ISSN: 1948-5948
Journal of Microbial & Biochemical Technology

OPEN ACCESS Freely available online

Review Article

The Technique for Excessive-Results of Recombinant Protein Purification led to GST-tag Affinity Chromatography: A Review

Lutfun Nahar*, Md. Bakhtiar Lijon

Department of Bacteriology, Graduate School of Medicine Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, 700-8558, Japan

ABSTRACT

Affinity Tags have been performed as the potential tools in the basic biological research field especially for production of recombinant protein and functional proteomics. Those affinity tags were wildly applied to simplify the purification of recombinant protein as well as differentiation of protein complex. Glutathione-S-Transferase (GST) tag has been extensively used in affinity chromatography for purification of fusion/recombinant protein to analysis of structure and function of protein, protein-protein interaction and to produce pharmaceutical product. In this review we describe the advantage of GST-tag in affinity chromatography technique as a method for inducible, high level protein expression and purification of recombinant protein. Recombinant protein which is expressed in a pGEX or pET vectors and that protein with GST-tag encoded at the NH_2 or COOH- region of genome sequence. There are some expression vectors which has different site to approve for unidirectional insertion of the coding region DNA, promoter, primers, antibiotic, Ori and GST-tag into pGEX vectors. By used reduced Glutathione (GSH) during affinity chromatography the GST-tag with recombinant protein is eluted and stored it. Displacement of the GST-tag from the recombinant protein performed by protease enzyme for digestion which is purified by the application of another affinity technique.

Keywords: Glutathione S-transferase (GST)-tag; Glutathione (GHS); pGEX/pET vectors; Recombinant protein expression; Protein purification

Abbreviations: GST: Glutathione S-transferase; GSH: Glutathione; SDS-PAGE: Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis; IPTG: Isopropyl β- d-1-Thiogalactopyranoside; PMSF: Phenylmethylsulphonyl Fluoride; CBB: Coomassie Brilliant Blue; FPLC: Fast Protein Liquid Chromatography; PCR: Polymerase Chain Reaction; LIC: Ligation-Independent Cloning; SUMO: Small Ubiquitin-like Modifier; HPLC: High Performance Liquid Chromatography; DNA: Deoxyribonucleic Acid; RNA: Ribonucleic Acid; ORF: Open Reading Frame

INTRODUCTION

Excessive result refers as research that permits thousands of running estimation a macromolecule to achieve and in this way makes large scale duplication probably. In the nineteenth century this innovation was established when genome sequence started, and the computerized DNA sequence was developed [1]. Some biomolecules such as lipid, protein, DNA, RNA, and other endogenous compounds have been expanded drastically utilize of high throughput or excessive method [2]. Those kinds of method have been effectively connected reply to different types of organic question associated to various biological and medical science research fields [3].

Glutathione (GSH) affinity chromatography is a skillful technology for purification of fusion protein fused to GST-tag. This tag used to purify fusion/recombinant protein due to ability bind with immobilized glutathione (GSH in Agarose beads and promotes the solubility of fusion protein which expressed in *Escherichia coli* [4]. The molecular weight of GST is naturally occurring 26000 da/26 kda found in eukaryotic cells and the source, organisms and function of GST are showed in Table 1 [5-15]. The gene from the *S.japonicum* was utilized within the advancement of commercially available vectors such as pGEX (GE Healthcare) or pET (Novagen) [16]. Those vectors were utilized for expression to help of recombinant protein purification. The 26000 da/26 kda GST-tag ties with high affinity to Glutathione (GSH) linked to Sepharose resin. This type of linking is reversible and after adding reduced glutathione to the elution buffer, protein can be eluted as a non-

Correspondence to: Lutfun Nahar, Department of Bacteriology, Graduate School of Medicine Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, 700-8558, Japan.

Received: 21-Aug-2023, Manuscript No. JMBT-23-22676; **Editor assigned:** 24-Aug 2023, PreQC No. JMBT-23-22676 (PQ); **Reviewed:** 07-Sep-2023, QC No JMBT-23-22676; **Revised:** 14-Sep-2023, Manuscript No. JMBT-23-22676 (R); **Published:** 21-Sep-2023 DOI: 10.35248/1948-5948.23.15.568

Citation: Nahar L, Lijon Md B, (2023) The Technique for Excessive-Results of Recombinant Protein Purification led to GST-tag Affinity Chromatography: A Review. J Microb Biochem Technol.15:568.

denaturing condition. Following purification, it may be eligible to transfer the GST-tag from the target recombinant protein. Most of the vectors such as pET or pGEX plasmid series encoded for an end protease site for cleavage between GST and target recombinant protein. By the application of re-chromatography technique GST-tag can be removed from target recombinant protein. The recombinant protein which has various application field such as determination of protein microarrays, DNA-protein interaction, immunological studies, crystallography structure, and protein-protein interaction through GST pull-down assay [17-21]. Pull-down assay technique implies physical interaction between two or more protein and identifying unfamiliar protein-binding partners *in vitro*. For effective purification of GST fusion protein requires a few key choices and optimization of strategies and agreement for target recombinant protein. A stream chart presenting the fundamental steps of the vector plan, expression, and purification method showed in Figure 1. All steps include different types of choice that are repeatedly interrelation and might influence the final production and integrity. The measure of purification of GST-tagged protein is following on the amount of protein preparation. The GST-tag affinity chromatography column size and whole the binding capability should be approved on an average equal the amount of protein to be purified to inhibit binding of needless protein and save the Agarose beads. Some of non-tagged proteins are generally keep up on the Agarose beads if the target protein be placed almost all the obtainable GSH-binding site. In case the high amount of Agarose beads is applied, various kinds of protein attached which is not specific unoccupied location and protein eluted as like contaminants. The excessive result of GST-tag fusion protein expression is carried out by a fundamental genomic purpose to test expression clones, generally including minimum bacteria culture in antimicrobial plate [22]. After culture some important steps as like recombinant protein expression in *E. coli*, purification and analyze molecular mass of protein specifically done by SDS-PAGE, CBB staining or another electrophoresis technique [23]. Some research reported that they purified excessive result of GST (Glutathione S-transferase) tag fusion protein by commercially available instruments [24]. Another research reported that they purified excessive result of GST-tag fusion protein and His-tag protein by used a conventional method such as 96-well filter plate format [25].

Table 1: Classification, origin, and purpose of Glutathione S-transferase (GST).

Number	Class of GST	Organisms	Origin	Ligand	Purpose (Function)	Reference
1	Alpha	Mammalian	Human (liver)	S-benzylGSH	Peroxide reduction	[5]
2	Beta	Bacteria	*Sogatella furcifera*	MiGSTU	Haloalkane Conjugation	[6]
3	Delta	Insects	*Periplaneta americana*	adGSTD4-4	Insecticide resistance	[7]
4	Epsilon	Insects	*Nilaparvata lugens*	DmGSTE6	Insecticide resistance	[8]
5	Theta	Bacteria	*Schistosoma japonica*	HIV gp41 epitope	Haloalkane	[9]
6	Zeta	Mammalian and Bacteria	Hepatocytes	Maleylacetoacetate isomerase	Meleylpyruvate isomerases tetrachlodo hydroquinone dehalogenase	[10]
7	Mu	Mammalian	Rat liver	S-(p-nitrobenzyl) GSH	Function of Detoxification	[11]
8	Pi	Mammalian	Pig lung	GSH sulphonate	Function of Detoxification	[12]
9	Sigma	animals and insects	Invertebrate	GSH-DNB product	Prostaglandin D synthesis	[13]
10	Omega	Mammalian and Bacteria	Endocrine cells	Thioltransferase	Dehydroascorbate reduction	[14]
11	Plant	Plants	*Arabidopsis thaliana*	S-hexylGSH	Anthocyanin synthesis	[15]

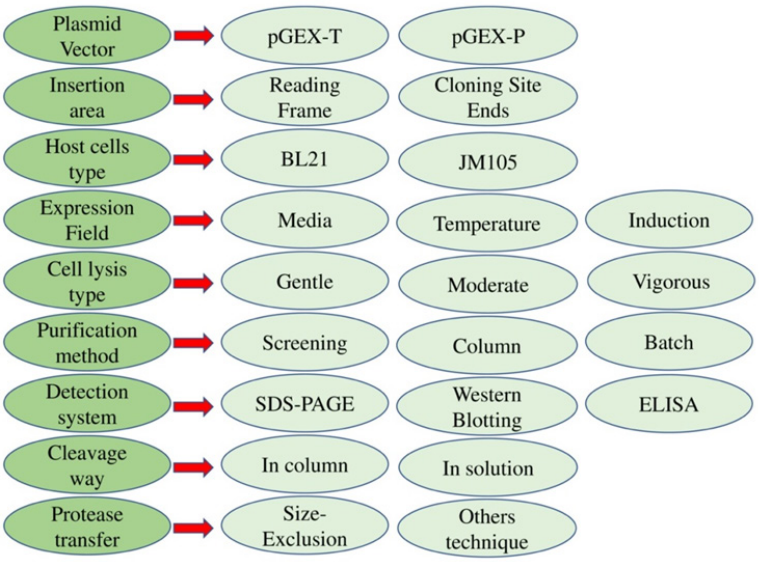

Figure 1: Stream graph outlining the indicator judgment-formin steps for planning and performing of GST-tag recombinant protein purification. Determined some expression vector and *E. coli* is used as a host cell for over-expressed of GST-tag recombinant protein. The expression conditions ought to be optimized to produce the most elevated level of dissolvable fusion protein. Glutathione affinity chromatography column is used purify GST-tag recombinant protein, cleavage with protease inhibitor enzyme, used further chromatography for removal of GST-tag to purify target protein.

