

**Risk Assessment made under the**

[**Genetically Modified Organisms**](http://www.hse.gov.uk/pubns/books/l29.htm)

[**(Contained Use) Regulations 2014**](http://www.hse.gov.uk/pubns/books/l29.htm)

### Supervisor and Assessor Details

**Supervisor of this project**

Name: Abigail Wood

Signature of supervisor: Abigail Wood

Date: 2 March 2020

**Assessed By**

Name:

Signature: Date:

(Has to be a different from person above)

### Risk Assessment approved by GM/Bio Safety Committee

Name:

Signature: Date:

(Formally appointed Biomakespace Safety Officer - BSOs cannot assess there own RAs)

**HSE notification required? Yes☐ / No☐**

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### Risk Assessment Review

**Risk Assessment to be reviewed on [DATE]**

**Name and signature of reviewer(s):**

**This is the BMS\_GM1 Form**: For the production and use of genetically modified of non-pathogenic E. coli and derivatives, and other low risk micro-organisms

### Project Details

**Project** [**Title**](#bookmark=id.tyjcwt)**:**

Hands-on CRISPR gene editing workshop (2020)

**Project** [**SUMMARY**](#bookmark=id.3dy6vkm) **(Aims and Objectives)**

Produce a basic introductory CRISPR-cas9 gene editing workshop, using William Shaw’s two plasmid system in yeast. Also perform DNA sequencing of yeast using an Oxford Nanopore MinION.

### Biological Details

**Give details of host Micro-organism(s):**

Three types of Saccharomyces cerevisiae yeast: wild-type BY4741, BY4741 + genomic insert GFP, BY4741 + genomic insert BFP

**Vector(s):**

3 Cas9 plasmids (pWS2081-2083)

1 vector for making new gRNA expression constructs (pWS2069)

2 pre-made gRNA expression plasmids, one for cutting GFP (gWS152) and another for cutting BFP (gWS153)

**Expected biological action of altered DNA/RNA or transcribed/translated gene product:**

There are a few possible experiments with these materials, with these possible results:

Conversion of GFP to BFP expression

Conversion of BFP to GFP expression

Introduction of GFP expression to wild-type strain

Introduction of BFP expression to wild-type strain

We intend to run the BFP to GFP expression conversion experiment, using pWS2081 and gWS153 in BY4741 + genomic insert BFP.

**Technique used to introduce** [**modification**](#bookmark=id.4d34og8) **or vector into host:**

High-efficiency yeast transformation with PEG-3350, LiOAc and salmon sperm DNA (boiled) .

### Nature of Work to be Undertaken

**Give brief description of types of laboratory procedures including maximum culture volumes at any time (show as multiples of unit volumes).** See [Guidance](#bookmark=id.3rdcrjn) 1 and. [Guidance 2](#bookmark=id.3j2qqm3)

Glycerol stock preparation from yeast streaked on agar plates. Maximum culture volume of BY4741 is likely to be 15 mL in YPD for preparation of cells to be transformed; edited variants are likely to be up to 5000 transformants forming colonies on minimal media plates.

**Provide details of any non-standard laboratory operations**

None expected.

**Additional control measures** required for specific risks:

None expected.

