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

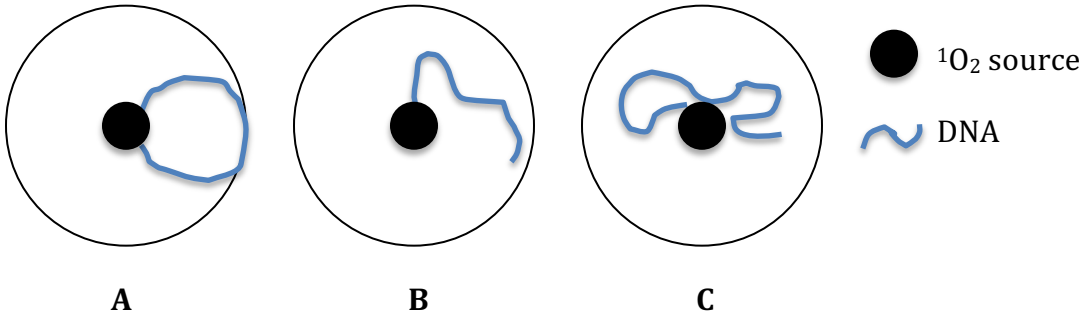
LOOP: Distance Measurements in the Genome

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***



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?

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.

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?