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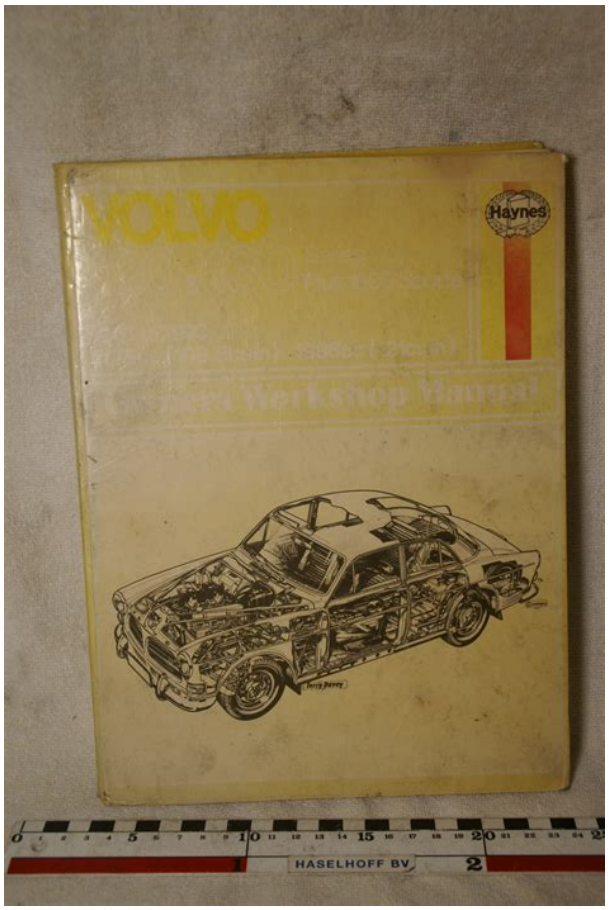
Bugbuster novagen manual



Training programs and seminars designed for you. Explore, interact. The product quantity has been adjusted. Please enter a quantity of 1 or more to add items to your cart. The product quantity has been adjusted. Please enter a quantity of 1 or more to add items to your cart. We are a leading supplier to the global Life Science industry solutions and services for research, development and production of biotechnology and pharmaceutical drug therapies. Training programs and seminars designed for you. Explore, interact. The product quantity has been adjusted. Please enter a quantity of 1 or more to add items to your cart. With the Master Mix, there is no need for dilution or separate addition steps. The two available package sizes provide sufficient reagents for protein extraction from 20 g and 100 g cell paste. We are a leading supplier to the global Life Science industry solutions and services for research, development and production of biotechnology and pharmaceutical drug therapies. The inclusion bodies were were centrifuged and washed with BugBuster reagent, followed by CelLytic B reagent Sigma diluted 110 with water, and then finally 1x HisBind buffer Novagen. The inclusion bodies were solubilized by sonication in solubilization buffer. The protein was then refolded by a 2step dialysis process, firstly for 20h against 20 volumes of refolding buffer 1, then for 24h against 100 volumes of refolding buffer 2.Other constructs also purified. HuS aa.18125 or 18127 see separate protocols, records no.1507, 7k9h3. Flow through the columns were efficient and got good yields of proteins. Followed protocol given in the kits handbook. Then followed remaining steps for final separation of proteins using size exclusion column chromatography. All rights reserved. Find products. Free subscription. Find articles by Sammy Patry John L. Markley a Biochemistry Department, University of WisconsinMadison, 171a Biochemistry Addition, 433 Babcock Dr, Madison, WI 53706,

USA.http://www.vds-construct.pl/userfiles/debian-get-list-of-manually-installed-packages.xml

