Application of Linear Polyacrylamide (LPA) Matrix in Cotton Chromatin Immunoprecipitation to Increase Sheared DNA Isolation Efficiency

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Abstract: Linear Polyacrylamide (LPA) is one of the polymers used as a yield-enhancing agent in DNA isolation of short and small molecular weight DNA molecules. The Chromatin immunoprecipitation (ChIP) method is widely used to elucidate DNA-Protein interactions. In ChIP, which consists of many successive steps, it is an important problem to experience losses due to the small size of the DNA fragments during the re-isolation of the sheared DNA fragments. In this study, the effect of using LPA matrix on the isolation of small molecular weight DNA fragments with higher efficiency in the ChIP method applied to cotton plant, which is a eukaryotic organism with high commercial value in our country and in the world, was investigated. Here, the ChIP method was adapted and applied till the DNA fragmentation process by ultrasonication and the isolation of the fragmented DNA molecules. In statistical analyzes, the concentrations of DNA isolates used at the beginning of the ChIP experiment and the DNA molecules mechanically fragmented by ultrasonication, measured by the Nanodrop, were compared in ng/µl. Experimental and statistical analyzes showed that LPA application increased DNA isolation efficiency by 0.59 times. Based on these data, it has been proven that using LPA in ChIP method with cotton will directly affect the success of the experiment and contribute to get much more efficient results. This study is unique in that it focuses on improving the yield of sheared DNA isolation in direct cotton plant-specific ChIP application.

Keywords: Linear polyacrylamide, chromatin immunoprecipitation, Cotton, Sheared DNA isolation, Efficiency

Pamukta Kromatin İmmünopresipitasyon Yönteminde Kırpılmış DNA İzolasyon Verimini Arttırmak için Lineer Poliakrilamid (LPA) Matriksinin Uygulanması

Öz: *Lineer Poliakrilamid (LPA), kısa ve küçük moleküler ağırlıklı DNA moleküllerinin DNA izolasyonunda verimi arttırıcı ajan olarak kullanılan polimerlerden biridir. Kromatin imminopresipitasyon yöntemi DNA-Protein etkileşimlerinin aydınlatılmasında yaygın olarak kullanılan etkili ve verimli bir yöntemdir. Ardışık birçok aşamadan oluşan ChIP yönteminde kırpılmış DNA parçalarının tekrar izole edilmesi aşamasında DNA parçalarının boyutlarının küçük olmasına bağlı olarak kayıplar yaşanması önemli bir problem oluşturmaktadır. Bu çalışmada ülkemizde ve dünyada ticari değeri çok yüksek bir ökaryot organizma olan pamuk bitkisine yönelik uygulanan ChIP yönteminde kırpılmış küçük moleküler ağırlıklı DNA parçalarının daha yüksek verimle izole edilebilmesinde LPA matriksi kullanılmasının etkisi araştırılmıştır. Burada deneysel olarak ChIP yöntemi uyarlanarak ultrasonikasyon yöntemiyle DNA parçalama işlemi ve ardından parçalanmış DNA moleküllerinin izolasyonu aşamalarına kadar uygulanmıştır. İstatistiksel analizlerde ChIP deneyinin başlangıcında kullanılan DNA izolatlarının ve ultrasonikasyonla mekanik olarak parçalanmış DNA moleküllerinin Nanodrop cihazıyla ng/µl cinsinden konsantrasyonları karşılaştırılmıştır. Deneysel ve istatistiksel analizler, LPA uygulamasının DNA izolasyon etkinliğini 0,59 kat arttırdığını göstermiştir. Bu verilere dayanarak, pamuk bitkisine yönelik ChIP yönteminde LPA kullanılmasının deney başarısını doğrudan etkileyeceği ve çok daha verimli sonuçlar alınmasına katkı sağlayacağı ispatlanmıştır. Bu çalışma doğrudan pamuk bitkisine özel ChIP uygulamasında kırpılmış DNA izolasyonundaki verimi arttırmaya odaklanılması açısından özgündür.*

Anahtar Kelimeler: *Lineer poliakrilamid, Kromatin immünopresipitasyonu, Pamuk, Kırpılmış DNA izolasyonu, Verimlilik*

