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Transformation of *Magnaporthe oryzae*

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Magnaporthe Protocols



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We use this protocol and it's working

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Abstract

Magnaporthe oryzae has been used as a genetically tractable model system for plant-pathogen interactions for more than 30 years. Genetic modifications such as gene deletions, complementations or fluorescence-tagging of proteins are regularly used to investigate the function of genes. To date, there are multiple strategies for transformation of *M. oryzae*. While *Agrobacterium tumefaciens* mediated transformation has been successfully adopted for this system, Polyethyleneglycol (PEG)-mediated protoplast transformation with a linearized DNA fragment remains the most commonly used method. The PEG-mediated transformation approach can be combined with co-transformation of preassembled Cas9/small guide RNA (sgRNA) ribonucleoproteins (RNPs) and a donor-DNA to increase homologous recombination efficiency. Here, we provide a step-by-step protocol for standard PEG-mediated transformation and subsequent identification of positive transformants.

Guidelines

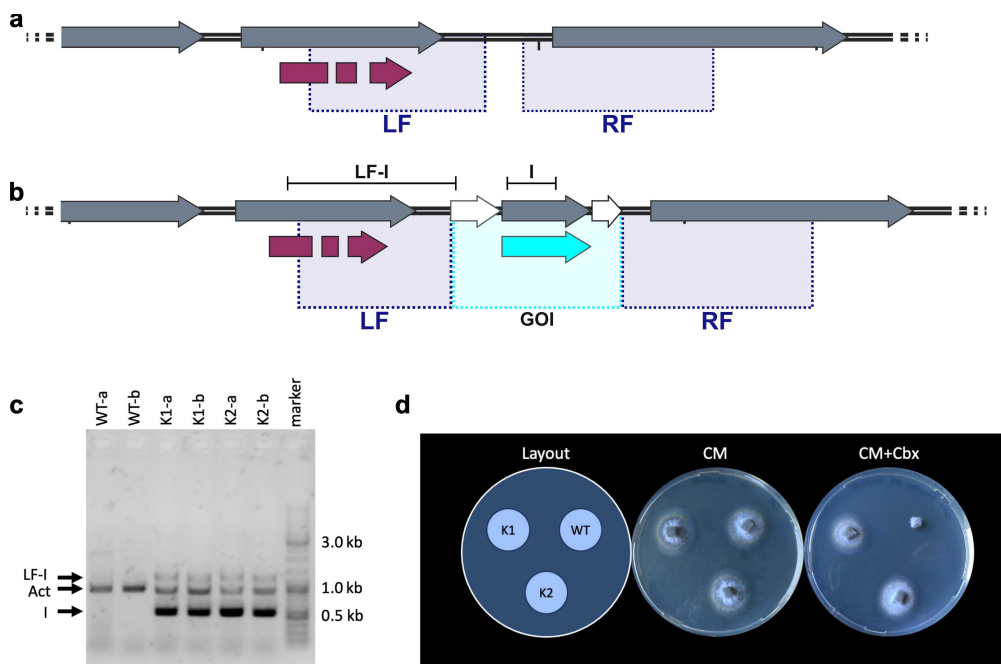
The protocol was tested for *M. oryzae* which was transformed with circular plasmids or linearized DNA fragments. Both, ectopic integration and insertion by homologous recombination at a specific locus are possible with this protocol.

Colony PCR is used to confirm positive transformants. Primer pairs are typically chosen to amplify the following:

- a control locus (e.g. Actin) as a positive control for DNA extraction
- a part of the insert (e.g. the resistance cassette) to test for any integration event
- a region spanning the borders between the native locus and the newly integrated DNA (only if transformation is carried out at a specific locus by homologous recombination)
- a region that is lost due to transformation (e.g., if a gene is deleted)

The parental, non-transformed strain should be included as a control; the DNA used for transformation can be included as a positive control.

When all primers have the same annealing temperature and the PCR products have different sizes, all PCR reactions can be run as a single multiplex reaction. The figure below shows a multiplex PCR amplifying the left flank (1.3 kb), in the actin locus (1.0 kb) and the insert (0.5 kb).



