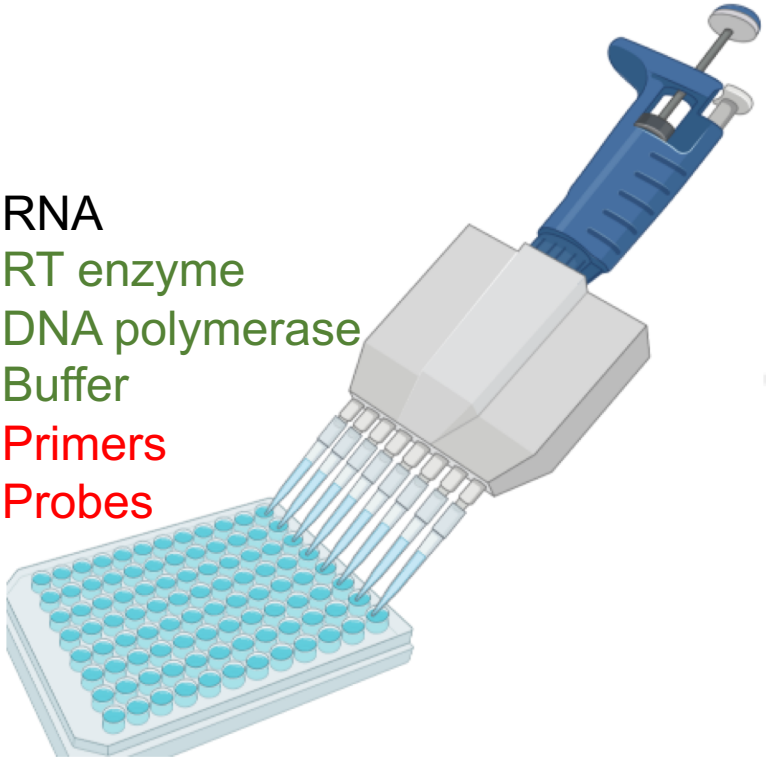
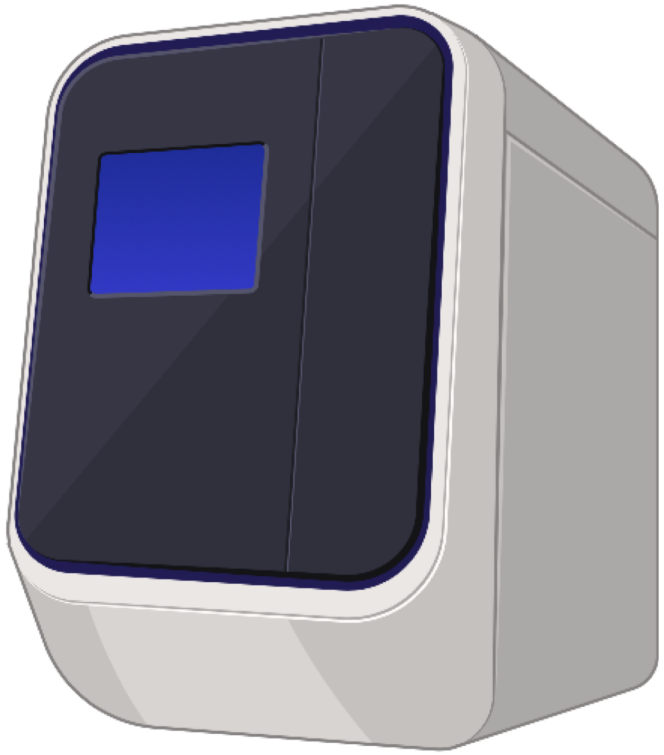
