Worm Culturing, Weekly Notes

Week 1 (11/6-15/6)
Preparation of LB, M9, saturated NaCl-solution, physiological salt solution.
Tried two different protocols for egg purification, one suggested by Vidilab and a modified version.

Week 2 (18/6-22/6)
Optimizing the egg recovery protocol by changing centrifugation speed, centrifugation time and with or without a glass slide lid. We determined that the best method was to use a glass slide lid at 1000 rpm for 2 minutes. Eggs have been recovered and sterilized by washing them with bleach. Eggs have been incubated in eppendorfs with LB or M9 at 29 degrees.

Week 3 (25/6-29/6)
After microscope analysis we determined that M9 does not work and that LB will be used exclusively. Tried out new protocol of recovering L3 worms. A cup with feces and physiological water was placed upside down on a petri dish and incubated at 29 C for 7 days. Performed more egg recoveries using glass slides on falcon tubes. Started setting up a system for separating large and small strongyles using peristaltic pumps and bent pasteur pipettes.

Week 4 (2/7-6/7)
Continued to improve the machine for worm separation. Analysed worm samples to check for worms, results were mixed. The cup left for incubation during week 3 was taken out for analysis which revealed a problem, the water should be added after the incubation not before. Started testing different protocol for sterilizing the worms. These included bleach, ampicillin and a combination of both.

Week 5 (9/7-13/7)
Continued to test sterilization protocol for the worms. Results indicate that the best approach would be to use a 1 % bleach solution. Discovered problems with debris when recovering the worms after one week of incubation. Set up a filtration system using cotton and pasteur pipettes to get rid of unwanted particles.

Week 6 (16/7-20/7)
Worm sterilization protocol has been ultimately defined, and its efficiency tested. The worm separating machine has been tested, with different conditions, but with limited success. Two co-culturing experiment has been conducted, but both led to the identification of an issue in the removal of the worms from the solution after the end of the culturing.

Week 7 (23/7-27/7)
A further sterilization and co-culturing have been performed, and Whatman n°1 filters with a vacuum filter have been tested for the removal of the worms. This system appeared to work with a high degree of success. An ulterior recovery of nematodes from faecal samples has led to a high content of faecal debris in the solutions. To remove it the nematode purification process has been introduced, involving the use of a cotton filter.

Week 8 (30/7-3/8)
A new co-culturing experiment has been performed, introducing the improvements developed the previous week. The microfluidics chip for the worm separation has been designed and has been printed for the first time. Bonding of the chip proved to be an issue. A new worm recovery has been started

Week 9 (6/8-10/8)
2 more microfluidics chips have been printed. This presented problems with the bonding to the glass slide as well. A problem with the corona discharger has been identified. A new chip has been designed, where a vacuum source provides adhesion to the glass slide.

Week 10 (13/8-17/8)
One more co-culturing has been performed after nematode sterilization. The testing of the vacuum chip showed limited success. Leakage of fluid from the inner channels to the vacuum channels has been detected. An attempt to obtain a whole protein content from a worm sample has been made. The extract has been analysed with an SDS-PAGE, that indicated no success. A functioning corona discharger has been obtained.

Week 11 (20/8-24/8)
Different lysis techniques to obtain whole protein extract from small strongyles have been tested, with no success. A new microfluidics chip has been cast and successfully bound to a microscope slide. Testing has begun.

Week 12 (27/8-31/8)
More techniques for nematode lysis have been tested, with limited success. Microfluidics chip has been tested with perisaltic pump, with very limited success.