Liquidator 96

RAININ
Pipetting 360°

CCDB Simplifies Genomic Workflow

Liquidator 96 used in DNA Barcoding

The Canadian Centre for DNA Barcoding (CCDB) uses the Liquidator 96 throughout its barcoding workflow. The CCDB is developing a barcode reference database for short, standardized gene regions to assist in the discovery of 5 million new specimens from more than 500,000 plant and animal species. The CCDB typically processes between 20 and 40 96-well plates containing animal or plant tissue per day.

Biological research is increasingly reliant on the ability of researchers to collect data from large numbers of samples. The faster numerous data points can be collected, the more quickly conclusions can be drawn and the sooner publications and products can reach the public.

With liquid handling, the push for high-throughput data collection is a major reason modern researchers moved from manual pipettes to electronic multichannel pipettes and robotic pipetting systems. Liquid handling devices increase in cost, oftentimes dramatically as their complexity increases and this is particularly true for the transition from multichannel pipettes to robotic systems.

METTLER TOLEDO

For genomics applications, such as PCR and sequencing, the need for pipetting accuracy and precision in combination with increased throughput is significant. In traditional sequencing, which uses Sanger dye termination chemistries and resolution of DNA extension products of 50 to 700 bases in length, the need to manipulate complex samples in parallel is common, either for assembly of an entire genome or for sequencing of numerous unrelated samples.

Some laboratories rely on robotic pipetting to meet the demands for throughput and accuracy and precision, but for most, the cost to acquire and maintain automated equipment is prohibitive. Yet the only alternative – multichannel pipetting – is not appropriate for laboratories where the number of samples is too great and the tolerance for errors, like skipping of rows in a microplate, is low.

The Liquidator 96 is a 96-channel manual pipetting system (Figure 1) that is useful for genomics applications because it provides:

- Faster sample preparation and significantly reduced likelihood for error than a multichannel pipette.
- Medium to high-throughput sample setup without the cost, training or footprint associated with robotic pipetting.
- High accuracy and precision compared to bulk liquid dispensers.

Because of the accuracy and precision with which it can process up to 96 samples in parallel, the Liquidator 96 has proven useful in a broad number of applications[1, 2], including portions of traditional genomics workflows, such as DNA amplification[3, 4]. This paper describes the role of the Liquidator 96 in a full genomics workflow.

Figure 1 Liquidator 96

The Liquidator 96 is a manual 96-channel pipetting station that enables users to easily load pipette tips, aspirate and dispense liquids, and perform other necessary manipulations, like "touching-off." The Liquidator offers higher throughput and less risk of error than multichannel pipettes, greater versatility and affordability than robotic pipetting stations, and higher accuracy and precision than bulk liquid dispensers.

Liquidator 96 in DNA Barcoding

Compared with multichannel pipettes, the Liquidator 96 saves the CCDB considerable time. According to Dr. Natalia Ivanova (Figure 2), lead DNA scientist at the CCDB, the Liquidator 96 plays an important role in four major steps in the DNA barcoding process (Figure 3):

- DNA extraction from plant and animal tissue
- PCR amplification of extracted DNA
- Cycle sequencing: reaction setup
- Cycle sequencing: product clean-up

According to Dr. Ivanova, the Liquidator 96 is used in different ways depending upon whether DNA is extracted from animal or plant tissue.

Figure 2 Dr. Natalia Ivanova and the Liquidator 96

Dr. Ivanova uses the Liquidator for numerous steps in the DNA barcoding process, such as DNA extraction for PCR amplification, cycle sequencing-reaction setup and cycle sequencing-product clean-up. The Liquidator has a small footprint and no need for electricity, so she can place it in a laminar flow cabinet.

