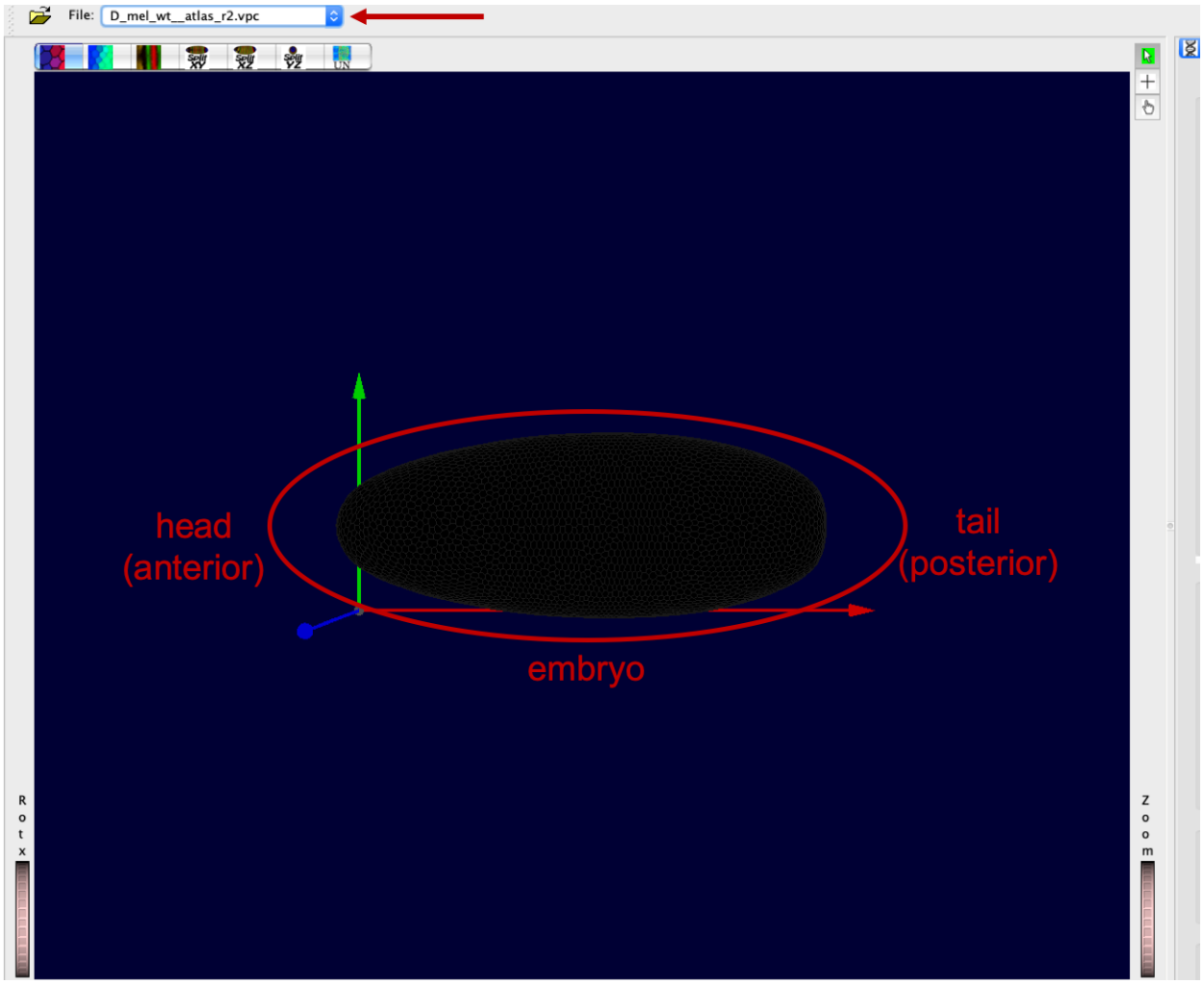
Part 1: Visualizing gene expression in *Drosophila melanogaster* embryos

