BRC-Seq Customers Benefit from Streamlined BaseSpace™ Integrated Workflow

BaseSpace workflow enables tracking of samples, from library prep to data analysis, helping BRC-Seq deliver high-quality sequencing data and additional value to its customers.

Introduction

Ryan Vander Werff is the Sequencing Manager at BRC-Seq, the Biomedical Research Centre core sequencing facility at the University of British Columbia (UBC). He has been using Illumina sequencing systems for more than three years and has acquired experience with instrumentation, software, reagents, and consumables for sequencing on HiSeq™, MiSeq™, and NextSeq™ 500 Systems. At the BRC-Seq core facility, Ryan uses NextSeq 500, MiSeq, and NeoPrep™ Library Prep Systems routinely to sequence samples submitted by research groups in Canada and the United States. He uses BaseSpace software*, the Illumina genomics computing environment, for sample tracking, data analysis, data management, and data sharing with his customers.

iCommunity spoke with Ryan about his experience with Illumina systems and BaseSpace software at BRC-Seq, which is becoming an important sequencing resource in Canada and the Pacific Northwest.

Q: What sparked your interest in molecular and cellular biology?
Ryan Vander Werff (RVW): Like many in the field, I had an early interest in science, and I entered college at the University of California, San Diego (UC San Diego) with the intention of studying chemistry. As I progressed through my classes, a series of small events and circumstances pulled me towards an academic emphasis in biology, and ultimately to pursue it as my major. I admired my biology professors, and when I had the opportunity to contribute in the lab, I really enjoyed it.

Q: When did you begin performing sequencing?
RVW: My introduction to sequencing came about as a result of my interest in laboratory automation and liquid handling systems. Experience with those systems was in demand at research institutions and commercial biotech companies near the UC San Diego campus. Sequenom is only a block away from campus, and when I joined I became a part of a project group that used the HiSeq System. We were performing prenatal testing for chromosome abnormalities. The studies could have been accomplished on other sequencing systems, but the HiSeq System provided flexibility for growth into other diagnostic studies or strategies without changing platforms. My experience with lab automation and the HiSeq System at Sequenom provided the background I needed for my role at BRC-Seq.

Q: What is the history of BRC-Seq?
RVW: BRC-Seq is the core sequencing facility in the Biomedical Research Centre at UBC. When I joined in late 2013, the facility had a MiSeq System that they had used for low-throughput sequencing for several years. I began working with a research group, prepping some of the samples and providing data. That led to a demand for more sequencing from that group and their collaborators. We received a Canadian Foundation for Innovation (CFI) grant for a NextSeq 500 System and became an official Illumina Core lab in early 2015. We also hired Tara Stach, an accomplished genotyping and sequencing technician.

As we became more experienced and confident in our sequencing data throughput, we told members of the local research community about our capabilities and started accepting more samples. We've now run samples for labs in British Columbia, other Canadian provinces, and from the state of Washington in the US.

Q: What sequencing studies do you perform at BRC-Seq?
RVW: About 98% of what we do is mRNA sequencing to analyze the impact of different experimental treatments on the transcriptome. We are performing a few ChIP-Seq studies and have begun some methylation sequencing studies. We're also working on a protocol for transposon sequencing to use in a new lineage tracing method.

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*BaseSpace software is currently known as BaseSpace Sequence Hub.

Q: How do you handle challenging samples?
RVW: Some of the researchers we support are working with quiescent stem cell populations that have very low RNA. The NeoPrep™ Library Prep System has enabled us to successfully prepare low-input samples that would otherwise be impossible for us to sequence.

In an ongoing study, researchers are pooling 20 animals to obtain enough material for a single sequencing sample. It is very expensive and ethically unfavorable to use these numbers of animals in studies. By preparing low-input samples on the NeoPrep System, we obtain high-quality data while limiting animal use.

> "The BaseSpace graphical user interface (GUI) provides us with access to all the features we need."

Q: Which library preparation kits are you using?
RVW: We use the TruSeq™ Stranded mRNA Library Prep Kit routinely. We also use the TruSeq Stranded Total RNA Kit with the Ribo-Zero™ Gold rRNA Removal Kit. Less frequently, we use the TruSeq Nano DNA, Nextera™ DNA, and Nextera XT Library Prep Kits for other exploratory studies.

Q: How would you compare your experience with MiSeq, HiSeq, and NextSeq 500 Systems?
RVW: Our workflow with the NextSeq System is straightforward. It's simpler to use than the HiSeq System. While the MiSeq System is easy to use, the NextSeq 500 System offers the distinct advantage of integrating with BaseSpace software and having the sample information neat and organized. We've moved most of our sequencing throughput over to the NextSeq 500 System because it's efficient in terms of cost per gigabyte of data. We still use the MiSeq System when we want to answer a specific question or as an independent quality control tool.

