



## Five-minute purification of PCR products by new-freeze-squeeze method

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### Abstract

Here, we describe a new-freeze method (NFM) for rapid purification of PCR products. After PCR products were separated by horizontal common agarose electrophoresis, gel fragments containing the desired PCR products were directly subjected to NFM. The resulting gel was frozen in nitrogen canister for 60 s and thawed in 70°C for 2 min and then centrifuged. DNA in the supernatant was stored at -20°C until further use. PCR products in the size range of 100-2000 bp were purified within nearly 5 min. NFM could separate 2 DNA fragments that differed in length by 50 bp. As judged by the purification efficiency, the quality of PCR products separated by NFM was equal to that of the method of DNA purification by low-melting-agarose gel method (LMM) and freeze-squeeze method (FSM). NFM reduces the operational time and cost of recombinant DNA technology, in which purification of PCR products is a repetitive process. With no complicated equipment and technical training, NFM is very simple and easy to operate.

**Key words:** Agarose gel electrophoresis, new-freeze method, PCR, purification.

### Introduction

Purification of polymerase chain reaction (PCR) products and nucleic acids is one of the fundamental and repetitive processes in the field of biotechnology and all disciplines of life sciences for isolating an appropriate length of amplified DNA<sup>1,2</sup>. The purified products are widely used for direct DNA sequencing and for recombinant DNA technologies<sup>3</sup>. There are a variety of methods for the purification of PCR products at different scales and with different levels of quality. Among them, the method in which the PCR products are separated by electrophoresis on a horizontal agarose gel, and the desired PCR products are excised from the gel for the isolation of DNA by melting the gel piece is commonly used<sup>4,5</sup>. However, the low-melting-agarose gel method (LMM), which is considered as the gold standard for DNA extraction and purification, requires low-melting-point agarose. Although the commercial DNA purification kit method is convenient, rapid and highly efficient, it is not widely applied on account of its high cost<sup>6</sup>. There is a report on the purification of PCR products by continuous elution electrophoresis, which requires special equipments<sup>7</sup>, and all methods above employ the common protocol that is used to detect DNA fragments after PCR by horizontal agarose gel electrophoresis. Freeze-squeeze methods (FSM) were also reported as the efficient method<sup>6,8</sup>. We improved the FSM that could purify PCR products after agarose gel electrophoresis within nearly 5 min by using common agarose and common equipments, it is a more simple and rapid method for the purified PCR production, compared to the FSM which lasts at least 2-3 h.

The method required the following standard laboratory equipments: (i) a centrifugation parameter, (ii) a nitrogen canister, and (iii) a waterbath.

### Experimental

First, DNA samples amplified by PCR were separated by horizontal agarose electrophoresis as described by Wieslander<sup>4</sup>, but instead of using the low-melting-point agarose gel, common agarose gel was used. The band on the gel corresponding to the desired PCR product was cut out, grinded and suspended in 150 µL of TE buffer [10 mmol/L Tris-HCl (pH 8.0) and 1 mmol/L ethylenediaminetetraacetic acid (EDTA)]<sup>9</sup> in a sterile Eppendorf tube. Next, the tube was subjected to one freeze-thaw cycle using a nitrogen canister and waterbath, alternating between -196°C for 60 s and 70°C for 2 min. The sample was then centrifuged at 10,000 rpm for 20 s at 4°C. Finally, a 100 µL aliquot of the supernatant as purified production was transferred to a sterile tube. DNA in the supernatant was precipitated, and resuspended in a small volume of buffer and stored at -20°C until further use.

PCR was conducted in a mixture (25 µl) containing 2.5 mM deoxynucleoside triphosphates (dNTPs), 0.5 µM primers, and 10 ng/ml template DNA<sup>10</sup>. The DNA fragments of 100-, 150-, 300-, 500-, 700-, 1200- and 2000-bp lengths were amplified by 35 PCR cycles using Taq DNA polymerase, and 5 µl of the amplified mixture was separated and purified using NFM, FSM and LMM. Fragment patterns of separated and purified amplified products obtained by NFM, FSM or LMM were visualized by ultraviolet (UV) transillumination, and photographs were taken using a charge-coupled device camera (TILL Photonics LLC, Martinsreid,

Abbreviations used: dNTP, deoxynucleoside triphosphate; EDTA, ethylenediaminetetraacetic acid; FSM, freeze-squeeze methods; LMM, low melting-temperature gel method; LB, Luria-Bertani; NFM, new-freeze-squeeze method.

