Green Chemistry in Common Bioresearch Procedures

Encouraging Substitution with Less Toxic Chemicals

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Green Labs Program
UNIVERSITY OF COLORADO **BOULDER**

In partnership with the Environmental Center, Environmental Health and Safety, and Facilities Management

Green your lab!

LEARNING OBJECTIVES

1. Be aware of a few chemical substitutions that have already been proven to be successful for many years in common biological research procedures.
2. Gain knowledge about our successes and challenge with implementing this project.
3. Know where to go to find the resources that CU Boulder Green Labs has for this project and are available for other nonprofit research institutions to use.
4. Consider having students or staff implement this green chemistry project on your research campus.

OVERVIEW OF TARGETED BIO-PROCEDURES

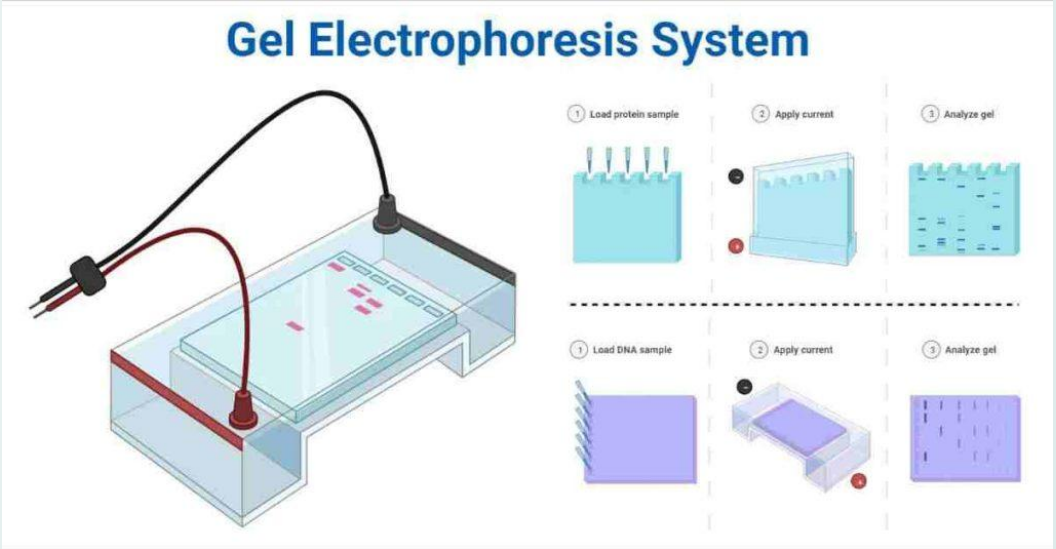
Staining of DNA and RNA in gels

Staining of protein in gels

Western blots

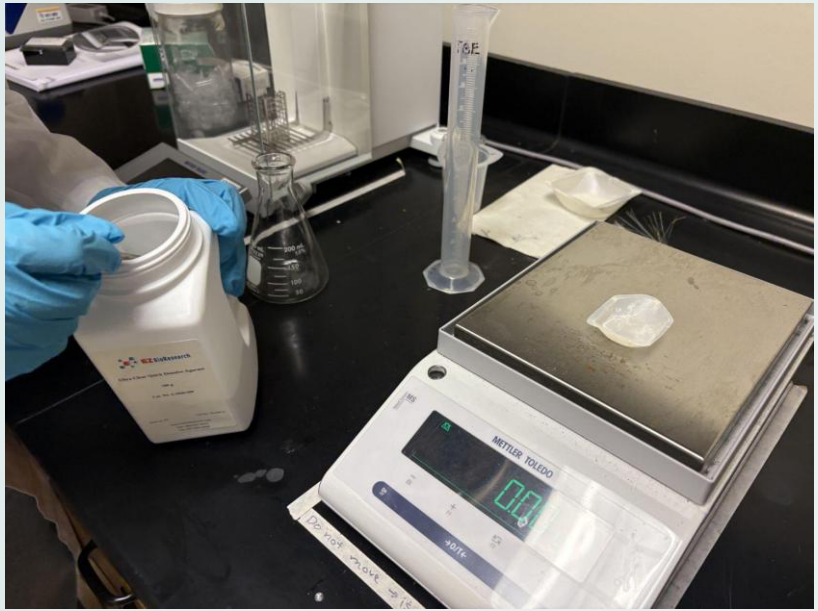
Gel Electrophoresis

- Charge is applied across the gel
- Separates DNA (or RNA) and proteins primarily by size or charge in the gels
- Then stains visualize different sizes in the gels
- Larger ones moving less distance and smallers ones moving longer distances

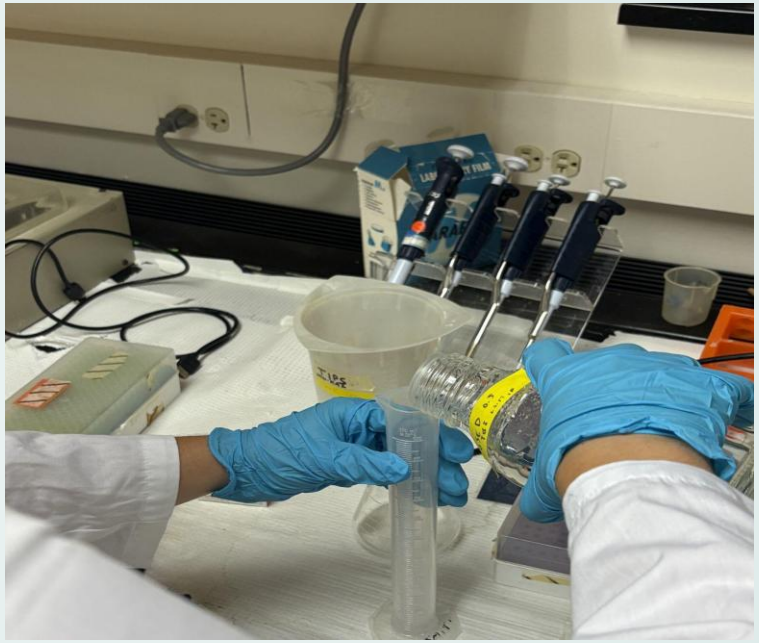


https://www.drawellanalytical.com/2023-latest-gel-electrophoresis-system-knowledge-guide/

