



# Long-read Native RNA Sequencing of Multiple Pathogenic RNA Viruses

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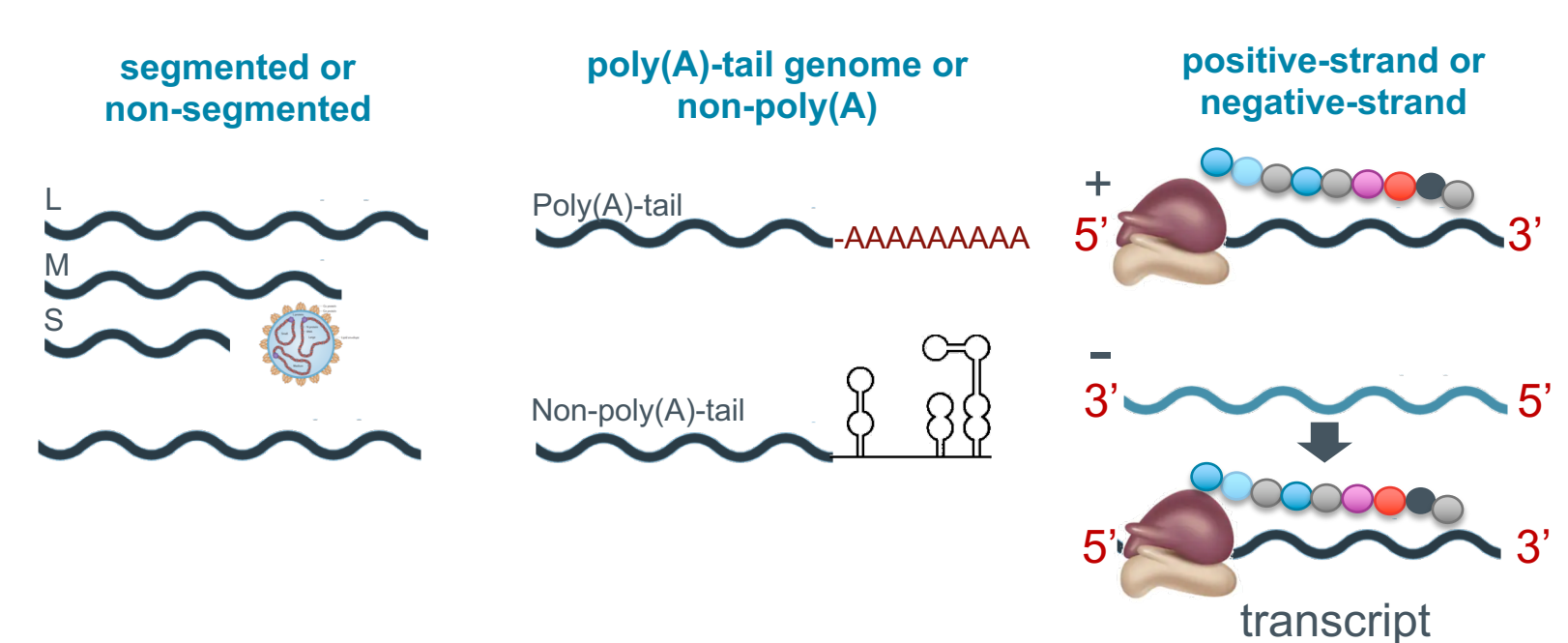


## Background

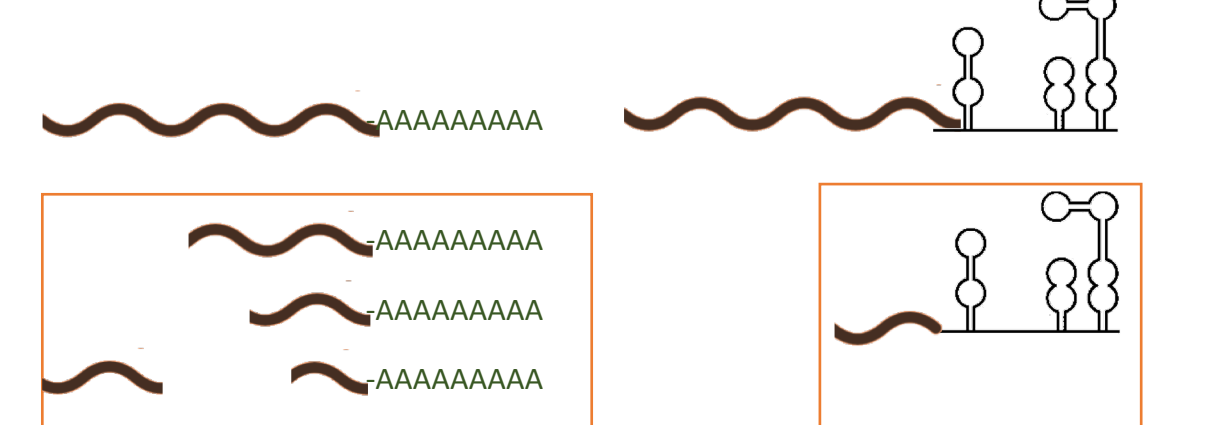
Single stranded (ss)RNA virus is the major cause of emerging diseases in humans



Genome of ssRNA virus  
RNA genome can be either...



