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Batrachochytrium dendrobatidis electroporation

Scientific Reports

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1 Works for me dx.doi.org/10.17504/protocols.io.88xhzxn

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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.

EXTERNAL LINK

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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

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

SAFETY WARNINGS

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

BEFORE STARTING

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 Milimolar (mM) KCL (Autoclaved)**
- **750 µl** pH**7.2** [M]**1 Molarity (M) Sodium Phosphate Buffer (Filter Sterilized)**
- **7.5 mL** [M]**100 Milimolar (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

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3 Make 2mg/mL dextrans in SM buffer (**Be sure it is Cat No. D3305 from Invitrogen by Thermo Fisher Scientific)** 5m
- dextrans is sticky, measure in the tube you will be adding buffer to.
- 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 $2x10^7$ - $1x10^7$ cells/mL 10m

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14.1 If the spores are overly concentrated, dilute to the appropriate concentration. 2m

15 Prepare cuvettes & tubes.
E- = electroporation control (no voltage)
D- = Dextrans control (no dextrans)

15.1 Prepare E-D- tubes. 1.5mL eppendorfs: 1m
- **100 µl SM**
- **100 µl Cell suspension**

15.2 Preprare E-D+ tubes: 1.5ml eppendorfs 1m
- **100 µl Dextrans solution**
- **100 µl Cell suspension**

15.3 Prepare E+D- cuvettes: 0.2cm electroporation cuvettes 1m
- **100 µl SM**
- **100 µl Cell suspension**

15.4 Prepare E+D+ cuvettes: 0.2cm electroporation cuvettes 1m
- **100 µl Dextrans solution**
- **100 µl Cell suspension**

16 Set samples on ice for ~ 10 minutes 10m

Electroporation

17 Set electroporation protocol

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

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

19 Insert cuvette

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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 µl 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

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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 µl Bonner's salts**

41 Immediately quantify via flow cytometry or wrap in foil and store at **4 °C** for up to 4 hours.

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