



Australian Government

Department of Health, Disability and Ageing

Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan (Consultation version)

for

DIR 230

Fermentation of straw-derived sugars using
genetically modified bacterium for hydrogen
production

Applicant: Hydgene Renewables Pty Ltd

20 October 2026

This RARMP is open for consultation until 7 December 2026.

Written comments on the risks to human health and safety and the environment posed by this proposed release are invited. You may make your submission

Via the consultation hub: <https://consultations.health.gov.au> (search for DIR 230)

via email to: ogtr@health.gov.au

or

via mail to: The Office of the Gene Technology Regulator, MDP 54 GPO Box 9848, Canberra ACT 2601

Please note that issues regarding food safety and labelling, the use of agricultural chemicals, and marketing and trade implications **do not** fall within the scope of these evaluations as they are the responsibilities of other agencies and authorities.

Summary of the Risk Assessment and Risk Management Plan

(Consultation Version) for

Licence Application DIR 230

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application to use genetically modified (GM) *Escherichia coli* as a biocatalyst to produce hydrogen (H₂) gas as an energy source from straw-derived or dextrose based liquid sugar extract. It qualifies as Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment under the *Gene Technology Act 2000* (the Act).

The applicant, Hydgene Renewables Pty Ltd, proposes to produce a GMO able to produce higher yield of H₂ than the parental strain from straw-based sugars. The parent strain is *E. coli* K-12 strain, *DH5α*, a non-pathogenic strain, retaining most of the characteristics of K-12. The proposed application is for a proof of principle project to optimise the fermentation process in closed system on a large scale. The proposed dealings are set to take place in a demonstration plant building in New South Wales (NSW).

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed trial poses negligible risks to human health and safety and the environment. Licence conditions have been drafted for the proposed trial. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether to issue a licence.

The application

Project Title	Fermentation of straw-derived sugars using genetically modified bacterium for hydrogen production ¹
Parent organism	<i>Escherichia coli</i> DH5α (<i>E. coli</i> K-12 derivative)- non pathogenic GM strain
Genetic modifications	Additional modifications have been made to <i>DH5α</i> to produce the GMOs to improve the ability of the bacteria to produce hydrogen and use a broader source of sugars, including: 1. Insertion of genes from <i>Chlamydomonas reinhardtii</i> encoding for high efficiency enzymes for production of hydrogen gas. 2. Upregulation of one or more of <i>E. coli</i> K-12 native genes to facilitate the following: • efficient metabolism of sugars towards hydrogen gas production rather than alternative products; • temperature tolerance; • use of broad range of sugars; 3. Deletion of: • genes encoding proteins that would promote glucose use over other sugars;

¹ The original titles supplied by the applicant was: "Deployment of encapsulated genetically engineered Escherichia Coli in a straw-to-hydrogen demonstration plant". The title has been modified for clarity and consistency.

	genes that encode proteins that would interfere with hydrogen production and use hydrogen for other cellular processes; 4. Insertion of selectable markers such as antibiotic resistance genes;
Principal purpose	The principal purpose of the trial is to conduct experiments to determine the efficiency of the biocatalyst cartridge system containing <i>E. coli</i> using straw-based sugars as a nutrition source for H ₂ production.
Previous releases	There has been no prior approval for this type of release of these GMOs
<i>Proposed limits and controls</i>	
Proposed duration	5 years
Proposed location	Demonstration plant in Local Government Area (LGA) of Blayney Shire Council, Lyndhurst, NSW 2797
Proposed controls	- access to the trial site will be restricted; - demonstration plant will be located within an existing farm machinery shed on a dedicated sealed concrete slab surrounded by a bunded area which is designed to capture accidental leaks or spills; - GMO will be trapped in solid hydrogel beads, which will be packaged within sealed cartridges; - only trained personnel will handle the GMO-containing cartridges; - all liquid waste containing the GMO will undergo heat and/or chemical treatment on-site to destroy the GMO; - a contingency plan to allow containment and decontamination of liquid in the event of a spill; - upon completion of an experiment, the cartridges are returned to the OGTR Certified PC2 large-scale manufacturing facility (Cert-5144) for decontamination of hydrogel beads and cartridges via autoclaving or chemical means before disposal; - transport of the GMO to the location of GMO deployment will follow the Regulators <i>Guidelines for Transport, Storage and Disposal of GMOs</i>.

Risk assessment

The risk assessment process considers how the genetic modifications and proposed activities conducted with the GMO might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application (including proposed controls), relevant previous approvals and current scientific/technical knowledge. Both the short- and long-term impacts are considered.

Credible pathways to potential harm that were considered included exposure of people or other organisms to the GM *E. coli*, and the potential for persistence or dispersal of the GM *E. coli*. Potential harms associated with these pathways included health hazards to people and animals from accidental ingestion of GMOs, and environmental harms due to the potential for the GMO to spread in the environment, including horizontal gene transfer of transgenes including antibiotic resistance genes to other bacteria in the environment.

The risk assessment concludes that the proposed dealings pose negligible risks to human health and safety and the environment. No specific risk treatment measures are required to manage these negligible risks.

The principal reasons for the conclusion of negligible risks are that the GMO does not contain protein-encoding transgenes that are not already present in the environment, the GMO is derived from a non-pathogenic organism, the GMO will not be used for human food or animal feed, and proposed limits and controls will effectively minimise exposure to and dispersal of the GMO.

Risk management

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions. Draft licence conditions are detailed in Chapter 4 of the RARMP.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the licence includes limits on the location and duration of the release. Controls are included to restrict the location and access to the site, to provide a contingency plan in case of GMO spill, multiple levels of GMO containment, and filtration and/or decontamination of the liquid waste to remove the GMO prior discharge.

In addition, there are several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects.

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Abbreviations

ARGs	Antibiotic resistance genes
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
GM	Genetically modified
GMO	Genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
HGT	Horizontal gene transfer
IBC	Institutional Biosafety Committee
L	Litre
OGTR	Office of the Gene Technology Regulator
ppm	parts per million
v/v	Volume per volume
RAF	Risk Assessment Framework
RARMP	Risk Assessment and Risk Management Plan
the Act	The <i>Gene Technology Act 2000</i>
the Regulations	The Gene Technology Regulations 2001
the Regulator	The Gene Technology Regulator
TSD guidelines	Regulator's <i>Guidelines for Transport, Storage and Disposal of GMOs</i>

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.
2. The Act and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.
3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.
4. The *Risk Analysis Framework* (RAF) (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator ([OGTR website](#)).
5. Figure 1 shows the information that is considered, within the regulatory framework, in establishing the risk assessment context. This information is specific for each application. Risks to the health and safety of people or the environment posed by the proposed dealings are assessed within this context. Chapter 1 describes the risk assessment context for this application.

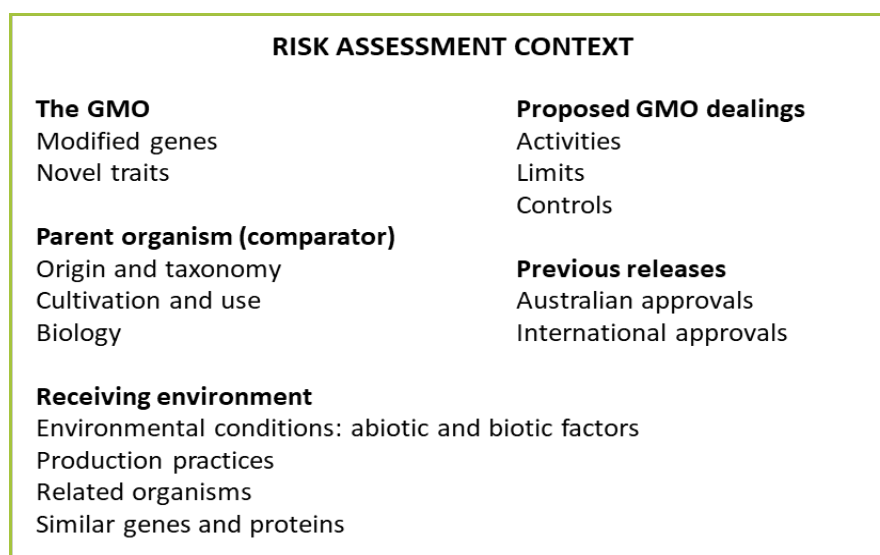


Figure 1 Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

6. In accordance with Section 50A of the Act, this application is considered to be a limited and controlled release application, as the Regulator was satisfied that it meets the criteria prescribed by the Act. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

7. Section 52 of the Act requires the Regulator to seek comment on the consultation RARMP from agencies - the Gene Technology Technical Advisory Committee, State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils and the Minister for the Environment - and from the public.

1.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority, the Therapeutic Goods Administration, the Australian Industrial Chemicals Introduction Scheme and the Department of Agriculture, Fisheries and Forestry (DAFF).

9. The DAFF regulates products imported into Australia to protect Australia from biosecurity risks. Under the *Biosecurity Act 2015*, the importation of biological material such as live GMOs requires a permit from the DAFF.

Section 2 The proposed dealings

10. Hydgene Renewables Pty Ltd (Hydgene) is seeking authorisation to carry out a trial of fermentation of sugars by genetically modified (GM) *E. coli* for the production of H₂ to be used as biofuel. The overall aim of the project is to demonstrate effectiveness of the biocatalyst system. For this, the applicant will:

- transport the canisters containing the GMOs from a PC2 certified facility to the demonstration plant building; and
- deploy canisters on-site and evaluate effectiveness of fermentation of sugars in the production of H₂.

11. The dealings involved are to:

- (a) culture the GMOs in volumes exceeding 25 litres per vessel;
- (b) conduct the following experiments with the GMOs:
 - i. optimise the conditions for hydrogen production;
 - ii. test samples for quality control purposes; and
- (c) use the GMOs in the course of manufacture of a thing that is not the GMOs;
- (d) transport the GMO;
- (e) dispose of the GMO;

and the possession (including storage), supply and use of the GMO for the purposes of, or in the course of, any of these dealings.

2.1 Proposed limits of the dealings (duration, location, scale, people)

- Proposed location is a demonstration plant in Local Government Area (LGA) of Blayney Shire Council, Lyndhurst, NSW 2797.
- A maximum of 60 cartridges (each having a 60L capacity and holding 60 kg of hydrogel beads with approximately 18 kg of GMO) will be deployed at the above location at any given time.
- Proposed period of release is 5 years.

2.2 Proposed controls to restrict spread and persistence of the GMO in the environment

12. The applicant has proposed controls to restrict the spread and persistence of the GMO in the environment. These include the following:

- access to the trial site will be restricted;
- the demonstration plant will be located within an existing farm machinery shed on a dedicated sealed concrete slab surrounded by a bunded area which is designed to capture accidental leaks or spills;
- the GMO will be trapped in solid hydrogel beads, which will be packaged within sealed cartridges;
- only trained personnel will handle the GMO-containing cartridges;
- all liquid waste containing the GMO will undergo heat and/or chemical treatment on site to destroy the GMO;
- a contingency plan will be developed and implemented in the event of an incident to allow containment and decontamination of liquid;
- upon completion of an experiment, the cartridges will be returned to Hydgene's OGTR Certified PC2 large-scale manufacturing facility (Cert-5144) for decontamination of hydrogel beads and cartridges via autoclaving or chemical means before disposal;
- transport of the GMO to the location of GMO deployment will follow the Regulators Guidelines for Transport, Storage and Disposal of GMOs ([TSD Guidelines](#)).

2.3 Details of the proposed dealings

2.3.1 Manufacturing and packaging of the GMOs

13. The GMOs will be manufactured, encapsulated into beads and packaged into canisters in an OGTR certified PC2 large scale facility, under a Notifiable Low Risk Dealings (NLRD) approval and will undergo in-house quality control and performance evaluation prior to the packaging for transport to the deployment site.

14. Quality control will include genotypic profiling to confirm strain identify and verification of culture purity.

15. The GMOs will be encapsulated in hydrogel beads which serves as the primary containment. These will be further packaged into cages which both contain and evenly distribute beads to allow gas escape. Each cage can contain 5-8 L of beads with 6-12 cages loaded per cartridge (Figure 2). Up to 60 cartridges will be deployed on site at any given time.

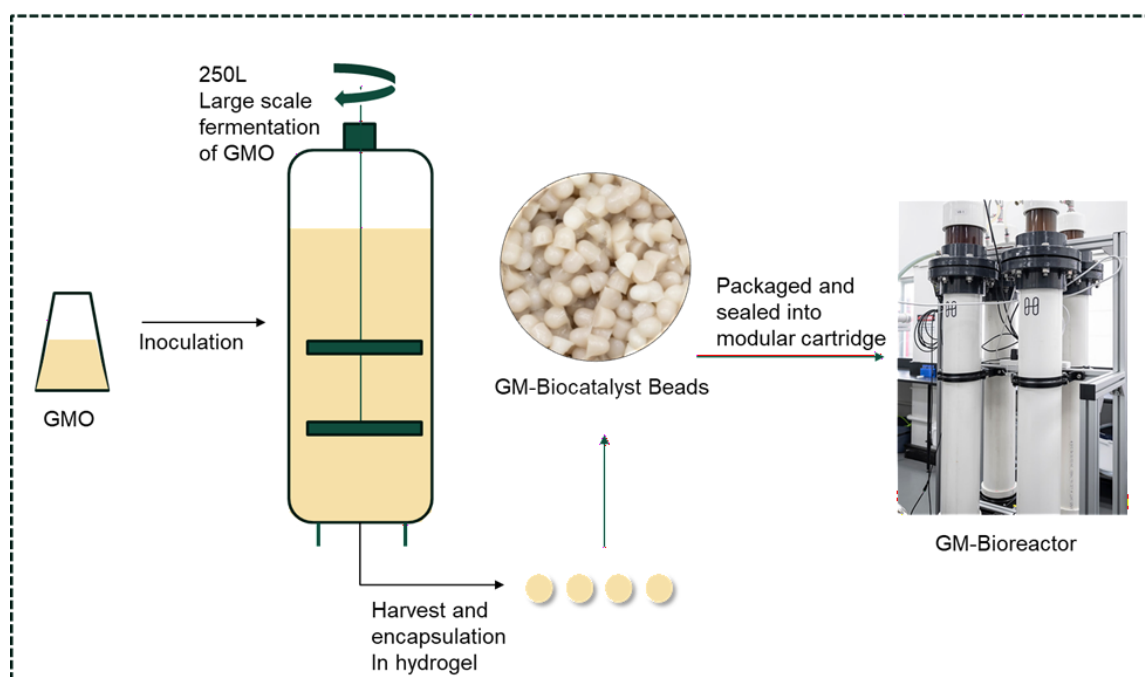


Figure 2 Preparation of the GMO under NLRD prior to deployment at the field site (provided by applicant)

2.3.2 Properties of the biocatalyst system (bioreactor)

16. The system consists of cartridges containing the GMOs and associated tubing and equipment, all of which are considered part of the closed system. The cartridges are secured on a skid for stability both during transport and for on-site deployment, forming the bioreactor (Figure 3).
17. Sugar for fermentation is supplied to the closed system via tubing from hydrolysis of straw, or dextrose may also be supplied.
18. Only one GMO type will be contained per cartridge, but cartridges containing different GMOs could be deployed at the same time.

