



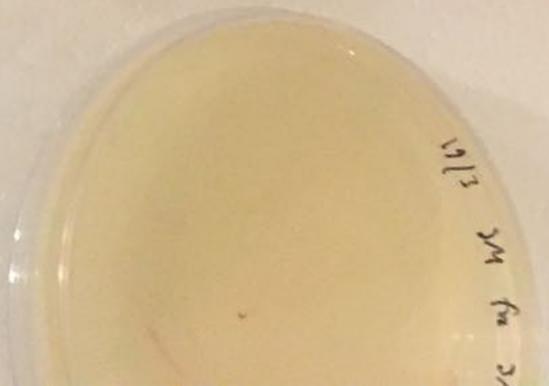
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Publication date: June 2021

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# Contents

<b>1 General guidelines</b>	<b>5</b>
How to use this book	7
Laboratory hierarchy	9
The DSKD biolab	11
Safety rules in the biolab	13
Keeping a lab protocol	15
Sterile working area	17
<b>2 Bacteria descriptions</b>	<b>19</b>
Microorganisms metabolism	21
Isolating microorganisms	23
Growth phases	25
Pigment production	27
Bacteria collection	29
Storing bacteria	31
<b>3 Recipes</b>	<b>33</b>
Preparing LB liquid media	35
Preparing LB agar plates	37
Bacteria inoculation to LB liquid media	39
Bacteria inoculation to LB agar plates	41
Coloring textile with live bacteria	43
Coloring BIG pieces of textile with live bacteria	45
Stamping pieces of textile with live bacteria	47
Coloring textile with harvested pigment	49
Coloring biomaterials with harvested pigment	51
Printing on textile with harvested pigment	53
Waste management	55
Storing bacteria	57
<b>4 Video tutorials</b>	<b>59</b>
<b>5 Glossary</b>	<b>63</b>
<b>6 Suggested readings</b>	<b>69</b>
<b>7 References</b>	<b>73</b>



# 1. General guidelines



# How to use this book

**This book explores the biolab at DSKD and the methods applied within the biolab at DSKD to teach designers how to work within it, question and reflect upon the possibilities presented to expand, explore and inspire future workshop practice. The book is not a manual on how to set up a biolab elsewhere, as safety regulations have to be followed.**

The first chapter covers a general guidelines to working in the biolab primarily covering safety issues and.

The second chapter gives an overview of how naturally pigments producing bacteria lifecycle works and how they produce pigments as well as an overview of the microorganisms currently available in the biolab.

The third chapter describes the recipes used in the biolab e.g. how to prepare media and petri dishes for growing the bacteria.

The fourth chapter is a collection of the different video tutorials showing how to carry out the different steps described in the recipes in chapter three.

The remaining chapters five, six and seven contains a glossary for words connected to working in the biolab, a list of suggested readings if you wish to explore a bit more about this area of design and a reference list used for the factual descriptions. In addition to the references several interviews with different biolabs and community labs around Europe have contributed with knowledge to this book as well as sharing relevant resources.

A special thanks to Shem Johnson from the Central Saint Martins Growlab, Roland van Dierendonck from Waag Amsterdam and Utrecht University of the arts, Danial Gruskin from Biodesign Challenge (BDC), Anke Pasold from KEA, Maria Boto Ordonez & Heleen Sintobi from the biolab at KASK Ghent, Adrian Rigobello from CITA at the Royal Danish Academy and VTT in Finland for sharing past and present experiences, valuable insights all helping and guiding the contents of this book.

**BEFORE WORKING IN THE  
BIOLAB YOU NEED TO GET  
AN INTRODUCTION**



# Laboratory hierarchy

DSKD BIOLAB	NATURAL SCIENCE LAB	
Biosafety level 1	Biosafety level 2	Biosafety level 3
Grow known BSL-1 microorganism at max. 25°C	Grow known BSL-1 and BSL-2 microorganisms at all temperatures	Grow known BSL-1, BSL-2 and BSL-3 microorganisms at all temperatures
Prepare media for growth of microorganisms	Prepare media for growth of microorganisms	Prepare media for growth of microorganisms
Look at unknown microorganisms in a closed container wrapped with parafilm	Look at unknown microorganisms in a closed container wrapped with parafilm	Look at unknown microorganisms in a closed container wrapped with parafilm
Open access	Isolate an unknown microorganism under a laboratory fume hood	Isolate an unknown microorganism under a fume hood
No prior training	Extract pigment or other molecules produced by the microorganism	Extract pigment or other molecules produced by the microorganism
Uncontrolled and unreproducible	Some GMO work	GMO work
Small scale	Restricted access	Human tissue and cells
	Some prior training is advantageous	VERY restricted access due to laboratory costs
	Controlled and reproducible	Prior training is required
	Bigger scale	Controlled and reproducible
		Small scale



# DSKD Biolab

**Welcome to the DSKD Biolab. Before starting any work in the biolab you need to get an introduction. Please familiarize yourself with this book and its contents.**

## **Biosafety level 1**

The DSKD biolab is classified as a biosafety level 1 lab (BSL 1), which means we are allowed to work with any biological material, so long as it is not a pathogenic (disease causing) agent, organism, parasite or virus that is hazardous to humans and/or the environment. Ask Monica Hartvigsen before bringing any microorganisms into the lab.

## **Human tissues not allowed**

Please note that working with human tissues and fluids are not permitted because they can contain disease-causing agents.

## **Be aware of health hazards**

There is a LARGE diversity of microbes that exist, each may have its own health hazards. One should be aware of what is being handled and what risks, if any, it may pose. If the organism is unknown, precautions should be taken to minimise contact/interaction with humans as the microbe(s) could pose health hazards.

## **Use sterile and aseptic techniques**

Sterile/aseptic techniques should be practised wherever and whenever possible to avoid exposure to one's self and avoid the growth of unwanted microbes. Sterilising for microbial work often uses a high temperature oven or a pressure vessels containing hot steam, such as an autoclave (or pressure cooker). Sterilizing is especially important for waste management.

## **Working with Genetically Modified Organisms (GMOs)**

BSL 1 laboratories cannot work with genetically modified organisms (GMOs). GMOs are considered any organism (including cloning bacteria) that contains any foreign genetic material (DNA or RNA) or unnatural rearrangement of genetic material. Ask Monica Hartvigsen if you have questions regard working witg GMO's.

## **A shared space**

Be mindful of others and their work, since it is a shared space. Try to minimize clutter and practice a good hygiene in the lab. In this way more people get to enjoy the space.

## **For introduction and questions please contact:**

Monica Hartvigsen, mlh@dskd.dk, +4561401027



**Wash hands**



**Ask for help and guidance**



**Never eat or drink**



**Mark your samples**



**Use protective equipment**



**Clean up after experiments**



**Read all instructions**



**Sterilize biological waste**

# Safety rules in the biolab

## Wash hands

Before entering and leaving the biolab please wash your hand, to make sure you do not bring any unwanted chemicals or microorganisms with you on your skin.

