Application to amend the Australia New Zealand Food Standards Code to permit a new genetically modified source organism – *Eschericia coli* K12 strain E997 – for the production of 2’-fucosyllactose (2’-O-fucosyllactose, 2’-FL).

Date: 1 June 2021

Executive Summary

[see separate document]

APPLICATION TO AMEND THE AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE TO PERMIT A NEW GENETICALLY MODIFIED SOURCE ORGANISM – *ESCHERICIA COLI* K12 STRAIN E997 – FOR THE PRODUCTION OF 2'-FUCOSYLLACTOSE (2'-FL). 1

EXECUTIVE SUMMARY ..... A

PART 1 - APPLICANT DETAILS 1

PART 2 – INFORMATION ABOUT THE APPLICATION 2

2.1 Purpose of the application 2

2.2 Justification for the application 2

2.3 Regulatory impact information 2

2.3.1 Costs and benefits of the application 2

2.3.2 Impact on international trade 2

PART 3 INFORMATION ABOUT *E. COLI* K12 STRAIN E997 3

3.1 Exclusive use of Escherichia coli K12 containing the gene for alpha-1,2-focusyltransferase from Bacteroides vulgatus 3

3.2 Technical information on 2'-FL 3

3.2.1 Information on the physical and chemical properties of 2'-FL produced by microbial fermentation using E. coli K12 strain E997 3

3.2.1.1 Chemical Identity of 2'-FL 3

3.2.1.2 Chemical Structure of Purified 2'-FL 3

Figure 1: - Chemical structure of 2'-FL 4

3.2.2 Information on the impurity profile for a typical preparation of 2'-FL 4

3.2.3 Manufacturing process for 2'-FL using E. coli K12 strain E997 4

Table 3 - Overview of the FrieslandCampina Production Process for 2'-FL using E. coli K12 strain E997 5

Figure 2 - Flowchart of the FrieslandCampina 2'-FL Manufacturing Process 6

3.2.4 Specification for identity and purity of 2'-FL 7

3.2.5 Analytical method for detection of 2'-FL 7

PART 4 - INFORMATION SPECIFIC TO THE SOURCE ORGANISM USED THE MANUFACTURE OF 2'-FL 8

4.A.1 Information on the source organism used the manufacture of 2'-FL 8

Table 1. Specific Genetic Modifications Contained Within E. coli K12 strain E638 8

Figure 3. - 2'-Fucosyllactose Production in Engineered E. coli K12 10

Figure 4. Map of the 2'-Fucosyllactose Production Plasmid, pG217 11

Table 2. Details of the Genes and Elements Carried on Plasmid pG217[a] 11

4.A.2 History of use of the host and donor organisms 12

4.A.3 The nature of the genetic modification 13

4.B Characterisation and safety assessment of new substances 13

4C Other information 13

PART 5 ASSESSMENT PROCEDURE 14

PART 6 CONFIDENTIAL INFORMATION 14

6.1 Confidential commercial information (CCI) 14

6.2 Other confidential information 14

PART 7 EXCLUSIVE CAPTURABLE COMMERCIAL BENEFIT (ECCB) ... 14
PART 8 INTERNATIONAL AND OTHER NATIONAL STANDARDS ... 14
8.1 International Standards ... 14
8.2 Other national standards or regulations ... 14
PART 9 STATUTORY DECLARATION ... 15
PART 11 CHECKLISTS ... 16
Checklist for General requirements ... 16
Checklist for applications for new foods ... 17
ATTACHMENTS AND APPENDICES ... 18
Attachment 1 ... 18
Attachment 2 ... 18
Attachment 3 ... 18
Attachment 4 ... 18
Appendix 1 – primary references cited in respect of the function and safety of 2’-FL ... 18
Appendix 2 – Letters of support and statutory declarations ... 18

Part 1 - Applicant details

(a)	applicant name	FrieslandCampina Ingredients
(b)	name of contact person	
(c)	address	
(d)	telephone number	
(e)	email address	
(f)	nature of applicant's business	
(g)	details of other individuals, companies or organisations associated with the application	

Part 2 – Information about the application

2.1 Purpose of the application

This application seeks an amendment to Schedule 26 to permit an alternative genetically modified source organism – *Escherichia coli* K12 strain E997 - for the production of 2’-fucosyllactose (2’-O-fucosyllactose, 2’-FL) by fermentation and recognise the applicable specification for 2’-FL published by the European Union (EU).

2.2 Justification for the application

Schedule S29-5 permits the addition of 2’-O-fucosyllactose (2’-fucosyllactose), as a nutritive substance, to infant formula products, as a result of Application A1155.

FrieslandCampina produces 2’-FL by microbial fermentation, using the non-pathogenic *E. coli* K12 strain E997, through the addition of fucose to the galactose moiety of lactose in an α-1,2-linkage. This organism in not currently listed in Schedule 26 as a permitted source organism for 2’-FL.

Letters of support from New Zealand businesses for the approval of 2’-FL as proposed are included in Appendix 2.

2.3 Regulatory impact information

2.3.1 Costs and benefits of the application

Consumers

There are no costs to consumers from this application. Consumers may benefit from the greater availability of foods containing 2’-FL through the approval of an additional source organism.

Food Industry

There are no costs to food manufacturers and suppliers from this application. Food manufacturers may benefit from the opportunity to source 2’-FL from an alternative supplier.

