

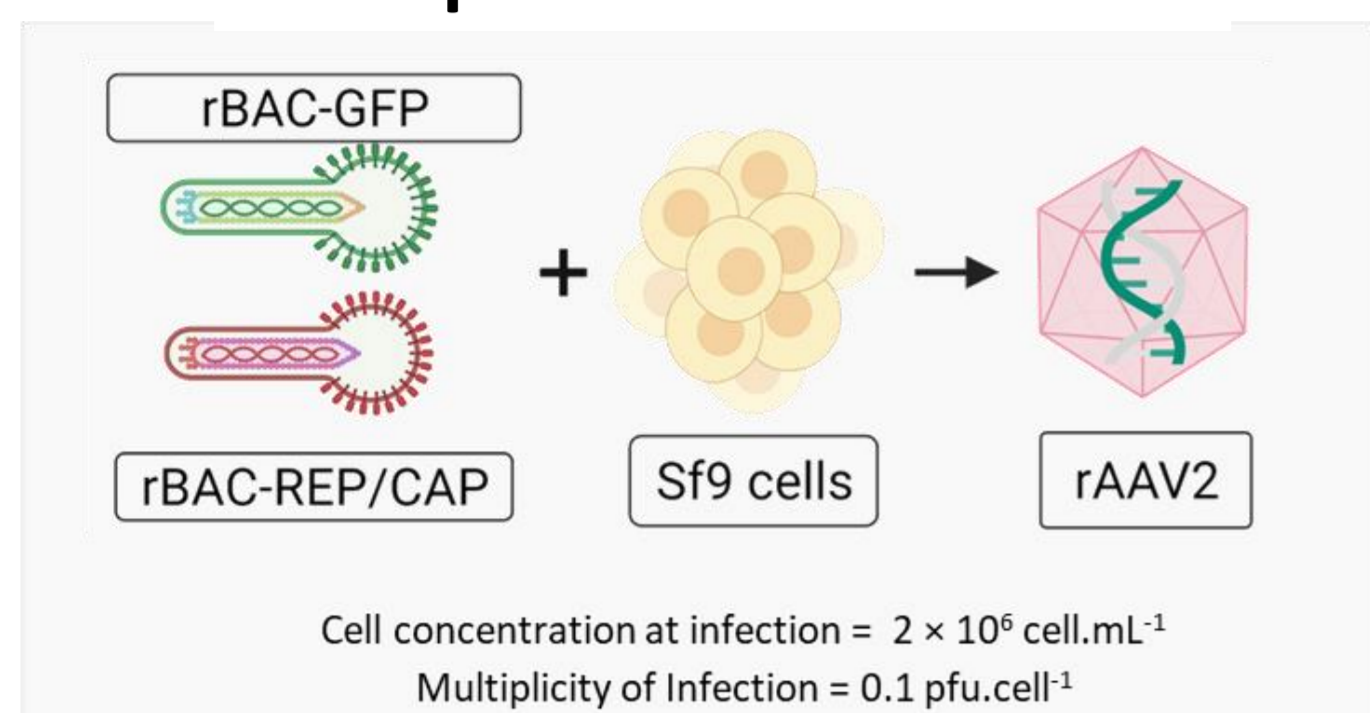
Background

The insect cell-baculovirus expression vector system (IC-BEVS) has emerged as a versatile cell factory, allowing production of biopharmaceuticals in a time- and cost-efficient manner. Nevertheless, the underlying biological mechanisms of the system and the impact of baculovirus infection on the cell transcriptome and population heterogeneity are still not extensively studied. In this work, we present single-cell and bulk transcriptome analysis of insect Sf9 cells during low multiplicity of infection (MOI), dual baculovirus infection for the production of recombinant Adeno-associated Virus of serotype 2 (rAAV2)^[1].

