香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

40th Anniversary
四十週年

香港中文大學
The Chinese University of Hong Kong

Rapid Detection of COVID-19 by CRISPR-Cas Systems

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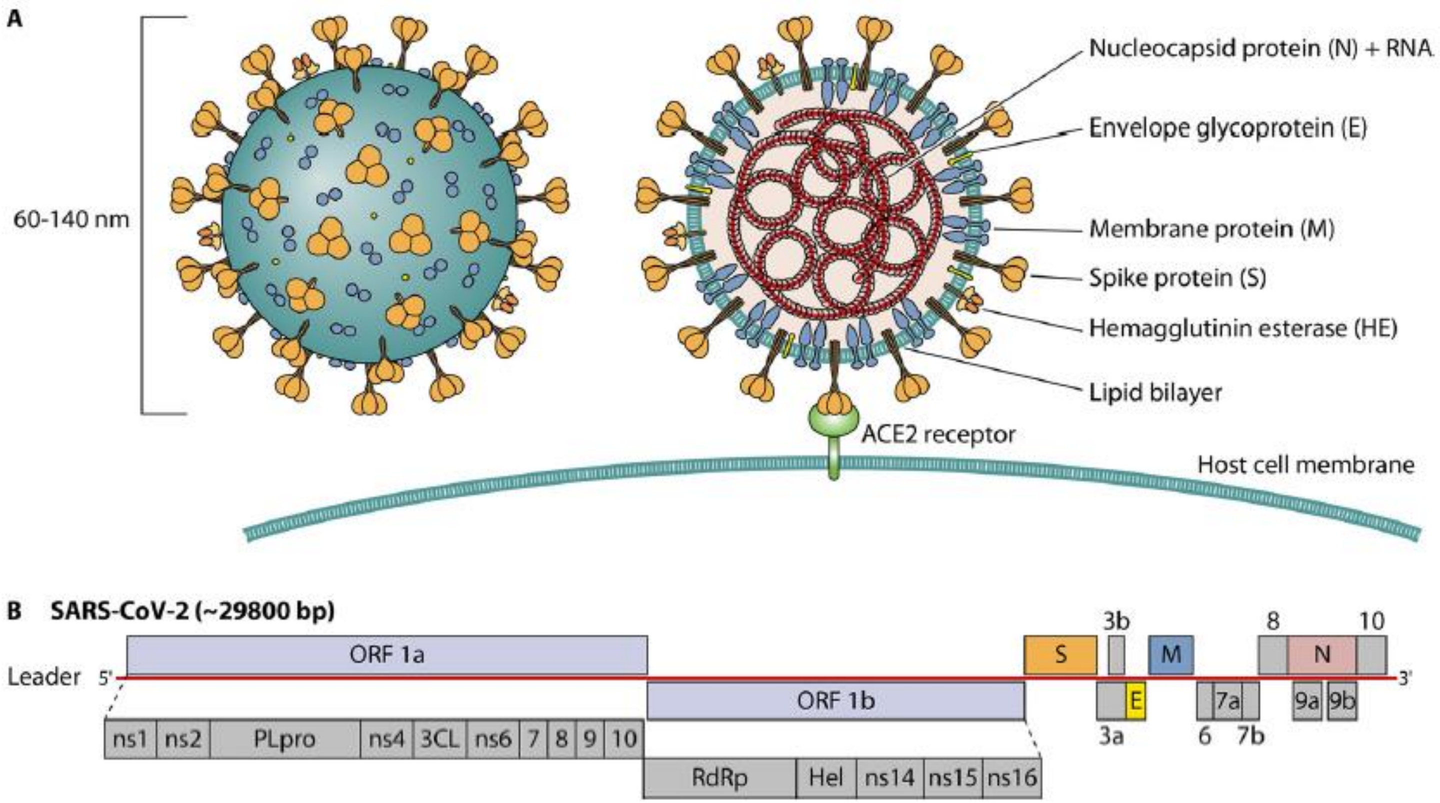
Supervisor: **Prof. Margaret Ip**

Joint Graduate Student Seminar
Department of Microbiology, Faculty of Medicine, CUHK
14 December 2021

Introduction of COVID-19



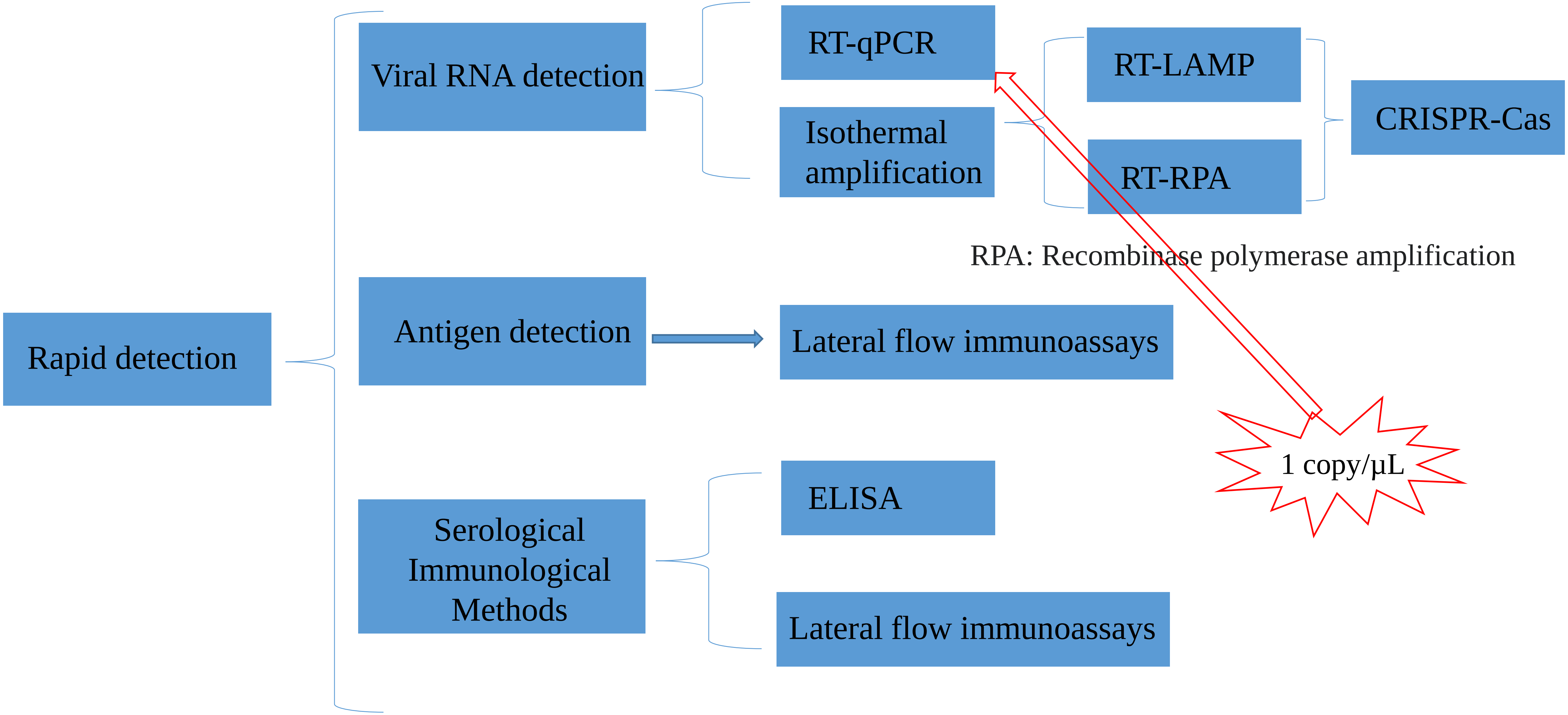
Physical and genome structure of SARS-CoV-2
(A) Diagram of the SARS-CoV-2 virion.
(B) Genome organization and proteins with known or unknown functions.

Safiabadi TaliSH *et al*, Clin Microbiol Rev, 2021

The coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute respiratory disease coronavirus 2 (SARS-CoV-2), which has led to 270 million confirmed cases and 5.3 million deaths globally as of 13 December, 2021 (https://www.worldometers.info/coronavirus/).

Rapid and massive detection plays vital role in patient management and curbing disease transmission.

