Isolation of genomic DNA from chicken feathers 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 feathers. The purifications were carried out following the kit instructions with minor changes.

INTRODUCTION

Feathers are a difficult material to isolate DNA from, since only the feather tip contains the DNA. Attached to the outside are old skin cells, while inside are old blood cells, from when the feather was still growing. However, feathers offer advantages compared to blood samples because they can be collected much earlier from young chicks and DNA analysis can be performed at a very early stage.

For some downstream applications the crude feather lysate can be used directly, but when several different analyses from the same sample are needed the pure DNA should be isolated. Pure DNA is needed also for long term storage. The QuickPick gDNA kit, although specified for human blood, has been shown to give good results also when purifying blood from other sample materials. DNA purification results from chicken feathers are described below.

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 feather application:

Feathers were plucked so that some cells /tissue from the feather follicle remained attached to the tip (*calamus*). Feathers were stored at room temperature for a few weeks before analysis. For DNA purifications the tips of the feathers were diced up and placed in a tube containing Proteinase K and Lysis Buffer. One to five tips were used per sample. When working with the tiny feathers from a one day old chick, the whole feather was used.

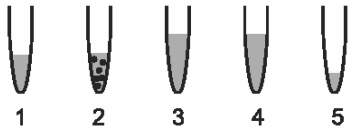
Picture 1: The part of the feathers that was used for DNA analysis is shown inside the circle.

The QuickPick gDNA kit insert protocol was followed with minor exceptions:

1. The lysis step was extended from 10 to 30 minutes.
2. DTT was added to the Lysis Buffer to a final concentration of 1 %.
3. Elution volume was reduced to 100 µl to obtain sufficient DNA concentrations for downstream applications.

The DNA isolation protocol is as follows:

Predispense reagents and sample into tubes:

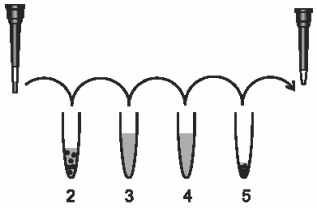


Tube 1: Lysis Buffer, sample, Proteinase K Solution
Tube 2: MagaZorb* Magn. Particles, Binding Buffer
Tube 3: gDNA Wash Buffer
Tube 4: gDNA Wash Buffer
Tube 5: gDNA Elution Buffer

Incubate the sample, Proteinase K Solution and Lysis Buffer for 30 minutes at 56 ºC.

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.

The protocol was carried out in 40 minutes including the lysis step.

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

RESULTS

DNA from chicken feathers:

From 0.4 µg (1 feather) to 0.9 µg (2 feathers from a day old chick) of DNA was isolated using the QuickPick gDNA kit. Yields and purities were determined spectrophotometrically and the purities were ≥ 1.8 (A_{260}/A_{280}).

Sample amount	DNA concentration in the eluate	Total yield
1 feather	2.1 ng/µl	0.4 µg
3 feathers	2.9 ng/µl	0.6 µg
5 feathers	2.5 ng/µl	0.5 µg
2 feathers (one day old chick)	8.8 ng/µl	0.9 µg

Table 1: Concentrations and total yields of the DNA isolated from chicken feathers.

The usefulness of the QuickPick gDNA method in isolating DNA from chicken feathers was demonstrated.