# DIGITAL, INNOVATION, AND GREEN TECHNOLOGY PROJECT (DIGIT PROJECT)


DIGIT
DIGITAL, INNOVATION, AND GREEN TECHNOLOGY PROJECT

REPUBLIKA HRVATSKA
Ministry of Science, Education and Youth

THE WORLD BANK
IBRD • IDA | WORLD BANK GROUP

**REPUBLIC OF CROATIA**
**MINISTRY OF SCIENCE, EDUCATION AND YOUTH**
Donje Svetice 38, Zagreb 10 000, Croatia

**DIGITAL, INNOVATION, AND GREEN TECHNOLOGY PROJECT (DIGIT PROJECT)**

IBRD LOAN NO. 9558-HR

PROJECT ID: P180755

# ESMP CHECKLIST

## CALL FOR PROPOSALS "SEAL OF EXCELLENCE UNDER THE SYNERGIES PROGRAM"

CALL REFERENCE NUMBER: DIGIT.2.2.01

June 2025

# ESMP CHECKLIST

The template presented below will be revised for specific projects to reflect scope of works and E&S concerns.

The ESMP Checklist provides "pragmatic good practice" and it is designed to be user friendly and compatible with WB safeguard requirements. The checklist-type format attempts to cover typical mitigation approaches to common civil works contracts with localized impacts.

This document will help assess potential environmental impacts associated with the proposed project, identify potential environmental improvement opportunities and recommend measures for to the prevention, minimization and mitigation of adverse environmental and social impacts.

ESMP Checklist is a document prepared and owned by final beneficiary. The Beneficiary is responsible for the implementation of the ESMP Checklist as well as any subsequent corrective measures prescribed by PIU and WB.

The checklist has one (1) introduction section and three (3) main parts:

**Introduction** or foreword part consisted of following sections:

- *Introduction* (project description),
- *Environmental and social category* (environmental and social category is defined),
- *Potential environmental and social impacts* (potential impacts are defined)
- *ESMP Checklist* (concept and application of Checklist are explained),
- *Monitoring and reporting* (brief description of the monitoring and reporting process including responsibilities of involved stakeholders)

**Part 1** - constitutes a descriptive part ("site-passport") that describes the project specifics in terms of physical location, the institutional and legislative aspects, the project description, inclusive of the need for a capacity building program and description of the public consultation process.

**Part 2** - includes the environmental and social screening in a simple Yes/No format followed by mitigation measures for any given activity.

**Part 3 -** is a monitoring plan for activities during project construction and implementation. It retains the same format required for standard World Bank ESMPs.

ESMP Checklist implementation report will be submitted to WB semi-annually if not agreed differently.

Workers code of conduct (subject to WB approval) will be a part of bidding documentation and contracts with Contractors. Code of conduct will extend to sub-contractors and be a part of Contractor's contractual agreements.