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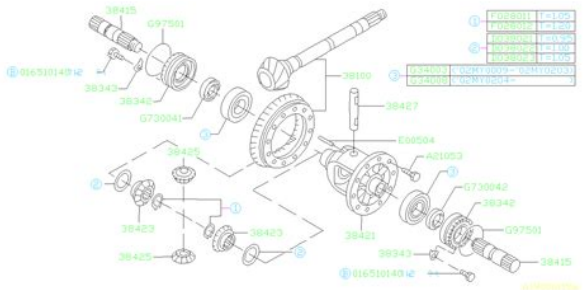
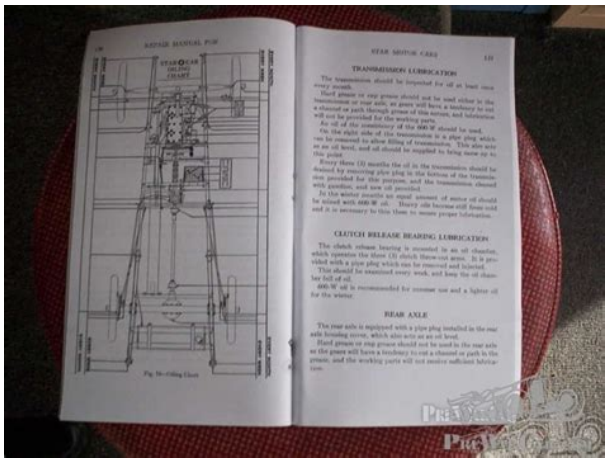
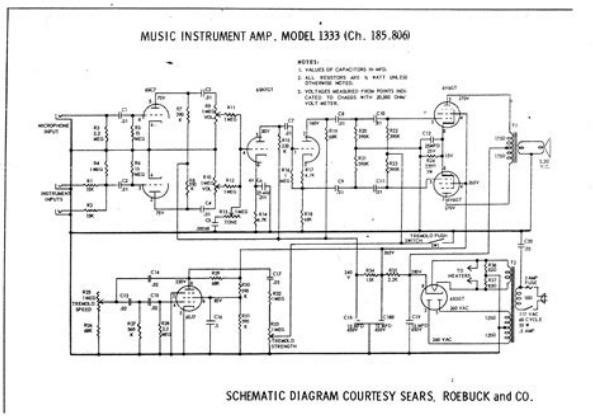


Find articles by John L. Markley Author information Copyright and License information Disclaimer a Biochemistry Department, University of WisconsinMadison, 171a Biochemistry Addition, 433 Babcock Dr, Madison, WI 53706, USA.Abstract Brazzein protein comes from an edible fruit, which has a long history of being a staple in the local human diet in Africa. The attractive features of brazzein as a potential commercial sweetener include its small size 53 amino acid residues, its stability over wide ranges of temperature and pH, and the similarity of its sweetness to sucrose. Heterologous production of brazzein is complicated by the fact that the protein contains four disulfide bridges and requires a specific Nterminal sequence. Our previous protocol for producing the protein from Escherichia coli involved several steps with low overall yield expression as a fusion protein, denaturation and renaturation, oxidation of the cysteines, and cleavage by cyanogen bromide at an engineered methionine adjacent to the desired Nterminus. The new protocol described here, which is much faster and leads to a higher yield of native protein, involves the production of brazzein in E. coli as a fusion with SUMO. The isolated protein product contains the brazzein domain folded with correct disulfide bonds formed and is then cleaved with a specific SUMO protease to liberate native brazzein. This protocol represents an important advancement that will enable more efficient research into the interaction between brazzein and the receptor as well as investigations to test the potential of brazzein as a commercially viable natural low calorie sweetener. In our evolutionary past, a strong drive to find rich energy sources and high carbohydrate foods was an advantage for survival.http://tunisie.mawarids.org/mpfiles/debian-etc-network-interfaces-manual.xml



