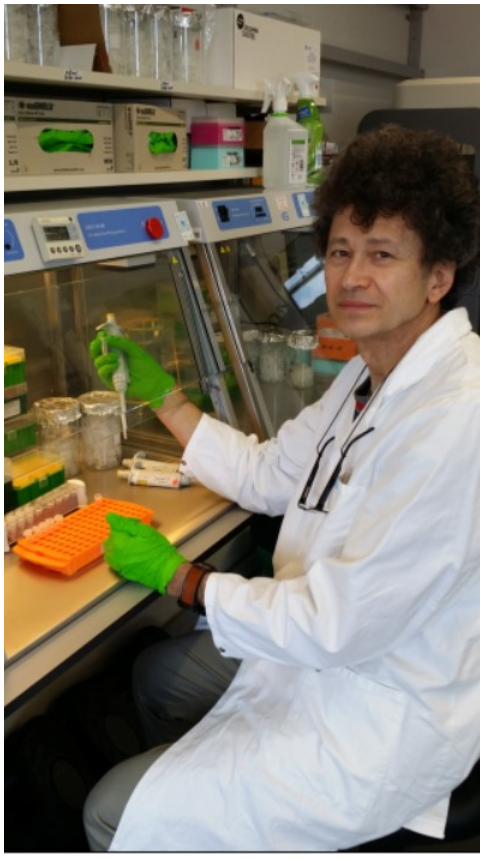
Full genome sequencing of ASFV using myBaits capture probes and Illumina MiSeq platform



The aim of the EPIZONE short-term mission (STM, 16-22 February 2020) at the Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Greifswald - Insel Riems, Germany was:

- To learn laboratory techniques of full genome sequencing (170-190 kbp) of ASFV using specific myBaits hybridization probes and Illumina MiSeq high-throughput sequencing platform.
- Technology transfer to increase preparedness and control of potential threat of ASFV spreading in Sweden, which at present is free from the virus.

The work was conducted during seven-day research mission at the Friedrich Loeffler Institute (FLI) and followed the detailed plan of the professional activities submitted in the application form to Epizone.

Four ASFV samples (Table 1) were chosen to learn library preparation methodology using myBaits hybridization probes from Arbour Biosciences (Ann Arbor, MI) for the sequencing on Illumina MiSeq platform. Samples were used in duplicates (ASFV Bulg, ASFV 4vb) or triplicates (ASFV Ukr12, ASFV Ukr26) to test different ASFV baits probes and influence the number of PCR cycles (14x, 18x, and 22x) on library amplification. During the laboratory work at FLI samples were divided on to two batches for easy processing.

Samples were tested with ASFV real-time PCR which contained swine beta-actin as an endogenous control according to published reference (Tignon et al., 2011) to determine Ct-values and DNA concentration (Table 1).

Table 1. ASFV samples tested and qPCR Ct-values

Sample	Country of origin	Supplier	To use as	Conc., ng/µl	Ct-value
ASFV Bulg	Bulgaria	FLI	Positive control	177.5*	19.08
ASFV Ukr12	The Ukraine	FLI	Test sample	60.0	15.22
ASFV Ukr26	The Ukraine	FLI	Test sample	41.0	28.39
ASFV 4vb	Spain	SVA	Test sample	17.2	31.10

* ASFV Bulg. concentration was measured with NanoDrop (Nanodrop, Thermo Fisher Scientific, Wilmington, DE), while for the rest of the samples with Qubit dsDNA HS assay kit (Invitrogen, Thermo Fisher Scientific, Eugene, OR).

PCR reaction components and thermodynamic profile were following:

Reagents	1x, µl
RNase-free water	1.75
2x QuantiTect Multiplex PCR Master Mix	6.25
ASFV primer/probe mix	1.00
actB mix	1.00

Step	Time	Temp.	
Enzyme activation	15 min	95 °C	
Denaturation	1 min	95 °C	
Annealing	30 s Data	60 °C	44X
Extension	30 s	72 °C	
Hold	Forever	8 °C	

DNA	2.5
Total	**12.5**

Qiagen GeneRead Library Prep kit (Qiagen, Hilden, Germany) was used for preparation of DNA libraries for the next-generation sequencing (NGS) with minor modifications. MyBaits Hybridization Capture for Targeted NGS kit, Version 4.01 (Arbor Biosciences, Ann Arbor, MI) was used to enrich NGS library.

The following library preparation workflow using FLI standard operation procedures (SOPs) were used:

- DNA fragmentation aiming at a fragment length of 500 bp (SOP_070104).
- Clean up and concentration the fragmented DNA using AMPure XP beads (SOP_080103)
- Qiagen GeneRead Library preparation (for Illumina sequencing) according to manufacturer's instructions. This step included:
  - ✓ End-repair (30 min at 25 ºC; 20 min at 75 ºC; ∞ at 8 ºC)
  - ✓ A-addition (30 min at 37 ºC; 10 min at 75 ºC; ∞ at 8 ºC)
  - ✓ Adapter ligation (10 min at 25 ºC)
- Extra clean up and concentration the end-repaired and adapter -ligated DNA library using AMPure XP beads (SOP_090303)
- Size selection using AMPure XP beads (SOP_100102)
- Clean up the fragmented DNA using AMPure XP beads (SOP_080103)
- Second clean up the fragmented DNA using AMPure XP beads and elution the final library in 9 µl (SOP_080103)
- MyBaits hybridization capture for targeted NGS according to the MyBaits protocol (v 4.01)
- Elution the enriched library from the baits and library amplification for 14x, 18x, and 22x cycles using ACCU prime *Taq* Polymerase with NetFlex universal primer mix
- Double purification of PCR amplified product and subsequent dilution of DNA in 50 µl and 30 µl of EB-buffer (SOP_090303)
- Library quality check on a 2100 Agilent Bioanalyzer and High Sensitivity DNA Chip (SOP 110102)
- Library quantification using the KAPA PCR system (SOP 120102)
- Illumina MiSeq sequencing according to Illumina instructions.

The last step of the protocol workflow (MiSeq sequencing) still have to be completed after the STM by colleagues at FLI. Illumina MiSeq sequencing of ASFV libraries is planned on the next available scheduled sequencing of the instrument at FLI.

In summary, ASFV library preparation protocol workflow using myBaits capture probes and laboratory practice at FLI was followed carefully. This will ensure the technology transfer and successful implementation of this method at SVA. Thanks to Epizone STM the task successfully performed (learning a new method and laboratory practice) and a new collaboration established between FLI and SVA.

References

Tignon M, Gallardo C, Iscaro C, Hutet E, Van der Stede Y, Kolbasov D, De Mia GM, Le Potier MF, Bishop RP, Arias M, Koenen F. 2011. Development and inter-laboratory validation study of an improved new real-time PCR assay with internal control for detection and laboratory diagnosis of African swine fever virus. J Virol Methods **178:**161-170.