LenioBio®
Applied Nature for Better Science

ALiCE®
Protein Expression Made Easy

Instruction Manual for Cell-Free Protein Expression with ALiCE®

Product numbers AL0103050 (ALiCE® Mini Kit)
AL0103200 (ALiCE® Midi Kit)
AL0103500 (ALiCE® Maxi Kit)

LenioBio GmbH
Headquarters
c/o Factory Campus
Erkrather Straße 401
40231 Düsseldorf
GERMANY

LenioBio GmbH
Technology and Innovation Center
Forckenbeckstr. 6
52074 Aachen
GERMANY

Contents

1. Introduction

Cell-free protein expression (CFPE) systems derived from crude cell extracts have been used for decades as a research tool, but so far were lacking the necessary protein yield which would make the technology feasible and economically superior to cell-based protein expression. This changed with the introduction of our ALiCE® system to the research community in 2018.

To address this problem, LenioBio has leveraged plant cell biology to introduce a novel eukaryotic cell-free expression system called ALiCE® with unprecedented yields of up to 3 mg/mL of protein (internal standard is. eYFP). ALiCE® can overcome the limitations that are imposed by cell-based systems on the overexpression of challenging proteins while exceeding the yield expectations of cell-free systems in a single reaction.

The basis of ALiCE® is a plant cell lysate of the *Nicotiana tabacum* strain BY-2 to which we add components to maximize the efficiency of the system. All factors required for *in vitro* transcription (RNA polymerases, NTPs) and translation reactions (ribosomes, translation initiation/elongation factors, tRNA, etc.) are contained in the reaction mixture and no further intervention is needed by the user. On the other side, ALiCE® is an open system which can be modified according to your experimental needs either by us or by you, the user.

Protein expression is initiated by the addition of template DNA to the ALiCE® reaction mix. We recommend a reaction duration of 48 h for maximum protein yields. The DNA concentration and expression time can be modified according to your protein's needs.

Our ALiCE® kit includes two expression vectors, pALiCE01 and pALiCE02. These allow the expression of your protein-of-interest in different cellular compartments so you can benefit from the full potential of our system. Insertion of your gene-of-interest into pALiCE01 will keep the protein expression inside the cytosolic fraction, enabling high yields for standard proteins. For more complex proteins which require post-translational modifications like disulfide bonds, phosphorylation and/or glycosylation, we recommend the use of pALiCE02. pALiCE02 contains the honeybee melittin signal peptide (MSP) on the N-terminal end of your protein-of-interest which directs the protein expression towards the microsomes.

2. Kit Contents

Our ALiCE® reaction mix is provided in 2 mL screw cap micro tubes (Sarstedt, 72.609.711).

Mini-Kit (6 x 50 µL ALiCE®)

Component	Quantity	Concentration	Volume	Storage
ALiCE® reaction mix	6	n/a	50 µL	-80 °C
Vector pALiCE01	1	250 ng/µL	15 µL	-20 °C
Vector pALiCE02	1	250 ng/µL	15 µL	-20 °C
ALiCE® Tube caps, perforated	6	n/a	n/a	Room temp.

Midi-Kit (6 x 200 µL ALiCE®)

Component	Quantity	Concentration	Volume	Storage
ALiCE® reaction mix	6	n/a	200 µL	-80 °C
Vector pALiCE01	1	250 ng/µL	50 µL	-20 °C
Vector pALiCE02	1	250 ng/µL	50 µL	-20 °C
ALiCE® Tube Set, 12 pcs.	2	n/a	n/a	Room temp.

Maxi-Kit (6 x 500 µL ALiCE®)

Component	Quantity	Concentration	Volume	Storage
ALiCE® reaction mix	6	n/a	500 µL	-80 °C
Vector pALiCE01	1	250 ng/µL	50 µL	-20 °C
Vector pALiCE02	1	250 ng/µL	50 µL	-20 °C
ALiCE® Tube Set, 12 pcs.	5	n/a	n/a	Room temp.

Equipment and material to be provided by user:

- Gloves
- Pipette filter tips (RNase-free)
- RNase-free water
- Plasmid purification kit for isolating RNase-free transfection-grade plasmid DNA. We recommend using the Macherey-Nagel™ NucleoBond™ Xtra kit.
- For reactions performed in tubes: Eppendorf ThermoMixer® series or similar heated table-top shaker with an equivalent shaking diameter of 3 mm.
- **Recommended** For reactions performed in microtiter plates: 96-well half area microplates and a shaker system with a humidity above 70%, run at 500 rpm with a shaking diameter of 12.5 mm. Additionally, we recommend using the Sandwich cover system “Duetz” from EnzyScreen BV (Heemstede, Netherlands) together with their universal clamp holder system. Please follow the recommendations for the microtiter plate setup if humidity control is missing.
- For proteins expressed in the microsome: n-dodecyl-β-maltoside (DDM)

Important Notices

RNase contamination leads to lower or no protein yields. Only use **RNase-free filter-tips** and **wear gloves at all times!**

ALiCE® requires oxygen during the whole reaction time for a successful reaction. **Do not seal the reaction vessels!**

Shipping and Storage

ALiCE® kits are shipped on dry ice. Upon arrival, immediately store the components as indicated in the table above. The Reaction mix is stable for at least 12 months under these conditions. More than one freeze-thaw cycle should be avoided. Please aliquot lysate according to your needs.

Product Use Limitations

This kit is for research purposes only. It is not to be used for diagnostic or preventive action or treatment of a disease, nor to be administered to humans. Please refer to the Limited Use Label License (LULL) on our website: www.leniobio.com/lull

Product Warranty

The kit is shipped frozen on dry ice. If there is no dry ice remaining in the package upon delivery, or if the package is damaged, the quality of the kit may be compromised. Contact us immediately if any issues with the delivery have occurred. The warranty remains in effect up to the expiration date indicated on the product label.

Quality Assurance

High quality chemicals and materials have been used to manufacture the components of this kit. Each lot is carefully tested to ensure that all components meet the stated specifications (see Certificate of Analysis).

