

Protein Expression and Purification in *E.coli*

FROM PCR TO LIGATION:

Read More: <http://www.scientifix.com.au/pdf/appfocus/Whole%20Successful%20cloning%20step%20by%20step%2015%20Feb%2007.pdf>

PLASMID MINIPREPS:

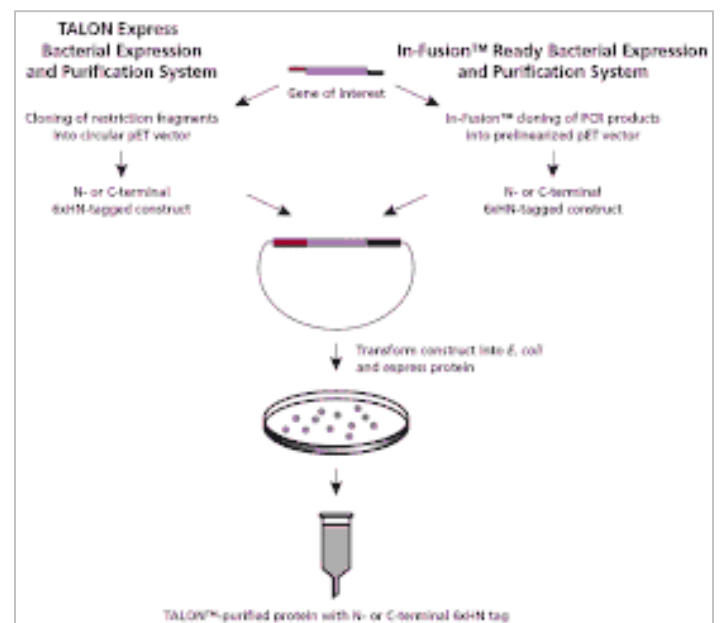
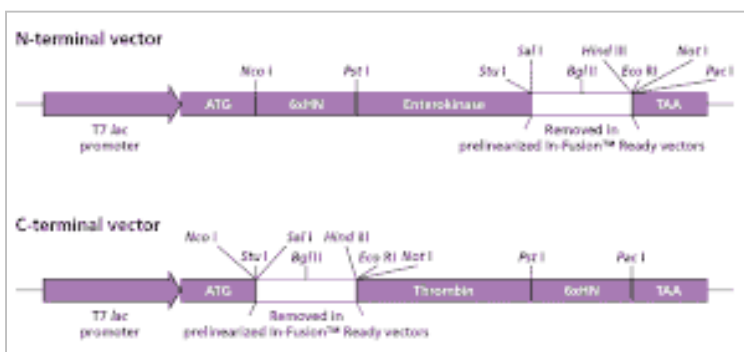
Macherey- Nagel Nucleospin Plasmid: up to 40µg of plasmid from 5ml of culture.

Read More: [https://www.macherey-nagel.com/web/MN-WEB-BioKatalog.nsf/web/PLASMID3/\\$File/Plasmid_DNA_NS_R03.pdf](https://www.macherey-nagel.com/web/MN-WEB-BioKatalog.nsf/web/PLASMID3/$File/Plasmid_DNA_NS_R03.pdf)

VECTORS:

Clontech Talon Express Bacterial Expression and Purification kit

- The vectors are based on the pET system, which uses the IPTG-inducible T7 polymerase gene and promoter to produce very high levels of protein, as much as one third of the total cellular protein.
- Two vector formats are available to generate an N- or C-terminal-tagged construct with 6xHN as fusion tags.
- The kits come with either circular vectors for traditional restriction enzyme cloning or prelinearized vectors (TALON Express In-Fusion™ Ready Bacterial Expression and Purification Kit) enabling easy, directional cloning of PCR products with very low background. In-Fusion cloning provides several advantages over traditional cloning, including the ability to clone PCR products without restriction digestion, saving valuable time. Significantly, In-Fusion cloning is both precise and directional and is ideally suited for the generation of in-frame fusions to purification tags. In the case of the In-Fusion Ready Kit, the same PCR insert can be used to generate both N- and C-terminal-tagged constructs.



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Clontech Pro Tet Bacterial Expression System

- Achieve high protein expression and induction levels with tight control of expression in response to the level of inducer (anhydrotetracycline).
- The kit comes with a PROTet 6xHN Vector in one of 3 reading frames, and encodes an N-terminal polyhistidine affinity tag that allows proteins to be easily and efficiently purified. These vectors also contain an enterokinase (EK) site so that the tag can be excised from the protein of interest by proteolytic cleavage.
- The PROTet 6xHN System is compatible with the Creator™ System. This system comes with the pLP-PROTet-6xHN Acceptor Vector, for highly inducible, tet-regulated protein expression of any gene of interest cloned with the Creator™ pDNR Cloning Kits. pLP-PROTet-6xHN has all the features of the PROTet Vectors, but also includes a *loxP* site for easy gene transfer from a Creator™ Donor Vector.

