Bacillus megaterium
Protein Production System

21.03.2016

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

Content

1. Features

1.1. Recombinant protein production with *Bacillus megaterium*

- Stable, high-yield production of recombinant proteins using *Bacillus megaterium*
- Growth rate comparable to that of *Escherichia coli*, but reaching higher final optical density (OD)
- No alkaline proteases activity even up to 5 hours after induction
- No endotoxins
- MoBiTec host strains are asporogenic on common media.
- *B. megaterium* is non-pathogen.

1.2. Vectors for xylose-inducible gene expression

- Vectors structurally and segregationally stable
- Ideal for both small- and large-scale protein production
- Convenient cloning due to *B. megaterium*/ *E. coli* shuttle vectors with an extended multiple cloning site (MCS) downstream of the promoter
- Tightly regulated promoter and efficiently inducible expression (up to 350-fold)
- Vectors for intracellular or extracellular protein production available
- Diverse tags allow easy purification of the recombinant proteins (6xHis-tag, Strep-tag II, Strep II/6xHis double-tag)
- Removable tag versions available (TEV or Xa protease cleavage site included)
- System is compatible for other *Bacillus* ssp.

2. Introduction

Bacillus megaterium is a rod-shaped, non-pathogenic Gram-positive bacterium, which is able to grow on a wide variety of carbon sources. Like other soil bacteria, it is able to secrete high amount of protein into the culture medium (up to 0.5 g/L of recombinant protein). This property, together with its ability to grow in many ecological niches such as waste from meat industry, melasse or petrochemical effluents makes it an interesting organism for industrial-scale production of recombinant proteins. In addition to *B. subtilis*, it has been used as Gram-positive model organism for studying several research topics such as biochemistry, sporulation, and bacteriophages.
The name "*megaterium*" is due to its large size of vegetative cells (>10 µm). *B. megaterium* cells often occur in pairs and chains where they are joined together by polysaccharides on their cell walls. An overview about the features of this unique organism has been described in the following review articles: Vary (1994), Vary *et al.* (2007), Bunk *et al*. (2010), and Schulz *et al*. (2014).

One of the genetic regulatory elements for carbon metabolism of *B. megaterium* is the xylose operon that has been investigated and well characterized by Rygus and Hillen (1991) many years ago.
MoBiTec is offering diverse xylose-inducible *E. coli*/ *B. megaterium* shuttle vectors and different *B. megaterium* strains, which are provided as protoplasts ready for

transformation. All vectors are multi-copy plasmids, well suited for both molecular cloning in *E. coli* and xylose-induced protein expression in *B. megaterium*. Different signal peptides for protein secretion and affinity tags for purification (including removable tag versions) arranged in a high variety of combinations are available. By using this system, recombinant proteins can be produced, secreted, and purified in the g/L scale.
A large number of proteins were successfully produced using *B. megaterium* system, e.g.:

- Antibody fragments (scFv, scFab)
- β-Galactosidase
- Catabolite control protein (CcpA)
- *Clostridium difficile* toxin A
- Cobaltochelatase (CbiX)
- Dextransucrase (DsrS) - secreted
- Endolevanase (LevB) - secreted
- Formiate dehydrogenase (Fdh)
- Glucose dehydrogenase (GdhA)
- Green fluorescent protein (GFP)
- Heat shock protein (HPr) from PTS (phosphotransferase sugar transport system)
- Human single-chain urokinase-like plasminogen activator (rscuPA)
- Hydrolase of *Thermobifida fusca* (Tfh) - secreted
- Levansucrase (LevΔ773, SacB) - secreted
- Mannitol dehydrogenase (Mdh)
- Mutarotase (Mro)
- Neopullulanase
- Translocation ATPase of the preprotein translocase (SecA)
- Trehalose repressor (TreR)

Protein yields vary depending on the produced protein itself. Rygus and Hillen (1991) have observed that, e.g., GdhA and Mro accumulated to 20% and 30% of the total soluble protein, respectively. The time dependence of the induced expression of these enzymes is shown in Fig. 1.

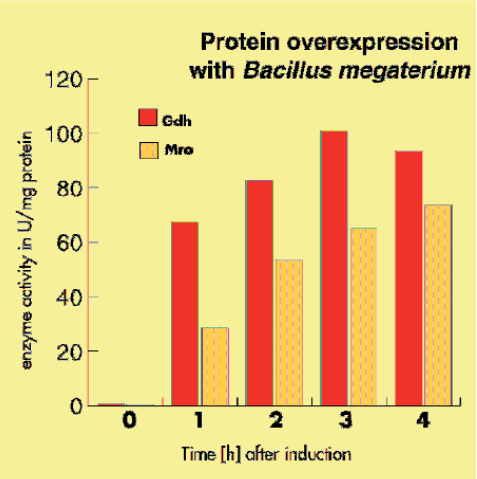

Fig. 1: Time dependence production of the enzymes GdhA (glucose dehydrogenase) and Mro (mutarotase) in *B. megaterium*. Enzymatic activity is indicated in U/mg protein.

3. Expression Vectors

With exception of pMGBm19, all shuttle vectors are derived from the original pWH1520 vector (Rygus and Hillen, 1991) with following features:

- Xylose-inducible expression system: strong P_{xylA} promoter and repressor gene *xylR*; after xylose addition, the repressor is released from the P_{xylA} that activates transcription initiation
- Ribosomal binding site (RBS) and start codon (ATG) upstream of the multiple cloning site (MCS)
- The MCS is located within the *xylA'* reading frame and allows easy cloning due to identical restriction sites in all vectors (from BglII to NaeI, except for the pWH1520)
- Replication origins: ColE1 ori (*E. coli*) and pBC16 ori (*Bacillus*)
- *repU* gene encoding for the replication protein RepU of *Staphylococcus aureus*, necessary for replication in *Bacillus*
- Resistance genes for selection: *β-lactamase* (ampicillin resistance for *E. coli*), *tetL* encoding a tetracycline efflux pump (tetracycline resistance for *Bacillus*)

- There are two kinds of vector series available:
  → **vectors of the 1520 series:** with a) far-off located stop codon (> 120 bp downstream of start codon or b) stop codon directly downstream of an existing C-terminal tag
  → **vectors of the 1622 series:** size-reduced vector variants with a) closely located stop codon directly downstream of the MCS or b) stop codon directly downstream of an existing C-terminal tag (Malten *et al*., 2006)

Table 1: Overview of vector features including tag and secretion options. Highlighted in blue: secretion vectors

Vector	Secretion	N-terminal tag		C-terminal tag		tag cleavable
		6xHis	StrepII	6xHis	StrepII	
pWH1520	–	–	–	–	–	n/a
pMM1522	–	–	–	–	–	n/a
pMM1525	✓	–	–	–	–	n/a
pHIS1522	–	–	–	✓	–	–
pHIS1525	✓	–	–	✓	–	–
pSTREP1525	✓	–	✓	–	–	✓
pSTREPHIS1525	✓	–	✓	✓	–	✓ (**StrepII**)
pSTOP1622	–	–	–	–	–	n/a
pC-His1622	–	–	–	✓	–	–
pC-Strep1622	–	–	–	–	✓	–
pN-His-TEV1622	–	✓	–	–	–	✓
pN-Strep-TEV1622	–	–	✓	–	–	✓
pN-Strep-Xa1622	–	–	✓	–	–	✓

Vectors of the 1520 series

pWH1520
Original shuttle vector pWH1520 (Rygus and Hillen, 1991). Features as described above.

pMM1522
Like pWH1520 with additional restriction site BsrGI upstream of the ATG. The BsrGI site can be used to clone the gene of interest with its native start codon (corresponding to the native N-terminus of the protein of interest). The vector contains a stop codon downstream of the ATG (> 120 bp).

pMM1525
Like pMM1522 with additional signal sequence for protein secretion (SP_{lipA}) upstream of the MCS (the native *lipA* encodes for an extracellular esterase).

pHIS1522
Like pMM1522 with additional sequence for C-terminal 6xHis-tag fusion (including stop codon).

pHIS1525
Like pMM1525 with additional sequence for C-terminal 6xHis-tag fusion (including stop codon).

pSTREP1525
Like pMM1525 with additional sequence for N-terminal Strep-tag fusion (cleavable with Factor Xa protease) and stop codon downstream of the MCS.
Note: this vector does not contain BsrGI restriction site.

pSTREPHIS1525
Like pMM1525 with additional sequences for N-terminal Strep-tag (cleavable with Factor Xa protease) and C-terminal His-tag fusion (including stop codon).
Note: this vector does not contain a BsrGI restriction site.

Vectors of the 1622 series

pSTOP1622
Size-reduced pMM1522 variant with a stop codon directly downstream of the MCS.

pC-His1622
Size-reduced pHIS1522 variant with sequence for C-terminal 6xHis-tag fusion (including stop codon right downstream of the tag).

pC-Strep1622
Size-reduced pHIS1522 variant with sequence for C-terminal StrepII-tag fusion (including stop codon directly downstream of the tag).

pN-His-TEV1622
Like pSTOP1622 with additional sequence for N-terminal 6xHis-tag fusion, cleavable with TEV (tobacco etch virus) protease.

pN-Strep-TEV1622
Like pSTOP1622 with additional sequence for N-terminal fusion of StrepII-tag, cleavable with TEV (tobacco etch virus) protease.

pN-Strep-Xa1622
Like pSTOP1622 with additional sequence for N-terminal fusion of StrepII-tag, cleavable with Factor Xa protease.

Vectors for special requirements

pMGBm19
pMGBm19 is an *E.coli*/ *Bacillus* shuttle vector with xylose-inducible promoter (P_{xylA}) that is designed for co-expression studies. It can be used in combination with any other vector of the 1520, 1622, and 1623hp series, since it contains an origin of replication of a different compatibility group (pMB100 replicon).

pMMEc4
Since the xylose-inducible P_{xylA} promoter is not tightly controlled in *E. coli*, cloning the toxic genes into vectors of the 1520, 1622, and 1623hp series, respectively, using *E. coli* as host may be difficult. In such cases, we recommend using the pMMEc4 helper plasmid. This *E. coli* vector (not replicating in *Bacillus*!) encodes xylose repressor XylR and is designed for blocking any expression starting from the P_{xylA} promoter while cloning gene of interest within *E. coli* (Jordan *et al.*, 2007).
In pMMEc4 the expression of *xylR* is controlled by the arabinose-dependent promoter P_{BAD} and the AraC protein. In the presence of 0.2% arabinose, the AraC protein binds to the operator sequence that activates the expression of the *xylR* gene, and additionally upregulates its own expression. The vector pMMEc4 carries the p15A origin of replication that is compatible with vectors from other incompatibility groups such as ColE1.

Control vectors

Vectors indicated below in the table are available as positive controls for expression in *B. megaterium* as well as for one-step affinity purification (except for pGFP1522). The vectors encode the GFP and different mutant proteins of levansucrase, an enzyme of *Lactobacillus reuteri*.

Vector backbone	Control vector	Encoded protein	Secretion	Order #
pMM1522	**pGFP1522**	**GFP**	**–**	**BMEG10C**
pHIS1525	**pRBBm15C**	**LevΔ773His**	**✓**	**BMEG13C**
pSTREP1525	**pRBBm13C**	**StrepLevΔ773**	**✓**	**BMEG14C**
pSTREPHIS1525	**pRBBm16C**	**StrepLevΔ773His**	**✓**	**BMEG15C**

Please note that these vectors are available only in combination with a regular *B. megaterium* expression vector. For background information regarding control vectors for secretion, please see Malten *et al*. (2006).

4. Strains

MoBiTec offers three different *B. megaterium* strains (WH320, MS941, and YYBm1) for protein production. All strains are supplied as protoplasts, ready-to-use for transformation.

