
BIOGRAPHICAL SKETCH

NAME: **Francesco Ferrari**

POSITION TITLE: Tenured senior researcher ("Dirigente di ricerca") - National Research Council (CNR)

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Modena and Reggio Emilia, Modena, Italy	Ms	09/2004	Medical Biotechnologies
University of Modena and Reggio Emilia, Modena, Italy	PhD	02/2008	Biotechnology and Molecular Medicine
University of Padova, Padova, Italy	Postdoctoral research fellow	04/2010	Computational biology
Harvard Medical School, Boston, MA, USA	Postdoctoral research associate	11/2014	Computational biology

A. Personal Statement

I established my "computational genomics" research group as independent principal investigator (PI) at IFOM, in Milan, in January 2015. Since 2019 I am also a tenured CNR senior researcher. My undergraduate, PhD and postdoctoral research training covered both molecular and computational biology. I have extensive expertise in the fields of computational biology, functional genomics, epigenetics and transcription regulation, with a substantial track record of more than 60 publications, including 11 as first/co-first author and 11 as last author.

My research group name "computational genomics" reflects our area of technical expertise in computational biology and genomics, but our research interests and biological expertise are focused on chromatin three-dimensional (3D) architecture. The 3D organization of chromatin within the cell nucleus is crucial for regulating genome functionality. The knowledge of its role has greatly advanced over the few years thanks to the development of novel imaging, molecular biology, computational data analysis and modelling techniques. My research group is especially interested in understanding how chromatin 3D architecture is connected to epigenetic and transcriptional regulation of genome functionality, and how this regulatory axis ultimately affects cell identity definition and homeostasis in physiological and pathological conditions, with a special focus on cancer. We can distinguish different levels of 3D structural organization of chromatin, that correspond to different molecular mechanisms acting on chromatin and the genome. Thus, chromatin architecture is studied at different levels of resolution:

- ***On a large scale***, we study mechanisms for the coordinated control of large chromatin domains in physiological and disease conditions. These involve, among the others, the compartmentalization of the genome in distinct structural domains, such as Topologically Associating Domains (TADs), or Lamina Associated Domains (LADs), as well as the functional and biochemical distinction between heterochromatin and euchromatin regions. We investigate their functional and structural reorganization dynamics in cancer and genetic diseases.

- ***On a finer scale***, instead, we study how chromatin 3D folding comes into play to control the physical association of distal regulatory elements (enhancers) and their target genes. In this context we combine biological knowledge, experimental data and computational approaches to reconstruct the broader regulatory network encompassing genes and their non-coding regulatory elements (promoters and enhancers). We use this complex network or physical (in the 3D space) and regulatory (epigenetics and transcriptional) connections as a lens to understand and characterize the multivariate effect of epigenetic or genetic alterations in cancer and other diseases.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

Jan 2023 - present – IFOM-ETS, the AIRC Institute of Molecular Oncology, Milan, Italy
Tenured Principal Investigator, Computational Genomics Laboratory.

Jan 2020 – present – CNR (National Research Council), IGM (Institute of Molecular Genetics), Pavia, Italy
Tenured senior researcher position upgraded to (“Dirigente di ricerca”).

Nov 2019–Dec 2019 –CNR (National Research Council), IGM (Institute of Molecular Genetics), Pavia, Italy
Tenured senior researcher (“Primo ricercatore” - "first researcher").

Jan 2015 - Dec 2022 – IFOM-ETS, the AIRC Institute of Molecular Oncology, Milan, Italy
Tenure track Principal Investigator, Computational Genomics Laboratory.

May 2010 - November 2014 – Harvard Medical School, Boston MA, USA
Postdoctoral research associate at the Center for Biomedical Informatics.
Supervisor prof. Peter J. Park.

Jan 2008 - Apr 2010 – University of Padova, Italy
Postdoctoral research fellow.
Supervisors prof. Silvio Biciato and Stefania Bortoluzzi.

