Unexpected developments during isolation

How a HEP physicist got involved in building a highly sensitive yet inexpensive COVID detection system in Argentina

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Fermilab - Joint Experimental-Theoretical Physics Seminar - May/20/2022

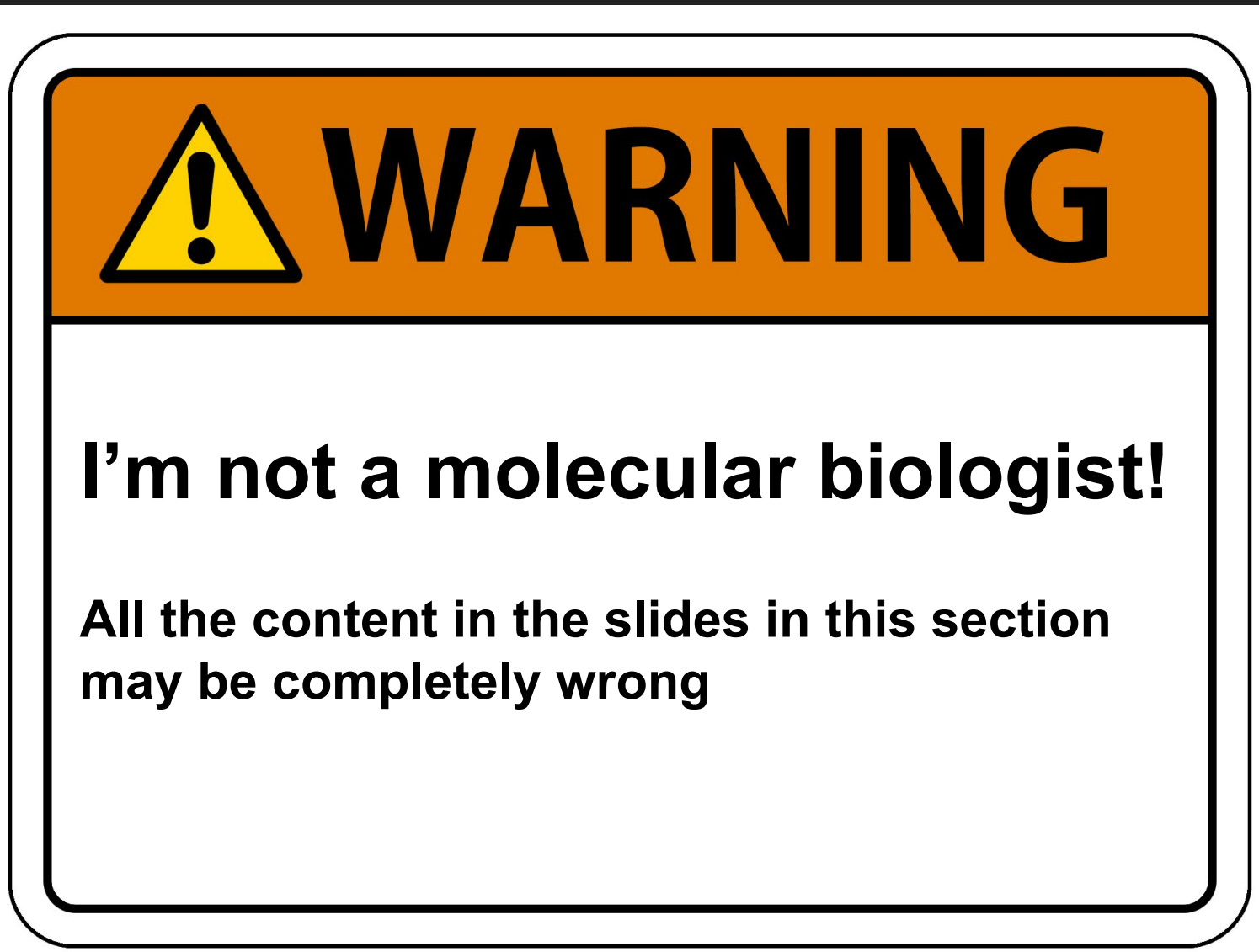
Talk summary

- What is this talk about?
- Brief introduction to COVID detection methods
- Testing situation in Argentina at the beginning of the pandemics
- Developing of NeoQ, a COVID detection equipment

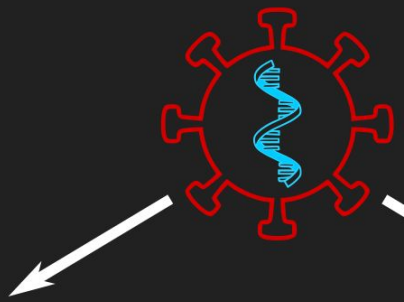
What is this talk about?

- During the early stages of the pandemics Argentina had challenges related to COVID-19 testing
- The only technology was PCR but it was not easily available
- A small group of Argentinian scientist from the University of Buenos Aires had an idea to improve the testing capabilities
- I got involved in the project working after hours and weekends during the many months of lock-down (I was in Argentina)
- We were able to build and deploy the NeoQ system that can greatly expand the capabilities of an Argentinian-developed genetic test technology (NeoKit)

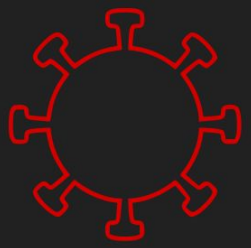
WARNING!


WARNING
I’m not a molecular biologist!
All the content in the slides in this section may be completely wrong

Very brief taxonomy of COVID detection techniques



Look for the virus shell



These techniques are more direct. They look for the materials from which the shell is made

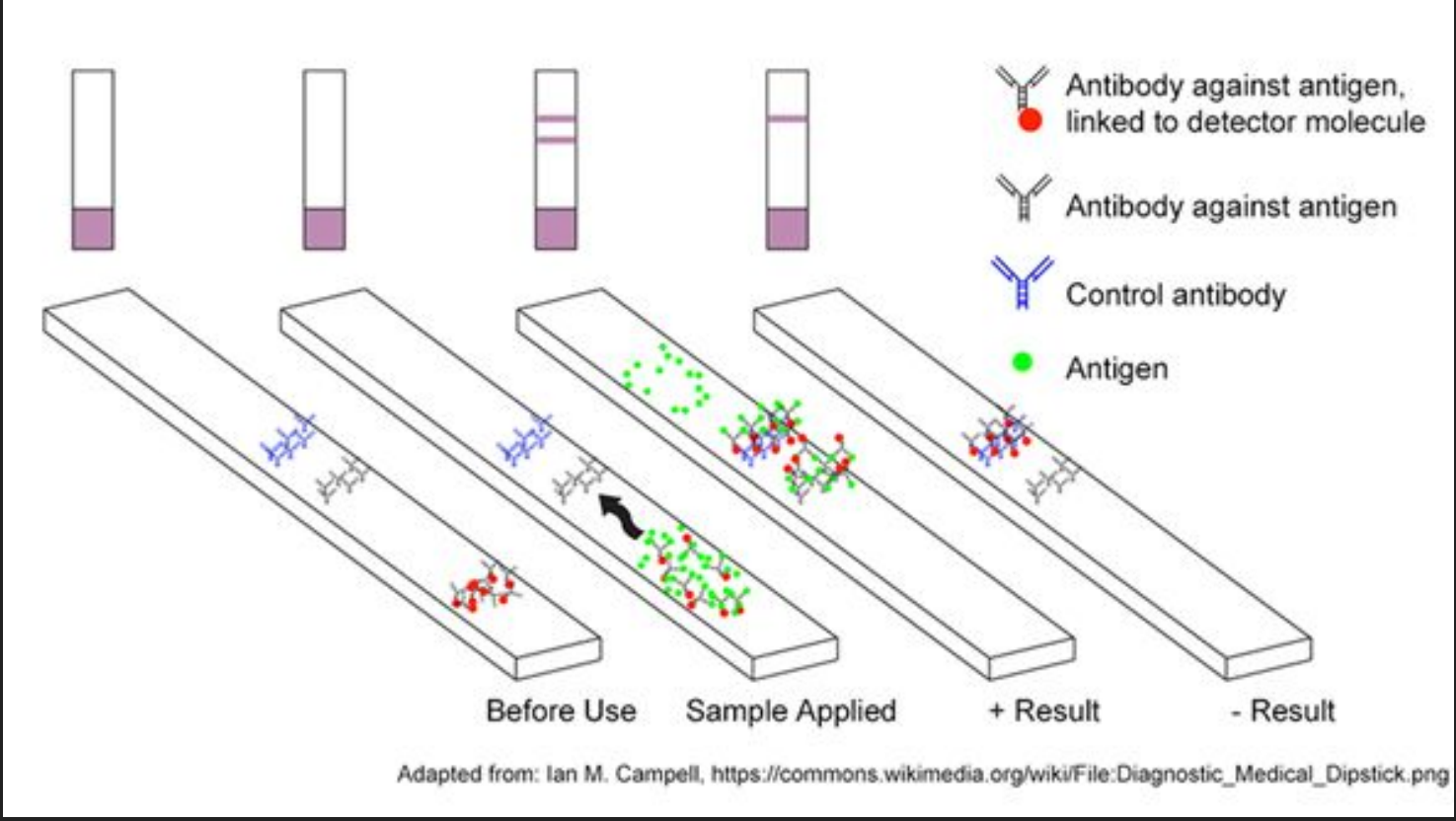
If the initial number of viral particles is small, they may not produce a positive signal

Look for genetic material of the virus

These techniques rely on producing many copies of the molecules they want to detect (amplification)

Thanks to amplification a single starting molecule can produce a positive signal

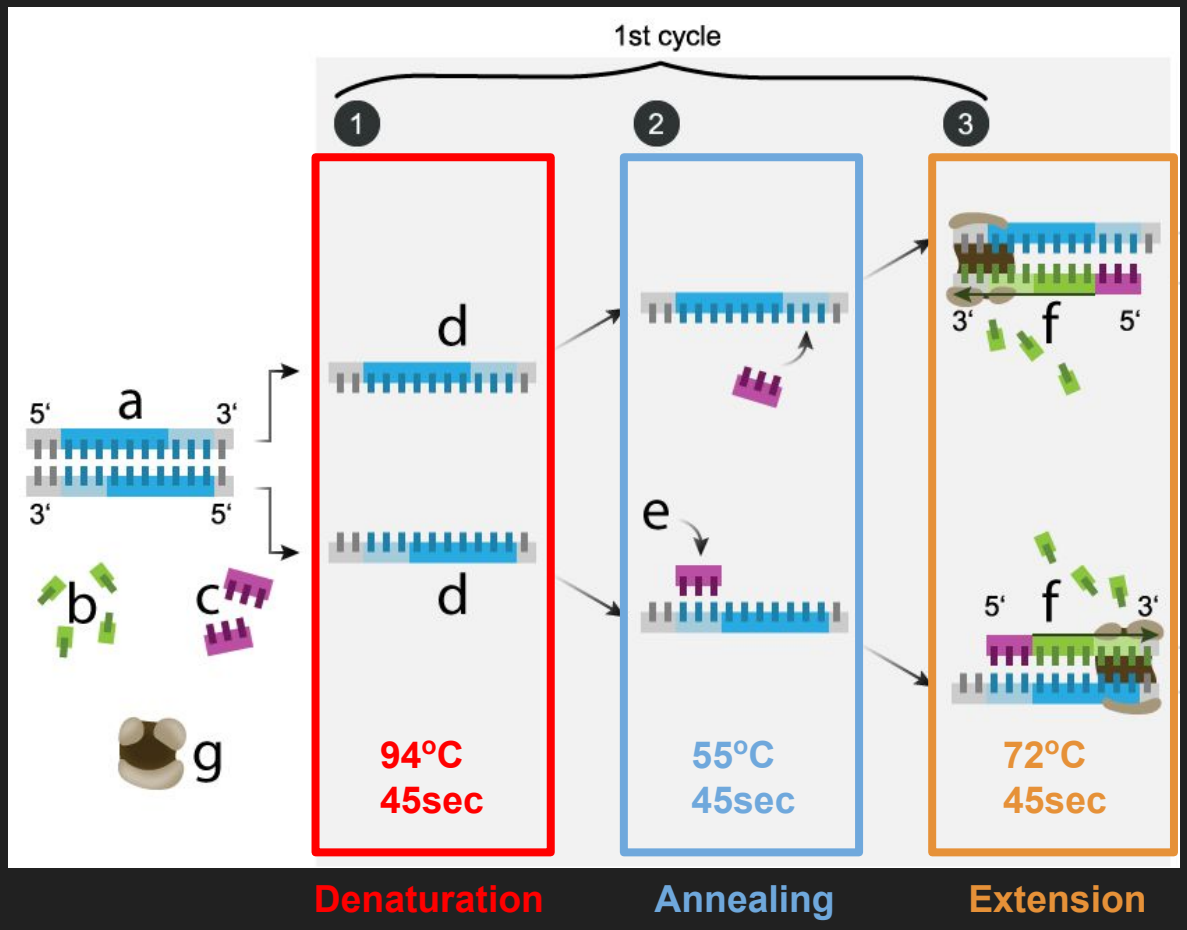
Antigen tests (direct, no amplification)