1. **The strain WH320** is a chemical mutant of strain DSM319, which is deficient in the production of β-galactosidase (Δ*lacZ*). It was described by Rygus and Hillen (1991).

2. **The strain YYBm1** carries the *nprM* deletion and an additional deletion of the xylose isomerase gene *xylA*. It is thus unable to metabolize xylose, which is used as inducer for gene activation (Yang *et al*. 2006).

3. **The strain MS941** was generated from the wild-type strain DSM319 by deletion of major extracellular protease gene *nprM* (Wittchen and Meinhardt 1995). Because of reduced extracellular protease activity, this strain is well suited for extracellular protein production.

5. Cloning Strategies

Based on the nature of your target gene it is important to choose an appropriate expression vector and cloning strategy.

Gene of interest with own RBS and ATG start codon
If the gene of interest (*goi*) provides its own RBS and start codon, the fragment can be inserted into any cloning site on the vector (located in the reading frame of *xylA'*). Transcription will start from the P_{xylA} promoter, forming one bicistronic transcript (*xylA'-goi*). This bicistronic mRNA will then be translated into two individual proteins: XylA' and the protein of interest.
Please consider the following for your cloning strategy: the insertion of the gene of interest into the *xylA'* gene may create a new stop codon for translation termination of *xylA'*. This may lead to translational coupling, positively or negatively influencing the translation efficiency of the gene of interest:

<u>Positive translational coupling</u>

If the stop codon of *xylA'* and the downstream located start codon of the gene of interest are close together, translational coupling may occur: ribosomes translating the *xylA'* reading frame would terminate at the newly created stop codon, generating a locally high concentration of ribosomes, so that translation initiation at the proximal start codon of the gene of interest would be more efficient compared to a construct in which the *xylA'* translation terminates farther away from the start codon.

<u>Negative translational coupling</u>

If a new stop codon of *xylA'* is located within the sequence of the gene of interest, the reading frame of *xylA'* will overlap with the reading frame to the gene of interest. Ribosomes translating the new created *xylA'* fusion gene might thereby inhibit initiation of translation of the gene of interest. This will lead to a reduced production of the protein of interest.

Gene of interest without RBS but with own start codon

Intracellular protein production

The BsrGI restriction site enables target gene cloning with its native start codon. Please note that there is no BsrGI site in the following vectors: pWH1520, pSTREP1525, and pSTREPHIS1525

Protein production by secretion

Target genes can be directly inserted downstream of the signal sequence encoding the signal peptide SP_{lipA} by using the following restriction sites: KasK, NarI, or SfoI. These restriction sites are suitable for cloning with the following vectors: pMM1525, pHIS1525.

Gene of interest without RBS and with/without ATG start codon

The gene of interest (in full length or as truncated version) can be fused in-frame to the *xylA'*-gene to create a translational fusion. Depending on the vector, this will result in expression of a fusion protein consisting of the protein of interest and additional amino acids:

- parts of the XylA' protein sequence
- the signal peptide SP_{lipA} (enabling secretion)
- tag sequence(s) (6xHis, Strep, or Strep/6xHis-tag)

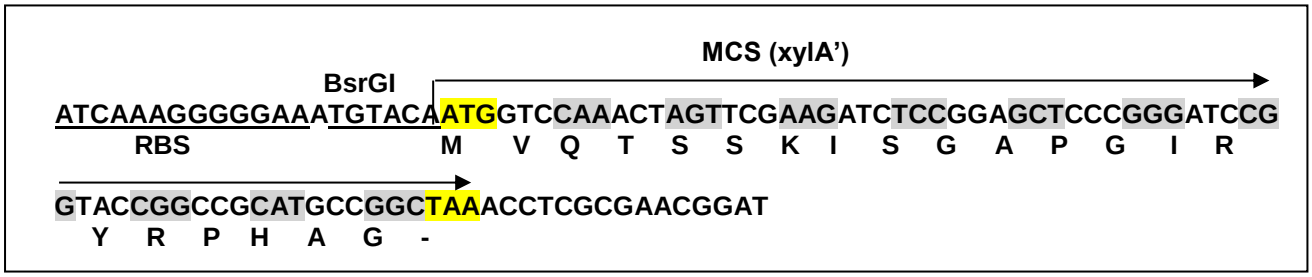

Fig. 2: Detailed graphic of the Multiple Cloning Site of pSTOP1622.
The MCS starts with the start codon of *xylA*' and ends with an early stop codon (both highlighted in yellow). For cloning the gene of interest with its native N-terminus, an additional restriction site (BsrGI) is provided upstream of the ATG. RBS: ribosomal binding site

6. Protocols

6.1. Cloning the DNA fragment of interest

The *E. coli*/ *B. megaterium* shuttle vectors are supplied as lyophilized DNA. Green and Sambrook (2012) describe all standard protocols for propagation of the plasmid in *E. coli*, plasmid DNA preparation, restriction endonuclease cleavages, and ligation of the DNA fragment of interest into the vector. After ligation of the insert the vectors should be propagated in *E. coli* (amp^R) before transforming into *Bacillus* protoplasts (tet^R). For all cloning purposes and propagation of plasmids we recommend to use *E. coli* strain DH10B.

6.2. General remarks on the handling of *B. megaterium*

B. megaterium strains grow well on rich media such as Luria-Bertani (LB) broth at 30 °C and 37 °C. Make sure to aerate liquid cultures well in baffled flasks by vigorous agitation.
We found MS941, WH320, YYBm1, and derived strains to be asporogenic on common growth media - they will die on the plates stored at 4 °C within two weeks, so prepare

glycerol stocks (30% w/v) as a backup and streak the working cultures on fresh plates every 7 to 10 days.
Positive clones carrying the plasmid with a gene of interest can be selected by adding 10 µg/ml of tetracycline to the growth medium.
To prove successful overexpression of the target gene harvest small samples of the culture just before, and at certain time intervals after induction of recombinant gene expression with xylose. To obtain crude extracts for protein analysis, cells have to be lysed using lysozyme. Simple boiling of cells in sample buffer (Laemmli, 1970), which is quite convenient for *E. coli*, does not lyse *B. megaterium* cells.

6.3. Transformation of *Bacillus megaterium* protoplasts

For recombinant protein production *B. megaterium* protoplasts are transformed with the plasmids coding for the protein of interest. After transformation it is advisable to screen at least three different clones for protein production as the yield can vary among different clones.
Since intact *B. megaterium* cells cannot be transformed, MoBiTec conveniently provides protoplasts of *B. megaterium* cells, which are ready for transformation (strains MS941, WH320, and YYBm1). They can be used at least 2 months after date of arrival and have to be stored at -80 °C. The protoplast suspension is supplied in 5 aliquots of 500 µl each to prevent multiple freezing and thawing of protoplasts that are not used immediately. One aliquot is provided per transformation. It is advisable to use two of the vials for the control experiments as described below.

Control experiments

1. Negative control: protoplasts without DNA

This is a test reassuring that the protoplasts are not only fully viable but also free of contaminations before using them for transformation. Perform this test according to the transformation protocol as demonstrated below. After incubation at 30 °C, apply CR5-top agar to the protoplasts and split the sample in two portions. You may plate one sample on a LB plate with antibiotic such as tetracycline or chloramphenicol, and another one on a plate without any drug. In this case, bacterial colonies will grow only on a solid medium without antibiotics.

2. Positive control: protoplasts transformed with an empty plasmid

This is a test control for a successful transformation and should yield lots of colonies on the plates supplemented with an antibiotic (here: tetracycline or chloramphenicol). If this transformation works well, but you have problems with the plasmid containing your target gene, the problem is most likely associated with your construct.

Transformation protocol:

1. Combine 500 µl of protoplast suspension and 3-5 µg of plasmid DNA in a 15 ml tube, one for each transformation. DNA should be purified using a commercial preparation kit. Elute the DNA from the column using water.
2. Add 1.5 ml of PEG-P, mix gently, and incubate for 2 minutes at RT.
3. Add 5 ml SMMP and mix carefully by rolling the tube.
4. Harvest cells by gentle centrifugation (1,300 x g for 10 minutes at RT), discard the supernatant immediately after centrifugation. Supernatant does not have to be removed completely.
   (*Note: do not check for a cell pellet - most of the time it will be invisible*)

5. Add 500 µl of SMMP to remaining supernatant (containing bacterial cells) and transfer to a 1.5 ml microcentrifuge tube.
6. Incubate at 30 °C for 90 minutes with gentle shaking or rolling of tubes (max. 100 rpm) or incubate for 45 min without shaking followed by another 45 min while shaking at 300 rpm.
7. Prepare 2.5 ml aliquots of CR5-top agar in sterile tubes.
8. After incubation at 30 °C add all cells to 2.5 ml top agar, mix gently by rolling the tube between both hands (do not vortex!), and pour onto a pre-warmed plate of LB containing desired antibiotic.
9. Incubate overnight at 30 °C - expect colonies of varying diameter because some will be covered with agar and others have easier access to air.
   (*Note: bacterial colonies on the top of the agar surface will be shiny*)
10. Streak several single colonies (at least 3) on fresh plates within two days.

Note: *Protein production may vary among the single colonies due to yet unknown reasons.*

6.4. Recombinant protein production and secretion

Test protein production

1. Prepare an overnight culture inoculated with *B. megaterium* single colonies from a plate (medium including antibiotic such as tetracycline or chloramphenicol) grown 14 h at 37 °C while agitation at 100 rpm.
2. Inoculate fresh medium with overnight culture in a dilution of 1:100.
3. Grow the recombinant *B. megaterium* cells in baffled flasks to an optical density (OD_{578nm}) of 0.3 - 0.4 at 37 °C under vigorous shaking (250 rpm).
4. Take a sample as control before induction.
5. Induce the xylose inducible promoter by addition of 0.5% (w/v) of (D)-xylose.
6. Incubate at 37 °C with vigorous shaking at 250 rpm.
7. Withdraw samples every 30 to 60 minutes for OD_{578nm}-measurement and protein analysis (up to 6 hours after induction). For extracellular protein analysis take 2 ml of cell culture. Intracellular protein analysis requires a higher volume than 2 ml of sample.
8. Centrifuge each sample to harvest cells and cell free supernatant.
9. For extracellular protein analysis store the cell free supernatant at 4 °C, and for intracellular protein analysis completely remove supernatant and freeze the cell pellet at -20 °C.

Analysis of intracellular proteins

1. Resuspend cells in 30 µl of lysis buffer.
2. Incubate for 30 min at 37 °C while shaking at 1,000 rpm in a thermomixer. An effective cell lysis can be obtained by whirling the samples every 10 minutes.
3. To separate insoluble fraction (pellet) from the soluble fraction (supernatant), centrifuge cell lysate for 30 min at 4 °C and 13,000 rpm.
4. Mix 27 µl of supernatant containing soluble proteins with 13 µl of SDS sample buffer.
5. Completely remove the supernatant and resuspend the pellet in 30 µl of 8 M urea (w/v). Centrifuge for 30 min at 4 °C and 13,000 rpm.
6. Mix 27 µl of the supernatant with 13 µl of SDS sample buffer.
7. Heat each sample for 5 min at 95 °C.

8. Load 7.5 µl of each sample onto an SDS-PAGE gel.

Ammonium sulfate precipitation of proteins in the cell-free supernatant

1. Add 600 mg of ground ammonium sulfate to 1.5 ml of cell-free supernatant and incubate for 2 hours at 4 °C while vigorous shaking.
2. Centrifuge for 30 minutes at 4 °C and 13,000 rpm.
3. Completely remove the supernatant, centrifuge for 1 min again, and make sure that the protein pellet remains free of any fluid.
4. Add 10 µl of 8 M urea (in 50 mM Tris-HCl, pH 7.5), and 5 µl of SDS sample buffer to dissolve the proteins.
5. Spin briefly at 13,000 rpm, heat at 95 °C for 5 minutes, and load onto an SDS-PAGE for analysis.
6. Determine enzymatic activities with the appropriate assays (not included in the kit).
7. Perform Western blot using appropriate antibodies (not included in the kit).