Example of Running a DNA Gel



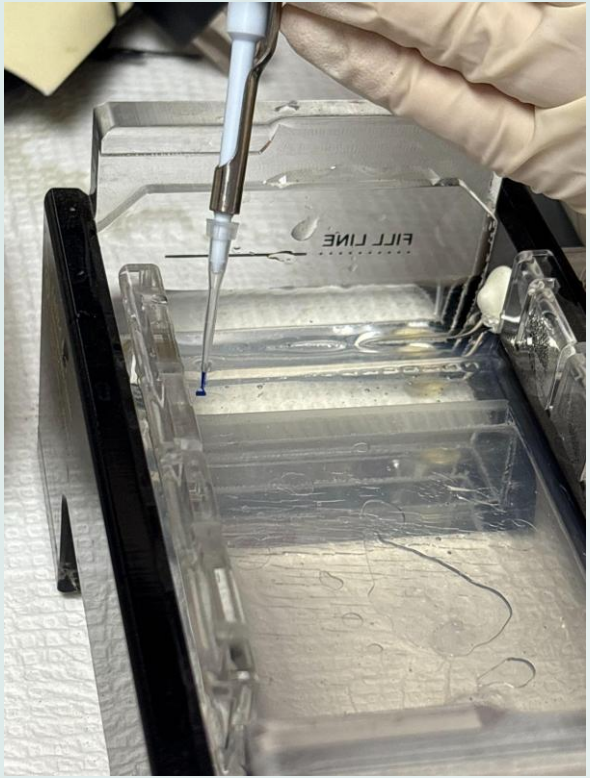
Measure powdered agarose



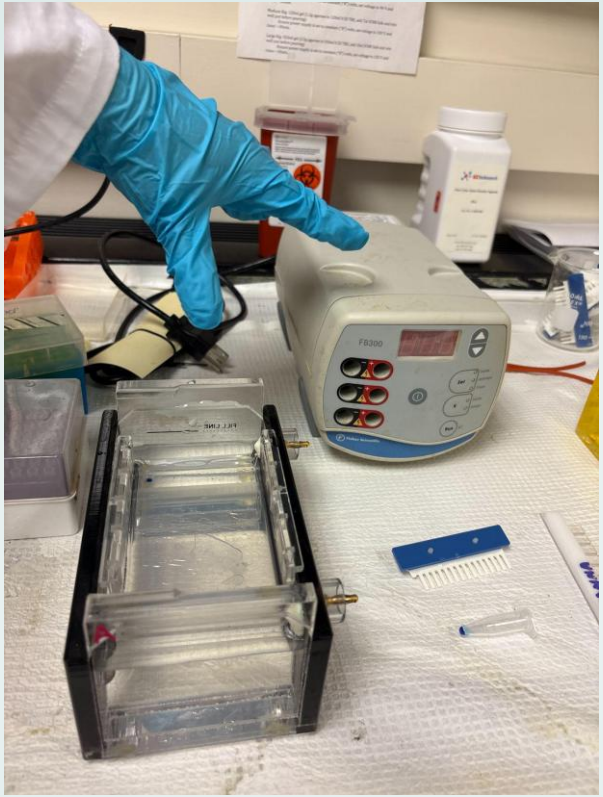
Add TBE buffer

Heat to dissolve agarose

Add EtBr to solution and pour into tray to solidify



Add DNA sample to grooves in solidified gel.



Use electricity to separate DNA into bands.

The imager will take a photo to visual the DNA bands via UV light.

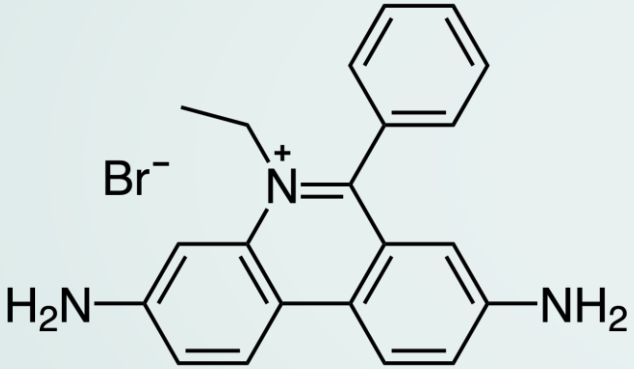
STAINING OF DNA/RNA GELS

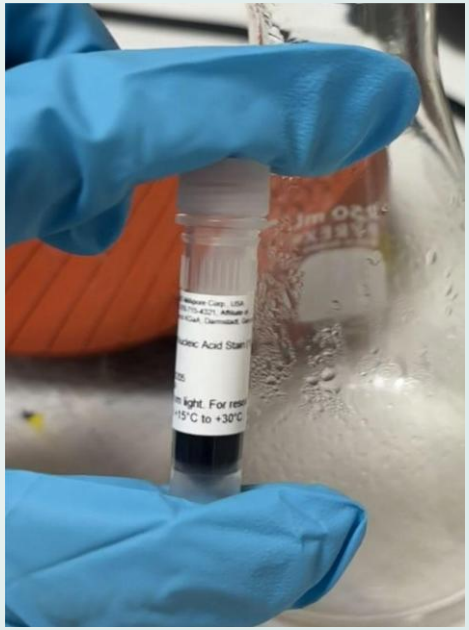
Purpose: Ethidium bromide (**EtBr**) is a dye used to **see DNA** in a gel.

