Evaluation of ampicon-based sequencing method for the European filovirus (Lloviu cuevavirus)

UNIVERSITY OF PÉCS
NATIONAL LABORATORY OF VIROLOGY

Ágota Ábrahám [1,2], Gábor Endre Tóth [1,2], Zsófia Tauber [1,2], Gábor Kemenesi [1,2] ✉
1. University of Pécs, National Laboratory of Virology, Hungary ; 2. University of Pécs, Faculty of Sciences, Institute of Biology, Hungary
✉kemenesi.gabor@gmail.com

INTRODUCTION

Lloviu virus (Lloviu cuevavirus, LLOV) is the first filovirus discovered in Europe in Schreibers's Bent-winged bats (Miniopterus schreibersii) during a die-off event in 2002 in Spain (1). In this last 20 years increasing amount of data suggest a wider circulation of LLOV amongst Schreibers's bats presumably across its whole geographic range (2). Characterization of LLOV virus at the genomic level is challenging because the limitation of sample quantity and quality.
Targeted, amplicon-based next generation sequencing gives us the opportunity to gather valuable knowledge on the evolution and epidemiology of the virus and overcome these limitations.
The purpose of this study was to test the efficiency of the previously developed amplicon-based sequencing method for the bat-related European filovirus on several recently collected samples and sample types (bat flies and bat whole blood).

METHODS

SAMPLE COLLECTION AND VIRUS DETECTION

Protocol for the field detection of the European filovirus (Lloviu cuevavirus)

This protocol is a draft, published without a DOI.

Gábor Tóth[1], Sándor Boldogh[2], Ágnes Balázs-Nagy[3], Tamás Görföl[1], Gabor Kemenesi[4]

[1]National Laboratory of Virology (Hungary);
[2]Aggtelek National Park Directorate;
[3]Hungarian Army Medical Centre; [4]University of Pecs

FEB 23, 2023

SHARE

IN DEVELOPMENT

Gábor Tóth

SEQUENCING OF THE VIRUS GENOME

Lloviu cuevavirus sequencing protocol
Forked from nCoV-2019 sequencing protocol v2 (GunIt)

This protocol is a draft, published without a DOI.

Gábor Tóth[1], Gabor Kemenesi[1]

[1]National Laboratory of Virology (Hungary)

Szentagothai Research Centre Virology

DEC 17, 2020

SHARE

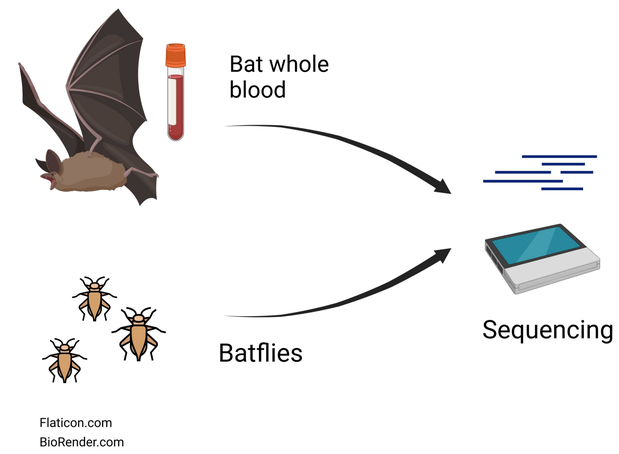
IN DEVELOPMENT

Gábor Tóth

Bioinformatics pipeline

400 bp set | 500 bp set | 2000 bp set

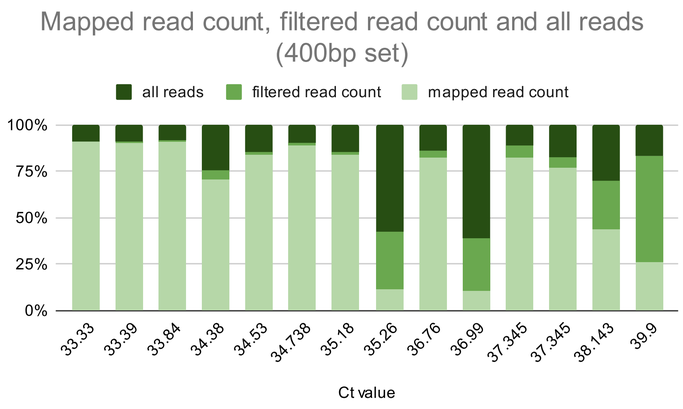
Guppy (v6.4.6)	Basecalling (dna9.4.1_450bps_sup.cfg) Demultiplexing (enable_trim_barcodes)
NanoPlot (v1.41.0)	Quality check
NanoFilt (v2.8.0)	400bp set : minimal length 300 bp 500bp set: minimal length 400 bp 2000bp set: minimal length 1400 bp Trim 25 bases from both ends of the reads
Minimap2 (v2.24)	Mapping to reference genome (GenBank MZ_541881)
SAMtools (v1.17)	Converting SAM files to BAM files and indexing
BCFtools (v1.17)	Generating consensus sequence
Medaka (v1.7.3)	Polishing (model: r941_min_sup_g507)

