

Supplementary Information

Inhibition of parasite invasion by monoclonal antibody against epidermal growth factor-like domain of *Plasmodium vivax* merozoite surface protein 1 paralog

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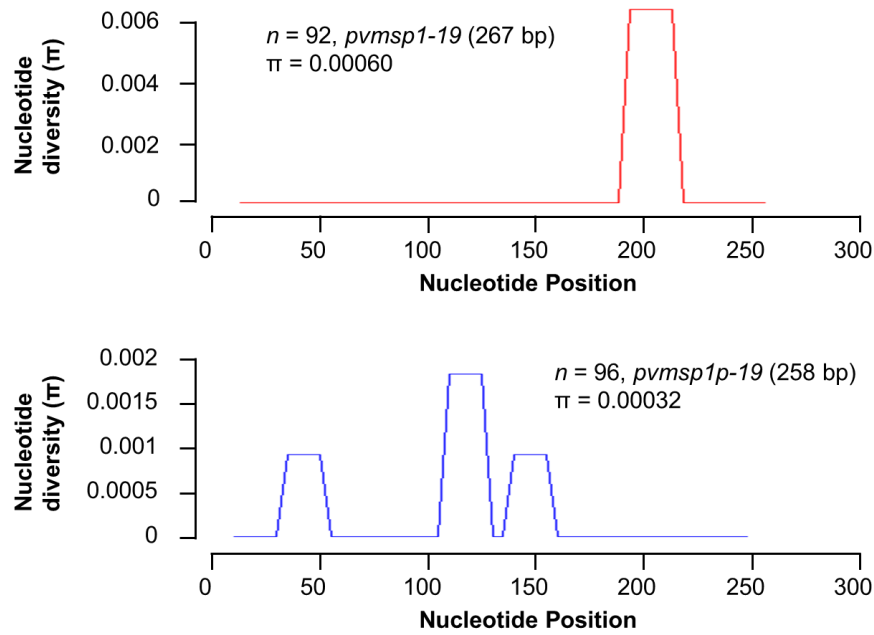
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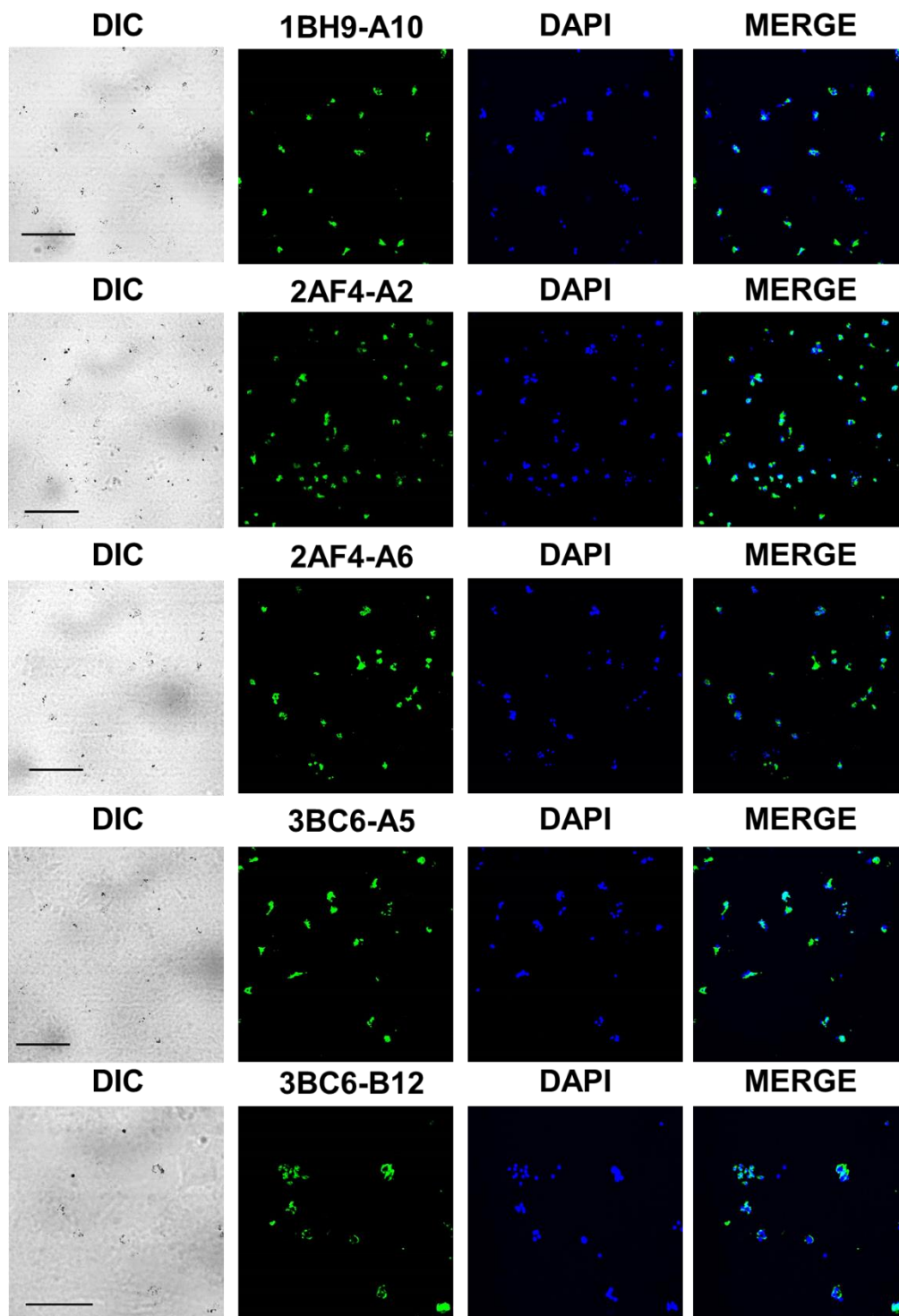
