

GENE-SWitCH

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

Deliverable D1.2
Raw sequences of WGBS and RRBS delivered to ENA and FAANG

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1 Summary

- **Objectives:** 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. One of the important marks of the functional genome is DNA methylation which is generally reverse correlated with gene expression. The objectives were to achieve high quality DNA methylation data from the tissues sampled from the different developmental stages (D1.1) and to make the DNA methylation data available to the GENE-SWitCH partners and the global (worldwide) research community.
- **Method:** DNA methylation data were achieved by the Bisulfite Sequencing (BS) methodology which is the golden standard for accessing high quality DNA methylation. Two BS methods were applied, 1) Whole genome Bisulfite Sequencing (WBBS) to access the DNA methylation level at all DNA methylation sites of a genome and 2) Reduced Representation Bisulfite Sequencing (RRBS) which is a cost-efficient method to access the DNA methylation in CpG rich regions of the genome. CpG rich regions are generally enriched in functional element regions.
- **Main Results:** WGBS data were obtained from one of each tissue sample of each developmental stage of pig and chicken (for a total of 21 samples per each species). RRBS data were obtained from three of each tissue sample of each developmental stage of pig and chicken (for a total of 63 samples per each species). The raw DNA methylation data have been submitted to ENA and to the FAANG Data Portal (<https://data.faang.org/home>) and hereby made public available.
- **Partners involved:** INRAE, UEDIN, WU



2 Introduction

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. One of the important marks of the functional genome elements is DNA methylation. CpG DNA methylation is a very informative regulator of gene expression and also one of the best-studied epigenetic mechanisms in farm animals. Generally, CpG methylation is reversely correlated with gene expression at the functional genome elements.

The objective of this task was to achieve high quality DNA methylation data from the tissues sampled from the different developmental stages (see D1.1) and to make the DNA methylation data available to the GENE-SWitCH partners and the global research community.

High-quality DNA methylation data were obtained by using two BS methods (WGBS and RRBS) allowing DNA base pair resolution of the level of methylation.

The public availability of the DNA methylation data has been done under the FAIR principle (Findable, Accessible, Interoperable, Reusable data) following the Fort Lauderdale principles, as confirmed in the Toronto Statement (Toronto International Data Release Workshop (Birney et al. 2009)).

3 Results

3.1 Outsourcing of RRBS and WGBS assays

Both DNA methylation assays (RRBS and WGBS) were outsourced to high quality sequence facilities that perform these assays on a regular basis. The aim was to obtain first class uniform data as needed to make reliable estimates of level of DNA methylation. Separate quotes for RRBS and WGBS of pig and chicken, respectively, were provided by three commercial companies as requested by the EU regulation for outsourcing. The requirements for RRBS were as follows: RRBS pig: 40-50 million reads – single end - 100bp long (or 150bp if this is standard setting) - DNA digestion by MspI with fragment size in the range of 20-500bp (preferably with the Ovation RRBS Methyl-Seq kit). RRBS chicken: 20-30 million reads – single end - 100bp (or 150bp if this is standard setting) - DNA digestion by MspI with fragment size 20-500bp (preferably with the Ovation RRBS Methyl-Seq kit). The requirements for the WGBS for both pig and chicken were as follows: 30x average genome coverage - PE - 150bp (or 100bp if this is standard setting) - max 20% PCR duplication. From the separate quotes the best price and data quality quotes were selected: The RRBS assays were outsourced to the Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign, US. The WGBS assays were outsourced to Novogene Europe, Cambridge Science Park, Cambridge, CB4 0FW, United Kingdom.

3.2 RRBS data

RRBS libraries were made with the Ovation RRBS Methyl-Seq kit and sequenced on a Novaseq 6000 single-end and 151bp long reads.

RRBS Data from pig was downloaded from the sequence provider and checked for correct file size (MD5sum method). The number of reads varied from 40 to 69 million reads with an average of 59 million reads (annex 1).

RRBS Data from chicken was downloaded from the sequence provider and checked for correct MD5sum. The number of reads varied from 45 to 70 million reads with an average of 55 million reads (annex 1).

