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Faster and economic detection of SARS-CoV-2 using isothermal amplification techniques

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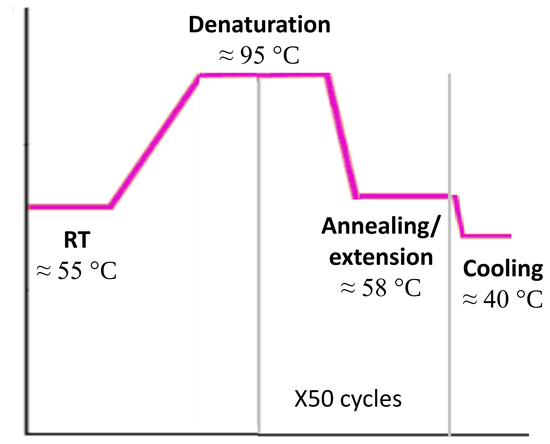
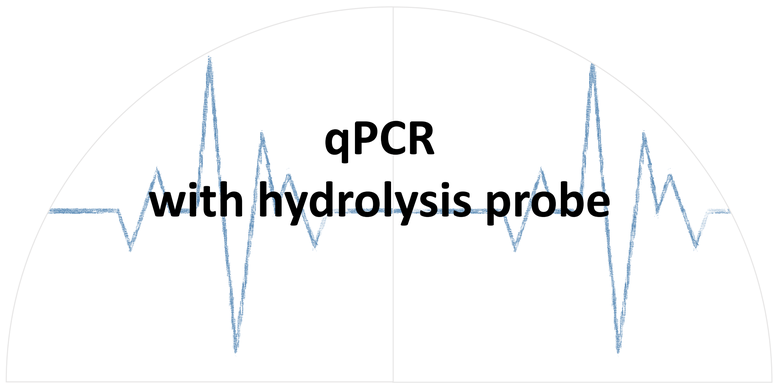
The identification of people infected with SARS-CoV-2 is key to break the transmission network, and DNA-based techniques, like PCR are mostly used for the detection of this type of pathogens [1]. This virus has single-stranded, non-segmented positive-sense RNA, and for this reason the addition of an upstream reverse transcriptase (RT) step to convert the viral RNA to cDNA needs to be performed, to be possible its amplification and detection by DNA-based methodologies.
Although RT-qPCR methods is used as the gold standard for the diagnosis of COVID-19, it presents several limitation. To overcome them and detect pathogen's RNA with high sensitivity, isothermal amplification methods have been developed as, for instance, Loop-mediated isothermal AMPlification (LAMP) and Recombinase Polymerase Amplification (RPA) [2]. This poster shows the difference between this two techniques, and compares the limitations and advantages over PCR analysis,



Standrad RT-qPCR for the detection of SARS-COV-2 (Institut Pasteur) (3)



Advantage

- High sensitivity and specificity

Disadvantage

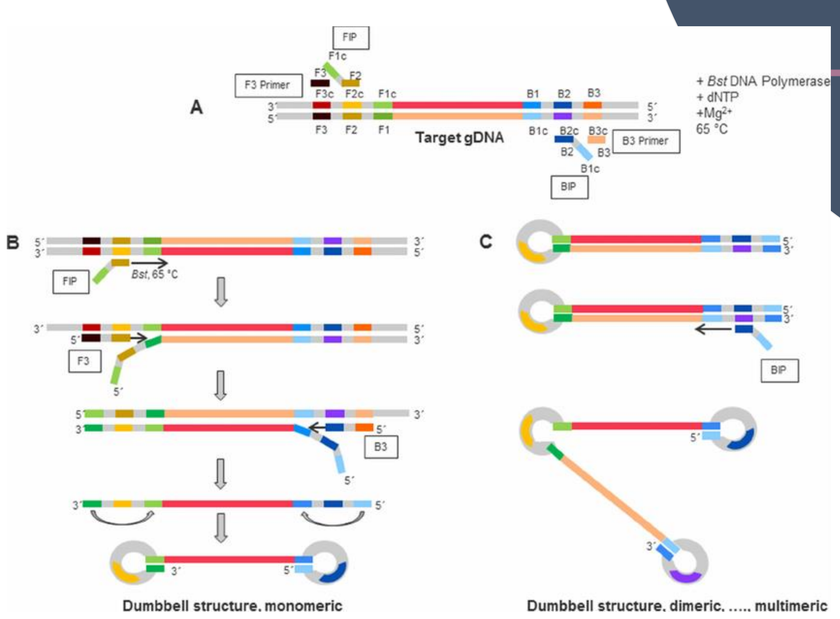
- Complex equipment
- Trained personnel
- Difficult to integrate in a miniaturized device
- Expensive