Staining:
EtBr **sticks to DNA** by slipping between its bases.

- Under **UV light**, DNA with EtBr **glows**, showing where the DNA is.

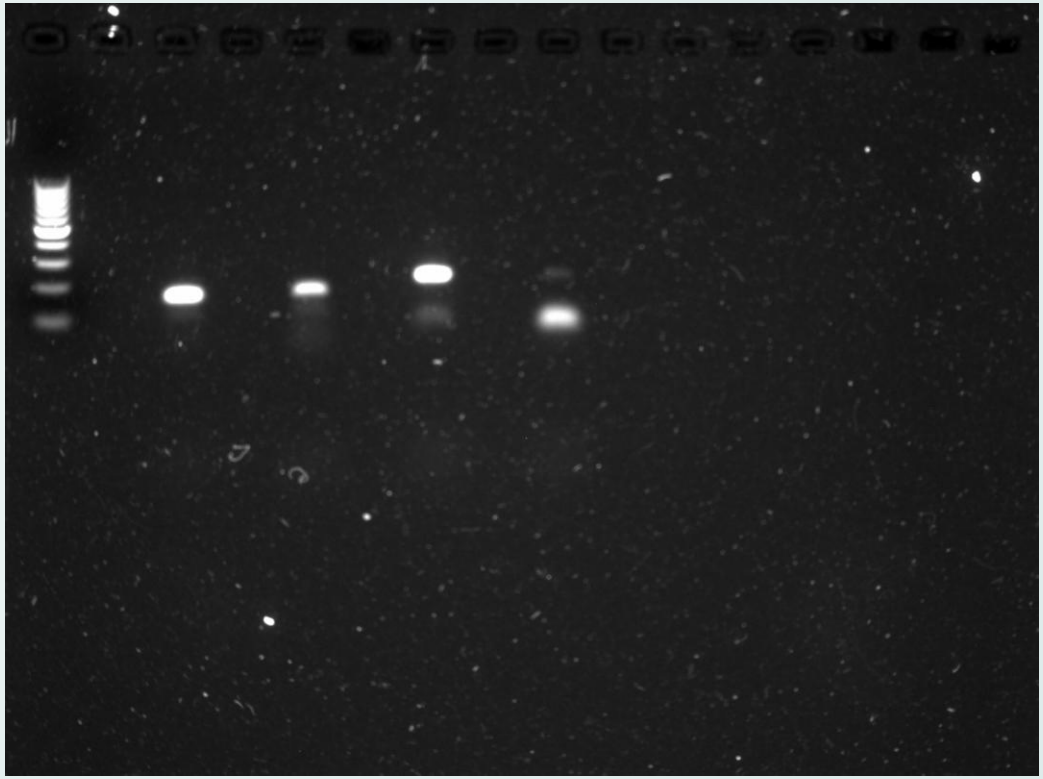
ETHIDIUM BROMIDE (EtBr) ⟶ SYBR SAFE OR GELRED STAIN





Agarose Gel

Using SYBR SAFE



ETHIDIUM BROMIDE (EtBr) ⟶ SYBR SAFE OR GELRED STAIN

01

LESS TOXIC

These are safer alternatives to EtBr

02

EASIER DISPOSAL

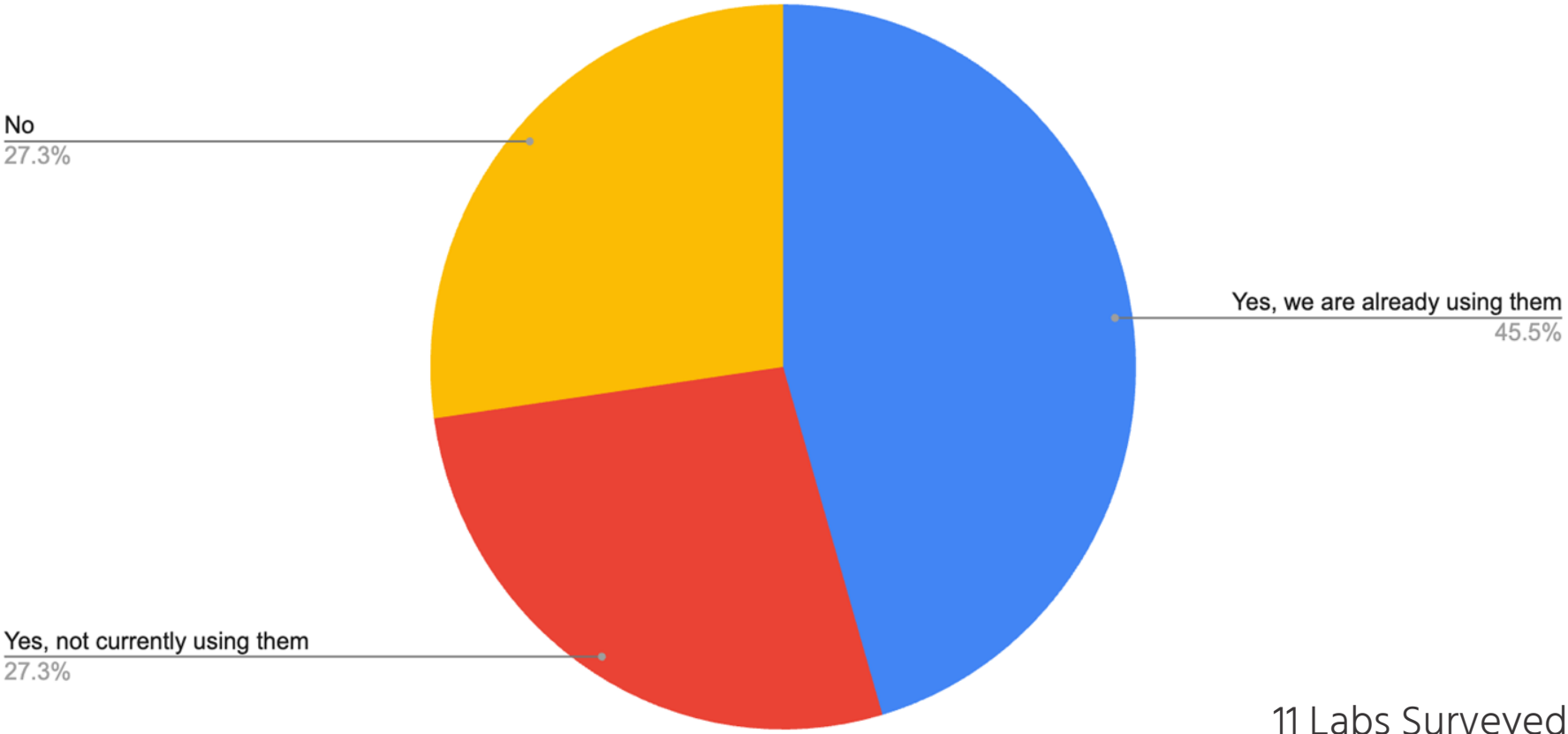
Not classified as a hazard waste, which saves cost for disposal.

