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 extra mRNA from frog retina, create cDNA libraries, and prepare sampled for sequence at the UT core facility under standard protocols.

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Ryan K Schott, Leah Perez, Matthew A Kwiatkowski, Vance Imhoff, Jennifer Gumm 2022. Protocols from: Evolutionary analyses of visual opsin genes in frogs and toads: diversity, duplication, and positive selection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b3wgqpbw

protocol

Schott RK, L Perez, MA Kwiatkowski, V Imhoff, JM Gumm. 2022. Evolutionary analyses of visual opsin genes in frogs and toads: diversity, duplication, and positive selection. Ecology and Evolution (in press).

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Frog retinas.

mRNA Extraction

1 Transfer sample into a 1.5ml 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

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

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

12 12. Transfer RNeasy column to new collection tube

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

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

15 15. Transfer RNeasy column to new collection tube

16 16. Elute with **30 µL** RNAse-Free H20- Spin **00:01:00** @ 13,00rpm 1m

17 17. Elute with **30 µL** RNAse-Free H20- Spin **00:01:00** @ 13,00rpm 1m

cDNA Synthesis 1h 6m

18 1. Combine mRNA and RNAse-free H20 to standardize all samples to aliquots containing 0.4µg mRNA total in 10µl.

19 2. Make 2 Master mixes

20 Master Mix 1: add **1 µL** dntp mix and **2 µL** dT primer per sample

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

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

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

24 5. Put samples **On ice** for **00:01:00** . 1m

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

26 7. Pipette **1 µL** Superscript into each sample.

27 8. Incubate samples **Room temperature** for **00:10:00** . 10m

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

PCR 17m 30s

29 1. Keep all reagents on ice at all times.

30 2. Make a master mix. Per sample add the following:

2.0 µL 10X Buffer

1.0 µL 50mM MgSO4

0.5 µL dNTP mix (10mM each)

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18.4 µL ddH2O

1 µL forward primer (10µM)

1 µL reverse primer (10µM)

0.5 µL taq polymerase

31 3. Mix well by spinning.

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

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

34 6. 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 °C - **50 °C** for **00:01:00** *temp depends on primer

72 °C for **00:02:00**

END Repeat

72 °C for **00:02:00**

4 °C hold

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