pColiExpress Bacterial Expression Kits

Manual for Cat. nº :

BE001: pColiExpress I Glue Enzyme Kit

BE005: pColiExpress II Glue Enzyme Kit

BE010: pColiExpress III Glue Enzyme Kit

BE015: pColiExpress IV Glue Enzyme Kit

Upon Receipt
Store Kits at -20ºC

PRODUCT MANUAL
Version 3.0
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www.canvaxbiotech.com

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Materials provided

Item	Quantity	Storage
pColiExpress™ linearized vector (50 ng/µL)	20 µL	-20ºC
Glue Enzyme (10U/µL)	40 µL	-20ºC
10X Glue Enzyme Buffer	50 µL	-20ºC
Insert Control (30 ng/µL)	10 µL	-20ºC
pColiExpress™ Control (50ng/µL)*	5µL	-20ºC

*Uncut vector.

Additional material required

For cloning

- Proofreading DNA polymerase (*Recommended FastPANGEA™ High Fidelity DNA Polymerase from Canvax Biotech*).
- High efficiency competent cells.
- LB agar plates for ampicillin selection.

For transformants analysis

- Taq DNA polymerase kit (*Recommended **Red-Taq DNA polymerase kit** (Cat.Nº:P0027) from Canvax Biotech*).
- Restriction enzymes.
- Sequencing primer .

For plasmid purification

- Plasmid Purification kit (*Recommended **WideUSE™ Plasmid Purification Kit** (Cat.Nº:AN0068) from Canvax Biotech*).

1. INTRODUCTION

1.1 Description

pColiExpress™ Glue-enzyme Kits is a highly efficient directional PCR cloning kit designed for rapid cloning of PCR fragments without the need for ligase or restriction enzyme. The cloning technology is based on Canvax´s proprietary enzyme system (Glue-Enzyme™).

The DNA cloning for protein expression in bacteria involve a first step for subcloning PCR in a vector base and then the restriction enzymatic digestion for cloning into protein expression vector. This fact involve two cloning step that can be tedious and lengthy. In Canvax Biotech we developed a DNA cloning system for protein expression in bacteria which enables the cloning of DNA for protein expression in **only one step cloning**.

pColiExpress™ DNA cloning vectors are a family of bacteria expression vectors designed for one step DNA cloning for protein expression. This vectors family has four DNA cloning vectors for protein expression with a polyhistidine-tag at N or C-terminus of the protein. The His-tag facilitates purification by immobilised metal affinity chromatography. One these DNA cloning vectors with a signal peptide to export to periplasm and another DNA cloning vector with a tag in N-terminus like cmyc for protein purification and protein detection. **(See Appendix 1. Selection guide)**

All these DNA vectors include the T7 RNA polimerse promoter IPTG inducible, lac Operator DNA sequence, T7 terminator, lacI coding sequence, bla (Ap) coding sequence and f1 origin.

1.2 The family of pColiExpress™ bacterial expression vectors

The family of pColiExpress™ bacterial expression vectors is a powerful system developed for the cloning and expression of recombinant protein in *E. coli*. Comprise vectors that contain the T7 cloning and expression region based on the T7 promoter-driven system (Moffatt 1986, Rosemberg 1987 and Studier 1990).

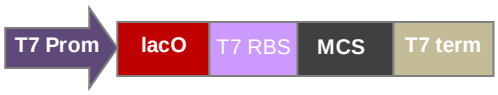


Figure 1: T7 cloning and expression region.

That system depends on the regulated expression of T7 RNA polymerase, an extremely active enzyme that is encoded in the DNA of bacteriophage T7.

After the pColiExpress™ with the gene of interest are established in a non-expression host, they are most often transformed into a host bearing the T7 RNA polymerase gene for expression of target proteins, like **BL21 λDe3** or **BL21DE3 pLYS**. In this host the T7 RNA polymerase gene expression is under the lacUV5 promoter. Transcription from the lacUV5 promoter occurs only when lactose, or a lactose analogue such as isopropylthiogalactoside (IPTG), is added to the culture medium competing with the lac repressor allowing binding of *E. coli* RNA polymerase witch catalyses transcription of T7 RNA

polymerase. IPTG generally is used because it cannot be metabolized, and therefore its concentration does not change as the cells grow.

