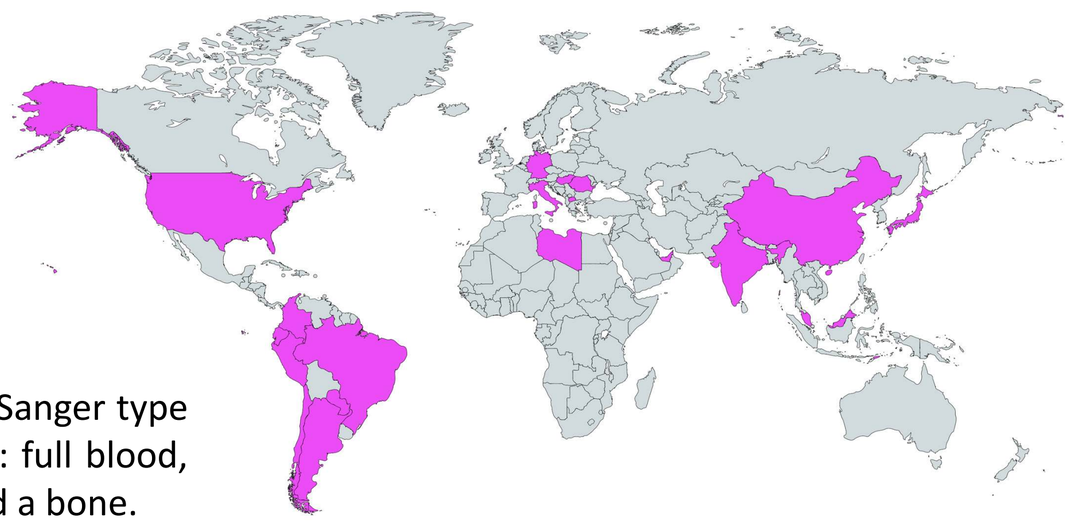
VALIDATION OF THE PRECISION ID MTDNA WHOLE GENOME PANEL IN A WORLDWIDE LINEAGE STUDY

C. Strobl[a], J. Churchill Cihlar[b], R. Lagacé[c], S. Wootton[c], C. Roth[c], N. Huber[a], L. Schnaller[a], B. Zimmermann[a], G. Huber[a], S. L. Hong[d], R. Moura-Neto[e], R. Silva[f], F. Alshamali[g], L. Souto[h], K. Anslinger[i], B. Egyed[j], R. Jankova-Ajanovska[k], A. Casas-Vargas[l], W. Usaquén[l], D. Silva[m], C. Barletta-Carrillo[n], D. H. Tineo[o], C. Vullo[p], R. Würzner[q], C. Xavier[a], L. Gusmão[m], H. Niederstätter[a], M. Bodner[a], B. Budowle[b], W. Parson[a,r]

Christina.Strobl@i-med.ac.at

Introduction

For evaluation of the Precision ID mtDNA Whole Genome Panel more than 500 samples were analyzed from 24 different populations of diverse phylogenetic backgrounds. Different forensically relevant tissues and DNA extraction methods were tested to assess the performance of the assay.