Today, with lives of a more sedentary nature, this sweetseeking behavior has now become a liability, and lowcalorie sweeteners with good taste properties are becoming more sought after, particularly naturally occurring ones, such as proteins. Over the last 30 years, high potency sweet proteins have been identified in a variety of African and South Asian fruits. It is thus likely that protein sweeteners share similar receptor binding interaction sites. Among the natural, lowcalorie sweeteners, brazzein is the most promising because of its superior taste quality and its physical properties. Brazzein contains no carbohydrate and bears no structural resemblance to sucrose. Although taste perception and taste preferences have a rich history of study, it is only in the last few years that the receptors thought to underlie this behavior have been identified. Brazzein is perceived as sweet by humans, old world monkeys, and apes, but not new world monkeys or other tested species. The major drawbacks of nuclease fusion system are lack of protein solubility, the requirement for refolding brazzein, and inefficiency in removal of the fusion tag. We report here a new method based on the SUMO fusion system that supports more efficient production of brazzein. The major advantage of the SUMO fusion system is that it yields brazzein in folded and soluble form in high yield. This method of production will allow us to more rapidly produce brazzein with lower cost for both research studies and future largescale production. By learning more about its mechanism of action and by developing more potent brazzein variants, we will be in a better position to evaluate it as a potential sugar substitute as a means for fighting problems related to obesity and diabetes. The pSUMO vector is derived from pET24d and contains T7 promotor induced with IPTG. The fusion gene coded for a His 6 tag at the Nterminus of the SUMO protein followed by brazzein at the Cterminus.

The correct gene sequence was confirmed by sequencing at the University of WisconsinMadison Biotechnology Center. Protein expression of SUMObrazzein A variety of expression strains were tested Rosetta DE3 from Novagen and BL21 pLysS, BL21CodonPlus DE3RI, BL21CodonPlus DE3RIP, and BL21CodonPlus DE3RIPL from Stratagene. Of the host cells studied, only BL21CodonPlus DE3 RIPL produced high amounts of the SUMObrazzein fusion protein as detected on TrisTricine SDSPAGE 16% Invitrogen , and this strain was used for high level protein production. Cell disruption, denaturation and disulfide reduction were allowed to proceed for 5 min before the sample was loaded onto the gel. The running buffer was Tristricine; gel staining was with R250 Coomassie blue. Purification of the His 6 SUMObrazzein fusion We used NiNTA superflow resin Qiagen, Valencia, CA under native conditions to quickly purify the soluble His 6 SUMObrazzein fusion from the cell lysate. The suspensions were kept on ice to facilitate cell lysis. For complete lysis, cells were sonicated on ice by 2 periods each of 4 min cycles of 10 s on followed by 30 s off. However, after cleavage, both the brazzein and SUMO fractions were white in dried powder form. RPHPLC column chromatography was used for the final brazzein purification step. TrisTricine SDSPAGE 16% was used to assay the extent of proteolysis protease activity and the recovery of brazzein product. Characterization of the brazzein product The protein product was further characterized by electrospray ionization mass spectroscopy ESIMS at the Biotechnology Center, University of WisconsinMadison, and by NMR spectroscopy at the National Magnetic Resonance Facility at Madison. Results Expression of the fusion protein We found that expression levels of His 6 SUMObrazzein fusion protein were very low in conventional E. coli strains, e.g. BL21DE3pLysS, possibly because of complications of codon bias, because the SUMO gene was derived from a eukaryotic system.



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In an attempt to improve expression levels, we tested several other bacterial strains recommended for T7 driven expression systems. Although we observed no expression of the fusion construct from Rosetta DE3pLysS cells, we found increasing levels of expression from the cell series, BL21CodonPlus DE3RP, BL21CodonPlus DE3RIL, BL21CodonPlus DE3RIPL RP Fig. 1 . Open in a separate window Figure 1 SDSPAGE showing the expression of the His 6 SUMObrazzein fusion in various host strains. Lane 1 Molecular weight markers Invitrogen. Lane 4 supernatant. Lane 10 purified fusion protein. Lane 11 SUMO 1 protease cleavage product after 30 min. Lane 12 cleavage product after 60 min. We used a Bradford protein assay BioRad to quantify the amounts of protein recovered in various steps in the protocol. The fusion protein was found to represent 26% of the total cell lysate protein, and the amount of brazzein produced represented 9% of the total soluble protein from the cells Table 2 . Then amount of fusion protein recovered through NiNTA column was high, and resulted native brazzein protein following cleavage as judged by RPHPLC. Furthermore, the protein had the sweetness of native brazzein. Discussion To date, no gene for brazzein has been isolated from its plant of origin. Because the nucleasebrazzein fusion protein was expressed in insoluble form, the fusion protein had to solubilized and refolded prior to cyanogen bromide cleavage. This procedure was time consuming, and the refolding and oxidation steps required manual effort. In addition, the yield from the CNBr cleavage step was only about 50% as the result of partial oxidation of the methionine residue during the oxidation processes. However, when we followed the standard recommended protocol, which specified Rosetta DE3pLysS host cells and NiNTA purification, the yield of the His 6 SUMObrazzein fusion protein was undetectable.

