K562 cell culture and formaldehyde cross-linking

1. Take out the K562 vial (1 million cells in 1 ml) from liquid nitrogen and thaw it in 37 degree waterbath. Suspend the washed cells in 10 ml RPMI with 10% FBS and GIBCO Antibiotic-antimycotic (Cat. No. 15240-062 5ml per 500 ml of culture.
2. Centrifuge at 700 rpm for 5 min.
3. Suspend the cells in 10 ml RPMI with 10%FBS and transfer them into a small cell culture flask (not a spinner flask) to be incubated in CO2 incubator.
4. These cells get into log phase in 5 to 7 days. Start counting at day 3. When the cell density reaches 0.7 to 0.8(x 10?)/ml. split the culture to about 0.4 million cells per ml with fresh RPMI with 10% FBS (this is the growth medium for K562). From this point on the cells should double every 24 hours,
5. From now on expect the cell density to double every 24 hours. (When the total cell number reaches 2X10>7, they can be stored as stock in liquid nitrogen at 1 million cells/ml (a total of 20 vials) in straight serum (FBS) containing 10% DMSO)
6. Split the cells when the density reaches around 0.75 million/ml.
7. Grow the cells to required numbers.

Note: For our labs purposes, we calculate the number of cells we will need for an experimental group, open a fresh vial of frozen cells, grow up the desired number of cells and then stop the culture-opening a fresh vial of cells for next experimental group. Therefore the cells are rarely passaged for more than 4 weeks.

Formaldehyde cross linking (all the solutions should be at room temp).

1. Centrifuge the required number of freshly growing K562 cells and suspend them in RPMI (without serum and antibiotics) to 2X10>7 cells /ml density. Add equal volume of freshly made 2% formaldehyde in RPMI to the cells. Rotate on end to end shaker for 10 minutes. Add 2M glycine stock to the final concentration of 125 mM and rotate on a shaker at room temp for 5 minutes followed by centrifuging at 300 rpm on a table top CS-6R centrifuge.
2. Discard the sup and process the pellet as per the ChIP protocol.