SUMMARY ~~OF THE INVENTION~~

The ~~invention~~disclosure provides a novel means for increasing production of heterologous proteins comprising use of novel translational initiation region (TIR) variants, including TIR variants comprising co-translational secretion signal peptides (signal peptides that direct translocation in a co-translational manner) and/or TIR variants comprising post-translational secretion signal peptides (signal peptides that direct translocation in a post-translational manner). In addition, demonstrated herein is increased antibody production using vectors comprising antibody light chain operably linked to a TIR comprising a co- or post-translational secretion signal peptide and an antibody heavy chain operably linked to a TIR comprising a co-translational secretion signal peptide for peak expression. Novel TIR variants are also provided herein.

In one aspect, there is provided a method of making an antibody, said method comprising culturing a host cell comprising polynucleotide comprising (1) a first translation initiation region (TIR) operably linked to a polynucleotide encoding an antibody heavy chain, wherein the TIR comprises a DsbA variant co-translation prokaryotic secretion signal sequence; and (2) a second TIR operably linked to a polynucleotide encoding an antibody light chain, wherein the second TIR comprises a co-translation or post-translation prokaryotic secretion signal sequence, whereby upon expression of the antibody in a host cell, the heavy and light chains are folded and assembled to form a biologically active antibody.

In another aspect, there is provided a polynucleotide comprising (1) a TIR operably linked to a polynucleotide encoding an antibody heavy chain, wherein the TIR comprises a DsbA variant co-translation prokaryotic secretion signal sequence; and (2) a second TIR operably linked to a polynucleotide encoding an antibody light chain, wherein the second TIR comprises a co-translation or post-translation prokaryotic secretion signal sequence, whereby upon expression of the antibody in a host cell, the heavy and light chains are folded and assembled to form a biologically active antibody.

In yet another aspect, there is provided a host cell comprising the polynucleotide as defined above.

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides variant translation initiation regions. In some embodiments, the variant comprises a variant translation initiation region (in some embodiments, a prokaryotic post-translational secretion signal sequence or a prokaryotic co-translational secretion signal sequence). In some embodiments, the variant comprises nucleic acid variants of a secretion signal sequence, such as PhoA, MalE, DsbA or STII. In some embodiments, the variant further comprises a *MlaI*, *BssHII*, or *XbaI* restriction site. In some embodiments, the variant comprises a translation initiation region variant comprising a sequence shown in Table 2.

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides variant secretion signal sequences. In some embodiments, the secretion signal sequence is a prokaryotic post-translational secretion signal sequence or a prokaryotic co-translational secretion signal sequence. In some embodiments, the secretion signal sequence is a eukaryotic post-translational secretion signal sequence or a eukaryotic co-translational secretion signal sequence. In some embodiments, the variants are nucleic acid variants of a PhoA, MalE, DsbA or STII secretion signal sequence. In some embodiments, the variants comprise a secretion signal sequence shown in Table 2. The variant secretion signal sequences of the ~~invention~~disclosure are suitable for use, for example, in any of the methods disclosed herein.

In another ~~aspect~~embodiment, the ~~invention~~disclosure provides a polynucleotide comprising a translation initiation region of the ~~invention~~disclosure. In some embodiments, the translation initiation region comprises sequence shown in Table 2 (e.g., one of SEQ ID NOs 1-42). In some embodiments, the translation initiation region comprises one of SEQ ID NOs. 1-14, 16-24,

26-39, 41-42. The polynucleotides are suitable for use, for example, in any of the methods disclosed herein.

In another ~~aspect~~embodiment, the ~~invention~~disclosure provides a polynucleotide comprising a secretion signal sequence of the ~~invention~~disclosure. In some embodiments, the secretion signal sequence comprises sequence shown in Table 2. (e.g., one of SEQ ID NOs 1-42). In some embodiments, the translation initiation region comprises one of SEQ ID NOs. 1-14, 16-24, 26-39, 41-42. The polynucleotides are suitable for use, for example, in any of the methods disclosed herein.

