

## Testis germ cell isolation

(reagents and buffers listed at end of protocol)

### Protocol

1. Dissect testes from adult male. Place in separate 3.5 cm dishes, remove tunica, and add a little PBS if there's a delay before dissociating.
2. When ready to dissociate, pipet off liquid.
3. Add 450 ul collagenase solution. Digest ~7 min. During digestion, gently tease apart tubules with forceps. Let sit without teasing 50-75% of the time. No swirling.
4. Pipet off liquid and wash 2x in 450 ul wash solution, 2-3 min each, with continued teasing.
5. Transfer to an Eppendorf, add another 450 ul wash solution, and allow to settle ~2 min. Remove supernatant.
6. Pipet off liquid and add 700 ul trypsin solution. Pipet up & down a few times with a P1000 and transfer to an Eppendorf.
7. Pipet up & down a few more times with a P1000 set at 300 ul, then with a P200 set at 200 ul.
8. Shake rapidly on a shaker at RT, pipetting up and down with a P200 ~every 2 min, for a total of 10-15 min. After 5-10 min, remove 2 ul and put on slide to check dissociation (on microscope in TC room). Should see mostly single cells, large tissue chunks gone.
9. If there are still too many tissue chunks, let settle for ~1 min, then transfer chunk-free cell suspension to a fresh eppendorf.
10. Add equal volume CCS to quench, then pipet up & down ~20x with a P200.
11. Spin 6000 rpm, 4 min, 4C.
12. Remove sup, resuspend in 1000 resuspension solution.
13. If staining with DyeCycle Green, add 2 ul dye, mix, and incubate in the dark at 37C, 30 min.
14. Filter cells over 40 um filter into a 50ml Falcon. Wash through with more resuspension solution.
15. If extracting RNA directly, spin 6000 rpm, 4 min, 4C. Resuspend in Trizol and continue with RNA isolation.

## **Solutions**

Cosmic calf serum (CCS)

### Collagenase solution

PBS

1:500 DNase I (Sigma, resuspended at 6820 U/ml)

0.1% (w/v) hyaluronidase (type I-S from bovine testes)

0.2% (w/v) collagenase (type IV)

### Trypsin solution

Trypsin (from TC fridge, 10 ml)

0.2% (w/v) collagenase

1:1000 DNase I (optional)

### Wash solution

PBS

1:1000 DNase I

### Resuspension solution

PBS

1% (w/v) BSA

## **References**

Lesch BJ, Tothova Z, Morgan EA, et al. Intergenerational epigenetic inheritance of cancer susceptibility in mammals. *Elife*. 2019;8:e39380. Published 2019 Apr 9.