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A sensitive two-color electrophoretic mobility shift assay for detecting both nucleic acids and protein in gels

DNA-binding proteins are key to the regulation and control of gene expression, replication and recombination. The electrophoretic mobility shift assay (or gel shift assay) is considered an essential tool in modern molecular biology for the study of protein-nucleic acid interactions. As typically implemented, however, the technique suffers from a number of shortcomings, including the handling of hazardous ^{32}P-labeled DNA probes, and difficulty in quantifying the amount of DNA and especially the amount of protein in the gel. A new detection method for mobility-shift assays is described that represents a significant improvement over existing techniques. The assay is fast, simple, does not require the use of radioisotopes and allows independent quantitative determination of: (i) free nucleic acid, (ii) bound nucleic acid, (iii) bound protein, and (iv) free protein. Nucleic acids are detected with SYBR® Green EMSA dye, while proteins are subsequently detected with SYPRO® Ruby EMSA dye. All fluorescence staining steps are performed after the entire gel-shift experiment is completed, so there is no need to prelabel either the DNA or the protein and no possibility of the fluorescent reagents interfering with the protein-nucleic acid interactions. The ability to independently quantify each molecular species allows more rigorous data analysis methods to be applied, especially with respect to the mass of protein bound per nucleic acid.

Keywords: DNA-binding protein / Gel shift assay / Multiprotein complex PRO 0438

1 Introduction

Many regulatory steps in cellular processes, such as replication and transcription, depend upon the binding of proteins to specific DNA sequences. Such protein-nucleic acid interactions are commonly studied by filter binding, DNA footprinting, methylation interference, and gel shift assays. Among these methods, the electrophoretic mobility shift assay (EMSA) is certainly one of the most frequently employed for a number of fundamental reasons. The gel shift assay is suitable for detecting small amounts of sequence-specific interaction, even in crude cell or tissue extracts. Since the gel shift assay is based upon differences in mobility between the stable protein-nucleic acid complex and free nucleic acids, experiments are not subject to the signal-to-background problems that plague filter binding methods [1]. In addition, the gel shift assay may be used to monitor stepwise assembly of multiprotein complexes onto a nucleic acid template and also for monitoring compositional changes in protein-nucleic acid complexes.

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Traditionally, mobility-shift assays have employed ^{32}P-labeled nucleic acids to detect the DNA component of protein-DNA complexes in polyacrylamide gels. Isotopic labeling offers high detection sensitivity, but also suffers from many disadvantages, including the short half-life of the label and extended exposure requirements for detection, as well as environmental and safety issues. Other labeling techniques require secondary detection methods, such as chemiluminescence detection [2, 3]. The primary advantage of secondary detection methods is the potential for enzyme-catalyzed signal amplification. However, the methods generally require prelabeling of the DNA, and additional steps associated with the blotting of the gel to a positively charged nylon or nitrocellulose membrane and detection with secondary amplification reagents. Both isotopic labeling and secondary detection methods are time-consuming and inconvenient. More importantly, they can only detect DNA band shifts without providing any direct information regarding the protein component of the complex.

In order to overcome the shortcomings of current techniques, we have developed a two-color fluorescence EMSA. The assay provides a simple, sensitive, and direct detection method for observing both the nucleic acid and protein components in a gel-shift assay displayed as green and orange-red fluorescence staining,

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respectively (Fig. 1). Fluorescence-based EMSA techniques have been reported previously that require cyanine dye-labeled oligonucleotides or pre-incubation of unmodified oligonucleotides with intercalating dyes, such as ethidium, thiazole orange or oxazole yellow homodimers, prior to electrophoresis [4, 5]. However, the procedure described in this manuscript relies upon the fluorescence staining being applied after the entire gel-shift experiment is completed. Hence, completely "native" protein-nucleic acid interactions are evaluated. The advantages of using unlabeled protein and nucleic acids was recently illustrated when a thiol-derivatization method was employed to label proteins with an iron chelator and radioactive ^{55}Fe prior to polyacrylamide gel electrophoresis [6] Unfortunately, the derivatized vaccina virus protein VP55 failed to interact with an appropriate oligonucleotide in EMSA experiments, demonstrating that the derivatized protein lost its ability to bind to nucleic acids.

The most important advantages of the new two-color fluorescent EMSA staining method relative to other labeling techniques are that the detection is rapid, requiring less than 20 min to stain the nucleic acids and 3 h to stain protein bands, and also omits any labeling steps for either nucleic acids or proteins. The ability to independently quantify each molecular species allows more rigorous data analysis methods to be applied, especially with respect to the mass of protein bound per nucleic acid. Thus, the method provides more comprehensive information concerning nucleic acid-protein interactions than the standard band shift methods.

