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 reference protein eYFP from pALiCE01 leads to yields of around 2mg/mL.**
  - **Microsomal expression of reference protein GrB-sfGFP leads to yields of around 0.5mg/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 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   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