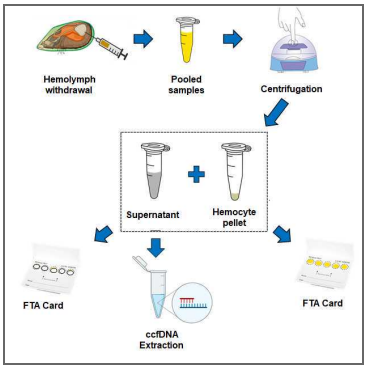


VERSION 2

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# Liquid biopsy in sentinel mussels V.2

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## ABSTRACT

This protocol has been optimized for sampling hemolymph in sentinel mussels in remote areas, such as polar regions. A detailed protocol can be found in Caza *et al.* (2019).

## MATERIALS

**A. Sampling Section**

1) Syringe (from 3 to 5 cc)
2) 25 gauge needle
3) Sterile 1.5 mL Eppendorf tubes
4) Multi Spin Battery-Powered Mini-Centrifuge (3000 x *g*) or equivalent.
5) Whatman 903™ FTA cards
6) Plastic ziplock bags
7) Silica gel desiccants (1gr/bag)
8) Pipette P200
9) Sterilized filter tips
10) Knife
11) Ethanol 70%

**B. Extraction of circulating cell-free DNA Section**

1) QIAamp DNA Investigator Kit (Qiagen - Cat #56504)
2) Ethanol 100%
3) Sterile 1.5 mL Eppendorf tubes
4) Pipettes (P1000, P100, P20, P2)
5) Sterilized filter tips

**Protocol status:** Working
We use this protocol and it's working

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**PROTOCOL integer ID:** 90415

**Keywords:** Mussels, liquid biopsy, remote area, circulating cell-free DNA

BEFORE START INSTRUCTIONS

**A. Sampling Section (Protocol optimized for freshly collected mussels)**
Be sure to gather all materials before collecting mussels.

**B. Extraction of circulating cell-free DNA Section**
1) Equilibrate samples to room temperature (15–25°C).
2) Equilibrate Buffer ATE to room temperature.
3) Set a thermomixer or heated orbital incubator to 56°C for use in step #13.
4) Ensure that buffers AW1 and AW2 have been prepared :
a) Add 25 mL ethanol (100%) to the bottle containing 19 mL Buffer AW1 concentrate.
b) Add 30 mL ethanol (100%) to the bottle containing 13 mL Buffer AW2 concentrate.
5) If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
6) Reconstitute carrier RNA by adding 310 µL of buffer ATE.

## Sampling

50m

1 Collect mussels (*Mytilus spp.*) with a shell length range between 50 to 70 mm. 10m

2 Remove intervalvar liquid by opening gently the valves with the tip of a knife. 5m

3 Withdraw hemolymph from the adductor muscle using a syringe fitted with a 25G needle. 10m

4 Transfer immediately the hemolymph to a sterile 1.5 mL Eppendorf tube (Optional: You can pool 3 or 4 hemolymph samples to obtain a total volume of 1.5 mL per tube). 1m

5 Centrifuge for 3 minutes at maximum speed (approx. 3000 x*g*) at room temperature using a battery-powered mini-centrifuge (TOMY, Japan) or equivalent. 3m

6 After centrifugation, transfer the supernatant (clear solution above pellet at bottom of tube) in a new 1.5 mL Eppendorf tube and gently resuspend the pellet. The pellets can be frozen (-20°C) or spotted on FTA cards until used use. Supernatants can also be frozen (-20°C) or spotted on FTA cards (using 70 µL aliquots) until use. We routinely use Whatman 903™ FTA cards with 13 mm discs to spot pellets or supernatants. 5m

**Note :** Once spotted, FTA cards are dried for 15 minutes at room temperature and stored individually in small ziplock plastic bags containing one silica gel desiccant moisture absorber.

**Note :** Unless otherwise indicated, hemolymph should be collected and processed within one hour after sampling.

7 Bring the mussels back to their respective mussel bed. 5m

## Extraction of circulating cell-free DNA 1h 13m

8 Unless otherwise indicated, you can use the QIAamp DNA Investigator Kit (Qiagen) for extraction of the hemolymphatic circulating cell-free DNA.

9 First, thaw frozen supernatants at room temperature and clarify by centrifugation for 10 minutes at 4500 x*g* at room temperature. 10m

10 Transfer 70 µL of the clarified supernatant into a new sterile 1.5 mL Eppendorf tube. 2m

11 Add 30 µL of buffer ATL and 10 µL of proteinase K (>600 mAU/mL) to the sample. 5m

12 Add 100 µL of buffer AL and 1 µL of reconstituted RNA Carrier (1µg/µL). Mix by pulse-vortexing for 15 seconds or until it is a homogeneous solution. 2m

13 Incubate at 56°C for 10 minutes on a lab orbital shaker set at 900 rpm. 10m

14 Add 50 µL of ethanol (100%) and mix thoroughly by pulse-vortexing for 15 seconds. Then, incubate for 3 minutes at room temperature. 5m

15 Transfer the entire lysate from step #14 to the QIAamp MinElute column. 5m

16 Centrifuge at 6000 x *g* for 1 minute. Place the QIAamp MinElute column in a clean 2 mL collection
tube and discard the collection tube containing the flow-through. 1m

17 Add 500 µL of buffer AW1 to the QIAamp MinElute column. 1m

18 Repeat Step #16. 1m

19 Add 700 µL of buffer AW2 to the QIAamp MinElute column. 1m

20 Repeat Step #16. 1m

21 Add 700 µL of ethanol (100%) to the QIAamp MinElute column. 1m

22 Repeat Step #16. 1m

23 Centrifuge at full speed (20,000 x *g*) for 3 minutes at room temperature. 3m

24 Place the QIAamp MinElute column in a clean 1.5 mL Eppendorf tube and discard the collection tube containing the flow-through. 1m

25 Open the lid and incubate at room temperature for 10 minutes. 10m

26 Apply 60 µL of buffer ATE to the center of the membrane. 1m

27 Close the lid and incubate at room temperature for 10 minutes. 10m

28 Centrifuge at full speed (20,000 x *g*) for 1 minute. 1m

29 Discard the QIAamp MinElute column and keep the eluate. 1m