

**Constitutive Gene Expression
System for *Lactococcus lactis*
and Other Lactic Acid Bacteria**



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Note: The products described herein are sold by MoBiTec GmbH solely to academic institutions for research & development. Commercial entities interested in these products are required to contact NIZO food research, BV, The Netherlands.



1 Introduction

The Constitutive Gene Expression System for *Lactococcus lactis* and Other Lactic Acid Bacteria, developed by NIZO food research BV, The Netherlands, is easy-to-operate and has advantages for the following applications:

- Overexpression of homologous and heterologous genes for functional studies and to obtain large quantities of specific gene products
- Metabolic engineering
- Suitability for protein secretion (Novotny, R. *et al.* 2005; Ravn, P. *et al.* 2003; van Asseldonk *et al.*, 1990; Vos, P. *et al.*, 1989) and anchoring in the cell envelope
- Large scale applications

Major advantages of this system over other expression systems are:

- Less endogenous and no exogenous proteases
- Endotoxin-free expression system
- Food grade protein expression possible
- No formation of inclusion bodies
- No formation of spores
- Simple fermentation, scale-up and downstream processing
- Suitable also for bacteria other than *Lactococcus lactis*

1.1 *Lactococcus lactis*

Lactococcus lactis is a homofermentative bacterium. Its primary function is rapid lactic acid production from saccharides. Functional characteristics that have extensively been studied in lactococci include the carbon metabolism, the extracellular and intracellular proteolytic system, the production of antibiotic substances, and their interaction with and resistance to bacteriophages. The genome information of many *L. lactis* strains is publicly available. This wealth of knowledge and experience has led to the use of lactococci in several fields of biotechnology, e.g., the expression of bacterial and viral antigens for safe vaccination via mucosal immunization, the production of human cytokines and other therapeutic agents for *in situ* treatments, the use of lactococci as a cell factory for the production of specific compounds, and the pilot production of pharmaceutical products (Mierau, 2005).

1.2 Use of *Lactococcus lactis* plasmids in other Gram-positive bacteria

Plasmids with a replication origin from *L. lactis* can be used in all lactic acid bacteria and in other Gram-positive bacteria such as *Bacillus subtilis* (Silke David, 1989, AEM 55:1483; Christ Platteeuw, 1994, AEM 60:587; Elisabeth Sorvig, 2005, Microbiology, 151:2439; Indranil Biswas, 2008, Microbiology, 154:2275).



1.3 Codon usage

Until very recently codon usage was an important issue in the possibility and efficiency to express heterologous genes in *L. lactis* (GC content of the DNA of 35-37%). When a gene donor organism is closely related to *L. lactis*, or the DNA GC content is similar to that of *L. lactis*, the probability that a gene can be successfully expressed is high. Nowadays, cheap and reliable tailor-made DNA synthesis is available, revealing restrictions as to the origin of a specific target gene, as from a known amino acid sequence, a gene can be designed that matches the codon usage pattern of the host organism. In addition to a general codon optimization, specific codon tables can be used, such as the codon table for the highly expressed ribosomal protein genes, to further increase product formation.

2 Products

2.1 Host strains

Lactococcus lactis subsp. *cremoris* MG1363

Plasmid-free progeny of the dairy strain NCDO712. Most widely used host strain for cloning and gene expression in *L. lactis* (Gasson, 1983).

Lactococcus lactis NZ3000

Standard strain for food grade selection is based on the ability to grow on lactose. The lactose operon, that is generally present on plasmids, has been integrated into the chromosome and the *lacF* gene was deleted. Deletion of the *lacF* gene makes this strain unable to grow on lactose unless *lacF* is provided on a plasmid (van Alen-Boerrigter and de Vos, unpublished data; de Ruyter *et al.*, 1996).

Lactococcus lactis NZ1330

This host strain is made for food grade expression systems based on an *alr* selection marker. It was generated by deleting the *alr* gene, an alanine racemase encoding gene, in the progenitor strain MG1363. Deletion of the *alr* gene results in auxotrophy for the essential component D-alanine, implying that the strain is unable to grow on media without D-alanine unless *alr* is provided on a plasmid, and thereby enables the conversion of L-Ala to D-Ala. The *alr* gene serves as food-grade complementation marker (Bron *et al.*, 2002).

Strains	Plasmids	Medium
<i>Lactococcus lactis</i> MG1363	non-food grade	M17 + 0.5% glucose + 10 µg/ml chloramphenicol
<i>Lactococcus lactis</i> NZ3000	food grade	see 3.2 & 3.6
<i>Lactococcus lactis</i> NZ1330	food grade	see 3.6



2.2 Plasmids

All plasmids are based on the pSH71 rolling circle replicon (de Vos, 1987).

pNZ124: General broad-host-range cloning vector with multiple cloning site for *L. lactis* and other lactic acid bacteria; chloramphenicol selection; (Platteeuw *et al.*, 1994)

pNZ2103: Broad-host-range vector for constitutive gene expression under control of the *lacA* promoter; for transcriptional fusion; Chloramphenicol selection; (Platteeuw *et al.*, 1996)

pNZ2105: General food grade cloning vector with multiple cloning site for *L. lactis* NZ3000; *LacF*-based selection; (Platteeuw *et al.*, 1996)

pNZ2122: Food grade vector for constitutive gene expression under control of the *lacA* promoter for *L. lactis* NZ3000; For transcriptional fusions; *LacF*-based selection; (Platteeuw *et al.*, 1996)

pNZ2123: Food grade vector; Identical to pNZ2122 but with inverted multiple cloning site *LacF*-based selection; (Platteeuw *et al.*, 1996)

pNZ2125: Food grade cloning vector with multiple cloning site appropriate for gene expression into *L. lactis* strain NZ1330; *alr*-based selection; (Platteeuw *et al.*, 1996)

pNZ7021: Broad-host-range vector for constitutive gene expression under control of a strong promoter *PpepN*; Chloramphenicol selection; (Wegkamp *et al.*, 2007)
This vector is designed for translational fusions. It contains the *pepN* ribosome binding site (RBS) and a start codon (ATG) followed by an *SphI* site which is essential for the cloning of a target gene without own ribosome binding site and start codon.

pNZ7025: Food grade vector for constitutive gene expression under control of the *pepN* promoter; *alr*-based selection; (Wegkamp *et al.*, 2007)
This vector is also designed for translation fusions as described above for the plasmid pNZ7021.