Safety

The user should observe all applicable regulations for handling chemicals and recombinant DNA. Lab coat, safety glasses and gloves should be worn when handling the kit components. When handling ultra-cold material wear additional protective gloves to avoid frostbite. Used components are to be disposed of in accordance to the local regulations.

Disclaimer

We assume no liability for any direct or indirect damages or loss arising from the use, misuse, or results of use of this kit.

3. Preparation for ALiCE® reaction

3.1. General ALiCE® workflow

Every protein has its unique expression requirements to be functional and active. ALiCE® is a highly versatile system that allows the expression of proteins which require highly complex post-translational modifications (like *N*-glycosylation). This can be achieved by expressing the protein-of-interest in the microsomes present in the lysate using our vector **pALiCE02**. If post-translational modifications are not required, the protein can be expressed in the cytosol using **pALiCE01**. This enables high-yield protein expression in a simple one-step reaction scheme. This workflow describes the decision making process for your protein-of-interest (**Figure 1**).

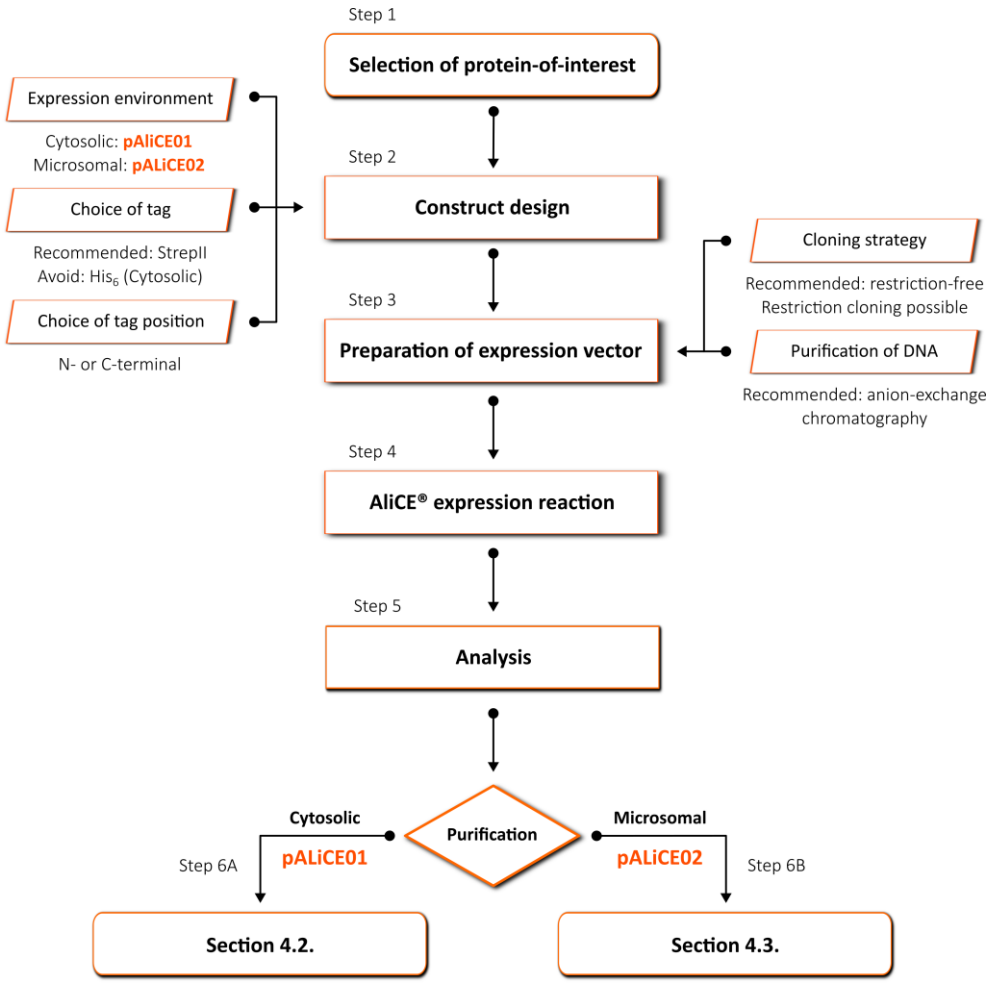


Figure 1. ALiCE workflow from protein selection to purification steps

Step 1: Selection of protein-of-interest

Choose your protein-of-interest (POI) for cell-free protein expression with ALiCE®.

Step 2: Construct design

Decide if your POI requires post-translational modifications: Choose **pALiCE01** for standard cytosolic expression and **pALiCE02** for microsomal expression with complex post-translational modifications. We recommend using a **StrepII**-tag (or variation thereof) for protein purification purposes. **Avoid** His_6-tag in **pALiCE01**. The purification tag position can be N- or C-terminal and should be tested for each protein construct anew.

Step 3: Preparation of expression vector

The expression of your POI in ALiCE® requires the insertion of the gene sequence at predefined locations in **pALiCE01** and **pALiCE02** to ensure the correct expression of your POI. The start and stop codon of the reference protein eYFP (already present in **pAliCE01** and **pALICE02**) should be used for guidance. We recommend restriction-free cloning methods for the insertion of your gene-of-interest. For your convenience, we also included restriction sites that enable cloning methods which use restriction enzymes. For **pALiCE01** and **pALiCE02**, the restriction enzymes *Nco*I-*Kpn*I can be used for insertion of your gene-of-interest. Alternatively, in **pALiCE02** only, one can use the restriction enzyme pair *Nco*I-*Xho*I to add a C-terminal His_6-tag to your POI.

We recommend to purify your plasmid DNA for the ALiCE® reaction by anion exchange chromatography (e.g. NucleoBond Xtra Maxi kit for transfection-grade plasmid DNA, Macherey-Nagel). Silica-based purification methods may leave impurities that can influence the expression performance.

