SERVICE MANUAL

Finnish Functional Genomics Centre

Details of the laboratory

Finnish Functional Genomics Centre
Turku Bioscience
University of Turku and Åbo Akademi University
Tykistökatu 6A, Biocity 5th floor
20520 Turku, Finland
ffgc@bioscience.fi

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1 Abbreviations

FFGC	Finnish Functional Genomics Centre
ISO	International Standardization Organization
IEC	International Electrotechnical Commission
NGS	Next Generation Sequencing
QC	Quality Control
RUO	Research Use Only
SFS	Finnish Standards Association
SOP	Standard Operating Procedure

2 Objectives and scope

Finnish Functional Genomics Centre (FFGC) is a national infrastructure with a mission to support high level research in Finland by providing open access to the latest technologies available for genome research in different fields. FFGC operates at the Turku Bioscience Centre, which is an independent department of University of Turku and Åbo Akademi University. FFGC belongs to the Biocenter Finland infrastructure.

FFGC is a testing laboratory No. T351 accredited by FINAS Finnish Accreditation Service (accreditation requirement SFS-EN ISO/IEC 17025:2017). The accredited services provided by FFGC, which are suitable for clinical use are described in section 3. In addition, FFGC provides a wide variety of services for research use only (RUO). These services are described in section 4 of this manual.

3 Accredited services and flexible scope

FFGC provides accredited services for the clinical testing, genetics, and molecular genetics with flexible scope. The flexible scope of accreditation at FFGC is production of sequence raw data from genomic DNA for various further analyses with Next Generation Sequencing. The detailed services which are currently within the boundaries of the flexible scope are listed in Table 1.

The flexible scope has been developed to enable quick accreditation of rapidly changing activities. Therefore, the flexible scope enables FFGC to make changes in the provided accredited services if the activities remain in the competence field of FFGC and the services remain within the boundaries of the existing flexible scope, and the processes and services follow the requirements of the SFS-EN ISO/IEC 17025:2017. Such changes do not require prior assessment by the accreditation body. The compliance of the changes with the requirements of the SFS-EN ISO/IEC 17025:2017 are evaluated in the next annual external evaluation following the change. The procedures for making changes in the flexible scope at FFGC are summarized in the following sections.

Table 1. Detailed list of services in flexible scope.

Biological material	Library preparation kit	Manufacturer	Test method	Sequencing instrument	Turnaround time
Genomic DNA	Illumina DNA PCR-free	Illumina	Whole genome sequencing	Illumina NovaSeq X	Agreed in contract
Genomic DNA	Twist WES core + refseq (comprehensive exome panel)	Twist Bioscience	Whole exome sequencing	Illumina NovaSeq X	Agreed in contract

3.1 Analysis of the needs for the flexible scope

The need for the flexible scope is primarily determined by the needs of the service users. Changes in the accredited services can be made upon request of the existing or potential service users or as a development and improvement of the methods and technologies when improved reagents, materials or instruments become available from the suppliers. The needs of the service users are continuously monitored through regular communication and collection and processing of feedback and suggestions. Standard operating procedures have been established at FFGC for management of these activities and making changes in the provided services.

3.2 Process for making changes in the flexible scope

To ensure compliance with the requirements of SFS-EN ISO/IEC 17025:2017 and flexible scope, FFGC has established processes for verification and validation of procedures for accredited services. These activities include internal validation/verification of the processes by using NIST's human genome reference materials and data (https://www.coriell.org/1/NIGMS/Collections/NIST-Reference-Materials). These reference materials can be used for benchmarking of DNA sample preparation and sequencing methods for clinical use. After initial validation/verification the same reference materials are used for continuous monitoring of the quality and performance of the processes. In addition to internal verification, the technical quality is assured through annual participation to interlaboratory proficiency comparison programs organized by European Molecular Genetics Quality Network, EMQN.

4 Services for research use only

In addition to accredited services, FFGC provides a wide variety of services for research use only (RUO), which are not accredited and do not belong to the flexible scope of FFGC. Although FFGC follows the same principles for all processes, the compliance of the RUO methods and processes with SFS-EN ISO/IEC 17025:2017 cannot be confirmed. The services available for research use are listed in Table 2.

