P01-57 170045

Systematic Evaluation of Massively Parallel STR Sequencing in the DNASeqEx Project

Petra Müller[1], Burkhard Berger[1], Martin Bodner[1], Antonio Alonso[2], Pedro Barrio[2], Pablo Martin[2], Steffi Köcher[3], Lutz Roewer[3], Sascha Willuweit[3], Bruce Budowle[4,5], The DNASEQEX Consortium, Walther Parson[1,6]

[1]Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria; [2]National Institute of Toxicology and Forensic Sciences, Madrid Department, Spain; [3]Institute of Legal Medicine and Forensic Sciences, Charité–Universitätsmedizin Berlin, Germany; [4]Center for Human Identification, University of North Texas Health Science Center, USA; [5]Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia; [6]Forensic Science Program, The Pennsylvania State University, PA, USA

✉ petra.mueller@i-med.ac.at

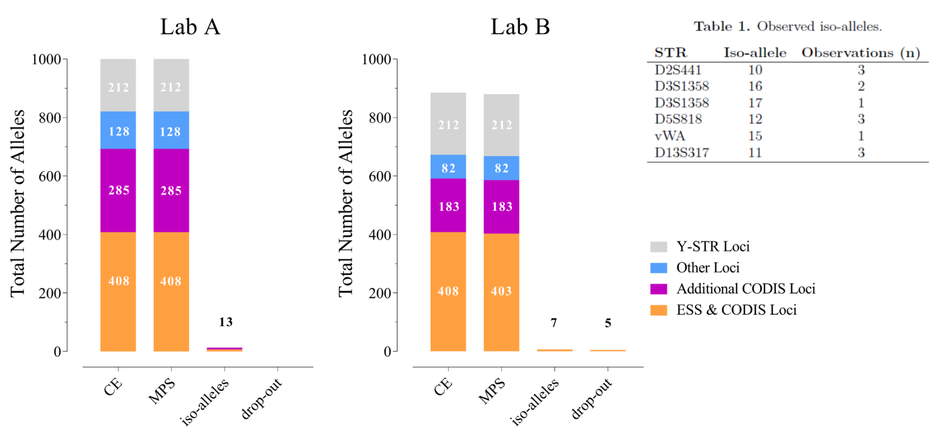
Introduction

The EU funded **DNASeqEx** (**DNA**-STR Massive **Seq**uencing & International Information **Ex**change) project evaluates current massively parallel sequencing (MPS) technologies in a collaborative manner. The aim of the project is to perform interlaboratory evaluation and comparison of forensically relevant parameters, such as allele calling, sensitivity, reproducibility, and concordance in STR analysis using single source and mixture samples.

Here, we present preliminary results from experiments that were performed in the first year of the collaboration among the participating laboratories using either the MiSeq (Illumina, CA) or the Ion S5 System (Thermo Fisher Scientific, CA).

Interlaboratory Comparison 1 – MiSeq FGx

Concordance & Reproducibility



1a. 1b.

Table 1. Observed iso-alleles.

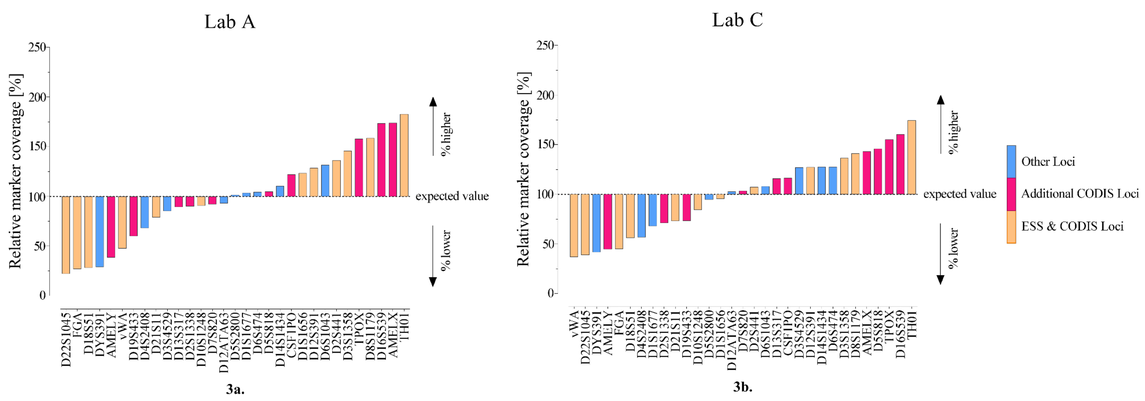
STR	Iso-allele	Observations (n)
D2S441	10	3
D3S1358	16	2
D3S1358	17	1
D5S818	12	3
vWA	15	1
D13S317	11	3

A drop-out indicates an allele that was not observed in two replicates. Differences in allele numbers and detected iso-alleles result from a smaller marker set (CE data from GEDNAP proficiency tests) that was analyzed in lab B. ESS & CODIS Loci: D1S1656, D2S441, D3S1358, FGA, D8S1179, D8S1179, TH01, vWA, D12S391, D18S51, D21S11, D22S1045. Additional CODIS Loci: AMEL, D2S1338, D16S539, D19S433, TPOX, D5S818, CSF1PO, D7S820, D13S317. Other Loci: D6S1043, PentaE, PentaD, D4S2408, D9S1122, D17S1301, D20S482. Y-STR Loci: DYS570, DYS576, DYS481, DYS19, DYS391, DYS635, DYS437, DYS439, DYS389I, DYS389II, DYS438, DYS390, DYS643, DYS533, Y-GATA-H4, DYS385a-b, DYS460, DYS549, DYS392, DYS448, DYF387S1.