2005-2007 – University of Modena and Reggio Emilia, Italy
Graduate student in the Biotechnology and Molecular Medicine program.
Supervisor prof. Sergio Ferrari

Other Experiences and appointments

Sept-Oct 2009 – Leiden University Medical Center, Leiden, The Netherlands
Visiting postdoctoral fellow – HPC Europa transnational access grant.

Nov-Dec 2007 – Weizmann Institute of Science, Rehovot, Israel
Visiting graduate student – Sergio Lombroso program for cancer research fellowship.

HONORARY APPOINTMENTS (unpaid)

Jan 2018 - Nov 2024 – Adjunct Lecturer.
Center for Chromosome Biology, National University of Ireland Galway, Galway, Ireland

Mar 2017 - Nov 2019 – Associated researcher ("Associatura con incarico di ricerca")
National Research Council (CNR), Institute of Molecular Genetics, Pavia, Italy

Honors

FELLOWSHIPS

2008-2010 – Postdoctoral fellowship ("assegno di ricerca") of the University of Padova

2005-2007 – Doctoral degree fellowship of the University of Modena and Reggio Emilia

SHORT TERM FELLOWSHIPS

2009 - HPC Europe transnational access program grant.

2007 - Visiting PhD student Fellowship of Sergio Lombroso Foundation for Cancer Research

C. Contributions to Science

1) Heterochromatin 3D architecture dynamics through the lens of chromatin solubility.

Lamina Associated Domains (LADs) are compacted and silenced (not transcribed) heterochromatic domains mostly located at the nuclear periphery and anchored to the nuclear lamina. The association of heterochromatin to lamina poses a constraint to the possible 3D conformations of chromatin, contributes to nuclear stiffness, and to preserve the epigenetic silent status of these domains.

LADs are mostly stable across cell types and conserved across species. Their crucial role in cell homeostasis is also attested by laminopathies, a class of genetic diseases with mutations in lamina or lamina-associated proteins. Detachment of LADs from lamina leads to loss of epigenetic homeostasis, with alterations on Polycomb epigenetic regulation and reactivation of transposable elements transcription.

When this interaction is disrupted in pathological or physiological conditions, chromatin undergoes an extensive rearrangement, but chromosome conformation capture techniques are not able to detect major changes. To map rearrangements of heterochromatin domains across nuclear compartments we recently developed SAMMY-seq, a method based on the biochemical separation of multiple chromatin fractions depending on their solubility and accessibility, then sequenced to map their genomic position.

Thanks to this technique, in our recent work we reported for the first time that the dynamics of heterochromatin domains interacting with the nuclear lamina (LADs) may occur with a decoupling of the chromatin state (linear chromatin domain compaction due to heterochromatin) with respect to its biochemical properties of solubility and accessibility (modulated by the presence or loss of interaction with lamina) (Sebestyen et al., Nature Communications 2020). From a practical point of view this means that the disruption of LADs interaction with the nuclear lamina is not sufficient to trigger an immediate loss of heterochromatin domains, nor transcriptional derepression. These results were obtained by developing a novel genomics experimental technique named SAMMY-seq, for genome-wide mapping of heterochromatin reorganization in the nucleus (EU patent "EP3640330 A1"). In a further collaboration with Giorgio Scita (IFOM) we showed that these observations apply also to cellular models of cell motility in metastases (Frittoli et al., Nature Materials 2023). We have now published a further development of the technique (Lucini et al Nucleic Acids Research 2024) named 4f-SAMMY-seq. We then applied 4f-SAMMY-seq on prostate cancer biopsies taken at the time of diagnosis, where we 1) stratify patients based on their chromatin compartments changes; 2) identify transcriptional changes in EMT and ECM remodeling pathways; 3) validate our signature as predictive of biochemical recurrence (i.e. PSA positivity after surgery, indicative of metastatic tumors) on more than 900 samples across 4 cohorts and we applied it to the stratification of prostate cancer patient biopsies (Rosti et al., Nature Communications 2025 - Accepted IN PRESS).

