iDEC Responsible Research Form

Dear iDEC Teams,

this is the iDEC 2024 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 2024!

Your Commission for Responsible Research

*Required

1. Team Name *

iTidetron

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

jiangao@tidetronbio.com, linhouliang@tidetronbio.com

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

Newly developed COMDEL model (Comparing and Optimizing Multiple DEep Learning) can effectively identify and classify antimicrobial peptides (AMPs) with high accuracy and broad-spectrum antibacterial activity.

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

Current antimicrobial peptide (AMP) prediction models face significant challenges due to their limited accuracy and narrow applicability, which restrict their industrial application. To address these issues, we developed and enhanced an AMP prediction model named COMDEL (Comparing and Optimizing Multiple DEep Learning) by leveraging integrated training approaches based on neural network algorithms and high-throughput AMP screening methods. COMDEL achieved an accuracy of 94.8% in tests and 88% in experimental verification, surpassing other state-of-the-art models. Additionally, we employed phage-assisted non-continuous evolution (PANCE) to enhance Sortase A (SrtA) mutants for efficient AMP synthesis and implemented a cell-free AMP synthesis (CFAS) system that increased AMP yields to 0.5–2.1 g/L within hours. Using multi-omics analysis, COMDEL identified Lactobacillus plantarum as a promising candidate for AMP production among 35 edible probiotics. We further developed a microdroplet sorting approach and successfully screened L. plantarum mutants with significantly increased antimicrobial capabilities. Our study provides a robust framework for the effective screening, synthesis, and industrial application of AMPs, demonstrating the potential for COMDEL to revolutionize AMP production.

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?

Yes (otherwise I would probably be wrong in this committee). The first time I was taught about biosafety risks was prior to my first practical course in the laboratory (first year of my master degree)

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?

Yes, when I was training at the National Laboratory of Virology at Wuhan University in my first year of master's degree, I learned about the difference between biosafety and biosecurity.

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

Yes, I learned about the "Dual-Use Research of Concern " when I was training at the National Laboratory of Virology at Wuhan University in my first year of master's degree.

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

Yes. I learned about them while researching about biosensor issues

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. Yi-Rui Wu, who is a senior scientist in microbial metabolic modification and directed evolution. Wu has all the by China law required qualifications to function as safety officer for BSL 1 and 2 labs and has lead countless research projects in the biotechnology field.

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

Our research institute and company allowed us to perform our experiments under supervision of our PI. We only allow experiments to be conducted under the supervision of a designated safety officer.

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

Laboratory biosafety (waste management, how to check the dangerous level of chemicals (R and S records), work and safety equipment, emergency showers, eye showers, what to do in case of emergency (fire, accident)
Biosecurity (Access Control and DURC)

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) *

Eye shower, emergency shower, list of phone numbers with contact persons, emergency passage and measures, first aid kit, designated area for working with acrylamide.

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

We autoclave all waste that leaves the laboratory that has been or may have come into contact with microorganisms.
We have strict rules to wear lab coats and sanitize hands before leaving the lab. The windows are not allowed to open and we have a special air conditioning system designed to filter the air from the laboratory and create low undervoltage.
The responsible government agency conducts safety and inspection of laboratories at least twice a year with safety measures.

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

No

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. *

We run the gel using acrylamide. We have a special designated area with extra security precautions to prevent accidental exposure to this chemical by researchers working in the lab. There is only a small amount (50 mL) of stock solution in our laboratory

