BIOCHEMISTRY AND MOLECULAR BIOLOGY EDUCATION
Vol. 38, No. 3, pp. 161–166, 2010

Laboratory Exercise

DNA Extraction Techniques for Use in Education

Received for publication, March 29, 2009

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DNA extraction provides a hands-on introduction to DNA and enables students to gain real life experience and practical knowledge of DNA. Students gain a sense of ownership and are more enthusiastic when they use their own DNA. A cost effective, simple protocol for DNA extraction and visualization was devised. Buccal mucosal epithelia provide a readily available source of cells for DNA extraction and can be harvested in a painless, noninvasive manner. Seven criteria were established to evaluate the protocol: Safety, DNA yield, DNA quality/stability, cost, user friendliness, reliability, and time. To identify the optimum conditions for each stage of the protocol (cell harvest, lysis, purification, and precipitation), each was investigated separately, and an adaptation of the fast-boiling protocol was used for the remaining stages. A validation study was undertaken with the optimized protocol to assess its performance when conducted by a group of students in a classroom setting. The optimum protocol used an isotonic Lucozade Hydro Active Fitness Water (HAFW) mouthwash. Lysis was achieved using a TE (10 mM Tris-HCl, 1 mM EDTA, pH 8) + 1% Sodium Dodecyl Sulphate (SDS) buffer. Protein was then digested using Proteinase K (Qiagen Inc., UK) at 56°C for 10 min. The DNA was then precipitated with sodium chloride and absolute ethanol. This protocol achieved an increase in DNA yield using readily available equipment and reagents at a lower per capita cost and is simple to use.

Keywords: DNA, extraction, buccal cells, education.

The importance of DNA and its structure and function are central to the UK science curriculum at every level of education from the age of 12 upward [1]. DNA extraction allows students to relate an abstract idea to a tangible product and gain a better understanding of the DNA molecule.

The International Centre for Life, Science Center offers DNA extraction workshops to school groups and members of the public. The aims of these sessions are to promote an interest in science and a deeper knowledge of genetics. At present, the workshops involve students extracting DNA using a "Genes in a bottle kit" (Bio-Rad, UK) which allows students to extract, prepare, and precipitate their own DNA from buccal mucosa. The current workshop protocol is prohibitively expensive (£4.23 GBP per person) and is therefore not used to its full potential. There is a need for an inexpensive, high-quality alternative which would allow students to extract and visualize their own DNA.

The stages involved in extracting DNA from cells can be categorized as: cell harvesting, lysis, protein digestion, and precipitation. The major variances in previously described protocols are in the cell harvesting stage. The stages that follow cell harvesting are fairly uniform throughout the literature.

Previous methods for DNA extraction have relied on either peripheral blood [2–4] or buccal cells [2, 3, 5–19] harvested using either mouthwash [5, 11, 20–25] or brushing methods [6–11, 13, 16, 25–28].

Blood sampling provides a high yield of excellent quality DNA [3, 4]. Methods for DNA extraction from blood rely primarily on venepuncture for harvest. A number of novel techniques have been tested such as finger prick harvesting [29]; however, this method yields low quantities of DNA due to the small amount of blood obtainable via this method.

As an alternative to invasive techniques, buccal cells provide a readily available, noninvasive source of DNA for crude extraction. Collecting buccal cells is painless, quick, easy to understand, and safe if conducted correctly. Previously described buccal cell harvesting techniques fall into two categories: mouthwash techniques (using, for example, saline, water, or alcohol-based mouthwashes) [20–25] and cytobrush techniques (using brushes, sticks, swabs, and other scraping methods) [6–11, 13, 16, 25–28]. Mouthwash techniques are advantageous as they produce higher quantities of DNA than did cytobrush methods [18].

It has been demonstrated that DNA yield can be increased by directly supervising and instructing students during the cell harvesting [16]. London *et al.* reported on the use of a soft toothbrush by school children which yielded on average 29 μg of DNA in comparison with 17 μg from a simple water mouthwash method alone. Similar effects regarding supervision were also reported for mouthwash and cytobrush methods [16].

