Integrated Practical

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Introduction

During my Integrated Practical course, I completed an internship at the Ecology, Evolution and Genetics Research Group within the Biology Department at the Vrije Universiteit Brussel (VUB), located at Pleinlaan 2 in Brussels. The research group, led by Prof. Dr. Paris Veltsos, focuses on evolutionary genetics and the study of species from the genus *Caenorhabtidis*, including *C. elegans*. In this lab, I had the opportunity to become familiar with the daily workflow of a professional research laboratory and to actively participate in several ongoing experiments and maintenance tasks.

Most of my work in the laboratory focused on the handling and maintenance of different worm strains from *C. remanei* and *C. brenneri*, as well as performing molecular biology techniques such as PCR and agarose gel electrophoresis. Through these activities, I gained hands-on experience with handling laboratory equipment, sterile working methods, sample preparation and DNA analysis whilst cojointly learning the importance of precision, organization and teamwork that is needed within a research environment.

This report provides an overview of the practical work that was carried out during my integrated practical course. First, a short theoretical background is given for the different techniques and methods that were executed in the lab. Followed by the methods and their obtained results. The report aims to show both the scientific relevance of the performed experiments and the practical skills that were developed throughout this experience.

Finally, I would like to take the opportunity to sincerely thank the entire lab team and in particular Prof. Dr. Paris Veltsos for giving me the opportunity to experience the workflow of several ongoing research projects and hereby also fulfilling my integrated practical course with the assistance of his expertise.

Methods & Results

DNA extraction with proteinase K

Principle

Proteinase K is an enzyme with a broad specificity for degrading proteins. This makes the enzyme perfect for DNA extractions as it can inactivate nucleases that degrade DNA. Proteinase K is a serine protease which breaks down proteins by hydrolyzing peptide bonds (Cronier et al., 2008). This proteolytic enzyme is mainly produced by the fungus *Tritirachium album* Limber owing the name of the enzyme due to its ability to digest keratin (Sweeney & Walker, 2003).

Method

Start by pipetting 20 µL of 10X PCR buffer in PCR tubes. Each strain should use a separate PCR tube. Secondly, add 1 µL of proteinase K in each tube. Label each tube with the strain that will be added to the tube.
Using a worm picker, pick 10-20 adults worms from each strain using the binocular microscope and add them to the tube. Freeze the tubes at -80°C for 20 min to break up the worms. Place the PCR Eppendorf tubes into a Bio Rad T100™ Thermal Cycler, ensuring they are positioned securely and the lids are sealed firmly. Select the program "WORM_DNA" and let it run.

The program will run firstly at 65°C for 1 h followed by 95°C for 10 min and keeps the samples at 12°C until removed from the machine.

Nanopore

Principle

NanoDrop microvolume sample retention system functions by combining fiber optic technology with the natural surface tension properties of liquids, allowing it to capture and retain minute amounts of sample without relying on traditional containment apparatus such as cuvettes or capillaries. This eliminates the need for making dilutions and reduces the sample volume that is required to make the measurements (Desjardins & Conklin, 2010).

Nanodrop detects the presence and concentration of DNA by determining the UV absorbance at three specific wavelengths; 230 nm, 260 nm and 280 nm. These three measurements allows for an easy determination of nucleic acid concentration and purity.
Doublestranded DNA maximally absorb light of a wavelength of 260 nm, whilst proteins of 280 nm. Lastly, contaminants such as salts, phenols and EDTA highly absorb light of a wavelength of 230 nm. (NanoDrop Microvolume Spectrophotometers And Fluorometers Applications | Thermo Fisher Scientific - US, z.d.).

To accurately represent the samples two ratios are analyzed; the 260/280 ratio and the 260/230 ratio. A pure sample of DNA has a 260/280 ratio of 1.8. If the ratio significantly differs from this result (>0.3), it may indicate the presence of proteins, phenol or other contaminants that absorb strongly at 280 nm. The second purity ratio, being 260/230, scores between 1.8-2.2 if the sample is pure. Purity ratios that score significantly lower than this can indicate that the DNA isolation technique may require an optimized protocol (Desjardins & Conklin, 2010c).

