Forensic Science International: Genetics 31 (2017) 118–125

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Research paper

Comparison of manual and automated AmpliSeq™ workflows in the typing of a Somali population with the Precision ID Identity Panel

Suzanne van der Heijden, Susanne Juel de Oliveira, Marie-Louise Kampmann, Claus Børsting*, Niels Morling

Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

ARTICLE INFO

Keywords:
Forensic genetics
Next-generation sequencing
Precision ID Identity Panel
Single nucleotide polymorphism
Somali population
Library building
Automation

ABSTRACT

The Precision ID Identity Panel was used to type 109 Somali individuals in order to obtain allele frequencies for the Somali population. These frequencies were used to establish a Somali HID-SNP database, which will be used for the biostatistic calculations in family and immigration cases. Genotypes obtained with the Precision ID Identity Panel were found to be almost in complete concordance with genotypes obtained with the SNP*for*ID PCR-SBE-CE assay. In seven SNP loci, silent alleles were identified, of which most were previously described in the literature.

The project also set out to compare different AmpliSeq™ workflows to investigate the possibility of using automated library building in forensic genetic case work. In order to do so, the SNP typing of the Somalis was performed using three different workflows: 1) manual library building and sequencing on the Ion PGM™, 2) automated library building using the Biomek®3000 and sequencing on the Ion PGM™, and 3) automated library building using the Ion Chef™ and sequencing on the Ion S5™. AmpliSeq™ workflows were compared based on coverage, locus balance, noise, and heterozygote balance. Overall, the Ion Chef™/Ion S5™ workflow was found to give the best results and required least hands-on time in the laboratory. However, the Ion Chef™/Ion S5™ workflow was also the most expensive. The number of libraries that may be constructed in one Ion Chef™ library building run was limited to eight, which is too little for high throughput workflows. The Biomek®3000/Ion PGM™ workflow was found to perform similarly to the manual/Ion PGM™ workflow. This argues for the use of automated library building in forensic genetic case work. Automated library building decreases the workload of the laboratory staff, decreases the risk of pipetting errors, and simplifies the daily workflow in forensic genetic laboratories.

1. Introduction

In our ISO17025 accredited laboratory, SNPs have been used as supplementary markers in relationship case work for more than a decade [1–4]. From 2007 to 2015, the SNPs were typed with a single base extension (SBE) assay developed by the SNP*for*ID consortium [2,5]. However, this assay demanded a lot of hands-on time in the laboratory, and the method was not suitable for typing large numbers of SNPs simultaneously. Furthermore, analysis of the data was often complicated by the variations in SBE signal intensities and the small sized peaks originating from single base extension of PCR products or primer-dimers [2,5–7]. In 2015, the SBE assay was replaced by a next generation sequencing (NGS) assay [4]. The Precision ID Identity Panel amplifies 90 autosomal SNPs from the SNP*for*ID [5] and Individual Identification SNP (IISNP) panels [8], and 34 upper-clade Y-chromosome SNPs in one multiplex PCR and the fragments are sequenced on an Ion Torrent platform [4,9–12].

In this work, allele frequencies for a Somali population were obtained by sequencing 109 individuals using the Precision ID Identity Panel. Furthermore, three different workflows (Table 1) were compared: 1) manual library building and sequencing on the Ion PGM™ (Thermo Fisher Scientific), 2) automated library building using the Biomek®3000 (Beckman Coulter) and sequencing on the Ion PGM™, and 3) automated library building using the Ion Chef™ (Thermo Fisher Scientific) and sequencing on the Ion S5™ (Thermo Fisher Scientific). An important and time consuming step of NGS methods is library building, where the PCR products are prepared for sequencing [13]. Automation of the library building process will reduce the hands-on time in the laboratory, decrease the risk of pipetting errors, and reduce the workload of the laboratory technicians. With the Biomek®3000

* Corresponding author at: Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, 11 Frederik V's Vej, DK-2100, Copenhagen, Denmark.
E-mail address: claus.boersting@sund.ku.dk (C. Børsting).

http://dx.doi.org/10.1016/j.fsigen.2017.09.009
Received 26 July 2017; Received in revised form 11 September 2017; Accepted 12 September 2017
Available online 14 September 2017
1872-4973/

Table 1
Overview of AmpliSeq™ workflows.

	Manual/Ion PGM™ (N = 109)	Biomek®3000/Ion PGM™ (N = 60)	Ion Chef™/Ion S5™(N = 49[a])
PCR	GeneAmp™ PCR system	GeneAmp™ PCR system	Ion Chef™
Library building	Manual	Biomek®3000	Ion Chef™
Quantification	Qubit™ 2.0	Qubit™ 2.0	Real-time PCR (pool only)
Library pooling	Manual	Manual	Ion Chef™/Manual
Template preparation and chip loading	Ion Chef™	Ion Chef™	Ion Chef™
Sequencing	Ion PGM™	Ion PGM™	Ion S5™

[a] A total of 14 samples were typed with a DNA input of 10 ng and 35 samples were typed with a DNA input of 1 ng.

protocol, all pipetting steps in the library building process were automated and were only interrupted by the incubation steps with FuPa Reagent and DNA Ligase. Template preparation and chip loading were subsequently performed on the Ion Chef™. The Ion Chef™/Ion S5™ workflow offered further reduction in hands-on time. The Ion Chef™ was used for PCR, library building, library pooling, template preparation, and chip loading, before sequencing on the Ion S5™. Furthermore, initialisation and post-run wash were automated as part of the Ion S5™ run protocol and were performed with prepared Ion S5™ sequencing reagents – in contrast to the Ion PGM™ protocol, where these steps can be time-consuming and the reagents are prepared manually.

