SYBR I GREEN STAINING OF LOW AMOUNT DNA

Adapted from BV's Cookbook, TCH 1/27/02

SYBR GREEN I (Molecular Probes Inc.) is a highly sensitive nucleic acid stain. The manufacturer claims that a 20 pg band can be detected in a SYBR I stained gel at 254 nm transillumination. SYBR I also stains RNA and single stranded oligonucleotides. So far we have seen that it is superior to ethidium bromide for detecting small PCR products and for gradient gel staining. Gels can be pre-cast with SYBR I, but the best sensitivity is achieved by post-staining:

1. The stock solution is located in the annex freezer, in aliquots. Keep the solution protected from light and thaw. Dilute 1:10,000 in your favorite buffer (Water works fine, but you can use TBE or TAEB if you like). Make just enough to cover the gel in the smallest container possible. 20 ul of stock in 200 ml of buffer is enough for all gels we standardly run.

2. Stain at room temperature for 10 - 20 min, depending on gel thickness, under ambient light (probably don't need a light-tight container, though manufacturer suggests it; never tested sensitivity with and without it), with gentle agitation. Longer staining does not seem to help.

3. Use the camera set-up with a SYBR I filter and the transilluminator has a 254 nm screen (which works better than 300 nm, though 300 nm screen also works OK; our screen on the Stratagene Eagle Eye). Typically a 2 to 10 second exposure with a fully open aperture works well.

Note that you must use the SYBR1 filter, not the ethidium bromide filter, for photographing your gel on the Eagle Eye, and you should change the filter back to ethidium after you use it.

4. The diluted stain can be reused 3-4 times, but is most sensitive fresh. It can be stored at 4°C, for several weeks. Handle and dispose of like ethidium bromide.