

ORIGINAL

Abstract:

SORF CONSTRUCTS AND MULTIPLE GENE EXPRESSION

Embodiments of the invention relate to vector constructs and methods for expression of polypeptides including multimeric products such as therapeutic antibodies. Particular constructs allow for the generation of expression products from a single open reading frame (sORF). An embodiment provides an isolated or purified expression vector for generating one or more recombinant protein products comprising a single open reading frame insert; said insert comprising a signal peptide nucleic acid sequence encoding a signal peptide; a first nucleic acid sequence encoding a first polypeptide; a first intervening nucleic acid sequence encoding a first protein cleavage site, wherein said first protein cleavage site is provided by an intein segment of a lon protease gene of *Pyrococcus* or a klbA gene of *Pyrococcus* or *Methanococcus*, or a modified intein segment derived therefrom; and a second nucleic acid sequence encoding a second polypeptide. Certain embodiments of constructs and methods employ an intein segment of a lon protease gene of *Pyrococcus abyssi*, *Pyrococcus furiosus*, or *Pyrococcus horikoshii* OT3; or an intein segment of a klbA gene of *Pyrococcus abyssi*, *Pyrococcus furiosus*, or *Methanococcus jannaschii*; or other intein segment.

CLAIMS:

WE CLAIM:

1. An isolated or purified expression vector for generating one or more recombinant protein products comprising a single open reading frame insert; said insert comprising:
 - (a) a signal peptide nucleic acid sequence encoding a signal peptide;
 - (b) a first nucleic acid sequence encoding a first polypeptide;
 - (c) a first intervening nucleic acid sequence encoding a first protein cleavage site, wherein said first protein cleavage site is provided by an intein segment of a lon protease gene of *Pyrococcus* or a klbA gene of *Pyrococcus* or *Methanococcus*, or a modified intein segment derived therefrom; and
 - (d) a second nucleic acid sequence encoding a second polypeptide;wherein said first intervening nucleic acid sequence encoding said first protein cleavage site is operably positioned between said first nucleic acid sequence and said second nucleic acid sequence;
wherein said signal peptide nucleic acid sequence encoding said signal peptide is operably positioned before said first nucleic acid sequence; and
wherein said expression vector is capable of expressing a single open reading frame polypeptide cleavable at said first protein cleavage site.
2. The expression vector of claim 1 wherein said first protein cleavage site is provided by an intein segment of a lon protease gene of *Pyrococcus abyssi*, *Pyrococcus furiosus*, or *Pyrococcus horikoshii* OT3; or an intein segment of a klbA gene of *Pyrococcus abyssi*, *Pyrococcus furiosus*, or *Methanococcus jannaschii*; or a modified intein segment derived respectively therefrom.
3. The expression vector of claim 1 wherein the intein segment or modified intein segment encodes a penultimate residue which is a lysine, serine or not a histidine.
4. The expression vector of claim 1 wherein said intein segment or modified intein segment is capable of cleavage but not complete ligation of said first polypeptide to said second polypeptide.

5. The expression vector of claim 1 wherein said first protein cleavage site is provided by an intein segment comprising a sequence selected from the group consisting of SEQ ID NO: 1, 3, 4, 6, 7, 55, 35, 37, and 39 and modified intein segments derived therefrom.
6. The expression vector of any of claims 1-5 wherein the first polypeptide and second polypeptide are capable of multimeric assembly.
7. The expression vector of any of claims 1-6 wherein at least one of said first polypeptide and second polypeptide are capable of extracellular secretion.
8. The expression vector of any of claims 1-7 wherein at least one of said first polypeptide and second polypeptide are of mammalian origin.
9. The expression vector of any of claims 1-8 wherein said first polypeptide comprises an immunoglobulin heavy chain or functional fragment thereof, and said second polypeptide comprises an immunoglobulin light chain or functional fragment thereof, and said first polypeptide is upstream of said second polypeptide.
10. The expression vector of any of claims 1-9 comprising only one of said signal peptide nucleic acid sequence.
11. The expression vector of any of claims 1-10 further comprising a third nucleic acid sequence encoding a third polypeptide, and a second intervening nucleic acid sequence encoding a second protein cleavage site; wherein the second intervening nucleic acid sequence and third nucleic acid sequence, in that order, are operably positioned after said second nucleic acid sequence.
12. The expression vector of any of claims 1-11 wherein said first and said second polypeptide comprise a functional antibody or other antigen recognition molecule; with an antigen specificity directed to binding an antigen selected from the group consisting of: TNF α (tumor necrosis factor-alpha), erythropoietin receptor, RSV, EL/selectin, interleukin-1, interleukin-12, interleukin-13, interleukin-18, interleukin-23, interleukin-33, CD81, CD19, IGF1, IGF2, EGFR, CXCL-13, GLP-1R, prostaglandin E2, and amyloid beta.