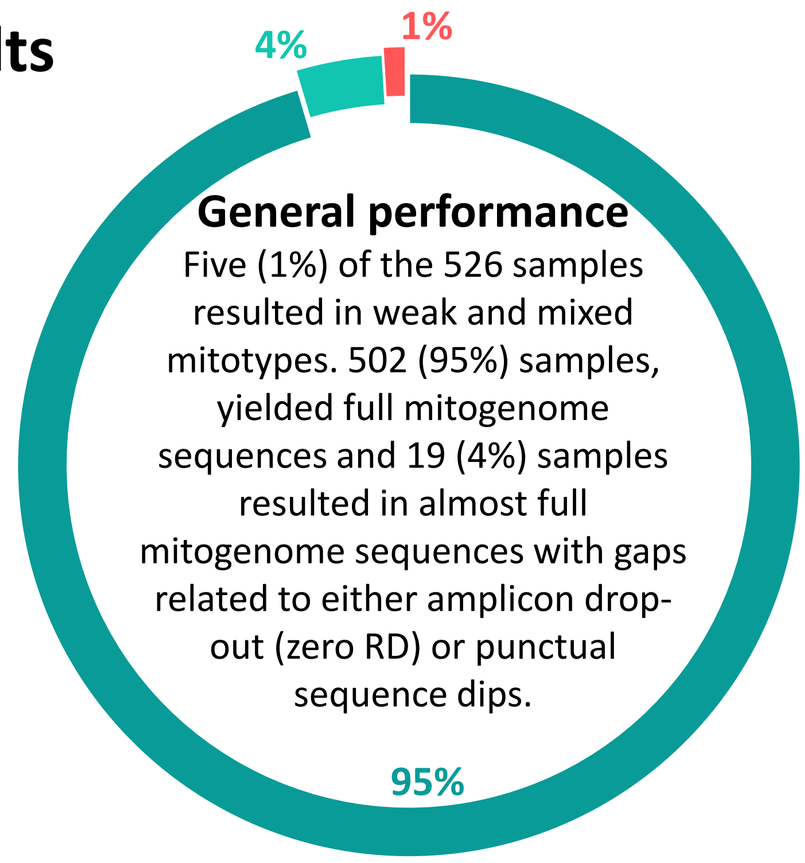
Materials and methods

Samples: 526 in total of which 256 also were analyzed in the Control Region (CR) with Sanger type Sequencing (STS) and 27 whole mitogenomes with Illumina, MiSeq. Samples included: full blood, FTA bloodstain cards, buccal swabs, mouth wash, post-mortem blood, blood plasma and a bone.

Process: sample libraries were processed with the 2-in-1 method using the AmpliSeq Precision ID Library Kit 2.0. Template preparation and sequencing were performed on the Ion Chef and S5 respectively. All samples were sequenced in sets of 32 libraries on 530 chips. A subset of the samples were sequenced in sets of 64 libraries on additional 530 chips.

Analysis: Torrent Suite Software (TSS) v5.2.1. and a customized version of IGV, which represents an earlier version of Converge Software v.2.1.

Results

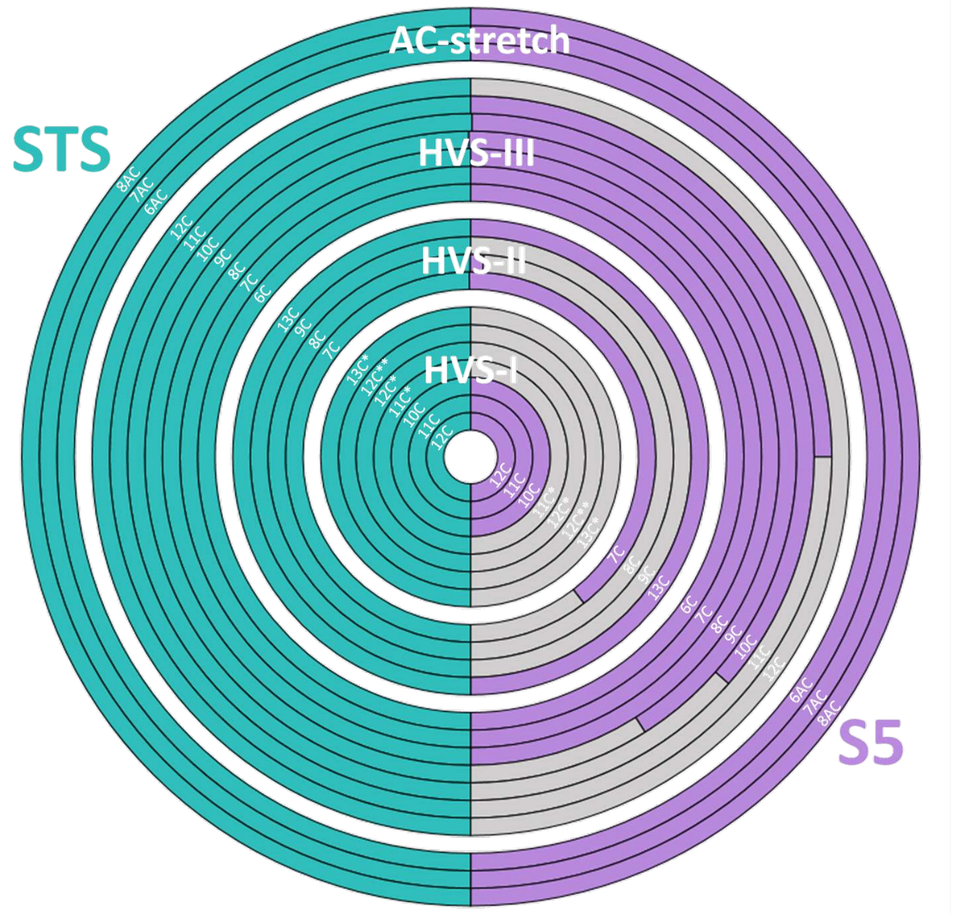


General performance

Five (1%) of the 526 samples resulted in weak and mixed mitotypes. 502 (95%) samples, yielded full mitogenome sequences and 19 (4%) samples resulted in almost full mitogenome sequences with gaps related to either amplicon drop-out (zero RD) or punctual sequence dips.

