

In one aspect, the present disclosure refers to a nucleic acid construct for expression of a protein of interest comprising the following elements in operable association in 5' to 3' order: optionally, a first promoter sequence; a selectable marker sequence; a second promoter sequence; a nucleic acid sequence encoding a first protein of interest that is operably linked to the second promoter sequence; and a poly A signal sequence; the nucleic acid construct further comprising at least one insertion element at a position or positions selected from the group consisting of 5' to the optional first promoter if present or selectable marker sequence, 3' to the poly A signal sequence, between the optional first promoter if present and the poly A signal sequence, between the selectable marker and the second promoter sequence, and both 5' to the optional first promoter sequence if present or the selectable marker sequence and 3' to the poly A signal sequence, wherein the insertion element is a recombinase insertion element. In some examples,

CLAIMS

What is claimed is:

- 5 1. A nucleic acid construct for expression of a protein of interest comprising the following elements in operable association in 5' to 3' order:
 - optionally, a first promoter sequence;
 - a selectable marker sequence;
 - a second promoter sequence;
 - 10 a nucleic acid sequence encoding a first protein of interest that is operably linked to the second promoter sequence; and
 - a poly A signal sequence;
 - the nucleic acid construct further comprising at least one insertion element at a position or positions selected from the group consisting of 5' to the optional first promoter if present or selectable marker sequence, 3' to the poly A signal sequence, between the optional
 - 15 first promoter if present and the poly A signal sequence, between the selectable marker and the second promoter sequence, and both 5' to the optional first promoter sequence if present or the selectable marker sequence and 3' to the poly A signal sequence
 - wherein the insertion element is a recombinase insertion element.
- 20 2. The nucleic acid construct of claim 1, wherein the nucleic acid construct does not comprise a poly A signal sequence between the selectable marker and the second promoter.
3. The nucleic acid construct of claim 1, wherein the selectable marker is adjacent to the
- 25 second promoter.
4. The nucleic acid construct of claim 1, wherein the second promoter is adjacent to the nucleic acid sequence encoding the first protein of interest.
- 30 5. The nucleic acid construct of claim 1, wherein the nucleic acid construct comprises an extending packaging region (EPR) between the first promoter and the selectable marker.
6. The nucleic acid construct of claim 5, wherein the EPR comprises multiple potential Kozak sequences and/or ATG translation start sites.

7. The nucleic acid construct of claim 1, wherein the first promoter sequence is a weak promoter sequence.

8. The nucleic acid construct of claim 1, wherein the first promoter sequence is selected from the group consisting of SIN-LTR, SV40, *E. coli* lac, *E. coli* trp, phage lambda PL, phage lambda PR, T3, T7, cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, alpha-lactalbumin, human elongation factor 1 alpha (hEF1alpha), and mouse metallothionein-I promoter sequences.

9. The nucleic acid construct of claim 1, wherein the selectable marker sequence is an amplifiable selectable marker sequence selected from the group consisting of the Glutamine Synthase (GS) sequence and the Dihydrofolate Reductase (DHFR) sequence.

10. The nucleic acid construct of claim 1, wherein the selectable marker sequence is an antibiotic resistance marker sequence selected from the group consisting of neomycin resistance gene (neo), hygromycin B phosphotransferase gene and puromycin N-acetyl transferase gene sequences.

11. The nucleic acid construct of claim 1, wherein the second promoter sequence is selected from the group consisting of SV40, *E. coli* lac, *E. coli* trp, phage lambda PL, phage lambda PR, T3, T7, cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, alpha-lactalbumin, human elongation factor 1 alpha (hEF1alpha), and mouse metallothionein-I promoter sequences.

12. The nucleic acid construct of claim 1, wherein the nucleic acid sequence encoding a protein of interest encodes a protein selected from the group consisting of heavy and light chain immunoglobulin sequences.

13. The nucleic acid construct of claim 1, wherein the recombinase insertion element is an attachment site (att).

14. The nucleic acid construct of claim 13, wherein the attachment site (att) is attB.

15. A host cell comprising the nucleic acid construct of claim 1.

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16. The host cell of claim 15, wherein the host cell comprises from 1 to 1000 copies of the nucleic acid construct.

17. A process for producing a protein of interest comprising culturing host cells according to claim 15 and purifying the protein of interest from the host cell culture.

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18. A system comprising:
a first nucleic acid construct according to claim 1; and
a second nucleic acid construct encoding an enzyme.

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19. The system of claim 18, wherein the enzyme is a recombinase.