Table 2. List of all the tests performed in the laboratory and which are available for research use

Application	Purpose	Turnaround time (working days per 96 samples) *
Whole exome sequencing (RUO)	Raw data for analysis of genetic variants in the exome regions of the human genome.	1-3 months
Whole genome sequencing, PCR-free (RUO)	Raw data for analysis of genetic variants in the genomic DNA of any organism.	1-3 months
Whole genome sequencing, PCR amplified (RUO)	Raw data for analysis of genetic variants in the genomic DNA of any organism.	1-3 months
Whole transcriptome sequencing (RUO)	Raw data for analysis of coding and non-coding transcripts with size > 200 bp.	1-3 months
PolyA+ transcriptome sequencing (mRNA) (RUO)	Raw data for analysis of coding transcripts or genes.	1-3 months
Small RNA sequencing (RUO)	Raw data for analysis of miRNAs or smallRNAs (<200 bp).	1-3 months
Reduced representation bisulfite sequencing (RUO)	Raw data for analysis of DNA methylation in the CpG rich regions of the genome. (unavailable at the moment)	1-3 months
16S rRNA-seq (RUO)	Metagenome analysis.	1-3 months
Targeted sequencing (RUO)	Raw data for targeted analysis of e.g. gene panels for gene expression or genetic variants.	1-3 months
Sequencing of ready-made libraries (RUO)	Sequencing service for libraries prepared by the service user.	4-6 weeks
Quality analysis (RUO)	Quality analysis of DNA, RNA or NGS libraries for samples that will be sequenced at FFGC.	1-4 weeks
Plate running service for QuantStudio** (RUO)	Plate running service for 96- or 384-well RT-qPCR plates prepared by the service user.	1-3 days

*Other turnaround times can be negotiated and agreed in a contract.

**Plate running service available from Monday to Wednesday, possibility for open-access use

5 Preanalytical phase

5.1 Project initiation

For initiation of a new project, please contact ffgc@biosience.fi. When needed, our personnel will help you to choose the most appropriate application for your project, help you with the experimental design and calculate the budget. This service is free of charge.

5.2 Sample submission form

Before sample delivery a filled sample submission form needs to be delivered in electronic form by email and signed in UTU electronic signature system by project PI. The sample submission form templates can be downloaded from our webpages https://bioscience.fi/services/functional-genomics/services or by contacting ffgc@biosience.fi. Please, note that all **the samples must be pseudonymised** before sending to FFGC.

5.3 Sample delivery

The starting material requirements for each application are listed in the Table 3. To secure turn-around times for the provided services, **the samples need to be delivered to FFGC in appropriate containers, volumes, concentrations and order.** Additional fee per sample (10 €) is invoiced and project may be delayed by at least 2-4 weeks if samples are not provided according to following instructions.

Containers

- Samples within the campus are recommended to be delivered either in 8-tube strips, 96-well PCR plates or screw capped barcoded tubes from FluidX (VingLab cat. No. FLU-68-0702-11).
- 1.5 ml tubes can be used only if the sample number is less than 10.
- If you use courier for the sample delivery, make sure the containers are properly sealed to prevent cross contamination. Use freezer compatible PCR plate seal.
- If 8-tube strips are used, each tube (not the removable cap) should be marked according the sample list numbering.

Sample normalization

- Concentration of the sample should be based on fluorometric measurement method when diluting the samples.
- Place the samples in defined well order column wise to the PCR plate and include max 94 samples per 96-well plate. Use plates with transparent wells.
- Do not leave empty wells or columns in the middle of plate. Full reaction cost is invoiced for empty wells.
- If you bring e.g. two type of samples which require different sample preparation protocol, samples should be delivered in separate plates.

Additional fee

For empty wells or columns in the middle of 8-tube strips or 96-well PCR plates full library preparation cost is invoiced.

Additional fee per sample (10 €) is invoiced if samples are not provided according to the sample delivery instructions in section 5.3. This extra charge is included if

- over 10 samples are delivered in 1.5 ml tubes.
- the sample order in plate must be changed at FFGC.
- sample concentration between the samples varies more than 20% when concentration is measured at FFGC.
- service user submits afterwards replacement or extra samples if replacement is not due to poor quality

Table 3. Description of the required starting material for each application.