2. Material and methods

2.1. Samples, DNA extraction and quantification

Samples from 109 unrelated Somali individuals (96 males and 13 females) were selected. All selected samples were previously typed with the PCR-SBE-CE version of the SNP*for*ID assay [1]. Genomic DNA was extracted from 200 µl of blood with the QIAamp® DNA Blood Mini kit (Qiagen) according to the protocol. DNA was quantified using the Qubit™ 3.0 Fluorometer with the Qubit™ dsDNA HS Assay kit (Thermo Fisher Scientific) according to the protocol. Samples were diluted in nuclease-free water to concentrations of 1 ng/µl. After DNA extraction and quantification, all samples were typed in duplicates. For all samples, one preparation was typed using the manual library building method. The second preparation was typed using either the Biomek®3000 or the Ion Chef™ library building method. The work was approved by the Danish ethical committee (H-1-2011-081).

2.2. Manual library building, template preparation using Ion Chef™ and sequencing using Ion PGM™

DNA libraries were constructed using the Ion AmpliSeq™ Library Kit 2.0 (Thermo Fisher Scientific) combined with the Precision ID Identity primer panel. PCR was performed using half of the volumes of the reagents mentioned in the manufacturer's protocol. The PCR contained 2 µl of 5X Ion AmpliSeq™ HiFi Mix (Thermo Fisher Scientific), 5 µl of Precision ID Identity primer panel and a DNA input of 1 ng per sample. One negative and one positive control (DNA extracted from a blood sample from a Danish male individual) were included in each experiment. PCR was performed with the GeneAmp™ PCR system (Thermo Fisher Scientific) using the following program: 2 min at 99 °C, 24 cycles of 15 s at 99 °C and 4 min at 60 °C, followed by a 10 °C hold. FuPa Reagent (Thermo Fisher Scientific) was diluted with low TE buffer (1:1) and 2 µl of the dilution was added to each amplified sample to digest primer sequences. The reaction was incubated at 50 °C for 10 min, 55 °C for 10 min and 60 °C for 20 min. Barcode adapter mix consisting of 2 µl Ion P1 Adapter (Thermo Fisher Scientific), 2 µl Ion Xpress™ barcode (Thermo Fisher Scientific), and 4 µl nuclease-free water was prepared. A total of 2 µl Switch Solution (Thermo Fisher Scientific), 1 µl barcode adapter mix and 1 µl DNA Ligase (Thermo Fisher Scientific) was added to each well. The plate was incubated at 22 °C for 30 min and at 72 °C for 10 min. Libraries were purified using 22.5 µl AMPure® XP Reagents (Agencourt) per sample. The rest of the purification process was performed according to the manufacturer's protocol, with the exception that the DNA was eluted from the beads using 25 µl instead of 50 µl of low TE buffer. Libraries were quantified using the Qubit™ 2.0 Fluorometer with the Qubit™ dsDNA HS Assay kit. Purified libraries were diluted to a concentration of 50 pg/µl and were pooled in equal molar ratios. 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 performed on the Ion Torrent PGM™ system with the Ion PGM™ IC 200 sequencing kit (Thermo Fisher Scientific) following the manufacturer's protocol. Sequencing was performed on Ion 316™ chips (Thermo Fisher Scientific).

2.3. Automated library building using the Biomek®3000, template preparation using Ion Chef™ and sequencing using Ion PGM™

DNA libraries were constructed using the Ion AmpliSeq™ Library Kit 2.0 combined with the Precision ID Identity primer panel. PCR was performed using the volumes of the reagents mentioned in the manufacturer's protocol. The PCR contained 4 µl of 5X Ion AmpliSeq™ HiFi Mix, 10 µl of Precision ID Identity primer panel and a DNA input of 1 ng per sample. PCR was performed with the GeneAmp™ PCR system using the following program: 2 min at 99 °C, 24 cycles of 15 s at 99 °C and 4 min at 60 °C, followed by a 10 °C hold.

Library building was performed using the Biomek®3000 with an in-house developed library building script that is available on request. A total of 24 libraries were constructed per run. One negative control was included in each run. FuPa Reagent was diluted with low TE buffer (1:1) and 2 µl of the dilution was added to each amplified sample using the Biomek®3000. The reaction was incubated at 50 °C for 10 min, 55 °C for 10 min and 60 °C for 20 min. Barcode adapter mix was prepared as in the manual library building method. A total of 4 µl Switch Solution, 2 µl barcode adapter mix and 2 µl DNA ligase was added to each well by the Biomek®3000. The plate was incubated at 22 °C for 30 min and at 72 °C for 10 min. Libraries were purified using the Biomek®3000 according to the manufacturer's protocol. Library quantification, dilution, pooling, emulsion PCR, chip loading, and sequencing was performed as described above. Sequencing was performed on Ion 318™ chips (Thermo Fisher Scientific).

2.4. Automated library building and template preparation using the Ion Chef™ and sequencing using Ion S5™

Library building was performed on the Ion Chef™ using the Ion AmpliSeq™ Kit for Chef DL8 (Thermo Fisher Scientific) according to the manufacturer's protocol. A total of eight libraries were constructed per run. One negative and one positive control (DNA extracted from a blood sample from a Danish male individual, or 007 or 9947a control DNA (Thermo Fisher Scientific)) were included in each run. For 14 samples, library building was performed with a DNA input of 10 ng per sample (15 µl of 0.67 ng/µl dilution), with 19 amplification cycles, and annealing and extension time of 4 min. For the other 35 samples, library building was performed with a DNA input of 1 ng per sample (15 µl of