

Risk Declaration

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Experiment: PCB degradation by yeast

1. Description of experiment:

Short and precise, explaining the different steps in your experiment.

Assemble genes in easy-clone vectors in *E.coli*. Extraction, restriction digestion and purification of gene cassettes. Yeast Transformation; Integration of PCB degradation genes in yeast. Verification of working degradation in yeast using dissolved PCB solution.

New experiment

✓ *Addition to previously made declarations (if so, state name and date of previous one)*

Tilia; Emma; Dharmik 13/8 **PCB degradation with yeast**

2. KLARA Risk Assessment:

Specify risks assessments that are relevant to your experiment, e.g. SB/IB Handling of bases. Use the information when you summarize the risks and how to minimize them under sections 4 and 5.

I have read the following risk assessments in KLARA/binder:

SB/IB - Rotary Shakers/Incubators;

SB/IB - -80°C Freezer

SB/IB - Heat Block

SB/IB - Water Bath

SB/IB - Sterile work

SB/IB - Small centrifuges

SB/IB - Gelelectrophoresis /w GelRed

SB/IB - Vertical Autoclave/Benchtop autoclave

SB/IB- Thermal Cycler; SB/IB PCR machine

















3. Microorganisms

Specify what species, if any, that you will handle during the experiment. Also clearly state, what biosafety level the organism is classified as, according to BIO microorganism list (available on the servers).

During this experiment E.coli DH5 alpha and S.cerevisiae IMX585 will be used. Both organisms have a biosafety level of 1, not hazardous.





4. Chemicals:

Specify MSDS read and safety information for all chemicals in your experiment. For every chemical, specify the chemical name, CAS-number, the highest concentration handled (if applicable), CLP hazard pictogram(s) (use table below) and hazard statement(s). If no pictograms are available, write "None".

CLP hazard pictograms in accordance to EG 1272/2008								
								
Gas under pressure	Explosive	Oxidizing	Flammable	Corrosive	Health hazard	Acute toxicity	Serious health hazard	Hazardous to the environment
Chemical name and [CAS-No]	Conc. of handled solution	Pictogram(s)		H statement(s)				
Ampicillin sodium salt [69-52-3]	100 mg/mL			H317: May cause an allergic skin reaction. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled.				
Agarose {9012-36-6}	Powder			Not Hazardous				
Aroclor 1260 (PCB) [11096-82-5]	200 µg/mL in methanol	 		H301: Toxic if swallowed H311: Toxic in contact with skin H331: Toxic if inhaled H370: Causes damage to organs H373: May cause damage to organs through prolonged or repeated exposure				
Betaine [590-47-6]	solid			Not Hazardous				
Chloramphenicol [56-75-7]	98%	 		H302 - Harmful if swallowed H350 – May cause cancer				
4-chlorobenzoate [1126-46-1]				Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008				
DMSO	100% pure			Remark H: Skin penetrating substance according to The Swedish Work Environment Authority's provisions and on occupational exposure limit values				
DreamTaq DNA Polymerase				Not Hazardous				
EDTA [60-00-4]	~100% pure	 		H332 - Harmful if inhaled				

			H319 - Causes serious eye irritation H373 - May cause damage to organs through prolonged or repeated exposure if inhaled
GelRed/GelGreen Nucleic Acid Gel Stain	100%		Not hazardous
G418 salt solution [108321-42-2]	50 mg/mL		H317 : May cause an allergic skin reaction H334 : May cause allergy or asthma symptoms or breathing difficulties if inhaled.
KOH [1310-58-3]	~100% pure		H290 : May be corrosive to metals. H302 : Harmful if swallowed. H314 : Causes severe skin burns and eye damage.
Lithium acetate [6108-17-4]	solid		H319 : Causes serious eye irritation
PEG3350 [25322-68-3]	solid		Not Hazardous
Phusion U Hot Start DNA Polymerase			Not Hazardous
PrimeStar DNA Polymerase			Not Hazardous
Restriction enzymes			Not Hazardous
Nourseothricin solution [96736-11-7]	100 mg/mL		Not Hazardous
NaOH [1310-73-2]	solid		H290 : May be corrosive to metals. H314 : Causes severe skin burns and eye damage.
Tris-HCl solution	1 M		
Methanol [67-56-1]			H225 : Highly flammable liquid and vapour H301 : Toxic if swallowed H311 : Toxic in contact with skin H331 : Toxic if inhaled H370 : Causes damage to organs
ssDNA Carrier			Not Hazardous
SDS [151-21-3]	Pure powder		H228 - Flammable solid. H302 - Harmful if swallowed H315 - Causes skin irritation. H318 - Causes serious eye damage. H332 - Harmful if inhaled. H335 - May cause respiratory irritation. H412 - Harmful to aquatic life with long lasting effects.

