INTRODUCTION

Recombinant protein production in bacteria started with cytoplasmic expression and gained a lot of interest by industries to manufacture protein that replicates original features. To achieve high requirements of industry, researchers induced protein leading to high-level expression in the cytoplasm forming inclusion bodies. Though cytoplasmic expression is majorly known and used, there are a lot of problems with it including (i) inactive protein, (ii) high contamination, (iii) complex purification and (iv) high protease activity in cytoplasm. While Escherichia coli has capability to over express recombinant protein in the cytoplasm, it is also found that high-level secretion of target proteins is possible in Escherichia coli. Periplasmic expression has opened doors for researchers to find middle ground among intracellular and extracellular expression. Advantages of recombinant protein expression in bacterial periplasm are not new, (i) N-terminal authenticity, (ii) eases downstream processes, (iii) good protein stability, (iv) better biological activity and (v) increased solubility are some of them. Escherichia coli periplasm is unique in many ways. Escherichia coli periplasm contains around 100 proteins that constitutes only 4% of the total protein, which makes recovery of a recombinant gene product simpler as secretion strategy minimises the contamination from host proteins. It also protects recombinant proteins from cytoplasmic proteases as there is very less protease activity in the periplasm. Oxidative environment of periplasmic space contains disulphide-binding proteins (DsbA-D) and peptideyl-prolyl cis-trans isomerases like SurA, RotA, FkB, and FkpA. This group of enzymes participate to promote the correct folding of thiol-containing proteins.

Regardless of these advantages, recombinant protein secretion in periplasm is a complex process and secretion of protein encounters many problems. The most persistent problems are (i) incomplete translocation, (ii) little or undetectable amounts of protein secretion, (iii) variable secretion efficiency.
depending on the nature of the proteins and (iv) insufficient capacity of the export machinery 18.

Several methods have been developed to improve protein secretion in bacteria, one of the solutions to deal with these problems is to work on modifying signal peptide until it is optimized to give better results 18. Improved translocation and accumulation of periplasmic protein could be accomplished by optimizing traditional signal peptides 19 and by comparing multiple signal peptides 11.

**Signal Peptide (SP)**

Signal peptide helps to translocate protein to either periplasm or outer medium. In bacteria, signal peptides are usually consist of a positively charged amino terminus N-domain, a central nonpolar hydrophobic H-domain, and a polar cleavage C-domain that is the cutting site for the signal peptidase. N-domain of Signal peptide is tentatively short from 2-10 amino acids, followed by H-domain, which is about 7-20 amino acids long and finally 3-9 amino acids long C-domain. Signal peptide is removed by signal peptidase during translocation of protein into periplasm giving rise to mature protein products 18.

While there are various traditionally used signal peptides, Table 1 contains list of most common signal peptides with their amino acid and DNA sequences. The Signal Peptide Website contains information on signal sequences and signal peptides (www.signalpeptide.de). [More information of various signal peptides can be found on this website].

The most important part of periplasmic protein expression is selection of appropriate signal peptide 12. Each domain of signal peptide plays an essential role in translocation. Understanding role of each domain and improving these regions have resulted in better translocation. It is observed that the positive charge on N-domain helps to target preprotein to the translocase and binding to the negatively charged surface of the membrane lipid bilayer. Increasing the positive charge of N-domain, hydrophobicity of the H-domain and cleavage efficiency of C-domain have resulted in increasing translocation rates 18. Hence, signal peptide selection is the key element of process design.