Attention: It is very important to take into consideration that expression vectors made for transcriptional fusion can only be used for cloning of target genes with own ribosome-binding site and own start codon using any possible cloning sites. However, the expression vectors designed for translational fusion supply both promoter and translation initiation regions that include the sequence of an RBS and a start codon followed by an essential cloning site. In this case, cloning the gene of interest must be carried out using corresponding defined restriction site that results in an in-frame insertion of the open reading frame (ORF).

All plasmids are lyophilized from water. Use TE buffer or water for reconstitution of plasmid DNA.



2.3 Propagation of plasmid DNA

Because of the rolling circle replication mechanism *L. lactis* plasmids can also replicate in *E. coli*. This replication mechanism is only functional if the strain harbors an intact *recA* gene, which is not the case in many standard commercially available *E. coli* strains. Therefore, *E. coli* strain MC1061 should be used as host. In some instances cloning in *E. coli* can be troublesome. In such an event, cloning can also be performed directly in *L. lactis* by using electroporation.

E. coli can be used for the propagation of all plasmids except for the food-grade plasmids, which only can be propagated in *L. lactis* NZ3000 or NZ1330. Since *E. coli* is *alr+* or *lacF+* the selection of food-grade vectors will fail after uptake of the DNA. For propagation in *E. coli* it is strongly recommended to use the *recA* positive strain MC1061, order# VS-ELS-10610-01 or VS-ELS10710-01 (10 µg/ml of chloramphenicol; growth of colonies may take 2 days):

MC1061 Genotype: *araD139*, $\Delta(ara, leu)7697$, $\Delta lacX74$, *galU-*, *galK-*, *hsr-*, *hsm+*, *strA*

Never thaw frozen bacterial glycerol stock. Use a sterile loop, sterile wooden stick, or sterile disposable pipette to scratch the surface of the stock. Streak it out on appropriate agar plate (TY) for single colonies. Recap the frozen stock and return to storage at -80°C. Incubate the plate overnight at 37 °C (*E. coli*). Use single colonies to prepare competent cells for transformation.

Important Notes!

Lactococcus lactis grows at 30 °C, without aeration!

L. lactis dies on agar plates within 1 week. Always use fresh frozen stock material for new cultures!

Do not thaw frozen stock. Scratch cell material from the surface with a sterile inoculating loop, or use prepared single-use aliquots!

Standard procedure to grow 100 ml cell material is as follows: inoculate 5 ml pre-culture with some cells from frozen glycerol stock or from single colonies on the agar plate and grow overnight. Inoculate 100 ml of growth medium with the pre-culture in a dilution of 1:100, and incubate at 30 °C overnight!

Plasmid preparations of *L. lactis* vectors have a lower yield compared to *E. coli*!



3 Protocols

3.1 Media and growth conditions for *L. lactis*

Various media are available for growth of lactococci. The most commonly used laboratory medium is M17 supplemented with glucose, lactose, or other sugars as carbon source, and a relevant antibiotic for plasmid selection. The basic ingredients for a large-scale medium are 1-3% peptone, 0.5-2% yeast extract, 1-10% carbon source, and small amounts of magnesium and manganese ions. Individual processes may require specific optimization of the medium components and fermentation conditions.

For experiments in which specific metabolites are addressed or cell components need to be labeled, a chemically defined medium can be used. Lactococci are auxotrophic for a number of amino acids that can be added in a labeled form and are then integrated into newly formed proteins.

At a laboratory scale, most commonly an overnight pre-culture (e.g., 5 ml inoculated from a frozen stock) is inoculated into fresh medium with a dilution of 1:100, and grown overnight. In this set-up pH is not controlled, and the culture will stop growing at low cell density (approx. $OD_{600} = 2-3$) because of lactic acid production and the consequent pH drop. Alternatively, the culture can be grown with pH control to much higher cell density ($OD_{600} \approx 12-15$).

3.2 Media and growth conditions for food grade selection in *L. lactis*

Lactococcus lactis vectors pNZ2105, pNZ2122, and pNZ2123 with *lacF* as food grade selection marker allow the host strain NZ3000 to grow on lactose. The host strain NZ3000 has all genes involved in lactose fermentation integrated into the chromosome, with a deletion of the *lacF* gene (Enzyme III of the Lac-phosphotransferase system, PTS). Strain NZ3000 can grow on glucose, but in the presence of a plasmid carrying the *lacF* gene, it also can grow on lactose.

A special medium that can be used for selection of Lac^+ colonies is Elliker medium. On this rich medium all cells can grow, Lac^+ or Lac^- (because of residual glucose from other medium components such as yeast extract), but when lactose is added as only carbon source the lactose fermenting cells grow to yellow colonies which typically are bigger than the white colonies obtained for Lac^- cells.

The host strain NZ1330 carries an *alr* deletion (Δalr) that results in auxotrophy for D-alanine. D-alanine auxotrophy can be complemented using a plasmid harboring *alr*. *Lactococcus lactis* vectors pNZ2125 and pNZ7025 containing *alr* as food-grade selection marker allow the strain NZ1330 to grow on M17 broth containing L-alanine and glucose as carbon source.

L. lactis, as a fermentative organism, is usually grown in liquid medium as standing culture. In case of larger cultures, stirring may be used.



3.3 Preparation of own stocks of *L. lactis*

Lactococcus lactis can grow on M17 broth containing 0.5% sugar (glucose or lactose). For stable propagation of plasmids containing antibiotic resistance marker such as *cat* gene growth medium requires addition of 10 µg/ml chloramphenicol. On agar plate or slant, the strain will survive for about one week.

