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RNA Extraction black fungi using the MP FastRNA Pro Kit with Lysing Matrix Y

! The Lysing Matrix Y is not in the kit –you can ask MP to exchange the vials from C to Y Matrix (yellow cap)! (https://www.fishersci.de/shop/products/mp-biomedicals-fastrna-pro-red-kit/11492380). No DEPC water was used as it often disturbs in the sequencing steps. We used Gibco water instead.

RNA Isolation steps:

- Clean all used materials, instruments, bench, digestor with RNase zap and use gloves always (also clean the gloves with RNase zap and use new ones if you touched anything else). Clean the centrifuge and set it to 4°C. Only use RNase free filter tips.
- Prepare matrix Y vials on ice
- Scrape off the biomass from the agar plates using a scalpel cleaned with RNase zap or any other cleaned device (avoid scraping off agar) and add it to the vials on ice
- Add 1 mL of RNAPro Solution to the vials in a digestor
- Process the tube on the FastPrepInstrument Ribolyzer for 40 seconds at a setting of 6
- Let the tube rest at room temperature for 3 minutes
- Centrifuge the tube at 12000 x g for 5 minutes at 4°C
- Transfer the upper layer liquid (approx. 750µl) to a new RNase free tube (e.g. Eppendorf tube Safe lock, low bind) without disturbing the lower layers in the tube. Avoid transferring the debris pellet and lysing matrix.
- Incubate the transferred liquid for 5 minutes at room temperature.
- Add 300 µl of Chloroform to the samples, vortex for 10 seconds and let it incubate for 5 minutes at room temperature.
- Centrifuge the tubes at 12000 x g for 5 minutes at 4°C
- Transfer the upper liquid phase without disturbing the interphase to a new RNase free Eppendorf tube.
- Add 200 µl of Chloroform to the new sample. Vortex for 10min and let it incubate for 5 min at room temperature.
- Centrifuge the tubes at 12000 x g for 5 minutes at 4°C
- Transfer the upper liquid phase without disturbing the interphase to a new RNase free Eppendorf tube. Add 500µl of cold absolute ethanol (Ethanol absolute ≥99.8%, e.g.VWR-Electran for Molecularbiology, be sure to have clean ethanol) and invert 5X to mix and store at -20°C for at least 30 min (better overnight).

- Centrifuge at max of centrifuge (min. 12000xg) for 15min at 4°C.

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- Remove the supernatant. You should see a pellet. Sometimes it is not visible - but with black yeasts most of the time it is. Try to not disturb the pellet in the following steps!
- Wash the pellet with 500 µl of cold 75% ethanol. Do not disturb the pellet.
- Centrifuge at max of centrifuge (min. 12000 x g) for 2min at 4°C.
- Remove wash Ethanol and repeat wash step with 500 µl of cold 75% ethanol. Do not disturb the pellet.
- Centrifuge at max of centrifuge (min. 12000 x g) for 2min at 4°C.
- Remove Ethanol completely without disturbing the pellet. If needed use a microcentrifugation step in-between to remove remaining ethanol.
- Air dry Pellet for 5 min (open vial) – if there is any remaining Ethanol, prolong the drying step.
- Resuspend the RNA in 100 µl of RNase free water (Gibco water) and incubate 5 min at room temperature.
- Check the quality on a Nanodrop instrument, Gel, Qbit, Bioanalyzer
- Store the RNA at -80°C!

Citations:

Poyntner, C; Mirastschijski, U; Sterflinger, K; Tafer, H Transcriptome Study of an Exophiala dermatitidis PKS1 Mutant on an ex Vivo Skin Model: Is Melanin Important for Infection? FRONT MICROBIOL. 2018; 9

Blasi, B; Tafer, H; Kustor, C; Poyntner, C; Lopandic, K; Sterflinger, K Genomic and transcriptomic analysis of the toluene degrading black yeast Cladophialophora immunda. SCI REP-UK. 2017; 7

Poyntner, C; Blasi, B; Arcalis, E; Mirastschijski, U; Sterflinger, K; Tafer, H The Transcriptome of Exophiala dermatitidis during Ex-vivo Skin Model Infection. FRONT CELL INFECT MI. 2016; 6