Subgenomic rna /transcript



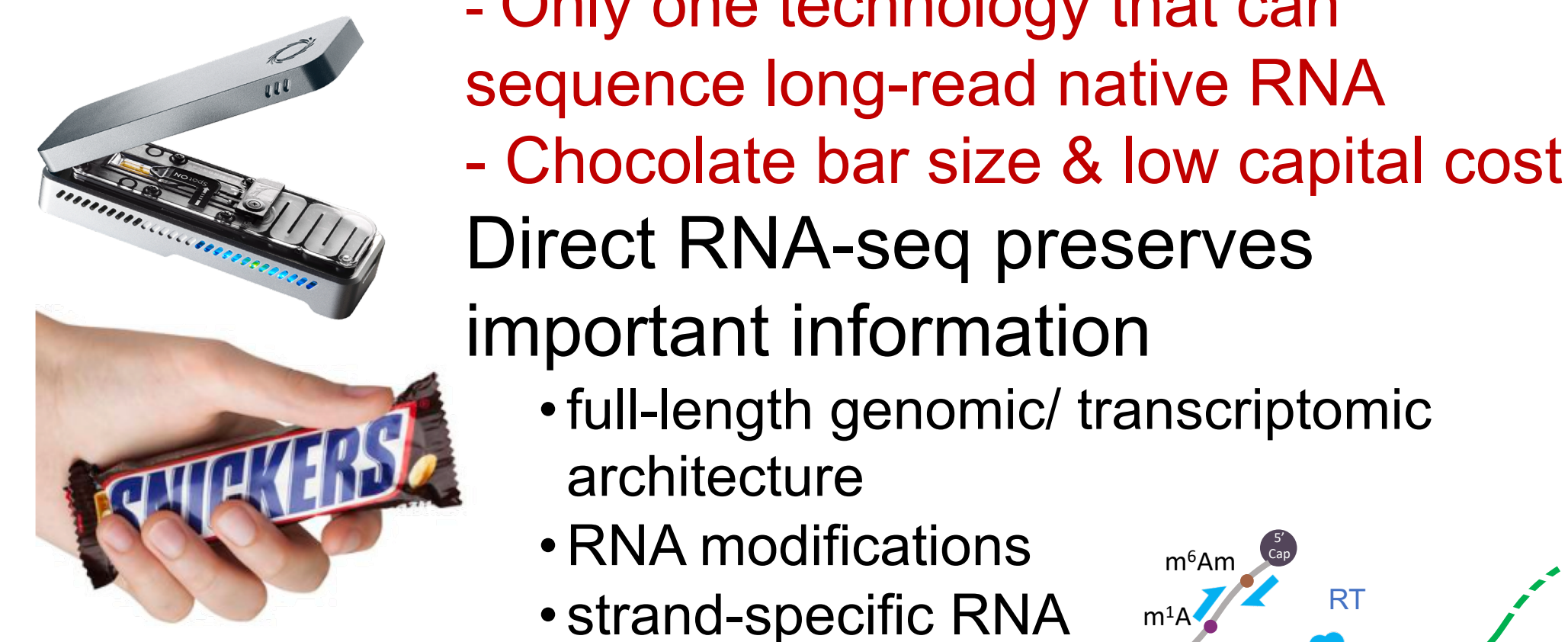
## Goal

Sequence everything in one run using Direct RNA-seq

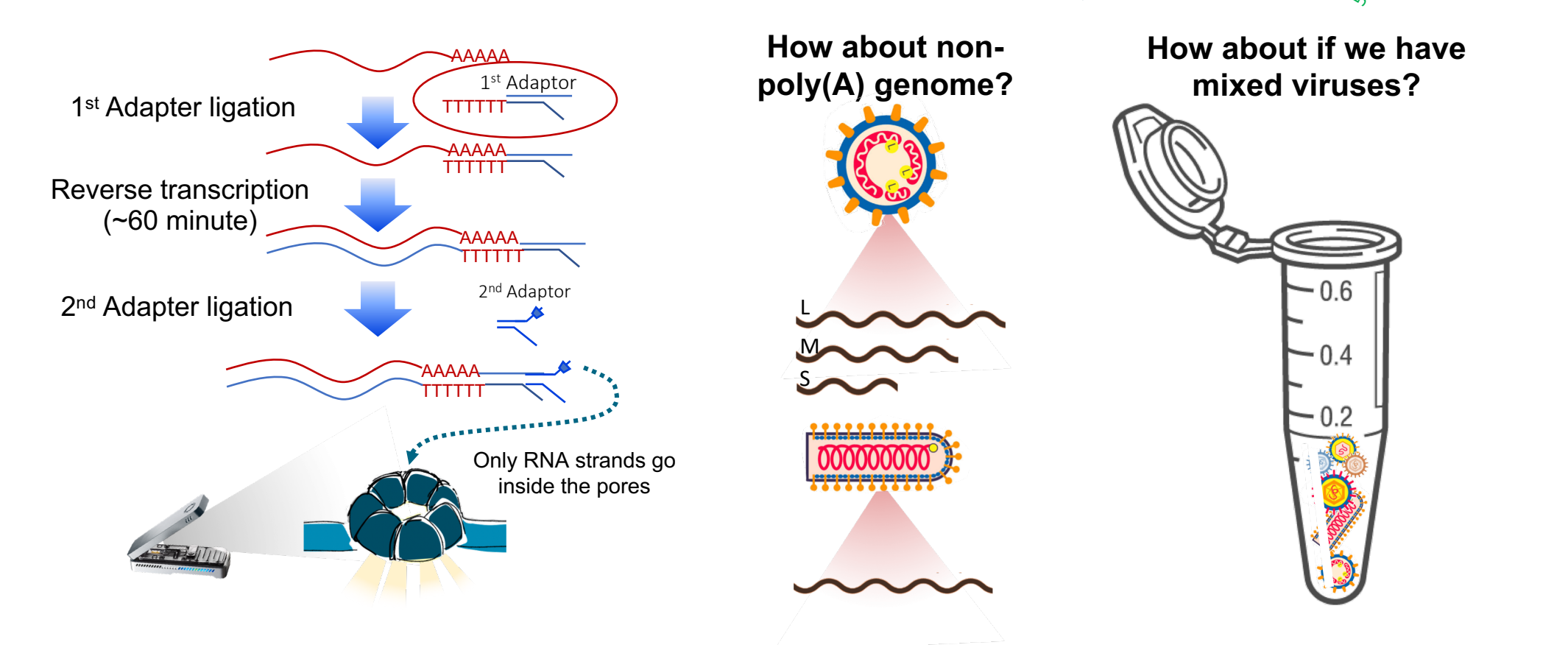
## Funding

Helen Adams & Arkansas Research Alliance Professor & Chair  