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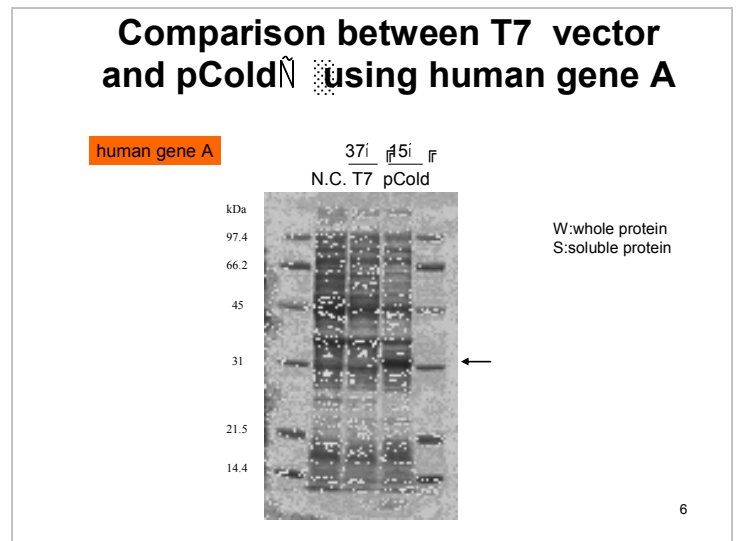
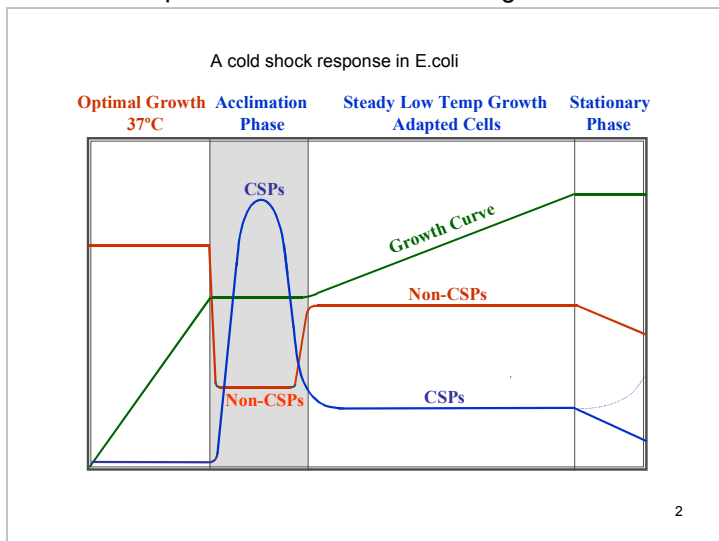
Clontech HAT Protein Expression & Purification System

- The HAT (histidine affinity tag) Vectors encode a novel polyhistidine epitope tag that allows proteins expressed in bacteria to be purified at neutral or physiological pH and in native or denaturing conditions.
- HAT-protein fusions exhibit solubility that more closely resembles that of wild-type proteins, while still possessing strong affinity for immobilized metal ions. Using the HAT System to purify proteins offers two major advantages:
 - The protein is soluble therefore does not form aggregates or reside within inclusion bodies.
 - The protein can be eluted under mild conditions, such as neutral pH or low imidazole concentration.
 - The HAT System includes three pHAT Vectors that have multiple cloning sites (MCS) in each of the three reading frames. An enterokinase (EK) cleavage site has been incorporated into each of the vectors, allowing removal of the HAT sequence from the purified protein. Restriction sites are present to allow the HAT sequence to be excised, with or without the EK site, for cloning into other vectors.

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TaKaRa Cold Shock Expression System

- Cold-shock expression vectors, pCold™ DNA, are designed utilizing the Cold Shock Protein promoter to perform efficient protein expression at lower temperatures with increased levels of soluble expression. Proteins that are insoluble in conventional expression systems can be expressed in soluble form.
- There are four kinds of pCold™ vectors, whose arrangements vary in the existence of TEE, His-Tag sequence and Factor Xa cleavage site.



- Wide Range of *E. coli* Hosts.
- Compatible with Chaperone Plasmids Vectors: When used in conjunction with one of TaKaRa's Chaperone Plasmids, the amount of recoverable soluble protein can be further increased.

