Slicprep™ 96 Device

High-Throughput Processing of Samples on Solid Supports Using the Slicprep™ 96 Device

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INTRODUCTION

Crime statistics now support the effectiveness of aggressively analyzing breaking-and-entering cases and developing genotype databases of these felons. Unfortunately, this approach has created an ever-expanding workload and increasing backlogs. Over the last few years, automation has played an important and expanding role in handling the increased work, but the upfront processing of these samples still creates a bottleneck. Most casework, reference and database samples are on solid supports that do not lend themselves to robotic manipulation. FTA® cards have provided one solution for reference and database samples. However, because of the high DNA-binding capacity, they typically give poor genotype profiles unless the DNA is removed. Although automated methods exist for buccal swabs, they can give variable results and are prone to clogged pipette tips. Until now, casework samples have had to be processed individually to separate the solid support from the eluted DNA solution.

Promega has solved this bottleneck by developing the Slicprep™ 96 Device. This device allows the simultaneous centrifugation of 96 samples and is designed so that both the digestion or lysis and centrifugation can be performed in the same device.

THE SLICPREP™ 96 DEVICE

The Slicprep™ 96 Device consists of 3 components: a 2.2ml 96 Deep Well Plate, a 96 Spin Basket and a U-Shaped Collar (Figure 1). The 7 holes in the rounded bottom of the baskets ensure good removal of DNA and cells from the solid support. Samples such as cotton swabs, blood punches or pieces of clothing are inserted into the baskets, which are big enough to accept an entire dried cotton swab. In the digestion position, the 96 Spin Basket is fully inserted into the 96 Deep Well plate, allowing space for approximately 165µl of solution below the basket in each well (Figure 2, Panel A). After the incubation, the baskets are raised approximately 1cm (the spin position, Figure 2, Panel B) to create space for an additional 500µl of solution.

MATERIALS AND METHODS

With the device in the digestion position, samples were placed in the baskets. For reference samples, 400µl of DNA IQ™ Lysis Buffer was added, and the device was sealed with a foil seal and heated in a 70°C water bath for 1 hour. For touch samples, 400µl of a 1.8µg/µl proteinase K solution was added, and samples were incubated in a 56°C oven for 1 hour. After incubation, the Slicprep™ 96 Device was removed from the water bath or oven, and the U-Shaped Collar was inserted. The device was then centrifuged at 1,450 × *g* for 5 minutes in a swinging plate rotor. The collar and baskets were then discarded, leaving the DNA-containing solution in the 96 Deep Well plate. The 96 Deep Well Plate was placed on a Beckman Coulter Biomek® 2000 workstation, and a BioWorks™ method directed DNA purification using the DNA IQ™ System[a]. DNA was eluted in 100µl (reference samples) or 40µl (touch samples) of TE^{-4} buffer (10mM Tris [pH 8.0], 0.1mM EDTA). Finally, a

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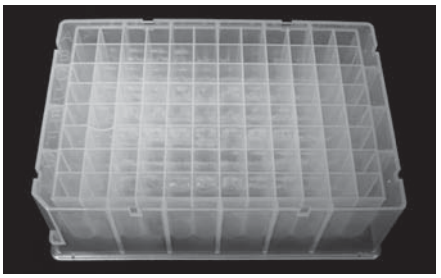
total of 1µl of the eluted DNA or 10µl of eluted control (blank) DNA was amplified with the PowerPlex® 16 System[b–d] and analyzed on an ABI PRISM® 3100 Genetic Analyzer.

TESTING FOR CONTAMINATION

The Slicprep™ 96 Device is made and packaged in a HEPA-filtered clean room to reduce the risk of DNA contamination. To detect any contamination introduced during the manufacturing process or the robotic method, the Slicprep™ 96 Device was incubated with DNA IQ™ Lysis Buffer, and the solution was then processed using the DNA IQ™ System and amplified with the PowerPlex® 16 System. To check for cross-contamination between samples, FTA® blood punches and cotton buccal swabs were placed in the baskets in a checkerboard pattern. Blank FTA® punches and cotton swabs were placed in the remaining baskets. Samples were processed as described in the Materials and Methods section. None of the blanks contained discernible peaks (data not shown).

96 Spin Basket

U-Shaped Collar



96 Deep Well Plate

5244TA

Figure 1. Components of the Slicprep™ 96 Device. The Slicprep™ 96 Device contains an ABgene 2.2ml, 96-well, deep-well plate (the 96 Deep Well Plate), a 96 Spin Basket and a U-Shaped Collar.

