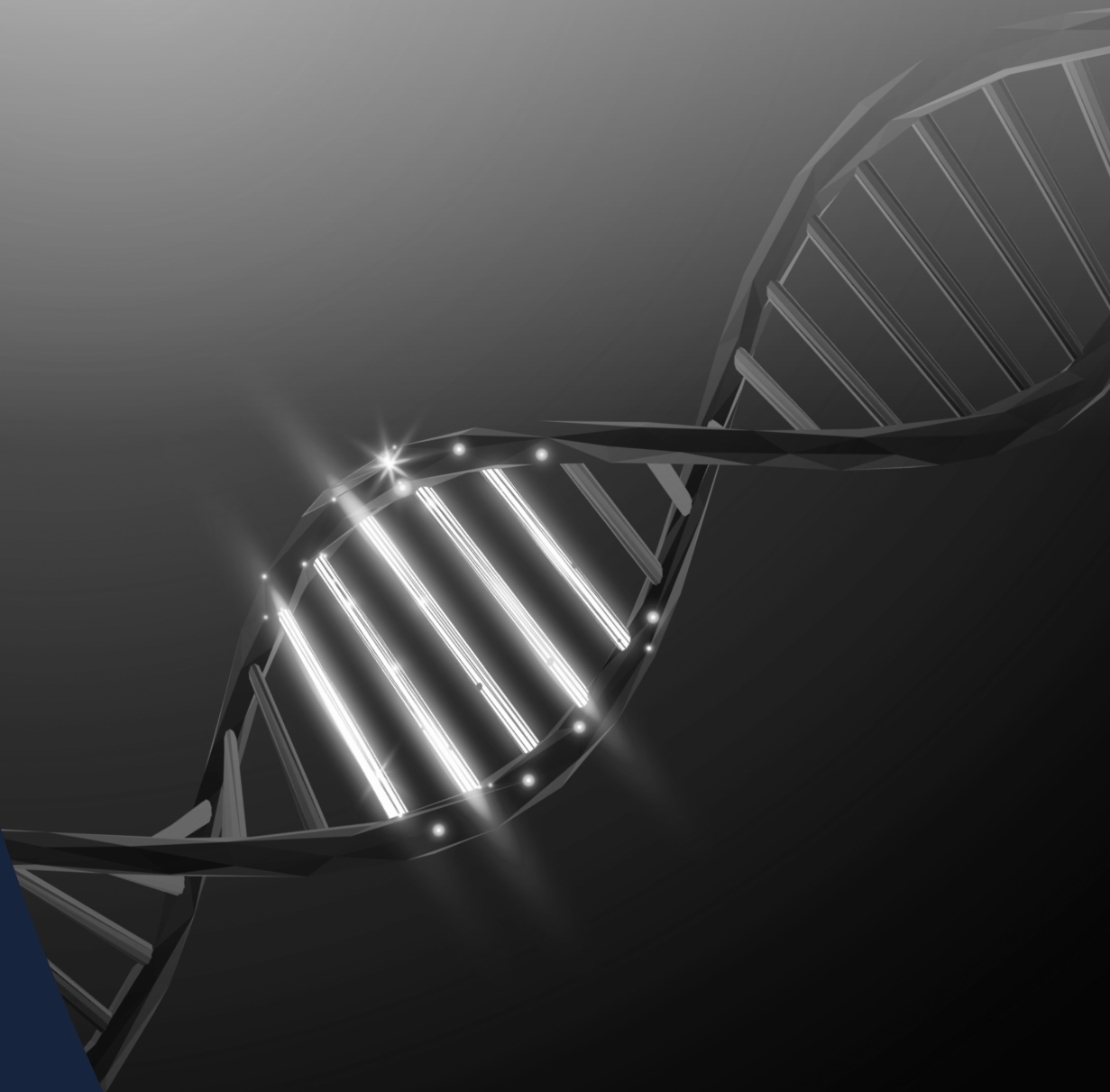


18



Enhancing Long-Read Sequencing Workflows for Plant & Animal DNA with LightBench® Discover





In this edition of Your Expert, YourGene Health interviewed **Dr. Alex Harkess** and Lab Manager, **Haley Hale**, at **HudsonAlpha** in the CoLab, where a major part of their work involves using genomics to better understand plant reproduction and investigate potential crop improvements for sustainable agriculture.

HudsonAlpha Institute for Biotechnology

HudsonAlpha Institute for Biotechnology is a non-profit organisation which is home to 10 genetics and genomics faculty investigators and 50 companies across the fields of biology, medicine, and health. The organisation is a global leader in genomic research and biotechnology.



