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*****WARNING!!!!!! THIS PROTOCOL IS UNDER CONSTANT COSTRUCTION*****
Modified from the "Isolation of DNA and proteins from QIAzol Reagent-lysed samples-User developed protocol" available online* (starts on step 20).

Protein extractions and electrophoretic gel for coral gametes protocol

The entire process takes approximately: 10 hours.
Protein extraction [method depandat] :1-2 hours
Gel running: 1-1.5 hours
Silver staining ~ 5 hours: (first step can be overnight)
Comassie: (overnight)

Protein extraction

Reagents

- 2x Modified Newrock & Raff Buffer A (Prepare Fresh Everytime)
  - Solutions on Bishoy/Ana's bench or on chemical shelf**.** For a 10 mL solution add:
  - 20 µl - Magnesium acetate 1M
  - 200 µl - 100x Protease inhibitor
  - 200 µl - Sodium phosphate buffer, 1M and pH 7.2
  - 1 ml - Triton X-100 (detergent), 10% (handle with care)
  - 8.58 ml - MilliQ water
- Qiazol (in fridge)
- Chloroform (in hood)
- Isopropanol (bench aliquot or under the hood)
- Laemmli buffer (small, and in hood)
- Beta-mercepto-ethanol (in hood)

Protocol
1. Clean bench with ethanol.
2. Set centrifuge at 0 ºC.
3. Get ice on a box from the 6th floor.
4. Change gloves and get the gamete samples. Thaw samples on ice.
5. Label new 1.75 ml tubes
6. When samples are thaw, they will be blobby, so cut the pipette tip with a new clean razor blade to suck gametes out and transfer easily to the new and labeled tubes (approx. 100 µl). Work fast. Save leftover gametes for later.

7. Centrifuge at 5000 g for 5 min at 0 ºC.
8. Remove water (supernatant).
9. Add equal parts of 2x Modified Newrock & Raff Buffer A.
10. Vortex.
11. If necessary, split samples in 2 new, 2 mL tubes. First, add Qiazol, approx 500 µl.
12. Add equal parts of sample-buffer mix.
13. Adjust Qiazol to 1.5 mL total and mix by pipetting.
14. Add 500 µl of chloroform.
15. Vortex
16. Centrifuge at max speed for 15 min, at cold temperature (0-4 ºC).
17. Label RNA tubes in the meantime.
18. Remove supernatant into new, labeled RNA tubes. Fill with tube isopropanol and store in -20 ºC freezer until RNA extractions.
19. DNA and protein are in the pellet.

20. Add 0.3 ml of 100% ethanol to the interphase and phenol phase, and carefully mix samples by inversion. Divide sample in 2 tubes again if necessary (2, 500 µl each)
21. Incubate samples at room temperature (15–25 °C) for 2–3 min.
22. Centrifuge at 2000 g at 4 °C for 2 min to sediment DNA.
23. Transfer the phenol/ethanol supernatant containing the protein fraction to a new safe-lock reaction tube. The DNA pellet can be washed in sodium citrate/acetate and stored in 75% ethanol at 4°C for over 3 months (see protocol*1, step 6–8).
24. Add 1.5 ml isopropanol to precipitate the protein, and mix by inversion for 15 s.
25. Incubate samples at room temperature (15–25 °C) for 10 min.
26. Centrifuge at 12,000 g for 10 min at 4 °C and remove the supernatant.
27. Add 2 ml ethanol solution to the pellet containing the protein. Shake, and incubate at room temperature for 20 min. The protein pellet can be stored in ethanol solution at 4 °C (for at least 1 month) or –20 °C (for at least 1 year).
28. Centrifuge at 7500 g for 5 min at room temperature, and remove the supernatant.
29. Repeat steps 9 and 10 twice. Skip incubation.
30. Air-dry the pellet for 5–10 min upside down on kimwipe first and then, in rack but covered with kimwipe. Do not dry under centrifugation, as the pellet will be more difficult to dissolve. Turn heat block on at 95 ºC. Get gels ready.
31. Make laemmli buffer according to bottle instructions (mix beta-mercapto-ethanol). Add laemmli buffer to sample and mix well.
32. Add laemmli buffer depending on the concentration usually 1:3, and break up the pellet using a needle or a pippette tip. Make sure 90% of the pellet is at least dissolved.
34. Incubate at 95-100°C for 5 min. Pipette in and out to mix. All proteins should be in solution.
35. Spin using benchtop centrifuge for 1 minute.
36. Load 10-15 µl on gel.

Protein electrophoretic gel

Reagents

Gel (BIORAD Mini-PROTEAN TGX, Cat #456-1089, in fridge)
TGS buffer 1x (Dilute 1:10 distilled water, keep a stock).
Ladder (Precision Plus Protein Standard Cat #161-0373, in -20 °C)
Feather pipette tips
Coomasie blue stain (BIORAD Cat #161-0786, on top chemical shelf)
Silver stain kit (BIORAD Cat #161-0481, on Bishoy/Ana shared top shelf)

Protocol

1. Thaw ladder at RT. Vortex.
2. Take 1 gel or 2 depending on how many samples you are running and remove the green tape at the bottom of each.
3. Place in gel box. Numbers go inwards. Make sure there are no leaks
4. Fill space between gels all the way, and box half way with TGS buffer.
5. Use feather tips to load protein samples and ladder, 10 µl each. Save leftover sample for other gels. Keep track of order.
6. Run approximately 15 min at 96 volts. Check for bubbles. Put stuff back and clean.
7. When it is apparent that the proteins are migrating and have crossed the first gel, increase the voltage to 150.
8. Run gel until migration reaches the bottom.
9. When the gels are ready turn off the power and take gels out for staining. Carefully open gel cast and place gel on a plate for staining. Rinse with distilled water three times.

Coomassie blue

Pour on gel and cover it (approx. 50 ml). Place on mixer. Let it sit overnight on mixer at max speed. Next day: Rinse with distilled water. Scan gel. Gel can be disposed or kept in distilled water in the fridge indefinitely.

Runs overnight.

Silver Stain

Follow the Quick reference protocol (small) from the Dodeca Silver Staining kit (Cat #161-0481). This kit requires solution preparation. Some are available on Bishoy/Ana shared bench. Occasionally it will be necessary to make more solutions. Pay attention to the units. Silver stain is in Bishoy's bench covered in aluminum foil.

Takes approximately 3 hours.