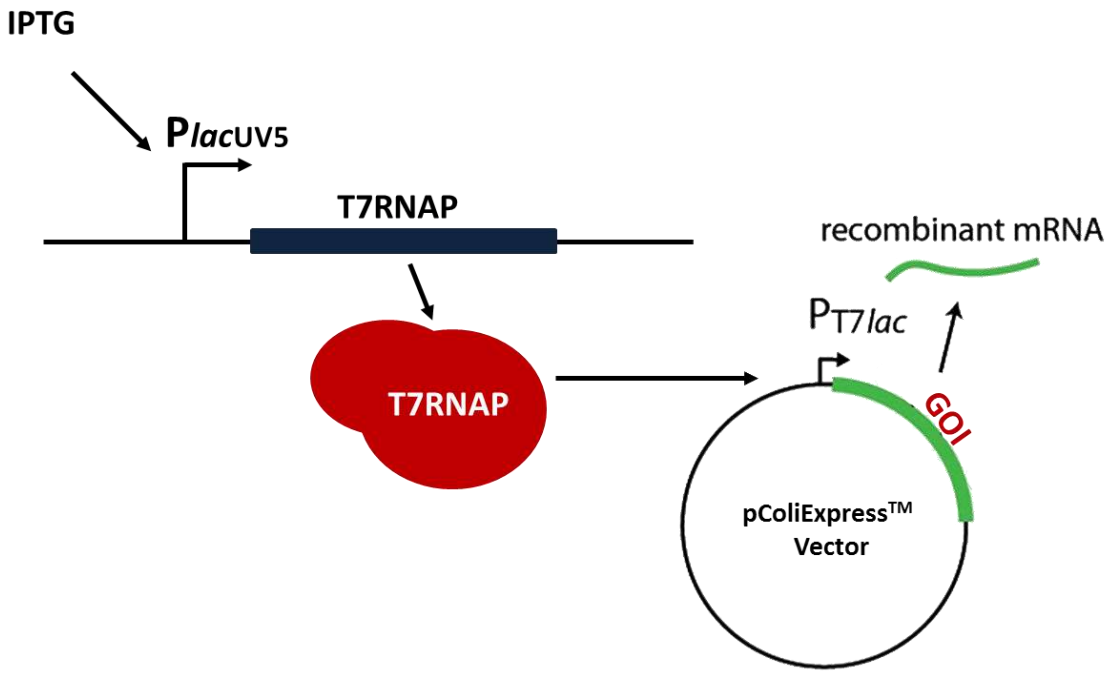


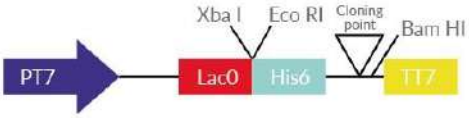
Figure 2: Schematic representation of T7 RNA polymerase system.

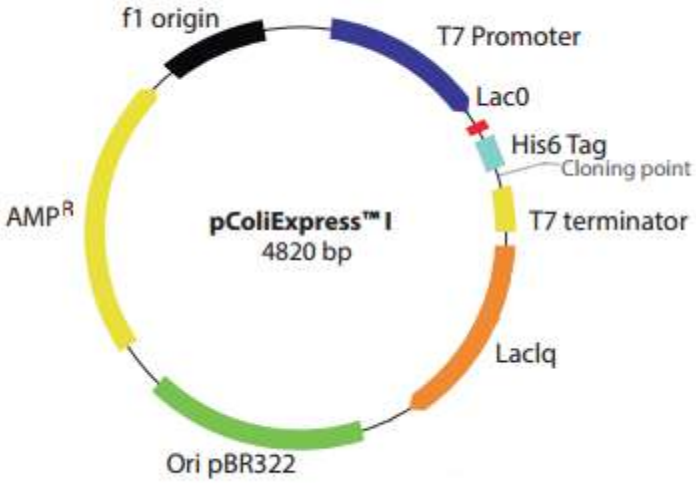
The T7 RNA polymerase transcribes DNA beginning within a specific 23-bp promoter sequence called the T7 promoter which is located before gene of interest. As a result **T7 RNA polymerase** catalyzes transcription of bulk of gene of interest.

1.3 Maps and sequences

Vectors that constitute pColiExpress™ family are represented below.

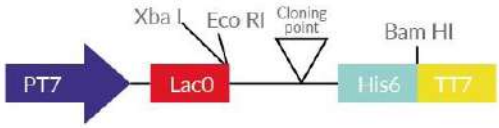
pColiEXpress™-I

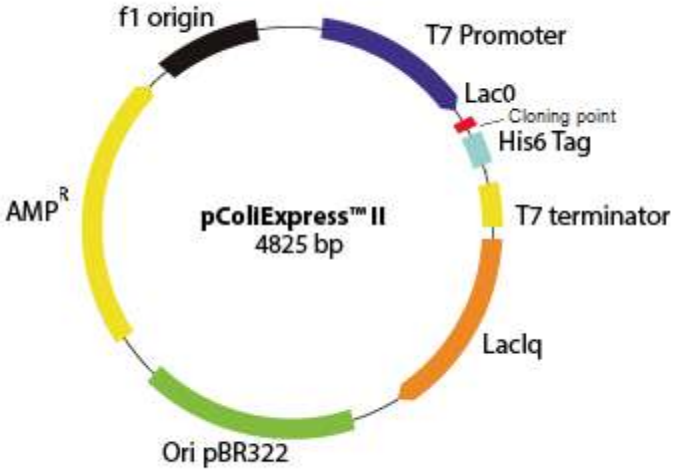




Features	
T7 Promoter	4605-4621
Lac O	4627-4645
T7 transcription start	4621
His Tag	4700-4717
T7 terminator	4743-4791
LacIq	96-1175
Ori pBR322	2970-2356
Ampicillin resistance gene (ORF)	3133-3990
F1 Origin	4562-4115

