

## **P2 2: Two-Plasmid System to Increase the Rescue Efficiency of Paramyxoviruses by Reverse Genetics: the example of rescuing Newcastle Disease Virus**

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Within paramyxoviruses, conventional reverse genetics require the transfection of a minimum of four plasmids: three to reconstruct the viral polymerase complex that replicates and expresses the virus genome delivered by a fourth plasmid. The successful transfection of four or more plasmids of different sizes into one cell and the subsequent generation of at least one viable and replicable viral particle is a rare event, which explains the low rescue efficiency, especially of low virulent viruses with reduced replication efficiency in cell lines. In this study, we report on an improved reverse genetics system developed for an avian paramyxovirus, Newcastle Disease Virus (NDV), in which the number of plasmids was reduced from four to two. Compared to the conventional method, the 2-plasmid system enables earlier and increased production of rescued viruses and, in addition, makes it possible to rescue viruses that it was not possible to rescue using the 4-plasmid system.