[0005] Glutamine synthetase has been used as an amplifiable, selectable marker for high level expression of recombinant proteins. See e.g., Bebbington C. R. et al., BioTechnology, 10:169-175 (1992). Glutamine synthetase is a multimeric protein responsible for the biosynthesis of glutamine by catalyzing the condensation of ammonia and glutamate. When a gene encoding a functional glutamine synthetase is introduced in a cell lacking endogenous glutamine synthetase (e.g., a glutamine synthetase knock out mammalian cell), the cell can grow in a glutamine-free medium. There have been efforts in developing expression systems that utilize intragenic complementation of glutamine synthetase, wherein each antibody chain is linked to each of two mutant glutamine synthetases that can complement each other to form a functional glutamine synthetase such that roughly equal amounts of each chain are expressed. Intragenic complementation is a phenomenon that occurs when a multimeric protein is formed from subunits produced by different mutant alleles of a gene (e.g., mutations that mapped to the 5' end of the glutamine synthetase gene could complement those in the 3' end). See e.g., Mitchell, A. P. Genetics 111, 243-258 (1985).

[0006] Despite these progresses, there is a continued need for vectors, expression systems and methods that are robust and express heteromeric recombinant proteins to high levels.

SUMMARY

[0007] Disclosed herein are vectors, expression systems and methods for producing heteromeric complexes comprising different polypeptides. Vectors and expression systems disclosed herein are based on the finding that the selectable marker glutamine synthetase can be divided into two fragments at selected amino acid positions of the glutamine synthetase polypeptide, and the two fragments can interact and/or associate to form a monomer and then a functional multimeric glutamine synthetase protein. Specifically, in the vectors and expression systems disclosed herein, the expression of each polypeptide of a heteromeric complex (e.g., heavy and light chain of an antibody) is linked to the expression of a glutamine synthetase fragment, and the expression of the heteromeric complex is accomplished by growing a recombinant cell comprising the vector or the expression system under conditions that require the expression of a functional glutamine synthetase. These vectors and expression systems are robust and express proteins to high levels. In addition, they reduce the time required to select for cells expressing high levels of proteins.

[0008] In one aspect, the present disclosure refers to a vector comprising a) a first nucleic acid encoding a first polypeptide, b) a second nucleic acid encoding a first fragment of glutamine synthetase, wherein the transcription of the first nucleic acid is operably linked to the transcription of the second nucleic acid, c) a third nucleic acid encoding a third polypeptide, the third polypeptide is capable of associating with the first polypeptide to form a heteromeric complex, and d) a fourth nucleic acid encoding a second fragment of glutamine synthetase, wherein the transcription of the third nucleic acid is operably linked to the transcription of the fourth nucleic acid, and wherein the first fragment and the second fragment of glutamine synthetase associate to provide a selectable activity, and wherein the vector is capable of being transfected into mammalian cells and improving selection of the transfected cells.

[0009] In another aspect, the present disclosure refers to an expression system comprising: a) a first vector comprising a first nucleic acid encoding a first polypeptide, wherein the transcription of the first nucleic acid is operably linked to the transcription of a second nucleic acid encoding a first fragment of glutamine synthetase, and b) a second vector comprising a third nucleic acid encoding a third polypeptide, wherein the transcription of the third nucleic acid is operably linked to the transcription of a fourth nucleic acid encoding a second fragment of glutamine synthetase, wherein the first polypeptide is capable of associating with the third polypeptide to form a heteromeric complex, wherein the first and second fragments of glutamine synthetase associate to provide a selectable activity, and wherein the expression system is capable of being transfected into mammalian cells and improving selection of the transfected cells.

[0010] In certain examples, the first fragment of glutamine synthetase is an N-terminal fragment of glutamine synthetase and the second fragment of glutamine synthetase is a C-terminal fragment of glutamine synthetase, or the first fragment of glutamine synthetase is a C-terminal fragment of glutamine synthetase and the second fragment of glutamine synthetase is an N-terminal fragment of glutamine synthetase.

[0011] In certain examples of the vector or the expression system disclosed herein, the glutamine synthetase comprising the amino acid sequence of SEQ ID NO: 1, and the first and second fragments of glutamine synthetase are generated by splitting the glutamine synthetase polypeptide at an amino acid position selected from K52, E55, D92, G187, G245, R262, K291, G302 and D311 of SEQ ID NO: 1. In certain examples, the first and second fragments of glutamine synthetase are generated by splitting the glutamine synthetase polypeptide at an amino acid position selected from K52, E55, D92, G187, and G245 of SEQ ID NO: 1, and in

certain examples, the first and second fragments of glutamine synthetase are generated by splitting the glutamine synthetase polypeptide at the amino acid position D92 or G187 of SEQ ID NO: 1.

