Estimation of the Rate of SNP Genotyping Errors From DNA Extracted From Different Tissues

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High density single nucleotide polymorphism (SNP) genotyping panels provide an alternative to microsatellite markers for genome scans. However, genotype errors have a major impact on power to detect linkage or association and are difficult to detect for SNPs. We estimated error rates with the Affymetrix GeneChip® SNP platform in samples from a family with a mixed set of monozygotic (MZ) and dizygotic (DZ) triplets using lymphocyte, buccal DNA and samples from whole genome amplification using the multiple displacement amplification (MDA) technique. The average call rate from 58,960 SNPs for five genomic samples was 99.48%. Comparison of results for the MZ twins showed only three discordant genotypes (concordance rate 99.995%). The mean concordance rate for comparisons of samples from lymphocyte and buccal DNA was 99.97%. Mendelian inconsistencies were identified in 46 SNPs with errors in one or more family members, a rate of 0.022%. Observed genotype concordance rates between parents, between parents and children, and among siblings were consistent with previously reported allele frequencies and Hardy–Weinberg equilibrium. Using the MDA technique, results for two samples had equivalent high accuracy to results with genomic samples. However, the SNP call rate for the remaining seven samples varied from 72.5% to 99.5%, with an average of 86.11%. Quality of the DNA sample following the MDA reaction appears to be the critical factor in SNP call rate for MDA samples. Our results demonstrate highly accurate and reproducible genotyping for the Affymetrix GeneChip® Human Mapping Set in lymphocyte and buccal DNA samples.

High density single nucleotide polymorphism (SNP) genotyping panels are now available as a result of advances in SNP discovery and development of high throughput SNP genotyping platforms (Kennedy et al., 2003; Oliphant et al., 2002). These high density SNP panels provide alternatives for genome scans (John et al., 2004) and are being further developed for genome-wide association studies. High throughput SNP genotyping methods with automated allele calling provide significant potential savings in time and cost compared to microsatellite typing.

The rate of genotype errors is an important factor and has a major impact on power to detect linkage and association, especially where genotype data from parents are not available (Abecasis et al., 2001; Cherny et al., 2001; Kang et al., 2004). Error rates are influenced by a number of factors, but are generally quoted between 0.25% and 1% for microsatellite genotyping (Ewen et al., 2000). Where family information is available, a proportion of genotyping errors can be detected as Mendelian inconsistencies, but this is more difficult for SNP markers with only two alleles (Gordon et al., 1999; Weale et al., 2003). The aim of the present experiment was to evaluate genotyping error rates with the Affymetrix GeneChip® SNP platform at the Australian Genome Research Facility (AGRF). One important control available in twin studies is the inclusion of monozygotic (MZ) twins to assess error rates. We therefore typed DNA samples from dizygotic (DZ) triplets (i.e., a set of twins in which one embryo has split to produce a pair of MZ twins).

Another concern for genetic research is the finite supply of DNA available from study participants. Although the Affymetrix SNP platform requires substantially less sample (0.25 μg per chip) than a conventional genome scan with microsatellites (10–20 μg), DNA sample volume can become a limitation where families participate in a number of studies. Recently methods have been developed to amplify genomic DNA samples using a rolling circle amplification method (Dean et al., 2002; Hosono et al.,

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2003). This whole genome amplification method is called multiple displacement amplification (MDA). One concern not adequately addressed by typing a small number of markers is the fidelity and reproducibility of results from whole genome amplification. Therefore, our study also compared reproducibility from genomic DNA extracted from lymphocytes, buccal DNA, and both lymphocyte and buccal DNA amplified using MDA (Dean et al., 2002).

Subjects and Methods

Participants were recruited from the ongoing Queensland adolescent twin family study as part of twin studies on the genetics of complex traits (Zhu et al., 2004); 58,960 SNP genotypes were obtained for samples from a set of triplets (a MZ pair plus a DZ sibling) and their parents. Data were also available from a ~10cM genome scan with microsatellite markers for 13 pairs of MZ twins from the same population. Study protocols were reviewed and approved by the Queensland Institute of Medical Research Human Research Ethics Committee and informed consent for genotyping was obtained from all participants and their parents prior to sample collection.