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| --- | --- |
| RISK ASSESSMENT FOR HUMAN HEALTH AND SAFETY **Human health hazard identification** - Identify any potentially harmful properties of:  i) the [recipient](#bookmark=id.lnxbz9) micro-organism(s) *(see definition)*  *The yeast strains used are Containment Level 1 organisms. Using standard microbiological practices it is not known to cause disease in immunocompetent adult humans, and presents minimal potential hazard to laboratory personnel.*  **Overall Risk:** *Effectively Zero*  ii) the altered genetic material(s)  No genetic material used contains or will express or produce material hazardous to human health e.g. toxins, viral sequences, allergen, oncogene etc. Proteins expressed are all reporter genes and have no function that could result in health hazards.  **Overall Risk:** *Effectively Zero*  iii) the donor micro-organism(s) *(where used/appropriate*)  N/A - transformations will use purified plasmid.  **Overall Risk:** *Effectively Zero*  iv) the vector(s)**\*** (see additional section below for viruses or viral vectors)  **Overall Risk:** *Effectively Zero*  v) the resulting genetically modified micro-organism(s) (including viral/cellular vectors)  The resulting GMO will express only a reporter protein that is not known to have any harmful effects on human health and its risk profile will not change.  **Overall Risk:** *Effectively Zero*  \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  **\* If genetic modification work will include the use of viruses or viral vectors, ensure that you have considered the following areas in your risk assessment.**  **IF NOT APPLICABLE, STATE HERE AND DELETE THE FOLLOWING TEXT**  VIRAL WORK IS NOT APPLICABLE  \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  **CONTROL MEASURES** – Assign provisional containment level- indicate the relevant CL  **Containment Level:** **1**  Applying the principles of[Good](http://www.hse.gov.uk/pubns/priced/l29.pdf) Microbiological Practice and Good Occupational Safety and Hygiene | **Guidance and Prompts**  State [ACDP](http://www.hse.gov.uk/pubns/misc208.pdf) hazard group if appropriate & GM class if already modified.  **\*** If using a plasmid vector - consider whether mobilisable, non-mobilisable or mobilisation defective and resultant risk level.  If using pre-existing genetically modified micro-organisms, eg pseudotyped lentiviruses, consider how their properties differ from the wild type parent.  Consider control measures? Eg vaccines/prophylaxis/ treatment?  [Assign](#bookmark=id.44sinio) a provisional CL.  **Only CL1 projects are possible at Biomakespace** |
| RISK ASSESSMENT FOR ENVIRONMENTAL HARM **Environmental hazard identification -** Identify any potentially harmful properties of:  i) the recipient micro-organism(s)  The yeast strains are Containment Level 1 organisms. Using standard microbiological practices they present minimal potential hazard to the environment.  **Overall Risk:** *Effectively Zero*  ii) the altered genetic material  No genetic material used contains or will express or produce material hazardous to the environment.  **Overall Risk:** *Effectively Zero*  iii) the donor micro-organism(s) *(where used/appropriate*)  Not applicable.  **Overall Risk:** *Effectively Zero*  iv) the vector\* (see additional section below for viruses or viral vectors)  **Overall Risk:** *Effectively Zero*  v) the resulting genetically modified micro-organism including viral/cellular vectors  The resulting GMO will express only a reporter protein that is not known to have any harmful effects on the environment and its risk profile will not change.  **Overall Risk:** *Effectively Zero*  \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  **\*If genetic modification work will include the use of viruses or viral vectors, ensure that you have answered the following questions in your assessment:** | **Guidance and Prompts** RA for Environmental Harm must be completed(See HSE [Regulation](http://www.hse.gov.uk/pubns/priced/l29.pdf) 18) [Potentially](#bookmark=id.z337ya) [harmful](#bookmark=id.1y810tw) effects include: (see hover guidance) |
| FINAL CLASSIFICATION AND ASSIGNMENT OF FINAL CONTROL MEASURES Work will be carried out at: **Containment Level 1**  ***Any work requiring Containment Level 2 or above CANNOT be performed at Biomakespace.***  **State waste procedures and inactivation methods for the GMM:**  Kill effectively 100% using final concentration of 5% chemgene for liquid culture, followed by autoclaving of affected vessels, with validation using testing strips to ensure correct temperature and hold are reached (121 C for at least 15 min).  **State contingency waste procedures (eg if autoclave is down):**  Sterilisation of liquid using final concentration of 5% chemgene; waste will be reserved in sealed containers clearly marked until autoclave becomes available again (or until temporary access to another autoclave is established.) | * Include expected degree of kill (eg ‘effectively 100 %’), and appropriate validation methods (eg run shown to reach and hold temp correctly). * **GM WASTE**-   Biomakespace requires all GM waste to be inactivated, by autoclave or other validated means prior to disposal. |

**If the project is risk assessed as Class 2 or above it cannot be performed at Biomakespace.** Please speak to the Safety officer or email safety@biomake.space if you have further questions.

Always remember - all GM waste must be inactivated before disposal!