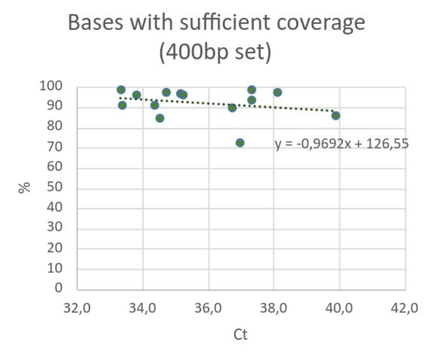


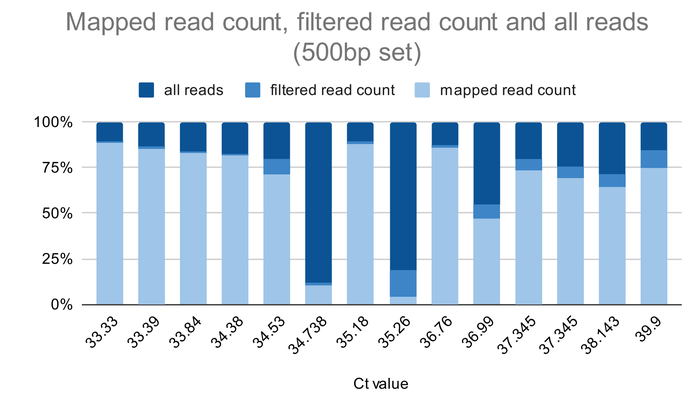
LIMITATIONS OF THE METHOD

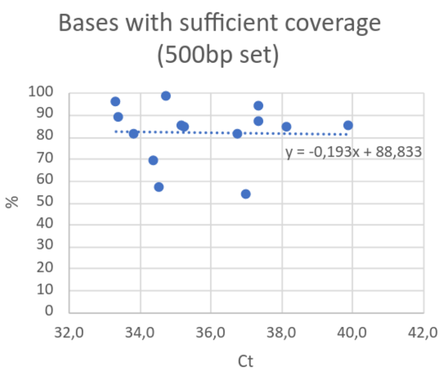
- Quality of the samples
- Quantity of the samples
- Additional optimization is needed for the optimal recovery of intergenic and genomic end regions.

