



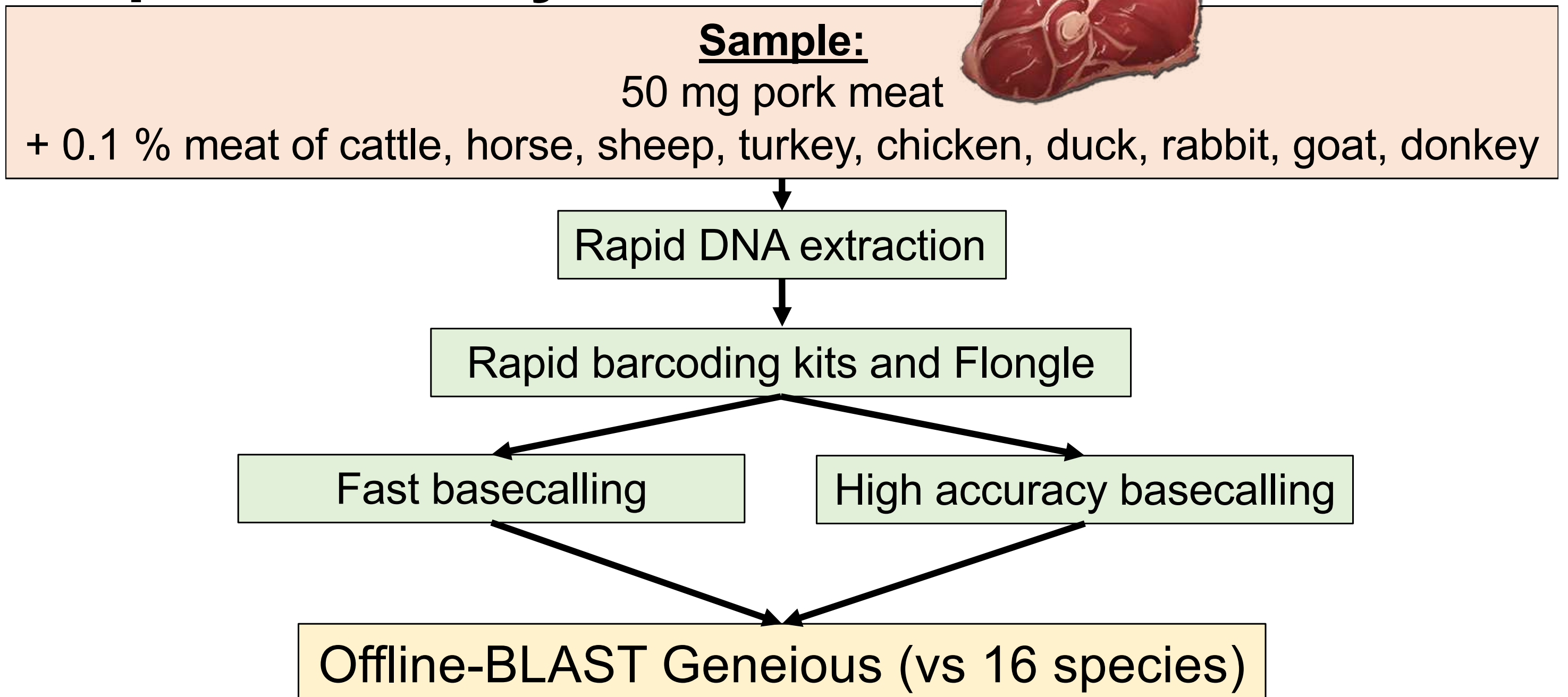
Introduction

- Detection of animal species in meat product is crucial to prevent adulterated and unnecessary contamination during processing, in addition to avoid allergy and religious consequences. Gold standard is the real-time PCR assays, which has a limited target capability.
- In this study, we have established a rapid sequencing protocol to identify animal species within hours.
- Sequencing was achieved by nanopore sequencing and data analysis *via* offline BLAST search.
- The whole procedure was conducted in a mobile suitcase lab. As per national and international regulations, the developed assay detected adulteration of pork meat with 0.1 % of Horse, Chicken, Turkey, Cattle, Sheep, Duck, Rabbit, Goat and Donkey.
- The developed test could be used on-site as a rapid and mobile detection system to determine contamination of meat products.

