# Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis

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It has been nearly three decades since the invention of DNA electrophoresis (1–6). Current conductive media for DNA electrophoresis remain largely restricted to legacy Tris-acetic acid-disodium EDTA (TAE) and Tris-boric acid-disodium EDTA (TBE) at substantial ionic strengths, leading to higher cost and excessive heat generation, and limiting the voltage and speed of electrophoretic runs. TAE and TBE usually contain between 40 to 90 mM Tris, corresponding anion concentrations, and trace amounts of different forms of EDTA (1–2 mM) (7).

Investigators have compared and analyzed TAE and TBE buffers in DNA electrophoresis; however, to our knowledge no one has substantially investigated the simplification and substitution of components of these buffers to achieve a more efficient and inexpensive conductive medium for DNA electrophoresis (8,9).

It is well established that heat generation is a primary source of problems in TAE and TBE gels, causing sample diffusion, convection, denaturation, and poor gel integrity, and limiting the ability to run gels at a high voltage (10). Ohm's law and the power law interrelate voltage (V), current (I), and power (P) (11,12). Power consumed in the electrophoresis system manifests as heat generation (P = VI). These interrelated variables are affected by ionic conductance due to choice of salts and ionized components in proportion to their particular concentrations in the media used in electrophoresis. A thorough analysis of these critical properties of widely used buffered media for DNA electrophoresis was performed. We found that TAE and TBE create a "runaway" positive feedback loop of current and temperature, which results in poor gel resolution at high voltage (Figure 1, lanes 3–4). This positive feedback loop makes low-voltage (5–10 V/cm) runs necessary and highlights the limitations of using Tris as a cation for DNA electrophoresis. Another component of currently used buffers, EDTA, is now largely superfluous, since most DNA samples are readily soluble and since commonly used enzymes today would not carry an undesirable enzymatic activity under electrophoretic conditions.

We thereby explored alternative, low-molarity, sodium-based conductive media that could mitigate this feedback loop. Extensive studies established that sodium concentrations between 7.5 and 12.5 mM, with borate as the counterion, provided the best resolution (data not shown). We therefore prepared a sodium boric acid (SB) conductive medium, where 1× SB consisted of 5 mM disodium borate decahydrate or 10 mM sodium hydroxide, pH adjusted to 8.5 with boric acid. A 20× stock solution of pH 8.0 produces the appropriate pH upon dilution to working strength; stock solutions of 10–50× can also be prepared and stored without difficulty.

Under high-voltage gel running conditions, SB showed superior resolution compared to TBE, yielding high-resolution separation on a 1.2% gel within 16 min (Figure 1). For the SB gel, current and temperature increased only slightly; current increased from 200 to 287 mA, and the final temperature was 41°C. By contrast, the current for the TBE gel doubled from 306 to 600 mA, and the final temperature was 60°C. A TAE gel run under identical conditions showed a current spike from 395 to 838 mA, and a final temperature of 70°C, resulting in partial melting of the gel (data not shown). Under standard electrophoretic conditions, SB provided resolution and separation as good as or better than TBE and TAE gels (Figure 1, lanes 5–7).

We next sought to address the comparative conductive capacities of SB, TBE, and TAE. To monitor electrolyte exhaustion, we presumed that an eventual drop in current over time was primary evidence of one electrolyte having migrated enough that it could no