Step 4: ALiCE® expression reaction

The ALiCE® reaction mix only requires the addition of plasmid DNA for protein expression. To avoid excess dilution of the reaction mix, we recommend a minimal DNA concentration of 250 ng/µL. The expression reaction performs best at a defined final DNA concentration. A good starting point for the final plasmid DNA concentration is 5 nM and can be further optimized if needed.

For larger experimental setup, we recommend using microtiter plates with the “Duetz” sandwich cover plate system from EnzyScreen BV (Heemstede, Netherlands) together with a humidity-controlled shaker with a humidity of above 70% at 500 rpm at a shaking diameter of 12.5 mm. The expression is performed over the course of 24-48 h.

Step 5: Analysis

Before attempting purification, successful expression of your POI can be checked by SDS-PAGE/Western Blot. We recommend to use a StrepII-tag for purification which also enables the detection of the POI using an antibody conjugated with horseradish peroxidase that binds to the StrepII-tag.

Step 6: Purification

A. Protein expressed in the cytosol

The protein is ready to use for purification steps. Follow the protocol description in section 4.3.

B. Protein expressed in the microsome

The protein needs further processing before purification. Follow the protocol description in section 4.4.

3.2. Sequence features of ALiCE® expression vectors

ALiCE® is provided with a set of two vectors to serve different needs regarding protein expression. The **pALiCE** vector backbone contains features needed for the expression of your protein-of-interest and is present in all **pALiCE** vectors (**Figure 2**).

A T7 promoter sequence enables the use of plasmid DNA in ALiCE® and is used as the reference point for the relative location of the other sequence featuresfpALC (i.e. the first nucleotide in the T7 promoter sequence is position 1). Two plant translational enhancer elements from the tobacco mosaic virus (TMV) are present in **pALiCE** which greatly impact the expression yield of your protein-of-interest. As a consequence, the distance of the TMV 5' untranslated region (UTR), also referred to as Omega, to the start codon, ATG, in all **pALiCE** vectors must not be changed. In **pALiCE02** only, a melittin signal peptide (MSP) from the honeybee is present in the sequence to target the protein-of-interest to the microsome.

For purification purposes, in both **pALiCE01** and **pALiCE02**, an N-terminal StrepII-tag is present in the vector already. The StrepII-tag can be cleaved off using the Factor Xa cleavage site. Additionally, in **pALiCE02**, a C-terminal His_6-tag is present in the vector that can be included in the final protein sequence. Note that due to current processing requirements, a His_6-tagged T7 polymerase is present in the lysate at low concentrations which may impact downstream detection and purification efforts when a His_6-tag is used in **pALiCE01**.

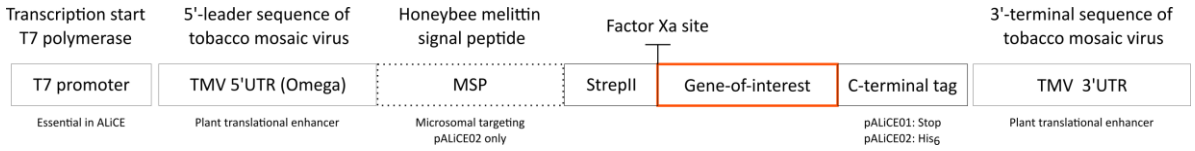


Figure 2. General sequence features of the pALiCE vector suite

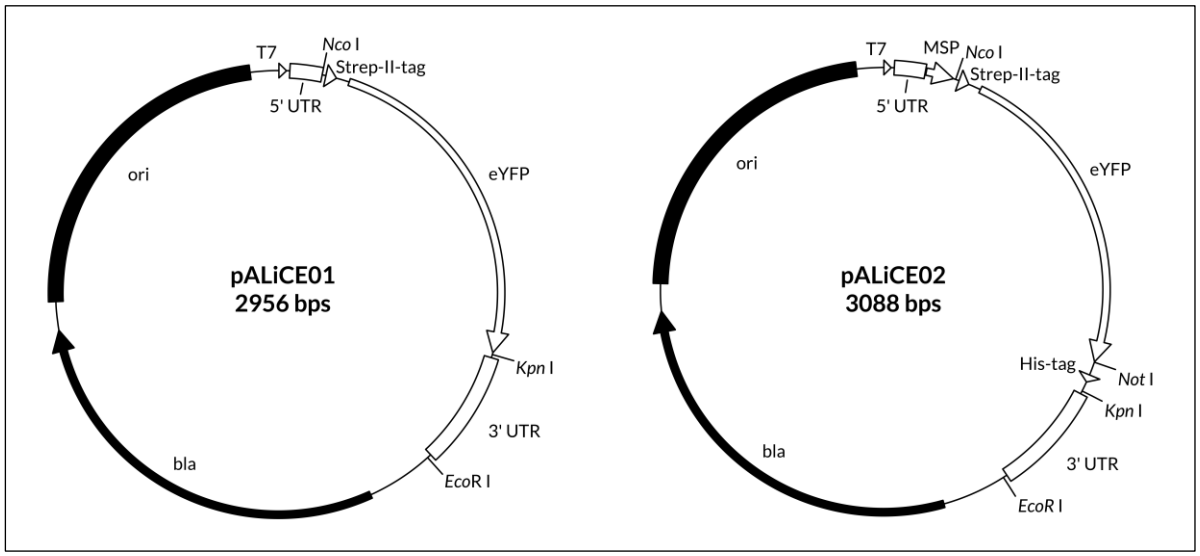


Figure 3. Plasmid maps of pALiCE01 and pALiCE02 including sequence features, vector size, and selected restriction sites.

a) pALiCE01 to express proteins in the cytosolic fraction

pALiCE01 is the vector of choice if no post-translational modifications are needed. It enables the high-yield production of your protein-of-interest. pALiCE01 contains a StrepII-tagged enhanced Yellow Fluorescent Protein (eYFP) sequence which can be used as an expression control (**Figure 3**). We recommend restriction-free cloning to insert your gene-of-interest but for your convenience, the *Nco*I-*Kpn*I restriction enzyme pair can be used for cloning purposes as well. A Factor Xa protease cleavage site is present to allow multi-step purification schemes.