Stock preparation

- Inoculate 5 ml broth with cells from frozen glycerol stock at -80 °C
- Grow the cells overnight at 30 °C
- Add 3 ml fully grown culture to 1 ml 60% glycerol and store at -80 °C

Materials

Medium: M17 broth with 0.5% lactose or glucose, 10 µg/ml chloramphenicol
Glycerol: Sterile (15 min, 121 °C) 60% glycerol in cryotubes

3.4 Plasmid DNA isolation from *L. lactis*, small scale

Method

- Use 5 ml full grown culture
- Spin down 10 min at 3000 x g or more (up to 6000 x g)
- Resuspend pellet in 250 µl of THMS-buffer + 2 mg/ml lysozyme in 1.5 ml reaction tube
- Incubate 10 min at 37 °C
- Add 500 µl of 0.2 N NaOH + 1% SDS, shake carefully (do not vortex)
- Incubate 5 min on ice
- Add 375 µl ice-cold 3 M potassium acetate pH 5.5, shake carefully
- Incubate 5 min on ice
- Spin 5 min in a benchtop centrifuge
- Transfer supernatant to a new reaction tube
- Fill the tube with 2-propanol
- Incubate 5-10 min at room temperature
- Spin 10 min in a benchtop centrifuge
- Carefully wash pellet with 70% ethanol
- Air or vacuum dry the pellet (avoid over-drying)
- Dissolve the pellet in 50 µl TE or sterile water

Materials

Medium: M17 broth with 0.5% lactose or glucose, 10 µg/ml chloramphenicol

THMS buffer: 30 mM Tris-HCl pH 8
3 mM MgCl₂
in 25% sucrose
add lysozyme before use



Others: 0.2 N NaOH + 1% SDS (not older than 3 months)
 3 M potassium acetate pH 5.5
 2-propanol
 70% ethanol
TE: 10 mM Tris-HCl pH 8
 1 mM EDTA pH 8

3.5 Transformation of *L. lactis*

Preparation of the cells

Day 1:

- Inoculate 5 ml G/L-SGM17B medium (s. below) with cells from a -80 °C stock, grow at 30 °C overnight

Day 2:

- Inoculate 50 ml G/L-SGM17B medium with the pre-culture in a dilution of 1:100 and grow at 30 °C overnight, without aeration

Day 3:

- Dilute 50 ml culture with 400 ml G/L-SGM17B
- Grow until OD₆₀₀ is 0.2-0.3 (ca. 3 h)
- Spin down cells for 20 min, 6000 x g, at 4 °C
- Wash cells with 400 ml 0.5 M sucrose + 10% glycerol (4 °C), spin down at 6000 x g (centrifugation speed may need to increase during successive washing steps)
- Resuspend the cells in 200 ml solution of 0.5 M sucrose + 10% glycerol + 50 mM EDTA (4 °C), keep the suspension on ice for 15 min and spin down
- Wash cells with 100 ml of 0.5 M sucrose + 10% glycerol (4 °C), spin down (6000 x g)
- Resuspend the cells in 4 ml 0.5 M sucrose + 10% glycerol (4 °C):
 - Use 40 µl per electroporation (keep on ice)
 - Or store the cells in small portions at -80 °C; let them defreeze on ice before use

Electroporation

- Place 40 µl cells in a pre-chilled electroporation cuvette with 1 µl DNA (100-500 ng vector DNA reconstituted in TE-, Tris-buffer or water; for transforming cells with ligation product use 500-1000ng DNA) and keep the cuvette on ice
- Use a Bio-Rad Gene Pulser, or similar, with following settings:
 - 2000 V
 - 25 µF
 - 200 Ω
- Pulse (normal reading is 4.5-5 ms)
- Add 1 ml G/L-M17B + 20 mM MgCl₂ + 2 mM CaCl₂
- Keep the cuvette for 5 min on ice and incubate 1-1.5 h at 30 °C
- Plate 10 µl, 100 µl, 900 µl on M17 agar with glucose or lactose and antibiotics (depends on plasmid)
- Incubate 1-2 days at 30 °C



Materials

Medium: G/L-SGM17B: M17-Bouillon with:

- 0.5 M sucrose
- 2.5% glycine
- 0.5% glucose or 0.5% lactose (strain dependent)

Add the sucrose and glycine to the M17-B and sterilize 20 min at 121 °C. Add sterile glucose or lactose after cooling down.

Others: 0.5 M sucrose + 10% glycerol
0.5 M sucrose + 10% glycerol + 0.05 M EDTA

L. lactis grows very slowly on the G/L-SGM17B. Leaving out the sucrose is possible (Wells *et al.*, 1993), but can decrease the transformation efficiency. The medium for cell recovery must contain MgCl₂ and CaCl₂.

3.6 Food grade selection of recombinant *L. lactis*

M17 medium

M17 medium is the commonly used growth medium for *L. lactis*. This medium is commercially available without carbon source.

Addition of carbon source for growth:

- 0.5% glucose or 0.5% lactose (all strains can grow on glucose; for growth on lactose *L. lactis* strain needs the lactose operon)

Elliker medium

Elliker medium is used for selection of Lac⁺ transformants. This medium is not commercially available without carbon source and needs to be prepared from its components.

Ingredients:

20 g/l	Tryptone
5 g/l	Yeast extract*
4 g/l	Sodium chloride
1.5 g/l	Sodium acetate (anhydrous)
0.5 g/l	L-(+) Ascorbic acid

For agar: 15 g/l agar

The pH is about 6.8, no adjusting necessary

Sterilization: 15 min at 121 °C

After sterilization: add 0.5% lactose or 0.5% glucose (stock: 20% solution)

* For less background of lactose-negative colonies, the yeast extract concentrations can be reduced to one half.



D-alanine auxotrophic *L. lactis* strain with *alr* gene-carrying plasmids can grow without alanine on M17 broth supplemented with 0.5% glucose.

Growth conditions

Lactococcus lactis grows at 30 °C, without aeration. Consult the literature in case of other organisms used in conjunction with the present plasmids.



4 Overview of Applications

Lactococci have been used by many laboratories for the expression of homologous and heterologous genes. The advantages are that they are food grade and do not produce endotoxins, they are relatively easy to manipulate, easy to grow and to process. Heterologous genes and operons come from other bacteria and eukaryotic organisms. Because of the possibility of *de-novo* gene synthesis virtually any protein of interest can be produced. There is a great interest in biotechnical application of lactococci for the development of live mucosal vaccines (Morello *et al.*, 2008; Pontes *et al.*, 2011). In recent years, they have also been used for production of milk protein-derived bioactive peptides in the human gastrointestinal tract (Korhonen and Pihlanto, 2006).

Constitutive gene expression can be scaled up to 3,000 liter, with almost identical fermentation characteristics and product yields (300 mg/l and more). The downstream processing is straightforward. With four unit operations - microfiltration, homogenization, second microfiltration, and chromatography - a product with 90% purity could be obtained. A substantial increase in yield can be achieved with careful optimization of the complete process (Mierau *et al.*, 2005b).

