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Establishment of a single-cell RNA sequencing platform for the investigation of immune responses against ASFV under high containment conditions

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Background

- Immunoassays are primarily developed for high-profile species like humans and mice
- Current methods limit the applicable extent of available (scarce) tools for veterinary species
- Methods are further restricted by biosafety protocols

Objective

- Enable detailed analyses of leukocyte responses in Suids against African swine fever virus (ASFV)
- Expand extent and resolution of current assays to the single-cell level
- Enable analysis in accordance with biosafety protocols

Single-cell RNA sequencing under high containment conditions

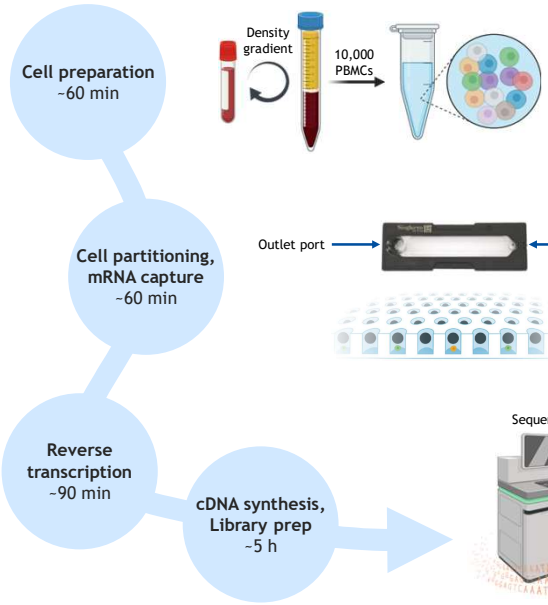

Figure 1. Cell preparation for scRNAseq experiments.
Single cell suspensions are prepared, e.g., by density gradient centrifugation or tissue dissociation, prior to chip loading. Depending on chip size, up to 30,000 PBMCs can be loaded and processed on the chip.

Figure 2. Microwell chip architecture.
The microwell chip has an inlet port for injection of cells, buffers, and beads, and an outlet port for removal of excess material (top). The diameters of wells and capture beads are 40/25 µm, respectively, ensuring presence of only a single bead per well (bottom).

Overview of the workflow for scRNAseq experiments.
Single cell suspensions are prepared and loaded onto the chip, where cells are seperated into microwells. After addition of capture beads, cells are lysed and mRNA is bound by capture beads. The beads are washed from the chip and the captured mRNA is purified for reverse transcription, cDNA synthesis, and library preparation. The libraries are then removed from the containment unit according to safety protocols, sequenced, and analyzed bioinformatically.

Application of single-cell RNA sequencing in an ASFV infection trial

Figure 3. Animal trial.
Individuals of four suid species, domestic pigs, wild boar, red river hogs, and warthogs (n = 6 each), were intramuscularly infected with the highly virulent ASFV strain ‚*Armenia08*' (10^4 HAD). 10,000 freshly isolated, pooled PBMCs of up to six individuals were subjected to scRNAseq 0, 2, 4, and 7 dpi.

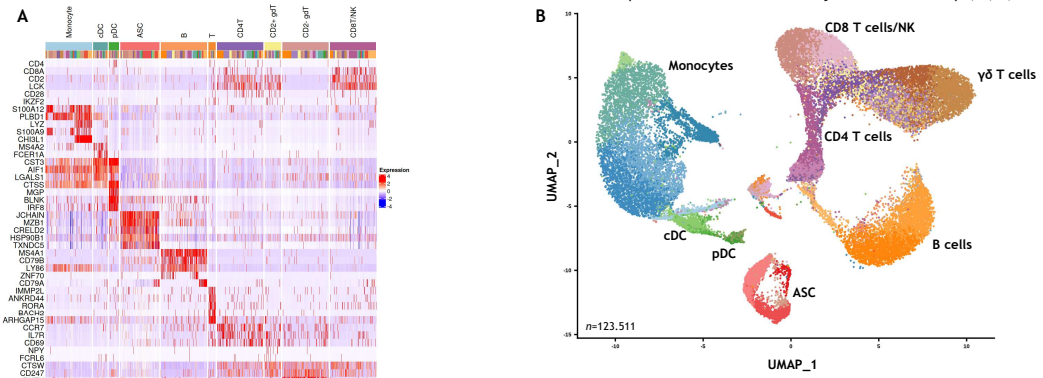

Figure 4. Immune cell landscape in ASFV-infected suids.
123,511 cells passed quality control measures and were used for further analysis. (**A**) Heatmap shows expression of lineage markers for cluster identification . (**B**) UMAP shows the immune cell landscape of all suid species in this study, with cluster identity annotated.

Acknowledgement

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Illustrations were created with BioRender. bio RENDER