Replacing Ethidium Bromide in an Undergraduate Laboratory: SYBR Safe® Case Study

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What is Ethidium Bromide and Why is it Used?

Ethidium bromide (CAS #1239-45-8), or $C_{21}H_{20}BrN_3$, is used in a number of laboratories, including those at MIT, for identifying DNA bands in samples that are loaded onto agarose gels. Ethidium bromide, commonly referred to as EtBr, binds to DNA. When placed under ultraviolet light, the EtBr-stained DNA bands fluoresce, allowing for the identification and visualization of nucleic acid bands. Ethidium bromide is considered an effective and relatively inexpensive technique for visualizing nucleic acid bands.

Drawbacks of Ethidium Bromide

Though effective and relatively inexpensive, ethidium bromide does have the following drawbacks for those handling the material in the lab:

- it can be absorbed through the skin, irritating the eyes, mouth, and upper respiratory tract;
- because of its tendency to intercalate in DNA bands, ethidium bromide is a powerful mutagen;
- if handled indiscriminately in the lab, ethidium bromide can easily contaminate a large work area. When lab spaces are prepared for a move or for renovation, the space must be decontaminated of chemical, biological and radiological hazards. Because individual laboratories bear most, if not all, of the cost of decontaminating a lab, widespread ethidium bromide contamination may unnecessarily increase either the time or cost of lab preparation for moves or renovations; and
- techniques for managing ethidium bromide waste are expensive - from a materials perspective, labor perspective, or both - or they beget more waste.

Management of Ethidium Bromide Waste

The United States Environmental Protection Agency (EPA) does not currently regulate ethidium bromide as a hazardous waste. Because of its highly mutagenic nature, however, many municipal wastewater treatment authorities either prohibit ethidium bromide discharges, or only allow it in very dilute concentrations. MIT's Environment, Health, and Safety (EHS) Office does not allow drain or trash disposal of ethidium bromide waste. Labs must segregate EtBr waste, which the EHS Office then collects and manages through its hazardous waste contractor.

In 2005, MIT generated approximately 4.7 tons of ethidium bromide waste. In an effort to manage this waste in an environmentally sound manner, MIT pays to have the waste incinerated rather than sending it to a landfill. Incineration is preferred because the ethidium bromide is destroyed. Incineration, however, comes with a price - $300 per 55-gallon drum. For the *pennies* that it costs to *purchase ethidium bromide*, MIT pays *dollars* for its safe *disposal***.** When one considers that the overhead charges on research grants pay for chemical waste disposal, it is clear that ethidium bromide disposal is an obscured, yet expensive and potentially avoidable cost of research.

The drive to create a safer laboratory environment, provide researchers with high performance options for conducting standard laboratory operations, and reduce MIT's hazardous chemical streams all motivate the search for an alternative to ethidium bromide.

SYBR Safe® Alternative to Ethidium Bromide

Recently, SYBR Safe® DNA gel stain has emerged as a safer substitute for EtBr. In a series of mutagenicity tests, including the Ames test, involving various strains of *Salmonella typhimurium* SYBR Safe® caused substantially fewer mutations than EtBr.[1] Whereas EtBr tested positive in the Syrian hamster embryo (SHE) cell assay, SYBR Safe® tested negative, indicating that SYBR Safe® is not suspected to be carcinogenic. SYBR Safe is not classified as hazardous waste under the Resource Conservation and Recovery Act (RCRA) and is not a regulated waste under the Commonwealth of Massachusetts Hazardous Waste Regulations.

To verify that SYBR Safe®'s safety copared with ethidium bromide, and to assess its accessibility for drain disposal, in Spring 2005, the MIT EHS Office contracted with an experienced industrial toxicologist to provide an independent assessment of the Invitrogen study which may be downloaded from (http://probes.invitrogen.com/media/publications/494.pdf). The purpose of the independent review, conducted by an experienced toxicologist, was to evaluate the assessment methods used in the Invitrogen study to determine whether it represented the state-of-the-art. Upon receiving confirmation that the methods used were indeed state-of-the-art and the data provided validated that SYBR Safe® was substantially less mutagenic than ethidium bromide, the MIT EHS Office proceeded to work with toxicologists at the Massachusetts Resources Authority (MWRA) to determine the potential for drain disposal of liquid SYBR Safe® waste. In August 2005, the Massachusetts Water Resources Authority (MWRA), which regulates MIT's wastewater discharges, granted MIT permission to dispose of SYBR Safe® by sewer.

