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Development of an improved recombinant NDV Clone 30

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Newcastle Disease Virus belongs to the genus *Avulavirus* within the family *Paramyxoviridae*, subfamily *Paramyxovirinae*. Virulent NDV strains cause significant morbidity and mortality of unvaccinated poultry. Vaccination against NDV is widely practised using live virus vaccines applicable by spray or drinking water. NDV Clone 30, an attenuated lentogenic vaccine strain, was the basis for the rescue of our first recombinant NDV (rNDV) by reverse genetics. Compared to wild type NDV Clone 30, recombinant NDV Clone 30 (GenBank accession number Y 18898) was even further attenuated as determined by intracerebral pathogenicity index and mean death time. In order to detect mutations responsible for this phenotype in the NDV Clone 30 genome, two Genome Sequencer (GS) FLX libraries were prepared from RNA extracted from purified virions and allantoic fluid, respectively, and sequenced. Mapping the reads along the reference sequence identified nine nucleotide exchanges, four of them resulting in an amino acid alteration, one in the P protein, one in the F protein and two in the L protein. Based on these results, new plasmid-cloned full-length NDV genomes were constructed by mutation of the indicated nucleotides and used for virus rescue. Resulting recombinant rNDVGu which exhibits all nine nucleotide alterations was characterized by a slight increase in pathogenicity.