FAQ

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1. What are the benefits of this method compared to classical cloning methods?

NZYEasy Cloning & Expression System relies on a simple reaction that enables the assembly of DNA inserts into a cloning or an expression vector in a precise and predetermined order. No insert phosphorylation, blunt-end polishing or digestion is required, thus making the NZYEasy Cloning & Expression System fast when compared to classical cut and ligation methods. Furthermore, cloning can be achieved either directly using blunt-ended or *Taq*-generated PCR products. The cloning is performed in a single reaction with high efficiencies compared to traditional methods, even when simultaneously clone multiple inserts into one vector.

2. Is it necessary to purify PCR products?

Spin-column purification of PCR products is highly recommended to remove dNTPs and impurities resulting from the amplification. Presence of non-specific amplification products and/or primer-dimers requires gel-purification of the amplified nucleic acid, which will enhance cloning efficiencies.

3. To amplify the insert, do I need to use PCR primers that have been purified by PAGE or HPLC?

No, you can use standard, desalted primers.

4. The insert will be cloned in reading-frame into pHTP vectors?

Yes. Inserts correctly cloned into pHTP1 expression vector will maintain reading frames starting on the ATG codon.

5. What type of competent cells is suitable to transform DNA clones obtained from the ligase-independent cloning reaction?

We recommend using *E. coli* DH5α (NZYTech, cat. No. MB004) as a host for cloning. Regarding expression, all *E. coli* strains expressing the gene that codifies for T7 RNA polymerase, such as BL21(DE3) (NZYTech, cat. No. MB006), are suitable to use.

6. Can other vectors be used instead of pHTP vectors?

No. pHTP vectors were designed to contain the required insert-complementary overhangs generated by

PCR, as well as the specific sequence recognized by the NZYEasy enzyme mix. In theory, any vector can be converted into a "pHTP vector" to function with the NZYEasy Cloning & Expression System. In case vectors available in our pHTP portfolio do not fit your needs, please contact us at *services@nzytech.com*. Other solubility tags can easily be included in the pHTP1 backbone and other vectors can be engineered to be used with this system. The NZYTech R&D team is available to assist you in case of need.

7. It is possible to clone many different inserts into one vector?

Yes, the NZYEasy Cloning & Expression System allows multiple fragment cloning into one pHTP vector. DNA fragments will be correctly annealed together in the desired order on the pHTP vector through the action of the highly efficient NZYEasy enzyme mix, free of any DNA ligase. Just combine the PCR-generated fragments with appropriate complementary overhangs, by allowing assembling in the order you choose, and a linearized pHTP vector. Both first and last inserts should have overhangs complementary with respective ends of the vector, thereby allowing efficient cloning. Each DNA fragment, encoding for an individual domain of the final multidomain construct, are kept in frame and are separated from each other by a linker sequence of 6 amino acids.

8. How many DNA fragments can be cloned in one reaction?

The number of inserts that can be joined in the same vector depends on their length and sequence. NZYEasy Cloning & Expression System has been tested efficiently for cloning 5 different genes of around 500-700 bp in size in the same vector. Note that the cloning efficiency decreases as the size of the final construct increases. However, if the simultaneous cloning of the desired inserts does not work, you can try a sequential cloning approach.

9. How can I reduce the number of false positive colonies containing the vector-backbone only?

Gel-purify the PCR band of interest in order to remove the template plasmid. Alternatively, digest PCR products with DpnI (NZYTech cat No. MB078) restriction enzyme to eliminate parental methylated DNA templates. When possible, you can also reduce the amount of template plasmid DNA to 0.1-0.5 ng per 50 µL PCR reaction.

10. The cloning reactions originate no colonies or a few number of colonies. What should I do?

Perform a cloning reaction using the PCR fragment provided as positive control with NZYEasy Cloning & Expression kits. Using this procedure, you can evaluate the functionality of cloning and transformation conditions.

If cloning and transformation result successfully using the positive control, then make sure that your DNA insert and the linearized pHTP vector share the required end-terminal complementarity by analysing primer sequences; verify the quality of the insert by gel electrophoresis; optimize the ligase-independent cloning reaction trying different vector:insert molar ratio, and/or repeat PCR and gel-purify the PCR product for a new cloning reaction and transformation. Alternatively, consider that the cloned insert may be toxic to *E. coli*. If this is the case, and if you are directly cloning into the expression vector, try to clone first into the low-copy number pHTP0 vector.

If cloning and transformation do not result successfully, check also the transformation efficiency of *E. coli* competent cells; verify if LB plates contain the appropriate antibiotic for the pHTP vector you are using; make sure you correctly handle the NZYEasy enzyme mix; verify if a specific component is missing in the cloning reaction.