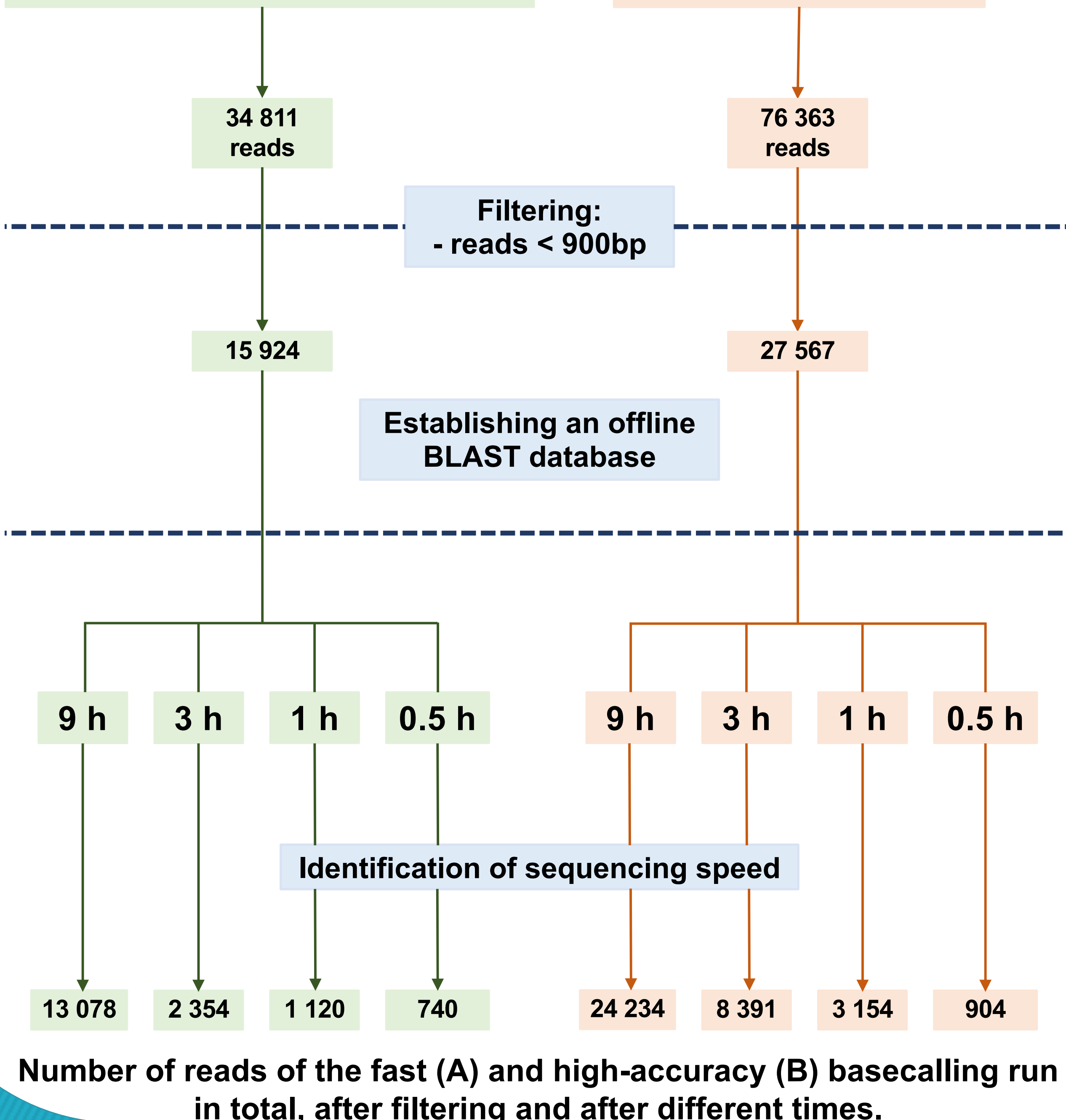
Mobile suitcase lab for nanopore sequencing