PROCESSING FTA® CARD PUNCHES AND BUCCAL SWABS

The Slicprep™ 96 Device is ideally suited for high-throughput extraction of DNA from database samples such as blood cards and buccal swabs. One 4mm-diameter or three 2mm-diameter FTA® punches containing blood, and whole cotton and paper (CEP) buccal swabs were processed as described in the Materials and Methods section using 400µl of DNA IQ™ Lysis Buffer (500µl for paper swabs).

The results are shown in Table 1, and representative genotypes from cotton swabs are shown in Figure 3. All three sample sets gave the expected yields with tight standard deviations. The FTA® blood cards yielded, on average, 70ng of DNA. Because DNA is most efficiently removed from FTA® paper by denaturation, heating this sample type to 90°C may give slightly higher yields. The buccal swabs gave average DNA yields of 151ng and 396ng for whole cotton and paper swabs, respectively. The higher DNA:protein ratios in these sample types (compared to blood) result in a higher DNA-binding capacity for the DNA IQ™ Resin. These yields are similar to those obtained using single spin baskets. Paper swabs can collect a large number of cells, forcing higher recovered yields. Using a fraction of the paper swabs would give yields closer to those obtained with cotton swabs but is less convenient when processing large numbers of this sample type.

Table 1. Average DNA Yields Using the Slicprep™ 96 Device.

Support Type	Average Yield (ng)	Standard Deviation (ng)
FTA® Blood Cards	70	10
Cotton Swabs	151	56
Paper Swabs	396	60

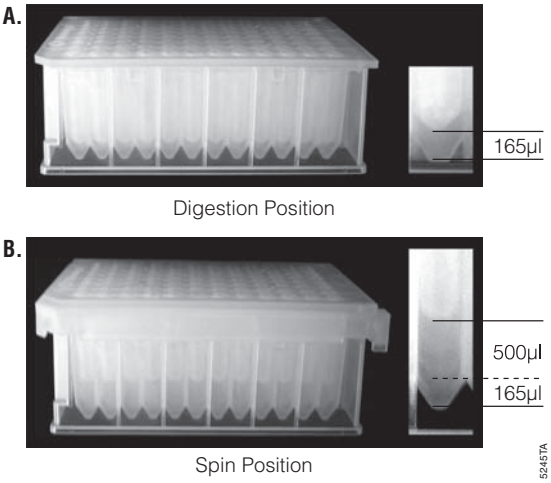


Figure 2. Operational modes of the Slicprep™ 96 Device. Panel A. The digestion position. The 96 Spin Basket is fully inserted into the 96 Deep Well Plate to allow incubation of solid supports with digestion or lysis buffer. **Panel B.** The spin position. The U-Shaped Collar is inserted between the 96 Deep Well Plate and the 96 Spin Basket. This raises the baskets and allows an additional 500µl below the baskets.

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To test whether touch samples can be processed in a high-throughput manner, samples were collected on cotton swabs from soda cans, phones and computer keyboards. The swabs were placed in the 96 Spin Basket, and the shafts were broken off and used to push the swab head to the bottom of the basket. Samples were processed as described in Materials and Methods. The resulting DNA solutions were purified manually using the DNA IQ™ chemistry. As expected, some samples provided more DNA than others, but many of the samples gave full profiles (Figure 4).

AUTOMATION WITH THE SLICPREP™ 96 DEVICE

The Slicprep™ 96 Device is designed to provide a higher throughput than individual spin baskets for upfront processing of samples on solid supports. For database and reference samples where the sample is soaked in DNA IQ™ Lysis Buffer, the extracted fractions in the 96 Deep Well Plate can be processed using existing methods. Touch samples are best processed with up to 500µl of proteinase K digestion solution, which must be diluted with two volumes of DNA IQ™ Lysis Buffer before DNA purification. The resulting large volumes are not efficiently processed by most workstations. However, Dr. Susan Greenspoon from the Virginia Division of Forensic Sciences has collaborated with Promega to develop a Biomek® 2000 method that can efficiently handle aqueous sample volumes up to 0.5ml as efficiently as manual DNA IQ™ System or phenol:chloroform purification. A method to incorporate the Slicprep™ 96 Device for processing large volumes is being developed.