3.3 WGBS

DNA was fragmented and treated with bisulfite using the EZ DNA Methylation Gold Kit (Zymo Research), followed by size selection and PCR amplification using KAPA HiFi Hot Start Uracil + Ready Mix (2x). PE 150 bp sequencing was done on a Novaseq 6000.



All the WGBS data were sent to WU on a hard disk support. For the pig, the average genome coverage varied from 33x to 40x with an average of 36x coverage (annex 2). For the chicken, the average genome coverage varied from 28x to 36x with an average of 31x coverage (annex 2).

3.4 Data submission RRBS

Each RRBS dataset was submitted to the ENA database website (<https://www.ebi.ac.uk/ena/browser/submit>), including MD5sum for each sample. A detailed spreadsheet with all the required metadata was submitted to the FAANG data portal (DCC) and validated by the established DCC procedure. The data are available with the accession numbers: PRJEB41822 (ERP125658): GENE-SWITCH Pig DNA methylation with Reduced representation bisulfite sequencing (RRBS) and PRJEB41829 (ERP125665): GENE-SWITCH Chicken DNA methylation with Reduced representation bisulfite sequencing (RRBS).

3.5 Data submission WGBS

Each tissue/developmental stage WGBS dataset was submitted to the ENA database website (<https://www.ebi.ac.uk/ena/browser/submit>), including MD5sum for each sample. A detailed spreadsheet with all the required metadata was submitted to the FAANG data portal (DCC) and validated by the established DCC procedure. The data are available with the accession numbers: PRJEB42772 (ERP126677): GENE-SWITCH Pig DNA methylation with Whole Genome bisulfite sequencing (WGBS) and PRJEB42775 (ERP126680): GENE-SWITCH Chicken DNA methylation with Whole Genome bisulfite sequencing (WGBS).

4 Conclusion

High quality DNA methylation data have been obtained from seven tissues at three developmental stages in pig and chicken. The DNA methylation data have been made available to project partners and the global community through ENA and the FAANG data sharing portal.

5 Deviations or delays

There has been a delay (from M12 to M19) for submitting the data to ENA and the FAANG portal, due to delays in animal sampling. This was caused by the breeding delays and restricted access to animals that occurred during the COVID-19 pandemics lockdown in UK (see also D1.1).

6 Acknowledgements

We would like to thank Alexey Sokolov (Part 5, EMBL) for his great help in submitting the data to ENA and the FAANG portal.

7 References

Toronto International Data Release Workshop Authors, Birney E, Hudson TJ, Green ED, Gunter C, Eddy S, Rogers J, Harris JR, Ehrlich SD, Apweiler R, Austin CP, Berglund L, Bobrow M, Bountra C, Brookes AJ, Cambon-Thomsen A, Carter NP, Chisholm RL, Contreras JL, Cooke RM, Crosby WL, Dewar K, Durbin R, Dyke SO, Ecker JR, El Emam K, Feuk L, Gabriel SB, Gallacher J, Gelbart WM, Granell A, Guarner F, Hubbard T, Jackson SA, Jennings JL, Joly Y, Jones SM, Kaye J, Kennedy KL, Knoppers BM, Kyrpides NC, Lowrance WW, Luo J, MacKay JJ, Martín-Rivera L, McCombie WR, McPherson JD, Miller L, Miller W, Moerman D, Mooser V, Morton CC, Ostell JM, Ouellette BF, Parkhill J, Raina PS, Rawlings C, Scherer SE, Scherer SW, Schofield PN, Sensen CW, Stodden VC, Sussman MR, Tanaka T, Thornton J, Tsunoda T, Valle D, Vuorio EI, Walker NM, Wallace S, Weinstock G, Whitman WB, Worley KC, Wu C, Wu J, Yu J. Prepublication data



sharing. Nature. 2009 Sep 10;461(7261):168-70. doi: 10.1038/461168a. PMID: 19741685; PMCID: PMC3073843.t al. 2009. Pre-publication data sharing. Nature 461:168-170

8 Annexes

Annex 1: Number of RRBS reads for Pig and Chicken libraries.

