

Aug 12, 2025

Isolation of *Pyricularia* and preparation of DNA for Illumina Sequencing

 [Microbiology Resource Announcements](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.14egnr7pl5d/v1>

Mark Farman¹

¹Farman Lab, Department of Plant Pathology, University of Kentucky, Lexington, KY 40506

FarmanLab



Mark Lewis Farman

University of Kentucky

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.14egnr7pl5d/v1>

External link: <https://doi.org/10.1128/mra.00091-25>

Protocol Citation: Mark Farman 2025. Isolation of *Pyricularia* and preparation of DNA for Illumina Sequencing. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.14egnr7pl5d/v1>

**Manuscript citation:**

Avery Meyer, Bram Dutch, Ashab Ahmed, Anna Baloh, Matt Bartholomai, John Boggess, Amelia Burnett, Colin Carver, Evan Courtwright, Chase Eastham, Darrin Egan, Knox Garland, Kelly Claire Gray, Audrey Harper, Megan Johar, Tanner Jones, Leighanne Lyvers, Jacob Marquez, Summer McCune, Matt Mitchell, Carson Moylan, Luke Olsen, Tucker Overton, Oluwatofunmi Oyetan, Khumbo Phiri, Kevin Ramirez, Gavin Robinson, Connor Talbot, Bruno Athie Teruel, Parker Thompson, Lark Wuetcher, Wesley Yang, Andrew Tapia, Jerzy Jaromczyk, Mark Farman (2025) Genome assemblies for *Pyricularia* species and related genera isolated from diverse host plants. Microbiology Resource Announcements doi: [10.1128/mra.00091-25](https://doi.org/10.1128/mra.00091-25)

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

This is an optimized protocol that has been used for years in the Farman lab. We have utilized the single spore isolation, liquid culture and DNA purification steps successfully for many different fungi.

Created: June 30, 2025

Last Modified: August 12, 2025

Protocol Integer ID: 221303

Keywords: fungus, purifying fungal cultures, fungal culture storage, DNA isolation, isolating pure *pyricularia* culture, pure *pyricularia* cultures from lesion, isolation of *pyricularia*, preparation of dna, typical yield of purified dna, purified dna, generating dna, dna of sufficient purity, *pyricularia*, illumina library production, dna, illumina, ml of culture

Abstract

This protocol describes procedures for isolating pure *Pyricularia* cultures from lesions in just 24 h and then generating DNA of sufficient purity for Illumina library production. The typical yield of purified DNA from 10 ml of culture is 5-10 µg.

Isolation of Pure, Single-Spored Pyricularia Cultures Directly from Diseased Plant Tissues

- 1 Collect host tissues and place in a plastic bag, along with a piece of paper towel to help absorb moisture. Tissues should be air-dried before long-term storage (i.e., if it will be a few days before spores will be harvested).
- 2 Place leaves in a moist chamber. A Petri dish containing a dampened paper towel will work.
- 3 Leave overnight. If the lesions were caused by *Pyricularia*, in the morning, you will see a mass of conidiophores, with an abundance of sparkling spore masses visible (Figure 1). The spores will look crystal clear - any pigment and it is not *Pyricularia*.

