PCR_Sequencing for genotyping SNPs PF3D7_1343700 Kelch protein propeller domain

Didier Menard (dmenard@pasteur.fr)
Malaria Molecular Epidemiology Unit
Frederic Ariey (frederic.ariey@pasteur.fr)
Unité d'Immunologie Moléculaire des Parasites, Institut Pasteur, Paris, France

Method Article

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Abstract

This procedure is designed to genotype point mutations on chromosome 13 \(PF3D7_1343700) in Kelch protein propeller domain of Plasmodium falciparum, identified in a study by Ariey et al, 2013.

Introduction

This procedure is intended for use in molecular studies of DNA extracted from dried blood spots or whole blood samples for genotyping of P. falciparum infections. It describes the genotyping procedure for SNPs detection in Kelch protein propeller domain of Plasmodium falciparum \(PF3D7_1343700). Full gene sequences are given in Appendix A and SNPs already observed are given in Appendix B. This procedure is applicable for well-equipped laboratories with staff familiar with PCR and sequencing.

Reagents

General - Micropipets and tips \(10 µL, 200 µL and 1000 µL) 1.5 mL centrifuge tubes - PCR tubes with caps - Disposable gloves - Fine tip marker pens - Nuclease-free water PCR - 10X PCR buffer \(MgCl2-free) - MgCl2 \(concentration varies) - dNTP \(concentration varies) - Taq DNA Polymerase \(5U/µL) - Primers for PCR and nested PCR \(see Table 1) - Parasite DNA standards: 3D7 at 25 pg/µL Agarose gel electrophoresis - 6X Xylene cyanol dye - Ethidium bromide \(10 mg/mL) - Agarose - 1X TBE \(Tris/Borate/EDTA) buffer - 50 bp or 100 bp or 200 bp size standard with xylene cyanol dye added or ready-to-use - SmartLadder MW-1700-10 \(Eurogentec) - Parafilm

Equipment

- Thermocycler - Gel electrophoresis apparatus including chamber and power pack - Microwave to melt agarose

Procedure

1. PCR 1.1. Prepare Primary PCR Master Mixes in a 1.5 mL centrifuge tube according to the volumes calculated using Table 2. Include enough reactions for DNA controls \(3D7) and negative \(no template) controls. 1.2. Label PCR tubes and add 20 µl Primary Master Mix to each tube. 1.3. Add 5 µl of template DNA to each tube. Seal and run PCR in thermocycler according to the conditions listed in Table 3. 2. Nested PCR 2.1. Prepare nested PCR Master Mixes in a 1.5 mL centrifuge tube according to the volumes calculated using Table 4. 2.2. Label PCR tubes and add 45 µl Secondary Master Mix to each tube. 2.3. Add 5 µl of Primary PCR product to each tube. Seal and run PCR in thermocycler according to the conditions listed in Table 5. 2.4. Run an agarose gel of Nested PCR product to ensure amplification has been successful \(See Section 3). NOTE: PCR product may be stored at 4 °C for up to 1 week or at – 20 °C or -80 °C for long-term storage. 3. Agarose gel electrophoresis 3.1. Make a 2% agarose gel: Dissolve 2 grams of agarose and 100 mL of 1X TBE in the microwave. Cool, then add 4 µL Ethidium bromide and

gently swirl to mix. Pour into assembled gel tray with comb\(s) and leave at room temperature for 30 minutes to set. 3.2. Load the gel: Place the gel in gel apparatus and fill to line with 1X TBE. Place 2 µL dots of xylene cyanol per sample on Parafilm. Carefully pipet 10 µL Nested PCR product to each dot of dye. Add 4-5 µL of size standard. 3.3. Run gel at 100-150 volts for 60 minutes and view using a UV transilluminator. Expected size: 849 bp \(Figure 1). 4. Sequencing Send 40 µl of N1 PCR products for sequencing, according to the company's instructions.

References

1. Ariey _et al._ A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. _Nature_ \(2013) doi: "10.1038/nature12876":http://dx.doi.org/10.1038/nature12876

