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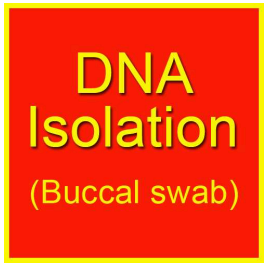
Rapid, environmentally friendly and cost effective human DNA isolation method from buccal swab for molecular analysis within 10 minutes



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We use this protocol and it's working

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Disclaimer

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Abstract

Isolation of human DNA from buccal swab is an important part of molecular biology. It is conducted to perform the different kinds of molecular assays e.g., detection of pathogens (including dental pathogens), oral microbiome analysis, genetic tests as well as genetic fingerprinting. There are different methods available in the literature e.g., mini column, magnetic beads as well as solution based. There are a number of commercial methods available on the market. The cost of one DNA isolation from each buccal swab with mini column method varies from Euro 2.50 to 7 depending upon the manufacturer and it needs around 40-55 minutes to be completed as there are many steps like lysis, washing and elution. We have developed a protocol, which will finish within 10 minutes and has only 3 major steps. It will give huge yield of isolated DNA. The spectrophotometric values of isolated DNA from such buccal swab is around 40 ng/µl and total volume of isolated DNA is around 400 µl. This is huge yield to perform the different tests on the same isolation against the yield of DNA isolated with magnetic beads and mini column method is between 10 to 25 ng/µl and the total volume is 100 µl. The isolated DNA can be stored in freezer for many years.

The cost of each isolation with our method is between 10 to 20 cents, which is 10 to 50 times cheaper than that of commercially available mini column method. Such methods should be part of teaching students about the molecular biology during the practical training. This protocol can help to save of a lot money during research work. This method has been used in many laboratories world worldwide for conducting molecular analysis.

The method is environmentally friendly as it does not contain phenol, chloroform as well as other toxic substance along do not produce any plastic waste as in case of mini column isolation method, hence this can be performed in any laboratory. The chemicals can be stored at room temperature.

This method has been used for different kinds of samples e.g., isolation from blood spots, cell cultures, bacterial colonies, sperm samples, pathological tissue samples etc. In this protocol, buccal swab is shown as an example.

Image Attribution

DNA isolation (Buccal swab)

Materials

Material needed: Heating block, microtubes, DNA isolation kit (Product: SB0072, Genekam Biotechnology AG), vortexer, scissor. pipettor and pipette tips.

Troubleshooting

Safety warnings

Precautions: Always take 2 buccal swab samples per person. In case one sample is accidentally destroyed, the second sample can be used.

2. Always do some exercise with the protocol before working with real and important samples as the experience will help to reduce the mistakes.

3. If the user is going to isolate the DNA from multiple number of buccal swabs from different persons, please use different scissors for cutting the heads of buccal swabs. NEVER use the single scissor for different buccal swabs for different persons as it will contaminate the samples and the results will be wrong.

Before start

Read the protocol carefully before using it.

1 Label the microtube.

2 Switch on the heating block and set the temperature to 88 ^{0}C.

3 Cut the head of buccal swab with a scissor in such a way that it falls in microtube.

4 Add 100 µl of 1:10 diluted tube A (lysis solution) to the microtube.

5 Put this microtube in the heating block at 88 ^{0}C for 8 minutes.

6 Vortex the microtube for 5 seconds. This is optional. User can shake the tube with hand.

7 Add 100 µl of first buffer (tube B) to the microtube.

8 Add 200 µl of second buffer (tube C) to the microtube. There will be around 400 µl of total volume.

9 Now the supernatant has DNA to be used in PCR analysis. 1 or 2 µl isolated DNA should be sufficient for conducting the experiments.

10 Store it at 4 ^{0}C for short term use e.g., 7-14 days or at -20 0C for long term use.