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## Transient production of VLPs in HEK 293 cells and the evaluation of parameters influencing transfection and expression

Transient transfections can serve as an attractive alternative to generating cell lines which are difficult to engineer, such as those producing virus-like particles (VLPs). Transient methods can also allow for the production of large quantities of material within short periods of time. However, in order to make the process viable on a steady schedule or a larger scale, a broad realization of the potential variations of the process and the resulting effect must be explored. Upon identifying the impactful variations, the transient process can be properly controlled and become less challenging to carry out and reproduce.

In this work we evaluate the transient expression of Chikunguyna VLPs using polyethylenimine (PEI) based transfection in HEK 293 cells. Utilizing the AMBR bioreactor system, various growth parameters were evaluated and interpreted to identify transfection and expression effectiveness. Additionally, flow cytometry was employed to assess the distribution of VLP expression among the cell population and draw correlations with plasmid uptake. This work offers valuable insight in realizing the importance of the cell state and process parameters and the influence they have on the efficacy of transfection and expression.