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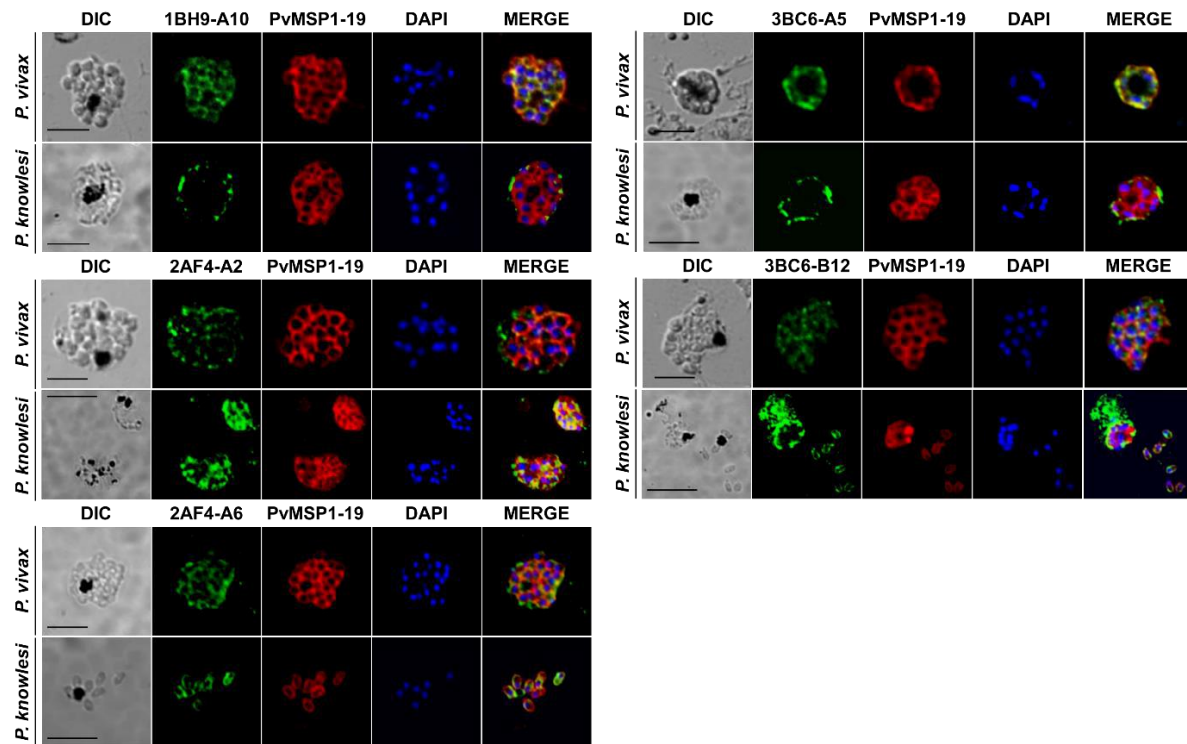
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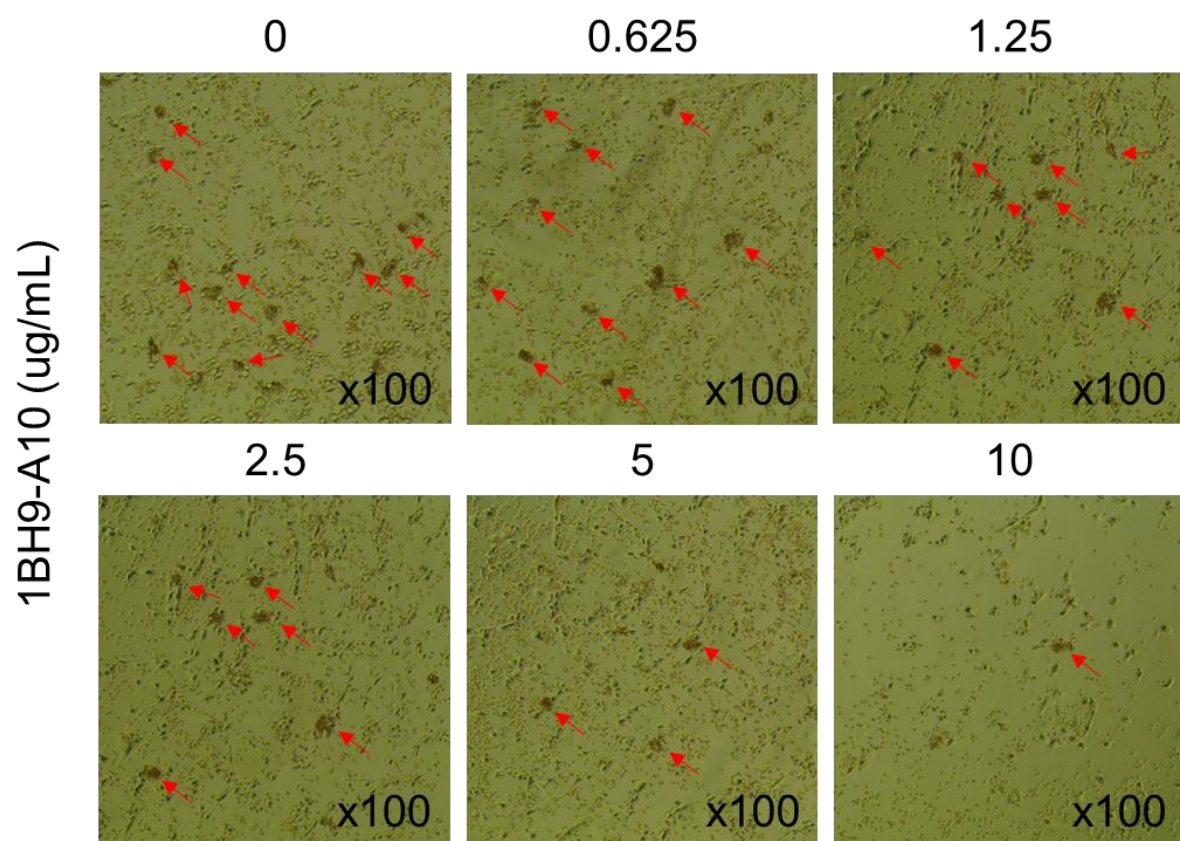
Supplementary Figure S1. The *pvmsp1-19* and *pvmsp1p-19* nucleotide diversity. *pvmsp1* ($n=92$) and *pvmsp1p* ($n=66$) isolates were obtained from PlasmoDB originating from 10 countries (Brazil, China, Columbia, India, Mauritania, Mexico, North Korea, Peru, Papua New Guinea, and Thailand) and *pvmsp1p-19* ($n=30$) sequence from ROK, Thailand and Myanmar were used for nucleotide diversity analysis. The nucleotide diversity (π) of *pvmsp1-19* was 0.00060 and *pvmsp1p-19* was 0.00032, thus indicating that *pvmsp1p-19* was limited polymorphism worldwide. Graphical visualization was analyzed using the sliding window option with window length 20 and step size 5 site in DNAsp ver. 5.0 software.



