

Jan 16, 2024

## External Quality Control for inter-Batch comparison (English)

DOI

[dx.doi.org/10.17504/protocols.io.dm6gp36ydvzp/v1](https://dx.doi.org/10.17504/protocols.io.dm6gp36ydvzp/v1)



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**Protocol Citation:** Ricardo M. Borges 2024. External Quality Control for inter-Batch comparison (English). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.dm6gp36ydvzp/v1>

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** January 15, 2024

**Last Modified:** January 16, 2024

**Protocol Integer ID:** 93561

**Keywords:** new sample of the strain code ccmr0280, strains of the ccmr, strain code ccmr0280, external quality control, term metabolomics reference material, culture collection of microorganism, strain, stock of ccmr0280, ccmr, biomass, ccmr0280, microorganism, analytical chemistry, batch

**Funders Acknowledgements:**

Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ

Grant ID: E-26/210.489/2019

Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ

Grant ID: E-26/201.260/2021

Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq

Grant ID: 304501/2021-2

## Abstract

**External Quality Control (QCExt):** The sample containing the strain code CCMR0280, cultivated in parallel with all batches, will serve as a reference for comparative studies between the strains of the CCMR (Culture Collection of Microorganisms at the Federal University of Rio de Janeiro). After drying the biomass, the new sample of the strain code CCMR0280 obtained in the current cultivation will be combined with the stock of CCMR0280-QCExt for equitable distribution of inter-batch Quality Control.

This document is based on the article: Long-Term Metabolomics Reference Material. G.J. Gouveia, A.O. Shaver, B.M. Garcia, A.M. Morse, E.C. Andersen, A.S. Edison\*, and L.M. McIntyre\*. Analytical Chemistry. 2021, 93, 26, 9193–9199. <https://doi.org/10.1021/acs.analchem.1c01294>

## Troubleshooting

## Safety warnings

- !** **Handling of Chemical Products and Biological Samples:** Ensure you are familiar with the risks associated with the chemicals used, as well as the biological samples, and follow appropriate safety guidelines. Use personal protective equipment, such as gloves, aprons, and safety glasses.
- Lyophilization:** Lyophilization involves freezing samples, which can pose risks of exposure to extremely low temperatures. Make sure to use lyophilization equipment in compliance with safety standards and avoid direct contact with cold surfaces.
- Storage:** When storing samples at -20°C, use a dedicated freezer for biological and chemical substances, ensuring clear identification of each sample. Ensure that the freezer is in good working condition and that the samples are properly sealed.
- Sample Identification:** Carefully label all samples with essential information, including batch number and production date. This is crucial to prevent confusion and ensure traceability.
- Proper Disposal:** Ensure you follow proper waste disposal procedures in accordance with local and international regulations.
- Training:** Ensure that all team members are adequately trained in the specific safety techniques and practices of this protocol.
- Emergency Procedures:** Have emergency action plans in place, including knowledge of how to deal with spills, fires, or other adverse situations.
- Risk Assessment:** Conduct specific risk assessments for this protocol and develop appropriate risk control measures.
- Documentation:** Keep detailed records of all protocol steps, including adopted safety measures, for future reference and traceability.



## Material

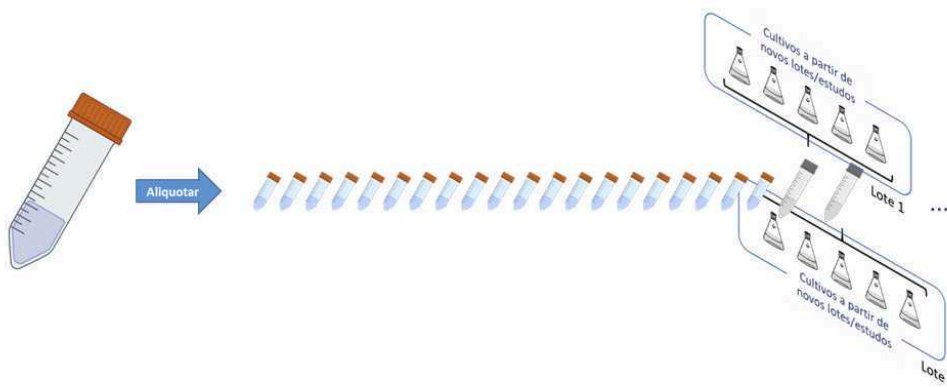
- 1
  - 2 mL microtubes with screw cap
  - 50 mL Falcon type centrifuge tube
  - Analytical balance (AUW220, Shimadzu)

## Material in Stock

- 2 Initially, a starting batch composed of 25 replicas of the cultivation of the strain code CCMR0280 was (i) cultivated, (ii) collected and combined, (iii) subjected to lyophilization, and (iv) subsequently ground into powder.
- 3 This ground sample is meticulously divided into 50, if possible, 50 mg aliquots in 2 mL microtubes with screw caps.