INTRODUCTION

Chromatin immunoprecipitation (ChIP) method is one of the most effective and efficient methods used to investigate DNA-protein interactions (Haring et al.,2007; Kuo and Allis, 1999; de Jonge et al., 2020). The ChIP method provides the explanation of DNA-protein centered events such as gene regulation, global regulator-regulation analysis in prokaryotic and eukaryotic organisms (Euskirchen et al., 2007; Wang et al., 2011; Barrett et al., 2011). Today, there are many commercial kits for microorganisms plants, animals and human cells, and each commercial ChIP kits or different manuel ChIP methods suggested in the literature have some superior features as well as negative aspects (Holliday et al., 2021; Greulich et al., 2021; Liu et al., 2012). Unfortunately, there is no fully optimized ChIP procedure or commercial product that we can call perfect for every living species yet (Martin et al., 2019; Collas, 2010). Therefore,

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new scientific approaches related to ChIP methods are increasing day by day (Harada et al., 2021; Spencer et al., 2003). A ChIP method is an experimental process consisting of an average of 10 consecutive steps (Avcı, 2015). It is essential for a ChIP experiment to be successful that each of these steps is 100% efficient (Sırma-ekmekçi et al., 2014).

For that reason, optimizing these stages one by one before starting ChIP applications increases the experimental efficiency and reliability in ChIP methods (He et al., 2021). In ChIP methods, both *in vivo* and *in vitro* processes can be monitored (Lukoseviciute et al., 2020; Sullivan et al., 2020; Das et al., 2004; Dey et al., 2012). During in vivo applications, when the target cell reaches a certain stage of growth and development or reproduction, the cell content is fixed by treatment with formaldehyde (Schoppee Bortz and Wamhoff, 2011). In this case, proteins such as transcription factors and histone proteins that are reversibly bound to DNA and released are bound to DNA by covalent bonds and cannot be separated from DNA (Sauvé et al., 2004; Posé and Yant, 2016; Aparicio et al., 2005).

These fixed cells are lysed by mechanical or enzymatic means until the DNA is fragmented (Oshino and Fujii, 2009). Protein-bound DNA fragments are isolated from the medium by first binding antibodies specific to that protein and then using the antibody-specific isolation matrix used (Ranawaka at al., 2020; Schoppee Bortz and Wamhoff, 2011). The isolated DNA-Protein-Antibody complex is first separated from the matrix with the appropriate isolation method and transformed into only DNA-protein complex, and finally, pure DNA molecule is obtained by denaturation and elimination of the protein (Weinmann and Farnham, 2002). After this stage, advanced molecular analysis and applications such as DNA sequence analysis and cloning can be performed (Schmidt et al., 2009; Ho et al., 2011; Pillai and Chellappan, 2009). During in vitro applications, DNA is first isolated from plant, animal or human cells by routine methods (Avcı, 2015). DNA isolates are sheared by enzymatic or mechanical applications until they reach a certain size (Hoshino and Fujii, 2009). On the other hand, the recombinant protein specific for the target gene is isolated. Then, the sheared DNA fragments and this protein are allowed to react in appropriate binding buffers in a test tube (Molle et al., 2003). It is waited for the appropriate time for the proteins to bind to the relevant target regions of the DNA (Fawcett et al., 2000). Then, with the application of formaldehyde, the DNA-Protein complexes are fixed by covalent bonds. After formaldehyde fixation, protein isolation is done with the help of protein-specific antibody or by utilizing the structural feature of the recombinant protein. Thanks to this special protein isolation, only DNA molecules included in DNA-Protein complexes are isolated (Avcı, 2015). DNA molecules that do not bind to any protein are discarded. Then the proteins bound to sheared DNA are eliminated by denaturation thermally or enzymatically and DNA isolation method (Singh and Szabó, 2012). Purely obtained DNA fragments are analyzed by DNA sequence analysis and molecular methods (Molle et al., 2003).

