

iDEC Responsible Research Form

Dear iDEC Teams,

this is the iDEC 2023 Responsible Research Form. Submission of this form is mandatory for your participation in iDEC. Please remember: safety and ethical considerations are an important part of modern research and we are not asking you these questions to waste your time, but to educate you, and protect you and those around you! Please take the time to answer the questions as a team, and if you need help, do not hesitate to contact the Commission for Responsible Research (vinke@idechq.org). We are here to help you with things like this!

The Responsible Research Form should be submitted prior to the start of the wetlab, but no later than July 1. If you find that you need to change parts of your projects (e.g., use additional genes), please contact us and we will approve those changes as soon as possible.

The responsible research form consists of 5 sections: General Information about your team, Laboratory Biosafety, Genes and Chassis, Biosecurity, and Ethical Considerations. We have gathered resources for each section to guide your team, and we have completed the entire form as an example on our website (https://idec.io/pages/ethics_biosafety_and_biosecurity.html).

Be aware that you are able to shape the values of your research community. In our ideal directed evolution research community, safety, security, and ethical considerations are valued and common place. We hope the iDEC responsible research project can teach you how important these values are. We hope you learn more about your project as you think about biosafety, biosecurity, and ethical considerations, and have a wonderful time during iDEC 2023!

Your Commission for Responsible Research

*Required

1. Team Name *

Ferroptosis Expedition-NMU_China

2. Email address for questions regarding your Responsible Research Form *

lizheng20201@163.com

3. What is the research hypothesis of your project? *

Genes upregulated by sorafenib induction may contribute to the differential activation of the ferroptosis pathway. Through directed evolution of sorafenib-resistant liver cancer cell lines, followed by transcriptional profiling and pathway enrichment analysis with wild-type cell lines, these genes may be screened and identified, thus their relationship with ferroptosis may be elucidated.

4. Please provide a detailed abstract of your project. *

Sorafenib is effective in treating hepatocellular carcinoma (HCC), though it may lead to acquired resistance. We sought to explore the key signaling pathway alterations and crucial regulatory molecules underlying sorafenib resistance. Through directed evolution of sorafenib-resistant liver cancer cell lines, followed by transcriptional profiling and pathway enrichment analysis with wild-type cell lines, these genes will be screened and identified, thus their relationship with ferroptosis may be elucidated.

State-of-knowledge

The following four questions will ask you how much you knew about risk assessment before iDEC. You don't have to answer them, but they would help our committee design better, more tailored educational projects. There are no wrong answers to these questions and it is okay if you answer "no" to all of them - it is not your fault if no one told you about these risks.

5. Have you ever assessed biosafety risks of a scientific project? When was the first time and place someone taught you about biosafety risks?

6. Have you ever assessed biosecurity risks of a scientific project? When was the first time and place someone taught you about the difference between biosafety and biosecurity risks?

7. Do you know what "Dual-Use Research of Concern " means? Where and when did you learn about dual-use research of concern?

Dual-Use Research of Concern often describes life sciences research with clear benefit, yet may cause harm if misapplied.

8. Do you know what "Gain of function experiment" means? Where and when did you learn about them?

In order to enhance biological functions of certain product, Gain of function experiment is applied to alter an organism genetically.

Laboratory Biosafety

Thank you for helping us by sharing your level of knowledge prior to iDEC! We will now start with the risks you have identified for your iDEC project. The first category of risks is "Laboratory Biosafety." In this section, you will need to indicate that your lab meets the criteria to be a safe place for you to work, and ensure no accidental release of your GMOs.

Biosafety describes personal protection and protection from accidental release of biological agents that have the potential to harm plants, animals, humans, or the environment. In this laboratory biosafety section of the Responsible Research Form, your teams will need to answer questions about safety equipment in your lab and safety countermeasures to prevent accidental release of GMOs.

If you are looking for resources or would like to learn more, we recommend you take a look at the WHO Laboratory Biosafety Manual:

<https://www.who.int/publications/i/item/9789240011311>

9. Mention the PI of your team and the relevant qualifications of your PI to function as your team's person responsible for safety. *

Our PI is Prof. Dr. Yu Yizhi, who serves as the Director of the Department of Immunology at Naval Medical University. With extensive experience in leading multi-term research projects in biotechnology, Yu possesses the necessary qualifications to serve as a safety officer for BSL 1 and 2 laboratories.

10. Did you get permission from your institute to perform the experiments necessary for your iDEC project? *

Our research institute and university allowed us to perform our experiments under supervision of our PI.

11. List all topics mentioned in your safety introduction. *

Laboratory biosafety, Personnel safety, Chemical agent safety, biosafety, laboratory animals Safety, Instrument safety.

