SalMotifDB: a tool for analyzing putative transcription factor binding sites in salmonid genomes

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Abstract

Background: Recently developed genome resources in Salmonid fish provides tools for studying the genomics underlying a wide range of properties including life history trait variation in the wild, economically important traits in aquaculture and the evolutionary consequences of whole genome duplications. Although genome assemblies now exist for a number of salmonid species, the lack of regulatory annotations are holding back our mechanistic understanding of how genetic variation in non-coding regulatory regions affect gene expression and the downstream phenotypic effects.

Results: We present SalMotifDB, a database and associated web and R interface for the analysis of transcription factors (TFs) and their cis-regulatory binding sites in five salmonid genomes. SalMotifDB integrates TF-binding site information for 3072 non-redundant DNA patterns (motifs) assembled from a large number of metazoan motif databases. Through motif matching and TF prediction, we have used these multi-species databases to construct putative regulatory networks in salmonid species. The utility of SalMotifDB is demonstrated by showing that key lipid metabolism regulators are predicted to regulate a set of genes affected by different lipid and fatty acid content in the feed, and by showing that our motif database explains a significant proportion of gene expression divergence in gene duplicates originating from the salmonid specific whole genome duplication.

Conclusions: SalMotifDB is an effective tool for analyzing transcription factors, their binding sites and the resulting gene regulatory networks in salmonid species, and will be an important tool for gaining a better mechanistic understanding of gene regulation and the associated phenotypes in salmonids. SalMotifDB is available at https://salmobase.org/apps/SalMotifDB.

Keywords: Transcription factor binding sites, Regulatory networks, Salmonid genomics, Gene regulation, Web tool

Background

Salmonid fish are widely studied due to their ecological importance, unique evolutionary history, and large economic impact as an aquaculture species [1]. Recently, the availability of genomic resources for salmonids has exploded, with chromosome level assemblies for several species including Atlantic salmon [2], Arctic charr [3], Chinook salmon [4] and Rainbow trout [5], and draft assemblies for other species including Coho salmon (GCF_002021735.1).

These new resources have sparked a number of studies to understand the genetic basis for life history trait variation in the wild [6–8], identify genes and genetic variation associated with economically important aquaculture traits [9–12], and shed light on consequences of the salmonid-specific whole genome duplication on gene regulatory evolution [2, 3, 13, 14]. However, reaching a mechanistic understanding of how regulatory DNA changes effect trait variation and give rise to novel genome regulation still remains a major challenge.
Regulatory DNA plays an important role in trait variation within populations [15] and also for evolution of novel traits at the evolutionary time scale [16]. Most causal variants underlying phenotypic variation in vertebrates are non-coding variants in regulatory regions [15]. Such variants likely alter transcription factor binding propensity with consequences for gene regulation [17]. Gene regulatory evolution is partly shaped by the birth and death of cis-regulatory elements [18], which in many cases are linked to transposable element insertions [19]. Despite the great genomic resources now available for salmonids, the gene regulatory landscape is poorly characterised and no resource so far exists for predicted cis-regulatory elements. This is a hindrance for further progress in understanding the mechanistic basis of salmonid traits and adaptations.

In this paper, we describe a new database containing predicted transcription factor binding sites in salmonid genomes, called SalMotifDB. The database is accessible through salmobase (http://www.salmobase.org/) [20], and can be queried both through a graphical user interface and an R package. It includes tools to extract gene regulatory network information, as well as tools to perform tests for overrepresented TF-binding sites in the cis-regulatory regions of user specified genes.

