ROY J. CARVER CENTER FOR GENOMICS

"ACCELERATING GENOMICS RESEARCH"

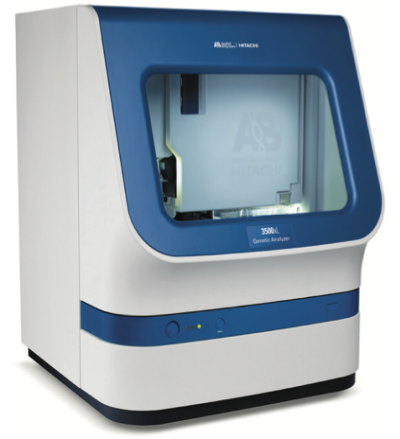
Newsletter: Volume 1- October 2023

RECENT NEWS:

The CCG and CCI will be at the UI Research Services Fair on October 12, 2023. The fair is being held at the IMU Main Lounge on the 1st Floor from 1pm-4:30pm. The fair is a vendor-style event to inform researchers about campus resources and services. Registration is required to attend and can be done through the following link:

https://research.uiowa.edu/research-development-office/collaboration/ui-research-services-fair

Registration deadline Oct. 9th



ABI 3500 Genetic Analyzer

CCG UPDATES

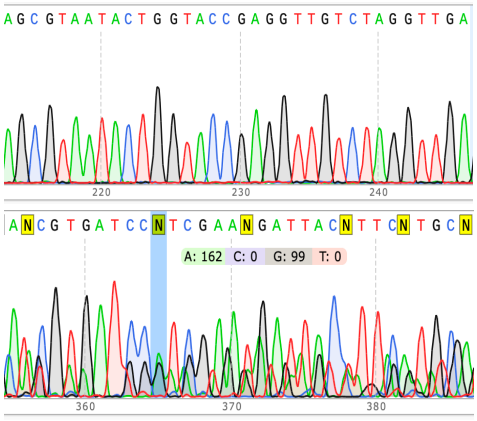
Hello everyone and Happy Fall Semester. I hope everyone's semester is off to a good start and we start getting nice, cooler weather.

- First off, please take a look at the "Recent News" box and mark your calendars for the Research Services Fair. This is a great event to check out and discover what resources are available on campus to support your research.
- **NEW EVENT**: The CCG will be hosting its first Applications Seminar on October 25, 2023. The seminar will focus on this newsletter's highlighted equipment: the ABI 3500 Genetic Analyzer. Yes, this machine has other applications than just Sanger sequencing. The seminar will be presented by an Applied Biosystems Applications Specialist at Noon with a free lunch in Room 106 BBE. Please register via email (ccg@uiowa.edu) by October 20 if you plan to attend, so we can plan accordingly.
- Please remember to sign-up for equipment using the online scheduling system: https://bioweb.biology.uiowa.edu/servicecenters/login.php. If you decide not to use the machine at that time, please email the CCG (ccg@uiowa.edu) to remove your reservation. This will allow others to sign-up in your canceled spot.
- A sign-in log next to the Cytation5 machine has been added in addition to scheduling time online. New pricing went into effect in September 2023. Also, please note on the sign-in sheet if you log into the machine to just download data (for example: Date, Name, Lab, "data download"). The machine logs are cross-checked and this will enable accurate counting of the number of runs done for billing purposes.
- Please remember to log out of the software on the LI-COR Fc machine before signing out of the system. The machine will **not** let other users access the software if someone else is still signed into the software.
- We have several items that are free to a good home and have been placed in the 2nd Floor hallway of BB. You are welcome to retrieve them for your lab.
- If you have any questions or would like to be added to our listserv for future newsletters, please email the CCG (ccg@uiowa.edu).