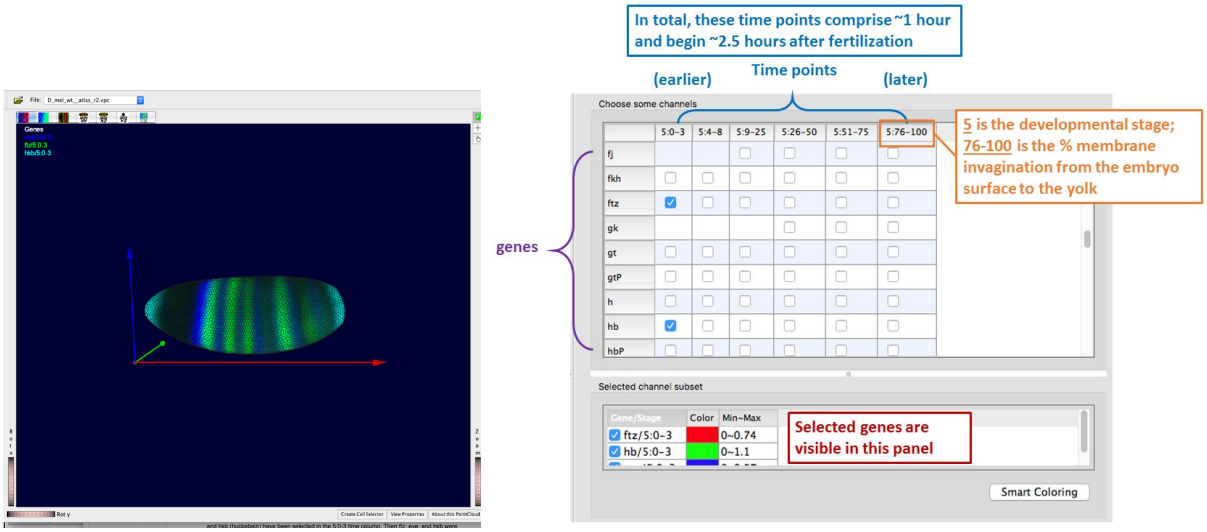
Please refer to the reference page on the back of the handout for a review of the genes mentioned during the activity.

PointCloudXplore (PCX) is a software tool developed to view embryonic gene expression in 3D and across time. Each VirtualEmbryo file (.vpc) describes average data for many genes, embryos, and developmental stages. To explore its capabilities, we will use PCX to look at the expression atlas for *D. melanogaster* (D_mel_wt_atlas_r2.vpc).

1. The wild-type expression atlas has been loaded on the PointCloudXplore app. The gene expression in this atlas starts at around 2 hours after fertilization. The embryo (the football shape in the center of the main screen) will start with no gene expression. Gain familiarity with the shape and manipulations of the 3D embryo by zooming in and out (scrolling) and rotating the embryo (click, hold, and move the cursor around).



2. The 99 genes in this atlas and the times in which they are expressed are listed in the left pane of PCX.
    - Select a couple of genes from the same time point (column) (ie. 5:0-3) to view their expression patterns.
    - The columns are labeled with the developmental stage (5) and the percent membrane invagination from the embryo surface to the yolk (ie 0-3) which refers to cellularization at this stage.
    - The time unit most related to this percentage is minutes, so the gene expression in this atlas takes place within 1 hour and starts ~2.5 hours post-fertilization.
    - The selected genes will show up in the “Selected channel subset” panel on the right.
    - Click the “Smart Coloring” button to make the image clearer, or manually pick the color for each gene by clicking on the color column under selected channel subset.



In this image, the genes ftz (fushi tarazu), eve (even-skipped), and hkb (huckebein) were selected in the 5:0-3 time column. Smart coloring was then used to give each gene its own color.

What categories of gene expression patterns do you notice?