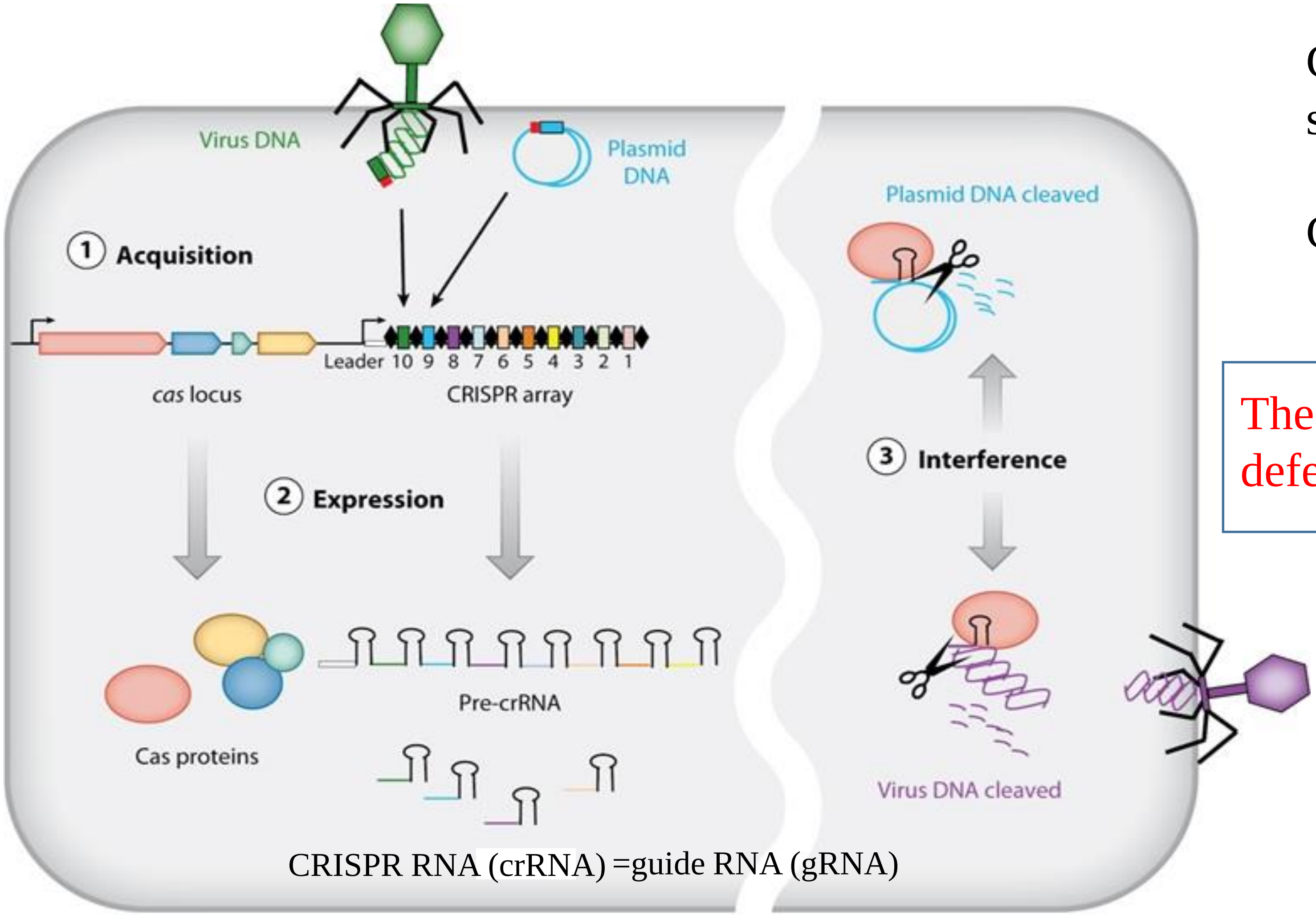
Rapid detection of COVID-19



Introduction of CRISPR-Cas

JazzIRT/Science Picture Co/Getty/Jackson Ryan

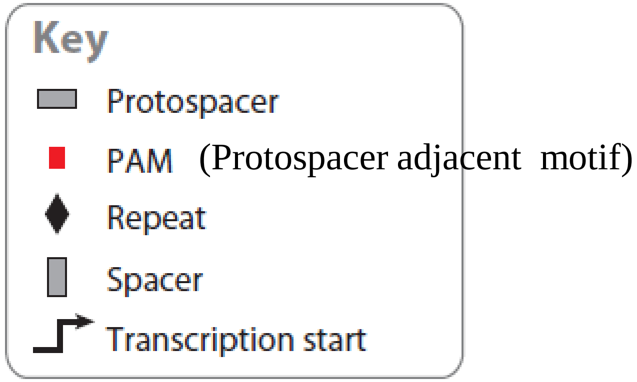
Features of the CRISPR-Cas adaptive immune system



CRISPR: clustered regularly interspaced short palindromic repeats

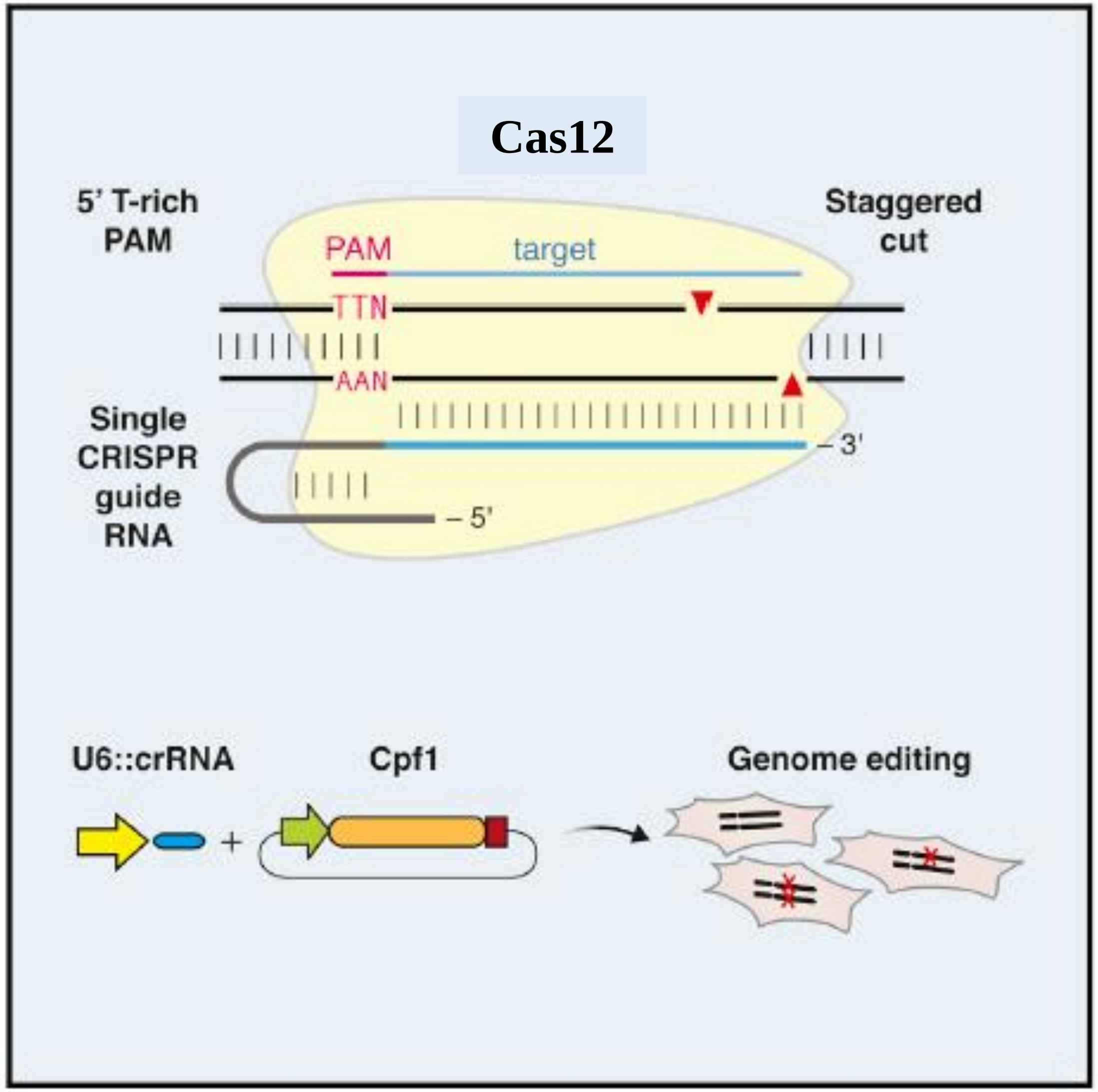
Cas: CRISPR-associated proteins

The CRISPR-Cas system with sequence-specific defense against virus and plasmid DNA



