pTYB21

Expresión 14
Resistance: Ampicillin 100 µg/mL

Description pTXB1 vector :
pTYB21 is an *E. coli* cloning and expression vector (7514 bp) used in the IMPACT™ Protein Purification System which allows the overexpression of a target protein as a fusion to a self-cleavable affinity tag (1,2). It is an N-terminal fusion vector designed for in-frame insertion of a target gene into the polylinker, downstream of the intein tag (the Sce VMA intein/chitin binding domain, 55 kDa)(3,4). This allows the N-terminus of the target protein to be fused to the intein tag. The self-cleavage activity of the intein allows the release of the target protein from the chitin-bound intein tag, resulting in a single column purification of the target protein.
This vector can be used in conjuction with a C-terminal fusion vector to test which fusion construction (N-terminal or C-terminal) maximizes the expression and yield of a target protein. For the fusion of the C-terminus of the target protein to the intein tag, use pTXB1 (NEB #N6707).

Complete sequence:
https://www.neb.com/~/media/NebUs/Page%20Images/Tools%20and%20Resources/Interactive%20Tools/DNA%20Sequences%20and%20Maps/Text%20Documents/ptyb21gbk.txt

Genotype of *E coli* strain BL21(DE3) pLysS : F– *ompT hsdS*(rB– mB–) *gal dcm* λ(DE3) pLysS (Camr)(λ(DE3): *lac*I, *lac*UV5-T7 gene 1, *ind*1, *sam*7, *nin*5)

Multiple Cloning Sites (MCS):

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      Intein     SapI      NdeI     NcoI      NotI     EcoRV    SalI     BamHI   EcoRI   SbfI/PstI
5´...CAGAACGGAAGAGCTCATATGTCCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCCTGCAGGTAATTAAATAA
       Q  N  G  R  A  H  M  S  M  G  G  R  D  I  V  D  G  S  E  F  P  A  G  N  *
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Features

- The multiple cloning site (MCS) is compatible with the multiple cloning sites of vectors in the pMAL Protein Fusion and Purification System (NEB #E8200) and the *K. lactis* Protein Expression Kit (NEB #E1000).
- When the SapI (or BspQI) site in the MCS is used for cloning the 5´ end of the target gene, the N-terminus of the target protein is immediately adjacent to the intein cleavage site. This results in the purification of a target protein without any extra vector-derived residues at its N-terminus. After cloning the target gene in the MCS using SapI, the recognition sequence of SapI is lost; therefore, the vector cannot be recut with SapI. For details, see the IMPACT Manual.

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- When NdeI is used for cloning the 5' end of the target gene, extra amino acids (Gly-Arg-Ala-His) will be added to the N-terminus of the target protein.
- A stop codon should be included in the reverse primer.
- A pBR322 derivative with a ColE1 replication origin.
- Expression of the fusion gene is under the control of the T7/*lac* promoter and can be induced by IPTG due to the presence of a *lac*I gene (5).
- Expression requires an *E. coli* host that carries the T7 RNA Polymerase gene [e.g., T7 Express Competent *E. coli*, (NEB #C2566) or BL21(DE3) Competent *E.coli* (NEB #2527) and derivatives].
- Ampicillin resistance.
- When pTYB21 or pTYB22 is used, a small peptide (15 amino acids, 1.6 kDa) is also cleaved from the intein tag and co-eluted with the target protein. It cannot be detected on a regular SDS-PAGE and can be dialyzed out.
- Origin of DNA replication from the bacteriophage M13 allows for the production of single-stranded DNA by helper phage superinfection of cells bearing the plasmid. M13K07 Helper Phage (NEB #N0315) is available.
- Other IMPACT vectors are available which allow for fusion of a target gene to N- or C- terminus of an intein and a cleavage reaction which can be induced by thiol reagent or temperature/pH shift.
- Intein Forward Primer (NEB #S1263) and T7 Terminator Reverse Primer (NEB #S1271) are available for sequencing the target gene.

