Supplemental Figure 1

Alignment of Native and Codon-Harmonized PfMSP8 (FVO) Gene Sequences

	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54
Native gene	ATG	GTG	TTC	AAA	AGT	TCG	GAC	ATA	TTT	TTC	TTT	CTC	TTC	CTT	GTG	ATA	CTT	TAT
Codon-harmonized	ATG	GTC	TTT	AAA	AGC	TCG	GAT	ATT	TTC	TTT	TTC	TTA	TTT	CTG	GTC	ATT	CTC	TAC

	57	60	63	66	69	72	75	78	81	84	87	90	93	96	99	102	105	108
Native gene	TTT	AAT	AAC	GTT	GTA	GAA	GGG	GAG	AAT	GGA	ACT	ACA	AAT	ATC	GAA	AAT	AAT	CCT
Codon-harmonized	TTC	AAC	AAT	GTT	GTA	GAA	GGG	GAG	AAC	GGT	ACT	ACC	AAC	ATA	GAA	AAC	AAC	CCA

	111	114	117	120	123	126	129	132	135	138	141	144	147	150	153	156	159	162
Native gene	GGA	AAT	AAT	GGT	AAT	ATG	GGA	CCA	TCA	GGA	CCA	AAA	GAT	AAA	GAC	AAG	AAT	ATT
Codon-harmonized	GGT	AAC	AAC	GGC	AAC	ATG	GGT	CCG	TCT	GGT	CCG	AAA	GAC	AAA	GAT	AAG	AAC	ATT

	165	168	171	174	177	180	183	186	189	192	195	198	201	204	207	210	213	216
Native gene	GAA	AAG	GAT	GTA	AAT	CAC	AAC	ATG	AGT	ATG	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT
Codon-harmonized	GAA	AAG	GAC	GTA	AAC	CAT	AAT	ATG	AGC	ATG	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC

	219	222	225	228	231	234	237	240	243	246	249	252	255	258	261	264	267	270
Native gene	AAT	GAC	AAT	AAT	AAT	AAT	ATA	AAT	AAC	AAT	AAT	AAT	AAT	AAT	ATA	AAT	AAC	AAC
Codon-harmonized	AAC	GAT	AAC	AAC	AAC	AAC	ATT	AAC	AAT	AAC	AAC	AAC	AAC	AAC	ATT	AAC	AAC	AAC

	273	276	279	282	285	288	291	294	297	300	303	306	309	312	315	318	321	324
Native gene	AAC	AAT	AAT	AAT	AAT	AAT	AAT	GGT	AAT	GGA	TTT	TCA	AAT	TTT	TTT	AAT	AAA	CTT
Codon-harmonized	AAT	AAC	AAC	AAC	AAC	AAC	AAC	GGC	AAC	GGT	TTC	TCT	AAC	TTC	TTC	AAC	AAA	CTC

	327	330	333	336	339	342	345	348	351	354	357	360	363	366	369	372	375	378
Native gene	TTT	GGA	AAA	AAA	AAA	GAT	AAC	AAA	AAA	GAG	GGA	GAA	GAA	AAA	AAT	GAA	GAA	GAT
Codon-harmonized	TTC	GGT	AAA	AAA	AAA	GAC	AAT	AAA	AAA	GAG	GGT	GAA	GAA	AAA	AAC	GAA	GAA	GAC

	381	384	387	390	393	396	399	402	405	408	411	414	417	420	423	426	429	432
Native gene	TTA	AAT	AGT	AAT	AAG	AAC	ATT	GAA	AGT	AAT	AAG	GGG	AGT	GCT	GTA	ACT	TCT	AAT
Codon-harmonized	CTG	AAC	AGC	AAC	AAG	AAT	ATT	GAA	AGC	AAC	AAG	GGG	AGC	GCT	GTA	ACT	AGC	AAC

	435	438	441	444	447	450	453	456	459	462	465	468	471	474	477	480	483	486
Native gene	GTA	GGG	GAT	ACA	AAT	AAT	GAC	GCA	AAA	GCA	AGG	GAC	AAC	AAT	AAT	AAT	GAT	GAT
Codon-harmonized	GTA	GGG	GAC	ACC	AAC	AAC	GAT	GCT	AAA	GCT	CGA	GAT	AAT	AAC	AAC	AAC	GAC	GAC

