Comparison of genotyping consistency between genomic and whole-genome amplified DNA using the Illumina GoldenGate and Infinium-II assays

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INTRODUCTION

High-throughput SNP genotyping has become an important research strategy in human genetics. Although most genotyping assays require minimal amounts of DNA, repeated use can lead to depletion of often irreplaceable samples. To address this problem whole-genome amplification technologies have recently been developed and are meanwhile commercially available. Albeit amplification seems to be successful for most genomic DNA samples, it is controversially discussed whether whole genome amplified DNA (wgaDNA) represents an exact copy of the genomic DNA (gDNA) template. This is particularly important when using samples of different age and quality.

In the present study we compared the genotyping consistency between 45 wgaDNAs amplified with the Qiagen Repli-G midi Kit and their corresponding gDNAs. 20 DNA wgaDNA/gDNA pairs were genotyped using the Illumina HumanHap550kV3.0 Beadchip, the other 25 DNA pairs using an Illumina 384 custom SNPs GoldenGate assay.

MATERIAL and METHODS

Samples. The 20 DNA samples used in the Infinium-II assay were extracted from frozen EDTA whole blood by using the Chemagic Magnetic Separation Module I according to the manufacturer's instructions (Berensmeier S., 2006).

The 25 DNA samples used in the GoldenGate assay were extracted from whole blood using the conventional salting out protocol according to Miller et al. , 1988.

Whole genome amplification. We amplified 10ng of the DNAs using the Repli-G midi Kit (Qiagen, Hilden) according to the manufacturer´s instructions.

Infinium-II assay. We performed a whole genome genotyping approach using the HumanHap550kV3.0 Beadchip from Illumina. This chip contains 561.466 SNPs spread over the whole genome at a median density of 4.7kb.

GoldenGate assay. We performed a custom Illumina Golden Gate assay with 384 custom SNPs chosen from different regions of the genome.

Data Analysis. The SNP genotypes were assessed using the Illumina BeadStudio V3.0 software. Comparison of consistency was done by using a standard spreadsheet calculation program.

RESULTS

The aim of our study was to evaluate to what extent wgaDNA represents an exact copy of the gDNA template and therefore is a suitable template for performing Illumina GoldenGate and Infinium-II assays.

To address these questions we compared callrate and genotyping consistency between wgaDNA and gDNA sample pairs using both above mentioned assays. The whole genome amplification was done through isothermal strand displacement amplification (Dean et al., 200) using the Repli-G midi kit (Qiagen, Hilden).

25 DNAs of different age (1 to 10 years) extracted from whole blood using the conventional salting out protocol were genotyped for 384 SNPs using a custom GoldenGate assay. Nine of the 384 SNPs failed in the complete assay due to technical reasons. All gDNA samples performed well with an average callrate (CR) of 99,75%. 19 of the wgaDNAs performed well with an average CR of 98,8%, 3 performed average (CR 96,7%) and 3 samples performed bad (CR 61%) (table 1). When analysing genotype consistency, only samples of the bad performing groups showed inconsistencies (table 1). All inconsistencies were found to be due to loss of one allele leading to a hemizygous genotype. The samples showing Inconsistencies after whole genome amplification belonged to the oldest samples and also appeared to have lower quality when analyzing the 260/280 and 260/230 ratios.

In a second setting we extracted DNA from 20 frozen whole blood samples, which had been stored at appropriate conditions for some years, using an automated extraction system (Chemagic Magnetic Separation Module I). Extracted DNAs were afterwards subjected to whole genome genotyping using the Illumina Infinium-II assay on HumanHap550kV3.0 BeadChips. These chips contain 561.466 SNPs spread over the whole genome. All gDNA and wgaDNA samples performed well, with a slightly higher average CR of 99,89% for the gDNA compared to 99,41% for the wgaDNA (table 2). The genotype consistency was 99,988% when comparing genotypes which were successfully called in both samples (table 2).

DISCUSSION

Our results show that whole genome amplified DNA represents an extremely similar copy of the genomic DNA template and showed comparable callrates when used in high-throughput SNP genotyping assays. The inconsistencies found in the Infinium-II Assay were at a rate which is also found when comparing repeated genotyping of genomic DNA samples. The six samples showing a reduced callrate also showed lower quality when analyzing the 260/280 and 260/230 ratios. This suggests that gDNA template quality is an important factor to achieve good results when using wgaDNA in high-troughput genotyping assays.

