PMA-seq Protocol

START A HEAT BLOCK AND HAVE A FROZEN ICE BLOCK READY.

Starting material: cell cultures, fecal samples, or environmental samples. Each sample must be triplate, with one sample going straight to DNA extraction, one being treated with just H_2O, and one being treated with H_2O and PMA. 50% Glycerol is ok to use, but consider a longer incubation time in the dark and centrifugation time.

PMA Treatment

1. Emulsify one sample in H_2O and PMA to a final PMA concentration of 50 µM. For filtered stool homogenate and cell cultures, add 1.25ul of 20 mM PMA to 498.75 µl of sample. Cover with aluminum foil, and dim lights to avoid photolysing the PMA stock or samples.

2. Vortex for 5 seconds to mix and incubate in the dark at room temperature for 5 minutes. Continue to mix the tubes by inversion/vortex every minute or place them on a rocker. During this time, prepare the light apparatus.

3. Place a layer of aluminum foil on top of ice underneath the LED light apparatus. Ice packs and wet ice work fine. The LED light from TaoTronics has both white and blue LEDs on separate circuits. It sits reasonably well on top of 2 1ml filter pipet tip boxes. See crudely drawn Figure 1.

4. Remove the samples from the dark, take off the aluminum foil, and place them under the light apparatus on top of the aluminum foil-covered ice block. Turn both the white and blue leds on to maximum power (you should hear and see the fans come on) and incubate the samples for 15 minutes underneath the aluminum foil covered box. Turn over the tubes at 7.5 min or more often for sensitive samples. I have found that turning them once will keep solution temperature below 25 °C.

5. Remove the samples from light, keeping them on ice. Pellet cells or sample by centrifuging the sample at 5,000g for 10 min or as needed (10,000g for 2 min for fecal samples, 10,000g for 7 min for 50% glycerol solutions
). Discard supernatant. Proceed to DNA extraction using the pelleted cells.

DNA extraction

1. For each sample, draw off the supernantant from a MoBio PowerSoil Tube.

2. Add the supernatant to the pelleted cells/fecal matter and pipet to mix.

3. Return the sample to the PowerSoil tube. Proceed with standard extraction protocol.

Notes

PMA stock solution: Dissolve PMA in H2O to a final concentration of 20 mM, or dissolving 1mg of PMA in 98 ul of H2O. Store covered, in the dark, at –20°C. This stock solution will last for ~6 months.

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