	489	492	495	498	501	504	507	510	513	516	519	522	525	528	531	534	537	540
Native gene	AAT	GAT	GAT	AAT	GAC	GAG	AAT	GAT	GAT	AAT	GAT	GAT	AAT	GAT	GAT	ATT	GAT	GAG
Codon-harmonized	AAC	GAC	GAC	AAC	GAT	GAG	AAC	GAC	GAC	AAC	GAC	GAC	AAC	GAC	GAC	ATT	GAC	GAG

	543	546	549	552	555	558	561	564	567	570	573	576	579	582	585	588	591	594
Native gene	ATT	GAT	GAA	CGT	GAT	GAT	AAT	GAT	GAT	AAT	GGT	GAT	GAT	AAT	GAT	GAT	AAT	GGT
Codon-harmonized	ATT	GAC	GAA	CGA	GAC	GAC	AAC	GAC	GAC	AAC	GGC	GAC	GAC	AAC	GAC	GAC	AAC	GGC

	597	600	603	606	609	612	615	618	621	624	627	630	633	636	639	642	645	648
Native gene	GAT	GAT	GAT	GAT	GAT	AAT	GAT	GAT	GAC	GAT	GAT	AAT	AAT	GAT	AAT	AAT	AAT	AAA
Codon-harmonized	GAC	GAC	GAC	GAC	GAC	AAC	GAC	GAC	GAT	GAC	GAC	AAC	AAC	GAC	AAC	AAC	AAC	AAA

	651	654	657	660	663	666	669	672	675	678	681	684	687	690	693	696	699	702
Native gene	AAT	AAT	AGT	AAT	AAT	TTA	ACG	GAT	ACA	AAA	AAG	GAA	GGG	GAG	AAA	ATT	GAT	TTA
Codon-harmonized	AAC	AAC	AGC	AAC	AAC	CTG	ACG	GAC	ACC	AAA	AAG	GAA	GGG	GAG	AAA	ATT	GAC	CTG

	705	708	711	714	717	720	723	726	729	732	735	738	741	744	747	750	753	756
Native gene	GGA	GTT	CAA	AAT	AAA	AAA	CAA	AAT	ATT	TTT	TCA	ACC	AAC	AAT	AAA	GGA	TTA	AAT
Codon-harmonized	GGT	GTT	CAG	AAC	AAA	AAA	CAG	AAC	ATT	TTC	TCT	ACT	AAT	AAC	AAA	GGT	CTG	AAC

	759	762	765	768	771	774	777	780	783	786	789	792	795	798	801	804	807	810
Native gene	AAA	TAT	AAT	ATA	GAT	AAT	GAA	TTA	AAA	GAA	GTA	GAT	GCA	CTT	TTG	AAA	AAT	GAT
Codon-harmonized	AAA	TAC	AAC	ATT	GAC	AAC	GAA	CTG	AAA	GAA	GTA	GAC	GCT	CTG	CTC	AAA	AAC	GAC

	813	816	819	822	825	828	831	834	837	840	843	846	849	852	855	858	861	864
Native gene	AAT	TAT	ATT	TTA	AAC	AAA	TAC	CAT	GTT	TCA	TTT	TTT	AAT	AAT	TTT	GAA	GAA	GAT
Codon-harmonized	AAC	TAC	ATT	CTG	AAT	AAA	TAT	CAC	GTT	TCT	TTC	TTC	AAC	AAC	TTC	GAA	GAA	GAC

	867	870	873	876	879	882	885	888	891	894	897	900	903	906	909	912	915	918
Native gene	ACA	TAT	AAT	AAG	AAG	AAA	TTT	ATA	AGA	CCG	TAT	GAT	CTT	AGC	TTG	TTA	AAA	AGT
Codon-harmonized	ACC	TAC	AAC	AAG	AAG	AAA	TTC	ATT	CGT	CCC	TAC	GAC	CTG	AGC	CTC	CTG	AAA	AGC