Government

There are no additional costs to governments from this application, beyond the normal costs of ensuring compliance with food laws.

2.3.2 Impact on international trade

This application will align Australian and New Zealand with the USA and The European Union, which both permit the addition of 2’-FL produced using *E. coli* K12 strain E997 to food products. This has the potential to enhance international trade in respect of both the import and export of fortified food products.

Parts 3 to 7 of this dossier have been collated to address:

- *the Application Handbook section3.1.1 E.1 General requirements - Data requirements, and*
- *3.5.1Foods Produced Using Gene Technology*

Part 3 Information about *E. coli* K12 strain E997

3.1 Exclusive use of *Escherichia coli* K12 containing the gene for alpha-1,2-focusyltransferase from *Bacteroides vulgatus*

Exclusive permission is sought for use of FrieslandCampina's *E. coli* K12 strain containing the gene for alpha-1,2-focusyltransferase from *Bacteroides vulgatus* to produce 2'-fucosyllactose (2'-O-fucosyllactose, 2'-FL) by microbial fermentation.

3.2 Technical information on 2'-FL

Complete information about FrieslandCampina's purified 2'-FL is provided in Attachment 1 - GRAS Notification of Purified 2'-Fucosyllactose (2'-FL) Food Usage Conditions for General Recognition of Safety on behalf of Glycosyn, LLC Woburn, MA and FrieslandCampina Domo B.V. Amersfoort, The Netherlands (GRAS Notice No. GRN 000735). The technical information provided below is extracted from the GRAS Notification report.

3.2.1 Information on the physical and chemical properties of 2'-FL produced by microbial fermentation using E. *coli K12 strain E997*

3.2.1.1 Chemical Identity of 2'-FL

2'-FL is a trisaccharide/fructooligosaccharide that is naturally occurring in human breast milk.

Chemical name: α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose.

Synonyms: 2'-*O*-fucosyllactose; 2'-*O*-L-fucosyl-D-lactose; fucosyl-α-1,2-galactosyl-β-1,4-glucose; Fuc-α-(1→2)-Gal-β-(1→4)-Glc; 2'-FL.

Chemical formula: $C_{18}H_{32}O_{15}$

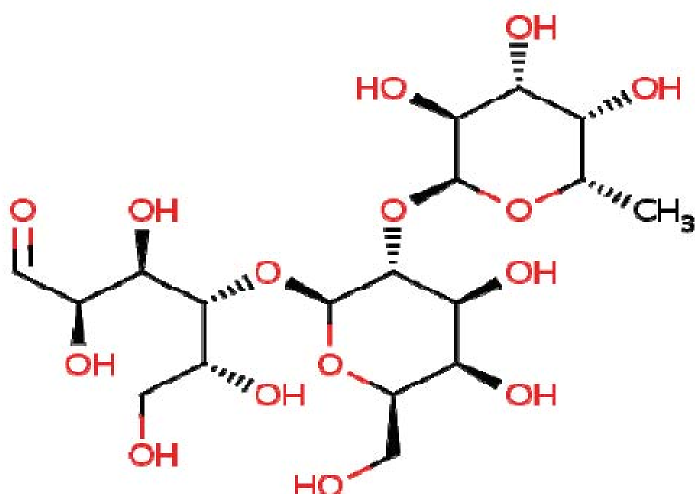
Molecular weight: 488.44 daltons

CAS Number: 41263-94-9

3.2.1.2 Chemical Structure of Purified 2'-FL

2'-FL is composed of L-fucose, D-galactose, and D-glucose (Figure 1). The monosaccharide L-fucose is linked to the disaccharide D-lactose by an α-(1→2) bond. The primary constituent of the subject ingredient is 2'-FL (> 90%), with minor concentrations (maximum 3% each) of related sugars, including lactose, allo-lactose, glucose, galactose, and fucose.

Figure 1: - Chemical structure of 2'-FL



3.2.2 Information on the impurity profile for a typical preparation of 2'-FL

The primary constituent of the subject ingredient is 2'-FL (> 90%), with minor concentrations (maximum 3% each) of related sugars, including lactose, allo-lactose, glucose, galactose, and fucose.

Analytical Results for 5 Nonconsecutive Lots of 2'-FL are attached (Attachment 1-1, Attachment 1 Table 7).