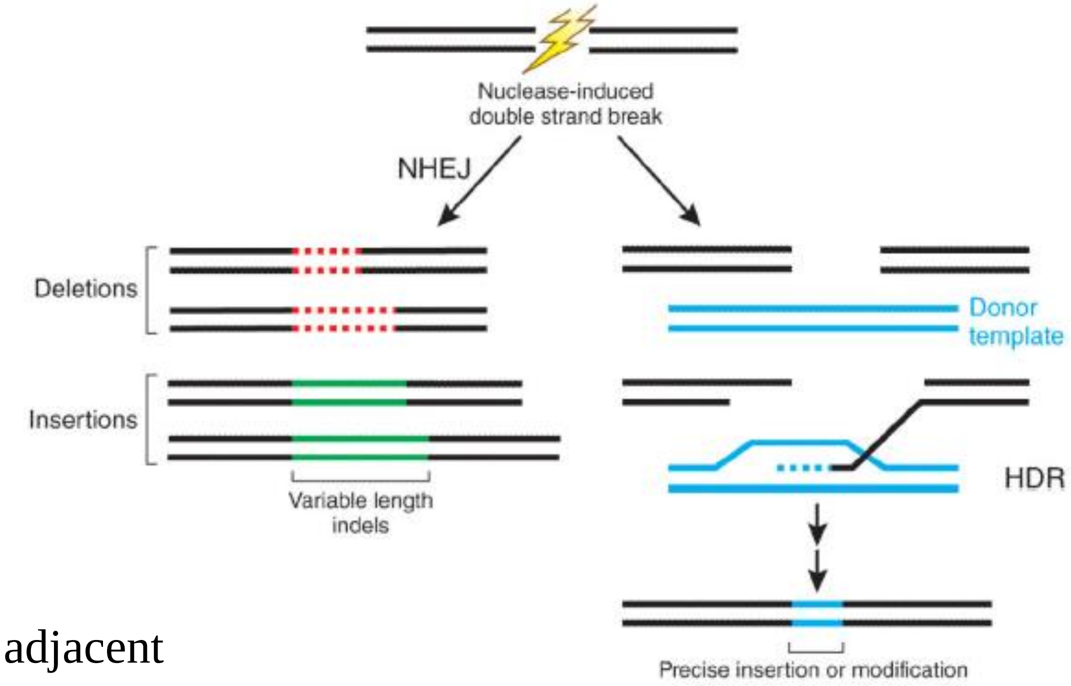
Devaki Bhaya *et al.*, Annu Rev Genet, 2011

Workflow of CRISPR-Cas9/Cas12 system



Zetsche *et al.*, Cell, 2015

Non-Homologous End Joining (NHEJ)
and Homology Directed Repair (HDR)

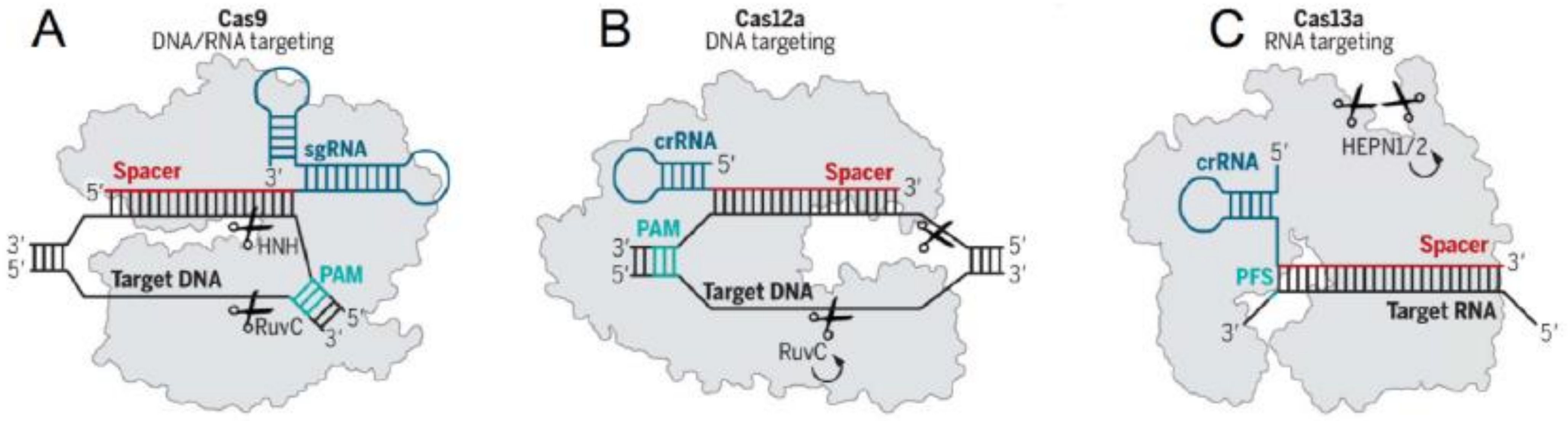


adjacent

) is necessary

Jeffry D Sander *et al.*, Nat Biotechnol, 2014

Introduction of CRISPR-Cas



Class 2, type II

Class 2, type V

Class 2, type VI

CRISPR-Cas systems have been developed as the next-generation POC testing for nucleic acid detection because of the high sensitivity and specificity of the CRISPR-Cas systems.

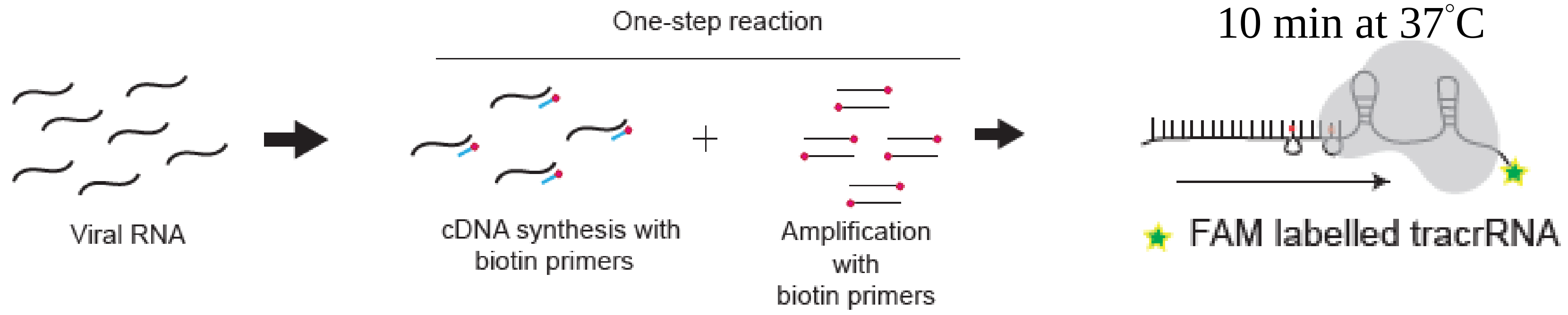
Knott GJ *et al*, Science, 2018

Mechanism of CRISPR-Cas9 detection (FELUDA)

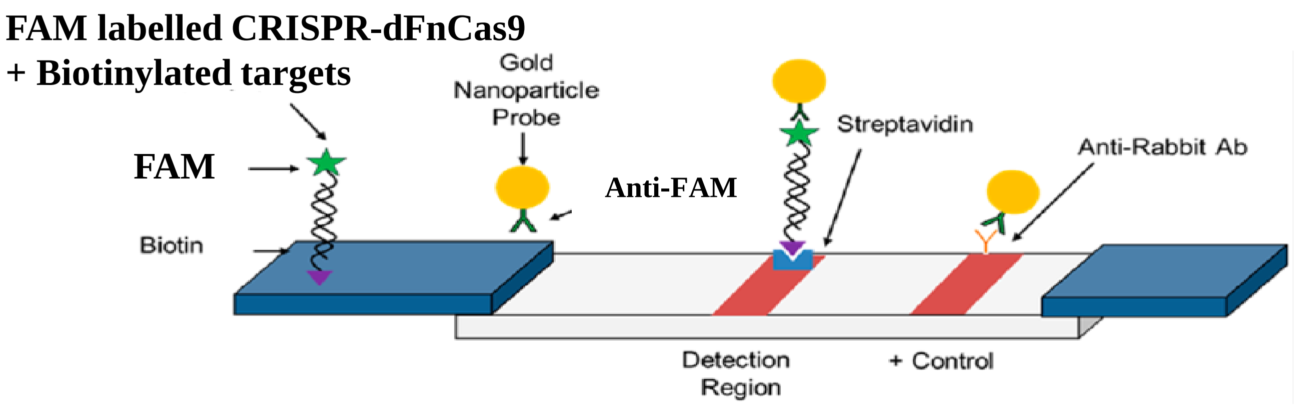
FELUDA: FnCas9 Editor Linked Uniform Detection Assay

