GENE-SWitCH

The regulatory GENomE of SWine and CHicken: functional annotation during development

Protocol WP4
Purification of Genomic DNA and RNA, from liver and skeletal muscle of adult pigs

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Workpackage: WP4

Version: 1.0

Protocol associated with Deliverable(s):	
Submission date to FAANG:	

Research and Innovation Action, SFS-30-2018-2019-2020 Agri-Aqua Labs
Duration of the project: 01 July 2019 – 30 June 2023, 48 months

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 817998

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1 Summary

The genetic variants that are significantly associated with a trait of interest are called quantitative trait loci (QTLs), and they are named expression-QTLs (eQTLs) if the association is found between the variant and the expression of a gene. In the GENE-SWitCH project, we aim to identify eQTLs in three porcine tissues (i.e., small intestine (duodenum), liver and muscle).

From 300 pigs (100 Duroc, 100 Landrace and 100 Large White), small intestine (duodenum), liver and muscle were sampled at the slaughter house and snapfrozen in liquid nitrogen or stored in RNAlater solution.

We performed DNA extraction from the liver of LW pigs (100 extractions) and RNA extraction from the skeletal muscle of LW and LD pigs (200 extractions)

Genomic DNAs with low 260/230 ratios have been purified a second time with the QIAamp DNA Mini Kit (250) (ref: 51306). We have partially modified the protocol "QIAamp® DNA Mini and Blood Mini Handbook" (cf : step 2.6 Second Purification DNA QIAamp).

2 Protocol description

2.1 Required Reagents and Instruments

- RNeasy FibrousTissue Mini (QIAGEN ref: 74704)
- QIAamp DNA Mini Kit (250) (Qiaen ref: 51306)
- RNA 6000 Nano Kit (Agilent ref: 5067-1511)
- Standard Sensitivity Genomic DNA Analysis Kit - 50Kb (Agilent ref: DNF-467-0500)
- 2 ml Tube and 2 ml centrifuge tubes
- Pipets and tips
- Fragment Analyzer (Agilent)
- BioAnalyzeur (Agilent)
- Nanodrop spectrophotometer (thermofischer)
- Thermoblock
- Tissuelyser II (Qiagen)
- Stainless steel beads with a diameter of 5 mm (Qiagen ref:69989)
- β-Mercaptoethanol
- Ethanol
- Reagent DX (reduce foam) (Qiagen ref:19088)
- Cryotable (or box of dry ice)
- Cryoprotection gloves
- Sterile disposable Petri dishes (100 mm and 60mm)
- Disposable scalpels
- Sterile clamps with smooth ends, 10cm long
- Racks for 2 mL tubes
- A permanent marker to label the zip lock bags
- Paper towels
- Waste bag
- A cleaning spray against RNAse
- Weighting scales

2.2 Preparatory Step

Before starting the purification of genomic DNA and total RNA we need to weight the adequate quantity of tissue and to perform tissue homogenization. Working on a cryotable at - 25°C (or above a box of dry ice), for each sample, we excise 20-30 mg of liver tissue, for muscle we cut pieces of 45-55 mg. Each piece of tissue is then placed into a centrifuge tube and stored at - 80°C before performing the homogenization step. Between each tissue, we took care to clean the forceps and scalpel with ethanol and to tare the pre-labelled centrifuge tube. To perform tissue homogenization, we used the Tissuelyser II.

2.3 DNA Purification from liver (**QIAamp DNA Mini Kit**)

2.3.1 Tissue lysis:

1. Recover pre-cut tissue pieces stored at -80°C (30 mg).
2. Keep the tissues on ice and add 190 µL of ATL buffer, 1µL of DX reagent and a 5mm stainless steel bead.
3. Place the microcentrifuge tubes in the TissueLyser adapters (2x24) (placed in cold room). Perform a cycle of 1 min at 25 Hz.
4. Rapidly centrifuge the microcentrifuge tubes (2-3 seconds).
5. Transfert 180 µL of samples in a 1.5 ml tube and add 20 µL of proteinase K, vortex the tube.
6. Incubate the tubes at 56°C in a thermomixer for about 1 hour.
7. Wait for the complete lysis of the tissues.
8. Proceed to the Fixation and Washing step.

2.3.2 Fixation and Washing:

9. Pipette the mixture into the QIAamp Mini spin column and centrifuge at 6000 g (8000 rpm) for 1 minute. Discard the collection tube.
10. Place the column in a new collection tube and add 500 µL of AW1 buffer. Centrifuge for 1 min at 6000 g (8000 rpm). Discard the filtrate and wipe the collection tube on paper towels to reuse it for the next step.
11. Place the column in the collection tube and add 500 µL of AW2 buffer. Centrifuge for 3 min at 20000 g (14000 rpm). Discard the filtrate and wipe the collection tube on paper towels to reuse it for the next step.

