Risk Declaration

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Date: 20190531
Addition date: 20190828
Experiment: PCB degradation with yeast.

1. Description of experiment:

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

☐ ***New experiment***

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

Previous: 20190531 PCB degradation with yeast.

Procedure: EasyClone plasmids will be assembled *E. coli*, gene cassetes from the plasmids will be extracted and purified and introduced into the genome of *Saccharomyces cerevisiae* using CRISPR/Cas9. Degradation will be measured by exposing the cells to a solution containing low concentrations of PCB. Standard protocols will be used throughout the experiment, including PCR, Gibson assembly, restriction digestion, purification, gel electrophoresis.

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).

The following microorganisms will be used during the experiment
Escherichia coli - Biosafety level 1
Saccharomyces cerevisiae - Biosafety level 1

4. Chemicals:

Specify MSDS read and safety information for <u>all</u> 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								
								
	Explosive	Oxidizing	Flammable	Corrosive	Health hazard	Acute toxicity		

Gas under pressure							Serious health hazard	Hazardous to the environment

Chemical name and [CAS-No]	Conc. of handled solution	Pictogram(s)	H statement(s)
Abscisic acid [21293-29-8]	solid	none	Not Hazardous
Agarose [9012-36-6]		none	Not Hazardous
Ampicillin [69-53-4]	100 mg/ml		**H315:** Causes skin irritation. **H317:** May cause an allergic skin reaction. **H319:** Causes serious eye irritation. **H334:** May cause allergy or asthma symptoms or breathing difficulties if inhaled. **H335:** May cause respiratory irritation. May cause allergic skin reactions, asthma symptoms and breathing problems
Aroclor 1260 [11096-82-5]	200 µg/ml		**H225:** Very flammable liquid and gas **H301:** Toxic if swallowed **H311:** Toxic in contact with skin **H331:** Toxic if breathed in **H302:** Harmful if swallowed **H370:** Causes damage to organs **H371:** May cause damage to organs **H373:** May cause damage to organs through prolonged exposure **H400:** Very toxic to aqueous organisms **H410:** Very toxic to aqueous organisms with long-term effects
Betaine [107-43-7]	solid	none	
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
Chloramphenicol [56-75-7]	98%		**H302 -** Harmful if swallowed **H350** – May cause cancer
DreamTaq DNA Polymerase		none	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%	none	Not hazardous
Glycerol [56-81-5]	99% pure, liquid	none	
Kanamycin/G418 [64013-70-3]	200mg/ml		**H360:** May damage fertility. May damage the unborn child
KOH [1310-58-3]	~100% pure		**H290:** May be corrosive to metals. **H302:** Harmful if swallowed. **H314:** Causes severe skin burns and eye damage.
Methanol [67-56-1]	100% pure		**H225:** Very flammable liquid and gas **H301:** Toxic if swallowed **H311:** Toxic in contact with skin **H331:** Toxic if breathed in **H370:** Causes damage to organs
NaOH	solid		**H290:** May be corrosive to metals. **H314:** Causes severe skin burns and eye damage.
Nourseothricin [96736-11-7]	100mg/ml		**H302:** Harmful if swallowed.
Lithium acetate [546-89-4]	Solid		**H319:** Causes serious eye irritation
PEG3350 [25322-68-3]	Solid	none	Not hazardous
Phusion U Hot Start DNA Polymerase		none	Not hazardous
Restriction enzymes		none	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.

ssDNA Carrier		none	Not hazardous
Tris-HCl solution	1 M	none	

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
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
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
Concentrated sulfuric acid	95-97%		**H290** May be corrosive to metals **H314** Causes severe skin burns and eye damage

1,3,5-trichlorobenzene			**H302:** Harmful if swallowed **H312:** Harmful in contact with skin. **H315:** Causes skin irritation. **H319:** Causes serious eye irritation. **H332:** Harmful if inhaled. **H335:** May cause respiratory irritation. **H412:** Harmful to aquatic life with long lasting effects.

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?

☐ **YES** ☑ **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** ☐ **NO**

If yes:

i. Which one(s)?

G418, Chloramphenicol**, Hexane**

ii. How frequently will you be handling them (times/month)?

Once per month, **twice per month, four times in total.**

c) Does any of the chemicals have the hazard statement H317 and/or H334? If yes:

i. Which one(s)?

Ampicillin

ii. How frequently will you be handling them (times/month)?

