QIAamp DNA Micro Kit 56304 extraction from dried fin clip punches

sample description

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Points before starting

- centrifugation done at room temperature. place columns in centrifuge in a consistent position so that fluid takes the same path each time
- reconstituted RNA not to exceed 3 freeze/thaw cycles
- thermomixer holds 24 tubes. set up lysis for all 24 at end of day. carry out bind/wash/elute the next morning in 3 rounds of 8 samples each
- protocol goes through 5 collection tubes per sample. ethanol (96-100%) is required

Things to do before starting

- Before using Buffers AW1 and AW2 for the first time, add the appropriate amount of ethanol (96-100%) to obtain working solutions
- Buffers ATL and AL may form precipitates upon storage. Warm at 56C with gentle agitation until dissolved. Can both check for precipitates by pouring off into beakers and pipette from same
- reconstitute lypholized RNA in 310 ul AE. aliquot into 0.5 ml tubes, store -20C
- set up single-level program on thermomixer for infinite time, 56C, and 1200 rpm for incubation

  set interval mix option to 30 s mixing time, 30 min. pause

Lysing with ATL:proteinase K

1. load dried fin clip punches into labelled 1.7 ml Safe-lock centrifuge tubes
2. add 180 ul ATL to each of all 24 centrifuge tubes
3. load ethanol-stored samples
4. add 20 ul pro-K to each tube in turn and pulse-vortex 6X
5. check that punches are submerged and lids are tight
6. incubate samples overnight on thermomixer

Binding with AL, ethanol

prep:label tubes, spin columns as you go

1. centrifuge 3 min. at ¿ 1200 x g, transfer lysate without debris to new 1.7 ml tube
2. make MM of 200 ul buffer AL and 1 ul RNA per. pulse-vortex 6X
3. NH dried fin clips only: add AL/RNA to each tube, pulse-vortex 6X
4. add AL only to IPOR samples, pulse-vortex 6X
5. add 200 ul chilled ethanol to each tube, cap, pulse-vortex each in turn 6X
6. incubate 5 min. at RT, spin down
7. transfer lysate to spin column in collection tube without wetting the rim
    - NH samples get micro spin columns, IPOR samples standard
    - avoid transferring foam from lysis or touching upper column area. apply sample only to membrane. close cap gently and move tubes carefully to avoid splashing
8. centrifuge 3 min at 1200 rpm
9. centrifuge 1 min at 8000 rpm (6000 x g). ensure lysate has cleared spin column

First wash with AW1

1. place spin column in a new 2 ml collection tube, discarding flow-through and collection tube
2. add 500 ul AW1
3. centrifuge for 1 min at 8000 rpm

Second wash with AW2

1. place spin column in a new 2 ml collection tube, discarding flow-through and collection tube
2. add 500 ul AW2
3. centrifuge 1 min at 14,000 rpm (20,000 g)
4. remove spin column from the collection tube, avoiding column contact with flow-through
5. place spin column in new collection tube, discarding flowthrough and collection tube
6. centrifuge 3 min at 14,000 rpm (20,000 g)

increased centrifugation dries the membrane, avoiding ethanol contamination

Elution with AE

1. place spin column in a new, labelled 1.7 ml microcentrifuge tube (not provided)
2. pipette 100 ul Buffer AE directly onto membrane
3. incubate 5 min. at room temp
4. centrifuge for 1 min at 14,000 rpm
5. aliquot 30 ul as working stock. store -80C, -20C respectively