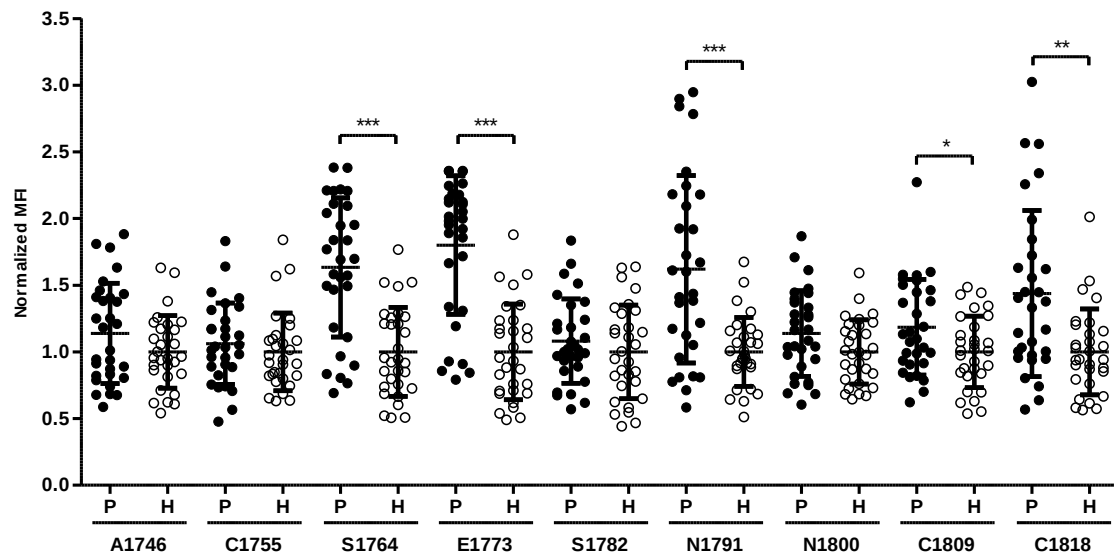
Supplementary Figure S2. The monoclonal antibodies recognition of *P. knowlesi* parasite on immunofluorescence assay. The mature schizont of *P. knowlesi* was labelled with PvMSP1P-19 monoclonal antibodies (green). Nuclei are visualized with DAPI (blue). Bars indicate 25 μm .



