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Evaluation of whole genome amplified DNA to decrease material expenditure and increase quality

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ABSTRACT

Aim: The overall aim of this study is to evaluate whole genome amplification of DNA extracted from dried blood spot samples. We wish to explore ways of optimizing the amplification process, while decreasing the amount of input material and inherently the cost. Our primary focus of optimization is on the amount of input material, the amplification reaction volume, the number of replicates and amplification time and temperature. Increasing the quality of the amplified DNA and the subsequent results of array genotyping is a secondary aim of this project.
Methods: This study is based on DNA extracted from dried blood spot samples. The extracted DNA was subsequently whole genome amplified using the REPLIg kit and genotyped on the PsychArray BeadChip (assessing > 570,000 SNPs genome wide). We used Genome Studio to evaluate the quality of the genotype data by call rates and log R ratios.
Results: The whole genome amplification process is robust and does not vary between replicates. Altering amplification time, temperature or number of replicates did not affect our results. We found that spot size i.e. amount of input material could be reduced without compromising the quality of the array genotyping data. We also showed that whole genome amplification reaction volumes can be reduced by a factor of 4, without compromising the DNA quality.
Discussion: Whole genome amplified DNA samples from dried blood spots is well suited for array genotyping and produces robust and reliable genotype data. However, the amplification process introduces additional noise to the data, making detection of structural variants such as copy number variants difficult. With this study, we explore ways of optimizing the amplification protocol in order to reduce noise and increase data quality. We found, that the amplification process was very robust, and that changes in amplification time or temperature did not alter the genotyping calls or quality of the array data. Adding additional replicates of each sample also lead to insignificant changes in the array data. Thus, the amount of noise introduced by the amplification process was consistent regardless of changes made to the amplification protocol. We also explored ways of decreasing material expenditure by reducing the spot size or the amplification reaction volume. The reduction did not affect the quality of the genotyping data.

1. Introduction

For decades, dried blood spots (DBS) samples have been used in neonatal screening [1]. In some countries, especially the Nordic, the DBS samples are collected and stored in biobanks. In Denmark, the excess DBS samples are stored in the Danish Neonatal Screening Biobank (DNSB). At this point DNSB holds samples from almost every Dane born after 1982 - presently well over 2 million samples [2,3]. The excess material is stored with the primary purpose of improving diagnostics for individuals and developing novel screening methods. However, the samples may to some extent be used for scientific purposes, following approval from local ethical committees, the data protection agency and the DNSB steering committee. At birth, every child is given a unique civil registration number. This identification number can be used to link the DBS sample to information from the Danish national registries on disease diagnosis and socio-economic factors [4]. DBS samples from the DNSB have previously been used in genetic studies aiming at elucidating genetic factors of various clinical

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endpoints, such as birth weight [5], childhood asthma [6], schizophrenia [7] and Psychosis [8].

One of the limitations of DBS samples is the very limited amount of accessible material. The amount of DNA, that can be extracted from a 3 mm disk is low, equivalent to 6 μl of whole blood and a DNA yield of 100–200 ng [9–11]. To ensure a sufficient amount of DNA for various genetic platforms, the extracted DNA may be whole genome amplified (WGA). We have previously shown, that the WGA DNA is robust with regard to array SNP genotyping [12]. However, WGA introduces a range of challenges. WGA by RepliG will inevitably introduce amplification bias [13]. Also genotyping array data from WGA DNA contains far more noise than array data from unamplified samples [14,15]. The genotyping noise limits our ability to identify structural variations such as copy number variants (CNV) [16].

In this study, we aim to optimize our whole genome amplification protocol in order to retrieve a better quality of WGA DNA and array genotyping calls. As the DBS samples are a very limited source of material, we also wish to elucidate whether the amount of input DNA might be downscaled without compromising the quality of the data.

2. Materials and methods

2.1. Sample

This study uses anonymized neonatal and adult DBS samples. All dried blood samples were stored frozen (− 20 °C/− 4 °F) limiting potential performance bias due to differences in age of the samples/storage time [12]. For comparison of amplified to genomic DNA, we also used a set of whole blood samples. The study is purely methodological and developmental and does not constitute a heath related research project - hence no ethical approvals from ethical committees were necessary.

2.2. DNA extraction, whole-genome amplification and genotyping

In our standard protocol, two 3.2-mm discs were punched from each DBS sample, and DNA was extracted using Extract-N-Amp Blood PCR Kit (Sigma-Aldrich, St. Louis, Missouri, United States). WGA DNA samples were produced in triplicates using the REPLIg kit (Qiagen, Hilden, Germany). WGA DNA concentrations were estimated using Quant-IT Picogreen dsDNA reagent (Invitrogen, Carlsbad, California, United States), and samples were normalized to a concentration of 60 ng/μl prior to Illumina PsychArray BeadChip genotyping. Array genotyping was conducted using standard Illumina protocol including a second WGA step. All setups were customized for subprojects. Supplementary Table S1 provides a summary of the samples included in each of the subprojects and the deviations made from the standard protocol. There are two main optimization aims in this study - one being reduction of the amount of material used, the other being improvement of the quality of the WGA DNA and the array genotyping data. With regard to material usage, we reduced disc size from 2 × 3.2 mm to 2 × 1.6 mm and we decreased the WGA reaction volume from 50 μl to 25 μl and 12.5 μl equivalent to a reduction of a factor 2 × and 4 ×. Concerning improvement of the WGA DNA quality and decreasing of the noise in the genotyping data, we explored the effect of an increased number of replicates as well as the effect of altered amplification time or temperature.

2.3. Data analyses

The quality of the genotyping array data was evaluated with Genome Studio (Illumina, San Diego, California, United States). The Illumina PsychArray BeadChip contains 571.000probes, for which we used custom build cluster file to call SNP variants and genotype. The custom cluster file was built on data from genotyping of DBS samples, but can also be applied to analysis of DNA from other sample types, such as whole blood samples. Two custom clusterfiles were used; one for WGA DNA samples and one for genomic DNA samples. In the WGA DNA clusterfile, 11,870 SNP-probes were excluded based on their poor performance. In the genomic DNA cluster file, 18,576 SNP-probes were excluded. The WGA DNA clusterfile was built on 4145 idat files and the genomic DNA cluster file was built on 384 idat files. Call rates for each sample were calculated as number of called SNPs divided by total number of SNPs defined by the clusterfile. Our threshold call rate was set to > 98%, and samples with a lower call rate were excluded from any subsequent analyses. Genome Studio was also used to calculate log R values, number of reproduction errors and GenCall scores as well as generating log R ratio and B allele frequency plots. Concordance rates were calculated using PLINK [17] and the command > plink –genome. Additional statistical analyses were conducted in STATA SE 13 (STATA Corp LP, College Station, Texas, United States).

3. Results and discussion

Our study has a dual aim. We wish to reduce the amount of input material used for genotyping analysis, reducing the WGA costs and preserving more of the DBS sample. The second aim is to optimize the WGA protocol in order to obtain a better quality of genotyping data with increased SNP call rates, reduced noise and improved ability to identify copy number variants. Our primary parameters for assessing the quality of the genotyping data, is call rate, log R ratio, B allele frequencies and number of reproduction errors. The call rate is calculated as the percentage of called SNPs and high call rates are correlated to high quality data. R is the normalized intensity value and log R ratio is log to the observed R divided by the expected R [18]. The log R ratio can be used as a measure of noise in the genotyping data [19]. Each genotyped SNP has a corresponding log R ratio. The ratio should ideally be 0, and an increase in noise will increase the amplitude of the log R ratio. The B allele frequency is calculated from the θ value of the sample and the expected cluster position and can be either 0, 0.5 or 1 for two allele samples. Copy number variations will result in shifted log R ratios, and an increase in possible B allele frequency levels with increased copy number. If the samples contain large amounts of noise, the shift will be difficult to observe and it will not be possible to call CNVs.

In addition to the reduction in input material and optimization of the WGA protocol, we also compared WGA samples and their unamplified genomic counterparts, in order to evaluate the change in DNA and data quality introduced by WGA in our samples.

3.1. Amplified vs genomic DNA

In many studies, the amount of DNA available for analysis is limited. This problem may be solved by WGA of the DNA. WGA DNA has been shown to perform reliably in regard to genotype calls on array genotyping assays [20,21]. However, the amplification process is potentially non-uniform, causing regions to be miss-represented. At the same time array data from amplified samples are far more noisy compared to array data from unamplified DNA [14,15,19]. To evaluate the noise introduced by the WGA procedure in our samples, we compared genotype array data from genomic DNA samples and their corresponding WGA DNA samples. We used both whole blood and DBS samples for this study, as we also wished to evaluate if the noise introduced by the WGA were independent of sample type. With regard to genotyping results, the WGA DNA call rates were slightly lower compared to unamplified genomic DNA. Call rates range between 98.38% and 99.37% in WGA DNA samples and between 99.45% and 99.93% in genomic DNA. Concordance rates between WGA DNA and genomic DNA samples was above 99.94% within identical samples and 50.72–55.18% between non identical samples. The number of reproduction errors between genomic DNA samples and their counterpart WGA DNA samples ranged from 48 to 405 (median = 192,

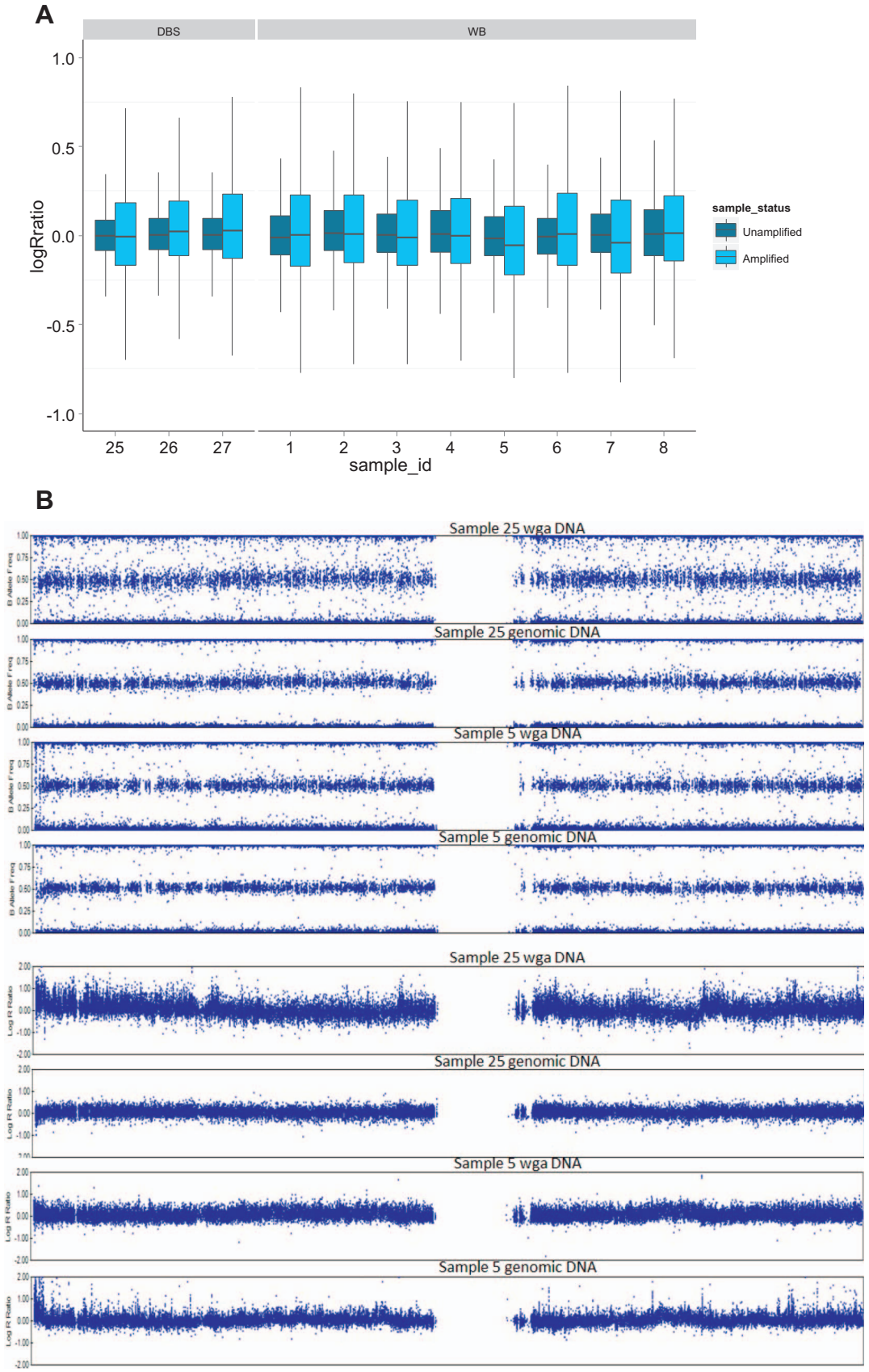

Fig. 1. A. Boxplot of log R ratios by sample and type. Boxes display median and interquartile range. Whiskers are the most extreme values within Q3 + 1.5 (Q3-Q1) and Q1–1.5 (Q3-Q1). Outliers are not displayed in the plot, but values were included in the data analysis. B. upper panels show B allele frequencies and lower panels show log R ratio for chromosome 1 of sample 5 and 15 genomic- and WGA DNA.

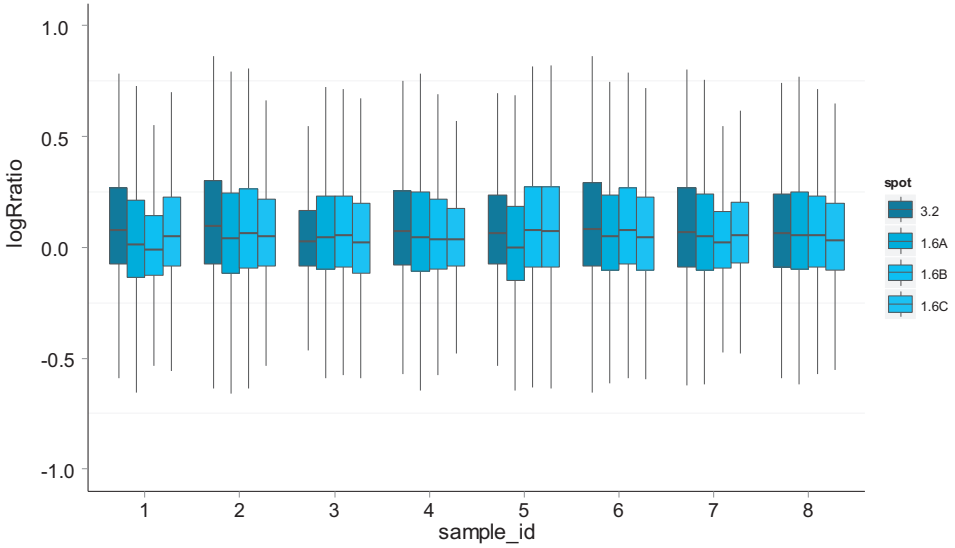

Fig. 2. Boxplot of log R ratios by sample and spot size. Boxes display median and interquartile range. Whiskers are the most extreme values within Q3 + 1.5 (Q3-Q1) and Q1–1.5 (Q3-Q1). Outliers are not displayed in the plot, but values were included in the data analysis.

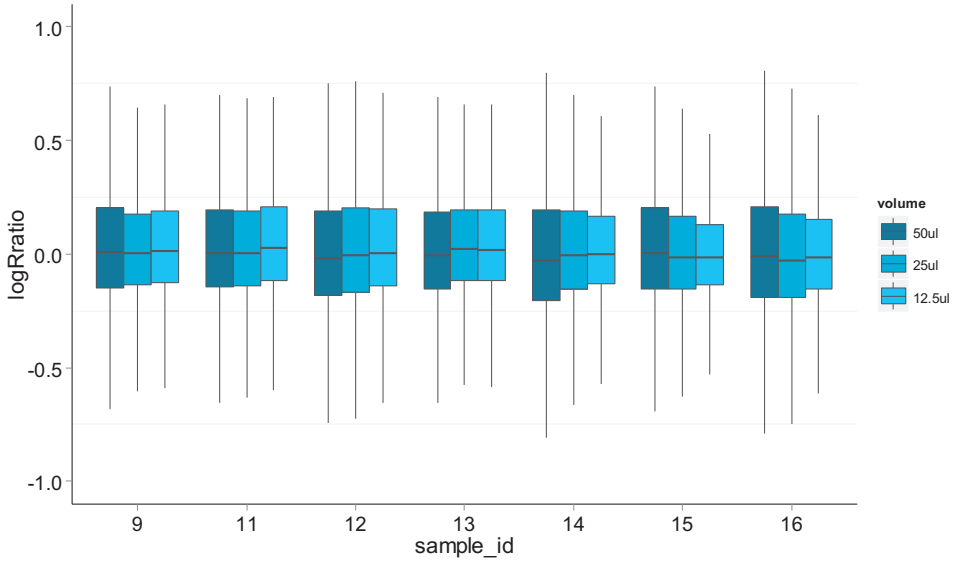

Fig. 3. Boxplot of log R ratios by sample and volume. Boxes display median and interquartile range. Whiskers are the most extreme values within Q3 + 1.5 (Q3-Q1) and Q1–1.5 (Q3-Q1). Outliers are not displayed in the plot, but values were included in the data analysis.

IQR = 178–214) in whole blood samples and 171–249 (median = 223, IQR = 197–236) in DBS samples. This corresponds to reproduction error rates < 1 in 1408 called variants. We compared log R ratios between amplified and genomic DNA from both whole blood samples (sample 1–8) and DBS samples (sample 25–27). Data is displayed in Fig. 1. Fig. 1A is a boxplot of the log R ratios for each probe by sample and amplification status. Fig. 1B displays the B allele frequency and log R ratios on chromosome 1 for sample 5 and 25. There is a clear difference between amplified and unamplified samples in the variation in log R ratio within samples and in the noise in the B allele frequency plot. Interestingly, it appears that the additional noise introduced by the amplification procedure is of similar magnitude regardless of the sample material i.e. whole blood or DBS samples. The same is observed for the number of reproduction errors. From Fig. 1, it might seem that DBS samples perform better than whole blood samples concerning noise. However, the cluster file used for both sample types were built on DBS samples, and thus excess noise in whole blood samples can most likely be attributed to the cluster file being less optimal for whole blood samples compared to DBS samples. In summary, WGA DNA performs well with regard to genotype calling and reproduction error. However, log R distributions of WGA DNA samples were wider and hence noisier than genomic DNA, thus limiting the ability of copy number variants (CNV) algorithms to identify structural variations. Amplification may introduce allele dropout. If this was the case for our samples, it should be visible in the b allele frequency plots, with a stretch of only homozygous probes. This does not appear to be the case. In addition, the number of reproduction errors between amplified and genomic samples is low. However, it cannot be ruled out that there are small areas of allele drop out that would only be covered by a few probes and hence not be detected by inspecting the B allele frequency plots. In this regard, one should also keep in mind, that the genomic DNA samples also undergo amplification as part of the Illumina array genotyping protocol.

3.2. Reduction of input material

After the comparison of genomic and WGA DNA, we explored ways of reducing the amount of material usage, by reducing either disc size

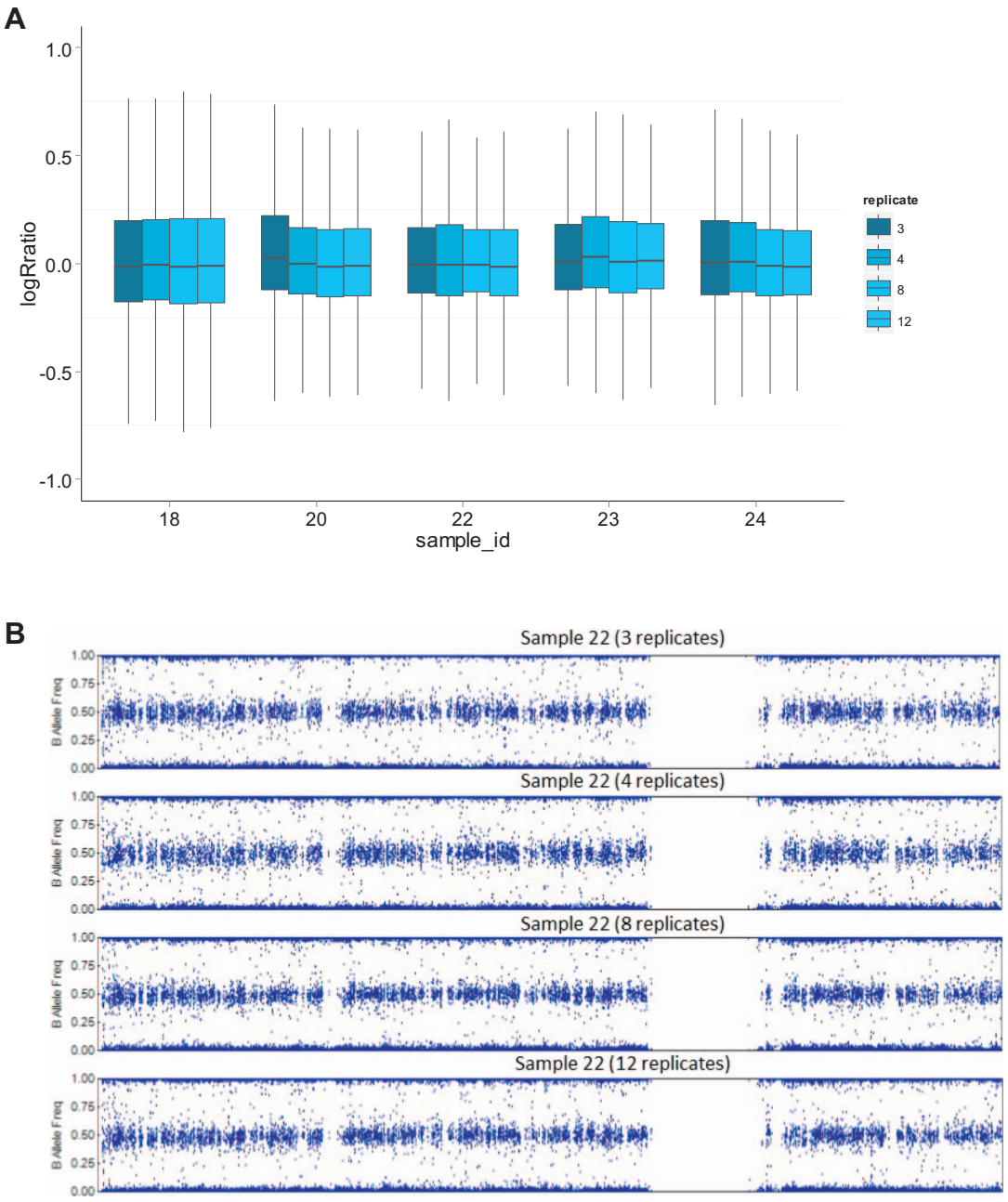

Fig. 4. A. Boxplot of log R ratios by sample and number of replicates. Boxes display median and interquartile range. Whiskers are the most extreme values within Q3 + 1.5 (Q3-Q1) and Q1–1.5 (Q3-Q1). Outliers are not displayed in the plot, but values were included in the data analysis. B. B allele frequency plot of chromosome 3 from sample 23 replicate 3, 4, 8 and 12.

or WGA volume.

3.3. Disc size

With this sub study, we wished to downscale the amount of input DNA material by downscaling the disc size from 2 × 3.2 mm to 2 × 1.6 mm. Eight samples were included in the sub project (samples 1–8). Each sample was punched in discs of 2 × 3.2 mm and in triplicates of 2 × 1.6 mm, denoted 2 × 1.6 A-C. DNA was extracted and WGA in accordance with the standard protocol. On average 49 ng of DNA was extracted from 2 × 3.2 mm discs and 22 ng DNA was extracted from 2 × 1.6 mm discs. The samples were genotyped on the Illumina PsychArray BeadChip. Overall, the samples performed well, with call rates between 98.82% and 99.85%. Thus, all samples passed the initial quality control step and were included in the subsequent analyses. Call rates varied less between samples of 2 × 3.2 mm discs (99.66%–99.85%) than call rates of samples of 2 × 1.6 mm (98.82%–99.84%). We evaluated the concordance rates between and within samples. All concordance rates are displayed in Supplementary Fig. 2. As expected, within sample concordance rates were very close to 100%, ranging from 99.97% to 100%. Between samples concordance rates ranged from 49.94% to 51.40%. Reproduction errors between 2 × 3.2 mm discs and 2 × 1.6 mm discs ranged from 14 to 287 (median = 169,5, IQR = 132–186). To evaluate whether reducing spot size affected the amount of noise in the genotyping data, we examined the log R ratios. Fig. 2 displays a boxplot of the log R ratio for each of the > 570,000 called genotypes for each sample. To evaluate the potential difference in the log R ratio variance between 2 × 3.2 mm discs and 2 × 1.6 mm discs we constructed a regression model. We calculated the log R ratio standard deviation (SD) for each sample. SD was introduced as the dependent variable in the regression model and spot size and sample id was entered as explanatory variables. SDs from

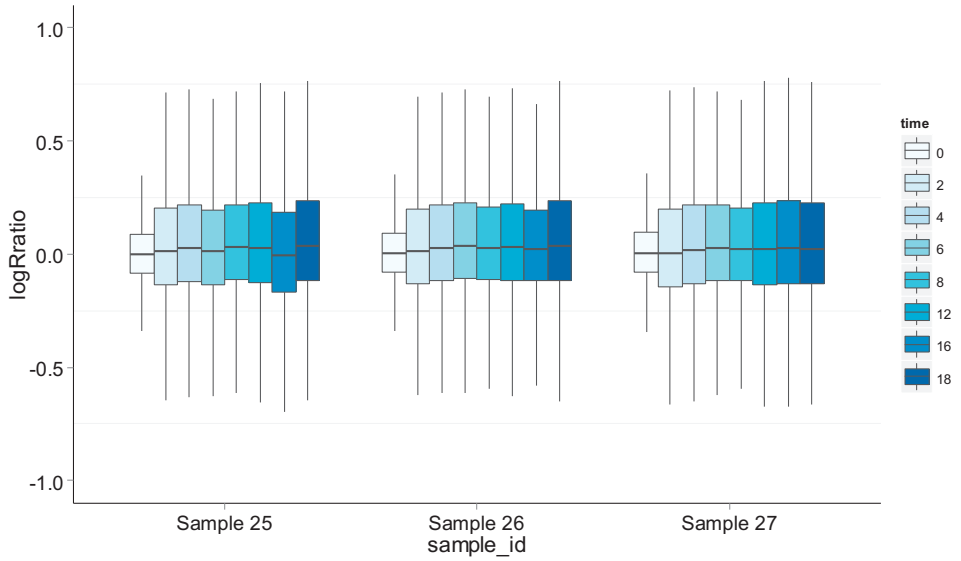

Fig. 5. Boxplot of log R ratios by sample and amplification time. Boxes display median and interquartile range. Whiskers are the most extreme values within Q3 + 1.5 (Q3-Q1) and Q1–1.5 (Q3-Q1). Outliers are not displayed in the plot, but values were included in the data analysis.

each of the triplicate 2 × 1.6 mm A-C samples were combined and entered in the model. Sample id was included in the model in order to account for any between sample variation. The model showed no statistically significant difference between spot size ($p = 0.76$) with regard to variability of the log R ratio. Finally, we compared the GenCall 50 (GC50) score. GC50 score > 0.70 indicates valid genotypes. All samples had a GC50 between 70.98 and 71.40. Thus, genotype data were of high quality for all samples.

3.4. Reducing WGA volume

Eight samples (9–16) were included in this sub study. Each sample was WGA in a total reaction volume of 50, 25 and 12.5 µl equivalent to standard protocol and a reduction by a factor 2 × and 4 ×. With the exception of reaction volume, standard protocol for WGA was followed. DNA quantification subsequent to amplification showed similar DNA concentrations regardless of the amplification volume (data not shown). All samples were genotyped on the Illumina PsychArray BeadChip. Sample 10, reaction volume 50 µl, had a call rate of 95.00% and therefore did not reach our predefined threshold of 98%. As we wanted to examine the performance and quality of array genotyping data across reaction volumes within the same sample, all three samples based on sample 10 were excluded from further analyses. Call rates of the remaining samples ranged between 98.08% and 99.43% with similar ranges across reaction volume groups. We calculated concordance rates between and within all samples. Within samples concordance rates were between 99.88% and 99.90%. Between samples concordance rates were between 50.36% and 52.08%. All concordance rates are displayed in Supplementary Fig. 3. We also evaluated the number of reproduction errors within the same samples, using samples with 50 µl reaction volume as the reference. The number of reproduction errors ranges from 348 to 1517 (median = 786, IQR = 507–1072). To evaluate whether reduction in the reaction volume had an apparent influence on the quality of genotyping data, we examined the log R ratios. Fig. 3 displays a boxplot of the log R ratios for each sample and reaction volume. From the plot, there appear to be a slight decrease in log R ratio variance when sample volume is reduced. To evaluate this tendency further, we calculated the log R ratio SD for each sample. We then constructed a regression model with log R ratio SD as depended variable and volume as explanatory variable. The model was also adjusted for sample id in order to account for any between sample variance. The model showed, that a decrease in volume was significantly associated with a smaller log R ratio SD ($p = 0.002$). As a final quality parameter, we looked at the GC50 score. All samples had GC50 above 70, ranging between 70.05 and 71.19.

In summary, it appears that reducing the spot size does not affect genotyping performance. Thus for future projects it seem valid to decrease the amount of input material by using spots of 1.6 mm size. This would preserve a greater amount of blood on the DBS samples, potentially enabling the performance of a greater number of analyses on each sample. Reduction of the WGA reaction volume from 50 µl to 12.5 µl does not alter the quality of the genotyping array data. On the contrary, it seems that lower volumes leads to a decrease in noise. Based on the above results, all WGA in the subsequent sub studies were carried out in total volumes of 12.5 µl.

3.5. Optimization of the WGA procedure

The second aim of our study was to optimize the WGA protocol. We addressed the issues of non-uniform amplification and amplification noise by increasing the number of amplification replications and altering amplification time and temperature.

3.6. Effect of number of replicates

WGA of DNA is by chance unequal, since some regions may be more or less amplified [13]. If the amplification process is random, replicating the amplification process and subsequent pooling of the sample replicates should even some of the unequal amplification, as each replicate should differ in the regions that are more or less amplified. We evaluated whether additional replicates might lead to a uniform amplification and decrease the noise seen in the array data. Eight samples (17–24) were included in this sub project. Samples were amplified in replicates of 3, 4, 8 or 12. Standard amplification protocol was applied; however total reaction volume per well was 12.5 µl. Based on DNA quantification results, two samples (19 and 21) were excluded from further analyses. Thus, six samples with four different numbers of replicates were genotyped on the PsychArray. Sample 17 had call rates < 91.00% for all four replicates and was therefore excluded. Overall call rates for the remaining samples were between 98.87% and 99.59%. There were no correlation between number of replicates and call rate. We calculated concordance rates between and within all samples. Within samples, concordance rates were between 99.74% and 99.97%. Between samples, concordance rates were between 44.36%

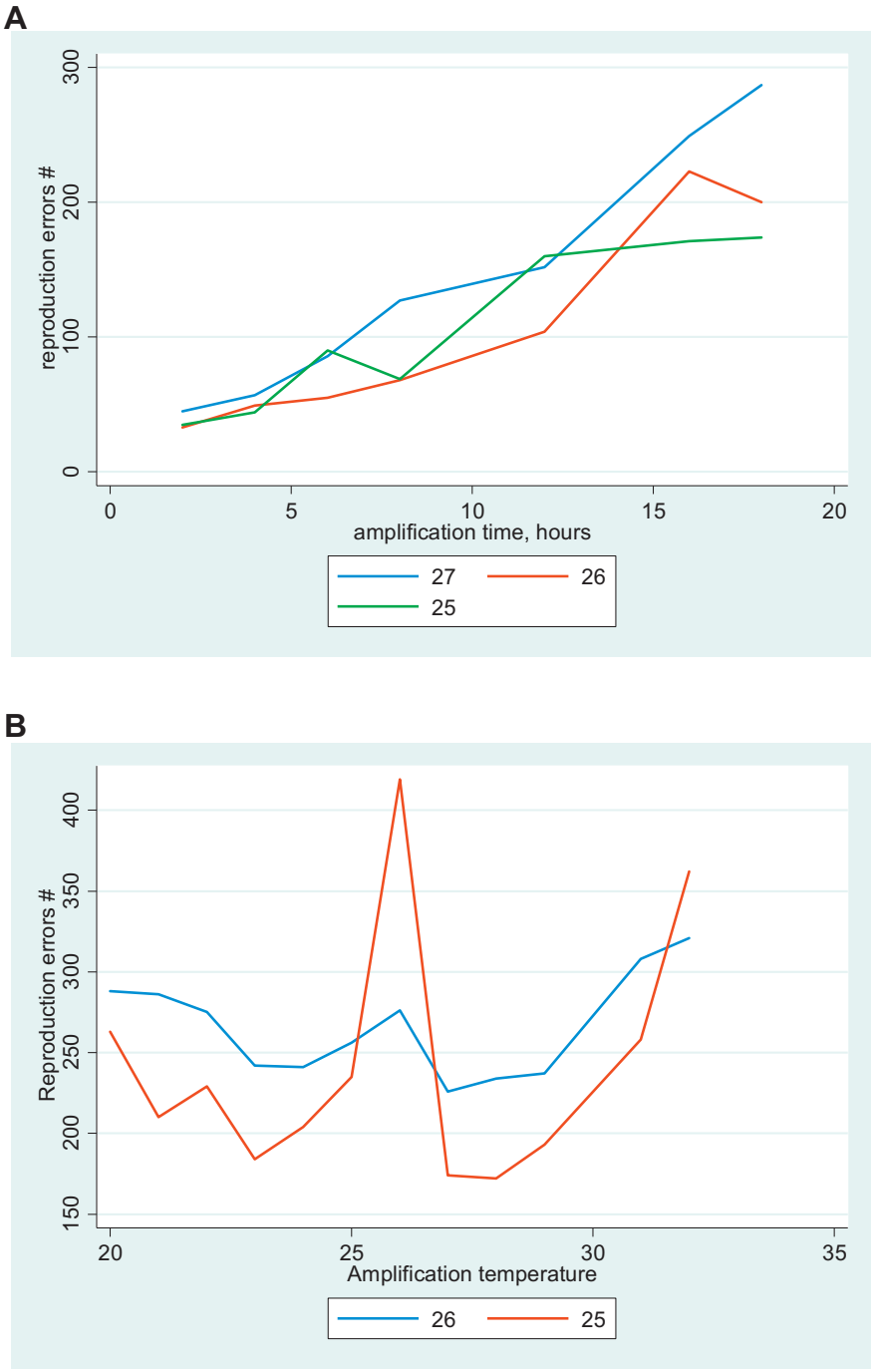

Fig. 6. A. Number of replication errors as a function of amplification time in hours. Amplification time 0, corresponding to unamplified DNA was used as the reference. B. Number of replication errors as a function of amplification temperature in °C. Amplification temperature of 30 °C was used as the reference.

and 53.24%. Sample 22 appears to differ slightly from the other samples with slightly lower between samples concordance rates. All concordance rates are displayed in Supplementary Fig. 4. We calculated reproduction errors with 12 replicates as the reference. The number of reproduction errors ranged from 201 to 3437 (median = 464, IQR = 340–782) across samples. The number of errors was consistent within the same sample with no apparent difference between the reference 12 replicates and 8, 4 and 3 replicates respectively. A boxplot of log R ratios for each sample replicate is displayed in Fig. 4A. There is no apparent difference in noise in array data between the standard three replicates and additional number of replicates. We made a regression model with log R ratio SD as dependent variable and number of replicates and sample id as explanatory variables. The model showed no association between number of replicates and log R ratio SD, p = 0.87. Fig. 4B display a B allele frequency plot for chromosome 3 of the four replicates of sample 22. There are no apparent differences in B allele frequencies between the replicates and thus no indication of a more uniform amplification when performing twelve replicates compared to our standard three replicates.

3.7. Effect of amplification time and temperature

We then evaluated whether noise in array data could be minimized by altering amplification time or temperature. We also examined if altering amplification temperature might favor amplification of regions otherwise difficult to amplify, such as GC rich regions. Three indivi-

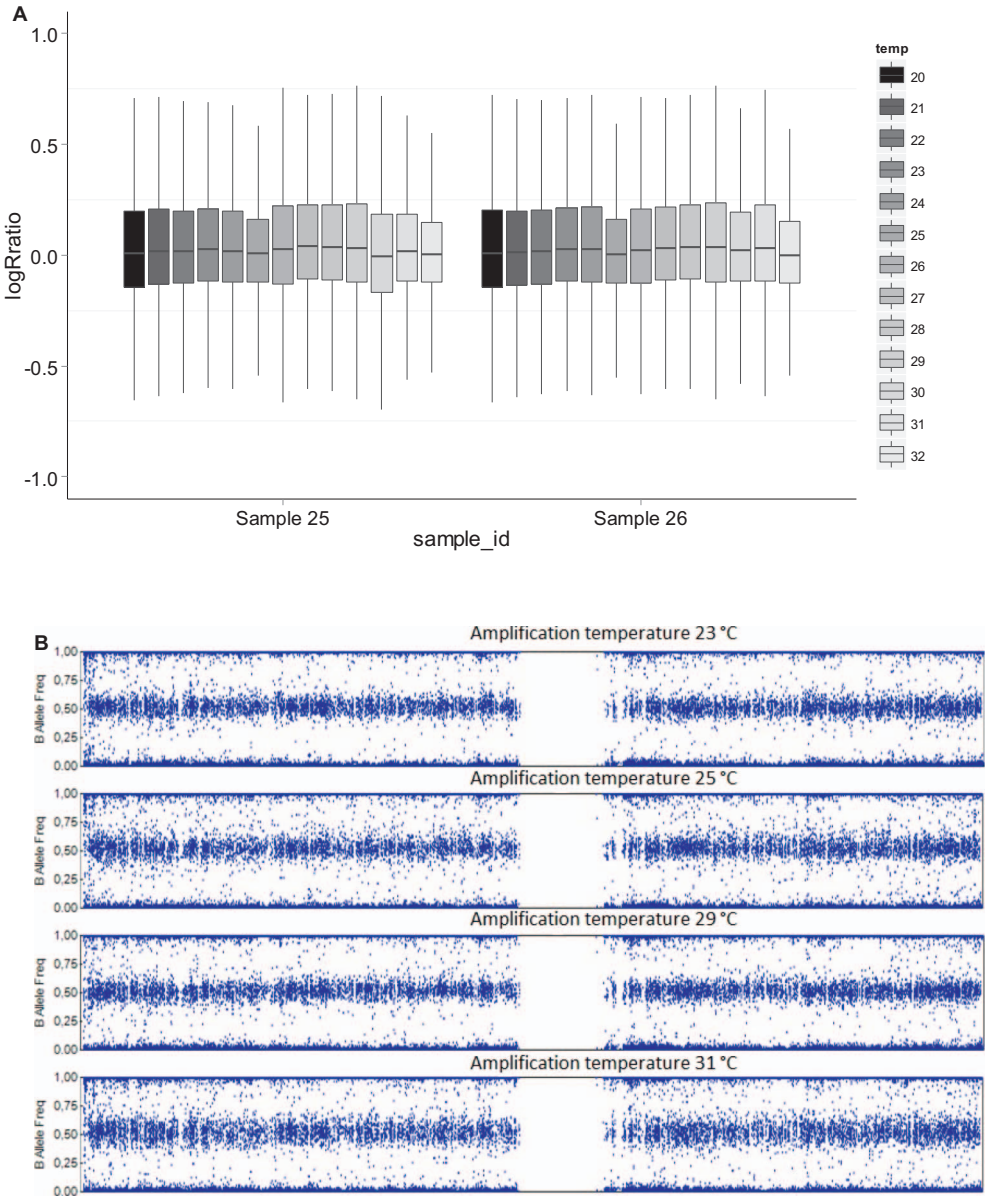

Fig. 7. A. Boxplot of log R ratios by sample and amplification temperature. Boxes display median and interquartile range. Whiskers are the most extreme values within Q3 + 1.5 (Q3-Q1) and Q1–1.5 (Q3-Q1). Outliers are not displayed in the plot, but values were included in the data analysis. B. B allele frequency plot of chromosome 1 for sample 26 amplification temperature 23, 25, 29 and 31 °C.

duals (25–27) were included in the sub study. With regards to time optimization, all three samples were WGA for 0 (unamplified genomic DNA), 2, 4, 6, 8, 10, 12, 14, 16 or 18 h. Supplementary Fig. 5 displays the WGA DNA concentration as a function of amplification time. As expected, longer amplification time led to higher WGA DNA yield, however longer amplification time might also lead to increased amplification of unspecific DNA and hence increase noise in the genotyping array. In addition, a greater fold of amplification would result in a greater fold of amplification bias [13]. We selected amplification time 0, 2, 4, 6, 8, 12, 16 and 18 h for genotyping on the PsychArray. All samples had call rates above 99.28%. Fig. 5 displays a boxplot of the log R ratios by sample and time. As already discussed in the previous section on amplified versus unamplified genomic DNA, the genomic DNA, amplification time 0, contains far less noise than data from the WGA DNA. From the plot, it appears that the amplification time does not affect the variability of the log R ratios within samples. To confirm the visual interpretation of the log R ratios statistically, we calculated log R ratio SD for each sample and made a regression model as previously described. Data from the genomic DNA samples, amplification time 0, were excluded from the analysis. The model showed no association between amplification time and log R ratio SD, $p = 0.98$. We calculated concordance rates between and within samples. Concordance rates are displayed in Supplementary Fig. 6. Within the sample, concordance was above 99.91% and between 54.50% and 55.31% between samples. The GC 50 was above 71.1 for all samples. We also evaluated the number of reproduction errors with amplification time 0 as the reference. The number of reproduction errors as a function of amplification time is displayed in Fig. 6A. There is a clear increase in errors with increased amplification time. I might therefore be reasonable to lower the amplification time, keeping in mind, that the DNA yield must be sufficient for array genotyping and that the number of reproduction errors ranges from an average of 38 between 0 and 2 h to 220 between 0 and 18 h. Thus the absolute number of errors is small even at 18 h of replication.

With regard to optimization of amplification temperature, samples were amplified at 20–32 °C in intervals of 1 °C, 34 °C and 36 °C.

Quantification of WGA DNA is displayed in Supplementary Fig. 7. For all three individuals, WGA DNA concentrations approached that of unamplified DNA for temperatures around 34 °C. We selected samples from individual 25 and 26 for genotyping. WGA temperatures of 20–32 °C were genotyped on the PsychArray. All samples passed the initial quality control step with call rates ranging between 98.87% and 99.63%. Fig. 7A displays a boxplot of the log R ratios for each temperature and sample. There is no apparent difference in the log R ratio distribution and a regression model of log R ratio SD, amplification temperature and sample id, did not find a statistically significant association between amplification temperature and log R ratio SD ($p = 0.39$). From the B allele frequency plot in Fig. 7B, there is no apparent differences between the different temperatures and noise. Concordance rates within identical samples were above 99.91% and between samples concordances ranged from 57.32% to 57.52%. All concordance rates are displayed in Supplementary Fig. 8. GC 50 scores were above 71.1 for all samples. Finally, we evaluated the number of reproduction errors between replicate samples amplified at different temperatures. We used amplification at 30 °C as the reference. Data is displayed in Fig. 6B. There appear to be a consistent number of replication errors between the reference samples and sample amplified at temperatures below 30 °C. Samples amplified at above 30 °C had a higher number of amplification errors, however this correlates with the reduced DNA concentration of samples amplified at above 30 °C and might likely be a result of sub optimal DNA input. In sample 25, there is a marked increase in number of reproduction errors at 26 °C. This correlates with the observed drop in DNA yield at 26 °C, see Supplementary Fig. 7.

In summary, it appears that the amplification process is very robust. Alteration in amplification time and temperature does not affect the call rate, log R ratio or B allele frequencies, but increased amplification time and temperature does increase the number of replication errors. However, compared to the total number of probes, this increase is very small. Adding of additional replicates does not seem to increase the quality of the WGA DNA or provide a more evenly amplified sample. Supplementary Tables S9 and 10 display the number of shared “no call” or genotype error probes between the samples in the different sub studies. In regard to “no call” and genotype error probes combined, the overlap was between 6.2% and 36.7%, with samples with a low amount of errors displaying the highest percentage of shared error probes. If “no call” variants were excluded, the overlap ranged from 0.3% to 19.1%. Thus, there seem to be a fair number of probes that often if not always fails and it might be reasonable to filter out these probes prior to analyzing the genotype data.

4. Conclusion

We find that that the amount of input material and the amplification volume can be reduced without compromising the quality of the DNA. We also find that alterations to the amplification protocol with regard to amplification temperature and length of amplification time slightly affected the quality of the WGA DNA. However, the increase in reproduction errors with increased amplification time were small and increased temperature only affected the number of reproduction errors when above 30 °C. Finally, we examined whether additional WGA replicates would even some of the amplification bias coursed by the non-uniform nature of the amplification. From our results, it appears that additional replicates do not reduce noise in the array data or affect the regional differences in amplification coverage.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgmr.2017.04.002.

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