MIT Undergraduate Laboratory Pilots SYBR Safe®

In the Spring 2005, Kendra Bussey, a teaching assistant (TA) for the laboratory class 7.02: Experimental Biology, conducted a side-by-side comparison of ethidium bromide and SYBR Safe® in one of the laboratory modules. One group of students carried out DNA staining using ethidium bromide, while the other used SYBR Safe®. In reviewing the photographs of the gels, Kendra determined that SYBR Safe® consistently produced clearer staining and resolution of the DNA bands than ethidium bromide *(Figure 1*) although SYBR Safe® was not effective for RNA staining. As a result of the superior performance and reduced health hazards, the Experimental Biology lab now uses SYBR Safe® instead of ethidium bromide for DNA staining.

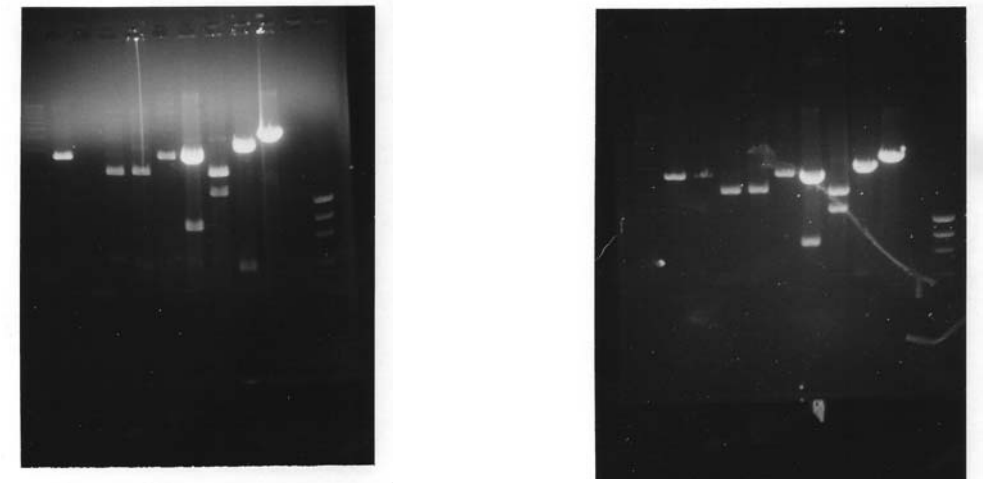

Figure 1

DNA staining with EtBr **DNA staining with SYBR Safe®**

[1] SYBR Safe™ DNA Gel Stain: Assessment of Mutagenicity and Environmental Safety**.** Compiled by Molecular Probes, Inc., from the results of two independent testing services: *Covance, Inc.* and *AMEC Earth & Environmental, Inc.*

The up-front cost of SYBR Safe is initially more expensive than ethidium bromide, as Table 1 illustrates.

TABLE 1:
Comparison of Ethidium Bromide vs. SYBR Safe® for DNA gel staining[2]
Experimental Biology Laboratory (7.02)
Based on 100 gels per module

	Ethidium Bromide	**SYBR Safe®**
Quantity stain per unit	10 mg/mL EtBr solution 10 mL tube	400 µL SYBR Safe® 10,000x (makes 4L)
Volume agarose per gel	40 mL	40 mL
Volume stain per mL agarose	0.05 µL EtBr (0.5 ug EtBr per mL agarose gel)	0.1 µL SYBR Safe®
Volume stain per 40 mL agarose gel	2 µL EtBr	4 µL SYBR Safe®
Number of gels per unit	5,000 gels/unit	100 gels/unit
Unit cost	$29	$40
Cost per gel	$0.0058/gel	$0.40/gel
Volume stain used per term at 100 gels per module, 1 module per term	200 µL EtBr	~400 µL SYBR Safe®
Cost per term (7.02)	$0.58	$40

Because of the small amounts of dye involved, stock solutions are prepared.

