BMB 174 2017
Problem Set: LNC RNA
Due: May 18, 2017 in class
100 points total

1. *Engreitz et al.* (2013) showed that the Xist lncRNA mediates X chromosome inactivation (XCI) by spreading across the chromosome in a fashion that utilizes the three-dimensional architecture of the chromosome. (40 points)

a. The authors developed a new method (RAP) to examine Xist's role in XCI. 12 points

i. Describe in your own words how RAP works. 6 points

ii. Describe the controls the authors used in Figure 1B and 1D. 6 points

b. Review a bit about what XCI is. 10 points

i. Why would the authors see Xist spread across the entire female X chromosome? 5 points

ii. What would you see in the male X? Which panel in the paper's figures would it look like? 5 points

c. The authors came up with two possible models for Xist propagation across the genome. 12 points

i. How did the authors test out the suggested models in Figure 3D? 4 points

ii. The authors explored whether these early sites had higher affinity (affinity transfer) for Xist, or were enriched because of the proximity to the Xist locus (proximity transfer) by data from the Hi-C technique.

1. Describe how Hi-C works and how it shows certain strands are close to each other. 4 points

2. How did the authors utilize the Xist transgene to further assess the idea of proximity transfer? 4 points

d. Comment on the general significance of this work. What does it mean for future studies? How could it be used for future investigations? (This is an open-ended question. Just meant explore future directions of new techniques.) 6 points.

2. *McHugh et al.* (2015) developed a new method to investigate the proteins involve in Xist-mediated X-chromosome inactivation. 40 points.

a. In this paper the authors used a modified RAP they call RAP-MS. 12 points.

i. What is different about the probes used in this paper versus the probes used in the previous paper? 4 points.

ii. Why did they make this change? 4 points.

iii. How did the authors distinguish between specific and background proteins? 4 points.

b. SHARP, LBR, and SAF-A are required for Xist-mediated gene silencing. 12 points.

i. If the authors were to somehow image the same cells in Figure 2 after getting rid of the siRNA for the siSHARP panel, what would you expect the images to look like? Why? 6 points.

ii. XCI has two main phases: maintenance and initiation. Why wouldn't maintenance-involved proteins show up in the Figure 2 assays? 6 points.

c. The authors observed that SHARP was required for PolII exclusion from Xist-coated territory. 8 points.

i. List the positive and negative controls in Figure 3b. 2 points.

ii. Why did the authors keep doing parallel experiments with SHARP and HDAC3? 3 points.

iii. If SHARP affects HDAC3 as the authors conclude, what would you expect to happen to the deacetylase activity of HDAC3 in cells with siSHARP? 3 points.

d. The authors also investigate the role of Xist and SHARP in recruiting PRC2. 8 points.

i. What feature of RAP-MS prevented the authors from identifying proteins that interact with SHARP? 3 point.

ii. How did the authors investigate whether SHARP was involved in PRC2 recruitment? 4 points.

3. Guttman et al. (2009) 20 points

i. Briefly discuss what are the problems when analyzing the repertoire of non-coding transcripts, and specifically lncRNA? 6 points

ii. Based on which property of non-coding transcripts did the authors believe it is functional? 4 points

iii. To find out functional lncRNA sequences, what is the method authors used to find out lncRNA sequences that are most likely to be functional? what is the molecular mechanism upon which this method is based? what is its relationship with chromatin state? 10 points