FR-AgENCODE

Protocol: purification of genomic DNA from tissues

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1 Summary

FR-AgENCODE is a FAANG pilot project for the functional annotation of livestock genomes.

As part of the FAANG action (Functional Annotation of ANimal Genomes), the FR-AgENCODE project aims at improving the genomic annotation of four livestock species:

- cattle (Bos taurus)
- goat (Capra hircus)
- chicken (Gallus gallus)
- pig (Sus scrofa)

This is achieved by performing molecular assays on tissues and on sorted primary cells (CD4+ and CD8+ T lymphocytes) from 2 males and 2 females of each species. These assays include RNA-seq, ATAC-seq, WGS and Hi-C to characterize the transcriptome, the chromatin accessibility and the genome 3D topology in these cells, respectively.

On-going results from the project are available here:

http://www.fragencode.org/results.html

A first publication is also available here:

Multi-species annotation of transcriptome and chromatin structure in domesticated animals.

Foissac S, Djebali S, Munyard K, Vialaneix N, Rau A, Muret K, Esquerré D, Zytnicki M, Derrien T, Bardou P, Blanc F, Cabau C, Crisci E, Dhorne-Pollet S, Drouet F, Faraut T, Gonzalez I, Goubil A, Lacroix-Lamandé S, Laurent F, Marthey S, Marti-Marimon M, Momal-Leisenring R, Mompart F, Quéré P, Robelin D, Cristobal MS, Tosser-Klopp G, Vincent-Naulleau S, Fabre S, der Laan MP, Klopp C, Tixier-Boichard M, Acloque H, Lagarrigue S, Giuffra E.

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2 Protocol description

Here we describe the protocol used to purify genomic DNA from liver for Whole Genome Sequencing of pig and cattle individuals.

To perform the extraction of genomic DNA from liver, we followed the User manual of the “Chemagic Star” machine and the Chemagic Star DNA Cell 12m (CMG-1769).

2.1 Required Reagents and Instruments

- Chemagic Star DNA Cell12M (Perkin Elmer CMG-1769)
- Chemagic Star (Chemagen)
- Standard Sensitivity Genomic DNA Analysis Kit - 50Kb (Agilent ref: DNF-467-0500)
- Qubit 1X dsDNA BR assay kit (Q33265)

- Qubit device (ThermoFisher)
- 2 ml Tube and 2 ml centrifuge tubes
- Pipets and tips
- Fragment Analyzer (Agilent)
- Nanodrop spectrophotometer (Thermofischer)
- Multi-Therm shaker (Benchmark)
- Proteinase K (Qiagen 19131)
- 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

2.2 Preparatory Step

Before starting the purification of genomic DNA we weighted the adequate quantity of tissue and performed tissue homogenization. Working on a cryotable at - 25°C (or above a box of dry ice), for each sample, we excise 10 mg of liver tissue. Each piece of tissue is then placed into a 2ml 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 add 200µl of lysis buffer (from the Chemagic kit) and 6µl of Proteinase K to each microtube containing liver sample. The tube is then placed into the Multi-therm shaker at 56°C and 700rpm overnight.
The following day, the tubes are centrifugated at 4000g during 20 minutes at room temperature. The lysate is then loaded into a deepWell 2ml plate (from the Chemagic kit) and processed using the Chemagic Star workstation.

2.3 Storage, Quantification, and Determination of Quality of total RNA

DNAs are stored at - 20°C. Quantification is performed with a Qubit device and a Nanodrop to determine the concentration of each purification and the 260/230, 260/280 ratios.
DNA Integrity Numbers (RINs) were determined using a FragmentAnalyzer.