Application Note

Quality control of genomic DNA using the QIAxcel® system

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This application note describes a rapid, reliable, and effective method for quality control of purified genomic DNA using the QIAxcel system. The results are highly reproducible and clearly show whether degradation products are present in a sample.

Introduction

The results of numerous molecular screening and assay methods rely on the quality of the genomic DNA (gDNA) that is used. Effective techniques for purification are crucial to secure gDNA that will give the best results in downstream procedures, but optimized quality control of purified gDNA is equally important. It helps to avoid time and money being wasted, particularly in the case of costly procedures, such as Next Generation Sequencing (1), where gDNA is fragmented and used for library preparation.

The sizes of purified gDNA fragments depend on the purification method. They fall between 20 and 30 kb when spin columns with silica-based membranes are used, and can be up to 100 kb or more when salting-out precipitation is applied. The silica-based method is the most commonly used, and it can be done manually or automated with a sample purification instrument such as the QIAcube®.

Using the QIAxcel system, quality control is possible for all gDNA that has been purified with silica membrane-based methods. It offers a straightforward and effective means for checking the quality of all samples purified with kits that use this approach (e.g., QIAamp® DNA Blood Kit, QIAamp DNA FFPE Tissue kit, QIAamp MinElute Virus Kits).

Materials and Methods

Genomic DNA was isolated from blood samples using QIAGEN's QIAamp DNA Blood Mini Kit, which is based on silica membrane DNA purification. The protocols were automated and run on the QIAcube.

The DNA samples were quantified with a NanoDrop® spectrophotometer. Concentrations of 10–100 ng/µl were chosen for testing. Samples were analyzed on 1% agarose gels in 30 mM TAE buffer for 2.5 h at 80 V. The same samples were analyzed using the QIAxcel system ▷

for capillary electrophoresis with a DNA Screening Cartridge. The AM900 method and QX Alignment Marker 15 bp were used. AM900 is a customized method with the following parameters: alignment marker injection at 4 kV for 20 sec, sample injection at 2 kV for 40 sec, and separation at 3.5 kV for 900 sec.

For degradation analyses, the samples were sonicated with 7, 14, 70 and 105 pulses. Ultra-sound degradation was performed in 500 µl (100 ng/µl) of DNA, sonicated on a Branson Sonifier® 250.

Results

Samples from the dilution series were electrophoresed on a 1% agarose gel (Figure 1A), as well as on the QIAxcel system using a DNA Screening Cartridge (Figure 1B). The results demonstrate that gDNA concentrations between 25 and 100 ng/µl are suitable when using this system.

Various running conditions, such as injection time, separation time and voltage, were tested (data not shown) and optimized. AM900 was identified as the optimal procedure for gDNA quality control with sample injections of 40 sec at 2 KV and a separation time of 900 sec at 3.5 KV.

The results of assessing sonicated gDNA samples with the QIAxcel system revealed that it is very simple to determine the degree of degradation by categorizing the type of peak yielded from each gDNA sample (Figure 2). A major peak with no signals for degradation products before it and a long tailing off after it indicates gDNA of very good quality that is suitable for downstream applications. Electropherograms for partially and highly degraded gDNA have no tailing off after the major peak. The level of degradation can be differentiated based on the signals for the degradation products in the broad peak before the major peak.

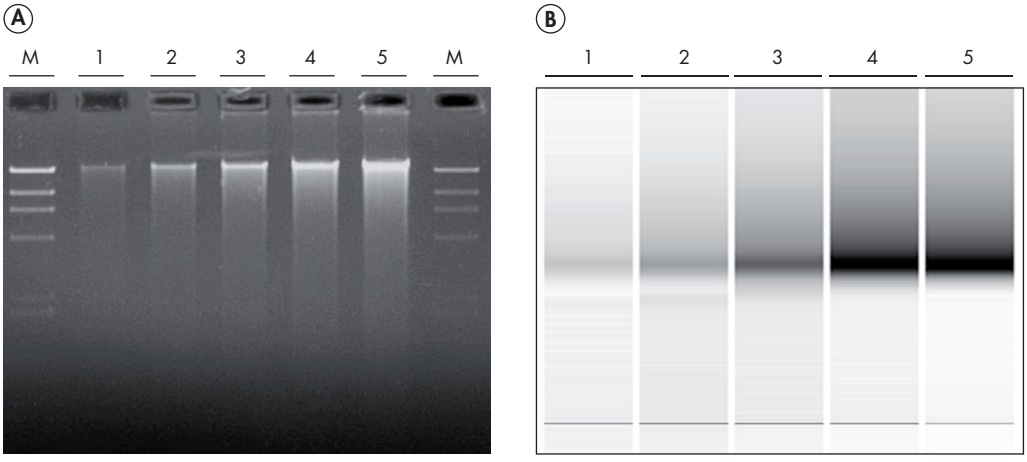

Figure 1. Electrophoresis of samples from a dilution series. A Lanes 1–5: gDNA samples at respective concentrations of 10, 25, 50, 75, and 100 ng/µl run on a 1% agarose gel for 150 min at 80 V. **Lane M:** lambda HindIII size marker. **B** The same samples run using a QIAxcel DNA Screening Cartridge and the AM900 method.

