METABOLOMICS INSTRUCTIONS FORM

Before filling out the Excel template please read the following instructions carefully:

1. Sample types and requirements for metabolomics

For cells: we need 500 µg protein equivalent per sample. Best is to send us cell pellets (stored and shipped at -80°C). Please realize that culturing has a large influence on the metabolome! When culturing take the following into account:

- For groups of samples that are to be compared to each other by metabolomics: **KEEP EVERYTHING PATHOLOGICALLY THE SAME**. For example: we have seen beautiful but meaningless differences because of different media! So:
    1. Culture in the same stove by the same person
    2. Culture with exactly the same medium/FCS etc.
    3. Try to harvest all cells on the same day by the same person.

We advise to harvest stationary/confluent cells (less variation). For fibroblasts, for instance, we use confluent cells that are left confluent for 4-7 days and then harvested (by trypsinization). Again, the most important aspect is to treat all the samples *in the same way*. Washing cells: generally, we wash twice with phosphate buffered saline and then a final wash with 0.9% NaCl (to remove phosphate). Alternatively, you can send us live cells and we can culture the cells for you (this should be discussed before sending cells), but this will result in additional costs that obviously depends on the number of samples.

For tissues: We use snap-frozen tissue (stored and shipped at -80°C). We need at least 10-20 mg of "wet" tissue. We will freeze dry the tissue, weight ~2 mg and use this for normalization purposes. Alternatively, we can receive homogenates and use a provided protein concentration (see below) or wet/dry weight for normalization.

For plasma: we need a minimum of 30 µl per sample, 50-100 µl preferred.

Other sample types: should be discussed with a representative of the Core Facility Metabolomics.

2. Samples per group

This is important. Metabolomics is a semi-quantitative technique suited for comparative discovery of biomarkers or biological effects between 2 or more groups. We therefore **cannot** have *less than 3* samples per group as this complicates the bioinformatics processing and statistical analysis. Depending on the situation we can refuse analysis of sample sets that do not meet these specifications or require an additional charge for the extra time spend on bioinformatics processing. How many samples per group is suitable depends on (not exhaustive): 1. the experimental question, 2. the amount of difference expected between the groups and 3. the type of samples and experimental setup (see below). This can be discussed with a representative of the Core Facility Metabolomics, preferably **before** sample collection. Our advice for the most frequent sample types/matrices:

- For isogenic or well controlled experimental setups (same cells treated +/- treatment, isogenic models (worm, yeast, mice), cultured cells of controls and patients) we advise n=6 samples per group (more is better, more than 10 is usually superfluous).
- For more heterogeneous samples (plasma/CSF/cells of different controls and different patients, e.g., clinical samples) we advise at least n=20 per group (more is better).

We do advise to –if possible– keep the total amount of samples below 50-100. For sample sets with more samples special arrangements needs to be made and a longer turn-around-time should be expected.

3. Shipment of samples

Please use our address label and click here for practical information.
Usually, samples are shipped on dry ice by express courier. Please inform us when samples have been shipped (with tracking information) at metabolomics@amsterdamumc.nl.

IMPORTANT: For animal material or animal by products please fill the EU Animal by-products commercial document (for EU) or NL vormvrij handelsdocument (NL only) and print this form in quadruplicate. Add an original signed form to the package, a copy for consignor, a copy for transporter and a copy for biosafety officer. Print the following two labels (DBP_cat1 and UN3373) and put this on the outside of the package. For genetically modified organisms (GMO) replace label UN3373 for the UN3245 label. The different forms can be found here.
Make sure that the material is shipped in a closed and leak free package.

4. Data and publication

If the data is published, we will provide reference(s) or materials and methods for the measurement(s) in questions. As a core facility we do ask the user to acknowledge the use of the core facility in any publication regarding the work we have done for you. We suggest the following phrase: "We thank the Core Facility Metabolomics of the Amsterdam UMC, location AMC, Amsterdam, the Netherlands for performing the metabolomics/lipidomics/…". We certainly can assist in the interpretation of the data and contribute to a publication and co-authorship(s) can be discussed depending on our contributions. If you publish the data, we produced we would appreciate to be notified about this (PMID number, PDF of the publication).

[see next page for Excel template instructions]

Fill out the Excel template according to the following instructions:

Project name and required information USE OUR METABOLOMICS_LIPIDOMICS TEMPLATE (tab1)

Projectnumber (will be provided by us)
Project name
Total number of samples
Date when samples are expected to ship
Requested analysis
Email contact (researcher)
Email responsible researcher (PI, if applicable)
Address of researcher/PI

We will provide you with a project number that should be included in all communication and will be used by our financial department as a reference.

Financial information USE OUR METABOLOMICS_LIPIDOMICS TEMPLATE (tab1)

To avoid unnecessary work, we need –**before processing any samples**– all the required information for smooth financial processing:

Email for financial department/officer
Address for invoice
Any additional financial information required (internal cost reference, order number etc.)