## Ask for help and guidance

Before you start working in the lab, you need to get an introduction. If you are in doubt with anything ask for guidance from more experienced students or staff.

## Never eat or drink in the biolab

For your own safety, it is not allowed to eat and drink in the biolab. Even though you might not be working with chemicals, treat your ingredients as if you were.

## Use protective equipment

In the biolab we need to wear protective gear, which in the biolab includes: labcoat, gloves (latex or nitril or the like). If you have long hair, you should tie it back. It is your responsibility to protect yourself from possible health hazards. The gloves and mask should be autoclaved before being disposed as waste.

## Familiarize yourself with the safety regulations

This booklet should provide the safety regulations needed for working in the biolab, but it is always good to think about and check the safety regulations before starting an experiment. For any chemical material safety data sheets (MSDS) are available online for almost all chemicals.

## Read all instructions carefully

Take time to think about what you are doing to make sure you are taking the right precautions. Be aware of hot water and steam from the autoclave and the gasflame from the bunsenburner, **especially together with ethanol (explosion danger)**.

## Mark your samples

Work must be clearly labeled with a name, date, and description, or it will be immediately tossed in the bin.

## Clean up after experiments

Do not take anything out of the biolab that has not been autoclaved or is properly wrapped. Ask for guidance if you are unsure on how to proceed. Wash your glassware and utensils, clean up, dispose of waste, and put everything in its right place. Wash your hands before leaving the biolab.

## Waste management

All waste which have been in contact with living microorganisms should be autoclaved for at least 30min before being thrown out.





ilot study

Author: Monica Louise Harvigsen

Set-up

Pigment production I

Author: Monica Louise Harvigsen

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# Keeping a lab protocol

## **What is a protocol?**

A protocol is a description of the different steps you are doing through out an experiment. There is no formal standard, so you can make yours in any way you want, as long as you understand how you did your experiment. It can be a good idea to prepare it in advance, so you have idea of what is going to happen.

You can document the steps by writing down all recipes and sample descriptions, as well as taking photos and videos.

## **Why should I keep one?**

Keeping some kind of protocol will make make it possible to reproduce your work and allow others to assess how to handle your samples safely. If you need guidance in further experiments, it will also be valuable to the supervisor to have an idea about, how you carried out your experiments.



# Sterile working area

## Aseptic techniques

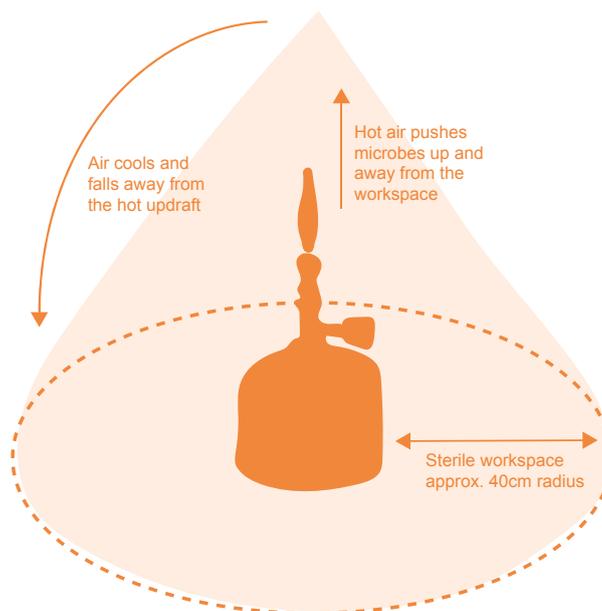
Researchers use aseptic technique to prevent contamination with unwanted bacteria, fungi, or viruses from the environment. The key elements of aseptic technique are a sterile work area, good personal hygiene, sterile reagents/media, and sterile handling (Thermofisher, 2021).

## Ethanol

Ethanol can be used to disinfect the workspace. The workspace should be disinfected before and after working with microorganisms. **Ethanol is highly flammable!!** so it should be handled with care, especially because it is used near the Bunsen burner.

## Bunsen burner

A Bunsen burner is a lab instrument that can be used to provide a single, continuous flame by mixing gas with air in a controlled fashion. The ratio of gas to air that is mixed together can be manually adjusted, allowing the user to control the intensity, temperature, and size of the flame. The Bunsen burner is the easiest way to create a relatively sterile environment on the lab bench. A major purpose of the open flame in aseptic technique is to create a cone of hot air above and around the lab bench to reduce the viability of organisms on suspended dust particles. The ability of the Bunsen burner flame to heat things very quickly also makes it an ideal choice for sterilizing inoculating loops or warming glass bottle necks (Thermofisher, 2021).





# 2 Bacterial descriptions

# Microorganism metabolism

using CO<sub>2</sub> as carbon source is called

using chemical compounds as carbon source is called

Autotrophy

Heterotrophy

and the organisms are

and the organisms are

Autotrophs

Heterotrophs

whose energy source is

whose energy source is

light

inorganic compounds

organic compounds

light

Photoautotrophs

Chemoautotroph

Chemoheterotroph

Photoheterotrophs

which include

which include

which include

Nitromonas and Nitrobacter

Many Bacteria, Fungi, Archaea and Eukarya

Green and purple nonsulfur bacteria

that use H<sub>2</sub>O to carry out

that do not use H<sub>2</sub>O to carry out

those feeding on dead organic matter

those feeding on living organic matter

Oxygenic photosynthesis

Anoxygenic photosynthesis

Saprobies

Parasites

which include

which include

Cyanobacteria, Plants and algae

Green and purple bacteria

# Microorganism metabolism

## Metabolism

Metabolism refers to all the biochemical reactions that occur in a cell or organism. The study of bacterial metabolism focuses on the chemical diversity of substrate oxidations and dissimilation reactions (reactions by which substrate molecules are broken down), which normally function in bacteria to generate energy. Also within the scope of bacterial metabolism is the study of the uptake and utilization of the inorganic or organic compounds required for growth and maintenance of a cellular steady state. These respective exergonic (energy-yielding) and endergonic (energy-requiring) reactions are catalyzed within the living bacterial cell by integrated enzyme systems, the end result being self-replication of the cell. The capability of microbial cells to live, function, and replicate in an appropriate chemical milieu (such as a bacterial culture medium) and the chemical changes that result during this transformation constitute the bacterial metabolism (Burgin et al, 2011).

## Heterotrophs

The natural pigment producing bacteria, we have available in the lab are all heterotrophs. They require preformed organic compounds, which we prepare for them using a standard bacterial growth medium called LB Broth, which consists of casein digest peptone, yeast extract and sodium chloride. These carbon- and nitrogen-containing compounds are growth substrates, which are used aerobically or anaerobically to generate reducing equivalents (e.g., reduced nicotinamide adenine dinucleotide;  $\text{NADH} + \text{H}^+$ ); these reducing equivalents in turn are chemical energy sources for all biologic oxidative and fermentative systems e.g. pigment production. Heterotrophs are the most commonly studied bacteria; they grow readily in media containing carbohydrates, proteins, or other complex nutrients (Baron, 1996).



