

PCR based kit to assess the biological quality and purity of isolated DNA samples following DNA extraction.

Introduction

DNA samples acquired from a number of sources such as buccal cells, blood, tissues and saliva are commonly used for genotyping, diagnostics, forensics, population studies and paternity testing. After DNA isolation it is imperative to assess the quality and purity of the DNA prior to moving into the PCR phase. Commonly used methods can have disadvantages of instrumental sensitivity¹ or lengthy practical steps. Furthermore none of these methods assess the native DNA's activity in the PCR environment². This application note presents the results obtained from an easy to use PCR based kit (DQC quality check kit) that beneficially provides a reliable method used within the actual PCR environment.

We describe the use of the DQC kit which has been specifically designed to test for the presence and biological quality of human DNA isolated from buccal swabs or other sampling methods.

The Isohelix DQC kit uses 6 sets of primers, 5 of which have been chosen to target areas on different chromosomes of the human genome which are particularly susceptible to degradation. Each pair of primers amplifies up a fragment of different size leading to the 100bp, 200bp, 300bp, 400bp and 600bp bands seen when the reaction is run out on an agarose gel. The 500bp fragment is an internal non human DNA control for the PCR reaction and should always be present. Failure to see this band indicates either that the PCR reaction itself has not worked, or that the sample was not loaded onto the gel successfully. The multiplex PCR reaction used by the Isohelix DQC kit requires the presence of high quality Human DNA to give a successful result. A positive DQC result indicates that the isolated DNA is of high quality and therefore suitable for use in downstream testing or research projects. The amount of intact human DNA present in the sample can also be estimated by comparison to known standards.

The DQC kit can also be used to compare different types of DNA isolation methods and to show the presence of PCR inhibitors in most DNA samples.

Method

After cycling in a thermal cycler, the PCR product is run on a 1.75% agarose gel containing Ethidium Bromide alongside a 100bp ladder (not supplied).

The expected band sizes are 100bp, 200bp, 300bp, 400bp, 500bp and 600bp.

The presence of all 6 fragments of equal intensities indicates that the DNA is of good quality and not denatured.

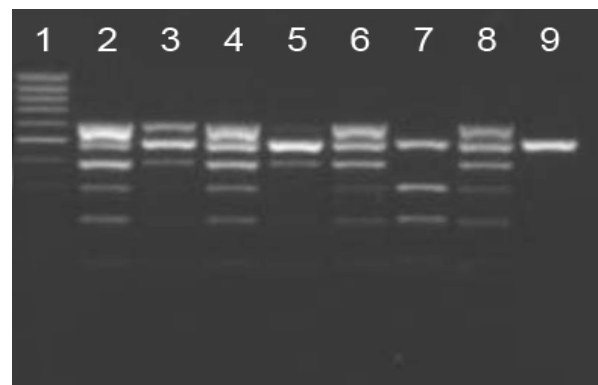
If all 6 bands are present but the intensities of the bands are lower, this indicates a lower yield of DNA in the sample.

If any degradation of the sample has occurred, the first band to disappear is normally the 400bp band and the absence of further bands indicates a more advanced state of degradation.

If only the 500bp internal control band is seen this indicates that either there is no human DNA present in the sample or that the DNA is completely degraded and unsuitable for further testing.

Below is an example of the results you would expect to see with both good quality and poor quality DNA when tested with the DQC quality check kit.

DQC Kit for checking quality of DNA



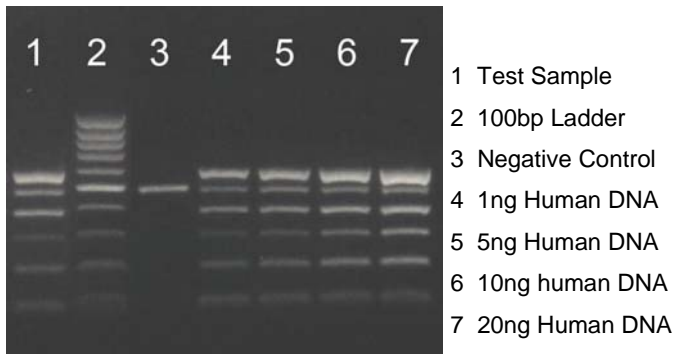
- 1 100bp ladder
- 2 Good Quality DNA - 6 bands present
- 3 Good Quality DNA - 6 bands present but lower yield so bands less intense
- 4 Good Quality DNA - 6 bands present
- 5 Poor Quality DNA - Uneven amplification indicates partially degraded DNA
- 6 Good quality DNA - 6 bands present
- 7 Poor Quality DNA - Some bands missing
- 8 Good Quality DNA - 6 bands present
- 9 Negative Control - 500bp internal control shows PCR worked

Conclusion

The DQC quality check kit provides a simple and reliable way to assess the quality and purity of isolated human DNA intended for use in downstream PCR based applications. It also enables the amount of DNA in the sample to be quantified when compared against specific human DNA standards.

By comparing the intensities of the bands in the test sample with those of the standards an estimated value of 7-9ng Human DNA is obtained.

DQC Kit for estimating yields of DNA



Notes:

¹ Although spectrophotometric analysis is traditionally used for quantifying DNA, the concentration of DNA in the isolated sample is likely to be at or below the minimum absorbance sensitivity for spectrophotometers and the results obtained are likely to be inaccurate and unreliable.

² The DQC Quality Check Kit is an appropriate method to determine the quality of isolated DNA prior to downstream PCR based applications because it relies on intact dsDNA to give a positive result and so demonstrates the biological activity of the sample rather than just giving a value for the nucleic acid concentration of that sample.

Other Cell Projects Products

- **Isohelix DNA Buccal Swabs.**
High yields, blood alternative, reproducible, easy to use, different formats for various extraction methodologies.
SK-1, SK-2, SK-3 and SK-4
- **Isohelix DNA Silica Gel Capsules**
For use with SK-1 swab kits, air-dries swab in tube giving extended storage times without loss of stability. SGC-3/50
- **Isohelix DNA Isolation and Handling kits**
DNA stabilising kits for the stable storage of DNA at room temperature for long periods DSK-3/50
DNA isolation kits optimised for high yields of intact DNA from buccal swabs. DDK-3/50
- **Isohelix Buccalyse Kit**
A quick and simple one tube method for extracting PCR-ready DNA from buccal swabs. BEK-25/SK1



Cell Projects specialise in buccal cell DNA sampling and isolation technologies and offer a range of Isohelix products together with full technical support in this area. Further technical application notes are available to download from www.isohelix.com
For further information on any of our products please contact Cell Projects technical support at info@isohelix.com