In another ~~aspect~~embodiment, the ~~invention~~disclosure provides a polynucleotide comprising a translation initiation region of the ~~invention~~disclosure operably linked to a polynucleotide encoding a heterologous polypeptide, whereby upon expression of the heterologous polypeptide in a host cell (e.g., a prokaryotic host cell, e.g., an E. coli host cell), the heterologous polypeptide is folded and assembled to form a biologically active heterologous polypeptide. Examples of heterologous polypeptides are further disclosed herein. In some embodiments, the heterologous polypeptide is an antibody heavy chain. In some embodiments, the heterologous polypeptide is an antibody light chain. In some embodiments, the heterologous polypeptide is an Fc polypeptide. In some embodiments, the heterologous polypeptide is a multimeric polypeptide. In some embodiments, the heterologous polypeptide is a heteromultimer. In some embodiments, the translation initiation region is any translation initiation region disclosed herein, e.g., a translation initiation region comprising sequence shown in Table 2. In some embodiments, the translation initiation region comprises sequence of one of SEQ ID NOs 1-42. In some embodiments, the translation initiation region comprises sequence of one of SEQ ID NOs 1-14, 36-39, 41-42. In some embodiments, the translation initiation region comprises a variant STII, DsbI, PhoA, or MalE signal sequence.

In another ~~aspect~~embodiment, the ~~invention~~disclosure provides a polynucleotide comprising (1) a first translation initiation region (TIR) operably linked to a polynucleotide encoding a first heterologous polypeptide, wherein the TIR comprises a co-translation prokaryotic secretion signal sequence; and (2) a second TIR operably linked to a polynucleotide encoding an second heterologous, wherein the second TIR comprises a co-translation or post-translation prokaryotic secretion signal sequence, whereby upon expression of the antibody in a host cell, the first and second heterologous polypeptides are folded and assembled to form a biologically active polypeptide complex.

In another ~~aspect~~embodiment, the ~~invention~~disclosure provides a polynucleotide encoding an antibody, said polynucleotide comprising (1) a first translation initiation region of the ~~invention~~disclosure operably linked to a polynucleotide encoding an antibody heavy chain and (2) a second translation initiation region operably linked to a polynucleotide encoding an antibody light chain, whereby upon expression of the antibody in a host cell (e.g., a prokaryotic host cell, e.g., an E. coli host cell), the heavy and light chains are folded and assembled to form a biologically active antibody.

In some embodiments, the first translation initiation region comprises a co-translational prokaryotic secretion signal sequence (e.g., a signal sequence that directs translation through the signal recognition peptide). In some embodiments, the first translation initiation region comprises a STII or DsbA signal sequence. In some embodiments, the first translation initiation region comprises a DsbA signal sequence. In some embodiments, the first translation initiation region comprises a PhoA or MalE signal sequence. In some embodiments, the first translation initiation region comprises sequence of one of SEQ ID NOs: 1-10 and 36-42. In some embodiments, the first translation initiation region comprises sequence of one of SEQ ID NOs: 1-10 and 36-29 and 41 and42. In some embodiments, the first translation initiation region comprises sequence of one of SEQ ID Nos 1-42. In some embodiments, the first translation initiation region comprises sequence of one of SEQ ID Nos. 1-14, 16-24, 26-39, 41-42.

In some embodiments, the second translation initiation region comprises (i) a co-translational prokaryotic secretion signal sequence or a post-translation prokaryotic secretion signal sequence (e.g., a signal sequence that directs translation through the sec pathway). In some embodiments, the second translation initiation region comprises a STII, DsbA, MalE or PhoA signal sequence. In some embodiments, the second translation initiation region comprises a PhoA or MalE signal sequence. In some embodiments, the second translation initiation region comprises sequence of one of SEQ ID NOs 1-42. In some embodiments, the second translation initiation region comprises sequence of one of SEQ ID NOs 1-14, 16-24, 26-39, 41-42.

In some embodiments, the polynucleotide encoding an antibody further comprises (3) a third translation initiation region operably linked to a polynucleotide encoding a Fc polypeptide. In some embodiments, the third translation initiation region comprises a STII, PhoA or DsbA signal sequence. In some embodiments, the third translation initiation

region comprises a DsbA signal sequence. In some embodiments, the third translation initiation region comprises a PhoA signal sequence.