In the chromatin immunoprecipitation methods mentioned above, especially in the in vitro ChIP method, the sheared DNA molecules are reduced to small units such as 300-1000 bases on average (Molle et al., 2003). Compared to normal DNA isolation, this situation negatively affects the DNA isolation efficiency due to the short chain lengths and small molecular weights (Avcı, 2015; Gaillard and Strauss, 1990). For this reason, the adaptation and application of alternative approaches to increase the yield in the isolation of small molecular weight DNA molecules to the ChIP method may be considered as an important strategy. Technically, each DNA strand obtained in the ChIP method is unique and each obtained ChIP-DNA strand theoretically carries a specific DNA sequence to which a protein directly binds (Avcı, 2015). Thus, it is an inevitable obligatory to make ChIP-DNA isolations without wastage and with 100% efficiency.

According to the IUPAC system, Polyacrylamide is a polymer (-CH2CHCONH2-) composed of acrylamide subunits called “poly(2-propenamide)” or “poly(1-carbamoylethylene”) (Erkekoglu and Baydar, 2014; Bovin, 1998). Linear polyacrylamide (LPA), a different form of polyacrylamide, is widely used in molecular biology research (Gaillard and Strauss, 1990). LPA is a special polymer obtained by mixing polyacrylamide molecules with Tris-HCl, Na-acetate, ammonium persulfate, ethanol, water, TEMED and EDTA (Gaillard and Strauss, 1990). LPA plays a role as a neutral carrier matrix in the precipitation of picogram nucleic acids with ethanol and is highly effective in isolation efficiency. Apart from LPA as carrier matrix, different macromolecules such as glycogen and tRNA are also used for the same purpose (Bartram et al., 2009). However, since these alternative macromolecules have some advantages and disadvantages, the use of LPA is more appropriate. Cotton is a plant of the genus Gossypium, belonging to the Malvaceae family, in the order Malvales (Erarslan and Koçyiğit, 2019; Güvercin and Sunulu, 2010; Samancı and Özkaynak, 2000; Ayaz and Emiroğlu, 2016). Cotton (Gossypium spp.) is a very important fiber crop cultivated in approximately 70 countries and forms the livelihood of 180 million people (Tian and Zhang, 2021; Wang et al., 2020). Cotton is included in both fiber and oil crops among industrial crops (Peláez-andérıca et al., 2018). Many structures of cotton such as fiber, seed, oil and pulp are used (Fidan and Ertaş, 2020; Haliloğlu et al., 2020). For these reasons, it is a plant with a very high economic value and both traditional and molecular breeding studies on cotton plant are increasing day by day (Tokel, 2021; Köken and İlker, 2020). The aim of this study is

to determine the effect of using LPA as a carrier molecule on yield in order to increase the yield of DNA isolation for "sheared DNA molecules" in chromatin immunoprecipitation methods to be applied in cotton plant.

MATERIAL AND METHODS

Plant Samples

The plant material used as a DNA source consists of cotton leaves collected from cotton plants grown in pots in Aydın Adnan Menderes University, Faculty of Agriculture, Department of Agricultural Biotechnology (Figure 1). The plant samples were brought to the laboratory by paying attention to the cold chain principles. Cotton plant samples, which were kept in cold room conditions and in an environment without light, were analyzed as quickly as possible.

Figure 1. Cotton samples used as DNA source.

DNA Isolation

DNA isolation was done manually using a method adapted from the CTAB (cetyltrimethylammonium bromide) method developed by Doyle and Doyle (1990). According to this method, after the cotton leaves were thoroughly crushed with liquid nitrogen in a porcelain mortar, they were weighed as 200-300 mg and transferred to microcentrifuge tubes. 0.7 ml of CTAB buffer (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, Ph 8.0) was added to the tubes and mixed well by vortexing. Afterwards, the samples were kept in a water bath at 65 °C for 1 hour by mixing every 10 minutes. After adding an equal amount of Phenol:chloroform:isoamyl alcohol (25:24:1) mixture to the tubes, they were mixed by inverting and centrifuged at 13500 rpm for 30 minutes.