3.2.3 Manufacturing process for 2'-FL using E. coli *K12 strain E997*

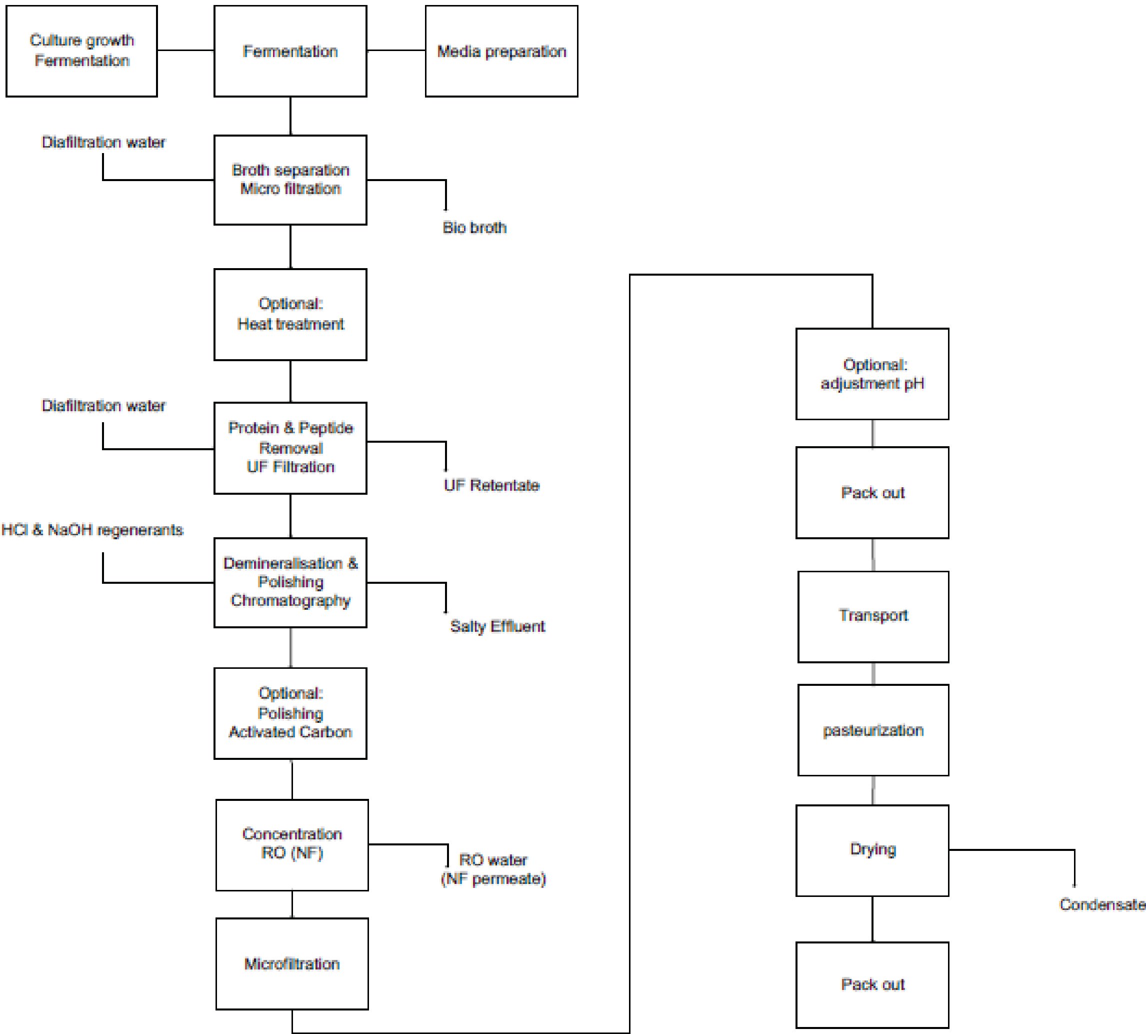
FrieslandCampina's 2'-FL is produced through the enzymatic transfer of fucose to lactose in an α-1,2 linkage. The reaction is catalysed by fucosyltransferase present in an engineered host strain of *E. coli* K12 bacteria, designated *E. coli* K12 strain E997, as described in Part 3.2.6. The 2'-FL production process consists of two stages: fermentation and purification (Table 1, Figure 2). All raw materials used in the manufacturing process, including processing aids, are food-grade or better materials (Attachment 1 Table 3). The fermentation and purification stages are described in detail in Attachment 1 pages 16-19).

Table 3 - Overview of the FrieslandCampina Production Process for 2'-FL using E. coli *K12 strain E997*

#	Step	Function
F1	Flask inoculation	Grow strain from cell bank
F2	Seed fermentation	Produce enough biomass to inoculate main fermenter
F3	Main fermentation	Biomass growth for 2'-FL production
P1	Microfiltration	Separation of cells from supernatant with product (2'-FL)
P2	Heat treatment	Optional: Improved processing behaviour: no impact on product
P3	Ultrafiltration	Removal of proteins and other larger cell debris
P4	Chromatography	Removal of ions, organic impurities like protein and DNA fragments, and colour by absorption
P5	Activated carbon	Optional: Removal of minor impurities
P6	Nanofiltration or reverse osmosis	Concentration of the product through the removal of water
P7	Microfiltration	Removal of potential microbiological contamination
P8	pH adjustment	Optional
P9	Sampling and packaging	Intermediate product is packaged
P10	Quality control	
P11	Transport	Transport to drying location
P12	Pasteurization	Removal of potential microbiological contamination
P13	Drying	Convert concentrate with a (spray) drying process into powder
P14	Sampling and packaging	Intermediate product is packaged
P15	Quality control	
P16	Release	

F represents fermentation steps; P represents purifications steps.

Figure 2 - Flowchart of the FrieslandCampina 2’-FL Manufacturing Process



3.2.4 Specification for identity and purity of 2’-FL

The European Union (EU) has published specifications for 2’-FL (Attachment 2-2 - 2’-FL EU novel food notification 2016), which are relevant to the current application. It is requested that this specification be referenced or included in Schedule 3 – Identity & Purity.

3.2.5 Analytical method for detection of 2’-FL

The analytical method for analysis for 2’-FL is described in Attachment 4 (and in GRAS Notice No. GRN 000735 Attachment 1).

Part 4 - Information specific to the source organism used the manufacture of 2’-FL

This part addresses the specific requirements of the Application Handbook section 3.5.1 Foods produced using gene technology.

