GENE-SWitCH

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

Deliverable D1.1
Biorepository of 3120 samples

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History of changes

VERSION	Date	Changes
1.0	30/09/2020	First submission
2.0	30/06/2021	Revised version to answer the comments of the rejection letter received on 1/06/2021

Table of contents

1 Summary

GENE-SWitCH aims at identifying functional elements located in the genomes of pig and chicken by targeting seven different tissues at three different developmental stages. This requires a collection of samples corresponding to the selected tissues and developmental stages with associated metadata describing accurately the samples and the sampling process. The samples are collected in a way to allow carrying out the identification of functional elements by several molecular assays (T1.3, T1.4, T1.5, T1.6).

The seven selected tissues are: cerebellum/hindbrain, lung, kidney, dorsal skin, small intestine/ileum, liver and skeletal muscle from the hindlimb. Six additional tissues were also sampled for biobanking: heart, gonads, brain cortex/telencephalon/forebrain, spleen, colon/large intestine, stomach/gizzard. The three developmental stages are: early fetal/embryonic organogenesis, late fetal/embryonic organogenesis, new-born. For each species, each developmental stage and each tissue, 4 biological replicates (2 males and 2 females) are sampled.

We used the Large White breed for pigs and an advanced intercross line (AIL) for chicken.

Pigs and chicken were respectively bred and sampled at INRAE and at the Roslin Institute (UEDIN), using dedicated protocols. All samples were placed at -80°C for long stem storage. Metadata associated with these samples are stored on the GENE-SWitCH sharepoint and registered in BioSamples through the FAANG Data Portal (https://data.faang.org/home).

2 Introduction

GENE-SWitCH aims at identifying functional elements located in the genomes of pig and chicken working on seven different tissues (cerebellum, lungs, kidney, dorsal skin, small intestine, liver and skeletal muscle) at three different developmental stages (newborn, early organogenesis and late organogenesis). This requires a collection of samples corresponding to the selected tissues and developmental stages with associated metadata describing accurately the samples and the sampling process. The samples are collected in a way to allow carrying out the identification of functional elements by several molecular assays (T1.3, T1.4, T1.5, T1.6).
Six additional tissues (biobanking) have also been sampled: brain cortex, heart, spleen, colon, gonads, stomach/gizzard.

3 Results

3.1 Pig sampling

3.1.1 D30 pig fetuses

Four pools of unrelated D30 Large White fetuses (2 pools of females and 2 pools of males) have been produced. To this goal, for each sow we sampled 6 fetuses (to get at least one female and one male from each sow), and each fetus was sexed by PCR (see Annexes for the detailed procedures). Subsequently, pools were made based on sexing. Metadata for the D30 fetuses and the four pools have been formatted using the Biosamples template and are available on the FAANG Data Portal (see Annexes).

Specifically, 6 sows were slaughtered the 21st of November 2019 at the slaughter house of INRAE Nouzilly Center. Sows were stunned by electronarcosis and bled. The uterus was recovered and the fetuses extracted from the amnios. Each fetus was weighted and photographed. For each sow, 6 fetuses were kept on ice and dissected under a stereomicroscope to take, in a pre-determined order: heart, lungs, liver, stomach, intestine, mesonephros (fetal kidney), skin, muscle from the hindlimb, hindbrain (fetal cerebellum), forebrain (embryonic brain cortex) and gonads. Spleen and colon were not sampled (as too small to be correctly identified). A piece of the fetus was also kept for sexing. After dissection, each organ was stored in previously labelled tubes, snapfrozen in liquid nitrogen and stored in dry ice for the transport.

Recorded metadata consists of the following information: Fetus ID, Weight of the fetus, picture of the fetus, sex of the fetus, date and hour of slaughtering, father ID, father birth date, father breed, mother ID, mother Birth date, mother breed, number of fetuses per mother, mother parity, insemination date.