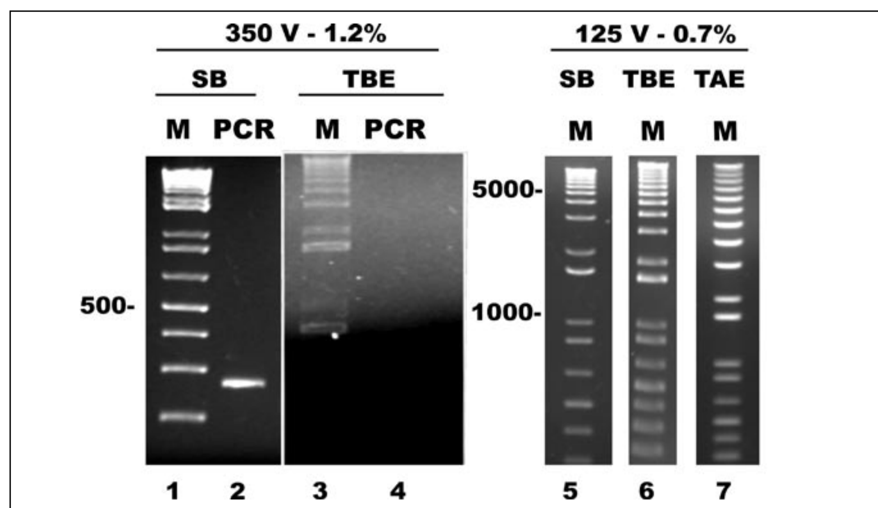


**Figure 1. Sodium boric acid (SB) performance in agarose DNA electrophoresis as compared to commonly used conductive media.** Gels comprised 1.2% (lanes 1–4) and 0.7% (lanes 5–7) agarose (Type I Low EEO; Sigma, St. Louis, MO, USA) and 0.2 µg/mL ethidium bromide (Fisher Scientific, Fair Long, NJ, USA). In lanes 1–4, SB and Tris-boric acid-disodium EDTA (TBE) gels were run fast (350 V) for 16 min. A Tris-acetic acid-disodium EDTA (TAE) gel run under the same conditions experienced poor gel integrity and melting (not shown). In lanes 5–7, SB, TBE, and TAE gels were run at legacy conditions (125 V) for 75 min. The power source (Model FB 570; Fisher Biotech, Pittsburg, PA, USA) displayed current flow at set voltage. All runs used a horizontal rig (MGU-500; CBS Scientific, Del Mar, CA, USA) and had 700 mL total volume of medium in the reservoirs and gels of 10 cm length. M, DNA marker (1 Kb Plus DNA Ladder™; Invitrogen, Carlsbad, CA, USA). PCR, 280-bp PCR-generated product.

**Table 1. Characteristics of Conductive Media for DNA Electrophoresis**

| Medium | Cost per Gel[c] ($) | Voltage Range (V/cm)[d] | Gel Resolution (High V) | Gel Resolution (Low V) |
|---|---|---|---|---|
| TAE[a] | 0.27 | 5–10 | Poor | Excellent |
| TBE[a] | 0.67 | 5–10 | Poor | Excellent |
| SB[b] | 0.07 | 5–35 | Excellent | Excellent |

Protocols from [a]Maniatis (2 mM diNaEDTA), [b]novel medium, sodium boric acid. [c]Cost and concentrations of all conductive media in an electrophoretic run (1 L total volume of conductive media). Prices were determined from US Biological (Swampscott, MA, USA), Invitrogen, and Sigma catalogs. A working estimate of cost of buffer used in the United States is greater than $37 million annually, derived from the above cost per gel, an estimate of agarose and buffer volume per gel, and an estimated agarose relevant consumption from a marketing survey of U.S. trends for 2002 (polyacrylamide gel-associated cost contributions were not included due to limited source data). [d]V/cm, voltage applied to electrophoretic rig, per cm of gel length.

longer serve its full role as a transporter of the current from one electrode to the other, as would be produced by a local depletion of an electrolyte. SB (pH 8–9) had a delayed electrolyte exhaustion at constant voltage as compared to other media tested (the pKa of borate is 9.2) (Figure 2). Electrolyte exhaustion occurred at 3 h for SB, but at less than 1 h for TAE, as determined by observing the current at a constant voltage (Figure 2). In this regard, SB outperformed TAE and TBE. Indeed, TBE outperformed TAE, most likely due to the boric acid, which by buffering the hydroxide production provides a continuing cathodic source of borate for conducting current. Along similar lines of explanation, SB at pH 9 may exhaust slightly earlier than SB at pH 8 (Figure 2). At the lower pH, boric acid represents a larger reserve to replenish ions at the cathode. One would expect that boric acid-containing media could be used for longer runs without recirculation.

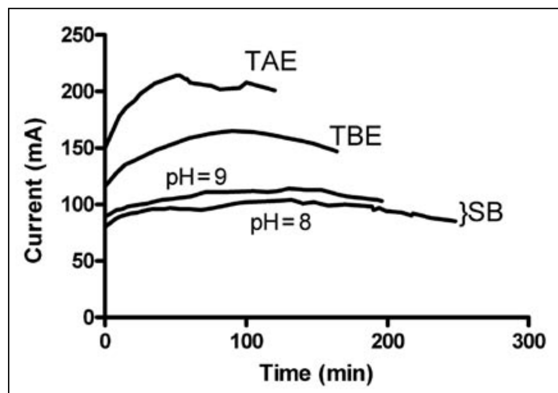


**Figure 2. Electrolyte exhaustion.** Conductive media were tested by plotting the current at constant voltage (200 V). TAE, Tris-acetic acid-disodium EDTA; TBE, Tris-boric acid-disodium EDTA; SB, sodium boric acid.

Within our genetics laboratory, we have completely switched over to SB media for routine DNA separation. In addition, we have performed gel extraction of restriction-digested plasmid DNA from SB gels and successfully subcloned the fragment (data not shown). SB also sufficed for DNA migration in vertical polyacrylamide gels (data not shown). We have not encountered a situation in which SB was not optimal.

Upon a re-examination of some fundamental properties of conductive media and a heightened attention to the self-amplifying nature of Joule heating, we can now strongly recommend that SB could replace TAE and TBE for many if not most standard DNA electrophoretic applications. It is possible to mitigate the runaway positive feedback loop with SB conductive medium, along with considerable savings in cost and time (Table 1). We estimate that SB could save over $30 million annually in the United States, based on cost per gel and an estimated agarose relevant consumption from a marketing survey of U.S. trends for 2002 (polyacrylamide gel-associated cost contributions were not included due to limited source data). SB is superior to conventional media in allowing better temperature control, improved portability and convenience by reduced power and amperage requirements of the power supply, an improved electrolyte exhaustion rate, and a decreased need for heat dissipation in the electrophoretic device. SB simply outperforms existing DNA electrophoretic media.


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