12. What is the biosafety level of your laboratory? *

Mark only one oval.

We have a computational project and don't use a laboratory

BSL1

BSL2

BSL3

Other: _____

13. Describe the safety measures in your laboratory (e.g. eye shower, emergency shower, etc) *

Protective equipment: Biohazard glove, laboratory coat, safety goggles, biological safety cabinet, reagent cabinet, SPF Animal Lab, etc. Ventilation equipment: Central supply and exhaust system, fume cupboard, etc. Sterilizing equipment: Ultraviolet lamp, high temperature-high pressure sterilization equipment, etc. Cleaning equipment: Eye shower, emergency shower, etc.

14. Describe how GMOs are prevented from being unintentionally released into the environment, including waste management *

We sterilize all waste leaving the laboratory that has come into contact with microorganisms, or may have done so. It is mandatory to wear lab coats and disinfect our hands before exiting the laboratory, and opening windows is strictly prohibited. Our specialized air conditioning system filters the air in the laboratory and creates a low-pressure environment.

15. Do you work with animals or samples (urine, blood, saliva, etc) derived from animals or humans? *

Yes. _____

16. Are you working with chemicals that are mutagenic, carcinogenic, explosives, or narcotics? If yes indicate what chemicals, in what amounts you use them, and what safety measures are in place. *

No. _____

Genes and Chassis

Genes and chassis of each team must be biosafety level 1 or 2. Any genes and chassis from organisms that fall into the biosafety level 3 or 4 category are prohibited to be used in iDEC. There will be a whitelist of chassis and all genes and chassis used beyond that must be listed here.

Whitelists are kind of outdated when it comes to synthetic biology, since the taxonomic classification of organisms into safety groups does not necessarily reflect the risks of an organism to cause harm (e.g., an organism might carry genes for toxins that would not be expressed under the conditions used to characterize that organism, so the recommended biosafety level would be low, but we can still derive the toxin from that organism and express it recombinantly). That said, we decided to provide a short whitelist for iDEC so you don't have to list the usual lab workhorses like your *Escherichia coli* lab strain. Our whitelist includes the FDA's GRAS organism as well as commonly used organisms for directed evolution projects. The iDEC whitelist can be found in this form and on the iDEC website and any organisms, genes from this white list do not need to be listed here unless you are evolving them (https://idec.io/pages/ethics_biosafety_and_biosecurity.html).

For us, it is very important that you really think about what additional risks might come from your evolved molecule (or pathway, or genome). The thing about directed evolution is that you are creating something novel, and that novel product may not have the same level of safety as the scaffold you started with.

17. List all chassis you plan to use that are not listed in the iDEC whitelist below, as well as their biosafety level (with reference) and a short explanation how you plan to use these. *

<i>Acetobacter suboxydans</i>	<i>Eisenia bicyclis</i>	<i>Lactococcus lactis</i>	<i>Porphyra suborbiculata</i>
<i>Acetobacter xylinum</i>	<i>Endothia parasitica</i>	<i>Laminaria angustata</i>	<i>Porphyra crispata</i>
<i>Actinoplane missouriensis</i>	<i>Eremothecium ashbyii</i>	<i>Laminaria cloustonia</i>	<i>Porphyra tenera</i>
<i>Anaëlis japonicus</i>	<i>Escherichia coli BL21 (DE3)</i>	<i>Laminaria digitata</i>	<i>Rhizopus niveus</i>
<i>Aspergillus niger</i>	<i>Escherichia coli DH5alpha</i>	<i>Laminaria japonica</i>	<i>Rhizopus oryzae</i>
<i>Aspergillus niger var. Awamori</i>	<i>Escherichia coli DH5alpha F'</i>	<i>Laminaria longiruris</i>	<i>Rhodymenis palmata</i>
<i>Aspergillus oryzae</i>	<i>Escherichia coli TOP10</i>	<i>Laminaria Longissima</i>	<i>Saccharomyces cerevisiae</i>
<i>Arabidopsis thaliana WT</i>	<i>Eucheuma cottonii</i>	<i>Laminaria ochotensis</i>	<i>Saccharomyces fragilis</i>
<i>Cooney et Emerson</i>	<i>Eucheuma spinosum</i>	<i>Laminaria saccharina</i>	<i>Scytosiphon lome</i>
<i>Bacillus cereus</i>	<i>Furcellaria fastigiata</i> of the class <i>Rodophyceae</i> (red seaweed)	<i>Leuconostoc citovorum</i>	<i>Streptococcus cremoris</i>
<i>Bacillus coagulans</i>	<i>Fusarium moniliforme</i>	<i>Leuconostoc dextranicum</i>	<i>Streptococcus lactis</i> subspecies <i>diacetylactis</i>
<i>Bacillus licheniformis</i>	<i>Gigartina acicularis</i>	<i>Leuconostoc mesenteroides</i> strain NRRL B-512(F)	<i>Streptococcus lactis</i>
<i>Bacillus stearothermophilus</i>	<i>Gigartina pistillata</i>	<i>Macrocystis pyrifera</i>	<i>Streptococcus thermophilus</i>
<i>Bacillus subtilis</i>	<i>Gigartina radula</i>	<i>Mortierella vinaceae</i> var.	<i>Streptomyces chattanoogensis</i>
brown algae	<i>Gigartina stellata</i>	<i>Raffinoseutilizer</i>	<i>Streptomyces griseus</i>
<i>Candida guilliermondii</i>	<i>Gloiopeltis furcata</i>	<i>Mucor miehei</i>	<i>Streptomyces natalensis</i>
<i>Candida lipolytica</i>	<i>Hizikia fusiforme</i>	<i>Mucor miehei</i> var. Cooney et Emerson	<i>Streptomyces olivaceus</i>
<i>Candida pseudotropicalis</i>	<i>Kjellmaniella gyrate</i>	<i>Mucor pusillus</i> Lindt	<i>Streptomyces olivochromogenes</i>
<i>Candida utilis</i>	<i>Kluyveromyces lactis</i>	<i>Penicillium roquefortii</i>	<i>Streptomyces rubiginosus</i>
<i>Chlamydomonas reinhardtii</i>	<i>Kluyveromyces marxianus</i> var. <i>lactis</i>	<i>Petalonia fascia</i>	<i>Xanthomonas campestris</i>
<i>Chondrus crispus</i>	<i>Lactobacillus bulgaricus</i>	<i>Porphyra deutat</i>	
<i>Chondrus ocellatus</i>	<i>Lactobacillus fermentum</i>	<i>Porphyra perforate</i>	