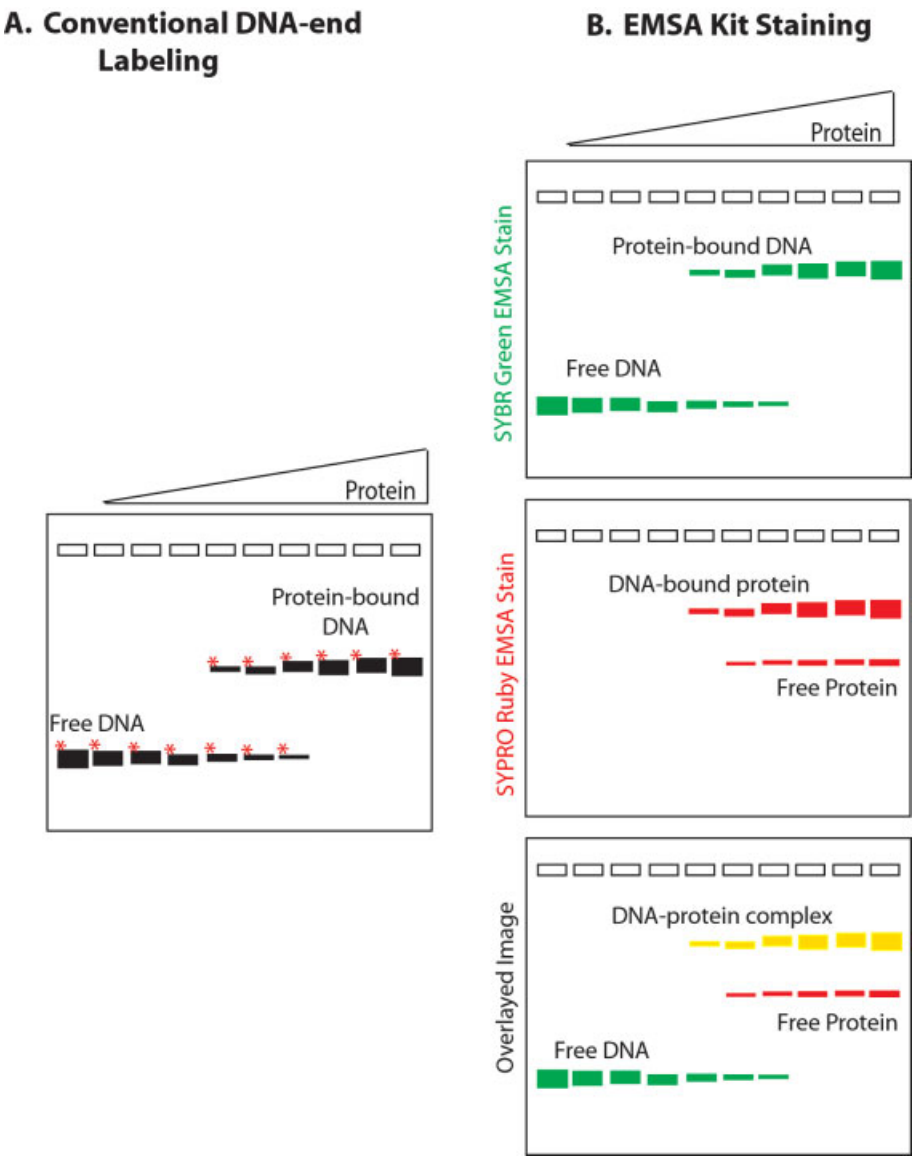

Figure 1. Schematic diagram of the EMSA. EMSA or band shift assay is based upon the observation that the migration rate of stable protein-DNA complexes through polyacrylamide gels is different from unbound DNA or protein. (A) Diagram of EMSA using conventional DNA-end labeling (radioactive or fluorescent labeling). (B) Diagram of EMSA using the EMSA Kit staining method (Molecular Probes). The EMSA gel is stained with SYBR Green EMSA stain first to visualize DNA (green color), then the same gel is stained with SYPRO Ruby EMSA stain to visualize protein (red color). The two images may be digitally overlaid and the regions where the green and red color corresponding to one another, which represents the DNA-protein complex, is displayed as yellow color.

2 Materials and methods

2.1 Oligonucleotides and proteins

The oligo dT 100-mers were purchased from Oligos *Etc*. (Wilsonville, OR, USA). *Lac* operator DNA was produced from single-stranded DNA sequences made by Oligos *Etc*. Two sequences of single-stranded operator oligonucleotides, 5'-TGTTGTGTGGAATTGTGAGCG GATAA CAATTTCACACAGG-3' and 3'-ACAACACACCTTAACA CTCGCCTATT GTTAAAGTGTGTCC-5', were annealed in 0.2 × SSC buffer (3 mM sodium citrate, pH 7, 30 mM NaCl, (Sigma Chemical, St. Louis, MO, USA) at 95°C for 5 min and then cooled slowly to room temperature for several hours. The absorbance values at 260 nm for all oligonucleotides were measured and the double-stranded character of paired *lac* operator 40-mer was evaluated by polyacrylamide gel electrophoresis. The *lac* repressor tetramer protein was a generous gift from Dr. Kathleen Matthews's laboratory (Rice University). The T4 phage single-stranded binding protein (gp32), was a generous gift from Dr. Peter von Hipel's laboratory (University of Oregon).

2.2 Gel electrophoresis

All the reactions were loaded onto precast 6% nondenaturing polyacrylamide gels (Invitrogen, Carlsbad, CA, USA). The gels containing either DNA, protein or both were stained using the EMSA kit (Molecular Probes, Eugene, OR, USA). This kit uses two fluorescent dyes for detection, SYBR Green EMSA stain and SYPRO Ruby EMSA stain. The DNA Molecular weight marker (12–1353 bp) was obtained from Roche (Indianapolis, IN, USA).