FnCas9: Cas9 from *Francisella novicida*

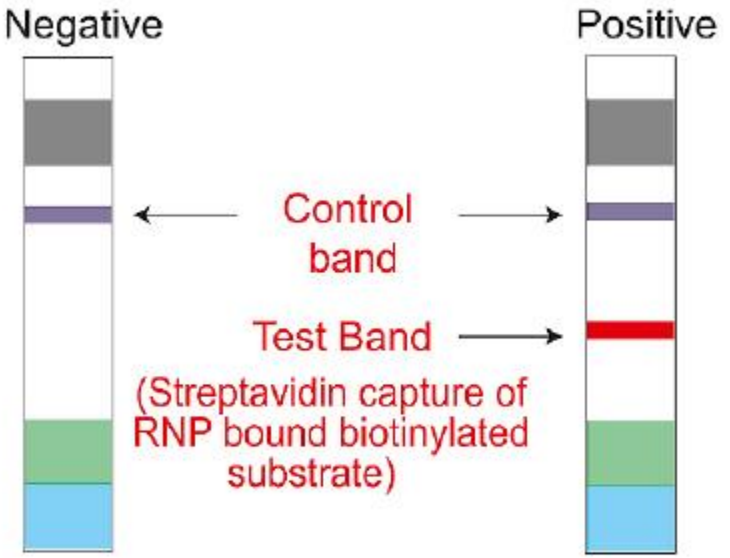
dFnCas9: catalytically dead FnCas9, can bind to targets but can not cleave



FAM labelled CRISPR-dFnCas9
+ Biotinylated targets



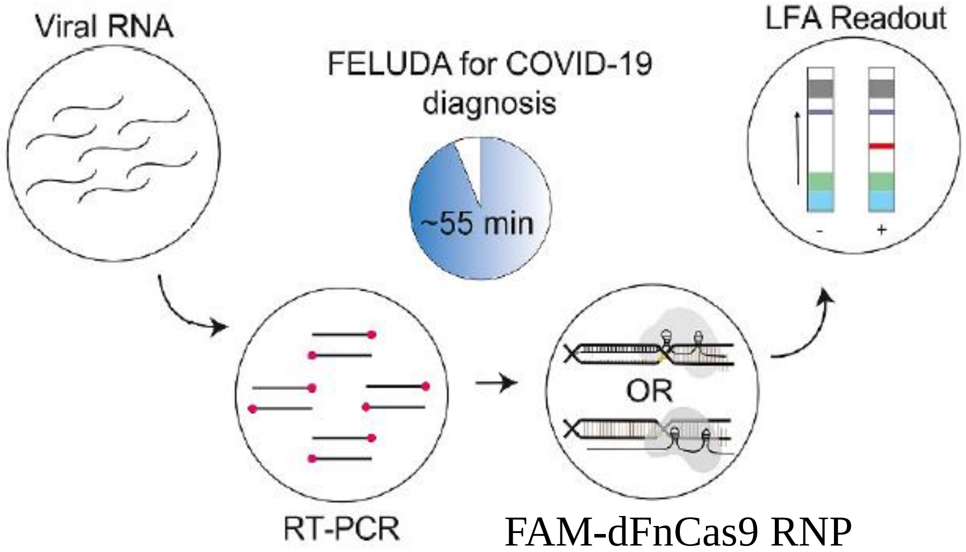
Milenia HybridDetect lateral flow strip



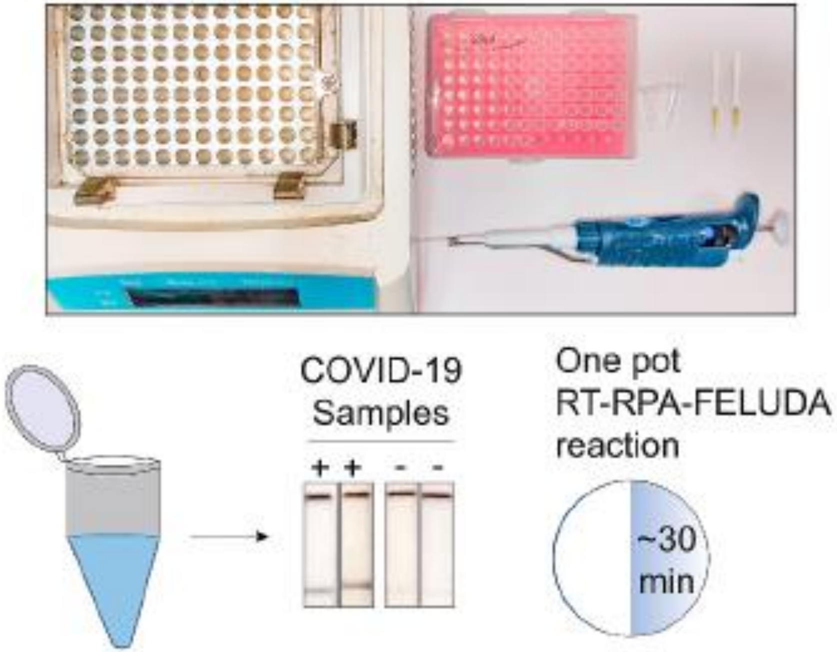
Azhar M, *et al*, Biosens Bioelectron, 2021

Crannell ZA *et al*, Anal. Chem. 2014

CRISPR-Cas9 detection (FELUDA)



FAM-dFnCas9 RNP



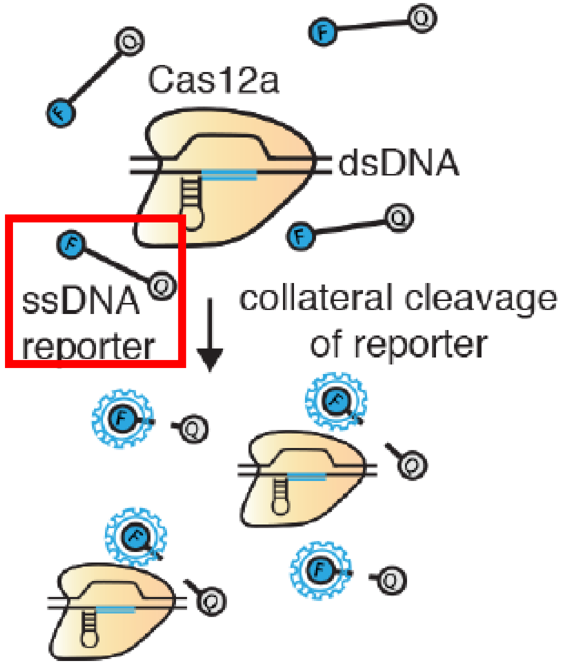
LoD: ~10 copies per reaction
85.3% sensitivity and 96.7% specificity
473 clinical samples (81 positive samples)

Azhar M *et al*, Biosens Bioelectron, 2021

Mechanism of CRISPR-Cas12/13 detection

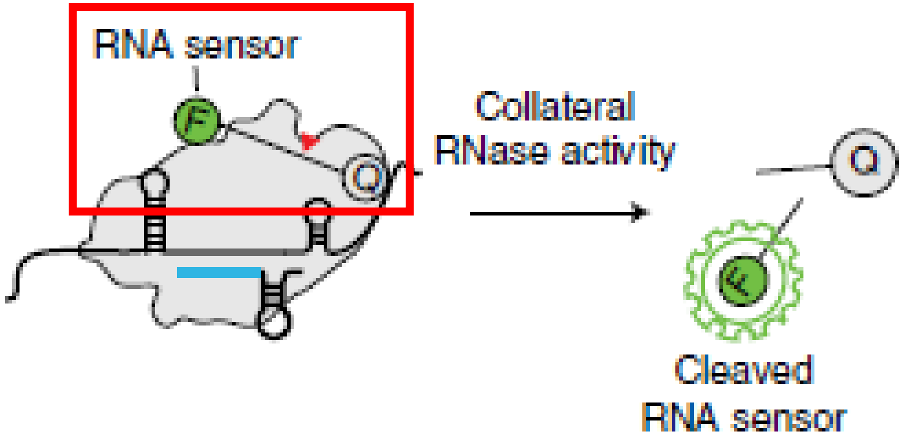
Collateral cleavage activity: RNA-guided target binding unleashes indiscriminate ssDNA or ssRNA cleavage activity that completely degrades ssDNA and ssRNA molecules.