For each fetus, we have finally recovered 4 aliquots for each of the 11 selected tissues. Aliquots were immediately snap-frozen in liquid nitrogen and stored in dry ice for shipment and then at -80ºC for long term storage. Pools of fetuses were then performed as follow:

First, for each sow, fetuses were sexed by PCR following the protocol in Annex (INRA_SOP_GENESWITCH_D30_FETUS_SAMPLING_20200221.pdf). Results are shown on the Table below (male in blue and female in pink):

Sow 1	Fetus1	Fetus2	Fetus3	fetus4	Fetus5	Fetus6
Sow 2	Fetus7	Fetus8	Fetus9	Fetus10	Fetus11	Fetus12
Sow 3	Fetus13	Fetus14	Fetus15	Fetus16	Fetus17	Fetus18

Sow 4	Fetus19	Fetus20	Fetus21	Fetus22	Fetus23	Fetus24
Sow 5	Fetus25	Fetus26	Fetus27	Fetus28	Fetus29	Fetus30
Sow 6	Fetus31	Fetus32	Fetus33	Fetus34	Fetus35	Fetus36

Pooling was done as follows to get 2 pools for males and 2 pools for females.

	Female 1	Female 2	Female 3	Female 4	Female 5	Female 6
Pool1	Fetus1 Fetus 6	Fetus8 Fetus 10	Fetus18	Fetus19 Fetus 22	Fetus25 Fetus 27	Fetus31 Fetus 33
Pool2	Fetus2 Fetus5	Fetus9 Fetus12		Fetus21 Fetus23 Fetus24	Fetus26	Fetus 32 Fetus34 Fetus35
Pool3	Fetus4	Fetus11	Fetus17 Fetus13	Fetus20	Fetus30	Fetus36
Pool6	Fetus3	Fetus7	Fetus14 Fetus15 Fetus16		Fetus28 Fetus29	

3.1.2 Fetuses D70

Four unrelated D70 Large White fetuses (2 females and 2 males) were sampled (see Annexes for the detailed procedures). Metadata for the D70 fetuses have been formatted using the Biosamples template and are available on the FAANG Data Portal (see Annexes).

Specifically, 4 sows were slaughtered the 21st of January 2020 at the slaughter house of INRAE Nouzilly Center. Sows were stunned by electronarcosis and bled. The uterus was recovered and the fetuses extracted from the amnios. Each fetus was weighed and photographed. For each sow, 2 fetuses (1 male and 1 female) were kept on ice and dissected under a stereomicroscope in a pre-determined order to take heart, lungs, liver, stomach, intestine, mesonephros (fetal kidney), skin, muscle from the hindlimb, hindbrain (fetal cerebellum), forebrain (embryonic brain cortex), gonads, spleen and colon. After dissection each organ was stored in previously labelled tubes, snapfrozen in liquid nitrogen and stored in dry ice for the transport.

Recorded metadata consists of the following information: Fetus ID, Weight of the fetus, picture of the fetus, sex of the fetus, date and hour of slaughtering, father ID, father birth date, father breed, mother ID, mother Birth date, mother breed, number of fetuses per mother, mother parity, insemination date.

For each fetus, we have finally recovered 5 aliquots for each of the 13 selected tissues. Aliquots were immediately snap-frozen in liquid nitrogen and stored in dry ice for shipment and at -80ºC for long term storage.

3.1.3 Newborn (1-day old) piglets

Four unrelated Large White piglets (2 males and 2 females) have been sampled the 19th of November 2019 at the Experimental Unit GenESI of INRAE Le Magneraud. Piglets were first weighed and photographed, then they were stunned by electronarcosis and bled. The tissues were successively dissected to take (in this pre-determined order): heart, lungs, liver, stomach, small intestine, colon, spleen, kidney, gonads, skeletal muscle (from the hind limb), dorsal skin, cerebellum and cerebral cortex. After dissection, each organ was cut in pieces, placed in labelled tubes, snap-frozen in liquid nitrogen and stored in dry ice for the transport. Metadata for the piglets have been recorded using the Biosamples template and are available on the FAANG Data Portal (see Annexes).