Method

Take the samples that have undergone DNA extraction, vortex and centrifuge them to mix the components. Turn on the Nanopore 2000 Spectrophotometer by ThermoFisher

Scientific™ and the computer next to it. Open the lid of the Nanodrop and clean carefully with paper tissue.

Pipet 1-2 µL of PCR buffer on the bottom lens (the small metallic bulge in the centre of the black circle). Close the lid carefully and press “run blanc”. This sets the background absorbance of the solution that contains DNA in the sample. Once run, open the lid and gently clean both top and bottom part of the Nanodrop with a paper tissue.

Take a new pipet tip and pipet 1-2 µL of the sample on the bottom lens. Close the lid carefully and press run on the computer. Open the lid and clean the Nanopore with paper tissues. Repeat these steps for all samples.

Results

As an example, DNA extractions from four strains that were selected for the third PCR are listed in the table below.

Strain	DNA conc. (µg/µL)	Absorbance (260/280)	Absorbance (260/230)
rD	227,7	1,41	0,8
rE	46,9	1,40	0,71
bV	184	1,25	0.37
bZ	250	1,31	0,62

PCR

Principle

PCR, short for polymerase chain reaction, is a technique used to amplify short DNA sequences. The process consists of three main steps.

First is the denaturation step. Here, the temperature is raised to around 95 °C, causing the double-stranded DNA to separate into two single strands. Next comes the annealing phase, where the temperature is lowered to about 60 °C. This allows primers to bind to their complementary sequences on each of the single-stranded DNA templates.

After that, the elongation phase begins. The temperature is increased to roughly 72 °C, which is optimal for Taq polymerase function. This enzyme extends the primers by adding complementary nucleotides, using free dNTPs present in the PCR mixture, to copy a new strand working in the 5’ direction to the 3’.

These three steps are repeated multiple times, leading to a large number of copies of the target DNA sequence (Khehra et al., 2025).

Method

Begin by preparing a mastermix containing the primer pair(s), dNTPs, 10× PCR buffer, H_2O, and Taq polymerase. Before starting, calculate the required volume of each component based on the total mastermix volume needed for all reactions and include a small excess to account for inaccurate pipetting.

Once all reagents have been added, vortex the mastermix and briefly centrifuge to ensure all components are well mixed and collected at the bottom of the eppendorf.

After the mastermix has been prepared, aliquot the appropriate volume into individual PCR Eppendorf tubes according to the reaction setup. Add the specific target DNA to each tube that needs to be amplified, making sure the correct sample is added to the corresponding reaction. Once all samples have been added, clearly label each Eppendorf tube to avoid confusion during analysis. Once labelled, mix the contents gently by pipetting up and down or by briefly vortexing if appropriate, followed by a short centrifugation step to collect all liquid at the bottom of the tubes.

Finally, place the PCR Eppendorf tubes into the Bio Rad T100™ Thermal Cycler, ensuring they are positioned securely. Select the program labelled "WORM_ID," verify that the settings are correct, and press start to begin the amplification process.

The PCR cycle used for "WORM_ID" for a volume of 20 µL:

1. 95°C, 2:00 minutes
2. 95°C, 0:30 seconds
3. 60°C, 0:30 seconds
4. 72°C, 1:00 minute
5. Cycle back to step 2, 34X
6. 72°C, 10:00 minutes
7. 12°C, ∞

Results

PCR 1:

Both C. *remanei* and C. *breneri* primer pairs in one PCR mix:

Ingredients	**Per reaction (µL)**	**For 25 reactions (µL)**
10x PCR buffer ($MgCl_2$ 15mM)	2	50
CBR F (1 µM)	1	25
CBR R (1 µM)	1	25
CRE F (1 µM)	1	25
CRE R (1 µM)	1	25
dNTP's (100 mM)	0,4	10
H_2O	11,4	285

