CRISPR-Cas mediated detection of monkeypox virus

Towards point-of-care testing

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We would like to acknowledge the traditional custodians of the land on which the conference is taking place, the Gadigal people of the Eora Nation, and pay our respects to their elders past, present, and emerging.

2022 mpox outbreak – WHO declares Health Emergency

Outbreak: May - Nov 2022 – over 80,000 new cases of mpx in 110 countries

Cases: Predominately reported in men who have sex with men (MSM), with cases also described in women and infants

Monkeypox virus: Orthopoxvirus with **dsDNA** (~197 kb)

Diagnostics – predominately PCR, serologically cross-reactive with other orthopoxviruses - antigen and antibody detection methods lack specificity

Limited approved point-of-care (POC) tests for mpox – transmission prevention

Global Problem

Accurate diagnosis relies on expensive centralised laboratory tests (NAATs - PCR)

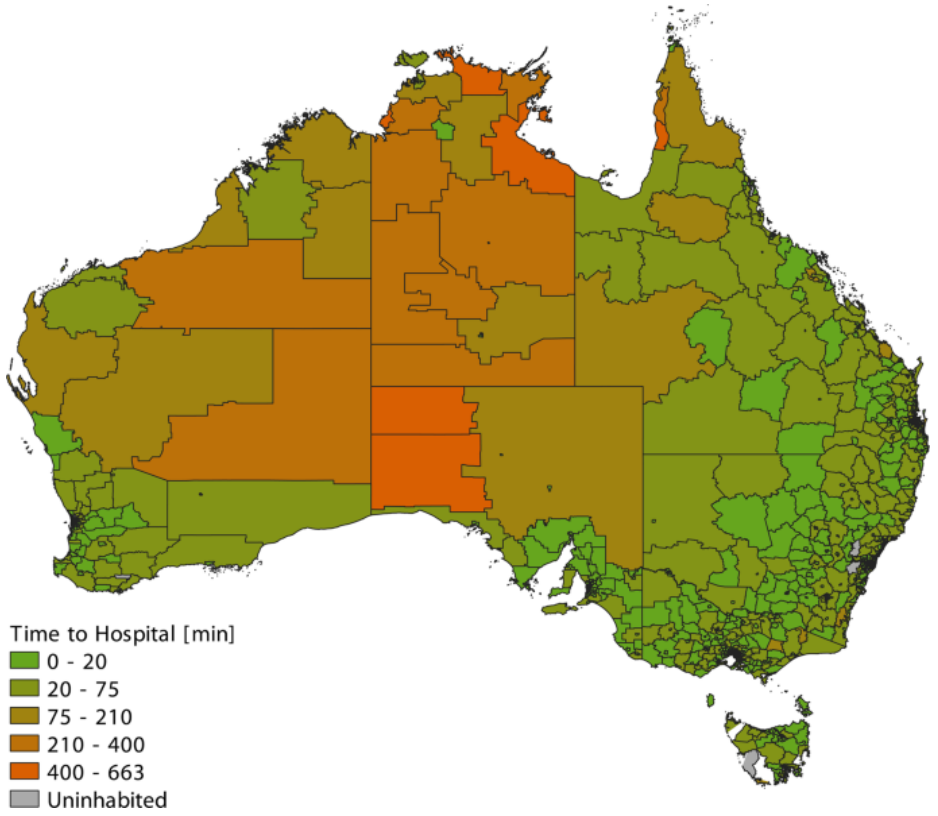
Impacts diagnostic strategies to interrupt transmission and prevent endemicity

Current challenges in Australia

Access & Duration - over two weeks for clinical samples to be processed from remote settings

Testing Platforms - small number of available testing platforms

Outbreak/Emerging Pathogen Response – critical shortages of reagents lead to extreme delays

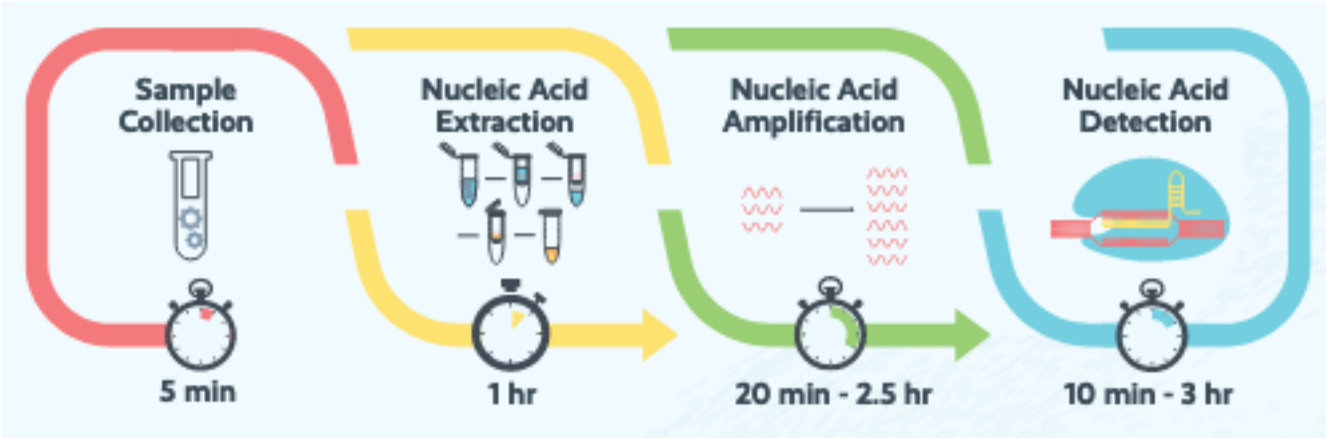


Barbieri & Jorm (2019) *Scientific Data*

Designing novel nucleic acid based POC tests for infectious diseases

The WHO ‘ASSURED’ criteria for diagnostic assays:

Affordable
Sensitive
Specific
User-friendly
Rapid and robust
Equipment-free
Deliverable to end users