Part I - General project and site information

<table>
<tr><th colspan="5">INSTITUTIONAL & ADMINISTRATIVE</th></tr>
<tr><td>Country</td><td colspan="4">Croatia</td></tr>
<tr><td>Project title</td><td colspan="4">CRISPR/dCas9 toolbox for epigenome and genome editing (EpiGen-toolbox)</td></tr>
<tr><td>Scope of project and activity</td><td colspan="4">The EpiGen-toolbox Proof of Concept (PoC) aims to develop a collaborative online platform that will make our upgradable CRISPR/dCas9 EpiGen-toolbox globally accessible. The platform will also allow further optimization and individual tailoring of the toolbox for both scientific and commercial purposes. By doing so, the project aims to advance epigenetic research and its application in various fields beyond current boundaries.<br><br>The main challenge we seek to address is the underutilized potential of epigenetic modifications in both research and therapeutic applications. This gap largely stems from the absence of a convenient, modular, and comprehensive CRISPR/dCas9-based toolbox. Existing solutions do not fulfil all requirements: while many toolboxes are available, none provide complete compatibility with the requirements of full epigenome editing. Most of them are designed primarily for genome editing, with limited or no functionality for precise epigenetic interventions such as targeted DNA methylation, histone post-translational modifications, or direct gene activation and repression. Furthermore, the majority of commercially available toolboxes are sold by US-based companies (e.g., IDT, GeneScript, VectorBuilder), which limits accessibility and customization for researchers worldwide.<br><br>Our modular CRISPR/dCas9 Epi-toolbox, built on the Golden Gate assembly approach, offers a flexible and standardized solution. It allows researchers to select from a wide range of modules, including multi-guide cassettes (supporting up to six gRNAs simultaneously), various eukaryotic promoters, effector domains, dCas9 orthologs, selection markers, transcriptional terminators, and backbone vectors suitable for both transient and stable transfections.<br><br>In addition to the predefined modules, the toolbox will support tailor-made customization to address specific scientific and commercial needs. Through the online platform, researchers and companies will gain access to:<br>- Standardized and validated on-demand assemblies of expression constructs.<br>- Custom assemblies incorporating new domains or novel purposes, enabling perfectly tailored solutions for diverse experimental setups.<br><br>By integrating these activities into a single online platform, the EpiGen-toolbox project will be a superior epigenome editing toolbox that will foster collaboration across the scientific community, and accelerate translational applications of epigenetics in medicine, biotechnology, and industry.</td></tr>
<tr><td>Institutional arrangements<br>(WB)<br>(Name and contacts)</td><td>(Task Team Leader)</td><td colspan="3">Environmental/Safeguards Specialists:</td></tr>
<tr><td>Implementation arrangements<br>(Borrower)<br>(Name and contacts)</td><td>Safeguard/Environment Supervision<br>Helena Deriš<br>hderis@genos.hr</td><td>Works supervisor<br>Goran Josipović<br>gjosipovic@genos.hr</td><td>Inspectorate Supervision<br>Ana Savanović<br>Ana.savanovic@adria-grupa.hr</td><td>Works Contactor<br>Filip Šoštarić<br>fsostaric@genos.hr</td></tr>
<tr><th colspan="5">RESEARCH DESCRIPTION</th></tr>
<tr><td>Objectives and Scope of the Research</td><td colspan="4">The main objective of the EpiGen-toolbox PoC project is to develop a collaborative online platform that will make our upgradable CRISPR/dCas9 EpiGen-toolbox globally accessible. In addition, the project seeks to further optimize the toolbox for individual tailoring, ensuring that it can be applied both for scientific research and commercial purposes. By doing so, we aim to expand the boundaries of epigenetic research and accelerate its</td></tr>
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| | implementation across multiple fields.<br>Specific objectives include:<br>- Developing a global sales and marketing strategy to promote the EpiGen-toolbox and make it widely available to global research society, biotech companies, and pharmaceutical industries.<br>- Demonstrating that the EpiGen-toolbox can be adapted to address specific scientific challenges as well as pharmaceutical needs.<br>- Continuously upgrading and expanding the toolbox with novel modules and functionalities, ensuring it remains at the forefront of epigenetic innovation.<br>- Building a digital platform that facilitates on-demand knowledge sharing, user feedback, and the integration of custom design requests.<br>By achieving these objectives, the project will not only provide a unique resource for researchers but also create new opportunities for translational and therapeutic applications of epigenetics.<br>The scope of the research will be focused on the development, optimization, and validation of the EpiGen-toolbox in a pilot setting. This pilot will be tailored to solve a specific scientific problem, thereby serving as a proof-of-concept for the toolbox's versatility and functionality. In parallel, the project will validate its relevance and applicability for pharmaceutical needs, demonstrating its potential impact in drug discovery and therapeutic development. The overarching scope is to provide evidence of feasibility and impact, showing that the EpiGen-toolbox can address unmet needs in epigenome research and therapy. |
| Research Methodology and Materials Used | The EpiGen-toolbox project will be conducted through a four-task approach designed to cover technological development, biological validation, and application for both scientific and pharmaceutical needs.<br>Activity 1: Developing sales and marketing approach for the EpiGen-toolbox global availability - we will develop the EpiGen-toolbox online platform, providing a modular CRISPR/dCas9-based toolbox that is easily accessible to the global research community and pharmaceutical companies. For this task, external programming expertise will be required to design and implement a secure, user-friendly, and scalable digital platform.<br>Activity 2: Adaptation for the needs of research society - a pilot study will be conducted to demonstrate the functionality of the toolbox by co-targeting DNMT3A with various epigenetic effector domains (G9a, G9a-me3, LSD1, KDM5A, and HDAC3). The aim is to identify combinations that provide persistent and synergistic effects on gene silencing. Cloning of parts for new toolbox modules and assembly of expression constructs will be performed in *E. coli*. These constructs will be tested in the commercially available HepG2 cell line. The study will include a time-course experiment on two gene loci to evaluate long-term stability of silencing effects.<br>Activity 3: Upgrade of our modular toolbox for novel purposes - to expand the toolbox, we will incorporate a new Cas9 ortholog (CjCas9), which will reduce the size of expression plasmids. This activity also includes repurposing the EpiGen-toolbox for genome editing applications and making it compatible with lentiviral transduction. Cloning of CjCas9 and its gRNA module will be carried out in *E. coli*, while functional validation will again be tested in HepG2 cells.<br>Activity 4: Adaptation for the needs of pharmaceutical companies - the EpiGen-toolbox will be applied to manipulate glycogene expression in order to restore proper IgG glycosylation in FreeStyle 293-F cells, even under altered environmental conditions. This will provide a proof-of-concept for long-term controllable antibody glycosylation. Furthermore, combinations of epigenetic effectors identified in Activity 2 will be repurposed for pharmaceutical applications to ensure durability and reproducibility of glycosylation control.<br>Materials Used –<br>- Cloning host: Escherichia coli (for cloning and module assembly).<br>- Cell lines: HepG2 (for validation of gene silencing and genome editing) and FreeStyle 293-F (for antibody glycosylation studies).<br>- Epigenetic effector domains: DNMT3A, G9a/G9a-me3, LSD1, KDM5A, HDAC3.<br>- New Cas9 ortholog: CjCas9. |

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| | - Platform development: External programming expertise for creation of the collaborative online platform.<br>Safety Measures and Precautions –<br>- The project will adhere to strict biosafety and bioethics guidelines throughout all stages of research. Key precautionary measures include:<br>- Training and education: All team members will undergo training on safe laboratory practices, biosafety standards, and ethical handling of genetic materials. Junior researchers will be mentored by senior staff to ensure compliance and prevent errors.<br>- Safe waste disposal: Special attention will be paid to the disposal of biological and chemical waste. Hazardous materials will be handled according to institutional biosafety regulations laws, minimizing risks to health and the environment.<br>- Containment procedures: All cloning and cell culture work will be conducted in designated biosafety level laboratories (BSL-2), using laminar flow cabinets and appropriate sterile techniques.<br>- Personal protective equipment (PPE): Researchers will be required to use lab coats, gloves, protective eyewear, and, when necessary, face masks or respirators.<br>- Emergency preparedness: Protocols for spill management, accidental exposure, and equipment malfunction will be established and regularly rehearsed.<br>Expected Impact -the results of the project will not only advance the field of epigenome editing but also enhance the credibility and visibility of our research outcomes. Through targeted communication, dissemination, and exploitation (CDE) strategies, we will increase global awareness of the EpiGen-toolbox. Ultimately, the platform will be available to the international research community and pharmaceutical industry, creating a standardized, validated, and customizable tool that will significantly accelerate innovations in epigenetics. |
| Research Setting and Duration | The research activities will be carried out at the Genos Research Laboratory, located at Borongajska cesta 83H, 10000 Zagreb, Croatia. The laboratory provides the necessary infrastructure, equipment, and expertise to successfully execute all planned activities of the project.<br>The EpiGen-toolbox project is planned to run over a period of 18 months, ensuring sufficient time for platform development, experimental validation, optimization, and dissemination of results. |
| Expected Outcomes and Ethical Considerations. | The primary outcome of the EpiGen-toolbox project is the development of an online platform that will make our modular CRISPR/dCas9-based toolbox accessible to the global research community. This resource will be particularly valuable for research groups engaged in epigenetic studies or those seeking to integrate epigenome editing into their experimental workflows. Through this platform, laboratories worldwide will gain access to a standardized and validated EpiGen-toolbox, available on-demand and adaptable to a wide range of scientific applications. Furthermore, the toolbox will also be made available commercially to pharmaceutical companies, thereby bridging the gap between fundamental research and therapeutic development.<br>The expected outcomes include:<br>- Shifting epigenetic research from correlative to causal studies, enabling researchers to directly test how specific epigenetic modifications influence gene expression and cellular function.<br>- Tailoring the toolbox to specific scientific problems, demonstrating its flexibility and ease of adaptation for different experimental setups.<br>- Conducting a pilot study to validate that the toolbox can be used to control antibody glycosylation, regardless of changing environmental conditions, thereby highlighting its pharmaceutical relevance.<br>- Establishing the EpiGen-toolbox platform as a global hub, fostering collaboration, knowledge exchange, and innovation across academic and industrial settings.<br>Ethical Considerations - to ensure the responsible dissemination and ethical use of the EpiGen-toolbox after the project's completion, we will implement a controlled-access strategy tailored for both academic and commercial users.<br>- Academic Research Community: The toolbox will be accessible through the online platform on a request basis. Principal Investigators (PIs) must submit an access request by completing a standardized online form, providing information on the intended application, |

