LA-UR-00-3985

97032

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*Title:* **Thermal Detection of DNA and Proteins During Gel Electrophoresis**

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*Submitted to:* DOE Office of Scientific and Technical Information (OSTI)

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Form 836 (10/96)
ST 2629

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# Thermal Detection of DNA and Proteins During Gel Electrophoresis

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## Abstract

This is the final report of a three-year, Laboratory-Directed Research and Development (LDRD) project at the Los Alamos National Laboratory (LANL). The goal of this project was to try to detect unstained, untagged, unlabeled DNA bands in real-time during gel electrophoresis using simple thermal measurements. The technical and ES&H advantages to this approach could potentially be quite significant, especially given the extreme importance of gel electrophoresis to a wide variety of practical and research fields. The project was unable to demonstrate sufficient thermal sensitivity to detect DNA bands. It is clear that we still do not understand the gel electrophoresis phenomenon very well. The temperature control techniques developed during the course of this project have other useful applications.

## Background and Research Objectives

Gel electrophoresis is one of the most important techniques in science and biotechnology [1]. It is frequently used for separating fragments of DNA (or proteins or other macromolecules) by size. Applications include biological and biomedical research, polymer science, forensics, law enforcement, anthropology, biometrics, genetic counseling, botany, counter-terrorism, agriculture, animal husbandry, and biotechnology.

The most common methods for detecting DNA (or proteins) in the gel are fluorescence staining, radioactive labeling, or ultraviolet absorption. Disadvantages of the first two methods include extra processing steps and delays; ES&H problems associated with handling hazardous materials (carcinogenic dyes and radioisotopes); changes in the DNA structure and its mobility through the gel; artifacts in quantification; partial loss of sample if the stains or radioactive labels have to be removed from the DNA after electrophoresis; and DNA damage [1-3]. The first two methods are also tricky to do in real-time. The disadvantages of ultraviolet absorption techniques include DNA damage and limited sensitivity [1,4].

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Schlieren optical techniques [2,3] and refractive index (RI) measurements [5-9] have also been used for the real-time detection of DNA or proteins bands during electrophoresis. Problems with Schlieren measurements include their limited sensitivity and difficulty in quantification [1-3]. Conventional RI detectors tend to have limited sensitivity, experimental complexity, and severe problems with noise and drift [1,5,8,9]. Some have serious problems with quantification or interpretation [5,8].

In a paper published in the Journal of Biochemical & Biophysical Methods (JBBM), we reported RI measurements during DNA gel electrophoresis which had unprecedented resolution, stability, and linearity [9]. The measurements consistently showed a significant decrease in the refractive index (RI) as a DNA band appeared at a given location in the gel. This was surprising given that DNA is known to have a much higher RI than the gel.

This anomalous decrease in RI hasn't been observed previously, apparently due to insufficient RI detector resolution and stability, insufficient temperature control, and problems with absolute quantification. In the JBBM paper, we speculated on 3 possible mechanisms that would cause a decrease in RI when a DNA band entered the probe region of the gel [9]. These possible mechanisms are:

(1) local Joule heating causing gel expansion that would decrease the gel RI,
(2) exclusion of the high-RI salt ions when the DNA entered a region of the gel,
(3) gel expansion caused by the presence of the large DNA molecules.

Postulated mechanism (1) seemed the most likely, especially given the time-lag seen in the RI change when a DNA band entered the monitored region of the gel [9]. Mechanisms (2) and (3) would seem unlikely to have such a time delay. In addition, experts on gel structure insisted that the rigidity of the gel structure would prohibit any substantial expansion, at least at modest temperatures.

We thought, therefore, that the most likely mechanism would be a temperature rise caused by Joule heating from the gel electrophoresis high voltage. The gel has a different electrical resistance when DNA is present than when it is not. The RI decreases we measured were consistent with temperature rises in excess of 1° C for DNA bands with high molecular weight.

