Life Sciences Outreach Faculty Speaker Series for High School Biology Teachers
How Biologists View Structure and Function
Fall 2018

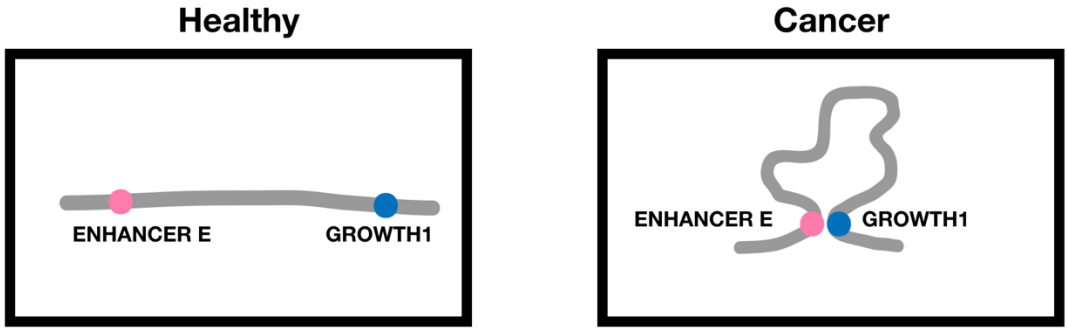
Worksheet on FISH (Fluorescence in situ Hybridization) and Chromosome Conformation Capture (e.g. 3C and Hi-C) Methods

Background: FISH and 3C-based technologies are used to study 3D genome organization. Through microscopy, FISH enables the visualization of where specific regions of the genome are located within the nucleus through the use of fluorescent probes that hybridize to a DNA sequence of interest. 3C-based technologies provide pairwise cross-linking frequencies detailing how often there are interactions between two disparate regions of DNA.

Case study: After performing gene expression profiling of patient A who has an extremely rare and aggressive form of melanoma, clinicians at Boston Research Hospital have found that a key gene GROWTH1 involved in cell proliferation is significantly upregulated. Researchers hypothesize that GROWTH1 might be driving tumor expansion and decide to investigate the molecular mechanism that leads to the upregulation of that gene.

The clinicians perform chromosome conformation capture (Hi-C) analysis and suspect that an enhancer E, which is known to drive the expression of very active genes, interacts with the promoter of GROWTH1 in the cancer cells but not the healthy cells.

1) Using the two Hi-C maps below, let's hypothesize how the folding of DNA near GROWTH1 is different in healthy individuals and individuals with this particular cancer. Draw one hypothetical cancer-causing DNA conformation, and label Enhancer E and GROWTH1 as was done for the healthy conformation. Do the drawings for the healthy cancer samples support the hypothesis of the clinicians above?

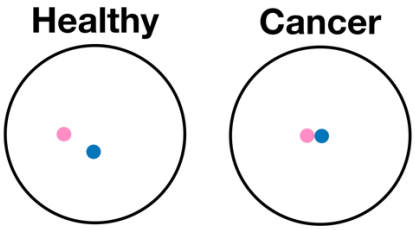


Yes, this is consistent with their hypothesis. In the heatmap for healthy cells the interaction frequency between GROWTH1 and ENHANCER E isn't above the background. By contrast, in the cancer cells, there appear to be a higher interaction frequency between these two regions, which is consistent with their hypothesis that the two regions physically interact.

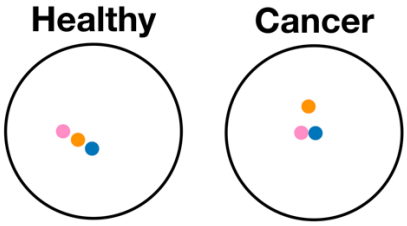
2) Look at the sequence below. The proposed regions of interaction are **ENHANCER E** and **GROWTH1** and are each highlighted in their respective colors. If one designed probes to image these regions through FISH, what would a plausible imaging result look like for

the Hi-C maps in question 1? Draw a diagram of the nucleus and the resulting FISH image.

