AN IMPROVED MODIFIED PROTOCOL FOR SILVER STAINING OF DS DNA IN AGAROSE GEL

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ABSTRACT

Objective: Present study was aimed to develop a reproducible, cheap and sensitive method for silver staining of double stranded DNA in agarose gel.
Study Design: Experimental, repeated measure design.
Place and Duration of Study: Department of Genetics, University of Karachi, Karachi. This experimental study was conducted, from Nov 2013 to Jan 2014.
Material and Methods: The new method is the modification and improvement in the original method proposed in the literature. Samples of ds genomic DNA was run on a nondenaturing 1.5% agarose/0.5X TBE. After electrophoresis gel was fixed in 10% acetic acid and staining was performed using 1 gm % silver nitrate. DNA bands were developed using 1.5% NaOH. At each step shaking was done manually with a circular movement. The modified method was also compared with the ethidium bromide staining of the same samples of DNA.
Results: The modified method was proved to be as sensitive as the ethidium bromide with the advantage of having long term conservation ability of the gel. The main advantage of the protocol is the consumption of far less concentrations of silver nitrate and sodium hydroxide and the avoidance of the use of sodium thiosulphate.
Conclusion: This method was easily reproducible, sensitive, and cheap with improved conservation ability of gel. It also avoids use of hazardous, expensive and time consuming radioactive and fluorescent detection.

Keywords: Agarose gel, dsDNA, Silver staining protocol.

INTRODUCTION

Gel electrophoresis is a technique in which the macromolecules like nucleic acids and proteins are forced to move through the pores of a gelatinous medium agarose or polyacrylamide by applying an electrical current[1]. The macromolecules are separated across the gel on the basis of size, electric charge, and other physical properties. Agarose is usually used at concentrations between 0.7% and 3% which determines the pore size. Lower concentrations result in larger pore sizes, whereas higher concentrations result in smaller pore sizes. Silver staining is one of the widely used methods for DNA fragment detection that have been optimized to improve sensitivity of detection as well as to reduce the time needed for the whole protocol[2-4]. DNA staining in gels has historically been carried out using silver staining and fluore-scent dyes like ethidium bromide and SYBR Green I (SGI)[5]. Silver staining has also been used for protein detection in polyacrylamide gel[6].

Silver staining of DNA (and other biological samples) has several advantages[7,8]. First, the whole protocol can be performed on laboratory bench with ambient light. No sophisticated equipment, darkroom or UV illumination facilities are needed though not compromising the quality of image development and visualization. Second, the image is resolved with the best possible sensitivity and detail as silver is deposited directly on the molecules within the transparent gel matrix. Thus visualization is from the primary source that is DNA and does not suffer any degradation or blurring that can accompany secondary imaging devices like fluorescence, autoradiography etc. Silver staining offers similar sensitivity to autoradiography, but

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avoids radioactive handling, delays from development times and waste disposal issues. However the method is performed and compared for quality mostly in polyacrylamide gel to detect denatured Single stranded DNA[9,10].

Silver staining of double stranded (ds) DNA in agarose gel have undergone various modifications since its introduction[11-13]. Silver staining for native and denatured eucaryotic DNA in 1% agarose gel is described by Gottlieb and Chavko in 1987[14].

When compared with the silver staining of ds DNA in polyacrylamide gel a high background of silver on agarose matrix is encountered, resulting from its higher avidity for the silver ions[15-17]. Even when the procedure is optimized for DNA in agarose, silver staining of DNA will only give sensitivity similar to ethidium bromide[18]. However the method described by Gottlieb and Chavko for denatured DNA is fivefold more sensitive than ethidium bromide staining, with a detection limit of 2.5 ng for total DNA[14].

Nevertheless silver staining is the only available method when a permanent record of the gel is required but one of the limitation of the method is the inhibition of silver staining by nuecleotide base Thymine (T) in cases where one wants to visualize oligonucleotide[8,19]. A sensitive and simplified procedure of silver staining of nucleic acids in agarose has been proposed by Prieto et al (1997)[20]. Later a modification in the protocol was proposed by Zalazar *et al* (2001)[21]. In the present study protocol for silver staining was modified in order to reduce cost and duration of the procedure and enhance the safety of the user.

MATERIAL AND METHODS

Experimental study design with repeated measure was used. Independent variable was the amount and concentration of the chemicals used. Dependent variable was the DNA bands on agarose gel. As the same DNA samples were visualized with the different amount and concentration of chemicals used for silver staining that's why the experiment is a repeated measure design. We manipulated the one of the independent variable silver staining that is the concentration of chemicals used for the protocol and observe when the DNA bands are more clearly stained with silver. In order to validate our results DNA bands with silver staining were compared with fluorescent staining.

The whole experiment was conducted at the Department of Genetics University of Karachi, Karachi, from Nov 2013 to Jan 2014.

