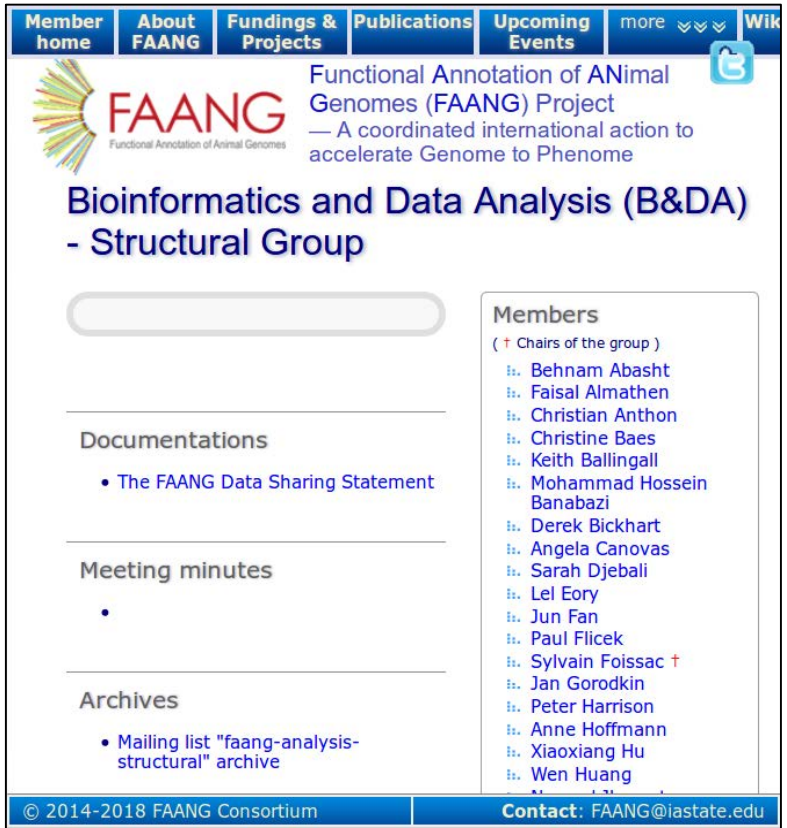
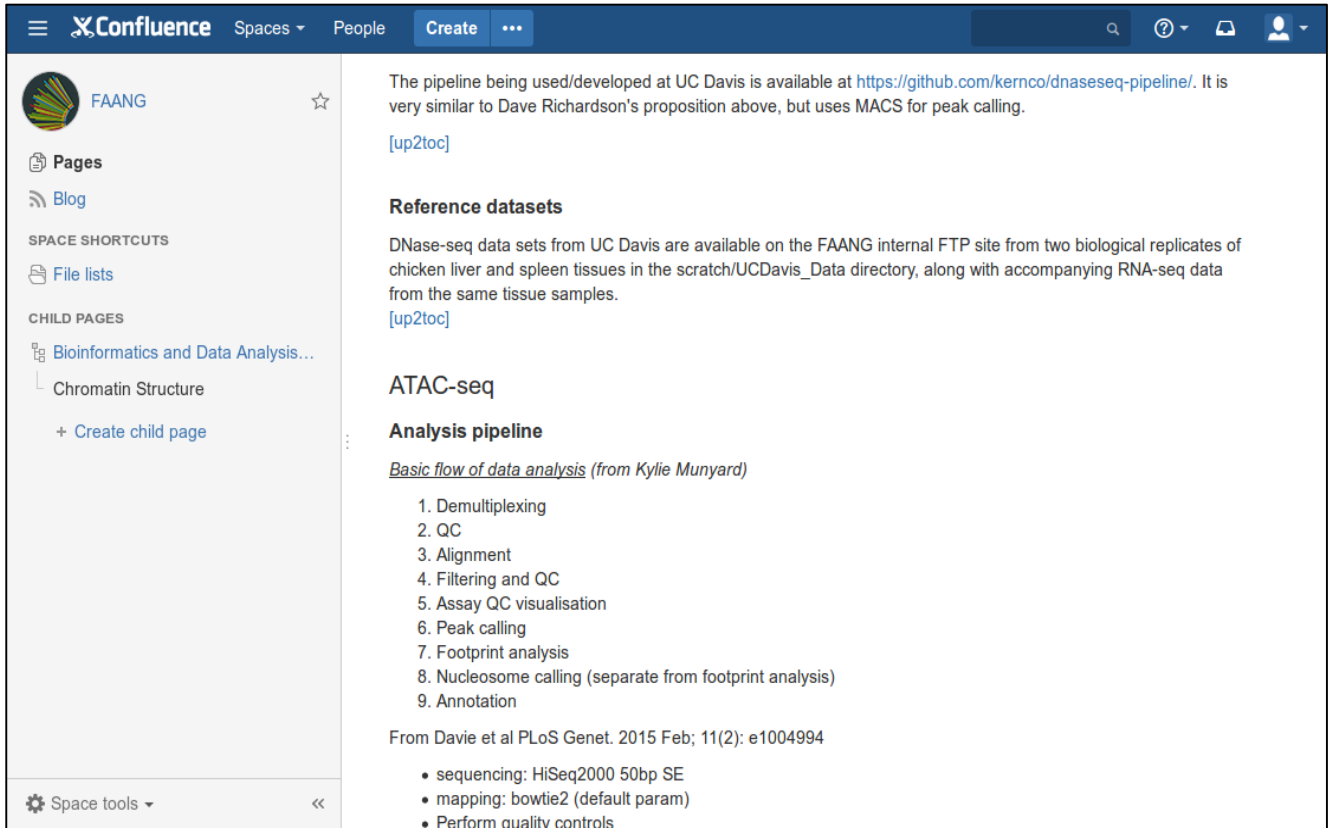
Bioinformatics and Data Analysis