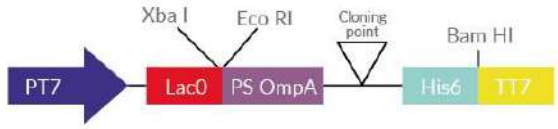
pColiEXpress-II

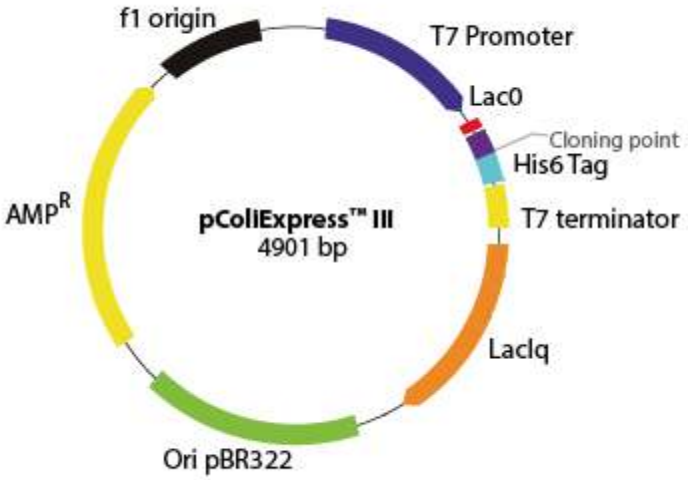




Features	
T7 Promoter	4605-4621
Lac O	4627-4645
T7 transcription start	4621
His Tag	4710-4727
T7 terminator	4749-4796
LacIq	96-1175
Ori pBR322	2970-2356
Ampicillin resistance gene (ORF)	3133-3990
f1 Origin	4562-4115

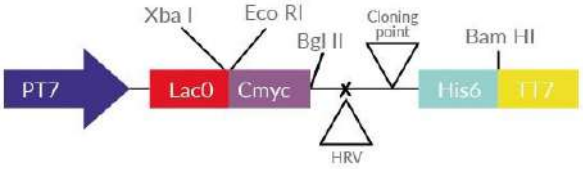
pColiExpress™ - III

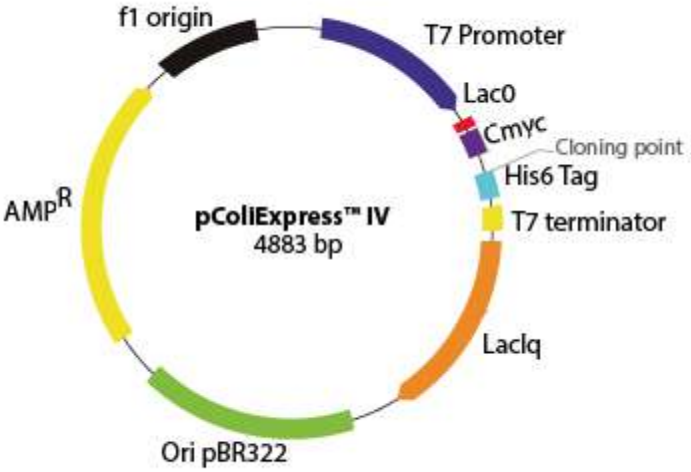




Features	
T7 Promoter	4605-4621
Lac O	4627-4645
T7 transcription start	4621
Signal Peptide OmpA	4696-4777
His Tag	4786-4803
T7 terminator	4825-4872
LacIq	96-1175
Ori pBR322	2970-2356
Ampicillin resistance gene (ORF)	3133-3990
f1 Origin	4562-4115

