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APPLICATION NOTE

RNA Extraction from Aspergillus parasiticus mycelium

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Introduction

Recent genomic efforts on toxigenic and nontoxigenic *Aspergillus* species have advanced our understanding of the biology and genetics of these filamentous fungi. However, it is clear that these complex experiments suffer greatly from the variability in the quality of RNA between each replicate and it is critical to establish a platform to isolate high quality RNA for use in both microarray and qRT-PCR. In that context we describe here RNA isolation of *A. parasiticus* during a simple carbohydrate shift.

Experiment

For this experiment *A. parasiticus* SRRC 143 (or SU-1; ATCC # 56775 and 201461) a wild-type strain used for biochemical and genetic studies in both B group (B_1 and B_2) and G group (G_1 and G_2) aflatoxins was chosen. Fresh spores were generated by plating 50 µL of 108 spores onto Difco Potato-Dextrose Agar (PDA) (American Scientific Products, Charlotte, NC) and incubated at 30°C. The spores were collected from 5-day cultures with sterile 0.05% Triton X-100 (EMD Chemicals Inc., Gibbstown, NJ) *A. parasiticus* spores were inoculated to a final concentration of 105 spores/mL in 200 mL yeast extract (YE) liquid medium consisting of 25 g/L of yeast extract (DIFCO) and incubated for 48 hours at 30°C with constant shaking at 150 rpm. The fungal mycelia were harvested by filtration in vacuo. The harvested mycelia were divided into 1 gram aliquots. One sample was frozen in liquid nitrogen and stored at - 80°C for RNA preparation and the remaining aliquots were used to inoculate 100 mL of YES medium (60 g/L of sucrose and 25 g/L of yeast extract) and incubated at 30°C with 150 rpm shaking until harvested at 3, 6, 12, 24, and 48 hours post-inoculation. The harvested fungal mycelium samples were flash frozen in liquid nitrogen and stored at -80°C for RNA preparation and aflatoxin extraction.

Sample Extraction

Before samples were processed in 24-well polyethylene vials (SPEX SamplePrep Cat. No. 2240-PE) containing one 3/822 (9.5 mm) stainless steel grinding ball per tube, they were arranged within a 24-well aluminum Cryo Adapter block (SPEX SamplePrep catalog 2263) and pre-chilled for 15 minutes in a Cryo-Station (SPEX SamplePrep Cat. No. 2600). Using a prechilled spatula 50 mg to 100 mg of the frozen fungal tissue was transferred to the pre-chilled tubes. The chilled samples and adapter block were clamped in the SPEX SamplePrep 2000 Geno/Grinder and ground at a rate of 1050 strokes per min (dial setting of 150) for 1 minute and 30 seconds. The samples and block combination were quickly removed from the Geno/Grinder and returned to the Cryo-Station. To each ground sample 1 mL of Puresol from the Aurum Total RNA fatty and fibrous tissue kit (Biorad) was added. To ensure that all ground tissue was suspended in Puresol the samples still housed within the adapter block was inverted 2-3 times and returned to the Cryo-Station. After addition of

- APPLICATION NOTE SP014: RNA Extraction from Aspergillus parasiticus mycelium
- APPARATUS: **Geno/Grinder® & Cryo-Station**
- APPLICATION: RNA Extraction

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Puresol the samples were homogenized in the SPEX SamplePrep 2000 Geno/Grinder for 2 minutes at 1050 strokes per minute. The sample tubes containing the homogenized sample buffer mix were transferred to a room temperature foam holder before homogenizing for an additional 2 minutes in the Geno/Grinder. During this final homogenization the Puresol buffer and ground mycelium thawed and mixed completely. Samples were removed from the Geno/Grinder and incubated at room temperature for 10 minutes before transferring to sterile 2 mL Eppendorf tubes. Total RNA was extracted according to manufacturer's protocols, using the Aurum Total RNA fatty and fibrous tissue kit (Biorad).

