Co-Extraction of RNA and DNA – Standard Operating Procedure
Simultaneous Isolation of Total RNA and Genomic DNA from Animal Tissues

Purpose:

Qiagen RNA/DNA Mini, Midi, and Maxi Kits are designed for the simultaneous isolation of up to 40, 200 or 1000 μg of total RNA and 20, 100 or 500 μg of genomic DNA, respectively, from animal cells or tissues, yeast, and Gram-positive or Gram-negative bacteria. Total RNA purified using the Qiagen RNA/DNA kit is suitable for use in Northern blotting, RT-PCR, Primer extension, Differential display, Poly A+ RNA selection, and RNase/S1 nuclease protection. Genomic DNA isolated with the Qiagen RNA/DNA kit ranges in size up to 40 kb, with an average length of 20-25 kb, and is suitable for downstream applications including PCR, AFLP analysis, RAPD analysis, Southern blotting and RFLP analysis.

Purification of nucleic acids is based on the selectivity of the Qiagen Resin, which efficiently separates RNA from DNA and eliminates contaminants by anion exchange in a 2-3 hour procedure. A highly denaturing cell-lysis buffer containing guanidine isothiocyanate (GITC) immediately generates an RNase-free environment, stabilizes the RNA, and simultaneously releases the DNA. The cellular extract is prepared, the conditions adjusted to allow separation of RNA and DNA, and the extract loaded onto the Qiagen-tip. Total RNA and a portion of the genomic DNA present in the sample bind to the Qiagen Resin while the remaining DNA passes through in the first flow-through fraction. The column is washed, and pure RNA is eluted in a high-salt buffer while DNA remains bound to the resin. The first flow-through is reapplied to the Qiagen-tip after elution of the RNA in order to bind the rest of the genomic DNA. The column is washed again, and the genomic DNA is eluted. Concentration and desalting of the RNA and DNA by isopropanol precipitation can then be performed in parallel.

The following protocol outlines the steps necessary to isolate total RNA and genomic DNA from tick (Ixodes scapularis) tissues, and is a modified version of the Qiagen protocol for RNA and DNA isolation from animal tissues (pgs. 27-31 in the Qiagen® RNA/DNA Handbook) that has been adopted by the Nelson lab. It is important to note that the disruption and homogenization procedures will vary based on the type of sample used. Please consult the Qiagen® RNA/DNA Handbook for more information.

I. Materials

- Qiagen RNA/DNA Mini Kit (Qiagen #14123) – 25 Qiagen tips
- QIAshredder column (Qiagen #79654) – 50 columns
- 14.3 M β-mercaptoethanol (β-ME)
- 100 mg/ml RNase A (optional)
- 2 ml polypropylene collection tubes
- 15 ml polypropylene collection tubes
- Equipment/supplies for disruption and homogenization
- Heating blocks or water baths (45°C and 60°C)
- Ice-cold isopropanol and 70% ethanol in water

II. Determining the Correct Amount of Starting Material
It is essential to use the correct amount of starting material in order to obtain high yields of pure RNA and DNA with Qiagen-tips. The maximum amount of tissue to be used depends on the specific RNA content of the tissue being studied, which varies greatly between tissue types. The maximum amount of tissue used is limited by two criteria:

- The volume of Buffer QRL1\( ^\beta \) required for efficient lysis
- The maximum RNA-binding capacity of the Qiagen-tip

Please consult the Qiagen-tip specifications table on pg. 10 of the Qiagen® RNA/DNA Handbook for choosing the correct amount of starting material and the correct Qiagen-tip (Mini, Midi or Maxi). The following protocol describes the use of the Qiagen RNA/DNA Mini Kit. Please note that the volumes of sample and buffers used in this protocol differ from those used for the Midi and Maxi kits.

III. Important Notes Before Starting

- If using the Qiagen RNA/DNA kit for the first time, please read “Important Points before Using Qiagen RNA/DNA Kits” on pg. 10 of the Qiagen® RNA/DNA Handbook.

- If working with RNA for the first time, read Appendix A on pg. 56 of the Qiagen® RNA/DNA Handbook.

- Fresh or frozen tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid N\( _2 \) and immediately transfer to \(-70^\circ C\). Tissue can be stored for several months at \(-70^\circ C\). To process, do NOT allow tissue to thaw (e.g. during weighing) prior to disruption in Buffer QRL1\( ^\beta \).

- Tissue lysates homogenized in Buffer QRL1\( ^\beta \) can be stored at \(-70^\circ C\) for several months. To process frozen lysates, thaw samples and incubate for 10-15 minutes at 37\( ^\circ C \) in a water bath to dissolve salts.

- Proper handling and storage of starting material is essential. Consult pg. 12 of the Qiagen® RNA/DNA Handbook.

