Adapted from Smilkstein et al,
Antimicrobial Agents and Chemotherapy, 2004

SYBR Green I DNA Fluorometry

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Purpose:

The SYBR Green assay is most useful as a measure of relative parasite levels between two parallel cultures (e.g., control vs. treatment). It is particularly well-suited to drug-efficacy and growth assays. The procedure results in cell lysis, so it should only be used after a period of growth under the desired experimental conditions.

Materials and Reagents:

- *P. falciparum* culture
- Sterile 96-well plate
- Tecan GENios microplate reader
- SYBR Green I DNA staining dye (Molecular Probes, Inc., Eugene, OR)
  NB: SYBR Green I is light-sensitive; keep it in the dark as much as possible

Buffers:

- Lysis buffer (20mM Tris-HCl, pH 7.5; 5mM EDTA; 0.08% Triton X-100; 0.008% saponin in PBS)

Culture preparation:

- For a 72 h growth assay, aim to have your culture at 1% parasitemia prior to incubation.[1]
- Adjust your culture's hematocrit to 1%.
- If your treatment involves a dropout, wash and resuspend your culture in the dropout medium.
- Split your culture by the number of treatments desired, and add any necessary drugs/nutrients/etc.

Plate preparation and incubation:

- Plan out your plate pattern in advance, keeping in mind the number of different treatments you want and the number of replicates.[2]
- Aliquot 100µl of water into the wells surrounding the wells you plan to use; this will prevent your samples from evaporating
- Aliquot 100µl of culture into each well according to your plan.
- NB: It is always helpful to have cultures growing in parallel so you can take smears to complement your data.[3]
- Store the plated cultures in the incubator for the desired time period (usually 48-96 h)
- After incubation, wrap the plate in parafilm and store O/N at -80ºC.

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[1] Assays lasting two life cycles may be more successful at a lower starting parasitemia to avoid starvation.

[2] On account of the evaporation-buffer wells, only the sixty interior wells are available for your experiment

[3] **Important: SYBR Green I measures DNA concentration, so it cannot distinguish between a single schizont and the viable parasites it may or may not ultimately produce. Ensure via smear inspection that your pre-lysis samples contain similar fractions of schizonts (preferably zero).**

SYBR Green I preparation and incubation

- You will need 100µl of buffered SYBR Green per well. Calculate the total volume needed and prepare the lysis buffer at this volume.
- Add 0.2µl SYBR Green I reagent per ml of total volume needed
- Thaw plate at room temperature
- Aliquot 100µl of buffered SYBR Green into each culture-containing well using a multichannel pipetman. Mix by pipeting up and down until no cell sediment remains.
- Wrap plate in tin-foil and store in incubator for 6 h.

DNA Fluorometry

- DNA quantification is performed using a Tecan GENios microplate detection device.
- Open the Tecan "Magellan 6" program.
- Select "Start Measurement" and hit the green arrow
- Hit the green arrow marked "Continue"
- Select "Use a predefined method" and scroll until you find "DNAfluorometerDW"; select it and hit the green arrow.
- Remove the top of your plate and orient it properly in the tray.
- Hit the green arrow marked "Start"

The program should create an Excel file containing your data after it is finished.

For non-lab members, the specific parameters for "DNAfluorometerDW" are as follows:

- Measurement mode: Fluorescent Top
- Excitation wavelength: 485 nm
- Emission wavelength: 535 nm
- Gain (Optimal): (blank)
- Number of flashes: 2
- Lag time: 0 µs
- Integration time: 40 µs
- Plate definition file: COS96fw.pdf
- Unit: RFU