STANDARD OPERATING PROCEDURE (SOP)

HIGH SALT DNA EXTRACTION

UiO : Department of Biology
University of Oslo

SOP #	**P-SOP-038-High salt DNA extraction**
Written by	Marianne HS Hansen
Approved by	
Issued by	
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Version	000

1. INTRODUCTION/PURPOSE

Standard Operating Procedure (SOP) to isolate DNA from animal tissue with use of a high salt method.

2. SAFETY

General laboratory safety applies.

User must read bullet points 6. "Procedures" and 7. "Risk assessment before starting the lab work". If the risk assessment is not complete, please report to person responsible for the lab or safety representative.

 **Risk assessment of this procedure**	There is a **small/minimal** overall risk associated with the use of this procedure, provided that this procedure is followed.

3. NECESSARY SAFETY EQUIPMENT

Gloves
Lab coat

4. RESPONSIBILITIES

Leader of Scientific programme/Head of CoE is responsible for all SOP's for the procedures performed in the unit's laboratories.

Leader of Scientific programme/Head of CoE is also responsible for making sure people using this procedure are 1) qualified and 2) have been through the necessary training.

The user is responsible for following this SOP.

5. EQUIPMENT, MATERIALS AND SOLUTIONS

Equipment:
Pipette
Pipette tips, assorted sizes
Eppendorf tubes (1,5mL)
50ml Falcon tube
Table top centrifuge
Micro centrifuge
Heating block
Scalpel blades
Weighing tray

Materials and solutions:

1M Tris, pH 7,5
5M NaCl
6M NaCl
0,5M EDTA
20% SDS (Sodium dodecyl sulphate)
20 mg/mlProteinase-K
96% Ethanol
70% Ethanol (Diluted from 96% Ethanol.)
Elution Buffer (Tris-EDTA or Qiagen EB-buffer)
milliQ water

CAS number	Chemical name	Supplier	MW	Catalog number
7647-14-5	Sodium Chloride (NaCl)	VWR	58,44g/Mol	N110
	1M Tris-HCl pH 7,5	Invitrogen		15567027
60-00-4	0,5M EDTA	Invitrogen		15575020
151-21-3	20%SDS	Ambion		AM9820
39450-01-6	Proteinase-K	Promega		V302B
64-17-5	Ethanol	Kemetyl		Kem-600051

6. PROCEDURES:

This method works well form fresh tissue of tissue that has been stored I absolute ethanol at room temperature.
If the tissue has been stored in ethanol, you can place it on some sterile tissue paper and drain off any excess ethanol before proceeding. Or you can let the sample piece you have cut off air dry in the tube before adding TNES-buffer.

Important notes before starting:

- Turn on heating block to 50◦C.
- If there are crystals in the TNES-buffer, put the tube containing the buffer in warm water. Keep it there until the crystals are gone before adding it to the samples.
- Thaw the Proteinase K.
- Make TNES-buffer:
  - Fill up ~30ml MQ-water in a Falcon tube
  - Add 500ul 1M Tris pH 7.5
  - Add 4ml 5M NaCl
  - Add 10 ml 0,5M EDTA
  - Add 1,5ml 20% SDS
  - Fill up to 50ml with MQ-water

Procedure:

1. **Put the tissue to be cut in a clean weighing tray, and cut a small piece of tissue (~0,5cm²) with a clean scalpel and put it in an eppendorf tube.** Repeat for all samples. If the tissue was stored in ethanol, let it air dry until the ethanol's gone.

2. **Add 600 µl of TNES buffer. Add 35µl of Proteinase K. Vortex and place on heating block at 50◦C with 750 rpm shaking. Leave on heat block overnight.**

3. **Optional:** if in a hurry, add more proteinase K to speed up tissue digestion and reduce the incubation time to 2-4 hours.

4. **Optional:** Add 5 µl of RNase A (100 mg/ml). Vortex and keep on bench for 5 min**.**

5. **Add 166,7 µl of 6M NaCl. Shake the samples vigorously for 20 sec.**
   Don't shake too hard, this may damage your DNA.

6. **Microfuge the samples at 12-14000 rpm at room temperature for 5-10 min**
   or 15 min if little DNA. Label new tubes during the centrifugation.

7. **Transfer supernatant to new labelled tubes.**
   Be very careful not to transfer any of the tissue debris to the new tube, unless there is very little tissue it is better to leave a little supernatant rather than transfer the cell debris.

8. **Add an equal volume (~800µl) of cold 100% ethanol and gently mix by inverting the tube a couple of times.** You should see white DNA precipitate out of the solution. But even if you don't see anything, DNA can be present.
   Better yields are achieved if the ethanol is kept at -20◦C before use, and then in an ice bucket when on the bench.