Scale up protein production

1. Grow larger culture and induce as described above.
2. Harvest cells at the time point of maximal protein overproduction, as determined by the test experiment.

7. Materials

2 x AB3 (Antibiotic Medium No. 3, DIFCO)

- 7 g AB3 (Difco) in 200 ml deion. water
- autoclave 15 min

2 × SMM (solubilize in the given order in 150 ml of deion. water!)

- 1.16 g maleic acid (40 mM)
- 800 mg NaOH (80 mM)
- 2.03 g $MgCl_2$ x $6H_2O$ (40 mM)
- 85.58 g sucrose (1 M)
- mix and fill with deion. water to 250 ml
- sterilize by filtration

SMMP

- 2 × AB3 and 2 × SMM 1:1 (freshly prepared!)

PEG-P

- solubilize 20 g PEG-6000 with 1 × SMM and fill to 50 ml
- autoclave 11 min

CR5-top agar
prepare separately for 500 ml:

solution A
- 51.5 g sucrose
- 3.25 g MOPS
- 300 mg NaOH
- add deionized water to 250 ml
- adjust pH to 7.3 with NaOH
- sterilize by filtration

solution B
- 2 g agar
- 100 mg casamino acids
- 5 g yeast extract
- add deion. water to 142.5 ml
- autoclave for 15 min

8 × CR5 salts
- 1.25 g K_2SO_4
- 50 g $MgCl_2 \times 6\ H_2O$
- 250 mg KH_2PO_4
- 11 g $CaCl_2$
- solubilize in 625 ml deion. water
- autoclave for 15 min

12% proline
- 3 g L-proline
- add deion. water to 25 ml
- sterilize by filtration

20% glucose
- 5 g glucose
- add deion. water to 25 ml
- sterilize by filtration

for a 2.5 ml portion of CR5-top agar add the following (in the given order!):
- 1.25 ml **solution A**
- 288 µl CR5 salts
- 125 µl 12% proline
- 125 µl 20% glucose

90 minutes after transformation:
- boil **solution B**
- add 713 µl to the provided CR5-top agar

- immediately add the regenerated protoplasts, and put onto prewarmed agar plates containing appropriate antibiotic (tetracycline or chloramphenicol).

lysis buffer

- 100 mM Na_3PO_4
- 5 mg/ml lysozyme
- pH 6.5 (adjust with H_3PO_4)
- add 1 µl of a 1 M $MgSO_4$ solution and
- 2 µl HS-Nuclease* (250 U/µl, cat.# GENUC10700-01, final concentration of 500 U/ml) per ml lysis buffer shortly before use.

* HS-Nuclease is not available in the US and Canada, but it can be purchased as TurboNuclease at Accelagen.

8. References

General

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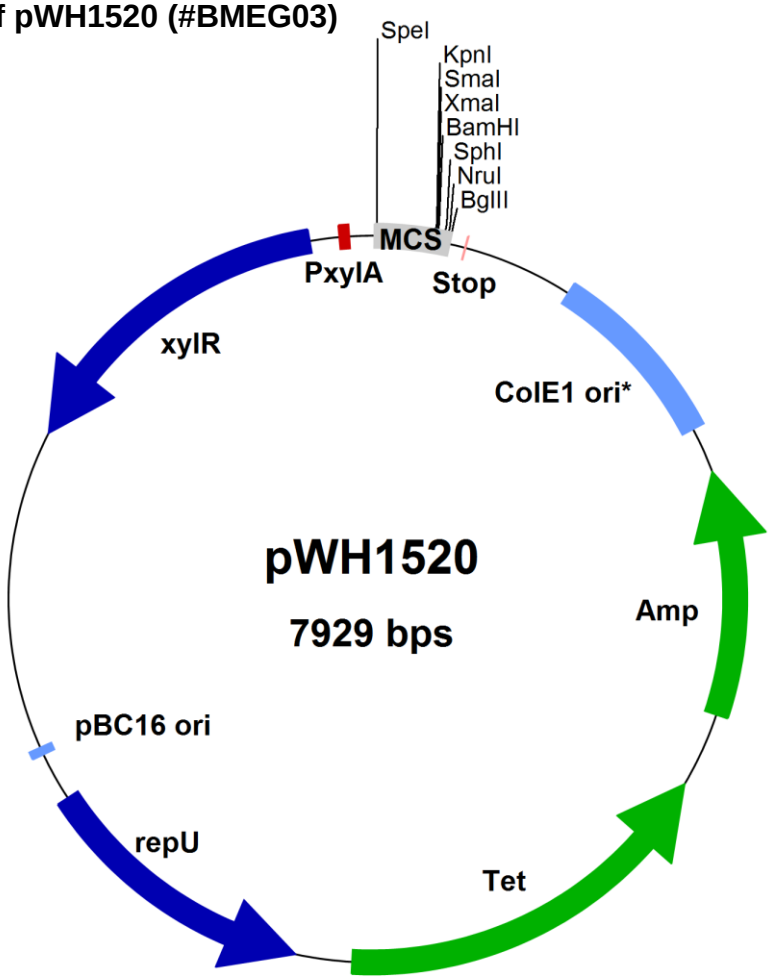
9. Vector Maps

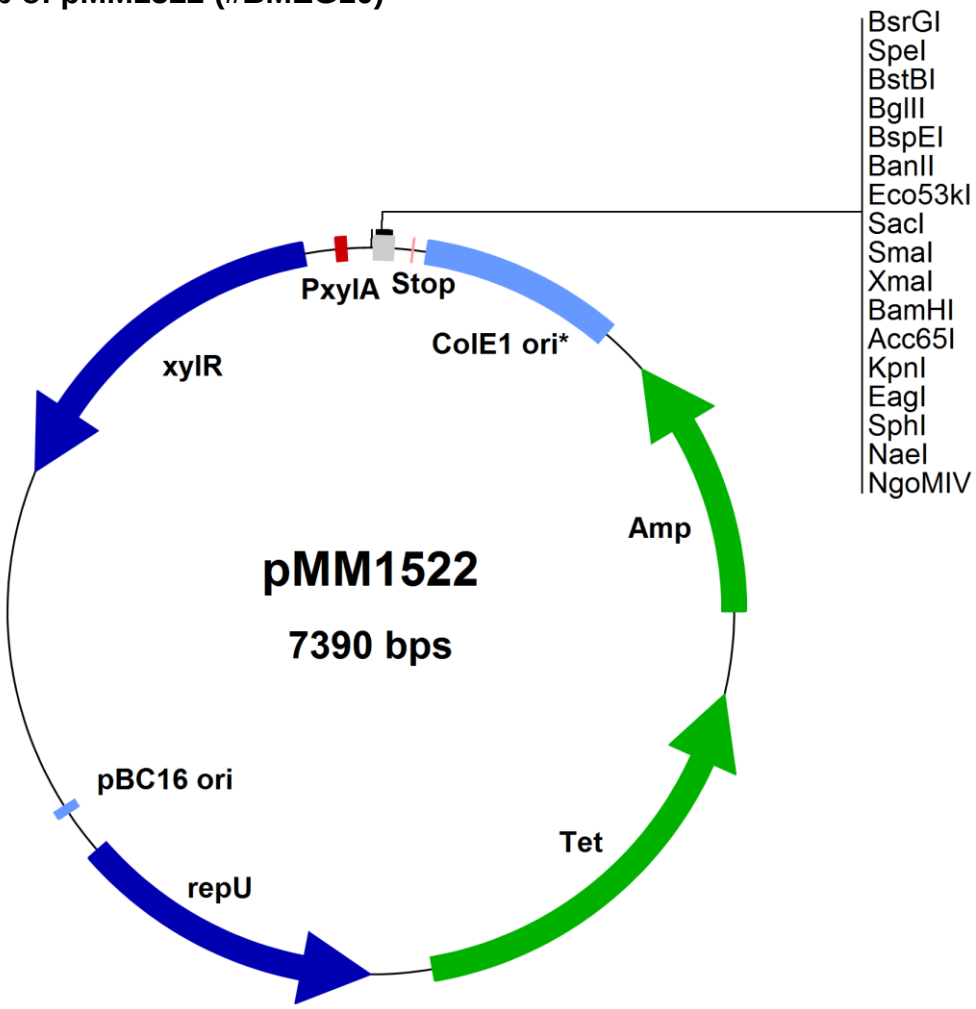
All vector maps and the complete DNA sequences are available for download on our web site at http://www.mobitec.com.

9.1. Vector map of pWH1520 (#BMEG03)



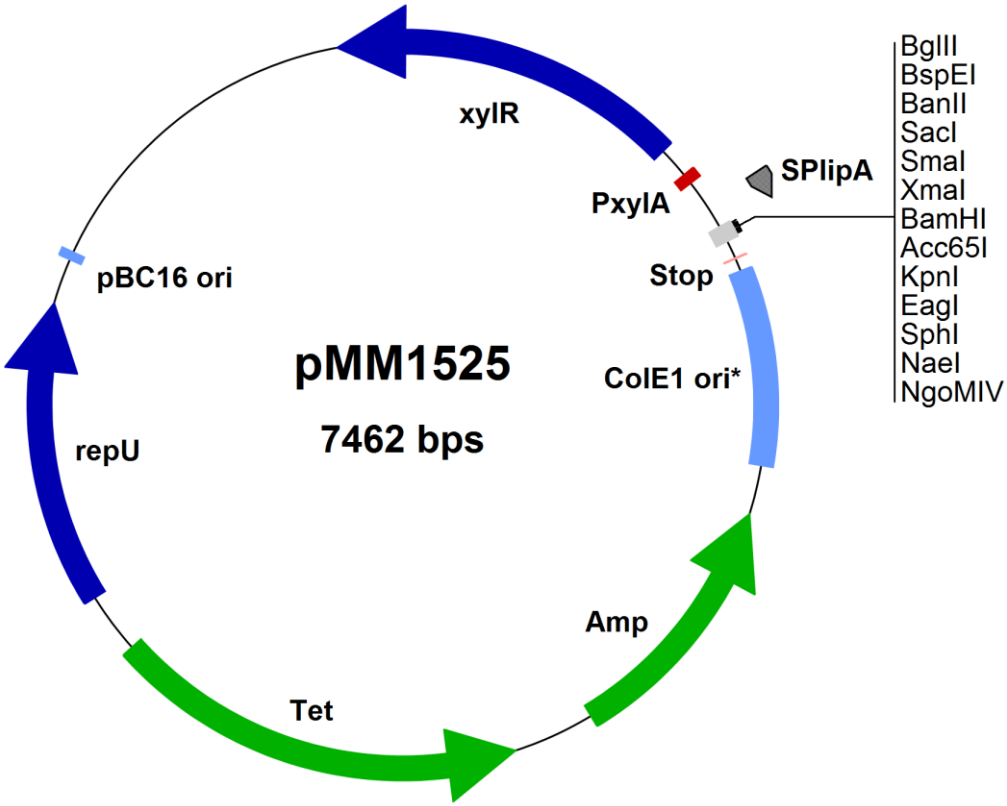
	Type	Start	End	Name	Description
	MCS	9	200	MCS	Multiple Cloning Site
	Region	327	329	Stop	Stop Codon
	Origin of replication	720	1368	ColE1 ori*	origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	2391	1531	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	4033	2657	Tet	Tetracycline resistance (*Bacillus*)
	Gene	5216	4233	repU	Gene of replication protein RepU
	Origin of replication	5389	5415	pBC16 ori	Origin of replication (*Bacillus*)
	Gene	7712	6546	xylR	Xylose repressor gene
	Promoter	7816	7850	PxylA	Xylose inducible promoter