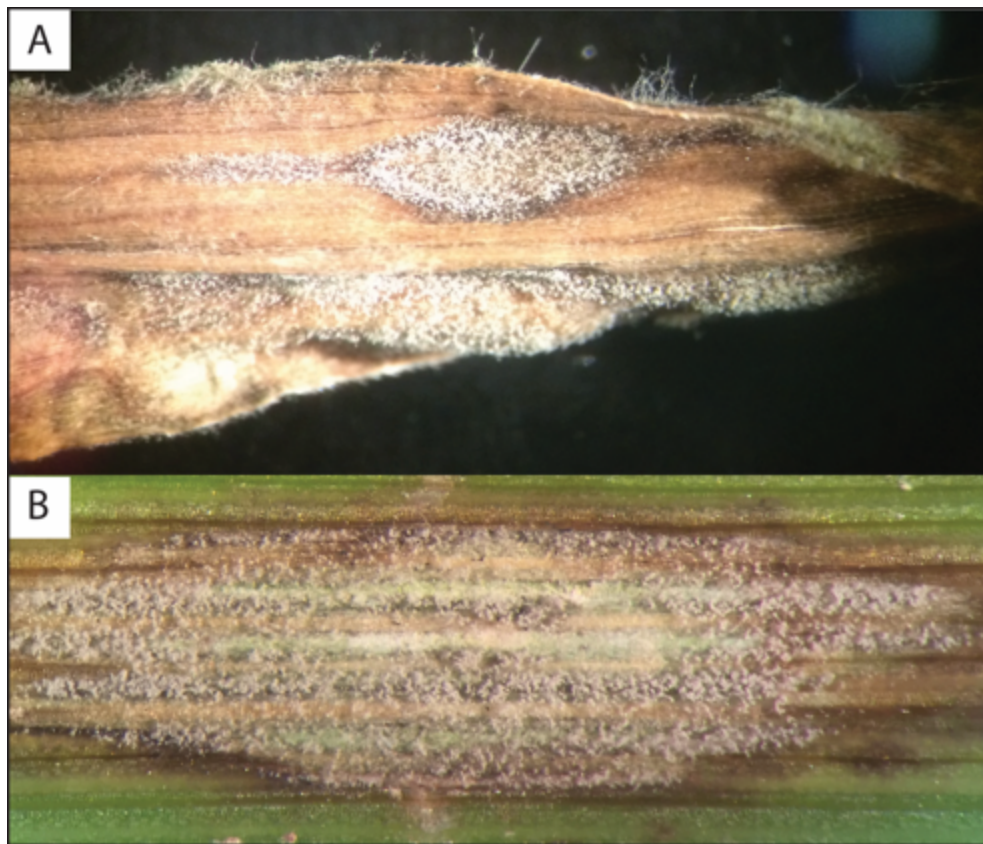


Figure 1. *Pyricularia* spores after overnight culture. A - dried tissue; B - fresh lesion

- 4 Under a dissecting microscope, rub a flame-sealed, glass Pasteur pipette gently across the tips of the conidiophores (Figure 2). Spores will adhere to the glass surface (inset).



Figure 2. Harvesting spores with a sterilized, sealed, glass Pasteur pipette

- 5 Gently run the glass pipette across the surface of 4% water agar, supplemented with 100 $\mu\text{g/ml}$ of ampicillin antibiotic. Note: 12 single-spore isolations can be performed using a single, 8.5 cm dia. Petri dish (Figure 3).

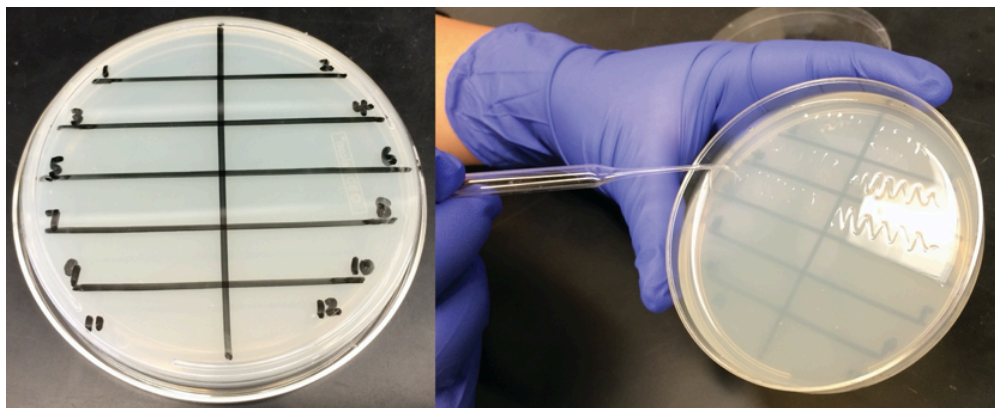


Figure 3. Streaking spores on water agar plates

- 6 After 12 to 24 h, scan the plate under the dissecting microscope to identify a germinated spore that is well separated from any others. Spores will be most densely distributed at the point where the glass pipette initially touched the agar. Follow the streak to find an isolated germling (note: it is critically important to be sure that a single spore has been picked, so do not pick any where the characteristic spore shape is not perfectly clear - sometimes two spores can adhere together).
- 7 Using a scalpel with a no. 11 blade, carefully cut around the germinated spore (Figure 4). Lift the agar block, being careful not to let it roll across the agar surface where it might pick up additional spores/hyphal fragments.

