Description of tetracistronic trVLP systems available on EVAg

Tetracistronic transcription and replication-competent virus-like particle (trVLP) systems for filoviruses allow modelling of almost every aspect of the virus life cycle without the need for a maximum containment laboratory. These systems are based on minigenomes, i.e. miniature versions of the viral genome, in which viral genes have been removed and replaced with a reporter gene, e.g. luciferase or GFP. Terminal non-coding regions (so called leader- and trailer-regions) are retained and carry important signals for the viral polymerase to recognize minigenomes as authentic templates for viral RNA synthesis (i.e. genome replication and transcription). Besides a reporter gene, tetracistronic minigenomes contain also the viral genes for VP40, $GP_{1,2}$, and VP24, which are required for trVLP formation, entry of trVLPs into target cells, and incorporation of minigenomes into trVLPs, respectively.

Minigenome-encoding plasmids are transfected into so-called p0 (or producer) cells together with expression plasmids for the viral proteins required for viral RNA synthesis (i.e. the ribonucleoprotein complex (RNP) proteins NP, VP35, VP30, and L), and expression plasmids for accessory proteins (i.e. the DNA-dependent RNA polymerase T7, and a firefly luciferase to control for transfection efficiency and effects on general cell health and plasmid-driven gene expression). The minigenome is initially transcribed by the DNA-dependent RNA polymerase into a minigenome RNA, which is then recognized by the viral RNP proteins, and replicated and transcribed into mRNAs. These mRNAs result in reporter activity in p0 cells reflecting viral RNA synthesis and protein expression. Further, they result in expression of VP40, $GP_{1,2}$, and VP24, leading to the formation of trVLPs that incorporate minigenomes. These trVLPs can be harvested and used to infect p1 (target) cells. Usually, these target cells have been pretransfected with expression plasmids for the RNP proteins as well as the accessory Tim1 protein, which serves as an attachment factor for filoviruses. After infection trVLPs will enter the p1 cells and deliver their minigenome, which is then recognized by the RNP proteins, resulting in reporter activity in these cells as well as the production of new trVLPs, which can be used to infect another generation of target cells (i.e. p2 cells). Reporter activity in p1 cells is dependent on viral RNA synthesis and protein expression as well as trVLP budding in p0 cells, and trVLP entry as well as viral RNA synthesis and protein expression in p1 cells.

trVLP systems are particularly well suited for testing of antivirals or neutralizing antibodies, as well as for studies of proviral host factors and studies addressing the basic molecular biology of filoviruses (especially for reverse genetics-driven approaches). Due to limitations inherent in the systems (i.e. the need for overexpression of viral RNP proteins and limited efficiency of the system in comparison to viral infections), imaging studies (e.g. electron microscopy studies of trVLPs or microscopy studies in infected cells) and studies of filovirus interactions with the innate immune system are more challenging.

Chimeric tetracistronic trVLP systems featuring glycoproteins from various filovirus species (e.g. Sudan virus, Reston virus, Marburg virus, or Lloviu virus) allow studying of the entry process of these viruses, as well as testing of neutralizing antibodies against these viruses. Tetracistronic minigenomes lacking the glycoprotein open reading frame can be used as negative controls, or to insert the open reading frames of glycoproteins of interest into these minigenomes using two BsmBI restriction sites.

The different reporters (e.g. Renilla luciferase, Nano luciferase, and eGFP) all have advantages and disadvantages regarding their use. In general, luciferase reporters allow easy and precise quantification of reporter activity, but do not provide information about the number of infected cells, or allow identification of individual cells that are infected. Nano luciferase provides significantly stronger signals than Renilla luciferase, and is the reporter of choice for applications where very low infection rates are to be expected, or where primary transcription in naïve target cells (i.e. cells that have not been pretransfected to express the RNP proteins) has to be assessed. However, Nano

luciferase is unspecifically secreted into the supernatant by p0 cells, and again taken up by p1 cells, so that there is a higher risk for unspecific background than when using Renilla luciferase. This background signal can be reduced or eliminated by purifying trVLP preparation via centrifugation through a sucrose cushion prior to infection. eGFP is the reporter of choice when individual cells that are infected with trVLPs have to be identified, or when the number of infected cells has to be determined. Precise quantification of the overall infection efficiency is more challenging, but can be achieved by flow cytometry.

Tetracistronic trVLP systems continue to be improved by the Laboratory for Molecular Biology of Filoviruses of the Friedrich-Loeffler-Institut (FLI), and are distributed through the European Virus Archive (EVAg). New versions of these systems might become available on EVAg from time to time.

For further information on trVLP systems and their use please contact the head of the Laboratory for Molecular Biology of Filoviruses, Dr. Thomas Hoenen (https://www.fli.de/en/institutes/institute-of-molecular-virology-and-cell-biology-imvz/scientists/dr-t-hoenen/).

For further information about obtaining the trVLP system please see EVAg (https://www.european-virus-archive.com/).

Systems available through EVAg:

order number	system species	glycoprotein species	reporter protein
022N-03908	Ebola virus	Ebola virus	Renilla luciferase
022N-03909	Ebola virus	Ebola virus	Nano luciferase
022N-03910	Ebola virus	Ebola virus	eGFP
022N-03911	Ebola virus	Sudan virus	Renilla luciferase
022N-03912	Ebola virus	Sudan virus	Nano luciferase
022N-03913	Ebola virus	Reston virus	Renilla luciferase
022N-03914	Ebola virus	Reston virus	Nano luciferase
022N-03915	Ebola virus	Marburg virus	Renilla luciferase
022N-03916	Ebola virus	Marburg virus	Nano luciferase
022N-03917	Ebola virus	Lloviu virus	Renilla luciferase
022N-03918	Ebola virus	Lloviu virus	Nano luciferase
022N-03919	Ebola virus	no glycoprotein	Renilla luciferase
022N-03920	Ebola virus	no glycoprotein	Nano luciferase

Recommended literature:

https://www.ncbi.nlm.nih.gov/pubmed/21699921: general review on reverse genetic systems for filoviruses

https://www.ncbi.nlm.nih.gov/pubmed/24965473: first description of a tetracistronic trVLP system

https://www.ncbi.nlm.nih.gov/pubmed/25285674: detailed instructions on the use of tetracistronic trVLP systems, including an instructional video

https://www.ncbi.nlm.nih.gov/pubmed/30053054: description of optimized protocols and of an eGFP-encoding tetracistronic trVLP system