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| | institutional affiliation, and biosafety infrastructure of their laboratory. This vetting process will ensure access is granted only to qualified and responsible users.<br>Material Transfer Agreements (MTA): All academic users will be required to sign an MTA before receiving our tools. The agreement will clearly define the scope of permissible use, prohibit redistribution to third parties, and outline biosafety and ethical responsibilities.<br>Pharmaceutical and Commercial Partners: Access will be formalized through a purchase and licensing framework. Companies will be required to sign a commercial MTA that specifies intellectual property rights (IPR), licensing terms, and compliance with regulatory and ethical standards.<br>By implementing these safeguards, the project will uphold the highest ethical standards, protect human health and the environment, and support responsible innovation. |
| **LEGISLATION** | |
| Identify national &local legislation & permits that apply to project activity(s) | National and Local Legislation<br>- Act on Genetically Modified Organisms (Zakon o genetski modificiranim organizmima, Narodne novine)<br>- Occupational Safety Act (Zakon o zaštiti na radu, Narodne novine)<br>- Act on Sustainable Waste Management (Zakon o održivom gospodarenju otpadom, Narodne novine)<br>- Chemicals Act (Zakon o kemikalijama, Narodne novine)<br>- Law on Health Care, Official Gazette no. 100/18 (Zakon o zdravstvenoj zaštiti, Narodne novine br. 100/18)<br>- Law on Application of Human Tissues and Cells, Official Gazette no. 144/12 (Zakon o primjeni ljudskih tkiva i stanica, Narodne novine br. 144/12)<br>- Act on the Right of Access to Information (Official Gazette no. 25/13, 85/15, 69/22)<br>- Labor Act (Official Gazette no. 93/14, 127/17, 98/19, 151/22, 46/23, 64/23);<br>- Gender Equality Act (Official Gazette no. 82/08, 69/17);<br>- Anti-discrimination Act (Official Gazette no. 85/08, 112/12);<br>Relevant EU Legislation and Directives<br>- Directive 2009/41/EC on the contained use of genetically modified micro-organisms<br>- Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work<br>- Regulation (EU) 2016/679 (General Data Protection Regulation – GDPR)<br>- Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 setting standards of quality and safety for the donation, procurement, testing, preservation, processing, storage and distribution of human tissues and cells<br>- Universal Declaration on the Human Genome and Human Rights (UNESCO)<br>- Convention on Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters (Aarhus Convention) |
| **PUBLIC CONSULTATION** | |
| Identify when / where the public consultation process took place and what were the remarks from the consulted stakeholders | There was no formal public consultation process. The project is grounded in the long-standing expertise of the Genos research team in targeted epigenetic modulation and the development of modular CRISPR/dCas9-based tools for epigenome editing. Its direction and objectives were shaped through internal discussions, reviews by the Institutional Biosafety and Ethics Committees, and informal consultations with academic collaborators and potential pharmaceutical partners. Stakeholder feedback emphasized the importance of strict biosafety compliance, clear access procedures (EpiGen toolbox platform and MTAs), and ensuring that the toolbox remains adaptable for both scientific research and industrial applications. |
| **INSTITUTIONAL CAPACITY BUILDING** | |
| Will there be any capacity building? | **YES**<br>Although Genos has solid scientific and laboratory expertise, we have identified several areas where additional capacity building is needed. In particular, the team has limited experience with World Bank ESF requirements and DIGIT-specific procedures, and some |

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| | of our existing biosafety, OHS and waste management practices need to be better documented and aligned with project-specific monitoring and reporting. In addition, we see a need to strengthen our understanding of GDPR and data protection in relation to the new EpiGen-toolbox online platform.<br>To address these gaps, the project will organize:<br><br>• A practical training for the PI, works supervisor, biosafety officer and administrative staff on World Bank/DIGIT environmental and social requirements, the ESMP Checklist, monitoring and reporting, and the operation of the project-level grievance mechanism.<br>• Refresher trainings for laboratory staff on biosafety, OHS, handling of GMOs and human cell lines, and segregation and disposal of chemical and biomedical waste in line with Croatian and EU rules.<br>• Targeted training for the project team and IT collaborators on GDPR-compliant data handling for the EpiGen-toolbox platform (data minimisation, secure storage, access rights and incident reporting).<br><br>As part of this process, we will also prepare and formally adopt short written SOPs, checklists and templates, and provide on-the-job guidance to junior staff. This will help us systematically address the identified gaps and ensure that environmental, social, health and safety aspects are managed consistently throughout implementation. |
| **ATTACHEMENTS** | |
| - Letter of support from Dr. Claudio Mussolino – for conducting a pilot study to define the optimal combination of epigenetic modifications for long-term gene silencing, tailored to his specific scientific problem, with particular relevance to challenges in CAR-T cell therapies.<br>- Letter of support from Prof. Gioacchino Natoli – confirming his role in leading the production and validation of lentiviruses using our mutated lentiviral backbone. | |