**Construction and content**

**The motifs**

A total of 19,845 metazoan transcription factors (TF) and their DNA binding sites were obtained from CISBP [21], JASPAR [22], 3D-footprint [23], UniPROBE [24], HumanTF [25], HumanTF2 [26], HT-SELEX2 [27], SMILE-seq [28], FlyZincFinger [29], HOCOMOCO [30], DrosophilaTF [31] through footprintDB [29] and TRANSFAC© [32]. Binding sites are represented as Position Specific Scoring Matrices (PSSMs), henceforth referred to as motifs. To mitigate motif redundancy, we employed the standard approach of RSAT (Regulatory Sequence Analysis Tool) [33]. Specifically, we used the RSAT matrix-clustering tool, with parameters -quick, -cor = 0.65 and -Ncor = 0.8. We first clustered motifs within each database (Fig. 1a) and then clustered the central motif of these database-specific clusters across databases (Additional file 1: Figure S1). Here, the central motif of a cluster is the motif with the highest similarity to other motifs in that cluster, as calculated by matrix-clustering. The final clustering resulted in 3092 motif clusters, and while all the original motifs are retained in SalMotifDB, the motif clusters and their representative (central) motifs are used to organize and remove redundancy in results throughout our tools. Different databases contributed with widely different numbers of motifs (Fig. 1a), as well as with different numbers of exclusive motifs not found in other databases (Fig. 1b). TRANSFAC was clearly the dominating database in both respects.

FIMO (Finding Individual Motif Occurrence) [34] is a widely used tool for mapping known motifs to genomes and has performed well in comparative studies [35]. We used FIMO to obtain statistically significant motif matches (compared to a zero-order background model, \( P \)-value < 0.0001) to cis-regulatory promoter regions in five salmonid species (Table 1). Similarly to other studies (e.g. [36, 37]), we defined cis-regulatory regions to extend from 1000 bps upstream to 200 bps downstream of transcription start sites of protein-coding genes, thus including the entire untranslated region (UTR) of most genes (Additional file 2: Figure S2). When several motifs matched identical genomic locations (i.e. same start and stop position), we only kept the motif with the lowest \( p \)-value. Mapping statistics were...
comparable across salmonid species, with each gene on average harboring from 311 to 439 different non-redundant motif matches in its promoter. Each non-redundant motif matched the promoter of 6062 to 8255 genes on average (corresponding to 10–14% of the genes in these species), with an average of ~1.3 matches per gene. Although the Atlantic salmon genome harbors a considerably higher absolute number of motif matches than other species, the per gene count is comparable to the other salmonids. For all species, motif matches are clearly enriched around the transcription start site in what is generally referred to as the core promoter (Fig. 2), which is consistent with observations in other species [37–39].

In addition to raw motif matches, SalMotifDB also allows the user to filter out motif matches that are in repeat regions or in unconserved regions. A repeat library database was built for each salmonid species using RepeatModeler v4.0.3 (http://www.repeatmasker.org) and the genomes were repeat masked using RepeatMasker v4.0.3 (http://www.repeatmasker.org). Genome wide multi-species homeologous block alignments across all the species in the database were generated using Mugsy [40] with Atlantic salmon genome as the reference. These alignments were then used by phastCons [41] to calculate a conservation scores and the most conserved elements in each salmonid species.

The transcription factors
We extracted the amino acid sequence for all 19,845 metazoa TFs associated to a motif in the motif databases and performed BLAST searches against each salmonid species (NCBI blast+ with evalue = 0.0001 and max_target_seqs = 5). The TFs and their hits to salmonid proteins were then both blasted against the NCBI Conserved Domain Database (CDD) (delta-blast evalue = 0.0001) [42], and protein domain similarity was computed using the Jaccard index. Salmonid genes with both a significant BLAST hit and a CDD Jaccard index ≥ 0.8 to a TF were considered putative salmonid TFs (Table 2). Considering that the number of genes vary substantially in these species (Table 1), the number of predicted TFs varied much less ranging from 2008 in Arctic char to 2194 in Chinook salmon.

