

# Running DNA Mini-Gels

in 20 Minutes or Less Using Sodium Boric Acid Buffer

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Providing a biotechnology experience for students can be challenging on several levels, with time becoming a real constraint in many experiments. Many techniques for DNA isolation and analysis involve a gel electrophoresis step that usually requires a large block of time. In addition to the time needed for loading the samples, which can be substantial for novices, DNA gels typically require 45 minutes to run using standard TBE (Tris-boric acid-disodium EDTA) or TAE (Tris-acetic acid-disodium EDTA) buffers. In a 50-minute class period it is virtually impossible for students to load samples, run and analyze their gels. Therefore any procedure involving gel electrophoresis becomes a challenge.

At the BioPharmaceutical Technology Center Institute (BTCI) we offer a variety of biotechnology field trips including a polymerase chain reaction (PCR) and a restriction enzyme digest, where we are often pressed for time both running and analyzing gels. Recently a paper was published (Brody & Kern, 2004) in which a sodium boric acid buffer was used to run gels much faster without loss of resolution. We tested this sodium boric acid buffer in our field trip program and were able to reduce the time required to run gels from 45 minutes to 20 minutes or less. This sodium boric acid buffer can be made relatively easily with common chemicals, or the stock solution can be ordered (Faster Better Media).

## Materials

### Chemicals

NaOH/sodium hydroxide (Mallinkrodt Cat#7708)

H<sub>3</sub>BO<sub>3</sub>/boric acid (FisherBiotech BP168-500, electrophoresis grade)

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0.5X TBE, agarose (Promega, cat#V3121)

ethidium bromide (Promega, cat#H5041)

6X loading dye (Promega cat#G1881)

lambda DNA HindIII/EcoRI molecular weight markers (Promega cat#G1731)

5X SB™ loading medium (Faster Better Media cat #SB5N-8)

1XSB Hi-Lo DNA Ladder (Faster Better Media)

EcoRI (Promega cat#R6011)

HindIII (Promega cat#R6041)

PstI (Promega cat#R6111)

**Safety Note:** Ethidium bromide can detect smaller quantities of DNA than other DNA dyes because of its high affinity for DNA. However, that high affinity for DNA makes ethidium bromide a strong mutagen, and possibly a teratogen and carcinogen. In addition, a UV light source is required to detect DNA stained with ethidium bromide. UV light can damage skin and eyes. As a consequence of these two potential health hazards, many school districts do not allow the use of ethidium bromide in classrooms. In districts where ethidium bromide use is permitted, safety and disposal protocols vary. Teachers should check with their school districts before using ethidium bromide.

There are many other dyes for detection of DNA, however the more common dyes require much larger quantities of DNA for visualization and shorter pieces of DNA may be difficult to visualize at all. Carolina Blu (Carolina Biological Supply Company) worked as well in sodium borate as in TBE buffer when tested in our lab, however it is three to four times less sensitive than ethidium bromide and requires at least an hour to stain and destain before samples can be viewed. SYBR Gold (Invitrogen) and GelStar (Cambrex) stains are sensitive and both work with sodium borate (Kern, personal communication), however they require UV transilluminators for detection. Other dyes may vary in their effectiveness with the sodium borate system, thus teachers are encouraged to test other dyes prior to use in class.

## Equipment

Stir plate

pH meter (for making buffer stock, not necessary if ordering buffer)

## Electrophoresis Equipment

FOTO/Force 250 power supply (cat#E7-4297)

a high voltage FOTODYNE power supply and mini-single cell electrophoresis chamber (cat# E1-1408)

FOTO/Phoresis UV transilluminator (Model 1-1430)

FOTODYNE Polaroid camera (cat# 1-1440) from FOTODYNE Inc., 950 Walnut Ridge Drive, Hartland WI 53029; Tel. 800-362-3686 or 262-369-7000; Fax: 262-369-7017; e-mail: [technical.service@fotodyne.com](mailto:technical.service@fotodyne.com); Web site: [www.fotodyne.com/education](http://www.fotodyne.com/education).

## Methods

A 20X sodium boric acid buffer stock was made by dissolving 8g NaOH in 700ml ddH<sub>2</sub>O and adding boric acid (H<sub>3</sub>BO<sub>3</sub>) to bring the pH to 8.0 (approximately 53g). ddH<sub>2</sub>O was added to a final volume of 1L and boric acid was added to adjust the final pH to 8.0. The stock was stored at room temperature. The stock was diluted to 1X with ddH<sub>2</sub>O for use at a final concentration of 10mM Na. (Faster Better Media offers a 20X stock buffer.)