**Fig 1** Signal peptide with domains.

**Table 1** Commonly used traditional signal peptides with amino acid and DNA sequences

<table>
<thead>
<tr>
<th>SP</th>
<th>Length</th>
<th>Amino acid Sequence</th>
<th>DNA Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>OmpA</td>
<td>21</td>
<td>MKKVDIAVALGGFATV72</td>
<td>ATG AAA AAG AGA GCA ATC ATC CCG ATT GCA GTC GCA CTG CCT GCT TCT TCT GCT GAC GCC ACC ATT GGC CAG GCC</td>
</tr>
<tr>
<td>PelB</td>
<td>22</td>
<td>MKT27TAAAGGLLLAMQ29</td>
<td>ATG AAA AAG AGA GCA ATC ATC CCG ATT GCA GTC GCA CTG CCT GCT TCT TCT GCT GAC GCC ACC ATT GGC CAG GCC</td>
</tr>
<tr>
<td>DsbA</td>
<td>19</td>
<td>AGYLFLSA</td>
<td>ATG AAA AAG AGA GCA ATC ATC CCG ATT GCA GTC GCA CTG CCT GCT TCT TCT GCT GAC GCC ACC ATT GGC CAG GCC</td>
</tr>
<tr>
<td>MalE</td>
<td>26</td>
<td>MKKGVAILALEnteritidis floridensis</td>
<td>ATG AAA AAG AGA GCA ATC ATC CCG ATT GCA GTC GCA CTG CCT GCT TCT TCT GCT GAC GCC ACC ATT GGC CAG GCC</td>
</tr>
<tr>
<td>TorA</td>
<td>39</td>
<td>MNNCLDLFLQ39</td>
<td>ATG AAA AAG AGA GCA ATC ATC CCG ATT GCA GTC GCA CTG CCT GCT TCT TCT GCT GAC GCC ACC ATT GGC CAG GCC</td>
</tr>
</tbody>
</table>

*Region in (i) bold - N-domain, (ii) italic - H-domain and (iii) underline - C-domain.

**Need for Signal Peptide optimization**

As Escherichia coli periplasm contains fewer proteins, the bacteria do not require to transfer more proteins to its periplasm. Thus, it is found out that Signal peptide of Escherichia coli has the highest frequency of non-optimodal codons resulting in translational pausing at an early stage in the export of proteins 13.

The more frequent occurrence of rare codons in the signal peptide play key part in controlling speed of translation and protein folding 15. Expression of periplasmic protein is inhibited by sequence in the early coding signal peptide region 14. Codon usage at the beginning of the translation i.e. at 5’ end can affect translation initiation rate. Different start codons lead to different levels of protein concentration, highest to lowest from AUG to GUG to UUG 16. The mRNA encoding structure of signal peptide also affects the rate of translation and can cause translational pausing, which proves that sequence of signal peptide influence protein translation and translocation by many mechanisms 15. Signal peptides have impact on the biosynthesis, stability and folding kinetics 17. Hence, amino acid sequence of the Signal peptide of the target protein is essential for high secretion.

Signal peptides play a more critical role in expression of recombinant protein than thought earlier 18, optimization becomes necessary to replace non-optimized codons in order to get (i) right mRNA encoding structure, (ii) quality biosynthesis (iii) fast and high translocation, (iv) good protein stability, and (v) high protein accumulation.

**Optimization**

While traditional signal peptides are capable of translocating some amount of recombinant protein into the periplasm, this amount may not be sufficient as per industrial requirements. In addition, little amount of protein is lost during protein isolation and purification process leading to even smaller amounts of final protein. Selection of better signal peptide and its optimization for improved translocation of recombinant proteins to the periplasm has been the topic of interest for many researchers 19,20.

Studies showed that codon-optimization 14, mutagenesis 21,22 and mixing regions of established signal peptides 15 can be a
practical solution for optimization. In some of the studies, high-level production is achieved through genetic optimizations. At few circumstances, non-optimal codons in a signal peptide are found to be needed for the folding of the mature protein, however for improving the periplasmic expression optimal codons may be beneficial.

Optimization can be required at multiple regions of the Single peptide. Combined optimization can be advantageous to accomplish target protein efficient secretion. There are various examples where optimization has led to improvement at different levels of expression, such as protein translocation, mRNA secondary structure, recombinant protein secretion, cell viability and achieving LC:HC balance.

**Codon Optimization**

Need for codon optimization started as understanding of the role of signal peptide in recombinant protein secretion increased. Profound study on native signal peptides reveal that there is a lot of improvement required at codon level. Maximum occurrences of rare codons are found in the signal peptides of secretory proteins in *Escherichia coli*. To reduce translational pausing caused by presence of non-optimal codons and to tackle the problem of inefficient translocation across the inner membrane, codon optimization is necessary. It is observed that codon usage in the signal peptide coding region is not only critical to obtaining good levels of expression but it is also important to enhance activity of heterologous protein.