The light colored supernatant formed in the tubes was taken and transferred to a new 1.5 ml microcentrifuge tube. After adding in a volume of 3M NaAc (pH 5.2) solution (~50 µl) corresponding to 10% of the transferred supernatant volume, the tubes were mixed by inverting. An equal volume of cold isopropanol (~500 µl) was added to the same mixture and mixed by inverting, and then it was kept at -20 ˚C for 15 minutes. Then, it was centrifuged at 12.000 rpm for 10 minutes, allowing the DNA molecules to settle to the bottom and the supernatant was poured. After washing the tubes with a solution containing 1 ml of ethanol (76%)/Ammonium acetate (10 mM), they were dried in a thermomixer and 500 µL of TE solution (10 mM Tris-HCl, 0.1 mM EDTA) was added. After this step, the pellet was dissolved by pipetting to remove the RNA residues and 5 µL of RNase A (10mg/ml) was added to it. After ensuring homogeneous dispersion of RNase A by inverting, it was incubated at 37 ˚C for 30 minutes and 50 µl of NaAc (3M) was added on it and turned upside down again.

1 ml of 90% ethanol was added and mixed by inverting again. DNA was precipitated by centrifugation at 13000 rpm for 10 min. After discarding the ethanol in the upper part, it was centrifuged again at 12000 rpm for 1 minute to remove the ethanol thoroughly. Ethanol was allowed to evaporate at 37˚C in a thermomixer device, 200 µL of TE was added to the pellet and the pellet was resuspended. The isolated DNA isolates obtained were stored at +8 ˚C for downstream processes.

Qualitative DNA Analysis

DNA molecules were visualized by agarose gel electrophoresis after isolation and in CHIP applications. Since the target DNA size should be 300-1000 bp in the ChIP method, the density of the agarose gel to be used was adjusted as 1.25-1.5%. Tris-Acetic acid-EDTA (TAE) and Tris-Boric acid-EDTA (TBE) buffers were used as the running buffer. DNA samples run at 100 Volts for 40 minutes were visualized in a UV transilluminator.

Quantitative DNA Analysis

Thermo Scientific NanoDrop device, a full spectrum UV-Vis spectrophotometer, was used to calculate and evaluate the purity of DNA samples. Measurements were made for 1 microliter volumes and obtained in ng/ul.

DNA Shearing Optimisation

In ChIP experiments, whether in vivo or in vitro, DNA molecules are recommended to be in the range of 300-500 bp (Molle et al., 2003). In this study, instead of performing a normal whole ChIP application, a partial ChIP adaptation was made to focus only on the LPA application. In order to make an ideal modeling, DNA molecules obtained from plant samples were first sheared by sonication method. Preliminary testing was done to determine the ideal shearing conditions. In this process, the number of pulses and rests and their duration were determined. For each consecutive pulse/rest period, 10 µl of sample was taken during rest and stored for analysis in agarose gel. The collected samples were labeled sequentially and loaded onto a single gel, and it was determined how long the 300-500 bp range was obtained. After this stage, all sonication processes were performed according to this determined standard.

Preparation of Lineer Polyacrylamid (LPA) Solution

Linear polyacrylamide (LPA) solution was prepared according to the experimental protocol used by Avci (2015) and Gaillard and Strauss (1990). Accordingly, 5% acrylamide solution was prepared in 1 mM EDTA, 20 mM Na-acetate and 40 mM Tris-HCl solutions without using bis-acrylamide and the pH was adjusted to 7.8. Then, 1/1000 volume of TEMED was added to the mixture along with 1/100 volume of 10%

ammonium persulfate. The mixture was allowed to become viscous by waiting 30 minutes for it to polymerize. For precipitation of the mixture, 2.5 volumes of ethanol was added and centrifuged. The resulting pellet was dissolved overnight by shaking with 20 volumes of dH2O overnight. The resulting 0.25% LPA solution was stored at +4˚C to be used in DNA isolation and ChIP analysis.

ChIP Semi-application

In order to establish a connection between normal ChIP methods and this research, and for the reliability of the results, it is important to perform the ChIP steps up to the stage where LPA will be applied (Avci, 2015). Therefore, different strategy possibilities can be evaluated. One of these approaches is to break up the cells of the same plant by ultrasonication to obtain a supernatant after DNA isolation and to provide sufficient incubation environment for the interaction of the proteins in the supernatant with the isolated DNA. Another approach is to treat with formaldehyde before starting the DNA isolation and after the binding process, break up the DNA molecules by ultrasonication and then apply LPA. A third and simpler approach is, without any protein treatment, to shear the isolated DNA molecules directly by ultrasonication and then applicate the LPA. The third is the approach applied in this study.

