Australian Government
Department of Health, Disability and Ageing
Office of the Gene Technology Regulator

September 2025

Risk Assessment and Risk Management Plan (consultation version) for

DIR 218

Commercial release of tomato genetically modified for purple fruit colour

Applicant: All Aussie Avocados Pty Ltd (trading as All Aussie Farmers)

This RARMP is open for consultation until 3 November 2025.

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/ogtr/dir-218-consultation

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, benefits, 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 No. DIR 218

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application for the intentional release of a genetically modified organism (GMO) into the environment. The Regulator has prepared a draft Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed release poses negligible risk to the health and safety of people and the environment. Licence conditions have been drafted for the proposed release. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether or not to issue a licence.

The application

Applicant	All Aussie Avocados Pty Ltd (trading as All Aussie Farmers)
Project Title	Commercial release of tomato genetically modified for purple fruit colour[1]
Parent organism	Tomato (*Solanum lycopersicum*)
Genetic modifications	
Introduced genes	Introduced genes conferring purple fruit colour, sourced from garden snapdragon (*Antirrhinum majus*): • *Delila* gene • *Rosea1* gene These 2 genes switch on production of natural purple/blue pigments, anthocyanins, in the ripening fruit. Introduced marker gene: • *nptII* gene from the bacterium *Escherichia coli* conferring resistance to the antibiotic kanamycin and structurally related antibiotics
Genetic modification method	*Agrobacterium*-mediated transformation
Identifier	Developer's event name: Del/Ros1-N Commercial name: The Purple Tomato™ OECD Unique Identifier: NPS-01201-8
Principal purpose	Commercial cultivation of the GM Purple Tomato in greenhouses
Previous releases	**Australia** The GM Purple Tomato has not been previously grown in Australia. **United States (US)** The US Department of Agriculture Animal and Plant Health Inspection Service deemed the GM Purple Tomato not a regulated article. Seed has been sold to home gardeners since 2024. The US Food and Drug Administration authorised the GM Purple Tomato as food in 2023. In 2024 and 2025, commercially produced fruit was sold in grocery stores.

[1] The original title for the application was *Commercial release of Lycopersicon esculentum genetically modified for purple anthocyanin pigment in ripe fruit*.

Risk assessment

The risk assessment process considers how the genetic modification and proposed activities conducted with the GMOs 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 current scientific/technical knowledge, information in the application (including proposed limits and controls) and relevant previous approvals. Both the short- and long-term impacts are considered.

Credible pathways to potential harm that were considered included exposure of people or other desirable organisms to the GM plant material, horizontal gene transfer of the antibiotic resistance gene, potential for persistence or dispersal of the GMOs, and transfer of the introduced genetic material to non-GM tomato plants. Potential harms associated with these pathways included increased allergenicity or toxicity to people, toxicity to other desirable organisms, increased antimicrobial resistance and environmental harms due to weediness.

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings are negligible. The principal reasons for the conclusion of negligible risks are that the introduced proteins are not expected to be toxic or allergenic, the *nptII* gene is not expected to increase antimicrobial resistance, tomatoes are not considered to be weedy and the genetic modifications are not expected to make the GM Purple Tomato weedier, and tomatoes have limited ability to naturally hybridise with sexually compatible species.

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 assessed as negligible, specific risk treatment is not required. However, licence conditions are proposed regarding post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP. The draft licence, detailed in Chapter 4 of the consultation RARMP, also contains 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

AICIS	Australian Industrial Chemicals Introduction Scheme
APHIS	United States Department of Agriculture Animal and Plant Health Inspection Service
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARTG	Australian Register of Therapeutic Goods
ASPCA	American Society for the Prevention of Cruelty to Animals
ASTAG	Australian Strategic and Technical Advisory Group on Antimicrobial Resistance
DAFF	Department of Agriculture, Fisheries and Forestry
Del	Delila
DIR	Dealings involving intentional release
DPA	Days post anthesis (flower opening)
FSANZ	Food Standards Australia New Zealand
GM(O)	Genetically modified (organism)
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare(s)
HGT	Horizontal gene transfer
HLH	Helix loop helix
kg	kilogram
m	Metre
mg	Milligram
NPTII	Neomycin phosphotransferase type II
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
PRR	Post release review
RARMP	Risk Assessment and Risk Management Plan
ROS	Reactive oxygen species
Ros1	Rosea1
TGA	Therapeutic Goods Administration
the Act	The *Gene Technology Act 2000*
the Regulations	The Gene Technology Regulations 2001
the Regulator	The Gene Technology Regulator
US	United States
USDA	United States Department of Agriculture
WDR	Tryptophan-aspartic acid-40 repeat
WHO	World Health Organization

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* (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. Potential risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

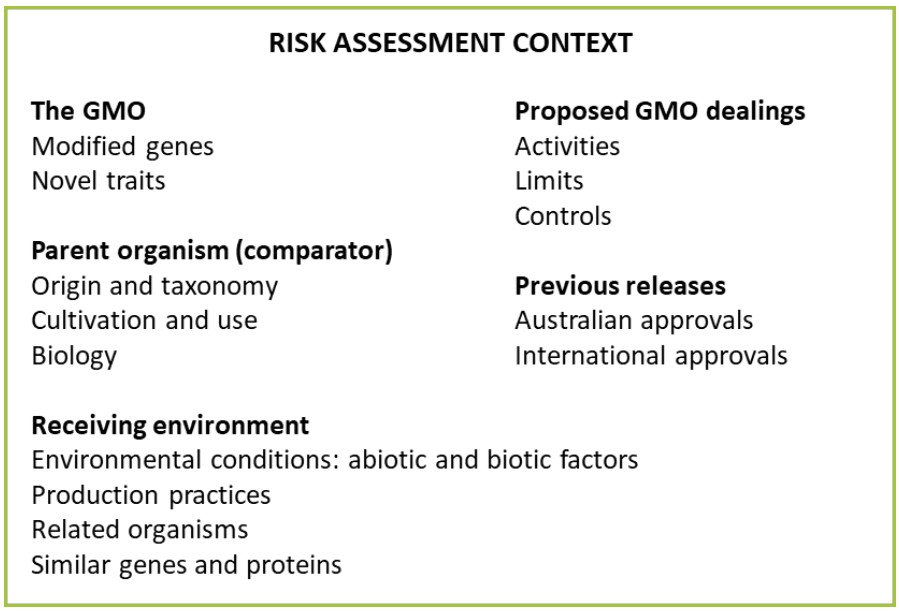

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR, and the Risk Analysis Framework

6. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. Therefore, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, all Australian local councils and the Minister for the Environment. A summary of issues contained in submissions received is provided in Appendix A.

1.1 Interface with other regulatory schemes – defining the scope of this evaluation

7. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings 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 (APVMA), the Therapeutic Goods Administration (TGA), the Australian Industrial Chemicals Introduction Scheme (AICIS) and the Department of Agriculture, Fisheries and Forestry (DAFF). These dealings may also be subject to the operation of State legislation recognising an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.

8. To avoid duplication of regulatory oversight, risks that will be considered by other regulatory agencies would not be assessed by the Regulator.

9. FSANZ assesses the safety of food produced using gene technology through administration of the *Australia New Zealand Food Standards Code*. FSANZ has received an application related to the GM Purple Tomato, A1333, and is currently assessing the safety of the GM Purple Tomato and its products as food for human consumption. More information is available on the FSANZ website.

10. DAFF is responsible for administering various biosecurity measures. The applicant has proposed to import the GM Purple Tomato seeds into Australia from the United States (US). These imports would be subject to permits obtained from DAFF.

11. The applicant has stated that the increased anthocyanins in the GMO could have health-promoting effects. Claimed benefits of the GMO are outside the scope of the Gene Technology legislation. The Regulator's responsibility is to identify and manage risk as a result of gene technology.

Section 2 The proposed dealings

12. All Aussie Avocados Pty Ltd (the applicant), trading as All Aussie Farmers, is seeking approval to commercially grow a GM Purple Tomato (Organisation for Economic Co-operation and Development/OECD Unique Identifier: NPS-01201-8). The proposed release would be Australia-wide, subject to restrictions imposed under any applicable legislation other than the Gene Technology legislation. The GM Purple Tomato and its products would enter general commerce including use in human food.

13. The dealings involved in the proposed intentional release are to:

- conduct experiments with the GMO
- breed the GMO
- propagate the GMO
- use the GMO in the course of manufacture of a thing that is not a GMO
- grow, raise or culture the GMO
- import the GMO
- transport the GMO
- dispose of the GMO

and the possession, supply or use of the GMO in the course of any of these dealings.

Section 3 The parent organism

14. In establishing the risk context, details of the parent organism form part of the baseline for a comparative risk assessment (OGTR, 2013). Non-GM tomato is the standard baseline for biological comparison in this RARMP.

15. The parent organism is tomato, *Solanum lycopersicum,* also known as *Lycopersicon esculentum*. Tomato plants are exotic to Australia. Modern cultivated tomatoes (*S. lycopersicum* var*. lycopersicum*)

originated from wild tomatoes in Peru, Ecuador, and other parts of South America, with domestication then occurring in Mexico. The wild cherry tomato *S. lycopersicum* var. *cerasiforme* is a weedy variety of tomato that is recognised to be closely related to cultivated tomatoes (Nesbitt and Tanksley, 2002). In this RARMP, *S. lycopersicum* and tomato are both terms that are used to refer to modern cultivated tomato *S. lycopersicum* var. *lycopersicum,* unless otherwise specified. Wild relatives include *S. cheesmaniae*, *S. pennellii, S. pimpinellifolium*, and *S. chilense* (Peralta et al., 2008).

16. Tomato plants belong to the family *Solanaceae*, also known as the nightshade family, which includes other edible crops like eggplants, capsicums and potatoes.

17. Botanically, the fruit of the tomato are berries, i.e. a simple fruit with many seeds in its flesh. In a culinary context, tomatoes are considered to be vegetables and are commonly used in a variety of savoury dishes.

3.1 Production and consumption

18. Tomatoes are grown both commercially and by individuals. Relevant agricultural practices for tomatoes in Australia are discussed further in Section 5.3.

19. Globally, over 192 million tonnes of tomatoes were produced commercially in 2023, with Australia accounting for approximately 0.17% of global production (Table 1). The ripe fruit of tomatoes are consumed either raw or cooked. Tomatoes may be processed and preserved as products such as juice, soups, dehydrated powder, salsas or sauces. Tomatoes are a popular food around the world, second only to potatoes when considering vegetable consumption. The average global consumption of tomatoes and tomato products is estimated at 22.25 kg/person/year for 2022, with Australia having an above average consumption at 37.16 kg/person/year (FAOSTAT website; accessed 10 July 2025).

Table 1. Production of tomatoes (2023)

Region	Area harvested (ha)	Yield (kg/ha)	Production (t)
World	5,412,458	35,533	192,317,973
Australia	4,055	79,340	321,736

Source: FAOSTAT website (accessed 10 July 2025). Data rounded to nearest whole number.

3.2 Reproduction

20. Tomatoes reproduce sexually and are primarily propagated through seed, although the plants also grow readily from cuttings and have a high capacity for the formation of adventitious roots (roots that form at the base of cuttings, stems in contact with soil etc.) (Guan et al., 2019; Nkongho et al., 2023).

21. After a seedling emerges, tomatoes undergo a vegetative growth period of approximately 2 to 3 months before flowering (Fullelove et al., 1998).

22. Most wild tomatoes and some intermediates to domesticated varieties have an exserted/elongated stigma (the pollen-receiving part of the female reproductive organ) which facilitates cross pollination by other sexually compatible plants. Domestication of wild tomato varieties has resulted in selection for a shortened stigma. As such, modern tomatoes are self-compatible and primarily self-pollinating (Peralta et al., 2008). A small amount of cross pollination is observed (usually about 0 – 1.75% at 1 m or less between plants), although cross pollination rates may be increased based on cultivar and environmental conditions (Horneburg et al., 2018; Quirós and Marcías, 1978; Reeves, 1973). Some heirloom varieties of tomatoes display a partially exserted stigma, which increases the likelihood of cross-pollination (McCormack. J, 2004).

23. Movement of the flower is important for releasing tomato pollen. In a field setting, vibration of the flower is provided by wind and insects, particularly bees, also known as buzz pollination (Bashir et al., 2018; OECD, 2017). In a greenhouse setting, pollination is aided artificially by methods such as vibrating the

flowers with an electric toothbrush, tapping the flower cluster several times with a pencil or using fans to create air movement across the flowers (McCormack. J, 2004).

24. Following pollination, tomato fruit then forms. In a commercial setting, it normally takes 6 – 8 weeks from first flower to fruit harvest (Fullelove et al., 1998).

25. As described in the United States Standards for Grades of Fresh Tomatoes (USDA, 1991), there are 6 main phases in red tomato fruit ripening (Figure 2);

- **Mature green**: the surface of the tomato is completely green in colour
- **Breaker**: there is a definite break in colour from green to tannish-yellow, pink or red on not more than 10% of the surface
- **Turning**: more than 10% but not more than 30% of the surface shows a definite change in colour from green to tannish-yellow, pink, red, or a combination thereof
- **Pink**: more than 30% but not more than 60% of the surface shows pink or red colour
- **Light red**: more than 60 percent of the surface shows pinkish-red or red AND not more than 90% of the surface is red
- **Red**: more than 90 percent of the surface shows red colour.

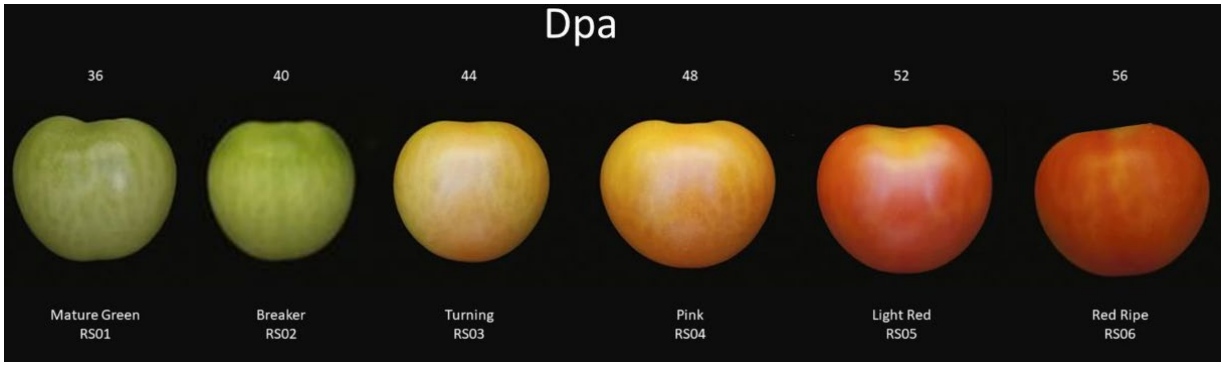

Figure 2. Tomato ripening phases

Source: Skolik et al. (2019). Ripening phases of *Solanum lycopersicum* cv. MoneyMaker. Dpa = days post anthesis (flower opening).

26. Fruit species can be classified on the basis of whether they continue to ripen after harvesting (climacteric) or must ripen fully on the plant (non-climacteric). Climacteric fruit, like tomatoes, bananas, and avocados, demonstrate increased respiration rates and ethylene synthesis during ripening and will continue to ripen off the plant (Giovannoni, 2004).

27. Each tomato fruit typically contains 150 to 300 seeds (McCormack. J, 2004).

28. Tomatoes have evolved mechanisms to prevent the germination of seeds while the fruit is still on the plant, also known as precocious germination. Tomato seeds are coated with a gel-like mucilage layer that acts as a germination inhibitor. The osmotic environment around the seeds is known to be important for germination inhibition, as is the production of endogenous abscisic acid (Berry and Bewley, 1992). In commercial seed production, seeds must be clean and dry for long-term storage. Fermentation of the seed pulp mix for 2 to 4 days is used to separate the seeds from the surrounding gel, then the seeds are washed and dried (McCormack. J, 2004).

29. Tomato seeds do not display strong dormancy, generally germinating whenever conditions (moisture and temperature) are suitable (Foolad et al., 2007). Seeds can remain viable for well over 10 years if stored under cool, dry conditions (Guadalupe et al., 2022) but lose viability rapidly in hot and humid conditions (Ariyarathna et al., 2020).

3.3 Phenotypic characteristics

30. Modern tomatoes display a wide range of phenotypic characteristics, including plant size, growth habit, fruit size, and fruit colour.

31. The two main growth habits of tomato plants are determinate or indeterminate. Determinate varieties display bushy growth, growing to approximately 1 m in height and flowering and setting fruit in a shorter period of time. This more concentrated harvest makes determinate varieties popular for processing as there are a large number of ripe tomatoes at one time. In contrast, indeterminate varieties continue to grow, flower and fruit over a much longer time, potentially reaching over 5 m in height when fully mature and giving a harvest window of approximately 12 to 20 weeks (Fullelove et al., 1998). One of the largest indeterminate tomato varieties is Giant Tree, which can reach up to 6 m in height (e.g. Seeds of Plenty). Because of their size, indeterminate tomatoes are more suited to commercial cultivation in greenhouses, where the plants are usually pruned to a single stem and trained up twine connected to overhead wiring.

32. In contrast to wild tomatoes which produce small, round fruit, modern tomatoes display a wide variety in fruit size and shape. On the smaller end of fruit size are cherry tomatoes and the larger end are beefsteak-style tomatoes which can reach over 1 kg per fruit. Shapes range from round, elongated, oblate (flattened at the poles), to pear shaped (reviewed in Tanksley, 2004).

33. Tomato fruit contain different classes of plant pigments, including carotenoids (typically red, orange or yellow), such as lycopene and β-carotene, chlorophyll (usually green) and anthocyanins (red, purple or blue) which result in a range of fruit colours from red, orange, yellow, green to purple (Li et al., 2025). Anthocyanins are discussed further in Section 4.1.

3.4 Toxicity and allergenicity of tomatoes

3.4.1 Toxicity

34. Plants in the Solanaceous family produce a number of alkaloids, which act as defence molecules to deter pests and pathogens. Glycoalkaloids inhibit cholinesterase and if enough is consumed can cause gastrointestinal symptoms, haemolysis, and kidney inflammation in people (Novak and Haslberger, 2000). The green tissues of tomatoes are listed in a report on harmful garden plants in Western Australia (Department of Agriculture and Food Western Australia, 2005). This report classifies green tomato tissues as an irritant when eaten and harmful to stock and other animals, presumably due to alkaloid concentrations. The American Society for the Prevention of Cruelty to Animals (ASPCA) lists tomato plants as toxic to dogs, cats and horses (ASPCA Toxic and Non-Toxic Plants List, accessed 10 July 2025). Clinical signs of toxicity are listed as hypersalivation, inappetence, severe gastrointestinal upset, depression, weakness, dilated pupils, and slow heart rate.

35. The main alkaloid in tomatoes is tomatine, a mixture of α-tomatine and dehydrotomatine. Dehydrotomatine contributes less than 10-20% of total tomatine in tomatoes, depending on the tissue type, therefore the following information focuses on α-tomatine. α-tomatine is present in all parts of the tomato plant but is predominately found in flowers (1100 mg/kg), leaves (975 mg/kg) and developing fruit (465 mg/kg), with levels decreasing significantly in the fruit during ripening (Friedman, 2004; Kozukue et al., 2004; Novak and Haslberger, 2000). Senescent leaves contain the highest levels at 4900 mg/kg (Friedman, 2004).

36. Modern tomato fruit have between negligible to 23 mg α-tomatine/kg fresh weight, typically about 1 mg/kg (OECD, 2008), however wild tomatoes growing in Peru have a much higher α-tomatine concentration of 500–5000 mg/kg of dry weight (approximately 30-300 mg/kg of fresh weight) and appear to be consumed by people with no ill effects (Rick et al., 1994).

37. In a laboratory study in hamsters, a diet of up to 20 mg α-tomatine/day for 21 days did not affect body weight gain or liver weight. The majority of the tomatine consumed was passed in the faeces as an insoluble complex with cholesterol, indicating that negligible tomatine was absorbed in the gastrointestinal tract (Friedman et al., 2000).

3.4.2 Allergenicity

38. The prevalence of tomato fruit allergy averages approximately 4.91% across Europe (Burney et al., 2014). There is limited information on the prevalence of tomato allergy in Australia. Tomato allergy appears to be relatively uncommon in Australia, as it is not one of the top 10 food allergies, which account for

approximately 90% of all food allergies, namely allergies to wheat, peanuts, tree nuts, shellfish, eggs, milk, fish, soy, sesame and lupin (Australian Institute of Food Safety blog, accessed 25 June 2025).

39. A group of tomato proteins known as Sola I are known to cause allergic reactions in some individuals. These include profilin Sola l 1, beta-fructofuranosidase Sola I 2, pathogenesis-related protein 10 (PR-10) Sola I 4, cyclophilin Sola I 5, and lipid transfer proteins Sola l 3, Sola l 6, and Sola l 7 (WHO/IUIS Allergen Nomenclature database, accessed 23 June 2025).