The first step in codon optimization is to search for non-optimal codons present in the concerned signal peptide. These non-optimal codons are then replaced with optimal codons as per codon usage of the host cell. For the purpose of codon optimization, Codon Adaptation Index (CAI), the tRNA Adaptation Index (tAI), and the ribosomal overhead cost formulation of the stochastic evolutionary model of protein production rates (ROC-SEMMPR) have been used.

**Mutagenesis**

Replacing non-optimal codons with optimal codons at times are not enough for improvement of signal peptide to its maximum potential. In such cases, changing one or more amino acids with the help of mutagenesis can be beneficial. Mutagenesis can be site-directed based on the characteristics of original sequence or completely random. Modification with mutagenesis is better alternative to increase secretion instead of changing complete signal peptides. Use of random mutagenesis followed by screening and selection, was initially developed to advance enzymes.

Mutagenesis is performed at the various regions of the signal peptide. As coding region at first position can be inhibiting, silent base mutations at this region caused a large increase of the protein level in the periplasm. Li et al. performed site-directed and site-saturation mutagenesis to find out charge distribution in the N-domain of the signal peptide was more important than the net charge. By combinatorial mutagenesis approach, consensus signal peptide (CSP) mutant libraries have been generated and it is shown that the mutagenesis can be focused to specific genetic region by using synthetic oligonucleotide mixtures.

For secretion of *Cyclodextrin glucanotransferase* (CGTase), site directed mutagenesis has been used in several cases, to optimize G1 signal peptide from Bacillus sp. and L-asparaginase II signal peptide. These improved signal peptides have resulted in the better secretion of recombinant GTase. In all three cases, mutagenesis were done on H-domain of the signal peptides to either decrease hydrophobicity in the first case and to increase hydrophobicity in the cases of rest two.

Using codon-based mutagenesis of the signal peptide-coding region, an attempt was made to optimize eukaryotic signal peptides and surprisingly these signal peptides worked very efficiently in *Escherichia coli*. Swiping regions/domains

Swiping or mixing the regions or domains of the well-characterised signal peptides can give rise to advance version of the signal peptide. In one such effort first codons of the cpxP gene encoding the signal peptide was replaced by MalE and DsbA signal peptides, leading to increase of the CpxP level in the periplasm. In another studies, Han et al. have developed arials of novel signal peptides by swapping individual domains and Leyun Yang et al. created a new synthetic signal peptide OmpA’ using N-domain of OmpA and H-domain of PelB.

**Software based optimization**

A number of softwares have been developed and employed to study, optimize and design signal peptides. Softwares such as TMHMM and Signa IP can be used to predict protein localization. The computational prediction of signal peptides and the cleavage site can be done by a neural net-work and hidden Markov model. The mRNA secondary structure can be studied using the mfold program. Ghahremanifard et al. designed and constructed a suitable signal peptide for secretion of lipase using a newly developed software and used response surface methodology (RSM) to improve secretion.

**Synthetic Signal peptides**

By analysing and comparing various existing signal peptides, researchers have developed new synthetic signal peptides instead of optimizing existing one and resulted products have shown increase in secretion. Construction of these de novo signal peptides is done by analysing most frequent amino acids at respective positions of signal peptides. Jeirankhahmeh et al. designed two new signal peptides of 24 and 29 amino acids with change in their H-domains lengths by understanding key features of class I sec-type signal peptide and the N-terminal sequence of mature hGH. Synthetically made signal peptides have also been employed to understand how proteins are targeted to protein machinery to facilitate translocation across membrane.

**Region**

Optimization starts with selecting region to modify. Most of modifications has been done on the regions that play key roles in secretion. Most of the modifications in the N-domain are...
done to replace biased codons and to increase positive charge\textsuperscript{[16,35]}, most of the modifications in the H-domain are done to increase or decrease hydrophobicity\textsuperscript{[19,23]} and most of the modifications in the C-domain are done at cleavage junction for efficient cutting\textsuperscript{[23,36]}. Table 2A, 2B and 2C contains information of region specific optimization studies performed on N, H and C domain respectively. Region specific modification of signal peptide have facilitated the expression of a functional antibody in both prokaryotic and eukaryotic cells\textsuperscript{[39]}.