2) Dysregulation of the enhancer-gene regulatory network

On a fine scale, chromatin achieves a more local level of 3D organization with the formation of loops, such as those bringing in close physical proximity regulatory elements. The textbook example of this structure is the loop allowing the interaction between distal non-coding regulatory elements (enhancers) and their target gene promoters, which are fundamental for cell-type specific regulation of transcription. Enhancers are non-coding regulatory regions modulating the transcription of target genes that can be distant along the genomic sequence. Enhancers notably harbor several risk associated variants (from GWAS studies) for cancers or other diseases. They exert their action at the level of an extended regulatory network encompassing enhancers and target genes (ETG). Multiple examples in literature support the potential role of genetic or epigenetic disruptions in the ETG network in pathological mechanisms. Tumors and genetic conditions may arise from the alteration of chromatin remodeling complexes which operate by modulating the epigenetic status of non-coding regulatory elements. However, the identification and characterization of somatic mutations in non-coding regulatory regions with a functional effect on tumorigenesis and prognosis remain a major challenge.

In this context we recently proposed a change of perspective on the identification of physical regulatory interactions between enhancers and target genes. Namely, building on the technical insights coming from our earlier works and on the consensus emerging from literature on the role of chromatin loops extrusion in the formation of insulated topological domains, we proposed a new statistical and computational framework for the genome-wide reconstruction of enhancer-target genes (ETG) regulatory interactions (Salviato et al., Nucleic Acids Res 2021). In this work we showed that the dynamic and hierarchical structure of topological domains formation defines the likelihood of cell-type specific enhancers interaction with their target genes.

We then applied this framework for detecting and characterizing enhancer mutations in a genome-wide analysis of patient cohorts covering three lung cancer subtypes. We then used this network to interpret the downstream impact of enhancer somatic mutations. We observed that mutated enhancers recurrently converge

on the regulation of key biological processes and pathways relevant to tumor biology (Hariprakash *et al.*, Cancer Research 2024).

3) Chromatin 3D architecture dynamics in response to epigenetic regulation of transcription

A crucial open question in the field is how can we reconcile the large-scale organization of chromatin into structural domains (TADs) and compartments with the fine-scale molecular dynamics of transcription. Clarifying the mechanisms of the interplay between molecular motors acting on the DNA, especially the transcription and replication machineries, and the structural organization of chromatin would be crucial to clarify mutagenic and DNA damage processes connected to this interplay.

In this context we recently demonstrated for the first time that coordinated transcriptional up-regulation over a very large genomic region (the entire chrX in male *Drosophila*) changes its global 3D architecture in terms of increased long-range interactions and local disturbances of TADs insulation (Pal *et al.*, Nature Communications 2019). Our work addressed fundamental biology questions with implications in several physiological and pathological processes. Progress in this field has been driven by methodological improvements. Indeed, our work relied on the development of innovative data analysis procedures that allowed addressing such non-standard biological questions. We also used polymer folding modelling techniques to rule out the confounding effects of Dipteran homologous chromosomes pairing.

On this same biological model, during my work in my previous research positions, in collaboration with Mitzi Kuroda's group (Harvard Medical School) I investigated the epigenetic regulation of transcription for compensating copy number differences between sex chromosomes in *Drosophila melanogaster*. Dosage compensation is an interesting model of coordinated epigenetic regulation of an entire chromosome. Different species have evolved distinct mechanisms, yet all are based on various level of epigenetic regulation, eventually resulting in differential transcriptional activity of sex chromosomes genes, as reviewed in (Ferrari *et al.*, Nat Struct Mol Biol 2014). In *D. melanogaster* the single copy of X in male is up-regulated, yet it has been debated which stages of the RNA Polymerase II (Pol II) transcription cycle are involved affected in the boost of gene expression associated to dosage compensation: Pol II recruitment, initiation, pausing release or elongation. Indeed, we challenged a previously proposed model suggesting that the two-fold up-regulation of chromosome X is achieved at the initiation stage (Ferrari*, Jung* *et al.*, Science 2013). In our work we combined transcription dynamics data obtained from several genome-wide techniques, to gather information on distinct stages of transcription (Ferrari*, Plachetka*, Alekseyenko* *et al.*, Cell Reports 2013).