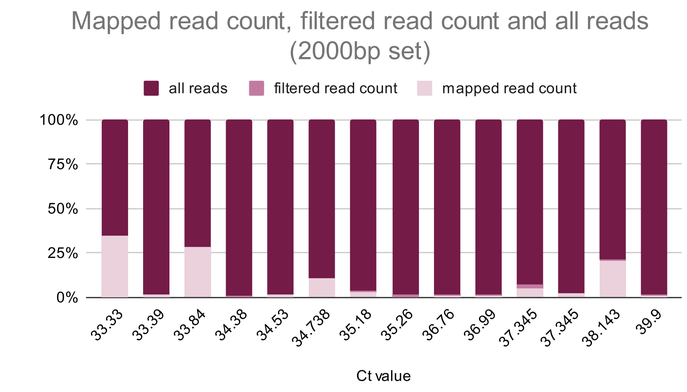
RESULTS

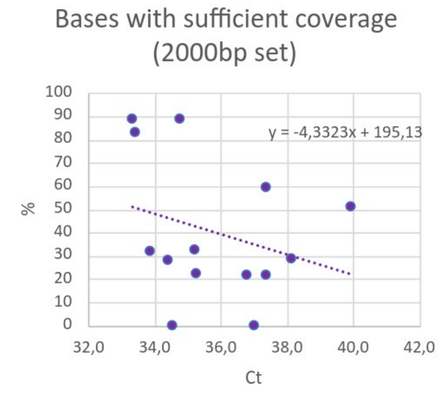


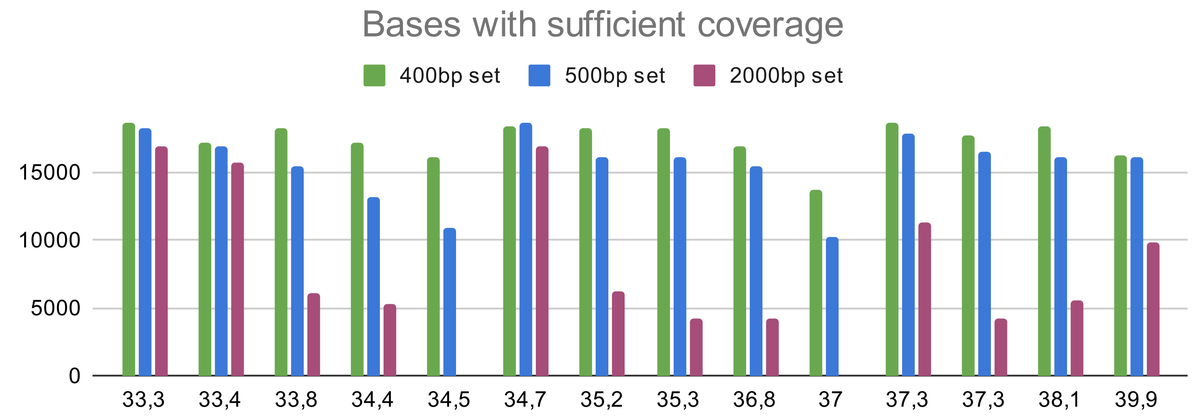


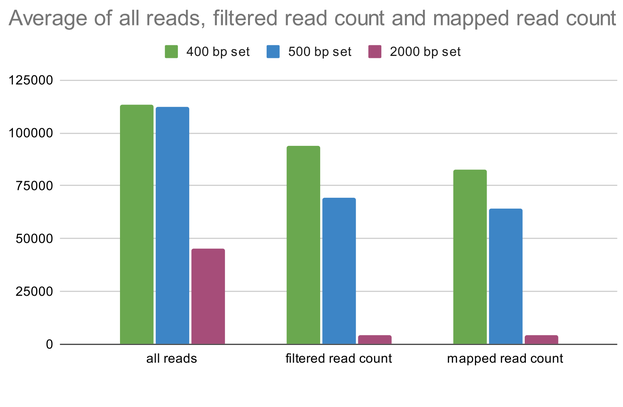


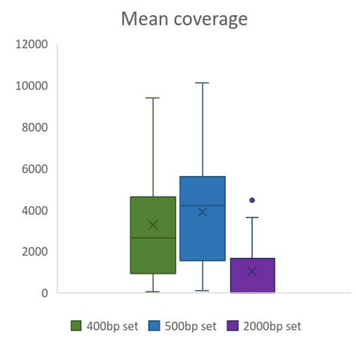


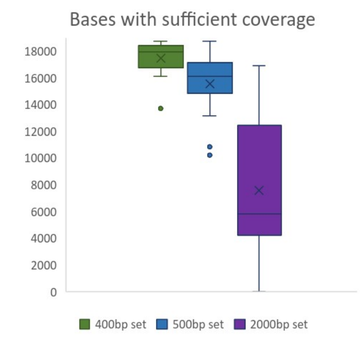












NOTES

Sufficient coverage here is defined by a base having at least 20X coverage.
All samples used for this study were processed with the same methods and sequenced in a single run, to avoid additional bias.

CONCLUSIONS

- We obtained less reads from the 2000bp amplicon set, possibly due to the low copy number, enzymatic limitations of the amplification or nucleic-acid fragmentation
- Sufficient recovery of the coding region of LLOV is most supported with shorter fragments
- This method is efficient for samples with high Ct values; for example 85% genome recovery with the 400bp and 500bp amplicon sets for a sample with 39.9 Ct
- This method is efficient for multiple sample types of samples – here we presented sequencing from whole blood and insects
- Higher ratio of LLOV specific reads with the 400bp and 500bp amplicon sets compared to the 2000bp amplicon set

- Negredo et al. 2011, PLoS Pathogens
- Kemenesi et al. 2022, Nature Communications