

# The Strategy for High Amount of Recombinant Protein Purification Guided to GST-Tag Affinity Chromatography: A Review

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## 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 widely 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<sub>2</sub> - 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 etc.

**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 has 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 field (Figure 1), [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

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**Received:** 16 October, 2023, Manuscript No. jbabm-23-116852; **Editor Assigned:** 18 October, 2023, PreQC No. P-116852; **Reviewed:** 01 November, 2023, QC No. Q-116852; **Revised:** 07 November, 2023, Manuscript No. R-116852; **Published:** 15 November 2023, DOI: 10.37421/1948-593X.2023.15.399

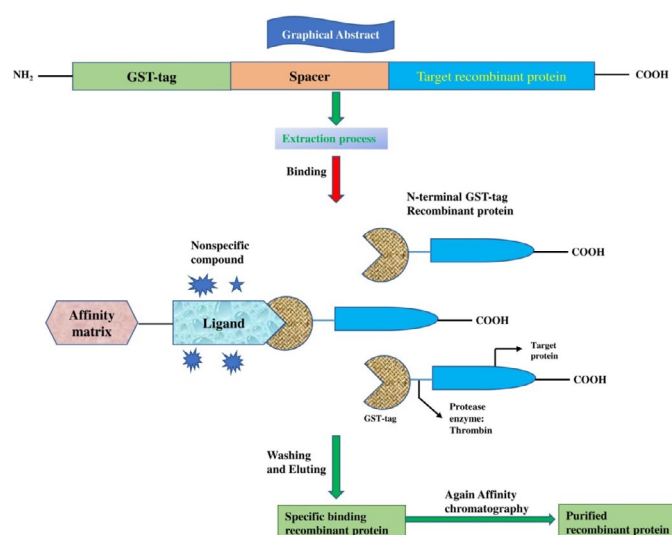


Figure 1. Graphical abstract.

agarose beads and promotes the solubility of fusion protein which expressed in *Escherichia coli* (*E.coli*) [4]. The molecular weight of GST is naturally occurring 26000da/26kda found in eukaryotic cells and the source, organisms and function of GST are showed in (Table 1). The gene from the *S.japonicum* was utilized within the advancement of commercially available vectors such as pGEX (GE Healthcare) or pET (Novagen) [5-16]. Those vectors were utilized for expression to help of recombinant protein purification. The 26000da/26kda GST-tag ties with high affinity to glutathione (GSH) linked to Sepharose

**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	<i>Sogatella furcifera</i>	MIgSTU	Haloalkane Conjugation	[6]
3	Delta	Insects	<i>Periplaneta americana</i>	adGSTD4-4	Insecticide resistance	[7]
4	Epsilon	Insects	<i>Nilaparvata lugens</i>	DmGSTE6	Insecticide resistance	[8]
5	Theta	Bacteria	<i>Schistosoma japonica</i>	HIV gp41 epitope	Haloalkane	[9]
6	Zeta	Mammalian and Bacteria	Hepatocytes	Maleylacetoacetate isomerase	Melelypyruvate isomerases tetrachloro 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	<i>Arabidopsis thaliana</i>	S-hexylGSH	Anthocyanin synthesis	[15]

resin. This type of linking is reversible and after adding reduced glutathione to the elution buffer, protein can be eluted as a non-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 endoprotease 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 [17], DNA-protein interaction [18], immunological studies [19], crystallography structure [20], and protein-protein interaction through GST pull-down assay [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 2). 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]. 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 and first time this study estimated the 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 (Table-3) proved the novelty of this manuscript.

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.

