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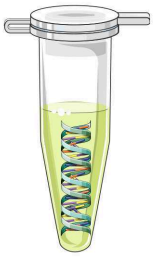
DNA Extraction from Biopsy Samples (Ph:Chl & Microcon) V.2



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We use this protocol and it's working

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Abstract

This protocol describes our latest protocol for extracting DNA from standard biopsy samples of whale skin. It uses a combination of phenol:chloroform to separate the DNA from other cellular material, and then Microcon filters to collect the DNA and then dissolve in the desired volume. This approach was motivated by original phenol:chloroform protocols described in Sambrook & Russell (2001), but modified based on organic extraction protocols used in the forensic sciences. From this protocol, we usually obtain ~250ng of DNA per milligram of starting tissue (a higher yield than that obtained from Qiagen columns, as well as phenol:chloroform followed by ethanol precipitation). The protocol takes place over 8 days.

Guidelines

When possible, extract DNA from the skin right at the skin-blubber interface. These cells were still dividing (prior to collection) and will result in DNA that is less degraded than DNA from more outer layers (Patenaude & White 1995).

We clean all of our work spaces and pipettes with a 10% bleach solution. This has been shown to effectively clean cells off of laboratory equipment (Merritt et al. 2000), as well as to degrade DNA to a degree where it is no longer available to act as a template in PCR (Prince & Andrus 1992; Kemp & Smith 2005).

Previous experimentation in our lab has indicated that proteinase K digests proteins effectively at 0.5 Units of proteinase K per milligram of tissue. Our stock proteinase K is at 600 U/ml. If we are extracting from ~40 mg of tissue, and we want each spike of proteinase K to be 0.5 U/mg, then each spike should be (0.5 U/mg) x (40 mg) = 20 Units of proteinase K. This means that each spike should be (20 U) / (0.6 U/µl) = **33.3 µl of proteinase K**.

We make our own lysis buffer solution as described below. Details can be found in our associated **Solutions** protocols.
10 mM Tris (pH 8.0) - from 2M liquid solution
10 mM EDTA (pH 8.0) - from 0.5M solution
2% SDS - from 20% solution
0.1M NaCl
40 mM DTT

Materials

Most of the supplies needed for this protocol are general/generic lab supplies. However, a few key materials for which it is important to know the details are below.

	Material	Supplier	Catalogue #	Comments
	Proteinase K	Fisher Scientific	LSG17916	Comes as a powder. We make into a solution of 600 U/ml
	Phenol:Chloroform	Fisher Scientific	BP1752I-400	
	Chloroform	Fisher Scientific	J67241.K2	
	Microcon Filters	Millipore Sigma	MRCFOR100ET	
	HPLC Water	Fisher Scientific	270733-4L	
	Lysis Buffer	Home Made		Recipe is in "Guidelines" section, and details are in our associated Solutions protocol.
	TE	IDT	11-05-01-09	

Safety warnings

Phenol and Chloroform are both hazardous chemicals that can cause damage to the liver or kidneys, can have genetic and reproduction effects, and can have carcinogenic effects. Read the SDS prior to working with them. They should only be handled in a fume hood. Neoprene gloves over nitrile gloves must be worn when working directly with these chemicals and should be immediately changed if a substantial spill on them occurs. Always wear other proper PPE including a lab coat, long pants, closed toe shoes, and safety glasses. When moving the bottles to different locations always use the rubber pail. All waste should stay in the fume hood with open lids/bags to allow chemicals to evaporate for at least 2 weeks prior to throwing them away. Waste fluids from the process are poured into a brown Ph:Chl waste bottle in the fume hood.

Ensure to wear proper PPE and carefully follow protocols to avoid contamination of DNA samples. Use caution when using scalpel to cut tissue and always dispose blades into a designated sharps container.

Day 1

1 Clean work area, supplies, and pipettes with a 10% bleach solution

2 Set up your work area. Put down a fresh piece of paper towel and get the following supplies:

- Two weigh boats for each sample
- One scalpel blade for each sample
- One 1.5 ml Eppendorf tube for each sample (plus one negative and one positive)
- A sharpie for labeling
- Kimwipes
- Lab book
- A 1,000 µl pipette (cleaned with 10% bleach)
- Tweezers (at least 3)
- Scalpel handles (at least 3)
- Bovine tissue sample (positive control)

3 Prepare 3 beakers for cleaning tweezers and scalpel handles in between samples. One beaker should contain soap (alconox) and HPLC water, the second should contain a fresh 15% bleach solution made with HPLC water, and the third should just contain HPLC water. After tweezers and scalpel handles have been used, they should be briefly rinsed in the soapy water beaker, then left to soak in the bleach solution. They should then be rinsed well in the clean water before use on the next sample. In this way, you can have a rotation going. The idea is that each pair should have adequate time in the bleach to become decontaminated but also rinsed adequately prior to touching the next sample.