PColiExpress™ - IV



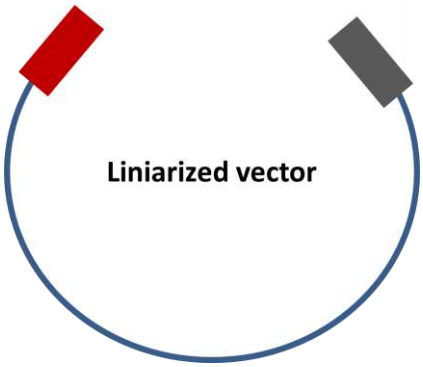


Features	
T7 Promoter	4605-4621
Lac O	4627-4645
T7 transcription start	4621
Cmyc	4697-4735
Prescission cleavage site	4736-4760
His Tag	4768-4785
T7 terminator	4807-4855
LacIq	96-1175
Ori pBR322	2970-2356
Ampicillin resistance gene (ORF)	3133-3990
f1 Origin	4562-4115

1.4 Vector consideration

In order to guarantee optimal cloning results, pColiExpress™ DNA cloning vectors are supplied linearized with a proved low background of non-recombinants. With the analysis of a minimal number of clones are achieved successful cloning processes, for an efficiency reported higher than 97%.

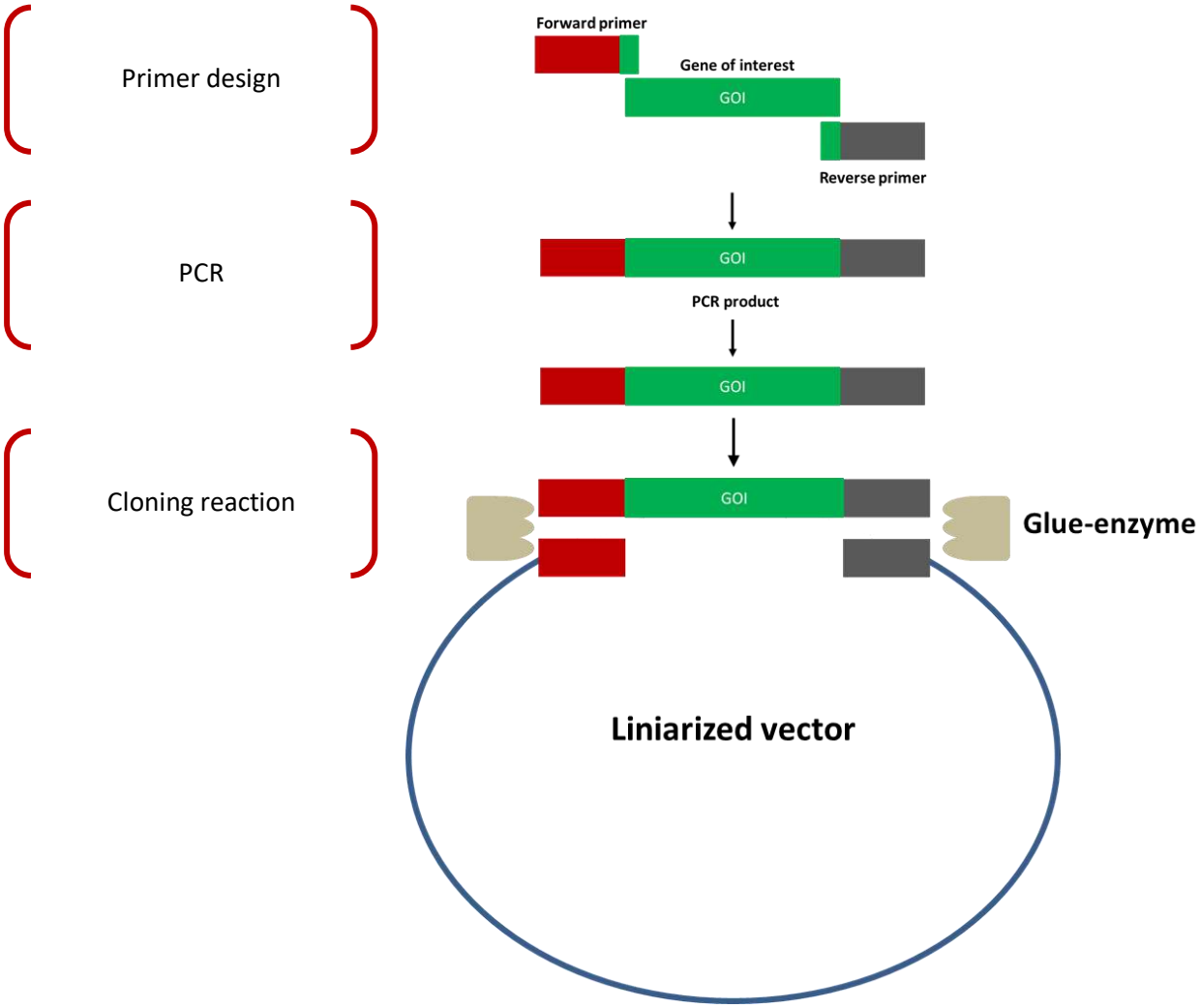
Selecting cloned inserts using pColiExpress™ linearized vectors are extremely powerful. The vectors are design and prepared to prevent self-ligation without need phosphatases, special bacterial strains or other singular components.