Sample Preparation

A 30mL blood sample and a single, cytology brush buccal sample were obtained from study participants. DNA was extracted from the buffy coat of one 10mL EDTA blood tube using a modification of the salt method (Miller et al., 1988). DNA from buccal cells collected from cheek swabs was extracted by organic phase extraction followed by filter concentration. All DNA samples were quantitated by a Picogreen conjugated fluorescence detection assay, and standardized to a working concentration of 50ng/µl in sterile 1 × TE buffer.

Whole Genome Amplification

DNA samples were amplified by MDA using the GenomiPhi DNA Amplification Kit (Amersham Biosciences Limited) using a protocol modified from the manufacturer's recommendations. The initial DNA denaturation step was omitted and 0.5µl of DNA at 5ng/µl was added to 2µl of sample buffer in a 96-well reaction plate. An enzyme/reaction buffer consisting of 2.25µl of reaction buffer and 0.25µl of enzyme mix per sample was prepared on ice and 2.5µl added to each well and the plate mixed gently. The plate was heat-sealed and incubated for 16 hours at 30°C, followed by heat inactivation of 65°C for 10 minutes, and storage at 4°C. Each 5µl reaction was diluted 1:25 by addition of 120µl of sterile 1 × TE buffer (pH 8.0).

Zygosity Testing

Zygosity of participants had been previously determined through their participation in the Queensland adolescent twin study, by typing nine independent DNA microsatellite polymorphisms plus the sex marker amelogenin using the Profiler multiplex marker set (AmpFLSTRR Profiler PlusT, Applied Biosystems, Foster City, CA). All twins had also been typed for ABO, Rh and MNS blood groups by the Red Cross Blood Service, Brisbane.

SNP Typing

SNP genotyping was carried out at the AGRF Melbourne on DNA samples from the family using the Affymetrix 50K XbaI SNP GeneChip®. Each chip has 2.5 million 8µm × 8µm features containing 40 different 25 base pair oligonucleotides for each of the ~60,000 SNPs. DNA (250ng) was digested using the restriction enzyme XbaI and adaptors containing a generic primer sequence were ligated to these fragments following the Affymetrix protocol (GeneChip® Mapping 100K assay manual Rev2). Three rounds of PCR provided 40µg of product for each sample. The PCR product was purified using Qiagen Mini Elute plates and quantitated via optical density assessment. Each sample was then fragmented using DNAse, labeled and hybridized to the oligonucleotides on each 50K XbaI SNP GeneChip® for 18 hours at 48°C. Each chip was washed using the Affymetrix fluidics station FS450 and scanned using the Affymetrix GC3000. Allele calls were made by the Affymetrix GDAS software with no human intervention.

SNP data were obtained for DNA samples extracted from white blood cells (WBCs) for all five family members and buccal DNA samples from both MZ twins. The buccal sample from MZ triplet (02) was typed twice. WBC DNA samples from all five family members and buccal DNA samples from all three triplets were amplified by MDA. A summary of the design is shown in Table 1.

Genome Scan

A ~10 cM microsatellite genome scan in 13 additional MZ twin pairs was also carried out at the AGRF on DNA samples collected as part of a study on adolescent twins and their families (Wright et al., 2002; Zhu et al., 2004), allowing a comparison of MZ marker concordance rates between SNPs and microsatellites. PCR was performed on 30ng of DNA and genotyping completed using ABI PRISM® 377 sequencing machines (Ewen et al., 2000). Dye-labeled PCR products were detected using GENESCAN software (PE Applied Biosystems) and allele calling performed using Genotyper 2.1 (PE Applied Biosystems). The scan consisted of 382 autoso-

Table 1

Samples Prepared for SNP Genotyping

Samples	SEX blood cell	White blood cell	Buccal Buccal	MDA White	MDA
MZ1	F	Y	Y	Y	Y
MZ2	F	Y	Y	Y	Y
DZ	F	Y		Y	Y
Father	M	Y		Y	
Mother	F	Y		Y	

mal and 18 X-chromosomal markers at an average spacing of 9.1 cM.

