INSECT CELL PLATFORMS FOR PRODUCTION OF PSEUDO-TYPED VLPs FOR DRUG AND VACCINE DEVELOPMENT

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Conformational-complex membrane proteins (MPs) are vaccine/drug targets in many diseases, but drug and vaccine development has been slowed down by the lack of efficient production tools. Co-expression of MPs with matrix proteins from enveloped viruses is a promising approach to obtain correctly folded proteins at the surface of ordered nanoscale architectures such as virus-like particles (VLPs), preserving their native lipidic environment.

In this work, we implemented an innovative site-specific recombination strategy based on flipase-mediated cassette exchange technology to establish reusable insect cell platforms for fast production of enveloped VLPs pseudo-typed with target MPs. Influenza M1 and HIV Gag proteins were evaluated as scaffolds, and proof-of-concept (PoC) demonstrated using two membrane proteins, the influenza HA protein (e.g. for vaccines) and the human beta-2 adrenergic receptor (e.g. for drug screening or antibody discovery). Bioprocess engineering schemes were designed (adaptive laboratory evolution to hypothermic culture conditions and supplementation with productivity enhancers), allowing to improve HIV Gag-VLPs production in the developed stable insect cells. Under hypothermic culture conditions, adapted cells expressed up to 30-fold more HIV Gag-VLPs than non-adapted cells. Noteworthy, the element driving such increase in productivity is the adaptation process and not the temperature shift as the later alone leads to lower production yields. A more modest increase in productivity (up to 7-fold) was observed when supplementing non-adapted cell cultures with productivity enhancers NaBu and DMSO. PoC was successfully demonstrated in 0.5 L stirred-tank bioreactors.

Profiting from the platforms developed above, a modular system comprising stable and baculovirus-mediated expression in insect cells was established for the production of a multi-HA influenza VLP as vaccine candidate that otherwise could not be obtained due to baculovirus vector instability. By combining stable with transient expression systems, we could rationally distribute the number of genes to be expressed per platform and thus generate the target VLP for subsequent animal studies. In addition, a tailor-made refeed strategy was designed based on the exhaustion of key nutrients during cell growth resulting in a 4-fold increase in HA titers per mL. PoC was successfully demonstrated in 2 L stirred-tank bioreactors.

Overall, the insect cell platforms and bioprocess engineering strategies herein assembled have the potential to assist/accelerate drug and vaccine development.

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