18 September 2023

Summary of Licence Application DIR 200

Cauldron Molecules Pty Ltd has made an application under the *Gene Technology Act 2000* (the Act) to produce a range of recombinant proteins using various strains of *Komagataella phaffii* (previously known as *Pichia pastoris*[1]).

Project Title	Fermentation and processing of recombinant proteins using genetically modified *Pichia pastoris*[2]
Parent organism	*Pichia pastoris*
Genetic modifications	Insertion of expression cassette for bovine milk, chicken egg and silk fibre proteins. The expression cassette may also contain: • antibiotic selectable marker gene that confers resistance to a specific antibiotic to enable selection for the GMO • secretion signal peptide to facilitate secretion of proteins • constitutive or inducible promoter to facilitate expression of introduced sequences • tags such as epitope or polyhistidine to detect and purify the recombinant proteins
Principal purpose	To optimise the scale-up fermentation process and characterise genetically modified (GM) yeast (*P. pastoris)* during production of animal proteins.
Previous releases	There has been no previous application in Australia for these GMOs.
Proposed limits and controls	
Proposed duration	5 years
Proposed location	Cauldron Molecules Pty Ltd facilities in Borenore
Proposed controls	• Laboratory strains of *P. pastoris* will be used in the production which require specific media and growth conditions. • Molecular characterisation for multiple generations to assess genetic stability and copy number. • Transport of viable *GM P. pastoris* will follow the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. • Viable GM *P. pastoris* will be decontaminated via high temperature steam sterilisation or chemical disinfection. • Staff handling the GMOs will undergo licence specific training. • Stringent manufacturing practices and quality control procedures will be followed to ensure that GM yeast does not remain in the environment

The application

P. pastoris is a non-pathogenic yeast that is widely used in the biotechnology industry to produce recombinant proteins for pharmaceutical or food enzyme use. The proposed application is to optimise the scale-up fermentation process and characterise GM yeast used to produce recombinant animal proteins from

[1] Host name *Pichia pastoris* will be used for this application.

[2] The title of the project as supplied by the applicant is 'Precision fermentation of alternative proteins'.

a non-animal source. The GM yeast will incorporate a protein expression cassette to produce a recombinant protein. The incorporated genes will encode for proteins in their native form. The production process will involve fermentation of GM yeast cultures in large volumes (up to 10,000 L per tank) at Cauldron's purpose-built protein production facility in Borenore, New South Wales. The recombinant proteins will be purified and will not contain any GM yeast.

The application is for limited and controlled release under section 50A of the Act, as the Regulator was satisfied that its principal purpose is to enable the applicant to optimise the scale-up fermentation process and characterise the GM yeast during the production of animal proteins, and the proposed limits and controls are such that consultation with prescribed experts, agencies and jurisdictions is not required at this stage.

Next steps

The gene technology legislation sets out what the Regulator must do, as well as what the Regulator can or must consider, before deciding whether or not to issue a licence for this application.

The Regulator's staff will prepare a consultation version of the Risk Assessment and Risk Management Plan (RARMP) considering aspects of the application including the proposed limits and controls in accordance with the legislation.

The Regulator will seek comment on the consultation RARMP from the public, as well as a wide range of experts, agencies and authorities. The public and experts will be invited to provide submissions on the risks to human health and safety, and on risks to the environment from the proposed release.

At this stage, the consultation RARMP is expected to be released for comment in **November 2023**.

After consultation, the Regulator's staff will finalise the RARMP, taking into account advice on relevant matters. The finalised RARMP will form the basis of the Regulator's decision whether or not to issue a licence. The consultation and final versions of the RARMP and associated documents will be available on the OGTR website when they are released.

Other information available from the OGTR website:

- Information on selectable marker genes
- information on Australia's national scheme for regulation of gene technology and
- information on the DIR application process.

Please use the contact details below, if you

- would like a copy of the application. Please include the identifier DIR 200.
- have any questions about the application or the legislated evaluation process or
- wish to register on the mailing list.

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