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Protocol status: Working

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Batrachochytrium dendrobatidis electroporation

Scientific Reports

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ABSTRACT

Batrachochytrium dendrobatidis (BD) is a parasitic symbiote with many frogs. BD infection is devastating to most frog species, causing mass mortality in ~6.5% of amphibian species. To understand the cellular biology of BD and its interaction with amphibians, we have developed an method of electroporation, allowing for efficient deliver of extracellular constructs to infectious BD zoospores. The current method reliably loads 95% of treated cells with extracellular dextrans.

GUIDELINES

Keep everything cold and work quickly when off ice.
 Dispose of supernatant as quickly as possible as zoopores will swim up from out of the pellet after centrifugation.

MATERIALS

MATERIALS

- Magnesium Chloride **Fisher Scientific Catalog #AC223210010**
- Sodium phosphate monobasic monohydrate **Sigma Aldrich Catalog #S9638**
- Gene Pulser Electroporation Cuvettes, 0.2 cm gap **Bio-rad Laboratories Catalog #1652086**
- D-Mannitol **Bio Basic Inc. Catalog #MB0335.SIZE.100g**
- Sodium phosphate, dibasic, anhydrous **Bio Basic Inc. Catalog #S0404.SIZE.500g**
- Sodium succinate, dibasic, hexahydrate **Bio Basic Inc. Catalog #SB0889.SIZE.500g**
- Paraformaldehyde **Sigma Aldrich Catalog #P6148**
- Potassium Chloride **Sigma Aldrich Catalog #P9541**
- sucrose **Fisher Scientific Catalog #sucrose**
- Dextran, Fluorescein, 3000 MW, Anionic **Thermo Fisher Catalog #D3305**

PROTOCOL integer ID:
29687

Keywords: chytrid,
electroporation, bd,
chytridiomycosis

This protocol requires the use of dangerous chemicals and biological hazards. Follow appropriate PPE, safety, and biological waste guidelines.

BEFORE START INSTRUCTIONS

Synchronize cultures 2 hrs before counting zoospores

Media & Material Prep

10m

- 1 Make 50 mL SM buffer (SM buffer should not be more than 7 days old).

10m

1.1 SM Buffer:

5 mL	[M] 50 millimolar (mM) KCL (Autoclaved)
750 µL	7.2	[M] 1 Molarity (M) Sodium Phosphate Buffer (Filter Sterilized)
7.5 mL	[M] 100 millimolar (mM) MgCL2 (filter sterilized)
.338 g	Sodium Succinate dibasic acid hexahydrate
.228 g	D-Mannitol
36.75 mL	Sterile water	

- 2 Make an appropriate volume of Paraformaldehyde fixation buffer.

10m

- 2.1 Final Concentration of PFA fixation buffer:
4.8% PFA
9mM Sucrose
50mM Sodium Phosphate Buffer, pH 7.2

- 3 Make 2mg/mL dextrans in SM buffer (**Be sure it is Cat No. D3305 from Invitrogen by Thermo Fisher Scientific**)
 - dextrans is sticky, measure in the tube you will be adding buffer to.

5m

- Spin down at high speed, then use supernatant.

4 Measure out the required amount of autoclaved growth media into an appropriate flask.

2m

5 Place fixation buffer, growth media, and bonner's salts on ice.

15m

6 Turn Centrifuge on to 4 °C

Zoospore Prep

7 Transfer synced cells to 50mL conicals

5m

8 Spin down cells and discard supernatant 2500 rpm @ 4 °C for 00:05:00

6m

9 Resuspend in 10 mL of SM buffer, combine after resuspension if multiple tubes were needed to hold all the synced spores.

2m

10 2500 rpm @ 4 °C for 00:05:00 . Discard supernatant immediately.

6m

11 Resuspend in 5mL of SM

2m

- 12 2500 rpm @ 4 °C for 00:05:00 . Discard supernatant immediately. 5m
- 13 Resuspend cells in the appropriate volume of SM buffer needed for the # of cuvettes and tubes that will be used (100 µL SM for each cuvette) 2m
- 14 Count cell concentration. Aiming for 2×10^7 - 1×10^7 cells/mL 10m
- 14.1 If the spores are overly concentrated, dilute to the appropriate concentration. 2m
- 15 Prepare cuvettes & tubes.
E- = electroporation control (no voltage)
D- = Dextran control (no dextrans)
- 15.1 Prepare E-D- tubes. 1.5mL eppendorfs: 1m
- 100 µL SM
 - 100 µL Cell suspension
- 15.2 Prepare E-D+ tubes: 1.5ml eppendorfs 1m
- 100 µL Dextran solution
 - 100 µL Cell suspension
- 15.3 Prepare E+D- cuvettes: 0.2cm electroporation cuvettes 1m
- 100 µL SM

🧪 100 μ L Cell suspension

15.4 Prepare E+D+ cuvettes: 0.2cm electroporation cuvettes

1m

🧪 100 μ L Dextran solution

🧪 100 μ L Cell suspension

16 Set samples on ice for ~ 10 minutes

10m

Electroporation

17 Set electroporation protocol

- 17.1**
- Squarewave protocol
 - 1000V
 - 2 pulses
 - 5 sec intervals
 - 2mm cuvette

2m

18 Gently pipette liquid in cuvette up and down to mix cells just before electroporation

19 Insert cuvette

20 Hit the PULSE button, record percent droop

21 Remove cuvette and place immediately on ice.

22 Repeat steps 18-21 for all cuvettes, be sure to pipette control eppendorf tubes even though they will not be exposed to a pulse.

23 00:10:00 allow samples to rest on ice

10m

24 Add 200 μ L chilled growth media to each cuvette

25 00:10:00 allow samples to rest on ice

Wash cells

26 Transfer contents of each tube/cuvette to 15mL conicals.

27 5 mL chilled growth media to each sample

28 2500 rpm 4 °C 00:05:00 discard supernatant

29 go to step #27 Unless supernatant has been discarded three times.

30 add 5 mL Bonner's salts 4 °C Bonner's Salts

31 2500 rpm 4 °C 00:05:00 discard supernatant

32 go to step #30 until supernatant as been discarded 2 times

33 add 600 µL Bonner's salts 4 °C

34 Aliquot 100 µL cells for imaging

35 Aliquot 100 µL cells for counting

36 Fix remaining cells for flow cytometry

Cell Fixation

- 37** Add an equal volume of fixation buffer (see go to step #2) to each remaining volume of cells.
- 38** Immediately place tubes on ice. 00:15:00 (centrifugation time in the following step is IN ADDITION to this time). Leave alone for 15 minutes.
- 39** Centrifuge 2200 rpm @ Room temperature 00:05:00
- 40** Properly dispose of supernatant and resuspend in 400 μ L Bonner's salts
- 41** Immediately quantify via flow cytometry or wrap in foil and store at 4 °C for up to 4 hours.