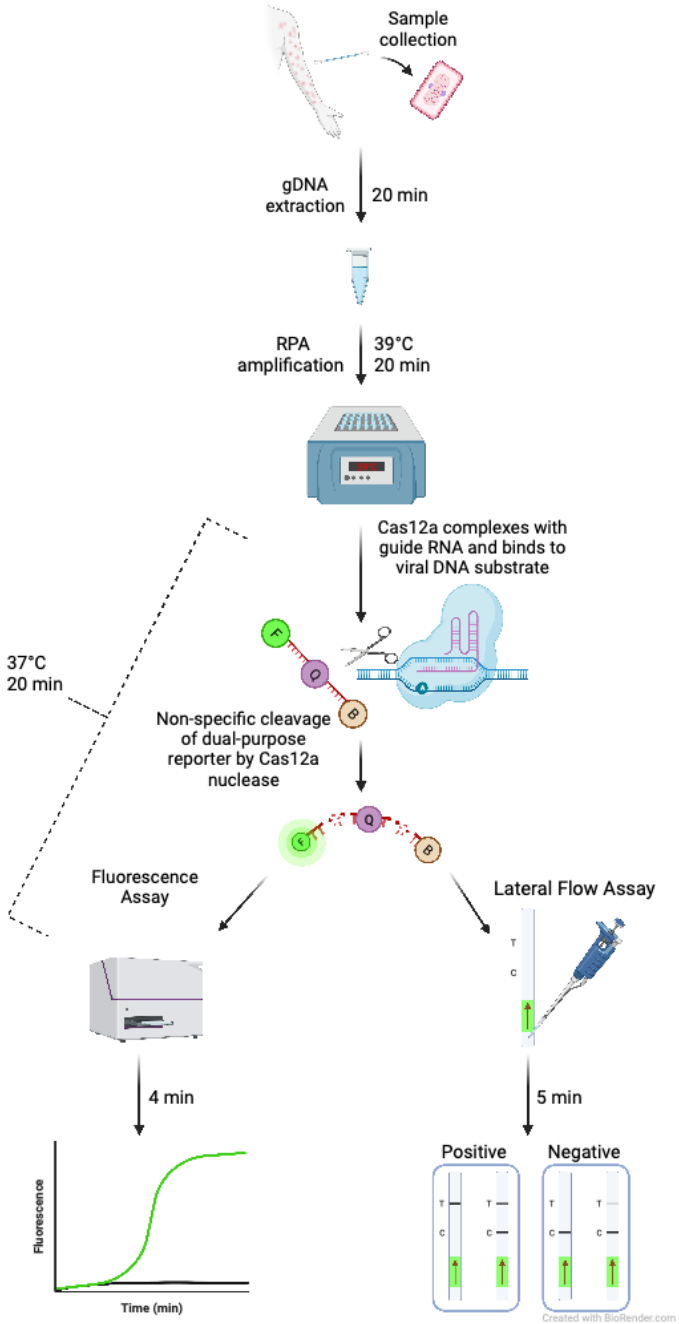


Kosack CS, Page AL, Klatser PR. A guide to aid the selection of diagnostic tests. Bull World Health Organ. 2017

Monkeypox virus detection using the MPV-CRISPR assay

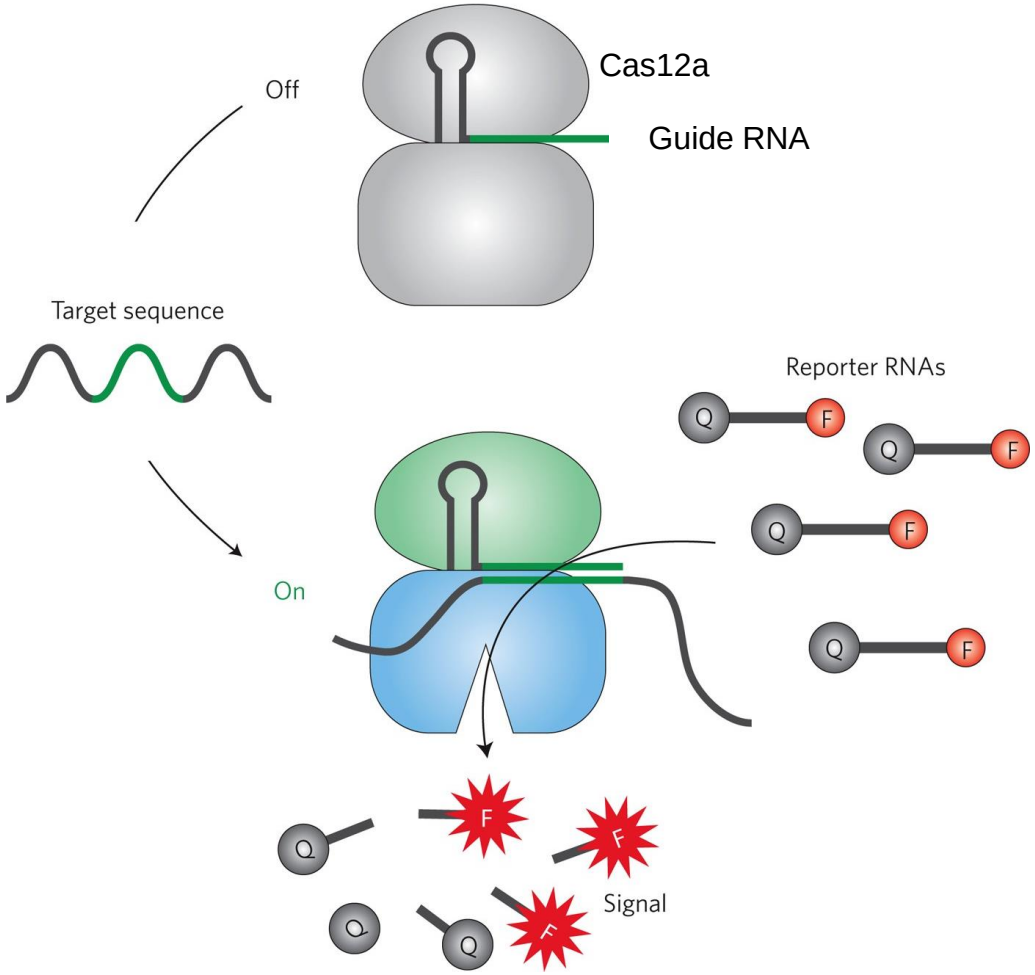
MPV-CRISPR workflow:

1. Genomic DNA extraction
2. Pre-amplification using RPA (20 min)
3. CRISPR-Cas12a assay (20 min)
4. Two possible readouts:
    1. Fluorescence OR
    2. Lateral flow



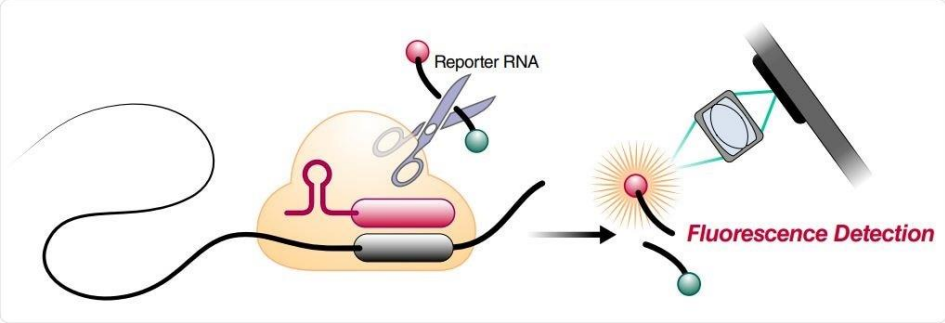
Exploiting the CRISPR-Cas system for diagnostics

