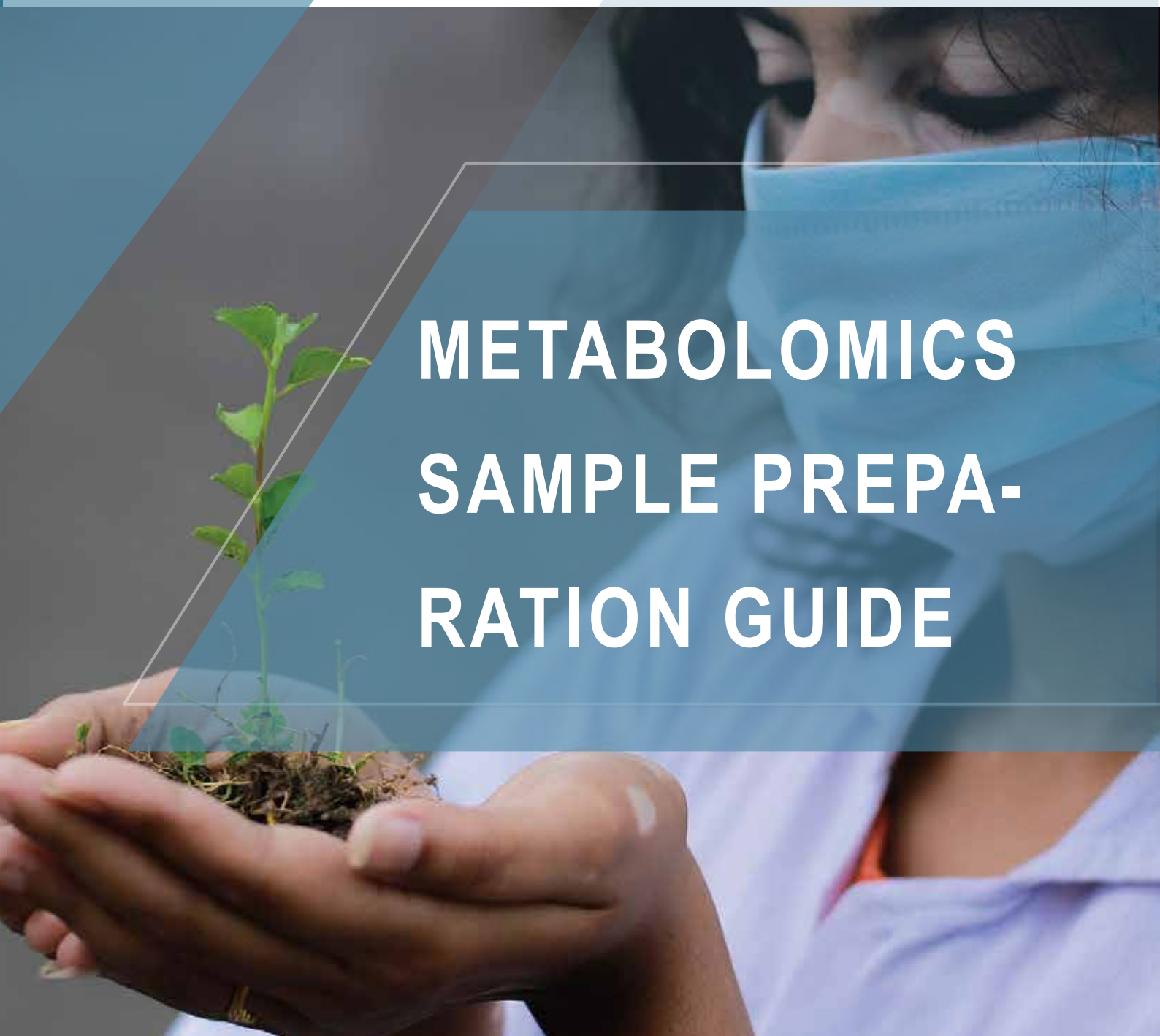
LEADER OF INNOVATIVE
METABOLOMIC AND
MULTI-OMICS STUDIES

METAVARE



METABOLOMICS SAMPLE PREPA-RATION GUIDE

Table of Contents

General principles of sample preparation

1. Representativeness

To ensure that the final results of the experiments are of scientific significance, the collected samples should pass strict phenotype analyses, which may include cytology, histology, pathology and other related analyses.