Application	Protocol/kit	Starting material	Total ng (library prep starting ng)	Min. volume µl*	Concentration ng/µl*
Whole exome sequencing (accredited/RUO)	Twist Bioscience comprehensive exome	Genomic DNA	500 (50)	10	20-50
Whole genome sequencing (accredited/RUO)	Illumina DNA PCR-free, standard input	Genomic DNA	700 (300)	10	>15
	Illumina DNA PCR-free, low input	Genomic DNA	60 (25)	10	>1
	Illumina DNA Prep (PCR-based method) standard input	Genomic DNA	500 (150)	10	>10
	Illumina DNA Prep (PCR-based method) low input	Genomic DNA, small genomes	10 (1-100)	10	>1
PolyA+ transcriptome sequencing (RUO)	Illumina stranded mRNA Prep	Total RNA	500 (25-100)	10	10-100
Whole transcriptome sequencing (RUO)	Illumina stranded total RNA Prep	Total RNA	500 (10-100)	10	10-100
Small RNA sequencing (RUO)	QIAseq miRNA library kit	Total RNA	300 (10-100)	10	10-100
	QIAseq miRNA library kit	Small RNA	30 (1-10)	10	10-100
16S rRNA-seq (RUO)	Illumina 16S Metagenomics	Microbial genomic DNA	12.5	10	5
Sequencing of ready-made libraries (RUO)	NovaSeq X	NGS library pool	4 nM**	25**	4-10 nM
	NovaSeq 6000	NGS library pool	4 nM**	50-150**	4-10 nM
	MiSeq i100 Plus	NGS library pool	4 nM**	20**	4-10 nM
	MiSeq	NGS library pool	2-4 nM**	20**	2-10 nM
Quality analysis (RUO)	Fragment Analyzer	Genomic DNA	-	5	0.5-12 (HS) 25-250 (STD)
	Bioanalyzer, Fragment Analyzer	Total RNA	-	5	0.5-5 (HS) 5-500 (STD)
	Bioanalyzer, Fragment Analyzer	NGS library	-	5	0.1-5 (HS) 5-50 (STD)
Plate running service for QuantStudio (RUO)		qPCR reactions on 96/384 well plate***			

*Volume and concentration must be the same for all the samples.
**The concentration and volume depend on the flow cell and application, please contact ffgc@bioscience.fi for detailed instructions.
***For plate requirements, please contact taqman@utu.fi for detailed instructions.

Sample delivery

Samples can be delivered to FFGC office in Biocity 5th floor on **Mondays 9-11** or on **Thursdays 13-15**. Please agree beforehand more precise delivery time for samples.

Samples should be sent to us well packed with enough dry ice using the courier service of your choice. We recommend you to ship the samples at the beginning of the week in order to avoid them to be left in transit over the weekend.

Delivery address:
Finnish Functional Genomics Centre
Turku Bioscience
Tykistökatu 6A, 5th floor
20520 TURKU
FINLAND

When the samples arrive to FFGC, you will be notified, and your project will be given a project number. Please, contact us, if there are questions or you need additional support with the sample delivery and containers.

5.4 Sample acceptance/rejection criteria

When samples arrive at FFGC the following things are inspected to define sample acceptance/rejection:

- Correct packaging of the sample
- Package is free from leakages
- Sample was transported under proper conditions and temperature
  - Pure DNA at room temperature or frozen
  - RNA short-term delivery (hours) in cold or long-term delivery (days) frozen, dry ice.
- In dry ice delivery, there is still dry ice left
- Sample container/tube is not broken or otherwise damaged
- Sample container/tube markings are clear and unambiguous
- Sample is of adequate volume
- Sample Submission form is adequately filled and in electronic format (excel format)
- Sample Submission form is signed electronically
- Concordance of the Sample Submission form details with the sample details

The FFGC personnel member responsible for your project will inform you whether your samples have passed the initial inspection. If the samples are rejected, you can replace them with new ones, if possible.

6 Analytical phase

After initial inspection, if the samples are accepted, they enter the analytical phase during which the samples are processed according to the SOPs established at FFGC.

6.1 QC analysis of the starting material

After initial inspection the quality and quantity of the nucleic acids or libraries will be analyzed.

The criteria for acceptance:

Ready-made libraries

- Library fragments should have compatible size distribution with selected read length and sequencing instrument according to the library preparation protocol recommendations.
- When several libraries are pooled in one run, it is important to maintain color balance for each base of the index read for optimal de-multiplexing. FFGC personnel can assist in the selection of color-balanced indexes before library preparation.

