Dear Customer,

Thank you for your interest in NRGene™ TraitMAGIC™ services.

We look forward to establishing our collaboration and provide you with a fast, accurate, and cost-effective genome assembly. Our genome assemblies are well known in the scientific community as well as in the commercial market for their high standards and quality.

Optimal genome analysis relies on high quality gDNA extraction and a well-designed library preparation and sequencing. In order to make sure you will get the most satisfying results, kindly adhere to the following standard instructions and specifications.

Please feel free to address us with any inquiry with the bellow email address,

Thank you for the collaboration,

NRGene professional services team.

gas\_support@nrgene.com

1. **Sequencing data specifications**

It is recommended to use NRGene’s certified sequence providers to prepare the sequencing data. NRGene has invested many efforts to provide you with the best available services with most affordable prices.

Nonetheless, whether you choose to prepare the sequencing data in-house or with the aid of a 3rd party contractor, the following criteria and instructions shall be met in order to allow NRGene to provide the best assembly results

1. **Coverage Calculation**

Coverage calculation depend on the zygosity of the organism being sequenced:

1. **Homozygous genome** – coverage should be calculated considering the haploid size of the genome[[1]](#footnote-1).
2. **Heterozygous genome** - coverage should be calculated considering the **double** haploid size of the genome.
3. **Sequencing Data Specifications**

The following table shows the libraries that are required to be constructed and the sequencing machines that can be used. Minimal coverage required from the library is an estimate that should be calculated considering the genome size[[2]](#footnote-2):

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| # | Library type | Insert size | Sequencing Instrument | Read length | Minimal Coverage |
| 1 | Standard PE Shotgun library  (Preferably PCR-Free) | 450-700bp | HiSeq 3000/4000, HiSeqX, NovaSeq6000 | PE150-160bp | X30 for parental lines |
| 2 | Standard PE Shotgun library  (Preferably PCR-Free) | 450-700bp | HiSeq 3000/4000, HiSeqX, NovaSeq6000 | PE150-160bp | X0.2-3[[3]](#footnote-3) |

**All output fastq files and relevant output analysis files should be referred to NRGene for QC and approval.**

1. **Quality Measurements**

The minimal quality thresholds required from the sequencing data produced[[4]](#footnote-4) is Q30>80%.

***Please note:*** *Should you encounter any difficulties, please contact NRGene’s Genomic Analysis Services team; the team will do its utmost to assist you with its own expertise.* (e-mail: [gas\_support@nrgene.com](mailto:gas_support@nrgene.com)).

1. Haploid genome size= flow-cytometry 1c and/or amount of DNA in a gamete cell; Genome size estimates should be rounded up to avoid missing coverage in the sequencing calculations. [↑](#footnote-ref-1)
2. For example: a heterozygous genome of 3Gbp haploid genome size requires 360Gbp (2\*3\*60) of data from the PCR-free PE library with 450bp insert size, sequenced on HiSeq 2500/NovaSeq6000, read length 250bp. Coverage may be extended where data quality does not suffice. [↑](#footnote-ref-2)
3. Depended on the genome and population complexity, please consult our Genomic Analysis Services team [↑](#footnote-ref-3)
4. Other quality criteria may apply, NRGene will conduct additional QC procedures to ensure data compatibility. [↑](#footnote-ref-4)