9.2. Vector map of pMM1522 (#BMEG10)



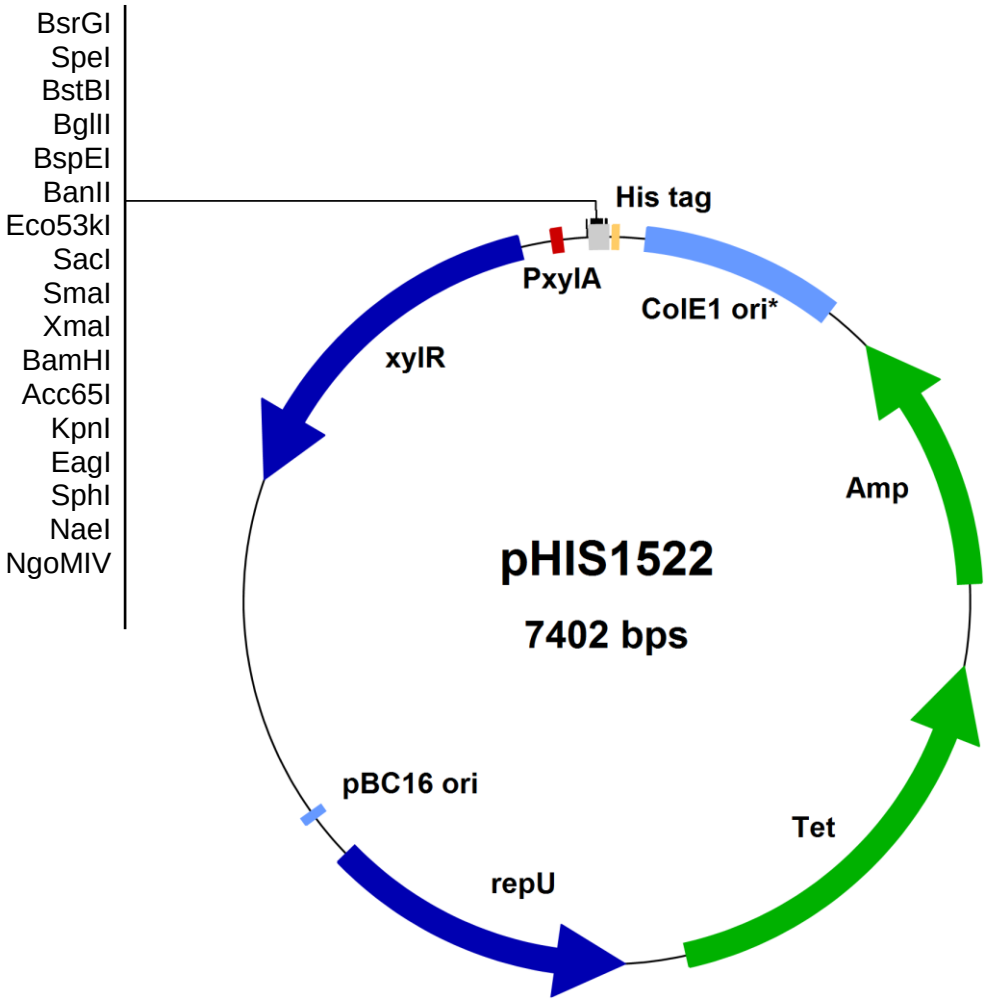
	Type	Start	End	Name	Description
	MCS	9	74	MCS	Multiple Cloning Site
	Region	135	137	Stop	Stop Codon
	Origin of replication	181	827	ColE1 ori*	origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	1849	992	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	3494	2121	Tet	Tetracyclin resistance (*Bacillus*)
	Gene	4698	3697	repU	Gene of replication protein RepU
	Origin of replication	4850	4876	pBC16 ori	Origin of replication (*Bacillus*)
	Gene	7173	6007	xylR	Xylose repressor gene
	Promoter	7277	7311	PxylA	Xylose inducible promoter

9.3. Vector map of pMM1525 (#BMEG11)



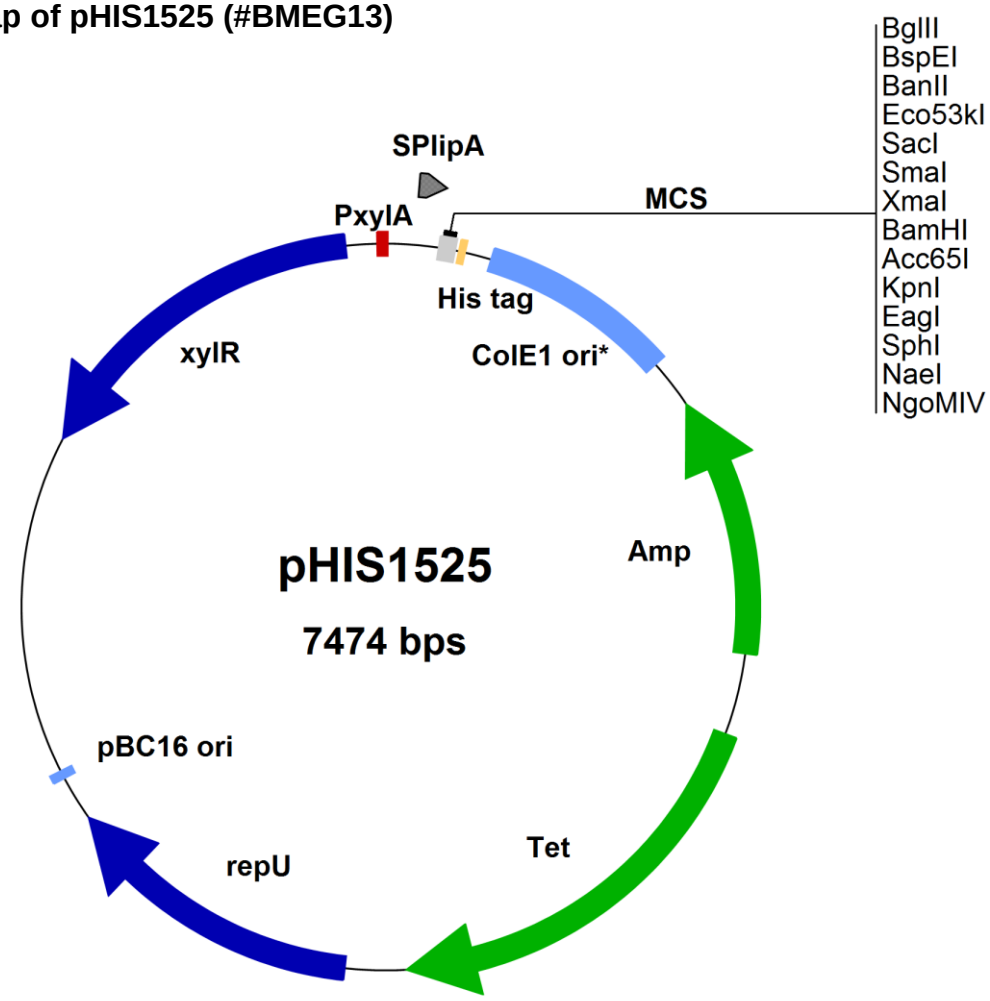
	Type	Start	End	Name	Description
	Gene	947	7246	xylR	Xylose repressor gene
	Promoter	1051	1085	PxylA	Xylose inducible promoter
	Signal peptide	1173	1256	SPlipA	
	MCS	1257	1310	MCS	Multiple Cloning Site
	Region	1371	1373	Stop	Stop Codon
	Origin of replication	1417	2063	ColE1 ori*	origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	3085	2228	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	4730	3357	Tet	Tetracyclin resistance (*Bacillus*)
	Gene	4933	4934	repU	Gene of replication protein RepU
	Origin of replication	6086	6112	pBC16 ori	Origin of replication (*Bacillus*)

9.4. Vector map of pHIS1522 (#BMEG12)



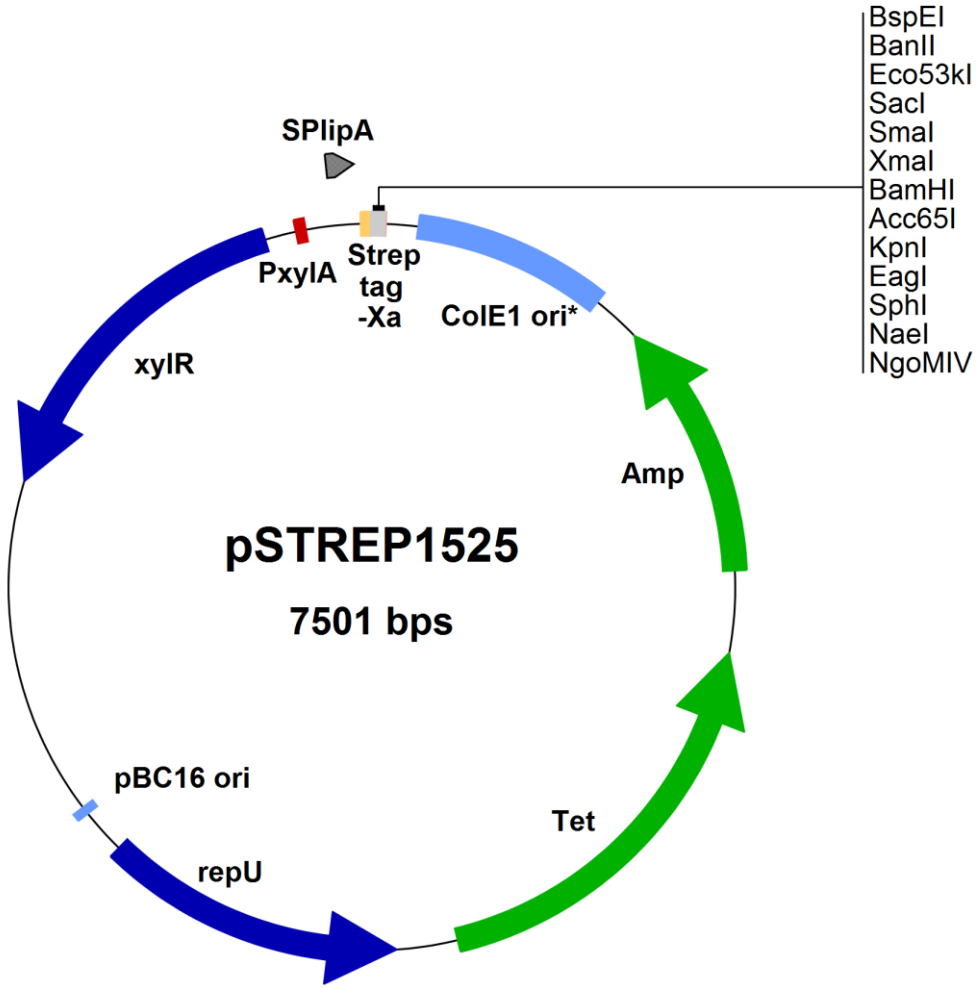
	Type	Start	End	Name	Description
	Tag	18	35	His Tag	6x histidine tag
	Region	36	38	Stop	Stop codon
	Origin of replication	127	773	ColE1 ori*	origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	1795	938	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	3440	2067	Tet	Tetracyclin resistance (*Bacillus*)
	Gene	4644	3643	repU	Gene of replication protein RepU
	Origin of replication	4796	4822	pBC16 ori	Origin of replication (*Bacillus*)
	Gene	7119	5953	xylR	Xylose repressor gene
	Promoter	7223	7257	PxylA	Xylose inducible promoter
	MCS	7345	5	MCS	Multiple Cloning Site