The National Institute of General Medical Sciences of the NIH award no. P20GM125503  
The NIH award no. R01AI103053

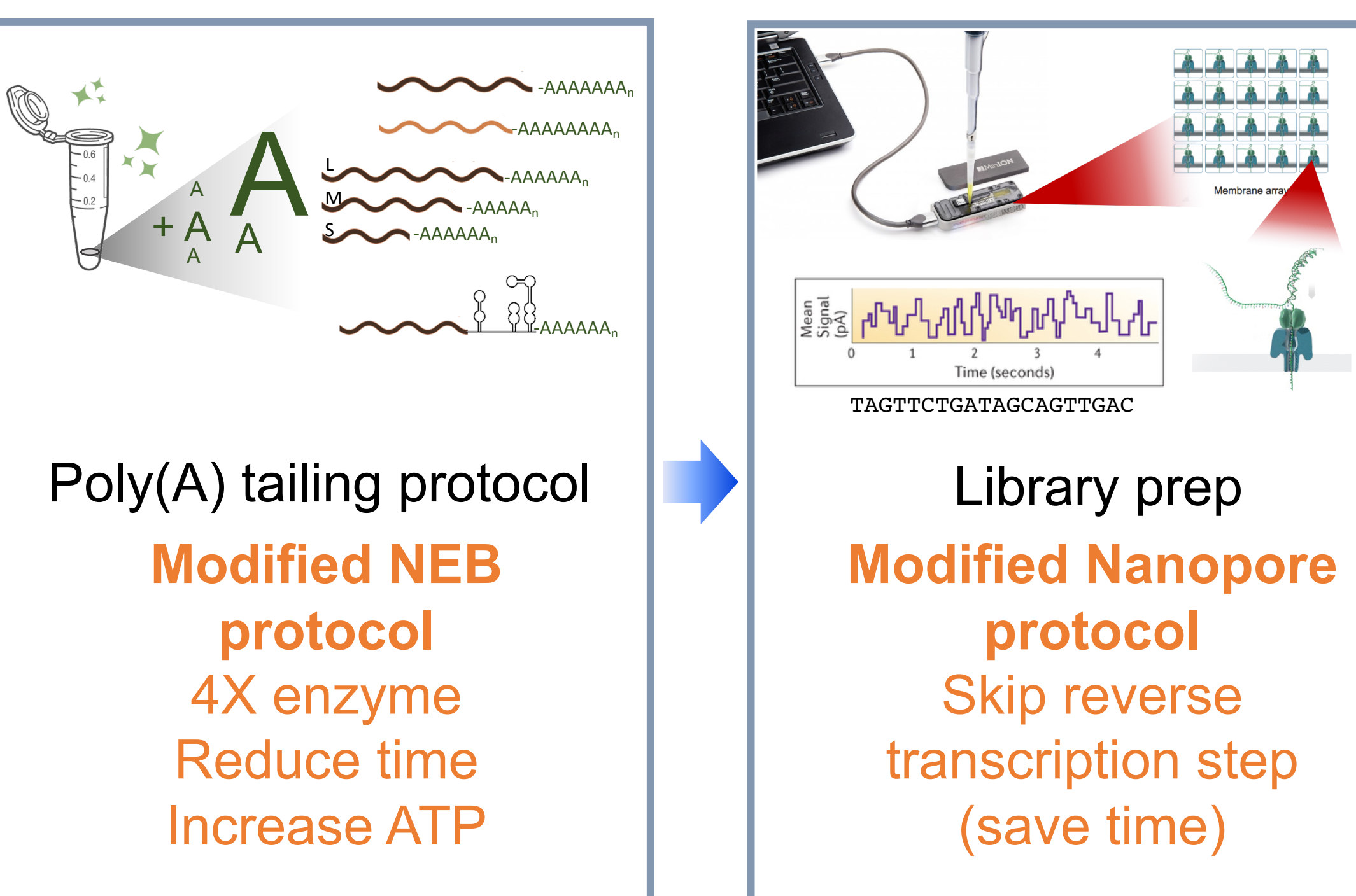
## Why nanopore sequencing technology?



