suggesting that mutations in the aspartoacylase gene are likely to be highly specific for Canavan disease. Mutational analysis for Canavan disease meets the previously defined characteristics of Tay-Sachs disease heterozygote-detection programs (Kaback et al. 1977) and could be used for screening in this population. Given the experience with Tay-Sachs disease–carrier programs, it is likely that carrier screening for Canavan disease will be widely accepted in the Ashkenazi community.

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High-Yield Noninvasive Human Genomic DNA Isolation Method for Genetic Studies in Geographically Dispersed Families and Populations

To the Editor:

Human genomic DNA is commonly isolated from peripheral blood samples for genetic studies of families and populations. Blood sampling, however, is expensive and an invasive procedure to which, for ethical reasons, objections may be raised, especially in studies involving older individuals and babies. We have developed a new noninvasive DNA sampling and isolation method involving oral samples taken with cotton swabs. Participants can take mouth swabs themselves, and can send these by mail to the research center, where DNA can be isolated at least up to 3 wk after sampling. DNA isolation from 20 cotton swabs resulted in an average yield of 40 μg of high-molecular-weight DNA per individual, sufficient for complete genome searches with ~800 polymorphic DNA markers when using PCR. Compared with blood sampling, which involves clinically trained personnel, this procedure is fast, less expensive, and suitable especially for DNA collection from geographically scattered subjects.

We have isolated human genomic DNA of family members (ages between 4 and 72 years), young twins (ages between 2 mo and 5 years), and random controls by using mouth swabs. Mouth swabs of the family members and random controls were taken by the participants themselves, and parents took mouth swabs of their twins, following a written protocol. Although twin pairs were sometimes only a few months old, problems were not encountered by parents obtaining samples from their children. At least 10 consecutive samples can be taken from one subject without a significant loss in yield per cotton swab. A second round of 10 cotton swabs can be taken after ~4 h, giving a maximal result of 20 samples in 1 d. The mouth swab sample should be taken in a clean mouth without food remains. After rubbing, the cotton swab, sample-end first, should be placed in a Falcon tube, containing 0.5 ml of STE buffer (100 mM NaCl, 10 mM TrisHCL [pH 8.0] and 10 mM EDTA)

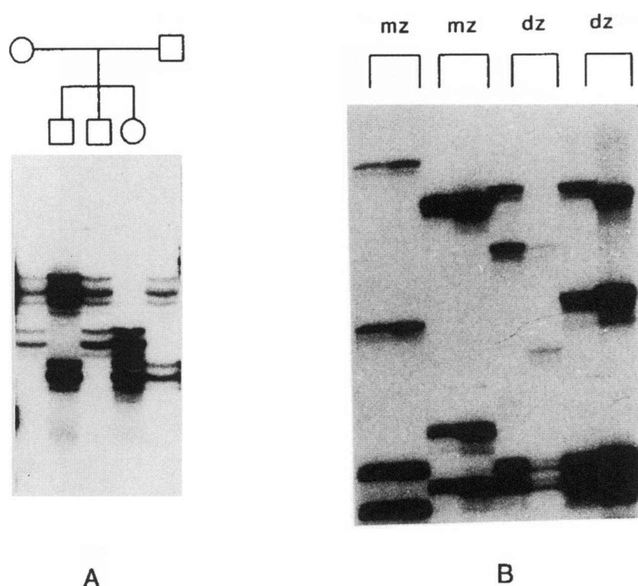

Figure 1 To isolate human genomic DNA, Falcon tubes containing the cotton swabs and STE buffer with proteinase K and SDS, are placed in a 65°C water bath for 2 h. To collect a maximal amount of buffer from the soaked cotton swabs after lysis, sticks are placed in a syringe, which is placed upside-down in the Falcon tube. Falcon tubes containing the syringes are centrifuged for 5 min at 1,000 rpm in a Beckman centrifuge. Genomic DNA is subsequently isolated from the collected lysis buffer using phenol:chloroform:isoamylalcohol (24:24:1) and chloroform:isoamylalcohol (24:1) extractions followed by isopropanol precipitation as described by Sambrook et al. (1990). RNAse treatment was necessary to remove low-molecular-weight RNA bands and smears. PCR reactions were performed in 25 μl containing 25–50 ng genomic DNA, 2.5 pmol of each primer, 1 × *Taq* buffer (Sphaero Q), 2 μCi α[^{32}P]-dCTP, 200 μM each of dCTP, dGTP, dTTP, and dATP, and 0.05 U of Super *Taq* DNA polymerase. Amplification was initiated with 3 min denaturation at 94°C followed by 35 cycles of 15 s at 94°C, 30 s at 60°C, and 30 s at 72°C. The amplification was finished by a final incubation at 72°C for 3 min. Alleles were separated by electrophoresis through a denaturing 6% polyacrylamide gel, and analyzed by autoradiography. *A*, Autoradiograph showing dinucleotide polymorphism D6S276 screened in a subset of a family using genomic DNA isolated from mouth swabs. *B*, Autoradiograph showing multiplex PCR of four twin pairs (two MZ and two DZ), using dinucleotide repeats ACTBP2 with D21S11.

with proteinase K (0.2 mg/ml) and SDS (0.5%) per cotton swab. In this way, samples can be kept at least 3 wk without decrease in yield or quality of DNA. High-molecular-weight human genomic DNA (>50 kb) was isolated with an average yield of 2.1 ± 0.2 μg per cotton swab from 81 individuals by using mouth swab samples that were placed in lysis buffer immediately after wiping. DNA isolations of another 181 individuals were performed with mouth swab samples that were kept dry after wiping, that is, without lysis buffer. High-molecular-weight human genomic DNA was also isolated from these individuals. The average DNA yield of these sam ples, however, was 1.3 ± 0.05 μg per cotton swab. Mouth swab samples which were kept dry for >7 d before isolation gave an extra decrease in DNA yield. The storage of cotton swabs in lysis buffer after wiping is therefore the better procedure. Variation of DNA yield within each method is primarily caused by the difference in pressure exerted during the mouth swab sampling.