9.5. Vector map of pHIS1525 (#BMEG13)



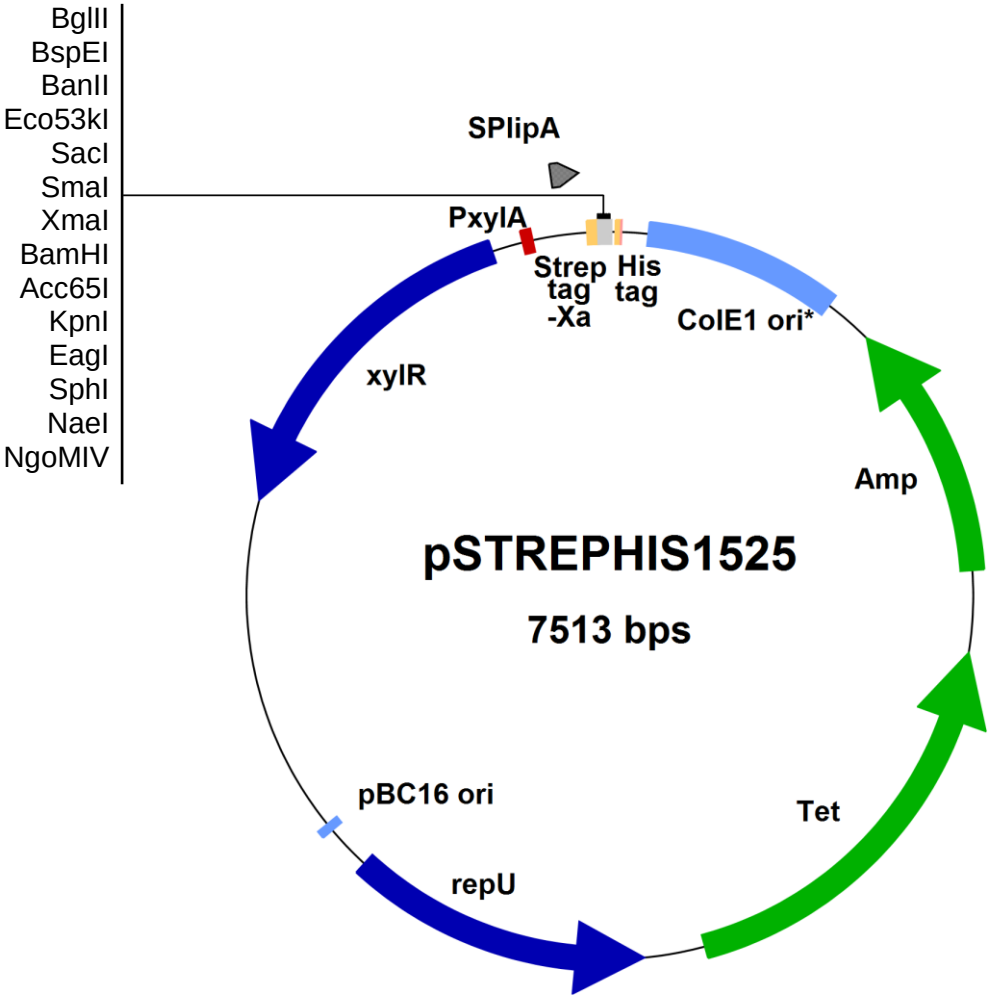
	Type	Start	End	Name	Description
	Signal peptide	97	180	SPlipA	Signal sequence of *lipA* gene
	MCS	181	231	MCS	Multiple Cloning Site
	Tag	244	261	His Tag	6x histidine tag
	Region	262	264	Stop	Stop codon
	Origin of replication	353	999	ColE1 ori*	origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	2021	1164	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	2293	3666	Tet	Tetracyclin resistance (*Bacillus*)
	Gene	3869	4870	repU	Gene of replication protein RepU
	Origin of replication	5022	5048	pBC16 ori	Origin of replication (*Bacillus*)
	Gene	7345	6179	xylR	Xylose repressor gene
	Promoter	7449	9	PxylA	Xylose inducible promoter

9.6. Vector map of pSTREP1525 (#BMEG14)



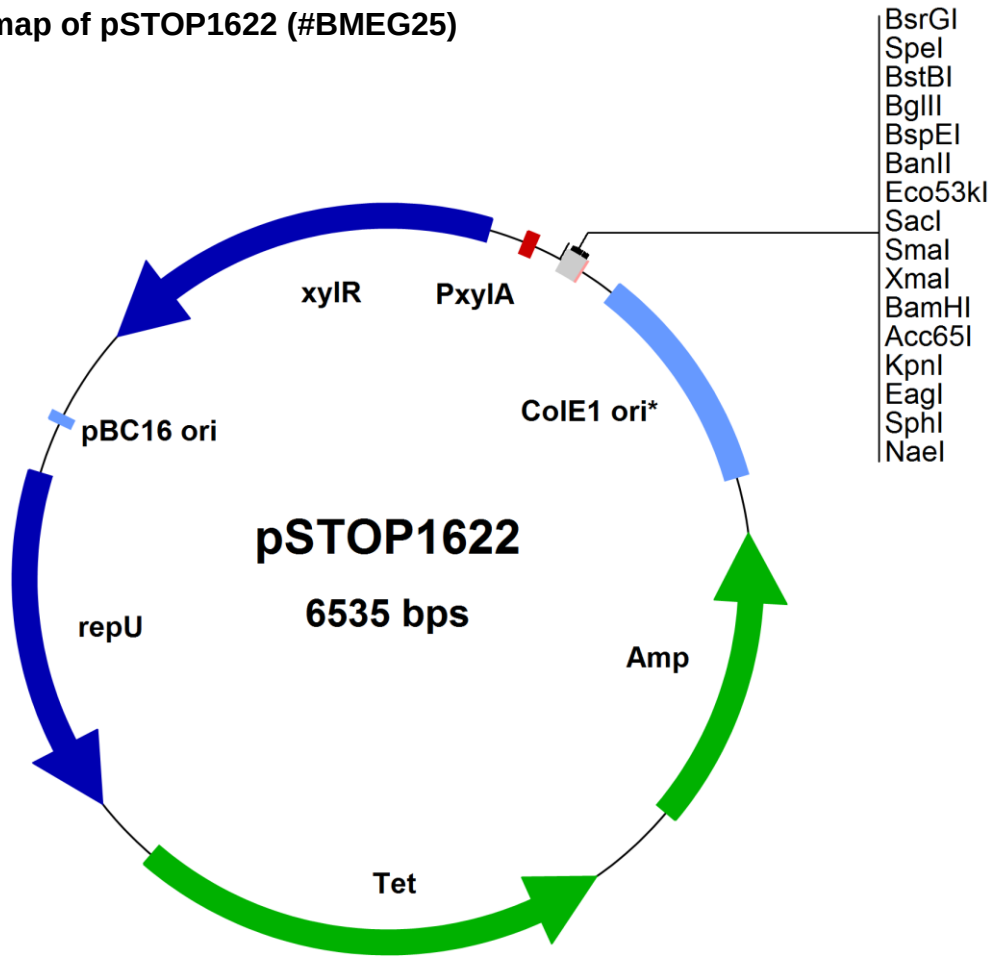
	Type	Start	End	Name	Description
	Region	44	46	Stop	Stop codon
	Origin of replication	153	799	ColE1 ori*	origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	1821	964	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	3466	2093	Tet	Tetracyclin resistance (*Bacillus*)
	Gene	4670	3669	repU	Gene of replication protein RepU
	Origin of replication	4822	4848	pBC16 ori	Origin of replication (*Bacillus*)
	Gene	7145	5979	xylR	Xylose repressor gene
	Promoter	7249	7283	PxylA	Xylose inducible promoter
	Signal peptide	7371	7454	SPlipA	Signal sequence of *lipA* gene
	Tag	7464	7487	Strep tag	Streptavidin tag II
	+ cleavage site	7488	7499	Xa	Factor Xa cleavage site
	MCS	7501	43	MCS	Multiple Cloning Site

9.7. Vector map of pSTREPHIS1525 (#BMEG15)



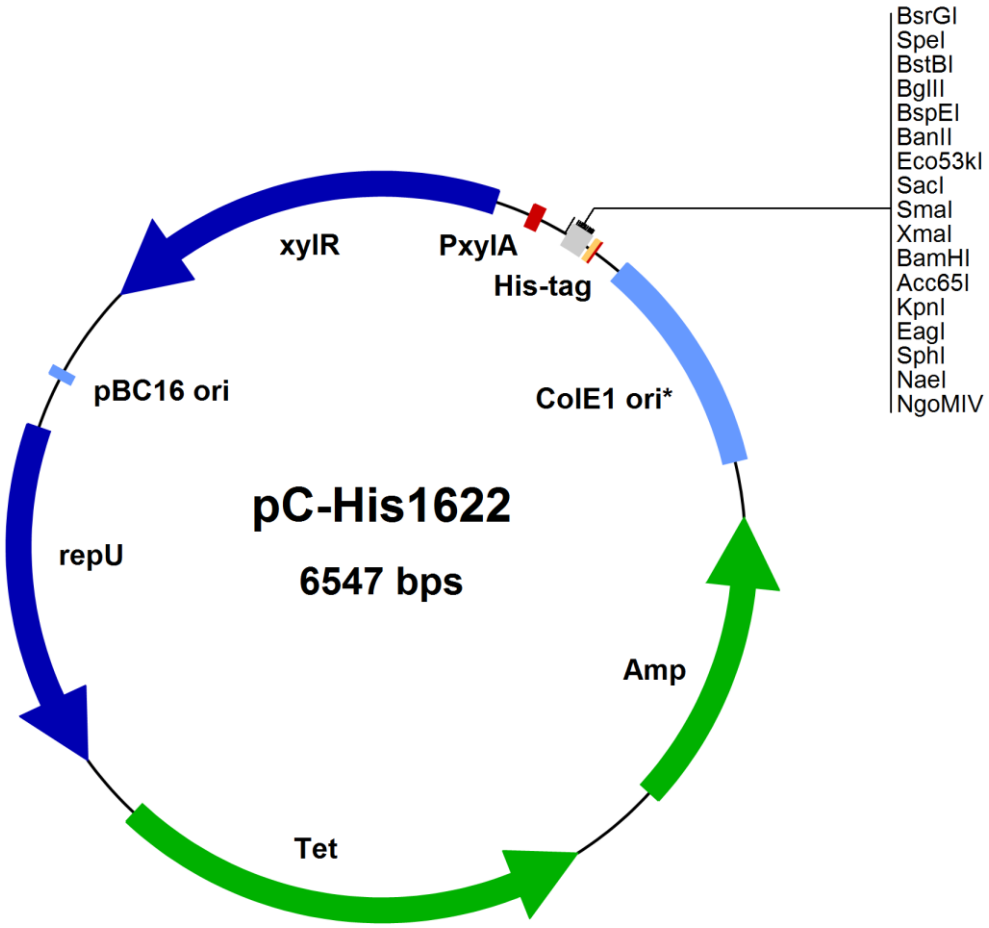
	Type	Start	End	Name	Description
	Tag	18	35	His tag	6x histidine tag
	Region	36	38	Stop	Stop codon
	Origin of replication	127	773	ColE1 ori*	origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	1795	938	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	3440	2067	Tet	Tetracyclin resistance (*Bacillus*)
	Gene	4644	3643	repU	Gene of replication protein RepU
	Origin of replication	4796	4822	pBC16 ori	Origin of replication (*Bacillus*)
	Gene	7119	5953	xylR	Xylose repressor gene
	Promoter	7223	7257	PxylA	Xylose inducible promoter
	Signal peptide	7345	7428	SPlipA	Signal sequence of *lipA* gene
	Tag	7438	7461	Strep tag	Streptavidin tag II
	+ cleavage site	7462	7473	Xa	Factor Xa cleavage site
	MCS	7474	5	MCS	Multiple Cloning Site