2. Precision

The tissue sampling site must be precise. One should correctly identify the tissue site to be studied and remove tissues unrelated to the research. If the conditions permit, strive to collect the samples of the experimental group and the control group as consistent as possible in terms of sampling time, location, and processing conditions. For example, the root system of plants is divided into main root and lateral root and should be precisely selected according to specific experimental purposes.

3. Avoid contamination

Use clean experimental equipment and containers. In the case of samples for RNA studies, the samples should be processed at a clean bench with the bench top and equipment for handling RNA samples wiped with RNase removal agents (such as RNaseZap from ThermoFisher). Any reagents used for RNA samples should be prepared using RNase-free water.

4. Timeliness

Sample quality is the most critical factor in the experiment and affects all subsequent results. The samples should be collected, prepared, stored, and transported as quickly as possible to minimize the time between sample collection and experimentation.

5. Keeping the sample cold

Once the sample has been isolated, any dissection steps should be processed on ice or at 4°C. Samples ready for experimentation should be snap frozen using liquid nitrogen and stored at -80°C before experimentation begins.

6. Storage

The preferred way for sample storage is to snap freeze in liquid nitrogen and store at -80°C. Pay attention to the condition of the tissue at the time of shipment and prevent thawing.

Sample preparation from fresh plant tissues

General notes on sample size

The recommended sample size is at least 0.6g and this is the size that we can perform the tests under normal conditions. The absolute minimum sample is is 0.3g and we do not recommend experimentation below this volume.

General notes on biological replicates

Biological replicates are important part of the experimental design and is required for subsequent statistical analysis. We typically recommend having at least 3 biological replicates. For metabolomics, one should mix 3-6 samples as 1 biological replicates to minimize the differences stemming from different individuals.

1. Sample preparation from leaves and stems

Sample selection: Preferentially select the young and tender parts of the plant under normal growth conditions. You may also select samples in poor states due to stress treatment experiments, however, avoid sampling tissues that have died due from long treatment or growth time. Select 3-6 individual plants with the same growth condition and combine as 1 biological replicate to minimized differences stemming from different individual.

Sample collection: Select 3-4 leaves (adjust the number according to the size) or 1-2 node stem tissues from each individual. Samples from different individuals should be collected at the same position (including same direction of light). We recommended rinsing samples with PBS or RNase-free water during collection, and then blot the surface of the sample with absorbent paper. The stalks or stem should be cut to 1-2cm lengths. Large leaves can be broken up after snap frozen with liquid nitrogen. Samples for 1 biological replicate should be mixed together.

Sample storage: Collect the samples in a suitable centrifuge tube (10ml, 15ml, or 50ml tubes) and snap freeze the samples in liquid nitrogen for 5-10 minutes. Store the samples in -80oC freezer. It is important to note NOT to use ziplock bags, tin foil, plastic wraps, or other airtight plastic bags. These packaging break easily in liquid nitrogen or dry ice.

Sample delivery: Make an appointment to ship the samples with dry ice. Depending on shipping time, we generally recommend having 5kg of dry ice per day. For example, if the shipment will take three days, we recommend having a minimum of 15kg of dry ice along with your samples.

Sample backup: We recommend that you have 1-2 backup samples. Should the experimentation require additional samples, these can be used without additional sample preparation.

Below are a few references that have collected leaf or node samples:

[1] Guo Q et al. Transcription-associated metabolomic adjustments in maize occur during

combined drought and cold stress. Plant Physiology, 2021. (Maize leaves)
[2] Fan K et al. Metabolomic and transcriptional analyses reveal the mechanism of C, N allocation from source leaf to flower in tea plant (Camellia sinensis. L). Journal of Plant Physiology, 2019, 232:200-208. (Tea leaves)
[3] Chen Z et al. Integrating transcriptomic and metabolomic analysis of hormone pathways in Acer rubrum during developmental leaf senescence. 2020. (American Red Maple)
[4] Rienzo J et al. Integrating transcriptomic and metabolomic analysis to understand natural leaf senescence in sunflower. Plant Biotechnology Journal, 2015, 14(2):1-16. (Sunflower leaves)

2. Sample preparation from root tissues

Sample selection: Preferentially select the young and tender parts of the plant under normal growth conditions. You may also select samples in poor states due to stress treatment experiments, however, avoid sampling tissues that have died due from long treatment or growth time. Select 3-6 individual plants with the same growth condition and combine as 1 biological replicate to minimized differences stemming from different individual.