Recorded information consists of the following information: piglet ID, weight at birth, picture of the piglet, sex of the piglet, date and hour of slaughtering, father ID, father birth date, father breed, mother ID, mother Birth date, mother breed, number of piglets per litter, mother parity, insemination date.

We have finally recovered 6 aliquots for each of the 13 selected tissues. Aliquots were immediately snap-frozen in liquid nitrogen and stored in dry ice for shipment and at -80ºC for long-term storage.

3.2 Chicken sampling

3.2.1 E8 chick embryos

Four pools of unrelated E8 chick embryos (2 pools of females and 2 pools of males) have been produced. Each embryo has been sampled individually and sexed by PCR (detailed procedures on the FAANG DCC, see Annexes). We used chick embryos from 4 pens composed of 4 hens and 1 cock. Eggs from each pen are traced but cannot be associated to one specific hen from the pen.

Fertilized eggs were incubated for 8 days at 37.4°C in a humidified incubator with automatic egg turning. The incubated egg was opened through a small window in the shell at the level of the air chamber. The external membranes were removed and the embryo was recovered using a perforated spoon and placed into a 100mm Petri dish. Each embryo was immediately weighed, photographed and dissected under the stereomicroscope in a pre-determined order : heart, lungs, liver, spleen, gizzard, small intestine, large intestine, mesonephros (embryonic kidney), gonads, skin, muscle from the hindlimb, forebrain (embryonic brain cortex) and hindbrain (embryonic cerebellum). A piece of the embryo was also kept for sexing. After dissection each organ or tissue was cut in half and was immediately stored into previously labelled tubes placed on dry ice.

Recorded information consists of the following information: Embryo ID, Weight of the embryo, sex of the embryo, date of dissection, father ID, father breed, mother breed.

First, for each pen, embryos were sexed by PCR (see Table, male in blue and female in pink):

Pen 1	Embryo 13				
Pen 2	Embryo 2	Embryo 6	Embryo 7	Embryo9	Embryo10
		Embryo 15	Embryo 16		
Pen 3	Embryo 3	Embryo 4	Embryo 5	Embryo 11	Embryo 12
			Embryo 14		

Pen 4	Embryo 1	Embryo 8			

After sexing, four pools of E8 chick embryos (2 pools of females and 2 pools of males) have been produced.

We determined the pools according to the following table, in order to balance the genetic diversity. Pooling was done to produce 2 pools of male and 2 pools of female samples for each tissue type as follows:

	Pen 1	Pen 2	Pen 3	Pen 4
Pool1		Embryo 6 Embryo 16	Embryo 3 Embryo 4	
Pool2		Embryo 9 Embryo 10	Embryo 12	Embryo 1
Pool3	Embryo 13	Embryo 2	Embryo 5 Embryo 11	
Pool4		Embryo 7 Embryo 15	Embryo 14	Embryo 8

3.2.2 E15 chick embryos

Fertilized eggs were incubated for 15 days at 37.4°C in a humidified incubator with automatic egg turning. The incubated egg was opened through a small window in the shell at the level of the air chamber. The external membranes were removed and the embryo was recovered using a perforated spoon and placed into a 100mm Petri dish. Each embryo was immediately weighed, photographed and dissected under the stereomicroscope in a pre-determined order: heart, lungs, liver, spleen, gizzard, small intestine, large intestine (immature colon), kidney, gonads, skin of the back, muscle from the hindlimb, telencephalon (embryonic brain cortex) and cerebellum). After dissection each organ or tissue small piece (20-40mg) was immediately stored into previously labelled tubes placed on dry ice.

