

Standard operating procedure for tissue collection for Chromatin isolation by RNA precipitation sequencing (ChIRP-seq)

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General:

- The animal tissue samples have to be collected and stored on wet ice as soon as possible in order to prevent degradation of Chromatin complexes.

Materials and Preparation:

- Sterilized surgical tools and instruments and other equipment used for sample preparation and cryo-freezing (e.g., preparation plates, knives)
- Sterilized and pre-labelled (tissue type) petri dishes
- Pre-labelled (animal ID, tissue type, date) 2 ml cryovials (PP)

Protocol for tissue sample collection and preparation and cryo-freezing

- The fed animals are killed according to regular slaughtering protocol including stunning and exsanguination by expert staff in an experimental slaughterhouse of the institute.
- A specific animal dissection protocol is applied to enable rapid tissue sample collection.
- Tissue samples are collected immediately (30 min) after slaughter by a veterinarian and distributed to trained lab technicians for further sample preparation.
- Preparation of tissues samples is taking place in a separate laboratory adjacently located to the slaughtering facility. The preparation team consists of 3 - 4 persons depending on the number of tissues to be sampled and collected.
- The tissue samples are meticulously dissected and cut in small cubes (2-3 mm each edge length) using surgical instruments and immediately collected pre-cooled 2 ml cryovials (PP) and stored on wet ice.
- Upon completion of sample collection and preparation the cryovials have to be transferred to the lab immediately for subsequent ChIRPseq protocol steps
- The experimental procedures were carried out according to the animal care guidelines with respect to welfare and health of the animals and were approved by the relevant authorities of the State Department of Agriculture, Food Security and Fisheries, Mecklenburg-Western Pomerania, Germany.