Figures

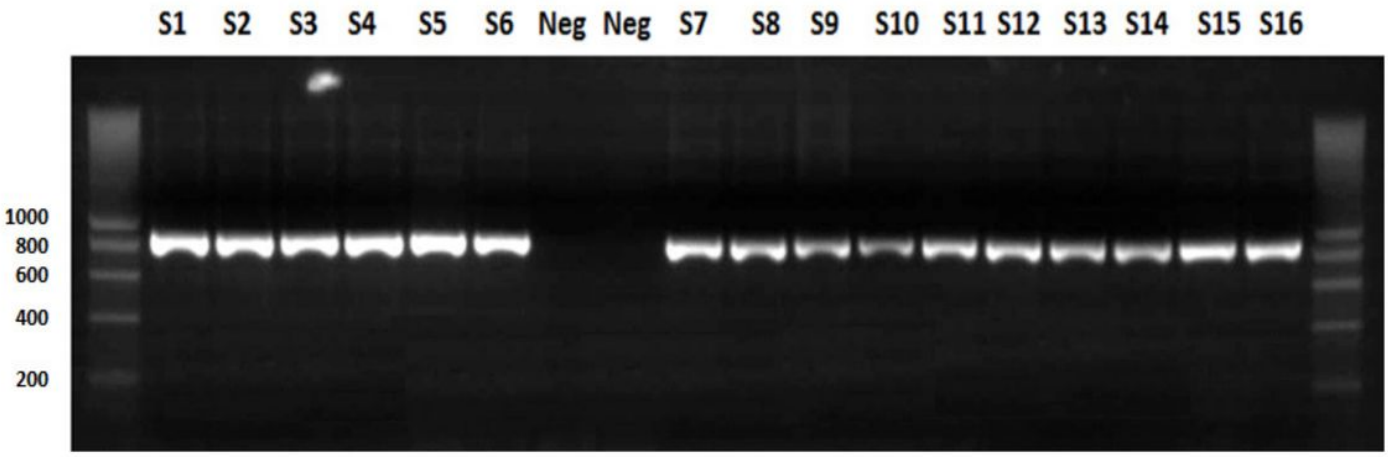
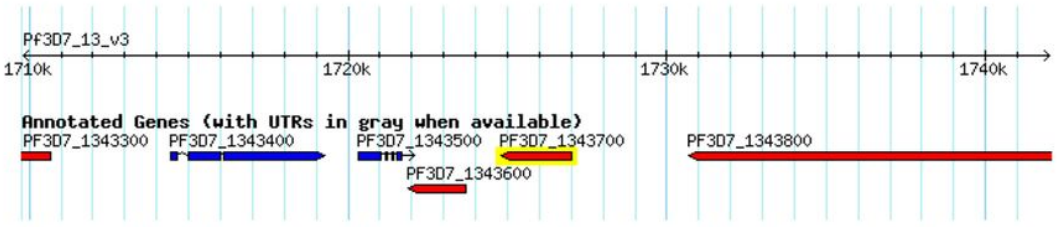


Figure 1

PCR products for Nested PCR for PF3D7_1343700. S1: 3D7, S2-S16: tested samples, Neg: PCR negative controls Image source: Didier Ménard, Institut Pasteur du Cambodge.



2181 bp sequences flanking candidate marker SNPs from 3D7 complete genome are given. Positions of primary primers (yellow) and secondary primers (green) are shown.

>gi|124513603|ref|XM_001350122.1| Plasmodium falciparum 3D7 kelch protein, putative (PF13_0238) mRNA, complete cds

ATGGAAGGAGAAAAAGTAAAAACAAAAGCAAATAGTATCTCGAATTTTTCTATGACGTATGATAGGGAAT
CTGGTGGTAACAGCAATAGTGATGATAAAAGCGGAAGTAGTAGCGAGAATGATTCTAATTCATTTATGAA
TCTAACTAGTGATAAAAATGAGAAAACGGAAAATAATAGTTTCCTTTTAAATAATAGTAGTTATGGAAAT
GTTAAAGATAGCCTATTAGAATCCATTGATATGAGTGTATTAGATTCGAACTTTGATAGTAAAAAAGATT
TTTTACCAAGTAATTTATCAAGAACATTTAATAATATGTCTAAAGATAATATAGGAAATAAATATTTAAA
TAAATTGTTAAATAAAAAAAAAGATACTATTACAAATGAAAATAATAATATTAATCATAATAATAATAAT
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CTTAATCAAATATATGTTGTTGGAGGTATTGATAATGAACATAACATATTAGATTCCGTTGAACAATATC
AACCATTTAATAAAAGATGGCAATTTCTAAATGGTGTACCAGAGAAAAAAATGAATTTTGGAGCTGCCAC
ATTGTCAGATTCTTATATAATTACAGGAGGAGAAAATGGCGAAGTTCTAAATTCATGTCATTTCTTTTCA
CCAGATACAAATGAATGGCAGCTTGGCCCATCTTTATTAGTTCCCAGATTTGGTCACTCCGTTTTAATAG
CAAATATATAA

Figure 2

P. falciparum 3D7 protein coding gene on Pf3D7_13_v3 from 1,724,817 to 1,726,997 (Chromosome: 13) 2181 bp sequences flanking candidate marker SNPs from 3D7 complete genome are given. Positions of primary primers (yellow) and secondary primers (green) are shown.