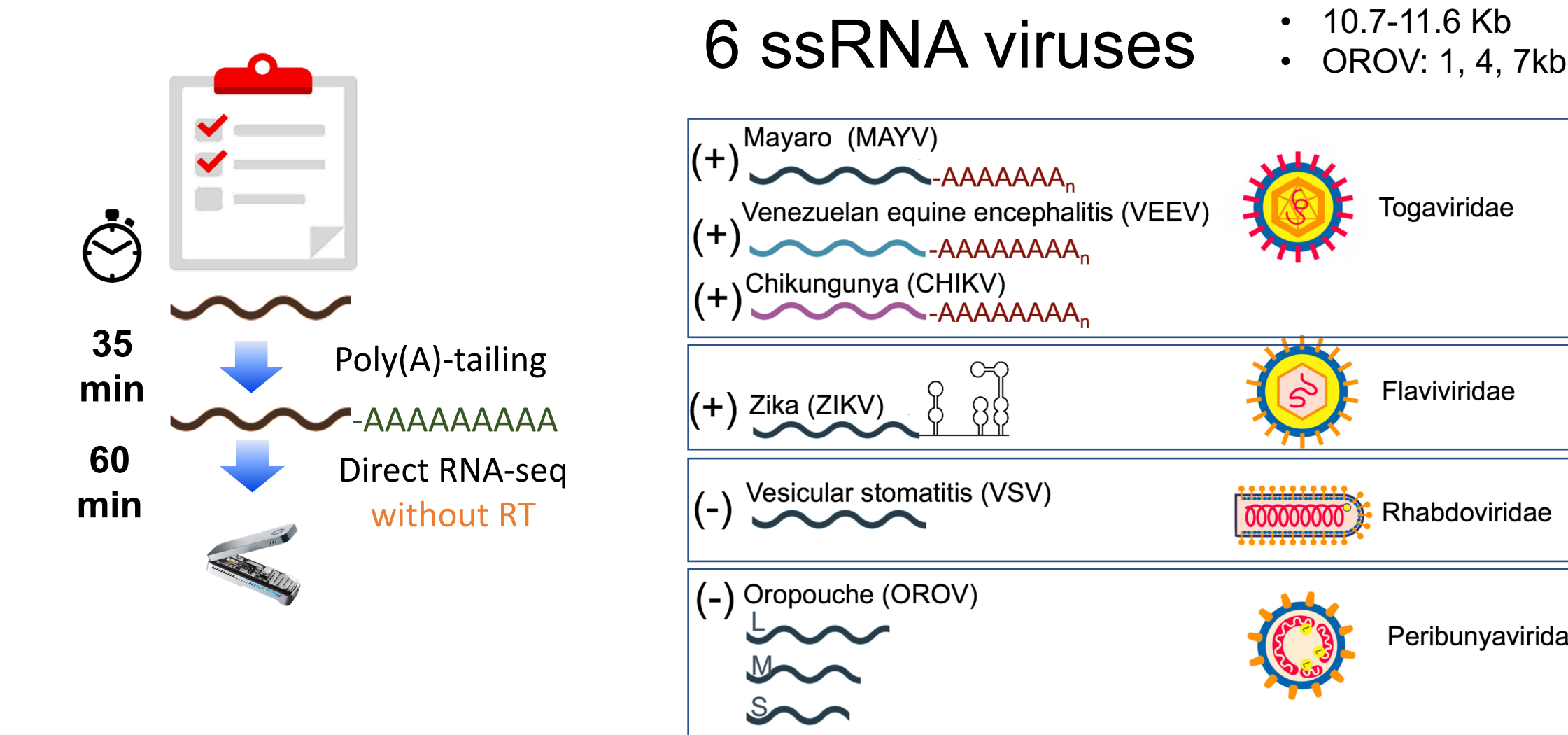
Short-read sequencing method relies on reverse transcription (RT) process which requires primers & amplification. → introduce biases & artifacts



