Sample Acceptance Criteria

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Sample Acceptance Conditions

The acceptance criteria of your samples to be sequenced are listed below. Please read these criteria carefully and fill in the section at the bottom of the form. ONT quality criteria are listed in the following part of the file. The specified criteria must be met for optimum results.

Concentrations must be measured by spectrofluorimetric methods (Qubit). Spectrophotometric measurements are not accepted (Nanodrop).

Data retention period is 3 months after analysis. After this period, your data will be deleted.

Crude Sample Acceptance Conditions:

- Before shipping your samples, please contact us with detailed information about the sample you want to send. The required amount of the sample is determined according to sample type.
- Samples must be sent in proper conditions where the target nucleic acid type is stable (for instance, fecal samples must be sent in a tube containing storage solution suitable for providing DNA stabilization.)
- The samples must be shipped with an ice pack at +4°C.

DNA Acceptance Conditions:

- DNA concentration must be 50 ng/µl and above, and DNA volume must be 40 µl minimum.
- DNA must be sent with ice pack at +4°C or with dry ice at -20°C.
- Gel confirmation is required.
- A260/280 value must be in the interval of 1.8-2.2.

RNA Acceptance Conditions:

- RNA amount must be 2000 ng and volume of RNA should not exceed 40 µl.
- RNA must be shipped with dry ice at -20°C storage conditions.
- A260/280 value must be between 1.8-2.2.

cDNA Acceptance Conditions:

- cDNA amount must be 2000 ng and above. The volume of cDNA should not exceed 40 µl.
- cDNA must be sent with dry ice at -20°C storage conditions.
- A260/280 value must be between 1.8-2.2.

Amplicon (PCR product) Acceptance Conditions:

- PCR products must be purified.
- A gel image showing the amplicon length of each sample is required.
- After purification, the amplicon concentration must be 50 ng/µl and above, and DNA volume must be 40 µl minimum.
- Samples must be sent with ice pack at +4°C or with dry ice at -20°C.
- Information about the primer sequence and the amplicon length are required.

Services

BARCODE GENE BASED METAGENOMIC SEQUENCING (16S, 18S and ITS)

- Sample, DNA or amplicon is accepted.

SHOTGUN SEQUENCING

1) Whole Covid-19 Shotgun Sequencing

- RNA or cDNA is accepted.
- Ct value must be lower than 20.

2) Genome Alignment (WGS)

a) Bacterial whole genome
b) Eukaryotic whole genome
c) Fungal whole genome
d) Plasmid sequencing
e) Viral whole genome (RNA, DNA)

- Crude sample is accepted (Viral samples must be in an inhibition fluid.)
- DNA, RNA or cDNA is also accepted.
- Nucleic acid extraction must be done with kits or methods that produce high molecular weight DNA.

3) Metagenomic analysis (Shotgun sequencing)

- Crude sample, DNA, RNA or cDNA is accepted.

AMPLICON-BASED SEQUENCING

1) Covid-19 ARTIC - WGS

- **RNA or cDNA is accepted:**
  1. When sending RNA; Ct values must be specified.
  2. When sending cDNA; spectrofluorometric measurement values must be specified.
- Ct value must be lower than 30.

2) Short/long amplicon sequencing

- Amplicons are accepted. The target region must be amplified before sending the sample (length of the target region must be more than 200 base pairs.)

Oxford Nanopore (ONT) Quality Criteria for DNA/RNA

For DNA sequencing run:

Checking the quality of DNA before library preparation is important for maximum efficiency with the ONT sequencing platform. Incorrect determination of DNA amount and/or contamination of DNA with salt, EDTA, protein or organic solvents will affect further steps of the experimental process.

- Purity measurement must be done with Nanodrop:

1. OD 260/280: 1.8 : If this value is above 1.8, it indicates RNA contamination; if this value below 1.8, it indicates protein or phenol contamination.

2. OD 260/230: 2.0 - 2.2 : If this value is below the stated range, it indicates presence of contaminant. Additional purification steps are advised.

- Determination of average fragment length by pulse-field gel electrophoresis (>30 kb)
- Measurement of DNA amount by Qubit.
- Storage of high molecular weight DNA in TE.
- Confirmation of DNA weight by Qubit fluorometer, fragment length by gel electrophoresis, Agilent Bioanalyzer or FEMTO Pulse for ensuring the use of the starting material with accurate amount.
- Performing quality controls at certain points during the library preparation (quantification of DNA with Qubit and purity measurement with Nanodrop)

Use of detergents, denaturants, chelating agents and salt with high concentration must be avoided during preparation phases.

- **Ethanol:** Presence of ethanol in isolated DNA can cause DNA concentration to be measured higher than its real value and a deviation on purity values. Contamination with more than 7.5% ethanol can prevent ONT library preparation.
- **Isopropanol:** Similar to ethanol contamination, isopropanol contamination directly affects the performance of runs with ligation chemistry.
- **EDTA:** : It causes perturbation in concentration and purity measurements with Nanodrop, showing the concentration to be higher. Contamination with more than 5 mM EDTA can prevent ONT library preparation.
- **NaCl:** Contamination over 100 mM can prevent ONT library preparation.
- **Guanidinium chloride:** As a denaturant, it affects Nanodrop measurements, especially, 260/230 ratio. Guanidinium chloride contamination over 100 mM can prevent ONT library preparation.
- **Guanidinium isothiocyanate:** As a denaturant, it disrupts concentration and purity measurements done with Nanodrop. Guanidinium isothiocyanate contamination over 50 mM can prevent ONT library preparation.
- **Phenol:** Presence of phenol in isolated DNA can cause DNA concentration to be measured higher than the real value and deviation on purity measurements. Contamination with more than 1% phenol can prevent ONT library preparation.

Oxford Nanopore (ONT) Quality Criteria for DNA/RNA

Recommendations for using high molecular weight DNA without disruption:

- Use wide orifice pipette tips when working with genomic DNA.
- Avoid mixing samples by pipetting and vortex, instead, prefer flicking the bottoms of the tubes.
- Avoid unnecessary freeze-thaw cycles.
- Make sure the pH value of the sample is between 6 and 9.
- Keep the samples away from high temperatures.

Gel electrophoresis with low percentage of agarose can be used to check whether DNA is degraded or not.

For RNA sequencing run:

The DNA Quality Criteria are valid for RNA as well. Reference values of purity determination with Nanodrop measurement are as follows:

- OD 260/280: 2.0 : If this value is below 2, it indicates DNA contamination; if this value is below 1.8, it indicates protein of phenol contamination.

Determination of RNA degradation by RNA analyse kits of Agilent Bioanalyzer and being RNA Integrity Number (RIN) at appropriate range are advised.

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