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## ACCEPTED MANUSCRIPT

Comparison of the Efficiency of Different Newcastle Disease Virus Reverse Genetics Systems

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### Highlights

- Six NDV reverse genetics including different numbers of plasmids were established.
- Fewer plasmids did not always induce higher viral rescuing efficiency.
- Only the 2-plasmid system improved efficiency compared to the traditional system.

#### **ABSTRACT**

Rescue of negative-sense single-stranded RNA viruses ((-)ssRNA virus), generally requires the handling of a large number of plasmids to provide the virus genome and essential components for gene expression and genome replication. This constraint probably renders reverse genetics of (-)ssRNA virus more complex and less efficient. Some authors have shown that the fewer the plasmids, the more efficient reverse genetics is for segmented RNA virus. However, it is not clear if the same applies for (-)ssRNA, such as Newcastle disease virus (NDV). To address this issue, six variants of NDV reverse genetic systems were established by cloning combinations of NP, P and L genes, mini-genome or full-genome in 4, 3, 2 and 1 plasmid. In terms of mini-genome and full-genome rescue, we showed that only the 2-plasmid system, assembling three support plasmids together, was able to improve the rescue efficiency over that of the conventional 4-plasmid system. These results may help establish and/or improve reverse genetics for other mononegaviruses.

**Keywords:** Newcastle disease virus; reverse genetics; higher rescuing efficiency.

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