

Freezing cells for ChIP

Last updated: 5/29/20 (Bluma Lesch)

(reagents and buffers listed at end of protocol)

Protocol A: freezing in ChIP lysis buffer (preferred)

1. Resuspend cells in 500 ul cold PBS
2. Add 10 ul 11% FA for every 100 ul cells (e.g., 50 ul for 500 ul cells). Flick tube to mix and incubate 10 min, room temp.
3. Add 5 ul 2.5M glycine for every 100 ul sorted cells (e.g., 25 ul for 500 ul cells). Flick tube to mix and incubate 5 min, room temp.
4. Wash 2x in 500 ul cold PBS.
(For mammalian germ cells, I spin spin ~6000xg, 3 min for each wash. If there are low numbers of cells, you can't see a pellet – just pipet carefully around where you think the pellet should be and leave a little liquid behind.)
5. Resuspend in 100 ul ChIP lysis buffer, poke hole in lid of tube with a needle (to prevent tubes from exploding – it's also ok to skip this), flash freeze in liquid nitrogen and store at -80C.

Protocol B: freezing the pellet (also ok if you don't have ChIP lysis buffer)

1. Resuspend cells in 500 ul cold PBS
2. Add 10 ul 11% FA for every 100 ul cells (e.g., 50 ul for 500 ul cells). Flick tube to mix and incubate 10 min, room temp.
3. Add 5 ul 2.5M glycine for every 100 ul sorted cells (e.g., 25 ul for 500 ul cells). Flick tube to mix and incubate 5 min, room temp.
4. Wash 1x in 500 ul cold PBS.
(For mammalian germ cells, I spin spin ~6000xg, 3 min for each wash. If there are low numbers of cells, you can't see a pellet – just pipet carefully around where you think the pellet should be and leave a little liquid behind.)
5. Remove most of the supernatant, leaving a little liquid behind. Flash freeze in liquid nitrogen and store at -80C.

Reagents

11% formaldehyde in PBS:
297 ul 37% formaldehyde
703 ul PBS

2.5M glycine

cold PBS

ChIP lysis buffer:

1% SDS
10 mM EDTA
50 mM Tris-HCl (pH 8.0-8.1)
make up remaining volume with ddH₂O

References

Lesch BJ, Dokshin GA, Young RA, McCarrey JR, Page DC. A set of genes critical to development is epigenetically poised in mouse germ cells from fetal stages through completion of meiosis. *Proc Natl Acad Sci U S A*. 2013;110(40):16061-16066.

Lesch BJ, Silber SJ, McCarrey JR, Page DC. Parallel evolution of male germline epigenetic poising and somatic development in animals. *Nat Genet*. 2016;48(8):888-894.