Targeted amplicon based rapid sequencing of infectious viruses using portable Nanopore sequencer

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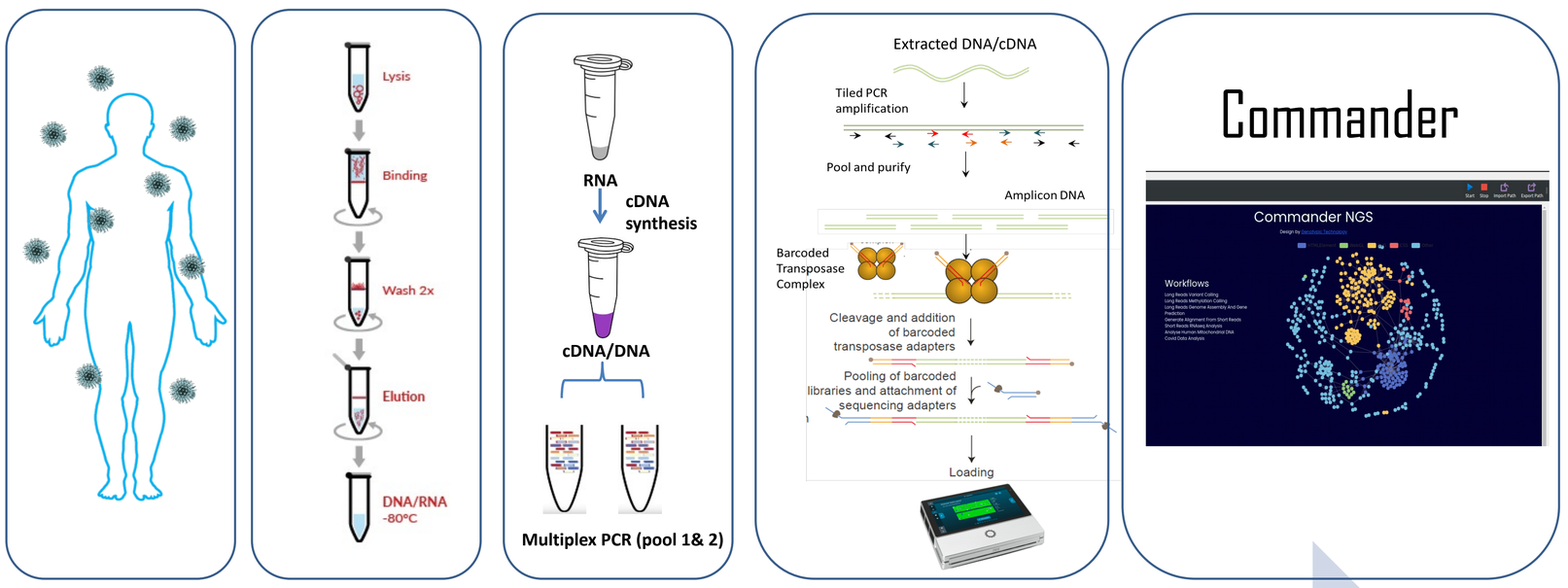
Background

- Human Viruses have been known to show genetic diversity, a consequence of ongoing evolution leading to novel serotypes.
- Rapid detection by whole viral genome sequencing is crucial for understanding of the diversity and can aid in predicting the vaccine efficacy of human viruses and of their relatives infecting other species.
- Rapid cost-effective sequencing approaches using tailed primers for SARS - CoV2 enabled surveillance across the globe, guided policy decisions, vaccine development and treatment of COVID19[1,2].

Aim

- Design a tailed primer approach[1] for targeted rapid and cost-effective method for sequencing of DNA and RNA viruses using Nanopore sequencing technology across multiple infectious viruses including Human Adenovirus, Measles, Kyasanur Forest disease virus (KFDV), Rabies virus and Norovirus.

Methodology



Reagents from Oxford Nanopore Technology (ONT) : Rapid Barcoding Kit 96 (SQK-RBK110.96**), Flow Cell (R9.4.1):** FLO-MIN106D
Reagents from NEB: LunaScript® RT SuperMix Kit (E3010L), Q5® High-Fidelity 2X Master Mix **(**M0492L**)**

Results

- Primers were designed using reference viral genomes to amplify approximately 1.2 to 3.5 kb of the target regions decreasing the number of primers required and thus reducing possible mismatches and/or undesired interactions (figure 1&Table 1).
- A total of 6, 11 and 8 pairs of tailed primers were designed to amplify complete genomes of rabies virus (11.8 kb), Human Adenovirus and KFDV viruses respectively (Figure 1 and table 1). Data not shown for Norovirus and Measles.
- A minimum of 40,000 reads per sample was generated on MinION Mk1c and rawdata was aligned to respective genomes using in-house Commander™ software, which has integrated analysis pipeline in the backend and uses minimap2 aligner to generate SNP and indel calls and subsequently a consensus sequence (Figure 2)