Read More: http://bio.TaKaRa.co.jp/bio_en/catalog_d.asp?C_ID=C1278

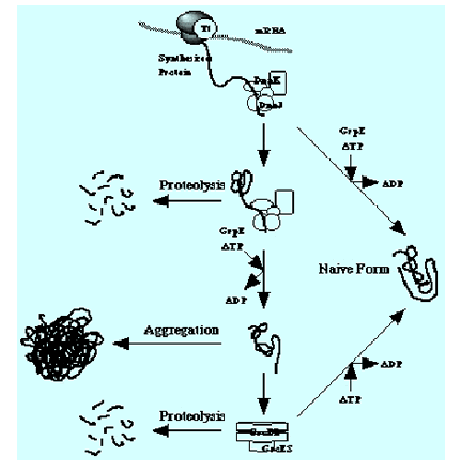
TaKaRa Chaperone Plasmid Set

- It is known that molecular chaperones are involved in the protein folding process, and numerous studies have been conducted to elucidate the mechanisms of in vivo protein folding. This set contains five types of plasmids each of which is designed to enable efficient expression of multiple molecular chaperones that are known to work in cooperation as a "chaperone team" for the protein folding process. It has been reported that co-expression of a target protein with one of these chaperone teams increases recovery of proteins in the soluble fraction.

Read More: http://bio.takara.co.jp/BIO_EN/Catalog_d.asp?C_ID=C1262

- Chaperone Competent cells: This product consists of the Escherichia coli strain BL21 transformed by each 5 types of plasmids included in Chaperone Plasmid Set. E.coli BL21 is a strain derived from B strain, which have a lon defect of protease and ompT Outer membrane protease. E.coli BL21 is commonly used for recombinant protein expression because it often brings a high stability in expressed protein.

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TaKaRa pCold TF DNA Vector

- TaKaRa's pCold TF DNA Vector is a fusion cold shock expression vector that expresses Trigger Factor (TF) chaperone as a soluble tag. Trigger Factor is a prokaryotic ribosome-associated chaperone protein (48 kDa), which facilitates co-translational folding of newly expressed polypeptides. Most E. coli strains can serve as expression hosts. Note: This product is not intended for protein expression system utilizing T7 promoter, such as the pET system, because the BL21 strain as a host doesn't express T7 RNA polymerases.

Read More: http://bio.TaKaRa.co.jp/bio_en/catalog_d.asp?C_ID=C1319

TaKaRa SPP System™ (Single Protein Production System)

- Ideal for isotope pulse labeling.

Read more: http://bio.takara.co.jp/BIO_EN/catalog_d.asp?C_ID=C1324

In Vitro REFOLDING

TaKaRa Refolding CA kit

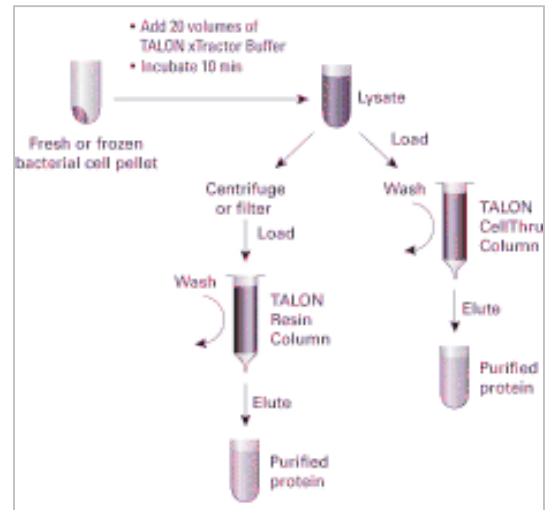
- The Refolding CA Kit uses a novel artificial chaperone in an easy 2-step procedure for optimizing the refolding conditions of inclusion body proteins. Optimization results in correct protein folding and restoration of protein activity.
- The small kit is supplied with guanidine hydrochloride and DTT for protein unfolding, four different surfactants that can be added independently to the unfolded protein solution to provide protection against molecular aggregation, and highly polymerized cycloamylose (CA), an artificial chaperone, for surfactant removal and recovery of protein activity. Overnight incubation of the CA-treated protein is followed by a quick 10-minute centrifugation and collection of the supernatant containing the refolded protein.
- The large kit is used for large scale refolding after the reaction conditions have been determined using the small Refolding CA kit and consists of only denaturant and CA.

