
Preface

Retroviruses are a large and diverse family of enveloped, single-strand RNA viruses characterized by the unique replicative strategy that includes reverse transcription of the virion RNA into linear double-stranded DNA and the subsequent integration of this DNA into the genome of the cells. Human retroviruses are subdivided into three groups: oncoviruses, lentiviruses, and spumaviruses. Oncoviruses have been associated with a variety of cancers. The first human retrovirus, human T-lymphotropic virus type 1 (HTLV-1), was discovered in the late 1970s, and was shown to cause adult T-cell leukemia and chronic neurological conditions. A relative of HTLV-1, HTLV-II, has also been associated with human leukemias. The most significant disease resulting from human retrovirus infection is the acquired immunodeficiency syndrome (AIDS), which is caused by lentiviruses, human immunodeficiency viruses type 1 and type 2 (HIV-1 and HIV-2). Spumaviruses cause no known disease in humans and interest in this category is relatively recent.

Although there are differences in the genetic composition of different types of retroviruses, all carry three basic coding genes in the same order: *gag*, encoding internal structural proteins that form the matrix, the capsid, and the nucleoprotein structures; *pol*, encoding the reverse transcriptase and integrase enzymes; and *env*, encoding the surface and transmembrane components of the viral envelope protein. These three genes and their coded proteins are the major focus of studies on the virology, serology, and molecular biology of human retroviruses.

In the last two decades, research on human retroviruses has been progressing at a rapid pace. Not surprisingly, recent studies of human retroviruses have focused largely on HIV-1 and HIV-2. Therefore, most of our present knowledge of human retroviruses is derived from studies on HIV-1. In addition, studies on HIV-1 have significantly advanced our understanding of biology and development of new biotechnologies. The prospect of acquiring a broad view of a field with so many viruses and associated technologies may seem daunting. In hopes of attenuating such concerns, we have organized *Human Retrovirus Protocols: Virology and Molecular Biology* by focusing on methodologies of the virology and molecular biology of human retroviruses. The first of two sections primarily explores methods for the isolation and detection of human retroviruses; the second looks at the interplay between the viruses and the host, focusing on the phenotype of human retroviruses. This division is not intended to be rigid or absolute; the PCR-based assays in the first section are typically molecular techniques.

Part I begins with a chapter on the quantitative isolation of HIV-1 from latently infected resting T-cells from the laboratory that developed this revolutionary technique. Nine chapters follow on methods for the isolation and propagation of HIV, HTLV, and foamy virus from the peripheral blood mononuclear cells (PBMC), blood monocytes, brain tissues, cerebrospinal fluids, semen, vagina, and lymph nodes. The succeeding chapters constitute protocols for detection and quantification of different retrovirus genes, antigens, and antibodies, for which we have placed greater emphasis on “universal” assays for detection of different subtypes of HIV-1 and HIV-2.

Part II begins with biological assays for determining cell tropism of HIV-1 from the laboratory that initially developed the techniques. The next two chapters describe “popular” assays for the determination of co-receptor usage of HIV-1. Three chapters follow on assays recently developed in each author’s laboratory for phenotyping HIV-1 infected monocytes and examining HIV-1 fitness with an effective cloning system. The next five chapters are on cloning, construction, and characterization of full-length HIV-1, HIV-2, HTLV-II, and spumaretrovirus. A chapter for assessing drug efficacy follows. *Human Retrovirus Protocols: Virology and Molecular Biology* concludes with two chapters describing new technologies for determining human gene expression with HIV-1 infection by microarrays and assessing genetic polymorphisms in two recently identified HIV-1 co-factors, DC-SIGN and DC-SIGNR.

All chapters offer the detailed steps necessary to carry out the assays, and provide discussion of problems and pitfalls that may be encountered. Most of these protocols can be applied directly, or can be adapted easily.

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