PCR results for all samples were checked on agarose gels and compared to a control sample prior to SNP genotyping.

Analyses

The average call rate for each sample was computed as the proportion of SNP markers that a genotype was able to be determined for. Genotype concordance rates were computed for parents, for parents and progeny and for siblings. Comparisons were made between samples containing the same genetic information, including the MZ twins and the repeated buccal sample. For each pairwise comparison, the fail rate was calculated where one or both samples failed at a SNP locus. Genotype concordance was calculated only when both samples had a successful call. A genotyping error was declared if, for a particular marker locus, the genotype of a child was inconsistent with the parental genotypes and Mendelian inheritance.

Results

Preliminary results for PCR products for all experimental samples and a control sample on an agarose gel are shown in Figure 1. The five WBC DNA samples (lanes 1 to 5) all showed similar profiles consistent with high quality DNA and the excellent results (see below) obtained from SNP typing with these samples. The WBC MDA sample from the DZ triplet (01) showed obvious poor quality and was not run for the SNP analysis. MDA samples (lanes 7 and 10) also showed reduced high molecular weight fragments. The SNP call rates for these two samples were 90.4 and 85.8 respectively.

The average call rate from 58,960 SNPs for the five genomic samples was 99.48% (Table 3). Nonidentical siblings had approximately 75% of their genotypes identical, while parents and progeny shared approximately 71% of genotypes. The proportion of concordant genotypes between the unrelated parents was 58% (Table 2). Call rates for the two buccal samples were 99.35% and 98.70% respectively (Table 3). For samples with an average call rate of less than 95% there was a deficit of heterozygous calls (18.00%) relative to samples with an average call rate of at least 95% (29.07%).

Across the comparisons the average fail rate was 1.23% (Table 4). The degree of concordance for the different WBC and buccal comparisons was very high. The comparison between WBC samples from the MZ twins showed only three discordant genotypes, a concordance rate of 99.995% (Table 4). Overall the mean concordance rate across different DNA templates was 99.97% (Table 4). Highest rates of discordance were observed for comparisons including the buccal sample with the lowest genotype call rate (for the first MZ

Table 2

Genotype Concordance Rates for 58,960 SNPs from Genomic Samples

	MZ 1	MZ 2	DZ	Father	Mother
MZ1	**99.43**				
MZ2	99.99	**99.43**			
DZ	***75.36***	***75.35***	**99.72**		
Father	*70.64*	*70.64*	*70.76*	**99.32**	
Mother	*70.48*	*70.48*	*70.73*	**58.02**	**99.49**

Note: The percentage of successful SNP calls is shown on the diagonal (bold). Below the diagonal the genotype concordance rates between the MZ twins, sibs (bold italics), parent–offspring (italics) and parents (bold).