Please note that FFGC does not take any responsibility of the quality of sequencing results for ready-made libraries.

Total RNA samples

- Total RNA should be intact and pure.
- The Agilent Bioanalyzer RIN or Fragment Analyzer RQN value should be > 7.0, when applicable.
- The ratio of absorbance 260 nm/280 nm is 2.0 (+/-0.2).
- All samples should have similar quality.
- RNA that has DNA contamination will result in underestimation of the amount of RNA used. To prevent this, DNase step is recommended, but not required, to be included with the RNA isolation method.

If good quality material is not available, alternative workflows exist, and can be discussed separately. When using low quality total RNA samples (RIN value < 7.0) for library preparation, all samples should be similar and equally degraded. FFGC does not guarantee results for samples with low RNA quality.

Genomic DNA samples

- Genomic DNA should be intact and pure, and free of RNA or small nucleic acid fragments, such as nucleotides, or other contaminants.
- The ratio of absorbance 260 nm/280 nm is of 1.8 (+/-0.2)
- DNA sample should contain < 1 mM EDTA and be free of phenol, ethanol and other organic contaminants.
- DNA that has RNA contamination will result in underestimation of the amount of DNA used. To prevent this, RNase step is recommended, however, not required to be included with the DNA isolation method.

If there is not enough starting material or enough good quality material available, alternative workflows exist and can be discussed separately. However, FFGC does not guarantee results for samples with low DNA quality.

FFGC personnel will contact you about the results of the QC analysis. If samples with low quality are detected, you can replace them with new ones, if possible.

6.2 Library preparation and next-generation sequencing

After your samples have passed the QC analysis, they will be placed in the queue for library preparation, which normally is approximately 1-30 days for research use only samples. Samples for clinical diagnostics are prioritized and processed immediately.

Depending on the protocol the library preparation takes approximately 1-5 days for a set of max 94 samples. The ready-made libraries will be analyzed for quality and quantity. If the libraries do not pass the QC they will be prepared again if enough starting material is available. If the libraries pass the QC they will be placed in the queue for next-generation sequencing, which is normally 1-30 days for research use samples. The samples for clinical diagnostics are prioritized and sequenced immediately.

Depending on the application, the next-generation sequencing run takes 1-3 days. The processing and delivery of the raw data usually takes approximately one week. The FFGC personnel will inform you about the results and progress.

The library preparation is sensitive to impurities present in the starting material, presence of which is controlled by the QC analysis described in 6.1. The library preparation can also be affected by defective reagents or instruments. Regular preventive maintenance and calibration program of the instruments and pipettes is followed to minimize risk of errors. In addition, quality controlled commercial reagents are utilized in the sample preparation to ensure high quality. Human error is also possible. The standardized workflows, automated procedures, when possible, as well as internal quality controls are utilized at FFGC to minimize risk of errors and monitor quality performance of the consumables, equipment, software, and personnel.

7 Post-analytical phase

7.1 Data for research use only

The research use only data is delivered to the service users in FASTQ format through selected route of delivery:

- internet link
- Illumina EU BaseSpace hub
  - Account can be acquired/purchased from Illumina
  - email address for the account needed
  - contains up to 1 TB storage space per account

For routine service applications, the quality of the raw data is monitored and service user is informed without delay, if any deviations from the quality criteria is observed.

7.2 Project closure

After data delivery we will send you a report of the service workflow and invoice of the used services.

After data delivery, **FFGC stores the data for 3 months**. The service user has the responsibility for the long-term data storage, e.g. in data repositories maintained by the host universities, institutes, CSC or public data archives.

If you want your original DNA/RNA samples back, please inform us within 3 months. After that FFGC does not guarantee the sample storage. If DNA/RNA samples are returned using courier service, the delivery costs are covered by the service user.

8 Policy for information and data protection

All the information and data processed at FFGC is managed confidentially and by using secured local electronic data management and storage resources protected from unauthorized access.

9 Complaint procedure

In case you wish to complain about the services we kindly ask you to contact us either through phone, direct discussion or through email (ffgc@bioscience.fi). Your complaint is then processed according to our established standard operating procedure.