Application 1A Animal DNA Purification

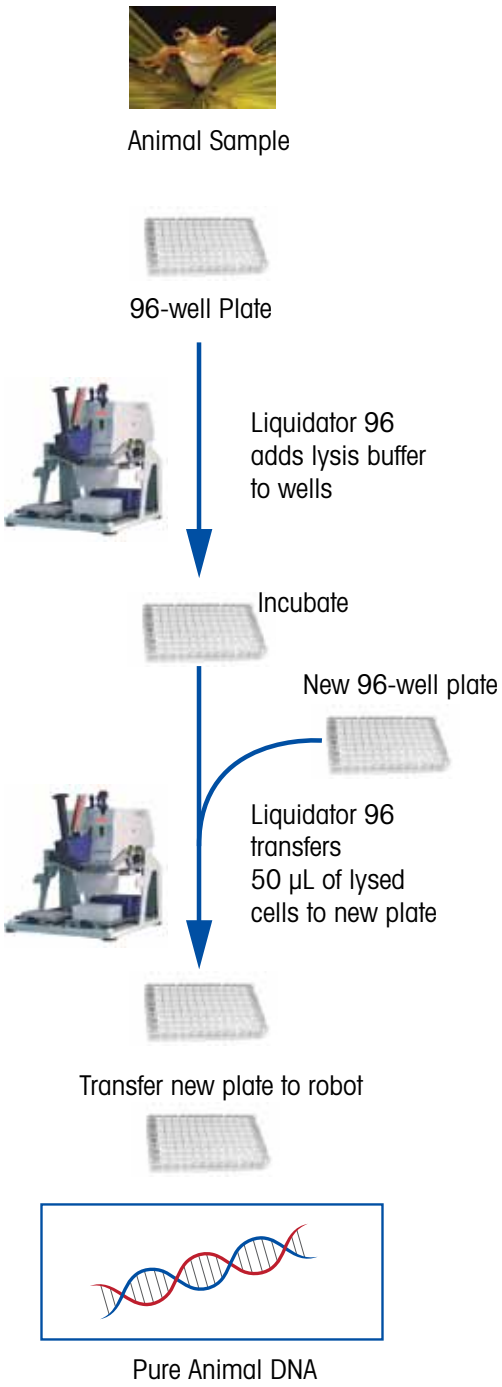


Figure 3a Four application steps in DNA Barcoding Animal and or Plant DNA Purification

When animal tissue is being treated (Application 1a), the Liquidator 96 is first used to deliver lysis solution containing protease to tissue samples. After incubating samples overnight, the Liquidator 96 is used to transfer lysate to a clean microplate for DNA extraction. The microplate is then delivered to a robotics system which completes the DNA extraction protocol. For plant samples (Application 1b), the Liquidator 96 is used to purify DNA using glass fiber membrane binding. First, the Liquidator 96 is used to transfer plant extract in lysis buffer from a tube rack to a 96-well plate containing a buffer that will bind it to a glass fiber membrane. The sample is then transferred to a 96-well glass fiber membrane purification plate. After centrifugation, the buffer reservoir of the plate is switched and two subsequent wash steps are carried out using the Liquidator to pipette buffer in both steps. After changing the sample reservoir and oven drying the sample, the Liquidator is used to pipette warm water into the plate for elution. Centrifugation leads to DNA being eluted into the sample reservoir plate where it is then transferred to tubes or plates for further processing.

Application 1B Plant DNA Purification

Plant Sample

Tube Rack

New 96-well plate
containing 100 µL
binding buffer

Liquidator 96
transfers
50 µL extract to new plate

96-well purification plate

Liquidator 96
transfers 150 µL
to purification plate

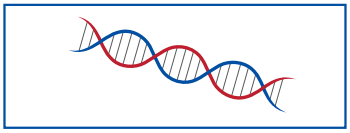
Bind to purification plate

Wash with 180 µL buffer

Wash with 750 µL buffer

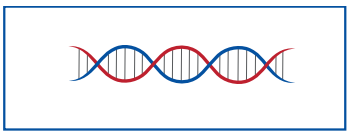
Elute in 40-50 µL

Centrifuge



Pure Plant DNA

Application 2 PCR Amplification

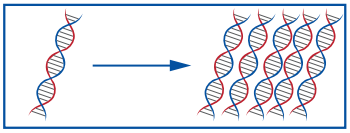


Animal or Plant DNA

New PCR plate with
10.5 µL mastermix

Liquidator 96
pipettes 2 µL DNA

Place plate in the
thermocycler and amplify



Amplified DNA

Figure 3b Four application steps in DNA Barcoding PCR Amplification

The next step is PCR amplification of the DNA regions to be sequenced for barcode analysis. Here, Dr. Ivanova uses the Liquidator 96 to pipette extracted DNA sample to a PCR plate containing PCR mastermix. The plate is centrifuged, heat-sealed, centrifuged again and then placed into a thermocycler for PCR amplification.