ChIP DNA Isolation

In the ChIP method, genomic DNA molecules are sheared mechanically by ultrasonication or enzymatically by various restriction endonuclease enzymes. LPA solution was used to isolate these small-sized DNA molecules, which were re-isolated at a certain stage of the ChIP method, with higher performance. In practice, while LPA was not added to the tubes in the control group (0 ng/µl), 20 (0 ng/µl) LPA solution was added to the tubes in the LPA group.

Statistical analysis

In the statistical analysis of the data obtained, the concentrations of DNA isolates measured in ng/ul in the Nanodrop device were taken into account. One-Way ANOVA, TUKEY and Independent-Sample T-Test analyzes were made with SPSS 16.0 program.

RESULTS

In order to investigate the potential of linear polyacrylamide to increase the yield during the isolation of sheared DNA fragments in the CHIP method, DNA isolation was first made by grinding the leaves of the cotton plant. For this purpose, the ground leaf samples were weighed 200-300 mg and transferred to 2 ml microcentrifuge tubes and DNA-isolation was performed from plenty of microcentrifuge tubes (Figure 2).

To mimic ChIP application, DNA isolates were sheared to 1000-500 bp by ultrasonication (Figure 3). Linear polyacrylamide was prepared according to the formulation and application specified in Gaillard and Strauss (1990) and Avci (2015) (Figure 4).

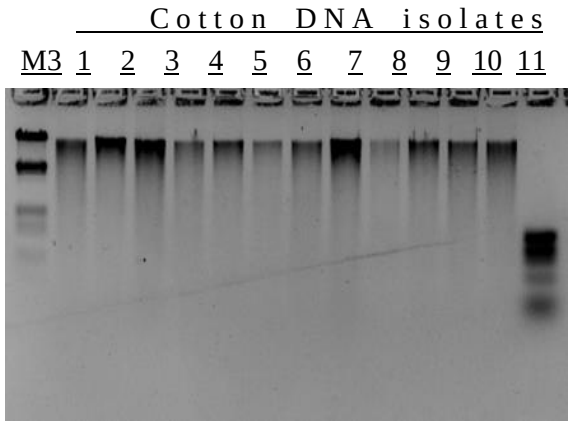


Figure 2. Agarose gel electrophoresis analysis of Cotton DNA isolates. M3 : Marker-3 (Thermo), M9: Marker-9 (Thermo) 1-12: DNA isolates.

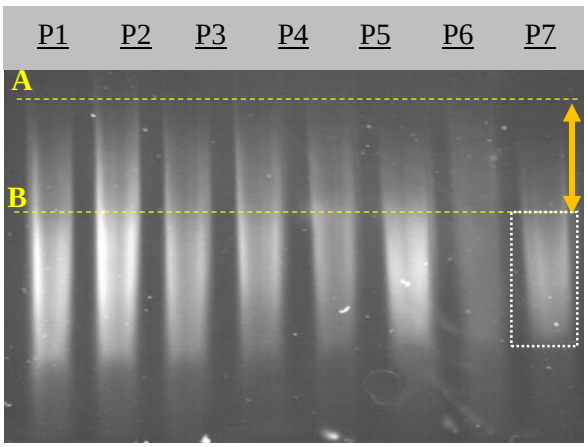


Figure 3. DNA shearing optimizasion by ultrasonication. P1-P8: Refers to each sample taken consecutively after the pulse and rest period. A and B: Shows us the shortening in DNA size due to fragmentation. White rectangle: DNA fragments in the range of 500-1000 bp.