# Isolating microorganisms

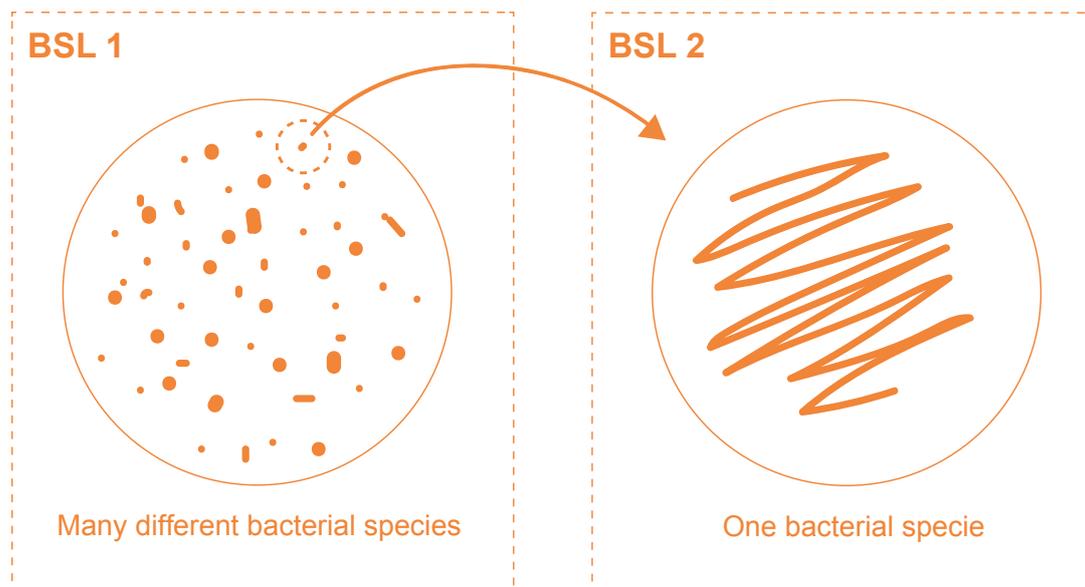
## - and why we cannot do it the lab

### The diverse nature of microorganisms

When taking a sample and streaking it onto an agar plate with prepared media from outside e.g. the soil, the air, your own skin, many different kind of microorganisms will start growing on the plate.

We do not know which kind of microorganisms grow on the agar plate (sadly, they cannot tell us). It could be something potentially very dangerous e.g. anthrax and we need to think of the health hazard we are exposing ourselves to as well as colleagues.

Once we have grown an unknown species on an agar plate, we can look at it in a microscope, but we cannot open it. If we need to know what kind of microorganism we have found, we need to take our agar plate to another lab, which is categorized as a biosafety level 2 lab (BSL 2). Here they can help us sequence the RNA or DNA to tell us, if it is safe to continue working with.





# Growth phases

## The growth curve

In a closed system or batch culture (no food added, no waste removed) bacteria will grow in a predictable pattern, resulting in a growth curve composed of four distinct phases of growth: the lag phase, the exponential or log phase, the stationary phase, and the death or decline phase. In the lab we do not work with a 100% closed system, so our growth curves would not look exactly as drawn below, but it can still give an indication of how the bacterial lifecycle works (Wang et al., 2015).

## Lag phase

An adaptation period, where the bacteria are adjusting to their new conditions. Cells in the lag period are synthesizing RNA, enzymes, and essential metabolites that might be missing from their new environment (such as growth factors or macromolecules), as well as adjusting to environmental changes such as changes in temperature, pH, or oxygen availability. This phase is also where pigment is generated.

## Exponential growth phase

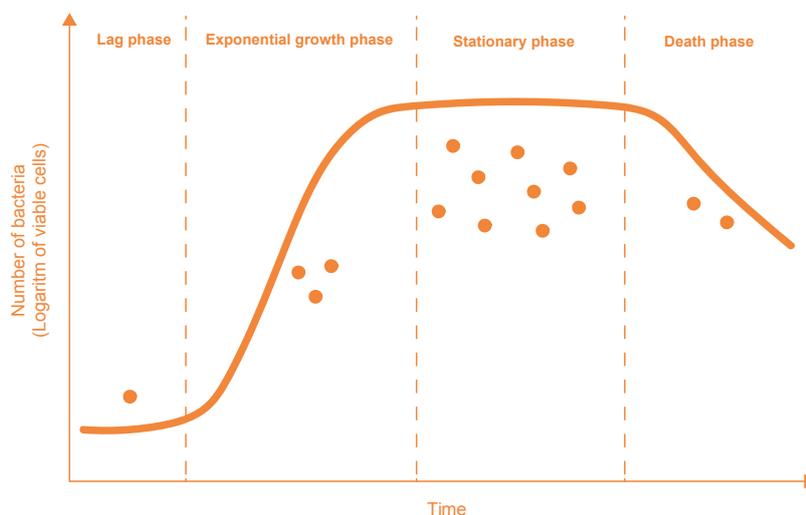
Once cells have accumulated all that they need for growth, they proceed into cell division, where 1 cell becomes 2 cells, becomes 4, becomes 8 etc. Pigment production can also take place in this phase.

## Stationary phase

At some point the bacterial population runs out of an essential nutrient/chemical or its growth is inhibited by its own waste products or lack of physical space. Pigment production can also take place in this phase.

## Death phase

The number of viable cells decreases in a predictable (or exponential) fashion. The steepness of the slope corresponds to how fast cells are losing viability.





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# Pigment production

## Quorum sensing

The regulation of pigments production is called quorum sensing. Quorum sensing uses signaling molecules (or autoinducers) as a form of communication. In this process bacteria detects and responds to changes in cell population density upon changes in its environment and produce pigment.

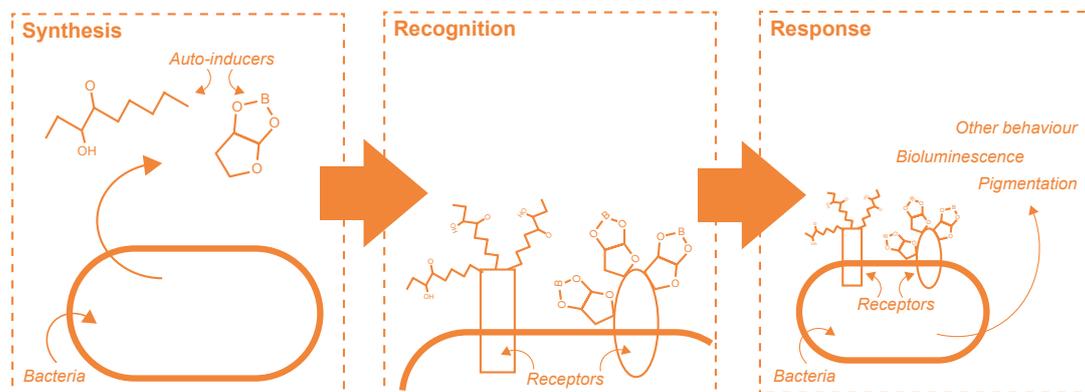
Bacteria and their pigment inducing capabilities are not just a form of communication but are indicators of compatibility and of multispecies living. Bacterial pigmentation is a result of an autopoietic system, one that is organized to continuously reproduce its own parts and structure (Mohammadi et al., 2012).