The name of the library indicates the species (SSR: pig and GGA: chicken), the partner in charge of sampling (INRA, UEDIN), the project (GS: GENE-SWitCH), the work package (WP1), the developmental stage (30dpf: foetuses of 30 days, 70dpf: foetuses of 70 days, NB : newborn, E8: embryos of 8 days, E15: embryos of 15 days, HC: hatched chick) and the replicate number.

| GENE SWitCH ID Pig RRBS | # reads | Geneswitch ID RRBS Chicken | # reads |
|---|-------------|---|-----------------|
| SSR INRA GS WP1 Liver FT 30dpf POOL 2 | 64,400,818 | GGA UEDIN GW WP1 Liver E8 POOL1 | 57,633,297 |
| SSR INRA GS WP1 Liver FT 30dpf POOL 3 | 63,626,014 | GGA UEDIN GW WP1 Liver E8 POOL2 | 52,530,707 |
| SSR INRA GS WP1 Liver FT 30dpf POOL 6 | 62,754,259 | GGA UEDIN GW WP1 Liver E8 POOL3 | 56,765,385 |
| SSR INRA GS WP1 Small Intestine FT 30dpf POOL 1 | 49,687,818 | GGA UEDIN GW WP1 small intestine E8 POOL1 | 52,431,055 |
| SSR INRA GS WP1 Small Intestine FT 30dpf POOL 3 | 59,999,494 | GGA UEDIN GW WP1 small intestine E8 POOL2 | 60,664,503 |
| SSR INRA GS WP1 Small Intestine FT 30dpf POOL 6 | 52,893,607 | GGA UEDIN GW WP1 small intestine E8 POOL4 | 52,701,975 |
| SSR INRA GS WP1 Lung FT 30dpf POOL 2 | 60,090,492 | GGA UEDIN GW WP1 kidney E8 POOL1 | 59,620,519 |
| SSR INRA GS WP1 Lung FT 30dpf POOL 3 | 60,134,610 | GGA UEDIN GW WP1 kidney E8 POOL2 | 53,771,485 |
| SSR INRA GS WP1 Lung FT 30dpf POOL 6 | 59,328,413 | GGA UEDIN GW WP1 kidney E8 POOL3 | 50,913,089 |
| SSR INRA GS WP1 Kidney FT 30dpf POOL 1 | 60,497,417 | GGA UEDIN GW WP1 lung E8 POOL1 | 52,945,187 |
| SSR INRA GS WP1 Kidney FT 30dpf POOL 3 | 66,220,107 | GGA UEDIN GW WP1 lung E8 POOL2 | 55,934,229 |
| SSR INRA GS WP1 Kidney FT 30dpf POOL 6 | 61,354,567 | GGA UEDIN GW WP1 lung E8 POOL3 | 56,011,342 |
| SSR INRA GS WP1 Hindbrain FT 30dpf POOL 2 | 49,568,493 | GGA UEDIN GW WP1 hindlimb muscle E8 POOL1 | 45,041,008 |
| SSR INRA GS WP1 Hindbrain FT 30dpf POOL 3 | 61,524,661 | GGA UEDIN GW WP1 hindlimb muscle E8 POOL2 | 56,745,930 |
| SSR INRA GS WP1 Hindbrain FT 30dpf POOL 6 | 61,910,276 | GGA UEDIN GW WP1 hindlimb muscle E8 POOL4 | 50,304,045 |
| SSR INRA GS WP1 Skin FT 30dpf POOL 1 | 49,213,471 | GGA UEDIN GW WP1 hindbrain E8 POOL1 | 48,397,610 |
| SSR INRA GS WP1 Skin FT 30dpf POOL 3 | 67,576,497 | GGA UEDIN GW WP1 hindbrain E8 POOL2 | 51,470,164 |