We exclusively employ E. coli DH5alpha, which is included in the approved list.

18. List all genes and plasmids you plan to use that are not derived from one of the organism on the iDEC whitelist above, as well as their original host/lab and their biosafety level of their original host (with references) and a short explanation on how you plan to use these. *

The pcDNA3.1(-) plasmid scaffold is utilized for the overexpression plasmids, while the pSpCas9 (Addgene plasmid ID: 48137) plasmid scaffold is employed for the gene knockout plasmids.

19. Which genes are evolved in your experiment? What kind of improvement do you want to achieve by your evolution (e.g. increase activity, increase stability)?*

Netrin receptor UNC5B (<https://www.uniprot.org/uniprotkb/Q8IZJ1/entry>) was cloned from mouse cDNA. We aim to manipulate the expression of the gene in HepG2 cells through molecular cloning and CAS9-mediated knockout techniques.

Biosecurity

Biosecurity describes the prevention of biological agents from unauthorized access, theft, misuse or loss. In the biosecurity section of the responsible research form, your team is required to evaluate potential biosecurity risks and their countermeasures. In this section we will ask for the dual-use research of concern potential. Dual-use research of concern in the life sciences, describes the potential of your project, generated technologies and knowledge to be directly misused by someone else to cause serious harm to animals, humans, plants, national security or the environment. Please take this section seriously. We have seen a lot of teams becoming very creative when it comes to envision 10,000 benefits of their project, but we almost never become creative when it comes to envision the risks. That is something we need to change! For most risks you identify, you will notice that countermeasures can be easily integrated and that makes your project better and more likely to be used in a project beyond your labbench!

If you would like to learn more, take a look at the WHO biorisk management guidance (https://www.who.int/ihr/publications/WHO_CDS_EPR_2006_6/en/), the NIH dual-use research of concern information page (<https://osp.od.nih.gov/biotechnology/dual-use-research-of-concern/>) or attend the free FutureLearn "Next Generation Biosecurity: Responding to 21st Century Biorisks" course (<https://www.futurelearn.com/courses/biosecurity>).

20. Describe the access control measures of your laboratory. *

Every individual seeking entry into the laboratory must possess an access card for both building and laboratory access, which is administered by the Laboratory Administration.

21. Evaluate if your project has Dual Use Research of Concern potential. *

Our project is solely focused on investigating the fundamental mechanisms that underlie sorafenib resistance through laboratory studies, with no associated adverse effects.

22. If yes, indicate what measures your team takes to minimize the risk of misuse. *

Not involve.

Ethical Considerations

Identifying ethical issues of scientific research projects is a big part of responsible research. To be able to identify possible unethical practices, teams need to evaluate who is impacted by their project and what this influence means to the person, group or society as a whole.