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 DOI 10.1002/bmb.20351

Box 1

Criteria for optimum DNA extraction protocol

- *Safety*: The protocol must be appropriate for use by children from the age of eleven upward.
- *DNA yield*: The main objective of the DNA workshop is to produce an easily visible quantity of DNA. Unlike many clinical techniques, the precise quantification of DNA yield is not relevant in this context as the DNA will not be processed further.
- *DNA quality/safety*: The appearance of precipitated DNA is stereotypically characterized as "white fluffy strands" and it would be useful for students if the product conformed to this stereotype.
- *Cost*: The cost of running DNA extraction experiments can be relatively high, prohibiting the regular use of the practical in educational settings. The primary purpose of this research is to minimize the cost per participant, allowing wide use of the protocol.
- *User friendliness*: The protocol should be easy to follow and quick to carry out as this allows more time to explain the science behind each step. The protocol should be easily carried out without any previous knowledge of practical laboratory techniques.
- *Time*: This is the biggest constraint since the workshop must run in 1 hr, including time to explain background to the practical as well as the practical itself. The protocol is only feasible if it can be conducted in approximately 30 min as this allows the required time for teaching and logistics.

The objectives of this study were to devise a DNA extraction protocol that is both effective in terms of the DNA produced and cost-effective for large-scale use in an educational setting.

INVESTIGATING A NEW OPTIMUM DNA EXTRACTION PROTOCOL

Six criteria were identified as important for the context of a DNA extraction protocol for use in a classroom. The criteria were as follows: Safety, DNA yield, DNA quality/stability, cost, user friendliness, and time (See Box 1). Possible protocols that were in keeping with the criteria were identified for each stage of the extraction. Some possibilities were ruled out because of safety concerns, lack of equipment, or cost. For example, safety concerns surrounding the age range of the practical participants ruled out phenol–chloroform extraction methods and lysis buffers with particularly harmful reagents.

The stages needed for successful DNA extraction were identified as: cell harvesting, cell preparation, cell lysis, purification, and precipitation. Protocol variations were tested by the author by following a default protocol, an adaptation of a fast boiling technique [30], and changing one variable from these stages at a time. Table I summarizes the different variables. Products of each trial were stored for 14 days at room temperature in sealed containers, exposed to natural ambient light. Samples were scored against the six criteria to ascertain the optimum variables for a new DNA extraction protocol.

CELL HARVESTING

The greater the amount of human material initially obtained the greater the amount of precipitated DNA. Although DNA yields from venepuncture are high, it is not feasible to use blood sampling as a method in children. Because of obvious practical advantages, buccal cells were selected as the source for genetic material. Harvesting can be carried out in one of two following ways.

Mouthwash Techniques

The mouthwash serves to maintain cells until DNA is extracted from them. Alcohol mouthwashes can be harmful if swallowed and many taste unpleasant. For these reasons mouthwashes containing alcohol were not considered. Other isotonic solutions are available for use in this context. Lucozade produce drinks at isotonic concentrations, such as Lucozade Sport and Lucozade Hydro Active Fitness Water (HAFW), these are similar to other brands of isotonic sports drinks, such as Poweraid

Table I

Variables used in trials to ascertain optimum DNA extraction protocol

Stage		Variable tested
Cell harvest	Mouthwash	Lucozade Orange, Lucozade HAFW, 0.9% saline, 5% dextrose, distilled water Each trialled with 30, 60, and 90 sec in the mouth
	Sample size	1.5 mL, 3 mL, 4.5 mL centrifuged at 10,000 rpm for 30 sec
	Centrifuge length	0.5 min, 1 min, 5 min, 10 min at 10,000 rpm with 1.5 mL samples
	Cytobrush	1 or 2 brushes, lolly stick Brush for 30, 60, or 90 sec Brush both sides of mouth, one side of mouth or upper and lower gutter
Cell lysis	Buffers	TE buffer, 0.9% saline, sterile water, TAE buffer, TBE buffer. Cells lysed using 1 or 2% SDS or 3 drops per milliliter household washing up liquid
	Protease	Batches tested using 20, 40, and 80μL of Proteinase K
	Temperature	40, 50, 56[a], 60°C
	Incubation	Samples incubated for 2, 5, 10, 15, and 20 min at 50°C
	Precipitation	Concs ranging from 0.05 to 0.4 mM NaCl; 100% and 70% ethanol compared at volumes of 0.5–2 mL per milliliter of cell extract. Solutions stood for 5 min and either inverted or agitated by shaking

[a] This was the enzyme manufacturer's reported optimum temperature.