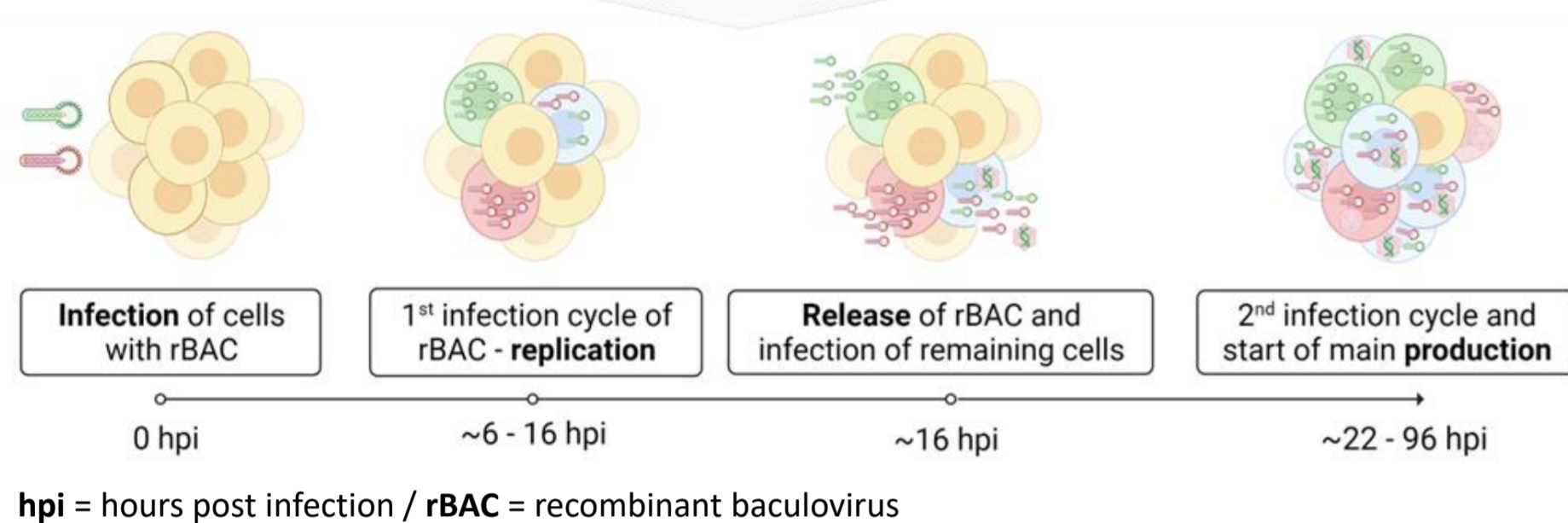
Aim

Understanding the underlying biological mechanisms of IC-BEVS during rAAV production using transcriptome analysis

