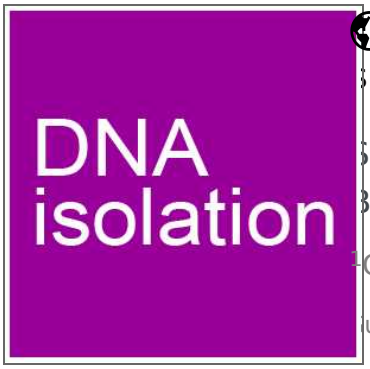

Isolation of DNA from spots (smears) of old microscopic glass slides with mini column isolation kit for molecular analysis

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

Many institutes have old fixed slides for microbiological purposes. These slides have valuable material for a particular
pathogen, which can be used for molecular analysis e.g., as reference control during conventional as well as real time PCR along with the gene sequencing. The problem is that user has fixed slides with spots, but they have to develop and standardize the method of isolation leading to wastage of time. Therefore, in this procedure, we are going to show step by step method to isolate DNA with Genekam DNA isolation (mini column) kit from slides. In our laboratory, we have used this method for DNA isolation from microscopic glass slides, which are many years old, very successfully. Sometimes, many laboratories have slides with fixed material for conducting the fluorescent microscopy or slides for conducting student practical, where our protocol can be used. This method is also suitable for histological as well as pathological slides for isolation of tissue DNA for biomarkers analysis. In parasitology, it is common to make the blood smears to examine them under microscope for the presence of parasites in the blood e.g. Plasmodium falciparum, hence such slides can be sent to reference laboratories for molecular analysis, where the reference lab can isolate the DNA with the method in this protocol as this is an innovative idea.

IMAGE ATTRIBUTION

Genekam Biotechnology AG

MATERIALS

Materials needed: Genekam DNA isolation kit (Mini
column) Product number: SB0001, Heating block, Centrifuge, Pipettors with pipettor tips, Edding pen, Hand gloves, Microtubes and Molecular ethanol.

SAFETY WARNINGS

Please read the protocol before use properly It is recommended to do some exercise with this protocol before using it on important samples.

BEFORE START INSTRUCTIONS

Check the followings before start:

Materials needed: Genekam DNA isolation kit (Mini column) Product number: SB0001, Heating block, Centrifuge, Pipettors with pipettor tips, Edding pen, Hand gloves, Microtubes and Molecular ethanol.

1 1. Mark the spot from the glass slide from which DNA is to be isolated but from other side. This can be done with normal Edding pen.

Now switch on the heating block and adjust the temperature to 56°C.

2 Put the slide on heating block but the marked area must touch the heating block.

3 Prepare a microtube with 300 µl of lysis buffer (Tube A) with proteinase K and label it as tube 1. Keep it at 56°C. From this step, user is going to work with Genekam DNA isolation kit (Mini Column)

4 Add 20 µl of lysis buffer (Tube A) from Genekam DNA isolation kit on the spot and keep it for 5 minutes at heating block at 56°C. The time may vary between 5-10 minutes, please take care that lysis buffer does not dry up.

5 Now take pipettor with pipette tips with filter (10-200 µl) and try to scrape off the spot with it. Usually, spot is likely to be dissolved in lysis buffer partial or whole. Now take the fluid from the spot out in tube 1. Repeat the step 5 and 6 to get maximum yield.

6 Keep the tube 1 for 20-30 minutes in the heating block at 56°C.

7 Add 400 µl of tube G (second lysis buffer) to tube 1 and keep it for 5 minutes at 70°C in the heating block. Prepare second microtube called EL and add 150 µl of Tube E (elution buffer) to it.

Keep it at 70°C till its use later on.

8 Take out the tube 1 from heating block. Add 400 µl of molecular ethanol to it.

9 Label the mini column with a receiver tube as MC1 and put 600 µl of fluid from tube 1 in it. Centrifuge it at 10000 rpm for 1 minute. Collect the fluid in microtube called tube 2. * -Repeat step 9-There should be no fluid after the above step in MC1 now.

10 Add 500 µl of tube B (washing buffer 1) and centrifuge it at 10000 rpm for 1 minute. Discard the fluid from MC1.

11 Add 500 µl of tube C (washing buffer 2) and centrifuge it at 10000 rpm for 1 minute. Discard the fluid from MC1.

12 Add 200 µl of tube C (washing buffer 2) and centrifuge it at 10000 rpm for 1 minute. Discard the fluid from MC1. Now there should be no fluid in mini column.

13 Centrifuge MC1 at 13000 rpm for 3 minutes to remove the rest of fluid in it. Discard the fluid.

14 Label a new collection tube called COL 1. Put the mini column MC1 into this tube COL1 now. To this mini column, add 100 µl elution buffer from microtube called EL.

15 Keep the MC1 at room temperature for 2 minutes. Centrifuge at 13000 rpm for one minute to collect isolated DNA. Your microtube COL1 has now isolated DNA, which can be used for different analysis.

16 Store the isolated DNA at -20°C for further use.

* Important Hints: Fluid from tube 2 from step 9 can be reused to isolate more DNA, if user needs more DNA. User needs to repeat the steps 9 to 14 with a new mini column. Donot throw the slide in waste as this may contain more DNA, therefore store the used slide to get DNA again. The slide can be used twice or more times.