**KLP61F RNAi protocol (this is 2 pages long)**

**You will need:**

1. Happy GFP-tubulin-expressing S2 cells (you’ve been maintaining these!)
2. Double-stranded RNAi complementary to the KLP61F gene (you made this in week 4!)
3. Complete Schneider’s Medium (what you’ve been splitting your cells with)
4. Serum-free Schneider’s Medium (contains no FBS – you haven’t used this yet)
5. Sterile 6-well plate (see below)



This protocol must be done under sterile conditions in the biosafety cabinet (aka the hood you split your cells in). You will set up two conditions:

1. one well of cells treated with the KLP61F dsRNA (experimental)
2. one well of cells that you will NOT add dsRNA to but will otherwise subject to the same steps as the experimental (untreated control)

**Steps:**

1. Label the lid above one well “KLP61F dsRNA” and a 2nd well “Untreated”



1. Add 1 ml of complete Schneider’s medium (containing FBS) to each of the labeled wells
2. After you split your cells, add ~250 uls of the concentrated cells to each of the wells

(we are aiming for ~50% confluency, check after ~10 minutes and - if necessary - add more cells to each well)

1. Let cells adhere to the plastic for ~30 minutes
2. While the cells are adhering add 5 ug of the KLP61F dsRNA to a labeled 1.5 mL Eppendorf tube
3. Once the cells are adhered (after ~30 mins), add 1 mL of serum-free medium to the 5 ug of dsRNA and mix well
4. Carefully remove the medium from the “KLP61F dsRNA”-labeled well - being sure to limit the removal of the semi-adhered cells (tilting the plate helps here)
5. Add the serum-free medium + KLP61F dsRNA to the “KLP61F dsRNA”-labeled well
6. Carefully remove the medium from the “Untreated”-labeled well
7. Add 1 mL of serum-free medium to the “Untreated” well
8. After 1 hour add 1 ml of serum-containing complete Schneider’s medium to each well.
9. Place the plate at 24C for 2 days

[Here](https://www.jove.com/v/53594/generating-humanized-drosophila-s2-cell-line-sensitive-to) is a video from my lab that was published in a journal called JOVE a few years back. You could watch the whole thing if you are interested or you could skip ahead to Part III (RNA Interference) at 3:29 to hear about how to set up a KLP61F RNAi.