Using a commercially available DNA extraction kit to obtain high quality human genomic DNA suitable for PCR and genotyping from 11-year-old saliva saturated cotton spit wads

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Abstract

Background: We sought to describe the integrity of human genomic DNA extracted from saliva saturated cotton spit wads stored at -20°C for approximately 11 years. 783 spit wad samples were collected from an ADHD sample population (Vermont Family Study) during 1996–2000. Human genomic DNA was extracted from the spit wads using a commercially available kit; QIAamp DNA Blood Midi Kit (Qiagen, Inc., Valencia, CA.) with a few modifications.

Results: The resulting DNA yield was more than adequate for genetic analysis and ranged from approximately 1 µg to a total of 80 µg (mean 17.3 µg ± 11.9 µg). A260/A280 ratios for the human genomic DNA extracted from the spit wads was consistently within the generally acceptable values of 1.7–2.0, with the lowest purity being 1.70, and a mean value of 1.937 ± 0.226 for the 783 samples. The DNA also was suitable for PCR reactions as evidenced by the amplification of the serotonin-transporter-linked polymorphic region, 5HTTLPR. 5HTTLPR is a functional polymorphism in the promoter region of the serotonin transporter gene (HTT, SLC6A4, or SERT), consisting of two intensively studied alleles. 770 of the 783 samples (98.3%) produced fragments after PCR of the expected size with primers specific for 5HTTLPR.

Conclusion: High quality and abundant genomic DNA can be successfully retrieved from saliva saturated cotton spit wads using the commercially available kit, QIAamp DNA Blood Midi Kit from Qiagen, Inc. Furthermore, the DNA can be extracted in less than 3 hours and multiple samples can be processed simultaneously thus reducing processing time.

Background

Given the increasing emphasis the study of molecular genetic influences on the development of psychiatric disorders; simple, noninvasive and cost-effective methods of collecting DNA for large-scale studies are needed for real time and remote (after years of storage) genetic analyses. Whole blood, serum, and plasma have long been the gold standard for obtaining high quality, abundant genomic
DNA suitable for genetics studies; however, research has shown that a blood draw may be a significant barrier for study participation, especially those studies involving pediatric patients [1] with complex psychiatric disorders such as attention deficit hyperactivity disorder (ADHD) and autism spectrum illness. Buccal cells have proven to be an effective painless procedure as a means to DNA collection from large sample populations [2]. In addition, the procedure is relatively quick, cost effective, and a non-invasive means to collect genomic DNA. Buccal cells can be collected using a variety of different methods including cytobrushes, clean sterile swabs, mouthwash, saliva alone, and in the case of the Vermont Family Study with spit wads.

Genetic analysis experiments require the genomic DNA from the study sample to be of adequate quantity and quality. The affymetrix 6.0 chips, which enable genotyping of up to 1.8 million genetic markers, requires 500 ng of total genomic DNA [3]. In addition, PCR reactions required to amplify microsatellite markers in candidate gene and linkage studies typically require 50 ng or more of genomic DNA per marker [4,5]. The quality of genomic DNA extracted for experiments are typically measured using spectrophotometric absorbance ratios of 260 nm/280 nm. High quality DNA is considered to have an A260/A280 ratio of 1.7–2.0. The A260/A280 ratio is that pure genomic DNA will have a ratio between 1.7 and 2.0. The mean A260/A280 Ratio for the 783 samples was determined to be 1.937 ± 0.226. Interestingly, samples concentrated using Microcon YM-100 centrifugal filter devices generally increased in purity as measured using the A260/A280 ratio. As a result, the adjusted mean A260/A280 ratio of the sample population after concentrating samples that fell below our selected 50 ng/ul cutoff was 1.965 ± 0.124.

Using PCR analysis of the 5HTTLPR polymorphism we identified that 770 DNA samples (98.3%) were successfully amplified and detected utilizing fragment analysis on the ABI 3130 Genetic Analyzer. 48 DNA samples were randomly selected and PCR was repeated under the same conditions and fragments were identified as concordant using gel electrophoresis. A representative gel is shown in figure 1.

**Results**

**DNA Yield**

The quantity of genomic DNA extracted from the saliva saturated cotton spit wads was determined using the conventional method of absorbance at 260 nm (A260). We found the DNA yield from the 783 DNA extractions to be highly variable from sample to sample, with an average yield of 17.3 µg/s with a standard deviation of ± 11.9 µg/s. Genomic DNA yield ranged from 1 µg to as high as 80 µg/s. By our own empirical observation, DNA yield seem to correlate with the saturation level of the spit wad. Spit wads heavily saturated with saliva generally produced a higher genomic DNA yield in comparison with drier spit wads.