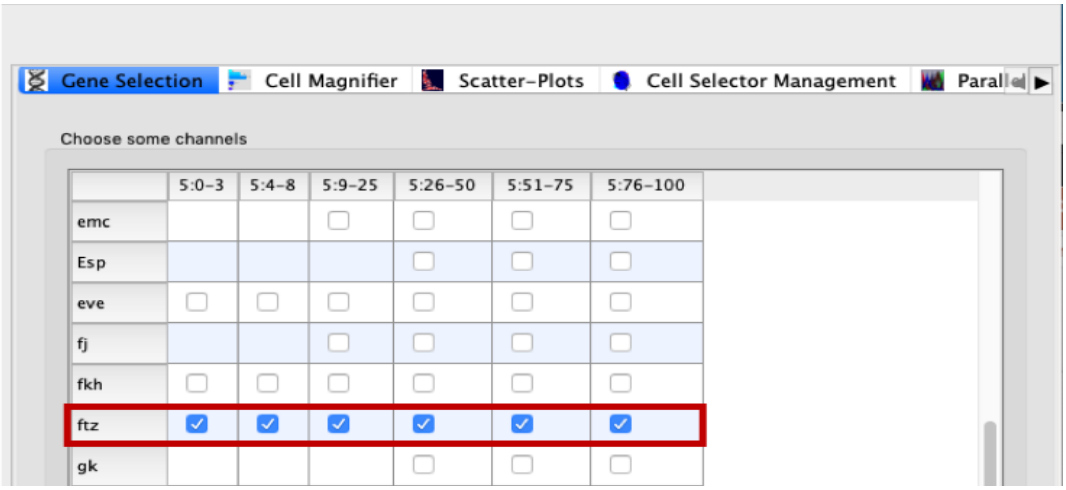
3. Try the unrolled view (UN in the top left corner) for a different method to look at the levels of gene expression. The unrolled view is equivalent to slicing the embryo from head to tail and flattening it. This allows you to view the complete surface area of the embryo along with gene expression levels.

Is gene expression typically symmetrical at the head and the tail? How about in other planes? What do you notice about the levels of gene expression as you circumnavigate the embryo?

How does this compare with what you may have expected?

4. By clicking on different genes to visualize their gene expression patterns, can you identify some genes that are gap genes? How about pair-rule genes?

5. To see how an expression pattern evolves over time, view the expression patterns for single gene at different time points. A good example is the gene fushi tarazu (ftz) that is expressed in a pattern of stripes.



Discuss with a partner: how does expression of ftz change over time?

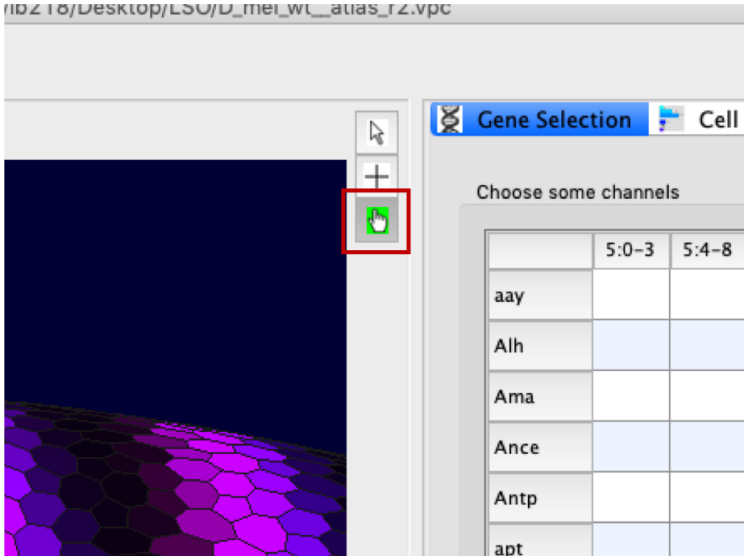
Do the bands shift in the anterior or posterior direction? If so, which?

Do the bands change in width? Do they become wider or thinner?

Do the bands change in number?

Considering the downstream roles of these gene spatial patterns in segmentation, would you expect genes to be more or less specifically defined in space over time?

6. In the center of the screen, click on the hand icon, and click on a cell in the visualization window. Expression values for all genes in the selected cell are displayed on the right.