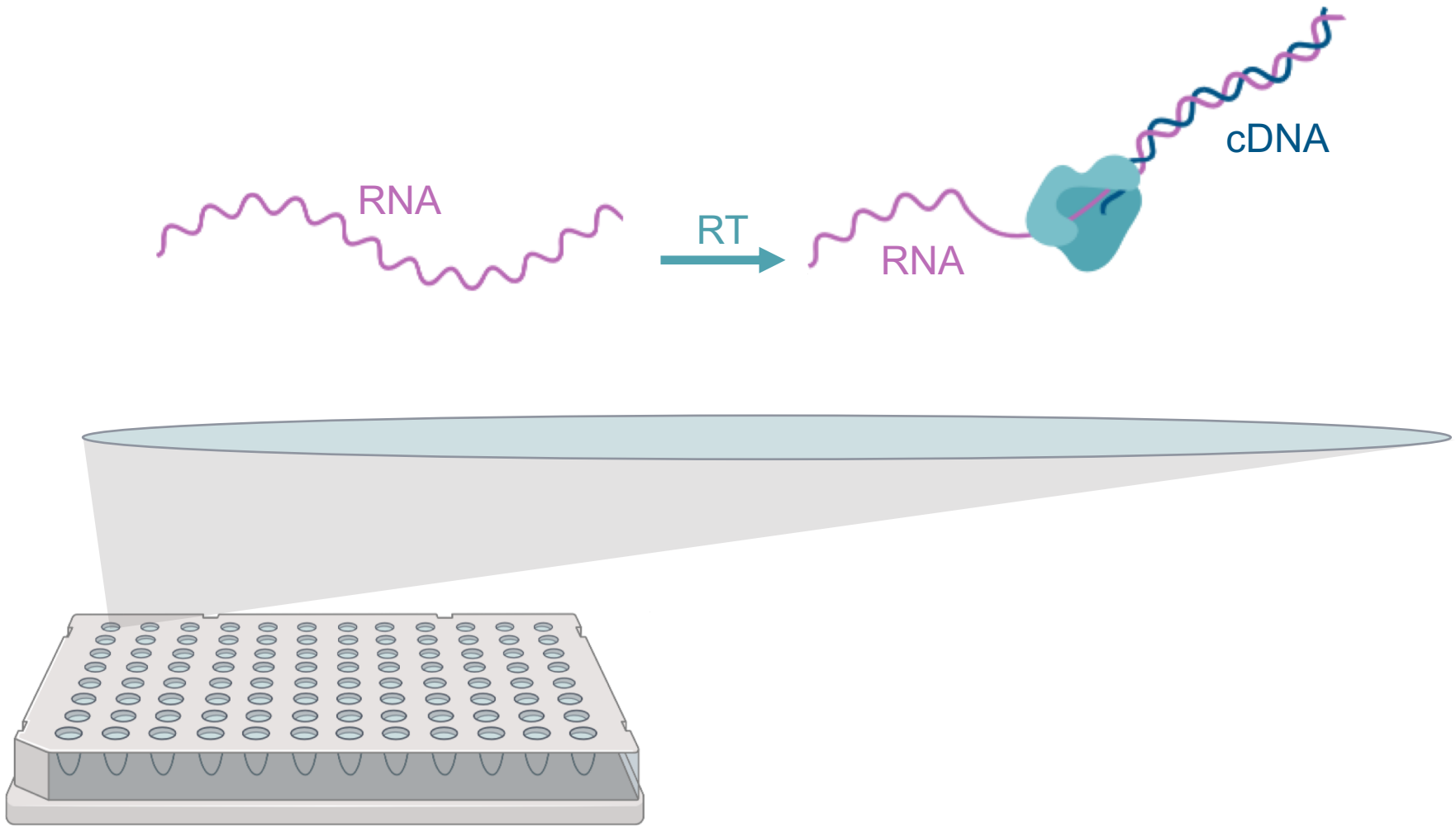
RT-PCR