Supplementary Figure S3. *P. vivax* and *P. knowlesi* Immunofluorescence assay. The recognition of monoclonal antibodies with naïve PvMSP1P and PkMSP1P by immunofluorescence assay. The mature schizont of *P. vivax* and *P. knowlesi* was dual labelled with PvMSP1P-19 monoclonal antibodies (green) and rabbit immune sera against PvMSP1-19 (red, merozoite surface marker). Nuclei are visualized with DAPI (blue). Bars indicate 5 µm.



Supplementary Figure S4. The rosette formation of PvMSP1P-19. Erythrocyte binding rosettes formed on the surfaces of COS-7 cells expressing PvMSP1P-19 under the serial diluted 1BH9-A10. The red arrow indicates positive rosette formation.



Supplementary Figure S5. B-cell epitope of PvMSP1P-19 on vivax patient. Thirty vivax malaria patient serum and healthy individual serum was used for PvMSP1P-19 B-cell epitope screening. The normalized MFI was calculated by each peptide with the patient MFI divided by the average value of healthy individual MFI for comparison of each peptide reactivity. Significant differences are shown as single asterisks, $p < 0.05$, double asterisks, $p < 0.01$ and triple asterisks, $p < 0.0001$ by student *t*-test. P, vivax-infected patient serum; H, healthy individual serum.

Supplement Dataset 1. Thirty of *pvmSP1p-19* sequence from Republic of Korea (KOR), Myanmar (MYA) and Thailand (THA). *PvmSP1p-19* nucleotide sequences are available under GenBank accession numbers MF968906 to MF968935.

		10	20	30	40	50	60	70	80
(PVX_099975) PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(KOR) 201401_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(KOR) 201402_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(KOR) 201404_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(KOR) 201502_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(KOR) 201503_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(KOR) 201504_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(KOR) 201505_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(KOR) 201602_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(KOR) 201603_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(KOR) 201604_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(KOR) 201605_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV01_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV02_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV03_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV04_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV07_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV08_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV09_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV10_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV13_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV14_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV15_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(MYA) KV16_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(THA) LS02_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(THA) PR01_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(THA) TS02_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(THA) TS03_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(THA) TS04_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(THA) TS05_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								
(THA) TS06_PvMSP1P-19	GACCGTGTGAAAAAGAACTGCAGAAATAGGAAGTGCCCACTCAACTCCTTCTGCTTCATTCAAACGATTAATGAAGAGTG								

[illegible]

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(KOR) 201401_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(KOR) 201402_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(KOR) 201404_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
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(KOR) 201503_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(KOR) 201504_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(KOR) 201505_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(KOR) 201602_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(KOR) 201603_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(KOR) 201604_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(KOR) 201605_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(MYA) KV01_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(MYA) KV02_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(MYA) KV03_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(MYA) KV04_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
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(THA) LS02_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(THA) PR01_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
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(THA) TS03_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(THA) TS04_PvMSP1P-19	GGTG	CGATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC	
(THA) TS05_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC
(THA) TS06_PvMSP1P-19	GGT	GCG	ATT	TGA	AGG	CAAC	GTG	CAG	TTG	AAAA	AAAA	AT	CGC	GTA	AA	TG	CATT	TG	CCCC	AAG	GGC	ACG	AA	ACCC	AT	GC	AC

(PVX_099975) PvMSP1P-19 GAGGGGGTCGTGTCAGC
 (KOR) 201401_PvMSP1P-19 GAGGGGGTCGTGTCAGC
 (KOR) 201402_PvMSP1P-19 GAGGGGGTCGTGTCAGC
 (KOR) 201404_PvMSP1P-19 GAGGGGGTCGTGTCAGC
 (KOR) 201502_PvMSP1P-19 GAGGGGGTCGTGTCAGC
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 (KOR) 201504_PvMSP1P-19 GAGGGGGTCGTGTCAGC
 (KOR) 201505_PvMSP1P-19 GAGGGGGTCGTGTCAGC
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 (KOR) 201603_PvMSP1P-19 GAGGGGGTCGTGTCAGC
 (KOR) 201604_PvMSP1P-19 GAGGGGGTCGTGTCAGC
 (KOR) 201605_PvMSP1P-19 GAGGGGGTCGTGTCAGC
 (MYA) KV01_PvMSP1P-19 GAGGGGGTCGTGTCAGC
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 (THA) TS05_PvMSP1P-19 GAGGGGGTCGTGTCAGC
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