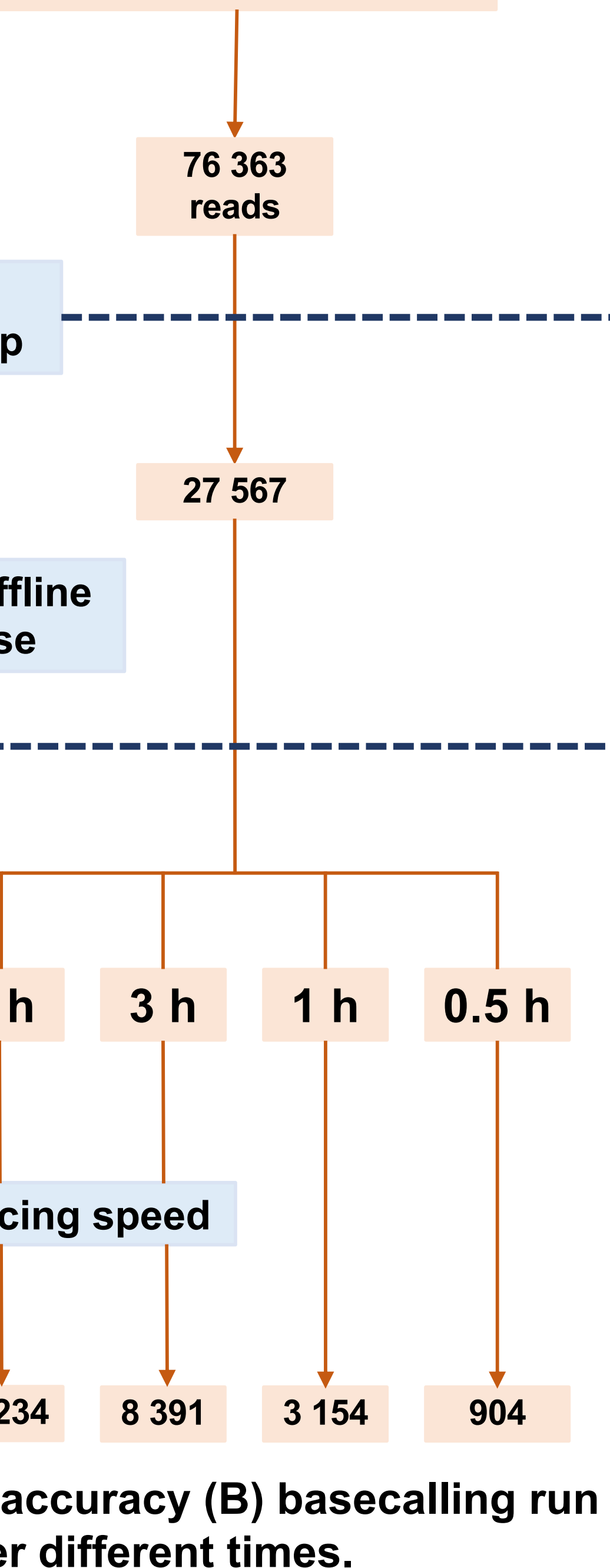
Experiment Layout



