Jan 18, 2022

Protocols from: Evolutionary analyses of visual opsin genes in frogs and toads: diversity, duplication, and positive selection

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Protocols used to extract mRNA from frog retinas, create cDNA libraries, and amplify opsins by PCR for sequencing at the UT core facility under their standard protocols. These protocols were used to obtain the opsin sequences in the paper: Evolutionary analyses of visual opsin genes in frogs and toads: diversity, duplication, and positive selection.

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Frog retinas, RNAEasy Kit, Qiashredder.

RNA Extraction

1 Transfer sample into a **1.5 mL** l collection tube.

2 Pipette off RNALATER.

3 Add **600 µL** Buffer RLT.

4 Add **6 µL** Beta-mercaptoethanol.

5 Disrupt tissue with sterile pestle.

6 Pipette into Qiashredder column. Spin **00:02:00** @ 8,000rpm. 2m

7 Remove Qiashredder column; Add cap; Spin **00:03:00** @ max speed. 3m

8 Add **600 µL** 70% Ethanol to new collection tube.

9 Transfer lysate to the collection tube; mix lysate and 70% Ethanol by pipetting.

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10 Transfer lysate to RNeasy column (**700 µL** at a time). Spin **00:00:15** @ 9,800rpm; Discard flow through. Add rest of lysate; Spin **00:00:15** @ 9,800rpm; Discard flow through. 30s

11 Add **700 µL** Buffer RWI. Spin **00:00:15** @ 9,800rpm. 15s

12 Transfer RNeasy column to new collection tube.

13 Add **500 µL** Buffer RPE. Spin Spin **00:00:15** @ 9,800rpm; Discard flow through. 15s

14 Add **500 µL** Buffer RPE – Spin **00:01:00** @ 9,800rpm; Discard Flow through/ Spin **00:02:00** @ 13,000rpm. 3m

15 Transfer RNeasy column to new collection tube.

16 Elute with **30 µL** RNAse-Free H_20. Spin **00:01:00** @ 13,000rpm. 1m

17 Elute with **30 µL** RNAse-Free H_20- Spin **00:01:00** @ 13,000rpm. 1m

CDNA Synthesis

18 Combine mRNA and RNAse-free H_20 to standardize all samples to aliquots containing **0.4 µg** mRNA total in **10 µL** .

19 Make 2 Master mixes:

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19.1 Master Mix 1: add **1 µL** dNTP mix and **2 µL** dT primer per sample.

19.2 Master Mix 2: add **4 µL** Buffer, **2 µL** DTT and **0.5 µL** RNAase inhibitor per sample.

20 Pipette **3 µL** of Master Mix 1 into each sample.

21 Place sample on dry bath at **65 °C** for **00:05:00** . 5m

22 **6.5 µL** Put samples on ice for **00:01:00** . 1m

23 Pipette **6.5 µL** of Master Mix 2 into each sample.

24 Pipette **1 µL** Superscript into each sample.

25 **65 °C** Incubate samples at room temp for **00:10:00** . 10m

26 Incubate samples at **42 °C** for **00:50:00** . 50m

PCR 17m 30s

27 Keep all reagents on ice at all times.

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28 Make a master mix. Per sample add the following:

- **2.0 µL** 10X Buffer
- **1.0 µL** **50 millimolar (mM)** $MgSO_4$
- **0.5 µL** dNTP mix (**10 micromolar (µM)** each)
- **18.4 µL** ddH_2O
- **1 µL** forward primer (**10 micromolar (µM)**)
- **1 µL** reverse primer (**10 micromolar (µM)**)
- **0.5 µL** Taq polymerase

29 Mix well by spinning.

30 Add **24 µL** of Master Mix to each PCR tube.

31 Add **1 µL** of sample for a total of **25 µL** per tube.

32 Program the thermocycler for the following program: 17m 30s

- **95 °C** for **00:10:00**
- **94 °C** for **00:02:00**
- REPEAT FOLLOWING 3 steps 35-50 times:
- **94 °C** for **00:00:30**
- **45-50 °C** for **00:01:00** *temperature depends on primer
- **72 °C** for **00:02:00**
- **72 °C** for **00:02:00**
- **4 °C** hold

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