9.8. Vector map of pSTOP1622 (#BMEG25)



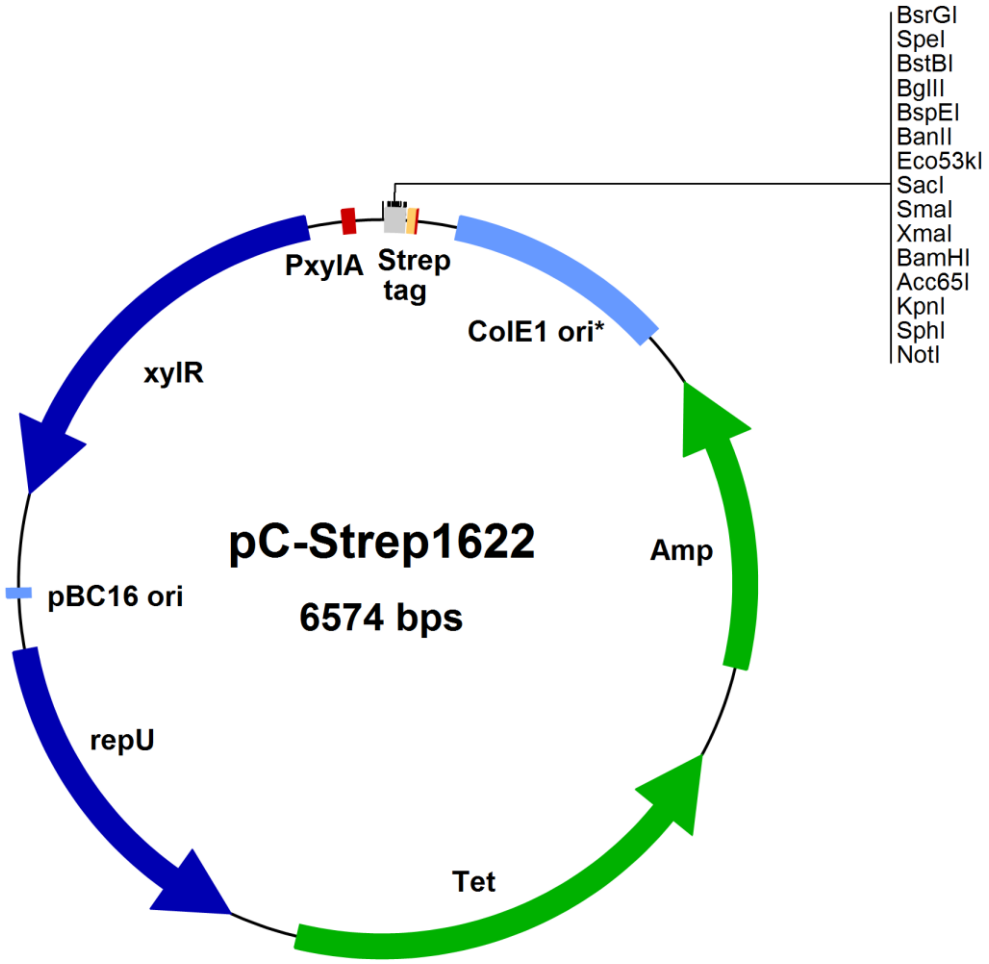
	Type	Start	End	Name	Description
	Gene	294	5663	xylR	Xylose repressor gene
	Promoter	398	432	PxylA	Xylose inducible promoter
	MCS	520	582	MCS	Multiple Cloning Site
	Region	583	585	Stop	Stop Codon
	Origin of replication	692	1338	ColE1 ori*	Origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	2360	1503	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	4005	2632	Tet	Tetracyclin resistance (*Bacillus*)
	Gene	5209	4208	repU	Gene of replication protein RepU
	Origin of replication	5361	5387	pBC16 ori	Origin of replication (*Bacillus*)

9.9. Vector map of pC-His1622 (#BMEG20)



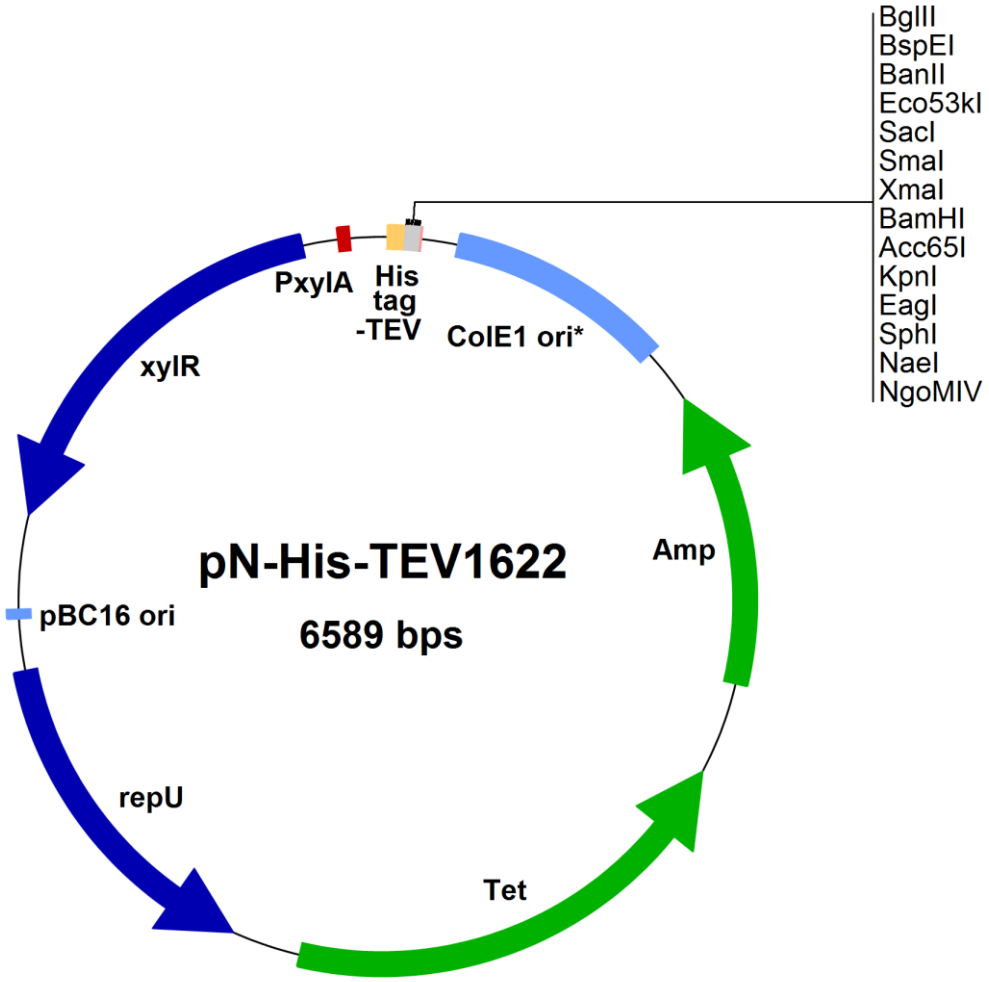
	Type	Start	End	Name	Description
	Gene	332	5713	xylR	Xylose repressor gene
	Promoter	436	470	PxylA	Xylose inducible promoter
	MCS	558	620	MCS	Multiple Cloning Site
	Tag	633	650	His Tag	6x histidine tag
	Region	651	653	Stop	Stop codon
	Origin of replication	742	1388	ColE1 ori*	Origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	2410	1553	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	4055	2682	Tet	Tetracyclin resistance (Bacillus)
	Gene	5259	4258	repU	Gene of replication protein RepU
	Origin of replication	5411	5437	pBC16 ori	Origin of replication (*Bacillus*)

9.10. Vector map of pC-Strep1622 (#BMEG21)



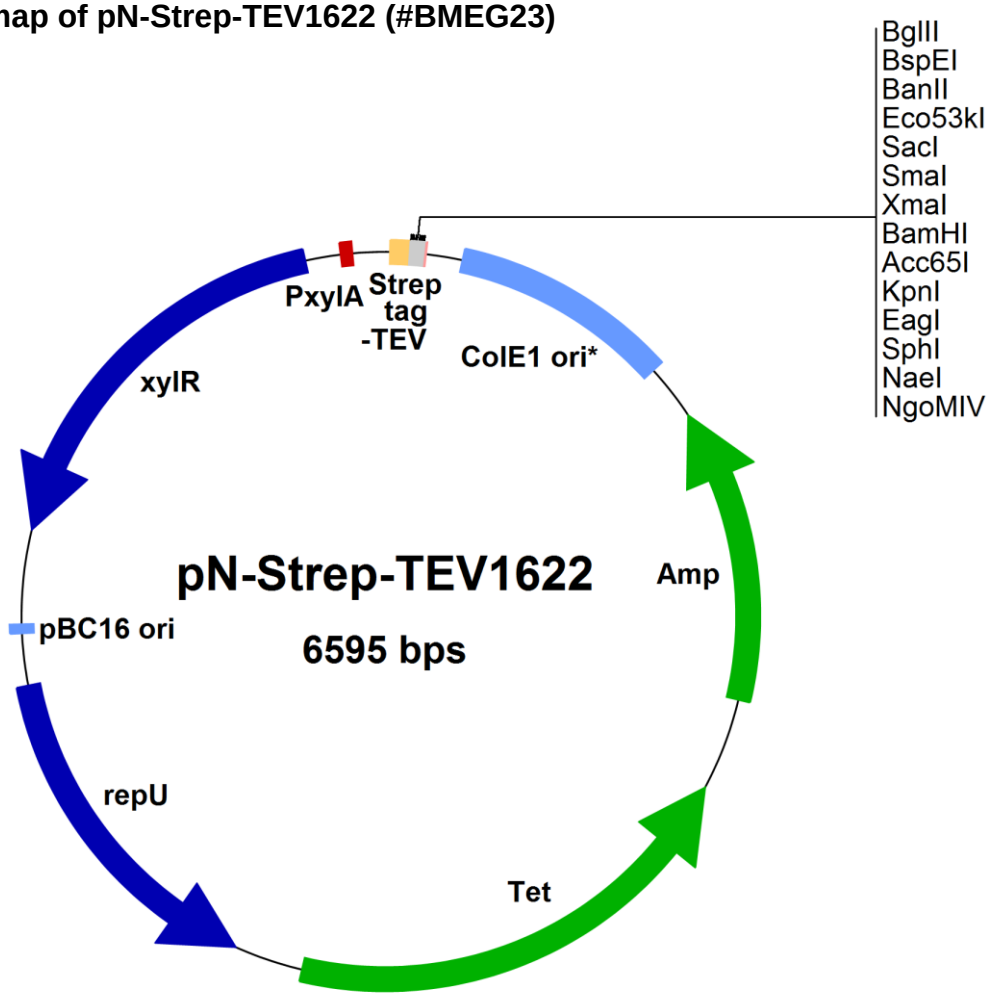
	Type	Start	End	Name	Description
	MCS	9	66	MCS	Multiple Cloning Site
	Tag	75	98	Strep Tag	Streptavidin tag II
	Region	99	101	Stop	Stop codon
	Origin of replication	220	864	ColE1 ori*	origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	1888	1031	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	3533	2160	Tet	Tetracyclin resistance (*Bacillus*)
	Gene	4737	3736	repU	Gene of replication protein RepU
	Origin of replication	4889	4915	pBC16 ori	Origin of replication (*Bacillus*)
	Gene	6357	5191	xylR	Xylose repressor gene
	Promoter	6461	6495	PxylA	Xylose inducible promoter