	921	924	927	930	933	936	939	942	945	948	951	954	957	960	963	966	969	972
Native gene	ATA	TTA	ATA	TAT	AGA	CAA	AGA	GTA	ACA	AGA	AAT	TGT	GTT	AAT	GTT	TTT	CAA	GAT
Codon-harmonized	ATT	CTG	ATT	TAC	CGT	CAG	CGT	GTA	ACC	CGT	AAC	TGC	GTT	AAC	GTT	TTC	CAG	GAC

	975	978	981	984	987	990	993	996	999	1,002	1,005	1,008	1,011	1,014	1,017	1,020	1,023	1,026
Native gene	CTC	AAT	GCT	GTT	TTT	GGT	AAA	TGT	TAT	AAT	AAA	GAT	GAC	ACT	AAA	TTA	AGT	ATT
Codon-harmonized	TTA	AAC	GCT	GTT	TTC	GGC	AAA	TGC	TAC	AAC	AAA	GAC	GAT	ACT	AAA	CTG	AGC	ATT

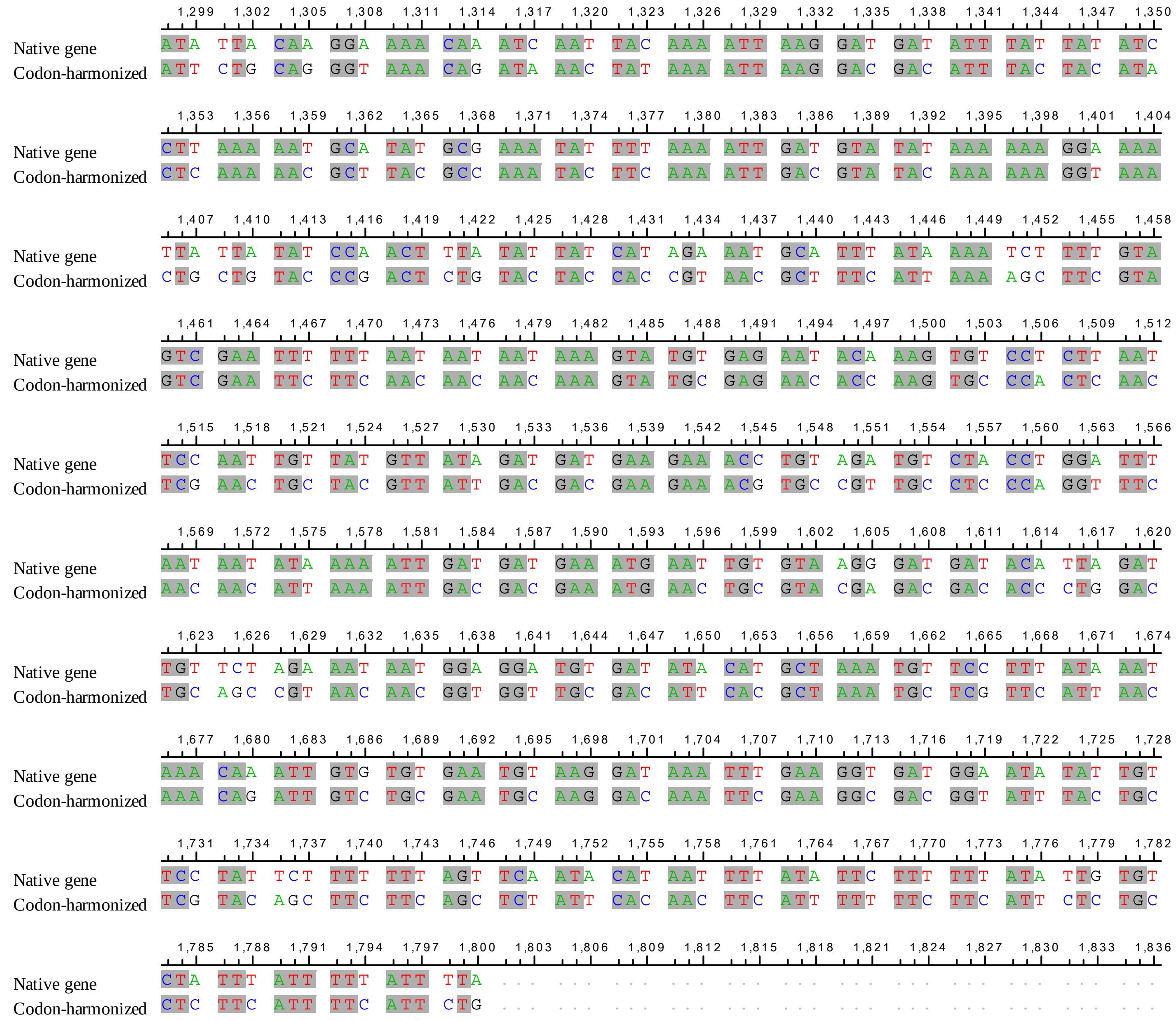
	1,029	1,032	1,035	1,038	1,041	1,044	1,047	1,050	1,053	1,056	1,059	1,062	1,065	1,068	1,071	1,074	1,077	1,080
Native gene	ACT	CGT	GAT	AAA	GTT	AAA	AAA	GAG	TTA	AGT	AGG	AAA	AAT	AGA	AAT	TTT	GTA	GAA
Codon-harmonized	ACT	CGA	GAC	AAA	GTT	AAA	AAA	GAG	CTG	AGC	CGA	AAA	AAC	CGT	AAC	TTC	GTA	GAA