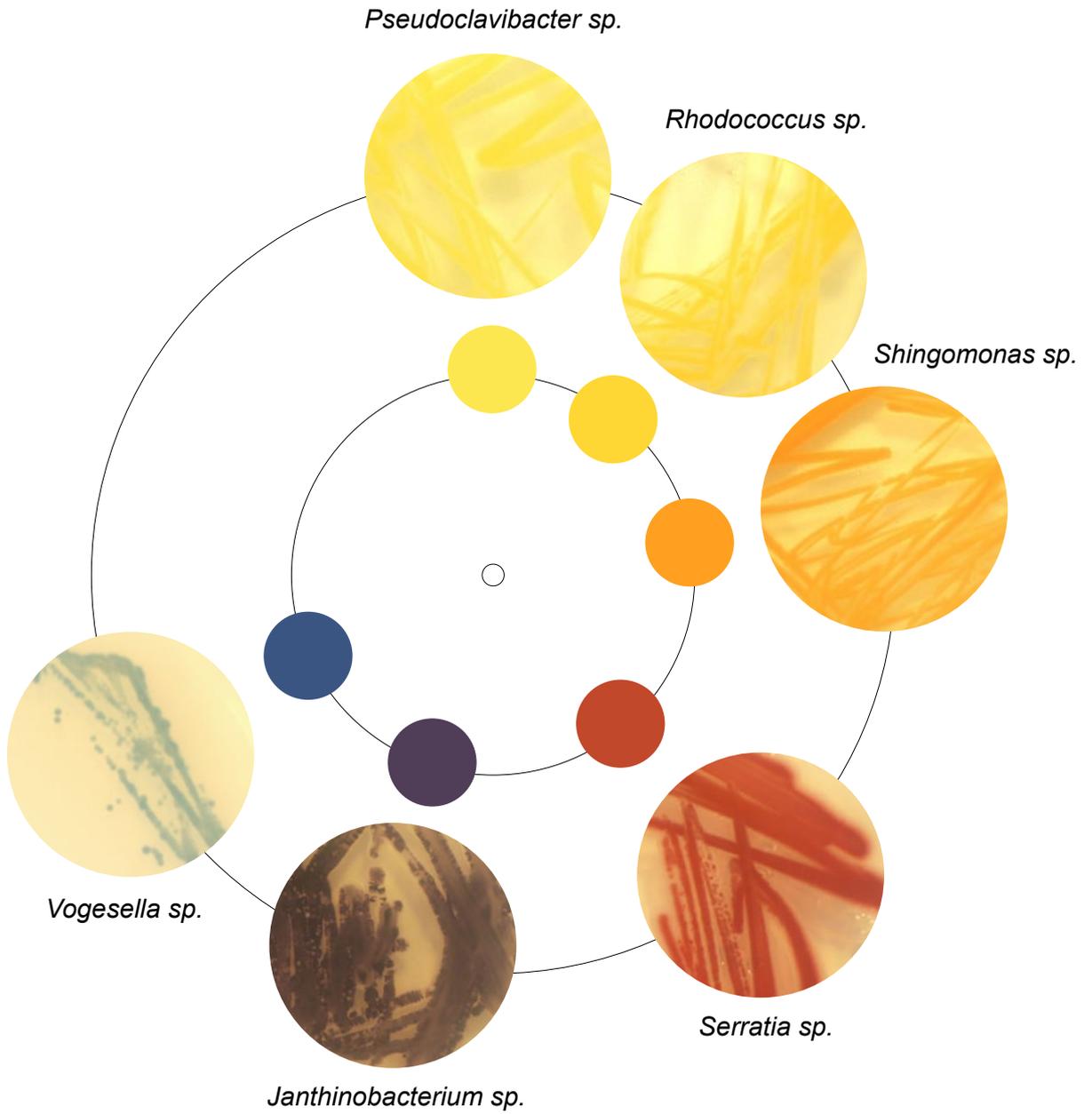
For the bacteria to use quorum sensing, they must possess three characteristics (Rutherford et al., 2012; iGEM, 2021):

**Synthesis:** to secrete a signaling molecule, an auto-inducer.

**Recognition:** detect the change in concentration of signaling molecules.

**Response:** regulate gene transcription as a response.





# Bacteria collection

In the biolab we only have microorganism categorized as BSL-1, thus being relatively safe to work with, when taking the right safety precautions.

## **Janthinobacterium lividum**

This bacteria is an aerobic, Gram-negative, soil-dwelling bacterium that has a distinctive dark-violet (almost black) color, due to a compound called violacein. Violacein has antibacterial, antiviral and antifungal properties. It has an optimal growth temperature for pigment production at 25-30 C<sup>0</sup> (Oh et al., 2019).

## **Serratia marcescens**

This bacteria is a rodshaped, anaerobic, Gram-negative, soil-dwelling bacterium that has a distinctive red color, due to a compound called prodigiosin. Prodigiosin has biological activities including antimalarial, antifungal, immunosuppressant and antibiotic agents. It has an optimal growth temperature for pigment production at 25-30 C<sup>0</sup> (Haddix & Shanks, 2018).

## **Rhodococcus sp.**

This bacteria is an aerobic, nonsporulating, nonmotile, Gram-positive, soil-dwelling bacteria that has a distinctive yellow-orange color, due to a compound called carotenoid. It has an optimal growth temperature for pigment production at 25-30 C<sup>0</sup> (Cappelletti et al., 2020).

## **Pseudoclavibacter sp.**

This bacteria is a Gram-positive, non-spore-forming, strictly aerobic and non-motile genus, soil-dwelling bacteria that has a distinctive yellow color, due to a compound called carotenoid. It has an optimal growth temperature for pigment production at 25-30 C<sup>0</sup> (Oyaert et al, 2013).

## **Shingomonas sp.**

This bacteria is an Gram-negative, rod-shaped, chemoheterotrophic, strictly aerobic bacteria, soil-dwelling bacteria that has a distinctive orange color, due to a compound called carotenoid. It has an optimal growth temperature for pigment production at 25-30 C<sup>0</sup> (Feng et al, 2014).

## **Vogesella indigofera**

This bacteria is an aerobic, Gram-negative, soil-dwelling bacterium that has a distinctive blue (dark blue) color, due to a compound called indigoidine. It has an optimal growth temperature for pigment production at 25-30 C<sup>0</sup> (Yu et al., 2020).



