AFFINITY PURIFICATION SYSTEMS

Profinity eXact™ Cloning and Expression Kits

- Downstream generation of native, tag-free proteins
- Trouble-free ligation of prepared inserts
- Generation of fusion proteins free of any linker residue between the tag and N-terminus of target
- Generation of tag-free target proteins containing a short (2 or more) N-terminal amino acid extension

An Unrivaled, Novel Tag System for the Expression of Fusion-Tagged Proteins

Introduction

Profinity eXact cloning and expression kits (Figure 1) are part of the Profinity eXact fusion-tag system, a patent-pending *E. coli*-based expression and purification system intended to address the bottlenecks in procedures for purification and tag-removal of fusion-tagged proteins. The use of protein fusion technologies have greatly simplified the process of purifying recombinant-tagged proteins, but a major disadvantage of working with protein fusions lies in the inconsistencies related to cleavage of the fusion partner, or tag. Cleavage of the tag is necessary when it poses a problem or disadvantage for the intended application of the protein. A system that improves the efficiency of existing methods of tag cleavage, while reducing the numbers of purification steps and reagents involved, is invaluable to life science researchers.

Fig. 1. Profinity eXact cloning and expression kits.

Profinity eXact Fusion-Tag Technology

The Profinity eXact system utilizes a modified form of the subtilisin protease, which is immobilized onto a chromatographic support and used to generate pure, tag-free target protein in a single step. The tag in this system is the prodomain of the subtilisin protease, a 75-amino acid sequence that fuses with the N-terminus of a target protein of interest. A high-affinity interaction exists between the Profinity eXact tag and the immobilized subtilisin protease ligand. The ligand both recognizes and binds the tag and, triggered by application of fluoride-containing elution buffer, quickly and precisely cleaves the target protein from the bound tag. With cleavage incubation times as short as 30 minutes, the Profinity eXact fusion-tag system provides a novel alternative to purify and process fusion proteins in a single step, without the addition of protease.

BIO-RAD

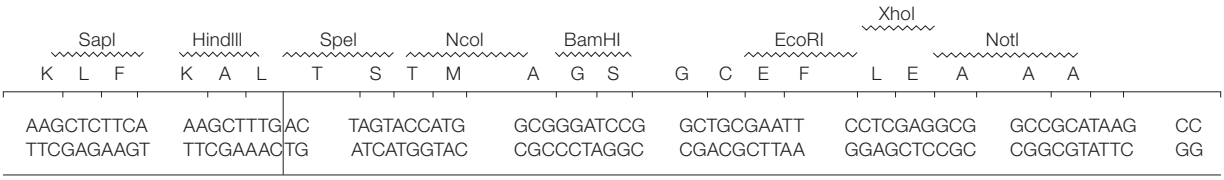

Fig. 2. Unique tag sequence of pPAL7 vector indicating cleavage recognition sequence ending in FKAL and location of restriction enzymes.

Profinity eXact pPAL7 Expression Vectors

Profinity eXact vectors facilitate the expression of a unique tag sequence (Figure 2) that not only participates in highly specific, tagged protein binding, but because of a modification at its cleavage recognition site (Figure 3), is also involved with precise, on-column processing in order to generate a native protein. The pPAL7 vectors contain the sequence that encodes the engineered subtilisin prodomain and a unique multiple cloning site (MCS), allowing creation of N-terminal Profinity eXact fusion-tagged proteins.