Confirmation of *M. oryzae* transformants generated using this protocol. **a** Wild-type locus before transformation. Left and right flank for homologous recombination are indicated with light blue shaded boxes. **b** Locus after transformation with a gene of interest inserted by homologous recombination. LF-I and I indicate fragments that are amplified in colony PCR. **c** Colony multiplex PCR of two positive transformants compared to Guy11-wildtype DNA (duplicates). Act represents a fragment from the actin locus (control), LF-I represents a fragment spanning the entire left flank, I represents a fragment from the insert. The results show that the two transformants carry the insert (I) and that it was integrated by homologous recombination (LF-I). **d** Growth of two positive transformants as well as the WT Guy11 strain on normal CM and CM supplied with 100 µg/ml Carboxin (Cbx). Note that the transformants grow well on both plates while Guy11 does not grow on CM + Cbx.



Materials

--- Transformation ---

0.7 M NaCl

40.9 g NaCl

ddH₂O to 1 L

Autoclave.

Lytic enzyme solution

100 ml 0.7 M NaCl

3 g Extralyse[®] (Laffort) or Vinotaste[®] Pro (Novozymes)

Filter-sterilize.

STC buffer

109.32 g Sorbitol (1.2 M final)

5 ml 1 M Tris-HCl pH 7.5 (10 mM final)

5 ml 1 M CaCl₂ (10 mM final)

ddH₂O to 500 ml

Autoclave.

PTC buffer

60 g PEG 4000

1 ml 1 M Tris-HCl pH 7.5 (10 mM final)

1 ml 1 M CaCl₂ (10 mM final)

ddH₂O to 100 ml

Microwave to dissolve the PEG.

Autoclave.

--- Media ---

Complete medium (CM, as in dx.doi.org/10.17504/protocols.io.9t7h6rn)

50 ml 20X Nitrate Salts (See Below)

1 ml Trace Elements (See Below)

10 g D-Glucose

2 g Peptone

1 g Yeast Extract

1 g Casamino Acids

1 ml Vitamin Solution (See Below)

ddH₂O to 1 l

Adjust pH to 6.5 with NaOH.

Autoclave.

20 X Nitrate Salts:

120 g NaNO₃

10.4 g KCl

10.4 g MgSO₄*7H₂O (5.2 g if anhydrous)

30.4 g KH₂PO₄

ddHOH to 1 L

Autoclave.

Store at 4°C.

1000 X Trace Elements:

Add the compounds in order!

80 ml ddH₂O

2.2 g ZnSO₄*7H₂O

1.1 g H₃BO₃

0.5 g MnCl₂*4H₂O

0.5 g FeSO₄*7H₂O

0.17 g CoCl₂*6H₂O

0.16 g CuSO₄*5H₂O

0.15 g Na₂MoO₄*2H₂O

5 g Na₄EDTA

Boil briefly and let cool to 60°C

Adjust pH to 6.5 with KOH

Cool to room temperature.

ddH₂O to 100 ml.

Store at 4°C

1000x Vitamin Solution:

0.01 g Biotin

0.01 g Pyridoxin

0.01 g Thiamine

0.01 g Riboflavin

0.01 g PABA (p-aminobenzoic acid)

0.01 g Nicotinic Acid

100 ml ddH₂O

Store in a dark glass bottle at 4°C.



Complete Medium agar

Add 15 g / 1 L CM.

Autoclave.

Osmotically stabilized complete medium (OCM) and OCM agar

As CM and CM-agar but with 0.8 M Sucrose (273.84 g for 1 L).

Bottom Agar (BA) and bottom broth

Melt 2.5 M Na_2HPO_4 in the microwave.

For 1 L of BA combine:

273.84 g Sucrose (0.8 M final)

1.7 g Yeast Nitrogen Base without amino acids and without ammonium sulphate

2.0 g Ammonium Nitrate

1.0 g L-Asparagine

10.0 g Glucose

Adjust pH to 6.0 with 2.5 M Na_2HPO_4 .

ddHOH to 1 L.

Aliquot 150 ml in 250 ml bottles.

Add 2.25 g Agar per bottle (for bottom agar; bottom broth without agar). Bacteriological (European Type) No. 2 Agar is recommended for preparing media for transformation due to its low gel point.

Autoclave.

Top Agar (TA)

Melt 2.5 M Na_2HPO_4 in the microwave.

For 1 L of TA combine:

1.7 g Yeast Nitrogen Base without amino acids and without ammonium sulphate

2.0 g Ammonium Nitrate

1.0 g L-Asparagine

10.0 g Glucose

Adjust pH to 6.0 with 2.5 M Na_2HPO_4 .

ddHOH to 1 L

Add 10 g/L agar (in smaller aliquots).

Autoclave.