7. Now we'll compare this average expression to an individual embryo's expression pattern by selecting "v5_s12781-29fe08-23.pce" from the file menu in the top left corner.
    - The gene expression patterns we can visualize in this embryo are hb (hunchback) and eve (even-skipped).
    - To compare between the atlas and the individual embryo, begin by selecting eve and hb in the .vpc file from one time category.
    - In the .pce file, select Coumarin_cell (hb) and Cy3_cell for eve.

How does the cell geometry compare between the individual embryo and the average?

How do the patterns of eve and hb compare between the individual embryo and the average? Consider the widths of eve stripes (number of cells wide) and the boundaries of the expression domains.

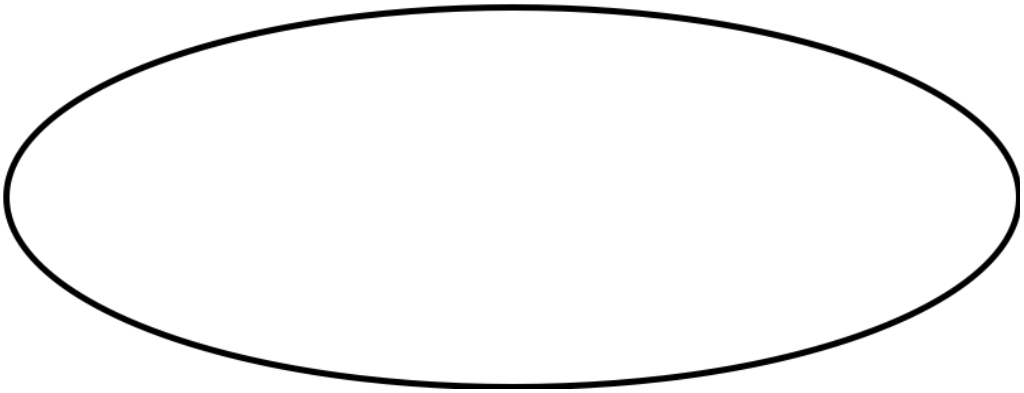
Note: For future reference, the PCX software is available online at the link below and more PCX capabilities are described in its online documentation.
http://bdtnp.lbl.gov:8080/Fly-Net/bioimaging.jsp?w=summary

Part 2: Visualizing gene expression in mutant *Drosophila melanogaster* embryos

From the paper that created and described the bicoid RNAi atlas: “We studied the behavior of the anterior-posterior segmentation network in *Drosophila melanogaster* embryos by depleting a key maternal input, *bicoid* (*bcd*), and measuring gene expression patterns of the network at cellular resolution. This method results in a gene expression atlas containing the levels of mRNA or protein expression of 13 core patterning genes over six time points for every cell of the blastoderm embryo. This is the first cellular resolution dataset of a genetically perturbed *Drosophila* embryo that captures all cells in 3D.” (Staller et al., *Development* 2015)

The bicoid RNAi atlas was created by combining the expression of many embryos stained for 1 of the 13 genes in the atlas. The stained embryos that we will look at under the fluorescence microscope in Part 3 are examples of the embryos that made up the final atlas. Compare the wt expression atlas (D_mel_wt_atlas_r2.vpc) to the bcd RNAi atlas (bcdrnai_20140807_v1.1.vpc).

From the wt atlas: Where is bicoid expressed at time 0-3? Draw the expression pattern.



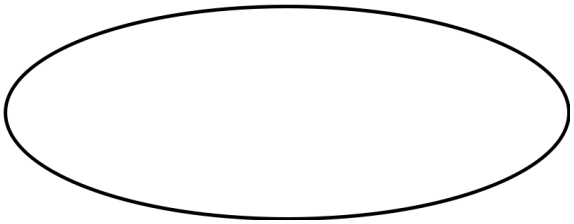
Since bicoid is an activator and drives expression of the gap genes, what would you expect to happen if bicoid was missing (ie. knocked down with RNAi)?