Part II - Environmental/Social screening

| **PART 2: ENVIRONMENTAL /SOCIAL SCREENING** | | | |
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| Will the site activity include / involve any of the following potential issues / risks: | **Activity** | **Status** | **Additional references** |
| | A. Social risk management<br>• Ethical Compliance<br>• Informed Consent and Voluntary Participation<br>• Privacy, Data Protection and Confidentiality<br>• Benefit Sharing and Fairness<br>• Management of Findings and Traceability<br>• Intellectual Property Management<br>• Dissemination<br>• Grievance Redress Mechanism | YES | See Section **A** |
| | B. Environmental risk management<br>• Generation of waste<br>• Sustainable Laboratory Practices<br>• Green Procurement and Materials<br>• Use of energy resources<br>• Sustainable Research Design | YES | If "Yes", See Section B below |
| | C. Occupational health and safety<br>• PPE<br>• Handling and storage of plasma<br>• Hygiene | YES | If "Yes", See Section C below |
| | D. Other issues, not mentioned above (e.g. AI related) | YES | If "Yes", See Section D below |

***Mitigation measures***

A. General conditions and social risk management

B. Social risk management

C. Environmental risk management

D. Other issues

Part III - Environmental and social mitigation measures

| Activity | Parameter | Mitigation measures checklist |
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| A Social risk management | Ethical Compliance and Legal Framework | a) Project activities shall comply with the ethical provisions outlined in the Code of Ethics and uphold the highest ethical standards.<br>- All experimental work with GMOs, human cell lines, and molecular cloning will be performed in accordance with institutional and national ethical standards. |
| | | b) International, EU, and national laws, particularly EU Directive 2004/23/EC, shall be applied during project-related research activities.<br>- Project activities involve contained use of GMOs, biosafety-level 2 laboratory work, and GDPR compliance for the online platform, which must follow EU and Croatian legislation. |
| | Informed Consent and Voluntary Participation | c) Consent protocols must be in full compliance with EU Regulation 536/2014 and the General Data Protection Regulation (GDPR).<br>- Online EpiGen-toolbox platform will collect personal and institutional data from users submitting access requests, which must be handled in compliance with GDPR. |
| | Privacy, Data Protection and Confidentiality | d) Confidentiality of personal data shall be ensured, and tissue samples shall be anonymized or de-identified.<br>- Personal and institutional information collected via the online platform must be protected, stored securely, and processed according to GDPR principles. |

| Activity | Parameter | Mitigation measures checklist |
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| | Benefit Sharing and Fairness | e) Research benefits shall be distributed fairly, and all populations shall have access to potential benefits.<br>- EpiGen-toolbox platform is designed to be globally accessible to the research community (at cost of production and shipping) and commercially available to pharmaceutical companies, ensuring fair and broad access. |
| | Management of Findings and Traceability | f) The origin of human tissue and cell samples shall be clearly documented, including those from commercial sources, biobanks, or other institutions.<br>- The project will clearly document the use of commercially available human cell lines (HepG2 and FreeStyle 293-F), ensuring transparency of sample origin. |
| | | g) Validation in Real-World Settings shall be clearly documented.<br>- Validation will be demonstrated through pilot studies (e.g., persistent gene silencing, IgG glycosylation control, lentiviral compatibility), which will be clearly documented as proof-of-concept for both scientific and pharmaceutical applications. |
| | Intellectual Property Management | h) Identifying and implementing appropriate protection measures such as copyright registration, trade secrets, or patents, where applicable.<br>- The project foresees a patent application for the newly developed Golden Gate–compatible lentiviral backbone and will implement appropriate intellectual property protection measures to safeguard innovations and ensure freedom to operate for both academic and commercial users. |
| | Dissemination | i) Open science practices will be applied whenever appropriate to support early dissemination and transparency, while ensuring that sensitive or potentially exploitable project outputs are protected prior to release.<br>- The project commits to open science practices by making the EpiGen-toolbox platform globally accessible to researchers, while protecting sensitive components (e.g., the lentiviral backbone patent application and commercial licensing terms) prior to public release. |
| | Grievance Redress Mechanism (GRM) | j) Grievance Redress Mechanism (GRM) shall be established by providing and publishing on the website e-mail address where the interested public, either groups or individuals, could send complaints, comments and/or suggestions. The e-mail address shall be reported to the DIGIT GRM of the CSF at grmdigit@hrzz.hr |

| Activity | Parameter | Mitigation measures checklist |
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| | | - The EpiGen-toolbox project will establish and publish a dedicated e-mail address on the platform's website to enable the public and stakeholders to submit complaints, comments, or suggestions, and this contact will be communicated to the DIGIT GRM of the CSF.<br>k) Grievance Redress Mechanism (GRM) shall be established by providing and publishing on the website e-mail address where the interested public, either groups or individuals, could send complaints, comments and/or suggestions. The e-mail address shall be reported to the DIGIT GRM of the CSF at grmdigit@hrzz.hr.<br>- The EpiGen-toolbox project will establish and publish a dedicated e-mail address on the platform's website to enable the public and stakeholders to submit complaints, comments, or suggestions, and this contact will be communicated to the DIGIT GRM of the CSF.<br><br>l) Information on such received complaints, comments, and suggestions should be archived in a logical framework database and reported to the DIGIT Project GRM of the CSF on a monthly basis, together with information on the measures taken following received complaints, comments, and/or suggestions.<br>- All received communications will be systematically archived and reported to the DIGIT Project GRM of the CSF, including details of measures taken in response, ensuring transparency and accountability.<br><br>m) A worker grievance mechanism shall be available for all workers engaged on the project (employees and external collaborators), with the option to submit concerns anonymously.<br>- Genos operates an internal worker grievance mechanism that is accessible to all staff involved in the project. Workers can raise concerns or complaints related to working conditions, occupational health and safety, ethics or integrity through several channels (direct supervisor, HR, dedicated e-mail/online form), including at least one anonymous reporting option. Information on these channels is provided during onboarding and posted on internal notice boards. All grievances are handled confidentially, without retaliation, and are recorded, reviewed and followed up by designated staff. Serious OHS or ethical issues raised by workers are also escalated, where appropriate, through the project GRM and relevant institutional bodies. |