5 Bottlenecks for Gene Expression in *Lactococcus lactis*

Aerobic bacteria can normally be grown to a cell density that yields a dry biomass of far above 100 g/l. Because of the fermentative metabolism this is not possible for *L. lactis*. In a simple acidic culture medium, for instance, M17 the maximum cell density representing an OD₆₀₀ of 3 gives a dry cell mass of 1 g/l. In such condition, bacterial cells will stop growing when the pH of culture has nearly reached to 5. With neutralization using NaOH or NH₄OH the cell density can increase to an OD₆₀₀ of 15 that yields a dry cell mass of 5 g/l. The main reason for this limitation is the accumulation of lactic acid which consequently inhibits the growth. There have been attempts to develop high cell density cultivation methods for lactic acid bacteria and to apply those methods to recombinant protein production; however this technology is still cost-intensive → www.jurag.dk.

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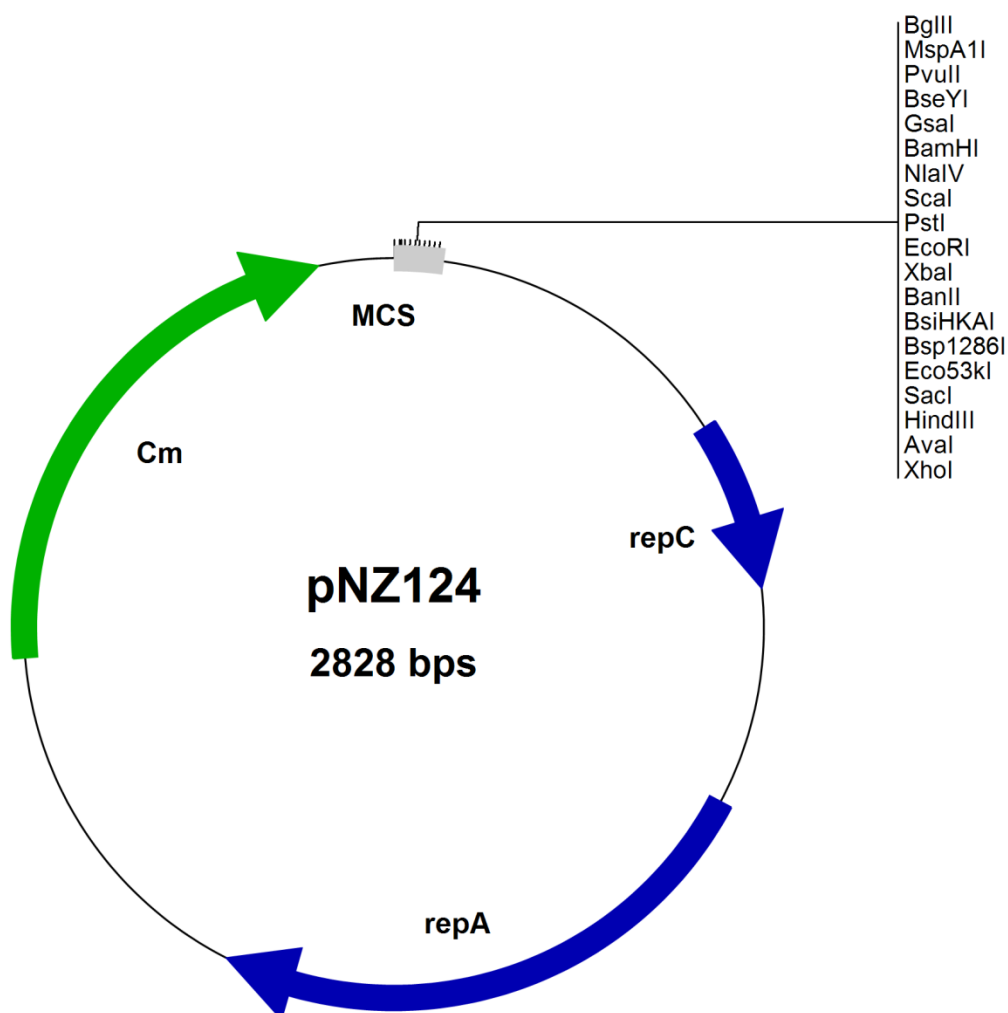
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7 Vector Maps

All vector maps and the complete DNA sequences are available for download on our website <http://www.mobitec.com>.

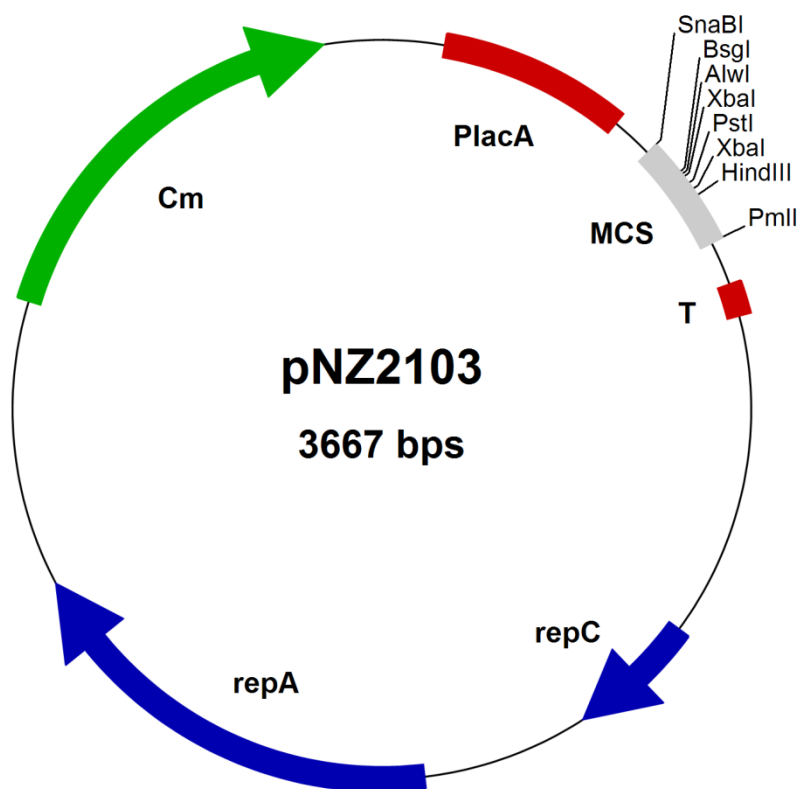
7.1 Vector map pNZ124



	Type	Start	End	Name	Description
	Region	1	60	MCS	Multiple Cloning Site
	Gene	449	658	<i>repC</i>	Replication gene C
	Gene	927	1625	<i>repA</i>	Replication gene A
	Selectable Genetic Marker	2085	2735	<i>Cm (cat)</i>	Chloramphenicol resistance (<i>L. lactis</i> / <i>E. coli</i>)



