Next Generation Sequencing Service Guidelines

Open Facility Center
Laboratory of Genome Sequencing and Analysis

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1. Procedure for Utilization

Next Generation Sequencing (NGS) Service will be conducted at the OFC Laboratory of Genome Sequencing and Analysis using the NextSeq 2000 sequencer. The sequencing service applications include **Whole Exome Sequencing (WES)** and **RNA sequencing (RNA-Seq), along with respective data analysis** and **sequencing run**. We will proceed with your request according to the following procedure:

① **Discussion of Sequencing Service Contents**
② **Completion and Submission of Sequencing Service Request Form**
③ **Preparation and Submission of Samples**
④ **Reporting of Results**
⑤ **Delivery of Data**

① Discussion of Sequencing Service Contents

Please contact us via email in advance to discuss the details about the sequencing service. We will contact you shortly to arrange a suitable date and time for the follow-up discussion. Please prepare as much specific information as possible regarding **the type of samples, the number of samples, and the desired data delivery timeframe** for our follow-up discussion.

② Completion and Submission of Sequencing Service Request Form

Please fill in the required information on the **Next Generation Sequencing Service Request Form** (Excel file) located on the Open Facility Center Sequencing Service site (https://www.fujita-hu.ac.jp/~kyoriken/jyutaku/index.html) and attach it to an email for submission.

③ Preparation and Submission of Samples

Please bring DNA samples refrigerated or frozen, and RNA samples preferably frozen, to room 106 in building 4. Bringing RNA samples refrigerated may potentially degrade their quality.

④ Reporting of Results

We will report the sequencing results via email. For those who have requested data analysis, we will report the results after the analysis has been completed. If there are any issues with the samples during library preparation, we will contact you in advance.

⑤ Delivery of Data

We will provide the analysis data on a USB memory stick. Please let us know whether you would like to receive the USB memory stick directly or prefer delivery via campus mail. Additionally, if you require other sequence files, we can copy them onto a separate HDD or SSD if you provide one.

2. Submitted Samples

The requirements for submitting samples for NGS analysis library preparation are as follows:

DNA

Total amount	≧ 1 μg
Concentration	≧ 20 ng/μL
Volume	≧ 20 ng/μL
Suspension solution	Pure water, EB buffer (10 mM Tris-HCl, pH8.5), TE=NG*
OD260/280	≧1.6
OD260/230	≧1.6

RNA

Total amount	≧ 1 μg
Concentration	≧ 20 ng/μL
Volume	≧ 20 ng/μL
Suspension solution	Pure water (RNase free)
OD260/280	≧1.8
OD260/230	≧1.8

*Samples containing EDTA-containing TE buffer are generally not accepted. If it's difficult, please feel free to contact us.
*Prior to library preparation, DNA and RNA samples undergo quality assessment using TapeStation to measure DIN and RIN values. Samples with **DIN values less than 3 and RIN values less than 7** do not meet the quality criteria for library preparation. In such cases, we will consult with you for further guidance.

Sequencing Run Library

Concentration	≧ 2 nM
Volume	≧ 15 μL

3. Details of Sequencing Service

For each analysis application, we will perform sequencing using the following reagents or kits on the NextSeq 2000.

■WES

Library preparation: Twist Library Preparation EF Kit 2.0 (Twist bioscience)
xGen Exome Research Panel ver 2 (IDT)
Sequencing: NextSeq 2000 Reagents (200 or 300 cycle)
100 bp or 150 bp paired-end sequencing

■RNA-Seq

Library preparation: NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs)
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs)
Sequencing: NextSeq 2000 Reagents (100 cycle)
50 bp paired-end sequencing

■Data Analysis
WES: We will conduct basic NGS data analysis using CLC Genomics Workbench (QIAGEN) and variant analysis using VarSeq (Golden Helix).
RNA-Seq: We will perform basic NGS data analysis using CLC Genomics Workbench and, upon request, conduct variant gene analysis, clustering analysis, PCA analysis, and other analyses as needed.
■ Sequencing Run
If you are preparing the library yourself, we will only handle the sequencing run on the NextSeq 2000. You will need to purchase sequencing cartridge reagents separately. If you have any questions about the types, prices, or ordering methods of cartridge reagents, please feel free to inquire.

4. Data Delivery

If you have requested sequencing only, we will copy **the Fastq files** onto an HDD (owned by the requester) and provide them to you. If data analysis has also been requested, we will provide the following files outputted from the data analysis on **a USB memory stick**: **Please consult with us in advance regarding how you receive the data**.
■WES
Excel file (containing gene mutations and annotation information, etc.)
■RNA-Seq
Excel file (containing gene expression values and comparative differential data), PowerPoint file (containing gene expression clustering and PCA analysis graphs, etc.)
*Fastq, Bam, and Vcf files are also available, so please let us know if you need data in formats other than those mentioned above.

5. Sequencing service fees and payment methods

① **Sequencing service fees**

Sequencing application		Price (tax included)
Whole Exon Sequencing (WES) for human : approx. 10 Gb	Per 1 sample	¥49,000
Data analysis for WES : (CLC Gx, VarSeq) mutation analysis	Per 1 sample	¥5,000
RNA Sequencing (RNA-Seq) : approx. 40M reads	Per 1 sample	¥28,500
Data analysis for RNA-Seq : (CLC Gx) Gene expression analysis	Per 1 sample	¥2,500
NGS sequencing run	Per 1 run	¥20,000

② **Payment methods**

We **bill the usage fees at the end of the month, and payments are due the following month**. Around the beginning of the following month after delivery, we will send a billing request from the Research Support Division of the Research Support Promotion Headquarters Office to your department. Please feel free to contact us if you need the invoice urgently. Payment can be made using university research funds, public research grants, etc. Please direct any inquiries to the Research Support Division.

6. Disclaimer and Notes

This sequencing service does not guarantee the results. Our lab assumes no responsibility if analysis results are not obtained due to the quality of the submitted samples or other factors. Please be aware that usage fees will be charged even if no results are obtained.

Analysis of human specimens (other than cell lines) requires **prior approval from the Ethics Review Committee**. Please note that we cannot analyze specimens that have not received approval from the Ethics Review Committee.

7. Research Consultation

For inquiries about sequencing service, research and analysis using next-generation sequencers, or specific methodologies, please feel free to contact us below.

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