| Activity | Parameter | Mitigation measures checklist |
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| | | k) Information on such received complaints, comments, and suggestions should be archived in a logical framework database and reported to the DIGIT Project GRM of the CSF on a monthly basis, together with information on the measures taken following received complaints, comments, and/or suggestions<br>- All received communications will be systematically archived and reported monthly to the DIGIT Project GRM of the CSF, including details of measures taken in response, ensuring transparency and accountability. |
| | Internal Procedure for Reporting of Irregularities | a) The adopted regulation on the procedure for internal reporting of irregularities and the method of appointing a confidential person and the protection of whistleblowers shall be published one the website with the reporting instructions. |
| B Environmental risk management | Sustainable Laboratory Practices | b) Energy-efficient technologies such as LED lighting, energy-saving equipment, and modern HVAC systems shall be implemented to reduce energy consumption.<br>- The Genos research laboratory already uses modern laboratory infrastructure, and energy-efficient technologies (such as LED lighting and optimized HVAC systems) will be applied to minimize energy consumption during the project. |
| | | c) Internal sustainability measures (waste segregation, efficient use of resources) are in place to reduce environmental footprint. |
| | | d) The use of hazardous chemicals shall be minimized, and safer alternatives shall be explored.<br>- The project involves molecular cloning and cell culture, so hazardous chemical use will be minimized wherever possible, and safer alternatives will be considered to reduce environmental impact. |
| | | e) Energy-efficient laboratory equipment (e.g., centrifuges, incubators, freezers) shall be used without compromising research quality.<br>- The laboratory operates with modern energy-efficient equipment (e.g., $CO_2$ incubators, centrifuges, safety cabinets), ensuring reduced energy consumption without affecting the quality of experimental results. |
| | Waste Management | f) Strict waste segregation protocols shall be implemented to separate hazardous from non-hazardous waste.<br>- The laboratory applies strict segregation protocols, ensuring hazardous biological and chemical waste is separated from non-hazardous waste in compliance with biosafety and environmental regulations. |

| Activity | Parameter | Mitigation measures checklist |
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| | | g) Single-use plastic items shall be replaced with reusable or biodegradable alternatives where possible.<br>- While certain single-use plastics are unavoidable for biosafety reasons (e.g., sterile consumables), reusable and biodegradable alternatives will be adopted wherever feasible to reduce environmental impact. |
| | | h) Hazardous biomedical waste shall be disposed of through licensed waste management contractors.<br>- All hazardous biomedical and chemical waste from the project will be collected and disposed of exclusively by certified waste management contractors, in line with Croatian and EU legislation. |
| | | i) Waste-to-energy technologies, such as incineration, shall be used where appropriate to reduce landfill waste.<br>- Hazardous biomedical waste will be incinerated via licensed contractors, contributing to safe disposal and reduced landfill burden. |
| | Green Procurement and Materials | j) Sustainably sourced materials (e.g., recycled paper, biodegradable supplies, eco-friendly packaging) shall be used.<br>- The project will prioritize sustainably sourced materials, including recycled paper for documentation, biodegradable lab supplies when available, and eco-friendly packaging for shipments. |
| | | k) Reusable lab tools and equipment (e.g., glassware) shall be used whenever feasible to minimize disposable waste.<br>- Standard laboratory practice at Genos already includes the use of reusable glassware and durable equipment wherever biosafety and experimental requirements permit, reducing reliance on disposable plastics. |
| | Reduction of Water Usage | l) Water-efficient laboratory practices shall be implemented, including water-saving equipment and process water recycling.<br>- The laboratory already applies water-efficient practices, such as using water-saving autoclaves and dishwashers, and will continue exploring options for process water recycling to minimize overall consumption. |
| | Sustainable Research Design | m) Sustainable methods and less resource-intensive technologies shall be integrated into the research design.<br>n) The project integrates modular cloning (Golden Gate assembly), which reduces |

| Activity | Parameter | Mitigation measures checklist |
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| | | time, reagents, and plastic use compared to traditional cloning, and prioritizes streamlined experimental workflows to lower resource consumption. |
| | | o) Collaboration with institutions and companies that develop sustainable lab technologies shall be promoted.<br>- The project will seek collaborations with external IT developers for the online platform and with partners working on efficient molecular biology tools, thereby encouraging integration of sustainable technologies through cooperative networks. |
| | Researcher Training and Awareness | p) Continuous training for researchers and lab staff on environmental best practices shall be provided.<br>- Genos already provides regular training on biosafety and laboratory protocols, and these sessions also include environmental best practices such as waste segregation, energy efficiency, and safe chemical handling. |
| | | q) Awareness within the research community about the environmental impacts of biomedical research shall be promoted.<br>- The project will promote awareness of sustainability and environmental responsibility through internal discussions, collaboration with partners, and dissemination activities highlighting responsible laboratory practices. |
| | Compliance and Alternatives | r) Research activities shall comply with a local, national, and international environmental regulations.<br>- All project activities (GMO work, use of human cell lines, biosafety-level 2 experiments, and chemical waste handling) will be carried out in full compliance with Croatian law, EU directives, and international standards. |
| | Community Health and Safety (Residual Risks in Case of Accidents/Mishandling) | s) Even with established biosafety and OHS procedures, there may still be residual risks to community health and safety in the event of accidents or mishandling, including: (i) accidental spills or releases of biological material or chemicals; (ii) unintended exposure of cleaning and maintenance staff, visitors or external waste contractors; and (iii) short-term disturbance related to emergency response or transport of hazardous waste.<br><br>**Prevention and mitigation measures:** |