Cas12 detection



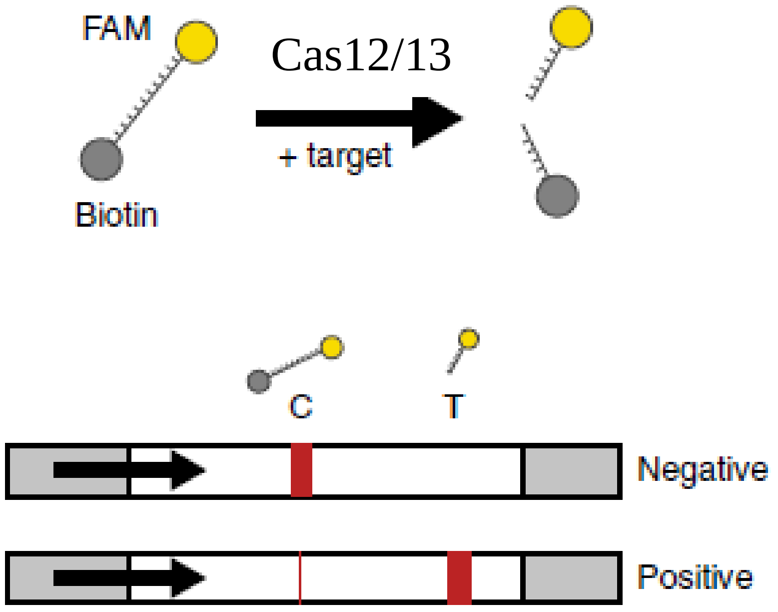
Gootenberg JS *et al*, Science, 2018.

Cas13 detection



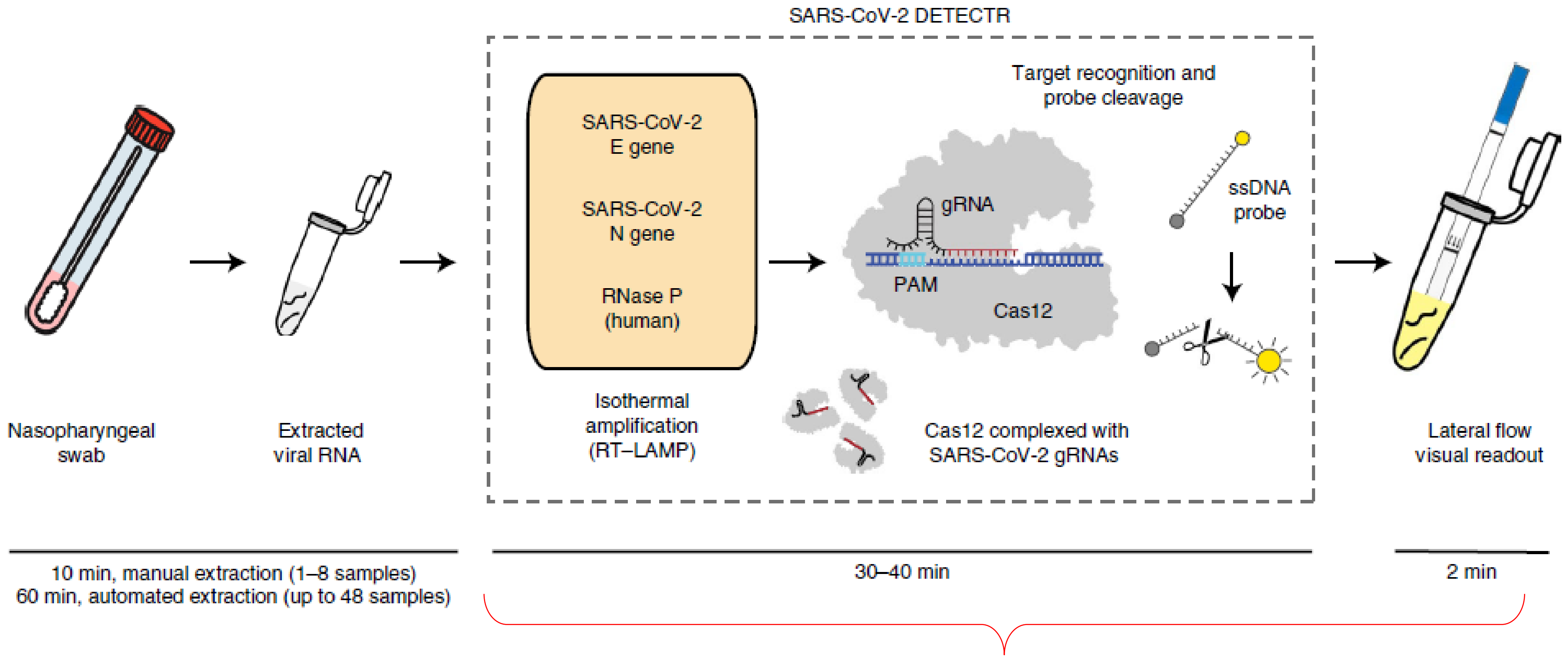
Kellner MJ *et al*, Nat Protoc, 2019

Lateral Flow Assay



Broughton JP *et al*, Nat Biotechnol, 2020

DETECTR: DNA endonuclease-targeted CRISPR *trans* reporter

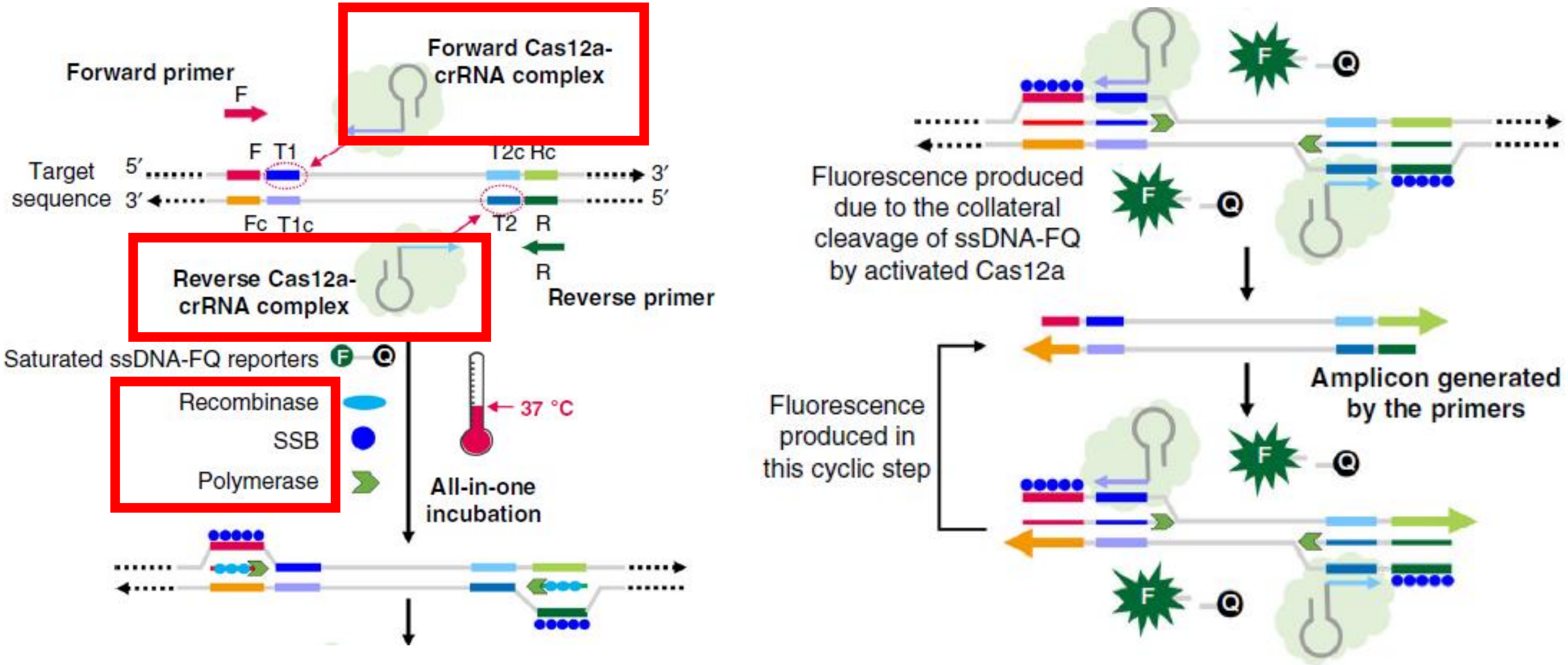


32-42 min from RNA to result

LOD: ~10 copies/µL
95.8% sensitivity and 100% specificity
78 clinical samples (36 positive samples)