2. PROCEDURES

2.1 Primer design

pColiExpress™ bacterial expression cloning vectors permits directional cloning using specific sequence primers. This special consideration in the primers design will guarantee cloning your gene of interest in the correct orientation.



PCR Primer Design and quality are critical for the success of cloning reaction. Each primer should contain a homologous sequence and be gene specific. The 16 base pairs towards the 5' end of the primer must match the 16 base pairs at the linear end of the linearized vector to which it will be joined. The 3' end of the primer is the gene specific portion of the primer. The 3' end of the primer must have a melting temperature (Tm) suitable for PCR.

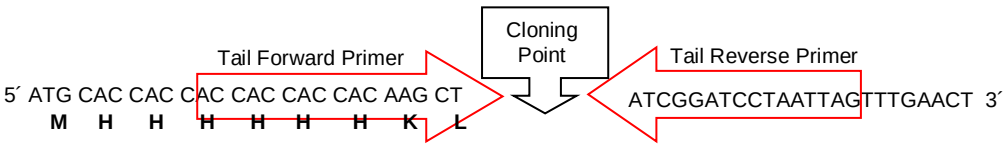
Please note that the Tm should be calculated based upon the 3' (gene-specific) end of the primer, and not the entire primer. If the calculated Tm is too low, increase the length of the gene-specific portion of the primer until a Tm of between 58º–65º C is reached. The Tm difference between the forward and reverse primers should be ≤ 4º C, otherwise, a good amplification will not be achieved. Desalted oligos in PCR reactions are recommended to use.

The **forward primer and reverse primer** for pColiExpress™ Glue-Enzyme Kit depend on the vector selected. **The primers must include the next sequences:**

For pColiExpress™ I

Fw: 5´ ACCACCACCACAAGCT...3´

Rev: 5´ CTAATTAGGATCCGAT….3´

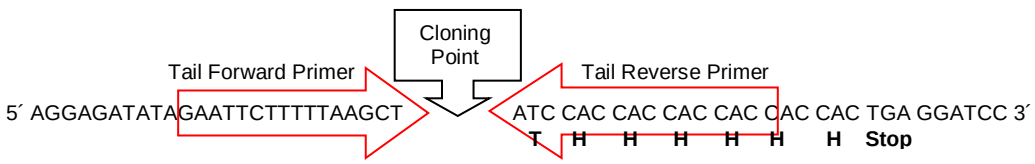


- *Must include the stop codon at the 3´end of your gene sequence in the reverse primer.*
- *Place the target sequence in frame with the His6 Tag.*
- ***You can see the His6 ORF and the tails in Forward and Reverse primers .***

For pColiExpress™ II

Fw: 5´ GAATTCTTTTTAAGCT….3´

Rev: 5´ GGTGGTGGTGGTGGAT.3´

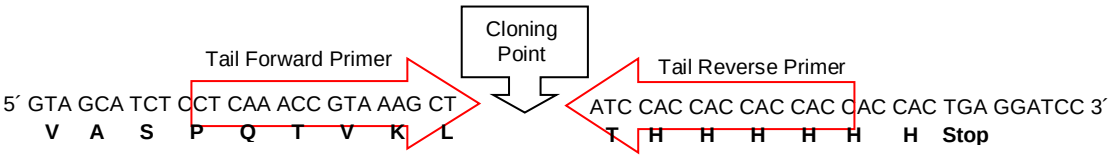


- *Must include the ATG at 3´ end of tail sequence in the forward primer.*
- *Place the target sequence in frame with the His6 Tag.*
- *Do not include the stop codon of your gene at the 3´end of insert specific sequence of the primer – this would prevent the expression of His6 Tag.*
- ***You can see the His6 ORF and the tails in Forward and Reverse primers.***

For **pColiExpress™ III**

Fw: 5´ CTCAAACCGTAAAGCT….3´

Rev: 5´ GGTGGTGGTGGTGGAT..3´

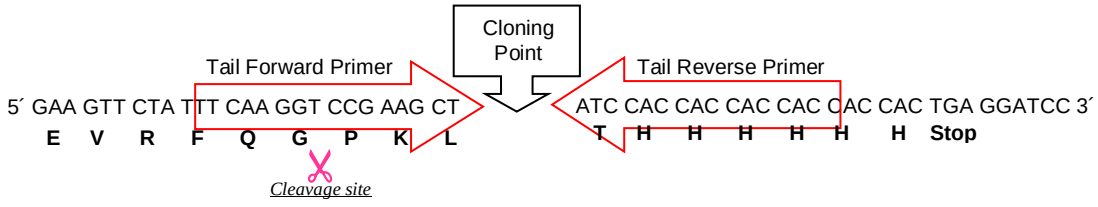


- *Place the target sequence in frame with the Signal peptide and His6 Tag.*
- *Do not include the stop codon of your gene at the 3´end of insert specific sequence of the primer – this would prevent the expression of His6 Tag.*
- ***You can see part of signal peptide OmpA and His6 ORF.***