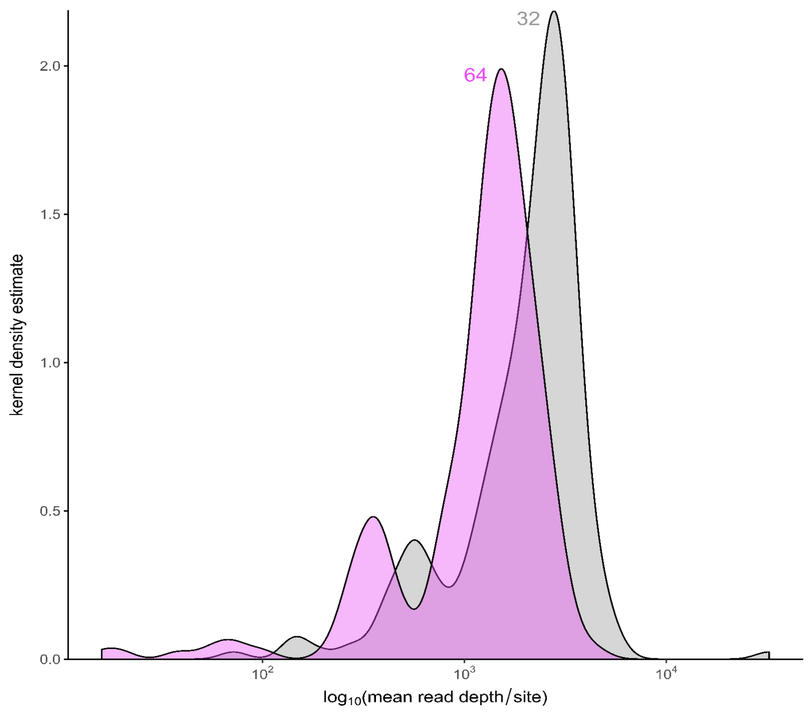
Length heteroplasmy

The MPS technology and its current software algorithm tend to underestimate the number of Cs in polycytosine stretches when more than 7-9 Cs were present, but only in regions where the algorithm was not adapted to the specific sequence context (lower number of Cs represented in grey, see figure below).



Amplicon analyses

On average, pool 1 amplicons yielded significantly higher median RD values than pool 2 amplicons. No statistically significant correlation was observed between median RD and amplicon size for the data set of the two pools.



Point heteroplasmy

The MPS approach proved to be more sensitive than STS. 29 PHPs were found in both datasets. Four of these positions were initially not called with STS and only found at very low levels after repeated examination of the respective positions in the electropherograms.

Read depth

The sequencing runs yielded mean read depth values of 2,353 and 1,417 for 32 and 64 sample batches, respectively. A direct comparison of the same 530 chips loaded with different sample numbers (32 or 64 samples per chip) showed a correlation with sequence information loss when sequencing twice as many samples per chip. See figure on the left.

Discussion

Despite the fact that challenging samples also were included, only a low number of drop-out events was observed. These findings suggest that this assay is a powerful tool in terms of sequence coverage across the entire mtDNA phylogeny and suitable for a broad application in forensic genetics.

Acknowledgements: This study was partly funded by the Tyrolean Science Fund (TWF (UNI-404/1998)) and supported by award no. 2016-DN-BX-K001, awarded by the National Inst. of Justice, Office of Justice Programs, U.S. Dep. of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the U.S. Dep. of Justice.

Affiliations: [a]Inst. of Legal Medicine, Medical Univ. of Innsbruck, Austria; [b]Center for Human Identification, UNT, TX, USA; [c]Human Identification Group, TFS, San Francisco, CA USA; [d]School of Health Sciences, Univ. Sains Malaysia; [e]Lab. de Biologia Molecular Forense, Inst. de Biologia, Univ. Federal do Rio de Janeiro, Brazil; [f]Inst. de Biofisica Carlos Chagas Filho, Univ. Federal do Rio de Janeiro, Brazil; [g]Dubai Police, Gen. Dept. Forensic Science & Criminology, Dubai, UAE; [h]Lab. de Genética Aplicada, Dep. de Biologia, Univ. de Aveiro, Portugal; [i]Inst. of Legal Medicine, Univ. of Munich, Germany; [j]Dep. of Genetics, ELTE Eötvös Loránd Univ., Budapest, Hungary; [k]Inst. of Forensic Medicine, Criminalistic and Med. Deontology, Medical Faculty, Univ. "St. Cyril and Methodius", Skopje, Macedonia; [l]Group of Population Genetics and Identification, Genetics Inst., National Univ. of Colombia, Bogotá, Colombia; [m]DNA Diagnostic Lab. (LDD), State Univ. of Rio de Janeiro (UERJ), Brazil; [n]Lab. de Genética Humana, Univ. Nacional Mayor de San Marcos, Lima, Peru; [o]Univ. Nacional Mayor de San Marcos, Inst. de Medicina Legal del Perú, Lima, Peru; [p]DNA Forensic Lab., Argentinean Forensic Anthropology team (EAAF), Córdoba, Argentina; [q]Division of Hygiene & Med. Microbiology, Medical Univ. of Innsbruck, Austria; [r]Forensic Science Program, The Pennsylvania State Univ., University Park, PA, USA