University of Glasgow | College of Medical, Veterinary & Life Sciences

Glasgow Polyomics

Metabolomics Service Protocol

Experimental design

1 Aims

To provide some guidance on the kinds of controls and metadata required for a range of metabolomics investigations. This list is not exhaustive, so please get in touch if your experimental design isn't covered below or if you have any questions about experimental design.

- ⇨ A **positive control** is a sample where the variable you wish to measure will definitely occur. Examples include a sample of a processed drug, a cell line with a known effect, a group of people with a known condition etc.
- ⇨ A **negative control** is a sample where the effect will definitely not occur. Examples include a sham operation on an animal, a vehicle control of an RNAi, a DMSO treated time course etc.

- All experimental setups are different so please think carefully about your own, individual needs from the data and controls that would help to rule out confounding factors.

2 Treatment versus control

- ⇨ Treatment with an external factor, such as a **drug** or a **different food source**, usually requires a control with that factor in extraction solvent. This control is required to determine whether signals are from the factor itself or from a biological reaction to the factor.
- ⇨ If you are treating with an external factor, you may wish to ensure that the external factor isn't **degrading or metabolising over time**, in which case you will need a control of the external factor in medium throughout your time course. This will account for drug degradation or metabolism in the absence of your organism of interest and mass spectrometry artefacts.
- ⇨ If you wish to perform a knockdown, overexpression or knockout of a gene of interest, you will need to include **vehicle controls**. Vehicle controls ensure any metabolic changes are due to the effects on the gene, rather than the effects of the vector or antibiotic used for induction. A **vehicle control** may also be the solvent that a drug is dissolved in, such as **DMSO**.
- ⇨ If a **time course** is performed, you will need to include controls to assess fluctuations in metabolites over the cell cycle. Controls in time courses can also account for cell density changes over time.
- ⇨ If you are comparing **disease** to **healthy** samples or trying to work out the **mode of action** of a drug, make sure to add a positive control so that effects specific to that disease can be monitored. The choice of positive control is very difficult under these circumstances, as ideally the positive control should have a known closely related effect. One example might be LPS to induce the effects of inflammation.

3 Biomarker discovery

- ⇨ Human studies generally require many more **replicates** than in bred animal studies which in turn require many more than cell culture studies. Please get in touch if you need advice on how many replicates is enough for your experiment.
- ⇨ Collect as much **metadata** as you can on your samples to correct for confounding factors. Common metadata are:
    - Age
    - Sex
    - Diet
    - Time of sample collection
    - Location of sample collection
    - Time from sample collection to freezing
    - Co-morbidities
    - Who collected the sample
    - Who extracted the sample
    - Method of sample collection
    - Method of sample extraction
    - What plasticware was used for sample processing
    - Socioeconomic status
    - Cell number
    - Protein content
    - Cell size
- ⇨ It is always better to **control for these confounding factors** than to correct for them. Try to use the same plasticware for all samples and the same extraction methods.
- ⇨ Some biofluids (such as urine) are much more difficult to analyse, so it's worth considering carefully **which biofluid** will be most useful to you.

4 Correlations

- ⇨ Correlations between a score of some kind (e.g. disease level, drug concentration, age, time etc) require good **metadata** to rule out confounders and dependent variables (see above).
- ⇨ It may be that this type of study requires a different kind of **randomisation** (e.g. randomising in blocks) during sample run so please let us know if you think this is the case.

5 Detecting anomalies

- ⇨ Analysing a product to see if it is beyond a "normal" range (e.g. to detect counterfeit whiskies) will require a broad range of **"normal" samples** from different batches and stored in different ways. Please speak to the Polyomics team if you need help deciding how many "normal" samples you require.

6 Profiling what metabolites are present

- ⇨ Experiments where you wish to determine what metabolites are present in a given sample are usually compared to blank extraction solvent.
- ⇨ It is important that he **blank sample is treated in the same way as the sample**. Use the same tubes and pipette the same number of times to reduce the risk of plasticisers or other contaminants entering the sample but not the blank.
- ⇨ This type of analysis suffers from a lot of **noise** so care must be taken when interpreting the results.

7 Metabolite degradation

- ⇨ This type of analysis is often done to assess the stability of drugs or other metabolites in solution.
- ⇨ This analysis will be similar to a time course experiment in that you should have **untreated (or vehicle treated), time matched samples as a control.**
- ⇨ Metabolite degradation is dependent on the conditions, so be careful to **record variables** such as temperature, pH, time and matrix content.

8 Heavy isotope tracking

- ⇨ People usually want to track a heavy precursor metabolite through a pathway to **interrogate new pathways** or to determine what the **source of a metabolite** is.
- ⇨ It is useful to provide a **control of your heavy label** so that we can ensure that any labelling we see is produced by the cells and not a consequence of contamination of the precursor label.
- ⇨ **Standards** of metabolites in your pathway of interest will also be useful to provide more evidence of a metabolite's identification. These do not need to be isotopically labelled.

9 Working with animals

- ⇨ If anaesthetics are used during the sampling process, then ensure that each sample is treated the same (e.g. perform a **sham treatment** on your control group).
- ⇨ You should also consider providing a **control of the anaesthetic** to determine which metabolites result from the anaesthetic itself.