

falciparum CSP as described in the preceding paragraph or elsewhere in this specification. Suitable nucleotide sequences include nucleotide sequences comprising SEQ ID NO:1 or sequences that are at least 85% homologous to SEQ ID NO:1, alternatively at least 90% homologous to SEQ ID NO:1; alternatively at least 95% homologous to SEQ ID NO:1. The nucleotide sequences can include at least one expression tag, such as the sequence of SEQ ID NO:5.

[0010] As another aspect of the present technology, novel expression vectors are provided for *E. coli* comprising a nucleotide sequence which encodes a recombinant *P. falciparum* CSP as described herein. The expression vectors can be stably cloned into a bacterial cell. A suitable bacterial cell can be transformed with such an expression vector. Preferably the bacterial cell is an *E. coli* cell, more preferably the SHUFFLE™ strain of *E. coli*. (New England Biolabs, Inc., Ipswich, Mass., described in U.S. Pat. No. 6,569,669, incorporated by reference). Surprisingly, the transfected *E. coli* cell expresses a recombinant *P. falciparum* CSP as a soluble protein.

[0011] As yet another aspect of the present technology, anti-malaria vaccines suitable for human administration are provided. The vaccines comprise a recombinant *Plasmodium falciparum* CSP as described herein, and one or more adjuvants. Preferred embodiments of the vaccines have an endotoxin level less than about 5 endotoxin units per microgram of protein, and/or less than about 1 ng/ml of bacterial host proteins. In some embodiments, the vaccines have a soluble protein content, and the soluble protein content is greater than 95%, alternatively greater than 99%, pure recombinant *P. falciparum* CSP as measured by gel densitometry.

[0012] As another aspect of the present technology, methods of eliciting an immune response against malaria in an animal or human comprise administering a vaccine or rCSP as described herein. Methods of immunizing an animal or human against malaria or a pathogen that causes malaria are also provided. The methods comprise administering to the animal or human a vaccine or rCSP as described herein. In these methods, the vaccine can be administered intramuscularly or by another route.

[0013] As still another aspect of the present technology, processes of producing recombinant *P. falciparum* CSP are provided, including processes of producing rCSP in soluble form from *E. coli*. The processes comprise the steps of providing cells, preferably bacterial cells such as *E. coli*, containing a nucleotide sequence that expresses one of the recombinant *P. falciparum* CSPs described herein (such as a transfected *E. coli* that expresses the peptide sequence of SEQ ID NO:2). The cells may be provided in a cell culture. The processes also comprise inducing expression of the recombinant CSP in the cells, and collecting the cells after a period of expression, such as by centrifuging to obtain a pellet containing the cells. The processes also comprise lysing the cells to obtain a cell lysate, collecting supernatant from the cell lysate, and purifying the recombinant *P. falciparum* CSP from the supernatant of the cell lysate without denaturing and refolding the recombinant *P. falciparum* CSP. Preferably, the bacterial cell is cultured in that is media free or substantially free of animal-derived components, such as media containing one or more of Phytone, yeast extract, ammonium sulfate, potassium phosphate monobasic, sodium phosphate dibasic, MgSO₄, glycerol, dextrose or kanamycin. In some embodiments, the recombinant *P. falciparum* CSP includes one or more expression tags at one or both ends to facilitate

the recovery or purification of the protein. The processes can include one or more purification steps, such as purifying the soluble protein over an affinity column and purifying the soluble protein over an anion exchange column, for example, a nickel affinity column and a Q-sepharose anion exchange column. The present technology also includes purified protein made by the processes described herein.

[0014] In preferred embodiments of the production processes, purified protein is recovered from a two-step purification procedure over the affinity column and the anion exchange column, and the purification procedure does not include any other chromatographic separation or consists essentially of an affinity column separation on an anion exchange column separation. Alternatively, the production process has no more than two purification steps. The purified protein of the present technology can contain at least about 90% recombinant *P. falciparum* CSP, alternatively at least about 95% or at least about 99%, as measured by gel densitometry. The processes can also include the step of filtering the purified protein. The protein can contain less than 1 ng/ml of *E. coli* host proteins and/or less than about 5 endotoxin units per microgram protein. Preferably the production process meets or exceeds current good manufacturing practices (for example, as described in the US Code of Federal Regulations, Title 21) for vaccine products. The present technology also includes a vaccine comprising a purified protein produced by the foregoing process.

[0015] As another aspect, the present technology provides novel CS gene constructs that encode an amino acid sequence having a start site at Tyr₂₆, 19 copies of the NANP amino acid repeat, and 3 copies of the NVDP repeat. On the C-terminal region, the glycosylphosphatidylinositol (GPI) anchor sequence can be excluded from the CS gene construct and excluded from the rCSP; in other words the CS gene construct does not include a nucleotide sequence that encodes the GPI anchor sequence, and the rCSP does not include the GPI anchor sequence. The novel soluble recombinant proteins of the present technology can comprise a protein sequence with at least 85% homology to SEQ ID NO:2, preferably 90% homology to SEQ ID NO:2, preferably at least 95% homology to SEQ ID NO:2, more preferably about 99% homology to SEQ ID NO:2, most preferably includes SEQ ID NO:2 and in some embodiments include at least one expression tag, preferably at least two expression tags. In some aspects, at least one expression tag is a histidine tag, for example, a 6×HIS tag, preferably two 6×HIS tags, such as in SEQ ID NO:8. Novel nucleotide sequences are provided that encode the soluble CSPs of the present technology, which can be expressed in *E. coli* and have at least 85% homology, preferably at least 90% homology, preferably at least 95% homology, more preferably at least 99% homology to SEQ ID NO:1. In some aspects, the nucleotide sequence encoding the CSP of the present technology comprises nucleotide sequences for at least one expression tag, preferably at least two expression tags (for example, expression tags SEQ ID NO: 3 and SEQ ID NO: 4), and includes SEQ ID NO:5.

[0016] In another aspect, the present technology provides vaccines comprising the soluble rCSP disclosed herein and at least one adjuvant. The vaccines can be used to vaccinate a subject (such as a human or animal) and elicit an immune response. In some aspects, the vaccine produces high titer antibodies in the subject.

[0017] In some aspects, the novel rCSP induces high titer antibodies when formulated with at least one adjuvant, pref-