Taq Polyemerase (5 U/µL)	0,2	5

+ 2 µL of DNA in each Eppendorf

C. *remanei* and C. *breneri* primer pairs in seperate PCR reactions

Ingredients	**Per reaction (µL)**	**For 25 reactions (µL)**
10x PCR buffer ($MgCl_2$ 15mM)	2	50
CBR F (1 µM)	1	25
CBR R (1 µM)	1	25
dNTP's (100 mM)	0,4	10
H_2O	13,4	335
Taq Polyemerase (5 U/µL)	0,2	5

+ 2 µL of DNA in each Eppendorf

Ingredients	**Per reaction (µL)**	**For 25 reactions (µL)**
10x PCR buffer ($MgCl_2$ 15mM)	2	50
CRE F (1 µM)	1	25
CRE R (1 µM)	1	25
dNTP's (100 mM)	0,4	10
H_2O	13,4	335
Taq Polyemerase (5 U/µL)	0,2	5

+ 2 µL of DNA in each Eppendorf

The strains rB, rD, rE, bR, bV, bW were tested for PCR.

PCR 2:

Both C. *remanei* and C. *breneri* primer pairs in one PCR mix:

Ingredients	**Per reaction (µL)**	**For 5 reactions (µL)**
10x PCR buffer ($MgCl_2$ 15mM)	2	10
CBR F (1 µM)	1	5
CBR R (1 µM)	1	5
CRE F (1 µM)	1	5
CRE R (1 µM)	1	5
dNTP's (100 mM)	0,4	2
H_2O	11,4	57
Taq Polyemerase (5 U/µL)	0,2	1

+ 2 µL of DNA in each Eppendorf

C. remanei and *C. breneri* primer pairs in seperate PCR reactions

Ingredients	**Per reaction (µL)**	**For 5 reactions (µL)**

10x PCR buffer ($MgCl_2$ 15mM)	2	10
CBR F (1 µM)	1	5
CBR R (1 µM)	1	5
dNTP's (100 mM)	0,4	2
H_2O	13,4	67
Taq Polyemerase (5 U/µL)	0,2	1

+ 2 µL of DNA in each Eppendorf

Ingredients	**Per reaction (µL)**	**For 5 reactions (µL)**
10x PCR buffer ($MgCl_2$ 15mM)	2	30
CRE F (1 µM)	1	15
CRE R (1 µM)	1	15
dNTP's (100 mM)	0,4	6
H_2O	13,4	67
Taq Polyemerase (5 U/µL)	0,2	1

+ 2 µL of DNA in each Eppendorf

In this PCR only four strains (rD, rE, bV and bW) have been used.

Agarose gelectrophoresis

Principle

Agarose gelelectrophoresis is a very effective way of separating DNA fragments based on size. When the gel is poured and the agarose solidifies, it forms a sort of sieve where the DNA fragments can pass through if an electric field is applied along the gel.
Once the DNA fragments are loaded in precast wells with the loading dye, applied current creates an electric field which is negative at the precast wells' side and positive at the bottom of the agarose gel. The phosphate backbone of the DNA molecule is negatively charged and thus will migrate towards the bottom of the agarose gel.
The bigger the DNA fragment is the longer it will take to move through this sieve that is created by the agarose, depending on the gel concentration. When the gelelectropheresis is terminated by turning off the current, the DNA fragments will stop moving through the gel. The fragments that are furthest from the precast wells are the smallest fragments whilst those closest are the biggest.
The gelelectrophoresis is not only run with the DNA fragments acquired from a previous PCR, but also with a DNA ladder. This ladder has predetermined DNA fragments of which the length is already known. The unknown DNA fragments can then be compared to the ladder to calculate their size (Lee et al., 2012).

Method

Begin by setting up the pouring square and determine the amount of wells will be needed. Once this is set up, add 1 gram to 100 mL of 1X TAE buffer. Stir the agarose into the TAE buffer by gently swirling the Erlenmeyer.

