A protocol of nuclei extraction from germinating spores of the wheat stripe rust pathogen

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We use this protocol and it's working

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ABSTRACT

Extracting nuclei from rust fungi is important because it allows for the study of the genetic material of these pathogens, which can provide insights into their biology, evolution, and interactions with their hosts. The nuclei contain the DNA of the rust fungus, which encodes the genes and genetic pathways that govern the organism's growth, development, and pathogenicity.
Nuclei extraction is often a prerequisite for downstream applications such as DNA sequencing, genome assembly, transcriptomics, and proteomics. For example, sequencing the DNA extracted from the nuclei can enable the assembly and annotation of the rust fungus genome, which can provide insights into the genes and genetic pathways that contribute to pathogenicity, host specificity, and virulence.
Additionally, the isolation of nuclei from rust fungi can facilitate the identification and characterization of effector proteins, which are secreted by the pathogen to manipulate the host plant and promote infection. The analysis of effector genes and their functions can help in the development of strategies to control rust diseases and mitigate their impact on crop yields and food security.
In summary, extracting nuclei from rust fungi is an essential step in the study of these pathogens and their interactions with their hosts. The genetic material contained in the nuclei can provide insights into the biology, evolution, and pathogenicity of rust fungi, and enable the development of new approaches to control these devastating plant diseases.

Rust spores are small, parasitic fungi that can infect a wide range of plant species. Isolating nuclei from rust spores is an essential step for various downstream applications such as genome sequencing, transcriptome analysis, and epigenetic studies. Here are some general steps for nuclei extraction from rust spores:

MATERIALS

BUFFERS & REAGENTS

Incubation buffer:

1. 0.1 M phosphate buffer (NaH2PO4- Na2HPO4, pH 7.0)
2. 40 mM EDTA
3. 0.2 % 2-mercaptoethanol

Wash Buffer:

1. ·0.1 M phosphate buffer (NaH2PO4- Na2HPO4, pH 7.0)
2. ·0.8 M KCl

Digestion Buffer:

1. 0.1 M phosphate buffer (NaH2PO4- Na2HPO4, pH 5.8)
2. 0.8 M KCl
3. 50 mM EDTA
4. Gulcanex 2.5 %

Nuclei extraction Buffer:

1. Hexylene Glycol 1M
2. PIPES-KOH (pH-7): 10 mM
3. Sucrose: 250 mM
4. EDTA: 50 mM
5. MgCl2: 10 MM
6. KCL: 10mM
7. DTT: 2mM
8. 2% Triton X-100 (added freshly)

Re-suspension Buffer:

1. Hexylene Glycol: 0.5 M
2. PIPES-KOH (pH-7): 10 mM
3. Sucrose: 250 mM
4. EDTA: 50 mM
5. MgCl2: 10 MM
6. KCL: 10mM
7. DTT: 2mM
8. 0.5% Triton X-100 (added freshly)

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Spore germination

1.1 Collect fresh spores ~300 mg for a Pyrex baking tray of size 33X23XX5 cm and put in a spore dispenser. The spore dispenser was made out of a 50 mL falcon tube by cutting the cap of it as shown in Figure 1.2

Fig 1.1 A spore dispencer made out of falcon tube

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1.2 Keep a Pyrex baking tray of size 33X23XX5 in a plastic container of size ?? Add ddH20 in the Pyrex tray for 3-4 cm and finely dust spores on the surface of the tray by gently taping the falcon tube with a finger (See guidelines)

1.3 Now with the help of a sprayer, sprinkle fine water mist on the layer of the spores and in the container container to create a humid condition inside the chamber close the lid and make it airtight by a tap around the opening of the box. Keep the box in a controlled growth chamber at the 16 C for 48 hrs

1.4 Harvesting the germinated spores :
Harvest the germinated spores scooping the layer on the plate with the help of a loop. the germinating spore germ tube intertwined with other germinating spores making a mash of spores on the surface of the water which can be easily scooped with the help of a loop. If the spores do not form a mash indicates poor germination.

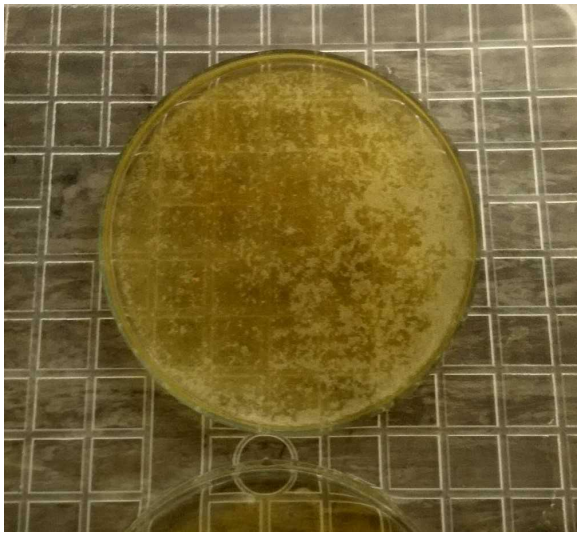

Fig 1.2 Spores put for germination after 48 hours

Fig 1.2 Mycelium mass scooped out of plate

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Fig 1.2 Mycelium mass under 10 X resolution

2 Nuclei extraction

2.1 Take the mycelium mass into a 50 mL falcon tube containing 10 mL 0.1% Triton X-100 and wash thoroughly by inverting the tube multiple times. No filer it through a 30-micron sieve to

get rid of ungerminated spores.