Figure 4. Sequence features of pALiCE01 containing a StrepII-tagged eYFP

b) pALiCE02 to express proteins with post-translational modifications in the microsomes

Microsomes are organelle-like structures formed from the endoplasmic reticulum during the manufacturing process of the ALiCE® reaction mix. Guided by the 20-residue long honeybee melittin signal peptide (MSP) present only in pALiCE02 (**Figure 4**), proteins are translocated into these organelles (Buntru et al., 2015), enabling post-translational modifications like glycosylation or disulfide bridge formation.

Similar to pALiCE01, pALiCE02 contains an N-terminal StrepII-tag with a Factor Xa cleavage site. Additionally, a C-terminal His_6-tag is present in the sequence and can be used for detection and purification purposes. The restriction enzyme pair *Nco*I-*Kpn*I can be used for insertion of your gene-of-interest. Additional restriction enzyme recognition sites are present at the C-terminal end of the insert sequence enabling the use of *Not*I, *Xho*I, and *Xma*I for cloning access.

After the expression reaction is complete, the microsomes can be disrupted to recover your protein-of-interest using a mild detergent like Dodecyl-β-D-maltoside (DDM).

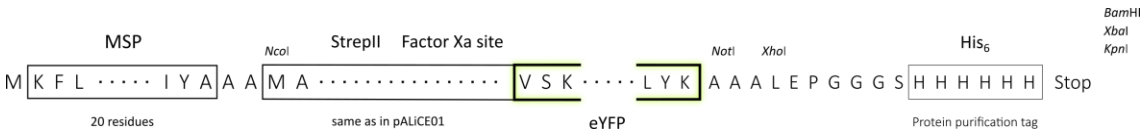


Figure 5. Sequence features of pALiCE02 containing an eYFP sequence with both an N-terminal StrepII-tag and a C-terminal His_6-tag

pALiCE01 can be used as a **positive control** for expression, expressing eYFP when added to the ALiCE® reaction mix and enabling an easy fluorescence readout (excitation peak at 513 nm, emission peak at 530 nm). The successful expression of eYFP can also be seen by eye with an intensive yellow colour. The eYFP construct expressed in pALiCE01 also contains a StrepII-tag which can be used for detection via a HRP-conjugated antibody against StrepII in a Western Blot.

3.3. Template preparation for ALiCE® reaction by cloning into pALiCE vectors

Insertion of your gene-of-interest into **pALiCE** vectors can be achieved by common cloning methods. We recommend restriction-free cloning to allow the insertion of your gene-of-interest without additional cloning artifacts in your protein sequence that may have an adverse effect on your protein function or expression. However, restriction sites have been carefully placed at specific positions within the insert region to allow for efficient restriction enzyme based cloning if so desired.

For your convenience, **pALiCE** vectors are available at third party suppliers which can synthesize your gene-of-interest and perform all the cloning steps including plasmid amplification for you. Please contact us for further information.

pALiCE vectors have been optimized for the ALiCE expression reaction and are our preferred vector of choice. Nonetheless, other plasmids can be used, but they should contain a T7 promoter sequence and the TMV 5' and 3' UTRs described in section 3.2.

Insertion of your gene-of-interest using restriction enzymes

For restriction-based cloning, several restriction sites are available to insert your gene-of-interest: In **pALiCE01**, the restriction enzyme pair *Nco*I-*Kpn*I can be used for insertion (Figure 6). Make sure to include additional nucleotides after the *Nco*I recognition site to avoid a frame shift. A stop codon needs to be included upstream of *Kpn*I when amplifying your gene-of-interest.

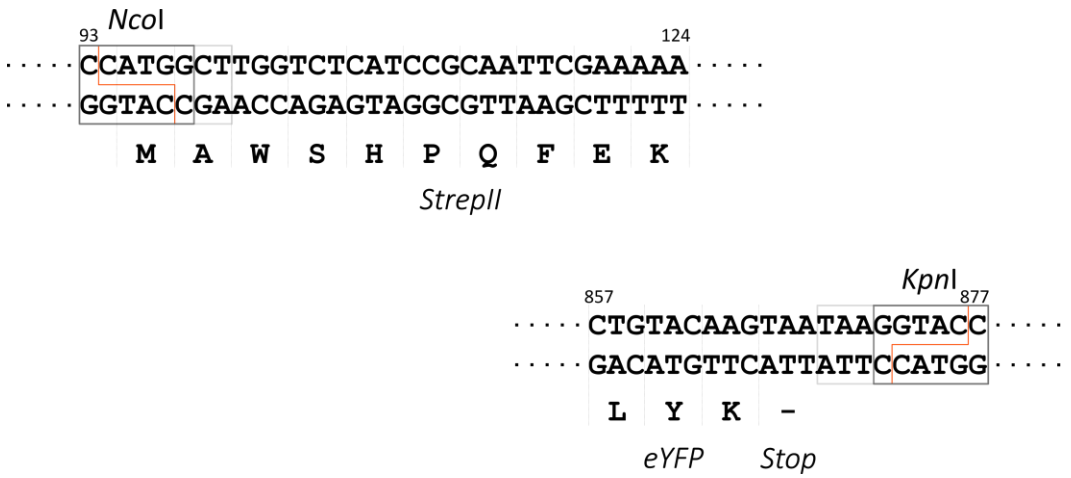


Figure 6. Suggested restriction enzyme recognition sites in pALiCE01 for insertion of gene-of-interest. Light grey box near *Nco*I indicates the extra codons that should be included in the primer design to avoid a frame shift. Note that a stop codon needs to be included on the 3' end of your amplified gene segment before *Kpn*I.