For comparison, 0.8% agarose horizontal mini-gels were made with either 1X sodium borate buffer and run in 1X sodium borate buffer at room temperature, or 0.5X TBE and run in 0.5X TBE at room temperature. Fragments from restriction digests ranging from 23kbp-564bp were electrophoresed for 20 minutes at 240V on the sodium borate gel (Figure 1a) and at 120V on the 0.5X TBE agarose gel (Figure 1b). Restriction digest samples contained approximately 300ng each and were loaded in 2 $\mu$ l 6X loading dye (Promega cat#G1881). Ethidium bromide was used in the

gels at a concentration of 0.1%. While bands are visible on both gels, the separation on the sodium borate gel is substantially better, allowing more effective analysis of the digests. A 0.5X TBE gel run at 240V for 20 minutes over-

heats and does not provide useful data. PCR samples ranging from 950-400bp were electrophoresed on 2% gels at 250V for 20 minutes with excellent separation as well (data not shown).

Although clear bands were visible in both the restriction digest and PCR reactions, traditional loading dyes may result in bands bending in the SB buffer due to salts from the loading dye and reactions (Kern, personal communication). Loading dyes and molecular weight markers designed to address this issue are available through Faster Better Media. In our experience, band clarity is reduced when using traditional loading dye with increasing voltage. For example, in a 1% gel run at 250V for 20 minutes, band clarity in traditional loading dye was fine. However in a comparison of molecular weight markers run on a 1% gel at 400V for 10 minutes, the Faster Better Media materials resulted in substantially clearer bands (Figure 2). The drawback to using the Faster Better Media loading dye and molecular weight markers is that they are light orange, which will be more difficult for students to see as they load their gels. Other options to address bending or smearing bands include using less loading dye, or purifying samples before gel electrophoresis to remove residual salts from the reaction.

Two gels may be run on a single power supply with no reduction of resolution. Gels and buffer may be reused. Buffer has been used twice in a short period, although the temperature of the second gel is higher than the first gel. Gels have been used two to three times. Gels were stored in a covered container at room temperature and the agarose was melted again to pour new gels. Although the concentration of the gel changes with this treatment, it does not change significantly and the gels continue to work well for the students.

### Figure 1a.

0.8% agarose gel made and run in sodium borate buffer at 240V for 20 minutes at room temperature.

Lanes (from left to right):

EcoRI digest of lambda DNA

HindIII- EcoRI digest of lambda DNA

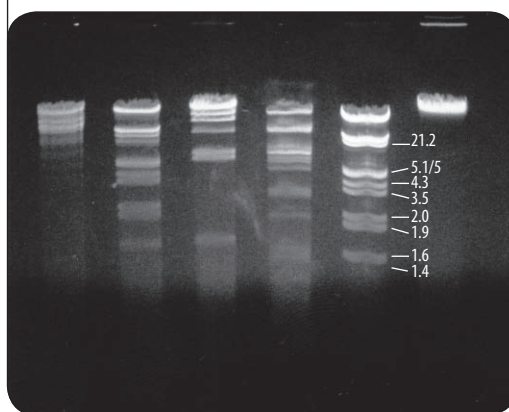
HindIII digest of lambda DNA

PstI digest of lambda DNA

HindIII-EcoRI lambda DNA molecular weight marker (Promega)

uncut lambda DNA

Molecular weights are indicated in kilobases.



### Figure 1b.

0.8% agarose gel made and run in 0.5X TBE buffer at 120V for 20 minutes at room temperature. Lanes (from left to right):

EcoRI digest of lambda DNA

HindIII- EcoRI digest of lambda DNA

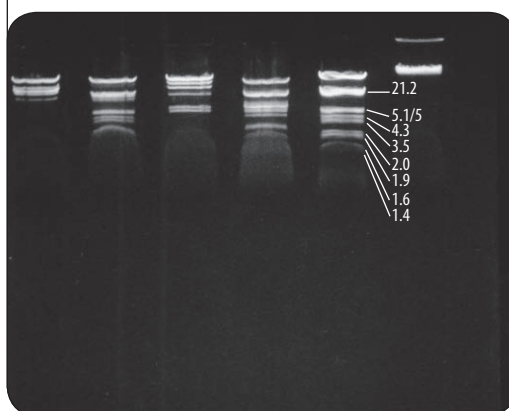
HindIII digest of lambda DNA

PstI digest of lambda DNA

HindIII-EcoRI lambda DNA molecular weight marker (Promega)

uncut lambda DNA

Molecular weights are indicated in kilobases.