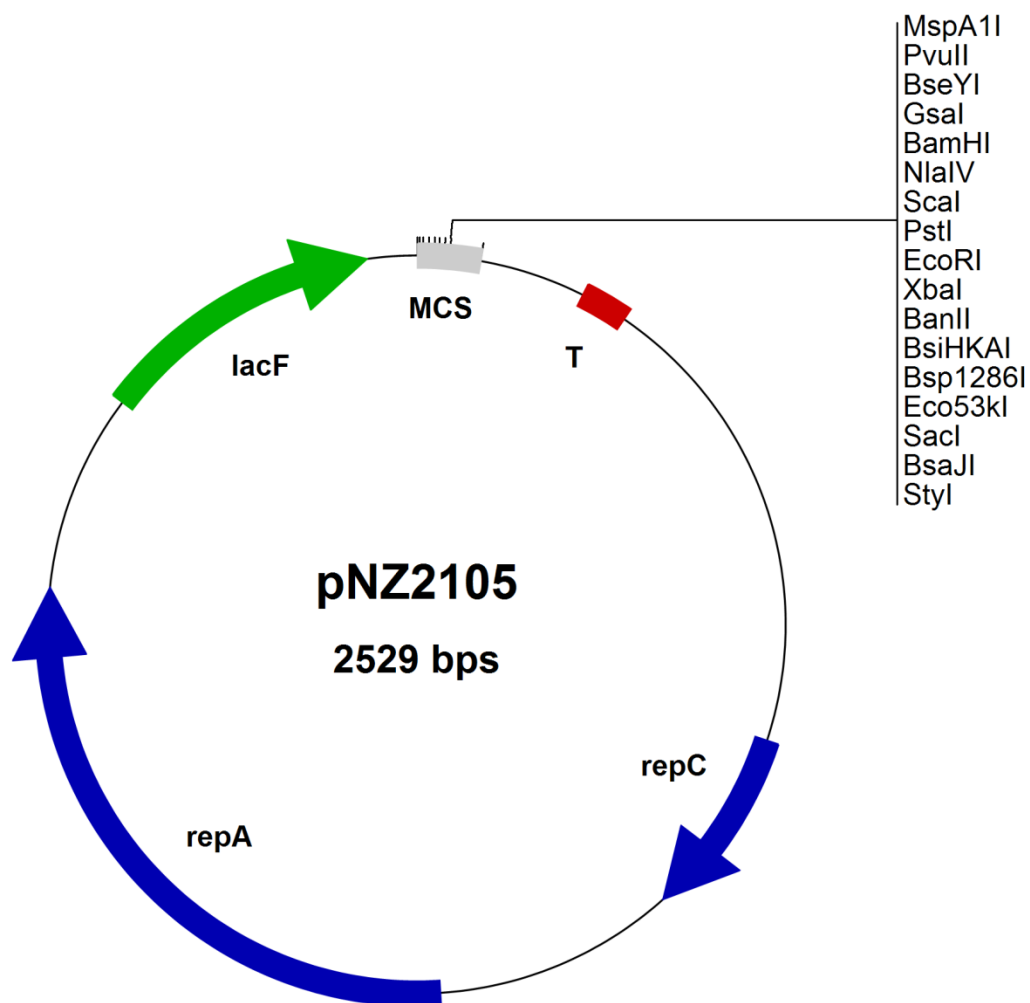
7.2 Vector map pNZ2103



	Type	Start	End	Name	Description
	Promoter	100	400	PlacA	<i>lacA</i> promoter region
	Region	469	644	MCS	Multiple Cloning Site
	Terminator	715	767	T	Terminator
	Gene	1288	1497	<i>repC</i>	Replication gene C
	Gene	1766	2464	<i>repA</i>	Replication gene A
	Selectable Genetic Marker	2924	33574	<i>Cm (cat)</i>	Chloramphenicol resistance (<i>L. lactis</i> / <i>E. coli</i>)



