Rapid Detection of live *Plasmodium falciparum* strains by PCR

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Reagents

- Parasitized culture
- 0.1% saponin/1XPBS
- 1X PBS

Materials

- Centrifuge tubes
- Allegra® 6 Series and GH-3.8 Swinging Bucket Rotor
- Eppendorf tubes
- Heat block

A. Saponin Lysis

1. Take between 100 µl and 10 ml of 2% hematocrit culture.
2. Add equal volume of 0.1% saponin in 1X PBS and mix until dark red.
3. Spin 3150 rpm (2300g), 5 min, room temperature, low brake. Aspirate supernatant being careful not to suck up any parasites! (2000 rpm, 10 min for 96-well plates; 5000 rpm, 5 min for microcentrifuge tubes).

B. Wash

4. Resuspend parasites in 1X PBS using the original culture volume taken.
5. Spin 3150 rpm (2300g), 5 min, room temperature, low brake. Aspirate supernatant being careful not to suck up any parasites!
6. Resuspend parasites in an appropriate volume of 1X PBS, which is ~ 1/20 - 1/40 original volume of culture used, depending on parasitemia.

C. Parasite lysis

7. Place samples in a heat block at 100ºC for 4 minutes.
8. Put on ice, or in fridge until ready for PCR.

D. Suggested PCR setup and Protocol

PCR Setup

0.5 µl Forward Primer 100 picomol/µl
0.5 µl Reverse Primer 100 picomol/µl
0.5 µl 25 mM dNTPs
2.5 µl 25 mM $MgCl_2$
5.0 µl 10X Taq Buffer
1.0 µl cell lysate
39.0 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

For additional PCR programs and troubleshooting, please see *PCR procedures* on Llinás Lab website (http://www.molbio1.princeton.edu/labs/llinas/protocols.html).