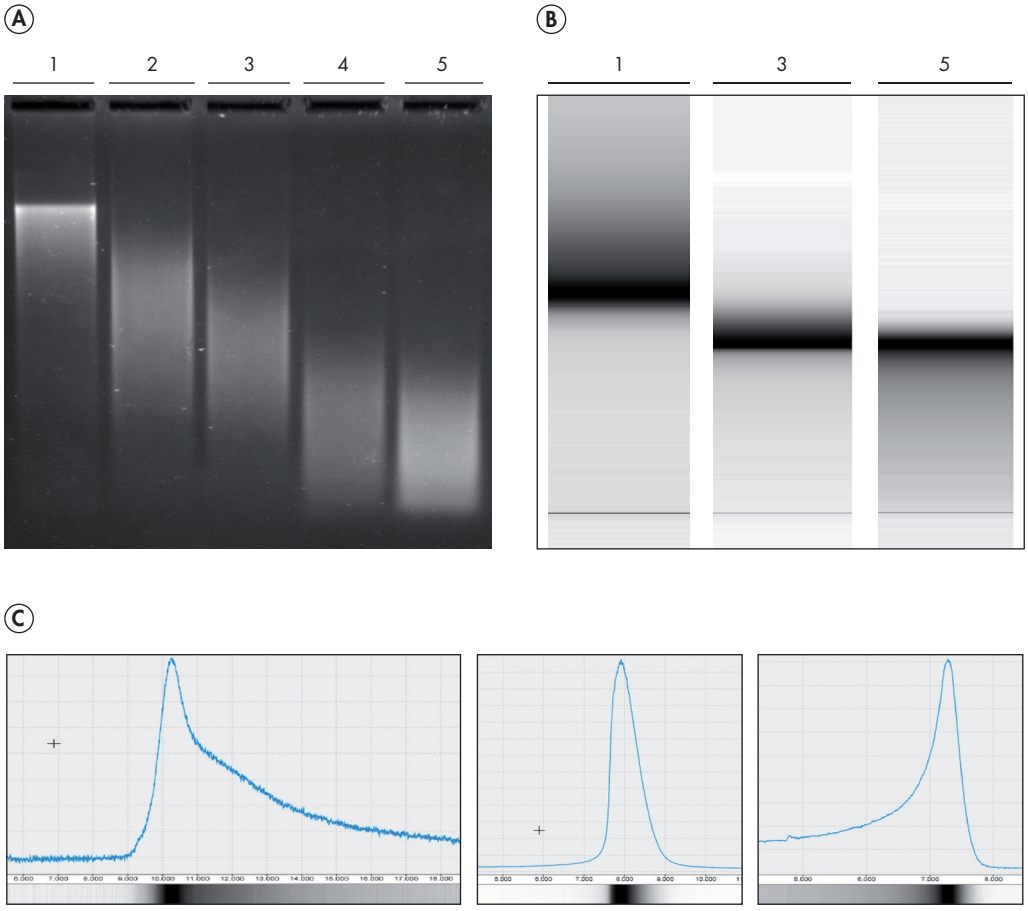

Figure 2. Assessment of DNA degraded by sonication. A Agarose gel photo showing gDNA in various states of degradation. **Lane 1** non-degraded gDNA samples. **Lanes 2 and 3:** gDNA samples partially degraded by sonication with 7 and 14 pulses, respectively. **Lanes 4 and 5:** gDNA samples fully degraded with 70 and 105 pulses, respectively. **B** QIAxcel gel photo showing gDNA samples in three states of degradation. 1, 3, and 5 correspond to lanes 1, 3, and 5 in **A**. **C** Electropherograms and a gel photo of gDNA samples from lanes 1, 3, and 5 in **A**. **Lane 1:** Electropherogram indicates very good quality gDNA (no degradation products before the major peak, long tailing off). **Lane 3:** Partially degraded gDNA (some degradation product signals, no tailing off). **Lane 5:** Highly degraded gDNA (many degradation product signals, no tailing off).

Conclusions

Quality control of genomic DNA purified with silica membrane-based methods can routinely and effectively be performed using the QIAxcel system. The optimal procedure uses the DNA Screening Cartridge and the AM900 method.

The results are highly reproducible, as shown in repeated runs. Using the electropherogram data as well as the results from a gel image allows straightforward verification of the integrity of the purified gDNA. If degradation products are present, they are visible as a broad peak preceding the major gDNA peak.

In order to detect even minute quantities of unwanted degradation products, a long sample injection time of 40 seconds is suitable. It is recommended that gDNA concentrations ranging between 25 and 100 ng/µl are used.

This novel method using the QIAxcel system is optimal for gDNA quality control.

References

1. Shendure, J. and Hanlee, J. (2008) Next Generation Sequencing. Nature Biotechnology **26(10)** 1135.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QX RNA Alignment Marker (1.5 ml)	RNA Alignment marker	929510

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