# Local implementation and optimization of plasmid-only rotavirus reverse genetics systems

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The use of reverse genetics (RG) to generate recombinant viruses from cDNA has revolutionized virology and remains the most definitive way to study the functions of viral genome elements to date. The applications of a robust RG system range from gene function assays to the generation of rationally designed vaccine candidates to the identification and development of viral treatment targets and strategies. The local development and implementation of the dsRNA virus RG systems for bluetongue virus (BTV) and African horsesickness virus (AHSV) has already delivered a wealth of new information in terms of immunogenicity, viral replication and recombinant vaccine development.

Until very recently the only RG systems available for rotaviruses (RV) were helper-virus based systems that allowed the exchange of a single genome segment at a time. These systems relied on the capacity of RV to undergo reassortment during viral replication and were notoriously dependent on a strong selection system to distinguish between unaltered helper virus and recombinant viral progeny. These type of systems have now been superseded by a plasmid-based RV RG system (Kanai, Y., *et al*. 2017. PNAS, 114:2349-2354) that is independent of any selection system and allows the generation of entirely recombinant RV from cDNA. This new pT7_SA11-L2 RV RG system was made commercially available in 2017 and was purchased by various institutions, including our laboratory at the NWU.

We implemented the pT7_SA11-L2 RV RG system alongside our own locally developed, consensus sequence, plasmid-based RV SA11-N5 RG system. We improved both systems based on our experience with the dsRNA virus RG systems for BTV and AHSV. Through implementation of various modifications we could recover recombinant virus from both systems by improving the overall viral yield and repeatability of both systems. These optimized RG systems now form the basis for further research into RV replication, reassortment and the generation of rationally designed and regionally specific RV vaccine candidates using the non-pathogenic SA11 as backbone.