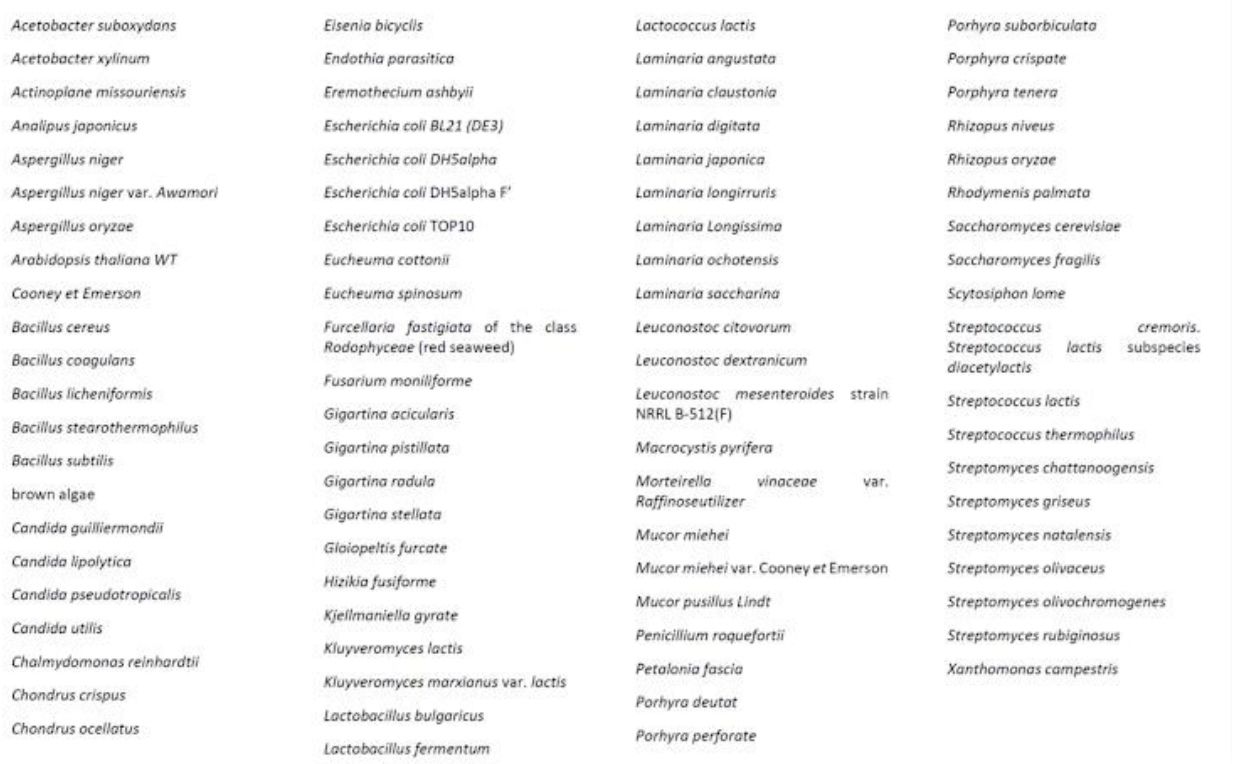
Genes and Chassis

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. *



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

In addition to the strains on the white list, we will use Lactobacillus plantarum for high AMP production using COMDEL and FADS technologies.

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. *

- Escherichia coli Strain S1030 purchased from Addgene (Cat. No.# 105063), biosafety level 1, used as a host for the expression of AMP candidates and in the phage-assisted non-continuous evolution (PANCE) process to enhance Sortase A (SrtA) mutants.

- Mutagenesis Plasmid MP4 from Addgene (Cat. No. #69652), used in the PANCE process for continuous mutation of the M13 phage containing the SrtA gene.

- GIII Expression Vector pJC175e from Addgene (Cat. No. #79219), used for expressing the gIII gene in the PANCE process.

- pBAD18-GFP and pT7-GFP Plasmids from Biofeng, used for AMP candidate expression in vivo and invitro, replacing the GFP gene with AMP candidates.

- M13 phage purchased from Guangzhou Zymostar Biotech (Cat. No. ZS1004), used in the PANCE process fro the evolution of SrtA.

- pTET-GFP Plasmid provided by the Liu lab at the Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, Jiangnan University, China. Used to construct split GFP sensors, with GFP1-10 and GFP11 inserted for validation and monitoring purposes.

- Codon-Optimized Genes for AMP Candidates, SrtA, gIII-R-pSPO-RBS-gIII-F, GFP1-10, and GFP-11, synthesized by GUANGZHOU IGE BIOTECHNOLOGY LTD. Used for codon-optimized for expression in E. coli, used for

validating and expressing AMP candidates, and in constructing split GFP sensors.

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)? *

Sortase A (SrtA). Targeted improvements: Enhanced ligation activity and increased AMP yield.

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 notive 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. *

Everyone who wants to enter the lab needs an access card and facial recognition to enter the building and lab. Access cards are managed by the administration.

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

The research project does not involve hazardous chemicals and the use of probiotics and crops on the iDEC whitelist mitigates some risks. However, the advanced techniques for AMP production and enhancement necessitate the careful consideration of biosafety and biosecurity measures to prevent misuse.

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

Like stated in the question below, we do not see our research as research with a high DURC potential. However, we will evaluate how we communicate our project and won't publish the potential misuse scenarios.

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 questionaire 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. *

Biotechnology companies. They could benefit from advanced screening methods and enhanced production techniques invented by our research.
The pharmaceutical industry could benefit from development of new antibiotic and broad-spectrum antimicrobials.
The use of AMPs can enhance food preservation and safety, reducing spoilage and contamination.

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 will interview some of those working in the medical and skincare fields to find out their views on the application and performance of recombinant full-length collagen.

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)		
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		
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		
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)		
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		
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		
7. Is there a risk that the study may cause participants to experience pain or more than mild discomfort?	3 (d), 9		
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		
9. Will video or audio recordings be taken ***without prior consent by the participants?***	3, 4		
10. Will any bodily substances of participants be sampled (blood, saliva, etc.)?	3, 4		
11. Will participants receive a payment of more than 10 Euros *per hour* for their participation?	7		
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?	–		

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.

All questions can be answered with "no" for our planned interviews. We furthermore got the permission of our ethics council to conduct our interviews.

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?

Adequate. It took me a approx. 6 h, including coming up with this project.

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