Claims

1. An expression construct comprising in a 5' to 3' direction:
a promoter;
a first flanking intron;
a first splice acceptor site;
a first exon encoding a first polypeptide;
a second flanking intron;
a second splice acceptor site; and
a second exon encoding a second polypeptide,
wherein upon entry into a host cell, transcription of the first exon results in expression of the first polypeptide and/or transcription of the second exon results in expression of the second polypeptide; and
wherein said first and said second flanking introns have a nucleic acid sequence homology of at least 95% for at least 450 nucleotides.

2. An expression construct according to claim 1, wherein said first and said second flanking introns are selected from the group consisting of: chicken troponin (cTNT) intron 4, cTNT intron 5 and first intron of the human EF1 alpha gene.

3. An expression construct according to claim 1 or claim 2, further comprising a first splice donor site and/or a second splice donor site.

4. An expression construct according to claim 1 or 2, further comprising at least one polypyrimidine (poly(Y)) tract.

5. An expression construct according to claim 4, wherein said poly(Y) tract comprises less than 30 pyrimidine bases.

6. An expression construct according to any one of claims 1, 2, 4 or 5, wherein said expression construct lacks a second splice donor site.

7. An expression construct according to claim 3, wherein said expression construct further comprises a third splice donor site, an intron and a third splice acceptor site located downstream of said promoter.

8. An expression construct according to claim 7, wherein said third splice donor site is preceded by a 5'UTR and/or said third splice acceptor site is followed by a 5'UTR.

9. An expression construct according to any one of the preceding claims, wherein said flanking intron sequences are selected from the group consisting of: SEQ ID Nos: 129 to 175.

10. An expression construct according to any one of the preceding claims, wherein said first polypeptide is an antibody heavy chain or fragment thereof and said second polypeptide is an antibody light chain or fragment thereof, or wherein said first polypeptide is an antibody light chain or fragment thereof and said second polypeptide is an antibody heavy chain or fragment thereof.

11. A host cell comprising an expression construct of any one of claims 1 to 9 encoding the expression of an antibody light or heavy chain.

12. A method of producing a polypeptide comprising culturing the host cell of claim 11 in a culture and isolating the polypeptide expressed from the culture.

13. A method of producing a bispecific antibody comprising culturing the host cell of claim 12 and isolating the polypeptide expressed from the culture.

14. A method of optimizing the expression level of a protein of interest encoded by one or more expression constructs according to any one of claims 1 to 10, comprising:

(i) using first and second flanking introns having a nucleic acid sequence homology of at least 80% for at least 50 nucleotides;

(ii) reducing the number of pyrimidine bases in a poly(Y) tract upstream of the first exon or increasing the number of pyrimidine bases in a poly(Y) tract downstream of the first exon; and/or

(iii) deleting a splice donor site upstream of the second flanking intron.

15. A method of optimizing the heterodimerisation level of a protein of interest encoded by one or more expression constructs according to any one of claims 1 to 10, comprising:

(i) using first and second flanking introns having a nucleic acid sequence homology of at least 80% for at least 50 nucleotides;

(ii) reducing the number of pyrimidine bases in a poly(Y) tract upstream of the first exon or increasing the number of pyrimidine bases in a poly(Y) tract downstream of the first exon; and/or

(iii) deleting a splice donor site upstream of the second flanking intron.