Genotyping Accuracy for Whole-Genome Amplification of DNA from Buccal Epithelial Cells

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We compared the accuracy of genotyping for DNA extracted from lymphocytes to that of DNA amplified from buccal epithelial cells. Amplification was via a rolling circle/ϕ29 DNA polymerase commercial kit. Paired buccal and lymphocyte DNA samples were available from 30 individuals. All samples were genotyped for 12 SNPs, 5 microsatellites and 2 VNTRs. The accuracy of genotyping (no-call proportions, reproducibility, and concordance) was similar for DNA from lymphocytes in comparison to amplified DNA from buccal samples. If used with caution, these data suggest that rolling-circle whole-genome amplification can be used to increase the DNA mass available for large-scale genotyping projects based on DNA from buccal cells.

Many twin registries have collected DNA via buccal epithelial cell-brush or mouthwash protocols. The choice of this method of DNA collection was due to its low subject burden and relatively small expense (10–20% of the cost of collection of a peripheral venous sample). However, the disadvantage of buccal DNA collection is the lesser quantity and poorer quality of DNA in comparison to DNA derived from lymphocytes. Many investigators now wish to genotype their twin samples for a large number of markers and the DNA mass required for these genotyping reactions often exceeds the DNA yield of most buccal DNA extraction protocols.

Recently, several whole-genome amplification protocols have been described using rolling circle amplification with Φ29 DNA polymerase (Dean et al., 2002; Nelson et al., 2002). These approaches appear to be accurate and robust for DNA from lymphocytes (Hosono et al., 2003; Tranah et al., 2003). There are no published data on how these methods perform with buccal-derived DNA samples.

The goal of this study was to examine the accuracy of whole-genome amplification of DNA from buccal samples in relation to lymphocyte DNA from the same individuals.

Methods

Paired DNA samples from 30 individuals (DNA from blood versus amplified buccal DNA) were compared for five microsatellites, two Variable Number of Tandem Repeats (VNTRs), and 12 Single Nucleotide Polymorphisms (SNPs). All SNPs and one VNTR were genotyped twice to assess replication error.

Thirty volunteers ascertained by convenience sampling donated blood and buccal samples. Genomic DNA was extracted from approximately 8 ml of whole blood using a Puregene DNA Purifcation Kit for whole-blood samples (Gentra Systems). Buccal-cell DNA was extracted from three sterile cytology brushes using the Puregene Genomic DNA Purification Kit for buccal samples (Gentra Systems) within a week of sampling. Extracted buccal-cell DNA was amplified (800–1000 ng total yield) using the GenomiPhi DNA Amplification Kit (Amersham Biosciences) via the manufacturer's protocol and using ~10 ng of DNA to seed the reaction.

Blood and amplified buccal samples from each of the 30 volunteers were genotyped for five microsatellites, two VNTRs, and 12 SNPs. These markers were scattered across the genome, and most are pertinent to neuropsychiatric disorders. The five microsatellites were D10S526, D5S592, FES/FPS, vWA31, and D22S417. The two VNTRs were located in the dopamine transporter (SLC6A3, 3' DATVNTR; Kang, Palmatier, & Kidd, 1999) and serotonin transporter (SLC6A4, 5-HTTLPR; Lesch et al., 1996). The SNPs were from dbSNP (rs1042713, rs6277, rs6265, rs4680, rs2619539, rs1801282, and rs1801133), Applied Biosystems Inc. (ABI) "Assay-on-Demand" library (C_7586657, C_8878813, C_304219, and C_2270166), and deCODE (NRG225133; Stefansson et al., 2002).

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Table 1

Comparison of Genotyping Accuracy Between Lymphocyte and Amplified Buccal DNA

Metric	SNPs		Microsatellites/VNTRs	
	Lymphocyte	Buccal	Lymphocyte	Buccal
Percent no-calls	0.28%	0.69%	1.25%	2.50%
Reproducability	100%	99.15%	100%	100%
Concordance	99.58%		100%	

Note: Microsatellite/VNTR reproducibility data includes DATVNTR only.

All PCR reactions were completed on a MJ Research PTC-200 DNA Engine. Microsatellite and VNTR markers were run on an ABI 3730 DNA Analyzer. ABI GeneMapper (v3.5) was used for semi-automated genotype calls with verification by an experienced operator and preestablished quality-control procedures. SNPs were genotyped using TaqMan predeveloped assay reagents for allelic discrimination and run on an ABI Prism 7900HT Sequence Detection System. Genotypes that could not be easily determined by an experienced operator were termed *no-calls*.

This study was reviewed and approved by the UNC Medical IRB. All samples were anonymized following collection.

Results

We considered three metrics by which to gauge genotyping accuracy (Table1): no-call proportions, reproducibility (test–retest), and concordance (blood versus amplified buccal DNA; Oliphant, Barker, Stuelpnagel, & Chee, 2002). No-calls were considered missing data for all reproducibility and concordance calculations.

No-call Proportions

For SNPs, the overall no-call proportions were 0.28% (2/720) for DNA from lymphocytes and 0.69% (n = 5/720) for DNA from buccal cells (Fisher's Exact Test $p = .45$). For the remaining genotypes, the overall no-call proportions were 1.25% (3/240) for DNA from lymphocytes and 2.50% (n = 6/240) for DNA from buccal cells (Fisher's Exact Test $p = .50$). No individual sample was consistently not called.

Reproducibility

Reproducibility was determined by repeating genotyping for 12 SNPS and 1 VNTR (DATVNTR). SNP reproducibility was 100% (358/358) for DNA from blood and 99.15% (352/355) for amplified buccal DNA (Fisher's Exact Test $p = .12$). For the one VNTR, reproducibility was 100% for DNA from blood (29/29) and amplified buccal DNA (27/27; Fisher's Exact Test $p = 1$).

Concordance

Concordance was assessed by pairwise comparison of blood versus amplified buccal DNA from the same subjects. For the 12 SNPs, the concordance was 99.58% (710/713). For the remaining markers, concordance was 100% (232/232).

Discussion

Our results suggest that whole-genome amplification of buccal DNA samples via a Φ29 DNA polymerase/rolling-circle method produces genotypes that are of comparable quality to those from genomic DNA from lymphocytes. Specifically, for 12 SNPs, 5 microsatellites, and 2 VNTRs, the no-call proportions and reproducibility were not significantly different for lymphocyte and amplified buccal DNA. In addition, the pairwise concordance for blood and amplified buccal samples was very high. Previous work suggested that Φ29 DNA polymerase whole-genome amplification can reasonably be used to amplify DNA extracted from lymphocytes (Barker et al., 2004; Paez et al., 2004); our results suggest that these results also apply to DNA extracted from buccal epithelial cells.

We thought it reasonable to consider no-call genotypes as missing for two reasons. First, inspection of the dataset strongly suggested that these were missing at random with respect to the individual DNA samples. Second, these no-calls can generally be resolved with additional genotyping.

These results do not remove the necessity to evaluate the adequacy of buccal whole-genome amplification for every marker assessed. For example, markers requiring long PCR may be more likely to fail on buccal DNA (Roberts, Sullivan, Joyce, & Kennedy, 2000). Moreover, even if whole genome amplification works well on average across the genome, there are likely to be discrete regions or markers for which it does not work as well.

However, if investigators use appropriate experimental and design precautions, our results suggest that Φ29 DNA polymerase whole-genome amplification can be used to increase the numbers of markers that can be genotyped on DNA from buccal samples collected from twin and other large population registries.

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