9.11. Vector map of pN-His-TEV1622 (#BMEG22)



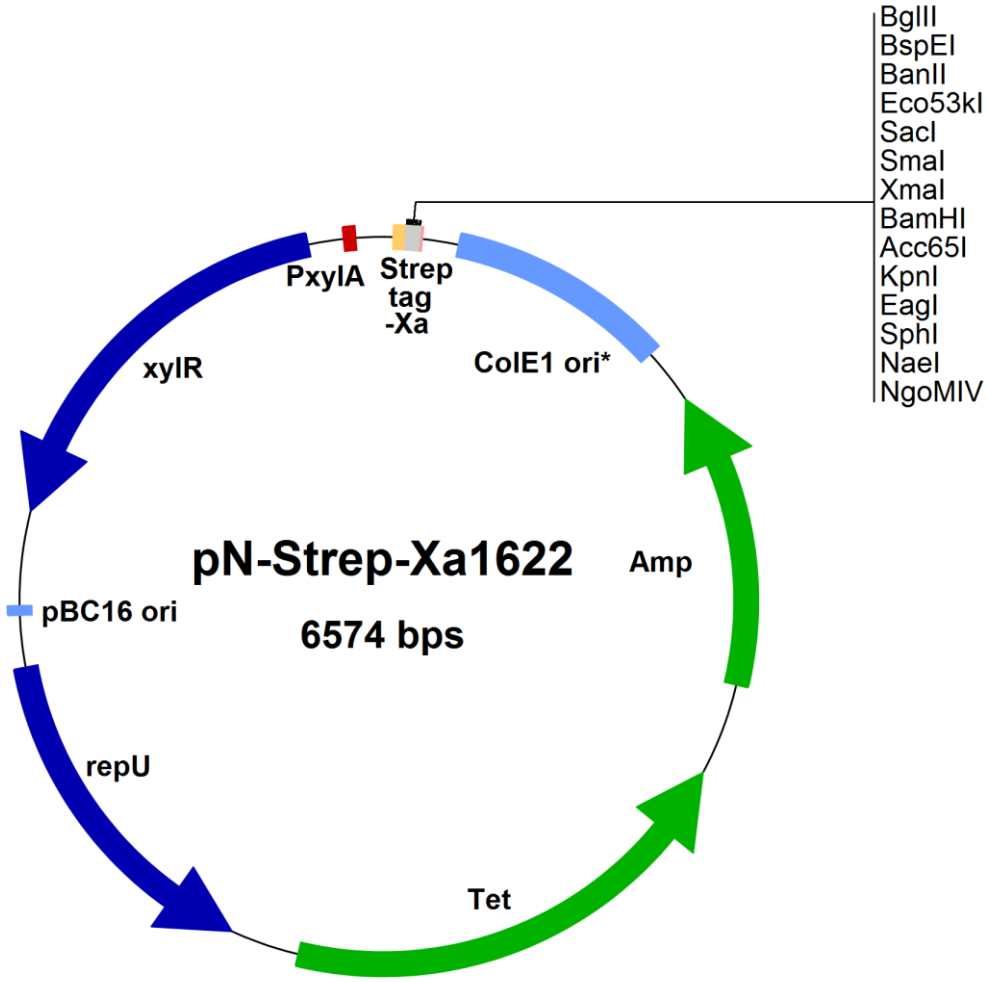
	Type	Start	End	Name	Description
	Tag	16	33	His Tag	6x histidine tag
	+ cleavage site	34	42	TEV	TEV cleavage site
	MCS	43	63	MCS	Multiple Cloning Site
	Region	112	114	Stop	Stop codon
	Origin of replication	221	867	ColE1 ori*	origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	1889	1032	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	3534	2161	Tet	Tetracyclin resistance (*Bacillus*)
	Gene	4738	3737	repU	Gene of replication protein RepU
	Origin of replication	4890	4916	pBC16 ori	Origin of replication (*Bacillus*)
	Gene	6358	5192	xylR	Xylose repressor gene
	Promoter	6462	6496	PxylA	Xylose inducible promoter

9.12. Vector map of pN-Strep-TEV1622 (#BMEG23)



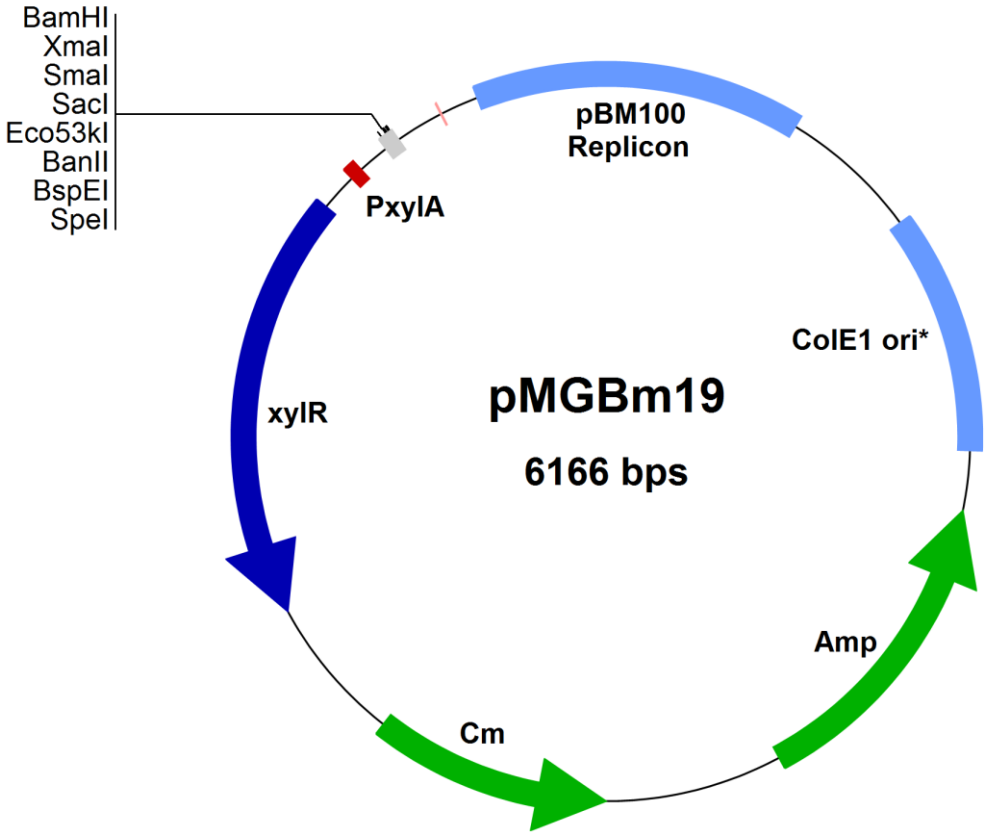
	Type	Start	End	Name	Description
	Tag	15	38	Strep tag	Streptavidin tag II
	+ cleavage site	48	68	TEV	TEV cleavage site
	MCS	72	116	MCS	Multiple Cloning Site
	Region	117	119	Stop	Stop codon
	Origin of replication	226	872	ColE1 ori*	origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	1894	1037	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	3539	2166	Tet	Tetracyclin resistance (*Bacillus*)
	Gene	4743	3742	repU	Gene of replication protein RepU
	Origin of replication	4895	4921	pBC16 ori	Origin of replication (*Bacillus*)
	Gene	6363	5197	xylR	Xylose repressor gene
	Promoter	6467	6501	PxylA	Xylose inducible promoter

9.13. Vector map of pN-Strep-Xa1622 (#BMEG24)



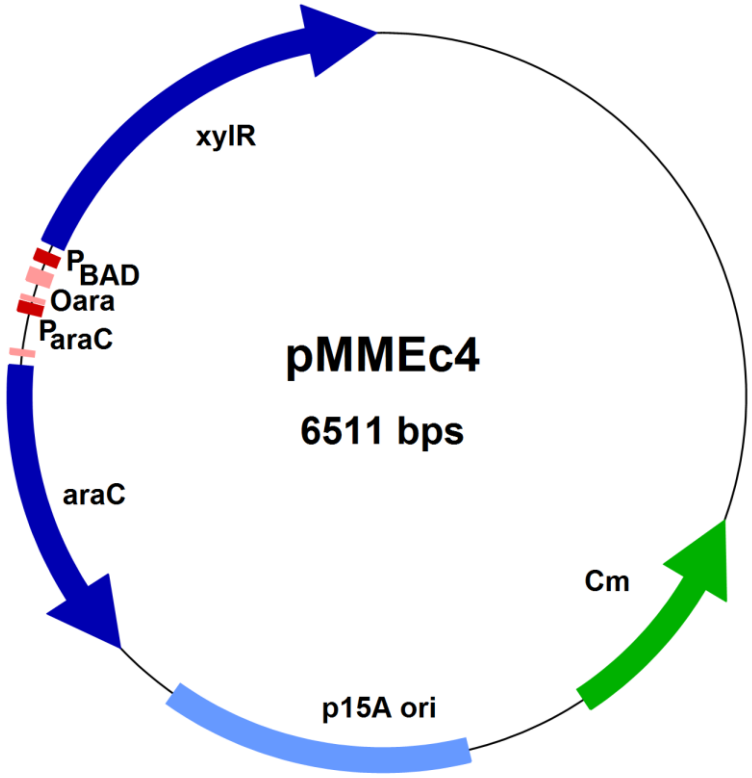
	Type	Start	End	Name	Description
	Tag	30	53	Strep tag	Streptavidin tag II
	+ cleavage site	54	65	Xa	Factor Xa cleavage site
	MCS	66	110	MCS	Multiple Cloning Site
	Region	111	113	Stop	Stop codon
	Origin of replication	220	864	ColE1 ori*	origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	1888	1031	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	3533	2160	Tet	Tetracyclin resistance (*Bacillus*)
	Gene	4737	3736	repU	Gene of replication protein RepU
	Origin of replication	4889	4915	pBC16 ori	Origin of replication (*Bacillus*)
	Gene	6357	5191	xylR	Xylose repressor gene
	Promoter	6461	6495	PxylA	Xylose inducible promoter