### Affinity tags and GST-Tag properties

Recombinant protein expression perform in *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 has 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 3). 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 *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 general, development of genes is expressed that presently cloned from cDNA which carries information of genome into vector such as pGEX or pET (Figure 4-I). This strategy was utilized by some researcher who developed a cDNA expression library expressed in *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 were 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

**Table 2.** Some expression plasmid used by GST-tag affinity chromatography.

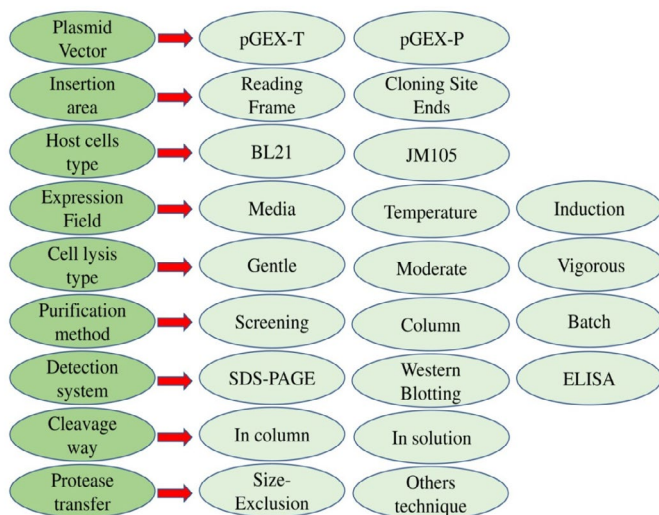
Region of GST-tag	Protein	Sequence of bp which inserted	Vector	Restriction site	Desired molecular weight of recombinant protein (kDa)	Reference
N-terminal GST	BAK	636	pCold-GST	EcoRI	53	[55]
N-terminal GST	ESP1	234	pGEX 6P-3 PRESAT	-	36	[56]
N-terminal Hexa-His and GST	OSBP	1173	pCold-GST	XhoI, Hind III	75	[57]
N-terminal Hexa-His and GST	FD	429	pCold-GST	NdeI, EcoRI	45	[58]
N-terminal GST	HY2	858	pCold-GST	XhoI, PstI	63	[59]
N-terminal GST	pHtrII	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	XhoI, NdeI	47	[62]
N-terminal GST	FT	525	pCold-GST	XhoI, PstI	49	[63]
N-terminal Hexa-His and GST	NHE1	126	pET200 D-TOPO	PstI, NdeI	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, XhoI	34	[67]
N-terminal Hexa-His and GST	PLB1	1818	pGEX-GST	EcoRI, XhoI	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 smal.	20.7	[74]
N-terminal GST	XYL-6	74	pPicH	XhoI, Hind III	47	[75]
N-terminal GST	XynG1	725	pNAN8142	Scal-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]

**Table 3.** 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 musculus	Pur- $\alpha$	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 musculus	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]

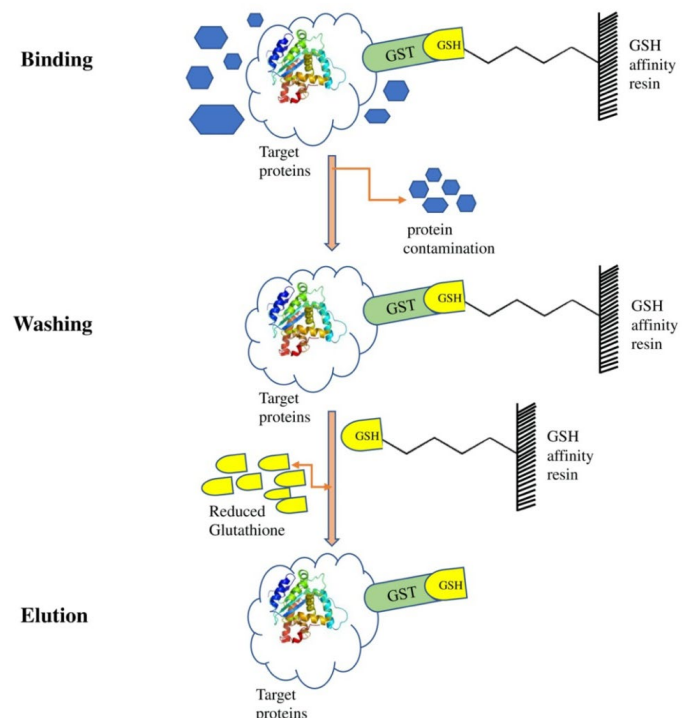
Eukaryotic cells	$\beta$ -tubulin	Full length	43	81	Microtubules are one of the major components of the cytoskeleton, and perform structural support	[100]
Plant cells	2-cys Prx	Full length	22	87	Works on peroxide-mediated inactivation, in hydrogen peroxide signaling pathway	[101]
Eukaryotic cells	NOS	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	94	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]