Highly specific target cleavage



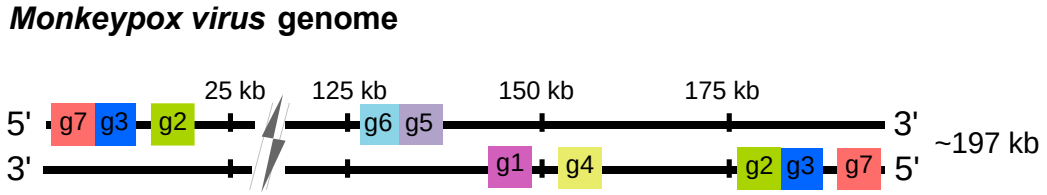
Adapted from Zuo et al. (2017) Nature biomedical engineering

Step 1: Design and evaluate performance of guide RNAs

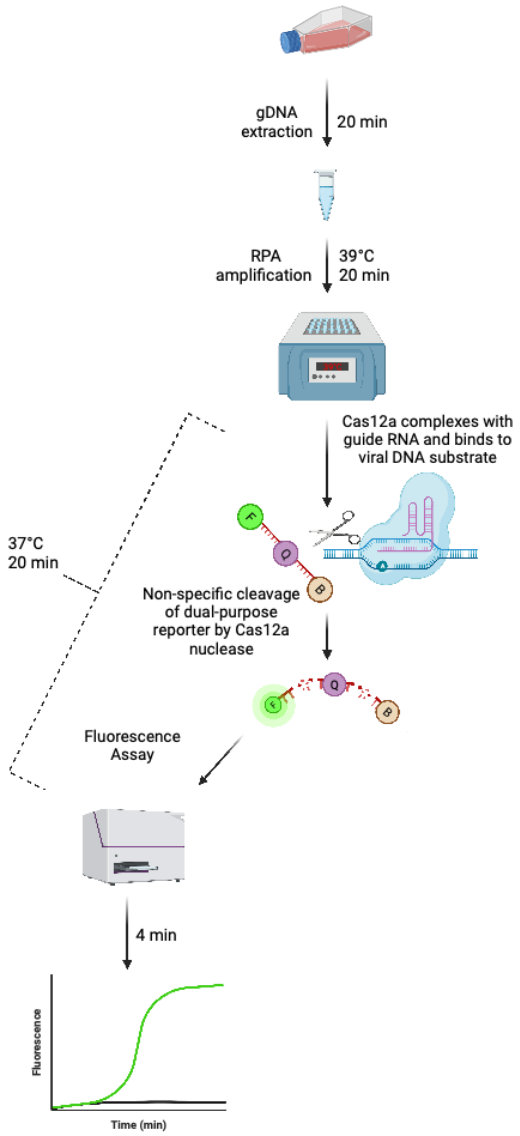


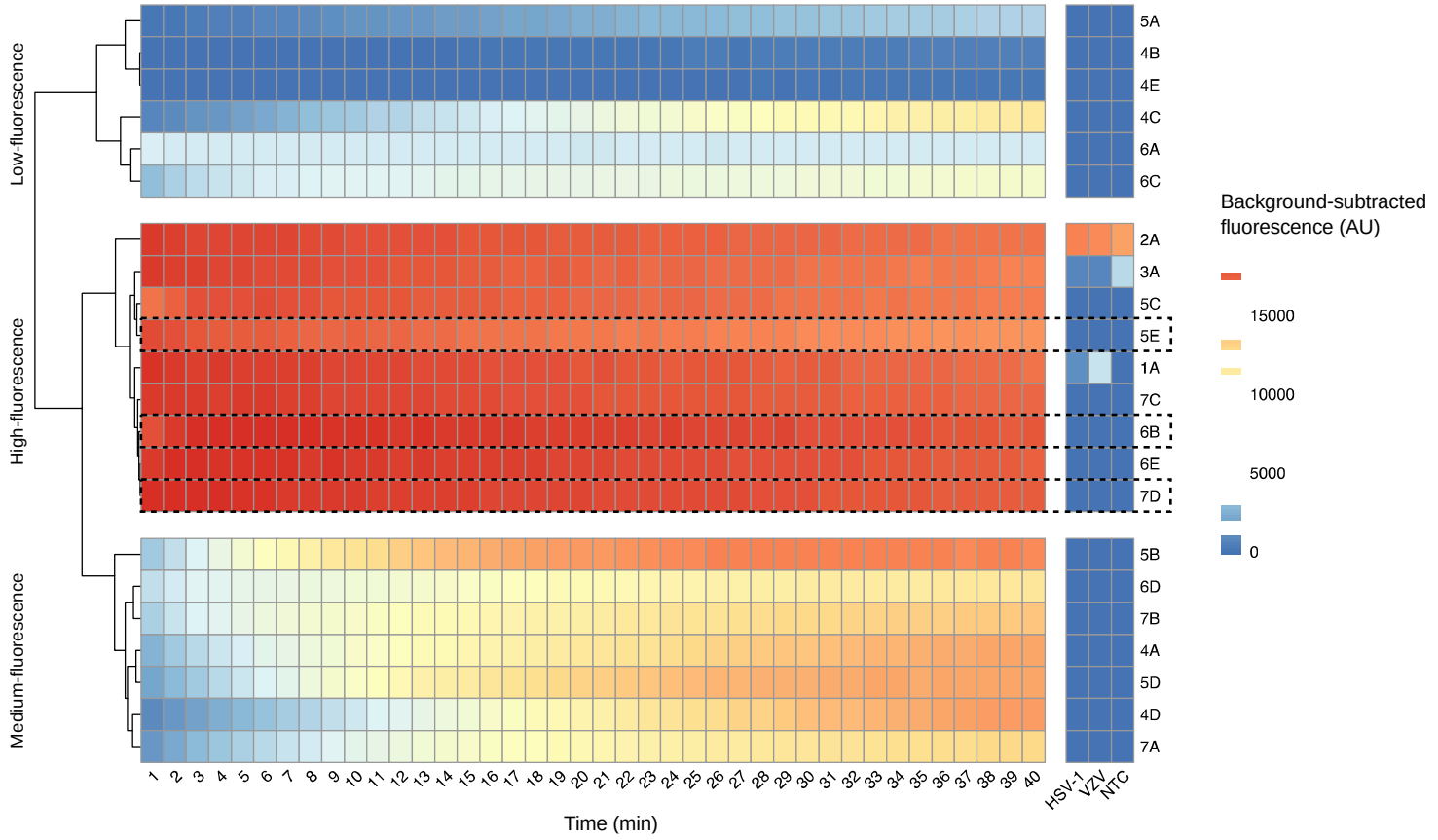
https://www.news-medical.net/news/20201004/

- CRISPR gRNAs and RPA primers were designed using a database of 523 MPV genomes
- 7 guides - 22 sets of primer/gRNA were tested