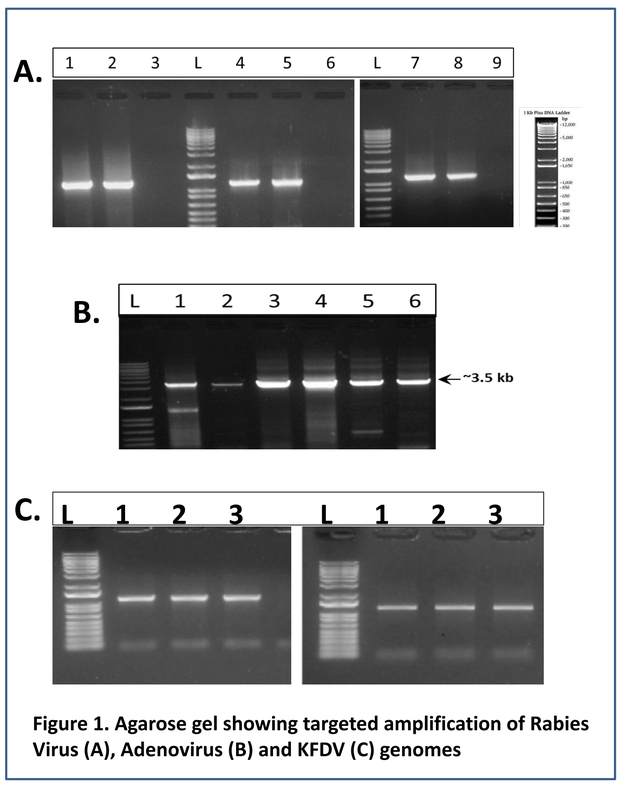


Figure 1. Agarose gel showing targeted amplification of Rabies Virus (A), Adenovirus (B) and KFDV (C) genomes

Table 1. Details on viruses targeted in this study

Viruses	Type	Genome size (Kb)	# Primers pairs	Amplicon size (bp)
Rabies	RNA	11.8	11	1200
Human Adenovirus	DNA	35	11	3500
KFDV	RNA	11	10	1200
Measles	RNA	16	15	1200
Norovirus	RNA	7.5	7	1200

Table 2. Genome coverage vs read depth

Depth	Rabies Virus (Genome Coverage %)		Human Adenovirus (Genome Coverage %)	KFDV virus (Genome Coverage %)	
	Sample _RV1	Sample_RV2	Sample_HadV1	Sample _KFDV1	Sample _KFDV2
at 1X	100	100	99.85	94.02	92.33
at 10X	97.28	99.5	99.80	93.95	89.65
at 100X	94.11	98.97	99.73	93.94	89.63

Table 3. Number of mutations identified in Rabies, Human adenovirus and KFDV viral genomes

Rabies	Average read depth	Number of Mutations
Sample_RV1	2000	19
Sample_RV2	2000	21
Human Adenovirus		
Sample_HadV1	3000	518
KFDV virus		
Sample_KFDV1	500	19
Sample_KFDV2	2000	21

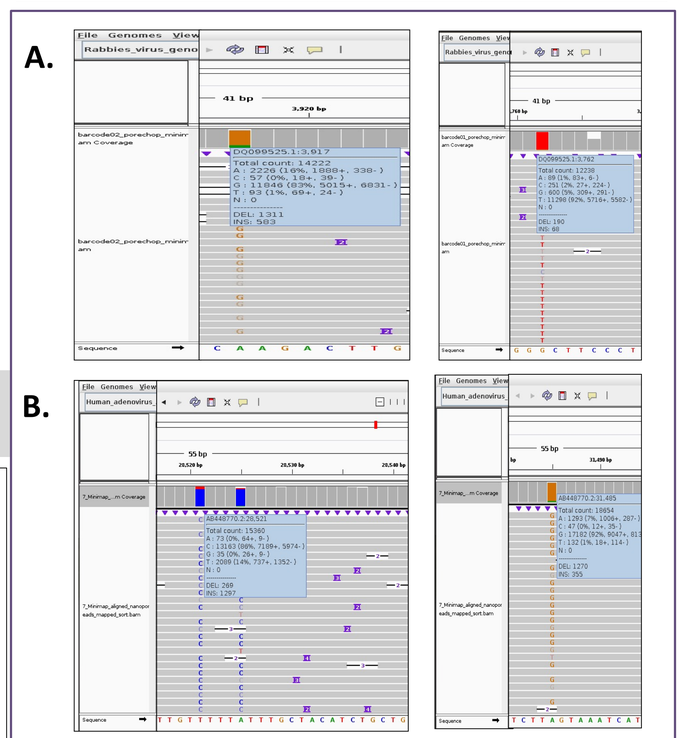


Figure 2. IGV images showing variants/mutations identified in Rabies Virus (A), and Adenovirus (B) genomes

Summary

- Our primer pools showed specific amplification with the very low input DNA/RNA samples across multiple viruses including Human Adenovirus, Measles, Kyasanur Forest disease virus (KFDV), Rabies virus and Norovirus.
- The complete workflow from multiplex PCR to sequence analysis was completed in less than 12 hours' time, aided by automated analysis of sequence data using Commander software developed in-house
- From our analysis minimum of 20,000 reads per sample with amplicon size of 1.2 kb is ideal to achieve 1000x coverage for viral genome size ranging from 10-15 kb and cost per sample would be $20 to $25.
- This approach can be employed during several other viral outbreaks like, Influenza, Dengue, Ebola etc. using Nanopore technology with reduced turnaround time.

References:

1. Nikki E Freed, Markéta Vlková, Muhammad B Faisal, Olin K Silander, Rapid and inexpensive whole-genome sequencing of SARS-CoV-2 using 1200 bp tiled amplicons and Oxford Nanopore Rapid Barcoding, *Biology Methods and Protocols*, Volume 5, Issue 1, 2020, bpaa014, https://doi.org/10.1093/biomethods/bpaa014
2. Quick, J., Grubaugh, N., Pullan, S. *et al.* Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. *Nat Protoc* **12,** 1261–1276 (2017). https://doi.org/10.1038/nprot.2017.066
3. https://www.protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bp2l6n26rgqe/v3