Comparing gene expression in WT and bicoid RNAi atlas:

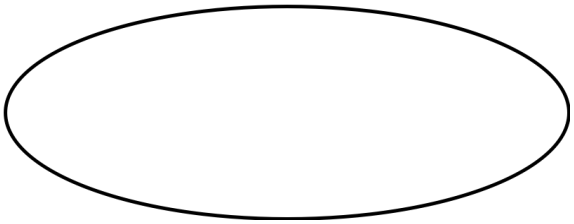
To visualize the effect of bicoid knockdown on the gap genes, let's compare the expression of two gap genes, knirps (kni) and tailless (tll), in the WT and the bicoid RNAi atlas. Draw the expression pattern of these genes in the WT and the bicoid knockdown embryos at an early time point (0-3 in WT or 4 in bcd).

Knirps (kni):

WT

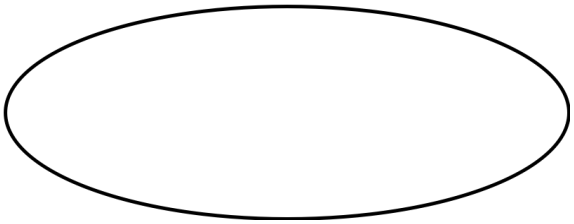


bcd

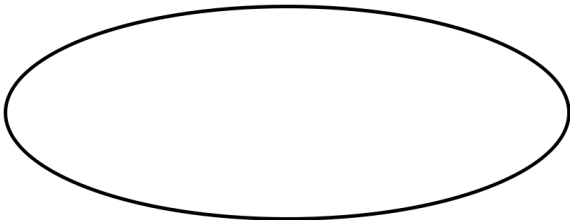


Tailless (tll):

WT



bcd



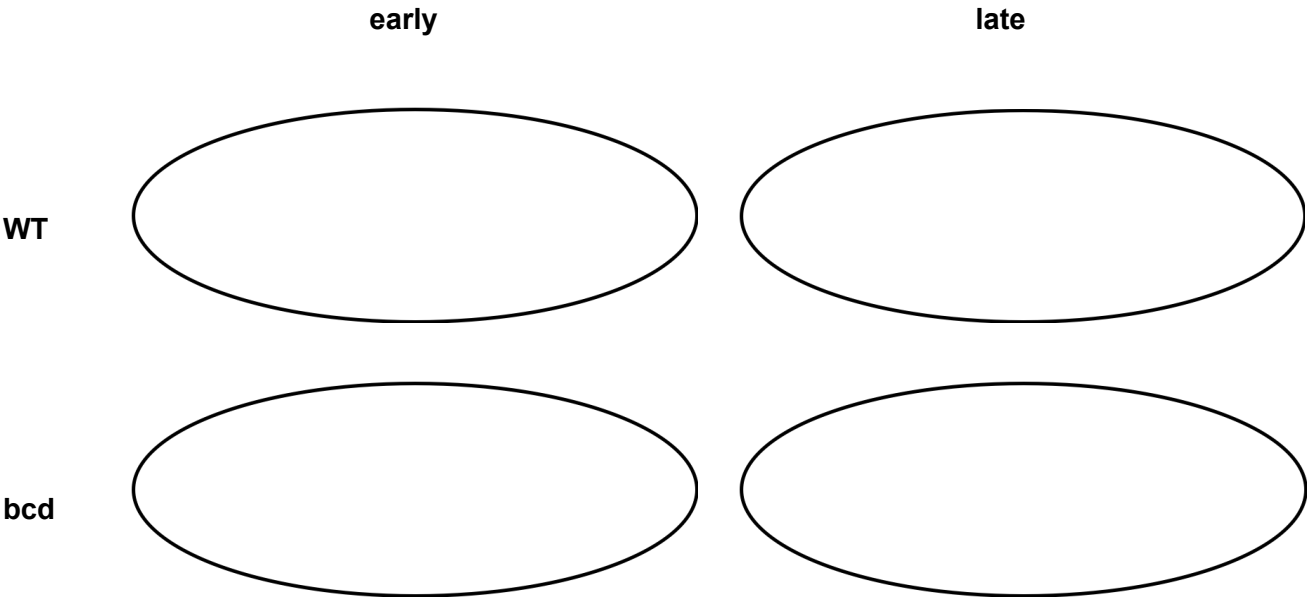
What can you conclude about how the lack of bcd affects the expression of these genes?