- Efficient disruption and homogenization of the starting material is required, and procedures will vary depending on the material used. Consult pgs. 13 and 14 of the Qiagen® RNA/DNA Handbook.

- Do NOT overload the Qiagen-tip. Overloading will significantly reduce yield and purity.

- Buffer QRL1 may form a precipitate upon storage. If necessary, warm to redissolve.

- \( \beta \)-ME must be added to Buffer QRL1 before use (making Buffer QRL1\( ^\beta \)). Add 10 \( \mu \)l of \( \beta \)-ME per 1 ml of Buffer QRL1. Buffer QRL1\( ^\beta \) is stable for 1 month after addition of \( \beta \)-ME.

- Buffer QRL1\( ^\beta \) contains a guanidine salt and is therefore NOT compatible with disinfecting reagents containing bleach. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.

- A working solution of Buffer QRU\(^R\) must be prepared by dissolving 29 g of urea in 60 ml of Buffer QRU (both supplied with the kit). Simply pour Buffer QRU into the bottle containing the urea powder and dissolve at 45\( ^\circ C \). This ready-to-use buffer is stable for 2 weeks at room temperature. If stored for longer periods, the pH of Buffer QRU\(^R\) should be adjusted with HCl to pH 7.0 immediately prior to use. Do NOT autoclave.
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- Heat Buffer QRU and Buffer QF to 45°C.

IV. Simultaneous Isolation of RNA and DNA

Dissection

1. Add 10 µl of β-ME to 990 µl of Buffer QRL1 (QRL1β).
   
   **Note:** Buffer QRL1β is stable for 1 month after addition of β-ME.

2. Add 500 µl of Buffer QRL1β to a 2 ml collection tube and place on ice.

3. Carefully remove midgut and/or salivary gland pairs from infected ticks.

4. Immediately transfer tissue to the appropriately labeled tube containing Buffer QRL1β on ice.

Disruption and Homogenization of Tissues

5. To homogenize, pass the lysate 3 times through a 25-gauge needle fitted to an RNase-free syringe.

   **Notes:**
   - Adjust the volume if part of the lysate is trapped in the syringe.
   - If the tissue does not pass through a 25-gauge needle, it may be necessary to homogenize with a pestle.

6. Then pass the lysate 3 times through a 27-gauge needle fitted to an RNase-free syringe.

7. Transfer lysate to a QIAshredder column. Spin at full speed for 2 minutes at room temperature.

8. Collect supernatant and store at –70°C for up to 3 months or proceed with RNA/DNA extraction procedure.

RNA/DNA Extraction

9. Add 500 µl of Buffer QRV1 to the lysate.

10. Mix thoroughly by vortexing and centrifuge at 15,000 x g for 20 minutes at 4°C.

   **Note:** Much of the protein will be precipitated in the presence of Buffer QRV1 and eliminated by centrifugation.

11. Carefully transfer the supernatant to a new RNase-free 2 ml collection tube.

12. Add 800 µl of ice-cold isopropanol.

13. Mix thoroughly by vortexing and incubate for 5 minutes on ice.

14. Centrifuge at 15,000 x g for 30 minutes at 4°C.

   **Note:** Nucleic acids will be pelleted during centrifugation.

15. Pipet 1 ml of Buffer QRE into the Qiagen-tip to equilibrate, and allow the buffer to enter the column by gravity flow.
Notes:
- Place Qiagen-tip over a 15 ml polypropylene tube using the tip holder provided.
- The buffer will begin to flow automatically – allow the Qiagen-tip to drain completely.
- The flow of buffer will stop when the meniscus reaches the upper frit in the Qiagen-tip.
- This step prevents drying out of the tip – do NOT force out the remaining buffer.
- After equilibration, place a 15 ml polypropylene collection tube under the Qiagen-tip to collect the flow-through containing DNA.

16. Carefully remove and discard the supernatant from step 14.
17. Add 150 µl of Buffer QRL1β to the nucleic-acid pellet.
18. Dissolve the nucleic-acid pellet by heating the tube for 3 minutes at 60°C followed by vortexing for 5 seconds and sharply flicking the tube.
19. Repeat step 18 at least twice.

Notes:
- Both vortexing and flicking the tube are important to completely dissolve the pellet.
- If RNA and DNA are not fully dissolved, the yield will be significantly reduced.

20. Add 1.35 ml of Buffer QRV2 and mix thoroughly by vortexing.
21. Centrifuge at 5,000 x g for 5 minutes at 4°C.

Notes:
- Dilution with Buffer QRV2 creates optimal conditions for binding RNA to Qiagen Resin.
- Undissolved particles must be removed by centrifugation prior to loading the Qiagen-tip.

22. Apply supernatant from step 21 to the Qiagen-tip, and allow it to enter the resin by gravity flow.

Collect the flow-through in a 15 ml collection tube for later DNA isolation.

