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DIRECT SORTING OF RECOMBINANT MVA BY FLOW VIROMETRY

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In the field of non-replicating virus-vectored vaccines, recombinant Modified Vaccinia Ankara (rMVA) production is a crucial translational technology for both infectious diseases and cancer. Methods based on cell sorting, although better than all other published methods, are still complex and time consuming, since involve the selection of cells infected by recombinant constructs, virus lysates production, multiple rounds of infections and sorting and of virus terminal dilution in order to produce recombinant untagged rMVAs. In this work, we have moved the selection step from the infected cells to the virions themselves, tagging the surface of recombinant virions by a fluorescent protein which allows direct sorting by a high-resolution flow cytometer and an appropriately modified protocol. Thus, the term “flow virometry” defines virus analysis and subsequent sorting. Advances in sorting technology allow the distinction between fluorescent and non-fluorescent micro-vesicles down to 100 nm in diameter and thus the direct sorting of large viruses, such as vaccinia viruses, distinguishing fluorescent from non-fluorescent virions. To exploit this opportunity, the gene of a fluorescent fusion protein (Flu hemagglutinin-EGFP) is associated to the transgene in the DNA construct that leads to the formation of recombinant virus. The fluorescent protein is integrated in the external membrane of the extracellular MVA virions (EEVs) so that the fluorescent sorted virions would also carry the transgene. A second (programmed) recombination event eliminates the DNA of the fluorescent fusion protein and untagged rMVA can be sorted by flow virometry and cloned by terminal dilution. In summary, two rounds of virus sorting separated by a single round of infection lead to the production of untagged rMVA, whose genotype and phenotype can be easily analyzed by PCR and Western blot analysis. Compared to our previous method based on the sorting of infected cells, the flow virometry method is more reliable and reproducible and allows to save time and labor. In practice, the schedule is cut down from 24 working days (and 57 hours of bench work) to 13 working days (and 38 hours of bench work). Morover, cutting down the number of cell cultures involved in the process, a larger number of recombinant viruses can be processed in the same facility, thus allowing the production of more vaccine candidate rMVAs at the same time.