iDEC Responsible Research Form

Dear iDEC Teams,

this is the iDEC 2021 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 June 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 reserach 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 2021!

Your Commission for Responsible Research

Team Name *

Svenja's imaginary iDEC Team

Email address for questions regarding your Responsible Research Form *

vinke@cebitec.uni-bielefeld.de

What is the research hypothesis of your project? *

A combination of computational design and directed evolution can be used to design tRNA/aminoacylsynthetases capable of incoporating D-amino acids

Please provide a detailed abstract of your project. *

The endogenous translational machinery incorporates only L-amino acids, thus the cellular machinery is specialized in degrading these and in producing immune responses against them. If we incorporate D-amino acids into peptides, these would be resistant against degradation by proteases and would persist longer in the system, making them powerfull new tool in pharmacy, e.g. vaccine development.
We want to harness this power by designing enzymes which are able to incorporate a specific D-amino acid during translation through the amber stop codon.
To incorporate a non-canonical amino acid through the amber stop codon, an orthogonal tRNA/aminoacylsynthetase (aaRS) and an amber suppressor tRNA are needed. The aaRS needs to be able to recognize the novel amino acid and charge it to the tRNA while beeing orthogonal to the endogenous translational machinery. Commonly used aaRS are the TyrRS aaRS from Methanococcus jannaschii and the PylRS aaRS from Methanosarcia mazei.
We want to use these two enzymes as scaffolds to perform compuational modelling and directed evolution to change these enzymes to be able to recognize and incorporate a specific D-amino acid. For our modelling we want to use the Rosetta 3 enzyme design protocol. The enzymes modelled through this will be ordered as gene synthetsis and tested towards their activity and specifity.
Furthermore, we want to use error-prone PCR to generate libraries based on our modelled enzymes and the wildtype and select these using the positive/negative selection described by Liu&Schultz 2010.
We decided to try to incorporate the non-canonical amino acid D-alanine as proof-of-concept for our project. Incorporation will be tested through fluorescence (GFP with an amber stop codon) and MALDI TOF analysis.

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.

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 bachelor degree).

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, but I have never learned about them during my university education, but in the first year supervising an iGEM team. Since the translations for biosafety and biosecurity are the same in German, I was not aware that there is a difference before I started working on these issues with my iGEM team.

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 DURC in 2018, during my first year as an iGEM supervisor. My team identififed a potential of misuse in their project idea and started to work on outreach projects regarding DURC issues and their communication.

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 DURC 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

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. Jörn Kalinowski, who is the head of the microbial genomics and biotechnology research group at the Center for Biotechnology in Bielefeld. Jörn has all the by German law requiered qualifications to function as safety officer for BSL 1 and 2 labs and has lead countless research projects in the biotechnolgy field.

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. We do not have a designated biosafety board which allows experiments.

List all topics mentioned in your safety introduction. *

Laboratory biosafety (waste management, how to check for the hazard level of chemicals (R and S records), work and safety equipment, emergency shower, eye shower, what to do in case of an emergency(fire, accident)
Biosecurity (access control and DURC)

What is the biosafety level of your laboratory? *

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

⦿ BSL1

○ BSL2

○ BSL3

○ Other: _____

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, first aid kit, designated area for working with ethidium bromide

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

We autoclave all waste leaving the lab which has or might have been in contact with microorganisms. We have a strict rule to wear labcoats and desinfect our hands before leaving the lab. Windows are not allowed to be opend and we have a special AC system designed to filter the air from the lab and creating a low underpressure.

The labratory is checked at least once a year by the responsible governmental body regarding safety and security measures.

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

No

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 use ethidiumbromide to stain our gels. We have a special designated area with additional safety precautions to prevent accidental exposure of the researchers working in the lab to this chemical. We only have a small (5mL) stock of the stock solution in the lab.

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.

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

We only use E. coli DH5alpha which is on the white list.

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. *

TyrRS (tyrosine aaRS) from Methanococcus jannaschii BSL1 (https://www.jcm.riken.jp/cgi-bin/jcm/jcm_number?JCM=10045) Scaffold for the generation of a D-alanine aaRS. This enzyme will be used for the computational modelling and evolution.