4 Calculate the amount of lysis buffer that you will need to extract all of your samples, plus 1 positive and 1 negative control. Aliquot needed volume into a separate tube. You will be adding 400 µl of lysis buffer to each sample. Record this calculation in your lab book.

5 Label all of your 1.5 ml tubes with the appropriate lab code and sample ID, and add 400 µL **lysis buffer** to each tube.

6 Prepare new weigh boats, a scalpel blade, and clean tweezers. Place the second weigh boat on the scale and zero it.

7 Select the first sample, write down the appropriate sample and lab codes in your lab book, and any other pertinent information. This should be created in a table-like format, as you will record the tube label/field code, lab code, DNA ID, mg of tissue used, and the

condition of each sample. **See Appendix for example of what should be written in your lab book.**

8 Using the tweezers, pull out the tissue, place it in the weight boat, and then close the sample tube. Dab the tissue dry with a Kim wipe.

9 Weigh out approximately 40 mg of tissue, place the rest back into the original sample tube. Try to get the skin as close to the blubber-skin interface.

10 Record the actual weight of the tissue used in your lab book next to the sample information. Include any information about the quality or condition of the sample.

11 After weighing the sample and recording all information, use the scalpel to finely chop the tissue into very small pieces. This will help the lysis buffer to break down the tissue more effectively.

12 Place tissue pieces into the new 1.5 ml Eppendorf tube with lysis buffer that matches the lab number of the sample you are working with. Always be sure to double check the IDs to ensure that they are the same on both tubes.

13 Discard weigh boats, Kimwipes, and the scalpel blade. Swish tweezers and scalpel handle in the soapy water, tap to remove excess liquid, and then place in the bleach solution.

14 **Repeat steps 6-13 for each sample, ensuring the tweezers and scalpel handle are cleaned properly between samples.**

15 Weight out approximately 40 mg of bovine tissue (in DMSO) and roughly chop it into pieces. Place the pieces into the positive control tube with lysis buffer. Treat both this positive control, and the negative control tube (that just contains lysis buffer), the same as all other samples for the rest of this extraction.

16 Let samples sit for **5 days** at Room temperature in the lysis buffer, rotating/mixing them a few times a day. This gives the lysis buffer plenty of time to lyse the cells.

17 Once all samples have been recorded into your lab book, go to the MySQL database and add these samples to the DNA table of the species you are working with. This will self-propagate a DNA ID number for each sample. The DNA ID for each sample should then also be recorded in your lab book under the DNA ID column. **It is vital that from this point forward that the DNA ID is tracked for each sample**.

Day 6

18 Calculate the necessary amount of proteinase K that you will need for all of your samples (plus controls) and aliquot into a new tube. You will be giving each sample **3 shots** of proteinase K, **each of** 33.33 µL . Record this calculation in your lab book.

19 Add the first spike (33.3 µL) of **proteinase K** to each sample, mix well, and leave at Room temperature **overnight**, mixing occasionally.

Day 7

20 Add the second spike (33.3 µL) of **proteinase K** to each sample, mix well, and place in a 55 °C water bath for **1 hour** .

21 Transfer samples to a rack on the lab bench. Leave samples on bench for **1 hour** to allow them to slowly cool to room temperature.

22 Add a third spike (33.3 µL) of **proteinase K** and leave the samples at Room temperature **overnight**, mixing occasionally.

Day 8

23 **All phenol:chloroform and chloroform steps should take place in the fume hood.**

Calculate the needed amount of reagents/solutions and aliquot into new tubes (e.g., 15 ml or 50 ml falcon tubes) to prevent repeatedly going back into stock bottles. Include all calculations in your lab book.