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



**Figure 2.** Stream graph outlining the indicator judgement-forming 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 to purify GST-tag recombinant protein, cleavage with protease inhibitor enzyme, used for further chromatography for removal of GST-tag to purify target protein.

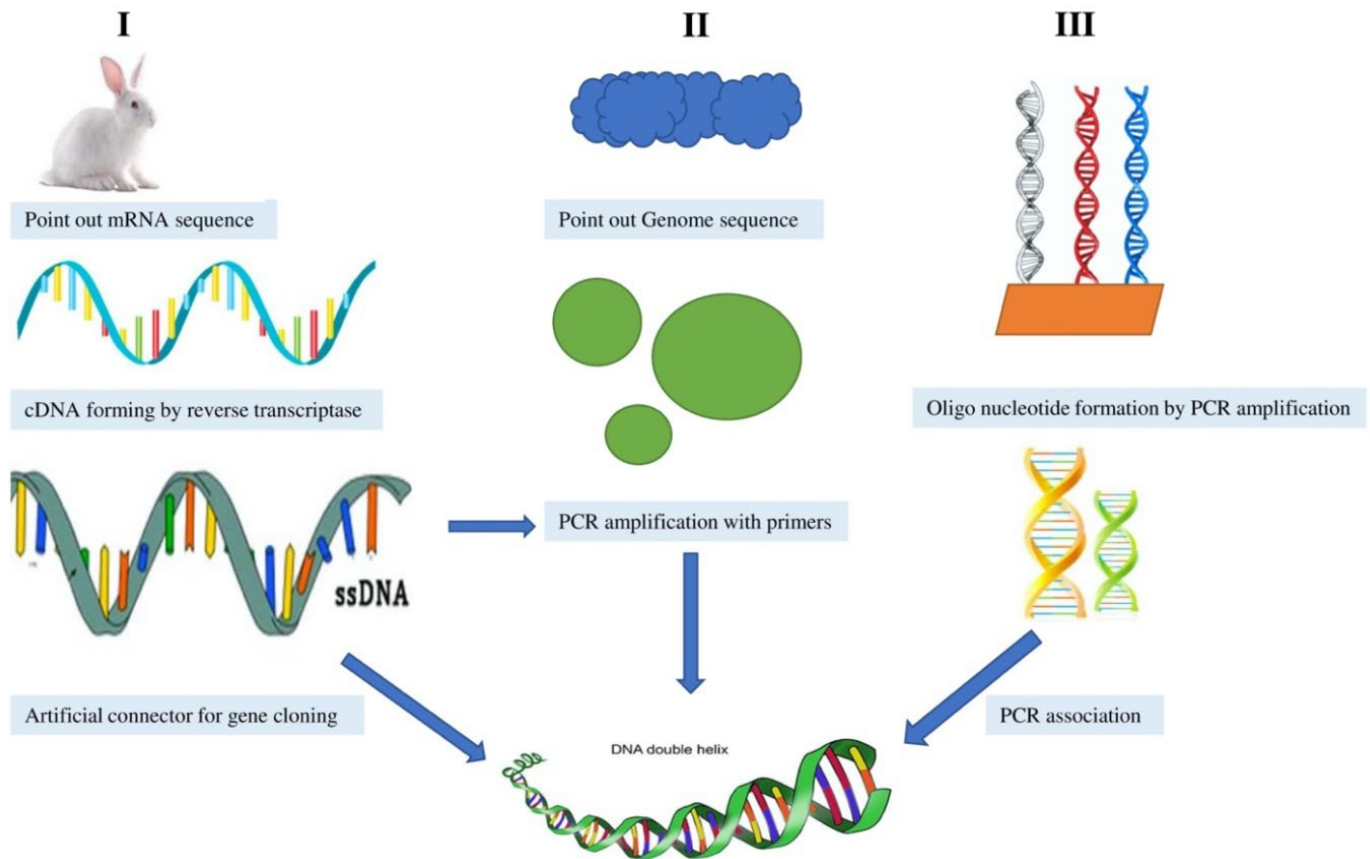
mammalian cells, there are some faults to the conformation of expression libraries. To the beginning the approach of non-translocate regions at both ends of clone for attaching fusion tag become critical situation. Another fault, although the method is difficult genes of interest must be habitually angled 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 4-II).