	1,083	1,086	1,089	1,092	1,095	1,098	1,101	1,104	1,107	1,110	1,113	1,116	1,119	1,122	1,125	1,128	1,131	1,134
Native gene	TAC	TTA	ATT	GAA	ATG	TTA	GAA	AAT	ACC	CTT	AAT	AGT	ATG	AAT	GAT	GAT	TTT	ATT
Codon-harmonized	TAT	CTG	ATT	GAA	ATG	CTG	GAA	AAC	ACG	CTC	AAC	AGC	ATG	AAC	GAC	GAC	TTC	ATT

	1,137	1,140	1,143	1,146	1,149	1,152	1,155	1,158	1,161	1,164	1,167	1,170	1,173	1,176	1,179	1,182	1,185	1,188
Native gene	AAT	AAA	GAT	AAT	TTT	GAT	TTA	AAT	AAT	TAT	GTT	AAA	GAA	TTT	GAA	TTA	ATA	AAT
Codon-harmonized	AAC	AAA	GAC	AAC	TTC	GAC	CTG	AAC	AAC	TAC	GTT	AAA	GAA	TTC	GAA	CTG	ATT	AAC

	1,191	1,194	1,197	1,200	1,203	1,206	1,209	1,212	1,215	1,218	1,221	1,224	1,227	1,230	1,233	1,236	1,239	1,242
Native gene	TAT	TTA	TTA	ATA	CAT	GAA	GAT	TCA	GAT	ATA	TTT	TTA	GAA	ACA	TAT	AAT	TTA	ATA
Codon-harmonized	TAC	CTG	CTG	ATT	CAC	GAA	GAC	TCT	GAC	ATT	TTC	CTG	GAA	ACC	TAC	AAC	CTG	ATT

	1,245	1,248	1,251	1,254	1,257	1,260	1,263	1,266	1,269	1,272	1,275	1,278	1,281	1,284	1,287	1,290	1,293	1,296
Native gene	AGT	GGA	TTA	AAT	TCA	AAC	ATA	GAA	GAA	ACA	TCT	ATT	GAA	AAG	CTT	AAA	TAT	GCA
Codon-harmonized	AGC	GGT	CTG	AAC	TCT	AAT	ATT	GAA	GAA	ACC	AGC	ATT	GAA	AAG	CTC	AAA	TAC	GCT



1,299 1,302 1,305 1,308 1,311 1,314 1,317 1,320 1,323 1,326 1,329 1,332 1,335 1,338 1,341 1,344 1,347 1,350
Native gene ATA TTA CAA GGA AAA CAA ATC AAT TAC AAA ATT AAG GAT GAT ATT TAT TAT ATC
Codon-harmonized ATT CTG CAG GGT AAA CAG ATA AAC TAT AAA ATT AAG GAC GAC ATT TAC TAC ATA

1,353 1,356 1,359 1,362 1,365 1,368 1,371 1,374 1,377 1,380 1,383 1,386 1,389 1,392 1,395 1,398 1,401 1,404
Native gene CTT AAA AAT GCA TAT GCG AAA TAT TTT AAA ATT GAT GTA TAT AAA AAA GGA AAA
Codon-harmonized CTC AAA AAC GCT TAC GCC AAA TAC TTC AAA ATT GAC GTA TAC AAA AAA GGT AAA

