# Rapid screening and scaled manufacture of immunogenic virus-like particles in the ALiCE cell-free protein synthesis system

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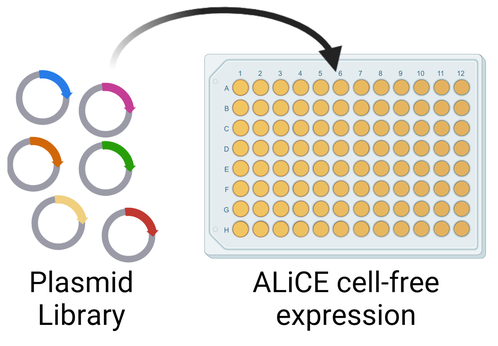
## INTRODUCTION

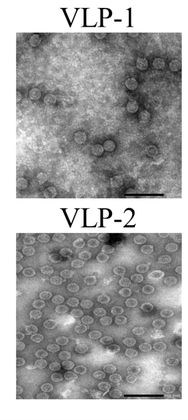
The recent SARS-CoV-2 pandemic brought renewed attention to the susceptibility of mankind to new and re-emerging diseases, and highlighted the necessity for timely vaccine development to slow the spread of disease (1). Virus-Like Particles (VLPs) are a very promising alternative to traditional vaccine platforms as they trigger strong and lasting humoral and cellular immune responses in humans, with fewer safety concerns and higher stability than other platforms such as inactivated viruses and RNA vaccines (2). Here we present the use of ALiCE®, a commercially available tobacco-based cell-free protein synthesis system, to rapidly screen and produce highly immunogenic Hepatitis B-core virus-like particles. We show that VLP production was successfully scaled to 1L, resulting in a 20,000 fold scaling with no loss in yield, leading to the potential production of up to 100,000 vaccine doses.

## 1. CANDIDATE SCREENING

**ALiCE® cell-free protein expression:**

1. **Rapid screening and parallelization**
2. **High VLP yields**
3. **Quick analysis of solubility and assembly**

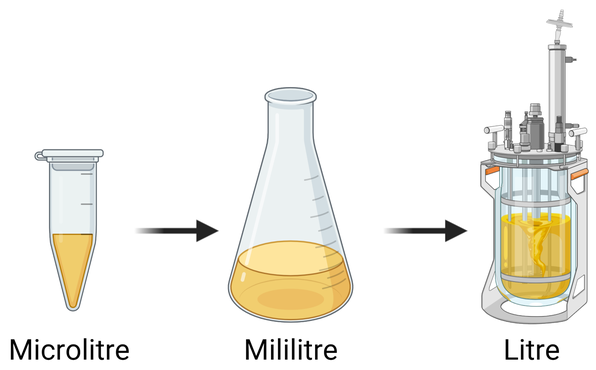


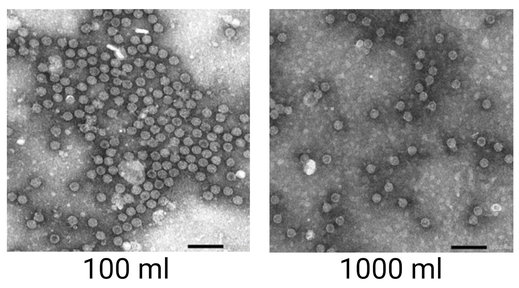



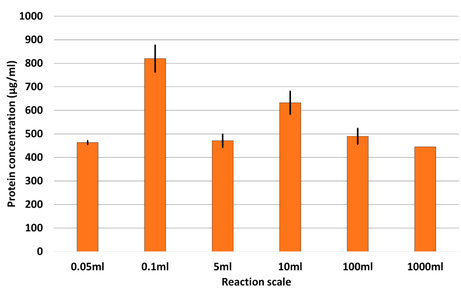

## 2. REACTION SCALE-UP

**Scaling up the ALiCE® reaction**

1. **Simple scale-up: batch mode**
2. **Linear scaling in a 20.000x fold range**
3. **Room for optimization**



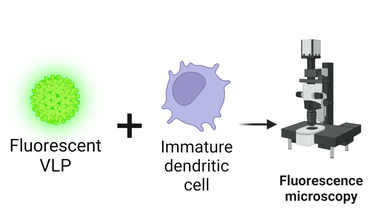



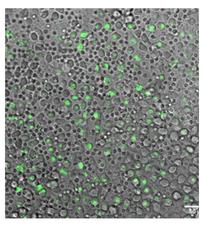



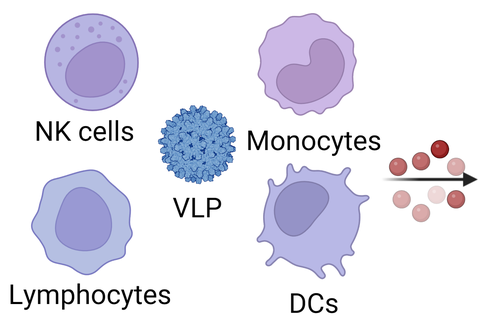

## 3. IMMUNOGENICITY TESTING

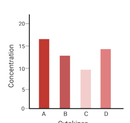
**Testing the immunogenicity of ALiCE®-produced VLPs:**

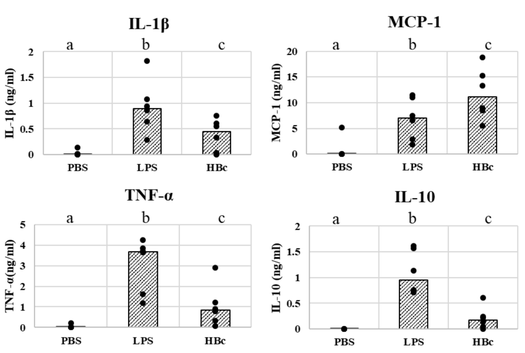
1. **VLPs were recognized and internalized by humand iDCs**
2. **Pro-inflammatory cytokines were produced upon VLP stimulation**






## CONCLUSIONS

1. **ALiCE® was successfully utilized to rapidly screen the production and assembly of different VLP variants. The production of a chosen candidate was scaled up to 1L scale without a major effect in protein yield. VLPs produced in ALiCE® were promptly recognized by human iDCs and triggered a pro-inflammatory cytokine response**
2. **ALiCE® thus is a promising cell-free protein synthesis platform with the potential to be used for both, high-throughput screening of novel VLP candidates, as well as their production at high-scale, bridging the gap between discovery and production**

**References**
1. Armero Gimenez et al., 2022 [Submitted for publication]
2. Wu et al., J. of Biosafety and Biosecurity, 2022, https://doi.org/10.1016/j.jobb.2021.09.003
3. Tariq et al., Front. in Microbiology., 2021, https://doi.org/10.3389/fmicb.2021.790121

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