Animals Agroscope Fact Sheet May 2019

Protocol sample collection RNA-seq

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This protocol describes the isolation of liver, muscle and jejunum samples for storage in RNAlater or RLT buffer.

Slaughter

At the Agroscope abattoir, pigs are stunned for 180 sec with CO₂ (87% CO₂; Samson C1 L 803; MPS Group, Holbaek, Denmark), weighed and immediately exsanguinated and weighed again. Pigs are scalded (62°C, 3 min) and subsequently carcasses are eviscerated. In the course of evisceration samples are taken. Slaughtering and the isolation of organs is carried out by professional butchers.

Tissue samples

All samples are obtained approximately 20 min *post mortem* by two teams (liver and muscle team and intestine team) and stored in duplicates in RNAlater®. Eppendorf tubes containing samples in RNAlater are stored in the fridge overnight and transferred to -20°C thereafter. Sample preparation is carried out by trained laboratory staff of the Agroscope Animal Biology laboratory.

Preparation:

- four 2ml-Eppendorf tubes with 1.5 ml RNAlater, labelled with the animal's ID
- two 2ml-Eppendorf tubes with 1.5 ml RLT buffer, labelled with the animal's ID, for randomly selected individuals

Liver

- 1. Place four pieces of liver, cut into cubes of approx. 2 mm side length, into each Eppendorf tube.
- 2. Ensure that the tissue is well covered with liquid



Muscle

Same procedure as above for liver; the sample is taken via carottage and immediately processed



Jejunum

- 1. Take a piece of jejunum 3m away from the pylorus valve and rinse with PBS buffer (phosphate-buffered saline)
- 2. Cut a piece of jejunum (approx. 2-3 cm)
- 3. Scrape the mucosa
- 4. Place mucosa in Eppendorf tubes filled with RLT buffer (Qiagen)
- 5. Transfer Eppendorf with jejunum samples in RLT buffer directly to -20°C

