
Preface

The International Committee on Taxonomy of Viruses (ICTV) classifies RNA viruses as those that belong to Group III, Group IV, or Group V of the Baltimore classification system and contain ribonucleic acid (RNA) as genetic material throughout their entire life cycle. Group III includes double-stranded RNA viruses (dsRNAs), whereas Groups IV and V contain single-stranded RNA viruses (ssRNAs) of positive and negative polarity, respectively. Positive sense RNA viruses (+ssRNAs) are those in which the RNA itself is translated by the host cell translation machinery and initiates an infectious cycle *de novo*. In contrast, negative sense RNA viruses (–ssRNAs) cannot be translated directly and require copying of the negative sense RNA into a positive sense RNA strand before the infection can proceed.

In biology, the term “forward genetics” is used to define an approach that seeks to find the genetic basis of a phenotype or trait. Forward genetics of RNA viruses implies imposing them to various stress conditions and then defining the genetic changes that occurred in the process. The term “reverse genetics” is an approach to unravel the function of a gene by establishing and analyzing the phenotypic effects of (artificially) engineered gene sequences. In case of RNA viruses, reverse genetics invariably requires the *de novo* reconstitution of the virus from a cDNA copy. Using molecular biology, cDNA copies of RNA viruses are cloned into a variety of vectors, most typically and in order of preference, plasmids, bacterial artificial chromosomes or bacmids, or recombinant viral vectors. The ability to further manipulate DNA elements encoding portions or entire cDNA copies of RNA viruses has revolutionized the manner in which these viruses can be studied and understood. Thanks to reverse genetics, it is possible to better define the molecular mechanisms that modulate pathogenesis, transmission, and host range of RNA viruses, to study virus evolution, receptor binding characteristics, virus entry, replication, assembly, and budding. Reverse genetics allows the development of novel vaccine strategies and to better test and/or develop alternative intervention strategies such as novel antivirals. Perhaps the initial perception is to think that reverse genetics of dsRNAs and +ssRNAs is easier than –ssRNAs; however, genome size, secondary RNA structures, genome segmentation, cryptic signal sequences, among other issues, make reverse genetics of all kinds of RNA viruses equally challenging.

This book *Reverse Genetics of RNA Viruses: Methods and Protocols* is a compilation of 16 chapters summarizing reverse genetics breakthroughs and detailed reverse genetics protocols. The book does not cover every reverse genetics protocol for every RNA virus. Instead, it does provide comprehensive protocols for those RNA viruses that were initially the most challenging to obtain and/or that were developed most recently. This book, of course, would not have been possible without the outstanding and most generous contributions of our authors who are leaders in their respective fields and that have shared their insights and step-by-step protocols to help you, our colleagues, with your own research endeavors. I hope you find this book helpful.

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Methods and Protocols

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