They established that GST-tag fusion protein showed maximum outcome of protein purification and expression than His-tag protein purification technology. There are also different types of column chromatography for purification of GST-tag fusion protein which is known as HiTrap and HiPrep column were utilized with a medium pressure chromatography system, example: FPLC and syringe. Recombinant protein purification and expression methodology selected rely on a final application of recombinant protein and comfort of the item selection. There are several contributions of this study as like 1st: It is the first study to analyze amount of recombinant protein used by GST-tag affinity chromatography in plant science, animal science and microbes. Those data clearly show that by used GST-tag affinity chromatography purification is more suitable than others tag, and higher amount of protein purified. Those results proved the novelty of this manuscript (Table 2).

In this review, we summarize the feature and application of GST-tag affinity chromatography such as: Advantage of modern technology-specially, a high-throughput gene cloning method, vector construction expression and GST-tag fusion protein purification and characterization.

LITERATURE REVIEW

Affinity tags and GST-tag properties

Recombinant protein expression performs in *Escherichia coli* (*E. coli*) where fusion protein binding with affinity tag is the most famous technique for purification of protein. Affinity tags are manufactured polypeptide chain which generally designed either N- or C-terminal sequence, by placing the cDNA sequence that encoded with tag peptide into an equal ORF of protein. Affinity tags have been promoted the folding of protein, solubility of protein and production of output in coupling to simplify purification of the recombinant process [26]. There are some small size tags alike poly-Arg-, FLAG-, poly-His-, c-Myc-, S-, and Strep II- tags etc have the potential of decrease the impact of recombinant protein properties, activities and other function generally remove the affinity tag is not mandatory. Even more NusA, MBP, and Trx, GST and MBP show the leading solvency improving properties and protein expression efficiency as a large size tag in affinity chromatography technique. There are also more utilization of large size tags such as cell metabolic process, over-expression process and expression efficiency [27]. The Glutathione S-Transferase (GST) tag a is major founded and well-built up affinity tag based on the strong affinity of GST for immobilized glutathione during affinity chromatography technique when execute purification of recombinant protein [28]. Glutathione affinity resins are easy to get from many commercial sources for purification of GST-tag fusion protein through their appropriate binding site to ligand, glutathione which is well-established immobilized on bead-based maintained. Glutathione affinity purification as like simple bind-wash-elution representation is showed in the flowchart (Figure 2). GST-tag is best appropriate for utilize in prokaryotic expression since GSTs are a family of multifunctional cytosolic proteins that are show in eukaryotic living beings [29]. In *Escherichia coli* (*E. coli*) some large size tags in affinity chromatography technique such as MBP tag and GST tags were utilized to enhance the analyzable of purified recombinant protein [30]. By using reduced glutathione protein are eluted which carried GST-tag and captured by immobilized glutathione under non denaturing condition [31].

High efficiency construction of the target gene and pGEX gene fusion construction

In Figure 3, development of genes is expressed that presently cloned from cDNA which carries information of genome into vector such as pGEX or pET (Figure 3-I). This strategy was utilized by some researcher who developed a cDNA expression library expressed in *Escherichia coli* (*E. coli*) [32]. A small amount of clone expressed protein from cDNA expression library. Most of the inquiry reported that a few of gene were able to be coding partial gene sequence and some gene was not able to code the correct reading frame. In yeast, along these lines a new human cDNA expression library empowering

the choice of the ORF establishment on histidine prototrophy was created and here this library around half of the clones were within the correct ORF [33]. Especially in the mammalian cells, there are some faults to the conformation of expression libraries. To the beginning the approach of non-translocate regions at both ends of clone for attaching fusion tag become critical situation. Another fault, although the method is difficult genes of interest must be habitually angled out of a library for utilize in test [34]. For getting the target gene, Polymerase Chain Reaction (PCR) is the most broadly used methodology and is always the primary step in any exertion to precise recombinant protein (Figure 3-II). Now-a-days by utilizing some online instruments approval of the gene and all primers can be designed [35,36]. The PCR product refinement are presently developing the procedure that can be completed a computerized research facility in the laboratory [37]. Be that as it may, difficulty as a like band looks like nonappearance in Agarose gel electrophoresis, non-specific groups and so on happen after PCR and moderate the experimental handle. By altering PCR parameter like change the annealing temperature, revised the concentrating of kits those issues can be overcome. There are some methodologies known as *de novo* synthesis of DNA which utilizing for getting the target gene (Figure 3-III). The DNA synthesis process as like solid phase which including chemical strategies has been customarily utilized but in this process, there are some difficulties arising. In addition, this process of DNA synthesis can reduce cost and impressively more for high efficiency about DNA construction. For synthesizing a long DNA sequence with expanded accuracy has been developed by used new array-based technique which showed significantly lower cost [38,39]. *De novo* synthesis has some advantage, one of them that researcher can freely design the target gene without confinements utilize of common formats [40]. The reliable gene expression, increased protein products, protein solubility etc. are ensured quality using codon optimize gene [41]. The development of this technique again and again, the pertinence of *de novo* DNA synthesis to the high efficiency measure is expected to extend. From GE Healthcare vectors such as *pGEX* gene fusion vectors has properties like the availability of a tac promoter for chemically inducible for bacterial growth and high-level protein expression with IPTG. Some interior gene monitoring over an expression of insert by binding to the tac promoter until induction with IPTG. The polypeptide of interest can be inserted soon after the *GST* gene using a multiple linker site as like the *pGEX* gene fusion vectors are designed. Between the GST sequence and linker site there are an enzymatically digested site which is known as protease a cleavage site. *GST* gene molecule can be removed after digestion of protease enzyme as like thrombin. The linker site is carried by stop codon in each reading frame where encoded the stop codon sequence. One of the significances of using the thrombin cleavage site that is ordinary cost effective and during incubation at 25°C-37°C is enough for cleave between GST-tag and target protein [42]. Moreover, when selecting a vector and picked up the restriction site that will be taken cleavage site of N-terminus sequence of the protein.

Table 2: Summary of recombinant protein purification and purity by used GST-tag affinity chromatography

Origin	Proteins names	Manufacture	Molecular weight (kDa)	Purity (%)	Functions	Reference
Bacteria (*E. coli*)	Lep	Full-length	28	95-97	Digest of peptide chain	[84]
Mus muscullus	Pur-α	Full-length	39	98	Regulator of transcription process	[85]
Bacteria	Prow	Full-length	38	91-95	ATP-binding cassette and enhance some biological function	[86]
Mus muscullus	Translin	Full-length	25	90-96	Regulator of recombination process	[87]
Bacteria	ecoKch	Full-length	46	92-95	Act as an ion-exchange process	[88]
Spinacia oleraceae	TLP40	Protein data bank	8	90-97	The method of signal transduction	[89]
Mammalian	COUP-TFI	(57-423) amino acids	46	96	Act as a function of hormone receptor	[90]
Mammalian	Thioredoxin (Trx)	1276 bp	12	91-94	reduction of oxidized cysteine residues and the cleavage of disulfide bonds	[91]
Taenia solium	Calreticulin (CRT)	417 amino acids	46	93	Cell-cell communication, immune responses, and recognition	[92]
Mo-MLV	p12	Full-length	40	90	Involved in directing the viral pre-integration complex to chromatin ready for integration.	[93]

