

Isolation of genomic DNA from chicken blood using QuickPick™ gDNA kit

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ABSTRACT

The QuickPick™ gDNA purification kit together with the PickPen® magnetic tool provides a fast and simple means of isolating genomic DNA. The technique does not require any organic solvents and eliminates the need for repeated centrifugation, vacuum filtration or column separation. The purified genomic DNA is of high quality, suitable for all commonly used downstream applications.

The QuickPick gDNA kit is intended for use with human whole blood and blood components such as leukocytes and buffy coat, as well as human cultured cells. However, the chemistry may be applied to other starting materials with good results as well. Below is described the purification of genomic DNA from chicken blood. The purifications were carried out following the kit instructions, and no additional reagents were required.

INTRODUCTION

Chicken blood differs from human blood in that also the red blood cells are nucleated, and thus contain DNA. In consequence, when purifying DNA from chicken blood, there is more DNA material than when working with human blood. With conventional column-based purification methods, clogging of the column bed is a major problem. However, if sample volumes are reduced to counteract this problem, the yield will be reduced, and the final product may not be pure enough.

The QuickPick gDNA kit, although specified for human blood, has been shown to give good results also when purifying blood from other animal

species. DNA purification results from chicken blood are described below. In brief, DNA was isolated from chicken blood cells with QuickPick gDNA kit using various amounts of blood as starting material.

PRINCIPLE OF THE QuickPick gDNA KIT

DNA in the sample is released from cells using Proteinase K and Lysis Buffer. The released DNA is bound specifically to the magnetic particles in the presence of Binding Buffer. PickPen® 1-M or PickPen® 8-M is used to capture the magnetic particles with bound DNA, and to carry out subsequent washes to remove contaminants. Finally, DNA is eluted from the particles using Elution Buffer, and DNA is ready for use in downstream applications.

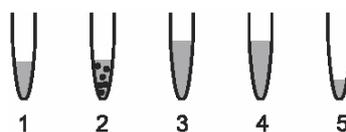
MATERIALS & METHODS

Chicken blood application:

Chicken (*Gallus sp*) blood was collected into Heparin tubes. The blood had been stored at -18 °C for more than 6 months and then a few days at room temperature before DNA purification. The blood was mixed thoroughly before use. The QuickPick gDNA kit insert protocol was followed. The Lysis step took 10 minutes, the binding and elution steps 2 minutes each. 5, 10, 15, 25 and 50 µl of whole blood from the same chicken was used as samples.

The DNA isolation protocol is as follows:

Predispense reagents and sample into tubes:



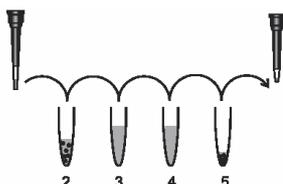
Tube 1: Lysis Buffer, sample, Proteinase K Solution
Tube 2: Magn. Particles, Binding Buffer
Tube 3: gDNA Wash Buffer
Tube 4: gDNA Wash Buffer
Tube 5: gDNA Elution Buffer

Incubate the sample, Proteinase K Solution and Lysis Buffer for 10 minutes at 56 °C.

Pipet the contents of tube 1 into tube 2 and incubate for 2 minutes at room temperature.



Wash the DNA bound to the magnetic particles twice in Wash Buffer using PickPen® to carry out the transfers.



Transfer the sample into Elution Buffer with PickPen® and elute DNA by incubating for 2 minutes at room temperature. Collect the magnetic particles from tube 5 and discard them and the PickPen® tip. The eluate containing DNA is now ready for downstream applications.

Purity of the DNA was determined spectrophotometrically as a ratio of absorbance at 260/280 nm.

RESULTS

DNA from chicken blood:

From 4.4 µg (using 5 µl sample) to 42 µg (using 50 µl sample) of DNA was isolated using the QuickPick gDNA kit and purities were in the range of 1.71 - 1.80 (see table 1).

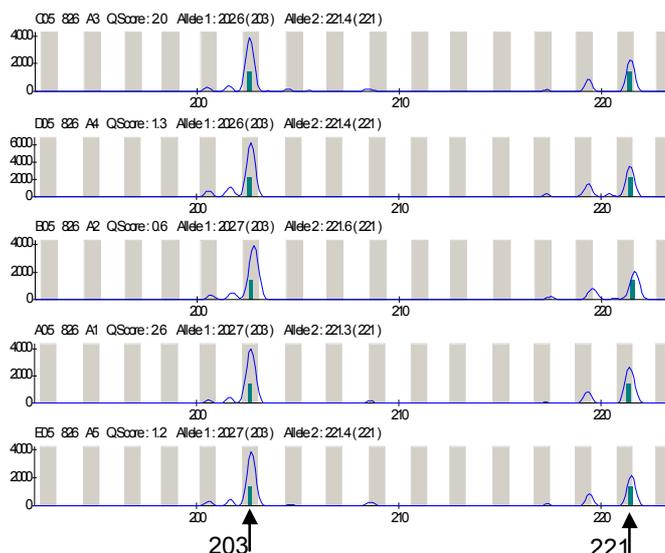
Table 1: Yields and purities of the DNA isolated from chicken blood.

Sample number	Sample volume	Total yield	Purity
A1	5 µl	4.4 µg	1.80
A2	10 µl	7.5 µg	1.78
A3	15 µl	45 µg	1.71
A4	25 µl	29 µg	1.73
A5	50 µl	42 µg	1.71

The DNA was used in microsatellite marker PCR amplification as follows:

PCR was carried out in a total of 20 µl, containing 50 ng genomic DNA, PCR buffer (10 nM Tris-HCl, pH 8.8; 1.5 mM MgCl₂; 50 mM KCl, and 0.1% Triton-X), 200 mM dNTP, 0.25 U Dynazyme II DNA

Polymerase (Finnzymes Oy) and 10 pmol of both primers. PCR was performed at an annealing temperature of 55 °C. Amplified PCR products were analysed with MegaBACE 500 (Amersham Biosciences) capillary electrophoresis sequencing apparatus. PCR reactions were performed with each sample A1 – A5 (see picture 1).



Picture 1: PCR amplification results from MegaBACE 500 capillary electrophoresis sequencing apparatus. Samples from top to bottom: A3, A4, A2, A1 and A5.

As seen from the picture 1, the DNA isolated from this particular chicken was shown to be heterozygous (peaks 203 and 221) for the marker MCW247.

The PCR results show that the DNA isolated from the various amounts of blood support PCR amplification, and there are no signal/noise amplification differences at any appreciable level between the samples.

The usefulness of the QuickPick gDNA method in isolating DNA from chicken blood was demonstrated; in addition, the method was rapid, and the DNA was ready for use immediately after purification without long solubilization, and suitable for microsatellite PCR.