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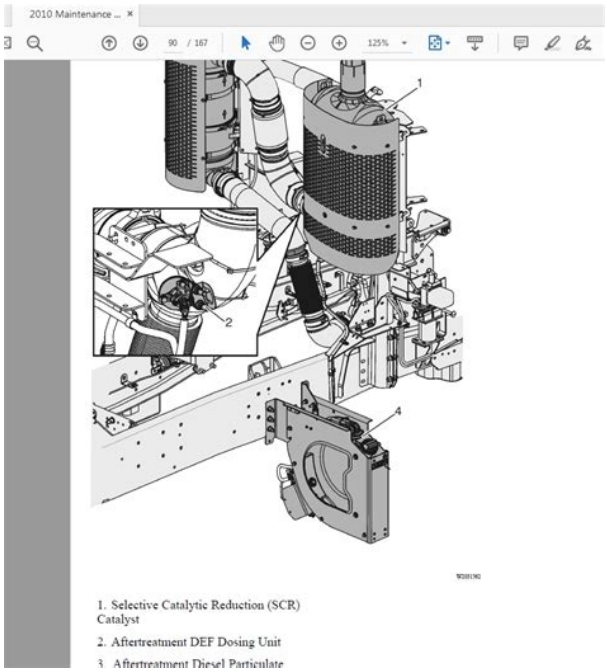
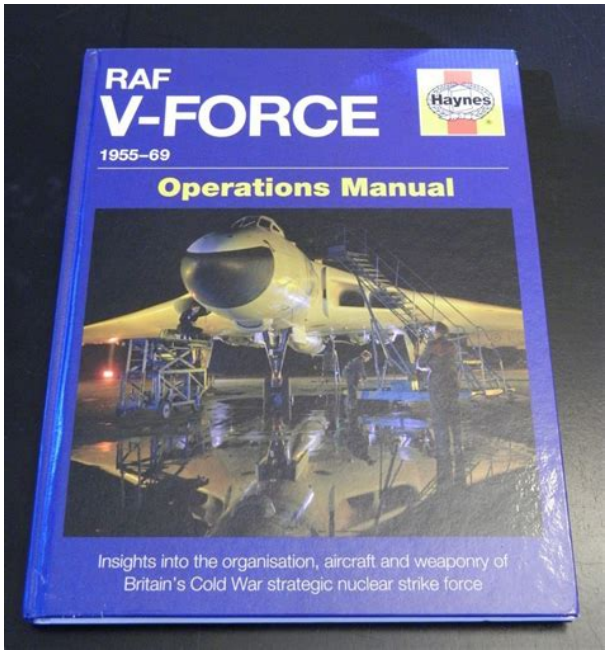


Thus, we introduced a series of changes that led to highlevel production and purification of the fusion protein. Expression of recombinant proteins in E. coli is generally difficult when the codon use in the recombinant gene differs from the codon use in the host cells. Our studies showed that the best host for expressing the His 6 SUMObrazzein fusion was BL21CodonPlus DE3RIPL. This strain provides an increased supply of rare E. coli tRNAs argU, ileY, leuW, and proL that correspond to codons used more frequently by eukaryotes. Also, whereas brazzein has been optimized to use more abundant E. coli codons for these amino acids, the gene coding for SUMO utilizes primarily rare codons Table 1 . This explains why the BL21CodonPlus DE3RIPL was more successful than Rosetta DE3pLysS, which is not optimized for these particular amino acids. Table 1 Protein determined by Bradford protein assay from 10g wet cells. Following RPHPLC and cleavage. Fraction Mass mg Total soluble protein 636 Flowthrough 376 Wash 53 Insoluble 1 44 His 6 SUMObrazzein 163 Brazzein 54 Open in a separate window 1 Precipitate formed following salt removal and concentration. We found it necessary to reduce the amount of DTT present from the recommended level of 5 mM to less than 0.5 mM to prevent reduction of the disulfide bridges of brazzein leading to misfolded, inactive product. The recombinant brazzein after purification was characterized by SDSPAGE for its purity, and its molecular weight was confirmed by mass spectrometry. Although the isolated His 6 SUMObrazzein fusion protein had a brownish cast, this color disappeared completely following cleavage and final purification. The purified brazzein product was in native form as judged from RPHPLC, 1D 1 H NMR and 2D 1 H 1 H NOESY analysis and taste tests. In summary, SUMO fusion system and customized expression and purification protocol described here have greatly improved the efficiency and lowered the costs of producing brazzein.