Step 1: Design and evaluate performance of guide RNAs

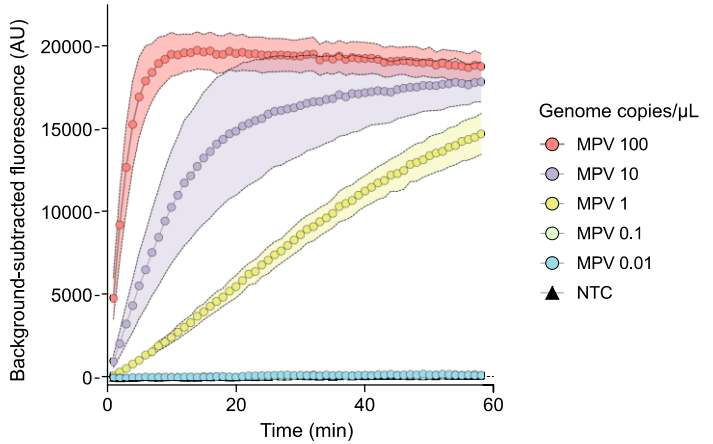




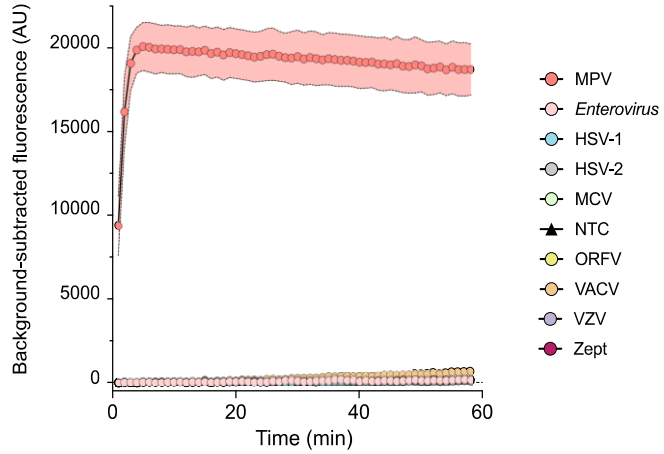
Step 2: Testing analytical sensitivity (LOD) and specificity

Detects single genome copies/ µL with no cross-reactivity

Sensitivity - Assay can detect single 1 copy/µL of spiked monkeypox virus gDNA

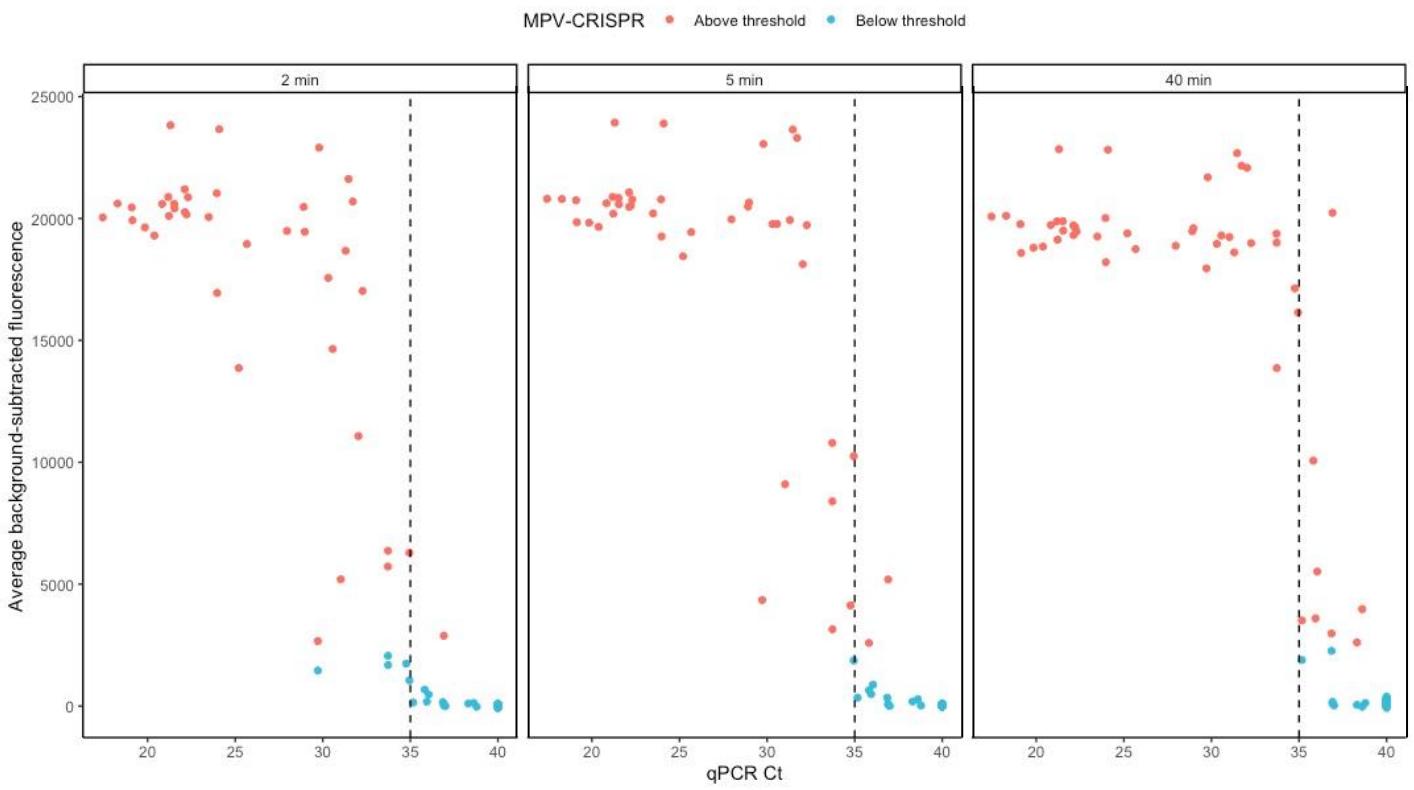


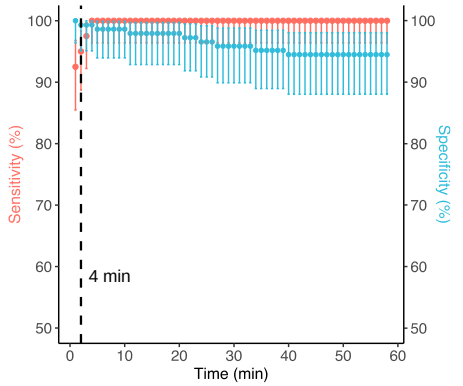
Specificity - No cross-reactivity against a panel of viruses

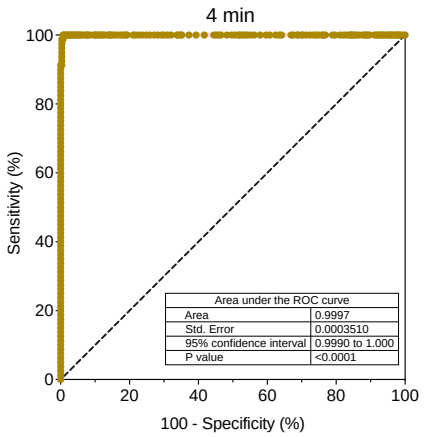


Step 3: Validation of MPV-CRISPR assay using clinical samples

Blind test of 185 clinical samples including oral, anal and skin lesion specimens