Mammalian, Bacteria	Vimentin	464	57	90-95	Plays a significant role in supporting and anchoring the position of the organelles in the cytosol	[94]
Mammalian	PTEN	403	47	91-93	Work as a tumor suppressor, which means that it helps regulate cell division	[95]
Bacteria (*E. coli*)	Pyruvate dehydrogenase	1,065 bp	38	88-90	Worked at key position in the oxidation of glucose by linking the glycolytic pathway	[96]
Eukaryotic cells	Actin	Full length	42	80-85	Actin work in muscle contraction	[97]
Mammalian	Myosin	Full length	20	88	converts chemical energy in the form of ATP to mechanical energy	[98]
Mammalian	P53	Full length	44	95	tumor suppressor, which means that it regulates cell division by keeping cells from growing and dividing	[99]
Eukaryotic cells	β-tubulin	Full length	43	81	Microtubules are one of the major components of the cytoskeleton, and perform structural support	[100]
Plant cells	2-cys Prx	Full length	22	87	Works on peroxide-mediated inactivation, in hydrogen peroxide signaling pathway	[101]
Eukaryotic cells	NOS	356 amino acids	43	91	Works on regulation of vasomotor tone and cell adhesion to the endothelium	[102]
Mammalian	Carbonic anhydrase	Full length	30	92	Perform on rapid inter-conversion of carbon dioxide and water into carbonic acid, protons and bicarbonate ions.	[103]
Eukaryotic cells	PDI	503 amino acids	62	62	It has multiple roles, acting as a chaperone, a binding partner of other proteins, and a hormone reservoir	[104]
Eukaryotic cells	HSP60	547 amino acids	61	93	as chaperones to assist in the proper folding of newly synthesized proteins and to protect the cell from denatured proteins	[105]
Mammalian	STAT3	Full length	83	94	It has signals for the maturation of immune system cells, especially T cells and B cells.	[106]
Mammalian	SerpinA1	418 amino acids	46	95	Works as a serine protease inhibitor (serpin). Serpins help control several types of chemical reactions by blocking (inhibiting) the activity of certain enzymes.	[107]

Mammalian	Enolase 1 (Eno1)	434 amino acids	48	96	Responsible for reversible conversion in glycolysis process and other's cellular function	[108]
Mammalian	HMGB1	215 amino acids	25	95	Works as an extracellular inflammatory cytokine, DNA replication and repair	[109]
Mammalian	Momordica anti-HIV protein (MAP30)	286 amino acids	30	92	Works on immunomodulatory, anti-tumor, anti-viral, and anti-human immunodeficiency virus (HIV) activities	[110]
Eukaryotic cells	SelenoproteinW	11,139 bp	370	95	It has down-regulation activity which impact on muscle disease.	[111]
Mammalian	E7	Full length	11	96	Plays a role in the human papillomavirus life cycle, reprogramming the cellular environment	[112]
Eukaryotic cells	CAP	209 amino acids	22.5	93	Perform as an effector cAMP, to specific DNA sites in or near target promoters	[113]
Escherichia coli	Orf135	Full length	15.4	96	Its play a role in pyrimidine biosynthesis, lipid biosynthesis and others biological process	[114]

Note: A chart summarizing protein production and purity yield for purification used by GST-tag chromatography.

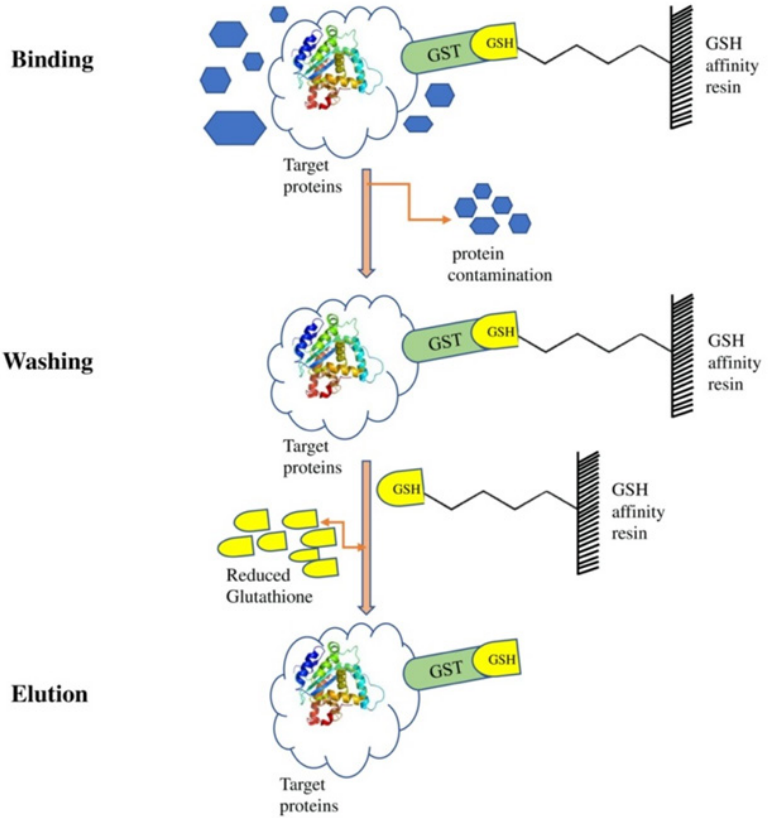

Figure 2: A Diagrammatic demonstration of Glutathione Affinity chromatography. Cleared lysate which carried GST fusion proteins that attach to immobilized glutathione (GSH, Bind). Non-binding proteins are discharge (washed) from the resin (Wash) and by the addition of excess reduced glutathione bound GST fusion proteins are eluted (Elute).

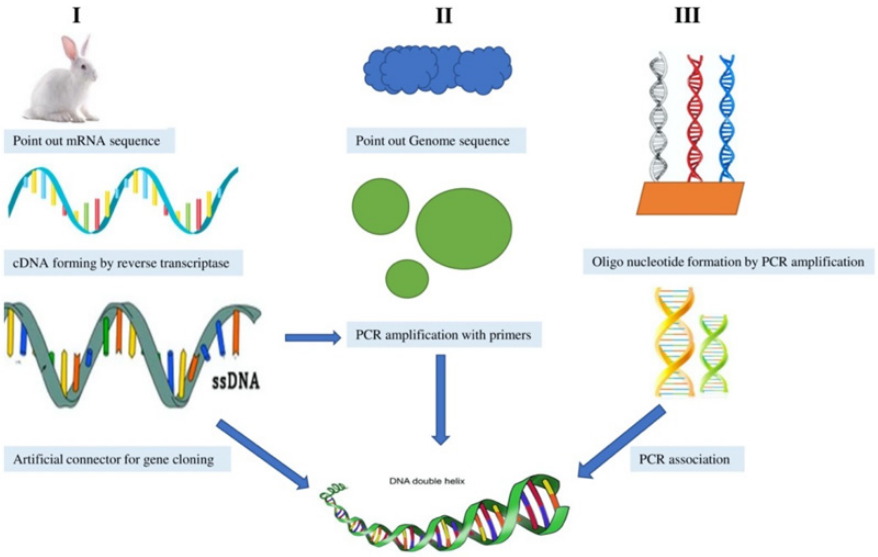

Figure 3: For making target genes it needs three types of different technique. **Note:** (I) After reverse transcription target genes can be acquired from a cDNA library (II) Amplify of genes from a cDNA library by utilization of PCR technique (III) Put together of small size of oligo can be employ to produce customized genes by the application of array-based oligonucleotide manufacture.

High efficiency cloning the GST fusion protein

Now days there are different kinds of process to create particular constructs for gene cloning and protein expression led to in a wide extend of living organism. Some cloning methods such as restriction enzyme based, ligation independent based and recombination-based cloning has empowered high-throughput protein expression for structural and functional analysis. These methods are moreover playing an important role by modification of the target gene, mutagenesis process and exchange of domain. Here we explain foremost common cloning procedures those are right now template for studies of gene cloning and recombinant protein expression, together with a brief outline of procedures related to co-expression technique.

Restriction enzyme-based cloning: Based on Restriction enzyme and DNA ligase it was the first convention accessible methodology is DNA cloning and in this method the restriction enzyme commonly utilized which recognition sequence that happen habitually inside open reading frames [43]. The method of restriction enzyme-based cloning was already considered to be unacceptable for high efficiency although this cloning method performed with DNA ligation has been utilized for last some years [44]. The most technique Flexi Cloning systems which was created by Promega Madison in the USA, where the two important rare cutting SgfI and PmeI were utilized and this technique has received expanded consideration since last few years. The two important link-up rare cutting SgfI and PmeI has been recommended to permit the cloning of maximum genes of chosen demonstrate life forms [45]. The target genes are amplified utilizing primers containing adapter sequence and after that digested by two enzymes; this technique is following to that utilize for a common restriction enzyme-based cloning method.