In another ~~aspect~~embodiment, the ~~invention~~disclosure provides polynucleotide comprising (1) a first translation initiation region (TIR) operably linked to a polynucleotide encoding an antibody heavy chain, wherein the TIR comprises a co-translation prokaryotic secretion signal sequence; and (2) a second TIR operably linked to a polynucleotide encoding an antibody light chain, wherein the second TIR comprises a co-translation or post-translation prokaryotic secretion signal sequence, whereby upon expression of the antibody in a host cell, the heavy and light chains are folded and assembled to form a biologically active antibody.

In another ~~aspect~~embodiment, the ~~invention~~disclosure provides a polynucleotide encoding an antibody fragment (such as a monovalent antibody fragment), said polynucleotide comprising (1) a first translation initiation region of the ~~invention~~disclosure operably linked to a polynucleotide encoding an antibody heavy chain; (2) a second translation initiation region operably linked to a polynucleotide encoding an antibody light chain; and (3) a third translation initiation region operably linked to a polynucleotide encoding a Fc polypeptide, whereby upon expression of the antibody in a host cell (e.g., a prokaryotic host cell), the heavy chain, light chain and Fc polypeptide are folded and assembled to form a biologically active antibody (such as an one-armed antibody). In some embodiments, the third translation initiation region comprises a co-translational prokaryotic secretion signal sequence or a post-translational prokaryotic secretion signal sequence. In some embodiments, the third translation initiation region comprises a STII, PhoA, MalE, or DsbA signal sequence. In some embodiments, the third translation initiation region comprises a DsbA signal sequence. In some embodiments, the third translation initiation region comprises a PhoA signal sequence. In some embodiments, the third translation initiation region comprises sequence of one of SEQ ID Nos 1-42. In some embodiments, the third translation initiation region comprises sequence of one of SEQ ID Nos. 1-14, 16-24, 26-39, 41-42.

In another ~~aspect~~embodiment, the ~~invention~~disclosure provides a polynucleotide encoding an antibody, said polynucleotide comprising (1) a first translation initiation region of the ~~invention~~disclosure operably linked to a polynucleotide encoding an antibody heavy chain, wherein the first translation initiation region comprises a STII or DsbA signal sequence and (2) a second translation initiation region operably linked to a polynucleotide encoding an antibody light

chain, wherein the second translation initiation region comprises a STII, DsbA, MalE or PhoA signal sequence, whereby upon expression of the antibody in a host cell (e.g., a prokaryotic host cell), the light and heavy chains are folded and assembled to form a biologically active antibody. In some embodiments, the first translation initiation region comprises a DsbA signal sequence and the second translation initiation region comprises a MalE or PhoA signal sequence. In some embodiments, the polynucleotide encoding an antibody further comprises (3) a third translation initiation region operably linked to a polynucleotide encoding a Fc polypeptide. In some embodiments, the third translation initiation region comprises a STII, PhoA or DsbA signal sequence. In some embodiments, the third translation initiation region comprises a PhoA signal sequence. In some embodiments, the third translation initiation region comprises a DsbA signal sequence.

In some embodiments, the translational strength of said variant translation initiation region is less than the translational strength of the wild-type translation initiation region. In some embodiments, the translational strength of said variant translation initiation region is greater than the translational strength of the wild-type translation initiation region. In some embodiments, the amino acid sequence of the translation initiation variant is not altered relative to wild-type amino acid sequence. In some embodiments, the amino acid sequence of the translation initiation variant is altered relative to wild-type amino acid sequence. In some embodiments, the translation initiation region includes a prokaryotic secretion signal sequence. In some embodiments, the first and second translational initiation regions (and in some embodiment, the third translational initiation region) provide approximately equal translational strengths. In some embodiments, the relative translation strength is about one or two. In some embodiments the relative translation strength is about one. In some embodiments, the relative translation strength is about two. In some embodiments, the relative translation strength is one and/or two. In some embodiments, the relative translation strength is about three or about four. In some embodiments, the relative translation strength is selected from one or more of one, two, three, four, five, or more (such as six or seven or more).

