Rapid Isolation of Genomic DNA from Small Quantities of Human Tissue

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INTRODUCTION

Many molecular biological techniques require genomic DNA as a starting material for manipulation. This is especially true for forensic disciplines, where the rapid and robust isolation of small quantities of DNA from human tissues is critical. This report demonstrates an extremely simple and rapid modification of the versatile SV Total RNA Isolation System[(a)] that enables the isolation of genomic DNA from a variety of fresh and dried human samples in as little as 15 minutes. The DNA isolated using this technique is suitable for analysis with standard techniques, including STR typing using the *GenePrint*™ PowerPlex™ Systems[(b)].

The techniques presented in this report can be used to isolate genomic DNA from a variety of sample media, including fresh or dried human buccal swabs, dried blood and as little as 10µl of fresh blood.

The isolation of human genomic DNA is a fundamental first step in an array of molecular techniques involved in genetic identity analysis. The initial isolation of DNA from a sample source can be tedious, involving steps that yield the highest quality DNA at the expense of speed and convenience. Many techniques currently used for genomic DNA isolation lead to significant dilutions of the sample material or require the precipitation of genomic material, making the isolation of nucleic acids from small amounts of material especially difficult. This consideration is especially important in the field of forensic analysis and genetic identity where DNA is extracted from extremely small amounts of starting material often recovered from suboptimal storage conditions.

The techniques presented in this report can be used to isolate genomic DNA from a variety of sample media, including fresh or dried human buccal swabs, dried blood and as little as 10µl of fresh blood. In addition, the speed and throughput of the technique enable large numbers of samples to be processed quickly. This report outlines the basic protocol used to isolate DNA from a variety of human sources. The quality of the isolated DNA is demonstrated by spectrophotometric analysis and performance in the *GenePrint*™ PowerPlex™ 1.1 System.

HUMAN GENOMIC DNA ISOLATION

The basic DNA isolation technique is outlined in Figure 1. Slight variations in sample preparation are required depending on the nature of the starting material. However, once the samples are lysed in the SV RNA Lysis Buffer provided with the SV Total RNA Isolation System, all subsequent steps in the isolation technique are identical. The details of sample preparation are given below. An optional RNase step can be performed while the DNA is bound to the SV membrane (Figure 1); however, omission of this step did not affect the performance of the isolated genomic DNA in any of the analyses. Therefore, this step was not performed in these studies.

This report demonstrates a simple modification of the SV Total RNA Isolation System protocol that allows rapid and robust isolation of human genomic DNA.

Human blood. Whole human blood was obtained by venipuncture into EDTA-coated Vacutainer® tubes. Blood (300µl) was placed into a microcentrifuge tube, and 900µl of SV RNA Red Blood Cell Lysis Solution (Cat.# Z3141) were added. The sample was mixed by inversion and incubated at room temperature for 5 minutes. The sample was centrifuged at 14,000 x *g* for 20 seconds, and 1ml of supernatant was removed carefully (so as not to disturb the cell pellet) and discarded. SV RNA Lysis Buffer (175µl) was added, and the tube was vortexed for 10 seconds to resuspend the cell pellet. The sample was then processed as outlined in Figure 1.

For blood samples obtained on cotton swabs, the swab was immersed in 350µl of SV RNA Lysis Buffer contained in a microcentrifuge tube. The swab was incubated in the SV RNA Lysis Buffer for 5 minutes with occasional agitation. Dried blood on cotton swabs was treated identically to freshly soaked cotton swabs. (Paper swabs may be used; however, DNA yields will be lower due to retention of the DNA in the paper matrix.) For dried blood spots on nonporous

The excellent performance of the isolated DNA as determined by spectrophotometric and STR analyses indicate that the technique may be used to isolate DNA for a variety of forensic and research purposes.