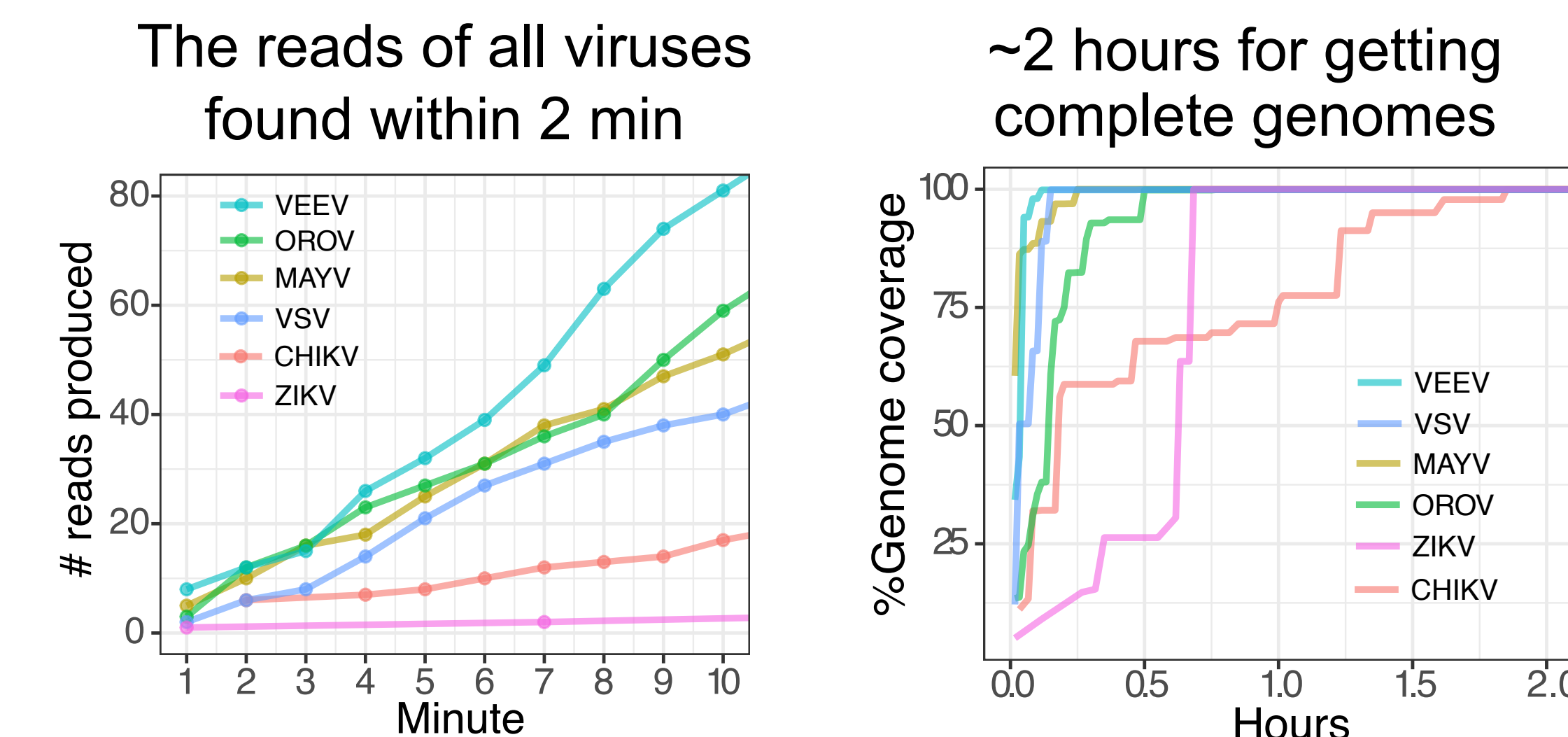
## Strategy using E. coli Poly(A) Polymerase (NEB)