4.A.1 Information on the source organism used the manufacture of 2’-FL

[*E. coli* K12 E638 Host Strain and E997 Fermentation Production Strain[1]]

The background organism from which *E. coli* K12 strain E638 was derived is the non-pathogenic bacterium *E. coli* K12, a host organism that is widely used to produce many oral and injectable human drugs. A related non-pathogenic *E. coli* strain (*E. coli* “Nissle”) has been used safely as a probiotic for 100 years. There are no virulence genes in *E. coli* K12, and in particular the specific strain of *E. coli* K12 used by the applicant (ATCC 55151; *E. coli* K12 GI724), as confirmed by whole genome sequencing. All genetic modifications are well documented in filed patents. Extensive purification steps remove viable cells, cell debris, and protein and peptide particles. Extensive qPCR testing demonstrated that no residual DNA from the genetically modified *E. coli* production strain was present in the final 2’-FL product.

The specific production *E. coli* host is derived directly from strain “GI724” which has been well defined. The strain is in the W3110 lineage of *E. coli* K12 (Bachmann, 1972; Hayashi et al., 2006). Strain RB791 passed through Mark Ptashne’s laboratory at Harvard University in the early 1980s (Brent and Ptashne, 1981), and then strain GI724 passed through Genetics Institute (LaVallie et al., 1993). A close relative of GI724 was developed for, and is currently used in, the commercial manufacture of the FDA-approved biopharmaceutical injectable protein Oprelvekin, a recombinant human interleukin 11, and the active ingredient in “Neumega” (Genetics Institute, 2002). Glycosyn obtained *E. coli* strain GI724 as the parent organism from the American Type Culture Collection (ATCC® number 55151).

GI724 is a prototroph, capable of growth on defined media containing only inorganic salts and a suitable carbon source. Complete deoxyribonucleic acid (DNA) sequencing of GI724 performed by Glycosyn confirms the strain to be completely free of any virulence genes, and very closely related to both the MG1655 and W3110 lineages of *E. coli* K12 (Bachmann, 1972; Blattner et al., 1997; Schultz, 2008).

Several specific genetic manipulations were performed on *E. coli* strain GI724 to develop a new background host for the commercial production of fucosylated oligosaccharides. These genetic changes are summarized in Table 1, and the newly engineered host carries the strain designation “E638”.

Table 1. Specific Genetic Modifications Contained Within E. coli *K12 strain E638*

E. coli Gene	Gene Function	Modification Introduced	New Locus Designation	Purpose
lacA	Lactose acetylase	Complete deletion	Δ*lacA*398	Eliminates the production of acetyl-lactose in the cell.
lacI	*lac* repressor	Complete deletion	Δ(*lacI*-*lacZ*)158	Removes the endogenous *lacI* gene encoding wild-type levels of β-galactosidase activity, enabling
lacZ	β-galactosidase	Complete deletion		

[1] Full details of the genetic construct of *E. coli* K12 strain E638 are also described in detail in Attachment 1 Part 2.A.2 (pages 7-13).

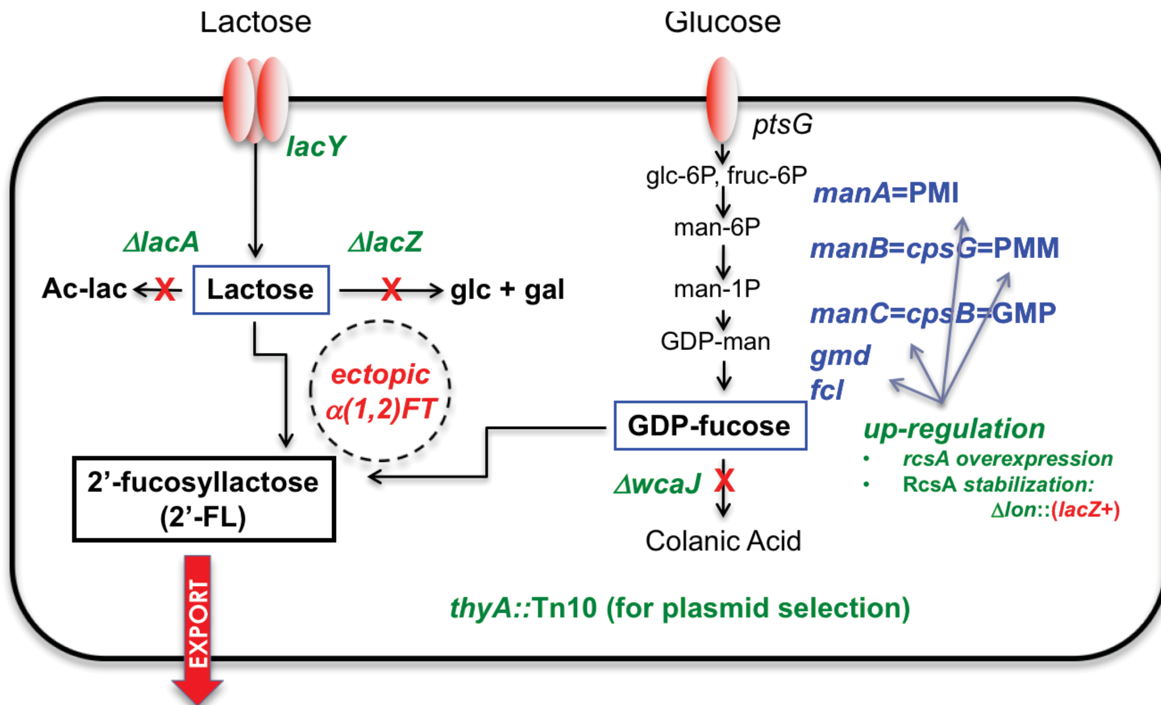
				development of a cytoplasmic lactose pool. Positions a strong constitutive promoter upstream of *lacY*, the lactose permease gene.
lacY	Lactose permease	Gene translocation	P*lacIq*-*lacY*	Positions the *lacY* gene downstream from a strong constitutive promoter.
lon	ATP-dependent protease	Complete deletion and replacement with a gene cassette containing a promoterless *lacZ* CDS and a kanamycin resistance marker	Δ*lon*::(*lacZ*$^{+}$, KAN)	Deletes Lon protease function and also re-introduces a low but useful level of β-galactosidase activity into the cell.
wcaJ	UDP-glucose:undecaprenyl-phosphate glucose-1-phosphate transferase	Complete deletion	Δ*wcaJ*134	Eliminates the production of colanic acid, and enables accumulation of a cytoplasmic GDP-fucose pool.
thyA	Thymidylate synthase	Insertional inactivation	*thyA*748::Tn10	Introduces an auxotrophy to be used later for plasmid maintenance. Note: Tn10 carries a tetracyline resistance marker.
ampC	*E. coli* K12 endogenous β-lactamase	Insertional inactivation	*ampC*::($P_{trp}\lambda cI^{+}$)	Introduces a wild-type lambda repressor gene under the control of a tryptophan promoter.