Simplified Expression and Purification Protocol:

1. Transformation: Transform the plasmid bearing the target gene into competent T7 Express or competent cells prepared from ER2566.
2. Cell Culture: Inoculate a freshly grown colony in LB medium containing 100 µg/ml ampicillin and grow the cells at 37°C. When the OD600 reaches 0.5, induce protein expression by adding IPTG to a final concentration of 0.4 mM, and incubate at 30–37°C.
3. Column Preparation: Equilibrate a chitin column (20 ml slurry for 1 liter culture) with 10 column volumes of Column Buffer [20 mM Tris-HCl (pH 8.5), 500 mM NaCl].
4. Cell Harvest: Centrifuge cell culture at 5,000 x g for 15 minutes at 4°C. Discard supernatant. Resuspend cell pellet in column buffer.
5. Loading: Break cells by sonication in Column Buffer, and centrifuge at 15,000 g for 30 minutes at 4°C. Slowly load the clarified lysate onto the chitin column (0.5–1.0 ml/minute).
6. Washing: Wash the column with at least 20 bed volumes of Column Buffer to thoroughly remove the unbound proteins (up to 2.0 ml/minute).
7. Adding Thiols: Quickly wash the column with 3 column volumes of Cleavage Buffer [Column buffer containing 50 mM DTT (for purification) or 50 mM MESNA (for IPL)].

8. On-column Cleavage: Stop the flow and incubate the column at 4°C–23°C for 16–40 hours. The temperature and duration of the cleavage reaction are dependent on the on-column cleavage efficiency which can be checked by analyzing samples of chitin resin before and after cleavage.
9. Elution: Elute the target protein with Column Buffer by continuing the column flow.
10. Dialysis: Dialyze the target protein in to an appropriate storage buffer; this will also remove the excess thiol reagent used in the Cleavage Buffer and the co-eluted small peptide (when using pTYB21).
11. Cleavage: To examine cleavage efficiency remove 100 µl of chitin resin and mix with 50 µl of 3X SDS Sample Buffer. After boiling for 5 minutes, analyze the supernatant on a Coomassie stained SDS-PAGE gel to determine the cleavage efficiency.
12. Regeneration of Chitin Resin: Wash the column with 3 bed volumes of the 0.3 M NaOH (Stripping Solution). Allow the resin to soak for 30 minutes and wash the resin with an additional 7 bed volumes of 0.3 M NaOH. Wash with 20 bed volumes of water, followed by 5 bed volumes of column buffer.

Media and Solutions:
The following are suggested media for cell culture, cell lysis and protein purification. They can be modified according to the specific properties of the target protein.
LB broth (per liter)
10 g tryptone
5 g yeast extract
10 g NaCl
Adjust pH to 7.0 with NaOH
Column Buffer
20 mM Na-HEPES (or Tris-HCl), pH 8.5
500 mM NaCl (or 50–1,000 mM NaCl)
1 mM EDTA (optional)
Nonionic detergents (0.1–0.5% Triton X-100 or 0.1–0.2% Tween 20) and protease inhibitors [e.g., PMSF (20 µM)] can also be included. For a target protein sensitive to oxidation, 1 mM of TCEP [tris-(2-carboxyethyl)phosphine] or TCCP [tris-(2-cyanoethyl)phosphine] may be used.
Cleavage Buffer
20 mM Na-HEPES (or Tris-HCl), pH 8.5
500 mM NaCl (or 50–1,000 mM NaCl)
50 mM DTT or β-mercaptoethanol or cysteine*
1 mM EDTA (optional)
Stripping Solution
0.3 M NaOH