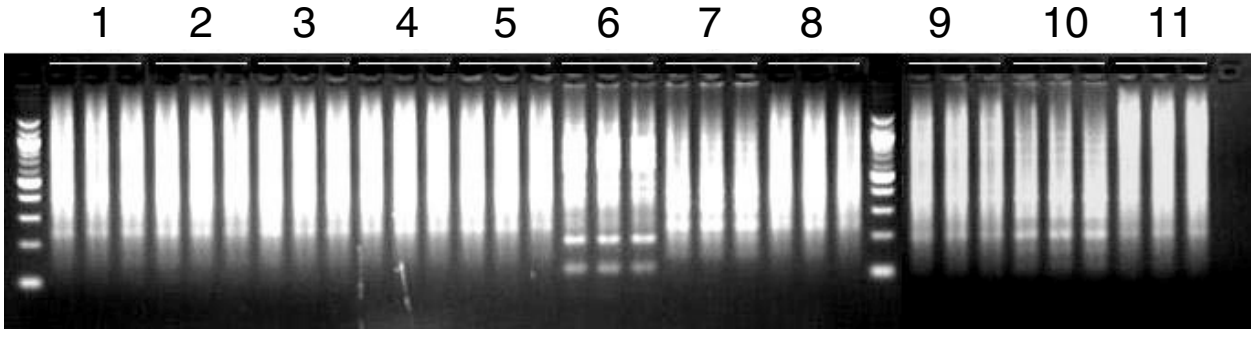

Figure 1:

Agarose gel analysis of PCR products following restriction enzyme digestion with XbaI ligation with adaptors containing a generic primer sequence and four rounds of PCR; lanes 1–5 genomic DNA (DZ, MZ1, father mother and MZ2), MDA samples lanes 6–10 (DZ, MZ1, father mother and MZ2), lane 11 Affymetrix Reference DNA.

1. DZ — gDNA 2. MZ1 — gDNA 3. Father — gDNA
4. Mother — gDNA 5. MZ2 — gDNA 6. DZ — MDA gDNA
7. MZ1 — MDA gDNA 8. Father — MDA gDNA 9. Mother — MDA gDNA
10. MZ2 — MDA gDNA 11. Affymetrix Reference DNA

Table 3

SNP Call Rates for All Samples Genotyped

Sample	Called gender	SNP call rate	AA call	AB call	BB call
MZ1 genomic**	F	99.43%	35.63%	29.26%	35.11%
MZ2 genomic**	F	99.43%	35.62%	29.28%	35.10%
DZ genomic**	F	99.72%	35.48%	29.61%	34.91%
Father genomic**	M	99.32%	36.16%	28.42%	35.42%
Mother genomic**	F	99.49%	35.42%	29.37%	35.20%
MZ1 buccal**	F	98.70%	35.80%	29.01%	35.19%
MZ2 buccal**	F	99.35%	35.65%	29.25%	35.11%
MZ1 genomic mda^	F	90.40%	37.54%	27.26%	35.20%
MZ2 genomic mda^	F	85.79%	43.19%	16.25%	40.56%
Father genomic mda**	M	99.27%	36.24%	28.31%	35.45%
Mother genomic mda**	F	99.16%	35.56%	29.10%	35.34%
MZ1 buccal mda^	F	88.90%	43.28%	15.74%	40.98%
MZ2 buccal mda^	F	92.96%	40.57%	20.61%	38.82%
DZ buccal mda^	F	72.49%	48.32%	10.14%	41.54%
Note: ** Average call rates for samples with call rate > 95%		99.32%	35.73%	29.07%	35.20%
^ Average call rates for samples with call rate < 95%		86.11%	42.58%	18.00%	39.42%

twin). Excluding these comparisons gives a concordance rate of 99.98%.

Mendelian errors were identified for 46 SNPs in one or more family members, a rate of 0.022% similar to estimates of error rates from comparing MZ twins and duplicate samples. SNP genotyping results for good quality DNA samples compared favorably with results from microsatellite genotyping on MZ twins from the same study (Table 5). Across 13 pairs of MZ twins, 0.19% of microsatellite markers showed discordance with a trend to higher rates of discordance in samples with lower call rates (Table 5).

WBC DNA samples from all five family members and buccal DNA samples from all three triplets were amplified by MDA. Results of the SNP genotyping were more variable. One WBC MDA sample was judged to be of poor quality from preliminary tests and excluded before the SNP analysis. The SNP call rate for the remaining seven samples varied from 72.5% to 99.3% with an average of 86.11% (Table 3). Call rates below 95% were considered unacceptable and data from these samples were not analysed further. Two successful MDA samples did show high concordance rates when compared with the corresponding WBC samples (Table 4). The buccal MDA samples gave SNP call rates ranging from 72% to 93% (Table 3).