4) Computational methods to study chromatin 3D organization

As a computational biology group, a part of our research activity is devoted to the development of methods for genomics data analysis. In particular, we are expert in bioinformatics solutions for the analysis of data from a variety of high-throughput sequencing-based techniques for the study of chromatin architecture.

In this context, genome-wide chromosome conformation capture (Hi-C) is a molecular biology technique to measure the frequency of physical interactions between any pair of genomic loci, which has been widely adopted to study chromatin 3D architecture in several experimental models and conditions.

We contributed to this field with publications about methods for data analysis (Forcato *et al.*, Nature Methods 2017; Pal *et al.*, Biophysical Reviews 2019), as well as with novel algorithms. Among the currently open challenges in this field, we worked on solutions to handle and analyze Hi-C data at high resolution (Pal and Ferrari, Methods in Molecular Biology 2022; Pal *et al.*, Bioinformatics 2019).

5) Chromatin architecture, epigenetics and transcriptional regulation of pluripotency and disease

As part of my past work, in collaboration with Konrad Hochedlinger's group (MGH and Harvard Stem Cell Institute), I studied the 3D-organization of the genome around a key pluripotency gene (Nanog) during reprogramming of somatic cells to induced pluripotent stem cells (Apostolou*, Ferrari* *et al.*, Cell Stem Cell 2013). We used a modified 4C (Circular Chromosome Conformation Capture) protocol coupled with deep sequencing (m4C-seq) to identify genome-wide chromatin interactions of the Nanog locus. We identified a pluripotency-specific interactome for Nanog, which is lost upon differentiation, and re-established during reprogramming. We also found that the genome architecture reorganization precedes gene expression changes associated to pluripotency, thus being pivotal for cell identity change. Our work was one of the first demonstrating that the re-organization of chromatin is a key molecular event associated to cell identity transition, as later confirmed also by others (Ferrari*, Apostolou* *et al.*, Cell Cycle 2014).

Connected to this line of research, in collaboration with George Daley's group (Harvard Medical School), I defined computational genomics approaches to verify the origin of pluripotent stem cells (De Los Angeles*, Ferrari* *et al.*, Nature, 2015). This addressed a relevant problem in the field of stem cells research as a number of controversial claims have arisen during the years. I collaborated also with Marcy MacDonald's lab (MGH

and Harvard Medical School) for the characterization of epigenetics alterations associated to Huntington disease (Biagioli*, Ferrari* et al., Human Molecular Genetics 2015).

Relevant publications (selected)

I authored more than 60 publications, including 11 as first/co-first author and 12 as last/co-last author. Full list on ORCID <https://orcid.org/0000-0002-9811-3753>