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# Storing bacteria

## **Agar stock**

Bacteria on an LB agar plate can be stored at 4°C for a few weeks to be sure the bacteria are still alive. The agar plate is wrapped with parafilm around the edge to prevent contamination. We only have a fridge available in the lab, so for now, we store the bacteria here. It is important to mark your plate with name, date and content, otherwise it will be removed.

## **Glycerol stock**

In case you want to store bacteria for a longer time, you will need to make glycerol stocks by mixing bacteria, water and glycerol. The addition of glycerol stabilizes the frozen bacteria, preventing damage to the cell membranes and keeping the cells alive. A glycerol stock of bacteria can be stored stably at -80°C for several years. We cannot store glycerol stock at the biolab, so in the case you want to store a microorganism for a longer time, we will have to contact a natural science lab. Contact Monica Hartvigsen if you need help to contact a science lab in regards of making glycerol stocks.



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# 3 Recipes



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## Experiment #1: Preparing LB liquid media

Equipment: Ethanol  
Gloves  
Mask  
Sterile petri dishes  
Bunsen burner  
LB Broth  
Agar  
Demineralised water  
Pressure cooker  
Fridge  
Paper towel  
Autoclave tape

### Prepare LB medium:

1. Measure 20g LB broth powder and 1L demineralised water.
2. Put in glass container suitable for autoclaving and shake.
3. Put a piece of autoclave tape on the lid of the glass containers.
4. Place in pressure cooker with the lid placed loosely on the top and cook for minimum 30min.  
OBS! Remember to add water to the pressure cooker.
5. Let it cool a bit.
6. Close the lids to make it as airtight as possible.
7. Store until use.

### Make sterile LB liquid medium plates:

1. Put on gloves and a mask.
2. Wipe the table surface with ethanol. Wait for the ethanol to evaporate.
3. Turn on the bunsen burner.
4. Pour in sterile petridishes. If you use a bunsenburner, heat the edge of the glass between each pour. You should fill approx. 1/2 of the petri dish.
5. Turn off the bunsen burner.
6. Clean the table surface with ethanol.

### Storage:

Keep in fridge. Remember to write name, date and content on your petri dishes e.g. *Monica, 12.05.12, LB*.

If you keep the prepared medium in the fridge, do not place it together with food.





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**WELFORD**

**SUGAR** High Gel Strength, Powder

MOLECULAR BIOLOGY GRADE

## Experiment #2: Preparing LB agar plates

Equipment: Ethanol  
Gloves  
Mask  
Sterile petri dishes  
Bunsen burner  
LB Broth  
Agar  
Demineralised water  
Pressure cooker  
Fridge  
Paper towel  
Autoclave tape

### Prepare LB agar:

1. Measure 20g LB broth powder, 12-15g agar powder and 1L demineralised water.
2. Pur in glass and stir with a spoon.
3. Put a piece of autoclave tape on the lid of the glass containers.
4. Place in pressure cooker with the lid placed loosely on the top and cook for minimum 30min.  
OBS! Remember to add water to the pressure cooker.
5. Let it cool a bit.
6. Close the lids to make it as airtight as possible.

### Make sterile LB agar plates:

1. Put on gloves and a mask.
2. Wipe the table surface with ethanol. Wait for the ethanol to evaporate.
3. Turn on the bunsen burner.
4. Pour in sterile petridishes, when still hot. If you use a bunsenburner, heat the edge of the glass between each pour. You should fill approx. 1/2 of the petri dish.
5. When solidified place the plates upside down in a plastic bag.
6. Turn off the bunsen burner.
7. Clean the table surface with ethanol.

### Storage:

Keep the prepared petri dishes in the fridge. Remember to write name, date and content on your bag e.g. *Monica, 12.05.12, LB agar.*

If you keep the petridishes in a fridge, do not place it together with food.





## Experiment #3: Bacteria inoculation to LB liquid media

Equipment: Ethanol  
Gloves  
Mask  
Sterile petri dishes  
Sterile inoculations loop  
Lighter  
Bunsen burner  
Bacterial sample  
Sterile LB liquid medium  
Paper towel  
Incubator

### Bacterial inoculation:

1. Put on gloves and a mask.
2. Wipe the table with ethanol and let it dry.
3. Take your bacterial sample out of the fridge and take it to your sterile workarea.
4. Write name, date and content on the sterile petri dish.  
E.g. *Monica*, 12.05.12, LB, *S. Marcescens*.
5. Turn on the bunsen burner.
6. Find your sterile inoculation loop.
7. Take a small sample from the liquid sample and try to keep the lid as closed as possible.
8. Take the sample to your fresh petridish and shake the sterile loop in the liquid LB medium.
9. Turn off the bunsen burner.
10. Clean the table surface with ethanol.

### Growth conditions and storage:

1. The newly prepared bacterial samples are stored in the incubator (the white styrofoam boxes placed in the biolab) at room temperature and the light turned on.
2. Check the bacteria specie to find out about the exact growth conditions. The bacteria we have in the biolab grow at room temperature.
3. Leave it to grow for some days (3-5 days).
4. Wrap the bacterial sample in parafilm and store it in the fridge to keep it alive.

### Waste management:

5. The gloves should be autoclaved before thrown in the waste bin.
6. Find an autoclave bag and put the gloves in. Close it with a piece of striped autoclaved tape. Cook for 30min. in the pressure cooker





## Experiment #4: Bacteria inoculation to LB agar plates

Equipment: Ethanol  
Gloves  
Mask  
Sterile petri dishes  
Sterile inoculations loop  
Lighter  
Bunsen burner  
Bacterial sample  
Sterile LB liquid medium  
Paper towel  
Incubator

### Bacterial inoculation:

1. Put on gloves and a mask.
2. Wipe the table with ethanol and let it dry.
3. Take your bacterial strain sample out of the fridge and take it to your sterile workarea.
4. Write name, date and content on the sterile LB agar petri dish. E.g. *Monica*, 12.05.12, LB agar, *S. Marcescens*.
5. Find your sterile inoculation loop.
6. Take a small sample from the liquid sample with the sterile inoculation loop and try to keep the lid as closed as possible.
7. Take the sample to your prepared LB agar plate and smear the sample on to the surface of the plate.
8. Turn off the bunsen burner.
9. Clean the table surface with ethanol.

### Growth conditions and storage:

1. The newly prepared bacterial samples are stored in the incubator (the white styrofoam boxes placed in the biolab) at room temperature and the light turned on.
2. Check the bacteria species to find out about the exact growth conditions. The bacteria we have in the biolab grow at room temperature.
3. Leave it to grow for some days (3-5 days).
4. Wrap the bacterial sample in parafilm and store it in the fridge to keep it alive. Or kill the bacteria using the pressure cooker and autoclave bags.

