frontiers

Supplementary Material

Plasmodium falciparum var gene is activated by its antisense long noncoding RNA

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Supplementary Tables

Target	Sequence (5'-3')
5' RACE	
PF3D7_0617400 antisense lncRNA	GATTACGCCAAGCTTGAACCTGAAGCTGATAAAGGCCCAGTC
Primer pairs for mRNA qPCR detection	
var family	See reference (Salanti et al., 2003)
T7 RNA polymerase	GGAATACAAGAAGCCTAT TGTTGGTGTTAATGGTAG
PF3D7_0100300	CATCATCTAAAAATCTTAATATCTA CTTTATTTCATATTTATATTGTGAG
Rex1	AATCGGGTGCTCCATACAAG CGTCTTTTTGTCCCTGTTCTG
Primer pairs for *var* aslncRNA qPCR detection	
PF3D7_0617400 aslncRNA (episome, p1/p2)	TGTGTAGAACATTTGGCGCA CAAGAAAACAGATCCCCTAC
PF3D7_0617400 aslncRNA (endogenous, p3/p4)	GGAATTGGTTTTGCTGCAATC CATCCACATCCACATACATAC
PF3D7_0400400	CTACTATCCCGTTTGGTATTG CATCCACACGTAAACATATCC

PF3D7_0425800	TTATTTTCTACCATCCTCTGG TCCAAACATACACAACATACAC
PF3D7_1200400	TTGGCATTAGGATCCATTGCT AACGCTCAAACATACATATACAG
PF3D7_1100200	GCGTTGACTTACTTTTTACTC CATACCCAAACATACATAAGC
PF3D7_0300100	CCGTTTGGTATTGCATTGGC ACGTAAACATATCCCCCACAC
PF3D7_0223500	TGGCATTAGGATCCATTGCTT TATATCCAAACACACCCACAC
PF3D7_0413100	TATTGGTTTTGCTGCGTTCAC CCAAACATACCCCACAAATAC
PF3D7_1240600	ATCGGTTTTGCTGCATTCACT ACAATCATATCAAACACATCCAC
PF3D7_0711700	CATCACAACCAACAACCCC CACTCATACATACATATACACAC
pT7SE and pT7 construction	
T7 RNA polymerase	CACATTTCGAATAAACTCGAGATAACACGATTAACATCGCTAAGAAC GACCTGCAGGGTACCTTACGCGAACGCGAAGT
Nuclear localization signal of Gal4p (1-222 bp)	CACATTTCGAATAAACTCGAGATGAAGCTACTGTCTTCTATCG GATGTTAATCGTGTTCCCGGGTCGAGGAAAAATCAGTAGAAATAGC
Flag-tag	CTGATTTTTCCTCGAGACTACAAGGACGACGATGACAAGAACACGATT AACATC GATGTTAATCGTGTTCTTGTCATCGTCGTCCTTGTAGTCTCGAGGAAAA ATCAG
T7 promoter and T7 terminator (*)	Forward1: CTATAGGGAGACCCGGGTTCTTGCAAGATAACTAGCATAACCCCTTGGG GCCTCT Reverse1: TTTCAGCAAAAAACCCCTCAAGACCCGTTTAGAGGCCCCAAGGGGTTA TGCTAGTT Forward2: GCCAGCCTAGGAGTTCCATGGAAATTAATACGACTCACTATAGGGAGAC CCGGGT Reverse2: TTCATATCGATAACTATCCGGATATAGTTCCTCCTTTCAGCAAAAAACCC CTCAAG
pT7SE-as0617400 and pT7-as0617400 construction	
PF3D7_0617400 aslncRNA template	GACTCACTATAGGGAGAGAAACACATATACATCAACAGAT CAAGAAAACAGATCCCCTACTTAGCCAGTTCAGCAT
Deletion of NLS-T7RNP operon	AAGACAGATCTTCGGGCGGCCGCGAGTATTCTATAGTGTC GACACTATAGAATACTCGCGGCCGCCCGAAGATCTGTCTT
Templates for FISH probe synthesis	
PF3D7_1240600 FISH template	ACATGACGAGGTACAGAAAG GCTTGTGGTGTCACCTG
PF3D7_0617400 FISH template	CAATACTTTCCACTGATAGAGC CCAAACTTCTTTTCTGTTTGCCT
pT7SE-as0617400-exonI construction	
PF3D7_0617400 aslncRNA template (exonI region)	ACTCACTATAGGGAGGTAACTGATTGCAGCAAAACC GTTATCTTGCAAGAACCCGGGCTACTTAGCCAGTTCAGCAT
pUC15A-NLS-T7RNP construction (**)	
p15A replicon	CACCGCCGGACATCAGCG CGGGGCATGACTAACATG

pUC19 fragment lacking replicon	GGATCTCAAGAAGATCCTTTG GTGAGCTGATACCGCTCGC
NLS-T7RNP	ATGACCATGATTACGCCAAGCTTGATGAAGCTACTGTCTTCTATCG GAGTCGACCTGCAGGCATTTACGCGAACGCGAAG

Supplementary Table S1. All primers used in this study. The vectors were constructed by In-Fusion technology (Vazyme). The sequence underlined is the homologous fragment for cloning as commercial standard manuals described. *: To obtain this fragment, products were amplified by two steps. The first product was amplified by primer pair Forward1/Reverse1 without template. Then, the second PCR was performed by primer pair Forward2/Reverse2 and using the first step product as template. **: p15A replicon and NLS-T7RNP were prepared by PCR. To construct pUC15A, p15A replicon was phosphorylated with T4 polynucleotide kinase, and ligated to pUC19 fragment lacking replicon with T4 DNA ligase.

References

Salanti, A., Staalsoe, T., Lavstsen, T., Jensen, A. T., Sowa, M. P., Arnot, D. E., et al. (2003). Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering Plasmodium falciparum involved in pregnancy-associated malaria. *Mol. Microbiol.* 49, 179–191.