rAAV production in IC-BEVS

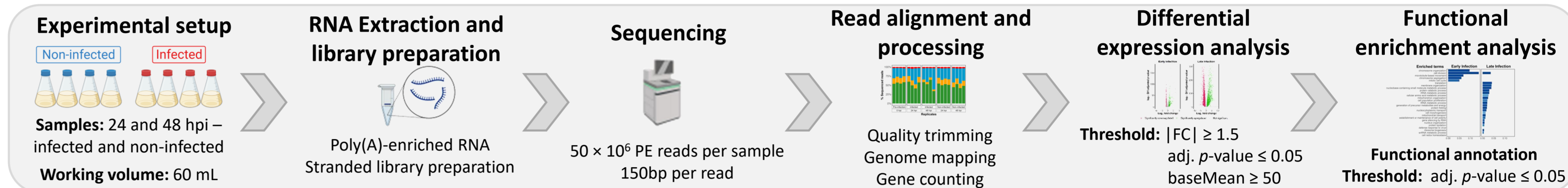


Snapshot of production process

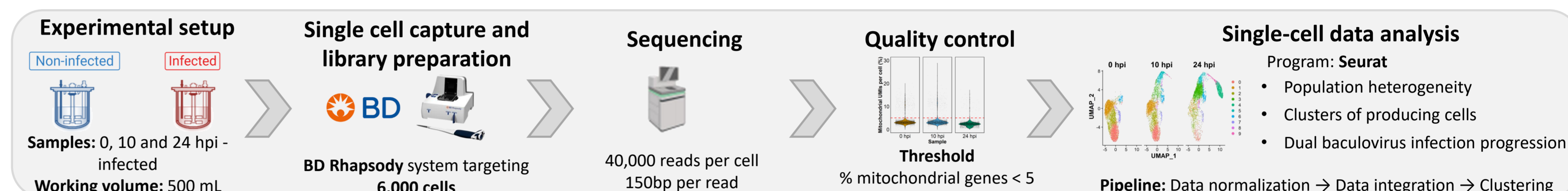


Strategy

Bulk RNA sequencing

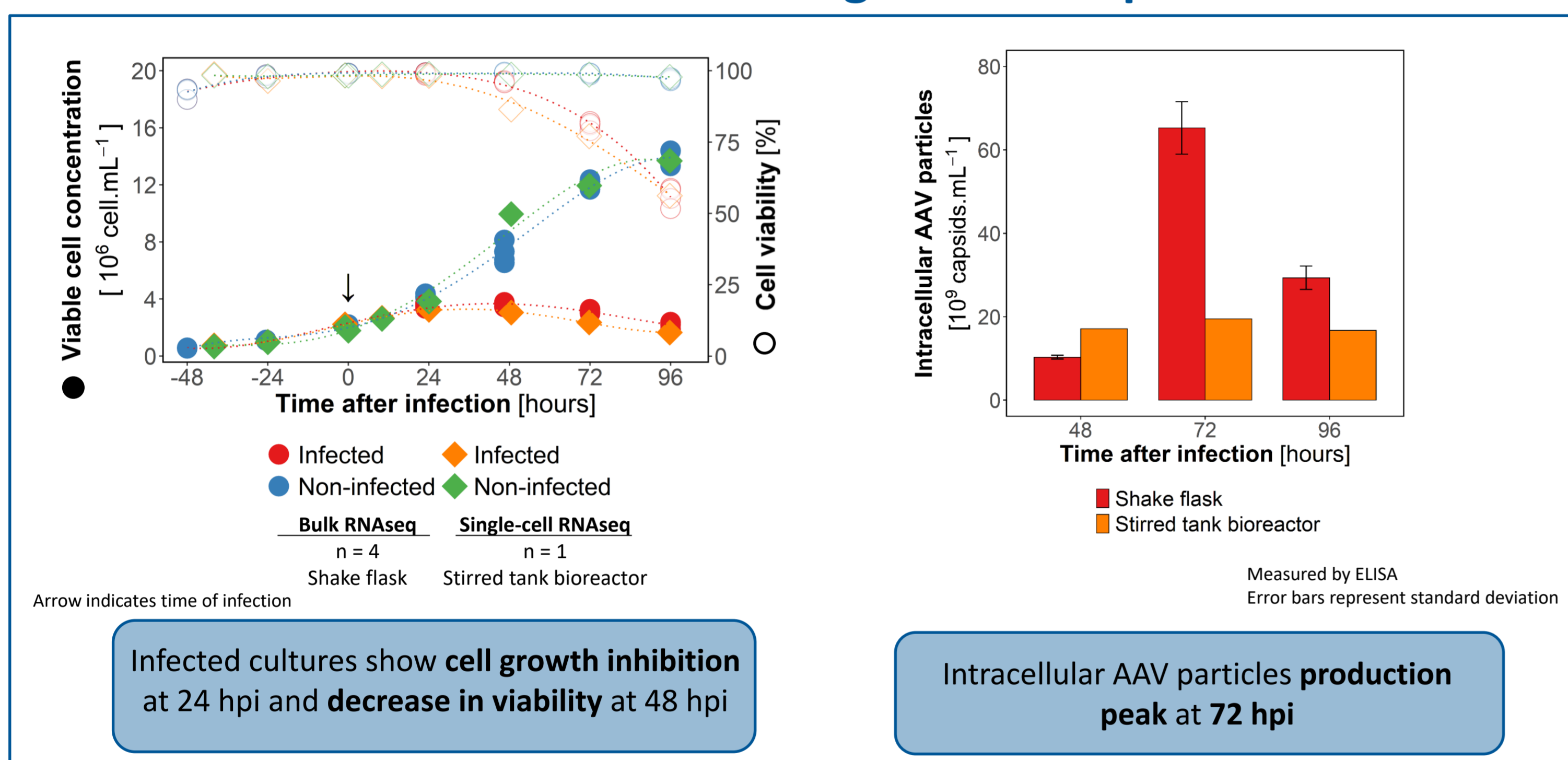


Single-cell RNA sequencing

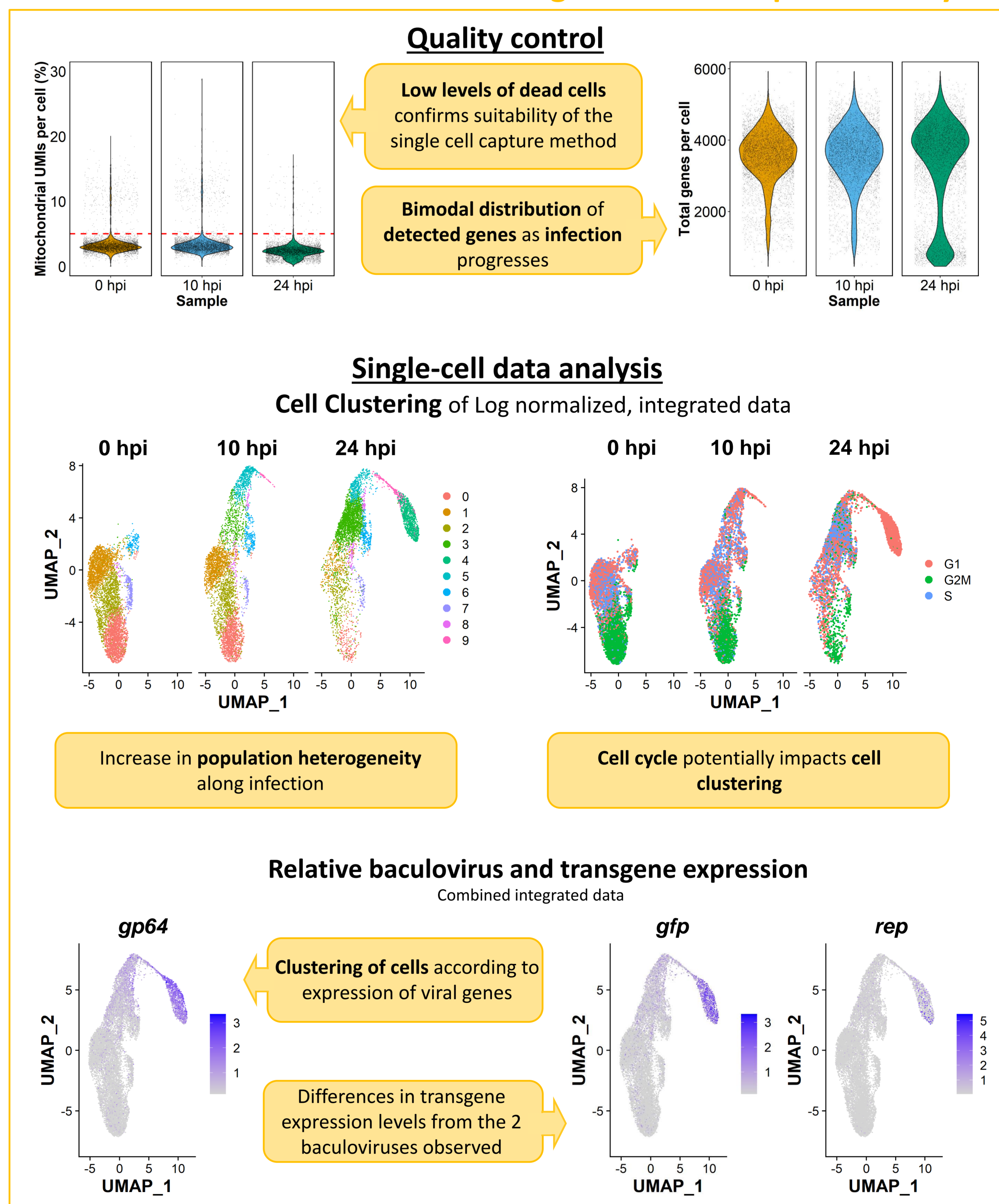


Results

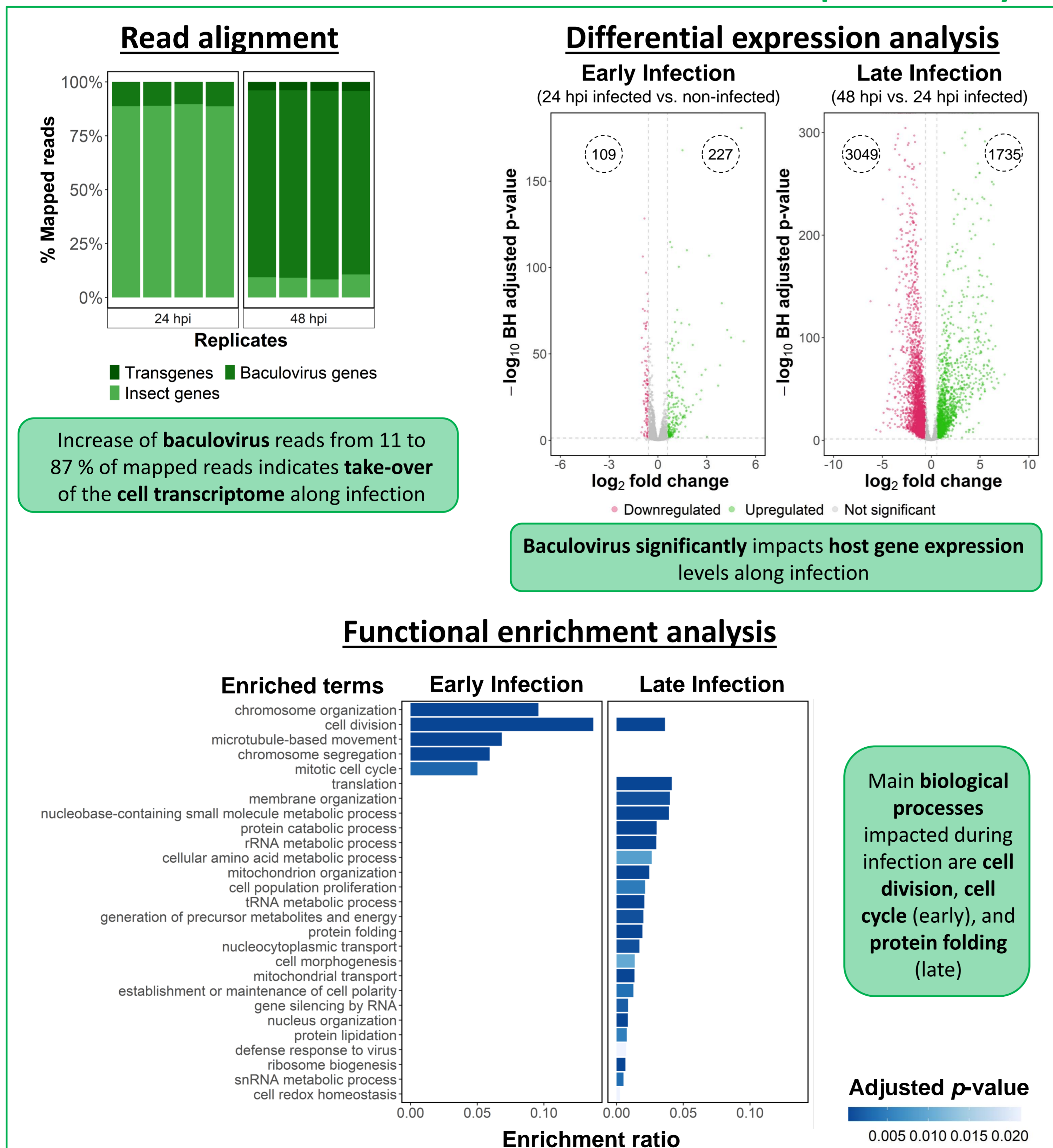
1. Cell growth and production kinetics



3. Single-cell transcriptome analysis



2. Bulk transcriptome analysis



Conclusions

- ✓ Sample processing and data analysis workflow established
- ✓ Successful implementation of scRNA-seq for IC-BEVS
- ✓ Baculovirus infection impacts the host cell transcriptome, thus altering specific biological processes
- ✓ Cell population heterogeneity increases throughout infection, with successful association of virus genes to specific clusters

References
[1] Pais, D.A.M., Portela, R.M.C., Carrondo, M.J.T., Isidro, I.A., Alves, P.M., 2019. Enabling PAT in insect cell bioprocesses: In situ monitoring of recombinant adeno-associated virus production by fluorescence spectroscopy. *Biotechnology and Bioengineering* 116, 2803–2814. <https://doi.org/10.1002/bit.27117>

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