19. The system operates as a batch system meaning that separate tanks are used for straw processing and H₂ production. The aim of the system is to produce up to 10 kg of H₂ per day.

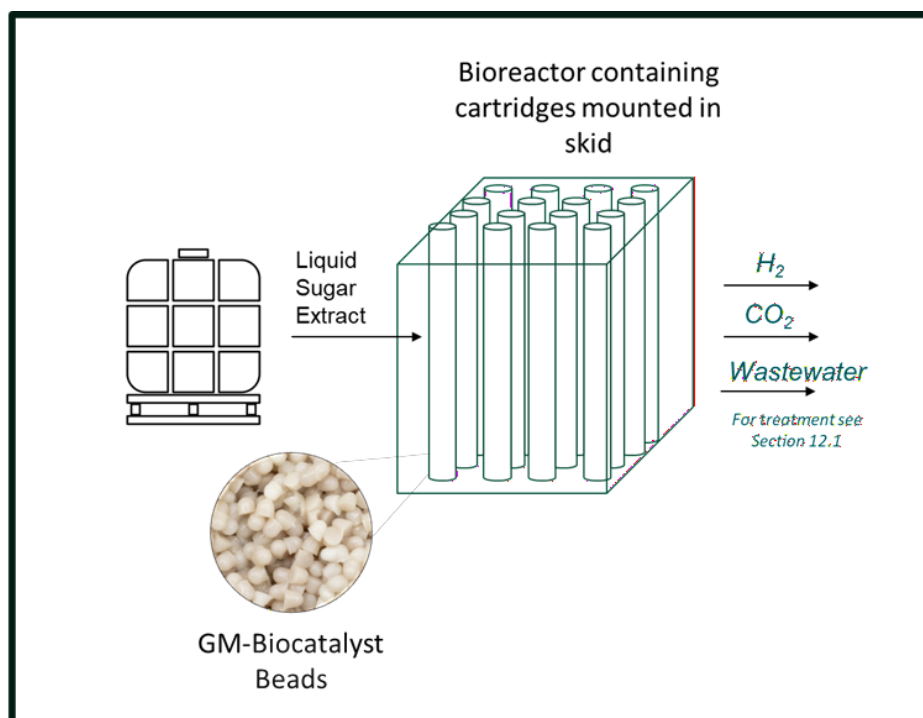


Figure 3 Overview of the system at deployment site (provided by applicant)

2.3.3 Deployment and operational set up

20. The demonstration plant (deployment site) is accessed via a 500 m compacted gravel driveway and consists of an existing farm machinery shed on a dedicated sealed concrete pad with bunding.

21. The applicant plans to install reinforced concrete flooring inside and outside the existing shed, to create hay and fodder storage areas and to construct an underground drainage tank (secondary waste tank) associated with the area as tertiary containment in the event of a leak, with 10 m³ capacity. The bioreactor is located within a bunded area of 12 m³ capacity, as is the primary waste tank for chemical or heat decontamination. The underground tank will hold decontaminated liquid from the primary waste tank or, in the event of a spill, will be used as part of the contingency plan to decontaminate GMO-containing liquid. Decontamination and disposal will be discussed further in Section 2.3.8.

22. Once the bioreactor is in place, the lines to liquid sugar extract feed are connected using quick-connect fittings.

23. The integrity of the system is checked before operation by performing a low-pressure and water circulation leak check.

24. Liquid sugar extract delivered to the cartridges is recirculated multiple times through the bioreactor system until all sugar is depleted. The liquid is filtered via a 0.2 micron filtration unit before re-circulation or diversion into the primary waste tank for chemical or heat decontamination.

25. The product stream leaving the bioreactor is received by the liquid-gas separator which separates residual liquid from the mixed gasses such as H₂/CO₂ produced by the system (Figure 4). The gas is vented into the atmosphere via a compressor. The separator incorporates a de-mister that removes droplets from the gas stream before the gas leaves the vessel. Any GMOs carried in droplets are retained in the liquid phase and returned to the system.

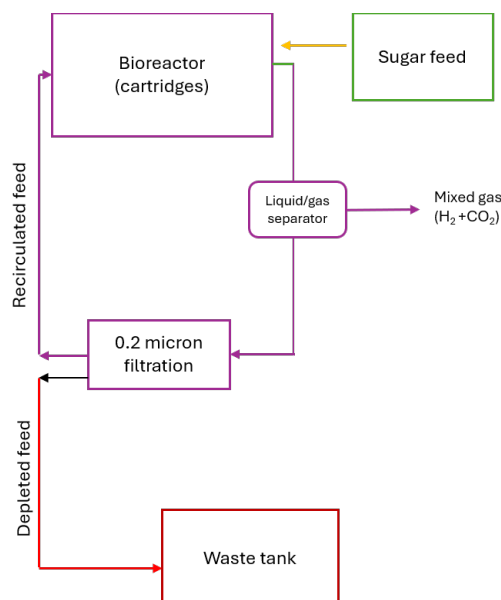


Figure 4 Process flow diagram

2.3.4 Sample testing

26. The 0.2 micron filtration unit, located after the liquid/gas separator, is used as a primary barrier to limit the concentration of the GMOs in transfer lines leaving the closed cartridge system. Weekly testing will be conducted to ensure that the primary effluent treatment filtration barrier is effective by collecting samples pre- and post-filtration and testing for live GMO (growth culture test). The system will have inlet and outlet ports for sampling. Dedicated PPE including gloves, eye protection, enclosed footwear and lab coat or disposable coveralls will be used. Although it is expected that some GMO will be present after filtration, this process is expected to reduce the concentration from approximately 2.6 log colony forming units (CFU)/mL to below the detection limit.

2.3.5 Liquid sugar extract

27. Straw-derived sugar solution is produced on-site by pre-treatment and hydrolysis of straw.

28. The pre-treatment set up is separated from the bioreactor and the two are not continuously connected. Transfer of new feed batch into the tank supplying the biocatalyst system occurs at high pH before pH adjustment to neutral pH. There is no hydraulic connection that would permit backflow.

2.3.6 Training

29. Training is required for the maintenance and operation of the cartridge system.

2.3.7 Transport and storage of the GMO

30. The bioreactor will be placed on a specially designed skid to secure the cartridges, and the skid will be placed within a robust secondary containment such as a bunded transport tray or pallet bund capable of retaining leaked liquid. Absorbent pads are placed under potential drip points during transport.

31. Transport will be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

32. Before transport, the cartridges are checked and confirmed to be sealed, labelled and have passed integrity testing.

33. Access is restricted to authorised persons and any loss of the GMO during transport initiates a leak response standard operating procedure (SOP) which refers to the requirements for immediate actions, containment measures and decontamination procedures.

34. Spill kits will be available and will include absorbent pads and granules, heavy-duty waste bags, disinfectants, disposable wipes/paper towels, an incident response instruction card and emergency contact list. General PPE to be worn while cleaning up a spill includes safety glasses, nitrile gloves, enclosed footwear and coveralls.

35. The contingency plan for loss of containment describes the actions to be taken in the event of an incident and distinguishes 4 levels of severity, from level 1 (a suspected leak confined within secondary containment, but no release of the GMO) to level 4 (major incident involving uncontrolled release and injuries).

36. The general outline of the plan includes using absorbent material to contain the GMO, and to ensure the GMOs are not released into waterways and drains. Collected liquid is to be treated with 1% sodium hypochlorite (v/v) for at least 30 minutes. Absorbent wipes, PPE and any other waste contaminated with the GMO should be collected into bags and returned to the PC2 large scale facility for decontamination.

37. Any GMO loss, spill or leak will be reported to the OGTR as soon as reasonably practicable.

2.3.8 Decontamination and disposal of the GMOs

38. The effluent management includes the filtration system, bunding, and decontamination/holding tanks. These are demonstrated in Figure 5.

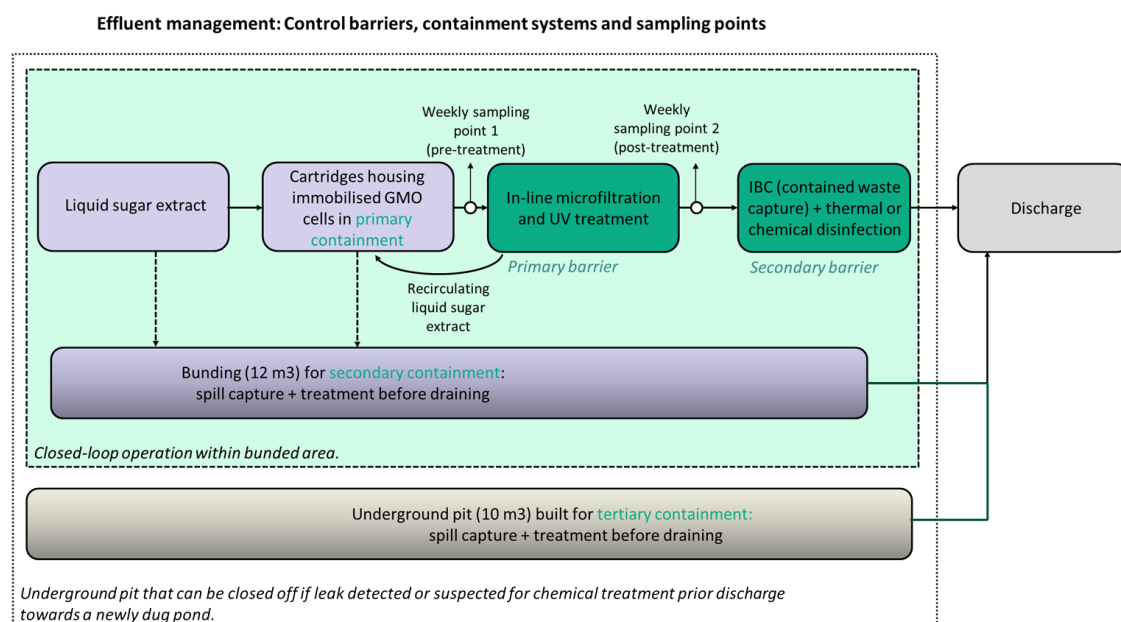


Figure 5 Effluent management (provided by applicant)

39. Liquid sugar extract that is no longer recirculated is passed through 0.2 micron filtration unit and collected in the waste holding tank located within the bunded area. The liquid undergoes inactivation using the most cost-effective combination of heat and/or chemical (chlorine) treatment to achieve complete inactivation of the GMOs.

40. Heat decontamination will be performed using time and temperature parameters that have been validated, or consistent with site-approved sterilisation conditions.

41. Chemical disinfection with sodium hypochlorite will be undertaken when chlorine addition is compatible with the intended recycling of the decontaminated liquid waste, and where verification

by free chlorine residual is practical. Effectiveness of the chlorine decontamination will be performed via achieving a free final chlorine residual of at least 2.5 ppm after a required contact time. The applicant has indicated that for wastewater treatment, 0.5 ppm of free chlorine was able to inactivate *E. coli* within 1 minute of exposure.

42. After inactivation in the waste tank, the liquid is transferred to the underground pit and stored to await off-site disposal by an external waste contractor. Decontaminated liquid may also be re-used for other processes. As a contingency in the case of overflow or accidental release from the bioreactors or waste tank, small spills can be decontaminated in situ using chemical decontamination or for larger volumes the GMO-containing liquid can be sent to the underground waste pit for chemical decontamination. If the volume of the liquid exceeds the waste pit capacity, pipes can then direct decontaminated liquid from the underground pit to a nearby evaporation pond.

43. At the conclusion of the trial, the cartridge module internal lines will be disinfected with 1% (v/v) sodium hypochlorite solution for 30 minutes before draining to the primary waste tank to undergo additional decontamination. This liquid is then disposed of by an external contractor as noted above. Ports will have protective caps for transport, to prevent leaks from the system. Used cartridges are returned to the OGTR certified PC2 Large scale facility, prior to disposal or reuse.

44. Solid waste generated during the operation of the bioreactor including PPE, absorbent material used to collect a spill if present, filters, damaged hoses and cartridge parts will be double contained and collected as biohazardous waste for disposal by approved contractors.

2.3.9 Accountability and Monitoring

45. Only trained staff and contractors will be allowed on site during operations, and the Project manager will remain near the site for the duration of the trial to supervise activities and ensure compliance.

46. All information relevant to the dealings with the GMOs will be included in a licence training package. This will be provided to all personnel and contractors who may handle or come into contact with the GM biocatalyst.

47. Training records will be signed confirming training and that they have read and understood the licence conditions and agree to comply with them.

2.3.10 Contingency plans

48. The GMOs will not be intentionally released into the environment.

Accidental release at primary site

49. In case of an unintentional release (e.g. spill, leak, treatment failure or suspected presence of viable GMOs outside of containment), physical containment of the system includes a bunded area around the bioreactor, a primary waste tank, and an underground secondary waste tank to contain large spills (Figure 5). The liquid can then be decontaminated in the primary waste tank or the underground secondary waste tank and either collected by external contractors for disposal or released via pipes to the nearby evaporation pond.

50. Contaminated equipment will be cleaned and disinfected using 80% (v/v) ethanol and/or commercial Pyroneg (sodium carbonate/benzenesulfonic acid) solution before being returned to service or removed from the site.

51. Any viable GMOs/GM waste will be packaged and transported to PC2 facility for decontamination or disposal.

52. Verification of whether GMOs have been released into the environment will include microbiological testing from samples collected from relevant locations (e.g. post-filtration effluent, primary waste tank, bunded area or underground waste tank, and any surfaces contacted by the

spill). Further molecular testing will occur if there is an indication of viable microbiological growth to identify the GMO.

53. Where the event meets reporting requirements, the incident will be reported to the Regulator.

Section 3 Parent organism

3.1 Risk Group and containment

54. The GMO is derived from gram-negative bacterium *Escherichia coli* (*E. coli*), strain K-12, isolated from a human patient and further modified to create DH5 α strain. K-12 has had multiple modifications during many decades of laboratory propagation (discussed in Section 3.4). The parent organism meets the criteria to be classified as a Risk Group 1 organism in accordance with the Australian/New Zealand Standard 2243.3:2022 (Standards Australia/New Zealand, 2022). The characteristics of the parent organism provide a baseline for comparing the potential for harm from dealings with GMOs. As such, the relevant biological properties of the non-GM *E. coli* K-12 strain and its GM derivative, DH5 α , will be discussed here.

3.2 Classification

55. *E. coli* belongs to the Family *Enterobacteriaceae*, Genus *Escherichia* and Species *coli*. *E. coli* is further divided into 14 phylogenetic groups (Abram et al., 2021) which are: A, B1, B2-1, B2-2, C, D1, D2, D3, E1, E2, F, G, Shig1 and Shig2, which includes the genus of *Shigella* as it has been shown to be a subspecies of *E. coli* (Abram et al., 2021).