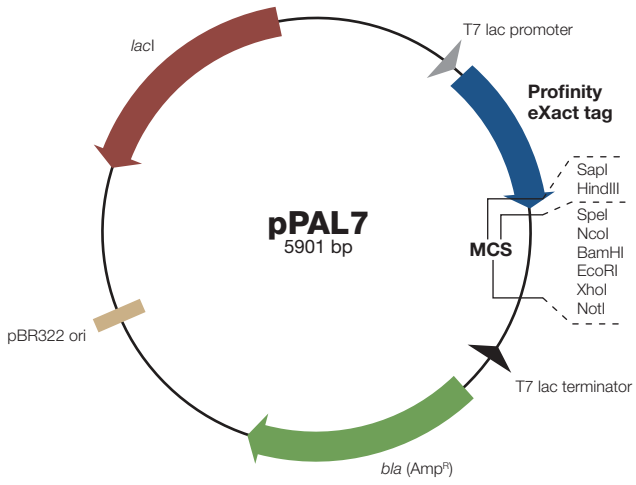

Fig. 3. Profinity eXact pPAL7 vector map.

The 5901 bp Profinity eXact pPAL7 vector offers a number of options for the cloning of DNA fragments and is available as a supercoiled plasmid and in a versatile predigested form for restriction enzyme-free cloning of any target gene.

The pPAL7 vectors are derived from a T7-based expression plasmid, and function through the T7 promoter and a T7 RNA polymerase expression host for inducible protein production. The plasmid confers ampillicin resistance, constitutively expresses the LacI repressor, and has a pMB1-derived ColE1 origin of replication (Table 1).

Table 1. Features pPAL7 expression vector.

Vector Feature	Vector Position
T7*lac* promoter	1–17
Profinity eXact tag	92–316
MCS	
SpeI	318
NcoI	325
BamHI	333
EcoRI	345
XhoI	351
NotI	358
T7 terminator	413–460
bla (Amp^R)	881–1,738
Ori	2,499
*lac*I	4,436–5,515

Generation of Precise-Cleaving Fusions

To preserve native N-termini of target proteins, fusions can be generated using either supercoiled or restriction enzyme-free plasmids. When using the Profinity eXact pPAL7 supercoiled expression vector, cloning is directed into the HindIII site and an appropriate restriction site in the MCS. The upstream PCR primer should contain a HindIII site, followed by two nucleotides, T and G, to restore the leucine codon at the cleavage recognition sequence terminus. In case of the predigested restriction enzyme-free vector, the gene of interest is produced following the restriction-independent cloning (RIC) protocol and is inserted into the SapI site. For all other insertions, primers can be designed according to the sites found in the MCS.

Utilizing Restriction-Independent Cloning

For a more flexible alternative, the pPAL7 vector is offered in a clever restriction-independent form that facilitates higher throughput cloning of insert fragments, regardless of internal restriction sites. The Profinity eXact pPAL7 RIC-ready expression vector (Figure 4) is supplied predigested with enzymes (SapI and EcoRI) in the multiple cloning site to provide precise cleavage upon purification. In this case, target insert preparation alone is needed prior to ligation with the RIC-ready vector. The target is PCR-amplified and treated with T4 DNA polymerase to create vector-compatible overhangs, and ligation is carried out according

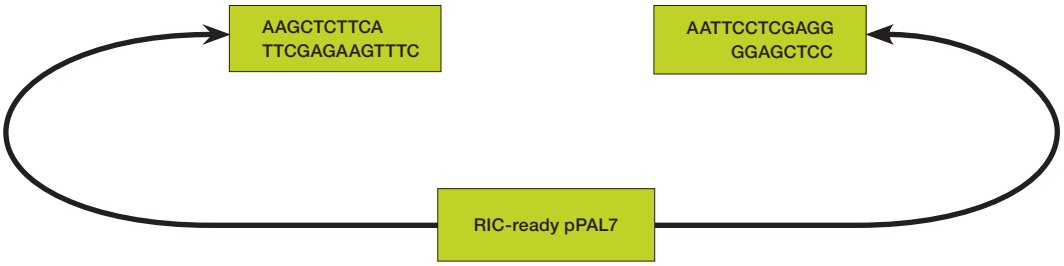

Fig. 4. Profinity eXact pPAL7 RIC-ready expression vector.

to a user-preferred protocol. When using the RIC-mediated protocol, ligation times of 16 hours (16°C) typically facilitate greater downstream transformation efficiencies.