When this is done put the Erlenmeyer into the microwave and let it cook. Keep a close eye on the microwave and do not let the agarose solution boil too long. When you see the solution boiling, gently give the Erlenmeyer another swirl and put it back in the microwave. Always stop the microwave when the solution is brought to a boil. If the solution has no more lumps, let it cool down until around 50-60°C, this to make sure the gel is still liquid and will not dry immediately when poured, but will not burn the plastic mould where the gel is poured into.

Pour the liquid gel in the square and make sure the wells are fitted correctly. Let the gel set. Once the gel has set, the gel can be put in the electrophoresis chamber with the power supply PowerPac 300 from BioRad. Make sure the gelelectrophoresis buffer is inside all the wells and covers the whole gel. If not add more buffer.

Start by carefully adding 5 µL of GeneRuler 100 bp DNA Ladder by ThermoFisher Scientific™ in the first well. Make sure to not pipet too high, too low or to add air bubbles in the well. Once the ladder is loaded, start by mixing 5 µL of the PCR product with 1 µL of loading dye. This can be accomplished by firstly adding 1 µL drops of loading dye on parafilm and then adding 5 µL of DNA. Pipet up and down several times to mix the loading dye with the DNA. Then, carefully add the DNA with loading dye inside the well, again making sure not to pipet inside the gel or getting the DNA outside the well.

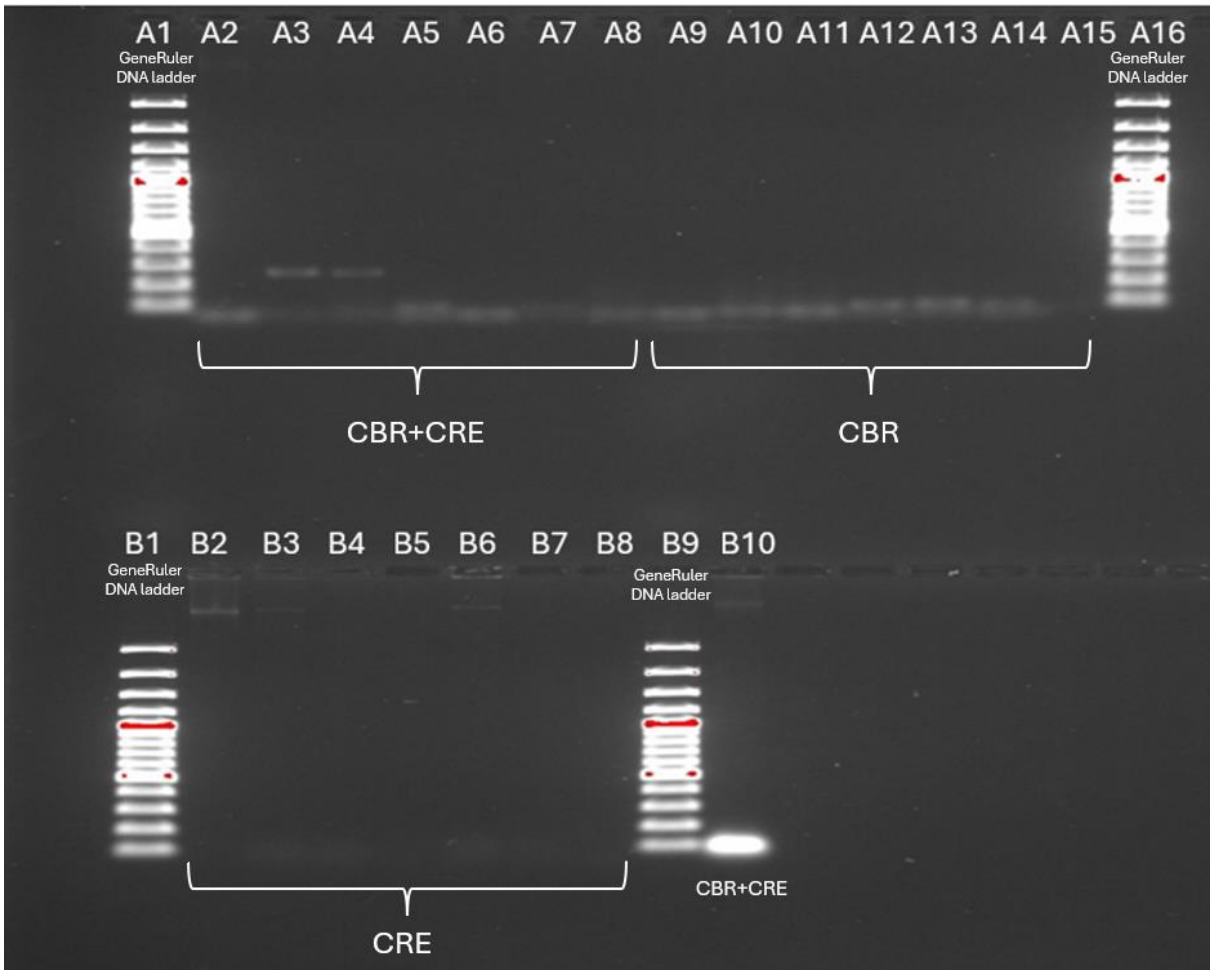
If necessary, add another DNA ladder at the end for easier visualization of the fragment sizes. Place the container lid correctly on the container. The negative anode (black cable) should be in the top left corner whilst the positive cathode (red cable) should be on the bottom right corner. Once the lid is correctly secured, turn on the power supply to 90 Volts and let it run for one hour and a half.