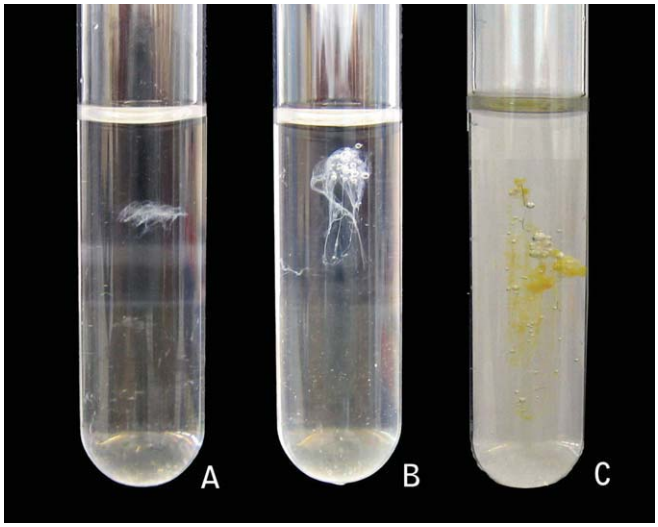

FIG. 1. **Comparison of precipitated DNA using different protocols.** Sample A was obtained using a standard DNA extraction protocol, sample B was obtained using the final revised protocol, and sample C was obtained using a protocol with Lucozade orange as a mouthwash and demonstrates the orange staining associated with such mouthwashes. The above samples were scored for DNA yield: (A) 2/7, (B) 7/7, (C) 7/7 and quality: (A) 2/7, (B) 3/7, (C) 7/7. Sample A demonstrates a minimal yield of poor quality DNA; sample B shows a high quantity yield but low quality as it has been died orange by Lucozade Orange; sample C shows a high yield and high quality and thus received the highest average score.

and Gatoraid, but HAFW is colourless. These are both pleasant to use and serve to maintain the cells during washing. The use of 0.9% saline, 5% dextrose, and water was also investigated.

Cytobrush Techniques

Cytobrush techniques as well as the possibility of using lolly sticks to harvest cells were investigated, as they are an inexpensive, readily available, familiar, and hygienic alternative to cytobrushes.

LYSIS BUFFERS

The buffer solution serves to maintain the DNA and prevent its degradation by heavy metal ions and extremes of pH. It also provides conditions conducive to proteinase action. The manufacturer's instructions list the relative activities of proteases in various buffers [31]. Cell lysis is required to gain access to the nuclear DNA. To lyse the cells either 1% or 2% Sodium Dodecyl Sulphate (SDS) solutions or Fairy Liquid were used (Procter and Gamble, UK).

PRECIPITATION

Sodium chloride is added as the negatively charged DNA molecules bind Na^+ and this encourages the free strands of DNA to group together and form larger strands of DNA, although there is some debate about this. This increases the size of the DNA product so that it is more easily visible. Ice cold alcohol is then added to form a layer on top of the aqueous solution. DNA is less soluble in alcohol and is precipitated out at the interface between the two liquids. The colder the alcohol the less soluble the DNA, therefore more is precipitated.

RESULTS

An analogue scoring system was used to assess the individual efficacies for variants at each stage of the protocol. This scoring system was chosen because the DNA extracted here was not to be used for genetic analysis, but rather for observation by students and as such the amount of DNA visible is of more importance than its precise quantification.