40. Oral allergy syndrome, where people with allergies to grass or tree pollens also have a rapid reaction in the lips, mouth, tongue and throat to certain foods, is known to occur with tomatoes (Kondo and Urisu, 2009). For example, tomato Sola I 4 is structurally homologous to major birch pollen allergen Bet v 1 (Wangorsch et al., 2015), as is tomato Sola I 1 to birch pollen profilin Bet v 2 (Westphal et al., 2004). The Australasian Society of Clinical Immunology and Allergy recommends avoiding oral allergy syndrome trigger foods in their raw, uncooked forms (ASCIA, 2024). Processing tomatoes may destroy the proteins that are responsible for oral allergy syndrome, although some of these proteins are resistant to processing (reviewed in Wlodarczyk et al., 2022).

41. "True" tomato allergy, i.e. not coming from a pollen cross reaction, is relatively rare. In a European allergy study, it was estimated that true tomato allergy occurs in 0.52% of the population (Burney et al., 2014).

42. Allergen levels have been shown to vary dependent on the tomato cultivar. Across 23 different tomato varieties Sola I 4 levels ranged from 0.24 and 1.71 µg/g fresh weight tomato fruit, a more than 7-fold difference (Kurze et al., 2018). This same study also noted differences in Sol I 4 levels when comparing processing techniques and seasonal differences across years.

43. There is limited literature on allergenicity of the vegetative tissue of the tomato plant. Some limited cases of contact dermatitis have been reported in people exposed to tomato plants or tomato plant extract (Paulsen et al., 2012).

3.5 Weed risk potential for tomato plants outside cultivation

44. Tomato is not recognised as a weed in Australia (Weeds Australia, accessed 3 July 2025).

45. Tomatoes are sensitive to extremes in abiotic conditions, including temperature and moisture (discussed further in Section 5.1) and susceptible to a variety of fungal, viral and bacterial diseases (discussed further in Section 5.2).

46. Tomatoes are heavy feeders that require high levels of nutrients for good growth, flowering and fruiting, including nitrogen, potassium, magnesium and calcium (Sainju et al., 2003). Competition for nutrients is one of the reasons that tomatoes are poor competitors with agricultural weeds, particularly during the early stages of tomato growth (Laude, 2023).

47. Tomatoes are considered to be herbicide-sensitive plants, including to 2,4-D, dicamba, glyphosate, saflufenacil, oxyfluorfen, and isoxaflutole, particularly from herbicide drift (Medeiros et al 2023).

48. Tomato seeds are not expected to survive the commercial composting process. In a laboratory experiment, commercial composting was simulated by adding tomato seeds to a fermenter system primed with pre-digested organic waste (Ryckeboer et al., 2002). After 1 day, tomato seeds had 0 – 0.2% viability. Anecdotally, tomato seeds can germinate in a home garden setting either when added to compost or from dropped fruit where tomatoes have been grown (see further discussion in Section 5.3.2).

49. Limited information is available on the potential for animals to disperse tomato seeds. Tomato seeds have no specific adaptations, such as hooks or burrs to facilitate their spread on the fur or feathers of animals. As producing fruit is an important mechanism by which plants can have their seeds dispersed following consumption by animals, it is expected that tomato seeds could be dispersed via fruit eating animals. Anecdotally, it is well known that tomato fruit are attractive food to animals such as birds, rats and possums (Birdlife Australia – a guide to feeding wild birds, Urban Food Garden – Protecting your crop at harvest time, accessed 17 July 2025). Tomatoes have a tough seed coat which resists digestion. Intact tomato seeds have been extracted from human stomach contents (Lee et al., 2006) and after passing

through the digestive system (Lee et al., 2005), however viability was not determined. In a study of seed dispersal by serrated tortoises, tomato seeds that had passed through the digestive tract of the tortoises had higher seed germination percentage (80%), than seeds that were mechanically extracted (55%) (Setlalekgomo and Sesinyi, 2014). In a nutrient availability trial, sheep fed whole, dried tomato seeds did not have any noticeable whole tomato seeds in their faeces, only parts of the seed coat (Heguy et al., 2015), although this may be more due to thorough chewing of the seeds rather than breakdown in the digestive processes.

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

50. The applicant proposes to release plants derived from one GM tomato event[2] modified for purple fruit colour, Del/Ros1-N. The GMO is known commercially as The Purple Tomato or by the OECD unique identifier NPS-01201-8. In this document, the GMO may also be referred to as the GM Purple Tomato as non-GM purple tomatoes are also discussed.

4.1 The genetic modifications in the GMO proposed for release

51. The GM Purple Tomato proposed for release has been genetically modified by the introduction of 2 transcription factors from garden snapdragon that switch on anthocyanin production during fruit ripening, resulting in purple fruit colour. The GMO also contains a selectable marker gene that confers antibiotic resistance (Table 2).

Table 2. Introduced genetic elements in the GM Purple Tomato

Gene (source)	Promoter (source)	Terminator (source)	Encoded protein	Intended function
Delila (Antirrhinum majus)	E8 promoter (*Solanum lycopersicum*) – activated during fruit ripening	Cauliflower mosaic virus (CMV) terminator (CMV)	Delila	Transcription factor – anthocyanin biosynthesis
Rosea1 (Antirrhinum majus)	E8 promoter (*Solanum lycopersicum*) – activated during fruit ripening	CMV terminator (CMV)	Rosea	Transcription factor – anthocyanin biosynthesis
Neomycin phosphotransferase type II (nptII) gene *(Escherichia coli)*	Nopaline synthase (NOS) promoter (*Agrobacterium tumefaciens*)	Octopine synthase 3 (Ocs 3) terminator (*Agrobacterium tumefaciens*)	Neomycin phosphotransferase type II (NPTII)	Antibiotic resistance, selectable marker

4.1.1 Introduced genes for anthocyanin production

52. The purpose of the 2 introduced genes *Delila* and *Rosea1* is to switch on anthocyanin production during fruit ripening, resulting in purple-coloured fruit, both in the skin and the flesh (Figure 3).

[2] An **event** is when DNA is inserted into the plant genome as a result of a single transformation process. Each time the transformation process occurs it is a new event, even if the same plasmid is used. The DNA may be inserted at a different location in the plant genome in a new event.

Figure 3. Cross section of the GM Purple Tomato fruit

Source: image supplied by applicant. GMO in the MoneyMaker x Goldkrone background.

53. In the GMO, Delila and Rosea1 cooperate to activate the existing anthocyanin biosynthesis pathway that is present in modern tomatoes but usually switched off (Figure 4).

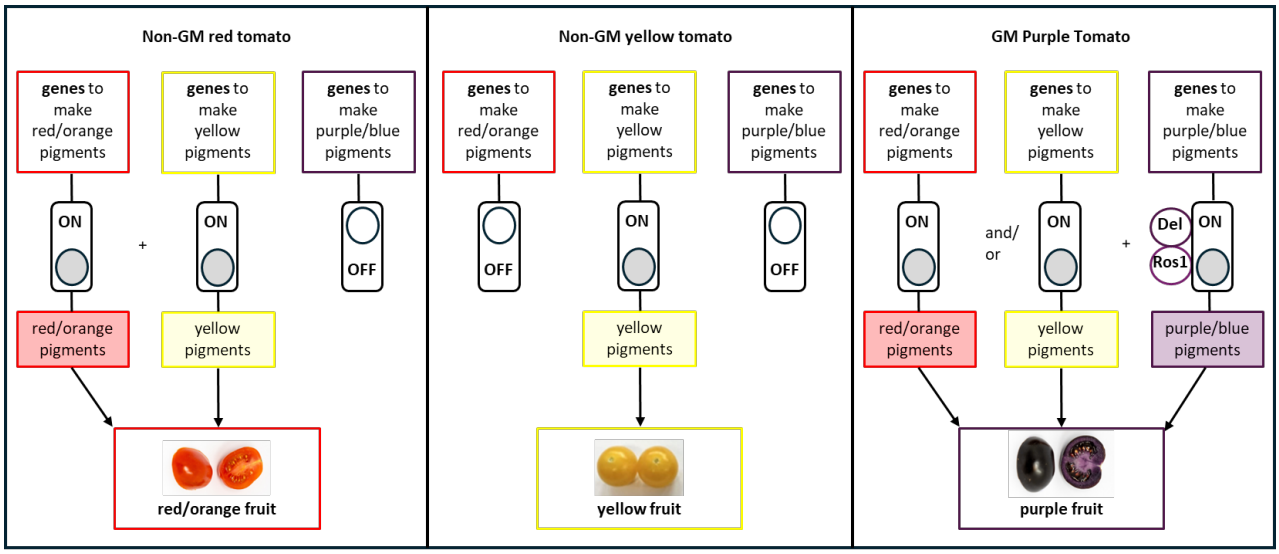

Figure 4. Comparison of pigment production in non-GM red tomatoes, non-GM yellow tomatoes and the GM Purple Tomato

Sources: Red/orange fruit and GM Purple Tomato fruit image provided by applicant. Yellow tomato fruit image from Martin and Butelli (2025).

4.1.1.1 Anthocyanin biosynthesis and function in plants

54. Anthocyanins (from the Greek *anthos* for flower and *kyáneos* for blue) are water soluble plant pigments that give red, blue, and purple colouring to plants, predominately their flowers and fruit. The colour of some anthocyanins is pH dependent, appearing red at lower pH, purple or violet at neutral pH, and blue at higher pH (reviewed in Glover and Martin, 2012; Khoo et al., 2017). Blue colouring is influenced by aromatic acylation and intense blue colouring can result from the association of anthocyanins with metals such as aluminium or iron (Glover and Martin, 2012; Takeda, 2006; Wahyuningsih et al., 2017).

55. The production of anthocyanins is regulated in response to a number of factors, including growth hormones, light, temperature, and nutrient availability (reviewed in Kapoor et al., 2022). Anthocyanins have a range of important functions in plants, including attracting pollinators through colouration of flowers (Glover and Martin, 2012), providing protection from the effects of high light conditions, whether through light-absorbing capacity or through antioxidant activity (Zhao et al., 2022; Zheng et al., 2021), and protecting against abiotic stresses such as cold stress (Xu et al., 2017) and drought stress (Cirillo et al.,

2021). Anthocyanins have strong antioxidant activity, reducing reactive oxygen species (ROS) and oxidative stress in plants (reviewed in Martín et al., 2017).

56. Although the most striking accumulation of anthocyanins is in flowers and fruit, anthocyanins also accumulate transiently in vegetative tissues in response to external stimuli such as light, cold and osmotic stress, and then are degraded when no longer needed (Hughes et al., 2007).

57. The non-sugar bound form of anthocyanins are known as anthocyanidins. The 6 most common anthocyanidins in plants are pelargonidin, cyanidin, delphinidin, petunidin, peonidin, and malvidin. Anthocyanidins are not stable and are readily converted to a sugar bound form, then stored in vacuoles (reviewed in Goncalves et al., 2021).

58. The anthocyanin biosynthesis pathway is well studied and is known to be conserved across different plant species. Anthocyanin biosynthesis is part of the phenylpropanoid pathway, which begins with the amino acid phenylalanine and produces a number of critical metabolites for plant growth, development, defence, and stress responses, including flavonoids, coumarins and lignins. The flavonoid biosynthesis pathway, which produces molecules such as anthocyanins, flavones, and flavonols, splits out from the phenylpropanoid pathway at the 4-coumaryl-CoA step. The genes encoding the enzymes involved in the anthocyanin biosynthesis pathway can be categorised into two groups; early biosynthetic genes which are also involved in the synthesis of flavonols and other flavonoid compounds (e.g. chalcone synthase; CHS, chalcone isomerase; CHI, flavanone 3-hydroxylase; F3H) and late biosynthetic genes which are involved in the downstream production of anthocyanins (e.g. dihydroflavonol-4 reductase; DFR, anthocyanidin synthase; ANS, UDP-glucose: flavonoid-3-O-glucosyltransferase; UFGT)(reviewed in Khusnutdinov et al., 2021)(Figure 5).

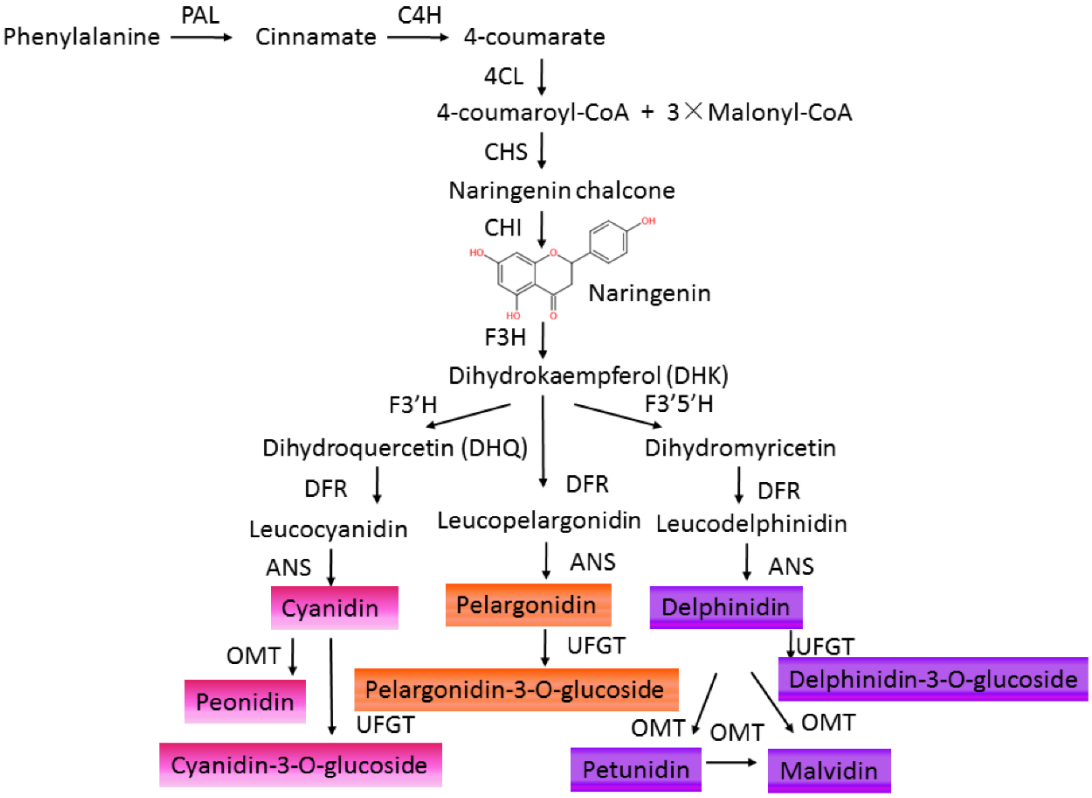

Figure 5. Anthocyanin biosynthesis pathway in plants

Source: Ma et al. (2021). PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 30 hydroxylase; F3'5'H, flavonoid 3050hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; UFGT, UDP-galactose flavonoid 3-O-galactosyltransferase; OMT, O-methyl transferase.

59. Across many plant species, anthocyanin biosynthesis is activated by an MBW regulatory complex consisting of 3 types of transcription factors (reviewed in Cappellini et al., 2021):

- R2R3-MYB
- basic helix-loop-helix (bHLH) and
- tryptophan-aspartic acid (WD)-40 repeat (WDR)-type proteins.

60. WDR genes are constitutively expressed in many plants (de Vetten et al., 1997; Walker et al., 1999), and therefore levels of the MYB and bHLH transcription factors are important for activating anthocyanin biosynthesis.

4.1.1.2 Regulation of anthocyanin biosynthesis in tomatoes

61. While modern tomatoes with red, orange and yellow fruit contain genes encoding the enzymes responsible for the anthocyanin biosynthesis pathway, activation of the pathway is incomplete in fruit. Anthocyanin biosynthesis is only normally activated in some vegetative tissues like the hypocotyl (the embryonic stem in a young seedling) in response to stress conditions, specifically derivatives of delphinidin, petunidin and malvidin (Roldan et al., 2014).

62. In tomatoes, anthocyanin biosynthesis is regulated by MYB transcription factors such as AN2, ANT1, ANT1-like, and AN2-like/Aft, bHLH transcription factors JAF13 and AN1, and the WDR transcription factor AN11 (reviewed in Menconi et al., 2024)(Figure 6). The transcription factors have different roles in activating anthocyanin biosynthesis in different tomato tissues. For example, the bHLH transcription factor AN1 (also known as Hoffman's anthocyaninless) has been shown to play an important role in accumulation of anthocyanin in young tomato seedlings in response to low temperatures (Qiu et al., 2016) and, in combination with AN11 and AN2, in fruit and vegetative tissues in response to high light conditions (Zhang et al., 2019). Overexpression of the MYB transcription factor ANT1 in MicroTom tomatoes resulted in purple-tinged areas in the leaves, stems and flowers, and purple spots on the skin of the fruit due to accumulation of anthocyanins, specifically derivatives of delphinidin, petunidin and malvidin (Mathews et al., 2003). In this same study, a purple leaf phenotype was also observed when tobacco was engineered to overexpress ANT1.

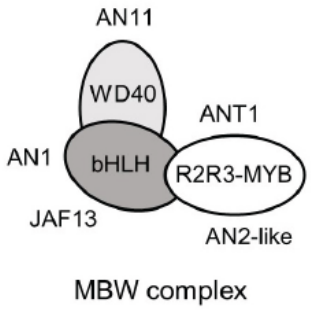

Figure 6. Anthocyanin biosynthetic regulatory genes in tomato

Source: Teo et al. (2022).

63. Some wild relatives of tomato have fruit with purple skin and have been crossed with domesticated non-GM tomatoes to give the non-GM purple fruit skin trait. Analysis of these wild relatives and their hybrids with modern tomatoes has revealed much about the regulation of anthocyanin biosynthesis in tomatoes. Three loci have been described that are derived from wild tomato and are responsible for increased anthocyanin production and purple colour in non-GM purple tomatoes (Mes et al., 2008).

64. The first is the *Aft* locus, which includes a gene that encodes the R2R3-MYB transcription factor, AN2-like (Sun et al., 2020; Yan et al., 2020). AN2-like contributes to the purple fruit skin colouring in the non-GM Indigo Rose tomato and is derived from a crossing of modern tomato with the wild tomato relative *S. chilense*. Mutations in this gene in modern tomato varieties have led to splicing defects and loss of function in activating anthocyanin biosynthesis. In Indigo Rose tomatoes, functional AN2-like activates the accumulation of anthocyanins in the skin of the fruit in a light-dependent manner. When the *AN2-like* gene was added to red-fruited variety Ailsa Craig under the control of the tomato E8 fruit-ripening specific

promoter, anthocyanins accumulated in both the skin and flesh of the fruit during ripening. Anthocyanin levels were 222 mg/100 g fresh fruit weight in the modified purple tomato compared to undetectable in the control (Sun et al., 2020).

65. The second locus is *atv*, also found in the Indigo Rose variety, which contains the gene *MYBATV* derived from *S. cheesmaniae*. *MYBATV* encodes a MYB transcription factor that is a negative regulator of anthocyanin biosynthesis in most modern cultivars and wild tomatoes, however *S. cheesmaniae* and Indigo Rose contain a mutated version that is non-functional (Sun et al., 2020).

66. The third locus is *Aubergine* (*Abg*), which is derived from a spontaneous cross with *S. lycopersicoides*. Fruit of heterozygous *Abg*-containing plants displays a blotchy purple colouring in response to light, as well as accumulating anthocyanins in the leaves and stem. Homozygous *Abg* plants fail to thrive and are sterile. It has been recently proposed that Abg is a splicing variant of AN2-like (Menconi et al., 2023).

4.1.1.3 Delila and Rosea1

67. Delila is a bHLH transcription factor that regulates anthocyanin biosynthesis in *A. majus*. *A. majus* with a loss of function *Delila* mutant displayed a loss of anthocyanin pigment in the tube of the flower, thought to be due to lack of activation of the later stage of the anthocyanin biosynthesis pathway (Martin et al., 1991). Further studies in snapdragon have indicated that Delila upregulates expression of an anthocyanin pathway late biosynthetic gene encoding the enzyme DFR (Albert et al., 2021).

68. Rosea1 is a MYB transcription factor that regulates anthocyanin biosynthesis in *A. majus*. In *A. majus*, Rosea1 has been shown to upregulate transcription of late biosynthetic genes for anthocyanin production, as well as an anthocyanin transporter and a gene involved in flavanol synthesis. Rosea1 had no effect on the expression of the early biosynthetic gene *CHS* and a minor effect on *CHI* (Schwinn et al., 2006).

69. Overexpression of either snapdragon *Rosea1* (Naing et al., 2018) or snapdragon *Delila* (Naing et al., 2017) alone throughout entire tobacco plants increased the production of anthocyanins, reduced reactive oxygen species, and increased tolerance to abiotic stresses such as cold, drought and salt tolerance.