Addition:

Chemical name and [CAS-No]	Conc. of handled solution	Pictogram(s)	H statement(s)
Acetone [67-64-1]			H225: Very flammable liquid and gas H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness
Biphenyl [92-52-4]			H315- Skin irritation H319- Causes serious eye irritation H335- May cause respiratory irritation. H410 - Very toxic to aqueous organisms with long-term effects
Hexane [110-54-3]			H225: Very flammable liquid and gas H304: H315: Causes skin irritation. H336: May cause drowsiness or dizziness H361F: Suspected of damaging fertility H373: May cause damage to organs through prolonged or repeated exposure H411: Toxic to aquatic life with long-lasting effects
BioRad Tris/Glycine/SDS buffer x10			H319: Causes serious eye irritation
Polyacrylamide			

5. Use of regulated chemicals

Use the chemical information in KLARA to answer the following questions. In KLARA you will find this information listed under the section "Regulations" or in Swedish "Regler och krav".

Note! If your chemical does **not** have a classification, this section will not show up on the KLARA information page.

- a) Are any of the chemicals classified as either a Group A or Group B chemical? If yes, which one(s), and do we have a valid permit?
No

- b) Are any of the chemicals classified as a CMR (Carcinogenic, Mutagenic or Reprotoxic) substance and/or marked with any of the following: H340, 341, 350, 351, 360, 361, 362? Note that there may be letters following the codes sometimes.

YES

If yes:

- i. **Which one(s)?** G418, Aroclor (PCB) Chloramphenicol, **Hexane**
 - ii. **How frequently will you be handling them (times/month)?** Twice a week / Five times in total / twice a month / **five times in total**
- c) **Does any of the chemicals have the hazard statement H317 and/or H334? Yes**
If yes:
- i. **Which one(s)?** Ampicilin, G418
 - ii. **How frequently will you be handling them (times/month)?** 2x a week
 - iii. **Do you have any allergies?** No

6. Comments on risks:

Identify and specify risks associated with reactions or combinations of chemicals, equipment used or other potential risks. Where is the actual element of risk? When do you need to take precautions to work in a safe way?

There might not be any risk associated with reactions of individual or combinations of the chemicals listed above. However, one should always consider the risk of sharp materials such as broken glass. There might also be a risk if the centrifuge is unbalanced, but this will be handled by a experienced coworker in the lab. The same will be applied to the autoclave and other electrical equipment that should be handled with care.

Different gloves should be used depending on what chemical is handled. Orange gloves when working with gel Red, gel Green and red gloves when working with concentrated acid and bases and antibiotics. One should always use proper glove removal and be careful not to touch any exposed skin. Red gloves when handling antibiotics, DMSO and **hexane will be handled in fumehood.**

7. Risk reductions:

7.1 Storage:

Some chemicals can be hazardous if they are not kept in a proper way (e.g. flammable compounds). Specify how you will store those chemicals safely.

Flammable compounds that will be used during the experiment includes Aroclor, Methanol and SDS. These will be handled with care to minimize the risk to oneself and others. Flammables need to be stored away from any open flames in a ventilated cupboard in the balance room. Working solution of corrosive compounds like NaOH, KOH and other acid and bases are stored and kept in fume hood when used. Bulk quantities of acids and bases are stored in cupboard 2 in the big lab. **Aroclor is particularly hazardous and should be handled very carefully. Always store in ventilated flammables cabinet in the balance room.** 4-chlorobenzoate is stored in cool, dry place with the container tightly closed. Biphenyl and DMSO will be stored with the container tightly closed in a dry place – in a ventilated cupboard in the balance room common storage place. **Hexane solutions (will also contain PCB) will be stored in ventilated cupboard).**

7.2 Chemical handling:

Specify how to minimize the risks in handling the chemical(s), (e.g. use of fume hood, ventilation arms, and which type of gloves you need to use). Use the glove guide to find appropriate gloves (outside Balance room at SysBio and on the solvents cupboard at IndBio).