When the run is complete, turn off the power supply and carefully remove the gel from the gelelectrophoresis chamber. Turn on the computer and Molecular Imager® ChemiDoc™ XRS+ from BioRad. Place the gel inside the dark chamber of this gel imaging system and align the gel correctly with the camera. Let the system run and save the picture of the gel.

Remove the gel from the imaging system and dispose. Lastly, clean the chamber where the gel was put in.

Results

Gel from PCR 2:

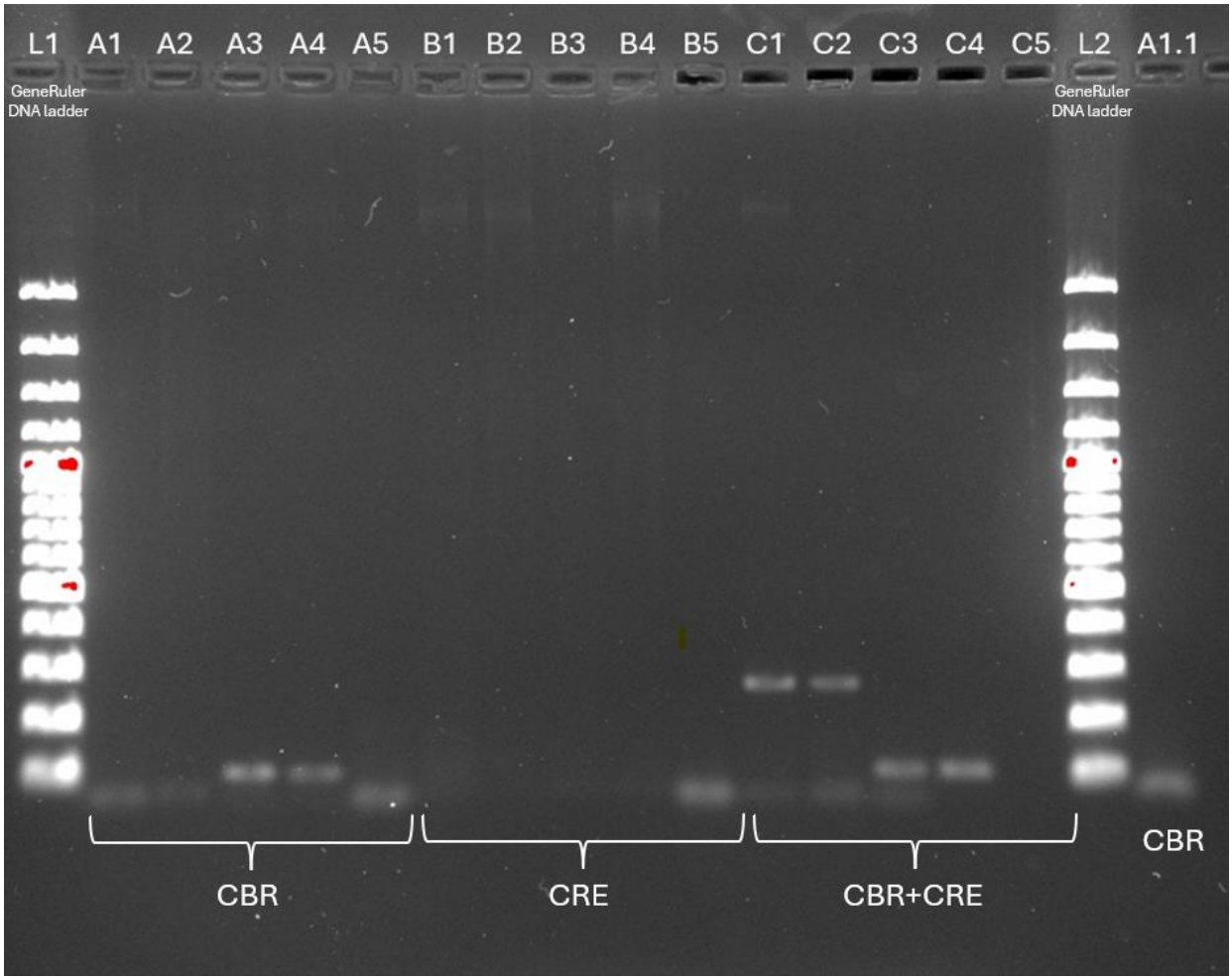


Well	**Sample**	**Primer(s)**
A1	100 bp ladder	/
A2	rB	CBR + CRE
A3	rD	CBR + CRE
A4	rE	CBR + CRE
A5	bR	CBR + CRE
A6	bV	CBR + CRE
A7	bW	CBR + CRE
A8	Negative control	CBR + CRE
A9	rB	CBR
A10	rD	CBR
A11	rE	CBR
A12	bR	CBR
A13	bV	CBR

A14	bW	CBR
A15	Negative control	CBR
A16	100 bp ladder	/
B1	100 bp ladder	/
B2	rB	CRE
B3	rD	CRE
B4	rE	CRE
B5	bR	CRE
B6	bV	CRE
B7	bW	CRE
B8	Negatieve controle	CRE
B9	100 bp ladder	/
B10	rB	CRE + CBR

In B10 another loading dye was used that was made by the lab technician.

Gel from PCR 3



Well	Sample	Primer(s)
L1	100 bp ladder	/
A1	rD	CBR
A2	rE	CBR
A3	bW	CBR
A4	bV	CBR
A5	Negative control	CBR
B1	rD	CRE
B2	rE	CRE
B3	bW	CRE
B4	bV	CRE
B5	Negative control	CRE
C1	rD	CRE + CBR
C2	rE	CRE + CBR
C3	bW	CRE + CBR
C4	bV	CRE + CBR
C5	Negative control	CRE + CBR
L2	100 bp ladder	/
A1.1	rD	CBR

In well A1.1 one extra µL of loading dye has been added by the PCR product.

Nemathode Growth Medium (NGM) and pouring plates

Principle

NGM (Nematode Growth Medium) plates are prepared to provide a nutrient-rich environment that supports the growth of *Escherichia coli*, which serves as a food source for *Caenorhabditis elegans*. The medium contains essential salts and supplements, including potassium phosphate buffer, calcium chloride, magnesium sulphate, and cholesterol, which are required for optimal nematode development and physiological function.
KPO_4 serves as a pH buffer to keep the pH stable at ~6. It also serves as an important source for phosphates. Cholesterol serves for the development of the nematodes as they can not synthesize their own sterols. $CaCl_2$ serves as an important Ca^{2+} source, which is needed for both optimal nematode growth and bacterial growth. $MgSO_4$ is crucial for the functioning of enzymes and thus also supports optimal growth.