2.3 Determining the sensitivity of SYPRO Ruby EMSA protein stain

A dilution series of SDS-PAGE molecular weight standards was generated by taking 5 μL of the molecular weight standards (2 μg/μL) and mixing it with 195 μL of 1 × Hi-Density TBE Sample Buffer (Invitrogen) to make a stock of the molecular weight standards at a concentration of 50 ng/μL. A 1:1 dilution of the molecular weight standards was prepared by combining 100 μL of the 50 ng/μL solution prepared above with 100 μL of the 1 × Hi-Density Tris-borate-EDTA (TBE) sample buffer. Further 1:1 dilutions were created six additional times, so that the concentrations of the dilution series of molecular weight standards ranged from 50 to 0.195 ng/μL. The dilution series of SDS-PAGE molecular weight standards was heated at 95°C for 5 min, 12 μL of each of the samples was applied onto a 6% nondenaturing polyacrylamide gel and the gels were run at 150 V for 50 min. The gels were stained in SYPRO Ruby EMSA stain (from the Molecular Probes EMSA kit) for 3 h with continuous and gentle agitation. The gel was then washed twice with dH_20 to remove excess stain and destained in 10% methanol, 7% acetic acid for about 60–90 min. The gel was washed again in deionized H_20 twice before acquiring an image. Occasionally, the gels were stained with SYPRO Ruby EMSA stain overnight with 2–3 h destaining. The stained protein gel was visualized using an FLA-3000 Fluorescent Imager (Fuji Photo, Tokyo, Japan) at 473 nm excitation and 580 nm of emission wavelength settings.

2.4 Generating *lac* operator DNA and repressor protein dilution series

The 40-mer of ds *lac* operator DNA was diluted to a final amount of 1, 5, 10, 15, 20, 25, 30, 35, 40, and 80 ng per lane in 1 × *lac* binding buffer (10 mM Tris-HCl, 150 mM KCl, 0.1 mM DTT, and 0.1 mM EDTA). Ten μL of diluted DNA with 2 μL of 6 × EMSA gel-loading solution (from Molecular Probes EMSA kit) was loaded onto a 6% nondenaturing polyacrylamide gel and the gel run at 200 V for 35 min in pre-chilled 0.5 × TBE buffer. The gel was then stained in SYBR Green EMSA stain (from Molecular Probes EMSA kit) at 1:10 000 dilution for 20 min at room temperature, then washed twice with deionized H_20 for 10 s to remove excess stain that could interfere with image analysis. The stained gel was visualized using an Fuji FLA-3000 Fluorescent Imager at 473 nm excitation and 520 nm of emission wavelength settings.

2.5 Generating a *lac* repressor protein dilution series

The *lac* repressor tetramer was diluted to final concentrations of 21, 43, 65, 87, 148, 173, 259, 346 nM in 1 × *lac* binding buffer. The reaction without *lac* repressor was included as a negative control. Approximately 150 nM of ds *lac* operator DNA was incubated with an increasing amount of *lac* repressor protein for 20 min in a final volume of 20 μL at room temperature. The reactions were loaded onto a 6% nondenaturing polyacrylamide gel and run at 200 V for 35 min. The gel was stained in SYPRO Ruby EMSA stain (from Molecular Probes EMSA kit) for 3 h, washed, destained, and imaged as described above.

2.6 Monitoring *lac* operator-repressor interaction

Approximately 151 nM of ds *lac* operator DNA 40-mer was added to the samples containing increasing concentrations of *lac* repressor at 21, 43, 65, 87, 148, 173, 259,

346 nM in *lac* binding buffer at a final volume of 20 μL. The reaction was incubated at room temperature for 20 min and the reaction mixture was separated by a 6% nondenaturing polyacrylamide gel electrophoresis as described above. The gel was stained with SYBR Green EMSA stain for 20 min and was visualized and documented using the FLA-3000 Fluorescent Imager first. The gel was then immersed in sufficient SYPRO Ruby EMSA protein gel stain to cover the gel and incubated for 3 h. Gels were washed, destained, and imaged as described above. Since incubation with SYPRO Ruby EMSA protein gel stain will wash away the SYBR Green EMSA stain, gels stained with SYBR Green EMSA stain must be imaged and documented before proceeding with protein staining.

2.7 Monitoring single-stranded DNA and single-stranded protein interactions

Increasing concentrations of T4 phage single-stranded protein, gp32, at 100, 300, 500, 700, 900, 1200, and 1500 nM were mixed with 50 nM of dT100 oligonucleotide in a final volume of 20 μL of TAMK buffer (33 mM Tris acetate, pH 7.6, 50 mM potassium acetate, 6 mM magnesium acetate). The zero protein concentration was used as a negative control. The reactions were incubated at room temperature for 5 min before loading onto a 6% nondenaturing polyacylamide gel. The gel was run at 150 V for 1 h in pre-chilled 0.5 × TBE buffer. The gel was stained with SYBR Green EMSA stain for 20 min first and the stained gel was visualized as described above. The same gel was then stained with SYPRO Ruby EMSA stain for 3 h and destained for 1.5 h before acquiring an image of the protein stain.