For **pALiCE02** the recommended restriction enzyme pairs are *Nco*I-*Not*I/*Xho*I if no C-terminal His6-tag is desired. If you want to include an additional C-terminal His6-tag together with the N-terminal StrepII-tag you should use the restriction enzyme pair *Nco*I-*Bam*HI/*Xba*I/*Kpn*I. In all cases remember to include additional nucleotides after *Nco*I to avoid a frame shift on that end and for the C-terminal end of your amplicon to include a stop codon before the restriction site.

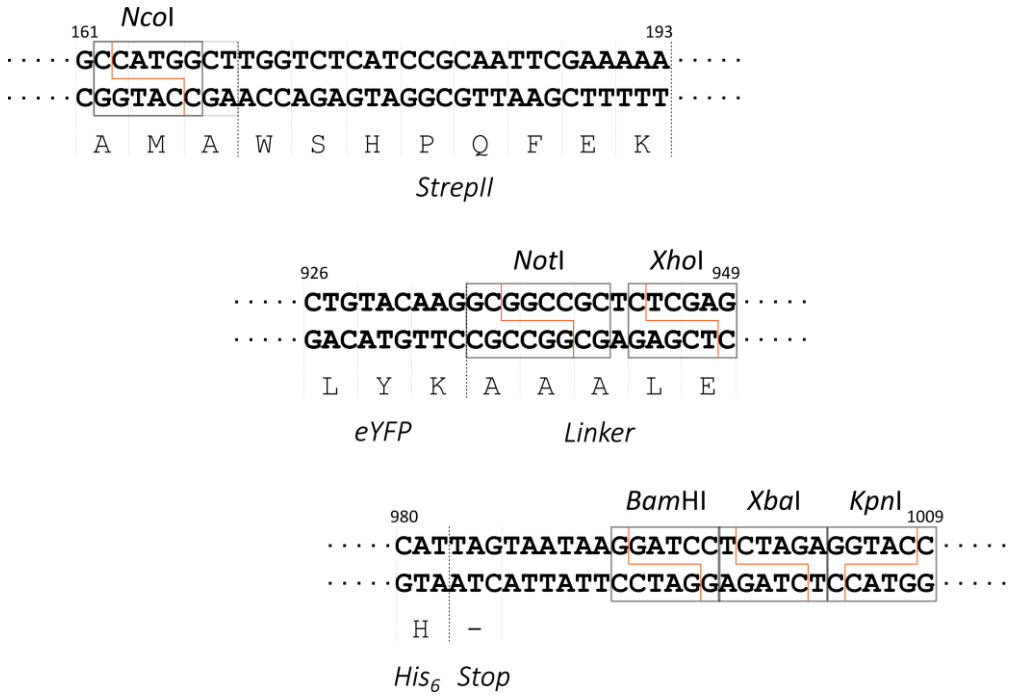


Figure 7. Suggested restriction enzyme recognition sites in pALiCE02 for insertion of gene-of-interest. An additional C-terminal His_6-tag is present which can be included in your desired construct using the *Not*I or *Xho*I restriction sites. *Bam*HI/*Xba*I/*Kpn*I can be used for insertion together with *Nco*I but a stop codon needs to be included in the primer design.

Once the gene of interest is cloned into **pALiCE** vectors (Green & Sambrook, 2012) they can be transformed into competent DH5α *E. coli* strain. Use of other *E. coli* strains may have an adverse effect on target protein yield due to diminished plasmid DNA quality. **pALiCE** vectors encode a β-lactamase (bla) gene which provides resistance against ampicillin for microbiological selection.

Template DNA Purification

To facilitate an efficient transcription-translation reaction, plasmid DNA with at least transfection-grade purity should be used in ALiCE®. It is therefore recommended to use a plasmid preparation procedure based on anion exchange chromatography. If you still decide to use a plasmid DNA preparation kit based on silica matrices, we recommend the addition of RNase inhibitors.

We recommend adjusting your final plasmid DNA concentration to 250-1000 ng/µL with 5 mM Tris-HCl buffer at a pH of 8.5. **Avoid** the use of EDTA as it can interfere with the expression reaction. Lower DNA concentrations will dilute the reaction mix and may impact the reproducibility of the expression reaction.

4. Protein expression with ALiCE®

4.1. Preparation of plasmid DNA

Each protein expressed in ALiCE® has a unique plasmid DNA concentration optimum. This depends on the accuracy of the DNA concentration determination, the size of your insert, and the overall efficiency of the transcription/translation reaction observed for the lysate. To allow a vector-independent comparison of DNA concentration, a conversion from mass per volume (here in ng/µL) to a molar DNA concentration (here nmol/L = nM) is needed.

A calculation scheme is provided which only requires the following input from you:

1) The full length of your plasmid (with insert).
2) The desired reaction volume (usually 50 µL).
3) The desired final molar concentration in the reaction mix (start with 5 nM).
4) The DNA mass concentration of your plasmid stock (>250 ng/µL).
5) A conversion factor to immediately receive a convenient volume output in µL.

The **formula** provided here provides you with a **volume output** in [µL] of the **plasmid stock** you need to add to your reaction mix. It assumes an average molecular weight of a double-stranded DNA (dsDNA) base pair (bp) of 618 g/mol for simplicity. A clarifying example is given for a vector with a size of 3000 bp:

$$V\,[\mu L] = \overbrace{\text{Plasmid length [bp]} \times 618\ \text{g/mol/bp}}^{\text{Average dsDNA molecular weight}} \times \text{Reaction volume [µL]} \times \frac{\text{Final concentration [nM]}}{\text{Plasmid stock [ng/µL]}} \times \overbrace{10^{-6}}^{\text{Conversion factor}}$$

Calculation example:

Plasmid length = 3000 bp
Reaction volume = 50 µL
Final concentration = 5 nM
Plasmid stock = 1000 ng/µL

$$V\,[\mu L] = 3000\ \text{bp} \times 618\ \text{g/mol/bp} \times 50\ \mu L \times 5\ \text{nM} / 1000\ \text{ng/}\mu L \times 10^{-6} = 0.5\ \mu L$$

Result: Add 0.5 µL of DNA to 50 µL of ALiCE reaction mix

Figure 7. Formula for calculating the volume of plasmid DNA needed for addition to ALiCE® reaction mix

4.2. ALiCE reaction protocol

The reaction volume for the ALiCE® expression reaction is ideally 50 µL per tube or well. The volume can be increased to 200 µL but comes with a decreased expression efficiency due to lower oxygen transfer rates.