## Study Design

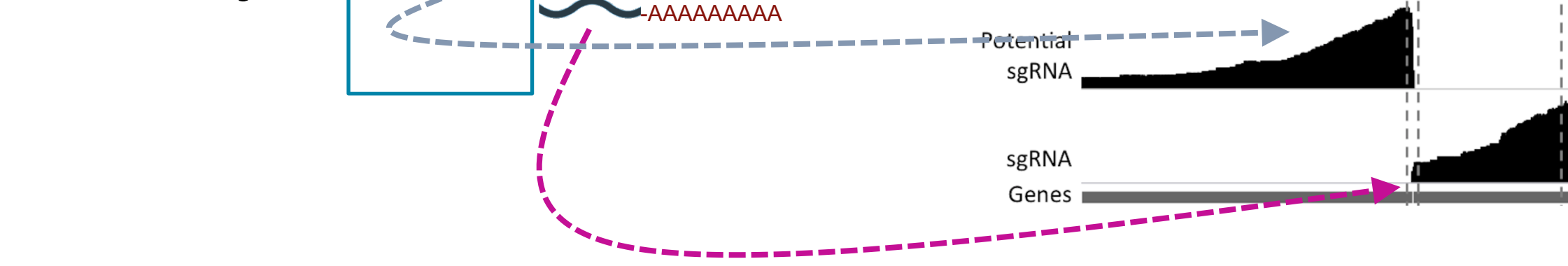


## Results



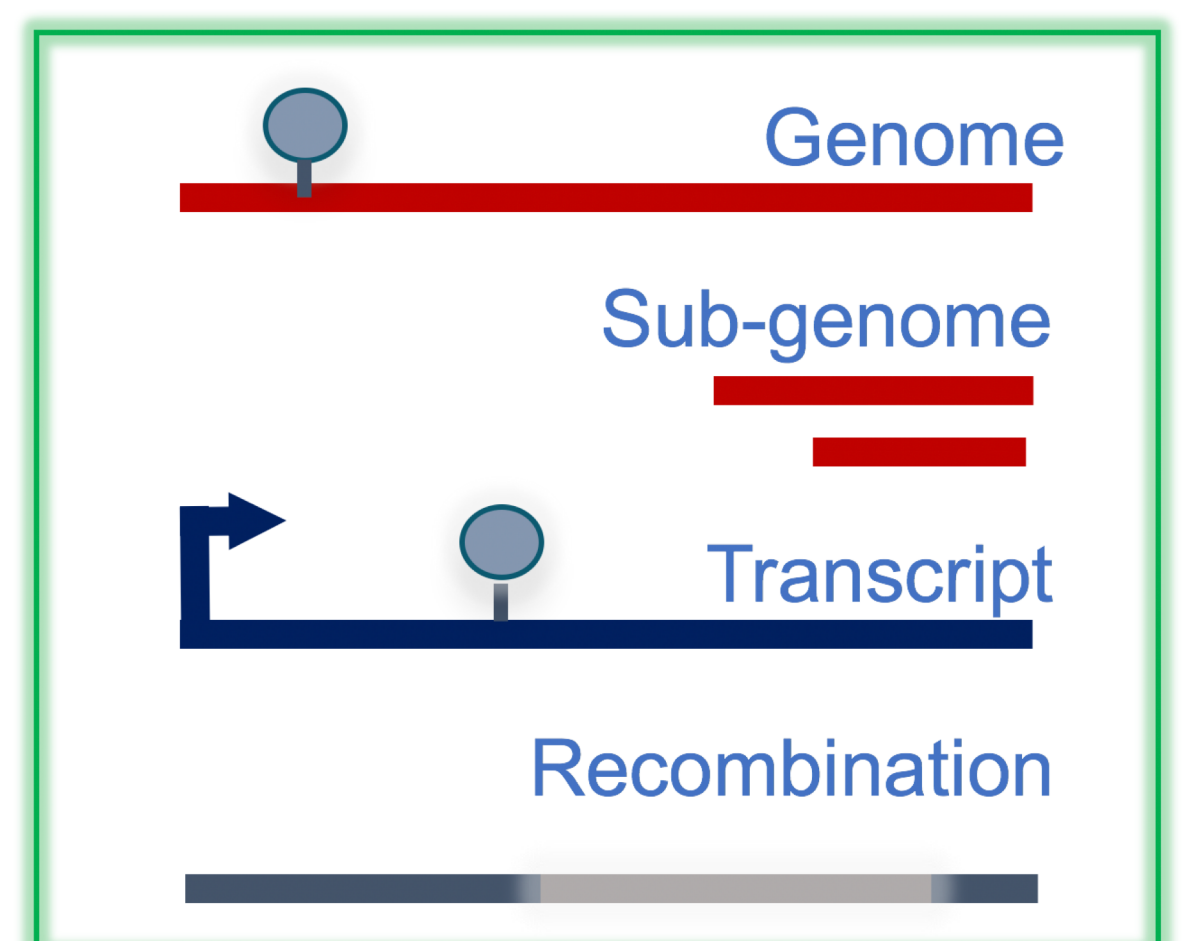
Virus*	(+) / (-) ssRNA	Genome size (Kb)	RNA Input (ng)	Coverage %	Mean coverage	Longest mapped length (kb)	Mapped virus (%)	Mapped host (%) (host rRNA)	Low quality seq (%)	Total number of reads
<b>Poly(A)-tail genome</b>										
MAYV	(+)	11.14	32	100	57x	11.11	0.93	72.40 (32.92)	21.76	85711
VEEV	(+)	11.45	35	100	218x	11.05	1.72			
CHIKV	(+)	12.04	48	100	49x	10.09	0.37			
<b>Non-poly(A)-tail genome</b>										
ZIKV	(+)	10.78	18	100	10x	7.29	0.04	72.40 (32.92)	21.76	85711
VSV	(-)	11.16	16	100	181x	10.87	1.16			
OROV**	(-)	0.96 (S) 4.39 (M) 6.85 (L)	66	100	81x 48x	4.31 6.72	0.95 1.08 0.24			