In some embodiments, the polynucleotide of the ~~invention~~disclosure further comprises a promoter operably linked to the heterologous polypeptide. In some embodiments, the promoter is a prokaryotic promoter selected from the group consisting of phoA, tac, lpp, lac-lpp, lac, ara, trp, and T7 promoter. In some embodiments, the promoter is a phoA promoter. In some embodiments involving expression of antibody heavy and light chain,

the polynucleotide further comprises (a) a first promoter, wherein the first promoter is operably linked to a light chain and (b) a second promoter, wherein the second promoter is operably linked to a heavy chain. In some embodiments, the first and second promoters are both phoA promoters. In some embodiments involving expression of antibody heavy and light chain and Fc polypeptide, the polynucleotide further comprises (c) a third promoter, wherein the third promoter is operably linked to a Fc polypeptide. In some embodiments, the third promoter is a Fc polypeptide.

When expressing polypeptides that comprise more than one polypeptide (e.g., an antibody comprising a heavy chain and light chain), the polynucleotide for expressing the polypeptide may be a polycistronic polynucleotide (ie, a single polynucleotide that contains and expresses multiple cistrons under the regulatory control of a single promoter). A common example of a polycistronic vector is a "dicistronic" vector that contains and expresses two different polypeptides under the control of one promoter. Upon expression of a dicistronic or poycistronic vector, multiple coding regions (eg, genes) are first transcribed as a single transcriptional unit, and then translated separately. A cistron refers to a genetic element broadly equivalent to a translation unit comprising the nucleotide sequence coding for a polypeptide chain and adjacent control regions (including, e.g., a TIR). In other embodiments, the polynucleotide may comprise separate cistrons, which refers to a single polynucleotide comprising at least two separate promoter-citron pairs, wherein each cistron is under the control of its own promoter. Upon expression of a separate cistron expression vector, both transcription and translation processes of different genes are separate and independent. In yet another embodiments, the polynucleotide may comprise a polycistronic portion and a separate cistron portion.

In yet another ~~aspect~~embodiment, the ~~invention~~disclosure provides vectors comprising polynucleotide of the ~~invention~~disclosure. In some embodiments, the vectors are expression vectors.

In a further ~~aspect~~embodiment, the ~~invention~~disclosure provides compositions comprising one or more polynucleotides of the ~~invention~~disclosure and a carrier. In one embodiment, the carrier is pharmaceutically acceptable.

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides host cells comprising polynucleotide or vector of the ~~invention~~disclosure. In some embodiments, the host cells comprise polynucleotide of the ~~invention~~disclosure encoding an antibody (in some embodiments, a bispecific or one-armed antibody). The host cell may comprise one or more polynucleotides collectively encoding the antibody. A vector can be of any type, for example, a recombinant vector such as an

expression vector. Any of a variety of host cells can be used. In one embodiment, a host cell is a prokaryotic cell, for example, *E. coli*. In some embodiments, the *E. coli* is of a strain deficient in endogenous protease activities. In some embodiments, the genotype of the *E. coli* lacks *degP* and *prc* genes and harbors a mutant *spr* gene.

In some embodiments, the host cell further comprises a polynucleotide encoding a prokaryotic chaperone protein (such as Dsb proteins (DsbA, DsbB, DsbC, DsbD, FkpA and/or DsbG). In some embodiments, chaperon protein is overexpressed in the host cell. In some embodiments, the chaperone protein is Dsb A and/or DsbC.

In one ~~aspect~~embodiment, the host cell comprises one or more polynucleotides collectively encoding a one-armed antibody. In one embodiment, a single polynucleotide encodes (a) the light and heavy chain components of the one armed antibody, and (b) the Fc polypeptide. In one embodiment, a single polynucleotide encodes the light chain and Fc polypeptide components of the one armed antibody, and a separate polynucleotide encodes the heavy chain polypeptide. In one embodiment, a single polynucleotide encodes the heavy chain and Fc polypeptide components of the one-armed antibody and a separate polynucleotide encodes the light chain component of the one-armed antibody. In one embodiment, separate polynucleotides encode the light chain component of the one-armed antibody, the heavy chain component of the one-armed antibody and the Fc polypeptide, respectively.