## Discussion

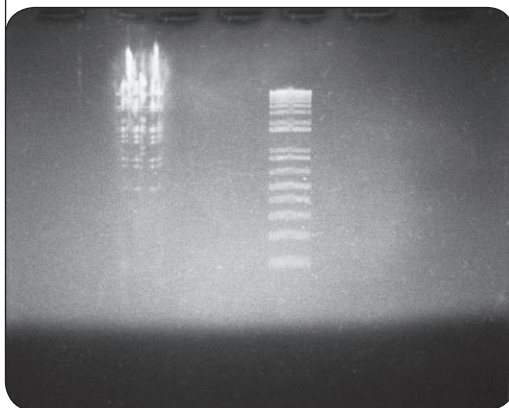
Although adjusting the pH of the buffer is a time consuming process, 1 L of 20X sodium borate stock provides enough 1X buffer to pour and run approximately 40 mini-gels. In addition, the ingredients are inexpensive, accessible, and easy to store. For example, Trizma is \$36 for 100g as opposed to sodium hydroxide at \$30 for 500g (Sigma 2003-2004). Reagents need to be electrophoresis grade. Just for fun, we tried off-the-grocery-store-shelf borax which was inexpensive, but also ineffective. We suspect it has too many impurities. An alternative is to purchase the pre-made 20X stock buffer solution from Faster Better Media.

By reducing the time involved in electrophoresis, we were able to increase the amount of time students had to analyze their gels. Previously, students would leave the field trip after a quick glance at their gels. While the experience of conducting a PCR or restriction digest was valuable, the experiment becomes more meaningful and has a more lasting effect on students' scientific development if they have an opportunity to analyze their results, draw conclusions, and troubleshoot problems.

Table 1 lists applicable National Science Education Standards. Sodium borate gels illustrate these standards and can be used to lead students through a discussion of how science develops, how the culture of science directs research, and how basic ideas can be revised with the introduction of new information. A popular lab manual (Sambrook, 1989) written by well respected researchers states that TAE is used in gel electrophoresis "for historical reasons." While TAE and TBE are good buffers for certain applications, this reliance on traditional methods was sufficient to prevent most researchers from investigating different buffer systems. Questioning traditional buffer systems provided an alternative that is faster and less expensive. However, students should consider the practical implications of constantly "reinventing the wheel." Science would come to a screeching halt if researchers were to question each aspect of every

**Figure 2.**

1% agarose gel made and run in sodium borate buffer at 400V for 10 minutes at room temperature. Left lane is Promega Lambda HindIII/EcoRI marker. Right lane is molecular weight marker from Faster Better Media.



method. Scientific progress is a balancing act between questioning and applying accepted methods. The sodium borate system is not appropriate for all electrophoretic applications, but is very useful for certain applications, particularly in the classroom.

**Note:** The BioPharmaceutical Technology Center Institute (BTCI) is a non-profit, tax exempt, independent organization contributing to the continued success of the biotechnology industry and to efforts aimed at enhancing the quality of education in the life sciences and of education overall. BTCI provides educational opportunities for K-12 students, teachers, college and graduate school students, professionals, and the general public. More information is available on

the Web at: [www.btc.org](http://www.btc.org).

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**Table 1. National Science Education Standards, grades 9-12 (from the National Research Council).**

Standards		
A	Understandings about scientific inquiry	Historical and current scientific knowledge influence the design and interpretation of investigations and the evaluation of proposed explanations made by other scientists.
B	Structure and properties of matter	Atoms interact with one another by transferring or sharing electrons that are furthest from the nucleus. These outer electrons govern the chemical properties of the element.
E	Understandings about science and technology	Solving technological problems often results in new scientific knowledge.
G	Science as a Human Endeavor	Scientists are influenced by societal, cultural, and personal beliefs and ways of viewing the world.