Anexos

Protocolos

QIAamp Circulating Nucleic Acid Kit (QIAGEN): Purification of Circulating Nucleic Acids from 2 ml Serum or Plasma

1) Pipet 200 µl QIAGEN Proteinase K into a 50 ml centrifuge tube (not provided).
2) Add 2 ml of serum or plasma to the 50 ml tube.
3) Add 1.6 ml Buffer ACL (containing 1.0 µg carrier RNA). Close the cap and mix by pulse-vortexing for 30 s. Make sure that a visible vortex forms in the tube. To ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.

Note: Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.

4) Incubate at 60°C for 30 min.
5) Place the tube back on the lab bench and unscrew the cap.
6) Add 3.6 ml Buffer ACB to the lysate in the tube. Close the cap and mix thoroughly by pulse-vortexing for 15–30 s.
7) Incubate the lysate–Buffer ACB mixture in the tube for 5 min on ice.
8) Insert the QIAamp Mini column into the VacConnector on the QIAvac 24 Plus. Insert a 20 ml tube extender into the open QIAamp Mini column. Make sure that the tube extender is firmly inserted into the QIAamp Mini column to avoid leakage of sample.

Note: Keep the collection tube for the dry spin in step 13.

9) Carefully apply the lysate–Buffer ACB mixture from step 7 into the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender. Please note that large sample lysate volumes (about 11 ml when starting with 3 ml sample) may need up to 10 min to pass through the QIAamp Mini membrane by vacuum force. For fast and convenient release of the vacuum pressure, the Vacuum Regulator should be used (part of the QIAvac Connecting System).

Note: To avoid cross-contamination, be careful not to move the tube extenders over neighboring QIAamp Mini Columns.

10) Apply 600 µl Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
11) Apply 750 µl Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
12) Apply 750 µl of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of ethanol has been drawn

through the spin column, switch off the vacuum pump and release the pressure to 0 mbar.

13) Close the lid of the QIAamp Mini column. Remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube, and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

14) Place the QIAamp Mini Column into a new 2 ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.

15) Place the QIAamp Mini column in a clean 1.5 ml elution tube (provided) and discard the 2 ml collection tube from step 14. Carefully apply 20–150 µl of Buffer AVE to the center of the QIAamp Mini membrane. Close the lid and incubate at room temperature for 3 min.

Important: Ensure that the elution buffer AVE is equilibrated to room temperature. If elution is done in small volumes (<50 µl) the elution buffer has to be dispensed onto the center of the membrane for complete elution of bound DNA.

Elution volume is flexible and can be adapted according to the requirements of downstream applications. The recovered eluate volume will be up to 5 µl less than the elution volume applied to the QIAamp Mini column.

16) Centrifuge in a microcentrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min to elute the nucleic acids.

AllPrep DNA/RNA FFPE Kit (QIAGEN)

Se extrajo únicamente ADN partiendo de 8 cortes de 5 µm y desparafinando mediante xileno:

1) Add 1 ml xylene, vortex vigorously for 10 s and centrifuge at full speed for 2 min.

2) Carefully remove the supernatant by pipetting without disturbing the pellet.

3) Add 1 ml ethanol (96–100%) to the pellet, mix by vortexing and centrifuge at full speed for 2 min. The ethanol extracts residual xylene from the sample.

4) Carefully remove the supernatant by pipetting without disturbing the pellet. Carefully remove any residual ethanol using a fine pipet tip. Keep the lid open, and incubate at room temperature or at up to 37°C. Incubate for 10 min or until all residual ethanol has evaporated. Proceed to step 5.

5) Resuspend the pellet by adding 150 µl Buffer PKD and flicking the tube to loosen the pellet. Add 10 µl proteinase K and mix by vortexing.

6) Incubate at 56°C for 15 min. Depending on the sample material, the sample may not be completely lysed. This does not affect the procedure. Proceed to step 7.

7) Incubate on ice for 3 min. Complete cooling is important for efficient precipitation in step 8.

8) Centrifuge for 15 min at 20,000 x g.

9) Keep the pellet for DNA purification.

Note: Depending on the amount and nature of the FFPE sample, the pellet may be very small or difficult to see. If the pellet is aspirated with the supernatant, allow the pellet to drop slowly to the bottom of the tube and use the pipet tip to reattach the pellet to the tube. Alternatively, centrifuge the supernatant again. The DNA-containing pellet can be stored for 2 h at room temperature, for up to 1 day at 2–8°C or for longer periods at –15 to –30°C.

10) Resuspend the pellet from step 9 in 180 µl Buffer ATL, add 40 µl proteinase K and mix by vortexing. The pellet should be equilibrated to room temperature prior to resuspension.

11) Incubate at 56°C for 1 h.

12) Incubate at 90°C for 2 h without agitation. This incubation step partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA. If using only one heating block, keep the sample at room temperature until the heating block has reached 90°C.

    Note: Agitation during this incubation step leads to lower DNA yields.

13) Briefly centrifuge the microcentrifuge tube to remove drops from the inside of the lid.

14) Add 200 µl Buffer AL, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix thoroughly again by vortexing or pipetting. It is essential that the sample, Buffer AL and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples. A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the AllPrep procedure.

15) Transfer the entire sample to a QIAamp MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 1 min at ≥8000 x g (≥10,000 rpm). Discard the collection tube with the flow-through. If the sample has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the QIAamp MinElute spin column is empty.

16) Place the QIAamp MinElute spin column in a new 2 ml collection tube (supplied). Add 700 µl Buffer AW1 to the spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane. Discard the flowthrough. Reuse the collection tube in step 17.

17) Add 700 µl Buffer AW2 to the QIAamp MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through. Reuse the collection tube in step 18.

18) Add 700 µl ethanol (96–100%) to the QIAamp MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm). Discard the collection tube with the flow-through.

19) Place the QIAamp MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the collection tube with the flow-through. To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise). It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during DNA elution.

20) Place the QIAamp MinElute spin column in a new 1.5 ml collection tube (supplied). Add 30–100 µl Buffer ATE directly to the spin column membrane. Close the lid gently, and incubate for 1 min at room temperature. Centrifuge at full speed for 1 min to elute the DNA.

Important: Ensure that Buffer ATE is equilibrated to room temperature. If using small elution volumes (<50 µl), pipet Buffer ATE onto the center of the membrane to ensure complete elution of bound DNA.

QIAamp MinElute spin columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. The volume of eluate will be up to 5 µl less than the volume of Buffer ATE applied to the column. Incubating the QIAamp MinElute spin column loaded with Buffer ATE for 5 min at room temperature before centrifugation can increase DNA yield.