Heterologous polypeptides are described herein. In some embodiments, the heterologous polypeptide is an antibody. In some embodiments, the antibody is a monoclonal antibody. In other embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is selected from the group consisting of a chimeric antibody, an affinity matured antibody, a humanized antibody, and a human antibody. In certain embodiments, the antibody is a bispecific antibody. In certain embodiments, the antibody is an antibody fragment. In some embodiments, the antibody is a monovalent antibody. In some embodiments, the antibody is a Fab, Fab', Fab'-SH, $F(ab')_2$, or scFv. In some embodiments, the antibody is a one-armed antibody (i.e., the heavy chain variable domain and the light chain variable domain form a single antigen binding arm) comprising an Fc region, wherein the Fc region comprises a first and a second Fc polypeptide, wherein the first and second Fc polypeptides are present in a complex and form a Fc region that increases stability of said antibody fragment compared to a Fab molecule comprising said antigen binding arm.

ASTRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYAYPWTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:46); and a third polypeptide comprising a Fc polypeptide, the polypeptide comprising the sequence DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK (SEQ ID NO: 47), wherein the heavy chain variable domain and the light chain variable domain are present as a complex and form a single antigen binding arm, wherein the first and second Fc polypeptides are present in a complex and form a Fc region that increases stability of said antibody fragment compared to a Fab molecule comprising said antigen binding arm.

In one embodiment, the anti-c-met antibody comprises a heavy chain variable domain comprising one or more of CDR1-HC, CDR2-HC and CDR3-HC sequence depicted in Figure 7 (SEQ ID NO:52-53 & 66). In some embodiments, the antibody comprises a light chain variable domain comprising one or more of CDR1-LC, CDR2-LC and CDR3-LC sequence depicted in Figure 7 (SEQ ID NOs: 49-51). In some embodiments, the heavy chain variable domain comprises FR1-HC, FR2-HC, FR3-HC and FR4-HC sequence depicted in Figure 7 (SEQ ID NOs: 62-65). In some embodiments, the light chain variable domain comprises FR1-LC, FR2-LC, FR3-LC and FR4-LC sequence depicted in Figure 7 (SEQ ID NO: 57-60).

In some embodiments, the antibody comprises at least one characteristic that promotes heterodimerization, while minimizing homodimerization, of the Fc sequences within the antibody fragment. Such characteristic(s) improves yield and/or purity and/or homogeneity of the immunoglobulin populations obtainable by methods of the ~~invention~~disclosure as described herein. In one embodiment, a first Fc polypeptide and a second Fc polypeptide meet/interact at an interface. In some embodiments wherein the first and second Fc polypeptides meet at an interface, the interface of the second Fc polypeptide (sequence) comprises a protuberance (also termed a "knob") which is positionable in a cavity (also termed a "hole") in the interface of the first Fc polypeptide (sequence). In one embodiment, the antibody comprises Fc mutations constituting "knobs" and "holes" as described in

WO2005/063816. For example, a hole mutation can be one or more of T366A, L368A and/or Y407V in an Fc polypeptide, and a knob mutation can be T366W.

The ~~invention~~disclosure also provides methods using the variant TIR and signal sequences of the ~~invention~~disclosure. It is understood that any of the variant TIR, signal sequences and polynucleotides disclosed herein are suitable for use in methods, e.g., methods of the ~~invention~~disclosure disclosed herein. In a further ~~aspect~~embodiment, the ~~invention~~disclosure provides methods of making a heterologous polypeptide of the ~~invention~~disclosure. For example, the ~~invention~~disclosure provides methods of making an a heterologous polypeptide (e.g., an antibody, which, as defined herein includes full length antibody and fragments thereof), said method comprising culturing a host cell comprising a polynucleotide of the ~~invention~~disclosure (e.g., a polynucleotide comprising a translation initiation region) so that the polynucleotide is expressed, whereby upon expression of said polynucleotide in a host cell (e.g. a prokaryotic host cell), the heterologous polypeptide is folded to form a biologically active heterologous polypeptide. In embodiments involving expression of antibodies, upon expression of said polynucleotide in a host cell, the light and heavy chains are folded and assembled to form a biologically active antibody. In some embodiments, the method further comprises recovering the heterologous polypeptide (e.g., an antibody) from the host cell culture. In some embodiments, the heterologous polypeptide is recovered from the host cell culture medium. In some embodiments, the method further comprises combining the recovered heterologous polypeptide (e.g., an antibody) with a pharmaceutically acceptable carrier, excipient, or carrier to prepare a pharmaceutical formulation comprising the heterologous polypeptide (e.g., antibody).