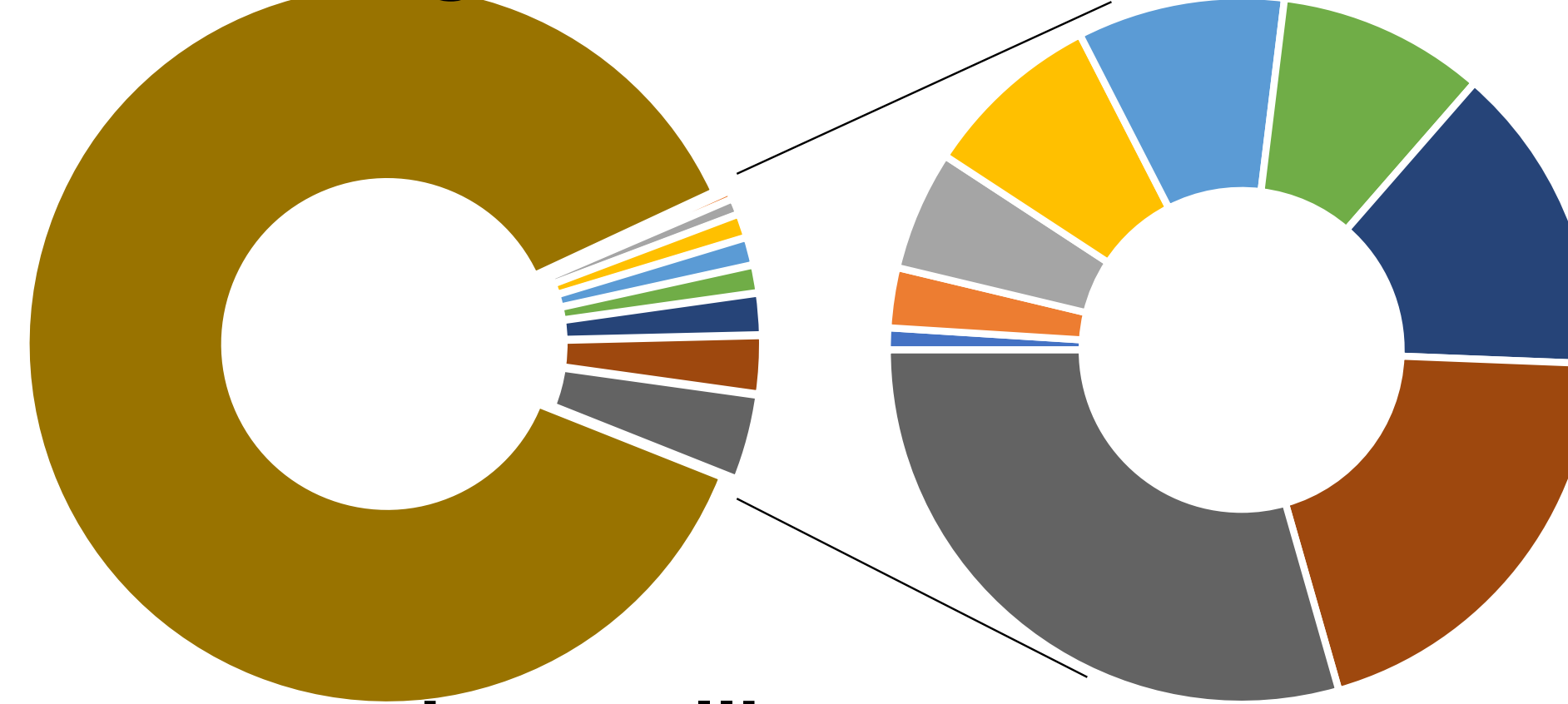
A) High - accuracy basecalling sequencing run



