GENE-SWitCH

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

Protocol WP1
PIG and CHICKEN cDNA Library Preparation and Iso-seq Sequencing

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1 Summary

GENE-SWitCH aims at identifying functional elements located in the genomes of pig and chicken working on seven different tissues at three different developmental stages. It requires a collection of samples corresponding to the selected tissues and developmental stages with associated metadata accurately describing the samples and the sampling process.

The seven tissues analysed in GENE-SWitCH are:

- Cerebellum
- Lung
- Kidney
- Dorsal skin
- Small intestine
- Liver
- Skeletal muscle

The three developmental stages are:

- Early organogenesis (E8 chick embryo and D30 pig foetuses)
- Late organogenesis (E15 chick embryo and D70 pig foetuses)
- Newborn piglets and hatched chicks

A set of molecular assays is then performed on the different samples. We described here the protocol used for pig and chicken cDNA Library Preparation and Iso-seq sequencing.

Total RNAs were extracted from the different tissues following the protocols: https://data.faang.org/api/fire_api/assays/INRA_SOP_GENESWITCH_WP1_PIG_EXTRACTION_DNA_RNA_20201111.pdf

https://data.faang.org/api/fire_api/assays/ROSLIN_SOP_GENESWITCH_WP1_CHICK_EXTRACTION_DNA_RNA_20201111.pdf

For Iso-seq sequencing 42 libraries (21 chickens and 21 pigs) were prepared using the TeloPrime Full-Length cDNA Amplification Kit V2. The detailed protocol is given below: https://data.faang.org/api/fire_api/experiments/ROSLIN_SOP_GENESWITCH_WP1_TeloPrime_cDNA_amplification_20210225.pdf

All samples were sent to Earlham Institute as a subcontractor for further processing.

2 Protocol description

The libraries were composed primarily of transcripts with an average size of ~2kb. Using a standard workflow for purification of amplified cDNA, samples were clean up with ProNex beads (NG2003). To ensure that the amplified and purified cDNA material had the expected size, the cDNAs were examined on an Agilent Bioanalyzer using a High Sensitivity DNA kit (5067-4626). The cDNAs were then converted into SMRTbell unbarcoded libraries using the following steps:

- DNA damage repair with 500ng input for every sample
- End Repair/A-Tailing
- Overhang adapter ligation

Prepared cDNA SMRTbell libraries were cleaned-up with ProNex beads with an extended elution time of 30 minutes. The samples were eluted in 21µl of PacBio EB buffer and in order to determine the final size of the libraries, they were examined again on the Agilent Bioanalyzer using a High Sensitivity DNA kit.

During the sequencing process the Sequel II instrument was updated to a Sequel IIe platform. Following the SMRT Link Sample Setup v8.0 instructions, the first eight libraries were sequenced with selected parameters: Sequencing Primer v4 Sequel II Binding Kit 2.1, 30 hour movies, default immobilisation, 2 hours Pre-extension time. Diffusion Loading. ProNex Bead Complex clean-up step. CCS Sequencing Mode. Sequel II DNA Internal Control 1.0. IsoSeq Experiment: Yes Primer:Template 20:1, Polymerase:Template 15:1 and the instrument and software details: SMRTLink Version: 9.0.0.92188,

Instrument Control Software version: 9.0.0.92233, Instrument Chemistry Bundle: 9.0.0.92017, Primary Analysis Software: 9.0.0.92233.

The remaining 34 libraries were sequenced on the Sequel IIe platform with similar parameters except: SMRTLink Version: 10.1.0.119588; Instrument Control Software version: 10.1.0.119549, Instrument Chemistry Bundle: 10.1.0.119528, Primary Analysis Software: 10.1.0.119549.

The detailed protocol is below:

https://www.pacb.com/wp-content/uploads/Procedure-Checklist-Iso-Seq-Express-Template-Preparation-for-Sequel-and-Sequel-II-Systems.pdf