Based on where bcd is expressed in WT, hypothesize why you see this movement in the bicoid knockdown.

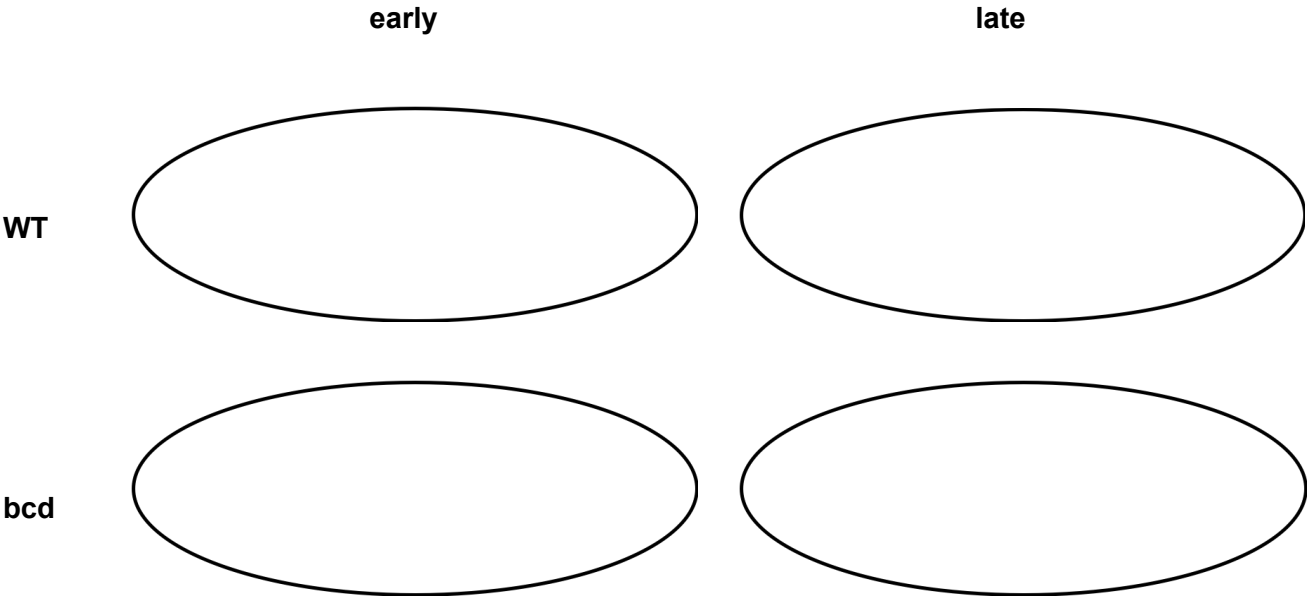
How would you expect this shift to affect downstream segmentation of the fly body plan?

To visualize the effect of bicoid knockdown on the pair-rule genes, compare the expression of hairy (h) and ftz (fushi tarazu) in the WT and the bicoid RNAi atlas. One of the benefits of having a gene expression atlas is that you can see how the expression pattern changes over time. Draw the expression pattern in the WT and the bicoid knockdown embryos at an early time point (4-8 in WT and 5 in bcd) and a late time point (51-75 in WT and 9 in bcd).

Hairy (h):



Fushi tarazu (ftz):



Note: Another good way to visualize expression changes over time is to select the two timepoints of your gene of interest and then visualize each timepoint in a different color in the selected channel subset section.

What do you notice about the expression pattern of both genes over time?

Since segmentation is critical for defining compartments of the embryo for organogenesis and for defining the segments of the body plan, how might you explain this correction over time?

Part 3: Visualizing gene expression in fixed embryos under the microscope

In this part, you will be looking at embryos that have been fixed and stained. Each microscope slide features a stain for a particular gene. Some stains show the wild-type (WT) gene expression of that gene, while other stains were done in mutant embryos that have decreased bicoid expression through RNAi. Since bicoid is a maternal effect gene, bicoid knockdown has many downstream effects in the expression of the gap genes and pair-rule genes.

One table at a time, we will view wild-type and mutant embryos with the fluorescence microscope and compare gene expression in the two conditions.