These are the Rapid-Tests

It takes O(1000) viral particles to produce a positive signal

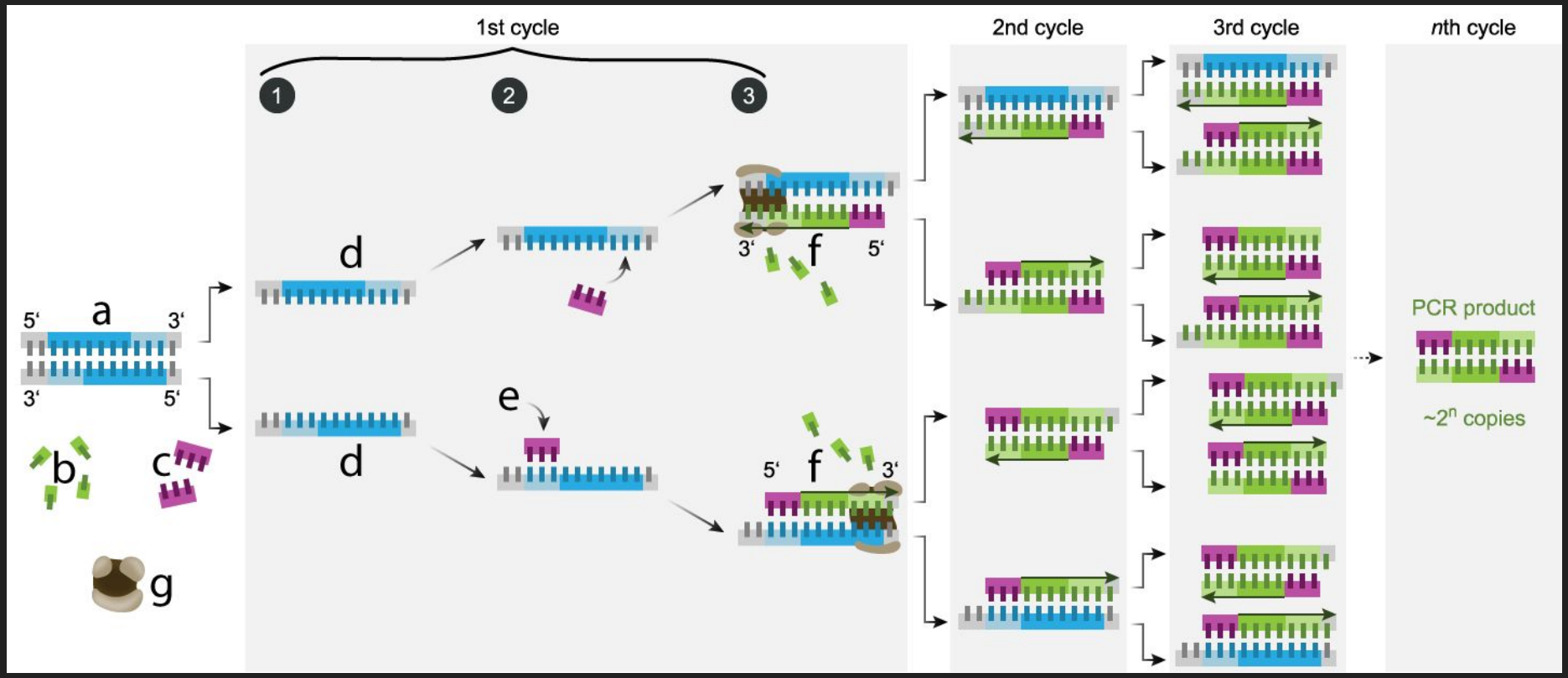
PCR amplification



The temperature needs to be controlled very precisely

The cycle takes around 2 minutes

PCR amplification



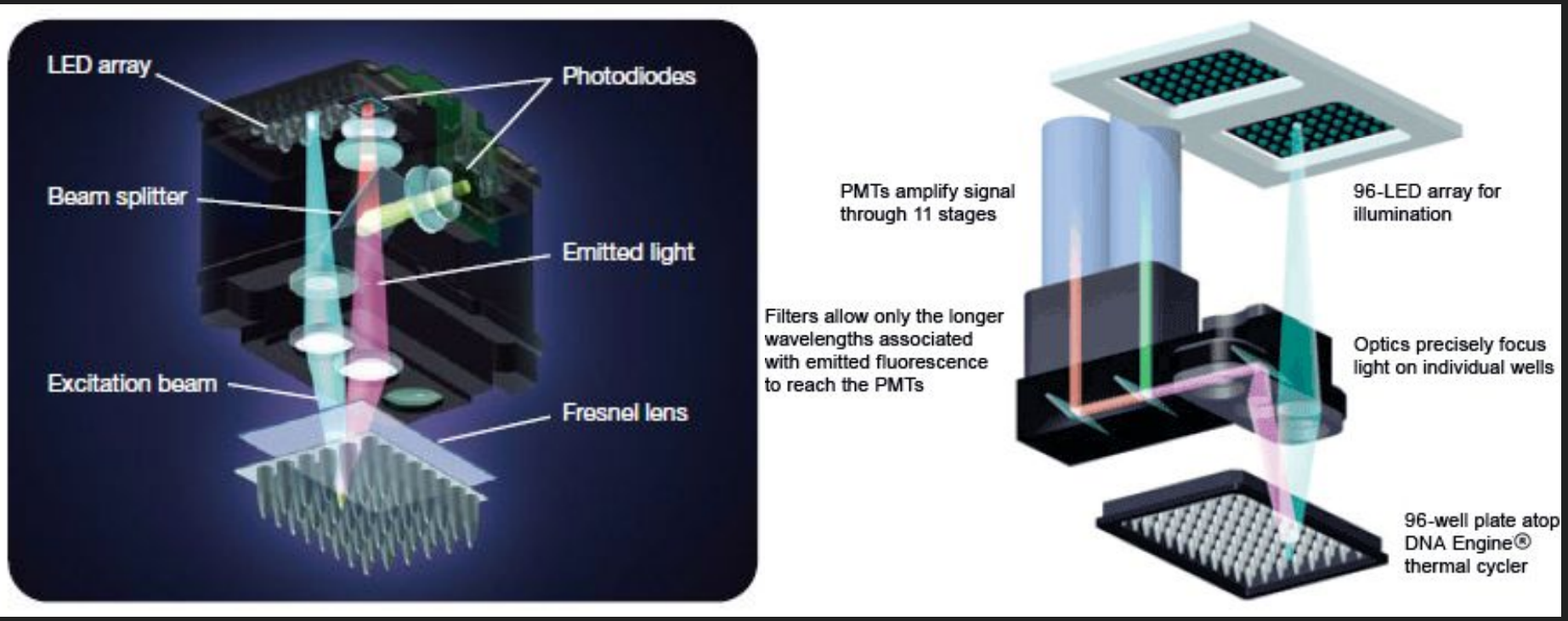
At the end of each cycle the number of DNA fragments is doubled

After *N* cycles the amplification factor is 2^N

Typically 35-40 cycles is the maximum used

In practice the detection limit is ~3 DNA copies

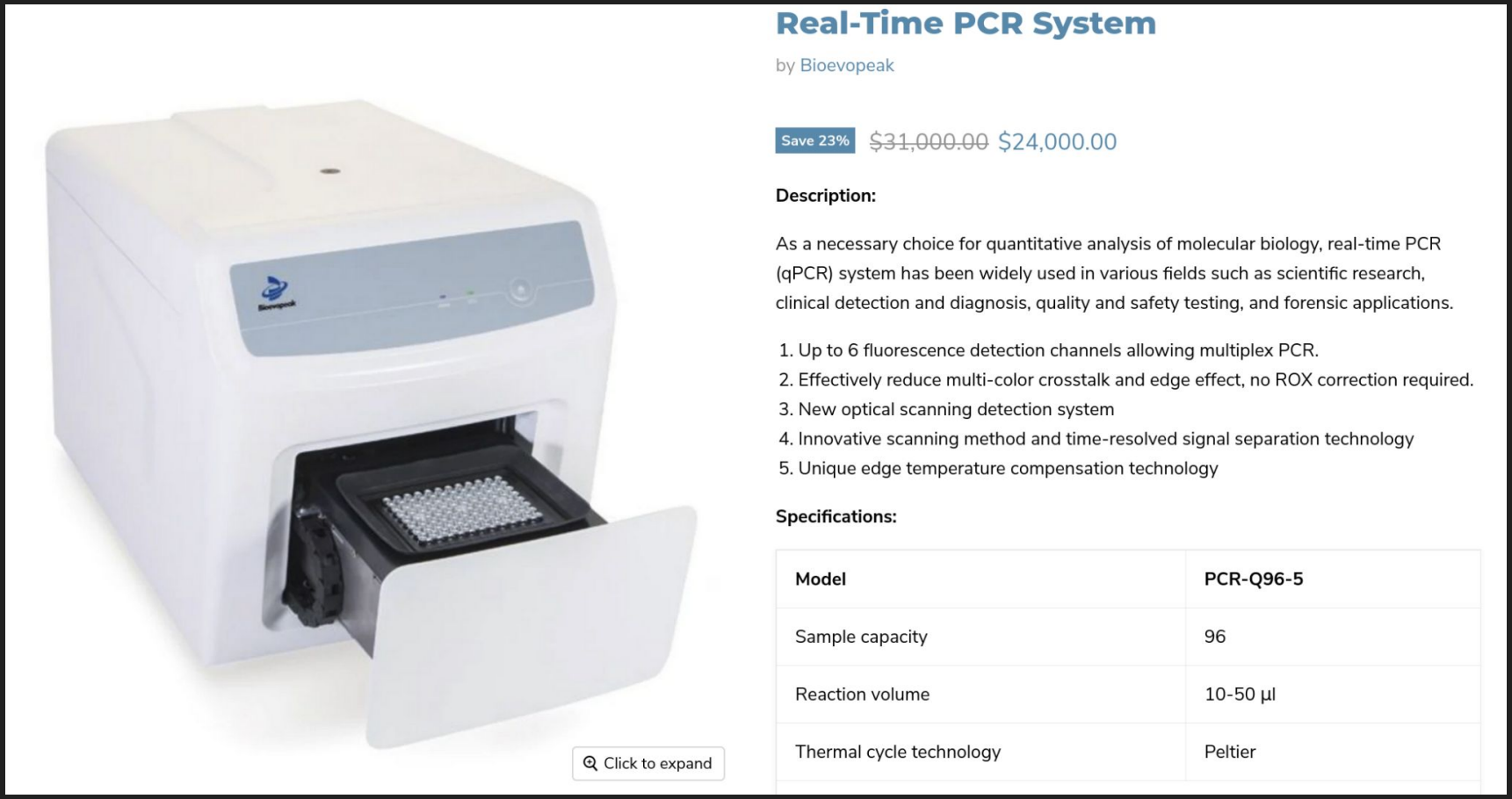
qPCR Machine



To measure the amount of DNA that is being produced in each cycle, qPCR systems excite a DNA-binding dye and measure the amount of emitted light. This is complex.

Many detection techniques are used (CCDs, PMTs, etc)

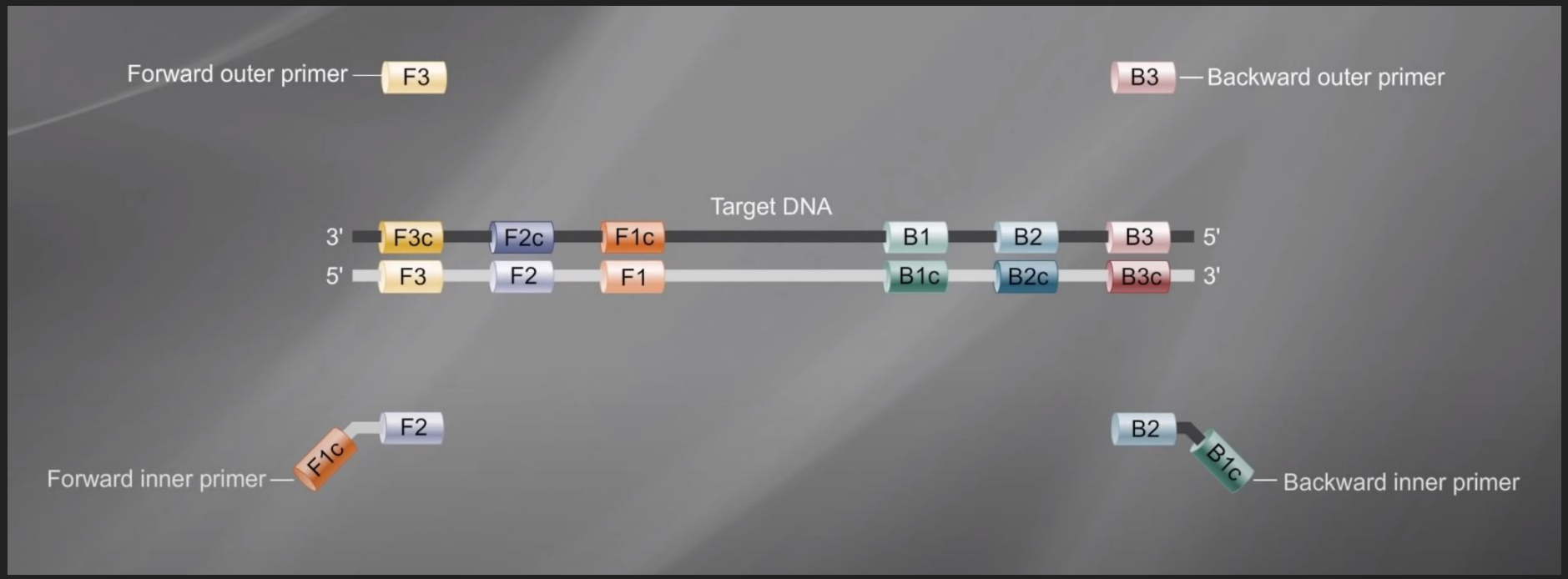
PCR machines



qPCR Systems are expensive and complex

Price is in the [$20k, $100k] range (this one is steal!)