PylRS (pyrrollysine aaRS) from Methanosarcia mazei BSL 1 (https://bacdive.dsmz.de/strain/7102#section_6) Scaffold for the generation of a D-alanine aaRS. This enzyme will be used for the computational modelling and evolution.

pSB1C3 plasmid scaffold for the selection plasmids (https://parts.igem.org/Part:pSB1C3)

Kanamycin resistance from Escherichia coli (Migula) Castellani and Chalmers (ATCC® 55244™) BSL 1 (https://www.lgcstandards-atcc.org/Products/All/55244.aspx?geo_country=de#generalinformation). Amber codons will be introduced and it will be used as selection marker for the positive selection.

Rnase Ba (Barnase) from Bacillus amyloliquefaciens BSL 1 (https://www.lgcstandards-atcc.org/Products/All/23350.aspx?geo_country=de). Amber codons will be introduced and it will be used as negative selection marker.

arabionse operon from Escherichia coli Bl21 (DE3) on white list. Will be used as promotor system for the negative selection.

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)? *

TyrRS (tyrosine aaRS) from Methanococcus jannaschii BSL1 (https://www.jcm.riken.jp/cgi-bin/jcm/jcm_number?JCM=10045) Scaffold for the generation of a D-alanine aaRS. This enzyme will be used for the computational modelling and evolution.

PylRS (pyrrollysine aaRS) from Methanosarcia mazei BSL 1 (https://bacdive.dsmz.de/strain/7102#section_6) Scaffold for the generation of a D-alanine aaRS. This enzyme will be used for the computational modelling and evolution.

We want to change the substrate affinity from both aaRS from their native substrate to the non-canonical amino acid D-alanine. The evolved aaRS will not increase the host's toxicity or it's ability to be inactivated and thus should not change the biosafety level of the host, nor can the recombinant expressed enzyme cause harm.

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).

Describe the access control measures of your laboratory. *

Every person who wants to enter the laboratory needs an access cards to enter the building and the laboratory. The access cards are administered by the CeBiTec administration.

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

The technology of genetic code expansion in genereal has DURC potential, since the incorporation of novel amino acids hinders detection, because detection systems are designt to work with the standard genetic code and canonical amino acids. Furthermore, peptides containing D-amino acids are more stable and the immune system reacts differently to peptides out of or containing these amino acids. This properties might be misused in the future. However, there are already aaRS sequences published which can be used to recombinantly express peptides containing non-canonical amino acids and we do not think that our experiments and their outcome pose an additional risk. Thus we would categorize our project as project with low DURC potential. It should still be noted that current technogies for the analysis of peptides and sequences need to be adapted to screen for sequences with an expanded genetic code and non-canonical amino acids to ensure safety.

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 reserach as research with a high DURC potential. However, we will evaluate how we communicate our project and won't publish the potential misuse scenarios like designing peptides which are more difficult for the immune system. We also plan to work on standard mass spektronomie protocols to analyse our peptides containing D-amino acids which might be a workflow to counter the risk of hindered detection.

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).

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. *

Science our project is more about designing a suitable incorporation technology to incorporate D-amino acids rather than a proposed real world application. Our research findings will mainly influence xenobiology researchers, because our research findings will hopefully be a valuable contribution to the field, and maybe the pharmaceutical industry which might use our research findings to produce therapeutics.

However, if we build up on our project by expressing peptides or proteins with a real world applications, we will review the ethical risks (e.g. when we think about producing a peptide with pharamceutical application). We do not plan to work on a application and will only work on a proof-of-concept on incorporating our non-canonical amino acid.

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

We do not see ethical issues arising from our project.

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

We will interview a few xenobiology researchers about their experiences with their work on non-canonical amino acids.

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.

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 (what else shall I say, that was the aim of my design). It took me a approx. 3 h, including coming up with this project.

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? *

◉ Yes

○ No

This form was created inside iDEC.

Google Forms