ORDERING INFORMATION

Slicprep™ 96 Device Cat.# V1391

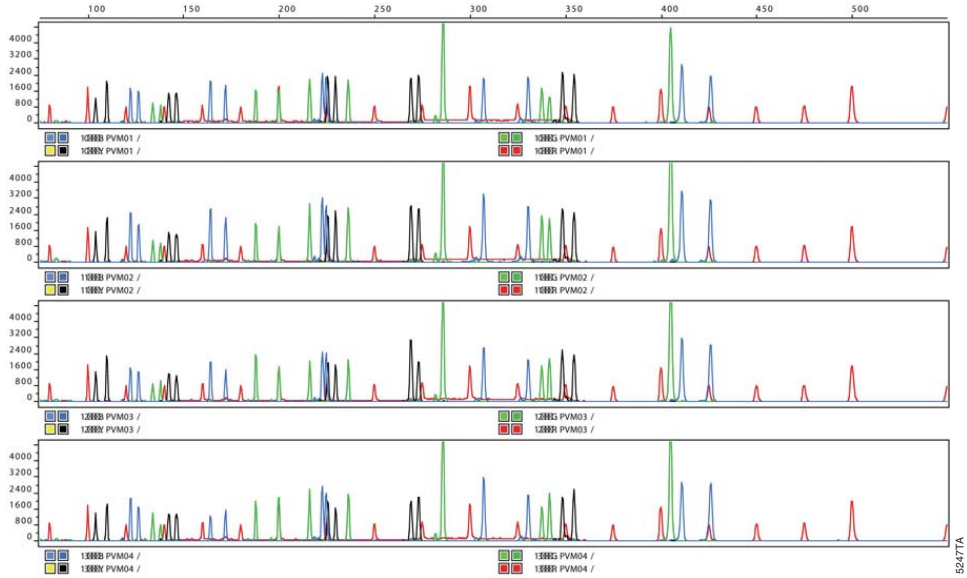

Figure 3. Analysis of buccal swabs. Cotton swabs were placed in a checkerboard pattern between buccal swabs in the Slicprep™ 96 Device. DNA IQ™ Lysis Buffer was added, and the device was sealed with a Beckman Coulter Biomek® seal & sample aluminum foil lid (Cat.# 538619) and incubated at 70°C for 1 hour. The device was centrifuged for 5 minutes at 1,450 × *g* (3,000rpm) in a Beckman Coulter Allegra 6R centrifuge with a GH-3.8 rotor containing Microplus plate carriers. The samples were processed on a Beckman Coulter Biomek® 2000 workstation using DNA IQ™ chemistry, and the DNA was eluted in 100µl of TE^{-4} buffer. One microliter was amplified with the PowerPlex® 16 System and analyzed on an ABI PRISM® 3100 Genetic Analyzer. Quantitation of the DNA was not necessary.

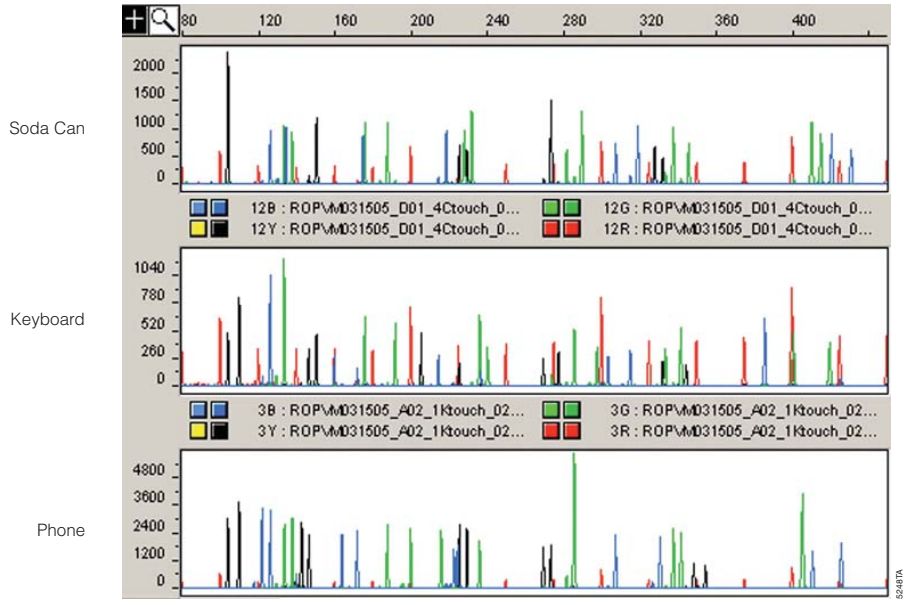

Figure 4. Analysis of touch samples. Soda cans **(Panel A)**, keyboards **(Panel B)** and phones **(Panel C)** were swabbed with cotton swabs, and the swabs were placed in a Slicprep™ 96 Device after drying. A total of 400µl of proteinase K digestion buffer was added, and the samples were incubated for 1 hour at 56°C in an oven with a foil seal. The device was centrifuged to recover the DNA. Samples were processed manually using the DNA IQ™ System, and DNA was eluted in 40µl of TE^{-4} buffer. The samples were amplified with PowerPlex® 16 and analyzed on an ABI PRISM® 3100 Genetic Analyzer.