

Risk Declaration

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Date: 2019 – 31/5

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Experiment: iGEM: PCB degradation by yeast

1. Description of experiment:

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

Gene transformation into yeast using Gibson assembly and easyclone system. Amplification of genes using E. coli.

☒ **New experiment**

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

Tim, Ellen 2019-08-13 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).











Saccharomyces cerevisiae IMX585 (1)

Escherichia coli DH5 alpha (1)

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)
PEG3350 [25322-68-3]	solid		Not Hazardous
Restriction enzymes			Not Hazardous
Phusion U Hot Start DNA Polymerase			Not Hazardous
PrimeStar DNA Polymerase			Not Hazardous
DreamTaq DNA Polymerase			Not Hazardous
Sodium Hydroxide [1310-73-2]	solid		H290: May be corrosive to metals. H314: Causes severe skin burns and eye damage.
Betaine [107-43-7]	solid		Not Hazardous
Tris-HCl solution [1185-53-1]	1 M		
EDTA [60-00-4]	~100% pure	 	H319: Causes serious eye irritation. H332: Harmful if inhaled H373: May cause damage to organs through prolonged or repeated exposure
Potassium Hydroxide [1310-58-3]	~100% pure	 	H290: May be corrosive to metals. H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage.
Agarose			Not Hazardous
Arcolor 1260 (PCB) [11096-82-5]	200 mg/L 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
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.
G418 disulfate 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.
PEG3350 [25322-68-3]	Solid		Not Hazardous
Nourseothricin [96736-11-7]	100 mg/mL		Not Hazardous

GelRed/GelGreen Nucleic Acid Gel Stain	100%		Not Hazardous
Lithium acetate [546-89-4]	Solid		H319: Causes serious eye irritation
Methanol [67-56-1]	Pure liquid	 	H225: Highly flammable liquid and vapor H301: Toxic if swallowed H311: Toxic in contact with skin H331: Toxic if inhaled H370: Causes damage to organs
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 long-term effects on aqueous organisms

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
Chloramphenicol [56-75-7]	98%	 	H302 - Harmful if swallowed H350 – May cause cancer
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-chlorobanazoate [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
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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)? Chloramphenicol, hexane
 - ii. How frequently will you be handling them (times/month)? twice in total/five times in total
- c) Does any of the chemicals have the hazard statement H317 and/or H334? If yes:
- i. Which one(s)? G418, ampicillin
 - ii. How frequently will you be handling them (times/month)? 9
 - 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?

Precautions always need to be taken when working in the lab. Safety goggles and lab coat will be worn at all time and the right type of gloves will be worn when needed. Chemicals that are harmful to inhale (Arcolor, EDTA, G418, Methanol) will be handled in a fume hood. 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.

All the chemicals need to be properly marked and stored in closed containers. SDS, and methanol are highly flammable and need to be stored away from hot surfaces and open flames. They will be stored in the flammables cabinet in the balance room. The strong acids need to be stored away from the

strong bases. NaOH, HCl, and KOH will be stored in the fume hood in the balance room when handling working solutions. Otherwise they will be stored in the Acids and Bases cabinets, respectively. **4-chlorobenzoate is stored in cool, dry place with the container tightly closed. Biphenyl and DMSO and hexane solutions will be stored with the container tightly closed in a dry place – in a ventilated cupboard in the balance room common storage place.**

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 and safety glasses will always be used during lab work. All work with chemicals that are harmful to inhale will be performed in fume hood. While handling antibiotics and Arcolor 1260 red gloves will be used. If Ampicillin in powder form is used to prepare a solution the powder will be handled inside the chemical hood in the balance room. While handling Gel red and Gel green gels, as well as strong bases and acids, orange gloves will be used. During other work in the lab grey gloves will be used as a safety measure. **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. Red gloves when handling antibiotics.**

Personal protection needed:

- ☒ Gloves and lab coat
- ☒ Safety glasses
- ☐ Facial mask
- ☒ Other, specify: Chemical Hood

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.

Glassware that have contained living cells will be rinsed with ethanol twice prior to being put in the dishwasher. The ethanol washout will be collected as biological waste.

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

Work bench will be cleared and wiped off with ethanol. Chemical containers will be closed and marked and those that can stay on the bench will be put in the specific tray. All instruments will be cleaned.

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.

The biological waste will be autoclaved and poured in the sink, unless it contains harmful chemical compounds. Ampicillin, G418, Lithium acetate, and Nourseothricin will be collected as hazardous waste. Lithium acetate and will be collected as hazardous waste as well. Waste containing Gel red/Gel green will be collected in a specific container. Containers that have contained living cultures will be washed twice with ethanol. The ethanol washout will be thrown in the liquid biological waste.

Agar plates will not be autoclaved but discarded as hazardous solid waste. Used pipette tips, cell spreaders etc will be discarded as solid biological waste if they have been in contact with biological material, and solid chemical waste if they have been in contact with hazardous chemicals. Liquids containing NaOH or KOH will be diluted until neutral with HCl prior to being poured out. Hexane will be treated as organic solvent. Uncontaminated gloves will be recycled. Contaminated gloves will be thrown in hazardous waste.

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

Ampicillin, Nourseothricin, and G418 will be turned in as hazardous waste. **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 then 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