RNA
RT enzyme
DNA polymerase
Buffer
Primers
Probes

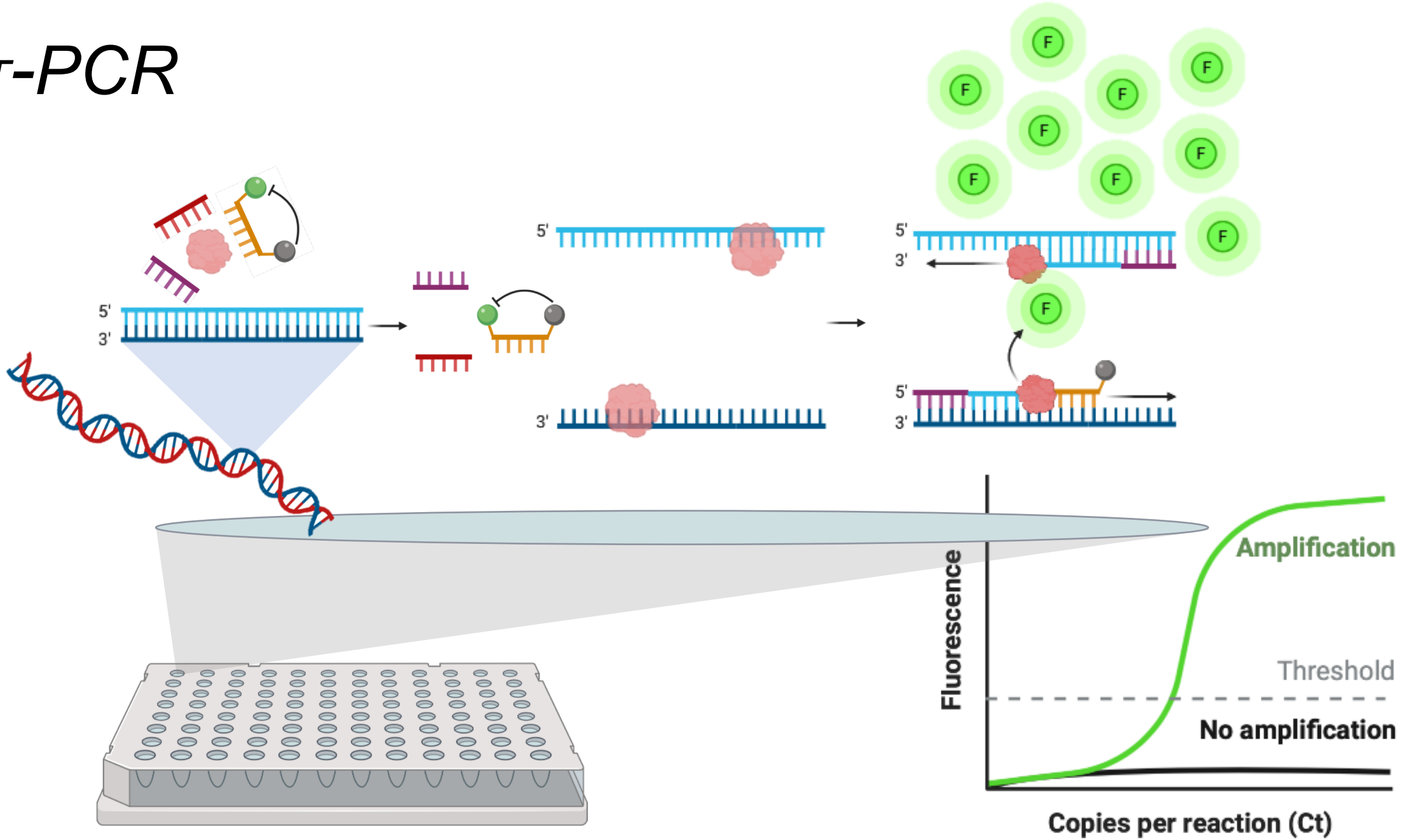




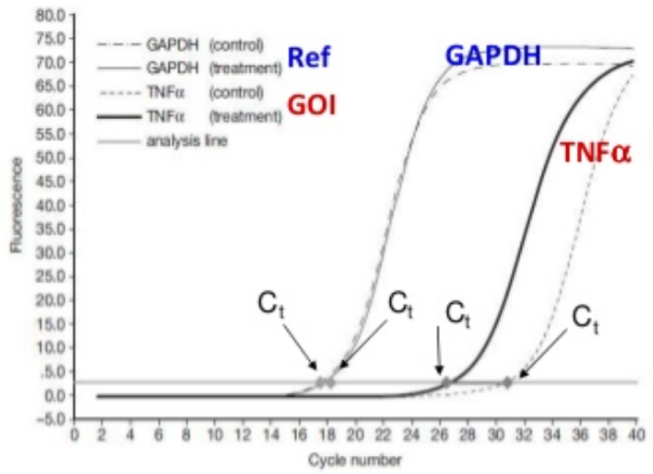
RT-PCR



RT-PCR


F
5'
3'
Amplification
Threshold
No amplification
Fluorescence
Copies per reaction (Ct)