LAMP amplification

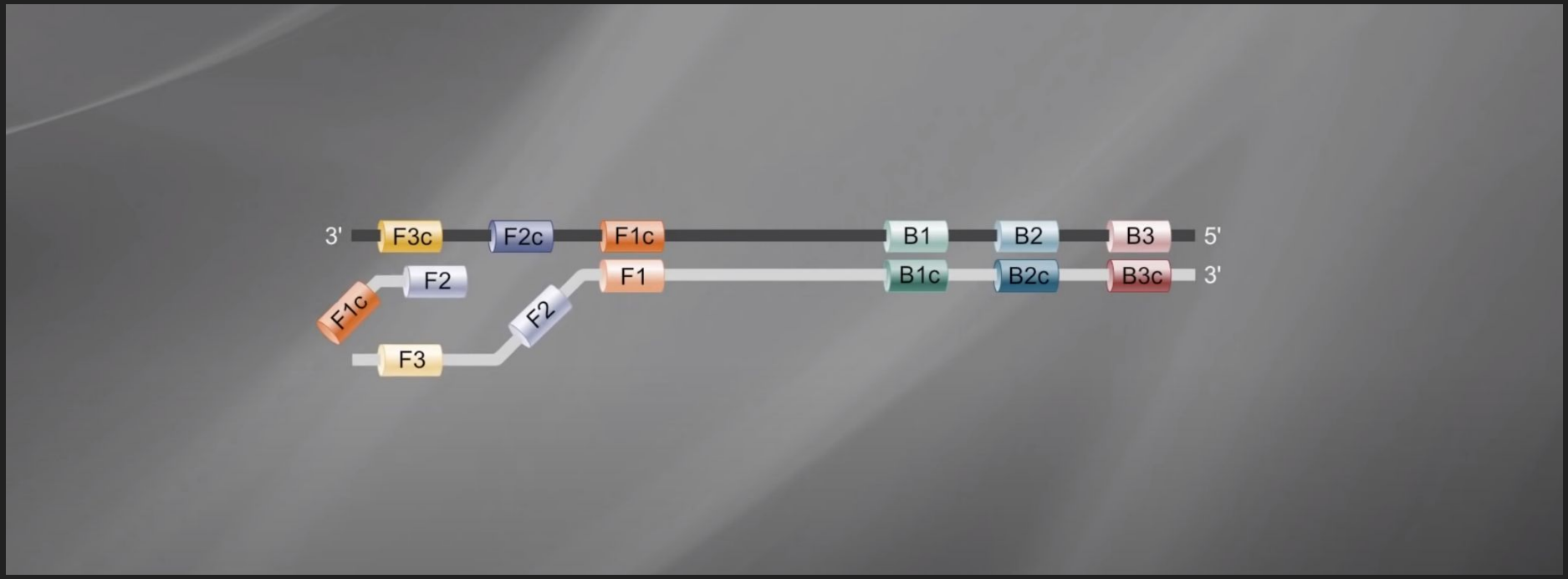


We start with a double stranded DNA fragment

In the medium there are four different primers

The temperature is constant at 64ºC

LAMP amplification



The medium is at 64ºC

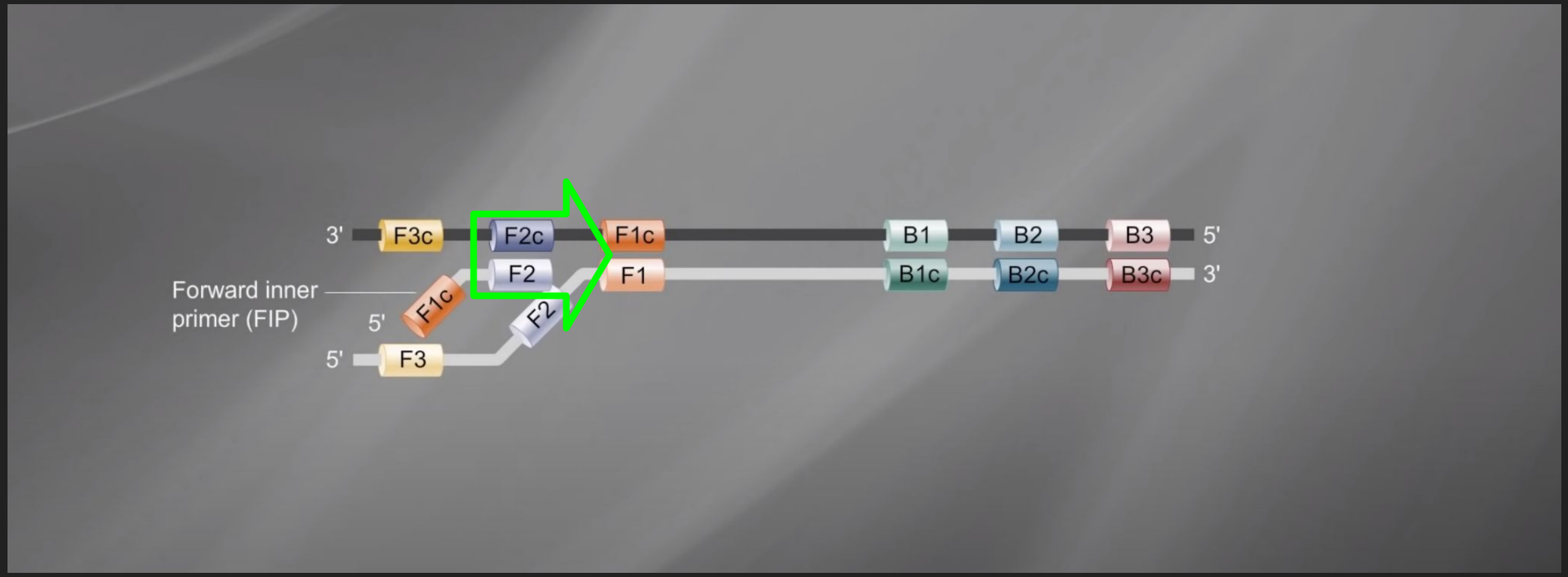
This is not enough to separate the strands

But.. there can be local openings that allow invasion

LAMP amplification

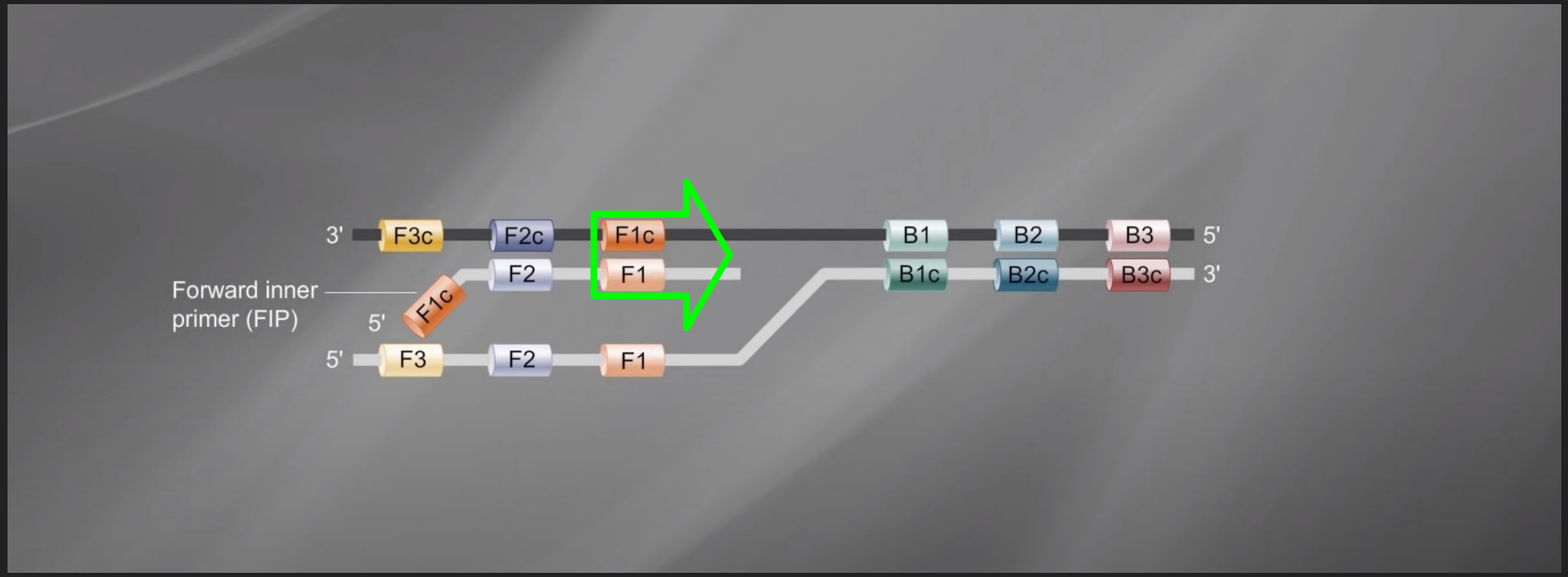
The FIP primer binds to the strand and provides a binding site for the polymerase

LAMP amplification



The FIP primer binds to the strand and provides a binding site for the polymerase (green arrow)

LAMP amplification



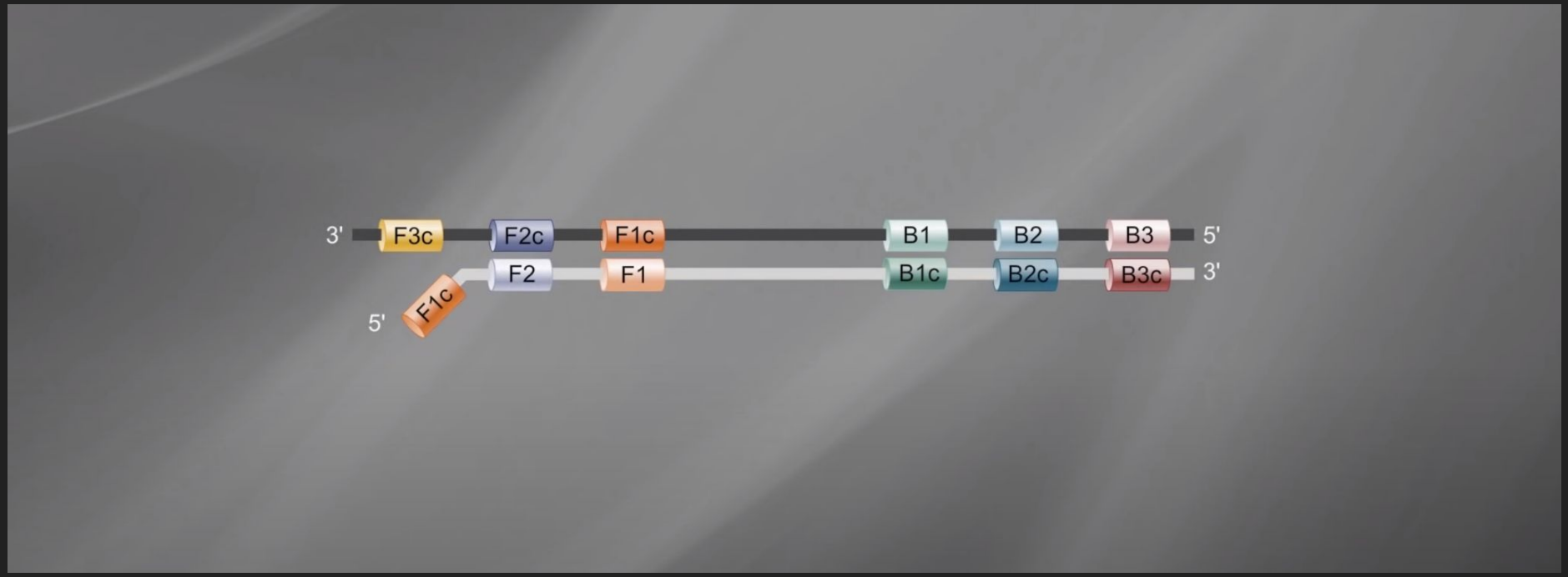
This is a special polymerase, it can separate the strands as it build the copy (strand displacing polymerase)

It's like a zipper

LAMP amplification

Once the strand is fully copied the displaced complementary one will also provide binding sites for the primers and will be copied

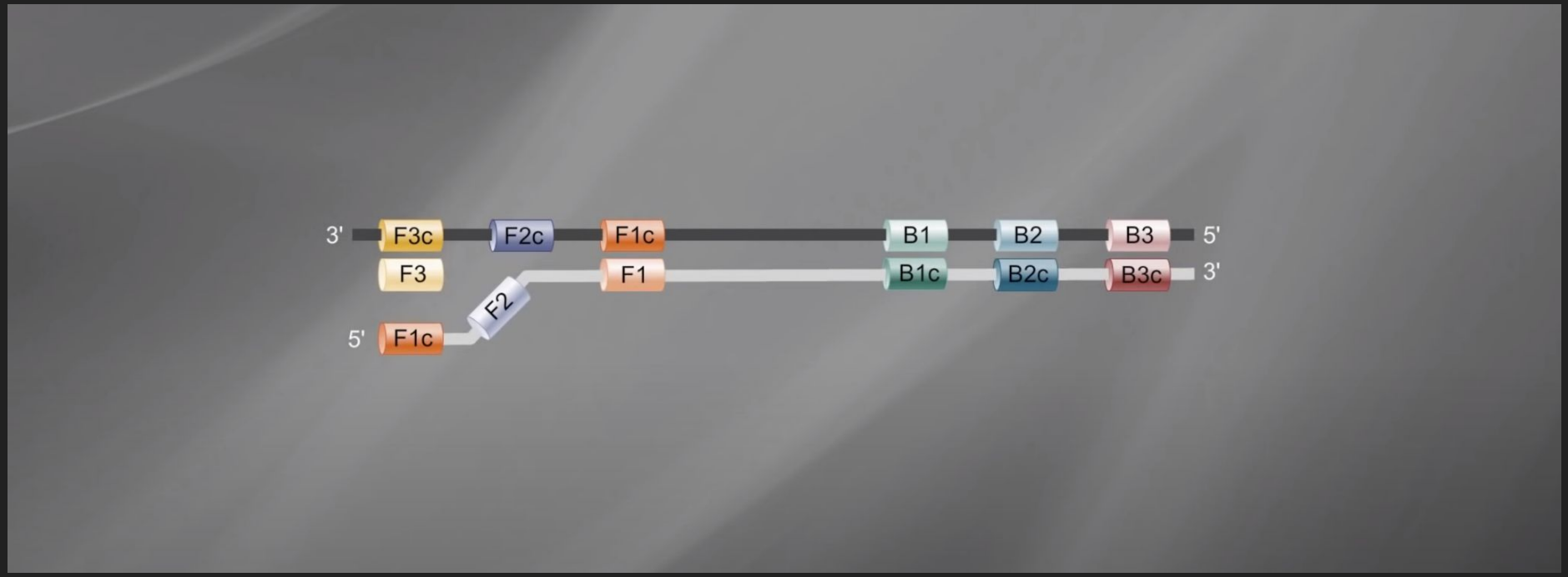
LAMP amplification



Note that the new strand (the one at the bottom) is not identical to the original complementary one

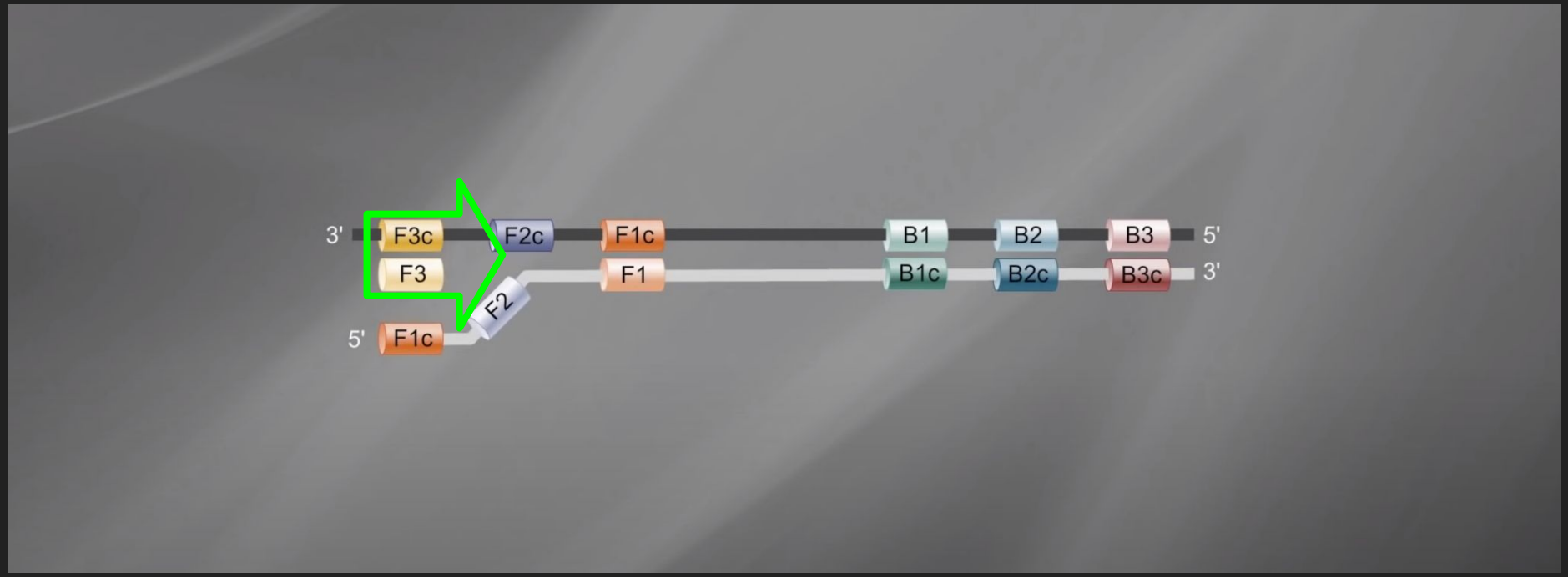
The first section is different F1c instead of F3c

