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

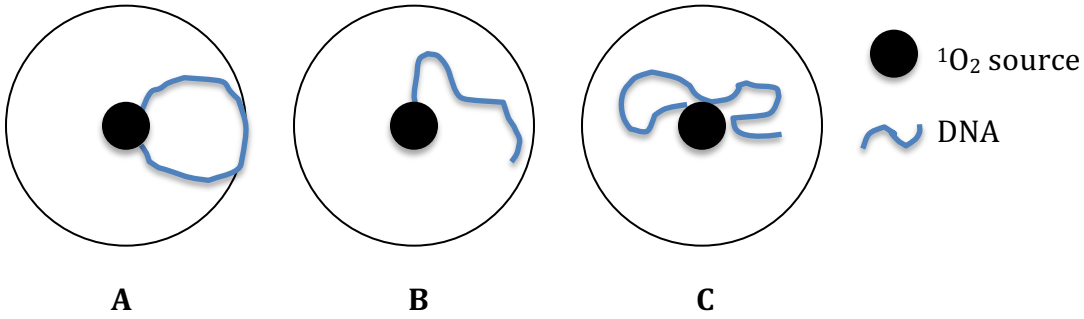
LOOP: Distance Measurements in the Genome: Answer Key

LOOP (Localized $^1O_2$ Oxidative Profiling) aims to study genome structure by using proximity-directed chemistry to "measure" the distance of genomic regions from proteins in the nucleus. Here, we will explore the principle behind LOOP and construct our own LOOP map.

Part 1) Mutation Rate Translates to Information about 3D Structure

The following is a short sequence of DNA that has been mutated by singlet oxygen ($^1O_2$), where regions closer to the singlet oxygen should be mutated more frequently than regions further away. Stars represent the sites of mutations, while B represents a normal nucleobase. Which map(s) could correspond to this mutation pattern?

BB**B*BBB*B*BBBB*BBBBBBBBBBBBBBBBBBBBB*BBBB*B*BB**BB*B*B***



Map A corresponds to this mutation pattern, because the strand has many mutations on the ends, but increasingly few as you reach the middle of the strand. This suggests a looping structure in which the two ends of the loop are closest to the O_2 source, which the middle of the loop is further from the mutagen source.

Part 2) Identifying Mutations and Inferring Distance

Loop relies on the fact that $^1O_2$ causes G nucleobases to mutate to T nucleobases upon amplification by many common DNA polymerases. The number of mutations corresponds directly to the distance of a DNA region from the singlet oxygen source (photosensitizer).

You want to use LOOP to understand how the genome of the melanoma cell line (the same melanoma as Patient A has) is organized. You decide to start by looking at which regions of the genome are close to the nuclear lamina. The lamina lines the periphery of the nuclear membrane, and DNA associated with the lamina is usually repressed.

The following is a sequence of DNA from a melanoma cell line with the same mutation as Patient A. The strands are all connected as one would read a paragraph.

CTTTTATGTCACAGTCATCTGTCATAGAGTCTTTCTTCTATCCAATTATAATTTTGAGTATTTT
GTAGAATGTCTGTGCAAGTTGCTGTGAAATACAAAAGTATAGAACAAGCTTTCTGATAATGCAT
ATCTTAGTCTCCTGGGGTTCCCAGAAAGTTAGCAGCTTACTTGTGTAACA
ACTGGAAAACATTGGAAAGCTTTTTTCAATAAAGTACAATTCTAAATGCTGTGCAGTTGGTCTA
CGAGCTGGAAAAAACTAAAAGTATTCTTTTTAAAAAAAAAACAAAACTAGAGGCT
GGTGTGGTGGCTCACACTTGTAATCCAGTACTTTGGGAGGCCCAAGCAGGAAAACCACTT
GAGGCCAGGAGTTTGAGACCAGCTTGGGCAGTAAATATAGTAA

Following irradiation of $^1O_2$ photosensitizers bound to the nuclear lamina, the DNA is removed, amplified by PCR, and sequenced. The following linear sequence is read out:

CTTTTATGTCACAGTCATCTGTCATAGAGTCTTTCTTCTATCCAATTATAATTTTGAGTATTTT
TTA**T**AATGTCT**T**TGCAA**T**TTGCT**T**TGAAATACAAAAGTATA**T**AACAAGCTTTCT**T**ATAATGCAT
ATCTTAGTCTCCTG**T**GGTTCCCAGAAAGTTAGCAGCTTACTT**T**TGTAACA
ACTGGAAAACATTGGAAAGCTTTTTTCAATAAAGTACAATTCTAAATGCTGTGCAGTTGGTCTA
C**T**A**T**CTGGAAAAAACTAAAA**T**TATTCTTTTTAAAAAAAAAACAAAACTA**T**A**T**GCT
TTTGT**TT**TG**T**CTCACACTT**T**TAATCCA**T**TACTTT**TT**GA**TT**CCCAA**T**CA**TT**AAAACCACTT
TAGGCCAGGAGTTT**T**AGACCAGCTTGG**T**CAGTAAATATA**T**TAA

How many G to T mutations are there on each line? Each mutated G→T is highlighted in red. What does this say about the distance of each line from the lamina?

Line 1:0/7 0%
Line 2:7/13 54%
Line 3:2/12 17%
Line 4:0/10 0%
Line 5:5/9 56%
Line 6:14/17 82%
Line 7:4/14 29%

The more mutations in a region (line), the closer that region is to the lamina. Thus, regions represented by lines 2, 5, and 6 are closest to the lamina because they have the most mutations.

Part 3) Constructing a LOOP map

Using the information from Part 2, take the pipe cleaner DNA strands and the hoop provided to construct a **possible** map of this DNA sequence in the nucleus. Remember that the source of singlet oxygen is located at the edge of the nucleus (the hoop). Each color pipe cleaner corresponds to a line of DNA from above.

You should use the number of mutations in each line to create a "map" using hoops and pipe cleaners, with more mutated regions closer to the lamina, and less mutated regions further inside. An important thing to note here is that there are many possibilities!

Part 4) Reflection

What are the strengths of this approach? What are the weaknesses?

How do other tools to study the 3D genome complement the information acquired from this method?

If you wanted to study the interaction of GROWTH1 and LOOP3 in the nucleus, would you use LOOP?

Strengths of LOOP include the following:
1. It is genome-wide and does not require super high sequencing depth.
2. It allows you to find distance-based measurements at a high-throughput scale, which you cannot otherwise do.
3. It could be interfaced with Hi-C or other exisiting technologies.

Weaknesses:
1. No primary samples. You need a knockin cell line. This also means you introduce a perturbation.
2. There are many possibilities when it comes to distance, so we will want to interface it with other technologies to build more reliable genome maps.