B) Fast basecalling sequencing run

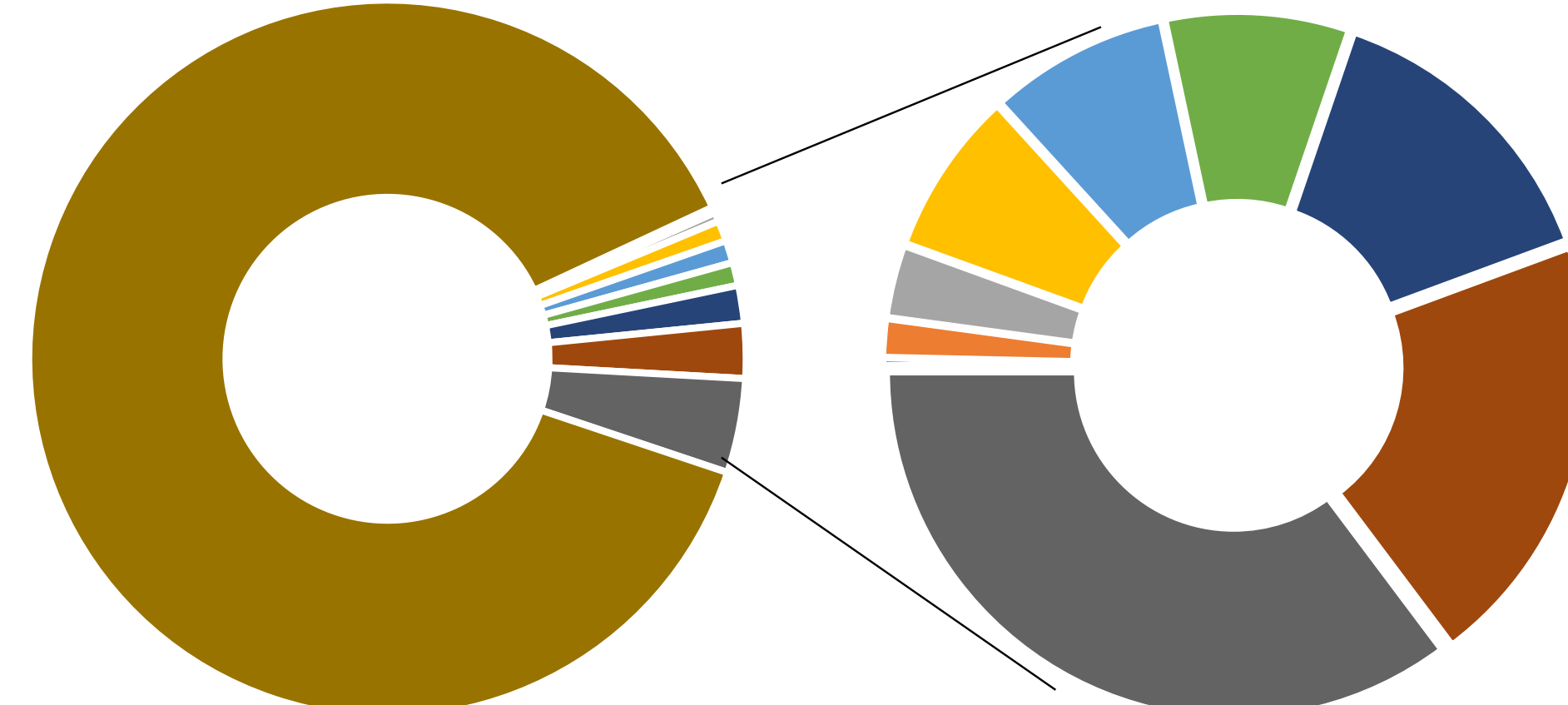


Fast basecalling



Speed: 9 hour

High accuracy basecalling



Speed: One hour

■ Chicken ■ Turkey ■ Duck ■ Horse ■ Rabbit ■ Goat ■ Cattle ■ Donkey ■ Sheep ■ Pig

Results of the offline BLAST-search by applying the Flesh-ID database to the sequencing data of the high-accuracy and fast basecalling run. In both sequence runs all 10 animal species could be identified using the Flesh-ID database.

Conclusion

- For the identification of animal species in meat products, nanopore sequencing was combined with a novel offline BLAST-search.
- The DNA was extracted in one hour using alkaline lysis buffer. Library preparation was conducted in 10 minutes and the sequencing run in 18 hours. The offline BLAST-search in Geneious was achieved in less than 20 Minutes.
- The whole procedure was conducted in a mobile suitcase lab, which facilitates the use at point of need.
- However, a highly trained person must operate the developed assay and the prices is still high. Furthermore, the stability of reagents must be improved to allow long storage at room temperature.
- In the long run, sequencing will be the standard of molecular diagnostics but data analysis and handling still a great obstacle.