Application 3 Cycle Sequencing Reaction Set-up

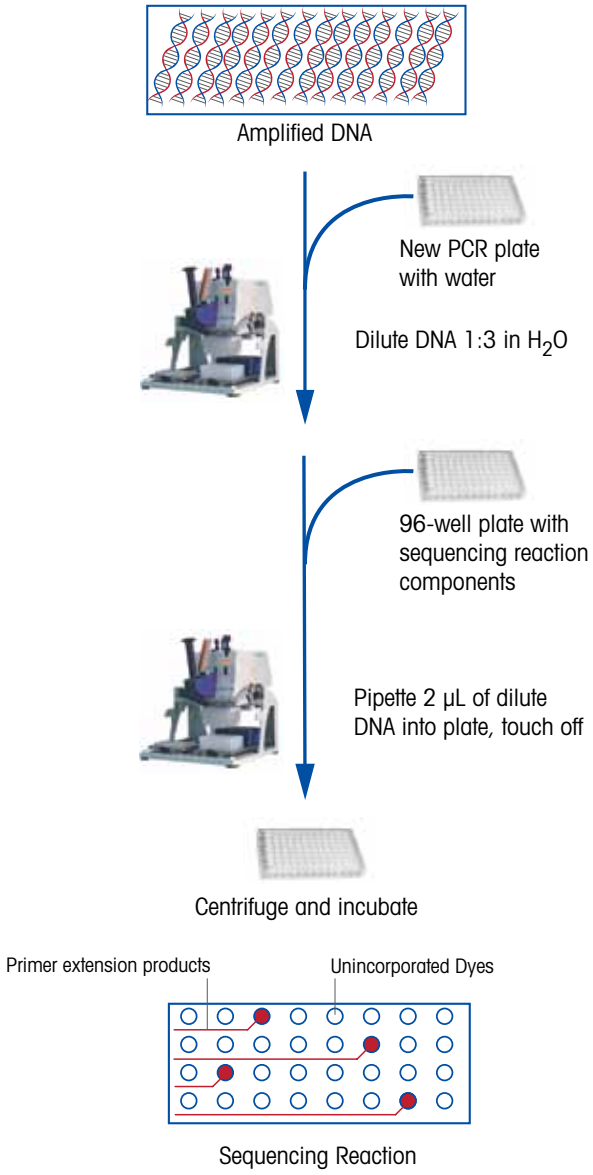


Figure 3c Four application steps in DNA Barcoding
Cycle Sequencing Reaction Set-up

Following amplification, cycle sequencing analysis is performed using an ABI 3730XL DNA analyzer to determine the nucleotide sequence of the amplified DNA barcode region. The DNA regions amplified by PCR are first diluted with water using the Liquidator 96, then pipetted into 96-well plates containing one of two different sequencing primers, reaction constituents and big dye terminators. After the initial pipetting step, the tips are "touched-off" to ensure that as much of the diluted PCR sample as possible is added to the sequencing reaction. Following centrifugation, the sequencing plate is placed in a thermocycler for the primer extension/termination reaction.