12. Place the column in the collection tube and centrifuge for 1 min at 20000 g (14000 rpm). This step removes excess AW2.

2.3.3 Elution:

13. Place the column in a 1.5 ml tube and add 200 µL of buffer AE. Leave at room temperature for 1 min then centrifuge for 1 min at 6000 g or 8000 rpm.
14. Recover the filtrate and return it to the column to perform a second elution.
15. Store the DNAs at -15°C to -30°C.

2.4 RNA Purification from muscle (**RNeasy** FibrousTissue Mini)

2.4.1 Tissue lysis:

1. Recover pre-cut tissue pieces stored at -80°C (50 mg muscle).
2. Keep the tissues on ice and in centrifuge tube of 2ml , add 600 µL of ATL buffer, 6µl of β-mercaptoethanol, 1µL of DX reagent and a 5mm stainless steel bead.
3. Place the microcentrifuge tubes in the TissueLyser adapters (2x24) (placed in cold room).
4. Perform two cycle of 2 min at 25 Hz.
5. Rapidly centrifuge the microcentrifuge tubes (2-3 seconds).
6. Transfert 450 µL of lysat in a new tube of 2ml and add 885 µL RNase-free water and 10µl of proteinase K, vortex the tube.
7. Incubate the tubes at 56°C in a thermomixer for about 10 min.
8. Wait for the complete lysis of the tissues.
9. Centrifuge at 15–25°C for 3 min at 10,000 x *g*.
10. Pipet 1000 µL of the supernatant (avoid to pipet the pellet of tissue debris) into a new 2 ml microcentrifuge.
11. Proceed to the Fixation and Washing step.

2.4.2 Fixation and Washing:

12. Add 0.5 volumes (usually 500 µl) of ethanol (96–100%) to the cleared lysate. Mix well by pipetting. Do not centrifuge.

13. Transfer 700 µl of the sample, including any precipitate that may have formed, to an RNeasy Mini spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge at 15–25°C for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through. Repeated this step until the entire sample has passed through the RNeasy Mini spin column. Discard the flow-through. Reuse the collection tube for the next step.

14. Add 350 µl Buffer RW1 to the RNeasy Mini spin column. Close the lid gently and centrifuge at 15–25°C for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the membrane. Discard the flow-through. Reuse the collection tube for the step.

15. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

16. Add the DNase I incubation mix (80 µl) directly to the RNeasy Mini spin column membrane and place on the benchtop (15–25°C) for 15 min.

17. Add 350 µl Buffer RW1 to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) at 15–25°C. Discard the flow-through. Reuse the collection tube for the next step.

18. Add 500 µl Buffer RPE to the RNeasy Mini spin column. Close the lid gently and centrifuge at 15–25°C for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the membrane. Discard the flow-through. Reuse the collection tube for the next step.

19. Add 500 µl Buffer RPE to the RNeasy Mini spin column. Close the lid gently and centrifuge at 15–25°C for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the membrane.

2.4.3 Elution:

20. Place the RNeasy Mini spin column in a new 2 ml collection tube and discard the old collection tube with the flow-through. Close the lid gently and centrifuge at full speed for 1 min.

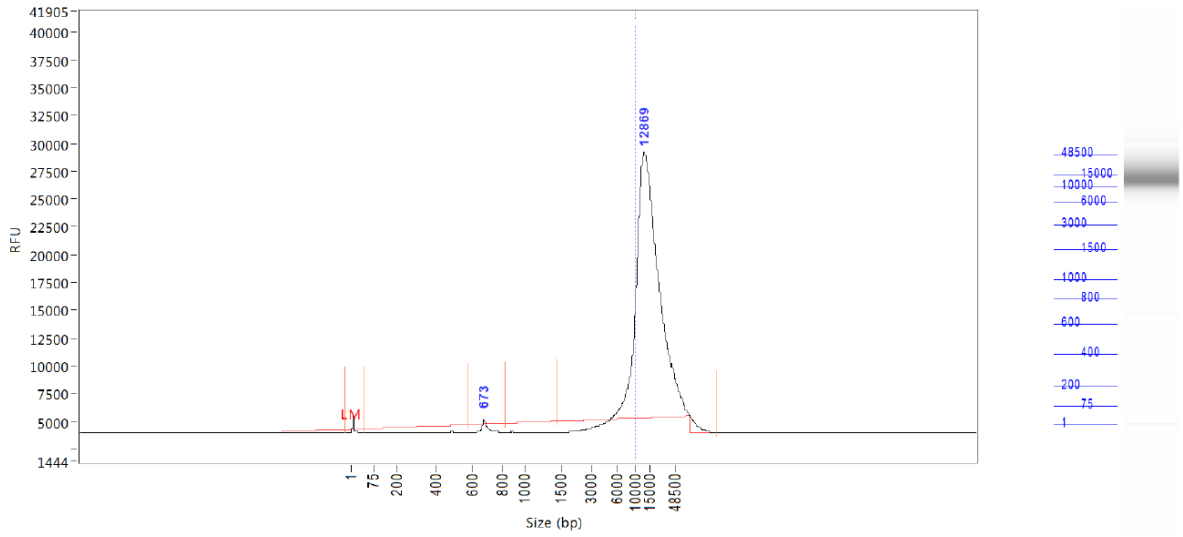
21. Place the RNeasy Mini spin column in a new 1.5 ml collection tube. Add 30 µl RNase-free water directly to the RNeasy Mini spin column membrane. Close the lid gently. To elute the RNA, centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) at 15–25°C. Repeat this step 19 using another 30 µl RNase-free water.

