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Fast and efficient generation of influenza A virus like particles from synthetic genes

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Microbial Cell Factories

Poster Presentation

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Fast and efficient generation of influenza A virus like particles from synthetic genes

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Background

With the recent emergence of the bird flu in many European countries, molecular biologists are challenged more then ever to advance the present methods of vaccine development. We have developed a method that is based on the following key elements: safety, efficacy and rapidity. Influenza A virus like particles (VLP) were generated in insect cells by co-transfection of especially designed plasmids. In order to produce VLPs, four recombinant baculoviruses were generated each containing two influenza genes under control of the *Autographa californica* multiple nuclear polyhedrosis virus (*Ac*MNPV) pH and p10 promotors for high level expression in *Sf*9 insect cells. VLPs contained 8 of 10 influenza A virus proteins of strain PR8, missing NS1 and NS2. Alternatively, VLPs were generated, by assembly of just three proteins, HA, NA and M1, which are responsible for induction of the immune system *in vivo*. All influenza genes have been produced from synthetical oligonucleotides, using a rapid thermocycler, the PCRJet®. Synthetic genes of new emerging influenza A variants can be produced accurately and rapidly by using this technology. Recombinant baculoviruses were generated using the Bac-to-Bac system by homologous recombination between a transfervector and the baculoviral shuttlevector (bacmid) in DH10Bac cells. By optimizing DNA synthesis and gene transfer into *Sf*9 cells, we anticipate major improvements in flexibility, speed and yield of influenza vaccine production as compared to available technologies.

Results

Co-transfection of bacmids resulted in the generation of influenza A virus like particles in the supernatant of *Sf*9 cells. VLPs were purified by means of Sucrose gradient centrifugation and the expected results were confirmed by Electron microscopy, Western Blot analysis and hemagglutination assays.