

**PacBio Sequel**

**Sequencing & Analysis**   
Report

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BallHort

ChrisBarbey



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# Service Information

|  |  |
| --- | --- |
| Company / Institute | BallHort |
| Client | ChrisBarbey |
| Service Type | SequencingOnly |
| Sequencing Platform | PacBio Sequel |
| Sample Name | Elburn-3 |
| Number of Cell | 1 |

# Workflow

1. Experiment
2. 15μg of DNA that has passed the quality control criteria is cut into 15Kb or larger using Covariis g-TUBE, purified using 0.45X AMPure XP magnetic beads, and then measured the size of shared DNA through bioanalyzer.
3. Add NAD+, DNA Prep Buffer, DNA Prep Enzyme, and DNA Prep Additive to Sheared DNA and react at 37°C for 15 minutes to remove single strand DNA at the end of the DNA strand.
4. Add DNA damage repair mix and react at 37°C for 30 minutes to proceed with DNA damage repair process.
5. After adding the End Repair Mix to the sample, react at 20°C for 10 minutes and 65°C for 30 minutes to proceed with the End Repair process.
6. Add adpater, Ligation Mix, Additive, and Enhancer to the End Repaired DNA and react at 20°C for 60 minutes and 65°C for 10 minutes to attach the Overhang adapter to the end of the DNA fragment.
7. After adding Enzyme clean up kit to the library to which the Overhang adapter is attached, it reacts at 37°C for an hour to remove the remaining unregistered products.
8. After purifying the library with 0.45X volume AMPureXP magnetic beads, measure the amount and size of DNA recovered through bioanalyzer.
9. The manufactured library 2 to 5 μg was placed in 1 lane of BluePippin 0.75% Gel, set BP end to 13,000bp at BP start 9,000bp, and electrophoresis to collect libraries over 9-13Kb and 15Kb.
10. Refine the recovered library with 0.5X AMPureXP magnetic bead and measure the size and density of the library cut through bioanalyzer.
11. SMRT Sequencing
12. ZMWs allow light to illuminate only the bottom of a well in which a DNA polymerase/template complex is immobilized.
13. Phospholinked nucleotides allow observation of the immobilized complex as the DNA polymerase produces a completely natural DNA strand.
14. This process occurs in parallel in up to thousands of ZMWs that make up the SMRT Cell.
15. Bioinformatic Analysis

|  |  |  |  |
| --- | --- | --- | --- |
| Step | Tool | Version | Reference |
| Subreads to HiFi Reads | Pacbio CCS | 6.2.0 | <https://ccs.how/> |
| Demultiplexing (Barcoding) | Lima | 2.3.99 | <https://lima.how/> |
| De novo Assembly (Bacteria) | Flye | 2.9-b1774 | <https://github.com/fenderglass/Flye> |
|  | HGAP.4 | pbcromwell 1.2.5 | <https://github.com/PacificBiosciences/pb-assembly> |
|  | pbmicrobial\_ assembly | pbcromwell 1.2.5 | <https://github.com/PacificBiosciences/pb-assembly> |
| De novo Assembly (Large Genome) | Falcon | 1.8.1 | https://github.com/PacificBiosciences/FALCON |
|  | IPA | pbcromwell 1.2.5 | https://github.com/PacificBiosciences/pbipa |
|  | NextDenovo | 2.4.0 | https://github.com/Nextomics/NextDenovo |
| Circulization | Circulator | 1.5.5 | <https://github.com/sanger-pathogens/circlator> |
| Polishing | Pilon | 1.23 | <https://github.com/broadinstitute/pilon/wiki> |
| Assembly Assessment | BUSCO | 5.3.1 | <https://gitlab.com/ezlab/busco> |
| Structural Annotation | Prokka | 1.14.6 | <https://github.com/tseemann/prokka> |
| Functional Annotation | DIAMOND | 0.9.30 | <https://github.com/bbuchfink/diamond> |
|  | Blast2GO | 4.1.9 | <https://www.blast2go.com/> |

CLR (Continuous Long Read) ResultLoadingProductive ZMWsProductivity 0Productivity 1Productivity 216,021,0829,703,4016,192,060133,881100.060.638.60.8Productivity 0: Empty ZMW, no signal detected.Productivity 1: ZMW with a high quality read detected.Productivity 2: Other, signal detected but no high quality read.AdapterAdapter Dimers (0-10bp) %Short Inserts (11-100bp) %0.00.0Adapter Dimer: The % of pre-filter ZMWs which have observed inserts of 0-10 bp. These are likely adapter dimers.Short Insert: The % of pre-filter ZMWs which have observed inserts of 11-100 bp. These are likely short fragment contamination.Polymerase ReadsPolymerase Read BasesPolymeraseReadsPolymerase Read Length (Mean)Polymerase Read N50490,997,458,3796,183,80079,401198,750Polymerase Read Length Mean: The mean high-quality read length of all polymerase reads. The value includes bases from adapters as well as multiple passes around a circular template.Polymerase Read Length N50: 50% of all read bases came from polymerase reads longer than this value.SubreadsSubread Length(Mean)Subread N50Longest Subread Length (Mean)Longest Subread N50Unique Molecular Yield15,08718,75052,989,120,512Subread Length (mean): The mean read length of all subreads in the DataSet.Subread N50: The length at which 50% of all subreads in the Data Set are longer than, or equal to, this value.Longest Subread Mean: The mean subread length, considering only the longest subread from each ZMW.Longest Subread N50: 50% of all read bases came from subreads longer than this value when considering only the longest subread from each ZMW.Unique Molecular Yield: The sum total length of unique single molecules that were sequenced. It is calculated as the sum of perZMW median subread lengths.Polymerase Read Length
Plots the number of reads against the polymerase read length.Subread Read LengthPlots the number of reads against the subread length.Base Yield DensityDisplays the number of bases sequenced in the collection, according to the length of the read in which they were observed. Values displayed are per unit of read length (i.e. the base yield density) and are averaged over 2000 bp windows to gently smooth the data. Regions of the graph corresponding to bases found in reads longer than the N50 and N95 values are shaded in medium and dark blue, respectively.

CCS (Circular Consensus Sequencing) ResultHiFi ReadsHiFi Read BasesHiFiReadsHiFi Read Length (Mean)HiFi Read QualityHiFi Number of Passes32,070,001,5902,176,54314,734Q3412HiFi Reads Bases: The total yield (in base pairs) of the CCS Reads whose quality value is equal to or greater than 20.HiFi Reads: The total number of CCS Reads whose quality value is equal to or greater than 20.HiFi Read Length (mean): The mean read length of the CCS Reads whose quality value is equal to or greater than 20.HiFi Read Quality: The mean number of CCS Reads whose quality value is equal to or greater than 20.HiFi Read Length Distribution
Displays a histogram distribution of HiFi Reads (QV>=20)Total Read Length Distribution
Displays a histogram distribution of HiFi Reads (QV>=20), other CCS Reads (three or more passes, but QV <20), and other reads, by read length.Number of Passes
Histogram of the number of complete subreads in CCS Reads, broken down by read type (HiFi Reads, other CCS Reads, other reads.)Read Quality Distribution
Histogram distribution of the CCS Reads by the read quality.

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