70. MBW complexes have also been shown to play a role in cell differentiation processes like root hair and trichome differentiation. When *Rosea1* and *Delila* were engineered into tomatoes under the control of a dexamethasone-inducible promoter, purple colouring was observed in vegetative tissues like the roots, stems and leaves, but not fruit or flowers (Outchkourov et al., 2018). Changes were also observed in root branching, root epithelial cell morphology, seed germination, and leaf conductance.

4.1.1.4 Comparison of non-GM purple tomatoes and the GM Purple Tomato

71. The purple fruit colour trait has also been achieved in tomatoes that have been conventionally bred (non-GM).

72. Some species of wild tomatoes have fruit with purple skin when exposed to light and this trait has been conventionally crossed into modern tomatoes. As discussed in Section 4.1.1.2, the non-GM variety Indigo Rose contains the *AN2-like* gene from *S. chilense*, encoding a positive regulator of anthocyanin biosynthesis that is non-functional in modern tomatoes, and *MYBATV* from *S. cheesmaniae*, encoding a repressor of anthocyanin biosynthesis that is non-functional in Indigo Rose and functional in modern tomato varieties. Indigo Rose fruit show light-dependent accumulation of petunidin, malvidin and delphinidin in the skin, but not in the flesh (Mes et al., 2008; Sun et al., 2020). Another purple-skinned non-GM variety that contains the same anthocyanin-related genes as Indigo Rose is called Sun Black (Mazzucato et al., 2013).

73. Purple fruit colouring that is not due to anthocyanins has also been found in tomatoes with various mutations in the *green flesh* gene. Mutations in this gene prevent chlorophyll from being degraded during fruit ripening, but there is still the normal accumulation of carotenoids (Cheung et al., 1993). This results in ripe fruit which have muddy purple or brown skin and may also have a darker tinge to the flesh, and includes varieties such as Cherokee Purple, Black Cherry, and Purple Russian (Barry and Pandey, 2009).

74. While these non-GM purple tomato varieties have purple skin, none of them have all purple flesh. This means they can be easily distinguished from the GM Purple Tomato which has all purple skin and flesh.

Pigments in fruit	GM Purple Tomato	Indigo Rose (non-GM)	Cherokee Purple (non-GM)
Chlorophyll	low/not present	low/not present	present
Red/orange and/or yellow pigments	present	present	present
Anthocyanins (skin)	present	present	low/not present
Anthocyanins (flesh)	present	low/not present	low/not present
			

Figure 7. Pigments in the ripe fruit of the GM Purple Tomato and non-GM purple tomatoes Indigo Rose and Cherokee Purple

Source: GM Purple Tomato image supplied by applicant. Indigo Rose image from Territorial Seed Company. Cherokee Purple image from Seeds of Plenty.

4.1.2 Selectable marker - nptII

75. The introduced *nptII* gene was used as a selectable marker during early stages of development. The *nptII* gene is derived from *Escherichia coli* strain K12 and encodes an enzyme, neomycin phosphotransferase (NPTII), also known as aminoglycoside 3'-phosphotransferase II enzyme (APH(3')-IIa). It provides resistance to neomycin, kanamycin, paromomycin and related aminoglycoside antibiotics by using ATP to phosphorylate and inactivate the antibiotic, preventing it from killing NPTII producing cells. More information on *nptII*, including information regarding its lack of toxicity or allergenicity, is available in the document *Risk Assessment Reference: Marker Genes in GM Plants* on the Risk assessment reference documents page on the OGTR website.

4.1.3 Regulatory sequences

76. Short regulatory sequences that control expression of the genes are also present in the GMO (Table 2). Expression of the *Delila* and *Rosea1* genes is driven by the tomato E8 promoter. The E8 promoter is a tissue specific promoter, which is active during fruit ripening, but not in unripe fruit or vegetative tissues (Hirai et al., 2011; Kurokawa et al., 2013). The *E8* gene encodes an enzyme involved in the detoxification of the glycoalkaloid α-tomatine to esculeoside A, which occurs during fruit ripening (Akiyama et al., 2021). The E8 promoter responds to ethylene, a gaseous plant hormone that initiates fruit ripening (Deikman et al., 1992). Expression of the *nptII* gene is driven by a constitutive NOS promoter from *Agrobacterium tumefaciens* which is active in a wide range of plant tissues and developmental stages (Ebert et al., 1987). Other short regulatory elements used include termination sequences.

4.2 Method of genetic modification

77. The GMO was generated by *Agrobacterium*-mediated transformation of the parental tomato variety MicroTom, a determinate dwarf cherry tomato. *Agrobacterium tumefaciens* is a species of soil bacteria that naturally infects plants and transfers some of its DNA, known as transfer DNA or T-DNA, into the plant during the infection process. By using molecular biology techniques to place genes of interest within this T-DNA, *Agrobacteria* can be used to transfer these genes into a plant. More information about this method can be found in the document *Methods of plant genetic modification*, available from the OGTR Risk Assessment References page.

78. After transformation, kanamycin was used to select for GM tomato cells. The resulting material was tested and confirmed to be free from *Agrobacteria*.

4.3 Toxicity and allergenicity of the proteins associated with the introduced genes

79. The introduced *Delila* and *Rosea1* genes are derived from *A. majus*, more commonly known as garden snapdragon. A comprehensive search of the literature yielded no information to suggest that either of the encoded proteins are toxic or allergenic to people, or toxic to other organisms. Snapdragon flowers are edible for humans (Chensom et al., 2019) and are considered non-toxic for animals, including dogs, cats and horses (ASPCA Toxic and Non-Toxic Plants List, accessed 10 July 2025).

80. The applicant has reported that bioinformatic analysis showed no amino acid sequence similarity of Delila or Rosea1 to known allergens or toxins (Martin and Butelli, 2025). The levels of the Delila and Rosea1 proteins were below the limit of detection in juice from the GM fruit (<0.5 ng Delila and <0.2 ng Rosea1 protein per mL juice). As the levels of Delila and Rosea1 were very low in the GM Purple Tomato fruit, the applicant expressed the proteins in *E.coli* to purify enough protein for proteolytic digestion analysis. Both *E.coli*-derived proteins were rapidly degraded by pepsin in simulated gastric fluid, indicating the proteins would be rapidly degraded in the digestive system if consumed.

81. There is no evidence that the *nptII* gene or the protein it encodes is toxic or allergenic (OGTR Risk Assessment document and references therein). GM foods containing the *nptII* gene have been assessed and approved for sale in Australia (FSANZ website, accessed 30 June 2025).

4.4 Toxicity and allergenicity of the end products associated with the introduced genes

82. Anthocyanin-containing foods are consumed by people around the world, with average daily intakes depending on many factors, including dietary patterns and food availability. In a US study of dietary flavonoid intake, it was estimated that daily average intake of anthocyanins was 11.6 mg/day, with berries contributing the most to anthocyanin consumption (Sebastian et al., 2015). The Australian intake of anthocyanins is estimated to be even higher at 24.17 mg/day, again with berries as the top source of anthocyanins (Igwe et al., 2019). In a clinical study of oxidative stress and inflammation, no adverse effects were reported when participants were given a blackcurrant extract daily containing approximately 240 mg/day anthocyanins for a 5 week period, predominately derivatives of delphinidin and cyanidin (Hurst et al., 2020).

83. The anthocyanin concentrations in the various GM tomato breeding varieties are 39.5 – 283.5 mg/100 g fresh fruit weight (discussed further in Section 4.5.4), which are within the ranges of total anthocyanins seen in common anthocyanin-containing foods such as blackberries, blueberries, black currant, elderberry and red cabbage (Table 3). In particular, blueberries and blackcurrants are specifically enriched in delphinidin and petunidin, which are the primary anthocyanidins found in the GMO. This indicates that people regularly consume food containing the same pigments produced in the GMO.

Table 3. Anthocyanin concentrations in common foods

	mg/100 g (of fresh weight or form consumed)			
Food	**Delphinidin**	**Petunidin**	**Total ACN**	**Total ACN/serving [a] (mg)**
Fruit				
Blackberry	-	-	245	353
Marion blackberry	-	-	300.5	433
Blueberry (cultivated)	120.7	71.9	386.6	529
Blueberry (wild)	141.1	87.6	486.5	705
Chokeberry	-	-	1480	2147
Black currant	333	7.3	476	533
Elderberry	-	-	1375	1993
Black raspberry	-	-	687	845
Vegetables				
Black bean	18.5	15.4	44.5	23.1
Eggplant	85.7	-	85.7	35.1
Red cabbage	-	-	322	113

	mg/100 g (of fresh weight or form consumed)			
Food	**Delphinidin**	**Petunidin**	**Total ACN**	**Total ACN/serving [a] (mg)**
Red onion	-	-	48.5	38.8
Red radish	-	-	100.1	116

Adapted from Wu et al. (2006). Select fruits and vegetables with the highest anthocyanin concentrations in each category are shown. The main 6 anthocyanidins were measured, only individual values for delphinidin and petunidin are shown here. [a] Serving size from the USDA National Nutrient Database for Standard Reference. ACN = anthocyanins.

84. Oral bioavailability of anthocyanins is low, estimated to be 0.26-1.8% in animal studies (reviewed in Fang, 2014).

85. Anthocyanins are not generally considered to be toxic, and are used as a dye and food colourant (reviewed in Khoo et al., 2017).

86. Anthocyanins are not generally considered to be allergenic, however there are some limited reports of hypersensitivity reactions and allergies when people are exposed dermally to concentrated anthocyanins (reviewed in Lis and Bartuzi, 2023). For example, when nasunin from eggplant extract was used at over 5% concentration in a skin patch test, 12% of participants experienced a moderate skin reaction and 3% experienced an allergic reaction. However, the concentration of this pigment does not normally exceed 1% as a dye in foods and cosmetics (Gallo et al., 2014).

4.5 Characterisation of the GMO

4.5.1 Breeding background of the GMO

87. Four primary transformants (C, N, Y, Z) were generated via Agrobacterium-mediated transformation of MicroTom tissue (Figure 8). The N transformant was selected for further development due to having the highest anthocyanin production in the fruit (Butelli et al., 2008). This transformant was self-pollinated for 6 generations (T6) to create the “Del/Ros1-N in MicroTom” variety. One individual plant in the T1 generation of the MicroTom variety was crossed to MoneyMaker (an indeterminate variety with medium sized fruit) to generate a purple fruited F1 population. One of the F1 plants was self-pollinated and then a F2 plant with dark purple fruit was self-pollinated through 8 generations propagated by single seed descent to develop the variety “Del/Ros1-N in MoneyMaker”.

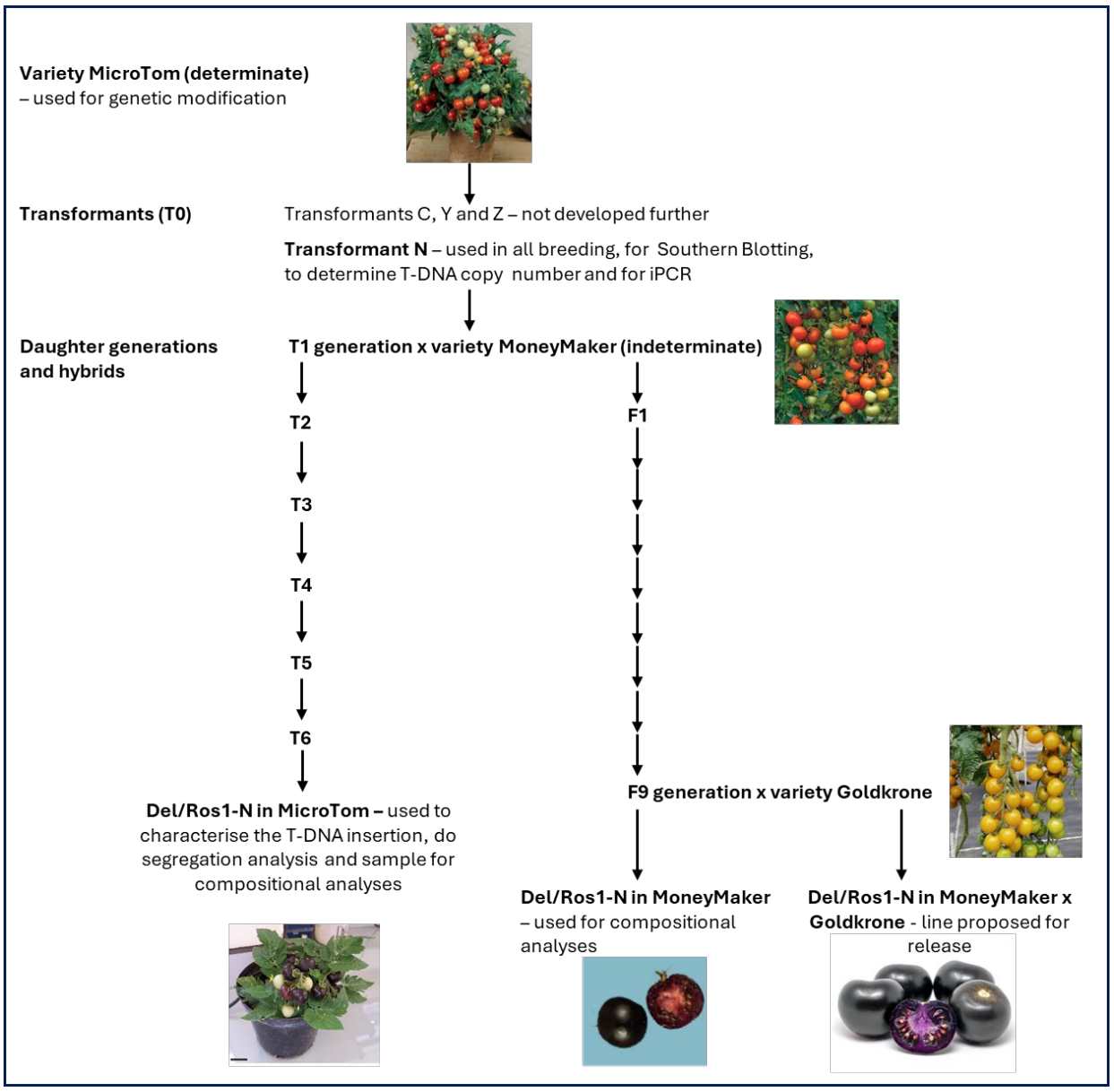

Figure 8. Breeding strategy of the GMO in the MicroTom, MoneyMaker and Goldkrone backgrounds

Source: adapted from diagram supplied by applicant. MicroTom image from Pan American Seeds. MoneyMaker image from Vasili's. Goldkrone image from Easyseeds. Del/Ros1-N in MicroTom image from Butelli et al. (2008). Del/Ros1-N in MoneyMaker image from Martin and Butelli (2025). Del/Ros1-N in MoneyMaker x Goldkrone image from applicant.

88. The commercial breeding variety released in the US is derived from MoneyMaker F9 x Goldkrone, an indeterminate cherry tomato variety with yellow fruit. This cross was chosen for commercial development as the combination of yellow pigments with the purple/blue anthocyanins results in a darker purple trait compared to crosses with red tomatoes, and the fruit was reportedly sweeter (Martin and Butelli, 2025). This is also the variety proposed for release in Australia.

89. Other successful crosses of the GMO resulting in purple fruit included to Ohio 8243, a processing tomato variety that is used for products such as tomato juice, Ailsa Craig, Maglia Rose, and Lucinda (Martin and Butelli, 2025).

4.5.2 Molecular characterisation

90. Southern blot analysis of the original N transformant in MicroTom revealed 2 inserts of the transfer DNA (T-DNA) containing the genes of interest; one at "Locus A" and one at "Locus B". Inverse polymerase chain reaction (iPCR) was used to identify the sequences flanking the left and right borders of the T-DNA. During subsequent breeding of the 2 varieties, the insert at Locus A was lost through segregation. This was

confirmed by polymerase chain reaction (PCR) analysis of the relevant regions of Locus A and Locus B in T6 Del/Ros1-N in MicroTom plants and also in Del/Ros1-N in MoneyMaker self-pollinated for 9 generations (F9). The insert at Locus B was confirmed to be present in both varieties.

91. iPCR and whole genome sequencing of both MicroTom and MoneyMaker varieties confirmed that the Locus B T-DNA insert was located at position 62904771 on chromosome 4 (Figure 9).

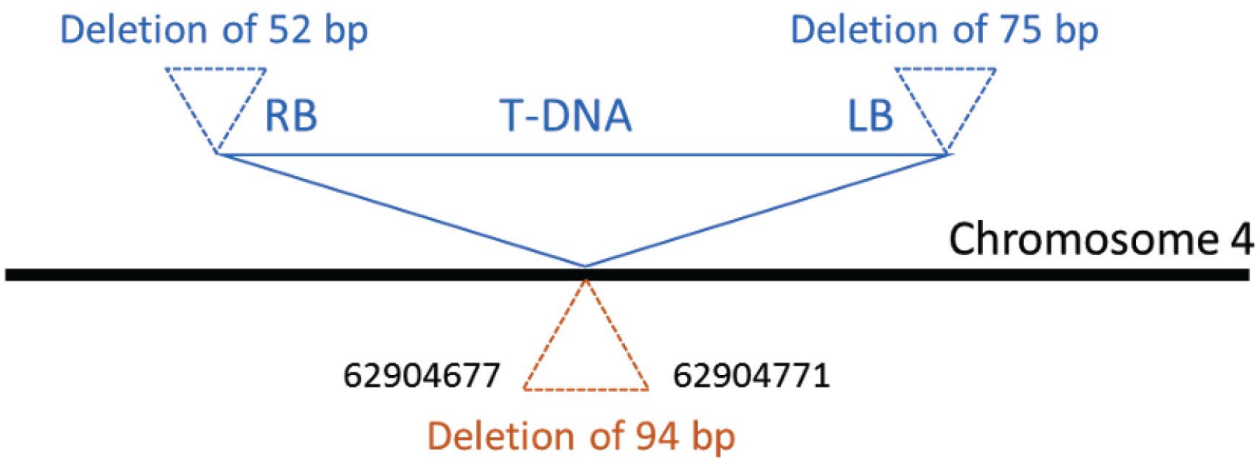

Figure 9. Location of the Locus B T-DNA insertion in the GM Purple Tomato genome

Source: image supplied by applicant. Insertion as determined by PCR analysis and confirmed by whole genome sequencing. The figure shows the relevant portion of chromosome 4 where the genes of interest have been inserted as a single T-DNA insert. Deletions that occurred as part of the insertions are shown. bp = base pairs, RB = right border of the T-DNA, LB = left border of the T-DNA.

92. Whole genome sequencing of the MicroTom and MoneyMaker x Goldkrone-derived varieties was used to confirm that all inserted genetic elements were intact in the single T-DNA insertion at Locus B. No mutations or mismatches were present, nor were any vector backbone sequences. The applicant has also stated that the insert on locus B did not interrupt any tomato genes/existing open reading frame (ORF) or create new functional ORFs.

93. In this RARMP "the GMO" refers to the event with the OECD identifier NPS-01201-8, being the N transformant containing the Del/Ros1 insert at Locus B and any varieties derived from it, including crosses with other *S. lycopersicum* cultivars. The following characterisation of the GMO includes data on Del/Ros1-N in MicroTom, Del/Ros1-N in MoneyMaker and Del/Ros1-N in MoneyMaker x Goldkrone, as indicated.

4.5.3 Inheritance and stability of the insert

94. Genotypic (presence of the *nptII* gene) and phenotypic (kanamycin resistance, colour of tomato fruit) characterisation of crosses of the GMO with non-GM tomatoes has shown that the insert is inherited in a dominant Mendelian manner.

95. As discussed in Section 4.5.2, whole genome sequencing of 2 varieties of the GMO has shown that the inserts have remained unchanged during the breeding program. In the case of the MoneyMaker x Goldkrone variety, the material used for sequencing is more than 7 generations of self-fertilised crosses from the original MoneyMaker and Goldkrone cross, and for the MicroTom variety it was more than 6 generations removed from the original N transformant, indicating stable inheritance of the insert.

96. The applicant has stated that, to date, no loss of the purple phenotype has been recorded, either in greenhouse breeding trials or in the commercial production of thousands of plants.

4.5.4 Anthocyanin content and gene expression analysis

4.5.4.1 Quantification and characterisation of anthocyanins

97. In the original hemizygous Del/Ros1-N MicroTom transformant, the anthocyanin concentration was measured at 283.5 mg/100 g of fresh fruit weight, compared to 0.8 – 1.6 mg/100 g in the MicroTom control (Butelli et al., 2008). The applicant has stated that the anthocyanin concentration in the MoneyMaker background, is 40 mg/100 g fresh weight. Literature indicates that non-GM MoneyMaker tomatoes have negligible anthocyanins (Sapir et al., 2008). In the MoneyMaker x Goldkrone variety, anthocyanin concentration ranges from 39.5 – 107.2 mg/100 g fresh fruit weight compared to none in non-GM Goldkrone (Martin and Butelli, 2025). The applicant has commented that the lower concentrations in the commercial variety are due to the higher water content in the globe-type tomatoes. The anthocyanin concentrations in the fruit of the MicroTom, MoneyMaker and MoneyMaker x Goldkrone varieties are within the ranges seen in common anthocyanin-containing foods such as blackberries, blueberries and red cabbage (Table 3). For comparison, anthocyanin concentrations in non-GM purple Indigo Rose tomatoes are 116.11 mg/100 g fresh weight skin (whole fruit was not analysed) (Mes et al., 2008).