In case you want to do research on human subjects (this includes for example interviews or surveys) we have included a questionnaire to identify possible issues that you need to evaluate if your experimental questions validates the ethical concern of pursuing this experiment. This questionnaire should give you an idea about the broad range of considerations necessary when you want to conduct research on human subjects. We do still encourage you to always get approval by your institute's ethic council if you would like to conduct such experiments.

If you would like to learn more about bioethics, we recommend to take a look at the NIH or UNESCO bioethic websites (NIH:

<https://www.niehs.nih.gov/research/resources/bioethics/index.cfm> UNESCO: <https://en.unesco.org/themes/ethics-science-and-technology/bioethics>).

23. List all people/groups that are potentially influenced by your research or outcomes of your research and describe the impact of your project on these people/groups. *

1. Patients with HCC. We sincerely hope that by revealing the genes and its role in ferroptosis, HCC resistance towards sorafenib can be weakened or even reduced one day. Thus, sorafenib, the well recognized part of systematic therapy can be revived and keep benefiting those suffered from HCC.
2. Fellow researchers. The minor work we have done may appear to be useful for scientists who have been dedicating in this area as well as newcomers. After all, science is boardless.

24. Do you see any ethical issues arising from your project? If so, indicate why. *

We do not see ethical issues arising from our project.

25. Do you plan to conduct research on humans or human samples (this includes questionnaires, interviews, public engagement)?*

We don not have such intentions right now.

26. If yes, does your experiment fulfil all requirements to be categorized as research with low potential to cause ethical issues? (A good guideline is the questionnaire below. If you can answer all questions on the questionnaire below with no, your research probably has a low potential to be the cause of ethical issues.) *

This questionnaire is derived from the basic questionnaire of the ethics council of Bielefeld University.

Please answer all 12 questions by ticking yes or no as appropriate:	DGPs*	yes	no
1. Will members of a vulnerable group or people who cannot give their own consent participate in this study (e.g., children and adolescents under 16 years of age, people with a learning disability, people in psychotherapeutic treatment)?	3 (b)		no
2. Will it be necessary that people participate in this study without having been informed about this previously or without having given their consent to participate (e.g., as in covert observation)?	6		no
3. Will the study involve covert observation or any other method that precludes informed consent, full debriefing, or the opportunity for participants to have their data deleted?	3, 9		no
4. Will the study feature questions about topics that are of an intimate nature or that participants may perceive as stigmatizing (e.g., questions pertaining to illegal or deviant behavior or to sexual preferences)?	3 (d)		no
5. Does the study include an active deception of participants or will information be deliberately withheld from participants? (This does not apply to withholding the study hypothesis.)	8		no
6. Is there a risk that the study may cause psychological stress, fear, exhaustion, or other negative effects in participants to an extent that they would not normally encounter in their daily life?	3 (d), 9		no
7. Is there a risk that the study may cause participants to experience pain or more than mild discomfort?	3 (d), 9		no
8. Will the participants be given drugs, placebos, or other substances to ingest (e.g., food, beverages, vitamins), or will the participants be subjected to any invasive or potentially harmful procedures?	3 (d), 3 (e), 8, 9		no
9. Will video or audio recordings be taken without prior consent by the participants?	3, 4		no
10. Will any bodily substances of participants be sampled (blood, saliva, etc.)?	3, 4		no
11. Will participants receive a payment of more than 10 Euros <i>per hour</i> for their participation?	7		no
12. Is there a conflict of interest for the applicant or applicants because of (a) economic or personal connections to a contracting entity or a collaborator whose interests may be affected positively or negatively by results of the research, or (b) any other factor(s) that might affect the applicants' independent scientific judgment?	–		no

Note: * This column points out particularly relevant subsections of section 7.3 of the document "Berufsethische Richtlinien der DGPs und des BDP". See website of the EUB.

Thank
you!

We hope this responsible research form gave you some idea how complex safety, security and ethical considerations for a research project are and that you learned a little bit more about your project by filling out this form. Two last questions:

We would like to ask you about the time your team needed to fill out the form and if you would consider the effort as to much.

Would it be okay if we use your answers to learn more about risk assessment of directed evolution projects? We might give a talk at the festival were we would like to talk about case studies or publish about the iDEC risk assessment resources. This does not include your email address or PI information and we would of course anonymize all answers, but someone might be able to connect projects to answers given in the form.

27. How much time did you and your team need to consider risks and to fill out the form. Would you consider this time as too long, too short or adequate?

We took about 1 day to fill out the form. We consider this time as adequate.

28. Do you give consent for your answers in this form to be anonymesly used for talks and publications by the iDEC Commission for Responsible Research? *

Mark only one oval.

Yes

No

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