LAMP amplification



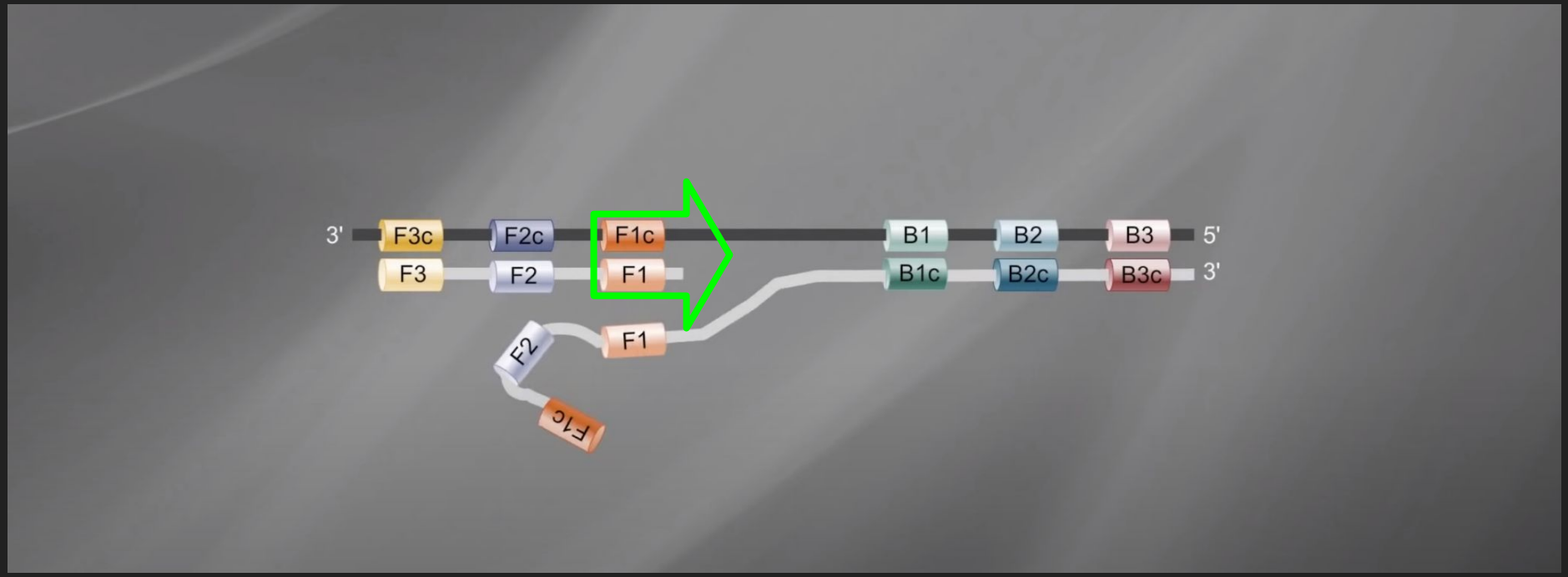
Because the F3c site is not paired it provides a binding site for the Forward Outer Primer F3

LAMP amplification



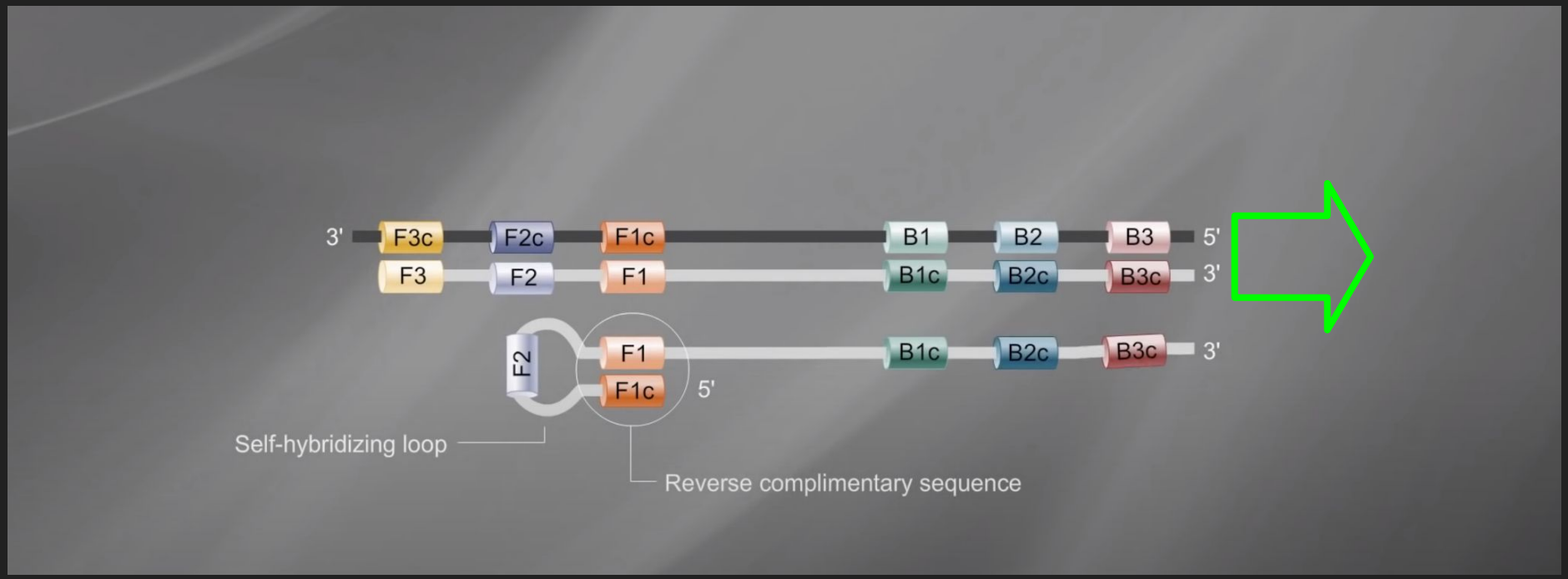
The polymerase can bind to this site and start a new copy

LAMP amplification



As it moves forward it releases the modified copy of the complementary strand (the one that starts with F1c)

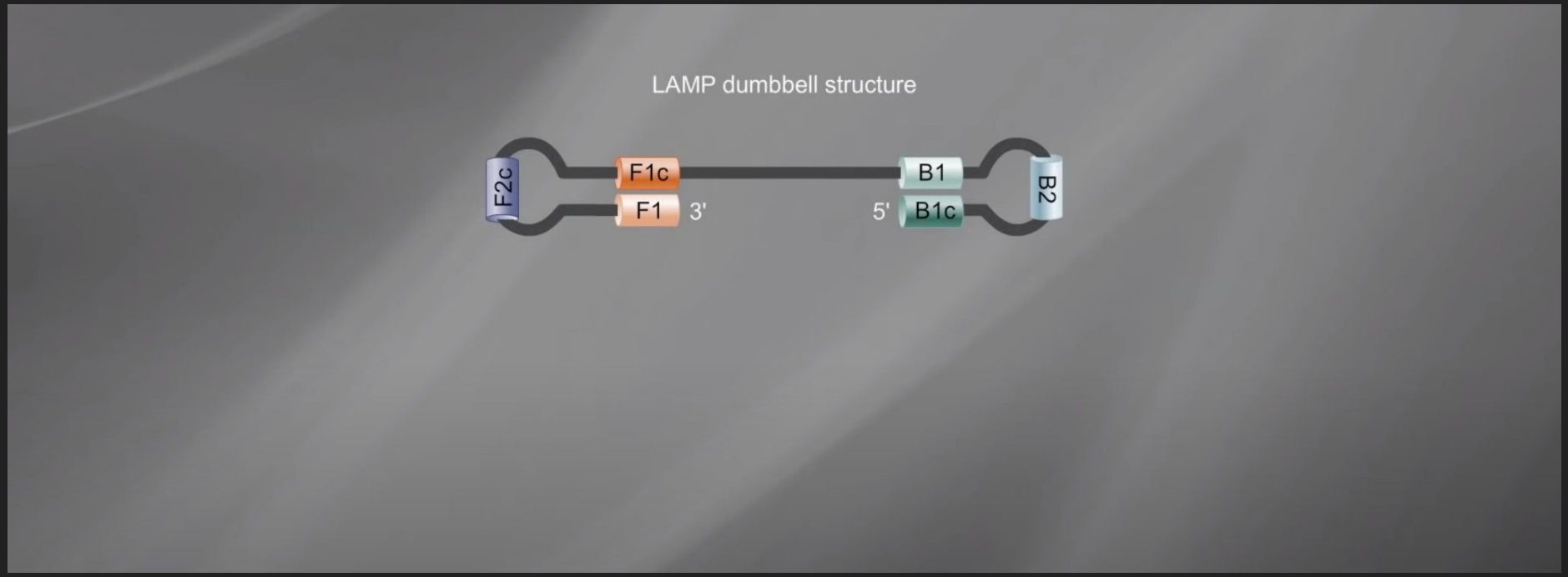
LAMP amplification



Note that F1c is complementary to F1!

This was not an accident, the inner primer was designed to do this

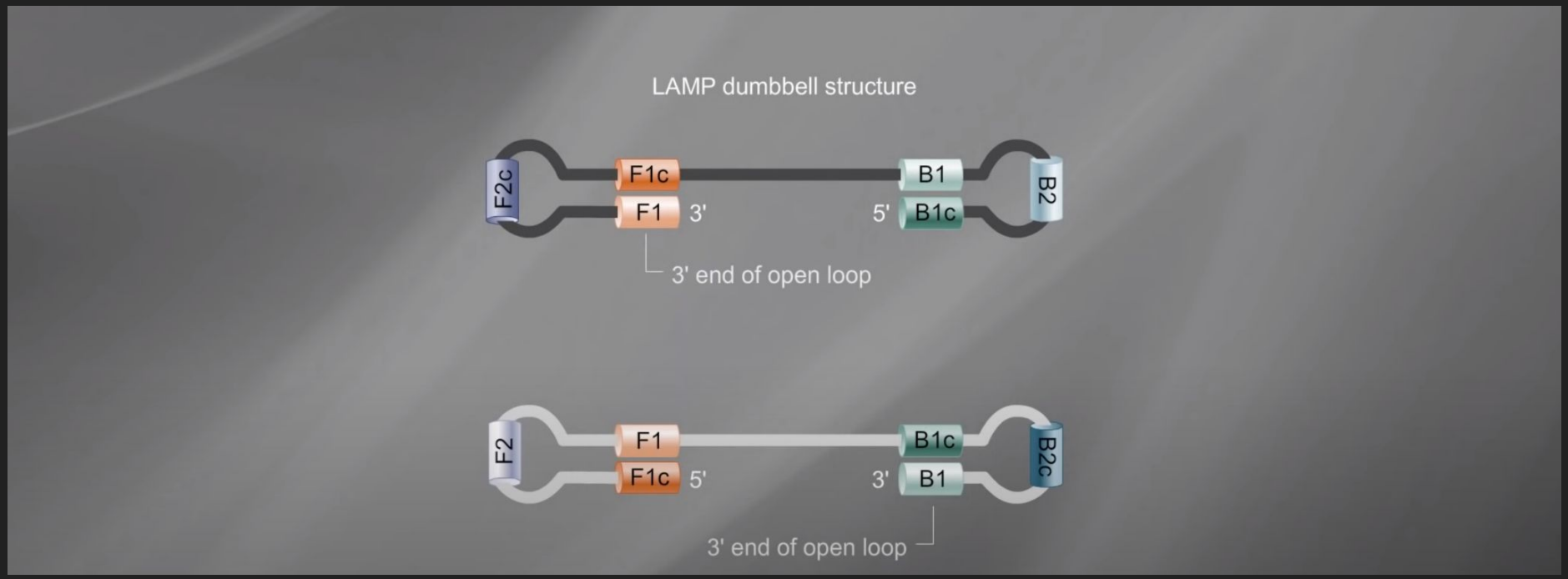
LAMP amplification



The same happens with the backward section

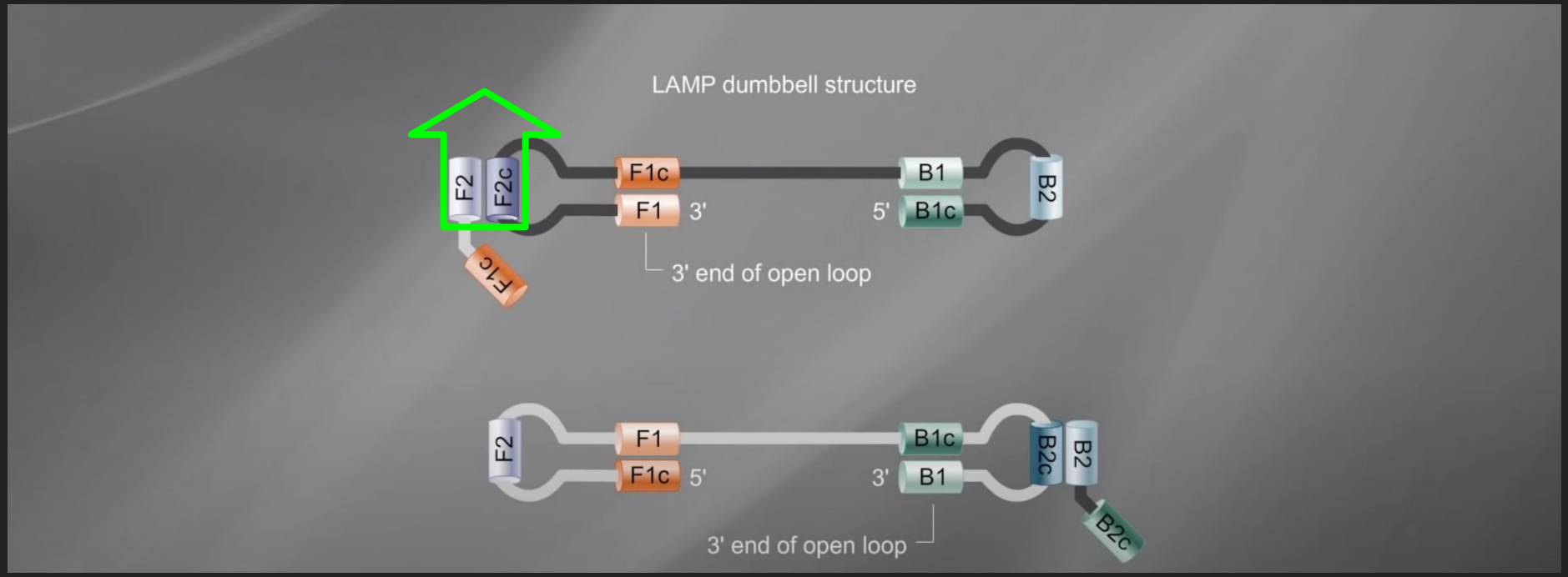
We end up with this double dumbbell structure