03

HIGH SENSITIVITY STAINS

2006 case study by MIT found that SYBR Safe provides clearer stains than EtBr.

Were you aware of alternatives to Ethidium Bromide (EtBr) before this trial opportunity?



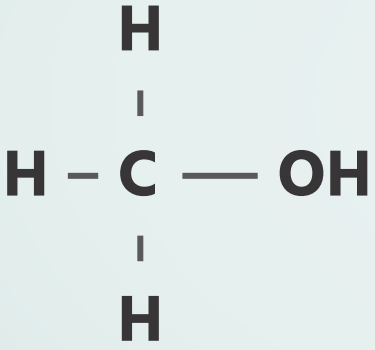
11 Labs Surveyed

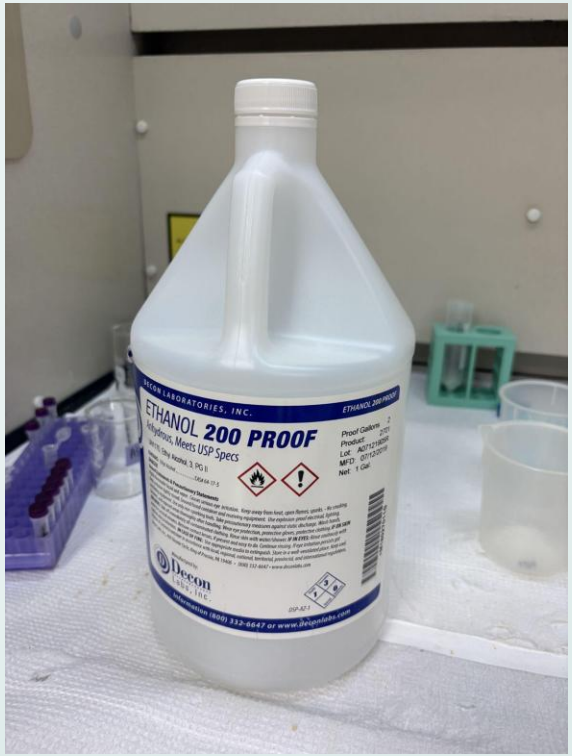
COST COMPARISON

Stain	Stock / unit size	Typical dilution (final in gel)	Volume per 40 mL gel (µL)	Unit price (USD)	Gels per unit	Cost per gel (USD)	Volume used per 100 gels (µL)	Cost per 100 gels (USD)	Source
Ethidium Bromide (EtBr)	10 mg/mL, 10 mL tube	0.5 µg/mL (0.05 µL/mL)	2	65.8	5000	0.0132	200	1.32	Thermo Fisher: Ethidium Bromide Solution, 10 mg/mL (cat. 17898, pack of 5×10 mL)
SYBR Safe®	400 µL, 10,000×	1× (0.1 µL/mL)	4	103.65	100	1.0365	400	103.65	Thermo Fisher: SYBR Safe DNA Gel Stain (cat. S33102)
GelRed®	500 µL (0.5 mL), 10,000× in water	1× (0.1 µL/mL)	4	143.82	125	1.1506	400	115.06	Fisher Scientific: GelRed Nucleic Acid Gel Stain, Biotium 41003