RT-qPCR



$\Delta\Delta Ct = \Delta Ct\ (TNF\alpha_{treat}\text{-}GAPDH_{treat}) - \Delta ct\ (TNF\alpha_{control}\text{-}GAPDH_{control})$

The fold change = $2^{(-\Delta\Delta Ct)}$

single data (n = 1) e.g. array results:

$$\text{relative expression} = \frac{E_{target}^{\Delta CP_{target}\ (control\ -\ sample)}}{E_{ref}^{\Delta CP_{ref}\ (control\ -\ sample)}}$$

Pfaffl, Nucleic Acids Research 2001

multiple data (1 < n < 16) e.g. experimental groups:

$$\text{relative expression} = \frac{E_{target}^{\Delta CP_{target}\ (MEAN\ control\ -\ MEAN\ sample)}}{E_{ref}^{\Delta CP_{ref}\ (MEAN\ control\ -\ MEAN\ sample)}}$$

Pfaffl et al., Nucleic Acids Research 2002

MIQE guidelines for RT-qPCR

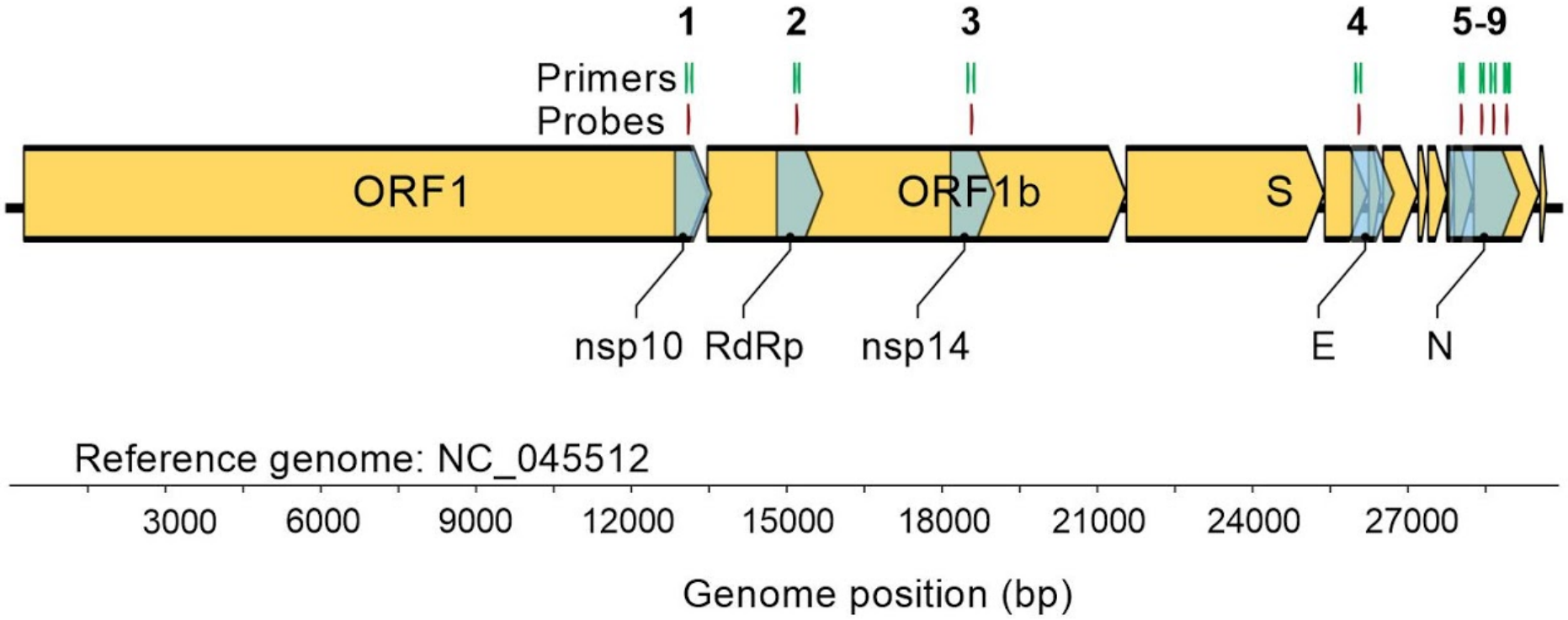
Clinical Chemistry 55:4
611–622 (2009)