LAMP amplification



And the equivalent for the complementary strand

LAMP amplification

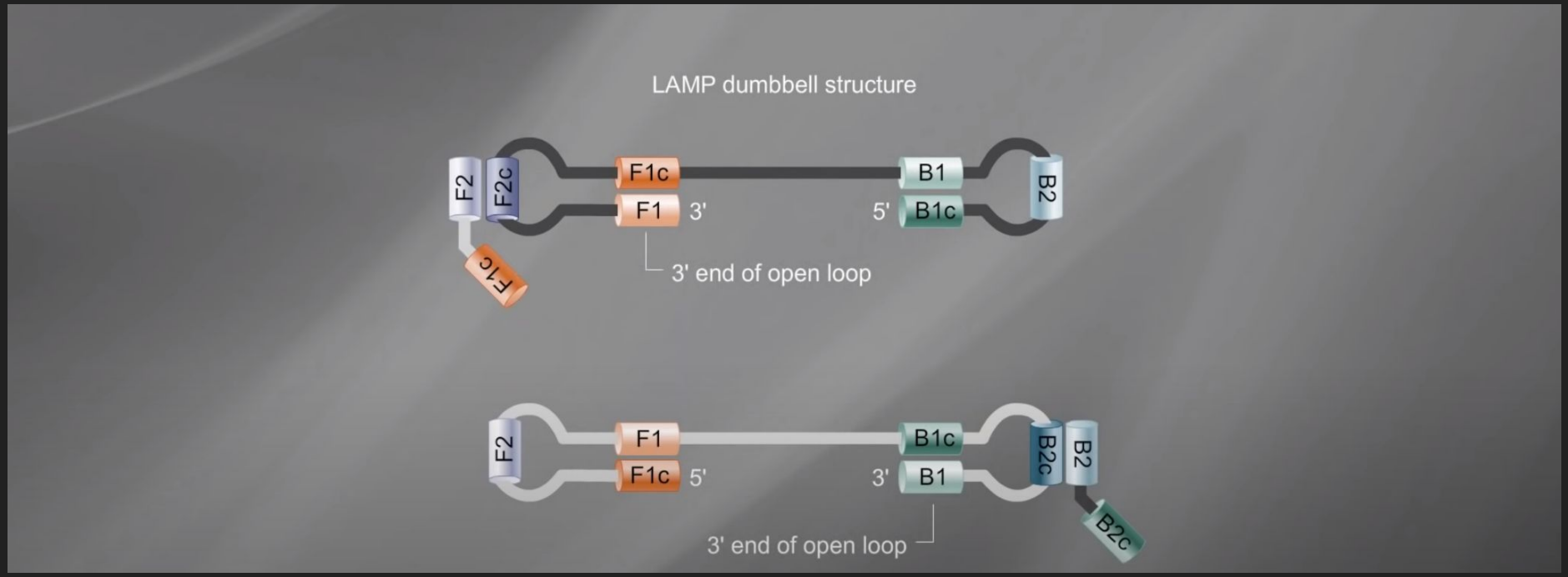


The process starts again!

This is happening continuously at fixed temperature (64°C)

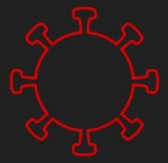
The dumbbell structures provide permanent binding sites for the primers where the polymerase can start a new copy

LAMP amplification



In practice this technique can successfully amplificate if we start with *O*(10) DNA fragments

Detection techniques summary



Antigen	**PCR**	**LAMP**
O(1000)	O(1)	O(10)
No additional equipment	Thermocycler machine	Isotermal bath (heat block)

Testing in Argentina at the beginning of the pandemics

- PCR test was the only technique available
- limited availability in Argentina (and only in the big cities)
- Countries restrict the export of COVID detection equipment and supplies
- PCR machines are expensive and require trained technicians
- Argentina strategy was to delay the spread of the virus to allow for the Health System to prepare
- Almost complete lock down. Only essential workers were allowed to leave their homes
    - **It was critical to be able to test them to keep essential services running**

NeoKit: LAMP test developed in Argentina

Developed by CONICET scientists at the Milstein Institute

Approved in May-2020 by the ANMAT (FDA equivalent)

Extracts the genome without purification of the virus RNA

Colorimetric test, it only requires constant temperature (64C)

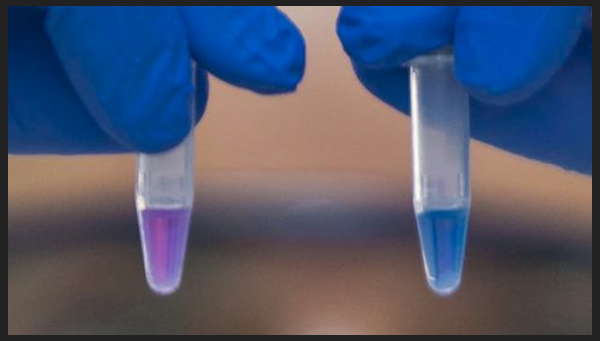
It was fully produced locally using supplies that were available

No PCR machines needed! Heat blocks or baths are enough

NeoKit: LAMP test developed in Argentina

Colorimetric tests:

NEGATIVE



POSITIVE

From taking the sample (swab) to result in one hour

Sensitivity: 95% detection limit is 12 viruses in the sample

NeoKit: LAMP test developed in Argentina

- **The production of testing kits ramped up and they were distributed and widely used**
- **The months that followed the early adoption of the kits provided a lot information on how it performed on the field.**
- **It was then possible to learned its advantages/limitations and what things could be improved**

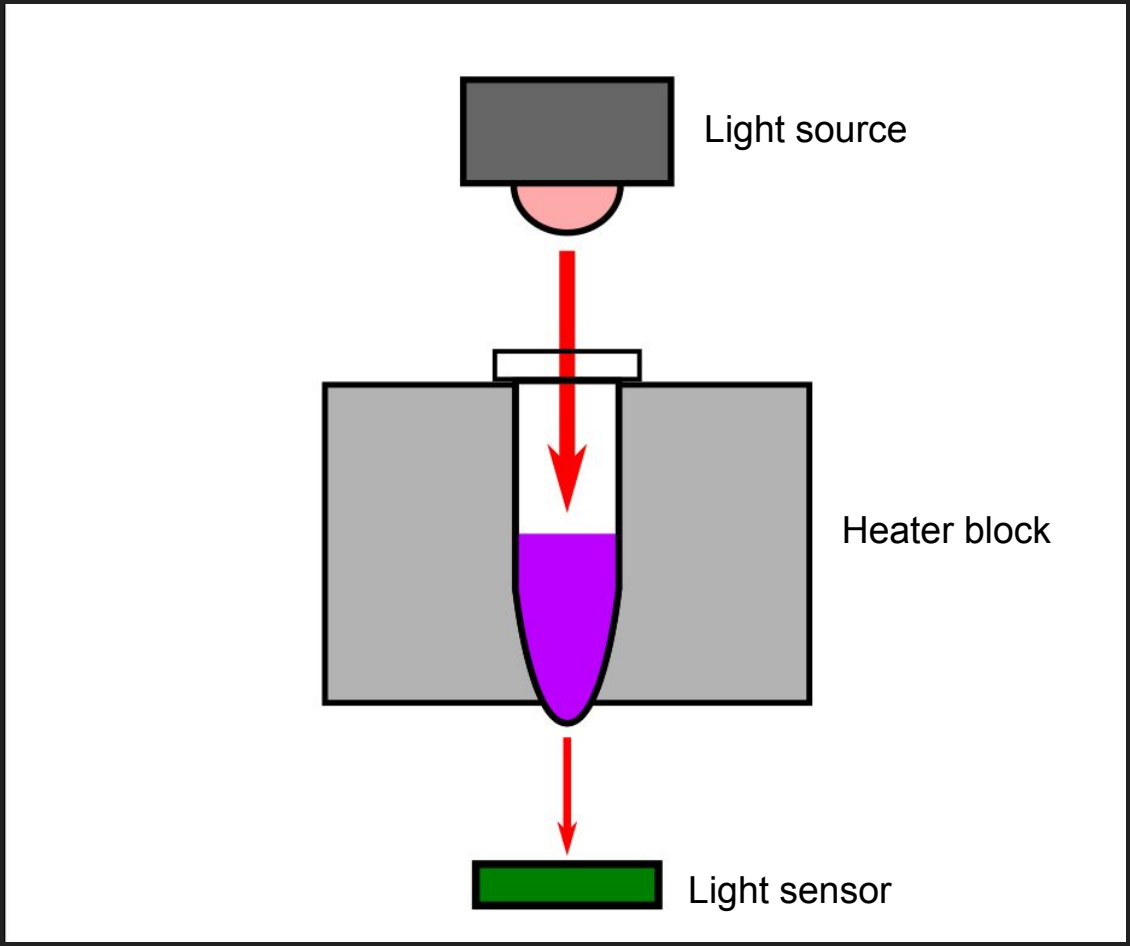
NeoQ project: genesis and motivation

R. Etchenique, Chemistry Professor at the UBA

- NeoKit result can be misread by the lab technician
  - color differences are hard to read and sometimes subjective
- It is critical to have a good temperature control to get consistent performance
- A real-time measurement of the color change could provide a quantitative measurement of the viral load (same as qPCR)
- If it was possible to push the sensitivity to 30+ CT PCR equivalent the kit could be used to do pool-testing

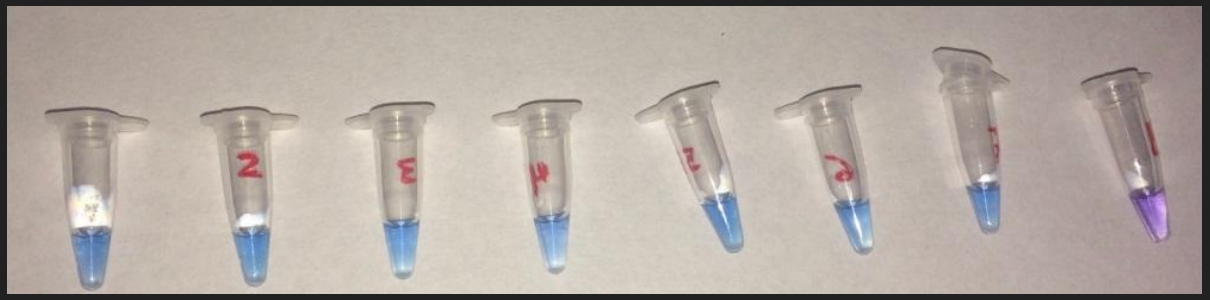
NeoQ project: initial concept

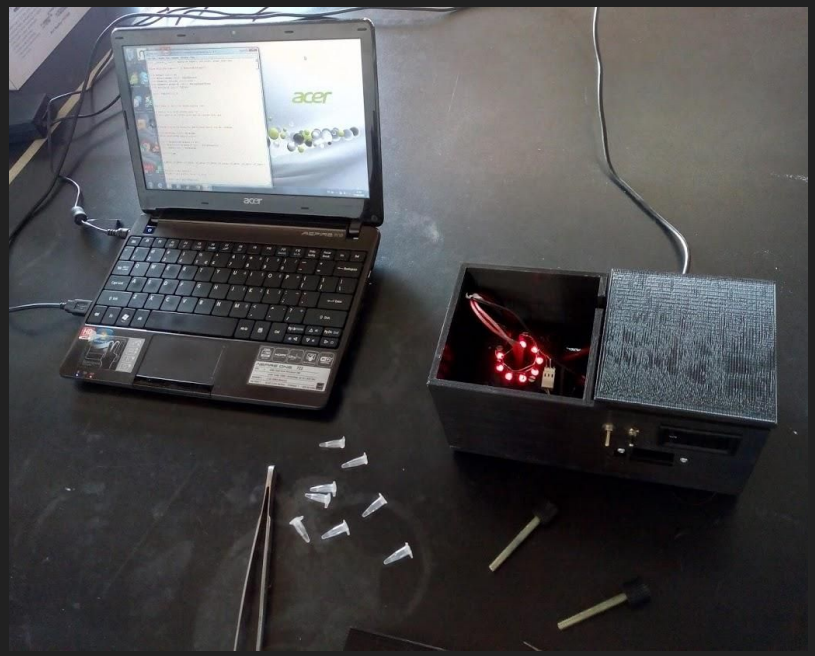
Simple idea: one real time spectrophotometer per tube