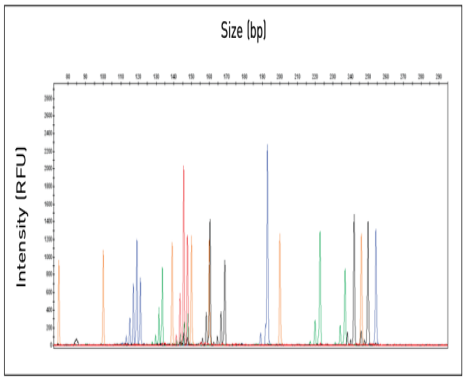
Equipment Highlighted:

ABI 3500 Genetic Analyzer is an 8-capillary electrophoresis instrument capable of DNA Sanger sequencing and fragment analysis applications including *de novo* sequencing and resequencing for mutational profiling, microsatellite analysis, MLPA, AFLP, LOH, and SNP screening or validation.

For Sanger sequencing, the machine determines the base-pair sequence of a DNA fragment from the formation of extension products of various lengths amplified by PCR by laying down a primer and incorporating labeled nucleotides. The figure shows example chromatograms of 1) a good read, and 2) a read with multiple calls for individual bases



Top graph: Good sequencing run! Bottom graph: Multiple base calls, highlighted N is being called as A & G equally. More than one base call (as observed by multiple peaks) can be seen for most of the bases in the read indicating more than one DNA sequence present in the sample.



Fragment analysis of fluorescently labeled fragments separated and sized.

Additional info:
https://assets.thermofisher.com/TFS-Assets/LSG/manuals/4474504.pdf

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indicating more than 1 DNA fragment in the sample or that the primer is annealing to multiple places in the sequence on the DNA fragment. To solve this issue of multiple base calls: 1) gel clean your DNA fragments, and 2) check your primer sequence to see where it should anneal and if there are multiple places in your sequence it will lay down. If your primer is annealing in multiple places, use a different primer. Remember you can use primers that go in both directions, sense and antisense. Sometimes reversing the primer direction will resolve problems with base calling and give you a clean read. Chromatograms of your sequencing reads can be viewed in the SnapGene program (https://www.snapgene.com/snapgene-viewer).

For fragment analysis, the machine can do up to 6 fluorescent dyes simultaneously which are detectable in a single sample. One of the dye colors is used to label a size standard in each sample for determination of base-pair sizes of the sample product peaks and to generate a standard curve for each sample. The labeled fragments are amplified by PCR and separated by size using capillary electrophoresis. The analysis software determines the relative size of each fragment in comparison to the standard curve and assigns allele calls based on user-defined markers, see figure. Some types of applications of fragment analysis include:

- Microsatellite (also known as short tandem repeats-STRs) analysis for linkage mapping, STR instability, Loss of Heterozygosity (LOH), Inter-Simple Sequence Repeat (ISSR), genetic diversity, Multi-Locus Variant Analysis (MLVA), etc.
- Single Nucleotide Polymorphism (SNP) genotyping
- Fingerprinting or AFLP includes microbial, animal or plant genome typing, genetic maps of new species, genetic diversity, and molecular phylogeny studies.
- Relative Fluorescence Quantitation (RFQ) compares peak height or area between two samples. Common techniques are Quantitative Multiplex PCR of Short Fluorescent Fragments (QMPSF), and Multiplex Ligation-dependent Probe Amplification (MLPA). A RFQ experiment can be used to determine copy number variation, LOH using STRs or SNPs, large chromosomal deletions, and for aneuploidy detection.
- Validation of CRISPR editing

For additional and more in-depth information, sign-up for the Applications Seminar on October 25 (register by sending an email to ccg@uiowa.edu by Oct. 20).

Important Use Information:

Please submit your samples on the top shelf of the “Sequencing Freezer” and the sequencing request sheet via email to CCG (ccg@uiowa.edu). All materials must be submitted by 8am on Tuesday and Thursday for samples to be run on those days. Results are usually available the next day.

Billing Information:

You are charged by sample run: $6.75 per sample. Total number of samples run are billed each month.