Allegato 1

CALL "TEST INTERLABORATORIO PER CORE FACILITIES NICo-BBMRI.it - STUDIO PILOTA"

Project PNRR IR "Strengthening BBMRI.it - Rafforzamento dell'Infrastruttura di Ricerca delle Biobanche e delle Risorse Biomolecolari in Italia"

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1. FLUORESCENT OPTICAL MICROSCOPY

Introduction

Quality proficiency tests are essential in optical microscopy Core Facilities (CFs) to guarantee the reliability, accuracy, and consistency of the instruments and acquisition services provided. These tests play a critical role in validating instrument performance and ensuring that the imaging services meet the highest standards of quality.

Interlaboratory test analyses

Here are listed the 6 types of proficiency quality tests considered necessary to ensure reliability for image acquisition services with wide-field and confocal microscopes (PMID: 36173380; PMID: 34021279; PMID: 34214188; https://quarep.org/) and that shall be performed by core facilities willing to participate in this type of interlaboratory test:

1) **Lateral and axial resolution:** measuring the microscope's lateral and axial resolution is essential for reporting size measurements of near-resolution limit objects or distances between them.
   Tests will be performed using sub-resolution fluorescent bead preparations to monitor resolution over time.
2) **Field illumination homogeneity:** the non-uniformity of illumination over the field of view may distort the images.
   Tests will be performed using fluorescent plastic slides to assess the field illumination flatness.
3) **System chromatic aberration and co-registration:** testing for system chromatic aberration is essential for correctly identifying and analyzing structures, especially in multi-channel fluorescence imaging.
   Tests will be performed using multi-coloured bead preparations to measure co-registration accuracy.
4) **Stage drift and positioning repeatability:** the performance of stage platforms and the optomechanical focus of the optical system relates to *X*, *Y*, *Z* movement, stability, reproducibility, and repeatability.
   Tests will be performed using fluorescent bead preparations to evaluate repositioning accuracy and stage drift.

5) **Detection system performance:** if the detection system is not functioning correctly, it can lead to poor image quality, with issues such as blurriness, noise, or low contrast.
   Tests will be performed for monitoring the detection system performance over time. The read noise is an inherent property of camera sensors. Thus, the measurements can be carried out without excitation light and sample.
6) **Illumination power stability and linearity:** comparison of fluorescence intensities between images requires measurements of the illumination power and stability of the excitation light source.
   Tests will be performed during both short- and long-term image acquisition sessions using a calibrated external power controller and slide sensor. The satisfaction of this last criterion is optional.

Reference material

The materials required for the execution of the tests (fluorescent beads, microscope glass slides, fluorescent glass slides, coverslips, mounting media, etc.) will be commercially purchased and provided by UniTrento.

Technical requirements and protocols

Wide-field and confocal systems compatible with the execution of the tests must be completely motorized in *X*, *Y*, and *Z* axes and thus equipped for multi-channel, multi-point, and z-stack acquisitions.
Protocols for sample preparation (if required) and image acquisition (for both wide-field and confocal systems) will be supplied for each of the six points mentioned above.
For analyzing the data obtained from the tests, detailed pipelines designed for the open-source software FIJI (PMID: 22743772) will be provided to minimize user actions and standardize the results. Consequently, basic expertise in using this software for image analysis is required.
The data collection will be standardized through the requirement to fill out a specifically created and shared table. This is to facilitate the comparison of test results between facilities and, over time, within the same facility. Regarding the data obtained from the measurements, the acceptable value ranges, referred to in the protocols as “tolerance values”, will also be indicated.

The protocols for executing and analyzing the tests were designed for specialized microscopists, such as the staff of optical microscopy core facilities. However, they can also be performed by any seasoned microscope user with a specialized interest in fluorescence light microscopy.

It will be required to perform the tests at least once a week for 1 month, both in the morning and evening, starting from the moment the reference materials are received.

2. RNA SEQUENCING (RNA-Seq)

Introduction

RNA sequencing (RNA-Seq) workflows consist of RNA purification, quality and quantity evaluation, library production, sequencing itself, and the analysis of sequenced fragments. Each of these steps can be accomplished through a large set of options in terms of kits, tools, parameters and algorithms. The participation of NGS Core Facilities in interlaboratory test analyses can indicate whether the adopted workflow meets the RNA seq analysis needs to approach a specific biological issue (PMID: 25150835).

Interlaboratory test analyses

The steps necessary for the Interlaboratory test analysis procedure, which should be carried out by core facilities that are willing to participate in this type of interlaboratory test, are outlined here:

- Quality controls on the standard sample provided.
- Library construction for 4 replicates and pool preparation for sequencing.
- Sequencing.
- Sequencing data submission for centralized analysis.

A data collection form will be provided to record the sequencing parameters, methods, instruments and kits used for qualitative or quantitative determinations performed on standard samples, libraries and pools.

Reference material

A standard sample consisting of a commercial human total RNA with a spike of synthetic RNA fragments (PMID: 16179916) will be distributed across the participants to identify the lower limit of detection and the dynamic range of the entire workflow.