2.5 Storage, Quantification, and Determination of Quality of DNA and RNA

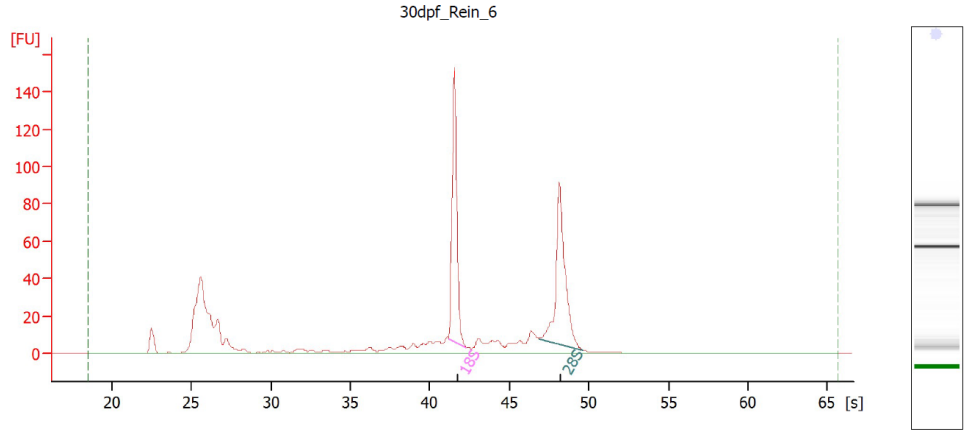
The DNAs and RNAs are stored at - 80°C. Quantification is performed with a Nanodrop to determine the concentration of each purification and the 260/230, 260/280 ratios. The DNAs with low concentration has been extracted a second time with the QIAamp DNA Mini Kit (ref: 51306).

To determine the quality of the DNAs we used a Fragment Analyser and "Standard Sensitivity Genomic DNA Analysis Kit - 50Kb" (ref: DNF-467-0500) while for the RNAs we used a BioAnalyser and performed RNA chips from the "RNA 6000 Nano Kit" (ref: 5067-1511).

Typical profile for genomic DNA on a Fragment Analyzer:



Typical profile for total RNA on a Bioanalyzer:



2.6 Second Purification DNA (QIAamp)

This protocol was carried out only for DNAs with low 260/230 ratios (lower than 1.8). This protocol follows the QIAamp® DNA Mini Kit handbook however, some steps have been modified to fit our experiments.

1. We pipetted 100µL of your DNA purification.
2. We added 80µl of buffer ATL.
3. We added 20µL of proteinase K and mixed by vortexing and incubated 20 min at 56°C.
4. We added 200µL of buffer AL and mixed thoroughly by vortexing for 15s.
5. We added 200µL ethanol (96-100%), vortex for 15s and briefly centrifuged the tube to remove drops from the lid.
6. We carefully applied the mixture from step 5 (including the precipitate) to the QIAamp Mini spin column. We centrifuged at 6000 x g (8000 rpm) for 1 min and discarded the tube containing the filtrate.

7. We added 500µl Buffer AW1 and centrifuged at 6000 x g (8000 rpm) for 1 min. We discarded the collection tube containing the filtrate.
8. We added 500µl Buffer AW2 and centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min.
9. We placed the QIAamp Mini spin column into a new 2 ml collection tube and discarded the old collection tube with the filtrate. We centrifuged at full speed for 1 min. This step helps to eliminate the chance of possible Buffer AW2 carryover.
10. We placed the QIAamp Mini spin column into a clean 1.5 ml microcentrifuge tube, and discarded the collection tube containing the filtrate. We added 50µl Buffer AE and incubated at room temperature for 1 min, and then centrifuged at 6000 x g (8000 rpm) for 1 min. We repeated this step.