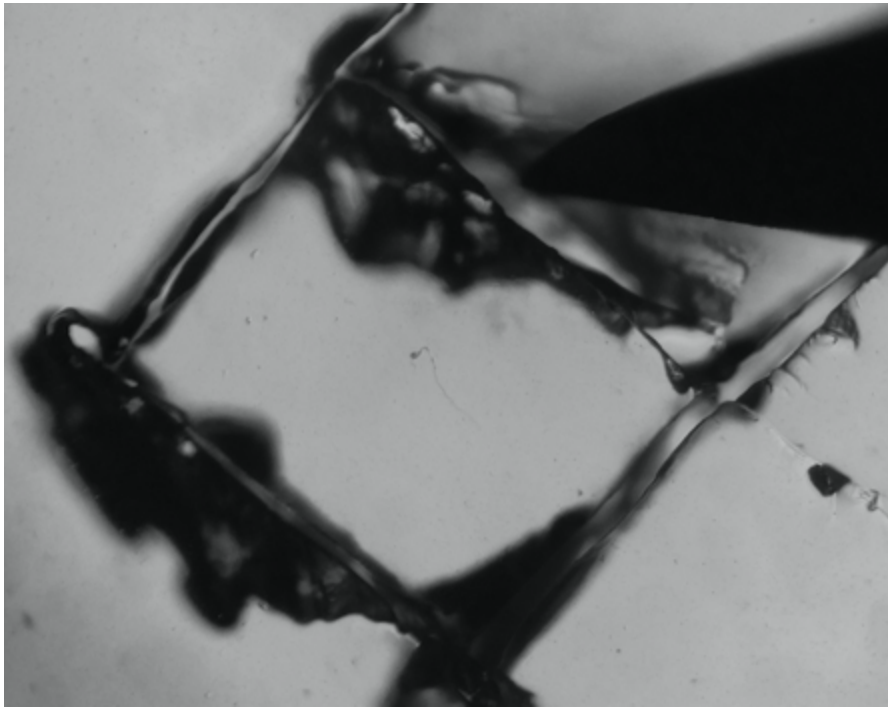


Figure 4. Using a scalpel to excise a single, germinated spore

- 8 Transfer the agar block to an oatmeal agar plate supplemented with 100 µg/ml ampicillin. Once you are sure that the spore is continuing to grow, place three or four sterilized Whatman 3M paper squares around the growing colony. Allow the fungus to colonize the paper completely.
- 9 If the mycelial culture appears to be contaminated with bacteria or yeast, remove the lid of the plate and place it upside down over a fresh oatmeal agar + ampicillin plate. *Pyricularia* spores will be released onto the agar below, while the sticky bacterial/yeast colonies will remain on the upper plate.
- 10 Transfer the paper pieces into a glassine/paper mini-envelope and air-dry in a containment hood for three to four days.
- 11 Heat-seal the envelopes in plastic bags. Include in each bag a sachet of silica gel crystals to absorb any residual moisture. Store cultures at -20°C. We also include an indicator paper, to ensure that the bag's integrity is not compromised (Figure 5).



Figure 5. Dried cultures sealed with silica gel and indicator papers

Culturing *Pyricularia* for DNA extraction

- 12 Inoculate test tube containing 10 ml Complete Medium with a single mycelial agar plug from actively growing culture. Incubate on a slant at room temperature, shaking at 150 rpm for approximately 5-7 days until the white mycelial mass has encompassed greater than 1/3 of the 10 ml culture volume (Figure 6).

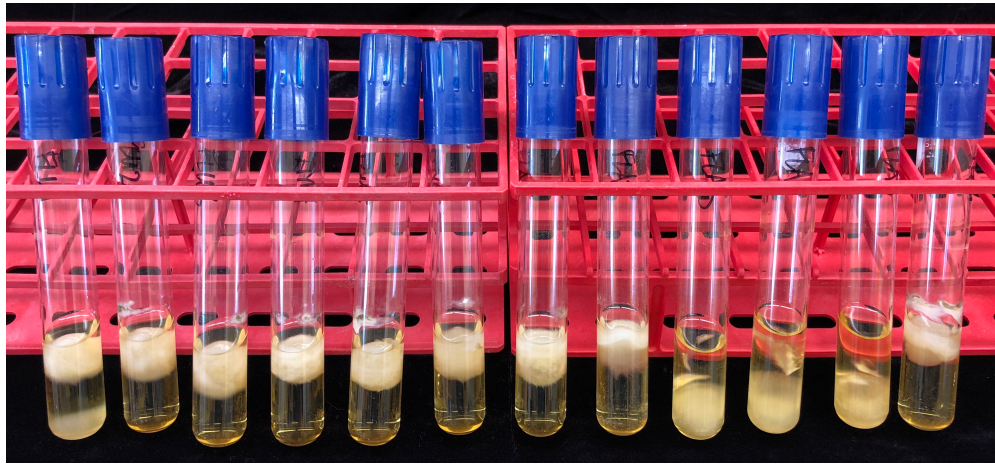


Figure 6. *Pyricularia* cultures ready for DNA extraction

- 12.1 *Complete Medium:*
0.6% casamino acids
0.6% yeast extract
1% sucrose
- 13 Harvest mycelial mass by removing from the culture tube with a glass rod or plastic spatula. Sandwich mycelial mass between stacks of paper towels and use pressure to blot dry. If visible, remove agar plug with glass rod/plastic spatula.
<https://www.youtube.com/watch?v=h9TZANDMnd8>
- 14 Transfer mycelium to a 15 ml conical tube. Freeze at -20°C and then lyophilize for 24 h.