Another important question which has not been addressed by us so far remains. Is wgaDNA also suitable for analyzing CNV data?

Table 1: Genotyping scores and reproducibility in the GoldenGate Assay.

	Genotyping				Callrate [%]		Reproducibility		
Sample	gDNA failed	wgaDNA failed	wgaDNA and gDNA failed	wgaDNA and gDNA succesful	gDNA	wgaDNA	genotypes consistent	genotypes inconsistent (one allele)	genotypes inconsistent (both allele)
1	0	2	0	373	100,00	99,47	373	0	0
2	0	2	0	373	100,00	99,47	373	0	0
3	0	3	0	372	100,00	99,20	372	0	0
4	0	3	0	372	100,00	99,20	372	0	0
5	1	2	0	372	99,73	99,47	372	0	0
6	0	3	1	371	100,00	99,20	371	0	0
7	1	3	0	371	99,73	99,20	371	0	0
8	0	3	1	371	100,00	99,20	371	0	0
9	3	2	0	370	99,20	99,47	370	0	0
10	0	4	1	370	100,00	98,93	370	0	0
11	0	5	0	370	100,00	98,67	370	0	0
12	1	4	1	369	99,73	98,93	369	0	0
13	0	5	1	369	100,00	98,67	369	0	0
14	0	6	1	368	100,00	98,40	368	0	0
15	0	7	0	368	100,00	98,13	368	0	0
16	1	7	0	367	99,73	98,13	367	0	0
17	3	5	0	367	99,20	98,67	367	0	0
18	1	5	2	367	99,73	98,67	367	0	0
19	1	7	0	367	99,73	98,13	367	0	0
20	0	11	0	364	100,00	97,07	364	0	0
21	0	12	0	363	100,00	96,80	363	0	0
22	0	13	1	361	100,00	96,53	361	0	0
23	0	65	0	310	100,00	82,67	307	3	0
24	0	89	0	286	100,00	76,27	281	5	0
25	0	283	2	90	100,00	24,53	63	27	0

Figure 1: Genotyping consistency compairing genomic and whole genome amplified DNA using the GoldenGate - Assay.

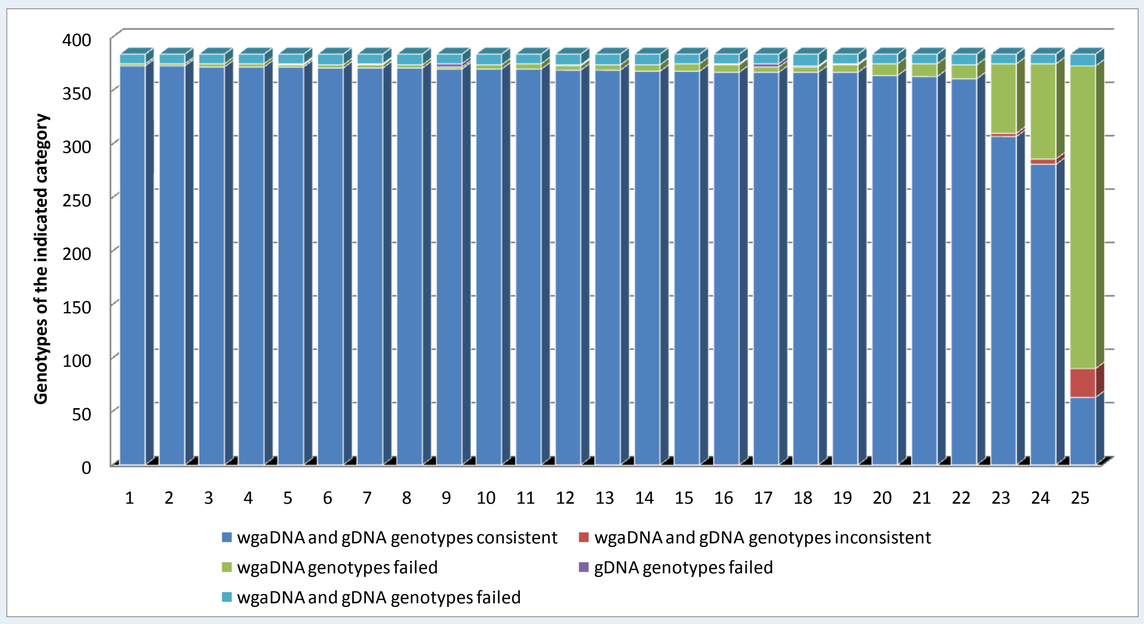
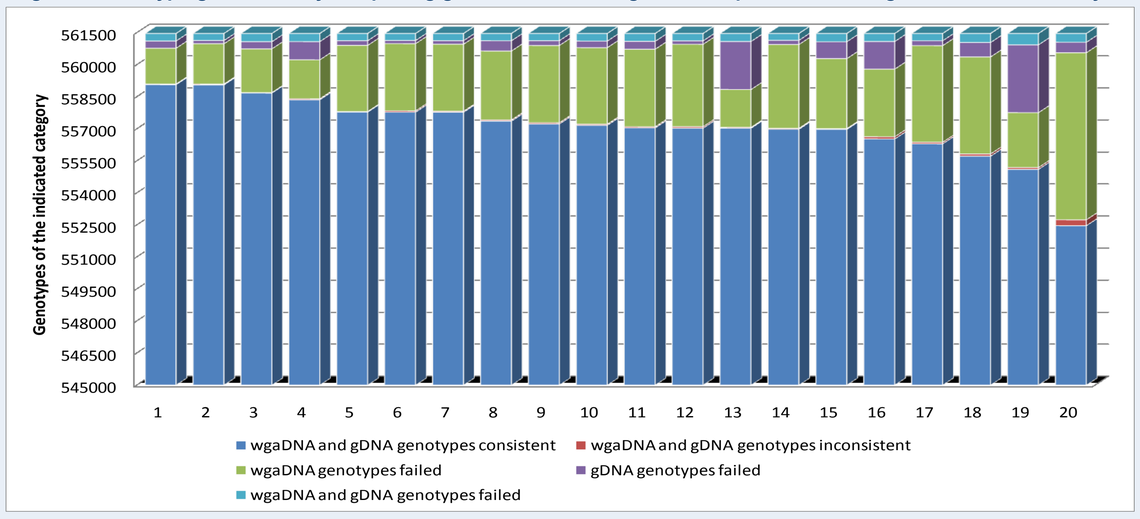


Table 1: Genotyping scores and reproducibility in the Infinium-II Assay.

	Genotyping				Callrate [%]		Reproducibility		
Sample	gDNA failed	wgaDNA failed	wgaDNA and gDNA failed	wgaDNA and gDNA succesful	gDNA	wgaDNA	genotypes consistent	genotypes inconsistent (one allele)	genotypes inconsistent (both allele)
1	340	1675	351	559100	99,94	99,70	559069	31	0
2	150	1887	329	559100	99,97	99,66	559060	40	0
3	334	2047	385	558700	99,94	99,64	558677	23	0
4	853	1823	380	558410	99,85	99,68	558362	48	0
5	217	3097	338	557814	99,96	99,45	557787	27	0
6	148	3147	326	557845	99,97	99,44	557783	62	0
7	167	3137	335	557827	99,97	99,44	557779	48	0
8	481	3227	339	557419	99,91	99,43	557363	56	0
9	226	3617	335	557288	99,96	99,36	557229	59	0
10	308	3582	355	557221	99,95	99,36	557167	54	0
11	371	3630	366	557099	99,93	99,35	557045	54	0
12	161	3857	344	557104	99,97	99,31	557034	70	0
13	2249	1772	374	557071	99,60	99,68	557030	41	0
14	197	3923	320	557026	99,96	99,30	556982	44	0
15	790	3276	387	557013	99,86	99,42	556977	36	0
16	1293	3168	375	556630	99,77	99,44	556528	102	0
17	228	4523	340	556375	99,96	99,19	556299	76	0
18	675	4545	422	555824	99,88	99,19	555721	103	0
19	3174	2570	532	555190	99,43	99,54	555104	86	0
20	489	7824	410	552743	99,91	98,61	552468	275	0

Figure 2: Genotyping consistency compairing genomic and whole genome amplified DNA using the Infinium-II - Assay.



References

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(3) Dean FB et al. (2002), Proc Natl Acad Sci USA 99: 5261-5266