2.5 M Na_2HPO_4

35.49 g Na_2PO_4

ddH₂O to 100 ml

Melt in the microwave.



Autoclave.

--- Quick DNA Extraction and colony PCR ---

Extraction solution

5 ml 1 M Tris-HCl pH 8.0 (100 mM final)

12.5 ml 1 M KCl (250 mM final)

1 ml 0.5 M EDTA pH 8.0 (10 mM final)

Adjust pH to 9.3 with KOH.

ddH₂O to 50 ml

Filter-sterilize.

Store at room temperature.

Dilution solution

3 % BSA powder in water

Adjust pH to 7.6 with KOH.

Filter-sterilize.

Store at -20 °C or at 4 °C for shorter periods of time.

PCR

Phire Hot Start II DNA Polymerase (F122/F124 Thermofisher,
or other polymerase of choice)

10 mM dNTPs

10 µM forward and reverse primer

--- General material ---

- Sterile Miracloth, ideally pre-assembled to a double-layer
- Steel forceps, spatula or blades
- Flame-sterilization unit
- Sterile toothpicks or pipette tips
- 96-well-PCR plate or 8-strips
- Ethanol for sterilization

Fungal growth in liquid culture

- 1 All steps are performed in a sterile biological safety cabinet.
- 2 Prepare a *M. oryzae* culture by placing a filter paper stock on complete-medium agar and let it grow for 7 days at 25 °C.
- 3 Place a forceps and a small (5 mm width) spatula into Ethanol, flame-sterilise and let cool down.
- 4 Add 150 ml of sterile complete medium to a sterilized 300 ml Erlenmeyer flask.
- 5 Cut approx. 5 cm along the outer edge of the colony (i.e., young vegetative hyphae) using the sterilized spatula and divide into small plugs (~0.5 × 0.5 cm).

Note

A sterilized blade can be used alternatively to only cut from the surface of the agar plate.

- 6 Transfer the agar plugs into liquid complete medium using a sterilized forceps or a sterilized spatula.

Note

Blending the culture in a sterile, stainless steel blender will strongly increase the surface of the solid culture and thus the amount of mycelium obtained. In case more mycelium is required, the amount of starting material can be increased.

- 7 Cover the Erlenmeyer flask with sterile aluminum foil and incubate the liquid culture on a rotary shaker (120 rpm, 25 °C) for 2 days. The mycelium grows out of the agar blocks during that time and forms small balls.

Polyethyleneglycol (PEG)-mediated transformation

- 8 Harvest the culture by filtering through two layers of sterile Miracloth. Wash the mycelium with sterile 0.7 M NaCl. Squeeze the mycelium with a pipette tip or sterile spatula to remove excess liquid without disrupting the Miracloth layers.

Note

The flowthrough of the harvested culture should be clear. Turbid medium indicates a bacterial contamination and a new liquid culture should be used.

- 9 Transfer mycelium to a 50 ml falcon tube. Add 45 ml of filter-sterilized lytic enzyme solution. Shake thoroughly to disperse hyphal clumps until homogenous. Cover the tubes with aluminum foil (as protoplasts can be light-sensitive) and incubate at 30 °C, 75 rpm for 3 - 4 h.

Note

The enzyme mix can be prepared with different enzymes. Glucanex or Trichoderma lysing enzymes have been standards for protoplast preparation for decades but have now been discontinued. Extralyse® (Laffort) or Vinotaste® Pro (Novozymes) enzyme mix can be used as alternatives. We have tested this protocol with both enzyme mixes at a concentration of 30 mg/ml.

- 10 Harvest protoplasts by filtering through 2 layers of sterile miracloth and collect flow through in a new, sterile 50 ml falcon tube.

Note

Carefully squeeze the mycelium with a pipette tip to increase yield. Avoid disrupting the miracloth to prevent release of undigested mycelium or cell debris. Keep protoplasts and buffers on ice for the following steps.

- 11 Pellet protoplasts by centrifugation (2000 rpm, 10 min, 4 °C).
- 12 Decant the supernatant. Resuspend protoplasts in 1 ml STC buffer by carefully pipetting up and down.

**Note**

Use wide-bore or cut pipette tips to reduce damage to protoplasts.

- 13 Pellet protoplasts by centrifugation (2000 rpm, 10 min, 4 °C).
- 14 Decant the supernatant. Resuspend protoplasts in 500 µl of STC buffer by carefully pipetting up and down or gently flicking the tube. Check the quality of the protoplasts under the microscope. Round-shaped, intact cells and no clumps should be present. The final number of protoplasts should be $\sim 5 \times 10^8$.