Samples were collected from students working at the Department of Genetics University of Karachi, Karachi. Only those students were included who were normal and healthy with no history of medical or familial disorder.

DNA samples were extracted from blood using protocol described in Sambrook *et al* with little optimization[22]. Peripheral blood (300 µl) was added to 900 µl of RBC lysis solution (0.829gm NH_4Cl (MERCK), 0.1 gm Disodium EDTA (SIGMA), 0.03 gm $NaHCO_3$ (MERCK) for 100 ml of solution, pH=7.4). It was mixed and incubated for ten minutes and then centrifuged for 1 minute at 16000g. A whitish pellet of DNA was formed. 300 µl of cell lysis solution (1M Tris Cl (RESEARCH ORGANICS) pH=8, 0.5M EDTA pH=8, 0.5% SDS (SIGMA)) was added and incubated for ten minutes to lyse the cells. Ammonium acetate (MERCK) 7.5 M was added to precipitate proteins. The mixture was vortexed and centrifuged for ten minutes at 16000g. Proteins were precipitated at the bottom as dark brown pellet. Supernatant having DNA was transferred to the tubes containing 100% isopropanol. It was mixed by inverting many times and then kept in freezer for at least 30 minutes. After 30 minutes it was centrifuged at 16000g for ten minutes. DNA was precipitated at the bottom of the tube as pearly white pellet. 70% ethanol was used to wash the pellets. Pellets were air dried and then stored in TE buffer with pH= 7.6 (1M Tris Cl pH=0.6, 0.5M EDTA pH=8, sterile deionized water) at -20°C.

Samples of ds genomic DNA was run on a non-denaturing 1.5% agarose in 0.5X TBE

solution. Thickness of the gel was 0.4-0.5 cm to ensure a low background. Electrophoresis was performed at 72 volts for two and a half hrs.

- After electrophoresis the gel was submerged in 10% acetic acid (MERCK) at room temp (RT) for 10 minutes, with continuous shaking.
- The gel was briefly (for 30 sec) rinsed with distilled water.
- The gel was then submerged in 1 gm% silver nitrate* (BDH CHEMICALS) solution at RT, with continuous shaking for 20 minutes. Then the gel was removed from silver nitrate and rinsed repeatedly with distilled water (5 changes, 30 sec each).
- After rinsing, the gel was submerged in 1.5% NaOH* (MERCK) solution with 37% formaldehyde (MERCK) (0.2 ml/100 ml) at RT. Shake the gel continuously till the bands (light

Table-I: Protocols and observations of silver staining of ds DNA in agarose gel.

	Method 1	Method 2	Method 3	Method 4	Method 5
Fixative (acetic acid)	10%	10%	10%	10%	10%
Rinse (distilled water)	Once (30 sec)	Once (30 sec)	Once (30 sec)	Once (30 sec)	Once (30 sec)
Staining solution ($AgNO_3$)	3 gm%	3 gm%	1.5gm%	1gm%	0.15 gm%
Rinse (distilled water)	5 times (30 sec each)	10 times (30 sec each)	10 times (30 sec each)	10 times (30 sec each)	5 times (30 sec each)
Developing solution (NaOH with 37% formaldehyde)	1.5 gm%	1.5 gm%	1.5 gm%	1.5 gm%	1.5 gm%
DNA Sample	2 µl	2 µl	2 µl	2 µl	2 µl
Observations					
Background Colour	**Gray**	**Gray**	**Light Gray**	**Dark brown**	**Yellow**
*Homogeneity of background	Not homogeneous	Not homogeneous	Less homogeneous	Homogeneous	Homogeneous
Gel Translucency	Not translucent	Not translucent	Not translucent	Translucent	Translucent
Colour of DNA bands	Black	Black	Black	Black	White
DNA bands on white light	Less visible	Less visible	Less visible	More prominent	More prominent

Table-II: Methods for silver staining of ds DNA in agarose gel.

Steps	Method 1[20]	Method 2[21]	Method 3 Present study
Fixation	10% acetic acid at RT for 10 min.	Gel is submerged in 18 mm Ag NO3 in 10% acetic acid for 10 min.	10% acetic acid at RT for 10 min.
Rinse	Once briefly Iin redistilled water at RT		Once for 30 seconds with continuous shaking
Staining	20 mM Ag NO_3 for 20 min. at 40°C		1 gm% $AgNO_3$ with continuous shaking for 20 min.
Rinse	10 changes 30 seconds each in redistilled water	3-5 changes 30 seconds each in redistilled water	5 changes 30 seconds each in redistilled water
Development	3% NaOH with 37% formaldehyde (1 ml/L) at RT	3% NaOH with 37% formaldehyde (0.5 ml/L) and 2 mg/L sodium thiosulphate at RT	1.5% NaOH with 37% formaldehyde (0.2 ml/100 ml) at RT with continuous shaking
Stop	-	Add 10% acetic acid to the developing solution	Remove as soon as the bands appear. Submerged in distilled water.

color against a yellow homogeneous background) appeared. It took 2-3 minutes for the bands to appear.