**DNA Quality**

The quality of DNA was assessed using two different methods. Initially, DNA quality was determined using an A260/A280 ratio. Additionally, because the main purpose for collecting the DNA was for future genotyping studies, we assessed DNA quality using PCR amplification of the 5HTTLPR polymorphism. The generally used convention of assessing DNA quality with an A260/A280 ratio is that pure genomic DNA will have a ratio between 1.7 and 2.0. The mean A260/A280 Ratio for the 783 samples was determined to be 1.937 ± 0.226. Interestingly, samples concentrated using Microcon YM-100 centrifugal filter devices generally increased in purity as measured using the A260/A280 ratio. As a result, the adjusted mean A260/A280 ratio of the sample population after concentrating samples that fell below our selected 50 ng/ul cutoff was 1.965 ± 0.124.

**Discussion**

This methodology report describes the effectiveness of a commercially available kit in extracting and purifying human genomic DNA from saliva saturated cotton spit wads. We found that DNA obtained from the spit wads was of adequate quantity and quality for use in downstream genetic studies.

The yield of genomic DNA from buccal cells from spit wads was highly variable, with yields ranging from 1 µg to as high as 80 µg/s. The variability observed with DNA yield is consistent with results from other studies using buccal cytobrushes in which yields are reported from 0.5 µg to 12.66 µg/s [8,9,6,10]. In our sample of 783 individuals, we generated a mean of 17.3 µg/s ± 11.9 µg/s of genomic DNA from the spit wads. The yield of genomic DNA achieved from the spit wads would be more than adequate for numerous genetics studies including microsatellite analysis, whole genome association studies, linkage analysis, copy number variation experiments, etc.

We also report an estimate of DNA quality extracted from the Vermont Family Study samples using an A260/A280 ratio to identify DNA purity and protein contamination. DNA is generally considered to be of adequate quality when the A260/A280 Ratio is between 1.70 and 2.0. The quality of genomic DNA from the saliva saturated cotton spit wads, assessed using spectrophotometer readings, was more than adequate as the mean A260/A280 ratio for...
the 783 samples was 1.937 ± 0.226. Since the DNA was to
be banked for future genetic studies, we imposed a 50 ng/μl concentration cut-off for each sample prior to being
stored at -20°C. Samples with concentrations below this
threshold were concentrated using Microcon YM-100 cen-
trifugal filter units. Interestingly, after concentrating these
samples through the filter unit, the purity as measured by
an A260/A280 ratio increased. The adjusted mean after con-
centration of samples falling below the 50 ng/μl cutoff
was 1.965 ± 0.124. Most likely, any contaminants or
impurities are removed during the concentration proce-
dure increasing purity of the genomic DNA prep.

In addition, the quality of genomic DNA was also assessed
by PCR success using primers specific for the 5HTTLPR
polymorphism. We identified a 98.3% success rate (770/
783) with the genomic DNA as a template for this PCR
reaction. A representative gel is shown in figure 1 showing
the fragments observed from a PCR reaction with
5HTTLPR specific primers.

Conclusion
This report describes a novel, cost effective, and efficient
way to collect genomic DNA from individuals enrolling in
genetic studies. This method should enable researchers to
obtain high quality and abundant genomic DNA that can
be successfully retrieved from saliva saturated cotton spit
wads using the commercially available kit, QIAamp DNA
Blood Midi Kit from Qiagen, Inc. Furthermore, the DNA
can be extracted in less than 3 hours and multiple samples
can be processed simultaneously thus reducing processing
time and cost.

Methods
Participants
The Vermont Family Study is a collection of samples from
207 families comprising 783 individuals. 167 families
were part of an ADHD sample with one member of each
family recognized as a Proband for the disorder. 40 fami-
lies were included as control families with no DSM-IV
diagnoses.

Sample Collection
Participants were asked to refrain from eating or drinking
1 hour prior to saliva collection. Each individual was
instructed to place a standard 2" × 2" piece of cotton gauze
in the buccal region of their cheek for 3 minutes. The
saliva saturated cotton spit wad was removed, rolled to fit
a collection tube, and stored at -20°C until the genomic
DNA was extracted. All data collected and analyzed with
approval of the UVM COM IRB Ethics Committee.