The optimal protocol design was drawn up based on a combination of the best individual steps. Products of each trial were assessed by the author based on a scale of 1 to 7 for each criterion: DNA yield and DNA quality. A scale of 1 to 7 was chosen as this gives a range of scores sufficient to differentiate between products and is more accurate/reproducible for a human observer than assessing on a larger scale such as 1 to 10. On the scale, 7 represents the highest attainable DNA yield/quality and 1 represents the lowest attainable DNA yield/quality. As an example, the samples in Fig. 1 received the following average scores for DNA yield and quantity: (A) 2/7, (B) 5/7, (C) 7/7. Sample A demonstrates a minimal yield of poor quality DNA; sample B shows a high quantity yield but low quality as it has been dyed orange by Lucozade Orange; sample C shows a high yield and high quality and thus received the highest score. Table II shows the final protocol that was chosen for use in the DNA extraction workshops.

CELL HARVEST

It was concluded that a mouthwash method was more appropriate than cytobrush techniques as the DNA yields are higher. Lucozade Orange was ruled out despite producing greater yield as the DNA was stained bright orange (see Fig. 1). Saline also produced a high yield, but was discounted due to its unpleasant taste. Of the remaining options, Lucozade HAFW performed best (scoring 6/7 for yield and 6/7 for DNA quality). The yield was only slightly lower than that obtained from saline or Lucozade Orange washes, and the resulting DNA was both stable and also characteristic in its appearance. Lucozade HAFW was also rated highly for taste. The only negative factor is its expense (£0.054 GBP per 10 mL), the highest of all the mouthwash options. Prepackaged, commercially supplied mouthwashes, such as Lucozade HAFW, are attractive for use with children as they are familiar to them, and present a more hygienic option, as a sealed bottle can be opened for each class.

Although a 90 sec mouthwash produces a higher yield, it was concluded that a 60 sec washing was easier for students, and the reduction in yield is minimal, the difference being a yield score of 6/7 for 90 sec and 5/7 for 60 sec.

Use of a lolly stick for preagitation of the mucosa was included in the final protocol due to the large increase in yield that it provides.

TABLE II
Optimized DNA extraction protocol from buccal mucosa epithelia

Stage	Description	Procedure
1	Cell harvest	1. Dispense 10 mL of Lucozade HAFW into a drinking cup. 2. Gently scrape the mucosa with a wooden lolly stick for 30 seconds. Avoid swallowing. 3. Harvest the cells by swishing the mouthwash around the mouth constantly for 60 sec; simultaneously massage the cheeks against the mouth to increase the yield of cells. 4. Expectorate the solution back into the cup.
2	Cell lysis	1. Whilst mixing the solution, pipette 1.5 mL of the cell suspension into a microtube. 2. Centrifuge at 10,000 rpm for 30 sec. 3. Pour off the supernatant, leaving behind a small amount of liquid to avoid losing the cell pellet. 4. Repeat steps 1–3 of the cell lysis stage, adding more cell solution to the tube each time to increase the cell pellet. 5. Add 1 mL of lysis buffer [TE pH 8 + 1% SDS] 6. Vortex for approximately 30 sec or until mixed.
3	Protein digestion	1. Add 20 μL of Proteinase K. 2. Incubate the lysate at 56°C for 10 min.
4	DNA precipitation	1. Add 100 μL of 2.5 M sodium chloride. 2. Mix gently by inverting the tube five times. 3. Transfer to a 5 mL acrylic test tube. 4. Slowly pipette approximately 1 mL (an equal volume to that of the lysate) of ice cold absolute ethanol down the side of the tube whilst holding it at 45° so that it forms a layer on top of the aqueous layer. 5. Allow the tube to stand undisturbed for 5 min at room temperature. 6. One may also encourage precipitation at the interface by briskly shaking the tube.

Smaller volumes of mouthwash resulted in the highest DNA concentrations, thus 10 mL of Lucozade HAFW was chosen as the appropriate volume. This also reduces the cost per person. The results suggest that the same number of cells is simply suspended in a greater volume of liquid.

The yield of DNA was found to increase greatly depending on the instructions given to participants: directly observed mouth washing, as well as concurrent verbal instructions, timing, and encouragement produced greatly increased yields. This is in keeping with the work of Heath *et al.* (2001) based on their comparison of yield between mailed samples collected on the basis of written instructions and directly supervised, verbally instructed collections [15].