| SSR INRA GS WP1 Skin FT 30dpf POOL 6 | 62,940,137 | GGA UEDIN GW WP1 hindbrain E8 POOL3 | 52,426,855 |
| SSR INRA GS WP1 Hindlimb muscle FT 30dpf POOL 2 | 61,024,889 | GGA UEDIN GW WP1 skin E8 POOL1 | 60,413,045 |
| SSR INRA GS WP1 Hindlimb muscle FT 30dpf POOL 3 | 63,601,544 | GGA UEDIN GW WP1 skin E8 POOL2 | 50,019,583 |
| SSR INRA GS WP1 Hindlimb muscle FT 30dpf POOL 6 | 61,806,067 | GGA UEDIN GW WP1 skin E8 POOL4 | 50,805,666 |
| SSR INRA GS WP1 liver FT 70dpf 1 | 58,763,216 | GGA UEDIN GW WP1 liver E15 3 | 65,517,777 |
| SSR INRA GS WP1 liver FT 70dpf 2 | 65,538,222 | GGA UEDIN GW WP1 liver E15 4 | 46,186,308 |
| SSR INRA GS WP1 liver FT 70dpf 3 | 59,255,477 | GGA UEDIN GW WP1 liver E15 1 | 45,506,598 |
| SSR INRA GS WP1 small intestine FT 70dpf 1 | 59,729,799 | GGA UEDIN GW WP1 small intestine E15 3 | 55,122,963 |
| SSR INRA GS WP1 small intestine FT 70dpf 2 | 58,779,460 | GGA UEDIN GW WP1 small intestine E15 4 | 62,314,188 |
| SSR INRA GS WP1 small intestine FT 70dpf 4 | 60,731,370 | GGA UEDIN GW WP1 small intestine E15 2 | 51,979,593 |
| SSR INRA GS WP1 Lung FT 70dpf 1 | 56,195,081 | GGA UEDIN GW WP1 kidney E15 3 | 51,685,657 |
| SSR INRA GS WP1 Lung FT 70dpf 2 | 56,953,066 | GGA UEDIN GW WP1 kidney E15 4 | 60,195,062 |
| SSR INRA GS WP1 Lung FT 70dpf 3 | 57,201,736 | GGA UEDIN GW WP1 kidney E15 2 | 48,021,604 |
| SSR INRA GS WP1 Cerebellum FT 70dpf 1 | 61,703,337 | GGA UEDIN GW WP1 lung E15 3 | 57,813,751 |
| SSR INRA GS WP1 Cerebellum FT 70dpf 2 | 61,546,853 | GGA UEDIN GW WP1 lung E15 4 | 63,617,630 |
| SSR INRA GS WP1 Cerebellum FT 70dpf 4 | 60,774,419 | GGA UEDIN GW WP1 lung E15 1 | 52,798,399 |
| SSR INRA GS WP1 Back skin FT 70dpf 1 | 66,249,176 | GGA UEDIN GW WP1 hindlimb muscle E15 3 | 48,235,038 |
| SSR INRA GS WP1 Back skin FT 70dpf 2 | 62,761,711 | GGA UEDIN GW WP1 hindlimb muscle E15 4 | 60,716,732 |
| SSR INRA GS WP1 Back skin FT 70dpf 3 | 58,090,473 | GGA UEDIN GW WP1 hindlimb muscle E15 2 | 54,637,757 |
| SSR INRA GS WP1 Kidney FT 70dpf 1 | 62,382,838 | GGA UEDIN GW WP1 cerebellum E15 3 | 63,386,689 |
| SSR INRA GS WP1 Kidney FT 70dpf 2 | 65,153,631 | GGA UEDIN GW WP1 cerebellum E15 4 | 57,620,310 |
| SSR INRA GS WP1 Kidney FT 70dpf 3 | 60,543,536 | GGA UEDIN GW WP1 cerebellum E15 1 | 53,287,100 |
