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Shotgun metagenomics as a tool for the rapid diagnosis and genotyping of dengue

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Background: Dengue virus (DENV) diagnosis can be performed by serological tests, isolation of the virus or through molecular methods. Especially, *q*RT-PCR and nested RT-PCR are widely used to detect DENV during the acute phase of illness. For genotyping, Sanger sequencing is an often used method that is time consuming and not suitable for the initial diagnosis of the virus. Therefore, new methodologies are required to improve rapid detection and genotyping of DENV (and other viruses) directly from clinical material. In this pilot study the use of shotgun metagenomics and sequence classification methods to identify and type DENV directly from sera and plasma samples was evaluated.

Material/methods: From five DENV-2 RT-PCR positive patients' samples from Venezuela (2015) viral RNA was isolated using the QIAmp® Viral RNA kit (Qiagen). cDNA libraries were prepared with TruSeq RNA v2 library prep kit (Illumina) and sequenced on a MiSeq instrument (Illumina) using a MiSeq Reagent Kit v2 (300-cycles, paired-end). The data was first analysed with Taxonomer (IDbyDNA), an ultrafast web-tool for comprehensive metagenomics data investigation. Subsequently, the raw reads were mapped against a human genome (hg18) and *de novo* assembled using CLC Genomics Workbench v9.5.4. Next, a BLAST analysis of the longest contig was performed. The phylogenetic analysis was performed in MEGA v7.0 using the maximum likelihood method, based on the general time reversible model, with a robustness of 1000 bootstrap replications.

Results: The mean total number of reads for the five samples was 3,145,628. Taxonomer could correctly identify DENV type 2 in all samples. About 85.23% of the reads were mapped against the human genome, while the proportion of reads that matched DENV were 4.02%. After *de novo* assembly, sequences with an average length of 10,700 bp and an average coverage of 1651.15 reads were obtained. Using BLAST, the closest strains identified were DENV 2 strains (99% identity) isolated in Venezuela between 2005-2007. Phylogenetic analysis showed the sequenced strains were closely related to strains from previous epidemic episodes in Venezuela (Figure 1).

Conclusions: Dengue identification and genotyping was possible directly from the patients' plasma. The whole workflow was performed in 3 days, which is approximately 2 times faster than what is needed for classical genotyping through standard isolation and identification (Peeling et al., 2010). A superior resolution on the dynamics of DENV epidemics is given by the phylogenetic analyses of the complete genome sequences, compared to partial genome sequencing. Furthermore, this approach also enables the detection and discrimination of other viruses causing similar clinical presentations, namely chikungunya and Zika.

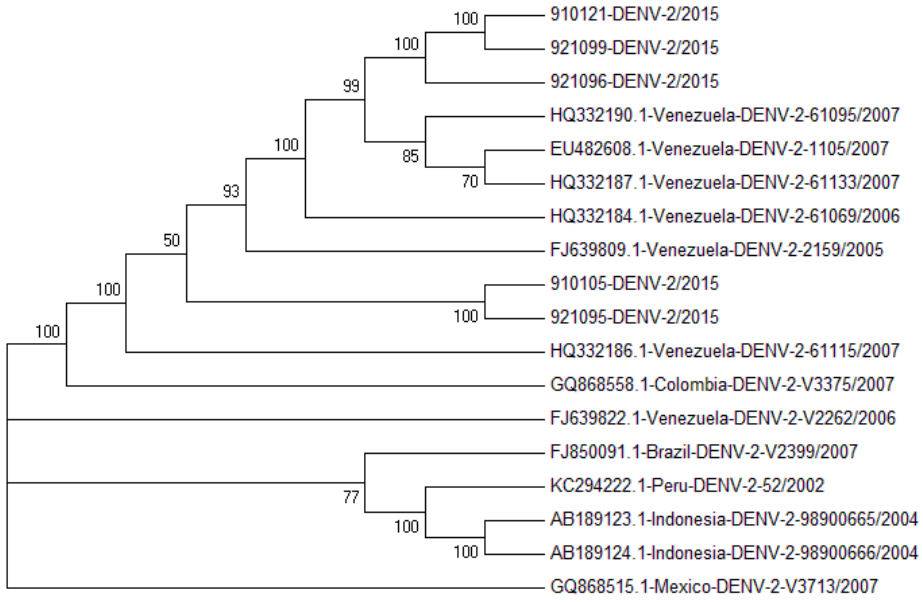

Figure 1. Molecular Phylogenetic analysis by Maximum Likelihood method. Maximum-likelihood phylogenetic tree constructed under the GTR + Γ model using software MEGA7