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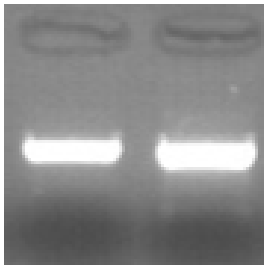
DNA isolation from cattle tissues: blood, semen or any kind of tissues, including ear biopsies



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We use this protocol and it's working

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Abstract

Here we describe a routine method for isolate DNA from different kinds of tissues: commercially available frozen semen straws, blood, ear biopsies, or other tissues.

This protocol is based on a salting-out method and uses several commercially available solutions.

It consists of several steps: washing of samples, lysis, removal of proteins and precipitation of genomic DNA. This protocol was used to isolate hundredsod samples for years.

Guidelines

For recovering DNA from blood, use K3-EDTA tubes.
Salting out is a good method to obtain DNA, as it avoids using phenol/chloroform and allows to recover DNA from different qualities of blood.

Materials

- Isopropanol
- 70% ethanol
- Puregene Tissue Kit **Qiagen Catalog #**158063
- Puregene blood kit **Qiagen Catalog #**158106
- Proteinase K, 2mL **Qiagen Catalog #**19131
- Tris(2-carboxyethyl)phosphine hydrochloride solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**646547-10X1ML
- Buffer RLT **Qiagen Catalog #**79216
- 1X PBS (Phosphate-buffered saline)
- DNA LoBind Tubes 2.0 mL **Eppendorf Catalog #**30108078

2mL tubes
2 X50mL tubes for each blood sample
Centrifuge for 2mL and 50mL tubes, blood tubes
3D heating rocker

Safety warnings

See Safety Data Sheets for warnings and safety hazards.

Before start

As we use commercial sperm straws to perform our extractions, we do not always know the composition of these straws, the quantity of material contained, the nature of the diluents and preservatives used. This is why it is sometimes necessary to use several straws to obtain enough material for sequencing. It is also sometimes wise to perform several washes (see step 3) to eliminate contaminants from diluents and preservatives.

Prepare reagents

10m

1 For semen straws, Immidiately before use, prepare a mix containing **RLT** buffer (Qiagen) and **TCEP** [Tris(2-carboxyethyl)phosphine hydrochloride] to a final volume of 500µL per sample as follow:
- 450 µL RLT
- 50 µL TCEP

Prepare samples

10m

2 Use DNA LoBind tubes at this stage (strongly recommended for sperm, not mandatory for other tissues).

2.1 For semen:
- Empty the 200 µL Straw in a 2 mL tube by cutting the two ends of the straw
- Rince the straw with 200 µL 1X **PBS** at Room temperature

For ear biopsy:
- Open the device
- Transfer the biopsy in a 2 mL tube
- Remove extra preservative liquid and the plastic ball

For blood:
- Pour the 5 mL blood in a 50 mL tube

For other tissues:
- Cut a 150 mg piece and put it in a 2 mL tube

2.2 Wash step

30m

For semen:
- Add 800 µL more **PBS** (up to 1 mL 1X **PBS**)
- Pellet 1000 x g at Room temperature during 00:05:00
- Discard the supernatant

Second wash is optional (no significant impact observed)

- Re-suspend in 1 mL 1X **PBS**
- Pellet 1000 x g at Room temperature during 00:05:00
- Discard the supernatant

For blood:

- Add 15 mL (i.e. three times the initial volume) **RBC** reagent from Puregene blood kit
- Shake vigorously and incubate 00:05:00 at Room temperature
- Pellet white blood cells 3000 x g, 10°C, 00:15:00

Lysis

3h 30m

3 ***Continue with Qiagen Puregene kit adapted as follow*** 5m

Step one

For semen:

- Add 500 µL of **RLT/TCEP** mix
- Vortex by pulsing at max speed
- Incubate 00:05:00 on ice
- Add 500 µL **CLS**

For tissues, including ear biopsies:

- Add 800 µL **CLS**
- Add 60 µL **Proteinase K**

For blood:

- Add 5 mL **CLS** (i.e. the initial blood volume)

4 **Step 2** 4h

- Mix by inversion (about 25 inversions)
- Incubate from 01:00:00 to 03:00:00, depending on the lysis process, 55 °C
- on a rotating shaker at 200 rpm

Check that no undigested parts remain after lysis

Protein precipitation

30m 30s

5

30m 30s

For semen and tissues:

- Add 200 µL of **Protein Precipitation Solution**
- Vortex 00:00:15 and incubate 00:05:00 on ice
- Pellet 16000 x g, 4°C, 00:05:00

For blood:

- Add 1.65 mL of **Protein Precipitation Solution** (for 5mL blood)
- Vortex 00:00:15 and incubate 00:05:00 on ice
- Pellet 3000 x g, 10°C, 00:15:00

DNA precipitation

6

5m

- Transfert the supernatant to a new tube containing
  - 600 µL of Isopropanol (semen) - 2 mL tube
  - 800 µL of Isopropanol (tissues) - 2 mL tube
  - 5 mL of Isopropanol (blood) - 50 mL tube
- Carrefully invert the tube 25-50X times to form the pellet
- Incubate 00:05:00 Room temperature

For semen and tissues:

- Centrifuge as previously
- Discard supernatant

For blood:

- Pick up the pellet with a lab pipet and transfer it into a 2 mL tube
- Discard supernatant left

Wash DNA

20m

7

20m

- Add 600 µL 70% Ethanol
- Centrifuge 16000 rpm, 4°C, 00:05:00
- Discard supernatant

- Allow the ethanol to evaporate, without drying the pellet 00:15:00

DNA resuspension

20m

8 1h

- Add **TE** or **EB** buffer, depending on the subsequent analysis and usual procedure

We recommend 50 µL for semen or tissue pellets, and 100 µL to 150 µL for pellets from 5mL blood

- Do not hesitate to heat for 01:00:00 at 50 °C on gentle agitation 100 rpm

9 1h

DNA quantity control

- Measure the O.D. with a Nanodrop® device to obtain the DNA concentration
- Dilute with extra **TE/EB** if you choose to standardize concentrations
- Measure until the concentration is as expected

DNA quality control

- Load the DNA on a 1% agarose gel in 0.5X TBE with Ethidium Bromide
- Perform an electrophoresis 01:00:00 at 70 V

Protocol references

See protocole for DNA isolation from cattle semen for long read sequencing at DOI **dx.doi.org/10.17504/protocols.io.j8nlkw1qwl5r/v1**