Discussion

Genotyping on the Affymetrix GeneChip® Human Mapping Set in samples from our family of MZ/DZ triplets showed high reproducibility and accuracy. Average concordance rates for comparisons with high quality DNA samples were similar to previously published error rates for this platform (Kennedy et al., 2003). Mendelian errors in the family were estimated at 0.022%, similar to the discordance rate for the high quality DNA samples. Errors using this platform were lower than our estimated error rate for SNP genotyping using a Sequenom MassARRAY platform (James et al., 2004; Zhao et al., 2005) and discordance rates were lower than those for microsatellite markers from 13 MZ twin pairs from our study of adolescent twins (Zhu et al., 2004). Accurate SNP genotyping is essential in genetic studies since SNP typing errors are difficult to detect using Mendelian error checks alone. For example, if both parents are heterozygous for a SNP then incorrect genotypes in a single child will never be detected. Consequently, for families consisting of parents and a single child, the true error rates are likely to be three to four times the apparent error rate (Gordon et al., 1999). Results of the present study demonstrate very low error rates using this platform. The true rate of errors estimated from detected Mendelian errors is approximately 0.066% (3×0.022), whereas the corresponding rate from the concordance measures is approximately 0.040% (100 – 99.96). These estimates are not significantly different from each other because the standard errors of the estimates are approximately 0.01% and 0.008%, respectively [$= 100^*\sqrt{(p/60{,}000)}$, with p the error probability]. Hence, the overall estimate of genotyping errors is approximately 0.05%.

The genotype concordance rate between the parents (58%) reflects the allele frequencies in the population, whereas the concordance rates among the siblings and between parents and progeny reflect population allele frequencies and identity-by-descent. For

a SNP with a minor allele frequency (q) and in Hardy–Weinberg equilibrium, the concordance probability is $q^4 + [2q(1-q)]^2 + (1-q)^4 = 1 - 2h(1-{}^3/_4h)$, with h the proportion of heterozygous genotypes [$h = 2q(1\text{-}q)$]. We calculated the average predicted genotype concordance rate for unrelated individuals from the allele frequencies in Caucasians for the SNPs in our study (Kennedy et al., 2003; retrieved March, 2005, from http://www.affymetrix.com/products/arrays/specific/100k.affx). The result was .583, very close to the observed value of .580 for the parents in our study (Table 2). Similarly, the average predicted concordance between parent and progeny genotypes [$= 1-2q(1-q) = 1-h$] was .707, close to the observed range of .705 to .708. Finally, the predicted average concordance rate between nonidentical siblings [$= 1- h(1-{}^3/_8h)$] was calculated from the allele frequencies as .749, again close to the observed values of .755. Hence, the observed concordance rates are consistent with allele frequencies estimated from an independent population and with Hardy–Weinberg equilibrium genotype proportions.

The quality of all the lymphocyte DNA samples was high and these samples gave consistently high genotype call rates (greater than 99%). The two buccal samples also gave high SNP call rates, 98.7% and 99.35% (Table 3), consistent with our extensive experience with microsatellite genotyping from lymphocyte and buccal DNA samples. Results from buccal DNA samples generally give high call rates, but the DNA quality is not always as high as that from lymphocyte DNA samples. Genotyping error rates across a range of platforms increase with poor quality DNA samples and with poor markers. Inspection of genotypes for the SNPs identified with Mendelian errors suggests the errors mainly arose from an excess of homozygous calls probably resulting from allele drop-out with consistent bias in allele calling for a small number of SNPs.