3 Results and discussion

3.1 Linear dynamic range of DNA and protein quantitation using SYBR Green EMSA stain and SYPRO Ruby EMSA stain in nondenaturing polyacrylamide gels

The ability to quantitatively analyze protein or DNA in an EMSA gel requires that there is a linear relationship between integrated fluorescence intensity of the dye stains and the amount of protein or DNA in the samples. For this purpose, we first examined whether SYBR Green EMSA stain and SYPRO Ruby EMSA stain and gel images provided a good linear measure of DNA and protein abundance. SYBR Green and SYBR Gold family of DNA stains are the most sensitive noncovalent dyes for detecting both double- and single-stranded DNA or RNA alone in nondenaturing electrophoretic gels, without staining protein [7, 8]. As little as 100 pg nucleotides per band can be detected using SYBR Green EMSA stain in a nondenaturing polyacylamide or agarose gel (data not shown). The linearity and detection limits of SYBR Green EMSA stain were determined by generating a series of dilutions ranging from 0.0 to 80 ng/lane of ds *lac* operator 40-mer oligonucleotides in *lac* binding buffer. The diluted amount of DNA was loaded onto a 6% nondenaturing polyacrylamide gel and the gel was stained with SYBR Green EMSA stain from the EMSA kit after gel electrophoresis. The results of the experiment are shown in Fig. 2A.

Quantitative data were manually determined with the Image Gauge Analysis software (Fuji Photo Film). The fluorescence intensity of stained DNA in each lane was estimated manually by drawing a box around the bands. Background, equivalent to the average intensity value of the gel, was subtracted from the intensity value of each DNA band. The background corrected intensity value of SYBR Green EMSA stain for each lane was plotted as a function of the amount of *lac* operator DNA as shown in Fig. 2B. As Fig. 2B demonstrates, the fluorescent intensity of SYBR Green EMSA DNA stain is a linear function of the amount of DNA in the gel with a linear correlation coefficient of greater than 0.98. The result shows that the amount of DNA and the fluorescence intensity of SYBR Green EMSA stain are linearly correlated over a 40-fold concentration range. Higher DNA concentrations were used to reveal any saturation effects in either the gel system or in the DNA staining. Stain saturation and limitations in the resolution of 6% nondenaturing polyacrylamide gels for double-stranded DNA start to occur at DNA concentrations exceeding 80 ng per lane (data not shown).

SYPRO Ruby EMSA protein stain is also one of the most sensitive noncovalent dyes for detecting proteins in a nondenaturing polyacrylamide gels that does not stain DNA or RNA [9]. In nondenaturing gels, the detection sensitivity of this fluorophore can be as low as 9.3 ng of a small protein (data not shown). The linear dynamic range and detection limits of DNA-bound protein were estimated in a similar manner as described above. Increasing amounts of *lac* repressor protein, in concentrations ranging from 0.0 to 346 nM of tetramer, were incubated with constant amounts of ds *lac* operator DNA (150 nM) for 20 min at room temperature before loading onto a 6% nondenaturing polyacrylamide gel as shown in Fig. 3A. After electrophoresis, the gel was stained with SYPRO Ruby EMSA stain for 3 h. In order to measure whether the SYPRO Ruby EMSA protein stain intensity is proportional to protein amount, the fluorescence signal of shifted *lac* repressor protein was quantified and plotted *versus* the protein amount. Fig. 3B demonstrates the depend-

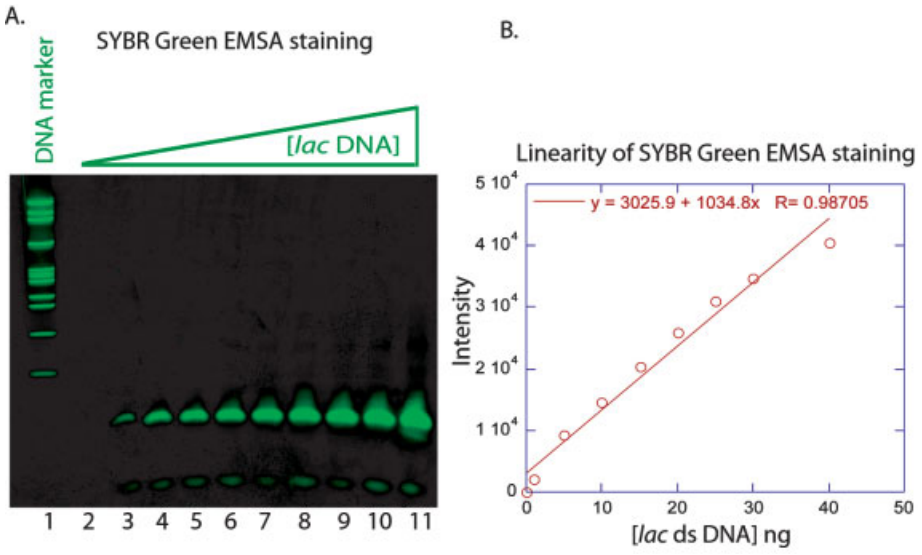

Figure 2. Sensitivity and linearity of SYBR Green EMSA stain for detecting a synthetic dsDNA 40-mer. (A) The 40-mer *lac* operator sequence was diluted and loaded onto a 6% nondenaturing polyacrylamide gel. The gel was stained with SYBR Green EMSA stain and imaged. Lane 1, DNA size markers 72–1353 bp, 2–11, increasing amounts of *lac* operator DNA 40-mer (0, 1, 5, 10, 15, 20, 25, 30, 40, and 80 ng). (B) Fluorescence intensity of SYBR Green EMSA stain for each gel lane was plotted as a function of the concentration of the *lac* operator.