Background / goal of the study USE OUR METABOLOMICS_LIPIDOMICS TEMPLATE (tab1)

It is very important for correct and efficient processing of samples in addition to getting the best results/interpretation possible that you describe the background and the goal of the study. If you want, we can discuss the experiment by online meeting to evaluate the potential of our techniques to your samples and their suitability. Also see below for samples per group, naming, etc.
The description of you study should be a short and concise paragraph concerning the background/goal of the study that also includes, if available, 1. expected results (if that can be projected), 2. classes of molecules or the part of metabolism that are/is of particular interest 3. a short description of the different groups that are to be compared (what is/does the treatment, what is the gene that is deficient, etc.). If the study design and goal of the experiment is clear this maximizes our efficiency in extracting relevant information and getting it right the first time. This prevents delay in reporting because of reiterations of the data analysis process.

Statistical setup / data design USE OUR METABOLOMICS_LIPIDOMICS TEMPLATE (tab1)

As part of the metabolomics analysis, we perform group comparisons and other statistical analyses on your data using a standardized bioinformatics pipeline. Our statistical analysis uses unpaired t-tests and VIP scores from the PLS-DA model but these **may not be suitable for your data**. We generate this statistical information using the normalized metabolomics data and do not take into account covariates that have been (independently) measured in your study. This statistical information is only provided **as a tool** to facilitate the interpretation of your data. For the bioinformatics processing of the data, we want to know what type of experimental setup applies to your study, you can indicate this in the provided template and choose from:

- Unpaired design
- Simple paired design (e.g., before/after treatment in the same/corresponding cell line/patient)
- Complex paired design (e.g., longitudinal data with more than 2 repeated measures, crossover design)
- Other, namely: ...

Please also indicate if you are using technical replicates as opposed to independent samples within groups (and make sure that this is reflected clearly in the Sample Name). This obviously affects the statistical analysis of the data. Depending on your experimental design we may accommodate simple modifications to the statistical processing but for more complex statistical analyses you will have to consult a statistician yourself.

Sample, group naming and required information: USE OUR METABOLOMICS_LIPIDOMICS TEMPLATE (tab2)

Please fill out the sample information in the Excel template according to the instructions below (it contains the example data which you can delete):

- **Sample ID:** samples should be clearly and unambiguously labeled with the Sample ID. The Sample ID is numbered from 001 to x number of samples. Please use chemical-resistant (preferably printed) stickers and also label the top of the tube with this Sample ID.
- **Sample Name:** A Sample Name (unique within data set) should be provided that can contain up to 15 characters but should be as short as possible, only contain letters and numbers, start with a letter, and not contain specials characters except for the minus "-" sign.
- **Group Name:** groups of samples should be defined and named similar to samples: as short as possible (<15 characters) should contain only letters and numbers, start with a letter and not contain special characters, except for the minus "-" sign.
- **Matrix:** type of sample; fibroblast, plasma, type of tissue, cells etc.
- **Normalization:** depending on the matrix *and what has been discussed with our core facility representative*, additional information can be provided. If samples are homogenates, protein concentration can be listed here, if samples are tissues, dry/wet weight can be listed here.
- **Unit:** Please provide the unit, if this applies.
- **Sample info:** Any sample information that you think is relevant for the processing of your project.

Structure of the Excel file and examples:

Sample ID	Sample Name	Group Name	Matrix	Normalization	Unit	Sample info
001	S1-min	Control	Plasma	10.3	mg/ml	Freeze dried
002	S1-plus	Treated	3T3 cells	11	mg	In 500 ul methanol
003	KO1-min	KO	Heart	125x10^6	cells	Pellet

Statistical comparison / reporting USE OUR METABOLOMICS_LIPIDOMICS TEMPLATE (tab3)

Our data analysis pipeline uses automated comparison/reporting of the different groups. It is important that you understand how to indicate this properly in the comparison matrix below:

		Group-01	Group-02
	Group name	**Controls**	**Patients**
Group-01	**Controls**		1 (A)
Group-02	**Patients**	1 (B)	

We compare column groups with row groups. You have to carefully fill out the table to get your desired comparisons. In the example table above, Controls are compared with Patients (A) and Patients are compared to Controls (B). This is not the same. If you compare Controls (row) with Patients (column) as in (A), Controls will be the reference to which the Patients will be compared (Controls vs Patients). So, if Patients have higher levels of a particular metabolite X, this will be seen as an elevation in Patients as compared to Controls. If you compare Patients (row) with Controls (column) as in (B), the Patients will be compared to Controls and metabolite X will decrease because it is high in Patients and low in Controls. If this is not properly filled out, re-processing of the statistics pipeline will be needed resulting in additional costs and report delays. You only have to fill out the group names in column B, the row 6 group names will be filled automatically.

- Rename the Excel file according to YYYYMMDD-projectname: So “20200801-XXXKOcomparison”. Please send the filled-out Excel template file to metabolomics@amsterdamumc.nl
- If you have any questions, please mail us on metabolomics@amsterdamumc.nl