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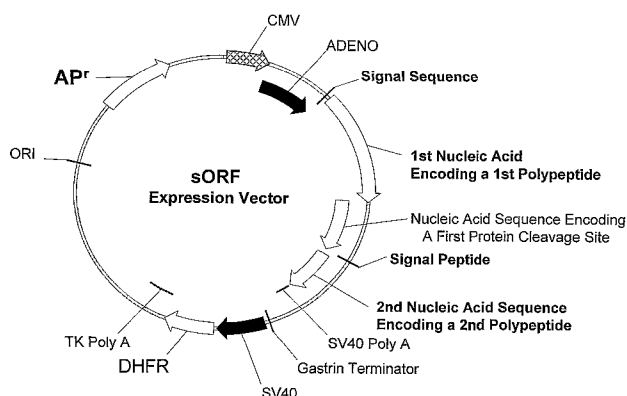
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(54) Title: MULTIPLE GENE EXPRESSION INCLUDING SORF CONSTRUCTS AND METHODS WITH POLYPROTEINS, PRO-PROTEINS, AND PROTEOLYSIS



(57) Abstract: Disclosed are useful constructs and methods for the expression of proteins using primary translation products that are processed within a recombinant host cell. Constructs comprising a single open reading frame (sORF) are described for protein expression including expression of multiple polypeptides. A primary translation product (a pro-protein or a polypeptide) contains polypeptides such as inteins or hedgehog family auto-processing domains, or variants thereof, inserted in frame between multiple protein subunits of interest. The primary product can also contain cleavage sequences such as other proteolytic cleavage or protease recognition sites, or signal peptides which contain recognition sequences for signal peptidases, separating at least two of the multiple protein subunits. The sequences of the inserted auto-processing polypeptides or cleavage sites can be manipulated to enhance the efficiency of expression of the separate multiple protein subunits. Also disclosed are independent aspects of conducting efficient expression, secretion, and/or multimeric assembly of proteins such as immunoglobulins. Where the polyprotein contains immunoglobulin heavy and light chain segments or fragments capable of antigen recognition, in an embodiment a selectable stoichiometric ratio is at least two copies of a light chain segment per heavy chain segment, with the result that the production of properly folded and assembled functional antibody is made. Modified signal peptides, including such from immunoglobulin light chains, are described.

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