

HiFi sequencing with the PacBio Revio in AgBiotech

From crop and livestock research to pest and disease control, the latest HiFi sequencing platform can bring many benefits to AgBiotech research.

Long-read sequencing in AgBiotech

Genomics is now at the core of AgBiotech research and requires highly adept sequencing technologies to decode and analyze the complex nature of plant genomes. With the development of long-read sequencing (LRS), such as PacBio's HiFi sequencing technology, exciting opportunities are now opening up in crop and livestock optimization.

While LRS has previously offered advantages over short-read sequencing (SRS), researchers had to contend with lower accuracy and increased costs. However, HiFi sequencing has addressed these limitations, enabling the sequencing and analysis of highly complex and repetitive genomes. Based on PacBio's Single Molecule Real-Time (SMRT) method, researchers can sequence the same molecule multiple times, generating HiFi reads that improve on previous LRS approaches with continuous reads of 10-25,000 bp while maintaining an average accuracy above 99.9%. Now, PacBio's Revio system offers up to a 15-fold increase in throughput compared to previous systems, delivering up to 360Gb of HiFi reads per day at the same 99.9% accuracy.

Here, we discuss the applications that the Revio system enables in AgBiotech using its combination of readlength, accuracy, and throughput.

The next agricultural revolution?

Growing crops poses a substantial global challenge, further exacerbated by the climate crisis and an everexpanding global population. In light of these circumstances, there is an urgent need for tools that can provide insights for crop improvement, disease management, food safety, and conservation efforts.

The emergence of Next Generation Sequencing (NGS) technology has fundamentally changed the AgBiotech research landscape, enabling researchers to leverage genomic and proteomic strategies to generate plants and livestock with resistance to various environmental and pathogenic threats.

While it has provided a deeper understanding of species diversity and evolution, SRS techniques have faced limitations in certain AgBiotech applications. For instance, the reads can be too short to accurately resolve structural variants (SVs). As a result, SRS often produces incomplete and ambiguous genome assemblies. SVs are defined as genomic variations of greater than 50 bp, and encompasses insertions, duplications, deletions, inversions, and translocations. Gaining deeper insight into SVs would be highly advantageous

Ask us how the Revio system can enhance your AgBiotech project for AgBiotech researchers since SVs play a substantial role in the evolution of crop and animal species, and impact on Mendelian traits and phenotypic variation ^[1].

Analysis of SVs at an appropriate scale for AgBiotech initiatives requires a long-read sequencing platform that can rapidly resolve SVs with the accuracy associated with SRS. SMRT sequencing can provide just this, delivering high-quality assemblies of complex genomes in a rapid and affordable manner. Researchers can leverage HiFi reads for marker discovery, enabling them to work towards improving nutrition, protecting crop and livestock health, and increasing agricultural yield. The Revio system can also excel at SRS applications – presenting a versatile platform for the determination of small variants, repeat expansions, methylation, and more.

Crop and livestock research

SMRT sequencing, with its superior read length and enhanced accuracy, empowers AgBiotech researchers with comprehensive whole genome sequencing capabilities that can bring substantial value to crop and livestock research. Being able to rapidly resolve and phase whole genomes is invaluable in marker-assisted selection. Given the complex nature of plant and animal biology, desirable traits like disease resistance or nutritional content are generally influenced by a multitude of genetic factors. As such, having access to sequencing data at the haplotype scale is much more relevant.

Thanks to the unparalleled read length and high accuracy provided by the Revio, it's possible to resolve haplotypes and rapidly assemble reference-quality genomes for plant and animal studies (Fig 1). This rich data can then be leveraged to gain a better understanding of processes behind key traits, such as disease resistance and climate adaptation.





HiFi sequencing can also inform data-driven strategies for selective breeding and gene therapy programs where the Revio could be used for marker identification, marker assisted selection, and validation of successful transgene integration.

Filling in the gaps

Crops present many challenges when it comes to sequencing at population scale – they often have large genomes that display wide heterozygosity and polyploidy ^[2]. As a result, reference genome assemblies have often been left incomplete, leaving out important non-coding and regulatory regions that contribute to phenotypic traits. SMRT sequencing can help AgBiotech researchers create complete genomic blueprints of complex individual crop lines, as well as 'pangenome' datasets that map genetic diversity across multiple strains.

It's not just crop research that could benefit from more complete reference genomes – livestock reference genomes have traditionally been far from comprehensive due to the limitations of short-read sequencing for resolving complex, gigabase-length mammalian genomes. The read length and throughput power of the Revio system could enable whole genome sequencing of livestock at population scale and enable pangenome reference assemblies to inform better breeding programs and disease protection measures.

Pest and disease control

Mitigating the risks of common pests and diseases is an ever-present challenge for the agricultural industry, but monitoring insect and pest populations at the molecular level has often proved difficult. From insects to microbes, agricultural pests generally have short life cycles and reproduce rapidly. This fosters highly variable genetics within single populations, making it difficult to identify genes responsible for immunity, metabolic detoxification, and resistance to pesticides in insects, fungi, and microbes.





The benefits of the Revio system for pest and disease control could be vast, providing data-driven strategies to improve the effectiveness and durability of environmentally sustainable pest management practices (Fig 2). Molecular-based management tools could be developed that target pests without causing knock-on damage to the environment or ecosystems, including damage to beneficial insect populations like pollinating bees.

Monitoring insect populations

Insects are no new threat to agriculture, but in recent years their impact on food production has become exacerbated by climate change and the misuse of pesticides. Insecticide resistance is now commonplace globally, while global warming has driven non-native species to new areas and increased the seasonal activity of native pests. This current situation presents a significant burden for farmers and will require innovative approaches to address these multifaceted challenges.

Similar to crops and livestock, there has traditionally been a lack of reference genomes for agricultural pests, especially insects. SMRT sequencing can provide the read length and coverage required for populationscale genome mapping and has already been successfully employed by the United States Department of Agriculture as part of the Ag100Pest Initiative ^[3]. When the project began in 2018, just six of 366 (1.6%) insect genomes available on NCBI met high standards of contiguity. Thanks to a HiFi-based approach, now at least 158 species are in the pipeline, and the Revio looks set to boost this number further.

The project has been a huge success, not only due to the high throughput and read length of SMRT sequencing, but also because of the low input DNA needed. By lowering DNA input requirements, PacBio's technology can be harnessed to monitor the smallest of target organisms. Unearthing pest genomes and transcriptomes with HiFi reads, novel genes responsible for immunity, disease, and resistance can be identified and inform data-driven strategies for pest prevention.

Time to accelerate your AgBiotech research?

Discuss a project with us today With its ability to perform full-length reads with high accuracy, HiFi sequencing can bring sweeping benefits to AgBiotech research. At Eremid[®], PacBio's latest Revio sequencing platform is now integrated into our genomics services lab, enhancing the capabilities of our world-class science team. We're fully equipped to support you in all aspects of your AgBiotech research, no matter how big or small your project.

References

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