Only use the supplied punctured caps to close ALiCE® tubes.

For 96-well half area microplate reactions, we recommend using the the Sandwich cover system "Duetz" from EnzyScreen BV (Heemstede, Netherlands) and a controlled humidity environment of >70%. Avoid using any wells on the edge of the plate.

If no humidity control is available, we recommend the addition of water in between the reaction compartments to maintain high humidity during the incubation.

1) Thaw ALiCE® reaction mix

a) Remove the ALiCE® reaction mix from storage and thaw in a water bath at room temperature.

b) Mix gently by inverting the tube. **Do not vortex!**

c) Spin tubes at 500 x g for a maximum of 5 seconds to collect all liquid.

d) Place on ice directly. Start the reactions within 30 min after thawing. If you would like to aliquot the remaining lysate, you can freeze it at -80°C. **Do not use liquid nitrogen!**

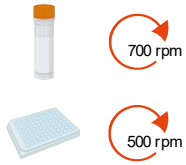
2) Reaction assembly and reaction

a) Prepare 50 µL of AliCE® reaction mix per tube/well.

b) For the 96-well half area microplates avoid pipetting into the wells on the edge of the plate and add water in between the wells if no humidity control is present in the shaker.

c) Add the calculated volume of plasmid DNA from **Step 1**. For the positive control (pALiCE01-eYFP), which is provided with each kit, use 2 µL of plasmid DNA.

d) Incubate the reaction mix at 25°C for 48 h. For ALiCE tubes, use a shaking speed of 700 rpm (Eppendorf ThermoMixer series or equivalent shaking diameter). For 96-well half area microplates, use a setting of 500 rpm on an orbital shaker (shaking diameter of 12.5-25 mm).



Do not seal reaction vessels or plates!

3) Analysis of expressed protein

- Perform an SDS-PAGE/Western Blot using 1 µL or less of reaction mix according to the manufacturers recommendations. We recommend a HRP-conjugated antibody against StrepII when using the tags included in **pALiCE01** and **pALiCE02**.

4.3. Downstream processing of proteins expressed in the cytosol

Your protein is ready for further downstream processing. We recommend affinity chromatography using the StrepII-tag included in **pALiCE** vectors for protein purification. **Avoid His_6-tag** in **pALiCE01** as the lysate contains His-tagged T7 RNA polymerase (about 100 kDa).

4.4. Downstream processing of proteins expressed in the microsome

Expressing proteins with ALiCE® using the **pALiCE02** vector will result in the target protein being produced in the microsomes of the reaction mix. The microsomes can be pelleted to separate the protein-of-interest from the proteins in the cytosol for a simple way of increasing the purity of the sample. The protein-of-interest is then released from the microsomes using a mild detergent like n-dodecyl-β-maltoside (DDM).

1) Transfer the reaction mix to a 1.5 mL Eppendorf tube.

2) Perform a centrifugation step at 4 °C at 16,000 x g for 20 min. Note that higher volumes (>50 µL) will require longer centrifugation times.

3) Carefully transfer the supernatant (sample S1) into a new tube for analysis.

4) Resuspend the pellet in at least 50 µL of 2 X concentrated buffer of your choice (e.g. PBS). Use higher volumes depending on the reaction mix volume you transferred in **Step 1**.

5) Add an equal volume of DDM stock (1% w/v) to the resuspended solution to achieve a final concentration of 0.5% (w/v) DDM with a 1 X buffer concentration. Take a small sample for later analysis (sample P1).

6) Incubate for 30 min at room temperature with a shaking speed of 300 rpm or less. **Do not agitate the solution excessively to avoid foaming!**

7) Centrifuge again at 16,000 x g for 30 min at 4 °C to pellet organelles and aggregated protein.

8) Transfer the supernatant (sample S2 for analysis) to a final tube. Resuspend the pellet in 1 X buffer for analysis purposes. Use the same volume as for the supernatant

9) Your protein is ready for further downstream processing. We highly recommend diluting the solution to <0.1% DDM as it may interfere with assays or affinity chromatography resin binding.

Note: Specific optimization may be required for certain proteins, especially membrane-bound proteins. A detergent with a higher critical micelle concentration may be used for convenient detergent removal by dialysis (not possible with DDM). For this we recommend n-decyl-β-maltoside (DM).

Troubleshooting

If the protein of interest is not produced or only produced in very small quantities, the following issues should be considered:

Problem	Possible cause	Recommended action
Low yield of target protein or positive control	Kit component deterioration	Make sure the expiration date on the label is not exceeded. Make sure all the components have been stored at the appropriate temperature.
	Poor plasmid DNA quality	Add RNase inhibitor to the purified DNA. If you used silica-based DNA purification methods, switch to anion exchange chromatography resin. Make sure to not heat-treat the purified template plasmids prior to protein expression reaction.
	Inappropriate plasmid DNA concentration	Check concentration of plasmid DNA. Titrate plasmid DNA concentration in a range of 1 to 20 nM in 2.5 nM steps. For some templates, lower or higher plasmid DNA concentration influences the level of protein production.
Problem	**Possible cause**	**Recommended action**
Low yield of target protein	Proteolytic degradation or precipitation	Check samples in regular intervals (every 12 h) for the presence of the target protein.
	Poor oxygenation	Ensure proper shaking of the samples. Do not seal the plate or the vial with gas-tight tape or film.
	Mechanical damage	Do not vortex the reaction mix, pipette carefully.
	Temperature fluctuation	Maintain a stable production temperature over the complete reaction cycle – lower temperature can lead to lower productivity and higher temperatures may possibly damage ALiCE®.
Evaporation of sample during the reaction	Wrong reaction vessel	Use ALiCE® Tubes with perforated caps provided by LenioBio. Ideally use 96-well half area microplates with the Duetz sandwich cover system and a controlled humidity of >70%.
	Environmental temperature too high	Make sure to control the environmental temperature to no higher than 25 °C.
	Environmental humidity too low	When using 96-well half area microplates, maintain a humidity level of optimally 70%. When using ALiCE® tubes in a tabletop shaker, control the room humidity to above 45%.
	Air circulation	Do not place ALiCE® next to a ventilator or a machine with exhaust air.