| Activity | Parameter | Mitigation measures checklist |
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| | | • Access to laboratories and waste storage rooms is controlled at all times. Only trained and authorised staff can enter BSL-2 areas, and any visitors, cleaners or service technicians are accompanied by Genos staff.<br>• Hazardous materials and waste are kept in closed, clearly labelled containers, often placed on spill trays if needed. They are not left in corridors or shared areas, and temporary storage spaces are locked and separated from offices and publicly accessible parts of the building.<br>• The laboratory has written procedures for dealing with spills and incidents involving biological or chemical agents. Spill kits, absorbent material and suitable disinfectants are available in the lab, and staff are trained to contain, clean up and report any incident immediately.<br>• Key equipment (biosafety cabinets, incubators, freezers, waste storage facilities) is subject to regular maintenance and safety checks to reduce the risk of leaks, failures or overheating.<br>• Hazardous waste is handed over only to licensed waste management companies. Waste is transferred in closed, labelled containers in designated areas, so that risks for transport workers and the wider community are kept to a minimum.<br>• Emergency procedures, evacuation routes and contact details for responsible staff and emergency services are displayed in visible places. Staff are trained, and regularly retrained, on how to react in case of larger spills, fire or other events that could have effects beyond the laboratory.<br>• Any incident that could be relevant for community health and safety (e.g. a larger spill or a near-miss involving external staff) is recorded and analysed, and followed by corrective measures such as updated procedures, extra training or technical improvements |

| Activity | Parameter | Mitigation measures checklist |
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| C Occupational health and safety | Personal Protective Equipment (PPE) | a) Appropriate PPE (e.g. lab coats, gloves, goggles, face shields, masks/respirators) must be provided and shall be worn by all personnel.<br>- All laboratory staff are required to use PPE (lab coats, gloves, protective eyewear, and masks/respirators when necessary) in accordance with institutional biosafety protocols and EU occupational health and safety standards. |
| | | b) PPE shall be regularly inspected**,** properly maintained**,** and safely disposed of after use.<br>c) PPE is routinely checked for integrity, maintained in good condition, and disposed of following biosafety level 2 (BSL-2) procedures and hazardous waste regulations to ensure worker safety and environmental protection. |
| | Handling and Storage of Plasma | d) The use of sharps shall be minimized**,** and injury prevention measures must be implemented**.**<br>- Although no plasma is used, standard laboratory practice minimizes sharps usage. When sharps are required (e.g., needles, glass Pasteur pipettes), strict injury prevention protocols and designated sharps containers are employed in compliance with occupational safety regulations. |
| | Decontamination and Disinfection | e) Laboratory surfaces and equipment must be disinfected before and after use with approved disinfectants.<br>- All laboratory benches and equipment are disinfected routinely before and after use with approved disinfectants (e.g., 70% ethanol, sodium hypochlorite), in accordance with BSL-2 biosafety procedures. |
| | | f) Reusable instruments must be sterilized using autoclaves or validated decontamination procedures.<br>- All reusable instruments are sterilized by autoclaving or other validated decontamination methods to prevent contamination and ensure compliance with biosafety standards. |
| | Hygiene and Laboratory Practices | g) Hands must be washed thoroughly with soap and water after handling samples or removing gloves.<br>- All personnel are trained to follow strict hygiene practices, including mandatory handwashing with soap and water after handling samples, chemicals, or removing |

| Activity | Parameter | Mitigation measures checklist |
|---|---|---|
| | | PPE. |
| | | h) Eating, drinking, smoking, and cosmetic application are to be prohibited in laboratory areas.<br>- These activities are strictly prohibited in all laboratory areas, in compliance with BSL-2 safety regulations and institutional policies. |
| | | i) Access to laboratories must be restricted to trained and authorized personnel.<br>- Laboratory access is restricted to trained and authorized staff only, with entry controlled through institutional policies and biosafety training requirements. |
| | Emergency Preparedness and Incident Response | j) Spill response kits and emergency eyewash stations must be made available in all laboratory spaces.<br>- All BSL-2 laboratories are equipped with appropriate spill response materials and emergency eyewash stations to ensure immediate response to accidents involving biological or chemical agents. |
| | | k) Staff must be trained to respond to spills and accidents, including containment, disinfection, reporting, and medical response.<br>- Personnel receive regular biosafety training that includes spill management, containment procedures, incident reporting, and medical emergency protocols. |
| | | l) Emergency procedures and contact details must be clearly posted, and exits must be marked and kept accessible<br>- Emergency contact numbers, evacuation procedures, and safety instructions are posted in all laboratory spaces, and exits are clearly marked and kept free of obstruction at all times. |
| | Health Surveillance and Protection | m) Exposure incidents must be managed according to post-exposure protocols, and medical treatment must be provided immediately.<br>- Any accidental exposure to biological or chemical agents is managed under established institutional post-exposure protocols, which include immediate medical evaluation, treatment, and formal incident reporting in line with occupational health and safety regulations. |
| | Training and Awareness | n) The project does not involve human plasma; however, all staff working with GMOs and immortalized human cell lines receive biosafety training covering PPE usage, safe handling, and waste management. |

| Activity | Parameter | Mitigation measures checklist |
|---|---|---|
| | | o) Refresher trainings must be conducted regularly and attendance must be documented**.**<br>- Regular refresher trainings on biosafety, occupational health, and laboratory safety are conducted at Genos, and participation is documented to ensure compliance and continuous staff awareness. |
| | Waste Management | p) All types of waste, including hazardous waste, will be collected by licensed waste collectors and subsequently disposed of or treated at authorized landfills and processing facilities specialized for each waste category. The disposal of infectious waste without prior neutralization treatment is strictly prohibited.<br>Any replaced equipment classified as electronic waste will be collected by certified e-waste handlers.Biomedical waste, including used PPE and plasma-contaminated materials, must be disposed of in clearly labeled biohazard containers.<br>- While the project does not involve plasma, all biomedical waste (e.g., used PPE, consumables from BSL-2 work, and materials exposed to GMOs or human cell lines) is disposed of in clearly labeled biohazard containers in accordance with biosafety regulations. |
| | | q) Licensed contractors must be engaged for the collection, transport, and final treatment of hazardous medical waste.<br>- All hazardous biomedical waste is collected, transported, and treated by licensed waste management contractors, ensuring compliance with Croatian and EU waste disposal regulations. |
| | Monitoring and Compliance | r) Regular inspections and audits must be performed to ensure compliance with OHS and biosafety requirements.<br>- Internal inspections and audits are conducted regularly at Genos to verify compliance with occupational health and safety (OHS) and biosafety standards, ensuring that procedures remain up to date and effective. |
| | | s) A designated OHS/biosafety officer must be appointed to oversee implementation and adherence to safety protocols.<br>- A designated institutional biosafety officer is responsible for overseeing adherence to biosafety and occupational health protocols, including training, risk assessment, and compliance monitoring. |