Read More: http://www.takarabiosa.com/cgi/showGroup.cgi?tableName=ProengGroup&op=showGroup&Group_ID=G7350

PROTEIN PURIFICATION SYSTEMS:

Clontech xTractor Buffer

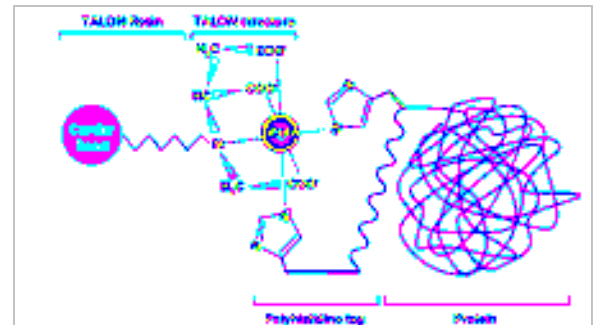
- The TALON xTractor Buffer Kit gently disrupts bacterial cells for protein purification. It has been optimized for extraction of polyhistidine-tagged proteins, so it is compatible with all TALON Resin applications.
- The extraction method is simple. Just re-suspend the cell pellet in the buffer (1:20 w/v) and mix gently for 10 minutes (Figure 1). At the end of this incubation, the buffer's mixture of salts and detergents produces a lysate that has no visible cell fragments or precipitates. The short incubation period is an advantage when your proteins are susceptible to proteases. After extraction, simply centrifuge or filter the lysate and load it on any TALON Resin Column to isolate your polyhistidine-tagged proteins. To save time, the resulting lysate can be loaded directly on a TALON CellThru Column without centrifugation or filtration.
- The biological activity of the protein obtained by xTractor Buffer extraction is higher than the activity of the protein obtained by sonication. This method works especially well for high molecular weight proteins, like β -galactosidase (LacZ), that cannot be extracted unless the membranes are completely disrupted.



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Clontech Talon His-Tag Purification Resins

- Better specificity than Ni: Talon resin is a durable, versatile immobilized metal ion affinity chromatography (IMAC) resin design for purifying poly-histidine tagged protein. The Talon ligand contains a Cobalt core that binds polyhistidine-tagged proteins more specifically than nickel-based IMAC resin. Only adjacent or specially positioned neighbouring histidines are able to bind this reactive core. Talon resins exhibit less metal ion leakage than Ni-NTA resins since Cobalt forms a more uniform complex with the chelating ligand than Nickel.
- Talon is available as single step columns. By utilizing TALON Superflow Resin with FPLC, purification can be achieved in less than 24 minutes. This ensures high yields of target proteins - which is particularly important if the protein of interest has limited stability.



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- Proteins can be produced under native or denaturing conditions using β mercaptoethanol.

Read More: http://www.clontech.com/images/ctq/JAN07UPD/UD712233_CTQJan07_26_IN.pdf

- Talon resin isolates protein with less contamination. Ni-NTA resin has been found to have a propensity for significant SlyD contamination – a common endogenous His-tagged protein found in E.coli. Talon doesn't bind E.coli SlyD.

Read More: http://www.clontech.com/images/brochures/AN6Z2212_TALON_IN.pdf

- Also available - Tag antibodies:
 - 6xHis Monoclonal Antibody (Albumin-free)
 - 6xHis Monoclonal Antibody-HRP Conjugate
 - 6xHN Polyclonal Antibody (Albumin-free)

Read More: http://www.clontech.com/products/detail.asp?product_family_id=1424&product_group_id=1459&product_id=10596

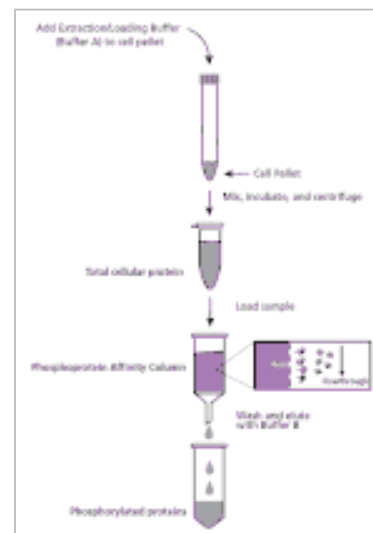
Clontech Talon Magnetic beads

- Combine the advantage of TALON chemistry with magnetic bead separation. Magnetic particles in the beads facilitate quick and easy separation of microscale quantities of protein when placed on a magnetic separator. Purified proteins are eluted in small volumes (50–200 μ l), resulting in concentrated sample (up to 3 mg/ml).