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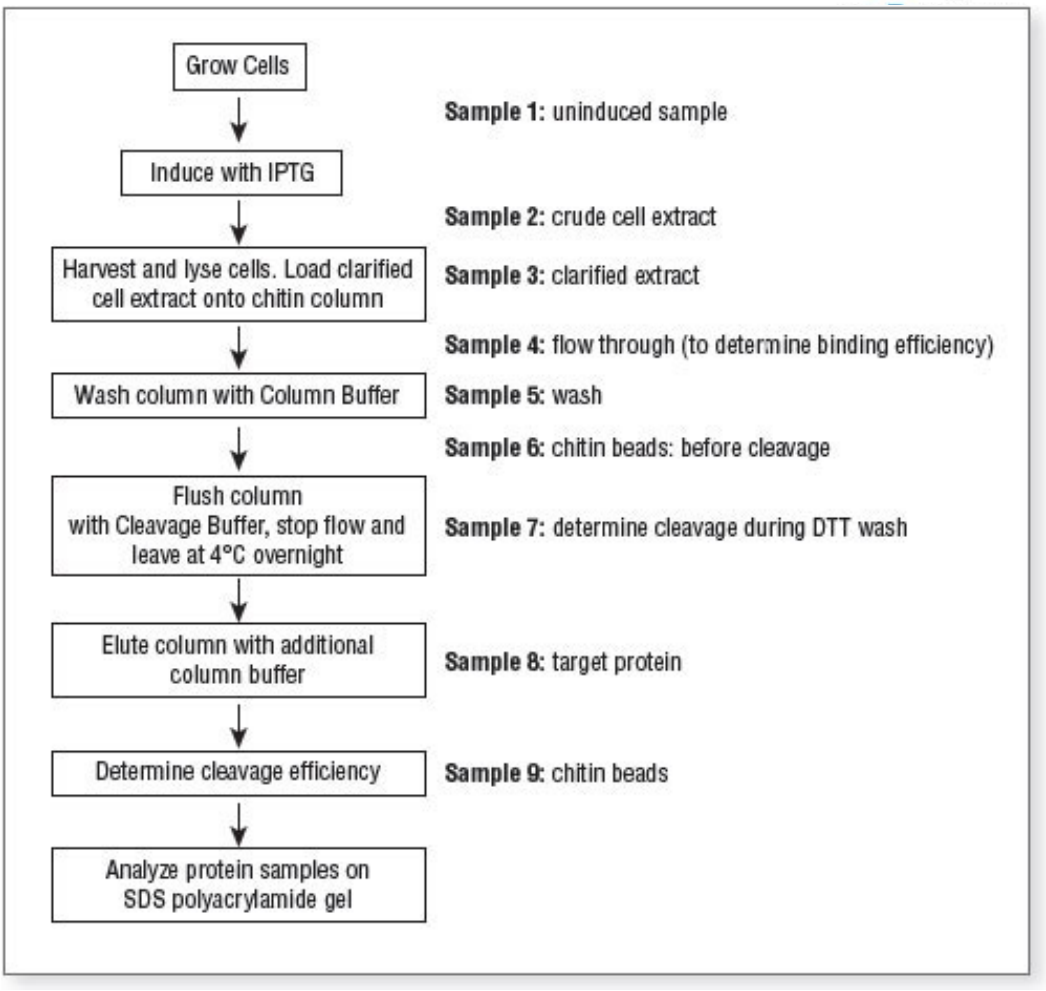

Flow chart for Protein Expression and Purification using the IMPACT System. Sample collection for analysis by SDS-PAGE is indicated.