Table 1 Summary of motif matches in SalMotifDB by species. Motifs were mapped to promoter regions spanning -1000/+200 bps up-/down-stream of transcription start sites (FIMO P-value < 0.0001). Numbers are given for all motifs as well as for central motifs (non-redundant) and are furthermore divided into numbers were all matches to a promoter is counted (Motif matches) and numbers were only one match per promoter is counted (Gene matches)

| Species                    | Genes | All motifs | Central motifs |
|----------------------------|-------|------------|----------------|
|                            |       | Motif matches | Gene matches   |
| Arctic char (Salvelinus alpinus) | 42,439 | 83,048,193 | 65,471,635 |
| Atlantic salmon (Salmo salar) | 81,586 | 118,076,727 | 89,320,019 |
| Chinook salmon (Oncorhynchus tshawytscha) | 48,724 | 92,515,215 | 71,056,812 |
| Coho salmon (Oncorhynchus kisutch) | 46,109 | 88,330,829 | 68,692,831 |
| Rainbow trout (Oncorhynchus mykiss) | 55,685 | 88,139,627 | 70,459,150 |

Table 2 Summary of TF prediction in the salmonids. The table shows the number of predicted TFs in each salmonid species. The second column contains the number of salmonid genes with significant BLAST hits to TFs with associated motifs in the motif databases. The last column contains the number of salmonid genes with both significant BLAST hits to TFs and a CDD Jaccard index ≥ 0.8.

| Species                  | No. genes predicted to be TFs |
|--------------------------|-------------------------------|
|                          | BLAST | BLAST + CDD |
| Arctic char              | 2480  | 2008        |
| Atlantic salmon          | 2430  | 2045        |
| Chinook salmon           | 2737  | 2194        |
| Coho salmon              | 2512  | 2035        |
| Rainbow trout            | 2761  | 2235        |
The putative regulatory networks
Through motif matching and TF prediction, we have effectively lifted over information on TF-binding site interactions from multi-species databases to salmonid species. By assuming that a TF regulates a gene if one of its associated motifs match in the promoter region of that gene, we have inferred putative global regulatory networks for the salmonid species. These directed networks are highly interconnected with each TF predicted to regulate on average ~ 6000 genes (network out-degree) and each gene predicted to be regulated by on average ~ 700 TFs (in-degree) (Fig. 3). Interestingly, these average numbers hide bimodal-like distributions (i.e. distributions with two peaks). For example, TFs belong to two groups with very different numbers of target genes (Fig. 3a), where the most highly connected group includes a long tail of extremely highly connected TFs with up to 25,000 targets (hubs). It should be noted, however, that these dense networks represent a potential for regulation, and can be made more realistic to a specific cellular context by integrating dynamic data such as expression data or open chromatin data from specific cell types, stress conditions or developmental processes.

The implementation
The backend of SalMotifDB consists of a MySQL database (database schema available in Additional file 3: Figure S3) and R scripts. The database schema and integrity is managed by the Django web framework. The frontend of SalMotifDB is hosted on an R shiny server that provides a user friendly interface for retrieving data from the database and performing different motif analysis. DNA binding site information such as motif logos, PSSMs and literature references is available through links to footprintDB (http://floresta.eead.csic.es/footprintdb) [29] for open source databases and geneXplain (http://genexplain.com/transfac/) for TRANSFAC© [32]. SalMotifDB is also accessible through an R packages. The R shiny web interface and R package code are publicly available in a GitLab repository accessible from the SalMotifDB web site (https://salmobase.org/apps/SalMotifDB).

Utility and discussion
The SalMotifDB web site and R package
We have implemented an R shiny web interface and an R package (https://salmobase.org/apps/SalMotifDB/) that provides access to the underlying SalMotifDB database through six tools (Fig. 4):

Gene search
The gene search tool allows the user to retrieve the motifs that match the promoter region of a query gene. Results include basic information about the gene, individual motif matches with distance from transcription start site, p-value and links to the external motif database as well as a graphical representation of where in the promoter the motifs match.

