

## A *PFE0565w* Full Length and Truncated Sequence Alignment

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FL      ATGAACATGATTAATATTGGATATTTGCTTTTAGTTTTATCTTTTTTTGTTATTGGAAAAGACATTTTATGGTAGTGATACC
Trunc  ATGAACATGATTAATATTGGATATTTGCTTTTAGTTTTATCTTTTTTTGTTATTGGAAAAGACATTTTATGGTAGTGATACC

FL      TTTAGAGTATTTGAAGATTTTGATGTTAGAAGAAAAACAAAAATATGGTGTGTAATTTAAATCATGAAAAAAGTTATTTT
Trunc  TTTAGAGTATTTGAAGATTTTGATGTTAGAAGAAAAACAAAAATATGGTGTGTAATTTAAATCATGAAAAAAGTTATTTT

FL      CATAACAAAACATAAAATACGTCATACAAAAATAGAGTTTTAAAAGAAAGCATTCTTAATTTAGATTTAGATATGAAG
Trunc  CATAACAAAACATAAAATACGTCATACAAAAATAGAGTTTTAAAAGAAAGCATTCTTAATTTAGATTTAGATATGAAG

FL      AAATAAAGGATAATATCATAACCAGAAAAGAAAACACCTGAAAATATTTATAAAGAAATATATGAAAATAATTATGAAATG
Trunc  AAATAAAGGATAATATCATAACCAGAAAAGAAAACACCTGAAAATAT-----

FL      AAATATGATGAAGATATTCCTAATAATATGAGTGAAGAAAAAGAGATGAAAAGGAAGTGATTGAACATCTAGAAATAGAC
Trunc  -----

FL      GAAAAAAACGGAAAACAATACAAAAGTGATATAAATAAACAGTTAGTTTATCACATCTTAAACAATATAAAAAATATTTAT
Trunc  -----

FL      GTTAATAACAATAATAAAATAAATAAAGTATAGACAAACATTTACCTTCATATAATTTAGAAAAGGAAAAATAAT
Trunc  -----

FL      AAGTATCTTAACTTTTTACTCGTAGATAATAGGAATGAATCTTATACCTTTATGGTGCCTATGAAATTTTATATAAATCAT
Trunc  -----

FL      GAAATGTATAATATATCAGATGAGGAATATAATAAATTAATGGAAGATAATAGTGTAGATGTTTATTTAAATAATATATTG
Trunc  -----

FL      GTTGAATACAAATATGAAAATTTGAAAATAAAGAAAGGAGAAGTTGATGGAGAAGTTGAAGGAGAAGGAGAAGTAGAAGGA
Trunc  -----
FL      GAAGTAGAAGGAGAAGTAAAAGGAAAAGTGGTAGAAGGAATAGAAAATAACATGAATGAGGAAGAAAATATAATAAAGAT
Trunc  -----

FL      AATAAAGATAAGGAAAATCAAAATAAATTCAAAACGGACAAGATGAAAACACTGAATTTCAAGAAAATGATAAACAATGATAGT
Trunc  -----

FL      GTAATTATGAAAATATACCATTATTATTTTCAGGATTAGTTCTCTTATTTTGTATCAGTTTTATTATTTATTATTTTGATATT
Trunc  -----CCATTATTATTTTCAGGATTAGTTCTCTTATTTTGTATCAGTTTTATTATTTATTATTTTGATATT

FL      ATACAAAAGGTAATAAATGAAGCTAAATAAAAAAAGAAAATCTAATGCAACCATGGCAATAAATAGAGACAAAATTCAGAG
Trunc  ATACAAAAGGTAATAAATGAAGCTAAATAAAAAAAGAAAATCTAATGCAACCATGGCAATAAATAGAGACAAAATTCAGAG

FL      GAATTTATGTGA
Trunc  GAATTTATGTGA

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## B Translation *PFE0565w* Truncated Sequence