Broughton JP *et al*, Nat Biotechnol, 2020

All-In-One Dual CRISPR-Cas12a (AIOD-CRISPR) assay

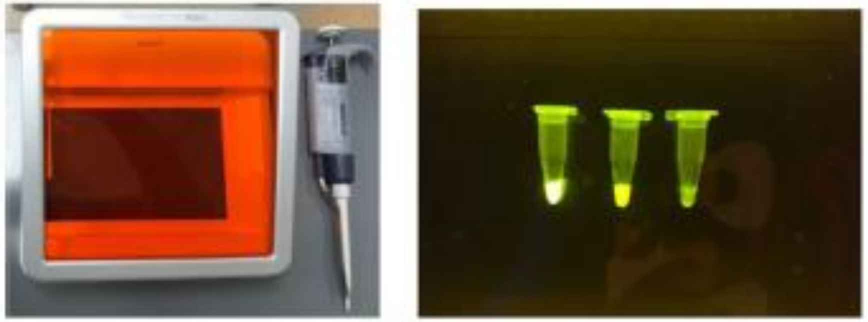


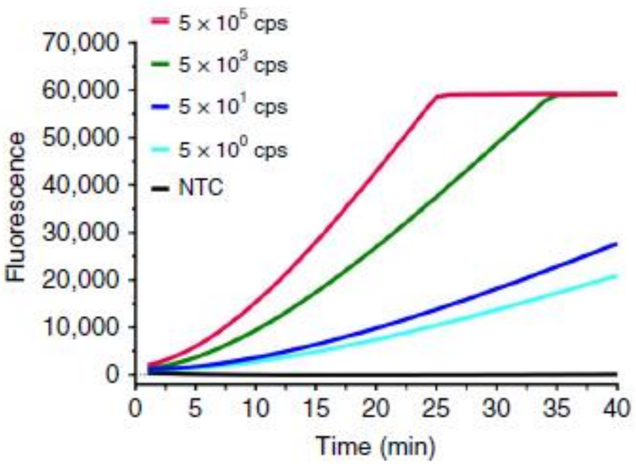
LbaCas12a: Cas12a from *Lachnospiraceae bacterium* ND2006

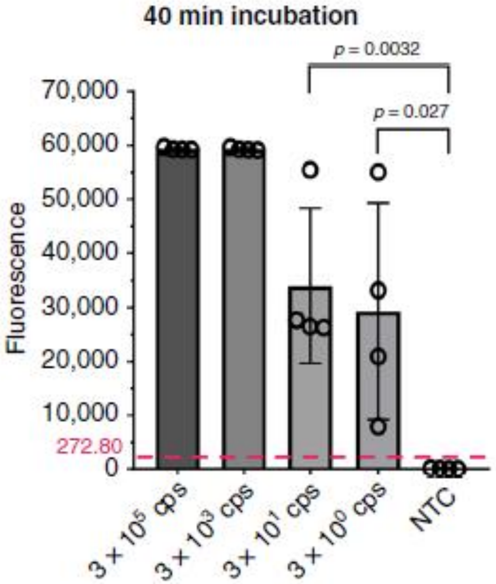
Ding X, *et al*, Nat Commun, 2020

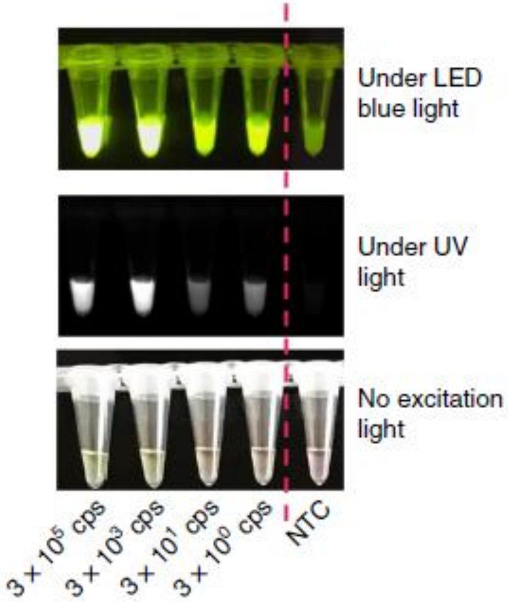
All-In-One Dual CRISPR-Cas12a (AIOD-CRISPR) assay

Visual detection in a portable LED blue transilluminator (**Immediately**)









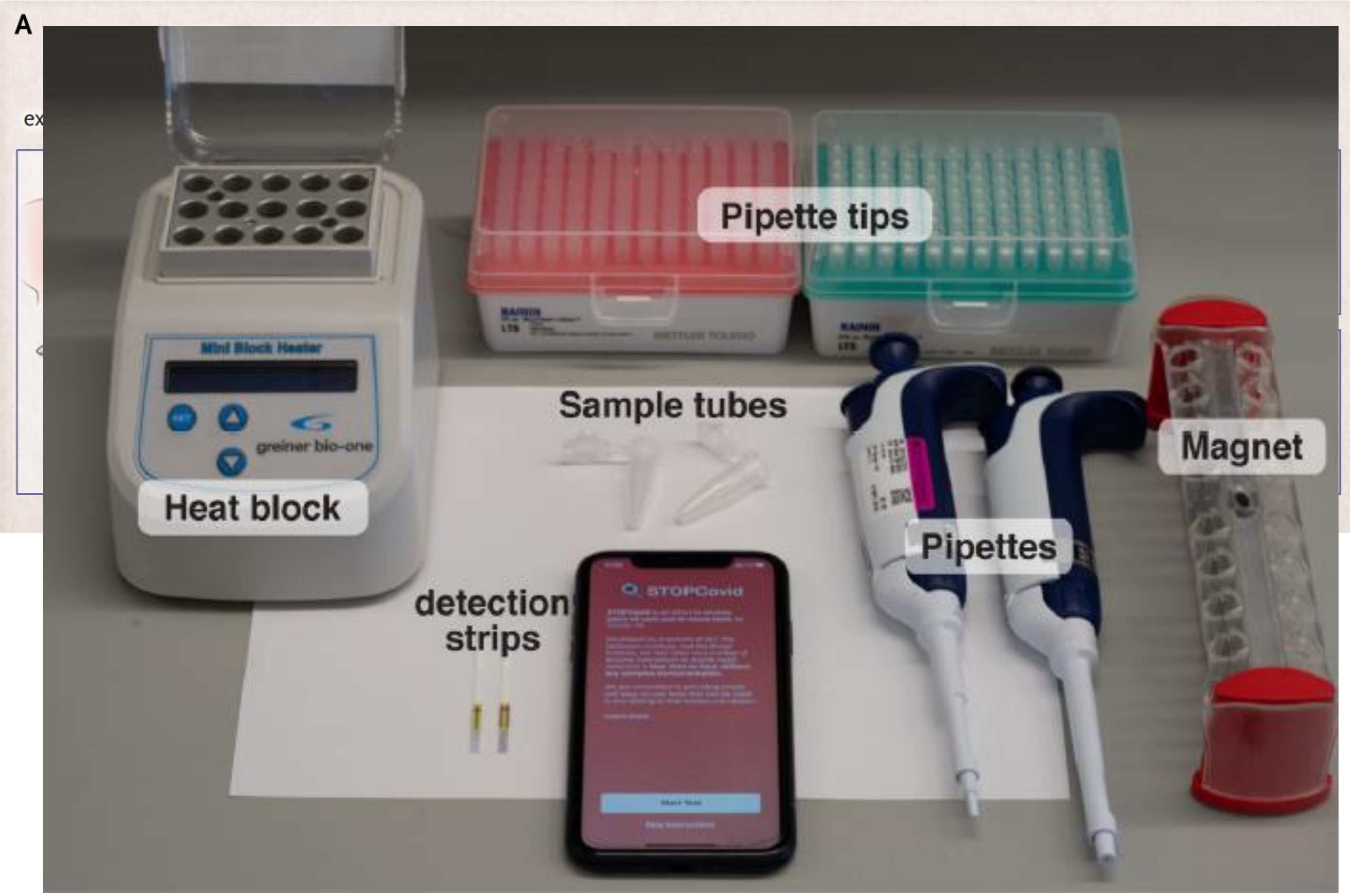
40 min from RNA to result

AIOD-CRISPR LOD: 3 copies

Consistent (100%) with result by RT-PCR 28 clinical swab samples (8 positive)

