Design and implementation of rotavirus reverse genetics systems expressing fluorescent reporter proteins

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Plasmid-only based reverse genetics (RG) is a powerful tool in the study of viruses where cDNA is used to generate rationally designed viable viruses. At NWU we have implemented two rotavirus reverse genetics systems Kanai *et al.* (2017. PNAS, 114:2349-2354), and our own. Our system uses an immunofluorescence microscopy assay to confirm viral rescue and takes 7-10 days.

The use of zsGreen1 reporter for tracking rotavirus viral growth after rescue has been implemented by Kanai *et al.* (2018. JVI.01774-18) and Komoto *et al.* (2018. JVI.00588-18). This allows tracking of viral growth in the initial stages without the need for fixation and visualisation. In Yuta Kanai's construct, the start codons before the fluorescent protein are deactivated and a segment of the 3' end of GS5 (NSP1) was deleted. Satoshi Komoto's construct expressed the N-terminal region of NSP1 and the fluorescent protein which is separated by a self cleaving peptide (P2A), the C-terminal region of NSP1 is not expressed.

We designed and generated a chimeric GS5, coding for a non-functional NSP1 protein and the reporter protein based on the both systems. The design of the construct expressing zsGreen1 is identical to the Kanai construct, but based on our SA11-N5 consensus sequence. The other construct was designed to express the N-terminal NSP1 and the fluorescent protein (Fresno) was separated by P2A, a deletion in the 3' region of GS5 was made to help improve stability and packaging. The 371 nucleotides at the end of GS5 was retained to preserve packaging signals.

The use of fluorescent proteins allows for the rapid detection of rescued viruses as early as 24hrs after transfection. The expression of the reporter proteins remains constant after several passages and thus the chimeric genome segment is stable. Quick evaluation of viral rescue allows for the timely termination of experiments as needed, saving time and money. Reporter proteins will speed up future research towards rationally designed chimeric rotaviruses carrying protective VP4/VP7 proteins of African strains.