98. Although not the subject of the current application, a study investigated further improvements to the anthocyanin content of Del/Ros1-N tomatoes. MYB12 is a transcription factor that regulates flavanol production in *Arabidopsis thaliana*, and has been shown to drastically increase flavanol content in tomato fruit under the E8 promoter, resulting in orange fruit (Luo et al., 2008). Del/Ros1 in MicroTom was crossed with AtMYB12 in MicroTom, which contains the MYB12 gene from *A. thaliana* under the control of the E8 promoter (Zhang et al., 2015). Fruit from Del/Ros1 x AtMYB12 (named Indigo) had an almost 2-fold increase in anthocyanin content compared to Del/Ros1 alone. The authors speculate that the dramatic increase in anthocyanins in the hybrid may be due to MYB12 upregulating earlier parts of the phenylpropanoid pathway, therefore increasing supply of metabolites into the anthocyanin pathway.

99. High-performance liquid chromatography (HPLC) and electrospray ionisation tandem mass spectrometry were used to analyse anthocyanin content in the flesh and peel of tomatoes from the MicroTom variety (Butelli et al., 2008). The major anthocyanins present in both the flesh and peel were:

- delphinidin 3-(cis-coumaroyl)-rutinoside-5-glucoside
- delphinidin 3-(trans-coumaroyl)-rutinoside-5-glucoside (also known as nasunin)
- delphinidin 3-(caffeoyl)-rutinoside-5-glucoside
- delphinidin 3-(feruloyl)-rutinoside-5-glucoside
- petunidin 3-(trans-coumaroyl)-rutinoside-5-glucoside (also known as petanin), and
- petunidin 3-(feruloyl)-rutinoside-5-glucoside.

100. Petanin and nasunin were the most abundant anthocyanins in the GM tomatoes and were not detected in the non-GM control fruit (Tohge et al., 2015). Petanin is also found in Indigo Rose tomato (Wang et al., 2020) and petunia (Schram et al., 1983), and nasunin is the main anthocyanin in eggplant skin (Panda et al., 2025; Sakamura et al., 1963).

101. A different laboratory used the methods described in Butelli et al. (2008) to create their own, different GM purple tomato variety (a GMO not covered in the current application) in the Rubion tomato cultivar background containing the same construct that was used to create the GM Purple Tomato (Lim et al., 2014). Anthocyanins were characterised in the ripe fruit and, like the GM Purple Tomato, petanin and nasunin were also the most abundant anthocyanins (Su et al., 2016).

102. As discussed in Section 4.1.1.1, anthocyanins are known to have strong antioxidant activity. The antioxidant activity of the GMO in the MicroTom background was 3 times higher than the control as measured by the Trolox Equivalent Antioxidant Capacity test, which determines how well a substance can neutralise free radicals in comparison to the vitamin E derivative Trolox (Butelli et al., 2008).

4.5.4.2 Expression of Delila and Rosea1 and components of the anthocyanin biosynthesis pathway

103. Expression of *Delila* and *Rosea1* in the GM Purple Tomato fruit has been confirmed by multiple methods, including northern blot (Butelli et al., 2008)(Figure 10). As discussed in Chapter 1 Section 4.3, the

levels of the Delila and Rosea1 proteins were below the limit of detection in juice from the GM fruit (<0.5 ng Delila and <0.2 ng Rosea1 protein per mL juice) (Martin and Butelli, 2025).

104. Expression of *Delila* and *Rosea1* in the GM Purple Tomato fruit lead to expression of multiple genes encoding enzymes involved in both the early and late stages of anthocyanin biosynthesis, including PAL, CHI, and F3’5’H, enzymes involved in side chain modification like a putative anthocyanin acyltransferase, and 2 genes that are expected to facilitate transport of the anthocyanins into vacuoles, putative anthocyanin permease and glutathione S-transferase (Figure 10) (Butelli et al., 2008).

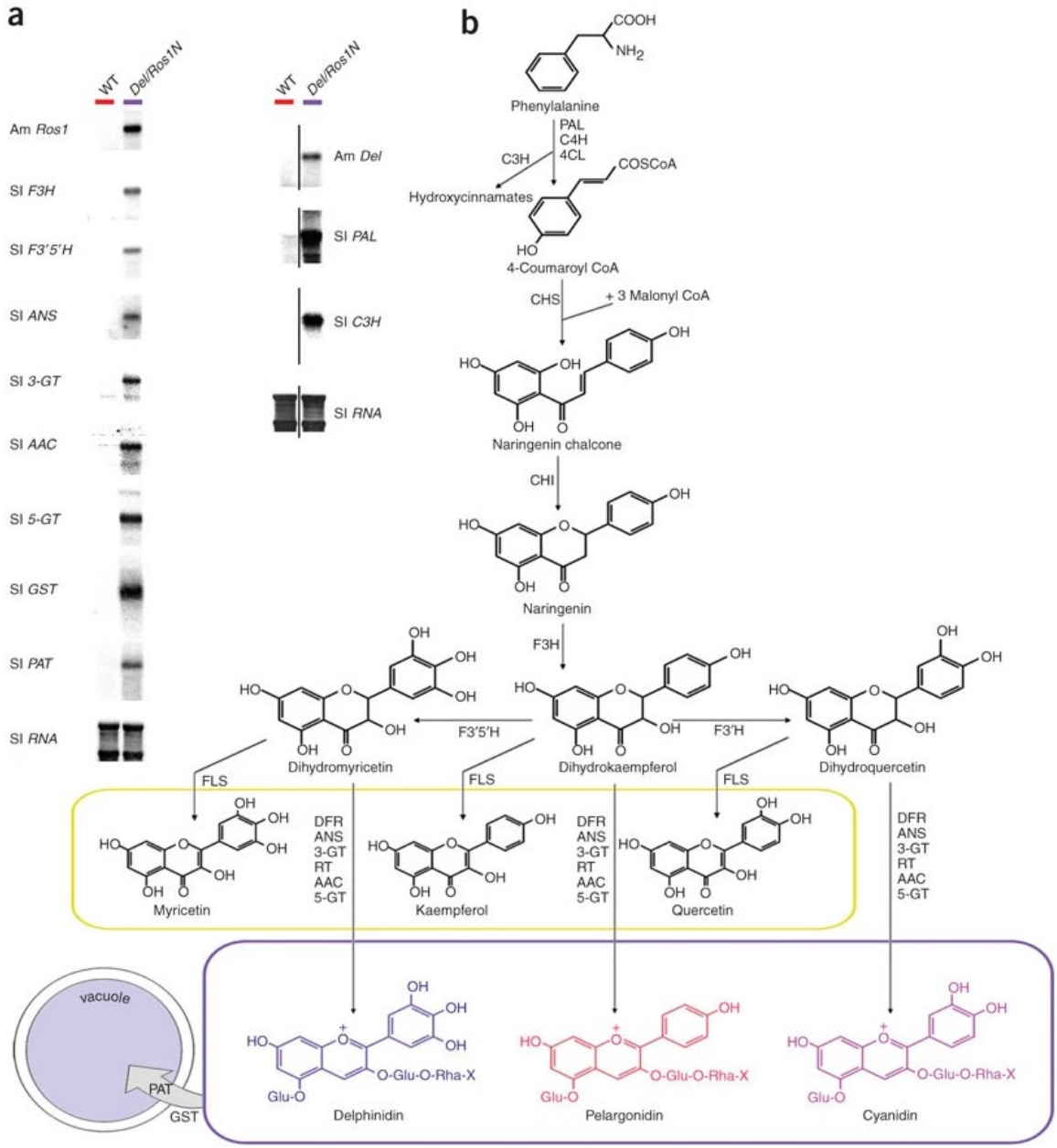

Figure 10. Anthocyanin biosynthesis genes upregulated in the GM Purple Tomato

Source: partial figure from Butelli et al. (2008). a) Northern blots showing the differential expression of several anthocyanin biosynthetic genes identified by suppression subtractive hybridisation. b) Schematic representation of the anthocyanin biosynthetic pathway. Relevant flavonoid classes are shown in boxes. Yellow box, flavonols; purple box, anthocyanins.

PAL, phenylalanine ammonia lyase; 4CL, 4-coumarate:coenzyme A ligase; C4H, cinnamate 4-hydroxylase; C3H, 4-coumarate 3-hydroxylase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone-3-hydroxylase; F3’H, flavonoid-3’-hydroxylase; F3’5’H, flavonoid-3’5’-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol reductase; ANS, anthocyanidin synthase; 3-GT, flavonoid 3-O-glucosyltransferase; RT, flavonoid 3-O-glucoside-

rhamnosyltransferase AAC, anthocyanin acyltransferase; 5-GT, flavonoid-5-glucosyltransferase; GST, glutathione S-transferase; PAT, putative anthocyanin transporter.

4.5.4.3 Other changes in the phenylpropanoid pathway

105. As Delila and Rosea1 are acting on common enzymes in the phenylpropanoid biosynthesis pathway, it is possible that other metabolites in this pathway would be increased. Increases in chlorogenic acid, flavonones and flavanols were observed in the GM Purple Tomato fruit (Tohge et al., 2015). This same study showed that pathways responsible for input into the phenylpropanoid pathway are also increased, including the shikimate pathway (essential for the synthesis of aromatic acids like phenylalanine) and phenylalanine biosynthesis.

106. Some phenylpropanoid metabolites were decreased in the GM Purple Tomato fruit, including phenylalanine. As phenylalanine is the first input to the phenylpropanoid pathway, which leads to anthocyanin biosynthesis, this decrease in phenylalanine is thought to be because of increased demands for inputs in the anthocyanin biosynthesis pathway, even outstripping the increases in phenylalanine biosynthesis (Tohge et al., 2015).

4.5.5 Upregulation of a plant defensin

107. Upregulation of expression of the endogenous *tgas118* gamma-thionin gene, which encodes a small defensin protein, was also observed in the GM Purple Tomato fruit (Butelli et al., 2008). This upregulation was further characterised at different ripeness stages with a 139-fold increase in RNA expression (13553 vs 98 count RNA reads) compared to wild-type seen in the GM tomatoes at 1 week after breaker stage, and a 285-fold increase (8289 vs 29 count RNA reads) seen at 4 weeks after breaker stage (Tohge et al., 2015). The applicant has not quantified the amount of TGAS118 defensin peptide in the GM Purple Tomato fruit, but given the increase in gene expression, it is expected that levels of TGAS118 would be increased compared to levels in the non-GM control.

108. Plant defensins are peptides that have antimicrobial activity, usually against fungi (reviewed in Stotz et al., 2009b).

109. The *tgas118* gene has been shown to be predominately expressed during tomato flower development and in non-GM tomatoes is induced by plant growth regulator gibberellin, tissue wounding and dehydration (van den Heuvel et al., 2001). TGAS118, also known as DEF1 or DF1, is predicted to be secreted from the cell and is upregulated in response to infection with soil-borne fungus *Verticillium dahlia*. The same study showed limited changes in expression after cold stress and viral infection of seedlings (Nikoloudakis et al., 2020). Another study showed that *tgas118* is expressed in the root, stem, leaf, flower and mature fruit of healthy tomato plants, predominately in the stem and mature fruit, and that it confers resistance to *Phytophthora infestans,* with overexpression of *tgas118* resulting in reduced ROS accumulation (Cui et al., 2018). Several other tomato defensins have been identified, including tomato defensin DEF2/DF2 (closely related to TGAS118/DEF1/DF1) which has been shown to have activity against fungal pathogen *Botrytis cinerea* (Stotz et al., 2009a) and fungal-like pathogen *Phytophthora infestans* (Cui et al., 2018), and tomato pistil predominant 3 defensin (TPP3) which has activity against fungus *Fusarium graminearum* (Baxter et al., 2015).

110. No direct link between anthocyanin biosynthesis and defensins could be found in the literature. It is possible that Delila and/or Rosea1 act directly to regulate expression of *tgas118*, however it is more likely that there is some complex regulatory interplay between anthocyanin biosynthesis, defensins, ROS and tomato stress responses.

111. Some defensins are known to be allergens, including those from peanut, celery, soybean and horse chestnut, and some allergenic proteins have a defensin domain, known as a defensin-polyproline–linked protein or DPLP, particularly plants from the *Artemisia* genus (Cosi and Gadermaier, 2023). TGAS118 is not known to be allergenic (Allergen Online database, WHO/IUIS Allergen Nomenclature database, accessed 22 July 2025).

112. Defensins from other plants have been reported to have toxicity in mammalian cells *in vitro*, including a defensin from the shrub *Pyrularia pubera* which displayed toxicity in cultured mouse cells (Vernon et al., 1985), a defensin from barley that inhibited translation in cell free systems derived from mammalian cells (Mendez et al., 1990) and the TPP3 defensin from tomato that is cytolytic to a human lymphoma cell lines (Baxter et al., 2015). No information about the potential toxicity of TGAS118 could be found in the literature.

113. Defensins are relatively abundant in seeds, which are vulnerable to fungal infection during germination (reviewed in Stotz et al., 2009b). For example, multiple types of defensins are strongly upregulated in wheat seeds, while having low expression throughout the rest of the plant (Shi et al., 2024).

114. Due to their anti-fungal anticity, plant defensins have been investigated *in vitro* for the treatment of a number of human fungal conditions, including defensin def1 from the plant *Picramnia pentandra* for the treatment of fungal nail infections (van der Weerden et al., 2023), and over 20 different plant defensins, including those from peas, rice, Indian mustard and corn, for the treatment of candidiasis (reviewed in Finkina et al., 2024). Two clinical trials have been conducted in Australia to test plant defensin HXP124 in fungal nail infections (Australian New Zealand Clinical Trials Registry, accessed 8 August 2025).

4.5.6 Compositional analysis of the GM Purple Tomato fruit

115. Compositional analysis of tomatoes from the Del/Ros1-N variety in MoneyMaker was conducted and compared to that of non-GM MoneyMaker tomatoes (Table 4). Approximately 1 kg of ripe fruit (10 fruits) was harvested from each of 5 Del/Ros1-N in MoneyMaker plants and 5 non-GM control MoneyMaker plants, then combined into 5 independent samples for each of the 2 varieties. Following the guidelines outlined in the *OECD Consensus Document on Compositional Considerations for New Varieties of Tomato* (OECD, 2008), the tomatoes were analysed for a number of nutrients and non-nutrients, including:

- proximates – protein, fat, total carbohydrates, fibre, ash
- minerals – magnesium and potassium
- carotenoids – β-carotene and lycopene
- vitamins – C, K and folate (B9).

Table 4. Nutrient composition of various tomato varieties

Analyte	unit	Non-GM red tomato fruit	GM Purple Tomato fruit	USDA (avg)	USDA (min)[a]	USDA (max)	McCance and Widdowson[b]
Moisture	g/100 g	94.72 ± 0.12	95.1 ± 0.12	94.52	92.7	95.73	94.6
Crude protein	g/100 g	0.64 ± 0.05	0.70 ± 0.09	0.88	0.59	1.06	0.5
Ash	g/100 g	0.32 ± 0.02	0.48 ± 0.02	0.50	0.37	0.60	n/a
Carbohydrates	g/100 g	3.06 ± 0.29	3.26 ± 0.27	3.89	n/a	n/a	3
Fructose	g/100 g	1.46 ± 0.05	1.22 ± 0.09	1.37	1.1	2.32	1.6
Galactose	g/100 g	<0.1 ± 0	0.1 ± 0	0.00	0.00	0.00	0.00
Glucose	g/100 g	1.26 ± 0.02	1.04 ± 0.08	1.25	0.49	2.67	1.4
Lactose	g/100 g	<0.1 ± 0	0.1 ± 0	0.00	0.00	0.00	0.00
Maltose	g/100 g	<0.1 ± 0	0.1 ± 0	0.00	0.00	0.00	0.00
Sucrose	g/100 g	<0.1 ± 0	0.1 ± 0	0.00	0.00	0.02	0.00
Total sugar	g/100 g	2.72 ± 0.06	2.24 ± 0.17	2.63	1.59	5.01	3.00
Total fibre [c]	g/100 g	1.1 ± 0.2	0.7 ± 0.1	1.2	0.7	2	1
Energy in kilocalories	kcal/100 g	18.4 ± 0.83	16.8 ± 0.77	18	n/a	n/a	4
Energy in kilojoules	kJ/100 g	81.8 ± 1.78	71 ± 2.95	74	n/a	n/a	61
Total fat	g/100 g	<0.3 ± 0.04	<0.3 ± 0	0.2	0.07	0.80	0.10
Salt	g/100 g	<0.025 ± 0	<0.025 ± 0	n/a	n/a	n/a	n/a
Monounsaturated FAs	g/100 g	<0.1 ± 0	<0.1 ± 0	0.031	n/a	n/a	0.03
Polyunsaturated FAs	g/100 g	<0.1 ± 0	<0.1 ± 0	0.083	n/a	n/a	0.05

Analyte	unit	Non-GM red tomato fruit	GM Purple Tomato fruit	USDA (avg)	USDA (min)[a]	USDA (max)	McCance and Widdowson[b]
Saturated FAs	g/100 g	<0.1 ± 0	<0.1 ± 0	0.028	n/a	n/a	0.03
Trans FAs	g/100 g	<0.1 ± 0	<0.1 ± 0	n/a	n/a	n/a	0
Magnesium	g/100 g	0.01 ± 0.00	0.01 ± 0.00	0.011	0.007	0.015	0.008
Potassium	g/100 g	0.16 ± 0.00	0.23 ± 0.01	0.237	0.144	0.385	0.0223
Sodium	g/100 g	<0.01 ± 0	0.01 ± 0.00	0.005	0.001	0.024	0.002
beta carotene	µg/100 g	451.6 ± 42.4	661.8 ± 64.8	449	184	572	349
Folate (vitamin B9)	µg/100 g	8.92 ± 0.28	14.22 ± 0.31	13.7	7.8	19.8	23
Ascorbate (vitamin C)	mg/100 g	6.86 ± 0.18	8.1 ± 0.86	15	1	36	22
Phylloquinone (vitamin K1)	µg/100 g	2.69 ± 0.14	1.99 ± 0.1	7.9	2.2	60	6
Lycopene	mg/kg	73.8*	59.9*	25.73	11.36	34.19	n/a

Source: * lycopene concentrations are unpublished information supplied by applicant, remaining data is from Martin and Butelli (2025).[a] Average and min/max values from USDA Food Composition Databases Show Foods - Tomatoes, Red, Ripe, Raw, Year-round; [b] McCance and Widdowson: The Composition of Foods Integrated Dataset 2019; [c] as determined using methods approved by the Association of Official Analytical Chemists. FAs = fatty acids.

116. The composition of the GM Purple Tomatoes is similar to the non-GM MoneyMaker control. Martin and Butelli (2025) noted that there was <25% difference compared to the non-GM control in most components measured, with folate (see table) and α-tomatine (see below) showing >25% difference to the non-GM control. The lycopene values for both the GM Purple Tomato and the non-GM MoneyMaker control are much higher than the USDA ranges shown in Table 4, but it appears this range is relatively low and narrow compared to the literature which includes ranges such as 8.8–77.4 mg lycopene/kg fresh weight (reviewed in Story et al., 2010).

117. As discussed in Section 3.4.1, plants in the Solanaceous family produce a number of alkaloids, with tomatine being the main alkaloid found in tomatoes. α-tomatine levels were measured in freeze-dried Del/Ros1-N in MoneyMaker tomatoes and MoneyMaker non-GM control. Del/Ros1-N in MoneyMaker tomatoes had more than double the levels of α-tomatine compared to the control (measured at 88 mg/kg vs 36.8 mg/kg dry sample, converted to 5.17 mg/kg vs 2.18 mg/kg fresh weight) but were well within the typical reported range for tomato varieties of negligible to 23 mg/kg fresh weight (OECD, 2008). The applicant has stated that increased tomatine levels may be a result of the slower ripening of the GMO (discussed further in Section 4.5.7), as tomatine levels decrease during ripening.

118. Compositional analysis was not conducted on the GMO in the MoneyMaker x Goldkrone background.

119. FSANZ is currently assessing the safety of the GM Purple Tomato and its products as food for human consumption under application A1333. As part of the assessment, FSANZ will examine the nutritional composition of the GM Purple Tomato.

4.5.7 Phenotypic characterisation

120. Phenotypic characteristics of the GMO from a greenhouse trial are shown in Table 5.

Table 5. Phenotypic characteristics of the GM MoneyMaker x Goldkrone variety

Seedling Characteristics	Seedlings (2-15 cm) have anthocyanin pigmentation on the hypocotyl at 3-5 weeks post sowing.
Plant Habit & Growth	Plants have an indeterminate, moderately sprawling growth habit with intermediate branching with 2-3 nodes between inflorescences along the length of the main stem.