Technical requirements and protocols

Core Facilities (CFs) can participate regardless of the type of quality control instruments, library construction methods, or NGS “short reads” sequencing platforms they use.

3. FLOW CYTOMETRY AND NANOPARTICLE TRACKING ANALYSIS OF EXTRACELLULAR VESICLES (EVs)

Introduction

Standardization/harmonization of flow cytometry and Nanoparticle Tracking Analysis (NTA) procedures for EV analysis is crucial for Core Facilities (CFs) to guarantee the reliability, accuracy, and consistency of the instruments and acquisition services provided. These tests play a critical role in validating instrument performance and ensuring that flow cytometry and NTA services meet the highest quality standards.

Therefore, standardized/harmonized operating procedures (SOPs) for identification, characterization and count of integer EVs for further biobanking purposes will be optimized. By sharing samples and protocols, we will improve the reproducibility of EV flow cytometry and NTA analyses across different laboratories and platforms, relying on current guidelines (PMID: 36222259; PMID: 31593368; PMID: 19546437; PMID: 36759917; PMID: 32128070).

Interlaboratory test analyses

Here the steps considered mandatory to ensure flow cytometry and NTA reliability for EV analysis services and that shall be performed by core facilities willing to participate in this type of interlaboratory test are listed:

- Standardisation of flow cytometry platforms (PMID: 29643468).
- Harmonization of procedures and EV analysis quality assessment to ensure adherence to a previously optimised protocol, allowing the flow cytometry and NTA identification and count of EVs (PMID: 33374539; PMID: 33114229).
- Application of the flow cytometry and NTA-optimized protocols across the CFs for the identification and count of intact EVs from reference plasma samples.
- Assessment of the EV limit of detection and dynamic range for each instrument (conventional, high-sensitivity and imaging flow cytometers) involved in the study (PMID: 33218198; PMID: 30765839).

Centralized data analysis using standardized templates and software to reduce inter-laboratory variability and improve the accuracy of flow cytometry and NTA measurements (PMID: 29095427; PMID: 15978127).
The final evaluation of analyzed data and establishment of standardized SOPs for identification, characterization and count of intact EVs will be shared among participants.

Reference material

The materials required for the tests (calibration and quality control beads, absolute count tubes, antibodies, dyes for EV detection and plasma samples) will be provided by UniTrento.

Technical requirements and protocols

CFs equipped with only flow cytometers, only NTA platforms or both can apply to the call. Flow cytometers or imaging flow cytometry systems compatible with the execution of the tests, equipped at least with two lasers (488 nm and 633 nm or similar) are required.

Protocols for sample preparation and instrument setting optimization (for both conventional and imaging systems, as well as for NTA analyses) will be supplied. These protocols were designed for specialized flow cytometry and NTA operators.
Consensus on standardized templates and software for data analysis and instrument calibration will be reached and analyses will be centralized to reduce the interlaboratory variability and improve the accuracy of flow cytometry measurements (PMID: 29095427; PMID: 15978127).

4. MASS-SPECTROMETRY-BASED PROTEOMIC ANALYSIS

Introduction

The current procedures for checking and evaluating the quality of proteomic data generated by the core facilities and research infrastructures are difficult because of the heterogeneous nature of methods, standards, tools, and software that are commonly used. Proteomics is based on complex methodologies, and laboratories often implement each step differently depending on the platforms and workflows in use (sample preparation, analysis, data processing).

In light of this, numerous proteomics platforms and core facility networks have raised the importance of launching initiatives to develop best practices for proteomic services and guarantee reproducibility, comparability, and data integrity across different proteomics infrastructures (PMID: 33241998, PMID: 29324744, PMID: 34009726).

Interlaboratory test

The steps necessary for the standardisation procedure, which should be carried out by core facilities that are willing to participate in this type of interlaboratory test, are outlined here:

- Equipment and expertise survey: a collection of technical information on the types of equipment and expertise of participating proteomics laboratories through the requirement to fill out a specifically created and shared table.
- Monitoring of performance and reproducibility of the nano liquid chromatography-mass spectrometry (nLC-MS) platforms.
  A commercial Hela Protein Digest Standard will provide a shared protocol for Label-Free MS data acquisition (DDA and/or DIA), allowing flexibility in the nano-LC settings to accommodate the different analytical setups across laboratories.
- Centralized data analysis:
  The anonymous data will be centrally analyzed using standardized software to reduce inter-laboratory variability.
- Evaluation, comparison, and harmonization of analyzed data, measuring intra- and inter-laboratory variability.
- Identification and mitigation of potential sources of performance drift.
- Definition of a common quality control framework within the MS-based proteomics laboratories.

- Elaboration of standardized operating procedures (SOPs), and developing shared methods for data acquisition and processing, to ensure high standards, reproducibility, and comparability in proteomics services.

Reference material

A commercial Hela Protein Digest Standard will be distributed across proteomics laboratories.

Technical requirements and protocols

For proteomics analysis, a nanoflow liquid chromatography system coupled to a high-resolution mass spectrometry (HR-MS) is mandatory.