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Universal cloning system independent of restriction sites and DNA ligation speeds up generation of recombinant influenza A viruses by reverse genetics

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Reverse genetics has become pivotal in influenza virus research. Basic research studies and vaccine development rely on the rapid generation of tailored recombinant influenza viruses. They are rescued from transfected plasmids encoding the eight influenza virus segments which have been cloned using restriction endonucleases and DNA ligation. However, in some cases suitable restriction cleavage sites are not available. Therefore, we established a cloning method which is universal for any influenza A virus strain and independent of restriction cleavage sites. This approach is based on an inverse PCR protocol using the amplicon of the influenza A gene segment as megaprimer. The prospective insert must contain termini homologous to the regions of the plasmid adjacent to the insertion site. In order to improve the efficiency, we modified the cloning vector by introducing the negative selection marker ccdB flanked by the highly conserved influenza A virus gene termini. Using this method, we cloned a complete set of gene segments representing viruses A/Thailand/1(KAN-1)/2004 (H5N1) and A/Swine/Belzig/2/2001 (H1N1). This approach allows fast cloning of all segments from any influenza A strain without knowledge of the genome sequence. Provided there are no other homologies between the ends of the PCR amplicon to its own internal regions and to plasmid vector regions beyond the annealing sites adjacent to the insertion point, this approach would be suitable for cloning of any insert with conserved termini.

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