Isothermal amplifications

Constant temperature

- 4 to 6 primers
- 60 – 65 °C
- 30 min to 1h

Loop-mediated isothermal amplification (LAMP)

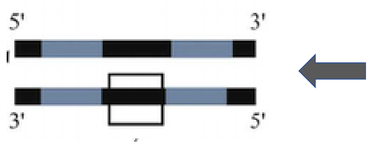


[4]

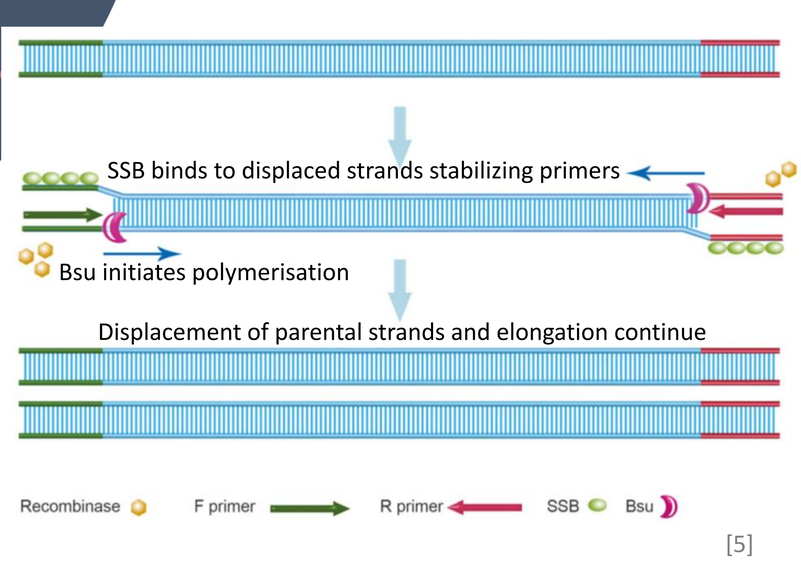
Produces complex loop structure

- 2 primers
- 37 - 42 °C
- 20 – 40 min

Recombinase polymerase amplification (RPA)



production of linear fragment (similar to PCR)

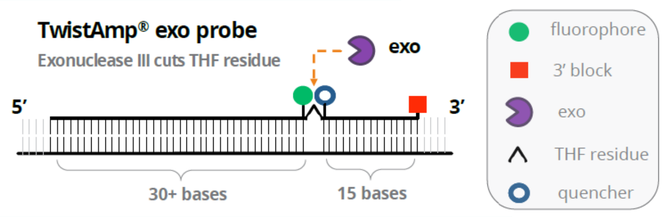


[5]

Possibility of real-time monitoring

- Intercalating dye
- Incorporation of a probe to improve specificity has been studied (more complex than PCR and RPA)

Easy to label with fluorescent probe



Lateral flow combination already developed

RT-LAMP already developed by several research groups for the detection of SARS-COV-2

Type of detection	Reagent	Reference
Colorimetric detection	WarmStart Colorimetric LAMP (NEB)	[6–9]
Fluorescent Detection	Reagent (Eiken)	[10]

Advantage of Isothermal Amplification over PCR

- Less complex equipment
- No need for specialized technicians
- Easier to miniaturized and integrate in automated systems
- Allows naked-eye detection

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