Metagenomics Service Guidance

Scout & Pioneer

We have prepared this document to help you decide if our metagenomics services are the right fit for you and to offer some guidance if you aren't sure about things like DNA extraction or analysis.

1. Which service is right for me?

- Here is a broad description of these services (for application guidance see our Service Description):
  - **Scout** - minimum yield 0.5 gigabases (or 1 million paired-end reads 2x250bp)
  - **Pioneer** - minimum yield 1.0 gigabases (or 2 million paired-end reads 2x250bp)
- When you have decided go to question 2

2. What type of sample do you have?

- Strain (go to question 3)
- DNA (go to question 4)

3. Sorry! At the moment we don't do extractions but this is something we plan to offer in the future.

- If you would like to see if we could do some R&D DNA extractions on your sample go to question 11
- If you would like some guidance on DNA extractions go to question 6

4. Have you already performed the DNA extraction?

- No (go to question 5)
- Yes! (go to question 7)

5. Do you know how you are going to do the extractions?

- No - (go to question 6) for some DNA extraction tips and guidance
- Yes! - (go to question 7)

6. DNA extraction tips and guidance (When you are happy go to question 7)

- We don't point to one kit or protocol, this is too dependent on your samples. However here are some considerations :
    - Physical lysis (e.g. bead beating). We recommend to include a bead beating or grinding step in your extraction, as many microorganisms require physical lysis steps in order to break the cell wall and release the DNA, particularly:
        - Spore forming bacteria
        - Fungi
        - Gram positive bacteria
    - Enrichment - Samples derived directly from a host often have a problems with the host DNA being too abundant, meaning that very little microbiome DNA is sequenced
        - If you anticipate a lot of host DNA in your sample this service might not be for you (unless you can enrich the microbiome DNA).
        - For pathogen enrichment, we recommend using physical methods that act on the sample to separate the microbiome from host cells rather than enzymatic kits. For example, see Marotz *et al*. Microbiome (2018)[1]
        - Environmental contaminants. Certain samples contain abundant contaminants (e.g soil samples with humic substances) that can inhibit enzymatic reactions (PCR, ligation)
        - If you are extracting from 'dirty' samples we recommend that you use an extraction method that has a inhibitor removal step
    - Negative controls
        - Sequencing negative controls can help you to account for background noise/contamination in reagents etc. To obtain robust results, metagenomics projects should include **extraction** negative controls of each lot number of the extraction kits used

[1] Marotz, C.A., Sanders, J.G., Zuniga, C. *et al.* Improving saliva shotgun metagenomics by chemical host DNA depletion. Microbiome **6**, 42 (2018). https://doi.org/10.1186/s40168-018-0426-3

as one of your samples (see Salter *et al*. (2014)[2] for a good demonstration of the effects of reagent contamination and the need of negative controls).

- We always include an internal **library preparation** negative controls in our batches to detect any potential reagent contamination during sample processing in our laboratories

7. Do you have someone with bioinformatics expertise that can perform the analysis? Do you feel comfortable doing the analysis?

- No, bioinformatics is scary!
  - That's no problem! As well as commonly used bioinformatics files we also give tables of the taxonomic abundances for each sample. These tables have been filtered to two different levels so that you have (a) one set where you can be really sure that there are few false positives but you might miss a few low abundance real taxonomies, and (b) the other set which might include false positives but will also include all of your real hits. (For a full description see our Metagenomics Service Description document.)
  - We do not offer interpretation, just the analysis but we hope that we have given you enough to be able to do your analyses/comparisons. (go to question 8)
- Yes
  - Welcome fellow data munger! We will give these files: fastqs, raw and filtered taxonomic distributions (sensitive and specific) and some basic visualisations (For a full description see our Metagenomics Service Description document.) (go to question 8)

8. Do you have any other questions?

- No! (go to question 9)
- Yes (go to question 10)

9. Great!

- It sounds like you are ready to decide if you want to embark on a project with us. Go here to fill out a quote form!

[2] Salter, S.J., Cox, M.J., Turek, E.M. *et al*. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol **12**, 87 (2014). https://doi.org/10.1186/s12915-014-0087-z

10. We are always looking for feedback and to improve our documentation.

- Please contact us on info@microbesng.com, someone will happily help you and you never know, maybe your question will appear in the next version of this documentation!

11. Would you consider using my sample for testing DNA extraction methods?

- We are always looking for opportunities to improve our services, and we are evaluating extraction methods for microbiome samples.
- If you would like to contribute to our extraction R&D with one of your samples please contact us on info@microbesng.com with subject "Metagenomics extraction R&D", and a description of the type of sample in the body of the email, and we will get back to you.