Dear Customer,

Thank you for your interest in NRGene™ DeNovoMAGIC™ 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 assembly rely on high quality gDNA extraction which is suitable for library preparation followed by sequencing and assembly. 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. **DNA extraction specification**
2. **High quality gDNA Extraction**

Isolation of intact, double stranded, highly concentrated, and not contaminated genomic DNA is prerequisite for successful and reliable project.

Below you will find NRGene™ specifications for gDNA extraction:

* 1. **Extraction Specifications and Quality**

***Please note each sample should be prepared from the same DNA batch this is essential for downstream analysis procedures***

|  |  |  |
| --- | --- | --- |
| ***Parameters*** | ***Thresholds*** | ***Recommended analysis method*** |
| ***DNA concentration*** | > 20 ng/µl | fluorometric methods as Qubit™ or Nanodrop  |
| ***DNA total mass[[1]](#footnote-1)*** | * Genome size <3Gb, >0.5 µg
* Genome size >3Gb, >1 µg
 |  |
| ***DNA fragmentation*** | **generation:** 70% of DNA is above 15kb subsampling of ~5% of samples should be sufficient in projects with sample number >96 | Agarose gel (0.8%, ladder above 15Kb); Fragment Analyzer™; TapeStation™; PFGE,  |
| ***Purity - RNA*** | without any traces of low molecular weight bands in gel | Agarose gel (0.8%, ladder bellow 15Kb); Fragment Analyzer™; TapeStation™; PFGE |
| ***Purity - protein & organic solvent*** | 260/280 ratio between 1.6- 2.2 260/230 ratio between 1.9- 2.2 | Nanodrop™ (Thermo-scientific) or equivalent |

Below you will find NRGene recommendation for High Molecular Weight DNA extractions methods:

|  |  |  |
| --- | --- | --- |
| ***Organism*** | ***Recommended protocol***  | ***Comments***  |
| ***Plant*** | CTAB based method | Use very young etiolated[[2]](#footnote-2) tissue, alternatively use leaves that have just finished budding |
| ***Human/Animal*** | Trysol based protocol /Dedicated DNA extraction kits |  |

***Please note:*** *Should you encounter difficulties in gDNA extraction, or adhering the standards mentioned above, kindly contact NRGene support team.*

*Our professional services may assist you with their own expertise or may refer you to a 3rd party service provider that might perform the DNA extraction for you.*

**e-mail:** **Gas\_support@NRGene.com**

1. **QC by NRGene**

NRGene will perform a preliminary assessment of the DNA samples prior shipment to the sequence providers. Please provide a table as in Annex A to NRGene’s personnel for review (Gas\_support@NRGene.com). The table should be signed and filled with the actual measured values and attached with a high-resolution picture of an agarose gel or other documents as stated therein.

1. **Samples Shipment Instruction**

DNA samples should be stored in a controlled temperature to avoid degradation and/or breakage of the high molecular weight DNA molecules, shipping should also be handled carefully to avoid spillage or shear stress.

* 1. **Storage**

Dissolve the DNA in TE buffer (10mM tris, with a maximum of 0.1mM EDTA). The samples may be stored at 4°C for up to 2 weeks, or at -20°C (on dry Ice) for up to 6 months.

***Important notice:*** *A*void freeze-thaw cycles as much as possible, it might cause a severe accelerated degradation and breakage damage to the gDNA

* 1. **Labelling**

All samples should include the following information:

1. Project number
2. Organism Name (including variety name)
3. Sample number (or name)

For example, label **Project\_85\_Wheat\_A.tauschii\_3** will be for:project number (85), organism name (Wheat) variety (A.tauschii) and sample number (3).

\*Label format should exclude spaces – please use only underscores “\_”

Some samples may require additional documentation as instructed by NRGene personnel such as a reservation number(s) or declarations of non-hazardous materials.

**Additional notes:**

1. Plates must be numbered and clearly labeled with smear-proof marker.
2. Provide an excel-based plate template file prior to shipping. Sample names will be entered in each well position for each plate. The files and plates should be named/labeled by the project name and plate number. For example, plate 8 of project 18001Abc should be labeled “18001Abc\_08” as well as the file with the sample IDs.
3. Any empty wells should be labeled “BLANK”
4. Keep the sample names simple without special characters (e.g. +, &, # etc.).
5. Shipping information will be provided by NRGene prior to shipment in accordance to providers availability
6. samples should be shipped with a project number
	1. **Shipping**
7. Use hard-shell 96-well skirted PCR plates (such as Perkin Elmer Cat. No. 6008870 or comparable) with a non-leaking tight seal or strip caps.
8. Samples must be shipped frozen on dry ice with sufficient amount to maintain the samples frozen until their arrival.

***Important notices:***

1. **Avoid freeze-thaw cycles as much as possible**, it might cause a severe accelerated degradation and breakage damage to the gDNA
2. Shipment is advised to take place at Monday-Wednesday to avoid long weekend storages.
3. Shipment is advised to be with a well-known currier company. Shipments via small companies might cause delays and get held in customs for longer than usual.
	1. **Tracking**

Samples should be shipped with a well-known (A class) carrier which will provide tracing information and coordinate their reception.

Annex A- samples positioning format

Open the attached excel file to fill in samples names and data, sheet2 is automatically filled according to Sheet1 content.







1. *DNA total mass =* Volume\*DNA concentration [↑](#footnote-ref-1)
2. *Ideally use seedlings that have been dark treated for 24-72 hours* [↑](#footnote-ref-2)