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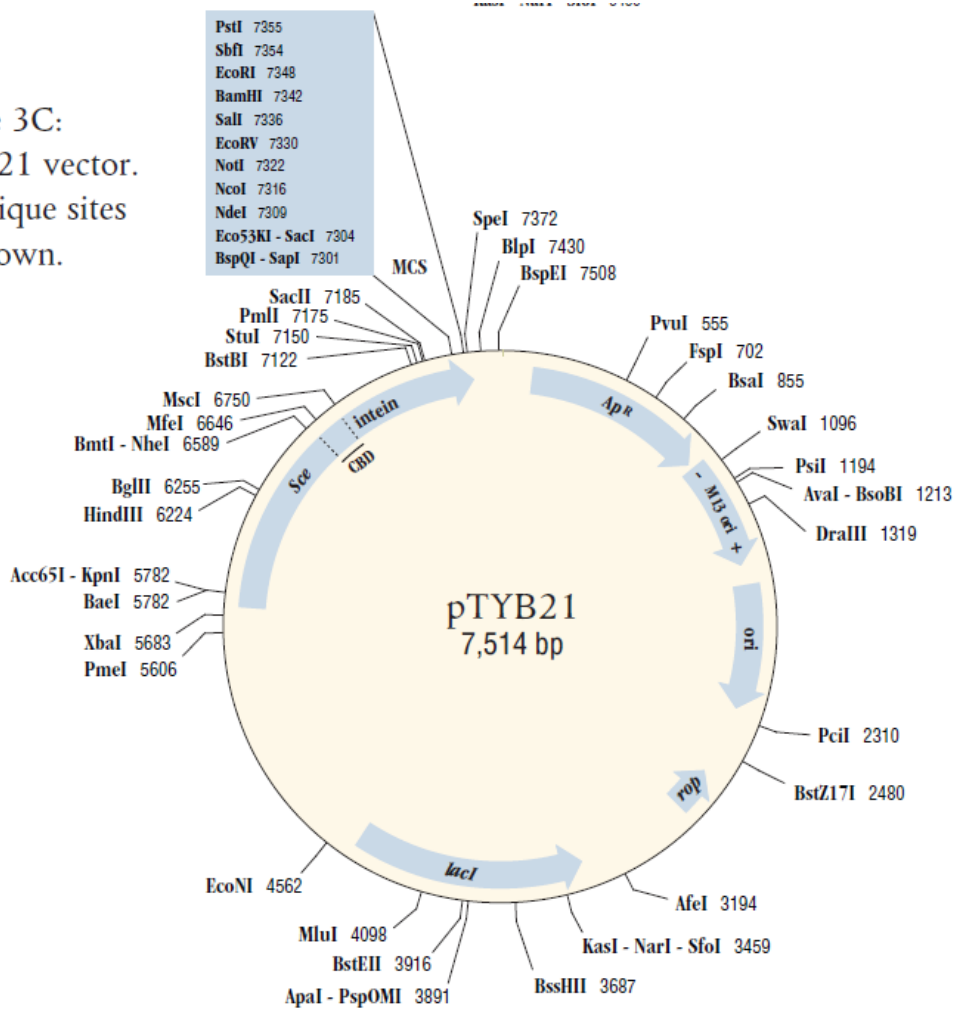
Figure 3C:
pTYB21 vector.
All unique sites
are shown.



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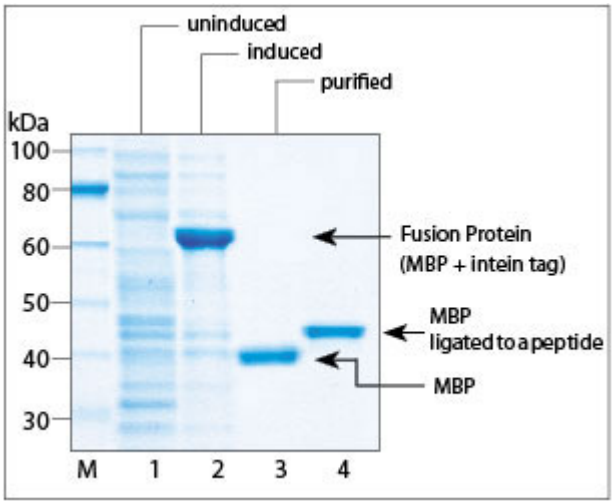

Purification of Maltose Binding Protein (MBP) in a single affinity purification step: Lane 1: uninduced cell extract. Lane 2: induced cell extract showing expressed fusion protein. Lane 3: MBP fractions eluted after inducing cleavage overnight at 4°C. Lane 4: MBP ligated to a peptide containing an N-terminal cysteine. Marker M is the Protein Ladder