As shown in **Figures 1a & 1b** a total of 36 samples were amplified in duplicates using the ForenSeq DNA Prep Kit (Illumina). Data analysis was performed using the ForenSeq Universal Analysis Software (Illumina) by applying the manufacturer's default settings. Both labs obtained fully concordant results compared to the reference capillary electrophoresis (CE) data.
All findings were reproducible among both labs, except of five drop-outs (lab B). Furthermore, thirteen alleles that appeared homozygous in CE displayed sequence variation when analyzed using MPS (**Table 1**).

Interlaboratory Comparison 3 – Ion S5 System

Performance

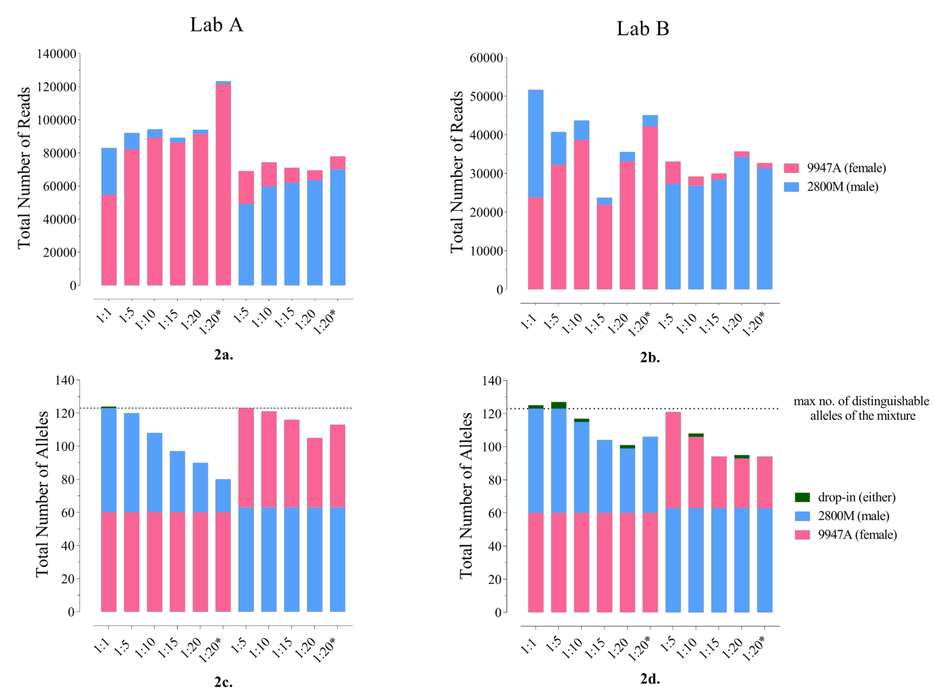


3a. 3b.

Figures 3a & 3b display the performance of the Early Access Globalfiler NGS STR Panel (Thermo Fisher Scientific). The sample sets used for performance and stutter ratio analyses consisted as follows: Lab A (n=22): NIST SRM 2391c (component A–C and E), control DNA (9947A), and single source GEDNAP stains. Lab C (n=21): NIST SRM 2391c (component A–C, E and F), control DNAs (2800M, 007 plus 9947A), single source GEDNAP stains and fresh buccal swab DNA samples from volunteers.
Samples were prepared fully automated using the Ion Chef System and sequenced on the Ion S5 System (both Thermo Fisher Scientific). Data analysis was performed using Converge software by applying the manufacturer's default settings. Both labs obtained similar relative coverage values for all analyzed markers, except D5S818, which showed a distinctly higher value in lab C.