TF search
The TF search tool allows the user to search with a TF name and retrieve its motifs from external databases as well as predicted salmonid orthologs with information on BLAST E-value score and shared CDD domains.

Fig. 3 Salmonid-specific putative regulatory networks. a The distribution of the number of genes predicted to be regulated by a TF in each species (out-degree). Note that the Atlantic salmon (Ssal) genome has a considerably higher gene count than the other species. b The distribution of the number of TFs predicted to regulate a gene (in-degree)
Motif search
The motif search tool allows the user to search with a motif name, and retrieve details about all matches of that motif to salmonid promoters. Results also include a graphical representation of the motif's similarity to other motifs.

Motif cluster
The motif cluster tool allows the user to explore clusters of similar motifs, and includes graphical representations of the number of motifs from each external database and the similarity structure of the motifs in the cluster. Results also include sequence logos for individual motifs.

Position based search
The position based search tool allows the user to specify a genomic region of interest and retrieve details about all motif matches to promoters of genes located in that region.

Motif enrichment analysis
The enrichment tool allows the user to input a list of genes (e.g. differentially expressed genes) and identify motifs that match the promoters of these genes more often than expected by chance. The tool gives details about enrichment p-values (using the hypergeometric distribution), as well as details about all individual motif matches to promoters of genes in the list. Results also include a visual representation of the regulatory network inferred from the motif enrichment analysis.

Each tool comes with a customized walkthrough explaining the input and output of that tool.

Examples of utility: lipid metabolism and duplicate divergence
We provide two examples of the utility of the SalMotifDB by testing the tool on two sets of genes. Gene set 1 contains genes that are differently regulated between Atlantic salmon that were given feed with contrasting lipid and fatty acid content in fresh water (67 genes with \( p < 0.05 \) from supplementary Table 8 in ref. [12]). Transcriptional regulation of hepatic lipid metabolism is extensively studied [43] and are known to be conserved across vertebrates, including salmon [44]. This gene set is thus expected to be enriched in motifs associated with the lipid metabolism regulatory network(s) in liver [10, 12]. Indeed, a test for enriched motifs in SalMotifDB showed that promoters in gene set 1 were significantly enriched (\( p < 0.05 \)) for motifs bound by key lipid metabolism regulators such as SREBP-1, PPAR, NF-Y, and SP (Additional file 5: Table S1). Next we tested the tool on a gene set of duplicated gene pairs (ohnologs) originating from the salmonid specific whole genome duplication 80–100 million years ago. Salmonids are used as a model system to understand consequences of whole genome duplication on genome regulatory evolution [2, 13] and we know that about 60% of the retained duplicates display diverged tissue expression profiles [2]. Using SalMotifDB to identify
motifs in promoters of duplicated genes in Atlantic salmon (identified using the same approach as in ref. [2]), we then tested the hypothesis that divergence in tissue expression is linked to divergence of the cis-regulatory landscape between gene duplicates. We observed a significant correlation of 0.20 \( (p < 2.2e-16 \text{ using Pearson Correlation Coefficient test and } p = 0.0 \text{ using randomization, Additional file 4: Figure S4}) \) between motif similarity (Jaccard index) and tissue expression correlation (Pearson Correlation Coefficient) for 10,515 ohnologs and a correlation of 0.21 \( (p < 2.2e-16) \) for 735 TF ohnologs (Fig. 5). Furthermore, the data included in SalMotifDB was also recently used to identify associations between groups of duplicated genes displaying similar regulatory evolutionary fates and their promoter motif divergence [14]. Taken together, these analyses demonstrates the utility of SalMotifDB as a tool to improve interpretations and support biological validity of gene expression analyses and help understand the mechanistic drivers of gene regulation evolution.