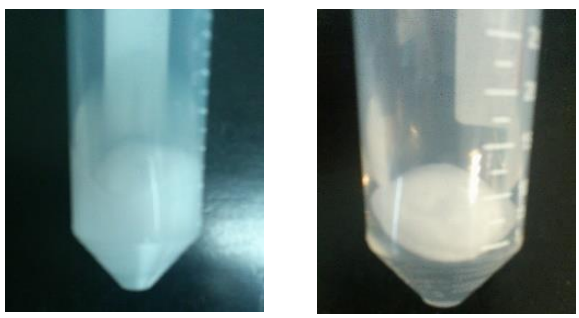
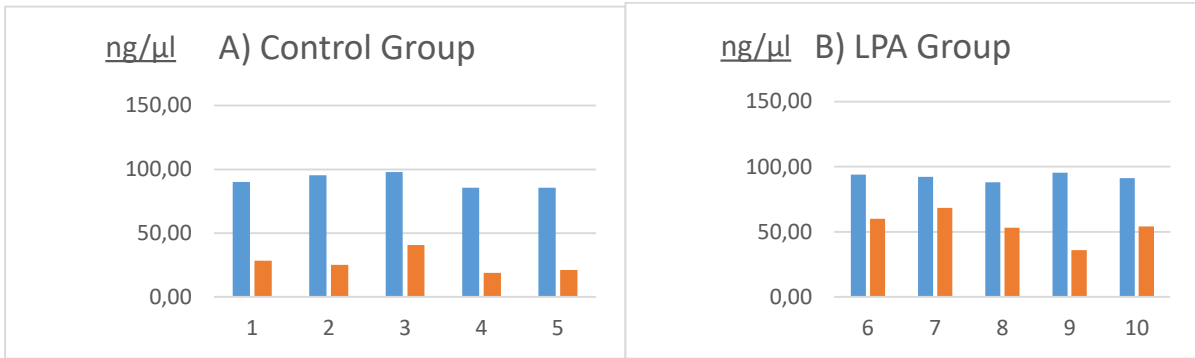


Figure 4. Appearance of the LPA pellet formed during the preparation of linear polyacrylamide. This pellet is completely dissolved in the last step of the LPA solution preparation.

DNA concentration measurements were made before and after ChIP application. In the concentration measurements made with the Nanodrop device in ng/ml (Table 1), the measurement results after the genomic DNA isolation process (before the ChIP application) were 90.15, 95.40, 98.00, 85.70, 85.60 ng/ml in the control group, while the results in the LPA group were 94.00, 92.20, 88.00, 95.45, 91.33 ng/ml. The mean DNA concentration of the control group was 90.97 ng/ml, while it was 92.20 ng/ml for the LPA group (Graph 1).



Graph 1. Comparison plot of DNA loss between ChIP initial DNA concentrations and sheared DNA concentrations obtained in ChIP adaptation. 1-5: Tube numbers

Table 1. Comparison of the concentrations obtained in DNA isolations before and after ChIP semi-application using LPA and without LPA.

	No	DNA concentration (ng/µl)					
		After DNA isolation	Avarage after DNA isolation	After ChIP DNA isolation	Avarage after ChIP DNA isolation	DNA loss	Avarage of DNA loss
Control	1	90,15		28,45		61,70	
	2	95,40		25,15		70,25	
	3	98,00	90,97	40,63	20,84	57,37	64,13
	4	85,70		18,8		66,90	
	5	85,60		21,17		64,43	
LPA	6	94,00		60		34,00	
	7	92,20	92,20	68,45	54,34	23,75	
	8	88,00		53,14		34,86	37,85
	9	95,45		36		59,45	
	10	91,33		54,12		37,21	

Table 2. Results of Tukey test

	(I) Grup	(J) Grup	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
TUKEY HSD	1	2	64,0240 0'	5,03517	.000	49,6183	78,4297
		3	-1,2260 0	5,03517	.995	-15,6317	13,1797
		4	36,6280 0'	5,03517	.000	22,2223	51,0337
	2	1	-64,0240 0'	5,03517	.000	-78,4297	-49,6183
		3	-65,2500 0'	5,03517	.000	-79,6557	-50,8443
		4	-27,3960 0'	5,03517	.000	-41,8017	-12,9903
	3	1	1,2260 0	5,03517	.995	-13,1797	15,6317
		2	-65,2500 0'	5,03517	.000	50,8443	79,6557
		4	-37,8540 0'	5,03517	.000	23,4483	52,2597
	4	1	-36,6280 0'	5,03517	.000	-51,0337	-22,2223
		2	27,3960 0'	5,03517	.000	12,9903	41,8017
		3	-37,8540 0'	5,03517	.000	-52,2597	-23,4483

*. The mean difference is significant at the 0.05 level

After DNA isolation for sheared DNA molecules during ChIP adaptation, DNA concentrations in the control group were determined as 28.45, 25.15, 40.63, 18.80, 21.17 ng/ml, and 60.00, 68.45, 53.14, 36.00, 54.12 ng/ml in the LPA group, respectively. After ChIP application, the mean DNA concentration of the control group was 20.84, while 54.34 ng/ml of the LPA group. In this case, when DNA concentrations loss due to ChIP application and DNA isolation is examined, the loss amounts were 61.70, 70.25, 57.37, 66.90, 64.43 ng/ml in the tubes of control group, 34.00, 23.75, 34.86, 59.45, 37.21 ng/ml in the tubes of LPA group.