Under green light, you will see the red expression pattern of the gene of interest. Under the blue light, you will see a green expression pattern which is the nuclear stain so that all the nuclei in the embryo are marked.

Fluorescence Example 1

Gene: _____

How is the expression of the gene affected in the bicoid knockdown?

Fluorescence Example 2

Gene: _____

How is the expression of the gene affected in the bicoid knockdown?

Part 4: Modeling the regulation of the pair-rule gene eve in the *Drosophila* embryo

The genes included in this expression atlas are largely composed of early regulators of anterior-posterior pattern development. We can use this data to create hypotheses about how these genes regulate each other, particularly how gap genes regulate pair-rule genes. In this exercise, each table will be assigned a gap gene. In groups of 2, you will use WT and mutant data to create a model of how your gap gene represses the pair-rule gene even-skipped (eve), particularly which stripe boundaries your gap gene defines. This information can be used to draw what the eve stripe pattern would look like in a mutant for your gap gene.

At the end, we will collect the puzzle pieces from each table (each gap gene) to construct the full model of how eve is patterned by the gap genes in embryogenesis.

My table's gap gene: _____

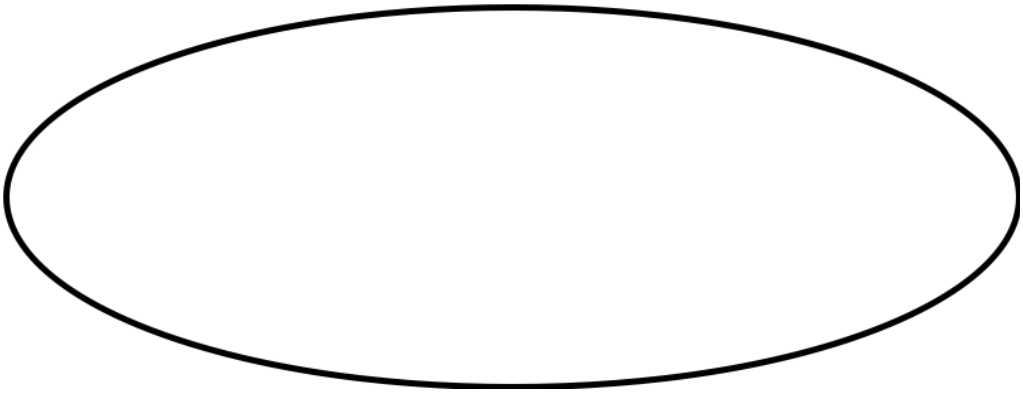
1. Display the expression pattern of *eve* from the 5:51-75 column in the WT atlas (D_mel_wt_atlas_r2.vpc).

   Bonus: Why do you think that the gene was named even-skipped? Hint: Add the gene expression pattern of another pair-rule gene fushi tarazu (ftz) in another color (or hit Smart Coloring when both are selected).

2. Add the expression pattern of your table's gap gene from the 5:0-3 column. The gap genes repress the expression of eve. Based on the expression pattern of this gap gene, generate a hypothesis about how this gene is regulating and defining the eve pattern. To generate this hypothesis, look at which stripes overlap with the expression of your gap gene (since the gap gene will repress eve expression wherever it is expressed).

   Additionally, the domains of gap gene expression dictate the boundaries of eve's stripe expression. Which stripe boundaries does this gene define (for example, [my gene] defines the anterior (left) side of the third stripe)?

3. The gap genes regulate eve expression by binding to specific sites on eve's enhancers to repress eve expression. Based on your conclusions from the WT regulation of eve, predict how the eve expression would change if eve's enhancers did not contain binding sites for your gap gene or if there was decreased or no expression of your gap gene (how would the stripe boundaries change - expand/shrink?).



After each pair at the table has made their conclusions and made their model for how this gap gene regulates eve, discuss as a table to corroborate or dispute the findings of your neighbors. As a table, come to a final conclusion and, on the large post-it board on the table, draw what eve expression would look like in a mutant of your gap gene. On your board, include what stripes and which stripe boundaries are regulated by your gap gene from question 2.