Future
SalMotifDB relies on transcription factor - motif interactions observed in different species, tissues and experimental conditions. Furthermore, we only consider matches of these motifs to relatively restricted upstream regions in our selected salmonid species. Despite these limitations, we here show the utility of this motif database in identifying biologically sound links between cis-regulatory landscapes and gene expression patterns in salmon. Future developments of this database include adding genome tracks for epigenetic profiling such as ATAC-Seq. This will greatly improve prediction of TF binding in celltype-, environment-, and developmental-stage-dependent contexts, as well as extending the use of the motif database to distal regulatory regions such as enhancers.

Conclusion
We show that the SalMotifDB is an effective tool for extracting information about transcription factor binding sites, transcription factors, and gene regulatory networks in salmonid species. This database is an important resource for future studies that aims to gain mechanistic understanding of regulation of transcription, and thereby salmonid evolution and physiology.

Additional files

Additional file 1: Figure S1. A radial tree displaying the similarity structure of a selected motif cluster. The inner node represent the cluster name (cluster-393), nodes in the second layer represent the motif databases, the third layer represent motif/TG names and the outer layer represent consensus motifs (not available for TRANSFAC). The central motifs of each motif database cluster are highlighted in red. (PDF 260 kb)

Additional file 2: Figure S2. Density plot of the lengths of the untranslated regions (UTRs) of genes in the salmonid genomes. (PDF 260 kb) (PDF 17 kb)
Additional file 3: Figure S3. SalMotifDB database schema for Atlantic salmon. (PDF 156 kb)

Additional file 4: Figure S4. Motif similarity (Jaccard index, y-axis) plotted against tissue expression correlation (Pearson correlation computed over 13 tissues) for 10,515 randomly selected ohnolog pairs. Quantile regression line are shown at 0.05, 0.25, 0.5, 0.75, and 0.95 and, in contrast to actual ohnologs, indicate no correlation. (PNG 207 kb)

Additional file 5: Table S1. Motifs in SalMotifDB that were significantly enriched (p < 0.05) in the promoters of genes in gene set 1: genes that are differentially regulated between Atlantic salmon that were given feed with contrasting lipid and fatty acid content in fresh water. (XLSX 35 kb)

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Authors’ contributions
S.R.S and T.R.H conceived of this study. T.D.M performed the data analysis. All authors discussed and interpreted results. All authors wrote, read and approved the final manuscript.

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Availability of data and materials
All data analysed in this study are publicly available through provided references. The code for the web tool and R package, as well as database resources were provided by the Norwegian National Infrastructure for Research Data (NIRD, project NS9055K). None of the funding bodies had any influence over study design, analysis and interpretation of the data, or in writing the manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Andreassen R, Worren MM, Hayheem B. Discovery and characterization of miRNA genes in Atlantic salmon (Salmo salar) by use of a deep sequencing approach. BMC Genomics. 2013;14:482. https://doi.org/10.1186/1471-2164-14-482.

2. Lien S, Koop BF, Sandve SR, Miller JR, Kent MP, Nome T, et al. The Atlantic salmon genome provides insights into re lipidization. Nature. 2016;533:200–5. https://doi.org/10.1038/nature17164.

3. Christensen KA, Rondeau EB, Minkey DR, Leong JS, Nugent CM, Danzmann RG, et al. The Arctic char (Salvelinus alpinus) genome and transcriptome assembly. PLoS One. 2018;13:e0204076. https://doi.org/10.1371/journal.pone.0204076.

4. Christensen KA, Leong JS, Sakhrani D, Biagi CA, Minkey DR, Wittler RE, et al. Chinook salmon (Oncorhynchus tshawytscha) genome and transcriptome. PLoS One. 2018;13:e0195461. https://doi.org/10.1371/journal.pone.0195461.

5. Bethelot C, Brunet F, Chalopin D, Juanchich A, Bernard M, Noël B, et al. The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. Nat Commun. 2014;5:3657. https://doi.org/10.1038/ncomms4657.