Alex Harkess, PhD

Alex Harkess, PhD is a Faculty Investigator at HudsonAlpha Institute for Biotechnology in Huntsville, AL. He was a PhD student at the University of Georgia with Jim Leebens-Mack, and identified the genes controlling sex determination on the young asparagus Y chromosome.

As an NSF PGRP Postdoctoral Fellow, he worked with Blake Meyers at the Danforth Center on the evolution and diversification of small RNA-producing machinery in angiosperms.

He has been a faculty member at HudsonAlpha since 2020, and now broadly works on the evolution and mechanisms underlying diverse reproduction mechanisms in plants, including dioecy and sex chromosomes, apomixis, dichogamy, and more.

He is a co-founder of Veil Genomics, a scaled long-read sequencing company unlocking the genetic variation within any species.



Haley Hale, MS

Haley Hale, MS is a Laboratory Manager in the Harkess Lab at HudsonAlpha. She received her MS degree from Texas Tech working in the genetics of dioecious Salicaceae species, and has been with Dr. Harkess' group for the last 5 years.

She leads major workflows in the laboratory spanning short-read and long-read isolation, library preparation, and sequencing, for a variety of both plant and animal species.



What technical challenges do you face in your laboratory and how does LightBench® Discover help you address these?



The projects we work on in our laboratory require the sequencing of diverse plant genomes – diploids, haploids, and complex polyploids.

It can be really challenging to isolate DNA from hundreds of species, many of which don't "play nice" during the extraction process.

As plant biologists, we're constantly working outside the bounds of standard kits, which means our main obstacle is obtaining a proficient amount of DNA that is both high quality and long enough for our sequencing needs. Typically, we're lucky if we get two out of the three of these.

Incorporating the LightBench® Discover into the PacBio® SMRTbell library preparation workflow for long-read sequencing has been instrumental in addressing these key challenges.

With the LightBench® Discover, **we can quickly size the DNA after extraction, as well as after mechanical shearing** – a process that varies significantly across plant species.

This QC step allows us to identify DNA libraries containing too many short DNA fragments. For example, if a DNA library contains a large proportion of fragments shorter than 10-13 kilobases (kb) after shearing, it would be unsuitable for long-read sequencing.

However, by **using the LightBench® Discover for size selection, we can remove these shorter fragments and rescue a library that was previously unsalvageable.** By doing a 10 to 13 kb lower cut off on whole genome shotgun libraries, we push the N50s higher on the read lengths and data quality.

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How do you use the LightBench® Discover at each stage of your workflow?

“We use the LightBench® Discover at least four times throughout our workflow, all the way from DNA extraction to long-read sequencing”.

- After initial DNA isolation, we check whether the fragment sizes are medium or large sized (40 to 60 kb or bigger).
- After shearing the DNA, we use the LightBench® Discover fragment length distribution to confirm whether shearing has been successful, or if we need to re-shear.
- Once libraries are prepared, we use the LightBench® Discover to assess the quality and cleanliness of the DNA library.
- We now default to performing a final size selection step because it removes all of the unwanted smaller fragments represented in the report – an issue which we saw using bead-based selection.

The LightBench® Discover has also **eliminated the need for additional bead clean-up steps** as the gel seems to clean things up. This is a wonderful benefit because cleans are such a pain to perform, **we’re saving money, and the quality is much better.**

