Biobank of human stool samples – sample processing

Material:

- Stomacher® 400 Classic Bags- Standard Bag Irradiated Sterile; VWR (BA6041/5)
- BD GasPak™ EZ Anaerobe Pouch System with indicator; Becton Dickinson (260683)
- Probengefäße White 1000 ml 130 mm(80), VWR (216-4278)
- Nitril Gloves
- Stool DNA Stabilizer; invitek molecular (1038111100)
- Eppendorf tubes 1.5 mL and 2 mL
- Water for molecular biology, DEPC-treated and filter-sterilised
- FMT medium (see separate protocol)
- 0.20-µm sterile syringe filter; Corning inc. (431219)
- 1 mL syringes
- 50 mL Falcon tubes
- Pipet tips with wide opening 1250µL; VWR (613-0751)

For each sample (001-NNN), label 50 x 1,5 mL Eppendorf Tubes as follows (example for project acronym "Cultimic"):

C Cultimic-001 15x (Cultivation)
S Cultimic-001 5x (Sequencing)
U Cultimic-001 5x (Untreated)
F Cultimic-001 20x (FACS)
M Cultimic-001 5x (Metabolomics)

Work hereon under the fume hood due to smell.

Take the stool sample in flexible plastic bag out of the plastic container and homogenise by kneading the bag manually (be careful to not let air inside).

1. **Cultivation**: In a 50 mL Falcon tube, mix thoroughly (vortexing) approx. 3 g of stool with 12 mL of FMT medium; let larger debris set for 1 min; distribute into **15 x C-tubes**, 1 mL each; use pipet tips with a wide opening to avoid clogging. (Alternatively, cut the end of 1000µL tips with a sterile scissor)

   [Store 100µL of FMT medium as such to serve as negative control].

2. **Sequencing**: mix approx. 1 g of stool with 6 mL DNA stabilizer; homogenize by vortexing and distribute into **5 x S-tubes**, 1 mL each; use tips with a wide opening to avoid clogging.

   [Store 600µL of DNA stabilizer as such to serve as negative control].

3. **Untreated (for any types of analysis for which additional reagents confound the results)**: With a sterile spatula, fill **5 x U-tubes** with ca. 600 mg of untreated stool sample.

4. **Flow Cytometry (equivalent to untreated):** With a spatula, fill **20 x F-tubes** with ca. 100 mg of untreated stool sample.

5. **Metabolomics (faecal water)**: Weigh a 50 mL falcon tube. Fill it with approx. 1 g of stool. Weigh the tube again and calculate the difference to determine the exact amount of stool. Dilute with 4 mL of water (molecular biology grade, DEPC treated, and filter sterilised). Distribute into 2 mL Eppendorf Tubes and centrifuge (12,000 x g, 3 min). Filter the supernatants using a syringe fitted with a 0.20 µm filter and distribute into **5 x M-tubes. Protect yourself (e.g. wear safety googles) during the filtration step, as the filter may disconnect abruptly from the syringe and faecal water be spilled.**

   [Store 2 negative controls: water only, and filtered water].

Transfer all samples to **-80°C** freezer immediately after preparation.

Collect questionnaires and enter metadata into the protected, study-specific spreadsheet.