H1 ES Cell Processing for ENCODE (Cross-linking protocol):

Cell Harvesting:

1. Remove media from 2 flasks via aspiration (***work with no more than 4 flasks at a time—if you choose to work with 4 instead of two, please keep a maximum of 2 flasks per tube)
2. Add 8 ml Trypsin/EDTA to each T175 flask. Incubate at 37°C for 5 minutes. If cells aren't coming off the plate, re-incubate for 2 additional minutes, but no longer (this is strict, as T/E will destroy cells if left on for too long!).
3. Tap flask against palm of hand to dislodge cells, and pipet gently in flask to break up to single cells. Add cells to conical tube. Immediately neutralize with 8 ml basal medium with 10% FBS for each flask.
4. Take a cell count.
5. Spin cells down at 1200 rpm for 3 ½ minutes.

Cell Cross-Linking:

6. Resuspend in 22 ml of DMEM/F12.
7. Add 581 ul formaldehyde (Sigma F8775) to tube, mix by inversion, and incubate at 37°C for 10 minutes. Mix by inversion every 3 minutes.
8. Add 1.1 ml Glycine solution, mix by inversion, and incubate at 37°C for 5 minutes. Mix by inversion every 2 minutes.

Cell Washing:

9. Spin cells at 1000 rpm for 4 minutes.
10. Aspirate media without disturbing cell pellet.
11. Resuspend pellet in 25 ml ICE COLD Protease Inhibitor Cocktail.
12. Spin cells at 1250 rpm for 4 minutes, then aspirate media.
13. Resuspend pellet in 25 ml ICE COLD Protease Inhibitor Cocktail.
14. Spin cells at 1250 rpm for 4 minutes, then aspirate media.

Cell Freezing/Storing:

15. Resuspend in Protease Inhibitor Cocktail at $2x10^7$ cells/ml. Cells should not be left at this stage for more than 20-30 minutes (keep on ice)!!!
16. Add 1 ml to each 2 ml Eppendorf tube.
17. Spin cells down at 1250 rpm for 4 minutes.
18. CAREFULLY remove liquid (ie. use 1 ml Rainin pipetter with a blue tip).
19. Freeze in liquid nitrogen about 20 seconds and move to -80C storage until shipment.