1,407 1,410 1,413 1,416 1,419 1,422 1,425 1,428 1,431 1,434 1,437 1,440 1,443 1,446 1,449 1,452 1,455 1,458
Native gene TTA TTA TAT CCA ACT TTA TAT TAT CAT AGA AAT GCA TTT ATA AAA TCT TTT GTA
Codon-harmonized CTG CTG TAC CCG ACT CTG TAC TAC CAC CGT AAC GCT TTC ATT AAA AGC TTC GTA

1,461 1,464 1,467 1,470 1,473 1,476 1,479 1,482 1,485 1,488 1,491 1,494 1,497 1,500 1,503 1,506 1,509 1,512
Native gene GTC GAA TTT TTT AAT AAT AAT AAA GTA TGT GAG AAT ACA AAG TGT CCT CTT AAT
Codon-harmonized GTC GAA TTC TTC AAC AAC AAC AAA GTA TGC GAG AAC ACC AAG TGC CCA CTC AAC

1,515 1,518 1,521 1,524 1,527 1,530 1,533 1,536 1,539 1,542 1,545 1,548 1,551 1,554 1,557 1,560 1,563 1,566
Native gene TCC AAT TGT TAT GTT ATA GAT GAT GAA GAA ACC TGT AGA TGT CTA CCT GGA TTT
Codon-harmonized TCG AAC TGC TAC GTT ATT GAC GAC GAA GAA ACG TGC CGT TGC CTC CCA GGT TTC

1,569 1,572 1,575 1,578 1,581 1,584 1,587 1,590 1,593 1,596 1,599 1,602 1,605 1,608 1,611 1,614 1,617 1,620
Native gene AAT AAT ATA AAA ATT GAT GAT GAA ATG AAT TGT GTA AGG GAT GAT ACA TTA GAT
Codon-harmonized AAC AAC ATT AAA ATT GAC GAC GAA ATG AAC TGC GTA CGA GAC GAC ACC CTG GAC

1,623 1,626 1,629 1,632 1,635 1,638 1,641 1,644 1,647 1,650 1,653 1,656 1,659 1,662 1,665 1,668 1,671 1,674
Native gene TGT TCT AGA AAT AAT GGA GGA TGT GAT ATA CAT GCT AAA TGT TCC TTT ATA AAT
Codon-harmonized TGC AGC CGT AAC AAC GGT GGT TGC GAC ATT CAC GCT AAA TGC TCG TTC ATT AAC

1,677 1,680 1,683 1,686 1,689 1,692 1,695 1,698 1,701 1,704 1,707 1,710 1,713 1,716 1,719 1,722 1,725 1,728
Native gene AAA CAA ATT GTG TGT GAA TGT AAG GAT AAA TTT GAA GGT GAT GGA ATA TAT TGT
Codon-harmonized AAA CAG ATT GTC TGC GAA TGC AAG GAC AAA TTC GAA GGC GAC GGT ATT TAC TGC

1,731 1,734 1,737 1,740 1,743 1,746 1,749 1,752 1,755 1,758 1,761 1,764 1,767 1,770 1,773 1,776 1,779 1,782
Native gene TCC TAT TCT TTT TTT AGT TCA ATA CAT AAT TTT ATA TTC TTT TTT ATA TTG TGT
Codon-harmonized TCG TAC AGC TTC TTC AGC TCT ATT CAC AAC TTC ATT TTT TTC TTC ATT CTC TGC

1,785 1,788 1,791 1,794 1,797 1,800 1,803 1,806 1,809 1,812 1,815 1,818 1,821 1,824 1,827 1,830 1,833 1,836
Native gene CTA TTT ATT TTT ATT TTA ... ...
Codon-harmonized CTC TTC ATT TTC ATT CTG ... ...

Supplemental Figure 1. Codon-harmonized *Pf*MSP8 gene sequence. Shown are the coding sequences of the native *P. falciparum* MSP8 (FVO) gene and the codon-harmonized synthetic gene used for the recombinant expression of *Pf*MSP8 in *E. coli*. Identical bases are shaded in gray.

