

Supplemental Appendix 1.

Design, production and characterization of recombinant *Plasmodium falciparum* and *Plasmodium vivax* MSP10 Proteins

Design

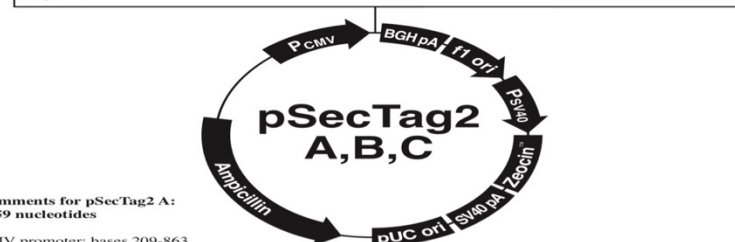
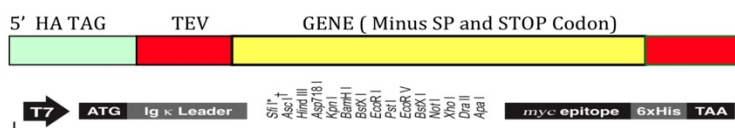
PLASMODB (Plasmodb.org) was the source for the amino sequences for recombinant protein design. The sequence of *P. falciparum* MSP10 was based on strain 3D7; that of *P. vivax* MSP10 was based on strain Sal I.

The post-signal amino acid sequences (**highlighted** in the complete predicted amino acid sequences below) of PfMSP10 (PF3D7_0620400) and PvMSP10 (PVX_114145) were synthesized in mammalian cell-optimized codons with predicted O- and N-glycosylation sites converted into alanine residues and cloned into the mammalian cell expression plasmid, pSecTag2. The recombinant protein was engineered to have an IgG Kappa secretory signal sequence, N-terminal hemagglutinin (HA) and C-terminal c-Myc epitope tags, with Tobacco Etch Virus (TEV) protease cleave sites inserted to release the otherwise-unmodified recombinant protein. Underlined amino acids were not included in final recombinant proteins.

Design of mammalian cell expression of recombinant *Plasmodium* PMS10

Gene for Synthesis

HA TAG: 9 a/a: Try-Pro-Try-Asp-Val-Pro-Asp-Try-Ala
TEV site: 6 a/a: Glu-Asn-Leu-Try-Phe-Gln-*Gly (*cleavage
RE sites (5' Bam H1 and 3' Xho I) followed by the following things:



Comments for pSecTag2 A:
5159 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
Murine Ig κ-chain VJ2-C signal peptide: bases 905-967
Multiple cloning site: bases 970-1081
c-myc epitope: bases 1082-1114
Polyhistidine tag: bases 1127-1144
BGH reverse priming site: bases 1167-1184
BGH polyadenylation sequence: bases 1166-1380
f1 origin: bases 1443-1856
SV40 promoter and origin: bases 1924-2245
EM-7 promoter: bases 2261-2327
Zeocin[™] resistance gene: bases 2328-2702
SV40 polyadenylation sequence: bases 2832-2961
pUC origin: bases 3345-4018
Ampicillin resistance gene: bases 4163-5023 (C)

* The Sfi I site is located before the variable region.

† Frame-dependent variations:
Asc I is only found in version A.

PF3D7_0620400 merozoite surface protein 1 amino acid sequence

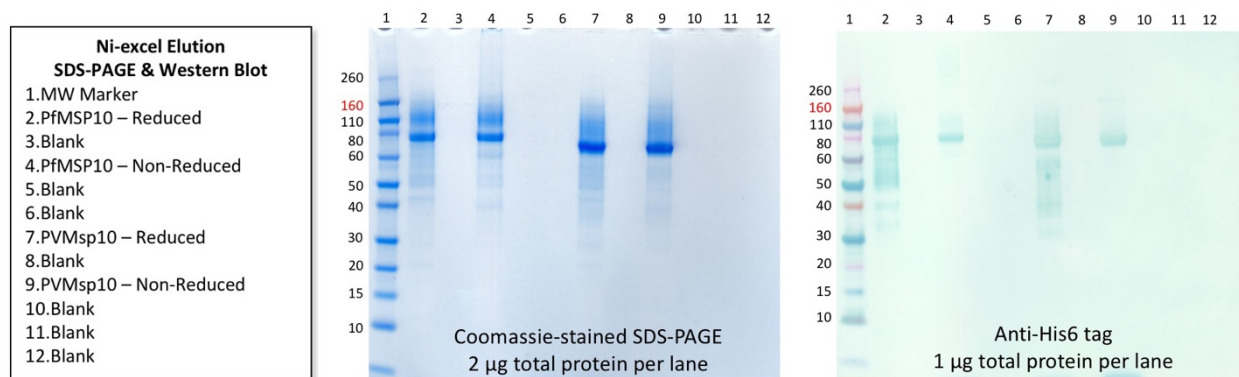
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KDNTQGSQHFNENIENNENVENNENIENNENNENIENIENNENNENNENIENNENNENN
ENSSIMNSESYNNIINSNEHNEEQIKKKEEDLIEAFFPFILKKLDNESLSLDNKYDDYYNLP
NDHNDTHKENSSDHNLLGYKLGNNLKSYLEENDVSQKKTDDINESASSDSENIQEILST
DSNTSHLKERKNQKAPPGEHKPEVKNALLNSQVASPKGEDEKKSQPQHPLVNSGDQLQ
HPKEIDENAEKIRRTLLKESRDIKNTTAIIDETVYKFEQLIMKGRYYATAVRNFVIFKVNY
ICEYSKCGPNSRCYIVEKDKEQCRCRPNYIVDMSVNYFKCIPMKDMNCSKNNGGCDVN
AECTIVEGAVKQCQSHLYFGDGVFCVKNSQTKQTLYILFIVILLVFQNFFI