Sample calculation:

- *(40 mL agarose/gel)*100 gels= 4,000 mL agarose = 4L agarose per term*
- *(0.5 µg EtBr/mL agarose)*(40 mL agarose /gel)*100 gels = 2,000 µg EtBr = 2 mg EtBr per term*
- *(2 mg EtBr per term)/(10 mg EtBr/mL solution)=0.2 mL solution per term=200µL solution per term*
- *(10 mg EtBr/mL solution)*10^{-3}=10µg EtBr/uL solution*
- *(10 µg EtBr/µL solution)=1µg EtBr/0.1 µL solution =0.5µg EtBr/0.05 uL solution*

From this analysis, it is obvious that those laboratories processing a high throughput of gels may find SYBR Safe® substantially more expensive than ethidium bromide. A laboratory that routinely runs 40 mini-gels (40 mL gels) per month for example, would spend $192 on SYBR Safe® per year, compared with about $3 using ethidium bromide. A lab that infrequently performs DNA gel staining may find the costs more comparable, as is the case with the Experimental Biology laboratory.

[2] Kendra Bussey, proposal submitted to Deb Kruzel to pilot SYBR Safe®. December 10, 2004. Numbers provided are based on running mini-gels, where the volume of agarose per gel is approximately 40 mL.

The Hidden Cost of Ethidium Bromide

MIT does not directly charge researchers for waste disposal, though a number of other major research universities do. Researchers are indirectly charged for waste disposal through indirect charges on research grants, and are directly charged for laboratory decontamination.

The table below summarizes recent estimated annual costs for managing ethidium bromide waste. Note that when ethidium bromide waste is collected from labs, it is overpacked in a drum, i.e., several 5-gallon containers are packed in a larger drum. This packaging entails additional use of packing material, which requires more labor.

TABLE 2:
Disposal Costs Associated with Managing Ethidium Bromide Waste

	Waste Generated	Average Charge per Drum	Annual Hazardous Material Disposal Cost
EtBr, 2003	40 drums	11 each $115[3] 29 each $135[4]	$ 5,180
EtBr, 2004	68 drums	39 each $115 29 each $135	$ 8,400
EtBr, 2005	30 drums	each $355[5]	$10,650[6]
SYBR Safe®	------	------	**$0**

While the substitution of ethidium bromide has proven successful in a teaching setting, the greatest reductions in the waste load can only be realized if laboratories are willing to try SYBR Safe®. Through the EPA's People, Prosperity and Planet (P3) grant, a collaboration of the Chemistry Department, Environmental Programs Office, and the Laboratory for Energy and Environment, research laboratories that are willing to try SYBR Safe® may be eligible to receive starter supplies.

SYBR Safe® may be ordered from Invitrogen at: http://probes.invitrogen.com/products/sybrsafe/.
In addition, Invitrogen has provided a filter selection guide for various visualization setups (geldoc station, etc.) and models at: http://probes.invitrogen.com/products/sybrsafe/filters.html.

If your laboratory has tried SYBR Safe®, and you would like to share your experiences or if you would to receive a startup supply of SYBR Safe®, please contact: **greenchem (at) mit.edu.**

[3] Plastic 30-gallon drums

[4] Metal 55-gallon drums

[5] In 2005, the EHS Office made a decision to incinerate ethidium bromide waste rather than landfill it. The EHS Office is currently exploring opportunities for waste-to-energy incineration of EtBr waste. Incineration costs are significantly higher, but the reduced transportation of the waste, and avoidance of solidification material required to prepare EtBr waste for landfilling make incineration a more environmentally preferable option.

[6] Consolidation in 55-gallon drums generates some efficiencies, but ethidium bromide waste itself increased.