

Risk Declaration

Name: iGEM

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Experiment: Detection and elimination of contaminants for future implementations in Bioreactors.

1. Description of experiment:

Short and precise, explaining the different steps in your experiment. If you are working with microorganisms, remember to specify species and the biosafety level required.

Amplification of different DNA parts by PCR. Enzyme digestion and ligation. Piecing together constructs using Gibson Assembly. Transformation of plasmids into *E. coli* (Biosafety level 1) and *Saccharomyces cerevisiae* (Biosafety level 1). Extraction of plasmid from *E. coli*. Cultivation on agar plates with ampicillin for *E. coli* and –Uracil for *S. cerevisiae*. Nickel based purification of produced proteins. Characterization of introduced genes through colony-PCR and cultivation in minimal media. *Schizosaccharomyces pombe* will be used in order to analyze whether the modified *S. cerevisiae* is able to detect contamination in a flask.

2. KLARA Risk Assessments read:

Specify risks assessments that are relevant to your experiment. Use the information when you summarize the risks and how to minimize them under sections 4 and 5.

SB/IB-Tillblandning av 1 liter YPD-medium

SB/IB-Gelelektrofores med GelRed

SB/IB-Vertikala autoklaven

SB/IB-Centrifug Sigma 4K15

SB/IB-Småcentrifuger

SB/IB-Sterile work/LAF-bench

SB/IB-Värmeblock

SB/IB-Bordsautoklav

SB/IB-Centrifuge (High speed) Avanti J-20 XP










SB/IB-Rotary Shakers / Inkubatorer








SB/IB-Thermal Cycler (PCR)




SB/IB-Diskmaskinen

3. Chemicals:

Specify MSDS read and safety information for each chemical in your experiment. For every chemical, specify the chemical name, CAS-number, the concentration of the final solution (if applicable), CLP hazard pictogram(s) (use table below) and hazard statement(s).

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 final solution	Pictogram(s)	H statement(s)
Ethanol (64-17-5)	70 %		H226
Ethanol (64-17-5)	96 %	 	H225, H319
Tris-HCl (1185-53-1)	0,1 M		
EDTA (6381-92-6)	10 mM		
Lithium acetate (6108-17-4)	1M		H319
Agarose (9012-36-6)	1%		
Sodium dodecyl sulfate (151-21-3)	1%	  	H228, H302, H311, H315, H318, H332, H335, H412
GelRed			
GelGreen			

Ampicillin (69-52-3)			H317, H334
Polyetylen glykol-3350 (25322-68-3)	50%		
Dithiothreitol (3483-12-3)			H302, H315, H319, H335
Coomassie Brilliant Blue (6104-58-1)			
Tryptone (91079-40-2)			
Yeast extract (8013-01-2)	10g/L		
Calcium chloride dihydrate (10035-04-8)			H319
D(+)-Glukos (50-99-7)	20g/L		
Glycerol (56-81-5)			

3.1 Use of restricted chemicals

Use the chemical information on KLARA to answer the following questions

- 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
- Are any of the chemicals classified as a CMR (Carcinogenic, Mutagenic or Reprotoxic) substance? If yes, which one(s)?
No
- Does any of the chemicals have the hazard statement H317 and/or H334? If yes, which one(s)?
Yes, Ampicillin (69-52-3).

4. 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?

Ethanol: Flammable, do not use close to open flame.

GelRed: Avoid inhalation of vapor or mist. Avoid direct contact with substance (binds to DNA).

GelGreen: Avoid inhalation of vapor or mist. Avoid direct contact with substance (binds to DNA).

Lithium acetate: Avoid contact with eyes.

Sodium dodecyl sulfate: Avoid contact with skin and eyes and avoid inhalation. Must not be exposed to heat, flame or sparks.

Ampicillin: Avoid inhalation and contact with skin.

Dithiothreitol: Avoid ingestion, inhalation and contact with skin and eyes.

Calcium chloride dehydrate: Avoid contact with eyes.

When running the centrifuges they should be equally balanced.

Handle gel electrophoresis with care, electric current is used.

Autoclaves: hot surfaces, use heatgloves when removing autoclaved equipment.

Heatblock: do not touch hot surface.

5. Risk reductions:

5.1 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.

Ethanol: Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Sodium dodecyl sulfate: Keep containers tightly closed in a dry, cool and well-ventilated place.

5.2 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).

The grey gloves will be used when handling all the chemicals.

Personal protection needed:

- Gloves and lab coat
- Safety glasses
- Facial mask
- Other, specify:

5.3 Waste handling:

Specify what kind of waste is produced, and how it is handled/disposed of. Consider every step in your experiment. Remember that you will likely generate both solid and liquid waste. If you are disposing of biological waste containing antibiotics, check and state whether or not the antibiotic is inactivated during autoclavation.

All waste that has been in contact with biohazard samples need to be autoclaved. All garbage, materials and liquids that potentially are biohazards will be thrown in a special garbage bag that will be autoclaved. Liquid biowaste is collected in plastic container and autoclaved before poured in the drain. Ampicillin is inactivated when autoclaved.

Materials that have been in contact with biohazard will be rinsed twice with 70% ethanol (also collected in plastic container) and placed in dishwasher.

Agar plates will be disposed in marked boxes.

Waste from chemicals will be placed in the correct "waste bucket". Gels containing GelRed or GelGreen will be placed in waste bin for gels.

6. 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 (see evaluation matrix in SOP).

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

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The risk declaration has been read by:

Signature of Research Engineer/Lab manager