Thus the new protocol provides a higher yield of brazzein with less time and effort. It is enabling us to rapidly produce brazzein variants for ongoing investigations of the interaction between the protein and the sweet receptor. In addition, the new approach may become a springboard for industrial level production of brazzein. Acknowledgements Work supported in part by NIH grant P41 RR02301. The authors thank Dr. Marco Tonelli for help with NMR data collection, Dr. James Radek for cloning brazzein into the SUMO plasmid, and Dr. Ronnie Fredrick for helpful discussions. Footnotes Publishers Disclaimer This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain. AssadiPorter FM, Aceti DJ, Cheng H, Markley JL. Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ, Zuker CS. Damak S, Rong M, Yasumatsu K, Kokrashvili Z, Varadarajan V, Zou S, Jiang P, Ninomiya Y, Margolskee RF. Malakhov MP, Mattern MR, Malakhova OA, Drinker M, Weeks SD, Butt TR. Luria SE, Burrous JW. Zuo X, Mattern MR, Tan R, Li S, Hall J, Sterner DE, Shoo J, Tran H, Lim P, Sarafianos SG, Kazi L, NavasMartin S, Weiss SR, Butt TR. The presence of a His tag enables the use of IMAC for purification. IMAC is a rapid affinity method based on binding of the adjacent histidines of the His tag to an immobilized divalent metal ion nickel is the most frequently used, followed by cobalt, but other divalent metals are also used. The method is quick, inexpensive, and straightforward, which has led to widespread adoption.

In fact, although many crystallographers cleave the tag before screening for crystallization conditions, 274,275 Carson et al.An additional benefit of the His tag is that its affinity for divalent metal cations is not dependent on a particular protein fold or secondary structure; therefore, IMAC can be performed under denaturing conditions 6 mol l 1 guanidine hydrochloride or 8 mol l 1 urea, for example. This can be useful when the protein cannot be expressed in a soluble form and refolding is being pursued see Section 9.19.5.7 . View chapter Purchase book Read full chapter URL Guide to Protein Purification, 2nd Edition Arun Malhotra, in Methods in Enzymology, 2009 3.1 Histag The Histag also called 6xHistag is one of the simplest and most widely used purification tags, with six or more consecutive histidine residues. IMAC is the preferred choice as a first step during the purification of Histagged proteins, though small batch reactions or spin columns with IMAC beads can be used for expression tests or smallscale preparations. For nickel binding media, the metal ion can often be stripped using buffers with EDTA and recharged for multiple use cycles. Some cobalt based resins such as Talon, Clontech Inc. use proprietary linkages that are more

durable and cannot be recharged; such resins can be reused three and four times, but offer the advantage of being more specific for polyhistidine tags and almost no metal leakage during protein elution. IMAC can also be used under denaturing conditions, since the Histag does not need a specific protein conformation for metal binding; indeed, binding to IMAC resins is stronger under denaturing conditions as the Histag becomes more exposed. While the mild elution conditions for IMAC is one of the positive aspects of Histags, care has to be taken to avoid EDTA or EGTA in any of the buffers.

