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Life Sciences

Australian Lab Achieves High-Throughput Genotyping by Amplicon Sequencing Using Echo 525 Acoustic Liquid Handler and Access Systems

Growth of genotyping services, enabled by Echo acoustic dispensing

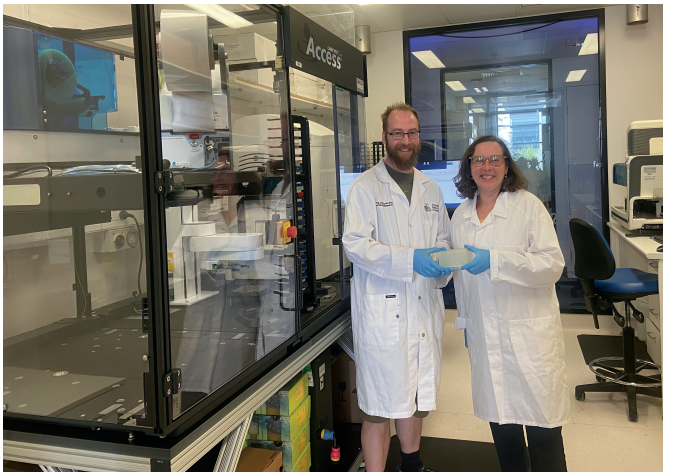
Since 1988, the **Australian Cancer Research Foundation Biomolecular Resource Facility (BRF) core lab** has provided researchers access to the latest techniques for molecular genetics including next generation sequencing (NGS), genotyping and bioinformatics.

Their services are available to researchers at The Australian National University (ANU), as well as to the regional scientific community and clients from across Australia's education, research and medical services community. The lab receives a variety of sample types, including medical bioscience, environmental and agricultural samples. A large proportion of the clients are external to ANU, and include commercial and government organizations.

The BRF lab typically analyzes 65,000+ mouse samples/year, which translates to 110,000+ genotypes annually, or an average of 2,000 genotypes each week.

They are currently developing a new highly multiplex amplicon sequencing method, both reducing cost while doubling their throughput - to 4,000 samples/run each week - a goal made feasible using an Echo 525 Acoustic Liquid Handler (LH).

BRF staff historically have used manual processes with only a few assays. That has grown to around 500 different assays in 2021 making manual polymerase chain reaction (PCR) set-up an increasingly difficult task. Implementing the Echo LH and assay miniaturization for their high-throughput amplicon sequencing genotyping services, the BRF should be able to screen 200,000 genotypes per year. This will allow the lab to continue to expand their offerings to researchers outside the institution.



Cameron Jack and Stephanie Palmer from the Australian Cancer Research Foundation (ACRF) Biomolecular Resource Facility (BRF). https://jcsmr.anu.edu.au/research/facilities/brf

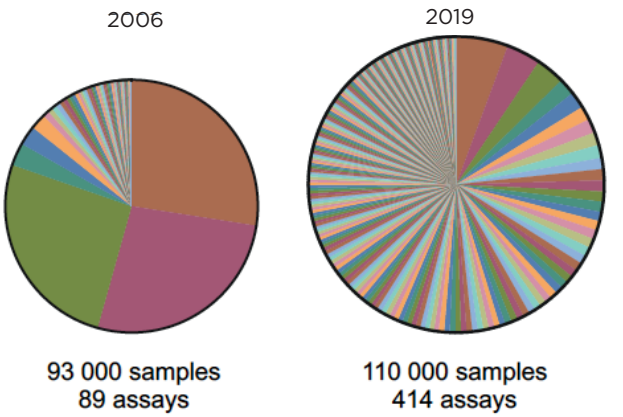


Figure 1. Number of samples covered by number of assays. In 2006, the facility generated around 93,000 genotyping results using 89 different assays with 3 assays accounting for more than 80% of all genotyping reactions. In 2019, the sample number increased to approximately 110,000 genotyping reactions with a total of 414 different genotyping assays with no single assay being required for more than 6% of samples.

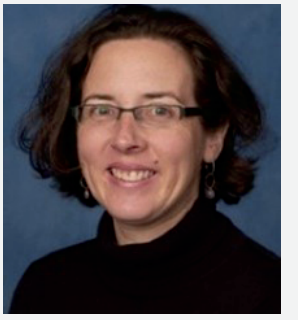
"(Our) genotyping methodology was changed to do amplicon sequencing using the Echo (LH)," says Anselm Enders, M.D., an Associate Professor at The John Curtin School of Medical Research (JCSMR), ANU. "(Because) we deal with a lot of different point mutations we require a vast array of different reactions, a task that was impossible to do using traditional methods due to the time it takes to run the cherry picking of primers."