3.3 Characteristics

56. *E. coli* is gram-negative, rod-shaped bacterium that is facultatively anaerobic, capable of both respiratory and fermentative metabolism (Clark, 1989). *E. coli* can live within a host as either commensal or pathogenic and is also found in the environment. Commensal strains reside in intestinal tract of humans and other animals as an important component of microbiota. It usually colonises intestine of humans within a few hours after birth. Pathogenic strains can cause intestinal or extra-intestinal disease relating to a variety of virulence genes.

57. Lipopolysaccharide (LPS) is found exclusively in the outer membrane of gram-negative bacteria and performs numerous functions. It consists of lipid A, core oligosaccharide and O-antigen. The O-antigen is a major surface antigen can be highly immunogenic but normally immune system tolerates intestinal bacterial LPS through low level LPS receptor expression (Tume et al., 2025).

58. K-12 was originally isolated in 1922 from a stool sample of a patient (Browning et al., 2023). It has since been used extensively in the laboratory research and has been extensively modified from the original strain. In contrast to the original isolate, the K-12 strain has lost the O antigen, and has lost the capacity to colonise the intestinal tract of humans and mice (Curtiss, 1978; Levy et al., 1980). It also no longer carries a conjugative F plasmid or infectious bacteriophage lambda (λ) (Browning et al., 2023).

59. A DH5 α -derivative, used for construct of the GMO has been sourced from Biolabs under name NEB5 α (NEB 5-alpha Biolabs competent *E. coli* strain) with features analogous to lab-adapted DH5 α (Anton and Raleigh, 2016).

60. This strain has several genetic modifications including:

- *recA1* gene- reduced homologous recombination and reduced plasmid integration into host genome;
- *endA1*- removal of nonspecific endonuclease I, retains high-quality plasmid DNA;

- *hsdR17*-disruption of endonuclease - effective transformation with unmethylated DNA that would otherwise be digested;
- *gyrA96* mutation conferring resistance to nalidixic acid antibiotic;
- *fhuA2* - resistance to bacteriophage T1;
- *thi-1* mutation requiring thiamine in growth media.

3.4 Genome

61. *E. coli* carries its genetic material in two forms: chromosomal and plasmid. Chromosomes contain essential genetic material and are generally passed vertically from parent to offspring. Plasmids are smaller packets of DNA that exist separately from chromosome and can replicate independently. *E. coli* bacteria often carry multiple plasmids simultaneously (Dionisio et al., 2019; García et al., 2007).

62. Several types of virulence plasmids have been described for intestinal *E. coli* encoded by the IncF family of plasmids (Johnson and Nolan, 2009) which commonly encode not only antibiotic resistance genes (ARGs) discussed below, but also genes that promote adherence and colonisation.

63. Genome size varies widely, with an average genome containing around 5000 genes. Only 1700 of these are conserved among all strains while another 3000 are conserved in at least 95% of strains (Kaas et al., 2012).

64. In addition to a large gene pool, *E. coli* can exchange genetic elements with other compatible bacteria in the environment. This contributes to evolution of bacterial variants, resulting in novel strains.

3.4.1 Horizontal gene transfer (HGT)

65. Four genetic mechanisms enable the horizontal transfer of genetic elements in *E. coli*: conjugation, transformation, transposition and transduction (Norman et al., 2009).

3.4.1.1 Conjugation

66. Conjugation refers to a direct transfer of DNA from one bacterium to another and is considered the most important mechanism of horizontal gene transfer in bacteria (Norman et al., 2009). This process involves filamentous pilus that allows a copy of the plasmid to travel across to the recipients. This process is limited by the proximity required for transfer of genetic material.

67. Conjugation occurs in bacterial communities present in the environment such as soil, water and sewage as well as in biofilms and bacterial communities associated with animal hosts.

Conjugative plasmids

68. Bacteria can transmit genes horizontally by conjugation and transfer of the conjugative plasmid. These plasmids encode all genes required for maintenance during transfer as well as genes necessary for the process of conjugation (Virolle et al., 2020) which offers an abundance of alternative hosts. The *Tra* gene encodes the complete transfer enzyme needed for this process (Liu et al., 2024).

69. Plasmid propagation by conjugation requires mating pair formation (mpf) and assembly and activity of a protein complex, the relaxosome, that allows the processing of the plasmid before DNA transfer (Norman et al., 2009; Virolle et al., 2020).

70. The conjugation window from *in vitro* experiments proposes a period shortly after *E. coli* cells divide but before they enter a nongrowing phase, indicating there is only a small window of opportunity for conjugation to take place (Headd and Bradford, 2020).

71. Colonisation of germ-free mice with *E. coli* containing an ARG plasmid led to loss of the plasmid unless selection pressure was applied via antibiotic treatment (Wilcks and Jacobsen, 2010).

3.4.1.2 Transformation

72. Transformation involves induction, uptake and stable maintenance of taken up DNA either by integration into the genome (recombination) or recircularization of plasmid DNA. The natural transformation mechanism requires competence of the bacterium such that it can take up extracellular DNA (Blokesch, 2016). The insertion of the DNA into the genome relies on DNA recombinase enzymes and can occur either via homologous recombination, or via so-called illegitimate recombination. In natural environments naked DNA becomes especially vulnerable to degradation from nucleases or heavy metals although sands and clay are known to increase DNA stability (Rumball et al., 2021; Verheijen et al., 2025). Inducers of competence include high cell density, antibiotic stress, DNA damage, and general starvation (Blokesch, 2016).

73. *E. coli* is not considered naturally transformable (Sinha and Redfield, 2012). DH5a lacks the RecA recombinase protein required for recombination and DNA integration into the genome.

3.4.1.3 Transduction

74. Transduction is movement of genetic material via bacteriophages, viruses that infect bacteria, and requires that a phage replicates within the donor organism, and in the process of DNA packaging erroneously incorporates bacterial DNA fragments which can then transfer to a new host. Most phages have a specific host range, and transduction is thought to provide intraspecies gene transfer (Liu et al., 2024).

3.4.1.4 Transposition

75. Transposition is a translocation of a discrete DNA segment from a donor site to another site. This usually involves translocation of transposon onto a plasmid upon conjugation and occasionally integration into genome (Huddleston, 2014). A study of ARGs in the human large intestine found multiple ARGs associated with integrase and transposase sequences, indicating a possibility that these ARGs are part of conjugative transposons (Huddleston, 2014).

3.5 Commensal *E. coli*

76. Approximately 10^{11} bacteria can be cultivated per gram of adult human colonic content (Conway et al., 2004). *E. coli* is largely non-pathogenic, commensal bacterium that resides in the large intestine (Martinson and Walk, 2020). *E. coli* in the human intestine helps in digestion of food, produces important vitamins and prevents against pathogenic bacteria colonisation. It represents 0.1%-5% of commensal microflora in the large intestine.

77. Human studies have demonstrated the poor ability of the lab propagated K-12 strain to colonise the intestine, even after ingestion of billions of organisms, and there was no evidence that it could transfer introduced plasmids to native bacteria isolated from faeces (Browning et al., 2023; Environmental, 1997; Levy et al., 1980). However, in the case of depleted microbiota (e.g. in germ-free mice), K-12 has been shown to have the capacity to colonise the intestine of mice (Levy et al., 1980). When these mice are exposed to intestinal *E. coli* they quickly displace K-12 (Environmental, 1997).

3.6 Pathogenicity

78. Pathogenic strains carry virulence factors that are not present in commensals, such as toxins, adhesins, protective coats and invasins and are estimated to cause hundreds of thousands of deaths per year (Russo and Johnson, 2003).

79. Most commonly, infections with *E. coli* cause diarrhoea or gastroenteritis and are often acquired through eating contaminated food. Some pathogenic *E. coli* produce a toxin called Shiga.

These bacteria can cause diarrhoea, abdominal cramps, vomiting and sometimes a serious condition called haemolytic uraemic syndrome (Freedman et al., 2023).

80. *E. coli* can cause diseases outside the intestine, such as urinary tract infections which are normally treated with antibiotics. Rarely, *E. coli* can cause respiratory disease, mainly in the elderly (Marrie et al., 1998). After a solid organ transplant, blood infections with *E. coli* are a common and life-threatening complication (Kritikos and Manuel, 2016).

81. A large study of skin and soft tissue infections from hospitalised patients found that *E. coli* was the third most common cause of infections (Moet et al., 2007). When these are not self-limiting, they are treated with antibiotics.

3.7 Transmission

82. *E. coli* is mostly spread via the faecal-oral route due to poor hygiene or food preparation practices. Most intestinal infections are due to ingestion of contaminated food or water. *E. coli* can also be acquired through direct contact with an infected person or animal.

3.8 Host range and reservoir

83. *E. coli* can be detected in many animals, including birds and mammals, but is more likely to be recovered from animals living in association with humans compared to the same species living in isolation. *E. coli* is not generally found in the intestines of fish.

3.9 Environmental stability and decontamination

84. *E. coli* can survive long periods of no growth in a variety of environments such as soil, water, sediments and food. Its presence in water was often used as an indicator of faecal pollution (Russell and Jarvis, 2001). However, more recent reports show that some *E. coli* is naturalised to soil, sand and sediments (Jang et al., 2017).

3.10 Fermentative metabolism of *E. coli*

85. *E. coli* can metabolise sugars for growth and in the process releases H₂ as a byproduct (Figure 6). Fermentation is a highly effective process in the production of H₂ (Maeda et al., 2012).

86. The basic pathway of glucose metabolism by *E. coli* leads to provision of pyruvate with downstream processes involving various enzymes, some of which lead to H₂ production (Figure 7). H₂ can be recycled for other metabolic pathways.

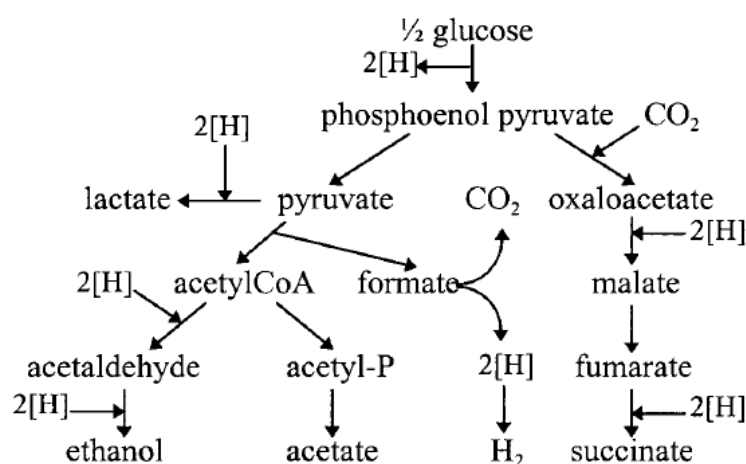


Figure 6 Fermentation pathways of *E. coli* (Riondet et al., 2000)

87. The enzyme complex involved in the production of H_2 from glucose consists of formate hydrogenlyase system (FHL) and includes formate dehydrogenase-H (encoded by *fdhF*) and hydrogenase (Hyd) (Figure 6, Figure 7).

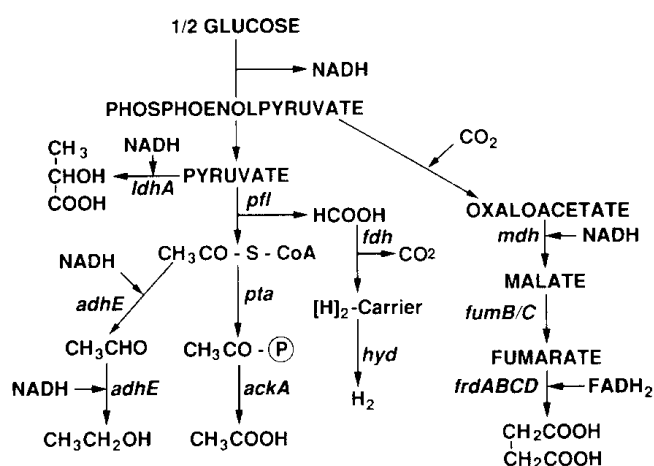
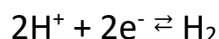


Figure 7 Genes responsible for fermentation pathway of *E. coli* (Clark, 1989). *ldhA*= lactate dehydrogenase; *pfl*= pyruvate formate lyase; *adh*= acetaldehyde CoA dehydrogenase; *pta*= phosphotransacetylase; *fdh*=formate dehydrogenase; *mdh*= malate dehydrogenase; *fumB/C*= fumarase B/C; *frd* fumarate reductase; *ack*= acetate kinase.

88. Biological synthesis of H_2 is catalysed by the hydrogenase (Hyd) enzyme (King et al., 2006), classified into several groups based on the type of iron sequestered by the active site of Hyd. The reaction which produces H_2 can be a reversible process:



89. H_2 production is highly dependent on factors such as carbon source, pH, temperature, redox potential, and presence of exogenous electron acceptors (Valle et al., 2019) and the amount of H_2 produced by non-GM *E. coli* during the fermentation of sugars such as glucose is considered relatively low.

3.10.1 Carbon catabolite repression

90. Carbon catabolite repression (CCR) controls the order in which different carbon sources are metabolised by *E. coli*. This is thought to result in maximising growth rate by utilising preferred

sugars first. In *E. coli*, CCR suppresses expression of genes that use secondary carbon sources until the primary carbon source has been depleted.

91. *E. coli*'s preference for glucose means that when provided with mixed sugars, glucose is consumed first, and genes involved in other sugar metabolic pathways are generally inhibited (Desai and Rao, 2010; Kaplan et al., 2024) until all glucose is exhausted. This sequential rather than simultaneous use of sugars can greatly reduce the efficacy of fermentation.

3.10.2 Straw-derived sugars

92. Straw is comprised of cellulose, hemicellulose and lignin. Hydrolysis of straw with enzymes can give rise to sugars such as glucose, xylose and arabinose which can then be used as fermentation substrates by *E. coli* (Zheng et al., 2018). Xylose and arabinose are 5-carbon sugars and are called pentose sugars. Glucose (dextrose) is a 6-carbon sugar. These sugars can be processed via similar metabolic pathways in *E. coli* (Tan et al., 2026).

Section 4 The GMO - nature and effect of the genetic modification

93. Numerous modifications are proposed to be made (alone or in combination) to NEB5α to create multiple GMOs. Some of the details of genetic modifications (methods and genes) have been declared Confidential Commercial Information (CCI). Under section 185 of the Act, the CCI is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

94. The genetic modifications are divided into 4 trait groups to broadly describe desired outcomes. All GMOs will express genes for Trait 1, and one or more of the modifications indicated for Traits 2-4.

95. The traits are as follows:

- Trait 1- improved H₂ yield by introduction of highly efficient hydrogenase and associated genes from algae *Chlamydomonas reinhardtii*;
- Trait 2- improved H₂ production by upregulation of native *E. coli* K-12 genes that enhance the availability of necessary components for H₂ production, assist hydrogenase function and provide stability at variable temperatures;
- Trait 3- improved use of straw-derived mixed sugars by upregulation of native *E. coli* K-12 genes;
- Trait 4- deletion of *E. coli* genes that bias sugar metabolism towards glucose preference and genes that use H₂ and its components for other metabolic pathways.

