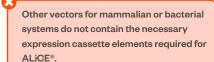
Getting started with ALiCE® - DNA preparation and cloning



Quick introduction to pALiCE vectors

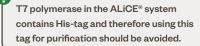
 The pALiCE vector backbone contains features needed for the expression of your protein-of-interest in the ALiCE® system and is present in all pALiCE vectors.



- pALiCE01 and pALiCE02 are high-copynumber plasmids.
- pALiCE02 carries the melittin signal peptide (MSP) which guides the protein to be produced to the microsomes, vesicles from formerly ER and Golgi. When the protein-of-interest requires post-translational modifications (PTMs), it needs to be produced in the microsomes.
 - We recommend replacing any native signal peptides with MSP.
- Expectations of yield pre-purification.
 - Cytosolic expression of simple proteins expressed using pALiCE01 requiring no ER/Golgi-specific post-translational modifications leads to yields of up to 2 mg/mL.
 - Microsomal expression of more complex proteins or membrane proteins expressed using pALiCE02 leads to yields between 0.05 and 0.5 mg/mL.
- The full vector sequences and additional information can be found in the Instruction Manual starting at page 18.

Designing the gene-of-interest

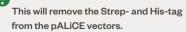
- Does the protein require PTMs?
 - If no: use pALiCE01.
 - If yes: use pALiCE02.
- When working with transmembrane proteins without PTMs, expression in the microsomes using pALiCEO2 can still be considered.
- When protein purification is needed the recommendations is:
- Strep-tag® for purification for proteins in general.
- Monoclonal antibody (mAb) purification via protein A (no special tag required).



- Are protease cleavage sites for removal of e.g. (affinity) tags required?
 - If so, cleavage sites can be added between protein of interest and tags.
- Host species for the ALiCE® system is
 Nicotiana tabacum and codon optimization
 is recommended.

Cloning

- · How to clone your gene-of-interest
 - Recommended: Restriction enzyme free e.g. using Gibson Assembly® Cloning Kit from NEB.
 - · Restriction enzymes.
 - Ncol/Kpnl restriction enzyme pair can be used.



- How to handle solubility problems for the protein of interest.
 - Solubility tags like SUMOstar or MBP could improve this.
- The ALiCE® system can work with linear DNA as template.
 - Please reach out directly to the LenioBio support team at <u>support@leniobio.com</u> to learn more.
- pALiCE01 and pALiCE02 vectors are also included in the portfolio of some third-party suppliers who prepare the complete pALiCE constructs of your choice containing your gene-of-interest for you.
 - Please reach out directly to the LenioBio support team at <u>support@leniobio.com</u> to learn more.

Preparation of plasmid DNA

 High quality transfection grade DNA is crucial.



Use anion-exchange columns, e.g.
NucleoBond Xtra Maxi kit for transfectiongrade plasmid DNA, from Macherey-Nagel.



Silica-based purification columns may leave impurities that will reduce the expression performance and in most cases prevent expression.

- Required DNA amounts.
- Start your experiments with 5nM.
- It's recommended to test other concentrations e.g. 7.5nM up to 20nM to maximise performance.
- When using multiple pALiCE plasmids with different inserts in one reaction (e.g. for bispecific antibodies) it is important to dilute or concentrate to the same final molarity of 5nM in the first experiment. Different ratios of the plasmids should be tested.



DNA concentration may significantly affect protein yield.

- Running a control.
 - pALiCE01 contains eYFP which can be used as positive control for cytosolic expression.



pALiCE02 with eYFP should not be used as control for microsomal expression. A proper control plasmid will be available shortly.

Additional information or support required?

All steps are described in

or

Follow this link to read our

for additional guidance.

If any questions come up while preparing the DNA template, don't hesitate to reach out to