	Mature plants can reach 300 cm or taller depending on pruning and season length.
Leaf & Flower Morphology	Mature plants have 'potato-type,' smooth (non-rugose) and bipinnate leaves. Flowers are yellow, non-fasciated, and remain fused in anther cone at anthesis.
Fruit & truss characteristics	Fruit is a round, 'Cherry' type tomato; individual fruit weight ranges from 10-18g (average 14.4 +/- 4.2g) and average fruit size approximately 33 mm at its widest point. As the fruit matures, fruit colour transitions from green to chocolate brown (late breaker stage) to a dark purple or 'black' when ripe. Ripe fruit have a dark purple skin and a purple/violet flesh and gel. Fruit can be harvested on the vine or loose as fruit readily detaches from the calyx without splitting or cracking at the fruit abscission zone. Truss architecture tends to be moderately branched with one or more forks per truss.

121. The GM Purple Tomato seedlings display anthocyanin pigmentation on the hypocotyl at 4-5 weeks post sowing (Table 5), indicating anthocyanin production in the vegetative tissues is functioning similar to non-GM tomatoes which also produce anthocyanins in the hypocotyl (as discussed in Section 4.1.1.2). The applicant has stated that the purple phenotype has not been observed in the mature plants. Flowers are a normal yellow colour, and the colour of the developing fruit is green before changing during ripening to a brown then dark purple colour. Together, these observations indicate that the E8 promoter is driving expression of the *Rosea1* and *Delila* genes, and therefore increasing anthocyanins, only in the fruit during ripening and not in other tissues or at other times. As discussed in Section 4.1.3, the E8 promoter responds to the presence of ethylene, which is produced during fruit ripening.

122. Purple fruit from the GMO in the MicroTom background have a similar size, shape, and number of seeds compared to non-GM MicroTom (Zhang et al., 2013).

123. The GM Purple Tomato fruit in the MicroTom background displayed delayed ripening after breaker stage compared to red fruit (Zhang et al., 2013). A statement on the Norfolk Health Produce website – Tips for Growing Purple Tomatoes (accessed 16 July 2025) says “Ready for harvest when fruit begins to feel soft and readily releases from the stem. Be patient - purple color develops before fruit is fully ripe.” It is well established that levels of ethylene increase rapidly in tomato fruit during the breaker stage, which is an early stage of fruit ripening (Huang et al., 2022). In the GMO, expression of *Delila* and *Rosea1* is controlled by the E8 promoter which responds to the presence of ethylene (Section 4.1.3). This may explain why anthocyanins, and therefore purple colouring, accumulate before fruit is fully ripe. In contrast, accumulation of anthocyanins in the non-GM Indigo Rose tomato is induced by light (Sun et al., 2020) and inhibited by ethylene (Xu et al., 2022).

124. The shelf life of the GMO in the MicroTom background was compared to the MicroTom control after harvesting at 14 days post breaker stage, with fruit softening during late stage ripening and pathogen susceptibility being important factors for shelf life (Zhang et al., 2013). The GM Purple Tomatoes displayed reduced softening during late-stage ripening and decreased susceptibility to the fungus *Botrytis cinerea*, resulting in double the shelf life compared to non-GM MicroTom tomatoes. The delay in late-stage fruit softening is thought to be due to the antioxidant effects of the anthocyanins reducing oxidative stress and tissue damage in the GM fruit. Susceptibility to the fungus *B. cinerea* is inversely related to the anthocyanin concentration and thought to be due to the antioxidant activity of the anthocyanins on reactive oxygen species (ROS), therefore reducing the spread of the ROS burst that is important for fungal infection by necrotrophic pathogens like *B. cinerea*. It is noted that the increased expression of a plant defensin (Section

4.5.5) could play a role in the reduced susceptibility of the GM fruit to fungal infection, as plant defensins commonly have activity against fungi including *B. cinerea*, however when agar plates were supplemented with fruit juice from the GM Purple Tomato or non-GM red tomato neither inhibited fungal growth. This indicates that the reduced susceptibility of the GM Purple Tomato fruit to fungal infection is likely the result of other components of the GM fruit such as anthocyanins modulating living cell stress responses rather than defensins directly killing the fungus.

4.5.8 Mouse feeding study

125. In order to assess any benefits of the increased anthocyanin production in the GM Purple Tomato, a survival study was conducted in cancer-prone p53 knockout mice, which have an average life expectancy of approximately 140 days (Butelli et al., 2008). From weaning (approximately 14 days old), mice were fed either standard diet mouse pellets, pellets supplemented with 10% red tomato powder or pellets supplemented with 10% Purple Tomato powder. Mice fed pellets supplemented with GM Purple Tomato powder had an increased average lifespan (average of 182.2 days survival and maximum of 260 days, n=20), compared to those fed pellets supplemented with non-GM red tomato powder (average 145.9 days, maximum 213 days, n=15) and standard pellets (average 142 days, maximum 211 days, n=24). Benefits are not in the scope of the Gene Technology legislation and will not be discussed further. Although not a toxicological evaluation, considering the increase in survival of the GM Purple Tomato-supplemented mice, this study provides some indication that mice fed a diet supplemented with GM Purple Tomato powder did not experience any observed toxicity or increased mortality following chronic exposure.

Section 5 The receiving environment

126. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. Relevant information about the receiving environment includes abiotic and biotic interactions of the crop with the environment where the release would occur; agronomic practices for the crop; presence of plants that are sexually compatible with the GMOs; and background presence of the gene(s) used in the genetic modification (OGTR, 2013).

127. The applicant has proposed to commercially grow the GM Purple Tomato. As discussed further in Section 5.3.2, individuals may also grow the tomatoes. Therefore, for this licence application, it is considered that the receiving environment is all of Australia. Relevant information about the receiving environment in Australia is presented below.

5.1 Relevant abiotic factors

128. In line with the warm climates from which tomatoes originated, tomato plants have an optimal growth temperature of approximately 24 to 26°C during the day and 15 to 17°C at night (Li et al., 2023). Tomato plants are frost sensitive and can become stressed at temperatures between 0 to 12°C (Liu et al., 2012), although gradual acclimation to lower temperatures can reduce cold stress (Barrero-Gil et al., 2016; Mesa et al., 2022). Higher temperatures (above 30°C) also negatively impact tomato plants, inducing heat stress and reducing pollination and fruit set (Pressman et al., 2002; Sato et al., 2001). Tomato fruit are also sensitive to temperature, with temperature extremes affecting fruit ripening (Adams, 2001). A temperature range of 15 to 35°C is required for seed germination with temperatures between 20 to 30°C optimal (Fullelove et al., 1998).

129. Tomato plants are prone to waterlogging (Umicevic et al., 2024). Excess moisture also makes tomatoes susceptible to disease, particularly through contact with wet soil and soil-borne pathogens. In climates with high rainfall it is recommended to trellis tomatoes to reduce contact of the plant with wet soil and/or avoid growing during the rainy season (Fullelove et al., 1998).

130. Considering these abiotic factors, in Australia tomatoes are usually grown throughout the cooler, drier conditions in autumn, winter and spring in warm climates to avoid excessive heat and moisture, and during spring and summer in colder climates to avoid frost (Fullelove et al., 1998).

5.2 Relevant biotic factors

131. Tomatoes are susceptible to a number of pests and diseases.

132. Common types of diseases include (McDougall et al., 2013):

- **fungal and fungal-like**: late blight (*Phytophthora infestans*), powdery mildew (*Leveillula taurica*, *Oidium lycopersici, O. neolycopersici*), grey mold (*Botrytis cinerea*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *lycopersici*), *Verticillium* wilt (*Verticillium dahliae*)
- **bacterial**: bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*), bacterial spot (*Xanthomonas euvesicatoria*, *X. vesicatoria*, *X. perforans*, *X. gardneri*), bacterial wilt (*Ralstonia solanacearum*)
- **viral:** tomato leaf curl (Begomoviruses, including tomato leaf curl virus), alfalfa mosaic (alfalfa mosaic virus), fern leaf (cucumber mosaic virus), tobacco mosaic (tobacco mosaic virus), tomato brown rugose fruit virus (ToBRFV)
- **other**: root knot nematode (*Meloidogyne* spp).

133. Soil-borne diseases like *Fusarium* have been shown to have significant impacts on the Australian tomato industry, with crop rotation, soil sterilisation and soil fumigation being some of the control measures (Ma et al., 2023). Imported tomato seeds also present a disease risk to the Australian tomato industry (Constable et al., 2019). ToBRFV is a highly contagious virus of tomatoes, chillies and capsicums that was detected in Australia in 2024 and has been recently declared not feasible to eradicate (Australian Government Outbreak website, accessed 4 August 2025). ToBRFV can reduce the commercial yield of tomatoes by 75% (PIRSA website, accessed 4 August 2025).

134. Common insect and mite pests include mites (e.g. Tomato russet mite *Aculops lycopersici*), thrips (e.g. tomato thrips *Frankliniella schultzei*), aphids (e.g. *Macrosiphum euphorbiae*), and potato moth (*Phthorimaea operculella*) (McDougall et al., 2013).

5.3 Relevant cultivation practices in Australia

135. It is anticipated that the cultivation practices for the proposed release will not differ from the standard practices used for current commercial non-GM tomatoes or for growing by home gardeners.

5.3.1 Commercial tomato production in Australia

136. The applicant has stated that the aim of this commercial release is to enable commercial greenhouse production of the GM Purple Tomato for the fresh market. The applicant has also stated that the GM Purple Tomatoes may be used to make processed products such as tomato paste, sauce, and soups.

137. According to the Australian Horticulture Statistics Handbook (Hort Innovation, 2024), 437,596 tonnes (t) of tomatoes were produced in Australia in the year ending June 2024. Only approximately 1000 t (<1%) was exported, with 51% going to the fresh supply market and 49% going to processing. No fresh tomatoes were imported.

138. Tomatoes are grown commercially in most States and Territories in Australia, with the biggest producers being Victoria (59.2%), then South Australia (14.0%), Queensland (11.4%), New South Wales (10.4%), Western Australia (4.4%) and Tasmania (0.5%). With the wide range of climates across Australia, production occurs year-round.

139. The handbook notes that conventional growing in fields is still the predominant production system (71.8% production), however technological improvements have seen in increase in glasshouse production (24.1%), followed by polyhouse and tunnel production (4.1%). Glasshouse production is highest in South Australia (94 hectares (ha)), Victoria (81 ha) and New South Wales (44 ha). Although the applicant has stated that the GM Purple Tomatoes are proposed to be grown in greenhouses, commercial production of the GM Purple Tomatoes in greenhouses as well as in the field will be considered.

140. An industry report by the Australian Processing Tomato Research Council Inc., the Annual Industry Survey 2024, indicates that a small number of specialist growers produce most of the tomatoes for processing across northern Victoria (70% of the entire crop for processing) and southern New South Wales

(remaining 30%) (Australian Processing Tomato Research Council, 2024). This report indicated that 3 organisations processed the entire crop grown on 2,741 ha of the 2023/24 season. Yields were at approximately 80.7 t/ha which is higher than the previous season but below the industry standard of approximately 100 t/ha. Adverse weather conditions have directly caused yield losses as has been observed previously. Two cultivars, H3402 and H1015 constituted 61% of the total area grown for processing tomatoes in 2023/24. The majority of plants were grown to seedling stage and then transplanted (85%) with the remaining 15% being direct sown.

141. In the year ending 2024, Australia imported 155,503 t of preserved tomatoes and 32,208 kilolitres of tomato juice (Hort Innovation, 2024).

142. Tomato pomace, the waste residue from the manufacture of tomato juice, sauce, puree, and paste, may be incorporated into animal feed in small quantities (<30%) as a byproduct energy concentrate (Agriculture Victoria -Unusual Feedstuffs, accessed 9 July 2025). Tomato pomace is approximately 60% seed and 40% peel (Kumar et al., 2022).

5.3.2 Cultivation by home gardeners

143. Although the applicant has not specifically indicated their intention to sell seed packets of the GM Purple Tomato to home gardeners in Australia, a commercial release may involve such sale in the future and seeds are being sold to home gardeners in the US. Tomatoes may also be cultivated from the seeds of fruit purchased commercially. Therefore, cultivation by home gardeners forms part of the risk context for this proposed release.

144. Growing edible produce at home is a common practice in Australian households, with an estimated 45% growing some of their own food (The Australia Institute, 2024). Seed packets and seedlings can be purchased from a number of sources, including plant nurseries and online stores. Tomatoes are one of the most popular choices, with well over 100 varieties available to Australian gardeners (e.g. Seeds of Plenty, Yates, The Seed Collection). Tomatoes can be grown in all climates across Australia. In tropical and subtropical climates, they are best grown in autumn and winter when the weather is drier, in cool or cold climates they are best planted in spring after last frosts to grow over summer (e.g. Yates – How to Grow Tomatoes, accessed 8 July 2025). Home gardeners are encouraged to hand pollinate tomato flowers using an electric toothbrush or paintbrush to boost pollination rates, particularly if growing in a home greenhouse (e.g. The Seed Collection - How to Hand Pollinate Tomatoes: Two Methods to Boost Your Harvest, accessed 1 August 2025).

145. Tomatoes are well known to grow as volunteer plants (self-seeded plants that have not been intentionally planted), either from kitchen waste being used for home compost or from dropped fruit from plants grown in the home garden. Gardeners may keep these volunteer plants as a source of new tomato seedlings (e.g. ABC Gardening, accessed 9 July 2025).

5.4 Presence of related species in the receiving environment

146. As discussed in Sections 5.3.1 – 5.3.2, tomatoes for commercial production are grown in most Australian States and Territories and home gardeners may grow tomatoes across Australia.

147. Tomatoes can hybridise with several wild relatives, including *S. pimpinellifolium, S. chilense*, *S. lycopersicoides*, *S. pennellii*, and *S. neorickii* (Brog et al., 2019; Mes et al., 2008; Sharma et al., 2008). Seeds of *S. pimpinellifolium*, also known as the currant tomato, can be purchased by home gardeners in Australia (e.g. Boondie Seeds, Seeds Station, accessed 9 July 2025) but are not grown commercially. Like domesticated tomato, *S. pimpinellifolium* primarily self-pollinates, but it also displays variation in floral structures which can either promote or reduce outcrossing (Georgiady et al., 2002). In a laboratory setting, *S. pimpinellifolium* has been successfully crossed bidirectionally (both as the female and male in the cross) with S*. lycopersicum* (Sharma et al., 2008). Natural hybrids between *S. lycopersicum* and *S. pimpinellifolium* are probably rare but possible. It is thought that the weedy *S. lycopersicum* var *cerasiforme* found in Peru and Ecudor may be a hybrid of *S. lycopersicum* with *S. pimpinellifolium* (Blanca et al., 2012; Nesbitt and Tanksley, 2002; Ranc et al., 2008). None of the other wild relatives of tomatoes are present in Australia (Altas of Living Australia, accessed 9 July 2025).

148. A number of native *Solanum* species are present in Australia, including bush tomato. In a laboratory setting, *S. orbiculatum*, a species of bush tomato found in the arid areas of Western Australia, South Australia, and the Northern Territory, was able to pollinate *S. lycopersicum* only if *S. orbiculatum* was the male parent, however there was no fruit or seed set due to post-zygotic barriers (Ahmad et al., 2023).

5.5 Presence of the introduced genes and their encoded proteins in the environment

149. All of the introduced genes are isolated from naturally occurring organisms that are already widespread and prevalent in the environment.

150. The *Delila* and *Rosea1* genes are derived from garden snapdragon (*A. majus)*. *A. majus* is a flowering plant that is native to the Mediterranean and has been bred to have flowers in a variety of colours and double flowered forms with increased petals (reviewed in Schwarz-Sommer et al., 2003). *A. majus* is grown throughout Australia for its ornamental qualities, with seeds or plants available from retailers (e.g. Bunnings, accessed 9 July 2025). Snapdragon flowers can also be purchased as part of edible mixes (e.g. Edible Flowers Melbourne, accessed 10 July 2025).

151. The *nptII* gene was isolated from *E. coli*, a common bacterium that is widespread in human and animal digestive systems and in the environment in Australia (reviewed in Jang et al., 2017). As such, it is expected that humans, animals and microorganisms routinely encounter the encoded protein.

5.6 Use of kanamycin, neomycin and related antibiotics in veterinary and human medicine

152. The *nptII* gene in the GM Purple Tomato encodes an enzyme that inactivates certain aminoglycoside antibiotics, including kanamycin, neomycin, paromomycin, ribostamycin, lividomycin, butirosin, gentamicin and isepamicin (Shaw et al., 1993). Of these antibiotics, only neomycin and gentamicin are approved for use in veterinary and human medicine in Australia (Table 6) so will be discussed further.

Table 6. Registration of veterinary and human medicines containing kanamycin and related antibiotics in Australia

Antibiotic	Registered as a veterinary medicine in Australia? [a]	Registered as a human therapeutic in Australia? [b]
Kanamycin	No	No
Neomycin	Yes	Yes
Paromomycin	No	No
Ribostamycin	No	No
Lividomycin	No	No
Butirosin	No	No
Gentamicin	Yes	Yes
Isepamicin	No	No
Geneticin	No	No

a. APVMA PubCRIS database, accessed 7 August 2025, b. Australian Register of Therapeutic Goods (ARTG), accessed 7 August 2025.

153. Aminoglycoside antibiotics like gentamicin and neomycin are mainly used to treat infections with aerobic gram-negative bacteria in both humans and animals. The World Organisation for Animal Health (OIE) lists aminoglycoside antibiotics, including gentamicin and neomycin, as Veterinary Critically Important

Antimicrobial Agents, as they are effective in a wide range of applications and noted that gentamicin is used in *Pseudomonas aeruginosa* infections with few available alternatives (OIE, 2021). The World Health Organization List of Medically Important Antimicrobials lists aminoglycosides, including neomycin and gentamicin, as Critically Important Antimicrobials (CIA), the second highest category (WHO, 2024). In the Australian context, the Australian Strategic and Technical Advisory Group on Antimicrobial Resistance (ASTAG) has listed neomycin individually as Low Importance in Australia (i.e. there are a reasonable number of alternative antibacterials in different classes available to treat or prevent most human infections even if antibacterial resistance develops) and gentamicin as Medium Importance (i.e. there are some alternative antibacterials in different classes available to treat or prevent human infections, but less than for those rated as Low Importance) (ASTAG, 2018).

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

6.1.1 Approvals by the Regulator

154. The GM Purple Tomato has not previously been approved for release in Australia.

155. There have been a number of previous approvals for commercial release of flowers with altered pigmentation via increased anthocyanin production (Table 7).

Table 7. Previous licences issued by the Regulator for the commercial release of GM flowers with altered flower colour and increased anthocyanins

Licence number	Project title	Licence holder	Licence status
DIR 030	Ongoing commercial release of colour modified carnations	Florigene Pty Ltd	Licence subsequently ceased and a determination issued by the Regulator as GMO Register Reg-001.
DIR 090	Commercial release of rose genetically modified for altered flower colour	Florigene Pty Ltd	Surrendered
DIR 134	Commercial import and distribution of genetically modified carnation cut-flowers with altered flower colour	International Flower Developments Pty Ltd	Licence subsequently ceased and a determination issued by the Regulator as GMO Register Reg-002.
DIR 191	Commercial import and distribution of chrysanthemum genetically modified for altered flower colour	International Flower Developments Pty Ltd	Current

156. Details of all GMOs approved by the Regulator for commercial release in Australia under a licence are available from the OGTR website. Dealings with GMOs authorised by the Regulator as safe for anyone to grow in Australia without a licence are listed on the GMO Register.

6.1.2 Approvals by other government agencies

157. The GM Purple Tomato has not previously been approved by any other government agencies in Australia.

158. FSANZ is currently assessing the food safety of the GM Purple Tomato and its products as food for human consumption as application A1333. More information is available on the FSANZ website. The application is being assessed under the Health Canada-FSANZ Shared Assessment Process.

6.2 International approvals

159. In 2022, the US Department of Agriculture Animal and Plant Health Inspection Service (APHIS) deemed the GM Purple Tomato to not be a regulated article as there was no plausible pathway identified by which the GMO would pose an increased plant pest risk compared to non-GM tomato (Martin and

Butelli, 2025; USDA, 2022). Seed has been sold to home gardeners since 2024. The applicant has stated that over 13,000 seed packets were sold to home gardeners in 2024.

160. The US Food and Drug Administration authorised the GM Purple Tomato as food in 2023. In 2024, commercially produced fruit began to be sold in US grocery stores. The applicant has stated that commercial cultivation of over 8000 GM Purple Tomato plants occurred at a single facility in Virginia and approximately 29 tonnes of fruit has been sold in hundreds of retail grocery stores. Some cultivation also occurred by regional growers to supply local farmers markets.