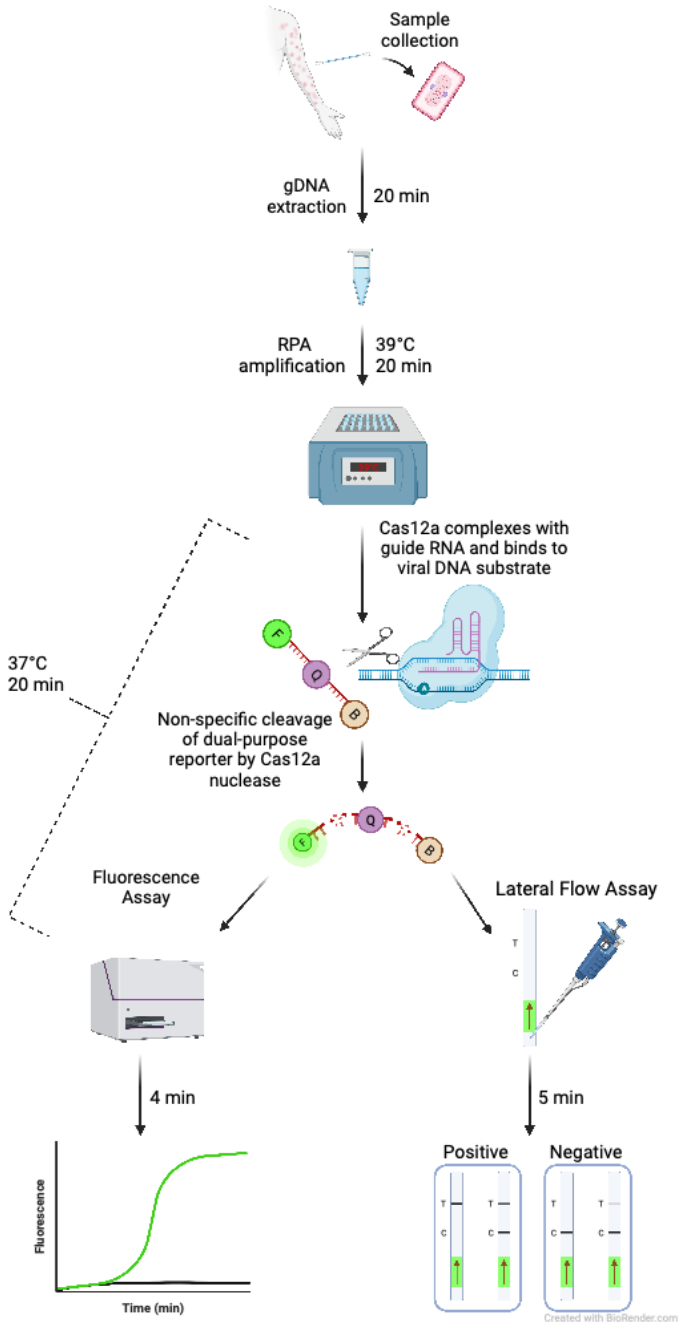
Step 3: Validation of MPV-CRISPR assay using clinical samples

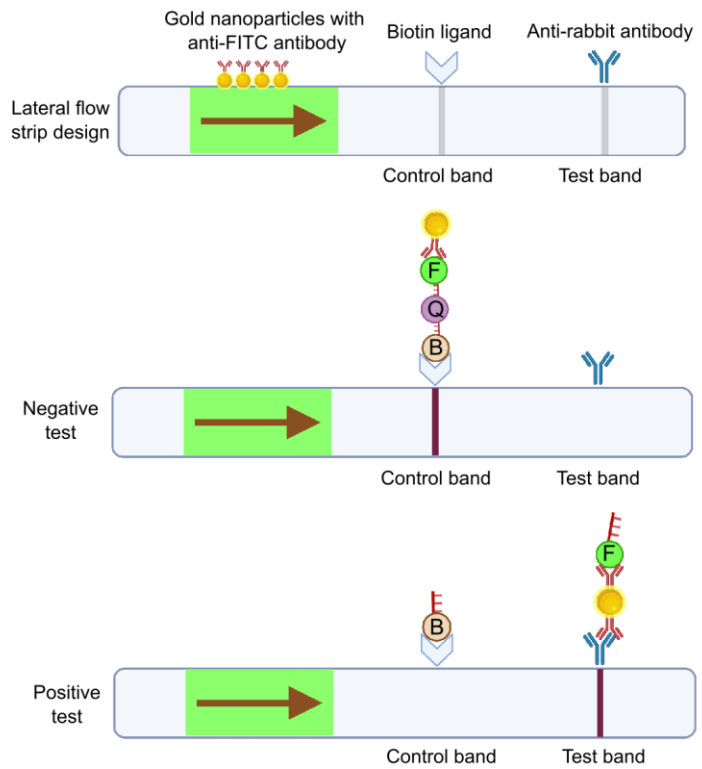
Strong concordance between MPV-CRISPR and gold-standard qPCR

Method	MPV-CRISPR
	fluorescence readout
	4 min (Ct ≤35)
True positive	40
False negative	0
Sensitivity (%)	100 (95% CI: 89-100)
Positive predictive value (%)	97.6 (95% CI: 86-100)
True negative	144
False positive	1
Specificity (%)	99.3 (95% CI: 96-100)
Negative predictive value (%)	100 (95% CI: 97-100)
MPV-CRISPR assay with fluorescence readout was performed with two experimental repeats, with at least one positive repeat interpreted as a positive result.	

- All MPV-positive samples detected within first 5 minutes
- 100% Sensitivity, 99.3% Specificity
- Discrepancies associated with low viral load samples

MPV-CRISPR assay with lateral flow readout capability





Validation of MPV-CRISPR assay using lateral flow readout

Strong concordance between MPV-CRISPR and gold-standard qPCR

Method	Concordance (Positive if Ct ≤35)	
	Visual (n=3)	Computational
True positive	40	40
False negative	0	0
Sensitivity (%)	100 (95% CI: 89-100)	100 (95% CI: 89-100)
Positive predictive value (%)	95.2 (95% CI: 83-99)	95.2 (95% CI: 83-99)
True negative	143	143
False positive	2	2
Specificity (%)	98.6 (95% CI: 95-100)	98.6 (95% CI: 95-100)
Negative predictive value (%)	100 (95% CI: 97-100)	100 (95% CI: 97-100)

- 100% sensitivity, 98.6% specificity
- Lateral flow readout showed 96% concordance with fluorescence readout
- Discrepancies associated with low viral load samples