**Optimization effect on mRNA**

It is observed that while producing recombinant protein in the periplasm, mRNA secondary structure, specifically in the N-terminal region, contribute in protein secretion and it is suggested that effect on mRNA secondary structure should be considered while optimizing signal peptide\textsuperscript{[9,23]}. Production of recombinant protein relies on translation and stability of mRNA, as RNAases activity varies with respect to protection through ribosomes, RNA folding and polyadenylation\textsuperscript{[6]}. When mRNA secondary structure predicted using mfold program and Vienna RNA secondary structure prediction program, different results could be seen for different signal peptides\textsuperscript{[23]}. Target protein with different signal peptide procedure mRNA transcript variants, with different secondary structure and stability, affecting the final concentration of recombinant preprotein\textsuperscript{[17]}.

<table>
<thead>
<tr>
<th>Table 2A Optimization analysis of N-domain</th>
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<tbody>
<tr>
<td>Optimization/region</td>
</tr>
<tr>
<td>MKK\textsuperscript{3} sequence in n N-domain</td>
</tr>
<tr>
<td>Each region of the PelB signal peptide mutated separately</td>
</tr>
<tr>
<td>Mutation at first codons of the cpxP gene encoding the SP with PelB and MalE SP</td>
</tr>
<tr>
<td>Charged residues in the N-domain</td>
</tr>
<tr>
<td>Positive charge on the N-terminal region</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2B Optimization analysis of H-domain</th>
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<tbody>
<tr>
<td>Optimization/region</td>
</tr>
<tr>
<td>Helix-breaking residue at the centre of H-domain</td>
</tr>
<tr>
<td>Fifth leucine position of the PelB</td>
</tr>
<tr>
<td>Optimization of signal peptide H-domain</td>
</tr>
<tr>
<td>Introduction of a G-turn motif or helix breaker in the H-domain</td>
</tr>
<tr>
<td>Polar amino acids in the middle of the signal peptide were replaced with large hydrophobic residues</td>
</tr>
<tr>
<td>Hydrophobicity of the central hydrophobic region</td>
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<table>
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<tr>
<th>Table 2C Optimization analysis of C-domain</th>
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<tbody>
<tr>
<td>Optimization/region</td>
</tr>
<tr>
<td>The cleavage site, in particular positions -3 and -1</td>
</tr>
<tr>
<td>Optimization of signal peptide cleavage site</td>
</tr>
<tr>
<td>Signal peptidase recognition site</td>
</tr>
<tr>
<td>Alanine residue in the C-domain of the signal peptide</td>
</tr>
<tr>
<td>Changing Serine to Alanine at two positions of C-domain</td>
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<tr>
<td>The secondary structure at the cleavage junction</td>
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</table>

**Comparative studies**

Signal peptides can be particular. There is no guarantee that one signal peptide optimized for expression of particular protein in *Escherichia coli* will translate for expression of another protein. For each protein, signal peptide need to be optimized as it is difficult to guess best signal peptide for target protein\textsuperscript{[17]}. One of the solutions to find the best signal peptide for targeted protein is to carry out comparative studies using multiple signal peptides. It is not possible to select better signal peptide for target protein using in silico tools. To try multiple signal peptides is one approach used by many\textsuperscript{[44]}. It is observed that, when scFv BL1 and hGH were expressed in periplasm with four signal peptides, yields for both targets significantly varied depending on the signal peptide\textsuperscript{[44]}. In another such study, pelB and ompA signal peptides were compared with consensus signal peptide mutants and results show that translocation efficiency and protein expression level changes as per signal peptide\textsuperscript{[18]}.

Comparative studies shows benefit of particular signal peptide over others\textsuperscript{[45,46]} and helps to find out better signal peptide for stability, folding\textsuperscript{[47]}, function and solubility\textsuperscript{[46]} of target protein. Selas Castiñeiras et al.\textsuperscript{[14]} developed a screening system for optimising signal peptides for translocation.

