**Monday March 2nd in-class “assignment”: the mystery of the *lacZ* transgene**

Assigned paper: Lonfat et al. (2013). Transgene- and locus-dependent imprinting reveals

allele-specific chromosome conformations. *PNAS* 110(29): 11947-51

You are welcome to work on this set of questions collaboratively or individually, and to request help as/if needed. You will hand in your answers at the end of class (one set of answers per group if you work collaboratively).

Before you start: spend about 4-5 minutes comparing your answers to the “background questions” within your group.

Specific questions about the article

1. Recall the general rule, “Figure 1 is often the most important figure in the paper”. Referring to Figure 1:
2. What transgenic lines did the author use (do these lines look somewhat familiar)?

They inserted Hoxd9 linked with lacZ

1. What do the data show (Panel C)?

-whole-mount staining of beta-gal activities of embryos heterozygous for the transgene before (HoxDrel5) and after the inversion (HoxDInv(rel5-Itga6).

- when the transgene is not inverted, the beta-gal activities staining is similar regardless of whether the transgene is inherited from mother or father. √

- Embryos with inverted transgene inherited from father also show betal-gal expression as the “non-inverted” embryos. But the inverted- transgene inherited from mother did not show beta-gal activities

1. What is striking/unexpected about the results, and why?

* Paternal inherited inverted-transgene embryos expressed heavier betal-gal expression because it is higher expression level than without inversion.
* Maternal inherited inverted-gransgene embryos expressed little to no beta-gal expression, this is suprsing because the paternal inherited inverted transgene embryos have much higher expression. √

1. What conclusions do you make from the data?

Depending on which parent donates the inverted-transgene, the beta-gal expression will be different. For maternal inhierted: there is no beta-gal expression ad for paternal inherited: there is high beta-gal expression. √

1. How did the authors show that the observed imprinting is *lacZ*-specific and position/site-specific? Do you agree with their data interpretation and with their conclusion?

-insert inverted transgene in many places that are upstream or down stream of Hga6. There was no imprinting, only found imprinting at Itga6 locus

* they remove lacZ from Hoxd/lacZ found through RT-QPCR insitu hybridization that there was no imprinting when there is no lacZ. This showed lacZ is required for imprinting at Itga6 site
* Replaced Hoxd9/lacZ insert with Hoxd11/lacZ and found there is imprinting in inverted transgene embryos.
* Yes these experiments showed that the imprinting is specific to lacZ, site specific, and imprinting is not due to Hoxd9 √

1. Refer to Figure 2.
2. Briefly explain how to “read” the diagrams shown (i.e. what do the rows of circles represent, what do the white vs. black circles represent).

The black dots represent methylated bases and the white circles represent The more black, the more methylation of the given sequence.

1. What do the data in Figure 2 show?
2. Shows the degree of methylation in parental methylatable cytosine nucleotides in the hoxd9/lacz reporter gene. The paternal data shows a lack of methylation and the maternal data shows high methylation of the transgene.
3. This figure shows the degree of methylation of “escapers”(which are from individuals with lesser degrees of methylation of the transgene) The higher degree of methylation implies a higher degree of imprinting. A ‘strong’ escaper has less methylation.
4. This figure shows that sperm (paternal) has less methylation on their transgene than oocytes (maternal)
5. This figure shows the degree of methylation with noninverted transgenic individuals. There is low methylation in both maternal and paternal transgenes
6. Why aren’t there a “paternal/+” and a “maternal/+” groups for sperm and oocytes?

Sperm is always paternal, oocytes are always maternal

1. What are “escaper” embryos, and how were they identified prior to bisulphite sequencing?

Escapers are referring to individuals that had a lesser degree of methylation than would be expected based of which parents donates the transgene. (EG maternally donated transgenes are expected to be high in methylation. A low degree of methylation would be an escaper.) …. But before bisulphite seq?

1. What was the purpose of the authors’ ChIP experiments, and why did they choose to look for specific histone modifications? What did they find? (Expected answer: max two sentences)

They were comparing the H3K9 trimethylation in paternally and maternally inherited transgenes, because H3K9me3 is a marker for transcriptional repression. Tri-methylation marker was highly present in maternal/+embryos but not in paternal/+embryos, which shows that maternal imprinting likely occurred in all embryonic cells.

1. Figure 3 depicts the results of a series of 3D chromatin conformation capture (aka “4C”) experiments. Try to “read” the figure and see if you can identify the information described in the text. You don’t need to answer this question in writing.
2. What does Figure 4 show? (Please describe/summarize its content including specific information).

A: The degree of interactions between the transgenes of maternal and paternal donated copies. Paternal copies of the transgene showed higher interaction between transgene with Dlxx1 and Dlx2 genes. Maternal transgene have lowers interaction between transgene and dlx& dlx2

B: the RT-qPCR on mRNA Maternal transgene have a significantly higher dix1/2 expression than WT. The paternal transgene have also a higher dix 1/2 expression than WT but not as high as maternal transgene.

C & D. diagrams show that possible models of the interactions between the inverted transgenes and the digit enhancer regions and the digit enhancers to dlx 1/2 locus. For maternal inherited inverted transgene, the highly methylated region chromatin compaction prevents interactions between the digits enhancers and invervted transgene. √

1. List any findings that you and your group found surprising.
   * They concluded that presence of LacZ reporter was necessary for maternal derived expression.
   * The inversion of the lacZ transgene has a different expression depending on which parent it is donated from.