161. The 2025 release in the US is still ongoing. Seeds and seedlings were available to home gardeners in 2025. It is estimated that approximately 60 tonnes of fruit will be sold in retail by the end of the year. As of August 2025, the applicant has stated that no adverse effects or unintended consequences of any kind have been reported from the US commercial release.

Chapter 2 Risk assessment

Section 1 Introduction

162. 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 11). 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.

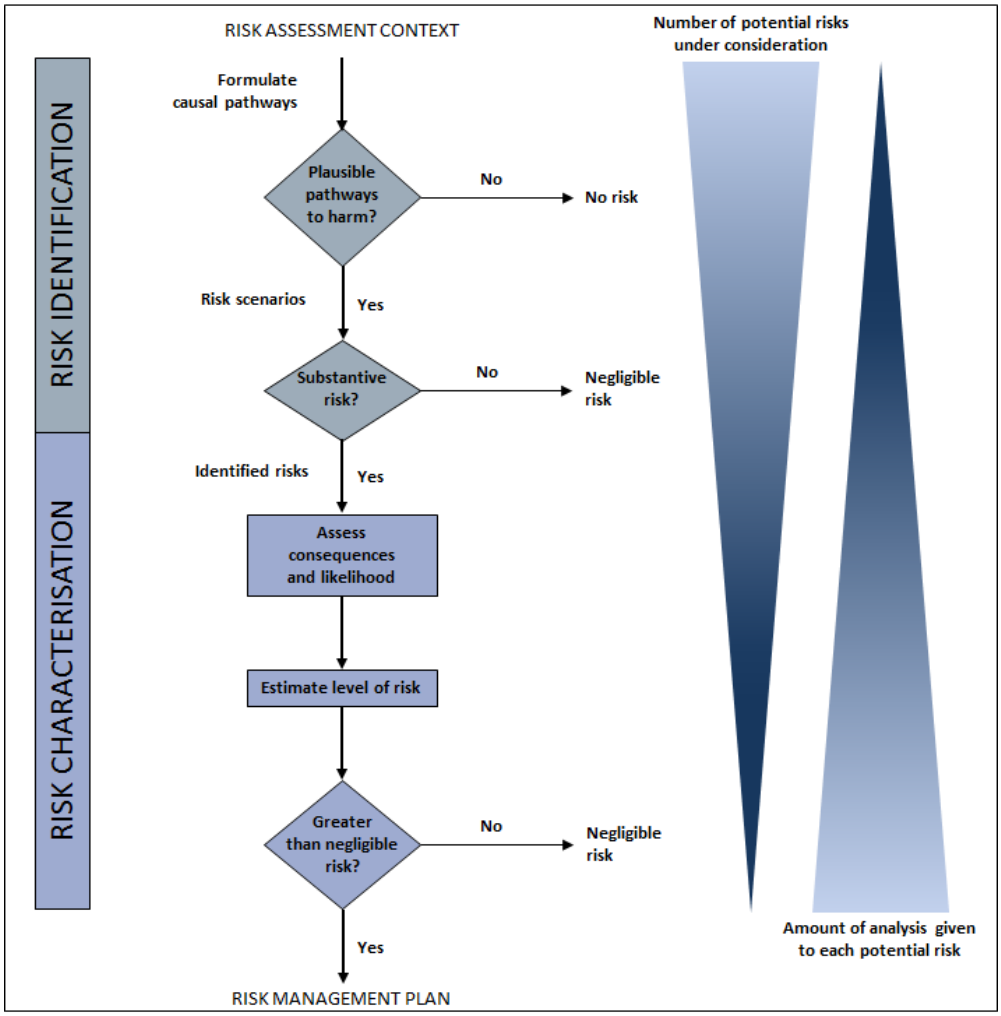

Figure 11. The risk assessment process

163. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, reported international experience and consultation (OGTR, 2013). Risk scenarios examined in RARMPs prepared for licence applications for the same or similar GMOs, are also considered.

164. 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. These risk scenarios are screened to identify those that are considered to have a reasonable chance of causing harm in the short or long term. Pathways that do not lead to harm, or those that could not plausibly occur, do not advance in the risk assessment process (Figure 11), that is, the risk is considered to be no greater than negligible.

165. Risks identified as being potentially greater than negligible are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood

assessment). Risk evaluation then combines the Consequence and Likelihood assessments to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

166. A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants, as this approach addresses the full range of potential adverse outcomes associated with plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are used to postulate risk scenarios (Keese et al., 2014). Risk scenarios postulated in previous RARMPs prepared for licence applications for the same or similar GMOs, are also considered.

Section 2 Risk identification

167. Postulated risk scenarios are comprised of three components (Figure 12):

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.

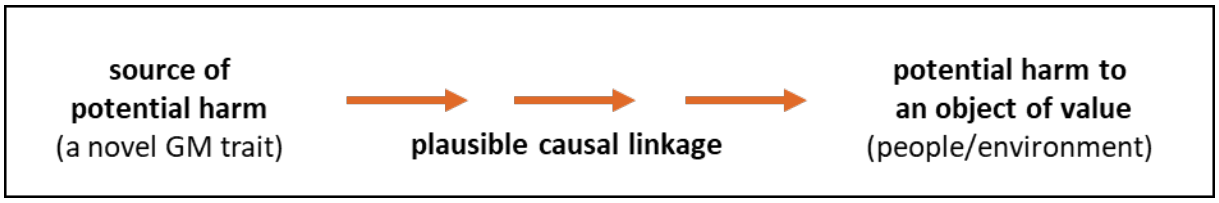

Figure 12. Risk scenario

168. 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 GMOs
- the characteristics of the parent organism(s).

2.1 Risk source

169. 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.

170. As discussed in Chapter 1, the GM Purple Tomato has been modified by the introduction of 2 genes from *Antirrhinum majus* intended to activate anthocyanin production during tomato fruit ripening and the *nptII* gene from *E. coli* which was used as a selectable marker during development of the GMO. These introduced genes will be considered further as a source of potential harm.

171. The introduced genes are controlled by regulatory sequences. These were originally derived from a plant, a plant virus and a bacterium (Table 2). Regulatory sequences are naturally present in all plants, and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary DNA has no toxicity (Delaney et al., 2018). Hence, potential harms from the regulatory sequences will not be considered further.

172. The genetic modifications involving introduction of genes have the potential to cause unintended effects in several ways. These include insertional effects such as interruptions, deletions, duplications or rearrangements of the genome, which can lead to altered expression of endogenous genes. There could also be increased metabolic burden due to expression of the introduced proteins, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels

in biochemical pathways. However, these types of effects also occur spontaneously and in plants generated by conventional breeding. Accepted conventional breeding techniques such as hybridisation, mutagenesis and somaclonal variation can have a much larger impact on the plant genome than genetic engineering (Schnell et al., 2015). While plants generated by conventional breeding have a long history of safe use, and there are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al., 2013), the upregulation of a defensin in the GM Purple Tomato was observed and so this is considered further below.

2.2 Causal pathway

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

- 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 environment at the site(s) of release
- agronomic management practices for the GMOs
- spread and persistence of the GMOs (e.g. reproductive characteristics, dispersal pathways and establishment potential)
- tolerance to abiotic conditions (e.g. climate, soil and rainfall patterns)
- tolerance to biotic stressors (e.g. pest, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organisms
- gene transfer by horizontal gene transfer (HGT)
- unauthorised activities.

174. Although all of these factors are taken into account, some are not included in risk scenarios because they have been considered in previous RARMPs and are not expected to give rise to substantive risks.

175. The potential for horizontal gene transfer (HGT) from GMOs to species that are not sexually compatible, and any possible adverse outcomes, have been reviewed in the literature (Keese, 2008; Philips et al., 2022) and assessed in previous RARMPs. No risk greater than negligible was identified, due to the rarity of HGT events and because the gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, HGT of *Delila* and *Rosea1* will not be assessed further. As antibiotic selectable markers are of public interest, HGT of the *nptII* gene will be considered further.

176. 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 harm

177. Potential harms from GM plants are based on those used to assess risk from weeds (Keese et al., 2014; Virtue, 2004) including:

- harm to the health of people or desirable organisms[3], including toxicity/allergenicity
- reduced biodiversity through harm to other organisms or ecosystems
- reduced establishment or yield of desirable plants
- reduced products or services from the land use
- restricted movement of people, animals, vehicles, machinery and/or water
- reduced quality of the biotic environment (e.g. providing food or shelter for pests or pathogens) or abiotic environment (e.g. negative effects on fire regimes, nutrient levels, soil salinity, soil stability or soil water table).

178. Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. A plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.4 Postulated risk scenarios

179. Four risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 8 and are examined in Sections 2.4.1 to 2.4.4.

180. In the context of the activities proposed by the applicant and considering both the short and long term, none of these risk scenarios gave rise to any substantive risks.

[3] Desirable organisms are those that are valued and should be protected, while undesirable organisms cause harm and should be controlled (OGTR, 2013). This is determined by legislation, government policies, national and international guidance material, and widely accepted community norms. Undesirable plants that cause economic, social or environmental harm, or harm to human/animal health, are called weeds. Animals that cause harm are known as pests.

Table 8. Summary of risk scenarios from the proposed dealings with the GM Purple Tomato

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reasons
1	The GM Purple Tomato	Cultivation of the GM Purple Tomato ⬇ Exposure of people and other desirable organisms to products of the introduced genes via ingestion, contact, or inhalation	Increased allergenicity or toxicity in people OR increased toxicity to other desirable organisms	No	• The introduced Ros1 and Del genes are from the edible snapdragon • The introduced proteins and their pigment products are not expected to be toxic or allergenic • The *nptII* gene and its product are present in other GM food and feed plants without causing adverse effects in people and other organisms • Food safety assessment is conducted by FSANZ • Tomato pollen is not wind borne • Expression of Ros1/Del is limited to the ripening fruit.
2	*nptII* gene	Cultivation of the GM Purple Tomato ↙ ↘ Consumption of GM Purple Tomato by humans or other desirable organisms AND Oral treatment with relevant antibiotics ⬇ NPTII protein impacts effectiveness of antibiotic therapy ⬇ Selection for antibiotic resistant bacteria in digestive tract Exposure of bacteria to the *nptII* gene in the gut when GM fruit is consumed by people or animals OR in the soil or aquatic environments when plant or fruit material decomposes ⬇ HGT of the *nptII* gene to gut, soil or aquatic bacteria	Increased antimicrobial resistance	No	• The NPTII protein is rapidly digested and therefore not expected to impact the effectiveness of relevant antibiotics taken orally • HGT of the *nptII* gene from a GM plant to bacteria is considered to be highly unlikely. • The *nptII* gene is already common in naturally-occurring bacteria.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reasons
3	The GM Purple Tomato	Cultivation of the GM Purple Tomato ⬇ Persistence of the GM seeds in cultivated areas or dispersal of the GM Purple Tomato to nature reserves or intensive use areas ⬇ Establishment of population of volunteer GM Purple Tomato in cultivated areas, nature reserves or intensive use areas	Increased allergenicity or toxicity in people or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable agricultural crops OR Reduced establishment or yield of desirable plants in the environment OR Reduced utility or quality of the environment OR Increased reservoir for pests or pathogens	No	• As discussed in Risk Scenario 1, no substantive risk was identified for increased toxicity or allergenicity. • The spread and persistence of tomatoes is restricted by a range of biotic and abiotic factors, which are not expected to be altered by the introduced genes. • GM volunteers can be controlled by standard weed management measures.
4	The GM Purple Tomato	Cultivation of the GM Purple Tomato ⬇ Outcrossing with sexually compatible plants ⬇ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment	As per Risk Scenario 3	No	• As discussed in Risk Scenario 1, no substantive risk was identified for increased toxicity or allergenicity. • As discussed in Risk Scenario 3, the genetic modifications are not expected to alter the persistence or competitiveness of the GMO Purple Tomato.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reasons
					• Tomato is predominantly self-pollinating and has limited ability to outcross • If a GM hybrid emerged, the purple fruit trait is inherited in a dominant manner and is easily visually identified. • GM hybrids can be easily controlled.

2.4.1 Risk Scenario 1

Risk source	The GM Purple Tomato
Causal pathway	⬇ Cultivation of the GM Purple Tomato ⬇ Exposure of people and other desirable organisms to products of the introduced genes via ingestion, contact, or inhalation ⬇
Potential harm	Increased allergenicity or toxicity in people OR increased toxicity to other desirable organisms

2.4.1.1 Risk source

181. The source of potential harm for this postulated risk scenario is the GM Purple Tomato.

2.4.1.2 Causal pathway

182. The applicant has stated that the GM Purple Tomato would be grown in commercial greenhouses in Australia. Although the applicant has not specifically indicated their intention to sell seed packets of the GM Purple Tomato to home gardeners in Australia, a commercial release may involve such sale in the future and GM tomatoes may also be cultivated by home gardeners from fruit purchased commercially.

183. The GM Purple Tomato fruit would enter general commerce and be used in the same way as non-GM tomato. The general public could be exposed to the fruit and other products derived from the fruit containing the introduced genetic changes and resulting proteins through contact and consumption. Workers in the greenhouse or home gardeners could be exposed to the GM Purple Tomato plants and pollen through inhalation or dermal contact. As tomato pollen is not wind borne, exposure to pollen is expected to be unlikely unless hand pollination is being conducted.

184. Desirable native and non-native organisms, such as birds, bats, possums, flying insects, and ground- or soil-dwelling species (e.g. earthworms, snakes or native rodents) could enter areas where the GM Purple tomato is being cultivated, whether commercially or in home gardens. These organisms could then feed on the fruit or other parts of the GM tomato plants or could come into contact with or consume GM plant material that falls to the ground or is left to decompose on the ground. Pollinators such as bees would be exposed to pollen from the GM Purple Tomato. Livestock may consume tomato pomace derived from processed GM Purple Tomato fruit. Pets, such as dogs, may consume the tomato fruit in the home or garden. Therefore, desirable organisms would be exposed to the GM tomato plants and fruit.

185. As fruit colour is important for attracting fruit eating animals, the purple colouration in the GM Purple Tomato may be more attractive to some animals than red, orange or yellow colouring in non-GM tomatoes. A study of fruit eating preferences in birds in Papua New Guinea found that red and purple artificial fruits were attacked at similar rates, and were strongly preferred over green fruit (Hazell et al., 2023). A different study in Sweden and Australia found that of the fruits commonly dispersed by birds, red fruit was the easiest for birds to detect compared to the background and black (dark purple or dark blue) was the least detectable (Tedore et al., 2022). A study of fruit colour preferences in Madagascar and Uganda found that fruits dispersed by birds tend to be reflect more in the red spectrum and fruits dispersed by mammals tend to reflect more in the green spectrum, with no significant difference between birds and mammals in the blue spectrum (Valenta et al., 2018). A study of New Zealand brushtail possums found no colour preference in fruit eating (Cowan, 1992). Therefore, it is possible that some animal species may find the GM Purple Tomato more attractive, while others may not, and some may not be able to distinguish a difference in colouration.

186. Regarding exposure of desirable organisms to anthocyanins, there are non-GM tomatoes with purple skin (Chapter 1 Section 4.1.1.1) and there are many other anthocyanin-rich fruits and vegetables (Chapter 1 Section 4.4).

2.4.1.3 Potential harm

187. If humans and other desirable organisms were exposed to the GMO, the potential harms are increased toxicity or allergenicity to humans or increased toxicity to other desirable organisms. Allergic reactions are an adverse effect resulting from sensitisation to a chemical, followed by an allergic response upon subsequent exposure (Klaassen and Watkins, 2010). Allergenicity is the potential for a substance to be recognised by a person's body as a foreign and to elicit a (disproportionate) immunological reaction. Toxicity is an adverse effect of exposure to a substance (Klaassen and Watkins, 2010). The effect of a toxic agent depends on the dose, duration of exposure and exposure route, e.g. inhalation, ingestion or via the skin. Responses may be either immediate or delayed.

188. The expression of *Rosea1* and *Delila* is under the control of the fruit ripening specific E8 tomato promoter (Chapter 1, Section 4.1.3). This means that expression of these transcription factors, and increase in anthocyanins, is limited to the ripening fruit and not throughout the GM Purple Tomato plant. Exposure of organisms to the non-fruit parts of the GM Purple Tomato plant, including pollen, green tissues and roots, is expected to be equivalent to exposure to a non-GM tomato. Therefore, this section will focus on potential harms from expression of *Delila* and *Rosea1* only in the GM Purple Tomato fruit. The expression of *nptII* is under the control of a constitutive promoter, so is expected to be expressed throughout the plant.

189. As discussed in Chapter 1, Section 6.2, the GM Purple Tomato has been commercially released in the United States since 2024, both as fruit sold in grocery stores and seeds sold to home gardeners, with no adverse effects reported from this release.

Food safety assessment by FSANZ

190. FSANZ is currently assessing the safety of the GM Purple Tomato and its products as food for human consumption under application A1333. As part of the assessment, FSANZ is examining the following:

- molecular characteristics of the GMO
- nutritional composition of the GM Purple Tomato fruit, including protein and fat, ash and minerals, carbohydrates and fibre, vitamins and carotenoids, and glycoalkaloids (α-tomatine)
- scientific evidence on the safety of Rosea 1, Delia, NPTII, anthocyanins, and chlorogenic acid for human consumption.

191. As these aspects are the regulatory responsibility of FSANZ, they will not be considered in detail in this RARMP as relates to human consumption of the GM Purple Tomato fruit. FSANZ's consultation version of the A1333 assessment notes that no public health and safety concerns were identified in the assessment of the GM Purple Tomato as food. Based on the data provided in the application and other available information, FSANZ have stated that food derived from GM Purple Tomato varieties is as safe for human consumption as food derived from conventional non-GM tomato varieties.

192. In coming to this conclusion FSANZ noted that:

- the Rosea1 and Delila proteins are unlikely to be toxic or allergenic to humans as:
    - *Rosea1* and *Delila* are derived from edible snapdragon flowers and their encoded proteins are homologous to proteins found in other plants, including commonly consumed foods
    - bioinformatic analysis showed that neither Rosea1 or Delila has any amino acid similarity with known allergens or toxins of relevance to humans
    - both the Rosea1 and Delila proteins are susceptible to digestion by pepsin and would be thoroughly degraded following ingestion.
- an extensive database demonstrating the safety of NPTII exists. Updated bioinformatic analyses provided for this application confirmed that the expressed protein is unlikely to be toxic or allergenic to humans
- regarding compositional analysis, there are no biologically meaningful differences in the levels of key constituents in fruit from the GM Purple Tomato compared to non-GM tomato varieties available on the market

- regarding nutritional impact, anthocyanins and associated metabolites are increased in the GM Purple Tomato fruit but within the natural rage of variation for anthocyanins in commonly consumed foods.

193. These conclusions align with the information presented in Chapter 1, Section 4 of this RARMP.

Potential for increased toxicity from exposure of other desirable organisms to the GM fruit

194. The Delila and Rosea1 proteins are not expected to be toxic to desirable organisms for the same reasons they are not expected to be toxic to humans.

195. Anthocyanins are found in a wide variety of vegetative tissues, fruits, and flowers. The anthocyanin content of the GM Purple Tomato fruit is within the range of commonly grown and consumed foods (Chapter 1 Section 4.4). There are also non-GM tomatoes with purple skin from increased anthocyanins (Chapter 1 Section 4.1.1.1). Therefore, it is expected that desirable organisms would already be exposed to anthocyanins from other sources in the environment and exposure to the increased anthocyanins in the GM Purple Tomato fruit is not expected to lead to harm.

196. As discussed in Chapter 1 Section 4.5.6, GM Purple Tomatoes in the MoneyMaker background had more than double the levels of α-tomatine compared to the control. However, these levels were well within the typical reported range for other tomato varieties and so are not expected to pose an increased toxicity risk to desirable organisms compared to non-GM tomatoes. As *Delila* and *Rosea1* are under the control of a fruit specific promoter, it is not expected that α-tomatine levels would be significantly altered in the vegetative tissue of the GM Purple Tomato. Therefore, exposure to the vegetative tissue is not expected to pose an increased toxicity risk to desirable organisms, including for ground- or soil-dwelling organisms that would feed on decaying plant tissues.

197. There is no evidence that the *nptII* gene or the encoded protein is toxic (OGTR Risk Assessment document and references therein). GM foods containing the *nptII* gene have been assessed and approved for sale in Australia (FSANZ website, accessed 30 June 2025). Therefore, it is expected that desirable organisms would already be exposed to NPTII from other sources in the environment and exposure to the NPTII protein in the GM Purple Tomato fruit is not expected to lead increased toxicity.

Potential harms from exposure to defensins in the GM fruit

198. Gene expression analysis of the GM Purple Tomato fruit has shown an almost 300-fold increase in expression of a tomato defensin gene *tgas118* compared to the non-GM control (Chapter 1 Section 4.5.4). The applicant has not quantified the amount of defensins in the GM Purple Tomato fruit, but given the increase in gene expression it is expected that levels of TGAS118 would be increased compared to levels in the non-GM control.