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides methods of secreting a heterologous polypeptide of interest from a cell, said method comprising culturing a host cell comprising a polynucleotide of the ~~invention~~disclosure so that the polynucleotide is expressed and the heterologous polypeptide is secreted.

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides methods of translocating a heterologous polypeptide of interest from a cell, said method comprising culturing a host cell comprising a polynucleotide of the ~~invention~~disclosure so that the polynucleotide is expressed and the heterologous polypeptide is translocated.

In another ~~aspect~~embodiment, the ~~invention~~disclosure provides method of optimizing secretion of a heterologous polypeptide of interest in a cell comprising comparing the levels of

expression of the polypeptide under control of a set of polynucleotide variants of a translation initiation region, wherein the set of variants represents a range of translational strengths, and determining the optimal translational strength for production of mature polypeptide. In some embodiments, the optimal translational strength is less than the translational strength of the wild-type translation initiation region. In some embodiments, the optimal translational strength is more than the translational strength of the wild-type translation initiation region. In some embodiments, the variants comprise polynucleotide variants of a secretion signal sequence. In some embodiments, the variant secretion signal sequences are sec pathway signal sequences and/or SRP pathway signal sequences. In some embodiments, the variant secretion signal sequences are PhoA, MalE, DsbA, or STII variant signal sequences. In some embodiments, the variant is one or more variant shown in Table 3. In some embodiments, the variant comprises sequence of one of SEQ ID Nos 1-14, 36-39, 41-42.

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides a heterologous polypeptide obtained by a method of the ~~invention~~disclosure as described herein. In some embodiments, the heterologous polypeptide is an antibody.

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides uses of a heterologous polypeptide generated using the methods of the ~~invention~~disclosure, in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder, and/or an immune (such as autoimmune) disorder. The heterologous polypeptide can be of any form described herein, including antibody, antibody fragment, polypeptide (e.g., an oligopeptide), or combination thereof.

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides use of a polynucleotide of the ~~invention~~disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder and/or an immune (such as autoimmune) disorder.

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides use of an expression vector of the ~~invention~~disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder and/or an immune (such as autoimmune) disorder.

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides use of a host cell of the ~~invention~~disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease,

such as a cancer, a tumor, a cell proliferative disorder and/or an immune (such as autoimmune) disorder.

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides use of an article of manufacture of the ~~invention~~disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder, an immune (such as autoimmune) disorder and/or an angiogenesis-related disorder (wound healing).

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides use of a kit of the ~~invention~~disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder and/or an immune (such as autoimmune) disorder).

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1: Translocation of indicated signal peptides across the inner membrane of bacteria. The maltose-binding periplasmic protein (MalE) and alkaline phosphatase (PhoA) signal peptides direct translocation from the cytoplasm to the periplasm in a post-translational manner with the aid of the molecular motor SecA. The heat-stable enterotoxin II (StII) and thiol:disulfide interchange protein (DsbA) signal peptides direct translocation in a co-translational manner with aid from the signal recognition particle (SRP).

FIGURE 2: Relative translocation initiation region strength of signal peptide variants. Normalized basal alkaline phosphatase activity of 27C7 cells carrying a vector with a fusion between either an STII, MalE, PhoA, or DsbA signal peptide and the mature domain of the *E. coli* alkaline phosphatase (BAP) gene. Each bar represents an individual culture incubated with the chromogenic substrate PNPP and enzymatic activity was determined as the absorbance of that culture at 410 nm less the absorbance of a culture carrying an empty vector (pBR322). Activities were normalized to the basal activity of 27C7 cells carrying the plasmid pPho41. White bars represent signal peptide variants with a *BssHII* restriction site at the -9 position relative to the first base pair of the initiation codon. Grey or striped bars represent an *MluI* or *XbaI* site at the -9 position, respectively. All activities are the mean of between seven and ten replicate experiments. Error bars are reported as the uncertainty in the mean with a 95% confidence limit. The differences in relative TIR strength between adjacent bars are all statistically significant ($P << 0.001$). Bars represent clones SH1.2, SH2.41, SH3.38, SH4.60, SH5.34, SH6.52, SH8.36, SL1.2, SL2.74, SL3.72, MH1.92, MH2.100, ML1.97, ML2.123, MX1.wt, MX2.15, MX3.12,