Note

If the yield of protoplasts is too low or too high, adjust the volume of STC buffer accordingly.

- 15 Combine 150 µl of the protoplast suspension with DNA (10 – 20 µg in 10-15 µl volume) in a 1.5 ml tube, tap to mix and incubate on ice for 25 min.

Note

The volume of DNA should not exceed 1/10 of the total volume as it reduces the osmolarity of the solution which can damage the protoplasts. In case the DNA volume is more than 15 µl, the volume of protoplasts should be increased accordingly. Transformation of linearized DNA is recommended to increase homologous recombination efficiency.

- 16 Prepare 50 ml falcon tubes with 1.0 ml of PTC buffer. Add the protoplast-DNA mixture, gently mix by swirling the tube and incubate at room temperature for exactly 15 min.

Note

Shorter incubation times decrease the efficiency of DNA uptake while longer incubation times might damage the protoplasts due to the toxicity of the PEG.



- 17 Add 10 ml of bottom broth or liquid osmotically stabilized CM (OCM) (see table in step 12) and mix by inverting. Cover the tubes with aluminum foil to keep them dark and shake overnight (75 rpm, 25 °C).

Note

When doing several transformation reactions at once, the protoplast-DNA-mixtures can be added to the respective PEG-containing falcon tubes every 30 s. The liquid medium is added exactly after 15 min every 30 s in the same order.

- 18 The next day, melt 125 – 150 ml bottom agar (BA) or OCM agar (see table in step 12) and let cool down to 45 °C. Pour the protoplast solution into the molten agar medium, gently mix while avoiding the formation of air bubbles. Immediately proceed to pour the mixture into 5 plates (25-30 ml per plate) and let solidify.

Note

Regularly mix and measure the temperature of the BA/OCM agar medium to avoid solidification in the bottle. The medium should be cool enough to hold it in the hand without feeling uncomfortable. Once the protoplast solution is added to the medium, mix by gently shaking and immediately proceed to pour the plates to avoid solidification of the agar.

- 19 Overlay the plates with a thin layer of medium containing antibiotics for selection (approx. 15 ml) according to the table below. Note that the fungicide concentration in the top layer is 2-3 x as high as the concentration required for inhibition on a normal plate as the top layer only makes up 1 half to 1 third of the total agar.

Note

The concentration required for selection may vary depending on the strain used. The following table shows the selection conditions for *M. oryzae* strain Guy11. As BASTA and Chlorimuron Ethyl interfere with amino acid biosynthesis pathways, selection is performed on minimal medium (BA/TA). Leftover medium for overlaying can be used to pour plates for selection of transformants.



	A	B	C	D
	Selection	Medium	Stock	Final concentration (µg/ml)
	BASTA	BA/TA	100 mg/ml in water	150-200
	Benomyl	OCM	10 mg/ml in DMSO	10
	Carboxin	OCM	100 mg/ml in DMSO	300
	Chlorimuron Ethyl	BA/TA	100 mg/ml in DMF	75
	Hygromycin B	OCM	50 mg/ml in PBS	600

- 20 Keep plates at 25 °C in the dark. First colonies are expected after 7 (BASTA, Hygromycin B) to 10 days (Carboxin, Sulfonylurea).

Note

Hygromycin seems to produce more false positive colonies, possibly due to incomplete selection. Hence, other selection markers should be used preferably.

Selection of transformants

- 21 After 7-10 days, many small colonies should be visible in the bottom of the transformation plate with some of them growing faster and penetrating through the top layer. Pick colonies that penetrate through the top layer by cutting out a 1-2 mm square using a sterile toothpick or pipette tip.
- 22 Place the colonies on a plate containing selection antibiotic (see Table below). Multiple possible transformants can be placed on the same plate (approx. 2 cm space in between).

A	B	C
Selection	Medium	Final concentration (µg/ml)



	A	B	C
	BASTA	TA	75
	Benomyl	CM	5
	Carboxin	CM	100
	Chlorimuron Ethyl	TA	25
	Hygromycin B	CM	200

- 23 Keep the selection plate at 25 °C for 2 days.
Resistant strains grow on the selection plate as quickly as on normal CM plates. False-positive strains exhibit slower growth and form more aerial hyphae.

