

Staining Slides with Sybr Green

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Sybr Green I is a double-stranded DNA dye with stronger affinity for DNA and greater fluorescent enhancement upon DNA binding than ethidium bromide. Sybr Green I (catalog # S-7563) is available from [Molecular Probes, Inc.](#).

Staining Protocol:

1. Rehydrate slides at room temperature over 0.3 M potassium phosphate 30 min.
2. UV cross link on setting 1,000 Energy in Stratalinker
3. Wash 30 sec. in 0.1% SDS
4. Rinse in 2 large volumes of ddH₂O
5. Stain for 3 min. in 1:20,000 dilution Sybr green in TE (pH 7.5)
6. Wash 5 min. in 0.2% Triton X-100
7. Rinse in 2 large volumes of ddH₂O
8. Wash 7 min. in Hexane
9. Scan Slide on Sybr Green protocol (Laser #4, Filter #2)

To remove Sybr green:

Boil 10 min. in ddH₂O and snap cool in ice-cold ethanol. This removes most of the dye. By the time I boil the slide again to denature the slide and hybridize a cDNA sample in the presence of formamide, there isn't any significant Sybr green signal that bleeds over into the Cy3 or Cy5 channels. You can do hybridization to slides after stripping the Sybr Green, provided that you don't scan the slide in the presence of the Sybr green too many times (< 3 scans). I've done a side-by side comparison and found no difference in hybridization efficiency: [see images](#).