Techniques of classical generalized phage transduction utilizing P1vir (Thomason et al., 2007) and λ Red recombineering (Datsenko and Wanner, 2000) were used in the construction of strain E638. Only three DNA segments in strain E638 are not formally derived from ancestral wild-type *E. coli* K12; the *cI*$^{+}$ repressor gene (from *E. coli* bacteriophage lambda), the transposon Tn10 (bearing a tetracycline resistance gene), and the kanamycin resistance gene. Each of these three non-*E. coli* K12 DNA segments are nevertheless found commonly in laboratory and wild-type isolates of non-pathogenic *E. coli*. Note that the *thyA* auxotrophy of E638 prevents endogenous DNA synthesis by the strain, and that this lesion is lethal unless the defect is

complemented by a plasmid-borne *thyA* gene, or unless the growth medium is supplemented with thymidine. Further details regarding the strain construction strategy of Glycosyn's *E. coli* K12 production host may be found in US patents 9,029,136 and 9,453,230 (Heidtman et al., 2015; Merighi et al., 2016).

The genetic changes that enable the production of 2'-FL in *E. coli* K12 are detailed in the schematic in Figure 3.

Figure 3. - 2'-Fucosyllactose Production in Engineered E. coli *K12*



The presence of two cytoplasmic precursor pools is required for 2'-fucosyllactose production:

1. The Lactose Pool: *E. coli* K12 naturally possesses the ability to transport lactose from its environment and to use the sugar as a carbon source for growth. Several modifications of the lactose (*lac*) utilization operon were performed in engineered strain E638 to prevent this lactose catabolism and to promote development of a robust cytoplasmic lactose pool. First, lactose degradation through the action of endogenous β-galactosidase was prevented by deletion of the endogenous *lacZ* gene. Second, lactose transport from the culture medium into the cell was enhanced by placing the endogenous lactose permease gene (*lacY*) under a strong constitutive promoter. Finally, the production of undesirable acetyl-lactose was eliminated by deleting the endogenous *lacA* gene (acetyl-lactose presents a downstream purification challenge); and

2. The GDP-Fucose Pool: Wild-type *E. coli* K12 produces GDP-fucose for the sole purpose of making colanic acid, a fucose-containing polysaccharide containing a repeat unit with D-glucose, L-fucose, D-galactose, and D-glucuronate that is used in extracellular capsule formation. Elimination of the ability to make colanic acid has no deleterious effects on *E. coli* K12 growth in the bioreactor. In engineered strain E638, colanic acid production is eliminated downstream of GDP-fucose by removal of the *wcaJ* gene, and this single mutation leads to an accumulation of cytoplasmic GDP-fucose. The concentration of this GDP-fucose pool is then enhanced by inducing the earlier steps of the colanic acid synthesis pathway. This is achieved by increasing the level of the pathway's positive transcriptional activator protein, RcsA, by: a) including the *rcsA* gene on a multicopy expression plasmid introduced into E638 (see below, the description of plasmid pG217); and b) eliminating the gene for Lon, the major protease responsible for RcsA turnover in the cell. As part of the *lon* deletion, a weak *lacZ* (β-galactosidase) allele was purposefully reintroduced into strain E638. The low level of β-galactosidase produced by this weak *lacZ* allele aids in downstream 2'-FL purification by removing residual lactose precursor at the end of fermentations, while not adversely impacting overall 2'-FL fermentation titres.

Lactose and GDP-fucose are efficiently and specifically converted into 2'-fucosyllactose by the action of the enzyme FutN, an α-1,2 fucosyltransferase first identified at Glycosyn in the genome of the gut commensal

organism, *Bacteroides vulgatus* ATCC 8482 (Heidtman et al., 2015). Production of FutN enzyme is achieved in E638 by first transforming the strain with plasmid pG217 to generate a new strain, designated as "E997". *E. coli* E997 is the actual production strain used for the manufacture of 2'-FL by the applicant. pG217 carries the *futN* gene in an expression construct downstream of the strong inducible transcriptional promoter, pL. This promoter maintains an "off" status in minimal medium due to the presence of the wild-type cI repressor protein, expressed in the host strain under these conditions from a single copy chromosomal gene under the control of a trp promoter. To induce 2'-FL synthesis, tryptophan is added to the growth medium, which turns off production of cI repressor and slowly changes the pL promoter status to "on." As a consequence, *futN* is expressed and, if lactose is present, 2'-FL synthesis begins.