Ligation-Independent Cloning (LIC): Ligation-Independent Cloning (LIC) is such kind of excessive result of cloning procedure that does not utilize restriction enzyme, which is available developed this process common and spontaneous [46]. This procedure depends on the efficiency of bacteria cells to close the gap in DNA and the annealing of complementary single strand DNA. Ligation-Independent Cloning (LIC) is created a long time ago empowers directional cloning of any insert as a like single stranded complementary end which recognized after the generation of DNA fragments [47]. Ligation-independent cloning is forming reasonable and effectively versatile to high efficiency due to need of necessity for DNA ligase and restriction enzyme. In any case, some enzyme such as T5 exonuclease and T4 DNA polymerase demand for the Ligation-independent cloning process to produce in vector or target gene using the single-stranded complementary ends.

Recombination-based cloning: Based on recombinant cloning technology the foremost popular high throughput cloning technology has been established [48], and development of expression libraries some researcher performed this innovation day by day [49-53]. In the site-specific of gene sequence recombinant cloning is negotiated by the genetic recombination enzyme and avoid utilize of restriction enzyme and DNA ligase. This type of genome areas is mostly made by long gene sequence which is makes extremely uncommon around 100 base pairs. The recombination area prepares to be cloned by insert DNA without anxiety of obstructing the coding sequence. Recombines-based frameworks with high proficiency rates led to strong properties to exclusively review each target sequence. The cloning procedure such as Echo cloning (Thermo Fisher Scientific), Gateway (Waltham, USA) and creator (CA, USA) are broadly utilized as a recombination-based cloning system. In this system without utilizing any restriction enzyme and ligases, a site-specific recombination enzyme is utilized to develop the specified recombinant vector. Excessive result of cloning innovation, the most prevalent recombinant-based cloning technology has been utilized as a Gateway method which performs high efficiencies to succeed for all clones, adding cost and time through the project [54-78].

Expression of GST fusion protein

The selection strains utilized to precise GST-tag recombinant proteins expression which leads to production and some expression plasmid used by GST-tag showed in Table 3. Sometimes a couple of *E. coli* strains and its subsidiaries are broadly utilized for recombinant protein expression technique. Another strategy refers that, *E. coli* strains influence the expression of proteins that are encoded by a gene containing uncommon codons and in that case

expression of proteins is dangerous to bacterial strain *E. coli*. In the post transitional modification process some gene makes strides by expression that can alter protein. A large strength behind the improvement of the GST fusion system is that the GST protein amasses in the cytoplasm and for a large size affinity tag solubility of GST moiety would be reproduced [16]. Production of GST recombinant protein which expressed in *E. coli* and performs manufacture of protein around 40 mg/liter, but during the different situation as like unstable fusion protein does not yield higher amount. When expression levels are some larger, bacterial culture grown in shaker flasks, protein will be achieved from 3-4 liters of culture media. The methodology of GST fusion proteins followed at 37°C or 25°C, the induction circumstance for each construct should be transformed to develop the maximum amount of protein production. The cultural media can be monitored Optical Density (OD) result 1.0 or 1.5 using A600 and finally, utilized of SDS-PAGE technique showed the molecular mass of expressed protein. During the bacterial culture added IPTG which induced the bacterial cell growth a particular time, as cell lysis can occur and in the cytoplasm release protease enzyme which can breakdown the GST fusion proteins.

Purification of GST-tag recombinant protein used by affinity chromatography

By using an immobilized glutathione Sepharose column, GST-tag recombinant proteins are purified from bacterial cell lysates which suspension by PBS-buffer [79]. Procedure of affinity chromatography is modifying assembled of glutathione Sepharose column using a roller/tube pump to control flow rates. Phenylmethylsulphonyl Fluoride (PMSF) is known as protease inhibitors and zinc metal (reducing agents) should be added to the buffers when suspension of bacterial cell pellets to decrease proteolysis of the GST-tag recombinant proteins. Protease inhibitors must be removed from the glutathione buffer prior to the enzymatic eradication of the GST-tag protein so that this kind of protease inhibitors will inhibit the proteolysis process. After purification of GST-tag recombinant protein to check out the position and molecular weight/mass by the analysis of SDS-PAGE. Sometimes another column chromatography used with a recombinant protein to play down potential cross defilement which is confirmation of molecular mass of recombinant protein. Normally In additional, batch purifications which is known as speedy and basic, but habitually the abdicate and virtue of the protein acquired but sometimes this procedure obtained lower amount of recombinant protein compare with others chromatographic technique [80]. For achieved purity of GST-tag recombinant protein purification result it would be better all experimental procedure carried out in cold room.

Enzymatic action to cleaved GST tag

After decided the expression vector, recombinant protein with GST-tag can be take off by the activity of protease enzyme (thrombin). This procedure happens at room temperature (25°C) either in protein solution in a falcon tube or attached to the chromatography column resin during chromatography running [81]. This protease enzyme cleavage GST-tag and some good qualifications are related for example as like buffer, temperature, enzyme-to-substrate proportion, pH, length of hatching and concentration of protein etc. When GST-tag recombinant protein digested during column chromatography technique that time yield of digested protein is less amount but when incubation at 25°C, digested in buffer solution between GST-tag recombinant protein and thrombin is more effective, produced comparatively high amount and convenient. By used re-chromatography technique as like glutathione Sepharose column, target recombinant protein and GST-tag are separated.

Further purification of remove GST tag and pooled recombinant protein

Without GST-tag pure recombinant protein purification steps, Glutathione Sepharose column used where recombinant protein carried non-functioning protease enzyme [82]. The purified recombinant protein carried a little volume of GST-tag or other compounds/fragments which did not attached glutathione resin during affinity chromatography technique. By used re-chromatography procedure, sometimes used ion exchange chromatography or S-100 (Size exclusion column) chromatography technique. The purified pooled fraction of recombinant protein is usually more than 95% pure and analyzed the purity of recombinant protein by SDS-PAGE experiment which is showed in Table 3. This recombinant protein can be stored below freezing for different purpose. An important strength of this research includes our review article (n=800). We can collect data consistently from last 10 years period, which increase the generalizability of the result. Furthermore, this study provides valuable information on protein technology which can be used to create new research question or hypothesis about GST-tag recombinant protein purification procedure although still now some limitation with this experiment [83-113].

Table 3: Some expression plasmid used by GST-tag affinity chromatography.

Region of GST-tag	Protein	Sequence of bp which inserted	Vector	Restriction site	Desired molecular weight of recombinant protein (kDa)	Reference
N-terminal GST	BAK	636	pCold-GST	EcoRI	53	[55]
N-terminal GST	ESP1	234	pGEX 6P-3 PRESAT	-	36	[56]
N-terminal Hexa-His and GST	OSBP	1173	pCold-GST	Xhol, Hind III	75	[57]
N-terminal Hexa-His and GST	FD	429	pCold-GST	Ndel, EcoRI	45	[58]
N-terminal GST	HY2	858	pCold-GST	Xhol, Pstl	63	[59]

N-terminal GST	pHtrll	54	pGEX 6p-3	BamHI, EcoRI	32	[60]
N-terminal GST	Tsr	165	pCold-GST	EcoRI, sall	34	[61]
N-terminal GST	CaM	235	pCold-GST	Xhol, Ndel	47	[62]
N-terminal GST	FT	525	pCold-GST	Xhol, Pstl	49	[63]
N-terminal Hexa-His and GST	NHE1	126	pET200 D-TOPO	Pstl, Ndel	10	[64]
N-terminal GST	CMPK	476	pGEX-3X/PTP	BamHI, EcoRI	83	[65]
N-terminal GST	TP	595	pGEX-3X/SH	EcoRI	70	[66]
N-terminal GST	rHLT-B	5000	pGEX-4T-1	EcoRI, Xhol	34	[67]
N-terminal Hexa-His and GST	PLB1	1818	pGEX-GST	EcoRI, Xhol	68	[68]
N-terminal GST	Cyan	779	pCY 3020-02	BamHI/BssHII	627	[69]
N-terminal GST	GFP	768	pCY 3040-01	BamHI/BssHII	28	[70]
N-terminal Hexa-His and GST	Tetcys	889	pCY 3130-02	XmaI/PacI	34	[71]
N-terminal GST	Xylanase	678	pGEM-T Easy	EcoRI, BamHI	21	[72]
N-terminal Hexa-His and GST	*Trichoderma reesei*	570	pET-28a	EcoRI, sall	24	[73]
N-terminal GST	XynA	721	pUC119	EcoRI and smaI.	20.7	[74]
N-terminal GST	XYL-6	74	pPicH	Xhol, Hind III	47	[75]
N-terminal GST	*XynG1*	725	pNAN8142	ScaI-BstEII	21	[76]
N-terminal GST	HXYN2	630	pHEN	EcoRI and PmlI	28	[77]
N-terminal GST	XYL3	1950	pXLB37-2	KpnI-XbaI	20.8	[78]