Sample collection: Wash the root with under running water after removing the plant from the soil. After that, wash the root with PBS or RNase-free water 2 times. Cut the desired root tissue with a scalpel and blot any excess liquid with paper. One should distinguish whether it is the main root or lateral root is needed for experimentation. For root tuber samples (such as potatoes, sweet potatoes, taro), please cut into 1-2cm blocks.

Sample storage: Collect the samples in a suitable centrifuge tube (10ml, 15ml, or 50ml tubes) and snap freeze the samples in liquid nitrogen for 5-10 minutes. Store the samples in -80^{o}C freezer. It is important to note NOT to use ziplock bags, tin foil, plastic wraps, or other airtight plastic bags. These packaging break easily in liquid nitrogen or dry ice.

Sample delivery: Make an appointment to ship the samples with dry ice. Depending on shipping time, we generally recommend having 5kg of dry ice per day. For example, if the shipment will take three days, we recommend having a minimum of 15kg of dry ice along with your samples.

Sample backup: We recommend that you have 1-2 backup samples. Should the experimentation require additional samples, these can be used without additional sample preparation.

Below are a few references that have collected root tissues:

[1] Liheng He et al. Transcriptomic and targeted metabolomic analysis identifies genes and metabolites involved in anthocyanin accumulation in tuberous roots of sweet potato (Ipomoea batatas L.), Plant Physiology and Biochemistry, Volume 156, 2020. (Sweet potato)

[2] Susann M et al. Plant-to-Plant Variability in Root Metabolite Profiles of 19 Arabidopsis thaliana Accessions Is Substance-Class-Dependent. International Journal of Molecular Sciences, 2016, 17(9). (Arabidospsis root system)

[3] Yasunori I et al. Transcriptomic and Metabolomic Reprogramming from Roots to Haustoria in the Parasitic Plant, Thesium chinense. Plant & Cell Physiology, 2018(4):729-738. (Thyme root system)

3. Sample preparation from fruit tissues

Sample selection: Preferentially select the young and tender parts of the plant under normal growth conditions. You may also select samples in poor states due to stress treatment experiments, however, avoid sampling tissues that have died due from long treatment or growth time. Select 3-6 individual plants with the same growth condition and combine as 1 biological replicate to minimized differences stemming from different individual.

Sample collection: Select 1-2 fruits from an individual with the same growth condition and position (including direction of light). If the fruit is from a fruit tree and there isn't many fruit trees to choose from, you may choose and mix different fruits from the same tree as a biological replicate. Use a scalpel to dissect the desired portion for experimentation. If you intend to study the skin, please be sure dissect with as little flesh as possible. This is especially important if the fruit exhibit different color between the skin and flesh. If you intend to study the flesh, dissect the flesh to 1-2cm blocks after removing the skin. If you do not intend to study the flesh with the core or seeds, be sure to remove the core or seeds from the flesh as much as possible. If the fruit of interest has excessive juice (such as tomato, orange, passion fruit), we recommend sampling and dissecting the entire fruit into 1-2cm blocks. Please do not snap freeze the fruit as a whole.

Sample storage: Collect the samples in a suitable centrifuge tube (10ml, 15ml, or 50ml tubes) and snap freeze the samples in liquid nitrogen for 5-10 minutes. Store the samples in -80^{o}C freezer. It is important to note NOT to use ziplock bags, tin foil, plastic wraps, or other airtight plastic bags. These packaging break easily in liquid nitrogen or dry ice.

Sample delivery: Make an appointment to ship the samples with dry ice. Depending on shipping time, we generally recommend having 5kg of dry ice per day. For example, if the shipment will take three days, we recommend having a minimum of 15kg of dry ice along with your samples.

Sample backup: We recommend that you have 1-2 backup samples. Should the experimentation require additional samples, these can be used without additional sample preparation.

Below are a few references that have collected fruit tissues:

[1] Qiu W et al. Combined Analysis of Transcriptome and Metabolome Reveals the Potential Mechanism of Coloration and Fruit Quality in Yellow and Purple Passiflora edulis Sims. Journal of agricultural and food chemistry, 68(43): 12096-12106. (Passion fruit skin).