Ding X, *et al*, Nat Commun, 2020

SHERLOCK One-Pot Testing (STOPCovid.v2)



AapCas12b: thermostable Cas enzyme from *Alicyclobacillus acidiphilus*, it can function up to 65 °C

It takes 45 min (fluorescence) or 80 min (lateral flow) from sample to result

STOPCovid.v2 LOD:
33 (fluorescence) or 83 (lateral flow) copies /mL

1/30 (fluorescence) or 1/12 (lateral flow) of RT-qPCR test (1000 copies/mL)

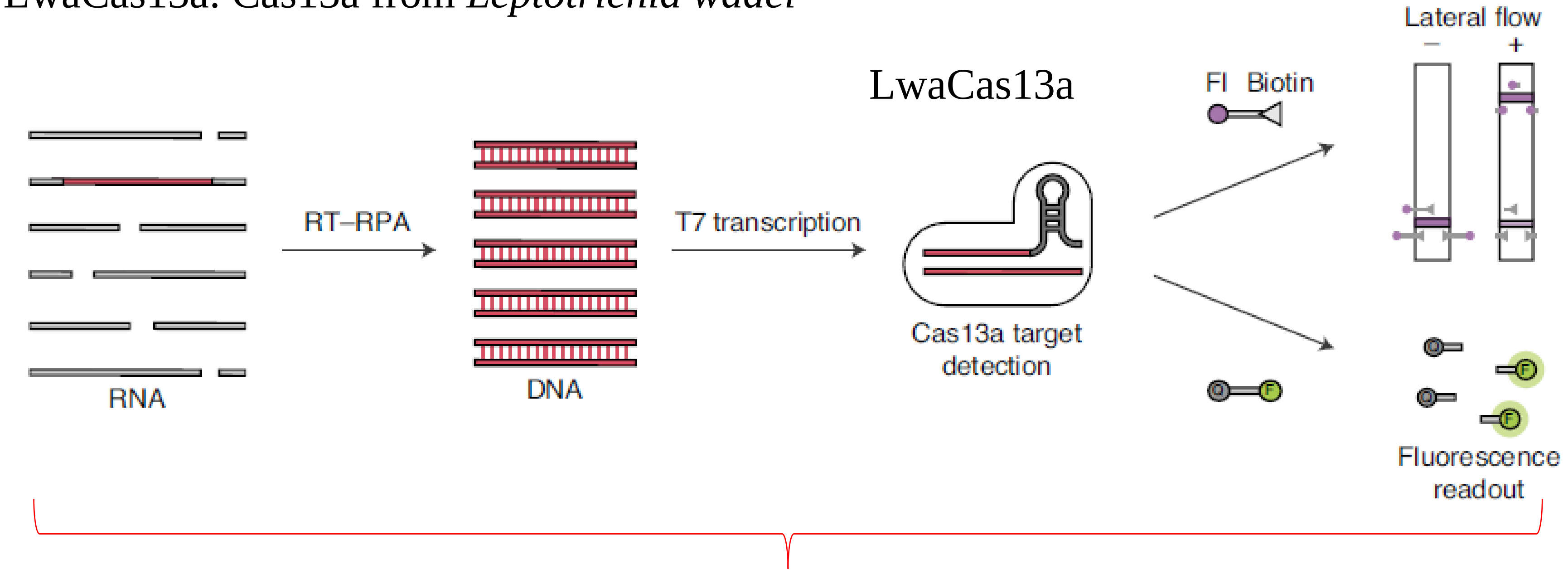
93.1% sensitivity and 98.5% specificity
402 clinical samples (202 positive samples)

Joung J *et al*, N. Engl. J. Med. 2020

CRISPR-Cas13-based assay

LwaCas13a: Cas13a from *Leptotrichia wadei*

LwaCas13a

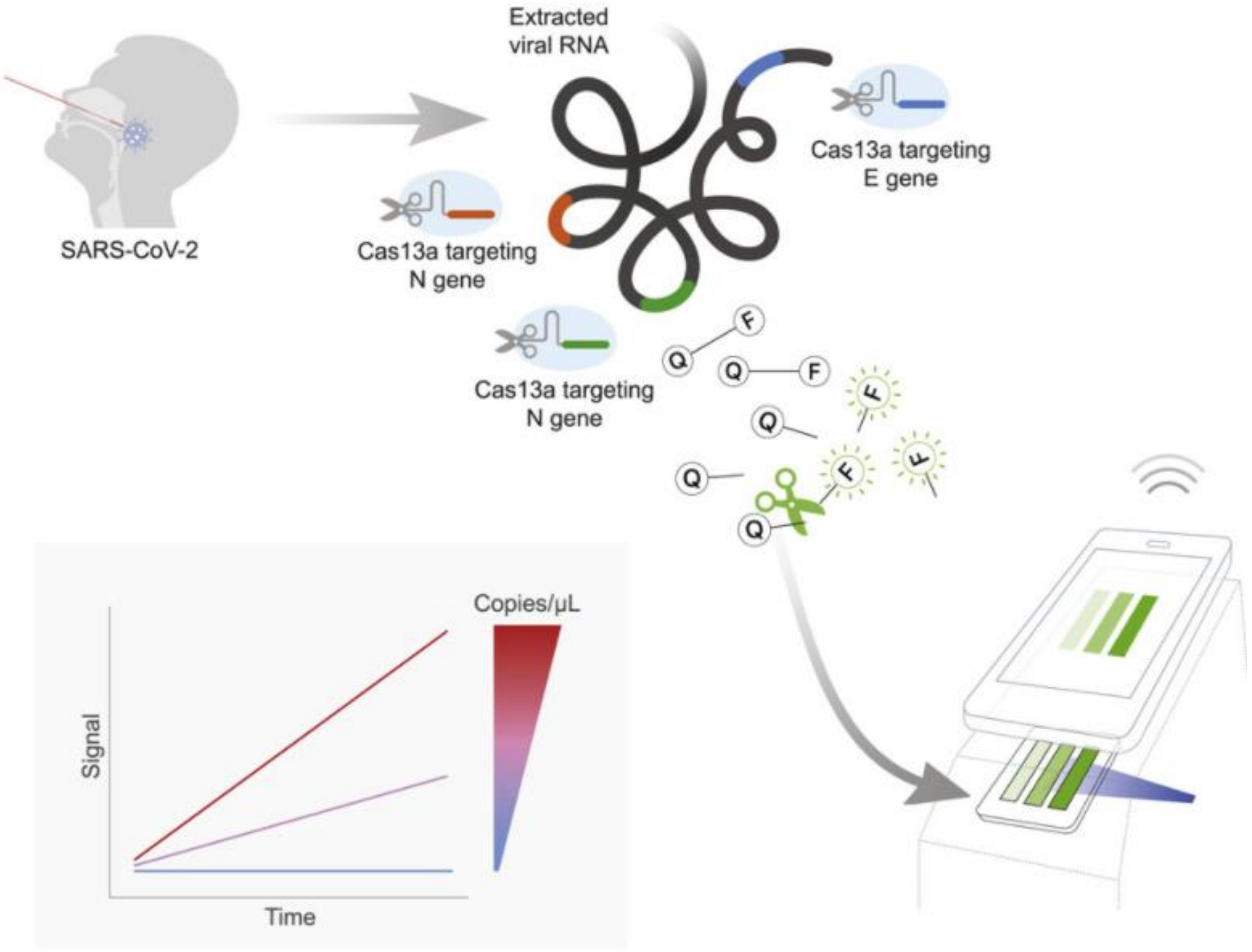


70 min

LoD: 42 copies per reaction
100% sensitivity and 100% specificity by fluorescence readout
97% sensitivity and 100% specificity by lateral-flow readout
154 clinical samples (81 positive samples)

Patchsung M *et al*, Nat Biomed Eng, 2020

Amplification-free detection by CRISPR-Cas13a



LbuCas13a: Cas13a homolog from *Leptotrichia buccalis*

The fluorescence signal can be amplified by using three different gRNAs

Direct detection of SARS-CoV-2 from extracted RNA with a mobile phone camera, it can be used to directly quantify viral load

LoD: 100 copies/μL within 30 min

Detect 5 positive clinical samples in under 5 min (ranging from 3.2×10^5–1.65×10^3 copies/μL)

Fozouni P *et al*, Cell, 2021

Conclusions and perspectives

1. The LoD, specificity, and sensitivity of CRISPR-Cas based detection platform is comparable with RT-qPCR and can be faster.