6. Boltfad GH, Hindar K, Robertsen G, Jonsson B, Sægrov H, Diserud OH, et al. Interplay of cis and trans mechanisms driving transcription factor binding across the salmonid genomes. S.R.S, T.R.H, T. N, D.I.V and D.J.M supervised the analysis. All authors discussed and interpreted results. All authors wrote, read and approved the final manuscript.

7. Czochralski Y, Akyanat K, Eriyel P, Primmer CR. Rapid sex-specific evolution of age at maturity is shaped by genetic architecture in Atlantic salmon. Nat Ecol Evol. 2017;1:124. https://doi.org/10.1038/s41559-017-01214.

8. Boistard GH, Hindar K, Robertsen G, Jonsson B, Sægrov H, Diserud OH, et al. Evolutionary dynamics of coding and non-coding transcriptomes. Nat Rev Genet. 2014;15:734–48. https://doi.org/10.1038/nrg3802.

9. Wong ES, Schmitt BM, Kazachenka A, Thybert D, Redmond A, Connor F, et al. Enhancer evolution across 20 mammalian species. Cell. 2015;160:554–66. https://doi.org/10.1016/j.cell.2015.01.006.

10. Sekulina A, Kaessmann H. Evolutionary dynamics of coding and non-coding transcriptomes. Nat Rev Genet. 2014;15:734–48. https://doi.org/10.1038/nrg3802.

11. Varadharajan S, Sandve SR, Gillard GB, Tørresen OK, Mulugeta TD, Hvidsten TR. Subfunctionalization versus individual motif occurrence. HOOCOMOCO: HOmologs Comprehensive MOfit Collection; HT-SELEX: High-Throughput Systematic Evolution of Ligands by Exponential enrichment; MySQL: My Structured Query Language; NCBI: National Center for Biotechnology Information; NF-Y: Nuclear transcription factor Y; PPAR: Peroxisome Proliferator-Activated Receptors; PSSM: Position Specific Scoring Matrices; RSAT: Regulatory Sequence Analysis Tool; SalMotifDB: Salmonid Motif DataBase; SMiLE-Seq: Selective Microfluidics-based Ligand Enrichment followed by Sequencing; SP: Specificity Protein; SREBP: Sterol Regulatory Element Binding Protein; TF: Transcription Factor; TRANSFAC: TRANScription FACTor database; UniIPROBE: Universal PBM Resource for Oligonucleotide-Binding Evaluation; UTR: UnTranslated Region.
19. Trizino M, Park Y, Hohbach-Beltrame M, Aracena K, Mika K, Calikan M, et al. Transposable elements are the primary source of novelty in primate gene regulation. Genome Res. 2017;27:1623–33. https://doi.org/10.1101/gr.218149.116.

20. Samy JKA, Mulugeta TD, Nome T, Sandve SR, Grammes F, Kent MP, et al. SalmiBase: an integrated molecular data resource for Salmonid species. BMC Genomics. 2017;18:482. https://doi.org/10.1186/s12864-017-3877-1.

21. Weiraua MT, Yang A, Albu M, Cote AG, Montenegro-Montero A, Dree P, et al. Determination and inference of eukaryotic transcription factor sequence specificity. Cell. 2014;158:1431–43. https://doi.org/10.1016/j.cell.2014.08.009.

22. Khan A, Fornez O, Stiglani A, Gheorghe M, Castro-Mondragon JA, van der Lee R, et al. JASPAR 2018: update of the open-access database of transcription factor binding profiles and its web framework. Nucleic Acids Res. 2018;46:D360–6. https://doi.org/10.1093/nkx126.

23. Contras-Moreira B. 3D-footprint: a database for the structural analysis of protein-DNA complexes. Nucleic Acids Res. 2011;38 Database issue:D91–7. https://doi.org/10.1093/nkx/jku081.

