Polymerase Chain Reaction for *Plasmodium falciparum*

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Reagents

- Primers 100 picomol/μl[1]
- 25 mM dNTPs (1:2:2:1, G:A:T:C)[2]
- 25 mM $MgCl_2$
- 10X Taq Buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3)
- 0.1-1 nanogram gDNA
- Taq or other DNA polymerase
- Milli Q water (or ddH_2O)
- Agarose
- Tris-Acetate EDTA (TAE) or other electrophoresis buffer
- DNA ladder
- Ethidum Bromide

Materials

- 0.2 ml PCR tubes
- Thermal cycler
- Microwave
- Electrophoresis apparatus
- Gel imaging apparatus

1. When designing primers stay within the lower end of the range for T_m [60-75ºC] keeping both the forward and reverse primers within 5ºC of one another. Measure this temperature only for the base pairs that actually will be annealing during the first annealing step of the PCR program.
2. dNTPs are usually sold separately as 100 mM. Use the above proportions and bring the final total concentration of dNTPs to 25 mM using Milli Q. One example is the following mix: 20 μl A, 20 μl T, 10 μl G, 10 μl C, 180 μl Milli Q.

PCR Setup

0.5 μl Forward Primer
0.5 μl Reverse Primer
0.5 μl dNTPs
2.5 μl $MgCl_2$
5.0 μl Taq Buffer
5.0 μl 0.1 ng/μl gDNA
35.0 μl Milli Q
1.0 μl Taq

PCR Program A

1. 92ºC 1 min
2. 92ºC 30 sec
3. 54ºC 30 sec
4. 62ºC 1 min 30 sec
5. Go to step 2, 29 times
6. 62ºC 4 min
7. 4ºC FOREVER

PCR Program B

1. 94ºC 1 min
2. 94ºC 30 sec
3. 65ºC 30 sec
4. 72ºC 30 sec
5. Go to step 2, 29 times
6. 72ºC 4 min
7. 4ºC FOREVER

*Run 4 ul of PCR reactions on an agarose gel using a DNA Standard to compare fragment sizes, ethidum bromide to stain DNA, and an UV gel imager to visualize the bands.