Purification of DNA fragments using QuickPick™ DNA Fragment kit and PickO™ gel exciser

SANNA PIETILÄ, M.Sc. PEKKA MATTSSON, Ph.D., MATTI KORPELA, M.Sc.
Bio-Nobile Oy

KEY WORDS: DNA fragment, agarose gel, TAE, TBE, magnetic particle separation, purification, PickPen®, PickO™

ABSTRACT

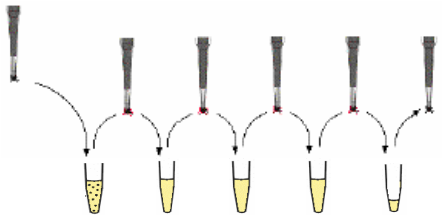
The QuickPick™ DNA Fragment kit together with the PickPen® magnetic tools provides a fast and simple means of purifying DNA fragments both from agarose gel and biological solutions. The method does not require any organic solvents and eliminates the need for repeated centrifugation, vacuum filtration or column separation. The purified DNA fragments are of high quality and are suitable for all commonly used downstream applications.

The QuickPick DNA Fragment kit with the PickO™ gel exciser is intended for purification of DNA fragments sized 60 bp to 50 kbp from any type and concentration of agarose gels prepared either in TAE or TBE buffers.

INTRODUCTION

The purification of DNA fragments from agarose gel is a standard method frequently used in molecular biology. A variety of purification methods are commercially available. The QuickPick™ DNA Fragment kit with the PickO™ gel exciser is advantageous to others methods for having a new solution both for sample preparation and for purification step.

Conventionally, the gel piece excising is time-consuming, laborious and clumsy with scalpel or razor blade which may lead to damaged DNA due to lengthened UV light exposure. The PickO™ gel exciser Bio-Nobile™ method is a straightforward one-hand process with high efficiency, leading to consistent gel piece sizes. The method saves time in allowing the pipetting of all DNA fragment purification reagents during gel electrophoresis.
DNA fragments in the gel are released using Gel melting/DNA binding Buffer which contains chaotropic salts. In the same step (a) the released DNA is bound to silica-based magnetic particles (MP). PickPen® is used to capture the MP with bound DNA and to carry out subsequent washes (b-c) to remove contaminants. Ethanol traces are eliminated by using a unique DipWash™ method, in which the magnetic particles are dipped a few times in the Wash buffer (d). Finally, DNA is eluted from the particles into the Elution Buffer (e).



(a) (b) (c) (d) (e)

MATERIALS & METHODS

DNA fragment excision with PickO™ gel exciser

pBAT4 plasmid (4176 bp) was linearizised using *Hin*dIII restriction enzyme. Linearized vector (3,5 µg) with SybrGreenI was loaded on 1 % low-melting agarose gels (both TAE and TBE buffered).

After electrophoresis separation DNA fragments of interest were excised from the agarose gel with the PickO™ gel exciser. The tool was positioned above the fragment by looking through the hole in PickO™. The tool was pressed through the gel and the gel piece was picked up by rotating and bending the PickO™. The gel piece was transferred to the microtube by pressing the upper part of the PickO™.

DNA fragment purification

The purification was performed according to the QuickPick™ DNA Fragment kit insert. The reagent volumes used were according to the amount of cut gel pieces (> 200 mg). The melting/binding time was 8 minutes and elution time 5 minutes. The

Copyright 2010
BN Products & Services Oy
www.bnpands.com / www.bionobile.com
All rights reserved

elution volume used was reduced to 20 µl to obtain sufficient DNA concentration for downstream applications. Reference purifications were performed with commercially available kit based on spin-column technology.

RESULTS

The purified DNA fragments were loaded onto a 1 % agarose gel, the same amount of each sample was used (Fig. 1). Clear, undegraded DNA fragments were observed from all of the purifications. The results show that corresponding recovery of DNA is achieved from both TAE or TBE buffered agarose gels.

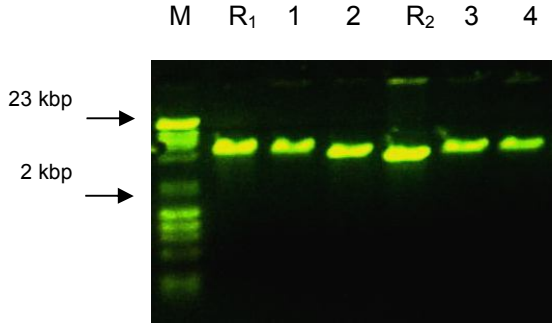


Figure 1: Purified DNA fragments run on 1 % agarose gel. M = Marker; R_1 = Reference DNA purified using spin-column technology (TBE-buffer) 1,2 = DNA purified using QuickPick™ DNA Fragment kit (TBE buffer); 3,4 = DNA purified using QuickPick™ DNA Fragment kit (TAE buffer); R_1 = Reference DNA purified using spin-column technology (TAE-buffer).

The quality of the purified DNA was determined with sensitive fluorescence sequencing method using Applied Biosystems (AB) ABI PRISM® 377-XL DNA Sequencer. No inhibition of the reactions can be seen from samples purified either from TAE or TBE buffered gels confirming the high quality of the purified DNA (Fig. 2). The sequences were readable to over 550 nucleotides.

CONCLUSION

The PickO™ gel exciser method is easy to learn. Wider or larger fragments can be picked up with PickO™ up to three pieces at a time or separately as many pieces as needed. Close DNA bands are also not a problem. Gel piece/pieces can be released from PickO™ in a simple way. With PickO™ gel exciser method the sizes of excised

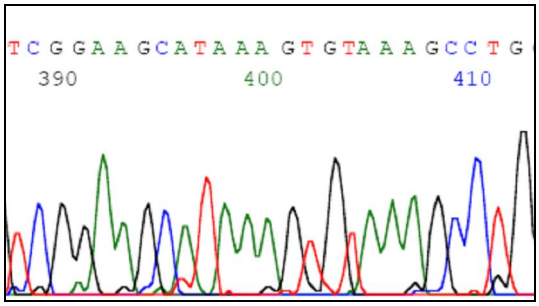


Figure 2: A segment of the sequence from the pBAT4 DNA purified with QuickPick™ DNA Fragment kit from TBE buffered 1% agarose gel.

gel pieces are consistent. This eliminates the need for laborious weighing of individual gel pieces and reagent volume adjustments.

Ethanol traces in the elution buffer is a common problem in DNA fragment purification methods. A unique and proprietary DipWash™ method made possible by the PickPen® technology is used in the QuickPick™ purification procedure to eliminate this problem. During the DipWash™ the magnetic particles are not released from the PickPen® tool, instead a few dips in the Wash Buffer is enough to rinse the ethanol away. The method is rapid and easy to use and guarantees the recovery of high quality DNA.

The purification of DNA using QuickPick™ DNA Fragment kit is quick and easy and the purity of the DNA is suitable for downstream applications such as RE digestions, cloning or sequencing.

The QuickPick™ DNA Fragment kit, although specified for DNA fragments in agarose gel, can also be applied to other starting materials such as PCR product, restriction enzyme digested product purification or to concentrate purified DNA.

The combination of PickO™ gel excision method with the QuickPick™ DNA Fragment purification kit forms an easy and fast overall process especially suitable for automation.

Copyright 2010
BN Products & Services Oy
www.bnpands.com / www.bionobile.com
All rights reserved