Advantages of the Profinity eXact RIC-ready vector are:

- Generation of native, tag-free protein
- Trouble-free ligation of prepared inserts, regardless of internal restriction sites
- Yield of fusion proteins that are free from linker residues between the tag and N-terminus of the target

Chemi-Competent Cells

For routine protein expression, *E. coli* BL21(DE3) chemi-competent expression cells are available individually or as part of the Profinity eXact cloning and expression starter kit. BL21(DE3) cells are the preferred hosts for T7 vector-based protein expression. They are DE3 λ lysogens with the T7 RNA polymerase gene under the control of the lacUV5 promoter. Induction with IPTG allows production of T7 RNA polymerase, which then directs expression of the target gene located downstream of T7 promoter in the expression vector. The strain is deficient in OmpT and Ion proteases for improved recombinant protein stability. BL21(DE3) chemi-competent cells are packaged in single-use aliquots for convenience and efficiency.

Storage, Shelf Life, and Stability

To maintain optimal performance, Profinity eXact consumables should be stored at temperatures specified on kit and reagent product labels. Instruction manuals and kit components sold individually also provide usage and exact expiration dates for reagents. Most consumables are guaranteed for up to one year after the date of manufacture unless otherwise indicated. Table 2 provides a reference guide for storing products to ensure reproducible results.

Table 2. Profinity eXact product storage guide.

Product	Storage
pPAL7 RIC-ready expression vector	–20°C
pPAL7 supercoiled expression vector	–20°C
Cloning and expression starter kit	–20°C (vector DNA) –70°C (cells)
Chemi-competent cells (includes BL21(DE3) and cloning cells)*	–70°C

* Six-month shelf life.

Ordering Information

Catalog #	Description
156-3000	**Profinity eXact Cloning and Expression Starter Kit**, includes 25 µl of 20 ng/µl RIC-ready pPAL vector, 100 µl of 100 ng/µl supercoiled pPAL vector, chemi-competent cells, SOC growth media, 20 reactions
156-3001	**Profinity eXact pPAL RIC-Ready Expression Vector Kit**, includes 25 µl of 20 ng/µl vector, 20 reactions
156-3002	**Profinity eXact pPAL Supercoiled Expression Vector Kit**, includes 100 µl of 100 ng/µl vector, 20 reactions
156-3003	**BL21(DE3) Chemi-Competent Expression Cells**, includes 10 x 0.05 ml BL21(DE3) cells, pUC19 control plasmid, 10 ml vial of SOC growth media
156-3004	**Profinity eXact Antibody Reagent**
156-3005	**Profinity eXact Purification Resin**, 10 ml
156-3006	**Profinity eXact Mini Spin Purification Starter Kit**, includes 10 spin columns, 2 ml capped tubes (10), 2 ml capless tubes (10), lyophilized control protein lysate, bacterial lysis reagent, 50 ml bind/wash buffer, 20 ml elution buffer
156-3007	**Profinity eXact Mini Spin Columns**, includes 10 spin columns, 2 ml capped tubes (10), and 2 ml capless tubes (10)
156-3008	**Profinity eXact Expression and Purification Starter Kit**, includes 1 Profinity eXact cloning and expression starter kit (20 reactions) and 1 Profinity eXact mini spin purification starter kit (10 spin columns)
732-4646	**Bio-Scale™ Mini Profinity eXact Cartridges**, 2 x 1 ml
732-4647	**Bio-Scale Mini Profinity eXact Cartridges**, 4 x 1 ml
732-4648	**Bio-Scale Mini Profinity eXact Cartridges**, 1 x 5 ml
170-8235	**Opti-4CN Substrate Kit**
170-8237	**Opti-4CN Goat Anti-Mouse Detection Kit**

Practice of the polymerase chain reaction (PCR) may require a license.

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