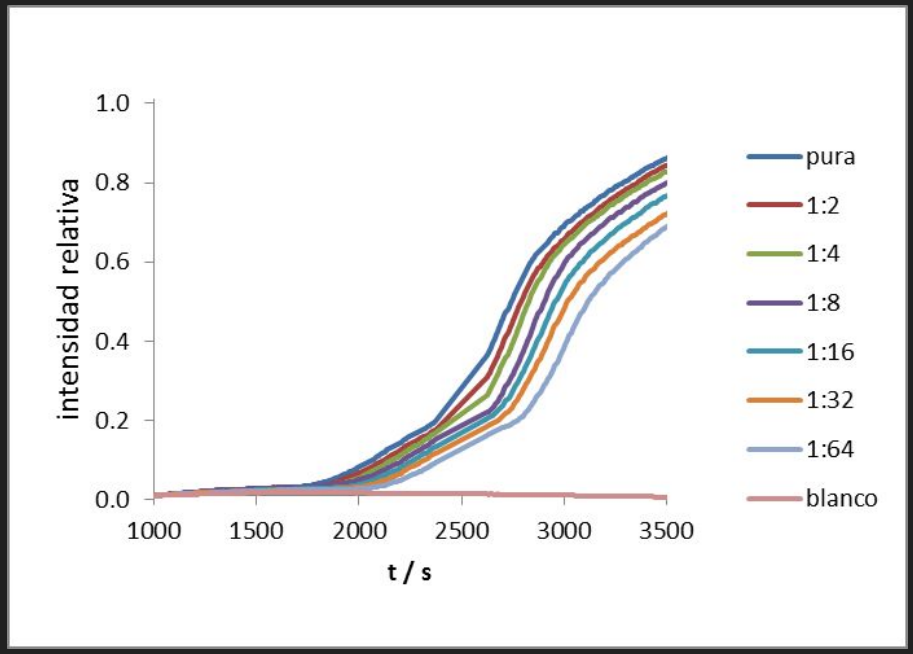


Day 0 (Aug-13-20): Proof of principle at the UBA

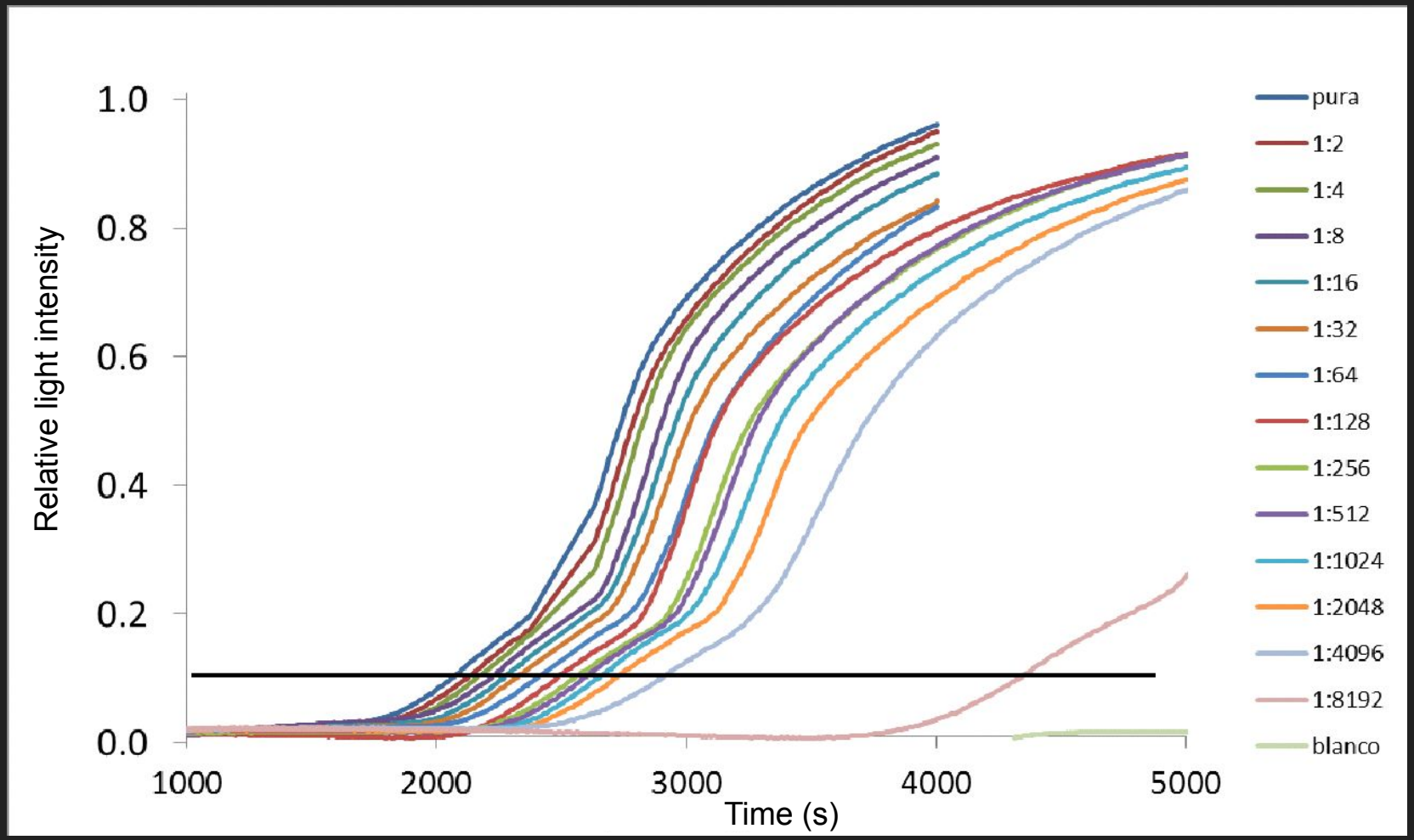
1:1 1:2 1:4 1:8 1:16 1:32 1:64 Neg



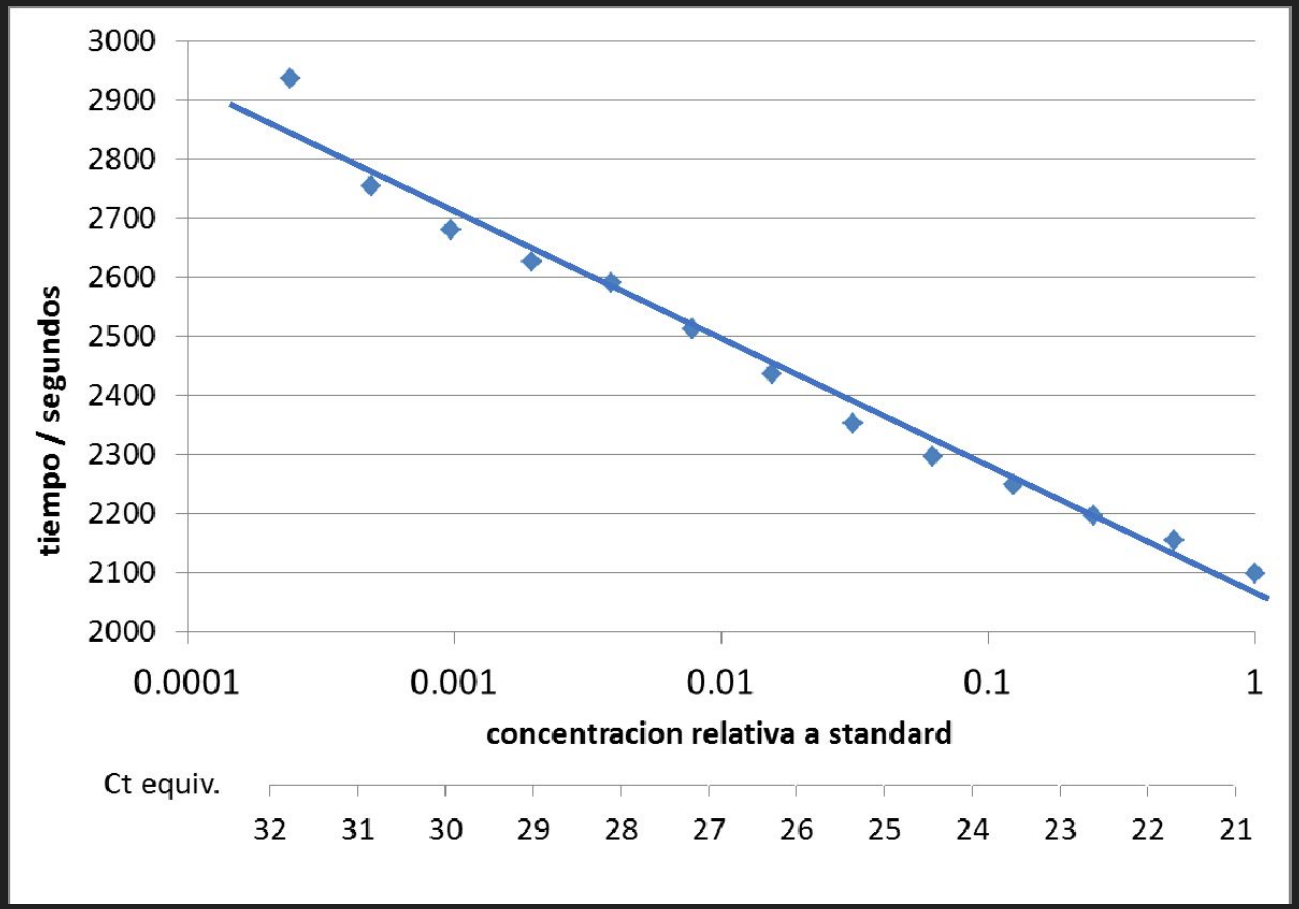




Day 0 (Aug-13-22): Proof of principle at the UBA



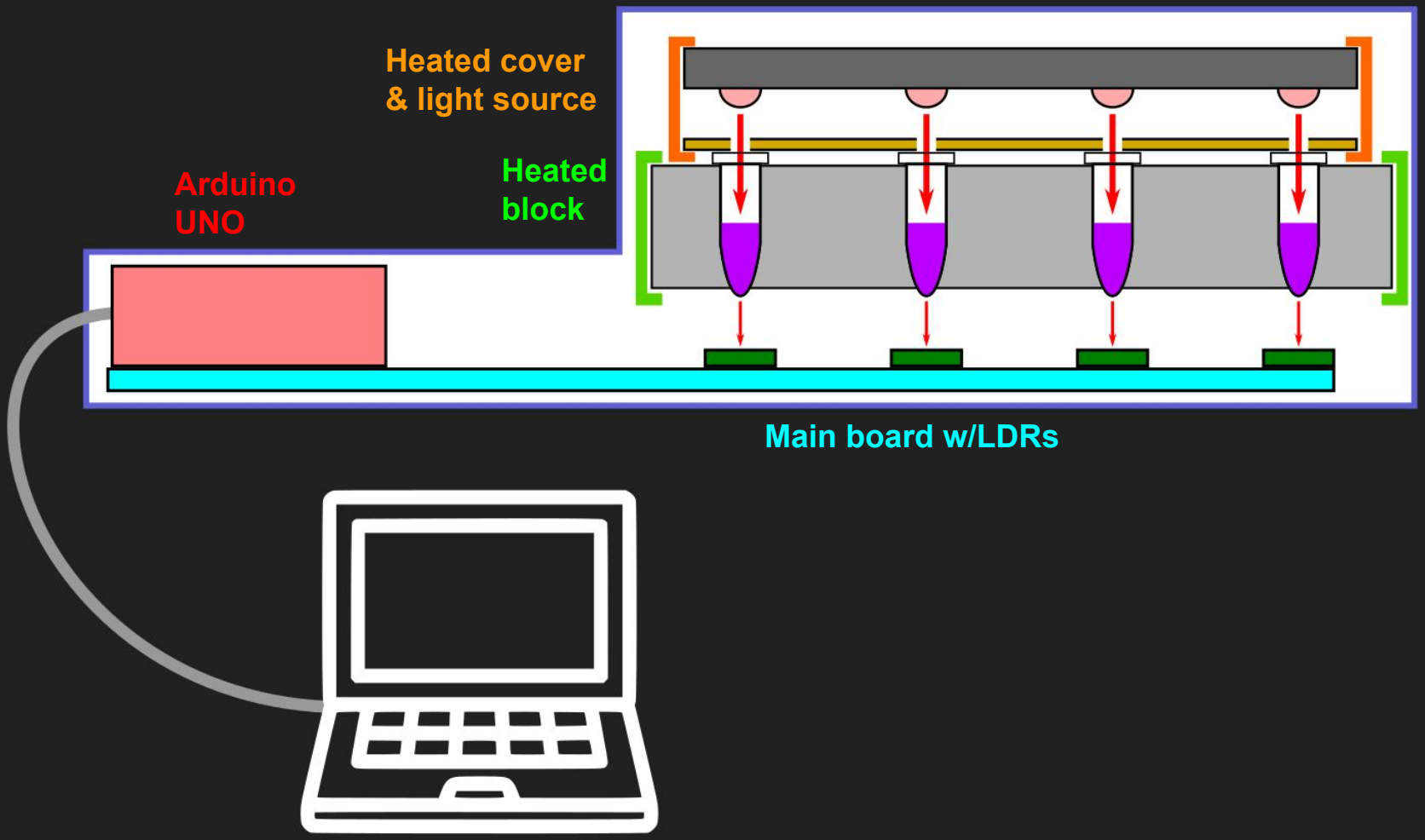
Day 0 (Aug-13-22): Proof of principle at the UBA