Handling methanol and aroclor will be carried out in fume hood, and red gloves, lab coat and safety glasses will be worn. During tasks when aroclor or methanol is not handled, gloves, lab coat and safety glasses will be worn. Orange gloves will be worn during work with gel Red and gel Green. The antibiotics will be in solution form which is less hazardous but will be handled cautiously. Safety procedures includes wearing red gloves, lab coat and glasses and handle the chemical in fume hood. For other laboratory work, grey gloves will be worn. When handling 4-chlorobenzoate grey gloves will be used and for biphenyl and DMSO we will use orange gloves. **Red gloves will be worn when handling hexane.** All will be handled in fume hood in the balance room and normal measures for preventive self harm and fire protection.

Personal protection needed:

✓ **Gloves and lab coat**

✓ **Safety glasses**

✓ **Chemical hood**

☐ Other, specify:

7.3 Cleaning & decontamination:

a. Specify if any special cleaning of lab-ware or instruments is required (i.e. sterilization).

Address how you will clean glassware from residues (biofilm formation) prior to putting things in the dishwasher.

Media not containing antibiotics can be autoclaved and poured in the sink. Media containing Ampicillin should be autoclaved and then collected separately as liquid biological and chemical waste. Glassware will be rinsed with 70% ethanol and then cleaned in dishwasher.

b. Clarify how and when you will perform a decontamination of your work environment and instruments.

The lab bench will be wiped with ethanol every day before and after laboration. Pipettes will be cleaned with 70 % ethanol if contaminated or dirty.

8. Waste handling:

a. Specify what kind of waste is produced, and how it is handled, labeled and disposed of.

Consider every step in your experiment. Remember that you will likely generate both solid and liquid waste.

Aroclor containing liquids will be collected in a separate container labelled as "PCB waste" and stored on work bench in small quantities. Larger quantities will be kept in the designated area in the balance room as chemical waste. The Green/Red gel and gel containing materials are collected in a separate designated box for gel - Green/red waste. Liquids containing lithium acetate and antibiotics will be considered as chemical waste. Liquids containing NaOH or KOH will be diluted and neutralized till pH 6-8 using HCL and can be poured in the sink (as long as they do not contain any hazardous substance). Solid media containing agar plates will be simply given to the recycle company. When working with Gelgreen/Gelred , orange gloves will be used to ensure safety from penetration through gloves.

Pipette tips, pipettes, cell spreaders and other plasticware will be handled as solid bio-waste ; which will be autoclaved and discarded as plastic waste. Gloves contaminated with hazardous waste can be treated as chemical waste. Otherwise gloves can be treated as a common waste since they are treated with 70% ethanol frequently. **Wastes containing 4-chlorobezoate, biphenyl and DMSO should be labled properly and treated as chemical waste and must be thrown in specific containers. Hexane will be treated as organic solvent. Glasswares containing these chemicals should be rinsed twice with 70% ethanol.**

b. If you have biological waste containing antibiotics, check and state if the antibiotic is inactivated during autoclaving.

Ampicillin and kanamycin are not inactivated during autoclaving and media where it is used must be collected as hazardous waste. The same applies to Nourseothricin. Chloramphenicol is inactivated during autoclaving.

9. Final evaluation of risks

Take into consideration the probability of an accident occurring and the severity of the possible consequences to evaluate the risk of your experiment. Use the matrix.

Probability <i>Of the accident</i> Different factors are taken into consideration <ul style="list-style-type: none"> • Frequency and duration. • Historic events. • Possibility of avoiding or limit the damage; training on the equipment, awareness of the risk, sudden - quick or slow event • Existing protection 	Consequence (Gravitas) <i>If the accident occurs.</i>					
	0. Safe or bagatelle	1. Short sick listing	2. long sick listing	3. Disablement	4. Casualties	5. Many casualties
5. Very common <i>Once a day.</i>	2	3	4	4	4	4
4. Common <i>Once a month.</i>	1	2	3	4	4	4
3. Rather common <i>Once a year.</i>	1	2	3	3	4	4
2. Rare <i>Once every ten years.</i>	1	1	2	3	4	4
1. Unlikely <i>Once every hundred years</i>	0	1	2	2	3	3
0. Very unlikely <i>Less than once every hundred years</i>	0	0	1	1	2	2
0. Negligible risk 1. Acceptable risk, no action needed 2. Some risk, action needed 3. Severe risk, action needed 4. Very severe risk, action needed Modellen framtagen av Previa						

- ☐ Choose one of the following: Acceptable risk
☒ **Some risk**
☐ Severe risk
☐ Very severe risk

I declare that I have read the Risk Assessments and MSDS stated above and that I am aware about the risks involved with this experiment. I will follow the guidelines concerning safety precautions to minimize the risks associated with this experiment.

Signature

The risk declaration has been read by:

Signature of Supervisor

Signature of Research Engineer