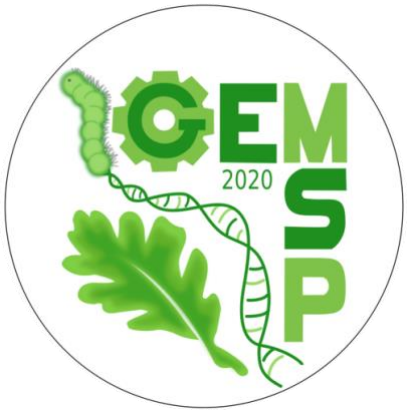
# LAB BOOK

Team MSP-Maastricht




2020

## Week 1

**01/09**

Alkaline lysis and neutralizing agents for oak processionary caterpillar (OPC) DNA extraction were prepared.

**02/09**

DNA was extracted from OPC's and the DNA was quantified. Quantification was done for future PCR reactions.

**03/09**

DNA extraction from OPC's was done as well as primer dilution. PCR was performed on the OPC DNA using these primers. Agarose gels were run to image the results.

**04/09**

PCR was run with different DNA concentrations with the previously prepared primers. To image the results, agarose gels were run.

## Week 2

**07/09**

PCR was run using different master mixes; Invitrogen™ Platinum™ SuperFi™ PCR Master Mix and 2x Phusion Master Mix. Agarose gels were run to see the bands.

**08/09**

PCR was run using different master mixes; Invitrogen™ Platinum™ SuperFi™ PCR Master Mix and 2x Phusion Master Mix and different DNA concentrations. Agarose gels were run to see the bands.

**09/09**

DNA extraction from OPC's was performed and was quantified. PCR was run with different DNA concentrations with the previously prepared primers. To image the results agarose gels were run.

**10/09**

PCR was done using different annealing temperatures specific for each of the primers to increase the efficiency of the reaction. Agarose gels were run to image the results.

**11/09**

DNA was extracted from the OPC's and was quantified as well.

**12/09**

PCR was run using different master mixes; Invitrogen™ Platinum™ SuperFi™ PCR Master Mix and 2x Phusion Master Mix and different DNA concentrations. Agarose gels were run to see the bands. Microscopy pictures were taken of the OPCs.

## Week 3

**15/09**

PCR was run with primers for different OPC genes. Gel electrophoresis was performed, and successful bands were cut out of the gels. DNA extraction was performed on the cut-out bands.

**16/09**

LB medium and glycerol stock was prepared. LB agar plates with ampicillin, tetracycline, ampicillin + tetracycline and without antibiotics were made. A second PCR was run on the samples extracted from the gels. In addition, gel electrophoresis was performed.

**17/09**

DNA was extracted from the OPC's and also was quantified. PCR was run on new DNA samples. Afterwards, gel electrophoresis was performed. Successful bands were cut out of the gels and the amplified DNA fragments were extracted from the gels.

**18/09**

Second amplification via PCR was done on samples cut out from gels the previous day. As well as, PCR was run with new DNA samples. To image the results, agarose gels were run.

## Week 4

**22/09**

New DNA samples were extracted and quantified. Successfully amplified fragments were prepared for sequencing. A second PCR was run on the samples extracted from the gels. In addition, gel electrophoresis was performed.

**24/09**

L4440 plasmids were isolated, glycerol stocks of 3 cultures (HT115/L4440, HT115(DE3)/L4440, HT115/L4440(GFP)) were prepared. PCR was run on new DNA samples. Afterwards, gel electrophoresis was performed.

## Week 5

**29/09**

New primer stock solutions were made. PCR was run on new DNA samples and bands were imaged using gel electrophoresis.

**30/09**

Overnight cultures of DH5α and DH5α+L4440 plasmid were prepared. PCR was run with new DNA samples. For result imaging, agarose gels were run.

**01/10**

TSS buffer was prepared. Competent HT115(DE3) cells were made. DH5α and HT115(DE3) liquid cell cultures were prepared. PCR on new DNA samples was run, gels were made for these samples.

## Week 6

**06/10**

DNA was extracted from OPC's. PCR was run on new DNA samples and bands were imaged using gel electrophoresis. 1x TAE buffer was prepared

**07/10**

Gel post staining was done. Liquid cultures of DH5α were prepared. PCR was run on new DNA samples. Afterwards, gels were run to image the bands and successful bands were cut out.

**08/10**

Gel extraction was done for bands obtained the previous day. Second PCR was performed for the extracted DNA samples, then gels were made with these samples. Minipreps of L4440 and L4440+GFP plasmids were prepared.

**09/10**

PCR on new DNA samples was run, gels were made for these samples and the relevant bands cut out afterwards. 1x TAE buffer was prepared. Multiple new LB agar plates were made.

## Week 7

**13/10**

Gel extraction was performed on previous samples and a second PCR was run for them. In addition, a new PCR was started with different primers. Gels were run on these samples, afterwards relevant bands were cut out. New liquid cultures were made for the DH5α cells. L4440 plasmids were isolated, restriction digestion was performed and left overnight.