- RNA Analysis working group – Lel Eory
- Methylation working group – Ole Madsen
- Structural working group – Sylvain Foissac
- DNA binging working group – Pablo Ross

Chromatin structure and 3D genomics

- Group organization
  - 53 registered members (including S. Foissac & J. Reecy)
  - Teleconf meetings
  - Reports on FAANG confluence website: www.ebi.ac.uk/seqdb/confluence





Methylation

PAG 2018 updates

Ole Madsen, University of Wageningen

Methylation Pipelines

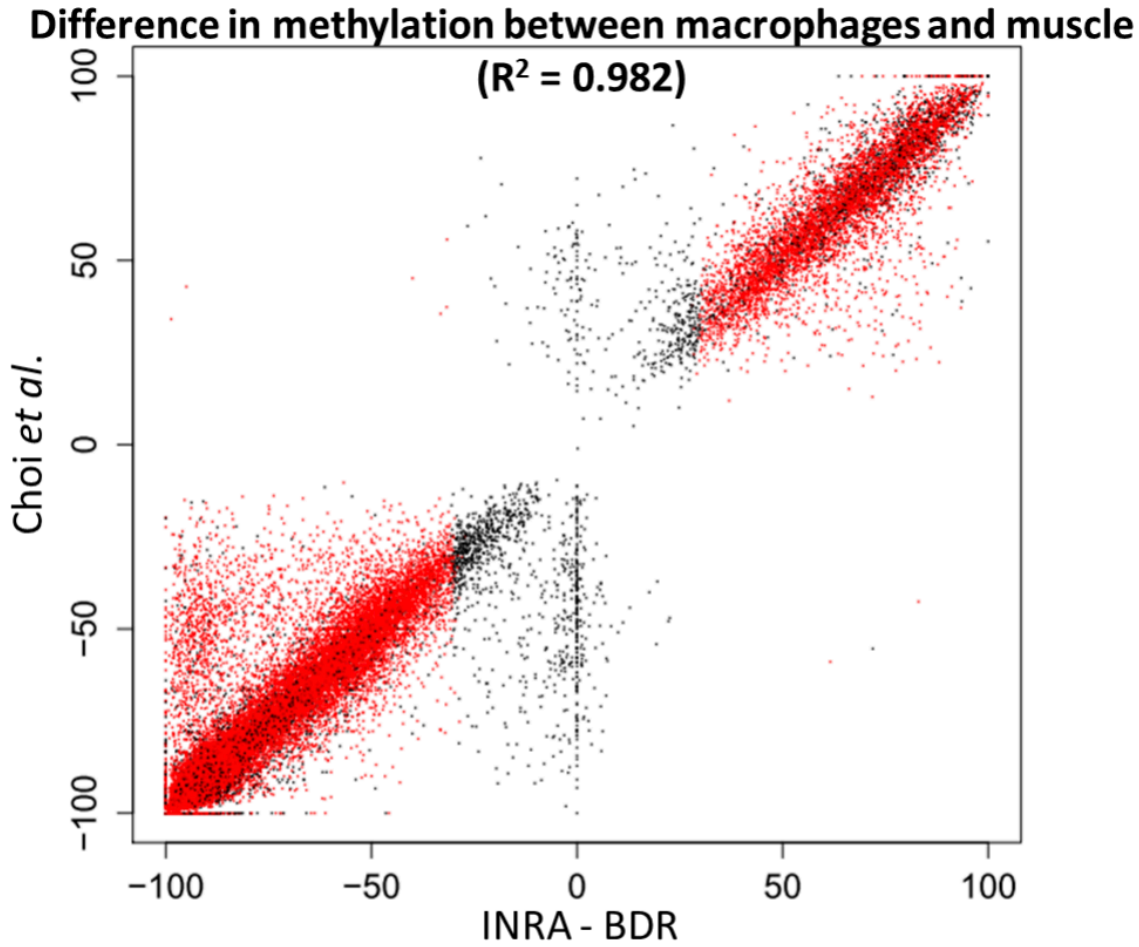
- The EBI Pipeline: https://www.ebi.ac.uk/seqdb/confluence/display/FAANG/Bisulfite+Sequencing+%28BS%29+pipeline
- Toulouse pipeline WorkflowBS: https://github.com/FAANG/faang-methylation/tree/master/workflowbs
- INRA Paris pipeline: link to poster https://www.ebi.ac.uk/seqdb/confluence/download/attachments/32192334/RRBS_pipeline_poster.pdf?version=1&modificationDate=1483369795000&api=v2

