



On-site laboratory techniques as a powerful toolkit for pandemic preparedness: the example of *Lloviu cuevavirus*

Gábor Endre Tóth¹, Tamás Görföl¹, Ágota Ábrahám¹, Zsófia Lanszki¹, Ferenc Jakab¹, Gábor Kemenesi¹

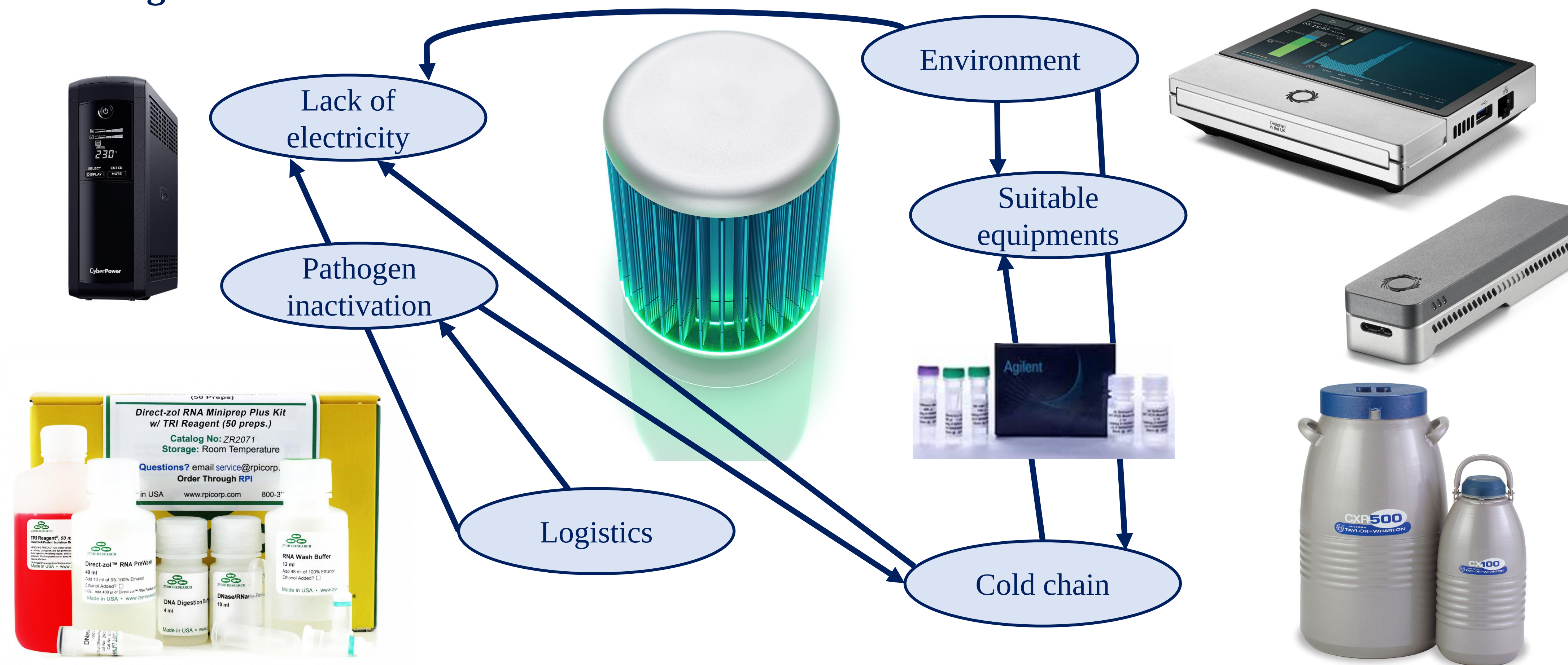
National Laboratory of Virology, University of Pécs, Pécs, Hungary¹

