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Short Communication: Evaluating the efficiency of ethanol precipitation method in purification of gDNA and PCR product

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Abstract: A numerous clean-up methods of nucleic acid were developed to achieve the requirements of downstream reactions like PCR and sequencing. The methods were varied in their mechanism, efficiency of purification and final product yield. The present study evaluated the efficiency of ethanol-sodium acetate (EOH-NaOAc3) precipitation method in purification of nucleic acid to satisfy downstream reactions requirements. The yield and purity of nucleic acid were considered as the main standard parameters to estimate the efficiency of method. Geneaid gel extraction kit DF100 was considered as a standard method for comparison. The results of methods comparison revealed that EOH-NaOAc3 method was significantly (P=0.000) surpassed the kit method in the yield of purified PCR product (93.24 and 18.37 ng/µl) with no significant differences (P=0.239) in quality (Absorbance ($A_{260/280+}$) = 1.816 and 1.843) respectively. To determine the productivity of EOH-NaOAc3 method, a specific amount of genomic DNA (G-DNA) (187.93 ng/ µl) was processed and the results showed that EOH-NaOAc3 method was efficiently conserved 89.6% of total processed G-DNA (168.51 ng/µl) accompanied by significant (P=0.03) elevation of DNA purity ($A_{260/280}$, 3.07 – 2.53).

Keywords: Ethanol-sodium acetate precipitation, purification, DNA drying.

Introduction

Nucleic acid isolation mostly associated with several contaminants like proteins, lipids, carbohydrates, and other molecular compounds resulted from cellular lysis processes, which are affecting downstream reactions like polymerase chain reaction (PCR), hybridization, cloning, and sequencing. These contaminants should be removed from genome samples to purify them and prevent any troubleshooting (Krsek & Wellington, 1999; Gallagher & Wiley, 2008; Tan & Yiap, 2009).

Many methods were developed to purify nucleic acid, which varied in their accuracy, productivity, and costs, but can nevertheless be grouped into two categories: the solution-based methods and the solid phase-based methods (column based methods) (Tan & Yiap, 2009). The solution-based methods

involve disruption of cells structure to get cell lysate, nucleases inactivation, clean-up of nucleic acid and precipitation, while the column based methods include adsorption of nucleic acid (from cell lysate) on solid matrix, extensive washing steps then elution with alkaline low ionic strength solution (Tan & Yiap 2009; Vandeventer *et al*., 2012; Maury *et al*., 2013; Vandeventer *et al*., 2013). The solid matrix mostly composed of silica, which is produced as a filter membrane or as magnetic particles coated with silica (Vandeventer *et al*., 2012). DNA adsorption to the silica matrix usually driven by chaotropic salts with organic solvent like ethanol or under low pH condition with kosmotropic salts (Boom *et al.*, 1990; Hourfar *et al.*, 2005). The elution of the DNA will then carrying out using high pH, low ionic buffer (Hourfar *et al.*, 2005). The disadvantage of silica-based method is the poor ability to collect small fragments or low quantities of DNA–especially when sample volume is limited or at low concentration (Koo *et al.*, 1998; Ali *et al.*, 2017).

Ethanol precipitation of DNA is one of the most frequently used methods in DNA purification and/or concentration (Fregel *et al.*, 2010). Most protocols involve cations addition like Na^+, NH_4^+ and Li^+ in a form of salts like sodium acetate, sodium chloride, ammonium acetate, and lithium chloride with ethanol (Clerget *et al.*, 2015). The mechanism of DNA precipitation can be explained depending on the fact that DNA is a polyanionic molecule due to huge negatively charged phosphate groups within phosphodiester-linked backbone, that dissolved very well in polar solvents especially those of high dielectric constant like water (Zumbo, 2013). Water molecules mostly form a hydration shell around DNA that avoid ionic bonds formation with cations in aqueous solution and then prevent DNA precipitation (Schneider *et al.*, 1998; Laage *et al.*, 2017). The DNA precipitation can be driven by ethanol in the presence of high concentration of chaotropic salt under high pH conditions (Tan & Yiap, 2009; Maurya *et al.*, 2013; Poh & Gan, 2014). Ethanol has less polarity than water, having dielectric constant of 24.3 comparing with 80.1 for water, when the concentration of ethanol being 64% or higher, the phosphodiester DNA backbone will be allowed to form ionic bonds with cations in the solution to precipitate DNA (Walker, 2015). The role of chaotropic salts in precipitation process involves breaking hydrogen bond network and neutralizing the PO_3^- group by Na^+ ions, making nucleic acid less hydrophilic and then less soluble in water to be fit for precipitation (Oswald, 2007; Dupont *et al.*, 2015; Till *et al.*, 2015).

The efficiency of any method used to isolate and/or purify ~~of~~ nucleic acid can also affected by sample condition (ex: fresh, old, dried, frozen, self-degraded, inhibitors content, *etc.*) and method selection to match the type of sample requirement (Khanuja *et al.*, 1999).

The present study aimed to evaluate the efficiency of ethanol precipitation as a recovery, purification and/or concentration method of nucleic acid before using it in downstream reactions like PCR amplification and sequencing or dehydration for long-term storage.

Material & Methods

Samples preparation

Eight samples of G-DNA were randomly obtained from multiple sources involved plant tissues, animal tissues and blood using Geneaid DNA extracting kit (gSYNCTM,

GS100, Geneaid Biotech Ltd. Taiwan) for blood and animal tissues; Plant Genomic DNA Mini Kit (GP100, Geneaid Biotech Ltd. Taiwan) for plant and fungal tissues. Ten samples of PCR products were amplified from plant, animal and fungal genes that were randomly collected. Eeach sample was divided to two sub-samples to measure purification impact on quality and quantity of PCR product due to their effect on downstream experiments. The samples in each group (DNA and PCR product) were considered as replicates. The sample volume was 20µl for each.