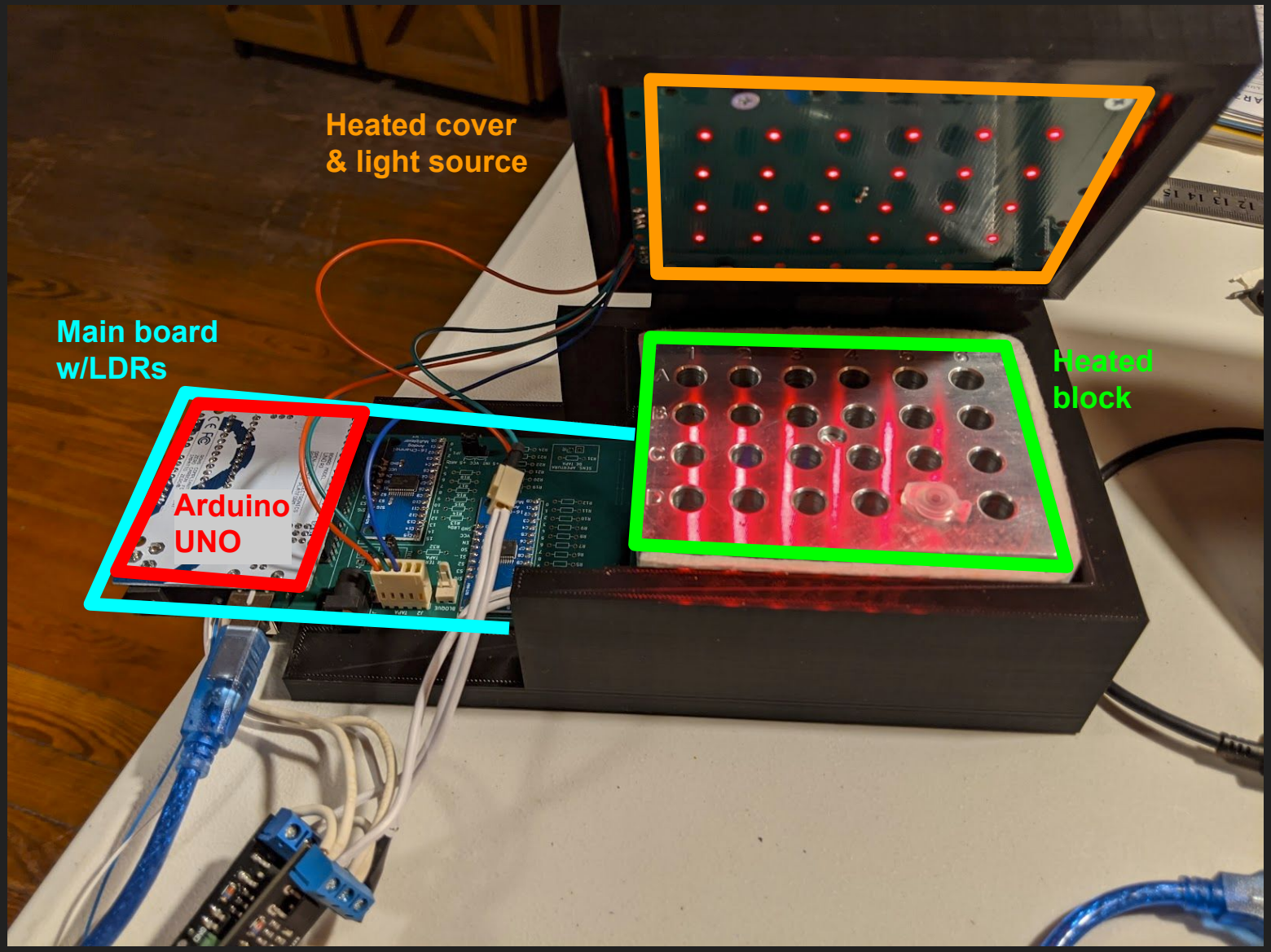
It was possible to get a quantitative measurement of viral load

Sensitivity was better than 30 CT PCR equivalent

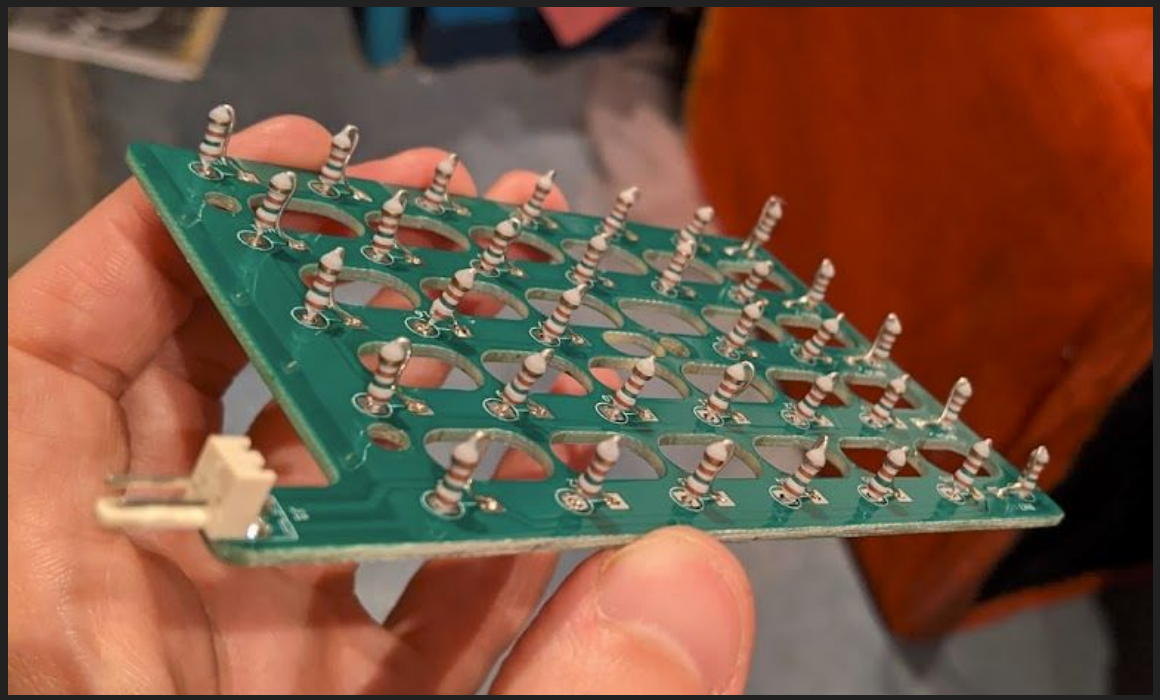
Day 10ish: conceptual design (around this time I joined the project)

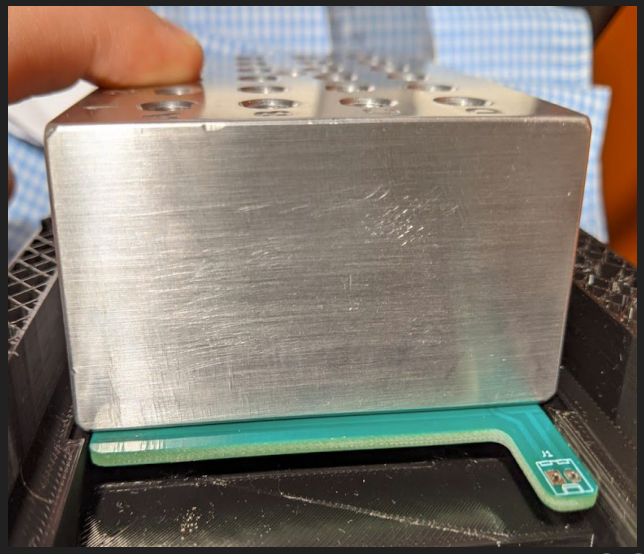


Day 64: Version 1.0



Version 1.0: heater block





Heating was done by resistors soldered to a PCB board

The block was machined to allow the resistors to go in it

It worked but was extremely cumbersome to assemble

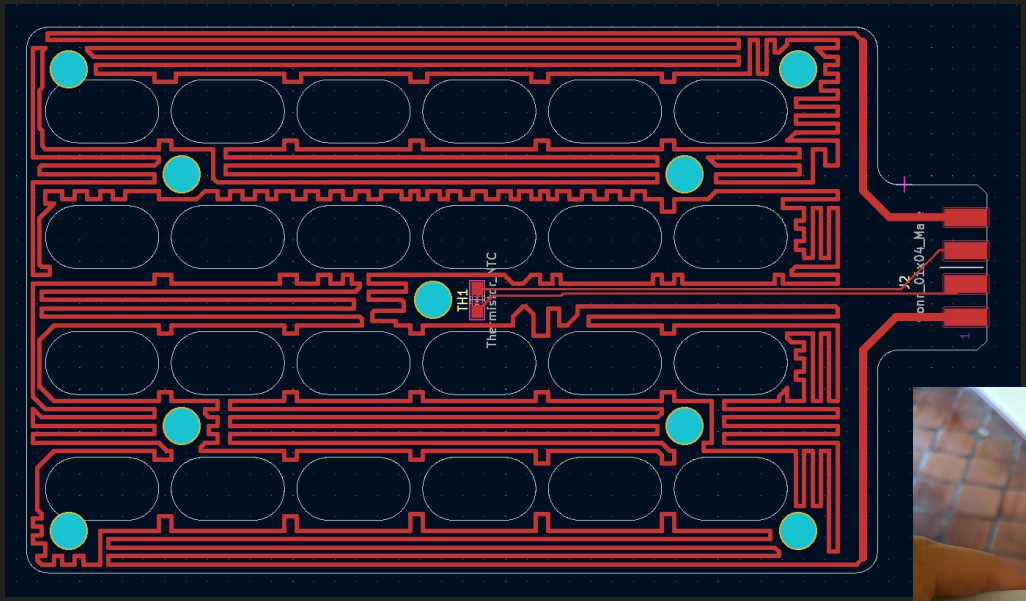
Towards NeoQ V2.0

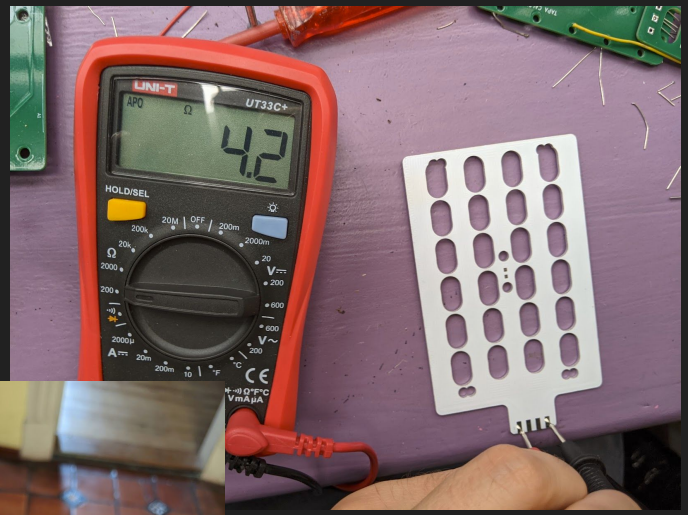
- **NeoQ V1 version was functional and allowed us to make a lot of progress**
- **We learned many things from it but..**
  - **The heater was too complicated and very cumbersome to assemble**
  - **The housing was hard to fabricate and too bulky**
  - **The inner cables were very hard to route and prone to stress**
  - **The ADC was limited by quantization effects (10 bits)**

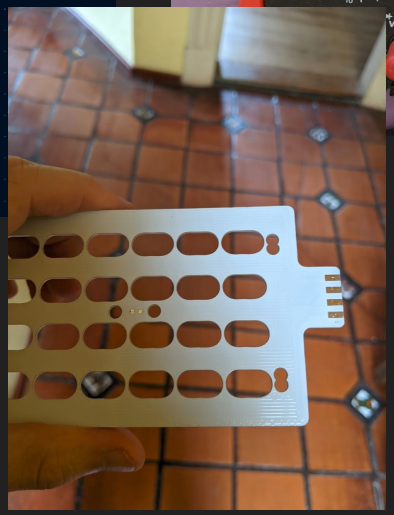
This system was hard to scale up. We decided to redesign it and build a new version with the goal of producing a field-deployable instrument in the shortest time possible

NeoQ V2.0: improving the heating

Instead of using heating elements (resistors) we build a single-layer copper circuit on top of an aluminum board (this is standard for circuits that need to dissipate a lot of heat)



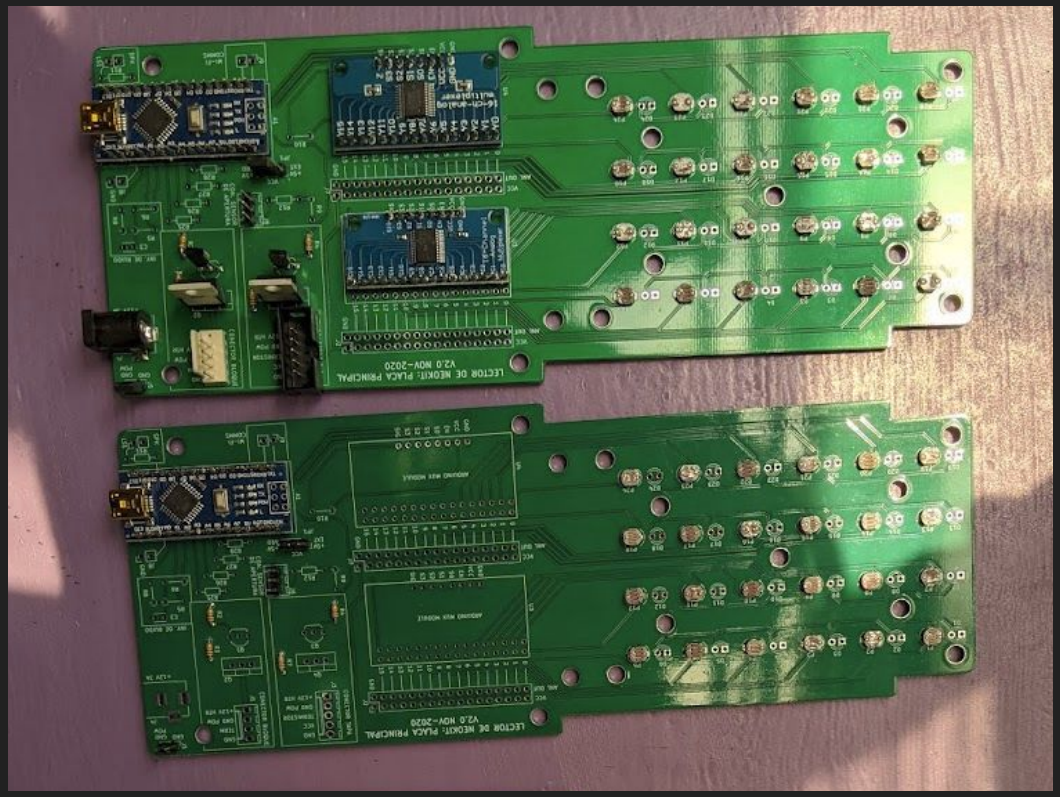




This circuit can be directly attached to the flat bottom of the heating block.

