ChIP Harvest and Crosslinking Protocol/ Fill-in – Oestreich Lab

1. Count cells for relevant samples. We routinely harvest $2x10^7$-$2.5x10^7$ cells per pellet. (These numbers are based on $5x10^6$ cells/IP.)

2. Calculate the <u>volume</u> of cells needed for each ChIP pellet.
   Note: Because we need space to add formaldehyde and also to invert the samples in a 50mL conical tube, the values below should not exceed 40mL per sample.

The volumes for both samples should be equivalent, after harvest you will add cIMDM to the lower-volume sample to compensate.

   a. Total volume of media for each sample: _____mL

3. Using the value from 2.a. calculate the amount of formaldehyde (1% final concentration) and glycine (125mM final concentration) you'll need to use for each pellet. Then, weigh out the glycine into sterile 50mL conical tubes (1 per pellet).

   a. Volume of formaldehyde needed per pellet preparation:
      0.027mL x value from 2.a. = _____mL

   b. Weight of glycine needed per pellet preparation:
      0.00909g x value from 2.a. = _____g

4. Harvest the cells based on the values you calculated in 2.a. and 2.b. in labeled 50mL conical tubes.

5. Bring the volume of the lower-volume sample up to match the volume in 2.a., using cIMDM.

6. In the fume hood, add the amount of formaldehyde you calculated in 3.a. to each tube of resuspended cells. Invert every minute for 10 minutes to ensure efficient exposure to the chemical. It may help to wrap the edges of each tube in parafilm to prevent leaking.

7. After the 10-minute incubation, pour the cells into the tubes you prepared in step 3 (containing glycine from 3.b.) Invert every minute for 5 minutes. It may help to wrap the edges of each tube in parafilm to prevent leaking.

8. Centrifuge the samples at 1800rpm for <u>5 minutes</u> @ 4°C, and aspirate off the supernatant in the fume hood.

9. Resuspend the cell pellet for each sample in 10mL of ice cold PBS and transfer to labeled 15mL conicals. *Note: This step can be used to combine samples, if necessary.*

10. Centrifuge the samples at 1800rpm for <u>5 minutes</u> @ 4°C, and aspirate off the supernatant in the fume hood. Resuspend the sample in 10mL of ice cold PBS.

11. Centrifuge the samples at 1800rpm for <u>5 minutes</u> @ 4°C, and aspirate the supernatant in the fume hood.

12. Use the cells immediately OR snap freeze them in either LN_2 or in a dry ice/ethanol bath.