Table 1. Spectrophotometric analysis of isolated human nucleic acids.*

Sample	A_{260}	A_{260}/A_{280}	Yield
Whole human blood (300µl)	2.21	1.54	11.1µg
Whole human blood (100µl)	1.05	1.23	5.25µg
Whole human blood (30µl)	0.465	1.40	2.33µg
Whole human blood (10µl)	0.186	1.48	0.93µg
HeLa cells (3.9 x 10^6)	1.65	1.86	8.25µg
Blood-soaked cotton swab	0.531	1.52	2.66µg
Blood-soaked cotton swab (dried)	0.255	1.60	1.28µg
Dried blood spot (approximately 50µl)	0.3	1.5	1.5µg
Oral cotton swab	0.279	1.62	1.4µg
Dried oral cotton swab	0.171	1.78	0.86µg

*Actual yields may vary depending on sample source and collection technique. Although RNA is present in the samples, it accounts for 1-1.5µg per 15-25µg of DNA from blood samples. This RNA does not affect performance of the DNA in PowerPlex™ Systems, but can be removed with an optional RNase step, if desired.

materials, the area was sampled using a cotton swab saturated with SV RNA Lysis Buffer. The swab containing the recovered blood material was then placed in 350µl of SV RNA Lysis Buffer and treated identically to the freshly soaked cotton swabs. After incubation at room temperature, the swab was removed and the samples were processed as shown in Figure 1.

Oral swabs. Oral (buccal) swabs were prepared by sampling the interior cheek of the subject with a cotton swab. Dried buccal swabs were prepared by drying the cotton swab overnight at ambient temperature. Freshly prepared and dried buccal swabs were immersed in a microcentrifuge tube containing 350µl of SV RNA Lysis Buffer and incubated at room temperature for 5 minutes with occasional agitation. The swab was removed, and the sample was processed as shown in Figure 1.

Other materials. Other materials, such as dried human teeth, were processed by placing the sample directly into 350µl of SV RNA Lysis Buffer and incubating at room temperature for 5 minutes with occasional vortexing. Samples were then processed as in Figure 1.

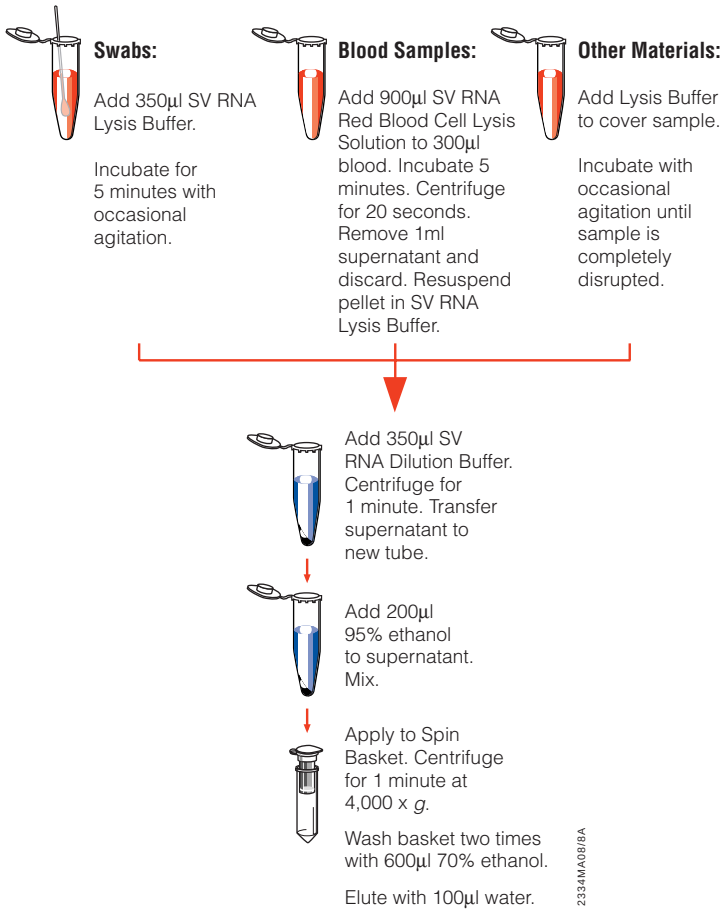

Figure 1. Schematic of genomic DNA isolation using the modified SV Total RNA Isolation System Spin Basket procedure.