Methylation Pipelines

For future analysis/next steps:

We (Wageningen University) recently made RRBS and WGBS from the same samples which will be used to compare the two methods (preliminary results indicate they seem to complement each other).

Conclusion



● : Difference of methylation between the two conditions for all tested CpGs

● : Difference of methylation between the two conditions for DMCs (q < 0.001)

Even if alignment softwares and parameters have a major influence on the set of CpGs selected for further analyses, the results obtained on the commonly found CpGs are in good correlation (see graph on the left).

RNA

PAG 2018 updates

Lel Rory, Roslin

Currently 140 members.

Linked with non-coding RNA subgroup led by Jan Gorodkin (at University of Copenhagen), with 56 member within lncRNA group

Web:
https://www.ebi.ac.uk/seqdb/confluence/display/FAANG/RNA-Seq

Email:
faang-analysis-rna@animalgenome.org
faang-analysis-lncrna@animalgenome.org

Aims:

- To improve annotation.
- Identify functional categories of novel genes.
- Quantify expression.
- Study gene interactions.

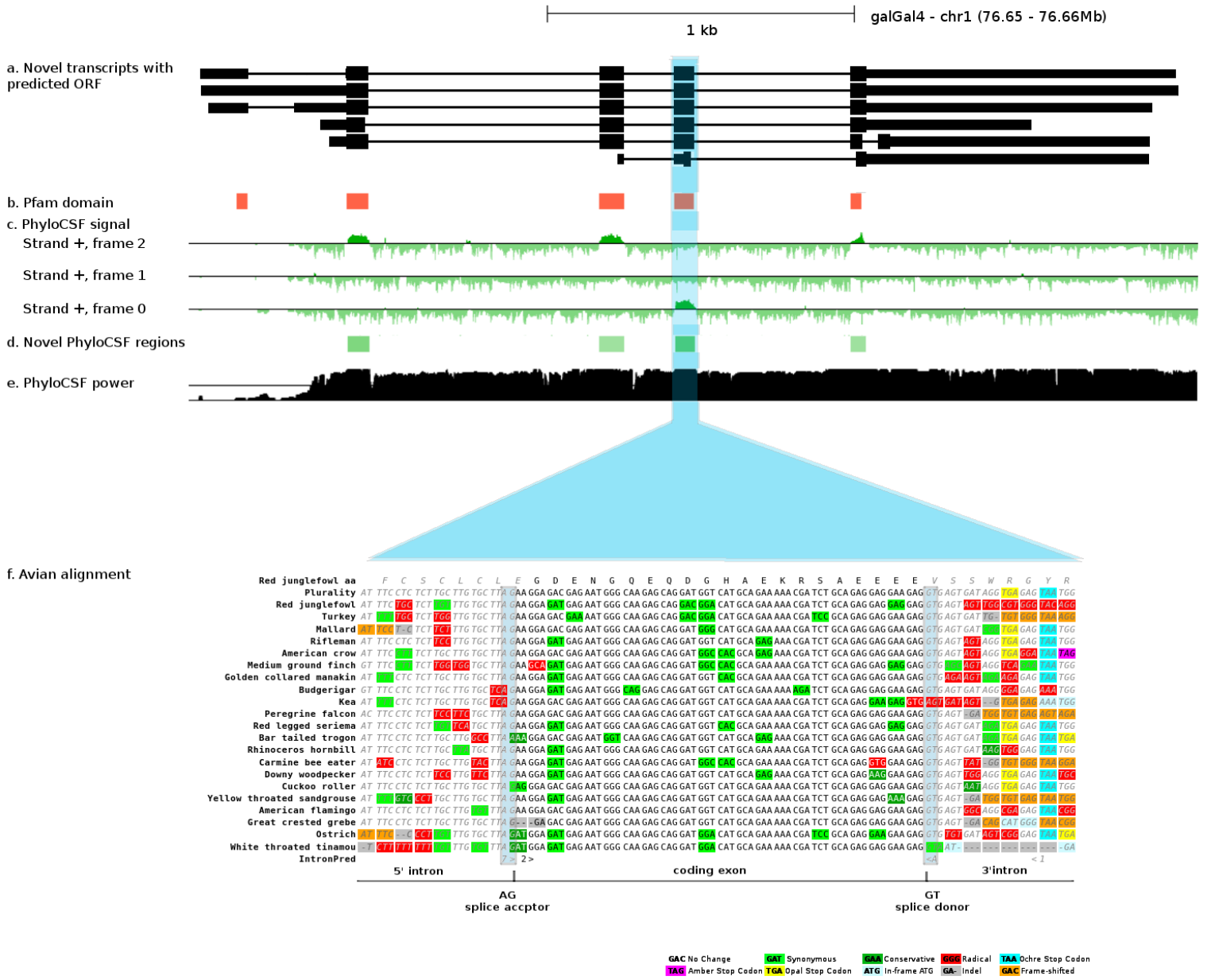
By:

- Contributing to experimental assay requirements and bioinformatics pipeline specifications.
- Benchmarking pipelines.
- Running assays: RNA-seq, small RNA-seq, PacBio Iso-Seq, CAGE.
- Providing reference datasets: for pig and chicken some of these from Roslin pilot projects.

