Melanin quantification assay for *Cryptococcus*

All *Cryptoccoccus* spp. isolates are grown on Sabouraud Dextrose Agar (SDA)(2% glucose, 2% peptone and 2% agar) for 2-3 days before conducting the melanin quantification assay.

After initial culture on SDA, each cryptococcal strain is grown in YPD broth (2% glucose, 2% peptone and 1% yeast extract) to saturation for 24 hr at 30°C. 100 µl (~10^7 CFU) of the saturated culture is then added into 10 ml of melanin induction media (1% glucose YNB without amino acid and ammonium sulfate) containing either 10 mM dopamine or 10 mM epinephrine at 30°C. Concentration of the initial culture (from YPD broth) of each strain is determined by conducting plate counts.

Supernatant of each culture in melanin induction media are taken at 48 hr and their optical density (OD) at 475 nm is measured.

To determine the effect of growth temperature on melanin production, the melanin assay is also carried out at 37°C and the OD is measured as described above after 48 hr incubation.