Interlaboratory Comparison 2 – MiSeq FGx

Mixtures

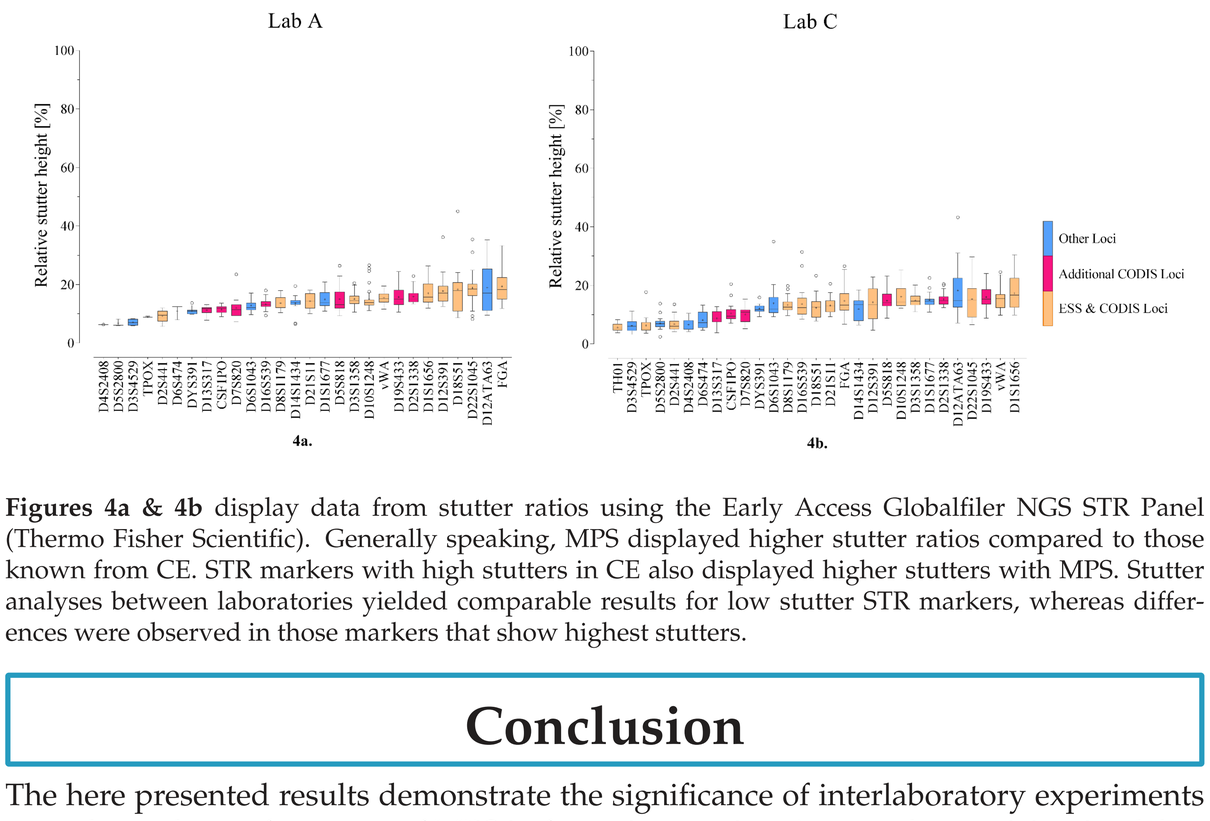


DNA input was 1 ng for all mixtures, except for tagged data. *Indicates the DNA input amount of 2 ng. Distinguishable markers: D1S1656, TPOX, D2S1338, D3S1358, D5S818, D8S1179, vWA, PentaE, D16S539, D18S51, D21S11, D22S1045.

Figures 2a & 2b display the total number of reads as a function of DNA mixture ratios (**male/female mixture ratios** 1:1, 1:5, 1:10, 1:15, 1:20 and **female/male mixture ratios** 1:5, 1:10, 1:15 and 1:20). As expected, the number of reads decreased with lowered DNA input. **Figures 2c & 2d** show the total number of distinguishable alleles as a function of DNA mixture ratios. For m/f mixtures, both labs were able to assign ≥95% of the included STR alleles to their contributors up to a mixture ratio of 1:5. For f/m mixtures, lab B was able to assign ≥95% of the included STR alleles up to a mixture ratio of 1:5 whereas lab A was able to call ≥95% of the alleles of the 1:10 mixture.

Interlaboratory Comparison 4 – Ion S5 System

Stutter Ratios



Figures 4a & 4b display data from stutter ratios using the Early Access Globalfiler NGS STR Panel (Thermo Fisher Scientific). Generally speaking, MPS displayed higher stutter ratios compared to those known from CE. STR markers with high stutters in CE also displayed higher stutters with MPS. Stutter analyses between laboratories yielded comparable results for low stutter STR markers, whereas differences were observed in those markers that show highest stutters.

Conclusion

The here presented results demonstrate the significance of interlaboratory experiments to evaluate the performance of MPS in forensic genetics. Among the participating laboratories we obtained fully concordant and reproducible results. In addition, we received comparable results in terms of performance and stutter analysis, albeit with individual differences that needs more experimental data. The generated data allow for the comparison of quality metrics that are crucial during this early evaluation stage to define the limitations of these technologies.
Additional experiments including optimized and newly developed STR-MPS kits will be performed to prepare a comprehensive overview on the current STR-MPS kits. Furthermore, a population study including approximately 250 samples will be performed in each laboratory.

Acknowledgements This project has been funded with support from the European Commission (grant **HOME/2014/ISFP/AG/LAWX/4000007135** under the Internal Security Funding Police program of the European Commission-Directorate General Justice and Home Affairs). This publication reflects the views only of the authors, and the European Commission cannot be held responsible for any use which may be made of the information contained herein. The authors would like to thank C. Xavier, C. Strobl, H. Niederstätter, M. Pircher, J. Kilgus, A. König, and C. Hölzl for technical support and helpful discussions. The authors would like to thank Illumina and Thermo Fisher Scientific for support. The authors declare no conflict of interest.