Prior to Cross Linking

1. **Prepare 4°C PBS with protease inhibitors, pre chill centrifuges and label tubes**

Collecting Cells

1. **For adherent cells, wash x 1 with PBS then trypsinize and collect cells in 50 ml conical tubes w complete media (the serum in the media will inactivate the trypsin) or PBS.**
2. **For cells in suspension, pipet cells and collect in appropriate vol. tube.**
3. **Centrifuge 5', RT, 1500 – 3000 rpm (300xg).**
4. **Re-suspend cells in a convenient volume of unsupplemented media or protein free PBS and count cell number. Note: the volume at this stage is optional and scaled to the number of cells you are working with. Typical volumes are between 1 and 45 mL, as detailed in the table below.**
5. **Optional: Remove 5 – 20 x 10^6 cells to snap-freeze (without fixation) for RNA and DNA extraction. Aliquot into tubes containing ~ 5e6 cells each. Snap Freeze.**

Cross Linking in Fume Hood

NOTE: change incubation temperatures accordingly

6. **Cross-link by adding 16% methanol free formaldehyde (important: single use ampoule; see part number below) to the cell suspension to a final conc. of 1% formaldehyde (see table for volumes). Once tube is sealed, it is safe to handle outside the fume hood.**
7. **Incubate at 37°C [manual agitation of cells in water bath, or using a rotating oven] for 10'**

uL of 16% Formaldehyde	Cell Suspension Vol.
63 uL	1.00 mL
250 uL	4.00 mL
625 uL	10.00 mL
938 uL	15.00 mL

Quench Cross Linking Reaction

NOTE: change incubation temperatures accordingly

8. **In fume hood, add <u>fresh</u> 2.5M glycine to a final conc. of 125mM (see table for volumes)**
9. **Incubate at 37°C for 5' [manual agitation in water bath, or rotating], then immediately place tubes on ice**

uL of 2.5 M Glycine	Cell Suspension Vol. including Formaldehyde
53 uL	1.063 mL
213 uL	4.250 mL
531 uL	10.625 mL
797 uL	15.938 mL

Rinsing and Aliquoting Cells

10. **Pellet cells 4°C for 5' @ 1500 – 3000 rpm and remove supt in fume hood.**
11. **Wash cells 2x with 1 vol. 4°C PBS (w/ protease inhibitor)***
12. **Spin each time at 4°C for 5' @ 1500 – 3000 rpm**
13. **Remove as much sup as possible in fume hood.**
14. **Re-suspend cells in limited volume (1ml for each 1 x 10^6 - 1 x 10^7 cells) of 4°C PBS* and transfer to 1.5 ml DNA low binding eppendorf tubes that have been pre chilled and labeled.**
    a. **Scale the number of cells per aliquot based on the intended use of the materials. If you anticipate a ChIP protocol with an antibody to a transcription factor, then 10^7 cells per aliquot is appropriate, since such ChIPs tend to require more cells.**
15. **Spin tubes 4°C , 3.5' ~ 4k and remove as much supt as possible**
16. **Snap freeze pellets and store at -80°C until use**

Items	Company	Cat#
[optional] Hybridization Oven (model 5430)	VWR	47746-130
Protease Inhibitor Cocktail- EDTA free	Roche	04693132001
Protease Inhibitor Cocktail mini-EDTA free	Roche	04693159001
16% Formaldehyde methanol free 10 x 1ml vials	Pierce/ Thermo	28906

NOTE: Experiment including liquid and solid waste needs to be contained in the fume hood