Centrifuge

After obtaining the mouthwash sample, a 3 mL sample spun at 10,000 rpm for 1 min produces a sufficient amount of precipitated DNA, obtaining a score of 6/7 for yield and quality of DNA produced. It was found that spinning the solution for 10 min required extensive vortexing with the lysis buffer to resuspend the cell pellet. This had the effect of shortening the strands of precipitated DNA, and reducing the quality of the final product. It is believed that this is because of damage to the DNA caused by extensive vortexing.

LYSIS

The optimum buffer for cell lysis and high Proteinase K activity was found to be the TE buffer, obtaining a score of 6/7 for yield and quality of DNA produced. This result is in keeping with the manufacturer's guidelines as having the optimum conditions for Proteinase K activity (31). TE buffer is also readily available and inexpensive.

One percent SDS is sufficient to lyse buccal cells in solution. Greater concentrations of SDS caused the solution to foam, and this caused problems when transferring the solution into the final 5 mL tube as DNA was left behind.

Proteinase K activity was greatest at 56°C. This is in agreement with the manufacturer's recommendations. An incubation time of 10 min produced a high quality final precipitate. These 10 min also provide a useful time in the workshop to explain the techniques used so far and the methods for the next stage.

PRECIPITATION

A 100 μL aliquot of 2.5 M NaCl provides conditions that allow DNA precipitation. Higher concentrations of Na^+ ions produce a cloudy solution on addition of alcohol. Less concentrated solutions caused less DNA to precipitate. Absolute alcohol kept on ice was better than 70% alcohol, gaining a score of 7/7 for quantity of DNA. After 5 min of standing at room temperature, DNA began to precipitate in visible amounts.

Shaking the tube containing the solution causes more DNA to be precipitated. By comparison, inverting the tube to mix the aqueous and alcoholic solutions precipitated less DNA. On shaking the tube, the solution began to vortex and the precipitate of DNA appeared to coil upward, more DNA is seen to precipitate from the interface and is wound up with the DNA already in the alcohol. This process led to the production of a larger amount of DNA.

TESTING THE REVISED PROTOCOL

The revised protocol for DNA extraction for use with school children was tested by two groups of students at the Life Science Centre, Newcastle. Both groups managed to conduct the protocol successfully and 30 of 31 participants obtained some DNA. Large amounts of DNA,

Box 2

Set up sheet for the protocol

To carry out the DNA extraction protocol, each student requires the following:

- 10 mL Lucozade Hydro Fitness Active Water
- 1 lolly stick
- 1 beaker or cup
- 5 mL pipette
- 1 micro centrifuge tube
- 1 mL buffer: TE (10 mM Tris-HCL, 1 mM EDTA, pH 8) + 1% Sodium Dodecyl Sulphate (SDS)
- 20 μL Proteinase K (20 mg/mL; activity >600 mAU/mL)
- 100 μL 2.5 M Sodium Chloride
- 5 mL acrylic test tube
- 1 mL absolute ethanol at freezer temperature

The class will also require the use of a micro-centrifuge, vortex and a water bath at 56°C.

scoring between 5/7 and 7/7 using the visual analogue scoring system explained above, were obtained by 25 of the 31 participants. To ascertain whether the DNA product could be used for further procedures such as PCR or restriction digestion, six unprecipitated samples of the DNA extracted using the revised protocol were used for PCR and all produced a good product.

Potential Pitfalls

From these trials it was found that the majority of the protocol was easy to follow. The cell harvesting stage was found to be the hardest step for participants. Those who did not obtain DNA or obtained small amounts of DNA failed to have a pellet after centrifugation. Supervision and encouragement during the preagitation and mouth washing were crucial in obtaining cells.

CONCLUSION

Table II shows the final revised protocol and Box 2 provides a set up sheet for lab technicians. Figure 1 compares the resultant DNA samples using a standard protocol and the final revised protocol. It demonstrates that the new protocol produces a greater quantity of DNA. The protocol produced larger quantities of DNA than the current cytobrush methods; it is inexpensive at a cost of £0.83 Pounds Sterling (GBP) per person. This new protocol can be used in classroom and group workshop situations as a cheap and easy to use method of DNA extraction. The protocol takes approximately 30 min allowing its use in a 1 hr session with time for explanation and exploration of further material.

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