The preparation and pouring of plates are carried out in a laminar flow chamber to maintain sterility and prevent microbial contamination. After the agar medium solidifies and dries, a standardized volume of OP50 *E. coli* in 2xYT medium is spread across the surface of the plates to create a uniform bacterial lawn. Incubation at room temperature allows the bacteria to grow and establish a consistent food source before the plates are used for nematode culture or experimental procedures (K. Corsi et al., 2015).

OP50 *E. coli* is a very broadly used strain as primary foodsource for *C. elegans*. This strain is an uracil auxotroph, meaning it can not synthesize its own uracil, which is crucial for the growth of organisms. This causes the strain to have a more restricted growth on NGM, which enables a more easier observation of C. elegans species (Kaiglová et al., 2025).
The E. coli are cultured in 2x yeast extract-tryptone (2xYT). Due to its double dose of yeast and tryptone the bacteria have a much faster growth rate and the medium thus contains a higher cell density (Kram & Finkel, 2015).

Method

To make a growth medium for a bottle of 400 mL start by weighing off 1,2 g of NaCl, 1 g of peptone and 8 g of agar. Place these powders inside a 400 mL glass bottle and fill up with water till 400 mL.
Autoclave for one hour. Once autoclaved, store the liquid media inside the medium storage oven. This oven will keep the medium liquid at 55°C for an indefinite time.

To pour the medium into the plate begin by turning on the laminar flow chamber and setting up the plates that need to be poured inside the cabinet. Once the setup is ready,

remove the bottle containing the liquid medium from the medium storage oven. To 400 mL of medium, add 10 mL of 1M KPO_4, 400 µL cholesterol, 400 µL of 1M $CaCl_2$, and 400 µL of 1M $MgSO_4$. Mix the medium thoroughly by gently stirring the bottle. All of these steps should be carried out inside the laminar flow chamber to maintain sterility.

Next, pour the prepared medium onto the plates, adding enough to completely cover the bottom surface of each plate. Allow the plates to dry completely before proceeding.

Once dry, retrieve the *E. coli* culture stored in liquid medium in the refrigerator. Using the Eppendorf Multipette® M4 - repeating pipette, aliquot 50 µL onto small plates and 200 µL onto large plates. After dispensing the bacterial suspension, gently sway the plates to distribute the *E. coli* evenly across the entire surface.

Finally, leave the plates at room temperature for 24 h to allow bacterial growth. If the plates need to be stored for a longer period, transfer them to 4oC.

Worm freezing protocol

Principle

Freezing *C. elegans* strains is done to store the worms for a long period while keeping the strain alive and genetically stable. L1 worms are the most resistant to stress caused by freezing and thawing. Plates that are close to starvation are preferred because worms in these conditions do not develop past the L1 stage.

The worms are collected in M9 buffer and mixed with a freezing solution containing glycerol. The tubes are cooled slowly in a polystyrene rack before being stored at −80 °C, which improves the survival of the worms after thawing (K. Corsi et al., 2015).

M9 consists 5,8 g of Na_2HPO_4 , 3 g of KH_2PO_4 , 0,5 g of NaCl and 1 g of NH_4Cl mixed in 1 L H_2O. M9 serves as a safe transfer medium to wash and transfer nematodes from NGM (Bar-Ziv et al., 2020).

Method

To freeze a strain, one of the plates of the strain must be starving. Prepare two Eppendorf tubes per strain by labelling these with the date of freezing and the strain that will be frozen.

Wash the plate with 1000 µL of M9 buffer by making sure most worms are loose from the agar plate and inside the M9 solution. Transfer the M9 buffer, now with worms inside it, to the two Eppendorf tubes. Add 350 µL of freezing solution to each tube. Shake the tubes briefly to mix. Place the tubes in a polystyrene rack and place another inverted polystyrene rack on top. Store the rack with the tubes inside in the freezer at -80°C for at least 12 h. Finally, fill in the excel file to indicate which strains are frozen at which date and how many are frozen.

To check if the freezing protocol was successful thaw one random tube of that freezing date on a NGM plate. Invert the tube on a new plate and observe if the worms are moving after 1 day.

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