PVX_114145 merozoite surface protein 10, putative

MKRAKCNKSLTFTIFLLLYVNGAVHVSANELNGTDGGDVPNQKDITKDYSIFERITSEGO
SASGKDDSPISQSNTQPQEEASDGKQGNTPLDEKNSAKDVEAHFIREGHDKVTHSDVGT
EEGKATGHVQKNANLRSTSYFSTQGAVSQAYNFVQENHPQLDNNGANVEQVARGQDD
VGSTENGEGNSGGEGNPLGSDKPGGKPEDASKDTPGNNPEESPNNRQEKGEKEKKEG
DTDNPNRGKTSEKADQQGGNHPNGLSPDEKNNPKTHNNHSESITNPGDVGALDGEENG
EGDDQTGISPTTEHPTGDAPPSDHAEKIKNTLLKEGIDLKETTSMDIDNAVYNMEQFILKT
KFYTTAIRNFVHFVKVNHICEYSKCGANARCYIVEKDKEECRCRANYMPDDSDYFKCIP
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ICLLHKFLF

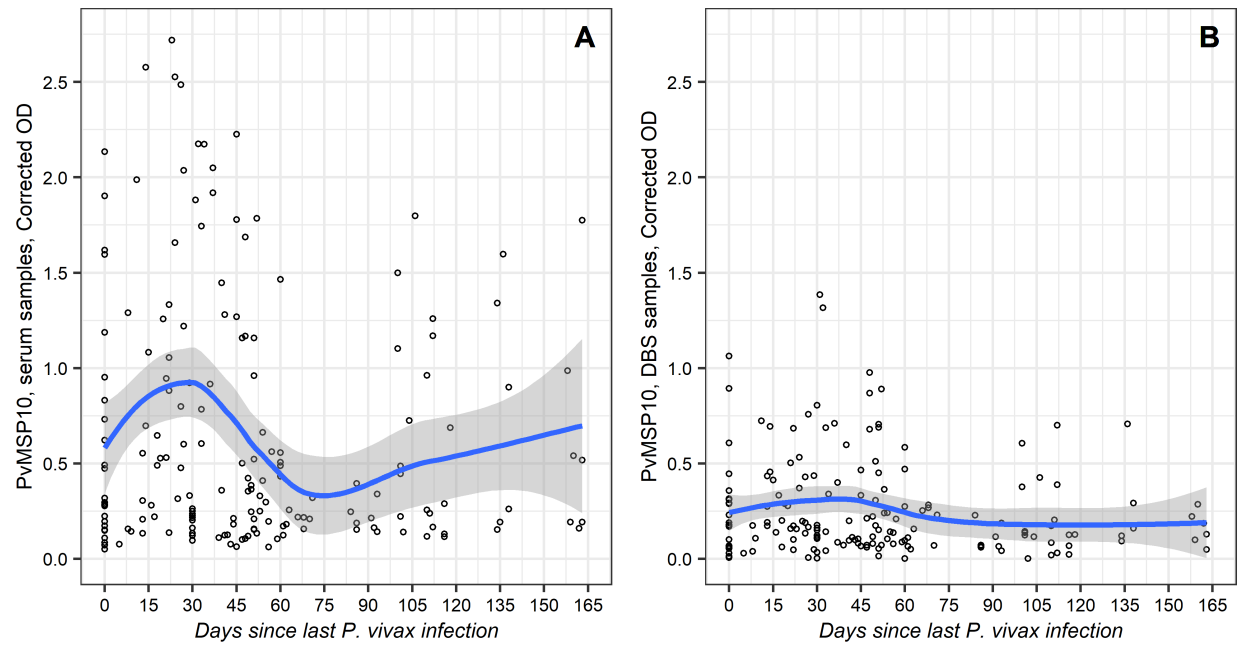
Production and characterization

Plasmid constructs were expressed in HEK-293 cells using transient transfection. Recombinant protein was affinity-purified from culture supernatants with nickel affinity chromatography. SDS-PAGE (Coomassie Blue staining) and Western blot (using anti-His₆ monoclonal antibody) analysis demonstrated proteins of the expected size. The identity of the recombinant proteins was further confirmed by anti-MSP10 monoclonal antibodies and mass spectrometry.



Supplemental Figure 1.

Relationship between corrected OD values and the time since last infection assessed through a LOESS smoothed fit curve with confidence region, A: serum samples, B: DBS samples.



FULL UNEDITED SDS-PAGE GEL and Western Blot

Ni-excel Elution
SDS-PAGE & Western Blot

1.MW Marker
2.PfMSP10 – Reduced
3.Blank
4.PfMSP10 – Non-Reduced
5.Blank
6.Blank
7.PVMsp10 – Reduced
8.Blank
9.PVMsp10 – Non-Reduced
10.Blank
11.Blank
12.Blank