Immediate goals

Dual-target assay design and moving from modular to one-pot

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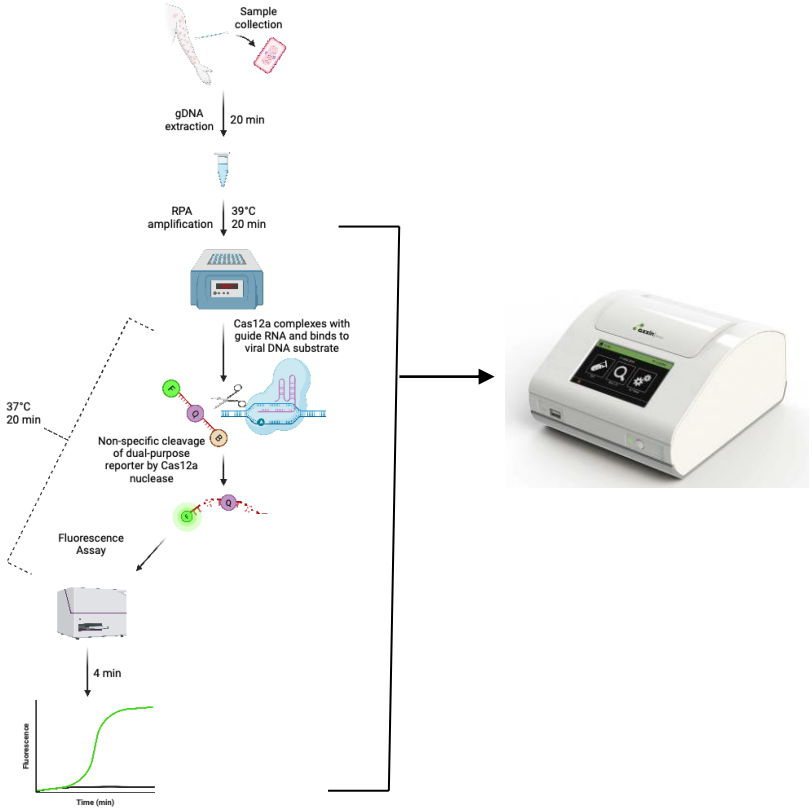

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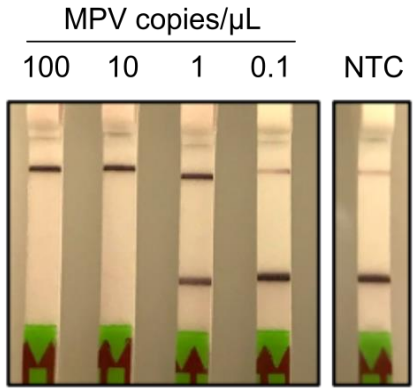
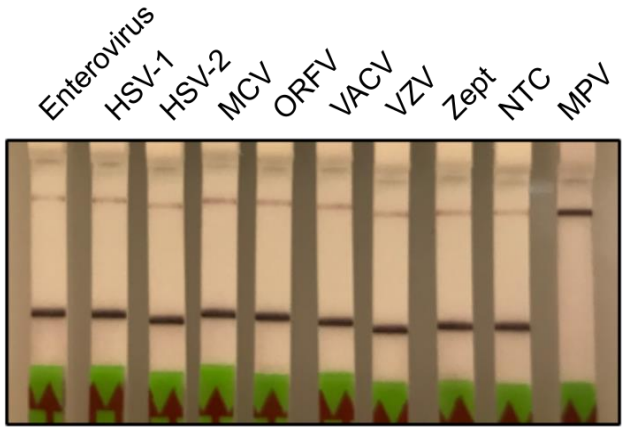
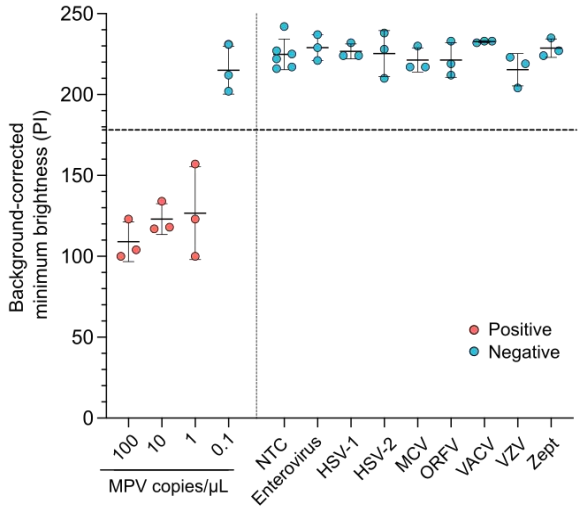
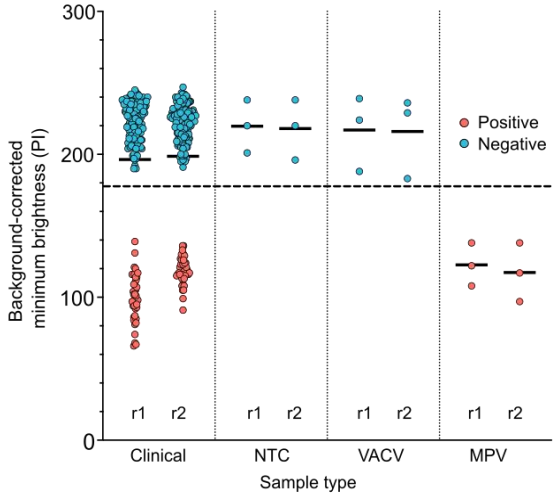
Validation of MPV-CRISPR assay using lateral flow readout