STAINING AND DESTAINING OF PROTEIN GELS & WESTERN BLOTS

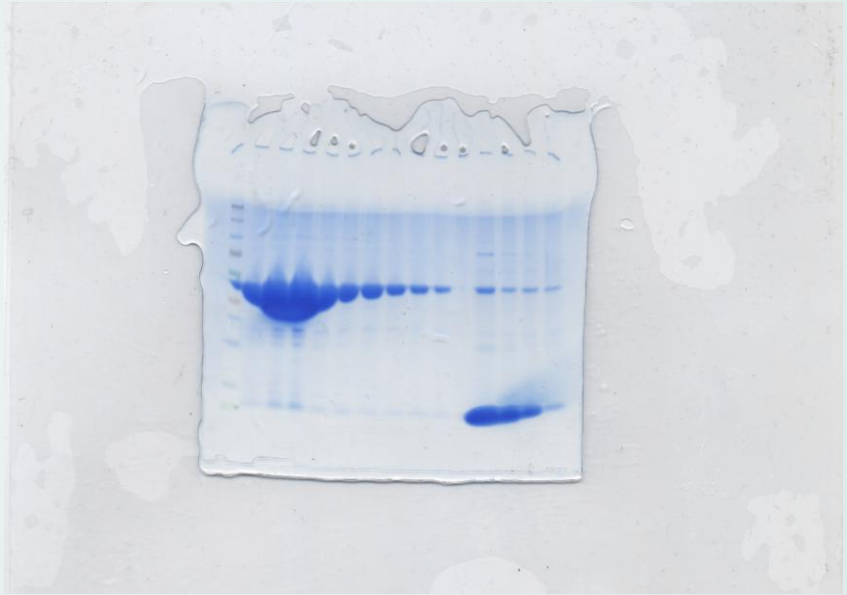
METHANOL ⟶ ETHANOL IN PROTEIN GEl STAIN/DESTAIN SOLUTIONS AND WESTERN BLOTS





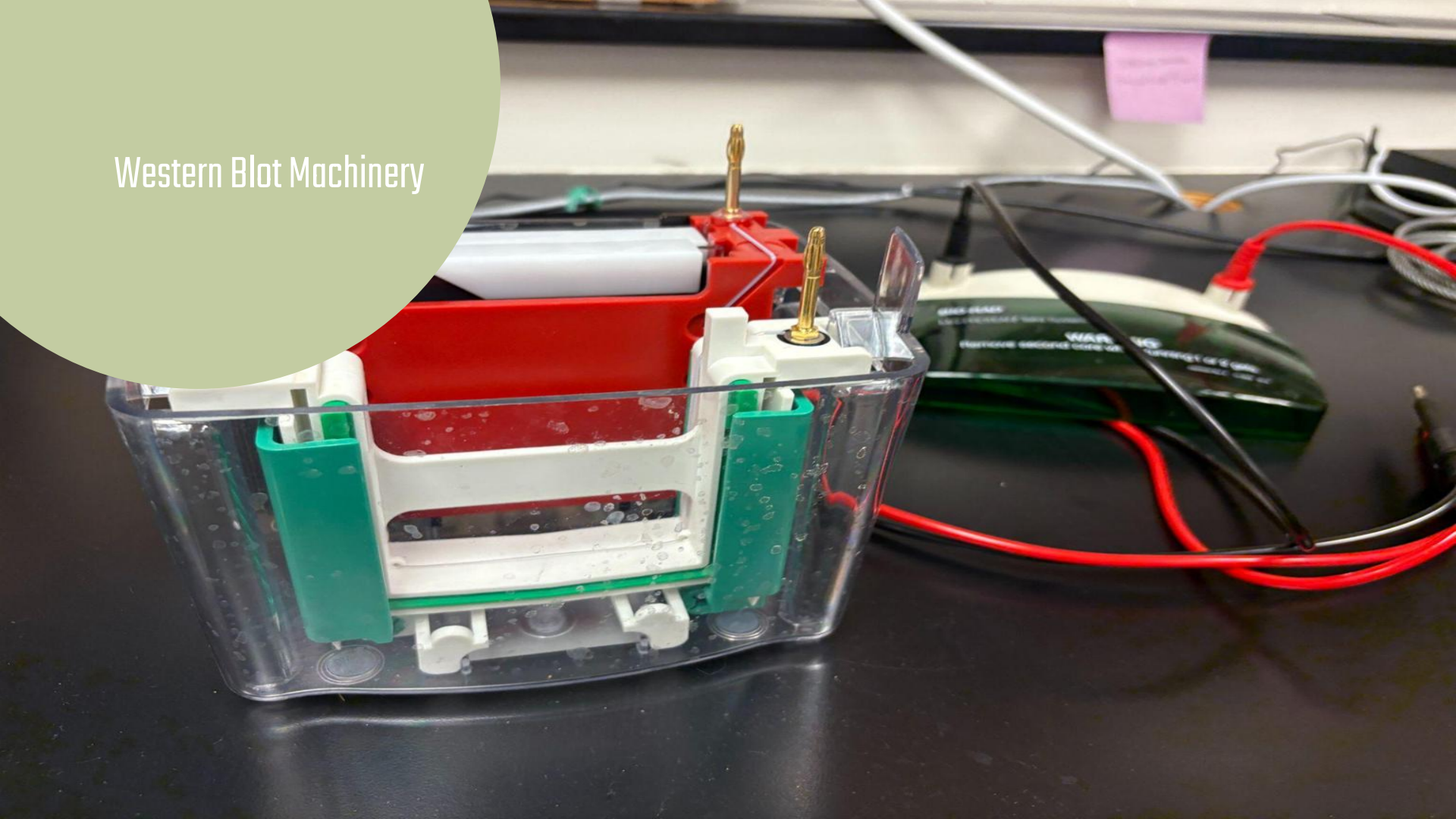
Protein Gel Staining/ Destaining

https://www.researchgate.net

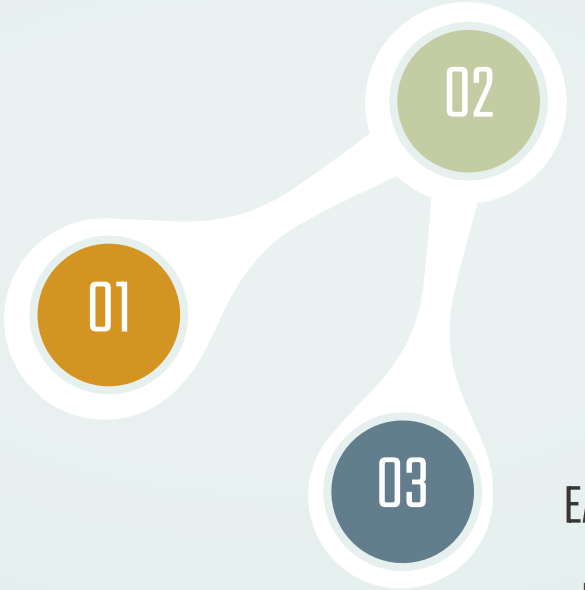


Shows proteins separated by their size

Western Blot Machinery



SWITCH FROM METHANOL TO ETHANOL



TOXICITY

Ethanol can be consumed (common in alcoholic beverages) Methanol should not even be exposed to bare skin.

COST EFFECTIVE

Methanol and Ethanol are the same cost.