**Figure 3.** 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).

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





**Figure 4.** For making target genes it needs three types of different technique. (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 oligos can be employ to produce customized genes by the application of array-based oligonucleotide manufacture.

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 4-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 have 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 25-37°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.

### High efficiency cloning the GST fusion protein

Now a 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

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 are makes extremely uncommon around 100 base pairs. The recombination area prepares to be cloned by insert DNA without anxiety of obstructing the coding sequence. Recombinase-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 perform high efficiencies to succeed for all clone, adding cost and time through the project [54].

### 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 2). 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 altered protein. A large strength behind the improvement of the GST fusion system is that the GST protein amass in the cytoplasm and for a large size affinity tag solubility of GST moiety would be reproduced [55-79]. Production of GST recombinant protein which expressed in *E. coli* and perform manufacture of protein around 40mg/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 A<sub>600</sub> 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 [80]. Procedure of affinity chromatography is modifying assembled of glutathione Sepharose column using a roller/tube pump to control flow rates. Phenyl Methyl Sulphonyl 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 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 [81]. 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 [82]. 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 glutathion 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 [83]. 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.

### Application of GST-tag affinity chromatography in plants sciences

Plants have been utilized as a 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 [84-115] and biopolymers [116], 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 [117]. Plants recombinant proteins stages were at first ruled by rural crops with built up generation foundation such as tobacco, corn, rice including vegetables [118]. A recent article reported that in plant molecular technique proposal has a handsome assembled pathway in short time that can achieve commercially acceptable recombinant protein production by GST-tag affinity chromatography [119]. 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 [120]. 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 [121]. Now a 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, transcriptomics, and metabolomics. In addition, as these plants are vulnerable to hereditary change, completely optimized conventions for recovery and development for perform of many important experiment and identify new idea is created for them [122]. 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 are 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 [123].

### 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 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 [124, 125]. 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 [126]. 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 chelators 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, barley) has been investigated [127–130].

### 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 are presently continue 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, biofertilizer, 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 analysis.

### Article highlights

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 (Table-3) proved the novelty of this manuscript. 2nd, This research contributes for the 1st time to better understanding of the reason behind such unique summaries the purification results which collected around 300 research articles. 3rd, the purification data proved that by the utilization of GST-tag affinity chromatography technique is high amount purification of recombinant protein compare with others tag affinity chromatography. Finally, by establishing the strong influence of affinity chromatography GST-tag affinity chromatography technique will be avenues for future research in protein chemistry. 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. This article is the first time evaluated the abridge experimental data about GST-tag affinity chromatography technique.

### 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 published 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.

## Conflict of interest

The authors declare that they have no conflict of interest. All the authors read and approved the final manuscript.

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**How to cite this article:** A Asaduzaman M, Nahar L, Lijon M, Imran S, et al. "The Strategy for high amount of recombinant protein purification guided to GST-tag affinity chromatography: A Review." *J Bioanal Biomed* 15 (2023): 399.