When the loss in DNA isolations is compared, it is seen that the DNA concentration in the tube decreased from 90.97 ng/ml to 20.84 ng/ml in the control group before the ChIP experiment, and the average DNA loss was 64.13 ng/ml. However, in the LPA group, the average DNA concentration of 92.20 ng/ml in tubes before ChIP application decreased to 54.34 ng/ml in isolation using LPA after ChIP adaptation, and the average DNA loss was 37.85 ng/ml.

In order to determine whether the usage of different amounts of LPA solution (0 and 20 µl) in the isolation of sheared DNA fragments in the ChIP method increases the DNA isolation efficiency, the differences between the DNA concentration averages were determined and variance analysis was performed. In other words, the concentrations of DNA isolates in the experimental groups were compared. Experimental groups were first divided into two according to the DNA isolation stage as "Genomic DNA isolation Group" and "ChIP DNA isolation Group". Secondly, both groups were divided into "Control Group" and "LPA Group" according to whether LPA solution is used or not (Table 3).

Table 3. Experimental groups

	Genomic DNA	**ChIP DNA**
CONTROL	1. Group	2. Group
LPA	3. Group	4. Group

In descriptive data, the number of Groups is (N)=5, since the experiments were performed in 5 parallels. Average DNA concentrations were determined as 90.97 ng/ul in Group 1, 92.19 ng/ul in Group 3, 26.94 ng/ul in Group 2 and 54.34 ng/ul in Group 3.

In the normality test, 'Shapiro-Wilk' sigma values were p(0.666)>0.05 for Genomic DNA group (Groups 1 and 3) and p(0.488)>0,05 for ChIP DNA group (Groups 2 and 4). Since they are greater than 0,05, the data were considered to be normally distributed with 95% confidence. While the 'Kolmogorov-Smirnov' sigma value (p. 0,200) was found to be greater than 0.05 for both groups, the Skewness and Kurtosis values were also found to be between -1.5 and +1.5.

After it was determined that the data were normally distributed and the variances were homogeneous, the relationship between the use of LPA and the increase in isolation efficiency in the DNA isolation method performed to obtain ChIP DNA fragments with one-way ANOVA test was investigated. For this purpose, the mean concentrations (ng/ul) of Genomic DNA and ChIP DNA isolates in both the control group and the LPA group were compared.

Since the one-way ANOVA test sigma values were greater than p(0.675)>0.05 between the 1. and 3. groups, it was determined that there was no statistically significant difference between the initial DNA concentrations in both groups. However, since sigma value p(0.003) was smaller then 0,05 between the 2. and 4. groups, it was determined that there was a statistically significant difference between the DNA concentrations obtained in ChIP DNA isolation between the two groups.

To correlate these results between LPA and DNA concentration efficiency, the concentrations between the four groups were compared separately. For this purpose, the "Tukey test" was performed since the Sigma value of the "variance homogeneity test" was greater than 0.05 (p>0.05).

In the Tukey test, 1st and 3rd groups, 2nd and 4th groups, 1st and 2nd groups, 3rd and 4th groups were compared separately (Table 3). Since Sig between 1. group and 3. group was greater than 0.005 (p(0.995)>0.005), initial DNA concentrations in both groups were considered close to each other (equal).

Since Sig is smaller than 0,05 between 1.Group and 2. group (p(0.000)<0.005), there is a statistically significant difference between the initial genomic DNA concentration of the experiment and the concentrations obtained as a result of re-isolation of sheared DNA fragments within CHIP adaptation. In other words, significant DNA loss was experienced.

Since Sigma was less than 0,05 between 3. group and 4. group (p(0.000)<0.005), DNA loss was experienced as between 1. group and 2. group. In other words, there is a significant difference between the concentration of genomic DNA at the beginning of the experiment in 3. Group and the concentrations obtained as a result of re-isolation of DNA fragments fragmented within the scope of CHIP adaptation.

