Risk Declaration

For further instructions on how to write your risk declaration, see SOP on the lifebio server.

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Date: 2017-06-15
Experiment: BIOSENSOR FOR LUNG CANCER IN YEAST, PART 2

1. Description of experiment:

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

The purpose is to design a biosensor with the ability to detect butanone and octanal respectively, using GPCRs integrated in yeast. When both molecules are present, the yeast cells will mate and expression of ADE2 will turn off.
In this part we will transform the plasmids to *Saccharomyces cerevisiae* and perform several experiments to investigate if our biosensor is functional. Following methods will be used:

- Transformation of *Saccharomyces cerevisiae* (Biosafety level 1) using the PEG/LiAc method and selection by a HIS/URA marker
- Colony PCR
- Flow cytometry for measurement of GFP activity of constructed yeast strains
- Replacement of native GPCR with our GPCR using CRISPR/Cas9
- Exposure of yeast to butanone and n-octanal in solution

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 – Rotary Shakers/Incubators; SB/IB --80°C Freezer; SB/IB – Heat Block; SB/IB – WaterBath; SB/IB – Sterile work; SB/IB – small centrifuges; SB/IB – Gelelectrophoresis /w GelGreen; SB/IB – Gelelectrophoresis /w GelRed; SB/IB – Vertical Autoclave/Benchtop autoclave; SB/IB – Thermal Cycler.

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 handled solution	Pictogram(s)	H statement(s)
EDTA disodium salt dihydrate [6381-92-6]	0.5M		**H332 – Harmful if inhaled** **H373 – May cause damage to organs through prolonged or repeated exposure if inhaled**
Tris-HCl [1185-53-1]	10mM	None	None
Butanone [78-93-3]	0.5-1 mM		EUH066 – Repeated exposure may cause skin dryness or cracking **H225 - Highly flammable liquid and vapour** **H319 - Causes serious eye irritation.** H336 - May cause drowsiness or dizziness.
Octanal [124-13-0]	0.1 mM		H226 - Flammable liquid and vapour. H315 - Causes skin irritation.
Lithium acetate [546-89-4]	1.0 M		**H319 – Causes serious eye irritation**
PEG3350 [25322-68-3]	50%	None	None
Potassium Hydroxide [1310-58-3]	1%		H290 – Corrosive to metals H302 – Harmful if swallowed **H314 – Causes severe skin burns and eye damage**
Ethanol [64-17-5]	99%		**H225 – Highly flammable liquid and vapor.** **H319 – Causes serious eye irritation.**
Agar [9002-18-0]		None	None
YPD broth {Peptone from meat Yeast extract D (+)-glucose Water}		None	None

3.1 Use of restricted 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? No**
   If yes, which one(s), and do we have a valid permit?
b) **Are any of the chemicals classified as a CMR (Carcinogenic, Mutagenic or Reprotoxic) substance? No**
   If yes:
   i. **Which one(s)?**
   ii. **How frequently will you be handling them (times/month)?**
c) **Does any of the chemicals have the hazard statement H317 and/or H334? No**
   If yes:
   i. **Which one(s)?**
   ii. **How frequently will you be handling them (times/month)?**
   iii. **Do you have any allergies?**

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?

In the experiments we intend to conduct, there will not be any risk associated with reactions or combination of chemicals. Since ethanol is flammable it should be kept away from fire or heat. However, when working with glass objects there is always a risk that they break; if this happens we should handle the broken glass carefully, since the glass shards are usually very sharp. Furthermore, when using a freezer or heat block/autoclave, there is always a risk of burn of freezing damages. All instruments we intend to use, including electrical equipment, should be used with great care and according to the SOP. There are some risks associated with the VOCs we are using, but no flame will be used close to the VOCs and safety gloves/glasses will be used during the handling of them. The VOCs will be handled under a fume hood all time.

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.

All chemicals that are used in any experiment should be kept in an organized manner to keep the risk for accidents at a minimum and stored in ventilated chemical cupboards. In this experiment, butanone, octanal and ethanol are flammable and will be stored in a fireproof cupboard. Temporary storage in the form of plastic containers will be used. Ethanol will be kept away from heat or flames.

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 chemicals that might be harmful to inhale will be handled in the fume hood and if needed, the scale will be moved to the fume hood. The flammable chemicals will not be handled anywhere near fire and heat.

Personal protection needed:

Gloves and lab coat ☑
Safety glasses ☑
☐
☑

Facial mask
Other, specify: Fume hood

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 autoclaving.

All containers with liquid biological or chemical waste will be labeled with content, name and date. The liquid biological waste containing hazardous compounds or lithium will be label (on a sticker what it contains) and sent to Ragn-sells for waste handling, while non-hazardous waste will be autoclaved and disposed in the sink. Solid biological waste is autoclaved in plastic bags and discarded as combustible waste unless it contains any hazardous or volatile chemicals. If this would be the case, a research engineer will be contacted. Gels and agar plates are collected in separate, labeled boxes. Aluminum foils and hard plastics, e.g. pipette tip racks and empty EtOH bottles, are collected in recycling bins. Liquid biological waste containing butanone and/or octanal and lithium will be classified as biological+chemical waste. The waste will be gathered in plastic bottles (HDPE) labelled with content, name, date and the appropriate pictograms, with emphasis on that the waste should not be autoclaved. The waste will be stored in ventilated cupboards. Solid chemical waste will be gathered in specific containers and placed on the chemical waste shelf in a ventilated cupboard.

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

Signature

Signature

Signature

Signature

Signature

Signature

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Signature

The risk declaration has been read by:

Signature of supervisor

Signature of Research Engineer