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Clontech Phosphoprotein Enrichment Kit

- The Phosphoprotein Enrichment Kit provides an effective affinity-based procedure for isolating phosphorylated proteins from mammalian cells and tissues. Each kit supplies a complete set of buffers along with six high-capacity columns for enrichment of both cytosolic and membrane-bound phosphoproteins regardless of the amino acid modified—including serine, tyrosine, or threonine.
- This enrichment procedure offers a number of advantages. The procedure is fast; the average cell-to-sample purification time is less than 2 hours. It is also straightforward, consisting of four main steps: adding Extraction/ Loading Buffer to the cell or tissue pellet to extract total cellular protein, loading the extract on an affinity column, washing, and finally eluting the bound phosphoprotein with a detergent-free Elution Buffer. A single buffer – Extraction / Loading Buffer - is used for both the protein extraction and affinity column steps, making buffer exchange unnecessary. Each column has a maximum binding capacity of ~4 mg of phosphorylated protein, and the procedure is nondenaturing, so phosphoproteins remain folded throughout the process, even during the extraction and elution steps.



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Clontech Talon PMAC Magnetic Phospho-Enrichment Kit

New!

- The Magnetic Phospho Enrichment Kit combines the phosphospecificity of TALON based Phosphoprotein Enrichment Kit with the convenience of magnetic bead separation to provide a simple, rapid, metal affinity-based method for isolating microgram quantities of phosphorylated proteins from mammalian cells and tissues. Each kit supplies a complete set of buffers along with the Phospho Magnetic Beads for group-specific enrichment of all types of phosphoproteins, both cytosolic and membrane-bound, that contain a phosphorylated amino acid side chain—including serine, tyrosine, or threonine.
- Phospho Magnetic Beads are supplied as a 5% suspension, with a demonstrated binding capacity of 400 μ g of α -casein per ml of suspension. Different amounts of beads may be used, depending on the initial sample concentration. Phosphoproteins can be eluted in small volumes (50–200 μ l) to yield concentrated samples.

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Clontech Glutathione Resin

- The large size of GST (35KDa) results in a higher potential for degradation by proteases than other smaller tags. Therefore, performing GST-protein purification as quickly as possible under non-degrading conditions is necessary in order to minimize sample loss. GST loses its ability to bind Glutathione resin when denatured, consequently strong denaturants such as guanidine-HCl or urea must not be used in the purification buffers.
- Glutathione-Superflow and -Uniflow Resins allow rapid affinity purification of GST-tagged proteins. These resins are based on 6% and 4% cross-linked agarose, respectively, with glutathione covalently bound to the resins.
- The GST Purification Kit provides sufficient stock buffers and pre-packed Glutathione-Uniflow Columns for performing five batch/gravity-flow purifications. Up to 10 mg of GST-tagged proteins per column can be purified using the GST Purification Kit.

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**Request a
Sample!**

Scientifix Resins

Scientifix offers Cobalt and Nickel resins for the purification of poly-His tagged proteins and Glutathione agarose for the purification of GST tagged proteins. For more information and pricing please contact Scientifix.

UNIVERSAL CHEMILUMINESCENCE HIS-WESTERN BLOT KIT

- The Universal His Western Blot Kit is the most specific Western blot kit for specific detection of polyhistidine-tagged proteins, including 6xHis, Histidine Affinity Tag (HAT), and the 6xHN tag for which there is no specific antibody using chemiluminescence.
- Detects as little as 1.0 mg of purified protein. This amount is less than other Western blot methods that typically require 2–4 mg of purified protein per lane. In addition, the chemiluminescent detection reagents yield highly sensitive results with low background. Film exposure times for these reagents range from 30 seconds to 10 minutes with relatively constant signal intensity over a six hour period.

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Protein Markers

- TaKaRa Protein Molecular Weight Markers are designed for use in SDS-Polyacrylamide gel electrophoresis. There are 3 kinds of Protein Molecular Weight Markers, Low (Molecular weight range: 14.3 - 97.2 kDa), High (Molecular weight range: 44.3 - 200 kDa) and Broad (Molecular weight range: 6.5 - 200 kDa).
- Each protein is proportioned to yield uniform band intensities with Coomassie Brilliant Blue R-250 staining on SDS polyacrylamide gel.
- 5µl of 20-fold diluted marker is loaded per lane of SDS-PAGE minigel, therefore each marker is sufficient for 200 lanes.

Read More: http://bio.takara.co.jp/BIO_EN/catalog_d.asp?C_ID=C1337

Please note that the price lists for TaKaRa, Clontech and Macherey-Nagel products are available on the Scientifix website:

www.scientifix.com.au

***This flyer is available for viewing on the Scientifix website,
Application Focus section.***