9. **Optional:** Keep the samples at -20◦C for a few hours, overnight or even over the weekend to obtain more DNA.

10. **Centrifuge the samples at 12-14 000 rpm for 10-20 minutes at 4◦C** *or 30 min if you feel like it's needed.* If there's plenty of DNA, room temperature will work.

    Tip: When pelleting the DNA, it is very helpful to place the hinge of the tube towards the outside of the rotor. The subsequent washing steps are made much easier since the DNA pellet can be readily found by looking at the bottom of the centrifuge tube below the hinge.

11. **Pour or pipette off the supernatant, taking care not to dislodge the pellet of DNA.**

12. **Wash the DNA pellet in 200-800 µl of 96% Ethanol (cold if wanted).**
    After adding the ethanol, close the cap of the tube, and invert gently. You can also gently roll the tube on its side. Pour or pipette off the ethanol and briefly spin the samples to keep the pellet at the bottom of the tube.

    If you're not worried about breaking up the DNA, you can vortex after adding cold ethanol to give the sample a better clean up. In that case you should spin the sample at 14 000rpm at 4◦C for 25 min.

13. **Wash the DNA pellet with 70% room tempered ethanol as above.** After removing the 70% ethanol, briefly centrifuge the samples to get the last of the ethanol to the bottom of the tube, and then pipette off the remaining ethanol. *If you have vortexed your sample ot just want to be sure you keep your pellet, centrifuge for 10 min at 14 000 rpm at room temperature.*

14. **Leave the sample to air dry, usually 10-30 min depending upon temperature.** Do not allow drying out too much or the DNA will be hard to re-suspend. However, any ethanol left in the tube will inhibit subsequent PCR.

15. **As soon as the sample is just dry, re-suspend the DNA in 100-200 µl**. This volume can be adjusted according to the size of the pellet. A small pellet should be re-suspended in less, a big in more. You can re-suspend in Tris-EDTA , sterile destilled water or elution buffer.

7. RISK ASSESSMENT[1]

The general risk factor of a SOP can be calculated using the part of the procedure with the assumed highest risk factor.
The risk assessment associated with this SOP is based on the user following the precautions stated in the step by step risk assessment below.

List of chemicals used in this procedure and their R and S (H and P) statements.

Chemical	Hazard symbol	R (H) statements	S (P) statements
20% SDS	Xn; Harmful	R36/37/38 Irritating to eyes, respiratory system and skin R42 May cause sensitisation by inhalation	S26 S36/37 S45 S60
Tris	Xi; Irritant	R36/37/38 Irritating to eyes, respiratory system and skin	S 26 S 37/39
NaCl	-	-	-
0,5M EDTA	Xn; Harmful	R-21 Harmful in contact with skin R-22 Harmful if swallowed	S24 S36 S37 S39
Proteinase K	Xn; Harmful	R36/37/38 Irritating to eyes, respiratory system and skin	S28 S26 S22
Ethanol 70% and 96%	F; Highly flammable	R11: Highly flammable	S16 S2 S7
EB buffer	-	-	-

Risk assessment; step by step

Part of procedure		Unwanted scenarios	Precautions	Emergency planning	S*K
1	Cutting tissue with scalpel	Finger cut	Be careful and put scalpel blade in special container for sharps	Bandages and plasters available in the lab.	3*3 (Green)
2	Adding Proteinase K	Proteinase K splash in eyes	Wear safety glasses.	Laboratory eyewash	2*3 (Green)
2	Placing samples on heat block	Burn from heat block	Don't touch the block	Cool in cold water	2*1 (green)
6 and 10	Centrifugation step	Unbalanced centrifuge	Get to know the instrument. Balance samples	-	1*1 (green)

Overall risk assessment for this SOP
If procedure specified above is followed there is MINIMAL RISK associated with the use of this SOP.

[1] Risk value: S*K See risk matrix in HSE handbook, chapter 4.3
S=Likelihood: 1=Rare, 2= Unlikely, 3=Likely, 4= Highly likely and 5= Near certainty.
K=Consequence: 1= Minimal, 2=Minor, 3=Major, 4=Serious and 5=Catastrophic.

8. WASTE DISPOSAL

All liquid waste from this SOP should be collected in a centrifuge tube and disposed of in the hazardous waste boxes.
Contaminated plastic waste should be disposed of in the hazardous waste boxes.

9. REFERENCES

http://www.genomics.liv.ac.uk/animal/RESEARCH/ISOLATIO.PDF
MSDS for all chemicals are in the MSDS folder and in EcoOnline.
HSE-handbook