4.1 Method of genetic modifications

96. Some genetic modifications will include provision of genes on non-conjugative plasmids. The genes are included on plasmids pBR322/pMB1 and p15A. Although pBR322 has a *bom* (basis of mobility) sequence, it does not contain transfer genes that would make it a conjugative plasmid. These genes may also be inserted into the genome. The plasmid maps have been declared CCI.²

97. Some genes may also be inserted into the bacterial genome. Details of the method of insertion have been declared CCI.²

² Under section 185 of the Act, the CCI is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

98. Other modifications include deletions and modification of the promoter regions to upregulate native genes. These will be performed using CRISPR/Cas system and λ -phage red operon system as described in Reisch and Prather (2015). This approach uses a 2-plasmid system encoding for Cas9, guide RNA and three bacteriophage lambda genes *exo*, *bet* and *gam* (λ -phage red operon system) under inducible promoters that facilitates insertion of target DNA into the *E. coli* genome. Once the process is completed, cells are cured from the plasmids.

99. Genetic modifications and manufacturing are performed under exempt dealings/NLRD authorisation and are not part of this application.

4.2 Genetic modifications

100. Genetic elements encoding for the proteins of interest are described in Table 1. Some of the genes have been declared CCI.²

101. In addition to the existing mutation in DH5 α which confers antibiotic resistance to nalidixic acid, other commonly used ARGs introduced as selection markers during making of the GMO may confer resistance to kanamycin, chloramphenicol and ampicillin. These are only present on plasmids. These genes have no role under the proposed trial.

102. Promoters and terminator sequences include bacterial lac promoters/T7 promoters and individual RBS elements associated with genes and transcription terminators including rrnB T1, T7Te.

4.2.1 *C. reinhardtii*-derived proteins that enhance H₂ production (Trait 1)

103. *C. reinhardtii* is a unicellular phototrophic green alga, studied extensively for fundamental processes such as photosynthesis and H₂ production especially under anaerobic conditions (Fields et al., 2020).

104. The [Fe-Fe] hydrogenase, A (HydA) from *C. reinhardtii* produces high yields of H₂ and hence holds interest in commercial bioproduction of H₂ (King et al., 2022). The activity of HydA requires a co-factor known as H-cluster comprised of iron (Fe) and sulphur (S) metals (Britt et al., 2020).

105. The maturation system both forms and inserts the H-cluster co-factor into [Fe-Fe] hydrogenase. The H-factor maturation system includes fusion protein HydEF and enzyme HydG (King et al., 2006; King et al., 2022) and these proteins are necessary for successful H-cluster function.

106. Transfer of electrons by ferredoxin (petF) to HydA is the key step in H₂ production. This process is assisted by ferredoxin reductase (fnr), enzyme that catalyses nicotinamide adenine dinucleotide phosphate (NADPH) formation (Housseini et al., 2025). These genes comprise Trait 1 and will be expressed by all the GMOs (Table 1).

4.2.2 *E. coli* derived proteins that reduce temperature sensitivity of the GMOs (Trait 2)

107. Temperature shock chaperone proteins from *E. coli* can help a cell deal with harmful effects of stresses and are considered stress adaptation proteins. The expression of native *E. coli* proteins will be enhanced via promoter modification. These genes have been declared CCI.²

4.2.3 *E. coli* derived proteins that enhance effectiveness of H₂ production (Trait 2)

108. HydA from *C. reinhardtii* requires the H-cluster to function. The iron-sulfur cluster (Fe-S) assembly genes from *E. coli* K-12 may be introduced to drive their increased expression in order to optimise HydA function. The specific identities of genes have been declared CCI.²

109. Pentose phosphate pathway (PPP) is involved in the processes that generate NADPH for biosynthetic pathways of the cell (Sprenger, 1995). NADPH is an important electron donor in the formation of H₂ and produced via PPP by glucose-6-phosphate dehydrogenase (G6PD) encoded by *zwf* and 6-phosphogluconate dehydrogenase (6PGD) encoded by *gnd* (Lee et al., 2011; Lim et al., 2002; TeSlaa et al., 2023). These genes from *E. coli* K-12 may be provided on a plasmid or integrated into the genome to enhance their expression.

110. Transaldolase plays a key role in the PPP and native TelA and TelB may be upregulated in the GMOs to assist with PPP efficiency.

4.2.4 *E. coli*-derived proteins for broad sugar use (Trait 3)

111. *AraE* is a transporter that allow arabinose entry into the cell to be metabolised. Introduction of this gene removes the CCR by glucose also allows simultaneous entry of arabinose sugar for metabolism.

112. Additional genes involved in xylose/pentose utilisation pathways that will be upregulated have been declared CCI.²

4.2.5 *Deleting E. coli* genes to improve H₂ production (Trait 4)

113. As noted in Figure 6, there are multiple pathways in the metabolism of glucose that consume hydrogen or NADHP that would otherwise lead to H₂ production. Deletion of key genes in these pathways may benefit H₂ production. GMOs may have some or all of these key genes deleted to enhance H₂ generation. Some of these genes have been declared CCI.²

Table 1 Traits and proposed inserted genes for increased hydrogen production

Trait 1: High yield hydrogen production -Inserted genes: Source organism - <i>C. reinhardtii</i> ; all genes will be present in all the GMOs			
Gene	Product	Properties and Function	Location
<i>hydA</i>	Hydrogenase	[Fe-Fe] hydrogenase, catalyses production of H ₂	Plasmid/genome insertion
<i>hydG</i>	Hydrogenase	Important in H cluster biosynthesis and maturation- relevant for HydA function.	Plasmid/genome insertion
<i>hydEF</i>	Fusion protein	Comprised of hydrogenase E and F; Important in H-cluster biosynthesis and maturation- relevant for HydA function.	Plasmid/genome insertion
<i>petF</i>	Ferredoxin	Electron donors to HydA-mediated H ₂ production	Plasmid/genome insertion
<i>fnr</i>	Ferredoxin NADP reductase	Biocatalyst - regenerates NADPH to provide electrons for H ₂ production	Plasmid/genome insertion
Trait 2: Optimisation of HydA function, temperature tolerance and improved availability of electrons for H₂ production. Source organism - <i>E. coli</i> K-12; some or all these modifications may be present in the GMOs			
<i>zwf</i>	glucose-6-phosphate dehydrogenase	Increases NADPH via PPP which provides additional electrons for H ₂ production	Plasmid/genome insertion
<i>gnd</i>	6-phosphogluconate dehydrogenase	Increases NADPH via PPP which provides additional electrons for H ₂ production	Plasmid/genome insertion
<i>talA</i>	Transaldolase A	Reroutes carbon through PPP to reinforce NADPH generation	Plasmid/genome insertion
<i>talB</i>	Transaldolase B	Alternative transaldolase isozyme; can contribute to PPP carbon routing and NADPH generation	Plasmid/genome insertion
6 x CCI genes	CCI	CCI	CCI

Trait 3: Expanded substrate utilisation - Source organism - <i>E. coli</i> K-12; some or all these modifications may be present in the GMOs			
<i>araE</i>	Arabinose:H ⁺ symporter	Assists in arabinose uptake; it is intended to support arabinose utilisation alongside glucose from straw hydrolysates	plasmid/genome insertion
5 x CCI genes	CCI	CCI	CCI
Trait 4: Reduced pathway competition to increase available components for H₂ production – Possible deletion of native <i>E. coli</i> genes			
<i>Glk</i>	Glucokinase	Acts to accumulate glucose inside the cell; deletion could reduce cellular preference for glucose as an energy source	Native gene deletion
<i>pfkA</i>	Phosphofructokinase I	Plays a role in glycolysis, deletion enhances pathways towards H ₂ production	Native gene deletion
<i>gpmA</i>	2,3-biphosphoglycerate-dependent phosphoglycerate mutase	Deletion removes competing pathways; contributes to redirecting carbon/electron distribution towards H ₂ production.	Native gene deletion
<i>gpmM</i>	2,3-biphosphoglycerate-independent phosphoglycerate mutase	As above for <i>gpmA</i>	Native gene deletion
<i>edd</i>	6-phosphogluconate dehydratase	Deletion reduces alternative pathways, supports redirection of carbon through PPP	Native gene deletion
<i>eda</i>	KDPG aldolase	Deletion reduces alternative pathways	Native gene deletion
<i>ppsA</i>	PEP synthase/pyruvate kinase	Deletion reduces competing pyruvate use for other pathways	Native gene deletion
<i>ldhA</i>	D-Lactate dehydrogenase	Deletion reduces lactate formation which is an alternative sugar processing pathway which does not promote H ₂ production	Native gene deletion
<i>dld</i>	Quinone dependent D-lactate dehydrogenase	Deletion reduces D-lactate formation which is an alternative pathway which does not promote H ₂ production	Native gene deletion

3 x CCI genes	CCI	CCI	Native gene deletion
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4.3 Expected properties of the GM *E. coli*

114. The genetic modifications are designed to enhance production of H₂ from *E. coli* and effectively co-utilise multiple sugars.

115. The GMOs tested thus far did not show a growth advantage in the laboratory culturing conditions compared to the parent DH5α (NEB 5α) strain.

116. Whereas bacteria can generally produce their own thiamine (vit B1) for growth, DH5α and hence the GMOs, require thiamine to be supplied in the growth media due to the mutation in *thi-1* gene which prevents synthesis of thiamine. Without this provision, the GMO cannot multiply. The applicant has stated that due to the absence of essential salts, trace elements and thiamine in the biocatalyst system, the GMO will go into a non-growing maintenance stage during hydrogen production at the site.

117. Due to the presence of ARGs in the GMO, the bacteria may have a selective advantage in the presence of antibiotics.

Section 5 The receiving environment

118. The receiving environment forms part of the context in which the risks associated with dealings with the GMOs are assessed (OGTR, 2013). Relevant information about the receiving environment includes interactions of the GMOs with the environment where the release would occur, presence of organisms that can receive or transfer genetic material with the GMOs and whether the gene(s) used in the genetic modification are already present in the environment.

119. The receiving environment is typical rural farmland with intermittent machinery activity and potential cattle movement on the property, with bore water also present on the site. An evaporation pond will also be dug nearby the deployment site as a contingency for release of excess decontaminated liquid from the underground waste pit.

5.1 Presence of related bacteria in the environment

120. The presence of related bacteria may offer an opportunity for genetic material to transfer between the GMO and other organisms in the receiving environment.

121. *E. coli* can be found in faeces of warm-blooded animals. It is a common commensal bacterium. As noted in Section 3.7, *E. coli* is commonly found in soil and water and can persist in the environment long-term.

5.2 Presence of similar genes in the environment

122. The balance of a system could be perturbed by the introduction of new genetic material through horizontal gene transfer or through release of GMO into the environment. However, the effect of perturbation would be relatively small if the genetic material was already present in the system and did not confer any selective advantage to an organism that gained this genetic material.

123. *C. reinhardtii* is a common alga widely distributed in soil and fresh water across the world and has been found in at least one location in the natural environment in Australia (MGnify, 2019). It is also a common lab organism. The genes encoding enzymes from this organism are therefore not novel to the environment. *E. coli* transgenes are common in native *E. coli* and are therefore already present in the environment.

5.2.1 Antibiotic resistance genes (ARG)

124. A common use of ARGs on plasmids in laboratory research is as selectable markers. This approach allows for survival of only those bacteria in the antibiotic-supplemented media that have taken up desired plasmids.

125. Some of the ARGs commonly used as selectable markers were originally isolated from *E. coli*. For example, the *nptII* gene encoding neomycin phosphotransferase II enzyme and provides kanamycin (and other structurally related antibiotics such as neomycin, geneticin) resistance (OGTR, Marker genes in GM Plants) was isolated from K-12.

126. Although ARGs are found naturally, they are much more prevalent now than in the pre-antibiotic use era (Cazares et al., 2025). The human intestinal tract is a reservoir of more ARGs than any other natural environments studied such as soil, marine or lake environments (Huddleston, 2014). It is proposed that this is due to extensive use of antibiotics that have biased the evolution of bacteria for those that naturally express ARGs (Dongre et al., 2025). To counteract this growing pool of ARGs in microorganisms, a variety of antibiotic families are currently available (Eyler and Shvets, 2019).

127. Soil is also a large reservoir of ARGs (Han et al., 2022) and natural antibiotic resistance is a means of competition for resources in soil. Although some ARGs are indigenous to soil bacteria, the selective pressure exerted by using antibiotics agriculturally to treat disease and promote animal growth has increased its prevalence. Antibiotic resistance to tetracycline is dominant in agricultural soil in countries such as China, possibly due to its extensive use in animal husbandry.

Section 6 Previous authorisations

128. The GMO has not been previously authorised for limited and controlled release in Australia. Manufacturing of the GMO is currently authorised under NLRD.

Chapter 2 Risk assessment

Section 1 Introduction

129. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 8). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

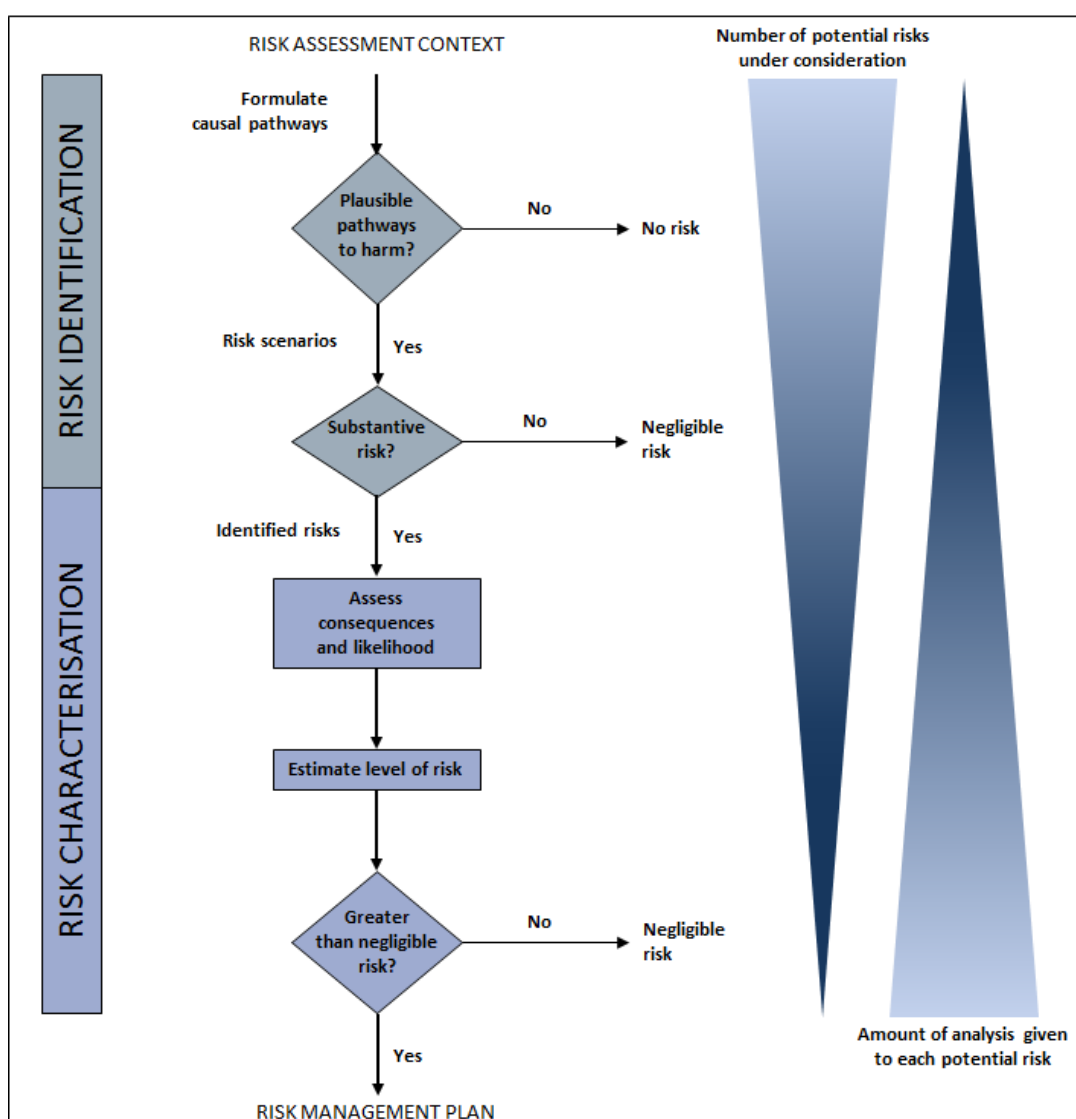


Figure 8 The risk assessment process

130. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, previous agency experience, reported international experience and consultation (OGTR, 2013).

131. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating plausible causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are risk scenarios.

132. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 8), i.e., the risk is considered no greater than negligible.

133. Risk scenarios identified as substantive risks are further characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The consequence and likelihood assessments are combined to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk identification

134. Postulated risk scenarios are comprised of three components (Figure 9):

- i. the source of potential harm (risk source)
- ii. a plausible causal linkage to potential harm (causal pathway)
- iii. potential harm to people or the environment.

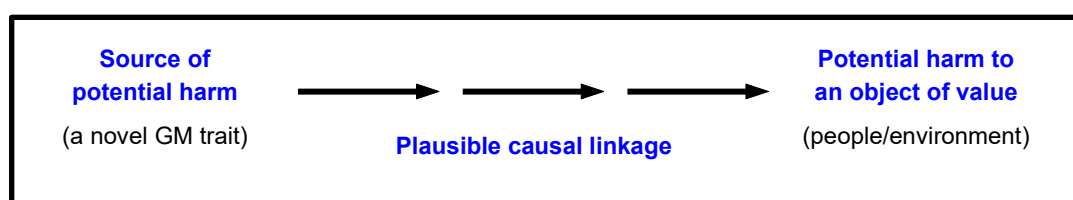


Figure 9 Components of a risk scenario

135. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors detailed in Chapter 1:

- the proposed dealings;
- the proposed limits including the extent and scale of the proposed dealings;
- the proposed controls to limit the spread and persistence of the GMO; and
- the characteristics of the parent organism(s).

2.1 Risk source

136. The sources of potential harms can be intended novel GM traits associated with one or more introduced genetic elements, or unintended effects/traits arising from the use of gene technology.

137. As discussed in Chapter 1Section 4, the introduced genes encoding proteins are sourced from *C. reinhardtii* and native *E. coli*. The introduced genes encode for bacterial and microalgae proteins, as well as ARG. The genes from *C. reinhardtii* as well as the ARGs are considered further as a source of risk. Some of the modifications include the introduction or deletion of genes derived from native *E. coli* K-12, or the modifications of promoters resulting in upregulation of native genes. These modifications do not introduce novel genetic material that is otherwise not present in humans, animals or the environment. However, they may introduce risks associated with modified traits. Therefore, risks from some native *E. coli* genes in isolation are therefore not considered further, however their contribution to the overall H₂ production and survival are considered further.

138. Control sequences such as promoters, transcriptional regulators present on standard plasmid backbones are DNA that is not expressed as a protein, so exposure to these will not be further considered as sources of potential harm for this application.

139. Genetic modifications have the potential to cause unintended effects in several ways. These include insertional effects, where the introduced genetic elements are randomly integrated into the

host's genome. The genome of the GMOs will be sequenced before packaging for transport and subsequent deployment. Hence the potential for the processes of genetic modification to result in unintended effects will not be considered further.

2.2 Causal pathway

140. The following factors are taken into account when postulating plausible causal pathways to potential harm:

- the proposed dealings with the GMO;
- proposed limits, including the extent and scale of the proposed dealings;
- characteristics of the parent organism;
- potential effects of introduced or deleted gene(s) on the properties of the organism;
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s);
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment;
- the receiving environment;
- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential);
- potential for gene transfer to sexually compatible organisms;
- gene transfer by horizontal gene transfer (HGT); and
- unauthorised activities.

141. Although all these factors were considered, some are not included in the risk scenarios below as a plausible pathway to harm could not be identified.

142. Unintended effects can arise through horizontal gene transfer (HGT), the transfer of genetic material from one organism to another without reproduction. All genes within an organism, including those introduced by gene technology, can be transferred to another organism by HGT. A gene transferred through HGT could confer a novel trait to the recipient organism. The novel trait may result in negative, neutral or positive effects on the fitness of the recipient organism. This pathway is further considered as a potential source of risk.

143. Previous RARMPs have considered the potential for unauthorised activities to lead to an adverse outcome. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires the Regulator to have regard to the suitability of the applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities, and no risk greater than negligible was identified in previous RARMPs. Therefore, unauthorised activities will not be considered further.

2.3 Potential harms

144. Risks encountered from the exposure to whole GMO, *C. reinhardtii* encoded genes, H₂, as well as ARG are considered as potential sources of harm.

145. The potential harms that will be considered are:

- risks to people undertaking this dealing from exposure to the GMOs; and
- risks to people and the environment from an unintentional release of GMOs.

2.4 Postulated risk scenarios

146. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 2 and examined in Sections 2.4.1 – 2.4.3

Table 2 Summary of risk scenarios from the proposed dealings with the GMOs

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reasons
1	GMOs	Exposure of people conducting dealings or animals via aerosols, spills and sprays, or sharps injury containing the GMOs during: a) set up of canisters and operation of the system; b) transport of the GMOs; c) disposal of the GMOs; ↓ Ingestion/ mucosal or blood exposure ↓ Exposure/colonisation of intestine, respiratory tract or internal organs ↓ Expression of genes	Immune response to the GMO; Ill health such as GI symptoms, inflammation, diarrhoea, increased H₂ levels, antibiotic resistance	No	- The GMOs are contained within hydrogel beads sealed within canisters with 0.2 micron filters on effluent lines making exposure to viable GMOs highly unlikely; - PPE is worn by trained staff conducting dealings where exposure to the GMOs is possible; - Transport follows the Regulators <i>Guidelines for Transport, Storage and Disposal of the GMOs</i>; - DH5α cannot colonise the intestines and is not pathogenic. Modifications are not expected to lead to colonisation or increased pathogenicity, therefore also highly unlikely to colonise other parts of the body; <u>In case of colonisation of intestines:</u> - K-12 is not pathogenic, and no immune response is anticipated as it would be similar to bacteria already present in the intestines;
2	GMOs	Exposure of people conducting dealings or animals via aerosols, spills and sprays, or sharps injury containing GMOs during: a) set up of canisters and operation of the system; b) transport of the GMOs; c) disposal of the GMOs; ↓	Establishment of a novel GMO in the intestine with increased virulence and antibiotic resistance.	No	- Non-conjugative plasmids used and cannot transfer to another bacterium via conjugation; - Transfer of plasmids from native <i>E. coli</i> is possible but requires direct contact; - As noted in Risk scenario 1. <u>In case of novel GMO:</u> - Most genes encoding proteins are already present in the environment, including ARGs;

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reasons
		Ingestion/ mucosal or blood exposure ↓ Exposure/colonisation of intestine, respiratory tract or internal organs ↓ Transfer of genetic material to or from the GMOs resulting in a novel bacterium			Generation of a virulent GMO would require multiple steps.
3	GMOs	Escape of the GMOs into the environment via: a) leaks from the canister during transport or on-site operation; b) improper decontamination of the liquid waste. c) accidental loss during transport; ↓ Presence of the GMOs in the environment (soil or local waterways) ↓ Establishment and persistence of GMOs in the environment; OR Transfer of genetic material to or from the GMOs resulting in novel GMOs.	Establishment of the GMO or <i>E. coli</i> expressing GMO-derived transgenes (novel GMO) in the environment that can negatively impact local environment.	No	- Laboratory <i>E. coli</i> is poorly competitive in soil and surface environments and is expected to decline rapidly outside controlled conditions; - GMO has non-conjugative plasmids that cannot be transferred to another bacterium via conjugation; - Transfer of plasmids from native <i>E. coli</i> via conjugation is possible but requires direct contact; - As noted in Risk Scenario 1, the GMOs are contained within hydrogel beads, any liquid effluent is filtered via 0.2 micron filter limiting spread of the GMOs; - Reliance on thiamine limits its survival in the environment. <u>In case of environmental survival:</u> - Unlikely for the GMO to establish itself in the environment; - Naturally adapted environmental bacteria are likely to outcompete GMO (which lacks thiamine);

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reasons
					• Sunlight is likely to inactivate the GMO reducing its presence in waterways and surface soil;

2.4.1 Risk scenario 1

Risk source	GMO
Causal pathway	Exposure of people conducting dealings or animals via aerosols, spills and sprays, or sharps injury containing GMOs during: a) set up of canisters and operation of the system; b) transport of the GMOs; c) disposal of the GMOs; ↓ Ingestion/mucosal or blood exposure ↓ Exposure/colonisation of intestine, respiratory tract or internal organs
Potential harm	Immune response to the GMO; Ill health such as GI symptoms, inflammation, diarrhoea, increased H ₂ levels, antibiotic resistance

Risk source

147. The source of potential harm for this postulated risk scenario is the GMO.

Causal PathwayLikelihood of exposure

148. People conducting dealings with the GMOs could be directly exposed to the GMOs in several ways. The GMO could be transmitted via aerosol droplets, sprays or accidental sharps injuries during deployment of canisters, including connecting hoses, changing filters, decontamination of liquid waste, or during experiments. These can allow entry of the GMOs via ingestion to intestine, via inhalation to respiratory tract or via sharps injury access to blood.

149. Animals can be exposed on site in case of an accidental failure of the system leading to overflow of the GMO into the surrounding soil/grazing areas. They can be also exposed if improperly decontaminated liquid waste is emptied into the evaporation pond, contaminating the surrounding area.

Exposure during deployment and operation, sample collection and analysis, and on-site decontamination of liquid waste

150. The GMOs are contained within hydrogel beads in sealed canisters with recirculation tubing outlets containing a 0.2 micron filtration system. This greatly limits the likelihood of exposure to the GMOs during normal operation and reduces the exposure concentration if exposed.

151. The system is regularly tested for integrity and leaks, limiting the possibility of the containment being compromised. The fermentation process is entirely contained in a closed system, the canisters sealed and never opened on site, therefore, the opportunity for exposure is minimised to steps such as maintenance and sample collection for testing or decontamination.

152. Staff will wear PPE during the normal operation of the demonstration plant including gloves, eye protection, closed footwear. In the event of a spill, the contingency plan requires that staff wear gloves and eye protection.

153. The pre- and post-filtration testing of the liquid effluent will be conducted using standard laboratory PPE which includes a lab gown or coverall, eye protection and gloves. These volumes are small and the precautionary PPE is considered sufficient for this type of GMOs.

154. Those measures will further reduce the risk of exposure of staff to the GMOs.

Exposure during transport, storage or disposal

155. The canisters containing the GMOs will be transported to and from the deployment site according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* (TSDs).

156. Decontamination of surfaces will be with 80% (v/v) ethanol. Decontamination of the waste will be as described in Chapter 12.3.8. The protocols for decontamination, operations of the system and transport would reduce the likelihood of exposure.

Likelihood of the GMO colonisation of intestines and other parts of the body

157. The K-12 strain (and its derivative DH5 α) cannot colonise human intestines. Clinical studies involving oral administration of K-12 to human participants was performed to investigate its colonisation capabilities. No colonisation was observed. In mice, only in exceptional circumstances, such as in germ-free animals devoid of commensal bacteria, was the bacterium shown to colonise the intestine (Freter et al., 1983; Levy et al., 1980). In both humans and non-germ-free mice, the bacterium was lost within 7 days. K-12 strain is easily displaced by commensal bacteria (Chapter 15.2.1). The proposed genetic modifications are not expected to increase the ability of the GMO to survive in the intestine compared to K-12. Without proliferation, the bacteria will be lost from the intestine due to rapid turnover of cellular and mucosal components.

158. In the event of aerosol exposure, the GMOs may transit to the lung or in extreme case enter the blood system and result in the GMOs being present in blood or organ. Colonisation is not expected to occur in these instances as these areas are not the typical habitat of intestinal *E. coli*. In addition, the modifications made to the parent strain negatively impact colonisation ability.

Likelihood of preferential survival due to co-consumption capacity of the GMO for broad sugars

159. As noted in Chapter 1 Section 13.10.1, CCR means that *E. coli* prefers glucose over xylose and arabinose as an energy source and the GMO has been modified to have co-consumption capacity to metabolise multiple straw-derived sugars simultaneously. The intestine is considered a nutrient rich environment and competition for nutrients is unlikely to be a contributing factor to increase the competitiveness of the GMOs over resident bacteria.

160. In addition, several metabolic pathways in the GMOs may be diverted in favour of the production of hydrogen, including through deletion of genes involved in glucose accumulation and glycolysis (see Chapter 1, Section 4.2 and Table 1 for further details). A reduction of activity through these pathways may have a negative impact on the ability of the GMO to compete with resident bacteria.

Potential harm

161. If people or animals are exposed to the GMOs, and the GMOs did establish in the intestine, or less likely, respiratory mucosa, it could cause respiratory issues or alter intestinal microbiota. The GMO may also enter the blood system.

162. K-12 shares traits with commensal bacteria. Therefore, humans and animals potentially exposed to the GMOs are likely colonised with similar *E. coli* as part of their normal gut flora. It is therefore unlikely that the GMOs would cause any immune responses beyond those already in place through exposure to native commensals. The GMOs do not encode for pathogenic proteins that would alter this.

163. Cattle are likely to be found on surrounding agricultural land. They carry *E. coli* as part of normal intestinal flora (Ramos et al., 2020). Infection of cattle with laboratory-adapted GMO derived from non-pathogenic strain is therefore unlikely to cause ill health in these animals.