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Met Asn Met Ile Asn Ile Gly Tyr Leu Leu Leu Val Leu Ser Phe Leu Leu Leu Glu Lys Thr Phe
Tyr Gly Ser Asp Thr Phe Arg Val Phe Glu Asp Phe Asp Val Arg Arg Lys Asn Lys Ile Trp Cys
Cys Asn Leu Asn His Glu Lys Ser Tyr Phe His Asn Lys Thr Ile Asn Thr Ser Tyr Lys Asn Arg
Val Leu Lys Glu Ser Ile Leu Leu Asn Leu Asp Leu Asp Met Lys Lys Tyr Lys Asp Asn Ile Ile
Thr Arg Lys Lys Thr Pro Glu Asn Ile His Tyr Tyr Phe Arg Ile Ser Ser Leu Ile Leu Tyr Gln
Phe Tyr Tyr Leu Leu Phe *** Tyr Tyr Thr Lys Gly Lys Asn Glu Ala Lys *** Lys Lys Lys Ile
*** Cys Asn His Gly Asn Lys *** Arg Gln Asn Ser Arg Gly Ile Tyr Val

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SUPPLEMENTAL FIGURE 1. An alternative *PFE0565w* transcript is present in the *P. falciparum* erythrocytic stages, including gametocytes; however, it does not translate into a full-length protein. DNA sequencing using *PFE0565w* gene specific primers was conducted to verify that the alternative transcript amplified during the RT-PCR experiments using mixed erythrocytic and mixed gametocyte stages was *PFE0565w*. (A) DNA sequencing results were aligned with the full-length *PFE0565w* sequence obtained from PlasmoDB using VectorNTI Contig Express (Invitrogen). (B) The truncated *PFE0565w* sequence obtained does not translate into a full length protein. FL = full length *PFE0565w* transcript; Trunc = truncated *PFE0565w* transcript.

**A Erythrocytic Stage Counts**

Bio Rep	Immune	Positive Control	Preimmune
1	4 / 324 = 1.2%	324 / 324 = 100%	0 / 318 = 0.0%
2	4 / 311 = 1.3%	311 / 311 = 100%	0 / 314 = 0.0%
3	3 / 310 = 0.97%	310 / 310 = 100%	0 / 319 = 0.0%

**B Gametocyte Counts**

Bio Rep	Immune	Positive Control	Preimmune
1	3 / 200 = 1.5%	200 / 200 = 100%	0 / 188 = 0.0%
2	2 / 200 = 1.0%	200 / 200 = 100%	0 / 200 = 0.0%
3	3 / 204 = 1.5%	204 / 204 = 100%	0 / 203 = 0.0%

**C Oocyst Sporozoite Counts**

Bio Rep	Immune	Positive Control	Preimmune
1	0 / 30 = 0.0%	30 / 30 = 100%	0 / 30 = 0.0%
2	0 / 183 = 0.0%	183 / 183 = 100%	0 / 175 = 0.0%

**D Salivary Gland Sporozoite Counts**

Bio Rep	Immune	Positive Control	Preimmune
1	158 / 160 = 98.7%	160 / 160 = 100%	0 / 160 = 0.0%
2	160 / 163 = 98.1%	163 / 163 = 100%	0 / 159 = 0.0%
3	189 / 193 = 97.9%	193 / 193 = 100%	0 / 188 = 0.0%

SUPPLEMENTAL FIGURE 2. *P. falciparum* mixed erythrocytic stage parasites (**A**), day 16 mixed gametocytes (**B**), oocyst sporozoites (**C**), and salivary gland sporozoites (**D**) were counted and the *PFE0565w* protein pattern was observed for each parasite during immunofluorescent assays. To verify *PFE0565w* protein presence in all erythrocytic stages and sporozoite stages examined, protein patterns were examined for each parasite counted. This was done for both *PFE0565w* immune and preimmune serum. All parasites counted labeled with the positive controls (parasites were triple-labeled - PfMSP1-19 for mixed erythrocytic stages, Pfs230 for gametocyte stages, and PfsCS for sporozoite stages). The very limited labeling of parasites with immune serum during the mixed erythrocytic and gametocyte stages likely reflects non-specific binding of the anti-*PFE0565w* antibodies.