FAQ

A current version of our list of frequently asked questions can be found on our website.

References

1) Buntru, M., Vogel, S., Stoff, K., Spiegel, H., Schillberg, S. 2015. A Versatile Coupled Cell-Free Transcription-Translation System Based on Tobacco BY-2 Cell Lysates. Biotechnology & Bioengineering, 112(5):867-78.

2) Green, M. R., Sambrook, J. 2012. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press.

3) Kumar, G., Chernaya, G. 2009. Cell-free protein synthesis using multiply primed rolling circle amplification products. Biotechniques, 47(1):637-9.

4) Wang, K., Ma, Q., Jiang, L., Shujuan, L., Lu, X., Hou, Y., Wu, C., Ruan, J. 2016. Ultra-precise detection of mutations by droplet-based amplification of cirucularized DNA. BMC Genomics, 17:214.

Contact

LenioBio Technology & Innovation Center
Forckenbeckstr. 6
52074 Aachen, GERMANY
Tel.: +49 (0)211 890 940 30 500
Email: support@leniobio.com
www.leniobio.com

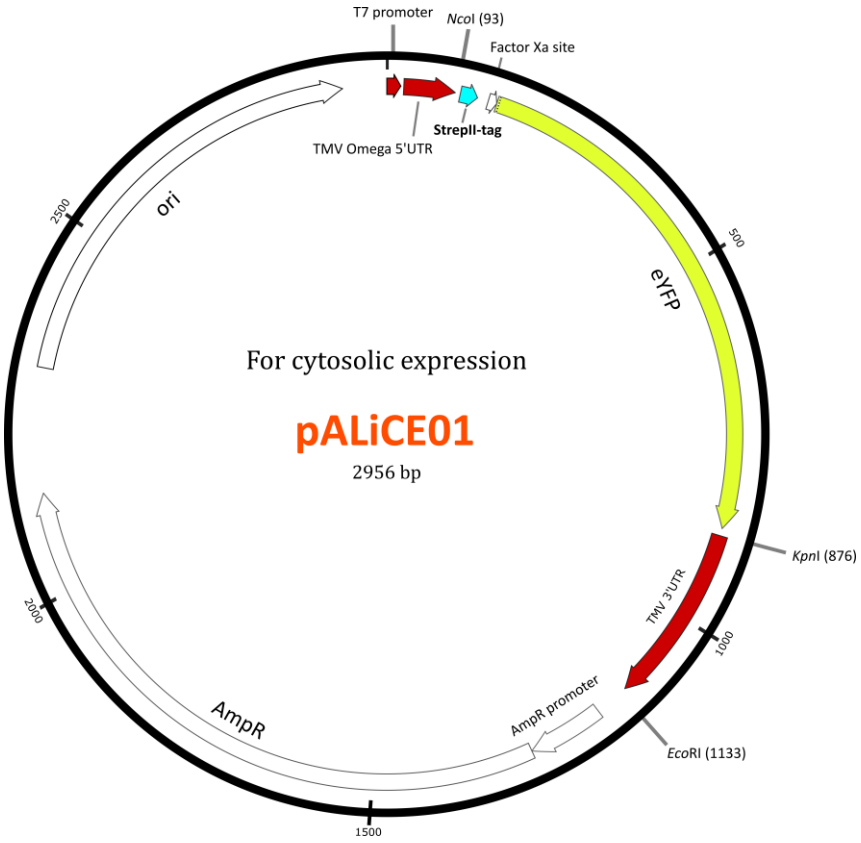
HB_ALiCE_V2_1022

Appendix

pALiCE01

Appendix Table 1. Reference elements of the pALiCE01 vector. Location of element is shown with relation to the T7 promoter.

Reference elements	Features	pALiCE01
T7 promoter	Transcription	1-17
TMV Omega 5' UTR	Translation enhancer	24-93
*Nco*I (contains start codon ATG)	Restriction enzyme	93
StrepII-tag	Protein purification	101-124
Factor Xa recognition site	Tag cleavage	140-151
eYFP from *Aequorea victoria*	Reporter	152-868
*Kpn*I	Restriction enzyme	876
TMV 3' UTR	Translation enhancer	879-1124
*Eco*RI	Restriction enzyme	1133
AmpR	Selection marker	1172-2137
Origin of replication (Ori)	Plasmid maintenance	2308-2896