24. Hume MA, Barrera LA, Gisselbrecht SS, Bulyk ML. UniPROBE, update 2015: new tools and content for the online database of protein-binding microarray data on protein-DNA interactions. Nucleic Acids Res. 2015;43(Database issue):D117–22. https://doi.org/10.1093/nkx/jku1045.

25. Jolma A, Yan J, Whitington T, Toivonen J, Nitta KR, Rastas P, et al. DNA-binding specificities of human transcription factors. Cell. 2013;152:327–39. https://doi.org/10.1016/j.cell.2012.12.009.

26. Jolma A, Yin Y, Nitta KR, Dave K, Popov A, Taipale M, et al. DNA-dependent formation of transcription factor pairs alters their binding specificity. Nature. 2015;527:384–8. https://doi.org/10.1038/nkx/15518.

27. Yin Y, Morgunova E, Jolma A, Kašinien S, Duh B, Khoud-Sayed E, et al. Impact of cytosine methylation on DNA-binding specificities of human transcription factors. Science. 2017;356. https://doi.org/10.1126/science.aaq2239.

28. Isakov A, Groux R, Imbeault M, Rainer P, Alpern D, Dainese R, et al. SMiLE-seq identifies binding motifs of single and dimeric transcription factors. Nat Methods. 2017;14:316–22. https://doi.org/10.1038/nmeth.4143.

29. Sebastian A, Contras-Moreira B. footprintDB: a database of transcription factors with annotated cis elements and binding interfaces. Bioinformatics. 2014;30:258–65. https://doi.org/10.1093/bioinformatics/btt663.

30. Kulakovskiy IV, Vorontsova IE, Yevshin IS, Sharipov RN, Fedorova AD, Rumyansky EI, et al. HOCOMOCO: towards a complete collection of transcription factor binding models for human and mouse via large-scale ChIP-Seq analysis. Nucleic Acids Res. 2018;46:D252–9. https://doi.org/10.1093/nkx/jky1106.

31. Shazman S, Lee H, Socol Y, Mann RS, Honig B. OnTheFly: a database of Drosophila melanogaster transcription factors and their binding sites. Nucleic Acids Res. 2014;42(Database issue):D167–71. https://doi.org/10.1093/nkx/jkt1165.

32. Matys V, Kel-Margoulis OV, Frickel E, Liebich I, Land S, Barre-Dimier A, et al. TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. Nucleic Acids Res. 2006;34(Database issue):D108–10. https://doi.org/10.1093/nkx/jkt1143.

33. Nguyen NTT, Contras-Moreira B, Castro-Mondragon JA, Santana-Garcia W, Osseo R, Robles-Espinoza CD, et al. RSAT 2018: regulatory sequence analysis tools 20th anniversary. Nucleic Acids Res. 2018;46:W209–12. https://doi.org/10.1093/nkx/gkw1126.

34. Angiuoli SV, Salzberg SL. Mugsy: fast multiple alignment of closely related whole genomes. Bioinformatics. 2011;27:334–42. https://doi.org/10.1093/bioinformatics/btr2665.

35. Siepel A, Bejerano G, Pedersen JS, Hinrichs AS, Hou M, Rosenbloom K, et al. Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. Genome Res. 2005;15:1034–50. https://doi.org/10.1101/gr.371505.

36. Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, et al. CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. Nucleic Acids Res. 2017;45:D200–3. https://doi.org/10.1093/nkx/gkw1129.

37. Reed BD, Charos AE, Szekely AM, Weissman SM, Snyder M. Genome-wide occupancy of SREBP1 and its partners NPY and SP1 reveals novel functional roles and combinatorial regulation of distinct classes of genes. PLoS Genet. 2008;4:e1000133. https://doi.org/10.1101/gkx1106.

38. Carmona-Antonanzas G, Tocher DR, Martinez-Rubio L, Leaver MJ. Conservation of lipid metabolic gene transcriptional regulatory networks in fish and mammals. Gene. 2014;534:1–9. https://doi.org/10.1016/j. gene.2013.10.040.

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