Codon Position	Amino Acid reference sequence	Amino Acid mutant-type sequence	Nucleotide reference sequence	Nucleotide mutant-type sequence
449	G	A	ggt	gCt
458	N	Y	aat	Tat
474	T	I	aca	aTa
476*	M	I	atg	atA
481	A	V	gct	gTt
493	Y	H	tac	Cac
508	T	N	act	aAt
527	P	T	cct	Act
533	G	S	ggt	Agt
537	N	I	aat	aTt
539	R	T	aga	aCa
543	I	T	att	aCt
553	P	L	ccg	cTg
561	R	H	cgt	cAt
568	V	G	gtg	gGg
574	P	L	cct	cTt
580	C	Y	tgt	tAt
584	D	V	gat	gTt
612**	E	D	gaa	gaT
623	S	C	agt	Tgt

Figure 3

Polymorphisms observed in the K13-propeller domain ==*== not observed in Cambodia, observed in F32-ART4 ==**== not observed in Cambodia, reported in The Gambia

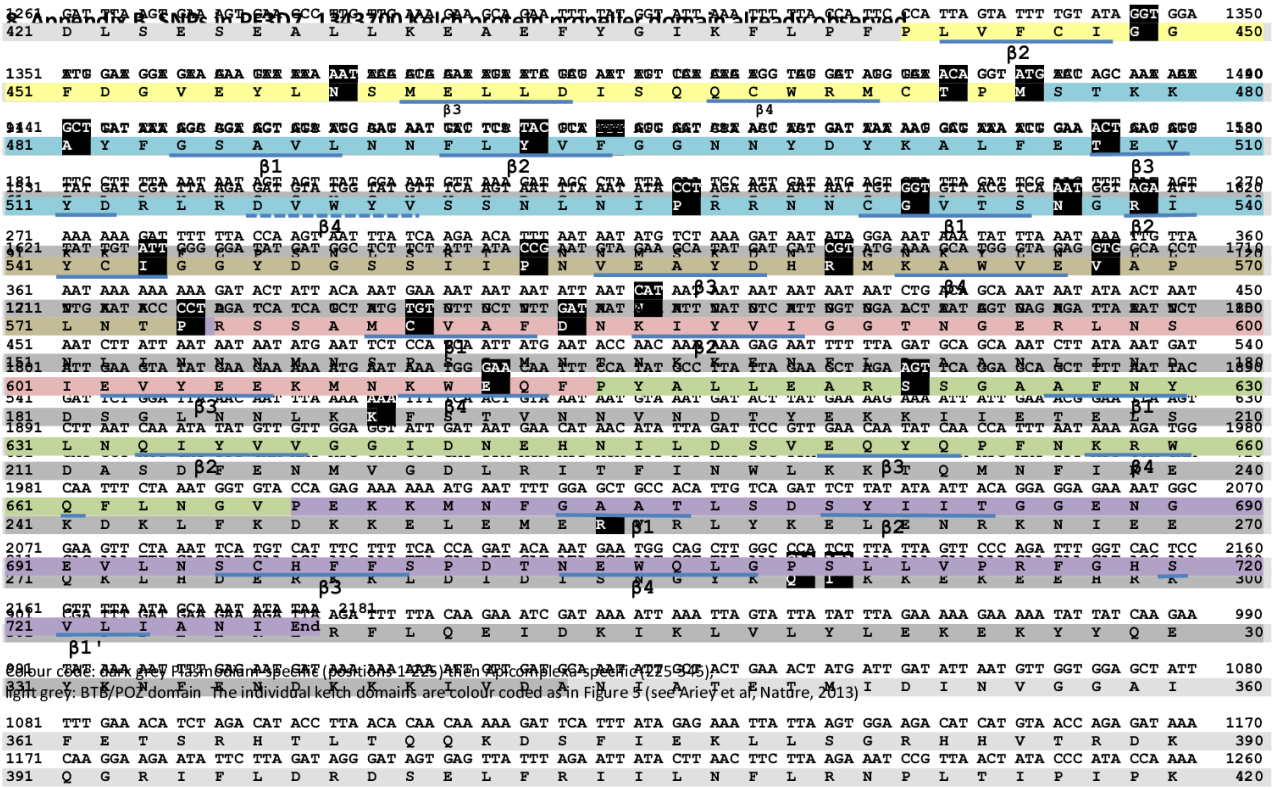

Figure 4

SNPs in PF3D7_1343700 Kelch protein propeller domain already observed

Supplementary Files

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