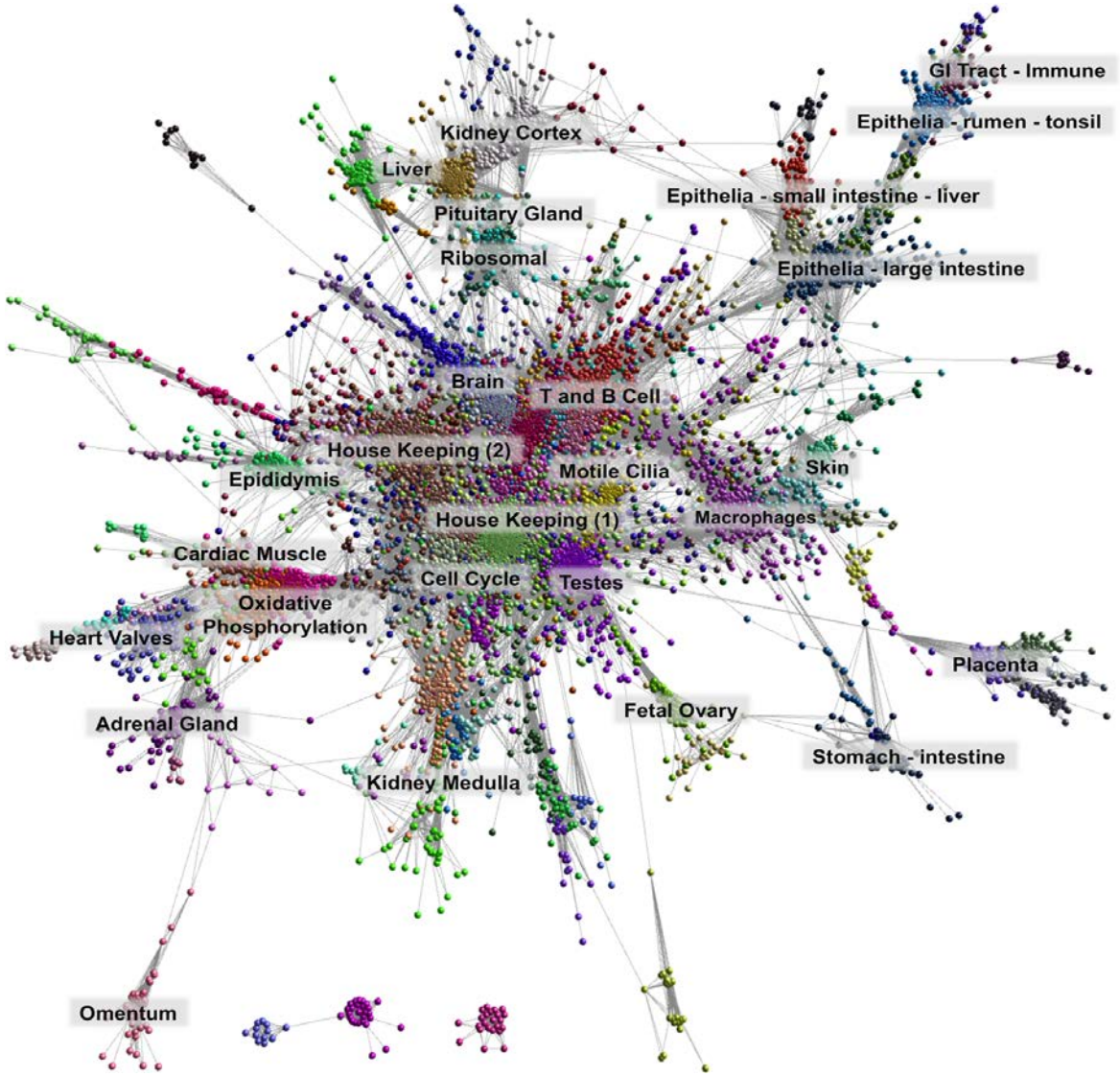
Pipelines:

- RNA-seq: quality control (FastQC), adapter trimming (cutadapt), assembly (Ensembl, HISAT2/StringTie2 or STAR/Cufflinks)
- PacBio Iso-Seq: Iso-Seq pipeline
- micro RNA-seq: read mapping (bowtie2), miRdeep2
- Protein coding potential: FEELnc, PhyloCSF, Pfam, BLAST
- CAGE: demultiplexing, trimming, quality control, mapping (bwa), promoter and expression analysis Paraclu/CAGEr
- Gene expression: RSEM, kallisto
- Gene interaction: Biolayout/Cytoscape

Example of novel transcripts from merged PacBio Iso-Seq and RNA-seq models.



Gene network from the sheep atlas project



Clark et al. 2017, PLoS Genetics

DNA Binding

PAG 2018 updates

Pablo Ross, University of California - Davis

ChIP-seq data Analysis

https://www.ebi.ac.uk/seqdb/confluence/display/FAANG/ChIP-Seq

- ChIP-Seq data acquisition standards
  - Sequencing: Single end 50bp
  - Read coverage: Narrow peaks: 20 million; Broad peaks: 40 million uniquely mapped reads
  - Input from same sonication batch
  - Biological replicates (at least 2 – same sex)
- Software tools for analysis and parameter
  - Quality Control and Trimming
  - Read Mapping
  - Peak calling
- Available Pipelines
  - ENCODE ChIP-Seq pipeline: https://github.com/ENCODE-DCC/chip-seq-pipeline
  - UC Davis ChIP-Seq pipeline: https://github.com/kernco/chipseq-pipeline
  - EpiDB pipeline: https://github.com/ercfrtz/epidb/blob/master/epidb.load.chipseq.pl

ChIP-seq data Analysis

- Currently investigating
  - ChIP-seq data quality metrics
    - Usable fragments (aligned, quality filtered, deduplicated)
    - Non-Redundant Fraction (NRF)
    - PCR Bottlenecking Coefficient (PBC)
    - Cross-Strand Correlation
  - Data reproducibility
    - IDR (mostly for TF)
    - Looking for good alternatives for Histone Marks
- Panned activities
  - Test a common set of parameters (initially defined by the ENCODE pipeline) under different conditions using a common sample dataset and consider modifications as needed.
  - Test the selected parameters in datasets from different animal species
  - Incorporate the selected parameters into easily accessible pipelines implemented in different platforms such as GitHub, CyVerse, Galaxy, etc.