Yields

Total RNA was quantified using a ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and visualized by gel electrophoresis (figure 1) to ensure quality. RNA concentration varied from 1.5 µg/uL to 3.8 µg/uL, in a final volume of 50 µL, with a A260/280 ratios varying from 2.01 to 2.19 or better. For a routine qRT-PCR 500 ng to 1 µg of total RNA is used as compared to 3 to 5 µg for the typical microarray experiment. More than adequate RNA for both microarray experiments and subsequent real time validation were isolated using this protocol.

Conclusion

Though several environmental factors can affect the final quality of RNA, which directly affects both microarray and real time experiments, we demonstrate here a highly reliable methodology capable of isolating high quality RNA from small quantities of fungal mycelium. This protocol allows simultaneous processing of large numbers of samples, thus demonstrating the suitability of the Geno/Grinder for high throughput processing of fungal mycelium.

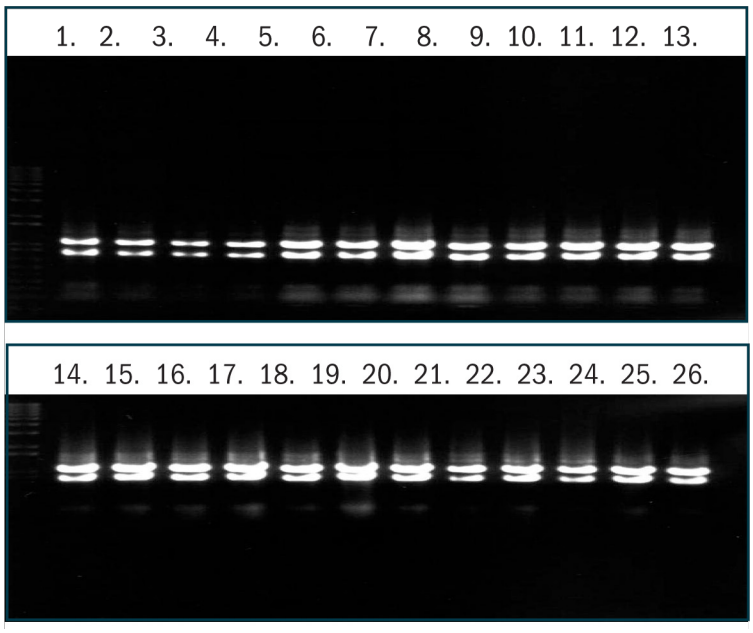

Figure 1.1.0% Agarose gel. Lanes 1 and 14, 1 Kb ladder (Invitrogen). Lanes 2-5, replicates A-D of *A. parasiticus* SU-1 900 ng of total RNA isolated from Mycelium grown 48 hours in Yeast Extract Media. Lane 6-9, 10-13, 15-18, 19-22, and 23-26 replicates A-D of *A. parasiticus* SU-1 total RNA isolated from Mycelium collected at 3, 6, 12, 24 and 48 hours after shift from Yeast Extract Method to Yeast Extract Sucrose Media.

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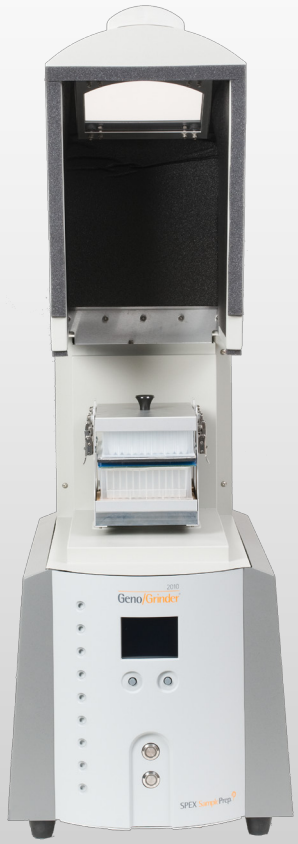

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