

Determination of the DNA Molecular Weight (MW) from different Norgen Columns and Isolation Methods

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INTRODUCTION

The isolation of large molecule weight DNA is required for certain sequencing applications such as paired-end sequencing of long-range DNA fragments for *de novo* assembly of mammalian genomes. It has been a belief that DNA isolated using a spin column is fragmented, and that the maximum size isolated is approximately 25 kb. Here, Norgen Biotek investigated the molecular weight (MW) of DNA purified using spin column chromatography based on five different column types (Micro Spin Column, Mini Spin Column, Midi Spin Column, Maxi Spin Column, 96-Well plate) as well as purification based on magnetic beads and alcohol precipitation.

MATERIALS AND METHODS

DNA Sample preparation

Fifty million HeLa cells were pelleted, and 15 mL of Buffer SK was added to lyse the cells (~3.3 M cells/mL). Different lysate volumes and centrifuge speeds were then applied according to the various column types and isolation methods as indicated in Table 1.

Table 1. Specifications of the type of column and method used for DNA isolation.

| | Method used for DNA isolation | Lysis Input volume* | Centrifuge speed (RPM) |
|----|-------------------------------|---------------------|------------------------|
| 1 | Micro Spin Column | 0.5 mL | 6000 rpm |
| 2 | | 0.5 mL | 6000 rpm |
| 3 | Mini Spin Column | 0.5 mL | 6000 rpm |
| 4 | | 0.5 mL | 6000 rpm |
| 5 | Midi Spin Column | 1.5 mL | 3000 rpm |
| 6 | | 1.5 mL | 3000 rpm |
| 7 | Maxi Spin Column | 3 mL | 3000 rpm |
| 8 | | 4 mL | 3000 rpm |
| 9 | 96-Well plate | 0.5 mL | 3000 rpm |
| 10 | | 0.5 mL | 3000 rpm |
| 11 | Magnetic bead | 1.5 mL | N/A |
| 12 | Alcohol ppt | 1.5 mL | N/A |

* 50 M HeLa cells in 15 mL of Buffer SK

Gel electrophoresis

For visual analysis, 10 µL of DNA from the elution was loaded onto a 1.2% 1x TAE agarose gel and run for 25 minutes at 150 V. The gel photo was taken using an Alphasampler™ IS-2200 (Alpha Innotech).

Pulsed-Field Gel Electrophoresis (PFGE)

The purified DNA was used for Pulsed-Field Gel Electrophoresis (PFGE) for better resolution of the MW range. One µg of DNA was loaded on the PFGE gel and run at 4 V for 16 hours followed by 6 V for 6 hours.

RESULTS AND DISCUSSION

The profile of DNA isolated using Norgen's different spin columns and isolation methods was compared between 1.2% 1x TAE gel (Fig. 1) and PFGE (Fig. 2).

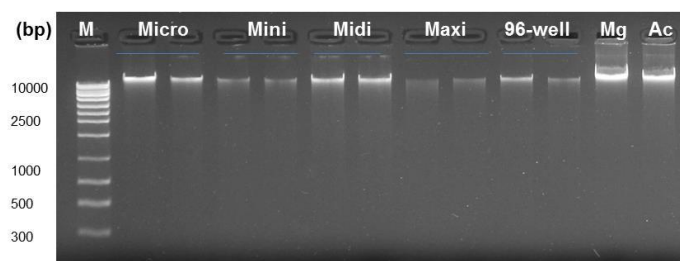


Figure 1. DNA resolution on 1x TAE 1.2% agarose gel. DNA was isolated using five column types (Micro Spin Column, Mini Spin Column, Midi Spin Column, Maxi Spin Column, 96-Well Plate) by spin column chromatography as well as two other isolation methods (Mg: Magnetic bead system, Ac: Alcohol precipitation). Lane M is Norgen's HighRanger 1 kb DNA Ladder (Cat# 11900).