Application of GST-tag affinity chromatography in plants sciences

Plants have been utilized as an originator of common items for last decades. It has various mechanical applications of plants that proceed to grow with the advancement of modern bioprocess and plant atomic science advances. For the expression of human therapeutics [114], and biopolymers [115], one kind of methodology has been developed of plant transformation which driven to the utilize different types of plants moderator. The preliminary effective transformation in plant science occurs many years ago and plants have been identified as a viable substitute for recombinant protein production by GST-tag affinity chromatography [116]. Plants recombinant proteins stages were at first ruled by rural crops with built up generation foundation such as tobacco, corn, rice including vegetables [117]. A recent article reported that in plant molecular technique proposal has a fine assembled pathway in short time that can achieve commercially acceptable recombinant protein production by GST-tag affinity chromatography [118]. By the utilization of affinity chromatography various types of recombinant protein in especial field are produced from tobacco, *Nicotiana benthamiana*, and *N. tabacum* because of good protein expression and manipulation. Plants recombinant protein purified by GST-tag affinity chromatography has been emerged and contributed for human growth hormone production from tobacco cells which nominated later as patent being issued [119]. Recombinant DNA innovation has made it conceivable to cross interspecies boundaries and achieved various types of plant assortments with alluring characteristics. It plays an important role that by the used of this technology genetic modifications of crop plants mainly concern progressing their resistance and challenge to changing natural circumstance [120]. Now days in plant molecular science field used some model plants for the purification of recombinant protein by

the utilization of GST-tag affinity chromatography so that scientist can acquire knowledge in the field of genomics, transcriptomic, and metabolomics. In addition, as these plants are vulnerable to hereditary change, completely optimized conventions for recovery and development for perform of much important experiment and identify new idea is created for them [121]. In any case, they have a few highlights that prohibit them from their planning utilize in different types of plant molecular research field. Some plants for example, the small size of *Nicotiana tabacum* or *Arabidopsis thaliana* seeds is more suitable and effective than leaves to produce recombinant proteins by the utilization of GST-tag affinity chromatography. Additionally, the extraction and purification of therapeutic proteins by GST-tag affinity chromatography from tobacco which is known as nicotine (one kind of alkaloid) that is toxic and harmful to human beings. There are multiple types of crops such as soybean, rapeseed, common bean, maize, wheat, and rice has been successfully utilized by recombinant protein purification for investigation of plants genetic engineering fields [122].

Update (modified) development of GST-tag affinity chromatography in plants sciences

The affinity tags such as GST-tag has been modified which explained so far were improved to be the supporting point for recombinant protein purification by affinity chromatography where the presence of a complementary affinity ligand, immobilized in a separation matrix are necessary for this experiment. However, GST-tag can be capable and auto-sufficient for enhanced fusion protein solubility, purification and further characterization. For most of the affinity chromatography technique GST-tag are harmless and do not obstruct with the structure and biological function of the purified recombinant protein. Some researcher reported that the elimination of GST-tag can be executed by enzymatic cleavage or others chemical treatments showed extensively reviewed of recombinant protein purification technique [123,124]. Sometime in plant science has some modified of GST-tag used in multimodal affinity chromatography which affinity ligands usually combine a hydrophobic moiety with ionic and hydrophilic groups, thus a perform on multi step purification schemes [125]. Moreover, another affinity ligand, anion-exchange and hydrophobic ligands are extremely popular as binding partners after changes/modify affinity GST-tags are widely employed for the purification of recombinant protein (Example: *Zea mays* and *Rosa bourboniana*). In addition, by the utilization of GST-tag affinity chromatography technique modified to structural ligands as binding partners of affinity tags or metal chelator as affinity ligands for His-tagged proteins perform a development of transformation and regeneration procedures for various plants recombinant proteins. The most implement of producing recombinant proteins in different fruits and vegetables (e.g., strawberries, bananas, potatoes, tomatoes, lettuce, spinach, rice, safflowers, and barley) has been investigated [126-129].

Future perspectives

Effective recombinant protein expression and decontamination is as often as possible vital for both fundamental investigate ponders and different types of biological implement. A large scale of protein expression and purification occurs in a bacterial strain named *E. coli* was started transformation in different investigate areas. Different types of laboratory work that were typically execute manually one molecular experiment at a time over 20-25 days can now be handled for the several molecular experiment in 7-8 days. In any case, some limitation is presently continued and in future advance changes are realizable with significantly. For example, analysis of specific molecule as like gene/DNA which used as a genetic sample for PCR, DNA microarray and next generation sequencing technique can be used in future for molecular diagnostics for drug design in pharmaceutical science, transformation of pathology and radiology field. Used by Gene cloning strategy a particular gene will be isolated that combine with vector genome for producing recombinant DNA which apply in future different agriculture field, plant disease, bio fertilizer, pesticides and pathogen identification with eradication. Others viewpoint such as gene cloning technology linked with post transitional modification technique has been developed for better understanding of molecular engineering in animal or plant genetic science that will apply as a great achieve in future at cell biology or others research field. However, the recombinant protein purification methodology will be used as medicinal research field such as vaccine, drugs or producing antibodies also in various biochemical analyses.

CONCLUSION

In this study we have demonstrated several key factors of GST-tag recombinant protein production and purification which frequently regularly vital for both essential investigate considers and various types of commercial applications. We have evaluated for the first-time summarized data of GST-tag affinity chromatography to describe recombinant protein purification technique. GST-tag recombinant protein was over-expressed in *E. coli* used by different expression vector and the recombinant protein was purified by affinity chromatography technique and then cleaved with protease enzyme known as thrombin to remove the GST-tag. The recombinant protein was further purified by Glutathione affinity chromatography or anion-exchange chromatography technique. This GST-tag promote the recombinant protein solubility and stability. This review paper suggests that the application of GST-tag recombinant protein technology used as many beneficial purposes as possible of protein chemistry, structure analysis in NMR, 3D structure measurement, detection of functional group etc. Moreover, this GST-tag produces high amounts of homogenous pure fusion protein in a convenient way.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included previous research article. The current review covers a literature across from 1988 to 2020.

AUTHOR CONTRIBUTIONS

Asaduzzaman Md, developed the concept, wrote the manuscript, and prepared the resources. Nahar Lutfun, took part in the manuscript writing and was responsible for the review. Lijon Md Bakhtiar, Imran Shahin and Rhaman Mohammad Saidur, have read and agreed to publish of this manuscript.

ACKNOWLEDGMENTS

I would like to appreciate the advice and intellectual suggestions

of all co-authors for their support, valuable suggestion and cordial companionship in making such an effort to submit the review manuscript. I am also express with deepest sense and thankful to all for their compatible manners, good assistance and unconditional co-operation.

FUNDING

This research received no external funding.