LFA showed 96.6% concordance with fluorescence readout









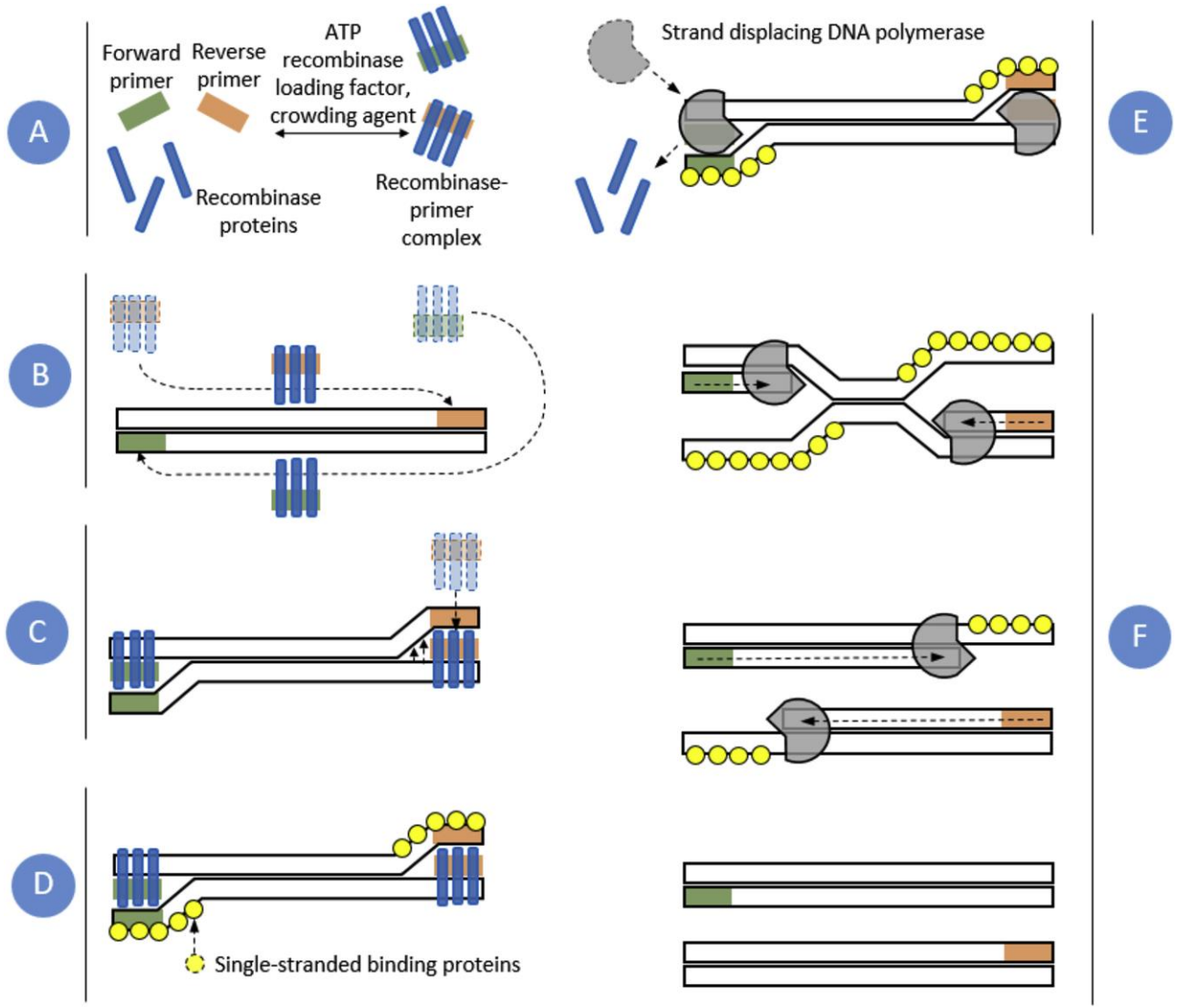
LF strips interpretated **manually by eye**

- 3 independent untrained observers.
- The overall verdict was based on the majority consensus of the three individuals

LF strips interpretated **computationally** using ImageJ

- Band intensity
- Sample defined as positive if at least one of the repeats had a minimum test line brightness of ≤177.6

RPA – Recombinase polymerase amplification



Labato & Sullivan (2018)
Recombinase proteins form complexes with each primer (A), which scans DNA for homologous sequences (B). The primers are then inserted at the cognate site by the strand-displacement activity of the recombinase (C) and single stranded binding proteins stabilise the displaced DNA chain (D). The recombinase then disassembles leaving the 3’-end of the primers accessible to a strand displacing DNA polymerase (E), which elongates the primer (F). Exponential amplification is achieved by cyclic repetition of this process.

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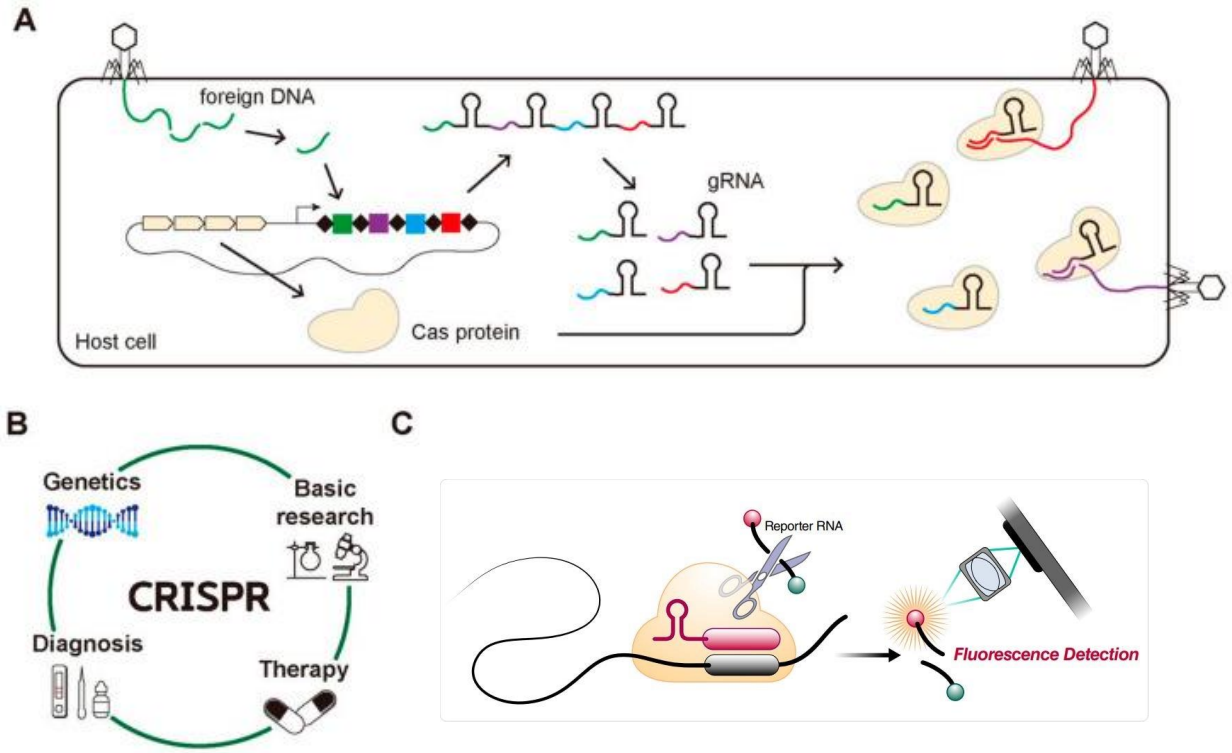
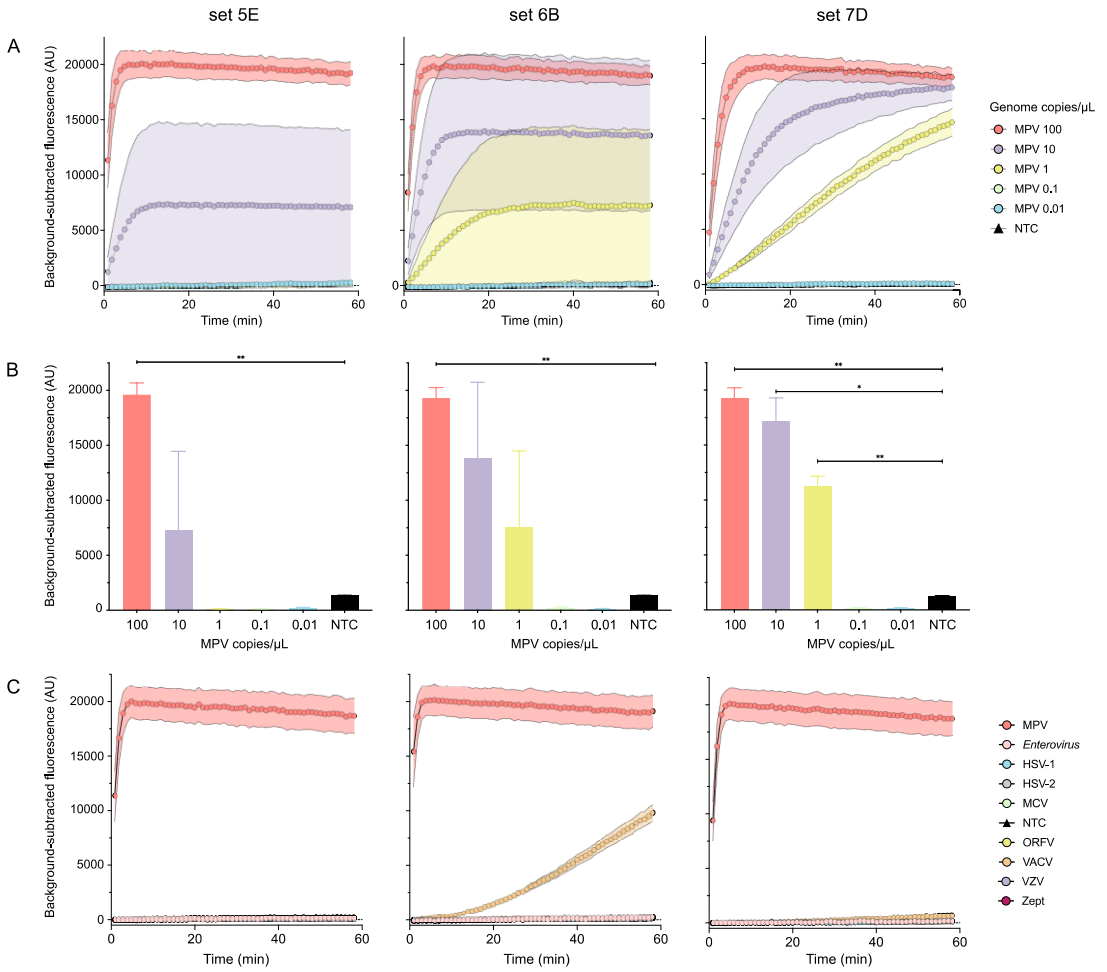
Highly specific target cleavage