2.2 Transfer in a falcon tube containing 15 mL incubation buffer for 20 min at room temperature.

2.3 Now wash the mycelium in the wash buffer three times.

2.4 Pellet down the mycelium mass by centrifugation at 2000g for 10 min

2.5 Prepare 15 mL 2.5% Glucanex solution in Wash Buffer (pH 5.6)

2.6 Resuspend the mycelium pellet in the 15 mL Glucanex solution by vertexing for 1-2 min at full speed.

2.7 Incubate the mycelium for digestion at 28 C for 2–3 hours; keep the tube inverting every 30 min

2.8 Pellet down the digested mycelium by centrifugation at 1000 g for 15 min

2.9 Filter the homogenate through a 20-micron nylon sieve and centrifuge the filtrate at 1000g for 10 min.

2.10 Remove the supernatant and resuspend the pellet in 10 mL Extraction Buffer containing 2%

Triton X-100 (freshly added every time)

2.11 Repeat the homogenization with Teflon piston homogenizer with 10–15 stocks and filter the homogenate through a 10-micron filter.

2.12 Pellet down the nuclei by centrifugation at 1000 g for 10 min

2.13 Remove the supernatant and dissolve the pellet in 2-3 mL Re-suspension Buffer without Triton X-100.

2.14 Filtration: Filter the spore suspension through a series of sterile nylon meshes with decreasing pore sizes (e.g., 70 µm, 40 µm, 20 µm and 10 um) to remove the cell debris and obtain a single-cell suspension of spores

2.15 Stain the crude nuclei extract with propidium iodide (5ug/mL) and look under a microscope

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2.17 Quality control: Assess the quality and quantity of the isolated nuclei using a fluorescent dye such as propidium iodide or DAPI and a fluorescence microscope or flow cytometer

3 FACS sorting nuclei

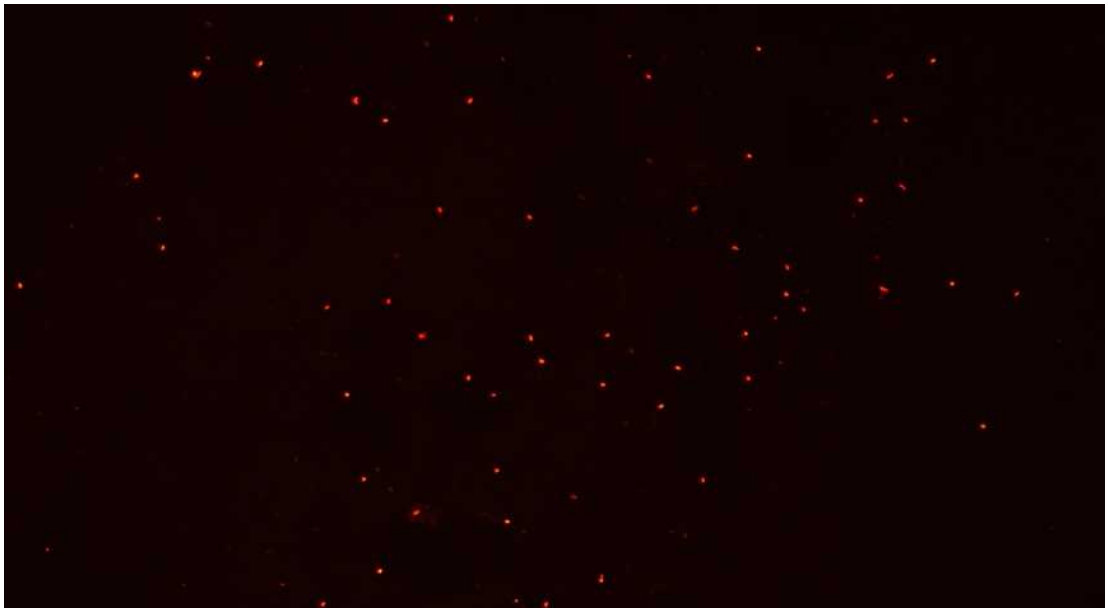

Fig FACS sorted PST nuclei