Application 4 Cycle Sequencing Clean-up

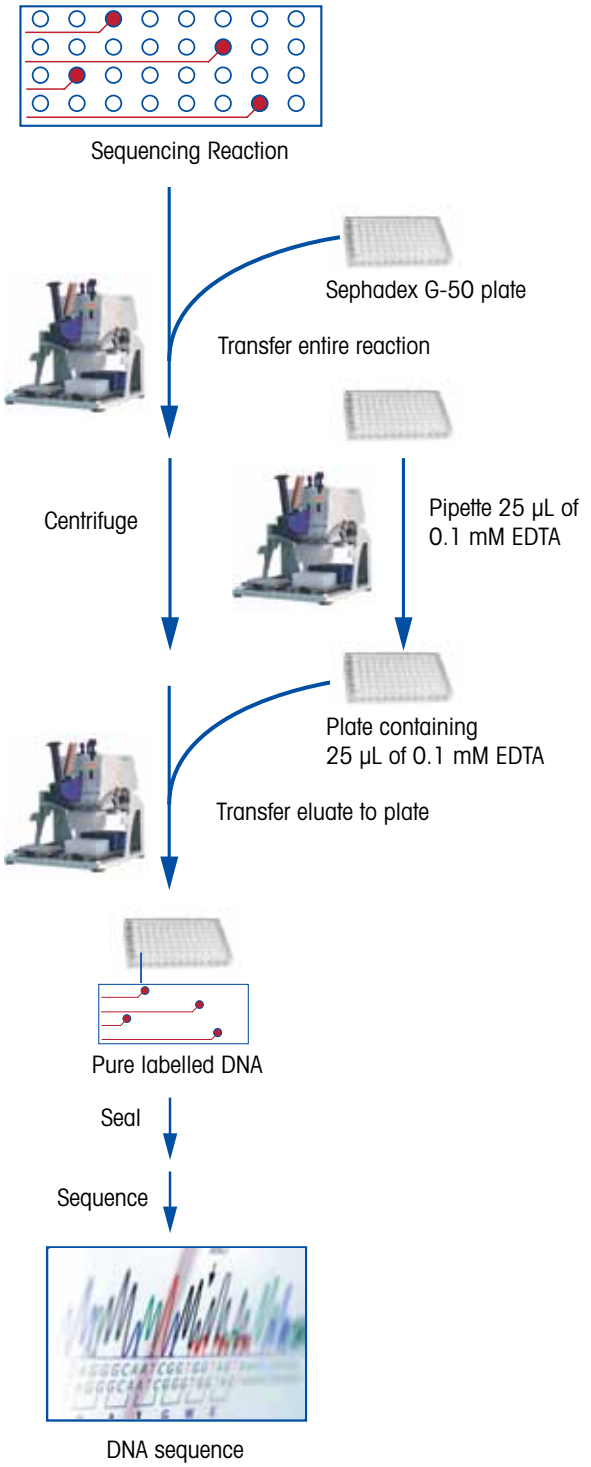


Figure 3d Four application steps in DNA Barcoding Cycle Sequencing Clean-up

Prior to determining the nucleotide sequence of the DNA barcode, the sequencing reaction undergoes a clean-up step to remove the big dye terminator. First, the Liquidator is used to pipette 0.1 mM EDTA solution to a collection plate. For purification, the entire volume of the sequencing reaction is pipetted via the Liquidator to a 96-well filter plate containing Sephadex G-50 resin. After centrifugation, the purified DNA sample is transferred in its entirety to the plate containing 0.1 mM EDTA. After the collection plate is sealed with septa, it is placed on a sequence assembly plate and loaded on an ABI 3730XL DNA analyzer for DNA sequencing. The final protocol and data are then uploaded on the CCDB's lab information management system for sample tracking.

Summary

Across Dr. Natalia Ivanova's DNA barcoding sequencing workflow, the Liquidator plays a critical role in DNA extraction for PCR, cycle sequencing reaction setup and cycle sequencing clean-up. The instrument is particularly useful for accurately and precisely pipetting 96 samples simultaneously, delivering different volumes without significant readjustments and performing delicate operations such as touching-off. Relative to Dr. Ivanova's protocol, here's how the Liquidator 96 differs from other liquid handling technologies:

- With the Liquidator 96, executing CCDB's DNA barcoding protocol generally requires 15 pipetting steps. Using a 12- or 8-channel multichannel pipette, the same protocol would require 120 or 180 pipetting steps, respectively, with the added concern of accidently skipping microplate rows. With multichannel pipettes, researchers might also have to time the buffer and reagent addition steps to ensure that all samples are treated equivalently. This latter disadvantage is avoided using the Liquidator 96[5].
- Compared with robotics, the Liquidator 96 offers lower upfront and maintenance costs. Unlike an automated pipetting stations, which have much larger footprints and require electricity and programming knowledge to operate, the Liquidator is easy and intuitive to use and can easily be moved around the laboratory from bench to bench, or to a Laminar flow cabinet. Another advantage of the Liquidator 96 over robotic systems is its ability to carry out delicate liquid-handling techniques, such as "touching-off."
- The Liquidator 96 offers much greater pipetting accuracy and precision than bulk liquid dispensers. Dr. Ivanova could not confidently dispense the volumes required for the barcoding protocols above with a bulk dispenser.

References

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