E-mail: toth.gabor.endre@gmail.com

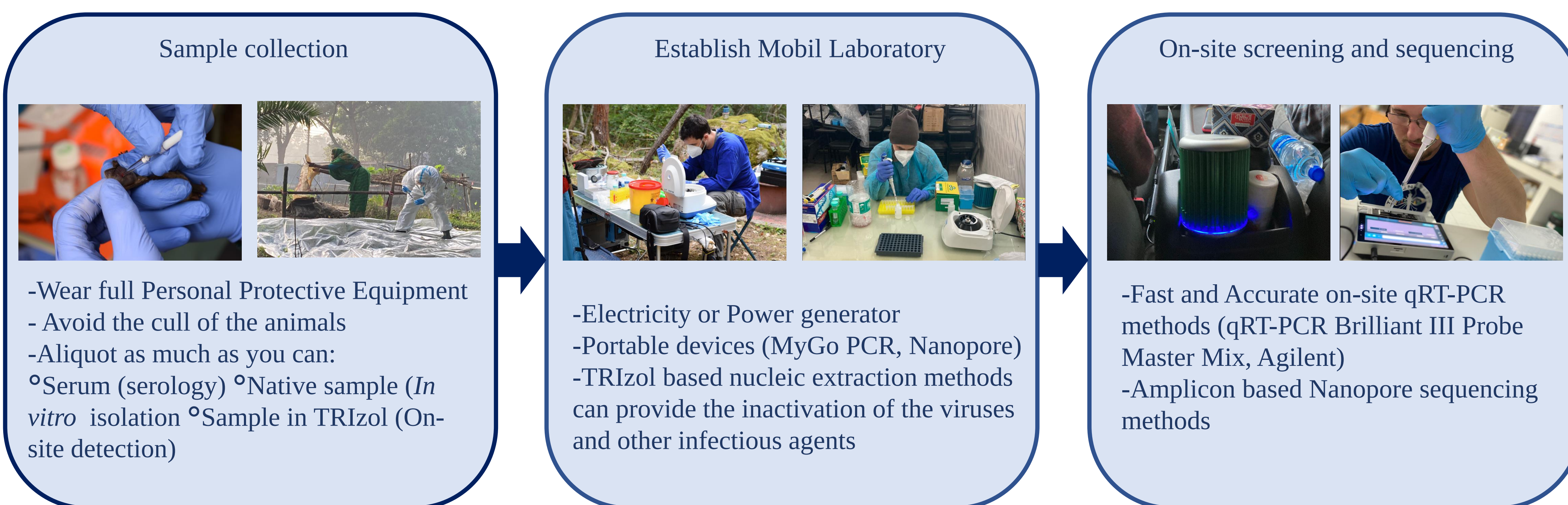
Introduction

Lloviu cuevavirus (LLOV) is the sole member of *Cuevavirus* genus and the only bat-associated filovirus discovered in Europe so far. LLOV genomic RNA was identified during the investigation of Schreiber's bats (*Miniopterus schreibersii*) die-off events in the Iberian-peninsula in 2002¹. No other reports were published with regards to the presence of this virus until 2016, when RT-PCR positive bat carcasses in Hungary confirmed a more widespread presence of LLOV in *M. schreibersii*². This was followed by a report on seropositivity among Schreiber's bats in Spain, suggesting the circulation of LLOV in that area³. Nearly two decades have now passed since the discovery of LLOV and many questions remain unresolved regarding the nature of the virus, the risk of zoonotic spillover, and most importantly, the pathogenic potential for bats and humans⁴. Our main goal was to develop novel sampling protocol and study design for viral discovery and monitoring in bats to support multi purpose research activities (ie. detection, sequencing, serology, isolation) with minimal conservational effect and disturbance. For this purpose, we started to implement on-site laboratory techniques which could accelerate research activity and serve as an intervention tool for the next pandemics. We tested this method within the framework of LLOV surveillance program, which is a barely known member of *Filoviridae*⁵.