[0012] In certain examples of the vector or the expression system disclosed herein, a) the first glutamine synthetase fragment comprises amino acid residues 1 to 51 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 52 to 373 of SEQ ID NO: 1; or b) the first glutamine synthetase fragment comprises amino acid residues 1 to 52 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 53 to 373 of SEQ ID NO: 1; or c) the first glutamine synthetase fragment comprises amino acid residues 1 to 54 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 55 to 373 of SEQ ID NO: 1; or d) the first glutamine synthetase fragment comprises amino acid residues 1 to 55 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 56 to 373 of SEQ ID NO: 1; or e) the first glutamine synthetase fragment comprises amino acid residues 1 to 91 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 92 to 373 of SEQ ID NO: 1; or f) the first glutamine synthetase fragment comprises amino acid residues 1 to 92 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 93 to 373 of SEQ ID NO: 1; or g) the first glutamine synthetase fragment comprises amino acid residues 1 to 186 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 187 to 373 of SEQ ID NO: 1; or h) the first glutamine synthetase fragment comprises amino acid residues 1 to 187 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 188 to 373 of SEQ ID NO: 1; or i) the first glutamine synthetase fragment comprises amino acid residues 1 to 244 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 245 to 373 of SEQ ID NO: 1; or j) the first glutamine synthetase fragment comprises amino acid residues 1 to 245 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 246 to 373 of SEQ ID NO: 1; or k) the first glutamine synthetase fragment comprises amino acid residues 1 to 261 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 262 to 373 of SEQ ID NO: 1; or l) the first glutamine synthetase fragment comprises amino acid residues 1 to 262 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 263 to 373 of SEQ ID NO: 1; or m) the first glutamine synthetase fragment comprises amino acid residues 1 to 301 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 302 to 373 of SEQ ID NO: 1; or

n) the first glutamine synthetase fragment comprises amino acid residues 1 to 302 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 303 to 373 of SEQ ID NO: 1; or o) the first glutamine synthetase fragment comprises amino acid residues 1 to 310 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 311 to 373 of SEQ ID NO: 1; or p) the first glutamine synthetase fragment comprises amino acid residues 1 to 311 of SEQ ID NO: 1 and the second glutamine synthetase fragment comprises amino acid residues 312 to 373 of SEQ ID NO: 1.

[0013] In certain examples, the glutamine synthetase is a mammalian glutamine synthetase having an amino acid sequence different from SEQ ID NO:1, and the first and second fragments of glutamine synthetase are generated by splitting the mammalian glutamine synthetase polypeptide at an amino acid position equivalent to an amino acid position selected from K52, E55, D92, G187, G245, R262, K291, G302 and D311 of SEQ ID NO: 1 according to sequence alignment; in certain examples, the first and second fragments of glutamine synthetase are generated by splitting the mammalian glutamine synthetase polypeptide at an amino acid position equivalent to an amino acid position selected from K52, E55, D92, G187, and G245 of SEQ ID NO:1 according to sequence alignment; in certain examples, the first and second fragments of glutamine synthetase are generated by splitting the mammalian glutamine synthetase polypeptide at an amino acid position equivalent to the amino acid position D92 or G187 of SEQ ID NO:1 according to sequence alignment.

[0014] In certain examples, the heteromeric complex is an antibody or an antigen binding molecule. In certain examples, a) the first polypeptide is a heavy chain of an antibody or a fragment thereof and the third polypeptide is a light chain of an antibody or a fragment thereof; or b) the first polypeptide is a heavy chain of an antibody or a fragment thereof and the third polypeptide is a light chain of an antibody or a fragment thereof. In certain examples, a) the first nucleic acid encodes an antibody heavy chain or a fragment thereof and the third nucleic acid encodes an antibody light chain or a fragment thereof; or b) the first nucleic acid encodes an antibody light chain or a fragment thereof and the third nucleic acid encodes an antibody heavy chain or a fragment thereof.

[0015] In certain examples, the vector disclosed herein, or one or both vectors of the expression system disclosed herein further comprises an internal ribosomal entry site (IRES) and/or an expression augmenting sequence element (EASE). In certain examples, the IRES occurs at a site selected from: a) a site between the first nucleic acid and the second nucleic acid; b) a site between the third nucleic acid and the fourth nucleic acid, and c) at sites between

both first and second, and third and fourth nucleic acids. In certain examples, the IRES comprises the sequence of GATGATAATACCCTCGAGATCCGTGCCATCATG.