| SSR INRA GS WP1 hindlimb muscle FT 70dpf 1 | 63,112,778 | GGA UEDIN GW WP1 skin E15 3 | 54,938,551 |
| SSR INRA GS WP1 hindlimb muscle FT 70dpf 2 | 62,614,533 | GGA UEDIN GW WP1 skin E15 4 | 53,965,083 |
| SSR INRA GS WP1 hindlimb muscle FT 70dpf 4 | 56,145,039 | GGA UEDIN GW WP1 skin E15 2 | 54,673,530 |
| SSR INRA GS WP1 Liver NB M 1 | 66,245,914 | GGA UEDIN GW WP1 liver HC 1 | 56,297,532 |
| SSR INRA GS WP1 Liver NB M 2 | 61,611,235 | GGA UEDIN GW WP1 liver HC 4 | 59,732,971 |
| SSR INRA GS WP1 Liver NB F 3 | 55,047,493 | GGA UEDIN GW WP1 liver HC 2 | 58,313,905 |
| SSR INRA GS WP1 Lung NB M 1 | 40,863,646 | GGA UEDIN GW WP1 small intestine HC 1 | 59,145,692 |
| SSR INRA GS WP1 Lung NB M 2 | 64,096,617 | GGA UEDIN GW WP1 small intestine HC 4 | 70,083,600 |
| SSR INRA GS WP1 Lung NB F 4 | 56,405,093 | GGA UEDIN GW WP1 small intestine HC 3 | 58,788,259 |
| SSR INRA GS WP1 kidney NB M 1 | 61,658,971 | GGA UEDIN GW WP1 kidney HC 1 | 49,417,834 |
| SSR INRA GS WP1 kidney NB M 2 | 69,444,868 | GGA UEDIN GW WP1 kidney HC 4 | 55,575,948 |
| SSR INRA GS WP1 kidney NB F 3 | 58,990,877 | GGA UEDIN GW WP1 kidney HC 2 | 50,338,948 |
| SSR INRA GS WP1 Ileum NB M 1 | 46,660,658 | GGA UEDIN GW WP1 lung HC 1 | 48,464,177 |
| SSR INRA GS WP1 Ileum NB M 2 | 67,474,098 | GGA UEDIN GW WP1 lung HC 4 | 61,166,071 |
| SSR INRA GS WP1 Ileum NB F 4 | 63,533,701 | GGA UEDIN GW WP1 lung HC 3 | 47,091,637 |
| SSR INRA GS WP1 cerebellum NB M 1 | 52,242,142 | GGA UEDIN GW WP1 hindlimb muscle HC 1 | 41,085,678 |
| SSR INRA GS WP1 cerebellum NB M 2 | 58,205,764 | GGA UEDIN GW WP1 hindlimb muscle HC 4 | 61,058,986 |
| SSR INRA GS WP1 cerebellum NB F 3 | 57,487,158 | GGA UEDIN GW WP1 hindlimb muscle HC 3 | 52,823,642 |
| SSR INRA GS WP1 oluteus medius NB M 1 | 49,701,281 | GGA UEDIN GW WP1 cerebellum HC 1 | 59,504,953 |
| SSR INRA GS WP1 oluteus medius NB M 2 | 55,637,033 | GGA UEDIN GW WP1 cerebellum HC 4 | 57,109,780 |
| SSR INRA GS WP1 oluteus medius NB F 4 | 59,237,372 | GGA UEDIN GW WP1 cerebellum HC 2 | 53,080,498 |
| SSR INRA GS WP1 back skin NB M 1 | 46,071,685 | GGA UEDIN GW WP1 skin HC 1 | 45,726,179 |
| SSR INRA GS WP1 back skin NB M 2 | 57,662,219 | GGA UEDIN GW WP1 skin HC 4 | 58,152,297 |
| SSR INRA GS WP1 back skin NB F 3 | 50,446,364 | GGA UEDIN GW WP1 skin HC 2 | 51,577,935 |
| Average | 59255612.56 | Average | 54671484.460318 |