DNA Extraction and Quantification
All DNA extractions were performed at the Avera Institute
for Human Behavioral Genetics. DNA was extracted from
saliva saturated cotton spit wads using a column-based
purification method. Rolled spit wads measured approxi-
mately, 5 cm × 1.5 cm, and DNA was extracted from buc-
cal cells using the QIAamp DNA Blood Midi Kit large
volume protocol (Qiagen) according to the manufac-
turer's instructions with a few modifications. The spit wad
was incubated at 70°C in a Protease/Lysis buffer mixture
(200 ul Qiagen Protease/2.4 ml buffer AL) for 30 minutes
in a 15 ml conical tube (Fisher Scientific). The lysate was
separated from the spit wad using centrifugal force by
placing the spit wad in the barrel of a 5 ml syringe (Becton
Dickson) that was seated in a conical 15 ml tube and cen-
trifuged for 10 minutes at 12,000 rpm. The spit wad was
discarded and 2 ml absolute ethanol was added to the
lysate and the tube was mixed by vigorous shaking (vor-
texing). Approximately one half of the lysate/ethanol mix-
ture was transferred to a Qiagen Midi column placed in a
clean 15 ml conical tube (Fisher Scientific). The lysate was
separated from the spit wad using centrifugal force by
placing the spit wad in the barrel of a 5 ml syringe (Becton
Dickson) that was seated in a conical 15 ml tube and cen-
trifuged for 10 minutes at 12,000 rpm. The spit wad was
discarded and 2 ml absolute ethanol was added to the
lysate and the tube was mixed by vigorous shaking (vor-
texing). Approximately one half of the lysate/ethanol mix-
ture was transferred to a Qiagen Midi column placed in a
clean 15 ml conical tube. The column was centrifuged @
1850 × g for 3 minutes. The filtrate was discarded and the
remaining lysate/ethanol mixture was applied to the same
column and centrifuged @ 1850 × g for 3 minutes. The
column was washed with 2 mls of buffer AW1 and centrifuged @
3220 × g for 2 minutes. The column was washed a second time with 2 mls of buffer AW2 and centrifuged @
3220 × g for 30 minutes to ensure complete drying.
DNA was eluted from the column into a clean 15 ml con-
ical tube by adding 200 μl buffer AE to the column, incu-
bating at room temperature for 5 minutes, and centrifuging @ 3220 x g for 4 minutes. For maximum DNA yield a second elution was performed as described above, yielding approximately 400 µl total volume. DNA concentration and purity were determined using UV spectrophotometry (Nanodrop). All genomic DNA was either diluted or concentrated to a final concentration of 50 ng/ul. DNA was diluted in a reduced EDTA buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and concentrated using Microcon YM-100 centrifugal filter devices (Millipore) according to the manufacturer’s instructions.

PCR Amplification
The Quality of DNA isolated from the spit wad was assessed by PCR amplification of the serotonin-transporter-linked polymorphic region (5HTTLPR). PCR products were visualized using 2% agarose gel electrophoresis or fragment analysis on an ABI 3130 Genetic Analyzer (Applied Biosystems, Inc.). Primer sequences for 5-HTTLPR were previously described; forward primer (5’-ATGCCCAGCCTAAACCCCTCCTG-3’) and the reverse primer (5’-GGACCGCAAGGTGGGGGA-3’) [7]. When running samples on the 3130 genetic analyzer a fluorescently tagged forward primer (6FAM, Applied Biosystems) was used to tag the PCR product for fragment analysis. This primer pair amplifies a 419 base pair product for the 16-repeat long (L) allele and a 375 base pair product for the 14-repeat short (S) allele. PCR reactions were performed using a PCR Master Mix (Promega) containing a final concentration of 1.5 mM MgCl₂, 1× reaction buffer, 200 µM of each dNTP, 40 ng purified genomic DNA, 1.25 units Taq DNA polymerase, and 5 pmols of each primer in a 25 µl reaction. PCR cycling conditions consisted of an initial denaturation at 95 °C for 15 minutes, 35 cycles each consisting of 30 s at 94 °C, 30 s at 66 °C, and 40 s at 72 °C. Elongation was continued for 15 min at 72 °C after the last cycle. S vs. L fragments were called using GeneMapper Software Version 4.0 (ABI), or fragments were separated on a 2% agarose gel supplemented with ethidium bromide (0.02%, Fisher).

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
EAE: Participated in design of study, manuscript preparation, DNA extraction, and PCR analysis, TLN: Participated in DNA extraction and PCR analysis, JJI: Designed and executed collection of spit wads in Vermont Family Study and manuscript preparation, GED: Participated in design of study and manuscript preparation

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