The three-dimensional genome conformation of *Mycoplasma pneumoniae*

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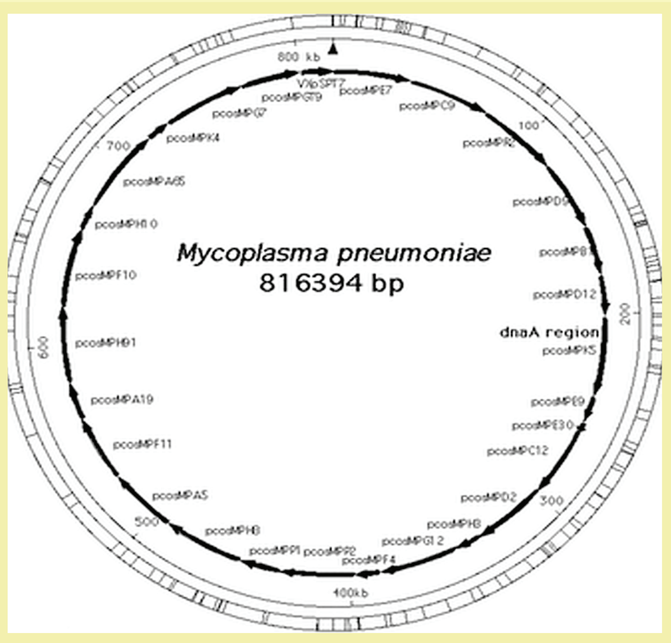
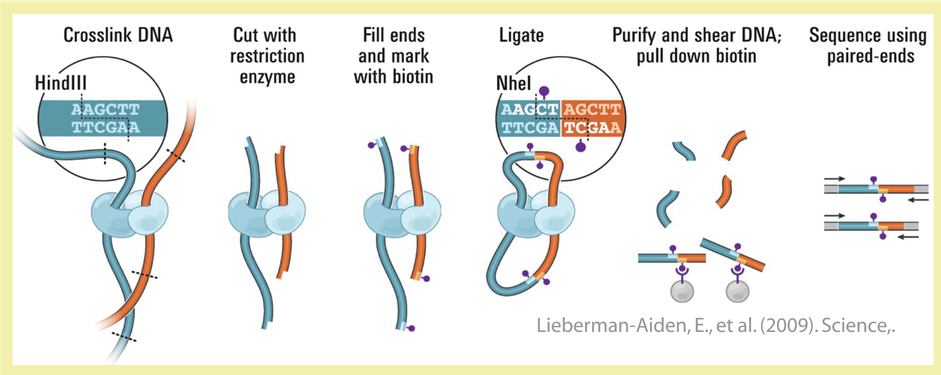
Abstract : A recent study, involving a genome-reduced bacterium, ***Mycoplasma pneumoniae***, one of the smallest self-replicating organism known to date, has revealed impressive transcriptome complexity[1]. Using the **Hi-C method**, which enables purification of ligation products followed by massively parallel sequencing, we are seeking to build the **three-dimensional (3D) architecture of the genome** of the *Mycoplasma pneumoniae* at a 8Kb resolution. Direct analysis of this genome-wide library of long-range interactions revealed numerous features of genomic conformation that change from exponential to stationary phases of growth. The resulting chromatin structure will help identify sequence elements that affect **supercoiling** and understand complex **transcriptional regulation** in this organism.