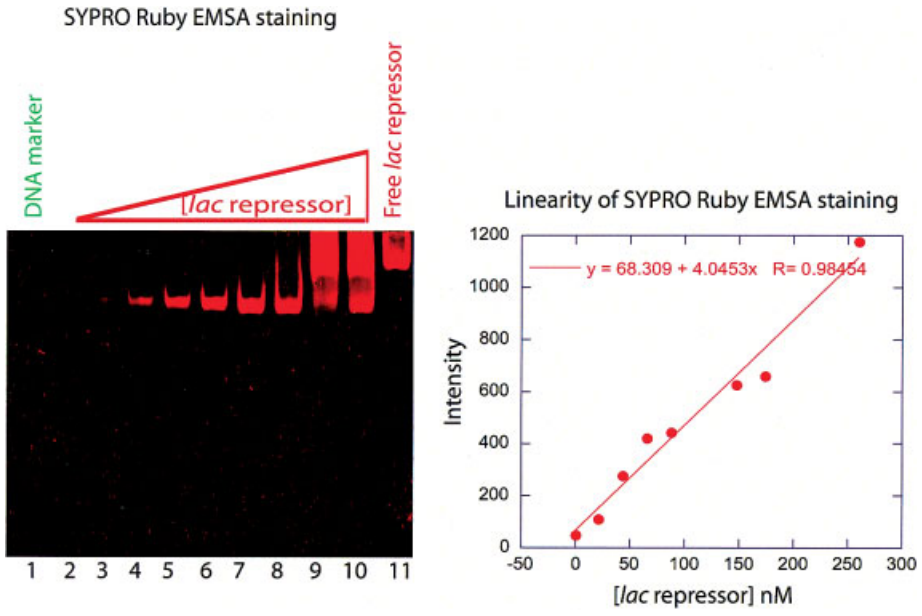

Figure 3. Sensitivity and linearity of SYPRO Ruby EMSA stain for detecting a DNA-bound *lac* repressor protein. (A) Approximately 150 nM of *lac* operator DNA was incubated with an increasing amount of *lac* repressor protein for 20 min. The reactions were then separated by electrophoresis using a 6% nondenaturing polyacrylamide gel. The gel was stained with SYPRO Ruby EMSA stain and destained as described in Section 2. Lane 1, DNA size markers; 2–10, 150 nM of *lac* operator interacted with increasing amounts of *lac* operon from 0, 21, 43, 65, 87, 148, 173, 259, to 346 nM in tetramer form; 11, 259 nM of *lac* operon tetramer only without any *lac* operator. (B) The fluorescence intensity of SYPRO Ruby EMSA stain for each gel lane was plotted as a function of concentration of the *lac* repressor protein.

ence of the ratio of normalized band intensities of SYPRO Ruby EMSA stain on added repressor. The figure shows that the intensity of SYPRO Ruby EMSA stain directly correlates with the amount of protein loaded on the gel with a linear correlation coefficient greater than 0.98 at *lac* repressor concentrations less than 259 nM. Further addition of *lac* repressor protein onto the gel produced very high molecular weight aggregates that could not be resolved by the gel system as shown in lanes 9 and 10 of Fig. 3A.

Figures 2B and 3B demonstrate that both SYBR Green EMSA DNA stain and SYPRO Ruby protein EMSA stain allow quantitation of DNA and protein concentrations,

respectively, on nondenaturing polyacrylamide gels, with a sensitivity limit of about 1 ng/band for DNA and 21 nM/band for *lac* repressor protein. A linear quantitation range of over 40-fold for the DNA stain and over 10 fold of DNA-bound *lac* repressor protein for the protein stain is obtained.

3.2 Double-stranded DNA and protein interaction system

lac repressor is a well-characterized tetrameric protein comprised of identical monomers with a molecular mass of 37.5 kDa [10–12]. The repressor binds to a 27 bp site on the DNA, the *lac* operator, and represses the transcription of the structural genes required for lactose utilization. In this study, we titrated the ds *lac* operator 40-mer with increasing amounts of *lac* repressor and the titration produces three bands. The free unannealed single-stranded oligomers migrated in the dye front and the free double-stranded *lac* operator 40-mers migrated slower than the single-stranded oligomer. The ds DNA-protein complexes were located near the top of the gel. The single-stranded oligonucleotide 40-mer serves as a control since it does not bind or binds very little to *lac* repressor at the concentrations used here. The DNA in the gel was stained with SYBR Green EMSA DNA stain first as shown in Fig. 4A, and then the same gel was stained with SYPRO Ruby EMSA protein stain (Fig. 4B). The pseudocolored image from Fig. 4A (green, represents *lac* operator) and 4B (red, represents *lac* repressor protein) were overlaid and the overlaid DNA-protein complexes were visualized as yellow color in the image, as shown in Fig. 4C.