13. The expression vector of any of claims 1-12 wherein the first and second polypeptides comprise a pair of immunoglobulin chains from an antibody of D2E7, EL246, ABT-007, ABT-325, or ABT-874.
14. The expression vector of any of claims 1-13, wherein the first and second polypeptide are each independently selected from an immunoglobulin heavy chain or an immunoglobulin light chain segment from an analogous segment of D2E7, EL246, ABT-007, ABT-325, ABT-874, or other antibody.
15. The expression vector of any of claims 1-14, wherein said vector further comprises a promoter regulatory element for said insert.
16. The expression vector according to claim 15, wherein said promoter regulatory element is inducible or constitutive.
17. The expression vector according to claim 15, wherein said promoter regulatory element is tissue specific.
18. The expression vector according to claim 15, wherein said promoter comprises an adenovirus major late promoter.
19. A host cell comprising a vector according to any of claims 1-18.
20. The host cell according to claim 19, wherein said host cell is a prokaryotic cell.
21. The host cell according to claim 20, wherein said host cell is *Escherichia coli*.
22. The host cell according to claim 19, wherein said host cell is a eukaryotic cell.
23. The host cell according to claim 22, wherein said eukaryotic cell is selected from the group consisting of a protist cell, animal cell, plant cell, and fungal cell.
24. The host cell according to claim 23, wherein said eukaryotic cell is an animal cell selected from the group consisting of a mammalian cell, an avian cell, and an insect cell.
25. The host cell according to claim 24, wherein said host cell is a mammalian cell line.

26. The host cell according to claim 24, wherein said host cell is a CHO cell or a dihydrofolate reductase-deficient CHO cell.
27. The host cell according to claim 24, wherein said host cell is a COS cell or HEK cell.
28. The host cell according to claim 23, wherein said host cell is a yeast cell.
29. The host cell according to claim 28, wherein said yeast cell is *Saccharomyces cerevisiae*.
30. The host cell according to claim 24, wherein said host cell is a *Spodoptera frugiperda* Sf9 insect cell.
31. A method for producing a recombinant polyprotein or a plurality of proteins, comprising culturing a host cell of claim 19 in a culture medium under conditions sufficient to allow expression of a vector protein.
32. The method of claim 31 further comprising recovering and/or purifying said vector protein.
33. The method of any of claims 31-32 wherein said plurality of proteins are capable of multimeric assembly.
34. The method of any of claims 31-33 wherein the recombinant polyprotein or plurality of proteins are biologically functional and/or therapeutic.
35. A method for producing a recombinant product, wherein the product is an immunoglobulin protein or functional fragment thereof, assembled antibody, or other antigen recognition molecule, comprising culturing a host cell according to claim 19 in a culture medium under conditions sufficient to produce the recombinant product.
36. A protein produced according to the method of any of claims 31-35.
37. A polyprotein produced according to the method of any of claims 31-36.
38. An assembled immunoglobulin; assembled other antigen recognition molecule; or individual immunoglobulin chain or functional fragment thereof produced according to the method of any of claims 31-37.

39. The immunoglobulin; other antigen recognition molecule; or individual immunoglobulin chain or functional fragment thereof according to claim 38, wherein there is a capability to effect or contribute to specific antigen binding to tumor necrosis factor- α , erythropoietin receptor, RSV, EL/selectin, interleukin-1, interleukin-12, interleukin-13, interleukin-18, interleukin-23, interleukin-33, CD81, CD19, IGF1, IGF2, EGFR, prostaglandin E2, CXCL-13, GLP-1R, or amyloid beta.

40. The immunoglobulin or functional fragment thereof according to claim 39, wherein the immunoglobulin is D2E7 or ABT-874 or wherein the functional fragment is a fragment respectively thereof.

41. A pharmaceutical composition comprising a therapeutically effective amount of a protein according to any of claims 36-40, and a pharmaceutically acceptable carrier.

42. The expression vector of any of claims 1-18, further comprising a nucleic acid sequence encoding a tag.

43. The expression vector of any of claims 1-18, wherein said intervening nucleic acid sequence additionally encodes a tag.

44. An expression vector, host cell with the vector, vector expression product, pharmaceutical composition, and/or method of making or using of any of the foregoing, wherein the vector is the vector of any of claims 1-18 and further comprises a segment encoding a light chain signal peptide.

45. The vector, host cell, vector expression product, pharmaceutical composition and/or method of making or using of claim 44 wherein the encoded light chain signal peptide is a kappa light chain signal peptide selected from the group consisting of A17, A18, A19, A26, and H2G.

46. The vector, host cell, vector expression product, pharmaceutical composition and/or method of making or using of claim 44 wherein the encoded light chain signal peptide is VKII kappa light chain signal peptide A18, SEQ ID NO:82 (amino acid sequence MRLPAQLLGLLMLWIPGSSA).

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