164. The proposed transgenes are derived either from native *E. coli*, and are therefore already present in the commensal microbiota, or from *C. reinhardtii*, with a very specific, intracellular role in enhancing H₂ production. Consumption of *C. reinhardtii* was reported to have no effect on commensal gut microbiome and positive association was made between consumption and improved gut health (Fields et al., 2020). There is therefore no anticipated harm that could be identified from proposed transgenic proteins.

165. *E. coli* can also cause respiratory infection in a very specific group of individuals, and community acquired infections were found mostly in the elderly with poor general health (Marrie et al., 1998). *E. coli* was also found to cause blood infections after solid organ transplantation due to the immuno-suppressive medication. In one study up to 30% of blood infections were caused by *E. coli* (Kritikos and Manuel, 2016). However, accidental exposure to a small number of GMOs would not hold a greater risk to cause harm than commensal *E. coli*.

166. As the GMOs are designed for an effective H₂ production, in a very unlikely scenario that the GMO can establish presence in the intestine, this could bias local microbiota through changes in the local concentration of H₂. Elevated intestinal H₂ production by commensal bacteria was found to support fitness of beneficial *Bacteroides* (Ruaud et al., 2020; Welsh et al., 2025). In diseases manifesting in intestinal inflammation such as Crohn's disease, a clear reduction in the expression of *E. coli* Hyd enzymes responsible for H₂ production was noted (Welsh et al., 2025). This indicates that intestinal output of H₂ varies between individuals and a slight increase due to the GMO is unlikely to cause ill health.

167. The GMOs may carry ARGs and therefore would be resistant to antibiotics such as chloramphenicol, kanamycin and ampicillin. This would only be of benefit to the GMOs in case of antibiotic use that would encourage its preferential survival. For example, kanamycin is not registered as a human therapeutic in Australia. Recent publications have reported that a large proportion of commensal bacteria in healthy individuals carry ARGs (Nji et al., 2021) and it is likely that there would not be preferential survival of the GMOs over already established resistant bacteria in case of antibiotic use.

Conclusion

168. The potential for exposure to the GMOs that would lead to harm via inducing immune responses or ill health is not identified as a risk that could be greater than negligible. The main reasons are that the GMO is unlikely to be able to colonise mammalian intestines and is unlikely to cause lung or blood infection, due to its attenuation. K-12 has traits of commensal bacterium, likely already present in healthy humans. Even if the establishment of the GMO occurred in the intestine, H₂ production, as the main outcome of genetic modifications is unlikely to cause ill health. The GMO does not encode pathogenic determinants or proteins that would otherwise allow increased fitness compared to K-12. Antibiotic resistance is already highly widespread in healthy individual's commensal flora so use of antibiotics is unlikely favour survival of the GMOs compared to these commensals. It is not likely that the GMOs would warrant antibiotic use as they are unlikely to cause harm. If they did, a broad range of antibiotics is available (as discussed in Chapter 15.2.1). Therefore, this risk scenario does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	GMO
Causal pathway	Exposure of people conducting dealings or animals via aerosols, spills and sprays, or sharps injury containing GMOs during: a) set up of canisters and operation of the system; b) transport of the GMOs; c) disposal of the GMOs; ↓ Ingestion/ mucosal or blood exposure ↓ Exposure/colonisation of intestine, respiratory tract or internal organs ↓ Transfer of genetic material to or from the GMOs resulting in novel bacteria
Potential harm	Establishment of a novel GMO in the intestine that can be spread to other people and to the environment

Risk source

169. The source of potential harm for this postulated risk scenario is the GMO.

Causal Pathway

Likelihood of exposure and colonisation

170. The likelihood of exposure and intestinal colonisation would be the same as discussed in Risk scenario 1. It is highly unlikely that a person or an animal would be exposed to the GMOs during standard operation of the system, transport or disposal, and is highly unlikely that the GMOs would colonise intestine. The colonisation of respiratory tract and internal organs is considered to be highly unlikely as these are not the sites intestinal *E. coli* is likely to colonise and the GMOs have modifications that negatively impact colonisation ability. Risk scenario 1 has addressed the harm that could come from exposure of these sites. Since these organs are unlikely to be colonised by the GMO, the likelihood of genetic exchange is no greater than that for the intestine.

Likelihood of gene transfer between GMOs and intestinal bacteria

171. Gene transfer between the GMO and intestinal bacteria could occur via conjugation, transformation, transduction or transposition.

Conjugation

172. Conjugation via conjugative plasmid is an important driver of bacterial evolution in many environments (Virolle et al., 2020). The GMOs lack conjugative plasmids hence cannot transfer genetic material via conjugation. If the genes are expressed on the chromosome, transfer via conjugation does not occur.

173. Conjugation could transfer plasmids from intestinal bacteria to the GMOs. Generally, conjugation occurs between similar bacteria, therefore this is likely to occur from another *E. coli*. There are several types of virulence plasmids in *E. coli* that may pose a concern for transfer of pathogenicity (Johnson and Nolan, 2009). As the GMO carries antibiotic resistance genes, this could

potentially create a pathogenic GMO that is resistant to some antibiotics. For this to occur, a person would have to be infected with a pathogenic bacterium at the time of exposure to the GMOs.

174. Conjugation requires physical contact between bacteria. The opportunity for physical contact between pathogenic bacteria and the GMO is low. Major commensal phyla in mammalian intestine are Firmicutes and Bacteroidetes (Rosenberg, 2024) and they maintain colonisation resistance in intestine, i.e. maintain low density of other bacteria (Stecher et al., 2012). Although pathogenic bacteria increase in number during active infection, the GMO is not able to colonise intestines, making its presence transient. The number of GMOs entering the intestine during accidental release and exposure is anticipated to be minimal, and it is therefore highly unlikely that the GMO could colonise the intestine in a way that would allow it to encounter a pathogenic bacterium that would then, via conjugation, transfer its virulence plasmid.

175. *E. coli* O157:H7 is a zoonotic foodborne pathogen living in cattle as commensal bacterium but are pathogenic to people (Stein and Katz, 2017). *E. coli* O157:H7 can be present in Australian cattle and can contain ARG as well as carry the shiga toxin gene (Mellor et al., 2016). If this bacterium is present in cattle at the trial location, the exposure of these animals to the GMOs may provide an opportunity for pathogenic *E. coli* to encounter the GMO in the intestine of an animal and transfer some virulence genes to the GMO. However, the dealing is proposed to be conducted in a closed system, offering limited opportunity for the GMO to escape. In the event a small number of GMOs are released due to ineffective decontamination, or accidental exposure during the operation of the system or during conduct of experiments, the number of GMOs likely to be present in the intestine via accidental exposure would be minimal. Compounded by the fact that the GMO possess similar characteristics to its parental strain and is unable to colonise the intestine, the likelihood of conjugation occurring is considered highly unlikely.

Transformation

176. Transformation involves uptake of free DNA fragments from the environment by competent bacteria. To be competent, bacteria must have specific genes to allow them to take in DNA (Blokesch, 2016). *E. coli* is generally thought not to be naturally easily transformable and develops competence only under artificial conditions. (Sinha and Redfield, 2012). *In vitro*, transformation via plasmid DNA does occur at low frequency. When exposed to pure water, treated wastewater or artificial lake water *in vitro*, the DH5 α strain acquired exogenous DNA at a frequency of 10^{-9} - 10^{-10} (Riva et al., 2020). *In vivo*, when germ-free mice were colonised with *E. coli* and then fed plasmids containing ARG, no transformation of the bacteria was observed (Wilcks and Jacobsen, 2010). There is currently no evidence that dietary DNA integrates into intestinal bacteria and the likelihood of successful transfer and functional maintenance of recombinant DNA by intestinal microorganisms would be highly unlikely (FSANZ; GM food: Safety of ingested recombinant DNA). It is therefore unlikely that DNA could be transferred between the GMO and intestinal bacteria in this way.

Transduction

177. Transduction is a movement of genetic material via bacteriophage. The GMOs are based on DH5 α that is resistant to T1 bacteriophage due to *fhuA2* allele that encodes for a receptor used by T1 phage to infect *E. coli* (Langenscheid et al., 2004). The GMO is not resistant to other phages and transfer of genetic material could occur via this method. The bacteriophage, however, would need to carry genetic material that would enable the GMO to become more virulent, for example by carrying virulence genes (Schroven et al., 2021). Although this is possible, given the low number of the GMOs that would be present in the intestine, the likelihood of a virulence transfer via phage would be highly unlikely.

Transposition

178. Transposable elements (TE) machinery can move genes from plasmids into the chromosome and vice versa. Transfer of genetic elements between bacteria would likely be via conjugation of a

plasmid. The GMOs lack conjugative plasmids which would limit movement of the transgene upon conjugation as noted above. Additional steps would be required to move transgenes from the GMO. The likelihood of genomic element movement from the GMO via this method is even less likely than conjugation. Acquisition of genes via transposition would not be of greater likelihood than acquisition via conjugation.

Potential harm

179. The GMO could transfer ARG, native *E. coli* genes or *C. reinhardtii* genes encoding specific metabolic pathway proteins to intestinal bacteria. These genes on their own would likely have little contribution to the metabolic pathways of the recipient, and it is likely a whole set of genes for a particular trait would be required to result in changes in the recipient metabolic pathways. Even if this occurs, the modifications are designed to encourage H₂ production which does not provide the host with survival advantage or increased capacity to cause harm. Therefore, it is unlikely that transfer of any individual gene or a set of genes encoding for metabolic pathways from the GMO to another bacterium would cause harm. Furthermore, the GMO is likely to have multiple pathways deleted which may disadvantage it compared to commensal bacteria which utilise glucose as their primary sugar source. Glucose is favoured by *E. coli* for proliferation.

180. Native *E. coli* may already carry ARG and therefore transfer of a ARG is unlikely to greatly contribute to the harm given abundance of ARG in commensal microbiota pool (Lee et al., 2023).

181. Another scenario involves transfer of a virulence plasmid from pathogenic *E. coli* to the GMOs via conjugation or transduction. This could give rise to a novel antibiotic-resistant pathogenic GMO. For this to occur, the GMO would need to acquire not only the plasmid virulence genes, but also likely chromosomal genomic regions that have been lost during *in vitro* propagation to allow the ability to colonise the intestine (Sansonetti et al., 1983). Even in this setting, a novel GMO would be posing a no greater risk than the pathogenic bacterium that has donated the virulence plasmid because a broad range of antibiotics is available.

Conclusion

182. Risk scenario 2 is not identified as a substantive risk due to the limited ability of the GMO to transfer or receive genomic sequences that would provide potential for harm. Multiple genes would need to be acquired by the GMO to increase virulence or colonisation capacity. Transfer of genetic material to other bacteria from the GMO that would be considered of relevance regarding potential harm would be limited to ARG, the transfer of which is unlikely to cause harm.

2.4.3 Risk scenario 3

Risk source	GMO
Causal pathway	Escape of GMOs into the environment via:
	a) Leaks from canisters during transport or on-site operation;
	b) Inadequate on-site decontamination of the liquid waste;
	c) accidental loss during transport;
	↓
	Presence of the GMOs outside the deployment site
	↓
	Establishment and persistence of GMOs in the environment; OR
	Transfer of genetic material to or from the GMOs resulting in novel bacteria
Potential harm	Establishment of the GMO or <i>E. coli</i> expressing GMO-derived transgenes in the environment that can negatively impact local environment.

Risk source

183. The source of potential harm for this postulated risk scenario is the GMO.

Causal Pathway

The loss of the GMOs into the environment could occur in multiple ways. This could happen during operation of the system on site, during transport of cartridges to the location, or it could be through release of incompletely decontaminated GMOs that are released in the environment either in the evaporative pond, during transport, or during disposal of the liquid via external contractor.

Likelihood of GMO loss from the containment

184. As noted in Risk scenario 1, the likelihood of GMO escape from the containment is limited by the proposed operational controls. This includes:

- the containment of the GMOs within hydrogel beads that reduces their loss during the fermentation;
- the recirculation system which contains a 0.2 micron filter reducing the number of GMOs present in the final effluent,
- the regular testing of the integrity of the system which further reduces the likelihood of the GMO escape through accidental release.

185. Maintaining a low concentration of GMO in the effluent reduces the amount that could be released in case of accidental spill or system integrity failure.

186. In the event of a significant failure of the closed system, the production area is bunded, and the underground containment tank is designed to collect liquid as a contingency plan to allow in situ chemical decontamination.

187. The likelihood of the GMO escape during transport has been discussed in Risk scenario 1 and this too was a highly unlikely scenario.

Likelihood of the GMO establishment in the environment

188. If GMO escape did occur, it could enter the environment via the evaporative pond, waterways or soil.

189. The GMO in waterways would be greatly diluted, and exposure to sun, particularly to ultraviolet wavelength, would further limit the number of GMOs in both water and soil. Bacteria however do survive in the environment, and more recent data indicates that *E. coli* may have adapted to grow in the environment (Brennan et al., 2013).

190. Assessment of *E. coli* isolated from soil shows unique traits such as effective utilisation of various substrates including carbon, nitrogen, phosphorus and sulphur in environmental conditions as opposed to the laboratory K-12, indicating environmental adaptation of environmental strains (Brennan et al., 2013) and confirming that the laboratory strain would be considerably less competitive than *E. coli* present in the environment.

191. Although the modification to the GMOs means it can effectively co-utilise different sugars, these genes are already present in *E. coli* and previous work has demonstrated that in nutritionally limiting environments bacteria do co-utilise multiple sugars simultaneously (Egli, 2010; Lendenmann et al., 1996). The modifications are therefore unlikely to provide a survival advantage to the GMO in the environmental setting as is a high H₂ output.

192. The GMO is based on DH5α which lacks the *thi-1* gene preventing it from synthesising its own thiamine and instead it relies on provision of this crucial vitamin from the environment. In the culture, *E. coli* growth media is supplemented with thiamine. In the large intestine a multitude of bacteria produce thiamine and so, this is unlikely to be a growth limiting factor in Risk scenario 2 (Rodionov et al., 2019). However, thiamine is likely more limited in the waterways and soil than in intestine which could impair to the capacity of the GMOs to establish outside a host.

193. In addition, experimental studies have found that DH5α does not survive long-term in non-sterile freshwater (Arana et al., 2003), or soil (Naganandhini et al., 2015).

194. Additional points include information which is declared CCI and is therefore made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

Likelihood of DNA transfer between the GMO and soil or water bacteria

195. Decontaminated effluent would be collected for off-site disposal/use by external service providers. Some decontaminated liquid effluent may be released into the evaporation pond after decontamination, but only in case of contingency upon accidental overflow or other failure of the system that allows decontaminated liquid to escape the closed system and after decontamination in the underground waste pit. Off-site use may also expose microorganisms to the DNA.

196. If the GMOs persist in the environment in soil or water, the transfer of ARGs to resident bacteria could happen. This type of transfer has been discussed in detail in Risk scenario 2 for intestinal bacteria. As the GMOs are laboratory strains, the likelihood of their survival and persistence in soil to provide sufficient opportunity for conjugation is highly unlikely.