Notes:
- Isolated DNA may have a slight RNA contamination. Therefore, add 1.5 µl of RNase A (100 mg/ml) to the flow-through (NOT supplied with kit).
- Mix flow-through containing DNA and RNase A by vortexing, and keep at room temperature for later DNA extraction.

23. Pipet 2 ml (2 x 1 ml) of Buffer QRW onto the Qiagen-tip and allow it to enter the resin by gravity flow.

Notes:
- The Qiagen-tip is almost completely filled by this volume.
- Nucleic acids remain bound to the Qiagen resin, while contaminants such as proteins, polysaccharides, carbohydrates and cellular metabolites are washed away.
- Do NOT force out residual wash buffer as traces of buffer will not affect the elution step.
24. Pipet 1 ml (or 2 x 1ml)** of preheated (45°C) **Buffer QRU** onto the **Qiagen-tip**, and elute the RNA by gravity flow into a 2 ml collection tube (supplied).

**Notes:**
- This step specifically elutes RNA, while any DNA remains bound to the Qiagen resin.
- **A 2nd** elution step with 1 ml of preheated (45°C) **Buffer QRU** will increase yields by 10-15%. Elute into a 10-15 ml RNase-free polypropylene tube if elution volume is 2 x 1 ml.
- **Preparing Buffer QRU:** Pour **Buffer QRU** (60 ml) into the bottle containing urea powder (29 g) and dissolve at 45°C.
- **The ready-to-use Buffer QRU is stable for 2 weeks at room temperature. If stored for longer periods, the pH of Buffer QRU should be adjusted with HCl to pH 7.0 immediately prior to use. Do NOT autoclave.

25. Add 1 volume of **ice-cold isopropanol** to the eluate. Mix thoroughly by vortexing and place on ice.

26. Pipet the flow-through from step 22 onto the same **Qiagen-tip**, and allow it to enter the resin by gravity flow. **Save the flow-through for the next step.**

27. Pipet the flow-through onto the same **Qiagen-tip**, and allow it to enter the resin by gravity flow.

**Note:** Omission of this step may result in less than quantitative recovery of genomic DNA.

28. Pipet 3 ml (3 x 1 ml) of **Buffer QC** onto the **Qiagen-tip**, and allow it to enter the resin by gravity flow.

**Note:** Do NOT force out residual **Buffer QC** as traces of the buffer will not affect the elution step.

29. Elute the genomic DNA with 1 ml or (2 x 1 ml) of preheated (45°C) **Buffer QF**.

**Notes:** A second elution with 1 ml of preheated (45°C) **Buffer QF** will increase yields by 10-15%. Elute into a 10-15 ml polypropylene tube if elution volume is 2 x 1 ml.

30. Add 0.7 volumes of **room temperature isopropanol**. Mix thoroughly by vortexing and incubate for 10 minutes at room temperature.

31. Centrifuge RNA from step 25 and DNA from step 30 at 15,000 x g for 30 minutes at 4°C to precipitate.

**Notes:**
- If a larger centrifuge is not available, aliquot the RNA and DNA to 1.5 ml tubes and use a microfuge.
- Pellets from isopropanol precipitation may be difficult to see – mark the expected location of the pellet on the tubes prior to centrifugation.

32. Carefully remove and discard the supernatants.

33. Add up to 500 µl of **ice-cold 70% ethanol**. Vortex briefly, and centrifuge at 15,000 x g for 20 minutes at 4°C. Carefully remove and discard supernatants.

34. Repeat step 33.
35. Air-dry the RNA and DNA pellets for approximately 10 minutes at room temperature with the tubes resting upside down on a paper towel.

   Note: Over drying the pellets will make the RNA and DNA difficult to dissolve.

36. Dissolve the DNA in a suitable volume (100-200 μl) of TE Buffer (pH 8.0) by heating the tube for 3 minutes at 60°C followed by vortexing for 5 seconds and sharply flicking the tube.

37. Repeat heating and mixing in step 36 at least twice.

   Notes:
   - Alternatively, dissolve the DNA overnight at room temperature on a shaker, or at 55°C for 1-2 hours.
   - DNA dissolves best under slightly alkaline conditions (pH 8.0-8.5).

38. Store dissolved DNA at –20°C.

39. Dissolve the RNA in a small volume of RNase-free water (50 μl) by heating the tube for 3 minutes at 60°C followed by vortexing for 5 seconds and sharply flicking the tube.

40. Repeat heating and mixing in step 39 at least twice.

   Notes:
   - Both vortexing and flicking the tube are important to completely dissolve the RNA.
   - Large RNA molecules will not be quantitatively recovered if the RNA pellet is not completely dissolved.

41. Store dissolved RNA at –70°C.

[Created by Ryan Rhodes; July, 2004]