For **pColiExpress™ IV**

Fw: 5´ TTCAAGGTCCGAAGCT…3´

Rev: 5´ GGTGGTGGTGGTGGAT.3´



- *Place the target sequence in frame with the Signal peptide and His6 Tag.*
- *Do not include the stop codon of your gene at the 3´end of insert specific sequence of the primer – this would prevent the expression of His6 Tag.*
- ***You can see part of protease 3C cleavage Site and His6 ORF.***

Additional important tips to consider in primer design are:

1. The size of specific annealing to target gene must be 18-30 bp for good target specificity.
2. Primers hairpins with a melting temperature higher than 46-48ºC should be avoided because of those structures decreasing PCR efficiency. Take care do not modify the specific sequence that guarantee directional cloning.

For PCR reaction, it is highly recommended to use proofreading enzymes or any other like enzymes to generate high fidelity amplification products.

2.2 Cloning

1. Spin pColiExpress™ DNA cloning vector to collect content at the bottom of the tubes.

2. Set up reaction as described below.

Match Reaction

	Cloning Reaction	Control Reaction	Background Reaction
pColiExpress™ vector (50 ng/µL)	1 µL	1 µL	1 µL
10x Match Enzyme Buffer	1.5 µL	1.5 µL	1.5 µL
PCR Product	X µL*	-	-
Control Insert DNA	-	2 µL	-
Water (*Molecular Biology grade*)	up 15 µL	up 15 µL	up 15 µL

* Insert ng = vector ng x insert bp/ 3 x vector bp (For 1/3 vector/insert).

3. Chill the reaction **without Glue-Enzyme** during 10 minutes. When all the components mix is chill, add 2 µL of Glue- Enzyme (10 U/µL).

4. Mix the reactions by pipetting.

5. Incubate 45 minutes on ice.

6. Proceed to transformation (see **Section 2.3**)

We strongly recommend:

To prepare a match-reaction with the supplied control insert to check that match reaction and transformation process are working properly. This positive control MUST be prepared and transformed at the same time than your samples.

2.3 Transformation using chemical competent cells

Use competent cells with a competence of at least $1x10^7$ colonies/ µg DNA following the protocol provided with the competent cells. Standard and fast protocol for transformation can be found below. In our hands the fast transformation protocol gives no less than 4-5 fold of the number of colonies obtained using the classical transformation protocol. Thus, for the most demanding cloning task the

classical protocol is recommended but for routine cloning the fast transformation protocol is suggested as it saves 1.5 hours.

2.3.1 Standard protocol for transformation

1. Prepare one LB ampicillin plate for each match reaction, plus one plate for determining transformation efficiency and one plate for control transformation (vector without insert). Equilibrate the plates to room temperature prior to plating (**Step 7**).
2. Centrifuge the tubes containing the reactions to collect content at the bottom of the tube. Add 7.5 µL of each reaction to a sterile 1.5 mL microcentrifuge tube on ice. Set up another tube on ice with 25 pg uncut plasmid for determination of the transformation efficiency of the competent cells.
3. Remove a tube of frozen Competent Cells (*non supplied*) from storage at -80ºC and place in an ice bath until just thawed (about 10 to 15 minutes). Mix the cells by **gently** flicking the tube with your fingertips.

IMPORTANT: *Do not thaw competent cells with your hands. Keeping competent cells out of an ice bath even for extremely short times strongly affects the transformation efficiency of cells. Also avoid excessive pipetting, as the competent cells are extremely fragile and thus mixing of DNA with competent cells should be made by gently flicking and not by pipetting.*

4. **Carefully** transfer 50 µL of cells into each tube prepared in **Step 2**.
5. **Gently** flick the tubes to mix and place them on ice for 30 minutes.
6. Heat-shock the cells for exactly 45 seconds in a water bath at exactly 42°C (***Do not shake nor heat shock more than 45 seconds***).
7. Immediately return the tubes to ice for 2 minutes and plate all transformation mix onto pre-warmed LB ampicillin plates
8. Incubate the plates overnight (12–16 hours) at 37°C.

2.3.2 Fast transformation protocol

An alternative transformation protocol of only 5 minutes is recommended. The main difference between this protocol and the standard protocol is that this fast protocol avoids the heat shock step and instead this essential step occurs directly on the plate. If you feel unfamiliar with this protocol, please use the standard transformation protocol.

