SUMMARY OF THE INVENTION

The invention 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, the invention 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 ~~Table 3~~in Table 2.

In one aspect, the invention 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~~3~~. The variant secretion signal sequences of the invention are suitable for use, for example, in any of the methods disclosed herein.

In another aspect, the invention provides a polynucleotide comprising a translation initiation region of the invention. In some embodiments, the translation initiation region comprises sequence shown in Table 2~~3~~ (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, the invention provides a polynucleotide comprising a secretion signal sequence of the invention. In some embodiments, the secretion signal sequence comprises sequence shown in Table 23. (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, the invention provides a polynucleotide comprising a translation initiation region of the invention 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 23. 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, the invention 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.