Introduction to phage microbiology techniques

All laboratory work will take place in the Institute of Technology (Nooruse 1), room 508b.

Day 1: Phage isolation from environmental water samples. 11.00-17.30

- 11.00 Meet up at Tartu Raekoja square
- Collection of environmental samples across the town (Botanical garden, Emajõgi etc.) (1-2h)
- Lunch
- Bus to the Institute at Nooruse 1. Lab work starts around 13.00-14.00.
- Preparation of the top agar and SM buffer (15-20 min)
- Isolation of phages infecting *E. coli* BW25113 strain from the environmental samples (3h)

Day 2: Phage isolation continued, phage immunity assays. 9.30 to 19.00

- Picking plaques from the isolation plates and re-streaking (30 min)
- Immunity assays: *E. coli* BW25113 + pBR322-EcorI and BW25113 transformed with pBR322-TA expression constructs vs BASEL phage collection (3-4h)
- 13.00 Lunch
- Break until 18.00
- 18.00 Second round of re-streaking the isolated phages; quick and easy working stock replication (30 min).

Day 3: Preparation of phage working stock, determination of phage the titer, confluence plates, phage escape mutants. 9.30 to 16.00

- Preparation of the phage lysate (working stock) from isolated phages (1-2h)
- Determination of the phage titer (2h + growth time)
- 12.00 Lunch
- Phage escape mutant assay (1h)
- Preparation of confluence plates (2h)

Day 4: Preparing the high titer stock and phage escape mutants continued. 9.30 to 12.30

- Preparation of the high titer phage stock (2h)
- Streaking the phage escape mutants into single plaques (1-2h)
- Lunch

Day 5: Verifying the phage escape mutants, analysis of results. 9.30 to 12.00

- Working stocks of escape mutants; verification of the escape mutants with a spot assay (2h)
- Discussion and data analysis (1-2h)
- Lunch