**14/10**

DNA extraction from large caterpillars was done using liquid nitrogen to make a powder. Afterwards, usual DNA extraction protocol was followed. Gels were run for linearized L4440 plasmids. DNA was extracted from the gel cut outs, a second PCR was run on these samples as well as PCR on the new DNA samples was performed. Afterwards, gels were run to identify the necessary bands. 1x TAE buffer was prepared.

**15/10**

Touchdown PCR was performed on Wg, RNase and Tha p 2. Restriction digestion was done for the L4440 plasmid. Gels were run both for the PCR samples and digested plasmids. Started testing the bacterial lifespan and growth, when sprayed on oak branches. A, B, C branches were prepared and swabs ($T_0$) of the leaves before spraying with bacteria were taken. Directly afterwards the spraying, another swab ($T_1$) was taken from the leaves and LB + tetracycline plates were made with the swabbed bacteria.

**16/10**

Gel extraction for linearised L4440 plasmids, as well as for EF1a and Tha p 2 samples was done. Second PCR was run on EF1a and Tha p 2 samples together with RNase and Wg. Afterwards, an agarose gel was prepared, samples were inspected with it and successful bands cut out. L4440 plasmid minipreps were prepared. LB medium and LB agar plates were made. Oligos that correspond to the designed shRNA sequences were annealed. Swabs ($T_2$) were taken from the A, B, C oak branches and LB + tetracycline plates made with these bacteria.

## Week 8

**19/10**

TSS buffer was prepared. Competent DH5α and HT115(DE3) cells were made. DNA extraction was performed from the cut-out gel pieces with EF1a and Tha p 2 primers. For branch testing, final swabs ($T_3$) were taken from branches A, B, C and LB + tetracycline plates made with these bacteria. New branches (D, E, F) were obtained for testing the bacteria lifespan on them. HT115(DE3) cells were sprayed on the branches. Fresh LB medium was made.

**20/10**

Additional amplicon samples were prepared for sequencing. Swabs were taken from the D, E as well as F branch after 24 h ($T_1$) and spread on LB + tetracycline plates. The expression cassette was inserted into the L4440 plasmid via Gibson assembly and transfected into DH5α cells.

**21/10**

Liquid cultures of DH5α + L4440 plasmid and the expression cassette cells from plates done the previous day were made. After sufficient growth of the liquid cultures, mini preps were done. Afterwards, the plasmid was linearized using EcoR1 and BamH1. Then agarose gels were run to isolate the cut plasmids from the uncut plasmids. For ligation of vectors and inserts, Pro2-A, Wg-A, Tha p 2-A and EF1a-A inserts were used. Afterwards, the new plasmids were transfected into competent DH5α cells and spread on LB + ampicillin plates. For branch testing, swabs after 48 h ($T_2$) were taken from the D, E, F branches and spread on LB + tetracycline plates.

**22/10**

Mini preps of DH5α + L4440 plasmid with the expression cassette liquid cultures were made, the DNA concentration was measured. Then, restriction digest was performed using EcoR1 and BamH1 restriction enzymes and a gel was run to separate the cut plasmid. Afterwards, the band containing these linearized plasmids were cut out of the gel and DNA gel extraction was performed. Overnight liquid cultures of DH5α cells with 4 different inserts were prepared.

**23/10**

Ligation of vector and inserts was done for all 14 shRNA inserts. Afterwards, these plasmids were transfected into competent DH5α cells and spread on LB + ampicillin plates.

Mini preps of DH5α cells with 4 different inserts were prepared. Afterwards, the plasmids were transfected into competent HT115(DE3) cells and spread onto LB plates with tetracycline and ampicillin. Fresh LB medium and additional LB plated were prepared.

**24/10**

Liquid cultures of DH5α + L4440 plasmids with the inserts as well as the HT115(DE3) with L4440 plasmid and inserts were prepared. After sufficient culture growth, mini preps of these cultures were prepared. Part of the mini prep was used for restriction digest with Pci1 and NgoMIV. For result imaging, gel electrophoresis was done. Other parts of DH5α + L4440 plasmid with the insert mini preps were used for transfection into competent HT115(DE3) cells. Afterwards, they were plated onto LB with tetracycline + ampicillin plates. IPTG induction was done for HT115(DE3) with shRNA insert liquid cell cultures. Additionally, overnight liquid cultures of DH5α with 14 different shRNA inserts as well as the HT115(DE3) with L4440 plasmid and 4 different shRNA inserts were prepared.

**25/10**

Mini preps of overnight cultures from the previous day were prepared. Restriction digest with EcoR1 and BamH1 enzymes was performed. The results were imaged via gel electrophoresis. From successful DH5α with insert liquid cell culture mini preps, transfection into competent HT115(DE3) cells was done. Afterwards, they were plated onto LB with tetracycline + ampicillin plates. 1x TAE buffer was prepared. Microscopy images were taken of grown OPC's.