We used the same strategy for the heated lid and it worked like a charm

NeoQ V2.0: improving the electronics



- **Cleaner layout, much easier to assemble**
- **Moved from Arduino Uno to Arduino nano**
- **Added a circuit to inject noise into the ADC to go beyond the 10bits resolution by taking many samples and averaging**

NeoQ V2.0: better housing

- **Much more compact**
- **The long direction extends towards the wall to take less space on the lab bench**
- **Easier to clean in case of contamination**
- **3D printed parts are simpler and require less material**
- **Minimal cable routing required**

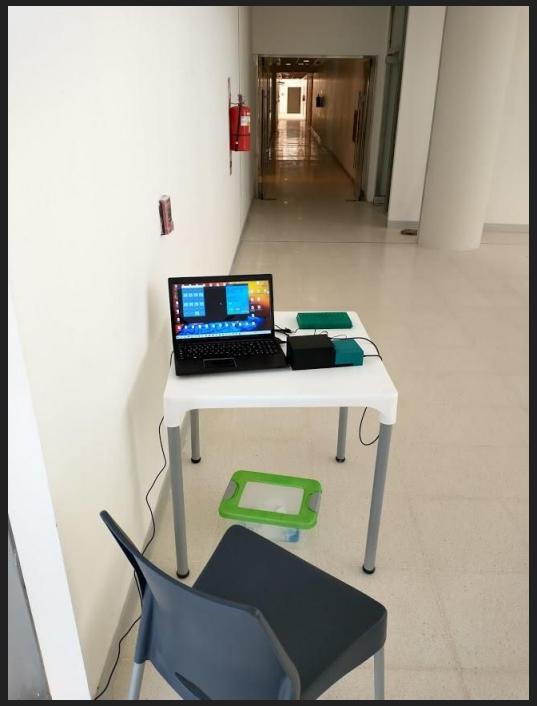
Day 130: Eight NeoQ V2.0 sent for certification

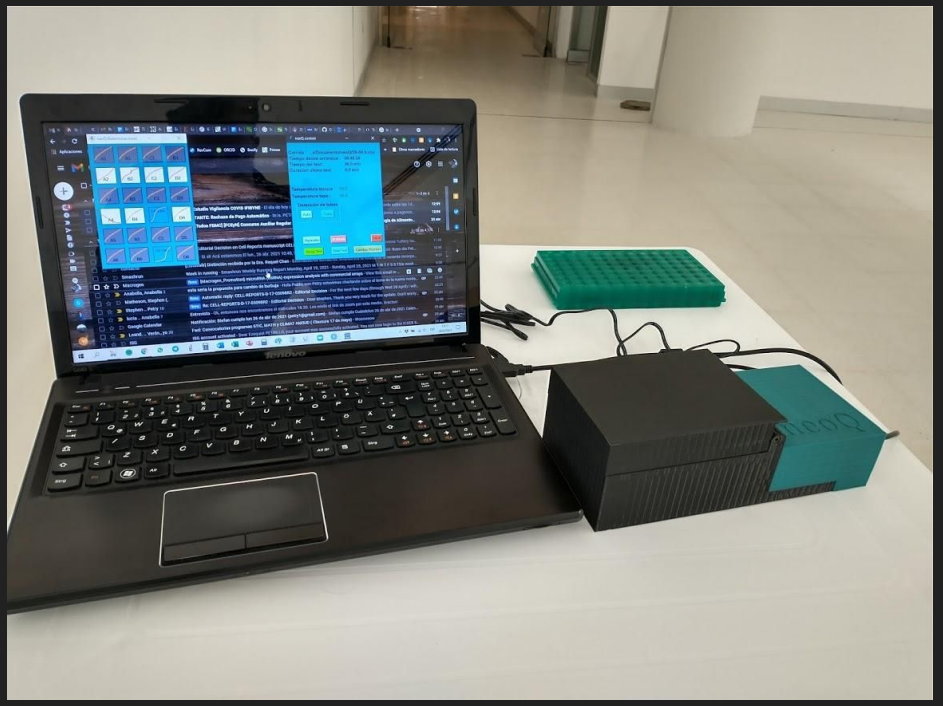
Eight V2.0 NeoQ systems sent for validation to:

Muñiz Hospital, National Infectious Diseases Center

Field Hospitals in Córdoba Province

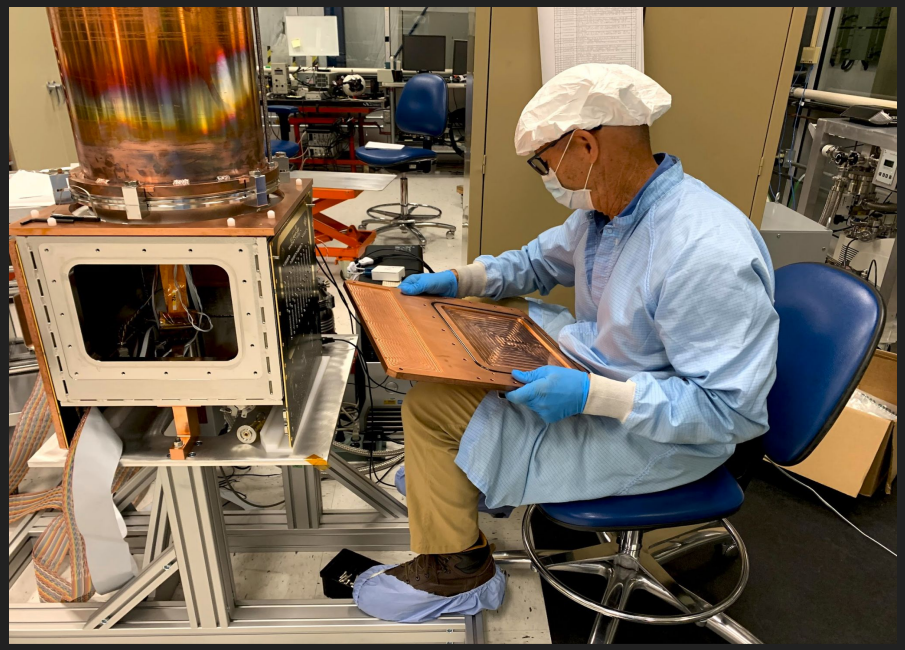
NeoQ is adopted for testing at the UBA





Screening and epidemiological monitoring program at the Institute for Physiology, Molecular Biology and Neurosciences (IFIByNE)

Meanwhile.. SENSEI



During this period we were working to have a remotely guided installation of the SENSEI vessel at Snolab

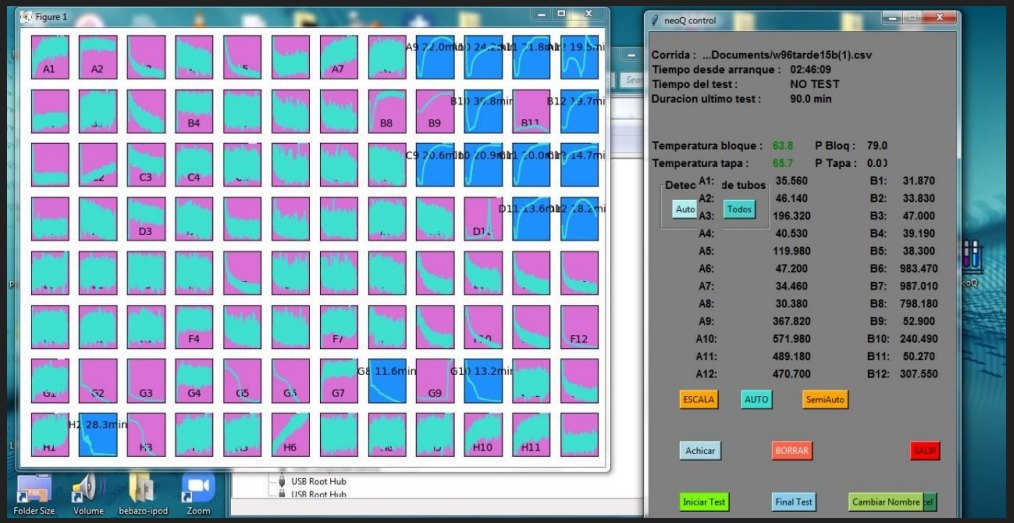
This another story.. but just wanted to thank all the people that worked really hard to help us do this successfully under very challenging circumstances

Thanks Kevin, Greg, Andrew, Michelle, Lee, Bert, Juan, Sho and Snolab team!

NeoQ V3: final version (March-2021)

- **Modified the PCB and heater block to be compatible with off-the-shelve housing**
- **Improved the software and electronics**
- **Easier to assemble and more robust**
- **Cheaper (less 3D-printed parts required)**

April 2021: Major milestone NeoQ-96

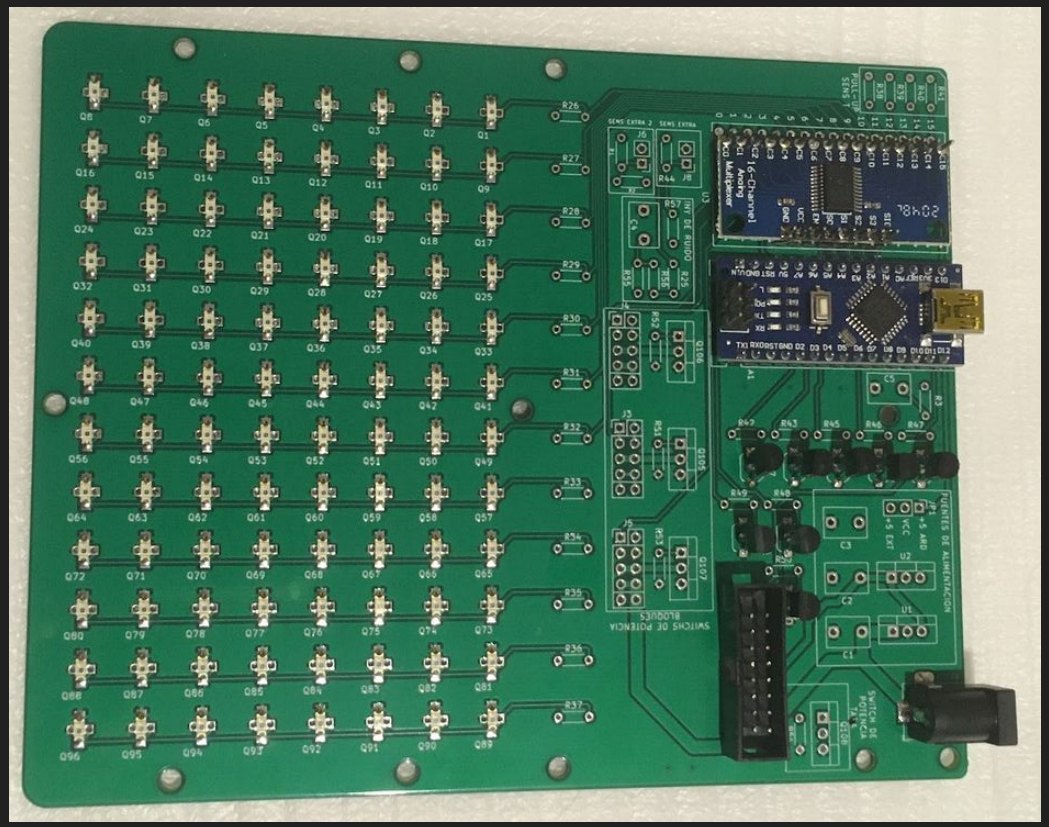


In parallel, we worked on a version of NeoQ to read 96-Well plates

This is the standard for mass testing

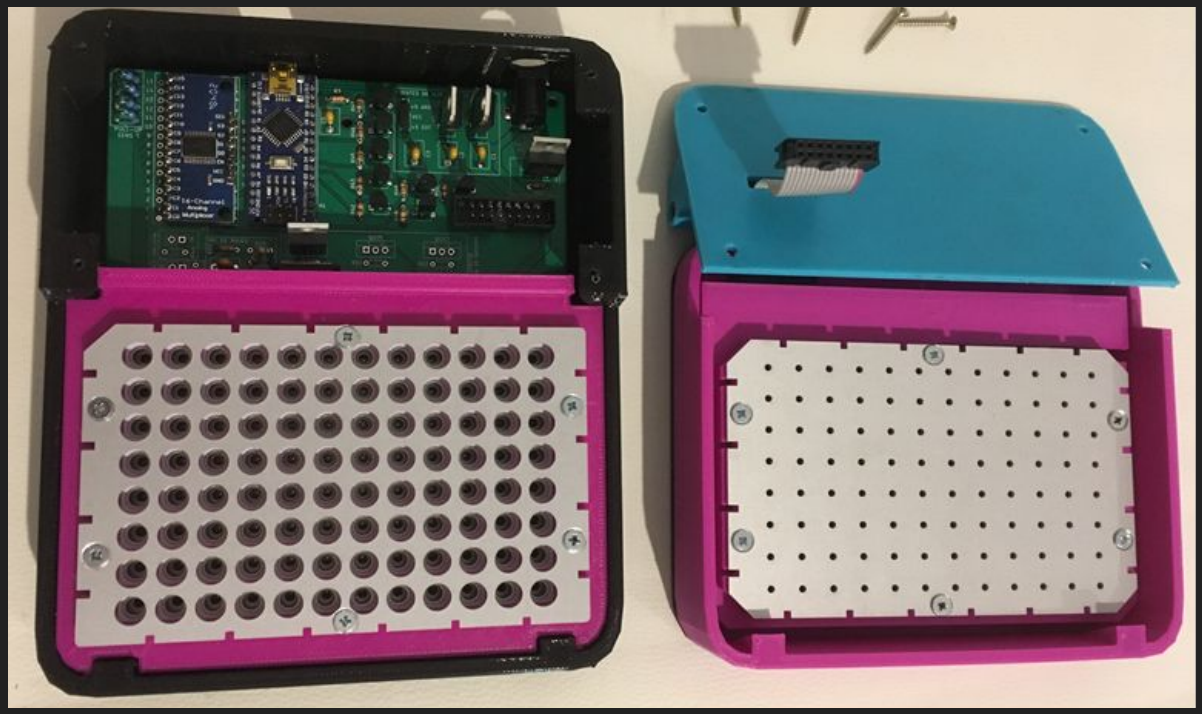
Cheaper and easier to assemble than the 24-tubes version

NeoQ-96: Main board



- **Replaced LDRs with SMD phototransistors**
- **Easy to populate in an automated processes by the company that fabricates de PCB**

NeoQ-96: Heating



- **Took the heating concept of NeoQ-V2.0 to the extreme and replaced the heater block with a stack of aluminum circuits**
- **The heating and mechanical support of the 96-well plate is provided by the circuits on an aluminium substrate**

NeoQ-96: finishing touches - 80’s inspired style

April-2022: NeoQ at INNOVAR 2021

NeoQ was awarded one of the 8 prizes in the *Applied Research* category at INNOVAR 2021 (National Innovations Competition organized by the Ministry of Science & Technology)

Costs an scalability

- **All the components we selected are inexpensive and readily available (~$30 total w/shipping)**
- **Custom PCB and aluminum circuits are easy to produce and low cost (~$25 total w/shipping)**
- **Assembly, 3D-printed parts: <$200**

Total fabrication cost ~$250

All the parts are in high supply (or easy to fabricate)

Closing remarks

- **In ~4 months we produced a working instrument that was validated in the field and ready to scale up (NeoQ-V2)**
  - **All these equipments are still being used**
- **NeoQ V3, the final 24-tubes version, completed on Mar-21**
- **NeoQ-96 for mass testing completed on Apr-21**

I'd like to deeply thank all my colleagues from the NeoQ team:

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Luciana Rocha Viegas
Javier Tiffenberg

Although we shared many, many zoom talks we've still never been all together in the same room!