Recorded information consists of the following information: Embryo ID, Weight of the embryo, sex of the embryo, date of dissection, father ID, father breed, mother breed. Embryos were sexed by PCR following the protocol in Annex:

ROSLIN_SOP_GENESWITCH_E8_EMBRYO_POOLING_20200915.pdf

3.2.3 Hatched chicks

Fertilized eggs were incubated for 21 days at 37.4°C in a humidified incubator with automatic egg turning. After hatching the chick was recovered and euthanized. Each chick was immediately weighed, photographed and dissected in a pre-determined order: heart, lungs, liver, spleen, gizzard, small intestine, large intestine, kidney, gonads, skin of the back, hindlimb muscle, telencephalon and cerebellum. After dissection each organ or tissue small piece (20-40mg) was immediately stored into previously labelled tubes placed on dry ice.

Recorded information consists of the following information: Chick ID, Weight, sex of the chick, date of dissection, father ID, father breed, mother breed. Hatched chicks were sexed by PCR.

4 Conclusion

We successfully sampled all the tissues necessary to perform the molecular assays described in the DoA and corresponding to Tasks 1.3 to 1.6.

4 animals/ replicates	Fetus 30D	Fetus 70D	Piglet	Embryo 8D	Embryo 15D	Chick
core tissues	hindbrain, lungs, kidney, skin, small intestine, liver, hindlimb muscle	cerebellum, lungs, kidney, dorsal skin, small intestine, liver, hindlimb muscle	cerebellum, lungs, kidney, dorsal skin, ileum, liver, gluteus medius	hindbrain, lungs, mesonephros, skin, small intestine, liver, hindlimb muscle	cerebellum, lungs, kidney, dorsal skin, small intestine, liver, hindlimb muscle	cerebellum, lungs, kidney, dorsal skin, small intestine, liver, gluteus medius
additional tissues (biobanking)	brain cortex, heart, stomach, spleen, large intestine, gonads			brain cortex, heart, gizzard, spleen, large intestine, gonads		
Tasks	T1.1			T1.2		
Deliverable	D1.1 Biorepository of 3120 samples submitted in September 2020 (M15)					
Sampling date	M5	M7	M3	M8	M8	M13
DNA and RNA extraction	Completed M8			Completed M15		
Delivery of tissue for ChIP/ATAC	Delivered to Diagenode M9			Delivered to Diagenode M15		

To summarize:

- pig sampling finally yielded 1584 samples from of D30 pig fetuses (36 fetuses, 11 tissues, 4 aliquots per tissue), 260 samples from D70 pig fetuses (4 fetuses, 13 tissues, 5 aliquots per tissue) and 312 samples (4 piglets, 13 tissues, 6 aliquots per tissue) of newborn piglets, representing a total of 2156 samples.

- chicken sampling finally yielded 416 samples from of E8 chicken embryos (16 embryos, 13 tissues, 2 aliquots per tissue), 104 samples from E15 chicken embryos (4 embryos, 13 tissues, 2 aliquots per tissue) and 104 samples from hatch chicken embryos (4 embryos, 13 tissues, 2 aliquots per tissue), representing a total of 624 samples.

The six additional tissues (heart, gonads, brain cortex/telencephalon/forebrain, spleen, colon/large intestine, stomach/gizzard) and the remaining samples not used for molecular assays of the seven main tissues (cerebellum/hindbrain, lung, kidney, dorsal skin, small intestine/ileum, liver and skeletal muscle from the hindlimb) are stored at -80°C at the CRB-Anim, the French National Biological Resource Center for Domestic Animals (https://crb-anim.fr/crb-anim_eng/).

5 Deviations or delays

We initially planned to sample 10 aliquots for each tissue, species and stage, representing a total of 3120 samples. However, the small sizes of tissues and organs (particularly at the

embryo/fetus stages) did not make it possible to always obtain 10 aliquots per tissue / organ. To ensure the quantities necessary for carrying out the molecular assays, we assessed that a minimum of 2 aliquots (20-40mg) was required per sample to perform DNA / RNA extractions and ATAC-seq / ChIP-seq libraries. We therefore decided to set a minimum of 2 aliquots per tissue., Moreover, additional E8 embryos (16 instead of 4) and D30 fetuses (36 instead of 4) were sampled. The number of samples obtained per tissue and stage is summarized in the following table:

	Chicken (number of samples)			Pig (number of samples)		
	E8	E15	Hatched	D30	D70	Newborn
Liver	32	8	8	144	20	24
Small intestine	32	8	8	144	20	24
Kidney	32	8	8	144	20	24
Lungs	32	8	8	144	20	24
Muscle	32	8	8	144	20	24
Cerebellum	32	8	8	144	20	24
Skin	32	8	8	144	20	24
Stomach / Gizzard	32	8	8	144	20	24
Large Intestine	32	8	8	0	20	24
Heart	32	8	8	144	20	24
Gonads	32	8	8	144	20	24
Spleen	32	8	8	0	20	24
Brain cortex	32	8	8	144	20	24
Total	416	104	104	1584	260	312
	624			2156		

This deliverable was expected on M6 and was delivered on M15.

This delay is due to:

1) insemination problems for the production of pig D70 fetuses and subsequent space availability in the breeding unit at INRAE GenESI.

2) fertility problems with hens for the AIL chicken line

3) Additional delays due to the COVID-19 lockdown in UK and the closure of the Roslin Institute until July 2020.

This 9-months delay is not affecting much the subsequent tasks (Tasks 1.3 to 1.6). RNAs/DNAs from pig samples were delivered in July 2020 for RNA-seq and RRBs/WGBS assays and RNAs/DNAs from chick samples were delivered in September 2020. All tissues have been sent to Diagenode for ATAC-seq and ChIP-seq optimization and libraries production on M9 for pig samples and M15 for chicken samples.

6 Acknowledgements

We particularly thanks people from the INRAE GenESI experimental unit and people from the INRAE GeMS team for their involvement and their help to perform pig sampling.

We particularly thanks people from the Development and Functional Genetics Department at the Roslin Institute for their involvement and their help to perform chicken sampling.

7 References

8 Annexes

The protocols for pig sampling are available on the FAANG data portal.

It contains the following files:

https://data.faang.org/api/fire_api/samples/INRA_SOP_GENESWITCH_D30_FETUS_SAMPLING_20200221.pdf

https://data.faang.org/api/fire_api/samples/INRA_SOP_GENESWITCH_D30_FETUS_POOLING_20200221.pdf.

https://data.faang.org/api/fire_api/samples/INRA_SOP_GENESWITCH_D70_FETUS_SAMPLING_20200221.pdf

https://data.faang.org/api/fire_api/samples/INRA_SOP_GENESWITCH_PIGLET_SAMPLING_20200221.pdf

The pictures for pig sampling are available on the following link:

http://vm-genobiotoul.toulouse.inra.fr/~hacloque/

It contains 35 *.jpg files for D30 fetuses, 4 *.jpg files for D70 fetus and 1 *.jpg file for newborns.

The metadata for pig sampling and the corresponding BioSamples IDs are available on the following links:

http://vm-genobiotoul.toulouse.inra.fr/~hacloque/faang_sample_WP1_GS_PIG.xlsx

http://vm-genobiotoul.toulouse.inra.fr/~hacloque/BiosamplesIDs_faang_GS_WP1_pig.txt

The protocols for chicken sampling are available on the FAANG data portal:

https://data.faang.org/api/fire_api/samples/ROSLIN_SOP_GENESWITCH_E8_EMBRYO_SAMPLING_20200915.pdf

https://data.faang.org/api/fire_api/samples/ROSLIN_SOP_GENESWITCH_E15_EMBRYO_SAMPLING_20200915.pdf

https://data.faang.org/api/fire_api/samples/ROSLIN_SOP_GENESWITCH_HATCHED_CHICK_SAMPLING_20200915.pdf

The metadata for chicken sampling and the corresponding BioSamples IDs are available on the following links:

http://vm-genobiotoul.toulouse.inra.fr/~hacloque/BiosamplesIDs_faang_GS_WP1_chicken.txt

http://vm-genobiotoul.toulouse.inra.fr/~hacloque/faang_sample_WP1_GS_CHICKEN.xlsx