REFERENCES

1. Hsiao A, Kuo MD. High-throughput biology in the postgenomic era. J Vasc Interv Radiol. 2006;7(17):1077-1085.
2. Cameron DE, Bashor CJ, Collins JJ. A brief history of synthetic biology. Nat Rev Microbiol. 2014;12(5):381-390.
3. Pepperkok R, Ellenberg J. High-throughput fluorescence microscopy for systems biology. Nat Rev Mol Cell Biol. 2006;7(9):690-696.
4. Smith DB, Rubira MR, Simpson RJ, Davern KM, Tiu WU, Board PG, et al. Expression of an enzymatically active parasite molecule in *Escherichia coli*: *Schistosoma japonicum* glutathione S-transferase. Mol Biochem Parasitol. 1988;27(2-3):249-256.
5. Moons A. Regulatory and functional interactions of plant growth regulators and plant glutathione S-transferases (GSTs). Vitam Horm. 2005;72:155-202.
6. Lallement PA, Meux E, Gualberto JM, Prosper P, Didierjean C, Saul F, et al. Structural and enzymatic insights into lambda glutathione transferases from *Populus trichocarpa*, monomeric enzymes constituting an early divergent class specific to terrestrial plants. Biochem J.2014;462(1):39-52.
7. Lan T, Wang XR, Zeng QY. Structural and functional evolution of positively selected sites in pine glutathione S-transferase enzyme family. J Biol Chem. 2013;288(34):24441-24451.
8. Townsend DM, Findlay VJ, Fazilev F, Ogle M, Fraser J, Saavedra JE, et al. A glutathione S-transferase π-activated prodrug causes kinase activation concurrent with S-glutathionylation of proteins. Mol Pharmacol. 2006;69(2):501-508.
9. Sylvestre-Gonon E, Law SR, Schwartz M, Robe K, Keech O, Didierjean C, et al. Functional, structural and biochemical features of plant serinyl-glutathione transferases. Front Plant Sci. 2019;10:608.
10. Thom R, Dixon DP, Edwards R, Cole DJ, Lapthorn AJ. The structure of a zeta class glutathione S-transferase from *Arabidopsis thaliana*: Characterisation of a GST with novel active-site architecture and a putative role in tyrosine catabolism. J Mol Biol. 2001;308(5):949-962.
11. Rundle A, Tang D, Hibshoosh H, Estabrook A, Schnabel F, Cao W, et al. The relationship between genetic damage from polycyclic aromatic hydrocarbons in breast tissue and breast cancer. Carcinogenesis. 2000;21(7):1281-1289.
12. Bagnoli F, Fady B, Fineschi S, Oddou-Muratorio S, Piotti A, Sebastiani F, et al. Neutral patterns of genetic variation and applications to conservation in conifer species. Genetics, Genomics and Breeding of Conifers. 2011:141-195.
13. Yang SH, Galanis A, Witty J, Sharrocks AD. An extended consensus motif enhances the specificity of substrate modification by SUMO. EMBO J. 2006;25(21):5083-5093.
14. Whitbread AK, Masoumi A, Tetlow N, Schmuck E, Coggan M, Board PG. Characterization of the omega class of glutathione transferases. Meth Enzymol. 2005;401:78-99.
15. Chronopoulou E, Ataya FS, Pouliou F, Perperopoulou F, Georgakis N, Nianiou-Obeidat I, et al. Structure, evolution and functional roles of plant glutathione transferases. Glutathione in plant growth, development, and stress tolerance. 2017:195-213.
16. Smith DB, Johnson KS. Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene. 1988;67(1):31-40.
17. Vikis HG, Guan KL. Glutathione-S-transferase-fusion based assays for studying protein-protein interactions. Methods Mol Biol. 2004:175-186.
18. Yip YL, Smith G, Ward RL. Comparison of phage pIII, pVIII and GST as carrier proteins for peptide immunisation in Balb/c mice. Immunol Lett. 2001;79(3):197-202.
19. Smyth DR, Mrozkiewicz MK, McGrath WJ, Listwan P, Kobe B. Crystal structures of fusion proteins with large-affinity tags. Protein Sci. 2003;12(7):1313-1322.
20. Singh CR, Asano K. Localization and characterization of protein–protein interaction sites. Meth Enzymol. 2007;429:139-161.
21. Yokoyama S. Protein expression systems for structural genomics and proteomics. Curr Opin Chem Biol. 2003;7(1):39-43.
22. Savchenko A, Yee A, Khachatryan A, Skarina T, Evdokimova E, Pavlova M, et al. Strategies for structural proteomics of prokaryotes: Quantifying the advantages of studying orthologous proteins and of using both NMR and X-ray crystallography approaches. Proteins. 2003;50(3):392-399.
23. Lesley SA. High-throughput proteomics: Protein expression and purification in the postgenomic world. Protein Expr Purif. 2001;22(2):159-164.
24. Braun P, Hu Y, Shen B, Halleck A, Koundinya M, Harlow E, et al. Proteome-scale purification of human proteins from bacteria. Proc Natl Acad Sci. 2002;99(5):2654-2659.
25. Waugh DS. Making the most of affinity tags. Trends Biotechnol. 2005;23(6):316-320.
26. Zhang Y, Olsen DR, Nguyen KB, Olson PS, Rhodes ET, Mascarenhas D. Expression of eukaryotic proteins in soluble form in *Escherichia coli*. Protein Expr Purif. 1998;12(2):159-165.
27. Liang S, Gong F, Zhao X, Wang X, Shen G, Xu Y, et al. Prokaryotic expression, purification of a new tumor-relative protein *FAM92A1-289* and its characterization in renal cell carcinoma. Cancer Lett. 2009;276(1):81-87.
28. Hammarström M, Hellgren N, Van Den Berg S, Berglund H, Härd T. Rapid screening for improved solubility of small human proteins produced as fusion proteins in *Escherichia coli*. Protein Sci. 2002;11(2):313-321.
29. Wang C, Castro AF, Wilkes DM, Altenberg GA. Expression and purification of the first nucleotide-binding domain and linker region of human multidrug resistance gene product: Comparison of fusions to glutathione S-transferase, thioredoxin and maltose-binding protein. Biochem J. 1999;338(1):77-81.
30. Schmidt TG, Koepke J, Frank R, Skerra A. Molecular interaction between the Strep-tag affinity peptide and its cognate target, streptavidin. J Mol Biol. 1996;255(5):753-66.
31. Büssow K, Nordhoff E, Lübbert C, Lehrach H, Walter G. A human cDNA library for high-throughput protein expression screening. Genomics. 2000;65(1):1-8.
32. Holz C, Lueking A, Bovekamp L, Gutjahr C, Bolotina N, Lehrach H, et al. A human cDNA expression library in yeast enriched for open reading frames. Genome Res. 2001;11(10):1730-1735.
33. Atanassov II, Atanassov II, Etchells J, Turner SR. A simple, flexible and efficient PCR-fusion/Gateway cloning procedure for gene fusion, site-directed mutagenesis, short sequence insertion and domain deletions and swaps. Plant Methods. 2009;5:1-1.
34. Klein JC, Lajoie MJ, Schwartz JJ, Strauch EM, Nelson J, Baker D, et al. Multiplex pairwise assembly of array-derived DNA oligonucleotides. Nucleic Acids Res. 2016;44(5):e43.
35. Cao Y, Sun J, Zhu J, Li L, Liu G. PrimerCE: Designing primers for cloning and gene expression. Mol Biotechnol. 2010;46:113-117.
36. Otto P, Larson B, Krueger S. Automated high-throughput purification of PCR products using Wizard® MagneSil™ paramagnetic particles. J Lab Autom. 2002;7(6):120-124.
37. Yehezkel TB, Linshiz G, Buaron H, Kaplan S, Shabi U, Shapiro E. *De*

novo DNA synthesis using single molecule PCR. Nucleic Acids Res. 2008;36(17):e107

38. Kosuri S, Church GM. Large-scale *de novo* DNA synthesis: Technologies and applications. Nat Methods. 2014;11(5):499-507.

39. Quan J, Saaem I, Tang N, Ma S, Negre N, Gong H, et al. Parallel on-chip gene synthesis and application to optimization of protein expression. Nat Biotechnol. 2011;29(5):449-452.

40. Presnyak V, Alhusaini N, Chen YH, Martin S, Morris N, Kline N, et al. Codon optimality is a major determinant of mRNA stability. Cell. 2015;160(6):1111-1124.

41. Qian W, Yang JR, Pearson NM, Maclean C, Zhang J. Balanced codon usage optimizes eukaryotic translational efficiency. PLoS Genet. 2012;8(3):e1002603.

42. Cohen SN, Chang AC, Boyer HW, Helling RB. Construction of biologically functional bacterial plasmids *in vitro*. Proc Natl Acad Sci. 1973;70(11):3240-3244.

43. Stevens RC. Design of high-throughput methods of protein production for structural biology. Structure. 2000;8(9):177-185.

44. Blommel PG, Martin PA, Wrobel RL, Steffen E, Fox BG. High efficiency single step production of expression plasmids from cDNA clones using the Flexi Vector cloning system. Protein Expr Purif. 2006;47(2):562-570.

45. Care S, Bignon C, Pelissier MC, Blanc E, Canard B, Coutard B. The translation of recombinant proteins in *E. coli* can be improved by *in silico* generating and screening random libraries of a− 70/+ 96 mRNA region with respect to the translation initiation codon. Nucleic Acids Res. 2008;36(1):e6.

46. Aslanidis C, De Jong PJ. Ligation-Independent Cloning of PCR products (LIC-PCR). Nucleic Acids Res. 1990;18(20):6069-6074.

47. Aslanidis C, de Jong PJ, Schmitz G. Minimal length requirement of the single-stranded tails for ligation-independent cloning (LIC) of PCR products. Genome Res. 1994;4(3):172-177.

48. Brizuela L, Richardson A, Marsischky G, Labaer J. The *FLEX* Gene repository: Exploiting the fruits of the genome projects by creating a needed resource to face the challenges of the post-genomic era. Arch Med Res. 2002;33(4):318-324.

49. Rolfs A, Montor WR, Yoon SS, Hu Y, Bhullar B, Kelley F, et al. Production and sequence validation of a complete full length ORF collection for the pathogenic bacterium *Vibrio cholerae*. Proc Natl Acad Sci. 2008;105(11):4364-4369.

50. Yamada K, Lim J, Dale JM, Chen H, Shinn P, Palm CJ, et al. Empirical analysis of transcriptional activity in the Arabidopsis genome. Science. 2003;302(5646):842-846.

51. Walhout AJ, Temple GF, Brasch MA, Hartley JL, Lorson MA, Van den Heuvel S, et al. GATEWAY recombinational cloning: Application to the cloning of large numbers of open reading frames or ORFeomes. Meth Enzymol. 2000;328:575-577.