EXTREMELY IMPORTANT: *Before starting this protocol you must pre-warm LB Agar-antibiotic-X-Gal-IPTG plates at 37ºC for at least 1 hour. Heat shock occurs on the pre-warmed plates when using this protocol, thus it is essential that plates are pre-warmed before transformation and withdrawn from incubator to laminar flow cabinet only just before transformation.*

1. Prepare one LB ampicillin plate for each match reaction, plus one plate for determining transformation efficiency and one plate for control transformation (vector without insert). Pre-warm plates at 37ºC in the incubator for at least one hour before transformation.
2. Centrifuge the tubes containing the match reactions and the control reaction to collect content at the bottom of the tube. Add 10 µL of each reaction to a sterile 1.5 mL microcentrifuge tube on ice. Set up another tube on ice with 25 pg uncut plasmid for determination of the transformation efficiency of the competent cells.
3. Remove a tube of frozen Competent Cells (non supplied) from storage at -80ºC and place in an ice bath until just thawed (about 10 to 15 minutes). Mix the cells by gently flicking the tube with your fingertips.

IMPORTANT: *Do not thaw competent cells with your hands. Keeping competent cells out of an ice bath even for extremely short times strongly affects the transformation efficiency of cells. Also avoid excessive pipetting, as the competent cells are extremely fragile and thus mixing of DNA with competent cells should be made by gently flicking and not by pipetting.*

4. Carefully transfer 50 µL of cells into each tube prepared in Step 2. For determination of transformation efficiency add 50 µL of competent cells to the tube prepared in Step 2 containing 25 pg of uncut plasmid DNA.
5. Gently flick the tubes to mix and place them on ice for 5 minutes.
6. Plate all transformation mix onto pre-warmed LB ampicillin plates
7. Incubate the plates overnight (12–16 hours) at 37°C.

2.3.3 Analysis of transformants

Colony PCR is a suitable approach for screening colonies before isolation of plasmid DNA. The principle behind colony PCR is the lysis of plasmid bearing bacteria (after saving a portion of the bacterial colony since the sample is destroyed by colony PCR) and PCR using as template the crude unpurified plasmid DNA released from bacteria. The most common method for bacterial lysis is boiling at 100ºC for 10 minutes. Some protocols use the initial DNA denaturation step of PCR as the bacterial lysis step and thus in this protocol the bacterial colony is added directly to a master mix of polymerase, buffer, dNTPs and primers.

The protocol below is one we have tested at Canvax but other protocols are also suitable.

Protocol for colony PCR

Previously, check the material required but not supplied with kit.

1. For each bacterial colony to be screened prepare a 1.5 mL microcentrifuge tube with 30 µL of water.
2. Pick one colony with a sterile toothpick or a sterile pipet tip and resuspend the colony in the 1.5 mL microcentrifuge tube with water.

3. Streak the toothpick from **Step 2** in either a plate with antibiotic or liquid media with antibiotic (e.g. LB with antibiotic) for growing positive colonies. Discard the toothpick and repeat **Steps 2** and **3** for each colony to be screened.
4. Boil the tubes of **Step 2** in a water bath at 100ºC for 10 minutes to lyse the cells and inactivate nucleases. Please make sure that the tubes are tightly closed because by boiling the lids can pop open.
5. While tubes are boiling, prepare a PCR master mix follow the guidelines of your Taq polymerase supplier for a 50 µL final volume reaction. Prepare at least one reaction master mix more than the total number of colonies to be screened. Distribute 30 µL of the master mix into sterile PCR tubes. You must use the insert primers in this reaction.
6. Spin boiled tubes from **Step 4** at 14500 rpm during 5 minutes in a microcentrifuge.
7. Add 20 µL of cleared lysate from **Step 6** to each PCR tube with 30 µL of master mix prepared in **Step 5**.
8. Running PCR program at thermocycler machine.
9. Load the tube contents on agarose gel and visualize it.
10. Choose positive clones and grow it in an appropriate amount of LB-Amp Broth, and purify the construct

Note

Recommended: *You could analyze the recombinant plasmids by restriction analysis and by sequencing.*

2.3.4 Sequencing

Confirm identity of your insert by sequence analysis of the construct. You may use one of the following sequencing primers which are located upstream and downstream of the cloning site:

Upstream 5´ TACGACTCACTATAGGGGAATT 3´ Tm = 66ºC

Downstream 5´ CAGCAAAAAACCCCTCAAGACC 3´ Tm = 64ºC

2.4 Expression

Once you have generated your expression clone, you will need to transform it into an *E. coli* strain for expression studies. For high-level protein production purposes, BL21(DE3) is an appropriate *E. coli* strain. It has the advantage of being deficient in both lon and ompT proteases and it is compatible with the T7 lacO promoter system33. For eukaryotic proteins, it is often important to use BL21(DE3) derivatives carrying additional tRNAs to overcome the effects of codon bias.

