Infection by flaviviruses such as dengue virus serotypes (DENV 1–4), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBE), yellow fever virus (YFV), and West Nile virus (WNV) impacts millions of lives and causes tens of thousands of mortalities each year. Recent studies on global dengue burden indicated that there are at least 100 million human symptomatic infections annually. This original estimate has recently been revised in 2013 to about three times higher than the dengue burden estimate of the World Health Organization. The urban-breeding *Aedes aegypti* mosquito has spread the DENV to more than 100 countries around the world and ~50% of the world’s population is now estimated to be at risk. Dengue is a global public health emergency especially since there is no preventative vaccine or antiviral treatment for dengue disease. Usually, infection with any one of the four DENV serotypes leads to mild self-limiting dengue fever (DF) with lifelong immunity to that specific serotype. Epidemiological evidence suggests that 90% of the severe and potentially fatal dengue diseases, dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS) occur during secondary heterotypic infections where the protective antibodies from a previous infection become pathogenic through the Antibody Dependent Enhancement (ADE) phenomenon. The co-circulation of multiple serotypes in dengue epidemic countries increases the risk of severe dengue diseases due to ADE. Dengue has also reappeared in the United States of America: the combination of a low immunity in the population, increased mosquito vector activity, and the continuous introduction of virus from the endemic countries forms the right ingredient for explosive epidemics.

This edition of methods and protocols for dengue research is aimed at providing the increasing number of dengue researchers a one-stop protocol book contributed by some of the leading laboratories working on dengue. Chapters on dengue virus isolation from clinical samples, quantification of human antibodies against the virus, and assays to quantify the virus particles are included. The widely used mouse model to study dengue pathogenesis, vaccine, and antiviral efficacies is also described. New technologies to study the conformation of *cis*-acting elements in dengue viral RNA genome that contribute to its function in translation and replication by novel computational and experimental methods are described in this book for the first time. The dynamic dengue RNA molecule from its initial biogenesis to its final most stable conformation through multiple intermediate folding pathways is analyzed by the predictive Massively Parallel Genetic Algorithm (MPGAfold) with frequencies of occurrence of each stage. Selective 2’-Hydroxyl Acylation analyzed by Primer Extension (SHAPE) analyzes the conformation of RNA experimentally. High-throughput SHAPE combines a novel chemical probing technology with reverse transcription, capillary electrophoresis, and secondary structure prediction software to determine RNA structure at a single nucleotide resolution. Next Generation Sequencing methodologies described here utilize high-throughput and massively parallel sequencing to track the viral genomes constantly changing under selective pressure imposed by environment. Cutting-edge cryo-electron microscopy technology reveals how the viruses also change their surface morphologies when they are subjected to environmental conditions under which the viruses...
grow and replicate their genomes. Moreover, the three-dimensional structures of the viral proteins are important for their function. One of the modern methods to achieve this objective, Small Angle X-ray Scattering (SAXS), is described here. Reverse genetic systems for different dengue virus serotypes to study viral replication using different reporter systems and virus-like particles to study viral entry, replication, and assembly are also described. The viral RNA codes for a number of enzymes that are important for its replication. Methods are described to measure quantitatively the various enzyme activities that are useful to screen for antivirals. Genome-wide screening methods and discovery of human and insect host cell proteins that are involved in virus life cycle are also included. The book contains 24 chapters, and we sincerely hope that the protocols contributed by the authors will form a valuable resource for dengue researchers.

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