### Waste management:

5. The gloves should be autoclaved before thrown in the waste bin.
6. Find an autoclave bag and put the gloves in. Close it with a piece of striped autoclaved tape. Cook for 30min. in the pressure cooker





## Experiment #5: Coloring textile with live bacteria

Equipment:	Ethanol	Textile
	Gloves	Autoclave bag
	Sterile petri dishes	Autoclave tape
	Sterile inoculations loop	Mask
	Lighter	
	Bunsen burner	
	Bacterial sample	
	Sterile LB liquid medium	
	Paper towel	
	Incubator	

### Prepare textile:

1. Cut the textile in the wanted size.
2. Place the textiles in an autoclave bag.
3. Close the bag with a piece of autoclave tape.
4. Place the autoclave bag in the pressure cooker.
5. Cook for 30min.

### Bacterial inoculation:

1. Put on gloves and a mask.
2. Wipe the table with ethanol and let it dry.
3. Take your bacterial strain sample out of the fridge and take it to you sterile workarea.
4. Find your sterile inoculation loop.
5. Take a small sample from the liquid sample with the sterile inoculation loop and try to keep the lid as closed as possible.
6. Take the sample to your prepared LB agar plate or LB medium and smear the sample on to the surface of the plate or into the liquid.
7. **PLACE THE STERILE TEXTILE IN/ON YOUR BACTERIAL SAMPLE.**
8. Turn off the bunsen burner.
9. Clean the table surface with ethanol.

### Growth conditions and storage:

1. The prepared bacterial samples **WITH TEXTILE** are stored in the incubator (the white styrofoam boxes placed in the biolab) and the light turned on.
2. The bacteria we have in the biolab grow at room temperature.
3. Leave it to grow for some days (3-5 days).
4. Kill the bacteria, petri dishes, sterile loops and gloves using the pressure cooker and autoclave bags.





## Experiment #6: Coloring BIG pieces of textile with live bacteria

Equipment:	Ethanol	Textile
	Gloves	Autoclave bag
	Sterile petri dishes	Autoclave tape
	Sterile inoculations loop	Mask
	Lighter	
	Bunsen burner	
	Bacterial sample	
	Sterile LB liquid medium	
	Paper towel	
	Incubator	

### Prepare textile:

1. Cut the textile in the wanted size.
2. Place the textiles in an autoclave bag.
3. Close the bag with a piece of autoclave tape.
4. Place the autoclave bag in the pressure cooker.
5. Cook for 30min.

### Bacterial inoculation:

1. Put on gloves and a mask.
2. Wipe the table with ethanol and let it dry.
3. Take your bacterial strain sample out of the fridge and take it to you sterile workarea.
4. Find your sterile inoculation loop.
5. Take a small sample from the liquid sample with the sterile inoculation loop and try to keep the lid as closed as possible.
- 6. Find your bag with the sterilized textile.**
7. Open the bag as close to the busen burner as possible.
8. Add some sterile LB liquid medium to the bag.
9. Take a small bacteria sample with the sterile inoculation loop and shake in the bag.
10. Turn off the bunsen burner.
11. Clean the table surface with ethanol.

### Growth conditions and storage:

1. The newly prepared bacterial samples **WITH TEXTILE** are stored in the incubator (the white styrofoam boxes placed in the biolab) at room temperature and the light turned on
2. The bacteria we have in the biolab grow at room temperature.
3. Leave it to grow for some days (3-5 days).
4. Kill the bacteria, petri dishes, sterile loops and gloves using the pressure cooker and autoclave bags.





## Experiment #7: Stamping pieces of textile with live bacteria

Equipment:	Ethanol	Textile
	Gloves	Autoclave bag
	Sterile petri dishes	Autoclave tape
	Sterile inoculations loop	Mask
	Lighter	
	Bunsen burner	
	Bacterial sample	
	Sterile LB liquid medium	
	Paper towel	
	Incubator	

### Stamping on to textile:

1. Cut the textile in the wanted size.
2. Put on gloves and a mask.
3. Wipe the table with ethanol and let it dry.
4. Take you bacterial strain sample to your sterile workarea.
5. Place the textile on top of the bacteria grown on the LB agar plate.
6. Use your fingers to transfer pigment from the plate to the textile.
7. Gently remove the textile.

### Fixating pigment to the textile:

1. Place the textiles in an autoclave bag. As well as petri dishes, sterile loops and gloves.
2. Close the bag with a piece of autoclave tape.
3. Place the autoclave bag in the pressure cooker.
4. Cook for 30min.  
OBS! Remember to add water to the pressure cooker.
5. Clean the table surface with ethanol.





## Experiment #8: Coloring textile with harvested pigment

Equipment:	Ethanol	Textile
	Gloves	Autoclave bag
	Sterile petri dishes	Autoclave tape
	Sterile inoculations loop	Mask
	Lighter	
	Bunsen burner	
	Bacterial sample	
	Sterile LB liquid medium	
	Paper towel	
	Incubator	

### Prepare textile:

1. Cut the textile in the wanted size.

### Killing bacteria to harvest pigment:

1. Put on gloves and a mask.
2. Wipe the table with ethanol and let it dry.
3. Take you bacterial samples, which have produced pigment in the liquid media, to your sterile workarea.
4. Put the liquid into a glass container.
5. Place the glass container in the pressure cooker.
6. Cook for 30min.
7. Clean the table surface with ethanol.

### Dyeing with the harvested pigment:

1. Find the amount of glass containers you need.
2. Place the prepared textiles into glass containers.
3. Add the harvested pigment from the previous step.
4. Place the glass containers in the pressure cooker. As well as petri dishes, sterile loops and gloves.
5. Cook for 30min.  
OBS! Remember to add water to the pressure cooker.
6. Clean the table surface with ethanol.





## Experiment #9: Coloring biomaterials with harvested pigment

### Equipment:

Ethanol  
Gloves  
Sterile petri dishes  
Sterile inoculations loop  
Lighter  
Bunsen burner  
Bacterial sample  
Sterile LB liquid medium  
Paper towel  
Incubator