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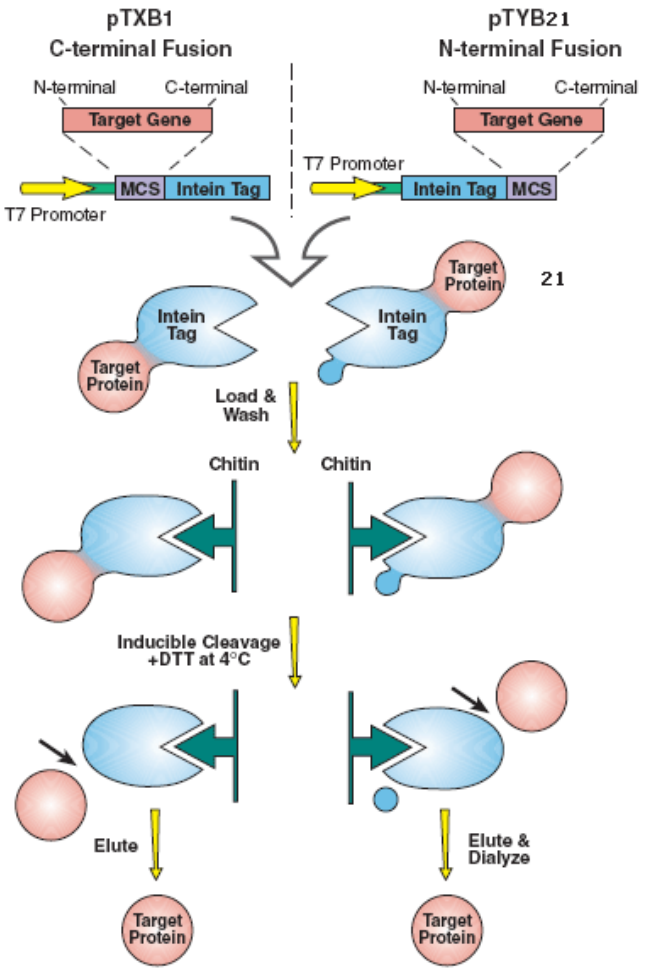

Schematic of the IMPACT System.

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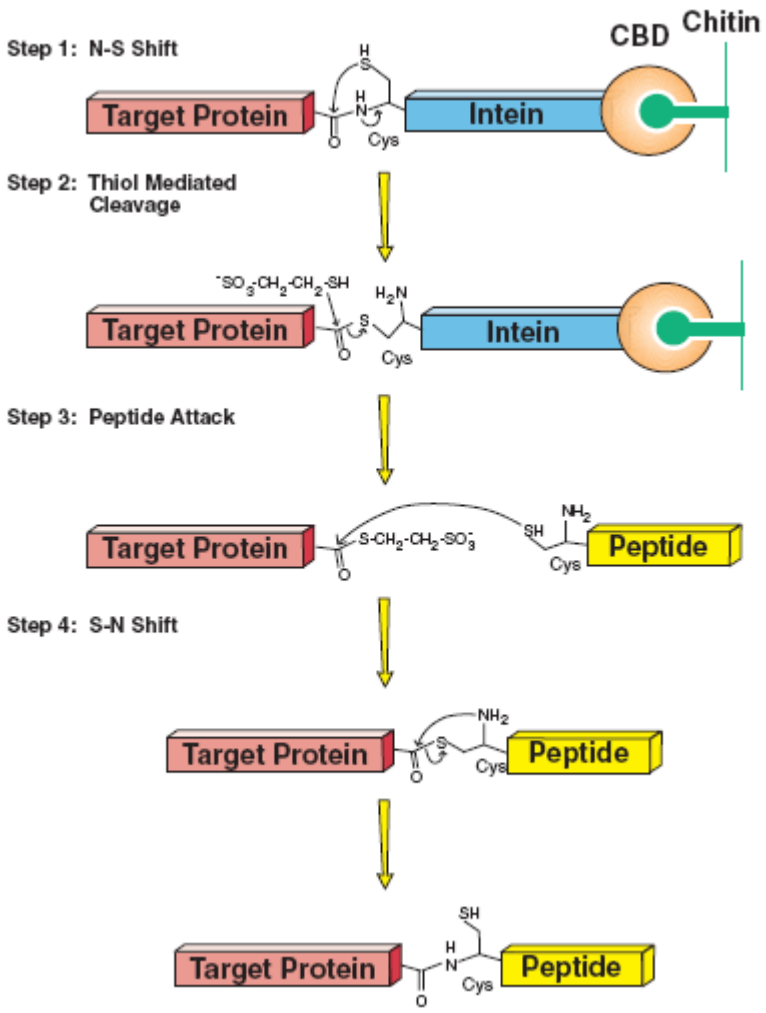

Intein-mediated Protein Ligation (IPL).