GENE-SWitCH

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

Protocol WP4
Purification of Genomic DNA from blood and RNA from liver, duodenum 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 blood of Duroc pigs (100 extractions) and RNA extraction from the skeletal muscle of Duroc pigs and liver and duodenum of LW and LD pigs (500 extractions)

2 Protocol description

2.1 Required Reagents and Instruments

- Ribopure RNA purification kit (Invitrogen ref: AM1924)
- Nucleospin blood (MN ref: 740951.250)
- Chemagic™ 360 (Perkin Elmer)
- Bromochloropropane (BCP)
- 2 ml Tube and 1.5 ml centrifuge tubes
- Pipets and tips
- Fragment Analyzer (Agilent)
- Nanodrop spectrophotometer (thermofischer)
- Thermoblock
- Fastprep (MP Biomedicals)
- Lysing matrix D beads (MP Biomedicals)
- β-Mercaptoethanol
- Ethanol
- Cryoprotection gloves
- Sterile disposable Petri dishes (100 mm and 60mm)
- Disposable scalpels
- Sterile clamps with smooth ends, 10cm long
- Racks for tubes
- A permanent marker to label the zip lock bags
- Paper towels
- Waste bag
- Rnase away
- Weighting scales

2.2 Preparatory Step

Before starting the purification of total RNA we need to weight the adequate quantity of tissue and to perform tissue homogenization. Working on a dry ice, we excise 15-20 mg of liver tissue and duodenum, 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 Fastprep instrument with the combination of bead tubes (Lysing matrix D).

2.3 DNA Purification from blood (**Nucleospin blood**)

2.3.1 Lyse blood sample:

1. Pipette 25 µL Proteinase K and up to 200 µL blood into 1.5 mL microcentrifuge tubes.
2. Add 200 µL Buffer B3 to the samples and vortex the mixture vigorously (10–20 s).
3. Incubate samples at 70 °C for 10–15 min.
4. Add 210 µL ethanol (96–100 %) to each sample and vortex again.

2.3.2 Fixation and Washing:

5. For each preparation, take one NucleoSpin® Blood Column placed in a Collection Tube and load the sample.
6. Centrifuge 1 min at 11,000 x g. Discard Collection Tube with flow-through.
7. Wash with 500 µL Buffer BW and centrifuge 1 min at 11,000 x g
8. Discard Collection Tube with flow-through.
9. Wash with 600 µL Buffer B5 and centrifuge 1 min at 11,000 x g
10. Discard Collection Tube with flow-through.
11. Place the NucleoSpin® Blood Column back into the Collection Tube and centrifuge 1 min at 11,000 x g.

2.3.3 Elution:

12. Place the NucleoSpin® Blood Column in a 1.5 mL microcentrifuge tube (not provided) and add 100 µL preheated Buffer BE (70 °C). Dispense buffer directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.
13. Store the DNAs at -20°C.

2.4 RNA Purification from liver and duodenum (chemagic RNA Tissue10)

2.4.1 Tissue lysis:

1. Recover pre-cut tissue pieces stored at -80°C.
2. Keep the tissues on dry ice and add to the 2ml beads tubes , add 800 µL of lysis buffer and 10µl of β-mercaptoethanol.

3. Place the tubes in the Fastprep adapters (2x24).
4. Perform one cycle of 30 sec at 6 m/s. If it's necessary perform a second round (incubate 2min on ice between rounds).
5. Rapidly centrifuge at 4°C for 5 min at 12,000 x *g*.
6. Transfer 600 µL of lysate in a new tube of 2ml.
7. Add the lysates in a 96 plate of the instrument chemagic™ 360 (Perkin Elmer).
8. Next steps of washing and elution are carried out with the robot protocol: Purification protocol for up to 10mg tissue using the chemagic 360 and the integrated chemagic dispenser.

2.5 RNA Purification from skeletal muscle (RiboPure RNA Purification Kit)

2.5.1 Tissue lysis:

9. Recover pre-cut tissue pieces stored at -80°C.
10. Keep the tissues on dry ice and add to the 2ml beads tubes , add 1 ml of lysis buffer.
11. Place the tubes in the Fastprep adapters (2x24).
12. Perform one cycle of 30 sec at 6 m/s. If it's necessary, perform a second round (incubate 2min on ice between rounds).
13. Incubate homogenates fro 5min at RT.
14. Centrifuge at 4°C for 10 min at 12,000 x g and transfer the supernatant to a new tube.
15. Add 100 µL of bromochloropropane (BCP) to the homogenate.
16. Cap tubes tightly and vortex at maximum speed for 15 seconds.
17. Incubate the mixture at room temperature for 5 min
18. Centrifuge at 12,000 x g for 10 min at 4°C and transfer 400 µL of the aqueous phase (top layer) to a new, labelled 1.5 mL microcentrifuge tube.

2.5.2 Fixation and Washing:

19. Add 200 µL of 100% ethanol and mix immediately.
20. Pass the sample through a Filter Cartridge and centrifuge the assembly at 12,000 x g for 30 seconds at room temperature.
21. Discard the flow-through and return the Filter Cartridge to the same Collection Tube.
22. Wash the filter twice with 500 µL of Wash Solution.
23. Centrifuge for 30 seconds at room temperature to remove residual Wash Solution.
24. Transfer the Filter Cartridge to a new Collection Tube

2.5.3 Elution:

25. Add 100 µL of Elution Buffer to the filter column.
26. Incubate at room temperature for 2 min.
27. Centrifuge for 30 seconds to elute the RNA from the filter.
28. Store the recovered RNA at -80ºC.

2.6 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.

To determine the quality of the DNAs and RNAs we used a Fragment Analyser (CNAG, Barcelona).

Typical profile for genomic DNA on a Fragment Analyzer:

Typical profile for total RNA on a Bioanalyzer: