Benchmarks

Development of an automated AmpliSeq™ library building workflow for biological stain samples on the Biomek® 3000

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ABSTRACT

Here, we present the development of an automated AmpliSeq™ (ThermoFischer, MA, USA) workflow for library building using the Biomek® 3000 Laboratory Automation Workstation (Beckman Coulter Inc., CA, USA), in which the total volume of PCR reagents and reagents for library preparation are reduced by one-half. The automated AmpliSeq workflow was tested using 43 stain samples (blood, bone, muscle tissue, semen, swab, nail scrape and cigarette butts) collected from crime scenes. The sequencing data were evaluated for locus balance, heterozygous allele balance and noise. The performance of libraries built with the automated AmpliSeq workflow using one-half of the recommended reagent volumes were similar to the performance of libraries built with the recommended (full) volumes of the reagents.

METHOD SUMMARY

Here, we developed an automated library building method for high-throughput sequencing of forensic stain samples on the Biomek® 3000 using one-half of the recommended reagent volumes by modifying parameters for automated liquid handling with special respect to low volume pipetting (1≤ µl). The automated workflow was tested for precision.

KEYWORDS

AmpliSeq™ workflow • Biomek® 3000 • library building • massively parallel sequencing

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Since the 1990s, short tandem repeats (STRs) have been the preferred loci worldwide for human identification in forensic genetic casework [1–3]. The standard genotyping methods involve amplification of the STRs by PCR and fragment length analysis of the PCR products by capillary electrophoresis (CE). Massively parallel sequencing (MPS) offers an alternative detection method with several advantages [4]. In addition to the length of the PCR product, MPS identifies the complete nucleotide variation of the STRs and its flanking regions. This allows detection of the true allelic diversity of the STRs. Furthermore, MPS allows simultaneous detection of STRs and other relevant loci (single nucleotide polymorphisms, insertion/deletions) with phenotypical or ancestral information [5–10]. Finally, the PCR amplicons in a PCR-MPS assay can be designed to be as short as possible because each locus may be identified by the DNA sequences and not by the size and color of the PCR product, as done in CE detection assays. This is an important advantage because short amplicons are more likely to be successfully amplified from highly degraded samples [11].

However, PCR-MPS assays also have disadvantages. The MPS workflows are complex and include numerous pipetting steps for the transfer and mixing of reagents. The steps are slow, labor intensive [12] and a significant source of experimental variability [13]. Accuracy and precision are crucial factors when analyzing unique stain samples because of the uncertain nature of this type of biological material. Additionally, the chemistries for MPS assays are relatively expensive, and the time from sample to result is 1–2 days longer than the traditional PCR-CE workflow. Lowering the cost per sample and automation of the workflows are attractive solutions and also necessary if PCR-MPS assays replace PCR-CE in high-throughput forensic laboratories in the future.

We setup and optimized the Biomek® 3000 Laboratory Automation Workstation (Beckman Coulter Inc., CA, USA) to build Ion AmpliSeq™ libraries (Thermo Fisher Scientific, MA, USA) using half volumes of reagents. The preparation of AmpliSeq libraries included pipetting of three viscous liquids; the FuPa, the switch solution and the DNA ligase (Thermo Fisher Scientific). The purification step included pipetting of beads and a volatile solution of ethanol. The Biomek 3000 was chosen as the liquid handler because it offered excellent control of the tip movement and dispensation speed. The optimization of the Biomek 3000 included lowering of the speed of aspiration and dispensation of viscous reagents, raising the tip from viscous stock solutions, and modifications of tip touch, prewetting and blowout. All the viscous reagents were dispensed while the tip was in solution and dispensation was followed by mixing up and down ten times (wet-dispense) with 60 µl/s. Dispensation of ethanol (60 µl/s) was performed while the tip was above the surface of the solution (air jet dispense). The aspiration of the purification beads was performed after mixing the stock solution up and down ten times. Dispensed volumes of reagents by the Biomek 3000 were controlled by measuring the volumes using manually calibrated pipettes. The accuracy and precision of 1 and 10 µl pipetting steps in the optimized Biomek workflow were tested with colorimetric pipetting precision test using Orange G (03756, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) [14]. Dispensation of 1 µl high-density liquid was tested by adding the aspirated FuPa to 10 µl of water (wet-dispense) and measuring the change in weight with respect to the density of the FuPa reagent (70% glycerin,

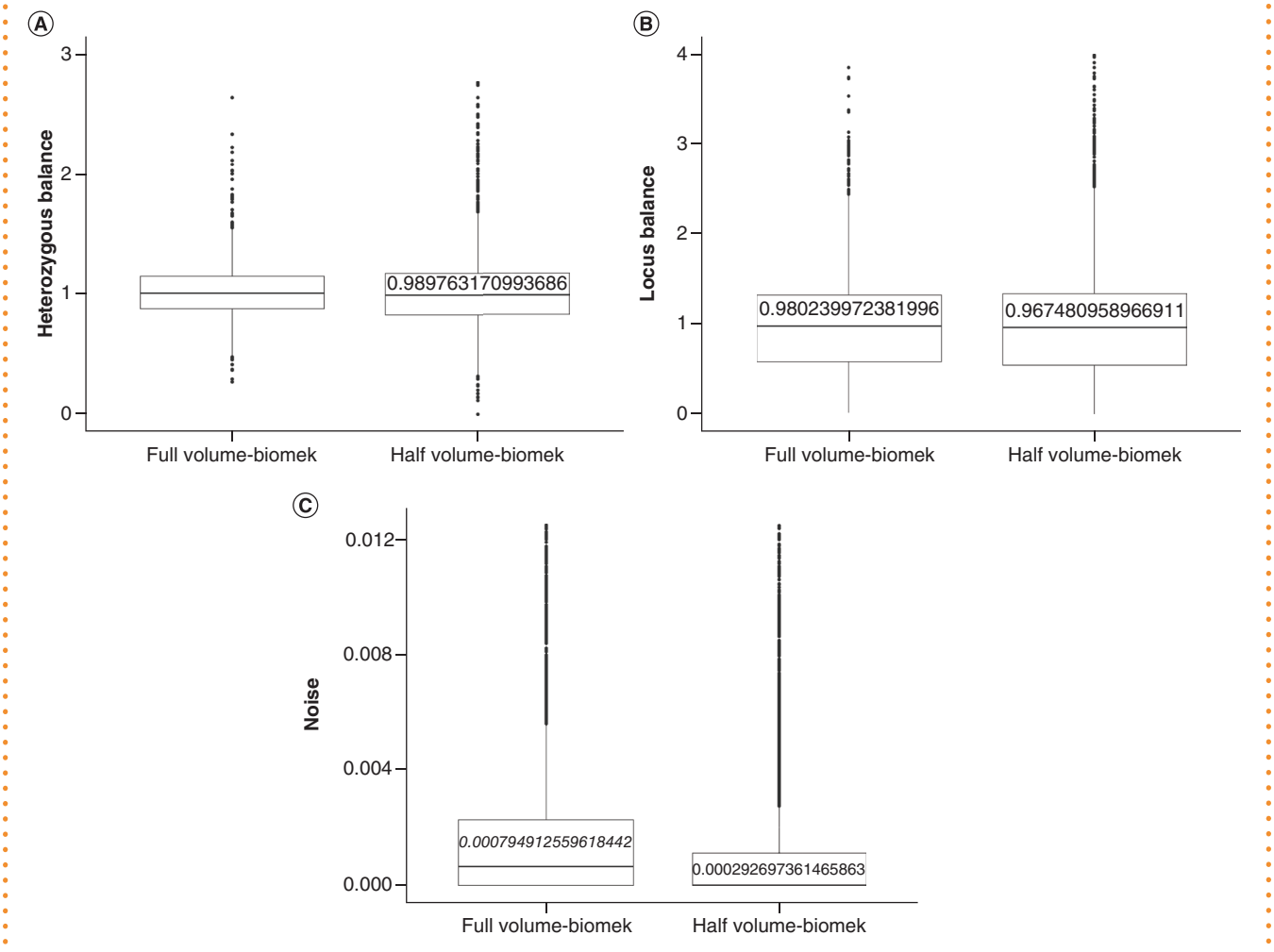

Figure 1. Automated preparation of libraries from forensic strain samples using full and half volumes of reagents. (A) Heterozygous balance. (B) Locus balance. (C) Noise ratio. The heterozygous balance was defined as the number of reads for one nucleotide divided by the number of reads for the other nucleotide among heterozygote genotypes. The locus balance was defined as the number of reads for a SNP locus divided by the average number of reads per SNP locus [10]. The noise levels were defined as the number of reads that were not identical to the genotype divided by the total number of reads for that SNP locus [10]. The numbers inside the boxes show the median values.

ρ = 1.26 g/cm^3) [15]. CSV files created in Microsoft Excel were used to generate documentation with information on sample name, volume of reagents and batch information that may be imported to laboratory information systems (Supplementary material; Biomek 3000 scripts are available in a .bmf binary file format that are readable with the Biomek 3000 software).

First, the precision test using 1 µl of Orange G and 1 µl of FuPa showed a coefficient of variation of 2 and 14.7%, respectively, and the accuracy (%d) was 8 and 17% (Supplementary Table 1). We found these values to be acceptable considering the challenging conditions of automated pipetting, low volumes and viscous liquids.

Second, genomic DNA was extracted from 43 different biological stain samples using in-house protocols (our laboratory is accredited according to the ISO 17025 standard). DNA quantification was done using the Quantifiler® Trio DNA Quantification kit (Thermo Fisher Scientific), which showed that 13 of the biological stain samples were degraded (Supplementary Table 2). The DNA concentrations ranged from 0.070 to 232 ng/µl. Initial PCR was performed using the Precision ID Ancestry Panel (Thermo Fisher Scientific) with half volumes of the PCR reagents. The amount of input DNA was 0.5–1 ng, and the number of PCR cycles was 25 [9]. DNA libraries were constructed using the automated Ampliseq workflow with one-half of the reagent volumes; 1 µl of FuPa, 2 µl of Switch solution and 1 µl of ligase. The volume of the barcode adaptor mix (2 µl) was unchanged because initial experiments had shown that higher concentrations of PCR products were obtained using half volume of the reagents. Automated purification of the generated libraries was performed using the Agencourt® AMPure® XP kit (Beckman Coulter, Inc.) with one-half volume of all solutions; 22.5 µl of magnetic beads, 150 µl of 70% ethanol and 50 µl of TE buffer. The volume of the magnetic beads was set to 22.5 µl after the initial experiments had shown that higher volumes of magnetic beads could lead to higher levels of primer-dimers in the purified libraries. The purified libraries were quantified using the Ion Library TaqMan™ quantification kit ▶

(Thermo Fisher Scientific). Dilution of purified libraries and subsequent pooling of diluted libraries in equimolar amounts (20 pM) were done with the Biomek 3000. A concentration of 20 pM was chosen after initial experiments had shown that this concentration would lead to the best balance between usable and polyclonal reads. Emulsion PCR and chip loading was performed on the Ion Chef™ system with the Ion PGM™ IC 200 Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. Sequencing was completed on the Ion Torrent PGM™ system with the Ion PGM IC 200 sequencing kit and Ion 316™ chips (Thermo Fisher Scientific). Data analysis was performed on the Torrent Suite Server (version 4.6) using the HID_SNP_Genotyper plugin version 4.2 (Thermo Fisher Scientific).

Automated library preparation using one-half of the recommended reagent volumes or the recommend (full) reagent volumes were compared. Noise levels, locus and heterozygous balances were calculated [10], and no significant differences between the full and half reagent volume reactions were found (Figure 1). Complete concordance was obtained for all samples typed with full and half reagent volume reactions (data not shown) [12].

SUPPLEMENTARY DATA

To view the supplementary data that accompany this paper, please visit the journal website at: www.future-science.com/doi/suppl/10.2144/btn-2019-0156

AUTHOR CONTRIBUTIONS

HS Mogensen designed the study. BM Pedersen modified the Biomek. MS Farzad analyzed the data and wrote the manuscript. C Børsting and HS Mogensen revised the manuscript.

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FINANCIAL & COMPETING INTEREST DISCLOSURES

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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ETHICAL CONDUCT OF RESEARCH

The section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Science, University of Copenhagen, Denmark has permission to store and use crime case samples for method development and quality control (Danish agency for data protection no. 2002-54-1080).

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