Germany). The Bio-Rad Quantity One software package (Bio-Rad Company, USA) was used according to the protocol described by the manufacturer. Fig. 1 shows the separation profile of the PCR products separated by horizontal agarose gel electrophoresis. Fig. 2 shows the purifications profile of 100-, 150- and 2000-bp long PCR products purified by using the NFM, FSM or LMM by horizontal agarose gel electrophoresis. The figure indicates that NFM, FSM and LMM provided satisfactory purification of each DNA fragment (Fig. 2). The recovery of PCR product was more than 80% for each length of DNA product (data not shown). In addition, NFM, FSM and LMM could separate 2 DNA fragments that differed in length by 50 bp (Fig. 2, Lanes 1-3 were purified by NFM; Lanes 4-6 were purified by FSM; Lanes 7-9 were purified by LMM). Theoretically, these methods would be able to purify any different lengths of DNA fragments that are separated by horizontal common agarose gel electrophoresis. We examined the purification efficiency of the NFM using the TA-cloning vector (Promega Corp., WI, USA) to purify a 100-bp long PCR product. The efficiency was compared with that of the DNA purified by LMM<sup>4</sup>. The number of transformants was counted on a Luria-Bertani (LB) plate containing 50 µg/ml ampicillin. Using the PCR

products purified from 5µl of the amplified PCR mixture, 14,000 and 13,500 colonies were obtained by the NFM and LMM, respectively. This result indicates that the quality of PCR products separated by NFM is equal to that purified by LMM.

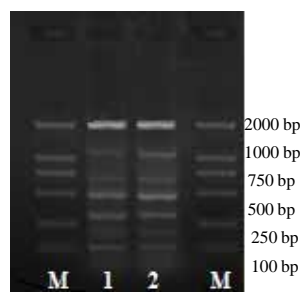
In summary, the NFM described here could purify 100- to 2000-bp long PCR products within nearly 5 min, and the PCR products obtained were sufficient for the separation of 2 DNA fragment differing in length by 50 bp, as is achieved using the low-melting-agarose gel method and freeze-squeeze method. The purified DNA fragments could be directly used for procedures such as DNA sequencing. With no complicated equipment and technical training, NFM is very simple and easy to operate. Separation speed, purification yield, and quality are the important factors that are considered while selecting a method for DNA purification. The NFM for the purification of PCR products satisfies all the 3 above-mentioned criteria. The NFM can reduce the time and cost required for the development of recombinant DNA, because the purification of PCR products is one of the most widely used methods in recombinant DNA technology<sup>6</sup>.

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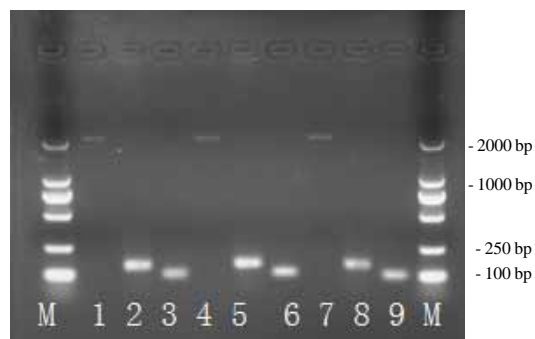
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**Figure 1.** Separation fingerprint of the polymerase chain reaction (PCR) products analyzed by horizontal common agarose gel electrophoresis.

Lanes 1 and 2: PCR products with lengths of 100, 150, 300, 500, 700, 1200, and 2000 bp. M: DL-2000 DNA marker (TaKaRa).



**Figure 2.** Purification fingerprint of polymerase chain reaction (PCR) products analyzed by horizontal common agarose gel electrophoresis.

Lanes 1-3 show the results for 100-, 150- and 2000-bp long PCR fragments, respectively, which were purified by the New-Freeze Method (NFM). Lanes 4-6 show the results for 100-, 150-, and 2000-bp long DNA fragments, respectively, which were purified by the Freeze-Squeeze method (FSM). Lanes 7-9 show the results for 100-, 150-, and 2000-bp long DNA fragments, respectively, which were purified by the low-melting-agarose gel method (LMM). M: DL-2000 DNA marker (TaKaRa).