Wellcome Genome Campus | OC4_2-13_Automation_Geetha

In this video, we will be demonstrating the use of automation in SARS-CoV-2 genome sequencing. There are several platforms available for genome sequencing with a wide range of instruments and service providers. But, still, the upstream steps of sample processing and laboratory preparation are done manually.

In this video, we will take you through the methods for performing different manual and automated sequencing. Even though there are several platform, they do share some common laboratory preparation procedures. Regardless of the platform, successful sequencing require high-quality RNA. The common RNA extraction method includes silica spin column based method and magnetic bead based particles.

Here we are showing the manual method of RNA extraction using silica spin column. In the first step, viral lysis buffer is aliquoted into 1.5 mL tubes and then mixed with 200 microliters of the primary sample in the viral transport medium. Here you can see the mixing procedure. Once the samples are mixed with the viral lysis buffer, the samples are kept at room temperature for 10 minutes and then mixed with 100% ethanol and mixed subsequently.

The samples are then loaded onto silica spin columns. 600 microliter of the mixture is loaded at one time. After loading the samples within the spin, silica spin columns are centrifuged at 10,000 RPM for one minute at room temperature. Since we have more amount of sample, we are loading the silica spin columns twice. And again it is centrifuged at 10,000 RPM for one minute at room temperature.

Then we will go with the washing steps. The silica spin columns are washed with 500 microliter of the wash buffer one and spin at 10,000 RPM for one minute. And then we'll go ahead with performing the wash buffer 2 step and spinning at 10,000 RPM for one minute.

And finally, after a dry spin, the empty spin columns are placed in elution tubes and 70 microliter of the elution buffer is added on to the spin column. The columns are then incubated with elution buffer at room temperature for one minute and centrifuged at 10,000 RPM for one minute. At the end, we will be getting the final eluted DNA which is ready for upstream processing.

The complete process of manual RNA extraction takes around one hour to complete 24 samples RNA extraction. Now let us see how the automated process works and how long it takes. Here we are demonstrating automated process using the extract kit. It consists of four plate-- lysis buffer plate, wash one, wash two, and elution plate. Here, also, sample in medium is mixed with the lysis buffer in the lysis plate, and the sample plate is loaded onto the automated extractor.

Here you can see sample is being aliquoted to the 96 well plate into the individual wells. The sample plate is then fitted with the magnetic comb and it is inserted into the extractor in its respective position. The other plates for wash one, wash two, and elution, is also placed in the extractor in their respective position.

After placing the plates, the programme is started. And you can see that the run approximately takes 22 minutes to complete the entire process. In the first step, the sample is mixed with the magnetic bead particles. And after 10 minutes, the magnetic head takes the magnetic particles from the lysis buffer, which contains RNA, and it is washed in plate two, containing wash one buffer, and then washed in wash two buffer. And subsequently, magnetic particles are placed in the elution buffer plate. And the final elution, containing the RNA is taken out.

RNA extracted is then taken through the process of viral whole genome sequencing method. For SARS-CoV-2, there are different type of methods used-- targeted sequencing approach and non-targeted approach. For COVID sequencing, amplicon sequencing is most commonly employed method.

In the first step, PCR amplicon is generated using different sets of primers-- either ARTIC primer or Midnight protocol primer. As a first step, cDNA synthesis is prepared from the total RNA. Here you can see the aliquoting LunaScript master mix, cDNA master mix, and then RNA is added into the plate. You can see that we are using 96 well plate and multichannel pipettes, which eases our process.

The cDNA step is then incubated in the conventional thermal cycler. During the incubation step, we are preparing the master mix for generating the PCR amplicons. This step consists of master mix preparation for Pool A separately and Pool B separately. Pool A specific primer and Pool B specific primers is mixed in different tubes and then added with Q5 master mix for performing PCR.

The master mix is then aliquoted individually into the different wells. Pool A separately, is aliquoted, and similarly, Pool B master mix is also aliquoted into different wells. Here you can see the aliquoting of Pool A and Pool B master mixes. This is done in between the cDNA synthesis step.

Once the cDNA synthesis is completed, the plate from the conventional PCR is removed and 2.5 microliter of the cDNA is added into each of the pre-aliquoted Pool A and Pool B vials. After aliquoting, the plate is then centrifuged and incubated in the thermal cycler at specified conditions.

This is done for a period of 35 cycles. After PCR, the PCR products are then loaded onto the agarose gel for confirming the successful amplification of the PCR amplicons. Here you can see the gel. You can see the electrophoresis.

PCR products are then visualised on the UV transilluminator to confirming the successful amplification. After the amplification, Pool B product is mixed with the Pool A using multichannel pipette. This pool is further processed for the corresponding library preparation protocol either using Illumina library preparation protocol or Nanopore protocol.

Here we are showing, demonstrating the use of library preparation using Nanopore rapid barcoding kit. The prime PCR pool is aliquoted separately into the 96 well plate and then mixed with the rapid barcode obtained from the Nanopore and incubated at specified condition. After incubation, all the barcoded amplicons are mixed into one single tubes and then purified using Beckman AMPure SPRI beads.

These are the magnetic particles where using the magnetic field, the sample is separated. At the finally, we will getting the elution. The eluted pool is quantified using Qubit instrument and processed 800 microliter of the library is further taken forward.

Now, let us see how these manual steps can be automated on a robotic platform. Here we are demonstrating automated liquid handler that is Opentron OT-2 platform for NGS library preparation. Here we can see the preparation of the cDNA using automated platform. 96 well plate is placed on the thermal cycler and cDNA is aliquoted individually into each of the well using a robotic handle.

This machine can also perform preparation of the PCR master mix and aliquoting into the PCR plate individually, either using a single channel pipette or using the multichannel pipette. And it can also perform PCR using on-deck thermal cycler.

Here you can see the PCR master mix preparation for Pool E and Pool B individually in different tubes. Once the PCR master mix is prepared, this master mix is mixed and aliquoted into the individual 96 well plate. And after the PCR gets over, the PCR Pool A and Pool B is mixed separately. Separately, and then added with the rapid barcode. And again, incubation step is performed using the on-deck thermal cycler.

In a similar way, library preparation protocol can be automated for Illumina library preparation kit, as well. To summarise, automation helps us to streamline the process where accurate and consistent pipetting helps to achieve uniformity in key processing steps. And it also leads to less manipulation and less contamination. And also increases the throughput and broadens the scope of the research.

And, moreover, it is time saving and it allows for more walkaway times. And also, it helps us to integrate the complete laboratory processes. Besides the above, automated extraction should also facilitate internal laboratory research since the impact of human intervention and error is greatly reduced, leading to greater data consistency and reliability.