- 1) Rosti V^{§,‡}, Lembo G[‡], Petrini C[‡], Gorini F, Quadri R, Cordiglieri C, Mutarelli M, Salviato E, Casari E, Di Patrizio Soldateschi E, Montanari E, Albo G, Ripa F, Fasciani A, Crosti M, De Lorenzis E, Maggioni M, Vaira V, Vivo M, **Ferrari F**^{*,§}, Lanzaolo C^{*,§}. Chromatin remodeling restrains oncogenic functions in prostate cancer. *Nature Communications* 2025 - Accepted IN PRESS ([‡] co-first authors, [§] co-corresponding authors, * co-last authors)
- 2) Lucini F, Petrini C, Salviato E, Pal K, Rosti V, Gorini F, Santarelli P, Quadri R, Lembo G, Graziano G, Di Patrizio Soldateschi E, Tagliaferri I, Pinatel E, Sebestyén E, Rotta L, Gentile F, Vaira V, Lanzaolo C*, **Ferrari F***. Biochemical properties of chromatin domains define genome compartmentalization. *Nucleic Acids Res.* 2024 May 29 (* co-corresponding/co-last authors)
- 3) Hariprakash JM, Salviato E, La Mastra F, Sebestyén E, Tagliaferri I, Silva RS, Lucini F, Farina L, Cinquanta M, Rancati I, Riboni M, Minardi SP, Roz L, Gorini F, Lanzaolo C, Casola S, **Ferrari F**. Leveraging tissue-specific enhancer-target gene regulatory networks identifies enhancer somatic mutations that functionally impact lung cancer. *Cancer Res.* 2024 Jan 2;84(1):133-153.
- 4) Salviato E, Djordjilović V, Hariprakash JM, Tagliaferri I, Pal K, **Ferrari F**. Leveraging three-dimensional chromatin architecture for effective reconstruction of enhancer-target gene regulatory interactions. *Nucleic Acids Research* 2021 Sep 27;49(17):e97.
- 5) Sebestyén E, Marullo F, Lucini F, Petrini C, Bianchi A, Valsoni S, Olivieri I, Antonelli L, Gregoret F, Oliva G, **Ferrari F***, Lanzaolo C*. SAMMY-seq reveals early alteration of heterochromatin and deregulation of bivalent genes in Hutchinson-Gilford Progeria Syndrome. *Nature Communications* 2020 Dec 8;11(1):6274. (* co-corresponding/co-last authors)
- 6) Pal K, Forcato M, Jost D, Sexton T, Vaillant C, Salviato E, Mazza EMC, Lugli E, Cavalli G, **Ferrari F**. Global chromatin conformation differences in the *Drosophila* dosage compensated chromosome X. *Nature Communications* 2019 Nov 25;10(1):5355.
- 7) Pal K, Tagliaferri I, Livi CM, **Ferrari F**. HiCBricks: building blocks for efficient handling of large Hi-C datasets. *Bioinformatics.* 2019 Nov 7. pii: btz808.
- 8) Forcato M, Nicoletti C, Pal K, Livi CM, **Ferrari F***, Bicciato S*. Comparison of computational methods for the analysis of Hi-C data. *Nature Methods*, 2017 Jul; 14(7):679-685. (* co-corresponding/co-last authors)
- 9) De Los Angeles A*, **Ferrari F***, Fujiwara Y, Mathieu R, Lee S, Lee S, Tu H, Ross S, Chou S, Nguyen M, Wu Z, Theunissen TW, Powell BE, Imsoonthornruksa S, Chen J, Borkent M, Krupalnik V, Lujan E, Wernig M, Hanna JH, Hochedlinger K, Pei D, Jaenisch R, Deng H, Orkin SH, Park PJ, Daley GQ. Failure to Replicate the STAP Cell Phenomenon. *Nature*, 2015 Sep 24;525(7570):E6-9. (* equal contribution)
- 10) Biagioli M*, **Ferrari F***, Mendenhall EM, Zhang Y, Erdin S, Vijayvargia R, Vallabh SM, Solomos N, Manavalan P, Ragavendran A, Oszlak F, Lee JM, Talkowski ME, Gusella JF, MacDonald ME, Park PJ, Seong IS. Htt CAG repeat expansion confers pleiotropic gains of mutant huntingtin function in chromatin regulation. *Hum Mol Genet.*, 2015 May 1;24(9):2442-57. Epub 2015 Jan 8. (* equal contribution)
- 11) Apostolou E*, **Ferrari F***, Walsh RM, Bar-Nur O, Stadtfeld M, Cheloufi S, Stuart HT, Polo JM, Ohsumi TK, Borowsky ML, Kharchenko PV, Park PJ, Hochedlinger K. Genome-wide interactions of the *Nanog* locus in pluripotency, differentiation and cellular reprogramming. *Cell Stem Cell*, 2013 Jun 6;12(6):699-712. (* equal contribution)