It was determined that Sigma was less than 0,05 between 2.Group and 4.Group (p(0.000)<0.005). In this case, it was determined that there was a statistically significant difference between the ChIP DNA isolation groups in terms of DNA concentrations. Another statistical analysis to determine the relationship between LPA and the isolation efficiency of small size DNA fragments is the Independent Sample T-Test (Table 4). Sigma (2-tailed) values were determined as less than 0.05 between Groups 1 and 2, (p(0.000)<0.05), greater than 0,05 between Groups 1 and 3 (p(0.675)>0.05), and less than 0.05 between Groups 2 and 4 (p(0.003)<0.05).

CONCLUSIONS

It is important that the yield is high in DNA isolation methods. While the isolation efficiency is generally high for large-size nucleic acid molecules such as genomic DNA, the yield is low because the amount of DNA lost is high in the isolation of some PCR products, restriction enzyme cut-off products or fragmented small-size DNA molecules.

For this reason, alternatives are being investigated to increase this efficiency with various approaches. One of these alternatives is the use of macromolecules such as glycogen, tRNA and LPA (Gaillard and Strauss, 1990). However, in using such matrices, it is expected that there will be no negative effects on downstream processes, the cost is low, and the application is easy and safe, and so on. In this respect, LPA is reported to be more advantageous than other macromolecules (Gaillard and Strauss, 1990).

Recovery of small-sized DNA molecules is one of the most important problems encountered in the ChIP method. In the ChIP method, which consists of many consecutive steps, the fact that the sheared DNA molecules cannot be re-isolated with 100% efficiency directly reduces the success of the ChIP method. Therefore, DNA isolation with minimal or no loss at this step is an extremely important strategy.

In this context, adapting LPA molecules, which are known to increase the isolation efficiency of small-sized DNA molecules, to the ChIP method may be an alternative approach to solving this problem. For this purpose, in this study, 5 parallel genomic DNA was isolated from cotton plant in two groups as "control" and "LPA" and ChIP application was started with equal amounts (~91.59 ng/µl) DNA molecule.

In the in vitro adaptation of ChIP without protein, DNA molecules were mechanically fragmented by ultrasonication and confirmed on agarose gel (Figure 3). As expected, DNA concentrations decreased to 20.84 ng/µl and 54.34 ng/µl in both the control group and LPA group, respectively, in the concentration measurements made as a result of the re-isolation of fragmented DNA molecules. According to these results, it was determined that there was 64.13 ng/µl DNA loss in the LPA-free control group and 37.85 ng/µl DNA loss in the LPA-treated group.When the statistical analysis of the obtained data was performed with the SPSS program, it was determined that the data were normally distributed and the variances were homogeneous.

Although the initial DNA molecules were statistically similar in the one-way ANOVA test, it was observed that the DNA concentrations obtained as a result of re-isolation of the sheared DNA molecules after ChIP adaptation were different from each other.

Similar results were obtained in the Independent-Sample T-Test, which is another statistical analysis to determine the effect of LPA use on DNA isolation efficiency. The absence of a statistically significant difference between the 1st and 3rd groups in the t-test analysis means that equal amounts of DNA molecules were used in both the control group and the LPA group. The difference between the 1st group and the 2nd group, it is understood that the control group without LPA experienced DNA loss in the re-isolation performed in the ChIP adaptation.

Similarly, the difference between the 3rd and 4th groups revealed that DNA loss was also experienced in the "LPA group" in which LPA was used. However, in addition to all these results, the statistically significant difference between the 2nd Group and the 4th Group reveals an important result. Accordingly, DNA loss occurs when using LPA matrix, just like not using LPA, but using LPA significantly reduces this loss.

Based on these data, it has been proven that the LPA matrix can be used in the ChIP method, which is widely used in the elucidation of DNA-protein interactions but encounters some problems at various stages. In the ChIP method, LPA solution can be used to increase the yield and efficiency of re-isolation of sheared DNA molecules. However, more extensive new research is needed to optimize the use of LPA solutions in ChIP methods applied with different procedures in vitro and in vivo for a wide variety of organisms.

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