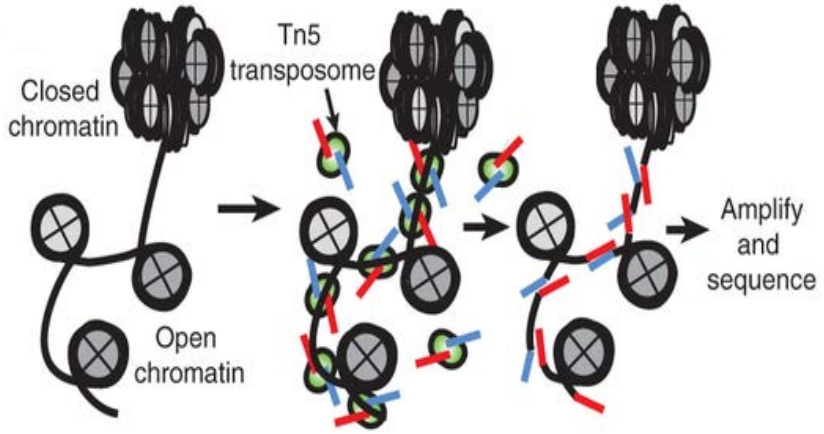
Chromatin structure and 3D genomics

PAG 2018 updates

Sylvain Foissac, INRA

Chromatin structure and 3D genomics

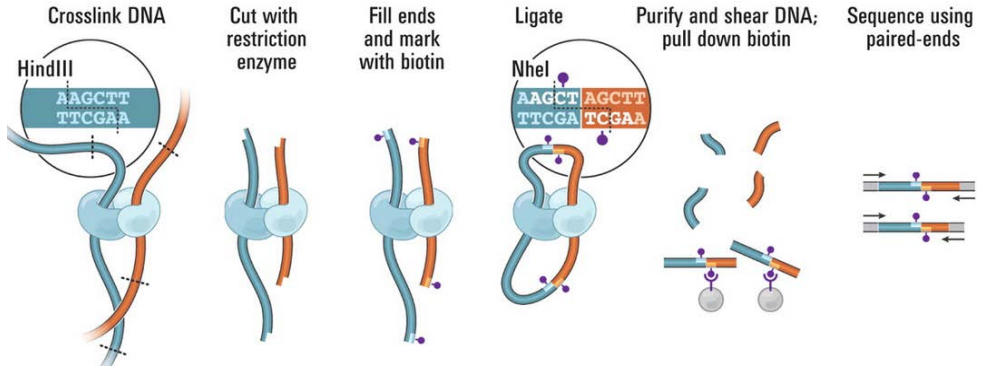
- Protocol & Pipelines
-



Buenrostro et al, 2013

ATAC-seq

- Read trimming (`trimgalore`)
- Read mapping (`Bowtie2`)
- PCR duplicate removal (`samtools`)
- Mitochondrial read removal (`samtools`)
- Peak calling (`MACS2`)
- Peak merging (`bedtools`)
- Peak quantification (`samtools`)
- LOESS normalization (`csaw`)
- Differential analysis (`edgeR`)