Special Report

The MIQE Guidelines: *M*inimum *I*nformation for Publication of *Q*uantitative Real-Time PCR *E*xperiments

Stephen A. Bustin,[1*] Vladimir Benes,[2] Jeremy A. Garson,[3,4] Jan Hellemans,[5] Jim Huggett,[6] Mikael Kubista,[7,8] Reinhold Mueller,[9] Tania Nolan,[10] Michael W. Pfaffl,[11] Gregory L. Shipley,[12] Jo Vandesompele,[5] and Carl T. Wittwer[13,14]

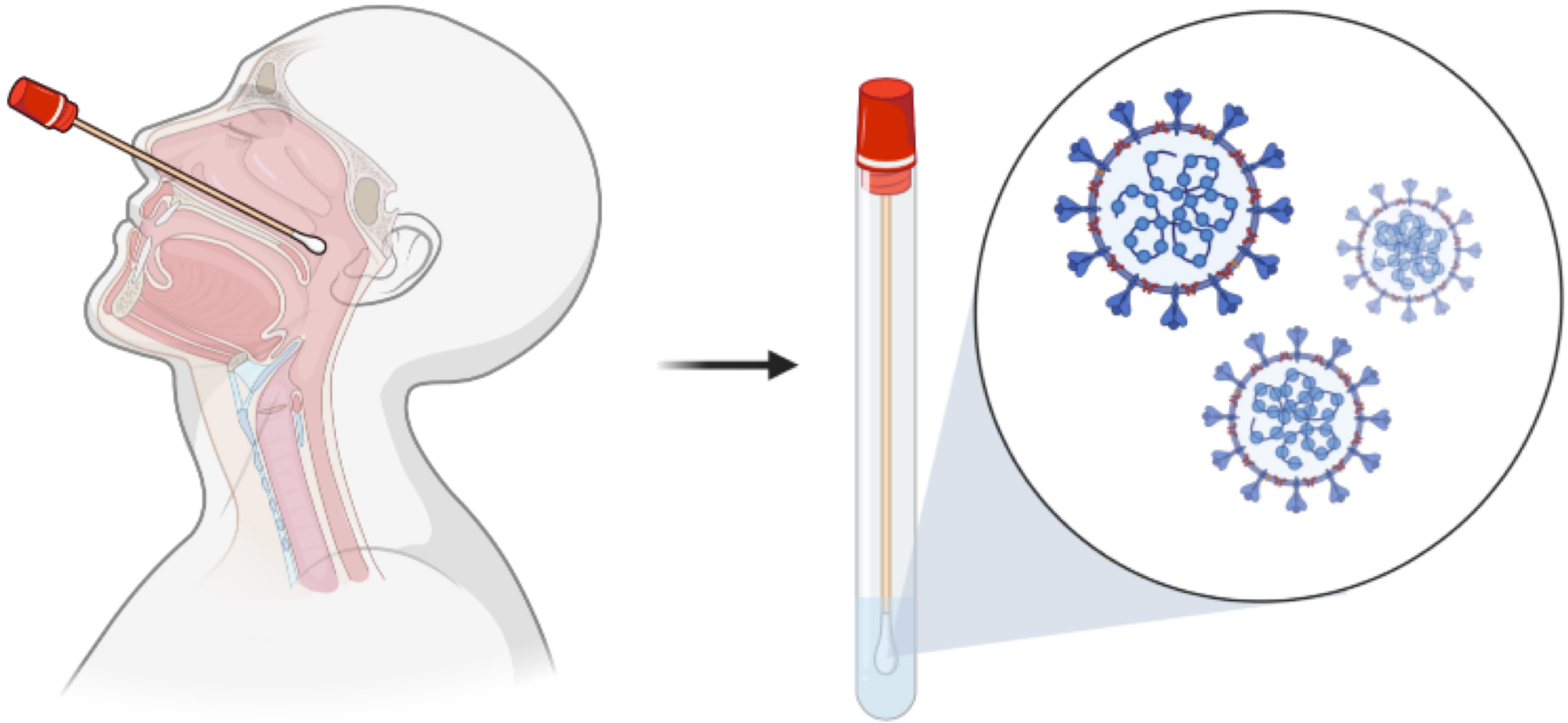
Applications: famous COVID19 tests



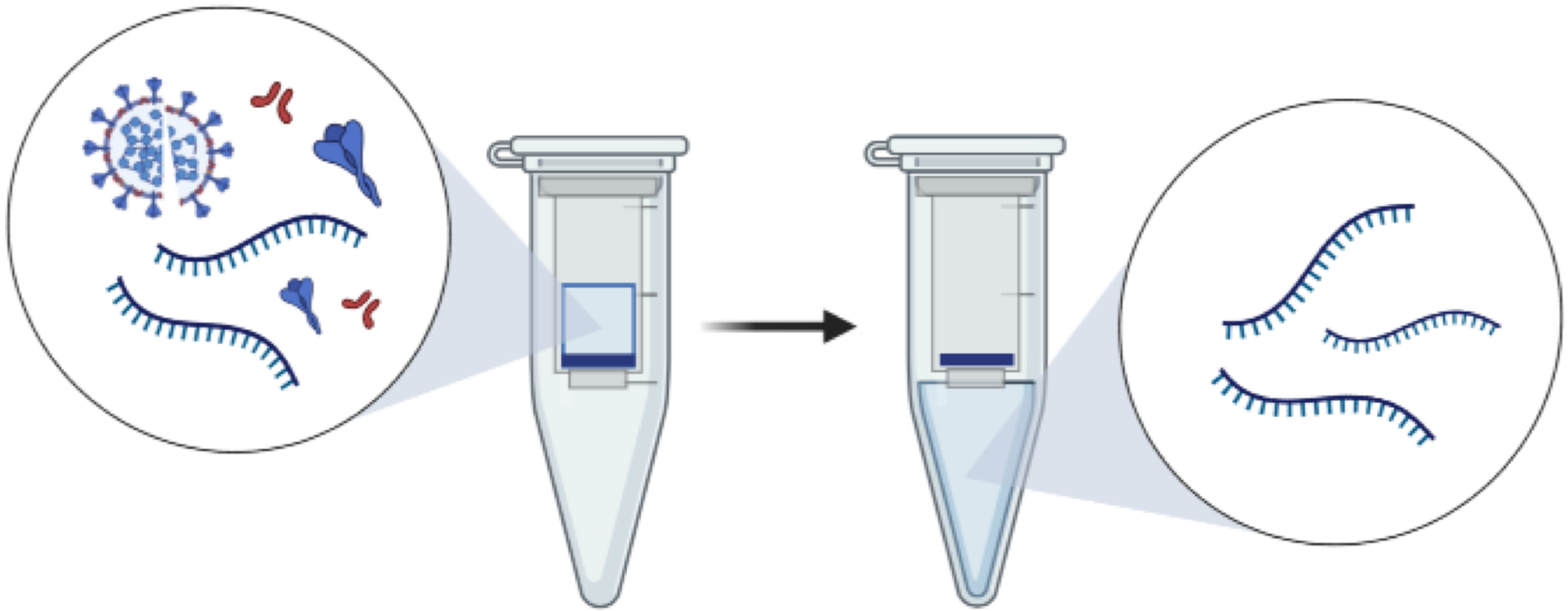
Design of Primers

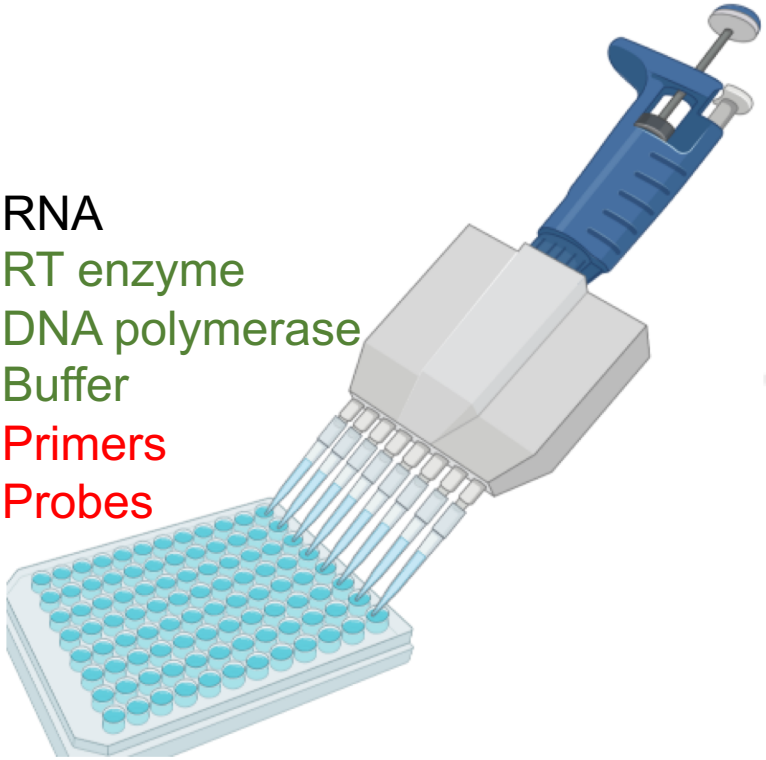
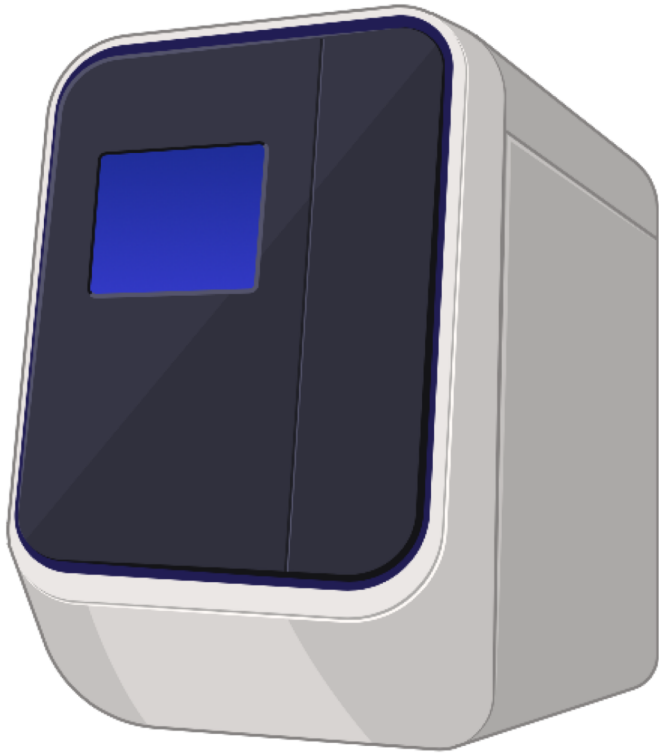
- Internal control primers:
  - GAPDH, RNAseP, ACTB, eEF-1 etc.
- Viral DNA primers: N region
  - N gene (CDC Panel, Hong Kong Panel, Japan National Institute of Infectious Diseases Panel)
  - E gene (Berlin-Charité Panel, Institut Pasteur Panel)
  - ORFab primers (Berlin-Charité Panel, China CDC panel)
  - RdRp (Berlin-Charité Panel, Institut Pasteur Panel)
  - S gene

Nasopharyngeal & Oropharyngeal swabs



RNA isolation



RT-PCR

RNA
RT enzyme
DNA polymerase
Buffer
Primers
Probes