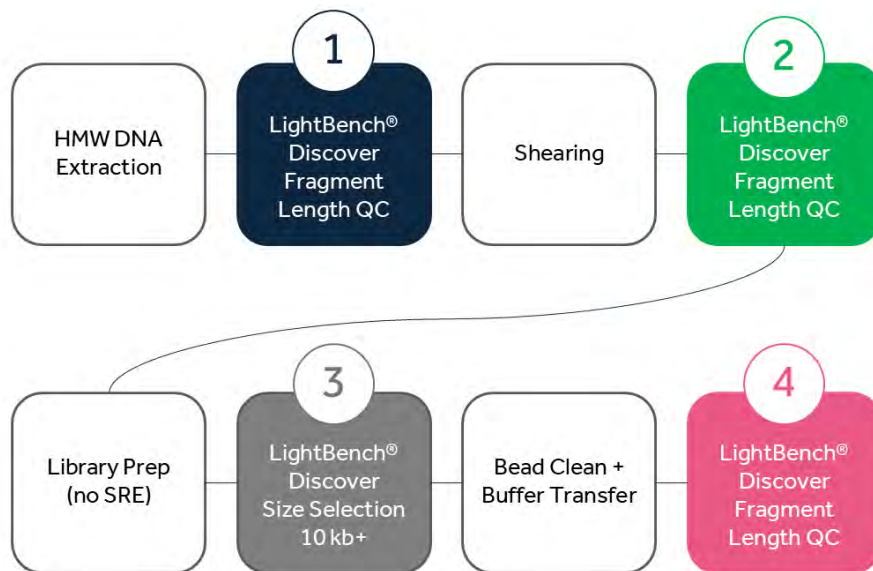


Figure 1 – Schematic of the integration of the LightBench® Discover in the CoLab's long-read sequencing workflow. Numbered positions indicate QC step performed by the LightBench® Discover.



Why is the LightBench® Discover a good fit for your workflow?

The LightBench® Discover allows our lab to manage the unpredictable workloads of both long-read and short-read sequencing projects simultaneously.

The LightBench® Discover can run partials gels for size selection and analytics cassettes, meaning we are not forced to burn any empty channels, or delay turnaround times whilst waiting for more samples to fill up a whole cassette.



We also love the flexibility of the LightBench® Discover. The instrument allows you to swap easily between short and long-read analytics and size selection which ensures we can “rescue” a library that is saturated with shorter fragments.

“A size selection cassette can run High Molecular Weight (HWM) DNA, it can run sheared DNA, and it can run anytime”.

“As a result, we are increasing our sequencing throughputs, in terms of read length and yield.

As a bonus, whenever you run a gel using the LightBench® Discover, you automatically clean the DNA too!”



How does the LightBench® Discover integrate with your PacBio Revio sequencing systems?

We currently have three Revio instruments in the building. We process thousands of HiFi libraries per year and multiplex anywhere from 1 to 24 libraries per cell depending on the application.



Almost every library we run goes through the LightBench® Discover at least three to four times for size selection, analytics and QC before sequencing.

We are pushing more than 10,000 lanes of LightBench® Discover annually, and the machine runs every day.

Is the Ranger® Technology software easy to use and to maintain?

The LightBench® Discover is straightforward to use – and you do pick it up quickly. The setup for both the size selection and analytics is very simple.

Because it's a three-in-one instrument, training is 1000 X easier. Staff only need to learn a single software platform to handle all three functions.

Now when we go into our lab, we see everyone using the LightBench® Discover - ranging from our technicians fresh out of undergrad, to our more senior members like the lab managers.

What DNA recovery rates do you see with the LightBench® Discover?

Many of our plant applications have been associated with poor DNA yield. However, the recovery that we get out of the LightBench® Discover has been better than any instruments we've previously used.

We are typically recovering 60-80% of the total DNA sample, depending on how aggressively we size select. Of course, naturally some sample loss is expected during size selection, however the LightBench® Discover ensures that we have sufficient DNA for sequencing - and without destroying our samples in the machine!



"The LightBench® Discover has become a workhorse in the laboratory"

Alex Harkess, Faculty Investigator, HudsonAlpha CoLab



LightBench®
Discover
powered by RANGER® TECHNOLOGY

"...the recovery that we get out of the LightBench® Discover has been better than any instruments we've previously used".

How do you utilise the LightBench® Discover's reporting functionality in your workflow?

The LightBench® Discover is super flexible with its reporting functionality. We can manually analyse at multiple times throughout the run when a full detail report isn't required. For instance, after High Molecular Weight extraction we visually assess and determine whether it needs to be sheared rather than using the analytics run to create a super detailed report.

After size selection, we use the full report function from the final analytics QC run to immediately feed data into SMRT Link for the Revio cell, generating accurate size distribution data. This is essential when pooling multiple libraries – particularly with High Molecular Weight DNA, where molarity can't be measured by qPCR.

This is one of the biggest advantages of using the LightBench® Discover. By helping us understand size distribution and tail diversity, the instrument lets us pool accurately, even with highly multiplexed library pools, and load more libraries per Revio cell without affecting data integrity.

We've also been working through all the options available during the analytics functions which allow you to refine the data, allowing us to generate some great images. We have added in information to smooth out the curves, generate sample traces and determine the average fragment sizes for the range specified.

"...the LightBench® Discover ensures that we have sufficient DNA for sequencing - and without destroying our samples in the machine!



Discover More

Scan the QR code to visit our Resources for LightBench® Discover, including Tech Notes, Videos and Your Expert articles.

Contact Us

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About YOURGENE HEALTH

Yourgene Health is an international molecular diagnostics group which develops integrated genomic technologies and services *enabling genomic medicine*

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