2. CRISPR-Cas based diagnostic platforms do not need expensive equipment, so they can be easily used in areas with poor resources.

3. LAMP and RPA are the most common isothermal amplification systems used in POC testing. When combine with CRISPR-Cas, the drawbacks of LAMP and RPA (primer dimer and nonspecific amplification) can be overcome.

4. CRISPR-Cas based diagnostic platforms are good for qualitative detection but can not provide quantitative information except the amplification-free detection by CRISPR-Cas13a.

References

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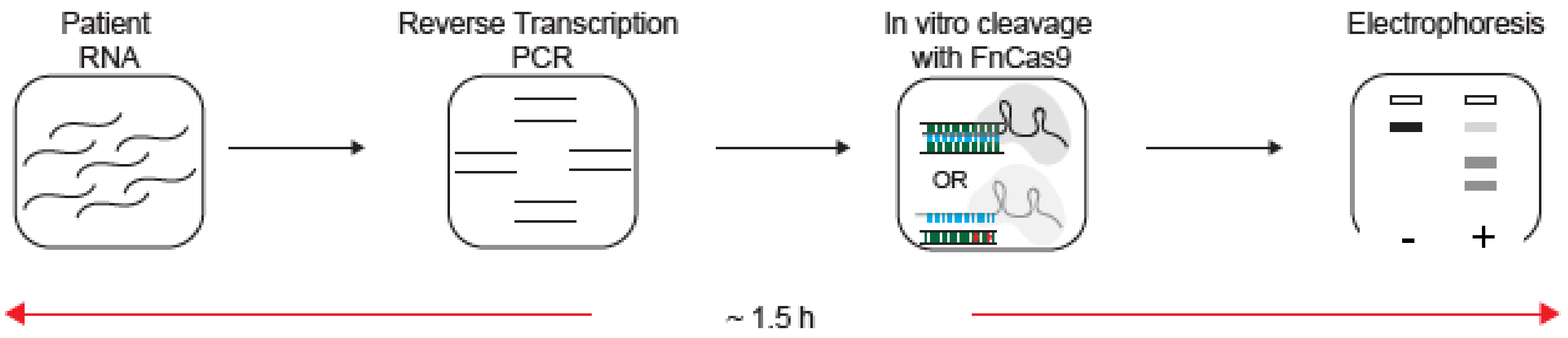
1. Fozouni P, Son S, Díaz de León Derby M, et al. Amplification-free detection of SARS-CoV-2 with CRISPR-Cas13a and mobile phone microscopy. Cell 2021; 184: 323-333.e9.

Thank you for your attention !

Mechanism of CRISPR-Cas9 detection (FELUDA)

FELUDA: FnCas9 Editor Linked Uniform Detection Assay

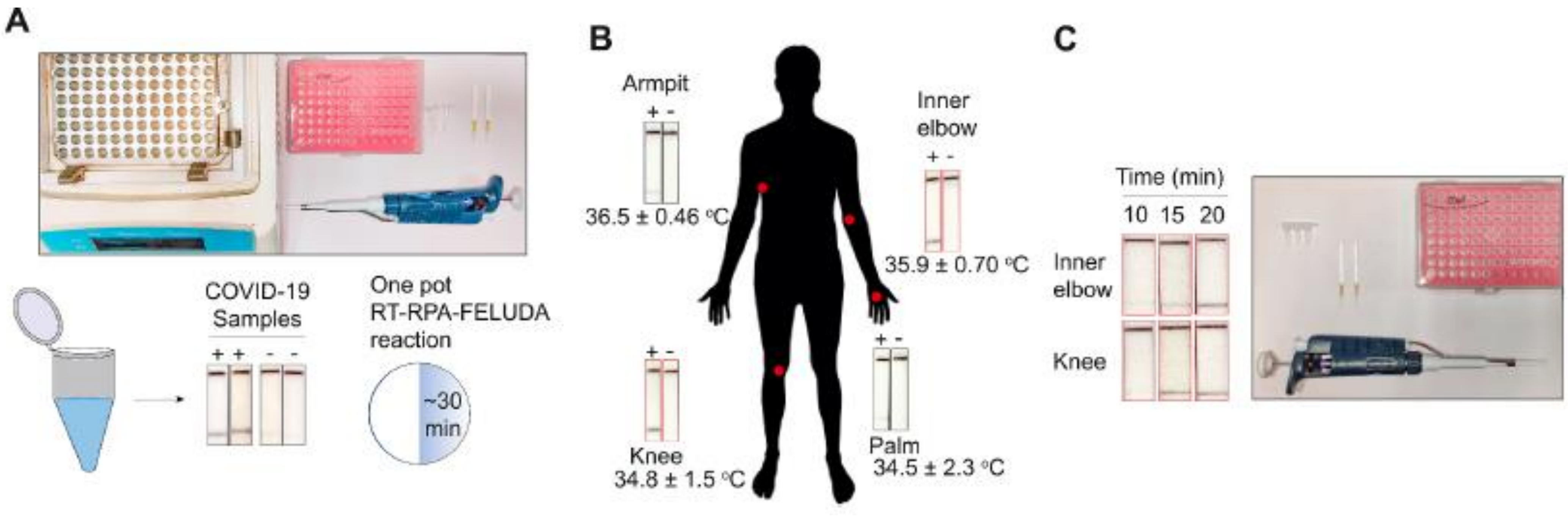
Gel Based Nucleic Acid Detection:



FnCas9 is one of Cas9 with high mismatch sensitivity both under *in vitro* and *in vivo* conditions.

Kumar M, *et al*, Elife, 2021

CRISPR-Cas9 detection (FELUDA)



Azhar M, *et al*, Biosens Bioelectron, 2021

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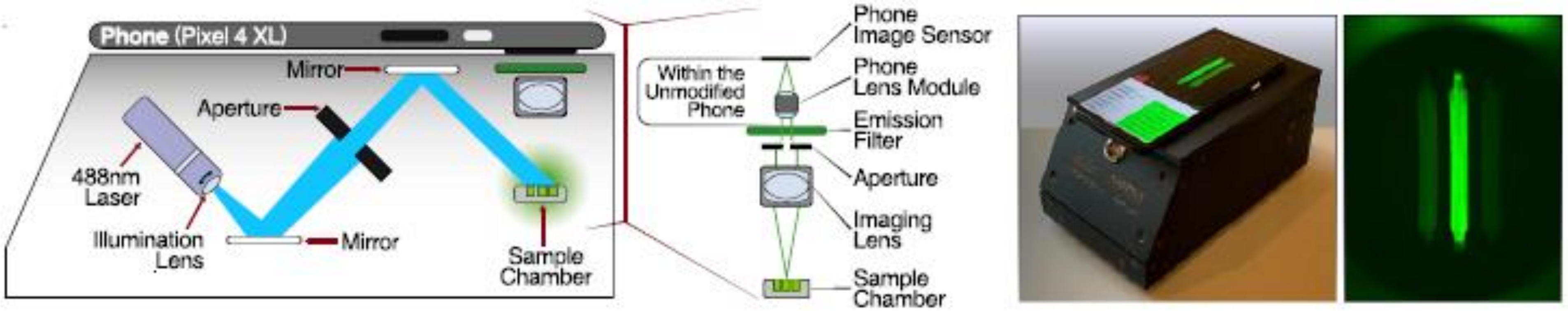

Schematic of mobile phone-based microscope for fluorescence detection

Fozouni P *et al*, Cell, 2021