2x per 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 are risks associated with handling different equipment, such as getting cuts from broken glass, freezing or burning damages from hot or cold equipment like heating blocks, autoclaves or -80°C freezer. There are also risks if other equipment is not handled properly, such as unbalanced centrifuges and electrical equipment. When using gloves, it is important to use proper procedures for glove removal, to prevent accidental skin contact with chemical spills or residues on the gloves. The right gloves must be used, depending on what chemicals are handled; orange gloves when handling NaOH, Gel Green, Gel Red and SDS; red gloves when handling antibiotics, PCBs (Aroclor 1260), KOH, DMSO, **concentrated sulfuric acid, hexane, 1,3,5-trichlorobenzene** and EDTA. Both acids and bases are used, so they need to be stored separately. Aroclor 1260, KOH, EDTA, **concentrated sulfuric acid, hexane** and DMSO **will be handled in fume hoods**. Safety glasses will be used when handling chemicals that can cause harm or irritation of the eyes (H314, H319, H318), including Ampicillin, Nourseothricin, EDTA, KOH, NaOH, Lithium acetate, **concentrated sulfuric acid, acetone, 1,3,5-trichlorobenzene** and SDS.

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.

Methanol, SDS and Aroclor are flammable compounds used in the experiment. These compounds are going to be carefully handled and kept away from open flames. The flammable compounds are going to be stored in the ventilated cupboard in the balance room. Chemicals such as NaOH, KOH, (working solution) and **concentrated sulfuric acid** are stored in fume hood when used. Bulk quantities of the acid and bases used is stored in cupboard 2 in small lab, **except for concentrated sulfuric acid which is stored in cupboard 12 in analytical room 2** . When working with Aroclor it should be handled carefully, the working procedure and working storage should be in the fume hood. Always use the right equipment depending on which chemicals is used. 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. **1,3,5-trichlorobenzene will be dissolved in hexane and therefore stored in a fume hood during use and in a ventilated cabinet for long term storage.**

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).

Lab coat will be worn at all times. Aroclor 1260, methanol, KOH, EDTA, **hexane, 1,3,5-trichlorobenzene, concentrated sulfuric acid** and DMSO will only be handled inside fume hoods, and red gloves will be worn whenever these chemicals are handled. For experimental procedures involving NaOH, SDS, Gel red and/or Gel green. orange gloves will be worn. During procedures not involving any of the above-mentioned chemicals, grey gloves will be worn. Safety glasses will be worn when handling Ampicillin, Nourseothricin, EDTA, KOH, NaOH, Lithium acetate and SDS. When handling 4-chlorobenzoate grey gloves will be used and for biphenyl we will use orange gloves. **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
- ☐ Facial mask
- ☐ Other, specify:

Comments:

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 containing antibiotics should be autoclaved and then collected separately (chemical waste or biological waste). Media not containing antibiotics should be autoclaved and can be poured into the sink. All glassware should be washed with 70% ethanol and then cleaned in the dishwasher.

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

The lab bench will be cleaned with ethanol (70%) every day as well as before the need to work in s sterile environment.

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.

Solutions containing PCB will be collected in a separate, labelled container which will be stored on the bench. Similarly, materials containing Gel red/green, lithium acetate or antibiotics will also be collected in designated containers. Consumables such as pipette tips will be handled as solid bio-waste; this will be autoclaved before being discarded as plastic waste. Gloves, if contaminated with hazardous chemicals will be treated as chemical waste, otherwise they can be treated as common waste. Media not containing any of the above-mentioned chemicals, or antibiotics, will be autoclaved and poured down the drain**. 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. 1,3,5-trichlorobenzene will be mixed with a combustible solvent and treated as chemical waste and stored in a separate container. 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, G418 and 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 *Of the accident* Different factors are taken into consideration • 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) *If the accident occurs.*					
	0. Safe or bagatelle	1. Short sick listing	2. long sick listing	3. Disablement	4. Casualties	5. Many casualties
5. Very common *Once a day.*	2	3	4	4	4	4
4. Common *Once a month.*	1	2	3	4	4	4
3. Rather common *Once a year.*	1	2	3	3	4	4
2. Rare *Once every ten years.*	1	1	2	3	4	4
1. Unlikely *Once every hundred years*	0	1	2	2	3	3
0. Very unlikely *Less then once every hundred years*	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

Signature

Signature

The risk declaration has been read by:

Signature of Supervisor

Signature of Research Engineer