **Incorporating IPTG and X-gal into the plates before pouring.** After autoclaving the media and cooling it to 65°C or less, add IPTG to a final concentration of 0.1 mM IPTG (1 ul IPTG stock solution per ml of media) and X-gal to a final concentration of 40 ug/ml (2 ul of X-gal stock solution per ml of media). Also be sure to add the selection antibiotics at this time: usually ampicillin to a final concentration of 100 ug/ml.

- **Putting IPTG and X-gal into top agar.** This method is generally used for bacteriophage, but also works for bacterial colonies. Use 3 ml of 0.7% agar (or agarose if you want DNA that can be cut with restriction enzymes) kept at 50°C. Add 10 ul IPTG stock and 40 ul of X-gal stock. Then add the bacteria and phage mixture, mix quickly by rolling the tube between your palms, and pour it onto the plate.