

serological testing; increased genetic information from diverse sources should enable PCR design that will reliably detect a wide range of strains thus facilitating diagnosis and epidemiological analysis of outbreaks.

A64 Overview of virus metagenomics classification tools

Sam Nooij,^{1,2} Dennis Schmitz,^{1,2} Annelies Kroneman,¹ Harry Vennema,¹ and Marion Koopmans^{1,2}

¹National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands and ²Erasmus University Medical Centre (EMC), Rotterdam, The Netherlands

The use of next-generation sequencing for discovery of viruses has yielded vast amounts of known and putative viral reads. The computational analysis of the reads, however, is quite a challenge, in particular the classification of reads to viral taxa. This is illustrated by the many computational tools that have been devised and new tools that appear monthly. These provide opportunities for other researchers, but the large numbers make it hard for virologists to pick a tool that suits their own study. To facilitate this choice and guide users through the forest of computational pipelines, we have surveyed publications describing fifty tools, inventoried their approaches and scored their methods, user-friendliness, validation, and other performance criteria for diagnostics, outbreak source tracing, virus discovery, and virome profiling. The tools have variable approaches to the classification of viral reads, but they all rely on searching (i.e., homology (44/50 pipelines) and composition search (8/50)) through reference databases (e.g., nucleotide databases, protein databases, or virus-specific databases). Some pipelines include quality control/pre-processing of reads (23/50), filtering non-viral reads (20/50), and de novo assembly (18/50) before the search, and checking and correcting classifications with phylogenetic or statistical methods after the search step (8/50). Furthermore, some are tailor-made for particular studies; others are more generally applicable. Few tools provide a graphical user interface, and when they do they are often online, which increases the ease of use. Reported runtimes vary greatly—from several minutes per sample to days; newer tools are often faster than old ones. Moreover, some tools have been validated in wet-lab experiments or compared to other tools with in silico benchmark tests. The overview of pipelines is presented on the COMPARE website (<https://compare.cbs.dtu.dk/inventory#pipeline>). A decision tree is provided separately to help virologists with any level of bioinformatics expertise select suitable analysis tools. The next step will be to benchmark the most promising tools with the COMPARE and VIROGENESIS projects to better assess their performance for diagnostics and surveillance studies.

A65 Mitochondrial DNA studies of Lisbon immigrants from Portuguese speaking African countries

A. Amorim,^{1,2,3,4} A. Afonso Costa,^{1,5} C. Vieira da Silva,¹ T. Ribeiro,¹ M. J. Porto,¹ N. Taveira,^{3,6} and T. Fernandes⁴

¹Instituto Nacional de Medicina Legal e Ciências Forenses, Portugal, ²Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, ³Instituto Superior de Ciências da Saúde Egas Moniz, Almada, Portugal, ⁴Escola de Ciências e Tecnologia da Universidade de Évora, Évora, Portugal, ⁵Faculdade de Ciências da Saúde da Universidade da Beira Interior, Covilhã, Portugal and ⁶Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal

Since the end of the 1970s, Portugal has had an important role in migratory movements, becoming a destination for immigrants of a wide range of nationalities, mainly from African countries. According to PORDATA, until the end of 2014 there

were ~40,000 immigrants from Cape Verde, 20,000 from Angola, 18,000 from Guinea-Bissau, and 3,000 from Mozambique living in Portugal, and of those, >80 per cent live in the Lisbon region. This may be one of the main contributors to genetic variation of Lisbon residents in the present and the future. Mitochondrial DNA (mtDNA) has features that make it desirable for forensics, namely, high copy number, lack of recombination, and matrilineal inheritance. These features are also important in evolutionary and population studies. We aim to characterize mtDNA diversity in immigrants from Portuguese Speaking African Countries (PALOP) living in Lisbon and their potential contribution to genetic variation of Lisbon population. Blood samples were collected from 439 PALOP immigrants living in Lisbon, of which 173 immigrants from Angola, 103 immigrants from Cape Verde, eighty-three immigrants from Mozambique and eighty immigrants from Guinea-Bissau, from January 2000 to December 2016. The control region of the mtDNA was amplified using two pairs of primers—L15971/H016 and L16555/H639, and sequenced by BigDye Terminator v.3.1 Cycle Sequence (AB). Sequenced products were detected in a sequencer Genetic Analyzer 3130 (AB). Finally the results were analysed by Sequencing Analysis v.5.2 software and also compared with Revised Cambridge Reference Sequence (rCRS) using SeqScape v.3 (AB) software. The haplogroups were determined based on Phylotree, build 17. Genetic distances and other genetic parameters were calculated with Arlequin software ver.3.5 and analysed and represented with PhyML 3.0. For each sample, the complete sequence of the control region was obtained. The comparison of the sequences obtained with the rCRS, among the 439 analysed individuals, allowed the identification of 319 different haplotypes, corresponding to 164 different haplogroups distributed by ten macrohaplogroups. Macrohaplogroup L was the most common with 386 haplotypes followed by U with fifteen haplotypes, H with twelve haplotypes, M and T with six haplotypes, K with five, R with four, X and J with two and HV with one. PALOP's immigrants presented a high number of unique haplotypes, most of them belonging to macrohaplogroup L, originating from sub-Saharan regions of Africa. This macrohaplogroup is uncommon in European and Portuguese populations. Consistent with this, phylogenetic analysis showed the establishment of two distinct groups, one composed of the Portuguese population and another of the African populations. In comparing the different immigrant populations living in Lisbon, the genetically closest community to the Portuguese population is Mozambique and the furthest is Cape Verde, followed by Guinea-Bissau and Angola. Our results show that the PALOP immigrants living in Lisbon are genetically heterogeneous. The increase in genetic diversity in Lisbon due to immigrants from PALOP countries may have a major impact on haplotypic and allelic frequencies, on which all forensic and medico-legal investigations are based.

A66 Multi-drug-resistant *Klebsiella pneumoniae* strains circulating in hospital setting: Whole-genome sequencing and Bayesian phylogenetic analysis for outbreak investigations

E. Cella,^{1,2} M. Ciccozzi,^{1,3} M. Fogolari,¹ T. Azarian,⁴ M. Prosperi,⁵ G. Dicuonzo,¹ M. Salemi,⁶ and S. Angeletti¹

¹Unit of Clinical Pathology and Microbiology, University Campus Bio-Medico of Rome, Italy, ²Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy, ³Department of Infectious Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy, ⁴Department of Epidemiology, Center for Communicable Disease Dynamics, Harvard's T.H. Chan School of Public Health, Boston, MA, ⁵Department of Epidemiology, University of Florida, Gainesville, FL, USA and ⁶Department of Pathology, Immunology, and