Purification methods

Two methods of DNA purification were studied to examine their efficiency in purification and/or drying of nucleic acid to prepare it for long-term storage and/or sequencing process. The purification methods included Gel extraction kit and ethanol (EOH-NaOAc3) precipitation method.

Gel extraction kit method

Geneaid gel extraction kit (Gel/PCR DNA Fragments Kit DF 100, Geneaid Biotech Ltd. Taiwan) was used to extract PCR product after running on 2% agarose gel. The bands were cut off carefully with sharp blade and the PCR product was extracted according to the manufacturer instructions. Nano drop measurements were performed to the samples to measure their quantity and quality after purification using nano-drop device (Termo ScientificTM, NanoDrop 2000).

EOH-NaOAc3 precipitation method

This method followed Shibayama *et al.* (2017) with little modification. A 0.1 volume of 3M Sodium acetate with 2.5-3 volume of ice-cold ethanol were added to the sample and vortex thoroughly then let to precipitate at -20º overnight (to give more precipitation time if the DNA amounts is low) (Zeugen & Hartley, 1985). The samples then centrifuged at full speed for 30 min at 4ºC and the supernatant was discarded. The pellet washed twice with 500µl of ice-cold 75% ethanol then spinned down at 4ºC for 10 min of each time. The supernatant was discarded and the samples were dried at 80ºC for 5 min (the samples can be stored for long time under room temperature at this step). The samples were re-hydrated with 20 µl of DNAse free distilled water (DD-Water) to estimate the quality and quantity of DNA after purification using nano drop devise (Termo Scientific™, NanoDrop 2000).

To evaluate the efficiency of ethanol precipitation method, a comparison was applied with standard purification method. The gel extraction kit (Gel/PCR DNA Fragments Kit DF 100, Geneaid Biotech Ltd. Taiwan) was considered as a standard control method. Both mentioned methods were used to purify 20µl of PCR product sub-samples separately (one sub-sample for each method) and the experiment was performed in ten replicates. T-test analysis was performed using SPSS Ver. 16 software to determine difference significances.

Evaluation of EOH-NaOAc3 efficiency in G-DNA clean up

To evaluate the efficiency of EOH-NaOAc3 as clean up method for G-DNA, the concentration and quality of G-DNA samples were determined before and after purification with this method. The experiment was performed in eight replicates.

Statistical analysis

The results data were analyzed statistically using T-test for independent variables at P≤

0.05 and the analysis was performed using the statistical package SPSS Ver. 16.

Results & Discussion

The purification efficiency of examined methods (Table 1) showed significant superiority (P=0.000) of ethanol precipitation method (93.24 ng/µl) on gel extraction method (18.37 ng/µl), while no significant difference was observed (P=0.239) between them on DNA quality ($A_{260/280}$= 1.816 and 1.843) respectively. The low yielded DNA in the kit method in comparison with ethanol precipitation was consistent with other studies (Maurya *et al.*, 2013; Poh & Gan, 2014), due to the efficiency of nucleic acid adsorption on silica membrane that likely to be buffer dependent (Vandeventer *et al.*, 2012, Vandeventer *et al.*, 2013). Furthermore, the composition of elution buffer and/or un-careful handling could cause the elution step to be not efficient enough to release all or most of the bound DNA from silica membrane (Vandeventer *et al.*, 2013).

Table (1): the effect of purification method on quality and quantity of pcr product.

	Conc. ($ng.\mu l^{-1}$)	Quality ($A_{260/280}$)
EOH-NaOAc3	93.24 ±11.01	1.81 ±0.06
Gel extraction kit	18.37 ±5.99	1.84 ±0.02
P-Value	0.000**	0.23

****High significant difference at 0.01 level**

***Significant difference, The sample size = 10 samples**

The concentration (Table 2) of the purified G-DNA samples (168.51 ng/µl) was not significantly (P=0.577) reduced after purification with EOH-NaOAc3 method comparing with raw samples (187.93 ng/ µl), while the quality of the raw and purified G-DNA samples ,(A260/280= 3.07, 2.53) respectively, showed a significant enhancement (P= 0.03) in G-DNA purity.

Table (2): The effect of EOH-NaOAc3 precipitation method on quality and quantity of treated G-DNA.

	Conc. (ng/µl)	Quality (A260/280)
Raw DNA	187.93 ±71.22	3.07 ±0.48
Purified DNA	168.51 ±64.84	2.53 ±0.41
P-Value	0.577	0.03*

***Significant difference, The samples number = 8 samples**

These results represent the efficacy of EOH-NaOAc3 method to conserve about 89.6% of G-DNA with elevation of purity level to the acceptable value ($A_{260/280} \approx 2$) that made it suitable enough for downstream reactions like PCR and sequencing (Sun, 2010). The high yield probably due to the mechanism of clean-up of this method depending on precipitation of DNA as a pellet under alkaline conditions using high concentration of chaotropic salts. In this method, DNA does not bound to any matrix that mostly decreases DNA quantity due to un-effective binding and/or elution step (Vandeventer *et al.*, 2013). Moreover, ethanol precipitation method is well-known as a very low cost method comparing with gel extraction method.

Conclusion

EOH-NaOAc3 precipitation protocol is highly efficient, simple, productive and low cost

method using for cleanup of nucleic acid to react with downstream reactions. Comparing with the silica-based method, this method can provide a perfect simple solution to recover the low quantities and/or fragments of DNA that could be critical issue in some experiments.

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