Challenges to overcome



On-site Laboratory Workflow



References

- ¹ Negredo A, Palacios G, Vázquez-Morón S, et al. Discovery of an ebolavirus-like filovirus in europe. *PLoS Pathog*. 2011;7(10). doi:10.1371/journal.ppat.1002304
- ² Kemenesi G, Kurucz K, Dallos B, et al. Re-emergence of Lloviu virus in Miniopterus schreibersii bats, Hungary, 2016 correspondence. *Emerg Microbes Infect*. Published online 2018. doi:10.1038/s41426-018-0067-4
- ³ Ramirez de Arellano E, Sanchez-Lockhart M, Perteguer MJ, et al. First Evidence of Antibodies Against Lloviu Virus in Schreiber's Bent-Winged Insectivorous Bats Demonstrate a Wide Circulation of the Virus in Spain. *Viruses*. 2019;11(4):360. doi:10.3390/v11040360
- ⁴ Görföl T, Tóth GE, Boldogh SA, Jakab F, Kemenesi G. Lloviu Virus in Europe is an Emerging Disease of Concern. *Ecohealth*. 2022;19(1):5-7. doi:10.1007/s10393-021-01574-4
- ⁵ Kemenesi G, Tóth GE, Mayora-Neto M, et al. Isolation of infectious Lloviu virus from Schreiber's bats in Hungary. *Nat Commun*. 2022;13(1):1706. doi:10.1038/s41467-022-29290-1



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

Gábor Tóth¹, Sándor Boldogh², Ágnes Balázs-Nagy³, Tamás Görföl¹, Gábor Kemenesi⁴

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

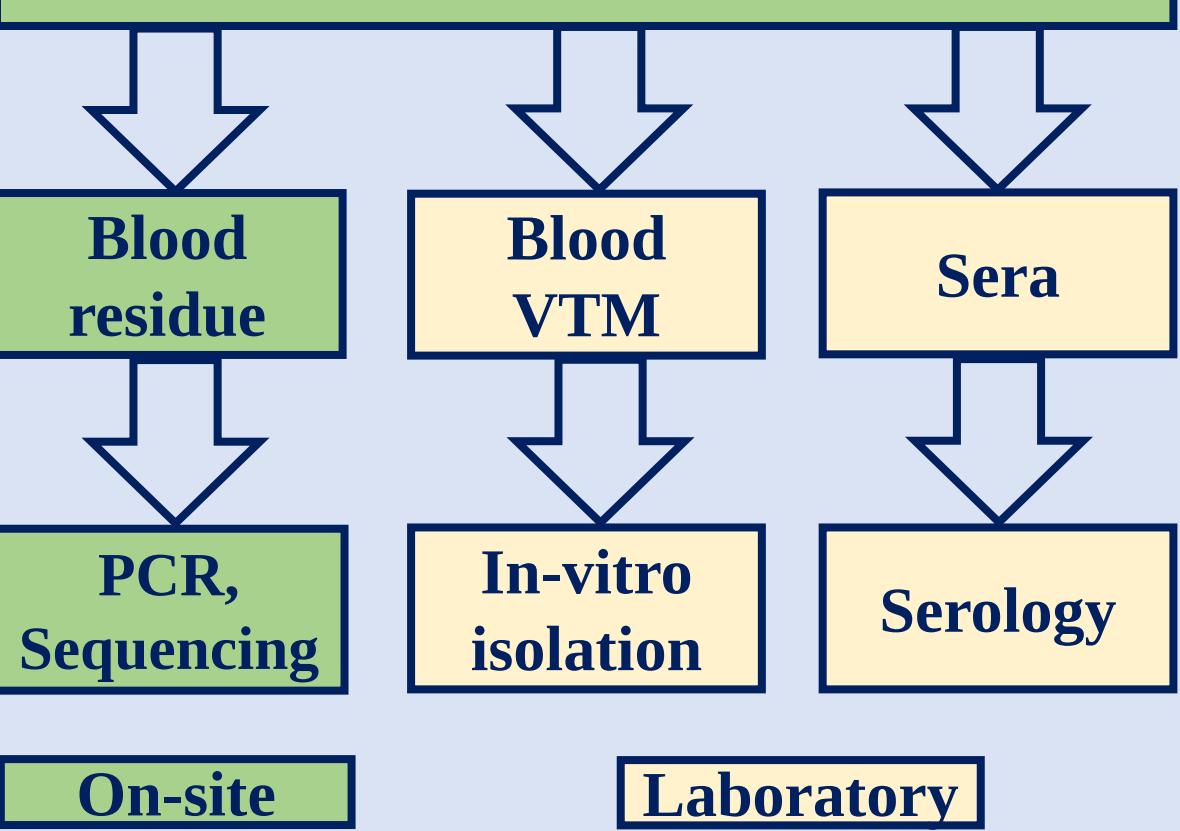


Lloviu cuevavirus sequencing protocol
Forked from nCoV-2019 sequencing protocol v2 (Gant)
Gábor Tóth¹, Gábor Kemenesi¹
National Laboratory of Virology (Hungary)
Szentagotai Research Centre Virology
Gábor Tóth