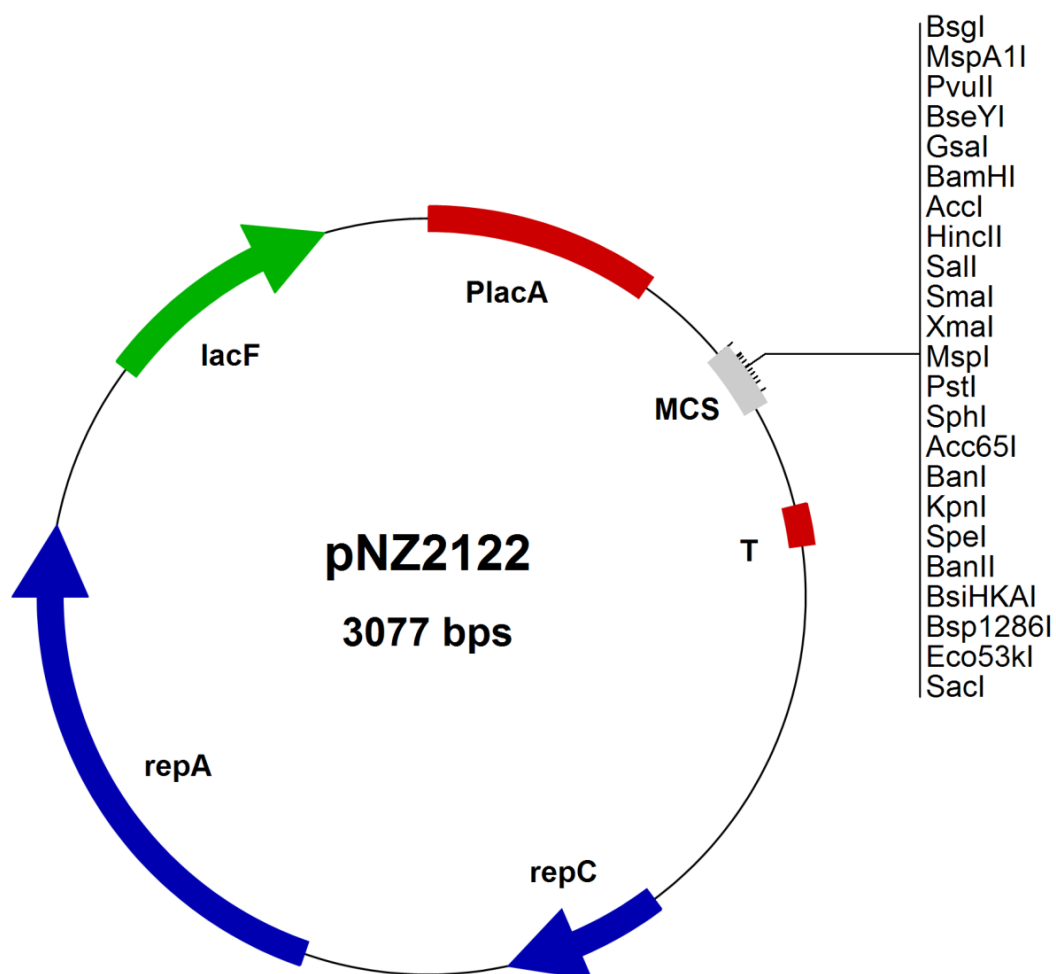
7.3 Vector map pNZ2105



	Type	Start	End	Name	Description
	Region	1	70	MCS	Multiple Cloning Site
	Terminator	188	240	T	Terminator
	Gene	761	970	<i>repC</i>	Replication gene C
	Gene	1239	1937	<i>repA</i>	Replication gene A
	Selectable Genetic Marker	2157	2474	<i>lacF</i>	Food-grade selection marker, <i>L. lactis lacF</i> gene



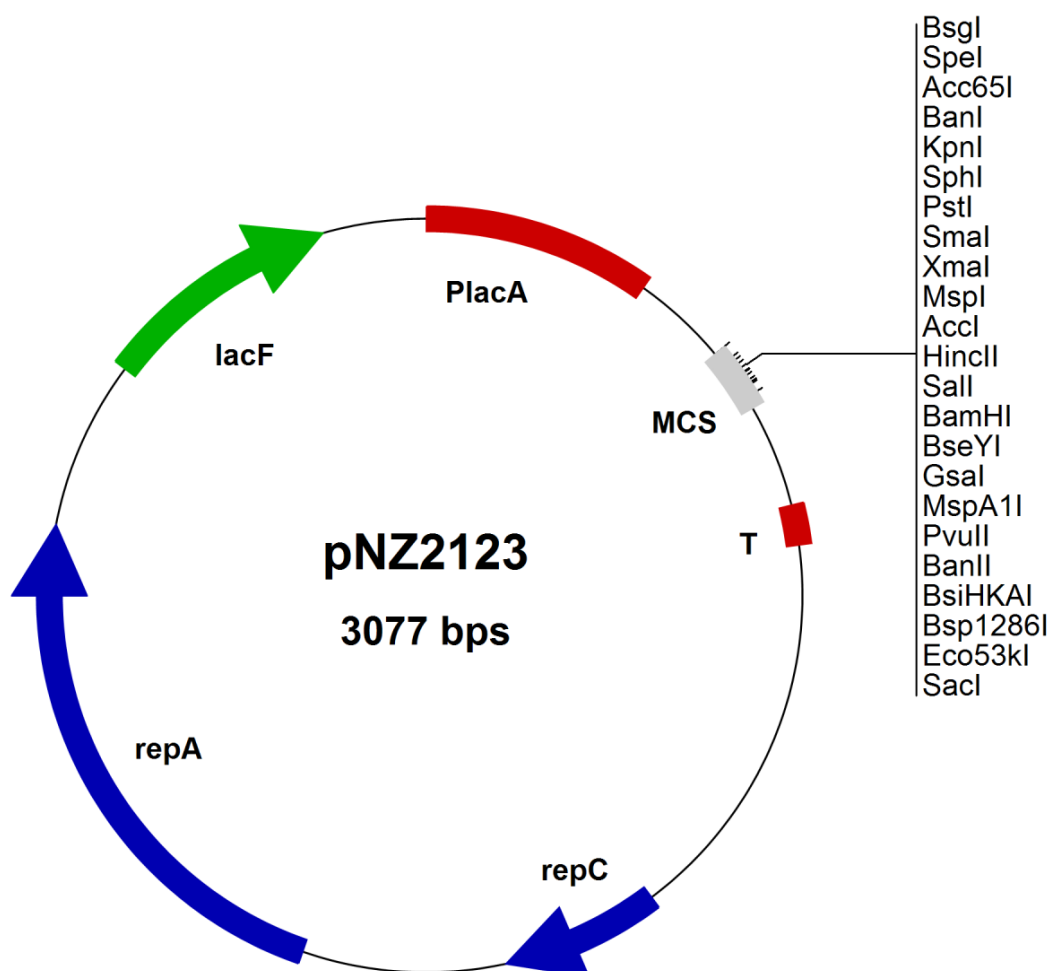
7.4 Vector map pNZ2122



	Type	Start	End	Name	Description
	Promoter	1	301	PlacA	<i>lacA</i> promoter region
	Region	429	514	MCS	Multiple Cloning Site
	Terminator	651	703	T	Terminator
	Gene	1224	1433	<i>repC</i>	Replication gene C
	Gene	1702	2400	<i>repA</i>	Replication gene A
	Selectable Genetic Marker	2624	2941	<i>lacF</i>	Food grade selection marker, <i>L. lactis lacF</i> gene