Q: How are you using BaseSpace?
RVW: We're using the cloud version of BaseSpace software, which has replaced Illumina Experiment Manager in our lab. The BaseSpace graphical user interface (GUI) provides us with access to all the features we need. We had to learn the BaseSpace nomenclature, but in the end, the advantages of having all our information online and traceable from NeoPrep library prep through to analysis has made it worth the adjustment.

Two of us handle the samples as they come into the BRC-Seq facility. We make it a priority to get everything into the BaseSpacesoftware and organized as quickly as possible, because it gives us the ability to keep track of everything from that point on, across the entire workflow. The value of maintaining that urgency on the front end becomes apparent as data comes off the NextSeq 500 System and into BaseSpace software for analysis.

Q: Which BaseSpace applications do you use?
RVW: For ChIP-Seq, we're using the BWA Whole Genome Sequencing App to align the data, and then we transfer it to our server for MACS2 and differential expression analysis. For RNA-Seq, we're using TopHat for alignment followed by assembly in Cufflinks. We can take the FPKM relative expression values directly into additional correlation analysis and from there we can do pathway and other types of analysis. We also use BWA Aligner for alignments to smaller FASTA sequences, FASTQ Toolkit for quality trimming data, Integrative Genomics Viewer for looking at the data on the reference genome, SRA Import for comparing data to previously published sets, and the SRA Submission App for submitting our data to the public databank. We also appreciate the open access to many of the other QC Apps and look forward to many of the Apps in development.

Q: How has BaseSpace software contributed to the sequencing studies you perform?
RVW: Our most comprehensive use of BaseSpace software has been for transcription profiling of hPSCs (human pluripotent stem cells) as part of a kidney disease modeling study. We prepared the cell transcriptomes with the TruSeq Stranded mRNA Library Prep Kit and performed sequencing on the NextSeq 500 System. We used TopHat2 for reference sequence alignment and calculated differential expression with Cuffdiff. We uploaded the data to the SRA (Sequence Read Archive) using the BaseSpace SRA Submission App, which saved quite a bit of time. The study was published in October 2015.[1]

> "BaseSpace software makes it easy to push all the data from a completed run through alignment, and share the output along with the new sequence data."

Q: How would you characterize the overall impact of BaseSpace software at BRC-Seq?
RVW: BaseSpace software is a tightly integrated element in our workflow and we use it for every RNA-Seq experiment to share data with our customers. Whether the customers are here at UBC, in Ottawa, or somewhere else, I can send them their results immediately after analysis.

What's potentially more important, is the way we use the integration of BaseSpace with alignment and other analysis tools. BaseSpace makes it easy to push all the data from a completed run through alignment, and share the output along with the new sequence data. If the customer wants to rerun the alignment on their own with different parameters, that's great. However, receiving a completed alignment back with the data can be valuable, especially for small research groups that are performing RNA sequencing. Often, these groups haven't performed much sequencing before and don't have a bioinformatics team to

support them, so they're at a loss if they only receive the BAM files. They can send us samples and a few weeks later, they receive high-quality results that include alignment output. That's huge for them. With BaseSpace and the BaseSpace Apps, we can provide that data depth easily and at no extra cost to BRC-Seq.

Learn more about the products and systems mentioned in this article:

BaseSpace Sequence Hub, www.illumina.com/informatics/research/sequencing-data-analysis-management/basespace.html

MiSeq System, www.illumina.com/systems/miseq.html

NextSeq 500 System, www.illumina.com/systems/nextseq-sequencer.html

HiSeq System, www.illumina.com/systems/hiseq_2500_1500.html

NeoPrep Library Prep System, www.illumina.com/systems/neoprep-library-system.html

TruSeq Stranded mRNA Library Prep Kit, www.illumina.com/products/truseq_stranded_mrna_library_prep_kit.html

TruSeq Stranded Total RNA Kit, www.illumina.com/products/truseq_stranded_total_rna_library_prep_kit.html

RiboZero Gold rRNA Removal Kit, www.illumina.com/products/ribo-zero-gold-rrna-removal-human-mouse-rat.html

TruSeq Nano DNA Library Prep Kit, www.illumina.com/products/truseq-nano-dna-library-prep-kit.html

Nextera DNA Library Prep Kit, www.illumina.com/products/nextera_dna_library_prep_kit.html

Nextera XT DNA Library Preparation Kit, www.illumina.com/products/nextera_xt_dna_library_prep_kit.html

References

1. Freedman BS, Brooks CR, Lam AQ, *et al*. Modeling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat. Commun.* 2015; 6:8715 doi: 10.1038/ncomms9715.

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