

**PacBio Sequel**

**Sequencing & Analysis**   
Report

{{date}}

{{company}}

{{client}}



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# Service Information

|  |  |
| --- | --- |
| Company / Institute | {{company}} |
| Client | {{client}} |
| Service Type | {{servicetype}} |
| Sequencing Platform | PacBio Sequel |
| Sample Name | {{samplename}} |
| Number of Cell | {{cell}} |

# 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) | Hifiasm | 0.16.1 | <https://hifiasm.readthedocs.io/en/latest/> |
| IPA | pbcromwell 1.2.5 | https://github.com/PacificBiosciences/pbipa |
| NextDenovo | 2.4.0 | https://github.com/Nextomics/NextDenovo |
| Polishing | Pilon | 1.23 | <https://github.com/broadinstitute/pilon/wiki> |
| Assembly Assessment | BUSCO | 5.3.1 | <https://gitlab.com/ezlab/busco> |
| Gene Prediction | Maker | 2.31.10 | <https://www.yandell-lab.org/software/maker.html> |
| Gene Annotation | Interproscan | 5.44-79.0 | <https://github.com/ebi-pf-team/interproscan> |
| DIAMOND | 0.9.30 | <https://github.com/bbuchfink/diamond> |
| Blast2GO | 4.1.9 | <https://www.blast2go.com/> |

{{CLRreport}}

{{CCSreport}}

{% if analysis\_assembly == “” %}

{{download}}

{% else %}

{{analysis\_assembly}}

{{download}}

{% endif %}