DNA isolation

- 15 Warm lysis buffer to 65°C .
- 15.1 *Lysis Buffer:*
50 mM Tris-HCl, pH 8.0
50 mM EDTA, pH 8.0
0.5 M NaCl
1% SDS
- 16 Use a glass rod to grind mycelial pellet against the sides of the 15 ml conical tube (not the bottom of the conical tube)
<https://www.youtube.com/watch?v=RR0qwc3liEI>



- 17 Add 1.5 ml of lysis buffer and use a glass rod to disperse mycelial clumps until a smooth slurry is formed. Cap the tube and place in a 65° C water bath for 30 min (shaking once halfway through). 
- 18 Add 1 ml of phenol/chloroform/isoamyl alcohol (25:24:1). Replace cap, carefully vortex briefly and place at 65° C for 30 min (shaking once halfway through). 
- 19 Centrifuge at max speed in a swinging bucket rotor for 30 min. 
- 20 Remove 1 ml of supernatant and transfer to a 2 ml microfuge tube. Add 600 µl of isopropanol, mix by inverting the tube several times. Centrifuge immediately for 10 min at full speed in a microfuge.
- 21 Discard supernatant, rinse pellet with 1 ml of 70% ethanol.
- 22 Centrifuge 5 min at 14,000 rpm. Discard ethanol wash using care not to discard the pellet. 
- 23 Allow the pellet to dry on the bench top.
- 24 Re-suspend in 100 µl of TE + RNase A (10 µl of 1 µg/ml stock solution per 15 ml TE). Allow 10 min for DNA to dissolve and disperse by using a finger to flick the tube.
- 25 Quantify the DNA using a Qubit machine. **Do not use NanoDrop as the readings will be wildly inaccurate.** 

Zymoclean purification of DNA for Illumina library preparation

- 26 Transfer 40 µl of DNA solution to a fresh microfuge tube. Add 80 µl of Zymo Binding Buffer and mix by pipetting up and down several times
- 27 Transfer solution to a Zymoclean column and centrifuge at 12,000 x g for 30 s. 
- 28 Add 0.2 ml of Zymo wash buffer to the top of the column and centrifuge at 12,000 x g for 30 s. 



- 29 Discard the flow-through, add another 0.2 ml of Zymo wash buffer to the column, and centrifuge at 12,000 x g for 1 min.
- 30 Transfer column to a fresh microfuge tube, add 40 µl of Zymo Elution Buffer, and incubate on the bench for 1 min. 
- 31 Centrifuge at 12,000 x g for 1 min and then discard the column.
- 32 Quantify the DNA using a Qubit machine. **Do not use NanoDrop as the readings will be wildly inaccurate.**

Protocol references

1. Ascari JP, Cazón LI, Rahn timer M, Lamour K, Fernandes JMC, Farman ML, Ponte EMD. *Pyricularia* Are Mostly Host-Specialized with Limited Reciprocal Cross-Infection Between Wheat and Endemic Grasses in Minas Gerais, Brazil. *Phytopathology*. 2024 Jan;114(1):226-240. doi: 10.1094/PHYTO-01-23-0024-R. Epub 2024 Feb 5. PMID: 37399001.
2. Farman ML, Ascari JP, Rahn timer M, Ponte EMD, Pedley KF, Martinez S, Fernandes JMC, Valent B. A Reevaluation of Phylogenomic Data Reveals that Current Understanding in Wheat Blast Population Biology and Epidemiology Is Obfuscated by Oversights in Population Sampling. *Phytopathology*. 2024 Jan;114(1):220-225. doi: 10.1094/PHYTO-01-23-0025-R. Epub 2023 Dec 23. PMID: 37486092.
3. Raeder U, Broda P, Rapid preparation of DNA from filamentous fungi, *Letters in Applied Microbiology*, Volume 1, Issue 1, 1 January 1985, Pages 17–20, <https://doi.org/10.1111/j.1472-765X.1985.tb01479.x>

Acknowledgements

Based on a protocol originally described by Raeder and Broda (1985)