A map of plasmid pG217 is provided in Figure 4, and a detailed listing of the genes and elements carried on this plasmid is provided in Table 2.

Figure 4. Map of the 2'-Fucosyllactose Production Plasmid, pG217

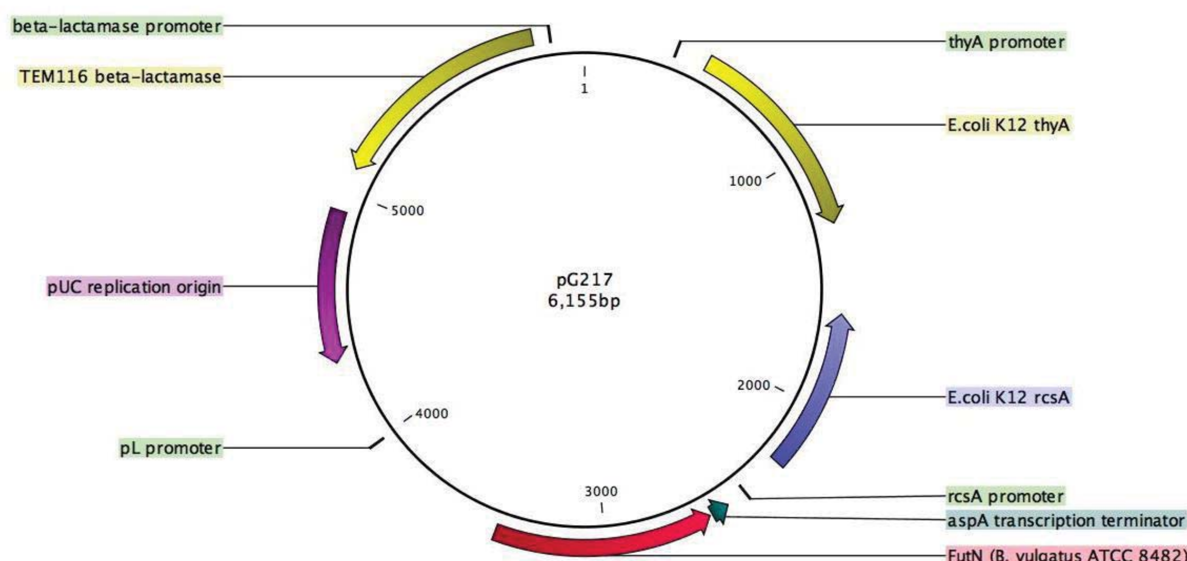


Table 2. Details of the Genes and Elements Carried on Plasmid pG217[a]

Nucleotide position	Gene or element name	Source	Purpose
1-242 bp	Vector DNA, no genes or elements	pUC19[b]	No function
243-1365 bp	Thymidylate synthase gene (*thyA*), includes the gene promoter	*E. coli* K12	Complements the *thyA* auxotrophy of E638, ensuring pG217 plasmid maintenance in minimal media
1366-1529 bp	Vector DNA, no genes or elements	pUC19	No function
1530-2495 bp	Colanic acid operon transcription activator protein gene (*rcsA*), includes the gene promoter	*E. coli* K12	Up-regulates genes responsible for production of GDP-fucose[c]
2496-2574 bp	*aspA* transcription terminator	*E. coli* K12	Terminates transcription from the upstream pL promoter
2574-3446 bp	Synthetic *futN* gene encoding an α-1,2-fucosyltransferase (YP_001300461)	*Bacteroides vulgatus* ATCC 8482	Synthesis of 2'-fucosyllactose from lactose and GDP-fucose
3447-4280 bp	DNA segment carrying the bacteriophage lambda strong leftwards promoter, pL	*E. coli* K12 phage lambda (l)	Controlled expression of the *futN* gene
4281-4335 bp	Vector DNA, no genes or elements	pUC19	No function

4336-4924 bp	pUC origin of replication	pUC19	Maintains pG217 plasmid copy number of ~25 copies per cell
4925-6155 bp	TEM116 β-lactamase gene, includes the gene promoter	pUC19	Encodes ampicillin resistance[d]

[a] pG217 is 6,155 base pairs (bp) long.
[b] Yanisch-Perron et al. (1985)
[c] The presence of multiple copies of the *rcsA* gene on pG217 boosts the cellular production of GDP-fucose.
[d] Inclusion of the TEM116 β-lactamase gene in pG217 was for operational convenience during the original construction of the plasmid. The gene is not utilized at any stage in the production of 2'-fucosyllactose, and at no stage is ampicillin or any other antibiotic present in the process (i.e. during the laying down of master and working cell banks, in the growth of seed cultures for production, or at any time in bioreactor fermentations).