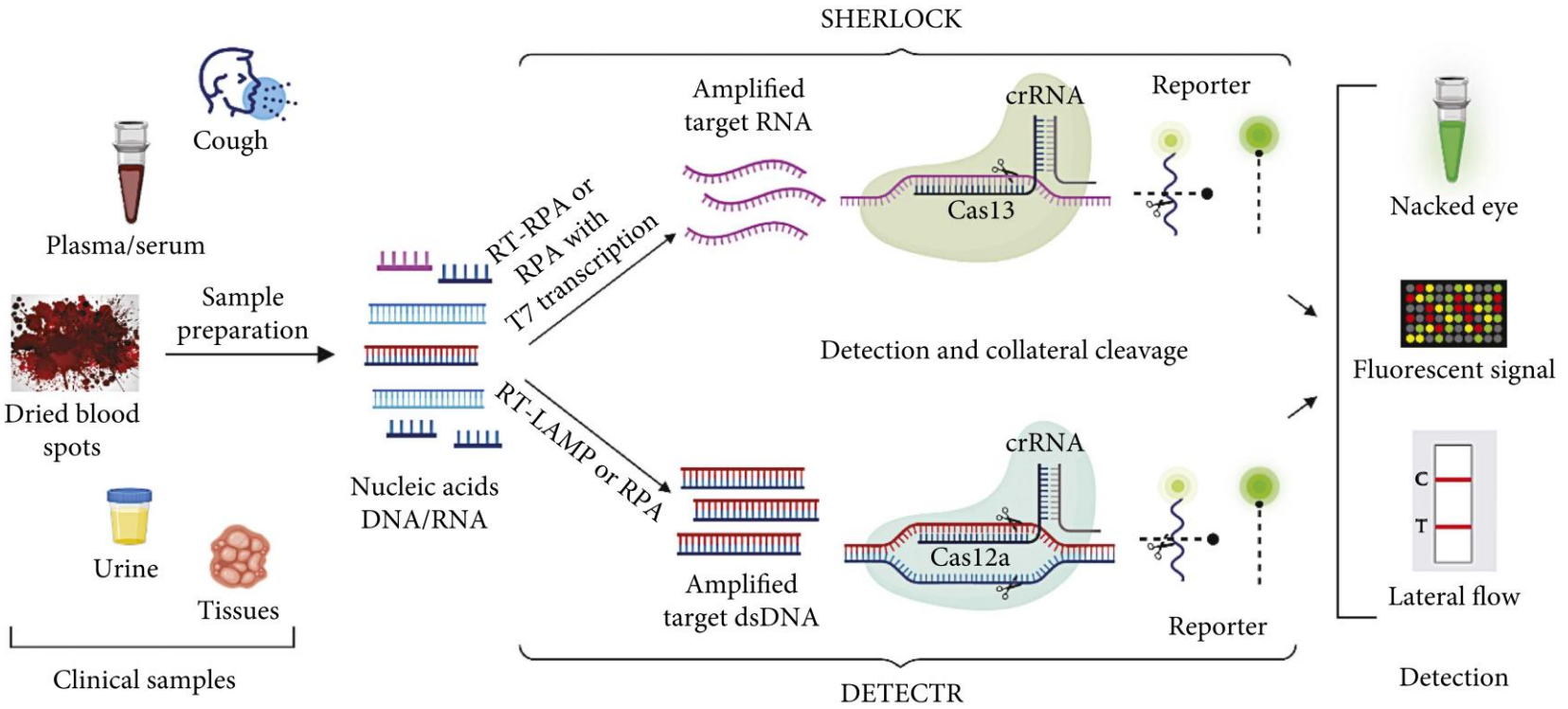


Adapted from Kim et al. 2021 (Biomolecules) & https://www.news-medical.net/news/20201004/Rapid-detection-of-SARS-CoV-2-with-portable-CRISPR-based-mobile-phone-diagnostic-test.aspx

Can the RPA reaction and the CRISPR reaction occur in the same tube?



CRISPR-diagnostic tools for point of care (POC) detection



- COVID-19 (DETECTR, AIOD-CRISPR, STOPCovid)
- Zika (SHERLOCK)
- Dengue (SHERLOCK)
- HPV (DETECTR)
- Ebola (SHERLOCK)
- Multiple other infectious diseases

Rahman et al. 2022 (Journal of tropical medicine)