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Cell extracts loaded on IMAC columns should not contain any EDTA, and only EDTAfree protease inhibitor cocktails should be used for sample preparation. TRIS salts weakly chelate metal ions as well, and the use of TRIS buffers should be minimized 50 m M or less. Most IMAC media is also very sensitive to reducing agents such as DTT or DTE, and low levels of mercaptoethanol M should be used instead. There are several variations on the standard 6xHis sequence used in Histags Terpe, 2003 . This assembly through the formation of a metal complex is only stable in aqueous medium and any extreme conditions i.e., extreme pH or ionic strengths should be properly tested to assure peptide conjugation to the particles. As the conjugation is not a covalent bond, extremely dilute solutions may also affect the stability of the construct, and samples should be kept in the n M range. The QD was selected to form a good FRET pair with the chosen organic label on the peptide, in this case a 540 nm emitting QD. M that is approximately an order of magnitude higher than the QD suspension. Left Before the presence of enzyme activity the substrate presents terminal FRET acceptors. Right In the presence of enzyme activity the substrate is cleaved, the acceptor then diffuses away from the QD. The extended distance reduces the FRET efficiency, increasing QD emission, and decreasing the acceptor emission. Remove any aggregates by centrifugation. Yehia Daaka, in Methods in Enzymology, 2013 4 HisTag Switch Technique The fundamental difference between the Histag switch technique and BST is that an irreversible Histag is bound to a cysteine thiol group instead of a biotin tag Camerini et al., 2007 . Free thiol groups in protein samples are first irreversibly blocked with Nethylmaleimide, and Snitrosylated cysteine residues are then reduced by ascorbate followed by labeling with Histagged peptides.

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This protein differs from MSP1 by a truncation, designated D1, and an insertion, designated E3 Table I . Initial versions carried a Factor X site between the 6histidine tag and the MSP. Newer and more efficiently cleaved versions carry a TEV protease site. Take 5 l samples during the incubation e.g., at 2, 4, 6, and 22 h. 3 Check progression of the proteolysis reaction by SDSPAGE and staining with Coomassie Brilliant Blue of samples taken before protease addition and during incubation, loading the same amount of MSP 1 g in each lane. Cleaved MSP is 2.6 kD smaller than intact protein and is resolved as a slightly more rapidly migrating band in SDSPAGE. 4 Remove EDTA and DTT by dialysis against 3 15 volumes 50 m M TrisHCl, pH 7.5, 100 m M NaCl in a cold box. Removal of these compounds is necessary in order to use Ni affinity chromatography. 5 Apply the dialyzed protein solution to a 1 cm diameter NiAgarose column e.g., 8 ml bed, for a 27 ml sample and wash with 4 bed volumes of 50 m M TrisHCl pH 7.5, 100 m M NaCl, 25 m M imidazole. Pool fractions collected during sample application and wash. These fractions should contain cleaved MSP but not the 6histidinetagged protease or uncleaved MSP, which should be retained by the column. 6 Concentrate the pooled fractions to 20 ml using an Amicon Ultra 15 concentrator and dialyze against 3 350 ml 20 m M TrisHCl pH7.5, 100 m M NaCl, 0.5 m M EDTA. View chapter Purchase book Read full chapter URL Radical SAM Enzymes Wen Zhu,. Judith P. Klinman, in Methods in Enzymology, 2018 2.3 PqqE Purification The expressed His 6 PqqE is purified by affinity chromatography from a nickel column. The following protocol is for anaerobic purification. A summary of PqqE purification

procedures is shown in Table 2. Table 2. Methods for Purification of K. pneumoniae or M. extorquens PqqE K. pneumoniae Wecksler et al., 2009 M. extorquens Barr et al., 2016; Latham et al.