## Novelty !!

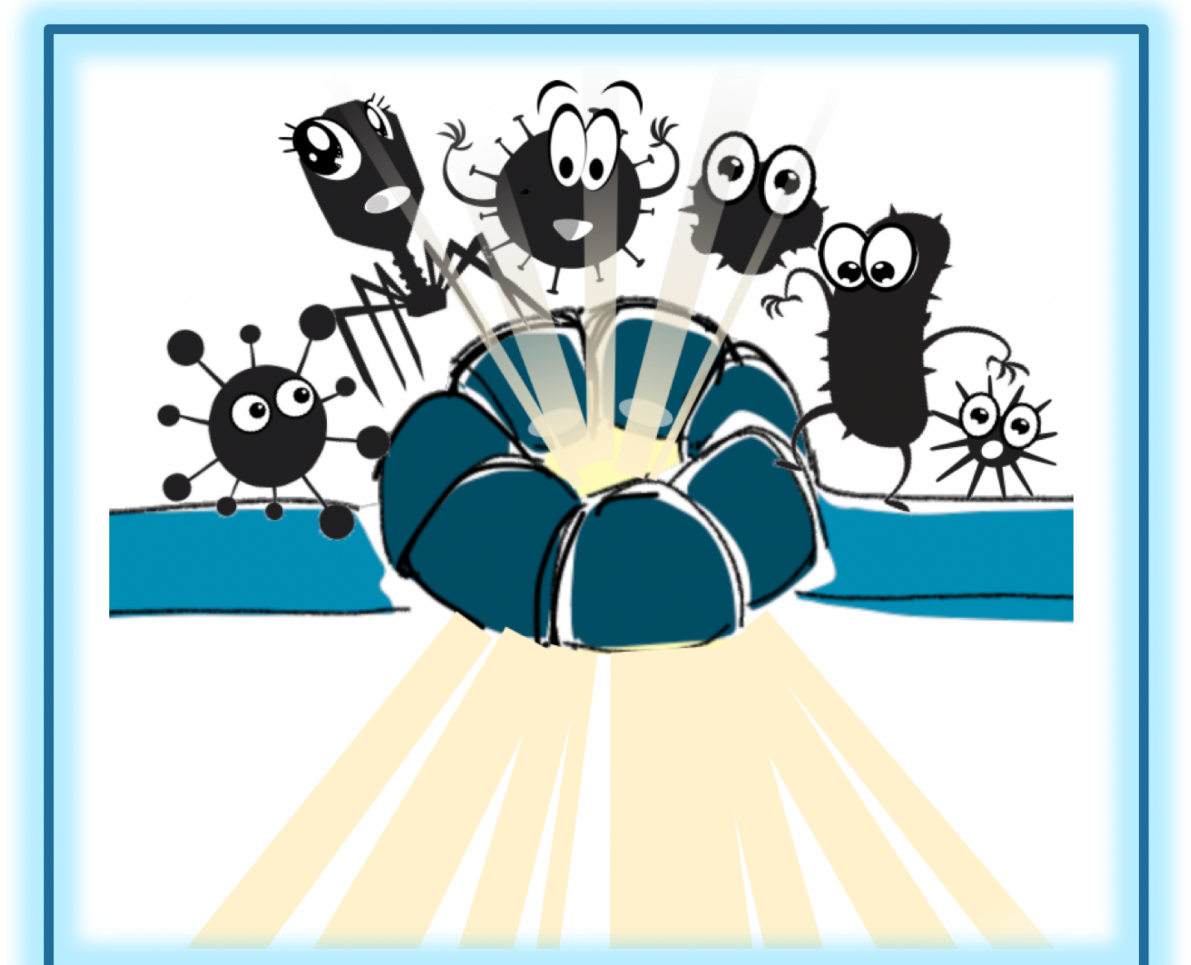


## Conclusion

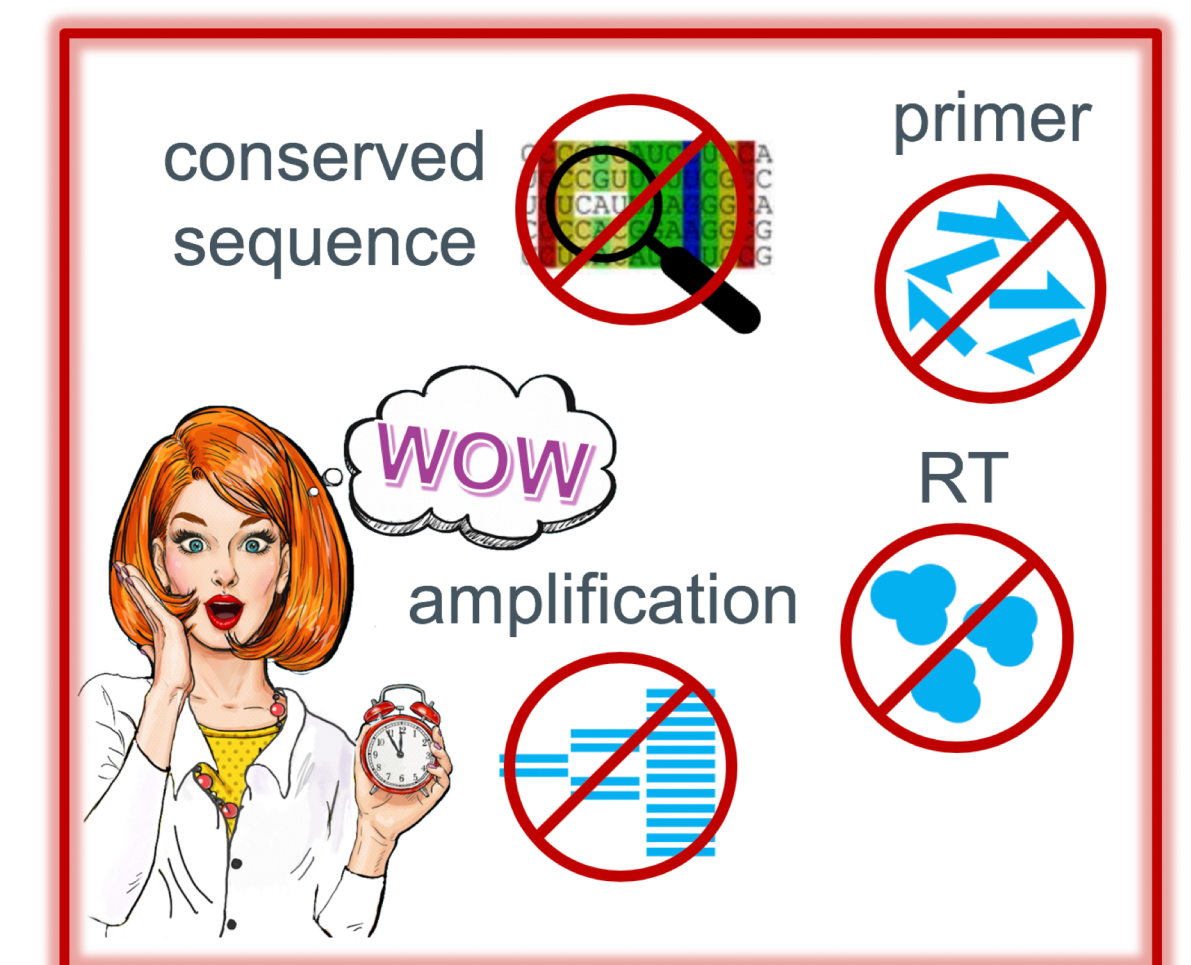
Multi-layers information "at a time"



Multiple RNA viruses "in parallel"



Eliminate complexity & "save time"



## Limitations of this protocol

- Requires high viral load
- Host RNA depletion strategy is needed
- Fully intact RNA is required
- Deep sequencing is needed for detection of RNA modifications

## Potential applications

- Viral transcriptomics/epitranscriptomics
- Comparison between cell lines/treatment
- Co-infection study
- Recombination detection
- Subgenomic identification

## Need more information?

Link to our publication  
February 2019 | Volume 10 | Article 260

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