

## Wellcome Genome Campus | OC4\_q1\_plus\_pablo\_edited\_v1 (1080p)

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So we have been sequencing SARS-CoV-2 samples from the very beginning of the pandemic, and some of the techniques have changed over time. But the most commonly used techniques is basically the ARTIC protocol. And now we have gradually moved on to our COVID seq protocol with Illumina, which I believe is based upon the ARTIC protocol also.

For sequencing, the Illumina platform, we usually use an iSeq 100 or a NextSeq 2000. However, when we had reagent shortages or other issues, we have also used the Oxford Nanopore technology to sequence some of our samples.

So we have used the well standardised in the popular ARTIC multiplex PCR sequencing protocol, and we have used this for both the Illumina and Nanopore platforms. The main reason was that these are the well-standardised and widely accepted protocols used worldwide. So we just used the same ones.

During the pandemic, we had experience with different methods, such as Sanger sequencing, Nanopore technology, Ion Torrent and Illumina. But Illumina is the methodology that we are using the most here in Brazil now in our lab, and in the Fiocruz genomic network.

And we tested these different --protocol-- platforms in special because in the beginning of pandemic, we developed in-house protocol to sequence the whole genome sequence of SARS-CoV-2. And we wanted, in that time, to standardise our protocol in these different technologies.

Because as a reference lab here in Americas, we could share the experience with other labs that had different technologies and also share our protocol. So we standardised it in the Nanopore methodology, in the Ion Torrent methodology, and first Sanger sequencig also. Because some labs didn't have NGS platform to use the protocol that we were using for NGS.

So this is-- this was the main reason that we established in these different platforms. But the Illumina platform was the main platform that we are using now.

While we have used two different sequencing methods, we use the method-- we started with metagenomics at the beginning, because at the time, that's what we had. We did the first genome sequence in Africa, and we used metagenomic to do that. And then after that, we were using the ARTIC's targeted genome amplification. So those are the two.

But we also use two different platforms for our --genome-- SARS-CoV-2 genome sequencing. We use Illumina platform. We also use Oxford Nanopore technology platform on the MinION and the GridION technology. So those are the platforms that we use.

For Illumina, we use the iSeq 100, the MiSeq, the NextSeq 2000, and the NovaSeq 6000. So those are the different platforms that we used.

Hello. We are at Cayetano Heredia University in Lima, Peru. And this is where my laboratory is based and where we have been doing all the COVID sequencing since 2020. Let's have a look.

Hi. I am Pablo Tsukayama, and I'm an assistant professor of microbiology at Cayetano University. And we are today in my laboratory on campus. So we are a small academic group that was working mostly on bacterial genomics prior to the pandemic, working on how to track antimicrobial resistance genes between clinical and community environments in Peru.

But we have been slowly increasing our capacity for NGS and bioinformatics since my return to Peru. And so around March 10 2020, we had to close down the lab because of COVID. This was a few days before the National emergency and lockdown was declared.

And we stayed in lockdown for a few weeks until April 2020 when, thanks to a grant from the National Council of Science and Technology, they were able to fund the first set of sequencing runs for SARS-CoV-2. Using the ARTIC primers, the ARTIC protocols on our Illumina platform.

So this took a couple of months. But eventually, we started generating around 200 genomes per month on one instrument. Again, very small scale, all manual extractions. But slowly, we were able to scale up our capacity by collaborating with other laboratories at universities outside Peru.

So we were slowly decentralising sequencing capacity, and we worked with folks in the Arequipa region to the South of Peru, and also in the Amazonas region to the North of Peru that were working already on sequencing. And they started generating sequencing data.

We trained them here in Italy. Then after a few months, we were able to visit their laboratories. And together, this small academic consortium ended up generating approximately 3,000 genomes, or about 10% of all the genome sequences from Peru.

Slowly, the National Institutes of Health, our National Public Health laboratories, have stepped up their capacity. And now, Peru is routinely sequencing around 1,200 genomes per month from 25 regions. So we're doing now weekly monitoring and, of course, our capacity has increased greatly.

Yet, we are still way below what other countries do. And so we're generating around 1,000 genomes per month. But this has enabled to track local variants. And in 2021, we were able to identify a novel variant that eventually was named the Lambda variant, and emerged in Peru and expand to 30-- to 30 countries around the world, and it was first identified here.

As mentioned earlier, my lab does mostly bacterial genomics, so we had to rely on local collaborators to find the best place to get our samples processed. So this is a neighbouring lab at Cayetano, the emerging diseases laboratory. Luckily, these folks had already been working with Zika, Chikungunya, and other emerging viruses in past years.

So they had some facilities already enabled that were suitable for SARS-CoV-2 work. So we had to do some repurposing of the labs to do the processing here. And this is the setup that we came up with. We had to build this anteroom. We had UV lights for disinfection.

And then this preliminary room where all the changing and washing and autoclaving happens. And then we also set up a negative pressure system, just to be extra sure. And we proceeded in enabling this laboratory. And this is where we do all the RNA extractions.

In the beginning, it was all manual extractions and column-based kits. And so daily, we were able to extract approximately 48 to 96 samples. And in recent months, we have moved to automated systems, which enables 48 samples at a time. It's still very low throughput, and we are reaching the maximum capacity of what we can extract here with available staff and machines.

But this has been working for us throughout the pandemic. And so far, we have processed and sequenced approximately 3,000 samples in this small laboratory.