Rao et al, 2014

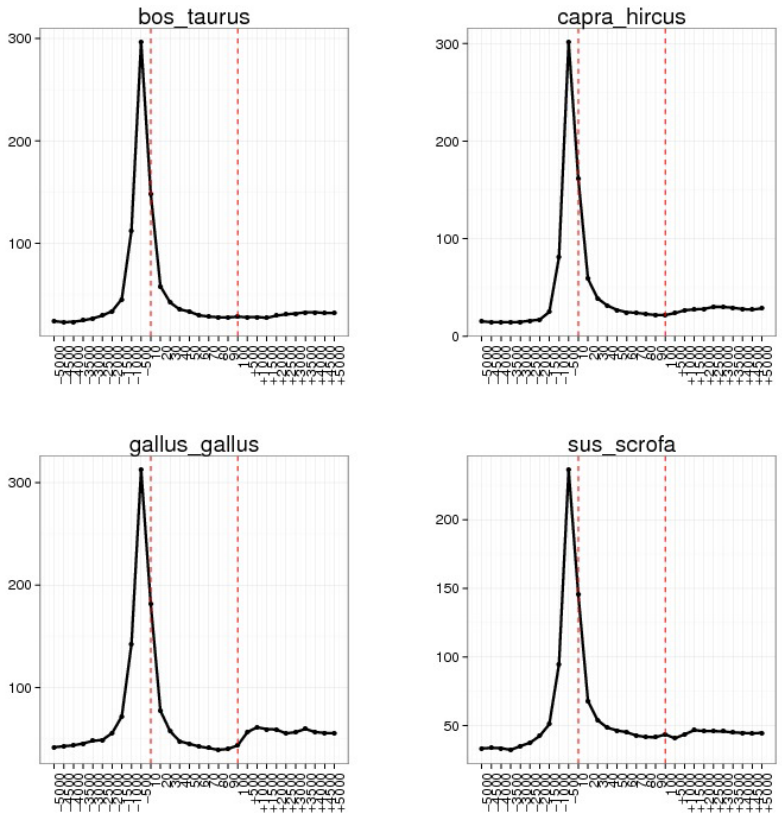
Hi-C

- Read trimming (`cutadapt`)
- Read mapping (`Bowtie2`)
- Invalid pairs filtering (`samtools`)
- Contact matrix generation (`HiC-Pro`)
- Matrix balancing normalization (`ICE`)
- TAD calling (`armatus`)
- A/B compartments calling (`HiT-C`)
- Visualization (`juicebox`)

Chromatin structure and 3D genomics

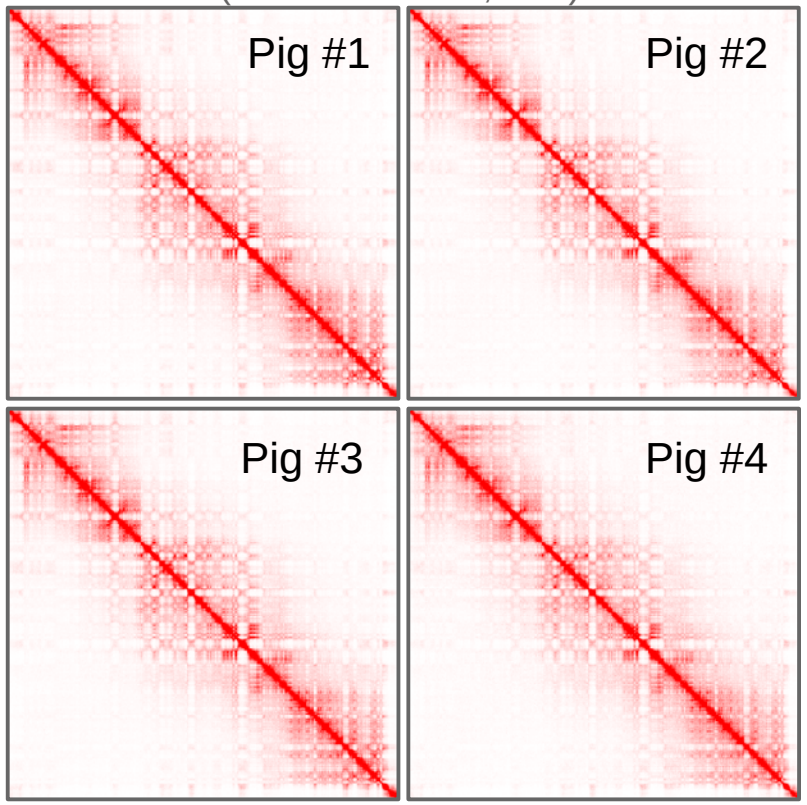
- Results: on Hi-C & ATAC-seq data from FR-AgENCODE pilot project

ATAC-seq gene coverage per species



ATAC-seq signal peaks at gene starts (TSS)

