# Fluorescent Semliki Forest Virus Proteins: Construction, Expression and Location within Cells

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Alphaviruses are a fascinating group of RNA viruses that belong to the Togaviridae family. Some cause severe diseases, while others have useful attributes as gene expression vectors. In some alphaviruses, however, the second non-structural protein (nsP2) has cytotoxic properties, as it shuts down the translation systems of infected cells (Garmashova et al. 2007). NsP2 increases the severity of diseases and limits the use of alphaviral expression vectors. Therefore, it is important to find ways to reduce the cytotoxicity of nsP2, for example by inhibiting its nuclear localization. The aim of my project was to construct a tool based on the Semliki Forest Virus (SFV), which can be used to study the effects of various factors on the localization of nsP2 within cells. I constructed two plasmids coding for the wild-type SFV nsP2 protein and a mutant nsP2, both fused with a fluorescent EGFP tag. The promoters in my plasmids can be used to produce a large amount of nsP2 in certain cell lines, in others the tag increases the visibility of the protein. Different compounds can then be added to media see if they affect the localization of nsP2. The mutant nsP2 does not localize in the nucleus and has been shown to be less cytotoxic to cells, so it can be used as a control. I tested the effectiveness of the tool on many levels. Both of the plasmids functioned as expected, having correct sequences and producing enough proteins to be visualized and to localize as their non-EGFP-fused counterparts. I conclude that the constructed plasmids can be used to study the effects of various factors on the subcellular localization of nsP2. These could potentially be used to find effective treatments against some alphaviral infections or simplify the use of alphaviral gene expression systems.