AGGAGTCCGAAACTTTTATTAAAGGAAAGGAGAGAAAGACTTACCAGAGACGCCGGGAAGGG
GGCCAGGAAGAAGATGCCTGCCACTT**ACCCCAGAACCAGACGGATGGGGGTGAGG**TGGTCCA
GGATGTCAACAGCAGTGTACAGATGGTGATGATGGAACAGCTGGACCCCACCCTTCTTCAGAT
GAAGACTGAAGTAATGGAGGGCACAGTGGCTCCAGAAGCAGAGGCTGCTGTGGACGATACCC
AGATTATAACTTTACAGGTTGTAAATATGGAGGAACAGCCCATAAACATAGGAGAACTTCAGCT
TGTTCAAGTACCTGTTCCTGTGACTGTACCTGTTGCTACCACTTCAGTAGAAGAACTTCAGGG**GG**
CTTATGAAAATGAAGTGTCTAAAGAGGGCCTTGCGGAAAGTGAACCCATGATATGCCACACCC
TACCTTTGCCTGAAGGGTTTCAGGTGGTTAAAGTGGGGGCCAATGGAGAGGTGGAGACACTAG
AACAAGGGGAACTTCCACCCCAGGAAGATCCTAGTTGGCAAAAAGACCCAGACTATCAGCCAC
CAGCCAAAAAAACAAAGAAAACCAAAAAGAGCAAACTGCGTTATACAGAGGA

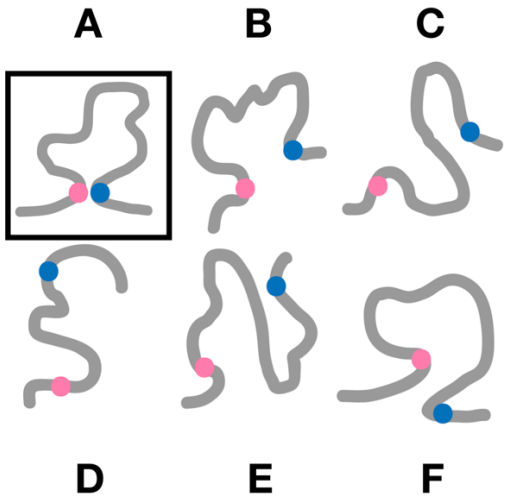


3) Refer back to your hypothetical structure in Q1. If you designed a third FISH probe, **MIDDLE**, (in addition to the ones labeling Enhancer E and GROWTH1), in between Enhancer E and GROWTH1, what would a plausible imaging result look like for the Hi-C maps in question 1. Add an orange dot to the diagram of the nucleus from Q2. How do the relative distances between the 3 probes vary in the healthy vs cancer conditions?



*In the healthy cells **MIDDLE** is closer to the **ENHANCER E** and **GROWTH1** than **ENHANCER E** and **GROWTH1** are from each other. In the cancer cells **ENHANCER E** and **GROWTH1** are closer to each other than they are to **MIDDLE.***

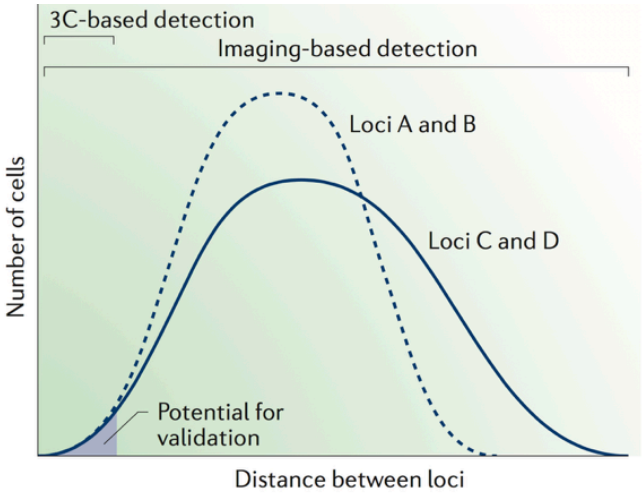
4) Which hypothetical conformations would it be possible to detect with FISH? How about 3C-based methods? Explain your reasoning. (Hint: 3C-based methods are only able to detect interactions close together.)



Fudenberg, Geoffrey, and Maxim Imakaev. "FISH-ing for captured contacts: towards reconciling FISH and 3C." *Nature methods* 14.7 (2017): 673.

5) In light of question 5, provide a potential explanation for why FISH and 3C-based measurements can often yield contradictory results. Explain what possible clinical implications these contradictory results could have?

They can yield different results because they measure different aspects of chromatin structure. If one were to calculate the average distance between the loci using FISH, one would average over all conformations. However, for 3C measurements, one would only be able to consider a small subset of the possible conformations, which would lead one to have a different picture of chromatin structure. The discrepancies between the two techniques is illustrated in the following figure where two regions could yield the same 3C signal but exhibit different average separation distances. In terms of clinical implications, one might falsely assume based off of a Hi-C map that two loci are drastically differentially located in cancer cells relative to healthy cells, when in reality these changes are driven by a couple of outliers.



Dekker, Job. "Mapping the 3D genome: aiming for consilience." *Nature Reviews Molecular Cell Biology* 17.12 (2016): 741.