| Activity | Parameter | Mitigation measures checklist |
|---|---|---|
| | (e.g. AI related) | t) Implement Code of Ethic for the preparation and implementation of the projects funded by the DIGIT Project<br>- The project will adhere to the Code of Ethics required under the DIGIT Project framework, ensuring that all activities during preparation and implementation are conducted with integrity, transparency, and accountability. |
| D Other issues, not mentioned above | Cybersecurity of the online platform | u) Cybersecurity measures will be applied to reduce the risk of unauthorised access to personal and institutional data on the EpiGen-toolbox platform (e.g. hacking attempts, weak passwords, software vulnerabilities).<br><br>- The platform will be hosted on secure infrastructure using HTTPS and firewall protection, with server and application software kept up to date. Together with the IT provider, we will carry out regular vulnerability scans of the platform and server environment and fix any identified issues as quickly as possible.<br><br>- User accounts will be protected with strong password rules and, in line with good practice under the EU Cybersecurity Act, multi-factor authentication (MFA) will be activated at least for administrator and other privileged accounts, and extended to other users where feasible. Administrative rights will be restricted to a small group of authorised staff.<br><br>- Access to data will be based on the "need-to-know" principle, using role-based permissions and individual user logins. Basic logging of access and changes, combined with regular back-ups, will help detect unusual activity and allow recovery if a problem occurs.<br><br>- An internal procedure for dealing with suspected data breaches will be in place, in line with GDPR. It will cover internal reporting, investigation of the incident, mitigation measures and, where required, informing affected users and the competent data protection authority. |

## WASTE MANAGEMENT PLAN

### Types of Waste Expected

- **Biological waste**: cell culture waste from HepG2 and FreeStyle 293-F lines; *E. coli* cultures; consumables contaminated with GMOs or human cell lines.
- **Chemical waste**: transfection reagents (e.g., PEI MAX), selection antibiotics (puromycin), buffers, and solvents used in cloning and cell culture experiments.
- **Municipal waste**: packaging, paper, non-contaminated consumables, and general office/lab waste.
- **Sharps/broken glass**: pipette tips, glass Pasteur pipettes, needles (minimized), and broken glassware.

### Procedures for Collection, Labeling, and Temporary Storage

- All biological and chemical waste will be collected in **clearly labeled, color-coded containers** (biohazard bags, sharps boxes, chemical-resistant containers).
- Contaminated materials will be **temporarily stored in designated waste areas** within BSL-1 and BSL-2 labs, with restricted access.
- Waste logs will be maintained to ensure traceability.

### Treatment Methods before Disposal

- **Biological waste**: autoclaved at 121 °C/1 bar for 20 min before disposal, rendering it non-infectious.
- **Chemical waste**: neutralized (if feasible) or collected separately for specialized disposal. Disinfectants, such as 10% bleach, are used for decontaminating surfaces/equipment.
- **Sharps**: disposed of in rigid, puncture-resistant containers, autoclaved, and sent for licensed disposal.
- **Municipal waste**: sorted according to recycling rules (paper, plastic, glass).

### Authorized Companies or Institutions Responsible for Final Handling

- Licensed biomedical and chemical waste management contractors, accredited by the Croatian Ministry of Economy and Sustainable Development, will be engaged for the collection, transport, and final treatment of hazardous waste.
- Non-hazardous recyclable waste will be handled by municipal waste services.

### Record-Keeping and Reporting Arrangements

- All waste generation, treatment, and disposal activities will be **documented in laboratory waste logs**.
- Autoclave cycle records will be maintained.
- Waste transfers to licensed contractors will be tracked with official documentation (waste transfer forms, invoices).
- Records will be retained for auditing and reporting under the DIGIT project requirements.

## EMERGENCY RESPONSE PROTOCOL (Spills, Exposure, Equipment Failure)

### Chemical Spills, Biological Material Release, or Lentiviral Sample Incidents

- All spills involving chemicals, biological material, or lentiviral samples must be addressed immediately.
- Small spills are contained by applying appropriate disinfectants (e.g., freshly prepared 10% bleach for lentiviral materials, 70% ethanol for general surfaces) and allowing sufficient contact time (minimum 30 minutes for bleach).
- For larger spills or potential aerosol release, work is stopped immediately, affected areas are evacuated, and senior staff (PI/biosafety officer) are informed.
- Following disinfection, surfaces and equipment are further cleaned, and the laboratory is UV-sterilized if required.

### Personnel Protection

- All responding staff must wear appropriate **PPE** (double gloves if handling lentiviruses, lab coat, face shield or protective goggles, and mask/respirator if required).
- Contaminated PPE is removed and disposed of in biohazard containers.
- Exposed skin or mucous membranes must be washed thoroughly with soap and water; eye exposures require 15 minutes of flushing at an eyewash station.

- Evacuation is initiated if there is an uncontrolled release, strong fumes, or risk of infection.

### Reporting Lines and Notification Procedures

- Any spill, exposure, or equipment failure is reported **immediately** to:
    1. **Principal Investigator (PI)** – responsible for initiating the incident response.
    2. **Biosafety Officer** – responsible for biosafety compliance and follow-up investigation.
    3. **Facility Manager** – responsible for coordinating technical support and infrastructure-related actions.
- An **incident report form** must be completed within 24 hours, including details of the incident, response, and follow-up measures.

### Exposure Protocols (Medical Treatment & Monitoring)

- Exposed personnel must seek **immediate medical evaluation**. First aid is administered on site, followed by transfer to occupational health services if required.
- All exposure incidents trigger a **post-exposure protocol**, including baseline medical testing (if applicable), prophylaxis, and scheduled follow-up monitoring.
- Medical records of exposure and treatment are confidentially archived in compliance with GDPR and occupational safety requirements.

### Equipment Failure

- Work is stopped immediately upon detection of an equipment malfunction.
- Contaminated instruments and surrounding surfaces are **decontaminated with appropriate disinfectants** before service or removal.
- The PI and facility manager are notified without delay; equipment is labeled as “out of service” until repaired or replaced.
- A maintenance and service request is logged, and incident documentation is archived.

