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

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We use this protocol and it's working

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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.

Materials

MATERIALS

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

Troubleshooting

Safety warnings

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

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.

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

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.

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

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).

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

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

7 Incubate at room temperature for 3 min.

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

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

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.

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

12 Repeat steps 10. and 11. twice.

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.

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

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

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

17 Air-dry the DNA pellet for 10 min.

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

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

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

21 Store DNA samples at -20°C until required.

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

23 Incubate at room temperature for 10 min.

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

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.

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

27 Repeat steps 25. and 26. twice.

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

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

30 Air-dry the pellet for 5 min.

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

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.

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

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.