Because each recombinant protein has different characteristics that may affect optimal expression, it is important to determine the best conditions for optimal expression of your particular protein.

3. TROUBLESHOOTING

For questions not addressed here, please contact us at www.lifescience.canvaxbiotech.com or alternatively contact your local Distributor.

PROBLEM	CAUSE	SOLUTION
No colonies (even in Control Insert Reaction)	Any component is missing in the match reaction	Repeat match reaction and transformation and don´t forget to include controls
	Competent cells are damaged or with very low efficiency	Check the transformation efficiency of *E. coli* competent cells. A transformation efficiency lower than $1x10^7$ cfu/µg is not recommended
Colonies only in the Control Insert Reaction	Any component is missing in the sample reaction only	Repeat match reaction and transformation and don´t forget to include controls
	PCR insert is degraded or damaged	Check quality of insert by gel electrophoresis
	A very low amount or no PCR insert have been used for match reaction. Alternatively, a very high amount of insert was used	Check by gel electrophoresis the yield of PCR or agarose purification. Purified products can also be quantified by Abs at 260/280 nm (eg. by Nanodrop™). If needed increase/decrease amount of insert in a new match reaction.
	Salts and/or ethanol present in the purified PCR insert	Repeat PCR and purification from agarose for a new match reaction and transformation.
	Match reaction is not optimal	Optimise the reaction by trying other insert to vector ratios
	The PCR insert product has multiple bands and is used unpurified directly for cloning	- Gel purify your PCR insert or screen more colonies by colony PCR. - Also is possible that the template complexity is high (eg. genomic DNA) then is preferably synthetized a couple of primers to obtain the gene of interest and other pair to clone in the expression vector. This fact avoid cloning artefacts due to the presence of additional long sequences for orientated cloning.
	PCR primers have a bad design	Check your primers designed according our recommendations.
	There is a negative correlation between the size of the fragments and the number of colonies after transformation (fewer colonies with increasing size insert	Adjust the reaction in order to have a molar ratios up to vector: insert 1:3.

4. REFERENCES

1. Alan H. Rosenberg, Barbara N. Lade, Chui Dao-shan, Shu-Wha Lin, John J. Dunn, *F.William (1987) Studier Vectors for selective expression of cloned DNAs by T7 RNA polymerase*. Gene Volume 56, Issue 1, Pages 125–135

2. Blaber M. (1998) Molecular Biology and Biotechnology

3. François Baneyx (1999) Recombinant protein expression in *Escherichia coli* Current Opinion in Biotechnology Volume 10, Issue 5, Pages 411–421

4. F.William Studier, Barbara A. Moffatt† (1986) *Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes.* Journal of Molecular Biology Volume 189, Issue 1, Pages 113–130

5. F.William Studier (1991) *Use of bacteriophage T7 lysozyme to improve an inducible T7 expression system*. Journal of Molecular Biology Volume 219, Issue 1, Pages 37–44

6. Gerhard Hannig and Savvas C. Makrides (1998) *Strategies for optimising heterologous protein expression in Escherichia coli.* Trends in Biotechnology Volume 16, Issue 2, Pages 54–60

7. Moffatt BA, Studier FW. (1986). *Use of Bacteriophage T-7 RNA Polymerase to Direct Selective High-Level Expression of Cloned Genes*. J Molecular Biology 189(1):113-130.

8. Song Tan (2001) *A Modular Polycistronic Expression System for Overexpressing Protein Complexes in Escherichia coli*. Protein Expression and Purification 21, 224 –234

9. Stanley Tabor and Charles C. Richardson, (1985). *A bacteriophage T7 RNA polymerase/ promoter system for controlled exclusive expression of specific genes*. Proc. Natl. Acac. Sci. USA Vol. 82, pp. 1074-1078

10. Unger TF. (1997). *Prokaryotic Expression Vectors and Purification Systems*. The Scientist 11(17).

APPENDIX 1: SELECTION GUIDE

Name		pColiExpress™ I	pColiExpress™ II	pColiExpress™ III	pColiExpress™ IV
Catalog Number		**BE001**	**BE005**	**BE010**	**BE015**
Features					
5´His⁶ Tag		✓			
3´His⁶ Tag			✓	✓	✓
Periplasmic expression				✓	
5´cmyc Tag					✓
HRV protease cut site					✓
Resistance Marker-Ampicillin		✓	✓	✓	✓
T7 promoter		✓	✓	✓	✓
Low copy number (ori pBR322)		✓	✓	✓	✓
Strain Protein Expression	BL21 (DE3)	✓	✓	✓	✓
	BL21 (DE3) pLys	✓	✓	✓	✓

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