Textile  
Autoclave bag  
Autoclave tape  
Mask

### Prepare material:

1. Find the material you want to dye e.g. PLA filament.

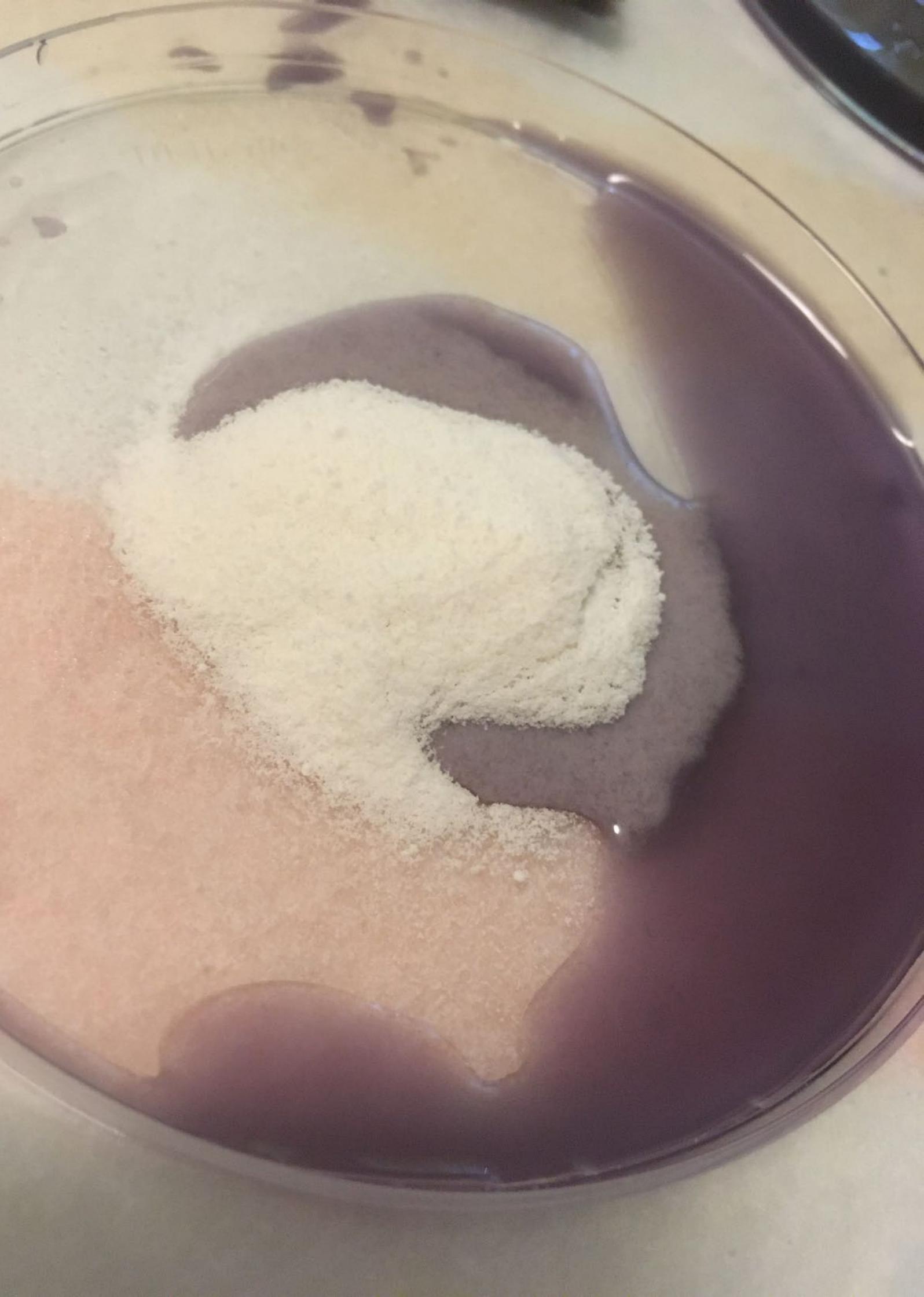
### Killing bacteria to harvest pigment:

1. Put on gloves and a mask.
2. Wipe the table with ethanol and let it dry.
3. Take your bacterial samples, which have produced pigment in the liquid media, to your sterile workarea.
4. Put the liquid into a glass container.
5. Place the glass container in the pressure cooker.
6. Cook for 30min.
7. Clean the table surface with ethanol.

### Dyeing with the harvested pigment:

1. Find the amount of glass containers you need.
2. Place the material(s) into glass containers.
3. Add the harvested pigment from the previous step.
4. Place the glass containers in the pressure cooker.
5. Cook for 30min.  
OBS! Remember to add water to the pressure cooker.
6. Clean the table surface with ethanol.





## Experiment #10: Printing on textile with harvested pigment

Equipment:	Ethanol	Textile
	Gloves	Autoclave bag
	Sterile petri dishes	Autoclave tape
	Sterile inoculations loop	Mask
	Lighter	Binder (Gummi arabicum)
	Bunsen burner	
	Bacterial sample	
	Sterile LB liquid medium	
	Paper towel	
	Incubator	

### Prepare textile:

1. Cut the textile in the wanted size.

### Killing bacteria to harvest pigment:

1. Put on gloves and a mask.
2. Wipe the table with ethanol and let it dry.
3. Take you bacterial samples, which have produced pigment in the liquid media, to your sterile workarea.
4. Put the liquid into a glass container.
5. Place the glass container in the pressure cooker.
6. Cook for 30min.
7. Clean the table surface with ethanol.

### Printing with the harvested pigment:

1. Add a binder to you harvested pigment e.g. gummi arabicum.
2. Mix it together to get a homogen paste.
3. Use the print paste to print your textile in your wanted pattern.

### Fixating the print to the textile:

1. Use a heat iron to fixate the print onto the textile. **THIS IS DONE IN THE BIOLAB and NOT IN THE TEXTILE WORKSHOP.**
2. Place the textiles in a pressure cooker and cook for 30min. to make sure all the bacteria are killed.  
OBS! Remember to add water to the pressure cooker.
3. Clean the table surface with ethanol.





## Experiment #11: Waste management

Equipment: Pressure cooker  
Textile  
Autoclave bag  
Autoclave tape  
Gloves  
Mask  
Bacterial samples  
Ethanol  
Paper towel  
Glass container

### Waste management:

Since you are working with biological material, it is important to kill the bacteria before throwing anything, that has been in contact with the living bacteria, in the trash.

### Safely kill bacteria:

1. Put on gloves and a mask.
2. Separate the textile and the petri dishes from each other.
3. Put the textiles in one autoclave bag
4. Put the petridishes, used sterile loops and gloves in another autoclave bag. If you have used anything else, when working with the bacteria, this should also be autoclaved.
5. Remember to open the petri dishes otherwise a vacuum can form and the petri dishes can explode in the pressure cooker.
6. If you have any liquid, put it in a glass container and place it in the pressure cooker as well.
7. Place the autoclave bags in the pressure cooker.
8. Cook for 30min.  
OBS! Remember to add water to the pressure cooker.
9. The waste is now safe to throw out in the normal trash bins.
10. The textile is also safe to touch without gloves.
11. Clean the workspace with ethanol.





PM-996

PARAFILM

PARAFILM  
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LABORATORY FILM

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Bemis

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Bemis



## Experiment #12: Storing bacteria - parafilming

Equipment: Pressure cooker  
Textile  
Autoclave bag  
Autoclave tape  
Gloves  
Mask  
Bacterial samples  
Ethanol  
Paper towel  
Glass container

### LB agar plates stock:

1. Put on gloves.
2. Cut a piece of parafilm.
3. Wrap it around the petri dish stretching the parafilm to reach all the way around the plate.
4. Put the parafilmed petri dish in the fridge.
5. Remember to mark the petri dish with date, content and name.