[2] Zhang J et al. A Comparative Metabolomics Study of Flavonoids in Radish with Different Skin and Flesh Colors (Raphanus sativus L.). Journal of Agricultural and Food Chemistry, 2020, 68(49): 14463-14470. (Radish skin and flesh)

[3] Xiong Y et al. Nutritional component analyses of kiwifruit in different development stages by metabolomic and transcriptomic approaches. Journal of the Science of Food and Agriculture, 2020, 100. (Kiwi exocarp tissue)

[4] Wan L et al. Transcriptome and metabolome reveal redirection of flavonoids in a white testa

peanut mutant. 2019. (Peanut seed coat)
[5] Zou S, Wu J, Shahid M Q, et al. Identification of key taste components in loquat using widely targeted metabolomics[J]. Food Chemistry, 2020, 323. (Loquat fruit)
[6] Zhu C, Lu Q, Zhou X, et al. Metabolic variations of organic acids, amino acids, fatty acids and aroma compounds in the pulp of different pummelo varieties[J]. LWT- Food Science and Technology, 2020, 130 :109445. (Grapefruit tissues)

4. Sample preparation from flower tissues

Sample selection: Preferentially select the young and tender parts of the plant under normal growth conditions. You may also select samples in poor states due to stress treatment experiments, however, avoid sampling tissues that have died due from long treatment or growth time. Select 3-6 individual plants with the same growth condition and combine as 1 biological replicate to minimized differences stemming from different individual. Please choose the appropriate flowering period (bud, first opening, blooming period) according to your study design.

Sample collection: Mix 5-6 petals from different plants as 1 biological replicate. Flower petals has higher water content and it is best to collect at least twice the required amount.

Sample storage: Collect the samples in a suitable centrifuge tube (10ml, 15ml, or 50ml tubes) and snap freeze the samples in liquid nitrogen for 5-10 minutes. Store the samples in -80^{o}C freezer. It is important to note NOT to use ziplock bags, tin foil, plastic wraps, or other airtight plastic bags. These packaging break easily in liquid nitrogen or dry ice.

Sample delivery: Make an appointment to ship the samples with dry ice. Depending on shipping time, we generally recommend having 5kg of dry ice per day. For example, if the shipment will take three days, we recommend having a minimum of 15kg of dry ice along with your samples.

Sample backup: We recommend that you have 1-2 backup samples. Should the experimentation require additional samples, these can be used without additional sample preparation.

Below are a few references that have collected flower tissues:

[1] Zhao H et al. PaACL silencing accelerates flower senescence and changes the proteome to maintain metabolic homeostasis in Petunia hybrida. Journal of Experimental Botany, 2020(16):16. (Petunia petals)

[2] Chen J et al. Integrated metabolomics and transcriptome analysis on flavonoid biosynthesis in safflower (Carthamus tinctorius L.) under MeJA treatment[J]. BMC Plant Biology, 2020, 20(1). (Safflower petals)

[3] Liu Y et al. Gene silencing of BnaA09.ZEP and BnaC09.ZEP confers orange color in Brassica napus flowers[J]. The Plant Journal, 2020. (Rapeseed flower petals)

5. Sample preparation from algal samples

Sample selection: Use algae samples grown in liquid cultures.

Growth cycle confirmation: Measure the OD reading with a spectrophotometer and select the optimal growth period. You may also use a cell counter to measure the cell concentration. We generally recommend selecting samples when it has reached 1×10^6-1×10^7 cells per 1ml;

Sample collection: Centrifuge the liquid culture in appropriate centrifuge tubes at 8000g in 4^{o}C for 5-10 minutes and remove the supernatant.

Sample washing: Add 2-3X volume of pre-cooled deionized water and shake vigorously to dislodge the pellet. Centrifuge as before and remove supernatant. Repeat the wash step 2 more time for a total of 3 times.

Sample storage: Weigh out 0.6g of sample for each biological replicate and store in an appropriate centrifuge tube. After snap freezing in liquid nitrogen, store the sample in -80^{o}C.

Sample delivery: Make an appointment to ship the samples with dry ice. Depending on shipping time, we generally recommend having 5kg of dry ice per day. For example, if the shipment will take three days, we recommend having a minimum of 15kg of dry ice along with your samples.