## PERSONNEL TRAINING AND SUPERVISION PLAN

### Initial and Periodic Training Program

- All staff will undergo **mandatory initial training** before commencing project activities, covering:
    - Biosafety and risk assessment procedures.
    - Safe handling of chemicals, including antibiotics and transfection reagents.
    - Work with immortalized human cell lines (HepG2, FreeStyle 293-F).

    - BSL-2 and BSL-2+ laboratory practices for work with lentiviral-compatible constructs.
- **Periodic refresher training** will be provided at least annually, or more frequently if new procedures, risks, or equipment are introduced.

### Responsible Persons for Training

- **Senior researchers** within Genos will serve as primary trainers for project-specific protocols (molecular cloning, cell culture, biosafety procedures).
- The **Institutional Biosafety Officer** will provide biosafety and emergency training.
- **External trainers** (if applicable) will be engaged for specialized modules, such as lentiviral vector handling or chemical safety updates.

### Documentation and Evidence

- Attendance at all training sessions will be **recorded in attendance lists** and signed by participants and trainers.
- Certificates of training (internal or external) will be issued and archived in institutional training records.
- Training documentation will be reviewed during internal audits to ensure compliance with OHS and biosafety requirements.

### Supervision System

- **Junior researchers** are supervised at all times by **senior researchers** until full competence is demonstrated.
- The **Principal Investigator (PI)** is ultimately responsible for ensuring compliance with safety procedures.
- The **Institutional Biosafety Officer** monitors adherence to biosafety rules and reports any non-compliance to the PI.
- Compliance is reinforced through routine laboratory inspections, regular team meetings, and corrective actions where needed.

## INCIDENT AND RISK MONITORING AND REPORTING

### System for Recording Incidents and Near-Miss Events

- All incidents, accidents, and **near-miss events** are recorded in a centralized **Incident Logbook** maintained by the Institutional Biosafety Officer.
- Records include date, location, personnel involved, type of incident, immediate response, and outcome.
- Each entry is assigned a reference number for traceability and follow-up.

### Methodology for Risk Assessment

- Risks are identified through **regular activity-specific risk assessments** before starting new procedures.
- Risks are ranked using a **likelihood × impact matrix** (low, medium, high) to prioritize mitigation measures.
- Re-assessments are conducted after incidents, protocol changes, or the introduction of new equipment/chemicals.

### Reporting Procedures

- All incidents are reported immediately to the **Principal Investigator (PI)** and the **Institutional Biosafety Officer**.
- Periodic summary reports (quarterly) are prepared and submitted to project management, and **annual safety summaries** can be shared with the donor if required.
- Serious incidents are escalated immediately to institutional management and regulatory authorities when applicable.
- Incident reports will be submitted to the Project Implemetation Unit (PIU) immediately, and not later than 24 hours, after any serious event which has, or is likely to have, a significant adverse effect on the environment, the affected communities, the public or workers. Incident reports will provide sufficient detail regarding the scope, severity, and possible causes of the incident or accident, indicating immediate measures taken or that are planned to be taken to address it. Reporting requirements will be in line with the Environmental and Social Commitment Plan (ESCP).

### Mechanism for Anonymous Reporting

- A confidential **anonymous reporting channel** (designated email) is available for staff to report safety concerns or near-miss events without risk of sanction.
- Anonymous reports are reviewed exclusively by the Biosafety Officer, who ensures appropriate action while protecting staff confidentiality.

### Corrective and Preventive Measures

- Following each incident, a **root cause analysis** is conducted.
- Corrective measures (e.g., changes in SOPs, additional training, equipment replacement) are implemented promptly.
- Preventive measures are incorporated into updated training, supervision, and risk assessments to reduce recurrence.
- Implementation of corrective actions is monitored by the PI and Biosafety Officer, and results are documented in follow-up reports.

## MONITORING PLAN

| Purpose of Monitoring | Parameters to be Monitored | Monitoring Methods & Frequency | Responsibilities | Reporting & Documentation | Corrective Actions | Monitoring Tools & Resources |
|---|---|---|---|---|---|---|
| Ensure reduced energy consumption and sustainable laboratory operations | Environmental risks – energy efficiency | Monthly utility checks; annual energy audits | Facilities Manager | Utility invoices, internal audit reports | Replace outdated equipment; improve HVAC efficiency | Energy meters, audit templates |
| Monitor water efficiency to minimize environmental footprint | Environmental risks - water usage | Monthly water consumption tracking; biannual audits | Facilities Manager | Water bills; facility maintenance logs | Repair leaks; install water-saving equipment | Water meters, inspection checklists |
| Ensure safe use and minimal reliance on hazardous substances | Chemical use & hazardous materials | Weekly inventory review; annual chemical risk assessment | Lab Manager; OHS Officer. | Updated chemical inventory; incident logs | Substitute safer reagents; reinforce training | MSDS sheets, chemical tracking system |
| Prevent environmental and health risks from improper waste handling | Biomedical & laboratory waste | Daily segregation checks; monthly disposal record reviews | Lab Managers; Procurement Officer | Waste logs; contractor manifests | Re-train staff; engage alternative contractor if needed | Waste containers, autoclaves, manifests |
| Ensure worker safety and compliance with OHS standards | Occupational health & safety (PPE, biosafety) | Daily PPE checks; monthly OHS inspections; annual safety audit | Lab Managers; OHS Officer | PPE inventories; inspection reports; training records | Replace damaged PPE; update SOPs; refresher training | OHS checklists, PPE logs |
| Minimize risks and ensure proper response to spills or exposures | Incident & exposure management | Immediate incident reporting; quarterly reviews of incident log | PI; OHS Officer | Incident logbook; medical follow-up records | Root cause analysis; corrective measures; re-training | Incident forms; emergency protocols |
| Ensure transparency, feedback collection, and controlled toolbox access | Stakeholder engagement & dissemination | Quarterly platform usage review; annual dissemination report | Project Team; PI | Platform request logs; dissemination reports; MTAs signed | Improve platform communication; adjust access protocols | GRM database; online platform; dissemination logs |