Comparative studies carried out on signal peptides are listed in Table 3.

**Libraries and Screening system**

Analysing small number of signal peptides can be useful but sometimes not enough to find probable candidate for better secretion. Also, analysis of short number of signal peptides is time consuming and yields low output\textsuperscript{[45]}. Performing comparative studies by developing libraries and screening methods can be effective for analysing more signal peptides. Many attempts to develop such screening systems to select better-quality signal peptides have been successful\textsuperscript{[10,14]}. For improved periplasmic yield, construction of signal peptide libraries can be useful technique\textsuperscript{[55,57]}. Generation of libraries using alkaline phosphatase as a reporter enzyme is common\textsuperscript{[10,38]}. Signal peptide libraries can be generated by using
error-prone PCR and screened by performing activity assay of tagged protein. Using bicistronic construct, codon usage was modulated and PelB signal peptide libraries were created by mutating each domain (N-domain, H-domain and C-domain) separately. Brockmeier et al. initially developed a systematic screening study to analyze the effects of signal peptide variation on the heterologous proteins secretory production. Optimal secretion of heterologous proteins secretory production is a critical factor for the success of expression in Gram-positive bacteria. A high and stable expression level is observed by signal peptide screenings which has been reported in several studies. However, no increase in expression level by signal peptide screenings was also observed for Gram-negative bacteria.

### Translocation pathway

Selection of particular signal peptide results in selection of pathway of translocation as protein translocation process differs according to amino acid sequence of the signal peptide. Bacterial protein can reach periplasm by three major pathways; SecB-dependent pathway, signal recognition particle (SRP) pathway and Twin Arginine Translocation (TAT) pathway. For SecB-dependent and SRP pathways, protein translocation and translation occurs simultaneously, while for TAT pathway, protein translocation occurs after translation. All three pathways are equally explored but first two pathways are more commonly used for expression of recombinant protein.