9.14. Vector map pMGBM19 (#BMEG39)



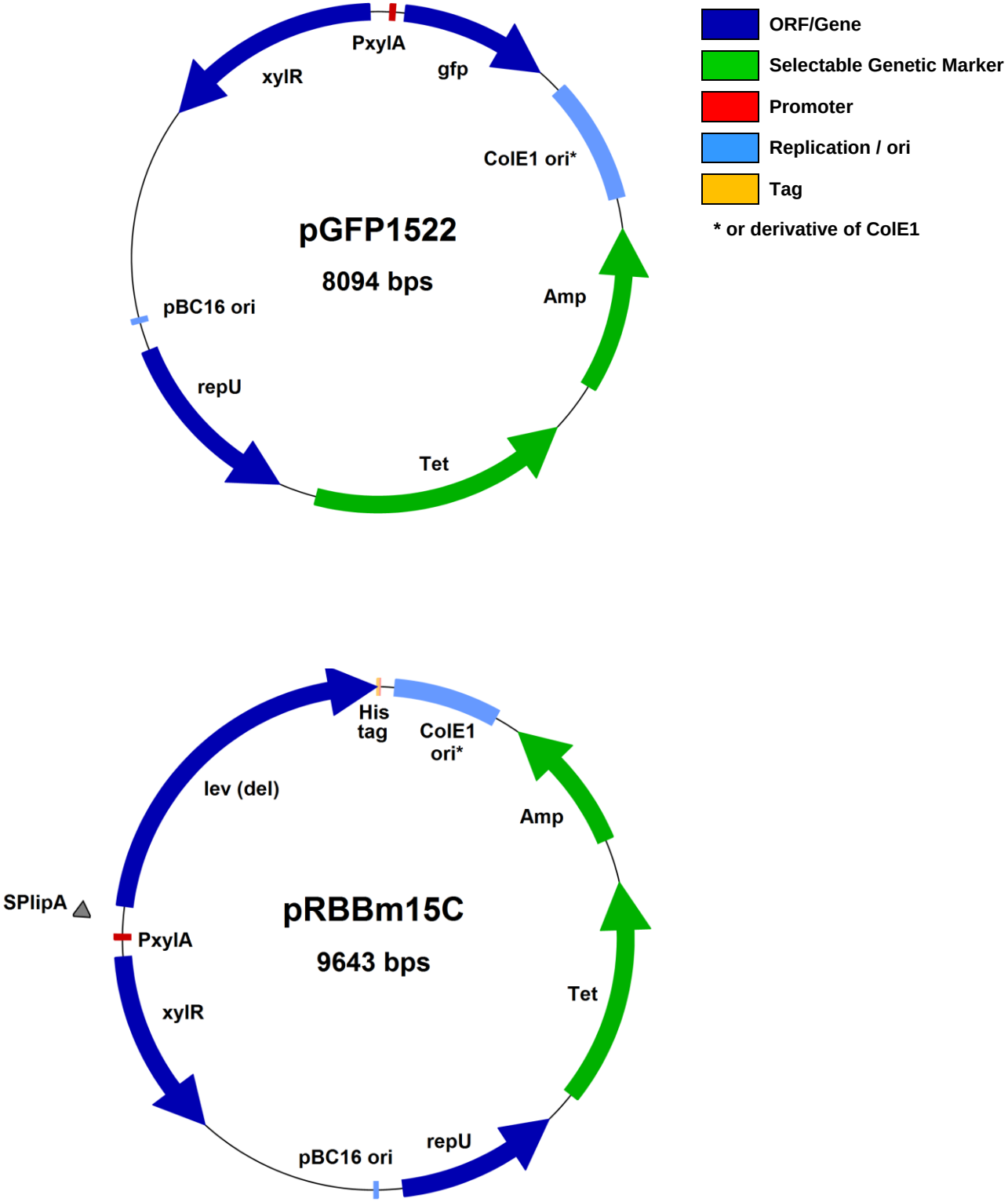
	Type	Start	End	Name	Description
	Origin of replication	922	1576	ColE1 ori*	Origin of replication (*E. coli*); ColE1 incompatibility group
	Selectable genetic marker	2598	1741	Amp	Ampicillin resistance (*E. coli*)
	Selectable genetic marker	3733	3086	Cm	Chloramphenicol resistance (*Bacillus*)
	Gene	5299	4136	xylR	Xylose repressor gene
	Promoter	5403	5436	PxylA	Xylose inducible promoter
	MCS	5525	5567	MCS	Multiple Cloning Site
	Region	5700	5702	Stop	Stop codon
	Origin of replication	5808	535	pMB100 replicon	Origin of replication (*Bacillus*)

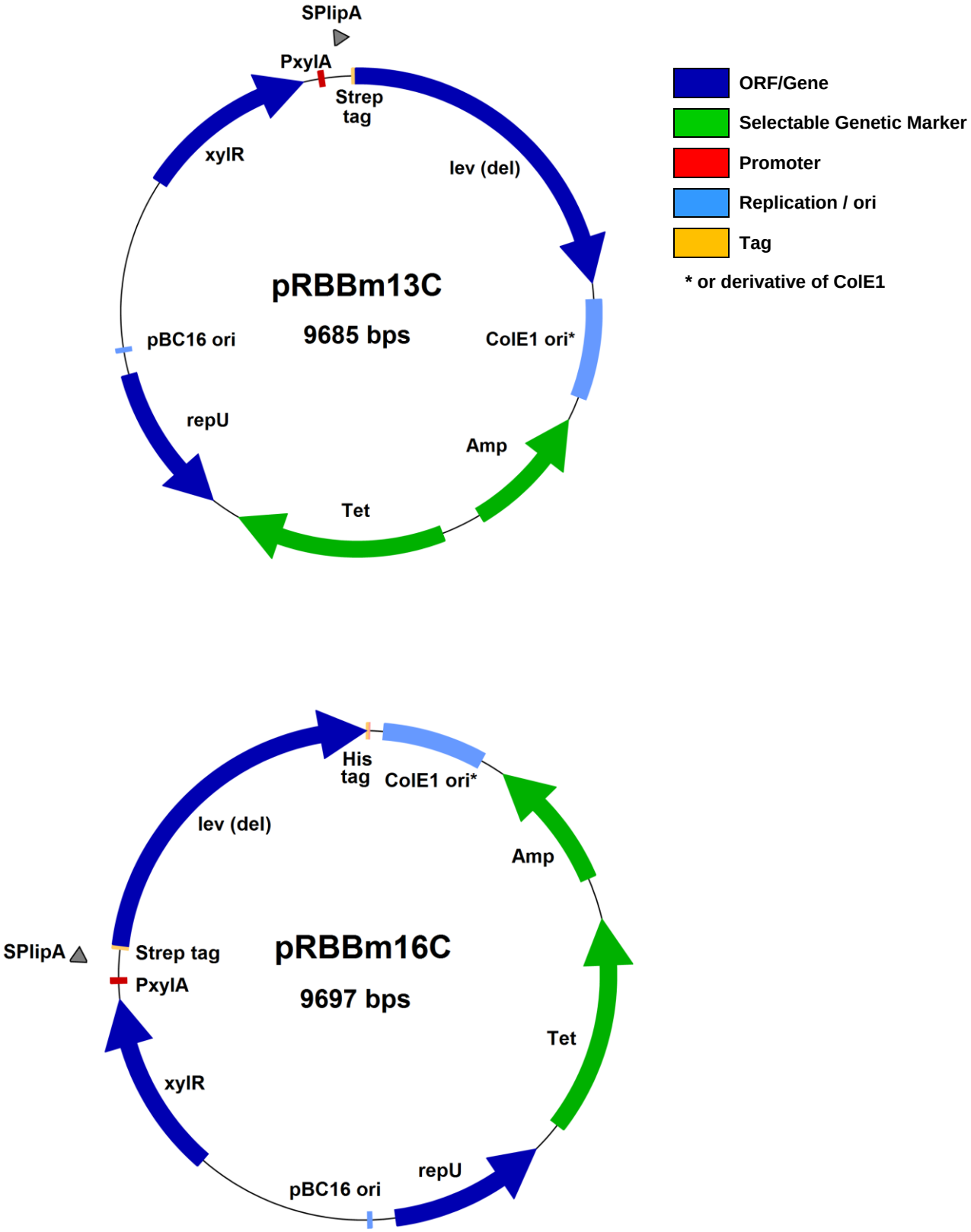
9.15. Vector map pMMEc4 (#PEC04)



	Type	Start	End	Name	Description
	Selectable genetic marker	2648	1989	Cm	Chloramphenicol resistance (*Bacillus*)
	Origin of replication	3010	3892	p15A ori	Origin of replication (*E. coli*)
	Gene	4971	4093	araC	Gene of AraC regulatory protein
	Operator	5001	5016	Oara	araO2, operator sequence
	Promoter	5122	5150	ParaC	Arabinose inducible promoter
	Operator	5158	5169	Oara	araO1, operator sequence
	Operator	5210	5248	Oara	araI1/2, operator sequence
	Promoter	5272	5302	pBAD	Arabinose inducible promoter
	Gene	5329	6492	xylR	Xylose repressor gene

9.16. Maps of Control Vectors




SPlipA
PxylA
Strep tag
lev (del)
xylR
pRBBm13C
9685 bps
pBC16 ori
ColE1 ori*
repU
Amp
Tet
ORF/Gene
Selectable Genetic Marker
Promoter
Replication / ori
Tag
* or derivative of ColE1
His tag
ColE1 ori*
lev (del)
Amp
SPlipA
Strep tag
PxylA
pRBBm16C
9697 bps
Tet
xylR
pBC16 ori
repU

10. Order Information, Shipping, and Storage

Order #	Description	Amount
BMEG02	*Bacillus megaterium* protoplasts, strain WH320	5 x 500 µl
BMEG04	*Bacillus megaterium* protoplasts, strain YYBm1	5 x 500 µl
BMEG50	*Bacillus megaterium* protoplasts, strain MS941	5 x 500 µl
shipped on dry ice; store at -80 °C		
BMEG03	*Bacillus megaterium* vector pWH1520, lyophilized DNA	5 µg
BMEG10	*Bacillus megaterium* vector, pMM1522, lyophilized DNA	10 µg
BMEG11	*Bacillus megaterium* vector, pMM1525, lyophilized DNA	10 µg
BMEG12	*Bacillus megaterium* vector, pHIS1522, lyophilized DNA	10 µg
BMEG13	*Bacillus megaterium* vector, pHIS1525, lyophilized DNA	10 µg
BMEG14	*Bacillus megaterium* vector, pSTREP1525, lyophilized DNA	10 µg
BMEG15	*Bacillus megaterium* vector pSTREPHIS1525, lyophilized DNA	10 µg
BMEG20	*Bacillus megaterium* vector pC-His1622, lyophilized DNA	10 µg
BMEG21	*Bacillus megaterium* vector pC-Strep1622, lyophilized DNA	10 µg
BMEG22	*Bacillus megaterium* vector pN-His-TEV1622, lyophilized DNA	10 µg
BMEG23	*Bacillus megaterium* vector pN-Strep-TEV1622, lyophilized DNA	10 µg
BMEG24	*Bacillus megaterium* vector pN-Strep-Xa1622, lyophilized DNA	10 µg
BMEG25	*Bacillus megaterium* vector, pSTOP1622, lyophilized DNA	10 µg
BMEG39	*Bacillus megaterium* vector, pMGBM19, lyophilized DNA	10 µg
PEC04	*Escherichia coli* P*xylA* repressing vector, pMMEc4, lyophilized DNA	10 µg
shipped at RT °C; store at 4 °C Once the DNA has been dissolved in sterile water or TE buffer we recommend storage at -20 °C.		

Control vectors:

Order #	Description	Amount
BMEG10C	pGFP1522, GFP expression vector, positive control	10 µg
BMEG13C	pRBBm15C, *lev* (Levansucrase) expression vector, positive control, His-Tag	10 µg
BMEG14C	pRBBm13C, *lev* (Levansucrase) expression vector, positive control, Strep-Tag	10 µg
BMEG15C	pRBBm16C, *lev* (Levansucrase) expression vector, positive control, StrepHIS-Tag	10 µg
shipped at RT °C; store at 4 °C Once the DNA has been dissolved in sterile water or TE buffer we recommend storage at -20 °C.		

Enzymes for removal of purification tags:

Order #	Description	Amount
EP0504	Factor Xa Protease (Ile-Glu-Gly-Arg)	250 µg
shipped at RT; store at 4 °C		
PR-ETA10010-01	MobiTEV Protease, recombinant, His-Tag	1000 U
PR-ETA10010-05	MobiTEV Protease, recombinant, His-Tag	10 x 1000 U
shipped at -20 °C; store at -70 °C		

Related Products

Order #	Product	Amount
For His-Tag Purification		
PR-HTK004	MoBiTec Ni-IDA Columns	4 columns
PR-HTK010	MoBiTec Ni-IDA Columns	10 columns
PR-HTK105	MobiSpin Ni-IDA Columns	5 columns
PR-HTK110	MobiSpin Ni-IDA Columns	10 columns
shipped at RT; store columns at RT		

11. Contact and Support

MoBiTec GmbH ◆ Lotzestrasse 22a ◆ D-37083 Goettingen ◆ Germany

Customer Service – General inquiries & orders
phone: +49 (0)551 707 22 0
fax: +49 (0)551 707 22 22
e-mail: order@mobitec.com

Technical Service – Product information
phone: +49 (0)551 707 22 70
fax: +49 (0)551 707 22 77
e-mail: info@mobitec.com

MoBiTec in your area: Find your local distributor at www.mobitec.com