## For each batch to be produced

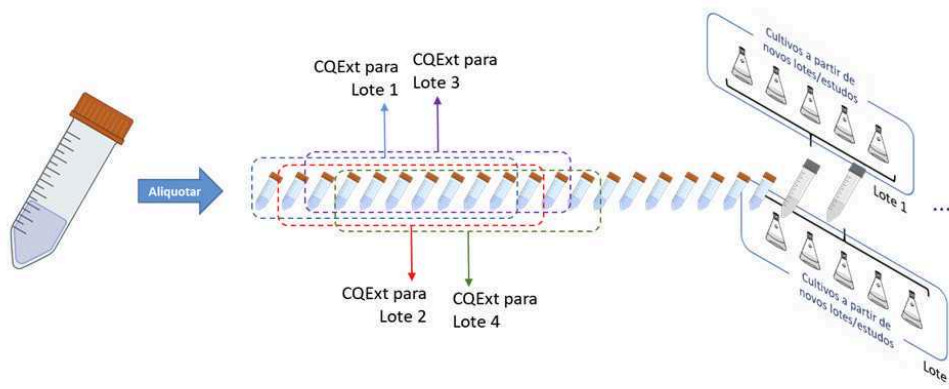
- 4
  - In each batch of cyanobacteria cultivated for the comparative study between the CCMR strains, the cultivation of the strain code CCMR0280 will be added, maintaining the same number of replicas as the study.
  - This sample will be used for intra-batch quality control and will also be part of the composition of the **CCMR0280-CQExt** sample, which, in turn, will be used for inter-batch quality control.
- 5 For each batch of strains to be cultivated, after completing the collection and lyophilization stage of the biomass, a sample of **CCMR0280-CQExt** will be added as a quality control sample.
  - The goal is to minimize variance between samples at all stages of batch analysis, facilitating the process of correction between different batches.
- 6 From the samples of the strain code CCMR0280 cultivated in each batch, after biomass collection and lyophilization, an aliquot will be combined in a 50 mL Falcon-type centrifuge tube to the equivalent amount of at least 100 mg.
- 7 This combined sample will be grounded and homogenized.
- 8 A 50 mg aliquot of this sample (of the strain code CCMR0280 cultivated in each batch) will be added to the sequence of **CCMR0280-CQExt** samples.



- The **CCMR0280-CQExt** samples will be stored in a freezer at -20°C in a properly identified box. Each new sample should be labeled with the batch number and production date.
- At this point, we will have a series of 50 mg aliquots of **CCMR0280-CQExt** samples, including those that will be produced with each new cultivation batch.

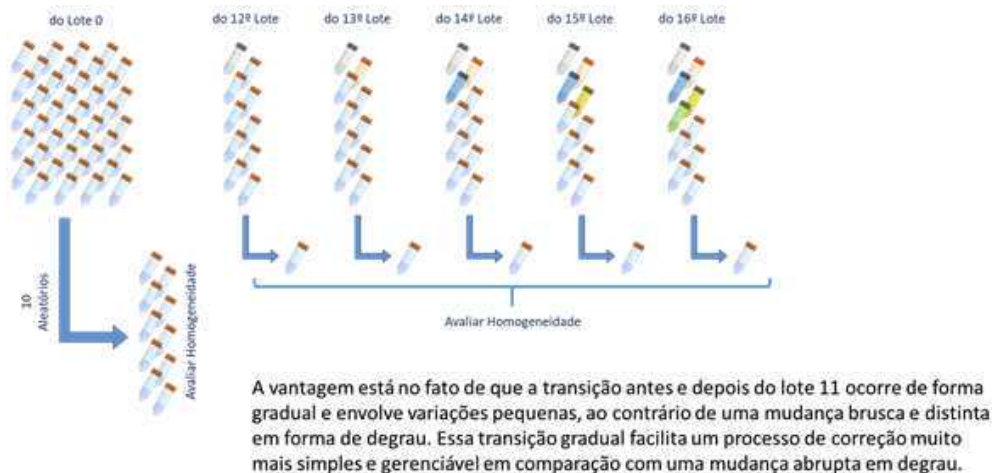
9 When the time comes to carry out the extraction and preparation stage of the samples from each batch, the first 10 **CCMR0280-CQExt** samples in the series will be (i) gathered, (ii) homogenized, and (iii) 5 representative aliquots of 50 mg will be added to the extraction sequence.

- For the first batch, samples 1-10 will be used.
- For the second batch, samples 2-11 will be used.
- For the third batch, samples 3-12 will be used.
- For the fourth batch, samples 4-13 will be used, and so on.



Esquema representativo considerando 40 alíquotas iniciais de **CCMR0280-CQExt**, onde a amostra do primeiro lote do estudo será adicionada apenas no 12º lote

- 10 The extraction protocol for the biomasses must be strictly followed with all the samples from each batch, including these 5 **CCMR0280-CQExt** samples.
- 11 All analytical data acquired from the **CCMR0280-CQExt** samples will be evaluated for their homogeneity.
  - 10 of the aliquots initially produced (step 3) will be separated and submitted to the extraction and preparation stage of the samples for the acquisition of analytical data.
  - These analytical data will be used to prove homogeneity.



Representative scheme considering 40 initial aliquots of **CCMR0280-CQExt**, where the sample from the first batch of the study will only be added in the 12th batch.