The fluorescence emission of SYBR Green EMSA stain bound to nucleic acid is centered at 520 nm, while the fluorescence emission of SYPRO Ruby EMSA stain bound to protein is centered at 610 nm. These spectral

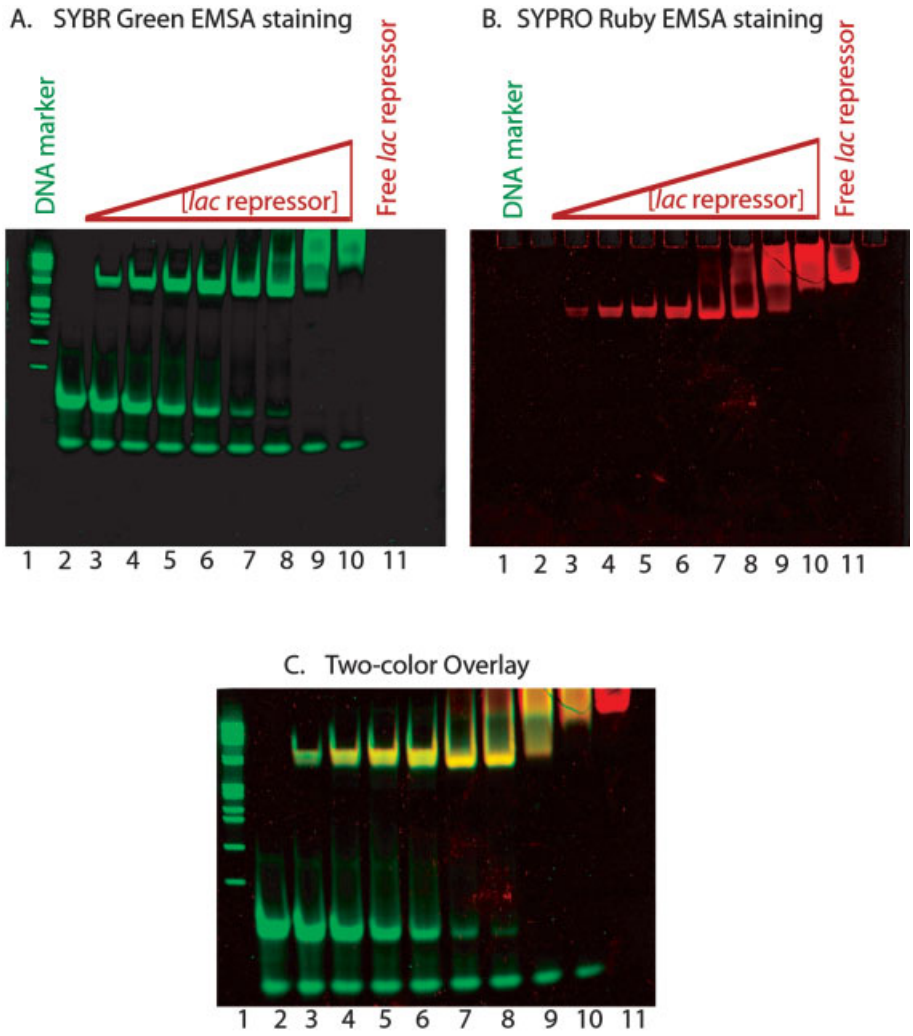

Figure 4. Titration of equal amounts of *lac* operator DNA with an increasing amount of *lac* repressor protein, imaged using a laser-based gel scanner. 150 nM *lac* operator DNA 40-mer was added to the samples containing different concentrations of *lac* repressor in *lac* binding buffer as described in Section 2. (A) Image of the EMSA gel stained with SYBR Green EMSA DNA stain. Lane 1, DNA size markers; 2–10, 150 nM of *lac* operator interacted with increasing amounts of *lac* operon from 0, 21, 43, 65, 87, 148, 173, 259, to 346 nM, in tetramer form; 11, 259 nM of *lac* operon tetramer only without any *lac* operator. (B) The same gel was stained with SYPRO Ruby EMSA protein stain and destained before taking an image. (C) The images in (A) and (B) were overlaid, with yellow color representing the DNA-protein complex. Gels were imaged using an FLA-3000 laser-based gel scanner.

characteristics make both stains compatible with a wide variety of gel imaging systems, ranging from conventional photography using UV epi- or transillumination to sophisticated laser-based or xenon arc-based scanning instruments. The gel shown in Fig. 4A was stained with SYBR Green EMSA stain and imaged using a Fujifilm FLA-3000 Fluorescent Imager first, then the same gel was placed onto a 300 nm-UV transilluminator (UVP, Upland, CA, USA) and an image was acquired using a Polaroid camera with black and white print film. For optimal sensitivity, a 490 nm long pass photographic filter, was used (Fig. 5A). The same gel was also imaged using a CCD camera system (Roche Lumi-Imager, Penzburg, Germany) as shown in Fig. 6A. After being imaged with the different instruments, the gel was stained with SYPRO Ruby EMSA protein stain and imaged again with the Fujifilm FLA-3000 Fluorescent Imager at excitation 473 nm and emission 580 nm first, followed by the UV transilluminator/ Polaroid camera system (Fig. 5B) and the CCD camera-based imaging system (Fig. 6B). Figures 4–6 demonstrate that the two-color EMSA staining kit may conveniently be used with most gel imaging instruments.