, 2015 Cell pellet disruption Lysis with BugBuster Novagen in 50 m M Tris pH 7.9, 1 m M DTT, 300 m M NaCl, 10 m M imidazole, 5 L benzonase nuclease; centrifugation 15,000. Washed with 100 mL of the same buffer, then with 100 mL of 25 m M imidazole in buffer, followed by 100 mL of 50 m M imidazole in buffer. Eluted with 200 m M imidazole in buffer. Fractions selected based on color, pooled and concentrated to 5 mL by ultrafiltration Amicon Ultra 30K membrane ii Gel filtration in PD10 column equilibrated with 50 m M Tris pH 7.9, 1 m M DTT, 300 m M NaCl to remove imidazole. Washed with the same buffer and eluted with 300 m M imidazole in buffer. Load the supernatant into the column manually with a pipette or using a peristaltic pump. 7. Upon protein binding, wash the column with three volumes of cold wash buffer. 8. Elute the protein with cold elution buffer. By continuing you agree to the use of cookies. No publication fee; no access fee. This protocol details the steps in production of a polyclonal antibody in rabbits using a bacterially expressed fusion protein as an antigen. The protocol is generated based on data presented in Wirschell et al.2013. The expressed protein sequence is shown in Figure 1. Figure 1. The DRCHis fusion protein. In bold is the sequence encoded by the pET28A vector including the 6His tag underlined followed by the sequences encoded by the EcoR1 insert from pMW199.1 containing 6 amino acids from the pCR2.1 cloning vector and DRC1 sequences italics. Resuspend the pellet in 0.5 volumes 15 ml of Bugbuster diluted 110 with water 0.1x Bugbuster. Vortex to resuspend the pellet.Resuspend the pellet in 15 ml of 0.1x Bugbuster. Vortex to resuspend the pellet.Save an aliquot for gel analyses IB. S1, S2 and S3 are the supernatants from the three washes of the insoluble cell pellet. IB is the inclusion body pellet. FT is the flow through from the Nickel column. IB is the inclusion body pellet. 110 are the fractions collected from the column.

These were tested on western blots for endogenous reactivity to axonemal proteins. 2 rabbits were selected that showed minimal to no reactive bands on western blots. The fusion protein was in the elution buffer used to elute the fusion protein from the Nickelchromatography column. Approximately 1 mg of total protein is used per rabbit for the immunizations. The sera was used at a 110,000 dilution for western blots or was further affinity purified as followsIsolated clones were sequence verified. The region of the membrane containing the fusion protein was cut out and used to purify DRC1specific antibodies. The region of the membranes containing the fusion protein were cut out and used to purify DRC1specific antibodies. The column was stored in 20% ethanol until ready to use for affinity purification. Incubate for 1 h. The process was repeated three more times. All samples were tested on western blots of wildtype and pf3 mutant axonemes. Aliquots with minimal background, but strong signals, were combined and used at the appropriate dilution Wirschell et al., 2013. The Chlamydomonas Sourcebook. E. Harris. Kidlington, Oxford, Academic Press. 1 241301. Nat Genet 453 262268. The authors will be requested to answer your questions at their earliest convenience. Once your questions are answered, you will be informed using the email address that you register with bioprotocol. You are highly recommended to post your data including images for the troubleshooting.By using our website, you are agreeing to allow the storage of cookies on your computer. In High Throughput Protein Expression and Purification Methods and Protocols, leading scientists detail the most successful protocols currently in use, including various high throughput cloning schemes, protein expression analysis, and production protocols. This volume describes the use of E.

coli, insect, and mammalian cells, as well as cellfree systems for the production of a wide variety of proteins, including glycoproteins and membrane proteins, in order to best represent strategies that create and exploit common features to enable simplified cloning, stable expression, and purification of proteins. The genes encoding CipA and CipB proteins from P. luminescens H06 were expressed respectively in Escherichia coli and these cells were used to feed the axenic first juveniles J1 of three

Steinernema nematode isolates in liquid cultures and on agar plates. In liquid cultures, the axenic J1 juveniles of all three test Steinernema nematode isolates were able to produce next dauer juveniles DJs in the E. coli cultures with at least one of the expressed Cip proteins, but unable to develop beyond the next J1 stage without expressed Cip proteins. For each target nematode isolate, addition of the supernatant of the bacterial culture of its Xenorhabdus symbiont to the tested liquid cultures did not induce the formation of DJs. However, on LB agar plates with different test E. coli cultures, all J1 juveniles of the three Steinernema strains finally developed into next DJs. It seemed that the metabolite pathway of the test bacteria in both culture systems was different. The presence of the Cip proteins has a significant influence on the DJ formation of the Steinernema nematodes in liquid culture system. Following several rounds of reproduction, the DJs disperse from the cadaver and search for new hosts. Two forms of Xenorhabdus and Photorhabdus spp. The genes encoding these crystal proteins in Xenorhabdus cells have not yet isolated. The crystal inclusion body is one of the important protein sources provided by primary form P. luminescens which Heterorhabditis sp. However, no feeding experiments with expressed Cip proteins were performed to demonstrate the nutrient significance of these proteins for the development of entomopathogenic nematodes.