[0016] In certain examples, each of the first and second fragment of glutamine synthetase further comprises an interaction domain. In certain examples, the interaction domain is a leucine zipper or an antiparallel leucine zipper polypeptide. In certain examples, the interaction domain is a leucine zipper polypeptide of GCN4, C/EBP, c-Fos, c-Jun, c-Myc or c-Max. In certain examples, the interaction domain is a leucine zipper polypeptide having the following sequence: ALKKELQANKKELAQLKWELQALKKELAQEQLEKKLQALEKKLAQLEWKNQALEKKLAQ. In certain examples, each of the first and second interaction domains comprise the amino acid sequence of ALKKELQANKKELAQLKWELQALKKELAQ or EQLEKKLQALEKKLAQLEWKNQALEKKLAQ.

[0017] In certain examples, each of the first and second interaction domains further comprises a linker linking the first or second interaction domain to an interaction domain. In certain examples, the linker comprises a sequence selected from GGPGG, GPGGG, GGGGSGGGGGS, GGGGS and GGGGSGGGGS.

[0018] In certain examples, a) the first interaction domain is fused to the N-terminal of the first glutamine synthetase fragment and has the amino acid sequence of EQLEKKLQALEKKLAQLEWKNQALEKKLAQGGGGSGGGGS and the second interaction domain is fused to the C-terminal of the second glutamine synthetase fragment and has the amino acid sequence of GGGGSGGGGSALKKELQANKKELAQLKWELQALKKELAQ; or b) the first interaction domain is fused to the C-terminal of the first glutamine synthetase fragment and has the amino acid sequence of GGGGSGGGGSALKKELQANKKELAQLKWELQALKKELAQ and the second interaction domain is fused to the N-terminal of the second glutamine synthetase fragment and has the amino acid sequence of EQLEKKLQALEKKLAQLEWKNQALEKKLAQGGGGSGGGGS.

[0019] In certain examples, the vector disclosed herein, or one or both vectors of the expression system disclosed herein further comprises a fifth nucleic acid encoding a selectable marker selected from Zeomycin, neomycin, puromycin, Blasticidin S, and GPT.

[0020] In yet another aspect, the present disclosure refers to a host cell comprising the vector or the expression system disclosed herein. In certain examples, the host cell is CHO, VERO, BHK, HeLa, Cos, MDCK, 293, 3T3, WI338, or NSO cells. In certain examples, the

host cell is CHO cells. In certain examples, the host cell lacks endogenous glutamine synthetase.

[0021] In yet another aspect, is the present disclosure refers to a method of producing an antibody heavy chain or a fragment thereof and an antibody light chain or a fragment thereof comprising culturing the host cell under conditions wherein the heteromeric complex is expressed by the host cell. In certain examples, the method further comprises isolating the heteromeric complex.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figure 1 shows sequence alignment of glutamine synthetase from difference species using Geneious Aligner.

[0023] Figure 2 shows the plasmid map of the vectors used in the Examples.

[0024] Figure 3 shows cell recovery data of various control cell lines (CS9, pGS) and cells transfected with plasmids comprising glutamine synthetase fragments generated by splitting the polypeptide at sites 1-55, 1-92, 1-187, and 1-244 of SEQ ID NO:1 in both linear (L1, L2) and circular format (C1, C2). Cells carrying split site 1-187 recovered in both the linear and circular format while the site 1-92 recovered in the linear format.

[0025] Figure 4 shows cells recovery data of cells carrying split site 1-186 in the linear format.

DETAILED DESCRIPTION

[0026] Disclosed herein are vectors, expression systems and methods for producing heteromeric complexes (e.g., monoclonal antibodies). The invention disclosed herein is advantageous in that they are robust, express each component of a heteromeric complex in proportion and to high levels. In addition, it reduces the time required to select for cells expressing high levels of proteins.

[0027] The invention disclosed herein utilizes that the selectable marker glutamine synthetase can be divided into two fragments at selected amino acid positions of the glutamine synthetase polypeptide, and the two fragments interact and form a functional glutamine synthetase protein when expressed together, thereby providing a selectable activity. The individual fragment does not have significant selectable activity when expressed alone, but they provide selectable activity when co-expressed. Polynucleotide encoding each glutamine