Getting started with ALiCE® – DNA preparation and cloning

LenioBio®

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.

Other vectors for mammalian or bacterial systems do not contain the necessary expression cassette elements required for ALiCE®.

- pALiCE01 and pALiCE02 are high-copy-number 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.

Open full ALiCE® Instruction Manual

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 pALiCE02 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).

T7 polymerase in the ALiCE® system contains His-tag and therefore using this tag for purification should be avoided.

- 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.
    - *NcoI/KpnI* restriction enzyme pair can be used.

This will remove the Strep- and His-tag from the pALiCE vectors.

- 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 **support@leniobio.com** 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 **support@leniobio.com** 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 transfection-grade 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 ALiCE® Instruction Manual or ALiCE® Short Reaction Protocol

Follow this link to read our FAQ | LenioBio: Answers to Common Questions for additional guidance.

If any questions come up while preparing the DNA template, don't hesitate to reach out to support@leniobio.com