199. Plant defensins are peptides that are part of the innate immune system of plants, targeting mostly fungi.

200. *Tga118* is an endogenous tomato gene that is normally expressed in the roots, stems, leaves, flowerers and mature fruit of healthy tomato plants, predominately either in the stem and mature fruit, or in the flowers, depending on the study (Chapter 1 Section 4.5.5).

201. It is noted that extracts of the GM Purple Tomato fruit did not have activity again *B. cinerea* (Zhang et al., 2013), which may indicate that although there is a strong upregulation of the *tgas118* gene, the levels of TGAS118 in the GM Purple Tomato are not sufficient to have anti-fungal activity. Although it is unknown whether TGAS118 has activity against *B. cinerea*, it is noted that the closely related tomato defensin DEF2 has been shown to have activity against that particular fungus (Stotz et al., 2009a).

202. As discussed in Chapter 1 Section 4.5.5, some defensins have been reported as allergenic. However, TGAS118 is not a known tomato allergen (for known tomato allergens, see Chapter 1 Section 3.4.2). Some defensins cause toxicity when applied to mammalian cells *in vitro*. However, this exposure pathway differs from ingestion where proteins are denatured and digested. Also, defensins are expressed in a wide range of plants, including in parts of plants that people consume e.g. wheat seeds. They are also being investigated for clinical anti-fungal applications.

203. Therefore, given that TGAS118 is an endogenous tomato defensin that is already expressed to some degree in tomato fruit and that plant defensins are expressed in many other plant species, it is not expected that the increased expression of the *tgas118* defensin in the GM Purple Tomato will lead to increased toxicity or allergenicity in people or other desirable organisms, however this is an area of some uncertainty. This expectation that upregulation of *tgas118* does not pose a harm has been confirmed by people in the US handling GM plant material and consuming GM Purple Tomato fruit without any reported ill effects (see Chapter 1 Section 6.2).

2.4.1.4 Conclusion

204. Risk Scenario 1 is not identified as a substantive risk for an increase in toxicity or allergenicity in people, or in toxicity in other desirable organisms because the introduced *Rosea1* and *Delila* genes are from the edible snapdragon, the introduced proteins and their pigment products are not expected to be toxic or allergenic, the *nptII* gene and its product are present in other GM food and feed plants without causing adverse effects in people and other organisms, a food safety assessment is being conducted by FSANZ, tomato pollen is not wind borne, and expression of *Rosea1* and *Delila* is limited to the ripening fruit. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk Scenario 2

Risk source	*nptII* gene
Causal pathway	⬇ Cultivation of the GM Purple Tomato ↙ ↘ Consumption of GM Purple Tomato by humans or other desirable organisms AND Oral treatment with relevant antibiotics ⬇ NPTII protein impacts effectiveness of antibiotic therapy ⬇ Selection for antibiotic resistant bacteria in digestive tract ⬇ Exposure of bacteria to the *nptII* gene in the gut when GM fruit is consumed by people or animals OR in the soil or aquatic environments when plant or fruit material decomposes ⬇ HGT of the *nptII* gene to gut, soil or aquatic bacteria ⬇
Potential harm	Increased antimicrobial resistance

2.4.2.1 Risk source

205. The source of potential harm for this postulated risk scenario is the *nptII* gene in the GM Purple Tomato.

2.4.2.2 Causal pathway

Activity of NPTII in mammalian gut on relevant antibiotics

206. As discussed in Risk Scenario 1, people may consume the GM Purple Tomato fruit and other desirable organisms may consume the GM Purple Tomato fruit or vegetative tissues. This part of the scenario focusses on humans and other mammals as these are the species that would receive the relevant antibiotics. As the expression of *nptII* is under the control of a constitutive promoter, it is expected that the NPTII protein would be produced throughout the plant.

207. If the NPTII protein resists digestion and has activity in the digestive tract, it may reduce the effectiveness of any oral antibiotics consumed by the person or animals. If the antibiotic is rendered less effective by NPTII this could increase selective pressure for the development of antibiotic resistance in the gut bacteria.

208. As discussed in Chapter 1 Section 5.6, of the antibiotics that NPTII confers resistance to, only neomycin and gentamicin are approved for use in veterinary and human medicine in Australia. For the NPTII protein in the gut to come into contact with the antibiotics it would need to be orally administered. Aminoglycoside antibiotics have poor absorption in the GI tract and are usually injected or administered topically. Several products containing neomycin are approved for oral administration to animals in Australia, including feed additive powders and oral suspensions (APVMA, 2024). Gentamicin products approved for veterinary use in Australia are predominately injected or used topically (APVMA PubCRIS database, accessed 7 August 2025). All neomycin products approved for human use in Australia are either for topical or ophthalmic use, not taken orally, and the gentamicin products are either used a part of orthopaedic cement or given as an intramuscular or intravenous injection (ARTG, accessed 7 August 2025).

209. NPTII is rapidly inactivated in simulated mammalian gastric juice (Fuchs et al., 1993). Therefore, under normal digestion, it would be expected that the NPTII protein would be degraded before it could inactivate the corresponding antibiotic, negating any possible interference with oral administration of the antibiotic.

Horizontal gene transfer of the nptII gene to microorganisms

210. Horizontal gene transfer (HGT) is the stable acquisition and heritability of genetic material that was not inherited from a parent organism. It should be noted that HGT per se is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome. A recent paper from staff at the OGTR outlines regulatory considerations for horizontal gene transfer from genetically modified plants (Philips et al., 2022). Key considerations from the paper and any newer publications will be discussed here.

211. For HGT to occur from a GM plant to a microorganism in the environment, the following factors need to be considered:

- Are naturally-occurring bacteria likely to come into contact with the genetic material from the GMO?
- Can the genetic material be transferred to naturally-occurring bacteria?
- Is the genetic material already present in the environment?
- Is there a selective advantage for microorganisms to contain the *nptII* gene?

212. As the GM Purple Tomato is proposed for commercial release it is expected that naturally-occurring bacteria could come into contact with the genetic material from the GMO, whether in the digestive system after consumption of GM Purple Tomato fruit or exposure of soil or aquatic microorganisms to decaying GM Purple Tomato plant material.

213. When considering the exposure of microorganisms to the *nptII* gene, it is important to consider the proportion of the introduced DNA in comparison to the total tomato genome as this influences the likelihood of the gene being transfer to a microorganism. The tomato genome is approximately 800 Mbp (Su et al., 2021) while *nptII* is approximately 800 bp (Ghanem, 2011). Therefore, as tomato is a diploid organism (i.e. that has two copies of *nptII*), the *nptII* gene is approximately 0.0002% of the GM Purple Tomato genome.

214. The main methods of HGT to microorganisms are conjugation (direct transfer of DNA between microorganisms), transfer via a phage, and update of naked DNA from the environment (reviewed in Good et al., 2025). Of the possible microorganisms for HGT to occur to from GM plants, bacteria are considered the most likely recipients because they possess several mechanisms facilitating DNA uptake and there are multiple ways that they can have physical proximity with plants and/or their DNA. Scenarios in which bacteria may come into contact with DNA from the GM Purple Tomato include in the gastrointestinal tract of humans and animals, in the soil and in an aquatic environment.

215. Philips et al. (2022) includes an extensive review of the potential for DNA from a GM plant to undergo HGT to bacteria and the findings can be summarised as:

- HGT from GM plants to bacteria has been rarely reported, likely due to the low proportion of introduced DNA in GM plants, the requirement for naked DNA to remain intact long enough for HGT to occur, and the low frequency of HGT from plants to microorganisms.
- Food preparation, cooking and digestion affect the integrity of DNA, so it is unlikely that an intact gene is able to participate in HGT in the digestive tract.
- DNA from GM plants could make its way to the aquatic environment and become available for HGT should the DNA maintain integrity.
- There is no evidence in the published literature of HGT from a GM plant to soil bacteria under field conditions.

216. Since the literature review for that paper, there have been several relevant studies published that discuss HGT to microorganisms. Three studies assessed soil bacteria around GM crops, specifically GM soybean (Oh et al., 2021), GM papaya (Thongrak et al., 2025), and GM maize (Jang et al., 2025). None of these studies found evidence of HGT of the introduced genes in the GMOs into soil bacteria. Regarding the potential for HGT from GM plant material to bacteria in the digestive tract, one new study reported HGT of 2 transgenic antibiotic resistance genes (including *nptII*) from a GM plant diet to gut bacteria in rats (Oraby et al., 2022). There are a number of limitations in this study regarding interpreting the potential for HGT to bacteria, including not testing for the full-length antibiotic resistance genes or confirming if antibiotic resistance genes were integrated into bacterial genomes or plasmids. In a rebuttal submission to Mexico regarding their measures for GM corn, the US included an analysis of the Oraby et al. (2022) paper, noting multiple experimental design issues that made conclusions difficult to interpret, including whether the bacteria in the rats digestive system naturally contained the antibiotic resistance genes (United States of America, 2024). Another study of HGT conducted a phylogenetic analysis of 1163 genomes from bacteria, 147 genomes from vascular plants and 456 genomes from other organisms was used to identify 16 genes that were likely transferred from plants to bacteria. This indicates that, while HGT from plants to bacteria is a very rare event, it is possible on a broader evolutionary scale.

217. Given this information, it is considered that HGT of introduced genes from GM plants to bacteria is theoretically possible, but is highly unlikely to occur.

218. If there is an advantage for microorganisms to contain the *nptII* gene, then this may exert selective pressure in favour of containing the *nptII* gene. Aminoglycoside antibiotics were originally isolated from fungi of either the *Streptomyces* genus (e.g. neomycin) or the *Micromonospora* genus (e.g. gentamicin) (reviewed in Serio et al., 2018). Therefore, it is expected that bacteria would come into contact with these fungi in the environment, but the selective pressure is not expected to be high based on random, localised interactions. Use of aminoglycoside antibiotics in humans and animals is discussed in the next section.

219. While not as prevalent in the environment as some other antibiotic resistance genes including *nptIII*, the *nptII* gene is found in bacteria around the world. In Australia, among 45 *E.coli* genomes sampled from pig faeces, 1 was positive for *nptII* (Messele et al., 2023). A study of soils in 100 fields in Austria where GMOs had never been grown showed 6% of the fields were positive for *nptII* DNA (Woegerbauer et al., 2015). Among paediatric and general hospitals in Iran, 14.4% of gram negative bacteria isolates contained the *nptII* gene, most of these being *Pseudomonas aeruginosa* (Azimi et al., 2022). In another study in Iranian hospitals, *nptII* was found in 61.8% of *P. aeruginosa* isolates (Aghazadeh et al., 2013). In China, of 205 *E. coli* strains collected from 9 different hospitals, 20 strains were positive for *nptII* (Xiao and Hu, 2012). Therefore, the *nptII* gene is already present in naturally-occurring bacteria in the environment and available for HGT to occur.

2.4.2.3 Potential harm

220. The *nptII* gene is a frequently used selectable marker in GM plants, with 150 commercial GM plant events approved in at least one country worldwide (ISAAA GM Approval database, accessed 7 August 2025). In Australia, approvals for the commercial release of GMOs containing *nptII* include GM banana (DIR 199), GM chrysanthemum (DIR 190), and GM cotton (DIR 124). There have been no adverse effects reported from these releases.

221. Antimicrobial resistance is being monitored in Australia, with antibiotic resistance being of particular concern for human and animal health, potentially leading to longer hospital stays for people, higher medical and veterinary costs, and possibly death (Australian Government Antimicrobial Resistance website, accessed 7 August 2025). The *nptII* gene confers resistance to a number of aminoglycoside antibiotics, including neomycin and gentamicin. As discussed in Chapter 1 Section 5.6, aminoglycoside antibiotics are globally recognised as a critically important class of antibiotics. Of the antibiotics that *nptII* confers resistance to, only neomycin and gentamicin are approved for both human and veterinary use in Australia while the others are approved for neither. In Australia, the Australian Strategic and Technical Advisory Group on Antimicrobial Resistance (ASTAG) has listed neomycin individually as Low Importance in Australia and gentamicin as Medium Importance. Therefore, an increase in antibacterial resistance to neomycin or gentamicin is considered to be a potential harm.

222. As discussed in Section 2.4.2.2, the causal pathways which may lead to increased antibacterial resistance are highly unlikely to occur. Therefore, the presence of the *nptII* gene in GM Purple Tomato plants is highly unlikely to lead to any of the potential harms listed above.

2.4.2.4 Conclusion

223. Risk Scenario 2 is not identified as a substantive risk because the NPTII protein is rapidly degraded in the digestive system and HGT of the *nptII* gene from a GM plant to bacteria is considered to be highly unlikely and the gene is already readily available in naturally-occurring bacteria for HGT to occur. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk Scenario 3

Risk source	The GM Purple Tomato
Causal pathway	⬇ Cultivation of the GM Purple Tomato ⬇ Persistence of the GM seeds in cultivated areas or dispersal of the GM Purple Tomato to nature reserves or intensive use areas ⬇ Establishment of population of volunteer GM Purple Tomato in cultivated areas, nature reserves or intensive use areas
Potential harm	⬇ Increased allergenicity or toxicity in people or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable agricultural crops OR Reduced establishment or yield of desirable plants in the environment OR Reduced utility or quality of the environment OR Increased reservoir for pests or pathogens

2.4.3.1 Risk source

224. The source of potential harm for this postulated risk scenario is the GM Purple Tomato.

2.4.3.2 Causal pathway

225. As per Risk Scenario 1, the GM Purple Tomato may be grown intentionally for commercial production or in the home garden.

226. If presence of the introduced genetic changes was to provide the GM Purple Tomato plants with a significant selective advantage over non-GM tomatoes, this may lead to persistence of the GM Purple Tomatoes in areas where they are cultivated (whether commercially or in the home garden). It is noted that

persistence of healthy tomato plants may a desirable outcome from tomato cultivation and persistence during tomato cultivation is not considered an adverse effect. However, if the GM Purple Tomato plants were dispersed outside the area they are cultivated in, and were able to establish and persist in environments, such as conservation and natural environments or intensive use areas, this may give rise to adverse outcomes. This assessment assumes that GM Purple Tomato plants have the potential to be present in all current and potential tomato growing areas in Australia and home gardens due to deliberate planting.

227. If GM tomato seed persisted in agricultural areas or the home garden after harvest and volunteer GM tomato plants emerged, it is not expected that expression of the introduced *Delila* and *Rosea1* genes would result in increased persistence of GM volunteers or reduced ability to control volunteer tomato plants. As discussed in Chapter 1 Sections 3.5, 5.1 and 5.2, tomatoes are sensitive to extremes in abiotic conditions, including temperature and moisture, are susceptible to a variety of fungal, viral and bacterial diseases, are poor competitors with agricultural weeds, and are not considered to be a weed in Australia. Anthocyanins are known to play a role in plant responses to a range of environmental stresses (Chapter 1, Section 4.1.1.1). As the *Delila* and *Rosea1* genes are under the control of a fruit ripening specific promoter and increasing anthocyanin in the fruit only, it is not expected that the GM Purple Tomato plants would have improved stress tolerance and ability to persist in the environment compared to non-GM tomatoes. Increased anthocyanin content is thought to contribute to the decreased susceptibility of the GM fruit to *Botrytis* infection. Again, as the increased anthocyanin production is limited to the ripening GM fruit, it is not expected that the GM Purple tomato plants would have altered susceptibility to pests and diseases.

228. The GM Purple Tomato could be dispersed in the environment by a number of mechanisms, including through consumption of fruit by animals, home composting and disposal of waste from commercial production and processing. It should be noted that as this is an application for commercial release, if approved, dealings with the GM Purple Tomato could occur across Australia and dispersal of the GM Purple Tomato into the environment is not considered an adverse outcome, but may be a step in a chain of events that may lead to an adverse outcome.

229. Producing fruit is an important mechanism by which plants can have their seeds dispersed by animals. While there is limited information available on the potential for animals to disperse tomato seeds, the information that is available indicates that tomato seeds can pass through the digestive system intact and viable (Chapter 1 Section 3.5). As discussed in Risk Scenario 1, there is no strong evidence that the GM Purple Tomato fruit would be more attractive to fruit eating animals than non-GM tomato fruit, so it is expected that GM Purple Tomato seeds would be dispersed by fruit eating animals at a similar rate as non-GM tomatoes.

230. Tomato pomace, a byproduct of commercial processing which is approximately 60% seed and 40% peel, may be used as a livestock feed, composted, or disposed of in landfill. The 2 main processing methods are hot break, where chopped tomatoes are heated to 90-95°C which is used for more viscous products such as tomato paste, and cold break, where chopped tomatoes are processed at ambient temperature or up to 65°C which is used for less viscous products such as tomato juice (reviewed in Shao et al., 2013). While it is likely that these processing methods will significantly affect the viability of the tomato seeds, it is possible that some may still be viable in the resulting pomace.

231. Vegetative material from the GM Purple Tomatoes may be composted or disposed of in landfill. As discussed in Chapter 1 Section 3.2, tomato plants grow readily from cuttings and have a high capacity for the formation of adventitious roots (roots that form at the base of cuttings, stems in contact with soil etc.), so it is possible that new tomato plants may grow from this waste vegetative material.

232. The *nptII* antibiotic resistance gene is under the control of a constitutive promoter and is expected to be expressed throughout the GM Purple Tomato plant. The *nptII* gene is only expected to provide a selective advantage to the GMO plant in the presence of neomycin, kanamycin and structurally related antibiotics. These antibiotics are used for medical and veterinary purposes, and are not used on tomato crops or on other plants.

233. The potential increase in TGAS118 defensin protein concentration in the fruit of the GM Purple Tomato may confer resistance to tomato diseases, such as specific fungal diseases. If the GM Purple Tomato seeds also have increased expression of the defensin they may be able to germinate in the presence of those specific fungal tomato pathogens, however the expression of TGAS118 is normal in other tissues and will not provide the GM Purple Tomato plants with an advantage over non-GM tomato plants.

234. Abiotic conditions, such as temperature, water availability and nutrient availability are the main limiting factors in the development of both GM and non-GM tomato plants. If the GM Purple Tomatoes were to spread into the environment, the purple fruit phenotype is easily identifiable in mature fruiting plants. It is expected that the GM volunteers would be controlled by standard management practice for control of non-GM tomato volunteers, such as uprooting or use of herbicides (Chapter 1, Section 3.5).

2.4.3.3 Potential harm

235. If the GM Purple Tomato remained as a volunteer population in agricultural areas or the home garden after cultivation, or was able to spread and persist outside in the environment, the postulated harms are increased toxicity or allergenicity to people, increased toxicity to other desirable animals, reduced establishment or yield of desirable agricultural crops or other desirable plants in the environment, reduced utility or quality of the environment, and an increased reservoir for pests or pathogens.

236. As discussed in Risk scenario 1, no substantiative risk was identified for increased toxicity or allergenicity of the GMO for people or increased toxicity to other desirable organisms.

237. If the GM Purple Tomato plants were to spread, establish and persist beyond growing areas, this could impact the environment, e.g. it could reduce establishment or yield of desirable agricultural crops; reduce establishment of desirable native vegetation; reduce utility of roadsides, drains, channels and other intensive use areas; or reduce the quality of the biotic environment by providing a reservoir for pathogens or pests.

238. If the GM Purple Tomato is more attractive to pollinators, then they may preferentially go to the GM Purple Tomato, changing their pollination habits at the expense of other desirable plants. Bees are the primary pollinators of tomatoes and are known to show a preference for purple flowers (Reverte et al., 2016), but it is noted that the GM Purple Tomato has the standard yellow flowers seen in non-GM tomatoes (Chapter 1 Section 4.5.7). Therefore, the GM Purple Tomato flowers are not expected to change the behaviours of usual tomato pollinators.

239. As discussed in Section 2.4.3.2, the causal pathways which may lead to increased spread and persistence of the GM Purple Tomato are highly unlikely to occur. Therefore, the presence of the introduced genetic changes in GM Purple Tomato plants is highly unlikely to lead to any of the potential harms listed above.

240. As discussed in Chapter 1, Section 6.2, the GM Purple Tomato has been commercially released in the United States since 2024, both as fruit sold in grocery stores and seeds sold to home gardeners, with no adverse effects reported from this release.

2.4.3.4 Conclusion

241. Risk Scenario 3 is not identified as a substantive risk because tomatoes have limited ability to establish outside cultivation due to abiotic and biotic factors, the introduced genes are not expected to affect the GMO's ability to respond to these limiting factors, and the GM tomato is not expected to be harmful. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk Scenario 4

Risk source	The GM Purple Tomato
Causal pathway	⬇ Cultivation of the GM Purple Tomato ⬇ Outcrossing with sexually compatible plants ⬇ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment
Potential harm	⬇ Increased allergenicity or toxicity in people or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable agricultural crops OR Reduced establishment or yield of desirable plants in the environment OR Reduced utility or quality of the environment OR Increased reservoir for pests or pathogens

2.4.4.1 Risk source

242. The source of potential harm for this postulated risk scenario is the GM Purple Tomato.

2.4.4.2 Causal pathway

243. As per Risk Scenario 1, the GM Purple Tomato may be grown intentionally for commercial production or in the home garden and may cross with sexually compatible species. It should be noted that vertical gene flow per se is not considered an adverse outcome, but may be a step in a chain of events that may lead to an adverse outcome.

