Simultaneous extraction of RNA, DNA and protein from canine mast cell tumour and skin RNAlater-preserved biopsies

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Abstract

This protocol describes a method to perform simultaneous extraction of RNA (using the Qiagen's miRNeasy Mini Kit), genomic DNA and protein from canine mast cell tumour or canine skin biopsies (in the form of 3mm cubes) preserved in RNA-Later.

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Before start

You will need to prepare the following solutions for this procedure:

Genomic DNA isolation (prepare using nuclease-free water, and 0.2µm filter-sterilise after preparation):

- 75% (v/v) Ethanol
- 0.1 M sodium citrate.2H_2O in 10% (v/v) ethanol (sodium citrate solution)
- 8 mM NaOH (pH is usually around 9)
- 0.1 M HEPES
- 100 mM EDTA

Protein isolation

- 0.3M Guanidine-hydrochloride in 95% (V/V) ethanol (guanidine-ethanol solution)
- 141.6mM β-mercaptoethanol in 10M urea (urea/ β-mercaptoethanol solution).

  Dissolve 0.6g of urea in 8mL of ‘ultrapure water’ by stirring. Heat gently to dissolve completely and adjust the volume to 10mL with ultrapure water. Add 1µl of 14.3M β-mercaptoethanol to 100µl of 10M urea shortly prior to use.

Materials

- Beta-mercaptoethanol by Contributed by users
- Isopropanol by Contributed by users
- Chloroform by Contributed by users
- Nuclease-Free Water by Contributed by users
- Ethanol (molecular biology grade, ≥99.8%) 51976-500ML-F by Sigma Aldrich
- 7mm stainless steel beads 69990 by Qiagen
- TissueLyzer LT 69980 by Qiagen
- miRNeasy mini Kit 217004 by Contributed by users
- Sample Tubes RB 2mL 990381 by Qiagen
- Qiazol 79306 by Qiagen
- guanidine hydrochloride by Contributed by users
- urea by Contributed by users
- sodium citrate by Contributed by users
- Sodium Hydroxide by Contributed by users
- HEPES by Contributed by users
- EDTA by Contributed by users

Protocol

Step 1.

Homogenise a 3mm tissue biopsy in 700µl of Qiazol (Qiagen) by shaking (at 30Hz) with 2 x 7mm stainless steel balls in a TissueLyser LT (Qiagen) for 10 min at room temperature.

Step 2.

Transfer the homogenate to a 1.5ml tube and add 140µl of chloroform. Shake vigorously for 15s. Allow the homogenate to sit at room temperature for 2 - 3 min.

Step 3.

Centrifuge at 12000 x g for 15 min at 4°C.

Step 4.

Carefully transfer the upper aqueous phase to a 2ml tube to proceed with RNA extraction. Store the interphase and organic phase at 4°C for subsequent DNA and protein extraction (conveniently performed a day later).

Step 5.

Proceed with RNA extraction using the miRNeasy Mini Kit (Qiagen), following the manufacturer's instructions.

Step 6.

Add 0.21ml of 100% (v/v) ethanol to the interphase and organic phases, and carefully mix by inversion.

Step 7.

Incubate at room temperature for 3 min.

Step 8.

Centrifuge at 2000 x g for 2 min at 4°C to precipitate DNA.

Step 9.

Carefully transfer the phenolic/ethanol supernatant to a new tube and store at 4°C for subsequent protein isolation.

Step 10.

For DNA isolation, add 0.7ml of sodium citrate solution to the DNA pellet. Incubate at room temperature for 30 min, and mix by inversion every 5 min.

Step 11.

Centrifuge at 2000 x g for 2 min at 4°C, and discard the supernatant.

Step 12.

Repeat steps 10. and 11. twice.

Step 13.

At this stage, the DNA pellet can be stored for up to 3 months in 2ml of 75% (v/v) ethanol at 4°C.

Step 14.

To proceed with the DNA isolation, add 1.4 ml of 75% (v/v) ethanol to the DNA pellet.

Step 15.

Incubate at room temperature for 20 min, and mix by inversion every 5 min.

Step 16.

Centrifuge at 2000 x g for 2 min at 4°C, completely remove the ethanol supernatant and discard it.

Step 17.

Air-dry the DNA pellet for 10 min.

Step 18.

Resuspend the DNA pellet in 150µl of 8mM NaOH.

Step 19.

Centrifuge at 14000 x g for 10 min at room temperature, and transfer the supernatant to a new tube.

Step 20.

To neutralise the DNA sample, add 18µl of 0.1M HEPES and 1.65µl of 0.1M EDTA.

Step 21.

Store DNA samples at -20°C until required.

Step 22.

For protein isolation, add 1.05ml of isopropanol to the phenolic/ethanol phase and mix by inversion for 15 s.

Step 23.

Incubate at room temperature for 10 min.

Step 24.

Centrifuge at 12000 x g for 10 min at 4°C to precipitate protein, and discard the supernatant.

Step 25.

Add 1.4ml of guanidine-ethanol solution to the pellet, and incubate at room temperature for 20 min. The protein pellet can be stored at 4°C for up to a month.

Step 26.

To proceed with the protein isolation, centrifuge at 7500 x g for 5 min at room temperature, and discard the supernatant.

Step 27.

Repeat steps 25. and 26. twice.

Step 28.

Add 1.4ml of 100% (v/v) ethanol to the pellet, vortex, and incubate at room temperature for 20 min.

Step 29.

Centrifuge at 7500 x g for 5 min at room temperature, and discard the supernatant.

Step 30.

Air-dry the pellet for 5 min.

Step 31.

Inside a fume hood, add 50µl of urea/β-mercaptoethanol solution, and break up the pellet by passing it through a syringe needle.

Step 32.

Inside a fume hood, add an additional 200µl of urea/2-mercaptoethanol solution, pass through the needle if the protein is not yet completely dissolved, and incubate at room temperature for 1 h.

Step 33.

Incubate at 95°C for 3 min, and then on ice for 5 min.

Step 34.

Centrifuge at 12000 x g for 10 min at room temperature. Transfer the protein-containing supernatant to a new tube and store at -20°C until required.

Warnings

β-mercaptoethanol should always be handled inside a fumehood, and the operator should be using the appropriate safety clothing and equipment.