- The developing step can be stopped by the addition of the fixative (10% acetic acid) which in turns helps in diminishing the high background on the gel.
- The gel was preserved in methanol: water: acetic acid (5:4:1) solution with 2.5% glycerine.

RESULTS

Results of the various modifications of protocol are shown in the table-I. It is evident that modifications used in Method 4 (table-I) is the most appropriate method as it gave out transparent and homogeneous background with dark and clear DNA bands. Therefore we recommend Method 4 for staining ds DNA in agarose with silver nitrate.

To compare the sensitivity of our silver staining protocol with the conventional fluorescent staining with ethidium bromide, the same preparations of DNA were stained by both the procedures. Result showed that the sensitivity of the silver stained procedure (fig-1) is equal to that of conventional fluorescent method (fig-2).

DISCUSSION

Silver staining of ds DNA is performed with various protocols, some of which are actually a modification of a single protocol. In this study we performed multiple experiments to get the most suitable method of silver staining ds DNA with less time consumption as well as less consumption of chemicals. The advantages of the proposed method are many folds. It is simple, inexpensive and reproducible. All the steps of the experiment were done at room temperature avoiding the need of special equipment to maintain temperature. Moreover, less concentration of staining and developing solutions is used making it feasible and economic. One of the main advantage of the proposed protocol from safety point of view is that it eliminates the use of sodium thiosulphate, carcinogenic ethidium bromide and UV transilluminator. For storage purpose silver stained gel can be immersed in 10% acetic acid solution for a long period of time. This does not affect the sharpness of the bands rather the chemical enhances it.

A comparison of methods proposed by Prieto *et al* and its modification Zalazar *et al* and

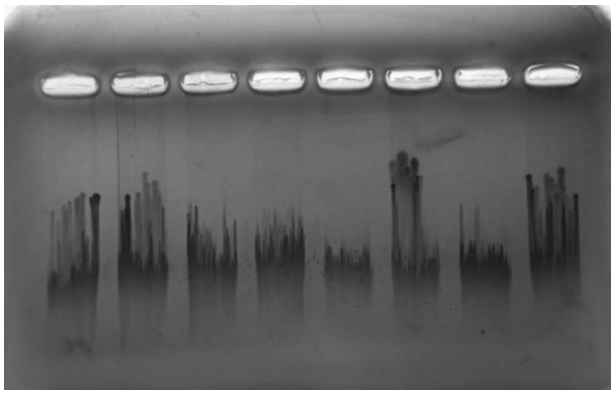

Figure-1: Silver Staining staining of ds DNA in 2% agarose gel.

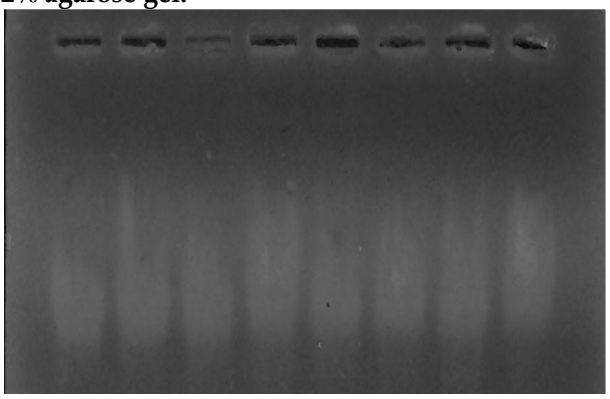

Figure-2: Ethidium Bromide staining of ds DNA in 2% agarose gel.

the modifications proposed by our lab is given in the table-II. When we compare our modified protocol with the above two mentioned studies, we observed that in the protocol proposed in the present study less concentrated solution of silver nitrate and NaOH were used. The concentration of silver nitrate described in protocol of Prieto *et al* is 20 mm, which is equivalent to 3.39 gm%. While NaOH solution used in Prieto *et al* protocol is 3%. Moreover a simple step that is likely to be neglected by the experimenters in the lab that is manual shaking with a circular movement in alternate clockwise and counter clockwise direction of the plate containing the gel

at each step, proved to be very effective for DNA bands visualization. It has been proposed that variation in color intensity of silver bands of DNA and Protein is due to diffractive scattering of silver grains of different sizes[23]. Manual shaking may help to disperse them uniformly.

CONCLUSION

This method is easily reproducible, sensitive, and cheap. It has the advantage of conservation ability as well as avoidance of hazardous, expensive and time consuming radioactive and fluorescent detection. The whole procedure of staining takes about 45-50 minutes. Moreover it does not require special equipments, therefore feasible to be used routinely in the lab to detect DNA in agarose gel.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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