197. The DNA from live or inactivated GMO could be taken up by resident soil bacteria, but there is no evidence that the widespread use of antibiotic resistance genes as markers for the selection of GM plants has led to a significant increase in clinical antibiotic resistance (Breyer et al., 2014). Antibiotic resistance in the clinical setting is instead associated with the high prevalence of antibiotic use.

198. Previous studies have investigated whether ARGs present in inactivated GMO biomass from fermentation, when used as a soil fertiliser, would transfer antibiotic resistance to microbial communities in the environment. Although ARGs could be detected from inactivated GMO biomass, this DNA was not able to transform bacteria *in vitro*. Furthermore, no differences was found in the ARG profiles between bacteria from the soil treated with GMO biomass and bacteria treated with inorganic fertilisers (Andersen et al., 2001).

199. Another study assessed degradation of heterologous DNA from inactivated GM *E. coli* in soil under field conditions. Relatively short heterologous DNA of 321 base pair (bp) in length were fully

degraded when tested at 14 days in non-autoclaved soil. The autoclaved soil allowed DNA integrity to be maintained for longer. Again, no horizontal gene transfer of the target sequence was observed in viable soil microorganisms (Halter and Zahn, 2017).

200. Naked, cell free plasmid DNA was shown to degrade in groundwater and river water within 48-96 hours, and DNA uptake was not detected when these water samples were used to transform bacteria in laboratory settings (Zhu, 2006). Uptake of genes from resident soil bacteria by the GMO is also unlikely and, if it did occur, would be unlikely to lead to genetic modifications as addressed in Risk scenario 2.

Potential harm

201. The GMO could establish in the environment and produce H₂ in water and soil. Diverse bacteria within soil, including those associated with plants, use H₂ as an energy source (Islam et al., 2023).

202. Antibiotic resistance genes are present naturally in a variety of bacteria, including environmental, soil and clinical bacteria (Peterson and Kaur, 2018) and GMOs are unlikely to significantly contribute to this in a way that would lead to harm.

Conclusion

203. Risk scenario 3 is not identified as a substantive risk due to the limited ability of the GMO to establish and persist outside of culture conditions at a density that would have an appreciable adverse effect on the biotic environment. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

204. Uncertainty is an intrinsic part of risk analysis and is present in all aspects of risk analysis. This is discussed in detail in the Regulator's Risk Analysis Framework document.

205. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimate the level of risk, the Regulator will take this uncertainty into account in making decisions.

206. For DIR-230, this is the first fermentation trial for this GMO and although the parent organism is extensively characterised, the possibility of gene transfer between the GMO and both commensal bacteria and environmental bacteria is possible. This uncertainty has been accommodated by taking a conservative approach to the risk analysis.

207. Additional data, including information to address these uncertainties may be required to assess possible future applications with reduced limits and controls, such as commercial release of these GMOs.

208. Chapter 3, Section 4 discusses information that may be required for future release.

Section 4 Risk evaluation

209. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

210. Factors used to determine which risks need treatment may include:

- risk criteria,
- level of risk,

- uncertainty associated with risk characterisation, and
- interactions between substantive risks.

211. Three risk scenarios were postulated whereby the proposed dealing might give risk to harm to people or the environment.

212. A risk is substantive only when the risk scenario may, because of gene technology, have some chances of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent a substantive risk and do not advance in the risk assessment process.

213. Three risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the control measures proposed by the applicant, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 2 and include:

- cultured GMOs are contained in hydrogel beads and packed inside canisters which will operate as a closed system;
- no GMO is planned on being released into the environment;
- limits on the duration of the proposed release;
- people conducting dealings will wear appropriate PPE;
- the waste that may contain the GMOs will be decontaminated before being released into the environment;
- the GMO is derived from a laboratory adapted strain and unlikely to survive in GI tract or environment; and
- suitability of proposed controls to restrict the spread and persistence of the GMOs.

214. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM yeast into the environment are considered negligible. The *Risk Analysis Framework* (OGTR, 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation.

215. Therefore, no controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment³.

³ As none of the proposed dealings are considered to pose a significant risk to people or the environment, Section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP.

Chapter 3 Risk management plan

Section 1 Background

216. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan addresses risks evaluated as requiring treatment and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through proposed licence conditions.

217. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

218. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: Section 64 requires the licence holder to provide access to premises to OGTR inspectors and Section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

219. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in Section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under Section 152 of the Act.

Section 2 Risk treatment measures for substantive risks

220. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed trial of the GMOs. The risk scenarios were considered in the context of the proposed release (Chapter 1Section 2), the proposed control measures (Chapter 1 Section 2.2) and the receiving environment (Chapter 14.3) and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

221. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible to low. Therefore, to maintain the risk context, draft licence conditions have been imposed to limit the location and duration of the trial, as well as a range of controls to restrict the spread and persistence of the GMOs in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in the draft licence.

Limits and controls on the release

222. Section 2.1 and Section 2.2 in Chapter 1 list limits and controls proposed by the applicant. Many of these are discussed in the 3 risk scenarios considered in Chapter 2. The appropriateness of the limits and controls is considered further in the following sections.

3.1.1 Consideration of limits proposed by Hydgene

223. The applicant has proposed limits to the number of locations (a single location), and a limit to the number of cartridges deployed on site at any given time, as well as the period of release, which is limited to 5 years. These limits are considered important to maintain the risk context and have been included in the licence.

3.1.2 Consideration of proposed controls to manage exposure, dispersal and persistence of the GMOs

224. Various controls have been proposed by the applicant, including use of hydrogel beads that entrap and immobilise the GMOs, sealed canisters for hydrogel beads containment and filtration of the liquid being passed through the system.

225. The applicant has also proposed that only trained individuals will have access to the GMOs and the access to the site is restricted.

226. Decontamination of the liquid on-site as well as transport of the solid waste back to the manufacturing facility for decontamination and transport for disposal will follow the Regulator's *Guidelines for Transport, Storage and Disposal of GMOs* (TSD guidelines) which will reduce the likelihood and the extent of exposure.

227. The applicant has proposed control measures to limit the dispersal of the GMO during canister deployment. The canisters are a part of the closed system as they are sealed vessels containing the GMO with connected closed transfer lines.

228. The applicant has also proposed that during general operation of the closed system effluent liquid will be filtered through a 0.2 micron filter. This limits the concentration of the GMOs, in the waste. To ensure that the filters are maintained in working order, a condition of the licence proposes that filters should be tested weekly for performance, as also proposed by the applicant.

229. The methodology for inactivation of the GMOs will be proven to be effective against the GMOs.

230. The applicant has proposed building a large underground collection tank connected to the above ground bunded area where the canisters will be deployed as a contingency containment of liquid containing the GMO in case of a leak.

231. A standard condition is included in the licence requiring the licence holder to ensure that dealings are conducted to not compromise the health and safety of people and minimise unintentional exposure to the GMOs.

232. A standard condition is included in the licence restricting access to the Deployment site to persons covered by this licence. In addition, the licence holder must have the authority to maintain the site and equipment and to admit or exclude persons from the site, or if not the owner, to have obtained such authority from the owner of the site.

3.1.3 Summary of licence conditions to be implemented to limit and control the dealings

233. Licence conditions have been drafted to limit and control the proposed dealings based on the above considerations. These include requirements to:

- limit the duration of the release to a maximum of 5 years from issue of the licence;
- limit the trial to a single location in NSW: Deployment site at Blayney Shire Council, Lyndhurst, NSW;
- limit the number of canisters on site at any given time;
- use a closed system for the fermentation process;
- restrict access to the GMOs;

- ensure the area where the cannisters are located will be bunded and able to be decontaminated and a contingency underground collection tank is present in case of a large spill;
- filter effluent liquid via 0.2 micron filter;
- decontaminate the GMOs and waste containing GMOs via heat-based or chemical methods validated as effective against the GMOs;
- return canisters to the PC2 large scale facility for decontamination;
- follow the Regulators *TSD Guidelines* for transport.

Other risk management considerations

234. All DIR licences issued by the Regulator contain conditions that relate to general risk management. These include conditions relating to:

- applicant suitability;
- contingency plans;
- identification of the persons or classes of persons covered by the licence;
- reporting requirements;
- access for the purpose of monitoring for compliance.

3.1.4 Applicant suitability

235. In deciding whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under Section 58 of the Act, matters that the Regulator must take into account include:

- any relevant convictions of the applicant;
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country;
- the capacity of the applicant to meet the conditions of the licence.

236. If a licence was issued, the conditions would include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

237. In addition, the applicant organisation must have access to an IBC and be an accredited organisation under the Act.

3.1.5 Contingency plans

238. The applicant has submitted a contingency plan that details measures to be undertaken in the event of unintentional spills of GMOs at the deployment site or release of the GMO into the environment. This includes accidental release during transport and operations of the system on-site. Licence conditions are implemented in case of accidental release of the GMOs.

3.1.6 Identification of the persons or classes of persons covered by the licence

239. If issued, the persons covered by the licence would be the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence.

240. Prior to deployment of the GMO, the applicant would be required to provide a list of people and organisations that are covered by the licence, or the function or position where names are not known at the time.

3.1.7 Reporting requirements

241. If issued, the licence would require the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the dealings;
- any contraventions of the licence by persons covered by the licence;
- any unintended effects of the dealings.

3.1.8 Monitoring for compliance

242. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

243. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

244. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to the health and safety of people or the environment could result.

Section 4 Issues to be addressed for future releases

245. Additional information has been identified that may be required to assess an application for a commercial release of the GMOs, or to justify a reduction in limits and controls. This includes:

- gene transfer capacity between the GMOs and other bacteria;
- effectiveness of the closed system in limiting the release of live GMOs into the environment.

Section 5 Conclusions of the consultation RARMP

246. The RARMP concludes that the proposed limited and controlled release of the GMOs poses negligible risks to the health and safety of people or the environment as a result of gene technology, and that these negligible risks do not require specific risk treatment measures.

247. If a licence were issued, conditions would be imposed to limit the proposed size, location and duration of the release, and to restrict the spread and persistence of the GMO in the environment, as these were important considerations in establishing the context for assessing the risks.

Chapter 4 Draft licence conditions

Section 1 Interpretations and definitions

1. In this licence:

- (a) unless defined otherwise in this licence, words and phrases used in this licence have the same meaning as they do in the Act and the Regulations;
- (b) words importing a gender include every other gender;
- (c) words in the singular number include the plural and words in the plural number include the singular;
- (d) expressions used to denote persons generally (such as “person”, “party”, “someone”, “anyone”, “no one”, “one”, “another” and “whoever”), include a body politic or corporate as well as an individual;
- (e) references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;
- (f) where a word or phrase is given a particular meaning, other grammatical forms of that word or phrase have corresponding meanings;
- (g) specific conditions prevail over general conditions to the extent of any inconsistency.

2. In this licence:

‘Act’ means the *Gene Technology Act 2000* (Commonwealth) or the corresponding State law under which this licence is issued.

‘Closed system’ means a system for culturing the GMOs consisting of sealed cartridges containing the GMO embedded in hydrogel beads, closed transfer lines and associated equipment (e.g. filtration, waste tank).

‘Cartridge’ means a sealed vessel that holds hydrogel beads containing the GMOs.

‘Decontaminate’ (or **‘Decontamination’**) means, as the case requires, kill the GMOs by one or more of the following methods:

- (a) chemical treatment;
- (b) heat;
- (c) autoclaving;
- (d) high-temperature incineration; or
- (e) a method approved in writing by the Regulator.

Note: ‘As the case requires’ has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate.

‘Deployment site’ means a purpose-built enclosed space as defined in condition 21.

‘External service provider’ means a person engaged by the licence holder solely in relation to transport, storage and/or disposal of the GMOs, and who is not undertaking any dealings with the GMOs that are not for those purposes.

‘GM’ means genetically modified.

‘GMO’ means the genetically modified organisms that are the subject of the dealings authorised by this licence.

‘NLRD’ is a Notifiable low risk dealing. Dealings conducted as an NLRD must be assessed by an institutional biosafety committee (IBC) before commencement and must comply with the requirements of the Gene Technology Regulations 2001.

‘Personal information’ has the same meaning as in the *Privacy Act 1988*. Personal information means information or an opinion about an identified individual, or an individual who is reasonably identifiable:

- (a) whether the information or opinion is true or not; and
- (b) whether the information or opinion is recorded in a material form or not.

‘Regulations’ means the Gene Technology Regulations 2001 (Commonwealth) or the corresponding State law under which this licence is issued.

‘Regulator’ means the Gene Technology Regulator.

‘Sample’ means any product (e.g. culture liquid or gas) collected from the Closed system for analysis.

‘Secondary containment’ means bunding and underground pit, designed as a contingency containment in case of a spill or a leak from the Closed system.

Section 2. General conditions and obligations

Holder of licence

3. The licence holder is Hydgene Renewables Pty Ltd.

Remaining an Accredited Organisation

4. The licence holder must, at all times, remain an accredited organisation.

Validity of licence

5. This licence remains in force until it is suspended, cancelled, or surrendered. No dealings with the GMOs are authorised during any period of suspension, or after the licence has been cancelled or surrendered.

Note: Although this licence has no expiry date, the duration of culture of the GMO at the Deployment site is restricted in accordance with Condition 21(c).

Persons covered by this licence

6. The persons covered by this licence are:
 - (a) the licence holder, and any employees, agents or External service providers engaged by the licence holder; and
 - (b) the project supervisor(s); and
 - (c) other persons who are, or have been, engaged or otherwise authorised by the licence holder or the project supervisor to conduct any of the dealings authorised by this licence.
7. The licence holder must keep a record of all persons covered by this licence, and must keep a record of the contact details of the project supervisor(s) for the licence.

Note: Where External service providers are used, it is sufficient to record the company name and the position or job title of the person(s) conducting the dealing.

8. The licence holder must provide information related to the persons covered by the licence when requested to do so in writing by the Regulator and must provide the information within a time period stipulated by the Regulator.

Description of GMOs covered

9. The licence authorises specified dealings in respect of the GMOs identified and described in **Attachment A**.

Dealings authorised by this licence

10. The licence holder and persons covered by this licence may conduct the following dealings with the GMOs during the period covered by this licence and in accordance with this licence:

- (a) culture the GMOs in volumes exceeding 25 litres per vessel;
- (b) conduct the following experiments with the GMOs:
 - i) optimise the conditions for hydrogen production;
 - ii) test Samples from the Closed system for quality control purposes.
- (c) use the GMOs in the course of manufacture of a thing that is not the GMOs;
- (d) transport the GMOs; or
- (e) dispose of the GMOs;

and may possess, use, or store, the GMOs for the purposes of, or in the course of, these dealings.

11. This licence does not apply to dealings with the GMOs conducted as a NLRD or pursuant to another authorisation under the Act.

Conditions imposed by the Act

Note: The Act mandates the following 3 conditions.