4.A.2 History of use of the host and donor organisms

(a) For the donor organism(s) from which the genetic elements are derived:

(i) any known pathogenicity, toxicity or allergenicity of relevance to the food;

There are five DNA segments in strain E638 are not formally derived from ancestral wild-type *E. coli* K12. Three of those reside on the chromosome, being the *cI*⁺ repressor gene (from *E. coli* bacteriophage lambda), the transposon Tn10 (bearing a tetracycline resistance gene), and the kanamycin resistance gene. The function of each of these element is understood due to their widespread use in laboratories and their occurrence in wild type *E. coli* strains. None of these genetic elements changes the status of the pathogenicity of the *E. coli* host strain as it is still unable to colonise or survive in the human gut.
The other two DNA segments not derived from the K12 strain are the pUC19 plasmid backbone (including the TEM116 β-lactamase gene) and the *futN* gene encoding the α-1-2-fucosyltransferase from *Bacteroides vulgatus*. The latter has no role in pathogenicity, toxicity or allergenicity of relevance to the food. The antibiotic resistance genes present in the strain are not utilised (*i.e*. no antibiotics are used) during any stage of strain's (MCB and WCB) maintenance, or production. The various DSP steps ensure that no protein or DNA of the production strain remains in the final product. There is no known pathogenicity, toxicity or allergenicity of relevance to the food. Refer Part 4.A.1

(ii) history of use of the organism in the food supply or history of human exposure to the organism through other than intended food use (e.g. as a normal contaminant).

The donor organism of the α-1,2-fucosyltransferase gene is *Bacteroides vulgatus*, a human gut commensal organism. The gene product was extensively characterised and is solely responsible for 2'-FL synthesis in the production strain. The cloned gene was made by gene synthesis so there is no risk of other uncharacterised sequences of this organism ending up in the production strain. All other introduced genetic elements, including those of phage lambda, are also part of naturally occurring *E. coli* strains. These normally inhabit the intestinal tract of humans and other animals. *E. coli* K12 contains no known pathogenic genes (either colonization factors or toxin genes) and is universally recognized as a safe, commercial manufacturing host. *E. coli* K12 is used globally in the commercial manufacturing of products ranging from amino acids and vitamins for foodstuff applications, to recombinant human proteins used in pharmaceutical applications, including protein products used as injectables. Pharmaceutical proteins expressed by *E. coli* include human insulin, growth hormones (somatostatin, somatotropin), immunomodulators (interferons, interleukins, TNFα), growth factors (G-CSF, EGF), blood factors, coagulation inhibitors (t-PA, staphylokinase), and enzymes. There is also precedent for the safe ingestion of live microbial non-pathogenic *E. coli* in probiotic preparations. Refer Part 4.A.1

(b) For the host organism into which the genes were transferred:

(i) its history of safe use for food

The background host organism is a non-pathogenic *E. coli* strain K12. It has a defective cell envelope that renders it incapable of colonizing or surviving in the human gut as described in

4.A.1.

E. coli are bacteria that normally inhabit the intestinal tract of humans and other animals. *E. coli* K12 contains no known pathogenic genes (either colonization factors or toxin genes) and is universally recognized as a safe, commercial manufacturing host.

It is important to underline here that the final product 2'-FL is devoid of the production host as demonstrated by both protein measurements and qPCR testing of three production strain specific genomic targets.

(ii) the part of the organism typically used as food

E. coli K12 is used globally in the commercial manufacturing of products ranging from amino acids and vitamins for foodstuff applications, to recombinant human proteins used in pharmaceutical applications, including protein products used as injectables. Pharmaceutical proteins expressed by *E. coli* include human insulin, growth hormones (somatostatin, somatotropin), immunomodulators (interferons, interleukins, TNFα), growth factors (G-CSF, EGF), blood factors, coagulation inhibitors (t-PA, staphylokinase), and enzymes (Schulze et al., 2006). There is also precedent for the safe ingestion of live microbial *E. coli* preparations. Molecular genetic differentiation and identification methods make it possible to unequivocally distinguish pathogenic *E. coli* variants from non-pathogenic strains. Mutaflor® is an example of a probiotic therapy in which the active ingredient consists of a viable non-pathogenic *E. coli* strain, *E. coli* Nissle 1917. It is used in Europe and Canada for inflammatory and chronic functional bowel diseases ((Mutaflor® website, https://aralez.com/Portfolio/mutaflor/). In the US, Mutaflor® was considered to be a "medical food," however, FDA classified Mutaflor® as a "biologic," and the product is currently discontinued from the US market pending a final decision (Mutaflor® website: https://aralez.com/Portfolio/mutaflor/). Probiotics are, by definition, living non-pathogenic micro-organisms that exert a positive effect on the host organisms when they enter the gastrointestinal tract in a viable condition in sufficiently large numbers (FAO/WHO, 2002). Mutaflor® has a 95-year record of being well tolerated and lacking adverse effects (Irrgang and Sonnenborn, 1988). In addition, non-pathogenic strains, unlike virulent *E. coli* variants, exhibit no harmful effects in toxicological studies in both conventional and germ-free animals (Schulze et al., 2006).

(iii) the types of products likely to include the food or food ingredient

The derived ingredient, 2'-FL, is already permitted to be used as nutritive substance in the Code.

(iv) whether special processing is required to render food derived from the organism safe to eat.

Food containing 2'-FL is safe for human consumption at the use levels already permitted in the Code.

4.A.3 The nature of the genetic modification

Refer Part 4.A1

4.B Characterisation and safety assessment of new substances

Not applicable

4C Other information

Not applicable. 2'-FL. Is already permitted to be used as nutritive substance in the Code. The application does not seek changes to the current approved uses.