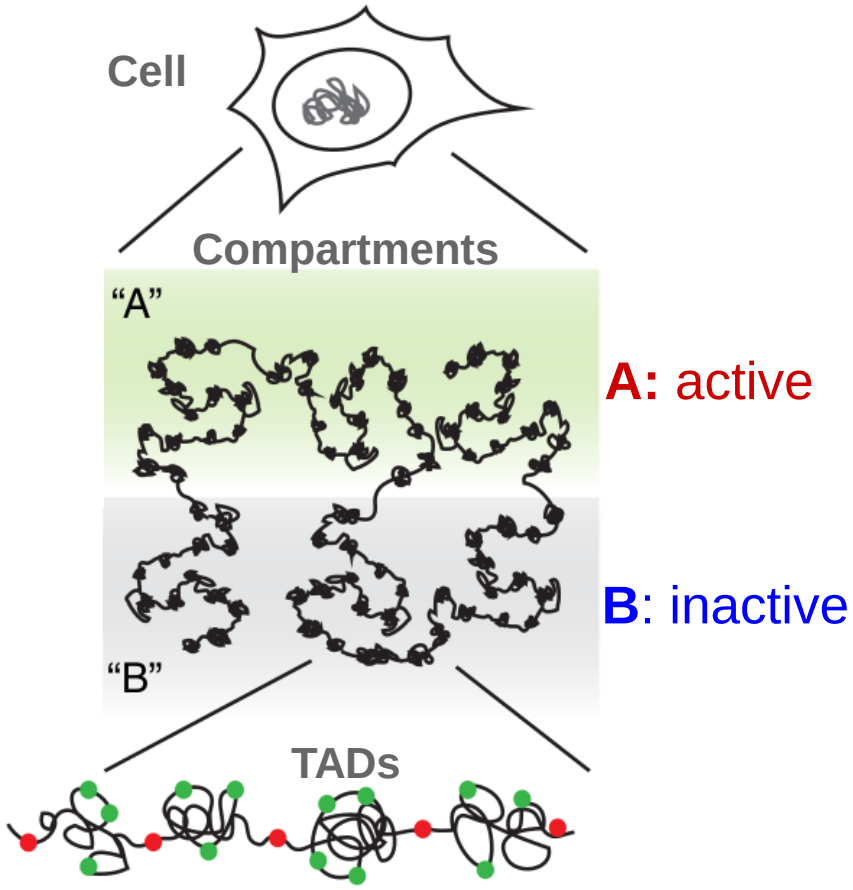
Hi-C interaction matrices per replicate
(*Sus scrofa* chr1, liver)



Hi-C signal is resolutive and reproducible

Chromatin structure and 3D genomics

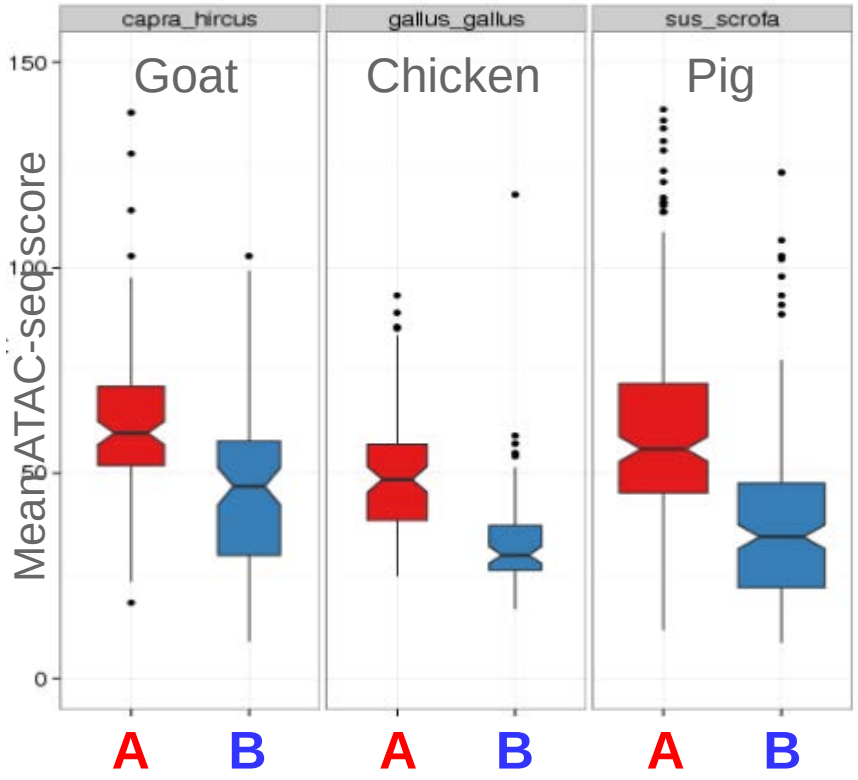
- Results: integrative analysis



A: active

B: inactive

Chromatin accessibility (from ATAC-seq) in A vs. B compartments (from Hi-C)



Chromatin is more accessible in active compartments
ATAC-seq and Hi-C pipelines generate consistent results
=> FR-AgENCODE manuscript in prep.