Sample backup: We recommend that you have 1-2 backup samples. Should the experimentation require additional samples, these can be used without additional sample preparation.

Below are a few references that have collected algal samples:

[1] Mangal V et al. An untargeted metabolomic approach for the putative characterization of metabolites from Scenedesmus obliquus in response to cadmium stress. Environmental Pollution, 2020:115123.

[2] Bromke MA et al. Metabolomic Profiling of 13 Diatom Cultures and Their Adaptation to Nitrate-Limited Growth Conditions. Plos One, 2015, 10(10):e0138965.

[3] Subramanian V et al. FDX5 deletion affects metabolism of algae during the different phases of S-deprivation. Plant Physiology, 2019, 181(2):pp.00457.2019.

6. Sample preparation from macrofungi

Sample selection: Select cultured or filed samples at different growth cycles. Mix 6 fungi samples as 1 biological replicate.

Sample collection: Wash the collected fungal samples with PBS or RNase-free water to remove any soil or contaminating materials. Dissect different parts of the fungi based on your study design (such as caps, scales, stripes, gills, rings, stalks, hyphae). Cut the fungi along the vertical axis into 1-2cm blocks.

Sample storage: Weigh 0.6g from the mixed samples and place in appropriate centrifuge tubes (10ml or 15ml). Snap freeze in liquid nitrogen for 15 minutes and store in -80^{o}C.

Sample delivery: Make an appointment to ship the samples with dry ice. Depending on shipping time, we generally recommend having 5kg of dry ice per day. For example, if the shipment will take three days, we recommend having a minimum of 15kg of dry ice along with your samples.

Sample backup: We recommend that you have 1-2 backup samples. Should the experimentation require additional samples, these can be used without additional sample preparation.

Below are a few references that have collected fungal samples:

[1] Caboni P et al. Multi-platform metabolomic approach to discriminate ripening markers of black truffles (Tuber melanosporum). Food Chemistry, 2020, 319:126573.

[2] Wang J et al. Quantitative proteomic and metabolomic analysis of Dictyophora indusiata fruiting bodies during post-harvest morphological development. Food Chemistry, 2020, 339.

7. Sample preparation from callus tissues

Sample selection: Select samples from different areas of the cultured medium under the same growth conditions. Mix 3-6 samples as 1 biological replicate. The sample can also come from different containers.

Sample collection: Take out the callus from the culture medium and remove any browning tissues or parts that have formed into leaves, stems, or other tissues.

Sample storage: Weigh out 0.6g of sample as 1 biological replicate and place in an appropriate centrifuge tube (5ml or 10ml). Snap freeze the sample in liquid nitrogen for 15 minutes and store in -80^{o}C.

Sample delivery: Make an appointment to ship the samples with dry ice. Depending on shipping time, we generally recommend having 5kg of dry ice per day. For example, if the shipment will take three days, we recommend having a minimum of 15kg of dry ice along with your samples.

Sample backup: We recommend that you have 1-2 backup samples. Should the experimentation require additional samples, these can be used without additional sample preparation.

Below are a few references that have collected callus tissues**:**

[1] Kumari A et al. Metabolomic homeostasis shifts after callus formation and shoot regeneration in tomato. Plos One, 2017, 12(5).

[2]Jing SA et al. Integrated metabolomic and transcriptomic strategies to understand the effects of dark stress on tea callus flavonoid biosynthesis. Plant Physiology and Biochemistry, 2020, 155:549-559.

8. Sample preparation from dried plant tissues

Sample selection: Select 3-6 plants (or seeds) with the same normal growth development as 1 biological replicate to minimize differences stemming from different individuals.

Sample processing: Process the samples under preset conditions (e.g. heat-dry, sun-dry, shade-dry). You may compare samples treated under different conditions for many traditional herbal medicine. The samples should aim to achieve water-loss of 90%-95% for subsequent experimentation. Samples not completely dry will require additional freeze-drying process.

Sample storage and shipment: Weigh 200mg of dried samples into appropriate centrifuge tubes (5ml or 15ml). You may store and ship the samples under room temperature. For samples underwent freeze-drying process or samples stored in seed storage units require shipment with dry ice. Depending on shipping time, we generally recommend having 5kg of dry ice per day. For example, if the shipment will take three days, we recommend having a minimum of 15kg of dry ice along with your samples.

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