Of 262 DNA samples isolated using mouth swabs (both dry and wet) 257 were successfully used in PCR reactions of 20 different human loci. Five DNA samples, kept without lysis buffer, did not work in the PCR. Figure 1*A* shows dinucleotide polymorphism D6S276 (Gyapay et al. 1994) which was screened in a family. DNA samples of baby twin pairs were used in multiplex PCR reactions (Kimpton et al. 1993) to determine zygosity. Two different multiplex PCR reactions, ACTBP2 (Polymeropoulos et al. 1992) with D21S11 (Sharma and Litt 1992) (fig. 1*B*) and D15S221 (Allamand et al. 1994) with D11S898 (Gyapay et al. 1994) and CSF1R (Hastbacka et al. 1992), were used. Together, these two multiplex PCR reactions determine the zygosity of each twin pair with 99.96% certainty.

We have used phenol/chloroform extractions to isolate uncontaminated genomic DNA, without yeast sporule or bacteria, that can be stored for many years and can be used also for DNA analysis methods other than PCR. Other DNA isolation procedures eliminating phenol/chloroform extractions as described, for example, by Richards et al. (1993) might be more efficient and cheaper. These methods may be tested in the near future. The presence of contamination with foreign genomic DNA such as yeast or bacteria might result in extra bands or completely nonmatching bands between MZ twin pairs. Using genomic DNA isolated from mouth swab samples in PCR reactions of 20 known human loci and in the zygosity determination of 50 twin pairs, we found no evidence of contamination. This fast and cost-effective method is being used in our laboratory in a genetic linkage study, various genetic population studies, and in zygosity determination of twin pairs.

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Contamination of cDNA Libraries and Expressed-Sequence-Tags Databases

To the Editor:

Partially sequenced cDNAs, or expressed sequence tags (ESTs), are claimed to represent an efficient strategy for characterizing an organism's genes (Grausz and Auffray 1993). By necessity, these sequences are incompletely characterized, and examples of contamination of cDNA libraries with sequences from other species have been described (Savakis and Doelz 1993; White et al. 1993). It has been suggested that a Human T-cell cDNA library (Clontech HL1963g) is contaminated by sequences from yeast (*Saccharomyces cerevisiae*) and an unknown bacterium.

We are characterizing human ESTs that represent new members of the ATP-binding cassette transporter superfamily (Allikmets et al. 1995). In examining human ESTs generated from the T-cell library, we have encountered one gene that was in fact a yeast sequence (Genbank Z15214 = SSH2 locus) and several genes that do not hybridize to human DNA or RNA. PCR primers from these sequences failed to amplify a product from human, yeast, or *Escherichia coli* DNA but did produce a product from a Clontech kidney cDNA library (HL1123a). To determine the source of the contamination, we amplified a conserved segment of the 16S rDNA (following a suggestion from Dr. C. Savakis) from the kidney library. The sequence of this product was nearly identical to that of the bacterium *Leuconostoc lactis* (300 of 304 bp). Leuconostoc species are commonly found in dairy products, fruits, vegetables, and wine and are nonpathogenic to humans (Buchanan and Gibbons 1994).

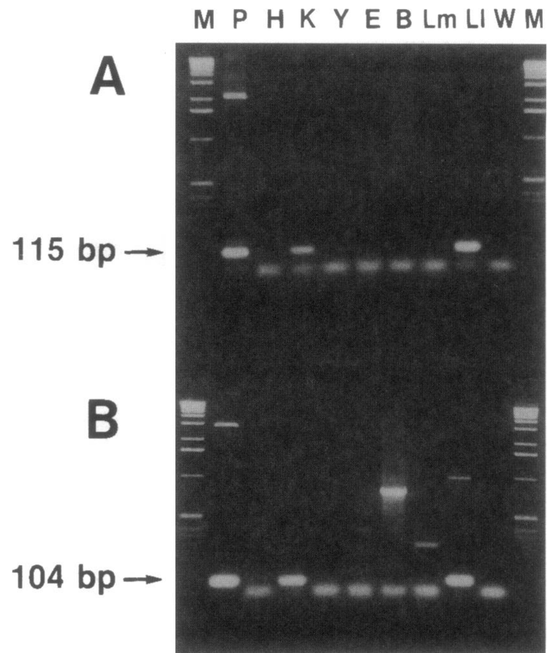

Figure 1 Agarose gel electrophoresis of PCR products, generated using primers designed from two EST sequences. *A*, Clone HSA51A111 (NCBI 4387, Z13478), yielded a 115-bp product. *B*, Clone HSA37A091 (NCBI 13134, z15753), product size 108 bp. Electrophoresis in 1.8% agarose gel in TAE buffer. Template DNAs are as follows: P = EST plasmid DNA (positive control); H = human genomic DNA; K = total DNA from Clontech kidney cDNA library; Y = yeast genomic DNA; E = *E. coli* DNA; B = *B. subtilis* DNA; Lm = *L. mesenteroides* DNA; Ll = *L. lactis* DNA; W = no DNA (negative control). M = marker lanes, 1-kb ladder (GIBCO BRL).

Several Leuconostoc species were obtained from the American Type Culture Collection and primers to several of the suspected EST sequences used in PCR reactions. Six of eight attempted sequences amplified a product from the kidney cDNA library and from *L. lactis* DNA but not from other leuconostoc species, human, yeast, *Bacillus subtilis,* or *E. coli* DNA (fig. 1). One sequence amplified a product only in the kidney cDNA