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Analysis of the dynamic transcriptome of a herpesvirus using full-length single molecule sequencing

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Full-length RNA sequencing is a powerful tool in identifying novel transcripts and isoforms. In this study we have shown the quantitative analysis of the dynamic transcriptome of the Pseudorabies virus (PRV). Poly(A)$^+$ RNA fraction was purified from PK-15 cells infected with PRV after 1-2-4-6-8-12 hours infection and was converted to dscDNA. SMRTbell libraries were prepared following the very low (10 ng) input protocol and then were sequenced on PacBio RSII single-molecule real-time (SMRT) platform. Raw reads were mapped to the PRV reference genome (KJ717942.1) using the BLASR and GMAP aligners. SMRT cells yielded 54,467 viral reads with a mean read length of 1,287 nucleotides and the majority of the PRV transcript isoforms was represented in each sample. The kinetics of the transcripts was characterized by the changes in the relative amounts of reads aligning to them in the different samples. Read counts were also normalized to the number of reads aligning to the *Sus scrofa* mitochondrial genome to show the overall increase in the relative copy number of PRV reads in the later stages of infection. Normalization by the changes of the relative amounts of viral DNA in our samples showed a drastic drop of the viral gene expression after 4 hours post infection. Our results are mostly concordant with previous kinetic characterizations of PRV transcripts using qRT-PCR analysis with the distinction that the latter one cannot distinguish between the transcript isoforms, while SMRT sequencing can. Our study shows that data from long-read sequencing can be used for quantitative analysis of transcripts.

Biography

Dora Tombacz has completed her MSc in Biology in 2006 and PhD in Medical Sciences from the University of Szeged, Hungary in 2010. She is working in the Department of Medical Biology as an Assistant Professor at the Faculty of Medicine at University of Szeged in the Boldogkoi's group. Her primary field of interest is the analysis of herpesvirus gene expression and utilization of herpesviruses as tools in various fields of biology including neurobiology and cardiology. She is currently working with next and 3rd generation sequencing techniques, focusing on virology and genomics of human diseases at the University of Szeged, Hungary and the Stanford University, USA.

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