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WHOLE-GENOME SEQUENCING
OF AFRICAN SWINE FEVER VIRUS IN PIG BLOOD SAMPLES

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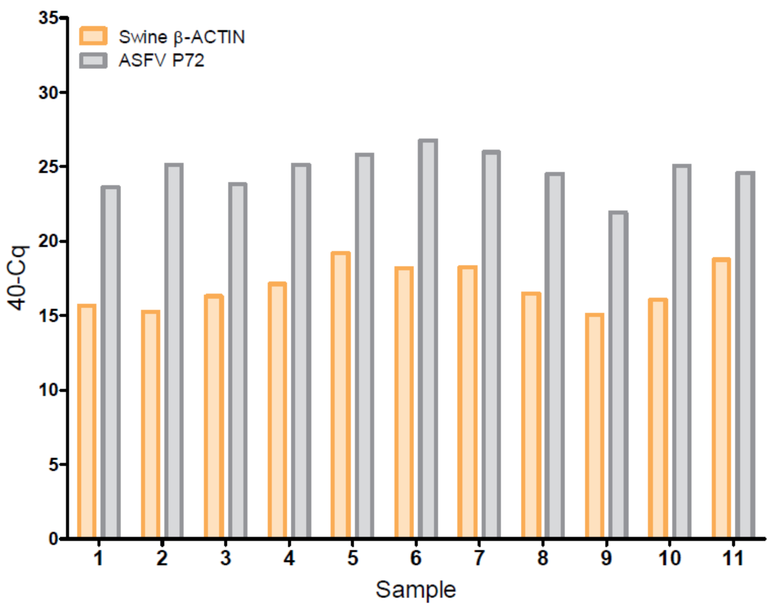
INTRODUCTION

African swine fever virus (ASFV) is a large (170-193 kbp) double-stranded DNA virus. Routine genome fragment analyses of the virus have shown low resolution power with regards to detailed analyses of variation among isolates, and whole-genome next generation sequencing (WG-NGS) might be a tool to achieve a higher resolution power. The aim of this study was to apply WG-NGS to obtain whole-genome ASFV sequences directly from erythrocyte-enriched pig blood samples.

RESULTS

Erythrocyte-enriched blood samples were analyzed by qPCR and 11 samples were chosen for NGS analysis (Fig. 1).

FIGURE 1: qPCR data for each selected sample



The 11 samples (two in duplicate) were mapped to the complete genome of ASFV Georgia 2007/1 (FR682468). The total no. of reads ranged from 0.8-5.6m of which 0.1-1.5 % could be mapped to the reference. From some of the best samples almost complete genome sequences were obtained with an average coverage up to 46 (Fig. 2).

When raw reads from all samples were analyzed together, 37m reads were obtained. Of these, 0.69 % could be mapped to FR682468, yielding a coverage of 200 and a consensus sequence of 189390 nucleotides.

The consensus was 99.9 % identical to the reference. Up to 80 % of the variation may be due to homopolymer artifacts, but further in-depth sequencing and analysis is needed.

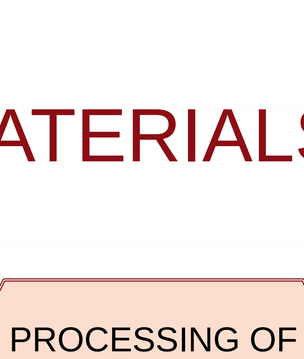
MATERIALS AND METHODS

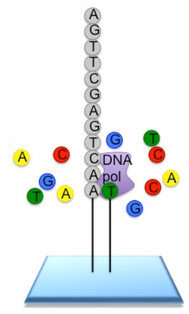
ASFV STUDY IN PIGS

EDTA-stabilized blood from pigs infected with an ASFV isolate from Poland

PROCESSING OF SAMPLES

- Lymphoprep™ density gradient medium
- MagNA Pure 96 system
- qPCR to select samples for NGS

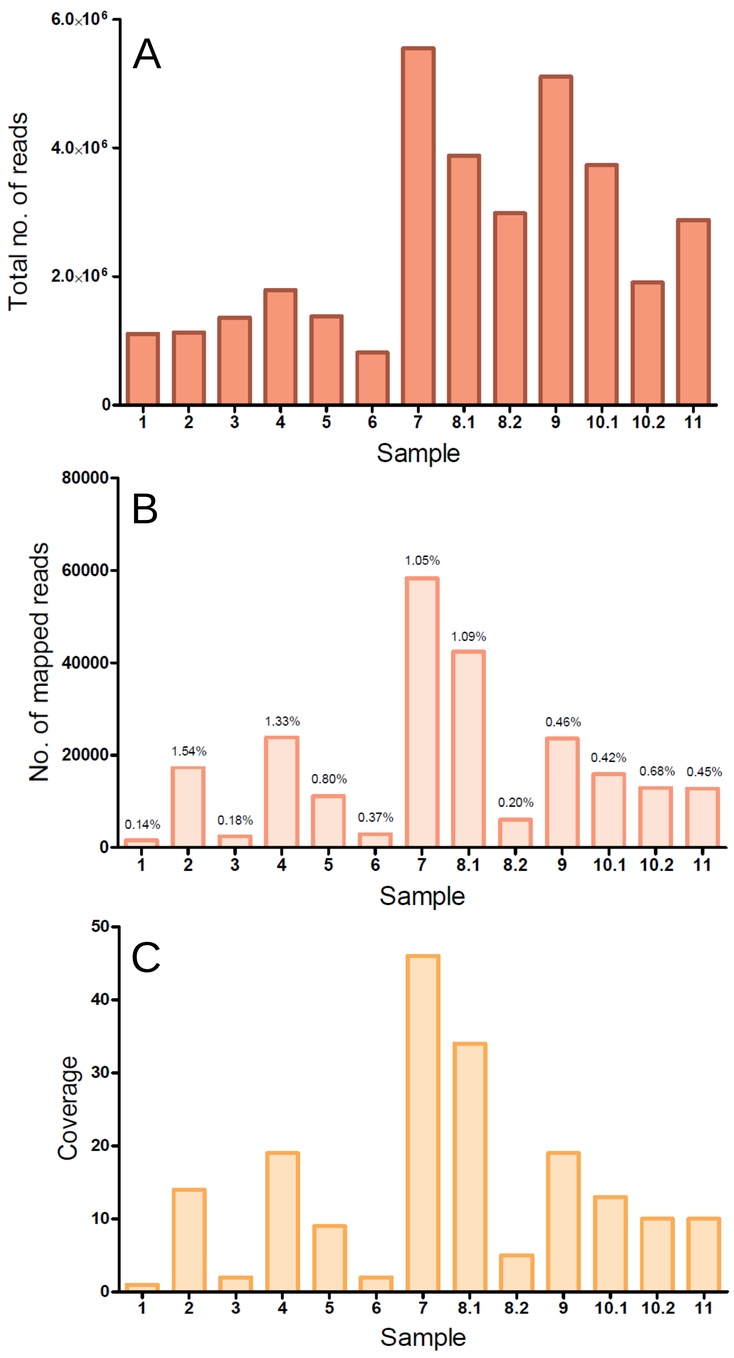




NGS ANALYSIS

- Illumina MiSeq platform
- Data analysis in CLC Genomics Workbench and Geneious

FIGURE 2: Statistics of NGS data for each sample. Total no. of reads (panel A), no. of mapped reads and % mapped reads (panel B), coverage (panel C)



CONCLUSION

This study shows that whole-genome ASFV sequences can be obtained directly from pig blood samples.

Still, deeper coverage is needed in order to obtain sufficient whole-genome data from such samples.

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