# 4 Video tutorials



**Preparing LB liquid media**  
[LINK TO VIDEO](#)



**Preparing LB agar plates**  
[LINK TO VIDEO](#)



**Bacteria inoculation to LB liquid media**  
[LINK TO VIDEO](#)



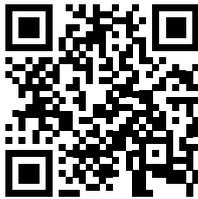
**Bacteria inoculation to LB agar plates**  
[LINK TO VIDEO](#)



**Coloring textile with live bacteria**  
[LINK TO VIDEO](#)



**Stamping textile with live bacteria**  
[LINK TO VIDEO](#)



**Coloring with harvested pigment**  
[LINK TO VIDEO](#)



**Printing textile with harvested pigment**  
[LINK TO VIDEO](#)



**Parafilming**  
[LINK TO VIDEO](#)



**Waste management**  
[LINK TO VIDEO](#)



# 5 Glossary

**Aerob:** an organism (such as a bacterium) that lives only in the presence of oxygen.

**Anaerob:** an organism (such as a bacterium) that lives in the absence of free oxygen.

**Agar:** a gelatinous colloidal extract of a red alga (as of the genera *Gelidium*, *Gracilaria*, and *Eucheuma*) used especially in culture media or as a gelling and stabilizing agent in foods.

**Agar plate:** a Petri dish that contains a growth medium solidified with agar, used to culture microorganisms. Sometimes selective compounds are added to influence growth, such as antibiotics.

**Aseptic:** preventing infection or free or freed from pathogenic microorganisms.

**Autoclave bag:** Autoclave or sterilisation bags are supplied for the secure containment of items intended for autoclaving, steam sterilisation, disposal or incineration.

**Autopoesis:** the property of a living system (such as a bacterial cell or a multicellular organism) that allows it to maintain and renew itself by regulating its composition and conserving its boundaries.

**Bacteria:** Microscopic single-celled organisms lacking a distinct nucleus are known as bacteria. They may be shaped like spheres, rods, or spirals. They inhabit virtually all environments, including soil, water, organic matter, and the bodies of animals.

**Biosafety level:** or pathogen/protection level, is a set of biocontainment precautions required to isolate dangerous biological agents in an enclosed laboratory facility. The levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4).

**Carbohydrate:** are the main source of energy for the body. They are the sugars, starches, and dietary fiber that occur in plant foods and dairy products. Carbohydrates are mainly found in plant foods. They also occur in dairy products in the form of a milk sugar called lactose.

**DNA:** Deoxyribonucleic Acid (DNA) is the chemical name for the molecule that carries genetic instructions in all living things. The DNA molecule consists of two strands that wind around one another to form a shape known as a double helix. Each strand has a backbone made of alternating sugar (deoxyribose) and phosphate groups. Attached to each sugar is one of four bases--adenine (A), cytosine (C), guanine (G), and thymine (T). The two strands are held together by bonds between the bases; adenine bonds with thymine, and cytosine bonds with guanine. The sequence of the bases along the backbones serves as instructions for assembling protein and RNA molecules.

**Endorgonic reaction:** (also called a heat absorbing nonspontaneous reaction or an unfavorable reaction) is a chemical reaction in which the standard change in free energy is positive, and an additional driving force is needed to perform this reaction.

**Enzyme:** a substance that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process.

**Ethanol:** (also called ethyl alcohol, grain alcohol, drinking alcohol, or simply alcohol) is an organic chemical compound.

**Exergonic reaction:** is a chemical reaction where the change in the free energy is negative (there is a net release of free energy).

**Fermentation:** is a metabolic process that produces chemical changes in organic substrates through the action of enzymes. In biochemistry, it is narrowly defined as the extraction of energy from carbohydrates in the absence of oxygen.

**Genetically Modified Organism:** (GMO) can be defined as organisms (i.e. plants, animals or microorganisms) in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination.

**Gram-positive bacteria:** bacteria with thick cell walls.

**Gram-negative bacteria:** bacteria with thin cell walls.

**Growth media:** or culture medium is a solid, liquid, or semi-solid designed to support the growth of a population of microorganisms or cells via the process of cell proliferation or small plants like the moss *Physcomitrella patens*. Different types of media are used for growing different types of cells.

**Incubator:** an apparatus with a chamber used to provide controlled environmental conditions especially for the cultivation of microorganisms or the care and protection of premature or sick babies.

**Inoculation:** to introduce (something, such as a microorganism) into a suitable situation for growth.

**Inorganic compound:** any substance in which two or more chemical elements (usually other than carbon) are combined, nearly always in definite proportions.

**LB Broth:** is a nutritionally rich medium primarily used for the growth of bacteria.

**Metabolism:** is a term that is used to describe all chemical reactions involved in maintaining the living state of the cells and the organism.

**Nutrients:** a nutrient is a substance used by an organism to survive, grow, and reproduce.

**Organic compound:** any of a large class of chemical compounds in which one or more atoms of carbon are covalently linked to atoms of other elements, most commonly hydrogen, oxygen, or nitrogen.

**Oxidative agent:** a substance that oxidizes something especially chemically (as by accepting electrons).

**Parafilm:** is a semi-transparent, flexible film composed of a proprietary blend of waxes and polyolefins. It is a ductile, malleable, non-toxic, tasteless and odorless, and self-sealing thermoplastic.

**Petri dish:** a Petri dish (alternatively known as a Petri plate or cell-culture dish) is a shallow transparent lidded dish that biologists use to hold growth medium in which cells can be cultured, originally, cells of bacteria, fungi and small mosses.

**Pigment:** A particle or substance that has a specific colour. a powdered substance that is mixed with a liquid in which it is relatively insoluble and used especially to impart color to coating materials (such as paints) or to inks, plastics, and rubber.

**Protein:** any of various naturally occurring extremely complex substances that consist of amino-acid residues joined by peptide bonds, contain the elements carbon, hydrogen, nitrogen, oxygen, usually sulfur, and occasionally other elements (such as phosphorus or iron), and include many essential biological compounds (such as enzymes, hormones, or antibodies).

**Protocol:** a detailed plan of a scientific or medical experiment, treatment, or procedure.

**RNA:** ribonucleic acid (RNA), complex compound of high molecular weight that functions in cellular protein synthesis and replaces DNA (deoxyribonucleic acid) as a carrier of genetic codes in some viruses. RNA consists of ribose nucleotides (nitrogenous bases appended to a ribose sugar) attached by phosphodiester bonds, forming strands of varying lengths. The nitrogenous bases in RNA are adenine, guanine, cytosine, and uracil, which replaces thymine in DNA.

**Sterile:** completely clean and free from dirt and bacteria.

**Streaking:** is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. Samples can then be taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested.

**Substrate:** the surface or material on or from which an organism lives, grows, or obtains its nourishment or the substance on which an enzyme acts.

**Reducing agent:** a substance that reduces a chemical compound usually by donating electrons.

**Tissue:** any of the distinct types of material of which animals or plants are made, consisting of specialized cells and their products.





# 6 Suggested readings



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