Aliquot your samples

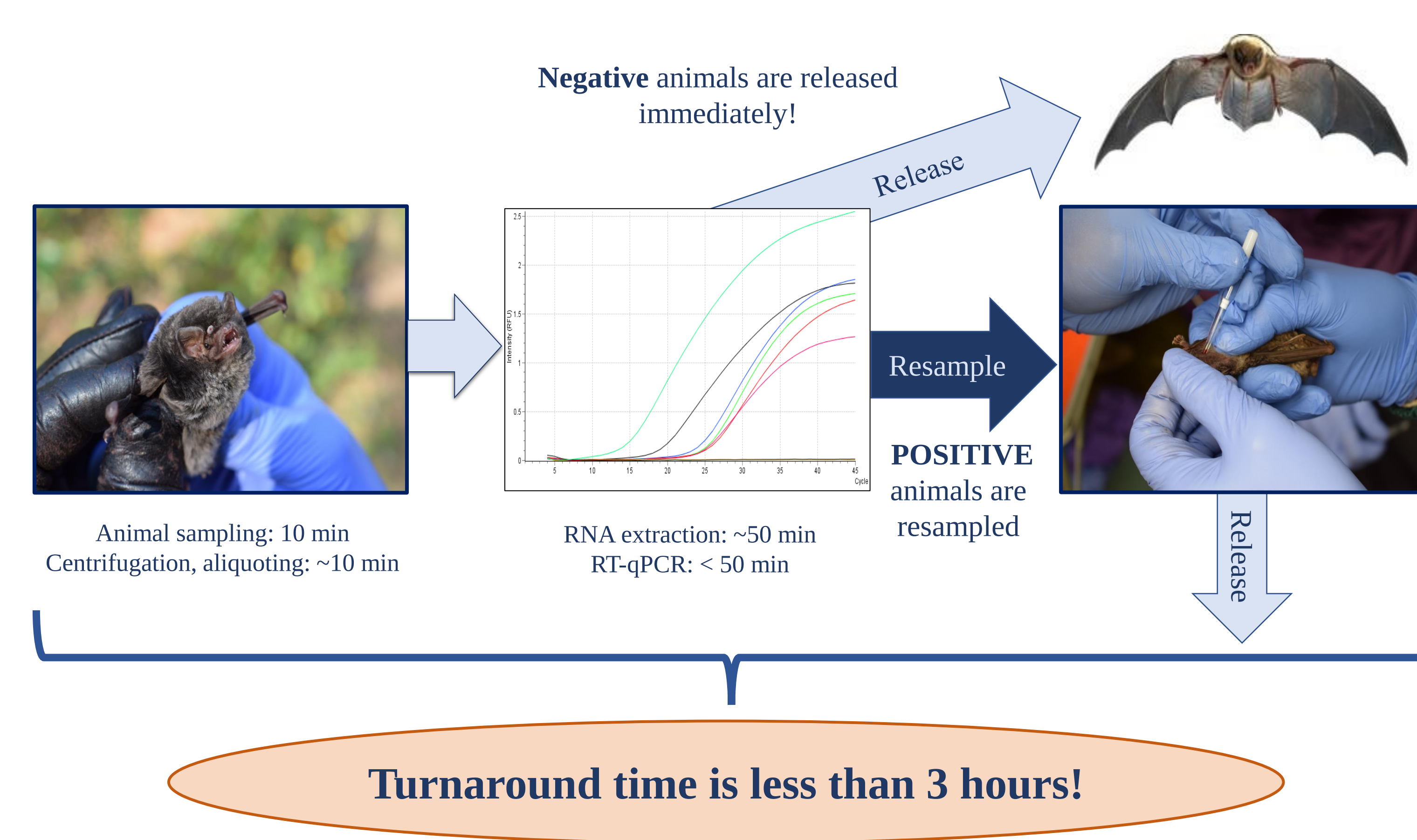


Centrifugation at 8000 rpm / 5 min

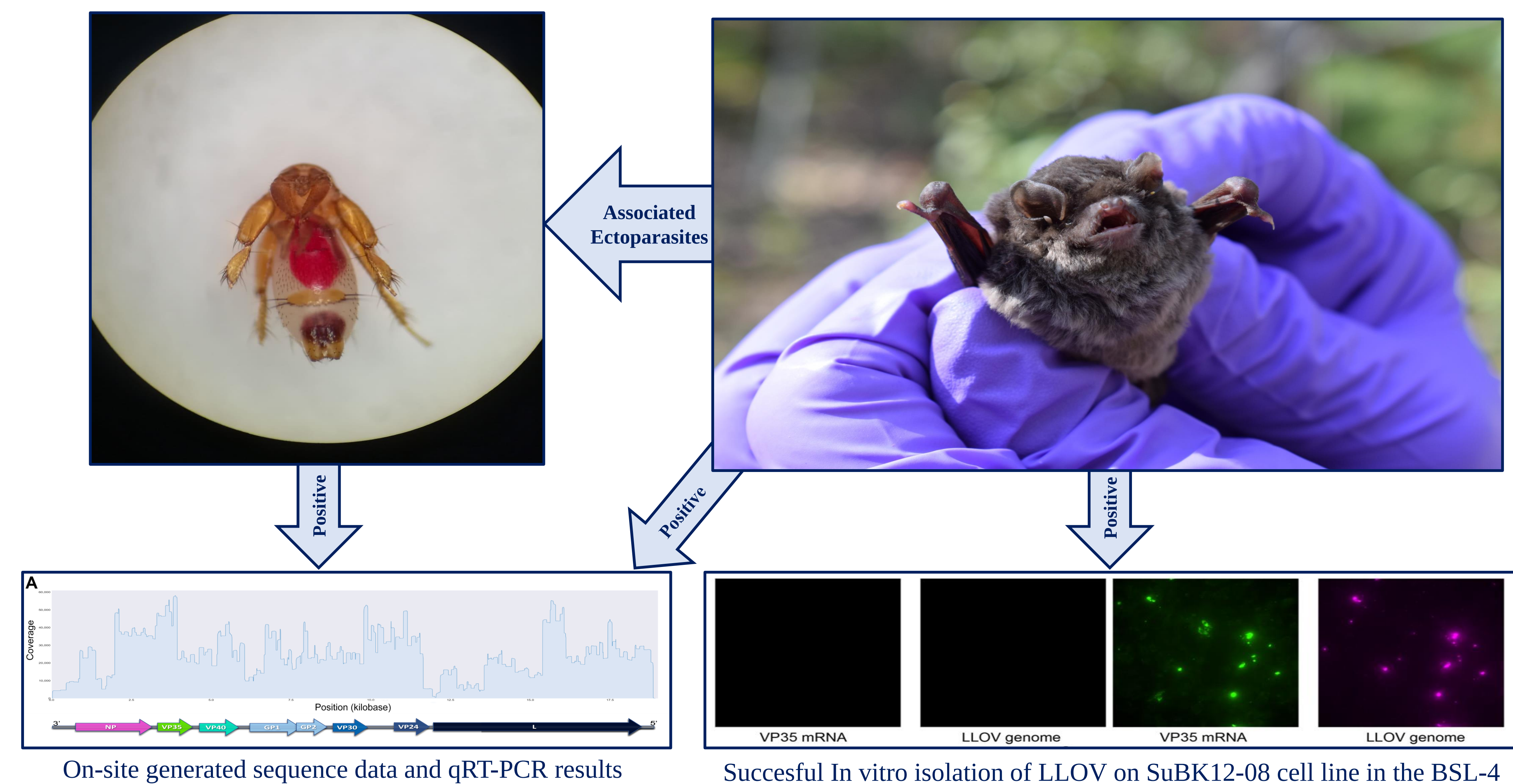


Reduce the freeze and thaw cycles!

Key feature - Concept of resampling



Lloviu cuevavirus (LLOV) on site surveillance



- Identification of active LLOV infection in bats
- Possibility to resample LLOV positive live animals
- On site generated sequence data from the complete coding region of LLOV
- Fresh sample for *in vitro* isolation
- Multipurpose sampling without any harm for the bats



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