Lower rates of heterozygosity were observed in samples with poor SNP call rates, suggesting that sample quality impacts on SNP detection in heterozygous individuals. Results for the MDA samples were more variable and showed lower average heterozygous call rates than genomic samples. MDA amplification of good quality DNA provides samples suitable for a range of DNA tests (Dean et al., 2002; Hosono et al., 2003; Tranah et al., 2003). We have previously achieved high success rates using the MDA protocol to amplify genomic DNA samples with limited DNA volumes for microsatellite analysis. It is unclear whether results with these samples and the Affymetrix SNP platform represent poor quality amplification for some samples or effects of the

Table 4

Summary of Concordance and Fail Rates for SNP Markers in Comparisons Between Pairs of Samples Expected to Have Identical Genetic Profiles

	Fail	Concordance	Discordance
MZ1 Genomic — MZ2 Genomic	534	58423	3
	0.91%	99.995%	0.005%
MZ1 Genomic — MZ1 Buccal	974	57948	38
	1.65%	99.934%	0.066%
MZ1 Genomic — MZ2 Buccal[1]	602	58345	13
	1.02%	99.978%	0.022%
MZ1 Genomic — MZ2 Buccal[2]	692	58252	16
	1.17%	99.973%	0.027%
MZ2 Genomic — MZ1 Buccal	970	57952	38
	1.65%	99.934%	0.066%
MZ2 Genomic — MZ2 Buccal[1]	606	58345	9
	1.03%	99.985%	0.015%
MZ2 Genomic — MZ2 Buccal[2]	703	58247	10
	1.19%	99.983%	0.017%
Subtotals	5081	407512	127
	1.23%	99.969%	0.031%
Father Genomic – Father Genomic MDA	695	58239	26
	1.18%	99.955%	0.045%
Mother Genomic – Mother Genomic MDA	670	58249	41
	1.14%	99.930%	0.070%
Combined Totals	9045	698214	261
	1.28%	99.963%	0.037%
Total #SNPs	58960		

Note: Two different buccal samples were compared from MZ twin 2 and these are indicated by superscripts in the table.

Table 5

Summary of Concordance and Fail Rates for Microsatellite Markers in Comparisons Between Pairs of Samples Expected to Have Identical Genetic Profiles

	Fail	Concord	Discord
MZ Pair 1	6	395	0
	1.50%	100%	0.00%
MZ Pair 2	4	396	1
	1.00%	99.75%	0.25%
MZ Pair 3	3	398	0
	0.75%	100%	0.00%
MZ Pair 4	4	397	0
	1.00%	100%	0.00%
MZ Pair 5	8	393	0
	2.00%	100%	0.00%
MZ Pair 6	6	395	0
	1.50%	100%	0.00%
MZ Pair 7	6	395	0
	1.50%	100%	0.00%
MZ Pair 8	14	387	0
	3.49%	100%	0.00%
MZ Pair 9	2	398	1
	0.50%	99.75%	0.25%
MZ Pair 10	9	391	1
	2.24%	99.75%	0.25%
MZ Pair 11	8	389	4
	2.00%	99.00%	1.00%
MZ Pair 12	7	392	2
	1.75%	99.5%	0.50%
MZ Pair 13	2	398	1
	0.50%	99.75%	0.25%
Combined	79	5124	10
	1.52%	99.805%	0.195%

Note: Total # markers = 410

method used here for the SNP genotyping. However, pooling DNA samples from replicate whole genome amplification has been found to reduce genotyping errors (Rook et al., 2004). For microsatellite analysis, we pooled replicate amplicons to provide sufficient DNA template for genome scans and this may have contributed to the successful genotyping results.

Running PCR products on an agarose gel prior to genotyping provided a useful quality control step. Results correlated well with the SNP call rates achieved for different samples. Two MDA samples with high call rates similar to genomic samples showed high molecular weight fragments on agarose gels similar to genomic samples. Samples that had a weak result on the agarose gels subsequently showed low final call rates.

In summary, results from this study showed highly accurate SNP genotyping on the Affymetrix GeneChip® platform for genomic samples, with an estimate of the genotyping error rate of approximately 1 in 2000 (0.05%). Success rates were influenced by sample quality and appropriate quality control steps could be employed to detect poor quality samples before genotyping.

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