If Joule heating is indeed the explanation for the anomalous RI decrease, it could potentially be exploited for real-time DNA detection during electrophoresis. Unlike electrical or electrochemical measurements, measurements of temperature will not interfere with DNA structure or the electrophoresis process. Temperature can also be measured quite accurately and inexpensively with small commercial thermistors.

The goal of this project was to attempt to determine if Joule heating was indeed occurring, and then, if so, to establish sensitivity levels and demonstrate the technique's efficacy for real-time DNA detection. The DNA bands could then be detected without the need for the fluorescent dyes, UV light, or radioisotopes.

## Importance to LANL's Science and Technology Base and National R&D Needs

Gel electrophoresis has a large number of important research and practical applications relevant to the Laboratory's core competencies and capabilities, as well as the Laboratory and DOE missions. Electrophoresis is a very powerful tool for identifying and characterizing biological warfare organisms, and determining the source of their origin. It has very important biometrics and security applications as well. The technique is also important in law enforcement, forensics, and medical screening. It can help us better understand populations of threatened or endangered species. It is often used for polymer and biochemistry research, to study the humane genome, and to characterize biological or molecular damage caused by ionizing radiation.

Any technique that has the potential to increase the speed, ease of use, detection sensitivity, and to reduce sample preparation time and DNA damage is of considerable potential interest. This is especially true if this can be done at low cost, and with minimal modifications to traditional electrophoresis methods.

The technique studied in this research also has the potential of eliminating the need for carcinogenic fluorescent dyes, UV radiation, and radioisotopes that are traditionally used for gel electrophoresis and that present significant ES&H problems, especially given the widespread use of gel electrophoresis in industry, government, and academia.

## Scientific Approach and Accomplishments

In FY97 (the first year of the project), we designed and constructed a novel gel electrophoresis apparatus that allowed careful control of the gel temperature during electrophoresis. This was important in order to accurately measure the hypothesized temperature rise caused by DNA Joule heating.

In FY98, we undertook a series of measurements using our gel electrophoresis apparatus. These measurements led to a redesign of the apparatus. This second generation apparatus, in turn, produced far more thermal temporal stability than we had thought could be readily achieved. The hypothesized Joule heating from DNA bands, however, was not substantially detected.

In FY99, we modified the gel apparatus so that more accurate temperature measurements could be made using newly available, high-sensitivity micro-thermistors, instead of the larger conventional thermistors used in FY98. This permitted us to make the most sensitive thermal measurements to date during gel electrophoresis. Despite this, we were unable to detect any significant local Joule-heating when a DNA band entered the probe region of the gel.

As a result of this work, we now reject Joule-heating as a likely mechanism for the anomalous large refractive index (RI) decrease seen when a DNA band enters the probe volume. The two remaining hypotheses, that the anomalous RI is caused by gel matrix expansion or by salt ion exclusion, remain to be tested.

Although this LDRD project has ended, one of the undergraduate researchers on this project (Anna Nogar) is now pursuing a research project at the University of New Mexico as part of a senior thesis in the Biochemistry Department. The goal of her project is to test if salt ion exclusion might be the actual mechanism. This will be done by using NaBr instead of NaCl in the buffer solution. The Br ion has a molar refractivity 50% greater than Cl. If salt exclusion is the mechanism, this should result in a 50% greater RI change.

In FY99, a third possible explanation for the anomalous RI was proposed based on collaborations with CIC Division. According to this argument, the anomalous RI decrease may be caused by a Frohlich resonance. This possibility was crudely modeled, though the hypothesis remains untested.

Despite its null result, this project helped us to develop techniques for controlling the gel temperature to better than 10mK during the course of gel electrophoresis, and temporal drifts in spatial gradients to less than 1 mK. The ability to maintain this (unprecedented) level of temperature control is potentially quite useful for conventional high-precision gel electrophoresis [1, 2, 5] , and also for high sensitivity RI measurements made using Zeeman interferometry [9]. The temperature control techniques we developed for this LDRD project also proved useful for a pollution and process monitoring research project unrelated to gel electrophoresis.

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