EASY TO PURCHASE

Readily available at chemical stores as it is already in use in many other procedures

TESTIMONIALS FROM CU BOULDER

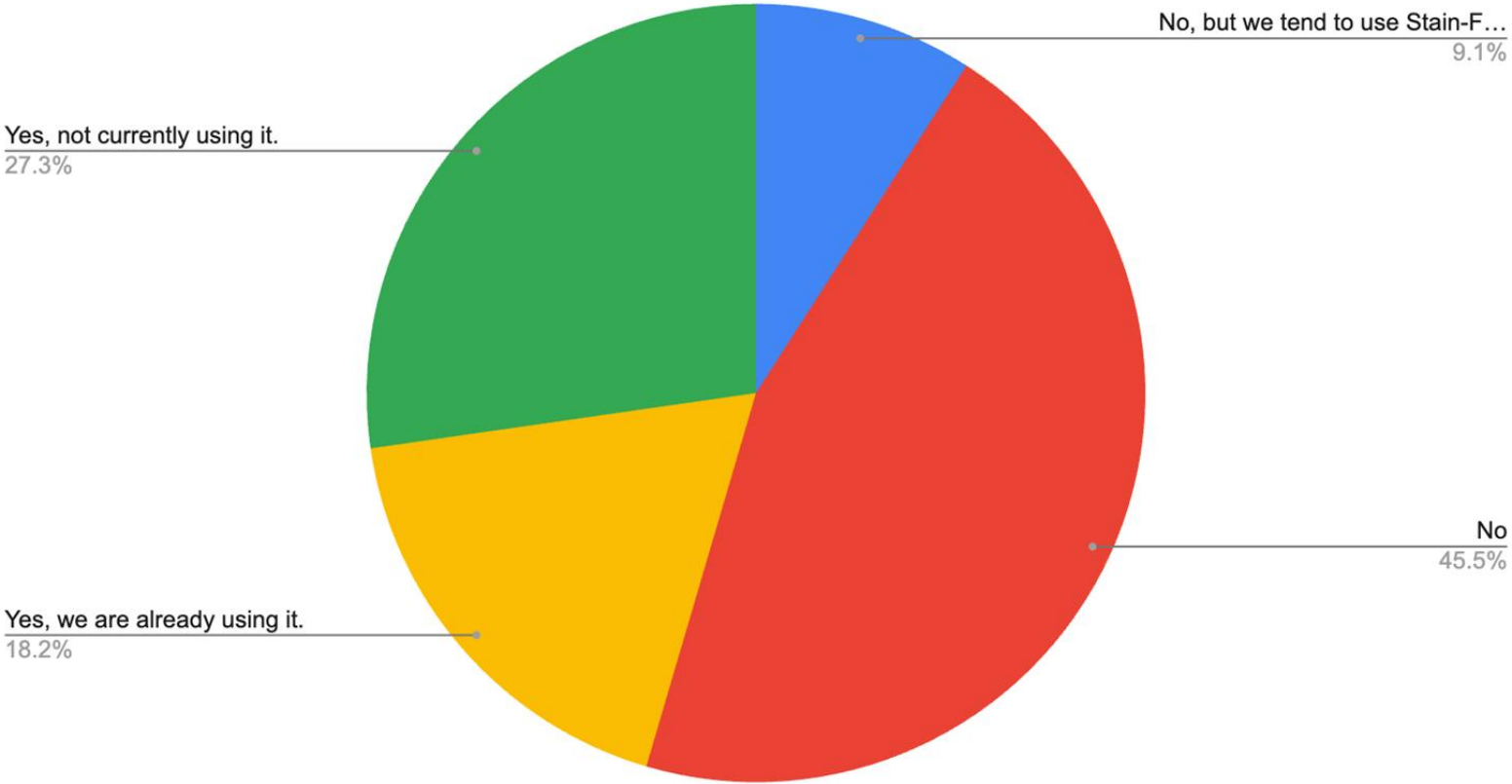
“For more than five years, the Luger Lab has been using ethanol in place of methanol for protein gel stain and destain solutions.”

Dr. Luger is recognized as a Distinguished Professor at CU.

TESTIMONIALS FROM CU BOULDER

The bio-chemistry teaching labs at CU Boulder are willing to make to switch to ethanol after our hearing about the green chemistry initiative.

Were you aware of using ethanol as an alternative to methanol in typical bio procedures?



IMPLEMENTING AT CU BOULDER

03

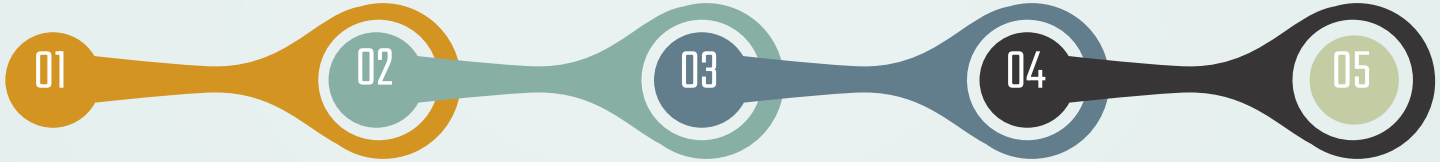
PROJECT FLOW

GRANT APPROVAL

Funding for providing samples.

IMPLEMENTATION

Delivering samples for labs to utilize.



IDENTIFIED NEED

OUTREACH

Methods and survey results.