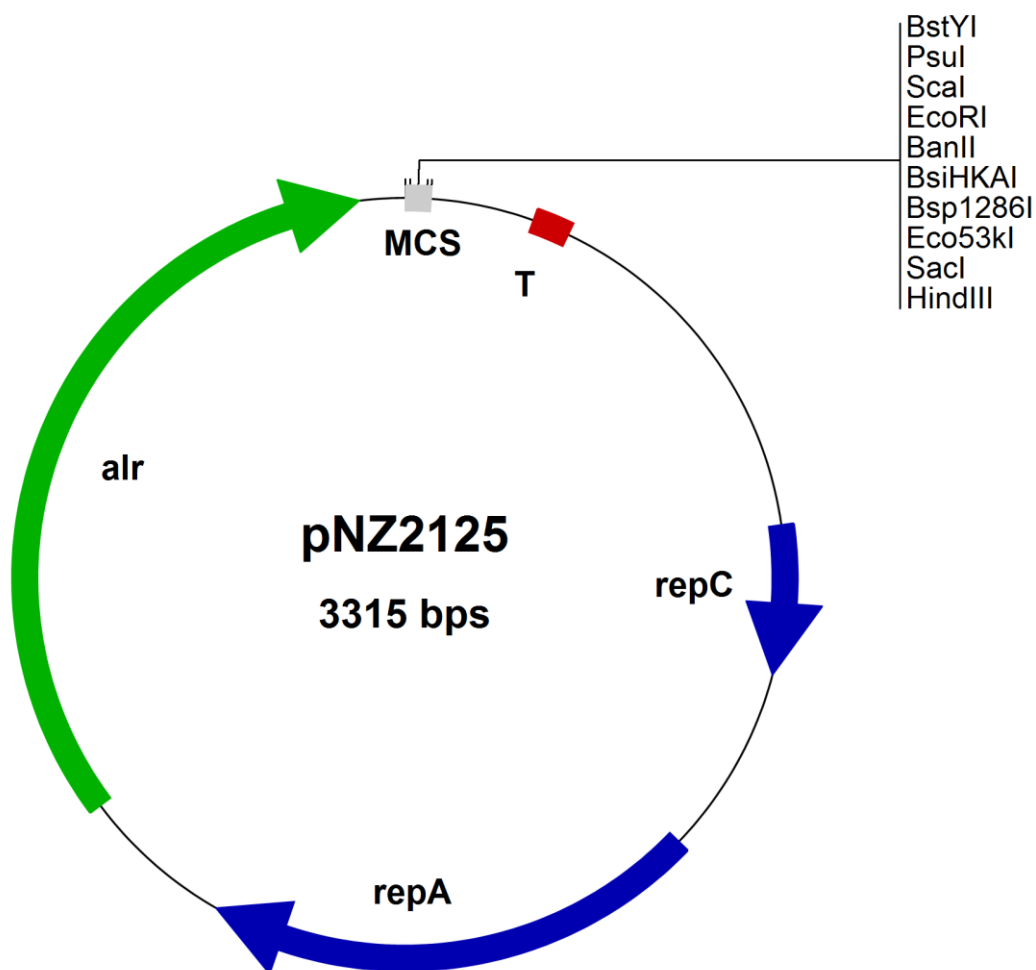
7.5 Vector map pNZ2123



	Type	Start	End	Name	Description
	Promoter	1	301	PlacA	<i>lacA</i> promoter region
	Region	429	514	MCS	Multiple Cloning Site
	Terminator	651	703	T	Terminator
	Gene	1224	1433	<i>repC</i>	Replication gene C
	Gene	1702	2400	<i>repA</i>	Replication gene A
	Selectable Genetic Marker	2624	2941	<i>lacF</i>	Food grade selection marker, <i>L. lactis lacF</i> gene



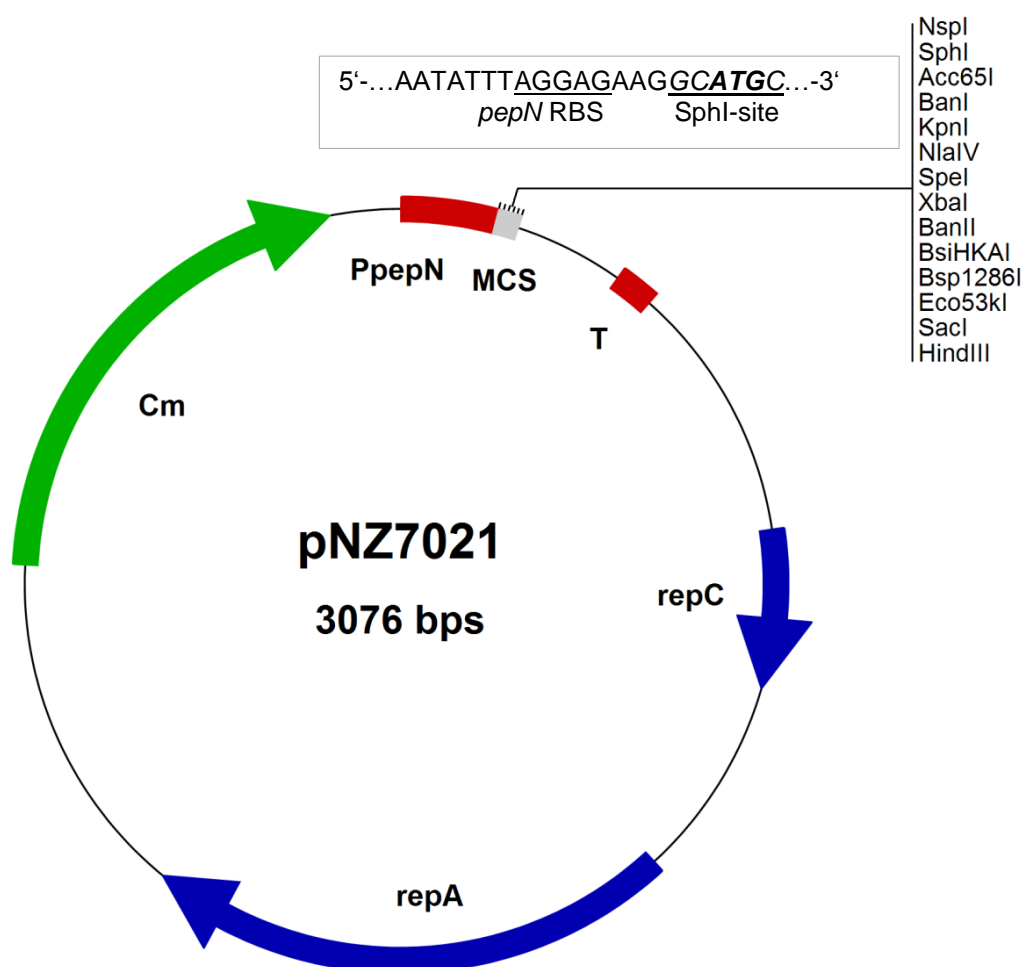
7.6 Vector map pNZ2125



	Type	Start	End	Name	Description
	Region	1	36	MCS	Multiple Cloning Site
	Terminator	182	234	T	Terminator
	Gene	755	964	<i>repC</i>	Replication gene C
	Gene	1233	1931	<i>repA</i>	Replication gene A
	Selectable Genetic Marker	2148	3251	<i>alr</i>	Food-grade selection marker, Alanine racemase gene