In order to better understand the functions of the crystalline inclusion proteins for nematode development, the genes encoding CipA and CipB proteins from P. luminescens H06 were respectively expressed in Escherichia coli and these cells were used to feed the J1 juveniles of several Steinernema nematode isolates. The development of the J1 nematodes was observed to verify the hypotheses that the presence of the Cip proteins has significant influence on the life cycle of the Steinernema nematodes in liquid cultures and on agar plates. Materials and methods SY5, Steinernema longicaudum X7 and Steinernema carpocapsae All, respectively. The primary form phase I of these bacterial isolates was obtained by selecting green or bluegreen colonies on NBTA medium Akhurst, 1980 . All medium components used in this study were purchased from Oxoid Company Basingstoke, Hampshire, UK. SY5 Strain SY5, phase I variant Hainan, China Xenorhabdus sp. SY5 Qiu, 2003 Host for Xenorabdus sp. SY5 Hainan, China Steinernema longicaudum X7 Qiu, 2003 Host for Xenorabdus sp. X7 Shandong, China Steinernema carpocapsae All Host for Xenorabdus nematophila All USA Open in new tab Construction and expression of recombinant Cip proteins Plasmids were extracted from E. coli with QIAprep Miniprep kit Qiagen, Hilden, Germany. When required, DNA fragments were extracted and purified from agarose gels using SpinPrep Gel DNA kit Novagen. The genomic DNA was isolated from P. luminescens H06 using a modified CTAB cetyltrimethylammonium bromide Genomic Extract Kit Shenergy Biocolor Co, Shanghai, China.The construct was verified by DNA sequence determination by BioAsis Co. Shanghai, China with appropriate primers. The obtained sequences were blasted in the NCBI database to check the homology of the amplified sequences. The insert DNA was ligated into pET15b vector Novagen encoding an Nterminal His 6 tag. The plasmid was transformed into E. coli strain BL21DE3.

The vector without the insert was transformed into the same E. coli strain as a control. The expression of recombinant Cip proteins was analyzed by GelPro software Media Cybernetics, Silver Spring, MD. Extraction of expressed Cip proteins. The percentage and content of inclusion proteins in purified insoluble protein were determined by GelPro software. The extraction of the Cip proteins from Photorhabdus luminescens H06 culture was performed as follows. The pellet was resuspended in a 0.5 original culture volume of dilute BugBuster after each successive wash.Freunds complete adjuvant and incomplete adjuvant Sigma Chemical Co. were used for the primary and secondary injections, respectively. Prior to emulsification in adjuvant, 2 mg per mL of Cip protein were suspended in sterile phosphatebuffered saline sPBS NaCl 137 mM, KCl 2.7 mM, Na 2 HPO 4 10 mM, KH 2 PO 4 2 mM, pH 7.4. For the additional immunizations, purified Cip proteins suspended in sPBS were used directly without any adjuvant. The quality of the antibodies in sera was monitored by indirect enzymelinked immunosorbent assay ELISA. A total of 2 mL of blood was abstracted from the large vein in the center of the ear before every immunization at weekly intervals. Using 3, 3, 5,

5Tetramebenaidine TMB as the color substrate, absorbance at 450 nm was read in a Model 550 Microplate Reader BioRad, Hercules, CA. The OD 450 of antiserum increased and was greater than 3.0 after five injections. Gel electrophoresis and Western blot analyses The gels were stained with Coomassie Brilliant Blue R250. A total of 5 L of Promega broad range protein molecular weight markers per lane were used. The gels were electroblotted for 3 h at 250 mA constant current in a tank blotter Liuyi Co.. The membrane was processed at room temperature in TBS 50 mM Tris, 150 mM NaCl, pH 7.5 using three 10min washes between all steps.