Appendix Figure 1. Plasmid map of pALiCE01

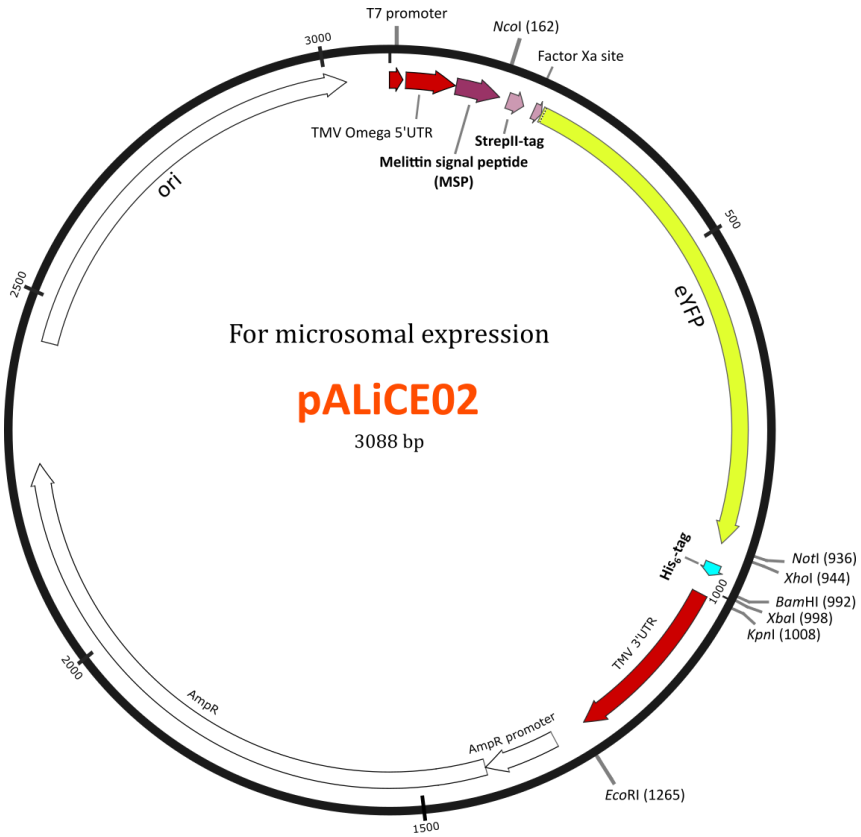
Full sequence:

TAATACGACTCACTATAGGGAGAGTATTTTTACAACAATTACCAACAACAACAACAAACAACAACAACATTACAT
TTTACATTCTACAACTACCATGGCTTGGTCTCATCCGCAATTCGAAAAAAGCGCTGAAAACCTGATCGAAGGCCG
TGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCA
CAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCAC
CGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCGCTACCC
CGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTT
CAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCT
GAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGT
CTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAG
CGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTA
CCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGAC
CGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATAAGGTACCAAGCTCTTCTGGTTTGGTTTGGA
CCTCTGGTCCTGCAACTTGAGGTAGTCAAGATGCATAATAAATAACGGATTGTGTCCGTAATCACACGTGGTGCG
TACGATAACGCATAGTGTTTTTCCCTCCACTTAAATCGAAGGGTTGTGTCTTGGATCGCGCGGGTCAAATGTATA
TGGTTCATATACATCCGCAGGCACGTAATAAAGCGAGGGGTTCGAATCCCCCCGTTACCCCCGGTAGGGGCCCAT
TATAGCCGAATTCGGCGCGCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTA
AATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAG
TATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCC
AGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAA
CAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATG
TGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTT
GGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCAT
AACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTT
GCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGA
GCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGC
TTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGC
TGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGTTCTCGCGGTATCATTGCAGCACTGGGGCCAGA
TGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGAT
CGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGA
TTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTA
ACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCT
GCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACC
AACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTT
AGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGC
CAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTG
AACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCT
ATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGA
GCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGA
GCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTT
CCTGGCCTTTTGCTGGCCTTTTGCTCACATG

pALiCE02

Appendix Table 2. Reference elements of the pALiCE02 vector. Location of element is shown with relation to the T7 promoter.

Reference elements	Feature	pALiCE02
T7 promoter	Transcription	1-19
TMV Omega 5' UTR	Translation enhancer	24-93
Melittin signal peptide (MSP)	Protein targeting	95-157
*Nco*I (contains start codon ATG)	Restriction enzyme	162
StrepII-tag	Protein purification	170-193
Factor Xa recognition site	Tag cleavage	209-220
eYFP from *Aequorea victoria*	Reporter	221-934
*Not*I	Restriction enzyme	936
*Xho*I	Restriction enzyme	944
His_6-tag	Protein purification	965-982
*Bam*HI	Restriction enzyme	992
*Xba*I	Restriction enzyme	998
*Kpn*I	Restriction enzyme	1004
TMV 3' UTR	Translation enhancer	1011-1256
*Eco*RI	Restriction enzyme	1265
AmpR	Selection marker	1304-2269
Origin of replication (Ori)	Plasmid maintenance	2440-3028



Appendix Figure 2. Plasmid map of pALiCE02

Full sequence:

TAATACGACTCACTATAGGGAGAGTATTTTTACAACAATTACCAACAACAACAACAAACAACAACAACATTACAT
TTTACATTCTACAACTACCATGAAATTCTTAGTCAACGTTGCCCTTGTTTTTATGGTCGTATACATTTCTTACAT
CTATGCGGCTGCCATGGCTTGGTCTCATCCGCAATTCGAAAAAAGCGCTGAAAACCTGATCGAAGGCCGTGTGAG
CAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTT
CAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAA
GCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCA
CATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGA
CGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGG
CATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATAT
CATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCA
GCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAG
CTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGC
CGGGATCACTCTCGGCATGGACGAGCTGTACAAGGCGGCCGCTCTCGAGCCCGGGGGGGGTTCTCATCATCATCA
TCATCATTAGTAATAAGGATCCTCTAGAGGTACCAAGCTCTTCTGGTTTGGTTTGGACCTCTGGTCCTGCAACTT
GAGGTAGTCAAGATGCATAATAAATAACGGATTGTGTCCGTAATCACACGTGGTGCGTACGATAACGCATAGTGT
TTTTCCCTCCACTTAAATCGAAGGGTTGTGTCTTGGATCGCGCGGGTCAAATGTATATGGTTCATATACATCCGC
AGGCACGTAATAAAGCGAGGGGTTCGAATCCCCCCGTTACCCCCGGTAGGGGCCCATTATAGCCGAATTCGGCGC
GCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTA
TCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTT
CCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGT
AAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGA
GAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCG
TATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGT
CACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACAC
TGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCA
TGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCC
TGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAAT
AGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGA
TAAATCTGGAGCCGGTGAGCGTGGTTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTAT
CGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTC
ACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTA
ATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCA
CTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTT
GCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGT
AACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAA
CTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTG
TCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCAC
ACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCT
TCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCC
AGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATG
CTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCC
TTTTGCTCACATG