Supplemental Table 1

18-mer overlapping peptides of *Pf*MSP8

Peptide Number	Peptide Sequence	Peptide Location in Full-Length *Pf*MSP8 (FVO)
1	NGTTNIENNPGNNGNMGP	27-44
2	PGNNGNMGPSGPKDKDKN	36-53
3	SGPKDKDKNIEKDVNHNM	45-62
4	IEKDVNHNMSMNNNNNNN	54-71
5	SMNNNNNNNNNDNNNNIN	63-80
6	NNDNNNNINNNNNNNINN	72-89
7	NNNNNNINNNNNNNNNNG	81-98
8	NNNNNNNNGNGFSNFFNK	90-107
9	NGFSNFFNKLFGKKKDNK	99-116
10	LFGKKKDNKKEGEEKNEE	108-125
11	KEGEEKNEEDLNSNKNIE	117-134
12	DLNSNKNIESNKGSAVTS	126-143
13	SNKGSAVTSNVGDTNNDA	135-152
14	NVGDTNNDAKARDNNNND	144-161
15	KARDNNNNDDNDDNDEND	153-170
16	DNDDNDENDDNDDNDDID	162-179
17	DNDDNDDIDEIDERDDND	171-188
18	EIDERDDNDDNGDDNDDN	180-197
19	DNGDDNDDNGDDDDDNDD	189-206
20	GDDDDDNDDDDDNNDNNN	198-215
21	DDDNNDNNNKNNSNNLTD	207-224
22	KNNSNNLTDTKKEGEKID	216-233
23	TKKEGEKIDLGVQNKKQN	225-242
24	LGVQNKKQNIFSTNNKGL	234-251
25	IFSTNNKGLNKYNIDNEL	243-260
26	NKYNIDNELKEVDALLKN	252-269
27	KEVDALLKNDNYILNKYH	261-278
28	DNYILNKYHVSFFNNFEE	270-287
29	VSFFNNFEEDTYNKKKFI	279-296
30	DTYNKKKFIRPYDLSLLK	288-305
31	RPYDLSLLKSILIYRQRV	297-314
32	SILIYRQRVTRNCVNVFQ	306-323
33	TRNCVNVFQDLNAVFGKC	315-332
34	DLNAVFGKCYNKDDTKLS	324-341
35	YNKDDTKLSITRDKVKKE	333-350
36	ITRDKVKKELSRKNRNFV	342-359
37	LSRKNRNFVEYLIEMLEN	351-368
38	EYLIEMLENTLNSMNDDF	360-377
39	TLNSMNDDFINKDNFDLN	369-386
40	INKDNFDLNNYVKEFELI	378-395

41	**NYVKEFELINYLLIHEDS**	**378-404**
42	**NYLLIHEDSDIFLETYNL**	**396-413**
43	**DIFLETYNLISGLNSNIE**	**405-422**
44	**ISGLNSNIEETSIEKLKY**	**414-431**
45	**ETSIEKLKYAILQGKQIN**	**423-440**
46	**AILQGKQINYKIKDDIYY**	**432-449**
46	**YKIKDDIYYILKNAYAKY**	**441-458**
48	**ILKNAYAKYFKIDVYKKG**	**450-467**
49	**FKIDVYKKGKLLYPTLYY**	**459-476**
50	**KLLYPTLYYHRNAFIKSF**	**468-485**
51	**HRNAFIKSFVVEFFNNNK**	**477-494**
52	**VVEFFNNNKVCENTKCPL**	**486-503**
53	**VCENTKCPLNSNCYVIDD**	**495-512**
54	**NSNCYVIDDEETCRCLPG**	**504-521**
55	**EETCRCLPGFNNIKIDDE**	**513-530**
56	**FNNIKIDDEMNCVRDDTL**	**522-539**
57	**MNCVRDDTLDCSRNNGGC**	**531-548**
58	**DCSRNNGGCDIHAKCSFI**	**540-557**
59	**DIHAKCSFINKQIVCECK**	**549-566**
60	**QIVCECKDKFEGDGIYCS**	**560-577**

Number, sequence and amino acid position of overlapping synthetic peptides of *Pf*MSP8 (FVO) used to map T cell epitopes recognized by mice immunized with r*Pf*MSP8 or r*Pf*MSP8 (ΔAsn/Asp).