Annex 2: Average coverage of WGBS for pig and chicken

| GENE-SwitCH ID - Pig | X coverage | GENE-SwitCH ID - Chicken | X coverage |
|---|-------------|---|-------------|
| SSR_INRA_GS_WP1_Liver_FT_30dpf_POOL_1 | 32,92592593 | GGA_UEDIN_GW_WP1_Liver_E8_POOL4 | 30,83333333 |
| SSR_INRA_GS_WP1_Small_Intestine_FT_30dpf_POOL_2 | 39,85185185 | GGA_UEDIN_GW_WP1_small_intestine_E8_POOL3 | 28,41666667 |
| SSR_INRA_GS_WP1_Lung_FT_30dpf_POOL_1 | 34,48148148 | GGA_UEDIN_GW_WP1_kidney_E8_POOL4 | 28 |
| SSR_INRA_GS_WP1_Kidney_FT_30dpf_POOL_2 | 33,18518519 | GGA_UEDIN_GW_WP1_lung_E8_POOL4 | 29 |
| SSR_INRA_GS_WP1_Hindbrain_FT_30dpf_POOL_1 | 36,11111111 | GGA_UEDIN_GW_WP1_hindlimb_muscle_E8_POOL3 | 33,41666667 |
| SSR_INRA_GS_WP1_Skin_FT_30dpf_POOL_2 | 39,44444444 | GGA_UEDIN_GW_WP1_hindbrain_E8_POOL4 | 30,66666667 |
| SSR_INRA_GS_WP1_Hindlimb_muscle_FT_30dpf_POOL_1 | 36,48148148 | GGA_UEDIN_GW_WP1_skin_E8_POOL3 | 36,25 |
| SSR_INRA_GS_WP1_liver_FT_70dpf_4 | 35,44444444 | GGA_UEDIN_GW_WP1_liver_E15_2 | 30,5 |
| SSR_INRA_GS_WP1_small_intestine_FT_70dpf_3 | 34,33333333 | GGA_UEDIN_GW_WP1_small_intestine_E15_1 | 33,83333333 |
| SSR_INRA_GS_WP1_Lung_FT_70dpf_4 | 35,14814815 | GGA_UEDIN_GW_WP1_kidney_E15_1 | 32,58333333 |
| SSR_INRA_GS_WP1_Cerebellum_FT_70dpf_3 | 37,77777778 | GGA_UEDIN_GW_WP1_lung_E15_2 | 30,66666667 |
| SSR_INRA_GS_WP1_Back_skin_FT_70dpf_4 | 38,74074074 | GGA_UEDIN_GW_WP1_hindlimb_muscle_E15_1 | 33,41666667 |
| SSR_INRA_GS_WP1_Kidney_FT_70dpf_4 | 36,88888889 | GGA_UEDIN_GW_WP1_cerebellum_E15_2 | 28,66666667 |
| SSR_INRA_GS_WP1_hindlimb_muscle_FT_70dpf_3 | 34,2962963 | GGA_UEDIN_GW_WP1_skin_E15_1 | 32,16666667 |
| SSR_INRA_GS_WP1_Liver_NB_F_4 | 40,33333333 | GGA_UEDIN_GW_WP1_liver_HC_3 | 29,75 |
| SSR_INRA_GS_WP1_Lung_NB_F_3 | 33,22222222 | GGA_UEDIN_GW_WP1_small_intestine_HC_2 | 34,58333333 |
| SSR_INRA_GS_WP1_kidney_NB_F_4 | 35,85185185 | GGA_UEDIN_GW_WP1_kidney_HC_3 | 27,5 |
| SSR_INRA_GS_WP1_Ileum_NB_F_3 | 33,03703704 | GGA_UEDIN_GW_WP1_lung_HC_2 | 28,16666667 |
| SSR_INRA_GS_WP1_cerebellum_NB_F_4 | 33,25925926 | GGA_UEDIN_GW_WP1_hindlimb_muscle_HC_2 | 29,08333333 |
| SSR_INRA_GS_WP1_gluteus_medius_NB_F_3 | 33,07407407 | GGA_UEDIN_GW_WP1_cerebellum_HC_3 | 28,33333333 |
| SSR_INRA_GS_WP1_back_skin_NB_F_4 | 36,18518519 | GGA_UEDIN_GW_WP1_skin_HC_3 | 27,5 |
| Average | 35,85740741 | Average | 30,63492063 |

The protocols for the production of RRBS and WGBS libraries are available on the GENE-SWitCH sharepoint:

https://sites.inra.fr/site/gene-switch/ layouts/15/start.aspx#/WP1_T15/Forms/AllItems.aspx

It contains the following files:

INRA_SOP_GENESWITCH_WP1_PIG_EXTRACTION_DNA_RNA_20201111.pdf

ROSLIN_SOP_GENESWITCH_WP1_CHICK_EXTRACTION_DNA_RNA_20201111.pdf

WU_SOP_GENESWITCH_WP1_RRBS_library_preparation_20201201.pdf

WU_SOP_GENESWITCH_WP1_WGBS_library_preparation_20210127.pdf

9 Glossary

BS – Bisulfite sequencing.

WGBS – Whole genome bisulfite sequencing.

RRBS - Reduced representation bisulfite sequencing.

CpG – Base combination in the genome which can be methylated (“C” is the base which methylation occurs)