3.3 Monitoring a single-stranded DNA-protein interaction system

The value of the gel mobility shift assay as a qualitative probe of DNA-protein interactions is clearly established. The usefulness of this technique for the quantitative

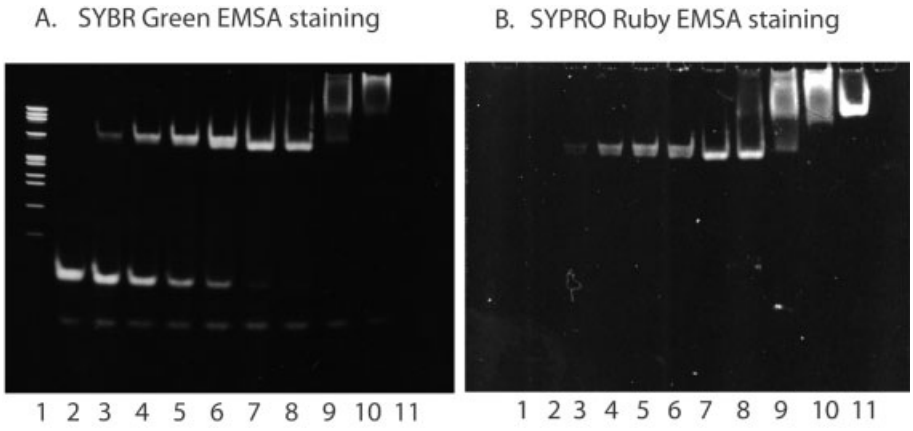

Figure 5. Titration of equal amounts of *lac* operator DNA with an increasing amount of *lac* repressor protein, imaged using a UV transilluminator/ Polaroid camera system. The same gel, which was stained with SYBR Green and SYPRO Ruby EMSA stains in Figs. 4A and B was placed on a UVP transilluminator and excited at 300 nm (UV-B). The image of DNA shown in (A) was obtained with a Polaroid camera and a 490 nm long pass filter (SYPRO photographic filter Molecular Probes, S-6656). The image of protein shown in (B) was acquired using the same instrument and filter. Exposure times for the black and white film were 2 s for (A) and 3 s for (B).

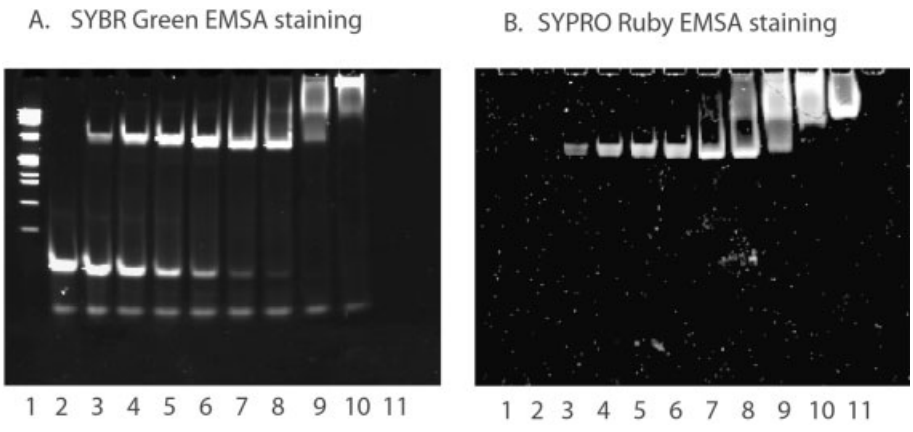

Figure 6. Titration of equal amounts of *lac* operator DNA with an increasing amount of *lac* repressor protein imaged using a UV transilluminator/ CCD camera-based system. Images of the same gel as in Figs. 4A and B, which were stained with SYBR Green EMSA stain and SYPRO Ruby EMSA stain, were acquired using the Lumi-imager (Roche Biochemicals) with a 2 s exposure time and using either (A) the instrument's 520 nm band pass emission filter in order to visualize DNA or (B) 600 nm band pass emission filter in order to visualize protein.

determination of cooperativity in the case of proteins that bind to multiple specific sites on DNA has also been demonstrated [13]. The EMSA is exceptional in its resolution of intermediate binding states (*i.e.*, states with some but not all sites bound) in a cooperative DNA-protein interaction system. T4 phage single-stranded DNA-binding protein, gp32, was used to demonstrate that the two-color EMSA kit is suitable for this task as well. Gp32 is the most studied single-stranded DNA binding-protein known to bind in a strongly cooperative fashion to single-stranded DNA.

The association constant ($K_c\omega_c$)) for gp32 has been established to be $\sim 10^8$ M^{-1} (in 0.1 M Na^+) and the binding site size is ~6 (±1) nucleotide residues/protein monomer [14]. Taking the neighboring effect as a consideration, there are roughly 10–13 gp32 proteins bound to a 100 nucleotide long dT oligonucleotide. A constant concentration (50 nM) of single-stranded dT100 oligomer was incubated with increasing amount of gp32 at room temperature. The reactions were separated by electrophoresis and the gel was stained with SYBR Green EMSA DNA stain first (Fig. 7A), then SYPRO Ruby EMSA protein stain (Fig. 7B). Fig. 7C is an overlay image of Figs. 7A and B and the DNA-protein complex is displayed as yellow color. The DNA-protein complexes in the gel are resolved as smears instead of a discrete single band, due to different intermediate bound states or different numbers of gp32 bound to different sites at a dT 100 oligomer. Figure 7 demonstrates a good example of the ability of the EMSA to resolve each step of binding of the DNA with protein.

4 Concluding remarks

EMSAs are often employed for the rapid characterization of sequence-specific DNA binding proteins. Typically, DNA and protein are mixed together, separated by gel electrophoresis, and DNA is detected by autoradiography of radiolabeled DNA. Covalent or noncovalent prelabeling of DNA with fluorophores is an alternative to using radiolabeled material, but the dyes can potentially interfere with the DNA-protein interactions under investigation [4, 5]. The EMSA procedure presented in this manuscript relies upon serial post-staining of gels with noncovalent fluorophores that are specific to DNA and to protein. Quantitative data concerning free nucleic acid, bound nucleic acid, bound protein, and free protein may be obtained

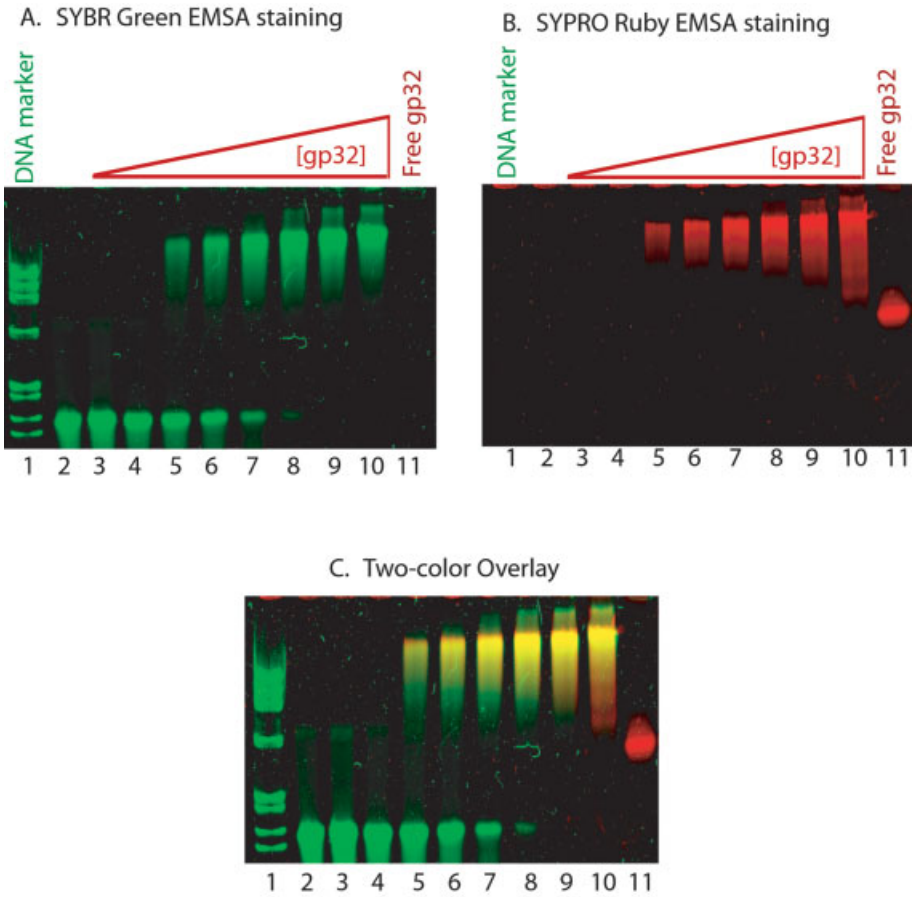

Figure 7. Titration of a single-stranded DNA against increasing concentrations of a single-stained binding protein (gp32). Fifty nM of dT100 oligonucleotide and various amounts of gp32 were incubated in 20 μL of TAMK binding buffer as described in Section 2. (A) The gel was stained with SYBR Green EMSA DNA stain and imaged. (B) Then, the same gel was stained with SYPRO Ruby EMSA protein stain and imaged. (C) The images of (A) and (B) were overlaid to visualize the DNA-protein complexes (yellow). Lane 1, DNA size marker; 2, 3, 0 nM gp32; 4–10, concentration of gp32 at 100, 300, 500, 700, 900, 1200, and 1500 nM; 12, 1200 nM gp32 but in the absence of oligo dT100. Gels were imaged using Fujifilm FLA-3000 Fluorescent Imager.

from a single experiment. The fluorescent EMSA method is rapid and easily analyzed on a broad spectrum of gel imaging platforms commonly available to molecular biologists.

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