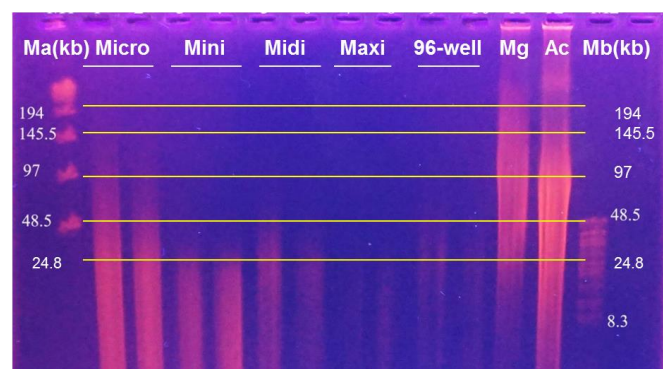


Figure 2. PFGE profile showing the range of the different DNA MW isolated using five column types (Micro Spin Column, Mini Spin Column, Midi Spin Column, Maxi Spin Column, 96-Well Plate) by spin column chromatography as well as two other isolation methods (Mg: Magnetic bead system, Ac: Alcohol precipitation). Lane Ma: NEB Lambda PFG ladder Cat # N0341. Lane Mb: Bio-Rad Chef DNA size standards: 8-48kb, Cat# 170-3707.

CONCLUSIONS

1. PFGE reveals that the molecular weight (MW) of the DNA isolated using Norgen's columns and methods is larger than 25 kb in general.
2. The centrifuge speed applied to the different size of columns, including the 96-well plate, did not affect the MW of the purified DNA.
3. Isolation using magnetic beads or alcohol precipitation isolated higher MW DNA.
4. A detailed summary of the variation in DNA MW is presented in Table 2.

Table 2. Overview of PFGE results showing variable DNA MW from different column types and isolation methods.

| Method used for DNA isolation | % of DNA molecular weight (MW) | | | |
|-------------------------------|--------------------------------|----------|---------|--------|
| | >100 kb | 50-100Kb | 25-50kb | <25 kb |
| Micro Spin Column | 3 | 35 | 35 | 27 |
| | 3 | 35 | 35 | 27 |
| Mini Spin Column | 0 | 5 | 45 | 55 |
| | 0 | 5 | 45 | 55 |
| Midi Spin Column | 0 | 10 | 50 | 40 |
| | 0 | 10 | 50 | 40 |
| Maxi Spin Column | 0 | 10 | 50 | 40 |
| | 0 | 10 | 50 | 40 |
| 96-Well plate | 0 | 10 | 50 | 40 |
| | 0 | 5 | 45 | 55 |
| Magnetic bead | 10 | 50 | 30 | 10 |
| Alcohol ppt | 5 | 40 | 40 | 15 |

Table 3. Examples of Norgen's kits that utilize the different types of columns and methods for DNA isolation from various sample types.

| | Method used for DNA isolation | Example of Norgen Kit | Cat. No |
|----|-------------------------------|---|---------|
| 1 | Micro Spin Column | Cells and Tissue DNA Isolation Micro Kit | 57300 |
| 2 | | Urine DNA Isolation Micro Kit | 18100 |
| 3 | Mini Spin Column | Saliva DNA Isolation Kit | RU45400 |
| 4 | | Stool DNA Isolation Kit | 27600 |
| 5 | Midi Spin Column | Blood DNA Isolation Midi Kit | 51400 |
| 6 | | Urine Cell-Free Circulating DNA Purification Midi Kit | 56700 |
| 7 | Maxi Spin Column | Soil DNA Isolation Maxi Kit | 62000 |
| 8 | | Urine Cell-Free Circulating DNA Purification Maxi Kit | 56800 |
| 9 | 96-Well plate | Soil DNA Isolation 96-Well Kit | 26560 |
| 10 | | Fungi/Yeast Genomic DNA Isolation 96-Well Kit | 27350 |
| 11 | Magnetic bead | Stool DNA Isolation Kit (Magnetic Bead System) | 55700 |
| 12 | Alcohol ppt | Saliva DNA Collection, Preservation and Isolation Kit | RU35700 |