52. Marsischky G, LaBaer J. Many paths to many clones: A comparative look at high-throughput cloning methods. Genome res. 2004;14(10b):2020-2028.

53. Lederberg EM. Lysogenicity in *Escherichia coli* strain K-12. Microbial Genetics Bulletin. 1950; 1:5–8.

54. Scientific TF. Gateway Technology: A universal technology to clone DNA sequences for function analysis and expression in multiple systems. 2003.

55. Hockings C, Anwari K, Ninnis RL, Brouwer J, O'Hely M, Evangelista M,etal. Bid chimeras indicate that most BH3-only proteins can directly activate Bak and Bax, and show no preference for Bak versus Bax. Cell Death Dis. 2015;6(4):17-35.

56. Di Guana C, Lib P, Riggsa PD, Inouyeb H. Vectors that facilitate the expression and purification of foreign peptides in *Escherichia coli* by fusion to maltose-binding protein. Gene. 1988;67(1):21-30.

57. Yasukawa T, Kanei-Ishii C, Maekawa T, Fujimoto J, Yamamoto T, Ishii S. Increase of solubility of foreign proteins in *Escherichia coli* by coproduction of the bacterial thioredoxin. J Biol Chem. 1995;270(43):25328-25331.

58. Bao WJ, Gao YG, Chang YG, Zhang TY, Lin XJ, Yan XZ, et al. Highly efficient expression and purification system of small-size protein domains in *Escherichia coli* for biochemical characterization. Protein Expr Purif. 2006;47(2):599-606.

59. Etchegaray JP, Inouye M. Translational enhancement by an element downstream of the initiation codon in *Escherichia coli*. J Biol Chem. 1999;274(15):10079-10085.

60. Walker PA, Leong LE, Ng PW, Tan SH, Waller S, Murphy D, et al. Efficient and rapid affinity purification of proteins using recombinant fusion proteases. Bio technology. 1994;12(6):601-605.

61. Cordingley MG, Callahan PL, Sardana VV, Garsky VM, Colonno RJ. Substrate requirements of human rhinovirus 3C protease for peptide cleavage *in vitro*. J Biol Chem. 1990;265(16):9062-9065.

62. Chong S, Mersha FB, Comb DG, Scott ME, Landry D, Vence LM, et al. Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element. Gene. 1997;192(2):271-281.

63. Forde GM. Preparation, analysis and use of an affinity adsorbent for the purification of GST fusion protein. Methods Mol Biol.2008:125-136.

64. Smith MC, Furman TC, Ingolia TT, Pidgeon C. Chelating peptide-immobilized metal ion affinity chromatography. A new concept in affinity chromatography for recombinant proteins. J Biol Chem. 1988;263(15):7211-7215.

65. Strambio-de-Castillia C, Tetenbaum-Novatt J, Imai BS, Chait BT, Rout MP. A method for the rapid and efficient elution of native affinity-purified protein A tagged complexes. J Proteome Res. 2005;4(6):2250-2256.

66. Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, Bertone P, et al. Global analysis of protein activities using proteome chips. Science. 2001 Sep 14;293(5537):2101-2105.

67. Kolodziej CM, Chang CW, Maynard HD. Glutathione S-transferase as a general and reversible tag for surface immobilization of proteins. J Mater Chem. 2011;21(5):1457-1461.

68. Voelker AE, Viswanathan R. Synthesis of a suite of bioorthogonal glutathione S-transferase substrates and their enzymatic incorporation for protein immobilization. J Org Chem. 2013;78(19):9647-9658.

69. Qu JB, Zhou WQ, Wei W, Su ZG, Ma GH. An effective way to hydrophilize gigaporous polystyrene microspheres as rapid chromatographic separation media for proteins. Langmuir. 2008;24(23):13646-13652.

70. Wang DM, Hao G, Shi QH, Sun Y. Fabrication and characterization of superporous cellulose bead for high-speed protein chromatography. J Chromatogr A. 2007;1146(1):32-40.

71. Guan K, Dixon JE. Eukaryotic proteins expressed in *Escherichia coli*: An improved thrombin cleavage and purification procedure of fusion proteins with glutathione S-transferase. Anal Biochem. 1991;192(2):262-267.

72. Krisana A, Rutchadaporng S, Jarupan G, Lily E, Sutipa T, Kanyawim K. Endo-1, 4-β-xylanase B from *Aspergillus cf. niger BCC14405* isolated in Thailand: Purification, characterization and gene isolation. BMB Reports. 2005;38(1):17-23.

73. Jun H, Bing Y, Keying Z, Xuemei D, Daiwen C. Expression of a *Trichoderma reesei* β-xylanase gene in *Escherichia coli* and activity of the enzyme on fiber-bound substrates. Protein Expr Purif. 2009;67(1):1-6.

74. Kimura T, Ito J, Kawano A, Makino T, Kondo H, Karita S, et al. Purification, characterization, and molecular cloning of acidophilic xylanase from *Penicillium sp*. 40. Biosci Biotechnol Biochem. 2000;64(6):1230-1237.

75. Wu SC, Halley JE, Luttig C, Fernekes LM, Gutiérrez-Sanchez G, Darvill AG, et al. Identification of an endo-β-1, 4-D-xylanase from Magnaporthe grisea by gene knockout analysis, purification, and heterologous expression. Appl Environ Microbiol. 2006;72(2):986-993.

76. Kimura T, Kitamoto N, Kito Y, Karita S, Sakka K, Ohmiya K. Molecular cloning of xylanase gene *xynG1* from *Aspergillus oryzae KBN 616*, a shoyu koji mold, and analysis of its expression. J Ferment Bioeng. 1998;85(1):10-16.

77. De Faria FP, Te'o VS, Bergquist PL, Azevedo MO, Nevalainen KM. Expression and processing of a major xylanase (*XYN2*) from the

thermophilic fungus *Humicola grisea var. thermoidea* in *Trichoderma reesei*. Lett Appl Microbiol. 2002;34(2):119-123.

78. Apel-Birkhold PC, Walton JD. Cloning, disruption, and expression of two endo-beta 1, 4-xylanase genes, *XYL2* and *XYL3*, from *Cochliobolus carbonum*. Appl Environ Microbiol. 1996;62(11):4129-4135.

79. Frangioni JV, Neel BG. Solubilization and purification of enzymatically active glutathione S-transferase (pGEX) fusion proteins. Anal Biochem. 1993;210(1):179-187.

80. Grieco F, Hay JM, Hull R. An improved procedure for the purification of protein fused with glutathione S-transferase. Biotechniques. 1992;13(6):856-858.

81. Jenny RJ, Mann KG, Lundblad RL. A critical review of the methods for cleavage of fusion proteins with thrombin and factor Xa. Protein Expr Purif. 2003;31(1):1-2.

82. Singh SM, Eshwari AN, Garg LC, Panda AK. Isolation, solubilization, refolding, and chromatographic purification of human growth hormone from inclusion bodies of *Escherichia coli* cells: A case study. Methods Mol Biol. Methods and Protocols. 2005:163-176.

83. Paetzel M, Dalbey RE, Strynadka NC. Crystal structure of a bacterial signal peptidase in complex with a β-lactam inhibitor. Nature. 1998;396(6707):186-190.

84. Kuo CH, Nishikawa E, Ichikawa H, Sadakata T, Niu SY, Miki N. Calmodulin functions as an activator of pur α binding to single-stranded purine-rich DNA elements (PURElements). Biochem Biophys Res Commun. 1999;255(2):406-411.

85. Nilsson I, Witt S, Kiefer H, Mingarro I, von Heijne G. Distant downstream sequence determinants can control N-tail translocation during protein insertion into the endoplasmic reticulum membrane. J Biol Chem. 2000;275(9):6207-6213.

86. Aoki K, Suzuki K, Sugano T, Tasaka T, Nakahara K, Kuge O, et al. A novel gene, *Translin*, encodes a recombination hotspot binding protein associated with chromosomal translocations. Nat. Genet. 1995;10(2):167-174.

87. Johansson M, Von Heijne G. Membrane topology of Kch, a putative K+ channel from *Escherichia coli*. J Biol Chem. 1996;271(42):25912-25915.

88. Rokka A, Aro EM, Herrmann RG, Andersson B, Vener AV. Dephosphorylation of photosystem II reaction center proteins in plant photosynthetic membranes as an immediate response to abrupt elevation of temperature. Plant Physiol. 2000;123(4):1525-1536.

89. Lee SK, Kim JH, Lee YC, Cheong J, Lee JW. Silencing mediator of retinoic acid and thyroid hormone receptors, as a novel transcriptional corepressor molecule of activating protein-1, nuclear factor-κB, and serum response factor. J Biol Chem. 2000;275(17):12470-12474.