FEEDBACK

It's ongoing

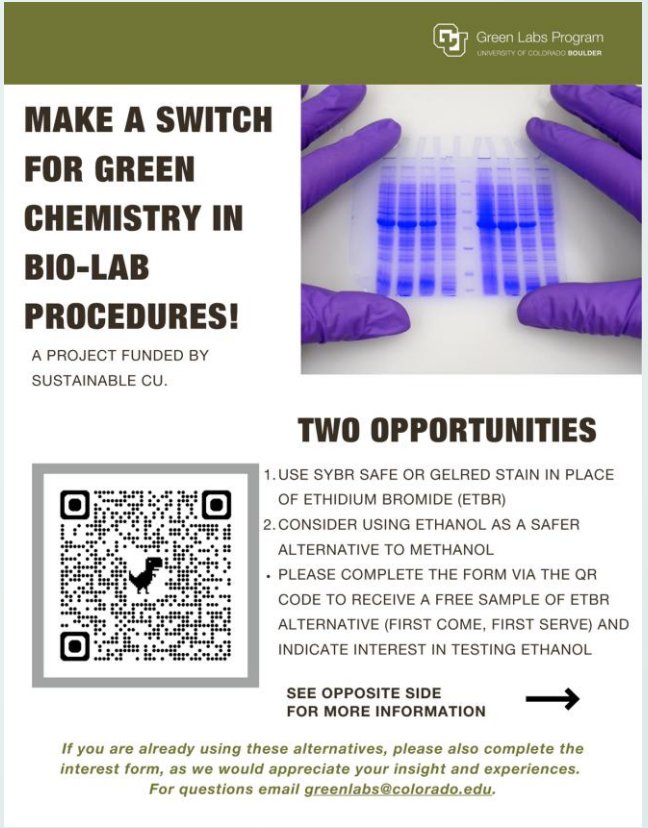
PROMOTION

Main Challenge:

SYBR safe/ GelRed stain cost

Solution:

Offer free sample of their choice of alternative chemical



SUSTAINABLE CU GRANT

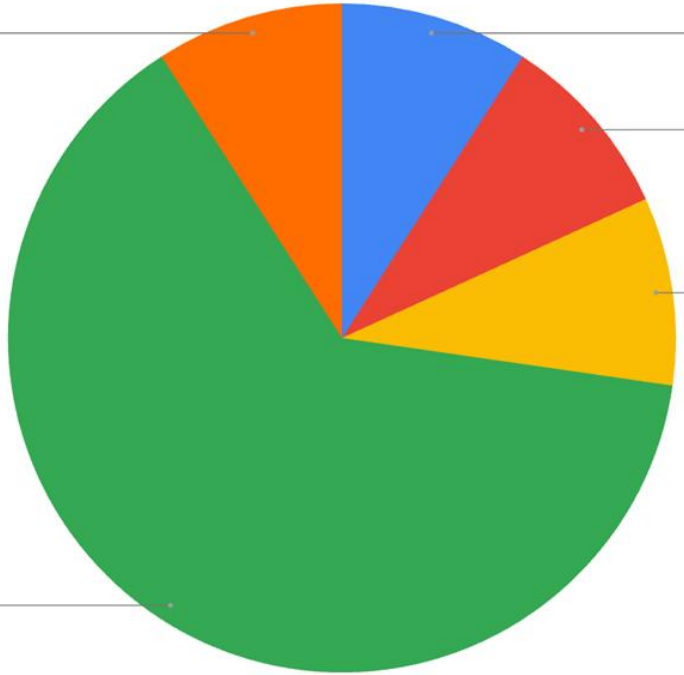
Administered by CU's student run Environmental Board

Focuses on projects that supports student-led, innovative capital projects that promote sustainability on the CU campus

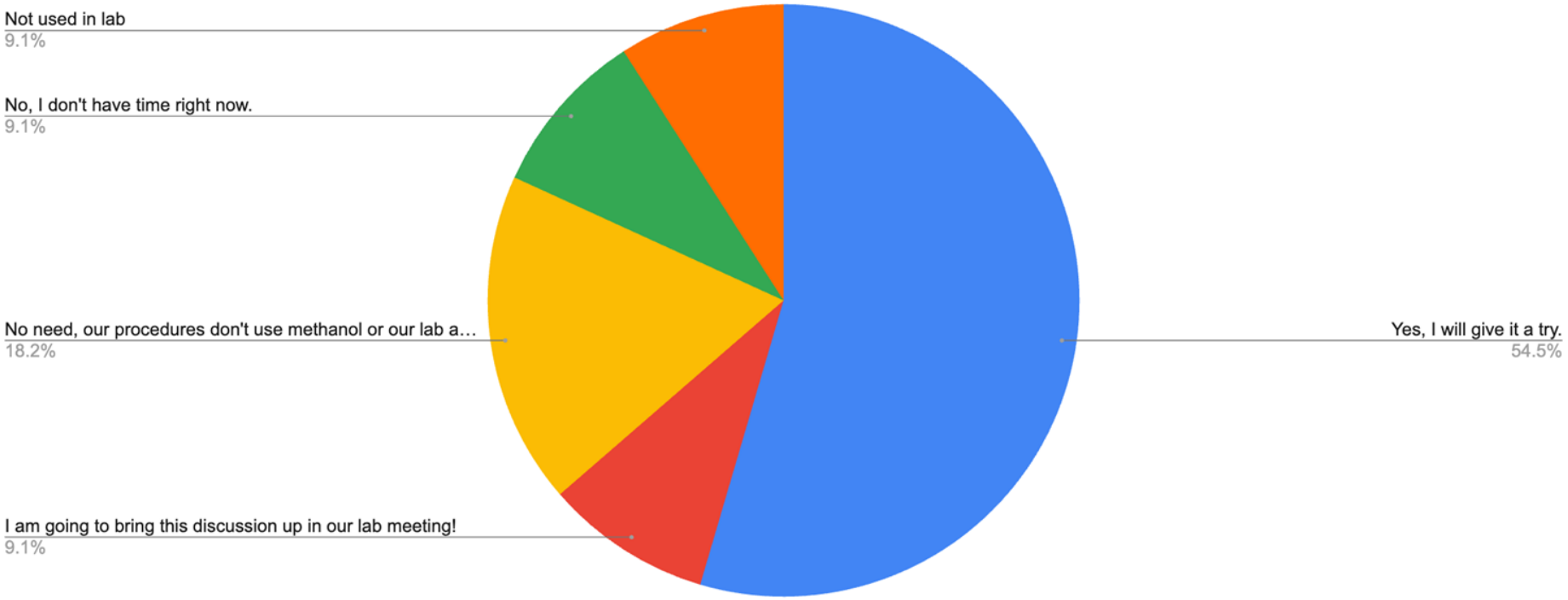
OUTREACH

1. Door to door outreach
2. Tabled in chemistry and biology buildings
3. Presented at bio-chem event
4. Outreach through email
5. Advertisements posted in lab buildings

Are you interested in testing out a free sample of GelRed stain or SYBR Safe?