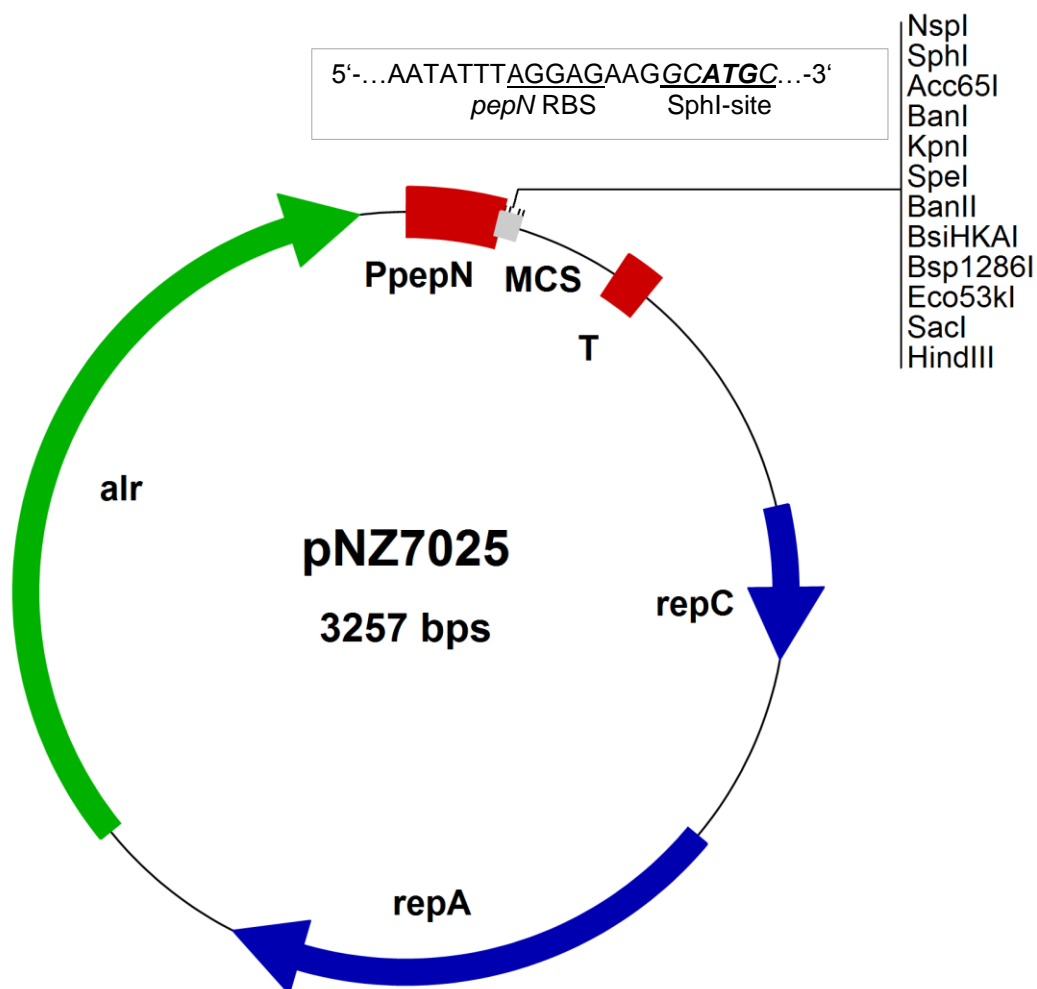
7.7 Vector map pNZ7021



	Type	Start	End	Name	Description
	Promoter	1	130	PpepN	Constitutive promoter
	Region	118	122	pepN RBS	pepN ribosome-binding site
	Region	126	156	MCS	Multiple Cloning Site
	Terminator	302	354	T	Terminator
	Gene	697	906	<i>repC</i>	Replication gene C
	Gene	1175	1873	<i>repA</i>	Replication gene A
	Selectable Genetic Marker	2333	2983	<i>Cm (cat)</i>	Chloramphenicol resistance (<i>L. lactis</i> / <i>E. coli</i>)



7.8 Vector map pNZ7025



	Type	Start	End	Name	Description
	Promoter	1	130	PpepN	Constitutive promoter
	Region	118	122	pepN RBS	pepN ribosome-binding site
	Region	126	156	MCS	Multiple Cloning Site
	Terminator	302	354	T	Terminator
	Gene	697	906	<i>repC</i>	Replication gene C
	Gene	1175	1873	<i>repA</i>	Replication gene A
	Selectable Genetic Marker	2090	3193	<i>alr</i>	Food-grade selection marker, Alanine racemase gene



8 Order Information, Shipping and Storage

Order#	Product	Quantity
VS-ELV01100	pNZ124 <i>Lactococcus lactis</i> cloning vector	10 µg
VS-ELV01150	pNZ2105 <i>Lactococcus lactis</i> cloning vector, food grade (lacF)	10 µg
VS-ELV01200	pNZ2103 <i>Lactococcus lactis</i> const. vector, lacA	10 µg
VS-ELV01250	pNZ7021 <i>Lactococcus lactis</i> const. vector, pepN	10 µg
VS-ELV01300	pNZ2122 <i>Lactococcus lactis</i> const. vector, mcs X-H, food grade (lacF)	10 µg
VS-ELV01350	pNZ2123 <i>Lactococcus lactis</i> const. vector, mcs H-X, food grade (lacF)	10 µg
VS-ELV01400	pNZ2125 <i>Lactococcus lactis</i> const. vector, food grade (alr)	10 µg
VS-ELV01450	pNZ7025 <i>Lactococcus lactis</i> const. vector, pepN, food grade (alr)	10 µg
lyophilized from water, shipped at RT; store at -20 °C		
VS-ELS01363	<i>Lactococcus lactis</i> expression strain MG1363	1 ml
VS-ELS03000	<i>Lactococcus lactis</i> expression Strain NZ3000, food grade, $\Delta lacF$	1 ml
VS-ELS01330-01	<i>Lactococcus lactis</i> expression Strain NZ1330, food grade, Δalr	1 ml
VS-ELS-10610-01	<i>E. coli</i> host strain MC1061	1 ml
VS-ELS10710-01	MC1061 Chemically Competent <i>E. coli</i>	5×100 µl
shipped on dry ice; store at -80 °C		

The Constitutive Gene Expression System was developed by NIZO food research BV.

9 Related Products

Also available from MoBiTec are:

- IPTG inducible gene expression systems for *Bacillus subtilis*
- Xylose inducible gene expression systems for *Bacillus megaterium*
- Nisin inducible gene expression system for *Lactococcus lactis*

For details please check www.mobitec.com.

10 Contact and Support

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