90. Spyrou G, Enmark E, Miranda-Vizuete A, Gustafsson JÅ. Cloning and expression of a novel mammalian thioredoxin. J Biol Chem. 1997;272(5):2936-2941.

91. Andrin C, Corbett EF, Johnson S, Dabrowska M, Campbell ID, Eggleton P, et al. Expression and purification of mammalian calreticulin in *Pichia pastoris*. Protein expression and purification. 2000; 20(2):207-215.

92. Alcami A, Angulo AN, Lopez-Otin C, Munoz M, Freije JM, Carrascosa AL, et al. Amino acid sequence and structural properties of protein p12, an African swine fever virus attachment protein. Virol J. 1992;66(6):3860-3868.

93. Nelson WJ, Traub P. Purification of the intermediate filament protein vimentin from Ehrlich ascites tumor cells. J Biol Chem. 1982;257(10):5536-5543.

94. Hu Y, An Y, Fang N, Li Y, Jin H, Nazarali A, et al. The optimization of soluble PTEN expression in *Escherichia coli*. Open Biochem J. 2015;9:42.

95. Neveling U, Klasen R, Bringer-Meyer S, Sahm H. Purification of the pyruvate dehydrogenase multienzyme complex of *Zymomonas mobilis* and identification and sequence analysis of the corresponding genes. J Bacteriol. 1998;180(6):1540-1548.

96. Kotila T, Wioland H, Selvaraj M, Kogan K, Antenucci L, Jégou A, et al. Structural basis of rapid actin dynamics in the evolutionarily divergent Leishmania parasite. Nat Commun. 2022;13(1):3442.

97. Bissonnette M, Kuhn D, De Lanerolle P. Purification and characterization of myosin light-chain kinase from the rat pancreas. Biochem J. 1989;258(3):739-747.

98. Lee SH, Ju SK, Lee TY, Huh SH, Han KH. *TIP30* directly binds p53 tumor suppressor protein *in vitro*. Mol Cell Biol.2012;34:495-500.

99. Hotta T, Fujita S, Uchimura S, Noguchi M, Demura T, Muto E, et al. Affinity purification and characterization of functional tubulin from cell suspension cultures of Arabidopsis and tobacco. Plant Physiol. 2016;170(3):1189-1205.

100. Bernier-Villamor L, Navarro E, Sevilla F, Lázaro JJ. Cloning and characterization of a 2-Cysperoxiredoxin from *Pisum sativum*. J Exp Bot. 2004;55(406):2191-2199.

101. Montgomery HJ, Dupont AL, Leivo HE, Guillemette JG. Cloning, expression, and purification of a nitric oxide synthase-like protein from *Bacillus cereus*. Biochem Res Int. 2010.

102. Yu S, Xia D, Luo Q, Cheng Y, Takano T, Liu S. Purification and characterization of carbonic anhydrase of rice (*Oryza sativa* L.) expressed in *Escherichia coli*. Prot express puri. 2007;52(2):379-383.

103. Townsend DM, Manevich Y, He L, Xiong Y, Bowers Jr RR, Hutchens S, et al. Nitrosative stress–induced S-glutathionylation of protein disulfide isomerase leads to activation of the unfolded protein response. Cancer Research. 2009;69(19):7626-7634.

104. Fratelli M, Demol H, Puype M, Casagrande S, Eberini I, Salmona M,et al. Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. Proc Natl Acad Sci. 2002;99(6):3505-3510.

105. Xie Y, Kole S, Precht P, Pazin MJ, Bernier M. S-glutathionylation impairs signal transducer and activator of transcription 3 activation and signaling. Endocrinology. 2009;150(3):112-231.

106. Grek CL, Townsend DM, Uys JD, Manevich Y, Coker III WJ, Pazoles CJ, et al. S-glutathionylated serine proteinase inhibitors as plasma biomarkers in assessing response to redox-modulating drugs. Cancer research. 2012;72(9):2383-2393.

107. Cianfruglia L, Perrelli A, Fornelli C, Magini A, Gorbi S, Salzano AM, et al. KRIT1 loss-of-function associated with cerebral cavernous malformation disease leads to enhanced S-glutathionylation of distinct structural and regulatory proteins. Antioxidants. 2019;8(1):27.

108. Hoppe G, Talcott KE, Bhattacharya SK, Crabb JW, Sears JE. Molecular basis for the redox control of nuclear transport of the structural chromatin protein Hmgb1. Exp Cell Res. 2006;312(18):3526-3538.

109. Chen WW, Zhang HR, Huang ZG, Zhou ZY, Lou QW, Jiang XY, et al. Expression and purification of a recombinant *ELRL-MAP30* with dual-targeting anti-tumor bioactivity. Prot Express Puri. 2021;185:105-893.

110. Shur I, Socher R, Hameiri M, Fried A, Benayahu D. Molecular and cellular characterization of SEL-OB/SVEP1 in osteogenic cells *in vivo* and *in vitro*. J Cell Physiol. 2006 ;206(2):420-427.

111. Zhu J, Chen L, Zheng Q, Han R, Chen X, Zhou Q, et al. Immunohistochemical detection of human papillomavirus 16 e7 oncoprotein in cervical lesions. J Cancer. 2021;12(23):7130-7137.

112. Lai GH, Lin YC, Tsai YL, Lien YY, Lin MK, Chen HJ, et al. High yield production of pigeon circovirus capsid protein in the *E. coli* by evaluating the key parameters needed for protein expression. BMC Vet Res. 2014;10(1):1-11.

113. Fujikawa K, Kasai H. The oxidized pyrimidine ribonucleotide, 5-hydroxy-CTP, is hydrolyzed efficiently by the *Escherichia coli* recombinant Orf135 protein. DNA repair. 2002;1(7):571-576.

114. Stoger E, Ma JK, Fischer R, Christou P. Sowing the seeds of success: Pharmaceutical proteins from plants. Curr Opin Biotechnol. 2005;16(2):167-173.

115. Sharma AK, Sharma MK. Plants as bioreactors: Recent developments and emerging opportunities. Biotechnol Adv. 2009;27(6):811-832.

116. Fraley RT, Rogers SG, Horsch RB, Sanders PR, Flick JS, Adams SP, et al. Expression of bacterial genes in plant cells. Proc Natl Acad Sci.

1983;80(15):4803-4807.

117. Basaran P, Rodríguez-Cerezo E. Plant molecular farming: Opportunities and challenges. Crit Rev Biotechnol. 2008;28(3):153-172.

118. Shanmugaraj B, I. Bulaon CJ, Phoolcharoen W. Plant molecular farming: A viable platform for recombinant biopharmaceutical production. Plants. 2020;9(7):842.

119. Barta A, Sommergruber K, Thompson D, Hartmuth K, Matzke MA, Matzke AJ. The expression of a nopaline synthase—human growth hormone chimaeric gene in transformed tobacco and sunflower callus tissue. Plant Mol Biol. 1986;6:347-357.

120. Gerszberg A, Hnatuszko-Konka K. Tomato tolerance to abiotic stress: A review of most often engineered target sequences. Plant Growth Regul. 2017;83:175-198.

121. Yonesi MA, Rezazadeh A, Mousai MM. The considerations and the required steps for production of the plant seeds vaccines with focus on the oral delivery. Plant Cell Biol Mol Biol. 2020;21:36-51.

122. Malik KA, Maqbool A. Transgenic crops for biofortification. Front sustain food syst. 2020;4:571402.

123. Arnau J, Lauritzen C, Petersen GE, Pedersen J. Current strategies for the use of affinity tags and tag removal for the purification of recombinant proteins. Protein Expr Purif. 2006;48(1):1-13.

124. Waugh DS. An overview of enzymatic reagents for the removal of affinity tags. Protein Expr Purif. 2011;80(2):283-293.

125. Zhao G, Dong XY, Sun Y. Ligands for mixed-mode protein chromatography: Principles, characteristics and design. J Biotechnol. 2009;144(1):3-11.

126. Lee YR, Lim CY, Lim S, Park SR, Hong JP, Kim J, et al. Expression of colorectal cancer antigenic protein fused to IgM Fc in chinese cabbage (*Brassica rapa*). Plants. 2020;9(11):14-66.

127. Webster DE, Smith SD, Pickering RJ, Strugnell RA, Dry IB, Wesselingh SL. Measles virus hemagglutinin protein expressed in transgenic lettuce induces neutralizing antibodies in mice following mucosal vaccination. Vaccine. 2006;24(17):3538-3544.

128. Chan HT, Chia MY, Pang VF, Jeng CR, Do YY, Huang PL. Oral immunogenicity of porcine reproductive and respiratory syndrome virus antigen expressed in transgenic banana. Plant Biotechnol J. 2013;11(3):315-324.

129. Park KY, Wi SJ. Potential of plants to produce recombinant protein products. J Plant Sci. 2016; 59:559-568.