244. The GM Purple Tomato is sexually compatible with other varieties of cultivated tomatoes. As discussed in Chapter 1, Section 4.5.1, the GMO has been bred into a number of different tomato backgrounds. The applicant has indicated that while most breeding activities are expected to take place outside Australia, some may take place in Australia. Therefore, the GMO may be intentionally hybridised with non-GM tomato varieties by the applicant.

245. Home gardeners may also intentionally cross the GM Purple Tomato with other tomato varieties (e.g. The Seed Collection – A Home Gardener's Guide to Breeding Your Own Tomatoes, accessed 4 August 2025).

246. As discussed in Chapter 1, Section 3.2, tomatoes are self-compatible and primarily self-pollinating, with this pollination facilitated by physical vibrating the tomato flowers. In a natural setting there are very low levels of outcrossing. It is not expected that the introduced genes would alter the pollen dispersal characteristics of the GM Purple Tomato and no changes to flower morphology have been noted by the applicant (Chapter 1 Section 4.5.7). Therefore, outcrossing between the GM Purple Tomato and non-GM tomato varieties would be expected to occur at similarly low levels as between non-GM tomato varieties.

247. As discussed in Chapter 1, Section 5.4, of the wild tomato varieties that *S. lycopersicum* is sexually compatible with, only *S. pimpinellifolium* (also known as the currant tomato) is present in Australia. It is not cultivated commercially but seed packets can be purchased by home gardeners. While unlikely, it is possible that the GM Purple Tomato could hybridise with *S. pimpellifolium* in a home garden setting if they are both grown simultaneously and close together. Some *S. pimpellifolium* plants display a more exserted stigma which improves outcrossing. In the laboratory, *S. pimpinellifolium* has been successfully crossed bidirectionally (both as the female and male in the cross) with S*. lycopersicum* (Sharma et al., 2008). In

addition, the weedy *S. lycopersicum* var. *cerasiforme* found in Peru and Ecudor may be a hybrid of *S. lycopersicum* with *S. pimpinellifolium* (Blanca et al., 2012; Nesbitt and Tanksley, 2002; Ranc et al., 2008).

248. If unintentional hybridisation was to occur, the insert containing the introduced genes is inherited in a dominant Mendelian manner (Chapter 1 Section 4.5.3), so hybrids with purple fruit could be easily visually identified when fruiting.

2.4.4.3 Potential harm

249. If the GM Purple Tomato were to hybridise with sexually compatible species, including other tomatoes or the wild relative currant tomato, the postulated harms are increased toxicity or allergenicity to people, increased toxicity to other desirable animals, reduced establishment or yield of desirable agricultural crops or other desirable plants in the environment, reduced utility or quality of the environment, and an increased reservoir for pests or pathogens.

250. As discussed in Risk Scenario 1, no substantiative risk was identified for increased toxicity or allergenicity of the GMO for people or increased toxicity to other desirable organisms. Similarly, in hybrids between the GM plants and sexually compatible plants, the same considerations as discussed in Risk Scenario 1 would apply.

251. As discussed in Risk Scenario 3, the GM Purple Tomato is not expected to be more able to spread and persist in the environment than non-GM tomatoes. Similarly, in the event of hybridisation with sexually compatible plants, the introduced genetic modifications are not expected to increase the ability of GM hybrid(s) to spread and persist, or to change their susceptibility to the abiotic and biotic factors that limit the survival of tomatoes in the environment. Also, standard weed management practices for tomato volunteers in agricultural settings would control GM hybrids.

2.4.4.4 Conclusion

252. Risk Scenario 4 is not identified as a substantive risk because tomatoes have limited ability to outcross and any hybrids between the GMO and sexually compatible species are not expected to show increased levels of toxicity or allergenicity, or increased ability to spread and persist in the environment. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

253. Uncertainty is an intrinsic property 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.

254. 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 estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

255. Uncertainty can arise from a lack of experience with the GMO. For this GMO, over 8000 GM Purple Tomato plants have been grown in the US and over 29 tonnes of fruit have been sold in US grocery stores (Chapter 1, Section 6.2). Relevant information from this release have been considered in relevant sections of Chapter 1 of this document and in the risk scenarios. No unintended effects or adverse events have been reported as part of those releases.

256. Although there is some uncertainty about the expression of a defensin gene (see Chapter 1 Section 4.5.5), the upregulation has been assessed as posing negligible risks and, overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.

257. Post release review (PRR) will be also used to address uncertainty regarding future changes to knowledge about the GMO or the receiving environment (Chapter 3, Section 4). PRR is typically required for commercial releases of GMOs, which generally do not have limited duration.

Section 4 Risk evaluation

258. 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.

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

- risk criteria
- level of risk
- uncertainty associated with risk characterisation
- interactions between substantive risks.

260. Four risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. The level of risk for each risk scenario was considered negligible, considering both the short and long term. The principal reasons for these conclusions are summarised in Table 8.

261. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM Purple Tomato into the environment are considered to be 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. Therefore, no additional 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 environment. [4]

[4] 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

262. 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 licence conditions.

263. 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.

264. All licences are subject to 3 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 must also be reported to the Regulator.

265. 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 and to manage risk to people or the environment. 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

266. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed commercial release of the GM Purple Tomato. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 2) and the receiving environment (Chapter 1, Section 6), 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.

Section 3 General risk management

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

- applicant suitability
- testing methodology
- identification of the persons or classes of persons covered by the licence
- reporting structures
- access for the purpose of monitoring for compliance.

3.1 Applicant suitability

268. In making a decision 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 and
- the capacity of the applicant to meet the conditions of the licence.

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

3.2 Testing methodology

270. If a licence were issued, the applicant would be required to provide a method to the Regulator for the reliable detection of the GMO. As part of the application, the applicant supplied appropriate detection methods to detect the DNA insert in the GMO. Therefore, a requirement to provide detection methods is not included in the draft licence conditions.

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

271. If a licence were issued, any person, including the licence holder, could conduct any permitted dealing with the GMO.

3.4 Reporting requirements

272. 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 and
- any unintended effects of the field trial.

273. The licence holder would also be obliged to submit an Annual Report containing any information required by the licence.

274. There are also provisions that would enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 4, below).

3.5 Monitoring for compliance

275. 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.

276. 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 Post release review

277. Paragraph 10 of the Regulations requires the Regulator to consider the short and the long term when assessing risks. The Regulator takes account of the likelihood and impact of an adverse outcome over the foreseeable future and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

278. The Regulator engages in ongoing oversight of licences to take account of future findings or changes in circumstances. If a licence was issued, this ongoing oversight would be achieved through post release review (PRR) activities. The 3 components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to collect additional specific information (Section 4.2)
- review of the RARMP (Section 4.3).

279. The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting system

280. Any member of the public can report adverse experiences/effects resulting from an intentional release of a GMO to the OGTR through the Free-call number (1800 181 030), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see Section 4.3 below) as well as the RARMPs of future applications involving similar GMOs.

4.2 Requirement to collect additional specific information

281. Collection of additional specific information on an intentional release provides a mechanism for 'closing the loop' in the risk analysis process and for verifying findings of the RARMP.

282. This may involve monitoring specific indicators of harm that have been identified in the risk assessment. The term 'specific indicators of harm' does not mean that it is expected that harm would necessarily occur if a licence was issued. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. Should a licence be issued, the licence holder would be required to monitor these specific indicators of harm as mandated by the licence.

283. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

284. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks greater than negligible. Therefore, they were not considered substantive risks that warranted further detailed assessment. No specific indicators of harm have been identified in this RARMP for application DIR-218. However, specific indicators of harm may also be identified during later stages, e.g. following the consideration of comments received on the consultation version of the RARMP, or if a licence were issued, through either of the other components of PRR.

285. Conditions have also been included in the draft licence to allow the Regulator to request further information from the licence holder about any matter to do with the release, including research to verify predictions of the risk assessment.

4.3 Review of the RARMP

286. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR, or by relevant new scientific information or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s) or identified new risks to people or to the environment that require management, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the consultation RARMP

287. The risk assessment concludes that the proposed commercial release of GM Purple Tomato plants poses negligible risks to the health and safety of people or the environment as a result of gene technology.

288. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, if a licence were to be issued, general conditions are proposed to ensure that there is ongoing oversight of the release.

Chapter 4 Proposed 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* (Cth) or the corresponding State legislation under which this licence is issued.

‘**GM**’ means genetically modified.

‘GMOs’ means the genetically modified organisms that are the subject of the dealings authorised by this licence. GMOs include live plants and viable seed.

‘OGTR’ means the Office of the Gene Technology Regulator.

‘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.

Section 2 General conditions and obligations

3. This licence does not authorise dealings with the GMOs that are otherwise prohibited as a result of the operation of State legislation recognising an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.
4. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with the GMOs are authorised during any period of suspension.
5. The licence holder is All Aussie Avocados Pty Ltd.
6. Any person, including the licence holder, may conduct any permitted dealing(s) with the GMO.
7. Except as restricted by condition 3, all dealings with the GMO are permitted.
8. Dealings with the GMO may be conducted in all areas of Australia.

9. The licence authorises dealings with the GMO described in **Attachment A**.

2.1 General obligations of the licence holder

10. The licence holder must notify the Regulator as soon as practicable if any of its contact details change.

Note: please address correspondence to OGTR.Applications@health.gov.au.

Prior to issuing a licence, the Regulator considers suitability of the applicant to hold a licence. The following condition addresses ongoing suitability of the licence holder.

11. The licence holder must:

    (a) inform the Regulator as soon as practicable after any of these events occur:

        i. any relevant conviction of the licence holder; or

        ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; or

        iii. any event or circumstances that would affect the capacity of the licence holder to meet the conditions of the licence; and

    (b) provide any information related to the licence holder's ongoing suitability to hold a licence, if requested by the Regulator, within the timeframe stipulated by the Regulator.

12. The licence holder must inform any person covered by this licence, to whom a particular condition of the licence applies, of the following:

    (a) the particular condition, including any variations of it;

    (b) the cancellation or suspension of the licence;

    (c) the surrender of the licence.

2.2 Provision of new information to the Regulator

Licence conditions are based on the risk assessment and risk management plan developed in relation to the application using information available at the time of assessment. The following condition requires that any new information that may affect the risk assessment is communicated to the Regulator.

13. The licence holder must inform the Regulator if the licence holder becomes aware of:

    (a) additional information as to 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: The Act requires, for the purposes of the above condition, that:

(a) *the licence holder will be taken to have become aware of additional information of a kind mentioned in condition 13 if he or she was reckless as to whether such information existed; and*

(b) *the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in condition 13, if he or she was reckless as to whether such contraventions had occurred, or such unintended effects existed.*

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

14. If the licence holder is required to inform the Regulator under condition 13, the Regulator must be informed without delay.

Note: An example of informing without delay is contact made within a day of becoming aware of new information via the OGTR free call phone number 1800 181 030 or email to OGTR.M&C@health.gov.au.

15. If at any time the Regulator requests the licence holder to collect and provide information about any matter to do with the progress of the dealings authorised by this licence, including but not confined to:

    (a) additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence, whether or not the licence holder has provided information to the Regulator under condition 13(a);

    (b) any contraventions of the licence by a person covered by the licence, whether or not the licence holder has provided information to the Regulator under condition 13(b);

    (c) any unintended effects of the dealings authorised by the licence, whether or not the licence holder has provided information to the Regulator under condition 13(c);

    (d) research, including by way of survey, to verify predictions of the risk assessment, or for any purpose related to risks to the health and safety of people, or to the environment;

    (e) scientific literature and reports in respect of the GMO authorised by this licence, for a nominated period;

    (f) details of any refusals of applications for licences or permits (however described) to deal with the GMO made pursuant to the regulatory laws of a foreign country;

and the request is reasonable, having regard to consistency with the Act and relevance to its purpose, then the licence holder must collect the information and provide it to the Regulator at a time and in the manner requested by the Regulator.

Note: The Regulator may invite the licence holder to make a submission on the reasonability of a request by the Regulator to collect and provide information relevant to the progress of the dealings with the GMO.

2.3 Obligations of persons covered by the licence

16. If a person is authorised by this licence to deal with the GMOs and a particular condition of the licence applies to the dealing by the 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.

Note: Under the Act, the definition of premises includes a building, area of land or vehicle.

Section 3 Reporting and documentation

3.1 Annual Report

17. The licence holder must provide an annual report to the Regulator by the end of September each year covering the previous financial year. An annual report must include:

    (a) information about any adverse impacts, unintended effects, or new information relating to risks, to human health and safety or the environment caused by the GMO or material from the GMO;

    (b) information about the volumes of the GMO grown for commercial purposes, including seed increase operations, in each State and Territory for each growing season in the period;

(c) information about the volumes of the GMO grown for research or breeding purposes in each State and Territory for each growing season in the period.

Note: Please address correspondence to OGTR.M&C@health.gov.au.

Note: nil plantings should also be reported under conditions 17(b) and 17(c).

ATTACHMENT A

DIR No: 218

Full Title: Commercial release of tomato genetically modified for purple fruit colour

Licence Holder

All Aussie Avocados Pty Ltd

GMO Description

GMO covered by this licence

Tomato varieties genetically modified by introduction of only the genes and genetic elements listed below as inserted at Locus B, known as The Purple Tomato or by the OECD unique identifier NPS-01201-8.

Parent Organism

Common Name: Tomato

Scientific Name: *Solanum lycopersicum* var. *lycopersicum*

Modified traits

Category: Altered fruit colour

Selectable marker – antibiotic resistance

Description: The tomato has been genetically modified by the introduction of 2 transcription factors that switch on anthocyanin production during fruit ripening, resulting in purple fruit colour. The GMO also contains a selectable marker gene that confers antibiotic resistance. The introduced genes and associated regulatory sequences are listed in Table 1.

Table 1. Introduced genetic elements in the GMO

Gene (source)	Promoter (source)	Terminator (source)	Encoded protein	Intended function
Delila (Antirrhinum majus)	E8 promoter (*Solanum lycopersicum*) – activated during fruit ripening	Cauliflower mosaic virus (CMV) terminator (CMV)	Delila	Transcription factor – anthocyanin biosynthesis
Rosea1 (Antirrhinum majus)	E8 promoter (*Solanum lycopersicum*) – activated during fruit ripening	CMV terminator (CMV)	Rosea	Transcription factor – anthocyanin biosynthesis
Neomycin phosphotransferase type II (nptII) gene *(Escherichia coli)*	Nopaline synthase (NOS) promoter (*Agrobacterium tumefaciens*)	Octopine synthase 3 (Ocs 3) terminator (*Agrobacterium tumefaciens*)	Neomycin phosphotransferase type II (NPTII)	Antibiotic resistance, selectable marker

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Appendix A: Summary of submissions

The Regulator received several submissions from prescribed experts, agencies, and authorities[5] on matters relevant to preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. These issues, and where they are addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	• Agreed that the following should be included in the RARMP: o the potential for the GM tomato to be harmful to the environment o the potential for the GM tomato to be harmful to people through toxicity or allergenicity o the potential for the GM tomato to be harmful to other organisms through toxicity o the potential for harm to result from gene flow to other tomatoes o whether commercial release is likely to result in changes to agricultural practices that may have an environmental impact.	These matters have been considered in Chapters 1 and 2 of the RARMP.
	• Advised that the Regulator should further consider the potential risks associated with horizontal gene transfer to the human gut microbiome from widespread consumption.	The potential for increased antimicrobial resistance resulting from horizontal gene transfer of the *nptII* gene is discussed in Risk Scenario 2.
	• Advised that the Regulator should further consider the potential risks to consumers from the off-target production of other compounds including toxic alkaloids.	Alkaloids levels in the GM fruit are discussed in Chapter 1 Section 4.5.5 and Risk Scenario 1.
	• Advised that the Regulator should seek more information related to tomato allergens in the GMO.	Allergenicity is discussed in Chapter 1 Sections 3.4.2, 4.3 and 4.4, as well as Risk Scenario 1.
	• Advised that the Regulator should seek more information related to the upregulation of other genes in the GMO.	Upregulation of other genes is discussed in Chapter 1 Sections 4.5.4.3 and 4.5.4.4.
2	States that it is not clear from the application what the public benefits are from the proposed approval for purple tomatoes, and that there are already purple style tomatoes in heritage varieties. States that if the application is just for cosmetic or marketing reasons, then does not support it.	Benefits are outside the scope of the Gene Technology legislation. The Regulator's responsibility is to identify and manage risk as a result of gene technology.

[5] Prescribed experts, agencies and authorities include GTTAC, State and Territory Governments, relevant local governments, Australian government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
3	Provides no comments at this stage and reserves any potential future comments to when the RARMP is prepared.	Noted.
4	Although the tomatoes will be grown in greenhouses, there is still potential for unintended release of modified plant material through waste disposal, or accidents like spilling during transport. Questioned whether waste management protocols should be considered for any sites growing these tomatoes to limit unintended environmental exposure.	As this application is for a commercial release of a GM plant, the licence application proposes an ongoing commercial release, with no restrictions on how the GM Purple Tomato is grown or used, and it is therefore assumed that there will be dispersal into the environment. Dispersal and persistence of the GMO is discussed in Risk Scenario 3.
	Questioned whether if the waste goes to a commercial composting site it needs to be separated so modified plant material doesn't contaminate compost.	As above.
	Advised that as some people in the community may be concerned about whether a GM plant is safe, it could be good to have clear communication materials about the new tomato and its safety in areas that the tomatoes are going to be grown and sold, while acknowledging that food safety is generally looked after by FSANZ.	As part of the consultation, the public will be notified that the RARMP is open for consultation via our subscriber list, our website, and newspaper advertisement. Other relevant documents including a Questions and Answers will also be made available at this time that includes a plain language summary.
5	The application does not comment on whether there is potential for the antimicrobial resistance gene in the GM tomato to spread into the environment and contribute to environmental antimicrobial resistance. Recommends that the applicant provide information in the RARMP on the likelihood of the antimicrobial resistance gene spreading to the environment and any mitigation activities that would be undertaken to minimise the risk.	The potential for increased antimicrobial resistance resulting from horizontal gene transfer of the *nptII* gene is discussed in Risk Scenario 2.

Submission	Summary of issues raised	Comment
6	• What technology was used to introduce the transgenes into the GMO?	The method of genetic modification is discussed in Chapter 1, Section 4.2 of the RARMP.
	• Other than the intended insert, were any sequence differences identified in the GMO when compared to the parent?	Characterisation of the insert in the GMO is discussed in Chapter 1, Section 4.5.2 of the RARMP.
	• Are the integration sites of the genes of interest stable over multiple generations?	Stability of the insert over multiple generations is discussed in Chapter 1, Section 4.5.3 of the RARMP.
	• Is expression of the *nptII* gene constitutive throughout the GM plant? • Is the protein produced from *nptII* gene present in the seeds of the GMO? • Is the protein produced from *nptII* gene present in the fruit of the GMO? • Has kanamycin resistance been reported in bacteria of medical importance in Australia? • What other aminoglycoside antibiotics are used in Australia and has resistance been reported?	The *nptII* gene and relevant antibiotics are discussed in Chapter 1 Sections 4.1.2 and 5.6, as well as Risk Scenario 2.
	• Discussed the potential benefits of the GM Purple Tomato as compared to conventional breeding.	Benefits are outside the scope of the Gene Technology legislation. The Regulator's responsibility is to identify and manage risk as a result of gene technology.
	• In considering risks posed by the GMO, commented that: o tomatoes present low risk o snapdragon flowers are edible o horizontal gene transfer between GM plants to bacteria and/or people doesn't appear to be an issue. Noted that most GM canola and some GM cotton that are widely grown in Australia contain the *nptII* gene.	Noted.
	• Although the application states that it is anticipated that the tomatoes will be grown in commercial glasshouses, recommends that the RARMP considers that the GMO, as a seeded fruit, could be grown more widely across Australia.	Cultivation of the GMO by home gardeners and commercial sale of seed packets has been considered as part of the risk context (see Chapter 1, Section 5.3.2 of the RARMP).

Submission	Summary of issues raised	Comment
7	• Discussed the intended purpose of the GM Purple Tomato, including the availability of dietary purple anthocyanin	Benefits are outside the scope of the Gene Technology legislation. The Regulator's responsibility is to identify and manage risk as a result of gene technology.
	• Commented that ○ the anthocyanin genes are well understood, and the pigments are a normal part of the diet ○ the product appears to have minimal impact in terms of GMO spread once in the food chain, as while it will be possible for plants to be spread by animal and human consumption, this is based on the seed being able to survive in the gut and excreted.	Noted.