- **Phenol:Chloroform**: You will be adding an equal volume to each sample, twice. The samples will be ~400 µl each, and therefore you will need to add 400 µl (x2) to each sample. Mix stock bottle well before aliquoting.
- **Chloroform**: You will be adding an equal volume of chloroform to each sample. The samples will be ~400 µl each, and therefore you will need to add 400 µl to each sample.
- **TE**: 50 µl is needed to activate the membrane. 200 µl of TE is used to wash the membrane (3X). 25-100 µl are used for eluting the DNA. TOTAL: 50 + (200 × 3) + 100 = ~750 µl of TE per sample.

24 Prepare and label 3 new 1.5 ml tubes per sample, all labeled with the appropriate lab codes. These tubes can have only simple lab codes, as they will be discarded. You will also need one labelled MicroCon column and 2 MicroCon collection tubes for each sample. The first set of collection tubes can have only simple lab codes. However, the final set of collection tubes should have **all pertinent lab codes including DNA ID**, as these will be used as the stock DNA tubes for each sample. See Appendix for an example.

25 Set up the fume hood by laying down fresh paper towels and making sure that all of the needed reagents and samples are there. You will need the labelled 1.5ml tubes here as well but the MicroCon columns and collection tubes do not need to be brought into the fume hood.

26 Add an equal volume (400 µL) of **phenol:chloroform** to each sample.

27 Upend samples (in rack) by hand for **5 minutes**.

28 Spin at 12000 x g for **2 minutes**

29 While samples are in the centrifuge, place 400 µL of **phenol:chloroform** into the next set of 1.5 ml tubes.

30 Carefully remove samples from the centrifuge and use a pipette to transfer the top (aqueous) layer from the samples to the fresh 1.5 ml tubes with phenol:chloroform. The aqueous layer should be clear, and the organic layer should be cloudy. If there is any question, place a drop of the bottom layer into a beaker of water. If it goes into solution, it is the aqueous layer. If it stays as a droplet, it is the organic layer. Always be sure to check the tube IDs to ensure that samples are added to the correct tubes.

31 Upend samples (in rack) by hand for **5 minutes**.

32 Spin at 12000 x g for **2 minutes**

33 While samples are in the centrifuge, place 400 µL of **chloroform** into the next set of 1.5 ml tubes.

34 Carefully remove samples from the centrifuge and use a pipette to transfer the top (aqueous) layer from the samples to the fresh 1.5 ml tubes with chloroform.

35 Upend samples (in rack) by hand for **5 minutes**.

36 Spin at 12000 x g for **2 minutes**

37 Carefully remove samples from the centrifuge and use a pipette to transfer the top (aqueous) layer from the samples to the last empty 1.5ml tube. At this point all following steps may be performed on the lab bench or continued in the fume hood.

38 Add 50 µL of **TE** to the microcon tubes to activate the membrane.

39 Transfer the aqueous layer collected in **step 37** to the MicroCon column.

39.1 **Note:** The MicroCon columns can hold up to 450 µL so 400 µL of the aqueous layer can be added. If there is more than 400 µL of sample, steps 39-41 should be repeated again until all of the sample has been run through the column.

40 Spin samples in the centrifuge at 1500 x g for **15 minutes**.

41 Carefully remove tubes from the centrifuge and discard the effluent in a waste beaker.

42 Add 200 µL of **TE** to the concentrated DNA solution in the upper reservoir of the microcon column.

43 Spin the samples in the centrifuge at 1500 x g for **15 minutes**.

44 Carefully remove tubes from the centrifuge and discard the effluent.

45 Repeat steps 42-44 **two more times**.

46 Add the desired elution volume (**30-100 µl**) of **TE** to the microcon column membrane.

47 Place the microcon filter inverted (upside down) into the new labelled collection tube (this is the final tube with the DNA ID).

48 Spin the samples in the centrifuge at 2500 x g for **3 minutes** to collect the DNA.

49 Aliquot 2 µL of DNA into Qubit tubes for quantification. This is done right away to limit freeze thaw cycles of the stock DNA.

50 Store samples at -20 °C

51 Record all relevant information in your lab book.

52 Add storage location of stock DNA samples to the DNA table of the appropriate species in the database.

Appendix

53 Example layout of how to record sample information in your lab book:

Tube Label/Field Code	Lab Code	DNA ID	Mg Tissue Used	Comments
Egl "A" Jan 13 2022	SID551090	123	40	Very mushy, hard to determine skin-blubber interface

54 Example of how to label final DNA stock tube:

Top: Write lab code. Always include letters at the beginning of the lab code, not just the numbers (i.e., SID for right whale, Bel for beluga, etc.).

Side: Write the appropriate DNA ID

Protocol references

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