Are you interested in trying the use of ethanol in the place of methanol in bio procedures such as protein gel stain/destain and western blots?



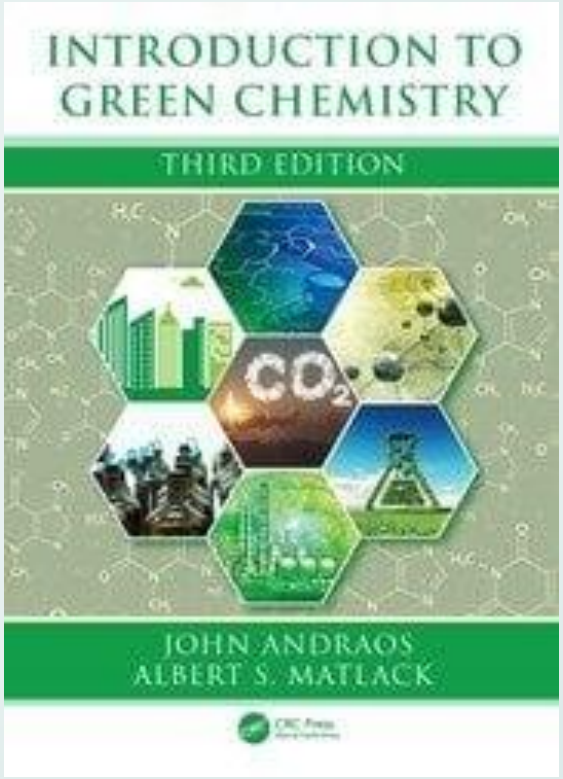
AVAILABLE RESOURCES

PROCEDURE RESOURCES

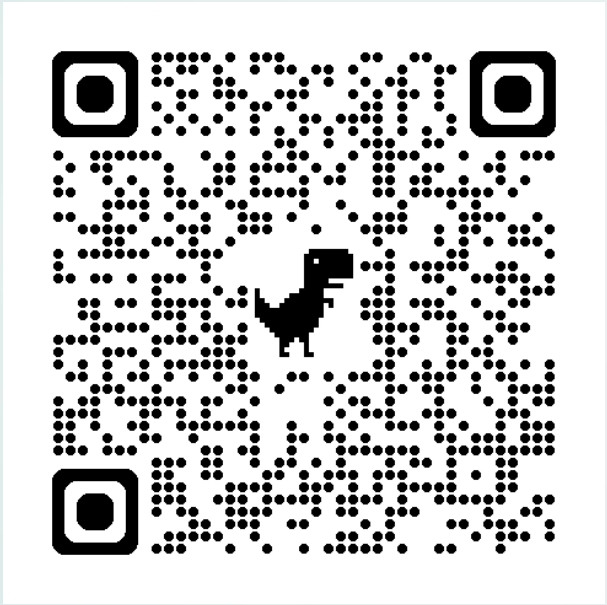
Information about making green chemical substitutions.

Introduction to Green Chemistry

By John Andraos, Albert Matlack



NIH CHEMICAL ALTERNATIVES



Thank you!

Questions?

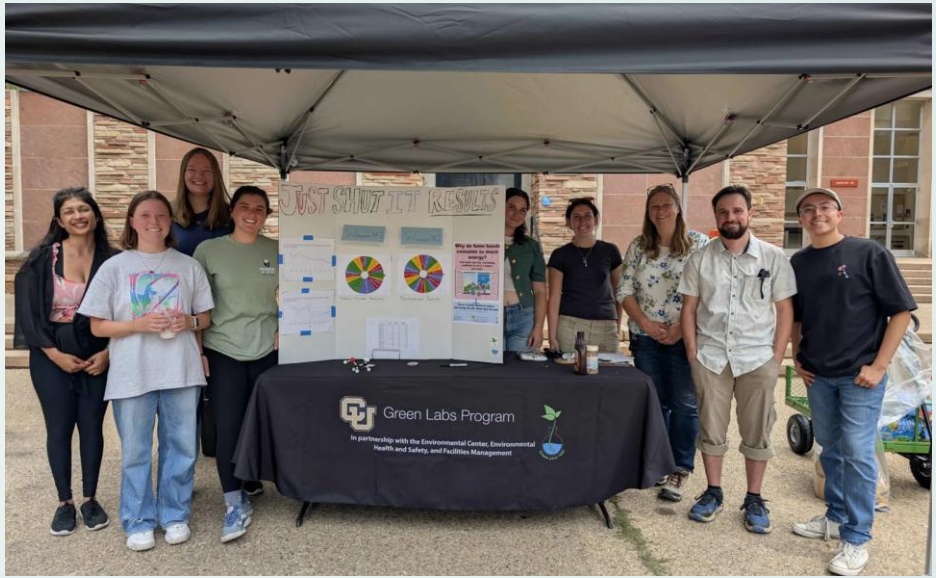
Summary:

Switching EtBr for SYBR safe or GelRed stain in the staining of DNA gels.

Switching to Ethanol from Methanol in western blot procedures.

Numerous labs expressed interest in alternative chemicals.

Consider having students or staff implement this green chemistry project on your research campus!



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