Dr. Enders explains that their process involves digesting mouse ear punches in 96-well plates, and then setting up multiple amplicon sequencing reactions for each sample on the Echo 525 LH, which was chosen due to its fast transfer speed for cherry-picking primers, as well as for cost savings on reagents and consumables. How was the cost savings achieved? Enders mentions that their lab has achieved a 4-fold reduction in reaction volumes—from 20 µL down to 5 µL – with a significant reduction in consumables per reaction.

The first PCR step to generate amplicons was reduced from 20 µL to 2 µL, and the second PCR step to add the DNA barcodes, adds an extra 3 µL for a total reaction volume of 5 µL. According to Enders, this workflow can be done in less than three days from sample receipt to results, due to the Echo (LH)'s fast transfer speed. Consequently, their lab has been able to establish a barcoding scheme and bioinformatics structure to process up to 4,000 amplicons per run of Illumina's MiSeq System.

Stephanie, manager at the BRF, JCSMR, ANU, elaborates: "the Echo (LH) being 'agnostic' to chemistry is of great value for us. In addition to amplicion sequencing, we can perform qPCR or other enzymatic based NGS library preparations. However, our main focus is the NGS amplicon genotyping, and our methodology can be run on any organism or sample type, an added flexibility which will increase our client base for this service. The Echo (LH) and NGS amplicon genotyping also improves the allele specific data for CRISPR mice and cell line generation. That would not have been possible using Sanger sequencing and traditional methods."

Anselm Enders, M.D.
Associate Professor at The John Curtin School of Medical Research, Australian National University



Stephanie Palmer,
Manager at the BRF, The John Curtin School of Medical Research, Australian National University

Advantages of Echo liquid handler in the age of COVID-19

"When COVID hit we were lucky to have an Echo (LH). We were able to pivot our service capability towards research-based COVID testing in our facility thanks to the Echo LH," says Stephanie Palmer.

"We were able to use the Echo (LH) for setting up RT-qPCR tests at 5 times smaller reaction volumes (10 µL instead of 50 µL), enabling us to provide 5 times more testing capacity with the same amount of kits, which were in short supply. (And) with a global shortage of pipette tips, Echo (LH)'s tip-less technology is a more sustainable solution during a pandemic." explains Stephanie Palmer.

Stephanie notes that their previous method, which included setting up 10 µL RT-qPCR reactions by hand, produced variability between technical replicates. Setting up reactions on the Echo LH, however, yielded highly reproducible results, and with the same limit of detection (LOD) as in large-volume reactions, which was cross-validated."

Doing more faster—with less hands-on time

Because the high-throughput assays at BRF require fast plate feeding, due to Echo LH quickly processing the plates, the lab added an Access System to automate their new process.

The Access system at BRF includes a plate handling robot, a peeler, sealer, and four random-access rack plate hotels, all from Beckman Coulter Life Sciences.

“In addition to building up our amplicon sequencing method,” Stephanie says. “We are also interested in developing other whole genome NGS method pipelines such as Nextera XT on the Echo LH and Access Systems.”

Access Laboratory Workstation

Advantages of using an Echo 525 acoustic liquid handler (LH) for high-throughput genotyping by amplicon sequencing.

1. **Expanded workflow options:** time-saving tip-less technology enables workflows that would be impossible with traditional methods.
2. **Cost savings:**
    - Plasticware costs were reduced by 75% (including 2.142 cubic meter volume of plastic tips) per year, accounting for approximately 20% reduction in total costs.
    - Reducing reaction volumes, without sacrificing accuracy and precision, resulting in up to a 4-fold savings on reagents and primers.
3. **Flexibility:** The Echo LH provided the facility with the flexibility to increase the number of genotyping assays as well as the number of samples, without any significant increase in running cost.
4. **Space savings:** Changing from 96- to 384-well plates, the BRF lab was able to replace 8 single-block thermocyclers with two double-block 384-well thermocyclers, providing much-needed space savings in their lab.
5. **Walkaway reaction setup** using the Access Workstation automation for the Echo LH, allowed for reduced hands-on time and confidence in the assay preparation.

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