<table>
<thead>
<tr>
<th>Signal Peptides</th>
<th>Number of SP compared</th>
<th>Protein used for expression</th>
<th>Method used for cloning SP</th>
<th>Method used for comparison</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>hPD1, mBlP, mLk</td>
<td>6 scfV and Fab’</td>
<td>Codon-based mutagenesis</td>
<td>scfV and Fab’ yield</td>
<td>High levels of scfV and Fab’ secretion achieved with codon optimized signal peptides</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>OmpA, OmpA’</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PeB, MalE, OmpA, TorA, DsbA, FluD, YcdO and MdcD</td>
<td>8 Malhogenic amylase</td>
<td>Coding codon-optimized signal peptides into the vector</td>
<td>MA activity assay</td>
<td>MA yields increased 18- and 50-fold with codon optimized signal peptides</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>PelB and DsbA</td>
<td>2 scfV 13R4</td>
<td>Two separate plasmids for PelB and DsbA</td>
<td>scfV concentration and solubility</td>
<td>PelB gives better results compared to DsbA in terms of scfV solubility and cell physiology</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>HlyA, TorA, GenEll, and PelB</td>
<td>4 GFP</td>
<td>BioBrick and BioFusion</td>
<td>GFP translocation and fluorescence</td>
<td>PelB signal peptide was inefficient in translocating GFP</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>STII, DsbA and PelB</td>
<td>3 scfV bound to TEM1 β lactamase</td>
<td>Chemically synthesised signal peptide library</td>
<td>β-lactamase activity assay</td>
<td>Improved signal peptides increased periplasmic scfV activity by ~40%</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>OmpA, OmpA’, PelB, OsmY, DsbA, MalE, TorA</td>
<td>7 PLD</td>
<td>Construction of recombinant PLD Plasmids</td>
<td>PLD extracellular expression</td>
<td>The mutant SP resulted in 110 and 94% increases in periplasmic and extracellular recombinant CGTase, respectively, compared to the wild-type SP</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>DsbA, Hbp, OmpA, and PhoA</td>
<td>4 scfV BL1 and hGH</td>
<td>Forward primers containing the signal peptide coding sequence</td>
<td>BL1 and hGH activity assay</td>
<td>Three alpha-amylase derived signal peptides processed partially and two modified BLA-derived signal peptides could increase the hGH precursor in the cytoplasm, but not in periplasm compared to the parental clone</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>PeB, OmpA, YcdO, FluD, MdcD and DsbA</td>
<td>6 BJ-10 lipase</td>
<td>Construction of recombinant plasmid pET-SigPFL</td>
<td>Relative expression levels and Lipase activity</td>
<td>OmpA’ was an efficient signal peptide to secrete the recombinant enzyme out of the cell</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>DsbA and MalE</td>
<td>2 LCI</td>
<td>Overlap extension PCR</td>
<td>Export efficiency and production of LCI</td>
<td>Highest periplasmic production yields for BL1 and hGH were achieved with the OmpA and Hbp respectively DsbA significantly decreased the formation of inclusion bodies and enhanced the function and solubility of lipBJ10</td>
<td>18</td>
<td></td>
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<tr>
<td>OmpA to DsbA</td>
<td>2 Fab’ co-expression with Staph. nuclease</td>
<td>Dicistronic expression cassette in plasmid</td>
<td>Growth performance and Fab’ levels</td>
<td>Better export and production obtained with DsbA DsbA-fused Staphylococcal nuclease co-produced with reduced Fab’ leakage relative to the original autotinolytic Fab’ strain with OmpA fused staphylococcal nuclease.</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>CgtS, PelB and DacD</td>
<td>3 CGTase</td>
<td>Codon-optimized PCR primers and synthesized SP</td>
<td>Enzyme activity and, extra- and intracellular yield of CGTases Gal2 scfV processing and yield</td>
<td>DacD increases activity of CGTase</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>OpeF, Pfp, PorE, Aza, Lip, Bp and Pfp</td>
<td>7 Gal2 scFv</td>
<td>PCR amplification</td>
<td>PCR yield varied from about 1 g/l to 10 g/l</td>
<td>PelB and CusF showed considerable increase in protein levels compared to wild type SmbP signal peptide</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>SmbP, CusF, PelB and TorA</td>
<td>4 RFP and GFP</td>
<td>PCR primers with signal peptide nucleotide sequence</td>
<td>Quantification of RFP and GFP</td>
<td>Better export and production obtained with DsbA DsbA-fused Staphylococcal nuclease co-produced with reduced Fab’ leakage relative to the original autotinolytic Fab’ strain with OmpA fused staphylococcal nuclease.</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3** Comparative studies on Signal peptides (SP).
Signal peptide interacts differently with components of the three pathways depending on its sequence and its route changes accordingly. Optimization of specific region of signal peptides can result in change in protein translocation pathway. It is reported that the pathway of the secretion of pre-secretory proteins could be switched by increasing the hydrophobicity of signal peptide. It is not fixed that all protein molecules will follow only one pathway of translocation. If designated pathway of signal peptide reaches its maximum ability, to rescue protein translocation, an alternative pathway would be used depending on the compatibility of signal peptide.

**CONCLUSION**

Escherichia coli is the first choice for recombinant protein expression owing to its short life cycle, simple culturing, less growth requirements, well studied genetics and simple genetic manipulation. Through the years of research, it has been clear that Escherichia coli is capable of expressing protein in cytoplasm, periplasm and medium. Target protein localization depends on many factors including required concentration, purity, activity etc.

Protein localization in all three compartments have their advantages and disadvantages, and selection of any compartment requires further optimization and standardization. When protein is required in active form with proper confirmation, periplasm or medium expression is chosen.

Selection of Signal peptide seems to play a bigger part for efficient secretion while periplasm protein expression. Modification of signal peptides by optimizing specific region have worked surprisingly well to increase accumulation by many folds. Comparative studies using multiple signal peptides provide extra support for deciding most proficient signal peptide in large pool. In this article, we review optimization and comparative studies done on Signal peptides, and some screening techniques that can be useful for finding better Signal peptide for periplasmic protein expression in Gram-negative bacteria, mainly Escherichia coli.

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