Sequencing a library of long-range interactings fragments

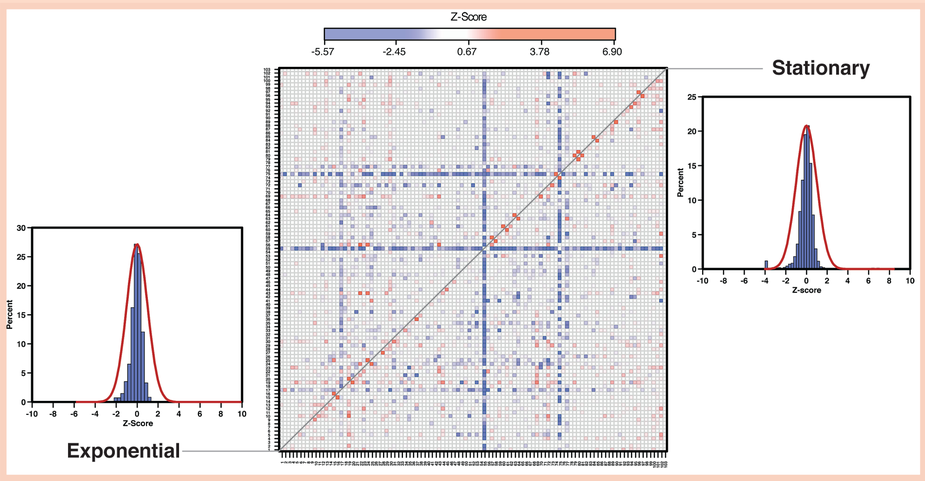




HiC : high-throughput method

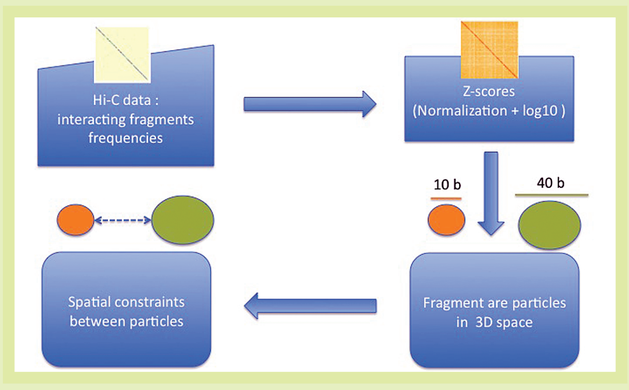
All cells are cross-linked with formaldehyde in order to link spatially nearby chromatin fragments that are then digested by **restrictions enzymes**. The resulting fragments ends are filled in with biotin before ligation. After DNA purification and shearing, the junctions are pulled down using streptavidin. This results in a **genome-wide library of ligation** products corresponding to pairs of DNA fragments that were **originally in close spatially proximity**.

Chromatin structure is more condensed in Stationnary phase than in Exponential phase



The mapped fragments are then filtered and normalized according to the restriction enzyme positions in order to build a **matrix of interactions**. Chromatin interactions can then be visualized as heat-maps constructed from the matrices of interactions, where the axis represent the **genome divided in bins**, and each pixel represents the number of observed interactions between them. Typically DNA regions that are very close to each other in the genome will have the tendency to interact frequently with each other[2].
To interrogate the mycoplasma pneumoniae genome at high density, we considerd 4,750,866 interactions in exponential phase and 11,456,360 interactions in stationnary phase enabling the measurement of contact frequencies with a resolution of **8,000 bp per bin**.

Modeling the 3D structure of whole genome



To generate the corresponding models, we will use the **Integrative Modeling Platform**[3] (IMP,) , inferring the 3D conformation of the Mycoplasma pneumoniae genome from the maps of interactions. The contact frequencies will be used to infer the 3D spatial distances.
Each bin from the map will represent a point, as a genomic region in the space, and each region will be **connected spatially according to its contact frequencies.**

Bibliography

1. Güell, M., et al. (2009). *Transcriptome complexity in a genome-reduced bacterium*. Science, 326(5957), 1268–1271

2. Lieberman-Aiden, E., et al. (2009). *Comprehensive mapping of long-range interactions reveals folding principles of the human genome*. Science, 326(5950), 289–293

3. Baù, D., et al. (2011). *The three-dimensional folding of the α-globin gene domain reveals formation of chromatin globules*. Nature Structural & Molecular Biology, 18(1), 107–114

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