Confirmation of transformants – quick DNA extraction and colony PCR

- 24 Tips and guidelines for primer design for colony PCR are given in the “guidelines” section of the protocol. Steps 25 and 26 are performed in a sterile hood.
- 25 Prepare a 96-well-plate or PCR-strips with 50 µl extraction solution.
- 26 Scrape 1-2 mm² of mycelium from the selection plate of resistant strains with a sterile forceps, toothpick or pipette tip and place into the extraction buffer.
- 27 Incubate at 95 °C for 15 min, keep on ice afterwards.
- 28 Add 100 µl dilution solution. Mix by flicking or pipetting up and down.
- 29 Use 1 µl of the diluted solution for colony PCR with Phire Hot Start II DNA Polymerase in a 20 µl reaction.

**Note**

The DNA may degrade rapidly and is not suitable for long-term storage. Degradation can be reduced by storage at 4 °C or -20 °C.

- 30 Set up the PCR reaction according to the manufacturer's instructions. For Phire Hot Start II Polymerase add 4 µl 5x Green Phire reaction buffer, 0.4 µl 10 mM dNTPs, 1 µl of each primer and water to 20 µl.

Note

When running a multiplex-PCR, use 1 µl of each primer and reduce the amount of water in the reaction.

- 31 Run the following program on a thermocycler:
- Initial denaturation: 98 °C, 5 min
 - Denaturation: 98 °C, 10 s
 - Annealing: (Annealing temperature), 10 s
 - Extension: 72 °C, 10-15s/kb
 - Go to step b 34 x
 - Final extension: 72 °C/kb
 - Hold 16 °C

- 32 Visualize the colony PCR on an agarose gel.

Note

If sequencing confirmation of the transformed locus is required, DNA extraction for PCR quality is recommended to obtain the template DNA for generating PCR products using a high-fidelity polymerase (see "DNA Extraction from *Magnaporthe oryzae* (PCR quality)" dx.doi.org/10.17504/protocols.io.x54v9rzd4v3e/v1)

Protocol references

- Arazoe, T. (2021). Genome Editing Using CRISPR/Cas9 in the Rice Blast Fungus. In S. Jacob (Ed.), *Magnaporthe oryzae: Methods and Protocols* (pp. 149-160). Springer US. https://doi.org/10.1007/978-1-0716-1613-0_12
- Arazoe, T., Miyoshi, K., Yamato, T., Ogawa, T., Ohsato, S., Arie, T., & Kuwata, S. (2015). Tailor-made CRISPR/Cas system for highly efficient targeted gene replacement in the rice blast fungus. *Biotechnology and Bioengineering*, 112(12), 2543-2549. <https://doi.org/https://doi.org/10.1002/bit.25662>
- Ebbole, D. J. (2007). Magnaporthe as a model for understanding host-pathogen interactions. *Annu Rev Phytopathol*, 45, 437-456. <https://doi.org/10.1146/annurev.phyto.45.062806.094346>
- Foster, A. J., Martin-Urdiroz, M., Yan, X., Wright, H. S., Soanes, D. M., & Talbot, N. J. (2018). CRISPR-Cas9 ribonucleoprotein-mediated co-editing and counterselection in the rice blast fungus. *Scientific Reports*, 8(1), 14355. <https://doi.org/10.1038/s41598-018-32702-w>
- Huang, J., & Cook, D. E. (2022). CRISPR-Cas12a ribonucleoprotein-mediated gene editing in the plant pathogenic fungus *Magnaporthe oryzae*. *STAR Protocols*, 3(1), 101072. <https://doi.org/https://doi.org/10.1016/j.xpro.2021.101072>
- Parsons, K. A., Chumley, F. G., & Valent, B. (1987). Genetic transformation of the fungal pathogen responsible for rice blast disease. *Proc Natl Acad Sci U S A*, 84(12), 4161-4165. <https://doi.org/10.1073/pnas.84.12.4161>
- Rho, H.-S., Kang, S., & Lee, Y.-H. (2001). Agrobacterium tumefaciens-mediated Transformation of the Plant Pathogenic Fungus, *Magnaporthe grisea*. *Molecules and Cells*, 12(3), 407-411. [https://doi.org/https://doi.org/10.1016/S1016-8478\(23\)17116-0](https://doi.org/https://doi.org/10.1016/S1016-8478(23)17116-0)
- Valent, B. (1990). Rice Blast as a Model System for Plant Pathology. *Phytopathology*, 80:33. <https://doi.org/10.1094/Phyto-80-33>