Informing people of licence conditions (section 63)

12. The licence holder must inform any person covered by the licence, to whom a particular condition of the licence applies, of the following:
 - (a) the particular condition, including any variations of it; and
 - (b) the cancellation or suspension of the licence; and
 - (c) the surrender of the licence.

Monitoring and audits (section 64)

13. If a person is authorised by this licence to deal with the GMOs and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Additional information to be given to the Regulator (section 65)

14. The licence holder must immediately inform the Regulator if they become aware of:
 - (a) additional information about any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or
 - (b) any contraventions of the licence by a person covered by the licence; or
 - (c) any unintended effects of the dealings authorised by the licence.

Note 1: For the purposes of this condition:

(a) *The licence holder is taken to have become aware of additional information if they were reckless as to whether such information existed; and*

(b) *The licence holder is taken to have become aware of contraventions, or unintended effects, if they were reckless as to whether such contraventions had occurred, or such unintended effects existed.*

Note 2: Contraventions of the licence may occur through the action or inaction of a person.

Note 3: An example of informing immediately is contact made at the time of the incident via the OGTR free call phone number 1800 181 030 or email to OGTR.M&C@health.gov.au.

Informing the Regulator of any material changes of circumstance

15. The licence holder must immediately, by notice in writing, inform the Regulator of:

- (a) any relevant conviction of the licence holder occurring after the commencement of this licence;
- (b) any revocation or suspension after the commencement of this licence, of a licence or permit held by the licence holder under a law of the Commonwealth, a State or a foreign country, being a law relating to the health and safety of people or the environment;
- (c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the licence holder to meet the conditions in it.

16. The licence holder must provide information related to the licence holder's ongoing suitability to hold a licence when requested to do so in writing by the Regulator and must provide the information within a time period stipulated by the Regulator.

Further conditions with respect to informing persons covered by the licence

17. The licence holder must inform External service providers of any condition relevant to the dealings they are engaged to conduct, or when applicable, any variation of the condition.

Note: Information required under Condition 17 may be provided to External service providers who are engaged solely for storage and transport of the GMO through labelling of the outermost container of the GMOs in accordance with Condition 32(a).

18. If a particular condition, including any variation of it, applies to a person with respect to any dealing, other than to an External service provider, the licence holder must not permit a person covered by this licence to conduct that dealing unless:

- (a) the licence holder has obtained from the person a signed and dated statement that the person:
 - i) has been informed by the licence holder of the condition and, when applicable, its variation; and
 - ii) has understood and agreed to be bound by the condition, or its variation; and
 - iii) has been trained in accordance with paragraph (b) below; and
- (b) the licence holder has trained that person in a manner which enables them to conduct the dealings in accordance with the conditions of this licence.

19. The licence holder must notify all persons covered by the licence, from whom Personal information relevant to the administration and/or enforcement of the licence is collected by the licence holder, that such Personal information may be disclosed to the Regulator.
20. The licence holder must ensure that a copy of the licence is readily available to all persons covered by the licence, other than External service providers, who are conducting dealings with the GMO.

Note: The licence may be made available electronically

Section 3. Limits and control measures

Limits on the release

21. The following conditions apply to dealings with the GMOs at the Deployment site:
 - (a) culturing of the GMO must take place in a purpose-built enclosed space (the Deployment site) in the Local Government Area (LGA) of Blayney Shire Council, Lyndhurst, NSW 2797 which includes:
 - i. walls, a roof and a concrete floor;
 - ii. a concrete bunded area where the Cartridges will be placed within;
 - iii. a waste treatment tank; and
 - iv. an underground waste collection pit.
 - (b) a maximum of 60 Cartridges, each containing up to 60 kg of hydrogel beads, may be used at the Deployment site at any given time; and
 - (c) culture of the GMOs must be completed within 5 years from the date of issuing of the licence.

Control measures

The following conditions relate to restricting the spread or persistence of the GMOs in the environment.

Conditions relating to the containment of the GMOs

22. The viable GMOs must not be used, sold or otherwise disposed of for any purpose, which is likely to result in their use as food for humans or animals.
23. Secondary containment must be provided to retain any leakage from the Closed system. It must be of sufficient capacity to contain:
 - (a) the volume of the fluid held in the Closed system; and
 - (b) the volume of any disinfectant that might be used;
 with additional capacity to prevent any expected general fluid movement from breaching the secondary containment.
24. During normal operations, liquid effluent leaving the Cartridge must be filtered through a filtration unit with a 0.2 micron filter.
25. While the dealings with the GMO are conducted at the Deployment site, trained individuals must inspect the Closed system and take actions as per the table below. The records must be kept and made available to the Regulator upon request.

Area	Period of inspection	Inspection frequency	Inspect for	Action
Deployment site	From the commencement of the GMO culture until culture of any GMOs has ceased and the Cartridges have been removed from Deployment site.	Closed system- at the start of each operation and daily during operation. Filtration unit- weekly if the Closed system is used within last 7 days or before each operation if the system is used infrequently.	Closed system- leaks, failure of the system including compromised integrity of the Cartridges; Filtration unit- integrity of the filter;	Implement Contingency plan as per condition 40. Filtration unit- replace the filter if the integrity is compromised;

Conditions relating to the work practices

26. While dealings with the GMOs are being conducted at the Deployment site, the licence holder must ensure that access to the GMOs is restricted to persons covered by this licence.
27. The licence holder must have authority, or obtain such authority in writing from the owner of the premises before GMO dealings commence, to:
- (a) maintain the Deployment site and associated equipment; and
 - (b) admit persons to the Deployment site and associated areas; and to exclude persons from these areas.
28. Persons conducting dealings with the GMOs at the Deployment site must wear personal protective equipment (PPE) including gloves, eye protection and closed footwear. In addition, while conducting dealings with samples potentially contaminated with the GMOs (e.g. during experiments, filter change), a lab gown or coverall must also be worn.
29. Hands must be decontaminated after any activity that involves handling samples or equipment potentially contaminated with the GMOs (e.g. sample collection, effluent handling, filter change).

Transport and storage of the GMOs

30. Unless covered by an NLRD, the licence holder must ensure that transport of the GMOs is conducted only for the purposes of, or in the course of, another dealing permitted by this licence.
31. Transport of the GMOs may be undertaken if it is:
- (a) between Cert-5144 and the Deployment site; or
 - (b) for the purposes of storage or disposal in compliance with Conditions 32 or 34-37.

No other transport is permitted.

32. The licence holder must ensure that transport and storage of the GMOs, unless conducted according to Condition 33, follows these sub-conditions:

- (a) GMO is contained within sealed, unbreakable primary container, with the outer packaging labelled to indicate at least:
 - i) that it contains GMOs;
 - ii) the contact details for the licence holder; and
 - iii) labelled with instructions to notify the licence holder in case of loss or spill of the GMOs; and
 - iv) instructions on how to clean up a spill, as per the contingency plan in Condition 40; and
- (b) External surface of the primary container must be decontaminated prior to transport; and
- (c) Procedures must be in place to ensure that GMOs can be accounted for and that a loss of GMOs during transport or storage or failure of delivery can be detected; and
- (d) Access to the GMOs during transport or storage is restricted to authorised persons for whom Condition 17 or Condition 18 have been met (i.e. the GMOs are within a locked unit or an area which has restricted access). This includes situations where containers are left for collection in a holding area, or left unattended prior to decontamination; and

Note: All stored GMOs remain the responsibility of the licence holder.

33. Transport by External service providers must be conducted in accordance with Condition 32(a).

Note: Any transport conducted by the licence holder for this purpose (i.e., getting it to the External service provider) is still subject to conditions under Condition 32.

Disposal of the GMOs

- 34. The GMO must be Decontaminated prior to disposal, unless the method of disposal is also a method of decontamination.
- 35. Unless dealings with the GMOs are covered by another authorisation under the Act, the GMOs and any material or waste containing the GMOs must be decontaminated before or upon suspension, cancellation or surrender of the licence.
- 36. Subject to condition 37, Decontamination of the GMOs or any material or waste containing the GMOs must be by autoclaving, heat, chemical treatment, high-temperature incineration or any other method approved in writing by the Regulator.
- 37. Disposal of solid waste (e.g. used PPE or filters) containing the GMOs by External service providers must be by autoclaving or high-temperature incineration.
- 38. Liquid waste must be decontaminated by a heat treatment or chemical inactivation that has been tested and validated for effectiveness in killing the GMOs. The results must be provided to the Regulator at least 2 weeks before the first run.
- 39. The liquid waste Decontamination method and test results from the first run using methods as validated in condition 38, must be documented and provided to the Regulator before proceeding with the next run.

Contingency plans

- 40. If there is an unintentional release of the GMOs, the following measures must be implemented:
 - (a) if aerosols have been generated, adequate time for them to settle must be allowed before decontaminating the spill;
 - (b) the GMOs must be contained to prevent further dispersal; and

- (c) persons cleaning up the GMOs must wear appropriate PPE as specified in condition 2828 including lab gown or coverall; and
- (d) the exposed area must be Decontaminated with an appropriate chemical disinfectant effective against the GMOs; and
- (e) any material used to clean up the spill and PPE worn during clean-up of the spill must be Decontaminated; and
- (f) as soon as practicable after taking the above measures, the licence holder must be notified of the spill.

Section 4 Reporting and Documentation

*Note: The following licence conditions are imposed to demonstrate compliance with other conditions and facilitate monitoring of compliance by staff of the OGTR. Notices and reports may be emailed to OGTR.M&C@health.gov.au. A summary of notification and reporting requirements is provided at **Attachment B**.*

41. At least 14 days prior to commencing dealings at the Deployment site, or a timeframe agreed to in writing by the Regulator, the licence holder must provide the Regulator with a Compliance Management Plan, specifying:
- (a) the role and contact details for key persons responsible for the management of the Deployment site;
 - (b) the proposed reporting structure for the trial at the site and how the reporting structure enables the licence holder to become aware of all reportable events including but not limited to Conditions 14, 15 and 42;
 - (c) details of how the persons covered by the licence (for that type of dealing) will be informed of licence conditions applicable to them and how they will be trained to safely conduct the dealings;
 - (d) the person(s) or class of persons working with the GMOs; and
 - (e) method of analysis of detecting the GMO in the environment. The detection method must be capable of identifying each GMO under this licence.

Note: For the purpose of finding out whether the Act has been complied with, an OGTR inspector may, if entry is at a reasonable time, enter a facility occupied by the licence holder or a person covered by the licence and exercise monitoring powers.

42. The licence holder must inform the Regulator as soon as reasonably possible:
- (a) in the event of a loss or spill of the GMOs;
 - (b) in the event of the exposure of a person to the GMOs.
43. Upon request from the Regulator, the licence holder must provide any signed records or documentation collected under a condition of this licence, within a time period stipulated by the Regulator.

Attachment A**DIR No: 230**

Title: Fermentation of straw-derived sugars using genetically modified bacterium for hydrogen production

Organisation Details Hydgene Renewables Pty Ltd

GMO Description**GMOs covered by this licence:**

Escherichia coli strain DH5 alpha (NEB 5 alpha) genetically modified by introduction or deletion of only the genes or genetic elements listed in Table 1 below.

Parent Organisms:

Common Name: *E. coli*

Scientific Name: *Escherichia coli* - DH5 alpha strain (NAB 5a)

Modified traits:

Categories: Biofuel production

Description: The GMO, modified for efficient H₂ production from a variety of sugars. Modified genes are listed in Table 1.

Table 1. Nucleic acid responsible for conferring the modified traits

Genetic modifications	
Source, identity, nature of modification	Modified trait description
- Introduction of the genes from <i>C. reinhardtii</i>: <i>hydA</i>, <i>hydG</i>, <i>hydEF</i>, <i>petF</i>, <i>fnr</i> One or more of the following modifications: - Introduction or promoter-modifications for genes from <i>E. coli</i>: <i>zwf</i>, <i>gnd</i>, <i>talA</i>, <i>talB</i> and 6 genes that have been declared CCI. - Introduction of the genes from <i>E. coli</i>: <i>araE</i> and 5 genes that have been declared CCI. - Deletion of: <i>glk</i>, <i>pfkA</i>, <i>gpmA</i>, <i>gpmM</i>, <i>edd</i>, <i>eda</i>, <i>ppsA</i>, <i>ldhA</i>, <i>dld</i> and 3 genes that have been declared CCI. - Additional non-coding sequences such as promoters, regulatory elements, introns and/or spacers - Common antibiotic resistance genes used in laboratory plasmids	Trait 1: high yield hydrogen production Trait 2. Optimisation of hydrogenase function, temperature tolerance and improve production of H₂. Trait 3: Broad sugars utilisation genes. Trait 4: Reducing pathway competition that would otherwise redirect H₂ and its products to other cellular pathways Selection marker providing resistance to chloramphenicol, kanamycin and/or ampicillin

Confidential commercial information (CCI)

Names of some *E. coli* genes upregulated or deleted to enhance H₂ production were declared CCI under Section 185 of the *Gene Technology Act 2000*.

Attachment B – Summary of reporting requirements*

Prior to the commencement of the field trial	Condition	Timeframe for reporting
A written Compliance Management Plan for the Deployment site: (a) the role and contact details for key persons responsible for the management of the Deployment site; (b) the proposed reporting structure for the trial at the site and how the reporting structure enables the licence holder to become aware of all reportable events including but not limited to Conditions 14, 15 and 42; (c) details of how the persons covered by the licence (for that type of dealing) will be informed of licence conditions applicable to them and how they will be trained to safely conduct the dealings; (d) the person(s) or class of persons working with the GMO; (e) method of analysis of detecting the GMO in the environment.	41	At least 14 days prior to deployment of the GMO on site
Information to be provided at any time during the trial	Condition	Timeframe for reporting
Any additional information related to the health and safety of people and the environment associated with the dealing covered by the licence, or any contravention of the licence by a person covered by the licence or any unintended effect of the dealing authorised by the licence	14	Immediately
Any relevant conviction of the licence holder	15(a)	Immediately
Any revocation or suspension of a licence or permit held by the licence holder under a law of the Commonwealth, a State or a foreign country	15(b)	Immediately
Any event or circumstances that would impact the licence holder capacity to meet the licence conditions.	15(c)	Immediately
Any loss or spill of the GMO or exposure of a person conducting dealing	42	As soon as reasonably possible
Information to be provided on request by the Regulator	Condition	Timeframe for reporting
Information related to the persons covered by the licence	8	Within a timeframe stipulated by the Regulator

Information related to the licence holder's ongoing suitability to hold a licence	16	Within a timeframe stipulated by the Regulator
Copies of signed and dated statements and training records	18	Within a timeframe stipulated by the Regulator
Records of filtration unit testing for effective filtration and of the closed system inspection for integrity	25	Within a timeframe stipulated by the Regulator
Any records or documentation collected under a condition of this licence	43	Within a timeframe stipulated by the Regulator
Information to be provided before or after the first run	Condition	Timeframe for reporting
Validation results of liquid waste decontamination before the first run	38	At least 2 weeks before the first run
Decontamination method and test results from the first run	39	Before the next run

* Notifications and documents to be sent to OGTR.M&C@health.gov.au

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