Part 5 Assessment procedure

2’-FL is already approved in the ANZ Food Standards Code. This application is limited to the approval a new source organism for its production, consequently, the general procedure, level 1 or 2, is the appropriate procedure to be adopted in assessing this application.

Part 6 Confidential Information

6.1 Confidential commercial information (CCI)

The application does not contain confidential commercial information.

6.2 Other confidential information

The application does not contain other information for which confidential treatment is requested.

Part 7 Exclusive capturable commercial benefit (ECCB)

As stated in Part 3.1, this application seeks exclusive permission for the use of a genetically modified *Escherichia coli* K12 containing the gene for alpha-1,2-focusyltransferase from *Bacteroides vulgatus* for the production of 2’-FL for the maximum allowed period.

Part 8 International and other national standards

8.1 International Standards

There are no Codex standards that expressly name the use of 2’-FL as an ingredient in foods. However, Codex Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (Codex Stan 72), permits “other ingredients may be added in order “to provide substances ordinarily found in human milk”.

8.2 Other national standards or regulations

FrieslandCampina’s 2’-FL), has received EU novel food approval and achieved GRAS status from the Food and Drug Administration in the U.S (Attachment 1).

EU

The Novel Foods Unit, the Ministry for Medical Care, The Netherlands has determined that the 2’-fucosyllactose (2’-FL) produced through fermentation with *E. coli* K12 strain E997 by FrieslandCampina is substantially equivalent to a (synthetic) 2’-FL previously authorised in the European Union (Attachment 1-1).

USA

The FDA has accepted a GRAS notification in relation to 2’-FL produced using *E. coli* K12 strain E997:

- Glycosyn, LLC and FrieslandCampina Domo B.V.(201 GRAS Notice No. GRN 735 2’-Fucosyllactose (Attachment 1);

Part 9 Statutory declaration

See Appendix 2 - Declaration Statement signature H.M.A. Ermens

Part 11 Checklists

Checklist for General requirements

General requirements (3.1.1)		
Check	**Page No.**	**Mandatory requirements**
✓		A Form of application ✓ *Application in English* ✓ *Executive Summary (separated from main application electronically)* ✓ *Relevant sections of Part 3 clearly identified* ✓ *Pages sequentially numbered* ✓ *Electronic copy (searchable)* ✓ *All references provided*
✓	1	B Applicant details
✓	2	C Purpose of the application
✓	2	D Justification for the application ✓ *Regulatory impact information* ✓ *Impact on international trade*
✓	3	E Information to support the application ✓ *Data requirements*
✓	13	F Assessment procedure ✓ *General* ☐ *Major* ☐ *Minor* ☐ *High level health claim variation*
✓	13	G Confidential commercial information *na CCI material separated from other application material* *na Formal request including reasons* *na Non-confidential summary provided*
✓	13	H Other confidential information *na Confidential material separated from other application material* *na Formal request including reasons*
✓	13	I Exclusive Capturable Commercial Benefit ✓ *Justification provided*

✓	42	J International and other national standards ✓ *International standards* ✓ *Other national standards*
✓	14 Appendix 2	K Statutory Declaration
✓	15 16	L Checklist/s provided with application ✓ *3.1.1 Checklist* ✓ *All page number references from application included* ✓*Any other relevant checklists for Chapters 3.2–3.7*

Checklist for applications for new foods

Foods produced using gene technology (3.5.1)		
Check	**Page No.**	**Mandatory requirements**
☑	3	A.1 Nature and identity
☑	8	A.2 History of use of host and donor organisms
☑	8	A.3 Nature of genetic modification
☒	12, 16	B.1 Characterisation and safety assessment
☒	-	B.2 New proteins
☒		B.3 Other (non-protein) new substances
☒	-	B.4 Novel herbicide metabolites in GM herbicide-tolerant plants
☑	7	B.5 Compositional analyses
☒		C Nutritional impact of GM food
☒	-	D Other information

Attachments and Appendices

Attachment 1 - GRAS Notification of Purified 2'-Fucosyllactose (2'-FL) Food Usage Conditions for General Recognition of Safety on behalf of Glycosyn, LLC Woburn, MA and FrieslandCampina Domo B.V. Amersfoort, The Netherlands (GRAS Notice No. GRN 000735).

Attachment 2 – EU considerations of 2'-FL

1. EFSA Journal 2015;13(7)4184_ 2'-FL
2. Appendix 3 - 2'-FL EU novel food notification 2016
3. EU Substantial Equivalence-2'-FL-FrieslandCampina

Attachment 3 - GRN 000735 Appendix 3 Analytical Methodology for Purified 2'-Fucosyllactose (2'-FL) Analysis.

Attachment 4 - Analytical Results for 5 Non-consecutive Lots of 2'-FL.pdf

Appendix 1 – primary references cited in respect of the function and safety of 2'-FL

Bachmann, 1972
Blattner et al., 1997
Datsenko and Wanner, 2000
FAO/WHO, 2002
Heidtman et al., 2015
Irrgang and Sonnenborn, 1988
Schultz, 2008
Schulze et al., 2006
Thomason et al., 2007
Yanisch-Perron et al. 1985

Appendix 2 – Letters of support and statutory declarations

Letter of Support Bodco Ltd, New Zealand

Letter of Support Yashili, New Zealand

Declaration Statement signature H.M.A. Ermens