ANALYSIS OF ISOLATED HUMAN GENOMIC DNA

Spectrophotometric data for duplicate isolated human nucleic acid samples are given in Table 1. Spectrophotometric analysis of teeth samples was not possible due to limiting amounts of purified nucleic acids. Isolated DNA was examined by Short Tandem Repeat (STR) analysis using the *GenePrint*™ PowerPlex™ 1.1 System (Cat.# DC6091). The *GenePrint*™ PowerPlex™ 1.1 System allows for the simultaneous, single-tube amplification and two-color detection of eight polymorphic STR loci. Each sample was analyzed following the GenePrint™ *PowerPlex™ 1.1 System Technical Manual* #TMD008 (2). Aliquots of each *GenePrint*™ PowerPlex™ reaction were analyzed by 4% denaturing acrylamide gel electrophoresis imaging of the resulting gel using a Hitachi FMBIO® II Fluorescent Scanner. Typical results demonstrating the excellent performance of the purified DNA for STR analysis are shown in Figure 2.

CONCLUSIONS

This report demonstrates a simple modification of the SV Total RNA Isolation System protocol that allows rapid and robust isolation of human genomic DNA. The technique was used for isolation of DNA from a variety of fresh and archived sample material including blood, buccal swabs and teeth. The excellent performance of the isolated DNA as determined by spectrophotometric and STR analyses indicate that the technique may be used to isolate DNA for a variety of forensic and research purposes.

REFERENCES

1. Kinzler, K.W. *et al.* (1991) *Science* **253**, 661.
2. GenePrint™ *PowerPlex™ 1.1 System Technical Manual* #TMD008, Promega Corporation.

[a]Patent Pending.

[b]U.S. Pat. No. 5,843,660 has been issued to Promega Corporation for multiplex amplification of STR loci.

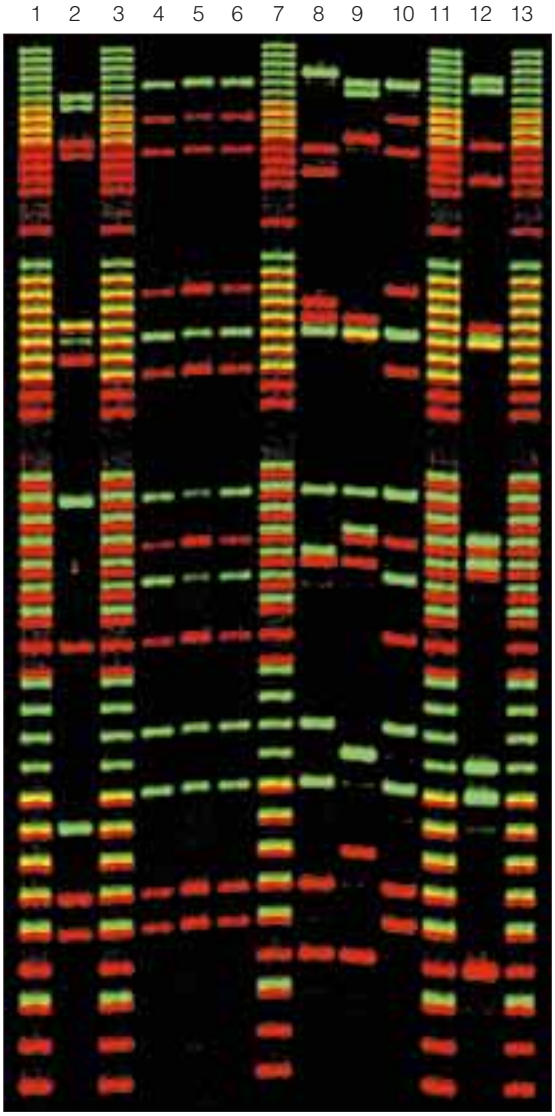

Figure 2. *GenePrint*™ PowerPlex™ 1.1 System analysis of isolated human genomic DNA. K562 Control DNA (1ng, lane 2) or isolated genomic DNA (2ng) from whole blood (lane 4), dried blood-soaked cotton swab (lane 5), dried blood spot (lane 6), buccal swabs (lanes 8-10) or 2.5µl of eluate from human tooth sample (lane 12) were amplified using the *GenePrint*™ PowerPlex™ 1.1 System. Aliquots of each amplification reaction were resolved by 4% denaturing acrylamide gel electrophoresis and analyzed using a Hitachi FMBIO® II Fluorescent Scanner. Lanes 1, 3, 7, 11 and 13, *GenePrint*™ PowerPlex™ 1.1 Allelic Ladder Mix (Cat.# DG3301).