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Selection system for rapid and efficient isolation of African swine fever virus recombinants

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African swine fever virus (ASFV), the sole member of the family Asfarviridae, genus Asfivirus, causes African swine fever in domestic pigs and wild boar. ASF is a highly contagious hemorrhagic disease with mortality rates up to 100 %. No efficacious vaccine has been obtained yet. Therefore it constitutes a major threat for pig husbandry worldwide, high-lighted particularly by the recent introduction of ASFV into Caucasian countries and the Russian Federation where it has become a large-scale epidemic involving both the domestic pig and wild boar population. The vicinity of some regions with circulating ASFV to the European Union (EU) borders (<150 km) has increased concerns about the potential economic consequences of an ASF incursion into the EU pig sector. The double stranded, up to 200 kbp ASFV DNA is not infectious per se and, therefore, isolation of recombinant viruses for molecular analyses of gene functions and generation of novel vaccine candidates requires the presence of ASFV helper virus which subsequently needs to be eliminated. With the hitherto used approaches, homogeneity of recombinant ASFV stocks usually requires seven to nine consecutive rounds of plaque purification which bears the risk of selecting viruses with unwanted second-site mutations that e.g. support replication in cell culture. To reduce significantly the number of plaque purifications, we established a procedure that permits isolation of recombinant ASFV after only two rounds of selection. To this end, an ASFV-permissive thymidine kinase (TK) -negative cell line was selected and used for the generation of an ASFV mutant in which the viral TK ORF was replaced by the ORF for GFP. Corresponding to the intended ASFV genome modification, helper virus can be eliminated by choosing an appropriate combination of cells, virus and HAT- or BudR-containing medium.

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